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(54) Title: METHODS FOR TREATING HETEROTOPIC OSSIFICATION

(57) Abstract: In part, the present disclosure relates methods for treating heterotopic ossification or one or more complications of heterotopic ossification. In particular, the disclosure provided methods for treating heterotopic ossification by administering to a patient in need thereof one or more TGF β antagonists, optionally in combination with one or more additional active agents or supportive therapies for treating heterotopic ossification. For example, in some embodiments, the disclosure provided methods for treating heterotopic ossifications by administering to a patient in need thereof an effective amount of a T β RII polypeptide.

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METHODS FOR TREATING HETEROTOPIC OSSIFICATION

Cross-Reference to Related Applications

This application claims the benefit of priority from U.S. Provisional Application No. 62/628,649, filed on February 9, 2018 and from U.S. Provisional Application No. 62/666,235, filed on May 3, 2018. The foregoing applications are incorporated herein by reference in their entirety.

Background of the Invention

Heterotopic ossification (HO) is the pathologic formation of extra-skeletal bone in soft tissues. Vanden Bossche and Vanderstraeten, J Rehabil Med 27, 129 (2005). In general, this process occurs in patient populations with severe trauma including large-surface area burns, musculoskeletal injury, orthopedic operations, and spinal cord injury, and in patient populations with a genetic disease known as fibrodysplasia ossificans progressiva (FOP). FOP is caused by a hyper-activating mutation in the type I bone morphogenetic protein (BMP) receptor ACVR1, and patients with FOP develop ectopic bone lesions in the absence of any substantial trauma. The clinical sequela of these pathologic ectopic bone formations, whether in the setting of trauma or genetic mutations, include non-healing wounds, chronic pain, and joint immobility. In the case of FOP, progressive ossification may lead to death due to loss of thoracic cage compliance.

Treatment options for HO are limited as bone often recurs following surgical resection, and some patients may have non-resectable HO due to its sensitive location. The risk of an operation may outweigh the benefits of excision, especially in the face of recurrence. Thus, there is a high unmet need for effective therapies for treating HO.

Summary of the Invention

In part, the present disclosure relates to the discovery that a TGF β antagonist (inhibitor) can be used to treat heterotopic ossification (HO). In particular, the data presented herein shows that a soluble T β RII polypeptide, which binds to TGF β 1 and TGF β 3, is effective at attenuating development of HO anlagen with decreased osseous deposition and marrow space formation in a mouse model of HO. Furthermore, treatment with the T β RII polypeptide significantly reduced the volume of mature HO in this animal model. While not wishing to be bound to any particular mechanism, it is expected that the effects of T β RII

polypeptides are caused primarily by TGF β signaling antagonist effect, particularly of TGF β 1 and/or TGF β 3. Therefore, it is expected that other TGF β antagonists [e.g., antagonists of the T β RII receptor, antagonists of one or more T β RII-binding ligand (e.g., TGF β 1, TGF β 2, and TGF β 3), antagonists of one or more T β RII-associated type I receptor (e.g., ALK5),
5 antagonists of one or more T β RII-associated co-receptor (betaglycan), antagonists of one or more T β RII downstream signaling components (e.g., Smads), or combination of such antagonists] will be useful in the treatment of HO. Such agents are collectively referred to herein as “TGF β antagonists” or “TGF β inhibitors”.

In certain aspects, the disclosure relates to methods for treating HO comprising
10 administering to a patient in need thereof an effective amount of a Transforming Growth Factor- β (TGF β) antagonist. In some embodiments, the methods prevent or reduce the severity and/or duration of one or more complications of HO. For example, treatment with a TGF β antagonist may prevent or reduce the severity and/or duration of one or more complications of HO selected from the group consisting of: joint contracture, ankylosis, pain,
15 spasticity, swelling fever, neurovascular compression, and pressure ulcers. In some embodiments, the methods relate to treating HO that is associated with one or more disorders or conditions selected from the group consisting of: spinal cord injury, trauma, brain injuries, burns, fractures, muscle contusion, joint arthroplasty/replacement, hip surgery/replacement, acetabular surgery/replacement, elbow fracture, fracture of the long bones of the lower leg,
20 combat-related trauma, amputation, neuromuscular blockade used to manage adult respiratory distress syndrome, nontraumatic myelopathies. In some embodiments, the disclosure relates to methods of treating fibrodysplasia ossificans progressiva (FOP) comprising administering to a patient in need thereof an effective amount of a TGF β antagonist. For example, the methods prevent or reduce the severity and/or duration of one
25 or more complications of FOP (e.g., heterotopic ossification). In some embodiments, the disclosure relates to methods of treating fibrodysplasia ossificans progressiva (FOP) comprising administering to a patient in need thereof an effective amount of a TGF β antagonist. For example, the methods prevent or reduce the severity and/or duration of one or more complications of FOP (e.g., heterotopic ossification). In some embodiments, the
30 disclosure relates to methods of treating fibrous dysplasia comprising administering to a patient in need thereof an effective amount of a TGF β antagonist. For example, the methods prevent or reduce the severity and/or duration of one or more complications of fibrous dysplasia (e.g., heterotopic ossification). In some embodiments, heterotopic ossification

occurs in one or more tissues selected from the group consisting of: bone, skin, subcutaneous tissue, skeletal muscle, fibrosis tissue adjacent to joints, walls of blood vessels, and ligaments. Optionally, such methods described herein further comprise administering to the patient an effective amount of one or more additional active agents or supportive therapies for treating HO. For example, such additional active agents or supportive therapies include isotretinoin, etidronate with oral corticosteroids, perhexiline maleate, ALK2 small-molecule inhibitors, palovarotene, retinoic acid receptor gamma agonists, retinoic acid receptor alpha agonists, activin antibodies (e.g., activin A antibodies such as REGN 2477), allele-specific RNA interference of ALK2, bisphosphonates, radiation therapy anti-inflammatory agents (e.g., indomethacin, ibuprofen and aspirin), and conservative treatments such as passive range of motion exercises or other mobilization techniques. In some embodiments, the additional active agent or supportive therapy is treatment with an activin antagonist. In some embodiments, the activin antagonist is an activin antibody. In some embodiments, the activin antibody is an activin A antibody. In some embodiments, TGF β antagonist is a multi-specific antibody that binds to and inhibits signaling mediated by one or more of TGF β 1, TGF β 2, and TGF β 3 and further binds to and inhibits activin (e.g., activin A).

In certain aspects, a TGF β antagonist, or combination of antagonists, to be used in accordance with methods and uses described herein is an agent that inhibits at least TGF β 1 (e.g., a TGF β 1 antagonist). Effects on TGF β 1 inhibition may be determined, for example, using a cell-based assay including those described herein (e.g., Smad signaling assay). Therefore, in some embodiments, a TGF β antagonist, or combination of antagonists, of the disclosure may bind to at least TGF β 1. Ligand binding activity may be determined, for example, using a binding affinity assay including those described herein. In some embodiments, a TGF β antagonist, or combination of antagonists, of the disclosure binds to at least TGF β 1 with a K_D of at least 1×10^{-7} M (e.g., at least 1×10^{-8} M, at least 1×10^{-9} M, at least 1×10^{-10} M, at least 1×10^{-11} M, or at least 1×10^{-12} M). As described herein, various TGF β antagonists that inhibit TGF β 1 can be used in accordance with the methods and uses described herein including, for example, ligand traps (e.g., T β RII polypeptides and variants thereof), antibodies, small molecules, nucleotide sequences, and combinations thereof. In certain embodiments, a TGF β antagonist, or combination of antagonists, that inhibits TGF β 1 may further inhibit one or more of: TGF β 2, TGF β 3, T β RII, ALK5, and betaglycan. In some embodiments, a TGF β antagonist, or combination of antagonists, that inhibits TGF β 1 further inhibits TGF β 3. In some embodiments, a TGF β antagonist, or combination of antagonists,

that inhibits TGF β 1 does not inhibit or does not substantially inhibit TGF β 2. In some embodiments, a TGF β antagonist, or combination of antagonists, that inhibits TGF β 1 further inhibits TGF β 3 but does not inhibit or does not substantially inhibit TGF β 2.

In certain aspects, a TGF β antagonist, or combination of antagonists, to be used in accordance with methods and uses described herein is an agent that inhibits at least TGF β 2 (e.g., a TGF β 2 antagonist). Effects on TGF β 2 inhibition may be determined, for example, using a cell-based assay including those described herein (e.g., Smad signaling assay). Therefore, in some embodiments, a TGF β antagonist, or combination of antagonists, of the disclosure may bind to at least TGF β 2. Ligand binding activity may be determined, for example, using a binding affinity assay including those described herein. In some embodiments, a TGF β antagonist, or combination of antagonists, of the disclosure binds to at least TGF β 2 with a K_D of at least 1×10^{-7} M (e.g., at least 1×10^{-8} M, at least 1×10^{-9} M, at least 1×10^{-10} M, at least 1×10^{-11} M, or at least 1×10^{-12} M). As described herein, various TGF β antagonists that inhibit TGF β 2 can be used in accordance with the methods and uses described herein including, for example, ligand traps (e.g., T β RII polypeptides and variants thereof), antibodies, small molecules, nucleotide sequences, and combinations thereof. In certain embodiments, a TGF β antagonist, or combination of antagonists, that inhibits TGF β 2 may further inhibit one or more of: TGF β 1, TGF β 3, T β RII, ALK5, and betaglycan.

In certain aspects, a TGF β antagonist, or combination of antagonists, to be used in accordance with methods and uses described herein is an agent that inhibits at least TGF β 3 (e.g., a TGF β 3 antagonist). Effects on TGF β 3 inhibition may be determined, for example, using a cell-based assay including those described herein (e.g., Smad signaling assay). Therefore, in some embodiments, a TGF β antagonist, or combination of antagonists, of the disclosure may bind to at least TGF β 3. Ligand binding activity may be determined, for example, using a binding affinity assay including those described herein. In some embodiments, a TGF β antagonist, or combination of antagonists, of the disclosure binds to at least TGF β 3 with a K_D of at least 1×10^{-7} M (e.g., at least 1×10^{-8} M, at least 1×10^{-9} M, at least 1×10^{-10} M, at least 1×10^{-11} M, or at least 1×10^{-12} M). As described herein, various TGF β antagonists that inhibit TGF β 3 can be used in accordance with the methods and uses described herein including, for example, ligand traps (e.g., T β RII polypeptides and variants thereof), antibodies, small molecules, nucleotide sequences, and combinations thereof. In certain embodiments, a TGF β antagonist, or combination of antagonists, that inhibits TGF β 3

may further inhibit one or more of: TGF β 1, TGF β 2, T β RII, ALK5, and betaglycan. In some embodiments, a TGF β antagonist, or combination of antagonists, that inhibits TGF β 3 further inhibits TGF β 1. In some embodiments, a TGF β antagonist, or combination of antagonists, that inhibits TGF β 3 does not inhibit or does not substantially inhibit TGF β 2. In some
5 embodiments, a TGF β antagonist, or combination of antagonists, that inhibits TGF β 3 further inhibits TGF β 1 but does not inhibit or does not substantially inhibit TGF β 2.

In certain aspects, a TGF β antagonist, or combination of antagonists, to be used in accordance with methods and uses described herein is an agent that inhibits at least T β RII (e.g., a T β RII receptor antagonist). Effects on T β RII inhibition may be determined, for
10 example, using a cell-based assay including those described herein (e.g., Smad signaling assay). Therefore, in some embodiments, a TGF β antagonist, or combination of antagonists, of the disclosure may bind to at least T β RII. Ligand binding activity may be determined, for example, using a binding affinity assay including those described herein. In some
15 embodiments, a TGF β antagonist, or combination of antagonists, of the disclosure binds to at least T β RII with a K_D of at least 1×10^{-7} M (e.g., at least 1×10^{-8} M, at least 1×10^{-9} M, at least 1×10^{-10} M, at least 1×10^{-11} M, or at least 1×10^{-12} M). As described herein, various TGF β antagonists that inhibit T β RII can be used in accordance with the methods and uses described herein including, for example, ligand traps (e.g., T β RII polypeptides and variants thereof), antibodies, small molecules, nucleotide sequences, and combinations thereof. In
20 certain embodiments, a TGF β antagonist, or combination of antagonists, that inhibits T β RII may further inhibit one or more of: TGF β 1, TGF β 2, TGF β 3, ALK5, and betaglycan. In some embodiments, a TGF β antagonist, or combination of antagonists, that inhibits T β RII does not inhibit or does not substantially inhibit TGF β 2.

In certain aspects, a TGF β antagonist, or combination of antagonists, to be used in
25 accordance with methods and uses described herein is an agent that inhibits at least ALK5 (e.g., an ALK5 antagonist). Effects on ALK5 inhibition may be determined, for example, using a cell-based assay including those described herein (e.g., Smad signaling assay). Therefore, in some embodiments, a TGF β antagonist, or combination of antagonists, of the disclosure may bind to at least ALK5. Ligand binding activity may be determined, for
30 example, using a binding affinity assay including those described herein. In some embodiments, an ALK5 antagonist, or combination of antagonists, of the disclosure binds to at least ALK5 with a K_D of at least 1×10^{-7} M (e.g., at least 1×10^{-8} M, at least 1×10^{-9} M, at

least 1×10^{-10} M, at least 1×10^{-11} M, or at least 1×10^{-12} M). As described herein, various TGF β antagonists that inhibit ALK5 can be used in accordance with the methods and uses described herein including, for example, ligand traps (e.g., T β RII polypeptides and variants thereof), antibodies, small molecules, nucleotide sequences, and combinations thereof. In certain embodiments, a TGF β antagonist, or combination of antagonists, that inhibits ALK5 may further inhibit one or more of: TGF β 1, TGF β 2, TGF β 3, T β RII, and betaglycan. In some embodiments, a TGF β antagonist, or combination of antagonists, that inhibits ALK5 does not inhibit or does not substantially inhibit TGF β 2.

In certain aspects, a TGF β antagonist, or combination of antagonists, to be used in accordance with methods and uses described herein is an agent that inhibits at least betaglycan (e.g., a betaglycan antagonist). Effects on betaglycan inhibition may be determined, for example, using a cell-based assay including those described herein (e.g., Smad signaling assay). Therefore, in some embodiments, a TGF β antagonist, or combination of antagonists, of the disclosure may bind to at least betaglycan. Ligand binding activity may be determined, for example, using a binding affinity assay including those described herein. In some embodiments, a betaglycan antagonist, or combination of antagonists, of the disclosure binds to at least betaglycan with a K_D of at least 1×10^{-7} M (e.g., at least 1×10^{-8} M, at least 1×10^{-9} M, at least 1×10^{-10} M, at least 1×10^{-11} M, or at least 1×10^{-12} M). As described herein, various TGF β antagonists that inhibit betaglycan can be used in accordance with the methods and uses described herein including, for example, ligand traps (e.g., T β RII polypeptides and variants thereof), antibodies, small molecules, nucleotide sequences, and combinations thereof. In certain embodiments, a TGF β antagonist, or combination of antagonists, that inhibits betaglycan may further inhibit one or more of: TGF β 1, TGF β 2, TGF β 3, T β RII, and ALK5. In some embodiments, a TGF β antagonist, or combination of antagonists, that inhibits betaglycan does not inhibit or does not substantially inhibit TGF β 2.

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

In part, the disclosure provides TβRII polypeptide fusion proteins and the use of such fusion proteins as selective antagonists for TGFβ1 or TGFβ3. As described herein, polypeptides comprising part or all of the TβRII extracellular domain (ECD), with or without additional mutations, bind to and/or inhibit TGFβ1 or TGFβ3 with varying affinities. In particular, TβRII polypeptides comprising a heterologous portion (*e.g.*, an Fc immunoglobulin domain) and a linker of at least 10 amino acids in length (*e.g.*, a linker having the amino acid sequence of SEQ ID NO: 6) are associated with surprisingly superior TGFβ1 and TGFβ3 binding properties as compared to TβRII polypeptides having a shorter linker. Thus, in certain aspects, the disclosure provides TβRII polypeptides for use in selectively inhibiting TGFβ superfamily associated disorders.

In some embodiments, the disclosure provides for a Transforming Growth Factor-β Receptor II (TβRII) fusion polypeptide comprising: a) an extracellular domain of a TβRII portion; b) a heterologous portion, and c) a linker portion; wherein the linker is at least 10 amino acids in length; and wherein the TβRII extracellular domain portion comprises an amino acid sequence at least 80% identical to: i) a sequence beginning at any of positions 23 to 35 of SEQ ID NO: 1 and ending at any of positions 153 to 159 of SEQ ID NO: 1 or ii) a sequence beginning at any of positions 23 to 60 of SEQ ID NO: 2 and ending at any of positions 178 to 184 of SEQ ID NO: 2. In some embodiments, the TβRII extracellular domain portion comprises an amino acid sequence at least 80% identical to a sequence beginning at any of positions 23 to 35 of SEQ ID NO: 1 and ending at any of positions 153 to 159 of SEQ ID NO: 1. In some embodiments, the TβRII extracellular domain portion comprises an amino acid sequence at least 90% identical to a sequence beginning at any of positions 23 to 35 of SEQ ID NO: 1 and ending at any of positions 153 to 159 of SEQ ID NO: 1. In some embodiments, the TβRII extracellular domain portion comprises an amino acid sequence at least 95% identical to a sequence beginning at any of positions 23 to 35 of SEQ ID NO: 1 and ending at any of positions 153 to 159 of SEQ ID NO: 1. In some embodiments, the TβRII extracellular domain portion comprises an amino acid sequence at least 97% identical to a sequence beginning at any of positions 23 to 35 of SEQ ID NO: 1 and ending at any of positions 153 to 159 of SEQ ID NO: 1. In some embodiments, the TβRII extracellular domain portion comprises an amino acid sequence beginning at any of positions 23 to 35 of SEQ ID NO: 1 and ending at any of positions 153 to 159 of SEQ ID NO: 1. In some embodiments, the TβRII extracellular domain portion comprises an amino acid sequence at least 80% identical to a sequence beginning at any of positions 23 to 60 of SEQ ID NO: 2 and ending at any of positions 178 to 184 of SEQ ID NO: 2. In some

embodiments, the T β RII extracellular domain portion comprises an amino acid sequence at least 90% identical to a sequence beginning at any of positions 23 to 60 of SEQ ID NO: 2 and ending at any of positions 178 to 184 of SEQ ID NO: 2. In some embodiments, the T β RII extracellular domain portion comprises an amino acid sequence at least 95% identical to a sequence beginning at any of positions 23 to 60 of SEQ ID NO: 2 and ending at any of positions 178 to 184 of SEQ ID NO: 2. In some embodiments, the T β RII extracellular domain portion comprises an amino acid sequence at least 97% identical to a sequence beginning at any of positions 23 to 60 of SEQ ID NO: 2 and ending at any of positions 178 to 184 of SEQ ID NO: 2. In some embodiments, the T β RII extracellular domain portion comprises an amino acid sequence beginning at any of positions 23 to 60 of SEQ ID NO: 2 and ending at any of positions 178 to 184 of SEQ ID NO: 2. In some embodiments, the T β RII extracellular domain portion comprises an amino acid sequence at least 80% identical to SEQ ID NO: 18. In some embodiments, the T β RII extracellular domain portion comprises an amino acid sequence at least 90% identical to SEQ ID NO: 18. In some embodiments, the T β RII extracellular domain portion comprises an amino acid sequence at least 95% identical to SEQ ID NO: 18. In some embodiments, the T β RII extracellular domain portion comprises an amino acid sequence at least 97% identical to SEQ ID NO: 18. In some embodiments, the T β RII extracellular domain portion comprises the amino acid sequence of SEQ ID NO: 18. In some embodiments, the T β RII extracellular domain portion consists of an amino acid sequence at least 80% identical to a sequence beginning at any of positions 23 to 35 of SEQ ID NO: 1 and ending at any of positions 153 to 159 of SEQ ID NO: 1. In some embodiments, the T β RII extracellular domain portion consists of an amino acid sequence at least 90% identical to a sequence beginning at any of positions 23 to 35 of SEQ ID NO: 1 and ending at any of positions 153 to 159 of SEQ ID NO: 1. In some embodiments, the T β RII extracellular domain portion consists of an amino acid sequence at least 95% identical to a sequence beginning at any of positions 23 to 35 of SEQ ID NO: 1 and ending at any of positions 153 to 159 of SEQ ID NO: 1. In some embodiments, the T β RII extracellular domain portion consists of an amino acid sequence at least 97% identical to a sequence beginning at any of positions 23 to 35 of SEQ ID NO: 1 and ending at any of positions 153 to 159 of SEQ ID NO: 1. In some embodiments, the T β RII extracellular domain portion consists of an amino acid sequence beginning at any of positions 23 to 35 of SEQ ID NO: 1 and ending at any of positions 153 to 159 of SEQ ID NO: 1. In some embodiments, the T β RII extracellular domain portion consists of an amino acid sequence at least 80% identical to a sequence beginning at any of positions 23 to 60 of SEQ ID NO: 2 and ending at any of

positions 178 to 184 of SEQ ID NO: 2. In some embodiments, the T β RII extracellular domain portion consists of an amino acid sequence at least 90% identical to a sequence beginning at any of positions 23 to 60 of SEQ ID NO: 2 and ending at any of positions 178 to 184 of SEQ ID NO: 2. In some embodiments, the T β RII extracellular domain portion

5 consists of an amino acid sequence at least 95% identical to a sequence beginning at any of positions 23 to 60 of SEQ ID NO: 2 and ending at any of positions 178 to 184 of SEQ ID NO: 2. In some embodiments, the T β RII extracellular domain portion consists of an amino acid sequence at least 97% identical to a sequence beginning at any of positions 23 to 60 of SEQ ID NO: 2 and ending at any of positions 178 to 184 of SEQ ID NO: 2. In some

10 embodiments, the T β RII extracellular domain portion consists of an amino acid sequence beginning at any of positions 23 to 60 of SEQ ID NO: 2 and ending at any of positions 178 to 184 of SEQ ID NO: 2. In some embodiments, the T β RII extracellular domain portion consists of an amino acid sequence at least 80% identical to SEQ ID NO: 18. In some embodiments, the T β RII extracellular domain portion consists of an amino acid sequence at

15 least 90% identical to SEQ ID NO: 18. In some embodiments, the T β RII extracellular domain portion consists of an amino acid sequence at least 95% identical to SEQ ID NO: 18. In some embodiments, the T β RII extracellular domain portion consists of an amino acid sequence at least 97% identical to SEQ ID NO: 18. In some embodiments, the T β RII extracellular domain portion consists of the amino acid sequence of SEQ ID NO: 18. In

20 some embodiments, the polypeptide comprises an N-terminal leader sequence. In some embodiments, the N-terminal leader sequence comprises the amino acid sequence of any one of SEQ ID NOs: 22-24. In some embodiments, the N-terminal leader sequence comprises the amino acid sequence of SEQ ID NO: 23. In some embodiments, the heterologous portion is an immunoglobulin Fc domain. In some embodiments, the immunoglobulin Fc domain is a

25 human immunoglobulin Fc domain. In some embodiments, the heterologous portion comprises an amino acid sequence that is at least 80% identical to SEQ ID NO: 49. In some embodiments, the heterologous portion comprises an amino acid sequence that is at least 90% identical to SEQ ID NO: 49. In some embodiments, the heterologous portion comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 49. In some embodiments,

30 the heterologous portion comprises an amino acid sequence that is at least 97% identical to SEQ ID NO: 49. In some embodiments, the heterologous portion comprises the amino acid sequence of SEQ ID NO: 49. In some embodiments, the linker is less than 25 amino acids in length. In some embodiments, the linker is between 10 and 25 amino acids in length. In some embodiments, the linker is between 15 and 25 amino acids in length. In some

embodiments, the linker is between 17 and 22 amino acids in length. In some embodiments, the linker is 21 amino acids in length. In some embodiments, the linker comprises (GGGGS)_n, wherein $n \geq 2$. In some embodiments, the linker comprises (GGGGS)_n, wherein $n \geq 3$. In some embodiments, the linker comprises (GGGGS)_n, wherein $n \geq 4$. In some

5 embodiments, the linker comprises (GGGGS)_n, wherein $n \geq 5$. In some embodiments, the linker comprises the amino acid sequence of SEQ ID NO: 21. In some embodiments, the linker comprises the amino acid sequence of any one of SEQ ID NOs: 4-7. In some

embodiments, the linker comprises the amino acid sequence of SEQ ID NO: 6. In some

embodiments, the polypeptide comprises an amino acid sequence that is at least 80%

10 identical to the amino acid sequence of SEQ ID NO: 11. In some embodiments, the polypeptide comprises an amino acid sequence that is at least 90% identical to the amino acid sequence of SEQ ID NO: 11. In some embodiments, the polypeptide comprises an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 11. In some embodiments, the polypeptide comprises the amino acid sequence of SEQ ID NO: 11.

15 In some embodiments, the polypeptide comprises an amino acid sequence that is at least 80% identical to the amino acid sequence of SEQ ID NO: 13. In some embodiments, the polypeptide comprises an amino acid sequence that is at least 90% identical to the amino acid sequence of SEQ ID NO: 13. In some embodiments, the polypeptide comprises an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 13. In

20 some embodiments, the polypeptide comprises the amino acid sequence of SEQ ID NO: 13. In some embodiments, the polypeptide comprises an amino acid sequence that is at least 80% identical to the amino acid sequence of SEQ ID NO: 63. In some embodiments, the polypeptide comprises an amino acid sequence that is at least 90% identical to the amino acid sequence of SEQ ID NO: 63. In some embodiments, the polypeptide comprises an amino

25 acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 63. In some embodiments, the polypeptide comprises the amino acid sequence of SEQ ID NO: 63. In some embodiments, the polypeptide comprises an amino acid sequence that is at least 80% identical to the amino acid sequence of SEQ ID NO: 48. In some embodiments, the polypeptide comprises an amino acid sequence that is at least 90% identical to the amino acid

30 sequence of SEQ ID NO: 48. In some embodiments, the polypeptide comprises an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 48. In some embodiments, the polypeptide comprises the amino acid sequence of SEQ ID NO: 48. In some embodiments, the polypeptide comprises an amino acid sequence that is at least 80% identical to the amino acid sequence of SEQ ID NO: 65. In some embodiments, the

polypeptide comprises an amino acid sequence that is at least 90% identical to the amino acid sequence of SEQ ID NO: 65. In some embodiments, the polypeptide comprises an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 65. In some embodiments, the polypeptide comprises the amino acid sequence of SEQ ID NO: 65.

- 5 In some embodiments, the polypeptide comprises an amino acid sequence that is at least 80% identical to the amino acid sequence of SEQ ID NO: 68. In some embodiments, the polypeptide comprises an amino acid sequence that is at least 90% identical to the amino acid sequence of SEQ ID NO: 68. In some embodiments, the polypeptide comprises an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 68. In some embodiments, the polypeptide comprises the amino acid sequence of SEQ ID NO: 68.
- 10 In some embodiments, the polypeptide comprises an amino acid sequence that is at least 80% identical to the amino acid sequence of SEQ ID NO: 15. In some embodiments, the polypeptide comprises an amino acid sequence that is at least 90% identical to the amino acid sequence of SEQ ID NO: 15. In some embodiments, the polypeptide comprises an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 15. In some embodiments, the polypeptide comprises the amino acid sequence of SEQ ID NO: 15.
- 15 In some embodiments, the T β RII polypeptide does not include amino acids 185-592 of SEQ ID NO: 2. In some embodiments, the T β RII polypeptide does not include amino acids 1-22 of SEQ ID NO: 2. In some embodiments, the polypeptide consists of or consists essentially of: a) a T β RII polypeptide portion comprising an amino acid sequence that is at least 85%, 90%, 95%, 97%, or 99% identical to the amino acid sequence of SEQ ID NO: 18 and no more than 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 additional amino acids; b) a linker portion comprising an amino acid sequence that is at least 85%, 90%, 95%, 97%, or 99% identical to the amino acid sequence of SEQ ID NO: 6 and no more than 5, 4, 3, 2 or 1 additional amino acids; c) a
- 25 heterologous portion comprising an amino acid sequence that is at least 85%, 90%, 95%, 97%, or 99% identical to the amino acid sequence of SEQ ID NO: 49 and no more than 25, 20, 15, 10, 5, 4, 3, 2, or 1 additional amino acids; and d) optionally a leader sequence (*e.g.*, SEQ ID NO: 23). In some embodiments, the polypeptide consists of or consists essentially of: a) a T β RII polypeptide portion comprising the amino acid sequence of SEQ ID NO: 18 and no more than 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 additional amino acids; b) a linker portion comprising the amino acid sequence of SEQ ID NO: 6 and no more than 5, 4, 3, 2 or 1 additional amino acids; c) a heterologous portion comprising the amino acid sequence of SEQ ID NO: 49 and no more than 25, 20, 15, 10, 5, 4, 3, 2, or 1 additional amino acids; and d) optionally a leader sequence (*e.g.*, SEQ ID NO: 23). In some embodiments, the
- 30

polypeptide comprises: a) an extracellular domain of a T β RII portion; wherein the extracellular domain comprises an amino acid sequence that is at least 85%, 90%, 95%, 97%, or 99% identical to the sequence of SEQ ID NO: 18; b) a heterologous portion, wherein the heterologous portion comprises an amino acid sequence that is at least 85%, 90%, 95%, 97%, or 99% identical to the sequence of SEQ ID NO: 49; and c) a linker portion connecting the extracellular domain and the heterologous portion; wherein the linker comprises an amino acid sequence that is at least 85%, 90%, 95%, 97%, or 99% identical to the amino acid sequence of SEQ ID NO: 6. In some embodiments, the polypeptide comprises: a) an extracellular domain of a T β RII portion; wherein the extracellular domain comprises the amino acid sequence of SEQ ID NO: 18; b) a heterologous portion, wherein the heterologous portion comprises the amino acid sequence of SEQ ID NO: 49; and c) a linker portion connecting the extracellular domain and the heterologous portion; wherein the linker comprises the amino acid sequence of SEQ ID NO: 6. In some embodiments, the polypeptide comprises an amino acid sequence that is at least 85%, 90%, 95%, 97%, or 99% identical to the amino acid sequence of SEQ ID NO: 48. In some embodiments, the polypeptide comprises the amino acid sequence of SEQ ID NO: 48. In some embodiments, the polypeptide does not include a leader sequence, or wherein the leader sequence has been removed. 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. In some embodiments, the polypeptide has a glycosylation pattern characteristic of expression of the polypeptide in CHO cells. In some embodiments, the polypeptide binds human TGF β 1 with an equilibrium dissociation constant (KD) less than 100 pM. In some embodiments, the polypeptide binds human TGF β 1 with an equilibrium dissociation constant (KD) less than 75 pM. In some embodiments, the polypeptide binds human TGF β 3 with an equilibrium dissociation constant (KD) less than 60 pM. In some embodiments, the polypeptide binds human TGF β 3 with an equilibrium dissociation constant (KD) less than 50 pM. In some embodiments, the polypeptide inhibits TGF β 1 with an IC₅₀ of less than 1.0 nM, as determined using a reporter gene assay. In some embodiments, the polypeptide inhibits TGF β 1 with an IC₅₀ of less than 0.25 nM, as determined using a reporter gene assay. In some embodiments, the polypeptide inhibits TGF β 1 with an IC₅₀ of less than 0.1 nM, as determined using a reporter gene assay. In some embodiments, the polypeptide inhibits TGF β 1 with an IC₅₀ of less than 0.05 nM, as

determined using a reporter gene assay. In some embodiments, the polypeptide inhibits TGFβ3 with an IC50 of less than 0.3 nM, as determined using a reporter gene assay. In some embodiments, the polypeptide inhibits TGFβ3 with an IC50 of less than 0.1 nM, as determined using a reporter gene assay. In some embodiments, the polypeptide inhibits TGFβ3 with an IC50 of less than 0.05 nM, as determined using a reporter gene assay. In some embodiments, the polypeptide inhibits TGFβ3 with an IC50 of less than 0.04 nM, as determined using a reporter gene assay. In some embodiments, the reporter gene assay is a CAGA reporter assay.

In some embodiments, the disclosure provides for a homodimer comprising any two of the polypeptides disclosed herein.

In some embodiments, the disclosure provides for an isolated polynucleotide comprising a coding sequence for any of the polypeptides disclosed herein. In some embodiments, the disclosure provides for a recombinant polynucleotide comprising a promoter sequence operably linked to any of the polynucleotides disclosed herein. In some embodiments, the polynucleotide comprises a nucleotide sequence that is at least 80%, 85%, 90%, 95%, 97% or 100% identical to any one of SEQ ID NOs: 10, 12, 14, 46 or 47.

In some embodiments, the disclosure provides for a cell transformed with any of the polynucleotides disclosed herein. In some embodiments, cell is a mammalian cell. In some embodiments, the cell is a CHO cell or a human cell.

In some embodiments, the disclosure provides for a pharmaceutical preparation comprising pharmaceutically acceptable excipient and any of the polypeptides disclosed herein or any of the homodimers disclosed herein.

In certain aspects, a TGFβ antagonist is an antibody, or combination of antibodies. In certain aspects, the antibody binds to at least TβRII. In some embodiments a TGFβ antagonist antibody that binds to TβRII inhibits TβRII signaling, optionally as measured in a cell-based assay such as those described herein. In some embodiments, a TGFβ antagonist antibody that binds to TβRII inhibits one or more TGF-beta superfamily ligands, TGFβ superfamily type I receptors, or TGFβ superfamily co-receptors from binding to TβRII. In some embodiments, a TGFβ antagonist antibody that binds to TβRII inhibits one or more TGF-beta superfamily ligands from binding to TβRII selected from the group consisting of: TGFβ1, TGFβ2, and TGFβ3. In certain aspects, the antibody binds to at least ALK5. In some embodiments a TGFβ antagonist antibody that binds to ALK5 inhibits ALK5 signaling, optionally as measured in a cell-based assay such as those described herein. In some

embodiments, a TGF β antagonist antibody that binds to ALK5 inhibits one or more TGF-beta superfamily ligands, TGF β superfamily type II receptors, or TGF β superfamily co-receptors from binding to ALK5. In some embodiments a TGF β antagonist antibody that binds to ALK5 inhibits one or more TGF-beta superfamily ligands from binding to ALK5 selected
5 from the group consisting of: TGF β 1, TGF β 2, and TGF β 3. In certain aspects, the antibody binds to at least betaglycan. In some embodiments a TGF β antagonist antibody that binds to betaglycan inhibits betaglycan signaling, optionally as measured in a cell-based assay such as those described herein. In some embodiments, a TGF β antagonist antibody that binds to betaglycan inhibits one or more TGF-beta superfamily ligands, TGF β superfamily type I
10 receptors, or TGF β superfamily type II receptors from binding to betaglycan. In some embodiments a TGF β antagonist antibody that binds to betaglycan inhibits one or more TGF-beta superfamily ligands from binding to betaglycan selected from the group consisting of: TGF β 1, TGF β 2, and TGF β 3. In certain aspects, a TGF β antagonist antibody binds to at least TGF β 1. In some embodiments, a TGF β antagonist antibody that binds to TGF β 1 inhibits
15 T β RII signaling, optionally as measured in a cell-based assay such as those described herein. In some embodiments, a TGF β antagonist antibody that binds to TGF β 1 inhibits TGF β 1-T β RII, TGF β 1-ALK5, and/or TGF β 1-betaglycan binding. In certain aspects, a TGF β antagonist antibody binds to at least TGF β 2. In some embodiments, a TGF β antagonist antibody that binds to TGF β 2 inhibits T β RII signaling, optionally as measured in a cell-based
20 assay such as those described herein. In some embodiments, a TGF β antagonist antibody that binds to TGF β 2 inhibits TGF β 2-T β RII, TGF β 1-ALK5, and/or TGF β 1-betaglycan binding. In certain embodiments, a TGF β antagonist antibody binds to at least TGF β 3. In some embodiments, a TGF β antagonist antibody that binds to TGF β 3 inhibits T β RII signaling, optionally as measured in a cell-based assay such as those described herein. In some
25 embodiments, a TGF β antagonist antibody that binds to TGF β 3 inhibits TGF β 3-T β RII, TGF β 1-ALK5, and/or TGF β 1-betaglycan binding. In some embodiments, a TGF β antagonist antibody is a multispecific antibody, or a combination of multispecific antibodies, inhibits signaling in a cell-based assay of one or more of: TGF β 1, TGF β 2, TGF β 3, T β RII, ALK5, and betaglycan. In some embodiments, antibody is a chimeric antibody, a humanized antibody,
30 or a human antibody. In some embodiments, the antibody is a single-chain antibody, an F(ab')₂ fragment, a single-chain diabody, a tandem single-chain Fv fragment, a tandem single-chain diabody, or a fusion protein comprising a single-chain diabody and at least a portion of an immunoglobulin heavy-chain constant region.

In certain aspects, a TGF β antagonist is a small molecule inhibitor or combination of small molecule inhibitors. In some embodiments, a TGF β antagonist small molecule inhibitor is an inhibitor of at least T β RII. In some embodiments, a TGF β antagonist small molecule inhibitor is an inhibitor of at least ALK5. In some embodiments, a TGF β antagonist small molecule inhibitor is an inhibitor of at least betaglycan. In some
 5 embodiments, a TGF β antagonist small molecule inhibitor is an inhibitor of at least TGF β 1. In some embodiments, a TGF β antagonist small molecule inhibitor is an inhibitor of at least TGF β 2. In some embodiments, a TGF β antagonist small molecule inhibitor is an inhibitor of at least TGF β 3.

10 In certain aspects, a TGF β antagonist is a nucleic acid inhibitor or combination of nucleic acid inhibitors. In some embodiments, a TGF β antagonist nucleic acid inhibitor is an inhibitor of at least T β RII. In some embodiments, a TGF β antagonist nucleic acid inhibitor is an inhibitor of at least ALK5. In some embodiments, a TGF β antagonist nucleic acid inhibitor is an inhibitor of at least betaglycan. In some embodiments, a TGF β antagonist
 15 nucleic acid inhibitor is an inhibitor of at least TGF β 1. In some embodiments, a TGF β antagonist nucleic acid inhibitor is an inhibitor of at least TGF β 2. In some embodiments, a TGF β antagonist nucleic acid inhibitor is an inhibitor of at least TGF β 3.

BRIEF DESCRIPTION OF THE DRAWINGS

20 **Figure 1** shows the amino acid sequence of native precursor for the B (short) isoform of human TGF β receptor type II (hT β 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).

25 **Figure 2** shows the amino acid sequence of native precursor for the A (long) isoform of human T β 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).

Figure 3 shows a comparison of the linker sequences of five different T β RII constructs.

30 **Figures 4A and 4B** show in tabular form the binding affinity between TGF β 1 and TGF β 3 and one of several different T β RII-Fc fusion protein constructs.

Figures 5A and 5C graph the results from reporter gene assays testing the affinity of TGFβ1 for one of several different TβRII-Fc fusion protein constructs. **Figures 5B and 5D** graph the results from reporter gene assays testing the affinity of the TGFβ3 for one of several different TβRII-Fc fusion protein constructs. **Figures 5E and 5F** provide IC₅₀ data
 5 from these same experiments in tabular form.

Figures 6A, 6B, and 6C show the effects of mTβRII-mFc in an *in vitro* inflammation model as well as an animal model of heterotopic ossification. **Figure 6A** shows that mTβRII-mFc can abrogate TGFβ1 secretion by regenerative M2 macrophages in an inflammatory environment *in vitro*. **Figure 6B** shows that treatment with mTβRII-mFc
 10 decreases osseous deposition and marrow space formation in an animal model of traumatic heterotopic ossification. **Figure 6C** shows that treatment with mTβRII-mFc significantly decreases the volume of mature HO in an animal model of traumatic heterotopic ossification.

Figure 7 shows multiple sequence alignment of Fc domains from human IgG isotypes using Clustal 2.1. Hinge regions are indicated by dotted underline. Double underline
 15 indicates examples of positions engineered in IgG1 Fc to promote asymmetric chain pairing and the corresponding positions with respect to other isotypes IgG2, IgG3 and IgG4.

Detailed Description of the Invention

1. Overview

20 Proteins described herein are the human forms, unless otherwise specified. NCBI references for the proteins are as follows: human TβRII isoform A (hTβRII_{long}), NP_001020018.1 and human TβRII isoform B (hTβRII_{short}), (NP_003233.4). Sequences of native TβRII proteins from human are set forth in Figures 1 and 2. In some embodiments, the TβRII proteins are from non-human animals, such as a mouse, rat, cow or monkey.

25 The TGFβ 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
 30 adipogenesis, myogenesis, chondrogenesis, cardiogenesis, hematopoiesis, neurogenesis, and epithelial cell differentiation. By manipulating the activity of a member of the TGFβ 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.,
5 N Engl J Med 2004, 350:2682-8.

TGF β signals are mediated by heteromeric complexes of type I (e.g. T β RI) and type II (e.g. T β 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-
10 binding extracellular domain with cysteine-rich region, a transmembrane domain, 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 β has three
15 mammalian isoforms, TGF β 1, TGF β 2 and TGF β 3, each with distinct functions in vivo. The binding of TGF β s to T β RII is a crucial step in initiating activation of the TGF β signaling pathway, leading to phosphorylation of SMAD2, and translocation of the activated SMAD2/SMAD4 complex to the nucleus to modulate gene expression.

Thus, in certain aspects, the disclosure provides T β RII polypeptides as antagonists of
20 TGF β 1 or TGF β 3 for use in treating various TGF β 1- or TGF β 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 TGF β 1 or TGF β 3 and inhibiting the ability of these ligands to form ternary signaling complexes.

The disclosure provides for fusion proteins comprising T β RII polypeptides and a
25 heterologous portion (e.g., an Fc portion). In particular embodiments, the T β RII portion and the heterologous portion are fused by means of a linker. As described in greater detail below, the disclosure demonstrates that T β RII-Fc fusion proteins comprising linkers of certain lengths (e.g., a linker having 21 amino acids) were surprisingly able to bind TGF β -1 and TGF β -3 with stronger affinity than T β RII-Fc fusion proteins having a linker of only four
30 amino acids.

As demonstrated herein, a soluble T β RII polypeptide, which binds to TGF β 1 and TGF β 3, is effective at substantially attenuating development of heterotopic ossification (HO) anlagen with decreased osseous deposition and marrow space formation in a mouse model of HO. Furthermore, treatment with the T β RII polypeptide significantly reduced the volume of

mature HO in this animal model. While not wishing to be bound to any particular mechanism, it is expected that the effects of T β RII polypeptides are caused primarily by TGF β signaling antagonist effect, particularly of TGF β 1 and/or TGF β 3. Regardless of the mechanism, it is apparent from the data presented herein the TGF β signaling antagonists do
5 reduce the severity of osseous deposition in HO. It should be noted that heterotopic ossification is dynamic, which changes depending on a balance of factors that increase HO and factors that decrease HO. HO can be decreased by increasing factors that reduce HO; decreasing factors that increase HO; or both. Therefore, the term decreasing (reducing) HO refers to the observable physical changes in osseous deposition and are intended to be neutral
10 as to the mechanisms by which the changes occur.

The animal model for HO that was used in the studies described herein are considered to be predicative of efficacy in humans, and therefore, this disclosure provides methods for using TGF β signaling antagonist to treat HO particularly preventing or reducing the severity or duration of one or more complications of HO, in humans. As disclosed herein, the term
15 TGF β antagonist refers a variety of agents that may be used to antagonize TGF β signaling including, for example, antagonists that inhibit one or more TGF β ligands [e.g., TGF β 1, TGF β 2, and/or TGF β 3]; antagonists that inhibit one or more TGF β -interacting type I-, type II-, or co-receptor (e.g., T β RII, ALK5 and betaglycan); and antagonists that inhibit one or more downstream signaling components (e.g., Smad proteins such as Smads 2 and 3). TGF β
20 antagonists to be used in accordance with the methods and uses of the disclosure include a variety of forms, for example, ligand traps (e.g., soluble T β RII polypeptides and betaglycan polypeptides), antibody antagonists (e.g., antibodies that inhibit one or more of TGF β 1, TGF β 2, TGF β 3, T β RII, and betaglycan), small molecule antagonists [e.g., small molecules that inhibit one or more of TGF β 1, TGF β 2, TGF β 3, T β RII, betaglycan, and one or more
25 Smad proteins (e.g., Smads 2 and 3)], and nucleotide antagonists [e.g., nucleotide sequences that inhibit one or more of TGF β 1, TGF β 2, TGF β 3, T β RII, betaglycan, and one or more Smad proteins (e.g., Smads 2 and 3)].

