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1       MPGQELRTVN GSQMLLVLLV LSWLPHGGAL SLAEASRASF PGPSELHSED  
51      SRFRELRLKRY EDLLTRLRAN QSWEDSNTDL VPAPAVRILT PEVRLGSGGH  
101     LHLRISRAAL PEGLPEASRL HRALFRLSPT ASRSWDVTRP LRRQLSLARP  
151     QAPALHLRLS PPPSQSDQLL AESSSARPQL ELHLPQAAAR GRRRARARNG  
201     DHCPPLGPGRC CRLHTVRASL EDLGWADWVL SPREVQVTMC IGACPSQFRA  
251     ANMHAQIKTS LHLRLKPDTVP APCCVPASYN PMVLIQKTD GVSLQTYDDL  
301     LAKDCHCI    (SEQ ID NO: 1)

FIGURE 1

(57) Abstract: In certain aspects, the present disclosure relates to polypeptides comprising a truncated, ligand-binding portion of the extracellular domain of T $\beta$ RII polypeptide useful to selectively antagonize a T $\beta$ RII ligand. The disclosure further provides compositions and methods for use in treating or preventing TGF $\beta$  associated disorders.

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## TGF-BETA RECEPTOR TYPE II VARIANTS AND USES THEREOF

### CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims priority from United States provisional application 61/868,713, filed on August 22, 2013; United States provisional application 61/906,270, filed 5 on November 19, 2013; and United States provisional application 61/906,849, filed November 20, 2013. The disclosure of the priority application is incorporated herein by reference in its entirety.

### BACKGROUND OF THE INVENTION

[0002] Members of the transforming growth factor-beta (TGF $\beta$ ) superfamily are 10 pleiotropic cytokines involved in essential cellular functions such as proliferation, differentiation, apoptosis, motility, extracellular matrix production, tissue remodeling, angiogenesis, immune response, cell adhesion, and also play a key role in pathophysiology of disease states as different as chronic inflammatory conditions and cancer. Members of the TGF $\beta$  superfamily have been classified into major family groupings, which include TGF $\beta$ s, 15 bone morphogenetic proteins (BMP), osteogenic proteins (OP), growth and differentiation factors (GDF), inhibins/activins, mullerian inhibitory substances (MIS) and glial derived neurotrophic factors (GDNF).

[0003] TGF $\beta$  superfamily members transduce their signals across the plasma 20 membrane by inducing the formation of heteromeric complexes of specific type I and type II serine/threonine kinase receptors, which in turn activate a particular subset of SMAD proteins (some inhibitory and some excitatory). The SMAD molecule compounds relay the signals into the nucleus where they direct transcriptional responses in concert with other proteins.

[0004] Dysfunctional TGF $\beta$  superfamily signaling has been linked to several 25 clinical disorders including cancer, fibrosis, bone diseases, diabetic nephropathy, as well as chronic vascular diseases such as atherosclerosis.

[0005] Thus, it is an object of the present disclosure to provide compositions and methods for modulating TGF $\beta$  superfamily signaling.

## SUMMARY OF THE INVENTION

**[0006]** In part, the disclosure provides T $\beta$ RII polypeptides and the use of such T $\beta$ RII polypeptides as selective antagonists for GDF15, TGF $\beta$ 1 or TGF $\beta$ 3. As described herein, polypeptides comprising part or all of the T $\beta$ RII extracellular domain (ECD), with or 5 without additional mutations, bind to and/or inhibit GDF15, TGF $\beta$ 1 or TGF $\beta$ 3 with varying affinities. Thus, in certain aspects, the disclosure provides T $\beta$ RII polypeptides for use in selectively inhibiting TGF $\beta$  superfamily associated disorders.

**[0007]** In certain aspects, the disclosure provides polypeptides comprising mutations and/or truncations in the extracellular domain of T $\beta$ RII. In certain aspects, the 10 disclosure provides a T $\beta$ RII fusion polypeptide comprising a first amino acid sequence from the extracellular domain of T $\beta$ RII and a heterologous amino acid sequence, wherein the first amino acid sequence comprises or consists of an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identical or identical to a) a sequence beginning at any of positions 23 to 35 of SEQ ID NO: 5 15 and ending at any of positions 153 to 159 of SEQ ID NO: 5 or b) a sequence beginning at any of positions 23 to 60 of SEQ ID NO: 6 and ending at any of positions 178 to 184 of SEQ ID NO: 6.

**[0008]** In certain aspects the disclosure provides polypeptides comprising a wild-type or altered and/or truncated extracellular domain of T $\beta$ RII fused to at least a portion of 20 the Fc domain of a human IgG2. Thus in certain aspects, the disclosure provides a T $\beta$ RII fusion polypeptide comprising a first amino acid sequence from the extracellular domain of T $\beta$ RII and a heterologous amino acid sequence, wherein the first amino acid sequence comprises or consists of an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identical or identical to a) a 25 sequence beginning at any of positions 23 to 35 of SEQ ID NO: 5 and ending at any of positions 153 to 159 of SEQ ID NO: 5 or b) a sequence beginning at any of positions 23 to 60 of SEQ ID NO: 6 and ending at any of positions 178 to 184 of SEQ ID NO: 6, and wherein the polypeptide comprises a second polypeptide sequence that comprises at least a constant domain of a human IgG2 and may optionally comprise or consist of an amino acid sequence 30 that is at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identical to SEQ ID NO: 19, and wherein an linker is optionally positioned between the first polypeptide and the second polypeptide. An example of the is provided as SEQ ID NO:50 and is encoded by the nucleic acid sequence of SEQ ID NO:51.

In certain embodiments, the disclosure provides polypeptides with an amino acid sequence that comprises or consists of an amino acid sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:50. In certain embodiments, the disclosure provides polypeptides that are encoded by a nucleic acid sequence that comprises or consists of a nucleic acid sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleic acid sequence of SEQ ID NO:51.

[0009] In some embodiments, the first amino acid sequence comprises or consists of the sequence beginning at position 23 of SEQ ID NO: 5 and ending at position 159 of SEQ ID NO: 5. In some embodiments, the first amino acid sequence comprises or consists of the sequence beginning at position 29 of SEQ ID NO: 5 and ending at position 159 of SEQ ID NO: 5. In some embodiments, the first amino acid sequence comprises or consists of the sequence beginning at position 35 of SEQ ID NO: 5 and ending at position 159 of SEQ ID NO: 5. In some embodiments, the first amino acid sequence comprises or consists of the sequence beginning at position 23 of SEQ ID NO: 5 and ending at position 153 of SEQ ID NO: 5. In some embodiments, the first amino acid sequence comprises or consists of the sequence beginning at position 29 of SEQ ID NO: 5 and ending at position 153 of SEQ ID NO: 5. In some embodiments, the first amino acid sequence comprises or consists of the sequence beginning at position 35 of SEQ ID NO: 5 and ending at position 153 of SEQ ID NO: 5.

20 NO: 5.

[0010] In some embodiments, the first amino acid sequence comprises or consists of the sequence beginning at position 23 of SEQ ID NO: 6 and ending at positions 184 of SEQ ID NO: 6. In some embodiments, the first amino acid sequence comprises or consists of the sequence beginning at position 29 of SEQ ID NO: 6 and ending at position 184 of SEQ ID NO: 6. In some embodiments, the first amino acid sequence comprises or consists of the sequence beginning at position 23 of SEQ ID NO: 6 and ending at position 178 of SEQ ID NO: 6. In some embodiments, the first amino acid sequence comprises or consists of the sequence beginning at position 29 of SEQ ID NO: 6 and ending at position 178 of SEQ ID NO: 6.

30 [0011] In some embodiments, the first amino acid sequence comprises or consists of a sequence that has a D at the position corresponding to position 36 of SEQ ID NO: 47 and/or a K at the position corresponding to position 76 of SEQ ID NO: 47.

[0012] In certain aspects, the disclosure provides a T $\beta$ RII fusion polypeptide comprising a first amino acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identical or identical to the sequence of SEQ ID NO: 7 or SEQ ID NO: 13, or active fragment thereof, and a second heterologous portion, wherein the first amino acid sequence has a D at the position corresponding to position 36 of SEQ ID NO: 47 and/or a K at the position corresponding to position 76 of SEQ ID NO: 47.

[0013] In some embodiments, the first amino acid sequence comprises an N-terminal truncation of 1-12 amino acids corresponding to amino acids 1-12 of SEQ ID NO: 7 or 1-37 amino acids corresponding to amino acids 1-37 of SEQ ID NO: 13. In some embodiments, the first amino acid sequence comprises an N-terminal truncation of 6 amino acids corresponding to amino acids 1-6 of SEQ ID NO: 7 or SEQ ID NO: 13. In some embodiments, the first amino acid sequence comprises an N-terminal truncation of 12 amino acids corresponding to amino acids 1-12 of SEQ ID NO: 7 or 37 amino acids corresponding to amino acids 1-37 of SEQ ID NO: 13. In some embodiments, the first amino acid sequence comprises a C-terminal truncation of 1-6 amino acids corresponding to amino acids 137-132 of SEQ ID NO: 7 or amino acids 162-157 of SEQ ID NO: 13. In some embodiments, the first amino acid sequence comprises a C-terminal truncation of 6 amino acids corresponding to amino acids 132-137 of SEQ ID NO: 7 or amino acids 157-162 of SEQ ID NO: 13. In some embodiments, the first amino acid sequence comprises an insertion corresponding to SEQ ID NO: 18 between the residues corresponding to positions 117 and 118 of SEQ ID NO: 47.

[0014] In some embodiments, the heterologous portion comprises one or more polypeptide portions that enhance one or more of: in vivo stability, in vivo half life, uptake/administration, tissue localization or distribution, formation of protein complexes, and/or purification. In some embodiments, the heterologous portion comprises a polypeptide portion selected from: an immunoglobulin Fc domain and a serum albumin. In a further embodiment, the immunoglobulin Fc domain is joined to the T $\beta$ RII polypeptide by a linker.

[0015] In some embodiments, the polypeptide includes one or more modified amino acid residues selected from: a glycosylated amino acid, a PEGylated amino acid, a farnesylated amino acid, an acetylated amino acid, a biotinylated amino acid, an amino acid

conjugated to a lipid moiety, and an amino acid conjugated to an organic derivatizing agent. In some embodiments, the polypeptide is glycosylated.

**[0016]** In certain aspects, the disclosure provides a T $\beta$ RII fusion polypeptide comprising a first amino acid sequence consisting of a portion of the extracellular domain of T $\beta$ RII that comprises an amino acid sequence that is at least 80%, at least 85%, at least 90%, or at least 95% identical to an amino acid sequence selected from SEQ ID NOS: 7-17 and 47-49, and a second heterologous portion. In certain aspects, the disclosure provides a T $\beta$ RII fusion polypeptide comprising a first amino acid sequence consisting of a portion of the extracellular domain of T $\beta$ RII that comprises an amino acid sequence that is at least 96% identical to an amino acid sequence selected from SEQ ID NOS: 7-17 and 47-49, and a second heterologous portion. In certain aspects, the disclosure provides a T $\beta$ RII fusion polypeptide comprising a first amino acid sequence consisting of a portion of the extracellular domain of T $\beta$ RII that comprises an amino acid sequence that is at least 97% identical to an amino acid sequence selected from SEQ ID NOS: 7-17 and 47-49, and a second heterologous portion. In certain aspects, the disclosure provides a T $\beta$ RII fusion polypeptide comprising a first amino acid sequence consisting of a portion of the extracellular domain of T $\beta$ RII that comprises an amino acid sequence that is at least 98% identical to an amino acid sequence selected from SEQ ID NOS: 7-17 and 47-49, and a second heterologous portion. In certain aspects, the disclosure provides a T $\beta$ RII fusion polypeptide comprising a first amino acid sequence consisting of a portion of the extracellular domain of T $\beta$ RII that comprises an amino acid sequence that is at least 99% identical to an amino acid sequence selected from SEQ ID NOS: 7-17 and 47-49, and a second heterologous portion. In certain aspects, the disclosure provides a T $\beta$ RII fusion polypeptide comprising a first amino acid sequence consisting of a portion of the extracellular domain of T $\beta$ RII that comprises an amino acid sequence is an amino acid sequence selected from SEQ ID NOS: 7-17 and 47-49 and a second heterologous portion.

**[0017]** In certain aspects, the disclosure provides a polypeptide comprising or consisting of an amino acid sequence that is at least 80%, at least 85%, at least 90%, or at least 95% identical to an amino acid sequence selected from SEQ ID NOS: 25, 27, 29, 31, 33, 35, 37, 39, 41 and 43 or the portion thereof with the leader sequence removed, e.g., a polypeptide comprising or consisting of an amino acid sequence that is at least 80%, at least 85%, at least 90%, or at least 95% identical to an amino acid sequence selected from SEQ ID

NOs: 53, 54, 55, 56, 57, 58, 59, 60, 61, and 62. In certain aspects, the disclosure provides a polypeptide comprising or consisting of an amino acid sequence that is at least 96% identical to an amino acid sequence selected from SEQ ID NOs: 25, 27, 29, 31, 33, 35, 37, 39, 41 and 43 or the portion thereof with the leader sequence removed, e.g., a polypeptide comprising or 5 consisting of an amino acid sequence that is at least 96% identical to an amino acid sequence selected from SEQ ID NOs: 53, 54, 55, 56, 57, 58, 59, 60, 61, and 62. In certain aspects, the disclosure provides a polypeptide comprising or consisting of an amino acid sequence that is at least 97% identical to an amino acid sequence selected from SEQ ID NOs: 25, 27, 29, 31, 33, 35, 37, 39, 41 and 43 or the portion thereof with the leader sequence removed, e.g., a 10 polypeptide comprising or consisting of an amino acid sequence that is at least 97% identical to an amino acid sequence selected from SEQ ID NOs: 53, 54, 55, 56, 57, 58, 59, 60, 61, and 62. In certain aspects, the disclosure provides a polypeptide comprising or consisting of an amino acid sequence that is at least 98% identical to an amino acid sequence selected from SEQ ID NOs: 25, 27, 29, 31, 33, 35, 37, 39, 41 and 43 or the portion thereof with the leader 15 sequence removed, e.g., a polypeptide comprising or consisting of an amino acid sequence that is at least 98% identical to an amino acid sequence selected from SEQ ID NOs: 53, 54, 55, 56, 57, 58, 59, 60, 61, and 62. In certain aspects, the disclosure provides a polypeptide comprising or consisting of an amino acid sequence that is at least 99% identical to an amino acid sequence selected from SEQ ID NOs: 25, 27, 29, 31, 33, 35, 37, 39, 41 and 43 or the 20 portion thereof with the leader sequence removed, e.g., a polypeptide comprising or consisting of an amino acid sequence that is at least 99% identical to an amino acid sequence selected from SEQ ID NOs: 53, 54, 55, 56, 57, 58, 59, 60, 61, and 62. In certain aspects, the disclosure provides a polypeptide comprising or consisting of an amino acid sequence selected from SEQ ID NOs: 25, 27, 29, 31, 33, 35, 37, 39, 41 and 43 or the portion thereof 25 with the leader sequence removed, e.g., a polypeptide comprising or consisting of an amino acid sequence selected from SEQ ID NOs: 53, 54, 55, 56, 57, 58, 59, 60, 61, and 62.

**[0018]** In certain aspects, the disclosure provides a T $\beta$ RII polypeptide comprising of an amino acid sequence encoded by a nucleic acid that hybridizes under stringent conditions to a complement of a nucleotide sequence selected from SEQ ID NOs: 26, 28, 30, 32, 34, 36, 30 38, 40, 42 and 44.

**[0019]** In each of the foregoing, the T $\beta$ RII polypeptide may be selected that it does not include a full-length T $\beta$ RII ECD. A T $\beta$ RII polypeptide may be used as a monomeric

protein or in a dimerized form. A T $\beta$ RII polypeptide may also be fused to a second polypeptide portion to provide improved properties, such as increased half-life or greater ease of production or purification. A fusion may be direct or a linker may be inserted between the T $\beta$ RII polypeptide and any other portion. A linker may be structured or unstructured and may 5 consist of 1, 2, 3, 4, 5, 10, 15, 20, 30, 50 or more amino acids, optionally relatively free of secondary structure.

**[0020]** In some embodiments, a T $\beta$ RII polypeptide of the disclosure binds human GDF15 with an equilibrium dissociation constant ( $K_D$ ) less than  $1 \times 10^{-8}$  M.

10 **[0021]** In some embodiments, a T $\beta$ RII polypeptide of the disclosure has a glycosylation pattern characteristic of expression of the polypeptide in CHO cells.

**[0022]** In some embodiments, the disclosure provides a homodimer comprising two T $\beta$ RII polypeptides of the disclosure.

15 **[0023]** In some embodiments, the disclosure provides an isolated polynucleotide comprising a coding sequence for the T $\beta$ RII polypeptides of the disclosure. In some embodiments, the disclosure provides a recombinant polynucleotide comprising a promoter sequence operably linked to the isolated polynucleotide. In some embodiments, the disclosure provides a cell transformed with an isolated polynucleotide or a recombinant polynucleotide of the disclosure. In some embodiments, the cell is a mammalian cell. In some embodiments, the cell is a CHO cell or a human cell. In some embodiments, the cell is 20 an HEK-293 cell.

**[0024]** In certain aspects, the disclosure provides a pharmaceutical preparation comprising the T $\beta$ RII polypeptides or homodimers of the disclosure and a pharmaceutically acceptable excipient.

25 **[0025]** In certain aspects, the disclosure provides a method of modulating the response of a cell to a TGF $\beta$  superfamily member, the method comprising exposing the cell to a T $\beta$ RII polypeptide or homodimer of the disclosure.

[0026] In certain aspects, the disclosure provides a method of treating a disease or condition associated with a TGF $\beta$  superfamily member in a patient in need thereof, the method comprising administering to the patient an effective amount of the T $\beta$ RII polypeptides or homodimers of the disclosure. In some embodiments, the TGF $\beta$  superfamily member is TGF $\beta$ 1, TGF $\beta$ 3 or GDF15.

[0027] In some embodiments, the disease or condition is a cancer. In some embodiments, the cancer is selected from stomach cancer, intestinal cancer, skin cancer, breast cancer, melanoma, bone cancer and thyroid cancer.

[0028] In some embodiments, the disease or condition is a fibrotic or sclerotic disease or disorder. In some embodiments, the fibrotic or sclerotic disease or disorder is selected from scleroderma, atherosclerosis, liver fibrosis, diffuse systemic sclerosis, glomerulonephritis, neural scarring, dermal scarring, radiation-induced fibrosis, hepatic fibrosis, and myelofibrosis.

[0029] In some embodiments, the disease or condition is heart disease.

[0030] In some embodiments, the disease or condition is selected from hereditary hemorrhagic telangiectasia (HHT), Marfan syndrome, Loeys-Dietz syndrome, familial thoracic aortic aneurysm syndrome, arterial tortuosity syndrome, pre-eclampsia, atherosclerosis, restenosis, and hypertrophic cardiomyopathy/congestive heart failure.

[0031] In certain aspects, the disclosure provides an antibody, or antigen binding fragment thereof, that binds to GDF15 and blocks the interaction between GDF15 and T $\beta$ RII.

[0032] In certain aspects, the disclosure provides a GDF15 polypeptide comprising the amino acid sequence of SEQ ID NO: 1 or a fragment thereof that binds T $\beta$ RII, wherein the GDF15 polypeptide is at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% pure, with respect to protein contaminants.

[0033] In certain aspects, the disclosure provides a GDF15 polypeptide comprising the amino acid sequence of SEQ ID NO: 1 or a fragment thereof that binds to a T $\beta$ RII

polypeptide of the disclosure, wherein the GDF15 polypeptide is at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% pure, with respect to protein contaminants.

[0034] In some embodiments, the GDF15 polypeptide binds T $\beta$ RII with an equilibrium dissociation constant ( $K_D$ ) of no greater than  $10^{-8}$  M. In some embodiments, the 5 GDF15 polypeptide binds to a T $\beta$ RII polypeptide of the disclosure with an equilibrium dissociation constant ( $K_D$ ) of no greater than  $10^{-8}$  M.

[0035] In some embodiments, the GDF15 polypeptide is produced by expression in CHO cells.

[0036] In certain aspects, the disclosure provides a method of concentrating or 10 purifying GDF15, comprising contacting a sample containing GDF15 with a T $\beta$ RII polypeptide of the disclosure.

## BRIEF DESCRIPTION OF THE DRAWINGS

[0037] **Figure 1** shows the amino acid sequence of native precursor for human GDF15 (NCBI reference seq: NP\_004855.2). Solid underline indicates mature GDF15 15 (residues 197-308), with N-terminus determined by sequencing. Dotted underline denotes leader (residues 1-29).

[0038] **Figure 2** shows a nucleotide sequence encoding native precursor for human GDF15. Solid underline indicates the sequence encoding mature GDF15 (nucleotides 589-924), and dotted underline denotes the sequence encoding the leader (nucleotides 1-87). A 20 silent mutation (G456A) used to disrupt a SfOI site in NM\_004864.2 is double underlined.

[0039] **Figure 3** shows the amino acid sequence of native precursor for murine GDF15 (NP\_035949.2). Solid underline indicates mature GDF15 (residues 192-303), with N-terminus determined by sequencing. Dotted underline denotes leader (residues 1-30).

[0040] **Figure 4** shows a nucleotide sequence encoding native precursor for murine 25 GDF15 (derived from NM\_011819.2). Solid underline indicates the sequence encoding mature GDF15 (nucleotides 574-909), and dotted underline denotes the sequence encoding the leader (nucleotides 1-90).

[0041] **Figure 5** shows the amino acid sequence of native precursor for the B (short) isoform of human TGF $\beta$  receptor type II (hT $\beta$ RII) (NP\_003233.4). Solid underline indicates the mature extracellular domain (ECD) (residues 23-159), and double underline indicates valine that is replaced in the A (long) isoform. Dotted underline denotes leader (residues 1-22).

[0042] **Figure 6** shows the amino acid sequence of native precursor for the A (long) isoform of human T $\beta$ RII (NP\_001020018.1). Solid underline indicates the mature ECD (residues 23-184), and double underline indicates the splice-generated isoleucine substitution. Dotted underline denotes leader (residues 1-22).

[0043] **Figure 7** shows N-terminal alignment of hT $\beta$ RII<sub>short</sub> truncations and their hT $\beta$ RII<sub>long</sub> counterparts. The 25-amino-acid insertion present in hT $\beta$ RII<sub>long</sub> truncations is underlined. Note that the splicing process causes the valine flanking the insertion site in the short isoform to be replaced by an isoleucine in the long isoform. Boxed sequence denotes leader.

## 15 DETAILED DESCRIPTION OF THE INVENTION

### 1. Overview

[0044] Proteins described herein are the human forms, unless otherwise specified. NCBI references for the proteins are as follows: human T $\beta$ RII isoform A (hT $\beta$ RII<sub>long</sub>), NP\_001020018.1; human T $\beta$ RII isoform B (hT $\beta$ RII<sub>short</sub>), NP\_003233.4; human GDF15, NP\_004855.2; murine GDF15, NP\_035949.2. Sequences of native T $\beta$ RII and GDF15 proteins from human and mouse are set forth in Figures 1-6.

[0045] The TGF $\beta$  superfamily contains a variety of growth factors that share common sequence elements and structural motifs. These proteins are known to exert biological effects on a large variety of cell types in both vertebrates and invertebrates. Members of the superfamily perform important functions during embryonic development in pattern formation and tissue specification and can influence a variety of differentiation processes, including adipogenesis, myogenesis, chondrogenesis, cardiogenesis, hematopoiesis, neurogenesis, and epithelial cell differentiation. By manipulating the activity of a member of the TGF $\beta$  family, it is often possible to cause significant physiological changes in an organism. For example, the Piedmontese and Belgian Blue cattle breeds carry

a loss-of-function mutation in the GDF8 (also called myostatin) gene that causes a marked increase in muscle mass. Grobet et al., *Nat Genet.* 1997, 17(1):71-4. Similarly, in humans, inactive alleles of GDF8 are associated with increased muscle mass and, reportedly, exceptional strength. Schuelke et al., *N Engl J Med* 2004, 350:2682-8.

5 [0046] TGF $\beta$  signals are mediated by heteromeric complexes of type I (e.g. T $\beta$ RI) and type II (e.g. T $\beta$ RII) serine/ threonine kinase receptors, which phosphorylate and activate downstream SMAD proteins upon ligand stimulation (Massagué, 2000, *Nat. Rev. Mol. Cell Biol.* 1:169-178). These type I and type II receptors are transmembrane proteins, composed of a ligand-binding extracellular domain with cysteine-rich region, a transmembrane domain, 10 and a cytoplasmic domain with predicted serine/threonine specificity. Type I receptors are essential for signaling; and type II receptors are required for binding ligands and for expression of type I receptors. Type I and II receptors form a stable complex after ligand binding, resulting in phosphorylation of type I receptors by type II receptors. TGF $\beta$  has three mammalian isoforms, TGF $\beta$ 1, TGF $\beta$ 2 and TGF $\beta$ 3, each with distinct functions in vivo. The 15 binding of TGF $\beta$ s to T $\beta$ RII is a crucial step in initiating activation of the TGF $\beta$  signaling pathway, leading to phosphorylation of SMAD2, and translocation of the activated SMAD2/SMAD4 complex to the nucleus to modulate gene expression.

20 [0047] Growth differentiation factor 15 (GDF15) is a member of the TGF $\beta$  family. Like other ligands in the TGF $\beta$  superfamily, which contain a characteristic cysteine knot motif, mature GDF15 is synthesized with a larger prodomain (Harrison et al., *Growth Factors* 29:174, 2011; Shi et al., *Nature* 474:343, 2011) that is removed through cleavage by a furin-like protease at the canonical RXXR site to generate mature dimeric GDF15. GDF15 has 25 been described in the literature as macrophage inhibitory cytokine-1 (MIC-1), placental bone morphogenic protein (PLAB), placental transforming growth factor beta (PTGF $\beta$ ), prostate derived factor (PDF), and non-steroidal anti-inflammatory activated gene-1 (NAG-1) reflecting the different functions that have been implied for this protein. GDF15 has been linked to several physiologic and pathologic conditions. For example, GDF15 is highly expressed in the placenta, and is necessary for the maintenance of pregnancy. GDF15 concentration is also notably increased in the serum of patients with prostate, colorectal, or 30 pancreatic cancer, as well as glioma. GDF15 has not been shown biochemically to bind or interact directly with any receptor. The present disclosure relates in part to the discovery that the TGF $\beta$  type II receptor, T $\beta$ RII, binds to GDF15 with high affinity and is a functional

receptor for GDF15. T $\beta$ RII fusion polypeptides, and other polypeptides containing a ligand-binding portion of T $\beta$ RII are demonstrated herein to inhibit GDF15-induced gene activation. The potent inhibition of GDF15 signaling provides evidence that T $\beta$ RII is a functional type II receptor for GDF15, opening a new avenue for therapeutic interventions in this signaling 5 pathway. Therefore, in part, the disclosure identifies a physiological, high-affinity receptor for GDF15 polypeptides.

[0048] Surprisingly, soluble T $\beta$ RII polypeptides are shown herein to have highly specific, high-affinity binding for GDF15. T $\beta$ RII is the known type II receptor for TGF $\beta$  and binds with high affinity to TGF $\beta$ 1 and TGF $\beta$ 3. Human T $\beta$ RII occurs naturally in at least two 10 isoforms – A (long) and B (short) – generated by alternative splicing in the extracellular domain (ECD) (Figures 6 and 5 and SEQ ID NOS: 6 and 5). The long isoform has a 25-amino-acid insertion and the splicing process causes the valine flanking the insertion site in the short isoform to be replaced by an isoleucine in the long isoform. Soluble receptor ectodomains can function as scavengers or ligand traps to inhibit ligand-receptor interactions. 15 Ligand traps such as soluble T $\beta$ RII-Fc fusion proteins incorporating the native T $\beta$ RII extracellular domain (ectodomain) will function as pan-inhibitors against T $\beta$ RII ligands, including, TGF $\beta$ 1, TGF $\beta$ 3 and based on the findings disclosed herein, GDF15. While in some therapeutic settings this broader spectrum of ligand-binding and signal inhibition may be advantageous, in other settings a more selective molecule may be superior. It is highly 20 desirable for ligand traps such as T $\beta$ RII ectodomain polypeptides to exhibit selective ligand-binding profiles. The present disclosure relates to the surprising discovery that polypeptides comprising a truncated portion of the extracellular domain of T $\beta$ RII and/or mutations within the extracellular domain have differential inhibitory effects on cell signaling by GDF15, TGF $\beta$ 1 or TGF $\beta$ 3. In part, the disclosure provides ligand traps, generated by a series of 25 mutations and/or truncations in the extracellular domain of T $\beta$ RII, that exhibit varying ligand-binding profiles distinct from that of the native T $\beta$ RII extracellular domain. The variant T $\beta$ RII polypeptides disclosed herein provide advantageous properties relative to the native full-length extracellular domain, and may be used to selectively inhibit pathways mediated by the different T $\beta$ RII ligands *in vivo*.

30 [0049] Thus, in certain aspects, the disclosure provides T $\beta$ RII polypeptides as antagonists of GDF15, TGF $\beta$ 1 or TGF $\beta$ 3 for use in treating various GDF15-, TGF $\beta$ 1- or TGF $\beta$ 3-associated disorders. While not wishing to be bound to any particular mechanism of

action, it is expected that such polypeptides act by binding to GDF15, TGF $\beta$ 1 or TGF $\beta$ 3 and inhibiting the ability of these ligands to form ternary signaling complexes.

5 [0050] The terms used in this specification generally have their ordinary meanings in the art, within the context of this invention and in the specific context where each term is used. Certain terms are discussed below or elsewhere in the specification, to provide additional guidance to the practitioner in describing the compositions and methods of the invention and how to make and use them. The scope or meaning of any use of a term will be apparent from the specific context in which the term is used.

## 2. T $\beta$ RII Polypeptides

10 [0051] Naturally occurring T $\beta$ RII proteins are transmembrane proteins, with a portion of the protein positioned outside the cell (the extracellular portion) and a portion of the protein positioned inside the cell (the intracellular portion). Aspects of the present disclosure encompass variant T $\beta$ RII polypeptides comprising mutations within the extracellular domain and/or truncated portions of the extracellular domain of T $\beta$ RII. As 15 described above, human T $\beta$ RII occurs naturally in at least two isoforms – A (long) and B (short) – generated by alternative splicing in the extracellular domain (ECD) (Figures 6 and 5 and SEQ ID NOS: 6 and 5). SEQ ID NO: 7, which corresponds to residues 23-159 of SEQ ID NO: 5, depicts the native full-length extracellular domain of the short isoform of T $\beta$ RII. SEQ ID NO: 13, which corresponds to residues 23-184 of SEQ ID NO: 6, depicts the native 20 full-length extracellular domain of the long isoform of T $\beta$ RII. Unless noted otherwise, amino acid position numbering with regard to variants based on the T $\beta$ RII short and long isoforms refers to the corresponding position in the native precursors, SEQ ID NO: 5 and SEQ ID NO: 6, respectively.

25 [0052] In certain embodiments, the disclosure provides variant T $\beta$ RII polypeptides. A T $\beta$ RII polypeptide of the disclosure may bind to and inhibit the function of a TGF $\beta$  superfamily member, such as but not limited to, GDF15, TGF $\beta$ 1 or TGF $\beta$ 3. T $\beta$ RII polypeptides may include a polypeptide consisting of, or comprising, an amino acid sequence at least 80% identical, and optionally at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a truncated ECD domain of a naturally occurring T $\beta$ RII polypeptide, whose 30 C-terminus occurs at any of amino acids 153-159 of SEQ ID NO: 5. T $\beta$ RII polypeptides may include a polypeptide consisting of, or comprising, an amino acid sequence at least 80%

identical, and optionally at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a truncated ECD domain of a naturally occurring T $\beta$ RII polypeptide, whose C-terminus occurs at any of amino acids 178-184 of SEQ ID NO: 6. Optionally, a T $\beta$ RII polypeptide does not include more than 5 consecutive amino acids, or more than 10, 20, 30, 40, 50, 52, 5 60, 70, 80, 90, 100, 150 or 200 or more consecutive amino acids from a sequence consisting of amino acids 160-567 of SEQ ID NO: 5 or from a sequence consisting of amino acids 185-592 of SEQ ID NO: 6. The unprocessed T $\beta$ RII polypeptide may either include or exclude any signal sequence, as well as any sequence N-terminal to the signal sequence. As elaborated herein, the N-terminus of the mature (processed) T $\beta$ RII polypeptide may occur at 10 any of amino acids 23-35 of SEQ ID NO: 5 or 23-60 of SEQ ID NO: 6. Examples of mature T $\beta$ RII polypeptides include, but are not limited to, amino acids 23-159 of SEQ ID NO: 5 (set forth in SEQ ID NO: 7), amino acids 29-159 of SEQ ID NO: 5 (set forth in SEQ ID NO: 9), amino acids 35-159 of SEQ ID NO: 5 (set forth in SEQ ID NO: 10), amino acids 23-153 of SEQ ID NO: 5 (set forth in SEQ ID NO: 11), amino acids 29-153 of SEQ ID NO: 5 (set forth 15 in SEQ ID NO: 48), amino acids 35-153 of SEQ ID NO: 5 (set forth in SEQ ID NO: 47), amino acids 23-184 of SEQ ID NO: 6 (set forth in SEQ ID NO: 13), amino acids 29-184 of SEQ ID NO: 6 (set forth in SEQ ID NO: 15), amino acids 60-184 of SEQ ID NO: 6 (set forth in SEQ ID NO: 10), amino acids 23-178 of SEQ ID NO: 6 (set forth in SEQ ID NO: 16), amino acids 29-178 of SEQ ID NO: 6 (set forth in SEQ ID NO: 49), and amino acids 60-178 20 of SEQ ID NO: 6 (set forth in SEQ ID NO: 47). Likewise, a T $\beta$ RII polypeptide may comprise a polypeptide that is encoded by nucleotides 73-465 of SEQ ID NO: 30, nucleotides 73-447 of SEQ ID NO: 34, nucleotides 73-465 of SEQ ID NO: 38, nucleotides 91-465 of SEQ ID NO: 38, or nucleotides 109-465 of SEQ ID NO: 38, or silent variants thereof or nucleic acids that hybridize to the complement thereof under stringent hybridization 25 conditions (generally, such conditions are known in the art but may, for example, involve hybridization in 50% v/v formamide, 5x SSC, 2% w/v blocking agent, 0.1% N-lauroylsarcosine, and 0.3% SDS at 65°C overnight and washing in, for example, 5x SSC at about 65°C). It will be understood by one of skill in the art that corresponding variants based on the long isoform of T $\beta$ RII will include nucleotide sequences encoding the 25-amino acid 30 insertion along with a conservative Val-Ile substitution at the flanking position C-terminal to the insertion. The T $\beta$ RII polypeptides accordingly may include isolated extracellular portions of T $\beta$ RII polypeptides, including both the short and the long isoforms, variants thereof (including variants that comprise, for example, no more than 2, 3, 4, 5, 10, 15, 20, 25, 30, or 35 amino acid substitutions in the sequence corresponding to amino acids 23-159 of SEQ ID

NO: 5 or amino acids 23-184 of SEQ ID NO: 6), fragments thereof, and fusion proteins comprising any of the foregoing, but in each case preferably any of the foregoing T $\beta$ RII polypeptides will retain substantial affinity for at least one of GDF15, TGF $\beta$ 1 or TGF $\beta$ 3. Generally, a T $\beta$ RII polypeptide will be designed to be soluble in aqueous solutions at 5 biologically relevant temperatures, pH levels, and osmolarity.

