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(54) **Title**: ALK7:ACTRIIB HETEROMULTIMERS AND USES THEREOF

(57) **Abstract**: In certain aspects, the disclosure provides soluble heteromeric polypeptide complexes comprising an extracellular domain of an ALK7 receptor and an extracellular domain of ActRIIB. In certain aspects, these ALK7:ActRIIB heteromultimers are can be used to improve metabolic parameters in a patient in need thereof. In certain aspects, these ALK7:ActRIIB heteromultimers are can be used to treat or prevent one or more kidney-associated disease or condition in a patient in need thereof.

ALK7:ACTRIIB HETEROMULTIMERS AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of priority to United States provisional application serial number 62/143,579, filed April 6, 2015. The disclosure of the foregoing application is hereby incorporated by reference in its entirety.

BACKGROUND OF THE INVENTION

The transforming growth factor-beta (TGF-beta) superfamily contains a variety of growth factors that share common sequence elements and structural motifs. These proteins are known to exert biological effects on a large variety of cell types in both vertebrates and invertebrates. Members of the superfamily perform important functions during embryonic development in pattern formation and tissue specification and can influence a variety of differentiation processes, including adipogenesis, myogenesis, chondrogenesis, cardiogenesis, hematopoiesis, neurogenesis, and epithelial cell differentiation. The family is divided into two general phylogenetic clades: the more recently evolved members of the superfamily, which includes TGF-betas, activins, and nodal and the clade of more distantly related proteins of the superfamily, which includes a number of BMPs and GDFs [Hinck (2012) FEBS Letters 586:1860-1870]. TGF-beta family members have diverse, often complementary biological effects. By manipulating the activity of a member of the TGF-beta family, it is often possible to cause significant physiological changes in an organism. For example, the Piedmontese and Belgian Blue cattle breeds carry a loss-of-function mutation in the GDF8 (also called myostatin) gene that causes a marked increase in muscle mass [Grobet *et al.* (1997) Nat Genet 17(1):71-4]. Furthermore, in humans, inactive alleles of GDF8 are associated with increased muscle mass and, reportedly, exceptional strength [Schuelke *et al.* (2004) N Engl J Med 350:2682-8].

Changes in fibrosis, muscle, bone, fat, red blood cells, and other tissues may be achieved by enhancing or inhibiting intracellular signaling (e.g., SMAD 1, 2, 3, 5, and/or 8) that is mediated by ligands of the TGF-beta family. Thus, there is a need for agents that regulate the activity of various ligands of the TGF-beta superfamily.

SUMMARY OF THE INVENTION

As described herein, it has been discovered that an ALK7:ActRIIB heterodimer protein complex is a unique antagonist of ligands of the TGF-beta superfamily, exhibiting a different ligand-binding profile/selectivity compared to corresponding ActRIIB and ALK7 homodimers. In particular, an exemplary ALK7:ActRIIB heterodimer displays enhanced binding to activin AC, activin C, and BMP5 compared to either homodimer, retains strong binding to activin B as observed with ActRIIB homodimer, and exhibits reduced binding to GDF11, GDF8, activin A, BMP10, BMP6, GDF3, and BMP9. In particular, ALK7:ActRIIB heterodimer displays low to no observable affinity for BMP9, whereas this ligand binds strongly to ActRIIB homodimer. See Figure 6. These results therefore demonstrate that ALK7:ActRIIB heterodimers are a more selective antagonists (inhibitors) of certain ligands of the TGF-beta superfamily compared to ActRIIB homodimers. Accordingly, an ALK7:ActRIIB heterodimer will be more useful than an ActRIIB homodimer in certain applications where such selective antagonism is advantageous. Examples include therapeutic applications where it is desirable to antagonize one or more of activin B, activin AC, activin C, and BMP5 with decreased antagonism of one or more of activin A, BMP10, BMP6, GDF3, and BMP9. Moreover, an ALK7:ActRIIB heterodimer was shown to have therapeutic effects in a mouse model of kidney disease as well as a beneficial catabolic effects on adipose cells. Therefore, while not wishing to be bound to a particular mechanism of action, it is expected that ALK7:ActRIIB heteromultimers, as well as variants thereof, that bind to/inhibit at least one or more of activin (e.g., activin A, activin B, activin AB, activin C, activin E, activin AC, activin AE, activin BC and activin BE), GDF8, GDF11, BMP10, BMP6, BMP5, GDF3, and/or nodal will be useful agents for treating kidney disease, particularly certain disorders of kidney disease such as inflammation and fibrosis, and/or promoting beneficial catabolic effects on adipose tissue. Furthermore, it is expected that other antagonists (inhibitors), or combinations of antagonists, that mimic the binding/inhibitory properties of the ALK7:ActRIIB heterodimers as well as agents that directly or indirectly antagonize ALK7 and/or ActRIIB receptors, agents that directly or indirectly antagonize ALK7 and/or ActRIIB-binding ligands, agents that directly or indirectly antagonize downstream signaling mediator (e.g., Smads), and/or agents that directly or indirectly antagonize TGFβ superfamily co-receptors (e.g., Cripto or Cryptic) will have similar biological effects. These antagonistic mimetic are collectively referred to herein as "ALK7:ActRIIB antagonists" or "ALK7:ActRIIB inhibitors".

Therefore, the present disclosure provides, in part, heteromultimer complexes (heteromultimers) comprising at least one ALK7 polypeptide and at least one ActRIIB polypeptide (ALK7:ActRIIB heteromultimers). Preferably, ALK7 polypeptides comprise a ligand-binding domain of an ALK7 receptor, for example, a portion of the ALK7 extracellular domain. Similarly, ActRIIB polypeptides generally comprise a ligand-binding domain of an ActRIIB receptor, for example, a portion of the ActRIIB extracellular domain. Preferably, such ALK7 and ActRIIB polypeptides, as well as resultant heteromultimers thereof, are soluble.

In certain aspects, an ALK7:ActRIIB heteromultimer comprises an ALK7 amino acid sequence that is at least 70% identical to a polypeptide that begins at any one of amino acids 21-28 of SEQ ID NO: 9 (e.g., amino acids 21, 22, 23, 24, 25, 26, 27, or 28) and ends at any one of amino acids 92-113 of SEQ ID NO: 9 (e.g., amino acids 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, or 113) of SEQ ID NO: 9. For example, ALK7:ActRIIB heteromultimers may comprise an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 28-92 of SEQ ID NO: 9. In other embodiments, ALK7:ActRIIB heteromultimers may comprise an ALK7 amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 21-113 of SEQ ID NO: 9. In other embodiments, ALK7:ActRIIB heteromultimers may comprise an ALK7 amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 10. In still other embodiments, ALK7:ActRIIB heteromultimers may comprise an ALK7 amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 20. In even other embodiments, ALK7:ActRIIB heteromultimers may comprise an ALK7 amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 39. In further embodiments, ALK7:ActRIIB heteromultimers may comprise an ALK7 amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 43. In further embodiments, ALK7:ActRIIB heteromultimers may comprise an ALK7 amino acid sequence that is at least 70%, 75%, 80%,

85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 46.

In certain aspects, an ALK7:ActRIIB heteromultimer comprises an ActRIIB amino acid sequence that is at least 70% identical to a polypeptide that begins at any one of amino acids 20-29 of SEQ ID NO: 1 (e.g., 20, 21, 22, 23, 24, 25 , 26, 27, 28, or 29) and ends at any one of amino acids 109-134 of SEQ ID NO: 1 (109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, or 134) of SEQ ID NO: 1. For example, ALK7:ActRIIB heteromultimers may comprise an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 29-109 of SEQ ID NO: 1. In further embodiments, ALK7:ActRIIB heteromultimers may comprise an ActRIIB amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 25-131. In other embodiments, ALK7:ActRIIB heteromultimers may comprise an ActRIIB amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 2. In still other embodiments, ALK7:ActRIIB heteromultimers may comprise an ActRIIB amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 3. In even other embodiments, ALK7:ActRIIB heteromultimers may comprise an ActRIIB amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 5. In still even other embodiments, ALK7:ActRIIB heteromultimers may comprise an ActRIIB amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 6. In certain preferred embodiments, ALK7:ActRIIB heteromultimers do not comprise an ActRIIB polypeptide comprising an acidic amino acid (e.g., the naturally occurring amino acids E or D or an artificial acidic amino acid) at the position corresponding to L79 of SEQ ID NO: 1.

Various combinations of the ALK7 and ActRIIB polypeptides described herein are also contemplated with respect to ALK7:ActRIIB heteromultimers. For example, in certain aspects, an ALK7:ActRIIB heteromultimer may comprise, consist essentially of or consist of a) a polypeptide comprising, consisting essentially of, or consisting of an ALK7 amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%,

94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 28-92 of SEQ ID NO: 9; and b) a polypeptide comprising, or consisting essentially of, or consisting of an ActRIIB amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 29-109 of SEQ ID NO: 1. In further aspects, an ALK7:ActRIIB heteromultimer may comprise, consist essentially of or consist of a) a polypeptide comprising, consisting essentially of, or consisting of an ALK7 amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 21-113 of SEQ ID NO: 9; and b) a polypeptide comprising, or consisting essentially of, or consisting of an ActRIIB amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 25-131 of SEQ ID NO: 1. In other aspects, an ALK7:ActRIIB heteromultimer may comprise, consist essentially of or consist of a) a polypeptide comprising, consisting essentially of, or consisting of an ALK7 amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 10; and b) a polypeptide comprising, or consisting essentially of, or consisting of an ActRIIB amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 2. In other aspects, an ALK7:ActRIIB heteromultimer may comprise, consist essentially of or consist of a) a polypeptide comprising, consisting essentially of, or consisting of an ALK7 amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 20; and b) a polypeptide comprising, or consisting essentially of, or consisting of an ActRIIB amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 2. In other aspects, an ALK7:ActRIIB heteromultimer may comprise, consist essentially of or consist of a) a polypeptide comprising, consisting essentially of, or consisting of an ALK7 amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 39; and b) a polypeptide comprising, or consisting essentially of, or consisting of an ActRIIB amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 2. In other aspects, an ALK7:ActRIIB heteromultimer may comprise, consist essentially of or consist of a) a

polypeptide comprising, consisting essentially of, or consisting of an ALK7 amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 43; and b) a polypeptide comprising, or consisting essentially of, or consisting of an ActRIIB amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 2. In other aspects, an ALK7:ActRIIB heteromultimer may comprise, consist essentially of or consist of a) a polypeptide comprising, consisting essentially of, or consisting of an ALK7 amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 46; and b) a polypeptide comprising, or consisting essentially of, or consisting of an ActRIIB amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 2. In even other aspects, an ALK7:ActRIIB heteromultimer may comprise, consist essentially of or consist of a) a polypeptide comprising, consisting essentially of, or consisting of an ALK7 amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 10; and b) a polypeptide comprising, or consisting essentially of, or consisting of an ActRIIB amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 3. In even other aspects, an ALK7:ActRIIB heteromultimer may comprise, consist essentially of or consist of a) a polypeptide comprising, consisting essentially of, or consisting of an ALK7 amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 20; and b) a polypeptide comprising, or consisting essentially of, or consisting of an ActRIIB amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 3. In even other aspects, an ALK7:ActRIIB heteromultimer may comprise, consist essentially of or consist of a) a polypeptide comprising, consisting essentially of, or consisting of an ALK7 amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 39; and b) a polypeptide comprising, or consisting essentially of, or consisting of an ActRIIB amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 3. In even other aspects, an

ALK7:ActRIIB heteromultimer may comprise, consist essentially of or consist of a) a polypeptide comprising, consisting essentially of, or consisting of an ALK7 amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 43; and b) a polypeptide comprising, or consisting essentially of, or consisting of an ActRIIB amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 3. In other aspects, an ALK7:ActRIIB heteromultimer may comprise, consist essentially of or consist of a) a polypeptide comprising, consisting essentially of, or consisting of an ALK7 amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 46; and b) a polypeptide comprising, or consisting essentially of, or consisting of an ActRIIB amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 3. In still other aspects, an ALK7:ActRIIB heteromultimer may comprise, consist essentially of or consist of a) a polypeptide comprising, consisting essentially of, or consisting of an ALK7 amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 10; and b) a polypeptide comprising, or consisting essentially of, or consisting of an ActRIIB amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 5. In still even other aspects, an ALK7:ActRIIB heteromultimer may comprise, consist essentially of or consist of a) a polypeptide comprising, consisting essentially of, or consisting of an ALK7 amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 10; and b) a polypeptide comprising, or consisting essentially of, or consisting of an ActRIIB amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 6.

As described herein, ALK7:ActRIIB heteromultimer structures include, for example, heterodimers, heterotrimers, heterotetramers, heteropentamers, and higher order heteromultimer complexes. See, e.g., Figures 1, 2, and 8-10. In certain preferred embodiments, ALK7:ActRIIB heteromultimers are heterodimers.

In certain aspects, ALK7 and/or ActRIIB polypeptides may be fusion proteins. For example, in some embodiments, an ALK7 polypeptide may be a fusion protein comprising an ALK7 polypeptide domain and one or more heterologous (non-ALK7) polypeptide domains. Similarly, in some embodiments, an ActRIIB polypeptide may be a fusion protein comprising an ActRIIB polypeptide domain and one or more heterologous (non-ActRIIB) polypeptide domains. Optionally, ALK7 polypeptides are connected directly (fused) to one or more heterologous domains, or an intervening sequence, such as a linker, may be positioned between the amino acid sequence of the ALK7 polypeptide and the one or more heterologous domains. Similarly, the ActRIIB polypeptide may be connected directly (fused) to one or more heterologous domains, or an intervening sequence, such as a linker, may be positioned between the amino acid sequence of the ActRIIB polypeptide and the one or more heterologous domains. Linkers may correspond to the roughly 15 amino acid unstructured region at the C-terminal end of the extracellular domain of ActRIIB or ALK7 (the "tail"), or it may be an artificial sequence of between 5 and 15, 20, 30, 50, 100 or more amino acids that are relatively free of secondary structure. A linker may be rich in glycine and proline residues and may, for example, contain repeating sequences of threonine/serine and glycines. Examples of linkers include, but are not limited to, the sequences TGGG (SEQ ID NO: 17), SGGG (SEQ ID NO: 18), TGGGG (SEQ ID NO: 15), SGGGG (SEQ ID NO: 16), GGGGS (SEQ ID NO: 58), GGGG (SEQ ID NO: 14), and GGG (SEQ ID NO: 13). In some embodiments, the one or more heterologous domains that provide a desirable property to the ALK7 and/or ActRIIB fusion proteins including, for example, improved pharmacokinetics, easier purification, targeting to particular tissues, etc. For example, a heterologous domain of a fusion protein may enhance one or more of in vivo stability, in vivo half-life, uptake/administration, tissue localization or distribution, formation of protein complexes, multimerization of the fusion protein, and/or purification. An ALK7 or ActRIIB fusion protein may include an immunoglobulin Fc domain (wild-type or mutant) or a serum albumin. In some embodiments, an ALK7 and/or ActRIIB polypeptides may comprise a purification subsequence, such as an epitope tag, a FLAG tag, a polyhistidine sequence, and a GST fusion.

In certain embodiments, ALK7:ActRIIB heteromultimers described herein comprise an ALK7 polypeptide covalently, or non-covalently, associated with an ActRIIB polypeptide wherein the ALK7 polypeptide comprises an ALK7 domain and an amino acid sequence of a first member (or second member) of an interaction pair and the ActRIIB polypeptide comprises an ActRIIB polypeptide and an amino acid sequence of a second member (or first

member) of the interaction pair. Interaction pairs described herein are designed to promote dimerization or form higher order multimers. See, e.g., Figures 1, 2, and 8-10. In some embodiments, the interaction pair may be any two polypeptide sequences that interact to form a complex, particularly a heterodimeric complex although operative embodiments may also employ an interaction pair that forms a homodimeric sequence. The first and second members of the interaction pair may be an asymmetric pair, meaning that the members of the pair preferentially associate with each other rather than self-associate (i.e., guided interaction pairs). Accordingly, first and second members of an asymmetric interaction pair may associate to form a heterodimeric complex. Alternatively, the interaction pair may be unguided, meaning that the members of the pair may associate with each other or self-associate without substantial preference and thus may have the same or different amino acid sequences. Accordingly, first and second members of an unguided interaction pair may associate to form a homodimer complex or a heterodimeric complex. Optionally, the first member of the interaction action pair (*e.g.*, an asymmetric pair or an unguided interaction pair) associates covalently with the second member of the interaction pair. Optionally, the first member of the interaction action pair (*e.g.*, an asymmetric pair or an unguided interaction pair) associates non-covalently with the second member of the interaction pair. Optionally, the first member of the interaction action pair (*e.g.*, an asymmetric pair or an unguided interaction pair) associates through both covalent and non-covalent mechanisms with the second member of the interaction pair.

In some embodiments, ALK7 polypeptides are fusion proteins that comprise an Fc domain of an immunoglobulin. Similarly, in some embodiments, ActRIIB polypeptides are fusion proteins that comprise an Fc domain of an immunoglobulin. Traditional Fc fusion proteins and antibodies are examples of unguided interaction pairs, whereas a variety of engineered Fc domains have been designed as asymmetric interaction pairs [Spiess et al (2015) Molecular Immunology 67(2A): 95-106]. Therefore, a first member and/or a second member of an interaction pair described herein may comprise a constant domain of an immunoglobulin, including, for example, the Fc portion of an immunoglobulin. For example, a first member of an interaction pair may comprise an amino acid sequence that is derived from an Fc domain of an IgG (IgG1, IgG2, IgG3, or IgG4), IgA (IgA1 or IgA2), IgE, or IgM immunoglobulin. Such immunoglobulin domains may comprise one or more amino acid modifications (e.g., deletions, additions, and/or substitutions) that promote ALK7:ActRIIB heteromultimer formation. For example, the first member of an interaction pair may comprise, consist essentially of, or consist of 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 any one of SEQ ID NOs: 23-37. Similarly, a second member of an interaction pair may comprise an amino acid sequence that is derived from an Fc domain of an IgG (IgG1, IgG2, IgG3, or IgG4), IgA (IgA1 or IgA2), IgE, or IgM. Such immunoglobulin domains may comprise one or more amino acid modifications (e.g., deletions, additions, and/or substitutions) that promote ALK7:ActRIIB heteromultimer formation. For example, the second member of an interaction pair may comprise, consist essentially of, or consist of 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 any one of SEQ ID NOs: 23-37. In some embodiments, a first member and a second member of an interaction pair comprise Fc domains derived from the same immunoglobulin class and subtype. In other embodiments, a first member and a second member of an interaction pair comprise Fc domains derived from different immunoglobulin classes or subtypes.

In some embodiments, an ALK7:ActRIIB heterodimer comprises i) an ALK7 polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 76, and ii) an ActRIIB polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 73. In other embodiments, an ALK7:ActRIIB heterodimer comprises i) an ALK7 polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 80, and ii) an ActRIIB polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 78.

Optionally, a first member and/or a second member of an interaction pair (*e.g.*, an asymmetric pair or an unguided interaction pair) comprise a modified constant domain of an immunoglobulin, including, for example, a modified Fc portion of an immunoglobulin. For example, protein complexes of the disclosure may comprise a first modified Fc portion of an IgG comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to an amino acid sequence selected from the group: SEQ ID NOs: 23-37 and a second modified Fc

portion of an IgG, which may be the same or different from the amino acid sequence of the first modified Fc portion of the IgG, comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to an amino acid sequence selected from the group: SEQ ID NOs: 23-37. In some embodiments, ALK7:ActRIIB heteromultimers comprise, consist essentially of, or consist of: a) an ALK7 (or ActRIIB) fusion protein comprising an immunoglobulin domain that comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 23, optionally wherein the immunoglobulin domain comprises a positively charged amino acid (e.g., K, R, or H) at the positions corresponding to residues 134 and 177 of SEQ ID NO: 23, and further optionally wherein the immunoglobulin domain does not comprise a positively charged amino acid (e.g., K, R, or H) at the position corresponding to residue 225 of SEQ ID NO: 23, and b) an ActRIIB (or ALK7) fusion protein comprising an immunoglobulin domain that comprises, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 24, optionally wherein the immunoglobulin domain comprises a negatively charged (e.g., D or E) amino acid at the positions corresponding to residues 170 and 187 of SEQ ID NO: 24, and further optionally wherein the immunoglobulin domain comprises a positively charged amino acid (e.g., K, R, or H) at the position corresponding to residue 225 of SEQ ID NO: 24. In other embodiments, ALK7:ActRIIB heteromultimers comprise, consist essentially of, or consist of: a) an ALK7 (or ActRIIB) fusion protein comprising an immunoglobulin domain that comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 27, optionally wherein the immunoglobulin domain comprises a C at the position corresponding to residue 132 of SEQ ID NO: 27 and a W at the position corresponding to residue 144 of SEQ ID NO: 27, and further optionally wherein the immunoglobulin domain does not comprise a positively charged amino acid (e.g., K, R, or H) at the position corresponding to residue 225 of SEQ ID NO: 27, and b) an ActRIIB (or ALK7) fusion protein comprising an immunoglobulin domain that comprises, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 28, optionally wherein the immunoglobulin domain comprises a S at the position corresponding to residue 144 of SEQ ID NO: 28, an A at the position corresponding to

residue 146 of SEQ ID NO: 28, and a V at the position corresponding to residue 185 of SEQ ID NO: 28, and further optionally wherein the immunoglobulin domain does not comprise a positively charged amino acid (e.g., K, R, or H) at the position corresponding to residue 225 of SEQ ID NO: 28.

Optionally, an ALK7 and/or ActRIIB 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. ALK7 and/or ActRIIB polypeptides may comprise at least one N-linked sugar, and may include two, three or more N-linked sugars. Such polypeptides may also comprise O-linked sugars. ALK7 and/or ActRIIB polypeptides may be produced in a variety of cell lines that glycosylate the protein in a manner that is suitable for patient use, including engineered insect or yeast cells, and mammalian cells such as COS cells, CHO cells, HEK cells and NSO cells. In some embodiments an ALK7 and/or ActRIIB polypeptide is glycosylated and has a glycosylation pattern obtainable from a Chinese hamster ovary cell line. Preferably ALK7:ActRIIB heteromultimer complexes of the disclosure exhibit a serum half-life of at least 4, 6, 12, 24, 36, 48, or 72 hours in a mammal (*e.g.*, a mouse or a human). Optionally, ALK7:ActRIIB heteromultimers may exhibit a serum half-life of at least 6, 8, 10, 12, 14, 20, 25, or 30 days in a mammal (*e.g.*, a mouse or a human).

In certain aspects, ALK7:ActRIIB heteromultimers of the disclosure bind to one or more TGF-beta superfamily ligands. Optionally, ALK7:ActRIIB heteromultimers bind to one or more of these ligands with a K_D of less than or equal to 10^{-8}, 10^{-9}, 10^{-10}, 10^{-11}, or 10^{-12} M. For example, in some embodiments, ALK7:ActRIIB heteromultimers bind to activin. In some embodiments, ALK7:ActRIIB heteromultimers bind to activin A. In some embodiments, ALK7:ActRIIB heteromultimers bind to activin B. In some embodiments, ALK7:ActRIIB heteromultimers bind to activin C. In some embodiments, ALK7:ActRIIB heteromultimers bind to activin AB. In some embodiments, ALK7:ActRIIB heteromultimers bind to activin AC. In some embodiments, ALK7:ActRIIB heteromultimers bind to activin BC. In some embodiments, ALK7:ActRIIB heteromultimers bind to activin E. In some embodiments, ALK7:ActRIIB heteromultimers bind to activin AE. In some embodiments, ALK7:ActRIIB heteromultimers bind to activin BE. In some embodiments, ALK7:ActRIIB heteromultimers bind to GDF11. In some embodiments, ALK7:ActRIIB heteromultimers bind to GDF8. In some embodiments, ALK7:ActRIIB heteromultimers bind to BMP6. In some embodiments, ALK7:ActRIIB heteromultimers bind to BMP10. In some embodiments,

ALK7:ActRIIB heteromultimers bind to BMP5. In some embodiments, ALK7:ActRIIB heteromultimers bind to GDF3. In some embodiments, ALK7:ActRIIB heteromultimers bind to nodal. In some embodiments, ALK7:ActRIIB heteromultimers do not bind to or do not substantially bind to BMP9. In some embodiments, the ALK7:ActRIIB heteromultimer binds to BMP10 with weaker affinity compared to a corresponding ActRIIB homomultimer. In some embodiments, the ALK7:ActRIIB heteromultimer binds to BMP9 with weaker affinity compared to a corresponding ActRIIB homomultimer. In some embodiments, the ALK7:ActRIIB heteromultimer binds to GDF3 with weaker affinity compared to a corresponding ActRIIB homomultimer. In some embodiments, the ALK7:ActRIIB heteromultimer binds to BMP6 with weaker affinity compared to a corresponding ActRIIB homomultimer. In some embodiments, the ALK7:ActRIIB heteromultimer binds to GDF8 with weaker affinity compared to a corresponding ActRIIB homomultimer. In some embodiments, the ALK7:ActRIIB heteromultimer binds to GDF11 with weaker affinity compared to a corresponding ActRIIB homomultimer. In some embodiments, the ALK7:ActRIIB heteromultimer binds to activin C with stronger affinity compared to a corresponding ActRIIB homomultimer. In some embodiments, the ALK7:ActRIIB heteromultimer binds to activin AC with stronger affinity compared to a corresponding ActRIIB homomultimer. In some embodiments, the ALK7:ActRIIB heteromultimer binds to BMP5 with stronger affinity compared to a corresponding ActRIIB homomultimer.

In general, ALK7:ActRIIB heteromultimers of the disclosure antagonize (inhibit) one or more activities of at least one TGF-beta superfamily ligand, and such alterations in activity may be measured using various assays known in the art, including, for example, a cell-based assay such as those described herein. In certain aspects, ALK7:ActRIIB heteromultimers may be used to inhibit signaling (e.g., Smad 2/3 and/or Smad 1/5/8 signaling) mediated by one or more TGFβ superfamily ligands in, for example, a cell-based assay. For example, in some embodiments, ALK7:ActRIIB heteromultimers inhibit activin signaling in a cell-based assay. In some embodiments, ALK7:ActRIIB heteromultimers inhibit activin A signaling in a cell-based assay. In some embodiments, ALK7:ActRIIB heteromultimers inhibit activin B signaling in a cell-based assay. In some embodiments, ALK7:ActRIIB heteromultimers inhibit activin C signaling in a cell-based assay. In some embodiments, ALK7:ActRIIB heteromultimers inhibit activin AB signaling in a cell-based assay. In some embodiments, ALK7:ActRIIB heteromultimers inhibit activin AC signaling in a cell-based assay. In some embodiments, ALK7:ActRIIB heteromultimers inhibit activin BC signaling in a cell-based

assay. In some embodiments, ALK7:ActRIIB heteromultimers inhibit activin E signaling in a cell-based assay. In some embodiments, ALK7:ActRIIB heteromultimers inhibit activin AE signaling in a cell-based assay. In some embodiments, ALK7:ActRIIB heteromultimers inhibit activin BE signaling in a cell-based assay. In some embodiments, ALK7:ActRIIB heteromultimers inhibit GDF11 signaling in a cell-based assay. In some embodiments, ALK7:ActRIIB heteromultimers inhibit GDF8 signaling in a cell-based assay. In some embodiments, ALK7:ActRIIB heteromultimers inhibit BMP6 signaling in a cell-based assay. In some embodiments, ALK7:ActRIIB heteromultimers inhibit BMP10 signaling in a cell-based assay. In some embodiments, ALK7:ActRIIB heteromultimers inhibit BMP5 signaling in a cell-based assay. In some embodiments, ALK7:ActRIIB heteromultimers may inhibit GDF3 signaling in a cell-based assay. In some embodiments, ALK7:ActRIIB heteromultimers may inhibit nodal signaling in a cell-based assay. In some embodiments, ALK7:ActRIIB heteromultimers do not inhibit or do not substantially inhibit BMP9 signaling in a cell-based assay. In some embodiments, ALK7:ActRIIB heteromultimers are weaker inhibitors of BMP9 signaling in a cell-based assay compared to a corresponding ActRIIB homomultimer. In some embodiments, ALK7:ActRIIB heteromultimers are weaker inhibitors of BMP10 signaling in a cell-based assay compared to a corresponding ActRIIB homomultimer. In some embodiments, ALK7:ActRIIB heteromultimers are weaker inhibitors of BMP6 signaling in a cell-based assay compared to a corresponding ActRIIB homomultimer. In some embodiments, ALK7:ActRIIB heteromultimers are weaker inhibitors of GDF3 signaling in a cell-based assay compared to a corresponding ActRIIB homomultimer. In some embodiments, ALK7:ActRIIB heteromultimers are weaker inhibitors of GDF11 signaling in a cell-based assay compared to a corresponding ActRIIB homomultimer. In some embodiments, ALK7:ActRIIB heteromultimers are weaker inhibitors of GDF8 signaling in a cell-based assay compared to a corresponding ActRIIB homomultimer. In some embodiments, ALK7:ActRIIB heteromultimers are weaker inhibitors of activin A signaling in a cell-based assay compared to a corresponding ActRIIB homomultimer. In some embodiments, ALK7:ActRIIB heteromultimers are stronger inhibitors of activin C signaling in a cell-based assay compared to a corresponding ActRIIB homomultimer. In some embodiments, ALK7:ActRIIB heteromultimers are stronger inhibitors of activin AC signaling in a cell-based assay compared to a corresponding ActRIIB homomultimer. In some embodiments, ALK7:ActRIIB heteromultimers are stronger inhibitors of BMP5 signaling in a cell-based assay compared to a corresponding ActRIIB homomultimer.