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.

30 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.

“Homologous,” in all its grammatical forms and spelling variations, refers to the relationship between two proteins that possess a “common evolutionary origin,” including proteins from superfamilies in the same species of organism, as well as homologous proteins from different species of organism. Such proteins (and their encoding nucleic acids) have sequence homology, as reflected by their sequence similarity, whether in terms of percent identity or by the presence of specific residues or motifs and conserved positions.

The term “sequence similarity,” in all its grammatical forms, refers to the degree of identity or correspondence between nucleic acid or amino acid sequences that may or may not share a common evolutionary origin. However, in common usage and in the instant application, the term “homologous,” when modified with an adverb such as “highly,” may refer to sequence similarity and may or may not relate to a common evolutionary origin.

“Percent (%) sequence identity” or “percent (%) identical” with respect to a reference polypeptide (or nucleotide) sequence is defined as the percentage of amino acid residues (or nucleic acids) in a candidate sequence that are identical to the amino acid residues (or nucleic acids) in the reference polypeptide (nucleotide) sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For purposes herein, however, % amino acid (nucleic acid) sequence identity values are generated using the sequence comparison computer program ALIGN-2. The ALIGN-2 sequence comparison computer program was authored by Genentech, Inc., and the source code has been filed with user documentation in the U.S. Copyright Office, Washington D.C., 20559, where it is registered under U.S. Copyright Registration No. TXU510087. The ALIGN-2 program is publicly available from Genentech, Inc., South San Francisco, Calif., or may be compiled from the source code. The ALIGN-2 program should be compiled for use on a UNIX operating system, including digital UNIX V4.0D. All sequence comparison parameters are set by the ALIGN-2 program and do not vary.

“Agonize”, in all its grammatical forms, refers to the process of activating a protein and/or gene (*e.g.*, by activating or amplifying that protein’s gene expression or by inducing an inactive protein to enter an active state) or increasing a protein’s and/or gene’s activity.

“Antagonize”, in all its grammatical forms, refers to the process of inhibiting a protein and/or gene (e.g., by inhibiting or decreasing that protein’s gene expression or by inducing an active protein to enter an inactive state) or decreasing a protein’s and/or gene’s activity.

The terms "about" and "approximately" as used in connection with a numerical value
5 throughout the specification and the claims denotes an interval of accuracy, familiar and acceptable to a person skilled in the art.

Numeric ranges disclosed herein are inclusive of the numbers defining the ranges.

The terms "a" and "an" include plural referents unless the context in which the term is used clearly dictates otherwise. The terms "a" (or "an"), as well as the terms "one or more,"
10 and "at least one" can be used interchangeably herein. Furthermore, "and/or" where used herein is to be taken as specific disclosure of each of the two or more specified features or components with or without the other. Thus, the term “and/or” as used in a phrase such as "A and/or B" herein is intended to include "A and B," "A or B," "A" (alone), and "B" (alone). Likewise, the term "and/or" as used in a phrase such as "A, B, and/or C" is intended to
15 encompass each of the following aspects: A, B, and C; A, B, or C; A or C; A or B; B or C; A and C; A and B; B and C; A (alone); B (alone); and C (alone).

Throughout this specification, the word “comprise” or variations such as “comprises” or “comprising” will be understood to imply the inclusion of a stated integer or groups of integers but not the exclusion of any other integer or group of integers. As used herein, the
20 term “comprises” also encompasses the use of the narrower terms “consisting” and “consisting essentially of.”

The term “consisting essentially of” is limited to the specified materials or steps and those that do not materially affect the basic and novel characteristics of the invention(s) disclosed herein.

25 The term “appreciable affinity” as used herein means binding with a dissociation constant (K_D) of less than 50 nM.

The terms "polypeptide", "oligopeptide", "peptide" and "protein" are used interchangeably herein to refer to chains of amino acids of any length. The chain may be linear or branched, it may comprise modified amino acids, and/or may be interrupted by non-
30 amino acids. The terms also encompass an amino acid chain that has been modified naturally or by intervention; for example, disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, or any other manipulation or modification, such as conjugation with a labeling component. Also included within the definition are, for example, polypeptides containing one or more analogs of an amino acid (including, for example,

unnatural amino acids, etc.), as well as other modifications known in the art. It is understood that the polypeptides can occur as single chains or associated chains.

2. TGF β Antagonists

5 As disclosed herein, the term TGF β antagonist refers a variety of agents that may be used to antagonize TGF β -mediated signaling including, for example, antagonists that inhibit one or more TGF β ligands [e.g., TGF β 1, TGF β 2, and/or TGF β 3]; antagonists that inhibit one or more TGF β -interacting type I-, type II-, or co-receptor (e.g., T β RII, ALK5 and betaglycan); and antagonists that inhibit one or more downstream signaling components (e.g.,
10 Smad proteins such as Smads 2 and 3). TGF β antagonists to be used in accordance with the methods and uses of the disclosure include a variety of forms, for example, ligand traps (e.g., soluble T β RII polypeptides and betaglycan polypeptides), antibody antagonists (e.g., antibodies that inhibit one or more of TGF β 1, TGF β 2, TGF β 3, T β RII, and betaglycan), small molecule antagonists [e.g., small molecules that inhibit one or more of TGF β 1, TGF β 2,
15 TGF β 3, T β RII, betaglycan, and one or more Smad proteins (e.g., Smads 2 and 3)], and nucleotide antagonists [e.g., nucleotide sequences that inhibit one or more of TGF β 1, TGF β 2, TGF β 3, T β RII, betaglycan, and one or more Smad proteins (e.g., Smads 2 and 3)].

A. T β RII and Betaglycan Polypeptides

20 Naturally occurring T β 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 β RII polypeptides comprising mutations within the extracellular domain and/or truncated portions of the extracellular domain of T β RII. As described above, human
25 T β RII occurs naturally in at least two isoforms – A (long) and B (short) – generated by alternative splicing in the extracellular domain (ECD) (Figures 1 and 2 and SEQ ID NOS: 1 and 2). SEQ ID NO: 27, which corresponds to residues 23-159 of SEQ ID NO: 1, depicts the native full-length extracellular domain of the short isoform of T β RII. SEQ ID NO: 18, which corresponds to residues 23-184 of SEQ ID NO: 2, depicts the native full-length extracellular
30 domain of the long isoform of T β RII. Unless noted otherwise, amino acid position numbering with regard to variants based on the T β RII short and long isoforms refers to the

corresponding position in the native precursors, SEQ ID NO: 1 and SEQ ID NO: 2, respectively.

In certain embodiments, the disclosure provides variant T β RII polypeptides. A T β RII polypeptide of the disclosure may bind to and inhibit the function of a TGF β superfamily member, such as but not limited to, TGF β 1 or TGF β 3. T β RII polypeptides may include a polypeptide consisting of, or comprising, an amino acid sequence at least 70% identical, and optionally at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a truncated ECD domain of a naturally occurring T β RII polypeptide, whose C-terminus occurs at any of amino acids 153-159 of SEQ ID NO: 1.

T β RII polypeptides may include a polypeptide consisting of, or comprising, an amino acid sequence at least 70% identical, and optionally at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a truncated ECD domain of a naturally occurring T β RII polypeptide, whose C-terminus occurs at any of amino acids 178-184 of SEQ ID NO: 2. In particular embodiments, the T β RII polypeptides comprise an amino acid sequence at least 70% identical, and optionally at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 18. Optionally, a T β RII polypeptide does not include more than 5 consecutive amino acids, or more than 10, 20, 30, 40, 50, 52, 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: 1 or from a sequence consisting of amino acids 185-592 of SEQ ID NO: 2. In some embodiments, the T β RII polypeptide does not include amino acids 160-567 of SEQ ID NO: 1. In some embodiments, the T β RII polypeptide does not include amino acids 1-22 of SEQ ID NO: 1. In some embodiments, the T β RII polypeptide does not include amino acids 1-22 and 160-567 of SEQ ID NO: 1. In some embodiments, the T β RII polypeptide does not include amino acids 185-592 of SEQ ID NO: 2. In some embodiments, the T β RII polypeptide does not include amino acids 1-22 of SEQ ID NO: 2. In some embodiments, the T β RII polypeptide does not include amino acids 1-22 and 185-592 of SEQ ID NO: 2. The unprocessed T β 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 β RII polypeptide may occur at any of amino acids 23-35 of SEQ ID NO: 1 or 23-60 of SEQ ID NO: 2. Examples of mature T β RII polypeptides include, but are not limited to, amino acids 23-159 of SEQ ID NO: 1 (set forth in SEQ ID NO: 27), amino acids 29-159 of SEQ ID NO: 1 (set forth in SEQ ID NO: 28), amino acids 35-159 of SEQ ID NO: 1 (set forth in SEQ ID NO: 29), amino acids 23-153 of SEQ ID NO: 1 (set forth in SEQ

ID NO: 30), amino acids 29-153 of SEQ ID NO: 1 (set forth in SEQ ID NO: 31), amino acids 35-153 of SEQ ID NO: 1 (set forth in SEQ ID NO: 32), amino acids 23-184 of SEQ ID NO: 2 (set forth in SEQ ID NO: 18), amino acids 29-184 of SEQ ID NO: 2 (set forth in SEQ ID NO: 33), amino acids 60-184 of SEQ ID NO: 2 (set forth in SEQ ID NO: 29), amino acids 23-178 of SEQ ID NO: 2 (set forth in SEQ ID NO: 34), amino acids 29-178 of SEQ ID NO: 2 (set forth in SEQ ID NO: 35), and amino acids 60-178 of SEQ ID NO: 2 (set forth in SEQ ID NO: 32). It will be understood by one of skill in the art that corresponding variants based on the long isoform of T β 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. The T β RII polypeptides accordingly may include isolated extracellular portions of T β 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: 1 or amino acids 23-184 of SEQ ID NO: 2), fragments thereof, and fusion proteins comprising any of the foregoing, but in each case preferably any of the foregoing T β RII polypeptides will retain substantial affinity for at least one of, or both of, TGF β 1 or TGF β 3. Generally, a T β RII polypeptide will be designed to be soluble in aqueous solutions at biologically relevant temperatures, pH levels, and osmolarity.

In some embodiments, the variant T β RII polypeptides of the disclosure comprise one or more mutations in the extracellular domain that confer an altered ligand binding profile. A T β 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 β 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: 1. In some embodiments, the mutation results in a substitution, insertion, or deletion at the position corresponding to position 110 of SEQ ID NO: 1. 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 110, respectively, of SEQ ID NO: 1. Examples of such variant T β RII polypeptides include, but are not limited to, the sequences set forth in SEQ ID NOs: 36-39. A T β RII polypeptide may comprise a polypeptide or portion thereof that is encoded by any one of SEQ ID NOs: 8, 10, 12, 14, 16, 46 or 47, or silent variants thereof or nucleic acids that hybridize to the complement thereof under stringent hybridization conditions. In particular embodiments, a T β RII polypeptide may comprise a polypeptide or portion thereof that is encoded by any one of SEQ ID NO: 12,

or silent variants thereof or nucleic acids that hybridize to the complement thereof under stringent hybridization conditions.

In some embodiments, the variant T β RII polypeptides of the disclosure further comprise an insertion of 36 amino acids (SEQ ID NO: 41) between the pair of glutamate residues (positions 151 and 152 of SEQ ID NO: 1, or positions 176 and 177 of SEQ ID NO: 2) located near the C-terminus of the human T β RII ECD, as occurs naturally in the human T β RII isoform C (Konrad et al., BMC Genomics 8:318, 2007).

It has been demonstrated that T β RII polypeptides can be modified to selectively antagonize T β RII ligands. The N70 residue represents a potential glycosylation site. In some embodiments, the T β RII polypeptides are aglycosylated. In some embodiments, the T β RII polypeptides are aglycosylated or have reduced glycosylation at position Asn157. In some embodiments, the T β RII polypeptides are aglycosylated or have reduced glycosylation at position Asn73.

In certain embodiments, a T β RII polypeptide binds to TGF β 1, and the T β RII polypeptide does not show substantial binding to TGF β 3. In certain embodiments, a T β RII polypeptide binds to TGF β 3, and the T β RII polypeptide does not show substantial binding to TGF β 1. Binding may be assessed using purified proteins in solution or in a surface plasmon resonance system, such as a BiacoreTM system.

In certain embodiments, a T β RII polypeptide inhibits TGF β 1 cellular signaling, and the T β RII polypeptide has an intermediate or limited inhibitory effect on TGF β 3 signaling. In certain embodiments, a T β RII polypeptide inhibits TGF β 3 cellular signaling, and the T β RII polypeptide has an intermediate or limited inhibitory effect on TGF β 1 signaling. Inhibitory effect on cell signaling can be assayed by methods known in the art.

Taken together, an active portion of a T β RII polypeptide may comprise amino acid sequences 23-153, 23-154, 23-155, 23-156, 23-157, or 23-158 of SEQ ID NO: 1, as well as variants of these sequences starting at any of amino acids 24-35 of SEQ ID NO: 1. Similarly, an active portion of a T β RII polypeptide may comprise amino acid sequences 23-178, 23-179, 23-180, 23-181, 23-182, or 23-183 of SEQ ID NO: 2, as well as variants of these sequences starting at any of amino acids 24-60 of SEQ ID NO: 2. Exemplary T β RII polypeptides comprise amino acid sequences 29-159, 35-159, 23-153, 29-153 and 35-153 of SEQ ID NO: 1 or amino acid sequences 29-184, 60-184, 23-178, 29-178 and 60-178 of SEQ ID NO: 2. Variants within these ranges are also contemplated, particularly those having at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the corresponding portion of SEQ ID NO: 1 or SEQ ID NO: 2. A T β RII

polypeptide may be selected that does not include the sequence consisting of amino acids 160-567 of SEQ ID NO: 1 or amino acids 185-592 of SEQ ID NO: 2. In particular embodiments, the TβRII polypeptides comprise an amino acid sequence at least 70% identical, and optionally at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 18.

The term “betaglycan polypeptide” includes polypeptides comprising any naturally occurring betaglycan protein (encoded by *TGFBR3* or one of its nonhuman orthologs) as well as any variants thereof (including mutants, fragments, fusions, and peptidomimetic forms) that retain a useful activity.

The human betaglycan isoform A precursor protein sequence (NCBI Ref Seq NP_003234.2) is as follows:

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1  MTSHYVIAIF ALMSSCLATA GPEPGALCEL SPVSASHPVQ ALMESFTVLS GCASRGTTGL
61 PQEVHVLNLR TAGQGPGLQ REVTLHLNPI SSVHIHHSKSV VFLNLSNPHPL VWHLKTERLA
121 TGVSRLFLVS EGSVVQFSSA NFSLTAETEE RNFPHGNEHL LNWARKEYGA VTSFTELKIA
15 181 RNIYIKVGED QVFPPKCNIG KNFLSLNYLA EYLQPKAAEG CVMSSQPQNE EVHIIELITP
241 NSNPYSAFQV DITIDIRPSQ EDLEVVKNL LILKCKKSVN WVKSFDVKG SLKIIAPNSI
301 GFGKESERSM TMTKSIRDDI PSTQGNLVKW ALDNGYSPIT SYTMAPVANR FHLRLNNAE
361 EMGDEEVHTI PPELRILLDP GALPALQNPP IRGGEGQNGG LPFFFPDISR RVWNEEGEDG
421 LPRPKDPVIP SIQLFPGLRE PEEVQGSVDI ALSVKCDNEK MIVAVEKDSF QASGYSGMDV
20 481 TLLDPTCKAK MNGTHFVLES PLNGCGTRPR WSALDGVVYY NSIVIQVPAL GDSSGWPDPGY
541 EDLESGDNGF PGDMDEGDAS LFTRPEIVVF NCSLQQVRNP SSFQEQPHGN ITFNMELYNT
601 DLFLVPSQGV FSVPENGHVV VEVSVTKAEQ ELGFAIQTCF ISPYSNPDRM SHYTIENIC
661 PKDES VKFYS PKRVHFP IPQ ADMDKKRFSF VFKPVFNTSL LFLQCELTLC TKMEKHPQKL
721 PKCVPPDEAC TSLDASIIWA MMQNKKTF FTK PLAVIHHEAE SKEKGPSMKE PNPISPPIFH
25 781 GLDTLTVMGI AFAAFVIGAL LTGALWYIYS HTGETAGRQQ VPTSPPASEN SSAAHSIGST
841 QSTPCSSSST A (SEQ ID NO: 50)

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The signal peptide is indicated by single underline, the extracellular domain is indicated in **bold font**, and the transmembrane domain is indicated by dotted underline. This isoform differs from betaglycan isoform B by insertion of a single alanine indicated above by double underline.

A processed betaglycan isoform A polypeptide sequence is as follows:

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GPEPGALCELSPVASHPVQALMESFTVLSGCASRGTTGLPQEVHVLNLRTAGQGPGLQREVTLHLNPISSVHI
HHKSVVFLNLSNPHPLVWHLKTERLATGVSRFLVSEGSVVQFSSANFSLTAETEERNFPHGNEHLLNWARKEYGA
VTSFTELKIARNIYIKVGEDQVFPPKCNIGKNFLSLNYLAEYLQPKAAEGCVMSSQPQNEEVHIIELITPNPNPY
35 SAFQVDITIDIRPSQEDLEVVKNLILILKCKKSVNWKSFVDVKGSLKIIAPNSIGFGKESERSMTMTKSIRDDI
PSTQGNLVKWALDNGYSPITSYTMAPVANRFHLRLNNAEEMGDEEVHTIPPELRILLDPGALPALQNPPIRGGE

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QONGGLPFPFPDISRRVWNEEGEDGLPRPKDPVIPSILQFPGLREPEEVQGSVDIALSVKCDNEKMIVAVEKDSF
 QASGYSGMDVTLDDPTCKAKMNGTHFVLESPLNGCGTRPRWSALDGVVYNSIVIQVPALGDSSGWPDPGYEDLES
 GDNGFPGDMDEGDASLFRPEIVVFNCSLQQVRNPSSFQEQPHGNITFNMELYNTDLFLVPSQGVFSVPENGHVY
 VEVSVTKAEQELGFAIQTCFISPYSNPDRMSHYTIIENICPKDES VKFYSPKRVHFPPIQADMDKKRFSFVFKPV
 5 FNTSLLFLQCELTILCTKMEKHPQKLPKCVPPDEACTSLDASIIWAMQNKKTFTKPLAVIHHEAESKEKGPSMKE
 PNPISPPIFHGLDTLTV
 (SEQ ID NO: 51)

A nucleic acid sequence encoding the unprocessed precursor protein of human
 betaglycan isoform A is shown below (SEQ ID NO: 52), corresponding to nucleotides 516-
 10 3068 of NCBI Reference Sequence NM_003243.4. The signal sequence is indicated by solid
underline and the transmembrane region by dotted underline.

ATGACTTCCCATTATGTGATTGCCATCTTTGCCCTGATGAGCTCCTGTTTAGCCACTGCAGGTCCAGAGCCTGGT
GCACTGTGTGAAGTGTACCTGTGTCAGTGCCTCCCATCCTGTCCAGGCCTTGATGGAGAGCTTCACTGTTTTGTCA
GGCTGTGCCAGCAGAGGCACAACCTGGGCTGCCACAGGAGGTGCATGTCTGAATCTCCGCACTGCAGGCCAGGGG
 15 CCTGGCCAGCTACAGAGAGAGGTACACCTTACCTGAATCCCATCTCCTCAGTCCACATCCACCACAAGTCTGTT
GTGTTCTCTGCTCAACTCCCCACACCCCTGGTGTGGCATCTGAAGACAGAGAGACTTGCCACTGGGGTCTCCAGA
CTGTTTTTGGTGTCTGAGGGTTCTGTGGTCCAGTTTTTCATCAGCAAACCTTCTCCTTGACAGCAGAAACAGAAGAA
AGGAACCTCCCCCATGGAAATGAACATCTGTTAAATTGGGCCCCGAAAAGAGTATGGAGCAGTTACTTCATTACAC
GAACTCAAGATAGCAAGAAACATTTATATTAAAGTGGGGGAAGATCAAGTGTTCCTCCAAAGTGCAACATAGGG
 20 AAGAATTTTCTCTCACTCAATTACCTTGCTGAGTACCTTCAACCCAAAGCAGCAGAAGGGTGTGTGATGTCCAGC
CAGCCCCAGAATGAGGAAGTACACATCATCGAGCTAATCACCCCAACTCTAACCCTACAGTGCTTTCCAGGTG
GATATAACAATTGATATAAGACCTTCTCAAGAGGATCTTGAAGTGGTCAAAAATCTCATCCTGATCTTGAAGTGC
AAAAAGTCTGTCAACTGGGTGATCAAATCTTTTGATGTTAAGGGAAGCCTGAAAATTATTGCTCCTAACAGTATT
GGCTTTGGAAGAGAGTGAAAGATCTATGACAATGACCAAATCAATAAGAGATGACATTCCTTCAACCCAAGGG
 25 AATCTGGTGAAGTGGGCTTTGGACAATGGCTATAGTCCAATAACTTCATACACAATGGCTCCTGTGGCTAATAGA
TTTCATCTTCGGCTTGAATAATGCAGAGGAGATGGGAGATGAGGAAGTCCACACTATTCTCTCTGAGCTACGG
ATCCTGCTGGACCTGGTGCCCTGCCTGCCCTGCAGAACCCGCCATCCGGGAGGGGAAGGCCAAAATGGAGGC
CTTCCGTTTCCTTTCCAGATATTTCCAGGAGAGTCTGGAATGAAGAGGGAGAAGATGGGCTCCCTCGGCCAAAG
GACCCTGTCAATCCAGCATACAACCTGTTTCTGGTCTCAGAGAGCCAGAAGAGGTGCAAGGGAGCGTGATATT
 30 GCCCTGTCTGTCAAATGTGACAATGAGAAGATGATCGTGGCTGTAGAAAAGATTCTTTTCAGGCCAGTGGCTAC
TCGGGGATGGACGTACCCCTGTTGGATCCTACCTGCAAGGCCAAGATGAATGGCACACACTTTGTTTTGGAGTCT
CCTCTGAATGGCTGCGGTACTCGGCCCGGTGGTCAAGCCTTGATGGTGTGGTCTACTATAACTCCATTGTGATA
CAGGTTCCAGCCCTTGGGGACAGTAGTGGTTGGCCAGATGGTTATGAAGATCTGGAGTCAGGTGATAATGGATT
CCGGGAGATATGGATGAAGGAGATGCTTCCCTGTTCAACCCGACCTGAAATCGTGGTGTAAATTGCAGCCTTCAG
 35 CAGGTGAGGAACCCAGCAGCTTCCAGGAACAGCCCCACGAAACATCACCTTCAACATGGAGCTATACAACACT
GACCTCTTTTTGGTGCCCTCCAGGGCGTCTTCTCTGTGCCAGAGAATGGACACGTTTATGTTGAGGTATCTGTT
ACTAAGGCTGAACAAGAACTGGGATTTGCCATCCAAACGTGCTTTATCTCTCCATATTCGAACCTGATAGGATG
TCTCATTACACATTATTGAGAATATTTGTCCTAAAGATGAATCTGTGAAATTCTACAGTCCCAAGAGAGTGCAC
TTTCTATCCCGCAAGCTGACATGGATAAGAAGCGATTACAGCTTTGTCTTCAAGCCTGTCTTCAACACCTCACTG

CTCTTTCTACAGTGTGAGCTGACGCTGTGTACGAAGATGGAGAAGCACCCCCAGAAGTTGCCTAAGTGTGTGCCT
 CCTGACGAAGCCTGCACCTCGCTGGACGCCTCGATAATCTGGGCCATGATGCAGAATAAGAAGACGTTCACTAAG
 CCCCTTGCTGTGATCCACCATGAAGCAGAATCTAAAGAAAAAGGTCCAAGCATGAAGGAACCAATCCAATTTCT
 CCACCAATTTTCCATGGTCTGGACACCCTAACCCTGATGGGCATTGCGTTTGCAGCCTTTGTGATCGGAGCACTC
 5 CTGACGGGGGCTTGTGGTACATCTATTCTCACACAGGGGAGACAGCAGGAAGGCAGCAAGTCCCCACCTCCCCG
 CCAGCCTCGGAAAACAGCAGTGTGCCCCACAGCATCGGCAGCACGCAGAGCACGCCTTGCTCCAGCAGCAGCACG
 GCC (SEQ ID NO: 52)

A nucleic acid sequence encoding a processed extracellular domain of betaglycan isoform A is shown below (SEQ ID NO: 53):

10 GGTCCAGAGCCTGGTGCACCTGTGTGAAGTGTACCTGTCAGTGCCTCCCATCCTGTCCAGGCCTTGATGGAGAGC
 TTCCTGTTTTGTGAGGCTGTGCCAGCAGAGGCACAACCTGGGCTGCCACAGGAGGTGCATGTCTGAATCTCCGC
 ACTGCAGGCCAGGGGCTGGCCAGCTACAGAGAGAGGTACACCTTACCTGAATCCCATCTCCTCAGTCCACATC
 CACCACAAGTCTGTTGTGTTCTGCTCAACTCCCCACCCCCCTGGTGTGGCATCTGAAGACAGAGAGACTTGCC
 ACTGGGGTCTCCAGACTGTTTTTGGTGTCTGAGGGTCTGTGGTCCAGTTTTTCATCAGCAAACCTTCTCCTTGACA
 15 GCAGAAACAGAAGAAAGGAACCTCCCCCATGGAAATGAACATCTGTTAAATTGGGCCGAAAAGAGTATGGAGCA
 GTTACTTCATTACCGAACTCAAGATAGCAAGAAACATTTATATTAAAGTGGGGGAAGATCAAGTGTTCCTCCA
 AAGTGCAACATAGGGAAGAATTTTCTCTCACTCAATTACCTTGCTGAGTACCTTCAACCCAAAGCAGCAGAAGGG
 TGTGTGATGTCCAGCCAGCCCCAGAATGAGGAAGTACACATCATCGAGCTAATCACCCCAACTCTAACCCTTAC
 AGTGCTTTCCAGGTGGATATAACAATTGATATAAGACCTTCTCAAGAGGATCTTGAAGTGGTCAAAAATCTCATC
 20 CTGATCTTGAAGTGCAAAAAGTCTGTCAACTGGGTGATCAAATCTTTTGATGTTAAGGGAAGCCTGAAAATTATT
 GCTCCTAACAGTATTGGCTTTGGAAAAGAGAGTGAAAGATCTATGACAATGACCAATCAATAAGAGATGACATT
 CCTTCAACCCAAAGGGAATCTGGTGAAGTGGGCTTTGGACAATGGCTATAGTCCAATAACTTCATACACAATGGCT
 CCTGTGGCTAATAGATTTTATCTTCGGCTTGAAAATAATGCAGAGGAGATGGGAGATGAGGAAGTCCACACTATT
 CCTCCTGAGCTACGGATCCTGCTGGACCCTGGTGCCTGCCTGCCTGCAGAACCCGCCCATCCGGGGAGGGGAA
 25 GGCCAAATGGAGGCCTTCCGTTTCTTTCCAGATATTTCCAGGAGAGTCTGGAATGAAGAGGGAGAAGATGGG
 CTCCCTCGGCCAAAGGACCCTGTCAATCCCAGCATACAACCTGTTTCTGGTCTCAGAGAGCCAGAAGAGGTGCAA
 GGGAGCGTGGATATTGCCCTGTCTGTCAAATGTGACAATGAGAAGATGATCGTGGCTGTAGAAAAAGATTCTTTT
 CAGGCCAGTGGCTACTCGGGGATGGACGTACCCCTGTTGGATCTACCTGCAAGGCCAAGATGAATGGCACACAC
 TTTGTTTTGGAGTCTCCTCTGAATGGCTGCGGTACTCGCCCCGGTGGTCCAGCCCTTGATGGTGTGGTCTACTAT
 30 AACTCCATTGTGATACAGGTTCCAGCCCTTGGGGACAGTAGTGGTTGGCCAGATGGTTATGAAGATCTGGAGTCA
 GGTGATAATGGATTTCCGGGAGATATGGATGAAGGAGATGCTTCCCTGTTACCCGACCTGAAATCGTGGTGT
 AATTGCAGCCTTCAGCAGGTGAGGAACCCAGCAGCTTCCAGGAACAGCCCCACGGAAACATCACCTTCAACATG
 GAGCTATACAACACTGACCTCTTTTTGGTGCCTCCAGGGCGTCTTCTCTGTGCCAGAGAATGGACACGTTTAT
 GTTGAGGTATCTGTTACTAAGGCTGAACAAGAACTGGGATTTGCCATCCAAACGTGCTTTATCTCTCCATATTG
 35 AACCTGATAGGATGTCTCATTACACCATTATTGAGAATATTTGTCTAAAGATGAATCTGTGAAATTCTACAGT
 CCAAGAGAGTGCATTTTCTATCCCGCAAGCTGACATGGATAAGAAGCGATTACGCTTTGTCTTCAAGCCTGTC
 TTCAACACCTCACTGCTCTTTCTACAGTGTGAGCTGACGCTGTGTACGAAGATGGAGAAGCACCCCCAGAAGTTG
 CCTAAGTGTGTGCTCCTGACGAAGCCTGCACCTCGCTGGACGCCTCGATAATCTGGGCCATGATGCAGAATAAG
 AAGACGTTCACTAAGCCCCCTTGCTGTGATCCACCATGAAGCAGAATCTAAAGAAAAAGGTCCAAGCATGAAGGAA
 40 CCAAATCCAATTTCTCCACCAATTTTCCATGGTCTGGACACCCTAACCCTG (SEQ ID NO: 53)

A human betaglycan isoform B precursor protein sequence (NCBI Ref Seq NP_001182612.1) is as follows:

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1  MTSHYVIAIF ALMSSCLATA GPEPGALCEL SPVSASHPVQ ALMESFTVLS GCASRGTTGL
61 PQEVHVLNLR TAGQGPGQLQ REVTLHLNPI SSVHIHHSV VFLNNSPHPL VWHLKTERLA
5 121 TGVSRLFLVS EGSVVQFSSA NFSLTAETEE RNFPHGNEHL LNWARKEYGA VTSFTELKIA
181 RNIYIKVGED QVFPPKCNIG KNFLSLNYLA EYLQPKAAEG CVMSSQPQNE EVHIIELITP
241 NSNPYSAFQV DITIDIRPSQ EDLEVVKNL LILKCKKSVN WVKSFDVKG SLKIIAPNSI
301 GFGKESERSM TMTKSIRDDI PSTQGNLVKW ALDNGYSPIT SYTMAPVANR FHLRLNNEE
361 MGDEEVHTIP PELRILLDPG ALPALQNPP IRGGEQNGGL PFPPFDISRR VWNEEGEDGL
10 421 PRPKDPVIPS IQLFPGLREP EEVQGSVDIA LSVKCDNEKM IVAVEKDSFQ ASGYSGMDVT
481 LLDPTCKAKM NGTHFVLESP LNGCGTRPRW SALDGVVYYN SIVIQVPALG DSSGWPDPGYE
541 DLESGDNGFP GDMDEGDASL FTRPEIVVFN CSLQQVRNPS SFQEOPHGNI TFNMELYNTD
601 LFLVPSQGVF SVPENGHVYV EVSVTAEQEL LGFAIQTCFI SPYSNPDRMS HYTIIENICP
661 KDESVKFYSP KRVHFPPIQA DMDKKRFSFV FKPVFNTSLL FLQCELTLC TCKMEKHPQKLP
15 721 KCVPPDEACT SLDASIIWAM MQNKKTFTK PLAVIHHEAES KEKGPSMKEP NPISPPIFHG
781 LDTLTVMGIA FAAFVIGALL TGALWYIYSH TGETAGRQQV PTSPPASENS SAAHSIGSTQ
841 STPCSSSSTA (SEQ ID NO: 54)

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The signal peptide is indicated by single underline, the extracellular domain is indicated in **bold** font, and the transmembrane domain is indicated by dotted underline.

A processed betaglycan isoform B polypeptide sequence is as follows:

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GPEPGALCELSPVSASHPVQALMESFTVLSGCASRGTTGLPQEVHVLNLRTAGQGPGQLQREVTLHLNPISSVHI
HHKS SVVFLNNSPHPLVWHLKTERLATGVSRFLVSEGSVVQFSSANFSLTAETEE RNFPHGNEHLLNWARKEYGA
VTSFTELKIA RNIYIKVGEDQVFPPKCNIGKNFLSLNYLAEYLQPKAAEGCVMSSQPQNEEVHIIELITPNSNPY
SAFQVDITIDIRPSQEDLEVVKNLILILKCKKSVNWVKSFDVKGSLKIIAPNSIGFGKESERSMTMTKSIRDDI
25 PSTQGNLVKWALDNGYSPITSYTMAPVANRFHLRLNNEEMGDEEVHTIPPELRILLDPGALPALQNPPIRGGEQ
QNGGLPFPPFDISRRVWNEEGEDGLPRPKDPVIPS IQLFPGLREPEEVQGSVDIALSVKCDNEKMIVAVEKDSFQ
ASGYSGMDVTLLDPTCKAKMNGTHFVLESPLNGCGTRPRWSALDGVVYYNSIVIQVPALGDSSGWPDPGYEDLESG
DNGFPGDMDEGDASLFTRPEIVVFNC SLQQVRNPS SFQEOPHGNI TFNMELYNTDLFLVPSQGVFSVPENGHVYV
EVSVTAEQELGFAIQTCFISPYSNPDRMSHYTIIENICPKDES VKFYSPKRVHFPPIQADMDKKRFSFVFKPVF
30 NTSLLFLQCELTLC TKMEKHPQKLPKCVPPDEACTSLDASIIWAMMQNKKTFTKPLAVIHHEAESKEKGPSMKEP
NPISPPIFHGLDTLTV
(SEQ ID NO: 55)

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A nucleic acid sequence encoding the unprocessed precursor protein of human betaglycan isoform B is shown below (SEQ ID NO: 56), corresponding to nucleotides 516-3065 of NCBI Reference Sequence NM_001195683.1. The signal sequence is indicated by solid underline and the transmembrane region by dotted underline.

ATGACTTCCCATTATGTGATTGCCATCTTTGCCCTGATGAGCTCCTGTTTAGCCACTGCAGGTCCAGAGCCTGGT
 GCACTGTGTGAACTGTCACCTGTCAGTGCCTCCCATCTGTCCAGGCCTTGATGGAGAGCTTCACTGTTTTGTCA
 GGCTGTGCCAGCAGAGGCACAACCTGGGCTGCCACAGGAGGTGCATGTCCTGAATCTCCGCACTGCAGGCCAGGGG
 CCTGGCCAGCTACAGAGAGAGGTACACTTCACCTGAATCCCATCTCCTCAGTCCACATCCACCACAAGTCTGTT
 5 GTGTTCTCTGCTCAACTCCCCACACCCCTGGTGTGGCATCTGAAGACAGAGAGACTTGCCACTGGGGTCTCCAGA
 CTGTTTTTGGTGTCTGAGGGTTCTGTGGTCCAGTTTTTCATCAGCAAACCTTCTCCTTGACAGCAGAAACAGAAGAA
 AGGAACTTCCCCCATGGAATGAACATCTGTTAAATTGGGCCCCGAAAAGAGTATGGAGCAGTTACTTCATTACCC
 GAACTCAAGATAGCAAGAAACATTTATATTAAAGTGGGGGAAGATCAAGTGTTCCTCCAAAGTGCAACATAGGG
 AAGAATTTTCTCTCACTCAATTACCTTGCTGAGTACCTTCAACCCAAAGCAGCAGAAGGGTGTGTGATGTCCAGC
 10 CAGCCCCAGAATGAGGAAGTACACATCATCGAGCTAATCACCCCCAACTCTAACCCTACAGTGCTTTCCAGGTG
 GATATAACAATTGATATAAGACCTTCTCAAGAGGATCTTGAAGTGGTCAAAAATCTCATCTGATCTTGAAGTGC
 AAAAAGTCTGTCAACTGGGTGATCAAATCTTTTGATGTTAAGGGAAGCCTGAAAATTATTGCTCCTAACAGTATT
 GGCTTTGGAAGAGAGTGAAAGATCTATGACAAATGACCAAATCAATAAGAGATGACATTCTTCAACCCAAAGGG
 AATCTGGTGAAGTGGGCTTTGGACAATGGCTATAGTCCAATAACTTCATACACAATGGCTCCTGTGGCTAATAGA
 15 TTTTCATCTTCGGCTTGAAAATAATGAGGAGATGGGAGATGAGGAAGTCCACACTATTCTCCTGAGCTACGGATC
 CTGCTGGACCTGGTGCCTGCCTGCCCTGCAGAACCCGCCCATCCGGGGAGGGGAAGGCCAAAATGGAGGCCTT
 CCGTTTCTTTCCAGATATTTCCAGGAGAGTCTGGAATGAAGAGGGAGAAGATGGGCTCCCTCGGCCAAAGGAC
 CCTGTCACTCCAGCATACAACCTGTTTCTGGTCTCAGAGAGCCAGAAGAGGTGCAAGGGAGCGTGGATATTGCC
 CTGTCTGTCAAATGTGACAATGAGAAGATGATCGTGGCTGTAGAAAAAGATTCTTTTCAGGCCAGTGGCTACTCG
 20 GGGATGGACGTCACCCTGTGGATCCTACCTGCAAGGCCAAGATGAATGGCACACACTTTGTTTTGGAGTCTCCT
 CTGAATGGCTGCGGTACTCGGCCCGGTGGTCTAGCCCTTGATGGTGTGGTCTACTATAACTCCATTGTGATACAG
 GTTCCAGCCCTTGGGGACAGTAGTGGTTGGCCAGATGGTTATGAAGATCTGGAGTCAGGTGATAATGGATTTCCG
 GGAGATATGGATGAAGGAGATGCTTCCCTGTTACCCGACCTGAAATCGTGGTGTTAATTGCAGCCTTCAGCAG
 GTGAGGAACCCAGCAGCTTCCAGGAACAGCCCCACGGAACATCACCTTCAACATGGAGCTATACAACACTGAC
 25 CTCTTTTTGGTGCCCTCCCAGGGCGTCTTCTGTGCCAGAGAATGGACACGTTTATGTTGAGGTATCTGTTACT
 AAGGCTGAACAAGAACTGGGATTTGCCATCCAAACGTGCTTTATCTCTCCATATTGAAACCCTGATAGGATGTCT
 CATTACACCATTATTGAGAATATTTGTCCTAAAGATGAATCTGTGAAATTCTACAGTCCCAAGAGAGTGACATTT
 CCTATCCCGCAAGCTGACATGGATAAGAAGCGATTACAGCTTTGTCTTCAAGCCTGTCTTCAACACCTCACTGCTC
 TTTCTACAGTGTGAGCTGACGCTGTGTACGAAGATGGAGAAGCACCCCCAGAAGTTGCCTAAGTGTGTGCCTCCT
 30 GACGAAGCCTGCACCTCGCTGGACGCTCGATAATCTGGGCCATGATGCAGAATAAGAAGACGTTCACTAAGCCC
 CTTGCTGTGATCCACCATGAAGCAGAATCTAAAGAAAAAGGTCCAAGCATGAAGGAACCAAATCCAATTTCTCCA
 CCAATTTTCCATGGTCTGGACACCCTAACCGTGATGGGCATTGCGTTTGCAGCCTTTGTGATCGGAGCACTCCTG
ACGGGGGCCTTGTGGTACATCTATTCTCACACAGGGGAGACAGCAGGAAGGCAGCAAGTCCCCACCTCCCCGCCA
 GCCTCGGAAAACAGCAGTGCTGCCCACAGCATCGGCAGCACGCAGAGCACGCCTTGCTCCAGCAGCAGCACGGCC
 35 (SEQ ID NO: 56)

A nucleic acid sequence encoding a processed extracellular domain of betaglycan isoform B is shown below (SEQ ID NO: 57):

GGTCCAGAGCCTGGTGCAGTGTGTGAACTGTCACCTGTCAGTGCCTCCCATCCTGTCCAGGCCTTGATGGAGAGC
 TTCACTGTTTTGTGAGGCTGTGCCAGCAGAGGCACAACCTGGGCTGCCACAGGAGGTGCATGTCCTGAATCTCCGC
 40 ACTGCAGGCCAGGGCCTGGCCAGCTACAGAGAGAGGTACACTTCACCTGAATCCCATCTCCTCAGTCCACATC

CACCACAAGTCTGTTGTGTTCTGCTCAACTCCCCACACCCCCTGGTGTGGCATCTGAAGACAGAGAGACTTGCC
 ACTGGGGTCTCCAGACTGTTTTTGGTGTCTGAGGGTTCTGTGGTCCAGTTTTTCATCAGCAAACCTTCTCCTTGACA
 GCAGAAACAGAAGAAAGGAACTTCCCCATGGAAATGAACATCTGTTAAATTGGGCCGAAAAGAGTATGGAGCA
 GTTACTTCATTACCGAACTCAAGATAGCAAGAAACATTTATATTAAAGTGGGGGAAGATCAAGTGTTCCTCCA
 5 AAGTGAACATAGGGAAGAATTTTCTCTCACTCAATTACCTTGCTGAGTACCTTCAACCCAAAGCAGCAGAAGGG
 TGTGTGATGTCCAGCCAGCCCCAGAATGAGGAAGTACACATCATCGAGCTAATCACCCCCAACTCTAACCCTAC
 AGTGCTTTCCAGGTGGATATAACAATTGATATAAGACCTTCTCAAGAGGATCTTGAAGTGGTCAAAAATCTCATC
 CTGATCTTGAAGTGAAAAAGTCTGTCAACTGGGTGATCAAAATCTTTTGATGTTAAGGGAAGCCTGAAAATTATT
 GCTCCTAACAGTATTGGCTTTGGAAAAGAGAGTGAAAGATCTATGACAATGACCAAATCAATAAGAGATGACATT
 10 CCTTCAACCCAAGGGAATCTGGTGAAGTGGGCTTTGGACAATGGCTATAGTCCAATAACTTCATACACAATGGCT
 CCTGTGGCTAATAGATTTTCATCTTCGGCTTGAAAATAATGAGGAGATGGGAGATGAGGAAGTCCACACTATTCCCT
 CCTGAGCTACGGATCCTGCTGGACCCCTGGTGCCCTGCCTGCCCTGCAGAACCCGCCCATCCGGGGAGGGGAAGGC
 CAAAATGGAGGCCTTCGGTTTCCCTTTCCAGATATTTCAGGAGAGTCTGGAATGAAGAGGGAGAAGATGGGCTC
 CCTCGGCCAAAGGACCTGTCAATCCCAGCATACAACCTGTTTCTGGTCTCAGAGAGCCAGAAGAGGTGCAAGGG
 15 AGCGTGGATATTGCCCTGTCTGTCAAATGTGACAATGAGAAGATGATCGTGGCTGTAGAAAAAGATTCTTTTCAG
 GCCAGTGGCTACTCGGGGATGGACGTCACCTGTTGGATCCTACCTGCAAGGCCAAGATGAATGGCACACACTTT
 GTTTTGGAGTCTCCTCTGAATGGCTGCGGTACTCGGCCCGGTGGTTCAGCCCTTGATGGTGTGGTCTACTATAAC
 TCCATTGTGATACAGGTTCCAGCCCTTGGGGACAGTAGTGGTTGGCCAGATGGTTATGAAGATCTGGAGTCAGGT
 GATAATGGATTTCCGGGAGATATGGATGAAGGAGATGCTTCCCTGTTTACCCGACCTGAAATCGTGGTGTTTAAT
 20 TGCAGCCTTCAGCAGGTGAGGAACCCAGCAGCTTCCAGGAACAGCCCCACGGAACATCACCTTCAACATGGAG
 CTATAACAACACTGACCTCTTTTTTGGTGCCCTCCAGGGCGTCTTCTCTGTGCCAGAGAATGGACACGTTTATGTT
 GAGGTATCTGTTACTAAGGCTGAACAAGAACTGGGATTTGCCATCCAAACGTGCTTTATCTCTCCATATTGCAAC
 CCTGATAGGATGTCTCATTACACCATTATTGAGAATATTTGTCTAAAGATGAATCTGTGAAATTCTACAGTCCC
 AAGAGAGTGCACCTTCTATCCCGCAAGCTGACATGGATAAGAAGCGATTACGCTTTGTCTTCAAGCCTGTCTTC
 25 AACACCTCACTGCTCTTTCTACAGTGTGAGCTGACGCTGTGTACGAAGATGGAGAAGCACCCCCAGAAGTTGCCT
 AAGTGTGTGCCTCCTGACGAAGCCTGCACCTCGCTGGACGCTCGATAATCTGGGCCATGATGCAGAATAAGAAG
 ACGTTCACTAAGCCCTTGCTGTGATCCACCATGAAGCAGAATCTAAAGAAAAAGGTCCAAGCATGAAGGAACCA
 AATCCAATTTCTCCACCAATTTTCCATGGTCTGGACACCCTAACCGTG (SEQ ID NO: 57)

In certain embodiments, the disclosure relates to betaglycan polypeptide, which
 30 includes fragments, functional variants, and modified forms thereof. Preferably, betaglycan
 polypeptides for use in accordance with inventions of the disclosure are soluble (*e.g.*,
 comprise an extracellular domain of betaglycan). In other preferred embodiments,
 betaglycan polypeptides for use in accordance with the inventions of the disclosure bind to
 and/or inhibit (antagonize) activity (*e.g.*, Smad signaling) of one or more TGF-beta
 35 superfamily ligands. In some embodiments, betaglycan polypeptide comprise an amino acid
 sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%,
 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NOs: 50, 51, 54, or 55.
 In some embodiments, betaglycan polypeptides comprise an amino acid sequence that is at

least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a polypeptide that begins at any one of amino acids of 21-28 of SEQ ID NO: 50, and ends at any one of amino acids 381-787 of SEQ ID NO: 50. In some embodiments, polypeptides of the disclosure comprise at least one betaglycan polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids of 21-381 of SEQ ID NO: 50. In some embodiments, polypeptides of the disclosure comprise at least one betaglycan polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids of 21-787 of SEQ ID NO: 50. In some embodiments, polypeptides of the disclosure comprise at least one betaglycan polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids of 28-381 of SEQ ID NO: 50. In some embodiments, polypeptides of the disclosure comprise at least one betaglycan polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids of 28-787 of SEQ ID NO: 50. In some embodiments, polypeptides of the disclosure comprise of at least one betaglycan polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids of 21-781 of SEQ ID NO: 50. In some embodiments, polypeptides of the disclosure comprise at least one betaglycan polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids of 28-781 of SEQ ID NO: 50. In some embodiments, polypeptides of the disclosure comprise at least one betaglycan polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a polypeptide that begins at any one of amino acids of 21-28 of SEQ ID NO: 54, and ends at any one of amino acids 380-786 of SEQ ID NO: 54. In some embodiments, polypeptides of the disclosure comprise at least one betaglycan polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids of 21-380 of SEQ ID NO: 54. In some embodiments, polypeptides of the disclosure comprise at least one betaglycan polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids of 21-786 of SEQ ID NO: 54. In some embodiments, polypeptides of the disclosure comprise at least one betaglycan polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids of 28-380 of SEQ ID NO: 54. In some embodiments, polypeptides of the disclosure comprise at least one betaglycan polypeptide that is at least 70%, 75%, 80%,

85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids of 28-786 of SEQ ID NO: 54. In some embodiments, polypeptides of the disclosure comprise at least one betaglycan polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids of 21-780 of SEQ ID NO: 54. In some embodiments, polypeptides of the disclosure comprise at least one betaglycan polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids of 28-780 of SEQ ID NO: 54. In some embodiments, polypeptides of the disclosure comprise at least one betaglycan polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a polypeptide that begins at any one of amino acids of 21-28 of SEQ ID NO: 50, and ends at any one of amino acids 730-787 of SEQ ID NO: 50. In some embodiments, polypeptides of the disclosure comprise at least one betaglycan polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids of 21-787 of SEQ ID NO: 50. In some embodiments, polypeptides of the disclosure comprise at least one betaglycan polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids of 28-730 of SEQ ID NO: 50. In some embodiments, polypeptides of the disclosure comprise at least one betaglycan polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a polypeptide that begins at any one of amino acids of 21-28 of SEQ ID NO: 50, and ends at any one of amino acids 730-787 of SEQ ID NO: 50. In some embodiments, polypeptides of the disclosure comprise at least one betaglycan polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids of 21-787 of SEQ ID NO: 50. In some embodiments, polypeptides of the disclosure comprise at least one betaglycan polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids of 28-730 of SEQ ID NO: 50. In some embodiments, polypeptides of the disclosure comprise at least one betaglycan polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a polypeptide that begins at any one of amino acids of 21-28 (e.g., amino acid residues of SEQ ID NO: 54, and ends at any one of amino acids 730-787 of SEQ ID NO: 54. In some embodiments, polypeptides of the disclosure comprise at least one betaglycan polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids of 21-786 of SEQ ID NO: 54. In some embodiments,

polypeptides of the disclosure comprise at least one betaglycan polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids of 28-729 of SEQ ID NO: 54.