[0053] In some embodiments, the variant T $\beta$ RII polypeptides of the disclosure comprise one or more mutations in the extracellular domain that confer an altered ligand binding profile. A T $\beta$ RII polypeptide may include one, two, five or more alterations in the amino acid sequence relative to the corresponding portion of a naturally occurring T $\beta$ RII polypeptide. In some embodiments, the mutation results in a substitution, insertion, or deletion at the position corresponding to position 70 of SEQ ID NO: 5. In some embodiments, the mutation results in a substitution, insertion, or deletion at the position corresponding to position 110 of SEQ ID NO: 5. Examples include, but are not limited to, an N to D substitution or a D to K substitution in the positions corresponding to positions 70 and 15 110, respectively, of SEQ ID NO: 5. Examples of such variant T $\beta$ RII polypeptides include, but are not limited to, the sequences set forth in SEQ ID NO: 8, SEQ ID NO:14, SEQ ID NO: 12 and SEQ ID NO: 17. A T $\beta$ RII polypeptide may comprise a polypeptide or portion thereof that is encoded by nucleotides 73-483 of SEQ ID NO: 26, nucleotides 73-465 of SEQ ID NO: 42 or silent variants thereof or nucleic acids that hybridize to the complement thereof under 20 stringent hybridization conditions.

[0054] In some embodiments, the variant T $\beta$ RII polypeptides of the disclosure further comprise an insertion of 36 amino acids (SEQ ID NO: 18) between the pair of glutamate residues (positions 151 and 152 of SEQ ID NO: 5, or positions 176 and 177 of SEQ ID NO: 6) located near the C-terminus of the human T $\beta$ RII ECD, as occurs naturally in 25 the human T $\beta$ RII isoform C (Konrad et al., BMC Genomics 8:318, 2007).

[0055] The disclosure further demonstrates that T $\beta$ RII polypeptides can be modified to selectively antagonize T $\beta$ RII ligands. Data presented here show that Fc fusion proteins comprising shorter N-terminally and C-terminally truncated variants of T $\beta$ RII polypeptides display differential inhibitory effects on cellular signaling mediated by GDF15, TGF $\beta$ 1 and 30 TGF $\beta$ 3. Specifically, N-terminally truncated variants beginning at amino acids 29 or 35 of SEQ ID NO: 5 and carrying, respectively, a 6- or 12-amino acid N-terminal truncation of the extracellular domain, were found to inhibit GDF15 most potently, TGF $\beta$ 3 least potently and

TGF $\beta$ 1 to an intermediate degree, compared to the full length extracellular domain of the short isoform of T $\beta$ RII. C-terminally truncated variants, ending at amino acid 153 of SEQ ID NO: 5 and carrying a 6-amino acid C-terminal truncation of the extracellular domain had no substantial effect on ligand binding and may therefore be used interchangeably with full length versions. An N to D substitution at the position corresponding to position 70 of SEQ ID NO: 5, was found to inhibit TGF $\beta$ 3 potently, have intermediate effect on GDF15 and negligible effect on TGF $\beta$ 1. The N70 residue represents a potential glycosylation site. Further, an Fc fusion protein comprising a D to K substitution at the position corresponding to position 110 of SEQ ID NO: 5, was found to inhibit GDF15 most potently, TGF $\beta$ 1 least potently and TGF $\beta$ 3 to an intermediate degree compared to compared to the full length extracellular domain of the short isoform of T $\beta$ RII. The region around position 110 has not been associated with selectivity for the known T $\beta$ RII ligands TGF $\beta$ 1, TGF $\beta$ 2 and TGF $\beta$ 3. Thus, unexpectedly, T $\beta$ RII polypeptides that contain mutations in the ECD, such as but not limited to, N70D and D110K (the numbering of the residues corresponds to that of SEQ ID NO: 5) and/or begin between amino acids 29 and 35 and/or terminate between amino acid 153 and amino acid 159 are all expected to be active and exhibit widely different inhibitory potencies towards the different ligands. Any of these truncated variant forms may be desirable to use, depending on the clinical or experimental setting.

**[0056]** In certain embodiments, a T $\beta$ RII polypeptide binds to GDF15, and the T $\beta$ RII polypeptide does not show substantial binding to TGF $\beta$ 1 or TGF $\beta$ 3. In certain embodiments, a T $\beta$ RII polypeptide binds to TGF $\beta$ 1, and the T $\beta$ RII polypeptide does not show substantial binding to GDF15 or TGF $\beta$ 3. In certain embodiments, a T $\beta$ RII polypeptide binds to TGF $\beta$ 3, and the T $\beta$ RII polypeptide does not show substantial binding to GDF15 or TGF $\beta$ 1. Binding may be assessed using purified proteins in solution or in a surface plasmon resonance system, such as a Biacore<sup>TM</sup> system.

**[0057]** In certain embodiments, a T $\beta$ RII polypeptide inhibits GDF15 cellular signaling, and the T $\beta$ RII polypeptide has an intermediate or limited inhibitory effect on TGF $\beta$ 1 or TGF $\beta$ 3. In certain embodiments, a T $\beta$ RII polypeptide inhibits TGF $\beta$ 1 cellular signaling, and the T $\beta$ RII polypeptide has an intermediate or limited inhibitory effect on GDF15 or TGF $\beta$ 3. In certain embodiments, a T $\beta$ RII polypeptide inhibits TGF $\beta$ 3 cellular signaling, and the T $\beta$ RII polypeptide has an intermediate or limited inhibitory effect on

GDF15 or TGF $\beta$ 1. Inhibitory effect on cell signaling can be assayed by methods known in the art.

[0058] Taken together, an active portion of a T $\beta$ RII polypeptide may comprise amino acid sequences 23-153, 23-154, 23-155, 23-156, 23-157, or 23-158 of SEQ ID NO: 5, 5 as well as variants of these sequences starting at any of amino acids 24-35 of SEQ ID NO: 5. Similarly, an active portion of a T $\beta$ RII polypeptide may comprise amino acid sequences 23- 10 178, 23-179, 23-180, 23-181, 23-182, or 23-183 of SEQ ID NO: 6, as well as variants of these sequences starting at any of amino acids 24-60 of SEQ ID NO: 6. Exemplary T $\beta$ RII polypeptides comprise amino acid sequences 29-159, 35-159, 23-153, 29-153 and 35-153 of 15 SEQ ID NO: 5 or amino acid sequences 29-184, 60-184, 23-178, 29-178 and 60-178 of SEQ ID NO: 6. Variants within these ranges are also contemplated, particularly those having at least 80%, 85%, 90%, 95%, or 99% identity to the corresponding portion of SEQ ID NO: 5 or SEQ ID NO: 6. A T $\beta$ RII polypeptide may be selected that does not include the sequence consisting of amino acids 160-567 of SEQ ID NO:5 or amino acids 185-592 of SEQ ID 15 NO:6.

[0059] As described above, the disclosure provides T $\beta$ RII polypeptides sharing a specified degree of sequence identity or similarity to a naturally occurring T $\beta$ RII polypeptide. To determine the percent identity of two amino acid sequences, the sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in one or both of a first and a 20 second amino acid or nucleic acid sequence for optimal alignment and non-homologous sequences can be disregarded for comparison purposes). The amino acid residues at corresponding amino acid positions are then compared. When a position in the first sequence is occupied by the same amino acid residue as the corresponding position in the second sequence, then the molecules are identical at that position (as used herein amino acid 25 "identity" is equivalent to amino acid "homology"). The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences.

[0060] The comparison of sequences and determination of percent identity and 30 similarity between two sequences can be accomplished using a mathematical algorithm (Computational Molecular Biology, Lesk, A. M., ed., Oxford University Press, New York, 1988; Biocomputing: Informatics and Genome Projects, Smith, D. W., ed., Academic Press,

New York, 1993; Computer Analysis of Sequence Data, Part 1, Griffin, A. M., and Griffin, H. G., eds., Humana Press, New Jersey, 1994; Sequence Analysis in Molecular Biology, von Heinje, G., Academic Press, 1987; and Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991).

5 [0061] In one embodiment, the percent identity between two amino acid sequences is determined using the Needleman and Wunsch (J Mol. Biol. (48):444-453 (1970)) algorithm which has been incorporated into the GAP program in the GCG software package (available at <http://www.gcg.com>). In a specific embodiment, the following parameters are used in the GAP program: either a Blosum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 10 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6. In yet another embodiment, the percent identity between two nucleotide sequences is determined using the GAP program in the GCG software package (Devereux, J., *et al.*, Nucleic Acids Res. 12(1):387 (1984)) (available at <http://www.gcg.com>). Exemplary parameters include using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. 15 Unless otherwise specified, percent identity between two amino acid sequences is to be determined using the GAP program using a Blosum 62 matrix, a GAP weight of 10 and a length weight of 3, and if such algorithm cannot compute the desired percent identity, a suitable alternative disclosed herein should be selected.

20 [0062] In another embodiment, the percent identity between two amino acid sequences is determined using the algorithm of E. Myers and W. Miller (CABIOS, 4:11-17 (1989)) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4.

25 [0063] Another embodiment for determining the best overall alignment between two amino acid sequences can be determined using the FASTDB computer program based on the algorithm of Brutlag *et al.* (*Comp. App. Biosci.*, 6:237-245 (1990)). In a sequence alignment the query and subject sequences are both amino acid sequences. The result of said global sequence alignment is presented in terms of percent identity. In one embodiment, amino acid sequence identity is performed using the FASTDB computer program based on the algorithm of Brutlag *et al.* (*Comp. App. Biosci.*, 6:237-245 (1990)). In a specific 30 embodiment, parameters employed to calculate percent identity and similarity of an amino acid alignment comprise: Matrix=PAM 150, k-tuple=2, Mismatch Penalty=1, Joining Penalty=20, Randomization Group Length=0, Cutoff Score=1, Gap Penalty=5 and Gap Size

Penalty=0.05.

[0064] T $\beta$ RII polypeptides may additionally include any of various leader sequences at the N-terminus. Such a sequence would allow the peptides to be expressed and targeted to the secretion pathway in a eukaryotic system. See, e.g., Ernst et al., U.S. Pat. No. 5,082,783 (1992). Alternatively, a native T $\beta$ RII signal sequence may be used to effect extrusion from the cell. Possible leader sequences include native leaders, tissue plasminogen activator (TPA) and honeybee mellitin (SEQ ID NOS. 22-24, respectively). Examples of T $\beta$ RII-Fc fusion proteins incorporating a TPA leader sequence include SEQ ID NOS: 25, 27, 29, 31, 33, 35, 37, 39, 41, and 43. Processing of signal peptides may vary depending on the 10 leader sequence chosen, the cell type used and culture conditions, among other variables, and therefore actual N-terminal start sites for mature T $\beta$ RII polypeptides may shift by 1, 2, 3, 4 or 5 amino acids in either the N-terminal or C-terminal direction. Examples of T $\beta$ RII-Fc fusion proteins include SEQ ID NOS: 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 53, 54, 55, 56, 57, 58, 59, 60, 61, and 62, as shown herein with the T $\beta$ RII polypeptide portion underlined (see 15 Examples). It will be understood by one of skill in the art that corresponding variants based on the long isoform of T $\beta$ RII will include the 25-amino acid insertion along with a conservative Val-Ile substitution at the flanking position C-terminal to the insertion.

[0065] In certain embodiments, the present disclosure contemplates specific mutations of the T $\beta$ RII polypeptides so as to alter the glycosylation of the polypeptide. Such 20 mutations may be selected so as to introduce or eliminate one or more glycosylation sites, such as O-linked or N-linked glycosylation sites. Asparagine-linked glycosylation recognition sites generally comprise a tripeptide sequence, asparagine-X-threonine (or asparagine-X-serine) (where "X" is any amino acid) which is specifically recognized by appropriate cellular glycosylation enzymes. The alteration may also be made by the addition 25 of, or substitution by, one or more serine or threonine residues to the sequence of the wild-type T $\beta$ RII polypeptide (for O-linked glycosylation sites). A variety of amino acid substitutions or deletions at one or both of the first or third amino acid positions of a glycosylation recognition site (and/or amino acid deletion at the second position) results in 30 non-glycosylation at the modified tripeptide sequence. Another means of increasing the number of carbohydrate moieties on a T $\beta$ RII polypeptide is by chemical or enzymatic coupling of glycosides to the T $\beta$ RII polypeptide. Depending on the coupling mode used, the sugar(s) may be attached to (a) arginine and histidine; (b) free carboxyl groups; (c) free

sulphydryl groups such as those of cysteine; (d) free hydroxyl groups such as those of serine, threonine, or hydroxyproline; (e) aromatic residues such as those of phenylalanine, tyrosine, or tryptophan; or (f) the amide group of glutamine. These methods are described in WO 87/05330 published Sep. 11, 1987, and in Aplin and Wriston (1981) CRC Crit. Rev.

5 Biochem., pp. 259-306, incorporated by reference herein. Removal of one or more carbohydrate moieties present on a T $\beta$ RII polypeptide may be accomplished chemically and/or enzymatically. Chemical deglycosylation may involve, for example, exposure of the T $\beta$ RII polypeptide to the compound trifluoromethanesulfonic acid, or an equivalent compound. This treatment results in the cleavage of most or all sugars except the linking sugar (N-acetylglucosamine or N-acetylgalactosamine), while leaving the amino acid sequence intact. Chemical deglycosylation is further described by Hakimuddin et al. (1987) Arch. Biochem. Biophys. 259:52 and by Edge et al. (1981) Anal. Biochem. 118:131. Enzymatic cleavage of carbohydrate moieties on T $\beta$ RII polypeptides can be achieved by the use of a variety of endo- and exo-glycosidases as described by Thotakura et al. (1987) Meth. 15 Enzymol. 138:350. The sequence of a T $\beta$ RII polypeptide may be adjusted, as appropriate, depending on the type of expression system used, as mammalian, yeast, insect and plant cells may all introduce differing glycosylation patterns that can be affected by the amino acid sequence of the peptide. In general, T $\beta$ RII polypeptides for use in humans will be expressed in a mammalian cell line that provides proper glycosylation, such as HEK293 or CHO cell 20 lines, although other mammalian expression cell lines, yeast cell lines with engineered glycosylation enzymes, and insect cells are expected to be useful as well.

[0066] This disclosure further contemplates a method of generating mutants, particularly sets of combinatorial mutants of a T $\beta$ RII polypeptide, as well as truncation mutants; pools of combinatorial mutants are especially useful for identifying functional 25 variant sequences. The purpose of screening such combinatorial libraries may be to generate, for example, T $\beta$ RII polypeptide variants which can act as either agonists or antagonist, or alternatively, which possess novel activities all together. A variety of screening assays are provided below, and such assays may be used to evaluate variants. For example, a T $\beta$ RII polypeptide variant may be screened for ability to bind to a T $\beta$ RII ligand, to prevent binding 30 of a T $\beta$ RII ligand to a T $\beta$ RII polypeptide or to interfere with signaling caused by a T $\beta$ RII ligand. The activity of a T $\beta$ RII polypeptide or its variants may also be tested in a cell-based or in vivo assay, particularly any of the assays disclosed in the Examples.

**[0067]** Combinatorially-derived variants can be generated which have a selective or generally increased potency relative to a T $\beta$ RII polypeptide comprising an extracellular domain of a naturally occurring T $\beta$ RII polypeptide. Likewise, mutagenesis can give rise to variants which have serum half-lives dramatically different than the corresponding wild-type 5 T $\beta$ RII polypeptide. For example, the altered protein can be rendered either more stable or less stable to proteolytic degradation or other processes which result in destruction of, or otherwise elimination or inactivation of, a native T $\beta$ RII polypeptide. Such variants, and the genes which encode them, can be utilized to alter T $\beta$ RII polypeptide levels by modulating the half-life of the T $\beta$ RII polypeptides. For instance, a short half-life can give rise to more 10 transient biological effects and can allow tighter control of recombinant T $\beta$ RII polypeptide levels within the patient. In an Fc fusion protein, mutations may be made in the linker (if any) and/or the Fc portion to alter the half-life of the protein.

**[0068]** A combinatorial library may be produced by way of a degenerate library of genes encoding a library of polypeptides which each include at least a portion of potential 15 T $\beta$ RII polypeptide sequences. For instance, a mixture of synthetic oligonucleotides can be enzymatically ligated into gene sequences such that the degenerate set of potential T $\beta$ RII polypeptide nucleotide sequences are expressible as individual polypeptides, or alternatively, as a set of larger fusion proteins (e.g., for phage display).

**[0069]** There are many ways by which the library of potential T $\beta$ RII polypeptide 20 variants can be generated from a degenerate oligonucleotide sequence. Chemical synthesis of a degenerate gene sequence can be carried out in an automatic DNA synthesizer, and the synthetic genes then be ligated into an appropriate vector for expression. The synthesis of degenerate oligonucleotides is well known in the art (see for example, Narang, SA (1983) Tetrahedron 39:3; Itakura et al., (1981) Recombinant DNA, Proc. 3rd Cleveland Sympos. 25 Macromolecules, ed. AG Walton, Amsterdam: Elsevier pp273-289; Itakura et al., (1984) Annu. Rev. Biochem. 53:323; Itakura et al., (1984) Science 198:1056; Ike et al., (1983) Nucleic Acid Res. 11:477). Such techniques have been employed in the directed evolution of other proteins (see, for example, Scott et al., (1990) Science 249:386-390; Roberts et al., (1992) PNAS USA 89:2429-2433; Devlin et al., (1990) Science 249: 404-406; Cwirla et al., 30 (1990) PNAS USA 87: 6378-6382; as well as U.S. Patent Nos: 5,223,409, 5,198,346, and 5,096,815).

[0070] Alternatively, other forms of mutagenesis can be utilized to generate a combinatorial library. For example, T $\beta$ RII polypeptide variants can be generated and isolated from a library by screening using, for example, alanine scanning mutagenesis and the like (Ruf et al., (1994) Biochemistry 33:1565-1572; Wang et al., (1994) J. Biol. Chem. 269:3095-3099; Balint et al., (1993) Gene 137:109-118; Grodberg et al., (1993) Eur. J. Biochem. 218:597-601; Nagashima et al., (1993) J. Biol. Chem. 268:2888-2892; Lowman et al., (1991) Biochemistry 30:10832-10838; and Cunningham et al., (1989) Science 244:1081-1085), by linker scanning mutagenesis (Gustin et al., (1993) Virology 193:653-660; Brown et al., (1992) Mol. Cell Biol. 12:2644-2652; McKnight et al., (1982) Science 232:316); by saturation mutagenesis (Meyers et al., (1986) Science 232:613); by PCR mutagenesis (Leung et al., (1989) Method Cell Mol Biol 1:11-19); or by random mutagenesis, including chemical mutagenesis, etc. (Miller et al., (1992) A Short Course in Bacterial Genetics, CSHL Press, Cold Spring Harbor, NY; and Greener et al., (1994) Strategies in Mol Biol 7:32-34). Linker scanning mutagenesis, particularly in a combinatorial setting, is an attractive method for identifying truncated (bioactive) forms of T $\beta$ RII polypeptides.

[0071] A wide range of techniques are known in the art for screening gene products of combinatorial libraries made by point mutations and truncations, and, for that matter, for screening cDNA libraries for gene products having a certain property. Such techniques will be generally adaptable for rapid screening of the gene libraries generated by the combinatorial mutagenesis of T $\beta$ RII polypeptides. The most widely used techniques for screening large gene libraries typically comprises cloning the gene library into replicable expression vectors, transforming appropriate cells with the resulting library of vectors, and expressing the combinatorial genes under conditions in which detection of a desired activity facilitates relatively easy isolation of the vector encoding the gene whose product was detected. Preferred assays include T $\beta$ RII ligand binding assays and ligand-mediated cell signaling assays.

[0072] In certain embodiments, the T $\beta$ RII polypeptides of the disclosure may further comprise post-translational modifications in addition to any that are naturally present in the T $\beta$ RII polypeptides. Such modifications include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation, pegylation (polyethylene glycol) and acylation. As a result, the modified T $\beta$ RII polypeptides may contain non-amino acid elements, such as polyethylene glycols, lipids, mono- or poly-saccharides, and phosphates.

Effects of such non-amino acid elements on the functionality of a T $\beta$ RII polypeptide may be tested as described herein for other T $\beta$ RII polypeptide variants. When a T $\beta$ RII polypeptide is produced in cells by cleaving a nascent form of the T $\beta$ RII polypeptide, post-translational processing may also be important for correct folding and/or function of the protein. Different 5 cells (such as CHO, HeLa, MDCK, 293, WI38, NIH-3T3 or HEK-293) have specific cellular machinery and characteristic mechanisms for such post-translational activities and may be chosen to ensure the correct modification and processing of the T $\beta$ RII polypeptides.

[0073] In certain aspects, functional variants or modified forms of the T $\beta$ RII polypeptides include fusion proteins having at least a portion of the T $\beta$ RII polypeptides and 10 one or more fusion domains. Well-known examples of such fusion domains include, but are not limited to, polyhistidine, Glu-Glu, glutathione S transferase (GST), thioredoxin, protein A, protein G, an immunoglobulin heavy chain constant region (Fc), maltose binding protein (MBP), or human serum albumin. A fusion domain may be selected so as to confer a desired property. For example, some fusion domains are particularly useful for isolation of the fusion 15 proteins by affinity chromatography. For the purpose of affinity purification, relevant matrices for affinity chromatography, such as glutathione-, amylase-, and nickel- or cobalt-conjugated resins are used. Many of such matrices are available in “kit” form, such as the Pharmacia GST purification system and the QIAexpress<sup>TM</sup> system (Qiagen) useful with (HIS<sub>6</sub>) fusion partners. As another example, a fusion domain may be selected so as to 20 facilitate detection of the T $\beta$ RII polypeptides. Examples of such detection domains include the various fluorescent proteins (e.g., GFP) as well as “epitope tags,” which are usually short peptide sequences for which a specific antibody is available. Well known epitope tags for which specific monoclonal antibodies are readily available include FLAG, influenza virus haemagglutinin (HA), and c-myc tags. In some cases, the fusion domains have a protease 25 cleavage site, such as for Factor Xa or Thrombin, which allows the relevant protease to partially digest the fusion proteins and thereby liberate the recombinant proteins therefrom. The liberated proteins can then be isolated from the fusion domain by subsequent chromatographic separation. In certain preferred embodiments, a T $\beta$ RII polypeptide is fused with a domain that stabilizes the T $\beta$ RII polypeptide in vivo (a “stabilizer” domain). By 30 “stabilizing” is meant anything that increases serum half life, regardless of whether this is because of decreased destruction, decreased clearance by the kidney, or other pharmacokinetic effect. Fusions with the Fc portion of an immunoglobulin are known to confer desirable pharmacokinetic properties on a wide range of proteins. Likewise, fusions to

human serum albumin can confer desirable properties. Other types of fusion domains that may be selected include multimerizing (e.g., dimerizing, tetramerizing) domains and functional domains.

[0074] As specific examples, the present disclosure provides fusion proteins comprising variants of T $\beta$ RII polypeptides fused to one of three Fc domain sequences (e.g., SEQ ID NOs: 19, 20, and 21). Optionally, the Fc domain has one or more mutations at residues such as Asp-265, Lys-322, and Asn-434 (numbered in accordance with the corresponding full-length IgG). In certain cases, the mutant Fc domain having one or more of these mutations (e.g., Asp-265 mutation) has reduced ability of binding to the Fc $\gamma$  receptor relative to a wildtype Fc domain. In other cases, the mutant Fc domain having one or more of these mutations (e.g., Asn-434 mutation) has increased ability of binding to the MHC class I-related Fc-receptor (FcRN) relative to a wildtype Fc domain.

[0075] It is understood that different elements of the fusion proteins may be arranged in any manner that is consistent with the desired functionality. For example, a T $\beta$ RII polypeptide may be placed C-terminal to a heterologous domain, or, alternatively, a heterologous domain may be placed C-terminal to a T $\beta$ RII polypeptide. The T $\beta$ RII polypeptide domain and the heterologous domain need not be adjacent in a fusion protein, and additional domains or amino acid sequences may be included C- or N-terminal to either domain or between the domains.

[0076] As used herein, the term "immunoglobulin Fc domain" or simply "Fc" is understood to mean the carboxyl-terminal portion of an immunoglobulin chain constant region, preferably an immunoglobulin heavy chain constant region, or a portion thereof. For example, an immunoglobulin Fc region may comprise 1) a CH1 domain, a CH2 domain, and a CH3 domain, 2) a CH1 domain and a CH2 domain, 3) a CH1 domain and a CH3 domain, 4) a CH2 domain and a CH3 domain, or 5) a combination of two or more domains and an immunoglobulin hinge region. In a preferred embodiment the immunoglobulin Fc region comprises at least an immunoglobulin hinge region a CH2 domain and a CH3 domain, and preferably lacks the CH1 domain.

[0077] In one embodiment, the class of immunoglobulin from which the heavy chain constant region is derived is IgG (Ig $\gamma$ ) ( $\gamma$  subclasses 1, 2, 3, or 4). Other classes of immunoglobulin, IgA (Ig $\alpha$ ), IgD (Ig $\delta$ ), IgE (Ig $\epsilon$ ) and IgM (Ig $\mu$ ), may be used. The choice of

appropriate immunoglobulin heavy chain constant region is discussed in detail in U.S. Pat. Nos. 5,541,087 and 5,726,044. The choice of particular immunoglobulin heavy chain constant region sequences from certain immunoglobulin classes and subclasses to achieve a particular result is considered to be within the level of skill in the art. The portion of the 5 DNA construct encoding the immunoglobulin Fc region preferably comprises at least a portion of a hinge domain, and preferably at least a portion of a CH<sub>3</sub> domain of Fc gamma or the homologous domains in any of IgA, IgD, IgE, or IgM.

[0078] Furthermore, it is contemplated that substitution or deletion of amino acids within the immunoglobulin heavy chain constant regions may be useful in the practice of the 10 methods and compositions disclosed herein. One example would be to introduce amino acid substitutions in the upper CH<sub>2</sub> region to create an Fc variant with reduced affinity for Fc receptors (Cole *et al.* (1997) *J. Immunol.* 159:3613).

[0079] In certain embodiments, the present disclosure makes available isolated and/or purified forms of the T $\beta$ RII polypeptides, which are isolated from, or otherwise 15 substantially free of (e.g., at least 80%, 90%, 95%, 96%, 97%, 98%, or 99% free of), other proteins and/or other T $\beta$ RII polypeptide species. T $\beta$ RII polypeptides will generally be produced by expression from recombinant nucleic acids.

[0080] In certain embodiments, the disclosure includes nucleic acids encoding 20 soluble T $\beta$ RII polypeptides comprising the coding sequence for an extracellular portion of a T $\beta$ RII protein. In further embodiments, this disclosure also pertains to a host cell comprising such nucleic acids. The host cell may be any prokaryotic or eukaryotic cell. For example, a polypeptide of the present disclosure may be expressed in bacterial cells such as *E. coli*, insect cells (e.g., using a baculovirus expression system), yeast, or mammalian cells. Other suitable host cells are known to those skilled in the art. Accordingly, some embodiments of 25 the present disclosure further pertain to methods of producing the T $\beta$ RII polypeptides.

### 3. Nucleic Acids Encoding T $\beta$ RII Polypeptides

[0081] In certain aspects, the disclosure provides isolated and/or recombinant 30 nucleic acids encoding any of the T $\beta$ RII polypeptides, including fragments, functional variants and fusion proteins disclosed herein. SEQ ID NO: 26, 28, 30, 32, 34, 36, 38, 40, 42, and 44 encode variants of T $\beta$ RII extracellular domain fused to an IgG2 Fc or an N-terminally truncated IgG1 Fc domain. The subject nucleic acids may be single-stranded or double

stranded. Such nucleic acids may be DNA or RNA molecules. These nucleic acids may be used, for example, in methods for making T $\beta$ RII polypeptides or as direct therapeutic agents (e.g., in an antisense, RNAi or gene therapy approach).

[0082] In certain aspects, the subject nucleic acids encoding T $\beta$ RII polypeptides are 5 further understood to include nucleic acids that are variants of SEQ ID NOS: 26, 28, 30, 32, 34, 36, 38, 40, 42, and 44. Variant nucleotide sequences include sequences that differ by one or more nucleotide substitutions, additions or deletions, such as allelic variants.

[0083] In certain embodiments, the disclosure provides isolated or recombinant 10 nucleic acid sequences that are at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NOS: 26, 28, 30, 32, 34, 36, 38, 40, 42, and 44. One of ordinary skill in the art will appreciate that nucleic acid sequences complementary to SEQ ID NOS: 26, 28, 30, 32, 34, 36, 38, 40, 42, and 44, and variants of SEQ ID NOS: 26, 28, 30, 32, 34, 36, 38, 40, 42, and 44 are also within the scope of this disclosure. In further embodiments, the nucleic acid 15 sequences of the disclosure can be isolated, recombinant, and/or fused with a heterologous nucleotide sequence, or in a DNA library.

[0084] In other embodiments, nucleic acids of the disclosure also include nucleotide 20 sequences that hybridize under highly stringent conditions to the nucleotide sequences designated in SEQ ID NOS: 26, 28, 30, 32, 34, 36, 38, 40, 42, and 44 complement sequences of SEQ ID NOS: 26, 28, 30, 32, 34, 36, 38, 40, 42, and 44, or fragments thereof. As 25 discussed above, one of ordinary skill in the art will understand readily that appropriate stringency conditions which promote DNA hybridization can be varied. For example, one could perform the hybridization at 6.0 x sodium chloride/sodium citrate (SSC) at about 45°C, followed by a wash of 2.0 x SSC at 50°C. For example, the salt concentration in the wash step can be selected from a low stringency of about 2.0 x SSC at 50°C to a high stringency of 30 about 0.2 x SSC at 50°C. In addition, the temperature in the wash step can be increased from low stringency conditions at room temperature, about 22°C, to high stringency conditions at about 65°C. Both temperature and salt may be varied, or temperature or salt concentration may be held constant while the other variable is changed. In some embodiments, the disclosure provides nucleic acids which hybridize under low stringency conditions of 6 x SSC at room temperature followed by a wash at 2 x SSC at room temperature.

**[0085]** Isolated nucleic acids which differ from the nucleic acids as set forth in SEQ ID NOS: 26, 28, 30, 32, 34, 36, 38, 40, 42, and 44 due to degeneracy in the genetic code are also within the scope of the disclosure. For example, a number of amino acids are designated by more than one triplet. Codons that specify the same amino acid, or synonyms (for example, CAU and CAC are synonyms for histidine) may result in “silent” mutations which do not affect the amino acid sequence of the protein. However, it is expected that DNA sequence polymorphisms that do lead to changes in the amino acid sequences of the subject proteins will exist among mammalian cells. One skilled in the art will appreciate that these variations in one or more nucleotides (up to about 3-5% of the nucleotides) of the nucleic acids encoding a particular protein may exist among individuals of a given species due to natural allelic variation. Any and all such nucleotide variations and resulting amino acid polymorphisms are within the scope of this disclosure.

**[0086]** It will be appreciated by one of skill in the art that corresponding variants based on the long isoform of T $\beta$ RII will include nucleotide sequences encoding the 25-amino acid insertion along with a conservative Val-Ile substitution at the flanking position C-terminal to the insertion. It will also be appreciated that corresponding variants based on either the long (A) or short (B) isoforms of T $\beta$ RII will include variant nucleotide sequences comprising an insertion of 108 nucleotides, encoding a 36-amino-acid insertion (SEQ ID NO: 18), at the same location described for naturally occurring T $\beta$ RII isoform C (see Exemplification).

**[0087]** In certain embodiments, the recombinant nucleic acids of the disclosure may be operably linked to one or more regulatory nucleotide sequences in an expression construct. Regulatory nucleotide sequences will generally be appropriate to the host cell used for expression. Numerous types of appropriate expression vectors and suitable regulatory sequences are known in the art for a variety of host cells. Typically, said one or more regulatory nucleotide sequences may include, but are not limited to, promoter sequences, leader or signal sequences, ribosomal binding sites, transcriptional start and termination sequences, translational start and termination sequences, and enhancer or activator sequences. Constitutive or inducible promoters as known in the art are contemplated by the disclosure.

The promoters may be either naturally occurring promoters, or hybrid promoters that combine elements of more than one promoter. An expression construct may be present in a cell on an episome, such as a plasmid, or the expression construct may be inserted in a

chromosome. In a preferred embodiment, the expression vector contains a selectable marker gene to allow the selection of transformed host cells. Selectable marker genes are well known in the art and will vary with the host cell used.

**[0088]** In certain aspects disclosed herein, the subject nucleic acid is provided in an expression vector comprising a nucleotide sequence encoding a T $\beta$ RII polypeptide and operably linked to at least one regulatory sequence. Regulatory sequences are art-recognized and are selected to direct expression of the T $\beta$ RII polypeptide. Accordingly, the term regulatory sequence includes promoters, enhancers, and other expression control elements. Exemplary regulatory sequences are described in Goeddel; *Gene Expression Technology*:  
5 *Methods in Enzymology*, Academic Press, San Diego, CA (1990). For instance, any of a wide variety of expression control sequences that control the expression of a DNA sequence when operatively linked to it may be used in these vectors to express DNA sequences encoding a T $\beta$ RII polypeptide. Such useful expression control sequences, include, for example, the early and late promoters of SV40, tet promoter, adenovirus or cytomegalovirus immediate early  
10 promoter, RSV promoters, the lac system, the trp system, the TAC or TRC system, T7 promoter whose expression is directed by T7 RNA polymerase, the major operator and promoter regions of phage lambda, the control regions for fd coat protein, the promoter for 3-phosphoglycerate kinase or other glycolytic enzymes, the promoters of acid phosphatase, e.g., Pho5, the promoters of the yeast  $\alpha$ -mating factors, the polyhedron promoter of the  
15 baculovirus system and other sequences known to control the expression of genes of prokaryotic or eukaryotic cells or their viruses, and various combinations thereof. It should be understood that the design of the expression vector may depend on such factors as the choice of the host cell to be transformed and/or the type of protein desired to be expressed. Moreover, the vector's copy number, the ability to control that copy number and the  
20 expression of any other protein encoded by the vector, such as antibiotic markers, should also be considered.