Any of the ALK7:ActRIIB heteromultimers as well as ALK7:ActRIIB antagonists described herein may be formulated as a pharmaceutical preparation (compositions). In some embodiments, pharmaceutical preparations comprise a pharmaceutically acceptable carrier. A pharmaceutical preparation will preferably be pyrogen-free (meaning pyrogen free to the extent required by regulations governing the quality of products for therapeutic use). A pharmaceutical preparation may also include one or more additional compounds such as a compound that is used to treat a disorder/condition described herein. In general, ALK7:ActRIIB heteromultimer pharmaceutical preparations are substantially free of ALK7 and/or ActRIIB homomultimers. For example, in some embodiments, ALK7:ActRIIB heteromultimer pharmaceutical preparations comprise less than about 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or less than about 1% ALK7 homomultimers. In some embodiments, ALK7:ActRIIB heteromultimer pharmaceutical preparations comprise less than about 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or less than about 1% ActRIIB homomultimers.

In certain aspects, the disclosure provides nucleic acids encoding an ALK7 or ActRIIB polypeptide as described herein. For example, an ActRIIB nucleic acid may comprise, consists essentially of, or consists of a nucleic acid that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence of 73-396 of SEQ ID NO: 7 or one that hybridizes under stringent conditions to the complement of nucleotides 73-396 of SEQ ID NO: 7. Such an nucleic acid may be one that comprises the sequence of SEQ ID NOs: 8 or 72. In some embodiments, an ActRIIB nucleic acids comprises, consists essentially of, or consists of a nucleotide sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID Nos: 7, 8, and 72. Similarly, an ALK7 nucleic acid may comprise, consists essentially of, or consists of a nucleic acid that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence of 61-339 of SEQ ID NO: 11 or one that hybridizes under stringent conditions to the complement of nucleotides 61-339 of SEQ ID NO: 11. Such an ALK7 nucleic acid may be one that comprises the sequence of SEQ ID NOs: 12, 22, 41, 45, or 75. In some embodiments, an ALK7 nucleic acids comprises, consists essentially of, or consists of a nucleotide sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID NOs: 11, 12, 22, 41, 45, or 75.

In certain aspects, the present disclosure provides nucleic acids sequence comprising a coding sequence for and ALK7 polypeptide and a coding sequence for the ActRIIB

polypeptide. For example, in some embodiments, nucleic acids of the disclosure a) comprises, consists essentially of, or consists of a nucleotide sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID NOs: 11, 12, 21, 22, 40, 41, 44, 45, or 75, and b) comprises, consists essentially of, or consists of a nucleotide sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID Nos: 7, 8, or 72. Preferably, ALK7 and/or ActRIIB nucleic acids are isolated and/or recombinant nucleic acids. Nucleic acids disclosed herein may be operably linked to a promoter for expression. The present disclosure further provides vectors comprising such ALK7 and/or ActRIIB polynucleotides as well as cells (e.g., CHO cells), preferably cells isolated from a human or other vertebrate species, comprising such ALK7 and/or ActRIIB polynucleotides as well as vectors comprising such ALK7 and/or ActRIIB polynucleotides.

In certain aspects, ALK7 polypeptides and/or ActRIIB polypeptides may be expressed in a mammalian cell line, optionally a cell line that mediates suitably natural glycosylation of the ActRIIB or ALK7 protein so as to diminish the likelihood of an unfavorable immune response in a patient (including the possibility of veterinary patients). Human and CHO cell lines have been used successfully, and it is expected that other common mammalian expression vectors will be useful. Thus the disclosure provides cultured cells comprising any of the nucleic acids disclosed herein. Such cells may be mammalian cells, including CHO cells, NSO cells, HEK cells and COS cells. Other cells may be chosen depending on the species of the intended patient. Other cells are disclosed herein. Cultured cells are understood to mean cells maintained in laboratory or other man-made conditions (e.g., frozen, or in media) and not part of a living organism.

In certain aspects, the disclosure provides methods for making any of the ALK7 and ActRIIB polypeptides described herein as well as ALK7:ActRIIB heteromultimer complexes comprising such polypeptides. Such a method may include expressing any of the nucleic acids disclosed herein in a suitable cell (*e.g.*, CHO cell or a COS cell). For example, in some embodiments a method of making a heteromultimer comprising an ALK7 polypeptide and an ActRIIB polypeptide comprises: a) culturing a cell under conditions suitable for expression of an ALK7 polypeptide and an ActRIIB polypeptide, wherein the cell comprises an ALK7 polynucleotide and an ActRIIB polynucleotide; and b) recovering the heteromultimer so expressed. Alternatively, a method of making a heteromultimer comprising an ALK7 polypeptide and an ActRIIB polypeptide may comprise: a) culturing a first cell under

conditions suitable for expression of an ALK7 polypeptide, wherein the first cell comprises an ALK7 polynucleotide; b) recovering the ALK7 polypeptide so expressed; c) culturing a second cell under conditions suitable for expression of an ActRIIB polypeptide, wherein the second cell comprises an ActRIIB polynucleotide; d) recovering the ActRIIB polypeptide so expressed; e) combining the recovered ALK7 polypeptide and the recovered ActRIIB polypeptide under conditions suitable for ALK7:ActRIIB heteromultimer formation; and f) recovering the ALK7:ActRIIB heteromultimer. In certain embodiments, ALK7 and/or ActRIIB polypeptides are expressed using a TPA leader sequence (e.g., SEQ ID NO: 70). In certain embodiments, ALK7 and/or ActRIIB polypeptides are expressed in a CHO cell. ALK7 and ActRIIB polypeptides described herein, as well as protein complexes of the same, may be recovered as crude, partially purified, or highly purified fractions using any of the well-known techniques for obtaining protein from cell cultures. In general, such methods result in ALK7:ActRIIB complexes that are substantially free of ALK7 and/or ActRIIB homomultimers. For example, in some embodiments, methods for producing ALK7:ActRIIB heteromultimers result in less than about 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or less than about 1% ALK7 homomultimers. In some embodiments, methods for producing ALK7:ActRIIB heteromultimers result in less than about 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or less than about 1% ActRIIB homomultimers. In some embodiments, methods for producing ALK7:ActRIIB heteromultimers result in less than about 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or less than about 1% ALK7 homomultimers and In some embodiments, methods for producing ALK7:ActRIIB heteromultimers result in less than about 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or less than about 1% ActRIIB homomultimers.

The disclosure further provides methods and ALK7:ActRIIB heteromultimers for use in the treatment or prevention of various activin C-associated diseases and conditions. For example, in some embodiments, ALK7:ActRIIB heteromultimers may be used to increase fertility, increase sperm production, increase seminiferous tubule volume, decrease prostate inflammation, decrease liver inflammation in a patient in need thereof. In some embodiments, ALK7:ActRIIB heteromultimers may be used to treat or prevent hepatic cancer, testicular cancer, or prostate cancer. In some embodiments, ALK7:ActRIIB heteromultimers may be used to decrease levels (e.g., serum levels) of activin C in a patient in need thereof.

The disclosure further provides methods and ALK7:ActRIIB antagonists (e.g., ALK7:ActRIIB heteromultimers) for use in the treatment or prevention of various ALK7:ActRIIB-associated diseases and conditions associated with, for example, kidney, fat,

fibrosis, and other tissues. Such diseases or conditions include, for example, chronic kidney disease or failure, acute kidney disease or failure, patients that have stage 1 kidney disease, patients that have stage 2 kidney disease, patients that have stage 3 kidney disease, patients that have stage 4 kidney disease, patients that have stage 5 kidney disease, non-diabetic kidney diseases, glomerulonephritis, interstitial nephritis, diabetic kidney diseases, diabetic nephropathy, glomerulosclerosis, rapid progressive glomerulonephritis, renal fibrosis, Alport syndrome, IDDM nephritis, mesangial proliferative glomerulonephritis, membranoproliferative glomerulonephritis, crescentic glomerulonephritis, renal interstitial fibrosis, focal segmental glomerulosclerosis, membranous nephropathy, minimal change disease, pauci-immune rapid progressive glomerulonephritis, IgA nephropathy, polycystic kidney disease, Dent's disease, nephrocytinosis, Heymann nephritis, autosomal dominant (adult) polycystic kidney disease, autosomal recessive (childhood) polycystic kidney disease, acute kidney injury, nephrotic syndrome, renal ischemia, podocyte diseases or disorders, proteinuria, glomerular diseases, membranous glomerulonephritis, focal segmental glomerulonephritis, pre-eclampsia, eclampsia, kidney lesions, collagen vascular diseases, benign orthostatic (postural) proteinuria, IgM nephropathy, membranous nephropathy, sarcoidosis, diabetes mellitus, kidney damage due to drugs, Fabry's disease, aminoaciduria, Fanconi syndrome, hypertensive nephrosclerosis, interstitial nephritis, Sickle cell disease, hemoglobinuria, myoglobinuria, Wegener's Granulomatosis, Glycogen Storage Disease Type 1, chronic kidney disease, chronic renal failure, low Glomerular Filtration Rate (GFR), nephroangiosclerosis, lupus nephritis, ANCA-positive pauci-immune crescentic glomerulonephritis, chronic allograft nephropathy, nephrotoxicity, renal toxicity, kidney necrosis, kidney damage, glomerular and tubular injury, kidney dysfunction, nephritic syndrome, acute renal failure, chronic renal failure, proximal tubal dysfunction, acute kidney transplant rejection, chronic kidney transplant rejection, non-IgA mesangioproliferative glomerulonephritis, postinfectious glomerulonephritis, vasculitides with renal involvement of any kind, any hereditary renal disease, any interstitial nephritis, renal transplant failure, kidney cancer, kidney disease associated with other conditions (e.g., hypertension, diabetes, and autoimmune disease), Dent's disease, nephrocytinosis, Heymann nephritis, a primary kidney disease, a collapsing glomerulopathy, a dense deposit disease, a cryoglobulinemia-associated glomerulonephritis, an Henoch-Schonlein disease, a postinfectious glomerulonephritis, a bacterial endocarditis, a microscopic polyangitis, a Churg-Strauss syndrome, an anti-GBM-antibody mediated glomerulonephritis, amyloidosis, a monoclonal immunoglobulin deposition disease, a fibrillary glomerulonephritis, an immunotactoid

glomerulopathy, ischemic tubular injury, a medication-induced tubulo-interstitial nephritis, a toxic tubulo-interstitial nephritis, an infectious tubulo-interstitial nephritis, a bacterial pyelonephritis, a viral infectious tubulo-interstitial nephritis which results from a polyomavirus infection or an HIV infection, a metabolic-induced tubulo-interstitial disease, a mixed connective disease, a cast nephropathy, a crystal nephropathy which may results from urate or oxalate or drug-induced crystal deposition, an acute cellular tubulo-interstitial allograft rejection, a tumoral infiltrative disease which results from a lymphoma or a post-transplant lymphoproliferative disease, an obstructive disease of the kidney, vascular disease, a thrombotic microangiopathy, a nephroangiosclerosis, an atheroembolic disease, a mixed connective tissue disease, a polyarteritis nodosa, a calcineurin-inhibitor induced-vascular disease, an acute cellular vascular allograft rejection, an acute humoral allograft rejection, early renal function decline (ERFD), end stage renal disease (ESRD), renal vein thrombosis, acute tubular necrosis, renal occlusion, acute interstitial nephritis, established chronic kidney disease, renal artery stenosis, ischemic nephropathy, uremia, drug and toxin-induced chronic tubulointerstitial nephritis, reflux nephropathy, kidney stones, Goodpasture's syndrome, normocytic normochromic anemia, renal anemia, diabetic chronic kidney disease, IgG4-related disease, von Hippel-Lindau syndrome, tuberous sclerosis, nephronophthisis, medullary cystic kidney disease, renal cell carcinoma, adenocarcinoma, nephroblastoma, lymphoma, leukemia, hyposialylation disorder, chronic cyclosporine nephropathy, renal reperfusion injury, renal dysplasia, azotemia, bilateral arterial occlusion, acute uric acid nephropathy, hypovolemia, acute bilateral obstructive uropathy, hypercalcemic nephropathy, hemolytic uremic syndrome, acute urinary retention, malignant nephrosclerosis, postpartum glomerulosclerosis, scleroderma, non-Goodpasture's anti-GBM disease, microscopic polyarteritis nodosa, allergic granulomatosis, acute radiation nephritis, post-streptococcal glomerulonephritis, Waldenstrom's macroglobulinemia, analgesic nephropathy, arteriovenous fistula, arteriovenous graft, dialysis, ectopic kidney, medullary sponge kidney, renal osteodystrophy, solitary kidney, hydronephrosis, microalbuminuria, uremia, haematuria, hyperlipidemia, hypoalbuminaemia, lipiduria, acidosis, and hyperkalemia. In some embodiments, the disclosure further provides methods and ALK7:ActRIIB antagonists (e.g., ALK7:ActRIIB heteromultimers) for use in delaying or preventing progression from: stage 1 to stage 2 kidney disease, stage 2 to stage 3 kidney disease, stage 3 to stage 4 kidney disease, or stage 4 to stage 5 kidney disease. In some embodiments, the disclosure further provides methods and ALK7:ActRIIB antagonists (e.g., ALK7:ActRIIB heteromultimers) for use in preventing or reducing kidney inflammation. In some embodiments, the disclosure further

provides methods and ALK7:ActRIIB antagonists (e.g., ALK7:ActRIIB heteromultimers) for use in preventing or reducing kidney damage. In some embodiments, the disclosure further provides methods and ALK7:ActRIIB antagonists (e.g., ALK7:ActRIIB heteromultimers) for use in preventing or reducing kidney fibrosis.

In some embodiments, the disclosure further provides methods and ALK7:ActRIIB antagonists (e.g., ALK7:ActRIIB heteromultimers) for use in lipolysis activity in a subject in need thereof. In some embodiments, the disclosure further provides methods and ALK7:ActRIIB antagonists (e.g., ALK7:ActRIIB heteromultimers) for use in decreasing body fat content or reducing the rate of increase in body fat content in a subject in need thereof. In some embodiments, the disclosure further provides methods and ALK7:ActRIIB antagonists (e.g., ALK7:ActRIIB heteromultimers) for use in treating a disorder or condition associated with undesirable body weight gain in a subject. Such disorders include, for example, obesity (*e.g.,* abdominal obesity); overweight; insulin resistance; metabolic syndrome and other metabolic diseases or conditions; a lipid disorder such as, low HDL levels, high LDL levels, hyperlipidemia, hypertriglyceridemia or dyslipidemia; lipoprotein aberrations; decreased triglycerides; fatty liver disease; non-alcoholic fatty liver disease; hyperglycemia; impaired glucose tolerance (IGT); hyperinsulinemia; high cholesterol (*e.g.,* high LDL levels and/or hypercholesterolemia); cardiovascular disease such as, heart disease including coronary heart disease, congestive heart failure, atherosclerosis; arteriosclerosis, and/or hypertension; Syndrome X; vascular restenosis; neuropathy; and/or other disorders/conditions associated with one or more of the above diseases or conditions, and/or with overweight (*e.g.*, BMI of $\geq$25 kg/m^2), or with too much body fat. In some embodiments, the disclosure provides methods for decreasing body fat content or reducing the rate of increase in body fat content in a subject, comprising administering to a subject in need thereof an effective amount of an ALK7:ActRIIB antagonist, or combination of ALK7:ActRIIB antagonists. In some embodiments, the disclosure provides methods for treating a disorder associated with undesirable body weight gain in a subject, comprising administering to a subject in need thereof an effective amount of an ALK7:ActRIIB antagonist, or combination of ALK7:ActRIIB antagonists. In some embodiments, the disorder associated with undesirable body weight gain in a subject is selected from the group consisting of: obesity, non-insulin dependent diabetes mellitus (NIDDM), cardiovascular disease, hypertension, osteoarthritis, stroke, respiratory problems, and gall bladder disease. In some embodiments, the disclosure provides methods for reducing cholesterol and/or

triglycerides in a patient, comprising administering to a subject patient in need thereof an effective amount of an ALK7:ActRIIB antagonist, or combination of ALK7:ActRIIB antagonists.

In some embodiments, the disclosure further provides methods and ALK7:ActRIIB antagonists (e.g., ALK7:ActRIIB heteromultimers) for use in treating and/or ameliorating cancer or a condition associated with cancer. In some embodiments, the subject has a cancer selected from the group consisting of melanoma, breast, colon, and endometrial, pancreatic, gastric, and uterine cancer. In some embodiments, the subject has myeloma (e.g., multiple myeloma, plasmacytoma, localized myeloma, and extramedullary myeloma). In some embodiments, the ALK7:ActRIIB antagonist is administered to treat or prevent lymphatic metastasis, bloodstream metastasis, tumor growth, or tumor invasion.

In some embodiments, the disclosure further provides methods and ALK7:ActRIIB antagonists (e.g., ALK7:ActRIIB heteromultimers) for use in treating or preventing fibrosis or a disorder or condition associated with fibrosis in a patient in need thereof. Such disorders or conditions include, for example, pulmonary fibrosis, hypersensitivity pneumonitis, idiopathic fibrosis, tuberculosis, pneumonia, cystic fibrosis, asthma, chronic obstructive pulmonary disease (COPD), emphysema, renal (kidney) fibrosis, renal (kidney) failure, chronic renal (kidney) disease, bone fibrosis, myelofibrosis, rheumatoid arthritis, systemic lupus erythematosus, scleroderma, sarcoidosis, granulomatosis with polyangiitis, Peyronie's disease, liver fibrosis, Wilson's disease, glycogen storage diseases (particularly types III, IV, IX, and X), iron-overload, Gaucher disease, Zellweger syndrome, nonalcoholic and alcoholic steatohepatitis, biliary cirrhosis, sclerosing cholangitis, Budd-Chiari syndrome, surgery-associated fibrosis, Crohn's disease, Duputren's contracture, mediastinal fibrosis, nephrogeneic fibrosis, retroperitoneal fibrosis, atrial fibrosis, endomyocardial fibrosis, pancreatic fibrosis.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1A and 1B show two schematic examples of heteromeric protein complexes comprising type I receptor and type II receptor polypeptides. Figure 1A depicts a heterodimeric protein complex comprising one type I receptor fusion polypeptide and one type II receptor fusion polypeptide, which can be assembled covalently or noncovalently via a multimerization domain contained within each polypeptide chain. Two assembled

multimerization domains constitute an interaction pair, which can be either guided or unguided. Figure 1B depicts a heterotetrameric protein complex comprising two heterodimeric complexes as depicted in Figure 1A. Complexes of higher order can be envisioned.

Figure 2 show a schematic example of a heteromeric protein complex comprising a type I receptor polypeptide (indicated as "I") (e.g. a polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 97%, 98%, 99% or 100% identical to an extracellular domain of an ALK7 protein from humans or other species such as those described herein, e.g., SEQ ID Nos: 9, 10, 19, 20, 38, 39, 42, 43, 46, 74, 76, 79, and 80) and a type II receptor polypeptide (indicated as "II") (e.g. a polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 97%, 98%, 99% or 100% identical to an extracellular domain of an ActRIIB protein from humans or other species as such as those described herein, e.g., SEQ ID Nos: 1, 2, 3, 4, 5, 6, 71, 73, 77, and 78). In the illustrated embodiments, the type I receptor polypeptide is part of a fusion polypeptide that comprises a first member of an interaction pair ("C_1"), and the type II receptor polypeptide is part of a fusion polypeptide that comprises a second member of an interaction pair ("C_2"). In each fusion polypeptide, a linker may be positioned between the type I or type II receptor polypeptide and the corresponding member of the interaction pair. The first and second members of the interaction pair may be a guided (asymmetric) pair, meaning that the members of the pair associate preferentially with each other rather than self-associate, or the interaction pair may be unguided, meaning that the members of the pair may associate with each other or self-associate without substantial preference and may have the same or different amino acid sequences. Traditional Fc fusion proteins and antibodies are examples of unguided interaction pairs, whereas a variety of engineered Fc domains have been designed as guided (asymmetric) interaction pairs [e.g., Spiess et al (2015) Molecular Immunology 67(2A): 95-106].

Figure 3 shows an alignment of extracellular domains of human ActRIIA (SEQ ID NO: 49) and human ActRIIB (SEQ ID NO: 2) with the residues that are deduced herein, based on composite analysis of multiple ActRIIB and ActRIIA crystal structures, to directly contact ligand indicated with boxes.

Figure 4 shows a multiple sequence alignment of various vertebrate ActRIIB precursor proteins without their intracellular domains (SEQ ID NOs: 50-55) human ActRIIA

precursor protein without its intracellular domain (SEQ ID NO: 56), and a consensus ActRII precursor protein (SEQ ID NO: 57).

Figure 5 shows multiple sequence alignment of Fc domains from human IgG isotypes using Clustal 2.1. Hinge regions are indicated by dotted underline. Double underline 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.

Figure 6 shows comparative ligand binding data for an ALK7-Fc:ActRIIB-Fc heterodimeric protein complex compared to ActRIIB-Fc homodimer and ALK7-Fc homodimer. For each protein complex, ligands are ranked by k_{off}, a kinetic constant that correlates well with ligand signaling inhibition, and listed in descending order of binding affinity (ligands bound most tightly are listed at the top). At left, yellow, red, green, and blue lines indicate magnitude of the off-rate constant. Solid black lines indicate ligands whose binding to heterodimer is enhanced or unchanged compared with homodimer, whereas dashed red lines indicate substantially reduced binding compared with homodimer. As shown, four of the five ligands with strong binding to ActRIIB-Fc homodimer (activin A, BMP10, GDF8, and GDF11) exhibit reduced binding to the ActRIIB-Fc:ALK7-Fc heterodimer, the exception being activin B which retains tight binding to the heterodimer. Similarly, three of four ligands with intermediate binding to ActRIIB-Fc homodimer (GDF3, BMP6, and particularly BMP9) exhibit reduced binding to the ActRIIB-Fc:ALK7-Fc heterodimer, whereas binding to activin AC is increased to become the second strongest ligand interaction with the heterodimer overall. Finally, activin C and BMP5 unexpectedly bind the ActRIIB-Fc:ALK7 heterodimer with intermediate strength despite no binding (activin C) or weak binding (BMP5) to ActRIIB-Fc homodimer. No ligands tested bind to ALK7-Fc homodimer.

Figure 7 shows a multiple sequence alignment of ALK7 extracellular domains derived from various vertebrate species (SEQ ID NOs: 59-64).

Figures 8A-8D show schematic examples of heteromeric protein complexes comprising an ALK7 polypeptide (e.g. a polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 97%, 98%, 99% or 100% identical to an extracellular domain of an ALK7 protein from humans or other species such as those described herein, e.g., SEQ ID Nos: 9, 10, 19, 20, 38, 39, 42, 43, 46, 74, 76, 79, and 80) and an ActRIIB polypeptide (e.g. a polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%,

94%, 95%, 97%, 98%, 99% or 100% identical to an extracellular domain of an ActRIIB protein from humans or other species such as those described herein, e.g., SEQ ID Nos: 1, 2, 3, 4, 5, 6, 71, 73, 77, and 78). In the illustrated embodiments, the ALK7 polypeptide is part of a fusion polypeptide that comprises a first member of an interaction pair ("C_1"), and the ActRIIB polypeptide is part of a fusion polypeptide that comprises a second member of an interaction pair ("C_2"). Suitable interaction pairs included, for example, heavy chain and/or light chain immunoglobulin interaction pairs, truncations, and variants thereof such as those described herein [e.g., Spiess et al (2015) Molecular Immunology 67(2A): 95-106]. In each fusion polypeptide, a linker may be positioned between the ALK7 or ActRIIB polypeptide and the corresponding member of the interaction pair. The first and second members of the interaction pair may be unguided, meaning that the members of the pair may associate with each other or self-associate without substantial preference, and they may have the same or different amino acid sequences. See Figure 8A. Alternatively, the interaction pair may be a guided (asymmetric) pair, meaning that the members of the pair associate preferentially with each other rather than self-associate. See Figure 8B. Complexes of higher order can be envisioned. See Figure 8C and 8D.

Figures 9A-9G show schematic examples of heteromeric protein complexes comprising two ALK7 polypeptides (e.g. polypeptide that are independently at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 97%, 98%, 99% or 100% identical to an extracellular domain of an ALK7 protein from humans or other species such as those described herein, e.g., SEQ ID Nos: 9, 10, 19, 20, 38, 39, 42, 43, 46, 74, 76, 79, and 80) and two ActRIIB polypeptides (e.g. two polypeptides that are independently at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 97%, 98%, 99% or 100% identical to an extracellular domain of an ActRIIB protein from humans or other species such as those described herein, e.g., SEQ ID Nos: 1, 2, 3, 4, 5, 6, 71, 73, 77, and 78).

In the illustrated embodiment 9A, the first ALK7 polypeptide (from left to right) is part of a fusion polypeptide that comprises a first member of an interaction pair ("C_1") and further comprises an additional first member of an interaction pair ("A_1"); and the second ALK7 polypeptide is part of a fusion polypeptide that comprises a second member of an interaction pair ("C_2") and further comprises a first member of an interaction pair ("A_2"). The first ActRIIB polypeptide (from left to right) is part of a fusion polypeptide that comprises a second member of an interaction pair ("B_1"); and the second ActRIIB polypeptide is part of a fusion polypeptide that comprises a second member of an interaction

pair ("B_2"). A_1 and A_2 may be the same or different; B_1 and B_2 may be the same or different, and C_1 and C_2 may be the same or different. In each fusion polypeptide, a linker may be positioned between the ALK7 or ActRIIB polypeptide and the corresponding member of the interaction pair as well as between interaction pairs. Figure 9A is an example of an association of unguided interaction pairs, meaning that the members of the pair may associate with each other or self-associate without substantial preference and may have the same or different amino acid sequences.

In the illustrated embodiment 9B, the first ActRIIB polypeptide (from left to right) is part of a fusion polypeptide that comprises a first member of an interaction pair ("C_1") and further comprises an additional first member of an interaction pair ("A_1"); and the second ActRIIB polypeptide is part of a fusion polypeptide that comprises a second member of an interaction pair ("B_2"). The first ALK7 polypeptide (from left to right) is part of a fusion polypeptide that comprises a second member of an interaction pair ("B_1"); and the second ALK7 polypeptide is part of a fusion polypeptide that comprises a second member of an interaction pair ("C_2") and further comprises a first member of an interaction pair ("A_2"). In each fusion polypeptide, a linker may be positioned between the ALK7 or ActRIIB polypeptide and the corresponding member of the interaction pair as well as between interaction pairs. Figure 9B is an example of an association of guided (asymmetric) interaction pairs, meaning that the members of the pair associate preferentially with each other rather than self-associate.