In certain aspects, the present disclosure relates to heteromultimers that comprise an
 5 ALK5 polypeptide. As used herein, the term “ALK5” refers to a family of activin receptor-like kinase-5 proteins from any species and variants derived from such ALK4 proteins by mutagenesis or other modification. Reference to ALK5 herein is understood to be a reference to any one of the currently identified forms. Members of the ALK5 family are generally
 10 transmembrane proteins, composed of a ligand-binding extracellular domain with a cysteine-rich region, a transmembrane domain, and a cytoplasmic domain with predicted serine/threonine kinase activity.

The term “ALK5 polypeptide” includes polypeptides comprising any naturally occurring polypeptide of an ALK5 family member as well as any variants thereof (including mutants, fragments, fusions, and peptidomimetic forms) that retain a useful activity.

15 The human ALK5 precursor protein, isoform 1 sequence (NCBI Ref Seq NP_004603.1) is as follows:

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  1  MEAAVAAPRP RLLLLVLAAA AAAAAALLPG ATALQCFCHL CTKDNFTCVT
    DGLCFVSVTE
  61  TTDKVIHNSM CIAEIDLIPR DRPFVCAPSS KTGSVTTTTC CNQDHCNKIE
20  LPTTVKSSPG
 121  LGPVELAAVI AGPVCFVCIS LMLMVYICHN RTVIHHRVPN EEDPSLDRPF
    ISEGTTLKDL
 181  IYDMTTSGSG SGLPLLQRT IARTIVLQES IGKGRFGEVW RGKWRGEEVA
    VKIFSSREER
25  241  SWFREAEIYQ TVMLRHENIL GFIAADNKDN GTWTQLWLVS DYHEHGSFLD
    YLNRYTVTVE
 301  GMIKLALSTA SGLAHLHMEI VGTQGKPAIA HRDLKSKNIL VKKNGTCCIA
    DLGLAVRHDS
 361  ATDTIDIAPN HRVGTKRYMA PEVLDD SINM KHFESFKRAD IYAMGLVFEW
30  IARRCSIGGI
 421  HEDYQLPYD LVPSDPSVEE MRKVCEQKL RPNIPNRWQS CEALRVMKI
    MRECWYANGA
 481  ARLTALRIKK TLSQLSQQEG IKM (SEQ ID NO: 69)
  
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The signal peptide is indicated by a single underline and the extracellular domain is indicated in **bold** font.

A processed extracellular ALK5 polypeptide sequence is as follows:

AALLPGATALQCFCHLCTKDNFTCVTDGLCFVSVTETTDKVIHNSMCIAEIDLIPDRPFVC
 5 APSSKTGSVTTTTYCCNQDHCNKIELPTTVKSSPGLGPVEL (SEQ ID NO: 70)

A nucleic acid sequence encoding the ALK5 precursor protein is shown below (SEQ ID NO: 32), corresponding to nucleotides 77-1585 of Genbank Reference Sequence NM_004612.2. The signal sequence is underlined and the extracellular domain is indicated in **bold** font.

10 ATGGAGGCGGCGGTGCTGCTCCGCGTCCCGGCTGCTCCTCCTCGTGCTGGCGGCGGCGGC
GGCGGCGGCG**GCGGCGCTGCTCCCGGGGCGACGGCGTTACAGTGTCTTCTGCCACCTCTGTA**
CAAAAGACAATTTTACTTGTGTGACAGATGGGCTCTGCTTTGTCTCTGTACAGAGACCACA
GACAAAGTTATACACAACAGCATGTGTATAGCTGAAATTGACTTAATTCCTCGAGATAGGCC
GTTTGTATGTGCACCTCTTCAAAAAGTGGGTCTGTGACTACAACATATTGCTGCAATCAGG
 15 **ACCATTGCAATAAAATAGAAGTTCCTCAACTACTGTAAAGTCATCACCTGGCCTTGGTCTGTG**
GAAGTGGCAGCTGTCATTGCTGGACCAGTGTGCTTCGTCTGCATCTCACTCATGTTGATGGT
 CTATATCTGCCACAACCGCACTGTCATTACCATCGAGTGCCAAATGAAGAGGACCCTTCAT
 TAGATCGCCCTTTTATTTTACAGAGGGTACTACGTTGAAAGACTTAATTTATGATATGACAACG
 TCAGGTTCTGGCTCAGGTTTACCATTGCTTGTTCAGAGAACAATTGCGAGAACTATTGTGTT
 20 ACAAGAAAGCATTGGCAAAGGTCGATTTGGAGAAGTTTGGAGAGGAAAGTGGCGGGGAGAAG
 AAGTTGCTGTAAAGATATTCTCCTCTAGAGAAGAACGTTTCGTGGTTCCGTGAGGCAGAGATT
 TATCAAACTGTAATGTTACGTCATGAAAACATCCTGGGATTTATAGCAGCAGACAATAAAGA
 CAATGGTACTTGGACTCAGCTCTGGTTGGTGTGAGATTATCATGAGCATGGATCCCTTTTTTG
 ATTACTTAAACAGATACACAGTTACTGTGGAAGGAATGATAAACTTGCTCTGTCCACGGCG
 25 AGCGGTCTTGCCCATCTTCACATGGAGATTGTTGGTACCCAAGGAAAGCCAGCCATTGCTCA
 TAGAGATTTGAAATCAAAGAATATCTTGGTAAAGAAGAATGGAAGTCTGCTGTATTGCAGACT
 TAGGACTGGCAGTAAGACATGATTCAGCCACAGATACCATTGATATTGCTCCAAACCACAGA
 GTGGGAACAAAAAGGTACATGGCCCTGAAGTTCTCGATGATTCCATAAATATGAAACATTT
 TGAATCCTTCAAACGTGCTGACATCTATGCAATGGGCTTAGTATTCTGGGAAATTGCTCGAC
 30 GATGTTCCATTGGTGGAAATTCATGAAGATTACCAACTGCCTTATTATGATCTTGTACCTTCT
 GACCCATCAGTTGAAGAAATGAGAAAAGTTGTTTGTGAACAGAAGTTAAGGCCAAATATCCC
 AAACAGATGGCAGAGCTGTGAAGCCTTGAGAGTAATGGCTAAAATTATGAGAGAATGTTGGT

ATGCCAATGGAGCAGCTAGGCTTACAGCATTGCGGATTAAGAAAACATTATCGCAACTCAGT
CAACAGGAAGGCATCAAAATG (SEQ ID NO: 71)

A nucleic acid sequence encoding an extracellular human ALK5 polypeptide is as follows:

5 GCGGCGCTGCTCCCGGGGGCGACGGCGTTACAGTGTTTCTGCCACCTCTGTACAAAAGACAA
TTTTACTTGTGTGACAGATGGGCTCTGCTTTGTCTCTGTACAGAGACCACAGACAAAGTTA
TACACAACAGCATGTGTATAGCTGAAATTGACTTAATTCCTCGAGATAGGCCGTTTGTATGT
GCACCCTCTTCAAAAACCTGGGTCTGTGACTACAACATATTGCTGCAATCAGGACCATTGCAA
TAAATAGAACTTCCAACACTACTGTAAAGTCATCACCTGGCCTTGGTCCTGTGGAACCTG
10 (SEQ ID NO: 72)

An alternative isoform of the human ALK5 precursor protein sequence, isoform 2 (NCBI Ref Seq XP_005252207.1), is as follows:

1 MEAAVAAPRP RLLLLVLAAA AAAAAALLPG **ATALQCFCHL CTKDNFTCVT**
15 **DGLCFVSVTE**
61 **TTDKVIHNSM CIAEIDLIPR DRPFVCAPSS KTGSVTTTYC CNQDHCNKIE**
LPTTGPFVSVK
121 **SSPGLGPVEL** AAVIAGPVCF VCISLMLMVY ICHNRTVIHH RVPNEEDPSL
DRPFISEGTT
20 181 LKDLIYDMMT SGSGSGLPLL VQRTIARTIV LQESIGKGRF GEVWRGKWRG
EEVAVKIFSS
241 REERSWFREA EIYQTVMLRH ENILGFIAAD NKDNGTWTQL WLVS DYHEHG
SLFDYLNRYT
301 VTVEGMIKLA LSTASGLAHL HMEIVGTQ GK PAIAHRDLKS KNILVKKNGT
25 CCIADLGLAV
361 RHDSATDTID IAPNHRVGTK RYMAPEVLDD SINMKHFESF KRADIYAMGL
VFWEIARRCS
421 IGGIHEDYQL PYYDLVPSDP SVEEMRKVVC EQKL RPNIPN RWQSCEALRV
MAKIMRECWY
30 481 ANGAARLTAL RIKKTLSQLS QQEGIKM (SEQ ID NO: 73)

The signal peptide is indicated by a single underline and the extracellular domain is indicated in **bold** font.

A processed extracellular ALK5 polypeptide sequence (isoform 2) is as follows:

AALLPGATALQCFCHLCTKDNFTCVTDGLCFVSVTETTDKVIHNSMCIAEIDLIPRDRPFVC
APSSKTGSVTTTTYCCNQDHCNKIELPTTGPFVSVKSSPGLGPVEL (SEQ ID NO: 74)

A nucleic acid sequence encoding human ALK5 precursor protein (isoform 2) is shown below (SEQ ID NO: 75), corresponding to nucleotides 77-1597 of Genbank Reference Sequence XM_005252150.1. The signal sequence is underlined and the extracellular domain is indicated in **bold** font.

ATGGAGGCGGCGGTGCTGCTCCGCGTCCCCGGCTGCTCCTCCTCGTGCTGGCGGCGGCGGC
GGCGGCGGCG**GCGGCGCTGCTCCCGGGGGCGACGGCGTTACAGTGTTCCTGCCACCTCTGTA**
CAAAAGACAATTTTACTTGTGTGACAGATGGGCTCTGCTTTGTCTCTGTACAGAGACCACA
10 **GACAAAGTTATACACAACAGCATGTGTATAGCTGAAATTGACTTAATTCCTCGAGATAGGCC**
GTTTGTATGTGCACCTCTTCAAAAAGTGGGCTCTGTGACTACAACATATTGCTGCAATCAGG
ACCATTGCAATAAAATAGAACTTCCAACACTGGCCCTTTTTCAGTAAAGTCATCACCTGGC
CTTGGTCTGTGAACTGGCAGCTGTCATTGCTGGACCAGTGTGCTTCGTCTGCATCTCACT
CATGTTGATGGTCTATATCTGCCACAACCGCACTGTCATTACCATCGAGTGCCAAATGAAG
15 AGGACCCCTTCATTAGATCGCCCTTTTATTTTCAGAGGGTACTACGTTGAAAGACTTAATTTAT
GATATGACAACGTCAGGTTCTGGCTCAGGTTTACCATTGCTTGTTTCAGAGAACAATTGCGAG
AACTATTGTGTTACAAGAAAGCATTGGCAAAGGTCGATTTGGAGAAGTTTGGAGAGGAAAGT
GGCGGGGAGAAGAAGTTGCTGTTAAGATATTCTCCTCTAGAGAAGAACGTTCTGGTTCCGT
GAGGCAGAGATTTATCAAACGTGAATGTTACGTCATGAAAACATCCTGGGATTTATAGCAGC
20 AGACAATAAAGACAATGGTACTTGGACTCAGCTCTGGTTGGTGTGAGATTATCATGAGCATG
GATCCCTTTTTGATTACTTAAACAGATACACAGTTACTGTGGAAGGAATGATAAACTTGCT
CTGTCCACGGCGAGCGGTCTTGCCCATCTTCACATGGAGATTGTTGGTACCCAAGGAAAGCC
AGCCATTGCTCATAGAGATTTGAAATCAAAGAATATCTTGGTAAAGAAGAATGGAACCTTGCT
GTATTGCAGACTTAGGACTGGCAGTAAGACATGATTCAGCCACAGATACCATTGATATTGCT
25 CCAAACCACAGAGTGGAACAAAAAGGTACATGGCCCCTGAAGTTCTCGATGATTCCATAAA
TATGAAACATTTTGAATCCTTCAAACGTGCTGACATCTATGCAATGGGCTTAGTATTCTGGG
AAATTGCTCGACGATGTTCCATTGGTGAATTCATGAAGATTACCAACTGCCTTATTATGAT
CTTGTACCTTCTGACCCATCAGTTGAAGAAATGAGAAAAGTTGTTTGTGAACAGAAGTTAAG
GCCAAATATCCCAAACAGATGGCAGAGCTGTGAAGCCTTGAGAGTAATGGCTAAAATTATGA
30 GAGAATGTTGGTATGCCAATGGAGCAGCTAGGCTTACAGCATTGCGGATTAAGAAAACATTA
TCGCAACTCAGTCAACAGGAAGGCATCAAAATG (SEQ ID NO: 75)

A nucleic acid sequence encoding an processed extracellular ALK5 polypeptide is as follows:

GCGGCGCTGCTCCCGGGGGCGACGGCGTTACAGTGTTTCTGCCACCTCTGTACAAAAGACAA
 TTTTACTTGTGTGACAGATGGGCTCTGCTTTGTCTCTGTACAGAGACCACAGACAAAGTTA
 TACACAACAGCATGTGTATAGCTGAAATTGACTTAATTCCTCGAGATAGGCCGTTTGTATGT
 GCACCCTCTTCAAAAACCTGGGTCTGTGACTACAACATATTGCTGCAATCAGGACCATTGCAA
 5 TAAATAGAACTTCCAACCTACTGGCCCTTTTTCAGTAAAGTCATCACCTGGCCTTGGTCCTG
 TGGAAGT (SEQ ID NO: 76)

In certain embodiments, the disclosure relates to heteromultimers that comprise at least one ALK5 polypeptide, which includes fragments, functional variants, and modified forms thereof. Preferably, ALK5 polypeptides for use in accordance with the disclosure (*e.g.*,
 10 heteromultimers comprising an ALK5 polypeptide and uses thereof) are soluble (*e.g.*, an extracellular domain of ALK5). In other preferred embodiments, ALK5 polypeptides for use in accordance with the disclosure bind to and/or inhibit (antagonize) activity (*e.g.*, Smad signaling) of one or more TGF-beta superfamily ligands. In some embodiments, heteromultimers of the disclosure comprise at least one ALK5 polypeptide that is at least
 15 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NOs: 69, 70, 73, or 74. In some embodiments, heteromultimers of the disclosure comprise at least one ALK5 polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a polypeptide that begins at any one of amino acids of 25-36 (*e.g.*, amino
 20 acid residues 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, or 36) of SEQ ID NO: 69, and ends at any one of amino acids 101-126 (*e.g.*, amino acid residues 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, or 126) of SEQ ID NO: 69. In some embodiments, heteromultimers of the disclosure comprise at least one ALK5 polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%,
 25 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids of 25-101 of SEQ ID NO: 69. In some embodiments, heteromultimers of the disclosure comprise at least one ALK5 polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids of 25-126 of SEQ ID NO: 69. In some embodiments, heteromultimers of the disclosure comprise at least one ALK5
 30 polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids of 36-101 of SEQ ID NO: 69. In some embodiments, heteromultimers of the disclosure comprise at least one ALK5 polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids of 36-101 of SEQ ID NO: 69. In some

100% identical to amino acids of 36-126 of SEQ ID NO: 69. In some embodiments, heteromultimers of the disclosure comprise at least one ALK5 polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a polypeptide that begins at any one of amino acids of 25-36 (e.g., amino acid residues 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, or 36) of SEQ ID NO: 73, and ends at any one of amino acids 101-130 (e.g., amino acid residues 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129 or 130) of SEQ ID NO: 73. In some embodiments, heteromultimers of the disclosure comprise at least one ALK5 polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids of 25-101 of SEQ ID NO: 73. In some embodiments, heteromultimers of the disclosure comprise at least one ALK5 polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids of 25-130 of SEQ ID NO: 73. In some embodiments, heteromultimers of the disclosure comprise at least one ALK5 polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids of 36-101 of SEQ ID NO: 73. In some embodiments, heteromultimers of the disclosure comprise at least one ALK5 polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids of 36-130 of SEQ ID NO: 73.

As described above, the disclosure provides T β R II or betaglycan polypeptides sharing a specified degree of sequence identity or similarity to a naturally occurring T β R II or betaglycan 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 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 "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.

The comparison of sequences and determination of percent identity and 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).

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, 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. 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.

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.

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 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.

Polypeptides of the disclosure (e.g., T β RII or betaglycan 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 signal sequence (e.g., native T β RII or betaglycan 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 β RII-Fc fusion proteins incorporating a TPA leader sequence include SEQ ID NOs: 11, 13, 15 and 17. Processing of signal peptides may vary depending on the leader sequence chosen, the cell type used and culture conditions, among other variables, and therefore actual N-terminal start sites for mature polypeptides may shift by 1, 2, 3, 4 or 5 amino acids in either the N-terminal or C-terminal direction. Examples of T β RII-Fc fusion proteins include SEQ ID NOs: 11, 13, 15 and 17. It will be understood by one of skill in the art that corresponding variants based on the long isoform of T β RII will include the 25-amino acid insertion along with a conservative Val-Ile substitution at the flanking position C-terminal to the insertion.

In some embodiments, any of the T β RII polypeptides disclosed herein are at least 80%, 85%, 90%, 92%, 94%, 95%, 97%, 99% or 100% identical to the amino acid sequence of any one of SEQ ID NOs: 18, 20, 27, 30, 34, 36, 37, 38, 39, or 48, but lack one or more N-terminal amino acids as compared to the amino acid sequences of SEQ ID NO: 18, 20, 27, 30, 34, 36, 37, 38, 39, or 48. In some embodiments, the T β RII polypeptide lacks the amino acid corresponding to the first amino acid (threonine) of any one of SEQ ID NOs: 18, 20, 27, 30, 34, 36, 37, 38, 39, or 48. In some embodiments, the T β RII polypeptide lacks the amino acids corresponding to the first and second amino acids (threonine and isoleucine, respectively) of any one of SEQ ID NOs: 18, 20, 27, 30, 34, 36, 37, 38, 39, or 48. In some embodiments, the T β RII polypeptide lacks the amino acids corresponding to the first, second and third amino acids (threonine, isoleucine, and proline, respectively) of any one of SEQ ID NOs: 18, 20, 27, 30, 34, 36, 37, 38, 39, or 48. In some embodiments, the T β RII polypeptide lacks the amino acids corresponding to the first, second, third and fourth amino acids (threonine, isoleucine, proline, proline, respectively) of any one of SEQ ID NOs: 18, 20, 27, 30, 34, 36, 37, 38, 39, or 48.

In some embodiments, any of the T β R β II polypeptides disclosed herein are at least 80%, 85%, 90%, 92%, 94%, 95%, 97%, 99% or 100% identical to the amino acid sequence of any one of SEQ ID NOs: 18 or 48, but lack the amino acid corresponding to the first amino acid (threonine) of SEQ ID NO: 18 or 48. In some embodiments, the T β R β II polypeptide lacks the amino acids corresponding to the first and second amino acids (threonine and isoleucine, respectively) of SEQ ID NO: 18 or 48. In some embodiments, the T β R β II polypeptide lacks the amino acids corresponding to the first, second and third amino acids (threonine, isoleucine, and proline, respectively) of SEQ ID NO: 18 or 48. In some embodiments, the T β R β II polypeptide lacks the amino acids corresponding to the first, second, third and fourth amino acids (threonine, isoleucine, proline, proline, respectively) of SEQ ID NO: 18 or 48.

In some embodiments, the disclosure provides for a composition comprising a mixture of T β R β II polypeptides, wherein the T β R β II polypeptides in the composition each comprise an amino acid sequence that is at least 80%, 85%, 90%, 92%, 94%, 95%, 97%, 99% or 100% identical to the amino acid sequence of any one of SEQ ID NOs: 18, 20, 27, 30, 34, 36, 37, 38, 39, or 48; but wherein at least a portion of the T β R β II polypeptides (*e.g.*, at least 1%, 3%, 4%, 5%, 10%, 15%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%) in the composition include the amino acids corresponding to the first, second, third and fourth amino acids (threonine, isoleucine, proline and proline, respectively) of any one of SEQ ID NOs: 18, 20, 27, 30, 34, 36, 37, 38, 39, or 48; and wherein at least a portion of the T β R β II polypeptides (*e.g.*, at least 1%, 3%, 4%, 5%, 10%, 15%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%) in the composition lack one or more of the amino acids corresponding to the first, second, third and fourth amino acids (threonine, isoleucine, proline and proline, respectively) of any one of SEQ ID NOs: 18, 20, 27, 30, 34, 36, 37, 38, 39, or 48. In some embodiments, the disclosure provides for a composition comprising a mixture of T β R β II polypeptides, wherein the T β R β II polypeptides are at least 80%, 85%, 90%, 92%, 94%, 95%, 97%, 99% or 100% identical to the amino acid sequence of any one of SEQ ID NOs: 18 or 48, but wherein at least 30% to 80% of the T β R β II polypeptides in the composition lack the amino acid corresponding to the first amino acid (threonine) of SEQ ID NO: 18 or 48. In some embodiments, the T β R β II polypeptides are at least 80%, 85%, 90%, 92%, 94%, 95%, 97%, 99% or 100% identical to the amino acid sequence of any one of SEQ ID NOs: 63-68. In some embodiments, the T β R β II polypeptides are at least 80%, 85%, 90%, 92%, 94%, 95%, 97%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 68.

In certain embodiments, the present disclosure contemplates specific mutations of the polypeptides (e.g., T β RII or betaglycan polypeptides) so as to alter the glycosylation of the polypeptide. Such 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 of, or substitution by, one or more serine or threonine residues to the sequence of the wild-type 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 non-glycosylation at the modified tripeptide sequence. Another means of increasing the number of carbohydrate moieties on a polypeptide is by chemical or enzymatic coupling of glycosides to the 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. Biochem., pp. 259-306, incorporated by reference herein. Removal of one or more carbohydrate moieties present on a polypeptide may be accomplished chemically and/or enzymatically. Chemical deglycosylation may involve, for example, exposure of the 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 polypeptides can be achieved by the use of a variety of endo- and exo-glycosidases as described by Thotakura et al. (1987) Meth. Enzymol. 138:350. The sequence of a 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, polypeptides (e.g., T β RII or betaglycan polypeptides) for use in humans will be expressed in a mammalian cell line that provides proper glycosylation, such as

HEK293 or CHO cell lines, although other mammalian expression cell lines, yeast cell lines with engineered glycosylation enzymes, and insect cells are expected to be useful as well.

This disclosure further contemplates a method of generating mutants, particularly sets of combinatorial mutants of a polypeptide (e.g., T β R II or betaglycan polypeptides), as well as
5 truncation mutants; pools of combinatorial mutants are especially useful for identifying functional variant sequences. The purpose of screening such combinatorial libraries may be to generate, for example, 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 β R II
10 polypeptide variant may be screened for ability to bind to a T β R II ligand, to prevent binding of a T β R II ligand to a T β R II polypeptide or to interfere with signaling caused by a T β R II ligand. The activity of a T β R II 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.

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

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 polypeptide (e.g., T β R II or betaglycan polypeptides) sequences. For instance, a mixture of synthetic oligonucleotides can be enzymatically ligated into gene sequences such that the
30 degenerate set of potential polypeptide nucleotide sequences are expressible as individual polypeptides, or alternatively, as a set of larger fusion proteins (e.g., for phage display).

There are many ways by which the library of potential polypeptide (e.g., T β R II or betaglycan polypeptide) 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. 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., (1990) *PNAS USA* 87: 6378-6382; as well as U.S. Patent Nos: 5,223,409, 5,198,346, and 5,096,815).

Alternatively, other forms of mutagenesis can be utilized to generate a combinatorial library. For example, polypeptide (e.g., T β R II or betaglycan 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 polypeptides.

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 polypeptides (e.g., T β R II or betaglycan 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 ligand binding assays and ligand-mediated cell signaling assays.

In certain embodiments, the polypeptides (e.g., T β RII or betaglycan polypeptides) of the disclosure may further comprise post-translational modifications in addition to any that are naturally present in the native polypeptides. Such modifications include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation, pegylation (polyethylene glycol) and acylation. As a result, the modified 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 polypeptide may be tested as described herein for other polypeptide variants. When a polypeptide is produced in cells by cleaving a nascent form of the 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 machinery and characteristic mechanisms for such post-translational activities and may be chosen to ensure the correct modification and processing of the polypeptides.

In certain aspects, the disclosure provides for fusion proteins (e.g., T β RII or betaglycan fusion proteins), and in some embodiments, a first portion (e.g., a T β RII or betaglycan polypeptide portion) is connected to a heterologous portion (e.g., Fc portion) by means of a linker. In some embodiments, the linkers are glycine and serine rich linkers. Other near neutral amino acids, such as, but not limited to, Thr, Asn, Pro and Ala, may also be used in the linker sequence. In some embodiments, the linker comprises various permutations of amino acid sequences containing Gly and Ser. In some embodiments, the linker is greater than 10 amino acids in length. In further embodiments, the linkers have a length of at least 12, 15, 20, 21, 25, 30, 35, 40, 45 or 50 amino acids. In some embodiments, the linker is less than 40, 35, 30, 25, 22 or 20 amino acids. In some embodiments, the linker is 10-50, 10-40, 10-30, 10-25, 10-21, 10-15, 10, 15-25, 17-22, 20, or 21 amino acids in length. In some preferred embodiments, the linker comprises the amino acid sequence GlyGlyGlyGlySer (GGGGS) (SEQ ID NO: 19), or repetitions thereof (GGGGS) n , where $n \geq 2$. In particular embodiments $n \geq 3$, or $n = 3-10$. The application teaches the surprising finding that proteins comprising a T β RII portion and a heterologous portion fused together by means of a (GGGGS) $_4$ linker were associated with a stronger affinity for TGF β 1 and TGF β 3 as compared to a T β RII fusion protein where $n < 4$. As such, in preferred embodiments, $n \geq 4$, or $n = 4-10$. The application also teaches that proteins comprising (GGGGS) n linkers in which $n > 4$ had similar inhibitory properties as proteins having the (GGGGS) $_4$ linker. As

such, in some embodiments, n is not greater than 4 in a (GGGS)_n linker. In some embodiments, n = 4-10, 4-9, 4-8, 4-7, 4-6, 4-5, 5-8, 5-7, or 5-6. In some embodiments, n = 3, 4, 5, 6, or 7. In particular embodiments, n = 4. In some embodiments, a linker comprising a (GGGS)_n sequence also comprises an N-terminal threonine. In some embodiments, the

5 linker is any one of the following:

GGGSGGGGS (SEQ ID NO: 21)

TGGGSGGGGS (SEQ ID NO: 4)

TGGGSGGGSGGGGS (SEQ ID NO: 5)

TGGGSGGGSGGGSGGGGS (SEQ ID NO: 6)

10 TGGGSGGGSGGGSGGGSGGGGS (SEQ ID NO: 25)

TGGGSGGGSGGGSGGGSGGGSGGGGS (SEQ ID NO: 26) or

TGGGSGGGSGGGSGGGSGGGSGGGSGGGGS (SEQ ID NO: 40).

In some embodiments, the linker comprises the amino acid sequence of TGGGPKSCDK (SEQ ID NO: 7). In some embodiments, the linker is any one of SEQ ID NOs: 21, 4-7, 25-26
15 or 40 lacking the N-terminal threonine. In some embodiments, the linker does not comprise the amino acid sequence of SEQ ID NO: 26 or 40.

In certain aspects, functional variants or modified forms of the T β R_{II} (or betaglycan) polypeptides include fusion proteins having at least a portion of the T β R_{II} (or betaglycan) polypeptides and one or more heterologous portions. Well-known examples of such
20 heterologous portions 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 heterologous portion may be selected so as to confer a desired property. For example, some heterologous portions are particularly useful for isolation of the fusion proteins by affinity
25 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™ system (Qiagen) useful with (HIS₆) fusion partners. As another example, a heterologous portion may be selected so as to facilitate
30 detection of the T β R_{II} (or betaglycan) 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 heterologous

portions have a protease 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 heterologous portion by subsequent chromatographic separation. In certain preferred embodiments, a T β RII (or betaglycan) polypeptide is fused with a domain that stabilizes the T β RII polypeptide in vivo (a "stabilizer" domain). By "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 heterologous portions that may be selected include multimerizing (e.g., dimerizing, tetramerizing) domains and functional domains.

As specific examples, the present disclosure provides fusion proteins comprising variants of T β RII polypeptides fused to an Fc domain sequence of SEQ ID NO: 49.

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 γ 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.

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 β RII (or betaglycan) polypeptide may be placed C-terminal to a heterologous domain, or, alternatively, a heterologous domain may be placed C-terminal to a T β RII (or betaglycan) polypeptide. The T β RII (or betaglycan) 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.

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. In some embodiments, the immunoglobulin Fc region is a human immunoglobulin Fc region.

In one embodiment, the class of immunoglobulin from which the heavy chain constant region is derived is IgG (Ig γ) (γ subclasses 1, 2, 3, or 4).

An example of a native amino acid sequence that may be used for the Fc portion of human IgG1 (G1Fc) is shown below (SEQ ID NO: 58). Dotted underline indicates the hinge region, and solid underline indicates positions with naturally occurring variants. In part, the disclosure provides polypeptides comprising, consisting essential of, or consisting of amino acid sequences with 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 58. Naturally occurring variants in G1Fc would include E134D and M136L according to the numbering system used in SEQ ID NO: 58 (see Uniprot P01857).

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1  THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE
51 VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK
101 VSNKALPAPI EKTISKAKGQ PREPQVYTL PSREEMTKNQ VSLTCLVKGF
151 YPSDIAVEWE SNGQPENNYK TTPVLDSDG SFFLYSKLTV DKSRWQQGNV
20 201 FSCSVMEAL HNHYTQKSLS LSPGK (SEQ ID NO: 58)

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Optionally, the IgG1 Fc domain has one or more mutations at residues such as Asp-265, lysine 322, and Asn-434. In certain cases, the mutant IgG1 Fc domain having one or more of these mutations (*e.g.*, Asp-265 mutation) has reduced ability of binding to the Fc γ receptor relative to a wild-type 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 wild-type IgG1 Fc domain.

An example of a native amino acid sequence that may be used for the Fc portion of human IgG2 (G2Fc) is shown below (SEQ ID NO: 59). Dotted underline indicates the hinge region and double underline indicates positions where there are data base conflicts in the sequence (according to UniProt P01859). In part, the disclosure provides polypeptides comprising, consisting essential of, or consisting of amino acid sequences with 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 59.

1 VECPPCPAPP VAGPSVFLFP PKPKDTLMIS RTPEVTCVVV DVSHEDPEVQ
 51 FNWYVDGVEV HNAKTKPREE QFNSTFRVVS VLTVVHQDWL NGKEYKCKVS
 101 NKGLPAPIEK TISKTKGQPR EPQVYTLPPS REEMTKNQVS LTCLVKGFYP
 151 SDIAVEWESN GQPENNYKTT PPMLDSDGSF FLYSKLTVDK SRWQQGNVFS
 5 201 CSVMHEALHN HYTQKSLSLS PGK (SEQ ID NO: 59)

Two examples of amino acid sequences that may be used for the Fc portion of human IgG3 (G3Fc) are shown below. The hinge region in G3Fc can be up to four times as long as in other Fc chains and contains three identical 15-residue segments preceded by a similar 17-residue segment. The first G3Fc sequence shown below (SEQ ID NO: 60) contains a short hinge region consisting of a single 15-residue segment, whereas the second G3Fc sequence (SEQ ID NO: 61) contains a full-length hinge region. In each case, dotted underline indicates the hinge region, and solid underline indicates positions with naturally occurring variants according to UniProt P01859. In part, the disclosure provides polypeptides comprising, consisting essential of, or consisting of amino acid sequences with 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%,
 15 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NOs: 60 or 61.

1 EPKSCDTPPP CPRCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD
 51 VSHEDPEVQF KQYVDGVEVH NAKTKPREEQ YNSTFRVVS V LTVLHQDWLN
 101 GKEYKCKVSN KALPAPIEKT ISKTKGQPRE PQVYTLPPSR EEMTKNQVSL
 151 TCLVKGFYPS DIAVEWESSG QPENNYNTTP PMLDSDGSFF LYSKLTVDKS
 20 201 RWQQGNIFSC SVMHEALHNR FTQKSLSLSP GK (SEQ ID NO: 60)

1 ELKTPLGDTT HTCPRCPEPK SCDTPPPCPR CPEPKSCDTP PPCPRCPEPK
 51 SCDTPPPCPR CPAPELLGGP SVFLFPPKPK DTLMISRTPE VTCVVVDVSH
 101 EDPEVQFKWY VDGVEVHNAK TKPREEQYNS TFRVVS VLTV LHQDWLNNGKE
 25 151 YKCKVSNKAL PAPIEKTISK TKGQPREPQV YTLPPSREEM TKNQVSLTCL
 201 VKGFYPSDIA VEWESSGQPE NNYNTTPPML DSDGSFFLYS KLTVDKSRWQ
 251 QGNIFSCSVM HEALHNRFTQ KLSLSLSPGK (SEQ ID NO: 61)

Naturally occurring variants in G3Fc (for example, see Uniprot P01860) include E68Q, P76L, E79Q, Y81F, D97N, N100D, T124A, S169N, S169del, F221Y when converted to the numbering system used in SEQ ID NO: 60, and the present disclosure provides fusion proteins comprising G3Fc domains containing one or more of these variations. In addition, the human immunoglobulin IgG3 gene (*IGHG3*) shows a structural polymorphism characterized by different hinge lengths [see Uniprot P01859]. Specifically, variant WIS is lacking most of the V region and all of the CH1 region. It has an extra interchain disulfide bond at position 7 in addition to the 11 normally present in the hinge region. Variant ZUC
 35

lacks most of the V region, all of the CH1 region, and part of the hinge. Variant OMM may represent an allelic form or another gamma chain subclass. The present disclosure provides additional fusion proteins comprising G3Fc domains containing one or more of these variants.

5 An example of a native amino acid sequence that may be used for the Fc portion of human IgG4 (G4Fc) is shown below (SEQ ID NO: 62). Dotted underline indicates the hinge region. In part, the disclosure provides polypeptides comprising, consisting essential of, or consisting of amino acid sequences with 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 62.

10 1 ESKYGPPCPS_CPAPEFLGGP SVFLFPPKPK DTLMISRTPE VTCVVVDVSQ
 51 EDPEVQFNWY VDGVEVHNAK TKPREEQFNS TYRVVSVLTV LHQDWLNGKE
 101 YKCKVSNKGL PSSIEKTISK AKGQPREPQV YTLPPSQEEM TKNQVSLTCL
 151 VKGFYPSDIA VEWESNGQPE NNYKTTTPVL DSDGSFFLYS RLTVDKSRWQ
 201 EGNVFSCSVM HEALHNHYTQ KSLSLSLGK (SEQ ID NO: 62)

15 A variety of engineered mutations in the Fc domain are presented herein with respect to the G1Fc sequence (SEQ ID NO: 58), and analogous mutations in G2Fc, G3Fc, and G4Fc can be derived from their alignment with G1Fc in Figure 7. Due to unequal hinge lengths, analogous Fc positions based on isotype alignment (Figure 7) possess different amino acid numbers in SEQ ID NOs: 58, 59, 60, 61, and 62. It can also be appreciated that a given
 20 amino acid position in an immunoglobulin sequence consisting of hinge, CH2, and CH3 regions (*e.g.*, SEQ ID NOs: 58, 59, 60, 61, and 62) will be identified by a different number than the same position when numbering encompasses the entire IgG1 heavy-chain constant domain (consisting of the CH1, hinge, CH2, and CH3 regions) as in the Uniprot database.

Other classes of immunoglobulin, IgA (Ig α), IgD (Ig δ), IgE (Ig ϵ) and IgM (Ig μ), may
 25 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 DNA construct encoding the immunoglobulin Fc region preferably
 30 comprises at least a portion of a hinge domain, and preferably at least a portion of a CH3 domain of Fc gamma or the homologous domains in any of IgA, IgD, IgE, or IgM.

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 methods

and compositions disclosed herein. One example would be to introduce amino acid substitutions in the upper CH2 region to create an Fc variant with reduced affinity for Fc receptors (Cole *et al.* (1997) J. Immunol. 159:3613).

In some embodiments, the disclosure provides for T β R II polypeptides fusion proteins comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of any one of SEQ ID NOs: 11, 13, 15 and 17, or biologically active fragments thereof. In some embodiments, the T β R II polypeptides fusion proteins comprise an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of any one of SEQ ID NOs: 11, 13, and 15, or biologically active fragments thereof. In some embodiments, the T β R II polypeptides fusion proteins comprise an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of any one of SEQ ID NO: 13, or a biologically active fragment thereof. In some embodiments, the T β R II polypeptides fusion proteins comprise an amino acid sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of any one of SEQ ID NO: 63, or a biologically active fragment thereof. In some embodiments, the T β R II polypeptides fusion proteins comprise an amino acid sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of any one of SEQ ID NO: 48, or a biologically active fragment thereof. In some embodiments, the T β R II polypeptides fusion proteins comprise an amino acid sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of any one of SEQ ID NO: 64, or a biologically active fragment thereof. In some embodiments, the T β R II polypeptides fusion proteins comprise an amino acid sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of any one of SEQ ID NO: 65, or a biologically active fragment thereof. In some embodiments, the T β R II polypeptides fusion proteins comprise an amino acid sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of any one of SEQ ID NO: 66, or a biologically active fragment thereof. In some embodiments, the T β R II polypeptides fusion proteins comprise an amino acid sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of any one of SEQ ID NO: 67, or a biologically active fragment thereof. In some embodiments, the T β R II polypeptides fusion proteins comprise an amino acid sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of any one of SEQ

ID NO: 68, or a biologically active fragment thereof. In some embodiments, the T β RII polypeptides fusion protein comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO: 49, or a biologically active fragment thereof.

5 In some embodiments, the fusion proteins described herein have improved binding affinity for TGF β 1 and TGF β 3. In some embodiments, a fusion protein comprising a linker at least 10 amino acids in length (*e.g.*, a fusion protein having the amino acid sequence of any one of SEQ ID NOs: 11, 13, 15, 48, and 63-68) has improved binding affinity for TGF β 1 and TGF β 3 as compared to a reference fusion protein (*e.g.*, a fusion protein having the amino acid
10 sequence of SEQ ID NO: 9). In some embodiments, the fusion protein binds to TGF β 1 with a K_D of less than 200 pM, less than 150 pM, less than 100 pM, less than 75 pM, less than 50 pM or less than 25 pM. In some embodiments, the fusion protein binds to TGF β 3 with a K_D of less than 75 pM, less than 70 pM, less than 60 pM, less than 50 pM, less than 40 pM, less than 35 pM, less than 25 pM, less than 15, less than 10, or less than 5 pM.