**[0089]** A recombinant nucleic acid included in the disclosure can be produced by ligating the cloned gene, or a portion thereof, into a vector suitable for expression in either prokaryotic cells, eukaryotic cells (yeast, avian, insect or mammalian), or both. Expression  
30 vehicles for production of a recombinant T $\beta$ RII polypeptide include plasmids and other vectors. For instance, suitable vectors include plasmids of the types: pBR322-derived

plasmids, pEMBL-derived plasmids, pEX-derived plasmids, pBTac-derived plasmids and pUC-derived plasmids for expression in prokaryotic cells, such as *E. coli*.

[0090] Some mammalian expression vectors contain both prokaryotic sequences to facilitate the propagation of the vector in bacteria, and one or more eukaryotic transcription units that are expressed in eukaryotic cells. The pcDNAI/amp, pcDNAI/neo, pRc/CMV, pSV2gpt, pSV2neo, pSV2-dhfr, pTk2, pRSVneo, pMSG, pSVT7, pko-neo and pHyg derived vectors are examples of mammalian expression vectors suitable for transfection of eukaryotic cells. Some of these vectors are modified with sequences from bacterial plasmids, such as pBR322, to facilitate replication and drug resistance selection in both prokaryotic and eukaryotic cells. Alternatively, derivatives of viruses such as the bovine papilloma virus (BPV-1), or Epstein-Barr virus (pHEBo, pREP-derived and p205) can be used for transient expression of proteins in eukaryotic cells. Examples of other viral (including retroviral) expression systems can be found below in the description of gene therapy delivery systems. The various methods employed in the preparation of the plasmids and in transformation of host organisms are well known in the art. For other suitable expression systems for both prokaryotic and eukaryotic cells, as well as general recombinant procedures, see *Molecular Cloning A Laboratory Manual*, 3rd Ed., ed. by Sambrook, Fritsch and Maniatis (Cold Spring Harbor Laboratory Press, 2001). In some instances, it may be desirable to express the recombinant polypeptides by the use of a baculovirus expression system. Examples of such baculovirus expression systems include pVL-derived vectors (such as pVL1392, pVL1393 and pVL941), pAcUW-derived vectors (such as pAcUW1), and pBlueBac-derived vectors (such as the  $\beta$ -gal containing pBlueBac III).

[0091] In certain embodiments, a vector will be designed for production of the subject T $\beta$ RII polypeptides in CHO cells, such as a Pcmv-Script vector (Stratagene, La Jolla, Calif.), pcDNA4 vectors (Invitrogen, Carlsbad, Calif.) and pCI-neo vectors (Promega, Madison, Wisc.). In a preferred embodiment, a vector will be designed for production of the subject T $\beta$ RII polypeptides in HEK-293 cells. As will be apparent, the subject gene constructs can be used to cause expression of the subject T $\beta$ RII polypeptides in cells propagated in culture, e.g., to produce proteins, including fusion proteins or variant proteins, for purification.

[0092] This disclosure also pertains to a host cell transfected with a recombinant gene including a coding sequence (e.g., SEQ ID NOS: 26, 28, 30, 32, 34, 36, 38, 40, 42, or

44) for one or more of the subject T $\beta$ RII polypeptides. The host cell may be any prokaryotic or eukaryotic cell. For example, a T $\beta$ RII polypeptide disclosed herein may be expressed in bacterial cells such as *E. coli*, insect cells (e.g., using a baculovirus expression system), yeast, or mammalian cells. Other suitable host cells are known to those skilled in the art.

5 [0093] Accordingly, the present disclosure further pertains to methods of producing the subject T $\beta$ RII polypeptides. For example, a host cell transfected with an expression

vector encoding a T $\beta$ RII polypeptide can be cultured under appropriate conditions to allow expression of the T $\beta$ RII polypeptide to occur. The T $\beta$ RII polypeptide may be secreted and isolated from a mixture of cells and medium containing the T $\beta$ RII polypeptide.

10 Alternatively, the T $\beta$ RII polypeptide may be retained cytoplasmically or in a membrane fraction and the cells harvested, lysed and the protein isolated. A cell culture includes host cells, and media. Suitable media for cell culture are well known in the art. The subject T $\beta$ RII polypeptides can be isolated from cell culture medium, host cells, or both, using techniques known in the art for purifying proteins, including ion-exchange chromatography,

15 gel filtration chromatography, ultrafiltration, electrophoresis, immunoaffinity purification with antibodies specific for particular epitopes of the T $\beta$ RII polypeptides and affinity purification with an agent that binds to a domain fused to the T $\beta$ RII polypeptide (e.g., a protein A column may be used to purify an T $\beta$ RII-Fc fusion). In a preferred embodiment, the T $\beta$ RII polypeptide is a fusion protein containing a domain which facilitates its purification.

20 As an example, purification may be achieved by a series of column chromatography steps, including, for example, three or more of the following, in any order: protein A chromatography, Q sepharose chromatography, phenylsepharose chromatography, size exclusion chromatography, and cation exchange chromatography. The purification could be completed with viral filtration and buffer exchange.

25 [0094] In another embodiment, a fusion gene coding for a purification leader sequence, such as a poly-(His)/enterokinase cleavage site sequence at the N-terminus of the desired portion of the recombinant T $\beta$ RII polypeptide, can allow purification of the expressed fusion protein by affinity chromatography using a Ni<sup>2+</sup> metal resin. The purification leader sequence can then be subsequently removed by treatment with enterokinase to provide the 30 purified T $\beta$ RII polypeptide (e.g., see Hochuli et al., (1987) *J. Chromatography* 411:177; and Janknecht et al., *PNAS USA* 88:8972).

[0095] Techniques for making fusion genes are well known. Essentially, the joining of various DNA fragments coding for different polypeptide sequences is performed in accordance with conventional techniques, employing blunt-ended or stagger-ended termini for ligation, restriction enzyme digestion to provide for appropriate termini, filling-in of 5 cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and enzymatic ligation. In another embodiment, the fusion gene can be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor primers which give rise to complementary overhangs between two consecutive gene fragments which can subsequently 10 be annealed to generate a chimeric gene sequence (see, for example, *Current Protocols in Molecular Biology*, eds. Ausubel et al., John Wiley & Sons: 1992).

[0096] Examples of categories of nucleic acid compounds that are antagonists of T $\beta$ RII, TGF $\beta$ 1, TGF $\beta$ 3 and GDF15 include antisense nucleic acids, RNAi constructs and catalytic nucleic acid constructs. A nucleic acid compound may be single or double stranded. 15 A double stranded compound may also include regions of overhang or non-complementarity, where one or the other of the strands is single-stranded. A single-stranded compound may include regions of self-complementarity, meaning that the compound forms a so-called “hairpin” or “stem-loop” structure, with a region of double helical structure. A nucleic acid compound may comprise a nucleotide sequence that is complementary to a region consisting 20 of no more than 1000, no more than 500, no more than 250, no more than 100 or no more than 50, 35, 30, 25, 22, 20 or 18 nucleotides of the full-length T $\beta$ RII nucleic acid sequence or ligand nucleic acid sequence. The region of complementarity will preferably be at least 8 nucleotides, and optionally at least 10 or at least 15 nucleotides, such as between 15 and 25 nucleotides. A region of complementarity may fall within an intron, a coding sequence, or a 25 noncoding sequence of the target transcript, such as the coding sequence portion. Generally, a nucleic acid compound will have a length of about 8 to about 500 nucleotides or base pairs in length, such as about 14 to about 50 nucleotides. A nucleic acid may be a DNA (particularly for use as an antisense), RNA, or RNA:DNA hybrid. Any one strand may include a mixture of DNA and RNA, as well as modified forms that cannot readily be 30 classified as either DNA or RNA. Likewise, a double-stranded compound may be DNA:DNA, DNA:RNA or RNA:RNA, and any one strand may also include a mixture of DNA and RNA, as well as modified forms that cannot readily be classified as either DNA or RNA. A nucleic acid compound may include any of a variety of modifications, including one

or modifications to the backbone (the sugar-phosphate portion in a natural nucleic acid, including internucleotide linkages) or the base portion (the purine or pyrimidine portion of a natural nucleic acid). An antisense nucleic acid compound will preferably have a length of about 15 to about 30 nucleotides and will often contain one or more modifications to improve 5 characteristics such as stability in the serum, in a cell or in a place where the compound is likely to be delivered, such as the stomach in the case of orally delivered compounds and the lung for inhaled compounds. In the case of an RNAi construct, the strand complementary to the target transcript will generally be RNA or modifications thereof. The other strand may be RNA, DNA, or any other variation. The duplex portion of double-stranded or single-stranded 10 “hairpin” RNAi construct will preferably have a length of 18 to 40 nucleotides in length and optionally about 21 to 23 nucleotides in length, so long as it serves as a Dicer substrate. Catalytic or enzymatic nucleic acids may be ribozymes or DNA enzymes and may also contain modified forms. Nucleic acid compounds may inhibit expression of the target by about 50%, 75%, 90%, or more when contacted with cells under physiological conditions and 15 at a concentration where a nonsense or sense control has little or no effect. Preferred concentrations for testing the effect of nucleic acid compounds are 1, 5 and 10 micromolar. Nucleic acid compounds may also be tested for effects on, for example, angiogenesis.

#### 4. Alterations in Fc-fusion proteins

[0097] The application further provides T $\beta$ RII-Fc fusion proteins with engineered or 20 variant Fc regions. Such antibodies and Fc fusion proteins may be useful, for example, in modulating effector functions, such as, antigen-dependent cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC). Additionally, the modifications may improve the stability of the antibodies and Fc fusion proteins. Amino acid sequence variants of the 25 antibodies and Fc fusion proteins are prepared by introducing appropriate nucleotide changes into the DNA, or by peptide synthesis. Such variants include, for example, deletions from, and/or insertions into and/or substitutions of, residues within the amino acid sequences of the antibodies and Fc fusion proteins disclosed herein. Any combination of deletion, insertion, and substitution is made to arrive at the final construct, provided that the final construct possesses the desired characteristics. The amino acid changes also may alter post- 30 translational processes of the antibodies and Fc fusion proteins, such as changing the number or position of glycosylation sites.

[0098] Antibodies and Fc fusion proteins with reduced effector function may be produced by introducing changes in the amino acid sequence, including, but are not limited to, the Ala-Ala mutation described by Bluestone et al. (see WO 94/28027 and WO 98/47531; also see Xu et al. 2000 Cell Immunol 200; 16-26). Thus, in certain embodiments, antibodies and Fc fusion proteins of the disclosure with mutations within the constant region including the Ala-Ala mutation may be used to reduce or abolish effector function. According to these embodiments, antibodies and Fc fusion proteins may comprise a mutation to an alanine at position 234 or a mutation to an alanine at position 235, or a combination thereof. In one embodiment, the antibody or Fc fusion protein comprises an IgG4 framework, wherein the 5 Ala-Ala mutation would describe a mutation(s) from phenylalanine to alanine at position 234 and/or a mutation from leucine to alanine at position 235. In another embodiment, the antibody or Fc fusion protein comprises an IgG1 framework, wherein the Ala-Ala mutation would describe a mutation(s) from leucine to alanine at position 234 and/or a mutation from 10 leucine to alanine at position 235. The antibody or Fc fusion protein may alternatively or 15 additionally carry other mutations, including the point mutation K322A in the CH2 domain (Hezareh et al. 2001 J Virol. 75: 12161-8).

[0099] In particular embodiments, the antibody or Fc fusion protein may be modified to either enhance or inhibit complement dependent cytotoxicity (CDC). Modulated CDC activity may be achieved by introducing one or more amino acid substitutions, 20 insertions, or deletions in an Fc region (see, e.g., U.S. Pat. No. 6,194,551). Alternatively or additionally, cysteine residue(s) may be introduced in the Fc region, thereby allowing interchain disulfide bond formation in this region. The homodimeric antibody thus generated may have improved or reduced internalization capability and/or increased or decreased 25 complement-mediated cell killing. See Caron et al., J. Exp Med. 176:1191-1195 (1992) and Shope, B. J. Immunol. 148:2918-2922 (1992), WO99/51642, Duncan & Winter Nature 322: 738-40 (1988); U.S. Pat. No. 5,648,260; U.S. Pat. No. 5,624,821; and WO94/29351.

## 5. GDF15- T $\beta$ RII signaling

[0100] The present disclosure relates in part to the discovery that the TGF $\beta$  type II receptor (T $\beta$ RII) binds to GDF15 with high affinity. Heretofore, GDF15 has not been shown 30 biochemically to bind or interact directly with a receptor. Inadequate or inappropriate ligand purification could be a potential reason for the inactivity of commercially available GDF15. Exemplary GDF15 polypeptides demonstrating a T $\beta$ RII binding activity and methods of

making and purifying such polypeptides are disclosed herein. Sequences of native precursor GDF15 proteins and nucleotides from human and mouse are set forth in Figures 1-4. Mature human GDF15 extends from residues 197 to 308 of SEQ ID NO: 1. Similarly, mature mouse GDF15 extends from residues 192 to 303 of SEQ ID NO: 3. In certain embodiments, the 5 present disclosure makes available isolated and/or purified forms of the GDF15 polypeptides or fragments thereof, which are isolated from, or otherwise substantially free of (e.g., at least 80%, 90%, 95%, 96%, 97%, 98%, or 99% free of), other proteins and/or other GDF15 polypeptide species. The GDF15 polypeptides of the disclosure bind to T $\beta$ RII with high affinity. Binding may be assessed using purified proteins in solution or in a surface plasmon 10 resonance system, such as a Biacore<sup>TM</sup> system. The GDF15 polypeptides will have an affinity (a dissociation constant) of about 10<sup>-6</sup>, 10<sup>-7</sup>, 10<sup>-8</sup>, 10<sup>-9</sup> M or less for T $\beta$ RII polypeptides. Preferably, the GDF15 polypeptides of the disclosure are isolated and purified according to methods described herein. GDF15 polypeptides will generally be produced by expression from recombinant nucleic acids.

15 [0101] GDF15 polypeptides may include a polypeptide consisting of, or comprising, an amino acid sequence at least 80% identical, and optionally at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the GDF15 polypeptide of SEQ ID NO: 1 or SEQ ID NO: 3, or a functional fragment thereof. GDF15 polypeptides may include a polypeptide consisting of, or comprising, an amino acid sequence at least 80% identical, and optionally at 20 least 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the GDF15 polypeptide comprising residues 197 to 308 of SEQ ID NO: 1 or residues 192 to 303 of SEQ ID NO: 3, or a functional fragment thereof. The unprocessed GDF15 polypeptide may either include or exclude any signal sequence, as well as any sequence N-terminal to the signal sequence. A GDF15 polypeptide may include variants of SEQ ID NO: 1 or SEQ ID NO: 3, or portions 25 thereof, corresponding to 197 to 308 of SEQ ID NO: 1 or residues 192 to 303 of SEQ ID NO: 3, respectively (including variants that comprise, for example, no more than 2, 3, 4, 5, 10, 15, 20, 25, 30, or 35 amino acid substitutions in the sequence of SEQ ID NO: 1 or SEQ ID NO: 3), fragments thereof, and fusion proteins comprising any of the foregoing, but in each case 30 preferably any of the foregoing GDF15 polypeptides will possess substantial affinity for a T $\beta$ RII polypeptide.

[0102] In certain embodiments, the GDF15 polypeptides of the disclosure may further comprise post-translational modifications in addition to any that are naturally present

in the GDF15 polypeptides. Such modifications include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation, pegylation (polyethylene glycol) and acylation. As a result, the modified GDF15 polypeptides may contain non-amino acid elements, such as polyethylene glycols, lipids, mono- or poly-saccharides, and phosphates.

5 Effects of such non-amino acid elements on the functionality of a GDF15 polypeptide may be tested as described herein for other GDF15 polypeptides. When a GDF15 polypeptide is produced in cells by cleaving a nascent form of the GDF15 polypeptide, post-translational processing may also be important for correct folding and/or function of the protein. Different cells (such as CHO, HeLa, MDCK, 293, WI38, NIH-3T3 or HEK-293) have specific cellular 10 machinery and characteristic mechanisms for such post-translational activities and may be chosen to ensure the correct modification and processing of the GDF15 polypeptides.

15 [0103] In certain embodiments, the disclosure includes nucleic acids encoding precursor and mature GDF15 polypeptides. In further embodiments, this disclosure also pertains to a host cell comprising such nucleic acids. The host cell may be any prokaryotic or eukaryotic cell. For example, a polypeptide of the present disclosure may be expressed in bacterial cells such as *E. coli*, insect cells (e.g., using a baculovirus expression system), yeast, or mammalian cells. Other suitable host cells are known to those skilled in the art. Accordingly, some embodiments of the present disclosure further pertain to methods of producing the GDF15 polypeptides.

20 [0104] In certain aspects, the disclosure provides isolated and/or recombinant nucleic acids encoding any of the GDF15 polypeptides, including fragments, functional variants and fusion proteins disclosed herein. The subject nucleic acids may be single-stranded or double-stranded. Such nucleic acids may be DNA or RNA molecules. These nucleic acids may be used, for example, in methods for making GDF15 polypeptides or as 25 direct therapeutic agents (e.g., in an antisense, RNAi or gene therapy approach).

[0105] In certain aspects, the subject nucleic acids encoding GDF15 polypeptides are further understood to include nucleic acids that are variants of SEQ ID NO: 1 or SEQ ID NO: 3. Variant nucleotide sequences include sequences that differ by one or more nucleotide substitutions, additions or deletions, such as allelic variants.

30 [0106] In certain embodiments, the disclosure provides isolated or recombinant nucleic acid sequences that are at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100%

identical to SEQ ID NO: 1 or SEQ ID NO: 3. One of ordinary skill in the art will appreciate that nucleic acid sequences complementary to SEQ ID NO: 1 or SEQ ID NO: 3 and variants of SEQ ID NO: 1 or SEQ ID NO: 3 are also within the scope of this disclosure. In further embodiments, the nucleic acid sequences of the disclosure can be isolated, recombinant, 5 and/or fused with a heterologous nucleotide sequence, or in a DNA library.

**[0107]** In other embodiments, nucleic acids of the disclosure also include nucleotide sequences that hybridize under highly stringent conditions to the nucleotide sequences designated in SEQ ID NO: 1 or SEQ ID NO: 3, complement sequences of SEQ ID NO: 1 or SEQ ID NO: 3, or fragments thereof. As discussed above, one of ordinary skill in the art will 10 understand readily that appropriate stringency conditions which promote DNA hybridization can be varied. For example, one could perform the hybridization at 6.0 x sodium chloride/sodium citrate (SSC) at about 45°C, followed by a wash of 2.0 x SSC at 50°C. For example, the salt concentration in the wash step can be selected from a low stringency of about 2.0 x SSC at 50°C to a high stringency of about 0.2 x SSC at 50°C. In addition, the 15 temperature in the wash step can be increased from low stringency conditions at room temperature, about 22°C, to high stringency conditions at about 65°C. Both temperature and salt may be varied, or temperature or salt concentration may be held constant while the other variable is changed. In some embodiments, the disclosure provides nucleic acids which hybridize under low stringency conditions of 6 x SSC at room temperature followed by a 20 wash at 2 x SSC at room temperature.

**[0108]** Isolated nucleic acids which differ from the nucleic acids as set forth in SEQ ID NO: 1 or SEQ ID NO: 3 due to degeneracy in the genetic code are also within the scope of the disclosure. For example, a number of amino acids are designated by more than one triplet. Codons that specify the same amino acid, or synonyms (for example, CAU and CAC 25 are synonyms for histidine) may result in “silent” mutations which do not affect the amino acid sequence of the protein. However, it is expected that DNA sequence polymorphisms that do lead to changes in the amino acid sequences of the subject proteins will exist among mammalian cells. One skilled in the art will appreciate that these variations in one or more nucleotides (up to about 3-5% of the nucleotides) of the nucleic acids encoding a particular 30 protein may exist among individuals of a given species due to natural allelic variation. Any and all such nucleotide variations and resulting amino acid polymorphisms are within the scope of this disclosure.

**[0109]** In certain embodiments, the recombinant nucleic acids of the disclosure may be operably linked to one or more regulatory nucleotide sequences in an expression construct. Regulatory nucleotide sequences will generally be appropriate to the host cell used for expression. Numerous types of appropriate expression vectors and suitable regulatory sequences are known in the art for a variety of host cells. Typically, said one or more regulatory nucleotide sequences may include, but are not limited to, promoter sequences, leader or signal sequences, ribosomal binding sites, transcriptional start and termination sequences, translational start and termination sequences, and enhancer or activator sequences. Constitutive or inducible promoters as known in the art are contemplated by the disclosure.

5      The promoters may be either naturally occurring promoters, or hybrid promoters that combine elements of more than one promoter. An expression construct may be present in a cell on an episome, such as a plasmid, or the expression construct may be inserted in a chromosome. In a preferred embodiment, the expression vector contains a selectable marker gene to allow the selection of transformed host cells. Selectable marker genes are well known in the art and will vary with the host cell used.

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**[0110]** In certain aspects disclosed herein, the subject nucleic acid is provided in an expression vector comprising a nucleotide sequence encoding a GDF15 polypeptide and operably linked to at least one regulatory sequence. Regulatory sequences are art-recognized and are selected to direct expression of the GDF15 polypeptide. Accordingly, the term regulatory sequence includes promoters, enhancers, and other expression control elements. Exemplary regulatory sequences are described in Goeddel; *Gene Expression Technology: Methods in Enzymology*, Academic Press, San Diego, CA (1990). For instance, any of a wide variety of expression control sequences that control the expression of a DNA sequence when operatively linked to it may be used in these vectors to express DNA sequences encoding a GDF15 polypeptide. Such useful expression control sequences, include, for example, the early and late promoters of SV40, tet promoter, adenovirus or cytomegalovirus immediate early promoter, RSV promoters, the lac system, the trp system, the TAC or TRC system, T7 promoter whose expression is directed by T7 RNA polymerase, the major operator and promoter regions of phage lambda, the control regions for fd coat protein, the promoter for 3-phosphoglycerate kinase or other glycolytic enzymes, the promoters of acid phosphatase, e.g., Pho5, the promoters of the yeast  $\alpha$ -mating factors, the polyhedron promoter of the baculovirus system and other sequences known to control the expression of genes of prokaryotic or eukaryotic cells or their viruses, and various combinations thereof. It should

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be understood that the design of the expression vector may depend on such factors as the choice of the host cell to be transformed and/or the type of protein desired to be expressed. Moreover, the vector's copy number, the ability to control that copy number and the expression of any other protein encoded by the vector, such as antibiotic markers, should also 5 be considered.

[0111] A recombinant nucleic acid included in the disclosure can be produced by ligating the cloned gene, or a portion thereof, into a vector suitable for expression in either prokaryotic cells, eukaryotic cells (yeast, avian, insect or mammalian), or both. Expression vehicles for production of a recombinant GDF15 polypeptide include plasmids and other 10 vectors. For instance, suitable vectors include plasmids of the types: pBR322-derived plasmids, pEMBL-derived plasmids, pEX-derived plasmids, pBTac-derived plasmids and pUC-derived plasmids for expression in prokaryotic cells, such as *E. coli*.

[0112] Some mammalian expression vectors contain both prokaryotic sequences to facilitate the propagation of the vector in bacteria, and one or more eukaryotic transcription 15 units that are expressed in eukaryotic cells. The pcDNAI/amp, pcDNAI/neo, pRc/CMV, pSV2gpt, pSV2neo, pSV2-dhfr, pTk2, pRSVneo, pMSG, pSVT7, pko-neo and pHyg derived vectors are examples of mammalian expression vectors suitable for transfection of eukaryotic cells. Some of these vectors are modified with sequences from bacterial plasmids, such as pBR322, to facilitate replication and drug resistance selection in both prokaryotic and 20 eukaryotic cells. Alternatively, derivatives of viruses such as the bovine papilloma virus (BPV-1), or Epstein-Barr virus (pHEBo, pREP-derived and p205) can be used for transient expression of proteins in eukaryotic cells. Examples of other viral (including retroviral) expression systems can be found below in the description of gene therapy delivery systems. The various methods employed in the preparation of the plasmids and in transformation of 25 host organisms are well known in the art. For other suitable expression systems for both prokaryotic and eukaryotic cells, as well as general recombinant procedures, see *Molecular Cloning A Laboratory Manual*, 3rd Ed., ed. by Sambrook, Fritsch and Maniatis (Cold Spring Harbor Laboratory Press, 2001). In some instances, it may be desirable to express the recombinant polypeptides by the use of a baculovirus expression system. Examples of such 30 baculovirus expression systems include pVL-derived vectors (such as pVL1392, pVL1393 and pVL941), pAcUW-derived vectors (such as pAcUW1), and pBlueBac-derived vectors (such as the  $\beta$ -gal containing pBlueBac III).

[0113] In a preferred embodiment, a vector will be designed for production of the subject T $\beta$ RII polypeptides in CHO cells, such as a Pcmv-Script vector (Stratagene, La Jolla, Calif.), pcDNA4 vectors (Invitrogen, Carlsbad, Calif.), pCI-neo vectors (Promega, Madison, Wisc.) and UCOE<sup>TM</sup>-derived vectors (Millipore). As will be apparent, the subject gene constructs can be used to cause expression of the subject GDF15 polypeptides in cells propagated in culture, e.g., to produce proteins, including fusion proteins or variant proteins, for purification.

[0114] This disclosure also pertains to a host cell transfected with a recombinant gene including a coding sequence (e.g., SEQ ID NO: 1 or SEQ ID NO:3) for one or more of the subject GDF15 polypeptides. The host cell may be any prokaryotic or eukaryotic cell. For example, a GDF15 polypeptide disclosed herein may be expressed in bacterial cells such as *E. coli*, insect cells (e.g., using a baculovirus expression system), yeast, or mammalian cells. Other suitable host cells are known to those skilled in the art. In a preferred embodiment, a GDF15 polypeptide disclosed herein is expressed in CHO cells.

[0115] Accordingly, the present disclosure further pertains to methods of producing the subject GDF15 polypeptides. For example, a host cell transfected with an expression vector encoding a GDF15 polypeptide can be cultured under appropriate conditions to allow expression of the GDF15 polypeptide to occur. The GDF15 polypeptide may be secreted and isolated from a mixture of cells and medium containing the GDF15 polypeptide.

Alternatively, the T $\beta$ RII polypeptide may be retained cytoplasmically or in a membrane fraction and the cells harvested, lysed and the protein isolated. A cell culture includes host cells and media. Suitable media for cell culture are well known in the art. The subject GDF15 polypeptides can be isolated from cell culture medium, host cells, or both, using techniques known in the art for purifying proteins, including ion-exchange chromatography, gel filtration chromatography, ultrafiltration, electrophoresis, immunoaffinity purification with antibodies specific for particular epitopes of the GDF15 polypeptides and affinity purification with an agent that binds to a domain fused to the GDF15 polypeptide (e.g., a protein A column may be used to purify an GDF15-Fc fusion). In a preferred embodiment, the GDF15 polypeptide is a fusion protein containing a domain which facilitates its purification. As an example, purification may be achieved by a series of column chromatography steps, including, for example, three or more of the following, in any order: protein A chromatography, Q sepharose chromatography, phenylsepharose chromatography,

size exclusion chromatography, and cation exchange chromatography. The purification could be completed with viral filtration and buffer exchange.

**[0116]** In a preferred embodiment, the subject GDF15 polypeptides are purified from culture media using a series of cation-exchange column chromatography steps.

5 Examples of the material used for the cation exchange column can be resins having substituents such as carboxymethyl (CM), sulfoethyl (SE), sulfopropyl (SP), phosphate (P) and sulfonate (S). Examples of the material used for the cation exchange column chromatography include SP Sepharose™ Fast Flow, Q Sepharose™ Fast Flow, DEAE Sepharose™ Fast Flow, Capto™ S, Capto™ DEAE (GE Healthcare), S HyperCel™ (Pall),  
10 TOYOPEARL GigaCap S-650 (TOSOH) or weak cation exchangers such as carboxymethyl. SP Sepharose™ Fast Flow and Q Sepharose™ Fast Flow are preferred.

**[0117]** To begin purification, parameters of the conditioned media from host cells stably expressing a GDF15 polypeptide, such as pH, ionic strength, and temperature may be adjusted if necessary. In some embodiments, a chromatography column is flushed and

15 equilibrated with one or more solutions prior to contact with a polypeptide containing supernatant. Such solutions can include, for example, a buffer (e.g., Tris, MES, HEPES, histidine, phosphate or sodium acetate, e.g., between 1-500 mM, 25-100 mM, 15-30 mM or 20 mM), and/or salt (e.g., NaCl, NaPO<sub>4</sub> sodium acetate, or CaCl<sub>2</sub>, e.g., between 0-2 M, 1-2 M or 500 mM-1M). The pH of an equilibration solution generally ranges from 3.5-10 (e.g.,  
20 between pH 3.5-6, 4.0-5.5, 4.5-4.8 or 4.7). After contacting a column with a polypeptide containing fluid, the bound column can be washed. Wash solutions can include a buffer (e.g., Tris, MES, HEPES, histidine, phosphate, or sodium acetate, e.g., between 1-500 mM, 25-100 mM, 15-30 mM or 20 mM), and/or salt (e.g., NaCl, NaPO<sub>4</sub>, sodium acetate, or CaCl<sub>2</sub>, e.g., between 0-2 M, 1-2 M, 100 mM-1M or 100 mM-500 mM), and/or an additive (e.g.,  
25 guanidine, urea, sucrose, arginine, or an arginine derivative), and/or a solvent (e.g., ethanol, acetonitrile, or polyethylene glycol). Wash solutions generally have a pH between 3.5 and 10 (e.g., a pH between 4.5-8.0). Polypeptides can be eluted from a column using a step or gradient change in pH, salt type, salt concentration, solvent type, solvent concentration, displacer type, displacer concentration, or a combination thereof. In general, to elute a  
30 polypeptide from a column, the medium is contacted with an elution buffer. In some embodiments, an elution buffer elution buffer contains a buffer (e.g., HEPES or Tris, e.g., 10-100 mM, 25-75 mM or 50 mM) and/or contains a salt (e.g., NaCl or CaCl<sub>2</sub>, e.g., 0-2 M, e.g.,

10-100 mM). In some embodiments, an elution buffer may contain glycine, acetic acid, or citric acid (e.g., 20-250 mM, or 150 mM). An elution buffer may also contain acetic acid (e.g., 20 mM to about 50 mM), an additive (e.g. guanidine, urea, or sucrose, e.g., 1-10 M, 2-8 M or 6 M), and/or a solvent (e.g., ethanol, acetonitrile, polyethylene glycol, e.g., 1-10% solvent, e.g., 5% solvent). The pH of the elution buffer may range from about 5.0 to about 5 10.0. In some embodiments, pH can be changed (e.g., gradually) to produce a gradient elution. In some embodiments, the pH of the elution buffer is about 8.0. In some embodiments, a series of column chromatography steps are performed.

15 [0118] The data presented herein demonstrates that T $\beta$ RII polypeptides act as antagonists of GDF15 signaling. Although soluble T $\beta$ RII polypeptides, and particularly T $\beta$ RII-Fc, are preferred antagonists, other types of GDF15 antagonists are expected to be useful, including anti-GDF15 antibodies, anti-T $\beta$ RII antibodies, antisense, RNAi or 10 ribozyme nucleic acids that inhibit the production of GDF15 or T $\beta$ RII and other inhibitors of GDF15 or T $\beta$ RII, particularly those that disrupt GDF15-T $\beta$ RII binding.

20 [0119] An antibody that is specifically reactive with a GDF15 polypeptide and which either binds to GDF15 polypeptide so as to compete with its binding to T $\beta$ RII polypeptide (binding competitively) or otherwise inhibits GDF15-mediated signaling may be used as an antagonist of GDF15 polypeptide activities. Likewise, an antibody that is specifically reactive with a T $\beta$ RII polypeptide and which disrupts GDF15 binding may be 15 used as an antagonist.

25 [0120] By using immunogens derived from a GDF15 polypeptide or a T $\beta$ RII polypeptide, anti-protein/anti-peptide antisera or monoclonal antibodies can be made by standard protocols (see, for example, Antibodies: A Laboratory Manual ed. by Harlow and Lane (Cold Spring Harbor Press: 1988)). A mammal, such as a mouse, a hamster or rabbit can be immunized with an immunogenic form of the GDF15 polypeptide, an antigenic fragment which is capable of eliciting an antibody response, or a fusion protein. Techniques for conferring immunogenicity on a protein or peptide include conjugation to carriers or other 30 techniques well known in the art. An immunogenic portion of a GDF15 or T $\beta$ RII polypeptide can be administered in the presence of adjuvant. The progress of immunization can be monitored by detection of antibody titers in plasma or serum. Standard ELISA or other immunoassays can be used with the immunogen as antigen to assess the levels of antibodies.

[0121] Following immunization of an animal with an antigenic preparation of a GDF15 polypeptide, antisera can be obtained and, if desired, polyclonal antibodies can be isolated from the serum. To produce monoclonal antibodies, antibody-producing cells (lymphocytes) can be harvested from an immunized animal and fused by standard somatic cell fusion procedures with immortalizing cells such as myeloma cells to yield hybridoma cells. Such techniques are well known in the art, and include, for example, the hybridoma technique (originally developed by Kohler and Milstein, (1975) *Nature*, 256: 495-497), the human B cell hybridoma technique (Kozbar et al., (1983) *Immunology Today*, 4: 72), and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole et al., (1985) *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc. pp. 77-96). Hybridoma cells can be screened immunochemically for production of antibodies specifically reactive with a GDF15 polypeptide and monoclonal antibodies isolated from a culture comprising such hybridoma cells.