Suitable interaction pairs included, for example, heavy chain and/or light chain immunoglobulin interaction pairs, truncations, and variants thereof as described herein [e.g., Spiess et al (2015) Molecular Immunology 67(2A): 95-106]. Complexes of higher order can be envisioned. See Figure 9C-9F. Using similar methods, particularly those that employ light and/or heavy chain immunoglobulins, truncations, or variants thereof, interaction pairs may be used to produce ALK7:ActRIIB heterodimers that resemble antibody Fab and $F(ab')_2$ complexes [e.g., Spiess et al (2015) Molecular Immunology 67(2A): 95-106]. See Figure 9G.

Figures 10A and 10B show schematic examples of a heteromeric protein complex comprising an ALK7 polypeptide (e.g. a polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 97%, 98%, 99% or 100% identical to an extracellular domain of an ALK7 protein from humans or other species as described herein, e.g., SEQ ID Nos: 9, 10, 19, 20, 38, 39, 42, 43, 46, 74, 76, 79, and 80), an ActRIIB polypeptide (e.g. a polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 97%,

98%, 99% or 100% identical to an extracellular domain of an ActRIIB protein from humans or other species as such as those described herein, e.g., SEQ ID Nos: 1, 2, 3, 4, 5, 6, 71, 73, 77, and 78), and a ligand-binding domain of an antibody (e.g., a ligand binding domain derived from an antibody that binds to one or more ALK7:ActRIIB-binding ligands). In the illustrated embodiments, the ALK7 polypeptide is part of a fusion polypeptide that comprises a first member of an interaction pair ("C_1"), and further comprises an additional first member of an interaction pair ("A_1"). The ActRIIB polypeptide is part of a fusion polypeptide that comprises a second member of an interaction pair ("B_1"). The variable heavy chain (V_H) polypeptide is part of a fusion polypeptide that comprises a second member of an interaction pair ("C_2"), and further comprises a first member of an interaction pair ("A_2"). The variable heavy chain (V_L) polypeptide is part of a fusion polypeptide that comprises a second member of an interaction pair ("B_2"). In each fusion polypeptide, a linker may be positioned between the ALK7 or ActRIIB polypeptide and the corresponding member of the interaction pair, between interaction pairs, and between the V_H and V_L polypeptides and a member of the interaction pair. A_1 and A_2 may be the same or different; B_1 and B_2 may be the same or different, and C_1 and C_2 may be the same or different. Suitable interaction pairs included, for example, constant heavy chain and/or light chain immunoglobulin interaction pairs, truncations, and variants thereof as described herein [e.g., Spiess et al (2015) Molecular Immunology 67(2A): 95-106]. Figure 10A is an example of an association of guided (asymmetric) interaction pairs, meaning that the members of the pair associate preferentially with each other rather than self-associate. Figure 10B is an example of an association of unguided interaction pairs, meaning that the members of the pair may associate with each other or self-associate without substantial preference and may have the same or different amino acid sequences.

Such antibody-ALK7:ActRIIB complexes may be useful in situations where it is desirable to further bind/antagonize an agent that is not an ALK7:ActRIIB ligand. Alternatively, such antibody-ALK7:ActRIIB complexes may be useful in situations where it is desirable to further enhance ALK7:ActRIIB ligand binding/antagonism. For example, as demonstrated by the examples herein, an ALK7:ActRIIB heterodimer has high affinity for several ligands including, e.g., activin B and activin AC. In addition, ALK7:ActRIIB heterodimers bind to GDF3 but with weaker affinity. In certain situations where it is desirable to antagonize GDF3 activity in addition to one or more of the high affinity-binding ligands (e.g., activin B and activin AC), GDF3 may be outcompeted for binding to an

ALK7:ActRIIB heterodimer. In these situations, addition of binding domain of an anti-GDF3 antibody to the ALK7:ActRIIB heteromultimer complex would improve the capacity of the protein complex to antagonize GDF3 in addition to one or more of the higher affinity ALK7:ActRIIB binding-ligands (e.g., activin B, and activin AC).

Figure 11 shows schematic examples of ALK7:ActRIIB single-trap polypeptides. ALK7:ActRIIB single-trap polypeptides may contain multiple ALK7 domains (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more domains), having the same or different sequences, and multiple ActRIIB domains (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more domains), having the same or different sequences. These ALK7 and ActRIIB domains may be arranged in any order and may comprise one or more linker domains positions between one or more of the ALK7 and ActRIIB domains. Such ligand traps may be useful as therapeutic agents to treat or prevent diseases or disorders described herein.

Figure 12A-12D show schematic examples of multimeric protein complexes comprising at least one ALK7:ActRIIB single-chain trap polypeptide. In the illustrated embodiments 12A and 12B, a first ALK7:ActRIIB single-chain trap polypeptide (from left to right) is part of a fusion polypeptide that comprises a first member of an interaction pair ("C_1"); and a second ALK7:ActRIIB single-chain trap polypeptide is part of a fusion polypeptide that comprises a second member of an interaction pair ("C_2"). C_1 and C_2 may be the same or different. The first and second ALK7:ActRIIB single-chain trap polypeptides may be the same or different. In each fusion polypeptide, a linker may be positioned between the ALK7:ActRIIB single-chain trap polypeptide and the corresponding member of the interaction pair. Suitable interaction pairs included, for example, heavy chain and/or light chain immunoglobulin interaction pairs, truncations, and variants thereof as described herein [e.g., Spiess et al (2015) Molecular Immunology 67(2A): 95-106]. Figure 12A is an example of an association of unguided interaction pairs, meaning that the members of the pair may associate with each other or self-associate without substantial preference and may have the same or different amino acid sequences. Figure 12B is an example of an association of guided (asymmetric) interaction pairs, meaning that the members of the pair associate preferentially with each other rather than self-associate. Complexes of higher order can be envisioned. In addition, such ALK7:ActRIIB single-chain trap polypeptides may be similarly be associated, covalently or non-covalently, with one or more ALK7 polypeptides and/or one or more ActRIIB polypeptides. See Figure 12C. Also, such ALK7:ActRIIB single-chain trap polypeptides may be similarly be associated, covalently or non-covalently, with one or more

ligand-binding domain of an antibody (e.g., a ligand binding domain of an antibody that binds to one or more ALK7:ActRIIB binding-ligands). See Figure 12D.

Figures 13A-13C shows gene expression profiles of fibrotic genes (Col1a1, Col3a1, Fibronectin, PAI-1, CTGF, and a-SMA), inflammatory genes (Tnfa, and MCP1), cytokine genes (Tgfb1, Tgfb2, Tgfb3, and activin A), kidney injury gene (NGAL), Hypoxia-inducible factor 1-alpha (HIF1a), and activin A receptor (Acvr2A) from mouse kidneys subjected to unilateral ureteral obstruction (UUO). Samples from the contralateral, non-surgery kidney were used as a control (Ctrl). Gene expression profiles were obtained at 3 days and 17 days post-surgery. Mice were administered either PBS or an ALK7-Fc:ActRIIB-Fc homodimer at days 3, 7, 10, and 14 post-surgery. Statistics was performed using a one-way ANOVA followed by Tukey analysis. (*) denotes a statistical difference between i) control samples compared to UUO kidneys at 3 days or ii) control samples compared to UUO kidneys at 17 days in mice administered the ALK7-Fc:ActRIIB-Fc homodimer. ($) denotes a statistical difference between UUO kidneys at 17 days in mice administered only PBS compared with UUO kidneys at 17 days in mice administered the ALK7-Fc:ActRIIB-Fc homodimer. (@) denotes that no transcript was detected.

DETAIL DESCRIPTION OF THE INVENTION

1. Overview

In part, the present disclosure relates to heteromultimers comprising a TGFβ superfamily type I receptor polypeptide and a TGFβ superfamily type II receptor polypeptide, uses thereof, and methods of making such heteromultimers. See, e.g., Figures 1 and 2. In certain preferred embodiments, heteromultimers comprise an extracellular domain of a TGFβ superfamily type I receptor polypeptide and an extracellular domain of a TGFβ superfamily type II receptor polypeptide. In particular, the disclosure provides heteromultimers comprising an ALK7 polypeptide and an ActRIIB polypeptide. Preferably ALK7 polypeptides comprise a ligand-binding domain of an ALK7 receptor, and ActRIIB polypeptides comprise a ligand-binding domain of an ActRIIB receptor. In certain preferred embodiments, ALK7:ActRIIB heteromultimers have an altered TGFβ superfamily ligand binding profile/specificity compared to a corresponding sample of a homomultimer (*e.g.*, an ALK7:ActRIIB heterodimer compared to an ActRIIB:ActRIIB homodimer or an ALK7:ALK7 homodimer).

The TGF-β superfamily is comprised of over 30 secreted factors including TGF-betas, activins, nodals, bone morphogenetic proteins (BMPs), growth and differentiation factors (GDFs), and anti-Mullerian hormone (AMH) [Weiss *et al.* (2013) Developmental Biology, 2(1): 47-63]. Members of the superfamily, which are found in both vertebrates and invertebrates, are ubiquitously expressed in diverse tissues and function during the earliest stages of development throughout the lifetime of an animal. Indeed, TGF-β superfamily proteins are key mediators of stem cell self-renewal, gastrulation, differentiation, organ morphogenesis, and adult tissue homeostasis. Consistent with this ubiquitous activity, aberrant TGF-beta superfamily signaling is associated with a wide range of human pathologies including, for example, autoimmune disease, cardiovascular disease, fibrotic disease, and cancer.

Ligands of the TGF-beta superfamily share the same dimeric structure in which the central 3-1/2 turn helix of one monomer packs against the concave surface formed by the beta-strands of the other monomer. The majority of TGF-beta family members are further stabilized by an intermolecular disulfide bond. This disulfide bonds traverses through a ring formed by two other disulfide bonds generating what has been termed a 'cysteine knot' motif [Lin et al. (2006) Reproduction 132: 179-190; and Hinck et al. (2012) FEBS Letters 586: 1860-1870].

TGF-beta superfamily signaling is mediated by heteromeric complexes of type I and type II serine/threonine kinase receptors, which phosphorylate and activate downstream SMAD proteins (*e.g.*, SMAD proteins 1, 2, 3, 5, and 8) upon ligand stimulation [Massagué (2000) Nat. Rev. Mol. Cell Biol. 1:169-178]. These type I and type II receptors are transmembrane proteins, composed of a ligand-binding extracellular domain with cysteine-rich region, a transmembrane domain, and a cytoplasmic domain with predicted serine/threonine kinase specificity. In general, type I receptors mediate intracellular signaling while the type II receptors are required for binding TGF-beta superfamily ligands. Type I and II receptors form a stable complex after ligand binding, resulting in phosphorylation of type I receptors by type II receptors.

The TGF-beta family can be divided into two phylogenetic branches based on the type I receptors they bind and the Smad proteins they activate. One is the more recently evolved branch, which includes, *e.g.*, the TGF-betas, activins, GDF8, GDF9, GDF11, BMP3 and nodal, which signal through type I receptors that activate Smads 2 and 3 [Hinck (2012) FEBS Letters 586:1860-1870]. The other branch comprises the more distantly related

proteins of the superfamily and includes, *e.g.*, BMP2, BMP4, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF1, GDF5, GDF6, and GDF7, which signal through Smads 1, 5, and 8.

TGF-beta isoforms are the founding members of the TGF-beta superfamily, of which there are 3 known isoforms in mammals designated as TGF-beta1, TGF-beta2 and TGF-beta3. Mature bioactive TGF-beta ligands function as homodimers and predominantly signal through the type I receptor ALK5, but have also been found to additionally signal through ALK1 in endothelial cells [Goumans *et al.* (2003) Mol Cell 12(4): 817-828]. TGF-beta1 is the most abundant and ubiquitously expressed isoform. TGF-beta1 is known to have an important role in wound healing, and mice expressing a constitutively active TGF-beta1 transgene develop fibrosis [Clouthier *et al.* (1997) J Clin. Invest. 100(11): 2697-2713]. TGF-beta1 is also involved in T cell activation and maintenance of T regulatory cells [Li *et al.* (2006) Immunity 25(3): 455-471]. TGF-beta2 expression was first described in human glioblastoma cells, and is occurs in neurons and astroglial cells of the embryonic nervous system. TGF-beta2 is known to suppress interleukin-2-dependent growth of T lymphocytes. TGF-beta3 was initially isolated from a human rhabdomyosarcoma cell line and since has been found in lung adenocarcinoma and kidney carcinoma cell lines. TGF-beta3 is known to be important for palate and lung morphogenesis [Kubiczkova et al. (2012) Journal of Translational Medicine 10:183].

Activins are members of the TGF-beta superfamily and were initially discovered as regulators of secretion of follicle-stimulating hormone, but subsequently various reproductive and non-reproductive roles have been characterized. There are three principal activin forms (A, B, and AB) that are homo/heterodimers of two closely related β subunits ($\beta_A\beta_A$, $\beta_B\beta_B$, and $\beta_A\beta_B$, respectively). The human genome also encodes an activin C and an activin E, which are primarily expressed in the liver, and heterodimeric forms containing β_C or β_E are also known. In the TGF-beta superfamily, activins are unique and multifunctional factors that can stimulate hormone production in ovarian and placental cells, support neuronal cell survival, influence cell-cycle progress positively or negatively depending on cell type, and induce mesodermal differentiation at least in amphibian embryos [DePaolo *et al.* (1991) Proc Soc Ep Biol Med. 198:500-512; Dyson *et al.* (1997) Curr Biol. 7:81-84; and Woodruff (1998) Biochem Pharmacol. 55:953-963]. In several tissues, activin signaling is antagonized by its related heterodimer, inhibin. For example, in the regulation of follicle-stimulating hormone (FSH) secretion from the pituitary, activin promotes FSH synthesis and secretion, while

inhibin reduces FSH synthesis and secretion. Other proteins that may regulate activin bioactivity and/or bind to activin include follistatin (FS), follistatin-related protein (FSRP, also known as FLRG or FSTL3), and α_2-macroglobulin.

As described herein, agents that bind to "activin A" are agents that specifically bind to the β_A subunit, whether in the context of an isolated β_A subunit or as a dimeric complex (*e.g.*, a $\beta_A\beta_A$ homodimer or a $\beta_A\beta_B$ heterodimer). In the case of a heterodimer complex (*e.g.*, a $\beta_A\beta_B$ heterodimer), agents that bind to "activin A" are specific for epitopes present within the β_A subunit, but do not bind to epitopes present within the non-β_A subunit of the complex (*e.g.*, the β_B subunit of the complex). Similarly, agents disclosed herein that antagonize (inhibit) "activin A" are agents that inhibit one or more activities as mediated by a β_A subunit, whether in the context of an isolated β_A subunit or as a dimeric complex (*e.g.*, a $\beta_A\beta_A$ homodimer or a $\beta_A\beta_B$ heterodimer). In the case of $\beta_A\beta_B$ heterodimers, agents that inhibit "activin A" are agents that specifically inhibit one or more activities of the β_A subunit, but do not inhibit the activity of the non-β_A subunit of the complex (*e.g.*, the β_B subunit of the complex). This principle applies also to agents that bind to and/or inhibit "activin B", "activin C", and "activin E". Agents disclosed herein that antagonize "activin AB" are agents that inhibit one or more activities as mediated by the β_A subunit and one or more activities as mediated by the β_B subunit. The same principle also applies to agents that bind to and/or inhibit "activin AC", "activin AE", "activin BC" or "activin BE".

Nodal proteins have functions in mesoderm and endoderm induction and formation, as well as subsequent organization of axial structures such as heart and stomach in early embryogenesis. It has been demonstrated that dorsal tissue in a developing vertebrate embryo contributes predominantly to the axial structures of the notochord and pre-chordal plate while it recruits surrounding cells to form non-axial embryonic structures. Nodal appears to signal through both type I and type II receptors and intracellular effectors known as SMAD proteins. Studies support the idea that ActRIIA and ActRIIB serve as type II receptors for nodal [Sakuma *et al.* (2002) Genes Cells. 2002, 7:401-12]. It is suggested that Nodal ligands interact with their co-factors (*e.g.*, Crypto or Cryptic) to activate activin type I and type II receptors, which phosphorylate SMAD2. Nodal proteins are implicated in many events critical to the early vertebrate embryo, including mesoderm formation, anterior patterning, and left-right axis specification. Experimental evidence has demonstrated that nodal signaling activates pAR3-Lux, a luciferase reporter previously shown to respond specifically to activin and TGF-beta. However, nodal is unable to induce pTlx2-Lux, a

reporter specifically responsive to bone morphogenetic proteins. Recent results provide direct biochemical evidence that nodal signaling is mediated by SMAD2 and SMAD3, which also mediate signaling by TGF-betas and activins. Further evidence has shown that the extracellular protein Cripto or Cryptic is required for nodal signaling, making it distinct from activin or TGF-beta signaling.

The BMPs and GDFs together form a family of cysteine-knot cytokines sharing the characteristic fold of the TGF-beta superfamily [Rider *et al.* (2010) Biochem J., 429(1):1-12]. This family includes, for example, BMP2, BMP4, BMP6, BMP7, BMP2a, BMP3, BMP3b (also known as GDF10), BMP4, BMP5, BMP6, BMP7, BMP8, BMP8a, BMP8b, BMP9 (also known as GDF2), BMP10, BMP11 (also known as GDF11), BMP12 (also known as GDF7), BMP13 (also known as GDF6), BMP14 (also known as GDF5), BMP15, GDF1, GDF3 (also known as VGR2), GDF8 (also known as myostatin), GDF9, GDF15, and decapentaplegic. Besides the ability to induce bone formation, which gave the BMPs their name, the BMP/GDFs display morphogenetic activities in the development of a wide range of tissues. BMP/GDF homo- and hetero-dimers interact with combinations of type I and type II receptor dimers to produce multiple possible signaling complexes, leading to the activation of one of two competing sets of SMAD transcription factors. BMP/GDFs have highly specific and localized functions. These are regulated in a number of ways, including the developmental restriction of BMP/GDF expression and through the secretion of several specific BMP antagonist proteins that bind with high affinity to the cytokines. Curiously, a number of these antagonists resemble TGF-beta superfamily ligands.

Growth and differentiation factor-8 (GDF8) is also known as myostatin. GDF8 is a negative regulator of skeletal muscle mass and is highly expressed in developing and adult skeletal muscle. The GDF8 null mutation in transgenic mice is characterized by a marked hypertrophy and hyperplasia of skeletal muscle [McPherron *et al.* Nature (1997) 387:83-90]. Similar increases in skeletal muscle mass are evident in naturally occurring mutations of GDF8 in cattle and, strikingly, in humans [Ashmore *et al.* (1974) Growth, 38:501-507; Swatland and Kieffer, J. Anim. Sci. (1994) 38:752-757; McPherron and Lee, Proc. Natl. Acad. Sci. USA (1997) 94:12457-12461; Kambadur *et al.* Genome Res. (1997) 7:910-915; and Schuelke *et al.* (2004) N Engl J Med, 350:2682-8]. Studies have also shown that muscle wasting associated with HIV-infection in humans is accompanied by increases in GDF8 protein expression [Gonzalez-Cadavid *et al.*, PNAS (1998) 95:14938-43]. In addition, GDF8 can modulate the production of muscle-specific enzymes (*e.g.*, creatine kinase) and modulate

myoblast cell proliferation [International Patent Application Publication No. WO 00/43781]. The GDF8 propeptide can noncovalently bind to the mature GDF8 domain dimer, inactivating its biological activity [Miyazono *et al.* (1988) J. Biol. Chem., 263: 6407-6415; Wakefield *et al.* (1988) J. Biol. Chem., 263; 7646-7654; and Brown *et al.* (1990) Growth Factors, 3: 35-43]. Other proteins which bind to GDF8 or structurally related proteins and inhibit their biological activity include follistatin, and potentially, follistatin-related proteins [Gamer *et al.* (1999) Dev. Biol., 208: 222-232].

GDF11, also known as BMP11, is a secreted protein that is expressed in the tail bud, limb bud, maxillary and mandibular arches, and dorsal root ganglia during mouse development [McPherron *et al.* (1999) Nat. Genet., 22: 260-264; and Nakashima *et al.* (1999) Mech. Dev., 80: 185-189]. GDF11 plays a unique role in patterning both mesodermal and neural tissues [Gamer *et al.* (1999) Dev Biol., 208:222-32]. GDF11 was shown to be a negative regulator of chondrogenesis and myogenesis in developing chick limb [Gamer *et al.* (2001) Dev Biol., 229:407-20]. The expression of GDF11 in muscle also suggests its role in regulating muscle growth in a similar way to GDF8. In addition, the expression of GDF11 in brain suggests that GDF11 may also possess activities that relate to the function of the nervous system. Interestingly, GDF11 was found to inhibit neurogenesis in the olfactory epithelium [Wu *et al.* (2003) Neuron., 37:197-207]. Hence, inhibitors of GDF11 may have in vitro and in vivo applications in the treatment of diseases such as muscle diseases and neurodegenerative diseases (*e.g.*, amyotrophic lateral sclerosis).

BMP7, also called osteogenic protein-1 (OP-1), is well known to induce cartilage and bone formation. In addition, BMP7 regulates a wide array of physiological processes. For example, BMP7 may be the osteoinductive factor responsible for the phenomenon of epithelial osteogenesis. It is also found that BMP7 plays a role in calcium regulation and bone homeostasis. Like activin, BMP7 binds to type II receptors, ActRIIA and ActRIIB. However, BMP7 and activin recruit distinct type I receptors into heteromeric receptor complexes. The major BMP7 type I receptor observed was ALK2, while activin bound exclusively to ALK4 (ActRIIB). BMP7 and activin elicited distinct biological responses and activated different SMAD pathways [Macias-Silva *et al.* (1998) J Biol Chem. 273:25628-36].

As described herein, comparative binding data demonstrated that an ALK7:ActRIIB heterodimer has an altered binding profile (ligand selectivity) compared to either corresponding ActRIIB or ALK7 homodimers. In particular, the ALK7:ActRIIB heterodimer

displays enhanced binding to activin C, activin AC, and BMP5 compared to either homodimer, and retains strong binding to activin B as observed with the ActRIIB homodimer. However, the ALK7:ActRIIB heterodimer exhibits reduced binding to GDF11, GDF8, activin A, BMP10, BMP6, GDF3, and BMP9 compared to the ActRIIB homodimer. In particular, BMP9 displays low or no observable affinity for the ALK7:ActRIIB heterodimer, whereas this ligand binds tightly to ActRIIB homodimer.

These results therefore demonstrate that ALK7:ActRIIB heterodimers are more selective antagonists of activin B, activin C, activin AC, and BMP5 compared to ActRIIB homodimers. Additionally, it is expected that such heterodimers will bind tightly to Activin BC, given the binding to Activins B and C. Additionally, it is expected that such heterodimers will bind to activin E, activin AE and activin BE given the structural similarity between activin C and E. Accordingly, an ALK7:ActRIIB heterodimer may be more useful than an ActRIIB homodimer in certain applications where such selective antagonism is advantageous. Examples include therapeutic applications where it is desirable to retain antagonism of one or more of activin B, activin C, activin AC, activin BC, activin E, activin AE, activin BE and BMP5 but minimize antagonism of one or more of activin A, BMP9, BMP10, GDF3, and BMP6.

Moreover, ALK7:ActRIIB heterodimers, as described herein, exert beneficial catabolic effects on adipose tissue. Additionally, the ALK7:ActRIIB heterodimers described herein are shown to have potent protective effects in a model of chronic kidney disease, with effects including the inhibition of fibrosis and inflammation. However, unlike ActRIIB homodimer, an ActRIIB:ALK7 heterodimer exhibits only low-affinity or transient binding to BMP9 and so will have little to no concurrent inhibition on processes mediated by BMP9, such as angiogenesis. This novel selectivity will be useful, for example, in treating patients in need of inhibitory effects on fat, or protective effects on the kidney, but not in need of altered angiogenesis.

The terms used in this specification generally have their ordinary meanings in the art, within the context of this disclosure and in the specific context where each term is used. Certain terms are discussed below or elsewhere in the specification to provide additional guidance to the practitioner in describing the compositions and methods of the disclosure 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 it is used.

The terms "heteromer" or "heteromultimer" is a complex comprising at least a first polypeptide chain and a second polypeptide chain, wherein the second polypeptide chain differs in amino acid sequence from the first polypeptide chain by at least one amino acid residue. The heteromer can comprise a "heterodimer" formed by the first and second polypeptide chains or can form higher order structures where one or more polypeptide chains in addition to the first and second polypeptide chains are present. Exemplary structures for the heteromultimer include heterodimers, heterotrimers, heterotetramers and further oligomeric structures. Heterodimers are designated herein as X:Y or equivalently as X-Y, where X represents a first polypeptide chain and Y represents a second polypeptide chain. Higher-order heteromers and oligomeric structures are designated herein in a corresponding manner. In certain embodiments a heteromultimer is recombinant (e.g., one or more polypeptide components may be a recombinant protein), isolated and/or purified.

"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. 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.

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.

"Percent (%) sequence identity" 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.

As used herein, unless otherwise stated, "does not substantially bind to *X*" is intended to mean that an agent has a K_D that is greater than about 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}, or greater (*e.g.*, no detectable binding by the assay used to determine the K_D) for "*X*", wherein "*X*" is a specified agent such as protein or nucleic acid.

The terms "about" and "approximately" as used in connection with a numerical value throughout the specification and the claims denotes an interval of accuracy, familiar and acceptable to a person skilled in the art. In general, such interval of accuracy is ± 10%. Alternatively, and particularly in biological systems, the terms "about" and "approximately" may mean values that are within an order of magnitude, preferably ≤ 5 -fold and more preferably ≤ 2-fold of a given value.

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," 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 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.

2. ActRIIB:ALK7 Antagonists

As described herein, it has been discovered that ALK7:ActRIIB heterodimers have a unique ligand-binding profile/selectivity compared to corresponding ActRIIB and ALK7 homodimers. Interestingly, four of the five ligands with the strongest binding to ActRIIB homodimer (activin A, BMP10, GDF8, and GDF11) exhibit reduced binding to the ALK7:ActRIIB heterodimer, the exception being activin B which retains tight binding to the heterodimer. Similarly, three of the four ligands with intermediate binding to ActRIIB homodimer (GDF3, BMP6, and particularly BMP9) exhibit reduced binding to the ALK7:ActRIIB heterodimer, whereas binding to activin AC is increased to become the second strongest ligand interaction with the heterodimer overall. Finally, activin C and BMP5 unexpectedly bind the ALK7:ActRIIB heterodimer with intermediate strength despite no binding (activin C) or weak binding (BMP5) to ActRIIB homodimer. Given the binding to activin B and activin C, it is expected that the ALK7:ActRIIB heterodimer binds tightly to activin BC. The net result is that the ALK7:ActRIIB heterodimer possesses a ligand-binding profile distinctly different from that of either ActRIIB homodimer or ALK7 homodimer, which binds none of the foregoing ligands.

These results therefore indicate that the ALK7:ActRIIB heteromultimers are more selective antagonist of activin B, activin AC and Activin BC compared to ActRIIB homomultimers. Moreover, ALK7:ActRIIB heterodimer exhibits the unusual property of robust binding to activin C. Accordingly, an ALK7:ActRIIB heteromultimers will be more useful than an ActRIIB homomultimers in certain applications where such selective antagonism is advantageous.

Moreover, ALK7:ActRIIB heteromultimers are surprisingly effective in ameliorating various complications of kidney disease (e.g., treating or preventing kidney injury,

inflammation, and damage) as well as exerting beneficial anabolic adipose cells. Although ALK7:ActRIIB heteromultimers may exert biological effects through a mechanism other than ligand inhibition [e.g., inhibition of one or more of activin B, activin AC, activin C, GDF11, GDF8, activin A, BMP10, BMP6, BMP5, nodal, and GDF3 may be an indicator of the tendency of an agent to inhibit the activities of a spectrum of additional agents, including, perhaps, other members of the TGF-beta superfamily, and such collective inhibition may lead to a desired effect on, for example, kidney disease or a metabolic disorder], other types of TGF-beta superfamily ligand antagonists as well as type I and type II receptor antagonists (e.g., natural ligand traps such as follistatin and Lefty, antibodies, inhibitory nucleic acids, and inhibitory small molecules), or combinations of such antagonists, are expected to be useful in accordance with the methods described herein, particularly those that that mimic the binding/inhibitory properties of the ALK7:ActRIIB heterodimers as well as agents that directly or indirectly antagonize ALK7 and/or ActRIIB receptors, agents that directly or indirectly antagonize ALK7 and/or ActRIIB-binding ligands, agents that directly or indirectly antagonize downstream signaling mediators (e.g., Smads), and/or agents that directly or indirectly antagonize TGFβ superfamily co-receptors (e.g., Cripto or Cryptic) will have similar biological effects. Collectively ALK7:ActRIIB heteromultimers and these alternative antagonists are referred to herein as "ALK7:ActRIIB antagonists" or "ALK7:ActRIIB inhibitors".