15 In some embodiments any of the polypeptides disclosed herein inhibits TGF β 1 and/or TGF β 3 in a measurable assay. In some embodiments, the polypeptide inhibits TGF β 1 with an IC₅₀ of less than 1.0, 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2, 0.1, 0.08, 0.09, 0.07, 0.06, 0.05, 0.04, 0.03, or 0.02 nM, as determined using a reporter gene assay. In some embodiments, the polypeptide inhibits TGF β 3 with an IC₅₀ of less than 1.0, 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2,
20 0.1, 0.09, 0.08, 0.07, 0.06, 0.05, 0.04, 0.03, or 0.02 nM, as determined using a reporter gene assay. In some embodiments, the reporter gene assay is a CAGA reporter assay. In some embodiments, the CAGA 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
25 is present in the promoters of TGF β -responsive genes (for example, PAI-1), so this vector is of general use for factors signaling through SMAD2 and SMAD3. *See, e.g.*, Example 2.

In some embodiments, the disclosure provides for T β RII (or betaglycan) containing fusion polypeptides. The fusion polypeptides may be prepared according to any of the methods disclosed herein or that are known in the art.

30 In some embodiments, any of the fusion polypeptides disclosed herein comprises the following components: a) any of the T β RII (or betaglycan) polypeptides disclosed herein (“A”), b) any of the linkers disclosed herein (“B”), c) any of the heterologous portions disclosed herein (“C”), and optionally a linker (“X”). In such embodiments, the fusion polypeptide may be arranged in a manner as follows (N-terminus to C-terminus): A-B-C or

C-B-A. In such embodiments, the fusion polypeptide may be arranged in a manner as follows (N-terminus to C-terminus): X-A-B-C or X-C-B-A. In some embodiments, the fusion polypeptide comprises each of A, B and C (and optionally a leader sequence such as the amino acid sequence of SEQ ID NO: 23), and comprises no more than 100, 90, 80, 70,
5 60, 50, 40, 30, 20, 10, 5, 4, 3, 2 or 1 additional amino acids (but which may include further post-translational modifications, such as PEGylation).

In some embodiments, the fusion polypeptide comprises a leader sequence (*e.g.*, SEQ ID NO: 23) positioned in a manner as follows (N-terminus to C-terminus): X-A-B-C, and the fusion polypeptide comprises 1, 2, 3, 4, or 5 amino acids between X and A. In some
10 embodiments, the fusion polypeptide comprises a leader sequence (*e.g.*, SEQ ID NO: 23) positioned in a manner as follows (N-terminus to C-terminus): X-C-B-A, and the fusion polypeptide comprises 1, 2, 3, 4, or 5 amino acids between X and C. In some embodiments, the fusion polypeptide comprises a leader sequence (*e.g.*, SEQ ID NO: 23) positioned in a manner as follows (N-terminus to C-terminus): X-A-B-C, and the fusion polypeptide
15 comprises an alanine between X and A. In some embodiments, the fusion polypeptide comprises a leader sequence (*e.g.*, SEQ ID NO: 23) positioned in a manner as follows (N-terminus to C-terminus): X-C-B-A, and the fusion polypeptide comprises an alanine between X and C. In some embodiments, the fusion polypeptide comprises a leader sequence (*e.g.*, SEQ ID NO: 23) positioned in a manner as follows (N-terminus to C-terminus): X-A-B-C,
20 and the fusion polypeptide comprises a glycine and an alanine between X and A. In some embodiments, the fusion polypeptide comprises a leader sequence (*e.g.*, SEQ ID NO: 23) positioned in a manner as follows (N-terminus to C-terminus): X-C-B-A, and the fusion polypeptide comprises a glycine and an alanine between X and C. In some embodiments, the fusion polypeptide comprises a leader sequence (*e.g.*, SEQ ID NO: 23) positioned in a
25 manner as follows (N-terminus to C-terminus): X-A-B-C, and the fusion polypeptide comprises a threonine between X and A. In some embodiments, the fusion polypeptide comprises a leader sequence (*e.g.*, SEQ ID NO: 23) positioned in a manner as follows (N-terminus to C-terminus): X-C-B-A, and the fusion polypeptide comprises a threonine between X and C.

30 In some embodiments, the fusion polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to any of the T β R β II polypeptide amino acid sequences disclosed herein (*e.g.*, SEQ ID NO: 18), wherein the T β R β II polypeptide portion of the fusion polypeptide comprises no more than 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 additional amino acids (but which may include further

post-translational modifications, such as PEGylation). In some embodiments, the fusion polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to any of the linker sequences disclosed herein (*e.g.*, SEQ ID NO: 6), wherein the linker portion of the fusion polypeptide comprises no more than 5, 4, 3, 2 or 1 additional amino acids (but which may include further post-translational modifications, such as PEGylation). In some embodiments, the fusion polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to any of the heterologous portion sequences disclosed herein (*e.g.*, SEQ ID NO: 49), wherein the heterologous portion of the fusion polypeptide comprises no more than 25, 20, 15, 10, 5, 4, 3, 2, or 1 additional amino acids (but which may include further post-translational modifications, such as PEGylation). In some embodiments, the fusion polypeptide comprises any of the T β R β II polypeptide amino acid sequences disclosed herein (*e.g.*, SEQ ID NO: 18), wherein the T β R β II polypeptide portion of the fusion polypeptide comprises no more than 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 additional amino acids (but which may include further post-translational modifications, such as PEGylation). In some embodiments, the fusion polypeptide comprises any of the linker sequences disclosed herein (*e.g.*, SEQ ID NO: 6), wherein the linker portion of the fusion polypeptide comprises no more than 5, 4, 3, 2 or 1 additional amino acids (but which may include further post-translational modifications, such as PEGylation). In some embodiments, the fusion polypeptide comprises any of the heterologous portion sequences disclosed herein (*e.g.*, SEQ ID NO: 49), wherein the heterologous portion of the fusion polypeptide comprises no more than 25, 20, 15, 10, 5, 4, 3, 2, or 1 additional amino acids (but which may include further post-translational modifications, such as PEGylation).

In some embodiments, the disclosure provides for a fusion polypeptide, wherein the fusion polypeptide consists or consists essentially of (and not necessarily in the following order): a) an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to any of the T β R β II polypeptide amino acid sequences disclosed herein (*e.g.*, SEQ ID NO: 18), wherein the T β R β II polypeptide portion of the fusion polypeptide comprises no more than 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 additional amino acids (but which may include further post-translational modifications, such as PEGylation); b) an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to any of the linker sequences disclosed herein (*e.g.*, SEQ ID NO: 6), wherein the linker portion of the fusion polypeptide

comprises no more than 5, 4, 3, 2 or 1 additional amino acids (but which may include further post-translational modifications, such as PEGylation); and c) an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to any of the heterologous portion sequences disclosed herein (*e.g.*, SEQ ID NO: 49), wherein the heterologous portion of the fusion polypeptide comprises no more than 25, 20, 15, 10, 5, 4, 3, 2, or 1 additional amino acids (but which may include further post-translational modifications, such as PEGylation); and d) optionally a leader sequence (*e.g.*, SEQ ID NO: 23). In some embodiments, the disclosure provides for a fusion polypeptide, wherein the fusion polypeptide consists or consists essentially of (and not necessarily in the following order): a) any of the T β R β II polypeptide amino acid sequences disclosed herein (*e.g.*, SEQ ID NO: 18), wherein the T β R β II polypeptide portion of the fusion polypeptide comprises no more than 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 additional amino acids (but which may include further post-translational modifications, such as PEGylation); b) any of the linker sequences disclosed herein (*e.g.*, SEQ ID NO: 6), wherein the linker portion of the fusion polypeptide comprises no more than 5, 4, 3, 2 or 1 additional amino acids (but which may include further post-translational modifications, such as PEGylation); and c) any of the heterologous portion sequences disclosed herein (*e.g.*, SEQ ID NO: 49), wherein the heterologous portion of the fusion polypeptide comprises no more than 25, 20, 15, 10, 5, 4, 3, 2, or 1 additional amino acids (but which may include further post-translational modifications, such as PEGylation); and d) optionally a leader sequence (*e.g.*, SEQ ID NO: 23).

In some embodiments, the disclosure provides for a fusion polypeptide consisting of or consisting essentially of (and not necessarily in the following order): a) a T β R β II polypeptide portion consisting of an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO: 18 and no more than 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 additional amino acids (but which may include further post-translational modifications, such as PEGylation); b) a linker portion consisting of an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO: 6 and no more than 5, 4, 3, 2 or 1 additional amino acids (but which may include further post-translational modifications, such as PEGylation); and c) a heterologous portion consisting of an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO: 49 and no more than 25, 20, 15, 10, 5, 4, 3, 2, or 1 additional amino

acids (but which may include further post-translational modifications, such as PEGylation); and d) optionally a leader sequence (*e.g.*, SEQ ID NO: 23). In some embodiments, the disclosure provides for a fusion polypeptide consisting or consisting essentially of (and not necessarily in the following order): a) a T β RII polypeptide portion consisting of the amino acid sequence of SEQ ID NO: 18 and no more than 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 additional amino acids (but which may include further post-translational modifications, such as PEGylation); b) a linker portion consisting of the amino acid sequence of SEQ ID NO: 6 and no more than 5, 4, 3, 2 or 1 additional amino acids (but which may include further post-translational modifications, such as PEGylation); and c) a heterologous portion consisting of the amino acid sequence of SEQ ID NO: 49 and no more than 25, 20, 15, 10, 5, 4, 3, 2, or 1 additional amino acids (but which may include further post-translational modifications, such as PEGylation); and d) optionally a leader sequence (*e.g.*, SEQ ID NO: 23).

In some embodiments, the fusion protein does not comprise a leader sequence. In some embodiments, the fusion protein comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO: 48.

TIPPHVQKSDVEMEAQKDEIICPSCNRHTAHLRHHINNDMIIVTDNNGAVKFPQLCKFCDVRFSTCDNQK
SCMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAAASPKCIMKEKKKPGETF
FMCSCSSDECNDNIIFSEYNTSNPDTGGGGSGGGSGGGSGGGSGGGSTHTCPPCPAPELLGGPSVFLF
PPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVHLQ
DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAV
EWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVSCSVMHEALHNHYTQKSLSLSPGK
(SEQ ID NO: 48)

In some embodiments, the disclosure provides for a T β RII fusion polypeptide wherein the polypeptide does not comprise an antibody or antigen-binding portion thereof. In some embodiments, the polypeptide does not bind with appreciable affinity to a cytokine other than a transforming growth factor beta superfamily ligand (*e.g.*, TGF β 1, TGF β 2 and/or TGF β 3). In some embodiments, the polypeptide does not bind with appreciable affinity to a cytokine other than TGF β 1, TGF β 2 and/or TGF β 3. In some embodiments, the polypeptide does not bind with appreciable affinity to a cytokine other than TGF β 1 and/or TGF β 3. In some embodiments, the polypeptide does not bind with appreciable affinity to CD4, CD8, CD25, CTLA-4, IL-10, TGF β Receptor, PD-1, PD-L1, PD-L2, RANK, RANKL, HER2/neu, EGFR1, CD20, VEGF, TNF- α , TNFR2, FoxP3, CD80, CD86, IFN- α , IFN- β , IFN- γ , GITR, 4-1BB, OX-40, TLR1-10, ErbB-1, HER1, ErbB-3/HER3, ErbB-4/HER4, IGFR, IGFBP, IGF-1R, PDGFR, FGFR, VEGFR, HGFR, TRK receptor, ephrin receptors, AXL receptors, LTK receptors, TIE receptors, angiopoietin1, 2, ROR receptor, DDR receptor, RET receptor,

KLG receptor, RYK receptor, MuSK receptor, IL β R, IL α R, TNFRSF, TRAIL receptor, ARTC1, alpha-actinin-4, Bcr-abl, B-Raf, caspases, beta-catenin, fibronectin, GPNMB, GDP-L, LDLR, HLA-A2, MLA-A11, HSP70, KIAA205, MART2, MUM-1, 2, 3, PAP, neo-PAP, NFYC, OGT, OS-9, pml-RARalpha fusion protein, PRDX5, PTPRK, KRAS2, NRAS, HRAS, RBAF600, SIRT2, SNRPD1, SYT-SSX1 or -SSX2 fusion protein, Triosephosphate Isomerase, BAGE, BAGE-1, BAGE-2, 3, 4, 5, GAGE-1, 2, 3, 4, 5, 6, 7, 8, GnT-V, HERV-K MEL, KK-LC, KM-HN-1, LAGE, LAGE-1, CAMEL, MAGE-1, MAGE-A2, MAGE-A3, MAGE-A4, MAGE-AS, MAGE-A6, MAGE-A8, MAGE-A9, MAGE-A10, MAGE-A11, MAGE-A12, MAGE-3, MAGE-B1, MAGE-B2, MAGE-B5, MAGE-B6, MAGE-C1, MAGE-C2, mucin 1 (MUC1), MART-1/Melan-A (MLANA), gp100, gp100/Pmel17 (S1LV), tyrosinase (TYR), TRP-1, HAGE, NA-88, NY-ESO-1, NY-ESO-1/LAGE-2, SAGE, Sp17, SSX-1, 2, 3, 4, TRP2-INT2, carcino-embryonic antigen (CEA), Kallikrein 4, mammaglobin-A, OA1, prostate specific antigen (PSA), prostate specific membrane antigen, TRP-1/, 75, TRP-2, AIM-2, BING-4, CPSF, cyclin D1, Ep-CAM, EpcA3, FGF-5, gp250, iCE), AFP, M-CSF, mdm-2, MUC1, p53 (TP53), PBF, FRAME, PSMA, RAGE-1, RNF43, RU2AS, SOX10, STEAP1, survivin (BIRC5), hTERT, telomerase, WT1, SYCP1, BRDT, SPANX, XAGE, ADAM2, PAGE-5, LIP1, CTAGE-1, CSAGE, MMA1, CAGE, BORIS, HOM-TES-85, AF15q14, HCA66I, LDHC, MORC, SGY-1, SPO11, TPX1, NY-SAR-35, FTHL17, NXF2 TDRD1, TEX 15, FATE, TPTE, estrogen receptors (ER), androgen receptors (AR), CD40, CD30, CD20, CD19, CD33, CD4, CD25, CD3, CA 72-4, CA 15-3, CA 27-29, CA 125, CA 19-9, beta-human chorionic gonadotropin, 1-2 microglobulin, squamous cell carcinoma antigen, neuron-specific enolase, heat shock protein gp96, GM2, sargramostim, CTLA-4, 707-AP, ART-4, CAP-1, CLCA2, Cyp-B, HST-2, HPV proteins, EBV proteins, Hepatitis B or C virus proteins, and/or HIV proteins.

In some embodiments, the disclosure provides for a T β RII fusion polypeptide wherein the polypeptide does not comprise an additional ligand binding domain in addition to the T β RII domain. In some embodiments, the polypeptide comprises a linear amino acid sequence comprising a T β RII domain and a heterologous portion (e.g., an Fc portion), but the linear amino acid sequence does not comprise any additional ligand binding domains. In some embodiments, the polypeptide comprises a linear amino acid sequence comprising a T β RII domain and an Fc portion, but the linear amino acid sequence does not comprise any additional ligand binding domains. In some embodiments, the disclosure provides for a T β RII fusion polypeptide wherein the polypeptide does not comprise multiple ligand binding domains in a single linear amino acid sequence. In some embodiments, the disclosure

provides for a T β R β II fusion polypeptide wherein the polypeptide does not comprise more than one continuous linker sequence in a single linear amino acid sequence. In some embodiments, the polypeptide does not comprise multiple continuous glycine and/or serine linkers (e.g., a linker comprising (GGGGS) n , wherein $n = > 4$) in a single linear amino acid sequence. In some embodiments, the disclosure provides for a T β R β II fusion polypeptide wherein the heterologous portion is an Fc domain, and wherein only one continuous linker is covalently bound to the Fc domain. In some embodiments, the only one continuous linker comprises or consists of a (GGGGS) n linker, wherein $n = > 4$.

The application further provides Fc fusion proteins with engineered or 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 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-translational processes of the antibodies and Fc fusion proteins, such as changing the number or position of glycosylation sites.

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, 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 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 leucine to alanine at position 235. The

antibody or Fc fusion protein may alternatively or additionally carry other mutations, including the point mutation K322A in the CH2 domain (Hezareh et al. 2001 J Virol. 75: 12161-8).

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, 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 complement-mediated cell killing. See Caron et al., J. Exp Med. 176:1191-1195 (1992) and Shopes, 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.

B. Nucleic Acids and Methods of Manufacture

In certain embodiments, the present disclosure makes available isolated and/or purified forms of polypeptides (e.g., T β RII or betaglycan polypeptides), 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 polypeptide species. Polypeptides will generally be produced by expression from recombinant nucleic acids.

In certain embodiments, the disclosure includes nucleic acids encoding soluble polypeptides (e.g., T β RII or betaglycan polypeptides) comprising the coding sequence for an extracellular portion of a protein (e.g., a T β RII or betaglycan 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 the present disclosure further pertain to methods of producing the polypeptides.

In certain aspects, the disclosure provides isolated and/or recombinant nucleic acids encoding any of the polypeptides (e.g., T β RII or betaglycan polypeptides), including fragments, functional variants and fusion proteins disclosed herein. SEQ ID NOs: 8, 10, 12, 14, 16, 46 or 47 encode variants of T β RII extracellular domain fused to an IgG 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 polypeptides or as direct therapeutic agents (*e.g.*, in an antisense, RNAi or gene therapy approach).

5 In certain aspects, the subject nucleic acids encoding polypeptides are further understood to include nucleic acids that are variants of SEQ ID NOs: 8, 10, 12, 14, 16, 46 or 47. Variant nucleotide sequences include sequences that differ by one or more nucleotide substitutions, additions or deletions, such as allelic variants.

10 In certain embodiments, the disclosure provides isolated or recombinant nucleic acid sequences that are at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NOs: 8, 10, 12, 14, 16, 46 or 47. In particular embodiments, the disclosure provides isolated or recombinant nucleic acid sequences that are at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 12, or fragments thereof. One of ordinary skill in the art will
15 appreciate that nucleic acid sequences complementary to SEQ ID NOs: 8, 10, 12, 14, 16, 46 or 47, and variants of SEQ ID NOs: 8, 10, 12, 14, 16, 46 or 47 are also within the scope of this disclosure. In further embodiments, the nucleic acid sequences of the disclosure can be isolated, recombinant, and/or fused with a heterologous nucleotide sequence, or in a DNA library.

20 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 NOs: 8, 10, 12, 14, 16, 46 or 47 complement sequences of SEQ ID NOs: 8, 10, 12, 14, 16, 46 or 47, or fragments thereof. As discussed above, one of ordinary skill in the art will understand readily that appropriate stringency conditions which promote
25 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 temperature in the wash step can be increased from low stringency conditions at
30 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.

Isolated nucleic acids which differ from the nucleic acids as set forth in SEQ ID NOs: 8, 10, 12, 14, 16, 46 or 47 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.

It will be appreciated by one of skill in the art that corresponding variants based on the long isoform of T β R II 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 β R II will include variant nucleotide sequences comprising an insertion of 108 nucleotides, encoding a 36-amino-acid insertion (SEQ ID NO: 41), at the same location described for naturally occurring T β R II isoform C.

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.

In certain aspects disclosed herein, the subject nucleic acid is provided in an expression vector comprising a nucleotide sequence encoding a polypeptide (e.g., T β R II or betaglycan polypeptide) and operably linked to at least one regulatory sequence. Regulatory sequences are art-recognized and are selected to direct expression of the T 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 polypeptide (e.g., T β R II or betaglycan 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 α -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 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 be considered.

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 polypeptide (e.g., T β R II or betaglycan 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*.

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 β -gal containing pBlueBac III).

In certain embodiments, a vector will be designed for production of the subject polypeptides (e.g., T β RII or betaglycan polypeptidea) in CHO cells, such as a Pcmv-Script vector (Stratagene, La Jolla, Calif.), pcDN4 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 polypeptides in HEK-293 cells. As will be apparent, the subject gene constructs can be used to cause expression of the subject polypeptides in cells propagated in culture, e.g., to produce proteins, including fusion proteins or variant proteins, for purification.

This disclosure also pertains to a host cell transfected with a recombinant gene including a coding sequence (e.g., SEQ ID NOS: 8, 10, 12, 14, 16, 46 or 47) for one or more of the subject polypeptides (e.g., T β RII or betaglycan polypeptides). The host cell may be any prokaryotic or eukaryotic cell. For example, a T β 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.

Accordingly, the present disclosure further pertains to methods of producing the subject polypeptides (e.g., T β RII or betaglycan polypeptides). For example, a host cell transfected with an expression vector encoding a polypeptide can be cultured under appropriate conditions to allow expression of the polypeptide to occur. The polypeptide may be secreted and isolated from a mixture of cells and medium containing the polypeptide. Alternatively, the 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 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 polypeptides and affinity purification with an agent that binds to a domain fused to the polypeptide (e.g., a protein A column may be used to purify an Fc fusion). In a preferred embodiment, the 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.

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 polypeptide (e.g., T β RII or betaglycan polypeptide), can allow purification of the expressed fusion protein by affinity chromatography using a Ni²⁺ metal resin. The purification leader sequence can then be subsequently removed by treatment with enterokinase to provide the purified polypeptide (e.g., see Hochuli et al., (1987) *J. Chromatography* 411:177; and Janknecht et al., *PNAS USA* 88:8972).

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 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 be annealed to generate a chimeric gene sequence (see, for example, *Current Protocols in Molecular Biology*, eds. Ausubel et al., John Wiley & Sons: 1992).

5

C. Antibodies

In certain aspects, a TGF β antagonist to be used in accordance with the methods and uses disclosed herein is an antibody (TGF β antagonist antibody) or combination of antibodies. A TGF β antagonist antibody, or combination of antibodies, may inhibit and/or
10 bind to, for example, one or more ligands (e.g., TGF β 1, TGF β 2, and TGF β 3), T β RII receptor, T β RII-associated type I receptor (e.g., ALK5), and/or T β RII co-receptor (e.g., betaglycan). In some embodiments, the ability for a TGF β antagonist antibody, or combination of antibody, to inhibit signaling (e.g., Smad signaling) and/or bind to a target is determined in an in vitro or cell-based assay including, for example, those described herein. As described
15 herein, a TGF β antagonist antibody, or combination of antagonist antibodies, may be used alone or in combination with one or more additional supportive therapies or active agents to treat heterotopic ossification, preferably preventing or reducing the severity or duration of one or more complications of heterotopic ossification

In certain embodiments, a TGF β antagonist antibody, or combination of antibodies, is
20 an antibody that inhibits at least TGF β 1. Therefore, in some embodiments, a TGF β antagonist antibody, or combination of antibodies, binds to at least TGF β 1. As used herein, a TGF β 1 antibody (anti-TGF β 1 antibody) generally refers to an antibody that is capable of binding to TGF β 1 with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting TGF β 1. In certain embodiments, the extent of binding
25 of an anti-TGF β 1 antibody to an unrelated, non-TGF β 1 protein is less than about 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or less than 1% of the binding of the antibody to TGF β 1 as measured, for example, by a radioimmunoassay (RIA). In certain embodiments, an anti-TGF β 1 antibody binds to an epitope of TGF β 1 that is conserved among TGF β 1 from different species. In certain preferred embodiments, an anti-TGF β 1 antibody binds to human
30 TGF β 1. In some embodiments, a TGF β 1 antibody may inhibit TGF β 1 from binding to a type I, type II, and/or co-receptor (e.g., T β RII, ALK5, and/or betaglycan) and thus inhibit TGF β 1 signaling (e.g., Smad signaling). It should be noted that TGF β 1 shares some sequence

homology to TGFβ2 and TGFβ3. Therefore antibodies that bind TGFβ1, in some embodiments, may also bind to TGFβ2 and/or TGFβ3. In some embodiments, the disclosure relates to a multispecific antibody (e.g., bi-specific antibody), and uses thereof, that binds to TGFβ1 and further binds to, for example, one or more additional ligands (e.g., TGFβ2, TGFβ3, or TGFβ2 and TGFβ3), one or more type I and/or type II receptors (e.g., TβRII and ALK5), and/or one or more co-receptors (e.g., betaglycan). In some embodiments, a multispecific antibody that binds to TGFβ1 further binds to TGFβ3 but does not bind or does not substantially bind to TGFβ2 (e.g., binds to TGFβ2 with a K_D of greater than 1×10^{-7} M or has relatively modest binding, e.g., about 1×10^{-8} M or about 1×10^{-9} M). In some embodiments, the disclosure relates to combinations of antibodies, and uses thereof, wherein the combination of antibodies comprises a TGFβ1 antibody and one or more additional antibodies that bind to, for example, one or more additional ligands (e.g., TGFβ2, TGFβ3, or TGFβ2 and TGFβ3), one or more type I and/or type II receptors (e.g., TβRII and ALK5), and/or one or more co-receptors (e.g., betaglycan). In some embodiments, a combination of antibodies that comprises a TGFβ1 antibody further comprises a TGFβ3 antibody but does not comprise a TGFβ2 antibody.

In certain embodiments, a TGFβ antagonist antibody, or combination of antibodies, is an antibody that inhibits at least TGFβ2. Therefore, in some embodiments, a Tβ TGFβ RII antagonist antibody, or combination of antibodies, binds to at least TGFβ2. As used herein, a TGFβ2 antibody (anti-TGFβ2 antibody) generally refers to an antibody that is capable of binding to TGFβ2 with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting TGFβ2. In certain embodiments, the extent of binding of an anti-TGFβ2 antibody to an unrelated, non-TGFβ2 protein is less than about 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or less than 1% of the binding of the antibody to TGFβ2 as measured, for example, by a radioimmunoassay (RIA). In certain embodiments, an anti-TGFβ2 antibody binds to an epitope of TGFβ2 that is conserved among TGFβ2 from different species. In certain preferred embodiments, an anti-TGFβ2 antibody binds to human TGFβ2. In some embodiments, a TGFβ2 antibody may inhibit TGFβ2 from binding to a type I, type II, and/or co-receptor (e.g., TβRII, ALK5, and/or betaglycan) and thus inhibit TGFβ2 signaling (e.g., Smad signaling). It should be noted that TGFβ2 shares some sequence homology to TGFβ1 and TGFβ3. Therefore antibodies that bind TGFβ2, in some embodiments, may also bind to TGFβ1 and/or TGFβ3. In some embodiments, the disclosure relates to a multispecific antibody (e.g., bi-specific antibody), and uses thereof, that binds to

TGFβ2 and further binds to, for example, one or more additional ligands (e.g., TGFβ1, TGFβ3, or TGFβ1 and TGFβ3), one or more type I and/or type II receptors (e.g., TβRII and ALK5), and/or one or more co-receptors (e.g., betaglycan). In some embodiments, the disclosure relates to combinations of antibodies, and uses thereof, wherein the combination of antibodies comprises a TGFβ2 antibody and one or more additional antibodies that bind to, for example, one or more additional ligands (e.g., TGFβ1, TGFβ3, or TGFβ1 and TGFβ3), one or more type I and/or type II receptors (e.g., TβRII and ALK5), and/or one or more co-receptors (e.g., betaglycan).

In certain embodiments, a TGFβ antagonist antibody, or combination of antibodies, is an antibody that inhibits at least TGFβ3. Therefore, in some embodiments, a TGFβ antagonist antibody, or combination of antibodies, binds to at least TGFβ3. As used herein, a TGFβ3 antibody (anti-TGFβ3 antibody) generally refers to an antibody that is capable of binding to TGFβ3 with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting TGFβ3. In certain embodiments, the extent of binding of an anti-TGFβ3 antibody to an unrelated, non-TGFβ3 protein is less than about 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or less than 1% of the binding of the antibody to TGFβ3 as measured, for example, by a radioimmunoassay (RIA). In certain embodiments, an anti-TGFβ3 antibody binds to an epitope of TGFβ3 that is conserved among TGFβ3 from different species. In certain preferred embodiments, an anti-TGFβ3 antibody binds to human TGFβ3. In some embodiments, a TGFβ3 antibody may inhibit TGFβ3 from binding to a type I, type II, and/or co-receptor (e.g., TβRII, ALK5, and/or betaglycan) and thus inhibit TGFβ3 signaling (e.g., Smad signaling). It should be noted that TGFβ3 shares some sequence homology to TGFβ2 and TGFβ1. Therefore antibodies that bind TGFβ3, in some embodiments, may also bind to TGFβ2 and/or TGFβ1. In some embodiments, the disclosure relates to a multispecific antibody (e.g., bi-specific antibody), and uses thereof, that binds to TGFβ3 and further binds to, for example, one or more additional TβRII ligands (e.g., TGFβ2, TGFβ1, or TGFβ2 and TGFβ1), one or more type I and/or type II receptors (e.g., TβRII and ALK5), and/or one or more co-receptors (e.g., betaglycan). In some embodiments, a multispecific antibody that binds to TGFβ3 does not bind or does not substantially bind to TGFβ2 (e.g., binds to TGFβ2 with a K_D of greater than 1×10^{-7} M or has relatively modest binding, e.g., about 1×10^{-8} M or about 1×10^{-9} M). In some embodiments, a multispecific antibody that binds to TGFβ3 further binds to TGFβ1 but does not bind or does not substantially bind to TGFβ2 (e.g., binds to TGFβ2 with a K_D of greater than 1×10^{-7} M or has

relatively modest binding, e.g., about 1×10^{-8} M or about 1×10^{-9} M). In some embodiments, the disclosure relates to combinations of antibodies, and uses thereof, wherein the combination of antibodies comprises a TGF β 3 antibody and one or more additional antibodies that bind to, for example, one or more additional ligands (e.g., TGF β 2, TGF β 1, or
5 TGF β 2 and TGF β 1), one or more type I and/or type II receptors (e.g., T β RII and ALK5), and/or one or more co-receptors (e.g., betaglycan). In some embodiments, a combination of antibodies that comprises a TGF β 3 antibody further comprises a TGF β 1 antibody but does not comprise a TGF β 2 antibody.

In some embodiments, the TGF β antagonist antibody binds to all three TGF β
10 isoforms, i.e., TGF β 1, TGF β 2, and TGF β 3. In some embodiments the TGF β antagonist antibody is fresolimumab.

In certain aspects, a TGF β antagonist antibody, or combination of antibodies, is an antibody that inhibits at least T β RII. Therefore, in some embodiments, a TGF β antagonist antibody, or combination of antibodies, binds to at least T β RII. As used herein, a T β RII
15 antibody (anti-T β RII antibody) generally refers to an antibody that binds to T β RII with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting T β RII. In certain embodiments, the extent of binding of an anti-T β RII antibody to an unrelated, non-T β RII protein is less than about 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or less than about 1% of the binding of the antibody to T β RII as measured, for example, by a
20 radioimmunoassay (RIA), Biacore, or other protein-protein interaction or binding affinity assay. In certain embodiments, an anti-T β RII antibody binds to an epitope of T β RII that is conserved among T β RII from different species. In certain preferred embodiments, an anti-T β RII antibody binds to human T β RII. In some embodiments, an anti-T β RII antibody may inhibit one or more ligands [e.g., TGF β 1; TGF β 2; TGF β 3; TGF β 1 and TGF β 3; TGF β 1 and
25 TGF β 2; TGF β 2 and TGF β 3; or TGF β 1, TGF β 2, and TGF β 3] from binding to T β RII. In some embodiments, an anti-T β RII antibody is a multispecific antibody (e.g., bi-specific antibody) that binds to T β RII and one or more ligands [e.g., TGF β 1, TGF β 2, and TGF β 3], type I receptor (e.g., ALK5), and/or co-receptor (e.g., betaglycan). In some embodiments, the disclosure relates to combinations of antibodies, and uses thereof, wherein the combination of
30 antibodies comprises an anti-T β RII antibody and one or more additional antibodies that bind to, for example, one or more ligands [e.g., TGF β 1, TGF β 2, and TGF β 3], type I receptors (e.g., ALK5), and/or co-receptor (e.g., betaglycan).

In certain aspects, a TGF β antagonist antibody, or combination of antibodies, is an antibody that inhibits at least ALK5. Therefore, in some embodiments, a TGF β antagonist antibody, or combination of antibodies, binds to at least ALK5. As used herein, an ALK5 antibody (anti-ALK5 antibody) generally refers to an antibody that binds to ALK5 with
5 sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting ALK5. In certain embodiments, the extent of binding of an anti-ALK5 antibody to an unrelated, non-ALK5 protein is less than about 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or less than about 1% of the binding of the antibody to ALK5 as measured, for example, by a radioimmunoassay (RIA), Biacore, or other protein-protein interaction or binding affinity
10 assay. In certain embodiments, an anti-ALK5 antibody binds to an epitope of ALK5 that is conserved among ALK5 from different species. In certain preferred embodiments, an anti-ALK5 antibody binds to human ALK5. In some embodiments, an anti-ALK5 antibody may inhibit one or more ligands [*e.g.*, TGF β 1; TGF β 2; TGF β 3; TGF β 1 and TGF β 3; TGF β 1 and TGF β 2; TGF β 2 and TGF β 3; or TGF β 1, TGF β 2, and TGF β 3] from binding to ALK5. In
15 some embodiments, an anti-ALK5 antibody is a multispecific antibody (*e.g.*, bi-specific antibody) that binds to ALK5 and one or more ligands [*e.g.*, TGF β 1, TGF β 2, and TGF β 3], type II receptor (*e.g.*, T β RII), and/or co-receptor (*e.g.*, betaglycan). In some embodiments, the disclosure relates to combinations of antibodies, and uses thereof, wherein the combination of antibodies comprises an anti-ALK5 antibody and one or more additional
20 antibodies that bind to, for example, one or more ligands [*e.g.*, TGF β 1, TGF β 2, and TGF β 3], type II receptors (*e.g.*, T β RII), and/or co-receptor (*e.g.*, betaglycan).

In certain aspects, a TGF β antagonist antibody, or combination of antibodies, is an antibody that inhibits at least betaglycan. Therefore, in some embodiments, a TGF β antagonist antibody, or combination of antibodies, binds to at least betaglycan. As used
25 herein, a betaglycan antibody (anti-betaglycan antibody) generally refers to an antibody that binds to betaglycan with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting betaglycan. In certain embodiments, the extent of binding of an anti-betaglycan antibody to an unrelated, non-betaglycan protein is less than about 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or less than about 1% of the binding of the
30 antibody to betaglycan as measured, for example, by a radioimmunoassay (RIA), Biacore, or other protein-protein interaction or binding affinity assay. In certain embodiments, an anti-betaglycan antibody binds to an epitope of betaglycan that is conserved among betaglycan from different species. In certain preferred embodiments, an anti-betaglycan antibody binds

to human betaglycan. In some embodiments, an anti-betaglycan antibody may inhibit one or more ligands [e.g., TGF β 1; TGF β 2; TGF β 3; TGF β 1 and TGF β 3; TGF β 1 and TGF β 2; TGF β 2 and TGF β 3; or TGF β 1, TGF β 2, and TGF β 3] from binding to betaglycan. In some
embodiments, an anti-betaglycan antibody is a multispecific antibody (e.g., bi-specific
5 antibody) that binds to betaglycan and one or more ligands [e.g., TGF β 1, TGF β 2, and
TGF β 3], type I receptor (e.g., ALK5), and/or type II receptors (e.g., T β RII). In some
embodiments, the disclosure relates to combinations of antibodies, and uses thereof, wherein
the combination of antibodies comprises an anti-betaglycan antibody and one or more
additional antibodies that bind to, for example, one or more ligands [e.g., TGF β 1, TGF β 2,
10 and TGF β 3], type I receptors (e.g., ALK5), and/or type II receptors (e.g., T β RII).

The term antibody is used herein in the broadest sense and encompasses various
antibody structures, including but not limited to monoclonal antibodies, polyclonal
antibodies, multispecific antibodies (e.g., bispecific antibodies), and antibody fragments so
long as they exhibit the desired antigen-binding activity. An antibody fragment refers to a
15 molecule other than an intact antibody that comprises a portion of an intact antibody that
binds the antigen to which the intact antibody binds. Examples of antibody fragments
include but are not limited to Fv, Fab, Fab', Fab'-SH, F(ab')₂; diabodies; linear antibodies;
single-chain antibody molecules (e.g., scFv); and multispecific antibodies formed from
antibody fragments. See, e.g., Hudson *et al.* (2003) Nat. Med. 9:129-134; Plückthun, in The
20 Pharmacology of Monoclonal Antibodies, vol. 113, Rosenberg and Moore eds., (Springer-
Verlag, New York), pp. 269-315 (1994); WO 93/16185; and U.S. Pat. Nos. 5,571,894,
5,587,458, and 5,869,046. Antibodies disclosed herein may be polyclonal antibodies or
monoclonal antibodies. In certain embodiments, the antibodies of the present disclosure
comprise a label attached thereto and able to be detected (e.g., the label can be a radioisotope,
25 fluorescent compound, enzyme, or enzyme co-factor). In preferred embodiments, the
antibodies of the present disclosure are isolated antibodies. Diabodies are antibody fragments
with two antigen-binding sites that may be bivalent or bispecific. See, e.g., EP 404,097; WO
1993/01161; Hudson *et al.* (2003) Nat. Med. 9:129-134 (2003); and Hollinger *et al.* (1993)
Proc. Natl. Acad. Sci. USA 90: 6444-6448. Triabodies and tetrabodies are also described in
30 Hudson *et al.* (2003) Nat. Med. 9:129-134. Single-domain antibodies are antibody fragments
comprising all or a portion of the heavy-chain variable domain or all or a portion of the light-
chain variable domain of an antibody. In certain embodiments, a single-domain antibody is a
human single-domain antibody. See, e.g., U.S. Pat. No. 6,248,516. Antibody fragments can

be made by various techniques, including but not limited to proteolytic digestion of an intact antibody as well as production by recombinant host cells (e.g., *E. coli* or phage), as described herein.

The antibodies herein may be of any class. The class of an antibody refers to the type of constant domain or constant region possessed by its heavy chain. There are five major classes of antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), for example, IgG₁, IgG₂, IgG₃, IgG₄, IgA₁, and IgA₂. The heavy-chain constant domains that correspond to the different classes of immunoglobulins are called alpha, delta, epsilon, gamma, and mu.

In general, an antibody for use in the methods disclosed herein specifically binds to its target antigen, preferably with high binding affinity. Affinity may be expressed as a K_D value and reflects the intrinsic binding affinity (e.g., with minimized avidity effects). Typically, binding affinity is measured *in vitro*, whether in a cell-free or cell-associated setting. Any of a number of assays known in the art, including those disclosed herein, can be used to obtain binding affinity measurements including, for example, surface plasmon resonance (Biacore™ assay), radiolabeled antigen binding assay (RIA), and ELISA. In some embodiments, antibodies of the present disclosure bind to their target antigens (e.g. TGFβ1, TGFβ2, ALK5, betaglycan, and TβRII.) with at least a K_D of 1×10^{-7} or stronger, 1×10^{-8} or stronger, 1×10^{-9} or stronger, 1×10^{-10} or stronger, 1×10^{-11} or stronger, 1×10^{-12} or stronger, 1×10^{-13} or stronger, or 1×10^{-14} or stronger.

In certain embodiments, K_D is measured by RIA performed with the Fab version of an antibody of interest and its target antigen as described by the following assay. Solution binding affinity of Fabs for the antigen is measured by equilibrating Fab with a minimal concentration of radiolabeled antigen (e.g., ¹²⁵I-labeled) in the presence of a titration series of unlabeled antigen, then capturing bound antigen with an anti-Fab antibody-coated plate [see, e.g., Chen *et al.* (1999) *J. Mol. Biol.* 293:865-881]. To establish conditions for the assay, multi-well plates (e.g., MICROTITER® from Thermo Scientific) are coated (e.g., overnight) with a capturing anti-Fab antibody (e.g., from Cappel Labs) and subsequently blocked with bovine serum albumin, preferably at room temperature (e.g., approximately 23°C). In a non-adsorbent plate, radiolabeled antigen are mixed with serial dilutions of a Fab of interest [e.g., consistent with assessment of the anti-VEGF antibody, Fab-12, in Presta *et al.*, (1997) *Cancer Res.* 57:4593-4599]. The Fab of interest is then incubated, preferably overnight but the incubation may continue for a longer period (e.g., about 65 hours) to ensure that equilibrium

is reached. Thereafter, the mixtures are transferred to the capture plate for incubation, preferably at room temperature for about one hour. The solution is then removed and the plate is washed times several times, preferably with polysorbate 20 and PBS mixture. When the plates have dried, scintillant (*e.g.*, MICROSCINT[®] from Packard) is added, and the plates
5 are counted on a gamma counter (*e.g.*, TOPCOUNT[®] from Packard).

According to another embodiment, K_D is measured using surface plasmon resonance assays using, for example a BIACORE[®] 2000 or a BIACORE[®] 3000 (Biacore, Inc., Piscataway, N.J.) with immobilized antigen CM5 chips at about 10 response units (RU). Briefly, carboxymethylated dextran biosensor chips (CM5, Biacore, Inc.) are activated with
10 N-ethyl-N'-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) according to the supplier's instructions. For example, an antigen can be diluted with 10 mM sodium acetate, pH 4.8, to 5 $\mu\text{g/ml}$ (about 0.2 μM) before injection at a flow rate of 5 $\mu\text{l/minute}$ to achieve approximately 10 response units (RU) of coupled protein. Following the injection of antigen, 1 M ethanolamine is injected to block
15 unreacted groups. For kinetics measurements, two-fold serial dilutions of Fab (0.78 nM to 500 nM) are injected in PBS with 0.05% polysorbate 20 (TWEEN-20[®]) surfactant (PBST) at a flow rate of approximately 25 $\mu\text{l/min}$. Association rates (k_{on}) and dissociation rates (k_{off}) are calculated using, for example, a simple one-to-one Langmuir binding model (BIACORE[®] Evaluation Software version 3.2) by simultaneously fitting the association and dissociation
20 sensorgrams. The equilibrium dissociation constant (K_D) is calculated as the ratio $k_{\text{off}} / k_{\text{on}}$ [see, *e.g.*, Chen *et al.*, (1999) J. Mol. Biol. 293:865-881]. If the on-rate exceeds, for example, $10^6 \text{ M}^{-1} \text{ s}^{-1}$ by the surface plasmon resonance assay above, then the on-rate can be determined by using a fluorescent quenching technique that measures the increase or decrease in
25 fluorescence emission intensity (*e.g.*, excitation=295 nm; emission=340 nm, 16 nm band-pass) of a 20 nM anti-antigen antibody (Fab form) in PBS in the presence of increasing concentrations of antigen as measured in a spectrometer, such as a stop-flow equipped spectrophotometer (Aviv Instruments) or a 8000-series SLM-AMINCO[®] spectrophotometer (ThermoSpectronic) with a stirred cuvette.

The nucleic acid and amino acid sequences of T β RII, ALK5, betaglycan, TGF β 1, TGF β 2, and TGF β 3, particularly human sequences, are well known in the art and thus
30 antibody antagonists for use in accordance with this disclosure may be routinely made by the skilled artisan based on the knowledge in the art and teachings provided herein.

In certain embodiments, an antibody provided herein is a chimeric antibody. A chimeric antibody refers to an antibody in which a portion of the heavy and/or light chain is derived from a particular source or species, while the remainder of the heavy and/or light chain is derived from a different source or species. Certain chimeric antibodies are described, for example, in U.S. Pat. No. 4,816,567; and Morrison *et al.*, (1984) Proc. Natl. Acad. Sci. USA, 81:6851-6855. In some embodiments, a chimeric antibody comprises a non-human variable region (*e.g.*, a variable region derived from a mouse, rat, hamster, rabbit, or non-human primate, such as a monkey) and a human constant region. In some embodiments, a chimeric antibody is a "class switched" antibody in which the class or subclass has been changed from that of the parent antibody. In general, chimeric antibodies include antigen-binding fragments thereof.