[0122] The term “antibody” as used herein is intended to include fragments thereof which are also specifically reactive with a subject polypeptide. Antibodies can be fragmented using conventional techniques and the fragments screened for utility in the same manner as described above for whole antibodies. For example, F(ab)<sub>2</sub> fragments can be generated by treating antibody with pepsin. The resulting F(ab)<sub>2</sub> fragment can be treated to reduce disulfide bridges to produce Fab fragments. The antibody of the present invention is further intended to include bispecific, single-chain, chimeric, humanized and fully human molecules having affinity for an T $\beta$ RII or GDF15 polypeptide conferred by at least one CDR region of the antibody. An antibody may further comprise a label attached thereto and able to be detected (e.g., the label can be a radioisotope, fluorescent compound, enzyme or enzyme co-factor).

[0123] In certain embodiments, the antibody is a recombinant antibody, which term encompasses any antibody generated in part by techniques of molecular biology, including CDR-grafted or chimeric antibodies, human or other antibodies assembled from library-selected antibody domains, single chain antibodies and single domain antibodies (e.g., human V<sub>H</sub> proteins or camelid V<sub>HH</sub> proteins). In certain embodiments, an antibody of the invention is a monoclonal antibody, and in certain embodiments, the invention makes available methods for generating novel antibodies. For example, a method for generating a monoclonal antibody that binds specifically to a GDF15 polypeptide or T $\beta$ RII polypeptide

may comprise administering to a mouse an amount of an immunogenic composition comprising the antigen polypeptide effective to stimulate a detectable immune response, obtaining antibody-producing cells (e.g., cells from the spleen) from the mouse and fusing the antibody-producing cells with myeloma cells to obtain antibody-producing hybridomas, and

5 testing the antibody-producing hybridomas to identify a hybridoma that produces a monoclonal antibody that binds specifically to the antigen. Once obtained, a hybridoma can be propagated in a cell culture, optionally in culture conditions where the hybridoma-derived cells produce the monoclonal antibody that binds specifically to the antigen. The monoclonal antibody may be purified from the cell culture.

10 [0124] The adjective “specifically reactive with” as used in reference to an antibody is intended to mean, as is generally understood in the art, that the antibody is sufficiently selective between the antigen of interest (e.g., a GDF15 polypeptide) and other antigens that are not of interest that the antibody is useful for, at minimum, detecting the presence of the antigen of interest in a particular type of biological sample. In certain methods employing the

15 antibody, such as therapeutic applications, a higher degree of specificity in binding may be desirable. Monoclonal antibodies generally have a greater tendency (as compared to polyclonal antibodies) to discriminate effectively between the desired antigens and cross-reacting polypeptides. One characteristic that influences the specificity of an antibody:antigen interaction is the affinity of the antibody for the antigen. Although the

20 desired specificity may be reached with a range of different affinities, generally preferred antibodies will have an affinity (a dissociation constant) of about  $10^{-6}$ ,  $10^{-7}$ ,  $10^{-8}$ ,  $10^{-9}$  or less. Given the high affinity between GDF15 and T $\beta$ RII, it is expected that a neutralizing anti-GDF15 or anti-T $\beta$ RII antibody would generally have a dissociation constant of  $10^{-9}$  or less.

25 [0125] In addition, the techniques used to screen antibodies in order to identify a desirable antibody may influence the properties of the antibody obtained. For example, if an antibody is to be used for binding an antigen in solution, it may be desirable to test solution binding. A variety of different techniques are available for testing interaction between antibodies and antigens to identify particularly desirable antibodies. Such techniques include ELISAs, surface plasmon resonance binding assays (e.g., the Biacore<sup>TM</sup> binding assay,

30 Biacore AB, Uppsala, Sweden), sandwich assays (e.g., the paramagnetic bead system of IGEN International, Inc., Gaithersburg, Maryland), western blots, immunoprecipitation assays, and immunohistochemistry.

**[0126]** Examples of categories of nucleic acid compounds that are GDF15 or T $\beta$ RII antagonists include antisense nucleic acids, RNAi constructs and catalytic nucleic acid constructs. A nucleic acid compound may be single- or double-stranded. A double-stranded compound may also include regions of overhang or non-complementarity, where one or the 5 other of the strands is single-stranded. A single-stranded compound may include regions of self-complementarity, meaning that the compound forms a so-called “hairpin” or “stem-loop” structure, with a region of double helical structure. A nucleic acid compound may comprise a nucleotide sequence that is complementary to a region consisting of no more than 1000, no more than 500, no more than 250, no more than 100 or no more than 50, 35, 30, 25, 22, 20 or 10 18 nucleotides of the full-length GDF15 nucleic acid sequence or T $\beta$ RII nucleic acid sequence. The region of complementarity will preferably be at least 8 nucleotides, and optionally at least 10 or at least 15 nucleotides, such as between 15 and 25 nucleotides. A region of complementarity may fall within an intron, a coding sequence or a noncoding 15 sequence of the target transcript, such as the coding sequence portion. Generally, a nucleic acid compound will have a length of about 8 to about 500 nucleotides or base pairs in length, such as about 14 to about 50 nucleotides. A nucleic acid may be a DNA (particularly for use as an antisense), RNA or RNA:DNA hybrid. Any one strand may include a mixture of DNA and RNA, as well as modified forms that cannot readily be classified as either DNA or RNA. Likewise, a double-stranded compound may be DNA:DNA, DNA:RNA or RNA:RNA, and 20 any one strand may also include a mixture of DNA and RNA, as well as modified forms that cannot readily be classified as either DNA or RNA. A nucleic acid compound may include any of a variety of modifications, including one or modifications to the backbone (the sugar-phosphate portion in a natural nucleic acid, including internucleotide linkages) or the base portion (the purine or pyrimidine portion of a natural nucleic acid). An antisense nucleic acid 25 compound will preferably have a length of about 15 to about 30 nucleotides and will often contain one or more modifications to improve characteristics such as stability in the serum, in a cell or in a place where the compound is likely to be delivered, such as the stomach in the case of orally delivered compounds and the lung for inhaled compounds. In the case of an RNAi construct, the strand complementary to the target transcript will generally be RNA or 30 modifications thereof. The other strand may be RNA, DNA or any other variation. The duplex portion of double-stranded or single-stranded “hairpin” RNAi construct will preferably have a length of 18 to 40 nucleotides in length and optionally about 21 to 23 nucleotides in length, so long as it serves as a Dicer substrate. Catalytic or enzymatic nucleic acids may be ribozymes or DNA enzymes and may also contain modified forms. Nucleic

acid compounds may inhibit expression of the target by about 50%, 75%, 90% or more when contacted with cells under physiological conditions and at a concentration where a nonsense or sense control has little or no effect. Preferred concentrations for testing the effect of nucleic acid compounds are 1, 5 and 10 micromolar.

5    6.    **Screening Assays**

[0127] In certain aspects, the present invention relates to the use of T $\beta$ RII polypeptides (e.g., soluble T $\beta$ RII polypeptides) and GDF15 polypeptides to identify compounds (agents) which are agonist or antagonists of the GDF15-T $\beta$ RII signaling pathway. Compounds identified through this screening can be tested to assess their ability to modulate 10 GDF15 signaling activity in vitro. Optionally, these compounds can further be tested in animal models to assess their ability to modulate tissue growth in vivo.

[0128] There are numerous approaches to screening for therapeutic agents for modulating tissue growth by targeting GDF15 and T $\beta$ RII polypeptides. In certain embodiments, high-throughput screening of compounds can be carried out to identify agents 15 that perturb GDF15 or T $\beta$ RII-mediated cell signaling. In certain embodiments, the assay is carried out to screen and identify compounds that specifically inhibit or reduce binding of a T $\beta$ RII polypeptide to GDF15. Alternatively, the assay can be used to identify compounds that enhance binding of a T $\beta$ RII polypeptide to GDF15. In a further embodiment, the compounds can be identified by their ability to interact with a GDF15 or T $\beta$ RII polypeptide.

20            [0129] A variety of assay formats will suffice and, in light of the present disclosure, those not expressly described herein will nevertheless be comprehended by one of ordinary skill in the art. As described herein, the test compounds (agents) of the invention may be created by any combinatorial chemical method. Alternatively, the subject compounds may be naturally occurring biomolecules synthesized in vivo or in vitro. Compounds (agents) to be 25 tested for their ability to act as modulators of tissue growth can be produced, for example, by bacteria, yeast, plants or other organisms (e.g., natural products), produced chemically (e.g., small molecules, including peptidomimetics), or produced recombinantly. Test compounds contemplated by the present invention include non-peptidyl organic molecules, peptides, polypeptides, peptidomimetics, sugars, hormones, and nucleic acid molecules. In a specific 30 embodiment, the test agent is a small organic molecule having a molecular weight of less than about 2,000 daltons.

**[0130]** The test compounds of the invention can be provided as single, discrete entities, or provided in libraries of greater complexity, such as made by combinatorial chemistry. These libraries can comprise, for example, alcohols, alkyl halides, amines, amides, esters, aldehydes, ethers and other classes of organic compounds. Presentation of test compounds to the test system can be in either an isolated form or as mixtures of compounds, especially in initial screening steps. Optionally, the compounds may be optionally derivatized with other compounds and have derivatizing groups that facilitate isolation of the compounds. Non-limiting examples of derivatizing groups include biotin, fluorescein, digoxxygenin, green fluorescent protein, isotopes, polyhistidine, magnetic beads, glutathione S transferase (GST), photoactivatable crosslinkers or any combinations thereof.

**[0131]** In many drug screening programs which test libraries of compounds and natural extracts, high throughput assays are desirable in order to maximize the number of compounds surveyed in a given period of time. Assays which are performed in cell-free systems, such as may be derived with purified or semi-purified proteins, are often preferred as “primary” screens in that they can be generated to permit rapid development and relatively easy detection of an alteration in a molecular target which is mediated by a test compound. Moreover, the effects of cellular toxicity or bioavailability of the test compound can be generally ignored in the in vitro system, the assay instead being focused primarily on the effect of the drug on the molecular target as may be manifest in an alteration of binding affinity between a T $\beta$ RII polypeptide and GDF15.

**[0132]** Merely to illustrate, in an exemplary screening assay of the present invention, the compound of interest is contacted with an isolated and purified T $\beta$ RII polypeptide which is ordinarily capable of binding to GDF15. To the mixture of the compound and T $\beta$ RII polypeptide is then added a composition containing a T $\beta$ RII ligand. Detection and quantification of T $\beta$ RII/GDF15 complexes provides a means for determining the compound's efficacy at inhibiting (or potentiating) complex formation between the T $\beta$ RII polypeptide and GDF15. The efficacy of the compound can be assessed by generating dose response curves from data obtained using various concentrations of the test compound. Moreover, a control assay can also be performed to provide a baseline for comparison. For example, in a control assay, isolated and a purified GDF15 is added to a composition containing the T $\beta$ RII polypeptide, and the formation of T $\beta$ RII/GDF15 complex is quantitated in the absence of the test compound. It will be understood that, in general, the order in which

the reactants may be admixed can be varied, and can be admixed simultaneously. Moreover, in place of purified proteins, cellular extracts and lysates may be used to render a suitable cell-free assay system.

[0133] Complex formation between the T $\beta$ RII polypeptide and GDF15 may be 5 detected by a variety of techniques. For instance, modulation of the formation of complexes can be quantitated using, for example, detectably labeled proteins such as radiolabeled (e.g.,  $^{32}\text{P}$ ,  $^{35}\text{S}$ ,  $^{14}\text{C}$  or  $^3\text{H}$ ), fluorescently labeled (e.g., FITC), or enzymatically labeled T $\beta$ RII polypeptide or GDF15, by immunoassay, or by chromatographic detection.

[0134] In certain embodiments, the present invention contemplates the use of 10 fluorescence polarization assays and fluorescence resonance energy transfer (FRET) assays in measuring, either directly or indirectly, the degree of interaction between a T $\beta$ RII polypeptide and its binding protein. Further, other modes of detection, such as those based on optical waveguides (PCT Publication WO 96/26432 and U.S. Pat. No. 5,677,196), surface plasmon resonance (SPR), surface charge sensors, and surface force sensors, are compatible with 15 many embodiments of the invention.

[0135] Moreover, the present invention contemplates the use of an interaction trap assay, also known as the “two hybrid assay,” for identifying agents that disrupt or potentiate interaction between a T $\beta$ RII polypeptide and its binding protein. See for example, U.S. Pat. No. 5,283,317; Zervos et al. (1993) Cell 72:223-232; Madura et al. (1993) J Biol Chem 20 268:12046-12054; Bartel et al. (1993) Biotechniques 14:920-924; and Iwabuchi et al. (1993) Oncogene 8:1693-1696). In a specific embodiment, the present invention contemplates the use of reverse two hybrid systems to identify compounds (e.g., small molecules or peptides) that dissociate interactions between a T $\beta$ RII polypeptide and its binding protein. See for example, Vidal and Legrain, (1999) Nucleic Acids Res 27:919-29; Vidal and Legrain, (1999) Trends Biotechnol 17:374-81; and U.S. Pat. Nos. 5,525,490; 5,955,280; and 5,965,368. 25

[0136] In certain embodiments, the subject compounds are identified by their ability 30 to interact with a T $\beta$ RII or GDF15 polypeptide of the invention. The interaction between the compound and the T $\beta$ RII or GDF15 polypeptide may be covalent or non-covalent. For example, such interaction can be identified at the protein level using in vitro biochemical methods, including photo-crosslinking, radiolabeled ligand binding, and affinity chromatography (Jakoby WB et al., 1974, Methods in Enzymology 46: 1). In certain cases,

the compounds may be screened in a mechanism based assay, such as an assay to detect compounds which bind to a GDF15 or T $\beta$ RII polypeptide. This may include a solid-phase or fluid-phase binding event. Alternatively, the gene encoding a GDF15 or T $\beta$ RII polypeptide can be transfected with a reporter system (e.g.,  $\beta$ -galactosidase, luciferase, or green

5 fluorescent protein) into a cell and screened against the library preferably by a high-throughput screening or with individual members of the library. Other mechanism-based binding assays may be used, for example, binding assays which detect changes in free energy. Binding assays can be performed with the target fixed to a well, bead or chip or captured by an immobilized antibody or resolved by capillary electrophoresis. The bound

10 compounds may be detected usually using colorimetric or fluorescence or surface plasmon resonance.

[0137] In certain aspects, the present invention provides methods and agents for modulating (stimulating or inhibiting) GDF15-mediated cell signaling. Therefore, any compound identified can be tested in whole cells or tissues, *in vitro* or *in vivo*, to confirm

15 their ability to modulate GDF15 signaling. Various methods known in the art can be utilized for this purpose.

## 7. Exemplary Therapeutic Uses

[0138] As used herein, a therapeutic that “prevents” a disorder or condition refers to a compound that, in a statistical sample, reduces the occurrence of the disorder or condition

20 in the treated sample relative to an untreated control sample, or delays the onset or reduces the severity of one or more symptoms of the disorder or condition relative to the untreated control sample. The term “treating” as used herein includes amelioration or elimination of the condition once it has been established. In either case, prevention or treatment may be discerned in the diagnosis provided by a physician and the intended result of administration

25 of the therapeutic agent.

[0139] The disclosure provides methods of treating or preventing a disease or condition associated with a TGF $\beta$  superfamily member by administering to a subject an effective amount of a T $\beta$ RII polypeptide, including a T $\beta$ RII-Fc fusion protein or nucleic acid antagonists (e.g., antisense or siRNA) of the foregoing, hereafter collectively referred to as

30 “therapeutic agents”. In some embodiments the disease or condition is associated with dysregulated GDF15, TGF $\beta$ 1 or TGF $\beta$ 3 signaling. Also provided are methods and

compositions for treating certain cardiovascular or vascular disorders. In addition, the disclosure provides methods and compositions for treating or preventing cancer. In addition, the disclosure provides methods and compositions for treating or preventing fibrotic disorders and conditions.

5 [0140] In particular, polypeptide therapeutic agents of the present disclosure are useful for treating or preventing chronic vascular or cardiovascular diseases. Exemplary disorders of this kind include, but are not limited to, heart disease (including myocardial disease, myocardial infarct, angina pectoris, and heart valve disease); renal disease (including chronic glomerular inflammation, diabetic renal failure, and lupus-related renal

10 inflammation); disorders associated with atherosclerosis or other types of arteriosclerosis (including stroke, cerebral hemorrhage, subarachnoid hemorrhage, angina pectoris, and renal arteriosclerosis); thrombotic disorders (including cerebral thrombosis, thrombotic intestinal necrosis); complications of diabetes (including diabetes-related retinal disease, cataracts, diabetes-related renal disease, diabetes-related neuropathology, diabetes-related gangrene, and diabetes-related chronic infection); vascular inflammatory disorders (systemic lupus erythematosus, joint rheumatism, joint arterial inflammation, large-cell arterial inflammation, Kawasaki disease, Takayasu arteritis, Churg-Strauss syndrome, and Henoch-Schoenlein purpura); diabetic vasculopathies; and cardiac disorders such as congenital heart disease, cardiomyopathy (e.g., dilated, hypertrophic, restrictive cardiomyopathy), and congestive

15 heart failure. Exemplary disorders further include, but are not limited to, hereditary hemorrhagic telangiectasia (HHT), Marfan syndrome, Loeys-Dietz syndrome, familial thoracic aortic aneurysm syndrome, arterial tortuosity syndrome, pre-eclampsia, and restenosis.

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25 [0141] The T $\beta$ RII polypeptide can be administered to the subject alone, or in combination with one or more agents or therapeutic modalities, e.g., therapeutic agents, which are useful for treating TGF $\beta$  associated cardiovascular disorders and/or conditions. In certain embodiments, the second agent or therapeutic modality is chosen from one or more of: angioplasty, beta blockers, anti-hypertensives, cardiotonics, anti-thrombotics, vasodilators, hormone antagonists, endothelin antagonists, calcium channel blockers, phosphodiesterase inhibitors, angiotensin type 2 antagonists and/or cytokine blockers/inhibitors

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[0142] In particular, polypeptide therapeutic agents of the present disclosure are useful for treating or preventing a cancer (tumor). The terms "cancer" and "cancerous" refer to or describe, the physiological condition in mammals that is typically characterized by unregulated cell growth/proliferation. Examples of cancer, or neoplastic disorders, include 5 but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia. More particular examples of such cancers include squamous cell cancer, cancer of the peritoneum, hepatocellular cancer, gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney cancer, 10 prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, stomach cancer, intestinal cancer, skin cancer, bone cancer, gastric cancer, melanoma, and various types of head and neck cancer, including squamous cell head and neck cancer. Other examples of neoplastic disorders and related conditions include esophageal carcinomas, theomas, arrhenoblastomas, 15 endometrial hyperplasia, endometriosis, fibrosarcomas, choriocarcinoma, nasopharyngeal carcinoma, laryngeal carcinomas, hepatoblastoma, Kaposi's sarcoma, skin carcinomas, hemangioma, cavernous hemangioma, hemangioblastoma, retinoblastoma, astrocytoma, glioblastoma, Schwannoma, oligodendrolioma, medulloblastoma, neuroblastomas, rhabdomyosarcoma, osteogenic sarcoma, leiomyosarcomas, urinary tract carcinomas, Wilm's tumor, renal cell carcinoma, prostate carcinoma, abnormal vascular proliferation associated 20 with phakomatoses, and Meigs' syndrome. A cancer that is particularly amenable to treatment with the therapeutic agents described herein may be characterized by one or more of the following: the cancer has elevated T $\beta$ RII levels detectable in the tumor or the serum, increased GDF15, TGF $\beta$ 1 or TGF $\beta$ 3 expression levels or biological activity, is metastatic or at risk of becoming metastatic, or any combination thereof.

25 [0143] In certain embodiments of such methods, one or more polypeptide therapeutic agents can be administered, together (simultaneously) or at different times (sequentially). In addition, polypeptide therapeutic agents can be administered with another type of compounds for treating cancer or for inhibiting angiogenesis.

30 [0144] In certain embodiments, the subject methods of the disclosure can be used alone. Alternatively, the subject methods may be used in combination with other conventional anti-cancer therapeutic approaches directed to treatment or prevention of proliferative disorders (e.g., tumor). For example, such methods can be used in prophylactic

cancer prevention, prevention of cancer recurrence and metastases after surgery, and as an adjuvant of other conventional cancer therapy. The present disclosure recognizes that the effectiveness of conventional cancer therapies (e.g., chemotherapy, radiation therapy, phototherapy, immunotherapy, and surgery) can be enhanced through the use of a subject 5 polypeptide therapeutic agent.

[0145] A wide array of conventional compounds have been shown to have anti-neoplastic or anti-cancer activities. These compounds have been used as pharmaceutical agents in chemotherapy to shrink solid tumors, prevent metastases and further growth, or decrease the number of malignant cells in leukemic or bone marrow malignancies. Although 10 chemotherapy has been effective in treating various types of malignancies, many anti-neoplastic compounds induce undesirable side effects. It has been shown that when two or more different treatments are combined, the treatments may work synergistically and allow reduction of dosage of each of the treatments, thereby reducing the detrimental side effects exerted by each compound at higher dosages. In other instances, malignancies that are 15 refractory to a treatment may respond to a combination therapy of two or more different treatments.

[0146] When a therapeutic agent disclosed herein is administered in combination with another conventional anti-neoplastic agent, either concomitantly or sequentially, such therapeutic agent may enhance the therapeutic effect of the anti-neoplastic agent or overcome 20 cellular resistance to such anti-neoplastic agent. This allows decrease of dosage of an anti-neoplastic agent, thereby reducing the undesirable side effects, or restores the effectiveness of an anti-neoplastic agent in resistant cells.

[0147] According to the present disclosure, the polypeptide therapeutic agents described herein may be used in combination with other compositions and procedures for the 25 treatment of diseases. For example, a tumor may be treated conventionally with surgery, radiation or chemotherapy combined with the T $\beta$ RII polypeptide, and then the T $\beta$ RII polypeptide may be subsequently administered to the patient to extend the dormancy of micrometastases and to stabilize any residual primary tumor.

[0148] In certain aspects of the invention, other therapeutic agents useful for 30 combination tumor therapy with a T $\beta$ RII polypeptide include other cancer therapies: e.g., surgery, cytotoxic agents, radiological treatments involving irradiation or administration of

radioactive substances, chemotherapeutic agents, anti-hormonal agents, growth inhibitory agents, anti-neoplastic compositions, and treatment with anti-cancer agents listed herein and known in the art, or combinations thereof.

**[0149]** The term "cytotoxic agent" as used herein refers to a substance that inhibits 5 or prevents the function of cells and/or causes destruction of cells. The term is intended to include radioactive isotopes (e.g., At<sup>211</sup>, 1<sup>131</sup>, 1<sup>125</sup>, Y<sup>90</sup>, Re<sup>186</sup>, Re<sup>188</sup>, Sm<sup>153</sup>, Bi<sup>212</sup>, P<sup>32</sup> and radioactive isotopes of Lu), chemotherapeutic agents e.g. methotrexate, adriamicin, vinca alkaloids (vincristine, vinblastine, etoposide), doxorubicin, melphalan, mitomycin C, chlorambucil, daunorubicin or other intercalating agents, enzymes and fragments thereof such 10 as nucleolytic enzymes, antibiotics, and toxins such as small molecule toxins or enzymatically active toxins of bacterial, fungal, plant or animal origin, including fragments and/or variants thereof, and the various antitumor or anticancer agents disclosed below. Other cytotoxic agents are described below. A tumoricidal agent causes destruction of tumor cells.

**[0150]** A "chemotherapeutic agent" is a chemical compound useful in the treatment 15 of cancer. Examples of chemotherapeutic agents include alkylating agents such as thiotepa and CYTOXAN® cyclophosphamide; alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, 20 triethylenephosphoramide, triethylenethiophosphoramide and trimethylololomelamine; acetogenins (especially bullatacin and bullatacinone); delta-9-tetrahydrocannabinol (dronabinol, MARINOL®); beta-lapachone; lapachol; colchicines; betulinic acid; a camptothecin (including the synthetic analogue topotecan (HYCAMTIN®), CPT-11 (irinotecan, CAMPTOSAR®), acetylcamptothecin, scopolactin, and 9-aminocamptothecin); 25 bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); podophyllotoxin; podophyllinic acid; teniposide; cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogues, KW-2189 and CBI-TMI); eleutherobin; pancratistatin; a sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlornaphazine, cyclophosphamide, 30 estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and

ranimnustine; antibiotics such as the enediyne antibiotics (e.g., calicheamicin, especially calicheamicin gammall and calicheamicin omegall (see, e.g., Agnew, Chem Intl. Ed. Engl., 33: 183-186 (1994)); dynemicin, including dynemicin A; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antibiotic

5 chromophores), aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabacin, carminomycin, carzinophilin, chromomycinis, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, ADRIAMYCIN® doxorubicin (including morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin and deoxydoxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins such

10 as mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, porfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprime, thioguanine; pyrimidine analogs such as

15 ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as folinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amsacrine; bestabucil; bisantrene; edatraxate; demecolcine;

20 diaiquone; el fornithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidamine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidanmol; nitraerine; pentostatin; phenamet; pirarubicin; losoxantrone; 2-ethylhydrazide; procarbazine; PSK® polysaccharide complex (JHS Natural Products, Eugene, OR); razoxane; rhizoxin; sizofiran; spirogermanium; tenuazonic acid;

25 triaziquone; 2,2',2"-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine (ELDISINE®, FILDESIN®); dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); thiotepa; taxoids, e.g., TAXOL® paclitaxel (Bristol-Myers Squibb Oncology, Princeton, N.J.), ABRAZAXANE™ Cremophor-free, albumin-engineered nanoparticle formulation of

30 paclitaxel (American Pharmaceutical Partners, Schaumberg, Illinois), and TAXOTERE® doxetaxel (Rhône-Poulenc Rorer, Antony, France); chlorambucil; gemcitabine (GEMZAR®); 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblastine (VELBAN®); platinum; etoposide (VP- 16); ifosfamide; mitoxantrone; vincristine (ONCOVIN®); oxaliplatin; leucovorin; vinorelbine

(NAVELBINE®); novantrone; edatrexate; daunomycin; aminopterin; ibandronate; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoids such as retinoic acid; capecitabine (XELODA®); pharmaceutically acceptable salts, acids or derivatives of any of the above; as well as combinations of two or more of the above such as

5 CHOP, an abbreviation for a combined therapy of cyclophosphamide, doxorubicin, vincristine, and prednisolone, and FOLFOX, an abbreviation for a treatment regimen with oxaliplatin (ELOXATINTM) combined with 5-FU and leucovorin.

[0151] Also included in this definition are anti-hormonal agents that act to regulate, reduce, block, or inhibit the effects of hormones that can promote the growth of cancer, and

10 are often in the form of systemic, or whole-body treatment. They may be hormones themselves. Examples include anti-estrogens and selective estrogen receptor modulators (SERMs), including, for example, tamoxifen (including NOLVADEX® tamoxifen), EVISTA® raloxifene, droloxifene, 4-hydroxytamoxifen, trioxifene, keoxifene, LY1 17018, onapristone, and FARESTON® toremifene; anti-progesterones; estrogen receptor down-

15 regulators (ERDs); agents that function to suppress or shut down the ovaries, for example, luteinizing hormone-releasing hormone (LHRH) agonists such as LUPRON® and ELIGARD® leuprolide acetate, goserelin acetate, buserelin acetate and triptorelin; other anti-androgens such as flutamide, nilutamide and bicalutamide; and aromatase inhibitors that inhibit the enzyme aromatase, which regulates estrogen production in the adrenal glands,

20 such as, for example, 4(5)-imidazoles, aminoglutethimide, MEGASE® megestrol acetate, AROMASIN® exemestane, formestane, fadrozole, RIVIS OR® vorozole, FEMARA® letrozole, and ARIMIDEX® anastrozole. In addition, such definition of chemotherapeutic agents includes bisphosphonates such as clodronate (for example, BONEFOS® or

25 OSTAC®), DIDROC AL® etidronate, NE-58095, ZOMET A® zoledronic acid/zoledronate, FOSAMAX® alendronate, AREDIA® pamidronate, SKELID® tiludronate, or ACTONEL® risedronate; as well as troxacicabine (a 1,3-dioxolane nucleoside cytosine analog); antisense oligonucleotides, particularly those that inhibit expression of genes in signaling pathways implicated in aberrant cell proliferation, such as, for example, PKC-alpha, Raf, H-Ras, and epidermal growth factor receptor (EGF-R); vaccines such as THERATOPE® vaccine and

30 gene therapy vaccines, for example, ALLOVECTIN® vaccine, LEUVECTIN® vaccine, and VAXID® vaccine; LURTOTECAN® topoisomerase 1 inhibitor; ABARELIX® rmRH; lapatinib ditosylate (an ErbB-2 and EGFR dual tyrosine kinase small-molecule inhibitor also

known as GW572016); and pharmaceutically acceptable salts, acids or derivatives of any of the above.

[0152] A "growth inhibitory agent" when used herein refers to a compound or composition which inhibits growth of a cell either in vitro or in vivo. Thus, the growth inhibitory agent may be one which significantly reduces the percentage of cells in S phase. Examples of growth inhibitory agents include agents that block cell cycle progression (at a place other than S phase), such as agents that induce G1 arrest and M-phase arrest. Classical M-phase blockers include the vincas (vincristine and vinblastine), taxanes, and topoisomerase II inhibitors such as doxorubicin, epirubicin, daunorubicin, etoposide, and bleomycin. Those agents that arrest G1 also spill over into S-phase arrest, for example, DNA alkylating agents such as tamoxifen, prednisone, dacarbazine, mechlorethamine, cisplatin, methotrexate, 5-fluorouracil, and ara-C. Further information can be found in *The Molecular Basis of Cancer*, Mendelsohn and Israel, eds., Chapter 1, entitled "Cell cycle regulation, oncogenes, and antineoplastic drugs" by Murakami et al. (WB Saunders: Philadelphia, 1995), especially p. 13.

13. The taxanes (paclitaxel and docetaxel) are anticancer drugs both derived from the yew tree. Docetaxel (TAXOTERE®, Rhone -Poulenc Rorer), derived from the European yew, is a semisynthetic analogue of paclitaxel (TAXOL®, Bristol-Myers Squibb). Paclitaxel and docetaxel promote the assembly of microtubules from tubulin dimers and stabilize microtubules by preventing depolymerization, which results in the inhibition of mitosis in cells.

[0153] In still other embodiments, T $\beta$ RII polypeptides may be useful in the treatment or prevention of fibrosis. As used herein, the term "fibrosis" refers to the aberrant formation or development of excess fibrous connective tissue by cells in an organ or tissue. Although processes related to fibrosis can occur as part of normal tissue formation or repair, dysregulation of these processes can lead to altered cellular composition and excess connective tissue deposition that progressively impairs to tissue or organ function. The formation of fibrous tissue can result from a reparative or reactive process. Fibrotic disorders or conditions include, but are not limited to, fibroproliferative disorders associated with vascular diseases, such as cardiac disease, cerebral disease, and peripheral vascular disease, as well as tissues and organ systems including the heart, skin, kidney, peritoneum, gut, and liver (as disclosed in, e.g., Wynn, 2004, *Nat Rev* 4:583-594, incorporated herein by reference). Exemplary disorders that can be treated include, but are not limited to, renal

fibrosis, including nephropathies associated with injury/fibrosis, e.g., chronic nephropathies associated with diabetes (e.g., diabetic nephropathy), lupus, scleroderma, glomerular nephritis, focal segmental glomerular sclerosis, and IgA nephropathy; gut fibrosis, e.g., scleroderma, and radiation-induced gut fibrosis; liver fibrosis, e.g., cirrhosis, alcohol-induced 5 liver fibrosis, biliary duct injury, primary biliary cirrhosis, infection or viral-induced liver fibrosis, congenital hepatic fibrosis and autoimmune hepatitis; and other fibrotic conditions, such as cystic fibrosis, endomyocardial fibrosis, mediastinal fibrosis, sarcoidosis, scleroderma, spinal cord injury/fibrosis, myelofibrosis, vascular restenosis, atherosclerosis, , injection fibrosis (which can occur as a complication of intramuscular injections, especially 10 in children), endomyocardial fibrosis, retroperitoneal fibrosis, and nephrogenic systemic fibrosis.

**[0154]** As used herein, the terms "fibrotic disorder", "fibrotic condition," and "fibrotic disease," are used interchangeably to refer to a disorder, condition or disease characterized by fibrosis. Examples of fibrotic disorders include, but are not limited to 15 sclerotic disorders (e.g., scleroderma, atherosclerosis, diffuse systemic sclerosis), vascular fibrosis, pancreatic fibrosis, liver fibrosis (e.g., cirrhosis), renal fibrosis, musculoskeletal fibrosis, cardiac fibrosis (e.g., endomyocardial fibrosis, idiopathic cardiomyopathy), skin fibrosis (e.g., scleroderma, post-traumatic, operative cutaneous scarring, keloids and cutaneous keloid formation), eye fibrosis (e.g., glaucoma, sclerosis of the eyes, conjunctival 20 and corneal scarring, and pterygium), myelofibrosis, progressive systemic sclerosis (PSS), chronic graft-versus-host disease, Peyronie's disease, post-cystoscopic urethral stenosis, idiopathic and pharmacologically induced retroperitoneal fibrosis, mediastinal fibrosis, proliferative fibrosis, neoplastic fibrosis, Dupuytren's disease, strictures, neural scarring, dermal scarring and radiation induced fibrosis.

25 **[0155]** As used herein, inhibition of the fibrotic response of a cell, includes, but is not limited to the inhibition of the fibrotic response of one or more cells within the liver (or liver tissue); one or more cells within the kidney (or renal tissue); one or more cells within muscle tissue; one or more cells within the heart (or cardiac tissue); one or more cells within the pancreas; one or more cells within the skin; one or more cells within the bone, one or 30 more cells within the vasculature, one or more stem cells, or one or more cells within the eye.

**[0156]** The present invention contemplates the use of T $\beta$ RII polypeptides in combination with one or more other therapeutic modalities. Thus, in addition to the use of

T $\beta$ RII polypeptides, one may also administer to the subject one or more "standard" therapies for treating fibrotic disorders. For example, the T $\beta$ RII polypeptides can be administered in combination with (i.e., together with) cytotoxins, immunosuppressive agents, radiotoxic agents, and/or therapeutic antibodies. Particular co-therapeutics contemplated by the present invention include, but are not limited to, steroids (e.g., corticosteroids, such as Prednisone), immune-suppressing and/or anti-inflammatory agents (e.g., gamma-interferon, cyclophosphamide, azathioprine, methotrexate, penicillamine, cyclosporine, colchicine, antithymocyte globulin, mycophenolate mofetil, and hydroxychloroquine), cytotoxic drugs, calcium channel blockers (e.g., nifedipine), angiotensin converting enzyme inhibitors (ACE) inhibitors, para-aminobenzoic acid (PABA), dimethyl sulfoxide, transforming growth factor beta (TGF $\beta$ ) inhibitors, interleukin-5 (IL-5) inhibitors, and pan caspase inhibitors.