A. ALK7:ActRIIB Heteromultimers

In certain aspects, the present disclosure relates to heteromultimers comprising one or more ALK7 receptor polypeptides (e.g., SEQ ID NOs: 9, 10, 19, 20, 38, 39, 42, 43, 46, 74, 76, 79, and 80) and one or more ActRIIB receptor polypeptides (e.g., SEQ ID NOs: 1, 2, 3, 4, 5, 6, 71, 73, 77, and 78), which are generally referred to herein as "ALK7:ActRIIB heteromultimer complexes" or "ALK7:ActRIIB heteromultimers". Preferably, ALK7:ActRIIB heteromultimers of the disclosure are soluble, for example, a heteromultimer may comprises a soluble portion (domain) of an ALK7 receptor and a soluble portion (domain) of an ActRIIB receptor. In general, the extracellular domains of ALK7 and ActRIIB correspond to a soluble portion of these receptors. Therefore, in some embodiments, heteromultimers of the disclosure comprise an extracellular domain of an ALK7 receptor and an extracellular domain of an ActRIIB receptor. Example extracellular domains of ALK7

and ActRIIB receptors are disclosed herein and these sequences, as well as fragments, functional variants, and modified forms thereof, may be used in accordance with the inventions of the disclosure (*e.g.*, ALK7:ActRIIB heteromultimers and uses thereof). ALK7:ActRIIB heteromultimers of the disclosure include, *e.g.*, heterodimers, heterotrimers, heterotetramers and higher order oligomeric structures. See, e.g., Figures 1, 2, and 8-10. In certain preferred embodiments, heteromultimers of the disclosure are ALK7:ActRIIB heterodimers.

Preferably, ALK7:ActRIIB heteromultimers of the disclosure bind to one or more TGF-beta superfamily ligands. In some embodiments, ALK7:ActRIIB heteromultimers bind to one or more of activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, and activin BE), GDF8, and GDF11. Optionally, in some embodiments, ALK7:ActRIIB heteromultimers further bind to BMP6. Optionally, in some embodiments, ALK7:ActRIIB heteromultimers further bind to BMP10. Optionally, in some embodiments, ALK7:ActRIIB heteromultimers further bind to BMP5. Optionally, in some embodiments, ALK7:ActRIIB heteromultimers further bind to GDF3. Optionally, in some embodiments, ALK7:ActRIIB heteromultimers further bind to nodal. Optionally, in some embodiments, ALK7:ActRIIB heteromultimers further bind to BMP10. Optionally, in some embodiments, ALK7:ActRIIB heteromultimers do not bind or do not substantially bind to BMP9. In certain preferred embodiments, ALK7:ActRIIB heteromultimers bind one or more of GDF11, GDF8, activin A, BMP10, BMP6, GDF3, and BMP9 with weaker affinity compared to a corresponding ActRIIB homomultimer. In other preferred embodiments, ALK7:ActRIIB heteromultimers bind one or more of activin C, activin AC, activin BC, and BMP5 with stronger affinity compared to a corresponding ActRIIB homomultimer. Optionally, ALK7:ActRIIB heteromultimers further bind to one or more of BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP7, BMP8a, BMP8b, GDF5, GDF6/BMP13, GDF7, GDF9b/BMP15, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty.

TGF-beta superfamily ligand-binding ALK7:ActRIIB heteromultimers may be used to inhibit (antagonize) signaling (*e.g.*, Smad 2/3 and/or Smad 1/5/8 signaling) mediated by one or more TGFβ superfamily ligands. In particular, ALK7:ActRIIB heteromultimers of the disclosure may be used to inhibit signaling by one or more TGFβ superfamily ligands in, for example, a cell-based assay such as those described herein. For example, ALK7:ActRIIB heteromultimers inhibit signaling mediated by one or more of *e.g.*, activin (e.g., activin A,

activin B, activin C, activin E, activin AB, activin AC, activin BC, activin AE and activin BE), GDF11, GDF8, BMP10, BMP6, BMP5, nodal, and GDF3 in a cell-based assay. Optionally, in some embodiments, ALK7:ActRIIB heteromultimers may further inhibit BMP6 signaling in a cell-based assay. Optionally, in some embodiments, ALK7:ActRIIB heteromultimers may further inhibit GDF3 signaling in a cell-based assay. Optionally, in some embodiments, ALK7:ActRIIB heteromultimers may further inhibit nodal signaling in a cell-based assay. Optionally, in some embodiments, ALK7:ActRIIB heteromultimers may further inhibit BMP5 signaling in a cell-based assay. Optionally, in some embodiments, ALK7:ActRIIB heteromultimers may further inhibit signaling BMP10 signaling in a cell-based assay. Optionally, in some embodiments, ALK7:ActRIIB heteromultimers do not inhibit or do not substantially inhibit signaling mediated by BMP9 in a cell-based assay. In certain preferred embodiments, ALK7:ActRIIB heteromultimers have a weaker inhibitory effect on signaling mediated by one or more of GDF11, GDF8, activin A, BMP10, BMP6, GDF3, and BMP9 compared to a corresponding ActRIIB homomultimer in a cell-based assay. In other preferred embodiments, ALK7:ActRIIB heteromultimers have a stronger inhibitory effect on signaling mediated by one or more of activin AC, activin BC, activin C, and BMP5 compared to a corresponding ActRIIB homomultimer in a cell-based assay. Optionally, ALK7:ActRIIB heteromultimers may further inhibit signaling by one or more of BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP7, BMP8a, BMP8b, GDF5, GDF6/BMP13, GDF7, GDF9b/BMP15, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty in a cell-based assay.

As used herein, the term "ActRIIB" refers to a family of activin receptor type IIB (ActRIIB) proteins from any species and variants derived from such ActRIIB proteins by mutagenesis or other modification. Reference to ActRIIB herein is understood to be a reference to any one of the currently identified forms. Members of the ActRIIB family are generally transmembrane proteins, composed of a ligand-binding extracellular domain comprising a cysteine-rich region, a transmembrane domain, and a cytoplasmic domain with predicted serine/threonine kinase activity.

The term "ActRIIB polypeptide" includes polypeptides comprising any naturally occurring polypeptide of an ActRIIB family member as well as any variants thereof (including mutants, fragments, fusions, and peptidomimetic forms) that retain a useful activity. Examples of such variant ActRIIB polypeptides are provided throughout the present disclosure as well as in International Patent Application Publication Nos. WO 2006/012627,

WO 2008/097541, and WO 2010/151426, which are incorporated herein by reference in their entirety. Numbering of amino acids for all ActRIIB-related polypeptides described herein is based on the numbering of the human ActRIIB precursor protein sequence provided below (SEQ ID NO: 1), unless specifically designated otherwise.

The human ActRIIB precursor protein sequence is as follows:

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  1 MTAPWVALAL LWGSLCAGSG RGEAETRECI YYNANWELER TNQSGLERCE
 51 GEQDKRLHCY ASWRNSSGTI ELVKKGCWLD DFNCYDRQEC VATEENPQVY
101 FCCCEGNFCN ERFTHLPEAG GPEVTYEPPP TAPTLLTVLA YSLLPIGGLS
151 LIVLLAFWMY RHRKPPYGHV DIHEDPGPPP PSPLVGLKPL QLLEIKARGR
201 FGCVWKAQLM NDFVAVKIFP LQDKQSWQSE REIFSTPGMK HENLLQFIAA
251 EKRGSNLEVE LWLITAFHDK GSLTDYLKGN IITWNELCHV AETMSRGLSY
301 LHEDVPWCRG EGHKPSIAHR DFKSKNVLLK SDLTAVLADF GLAVRFEPGK
351 PPGDTHGQVG TRRYMAPEVL EGAINFQRDA FLRIDMYAMG LVLWELVSRC
401 KAADGPVDEY MLPFEEEIGQ HPSLEELQEV VVHKKMRPTI KDHWLKHPGL
451 AQLCVTIEEC WDHDAEARLS AGCVEERVSL IRRSVNGTTS DCLVSLVTSV
501 TNVDLPPKES SI  (SEQ ID NO: 1)
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The signal peptide is indicated with a single underline; the extracellular domain is indicated in **bold** font; and the potential, endogenous N-linked glycosylation sites are indicated with a double underline.

The processed (mature) extracellular ActRIIB polypeptide sequence is as follows:

GRGEAETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWRNSSGTIELVKKGCWLDD FNCYDRQECVATEENPQVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPT (SEQ ID NO: 2)

In some embodiments, the protein may be produced with an "SGR..." sequence at the N-terminus. The C-terminal "tail" of the extracellular domain is indicated by a single underline. The sequence with the "tail" deleted (a Δ15 sequence) is as follows:

GRGEAETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWRNSSGTIELVKKGCWLDD FNCYDRQECVATEENPQVYFCCCEGNFCNERFTHLPEA (SEQ ID NO: 3)

A form of ActRIIB with an alanine at position 64 of SEQ ID NO: 1 (A64) is also reported in the literature. See, *e.g.*, Hilden *et al.* (1994) Blood, 83(8): 2163-2170. Applicants have ascertained that an ActRIIB-Fc fusion protein comprising an extracellular domain of ActRIIB with the A64 substitution has a relatively low affinity for activin and GDF11. By

contrast, the same ActRIIB-Fc fusion protein with an arginine at position 64 (R64) has an affinity for activin and GDF11 in the low nanomolar to high picomolar range. Therefore, sequences with an R64 are used as the "wild-type" reference sequence for human ActRIIB in this disclosure.

The form of ActRIIB with an alanine at position 64 is as follows:

```
  1 MTAPWVALAL LWGSLCAGSG RGEAETRECI YYNANWELER TNQSGLERCE
 51 GEQDKRLHCY ASWANSSGTI ELVKKGCWLD DFNCYDRQEC VATEENPQVY
101 FCCCEGNFCN ERFTHLPEAG GPEVTYEPPP TAPTLLTVLA YSLLPIGGLS
151 LIVLLAFWMY RHRKPPYGHV DIHEDPGPPP PSPLVGLKPL QLLEIKARGR
201 FGCVWKAQLM NDFVAVKIFP LQDKQSWQSE REIFSTPGMK HENLLQFIAA
251 EKRGSNLEVE LWLITAFHDK GSLTDYLKGN IITWNELCHV AETMSRGLSY
301 LHEDVPWCRG EGHKPSIAHR DFKSKNVLLK SDLTAVLADF GLAVRFEPGK
351 PPGDTHGQVG TRRYMAPEVL EGAINFQRDA FLRIDMYAMG LVLWELVSRC
401 KAADGPVDEY MLPFEEEIGQ HPSLEELQEV VVHKKMRPTI KDHWLKHPGL
451 AQLCVTIEEC WDHDAEARLS AGCVEERVSL IRRSVNGTTS DCLVSLVTSV
501 TNVDLPPKES SI (SEQ ID NO: 4)
```

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

The processed (mature) extracellular ActRIIB polypeptide sequence of the alternative A64 form is as follows:

```
GRGEAETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWANSSGTIELVKKGCWLDD
FNCYDRQECVATEENPQVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPT  (SEQ ID
NO: 5)
```

In some embodiments, the protein may be produced with an "SGR..." sequence at the N-terminus. The C-terminal "tail" of the extracellular domain is indicated by single underline. The sequence with the "tail" deleted (a Δ15 sequence) is as follows:

```
GRGEAETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWANSSGTIELVKKGCWLDD
FNCYDRQECVATEENPQVYFCCCEGNFCNERFTHLPEA (SEQ ID NO: 6)
```

A nucleic acid sequence encoding the human ActRIIB precursor protein is shown below (SEQ ID NO: 7), representing nucleotides 25-1560 of Genbank Reference Sequence NM_001106.3, which encode amino acids 1-513 of the ActRIIB precursor. The sequence as

shown provides an arginine at position 64 and may be modified to provide an alanine instead. The signal sequence is underlined.

```
   1 ATGACGGCGC CCTGGGTGGC CCTCGCCCTC CTCTGGGGAT CGCTGTGCGC
  51 CGGCTCTGGG CGTGGGGAGG CTGAGACACG GGAGTGCATC TACTACAACG
 101 CCAACTGGGA GCTGGAGCGC ACCAACCAGA GCGGCCTGGA GCGCTGCGAA
 151 GGCGAGCAGG ACAAGCGGCT GCACTGCTAC GCCTCCTGGC GCAACAGCTC
 201 TGGCACCATC GAGCTCGTGA AGAAGGGCTG CTGGCTAGAT GACTTCAACT
 251 GCTACGATAG GCAGGAGTGT GTGGCCACTG AGGAGAACCC CCAGGTGTAC
 301 TTCTGCTGCT GTGAAGGCAA CTTCTGCAAC GAACGCTTCA CTCATTTGCC
 351 AGAGGCTGGG GGCCCGGAAG TCACGTACGA GCCACCCCCG ACAGCCCCCA
 401 CCCTGCTCAC GGTGCTGGCC TACTCACTGC TGCCCATCGG GGGCCTTTCC
 451 CTCATCGTCC TGCTGGCCTT TTGGATGTAC CGGCATCGCA AGCCCCCCTA
 501 CGGTCATGTG GACATCCATG AGGACCCTGG GCCTCCACCA CCATCCCCTC
 551 TGGTGGGCCT GAAGCCACTG CAGCTGCTGG AGATCAAGGC TCGGGGGCGC
 601 TTTGGCTGTG TCTGGAAGGC CCAGCTCATG AATGACTTTG TAGCTGTCAA
 651 GATCTTCCCA CTCCAGGACA AGCAGTCGTG GCAGAGTGAA CGGGAGATCT
 701 TCAGCACACC TGGCATGAAG CACGAGAACC TGCTACAGTT CATTGCTGCC
 751 GAGAAGCGAG GCTCCAACCT CGAAGTAGAG CTGTGGCTCA TCACGGCCTT
 801 CCATGACAAG GGCTCCCTCA CGGATTACCT CAAGGGGAAC ATCATCACAT
 851 GGAACGAACT GTGTCATGTA GCAGAGACGA TGTCACGAGG CCTCTCATAC
 901 CTGCATGAGG ATGTGCCCTG GTGCCGTGGC GAGGGCCACA AGCCGTCTAT
 951 TGCCCACAGG GACTTTAAAA GTAAGAATGT ATTGCTGAAG AGCGACCTCA
1001 CAGCCGTGCT GGCTGACTTT GGCTTGGCTG TTCGATTTGA GCCAGGGAAA
1051 CCTCCAGGGG ACACCCACGG ACAGGTAGGC ACGAGACGGT ACATGGCTCC
1101 TGAGGTGCTC GAGGGAGCCA TCAACTTCCA GAGAGATGCC TTCCTGCGCA
1151 TTGACATGTA TGCCATGGGG TTGGTGCTGT GGGAGCTTGT GTCTCGCTGC
1201 AAGGCTGCAG ACGGACCCGT GGATGAGTAC ATGCTGCCCT TTGAGGAAGA
1251 GATTGGCCAG CACCCTTCGT TGGAGGAGCT GCAGGAGGTG GTGGTGCACA
1301 AGAAGATGAG GCCCACCATT AAAGATCACT GGTTGAAACA CCCGGGCCTG
1351 GCCCAGCTTT GTGTGACCAT CGAGGAGTGC TGGGACCATG ATGCAGAGGC
1401 TCGCTTGTCC GCGGGCTGTG TGGAGGAGCG GGTGTCCCTG ATTCGGAGGT
1451 CGGTCAACGG CACTACCTCG GACTGTCTCG TTTCCCTGGT GACCTCTGTC
1501 ACCAATGTGG ACCTGCCCCC TAAAGAGTCA AGCATC (SEQ ID NO: 7)
```

A nucleic acid sequence encoding processed extracellular human ActRIIB polypeptide is shown below (SEQ ID NO: 8). The sequence as shown provides an arginine at position 64, and may be modified to provide an alanine instead.

```
   1 GGGCGTGGGG AGGCTGAGAC ACGGGAGTGC ATCTACTACA ACGCCAACTG
  51 GGAGCTGGAG CGCACCAACC AGAGCGGCCT GGAGCGCTGC GAAGGCGAGC
 101 AGGACAAGCG GCTGCACTGC TACGCCTCCT GGCGCAACAG CTCTGGCACC
 151 ATCGAGCTCG TGAAGAAGGG CTGCTGGCTA GATGACTTCA ACTGCTACGA
 201 TAGGCAGGAG TGTGTGGCCA CTGAGGAGAA CCCCCAGGTG TACTTCTGCT
 251 GCTGTGAAGG CAACTTCTGC AACGAACGCT TCACTCATTT GCCAGAGGCT
 301 GGGGGCCCGG AAGTCACGTA CGAGCCACCC CCGACAGCCC CCACC
```

(SEQ ID NO: 8)

An alignment of the amino acid sequences of human ActRIIB extracellular domain and human ActRIIA extracellular domain are illustrated in Figure 3. This alignment indicates amino acid residues within both receptors that are believed to directly contact ActRII ligands. For example, the composite ActRII structures indicated that the ActRIIB-ligand binding pocket is defined, in part, by residues Y31, N33, N35, L38 through T41, E47, E50, Q53 through K55, L57, H58, Y60, S62, K74, W78 through N83, Y85, R87, A92, and E94 through F101. At these positions, it is expected that conservative mutations will be tolerated.

In addition, ActRIIB is well-conserved among vertebrates, with large stretches of the extracellular domain completely conserved. For example, Figure 4 depicts a multi-sequence alignment of a human ActRIIB extracellular domain compared to various ActRIIB orthologs. Many of the ligands that bind to ActRIIB are also highly conserved. Accordingly, from these alignments, it is possible to predict key amino acid positions within the ligand-binding domain that are important for normal ActRIIB-ligand binding activities as well as to predict amino acid positions that are likely to be tolerant of substitution without significantly altering normal ActRIIB-ligand binding activities. Therefore, an active, human ActRIIB variant polypeptide useful in accordance with the presently disclosed methods may include one or more amino acids at corresponding positions from the sequence of another vertebrate ActRIIB, or may include a residue that is similar to that in the human or other vertebrate sequences. Without meaning to be limiting, the following examples illustrate this approach to defining an active ActRIIB variant. L46 in the human extracellular domain (SEQ ID NO: 53) is a valine in *Xenopus* ActRIIB (SEQ ID NO: 55), and so this position may be altered, and optionally may be altered to another hydrophobic residue, such as V, I or F, or a non-

polar residue such as A. E52 in the human extracellular domain is a K in *Xenopus*, indicating that this site may be tolerant of a wide variety of changes, including polar residues, such as E, D, K, R, H, S, T, P, G, Y and probably A. T93 in the human extracellular domain is a K in *Xenopus*, indicating that a wide structural variation is tolerated at this position, with polar residues favored, such as S, K, R, E, D, H, G, P, G and Y. F108 in the human extracellular domain is a Y in *Xenopus*, and therefore Y or other hydrophobic group, such as I, V or L should be tolerated. E111 in the human extracellular domain is K in *Xenopus*, indicating that charged residues will be tolerated at this position, including D, R, K and H, as well as Q and N. R112 in the human extracellular domain is K in *Xenopus*, indicating that basic residues are tolerated at this position, including R and H. A at position 119 in the human extracellular domain is relatively poorly conserved, and appears as P in rodents and V in *Xenopus*, thus essentially any amino acid should be tolerated at this position.

Moreover, ActRII proteins have been characterized in the art in terms of structural and functional characteristics, particularly with respect to ligand binding [Attisano *et al.* (1992) Cell 68(1):97-108; Greenwald *et al.* (1999) Nature Structural Biology 6(1): 18-22; Allendorph *et al.* (2006) PNAS 103(20: 7643-7648; Thompson *et al.* (2003) The EMBO Journal 22(7): 1555-1566; as well as U.S. Patent Nos: 7,709,605, 7,612,041, and 7,842,663]. In addition to the teachings herein, these references provide amply guidance for how to generate ActRIIB variants that retain one or more normal activities (e.g., ligand-binding activity).

For example, a defining structural motif known as a three-finger toxin fold is important for ligand binding by type I and type II receptors and is formed by conserved cysteine residues located at varying positions within the extracellular domain of each monomeric receptor [Greenwald et al. (1999) Nat Struct Biol 6:18-22; and Hinck (2012) FEBS Lett 586:1860-1870]. Accordingly, the core ligand-binding domains of human ActRIIB, as demarcated by the outermost of these conserved cysteines, corresponds to positions 29-109 of SEQ ID NO: 1 (ActRIIB precursor). Thus, the structurally less-ordered amino acids flanking these cysteine-demarcated core sequences can be truncated by about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, or 28 residues at the N-terminus and/or by about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 residues a the C-terminus without necessarily altering ligand binding. Exemplary ActRIIB extracellular domains for N-terminal and/or C-terminal truncation include SEQ ID NOs: 2, 3, 5, and 6.

Attisano *et al.* showed that a deletion of the proline knot at the C-terminus of the extracellular domain of ActRIIB reduced the affinity of the receptor for activin. An ActRIIB-Fc fusion protein containing amino acids 20-119 of present SEQ ID NO: 1, "ActRIIB(20-119)-Fc", has reduced binding to GDF11 and activin relative to an ActRIIB(20-134)-Fc, which includes the proline knot region and the complete juxtamembrane domain (see, *e.g.*, U.S. Patent No. 7,842,663). However, an ActRIIB(20-129)-Fc protein retains similar, but somewhat reduced activity, relative to the wild-type, even though the proline knot region is disrupted.

Thus, ActRIIB extracellular domains that stop at amino acid 134, 133, 132, 131, 130 and 129 (with respect to SEQ ID NO: 1) are all expected to be active, but constructs stopping at 134 or 133 may be most active. Similarly, mutations at any of residues 129-134 (with respect to SEQ ID NO: 1) are not expected to alter ligand-binding affinity by large margins. In support of this, it is known in the art that mutations of P129 and P130 (with respect to SEQ ID NO: 1) do not substantially decrease ligand binding. Therefore, an ActRIIB polypeptide of the present disclosure may end as early as amino acid 109 (the final cysteine), however, forms ending at or between 109 and 119 (*e.g.*, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, or 119) are expected to have reduced ligand binding. Amino acid 119 (with respect to present SEQ ID NO: 1) is poorly conserved and so is readily altered or truncated. ActRIIB polypeptides ending at 128 (with respect to SEQ ID NO: 1) or later should retain ligand-binding activity. ActRIIB polypeptides ending at or between 119 and 127 (*e.g.*, 119, 120, 121, 122, 123, 124, 125, 126, or 127), with respect to SEQ ID NO: 1, will have an intermediate binding ability. Any of these forms may be desirable to use, depending on the clinical or experimental setting.

At the N-terminus of ActRIIB, it is expected that a protein beginning at amino acid 29 or before (with respect to SEQ ID NO: 1) will retain ligand-binding activity. Amino acid 29 represents the initial cysteine. An alanine-to-asparagine mutation at position 24 (with respect to SEQ ID NO: 1) introduces an N-linked glycosylation sequence without substantially affecting ligand binding [U.S. Patent No. 7,842,663]. This confirms that mutations in the region between the signal cleavage peptide and the cysteine cross-linked region, corresponding to amino acids 20-29, are well tolerated. In particular, ActRIIB polypeptides beginning at position 20, 21, 22, 23, and 24 (with respect to SEQ ID NO: 1) should retain general ligand-biding activity, and ActRIIB polypeptides beginning at positions 25, 26, 27, 28, and 29 (with respect to SEQ ID NO: 1) are also expected to retain ligand-biding activity.

It has been demonstrated, *e.g.*, U.S. Patent No. 7,842,663, that, surprisingly, an ActRIIB construct beginning at 22, 23, 24, or 25 will have the most activity.

Taken together, a general formula for an active portion (*e.g.*, ligand-binding portion) of ActRIIB comprises amino acids 29-109 of SEQ ID NO: 1. Therefore ActRIIB polypeptides may, for example, comprise, consist essentially of, or consist of an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a portion of ActRIIB beginning at a residue corresponding to any one of amino acids 20-29 (*e.g.*, beginning at any one of amino acids 20, 21, 22, 23, 24, 25, 26, 27, 28, or 29) of SEQ ID NO: 1 and ending at a position corresponding to any one amino acids 109-134 (*e.g.*, ending at any one of amino acids 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, or 134) of SEQ ID NO: 1. Other examples include polypeptides that begin at a position from 20-29 (*e.g.*, any one of positions 20, 21, 22, 23, 24, 25, 26, 27, 28, or 29) or 21-29 (*e.g.*, any one of positions 21, 22, 23, 24, 25, 26, 27, 28, or 29) of SEQ ID NO: 1 and end at a position from 119-134 (*e.g.*, any one of positions 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, or 134), 119-133 (*e.g.*, any one of positions 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, or 133), 129-134 (*e.g.*, any one of positions 129, 130, 131, 132, 133, or 134), or 129-133 (*e.g.*, any one of positions 129, 130, 131, 132, or 133) of SEQ ID NO: 1. Other examples include constructs that begin at a position from 20-24 (*e.g.*, any one of positions 20, 21, 22, 23, or 24), 21-24 (*e.g.*, any one of positions 21, 22, 23, or 24), or 22-25 (*e.g.*, any one of positions 22, 22, 23, or 25) of SEQ ID NO: 1 and end at a position from 109-134 (*e.g.*, any one of positions 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, or 134), 119-134 (*e.g.*, any one of positions 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, or 134) or 129-134 (*e.g.*, any one of positions 129, 130, 131, 132, 133, or 134) of SEQ ID NO: 1. Variants within these ranges are also contemplated, particularly those having at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity to the corresponding portion of SEQ ID NO: 1.

The variations described herein may be combined in various ways. In some embodiments, ActRIIB variants comprise no more than 1, 2, 5, 6, 7, 8, 9, 10 or 15 conservative amino acid changes in the ligand-binding pocket, and zero, one, or more non-conservative alterations at positions 40, 53, 55, 74, 79 and/or 82 in the ligand-binding pocket.

Sites outside the binding pocket, at which variability may be particularly well tolerated, include the amino and carboxy termini of the extracellular domain (as noted above), and positions 42-46 and 65-73 (with respect to SEQ ID NO: 1). An asparagine-to-alanine alteration at position 65 (N65A) actually improves ligand binding in the A64 background, and is thus expected to have no detrimental effect on ligand binding in the R64 background [U.S. Patent No. 7,842,663]. This change probably eliminates glycosylation at N65 in the A64 background, thus demonstrating that a significant change in this region is likely to be tolerated. While an R64A change is poorly tolerated, R64K is well-tolerated, and thus another basic residue, such as H may be tolerated at position 64 [U.S. Patent No. 7,842,663]. Additionally, the results of the mutagenesis program described in the art indicate that there are amino acid positions in ActRIIB that are often beneficial to conserve. With respect to SEQ ID NO: 1, these include position 80 (acidic or hydrophobic amino acid), position 78 (hydrophobic, and particularly tryptophan), position 37 (acidic, and particularly aspartic or glutamic acid), position 56 (basic amino acid), position 60 (hydrophobic amino acid, particularly phenylalanine or tyrosine). Thus, the disclosure provides a framework of amino acids that may be conserved in ActRIIB polypeptides. Other positions that may be desirable to conserve are as follows: position 52 (acidic amino acid), position 55 (basic amino acid), position 81 (acidic), 98 (polar or charged, particularly E, D, R or K), all with respect to SEQ ID NO: 1.