In certain embodiments, a chimeric antibody provided herein is a humanized antibody. A humanized antibody refers to a chimeric antibody comprising amino acid residues from non-human hypervariable regions (HVRs) and amino acid residues from human framework regions (FRs). In certain embodiments, a humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the HVRs (*e.g.*, CDRs) correspond to those of a non-human antibody, and all or substantially all of the FRs correspond to those of a human antibody. A humanized antibody optionally may comprise at least a portion of an antibody constant region derived from a human antibody. A "humanized form" of an antibody, *e.g.*, a non-human antibody, refers to an antibody that has undergone humanization.

Humanized antibodies and methods of making them are reviewed, for example, in Almagro and Fransson (2008) Front. Biosci. 13:1619-1633 and are further described, for example, in Riechmann *et al.*, (1988) Nature 332:323-329; Queen *et al.* (1989) Proc. Nat'l Acad. Sci. USA 86:10029-10033; U.S. Pat. Nos. 5,821,337, 7,527,791, 6,982,321, and 7,087,409; Kashmiri *et al.*, (2005) Methods 36:25-34 [describing SDR (a-CDR) grafting]; Padlan, Mol. Immunol. (1991) 28:489-498 (describing "resurfacing"); Dall'Acqua *et al.* (2005) Methods 36:43-60 (describing "FR shuffling"); Osbourn *et al.* (2005) Methods 36:61-68; and Klimka *et al.* Br. J. Cancer (2000) 83:252-260 (describing the "guided selection" approach to FR shuffling).

Human framework regions that may be used for humanization include but are not limited to: framework regions selected using the "best-fit" method [see, *e.g.*, Sims *et al.* (1993) J. Immunol. 151:2296]; framework regions derived from the consensus sequence of

human antibodies of a particular subgroup of light-chain or heavy-chain variable regions [see, *e.g.*, Carter *et al.* (1992) Proc. Natl. Acad. Sci. USA, 89:4285; and Presta *et al.* (1993) J. Immunol., 151:2623]; human mature (somatically mutated) framework regions or human germline framework regions [see, *e.g.*, Almagro and Fransson (2008) Front. Biosci. 13:1619-1633]; and framework regions derived from screening FR libraries [see, *e.g.*, Baca *et al.* (1997) J. Biol. Chem. 272:10678-10684; and Rosok *et al.* (1996) J. Biol. Chem. 271:22611-22618].

In certain embodiments, an antibody provided herein is a human antibody. Human antibodies can be produced using various techniques known in the art. Human antibodies are described generally in van Dijk and van de Winkel (2001) Curr. Opin. Pharmacol. 5: 368-74 and Lonberg (2008) Curr. Opin. Immunol. 20:450-459.

Human antibodies may be prepared by administering an immunogen (*e.g.* a T β R2, ALK5, betaglycan, TGF β 1, TGF β 2, or TGF β 3 polypeptide) to a transgenic animal that has been modified to produce intact human antibodies or intact antibodies with human variable regions in response to antigenic challenge. Such animals typically contain all or a portion of the human immunoglobulin loci, which replace the endogenous immunoglobulin loci, or which are present extrachromosomally or integrated randomly into the animal's chromosomes. In such transgenic animals, the endogenous immunoglobulin loci have generally been inactivated. For a review of methods for obtaining human antibodies from transgenic animals, see, for example, Lonberg (2005) Nat. Biotechnol. 23:1117-1125; U.S. Pat. Nos. 6,075,181 and 6,150,584 (describing XENOMOUSE™ technology); U.S. Pat. No. 5,770,429 (describing HuMab® technology); U.S. Pat. No. 7,041,870 (describing K-MOUSE® technology); and U.S. Patent Application Publication No. 2007/0061900 (describing VelociMouse® technology). Human variable regions from intact antibodies generated by such animals may be further modified, for example, by combining with a different human constant region.

Human antibodies provided herein can also be made by hybridoma-based methods. Human myeloma and mouse-human heteromyeloma cell lines for the production of human monoclonal antibodies have been described [see, *e.g.*, Kozbor J. Immunol., (1984) 133: 3001; Brodeur *et al.* (1987) Monoclonal Antibody Production Techniques and Applications, pp. 51-63, Marcel Dekker, Inc., New York; and Boerner *et al.* (1991) J. Immunol., 147: 86]. Human antibodies generated via human B-cell hybridoma technology are also described in Li *et al.*, (2006) Proc. Natl. Acad. Sci. USA, 103:3557-3562. Additional methods include those

described, for example, in U.S. Pat. No. 7,189,826 (describing production of monoclonal human IgM antibodies from hybridoma cell lines) and Ni, Xiandai Mianyixue (2006) 26(4):265-268 (2006) (describing human-human hybridomas). Human hybridoma technology (Trioma technology) is also described in Vollmers and Brandlein (2005) Histol. Histopathol., 20(3):927-937 (2005) and Vollmers and Brandlein (2005) Methods Find Exp. Clin. Pharmacol., 27(3):185-91.

Human antibodies provided herein may also be generated by isolating Fv clone variable-domain sequences selected from human-derived phage display libraries. Such variable-domain sequences may then be combined with a desired human constant domain.

10 Techniques for selecting human antibodies from antibody libraries are described herein.

For example, antibodies of the present disclosure may be isolated by screening combinatorial libraries for antibodies with the desired activity or activities. A variety of methods are known in the art for generating phage-display libraries and screening such libraries for antibodies possessing the desired binding characteristics. Such methods are reviewed, for example, in Hoogenboom *et al.* (2001) in *Methods in Molecular Biology* 178:1-37, O'Brien *et al.*, ed., Human Press, Totowa, N.J. and further described, for example, in the McCafferty *et al.* (1991) *Nature* 348:552-554; Clackson *et al.*, (1991) *Nature* 352: 624-628; Marks *et al.* (1992) *J. Mol. Biol.* 222:581-597; Marks and Bradbury (2003) in *Methods in Molecular Biology* 248:161-175, Lo, ed., Human Press, Totowa, N.J.; Sidhu *et al.* (2004) *J. Mol. Biol.* 338(2):299-310; Lee *et al.* (2004) *J. Mol. Biol.* 340(5):1073-1093; Fellouse (2004) *Proc. Natl. Acad. Sci. USA* 101(34):12467-12472; and Lee *et al.* (2004) *J. Immunol. Methods* 284(1-2): 119-132.

In certain phage display methods, repertoires of VH and VL genes are separately cloned by polymerase chain reaction (PCR) and recombined randomly in phage libraries, which can then be screened for antigen-binding phage as described in Winter *et al.* (1994) *Ann. Rev. Immunol.*, 12: 433-455. Phage typically display antibody fragments, either as single-chain Fv (scFv) fragments or as Fab fragments. Libraries from immunized sources provide high-affinity antibodies to the immunogen (*e.g.*, a T β RII, TGF β 1, TGF β 2, or TGF β 3 polypeptide) without the requirement of constructing hybridomas. Alternatively, the naive repertoire can be cloned (*e.g.*, from human) to provide a single source of antibodies directed against a wide range of non-self and also self-antigens without any immunization as described by Griffiths *et al.* (1993) *EMBO J*, 12: 725-734. Finally, naive libraries can also be made synthetically by cloning un-rearranged V-gene segments from stem cells and using

PCR primers containing random sequence to encode the highly variable CDR3 regions and to accomplish rearrangement in vitro, as described by Hoogenboom and Winter (1992) J. Mol. Biol., 227: 381-388. Patent publications describing human antibody phage libraries include, for example: U.S. Pat. No. 5,750,373, and U.S. Patent Publication Nos. 2005/0079574,
5 2005/0119455, 2005/0266000, 2007/0117126, 2007/0160598, 2007/0237764, 2007/0292936, and 2009/0002360.

In certain embodiments, an antibody provided herein is a multispecific antibody, for example, a bispecific antibody. Multispecific antibodies (typically monoclonal antibodies) have binding specificities for at least two different epitopes (*e.g.*, two, three, four, five, or six
10 or more) on one or more (*e.g.*, two, three, four, five, six or more) antigens.

Engineered antibodies with three or more functional antigen binding sites, including "octopus antibodies," are also included herein (see, *e.g.*, US 2006/0025576A1).

In certain embodiments, the antibodies disclosed herein are monoclonal antibodies. Monoclonal antibody refers to an antibody obtained from a population of substantially
15 homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical and/or bind the same epitope, except for possible variant antibodies, *e.g.*, containing naturally occurring mutations or arising during production of a monoclonal antibody preparation, such variants generally being present in minor amounts. In contrast to polyclonal antibody preparations, which typically include different antibodies directed
20 against different epitopes, each monoclonal antibody of a monoclonal antibody preparation is directed against a single epitope on an antigen. Thus, the modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the
25 present methods may be made by a variety of techniques, including but not limited to the hybridoma method, recombinant DNA methods, phage-display methods, and methods utilizing transgenic animals containing all or part of the human immunoglobulin loci, such methods and other exemplary methods for making monoclonal antibodies being described herein.

30 For example, by using immunogens derived from T β R2, anti-protein/anti-peptide antisera or monoclonal antibodies can be made by standard protocols [see, *e.g.*, Antibodies: A Laboratory Manual (1988) ed. by Harlow and Lane, Cold Spring Harbor Press]. A mammal,

such as a mouse, hamster, or rabbit can be immunized with an immunogenic form of the T β RII 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 techniques well known in the art. An immunogenic
5 portion of a T β 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 antibody production and/or level of binding affinity.

Following immunization of an animal with an antigenic preparation of T β RII, antisera
10 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 [see, *e.g.*, Kohler
15 and Milstein (1975) *Nature*, 256: 495-497], the human B cell hybridoma technique [see, *e.g.*, 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 T β RII
20 polypeptide, and monoclonal antibodies isolated from a culture comprising such hybridoma cells.

In certain embodiments, one or more amino acid modifications may be introduced into the Fc region of an antibody provided herein thereby generating an Fc-region variant. The Fc-region variant may comprise a human Fc-region sequence (*e.g.*, a human IgG1, IgG2,
25 IgG3 or IgG4 Fc region) comprising an amino acid modification (*e.g.*, a substitution, deletion, and/or addition) at one or more amino acid positions.

For example, the present disclosure contemplates an antibody variant that possesses some but not all effector functions, which make it a desirable candidate for applications in which the half-life of the antibody *in vivo* is important yet for which certain effector functions
30 [*e.g.*, complement-dependent cytotoxicity (CDC) and antibody-dependent cellular cytotoxicity (ADCC)] are unnecessary or deleterious. *In vitro* and/or *in vivo* cytotoxicity assays can be conducted to confirm the reduction/depletion of CDC and/or ADCC activities. For example, Fc receptor (FcR) binding assays can be conducted to ensure that the antibody

lacks FcγR binding (hence likely lacking ADCC activity), but retains FcRn binding ability. The primary cells for mediating ADCC, NK cells, express FcγRIII only, whereas monocytes express FcγRI, FcγRII and FcγRIII. FcR expression on hematopoietic cells is summarized in, for example, Ravetch and Kinet (1991) *Annu. Rev. Immunol.* 9:457-492. Non-limiting

5 examples of *in vitro* assays to assess ADCC activity of a molecule of interest are described in U.S. Pat. No. 5,500,362; Hellstrom, I. *et al.* (1986) *Proc. Nat'l Acad. Sci. USA* 83:7059-7063; Hellstrom, I *et al.* (1985) *Proc. Nat'l Acad. Sci. USA* 82:1499-1502; U.S. Pat. No. 5,821,337; and Bruggemann, M. *et al.* (1987) *J. Exp. Med.* 166:1351-1361. Alternatively, non-

10 radioactive assay methods may be employed (*e.g.*, ACTI™, non-radioactive cytotoxicity assay for flow cytometry; CellTechnology, Inc. Mountain View, Calif.; and CytoTox 96® non-radioactive cytotoxicity assay, Promega, Madison, Wis.). Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and natural killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed *in vivo*, for example, in an animal model such as that disclosed in Clynes *et al.* (1998) *Proc.*

15 *Nat'l Acad. Sci. USA* 95:652-656. C1q binding assays may also be carried out to confirm that the antibody is unable to bind C1q and hence lacks CDC activity [see, *e.g.*, C1q and C3c binding ELISA in WO 2006/029879 and WO 2005/100402]. To assess complement activation, a CDC assay may be performed [see, *e.g.*, Gazzano-Santoro *et al.* (1996) *J. Immunol. Methods* 202:163; Cragg, M. S. *et al.* (2003) *Blood* 101:1045-1052; and Cragg, M.

20 S, and M. J. Glennie (2004) *Blood* 103:2738-2743]. FcRn binding and *in vivo* clearance/half-life determinations can also be performed using methods known in the art [see, *e.g.*, Petkova, S. B. *et al.* (2006) *Int. Immunol.* 18(12):1759-1769].

Antibodies of the present disclosure with reduced effector function include those with substitution of one or more of Fc region residues 238, 265, 269, 270, 297, 327 and 329 (U.S.

25 Pat. No. 6,737,056). Such Fc mutants include Fc mutants with substitutions at two or more of amino acid positions 265, 269, 270, 297 and 327, including the so-called "DANA" Fc mutant with substitution of residues 265 and 297 to alanine (U.S. Pat. No. 7,332,581).

In certain embodiments, it may be desirable to create cysteine-engineered antibodies, *e.g.*, "thioMAbs," in which one or more residues of an antibody are substituted with cysteine

30 residues. In particular embodiments, the substituted residues occur at accessible sites of the antibody. By substituting those residues with cysteine, reactive thiol groups are thereby positioned at accessible sites of the antibody and may be used to conjugate the antibody to other moieties, such as drug moieties or linker-drug moieties, to create an immunoconjugate,

as described further herein. In certain embodiments, any one or more of the following residues may be substituted with cysteine: V205 (Kabat numbering) of the light chain; A118 (EU numbering) of the heavy chain; and S400 (EU numbering) of the heavy-chain Fc region. Cysteine engineered antibodies may be generated as described, for example., in U.S. Pat. No. 7,521,541.

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

In certain embodiments, amino acid sequence variants of the antibodies and/or the binding polypeptides provided herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibody and/or binding polypeptide. Amino acid sequence variants of an antibody and/or binding polypeptides may be prepared by introducing appropriate modifications into the nucleotide sequence encoding the antibody and/or binding polypeptide, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into, and/or substitutions of residues within, the amino acid sequences of the antibody and/or binding polypeptide. Any combination of deletion, insertion, and substitution can be made to arrive at the final construct, provided that the final construct possesses the desired characteristics, *e.g.*, target-binding (TβRII, ALK5, betaglycan, TGFβ1, TGFβ2, and/or TGFβ3).

Alterations (*e.g.*, substitutions) may be made in HVRs, for example, to improve antibody affinity. Such alterations may be made in HVR "hotspots," *i.e.*, residues encoded by codons that undergo mutation at high frequency during the somatic maturation process (see, *e.g.*, Chowdhury (2008) *Methods Mol. Biol.* 207:179-196 (2008)), and/or SDRs (a-CDRs), with the resulting variant VH or VL being tested for binding affinity. Affinity maturation by constructing and reselecting from secondary libraries has been described in the art [see, *e.g.*, Hoogenboom *et al.*, in *Methods in Molecular Biology* 178:1-37, O'Brien *et al.*, ed., Human Press, Totowa, N.J., (2001)]. In some embodiments of affinity maturation, diversity is

introduced into the variable genes chosen for maturation by any of a variety of methods (*e.g.*, error-prone PCR, chain shuffling, or oligonucleotide-directed mutagenesis). A secondary library is then created. The library is then screened to identify any antibody variants with the desired affinity. Another method to introduce diversity involves HVR-directed approaches, in which several HVR residues (*e.g.*, 4-6 residues at a time) are randomized. HVR residues involved in antigen binding may be specifically identified, *e.g.*, using alanine scanning mutagenesis or modeling. CDR-H3 and CDR-L3 in particular are often targeted.

In certain embodiments, substitutions, insertions, or deletions may occur within one or more HVRs so long as such alterations do not substantially reduce the ability of the antibody to bind to the antigen. For example, conservative alterations (*e.g.*, conservative substitutions as provided herein) that do not substantially reduce binding affinity may be made in HVRs. Such alterations may be outside of HVR "hotspots" or SDRs. In certain embodiments of the variant VH and VL sequences provided above, each HVR either is unaltered, or contains no more than one, two, or three amino acid substitutions.

A useful method for identification of residues or regions of the antibody and/or the binding polypeptide that may be targeted for mutagenesis is called "alanine scanning mutagenesis", as described by Cunningham and Wells (1989) *Science*, 244:1081-1085. In this method, a residue or group of target residues (*e.g.*, charged residues such as arg, asp, his, lys, and glu) are identified and replaced by a neutral or negatively charged amino acid (*e.g.*, alanine or polyalanine) to determine whether the interaction of the antibody or binding polypeptide with antigen is affected. Further substitutions may be introduced at the amino acid locations demonstrating functional sensitivity to the initial substitutions. Alternatively, or additionally, a crystal structure of an antigen-antibody complex can be used to identify contact points between the antibody and antigen. Such contact residues and neighboring residues may be targeted or eliminated as candidates for substitution. Variants may be screened to determine whether they contain the desired properties.

Amino-acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an antibody with an N-terminal methionyl residue. Other insertional variants of the antibody molecule include fusion of the N- or C-terminus of the antibody to an enzyme (*e.g.*, for ADEPT) or a polypeptide which increases the serum half-life of the antibody.

In certain embodiments, an antibody and/or binding polypeptide provided herein may be further modified to contain additional non-proteinaceous moieties that are known in the art and readily available. The moieties suitable for derivatization of the antibody and/or binding polypeptide include but are not limited to water-soluble polymers. Non-limiting examples of water-soluble polymers include, but are not limited to, polyethylene glycol (PEG), copolymers of ethylene glycol/propylene glycol, carboxymethylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone, poly-1,3-dioxolane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, polyaminoacids (either homopolymers or random copolymers), and dextran or poly(n-vinyl pyrrolidone)polyethylene glycol, propylene glycol homopolymers, polypropylene oxide/ethylene oxide co-polymers, polyoxyethylated polyols (e.g., glycerol), polyvinyl alcohol, and mixtures thereof. Polyethylene glycol propionaldehyde may have advantages in manufacturing due to its stability in water. The polymer may be of any molecular weight, and may be branched or unbranched. The number of polymers attached to the antibody and/or binding polypeptide may vary, and if more than one polymer are attached, they can be the same or different molecules. In general, the number and/or type of polymers used for derivatization can be determined based on considerations including, but not limited to, the particular properties or functions of the antibody and/or binding polypeptide to be improved, whether the antibody derivative and/or binding polypeptide derivative will be used in a therapy under defined conditions.

D. Small Molecules

In certain aspects, a TGF β antagonist to be used in accordance with the methods and uses disclosed herein is a small molecule (TGF β antagonist small molecule) or combination of small molecules. A TGF β antagonist small molecule, or combination of small molecules, may inhibit, for example, one or more ligands (e.g., TGF β 1, TGF β 2, and TGF β 3), T β RII receptor, T β RII-associated type I receptor (e.g., ALK5), T β RII-associated co-receptor (e.g., betaglycan), and/or downstream signaling component (e.g., Smads). In some embodiments, the ability for a TGF β antagonist small molecule, or combination of small molecules, to inhibit signaling (e.g., Smad signaling) is determined in a cell-based assay including, for example, those described herein. A TGF β antagonist small molecule, or combination of small molecules, may be used alone or in combination with one or more additional supportive

therapies or active agents to treat heterotopic ossification, preferably preventing or reducing the severity or duration of one or more complications of heterotopic ossification.

In certain aspects, a TGF β antagonist small molecule, or combination of small molecules, inhibits at least TGF β 1 (e.g., inhibition of Smad signaling). Therefore, in some
5 embodiments, a small molecule inhibitor of TGF β 1 binds to TGF β 1. In some embodiments, a small molecule inhibitor of TGF β 1 inhibits expression (e.g., transcription, translation, secretion, or combinations thereof) of TGF β 1. In some embodiments, a small molecule inhibitor of TGF β 1 further inhibits one or more of TGF β 2, TGF β 3, T β RII, ALK5, and betaglycan. In some embodiments, a small molecule inhibitor of TGF β 1 does not inhibit or
10 does not substantially inhibit TGF β 2. In some embodiments, a small molecule inhibitor of TGF β 1 further inhibits TGF β 3 but does not inhibit or does not substantially inhibit TGF β 2. In certain aspects, a TGF β antagonist small molecule, or combination of small molecules, inhibits at least TGF β 2 (e.g., inhibition of Smad signaling). Therefore, in some
embodiments, a small molecule inhibitor of TGF β 2 binds to TGF β 2. In some embodiments,
15 a small molecule inhibitor of TGF β 2 inhibits expression (e.g., transcription, translation, secretion, or combinations thereof) of TGF β 2. In some embodiments, a small molecule inhibitor of TGF β 2 further inhibits one or more of TGF β 3, TGF β 1, T β RII, ALK5, and betaglycan. In certain aspects, a TGF β antagonist small molecule, or combination of small molecules, inhibits at least TGF β 3 (e.g., inhibition of Smad signaling). Therefore, in some
20 embodiments, a small molecule inhibitor of TGF β 3 binds to TGF β 3. In some embodiments, a small molecule inhibitor of TGF β 3 inhibits expression (e.g., transcription, translation, secretion, or combinations thereof) of TGF β 3. In some embodiments, a small molecule inhibitor of TGF β 3 further inhibits one or more of TGF β 2, TGF β 1, T β RII, ALK5, and betaglycan. In some embodiments, a small molecule inhibitor of TGF β 3 does not inhibit or
25 does not substantially inhibit TGF β 2. In some embodiments, a small molecule inhibitor of TGF β 3 further inhibits TGF β 1 but does not inhibit or does not substantially inhibit TGF β 2. In certain aspects, a TGF β antagonist small molecule, or combination of small molecules, inhibits at least T β RII (e.g., inhibition of Smad signaling). Therefore, in some embodiments, a small molecule inhibitor of T β RII binds to T β RII. In some embodiments, a small molecule
30 inhibitor of T β RII inhibits expression (e.g., transcription, translation, secretion, or combinations thereof) of T β RII. In some embodiments, a small molecule inhibitor of T β RII further inhibits one or more of TGF β 1, TGF β 2, TGF β 3, ALK5, and betaglycan. In certain aspects, a TGF β antagonist small molecule, or combination of small molecules, inhibits

TGFβ1 from binding to TβRII. In certain aspects, a TGFβ antagonist small molecule, or combination of small molecules, inhibits TGFβ2 from binding to TβRII. In certain aspects, a TGFβ antagonist small molecule, or combination of small molecules, inhibits TGFβ3 from binding to TβRII. In certain aspects, a TGFβ antagonist small molecule, or combination of small molecules, inhibits TGFβ1 and TGFβ3 from binding to TβRII. In certain aspects, a TGFβ antagonist small molecule, or combination of small molecules, inhibits TGFβ1, TGFβ2, and TGFβ3 from binding to TβRII. In certain aspects, a TGFβ antagonist small molecule, or combination of small molecules, inhibits TGFβ1 from binding to TβRII but does not inhibit or does not substantially inhibit TGFβ2 from binding to TβRII. In certain aspects, a TGFβ antagonist small molecule, or combination of small molecules, inhibits TGFβ3 from binding to TβRII but does not inhibit or does not substantially inhibit TGFβ2 from binding to TβRII. In certain aspects, a TGFβ antagonist small molecule, or combination of small molecules, inhibits TGFβ1 and TGFβ3 from binding to TβRII but does not inhibit or does not substantially inhibit TGFβ2 from binding to TβRII. In certain aspects, a Tβ TGFβ RII antagonist small molecule, or combination of small molecules, inhibits at least ALK5 (e.g., inhibition of Smad signaling). Therefore, in some embodiments, a small molecule inhibitor of ALK5 binds to ALK5. In some embodiments, a small molecule inhibitor of ALK5 inhibits expression (e.g., transcription, translation, secretion, or combinations thereof) of ALK5. In some embodiments, a small molecule inhibitor of ALK5 further inhibits one or more of TGFβ1, TGFβ2, TGFβ3, TβRII, and betaglycan. In certain aspects, a TGFβ antagonist small molecule, or combination of small molecules, inhibits TGFβ1 from binding to ALK5. In certain aspects, a TGFβ antagonist small molecule, or combination of small molecules, inhibits TGFβ2 from binding to ALK5. In certain aspects, a TGFβ antagonist small molecule, or combination of small molecules, inhibits TGFβ3 from binding to ALK5. In certain aspects, a TGFβ antagonist small molecule, or combination of small molecules, inhibits TGFβ1 and TGFβ3 from binding to ALK5. In certain aspects, a TGFβ antagonist small molecule, or combination of small molecules, inhibits TGFβ1, TGFβ2, and TGFβ3 from binding to ALK5. In certain aspects, a TGFβ antagonist small molecule, or combination of small molecules, inhibits TGFβ1 from binding to ALK5 but does not inhibit or does not substantially inhibit TGFβ2 from binding to ALK5. In certain aspects, a TGFβ antagonist small molecule, or combination of small molecules, inhibits TGFβ3 from binding to ALK5 but does not inhibit or does not substantially inhibit TGFβ2 from binding to ALK5. In certain aspects, a TGFβ antagonist small molecule, or combination of small molecules, inhibits TGFβ1 and TGFβ3 from binding to ALK5 but does not inhibit or does not

substantially inhibit TGF β 2 from binding to ALK5. In certain aspects, a TGF β antagonist small molecule, or combination of small molecules, inhibits at least betaglycan (e.g., inhibition of Smad signaling). Therefore, in some embodiments, a small molecule inhibitor of betaglycan binds to betaglycan. In some embodiments, a small molecule inhibitor of
5 betaglycan inhibits expression (e.g., transcription, translation, secretion, or combinations thereof) of betaglycan. In some embodiments, a small molecule inhibitor of betaglycan further inhibits one or more of TGF β 1, TGF β 2, TGF β 3, T β RII, and ALK5. In certain aspects, a TGF β antagonist small molecule, or combination of small molecules, inhibits TGF β 1 from binding to betaglycan. In certain aspects, a TGF β antagonist small molecule, or
10 combination of small molecules, inhibits TGF β 2 from binding to betaglycan. In certain aspects, a TGF β antagonist small molecule, or combination of small molecules, inhibits TGF β 3 from binding to betaglycan. In certain aspects, a TGF β antagonist small molecule, or combination of small molecules, inhibits TGF β 1 and TGF β 3 from binding to betaglycan. In
certain aspects, a TGF β antagonist small molecule, or combination of small molecules,
15 inhibits TGF β 1, TGF β 2, and TGF β 3 from binding to betaglycan. In certain aspects, a TGF β antagonist small molecule, or combination of small molecules, inhibits TGF β 1 from binding to betaglycan but does not inhibit or does not substantially inhibit TGF β 2 from binding to betaglycan. In certain aspects, a TGF β antagonist small molecule, or combination of small
molecules, inhibits TGF β 3 from binding to betaglycan but does not inhibit or does not
20 substantially inhibit TGF β 2 from binding to betaglycan. In certain aspects, a TGF β antagonist small molecule, or combination of small molecules, inhibits TGF β 1 and TGF β 3 from binding to betaglycan but does not inhibit or does not substantially inhibit TGF β 2 from
binding to betaglycan.

TGF β antagonist small molecules can be direct or indirect inhibitors. For example, a
25 TGF β antagonist small molecule, or combination of small molecules, may inhibit the expression (e.g., transcription, translation, cellular secretion, or combinations thereof) of at least one or more of T β RII, ALK5, betaglycan, TGF β 1, TGF β 2, TGF β 3, and/or one or more downstream signaling factors (Smads). Alternatively, a direct TGF β antagonist small
molecule, or combination of small molecules, may directly bind to, for example, one or more
30 of T β RII, ALK5, betaglycan, TGF β 1, TGF β 2, and TGF β 3 or one or more downstream signaling factors. Combinations of one or more indirect and one or more direct TGF β antagonist small molecule may be used in accordance with the methods disclosed herein.

Binding organic small molecule antagonists of the present disclosure may be identified and chemically synthesized using known methodology (*see, e.g.*, PCT Publication Nos. WO 00/00823 and WO 00/39585). In general, small molecule antagonists of the disclosure are usually less than about 2000 daltons in size, alternatively less than about 1500, 750, 500, 250 or 200 daltons in size, wherein such organic small molecules that are capable of binding, preferably specifically, to a polypeptide as described herein (*e.g.*, T β RII, ALK5, betaglycan, TGF β 1, TGF β 2, and TGF β 3). Such small molecule antagonists may be identified without undue experimentation using well-known techniques. In this regard, it is noted that techniques for screening organic small molecule libraries for molecules that are capable of binding to a polypeptide target are well-known in the art (*see, e.g.*, international patent publication Nos. WO00/00823 and WO00/39585).

Binding organic small molecules of the present disclosure may be, for example, aldehydes, ketones, oximes, hydrazones, semicarbazones, carbazides, primary amines, secondary amines, tertiary amines, N-substituted hydrazines, hydrazides, alcohols, ethers, thiols, thioethers, disulfides, carboxylic acids, esters, amides, ureas, carbamates, carbonates, ketals, thioketals, acetals, thioacetals, aryl halides, aryl sulfonates, alkyl halides, alkyl sulfonates, aromatic compounds, heterocyclic compounds, anilines, alkenes, alkynes, diols, amino alcohols, oxazolidines, oxazolines, thiazolidines, thiazolines, enamines, sulfonamides, epoxides, aziridines, isocyanates, sulfonyl chlorides, diazo compounds, and acid chlorides.

E. Nucleic Acids

In certain aspects, a TGF β antagonist to be used in accordance with the methods and uses disclosed herein is a polynucleotide (TGF β antagonist polynucleotide) or combination of polynucleotides. A TGF β antagonist polynucleotide, or combination of polynucleotides, may inhibit, for example, one or more ligands (*e.g.*, TGF β 1, TGF β 2, and TGF β 3), T β RII receptor, T β RII-associated type I receptor (*e.g.*, ALK5), T β RII-associated co-receptor (*e.g.*, betaglycan), and/or downstream signaling component (*e.g.*, Smads). In some embodiments, the ability for a TGF β antagonist polynucleotide, or combination of polynucleotides, to inhibit signaling (*e.g.*, Smad signaling) is determined in a cell-based assay including, for example, those described herein. A TGF β antagonist polynucleotide, or combination of polynucleotide, may be used, alone or in combination with one or more additional supportive

therapies or active agents, to treat heterotopic ossification, preferably preventing or reducing the severity or duration of one or more complications of heterotopic ossification.

In certain aspects, a TGF β antagonist polynucleotide, or combination of polynucleotides, inhibits at least TGF β 1 (e.g., inhibition of Smad signaling). Therefore, in
5 some embodiments, a polynucleotide inhibitor of TGF β 1 binds to TGF β 1. In some embodiments, a polynucleotide inhibitor of TGF β 1 inhibits expression (*e.g.*, transcription, translation, secretion, or combinations thereof) of TGF β 1. In some embodiments, a polynucleotide inhibitor of TGF β 1 further inhibits one or more of TGF β 2, TGF β 3, T β RII, ALK5, and betaglycan. In some embodiments, a polynucleotide inhibitor of TGF β 1 does not
10 inhibit or does not substantially inhibit TGF β 2. In some embodiments, a polynucleotide inhibitor of TGF β 1 further inhibits TGF β 3 but does not inhibit or does not substantially inhibit TGF β 2. In certain aspects, a TGF β antagonist polynucleotide, or combination of polynucleotides, inhibits at least TGF β 2 (e.g., inhibition of Smad signaling). Therefore, in some embodiments, a polynucleotide inhibitor of TGF β 2 binds to TGF β 2. In some
15 embodiments, a polynucleotide inhibitor of TGF β 2 inhibits expression (*e.g.*, transcription, translation, secretion, or combinations thereof) of TGF β 2. In some embodiments, a polynucleotide inhibitor of TGF β 2 further inhibits one or more of TGF β 3, TGF β 1, T β RII, ALK5, and betaglycan. In certain aspects, a TGF β antagonist polynucleotide, or combination of polynucleotides, inhibits at least TGF β 3 (e.g., inhibition of Smad signaling). Therefore, in
20 some embodiments, a polynucleotide inhibitor of TGF β 3 binds to TGF β 3. In some embodiments, a polynucleotide inhibitor of TGF β 3 inhibits expression (*e.g.*, transcription, translation, secretion, or combinations thereof) of TGF β 3. In some embodiments, a polynucleotide inhibitor of TGF β 3 further inhibits one or more of TGF β 2, TGF β 1, T β RII, ALK5, and betaglycan. In some embodiments, a polynucleotide inhibitor of TGF β 3 does not
25 inhibit or does not substantially inhibit TGF β 2. In some embodiments, a polynucleotide inhibitor of TGF β 3 further inhibits TGF β 1 but does not inhibit or does not substantially inhibit TGF β 2. In certain aspects, a TGF β antagonist polynucleotide, or combination of polynucleotides, inhibits at least T β RII (e.g., inhibition of Smad signaling). Therefore, in some embodiments, a polynucleotide inhibitor of T β RII binds to T β RII. In some
30 embodiments, a polynucleotide inhibitor of T β RII inhibits expression (*e.g.*, transcription, translation, secretion, or combinations thereof) of T β RII. In some embodiments, a polynucleotide inhibitor of T β RII further inhibits one or more of TGF β 1, TGF β 2, TGF β 3, ALK5, and betaglycan. In some embodiments, a TGF β antagonist polynucleotide, or

combination of polynucleotides, inhibits TGF β 1 from binding to T β RII. In some embodiments a TGF β antagonist polynucleotide, or combination of polynucleotides, inhibits TGF β 2 from binding to T β RII. In some embodiments, a TGF β antagonist polynucleotide, or combination of polynucleotides, inhibits TGF β 3 from binding to T β RII. In some
5 embodiments, a TGF β antagonist polynucleotide, or combination of polynucleotides, inhibits TGF β 1 and TGF β 3 from binding to T β RII. In some embodiments, a TGF β antagonist polynucleotide, or combination of polynucleotides, inhibits TGF β 1, TGF β 2, and TGF β 3 from binding to T β RII. In some embodiments, a TGF β antagonist polynucleotide, or combination of polynucleotides, inhibits TGF β 1 from binding to T β RII but does not inhibit or does not
10 substantially inhibit TGF β 2 from binding to T β RII. In some embodiments, a TGF β antagonist polynucleotide, or combination of polynucleotides, inhibits TGF β 3 from binding to T β RII but does not inhibit or does not substantially inhibit TGF β 2 from binding to T β RII. In some embodiments, a TGF β antagonist polynucleotide, or combination of polynucleotides, inhibits TGF β 1 and TGF β 3 from binding to T β RII but does not inhibit or does not
15 substantially inhibit TGF β 2 from binding to T β RII. In certain aspects, a TGF β antagonist polynucleotide, or combination of polynucleotides, inhibits at least ALK5 (e.g., inhibition of Smad signaling). Therefore, in some embodiments, a polynucleotide inhibitor of ALK5 binds to ALK5. In some embodiments, a polynucleotide inhibitor of ALK5 inhibits expression (e.g., transcription, translation, secretion, or combinations thereof) of ALK5. In
20 some embodiments, a polynucleotide inhibitor of ALK5 further inhibits one or more of TGF β 1, TGF β 2, TGF β 3, T β RII, and betaglycan. In some embodiments, a TGF β antagonist polynucleotide, or combination of polynucleotides, inhibits TGF β 1 from binding to ALK5. In some embodiments, a TGF β antagonist polynucleotide, or combination of polynucleotides, inhibits TGF β 2 from binding to ALK5. In some embodiments, a TGF β antagonist
25 polynucleotide, or combination of polynucleotides, inhibits TGF β 3 from binding to ALK5. In some embodiments, a TGF β antagonist polynucleotide, or combination of polynucleotides, inhibits TGF β 1 and TGF β 3 from binding to ALK5. In some embodiments, a TGF β antagonist polynucleotide, or combination of polynucleotides, inhibits TGF β 1, TGF β 2, and TGF β 3 from binding to ALK5. In some embodiments, a TGF β antagonist polynucleotide, or
30 combination of polynucleotides, inhibits TGF β 1 from binding to ALK5 but does not inhibit or does not substantially inhibit TGF β 2 from binding to ALK5. In some embodiments, a TGF β antagonist polynucleotide, or combination of polynucleotides, inhibits TGF β 3 from binding to ALK5 but does not inhibit or does not substantially inhibit TGF β 2 from binding to ALK5. In some embodiments, a TGF β antagonist polynucleotide, or combination of

polynucleotides, inhibits TGF β 1 and TGF β 3 from binding to ALK5 but does not inhibit or does not substantially inhibit TGF β 2 from binding to ALK5. In certain aspects, a TGF β antagonist polynucleotide, or combination of polynucleotides, inhibits at least betaglycan (e.g., inhibition of Smad signaling). Therefore, in some embodiments, a polynucleotide inhibitor of betaglycan binds to betaglycan. In some embodiments, a polynucleotide inhibitor of betaglycan inhibits expression (e.g., transcription, translation, secretion, or combinations thereof) of betaglycan. In some embodiments, a polynucleotide inhibitor of betaglycan further inhibits one or more of TGF β 1, TGF β 2, TGF β 3, T β RII, and ALK5. In some embodiments, a TGF β antagonist polynucleotide, or combination of polynucleotides, inhibits TGF β 1 from binding to betaglycan. In some embodiments, a TGF β antagonist polynucleotide, or combination of polynucleotides, inhibits TGF β 2 from binding to betaglycan. In some embodiments, a TGF β antagonist polynucleotide, or combination of polynucleotides, inhibits TGF β 3 from binding to betaglycan. In some embodiments, a TGF β antagonist polynucleotide, or combination of polynucleotides, inhibits TGF β 1 and TGF β 3 from binding to betaglycan. In some embodiments, a TGF β antagonist polynucleotide, or combination of polynucleotides, inhibits TGF β 1, TGF β 2, and TGF β 3 from binding to betaglycan. In some embodiments, a TGF β antagonist polynucleotide, or combination of polynucleotides, inhibits TGF β 1 from binding to betaglycan but does not inhibit or does not substantially inhibit TGF β 2 from binding to betaglycan. In some embodiments, a TGF β antagonist polynucleotide, or combination of polynucleotides, inhibits TGF β 3 from binding to betaglycan but does not inhibit or does not substantially inhibit TGF β 2 from binding to betaglycan. In some embodiments, a TGF β antagonist polynucleotide, or combination of polynucleotides, inhibits TGF β 1 and TGF β 3 from binding to betaglycan but does not inhibit or does not substantially inhibit TGF β 2 from binding to betaglycan.

The polynucleotide antagonists of the present disclosure may be an antisense nucleic acid, an RNAi molecule [e.g., small interfering RNA (siRNA), small-hairpin RNA (shRNA), microRNA (miRNA)], an aptamer and/or a ribozyme. The nucleic acid and amino acid sequences of human T β RII, ALK5, betaglycan, TGF β 1, TGF β 2, and TGF β 3 are known in the art and thus polynucleotide antagonists for use in accordance with methods of the present disclosure may be routinely made by the skilled artisan based on the knowledge in the art and teachings provided herein.

For example, antisense technology can be used to control gene expression through antisense DNA or RNA, or through triple-helix formation. Antisense techniques are

discussed, for example, in Okano (1991) *J. Neurochem.* 56:560; Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, Fla. (1988). Triple helix formation is discussed in, for instance, Cooney *et al.* (1988) *Science* 241:456; and Dervan *et al.*, (1991) *Science* 251:1300. The methods are based on binding of a polynucleotide to a
5 complementary DNA or RNA. In some embodiments, the antisense nucleic acids comprise a single-stranded RNA or DNA sequence that is complementary to at least a portion of an RNA transcript of a desired gene. However, absolute complementarity, although preferred, is not required.

A sequence "complementary to at least a portion of an RNA," referred to herein,
10 means a sequence having sufficient complementarity to be able to hybridize with the RNA, forming a stable duplex; in the case of double-stranded antisense nucleic acids of a gene disclosed herein, a single strand of the duplex DNA may thus be tested, or triplex formation may be assayed. The ability to hybridize will depend on both the degree of complementarity and the length of the antisense nucleic acid. Generally, the larger the hybridizing nucleic acid,
15 the more base mismatches with an RNA it may contain and still form a stable duplex (or triplex as the case may be). One skilled in the art can ascertain a tolerable degree of mismatch by use of standard procedures to determine the melting point of the hybridized complex.

Polynucleotides that are complementary to the 5' end of the message, for example, the 5'-untranslated sequence up to and including the AUG initiation codon, should work most
20 efficiently at inhibiting translation. However, sequences complementary to the 3'-untranslated sequences of mRNAs have been shown to be effective at inhibiting translation of mRNAs as well [see, *e.g.*, Wagner, R., (1994) *Nature* 372:333-335]. Thus, oligonucleotides complementary to either the 5'- or 3'-untranslated, noncoding regions of a gene of the disclosure, could be used in an antisense approach to inhibit translation of an endogenous
25 mRNA. Polynucleotides complementary to the 5'-untranslated region of the mRNA should include the complement of the AUG start codon. Antisense polynucleotides complementary to mRNA coding regions are less efficient inhibitors of translation but could be used in accordance with the methods of the present disclosure. Whether designed to hybridize to the 5'-untranslated, 3'-untranslated, or coding regions of an mRNA of the disclosure, antisense
30 nucleic acids should be at least six nucleotides in length, and are preferably oligonucleotides ranging from 6 to about 50 nucleotides in length. In specific aspects, the oligonucleotide is at least 10 nucleotides, at least 17 nucleotides, at least 25 nucleotides, or at least 50 nucleotides.

In one embodiment, the antisense nucleic acid of the present disclosure is produced intracellularly by transcription from an exogenous sequence. For example, a vector or a portion thereof, is transcribed, producing an antisense nucleic acid (RNA) of a gene of the disclosure. Such a vector would contain a sequence encoding the desired antisense nucleic acid. Such a vector can remain episomal or become chromosomally integrated, as long as it can be transcribed to produce the desired antisense RNA. Such vectors can be constructed by recombinant DNA technology methods standard in the art. Vectors can be plasmid, viral, or others known in the art, used for replication and expression in vertebrate cells. Expression of the sequence encoding desired genes of the instant disclosure, or fragments thereof, can be by any promoter known in the art to act in vertebrate, preferably human cells. Such promoters can be inducible or constitutive. Such promoters include, but are not limited to, the SV40 early promoter region [see, *e.g.*, Benoist and Chambon (1981) *Nature* 29:304-310], the promoter contained in the 3' long terminal repeat of Rous sarcoma virus [see, *e.g.*, Yamamoto *et al.* (1980) *Cell* 22:787-797], the herpes thymidine promoter [see, *e.g.*, Wagner *et al.* (1981) *Proc. Natl. Acad. Sci. U.S.A.* 78:1441-1445], and the regulatory sequences of the metallothionein gene [see, *e.g.*, Brinster, *et al.* (1982) *Nature* 296:39-42].

In some embodiments, the polynucleotide antagonists are interfering RNA or RNAi molecules that target the expression of one or more genes. RNAi refers to the expression of an RNA which interferes with the expression of the targeted mRNA. Specifically, RNAi silences a targeted gene via interacting with the specific mRNA through a siRNA (small interfering RNA). The ds RNA complex is then targeted for degradation by the cell. An siRNA molecule is a double-stranded RNA duplex of 10 to 50 nucleotides in length, which interferes with the expression of a target gene which is sufficiently complementary (*e.g.* at least 80% identity to the gene). In some embodiments, the siRNA molecule comprises a nucleotide sequence that is at least 85, 90, 95, 96, 97, 98, 99, or 100% identical to the nucleotide sequence of the target gene.