**[0157]** Additional anti-fibrotic agents that may be used in combination with T $\beta$ RII polypeptides include, but are not limited to, lectins (as described in, for example, U.S. Patent No.: 7,026,283, the entire contents of which is incorporated herein by reference), as well as the anti-fibrotic agents described by Wynn et al (2007, *J Clin Invest* 117:524-529, the entire contents of which is incorporated herein by reference). For example, additional anti-fibrotic agents and therapies include, but are not limited to, various anti-inflammatory/immunosuppressive/ cytotoxic drugs (including colchicine, azathioprine, cyclophosphamide, prednisone, thalidomide, pentoxifylline and theophylline), TGF $\beta$  signaling modifiers (including relaxin, SMAD7, HGF, and BMP7, as well as TGF $\beta$ 1, T $\beta$ RI, T $\beta$ RII, EGR-I, and CTGF inhibitors), cytokine and cytokine receptor antagonists (inhibitors of IL-1 $\beta$ , IL-5, IL-6, IL-13, IL-21, IL-4R, IL-13R $\alpha$ 1, GM-CSF, TNF- $\alpha$ , oncostatin M, WISP-I, and PDGFs), cytokines and chemokines (IFN- $\gamma$ , IFN- $\alpha$ / $\beta$ , IL-12, IL-10, HGF, CXCL10, and CXCL11), chemokine antagonists (inhibitors of CXCL1, CXCL2, CXCL12, CCL2, CCL3, CCL6, CCL17, and CCL18), chemokine receptor antagonists (inhibitors of CCR2, CCR3, CCR5, CCR7, CXCR2, and CXCR4), TLR antagonists (inhibitors of TLR3, TLR4, and TLR9), angiogenesis antagonists (VEGF-specific antibodies and adenosine deaminase replacement therapy), antihypertensive drugs (beta blockers and inhibitors of ANG 11, ACE, and aldosterone), vasoactive substances (ET-1 receptor antagonists and bosentan), inhibitors of the enzymes that synthesize and process collagen (inhibitors of prolyl hydroxylase), B cell antagonists (rituximab), integrin/adhesion molecule antagonists (molecules that block  $\alpha$ 1 $\beta$ 1 and  $\alpha$ v $\beta$ 6 integrins, as well as inhibitors of integrin-linked kinase, and antibodies specific for ICAM-I and VCAM-I), proapoptotic drugs that target myofibroblasts, MMP inhibitors

(inhibitors of MMP2, MMP9, and MMP12), and TIMP inhibitors (antibodies specific for TIMP-1 ).

[0158] The T $\beta$ RII polypeptide and the co-therapeutic agent or co-therapy can be administered in the same formulation or separately. In the case of separate administration, 5 the T $\beta$ RII polypeptide can be administered before, after, or concurrently with the co- therapeutic or co-therapy. One agent may precede or follow administration of the other agent by intervals ranging from minutes to weeks. In embodiments where two or more different kinds of therapeutic agents are applied separately to a subject, one would generally ensure that a significant period of time did not expire between the time of each delivery, such that 10 these different kinds of agents would still be able to exert an advantageously combined effect on the target tissues or cells.

## 8. Pharmaceutical Compositions

[0159] The therapeutic agents described herein (e.g., T $\beta$ RII polypeptides) may be formulated into pharmaceutical compositions. Pharmaceutical compositions for use in 15 accordance with the present disclosure may be formulated in conventional manner using one or more physiologically acceptable carriers or excipients. Such formulations will generally be substantially pyrogen-free, in compliance with most regulatory requirements.

[0160] In certain embodiments, the therapeutic method of the disclosure includes administering the composition systemically, or locally as an implant or device. When 20 administered, the therapeutic composition for use in this disclosure is in a pyrogen-free, physiologically acceptable form. Therapeutically useful agents other than the T $\beta$ RII signaling antagonists which may also optionally be included in the composition as described above, may be administered simultaneously or sequentially with the subject compounds (e.g., T $\beta$ RII polypeptides) in the methods disclosed herein.

[0161] Typically, protein therapeutic agents disclosed herein will be administered 25 parentally, and particularly intravenously or subcutaneously. Pharmaceutical compositions suitable for parenteral administration may comprise one or more T $\beta$ RII polypeptides in combination with one or more pharmaceutically acceptable sterile isotonic aqueous or nonaqueous solutions, dispersions, suspensions or emulsions, or sterile powders which may 30 be reconstituted into sterile injectable solutions or dispersions just prior to use, which may contain antioxidants, buffers, bacteriostats, solutes which render the formulation isotonic with

the blood of the intended recipient or suspending or thickening agents. Examples of suitable aqueous and nonaqueous carriers which may be employed in the pharmaceutical compositions of the disclosure include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

[0162] The compositions and formulations may, if desired, be presented in a pack or dispenser device which may contain one or more unit dosage forms containing the active ingredient. The pack may for example comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration

[0163] Further, the composition may be encapsulated or injected in a form for delivery to a target tissue site. In certain embodiments, compositions of the present invention may include a matrix capable of delivering one or more therapeutic compounds (e.g., T $\beta$ RII polypeptides) to a target tissue site, providing a structure for the developing tissue and optimally capable of being resorbed into the body. For example, the matrix may provide slow release of the T $\beta$ RII polypeptides. Such matrices may be formed of materials presently in use for other implanted medical applications.

[0164] The choice of matrix material is based on biocompatibility, biodegradability, mechanical properties, cosmetic appearance and interface properties. The particular application of the subject compositions will define the appropriate formulation. Potential matrices for the compositions may be biodegradable and chemically defined calcium sulfate, tricalcium phosphate, hydroxyapatite, polylactic acid and polyanhydrides. Other potential materials are biodegradable and biologically well defined, such as bone or dermal collagen. Further matrices are comprised of pure proteins or extracellular matrix components. Other potential matrices are non-biodegradable and chemically defined, such as sintered hydroxyapatite, bioglass, aluminates, or other ceramics. Matrices may be comprised of combinations of any of the above mentioned types of material, such as polylactic acid and hydroxyapatite or collagen and tricalcium phosphate. The bioceramics may be altered in composition, such as in calcium-aluminate-phosphate and processing to alter pore size, particle size, particle shape, and biodegradability.

**[0165]** In certain embodiments, methods of the invention can be administered orally, e.g., in the form of capsules, cachets, pills, tablets, lozenges (using a flavored basis, usually sucrose and acacia or tragacanth), powders, granules, or as a solution or a suspension in an aqueous or non-aqueous liquid, or as an oil-in-water or water-in-oil liquid emulsion, or 5 as an elixir or syrup, or as pastilles (using an inert base, such as gelatin and glycerin, or sucrose and acacia) and/or as mouth washes and the like, each containing a predetermined amount of an agent as an active ingredient. An agent may also be administered as a bolus, electuary or paste.

**[0166]** In solid dosage forms for oral administration (capsules, tablets, pills, 10 dragees, powders, granules, and the like), one or more therapeutic compounds of the present invention may be mixed with one or more pharmaceutically acceptable carriers, such as sodium citrate or dicalcium phosphate, and/or any of the following: (1) fillers or extenders, such as starches, lactose, sucrose, glucose, mannitol, and/or silicic acid; (2) binders, such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinyl pyrrolidone, sucrose, 15 and/or acacia; (3) humectants, such as glycerol; (4) disintegrating agents, such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate; (5) solution retarding agents, such as paraffin; (6) absorption accelerators, such as quaternary ammonium compounds; (7) wetting agents, such as, for example, cetyl alcohol and glycerol monostearate; (8) absorbents, such as kaolin and bentonite clay; (9) lubricants, 20 such a talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof; and (10) coloring agents. In the case of capsules, tablets and pills, the pharmaceutical compositions may also comprise buffering agents. Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugars, as well as high molecular weight 25 polyethylene glycols and the like.

**[0167]** Liquid dosage forms for oral administration include pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups, and elixirs. In addition to the active ingredient, the liquid dosage forms may contain inert diluents commonly used in the art, such as water or other solvents, solubilizing agents and 30 emulsifiers, such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor, and sesame oils), glycerol, tetrahydrofuryl

alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof. Besides inert diluents, the oral compositions can also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, coloring, perfuming, and preservative agents.

5 [0168] Suspensions, in addition to the active compounds, may contain suspending agents such as ethoxylated isostearyl alcohols, polyoxyethylene sorbitol, and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth, and mixtures thereof.

10 [0169] The compositions of the invention may also contain adjuvants, such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of the action of microorganisms may be ensured by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It may also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like into the compositions. In addition, prolonged absorption of the injectable pharmaceutical 15 form may be brought about by the inclusion of agents which delay absorption, such as aluminum monostearate and gelatin.

20 [0170] It is understood that the dosage regimen will be determined by the attending physician considering various factors which modify the action of the subject compounds of the invention (e.g., T $\beta$ RII polypeptides). The various factors include, but are not limited to, the patient's age, sex, and diet, the severity disease, time of administration, and other clinical factors. Optionally, the dosage may vary with the type of matrix used in the reconstitution and the types of compounds in the composition. The addition of other known growth factors to the final composition, may also affect the dosage. Progress can be monitored by periodic assessment of bone growth and/or repair, for example, X-rays (including DEXA), 25 histomorphometric determinations, and tetracycline labeling.

30 [0171] In certain embodiments, the present invention also provides gene therapy for the in vivo production of T $\beta$ RII polypeptides. Such therapy would achieve its therapeutic effect by introduction of the T $\beta$ RII polynucleotide sequences into cells or tissues having the disorders as listed above. Delivery of T $\beta$ RII polynucleotide sequences can be achieved using a recombinant expression vector such as a chimeric virus or a colloidal dispersion system.

Preferred for therapeutic delivery of T $\beta$ RII polynucleotide sequences is the use of targeted liposomes.

**[0172]** Various viral vectors which can be utilized for gene therapy as taught herein include adenovirus, herpes virus, vaccinia, or, preferably, an RNA virus such as a retrovirus.

5 Preferably, the retroviral vector is a derivative of a murine or avian retrovirus. Examples of retroviral vectors in which a single foreign gene can be inserted include, but are not limited to: Moloney murine leukemia virus (MoMuLV), Harvey murine sarcoma virus (HaMuSV), murine mammary tumor virus (MuMTV), and Rous Sarcoma Virus (RSV). A number of additional retroviral vectors can incorporate multiple genes. All of these vectors can transfer  
10 or incorporate a gene for a selectable marker so that transduced cells can be identified and generated. Retroviral vectors can be made target-specific by attaching, for example, a sugar, a glycolipid, or a protein. Preferred targeting is accomplished by using an antibody. Those of skill in the art will recognize that specific polynucleotide sequences can be inserted into the retroviral genome or attached to a viral envelope to allow target specific delivery of the  
15 retroviral vector containing the T $\beta$ RII polynucleotide. In a preferred embodiment, the vector is targeted to bone or cartilage.

**[0173]** Alternatively, tissue culture cells can be directly transfected with plasmids encoding the retroviral structural genes gag, pol and env, by conventional calcium phosphate transfection. These cells are then transfected with the vector plasmid containing the genes of  
20 interest. The resulting cells release the retroviral vector into the culture medium.

**[0174]** Another targeted delivery system for T $\beta$ RII polynucleotides is a colloidal dispersion system. Colloidal dispersion systems include macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. The preferred colloidal system of this invention is a  
25 liposome. Liposomes are artificial membrane vesicles which are useful as delivery vehicles in vitro and in vivo. RNA, DNA and intact virions can be encapsulated within the aqueous interior and be delivered to cells in a biologically active form (see e.g., Fraley, et al., Trends Biochem. Sci., 6:77, 1981). Methods for efficient gene transfer using a liposome vehicle, are known in the art, see e.g., Mannino, et al., Biotechniques, 6:682, 1988. The composition of  
30 the liposome is usually a combination of phospholipids, usually in combination with steroids, especially cholesterol. Other phospholipids or other lipids may also be used. The physical

characteristics of liposomes depend on pH, ionic strength, and the presence of divalent cations.

[0175] Examples of lipids useful in liposome production include phosphatidyl compounds, such as phosphatidylglycerol, phosphatidylcholine, phosphatidylserine, 5 phosphatidylethanolamine, sphingolipids, cerebrosides, and gangliosides. Illustrative phospholipids include egg phosphatidylcholine, dipalmitoylphosphatidylcholine, and distearoylphosphatidylcholine. The targeting of liposomes is also possible based on, for example, organ-specificity, cell-specificity, and organelle-specificity and is known in the art.

[0176] The disclosure provides formulations that may be varied to include acids and 10 bases to adjust the pH; and buffering agents to keep the pH within a narrow range.

## EXEMPLIFICATION

[0177] The invention now being generally described, it will be more readily understood by reference to the following examples, which are included merely for purposes of illustration of certain embodiments of the present invention, and are not intended to limit 15 the invention.

### Example 1. Generation of bioactive GDF15

[0178] GDF15 (also known as macrophage-inhibitory cytokine-1) has not been shown biochemically to bind or interact directly with any receptor. Applicants first tried without success to identify a native receptor with high-affinity binding to GDF15 using 20 commercially available human GDF15 (R&D Systems) produced in mammalian CHO cells. Like other ligands in the TGF $\beta$  superfamily, which contain a characteristic cysteine knot motif, mature GDF15 is synthesized with a larger prodomain (Harrison et al., Growth Factors 29:174, 2011; Shi et al., Nature 474:343, 2011) that is removed through cleavage by a furin-like protease at the canonical RXXR site to generate mature dimeric GDF15. Since 25 inadequate or inappropriate ligand purification could be a potential reason for inactivity of commercially available GDF15, Applicants tested different purification procedures for GDF15.

#### *Stable Expression of GDF15 in CHO Cells*

[0179] Applicants used CHO cells to express human GDF15 (hGDF15) and murine 30 GDF15 (mGDF15) for further studies. The amino acid sequence of native precursor for

hGDF15 is shown in **Figure 1**, and a corresponding nucleotide sequence (with a silent, single nucleotide substitution compared to the native sequence) is shown in **Figure 2**. The native amino acid and nucleotide sequences for mGDF15 precursor are shown in **Figures 3** and **4**, respectively. For expression in CHO cells, UCOE<sup>TM</sup>-based constructs encoding human or 5 murine GDF15 precursor were stably transfected into a CHO-PACE cell line. Clones were selected in methotrexate levels of 10 nM, 20 nM, and 50 nM, and any clones that formed colonies (one or two per methotrexate concentration) were then pooled. No gene 10 amplification was performed since it is difficult to amplify UCOE<sup>TM</sup> pools while maintaining stability of expression. Instead of dilution cloning, high-expressing pools were identified and used for generating hGDF15 and mGDF15.

#### *Purification of Human GDF15*

[0180] To begin purification, conditioned media from CHO cells stably expressing hGDF15 was adjusted to pH 4.7 with acetic acid. After incubation of media for 10 min at ambient temperature, precipitate was removed by centrifugation. Supernatant was filtered 15 with a 0.8 µm disposable filter. An SP Sepharose<sup>TM</sup> Fast Flow column (GE Healthcare) was equilibrated with buffers A (20 mM sodium acetate, pH 4.7) and B (20 mM sodium acetate, 1M NaCl, pH 4.7). Loading was performed at 100 cm/hr. The column was washed with 20% B (200 mM NaCl) until no more protein eluted from the column and then washed back to 0% B to remove any residual salt. Protein was eluted with 50 mM Tris, 6M urea, pH 8.0 20 (Tris+urea pool) until no more protein eluted from the column, followed by elution with 50 mM Tris, 6M urea, 1M NaCl, pH 8.0 (Tris+urea+salt pool). Each pool was dialyzed in 50 mM 4-morpholinoethanesulfonic acid (MES, pH 6.5) overnight at 4°C.

[0181] GDF15 found in the Tris+urea+salt pool was degraded based on Western blot analysis, so this pool was discarded. The Tris+urea pool was loaded on a Q Sepharose<sup>TM</sup> 25 Fast Flow column (GE Healthcare) previously equilibrated with buffers A (50 mM MES, pH 6.5) and B (50 mM MES, 1M NaCl, pH 6.5). The flow-through was collected, and the column was washed with 10% B (100 mM NaCl), followed by a 10-50% B gradient (100-500 mM NaCl) over five column volumes at 120 cm/hr. After evaluation of the flow-through and wash fractions by Western blot, protein was found mainly in the flow-through. The flow- 30 through was injected on a reverse-phase preparative C4 column (Vydac) attached to a HPLC, with buffers A (water/0.1% TFA) and B (acetonitrile/0.1% TFA). A 25-40% B gradient over 1 h at 4.5 mL/min produced the best resolution. Collected fractions were evaluated by SDS-

PAGE gel (Sypro Ruby) and Western blot to select those for concentration in a centrifugal evaporator.

*Purification of Murine GDF15*

[0182] The pH of the conditioned media was adjusted to pH 4.7 with acetic acid.

5 After incubation of media for 10 min at ambient temperature, precipitate was removed by centrifugation. Supernatant was filtered with a 0.8  $\mu$ m disposable filter. An SP Sepharose<sup>TM</sup> Fast Flow column (GE Healthcare) was equilibrated with buffers A (20 mM sodium acetate, pH 4.7) and B (20 mM sodium acetate, 1M NaCl, pH 4.7). Loading was performed at 100-150 cm/hr, and the column was washed with buffer A until no more protein eluted from the

10 column. A wash was performed at 60% B (600 mM NaCl) for 3-4 column volumes, followed by elution with 100% B (1M NaCl) for 3-4 column volumes. Elution continued with 50 mM Tris, 6M urea, pH 8.0, to remove any protein still bound to the resin.

[0183] Non-reduced samples of SP-column fractions were analyzed by Western blot. Although most protein was found in the Tris-eluted fractions, previous experiments have indicated that mGDF15 found in these fractions is essentially inactive, so it was not used for further purification. Instead, purification was continued with protein found in the 100% B elution (salt-elution pool). This pool was injected on a reverse-phase preparative C4 column (Vydac) attached to a HPLC. Buffer A was water/0.1% TFA and buffer B was acetonitrile/0.1% TFA. Protein was eluted with a 25-40% B gradient over 1 h at 4.5 mL/min.

20 After evaluation of the reverse-phase column fractions by SDS-PAGE gel (Sypro Ruby) and Western blot, the fractions containing pure mGDF15 were pooled and concentrated in a centrifugal evaporator.

[0184] The identities of hGDF15 and mGDF15 were each confirmed by N-terminal sequencing. Both types of purified GDF15 stimulated SMAD2/3 phosphorylation in two different cell lines, thereby providing confirmation of ligand activity.

**Example 2. Identification of a TGF $\beta$  superfamily receptor with high-affinity binding to GDF15**

[0185] Once active GDF15 protein was obtained, receptor-Fc fusion proteins comprising TGF $\beta$  superfamily receptors were screened for binding to human or murine GDF15 that was generated and purified as described in Example 1. These fusion proteins incorporated an IgG1 Fc domain and were either purchased from R&D Systems or generated in-house. Among the five type II receptors (TGF $\beta$  receptor type II, activin receptor type IIA,

activin receptor type IIB, BMP receptor type II, and MIS receptor type II), only TGF $\beta$  receptor type II (T $\beta$ RII) exhibited detectable binding to GDF15 ( $k_a = 2.92 \times 10^5 \text{ M}^{-1}\text{s}^{-1}$ ;  $k_d = 0.001 \text{ s}^{-1}$ ), as determined by surface plasmon resonance with captured receptor-Fc fusion proteins. hGDF15 bound to captured hT $\beta$ RII-Fc at 37°C with an equilibrium dissociation constant ( $K_D$ ) of 9.56 nM. None of the seven type I receptors (ALK1, ALK2, ALK3, ALK4, ALK5, ALK6, and ALK7) displayed detectable binding to GDF15 (mGDF15 at 20 nM or 200 nM).

[0186] Human T $\beta$ RII occurs naturally in at least two isoforms – A (long) and B (short) – generated by alternative splicing in the extracellular domain (ECD) (Figures 5, 6).  
10 The hT $\beta$ RII-hG1Fc fusion protein (R&D Systems) used for the screening described above incorporates the wild-type T $\beta$ RII<sub>short</sub> isoform. In a follow-up analysis, the affinity of mGDF15 binding to a fusion protein incorporating the wild-type T $\beta$ RII<sub>long</sub> isoform (R&D Systems) was found by surface plasmon resonance to be very similar to that of the fusion protein incorporating the T $\beta$ RII<sub>short</sub> isoform ( $K_{DS}$  at 37°C were 2.7 nM and 4.8 nM,  
15 respectively). Having observed general equivalence of these short and long isoforms with regard to GDF15 binding, Applicants then generated a receptor-Fc fusion protein consisting of the wild-type ECD of hT $\beta$ RII<sub>short</sub> (SEQ ID NO: 7) fused at its C-terminus with a human IgG2 Fc domain via a minimal linker. Unless noted otherwise, amino acid position numbering with regard to variants based on the T $\beta$ RII short and long isoforms refers to the  
20 corresponding position in the native precursors, SEQ ID NO: 5 and SEQ ID NO: 6, respectively.

[0187] Given the high-affinity binding of T $\beta$ RII to GDF15, we tested whether T $\beta$ RII could be used as an inhibitor of GDF15. The fusion protein hT $\beta$ RII<sub>short</sub>(23-159)-hG2Fc was tested in A549 cells transfected with a reporter gene containing a CAGA-12  
25 promoter construct and was found to inhibit hGDF15-induced gene activation in such cells with an  $IC_{50}$  of 0.15-0.5 nM. Potent inhibition of GDF15 signaling by the hT $\beta$ RII<sub>short</sub> ECD provides additional evidence that T $\beta$ RII is the high-affinity receptor for GDF15. Even though GDF15 exhibited no detectable binding to ALK5 under cell-free conditions, suppression of endogenous ALK5 mRNA by siRNA methodology markedly reduced  
30 mGDF15-mediated signaling in A549 cells (a human pulmonary epithelial cell line) compared to control treatment. In contrast, suppression of other type I receptors (ALK2, ALK3, ALK4, and ALK7) by siRNA methodology failed to alter GDF15-mediated signaling in A549 cells. This result indicates that the GDF15 ternary signaling complex includes

ALK5 (TGF $\beta$  receptor type I) as its type I receptor and thus provides corroborating evidence for T $\beta$ RII as a functional type II receptor for GDF15.

**Example 3. Generation of receptor fusion protein variants**

*T $\beta$ RII ECD variants*

5 [0188] Since T $\beta$ RII also binds with high affinity to TGF $\beta$ 1 and TGF $\beta$ 3, native T $\beta$ RII-Fc fusion protein affects signaling of these ligands as well as GDF15. While in some therapeutic settings this broader spectrum of ligand binding may be advantageous, in other settings a more selective molecule may be superior. Therefore, Applicants sought polypeptides with enhanced or reduced selectivity for GDF15 by generating fusion proteins  
10 comprising variants of human T $\beta$ RII ECD. The wild-type hT $\beta$ RII<sub>short</sub>(23-159) sequence shown below (SEQ ID NO: 7) served as the basis for five receptor ECD variants listed below (SEQ ID NO: 8-12). A wild type hT $\beta$ RII<sub>short</sub>(23-159) was fused to an Fc portion of IgG2 to generate a novel, base Fc fusion construct. See SEQ ID Nos. 50, 51 and 52, below.

1 TIPPHVQKSV NNDMIVTDNN GAVKFPQLCK FCDVRFSTCD NQKSCMSNCS  
15 51 ITSICEKPQE VCVAVWRKND ENITLETVCH DPKL~~PYHDFI~~ LEDAASPKCI  
101 MKEKKKPGET FFMCSCSSDE CNDNIIIFSEE YNTSNPD (SEQ ID NO: 7)

(1) The hT $\beta$ RII<sub>short</sub>(23-159/D110K) amino acid sequence shown below (SEQ ID NO: 8), in which the substituted residue is underlined.

1 TIPPHVQKSV NNDMIVTDNN GAVKFPQLCK FCDVRFSTCD NQKSCMSNCS  
20 51 ITSICEKPQE VCVAVWRKND ENITLETVCH DPKL~~PYHDFI~~ LEDAASPKCI  
101 MKEKKKPGET FFMCSCSSDE CNDNIIIFSEE YNTSNPD (SEQ ID NO: 8)

(2) The N-terminally truncated hT $\beta$ RII<sub>short</sub>(29-159) amino acid sequence shown below (SEQ ID NO: 9).

1 QKSVNNNDMIV TDNNGAVKFP QLCKFCDVRF STCDNQKSCM SNC~~SITSICE~~  
25 51 KPQEVCVAW RKNDENITL~~E~~ TVCHDPKL~~PY~~ HDFILEDAA~~S~~ PKCIMKEKKK  
101 PGETFFMCSC SSDECNDNII FSEEEYNTSNP D (SEQ ID NO: 9)

(3) The N-terminally truncated hT $\beta$ RII<sub>short</sub>(35-159) amino acid sequence shown below (SEQ ID NO: 10).

1 DMIVTDNNNGA VKFPQLCKFC DVRFSTCDNQ KSCMSNCSIT SICEKPQEVC  
30 51 VAVWRKNDEN ITLET~~VCHDP~~ KLPYHDFILE DAASPKCIMK EKKKPGETFF  
101 MCSCSSDECN DNIIFSEEYN TSNPD (SEQ ID NO: 10)

(4) The C-terminally truncated hT $\beta$ RII<sub>short</sub>(23-153) amino acid sequence shown below (SEQ ID NO: 11).

1 TIPPHVQKSV NNDMIVTDNN GAVKFPQLCK FCDVRFSTCD NQKSCMSNCS  
 51 ITSICEKPQE VCVAVWRKND ENITLETVCH DPKLPYHDFI LEDAASPKCI  
 5 101 MKEKKKPGET FFMCSCSSDE CNDNIIIFSEE Y (SEQ ID NO: 11)

(5) The C-terminally truncated hT $\beta$ RII<sub>short</sub>(23-153/N70D) amino acid sequence shown below (SEQ ID NO: 12), in which the substituted residue is underlined.

1 TIPPHVQKSV NNDMIVTDNN GAVKFPQLCK FCDVRFSTCD NQKSCMSDCS  
 51 ITSICEKPQE VCVAVWRKND ENITLETVCH DPKLPYHDFI LEDAASPKCI  
 10 101 MKEKKKPGET FFMCSCSSDE CNDNIIIFSEE Y (SEQ ID NO: 12)

**[0189]** Applicants also envision five corresponding variants (SEQ ID NO: 14-17) based on the wild-type hT $\beta$ RII<sub>long</sub>(23-184) sequence shown below (SEQ ID NO: 13), in which the 25 amino-acid insertion is underlined. Note that splicing results in a conservative amino acid substitution (Val $\rightarrow$ Ile) at the flanking position C-terminal to the insertion.

15 Sequence relationships among several hT $\beta$ RII<sub>short</sub> variants and their hT $\beta$ RII<sub>long</sub> counterparts are indicated in **Figure 7**.

1 TIPPHVQKSD VEMEAQKDEI ICPSCNRTAH PLRHNNDMI VTDNNNGAVKF  
 51 PQLCKFCDVR FSTCDNQKSC MSNCSITSIC EKPQEVCVAV WRKNDENITL  
 101 ETVCHDPKLP YHDFILEDAA SPKCIMKEKK KPGETFFMCS CSSDECNDNI  
 20 151 IFSEEVNTSN PD (SEQ ID NO: 13)

(1) The hT $\beta$ RII<sub>long</sub>(23-184/D135K) amino acid sequence shown below (SEQ ID NO: 14), in which the substituted residue is double underlined.

1 TIPPHVQKSD VEMEAQKDEI ICPSCNRTAH PLRHNNDMI VTDNNNGAVKF  
 51 PQLCKFCDVR FSTCDNQKSC MSNCSITSIC EKPQEVCVAV WRKNDENITL  
 25 101 ETVCHDPKLP YHKFILEDAA SPKCIMKEKK KPGETFFMCS CSSDECNDNI  
 151 IFSEEVNTSN PD (SEQ ID NO: 14)

(2) The N-terminally truncated hT $\beta$ RII<sub>long</sub>(29-184) amino acid sequence shown below (SEQ ID NO: 15).

1 QKSDVEMEAQ KDEIICPSCN RTAHLRHN NDMIVTDNNG AVKFPQLCKF

51 CDVRFSTCDN QKSCMSNCSI TSICEKPQEVEV CVAVWRKNDE NITLETVCHD  
 101 PKLPYHDFIL EDAASPKCIM KEKKKPGETF FMCSCSSDEC NDNIIFSEYY  
 151 NTSNPD (SEQ ID NO: 15)

(3) The N-terminally truncated hT $\beta$ RII<sub>long</sub>(60-184) amino acid sequence shown below (same  
 5 as SEQ ID NO: 10).

1 DMIVTDNNGA VKFPQLCKFC DVRFSTCDNQ KSCMSNCSIT SICEKPQEVC  
 51 VAVWRKNDEN ITLETVCHDP KLKYHDFILE DAASPKCIMK EKKKPGETFF  
 101 MCSCSSDECN DNIIFSEEYN TSNPD (same as SEQ ID NO: 10)

(4) The C-terminally truncated hT $\beta$ RII<sub>long</sub>(23-178) amino acid sequence shown below (SEQ  
 10 ID NO: 16).

1 TIPPHVQKSD VEMEAQKDEI ICPSCNRTAH PLRHINNDMI VTDNNNGAVKF  
 51 PQLCKFCDVR FSTCDNQKSC MSNCSITSIC EKPQEVCVAV WRKNDENITL  
 101 ETVCHDPKLP YHDFILEDAA SPKCIMKEKK KPGETFFMCS CSSDECNDNI  
 151 IFSEYY (SEQ ID NO: 16)

15 (5) The C-terminally truncated hT $\beta$ RII<sub>long</sub>(23-178/N95D) amino acid sequence shown below  
 (SEQ ID NO: 17), in which the substituted residue is double underlined.

1 TIPPHVQKSD VEMEAQKDEI ICPSCNRTAH PLRHINNDMI VTDNNNGAVKF  
 51 PQLCKFCDVR FSTCDNQKSC MSDCSITSIC EKPQEVCVAV WRKNDENITL  
 101 ETVCHDPKLP YHDFILEDAA SPKCIMKEKK KPGETFFMCS CSSDECNDNI  
 20 151 IFSEYY (SEQ ID NO: 17)

**[0190]** Additional T $\beta$ RII ECD variants include:

(A) The N- and C-terminally truncated hT $\beta$ RII<sub>short</sub>(35-153) or hT $\beta$ RII<sub>long</sub>(60-178) amino acid  
 sequence shown below (SEQ ID NO: 47).

1 DMIVTDNNGA VKFPQLCKFC DVRFSTCDNQ KSCMSNCSIT SICEKPQEVC  
 25 51 VAVWRKNDEN ITLETVCHDP KLKYHDFILE DAASPKCIMK EKKKPGETFF  
 101 MCSCSSDECN DNIIFSEEY (SEQ ID NO: 47)

(B) The N- and C-terminally truncated hT $\beta$ RII<sub>short</sub>(29-153) amino acid sequence shown  
 below (SEQ ID NO: 48).

30 1 QKSVNNNDMIV TDNNNGAVKFP QLCKFCDVRF STCDNQKSCM SNCSITSICE  
 51 KPQEVCVAVW RKNDENITL TVCHDPKLPY HDFILEDAAS PKCIMKEKK

101 PGETFFMCSC SSDECNDNII FSEYY (SEQ ID NO: 48)

(C) The N- and C-terminally truncated hT $\beta$ RII<sub>long</sub>(29-178) amino acid sequence shown below (SEQ ID NO: 49).

5 1 QKSDVEMEAQ KDEIICPSCN RTAHLRHN NDMIVTDNNG AVKFPQLCKF  
 51 CDVRFSTCDN QKSCMSNCSI TSICEKPQEV CVAVWRKNDE NITLETVCND  
 101 PKLPYHDFIL EDAASPKCIM KEKKKPGETF FMCSCSSDEC NDNIIFSEYY  
 (SEQ ID NO: 49)

10 [0191] Any of the above variants (SEQ ID NO: 8-12, 14-17, and 47-49) could incorporate an insertion of 36 amino acids (SEQ ID NO: 18) between the pair of glutamate residues (positions 151 and 152 of SEQ ID NO: 5, or positions 176 and 177 of SEQ ID NO: 6) located near the C-terminus of the hT $\beta$ RII ECD, as occurs naturally in the hT $\beta$ RII isoform C (Konrad et al., BMC Genomics 8:318, 2007).

GRCKIRHIGS NNRLQRSTCQ NTGWESAHVM KTPGFR (SEQ ID NO: 18)

15 [0192] As an example, the paired glutamate residues flanking the optional insertion site are denoted below (underlined) for the hT $\beta$ RII<sub>short</sub>(29-159) variant (SEQ ID NO: 9).

1 1 QKSVNNDMIV TDNNGAVKFP QLCKFCDVRF STCDNQKSCM SNCSITSICE  
 51 51 KPQEVCVAWW RKNDENITLE TVCHDPKLPY HDFILEDAAS PKCIMKEKKK  
 101 101 PGETFFMCSC SSDECNDNII FSEYYNTSNP D (SEQ ID NO: 9)

20

#### *Fc domain variants*

[0193] hT $\beta$ RII-hFc fusion proteins were generated in which five hT $\beta$ RII<sub>short</sub> variants described above were each fused at their C-terminus (via a minimal linker) to a human IgG2 Fc domain, which has the following amino acid sequence (SEQ ID NO: 19):

25 1 VECPPCPAPP VAGPSVFLFP PKPKDTLMIS RTPEVTCVVV DVSHEPVEQ  
 51 51 FNWYVDGVEV HNAKTKPREE QFNSTFRVVS VLTVVHQDWL NGKEYKCKVS  
 101 101 NKGLPAPIEK TISKTGQPR EPQVYTLPPS REEMTKNQVS LTCLVKGFYP  
 151 151 SDIAVEWESN GQPENNYKTT PPMLSDGSF FLYSKLTVDK SRWQQGNVFS  
 201 201 CSVMHEALHN HYTQKSLSL PGK (SEQ ID NO: 19)

**[0194]** Applicants envision hT $\beta$ RII-hFc fusion proteins comprising alternative Fc domains, including full-length human IgG1 Fc (hG1Fc) (SEQ ID NO: 20, below) and N-terminally truncated human IgG1 Fc (hG1Fc<sub>short</sub>) (SEQ ID NO: 21, below). Optionally, a polypeptide unrelated to an Fc domain could be attached in place of the Fc domain.