In certain embodiments, the disclosure relates to heteromultimers that comprise at least one ActRIIB polypeptide, which includes fragments, functional variants, and modified forms thereof. Preferably, ActRIIB polypeptides for use in accordance with the disclosure are soluble (*e.g.*, an extracellular domain of ActRIIB). In other preferred embodiments, ActRIIB polypeptides for use in accordance with the disclosure bind to one or more TGF-beta superfamily ligands. Therefore, in some embodiments, ActRIIB polypeptides for use in accordance with the disclosure inhibit (antagonize) activity (*e.g.*, inhibition of Smad signaling) of one or more TGF-beta superfamily ligands. In some embodiments, heteromultimers of the disclosure comprise at least one ActRIIB polypeptide that comprises, consists essentially of, or consists of an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a portion of ActRIIB beginning at a residue corresponding to amino acids 20-29 (*e.g.*, beginning at any one of amino acids 20, 21, 22, 23, 24, 25, 26, 27, 28, or 29) of SEQ ID NO: 1 and ending at a position corresponding to amino acids 109-134 (*e.g.*, ending at any one of

amino acids 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, or 134) of SEQ ID NO: 1. In certain preferred embodiments, heteromultimers of the disclosure comprise at least one ActRIIB polypeptide that comprises, consists, or consists essentially of an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical amino acids 29-109 of SEQ ID NO: 1. In other preferred embodiments, heteromultimers of the disclosure comprise at least one ActRIIB polypeptide that comprises, consists, or consists essentially of an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical amino acids 25-131 of SEQ ID NO: 1. In some embodiments, heteromultimers of the disclosure comprise at least one ActRIIB polypeptide that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 71, 73, 77, and 78. In certain embodiments, heteromultimers of the disclosure comprise at least one ActRIIB polypeptide wherein the amino acid position corresponding to L79 of SEQ ID NO: 1 is not an acidic amino acid (*i.e.*, is not a naturally occurring D or E amino acid residue or artificial acidic amino acid).

In certain aspects, the present disclosure relates to protein complexes that comprise an ALK7 polypeptide. As used herein, the term "ALK7" refers to a family of activin receptor-like kinase-7 proteins from any species and variants derived from such ALK7 proteins by mutagenesis or other modification. Reference to ALK7 herein is understood to be a reference to any one of the currently identified forms. Members of the ALK7 family are generally 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 "ALK7 polypeptide" includes polypeptides comprising any naturally occurring polypeptide of an ALK7 family member as well as any variants thereof (including mutants, fragments, fusions, and peptidomimetic forms) that retain a useful activity. Numbering of amino acids for all ALK7-related polypeptides described herein is based on the numbering of the human ALK7 precursor protein sequence below (SEQ ID NO: 9), unless specifically designated otherwise.

There are various naturally occurring isoforms of human ALK7. The sequence of canonical human ALK7 isoform 1 precursor protein (NCBI Ref Seq NP_660302.2) is as follows:

1 MTRALCSALR QALLLLAAAA **ELSPGLKCVC LLCDSSNFTC QTEGACWASV**
MLTNGKEQVI
61 **KSCVSLPELN AQVFCHSSNN VTKTECCFTD FCNNITLHLP TASPNAPKLG**
PMELAIIITV
121 PVCLLSIAAM LTVWACQGRQ CSYRKKKRPN VEEPLSECNL VNAGKTLKDL
IYDVTASGSG
181 SGLPLLVQRT IARTIVLQEI VGKGRFGEVW HGRWCGEDVA VKIFSSRDER
SWFREAEIYQ
241 TVMLRHENIL GFIAADNKDN GTWTQLWLVS EYHEQGSLYD YLNRNIVTVA
GMIKLALSIA
301 SGLAHLHMEI VGTQGKPAIA HRDIKSKNIL VKKCETCAIA DLGLAVKHDS
ILNTIDIPQN
361 PKVGTKRYMA PEMLDDTMNV NIFESFKRAD IYSVGLVYWE IARRCSVGGI
VEEYQLPYYD
421 MVPSDPSIEE MRKVVCDQKF RPSIPNQWQS CEALRVMGRI MRECWYANGA
ARLTALRIKK
481 TISQLCVKED CKA (SEQ ID NO: 9)

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

The processed extracellular ALK7 isoform 1 polypeptide sequence is as follows:

ELSPGLKCVCLLCDSSNFTCQTEGACWASVMLTNGKEQVIKSCVSLPELNAQVFCHSSNNVT
KTECCFTDFCNNITLHLPTASPNAPKLGPME (SEQ ID NO: 10)

A nucleic acid sequence encoding human ALK7 isoform 1 precursor protein is shown below (SEQ ID NO: 11), corresponding to nucleotides 244-1722 of Genbank Reference Sequence NM_145259.2. The signal sequence is underlined and the extracellular domain is indicated in **bold** font.

ATGACCCGGGCGCTCTGCTCAGCGCTCCGCCAGGCTCTCCTGCTGCTCGCAGCGGCCGCC**GA**
GCTCTCGCCAGGACTGAAGTGTGTATGTCTTTTGTGTGATTCTTCAAACTTTACCTGCCAAA
CAGAAGGAGCATGTTGGGCATCAGTCATGCTAACCAATGGAAAAGAGCAGGTGATCAAATCC
TGTGTCTCCCTTCCAGAACTGAATGCTCAAGTCTTCTGTCATAGTTCCAACAATGTTACCAA

AACCGAATGCTGCTTCACAGATTTTTGCAACAACATAACACTGCACCTTCCAACAGCATCAC
CAAATGCCCCAAAACTTGGACCCATGGAGCTGGCCATCATTATTACTGTGCCTGTTTGCCTC
CTGTCCATAGCTGCGATGCTGACAGTATGGGCATGCCAGGGTCGACAGTGCTCCTACAGGAA
GAAAAAGAGACCAAATGTGGAGGAACCACTCTCTGAGTGCAATCTGGTAAATGCTGGAAAAA
CTCTGAAAGATCTGATTTATGATGTGACCGCCTCTGGATCTGGCTCTGGTCTACCTCTGTTG
GTTCAAAGGACAATTGCAAGGACGATTGTGCTTCAGGAAATAGTAGGAAAAGGTAGATTTGG
TGAGGTGTGGCATGGAAGATGGTGTGGGGAAGATGTGGCTGTGAAAATATTCTCCTCCAGAG
ATGAAAGATCTTGGTTTCGTGAGGCAGAAATTTACCAGACGGTCATGCTGCGACATGAAAAC
ATCCTTGGTTTCATTGCTGCTGACAACAAAGATAATGGAACTTGGACTCAACTTTGGCTGGT
ATCTGAATATCATGAACAGGGCTCCTTATATGACTATTTGAATAGAAATATAGTGACCGTGG
CTGGAATGATCAAGCTGGCGCTCTCAATTGCTAGTGGTCTGGCACACCTTCATATGGAGATT
GTTGGTACACAAGGTAAACCTGCTATTGCTCATCGAGACATAAAATCAAAGAATATCTTAGT
GAAAAAGTGTGAAACTTGTGCCATAGCGGACTTAGGGTTGGCTGTGAAGCATGATTCAATAC
TGAACACTATCGACATACCTCAGAATCCTAAAGTGGGAACCAAGAGGTATATGGCTCCTGAA
ATGCTTGATGATACAATGAATGTGAATATCTTTGAGTCCTTCAAACGAGCTGACATCTATTC
TGTTGGTCTGGTTTACTGGGAAATAGCCCGGAGGTGTTCAGTCGGAGGAATTGTTGAGGAGT
ACCAATTGCCTTATTATGACATGGTGCCTTCAGATCCCTCGATAGAGGAAATGAGAAAGGTT
GTTTGTGACCAGAAGTTTCGACCAAGTATCCCAAACCAGTGGCAAAGTTGTGAAGCACTCCG
AGTCATGGGGAGAATAATGCGTGAGTGTTGGTATGCCAACGGAGCGGCCCGCCTAACTGCTC
TTCGTATTAAGAAGACTATATCTCAACTTTGTGTCAAAGAAGACTGCAAAGCC (SEQ ID NO: 11)

A nucleic acid sequence encoding the processed extracellular ALK7 polypeptide (isoform 1) is as follows:

GAGCTCTCGCCAGGACTGAAGTGTGTATGTCTTTTGTGTGATTCTTCAAACTTTACCTGCCA
AACAGAAGGAGCATGTTGGGCATCAGTCATGCTAACCAATGGAAAAGAGCAGGTGATCAAAT
CCTGTGTCTCCCTTCCAGAACTGAATGCTCAAGTCTTCTGTCATAGTTCCAACAATGTTACC
AAAACCGAATGCTGCTTCACAGATTTTTGCAACAACATAACACTGCACCTTCCAACAGCATC
ACCAAATGCCCCAAAACTTGGACCCATGGAG (SEQ ID NO: 12)

The amino acid sequence of an alternative isoform of human ALK7, isoform 2 (NCBI Ref Seq NP_001104501.1), is shown in its processed form as follows (SEQ ID NO: 19), where the extracellular domain is indicated in **bold** font.

1 **MLTNGKEQVI KSCVSLPELN AQVFCHSSNN VTKTECCFTD FCNNITLHLP TASPNAPKLG**

61 **PME**LAIIITV PVCLLSIAAM LTVWACQGRQ CSYRKKKRPN VEEPLSECNL VNAGKTLKDL

121 IYDVTASGSG SGLPLLVQRT IARTIVLQEI VGKGRFGEVW HGRWCGEDVA VKIFSSRDER

181 SWFREAEIYQ TVMLRHENIL GFIAADNKDN GTWTQLWLVS EYHEQGSLYD YLNRNIVTVA

241 GMIKLALSIA SGLAHLHMEI VGTQGKPAIA HRDIKSKNIL VKKCETCAIA DLGLAVKHDS

301 ILNTIDIPQN PKVGTKRYMA PEMLDDTMNV NIFESFKRAD IYSVGLVYWE IARRCSVGGI

361 VEEYQLPYYD MVPSDPSIEE MRKVVCDQKF RPSIPNQWQS CEALRVMGRI MRECWYANGA

421 ARLTALRIKK TISQLCVKED CKA (SEQ ID NO: 19)

The amino acid sequence of the extracellular ALK7 polypeptide (isoform 2) is as follows:

MLTNGKEQVIKSCVSLPELNAQVFCHSSNNVTKTECCFTDFCNNITLHLPTASPNAPKLGPME (SEQ ID NO: 20).

A nucleic acid sequence encoding the processed ALK7 polypeptide (isoform 2) is shown below (SEQ ID NO: 21), corresponding to nucleotides 279-1607 of NCBI Reference Sequence NM_001111031.1. The extracellular domain is indicated in **bold** font.

ATGCTAACCAATGGAAAAGAGCAGGTGATCAAATCCTGTGTCTCCCTTCCAGAACTGAATGCTCAAGT CTTCTGTCATAGTTCCAACAATGTTACCAAAACCGAATGCTGCTTCACAGATTTTTGCAACAACATAA CACTGCACCTTCCAACAGCATCACCAAATGCCCCAAAACTTGGACCCATGGAGCTGGCCATCATTATT ACTGTGCCTGTTTGCCTCCTGTCCATAGCTGCGATGCTGACAGTATGGGCATGCCAGGGTCGACAGTG CTCCTACAGGAAGAAAAAGAGACCAAATGTGGAGGAACCACTCTCTGAGTGCAATCTGGTAAATGCTG GAAAAACTCTGAAAGATCTGATTTATGATGTGACCGCCTCTGGATCTGGCTCTGGTCTACCTCTGTTG GTTCAAAGGACAATTGCAAGGACGATTGTGCTTCAGGAAATAGTAGGAAAAGGTAGATTTGGTGAGGT GTGGCATGGAAGATGGTGTGGGGAAGATGTGGCTGTGAAAATATTCTCCTCCAGAGATGAAAGATCTT GGTTTCGTGAGGCAGAAATTTACCAGACGGTCATGCTGCGACATGAAAACATCCTTGGTTTCATTGCT GCTGACAACAAAGATAATGGAACTTGGACTCAACTTTGGCTGGTATCTGAATATCATGAACAGGGCTC CTTATATGACTATTTGAATAGAAATATAGTGACCGTGGCTGGAATGATCAAGCTGGCGCTCTCAATTG CTAGTGGTCTGGCACACCTTCATATGGAGATTGTTGGTACACAAGGTAAACCTGCTATTGCTCATCGA GACATAAAATCAAAGAATATCTTAGTGAAAAAGTGTGAAACTTGTGCCATAGCGGACTTAGGGTTGGC TGTGAAGCATGATTCAATACTGAACACTATCGACATACCTCAGAATCCTAAAGTGGGAACCAAGAGGT ATATGGCTCCTGAAATGCTTGATGATACAATGAATGTGAATATCTTTGAGTCCTTCAAACGAGCTGAC

ATCTATTCTGTTGGTCTGGTTTACTGGGAAATAGCCCGGAGGTGTTCAGTCGGAGGAATTGTTGAGGA
GTACCAATTGCCTTATTATGACATGGTGCCTTCAGATCCCTCGATAGAGGAAATGAGAAAGGTTGTTT
GTGACCAGAAGTTTCGACCAAGTATCCCAAACCAGTGGCAAAGTTGTGAAGCACTCCGAGTCATGGGG
AGAATAATGCGTGAGTGTTGGTATGCCAACGGAGCGGCCCGCCTAACTGCTCTTCGTATTAAGAAGAC
TATATCTCAACTTTGTGTCAAAGAAGACTGCAAAGCC (SEQ ID NO: 21)

A nucleic acid sequence encoding the extracellular ALK7 polypeptide (isoform 2) is as follows (SEQ ID NO: 22):

ATGCTAACCAATGGAAAAGAGCAGGTGATCAAATCCTGTGTCTCCCTTCCAGAACTGAATGCTCAAGT
CTTCTGTCATAGTTCCAACAATGTTACCAAAACCGAATGCTGCTTCACAGATTTTTGCAACAACATAA
CACTGCACCTTCCAACAGCATCACCAAATGCCCCAAAACTTGGACCCATGGAG (SEQ ID NO: 22)

The amino acid sequence of an alternative human ALK7 precursor protein, isoform 3 (NCBI Ref Seq NP_001104502.1), is shown as follows (SEQ ID NO: 38), where the signal peptide is indicated by a single underline.

1 MTRALCSALR QALLLLAAAA ELSPGLKCVC LLCDSSNFTC QTEGACWASV MLTNGKEQVI
61 KSCVSLPELN AQVFCHSSNN VTKTECCFTD FCNNITLHLP TGLPLLVQRT IARTIVLQEI
121 VGKGRFGEVW HGRWCGEDVA VKIFSSRDER SWFREAEIYQ TVMLRHENIL GFIAADNKDN
181 GTWTQLWLVS EYHEQGSLYD YLNRNIVTVA GMIKLALSIA SGLAHLHMEI VGTQGKPAIA
241 HRDIKSKNIL VKKCETCAIA DLGLAVKHDS ILNTIDIPQN PKVGTKRYMA PEMLDDTMNV
301 NIFESFKRAD IYSVGLVYWE IARRCSVGGI VEEYQLPYYD MVPSDPSIEE MRKVVCDQKF
361 RPSIPNQWQS CEALRVMGRI MRECWYANGA ARLTALRIKK TISQLCVKED CKA
(SEQ ID NO: 38)

The amino acid sequence of the processed ALK7 polypeptide (isoform 3) is as follows (SEQ ID NO: 39). This isoform lacks a transmembrane domain and is therefore proposed to be soluble in its entirety (Roberts et al., 2003, Biol Reprod 68:1719-1726). N-terminal variants of SEQ ID NO: 39 are predicted as described below.

1 ELSPGLKCVC LLCDSSNFTC QTEGACWASV MLTNGKEQVI KSCVSLPELN AQVFCHSSNN

61 VTKTECCFTD FCNNITLHLP TGLPLLVQRT IARTIVLQEI VGKGRFGEVW
HGRWCGEDVA
121 VKIFSSRDER SWFREAEIYQ TVMLRHENIL GFIAADNKDN GTWTQLWLVS
EYHEQGSLYD
181 YLNRNIVTVA GMIKLALSIA SGLAHLHMEI VGTQGKPAIA HRDIKSKNIL
VKKCETCAIA
241 DLGLAVKHDS ILNTIDIPQN PKVGTKRYMA PEMLDDTMNV NIFESFKRAD
IYSVGLVYWE
301 IARRCSVGGI VEEYQLPYYD MVPSDPSIEE MRKVVCDQKF RPSIPNQWQS
CEALRVMGRI
361 MRECWYANGA ARLTALRIKK TISQLCVKED CKA (SEQ ID NO: 39)

A nucleic acid sequence encoding the unprocessed ALK7 polypeptide precursor protein (isoform 3) is shown below (SEQ ID NO: 40), corresponding to nucleotides 244-1482 of NCBI Reference Sequence NM_001111032.1. The signal sequence is indicated by solid underline.

ATGACCCGGGCGCTCTGCTCAGCGCTCCGCCAGGCTCTCCTGCTGCTCGCAGCGGCCGCCGAGCTCTC
GCCAGGACTGAAGTGTGTATGTCTTTTGTGTGATTCTTCAAACTTTACCTGCCAAACAGAAGGAGCAT
GTTGGGCATCAGTCATGCTAACCAATGGAAAAGAGCAGGTGATCAAATCCTGTGTCTCCCTTCCAGAA
CTGAATGCTCAAGTCTTCTGTCATAGTTCCAACAATGTTACCAAAACCGAATGCTGCTTCACAGATTT
TTGCAACAACATAACACTGCACCTTCCAACAGGTCTACCTCTGTTGGTTCAAAGGACAATTGCAAGGA
CGATTGTGCTTCAGGAAATAGTAGGAAAAGGTAGATTTGGTGAGGTGTGGCATGGAAGATGGTGTGGG
GAAGATGTGGCTGTGAAAATATTCTCCTCCAGAGATGAAAGATCTTGGTTTCGTGAGGCAGAAATTTA
CCAGACGGTCATGCTGCGACATGAAAACATCCTTGGTTTCATTGCTGCTGACAACAAAGATAATGGAA
CTTGGACTCAACTTTGGCTGGTATCTGAATATCATGAACAGGGCTCCTTATATGACTATTTGAATAGA
AATATAGTGACCGTGGCTGGAATGATCAAGCTGGCGCTCTCAATTGCTAGTGGTCTGGCACACCTTCA
TATGGAGATTGTTGGTACACAAGGTAAACCTGCTATTGCTCATCGAGACATAAAATCAAAGAATATCT
TAGTGAAAAAGTGTGAAACTTGTGCCATAGCGGACTTAGGGTTGGCTGTGAAGCATGATTCAATACTG
AACACTATCGACATACCTCAGAATCCTAAAGTGGGAACCAAGAGGTATATGGCTCCTGAAATGCTTGA
TGATACAATGAATGTGAATATCTTTGAGTCCTTCAAACGAGCTGACATCTATTCTGTTGGTCTGGTTT
ACTGGGAAATAGCCCGGAGGTGTTCAGTCGGAGGAATTGTTGAGGAGTACCAATTGCCTTATTATGAC
ATGGTGCCTTCAGATCCCTCGATAGAGGAAATGAGAAAGGTTGTTTGTGACCAGAAGTTTCGACCAAG
TATCCCAAACCAGTGGCAAAGTTGTGAAGCACTCCGAGTCATGGGGAGAATAATGCGTGAGTGTTGGT
ATGCCAACGGAGCGGCCCGCCTAACTGCTCTTCGTATTAAGAAGACTATATCTCAACTTTGTGTCAAA
GAAGACTGCAAAGCC (SEQ ID NO: 40)

A nucleic acid sequence encoding the processed ALK7 polypeptide (isoform 3) is as follows (SEQ ID NO: 41):

GAGCTCTCGCCAGGACTGAAGTGTGTATGTCTTTTGTGTGATTCTTCAAACTTTACCTGCCAAACAGA
AGGAGCATGTTGGGCATCAGTCATGCTAACCAATGGAAAAGAGCAGGTGATCAAATCCTGTGTCTCCC
TTCCAGAACTGAATGCTCAAGTCTTCTGTCATAGTTCCAACAATGTTACCAAAACCGAATGCTGCTTC
ACAGATTTTTGCAACAACATAACACTGCACCTTCCAACAGGTCTACCTCTGTTGGTTCAAAGGACAAT
TGCAAGGACGATTGTGCTTCAGGAAATAGTAGGAAAAGGTAGATTTGGTGAGGTGTGGCATGGAAGAT
GGTGTGGGGAAGATGTGGCTGTGAAAATATTCTCCTCCAGAGATGAAAGATCTTGGTTTCGTGAGGCA
GAAATTTACCAGACGGTCATGCTGCGACATGAAAACATCCTTGGTTTCATTGCTGCTGACAACAAAGA
TAATGGAACTTGGACTCAACTTTGGCTGGTATCTGAATATCATGAACAGGGCTCCTTATATGACTATT
TGAATAGAAATATAGTGACCGTGGCTGGAATGATCAAGCTGGCGCTCTCAATTGCTAGTGGTCTGGCA
CACCTTCATATGGAGATTGTTGGTACACAAGGTAAACCTGCTATTGCTCATCGAGACATAAAATCAAA
GAATATCTTAGTGAAAAAGTGTGAAACTTGTGCCATAGCGGACTTAGGGTTGGCTGTGAAGCATGATT
CAATACTGAACACTATCGACATACCTCAGAATCCTAAAGTGGGAACCAAGAGGTATATGGCTCCTGAA
ATGCTTGATGATACAATGAATGTGAATATCTTTGAGTCCTTCAAACGAGCTGACATCTATTCTGTTGG
TCTGGTTTACTGGGAAATAGCCCGGAGGTGTTCAGTCGGAGGAATTGTTGAGGAGTACCAATTGCCTT
ATTATGACATGGTGCCTTCAGATCCCTCGATAGAGGAAATGAGAAAGGTTGTTTGTGACCAGAAGTTT
CGACCAAGTATCCCAAACCAGTGGCAAAGTTGTGAAGCACTCCGAGTCATGGGGAGAATAATGCGTGA
GTGTTGGTATGCCAACGGAGCGGCCCGCCTAACTGCTCTTCGTATTAAGAAGACTATATCTCAACTTT
GTGTCAAAGAAGACTGCAAAGCC (SEQ ID NO: 308)

The amino acid sequence of an alternative human ALK7 precursor protein, isoform 4 (NCBI Ref Seq NP_001104503.1), is shown as follows (SEQ ID NO: 42), where the signal peptide is indicated by a single underline.

1 MTRALCSALR QALLLLAAAA ELSPGLKCVC LLCDSSNFTC QTEGACWASV
MLTNGKEQVI
61 KSCVSLPELN AQVFCHSSNN VTKTECCFTD FCNNITLHLP TDNGTWTQLW
LVSEYHEQGS
121 LYDYLNRNIV TVAGMIKLAL SIASGLAHLH MEIVGTQGKP AIAHRDIKSK
NILVKKCETC
181 AIADLGLAVK HDSILNTIDI PQNPKVGTKR YMAPEMLDDT MNVNIFESFK
RADIYSVGLV
241 YWEIARRCSV GGIVEEYQLP YYDMVPSDPS IEEMRKVVCD QKFRPSIPNQ
WQSCEALRVM
301 GRIMRECWYA NGAARLTALR IKKTISQLCV KEDCKA (SEQ ID NO: 42)

The amino acid sequence of the processed ALK7 polypeptide (isoform 4) is as follows (SEQ ID NO: 43). Like ALK7 isoform 3, isoform 4 lacks a transmembrane domain