Additional RNAi molecules include short-hairpin RNA (shRNA); also short-interfering hairpin and microRNA (miRNA). The shRNA molecule contains sense and antisense sequences from a target gene connected by a loop. The shRNA is transported from the nucleus into the cytoplasm, and it is degraded along with the mRNA. Pol III or U6 promoters can be used to express RNAs for RNAi. Paddison *et al.* [*Genes & Dev.* (2002) 16:948-958, 2002] have used small RNA molecules folded into hairpins as a means to effect RNAi. Accordingly, such short hairpin RNA (shRNA) molecules are also advantageously

used in the methods described herein. The length of the stem and loop of functional shRNAs varies; stem lengths can range anywhere from about 25 to about 30 nt, and loop size can range between 4 to about 25 nt without affecting silencing activity. While not wishing to be bound by any particular theory, it is believed that these shRNAs resemble the double-stranded RNA (dsRNA) products of the DICER RNase and, in any event, have the same capacity for inhibiting expression of a specific gene. The shRNA can be expressed from a lentiviral vector. An miRNA is a single-stranded RNA of about 10 to 70 nucleotides in length that are initially transcribed as pre-miRNA characterized by a “stem-loop” structure and which are subsequently processed into mature miRNA after further processing through the RISC.

Molecules that mediate RNAi, including without limitation siRNA, can be produced *in vitro* by chemical synthesis (Hohjoh, FEBS Lett 521:195-199, 2002), hydrolysis of dsRNA (Yang *et al.*, Proc Natl Acad Sci USA 99:9942-9947, 2002), by *in vitro* transcription with T7 RNA polymerase (Donzeet *et al.*, Nucleic Acids Res 30:e46, 2002; Yu *et al.*, Proc Natl Acad Sci USA 99:6047-6052, 2002), and by hydrolysis of double-stranded RNA using a nuclease such as E. coli RNase III (Yang *et al.*, Proc Natl Acad Sci USA 99:9942-9947, 2002).

According to another aspect, the disclosure provides polynucleotide antagonists including but not limited to, a decoy DNA, a double-stranded DNA, a single-stranded DNA, a complexed DNA, an encapsulated DNA, a viral DNA, a plasmid DNA, a naked RNA, an encapsulated RNA, a viral RNA, a double-stranded RNA, a molecule capable of generating RNA interference, or combinations thereof.

In some embodiments, the polynucleotide antagonists of the disclosure are aptamers. Aptamers are nucleic acid molecules, including double-stranded DNA and single-stranded RNA molecules, which bind to and form tertiary structures that specifically bind to a target molecule, such as a T β RIL, TGF β 1, TGF β 2, and TGF β 3 polypeptide. The generation and therapeutic use of aptamers are well established in the art. See, *e.g.*, U.S. Pat. No. 5,475,096. Additional information on aptamers can be found in U.S. Patent Application Publication No. 20060148748. Nucleic acid aptamers are selected using methods known in the art, for example via the Systematic Evolution of Ligands by Exponential Enrichment (SELEX) process. SELEX is a method for the *in vitro* evolution of nucleic acid molecules with highly specific binding to target molecules as described in, *e.g.*, U.S. Pat. Nos. 5,475,096, 5,580,737, 5,567,588, 5,707,796, 5,763,177, 6,011,577, and 6,699,843. Another screening method to identify aptamers is described in U.S. Pat. No. 5,270,163. The SELEX process is based on the

capacity of nucleic acids for forming a variety of two- and three-dimensional structures, as well as the chemical versatility available within the nucleotide monomers to act as ligands (form specific binding pairs) with virtually any chemical compound, whether monomeric or polymeric, including other nucleic acid molecules and polypeptides. Molecules of any size or composition can serve as targets. The SELEX method involves selection from a mixture of candidate oligonucleotides and step-wise iterations of binding, partitioning and amplification, using the same general selection scheme, to achieve desired binding affinity and selectivity. Starting from a mixture of nucleic acids, which can comprise a segment of randomized sequence, the SELEX method includes steps of contacting the mixture with the target under conditions favorable for binding; partitioning unbound nucleic acids from those nucleic acids which have bound specifically to target molecules; dissociating the nucleic acid-target complexes; amplifying the nucleic acids dissociated from the nucleic acid-target complexes to yield a ligand enriched mixture of nucleic acids. The steps of binding, partitioning, dissociating and amplifying are repeated through as many cycles as desired to yield highly specific high affinity nucleic acid ligands to the target molecule.

Typically, such binding molecules are separately administered to the animal [see, *e.g.*, O'Connor (1991) *J. Neurochem.* 56:560], but such binding molecules can also be expressed *in vivo* from polynucleotides taken up by a host cell and expressed *in vivo* [see, *e.g.*, Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, Fla. (1988)].

3. Screening Assays

In certain aspects, the present invention relates to the use of T β RII polypeptides (*e.g.*, soluble T β RII polypeptides) to identify compounds (agents) which are agonist or antagonists of the TGF β 1, TGF β 3 and T β RII signaling pathway. Compounds identified through this screening can be tested to assess their ability to modulate TGF β 1 and TGF β 3 signaling activity *in vitro*. Optionally, these compounds can further be tested in animal models to assess their ability to modulate tissue growth *in vivo*.

There are numerous approaches to screening for therapeutic agents for modulating tissue growth by targeting TGF β 1, TGF β 3 and T β RII polypeptides. In certain embodiments, high-throughput screening of compounds can be carried out to identify agents that perturb TGF β 1, TGF β 3 or T β 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 β RII polypeptide to TGF β 1 or TGF β 3. Alternatively, the assay can be used to identify compounds that enhance binding of a T β RII polypeptide to TGF β 1 or TGF β 3. In a further embodiment, the compounds can be identified by their ability to interact with a TGF β 1, 5 TGF β 3 or T β RII polypeptide.

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 10 occurring biomolecules synthesized in vivo or in vitro. Compounds (agents) to be 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, 15 polypeptides, peptidomimetics, sugars, hormones, and nucleic acid molecules. In a specific embodiment, the test agent is a small organic molecule having a molecular weight of less than about 2,000 daltons.

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 20 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- 25 limiting examples of derivatizing groups include biotin, fluorescein, digoxigenin, green fluorescent protein, isotopes, polyhistidine, magnetic beads, glutathione S transferase (GST), photoactivatable crosslinkers or any combinations thereof.

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 30 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 β RII polypeptide and TGF β 1 or TGF β 3.

Merely to illustrate, in an exemplary screening assay of the present invention, the compound of interest is contacted with an isolated and purified T β RII polypeptide which is ordinarily capable of binding to TGF β 1 or TGF β 3. To the mixture of the compound and T β RII polypeptide is then added a composition containing a T β RII ligand. Detection and quantification of T β RII/TGF β 1 or T β RII/TGF β 3 complexes provides a means for determining the compound's efficacy at inhibiting (or potentiating) complex formation between the T β RII polypeptide and TGF β 1 or TGF β 3. 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 TGF β 1 or TGF β 3 is added to a composition containing the T β RII polypeptide, and the formation of T β RII/TGF β 1 or T β RII/TGF β 3 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.

Complex formation between the T β RII polypeptide and TGF β 1 or TGF β 3 may be 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}P , ^{35}S , ^{14}C or ^3H), fluorescently labeled (e.g., FITC), or enzymatically labeled T β RII polypeptide or TGF β 1 or TGF β 3, by immunoassay, or by chromatographic detection.

In certain embodiments, the present invention contemplates the use of fluorescence polarization assays and fluorescence resonance energy transfer (FRET) assays in measuring, either directly or indirectly, the degree of interaction between a T β 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 many embodiments of the invention.

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 β 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

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 β RII polypeptide and its binding protein. See for
5 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.

In certain embodiments, the subject compounds are identified by their ability to interact with a T β RII or TGF β 1 or TGF β 3 polypeptide of the invention. The interaction between the compound and the T β RII or TGF β 1 or TGF β 3 polypeptide may be covalent or
10 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 TGF β 1 or TGF β 3 or T β RII polypeptide. This may include
15 a solid-phase or fluid-phase binding event. Alternatively, the gene encoding a TGF β 1 or TGF β 3 or T β RII polypeptide can be transfected with a reporter system (e.g., β -galactosidase, luciferase, or green 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
20 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 compounds may be detected usually using colorimetric or fluorescence or surface plasmon resonance.

In certain aspects, the present invention provides methods and agents for modulating
25 (stimulating or inhibiting) TGF β 1- or TGF β 3-mediated cell signaling. Therefore, any compound identified can be tested in whole cells or tissues, in vitro or in vivo, to confirm their ability to modulate TGF β 1 or TGF β 3 signaling. Various methods known in the art can be utilized for this purpose.

30 4. Exemplary Therapeutic Uses

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 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 terms "treatment", "treating", "alleviation" and the like are used herein to generally mean obtaining a desired pharmacologic and/or physiologic effect, and may also be used to refer to improving, alleviating, and/or decreasing the severity of one or more symptoms of a condition being treated. The effect may be prophylactic in terms of completely or partially delaying the onset or recurrence of a disease, condition, or symptoms thereof, and/or may be therapeutic in terms of a partial or complete cure for a disease or condition and/or adverse effect attributable to the disease or condition. "Treatment" as used herein covers any treatment of a disease or condition of a mammal, particularly a human, and includes: (a) preventing the disease or condition from occurring in a subject which may be predisposed to the disease or condition but has not yet been diagnosed as having it; (b) inhibiting the disease or condition (e.g., arresting its development); or (c) relieving the disease or condition (e.g., causing regression of the disease or condition, providing improvement in one or more symptoms).

The terms "patient", "subject", or "individual" are used interchangeably herein and refer to either a human or a non-human animal. These terms include mammals, such as humans, non-human primates, laboratory animals, livestock animals (including bovines, porcines, camels, etc.), companion animals (e.g., canines, felines, other domesticated animals, etc.) and rodents (e.g., mice and rats). In particular embodiments, the patient, subject or individual is a human.

As used herein, "combination", "in combination with", "conjoint administration" and the like refers to any form of administration such that the second therapy is still effective in the body (e.g., the two compounds are simultaneously effective in the patient, which may include synergistic effects of the two compounds). Effectiveness may not correlate to measurable concentration of the agent in blood, serum, or plasma. For example, the different therapeutic compounds can be administered either in the same formulation or in separate formulations, either concomitantly or sequentially, and on different schedules. Thus, an individual who receives such treatment can benefit from a combined effect of different therapies. One or more TGF β antagonists of the disclosure can be administered concurrently with, prior to, or subsequent to, one or more other additional agents or supportive therapies. In general, each therapeutic agent will be administered at a dose and/or on a time schedule determined for that particular agent. The particular combination to employ in a regimen will

take into account compatibility of the antagonist of the present disclosure with the therapy and/or the desired therapeutic effect to be achieved.

In part, the disclosure provides methods of treating heterotopic ossification by administering to a patient in need thereof an effective amount of a TGF β antagonists. In some embodiments, the methods relate to preventing or reducing the severity and/or duration of heterotopic ossification in a patient in need thereof an effective amount of a TGF β antagonists. In some embodiments, the disclosure provides methods of treating a disease or condition associated with heterotopic ossification by administering to a patient in need thereof an effective amount of a TGF β antagonists. In some embodiments, the methods relate to preventing or reducing the severity and/or duration of a disease or condition associated heterotopic ossification in a patient in need thereof an effective amount of a TGF β antagonists. Optionally, such methods further include administering the TGF β antagonists in combination with one or more additional active agents or supportive therapies for treating heterotopic ossification.

In general, heterotopic ossification (HO) result from osteoid formation of mature lamellar bone in soft tissue sites outside the skeletal periosteum (skeletal system). Often, there is an inciting event prior to onset of HO, usually an episode of trauma which may result in hematoma. In general, HO progression requires a supply of pluripotent (multipotent) mesenchymal cells, which can differentiate into osteoblasts or chondroblasts. However, the mechanism of action behind HO development otherwise is largely unknown. Osteoid formation often is associated with an inflammatory phase characterized by local swelling, pain, erythema and sometimes fever. This pathological process may occur in sites such as the skin, subcutaneous tissue, skeletal muscle, and fibrous tissue adjacent to joints. Bone may also form in walls of blood vessels as well as in ligaments. Lesions range from small clinically insignificant foci to massive deposits throughout the body. HO may result in joint contracture and ankylosis, pain, spasticity, swelling fever, neurovascular compression, pressure ulcers, and significant disability. In some embodiments, the disclosure relates to methods of treating one or more complication associated with heterotopic ossification (e.g., osseous deposition, marrow space formation, joint contracture, ankylosis, pain, spasticity, swelling, fever, neurovascular compression, and pressure ulcers) by administering to a subject in need thereof an effective amount of a TGF β antagonists, optionally in combination with one or more additional active agents or supportive therapies for treating heterotopic ossification.

HO is most commonly it is associated with spinal cord injury, trauma, brain injuries,

burns, fractures, muscle contusion, and joint arthroplasty. In some embodiments, the disclosure relates to methods of treating heterotopic ossification associated with one or more disorders or conditions selected from the group consisting of: spinal cord injury, trauma, brain injuries, burns, fractures, muscle contusion, and joint arthroplasty by administering to a
5 subject in need thereof an effective amount of a TGF β antagonists, optionally in combination with one or more additional active agents or supportive therapies for treating heterotopic ossification.

HO is a severe complication of hip, acetabular, and elbow fracture surgery. About every third patient who has total hip arthroplasty (joint replacement) or a severe fracture of
10 the long bones of the lower leg will develop HO, but is uncommonly symptomatic. Between 50% and 90% of patients who developed HO following a previous hip arthroplasty will develop additional heterotopic ossification. In some embodiments, the disclosure relates to methods of treating heterotopic ossification associated with one or more of joint, hip, acetabular, and elbow fracture surgery (e.g., replacement surgery) by administering to a
15 subject in need thereof an effective amount of a TGF β antagonists, optionally in combination with one or more additional active agents or supportive therapies for treating heterotopic ossification.

HO often develops in patients with traumatic injury. In particular, HO is associated with brain or spinal cord injuries, which may account for the clinical observation that
20 traumatic brain injuries cause accelerated fracture healing. Morley et al. (2005) Injury 36(3): 363. HO is also associated with severe burns, combat-related trauma, and amputation. In traumatic heterotopic ossification, the patient may complain of a warm, tender, firm swelling in a muscle and decreased range of motion in the joint served by the muscle involved. There is often a history of a blow or other trauma to the area a few weeks to a few months earlier.
25 Patients with traumatic neurological injuries, severe neurologic disorders or severe burns who develop heterotopic ossification experience limitation of motion in the areas affected. In some embodiments, the disclosure relates to methods of treating heterotopic ossification associated with one or more of traumatic injury, brain or spinal cord injuries, burns, combat-related trauma, and amputation by administering to a subject in need thereof an effective
30 amount of a TGF β antagonists, optionally in combination with one or more additional active agents or supportive therapies for treating heterotopic ossification.

There are also rare genetic disorders causing heterotopic ossification such as fibrodysplasia ossificans progressiva (FOP). FOP is an extremely rare connective tissue disease. The disease is caused by a mutation of the body's repair mechanism, which causes fibrous tissue (including muscle, tendon, and ligament) to be ossified spontaneously or when
5 damaged. In many cases, injuries can cause joints to become permanently frozen in place. For unknown reasons, children born with FOP have deformed big toes, possibly missing a joint or simply presenting with a notable lump at the minor joint. The first "flare-up" that leads to the formation of FOP bones usually occurs before the age of 10. The bone growth progresses from the top downward, just as bones grow in fetuses. A child with FOP will
10 typically develop bones starting at the neck, then on the shoulders, arms, chest area and finally on the feet. Specifically, ossification is typically first seen in the dorsal, axial, cranial and proximal regions of the body. Later the disease progresses in the ventral, appendicular, caudal and distal regions of the body. However, it does not necessarily occur in this order due to injury-caused flare-ups. Often, the tumor-like lumps that characterize the disease
15 appear suddenly. This condition causes loss of mobility to affected joints, including inability to fully open the mouth limiting speech and eating. Extra bone formation around the rib cage restricts the expansion of lungs and diaphragm causing breathing complications. Since the disease is so rare, the symptoms are often misdiagnosed as cancer or fibrosis. This leads physicians to order biopsies, which can exacerbate the growth of these lumps. The
20 median age of survival is 40 years with proper management. However, delayed diagnosis, trauma and infections can decrease life expectancy. In general, FOP is caused by an autosomal dominant allele on chromosome 2q23-24. The allele has variable expressivity, but complete penetrance. Most cases are caused by spontaneous mutation in the gametes. A similar but less catastrophic disease is fibrous dysplasia, which is caused by a post-zygotic
25 mutation. A mutation in the gene ACVR1 (also known as activin-like kinase 2 (ALK2)) is responsible for the disease. ACVR1 encodes activin receptor type-1, a BMP type-1 receptor. The mutation causes substitution of codon 206 from arginine to histidine in the ACVR1 protein. This substitution causes abnormal activation of ACVR1, leading to the transformation of connective tissue and muscle tissue into a secondary skeleton. This
30 causes endothelial cells to transform to mesenchymal stem cells and then to bone. In some embodiments, the disclosure relates to methods of treating heterotopic ossification associated with fibrodysplasia ossificans progressiva by administering to a subject in need thereof an effective amount of a TGF β antagonists, optionally in combination with one or more additional active agents or supportive therapies for treating heterotopic ossification and/or

fibrodysplasia ossificans progressiva. In some embodiments, the disclosure relates to methods of treating heterotopic ossification associated with fibrous dysplasia by administering to a subject in need thereof an effective amount of a TGF β antagonists, optionally in combination with one or more additional active agents or supportive therapies for treating heterotopic ossification and/or fibrous dysplasia.

There is no cure or approved treatment for FOP. Attempts to surgically remove the bone result in explosive bone growth. Clinical trials of isotretinoin, etidronate with oral corticosteroids, and perhexiline maleate have failed to demonstrate effectiveness, though the variable course of the disease and small prevalence induces uncertainty. A handful of pharmaceutical companies focused on rare disease are currently in varying stages of investigation into different therapeutic approaches for FOP. In August 2015, U.S. Food and Drug Administration Office of Orphan Products Development granted La Jolla Pharmaceuticals orphan drug designation for two novel compounds for FOP. The compounds are small-molecule kinase inhibitors designed to selectively block ACVR1 (ALK2). In August 2015, Clementia Pharmaceuticals also began the enrollment of children into its Phase II clinical trial investigating palovarotene for the treatment of FOP. Preclinical studies demonstrated that palovarotene, a retinoic acid receptor gamma agonist, blocked abnormal bone formation in animal models via inhibition of secondary messenger systems in the BMP pathway. In September 2015, Regeneron announced new insight into the mechanism of disease involving the activation of the ACVR1 receptor by activin A. In 2016, the company initiated a phase 1 study of REGN 2477 (an activin A antibody) in healthy volunteers. Another potential therapeutic approach involves allele-specific RNA interference that targets mutated mRNA for degradation while preserving normal ACVR1 gene expression. J.W. Lowery et al. (2012) *Gene Therapy* 19(701-702): 701-702. A mouse model of FOP expressing a strong constitutively active ALK2 R206H mutant, was found to be useful in identifying a selective agonist to nuclear retinoic acid receptor- α (RAR- α) in mesenchymal cells. RAR- α agonists were found to partly inhibit HO, while an agonist to RAR- γ was found to be a potent inhibitor of intramuscular and subcutaneous HO in FOP models. Shimono et al., 2011, *Nature Medicine* 17:454-60). In some embodiments, the disclosure relates to methods of treating heterotopic ossification associated with FOP by administering to a subject in need thereof an effective amount of a TGF β antagonists in combination with one or more additional active agents or supportive therapies for treating FOP, wherein the additional active agent or supportive therapy is selected from the group consisting of:

isotretinoin, etidronate with oral corticosteroids, perhexiline maleate, ALK2 small-molecule inhibitors, palovarotene, retinoic acid receptor gamma agonists, retinoic acid receptor alpha agonists, activin antibodies (e.g., activin A antibodies such as REGN 2477, and allele-specific RNA interference of ALK2.

5 Another rare genetic disorder causing heterotopic ossification is progressive osseous heteroplasia (POH), which is a condition characterized by cutaneous or subcutaneous ossification. POH is associated with inactivating mutation in the GNAS gene, which encodes $G\alpha_s$, the alpha subunit of the stimulatory guanine nucleotide binding protein that acts downstream of many G protein-coupled receptors in activating adenylyl cyclase. Kaplan, et
10 al. 1994, J Bone Joint Surg Am 76, 425-436; Shore, et al., 2002, N Engl J Med 346, 99-106; and Eddy, et al., 2000, J Bone Miner Res 15, 2074-2083. Clinically, POH presents during infancy with dermal and subcutaneous ossifications that progress during childhood into skeletal muscle and deep connective tissues (e.g., tendon, ligaments, fascia). Over time these ossifications lead to joint stiffness, bone and joint fusions and growth retardation of the
15 affected limbs. Currently, patients with POH undergo aggressive surgical resection of ectopic bone to abrogate spreading of the lesion. This often results in partial or full amputation of limbs and lesions frequently return (Kaplan, et al., 2000, J Bone Miner Res 15, 2084-94; and Shehab, et al., 2003, J Nucl Med 43, 346-353), which underscores the importance of developing improved therapeutic interventions. Observations made in patients with POH
20 suggest that mesenchymal stem cells present in soft tissues inappropriately differentiate into osteoblasts and begin to deposit bone. Due to a lack of both in vitro and in vivo animal models, the pathogenesis of POH remains unknown and, like all forms of HO, lacks adequate treatments. In some embodiments, the disclosure relates to methods of treating heterotopic ossification associated with progressive osseous heteroplasia by administering to a subject in
25 need thereof an effective amount of a TGF β antagonists, optionally in combination with one or more additional active agents or supportive therapies for treating heterotopic ossification and/or progressive osseous heteroplasia.

HO also may occur in patients who are on neuromuscular blockade to manage adult respiratory distress syndrome and in patients with nontraumatic myelopathies. In some
30 embodiments, the disclosure relates to methods of treating heterotopic ossification associated with neuromuscular blockade used manage adult respiratory distress syndrome by administering to a subject in need thereof an effective amount of a TGF β antagonists, optionally in combination with one or more additional active agents or supportive therapies

for treating heterotopic ossification. In some embodiments, the disclosure relates to methods of treating heterotopic ossification associated with nontraumatic myelopathies by administering to a subject in need thereof an effective amount of a TGF β antagonists, optionally in combination with one or more additional active agents or supportive therapies
5 for treating heterotopic ossification.

There is no clear form of treatment for HO. Originally, bisphosphonates were expected to be of value after hip surgery but there has been no convincing evidence of benefit, despite having been used prophylactically. Prophylactic radiation therapy for the prevention of heterotopic ossification has been employed since the 1970s. A variety of doses
10 and techniques have been used. Generally, radiation therapy should be delivered as close as practical to the time of surgery. A dose of 7-8 Gray in a single fraction within 24–48 hours of surgery has been used successfully. Treatment volumes include the peri-articular region, and can be used for hip, knee, elbow, shoulder, jaw or in patients after spinal cord trauma. Single dose radiation therapy has been found to be well tolerated and is cost effective, without an
15 increase in bleeding, infection or wound healing disturbances. Certain anti-inflammatory agents, such as indomethacin, ibuprofen and aspirin, have shown some effect in preventing recurrence of heterotopic ossification after total hip replacement. Conservative treatments such as passive range of motion exercises or other mobilization techniques provided by physical therapists or occupational therapists may also assist in preventing HO. In some
20 embodiments, the disclosure relates to methods of treating heterotopic ossification by administering to a subject in need thereof an effective amount of a TGF β antagonists in combination with one or more additional active agents or supportive therapies for treating heterotopic ossification, wherein the one or more additional active agents or supportive therapies is selected from the group consisting of: bisphosphonates, radiation therapy anti-
25 inflammatory agents (e.g., indomethacin, ibuprofen and aspirin), and conservative treatments such as passive range of motion exercises or other mobilization techniques.

5. Pharmaceutical Compositions

The therapeutic agents described herein (e.g., T β RII fusion polypeptides) may be
30 formulated into pharmaceutical compositions. Pharmaceutical compositions for use in 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.

In certain embodiments, the therapeutic method of the disclosure includes administering the composition systemically, or locally as an implant or device. When administered, the therapeutic composition for use in this disclosure is in a pyrogen-free, physiologically acceptable form. Therapeutically useful agents other than the TGF β signaling antagonist 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 β RII polypeptides) in the methods disclosed herein.

Typically, protein therapeutic agents disclosed herein will be administered parentally, and particularly intravenously or subcutaneously. Pharmaceutical compositions suitable for parenteral administration may comprise one or more TGF β antagonists in combination with one or more pharmaceutically acceptable sterile isotonic aqueous or nonaqueous solutions, dispersions, suspensions or emulsions, or sterile powders which may 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.

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

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., TGF β antagonists) 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 TGF β antagonists. Such matrices may be formed of materials presently in use for other implanted medical applications.

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, 5 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 10 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.

In certain embodiments, methods of the invention can be administered for orally, e.g., 15 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 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 20 agent as an active ingredient. An agent may also be administered as a bolus, electuary or paste.

In solid dosage forms for oral administration (capsules, tablets, pills, 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 25 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, 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 30 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, such as 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 polyethylene glycols and the like.

5 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 emulsifiers, such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene
10 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.

15 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.

 The compositions of the invention may also contain adjuvants, such as preservatives,
20 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 form may
25 be brought about by the inclusion of agents which delay absorption, such as aluminum monostearate and gelatin.

 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., TGF β antagonists). The various factors include, but are not limited to, the
30 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), histomorphometric determinations, and tetracycline labeling.

In certain embodiments, the present invention also provides gene therapy for the in vivo production of TGF β antagonists. Such therapy would achieve its therapeutic effect by introduction of the TGF β antagonist polynucleotide sequences into cells or tissues having the disorders as listed above. Delivery of TGF β antagonist 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 TGF β antagonist polynucleotide sequences is the use of targeted liposomes.

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. 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 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 retroviral vector containing the TGF β antagonist polynucleotide. In a preferred embodiment, the vector is targeted to bone or cartilage.

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 interest. The resulting cells release the retroviral vector into the culture medium.

Another targeted delivery system for TGF β antagonist 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 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 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
 5 characteristics of liposomes depend on pH, ionic strength, and the presence of divalent cations.

Examples of lipids useful in liposome production include phosphatidyl compounds, such as phosphatidylglycerol, phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine, sphingolipids, cerebrosides, and gangliosides. Illustrative
 10 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.

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

15

Exemplification

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 the invention.

20

Example 1. Generation of receptor fusion protein variants

TβRII ECD variants

TβRII fusion proteins comprising a soluble extracellular portion of human TβRII and a human Fc portion were generated. For each fusion protein, a TβRII amino acid sequence
 25 having the amino acid sequence of SEQ ID NO: 18 was fused to an IgG Fc portion having the amino acid sequence of SEQ ID NO: 49 by means of one of several different linkers. Each of the fusion proteins also included a TPA leader sequence having the amino acid sequence of SEQ ID NO: 23 (below).

Tissue plasminogen activator (TPA): MDAMKRGLCCVLLLCGAVFVSP (SEQ ID
 30 NO: 23)

An illustration summary of several of the constructs designed is provided as Figure 3. A table detailing the sequences for the different constructs tested in the Exemplification section is provided below:

Construct Name	Construct Amino Acid Sequence	Linker Sequence
hTβRII-hFc	SEQ ID NO: 9	TGGG (SEQ ID NO: 3)
hTβRII (G4S)2-hFc	SEQ ID NO: 15	TGGGGS GGGGS (SEQ ID NO: 4)
hTβRII (G4S)3-hFc	SEQ ID NO: 11	TGGGGS GGGGS GGGGS (SEQ ID NO: 5)
hTβRII (G4S)4-hFc	SEQ ID NO: 13	TGGGGS GGGGS GGGGS GGGGS (SEQ ID NO: 6)
hTβRII extended hinge-hFc	SEQ ID NO: 17	TGGGPKSCDK (SEQ ID NO: 7)
hTβRII (G4S)5-hFc	SEQ ID NO: 44	TGGGGS GGGGS GGGGS GGGGS GGGGS (SEQ ID NO: 25)
hTβRII (G4S)6-hFc	SEQ ID NO: 45	TGGGGS GGGGS GGGGS GGGGS GGGGS GGGGS (SEQ ID NO: 26)

The amino acid sequences for the construct components and each of the constructs, along with the nucleic acid sequence used to express these constructs, are provided below.

5 *TβRII Portion: Amino Acid Sequence*

1 TIPPHVQKSD VEMEAQKDEI ICPSCNRTAH PLRHINNDMI VTDNNGAVKF
51 PQLCKFC DVR FSTCDNQKSC MSNCSITSIC EKPQEVCAV WRKNDENITL
101 ETVCHDPKLP YHDFILEDAA SPKCIMKEKK KPGETTFMCS CSSDECNDNI
10 151 IFSEYNTSN PD (SEQ ID NO: 18)

Fc Portion: Amino Acid Sequence

1 THTCP PCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE
51 VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK
15 101 VSNKALPAPI EKTISKAKGQ PREPQVYTL PPSREEMTKNQ VSLTCLVKGF
151 YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSKLTV DKSRWQQGNV
201 FSCSVMHEAL HNHYTQKSLS LSPGK (SEQ ID NO: 49)

hTβRII-hFc: Nucleic Acid Sequence

20 1 ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
51 AGTCTTCGTT TCGCCCGGCG CCACGATCCC ACCGCACGTT CAGAAGTCGG
101 ATGTGGAAAT GGAGGCCAG AAAGATGAAA TCATCTGCCC CAGCTGTAAT
151 AGGACTGCCC ATCCACTGAG ACATATTAAT AACGACATGA TAGTCACTGA
201 CAACAACGGT GCAGTCAAGT TTCCACAAC GTGTAAATTT TGTGATGTGA
25 251 GATTTTCCAC CTGTGACAAC CAGAAATCCT GCATGAGCAA CTGCAGCATC
301 ACCTCCATCT GTGAGAAGCC ACAGGAAGTC TGTGTGGCTG TATGGAGAAA
351 GAATGACGAG AACATAACAC TAGAGACAGT TTGCCATGAC CCAAGCTCC
401 CCTACCATGA CTTTATTCTG GAAGATGCTG CTTCTCCAAA GTGCATTATG
451 AAGGAAAAAA AAAAGCCTGG TGAGACTTTC TTCATGTGTT CCTGTAGCTC
30 501 TGATGAGTGC AATGACAACA TCATCTTCTC AGAAGAATAT AACACCAGCA
551 ATCCTGACAC CGGTGGTGGA ACTCACACAT GCCCACC GTG CCCAGCACCT
601 GAACTCCTGG GGGGACCGTC AGTCTTCCTC TTCCCCC CAA AACCCAAGGA
651 CACCCTCATG ATCTCCCGGA CCCCTGAGGT CACATGCGTG GTGGTGGACG

5 701 TGAGCCACGA AGACCCTGAG GTCAAGTTCA ACTGGTACGT GGACGGCGTG
 751 GAGGTGCATA ATGCCAAGAC AAAGCCGCGG GAGGAGCAGT ACAACAGCAC
 801 GTACCGTGTG GTCAGCGTCC TCACCGTCCT GCACCAGGAC TGGCTGAATG
 851 GCAAGGAGTA CAAGTGCAAG GTCTCCAACA AAGCCCTCCC AGCCCCATC
 901 GAGAAAACCA TCTCCAAAGC CAAAGGGCAG CCCCAGAAAC CACAGGTGTA
 951 CACCCTGCCC CCATCCCGGG AGGAGATGAC CAAGAACCAG GTCAGCCTGA
 1001 CCTGCCTGGT CAAAGGCTTC TATCCCAGCG ACATCGCCGT GGAGTGGGAG
 1051 AGCAATGGGC AGCCGGAGAA CAACTACAAG ACCACGCCTC CCGTGCTGGA
 1101 CTCCGACGGC TCCTTCTTCC TCTATAGCAA GCTCACCCTG GACAAGAGCA
 10 1151 GGTGGCAGCA GGGGAACGTC TTCTCATGCT CCGTGATGCA TGAGGCTCTG
 1201 CACAACCACT ACACGCAGAA GAGCCTCTCC CTGTCTCCGG GTAAATGA (SEQ
 ID NO: 8)

15 *hTβRII-hFc: Amino Acid Sequence*
 1 MDAMKRG LCC VLLLCGAVFV SPGATIPPHV QKSDVEMEAQ KDEIICPSCN
 51 RTAHLPLRHIN NDMIVTDNNG AVKFPQLCKF CDVRFSTCDN QKSCMSNCSI
 101 TSICEKPQEV CVAVWRKNDE NITLETVCHD PKLPYHDFIL EDAASPKCIM
 151 KEKKKPGETF FMCSCSSDEC NDNIIFSEY NTSNPDTGGG THTCP PCPAP
 20 201 ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE VKFNWYVDGV
 251 EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNKEYKCK VSNKALPAPI
 301 EKTISKAKGQ PREPQVYTLF PSREEMTKNQ VSLTCLVKFG YPSDIAVEWE
 351 SNGQPENNYK TTPPVLDSDG SFFLYSKLTV DKSRWQQGNV FSCSVMEAL
 401 HNHYTQKSL S LSPGK (SEQ ID NO: 9)

25 For the animal experiments described below, a mouse version of SEQ ID NO: 9 was
 generated by fusing the mouse extracellular domain of TβRII, which corresponds to isoform A of
 the human TβRII, to mouse G1Fc via a linker domain (TGGG). This fusion protein is
 designated herein as mTβRII-mFc.

30 *hTβRII (G4S)3-hFc: Nucleic Acid Sequence*
 1 ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
 51 AGTCTTCGTT TCGCCCGGCG CCACGATCCC ACCGCACGTT CAGAAGTCGG
 101 ATGTGGAAAT GGAGGCCAG AAAGATGAAA TCATCTGCCC CAGCTGTAAT
 35 151 AGGACTGCCC ATCCACTGAG ACATATTAAT AACGACATGA TAGTCACTGA
 201 CAACAACGGT GCAGTCAAGT TTCCACAAC GTGTAAATTT TGTGATGTGA
 251 GATTTTCCAC CTGTGACAAC CAGAAATCCT GCATGAGCAA CTGCAGCATC
 301 ACCTCCATCT GTGAGAAGCC ACAGGAAGTC TGTGTGGCTG TATGGAGAAA
 351 GAATGACGAG AACATAACAC TAGAGACAGT TTGCCATGAC CCCAAGCTCC
 40 401 CCTACCATGA CTTTATTCTG GAAGATGCTG CTTCTCCAAA GTGCATTATG
 451 AAGGAAAAAA AAAAGCCTGG TGAGACTTTC TTCATGTGTT CCTGTAGTCT
 501 TGATGAGTGC AATGACAACA TCATCTTCTC AGAAGAATAT AACACCAGCA
 551 ATCTTGACAC CGGTGGTGGA GGAAGTGCTG GAGGTGGTTC TGGAGGTGGT
 601 GGAAGTACTC ACACATGCCC ACCGTGCCCC GCACCTGAAC TCCTGGGGGG
 45 651 ACCGTCAGTC TTCTCTTCC CCCC AAAACC CAAGGACACC CTCATGATCT
 701 CCCGGACCCC TGAGGTCACA TGCGTGGTGG TGGACGTGAG CCACGAAGAC
 751 CCTGAGGTCA AGTTCAACTG GTACGTGGAC GGCGTGGAGG TGCATAATGC
 801 CAAGACAAAG CCGCGGGAGG AGCAGTACAA CAGCACGTAC CGTGTGGTCA
 851 GCGTCCTCAC CGTCTGCAC CAGGACTGGC TGAATGGCAA GGAGTACAAG
 50 901 TGCAAGGTCT CCAACAAAGC CCTCCCAGCC CCCATCGAGA AAACCATCTC
 951 CAAAGCCAAA GGGCAGCCCC GAGAACCACA GGTGTACACC CTGCCCCCAT
 1001 CCCGGGAGGA GATGACCAAG AACCAGGTCA GCCTGACCTG CCTGGTCAAA
 1051 GGCTTCTATC CCAGCGACAT CGCCGTGGAG TGGGAGAGCA ATGGGCAGCC

1101 GGAGAACAAAC TACAAGACCA CGCCTCCCGT GCTGGACTCC GACGGCTCCT
 1151 TCTTCCTCTA TAGCAAGCTC ACCGTGGACA AGAGCAGGTG GCAGCAGGGG
 1201 AACGTCTTCT CATGCTCCGT GATGCATGAG GCTCTGCACA ACCACTACAC
 1251 GCAGAAGAGC CTCTCCCTGT CTCCGGGTAA ATGA (SEQ ID NO: 10)

5

hTβRII (G4S)3-hFc: Amino Acid Sequence

1 MDAMKRGLCC VLLLCGAVFV SPGATIPPHV QKSDVEMEAQ KDEIICPSCN
 51 RTAHLPLRHIN NDMIVTDNNG AVKFPQLCKF CDVRFSTCDN QKSCMSNCSI
 101 TSICEKPQEV CVAVWRKNDE NITLETVCHD PKLPYHDFIL EDAASPKCIM
 10 151 KEKKKPGETF FMCSSSDEC NDNIIFSEY NTSNPD TGGG GSGGGSGGG
 201 GSTHTCPPCP APELLGGPSV FLFPKPKDT LMISRTPEVT CVVVDVSHED
 251 PEVKFNWYVD GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK
 301 CKVSNKALPA PIEKTISKAK GQPREPQVYT LPPSREEMTK NQVSLTCLVK
 351 GFYPSDIAVE WESNGQPENN YKTTTPVLDS DGSFFLYSKL TVDKSRWQQG
 15 401 NVFSCSVMHE ALHNHYTQKS LSLSPGK (SEQ ID NO: 11)

hTβRII (G4S)4-hFc: Nucleic Acid Sequence

1 ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
 20 51 AGTCTTCGTT TCGCCCGGCG CCACGATCCC ACCGCACGTT CAGAAGTCGG
 101 ATGTGGAAT GGAGGCCAG AAAGATGAAA TCATCTGCCC CAGCTGTAAT
 151 AGGACTGCCC ATCCACTGAG ACATATTAAT AACGACATGA TAGTCACTGA
 201 CAACAACGGT GCAGTCAAGT TTCCACAAC GTGTAAATTT TGTGATGTGA
 251 GATTTTCCAC CTGTGACAAC CAGAAATCCT GCATGAGCAA CTGCAGCATC
 25 301 ACCTCCATCT GTGAGAAGCC ACAGGAAGTC TGTGTGGCTG TAGGAGAAA
 351 GAATGACGAG AACATAACAC TAGAGACAGT TTGCCATGAC CCAAGCTCC
 401 CCTACCATGA CTTTATTCTG GAAGATGCTG CTTCTCCAAA GTGCATTATG
 451 AAGGAAAAAA AAAAGCCTGG TGAGACTTTC TTCATGTGTT CCTGTAGCTC
 501 TGATGAGTGC AATGACAACA TCATCTTCTC AGAAGAATAT AACACCAGCA
 30 551 ATCCTGACAC CGGTGGTGGG GGTCTGGAG GTGGAGGAAG TGGTGGAGGT
 601 GGTCTGGAG GTGGTGGAG TACTCACACA TGCCACCGT GCCCAGCACC
 651 TGAACCTCTG GGGGGACCGT CAGTCTTCCT CTTCCCCCA AAACCAAGG
 701 ACACCCTCAT GATCTCCCGG ACCCTGAGG TCACATGCGT GGTGGTGGAC
 751 GTGAGCCACG AAGACCCTGA GGTCAAGTTC AACTGGTACG TGGACGGCGT
 35 801 GGAGGTGCAT AATGCCAAGA CAAAGCCGCG GGAGGAGCAG TACAACAGCA
 851 CGTACCGTGT GGTCAGCGTC CTCACCGTCC TGCACCAGGA CTGGCTGAAT
 901 GGCAAGGAGT ACAAGTGCAA GGTCTCCAAC AAAGCCCTCC CAGCCCCCAT
 951 CGAGAAAACC ATCTCCAAAG CCAAAGGGCA GCGCCGAGAA CCACAGGTGT
 1001 ACACCCTGCC CCCATCCCGG GAGGAGATGA CCAAGAACCA GGTGAGCCTG
 40 1051 ACCTGCCTGG TCAAAGGCTT CTATCCAGC GACATCGCCG TGGAGTGGGA
 1101 GAGCAATGGG CAGCCGGAGA ACAACTACAA GACCACGCCT CCCGTGCTGG
 1151 ACTCCGACGG CTCCTTCTTC CTCTATAGCA AGCTCACCGT GGACAAGAGC
 1201 AGGTGGCAGC AGGGGAACGT CTTCTCATGC TCCGTGATGC ATGAGGCTCT
 1251 GCACAACCAC TACACGCAGA AGAGCCTCTC CTTGTCTCCG GGTAAATGA
 45 (SEQ ID NO: 12)

hTβRII (G4S)4-hFc: Amino Acid Sequence

1 MDAMKRGLCC VLLLCGAVFV SPGATIPPHV QKSDVEMEAQ KDEIICPSCN
 51 RTAHLPLRHIN NDMIVTDNNG AVKFPQLCKF CDVRFSTCDN QKSCMSNCSI
 50 101 TSICEKPQEV CVAVWRKNDE NITLETVCHD PKLPYHDFIL EDAASPKCIM
 151 KEKKKPGETF FMCSSSDEC NDNIIFSEY NTSNPD TGGG GSGGGSGGG
 201 GSGGGGSTHT CPPCPAPELL GGPSVFLFP KPKDTLMISR TPEVTCVVVD
 251 VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN
 301 GKEYKCKVSN KALPAPIEKT ISKAKQPRE PQVYTLPPSR EEMTKNQVSL
 55 351 TCLVKGFYPS DIAVEWESNG QPENNYKTP PVLDS DGSFF LYSKLTVDKS

401 RWQQGNVFSC SVMHEALHNNH YTQKSLSLSP GK (SEQ ID NO: 13)

hT8RII (G4S)4-hFc: Amino Acid Sequence lacking leader sequence

5 1 GATIPPHVQK SDVEMEAQKD EIICPSCNRT AHPLRHINND MIVTDNNGAV
 51 KFPQLCKFCD VRFSTCDNQK SCMSNCSITS ICEKPQEVCV AVWRKNDENI
 101 TLETVCHDPK LPYHDFILED AASPKCIMKE KKKPGETFFM CSCSSDECND
 151 NIIFSEELYNT SNPDTGGGGS GGGGSGGGGS GGGGSTHTCP PCPAPELLGG
 201 PSVFLFPPKP KDTLMISRTPEVTCVVVDVSH EDPEVKFNWY VDGVEVHNAK
 251 KTKPREEQYN TYRVVSVLT LHQDWLNGKE YKCKVSNKAL PAIEKTISK
 10 301 KAKGQPREPQ VYTLPPSREEM TKNQVSLTCL VKGFYPSDIA VEWESNGQPE
 351 ENNYKTTTPV LDSDGSFFLYS KLTVDKSRWQ QGNVFSCSV MHEALHNNHYT
 401 QKSLSLSPGK (SEQ ID NO: 63)

hT8RII (G4S)4-hFc: Amino Acid Sequence lacking leader sequence and lacking glycine prior to hT8RII portion

15 1 ATIPPHVQKS DVEMEAQKDE IICPSCNRTA HPLRHINNDM IVTDNNGAVK
 51 FPQLCKFCDV RFSTCDNQKS CMSNCSITSI CEKPQEVCAV VWRKNDENIT
 101 LETVCHDPKL PYHDFILED AASPKCIMKEK KKPGETFFMC SCSSDECNDN
 151 IIFSEELYNTS NPDTGGGGSG GGGSGGGSG GGGSTHTCPP CPAPELLGGP
 20 201 SVFLFPPKPK DTLMISRTPE VTCVVVDVSH EDPEVKFNWY VDGVEVHNAK
 251 TKPREEQYNS TYRVVSVLTV LHQDWLNGKE YKCKVSNKAL PAIEKTISK
 301 AKGQPREPQV YTLPPSREEM TKNQVSLTCL VKGFYPSDIA VEWESNGQPE
 351 NNYKTTTPVL DSDGSFFLYS KLTVDKSRWQ QGNVFSCSV MHEALHNNHYT
 25 401 KSLSLSPGK (SEQ ID NO: 64)

hT8RII (G4S)4-hFc: Amino Acid Sequence lacking leader sequence and lacking glycine, alanine, and threonine prior to hT8RII portion