5        1   GGPKSCDKTH TCPPCPAPEL LGGPSVFLFP PKPKDTLMIS RTPEVTCVVV  
51        51   DVSHEDEPEVK FNWYVDGVEV HNAKTKPREE QYNSTYRVVS VLTVLHQDWL  
101      101   NGKEYKCKVS NKALPAPIEK TISKAKGQPR EPQVYTLPPS REEMTKNQVS  
151      151   LTCLVKGFYP SDIAVEWESN GQPENNYKTT PPVLDSDGSF FLYSKLTVDK  
201      201   SRWQQGNVFS CSVMHEALHN HYTQKSLSLS PGK        (SEQ ID NO: 20)

10

1        1   THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDE  
51        51   VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK  
101      101   VSNKALPAPI EKTISKAKGQ PREPQVYTLPS PSREEMTKNQ VSLTCLVKGF  
151      151   YPSDIAVEWE SNGQPENNYK TPPVLDSDG SFFLYSKLTV DKSRWQQGNV  
15        201   FSCSVMHEAL HNHYTQKSLS LSPGK        (SEQ ID NO: 21)

*Leader sequence variants*

**[0195]** The following three leader sequences were considered:

(1) Native: MGRGLLRGLWPLHIVLWTRIAS (SEQ ID NO: 22)

(2) Tissue plasminogen activator (TPA): MDAMKRGCCVLLCGAVFVSP (SEQ ID

20        NO: 23)

(3) Honey bee melittin (HBML): MKFLVNVALVFMVVYISYIYA (SEQ ID NO:  
24)

*Expression of hT $\beta$ RII-hFc fusion proteins*

**[0196]** The selected hT $\beta$ RII-hFc protein variants incorporate the TPA leader and  
25 have the unprocessed amino-acid sequences shown in SEQ ID NOs: 25, 29, 33, 37, and 41  
(see Example 5). Corresponding nucleotide sequences for these variants are SEQ ID NOs:  
26, 30, 34, 38, and 42. Selected hT $\beta$ RII-hFc variants, each with a G2Fc domain (SEQ ID  
NO: 19), were expressed in HEK-293 cells and purified from conditioned media by filtration  
and protein A chromatography. Purity of samples for reporter gene assays was evaluated by  
30 SDS-PAGE and Western blot analysis.

**[0197]** Applicants envision additional hT $\beta$ RII-hFc protein variants with the unprocessed amino-acid sequences shown in SEQ ID NOs: 27, 31, 35, 39, and 43, and corresponding nucleotide sequences shown in SEQ ID NOs: 28, 32, 36, 40, and 44.

**[0198]** The amino acid sequence of the wild-type short construct hT $\beta$ RII<sub>short</sub>(23-5159)-hG2Fc (SEQ ID NO: 50 is shown below.

TIPPHVQKSV NNDMIVTDNN GAVKFPQLCK FCDVRFSTCD NQKSCMSNCS  
 ITSICEKPQE VCVAWRKND ENITLETVCH DPKLPYHDFI LEDAASPKCI  
 MKEKKKPGET FFMCSCSSDE CNDNIIFSEE YNTSNPDTGG GVECPPCPAP  
 PVAGPSVFLF PPKPKDTLMI SRTPEVTCVV VDVSHEDEPV QFNWYVDGVE  
 VHNAKTKPRE EQFNSTFRVV SVLTVVHQDW LNGKEYKCKV SNKGLPAPIE  
 KTISKTKGQP REPQVYTLPP SREEMTKNQV SLTCLVKGFY PSDIAVEWES  
 NGQPENNYKT TPPMLSDGS FFLYSKLTVD KSRWQQGNVF SCSVMHEALH  
 NHYTQKSLSL SPGK (SEQ ID NO: 50)

**[0199]** This protein was expressed from a construct including a TPA leader sequence, as shown below (SEQ ID NO:52). Dotted underline denotes leader, and solid underline denotes linker.

1 MDAMKRLGCC VLLLCGAVFV SPGATIPPHV QKSVNNDMIV TDNNNGAVKFP  
 51 QLCKFCDVRF STCDNQKSCM SNCSITSICE KPQEVCVAWW RKNDENITLE  
 101 TVCHDPKLPY HDFILEDAAS PKCIMKEKKK PGETFFMCSC SSDECNDNII  
 20 151 FSEEEYNTSNP DTGGGVECPP CPAPPVAGPS VFLFPPPKPD TLMISRTPEV  
 201 TCVVVVDVSHE DPEVQFNWYV DGVEVHNAKT KPREEQFNST FRVVSVLTVV  
 251 HQDWLNGKEY KCKVSNKGLP APIEKTISKT KGQPREPQVY TLPPSREEMT  
 301 KNQVSLTCLV KGFYPSDIAV EWESNGOPEN NYKTTPPMLD SDGSFFLYSK  
 351 LTVDKSRWQQ GNVFSCSVMH EALHNHYTQK SLSLSPGK  
 25 (SEQ ID NO: 52)

**[0200]** The nucleic acid sequence encoding SEQ ID NO:52 is shown below:

1 ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC  
 51 AGTCTTCGTT TCGCCCGGCG CCACGATCCC ACCGCACGTT CAGAAGTCGG  
 101 TTAATAACGA CATGATAGTC ACTGACAACA ACGGTGCAGT CAAGTTCCA  
 30 151 CAACTGTGTA AATTTGTGA TGTGAGATT TCCACCTGTG ACAACCAGAA  
 201 ATCCTGCATG AGCAACTGCA GCATCACCTC CATCTGTGAG AAGCCACAGG  
 251 AAGTCTGTGT GGCTGTATGG AGAAAGAATG ACGAGAACAT AACACTAGAG  
 301 ACAGTTTGCC ATGACCCCAA GCTCCCCTAC CATGACTTTA TTCTGGAAGA

351 TGCTGCTTCT CCAAAGTGCA TTATGAAGGA AAAAAAAAAG CCTGGTGAGA  
401 CTTTCTTCAT GTGTTCTGT AGCTCTGATG AGTGAATGA CAACATCATC  
451 TTCTCAGAAG AATATAACAC CAGCAATCCT GACACCGGTG GTGGAGTCGA  
501 GTGCCCACCG TGCCCAGCAC CACCTGTGGC AGGACCGTCA GTCTTCCTCT  
5 551 TCCCCCAAA ACCCAAGGAC ACCCTCATGA TCTCCCGGAC CCCTGAGGTC  
601 ACGTGCCTGG TGGTGGACGT GAGCCACGAA GACCCCGAGG TCCAGTTCAA  
651 CTGGTACGTG GACGGCGTGG AGGTGCATAA TGCCAAGACA AAGCCACGGG  
701 AGGAGCAGTT CAACAGCACG TTCCGTGTGG TCAGCGTCCT CACCGTCGTG  
751 CACCAAGGACT GGCTGAACGG CAAGGAGTAC AAGTGCAAGG TCTCCAACAA  
10 801 AGGCCTCCCA GCCCCCACATCG AGAAAACCCT CTCCAAAACC AAAGGGCAGC  
851 CCCGAGAACCC ACAGGTGTAC ACCCTGCCCT CATCCCGGGA GGAGATGACC  
901 AAGAACCAAGG TCAGCCTGAC CTGCCTGGTC AAAGGCTTCT ACCCCAGCGA  
951 CATCGCCGTG GAGTGGGAGA GCAATGGGCA GCCGGAGAAC AACTACAAGA  
1001 CCACACCTCC CATGCTGGAC TCCGACGGCT CCTTCTTCCT CTACAGCAAG  
15 1051 CTCACCGTGG ACAAGAGCAG GTGGCAGCAG GGGAACGTCT TCTCATGCTC  
1101 CGTGATGCAT GAGGCTCTGC ACAACCCTA CACGCAGAAC AGCCTCTCCC  
1151 TGTCTCCGGG TAAA (SEQ ID NO: 51)

**Example 4. Differential ligand inhibition by receptor fusion protein variants in cell-based assay**

20 [0201] A reporter gene assay in A549 cells was used to determine the ability of hT $\beta$ RII-hFc variants to inhibit activity of GDF15, TGF $\beta$ 1, TGF $\beta$ 2, and TGF $\beta$ 3. This assay is based on a human lung carcinoma cell line transfected with a pGL3(CAGA)12 reporter plasmid (Dennler et al, 1998, EMBO 17: 3091-3100) as well as a Renilla reporter plasmid (pRLCMV) to control for transfection efficiency. The CAGA motif is present in the 25 promoters of TGF $\beta$ -responsive genes (for example, PAI-1), so this vector is of general use for factors signaling through SMAD2 and SMAD3.

[0202] On the first day of the assay, A549 cells (ATCC<sup>®</sup>: CCL-185<sup>™</sup>) were distributed in 48-well plates at 6.5x10<sup>4</sup> cells per well. On the second day, a solution containing 10  $\mu$ g pGL3(CAGA)12, 100ng pRLCMV, 30  $\mu$ l X-tremeGENE 9 (Roche 30 Applied Science), and 970  $\mu$ l OptiMEM (Invitrogen) was preincubated for 30 min, then added to Eagle's minimum essential medium (EMEM, ATCC<sup>®</sup>) supplemented with 0.1% BSA, which was applied to the plated cells (500  $\mu$ l/well) for incubation overnight at room temperature. On the third day, medium was removed, and cells were incubated overnight at 37°C with a mixture of ligands and inhibitors prepared as described below.

[0203] Serial dilutions of test articles were made in a 48-well plate in a 200  $\mu$ l volume of assay buffer (EMEM + 0.1 % BSA). An equal volume of assay buffer containing the test ligand was added to obtain a final ligand concentration equal to the EC50 determined previously. Human GDF15 and murine GDF15 were generated in-house (see above), while 5 human TGF $\beta$ 1, human TGF $\beta$ 2, and human TGF $\beta$ 3 were obtained from PeproTech. Test solutions were incubated at 37°C for 30 minutes, then 250  $\mu$ l of the mixture was added to all wells. Each concentration of test article was determined in duplicate. After incubation with test solutions overnight, cells were rinsed with phosphate-buffered saline, then lysed with passive lysis buffer (Promega E1941) and stored overnight at -70°C. On the fourth and final 10 day, plates were warmed to room temperature with gentle shaking. Cell lysates were transferred in duplicate to a chemiluminescence plate (96-well) and analyzed in a luminometer with reagents from a Dual-Luciferase Reporter Assay system (Promega E1980) to determine normalized luciferase activity.

[0204] This assay was used to screen receptor fusion protein variants for potential 15 inhibitory effects on cell signaling by T $\beta$ RII ligands. Consistent with previous reports concerning wild-type T $\beta$ RII<sub>short</sub>-Fc and T $\beta$ RII<sub>long</sub>-Fc (del Re et al., J Biol Chem 279:22765, 2004), none of the variants tested were able to inhibit TGF $\beta$ 2, even at high concentrations. However, hT $\beta$ RII-hFc variants unexpectedly showed differential inhibition of cellular 20 signaling mediated by GDF15, TGF $\beta$ 1, and TGF $\beta$ 3. Compared with wild-type T $\beta$ RII<sub>short</sub>(23-159)-G2Fc, the T $\beta$ RII<sub>short</sub>(23-159/D110K)-G2Fc variant exhibited potent inhibition of GDF15 but loss of inhibition of TGF $\beta$ 1 and greatly reduced inhibition (~50 fold) of TGF $\beta$ 3 (see table below). Position 110 is located in the “hook” region of T $\beta$ RII (Radaev et al., J Biol Chem 285:14806, 2010) but has not been suggested to confer selectivity among the 25 recognized T $\beta$ RII ligands TGF $\beta$ 1, TGF $\beta$ 2, and TGF $\beta$ 3. Thus, this variant displays a profile of differential ligand inhibition in which GDF15 is inhibited most potently, TGF $\beta$ 1 least potently, and TGF $\beta$ 3 to an intermediate degree.

Construct	IC <sub>50</sub> (nM)			
	mGDF15 (35 ng/ml)	hTGF $\beta$ 1 (640 pg/ml)	hTGF $\beta$ 3 (270 pg/ml)	
Full-length wild-type ECD	T $\beta$ RII <sub>short</sub> (23-159)-G2Fc	~ 0.12	1.73	0.14
Full-length ECD with D110K substitution	T $\beta$ RII <sub>short</sub> (23-159/D110K)-G2Fc	~ 0.7	ND (> 73.6)	~ 6.9

ND, not determined

[0205] In a second experiment, potencies of variants with N-terminally truncated T $\beta$ RII ECD were compared with that of full-length wild-type T $\beta$ RII ECD. As shown in the table below, T $\beta$ RII<sub>short</sub>(29-159)-G2Fc and T $\beta$ RII<sub>short</sub>(35-159)-G2Fc displayed a greatly diminished ability to inhibit TGF $\beta$ 3 but an undiminished (N'Δ6) or only slightly diminished (N'Δ12) ability to inhibit GDF15 compared to T $\beta$ RII<sub>short</sub>(23-159)-G2Fc (wild-type). Effects of N-terminal truncation on inhibition of TGF $\beta$ 1 compared to wild-type were intermediate in magnitude. Thus, these two variants exhibit a profile of differential ligand inhibition in which GDF15 is inhibited most potently, TGF $\beta$ 3 least potently, and TGF $\beta$ 1 to an intermediate degree.

Construct	IC <sub>50</sub> (nM)			
	hGDF15 (70 or 112 ng/ml)	hTGF $\beta$ 1 (640 pg/ml)	hTGF $\beta$ 3 (270 pg/ml)	
Full-length wild-type ECD	T $\beta$ RII <sub>short</sub> (23-159)-G2Fc	0.14 – 0.53	0.52	0.37
N'Δ6 ECD	T $\beta$ RII <sub>short</sub> (29-159)-G2Fc	0.40	2.05	ND (> 7.5)
N'Δ12 ECD	T $\beta$ RII <sub>short</sub> (35-159)-G2Fc	0.92	2.51	ND (> 7.5)

10 ND, not determined

[0206] In a third experiment, we determined the effect on potency of a N70D substitution in a C-terminally truncated T $\beta$ RII ECD. This aspartate residue represents a potential glycosylation site. As shown in the table below, T $\beta$ RII<sub>short</sub>(23-153/N70D)-G2Fc displayed greatly diminished ability to inhibit TGF $\beta$ 1 and virtually undiminished ability to inhibit TGF $\beta$ 3 compared to T $\beta$ RII<sub>short</sub>(23-153)-G2Fc. Effects of N70D substitution on inhibition of GDF15 compared to both T $\beta$ RII<sub>short</sub>(23-153)-G2Fc and wild-type were intermediate in magnitude. Thus, the C-terminally truncated variant with N70D substitution exhibits a profile of differential ligand inhibition in which TGF $\beta$ 3 is inhibited most potently, TGF $\beta$ 1 least potently, and GDF15 to an intermediate degree.

Construct	IC <sub>50</sub> (nM)			
	hGDF15 (70 ng/ml)	hTGF $\beta$ 1 (640 pg/ml)	hTGF $\beta$ 3 (270 pg/ml)	
Full-length wild-type ECD	T $\beta$ RII <sub>short</sub> (23-159)-G2Fc	0.14	ND	ND
C'Δ6 ECD	T $\beta$ RII <sub>short</sub> (23-153)-G2Fc	0.18	2.62	0.14
C'Δ6 ECD with N70D substitution	T $\beta$ RII <sub>short</sub> (23-153/N70D)- G2Fc	2.42	17.7	0.28

20

[0207] Together, these results demonstrate that Applicants have generated truncations and mutations of the T $\beta$ RII ECD that exhibit widely different ligand binding profiles. Notably, this demonstration reveals that properly expressed and purified GDF15 interacts directly with T $\beta$ RII and can be differentially inhibited by fusion proteins comprising variants of the T $\beta$ RII ECD. Activity profiles of these variants can be summarized in the following table.

Summary of Ligand Selectivity				
Construct		Degree of Ligand Inhibition		
		Potent	Moderate	Negligible
Full-length wild-type ECD	T $\beta$ RII <sub>short</sub> (23-159)-G2Fc	GDF15 TGF $\beta$ 1 TGF $\beta$ 3	---	TGF $\beta$ 2
Full-length ECD with D110K substitution	T $\beta$ RII <sub>short</sub> (23-159/D110K)-G2Fc	GDF15	TGF $\beta$ 3	TGF $\beta$ 1 TGF $\beta$ 2
N'Δ6 ECD	T $\beta$ RII <sub>short</sub> (29-159)-G2Fc	GDF15	TGF $\beta$ 1	TGF $\beta$ 2 TGF $\beta$ 3
N'Δ12 ECD	T $\beta$ RII <sub>short</sub> (35-159)-G2Fc	GDF15	TGF $\beta$ 1	TGF $\beta$ 2 TGF $\beta$ 3
C'Δ6 ECD with N70D substitution	T $\beta$ RII <sub>short</sub> (23-153/N70D)-G2Fc	TGF $\beta$ 3	GDF15	TGF $\beta$ 1 TGF $\beta$ 2

[0208] We predict that the T $\beta$ RII<sub>long</sub> ECD counterparts of these T $\beta$ RII<sub>short</sub> ECD variants will exhibit similar ligand selectivity. In addition, a C'Δ6 truncated ECD (such as SEQ ID NOs: 11 and 16 for the T $\beta$ RII<sub>short</sub> and T $\beta$ RII<sub>long</sub> isoforms, respectively) can be used 10 as a base sequence for T $\beta$ RII<sub>short</sub> or T $\beta$ RII<sub>long</sub> in which to introduce mutations and N-terminal truncations.

#### Example 5. Exemplary hT $\beta$ RII-hFc nucleic acids and proteins

[0209] This example summarizes nucleic acid constructs that can be used to express T $\beta$ RII constructs in HEK-293 or CHO cells, according to the methods provided herein in 15 order to provide the proteins isolated from cell culture. In each case the mature protein isolated from cell culture will have the leader sequence (dotted underline in each sequence below) removed.

[0210] Item 1 shows the amino acid sequence of hT $\beta$ RII<sub>short</sub>(23-159/D110K)-hG2Fc (SEQ ID NO: 25). Double underline indicates D110K substitution. Dotted underline denotes 20 leader, and solid underline denotes linker.

1 MDAMKRGLCC VLLLCGAVFV SPGATIPPHV QKSVNNDMIV TDNNNGAVKFP

51 QLCKFCDVRF STCDNQKSCM SNCSITSICE KPQEVCVAWW RKNDENITLE  
 101 TVCHDPKLPY HKFILEDAAS PKCIMKEKKK PGETFFMCSC SSDECNDNII  
 151 FSEEVNTSNP DTGGGVECPP CPAPPVAGPS VFLFPPPKPD TLMISRTPEV  
 201 TCVVVVDVSHE DPEVQFNWYV DGVEVHNAKT KPREEQFNST FRVVSVLTVV  
 5 251 HQDWLNGKEY KCKVSNKGLP APIEKTISKT KGQPREPQVY TLPPSREEMT  
 301 KNQVSLTCLV KGFYPSDIAV EWESNGQOPEN NYKTTPPMLD SDGSFFLYSK  
 351 LTVDKSRWQQ GNVFSCSVMH EALHNHYTQK SLSLSPGK  
 (SEQ ID NO: 25)

[0211] Item 2 shows a nucleotide sequence encoding hT $\beta$ RII<sub>short</sub>(23-159/D110K)-  
 10 hG2Fc (SEQ ID NO: 26). Double underline indicates D110K substitution. Dotted underline  
 denotes leader, and solid underline denotes linker.

1 ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC  
 51 AGTCTTCGTT TCGCCCGGCG CCACGATCCC ACCGCACGTT CAGAAGTCGG  
 101 TTAATAACGA CATGATAGTC ACTGACAACA ACGGTGCAGT CAAGTTCCA  
 15 151 CAACTGTGTA AATTTGTGA TGTGAGATTT TCCACCTGTG ACAACCAGAA  
 201 ATCCTGCATG AGCAACTGCA GCATCACCTC CATCTGTGAG AAGCCACAGG  
 251 AAGTCTGTGT GGCTGTATGG AGAAAGAATG ACGAGAACAT AACACTAGAG  
 301 ACAGTTTGCC ATGACCCCAA GCTCCCCTAC CATAAGTTA TTCTGGAAGA  
 351 TGCTGCTTCT CCAAAGTGCA TTATGAAGGA AAAAAAAAAG CCTGGTGAGA  
 20 401 CTTTCTTCAT GTGTTCTGT AGCTCTGATG AGTGAATGA CAACATCATC  
 451 TTCTCAGAAG AATATAACAC CAGCAATCCT GACACCGGTG GTGGAGTCGA  
 501 GTGCCACCAG TGCCCAGCAC CACCTGTGGC AGGACCGTCA GTCTCCTCT  
 551 TCCCCCCAA ACCCAAGGAC ACCCTCATGA TCTCCGGAC CCCTGAGGTC  
 601 ACGTGCCTGG TGGTGGACGT GAGCCACGAA GACCCCGAGG TCCAGTTCAA  
 25 651 CTGGTACGTG GACGGCGTGG AGGTGCATAA TGCCAAGACA AAGCCACGGG  
 701 AGGAGCAGTT CAACAGCACG TTCCGTGTGG TCAGCGTCCT CACCGTCGTG  
 751 CACCAGGACT GGCTGAACGG CAAGGAGTAC AAGTGCAAGG TCTCCAACAA  
 801 AGGCCTCCCA GCCCCCACCG AGAAAACCCT CTCCAAAACC AAAGGGCAGC  
 851 CCCGAGAACCC ACAGGTGTAC ACCCTGCCCT CATCCGGGA GGAGATGACC  
 30 901 AAGAACCAAGG TCAGCCTGAC CTGCCTGGTC AAAGGCTTCT ACCCCAGCGA  
 951 CATCGCCGTG GAGTGGGAGA GCAATGGGCA GCCGGAGAAC AACTACAAGA  
 1001 CCACACCTCC CATGCTGGAC TCCGACGGCT CCTTCTTCCT CTACAGCAAG  
 1051 CTCACCGTGG ACAAGAGCAG GTGGCAGCAG GGGAACGTCT TCTCATGCTC  
 1101 CGTGATGCAT GAGGCTCTGC ACAACCACCA CACGCAGAAC AGCCTCTCCC  
 35 1151 TGTCTCCGGG TAAA (SEQ ID NO: 26)

**[0212]** Item 3 shows the amino acid sequence of hT $\beta$ RII<sub>short</sub>(23-159/D110K)-hG1Fc<sub>short</sub> (SEQ ID NO: 27). Double underline indicates D110K substitution. Dotted underline denotes leader, and solid underline denotes linker.

1 MDAMKRLGCC VLLLCGAVFV SPGATIPPHV QKSVNNDMIV TDNNNGAVKFP  
 5 51 QLCKFCDVRF STCDNQKSCM SNCSITSICE KPOEVCVAVW RKNDENITLE  
 101 101 TVCHDPKL $\underline{PY}$  HKFILEDAAS PKCIMKEKKK PGETFFMCSC SSDECNDNII  
 151 151 FSEEEYNTSNP DTGGGTHTCP PCPAPELLGG PSVFLFPPKP KDTLMISRTP  
 201 201 EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN STYRVVSVLT  
 251 251 VLHQDWLNGK EYKCKVSNKA LPAPIEKTI $\underline{S}$  KAKGQPREPQ VYTLPPSREE  
 10 301 301 MTKNQVSLTC LVKG $\underline{F}$ YPSDI AVEWESNGQP ENNYKTTPPV LDSDGSFFLY  
 351 351 SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPGK  
 (SEQ ID NO: 27)

**[0213]** Item 4 shows a nucleotide sequence encoding hT $\beta$ RII<sub>short</sub>(23-159/D110K)-hG1Fc<sub>short</sub> (SEQ ID NO: 28). Double underline indicates D110K substitution. Dotted underline denotes leader, and solid underline denotes linker.

1 ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC  
 5 51 AGTCTTCGTT TCGCCCGGCG CCACGATCCC ACCGCACGTT CAGAAGTCGG  
 101 101 TTAATAACGA CATGATAGTC ACTGACAACA ACGGTGCAGT CAAGTTCCA  
 151 151 CAACTGTGTA AATTTTGTGA TGTGAGATTT TCCACCTGTG ACAACCAGAA  
 20 201 201 ATCCTGCATG AGCAACTGCA GCATCACCTC CATCTGTGAG AAGCCACAGG  
 251 251 AAGTCTGTGT GGCTGTATGG AGAAAGAATG ACGAGAACAT AACACTAGAG  
 301 301 ACAGTTGCC ATGACCCCAA GCTCCCTAC CATAAGTTA TTCTGGAAGA  
 351 351 TGCTGCTTCT CCAAAGTGCA TTATGAAGGA AAAAAAAAAG CCTGGTGAGA  
 401 401 CTTTCTTCAT GTGTTCCGT AGCTCTGATG AGTGAATGA CAACATCATC  
 25 451 451 TTCTCAGAAG AATATAACAC CAGCAATCCT GACACCGGTG GTGGAACTCA  
 501 501 CACATGCCCA CCGTGCCAG CACCTGA $\underline{ACT}$  CCTGGGGGGA CCGTCAGTCT  
 551 551 TCCTCTTCCC CCCAAAACCC AAGGACACCC TCATGATCTC CCGGACCCCT  
 601 601 GAGGTCACAT GCGTGGTGGT GGACGTGAGC CACGAAGACC CTGAGGTCAA  
 651 651 GTTCAACTGG TACGTGGACG GCGTGGAGGT GCATAATGCC AAGACAAAGC  
 30 701 701 CGCGGGAGGA GCAGTACAAC AGCACGTACC GTGTGGTCAG CGTCCTCACC  
 751 751 GTCCTGCACC AGGACTGGCT GAATGGCAAG GAGTACAAGT GCAAGGTCTC  
 801 801 CAACAAAGCC CTCCCAGCCC CCATCGAGAA AACCATCTCC AAAGCCAAAG  
 851 851 GGCAGCCCCG AGAACACAG GTGTACACCC TGCCCCCATC CCGGGAGGAG  
 901 901 ATGACCAAGA ACCAGGT $\underline{CAG}$  CCTGACCTGC CTGGTCAAAG GCTTCTATCC  
 35 951 951 CAGCGACATC GCCGTGGAGT GGGAGAGCAA TGGGCAGCCG GAGAACAACT

1001 ACAAGACCAC GCCTCCGTG CTGGACTCCG ACGGCTCCTT CTTCCCTAT  
1051 AGCAAGCTCA CCGTGGACAA GAGCAGGTGG CAGCAGGGGA ACGTCTTCTC  
1101 ATGCTCCGTG ATGCATGAGG CTCTGCACAA CCACTACACG CAGAAGAGCC  
1151 TCTCCCTGTC CCCGGGTAAA (SEQ ID NO: 28)

[0214] Item 5 shows the amino acid sequence of hT $\beta$ RII<sub>short</sub>(29-159)-hG2Fc (SEQ ID NO: 29). Dotted underline denotes leader, and solid underline denotes linker.

1 MDAMKRLGCC VLLLGAVFV SPGAQKSVNN DMIVTDNNNGA VKFPQLCKFC  
51 DVRFSTCDNQ KSCMSNCSIT SICEKPQEVC VAVWRKNDEN ITLETVCHDP  
101 KLPYHDFILE DAASPKCIMK EKKKPGETFF MCSCSSDECN DNIIFSEEYN  
10 151 TSNPDTGGGV ECPPCPAPPV AGPSVFLFPP KPKDTLMISR TPEVTCVVVD  
201 VSHEDEXVQF NWYVDGVEVH NAKTKPREEQ FNSTFRVVS V LTVVHQDWLN  
251 GKEYKCKVSN KGLPAPIEKT ISKTKGQPR PQVYTLPPSR EEMTKNQVSL  
301 TCLVKGFYPS DIAVEWESNG QPENNYKTT P PMLDSDGSFF LYSKLTVDKS  
351 RWOQGNVFSC SVMHEALHNH YTOKSLSLSP GK (SEQ ID NO: 29)

15 [0215] Item 6 shows a nucleotide sequence encoding hT $\beta$ RII<sub>short</sub>(29-159)-hG2Fc (SEQ ID NO: 30). Dotted underline denotes leader, and solid underline denotes linker.

1 ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC  
51 AGTCTTCGTT TCGCCCGGCG CCCAGAAGTC GGTAAATAAC GACATGATAG  
101 TCACTGACAA CAACGGTGCA GTCAAGTTTC CACAACGTG TAAATTTGT  
20 151 GATGTGAGAT TTTCCACCTG TGACAACCAG AAATCCTGCA TGAGCAACTG  
201 CAGCATCACC TCCATCTGTG AGAAGCCACA GGAAGTCTGT GTGGCTGTAT  
251 GGAGAAAGAA TGACGAGAAC ATAACACTAG AGACAGTTG CCATGACCCC  
301 AAGCTCCCCT ACCATGACTT TATTCTGGAA GATGCTGCTT CTCCAAAGTG  
351 CATTATGAAG GAAAAAAAAGCCTGGTGA GACTTCTTC ATGTGTTCTC  
25 401 GTAGCTCTGA TGAGTGCAAT GACAACATCA TCTTCTCAGA AGAATATAAC  
451 ACCAGCAATC CTGACACCGG TGGTGGAGTC GAGTGCCAC CGTGCCAGC  
501 ACCACCTGTG GCAGGACCGT CAGTCTTCCT CTTCCCCCA AAACCCAAGG  
551 ACACCCCTCAT GATCTCCGG ACCCCTGAGG TCACGTGCGT GGTGGTGGAC  
601 GTGAGCCACG AAGACCCCGA GGTCCAGTTC AACTGGTACG TGGACGGCGT  
30 651 GGAGGTGCAT AATGCCAAGA CAAAGCCACG GGAGGAGCAG TTCAACAGCA  
701 CGTTCGGTGT GGTCAGCGTC CTCACCGTCG TGCACCAGGA CTGGCTGAAC  
751 GGCAAGGAGT ACAAGTGCAA GGTCTCCAAC AAAGGCCTCC CAGCCCCAT  
801 CGAGAAAACC ATCTCCAAAA CCAAAGGGCA GCCCCGAGAA CCACAGGTGT  
851 ACACCCCTGCC CCCATCCGG GAGGAGATGA CCAAGAACCA GGTCAGCCTG  
35 901 ACCTGCCTGG TCAAAGGCCTT CTACCCCAAGC GACATGCCG TGGAGTGGGA

951 GAGCAATGGG CAGCCGGAGA ACAACTACAA GACCACACCT CCCATGCTGG  
 1001 ACTCCGACGG CTCCTTCTTC CTCTACAGCA AGCTCACCGT GGACAAGAGC  
 1051 AGGTGGCAGC AGGGGAACGT CTTCTCATGC TCCGTGATGC ATGAGGCTCT  
 1101 GCACAACCAC TACACGCAGA AGAGCCTCTC CCTGTCTCCG GGTAAA  
 5 (SEQ ID NO: 30)

**[0216] Item 7** shows the amino acid sequence of hT $\beta$ RII<sub>short</sub>(29-159)-hG1Fc<sub>short</sub> (SEQ ID NO: 31). Dotted underline denotes leader, and solid underline denotes linker.

1 MDAMKRLGCC VLLLCGAVFV SPGAQKSVNN DMIVTDNNGA VKFPQLCKFC  
 51 DVRFSTCDNQ KSCMSNCSIT SICEKPQEVC VAVWRKNDEN ITLETVCHDP  
 10 101 KLPYHDFILE DAASPKCIMK EKKKPGETFF MCSCSSDECN DNIIIFSEEYN  
 151 TSNPDTGGGT HTCPPCPAPE LLGGPSVFLF PPKPKDTLMI SRTPEVTCVV  
 201 VDVSHEDPEV KFNWYVDGVE VHNAKTKPRE EQYNSTYRVV SVLTVLHQDW  
 251 LNGKEYKCKV SNKALPAPIE KTISKAKGQP REPQVYTLPP SREEMTKNQV  
 301 SLTCLVKGFY PSDIAVEWES NGQPENNYKT TPPVLDSDGS FFLYSKLTVD  
 15 351 KSRWQQGNVF SCSVMHEALH NHYTQKSLSL SPGK (SEQ ID NO: 31)

**[0217] Item 8** shows a nucleotide sequence encoding hT $\beta$ RII<sub>short</sub>(29-159)-hG1Fc<sub>short</sub> (SEQ ID NO: 32). Dotted underline denotes leader, and solid underline denotes linker.