and is therefore proposed to be soluble in its entirety (Roberts et al., 2003, Biol Reprod 68:1719-1726). N-terminal variants of SEQ ID NO: 43 are predicted as described below.

```
   1 ELSPGLKCVC LLCDSSNFTC QTEGACWASV MLTNGKEQVI KSCVSLPELN
AQVFCHSSNN
  61 VTKTECCFTD FCNNITLHLP TDNGTWTQLW LVSEYHEQGS LYDYLNRNIV
TVAGMIKLAL
 121 SIASGLAHLH MEIVGTQGKP AIAHRDIKSK NILVKKCETC AIADLGLAVK
HDSILNTIDI
 181 PQNPKVGTKR YMAPEMLDDT MNVNIFESFK RADIYSVGLV YWEIARRCSV
GGIVEEYQLP
 240 YYDMVPSDPS IEEMRKVVCD QKFRPSIPNQ WQSCEALRVM GRIMRECWYA
NGAARLTALR
 301 IKKTISQLCV KEDCKA   (SEQ ID NO: 43)
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A nucleic acid sequence encoding the unprocessed ALK7 polypeptide precursor protein (isoform 4) is shown below (SEQ ID NO: 44), corresponding to nucleotides 244-1244 of NCBI Reference Sequence NM_001111033.1. The signal sequence is indicated by solid underline.

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ATGACCCGGGCGCTCTGCTCAGCGCTCCGCCAGGCTCTCCTGCTGCTCGCAGCGGCCGCCGAGCTCTC
GCCAGGACTGAAGTGTGTATGTCTTTTGTGTGATTCTTCAAACTTTACCTGCCAAACAGAAGGAGCAT
GTTGGGCATCAGTCATGCTAACCAATGGAAAAGAGCAGGTGATCAAATCCTGTGTCTCCCTTCCAGAA
CTGAATGCTCAAGTCTTCTGTCATAGTTCCAACAATGTTACCAAAACCGAATGCTGCTTCACAGATTT
TTGCAACAACATAACACTGCACCTTCCAACAGATAATGGAACTTGGACTCAACTTTGGCTGGTATCTG
AATATCATGAACAGGGCTCCTTATATGACTATTTGAATAGAAATATAGTGACCGTGGCTGGAATGATC
AAGCTGGCGCTCTCAATTGCTAGTGGTCTGGCACACCTTCATATGGAGATTGTTGGTACACAAGGTAA
ACCTGCTATTGCTCATCGAGACATAAAATCAAAGAATATCTTAGTGAAAAAGTGTGAAACTTGTGCCA
TAGCGGACTTAGGGTTGGCTGTGAAGCATGATTCAATACTGAACACTATCGACATACCTCAGAATCCT
AAAGTGGGAACCAAGAGGTATATGGCTCCTGAAATGCTTGATGATACAATGAATGTGAATATCTTTGA
GTCCTTCAAACGAGCTGACATCTATTCTGTTGGTCTGGTTTACTGGGAAATAGCCCGGAGGTGTTCAG
TCGGAGGAATTGTTGAGGAGTACCAATTGCCTTATTATGACATGGTGCCTTCAGATCCCTCGATAGAG
GAAATGAGAAAGGTTGTTTGTGACCAGAAGTTTCGACCAAGTATCCCAAACCAGTGGCAAAGTTGTGA
AGCACTCCGAGTCATGGGGAGAATAATGCGTGAGTGTTGGTATGCCAACGGAGCGGCCCGCCTAACTG
CTCTTCGTATTAAGAAGACTATATCTCAACTTTGTGTCAAAGAAGACTGCAAAGCCTAA   (SEQ
ID NO: 44)
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A nucleic acid sequence encoding the processed ALK7 polypeptide (isoform 4) is as follows (SEQ ID NO: 45):

GAGCTCTCGCCAGGACTGAAGTGTGTATGTCTTTTGTGTGATTCTTCAAACTTTACCTGCCAAACAGA
AGGAGCATGTTGGGCATCAGTCATGCTAACCAATGGAAAAGAGCAGGTGATCAAATCCTGTGTCTCCC
TTCCAGAACTGAATGCTCAAGTCTTCTGTCATAGTTCCAACAATGTTACCAAAACCGAATGCTGCTTC
ACAGATTTTTGCAACAACATAACACTGCACCTTCCAACAGATAATGGAACTTGGACTCAACTTTGGCT
GGTATCTGAATATCATGAACAGGGCTCCTTATATGACTATTTGAATAGAAATATAGTGACCGTGGCTG
GAATGATCAAGCTGGCGCTCTCAATTGCTAGTGGTCTGGCACACCTTCATATGGAGATTGTTGGTACA
CAAGGTAAACCTGCTATTGCTCATCGAGACATAAAATCAAAGAATATCTTAGTGAAAAAGTGTGAAAC
TTGTGCCATAGCGGACTTAGGGTTGGCTGTGAAGCATGATTCAATACTGAACACTATCGACATACCTC
AGAATCCTAAAGTGGGAACCAAGAGGTATATGGCTCCTGAAATGCTTGATGATACAATGAATGTGAAT
ATCTTTGAGTCCTTCAAACGAGCTGACATCTATTCTGTTGGTCTGGTTTACTGGGAAATAGCCCGGAG
GTGTTCAGTCGGAGGAATTGTTGAGGAGTACCAATTGCCTTATTATGACATGGTGCCTTCAGATCCCT
CGATAGAGGAAATGAGAAAGGTTGTTTGTGACCAGAAGTTTCGACCAAGTATCCCAAACCAGTGGCAA
AGTTGTGAAGCACTCCGAGTCATGGGGAGAATAATGCGTGAGTGTTGGTATGCCAACGGAGCGGCCCG
CCTAACTGCTCTTCGTATTAAGAAGACTATATCTCAACTTTGTGTCAAAGAAGACTGCAAAGCCTAA
(SEQ ID NO: 45)

Based on the signal sequence of full-length ALK7 (isoform 1) in the rat (see NCBI Reference Sequence NP_620790.1) and on the high degree of sequence identity between human and rat ALK7, it is predicted that a processed form of human ALK7 isoform 1 is as follows (SEQ ID NO: 46).

1 LKCVCLLCDS SNFTCQTEGA CWASVMLTNG KEQVIKSCVS LPELNAQVFC
HSSNNVTKTE

61 CCFTDFCNNI TLHLPTASPN APKLGPME (SEQ ID NO: 46)

Active variants of processed ALK7 isoform 1 are predicted in which SEQ ID NO: 10 is truncated by 1, 2, 3, 4, 5, 6, or 7 amino acids at the N-terminus and SEQ ID NO: 46 is truncated by 1 or 2 amino acids at the N-terminus. Consistent with SEQ ID NO: 46, it is further expected that leucine is the N-terminal amino acid in the processed forms of human ALK7 isoform 3 (SEQ ID NO: 39) and human ALK7 isoform 4 (SEQ ID NO: 43).

In certain embodiments, the disclosure relates to heteromultimers that comprise at least one ALK7 polypeptide, which includes fragments, functional variants, and modified forms thereof. Preferably, ALK7 polypeptides for use in accordance with inventions of the disclosure (*e.g.*, heteromultimers comprising an ALK7 polypeptide and uses thereof) are soluble (*e.g.*, an extracellular domain of ALK7). In other preferred embodiments, ALK7 polypeptides for use in accordance with the disclosure bind to one or more TGF-beta superfamily ligands. Therefore, in some preferred embodiments, ALK7 polypeptides for use

in accordance with the disclosure inhibit (antagonize) activity (*e.g.*, induction of Smad 2/3 and/or Smad 1/5/8 signaling) of one or more TGF-beta superfamily ligands. In some embodiments, heteromultimers of the disclosure comprise at least one ALK7 polypeptide that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO: 9, 10, 19, 20, 38, 39, 42, 43, 46, 74, 75, 79, or 80. In some embodiments, heteromultimer of the disclosure consist or consist essentially of at least one ALK7 polypeptide that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO: 9, 10, 19, 20, 38, 39, 42, 43, 46, 74, 75, 79, and 80.

ALK7 is well-conserved among vertebrates, with large stretches of the extracellular domain completely conserved. For example, Figure 7 depicts a multi-sequence alignment of a human ALK7 extracellular domain compared to various ALK7 orthologs. Accordingly, from these alignments, it is possible to predict key amino acid positions within the ligand-binding domain that are important for normal ALK7-ligand binding activities as well as to predict amino acid positions that are likely to be tolerant to substitution without significantly altering normal ALK7-ligand binding activities. Therefore, an active, human ALK7 variant polypeptide useful in accordance with the presently disclosed methods may include one or more amino acids at corresponding positions from the sequence of another vertebrate ALK7, or may include a residue that is similar to that in the human or other vertebrate sequences. Without meaning to be limiting, the following examples illustrate this approach to defining an active ALK7 variant. V61 in the human ALK7 extracellular domain (SEQ ID NO: 59) is isoleucine in *Callithrix jacchus* ALK7 (SEQ ID NO: 62), and so the position may be altered, and optionally may be altered to another hydrophobic residue such as L, I, or F, or a non-polar residue such as A. L32 in the human extracellular domain is R in *Tarsius syrichta* (SEQ ID NO: 63) ALK7, indicating that this site may be tolerant of a wide variety of changes, including polar residues, such as E, D, K, R, H, S, T, P, G, Y, and probably a non-polar residue such as A. K37 in the human extracellular domain is R in *Pan troglodytes* ALK7 (SEQ ID NO: 60), indicating that basic residues are tolerated at this position, including R, K, and H. P4 in the human extracellular domain is relatively poorly conserved, appearing as A in *Pan troglodytes* ALK7 thus indicating that a wide variety of amino acid should be tolerated at this position.

Moreover, ALK7 proteins have been characterized in the art in terms of structural and functional characteristics [e.g., Romano et al (2012) Journal of Molecular Modeling 18(8):

3617-3625]. For example, a defining structural motif known as a three-finger toxin fold is important for ligand binding by type I and type II receptors and is formed by conserved cysteine residues located at varying positions within the extracellular domain of each monomeric receptor [Greenwald et al. (1999) Nat Struct Biol 6:18-22; and Hinck (2012) FEBS Lett 586:1860-1870]. Accordingly, the core ligand-binding domains of human ALK7, as demarcated by the outermost of these conserved cysteines, corresponds to positions 28-92 of SEQ ID NO: 9. The structurally less-ordered amino acids flanking these cysteine-demarcated core sequences can be truncated by 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, or 27 residues at the N-terminus and by 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or 21 residues at the C-terminus without necessarily altering ligand binding. Exemplary ALK7 extracellular domains for N-terminal and/or C-terminal truncation include SEQ ID NOs: 10, 20, 39, and 43.

Accordingly, a general formula for an active portion (e.g., a ligand-binding portion) of ALK7 comprises amino acids 28-92. Therefore ALK7 polypeptides may, for example, comprise, consists essentially of, or consists of an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a portion of ALK7 beginning at a residue corresponding to any one of amino acids 20-28 (*e.g.*, beginning at any one of amino acids 20, 21, 22, 23, 24, 25, 26, 27, or 28) of SEQ ID NO: 9 and ending at a position corresponding to any one amino acids 92-113 (*e.g.*, ending at any one of amino acids 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, or 113) of SEQ ID NO: 9.

Other examples include constructs that begin at a position from 21-28 (*e.g.*, any one of positions 21, 22, 23, 24, 25, 26, 27, or 28), 24-28 (*e.g.*, any one of positions 24, 25, 26, 27, or 28), or 25-28 (*e.g.*, any one of positions 25, 26, 27, or 28) of SEQ ID NO: 9 and end at a position from 93-112 (*e.g.*, any one of positions 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, or 112), 93-110 (*e.g.*, any one of positions 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, or 110), 93-100 (*e.g.*, any one of positions 93, 94, 95, 96, 97, 98, 99, or 100), or 93-95 (*e.g.*, any one of positions 93, 94, or 95) of SEQ ID NO: 9. Variants within these ranges are also contemplated, particularly those having at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity to the corresponding portion of SEQ ID NO: 9.

The variations described herein may be combined in various ways. In some embodiments, ALK7 variants comprise no more than 1, 2, 5, 6, 7, 8, 9, 10 or 15 conservative amino acid changes in the ligand-binding pocket. Sites outside the binding pocket, at which variability may be particularly well tolerated, include the amino and carboxy termini of the extracellular domain (as noted above).

In certain aspects, the present disclosure relates to heteromultimers comprising one or more ALK7 receptor polypeptides (*e.g.*, SEQ ID Nos: 9, 10, 19, 20, 38, 39, 42, 43, 46, 74, 76, 79, and 80) and one or more ActRIIB receptor polypeptides (*e.g.*, SEQ ID NOs: 1, 2, 3, 4, 5, 6, 71, 73, 77, and 78), which are generally referred to herein as "ALK7:ActRIIB heteromultimer complexes" or "ALK7:ActRIIB heteromultimers". Preferably, ALK7:ActRIIB heteromultimers are soluble, *e.g.*, a heteromultimer comprises a soluble portion (domain) of an ALK7 receptor and a soluble portion (domain) of an ActRIIB receptor. In general, the extracellular domains of ALK7 and ActRIIB correspond to soluble portion of these receptors. Therefore, in some embodiments, heteromultimers comprise an extracellular domain of an ALK7 receptor and an extracellular domain of an ActRIIB receptor. Example extracellular domains of ALK7 and ActRIIB receptors are disclosed herein and such sequences, as well as fragments, functional variants, and modified forms thereof, may be used in accordance with the inventions of the disclosure (*e.g.*, ALK7:ActRIIB heteromultimer compositions and uses thereof). In some embodiments, ALK7:ActRIIB heteromultimers comprise at least one ALK7 polypeptide that comprises, consists essentially of, or consists of a sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94% 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 9, 10, 19, 20, 38, 39, 42, 43, 46, 74, 76, 79, and 80. In some embodiments, ALK7:ActRIIB heteromultimers comprise at least one ALK7 polypeptide that comprises, consists essentially of, consists of a sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94% 95%, 97%, 98%, 99%, or 100% identical to a portion of ALK7 beginning at a residue corresponding to any one of amino acids 21-28, 22-28, 23-28, 24-28, 25-28, 26-28, or 27-28 of SEQ ID NO: 9 and ending at a position from 93-112, 93, 110, 93-100, 93-95 of SEQ ID NO: 9. In some embodiments, ALK7:ActRIIB heteromultimers comprise at least one ALK7 polypeptide that comprises, consists essentially of, consists of a sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94% 95%, 97%, 98%, 99%, or 100% identical to amino acids 28-92 of SEQ ID NO: 9. In some embodiments, ALK7-ActRIIB heteromultimers comprise at least one

ActRIIB polypeptide that comprises, consists essentially of, consists of a sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94% 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 71, 73, 77, and 78. In some embodiments, ALK7:ActRIIB heteromultimers comprise at least one ActRIIB polypeptide that comprises, consists essentially of, consists of a sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94% 95%, 97%, 98%, 99%, or 100% identical to a portion of ActRIIB beginning at a residue corresponding to any one of amino acids 20-29, 20-24, 21-24, 22-25, or 21-29 and end at a position from 109-134, 119-134, 119-133, 129-134, or 129-133 of SEQ ID NO: 1. In some embodiments, ALK7:ActRIIB heteromultimers comprise at least one ActRIIB polypeptide that comprises, consists essentially of, or consists of a sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94% 95%, 97%, 98%, 99%, or 100% identical to amino acids 29-109 of SEQ ID NO: 1. In some embodiments, ALK7:ActRIIB heteromultimers comprise at least one ActRIIB polypeptide that comprises, consists essentially of, consists of a sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94% 95%, 97%, 98%, 99%, or 100% identical to amino acids 25-125 of SEQ ID NO: 1. In certain preferred embodiments, ALK7:ActRIIB heteromultimers comprise at least one ActRIIB polypeptide wherein the position corresponding to L79 of SEQ ID NO: 1 is not an acidic amino acid (*i.e.*, not naturally occurring D or E amino acids or an artificial acidic acid residue). ALK7:ActRIIB heteromultimers include, *e.g.*, heterodimers, heterotrimers, heterotetramers and further higher order oligomeric structures. See, e.g., Figures 1, 2, and 8-10. In certain preferred embodiments, heteromultimers of the disclosure are ALK7:ActRIIB heterodimers.

In some embodiments, the present disclosure contemplates making functional variants by modifying the structure of an ALK7 polypeptide and/or an ActRIIB polypeptide. Variants can be produced by amino acid substitution, deletion, addition, or combinations thereof. For instance, it is reasonable to expect that an isolated replacement of a leucine with an isoleucine or valine, an aspartate with a glutamate, a threonine with a serine, or a similar replacement of an amino acid with a structurally related amino acid (*e.g.*, conservative mutations) will not have a major effect on the biological activity of the resulting molecule. Conservative replacements are those that take place within a family of amino acids that are related in their side chains. Whether a change in the amino acid sequence of a polypeptide of the disclosure results in a functional homolog can be readily determined by assessing the ability of the

variant polypeptide to produce a response in cells in a fashion similar to the wild-type polypeptide, or to bind to one or more TGF-beta superfamily ligands including, for example, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty.

In some embodiments, the present disclosure contemplates making functional variants by modifying the structure of an ALK7 polypeptide and/or an ActRIIB polypeptide for such purposes as enhancing therapeutic efficacy or stability (*e.g.*, shelf-life and resistance to proteolytic degradation *in vivo*).

In some embodiments, the present disclosure contemplates specific mutations of an ALK7 polypeptide and/or an ActRIIB polypeptide 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 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 sulfhydryl 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. 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 a 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. 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.* [Meth. Enzymol. (1987) 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, heteromeric complexes of the present disclosure for use in humans may be expressed in a mammalian cell line that provides proper glycosylation, such as HEK293 or CHO cell lines, although other mammalian expression cell lines are expected to be useful as well.

The present disclosure further contemplates a method of generating mutants, particularly sets of combinatorial mutants of an ALK7 and/or an ActRIIB polypeptide as well as truncation mutants. Pools of combinatorial mutants are especially useful for identifying functionally active (e.g., TGF-beta superfamily ligand binding) ALK7 and/or ActRIIB sequences. The purpose of screening such combinatorial libraries may be to generate, for example, polypeptides variants which have altered properties, such as altered pharmacokinetic or altered ligand binding. A variety of screening assays are provided below, and such assays may be used to evaluate variants. For example, ALK7:ActRIIB complex variants may be screened for ability to bind to one or more TGF-beta superfamily ligands, to prevent binding of a TGF-beta superfamily ligand to a TGF-beta superfamily receptor, and/or to interfere with signaling caused by an TGF-beta superfamily ligand.

The activity of a ALK7:ActRIIB heteromultimer may be tested, for example, in a cell-based or *in vivo* assay. For example, the effect of an ALK7:ActRIIB heteromultimer on the expression of genes or the activity of proteins involved in fat metabolism, kidney damage, kidney inflammation, and/or kidney fibrosis may be assessed. This may, as needed, be performed in the presence of one or more recombinant TGF-beta superfamily ligand proteins, and cells may be transfected so as to produce an ALK7:ActRIIB heteromultimer, and optionally, a TGF-beta superfamily ligand. Likewise, an ALK7:ActRIIB heteromultimer may be administered to a mouse or other animal, and one or more measurements, such as fat metabolism or kidney damage, inflammation, fibrosis and/or function may be assessed using art-recognized methods. Similarly, the activity of an ALK7:ActRIIB heteromultimer, or variants thereof, may be tested in, for example, adipocytes and/or glomerular cells for any effect on growth of these cells, for example, by the assays as described herein and those of

common knowledge in the art. A SMAD-responsive reporter gene may be used in such cell lines to monitor effects on downstream signaling.

Combinatorial-derived variants can be generated which have increased selectivity or generally increased potency relative to a reference ALK7:ActRIIB heteromultimer. Such variants, when expressed from recombinant DNA constructs, can be used in gene therapy protocols. Likewise, mutagenesis can give rise to variants which have intracellular half-lives dramatically different than the corresponding unmodified ALK7:ActRIIB heteromultimer. For example, the altered protein can be rendered either more stable or less stable to proteolytic degradation or other cellular processes which result in destruction, or otherwise inactivation, of an unmodified polypeptide. Such variants, and the genes which encode them, can be utilized to alter polypeptide complex levels by modulating the half-life of the polypeptide. For instance, a short half-life can give rise to more transient biological effects and, when part of an inducible expression system, can allow tighter control of recombinant polypeptide complex levels within the cell. In an Fc fusion protein, mutations may be made in the linker (if any) and/or the Fc portion to alter one or more activities of the ALK7:ActRIIB heteromultimer including, for example, immunogenicity, half-life, and solubility.

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 ALK7 and/or ActRIIB sequences. For instance, a mixture of synthetic oligonucleotides can be enzymatically ligated into gene sequences such that the degenerate set of potential ALK7 and/or ActRIIB encoding 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 homologs 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 can then be ligated into an appropriate vector for expression. The synthesis of degenerate oligonucleotides is well known in the art [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; and Ike *et al.* (1983) Nucleic Acid Res. 11:477]. Such techniques have been employed in the directed evolution of other proteins [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, ALK7:ActRIIB heteromultimers can be generated and isolated from a library by screening using, for example, alanine scanning mutagenesis [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; and 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 [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 ALK7 and/or ActRIIB 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 ALK7:ActRIIB heteromultimers. The most widely used techniques for screening large gene libraries typically comprise 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 TGF-beta superfamily ligand (*e.g.*, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, activin BC, activin AE, activin BE, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty) binding assays and/or TGF-beta superfamily ligand-mediated cell signaling assays.

In certain embodiments, ALK7:ActRIIB heteromultimers may further comprise post-translational modifications in addition to any that are naturally present in the ALK7 and/or ActRIIB polypeptide. Such modifications include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation, and acylation. As a result, ALK7:ActRIIB heteromultimers may comprise non-amino acid elements, such as polyethylene glycols, lipids, polysaccharide or monosaccharide, and phosphates. Effects of such non-amino acid elements on the functionality of a heteromultimer complex may be tested as described herein for other heteromultimer variants. When a polypeptide of the disclosure 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 (*e.g.*, CHO, HeLa, MDCK, 293, WI38, NIH-3T3 or HEK293) 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 ALK7 and/or ActRIIB polypeptide as well as heteromultimers comprising the same.

In certain preferred embodiments, heteromultimers described herein comprise at least one ALK7 polypeptide associated, covalently or non-covalently, with at least one ActRIIB polypeptide. Preferably, polypeptides disclosed herein form heterodimeric complexes, although higher order heteromultimeric complexes are also included such as, but not limited to, heterotrimers, heterotetramers, and further oligomeric structures (see, e.g., Figure 1, 2, and 8-10). In some embodiments, ALK7 and/or ActRIIB polypeptides comprise at least one multimerization domain. As disclosed herein, the term "multimerization domain" refers to an amino acid or sequence of amino acids that promote covalent or non-covalent interaction between at least a first polypeptide and at least a second polypeptide. Polypeptides disclosed herein may be joined covalently or non-covalently to a multimerization domain. Preferably, a multimerization domain promotes interaction between a first polypeptide (*e.g.*, an ALK7 polypeptide) and a second polypeptide (*e.g.*, an ActRIIB polypeptide) to promote heteromultimer formation (*e.g.*, heterodimer formation), and optionally hinders or otherwise disfavors homomultimer formation (*e.g.*, homodimer formation), thereby increasing the yield of desired heteromultimer (see, e.g., Figure 2).

Many methods known in the art can be used to generate ALK7:ActRIIB heteromultimers. For example, non-naturally occurring disulfide bonds may be constructed by replacing on a first polypeptide (*e.g.*, an ALK7 polypeptide) a naturally occurring amino acid with a free thiol-containing residue, such as cysteine, such that the free thiol interacts

with another free thiol-containing residue on a second polypeptide (*e.g.*, an ActRIIB polypeptide) such that a disulfide bond is formed between the first and second polypeptides. Additional examples of interactions to promote heteromultimer formation include, but are not limited to, ionic interactions such as described in Kjaergaard *et al.*, WO2007147901; electrostatic steering effects such as described in Kannan *et al.*, U.S.8,592,562; coiled-coil interactions such as described in Christensen *et al.*, U.S.20120302737; leucine zippers such as described in Pack & Plueckthun,(1992) Biochemistry 31: 1579-1584; and helix-turn-helix motifs such as described in Pack *et al.*, (1993) Bio/Technology 11: 1271-1277. Linkage of the various segments may be obtained via, *e.g.*, covalent binding such as by chemical cross-linking, peptide linkers, disulfide bridges, *etc.*, or affinity interactions such as by avidin-biotin or leucine zipper technology.

In certain aspects, a multimerization domain may comprise one component of an interaction pair. In some embodiments, the polypeptides disclosed herein may form protein complexes comprising a first polypeptide covalently or non-covalently associated with a second polypeptide, wherein the first polypeptide comprises the amino acid sequence of an ALK7 polypeptide and the amino acid sequence of a first member of an interaction pair; and the second polypeptide comprises the amino acid sequence of an ActRIIB polypeptide and the amino acid sequence of a second member of an interaction pair. The interaction pair may be any two polypeptide sequences that interact to form a complex, particularly a heterodimeric complex although operative embodiments may also employ an interaction pair that can form a homodimeric complex. One member of the interaction pair may be fused to an ALK7 or ActRIIB polypeptide as described herein, including for example, a polypeptide sequence comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence of any one of SEQ ID NOs: 2, 3, 5, 6, 10, 20, 39, 43, and 46. An interaction pair may be selected to confer an improved property/activity such as increased serum half-life, or to act as an adaptor on to which another moiety is attached to provide an improved property/activity. For example, a polyethylene glycol moiety may be attached to one or both components of an interaction pair to provide an improved property/activity such as improved serum half-life.

The first and second members of the interaction pair may be an asymmetric pair, meaning that the members of the pair preferentially associate with each other rather than self-associate. Accordingly, first and second members of an asymmetric interaction pair may

associate to form a heterodimeric complex (see, e.g., Figure 2). Alternatively, the interaction pair may be unguided, meaning that the members of the pair may associate with each other or self-associate without substantial preference and thus may have the same or different amino acid sequences. Accordingly, first and second members of an unguided interaction pair may associate to form a homodimer complex or a heterodimeric complex. Optionally, the first member of the interaction pair (*e.g.*, an asymmetric pair or an unguided interaction pair) associates covalently with the second member of the interaction pair. Optionally, the first member of the interaction pair (*e.g.*, an asymmetric pair or an unguided interaction pair) associates non-covalently with the second member of the interaction pair.

As specific examples, the present disclosure provides fusion proteins comprising ALK7 or ActRIIB fused to a polypeptide comprising a constant domain of an immunoglobulin, such as a CH1, CH2, or CH3 domain of an immunoglobulin or an Fc domain. Fc domains derived from human IgG1, IgG2, IgG3, and IgG4 are provided herein. Other mutations are known that decrease either CDC or ADCC activity, and collectively, any of these variants are included in the disclosure and may be used as advantageous components of a heteromultimeric complex of the disclosure. Optionally, the IgG1 Fc domain of SEQ ID NO: 31 has one or more mutations at residues such as Asp-265, Lys-322, and Asn-434 (numbered in accordance with the corresponding full-length IgG1). 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 Fcγ 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.

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: 31). Dotted underline indicates the hinge region, and solid underline indicates positions with naturally occurring variants. In part, the disclosure provides polypeptides comprising, consisting essentially 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: 31. Naturally occurring variants in G1Fc would include E134D and M136L according to the numbering system used in SEQ ID NO: 31 (see Uniprot P01857).

```
  1  THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE
 51  VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK
```

101 VSNKALPAPI EKTISKAKGQ PREPQVYTLP PSREEMTKNQ VSLTCLVKGF
151 YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSKLTV DKSRWQQGNV
201 FSCSVMHEAL HNHYTQKSLS LSPGK (SEQ ID NO: 31)

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: 32). 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: 32.

1 VECPPCPAPP VAGPSVFLFP PKPKDTLMIS RTPEVTCVVV DVSHEDPEVQ
51 FNWYVDGVEV HNAKTKPREE QFNSTFRVVS VLTVVHQDWL NGKEYKCKVS
101 NKGLPAPIEK TISKTKGQPR EPQVYTLPPS REEMTKNQVS LTCLVKGFYP
151 SDIAVEWESN GQPENNYKTT PPMLDSDGSF FLYSKLTVDK SRWQQGNVFS
201 CSVMHEALHN HYTQKSLSLS PGK (SEQ ID NO: 32)

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: 33) contains a short hinge region consisting of a single 15-residue segment, whereas the second G3Fc sequence (SEQ ID NO: 34) 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%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NOs: 33 and 34.

1 EPKSCDTPPP CPRCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD
51 VSHEDPEVQF KWYVDGVEVH NAKTKPREEQ YNSTFRVVSV LTVLHQDWLN
101 GKEYKCKVSN KALPAPIEKT ISKTKGQPRE PQVYTLPPSR EEMTKNQVSL
151 TCLVKGFYPS DIAVEWESSG QPENNYNTTP PMLDSDGSFF LYSKLTVDKS
201 RWQQGNIFSC SVMHEALHNR FTQKSLSLSP GK (SEQ ID NO: 33)

1 ELKTPLGDTT HTCPRCPEPK SCDTPPPCPR CPEPKSCDTP PPCPRCPEPK
51 SCDTPPPCPR CPAPELLGGP SVFLFPPKPK DTLMISRTPE VTCVVVDVSH
101 EDPEVQFKWY VDGVEVHNAK TKPREEQYNS TFRVVSVLTV LHQDWLNGKE

```
151  YKCKVSNKAL PAPIEKTISK TKGQPREPQV YTLPPSREEM TKNQVSLTCL
201  VKGFYPSDIA VEWESSGQPE NNYNTTPPML DSDGSFFLYS KLTVDKSRWQ
251  QGNIFSCSVM HEALHNRFTQ KSLSLSPGK        (SEQ ID NO: 34)
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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: 33, 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 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.

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: 35). 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: 35.

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  1  ESKYGPPCPS CPAPEFLGGP SVFLFPPKPK DTLMISRTPE VTCVVVDVSQ
 51  EDPEVQFNWY VDGVEVHNAK TKPREEQFNS TYRVVSVLTV LHQDWLNGKE
101  YKCKVSNKGL PSSIEKTISK AKGQPREPQV YTLPPSQEEM TKNQVSLTCL
151  VKGFYPSDIA VEWESNGQPE NNYKTTPPVL DSDGSFFLYS RLTVDKSRWQ
201  EGNVFSCSVM HEALHNHYTQ KSLSLSLGK      (SEQ ID NO: 35)
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A variety of engineered mutations in the Fc domain are presented herein with respect to the G1Fc sequence (SEQ ID NO: 31), and analogous mutations in G2Fc, G3Fc, and G4Fc can be derived from their alignment with G1Fc in Figure 5. Due to unequal hinge lengths, analogous Fc positions based on isotype alignment (Figure 5) possess different amino acid numbers in SEQ ID NOs: 31, 32, 33, 34, and 35. It can also be appreciated that a given amino acid position in an immunoglobulin sequence consisting of hinge, C_H2, and C_H3 regions (*e.g.*, SEQ ID NOs: 31, 32, 33, 34, and 35) will be identified by a different number than the same position when numbering encompasses the entire IgG1 heavy-chain constant

domain (consisting of the C_H1, hinge, C_H2, and C_H3 regions) as in the Uniprot database. For example, correspondence between selected C_H3 positions in a human G1Fc sequence (SEQ ID NO: 31), the human IgG1 heavy chain constant domain (Uniprot P01857), and the human IgG1 heavy chain is as follows.

Correspondence of C_H3 Positions in Different Numbering Systems		
G1Fc (Numbering begins at first threonine in hinge region)	IgG1 heavy chain constant domain (Numbering begins at C_H1)	IgG1 heavy chain (EU numbering scheme of Kabat et al., 1991*)
Y127	Y232	Y349
S132	S237	S354
E134	E239	E356
T144	T249	T366
L146	L251	L368
K170	K275	K392
D177	D282	D399
Y185	Y290	Y407
K187	K292	K409
* Kabat et al. (eds) 1991; pp. 688-696 in *Sequences of Proteins of Immunological Interest*, 5th ed., Vol. 1, NIH, Bethesda, MD.		

A problem that arises in large-scale production of asymmetric immunoglobulin-based proteins from a single cell line is known as the "chain association issue". As confronted prominently in the production of bispecific antibodies, the chain association issue concerns the challenge of efficiently producing a desired multichain protein from among the multiple combinations that inherently result when different heavy chains and/or light chains are produced in a single cell line [Klein et al (2012) mAbs 4:653-663]. This problem is most acute when two different heavy chains and two different light chains are produced in the same cell, in which case there are a total of 16 possible chain combinations (although some of these are identical) when only one is typically desired. Nevertheless, the same principle accounts for diminished yield of a desired multichain fusion protein that incorporates only two different (asymmetric) heavy chains.