30 1 IPPHVQKSDV EMEAQKDEII CPSCNRTAHP LRHINNDMIV TDNNGAVKFP
 51 QLCKFCDVRF STCDNQKSCM SNCSITSICE KPQEVCAVAV WRKNDENITL
 101 TVCHDPKLPY HDFILED AASPKCIMKEKKK PGETFFMCSC SDECNDNII
 151 FSEELYNTSNP DTGGGGSGGG GSGGGSGGG GSTHTCPPCP APPELLGGPSV
 201 FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNAKTK
 251 PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK
 301 GQPREPQVYT LPPSREEMTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN
 35 351 YKTTTPVLDS DGSFFLYSKL TVDKSRWQQG NVFSCSV MHEALHNNHYTQKS
 401 LSLSPGK (SEQ ID NO: 65)

hT8RII (G4S)4-hFc: Amino Acid Sequence lacking leader sequence and lacking glycine, alanine, threonine, and isoleucine prior to hT8RII portion

40 1 PPHVQKSDVE MEAQKDEIIC PSCNRTAHP LRHINNDMIV TDNNGAVKFPQ
 51 LCKFCDVRF STCDNQKSCM SNCSITSICE KPQEVCAVAV WRKNDENITL
 101 VCHDPKLPYH DFILED AASPKCIMKEKKK PGETFFMCSC SDECNDNIIF
 151 SEELYNTSNP DTGGGGSGGG GSGGGSGGG STHTCPPCPA PELLGGPSVF
 45 201 LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG VEVHNAKTKP
 251 REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC KVSNAKALPA IEKTISKAKG
 301 QPREPQVYTL PPSREEMTKN QVSLTCLVKG FYPSDIAVEW ESNQGPENNY
 351 KTTTPVLDS DGSFFLYSKL TVDKSRWQQG NVFSCSV MHEALHNNHYTQKSL
 401 SLSPGK (SEQ ID NO: 66)

hTβRII (G4S)4-hFc: Amino Acid Sequence lacking leader sequence and lacking glycine, alanine, threonine, isoleucine, and proline prior to hTβRII portion

5 1 PHVQKSDVEM EAQKDEIICP SCNRTAHPLR HINNDMIVTD NNGAVKFPQL
 51 CKFCDVRFST CDNQKSCMSN CSITSICEKP QEVCVAVWRK NDENITLETV
 101 CHDPKLPYHD FILEDAASPK CIMKEKKKPG ETFFMCSCSS DECNDNIIFS
 151 EEYNTSNPDT GGGGSGGGGS GGGGSGGGGS THTCPPCPAP ELLGGPSVFL
 201 FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE VKFNWYVDGV EVHNAKTKPR
 251 EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK VSNKALPAPI EKTISKAKGQ
 10 301 PREPQVYTLF PSREEMTKNQ VSLTCLVKGF YPSDIAVEWE SNGQPENNYK
 351 TTPVLDSDG SFFLYSKLTV DKSRWQQGNV FSCSVMHEAL HNHYTQKSLS
 401 LSPGK (SEQ ID NO: 67)

hTβRII (G4S)4-hFc: Amino Acid Sequence lacking leader sequence and lacking glycine, alanine, threonine, isoleucine, proline, and proline prior to hTβRII portion

15 1 HVQKSDVEME AQKDEIICPS CNRTAHPLRH INNDMIVTDN NGAVKFPQLC
 51 KFCDVRFSTC DNQKSCMSNC SITSICEKPQ EVCVAVWRKN DENITLETVC
 101 HDPKLPYHDF ILEDAASPKC IMKEKKKPGE TFFMCSCSSD ECNDNIIFSE
 20 151 EYNTSNPDTG GGGSGGGGSG GGGSGGGGST HTCPCCPAPE LLGGPSVFLF
 201 PPKPKDTLMI SRTPEVTCVV VDVSHEDPEV KFNWYVDGVE VHNAKTKPRE
 251 EQYNSTYRVV SVLTVLHQDW LNGKEYKCKV SNKALPAPIE KTISKAKGQP
 301 REPQVYTLPP SREEMTKNQV SLTCLVKGFY PSDIAVEWES NGQPENNYKT
 351 TPPVLDSDGS FFLYSKLTVD KSRWQQGNVF SCSVMHEALH NHYTQKSLSL
 25 401 SPGK (SEQ ID NO: 68)

hTβRII (G4S)2-hFc: Nucleic Acid Sequence

30 1 ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
 51 AGTCTTCGTT TCGCCCGGCG CCACGATCCC ACCGCACGTT CAGAAGTCGG
 101 ATGTGGAAAT GGAGGCCAGG AAAGATGAAA TCATCTGCCC CAGCTGTAAT
 151 AGGACTGCCC ATCCACTGAG ACATATTAAT AACGACATGA TAGTCACTGA
 201 CAACAACGGT GCAGTCAAGT TTCCACAACCT GTGTAAATTT TGTGATGTGA
 251 GATTTTCCAC CTGTGACAAC CAGAAATCCT GCATGAGCAA CTGCAGCATC
 35 301 ACCTCCATCT GTGAGAAAGC ACAGGAAGTC TGTGTGGCTG TATGGAGAAA
 351 GAATGACGAG AACATAACAC TAGAGACAGT TTGCCATGAC CCCAAGCTCC
 401 CCTACCATGA CTTTATTCTG GAAGATGCTG CTTCTCCAAA GTGCATTATG
 451 AAGGAAAAAA AAAAGCCTGG TGAGACTTTC TTCATGTGTT CCTGTAGCTC
 501 TGATGAGTGC AATGACAACA TCATCTTCTC AGAAGAATAT AACACCAGCA
 40 551 ATCCTGACAC CGGTGGAGGT GGTTCCTGGAG GTGGTGGAAG TACTCACACA
 601 TGCCCACCGT GCCCAGCACC TGAACCTCTG GGGGGACCGT CAGTCTTCCT
 651 CTTCCCCCCA AAACCCAAGG ACACCCTCAT GATCTCCCGG ACCCCTGAGG
 701 TCACATGCGT GGTGGTGGAC GTGAGCCACG AAGACCCTGA GGTCAAGTTC
 751 AACTGGTACG TGGACGGCGT GGAGGTGCAT AATGCCAAGA CAAAGCCGCG
 45 801 GGAGGAGCAG TACAACAGCA CGTACCGTGT GGTACGCGT CTCACCGTCC
 851 TGCACCAGGA CTGGCTGAAT GGCAAGGAGT ACAAGTGCAA GGTCTCCAAC
 901 AAAGCCCTCC CAGCCCCCAT CGAGAAAACC ATCTCCAAAG CCAAAGGGCA
 951 GCCCCGAGAA CCACAGGTGT ACACCCTGCC CCCATCCCGG GAGGAGATGA
 1001 CCAAGAACCA GGTGAGCCTG ACCTGCCTGG TCAAAGGCTT CTATCCCAGC
 50 1051 GACATCGCCG TGGAGTGGGA GAGCAATGGG CAGCCGGAGA ACAACTACAA
 1101 GACCACGCCT CCCGTGCTGG ACTCCGACGG CTCCTTCTTC CTCTATAGCA
 1151 AGCTCACCGT GGACAAGAGC AGGTGGCAGC AGGGGAACGT CTTCTCATGC
 1201 TCCGTGATGC ATGAGGCTCT GCACAACCAC TACACGCAGA AGAGCCTCTC
 1251 CCTGTCTCCG GGTAAATGA (SEQ ID NO: 14)

55

hTβRII (G4S)2-hFc: Amino Acid Sequence

1	MDAMKRGLCC	VLLLCGAVFV	SPGATIPPHV	QKSDVEMEAQ	KDEIICPSCN
51	RTAHPLRHIN	NDMIVTDNNG	AVKFPQLCKF	CDVRFSTCDN	QKSCMSNCSI
101	TSICEKPQEV	CVAVWRKNDE	NITLETVCHD	PKLPYHDFIL	EDAASPKCIM
5	151	KEKKKPGETF	FMCSCSSDEC	NDNIIFSEY	NTSNPDTGGG
	201	CPPCPAPELL	GGPSVFLFPP	KPKDTLMISR	TPEVTCVVVD
	251	NWYVDGVEVH	NAKTKPREEQ	YNSTYRVVSV	LTVLHQDWLN
	301	KALPAPIEKT	ISKAKQPRE	PQVYTLPPSR	EEMTKNQVSL
	351	DIAVEWESNG	QPENNYKTP	PVLDSDGSFF	LYSKLTVDKS
10	401	SVMHEALHNNH	YTQKSLSLSP	GK (SEQ ID NO: 15)	RWQQGNVFS

hTβRII extended hinge-hFc: Nucleic Acid Sequence

1	ATGGATGCAA	TGAAGAGAGG	GCTCTGCTGT	GTGCTGCTGC	TGTGTGGAGC
51	AGTCTTCGTT	TCGCCCCGGC	CCACGATCCC	ACCGCACGTT	CAGAAGTCGG
15	101	ATGTGGAAAT	GGAGGCCAG	AAAGATGAAA	TCATCTGCCC
	151	AGGACTGCCC	ATCCACTGAG	ACATATTAAT	AACGACATGA
	201	CAACAACGGT	GCAGTCAAGT	TTCCACAAC	GTGTAAATTT
	251	GATTTTCCAC	CTGTGACAAC	CAGAAATCCT	GCATGAGCAA
	301	ACCTCCATCT	GTGAGAAGCC	ACAGGAAGTC	TGTGTGGCTG
20	351	GAATGACGAG	AACATAACAC	TAGAGACAGT	TTGCCATGAC
	401	CCTACCATGA	CTTTATTCTG	GAAGATGCTG	CTTCTCCAAA
	451	AAGGAAAAAA	AAAAGCCTGG	TGAGACTTTC	TTCATGTGTT
	501	TGATGAGTGC	AATGACAACA	TCATCTTCTC	AGAAGAATAT
	551	ATCTTGACAC	CGGTGGTGGA	CCCAAATCTT	GTGACAAAAA
25	601	CCACCGTGCC	CAGCACTGA	ACTCCTGGGG	GGACCGTCAG
	651	CCCCCAAAA	CCAAGGACA	CCCTCATGAT	CTCCCGGACC
	701	CATGCGTGGT	GGTGGACGTG	AGCCACGAAG	ACCCTGAGGT
	751	TGGTACGTGG	ACGGCGTGGA	GGTGCATAAT	GCCAAGACAA
	801	GGAGCAGTAC	AACAGCACGT	ACCGTGTGGT	CAGCGTCCTC
30	851	ACCAGGACTG	GCTGAATGGC	AAGGAGTACA	AGTGCAAGGT
	901	GCCCTCCCAG	CCCCCATCGA	GAAAACCATC	TCCAAAAGCCA
	951	CCGAGAACCA	CAGGTGTACA	CCCTGCCCCC	ATCCCGGGAG
	1001	AGAACCAGGT	CAGCCTGACC	TGCCTGGTCA	AAGGCTTCTA
	1051	ATCGCCGTGG	AGTGGGAGAG	CAATGGGCAG	CCGGAGAACA
35	1101	CACGCCTCCC	GTGCTGGACT	CCGACGGCTC	CTTCTTCCTC
	1151	TCACCGTGGA	CAAGAGCAGG	TGGCAGCAGG	GGAACGTCTT
	1201	GTGATGCATG	AGGCTCTGCA	CAACCACTAC	ACGCAGAAGA
	1251	GTCCCCGGGT	AAATGA (SEQ ID NO: 16)		GCCTCTCCCT

hTβRII extended hinge-hFc: Amino Acid Sequence

1	MDAMKRGLCC	VLLLCGAVFV	SPGATIPPHV	QKSDVEMEAQ	KDEIICPSCN
51	RTAHPLRHIN	NDMIVTDNNG	AVKFPQLCKF	CDVRFSTCDN	QKSCMSNCSI
101	TSICEKPQEV	CVAVWRKNDE	NITLETVCHD	PKLPYHDFIL	EDAASPKCIM
151	KEKKKPGETF	FMCSCSSDEC	NDNIIFSEY	NTSNPDTGGG	PKSCDKTHTC
45	201	PPCPAPELLG	GPSVFLFPPK	PKDTLMISRT	PEVTCVVVDV
	251	WYVDGVEVHN	AKTKPREEQY	NSTYRVVSVL	TVLHQDWLNG
	301	ALPAPIEKTI	SKAKGQPREP	QVYTLPPSRE	EMTKNQVSLT
	351	IAVEWESNGQ	PENNYKTTPP	VLDSDGSFFL	YSKLTVDKSR
	401	VMHEALHNNH	TQKSLSLSPG	K (SEQ ID NO: 17)	WQQGNVFS
50					

hTβRII (G4S)5-hFc: Amino Acid Sequence

1	MDAMKRGLCC	VLLLCGAVFV	SPGATIPPHV	QKSDVEMEAQ	KDEIICPSCN
51	RTAHPLRHIN	NDMIVTDNNG	AVKFPQLCKF	CDVRFSTCDN	QKSCMSNCSI
55	101	TSICEKPQEV	CVAVWRKNDE	NITLETVCHD	PKLPYHDFIL

151 KEKKKPGETF FMCSCSSDEC NDNIIFSEY NTSNPD TGGG GSGGGGSGGG
 201 GSGGGGSGGG GSTHTCPPCP APELLGGPSV FLFPPKPKDT LMISRTPEVT
 251 CVVVDVSHED PEVKFNWYVD GVEVHNAKTK PREEQYNSTY RVVSVLTVLH
 301 QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT LPPSREEMTK
 5 351 NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTPPVLD SGSFFLYSKL
 401 TVDKSRWQQG NVFCSVMHE ALHNHYTQKS LSLSPGK (SEQ ID NO: 44)

hTβRII (G4S)6-hFc: Amino Acid Sequence

1 MDAMKRGLCC VLLLCGAVFV SPGATIPPHV QKSDVEMEAQ KDEIICPSCN
 10 51 RTAHLPLRHIN NDMIVTDNNG AVKFPQLCKF CDVRFSTCDN QKSCMSNCSI
 101 TSICEKPQEV CVAVWRKNDE NITLETVCHD PKLPYHDFIL EDAASPKCIM
 151 KEKKKPGETF FMCSCSSDEC NDNIIFSEY NTSNPD TGGG GSGGGGSGGG
 201 GSGGGGSGGG GSGGGGSTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR
 251 TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV
 15 301 LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSR
 351 EEMTKNQVSL TCLVKGFYPS DIAVEWESNG QPENNYKTP PVLDSDGSFF
 401 LYSKLTVDKS RWQQGNVFSC SVMHEALHNH YTQKSLSLSP GK (SEQ ID
 NO: 45)

hTβRII (G4S)5-hFc: Nucleotide Sequence

1 ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
 51 AGTCTTCGTT TCGCCCGGCG CCACGATCCC ACCGCACGTT CAGAAGTCGG
 101 ATGTGGAAAT GGAGGCCAG AAAGATGAAA TCATCTGCCC CAGCTGTAAT
 151 AGGACTGCCC ATCCACTGAG ACATATTAAT AACGACATGA TAGTCACTGA
 20 201 CAACAACGGT GCAGTCAAGT TTCCACAAC GTGTAAATTT TGTGATGTGA
 251 GATTTTCCAC CTGTGACAAC CAGAAATCCT GCATGAGCAA CTGCAGCATC
 301 ACCTCCATCT GTGAGAAGCC ACAGGAAGTC TGTGTGGCTG TATGGAGAAA
 351 GAATGACGAG AACATAACAC TAGAGACAGT TTGCCATGAC CCAAGCTCC
 401 CCTACCATGA CTTTATTCTG GAAGATGCTG CTTCTCCAAA GTGCATTATG
 30 451 AAGGAAAAAA AAAAGCCTGG TGAGACTTTC TTCATGTGTT CCTGTAGCTC
 501 TGATGAGTGC AATGACAACA TCATCTTCTC AGAAGAATAT AACACCAGCA
 551 ATCCTGACAC CGGTGGAGGA GGTCTGGTG GTGGAGGTTC TGGAGGTGGA
 601 GGAAGTGGTG GAGGTGGTTC TGGAGGTGGT GGAAGTACTC ACACATGCCC
 651 ACCGTGCCCC GCACCTGAAC TCCTGGGGGG ACCGTCAGTC TTCCTCTTCC
 35 701 CCCCCAAACC CAAGGACACC CTCATGATCT CCCGGACCCC TGAGGTCACA
 751 TGCCTGGTGG TGGACGTGAG CCACGAAGAC CCTGAGGTCA AGTTCAACTG
 801 GTACGTGGAC GCGGTGGAGG TGCATAATGC CAAGACAAAG CCGCGGGAGG
 851 AGCAGTACAA CAGCACGTAC CGTGTGGTCA GCGTCCTCAC CGTCCTGCAC
 901 CAGGACTGGC TGAATGGCAA GGAGTACAAG TGCAAGGTCT CCAACAAAGC
 40 951 CCTCCCAGCC CCCATCGAGA AAACCATCTC CAAAGCCAAA GGGCAGCCCC
 1001 GAGAACCACA GGTGTACACC CTGCCCCCAT CCCGGGAGGA GATGACCAAG
 1051 AACCAGGTCA GCCTGACCTG CCTGGTCAAA GGCTTCTATC CCAGCGACAT
 1101 CGCCGTGGAG TGGGAGAGCA ATGGGCAGCC GGAGAACAAC TACAAGACCA
 1151 CGCCTCCCGT GCTGGACTCC GACGGCTCCT TCTTCCTCTA TAGCAAGCTC
 45 1201 ACCGTGGACA AGAGCAGGTG GCAGCAGGGG AACGTCTTCT CATGCTCCGT
 1251 GATGCATGAG GCTCTGCACA ACCACTACAC GCAGAAGAGC CTCTCCCTGT
 1301 CTCCGGGTAA ATGA (SEQ ID NO: 46)

hTβRII (G4S)6-hFc: Nucleotide Sequence

1 ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
 50 51 AGTCTTCGTT TCGCCCGGCG CCACGATCCC ACCGCACGTT CAGAAGTCGG
 101 ATGTGGAAAT GGAGGCCAG AAAGATGAAA TCATCTGCCC CAGCTGTAAT
 151 AGGACTGCCC ATCCACTGAG ACATATTAAT AACGACATGA TAGTCACTGA
 201 CAACAACGGT GCAGTCAAGT TTCCACAAC GTGTAAATTT TGTGATGTGA
 55 251 GATTTTCCAC CTGTGACAAC CAGAAATCCT GCATGAGCAA CTGCAGCATC

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5   301  ACCTCCATCT GTGAGAAGCC ACAGGAAGTC TGTGTGGCTG TATGGAGAAA
    351  GAATGACGAG AACATAACAC TAGAGACAGT TTGCCATGAC CCCAAGCTCC
    401  CCTACCATGA CTTTATTCTG GAAGATGCTG CTTCTCCAAA GTGCATTATG
    451  AAGGAAAAAA AAAAGCCTGG TGAGACTTTC TTCATGTGTT CCTGTAGCTC
10  501  TGATGAGTGC AATGACAACA TCATCTTCTC AGAAGAATAT AACACCAGCA
    551  ATCCTGACAC CGGTGGAGGT GGAAGTGGTG GAGGAGGTTC TGGTGGTGGA
    601  GGTTCCTGGAG GTGGAGGAAG TGGTGGAGGT GGTTCCTGGAG GTGGTGGGAAG
    651  TACTCACACA TGCCCACCGT GCCCAGCACC TGAACCTCTG GGGGGACCGT
    701  CAGTCTTCCT CTTCCCCCCA AAACCCAAGG ACACCCTCAT GATCTCCCGG
15  751  ACCCCTGAGG TCACATGCGT GGTGGTGGAC GTGAGCCACG AAGACCCTGA
    801  GGTCAAGTTC AACTGGTACG TGGACGGCGT GGAGGTGCAT AATGCCAAGA
    851  CAAAGCCGCG GGAGGAGCAG TACAACAGCA CGTACCGTGT GGTGAGCGTC
    901  CTCACCGTCC TGCACCAGGA CTGGCTGAAT GGCAAGGAGT ACAAGTGCAA
    951  GGTCTCCAAC AAAGCCCTCC CAGCCCCCAT CGAGAAAACC ATCTCCAAAG
20 1001  CCAAAGGGCA GCCCCGAGAA CCACAGGTGT ACACCCTGCC CCCATCCCGG
    1051  GAGGAGATGA CCAAGAACCA GGTCAGCCTG ACCTGCCTGG TCAAAGGCTT
    1101  CTATCCAGC  GACATCGCCG TGGAGTGGGA GAGCAATGGG CAGCCGGAGA
    1151  ACAACTACAA GACCACGCCT CCCGTGCTGG ACTCCGACGG CTCCTTCTTC
    1201  CTCTATAGCA AGCTCACCGT GGACAAGAGC AGGTGGCAGC AGGGGAACGT
    1251  CTTCTCATGC TCCGTGATGC ATGAGGCTCT GCACAACCAC TACACGCAGA
    1301  AGAGCCTCTC CCTGTCTCCG GGTAAATGA (SEQ ID NO: 47)

```

The various constructs were successfully expressed in CHO cells and were purified to a high degree of purity as determined by analytical size-exclusion chromatography and SDS-PAGE. The hTβRII (G4S)2-hFc, hTβRII (G4S)3-hFc, hTβRII (G4S)4-hFc, hTβRII (G4S)5-hFc and hTβRII (G4S)6-hFc proteins displayed similarly strong stability as determined by SDS-PAGE analysis when maintained in PBS for 13 days at 37°C. The hTβRII (G4S)2-hFc, hTβRII (G4S)3-hFc, hTβRII (G4S)4-hFc proteins were also maintained in rat, mouse or human serum and displayed similarly strong stability.

TβRII ECD variants

In addition to the TβRII domains included in the fusion proteins described above (e.g., SEQ ID NO: 18), the disclosure also contemplates fusion proteins comprising alternative TβRII domains. For example, the fusion protein may comprise the wild-type hTβRII_{short}(23-159) sequence shown below (SEQ ID NO: 27) or any of the other TβRII polypeptides disclosed below:

```

1   TIPPHVQKSV NNDMIVTDNN GAVKFPQLCK FCDVRFSTCD NQKSCMSNCS
51  ITSICEKPQE VCVAVWRKND ENITLETVCH DPKLPYHDFI LEDAASPKCI
40 101  MKEKKKPGET FFMCS CSSDE CNDNIIFSEE YNTSNPD (SEQ ID NO:
    27)

```

(1) The hTβRII_{short}(23-159/D110K) amino acid sequence shown below (SEQ ID NO: 36), in which the substituted residue is underlined.

```

1   TIPPHVQKSV NNDMIVTDNN GAVKFPQLCK FCDVRFSTCD NQKSCMSNCS
51  ITSICEKPQE VCVAVWRKND ENITLETVCH DPKLPYHKFI LEDAASPKCI
5   101  MKEKKKPGET FFMCS CSSDE CNDNIIFSEE YNTSNPD (SEQ ID NO:
      36)

```

(2) The N-terminally truncated hTβRII_{short}(29-159) amino acid sequence shown below (SEQ ID NO: 28).

```

1   QKSVNNDMIV TDNNGAVKFP QLCKFCDVRF STCDNQKSCM SNCSITSICE
10  51  KPQEV VAVWRKNDENITLETVCHDPKLPY HFILEDAAS PKCIMKEKKK
      101  PGETFFMCSC SSDECNDNII FSEEYNTSNP D (SEQ ID NO: 28)

```

(3) The N-terminally truncated hTβRII_{short}(35-159) amino acid sequence shown below (SEQ ID NO: 29).

```

1   DMIVTDNNGA VKFPQLCKFC DVRFSTCDNQ KSCMSNCSIT SICEKPQEV
15  51  VAVWRKNDEN ITLETVCHDP KLPYHFILE DAASPKCIMK EKKKPGETFF
      101  MCSCSSDECN DNIIIFSEEYN TSNPD (SEQ ID NO: 29)

```

(4) The C-terminally truncated hTβRII_{short}(23-153) amino acid sequence shown below (SEQ ID NO: 30).

```

1   TIPPHVQKSV NNDMIVTDNN GAVKFPQLCK FCDVRFSTCD NQKSCMSNCS
20  51  ITSICEKPQE VCVAVWRKND ENITLETVCH DPKLPYHDFI LEDAASPKCI
      101  MKEKKKPGET FFMCS CSSDE CNDNIIFSEE Y (SEQ ID NO: 30)

```

(5) The C-terminally truncated hTβRII_{short}(23-153/N70D) amino acid sequence shown below (SEQ ID NO: 38), in which the substituted residue is underlined.