1 ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC  
 20 51 AGTCTTCGTT TCGCCCGGGCG CCCAGAACGT GGTAAATAAC GACATGATAG  
 101 TCACTGACAA CAACGGTGCA GTCAAGTTTC CACAACGTG TAAATTTGT  
 151 GATGTGAGAT TTTCCACCTG TGACAACCAG AAATCCTGCA TGAGCAACTG  
 201 CAGCATCACC TCCATCTGTG AGAAGCCACA GGAAGTCTGT GTGGCTGTAT  
 251 GGAGAAAGAA TGACGAGAAC ATAACACTAG AGACAGTTG CCATGACCCC  
 301 AAGCTCCCCT ACCATGACTT TATTCTGGAA GATGCTGCTT CTCCAAAGTG  
 351 CATTATGAAG GAAAAAAAAGCCTGGTGA GACTTTCTTC ATGTGTTCCCT  
 401 GTAGCTCTGA TGAGTGCAAT GACAACATCA TCTTCTCAGA AGAATATAAC  
 451 ACCAGCAATC CTGACACCGG TGGTGGAACT CACACATGCC CACCGTGCCC  
 501 AGCACCTGAA CTCCTGGGG GACCGTCAGT CTTCCCTCTTC CCCCCAAAAC  
 30 551 CCAAGGACAC CCTCATGATC TCCCGGACCC CTGAGGTAC ATGCGTGGTG  
 601 GTGGACGTGA GCCACGAAGA CCCTGAGGTC AAGTTCAACT GGTACGTGGA  
 651 CGGCGTGGAG GTGCATAATG CCAAGACAAA GCCGCGGGAG GAGCAGTACA  
 701 ACAGCACGTA CCGTGTGGTC AGCGTCCTCA CCGTCCTGCA CCAGGACTGG  
 751 CTGAATGGCA AGGAGTACAA GTGCAAGGTC TCCAACAAAG CCCTCCCAGC  
 35 801 CCCCCATCGAG AAAACCATCT CCAAAGCCAA AGGGCAGCCC CGAGAACAC

851 AGGTGTACAC CCTGCCCCA TCCCAGGAGG AGATGACCAA GAACCAGGTC  
 901 AGCCTGACCT GCCTGGTCAA AGGCTTCTAT CCCAGCGACA TCGCCGTGGA  
 951 GTGGGAGAGC AATGGGCAGC CGGAGAACAA CTACAAGACC ACGCCTCCG  
 1001 TGCTGGACTC CGACGGCTCC TTCTTCCTCT ATAGCAAGCT CACCGTGGAC  
 5 1051 AAGAGCAGGT GGCAGCAGGG GAACGTCTTC TCATGCTCCG TGATGCATGA  
 1101 GGCTCTGCAC AACCACTACA CGCAGAAGAG CCTCTCCCTG TCCCCGGGTA  
 1151 AA (SEQ ID NO: 32)

**[0218] Item 9 shows the amino acid sequence of hT $\beta$ RII<sub>short</sub>(35-159)-hG2Fc (SEQ ID NO: 33). Dotted underline denotes leader, and solid underline denotes linker.**

10 1 MDAMKRLGCC VLLLCGAVFV SPGADMIVTD NNGAVKFPQL CKFCDVRFST  
 51 CDNQKSCMSN CSITSICEKP QEVCVAVWRK NDENITLETV CHDPKLPYHD  
 101 FILEDAASPK CIMKEKKKPG ETFFMCSCSS DECNDNIIFS EEYNTSNPDT  
 151 GGGVECPPCP APPVAGPSVF LFPPPKPKDTL MISRTPEVTC VVVDVSHEDP  
 201 EVQFNWYVDG VEVHNAKTKP REEQFNSTFR VVSVLTVVHQ DWLNGKEYKC  
 15 251 KVSNKGLPAP IEKTISKTG QPREPQVYTL PPSREEMTKN QVSLTCLVKG  
 301 FYPSDIAVEW ESNQOPENNY KTPPPMLDSD GSFFFLYSKLT VDKSRWQQGN  
 351 VFSCSVMHEA LHNHYTQKSL SLSPGK (SEQ ID NO: 33)

**[0219] Item 10 shows a nucleotide sequence encoding hT $\beta$ RII<sub>short</sub>(35-159)-hG2Fc (SEQ ID NO: 34). Dotted underline denotes leader, and solid underline denotes linker.**

20 1 ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC  
 51 AGTCTTCGTT TCGCCCGGCG CCGACATGAT AGTCACTGAC AACAACGGTG  
 101 CAGTCAGATT TCCACAACTG TGTAATTGTT GTGATGTGAG ATTTTCCACC  
 151 TGTGACAACC AGAAATCCTG CATGAGCAAC TGCAGCATCA CCTCCATCTG  
 201 TGAGAAGCCA CAGGAAGTCT GTGTGGCTGT ATGGAGAAAG AATGACGAGA  
 25 251 ACATAACACT AGAGACAGTT TGCCATGACC CCAAGCTCCC CTACCATGAC  
 301 TTTATTCTGG AAGATGCTGC TTCTCCAAAG TGCATTATGA AGGAAAAAAA  
 351 AAAGCCTGGT GAGACTTCT TCATGTGTTG CTGTAGCTCT GATGAGTGCA  
 401 ATGACAACAT CATCTTCTCA GAAGAATATA ACACCAGCAA TCCTGACACC  
 451 GGTGGTGGAG TCGAGTGCC ACCGTGCCA GCACCACCTG TGGCAGGACC  
 30 501 GTCAGTCTTC CTCTTCCCCC CAAAACCAA GGACACCCCTC ATGATCTCCC  
 551 GGACCCCTGA GGTACGTGC GTGGTGGTGG ACGTGAGCCA CGAAGACCCC  
 601 GAGGTCCAGT TCAACTGGTA CGTGGACGGC GTGGAGGTGC ATAATGCCAA  
 651 GACAAAGCCA CGGGAGGAGC AGTCAACAG CACGTTCCGT GTGGTCAGCG  
 701 TCCTCACCGT CGTGCACCAG GACTGGCTGA ACGGCAAGGA GTACAAGTGC  
 35 751 AAGGTCTCCA ACAAAAGGCCT CCCAGCCCCC ATCGAGAAAA CCATCTCCAA

801 AACCAAAGGG CAGCCCCGAG AACCACAGGT GTACACCCTG CCCCCATCCC  
 851 GGGAGGAGAT GACCAAGAAC CAGGTCAGCC TGACCTGCCT GGTCAAAGGC  
 901 TTCTACCCC GCGACATCGC CGTGGAGTGG GAGAGCAATG GGCAGCCGGA  
 951 GAACAACTAC AAGACCACAC CTCCCATGCT GGACTCCGAC GGCTCCTTCT  
 5 1001 TCCTCTACAG CAAGCTCACC GTGGACAAGA GCAGGTGGCA GCAGGGGAAC  
 1051 GTCTTCTCAT GCTCCGTGAT GCATGAGGCT CTGCACAAACC ACTACACGCA  
 1101 GAAGAGCCTC TCCCTGTCTC CGGGTAAA (SEQ ID NO: 34)

**[0220] Item 11** shows the amino acid sequence of hT $\beta$ RII<sub>short</sub>(35-159)-hG1Fc<sub>short</sub> (SEQ ID NO: 35). Dotted underline denotes leader, and solid underline denotes linker.

10 1 MDAMKRLGCC VLLLCGAVFV SPGADMIVTD NNGAVKFPQL CKFCDVRFST  
 51 CDNQKSCMSN CSITSICEKP QEVCVAVWRK NDENITLETV CHDPKLPYHD  
 101 FILEDAASPK CIMKEKKKPG ETFFMCSCSS DECNDNIIFS EEYNTSNPD  
 151 GGGTHTCPPC PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVVDVSHE  
 201 DPEVKFNWYV DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY  
 15 251 KCKVSNKALP APIEKTISKA KGQPREPQVY TLPPSREEMT KNQVSLTCLV  
 301 KGFYPSDIAV EWESNGQOPEN NYKTTPPVLD SDGSFFLYSK LTVDKSRWQQ  
 351 GNVFSCSVMH EALHNHYTQK SLSLSPGK (SEQ ID NO: 35)

**[0221] Item 12** shows a nucleotide sequence encoding hT $\beta$ RII<sub>short</sub>(35-159)-hG1Fc<sub>short</sub> (SEQ ID NO: 36). Dotted underline denotes leader, and solid underline denotes linker.

20 1 ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC  
 51 AGTCTTCGTT TCGCCCGGCG CCGACATGAT AGTCACTGAC AACAACGGTG  
 101 CAGTCAAGTT TCCACAACTG TGAAATTG TGATGTGAG ATTTTCCACC  
 151 TGTGACAACC AGAAATCCTG CATGAGCAAC TGCAGCATCA CCTCCATCTG  
 201 TGAGAAGCCA CAGGAAGTCT GTGTGGCTGT ATGGAGAAAG AATGACGAGA  
 25 251 ACATAACACT AGAGACAGTT TGCCATGACC CCAAGCTCCC CTACCATGAC  
 301 TTTATTCTGG AAGATGCTGC TTCTCCAAAG TGCATTATGA AGGAAAAAAA  
 351 AAAGCCTGGT GAGACTTCT TCATGTGTTG CTGTAGCTCT GATGAGTGCA  
 401 ATGACAACAT CATCTTCTCA GAAGAATATA ACACCAGCAA TCCTGACACC  
 451 GGTGGTGGAA CTCACACATG CCCACCGTGC CCAGCACCTG AACTCCTGGG  
 30 501 GGGACCGTCA GTCTTCCTCT TCCCCCCAAA ACCCAAGGAC ACCCTCATGA  
 551 TCTCCCGGAC CCCTGAGGTC ACATGCGTGG TGGTGGACGT GAGCCACGAA  
 601 GACCCTGAGG TCAAGTTCAA CTGGTACGTG GACGGCGTGG AGGTGCATAA  
 651 TGCCAAGACA AAGCCGCGGG AGGAGCAGTA CAACAGCACG TACCGTGTGG  
 701 TCAGCGTCCT CACCGTCCTG CACCAAGACT GGCTGAATGG CAAGGAGTAC  
 35 751 AAGTGCAAGG TCTCCAACAA AGCCCTCCCA GCCCCCATCG AGAAAACCAT

801 CTCCAAAGCC AAAGGGCAGC CCCGAGAACCC ACAGGTGTAC ACCCTGCC  
 851 CATCCCGGGA GGAGATGACC AAGAACCAAGG TCAGCCTGAC CTGCCTGGTC  
 901 AAAGGCTTCT ATCCCAGCGA CATGCCGTG GAGTGGGAGA GCAATGGGCA  
 951 GCCGGAGAAC AACTACAAGA CCACGCCTCC CGTGCTGGAC TCCGACGGCT  
 5 1001 CCTTCTTCCT CTATAGCAAG CTCACCGTGG ACAAGAGCAG GTGGCAGCAG  
 1051 GGGAACGTCT TCTCATGCTC CGTGATGCAT GAGGCTCTGC ACAACCACTA  
 1101 CACGCAGAAG AGCCTCTCCC TGTCCCCGGG TAAA  
 (SEQ ID NO: 36)

[0222] Item 13 shows the amino acid sequence of hT $\beta$ RII<sub>short</sub>(23-153)-hG2Fc (SEQ ID NO: 37). Dotted underline denotes leader, and solid underline denotes linker.

1 MDAMKRGLCC VLLLCGAVFV SPGATIPPHV QKSVNNDMIV TDNNGAVKFP  
 51 QLCKFCDVRF STCDNQKSCM SNCSITSICE KPQEVCVAW RKNDENITL  
 101 TVCHDPKL PY HDFILEDAAS PKCIMKEKKK PGETFFMCSC SSDECNDNII  
 151 FSE~~EY~~TGGGV ECPPCPAPPV AGPSVFLFPP KPKDTLMISR TPEVTCVVVD  
 15 201 VSHEDPEVQF NWYVDGVEVH NAKTKPREEQ FNSTFRVVSV LTVVHQDWLN  
 251 GKEYKCKVSN KGLPAPIEKT ISKTKQPRE PQVYTLPPSR EEMTKNQVSL  
 301 TCLVKGFYPS DIAVEWESNG QPENNYKTPP PMLDSDGSFF LYSKLTVDKS  
 351 RWQQGNVFSC SVMHEALHNH YTQKSLSLSP GK (SEQ ID NO: 37)

[0223] Item 14 shows a nucleotide sequence encoding hT $\beta$ RII<sub>short</sub>(23-153)-hG2Fc (SEQ ID NO: 38). Dotted underline denotes leader, and solid underline denotes linker.

1 ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC  
 51 AGTCTTCGTT TCGCCCGGCG CCACGATCCC ACCGCACGTT CAGAAGTCGG  
 101 TTAATAACGA CATGATAGTC ACTGACAACA ACGGTGCAGT CAAGTTCCA  
 151 CAACTGTGTA AATTTGTGA TGTGAGATT TCCACCTGTG ACAACCAGAA  
 25 201 ATCCTGCATG AGCAACTGCA GCATCACCTC CATCTGTGAG AAGCCACAGG  
 251 AAGTCTGTGT GGCTGTATGG AGAAAGAACAT ACGAGAACAT AACACTAGAG  
 301 ACAGTTGCC ATGACCCCAA GCTCCCCTAC CATGACTTTA TTCTGGAAGA  
 351 TGCTGCTTCT CCAAAGTGCA TTATGAAGGA AAAAAAAAAG CCTGGTGAGA  
 401 CTTTCTTCAT GTGTTCCGT AGCTCTGATG AGTGAATGA CAACATCATC  
 30 451 TTCTCAGAAG AATATAACCGG TGGTGGAGTC GAGTGCCCAC CGTGCCCAGC  
 501 ACCACCTGTG GCAGGACCAGT CAGTCTTCCT CTTCCCCCA AAACCCAAGG  
 551 ACACCCCTCAT GATCTCCGG ACCCCTGAGG TCACGTGCGT GGTGGTGGAC  
 601 GTGAGCCACG AAGACCCGA GGTCCAGTTC AACTGGTACG TGGACGGCGT  
 651 GGAGGTGCAT AATGCCAAGA CAAAGCCACG GGAGGAGCAG TTCAACAGCA  
 35 701 CGTTCCGTGT GGTCAGCGTC CTCACCGTCG TGCACCAAGGA CTGGCTGAAC

751 GGCAAGGAGT ACAAGTGCAA GGTCTCCAAC AAAGGCCTCC CAGCCCCAT  
 801 CGAGAAAACC ATCTCCAAAA CCAAAGGGCA GCCCCGAGAA CCACAGGTGT  
 851 ACACCCTGCC CCCATCCCGG GAGGAGATGA CCAAGAACCA GGTCAGCCTG  
 901 ACCTGCCTGG TCAAAGGCTT CTACCCCCAGC GACATGCCG TGGAGTGGGA  
 5 951 GAGCAATGGG CAGCCGGAGA ACAACTACAA GACCACACCT CCCATGCTGG  
 1001 ACTCCGACGG CTCCTTCTTC CTCTACAGCA AGCTCACCGT GGACAAGAGC  
 1051 AGGTGGCAGC AGGGGAACGT CTTCTCATGC TCCGTGATGC ATGAGGCTCT  
 1101 GCACAACCAC TACACGCAGA AGAGCCTCTC CCTGTCTCCG GGTAAA  
 (SEQ ID NO: 38)

10 [0224] Item 15 shows the amino acid sequence of hT $\beta$ RII<sub>short</sub>(23-153)-hG1Fc<sub>short</sub> (SEQ ID NO: 39). Dotted underline denotes leader, and solid underline denotes linker.

1 MDAMKRLGCC VLLLCGAVFV SPGATIPPHV QKSVNNDMIV TDNNNGAVKFP  
 51 QLCKFCDVRF STCDNQKSCM SNCSITSICE KPQEVCVAVW RKNDENITLE  
 101 TVCHDPKLPY HDFILEDAAS PKCIMKEKKK PGETFFMCSC SSDECNDNII  
 15 151 FSE~~EY~~TGGGT HTCPPCPAPE LLGGPSVFLF PPKPKDTLMI SRTPEVTCVV  
 201 VDVSHEDPEV KFNWYVDGVE VHNAKTKPRE EQYNSTYRVV SVLTVLHQDW  
 251 LNGKEYKCKV SNKALPAPIE KTISKAKGQP REPQVYTLPP SREEMTKNQV  
 301 SLTCLVKGFY PSDIAVEWES NGQPENNYKT TPPVLDSDGS FFLYSKLTVD  
 351 KSRWQQGNVF SCSVMHEALH NYHTQKSLSL SPGK (SEQ ID NO: 39)

20 [0225] Item 16 shows a nucleotide sequence encoding hT $\beta$ RII<sub>short</sub>(23-153)-hG1Fc<sub>short</sub> (SEQ ID NO: 40). Dotted underline denotes leader, and solid underline denotes linker.

1 ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC  
 51 AGTCTTCGTT TCGCCCGGCG CCACGATCCC ACCGCACGTT CAGAAGTCGG  
 101 TTAATAACGA CATGATAGTC ACTGACAACA ACGGTGCAGT CAAGTTCCA  
 25 151 CAACTGTGTA AATTTGTGA TGTGAGATT TCCACCTGTG ACAACCAGAA  
 201 ATCCTGCATG AGCAACTGCA GCATCACCTC CATCTGTGAG AAGCCACAGG  
 251 AAGTCTGTGT GGCTGTATGG AGAAAGAATG ACGAGAACAT AACACTAGAG  
 301 ACAGTTGCC ATGACCCCAA GCTCCCCTAC CATGACTTTA TTCTGGAAGA  
 351 TGCTGCTTCT CCAAAGTGCA TTATGAAGGA AAAAAAAAAG CCTGGTGAGA  
 401 CTTTCTTCAT GTGTTCCCTGT AGCTCTGATG AGTGCAATGA CAACATCATC  
 451 TTCTCAGAAG AATATACCGG TGGTGGAACT CACACATGCC CACCGTGC  
 501 AGCACCTGAA CTCCTGGGG GACCGTCAGT CTTCCCTTTC CCCCCAAAC  
 551 CCAAGGACAC CCTCATGATC TCCCGGACCC CTGAGGTAC ATGCGTGGTG  
 601 GTGGACGTGA GCCACGAAGA CCCTGAGGTC AAGTTCAACT GGTACGTGGA  
 35 651 CGGCGTGGAG GTGCATAATG CCAAGACAAA GCCGCGGGAG GAGCAGTACA

701 ACAGCACGTA CCGTGTGGTC AGCGTCCTCA CCGTCCTGCA CCAGGACTGG  
 751 CTGAATGGCA AGGAGTACAA GTGCAAGGTC TCCAACAAAG CCCTCCCAGC  
 801 CCCCCATCGAG AAAACCATCT CCAAAGCCAA AGGGCAGCCC CGAGAACAC  
 851 AGGTGTACAC CCTGCCCTCA TCCCAGGGAGG AGATGACCAA GAACCAGGTC  
 5 901 AGCCTGACCT GCCTGGTCAA AGGTTCTAT CCCAGCGACA TCGCCGTGGA  
 951 GTGGGAGAGC AATGGGCAGC CGGAGAACAA CTACAAGACC ACGCCTCCCG  
 1001 TGCTGGACTC CGACGGCTCC TTCTTCCTCT ATAGCAAGCT CACCGTGGAC  
 1051 AAGAGCAGGT GGCAGCAGGG GAACGTCTTC TCATGCTCCG TGATGCATGA  
 1101 GGCTCTGCAC AACCAC TACA CGCAGAAGAG CCTCTCCCTG TCCCCGGGTA  
 10 1151 AA (SEQ ID NO: 40)

**[0226]** **Item 17** shows the amino acid sequence of hT $\beta$ RII<sub>short</sub>(23-153/N70D)-hG2Fc (SEQ ID NO: 41). Double underline indicates N70D substitution. Dotted underline denotes leader, and solid underline denotes linker.

1 MDAMKRLGCC VLLLCGAVFV SPGATIPPHV QKSVNNDMIV TDNNNGAVKFP  
 15 51 QLCKFCDVRF STCDNQKSCM SDCSITSICE KPQEVCVAVW RKNDENITLE  
 101 TVCHDPKLPY HDFILEDAAS PKCIMKEKKK PGETFFMCSC SSDECNDNII  
 151 FSE~~EY~~TGGGV ECPPCPAPPV AGPSVFLFPP KPKDTLMISR TPEVTCVVVD  
 201 VSHEDPEVQF NWYVDGVEVH NAKTKPREEQ FNSTFRVSV LTVVHQDWLN  
 251 GKEYKCKVSN KGLPAPIEKT ISKTKGQPRE PQVYTLPPSR EEMTKNQVSL  
 20 301 TCLVKGFYPS DIAVEWESNG QPENNYKTTP PMLSDGSFF LYSKLTVDKS  
 351 RWQQGNVFSC SVMHEALHNH YTQKSLSLSP GK (SEQ ID NO: 41)

**[0227]** **Item 18** shows a nucleotide sequence encoding hT $\beta$ RII<sub>short</sub>(23-153/N70D)-hG2Fc (SEQ ID NO: 42). Double underline indicates N70D substitution. Dotted underline denotes leader, and solid underline denotes linker.

25 1 ATGGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC  
 51 51 AGTCTTCGTT TCGCCCGGCG CCACGATCCC ACCGCACGTT CAGAAGTCGG  
 101 101 TTAATAACGA CATGATAGTC ACTGACAACA ACGGTGCAGT CAAGTTCCA  
 151 151 CAACTGTGTA AATTTTGTGA TGTGAGATT TCCACCTGTG ACAACCAGAA  
 201 201 ATCCTGCATG AGCGACTGCA GCATCACCTC CATCTGTGAG AAGCCACAGG  
 30 251 251 AAGTCTGTGT GGCTGTATGG AGAAAGAATG ACGAGAACAT AACACTAGAG  
 301 301 ACAGTTGCC ATGACCCCAA GCTCCCTAC CATGACTTTA TTCTGGAAGA  
 351 351 TGCTGCTTCT CCAAAGTGCA TTATGAAGGA AAAAAAAAAG CCTGGTGAGA  
 401 401 CTTTCTTCAT GTGTTCCGT AGCTCTGATG AGTGCAATGA CAACATCATC  
 451 451 TTCTCAGAAG AATATACCGG TGGTGGAGTC GAGTGCCCAC CGTGCCCAGC  
 35 501 501 ACCACCTGTG GCAGGACCGT CAGTCTTCCT CTTCCCCCA AAACCCAAGG

551 ACACCCTCAT GATCTCCGG ACCCCTGAGG TCACGTGCGT GGTGGTGGAC  
 601 GTGAGCCACG AAGACCCCGA GGTCCAGTTC AACTGGTACG TGGACGGCGT  
 651 GGAGGTGCAT AATGCCAAGA CAAAGCCACG GGAGGAGCAG TTCAACAGCA  
 701 CGTTCCGTGT GGTCAGCGTC CTCACCGTCG TGCACCAGGA CTGGCTGAAC  
 5 751 GGCAAGGAGT ACAAGTGCAA GGTCTCCAAC AAAGGCCTCC CAGCCCCAT  
 801 CGAGAAAACC ATCTCCAAA CCAAAGGGCA GCCCCGAGAA CCACAGGTGT  
 851 ACACCCTGCC CCCATCCCGG GAGGAGATGA CCAAGAACCA GGTCAGCCTG  
 901 ACCTGCCTGG TCAAAGGCTT CTACCCCCAGC GACATGCCG TGGAGTGGGA  
 951 GAGCAATGGG CAGCCGGAGA ACAACTACAA GACCACACCT CCCATGCTGG  
 10 1001 ACTCCGACGG CTCCTTCTTC CTCTACAGCA AGCTCACCGT GGACAAGAGC  
 1051 AGGTGGCAGC AGGGGAACGT CTTCTCATGC TCCGTGATGC ATGAGGCTCT  
 1101 GCACAACCAC TACACGCAGA AGAGCCTCTC CCTGTCTCCG GGTAAA  
 (SEQ ID NO: 42)

[0228] Item 19 shows the amino acid sequence of hT $\beta$ RII<sub>short</sub>(23-153/N70D)-hG1Fc<sub>short</sub> (SEQ ID NO: 43). Double underline indicates N70D substitution. Dotted underline denotes leader, and solid underline denotes linker.

1 MDAMKRLGCC VLLLCGAVFV SPGATIPPHV QKSVNNNDMIV TDNNGAVKFP  
 51 QLCKFCDVRF STCDNQKSCM SDCSITSICE KPQEVCVAVW RKNDENITLE  
 101 TVCHDPKL $\underline{PY}$  HDFILEDAAS PKCIMKEKKK PGETFFMCSC SSDECNDNII  
 20 151 FSE $\underline{EY}$ TGGGT HTCPPCPAPE LLGGPSVFLF PPKPKDTLMI SRTPEVTCVV  
 201 VDVSHEDPEV KFNWYVDGVE VHNAKTKPRE EQYNSTYRVV SVLTVLHQDW  
 251 LNGKEYKCKV SNKALPAPIE KTISKAKGQP REPQVYTLPP SREEMTKNQV  
 301 SLTCLVKGFY PSDIAVEWES NGQPENNYKT TPPVLDSDGS FFLYSKLTVD  
 351 KSRWQQGNVF SCSVMHEALH NHYTQKSLSL SPGK (SEQ ID NO: 43)

25 [0229] Item 20 shows a nucleotide sequence encoding hT $\beta$ RII<sub>short</sub>(23-153/N70D)-hG1Fc<sub>short</sub> (SEQ ID NO: 44). Double underline indicates N70D substitution. Dotted underline denotes leader, and solid underline denotes linker.

1 ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC  
 51 AGTCTTCGTT TCGCCCGGCG CCACGATCCC ACCGCACGTT CAGAAGTCGG  
 30 101 TTAATAACGA CATGATAGTC ACTGACAAACA ACGGTGCAAGT CAAGTTCCA  
 151 CAACTGTGTA AATTTGTGA TGTGAGATT TCCACCTGTG ACAACCAGAA  
 201 ATCCTGCATG AGCGACTGCA GCATCACCTC CATCTGTGAG AAGCCACAGG  
 251 AAGTCTGTGT GGCTGTATGG AGAAAGAATG ACGAGAACAT AACACTAGAG  
 301 ACAGTTGCC ATGACCCCAA GCTCCCTAC CATGACTTTA TTCTGGAAGA  
 351 TGCTGCTTCT CCAAAGTGCA TTATGAAGGA AAAAAAAAAG CCTGGTGAGA

401 CTTTCTTCAT GTGTTCTGT AGCTCTGATG AGTGCATGAA CAACATCATC  
 451 TTCTCAGAAG AATATACCGG TGGTGGAA CACACATGCC CACCGTGC  
 501 AGCACCTGAA CTCCTGGGG GACCGTCAGT CTTCCCTTTC CCCCCAAAC  
 551 CCAAGGACAC CCTCATGATC TCCCGGACCC CTGAGGTAC C ATGCGTGGT  
 5 601 GTGGACGTGA GCCACGAAGA CCCTGAGGTC AAGTCAACT GGTACGTGGA  
 651 CGGCGTGGAG GTGCATAATG CCAAGACAAA GCCGCGGGAG GAGCAGTACA  
 701 ACAGCACGTA CCGTGTGGTC AGCGTCCTCA CCGTCCTGCA CCAGGACTGG  
 751 CTGAATGGCA AGGAGTACAA GTGCAAGGTC TCCAACAAAG CCCTCCCAGC  
 801 CCCCCATCGAG AAAACCATCT CCAAAGCCAA AGGGCAGCCC CGAGAAC  
 10 851 AGGTGTACAC CCTGCCCTCA TCCCGGGAGG AGATGACCAA GAACCAGGTC  
 901 AGCCTGACCT GCCTGGTCAA AGGCTTCTAT CCCAGCGACA TCGCCGTGGA  
 951 GTGGGAGAGC AATGGGCAGC CGGAGAACAA CTACAAGACC ACGCCTCCCG  
 1001 TGCTGGACTC CGACGGCTCC TTCTTCCTCT ATAGCAAGCT CACCGTGGAC  
 1051 AAGAGCAGGT GGCAGCAGGG GAACGTCTC TCATGCTCCG TGATGCATGA  
 15 1101 GGCTCTGCAC AACCACTACA CGCAGAAGAG CCTCTCCCTG TCCCCGGGTA  
 1151 AA (SEQ ID NO: 44)

**[0230]** **Item 21** shows the mature amino acid sequence (i.e., without the leader sequence) of hT $\beta$ RII<sub>short</sub>(23-159/D110K)-hG2Fc (SEQ ID NO: 53). Double underline indicates D110K substitution. Single underline denotes linker.

20 TIPPHV QKSVNNDMIV TDNNNGAVKFP  
 QLCKFCDVRF STCDNQKSCM SNCSITSICE KPQEVCVAWW RKNDENITLE  
 TVCHDPKLPY HKFILEDAAS PKCIMKEKKK PGETFFMCSC SSDECNDNII  
 FSEEEYNTSNP DTGGGVECPP CPAPPVAGPS VFLFPPKPKD TLMISRTPEV  
 TCVVVVDVSHE DPEVQFNWYV DGVEVHNAKT KPREEQFNST FRVVSVLTVV  
 25 HQDWLNGKEY KCKVSNKGLP APIEKTISKT KGQPREPQVY TLPPSREEMT  
 KNQVSLTCLV KGFYPSDIAV EWESNGQOPEN NYKTTPPMLD SDGSFFLYSK  
 LTVDKSRWQQ GNVFSCSVMH EALHNHYTQK SLSLSPGK  
 (SEQ ID NO:53)

**[0231]** **Item 22** shows the mature amino acid sequence (i.e., without the leader sequence) of hT $\beta$ RII<sub>short</sub>(23-159/D110K)-hG1Fc<sub>short</sub> (SEQ ID NO: 54). Double underline indicates D110K substitution. Single underline denotes linker.

TIPPHV QKSVNNDMIV TDNNNGAVKFP  
 QLCKFCDVRF STCDNQKSCM SNCSITSICE KPQEVCVAWW RKNDENITLE  
 TVCHDPKLPY HKFILEDAAS PKCIMKEKKK PGETFFMCSC SSDECNDNII

5 FSEEVNTS SNP DTGGGTHTCP PCPAPELLGG PSVFLPPKP KDTLMIS RTP  
 EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN STYRVVSVLT  
 VLHQDWLNGK EYKCKVSNKA LPAPIEK TIS KAKGQP REPQ VYTLPPSREE  
 MTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTPPPV LDSDGSFFLY  
 SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPGK (SEQ ID  
 NO: 54)

[0232] **Item 23** shows the mature amino acid sequence (i.e., without the leader sequence) of hT $\beta$ RII<sub>short</sub>(29-159)-hG2Fc (SEQ ID NO: 55). Single underline denotes linker.

10 QKSVNN DMIVTDNNGA VKFPQLCKFC  
 DVRFSTCDNQ KSCMSNCSIT SICEKPQEVC VAVWRKNDEN ITLETVCHDP  
 KLPYHDFILE DAASPKCIMK EKKKPGETFF MCSCSSDECN DNIIIFSEEEYN  
 TSNPDTGGGV ECPPCPAPPV AGPSVFLFPP KPKDTLMISR TPEVTCVVVD  
 VSHEDPEVQF NWYVDGVEVH NAKTKPREEQ FNSTFRVVS V LTVVHQDWLN  
 GKEYKCKVSN KGLPAPIEKT ISKTKGQP RE PQVYTLPPSR EEMTKNQVSL  
 15 TCLVKGFYPS DIAVEWESNG QPENNYKTPP PMLSDGSFF LYSKLTVDKS  
 RWQQGNVFSC SVMHEALHNH YTQKSLSLSP GK (SEQ ID NO: 55)

[0233] **Item 24** shows the mature amino acid sequence (i.e., without the leader sequence) of hT $\beta$ RII<sub>short</sub>(29-159)-hG1Fc<sub>short</sub> (SEQ ID NO: 56). Single underline denotes linker.

20 QKSVNN DMIVTDNNGA VKFPQLCKFC  
 DVRFSTCDNQ KSCMSNCSIT SICEKPQEVC VAVWRKNDEN ITLETVCHDP  
 KLPYHDFILE DAASPKCIMK EKKKPGETFF MCSCSSDECN DNIIIFSEEEYN  
 TSNPDTGGGT HTCPPCPAPE LLGGPSVFLF PPKPKDTLM I SRTPEVTCVV  
 VDVSHEDPEV KFNWYVDGVE VHN AKTKP RE EQYNSTYRVV SVLTVLHQDW  
 LNGKEYKCKV SNKALPAPIE KTISKAKGQP REPQVYTLPP SREEMTKNQV  
 25 SLTCLVKGFY PSDIAVEWES NGQPENNYKT TPPVLDSDGS FFLYSKLTVD  
 KSRWQQGNVF SCSVMHEALH NYTQKSLSL SPGK (SEQ ID NO: 56)

[0234] **Item 25** shows the mature amino acid sequence (i.e., without the leader sequence) of hT $\beta$ RII<sub>short</sub>(35-159)-hG2Fc (SEQ ID NO: 57). Single underline denotes linker.

30 DMIVTD NNGAVKFPQL CKFCDVRFST  
 CDNQKSCMSN CSITSICEKP QEVCVAVWRK NDENITLEV CHDPKLPYHD  
 FILEDAASPK CIMKEKKPG ETFFMCSCSS DECNDNIIIFS EYNTSNPDT

5

GGGVECPPCP APPVAGPSVF LFPPKPKDTL MISRTPEVTC VVVDVSHEDP  
 EVQFNWYVDG VEVHNAKTKP REEQFNSTFR VVSVLTVVHQ DWLNGKEYKC  
 KVSNKGLPAP IEKTISKTKG QPREPQVYTL PPSREEMTKN QVSLTCLVKG  
 FYPSDIAVEW ESNGQOPENNY KTPPPMLDSD GSFFFLYSKLT VDKSRWQQGN  
 VFSCSVMHEA LHNHYTQKSL SLSPGK (SEQ ID NO: 57)

**[0235] Item 26** shows the mature amino acid sequence (i.e., without the leader sequence) of hT $\beta$ RII<sub>short</sub>(35-159)-hG1Fc<sub>short</sub> (SEQ ID NO: 58). Single underline denotes linker.

10

DMIVTD NNGAVKFPQL CKFCDVRFST  
 CDNQKSCMSN CSITSICEKP QEVCVAVWRK NDENITLETV CHDPKLPYHD  
 FILEDAASPK CIMKEKKPG ETFFMCSCSS DECNDNIIFS EYNTSNPDT  
GGGTHTCPPC PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDSHE  
 DPEVKFNWYV DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY  
 KCKVSNKALP APIEKTISKA KGQPREPQVY TLPPSREEMT KNQVSLTCLV  
 KGFYPSDIAV EWESNGQOPEN NYKTPPPVLD SDGSFFFLYSK LTVDKSRWQQ  
 GNVFSCSVMH EALHNHYTQK SLSPGK (SEQ ID NO: 58)

15

**[0236] Item 27** shows the mature amino acid sequence (i.e., without the leader sequence) of hT $\beta$ RII<sub>short</sub>(23-153)-hG2Fc (SEQ ID NO: 59). Single underline denotes linker.

20

25

25

TIPPHV QKSVNNDMIV TDNNGAVKFP  
 QLCKFCDVRF STCDNQKSCM SNCSITSICE KPQEVCVAVW RKNDENITLE  
 TVCHDPKLPY HDFILEDAAS PKCIMKEKKK PGETFFMCSC SSDECNDNII  
 FSEEEYTGGGV ECPPCPAPPV AGPSVFLFPP KPKDTLMISR TPEVTCVVVD  
 VSHEDPEVQF NWYVDGVEVH NAKTKPREEQ FNSTFRVSV LTVVHQDWLN  
 GKEYKCKVSN KGLPAPIEKT ISKTKQPRE PQVYTLPPSR EEMTKNQVSL  
 TCLVKGFYPS DIAVEWESNG QOPENNYKTPP PMLSDGSFF LYSKLTVDKS  
 RWQQGNVFSC SVMHEALHNH YTQKSLSLSP GK (SEQ ID NO: 59)

**[0237] Item 28** shows the mature amino acid sequence (i.e., without the leader sequence) of hT $\beta$ RII<sub>short</sub>(23-153)-hG1Fc<sub>short</sub> (SEQ ID NO: 60). Single underline denotes linker.