Various methods are known in the art that increase desired pairing of Fc-containing fusion polypeptide chains in a single cell line to produce a preferred asymmetric fusion

protein at acceptable yields [Klein et al (2012) mAbs 4:653-663; and Spiess et al (2015) Molecular Immunology 67(2A): 95-106]. Methods to obtain desired pairing of Fc-containing chains include, but are not limited to, charge-based pairing (electrostatic steering), "knobs-into-holes" steric pairing, SEEDbody pairing, and leucine zipper-based pairing [Ridgway et al (1996) Protein Eng 9:617-621; Merchant et al (1998) Nat Biotech 16:677-681; Davis et al (2010) Protein Eng Des Sel 23:195-202; Gunasekaran et al (2010); 285:19637-19646; Wranik et al (2012) J Biol Chem 287:43331-43339; US5932448; WO 1993/011162; WO 2009/089004, and WO 2011/034605]. As described herein, these methods may be used to generate ALK7-Fc:ActRIIB-Fc heteromultimer complexes. See Figures 8-10.

For example, one means by which interaction between specific polypeptides may be promoted is by engineering protuberance-into-cavity (knob-into-holes) complementary regions such as described in Arathoon *et al.*, U.S.7,183,076 and Carter *et al.*, U.S.5,731,168. "Protuberances" are constructed by replacing small amino acid side chains from the interface of the first polypeptide (*e.g.*, a first interaction pair) with larger side chains (*e.g.*, tyrosine or tryptophan). Complementary "cavities" of identical or similar size to the protuberances are optionally created on the interface of the second polypeptide (*e.g.*, a second interaction pair) by replacing large amino acid side chains with smaller ones (*e.g.*, alanine or threonine). Where a suitably positioned and dimensioned protuberance or cavity exists at the interface of either the first or second polypeptide, it is only necessary to engineer a corresponding cavity or protuberance, respectively, at the adjacent interface.

At neutral pH (7.0), aspartic acid and glutamic acid are negatively charged and lysine, arginine, and histidine are positively charged. These charged residues can be used to promote heterodimer formation and at the same time hinder homodimer formation. Attractive interactions take place between opposite charges and repulsive interactions occur between like charges. In part, protein complexes disclosed herein make use of the attractive interactions for promoting heteromultimer formation (*e.g.*, heterodimer formation), and optionally repulsive interactions for hindering homodimer formation (*e.g.*, homodimer formation) by carrying out site directed mutagenesis of charged interface residues.

For example, the IgG1 CH3 domain interface comprises four unique charge residue pairs involved in domain-domain interactions: Asp356-Lys439', Glu357-Lys370', Lys392-Asp399', and Asp399-Lys409' [residue numbering in the second chain is indicated by (')]. It should be noted that the numbering scheme used here to designate residues in the IgG1 CH3

domain conforms to the EU numbering scheme of Kabat. Due to the 2-fold symmetry present in the CH3-CH3 domain interactions, each unique interaction will represented twice in the structure (*e.g.*, Asp-399-Lys409' and Lys409-Asp399'). In the wild-type sequence, K409-D399' favors both heterodimer and homodimer formation. A single mutation switching the charge polarity (*e.g.*, K409E; positive to negative charge) in the first chain leads to unfavorable interactions for the formation of the first chain homodimer. The unfavorable interactions arise due to the repulsive interactions occurring between the same charges (negative-negative; K409E-D399' and D399-K409E'). A similar mutation switching the charge polarity (D399K'; negative to positive) in the second chain leads to unfavorable interactions (K409'-D399K' and D399K-K409') for the second chain homodimer formation. But, at the same time, these two mutations (K409E and D399K') lead to favorable interactions (K409E-D399K' and D399-K409') for the heterodimer formation.

The electrostatic steering effect on heterodimer formation and homodimer discouragement can be further enhanced by mutation of additional charge residues which may or may not be paired with an oppositely charged residue in the second chain including, for example, Arg355 and Lys360. The table below lists possible charge change mutations that can be used, alone or in combination, to enhance ALK7:ActRIIB heteromultimer formation.

Examples of Pair-Wise Charged Residue Mutations to Enhance Heterodimer Formation			
Position in first chain	Mutation in first chain	Interacting position in second chain	Corresponding mutation in second chain
Lys409	Asp or Glu	Asp399'	Lys, Arg, or His
Lys392	Asp or Glu	Asp399'	Lys, Arg, or His
Lys439	Asp or Glu	Asp356'	Lys, Arg, or His
Lys370	Asp or Glu	Glu357'	Lys, Arg, or His
Asp399	Lys, Arg, or His	Lys409'	Asp or Glu
Asp399	Lys, Arg, or His	Lys392'	Asp or Glu
Asp356	Lys, Arg, or His	Lys439'	Asp or Glu
Glu357	Lys, Arg, or His	Lys370'	Asp or Glu

In some embodiments, one or more residues that make up the CH3-CH3 interface in a fusion protein of the instant application are replaced with a charged amino acid such that the

interaction becomes electrostatically unfavorable. For example, a positive-charged amino acid in the interface (*e.g.*, a lysine, arginine, or histidine) is replaced with a negatively charged amino acid (*e.g.*, aspartic acid or glutamic acid). Alternatively, or in combination with the forgoing substitution, a negative-charged amino acid in the interface is replaced with a positive-charged amino acid. In certain embodiments, the amino acid is replaced with a non-naturally occurring amino acid having the desired charge characteristic. It should be noted that mutating negatively charged residues (Asp or Glu) to His will lead to increase in side chain volume, which may cause steric issues. Furthermore, His proton donor- and acceptor-form depends on the localized environment. These issues should be taken into consideration with the design strategy. Because the interface residues are highly conserved in human and mouse IgG subclasses, electrostatic steering effects disclosed herein can be applied to human and mouse IgG1, IgG2, IgG3, and IgG4. This strategy can also be extended to modifying uncharged residues to charged residues at the CH3 domain interface.

In part, the disclosure provides desired pairing of asymmetric Fc-containing polypeptide chains using Fc sequences engineered to be complementary on the basis of charge pairing (electrostatic steering). One of a pair of Fc sequences with electrostatic complementarity can be arbitrarily fused to the ALK7 or ActRIIB polypeptide of the construct, with or without an optional linker, to generate an ALK7:ActRIIB heteromultimer. This single chain can be coexpressed in a cell of choice along with the Fc sequence complementary to the first Fc to favor generation of the desired multichain construct (e.g., ALK7:ActRIIB heteromultimer). In this example based on electrostatic steering, SEQ ID NO: 23 [human G1Fc(E134K/D177K)] and SEQ ID NO: 24 [human G1Fc(K170D/K187D)] are examples of complementary Fc sequences in which the engineered amino acid substitutions are double underlined, and the TGF-beta superfamily type I or type II receptor polypeptide of the construct can be fused to either SEQ ID NO: 23 or SEQ ID NO: 24, but not both. Given the high degree of amino acid sequence identity between native hG1Fc, native hG2Fc, native hG3Fc, and native hG4Fc, it can be appreciated that amino acid substitutions at corresponding positions in hG2Fc, hG3Fc, or hG4Fc (see Figure 5) will generate complementary Fc pairs which may be used instead of the complementary hG1Fc pair below (SEQ ID NOs: 23 and 24).

```
  1  THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE
 51  VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK
101  VSNKALPAPI EKTISKAKGQ PREPQVYTLP PSRKEMTKNQ VSLTCLVKGF
```

```
151  YPSDIAVEWE SNGQPENNYK TTPPVLKSDG SFFLYSKLTV DKSRWQQGNV
201  FSCSVMHEAL HNHYTQKSLS LSPGK        (SEQ ID NO: 23)

  1  THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE
 51  VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK
101  VSNKALPAPI EKTISKAKGQ PREPQVYTLP PSREEMTKNQ VSLTCLVKGF
151  YPSDIAVEWE SNGQPENNYD TTPPVLDSDG SFFLYSDLTV DKSRWQQGNV
201  FSCSVMHEAL HNHYTQKSLS LSPGK        (SEQ ID NO: 24)
```

In part, the disclosure provides desired pairing of asymmetric Fc-containing polypeptide chains using Fc sequences engineered for steric complementarity. In part, the disclosure provides knobs-into-holes pairing as an example of steric complementarity. One of a pair of Fc sequences with steric complementarity can be arbitrarily fused to the ALK7 or ActRIIB polypeptide of the construct, with or without an optional linker, to generate an ALK7:ActRIIB heteromultimer. This single chain can be co-expressed in a cell of choice along with the Fc sequence complementary to the first Fc to favor generation of the desired multi-chain construct. In this example based on knobs-into-holes pairing, SEQ ID NO: 25 [human G1Fc(T144Y)] and SEQ ID NO: 26 [human G1Fc(Y185T)] are examples of complementary Fc sequences in which the engineered amino acid substitutions are double underlined, and the ALK7 or ActRIIB polypeptide of the construct can be fused to either SEQ ID NO: 25 or SEQ ID NO: 26, but not both. Given the high degree of amino acid sequence identity between native hG1Fc, native hG2Fc, native hG3Fc, and native hG4Fc, it can be appreciated that amino acid substitutions at corresponding positions in hG2Fc, hG3Fc, or hG4Fc (see Figure 5) will generate complementary Fc pairs which may be used instead of the complementary hG1Fc pair below (SEQ ID NOs: 25 and 26).

```
  1  THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE
 51  VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK
101  VSNKALPAPI EKTISKAKGQ PREPQVYTLP PSREEMTKNQ VSLYCLVKGF
151  YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSKLTV DKSRWQQGNV
201  FSCSVMHEAL HNHYTQKSLS LSPGK          (SEQ ID NO: 25)

  1  THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE
 51  VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK
101  VSNKALPAPI EKTISKAKGQ PREPQVYTLP PSREEMTKNQ VSLTCLVKGF
151  YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLTSKLTV DKSRWQQGNV
201  FSCSVMHEAL HNHYTQKSLS LSPGK          (SEQ ID NO: 26)
```

An example of Fc complementarity based on knobs-into-holes pairing combined with an engineered disulfide bond is disclosed in SEQ ID NO: 27 [hG1Fc(S132C/T144W)] and SEQ ID NO: 28 [hG1Fc(Y127C/T144S/L146A/Y185V)]. The engineered amino acid substitutions in these sequences are double underlined, and the TGF-beta superfamily type I or type II polypeptide of the construct can be fused to either SEQ ID NO: 27 or SEQ ID NO: 28, but not both. Given the high degree of amino acid sequence identity between native hG1Fc, native hG2Fc, native hG3Fc, and native hG4Fc, it can be appreciated that amino acid substitutions at corresponding positions in hG2Fc, hG3Fc, or hG4Fc (see Figure 5) will generate complementary Fc pairs which may be used instead of the complementary hG1Fc pair below (SEQ ID NOs: 27 and 28).

```
  1  THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE
 51  VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK
101  VSNKALPAPI EKTISKAKGQ PREPQVYTLP PCREEMTKNQ VSLWCLVKGF
151  YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSKLTV DKSRWQQGNV
201  FSCSVMHEAL HNHYTQKSLS LSPGK          (SEQ ID NO: 27)

  1  THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE
 51  VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK
101  VSNKALPAPI EKTISKAKGQ PREPQVCTLP PSREEMTKNQ VSLSCAVKGF
151  YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLVSKLTV DKSRWQQGNV
201  FSCSVMHEAL HNHYTQKSLS LSPGK          (SEQ ID NO: 28)
```

In part, the disclosure provides desired pairing of asymmetric Fc-containing polypeptide chains using Fc sequences engineered to generate interdigitating β-strand segments of human IgG and IgA C_H3 domains. Such methods include the use of strand-exchange engineered domain (SEED) C_H3 heterodimers allowing the formation of SEEDbody fusion proteins [Davis et al. (2010) Protein Eng Design Sel 23:195-202]. One of a pair of Fc sequences with SEEDbody complementarity can be arbitrarily fused to the ALK7 or ActIIB of the construct, with or without an optional linker, to generate an ALK7 or ActRIIB fusion polypeptide. This single chain can be co-expressed in a cell of choice along with the Fc sequence complementary to the first Fc to favor generation of the desired multi-chain construct. In this example based on SEEDbody (Sb) pairing, SEQ ID NO: 29 [hG1Fc(Sb_{AG})] and SEQ ID NO: 30 [hG1Fc(Sb_{GA})] are examples of complementary IgG Fc sequences in which the engineered amino acid substitutions from IgA Fc are double underlined, and the ALK7 or ActRIIB polypeptide of the construct can be fused to either SEQ ID NO: 29 or SEQ ID NO: 30, but not both. Given the high degree of amino acid sequence identity between native hG1Fc, native hG2Fc, native hG3Fc, and native hG4Fc, it can

be appreciated that amino acid substitutions at corresponding positions in hG1Fc, hG2Fc, hG3Fc, or hG4Fc (see Figure 5) will generate an Fc monomer which may be used in the complementary IgG-IgA pair below (SEQ ID NOs: 29 and 30).

```
  1  THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE
 51  VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK
101  VSNKALPAPI EKTISKAKGQ PFRPEVHLLP PSREEMTKNQ VSLTCLARGF
151  YPKDIAVEWE SNGQPENNYK TTPSRQEPSQ GTTTFAVTSK LTVDKSRWQQ
201  GNVFSCSVMH EALHNHYTQK TISLSPGK     (SEQ ID NO: 29)

  1  THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE
 51  VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK
101  VSNKALPAPI EKTISKAKGQ PREPQVYTLP PPSEELALNE LVTLTCLVKG
151  FYPSDIAVEW ESNGQELPRE KYLTWAPVLD SDGSFFLYSI LRVAAEDWKK
201  GDTFSCSVMH EALHNHYTQK SLDRSPGK     (SEQ ID NO: 30)
```

In part, the disclosure provides desired pairing of asymmetric Fc-containing polypeptide chains with a cleavable leucine zipper domain attached at the C-terminus of the Fc C_H3 domains. Attachment of a leucine zipper is sufficient to cause preferential assembly of heterodimeric antibody heavy chains [Wranik et al (2012) J Biol Chem 287:43331-43339]. As disclosed herein, one of a pair of Fc sequences attached to a leucine zipper-forming strand can be arbitrarily fused to the ALK7 or ActRIIB polypeptide of the construct, with or without an optional linker, to generate an ALK7 or ActRIIB fusion polypeptide. This single chain can be co-expressed in a cell of choice along with the Fc sequence attached to a complementary leucine zipper-forming strand to favor generation of the desired multi-chain construct. Proteolytic digestion of the construct with the bacterial endoproteinase Lys-C post purification can release the leucine zipper domain, resulting in an Fc construct whose structure is identical to that of native Fc. In this example based on leucine zipper pairing, SEQ ID NO: 36 [hG1Fc-Ap1 (acidic)] and SEQ ID NO: 37 [hG1Fc-Bp1 (basic)] are examples of complementary IgG Fc sequences in which the engineered complimentary leucine zipper sequences are underlined, and the ALK7 or ActRIIB polypeptide of the construct can be fused to either SEQ ID NO: 36 or SEQ ID NO: 37, but not both. Given the high degree of amino acid sequence identity between native hG1Fc, native hG2Fc, native hG3Fc, and native hG4Fc, it can be appreciated that leucine zipper-forming sequences attached, with or without an optional linker, to hG1Fc, hG2Fc, hG3Fc, or hG4Fc (see Figure 5) will generate an Fc monomer which may be used in the complementary leucine zipper-forming pair below (SEQ ID NOs: 36 and 37).

```
  1 THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE
 51 VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK
101 VSNKALPAPI EKTISKAKGQ PREPQVYTLP PSREEMTKNQ VSLTCLVKGF
151 YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSKLTV DKSRWQQGNV
201 FSCSVMHEAL HNHYTQKSLS LSPGKGGSAQ LEKELQALEK ENAQLEWELQ
251 ALEKELAQGA T           (SEQ ID NO: 36)

  1 THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE
 51 VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK
101 VSNKALPAPI EKTISKAKGQ PREPQVYTLP PSREEMTKNQ VSLTCLVKGF
151 YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSKLTV DKSRWQQGNV
201 FSCSVMHEAL HNHYTQKSLS LSPGKGGSAQ LKKKLQALKK KNAQLKWKLQ
251 ALKKKLAQGA T           (SEQ ID NO: 37)
```

As described above, various methods are known in the art that increase desired pairing of Fc-containing fusion polypeptide chains in a single cell line to produce a preferred asymmetric fusion protein at acceptable yields [Klein et al (2012) mAbs 4:653-663; and Spiess et al (2015) Molecular Immunology 67(2A): 95-106]. In addition, ALK7:ActRIIB heteromultimers may be generated using a combination of heavy and light chain fusion proteins comprising either an ALK7 or ActRIIB polypeptide. For example, in some embodiments, an ALK7 polypeptide may be fused, with or without a linker domain, to an immunoglobulin heavy chain (IgG1, IgG2, IgG3, IgG4, IgM, IgA1, or IgA2) that comprises at least a portion of the C_H1 domain. Similarly, an ActRIIB polypeptide may be fused, with or without a linker domain, to an immunoglobulin light chain (kappa or lambda) that comprises at least a portion of the light chain constant domain (C_L). In alternative embodiments, an ActRIIB polypeptide may be fused, with or without a linker domain, to an immunoglobulin heavy chain (IgG1, IgG2, IgG3, IgG4, IgM, IgA1, or IgA2) that comprises at least a portion of the C_H1 domain, and an ALK7 polypeptide may be fused, with or without a linker domain, to an immunoglobulin light chain (kappa or lambda) that comprises at least a portion of the light chain constant domain (C_L). This design takes advantage of the natural ability of the heavy chains to heterodimerize with light chains. In particular, heterodimerization of a heavy and light chain occurs between the C_H1 with the C_L, which is generally stabilized by covalent linking of the two domains via a disulfide bridge. Constructs employing the full-length heavy chain, or at least a portion of the heavy chain comprising the hinge region, could give rise to antibody-like molecules comprising two "light chains" and two "heavy chains". See Figure 9. A potential advantage of this design is that it may more

closely mimic the naturally occurring ALK7-ligand-ActRIIB complex and may display higher affinity for the ligand than comparable single heterodimers. In some embodiments, this design may be modified by incorporating various heavy chain truncations including, for example, truncations that comprise the C_H1 domain and some or all of the hinge domain (giving rise to F(ab')$_2$-like molecules) as well as truncations that only comprise the C_H1 domain or a fragment thereof (giving rise to Fab-like molecules). See Figure 9G. Various methods for designing such heteromultimer constructs are described in US 2009/0010879, Klein et al [(2012) mAbs 4:653-663], and Spiess et al [(2015) Molecular Immunology 67(2A): 95-106] the contents of which are incorporated in their entirety herein.

In some embodiments, it is desirable to generate antibody-like ALK7:ActRIIB heterodimers comprising at least one branch of the complex comprising an ALK7-C_L:ActRIIB-C_H1 heterodimer pair and at least a second branch comprising an ActRIIB-C_L:ALK7-C_H1 heterodimer pair. See, e.g., Figure 9B. Such heterodimer complexes can be generated, for example, using combinations of heavy chain and light chain asymmetrical pairing technologies [Spiess et al (2015) Molecular Immunology 67(2A): 95-106]. For example, in CrossMab technology, [Schaefer et al (2011). Proc. Natl. Acad. Sci. U.S.A. 108: 11187–11192] light chain mispairing is overcome using domain crossovers and heavy chains heterodimerized using knobs-into-holes [Merchant et al (1998) Nat. Biotechnol. 16: 677–681]. For the domain crossovers either the variable domains or the constant domains are swapped between light and heavy chains to create two asymmetric Fab arms that drive cognate light chain pairing while preserving the structural and functional integrity of the variable domain [Fenn et al (2013) PLoS ONE 8: e61953]. An alternative approach for overcoming light chain mispairing is designing heavy and light chains with orthogonal Fab inter-faces [Lewis (2014) Nat. Biotechnol. 32: 191–198]. This has been accomplished by computational modeling [Das et al (2008) Annu. Rev. Biochem.77: 363–382] in combination with X-ray crystallography to identify mutations at the V_H/V_L and C_H1/C_L interfaces. For the heterodimers generated using this methodology, it may be necessary to engineer mutations into both V_H/V_L and C_H1/C_L interfaces to minimize heavy/light chain mispairing. The designed orthogonal Fab interface may be used in conjunction with a heavy chain heterodimerization strategy to facilitate efficient IgG production in a single host cell. Electrostatic steering may also be used to generate orthogonal Fab interfaces to facilitate the construction of such heterodimers. Peptide linkers may be used to ensure cognate pairing of light and heavy chains in a format known as "LUZ-Y" [Wranik et al (2012) J. Biol. Chem.

287: 43331-43339], wherein heavy chain heterodimerization is accomplished using leucine zippers which may be subsequently removed by proteolysis in vitro.

Alternatively, ALK7:ActRIIB heteromultimers may comprise one or more single-chain ligand traps as described herein, optionally which may be covalently or non-covalently associated with one or more ALK7 or ActRIIB polypeptides as well as additional ALK7:ActRIIB single chain ligand traps [US 2011/0236309 and US2009/0010879]. See Figure 12. As described herein, single-chain ligand traps do not require fusion to any multimerization domain such as coiled-coil Fc domains to be multivalent. In general, single-chain ligand traps of the present disclosure comprise at least one ALK7 polypeptide domain and one ActRIIB polypeptide domain. The ALK7 and ActRIIB polypeptide domains, generally referred to herein as binding domains (BD), optionally may be joined by a linker region.

For example, in one aspect, the present disclosure provides heteromultimers comprising a polypeptide having the following structure:

$$(<BD1>\text{-linker1})_k\text{-}[<BD2>\text{-linker2-}\{<BD3>\text{-linker3}\}_f]_n\text{-}(<BD4>)_m\text{-}(\text{linker4-}BD5>_d)_h$$

where: n and h are independently greater than or equal to one; d, f, m, and k are independently equal to or greater than zero; BD1, BD2, BD3, BD4, and BD5 are independently ALK7 or ActRIIB polypeptide domains, wherein at least one of BD1, BD2, BD3, and BD4 is an ALK7 polypeptide domain, and wherein at least one of BD1, BD2, BD3, and BD4 is an ActRIIB polypeptide domain, and linker1, linker2, linker3, and linker 4 are independently greater than or equal to zero. In some embodiment, ALK7:ActRIIB single-chain traps comprise at least two different ALK7 polypeptides. In some embodiments, ALK7:ActRIIB single-chain traps comprise at least two different ActRIIB polypeptides. In some embodiment, ALK7:ActRIIB single-chain traps comprise at least two different linkers. Depending on the values of selected for d, f, h, k, m, and n, the heteromultimer structure may comprise a large number of repeating units in various combinations or may be a relatively simple structure.

In another aspect, the present disclosure provides heteromultimers comprising a polypeptide having the following structure:

<BD1>-linker1-<BD2>

In yet another aspect, the present disclosure provides heteromultimers comprising a polypeptide having the following structure:

<BD1>-(linker2-<BD2>)$_n$

where n is greater than or equal one.

Another aspect of the invention provides heteromultimers comprising a polypeptide having the following structure:

(<BD1>-linker1-<BD1>)$_f$-linker2-(<BD2>-linker3-<BD3>)$_g$

wherein f and g are greater than or equal to one.

In an embodiment where BD2 and BD3 are the same, and f and g are the same number, this can result in a substantially mirror symmetric structure around linker 2, subject to differences in the linkers. In instances where BD2 is different from BD3 and/or where f and g are different numbers, different structures will be produced. It is within the capacity of one of ordinary skill in the art to select suitable binding domains, linkers, and repeat frequencies in light of the disclosure herein and knowledge in the art. Specific, non-limiting examples of such single-chain ligand traps in accordance with the present disclosure are represented schematically in Figure 11.

The linkers (1, 2, 3, and 4) may be the same or different. The linker region provides a segment that is distinct from the structured ligand-binding domains of ALK7 and ActRIIB and thus can be used for conjugation to accessory molecules (e.g., molecules useful in increasing stability such as PEGylation moieties) without having to chemically modify the binding domains. The linker may include an unstructured amino acid sequence that may be either the same as or derived from conservative modifications to the sequence of a natural unstructured region in the extracellular portion of the receptor for the ligand of interest or another receptor in the TGF-β superfamily. In other instances, such linkers may be entirely artificial in composition and origin but will contain amino acids selected to provide an unstructured flexible linker with a low likelihood of encountering electrostatic or steric hindrance complications when brought into close proximity to the ligand of interest. Linker length will be considered acceptable when it permits binding domains located on each of the N- and C-termini of the linker to bind their natural binding sites on their natural ligand such that, with both binding domains so bound, the ligand is bound with a higher affinity than it would be bound by binding of only one of the binding domains. In some instances, the number of amino acid residues in the linker of either natural or artificial origin is selected to be equal to or greater than the minimum required distance for simultaneous (bridged) binding to two binding sites on the ALK7 and/or ActRIIB ligand. For example, and without wishing

to be limiting in any manner, the linker length may be between about 1- 10 amino acids, 10-20 amino acids, 18-80 amino acids, 25-60 amino acids, 35-45 amino acids, or any other suitable length.

Linkers may be designed to facilitate purification of the polypeptide. The exact purification scheme chosen will determine what modifications are needed, for example and without wishing to be limiting, additions of purification "tags" such as His tags is contemplated; in other examples, the linker may include regions to facilitate the addition of cargo or accessory molecules. When such additions affect the unstructured nature of the linker or introduce potential electrostatic or steric concerns, appropriate increases to the linker length will be made to ensure that the two binding domains are able to bind their respective sites on the ligand. In light of the methods and teachings herein, such determinations could be made routinely by one skilled in the art.

In addition, the present design permits linkage of other cargo molecules (for example imaging agents like fluorescent molecules), toxins, etc. For example, and without wishing to be limiting in any manner, single-chain polypeptides can be modified to add one or more cargo and/or accessory molecules (referred to collectively herein by R1, R2, R3, R4, etc.):

$$\begin{array}{ccccccccc} & R3 & & R4 & R5 & R6 & R7 & R8 & R9 \\ & | & & | & | & | & | & | & | \end{array}$$

$$R1 - (<BD1>\text{-linker1})_k\text{-}[<BD2>\text{-linker2-}\{<BD3>\text{-linker3}\}_f]_n\text{-}(<BD4>)_m\text{-}(\text{linker4-}BD5>_d)_h - R2$$

Without limiting the generality of R substituents available, R1, R2, R3, R4, R5, R6, R7, R8, R9, may or may not be present; when present, they may be the same or different, and may independently be one or more of: a fusion protein for targeting, for example, but not limited to such as an antibody fragment (e.g. single chain Fv) and/or a single domain antibody (sdAb); a radiotherapy and/or imaging agent, for example, but not limited to a radionuceotide (e.g. ^{123}I, ^{111}In, ^{18}F, ^{64}C, ^{68}Y, ^{124}I, ^{131}I, ^{90}Y, ^{177}Lu, ^{57}Cu, ^{213}Bi, ^{211}At), a fluorescent dye (e.g. Alexa Fluor, Cy dye) and/or a fluorescent protein tag (e.g. GFP, DsRed); a cytotoxic agent for chemotherapy, for example, but not limited to doxorubicin, calicheamicin, a maytansinoid derivatives (e.g. DM1, DM4), a toxin (eg. truncated Pseudomonas endotoxin A, diphteria toxin); a nanoparticle-based carrier, for example, but not limited to polyethylene glycol (PEG), a polymer-conjugated to drug, nanocarrier or imaging agent (e.g. of a polymer N-(2-hydorxylpropyl) methacrylamide (HPMA), glutamic acid, PEG, dextran); a drug (for example, but not limited to doxorubicin, camptothecin,

paclitaxel, palatinate); a nanocarrier, for example, but not limited to a nanoshell or liposome; an imaging agent, for example, but not limited to Supermagnetic Iron Oxide (SPIO); a dendrimer; and/or a solid support for use in ligand purification, concentration or sequestration (e.g. nanoparticles, inert resins, suitable silica supports).

In general, it will not be preferable to have cargo or accessory molecules in all possible positions, as this may cause steric or electrostatic complications. However, the effects of adding a cargo or accessory molecule to any given position or positions on the structure can be determined routinely in light of the disclosure herein by modeling the linker between the binding domains and carrying out molecular dynamics simulations to substantially minimize molecular mechanics energy and reduce steric and electrostatic incompatibility between the linker and the ALK7 and ActRIIB polypeptides as taught herein.