```

1   TIPPHVQKSV NNDMIVTDNN GAVKFPQLCK FCDVRFSTCD NQKSCMSDCS
25  51  ITSICEKPQE VCVAVWRKND ENITLETVCH DPKLPYHDFI LEDAASPKCI
      101  MKEKKKPGET FFMCS CSSDE CNDNIIFSEE Y (SEQ ID NO: 38)

```

Applicants also envision five corresponding variants (SEQ ID NOs: 37, 33, 34, 39) based on the wild-type hTβRII_{long}(23-184) sequence shown above and below (SEQ ID NO: 20), in which the 25 amino-acid insertion is underlined. Note that splicing results in a conservative amino acid substitution (Val→Ile) at the flanking position C-terminal to the insertion.

1 TIPPHVQKSD VEMEAQKDEI ICPSCNRTAH PLRHINNDMI VTDNNGAVKF
 51 PQLCKFCDVR FSTCDNQKSC MSNCSITSIC EKPQEV CVAV WRKNDENITL
 101 ETVCHDPKLP YHDFILEDAA SPKCIMKEKK KPGETFFMCS CSSDECNDNI
 151 IFSEEYNTSN PD (SEQ ID NO: 20)

- 5 (1) The hTβRII_{long}(23-184/D135K) amino acid sequence shown below (SEQ ID NO: 37), in which the substituted residue is double underlined.

1 TIPPHVQKSD VEMEAQKDEI ICPSCNRTAH PLRHINNDMI VTDNNGAVKF
 51 PQLCKFCDVR FSTCDNQKSC MSNCSITSIC EKPQEV CVAV WRKNDENITL
 101 ETVCHDPKLP YHKFILEDAA SPKCIMKEKK KPGETFFMCS CSSDECNDNI
 10 151 IFSEEYNTSN PD (SEQ ID NO: 37)

- (2) The N-terminally truncated hTβRII_{long}(29-184) amino acid sequence shown below (SEQ ID NO: 33).

1 QKSDVEMEAQ KDEIICPSCN RTAHPLRHIN NDMIVTDNNG AVKFPQLCKF
 51 CDVRFSTCDN QKSCMSNCSI TSICEKPQEV CVAVWRKNDE NITLETVCHD
 15 101 PKLPYHDFIL EDAASPKCIM KEKKKPGETF FMCSCSSDEC NDNIIFSEY
 151 NTSNPD (SEQ ID NO: 33)

- (3) The N-terminally truncated hTβRII_{long}(60-184) amino acid sequence shown below (same as SEQ ID NO: 29).

1 DMIIVTDNNGA VKFPQLCKFC DVRFSTCDNQ KSCMSNCSIT SICEKPQEV
 20 51 VAVWRKNDE NITLETVCHD KLPYHDFILE DAASPKCIM EKKKPGETFF
 101 MCSCSSDECN DNIIIFSEYN TSNPD (same as SEQ ID NO: 29)

- (4) The C-terminally truncated hTβRII_{long}(23-178) amino acid sequence shown below (SEQ ID NO: 34).

1 TIPPHVQKSD VEMEAQKDEI ICPSCNRTAH PLRHINNDMI VTDNNGAVKF
 25 51 PQLCKFCDVR FSTCDNQKSC MSNCSITSIC EKPQEV CVAV WRKNDENITL
 101 ETVCHDPKLP YHDFILEDAA SPKCIMKEKK KPGETFFMCS CSSDECNDNI
 151 IFSEY (SEQ ID NO: 34)

- (5) The C-terminally truncated hTβRII_{long}(23-178/N95D) amino acid sequence shown below (SEQ ID NO: 39), in which the substituted residue is double underlined.

30 1 TIPPHVQKSD VEMEAQKDEI ICPSCNRTAH PLRHINNDMI VTDNNGAVKF

51 PQLCKFCDVR FSTCDNQKSC MSDCSITSIC EKPQEVCAV WRKNDENITL
 101 ETVCHDPKLP YHDFILEDAA SPKCIMKEKK KPGETFFMCS CSSDECNDNI
 151 IFSEYY (SEQ ID NO: 39)

Additional T β RII ECD variants include:

- 5 (A) The N- and C-terminally truncated hT β RII_{short}(35-153) or hT β RII_{long}(60-178) amino acid sequence shown below (SEQ ID NO: 32).

1 DMIVTDNNGA VKFPQLCKFC DVRFSTCDNQ KSCMSNCSIT SICEKPQEV
 51 VAVWRKNDEN ITLETVCHDP KLPYHDFILE DAASPKCIMK EKKKPGETFF
 101 MCSCSSDECN DNIIIFSEYY (SEQ ID NO: 32)

10

- (B) The N- and C-terminally truncated hT β RII_{short}(29-153) amino acid sequence shown below (SEQ ID NO: 31).

1 QKSVNNDMIV TDNNGAVKFP QLCKFCDVRF STCDNQKSCM SNCSITSICE
 51 KPQEVCAVAV RKNDENITL TVCHDPKLPY HDFILEDAAAS PKCIMKEKKK
 15 101 PGETFFMCSC SSDECNDNII FSEYY (SEQ ID NO: 31)

- (C) The N- and C-terminally truncated hT β RII_{long}(29-178) amino acid sequence shown below (SEQ ID NO: 35).

1 QKSDVEMEAQ KDEIICPSCN RTAHPLRHIN NDMIVTDNNG AVKFPQLCKF
 20 51 CDVRFSTCDN QKSCMSNC SI TSICEKPQEV CAVAVWRKNDENITLETVCHD
 101 PKLPYHDFIL EDAASPKCIM KEKKKPGETF FMCSCSSDEC NDNIIFSEYY
 (SEQ ID NO: 35)

- Any of the above variants (SEQ ID NO: 36, 28, 29, 30, 38, 37, 33, 34, 39, 32, 31, and 35) could incorporate an insertion of 36 amino acids (SEQ ID NO: 41) between the pair of glutamate residues (positions 151 and 152 of SEQ ID NO: 1, or positions 176 and 177 of SEQ ID NO: 2) located near the C-terminus of the hT β RII ECD, as occurs naturally in the hT β RII isoform C (Konrad et al., BMC Genomics 8:318, 2007).

GRCKIRHIGS NNRLQRSTCQ NTGWESAHVM KTPGFR (SEQ ID NO: 41)

- As an example, the paired glutamate residues flanking the optional insertion site are denoted below (underlined) for the hT β RII_{short}(29-159) variant (SEQ ID NO: 28).

1 QKSVNNDMIV TDNNGAVKFP QLCKFCDVRF STCDNQKSCM SNCSITSICE
 51 KPQEVCAVAVW RKNDENITLE TVCHDPKLPY HDFILEDAAAS PKCIMKEKKK
 101 PGETFFMCSC SSDECNDNII FSE~~E~~YNTSNP D (SEQ ID NO: 28)

Fc domain variants

5 While the constructs described above were generated with an Fc domain having the amino acid sequence of SEQ ID NO: 49, the disclosure contemplates hT β RII-hFc fusion proteins comprising alternative Fc domains, including a human IgG2 Fc domain (SEQ ID NO: 42, below) or full-length human IgG1 Fc (hG1Fc) (SEQ ID NO: 43, below). Optionally, a polypeptide unrelated to an Fc domain could be attached in place of the Fc domain.

10 1 VECPPCPAPP VAGPSVFLFP PKPKDTLMIS RTPEVTCVVV DVSHEDPEVQ
 51 FNWYVDGVEV HNAKTKPREE QFNSTFRVVS VLTVVHQDWL NGKEYKCKVS
 101 NKGLPAPIEK TISKTKGQPR EPQVYTLPPS REEMTKNQVS LTCLVKGFYP
 151 SDIAVEWESN GQPENNYKTT PPMLDSGGSF FLYSKLTVDK SRWQQGNVFS
 201 CSVMHEALHN HYTQKSLSLS PGK (SEQ ID NO: 42)

15

1 GGPKSCDKTH TCPPCPAPEL LGGPSVFLFP PKPKDTLMIS RTPEVTCVVV
 51 DVSHEDPEVK FNWYVDGVEV HNAKTKPREE QYNSTYRVVS VLTVLHQDWL
 101 NGKEYKCKVS NKALPAPIEK TISKAKGQPR EPQVYTLPPS REEMTKNQVS
 151 LTCLVKGFYP SDIAVEWESN GQPENNYKTT PPVLDSGGSF FLYSKLTVDK
 20 201 SRWQQGNVFS CSVMHEALHN HYTQKSLSLS PGK (SEQ ID NO: 43)

Leader sequence variants

While the generated constructs described above included the TPA leader sequence, alternative leader sequences may be used, such as the native leader sequence (SEQ ID NO: 22- below) or the honey bee melittin (SEQ ID NO: 24- below) leader sequences.

Native: MGRGLLRGLWPLHIVLWTRIAS (SEQ ID NO: 22)

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

Example 2. Differential ligand inhibition by receptor fusion protein variants in cell-based assay

Affinities of TGF β 1, TGF β 2 and TGF β 3 for hT β RII (G4S)2-hFc; hT β RII (G4S)3-hFc; hT β RII (G4S)4-hFc; hT β RII-hFc; and hT β RII extended hinge-hFc proteins were evaluated in vitro with a Biacore™ instrument, and the results are summarized in Figures 4A

and 4B. Each of the fusion proteins was capable of binding TGF β 1 and TGF β 3 with high affinity, but the constructs having linker lengths longer than or equal to (G4S)₄ were surprisingly capable of binding to both TGF β 1 and TGF β 3 with higher affinity than constructs having linker lengths shorter than (G4S)₄. Binding between TGF β 2 and any of the
5 constructs was low or transient. Deglycosylation of the constructs did not change binding.

A reporter gene assay in A549 cells was used to determine the ability of hT β RII-hFc variants to inhibit activity of TGF β 1, TGF β 2 and TGF β 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
10 transfection efficiency. The CAGA motif is present in the promoters of TGF β -responsive genes (for example, PAI-1), so this vector is of general use for factors signaling through SMAD2 and SMAD3.

On the first day of the assay, A549 cells (ATCC[®]: CCL-185[™]) were distributed in 48-well plates. On the second day, a solution containing pGL3(CAGA)₁₂, pRLCMV, X-
15 tremeGENE 9 (Roche Applied Science), and OptiMEM (Invitrogen) was preincubated, then added to Eagle's minimum essential medium (EMEM, ATCC[®]) supplemented with 0.1% BSA, which was applied to the plated cells for incubation overnight at 37°C, 5% CO₂. On the third day, medium was removed, and cells were incubated overnight at 37°C, 5% CO₂ with a mixture of ligands and inhibitors prepared as described below.

Serial dilutions of test articles were made in a 48-well plate in assay buffer (EMEM +
20 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 TGF β 1, human TGF β 2, and human TGF β 3 were obtained from PeproTech. Test solutions were incubated at 37°C for 30 minutes, then a portion of the mixture was added to all wells. After
25 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 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
30 E1980) to determine normalized luciferase activity.

As illustrated in Figures 5A-5F, the hT β RII (G4S)₂-hFc; hT β RII (G4S)₃-hFc; hT β RII (G4S)₄-hFc; hT β RII (G4S)₅-hFc; hT β RII (G4S)₆-hFc; hT β RII-hFc; and hT β RII extended hinge-hFc proteins all were capable of inhibiting both TGF β 1 and TGF β 3. Interestingly, while there was a correlation between improved TGF β 1 and TGF β 3 inhibition and linker

length for the the hT β RII (G4S)2-hFc; hT β RII (G4S)3-hFc and hT β RII (G4S)4-hFc constructs (Figure 5E), this improvement trend appeared to have plateaued for hT β RII (G4S)5-hFc and hT β RII (G4S)6-hFc constructs (Figure 5F).

5 **Example 3. Effects of T β RII-Fc in a Heterotopic Ossification Animal Model**

Heterotopic ossification (HO) can commonly occur after severe trauma, burn injuries, and is a debilitating consequence of the congenital disease fibrodysplasia ossificans progressive (FOP). The etiology remains poorly understood, however, it is presumed that inflammation plays a critical role with several inflammatory cell types being recruited to the
10 site of HO development. The effects of mT β RII-mFc, as described above, on the progression of HO were investigated using a HO animal model.

Recruited macrophages are a known source of cytokines that influence the inflammatory microenvironment. Therefore, the secretion of TGF β 1 in cultured macrophages was initially assessed. Bone marrow derived macrophages were isolated and polarized into
15 M2 phenotype *in-vitro*. Secreted TGF β was measured in conditioned media using ELISA. Interestingly, it was observed that, while M0 macrophages secrete only minimal TGF β 1, the regenerative M2 macrophages, which are known to be recruited to inflammatory sites such as those occurring at sites of osseous deposition in HO, had a 500-fold increase in TGF β 1 secretion. Furthermore, the increase in TGF β 1 secretion was completely abrogated when the
20 mT β RII-mFc (5.44 μ g/ml) was present in the culture media (Figure 6A).

Next, the effect of systemically administered mT β RII-mFc were observed in a model of traumatic heterotopic ossification involving a 30% dorsal burn and Achilles tenotomy. Agarwal et al. (2016) Proc Natl Acad Sci USA 113(3): E338-347. Six-week old male C57BL/6 mice were induced for HO and then randomized into one of two treatment groups:
25 1) treatment with the mT β RII-mFc (10 mg/kg) (n=10) or 2) PBS control (n=10). This was administered subcutaneously twice weekly for 3 weeks. At 3 weeks, histology samples were collected, decalcified and stained with Safranin O to assess formation of HO anlagen. Volume of mature formed HO was quantified using micro CT analysis and imaging reconstruction at 9 weeks. Following treatment for 3 weeks, a substantially attenuated
30 development of HO anlagen in mice treated with mT β RII-mFc was observed upon histologic examination along with decreased osseous deposition and marrow space formation (Figure 6B). Furthermore, the volume of mature HO was significantly decreased in the mT β RII-mFc treatment group (Figure 6C).

In this study, it was demonstrated that TGF β 1 signaling plays a critical role in HO

formation and treatment with mTβRII-mFc is effective in attenuating ectopic bone deposition, thus resulting in decreased HO volume. Therefore, these data suggest that TβRII polypeptides, as well as other TGFβ antagonist, may be used to treat HO, revealing a new avenue for the treatment of this condition in human patients.

5

INCORPORATION BY REFERENCE

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.

10

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.

15

We claim:

1. A method for treating heterotopic ossification (HO) comprising administering to a patient in need thereof an effective amount of a Transforming Growth Factor- β (TGF β) antagonist.
5
2. The method of claim 1, wherein the heterotopic ossifications is associated with one or more disorders or conditions selected from the group consisting of: spinal cord injury, trauma, brain injuries, burns, fractures, muscle contusion, joint arthroplasty/replacement, hip surgery/replacement, acetabular surgery/replacement,
10 elbow fracture, fracture of the long bones of the lower leg, combat-related trauma, amputation, neuromuscular blockade used to manage adult respiratory distress syndrome, nontraumatic myelopathies.
3. A method for treating fibrodysplasia ossificans progressiva (FOP) comprising
15 administering to a patient in need thereof an effective amount of a TGF β antagonist.
4. A method for treating progressive osseous heteroplasia (POH) comprising administering to a patient in need thereof an effective amount of a TGF β antagonist.
- 20 5. A method for treating fibrous dysplasia comprising administering to a patient in need thereof an effective amount of a TGF β antagonist.
6. The method of any preceding claim, wherein the method prevents or reduces the severity or duration of one or more complications of HO selected from the group
25 consisting of: joint contracture, ankylosis, pain, spasticity, swelling fever, neurovascular compression, and pressure ulcers.
7. The method of any preceding claim wherein the method prevents or reduces the severity or duration of heterotopic ossification in the patient.
30
8. The method of any preceding claim, wherein the heterotopic ossification occurs in one or more tissues selected from the group consisting of: bone, skin, subcutaneous

tissue, skeletal muscle, fibrosis tissue adjacent to joints, walls of blood vessels, and ligaments.

- 5 9. The method of any preceding claim, wherein the method further comprises administration of an additional active agent or supportive therapy for treating heterotopic ossification, FOP, POH, or fibrous dysplasia.
- 10 10. The method of claim 10, wherein the additional active agent or supportive therapy for treating heterotopic ossification, FOP, POH, or fibrous dysplasia is selected from the group consisting of: isotretinoin, etidronate with oral corticosteroids, perhexiline maleate, ALK2 small-molecule inhibitors, palovarotene, retinoic acid receptor gamma agonists, retinoic acid receptor alpha agonists, activin antibodies (e.g., activin A antibodies such as REGN 2477), allele-specific RNA interference of ALK2, bisphosphonates, radiation therapy anti-inflammatory agents (e.g., 15 indomethacin, ibuprofen and aspirin), and conservative treatments such as passive range of motion exercises or other mobilization techniques.
- 20 11. The method of claim 10, wherein the additional active agent or supportive therapy is treatment with an activin antagonist.
12. The method of claim 11, wherein the activin antagonist is an activin antibody.
13. The method of claim 12, wherein the activin antibody is an activin A antibody.
- 25 14. The method of any preceding claim, wherein the TGF β antagonist is a Transforming Growth Factor- β Receptor II (T β RII) polypeptide.
15. The method of claim 14, wherein the T β RII polypeptide comprises an amino acid sequence selected from:
- 30 a. an amino acid sequence at least 75% identical to a sequence beginning at any one of positions 23 to 35 of SEQ ID NO: 1 and ending at any one of positions 153 to 159 of SEQ ID NO: 1; and

- b. an amino acid sequence at least 75% identical to a sequence beginning at any one of positions 23 to 60 of SEQ ID NO: 2 and ending at any one of positions 178 to 184 of SEQ ID NO: 2.

- 5 16. The method of claim 14, wherein the T β R β II polypeptide comprises an amino acid sequence at least 90% identical to a sequence beginning at any one of positions 23 to 35 of SEQ ID NO: 1 and ending at any one of positions 153 to 159 of SEQ ID NO: 1.
- 10 17. The method of claim 14, wherein the T β R β II polypeptide comprises an amino acid sequence at least 95% identical to a sequence beginning at any one of positions 23 to 35 of SEQ ID NO: 1 and ending at any one of positions 153 to 159 of SEQ ID NO: 1.
- 15 18. The method of claim 14, wherein the T β R β II polypeptide comprises an amino acid sequence at least 99% identical to a sequence beginning at any one of positions 23 to 35 of SEQ ID NO: 1 and ending at any one of positions 153 to 159 of SEQ ID NO: 1.
- 20 19. The method of claim 14, wherein the T β R β II polypeptide comprises an amino acid sequence beginning at any one of positions 23 to 35 of SEQ ID NO: 1 and ending at any one of positions 153 to 159 of SEQ ID NO: 1.
- 25 20. The method of claim 14, wherein the T β R β II polypeptide comprises an amino acid sequence at least 90% identical to a sequence beginning at any one of positions 23 to 60 of SEQ ID NO: 2 and ending at any one of positions 178 to 184 of SEQ ID NO: 2.
- 30 21. The method of claim 14, wherein the T β R β II polypeptide comprises an amino acid sequence at least 95% identical to a sequence beginning at any one of positions 23 to 60 of SEQ ID NO: 2 and ending at any one of positions 178 to 184 of SEQ ID NO: 2.
- 22. The method of claim 14, wherein the T β R β II polypeptide comprises an amino acid sequence at least 99% identical to a sequence beginning at any one of positions 23 to 60 of SEQ ID NO: 2 and ending at any one of positions 178 to 184 of SEQ ID NO: 2.
- 23. The method of claim 14, wherein the T β R β II polypeptide comprises an amino acid sequence beginning at any one of positions 23 to 60 of SEQ ID NO: 2 and ending at any one of positions 178 to 184 of SEQ ID NO: 2.

24. The method of claim 14, wherein the T β R β II polypeptide comprises an amino acid sequence at least 80% identical to SEQ ID NO: 18.

5 25. The method of claim 14, wherein the T β R β II polypeptide comprises an amino acid sequence at least 90% identical to SEQ ID NO: 18.

26. The method of claim 14, wherein the T β R β II polypeptide comprises the amino acid sequence of SEQ ID NO: 18.

10

27. The method of claim 14, wherein the T β R β II polypeptide is a fusion polypeptide comprising:

- a. an extracellular domain of a T β R β II portion; and
- b. a heterologous portion, and

15

wherein the T β R β II extracellular domain portion comprises an amino acid sequence at least 80% identical to:

- i) a sequence beginning at any of positions 23 to 35 of SEQ ID NO: 1 and ending at any of positions 153 to 159 of SEQ ID NO: 1 or
 - ii) a sequence beginning at any of positions 23 to 60 of SEQ ID NO: 2 and
- 20 ending at any of positions 178 to 184 of SEQ ID NO: 2.

28. The method of claim 27, wherein the T β R β II polypeptide comprises an amino acid sequence at least 80% identical to SEQ ID NO: 18.

25

29. The method of claim 27, wherein the T β R β II extracellular domain portion consists of an amino acid sequence at least 80% identical to a sequence beginning at any of positions 23 to 35 of SEQ ID NO: 1 and ending at any of positions 153 to 159 of SEQ ID NO: 1.

30

30. The method of claim 27, wherein the T β R β II extracellular domain portion consists of an amino acid sequence at least 80% identical to a sequence beginning at any of positions 23 to 60 of SEQ ID NO: 2 and ending at any of positions 178 to 184 of SEQ ID NO: 2.

31. The method of claim 27, wherein the T β R β II extracellular domain portion consists of an amino acid sequence at least 80% identical to SEQ ID NO: 18.

32. The method any one of claims 27-31, wherein the heterologous portion is an immunoglobulin Fc domain.

33. The method claim 32, wherein the immunoglobulin Fc domain is a human immunoglobulin Fc domain.

34. The method of claim 32, wherein the heterologous portion comprises an amino acid sequence that is at least 80% identical to SEQ ID NO: 49.

35. The method of claim 32, wherein the heterologous portion comprises the amino acid sequence of SEQ ID NO: 49.

36. The method of any one of claims 27-35, wherein the fusion protein further comprises a linker domain.

37. The method of claim 36, wherein the linker is between 10 and 25 amino acids in length.

38. The method of claim 36, wherein the linker comprises an amino acid sequence selected from:

- a. (GGGGS)_n, wherein $n \geq 2$;
- b. (GGGGS)_n, wherein $n \geq 3$;
- c. (GGGGS)_n, wherein $n \geq 4$; and
- d. the amino acid sequence of any one of SEQ ID Nos: 4-7, 19, 21, 25, 26, and 40.

39. The method of claim 36, wherein the linker comprises (GGGGS)_n, wherein $n \neq \geq 5$.

40. The method of claim 14, wherein the T β R β II polypeptide comprises an amino acid sequence that is at least 80% identical to the amino acid sequence of SEQ ID NO: 11.

41. The method of claim 14, wherein the T β R β II polypeptide comprises an amino acid sequence that is at least 80% identical to the amino acid sequence of SEQ ID NO: 13, 65, or 68.

42. The method of claim 14, wherein the T β R β II polypeptide comprises an amino acid sequence that is at least 80% identical to the amino acid sequence of SEQ ID NO: 15.

5 43. The method of any one of claims 14-42, wherein the T β R β II polypeptide or fusion polypeptide does not include amino acids 185-592 of SEQ ID NO: 2.

44. The method of any one of claims 14-43, wherein the T β R β II polypeptide or fusion polypeptide does not include amino acids 1-22 of SEQ ID NO: 2.

10

45. The method of claim 14, wherein the T β R β II polypeptide consists of or consists essentially of:

- 15 a) a T β R β II polypeptide portion comprising an amino acid sequence that is at least 85%, 90%, 95%, 97%, or 99% identical to the amino acid sequence of SEQ ID NO: 18 and no more than 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 additional amino acids;
- b) a linker portion comprising an amino acid sequence that is at least 85%, 90%, 95%, 97%, or 99% identical to the amino acid sequence of SEQ ID NO: 6 and no more than 5, 4, 3, 2 or 1 additional amino acids;
- 20 c) a heterologous portion comprising an amino acid sequence that is at least 85%, 90%, 95%, 97%, or 99% identical to the amino acid sequence of SEQ ID NO: 49 and no more than 25, 20, 15, 10, 5, 4, 3, 2, or 1 additional amino acids; and
- c) optionally a leader sequence (*e.g.*, SEQ ID NO: 23).

25 46. The method of claim 14, wherein the T β R β II polypeptide consists of or consists essentially of:

- a) a T β R β II polypeptide portion comprising the amino acid sequence of SEQ ID NO: 18 and no more than 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 additional amino acids;
- b) a linker portion comprising the amino acid sequence of SEQ ID NO: 6 and no more than 5, 4, 3, 2 or 1 additional amino acids;
- 30 c) a heterologous portion comprising the amino acid sequence of SEQ ID NO: 49 and no more than 25, 20, 15, 10, 5, 4, 3, 2, or 1 additional amino acids; and
- d) optionally a leader sequence (*e.g.*, SEQ ID NO: 23).

47. The method of claim 14, wherein the T β RII polypeptide comprises:

a) an extracellular domain of a T β RII portion; wherein the extracellular domain comprises an amino acid sequence that is at least 85%, 90%, 95%, 97%, or 99% identical to the sequence of SEQ ID NO: 18;

5 b) a heterologous portion, wherein the heterologous portion comprises an amino acid sequence that is at least 85%, 90%, 95%, 97%, or 99% identical to the sequence of SEQ ID NO: 49; and

10 c) a linker portion connecting the extracellular domain and the heterologous portion; wherein the linker comprises an amino acid sequence that is at least 85%, 90%, 95%, 97%, or 99% identical to the amino acid sequence of SEQ ID NO: 6.

48. The method of claim 14, wherein the T β RII polypeptide comprises:

a) an extracellular domain of a T β RII portion; wherein the extracellular domain comprises the amino acid sequence of SEQ ID NO: 18;

15 b) a heterologous portion, wherein the heterologous portion comprises the amino acid sequence of SEQ ID NO: 49; and

c) a linker portion connecting the extracellular domain and the heterologous portion; wherein the linker comprises the amino acid sequence of SEQ ID NO: 6.

20 49. The method of claim 14, wherein the T β RII polypeptide comprises an amino acid sequence that is at least 85%, 90%, 95%, 97%, or 99% identical to the amino acid sequence of SEQ ID NO: 48.

25 50. The method of claim 14, wherein the T β RII polypeptide comprises the amino acid sequence of SEQ ID NO: 48.

51. The method of any one of claims 14-50, wherein the T β RII polypeptide or fusion polypeptide does not include a leader sequence, or wherein the leader sequence has been removed.

30

52. The method of any one of claims 14-51, wherein the T β RII polypeptide or fusion 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, and an amino acid conjugated to a lipid moiety.

5 53. The method of claim 52, wherein the T β RII polypeptide or fusion polypeptide is glycosylated.

54. The method of claim 53, wherein the T β RII polypeptide or fusion polypeptide has a glycosylation pattern characteristic of expression of the polypeptide in CHO cells.

10 55. The method of any one of claims 14-54, wherein the T β RII polypeptide or fusion polypeptide binds to TGF β 1 and TGF β 3

15 56. The method of any one of claims 14-55, wherein the T β RII polypeptide or fusion polypeptide inhibits TGF β 1 and TGF β 3 signaling as determined using a reporter gene assay.

57. The method of any one of claims 14-56, wherein the T β RII polypeptide or fusion polypeptide is a homodimer.

20 58. The method of any one of claims 1-14, wherein the TGF β antagonist is an antibody or combination of antibodies that binds to one or more of TGF β 1, TGF β 2, TGF β 3, T β RII, ALK5, and betaglycan.

25 59. The method of any one of claims 1-14, wherein the TGF β antagonist is an antibody or combination of antibodies that binds to TGF β 1, TGF β 2, and TGF β 3.

60. The method of claim 59, wherein the antibody is fresolimumab.

30 61. The method any one of claims 1-14, wherein the TGF β antagonist is an antibody or combination of antibodies that binds to TGF β 1 and TGF β 3, but wherein the antibody or combination of antibodies does not bind to TGF β 2.

62. The method of any one of claims 1-14, wherein the TGF β antagonist is a small molecule or combination of small molecules that inhibit to one or more of TGF β 1, TGF β 2, TGF β 3, T β RII, ALK5, and betaglycan.
- 5 63. The method of any one of claims 1-14, wherein the TGF β antagonist is a nucleotide or combination of nucleotides that inhibit to one or more of TGF β 1, TGF β 2, TGF β 3, T β RII, ALK5, and betaglycan.
64. The method of claim 11, wherein the activin antagonists is selected from: a small molecule or nucleotide.
- 10 65. The method of claim 11, wherein the activin antagonists is a wherein the activin antagonists is a small molecule or nucleotide that inhibits activin A.
- 15 66. A method for treating heterotopic ossification (HO) comprising administering to a patient in need thereof an effective amount of a Transforming Growth Factor- β (TGF β) antagonist and an activin antagonist.
67. A method for treating fibrodysplasia ossificans progressiva (FOP) comprising administering to a patient in need thereof an effective amount of a TGF β antagonist and an activin antagonist.
- 20 68. A method for treating progressive osseous heteroplasia (POH) comprising administering to a patient in need thereof an effective amount of a TGF β antagonist and an activin antagonist.
- 25 69. A method for treating fibrous dysplasia comprising administering to a patient in need thereof an effective amount of a TGF β antagonist and an activin antagonist.
- 30 70. The method of any one of claims 66-69, wherein the TGF β antagonist corresponds any one as defined in claims 14-63.

71. The method of any one of claims 66-69, wherein the activin antagonists is selected from an antibody, small molecule, or nucleotide.

72. The method of claim 71, wherein the activin antagonist inhibits activin A.

5

73. The method of any one of claims 66-69, wherein the TGF β antagonist is an antibody that binds to one or more of TGF β 1, TGF β 2, or TGF β 3, and wherein the activin antagonist is an antibody that binds to activin.

10

74. The method of claim 73, wherein the TGF β antibody that binds to TGF β 1, TGF β 2, and TGF β 3.

75. The method of claim 73, wherein the TGF β antibody that binds to TGF β 1 and TGF β 3, but does not bind, or does not substantial bind to (e.g., greater than a K_D of at least 1×10^{-7} M), TGF β 2.

15

76. The method of any one of claims 73-75, wherein the antibody binds to activin A.

77. The method of any one of claims 66-69, wherein the TGF β antagonist and activin antagonist is a multi-specific antibody that binds to TGF β 1, TGF β 3, and activin A.

20

78. The method of any preceding claim, wherein the TGF β antagonist is a betaglycan polypeptide.

79. The method of any preceding claim, wherein the TGF β antagonist is a heteromultimer comprising a T β RII polypeptide and an ALK5 polypeptide.

25

80. The method of any preceding claim, wherein the TGF β antagonist is a heteromultimer comprising a T β RII polypeptide and a betaglycan polypeptide.

30

81. The method of any preceding claim, wherein the TGF β antagonist is a heteromultimer comprising an ALK5 polypeptide and a betaglycan polypeptide.

82. The method of claim 80 and 81, wherein the betaglycan polypeptide comprises an amino acid sequence that is at least 80%, 85%, 90%, 91%, 92%, 94%, 95%, 97%, 99% or 100% identical to SEQ ID NO: 51 or 55, or a biologically active fragment thereof.

5

83. The method of claim 79 or 81, wherein the ALK5 polypeptide comprises an amino acid sequence that is at least 80%, 85%, 90%, 91%, 92%, 94%, 95%, 97%, 99% or 100% identical to SEQ ID NO: 70 or 74, or a biologically active fragment thereof.

10

84. The method of any one of claims 79-81, wherein the heteromultimer is a heterodimer.

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1 mrglllrglw plhivlwtri astipphvqk synndmivtd nngavkfpql
51 ckfcdvrfst cdngkscmsn csitsicekp qevcvavwrk ndenitletv
101 chdpklpyhd filedaaspk cimkekkkpg etffmcscss decndniifs
151 eeyntsnpd1 llvifqvtgi sllpplgvai sviiifycyr vnrqqklsst
201 wetgktrklm efsehcaiil eddrdisst canninhnte llpieldtlv
251 gkgrfaevyk aklkqntseq fetvavkifp yeeyaswkte kdifsdinlk
301 henilqflta eerktelgkq ywlitafhak gnlqeyltrh viswedlrkl
351 gsslargiah lhsdhtpcgr pkmpivhrdl kssnilvknd ltccldcfdgl
401 slrldptlsv ddlansgqvg tarymapevl esrmnlenve sfkqtdvysm
451 alvlwemtsr cnavgevkdy eppfgskvre hpcvesmkdn vlrdgrpei
501 psfwnhqgi qmvcetltec wdhdpearlt aqcvaerfse lehldrlsgr
551 scseekiped gslnttk (SEQ ID NO: 1)

FIGURE 1

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1 mgrgllrglw plhivlwtri astipphvqk sdvemeaqkd eiicpscnrt
51 ahplrhinnd mivtdnngav kfpqlckfcd vrfstcdnqk scmsncsits
101 icekpqgevcv avwrkndeni tletvchdpk lpyhdfiled aaspkcimke
151 kkkpgetffm cscssdecnd niifseeynt snpdlllvif qvtgisllpp
201 lgvaisviii fycyrvnrrq klsstwetgk trklmefseh caiiledhrs
251 disstcanni nhntellpie ldtlvvgkgrf aevyakalkq ntseqfetva
301 vkifpyeeya swktekdifs dinlkhenil qfltaerkt elgkqywlit
351 afhakgnlqe yltrhviswe dlrklgssla rgiahllhsdh tpcgrpkmpi
401 vhrdlkssni lvkndltccl cdfglslrld ptlsvddlan sgqvgtarym
451 apevlesrmn lenvesfkqt dvysmalvlw emtsrcnavg evkdyepffg
501 skvrehpcve smkdnvlrdr grpeipsfwl nhqgiqmvce tltecwdhdp
551 earltaqcva erfselehld rlsgrscsee kipedgslnt tk
(SEQ ID NO: 2)

FIGURE 2

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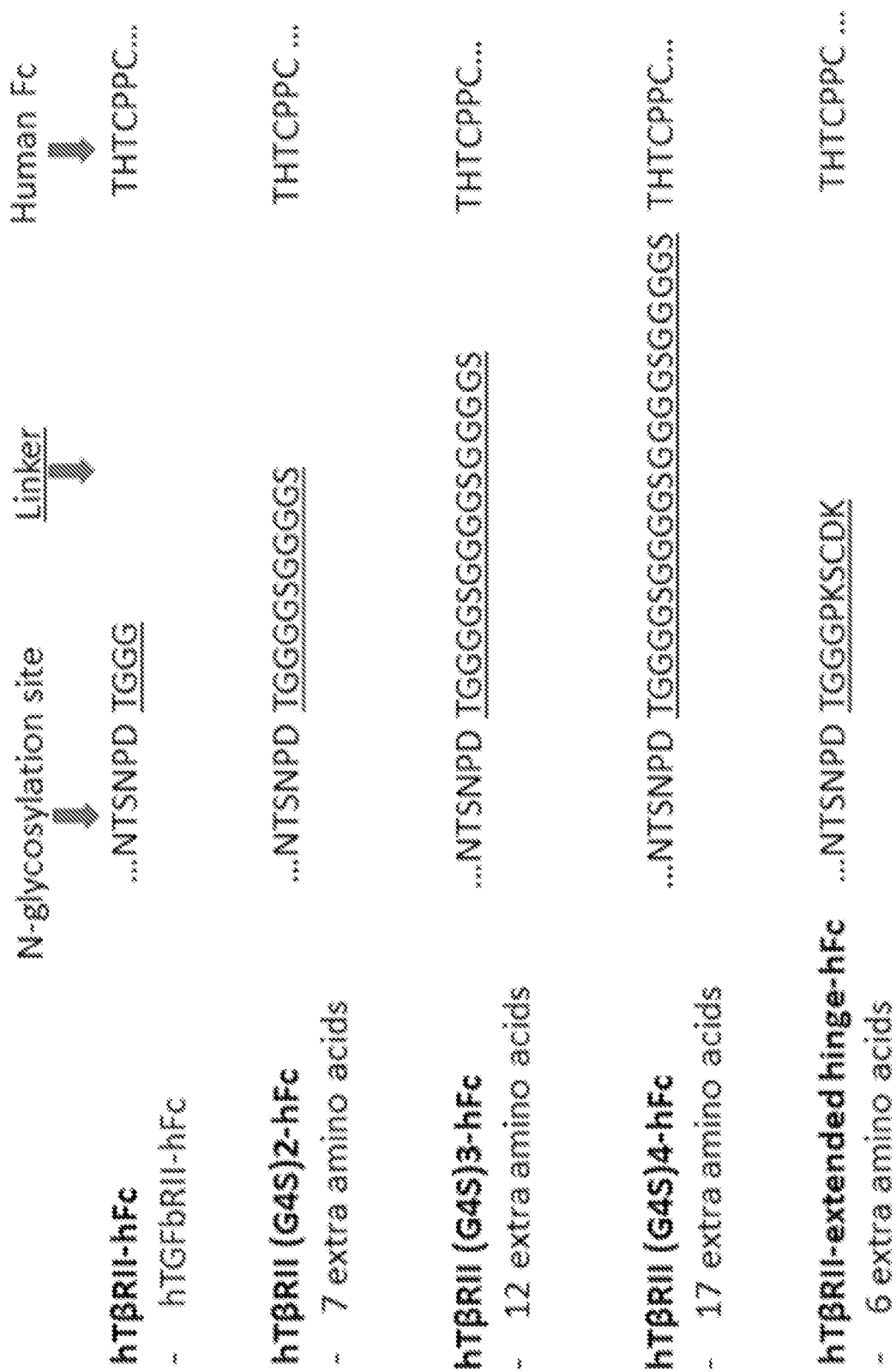


Figure 3

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	Fusion Protein	k_a (1/Ms)	k_d (1/s)	K_D (pM)
TGFβ1	hTβRII-hFc	2.01×10^6	4.16×10^{-4}	207.0
	hTβRII (G4S)2-hFc	2.91×10^6	4.83×10^{-4}	165.8
	hTβRII (G4S)3-hFc	3.89×10^6	5.10×10^{-4}	92.4
	hTβRII (G4S)4-hFc	6.69×10^6	4.57×10^{-4}	68.4
	hTβRII-extended hinge-hFc	2.38×10^6	4.64×10^{-4}	195.5
TGFβ3	hTβRII-hFc	1.99×10^7	1.57×10^{-3}	79.1
	hTβRII (G4S)2-hFc	1.74×10^7	1.81×10^{-3}	104.1
	hTβRII (G4S)3-hFc	2.09×10^7	8.32×10^{-4}	39.9
	hTβRII (G4S)4-hFc	8.80×10^6	2.76×10^{-4}	31.4
	hTβRII-extended hinge-hFc	1.51×10^7	1.39×10^{-3}	92.1

Figure 4A

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	TGFβ1		
Receptor	k_a (1/Ms)	k_d (1/s)	K_D (pM)
hTβRII (G4S)5-hFc	7.36×10^7	6.48×10^{-4}	8.8
hTβRII (G4S)6-hFc	1.66×10^8	6.32×10^{-4}	3.8

	TGFβ3		
Receptor	k_a (1/Ms)	k_d (1/s)	K_D (pM)
hTβRII (G4S)5-hFc	1.47×10^8	4.35×10^{-4}	2.96
hTβRII (G4S)6-hFc	5.99×10^7	2.75×10^{-4}	4.60

Figure 4B

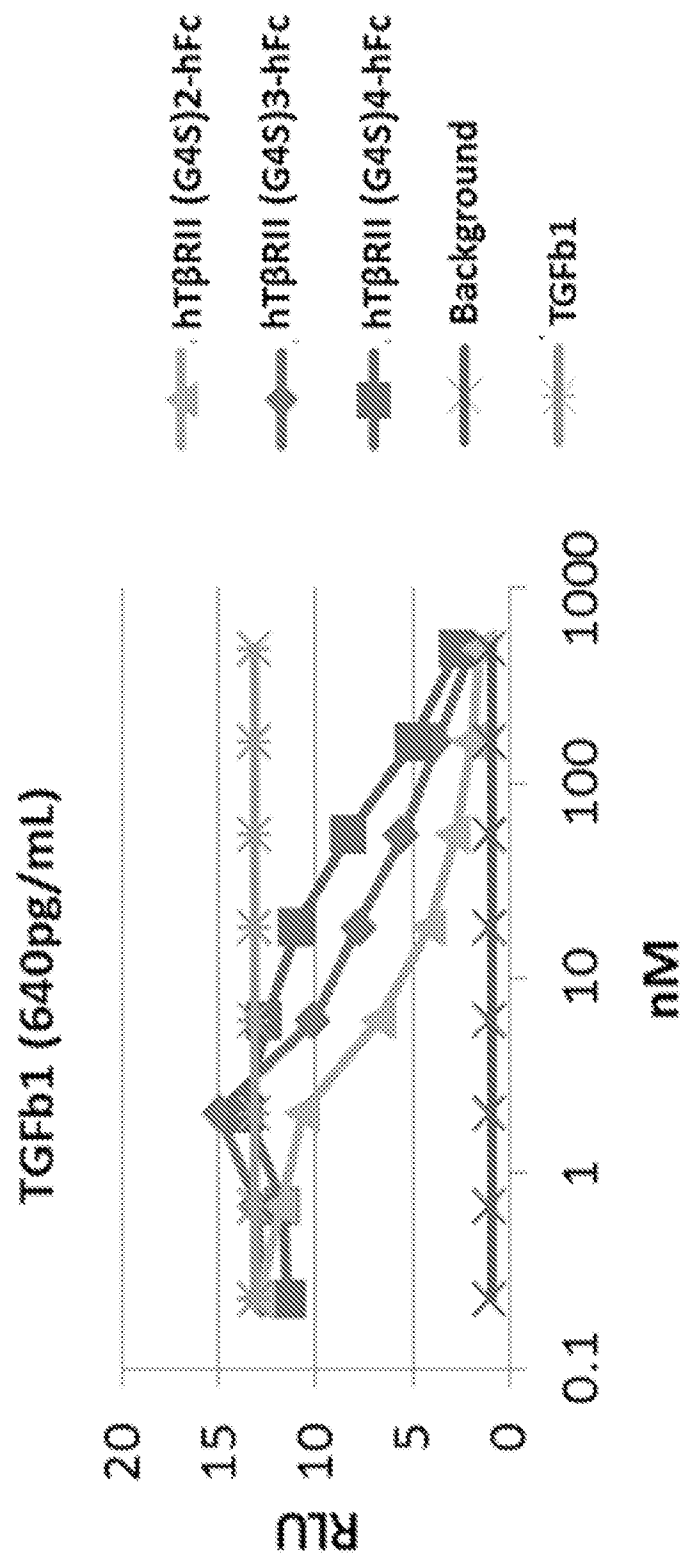


Figure 5A

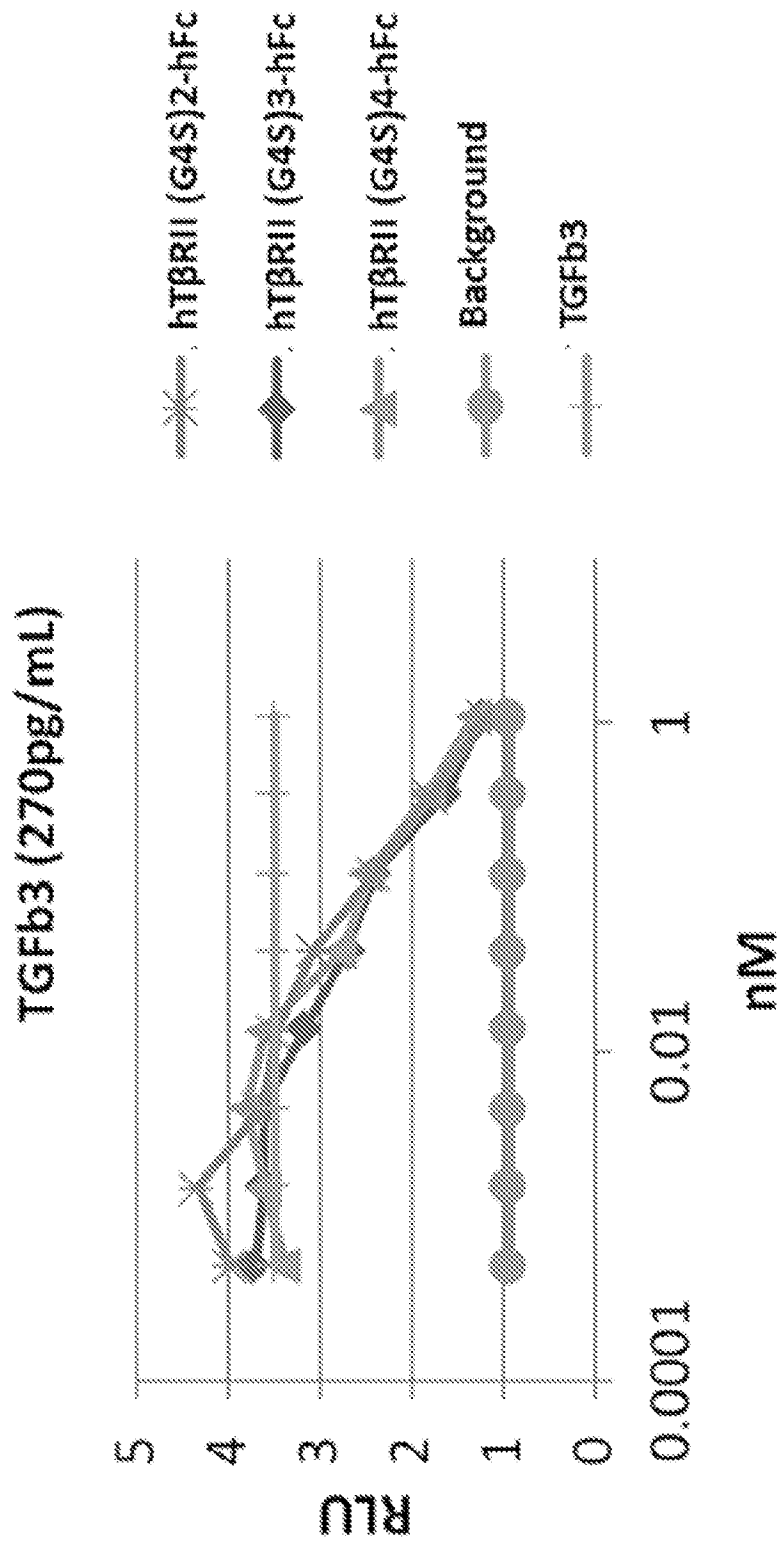


Figure 5B

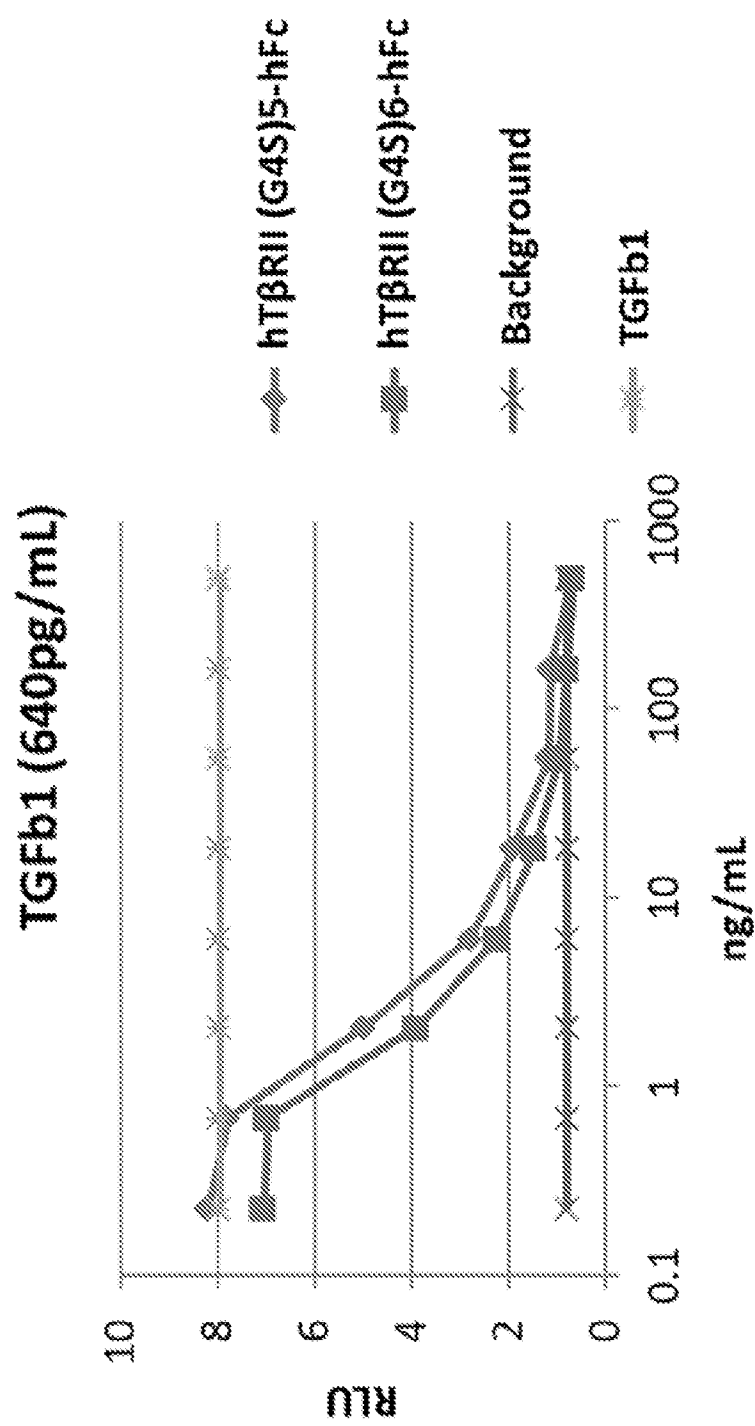


Figure 5C

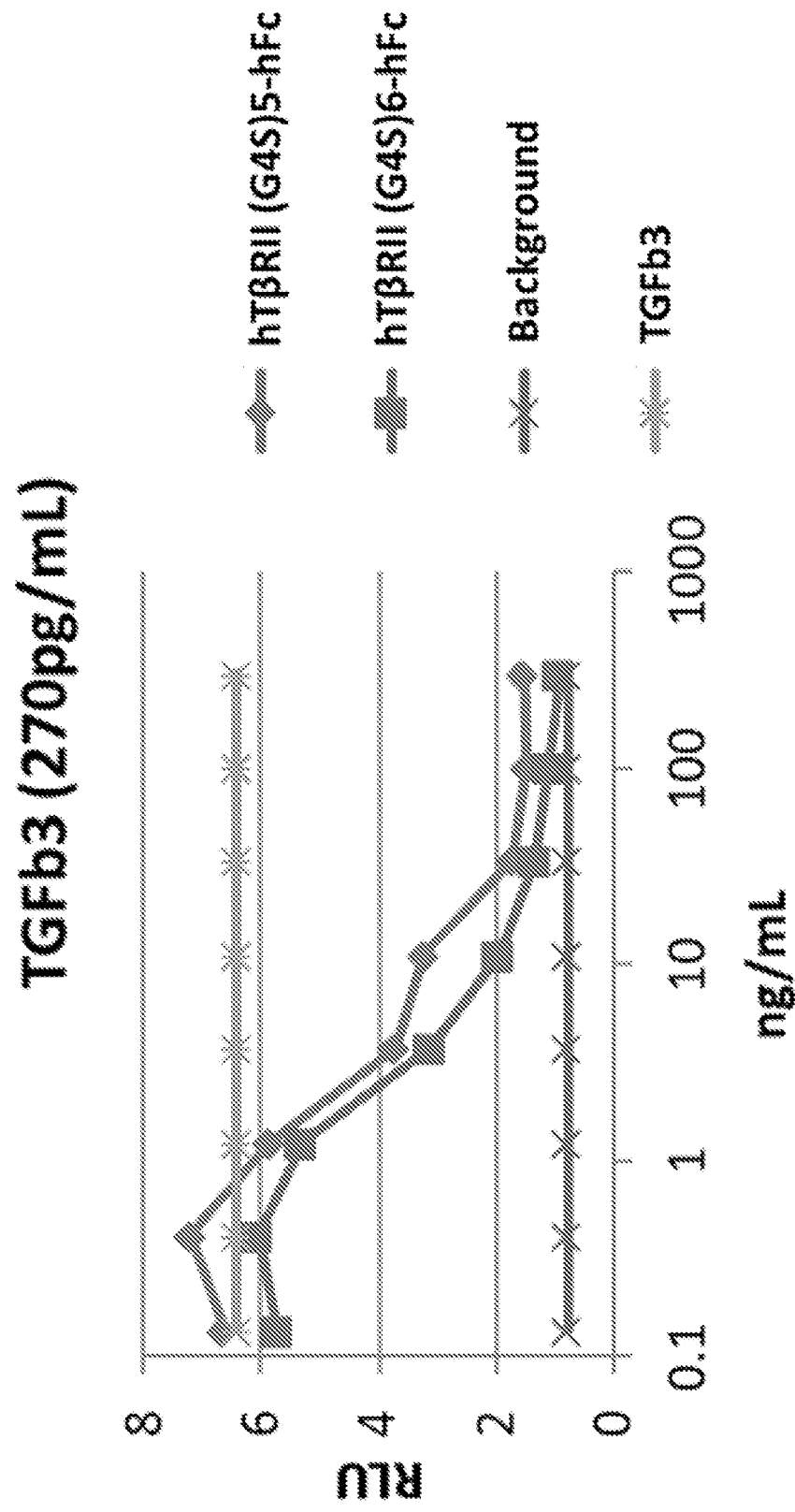


Figure 5D

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	IC ₅₀ (nM)		
	TGFβ1	TGFβ3	Ratio TGFβ1/TGFβ3
hTβRII-hFc	7.69	0.18	42
hTβRII (G4S)2-hFc	1.12	0.13	8.61
hTβRII (G4S)3-hFc	0.22	0.17	1.29
hTβRII (G4S)4-hFc	0.07	0.03	2.3
hTβRII extended hinge-hFc	5.67	0.11	52

Figure 5E

Construct	TGFβ1—IC50 (nM)	TGFβ3—IC50 (nM)	Ratio TGFβ1/ TGFβ3
hTβRII (G4S)5-hFc	0.03	0.04	0.75
hTβRII (G4S)6-hFc	0.02	0.04	0.5

Figure 5F

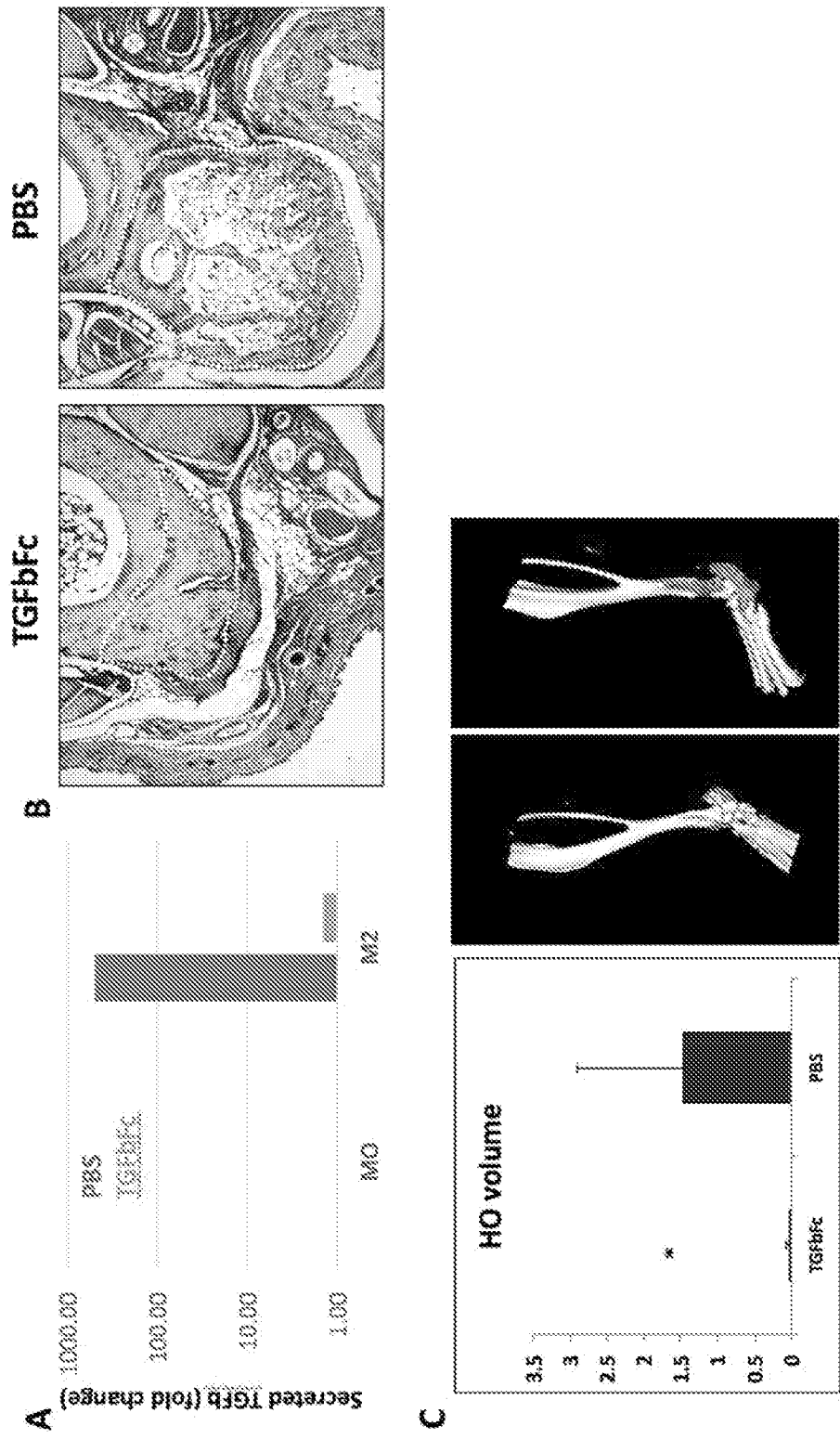


Figure 6A, Figure 6B, and Figure 6C

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IgG1  -----THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKF  53
IgG4  ----ESKYGPPCPSCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSQEDPEVQF  57
IgG2  -----VECPPCPAPPVAG-PSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVQF  51
IgG3  EPKSCDTPPPCPRCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVQF  60

      **  ****  .  *  *****,*****,*

IgG1  NQYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT  113
IgG4  NQYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKT  117
IgG2  NQYVDGVEVHNAKTKPREEQFNSTFRVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKT  111
IgG3  KQYVDGVEVHNAKTKPREEQYNSTFRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT  120

      :*****,***:*****,*****,*****,**:.***

IgG1  ISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGOPENNYK1TF  173
IgG4  ISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGOPENNYK1TF  177
IgG2  ISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGOPENNYK1TF  171
IgG3  ISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESSGOPENNYNT1TF  180

      ***:*****,*****,*****,*****,*****,***

IgG1  PVLDS2DG3SFFLY4SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK  225
IgG4  PVLDS2DG3SFFLY4SR5LTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK  229
IgG2  PMLDS2DG3SFFLY4SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK  223
IgG3  PMLDS2DG3SFFLY4SKLTVDKSRWQQGNIFSCSVMHEALHNRFTQKSLSLSPGK  232

      *:*****,*****,*:*****,*****,*:*****,**

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FIGURE 7

INTERNATIONAL SEARCH REPORT

 International application No.
PCT/US2019/017297

A. CLASSIFICATION OF SUBJECT MATTER

C07K 14/495 (2006.01) A61K 38/18 (2006.01) C07K 14/71 (2006.01) C07K 16/22 (2006.01) A61P 19/04 (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)

PATENW (Heterotopic ossification, fibrodysplasia ossificans progressive, Progressive osseous heteroplasia, Stone Man Syndrome, myositis ossificans, TGF β , ALK5, T β RII, betaglycan, fresolimumab)

MEDLINE, HCA, BIOSIS, EMBASE (Heterotopic ossification, fibrodysplasia ossificans progressive, Progressive osseous heteroplasia, Stone Man Syndrome, myositis ossificans, TGF β)

 Inventor and applicant name search: **Internal IP Australia databases, MEDLINE, HCA, BIOSIS, EMBASE:** (B. Levi, R. Kumar, S. Agarwal)

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

* "A"	Special categories of cited documents: 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 18 April 2019	Date of mailing of the international search report 18 April 2019
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA Email address: pct@ipaustalia.gov.au	Authorised officer Neal Dalton AUSTRALIAN PATENT OFFICE (ISO 9001 Quality Certified Service) Telephone No. +61262850740

INTERNATIONAL SEARCH REPORT		International application No.
C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		PCT/US2019/017297
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	Wang, X. et al. "Inhibition of overactive TGF- β attenuates progression of heterotopic ossification in mice" 2018 <i>Nature Communications</i> , Vol. 9, Article number: 551, published 7 February 2018. see abstract see abstract	1-4, 6-14, 58-68, 70-84 15-57
X	WO 2017/070194 A1 (THE CHILDREN'S MEDICAL CENTER CORPORATION et al.) 27 April 2017 see 0156-0169, claims 10 and 18	1, 3, 6-8, 58-60, 62-63
X	WO 2016/165808 A1 (MERCK PATENT GMBH) 20 October 2016 see abstract, Table 1, claims 11, 14	1, 3, 6-8, 62
Y	US 2016/0376341 A1 (Accelaron Pharma Inc.) 29 December 2016 see abstract, 0048, 0073-0078, 0139, claims 1, 21-22, 48	15-57
Y	US 2015/0056199 A1 (Accelaron Pharma Inc.) 26 February 2015 see abstract, 0048, 0073-0078, 0139, claims 1, 21-22	15-57

INTERNATIONAL SEARCH REPORT		International application No.	
Information on patent family members		PCT/US2019/017297	
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.			
Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	Publication Date	Publication Number	Publication Date
WO 2017/070194 A1	27 April 2017	WO 2017070194 A1	27 Apr 2017
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		JP 2016528295 A	15 Sep 2016
		US 2015056199 A1	26 Feb 2015
US 2015/0056199 A1	26 February 2015	US 2018179261 A1	28 Jun 2018
		WO 2015027082 A1	26 Feb 2015
		US 2015056199 A1	26 Feb 2015
		AU 2014308751 A1	17 Mar 2016
		CA 2921805 A1	26 Feb 2015
		CN 105658672 A	08 Jun 2016
		EP 3036262 A1	29 Jun 2016
		JP 2016528295 A	15 Sep 2016
		US 2016376341 A1	29 Dec 2016
		US 9809637 B2	07 Nov 2017
		US 2018179261 A1	28 Jun 2018
		WO 2015027082 A1	26 Feb 2015
End of Annex			
Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.			