30

TIPPHV QKSVNNDMIV TDNNGAVKFP  
 QLCKFCDVRF STCDNQKSCM SNCSITSICE KPQEVCVAVW RKNDENITLE  
 TVCHDPKLPY HDFILEDAAS PKCIMKEKKK PGETFFMCSC SSDECNDNII  
 FSEEEYTGGGT HTCPPCPAPE LLGGPSVLF PPKPKDTLMF SRTPEVTCVV  
 VDVSHEPEV KFNWYVDGVE VHNAKTKPRE EQYNSTYRVV SVLTVLHQDW

LNGKEYKCKV SNKALPAPIE KTISKAKGQP REPQVYTLPP SREEMTKNQV  
 SLTCLVKGFY PSDIAVEWES NGQPENNYKT TPPVLDSDGS FFLYSKLTVD  
 KSRWQQGNVF SCSVMHEALH NYTQKSLSL SPGK (SEQ ID NO: 60)

[0238] **Item 29** shows the mature amino acid sequence (i.e., without the leader sequence) of  
 5 hT $\beta$ RII<sub>short</sub>(23-153/N70D)-hG2Fc (SEQ ID NO: 61). Double underline indicates N70D  
 substitution. Single underline denotes linker.

TIPPHV QKSVNNDMIV TDNNNGAVKFP  
 QLCKFCDVRF STCDNQKSCM SDCSITSICE KPQEVCVAVW RKNDENITLE  
 TVCHDPKL PY HDFILEDAAS PKCIMKEKKK PGETFFMCSC SSDECNDNII  
 10 FSEEEYTGGGV ECPPCPAPPV AGPSVFLFPP KPKDTLMISR TPEVTCVVVD  
 VSHEDEPEVQF NWYVDGVEVH NAKTKPREEQ FNSTFRVSV LTVVHQDWLN  
 GKEYKCKVSN KGLPAPIEKT ISKTKGQPRE PQVYTLPPSR EEMTKNQVSL  
 TCLVKGFYPS DIAVEWESNG QPENNYKTPP PMLSDGSFF LYSKLTVDKS  
 RWQQGNVFSC SVMHEALHNH YTQKSLSLSP GK (SEQ ID NO: 61)

15 [0239] **Item 30** shows the mature amino acid sequence (i.e., without the leader sequence) of  
 hT $\beta$ RII<sub>short</sub>(23-153/N70D)-hG1Fc<sub>short</sub> (SEQ ID NO: 62). Double underline indicates N70D  
 substitution. Single underline denotes linker.

TIPPHV QKSVNNDMIV TDNNNGAVKFP  
 QLCKFCDVRF STCDNQKSCM SDCSITSICE KPQEVCVAVW RKNDENITLE  
 20 TVCHDPKL PY HDFILEDAAS PKCIMKEKKK PGETFFMCSC SSDECNDNII  
 FSEEEYTGGGT HTCPPCPAPE LLGGPSVLF PPKPKDTLMI SRTPEVTCVV  
 VDVSHEDEPEV KFNWYVDGVE VHNAKTKPREG EQYNSTYRVV SVLTVLHQDW  
 LNGKEYKCKV SNKALPAPIE KTISKAKGQP REPQVYTLPP SREEMTKNQV  
 SLTCLVKGFY PSDIAVEWES NGQPENNYKT TPPVLDSDGS FFLYSKLTVD  
 25 KSRWQQGNVF SCSVMHEALH NYTQKSLSL SPGK (SEQ ID NO: 62)

## INCORPORATION BY REFERENCE

[0240] All publications and patents mentioned herein are hereby incorporated by reference in their entirety as if each individual publication or patent was specifically and individually indicated to be incorporated by reference.

30 [0241] While specific embodiments of the subject matter have been discussed, the above specification is illustrative and not restrictive. Many variations will become apparent to those skilled in the art upon review of this specification and the claims below. The full

scope of the invention should be determined by reference to the claims, along with their full scope of equivalents, and the specification, along with such variations.

**We Claim:**

1. A T $\beta$ RII fusion polypeptide comprising a first amino acid sequence from the extracellular domain of T $\beta$ RII and a heterologous amino acid sequence, wherein the first amino acid sequence consists of an amino acid sequence at least 80% identical to:
  - 5 a) a sequence beginning at any of positions 23 to 35 of SEQ ID NO: 5 and ending at any of positions 153 to 159 of SEQ ID NO: 5 or
  - b) a sequence beginning at any of positions 23 to 60 of SEQ ID NO: 6 and ending at any of positions 178 to 184 of SEQ ID NO: 6.
- 10 2. The T $\beta$ RII fusion polypeptide of claim 1, wherein the first amino acid sequence consists of the sequence beginning at position 23 of SEQ ID NO: 5 and ending at position 159 of SEQ ID NO: 5.
- 15 3. The T $\beta$ RII fusion polypeptide of claim 1, wherein the first amino acid sequence consists of the sequence beginning at position 29 of SEQ ID NO: 5 and ending at position 159 of SEQ ID NO: 5.
- 20 4. The T $\beta$ RII fusion polypeptide of claim 1, wherein the first amino acid sequence consists of the sequence beginning at position 35 of SEQ ID NO: 5 and ending at position 159 of SEQ ID NO: 5.
- 25 5. The T $\beta$ RII fusion polypeptide of claim 1, wherein the first amino acid sequence consists of the sequence beginning at position 23 of SEQ ID NO: 5 and ending at position 153 of SEQ ID NO: 5.
6. The T $\beta$ RII fusion polypeptide of claim 1, wherein the first amino acid sequence consists of the sequence beginning at position 29 of SEQ ID NO: 5 and ending at position 153 of SEQ ID NO: 5.
- 30 7. The T $\beta$ RII fusion polypeptide of claim 1, wherein the first amino acid sequence consists of the sequence beginning at position 35 of SEQ ID NO: 5 and ending at position 153 of SEQ ID NO: 5.

8. The T $\beta$ RII fusion polypeptide of claim 1, wherein the first amino acid sequence consists of the sequence beginning at position 23 of SEQ ID NO: 6 and ending at positions 184 of SEQ ID NO: 6.

5 9. The T $\beta$ RII fusion polypeptide of claim 1, wherein the first amino acid sequence consists of the sequence beginning at position 29 of SEQ ID NO: 6 and ending at position 184 of SEQ ID NO: 6.

10 10. The T $\beta$ RII fusion polypeptide of claim 1, wherein the first amino acid sequence consists of the sequence beginning at position 23 of SEQ ID NO: 6 and ending at position 178 of SEQ ID NO: 6.

11. The T $\beta$ RII fusion polypeptide of claim 1, wherein the first amino acid sequence consists of the sequence beginning at position 29 of SEQ ID NO: 6 and ending at position 178 of SEQ ID NO: 6.

15 12. The T $\beta$ RII fusion polypeptide of any one of claims 1 to 11, wherein the first amino acid sequence consists of a sequence that has a D at the position corresponding to position 36 of SEQ ID NO: 47 and/or a K at the position corresponding to position 76 of SEQ ID NO: 47.

13. A T $\beta$ RII fusion polypeptide comprising a first amino acid sequence at least 80% identical to the sequence of SEQ ID NO: 7 or SEQ ID NO: 13, or active fragment thereof, 20 and a second heterologous portion, wherein the first amino acid sequence has a D at the position corresponding to position 36 of SEQ ID NO: 47 and/or a K at the position corresponding to position 76 of SEQ ID NO: 47.

14. The T $\beta$ RII fusion polypeptide of claim 13 wherein the first amino acid sequence comprises an N-terminal truncation of 1-12 amino acids corresponding to amino acids 1-12 of SEQ ID NO: 7 or 1-37 amino acids corresponding to amino acids 1-37 of SEQ ID NO: 13.

15. The T $\beta$ RII fusion polypeptide of claim 13 or 14, wherein the first amino acid sequence comprises an N-terminal truncation of 6 amino acids corresponding to amino acids 1-6 of SEQ ID NO: 7 or SEQ ID NO: 13.

16. The T $\beta$ RII fusion polypeptide of claim 13 or 14, wherein the first amino acid sequence comprises an N-terminal truncation of 12 amino acids corresponding to amino acids 1-12 of SEQ ID NO: 7 or 37 amino acids corresponding to amino acids 1-37 of SEQ ID NO: 13.

5 17. The T $\beta$ RII fusion polypeptide of any one of claims 13 to 16, wherein the first amino acid sequence comprises a C-terminal truncation of 1-6 amino acids corresponding to amino acids 137-132 of SEQ ID NO: 7 or amino acids 162-157 of SEQ ID NO: 13.

18. The T $\beta$ RII fusion polypeptide of any one of claims 13 to 17, wherein the first amino acid sequence comprises a C-terminal truncation of 6 amino acids corresponding to amino acids 132-137 of SEQ ID NO: 7 or amino acids 157-162 of SEQ ID NO: 13.

10 19. The T $\beta$ RII fusion polypeptide of any one of claims 1 to 18, wherein the first amino acid sequence comprises an insertion corresponding to SEQ ID NO: 18 between the residues corresponding to positions 117 and 118 of SEQ ID NO: 47.

20. The T $\beta$ RII fusion polypeptide of any one of claims 1 to 19, wherein the heterologous portion comprises one or more polypeptide portions that enhance one or more of: in vivo stability, in vivo half life, uptake/administration, tissue localization or distribution, formation of protein complexes, and/or purification.

15 21. The T $\beta$ RII fusion polypeptide of any one of claims 1 to 20, wherein the heterologous portion comprises a polypeptide portion selected from: an immunoglobulin Fc domain and a serum albumin.

20 22. The T $\beta$ RII fusion polypeptide of claim 21, wherein the immunoglobulin Fc domain is joined to the T $\beta$ RII polypeptide by a linker.

25 23. The T $\beta$ RII fusion polypeptide of any one of claims 1 to 22, wherein the polypeptide includes one or more modified amino acid residues selected from: a glycosylated amino acid, a PEGylated amino acid, a farnesylated amino acid, an acetylated amino acid, a biotinylated amino acid, an amino acid conjugated to a lipid moiety, and an amino acid conjugated to an organic derivatizing agent.

24. The T $\beta$ RII fusion polypeptide of claim 23, wherein the polypeptide is glycosylated.

25. A T $\beta$ RII fusion polypeptide comprising a first amino acid sequence consisting of a portion of the extracellular domain of T $\beta$ RII that comprises an amino acid sequence that is at least 95% identical to an amino acid sequence selected from SEQ ID NOs: 7-17 and 47-49, and a second heterologous portion.

26. A T $\beta$ RII fusion polypeptide comprising a first amino acid sequence consisting of a portion of the extracellular domain of T $\beta$ RII that comprises an amino acid sequence that is at least 97% identical to an amino acid sequence selected from SEQ ID NOs: 7-17 and 47-49, and a second heterologous portion.

10 27. A T $\beta$ RII fusion polypeptide comprising a first amino acid sequence consisting of a portion of the extracellular domain of T $\beta$ RII that comprises an amino acid sequence that is at least 98% identical to an amino acid sequence selected from SEQ ID NOs: 7-17 and 47-49, and a second heterologous portion.

15 28. A T $\beta$ RII fusion polypeptide comprising a first amino acid sequence consisting of a portion of the extracellular domain of T $\beta$ RII that comprises an amino acid sequence that is at least 99% identical to an amino acid sequence selected from SEQ ID NOs: 7-17 and 47-49, and a second heterologous portion.

20 29. A T $\beta$ RII fusion polypeptide comprising a first amino acid sequence consisting of a portion of the extracellular domain of T $\beta$ RII that comprises an amino acid sequence is an amino acid sequence selected from SEQ ID NOs: 7-17 and 47-49 and a second heterologous portion.

30. A polypeptide comprising an amino acid sequence that is at least 95% identical to an amino acid sequence selected from SEQ ID NOs: 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 53, 54, 55, 56, 57, 58, 59, 60, 61, and 62.

25 31. A polypeptide comprising an amino acid sequence that is at least 97% identical to an amino acid sequence selected from SEQ ID NOs: 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 53, 54, 55, 56, 57, 58, 59, 60, 61, and 62.

32. A polypeptide comprising an amino acid sequence that is at least 98% identical to an amino acid sequence selected from SEQ ID NOS: 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 53, 54, 55, 56, 57, 58, 59, 60, 61, and 62.

5 33. A polypeptide comprising an amino acid sequence that is at least 99% identical to an amino acid sequence selected from SEQ ID NOS: 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 53, 54, 55, 56, 57, 58, 59, 60, 61, and 62.

34. A polypeptide comprising an amino acid sequence selected from SEQ ID NOS: 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 53, 54, 55, 56, 57, 58, 59, 60, 61, and 62.

10 35. A T $\beta$ RII polypeptide comprising an amino acid sequence encoded by a nucleic acid that hybridizes under stringent conditions to a complement of a nucleotide sequence selected from SEQ ID NOS: 26, 28, 30, 32, 34, 36, 38, 40, 42 and 44.

36. The polypeptide of any one of claims 1 to 35, wherein the polypeptide binds human GDF15 with an equilibrium dissociation constant ( $K_D$ ) less than  $1 \times 10^{-8}$  M.

15 37. The polypeptide of any one of claims 1 to 36, wherein the polypeptide has a glycosylation pattern characteristic of expression of the polypeptide in CHO cells.

38. A homodimer comprising two polypeptides as defined in any of claims 1 to 37.

39. An isolated polynucleotide comprising a coding sequence for the polypeptide of any one of claims 1 to 37.

20 40. A recombinant polynucleotide comprising a promoter sequence operably linked to a polynucleotide of claim 39.

41. A cell transformed with an isolated polynucleotide of claim 35 or a recombinant polynucleotide of claim 40.

42. The cell of claim 41, wherein the cell is a mammalian cell.

43. The cell of claim 42, wherein the cell is a CHO cell or a human cell.

44. A pharmaceutical preparation comprising the polypeptide of any of claims 1 to 37 or the homodimer of claim 38 and a pharmaceutically acceptable excipient.

45. A method of modulating the response of a cell to a TGF $\beta$  superfamily member, the method comprising exposing the cell to a polypeptide of any one of claims 1 to 37 or the homodimer of claim 38.

5 46. A method of treating a disease or condition associated with a TGF $\beta$  superfamily member in a patient in need thereof, the method comprising administering to the patient an effective amount of a polypeptide of any of claims 1 to 37 or the homodimer of claim 38.

10 47. The method of claim 46, wherein the TGF $\beta$  superfamily member is TGF $\beta$ 1, TGF $\beta$ 3 or GDF15.

48. The method of claim 46 or 47, wherein the disease or condition is a cancer.

49. The method of claim 48, wherein the cancer is selected from stomach cancer, intestinal cancer, skin cancer, breast cancer, melanoma, bone cancer and thyroid cancer.

15 50. The method of claim 46 or 47, wherein the disease or condition is a fibrotic or sclerotic disease or disorder.

51. The method of claim 50, wherein the fibrotic or sclerotic disease or disorder is selected from scleroderma, atherosclerosis, liver fibrosis, diffuse systemic sclerosis, glomerulonephritis, neural scarring, dermal scarring, radiation-induced fibrosis, hepatic fibrosis, and myelofibrosis.

20 52. The method of claim 46 or 47, wherein the disease or condition is heart disease.

53. The method of claim 46 or 47, wherein the disease or condition is selected from hereditary hemorrhagic telangiectasia (HHT), Marfan syndrome, Loeys-Dietz syndrome, familial thoracic aortic aneurysm syndrome, arterial tortuosity syndrome, pre-eclampsia, atherosclerosis, restenosis, and hypertrophic cardiomyopathy/congestive heart failure.

54. An antibody, or antigen binding fragment thereof, that binds to GDF15 and blocks the interaction between GDF15 and T $\beta$ RII.

55. A GDF15 polypeptide comprising the amino acid sequence of SEQ ID NO: 1 or a fragment thereof that binds T $\beta$ RII, wherein the GDF15 polypeptide is at least 95% pure, with respect to protein contaminants.

56. A GDF15 polypeptide comprising the amino acid sequence of SEQ ID NO: 1 or a fragment thereof that binds to a polypeptide of any one of claims 1 to 37, wherein the GDF15 polypeptide is at least 95% pure, with respect to protein contaminants.

57. The GDF15 polypeptide of claim 55, wherein the GDF15 polypeptide binds T $\beta$ RII with an equilibrium dissociation constant (K<sub>D</sub>) of no greater than 10<sup>-8</sup> M.

58. The GDF15 polypeptide of claim 56, wherein the GDF15 polypeptide binds to a polypeptide of any one of claims 1 to 37 with an equilibrium dissociation constant (K<sub>D</sub>) of no greater than 10<sup>-8</sup> M.

59. The GDF15 polypeptide of any of claims 55 to 58, wherein the polypeptide is produced by expression in CHO cells.

60. A method of concentrating or purifying GDF15, comprising contacting a sample containing GDF15 with a polypeptide of any of claims 1 to 37.

61. A polypeptide that comprises an amino acid sequence that is at least 90% identical to the amino acid sequence of SEQ ID NO:50.

20 62. A polypeptide of claim 61 that comprises an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO:50.

63. A polypeptide of claim 61 that comprises an amino acid sequence that is at least 99% identical to the amino acid sequence of SEQ ID NO:50.

25 64. A polypeptide of claim 61 that comprises an amino acid sequence that is identical to the amino acid sequence of SEQ ID NO:50.

65. A polypeptide of claim 61 that consists of an amino acid sequence that is identical to the amino acid sequence of SEQ ID NO:50.
66. A nucleic acid that encodes a polypeptide of any of claims 61-65.
67. A nucleic acid comprising the nucleic acid sequence of SEQ ID NO: 51.
- 5 68. A polypeptide produced by expressing the nucleic acid of claim 67 in a mammalian cell.
69. The polypeptide of claim 68, wherein the mammalian cell is a Chinese Hamster Ovary cell (CHO cell).

10

1        MPGQELRTVN GSQMLLVLLV LSWLPHGGAL SLAEASRASF PGPSELHSED  
51      SRFRELRKRY EDLLTRLRAN QSWEDSNSDL VPAPAVRILT PEVRLGSGGH  
101     LHLRISRAAL PEGLPEASRL HRALFRLSPT ASRSWDVTRP LRRQLSLARP  
151     QAPALHLRLS PPPSQSDQLL AESSSARPQL ELHLRPQAAR GRRRARARNG  
201     DHCPLPGRC CRLHTVRASL EDLGWADWVL SPREVQVTMC IGACPSQFRA  
251     ANMHAQIKTS LHRLKPDTVP APCCVPASYN PMVLIQKTDT GVSLQTYDDL  
301     LAKDCHCI        (SEQ ID NO: 1)

## FIGURE 1

1 ATGCCCGGGC AAGAACTCAG GACGGTGAAT GGCTCTCAGA TGCTCCTGGT  
51 GTTGCTGGTG CTCTCGTGGC TGCCGCATGG GGGCGCCCTG TCTCTGCCG  
101 AGGCGAGCCG CGCAAGTTTC CGGGGACCCCT CAGAGTTGCA CTCCGAAGAC  
151 TCCAGATTCC GAGAGTTGCG GAAACGCTAC GAGGACCTGC TAACCAGGCT  
201 GCGGGCCAAC CAGAGCTGGG AAGATTGAA CACCGACCTC GTCCCAGGCC  
251 CTGCAGTCCG GATACTCACG CCAGAAGTGC GGCTGGGATC CGGCAGGCCAC  
301 CTGCACCTGC GTATCTCTCG GGCCGCCCTT CCCGAGGGGC TCCCCGAGGC  
351 CTCCCCCCTT CACCGGGCTC TGTTCGGCT GTCCCCGACG GCGTCAAGGT  
401 CGTGGGACGT GACACGACCG CTGCGCGTC AGCTCAGCCT TGCAAGACCC  
451 CAGGCACCCG CGCTGCACCT GCGACTGTGC CCGCCGCCGT CGCAGTCGGA  
501 CCAACTGCTG GCAGAATCTT CGTCCGCACG GCCCCAGCTG GAGTTGCACT  
551 TGCGGCCGCA AGCCGCCAGG GGGCGCCGCA GAGCGCGTGC GCGCAACGGG  
601 GACCACTGTC CGCTCGGGCC CGGGCGTTGC TGCCGTCTGC ACACGGTCCG  
651 CGCGTCGCTG GAAGACCTGG GCTGGGCCGA TTGGGTGCTG TCGCCACGGG  
701 AGGTGCAAGT GACCATGTGC ATCGGCGCGT GCCCCAGCCA GTTCCGGCG  
751 GCAAACATGC ACGCGCAGAT CAAGACGAGC CTGCACCGCC TGAAGCCC  
801 CACGGTGCCA GCGCCCTGCT GCGTGCCCGC CAGCTACAAT CCCATGGTGC  
851 TCATTCAAAA GACCGACACC GGGGTGTCAC TCCAGACCTA TGATGACTTG  
901 TTAGCCAAAG ACTGCCACTG CATA (SEQ ID NO: 2)

## FIGURE 2

1 MAPPALQAQP PGGSQLRFLL FLLLLLILS WPSQGDALAM PEQRPSGPES  
51 QLNADELRGR FQDLLSRLHA NQSREDSNSE PSPDPAVRIL SPEVRLGSHG  
101 QLLLRLVNRA S LSQGLPEAYR VHRALLLTP TARPWDITRP LKRALSRLGP  
151 RAPALRLRLT PPPDLAMLPS GGTQLELRLR VAAGRGRRSA HAHPRDSCPL  
201 GPGRCCHLET VQATLEDLGW SDWVLSPRQL QLSMCVGECP HLYRSANTHA  
251 QIKARLHGLQ PDKVPAPCCV PSSYTPVVLM HRTDSGVSLQ TYDDLVARC  
301 HCA (SEQ ID NO: 3)

## FIGURE 3

1 ATGGCCCCGC CCGCGCTCCA GGCCCAGCCT CCAGGCGGCT CTCAACTGAG  
51 GTTCTGCTG TTCCTGCTGC TGTTGCTGCT GCTGCTGTCA TGGCCATCGC  
101 AGGGGGACGC CCTGGCAATG CCTGAACAGC GACCCTCCGG CCCTGAGTCC  
151 CAACTCAACG CCGACGAGCT ACGGGGTCGC TTCCAGGACC TGCTGAGCCG  
201 GCTGCATGCC AACCAGAGCC GAGAGGACTC GAACTCAGAA CCAAGTCCTG  
251 ACCCAGCTGT CCGGATACTC AGTCCAGAGG TGAGATTGGG GTCCCACGGC  
301 CAGCTGCTAC TCCGCGTCAA CCGGGCGTCG CTGAGTCAGG GTCTCCCCGA  
351 AGCCTACCAGC GTGCACCGAG CGCTGCTCCT GCTGACGCCG ACGGCCCGCC  
401 CCTGGGACAT CACTAGGCCCT CTGAAGCGTG CGCTCAGCCT CGGGGGACCC  
451 CGTGCTCCCG CATTACGCCT GCGCCTGACG CCGCCTCCGG ACCTGGCTAT  
501 GCTGCCCTCT GGCGGCACGC AGCTGGAACT GCGCTTACGG GTAGCCGCCG  
551 GCAGGGGGCG CGAAGCGCG CATGCGCACC CAAGAGACTC GTGCCCACTG  
601 GGTCCAGGGC GCTGCTGTCA CTTGGAGACT GTGCAGGCAA CTCTTGAAGA  
651 CTTGGGCTGG AGCGACTGGG TGCTGTCCCC GCGCCAGCTG CAGCTGAGCA  
701 TGTGCGTGGG CGAGTGTCCC CACCTGTATC GCTCCCGCAA CACGCATGCG  
751 CAGATCAAAG CACGCCTGCA TGGCCTGCAG CCTGACAAGG TGCCTGCCCG  
801 GTGCTGTGTC CCCTCCAGCT ACACCCCGGT GGTTCTTATG CACAGGACAG  
851 ACAGTGGTGT GTCACTGCAG ACTTATGATG ACCTGGTGGC CCGGGGCTGC  
901 CACTGCGCT (SEQ ID NO: 4)

FIGURE 4

1 mrgllrglw plhivlwtri astipphvqk svnndmivtd nngavkfpql  
51 ckfcdvrfst cdnqkscmsn csitsicekp qevcvavwrk ndenitletv  
101 chdpk1pyhd filedaaspk cimkekkgpg etffmcscss decndniifs  
151 eeeyntsnpdl llvifqvtgi sllpplgvai sviiifycyr vnrrqqklsst  
201 wetgktrklm efsehcaiil eddrsdisst canninhnte llpieldtlv  
251 gkgrfaevyk aklkqntseq fetvavkifp yeeyaswkte kdifsdinlk  
301 henilqflta eerktelgkq ywlitafhak gnlqeyltrh viswedlrkl  
351 gsslargiah lhsdhtpcgr pkmpivhrdl kssnilvknd ltcclcdfgl  
401 slrlldptlsv ddlansgqvg tarymapevl esrmnlenve sfkqtdvysm  
451 alvlwemtsr cnavgevkdy eppfgskvre hpcvesmkdn vlrdrgrpei  
501 psfwlnhqgi qmvctltec wdhdpearlt aqcvaerfse lehldrlnsgr  
551 scseekiped gslnttk (SEQ ID NO: 5)

FIGURE 5

1 mrgllrglw plhivlwtri astipphvqk sdvemeaqkd eiicpscnrt  
51 ahplrhinnd mivtdnngav kfpqlckfcd vrfstcdnqk scmsncsits  
101 icekpqevcv avwrkndeni tletvchdpk lpyhdfiled aaspkcimke  
151 kkkpgetffm cscssdecnd niifseeeynt snpdlllvif qvtgisllpp  
201 lgvaisviii fycyrvnrqq klsstwetgk trklmefseh caiileddrs  
251 disstcanni nhntellpie ldtlvgkgrf aevykaklkq ntseqfetva  
301 vkifpyeeya swktekdifs dinlkhenil qfltaeerkt elgkqywlt  
351 afhakgnlqe yltrhviswe dlrklgssla rgiahlsdh tpcgrpkmpl  
401 vhrdlkssni lvkndlcc1 cdfglslrld ptlsvddlan sgqvgtarym  
451 apevlesrmn lenesfkqt dvysmalvlw emtsrcnavg evkdyeppfg  
501 skvrehpcve smkdnvlrdr grpeipsfwl nhqgiqmvce tltecwdhdp  
551 earltaqcva erfselehld rlsgrscsee kipedgslnt tk

(SEQ ID NO: 6)

FIGURE 6

Native Leader Sequence

Native T $\beta$ RII short

1 10 20 30

MGRGLLRGLWPLHIVLWTRIASTTIPPHVQKS

T $\beta$ RII<sub>short</sub>(23-X) 30 TIPPHVQKS

T $\beta$ RII<sub>short</sub>(29-X) 30 QKS

T $\beta$ RII<sub>short</sub>(35-X)

Native T $\beta$ RII long 1 10 20 30

MGRGLLRGLWPLHIVLWTRIASTTIPPHVQKS

T $\beta$ RII<sub>long</sub>(23-X) 30 TIPPHVQKS

T $\beta$ RII<sub>long</sub>(29-X) 30 QKS

T $\beta$ RII<sub>long</sub>(60-X)

## FIGURE 7

## Splice Insertion (25 aa)

35  
- - - - **V**NNNDMIV...

35  
- - - - **V**NNNDMIV...

35  
- - - - **V**NNNDMIV...

35  
- - - - DMIV...

40 50 60  
DVEMEAQKDEIIICPSCNRTAHPLRH**I**NNNDMIV...

40 50 60  
DVEMEAQKDEIIICPSCNRTAHPLRH**I**NNNDMIV...

40 50 60  
DVEMEAQKDEIIICPSCNRTAHPLRH**I**NNNDMIV...

60  
DMIV...

FIGURE 7 (continued)

**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.  Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
the subject matter listed in Rule 39 on which, under Article 17(2)(a)(i), an international search is not required to be carried out, including
2.  Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3.  Claims Nos:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

**Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

**See Supplemental Box for Details**

1.  As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2.  As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3.  As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4.  No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

- The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- No protest accompanied the payment of additional search fees.

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2014/052130

## A. CLASSIFICATION OF SUBJECT MATTER

**C07K 19/00 (2006.01)    C07K 14/475 (2006.01)    C07K 16/22 (2006.01)    A61K 38/16 (2006.01)    A61P 9/10 (2006.01)**  
**A61P 35/00 (2006.01)**

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)

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)

Databases EPODOC, MEDLINE, NPL, WPI, XPMISC, XPOAC and XPTK searched with keywords GDF15 (and synonyms) and TGF $\beta$ RII (and synonyms)

Databases EPODOC and MEDLINE searched with inventors' names

GenomeQuest: all protein databases searched with SEQ ID NO:1, and with a consensus sequence derived from the Fc region and amino acids 35-153 of SEQ ID NO: 5

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Documents are listed in the continuation of Box C	

 Further documents are listed in the continuation of Box C 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	"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
"E" earlier application or patent but published on or after the international filing date	"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
"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)	"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
"O" document referring to an oral disclosure, use, exhibition or other means	"&"	document member of the same patent family
"P" document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search  
30 September 2014Date of mailing of the international search report  
30 September 2014

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INTERNATIONAL SEARCH REPORT		International application No. <b>PCT/US2014/052130</b>
C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2011/109789 A2 (THE JOHNS HOPKINS UNIVERSITY) 09 November 2011 See SEQ ID NOS: 7, 12 and 13; paragraphs [0047], [0050], [0051], [0198] and [0208]; Figures 7, 10, 11 and 55	1-7, 20-36, 39-41, 44-49, 61, 62, 66
X	WO 2010/003118 A1 (TRUBION PHARMACEUTICALS, INC.) 07 January 2010 See SEQ ID NOS: 737 and 738; Paragraphs [0003], [0031], [00224]; Claims 13 and 14	1, 8-11, 23-28, 30, 35, 36, 39-41, 44-53, 61, 66
X	WO 2013/000234 A1 (HUABO BIOPHARM CO., LTD) 03 January 2013 See SEQ ID NO: 8; Page 17; Page 18 paragraph 2; Page 4, paragraphs 6 and 7; Examples 1 and 2; Figures 3 and 4B	25-29, 36-38, 42-51, 61, 66
X	WO 1998/048024 A1 (BIOGEN, INC.) 29 October 1998 See SEQ ID NO: 9, Claims 4 and 16-20	25-32, 35, 36, 44-47, 50, 51, 61-69
X	WO 2013/012648 A1 (EMORY UNIVERSITY) 24 January 2013 See SEQ ID NO: 1; Claim 21	54-59
X	CN 101852804 A (INSTITUTE OF PATHOGEN BIOLOGY, CHINESE ACADEMY OF MEDICAL SCIENCES) 06 October 2010 & Derwent Abstract Accession No. 2010-P08192 See SEQ ID NO: 1; Title; Abstract	54-59

**INTERNATIONAL SEARCH REPORT**

International application No.

**PCT/US2014/052130****Supplemental Box****Continuation of: Box III**

This International Application does not comply with the requirements of unity of invention because it does not relate to one invention or to a group of inventions so linked as to form a single general inventive concept.

This Authority has found that there are different inventions based on the following features that separate the claims into distinct groups:

- Claims 1-53 and 60-69 are directed to fusion proteins that comprise a TGF $\beta$ RII extracellular domain (ECD) fused to a heterologous protein (such as an immunoglobulin Fc domain), and to nucleic acids that encode these fusion proteins and therapeutic uses of these fusion proteins.
- Claims 54-59 relate to the GDF15 protein, and to antibodies that bind to GDF15.

PCT Rule 13.2, first sentence, states that unity of invention is only fulfilled when there is a technical relationship among the claimed inventions involving one or more of the same or corresponding special technical features. PCT Rule 13.2, second sentence, defines a special technical feature as a feature which makes a contribution over the prior art. When there is no special technical feature common to all the claimed inventions there is no unity of invention.

In the above groups of claims, the identified features may have the potential to make a contribution over the prior art but are not common to all the claimed inventions and therefore cannot provide the required technical relationship. Therefore there is no special technical feature common to all the claimed inventions and the requirements for unity of invention are consequently not satisfied *a priori*.

<b>INTERNATIONAL SEARCH REPORT</b> Information on patent family members		International application No. <b>PCT/US2014/052130</b>	
This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.			
<b>Patent Document/s Cited in Search Report</b>			<b>Patent Family Member/s</b>
Publication Number	Publication Date	Publication Number	Publication Date
WO 2011/109789 A2	09 November 2011	CA 2791383 A1 EP 2542590 A2 JP 2013521311 A US 2013039911 A1	09 Sep 2011 09 Jan 2013 10 Jun 2013 14 Feb 2013
WO 2010/003118 A1	07 January 2010	AU 2009266873 A1 CA 2729810 A1 CN 102203258 A EA 201170031 A1 EP 2310508 A1 JP 2011526794 A KR 20110044992 A MX 2011000039 A NZ 590667 A US 2011177070 A1	07 Jan 2010 07 Jan 2010 28 Sep 2011 30 Aug 2011 20 Apr 2011 20 Oct 2011 03 May 2011 31 May 2011 25 Jan 2013 21 Jul 2011
WO 2013/000234 A1	03 January 2013	CN 102850458 A	02 Jan 2013
WO 1998/048024 A1	29 October 1998	AU 737106 B2 AU 7120898 A BR 9808934 A CA 2286933 A1 CN 1257545 A EE 9900492 A EP 0975771 A1 EP 0975771 B1 IS 5217 A JP 2001515360 A JP 2008106076 A NO 994998 A NZ 500284 A PL 336306 A1 US 2005203022 A1	09 Aug 2001 13 Nov 1998 01 Aug 2000 29 Oct 1998 21 Jun 2000 15 Jun 2000 02 Feb 2000 11 Jul 2007 15 Oct 1999 18 Sep 2001 08 May 2008 20 Dec 1999 28 Sep 2001 19 Jun 2000 15 Sep 2005
WO 2013/012648 A1	24 January 2013	None	
CN 101852804 A	06 October 2010	CN 101852804 B	12 Jun 2013

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

Form PCT/ISA/210 (Family Annex)(July 2009)

<b>INTERNATIONAL SEARCH REPORT</b> Information on patent family members	International application No. <b>PCT/US2014/052130</b>
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This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

<b>Patent Document/s Cited in Search Report</b>		<b>Patent Family Member/s</b>	
<b>Publication Number</b>	<b>Publication Date</b>	<b>Publication Number</b>	<b>Publication Date</b>

**End of Annex**

## 摘要

在某些方面，本公开内容涉及可用于选择性地拮抗 T $\beta$  RII 配体的包含 T $\beta$  RII 多肽胞外域的截短的配体结合部分的多肽。本公开内容进一步提供用于治疗或预防 TGF $\beta$  相关病症的组合物和方法。