It may be preferable to add the cargo or accessory molecule to the linker portion of the agent, rather to the binding domain, to reduce the likelihood of interference in binding function. However, addition to the binding domain is possible and could be desirable in some instances and the effect of such an addition can be determined routinely in advance by modeling the binding agent and the linker with the proposed addition as described herein.

Conjugation methodologies may be performed using commercial kits that enable conjugation via common reactive groups such as primary amines, succinimidyl (NHS) esters and sulfhydral-reactive groups. Some non-limiting examples are: Alexa Fluor 488 protein labeling kit (Molecular Probes, Invitrogen detection technologies) and PEGylation kits (Pierce Biotechnology Inc.).

In certain aspects, ALK7:ActRIIB single-chain traps may be covalently or non-covalently associated with one or more ALK7 or ActRIIB polypeptides as well as additional ALK7:ActRIIB single chain ligand traps to form higher order heteromultimers, which may be used in accordance with the methods described herein. See, e.g., Figure 12. For example, an ALK7:ActRIIB single chain ligand trap may further comprise a multimerization domain as described herein. In some embodiments, ALK7:ActRIIB single chain ligand traps comprise a constant domain of an Ig immunoglobulin. Such immunoglobulins constant domains may be selected to promote symmetrical or asymmetrical complexes comprising at least one single-chain ALK7:ActRIIB trap.

In certain aspects, an ALK7:ActRIIB single-chain trap, or combinations of such traps, may be used as ALK7:ActRIIB antagonists to treat or prevent an ALK7:ActRIIB disorder or disease as described herein (e.g., kidney disease and/or a metabolic disorder or condition).

It is understood that different elements of the fusion proteins (*e.g.*, immunoglobulin Fc fusion proteins) may be arranged in any manner that is consistent with desired functionality. For example, an ALK7 and/or ActRIIB polypeptide domain may be placed C-terminal to a heterologous domain, or alternatively, a heterologous domain may be placed C-terminal to an ALK7 and/or ActRIIB polypeptide domain. The ALK7 and/or ActRIIB 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.

For example, an ALK7 and/or ActRIIB receptor fusion protein may comprise an amino acid sequence as set forth in the formula A-B-C. The B portion corresponds to an ALK7 or ActRIIB polypeptide domain. The A and C portions may be independently zero, one, or more than one amino acid, and both the A and C portions when present are heterologous to B. The A and/or C portions may be attached to the B portion via a linker sequence. A linker may be rich in glycine (*e.g.*, 2-10, 2-5, 2-4, 2-3 glycine residues) or glycine and proline residues and may, for example, contain a single sequence of threonine/serine and glycines or repeating sequences of threonine/serine and/or glycines, *e.g.*, GGG (SEQ ID NO: 13), GGGG (SEQ ID NO: 14), TGGGG (SEQ ID NO: 15), SGGGG (SEQ ID NO: 16), TGGG (SEQ ID NO: 17), SGGG (SEQ ID NO: 18), or GGGGS (SEQ ID NO: 58) singlets, or repeats. In certain embodiments, an ALK7 and/or ActRIIB fusion protein comprises an amino acid sequence as set forth in the formula A-B-C, wherein A is a leader (signal) sequence, B consists of an ALK7 and/or ActRIIB polypeptide domain, and C is a polypeptide portion that enhances one or more of *in vivo* stability, *in vivo* half-life, uptake/administration, tissue localization or distribution, formation of protein complexes, and/or purification. In certain embodiments, an ALK7 and/or ActRIIB fusion protein comprises an amino acid sequence as set forth in the formula A-B-C, wherein A is a TPA leader sequence, B is an ALK7 or ActRIIB receptor polypeptide domain, and C is an immunoglobulin Fc domain. Preferred fusion proteins comprise the amino acid sequence set forth in any one of SEQ ID NOs: 71, 73, 74, 76, 77, 78, 79 and 80.

In some embodiments, ALK7:ActRIIB heteromultimers further comprise one or more heterologous portions (domains) so as to confer a desired property. For example, some

fusion domains are particularly useful for isolation of the fusion proteins by affinity chromatography. Well-known examples of such fusion domains include, but are not limited to, polyhistidine, Glu-Glu, glutathione S-transferase (GST), thioredoxin, protein A, protein G, an immunoglobulin heavy-chain constant region (Fc), maltose binding protein (MBP), or human serum albumin. 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 (HIS_6) fusion partners. As another example, a fusion domain may be selected so as to facilitate detection of the ligand trap polypeptides. Examples of such detection domains include the various fluorescent proteins (*e.g.*, GFP) as well as "epitope tags," which are usually short peptide sequences for which a specific antibody is available. Well-known epitope tags for which specific monoclonal antibodies are readily available include FLAG, influenza virus haemagglutinin (HA), and c-myc tags. In some cases, the fusion domains have a protease cleavage site, such as for factor Xa or thrombin, which allows the relevant protease to partially digest the fusion proteins and thereby liberate the recombinant proteins therefrom. The liberated proteins can then be isolated from the fusion domain by subsequent chromatographic separation.

In certain embodiments, ALK7 and/or ActRIIB polypeptides may contain one or more modifications that are capable of stabilizing the polypeptides. For example, such modifications enhance the *in vitro* half-life of the polypeptides, enhance circulatory half-life of the polypeptides, and/or reduce proteolytic degradation of the polypeptides. Such stabilizing modifications include, but are not limited to, fusion proteins (including, for example, fusion proteins comprising an ALK7 and/or ActRIIB polypeptide domain and a stabilizer domain), modifications of a glycosylation site (including, for example, addition of a glycosylation site to a polypeptide of the disclosure), and modifications of carbohydrate moiety (including, for example, removal of carbohydrate moieties from a polypeptide of the disclosure). As used herein, the term "stabilizer domain" not only refers to a fusion domain (*e.g.*, an immunoglobulin Fc domain) as in the case of fusion proteins, but also includes nonproteinaceous modifications such as a carbohydrate moiety, or nonproteinaceous moiety, such as polyethylene glycol.

In preferred embodiments, ALK7:ActRIIB heteromultimers to be used in accordance with the methods described herein are isolated complexes. As used herein, an isolated

protein (or protein complex) or polypeptide (or polypeptide complex) is one which has been separated from a component of its natural environment. In some embodiments, a heteromultimer of the disclosure is purified to greater than 95%, 96%, 97%, 98%, or 99% purity as determined by, for example, electrophoretic (*e.g.*, SDS-PAGE, isoelectric focusing (IEF), capillary electrophoresis) or chromatographic (*e.g.*, ion exchange or reverse phase HPLC). Methods for assessment of antibody purity are well known in the art [Flatman *et al.*, (2007) J. Chromatogr. B 848:79-87]. . In some embodiments, ALK7:ActRIIB heteromultimer preparations are substantially free of ALK7 and/or ActRIIB homomultimers. For example, in some embodiments, ALK7:ActRIIB heteromultimers preparations comprise less than about 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or less than about 1% ALK7 homomultimers. In some embodiments, ALK7:ActRIIB heteromultimer preparations comprise less than about 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or less than about 1% ActRIIB homomultimers. In some embodiments, ALK7:ActRIIB heteromultimer preparations comprise less than about 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or less than about 1% ALK7 homomultimers and less than about 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or less than about 1% ActRIIB homomultimers.

In certain embodiments, ALK7 and/or ActRIIB polypeptides, as well as heteromultimers comprising the same, can be produced by a variety of art-known techniques. For example, polypeptides can be synthesized using standard protein chemistry techniques such as those described in Bodansky, M. Principles of Peptide Synthesis, Springer Verlag, Berlin (1993) and Grant G. A. (ed.), Synthetic Peptides: A User's Guide, W. H. Freeman and Company, New York (1992). In addition, automated peptide synthesizers are commercially available (Advanced ChemTech Model 396; Milligen/Biosearch 9600). Alternatively, the polypeptides and complexes, including fragments or variants thereof, may be recombinantly produced using various expression systems [E. coli, Chinese Hamster Ovary (CHO) cells, COS cells, baculovirus] as is well known in the art. In a further embodiment, the modified or unmodified polypeptides may be produced by digestion of recombinantly produced full-length ALK7 and/or ActRIIB polypeptides by using, for example, a protease, *e.g.*, trypsin, thermolysin, chymotrypsin, pepsin, or paired basic amino acid converting enzyme (PACE). Computer analysis (using commercially available software, *e.g.*, MacVector, Omega, PCGene, Molecular Simulation, Inc.) can be used to identify proteolytic cleavage sites.

B. Nucleic Acids Encoding ALK7 and/or ActRIIB polypeptides

In certain embodiments, the present disclosure provides isolated and/or recombinant nucleic acids encoding ALK7 and/or ActRIIB receptors (including fragments, functional variants, and fusion proteins thereof) disclosed herein. For example, SEQ ID NO: 11 encodes a naturally occurring human ALK7 precursor polypeptide, while SEQ ID NO: 12 encodes a mature extracellular domain of ALK7. 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 ALK7:ActRIIB heteromultimers as described herein.

As used herein, isolated nucleic acid(s) refers to a nucleic acid molecule that has been separated from a component of its natural environment. An isolated nucleic acid includes a nucleic acid molecule contained in cells that ordinarily contain the nucleic acid molecule, but the nucleic acid molecule is present extrachromosomally or at a chromosomal location that is different from its natural chromosomal location.

In certain embodiments, nucleic acids encoding ALK7 and/or ActRIIB polypeptides of the present disclosure are understood to include any one of SEQ ID NOs: 7, 8, 11, 12, 21, 22, 40, 41, 44, 45, 72, or 75, as well as variants thereof. Variant nucleotide sequences include sequences that differ by one or more nucleotide substitutions, additions, or deletions including allelic variants, and therefore, will include coding sequences that differ from the nucleotide sequence designated in any one of SEQ ID NOs: 7, 8, 11, 12, 21, 22, 40, 41, 44, 45, 72, or 75.

In certain embodiments, TGFβ superfamily ALK7 and/or ActRIIB polypeptides of the present disclosure are encoded by isolated or recombinant nucleic acid sequences that comprise, consist essentially of, or consists of a sequence that is least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NOs: 7, 8, 11, 12, 21, 22, 40, 41, 44, 45, 72, or 75. One of ordinary skill in the art will appreciate that nucleic acid sequences that comprise, consist essentially of, or consists of a sequence complementary to a sequence that is least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NOs: 7, 8, 11, 12, 21, 22, 40, 41, 44, 45, 72, or 75 also within the scope of the present 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.

In other embodiments, nucleic acids of the present disclosure also include nucleotide sequences that hybridize under stringent conditions to the nucleotide sequence designated in SEQ ID NOs: 7, 8, 11, 12, 21, 22, 40, 41, 44, 45, 72, or 75, the complement sequence of SEQ ID NOs: 7, 8, 11, 12, 21, 22, 40, 41, 44, 45, 72, or 75, or fragments thereof. One of ordinary skill in the art will understand readily that appropriate stringency conditions which promote DNA hybridization can be varied. For example, one could perform the hybridization at 6.0 x sodium chloride/sodium citrate (SSC) at about 45 °C, followed by a wash of 2.0 x SSC at 50 °C. For example, the salt concentration in the wash step can be selected from a low stringency of about 2.0 x SSC at 50 °C to a high stringency of about 0.2 x SSC at 50 °C. In addition, the temperature in the wash step can be increased from low stringency conditions at room temperature, about 22 °C, to high stringency conditions at about 65 °C. Both temperature and salt may be varied, or temperature or salt concentration may be held constant while the other variable is changed. In one embodiment, 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: 7, 8, 11, 12, 21, 22, 40, 41, 44, 45, 72, or 75 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.

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 some embodiments, 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, the subject nucleic acid is provided in an expression vector comprising a nucleotide sequence encoding an ALK7 and/or ActRIIB polypeptide and operably linked to at least one regulatory sequence. Regulatory sequences are art-recognized and are selected to direct expression of ALK7 and/or ActRIIB 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 ALK7 and/or ActRIIB polypeptides. 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 of the present 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 ALK7 and/or ActRIIB polypeptides include plasmids and other vectors. For instance, suitable vectors include plasmids of the following 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 [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 ß-gal containing pBlueBac III).

In a preferred embodiment, a vector will be designed for production of the subject ALK7 and/or ActRIIB polypeptides in CHO cells, such as a Pcmv-Script vector (Stratagene, La Jolla, Calif.), pcDNA4 vectors (Invitrogen, Carlsbad, Calif.) and pCI-neo vectors (Promega, Madison, Wisc.). As will be apparent, the subject gene constructs can be used to cause expression of the subject ALK7 and/or ActRIIB polypeptide 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 for one or more of the subject ALK7 and/or ActRIIB polypeptides. The host cell may be any prokaryotic or eukaryotic cell. For example, an ALK7 and/or ActRIIB polypeptide may be expressed in bacterial cells such as *E. coli*, insect cells (e.g., using a baculovirus expression system), yeast, or mammalian cells [*e.g.* a Chinese hamster ovary (CHO) cell line]. Other suitable host cells are known to those skilled in the art.

Accordingly, the present disclosure further pertains to methods of producing the subject ALK7 and/or ActRIIB polypeptides. For example, a host cell transfected with an expression vector encoding an ALK7 and/or ActRIIB polypeptide can be cultured under appropriate conditions to allow expression of the ALK7 and/or ActRIIB polypeptide to occur. The polypeptide may be secreted and isolated from a mixture of cells and medium containing the polypeptide. Alternatively, ALK7 and/or ActRIIB polypeptide may be isolated from a cytoplasmic or membrane fraction obtained from harvested and lysed cells. A cell culture includes host cells, media and other byproducts. 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 ALK7 and/or ActRIIB polypeptides and affinity purification with an agent that binds to a domain fused to ALK7 and/or ActRIIB polypeptide (*e.g.*, a protein A column may be used to purify ALK7-Fc and/or ActRIIB-Fc fusion proteins). In some embodiments, the ALK7 and/or ActRIIB polypeptide is a fusion protein containing a domain which facilitates its purification.

In some embodiments, purification is 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. An ALK7 and/or ActRIIB polypeptide, as well as fusion proteins and heteromeric complexes thereof, may be purified to a purity of >90%, >95%, >96%, >98%, or >99% as determined by size exclusion chromatography and >90%, >95%, >96%, >98%, or >99% as determined by SDS PAGE. The target level of purity should be one that is sufficient to achieve desirable results in mammalian systems, particularly non-human primates, rodents (mice), and humans.

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 ALK7 and/or ActRIIB polypeptide, can allow purification of the expressed fusion protein by affinity chromatography using a Ni^{2+} metal resin. The purification leader sequence can then be subsequently removed by treatment with enterokinase to provide the purified ALK7 and/or ActRIIB polypeptide, as well as heteromultimers thereof [Hochuli *et al.* (1987) *J. Chromatography* 411:177; and Janknecht *et al.* (1991) *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, *e.g.*, Current Protocols in Molecular Biology, eds. Ausubel *et al.*, John Wiley & Sons: 1992.

C. Antibody Antagonists

In certain aspects, an ALK7:ActRIIB antagonist is an antibody (ALK7:ActRIIB antagonist antibody), or combination of antibodies. An ALK7:ActRIIB antagonist antibody, or combination of antibodies, may bind to, for example, one or more ALK7 ligands, ActRIIB ligands, ALK7:ActRIIB-binding ligands, an ALK7 receptor, an ActRIIB receptor, and/or a TGF-beta superfamily co-receptor (e.g., Crypto or Cryptic). As described herein, ALK7:ActRIIB antagonist antibodies may be used, alone or in combination with one or more supportive therapies or active agents, to treat a patient in need thereof (e.g., patients having kidney disease and/or a metabolic disorder or condition).

In certain aspects, an ALK7:ActRIIB antagonist antibody, or combination of antibodies, is an antibody that inhibits one or more of the ligands bound or likely bound by an ALK7:ActRIIB heteromultimer, such as activin B, GDF11, activin A, BMP10, BMP6, BMP5,

GDF3, activin C, activin E, activin AC, activin BC, activin AE, nodal, or activin BE. Therefore, in some embodiments, an ALK7:ActRIIB antagonist antibody, or combination of antibodies, binds to at least one of such ligands. As an example, as used herein, an activin C antibody (or anti-activin C antibody) generally refers to an antibody that can bind to activin C with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting activin C. In certain embodiments, the extent of binding of a activin C antibody to an unrelated, non-activin C 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 activin C as measured, for example, by a radioimmunoassay (RIA), Biacore, or other protein interaction or binding affinity assay. In certain embodiments, an activin C antibody binds to an epitope of activin C that is conserved among activin C from different species. In certain preferred embodiments, an anti-activin C antibody binds to human activin C. In some embodiments, an activin C antibody may inhibit activin C from binding to a type I and/or type II receptor (*e.g.*, ActRIIB and/or ALK7) and thus inhibit activin C-mediated signaling (e.g., Smad signaling). In some embodiments, an activin C antibody may inhibit activin C from binding to a co-receptor and thus inhibit activin C-mediated signaling (e.g., Smad signaling). It should be noted that activin C shares some sequence homology to activin A, B and E and therefore antibodies that bind to activin C, in some instances, may also bind to and/or inhibit another activin. In some embodiments, the disclosure relates to a multispecific antibody (e.g., bi-specific antibody), and uses thereof, that binds to, for example, activin C and further binds to, for example, one or more additional TGF-β superfamily ligands that bind to ALK7:ActRIIB heteromultimer [*e.g.*, activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC, activin BC, activin AE, or activin BE), GDF11, GDF8, BMP10, BMP6, BMP5, nodal, and GDF3], one or more type I receptor and/or type II receptors (e.g., ActRIIB and/or ALK7), and/or one or more co-receptors. In some embodiments, a multispecific antibody that binds to activin C does not bind or does not substantially bind to BMP9 (e.g., binds to BMP9 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 combination of antibodies that bind to, for example, two or more TGF-β superfamily ligand that bind to ALK7:ActRIIB heteromultimer [*e.g.*, activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC, activin BC, activin AE, or activin BE), GDF11, GDF8, BMP10, BMP6, BMP5, nodal, and GDF] one or more type I receptor and/or type II receptors (e.g., ActRIIB and/or

ALK7), and/or one or more co-receptors. In some embodiments, a combination of antibodies does not comprise a BMP9 antibody.

In certain aspects, an ALK7:ActRIIB antagonist antibody, or combination of antibodies, is an antibody that inhibits at least GDF8. Therefore, in some embodiments, an ALK7:ActRIIB antagonist antibody, or combination of antibodies, binds to at least GDF8. As used herein, a GDF8 antibody (or anti-GDF8 antibody) generally refers to an antibody that binds to GDF8 with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting GDF8. In certain embodiments, the extent of binding of a GDF8 antibody to an unrelated, non- GDF8 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 GDF8 as measured, for example, by a radioimmunoassay (RIA), Biacore, or other protein interaction or binding affinity assay. In certain embodiments, a GDF8 antibody binds to an epitope of GDF8 that is conserved among GDF8 from different species. In certain preferred embodiments, an anti-GDF8 antibody binds to human GDF8. In some embodiments, a GDF8 antibody may inhibit GDF8 from binding to a type I and/or type II receptor (*e.g.*, ActRIIB and/or ALK7) and thus inhibit GDF8-mediated signaling (e.g., Smad signaling). In some embodiments, a GDF8 antibody may inhibit GDF8 from binding to a co-receptor and thus inhibit GDF8-mediated signaling (e.g., Smad signaling). It should be noted that GDF8 has high sequence homology to GDF11 and therefore antibodies that bind to GDF8, in some instances, may also bind to and/or inhibit GDF11. In some embodiments, the disclosure relates to a multispecific antibody (e.g., bi-specific antibody), and uses thereof, that binds to GDF8 and further binds to, for example, one or more additional TGF-β superfamily ligands [*e.g.*, activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC, activin BC, activin AE, activin BE), GDF11, BMP10, BMP6, BMP5, nodal, and GDF3], one or more type I receptor and/or type II receptors (e.g., ActRIIB and/or ALK7), and/or one or more co-receptors. In some embodiments, a multispecific antibody that binds to GDF8 does not bind or does not substantially bind to BMP9 (e.g., binds to BMP9 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 GDF8 antibody and one or more additional antibodies that bind to, for example, one or more additional TGF-β superfamily ligand [e.g., activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC, activin BC, activin AE, activin BE), GDF11, GDF3, BMP6, BMP10, nodal, and BMP5], one

or more type I receptor and/or type II receptors (e.g., ActRIIB and/or ALK7), and/or one or more co-receptors. In some embodiments, a combination of antibodies that comprises a GDF8 antibody does not comprise a BMP9 antibody.

In certain aspects, an ALK7:ActRIIB antagonist antibody, or combination of antibodies, is an antibody that inhibits at least GDF11. Therefore, in some embodiments, an ALK7:ActRIIB antagonist antibody, or combination of antibodies, binds to at least GDF11. As used herein, a GDF11 antibody (or anti-GDF11 antibody) generally refers to an antibody that binds to GDF11 with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting GDF11. In certain embodiments, the extent of binding of a GDF11 antibody to an unrelated, non-GDF11 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 GDF11 as measured, for example, by a radioimmunoassay (RIA), Biacore, or other protein interaction or binding affinity assay. In certain embodiments, a GDF11 antibody binds to an epitope of GDF11 that is conserved among GDF11 from different species. In certain preferred embodiments, an anti-GDF11 antibody binds to human GDF11. In some embodiments, a GDF11 antibody may inhibit GDF11 from binding to a type I and/or type II receptor (*e.g.*, ActRIIB and/or ALK7) and thus inhibit GDF11-mediated signaling (e.g., Smad signaling). In some embodiments, a GDF11 antibody may inhibit GDF11 from binding to a co-receptor and thus inhibit GDF11-mediated signaling (e.g., Smad signaling). It should be noted that GDF11 has high sequence homology to GDF8 and therefore antibodies that bind to GDF11, in some instances, may also bind to and/or inhibit GDF8. In some embodiments, the disclosure relates to a multispecific antibody (e.g., bi-specific antibody), and uses thereof, that binds to GDF11 and further binds to, for example, one or more additional TGF-β superfamily ligands [*e.g.*, activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC, activin BC, activin AE, activin BE), GDF11, BMP10, BMP6, BMP5, nodal, and GDF3], one or more type I receptor and/or type II receptors (e.g., ActRIIB and/or ALK7), and/or one or more co-receptors. In some embodiments, a multispecific antibody that binds to GDF11 does not bind or does not substantially bind to BMP9 (e.g., binds to BMP9 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 GDF11 antibody and one or more additional antibodies that bind to, for example, one or more additional TGF-β superfamily ligand [e.g., activin (e.g., activin A, activin B, activin C, activin E, activin AB,

activin AC, activin BC, activin AE, activin BE), GDF8, GDF3, BMP6, BMP10, nodal, and BMP5], one or more type I receptor and/or type II receptors (e.g., ActRIIB and/or ALK7), and/or one or more co-receptors. In some embodiments, a combination of antibodies that comprises a GDF11 antibody does not comprise a BMP9 antibody.

In certain aspects, an ALK7:ActRIIB antagonist antibody, or combination of antibodies, is an antibody that inhibits at least activin (activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC and/or activin BE). Therefore, in some embodiments, an ALK7:ActRIIB antagonist antibody, or combination of antibodies, binds to at least activin (activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC and/or activin BE). As used herein, an activin antibody (or anti-activin antibody) generally refers to an antibody that can bind to a form of activin with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting that form of activin. In certain embodiments, the extent of binding of an activin antibody to an unrelated, non-activin 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 activin as measured, for example, by a radioimmunoassay (RIA), Biacore, or other protein interaction or binding affinity assay. In certain embodiments, an activin antibody binds to an epitope of activin that is conserved among activin from different species. In certain preferred embodiments, an anti-activin antibody binds to human activin. In other preferred embodiments, a activin antibody may inhibit activin from binding to a type I and/or type II receptor (*e.g.*, ActRIIB and/or ALK7) and thus inhibit activin-mediated signaling (e.g., Smad signaling). In some embodiments, an activin antibody binds to activin B. In some embodiments, an activin antibody binds to activin A. In some embodiments, an activin antibody binds to activin A and activin B. In some embodiments, an activin antibody binds to activin AB. In some embodiments, an activin antibody binds to activin C. In some embodiments, an activin antibody binds to activin E. In some embodiments, an activin antibody binds to activin A and activin C. In some embodiments, an activin antibody binds to activin AC. In some embodiments, an activin antibody binds to activin A and activin E. In some embodiments, an activin antibody binds to activin AE. In some embodiments, an activin antibody binds to activin B and activin C. In some embodiments, an activin antibody binds to activin BC. In some embodiments, an activin antibody binds to activin B and activin E. In some embodiments, an activin antibody binds to activin BE. In some embodiments, an activin antibody binds to activin A, activin B, and activin C. In some embodiments, an activin antibody binds to activin A, activin B, and

activin E. Optionally, an activin antibody that binds to one or more of activin A, activin B, and activin C may further bind to activin E. In some embodiments, the disclosure relates to a multispecific antibody (e.g., bi-specific antibody), and uses thereof, that binds to activin and further binds to, for example, one or more additional TGF-β superfamily ligands [*e.g.*, GDF11, GDF8, BMP10, BMP6, BMP5, nodal, and GDF3], one or more type I receptor and/or type II receptors (e.g., ActRIIB and/or ALK7), and/or one or more co-receptors. In some embodiments, a multispecific antibody that binds to activin does not bind or does not substantially bind to BMP9 (e.g., binds to BMP9 with a K_D of greater than 1 x 10^{-7} M or has relatively modest binding, e.g., about 1 x 10^{-8} M or about 1 x 10^{-9} M. In some embodiments, the disclosure relates to combinations of antibodies, and uses thereof, wherein the combination of antibodies comprises an activin antibody and one or more additional antibodies that bind to, for example, one or more additional TGF-β superfamily ligand [e.g., GDF11, GDF8 GDF3, BMP6, BMP10, nodal, and BMP5], one or more type I receptor and/or type II receptors (e.g., ActRIIB and/or ALK7), and/or one or more co-receptors. In some embodiments, a combination of antibodies that comprises an activin antibody does not comprise a BMP9 antibody.

In certain aspects, an ALK7:ActRIIB antagonist antibody, or combination of antibodies, is an antibody that inhibits at least BMP6. Therefore, in some embodiments, an ALK7:ActRIIB antagonist antibody, or combination of antibodies, binds to at least BMP6. As used herein, a BMP6 antibody (or anti-BMP6 antibody) generally refers to an antibody that can bind to BMP6 with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting BMP6. In certain embodiments, the extent of binding of a BMP6 antibody to an unrelated, non-BMP6 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 BMP6 as measured, for example, by a radioimmunoassay (RIA), Biacore, or other protein interaction or binding affinity assay. In certain embodiments, a BMP6 antibody binds to an epitope of BMP6 that is conserved among BMP6 from different species. In certain preferred embodiments, an anti-BMP6 antibody binds to human BMP6. In some embodiments, a BMP6 antibody may inhibit BMP6 from binding to a type I and/or type II receptor (*e.g.*, ActRIIB and/or ALK7) and thus inhibit BMP6-mediated signaling (e.g., Smad signaling). In some embodiments, a BMP6 antibody may inhibit BMP6 from binding to a co-receptor and thus inhibit BMP6-mediated signaling (e.g., Smad signaling). In some embodiments, the disclosure relates to a multispecific antibody (e.g., bi-specific antibody), and uses thereof,

that binds to BMP6 and further binds to, for example, one or more additional TGF-β superfamily ligands [*e.g.*, activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC, activin BC, activin AE, activin BE), GDF8, BMP10, GDF11, BMP5, nodal, and GDF3], one or more type I receptor and/or type II receptors (e.g., ActRIIB and/or ALK7), and/or one or more co-receptors. In some embodiments, a multispecific antibody that binds to BMP6 does not bind or does not substantially bind to BMP9 (e.g., binds to BMP9 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 BMP6 antibody and one or more additional antibodies that bind to, for example, one or more additional TGF-β superfamily ligand [e.g., activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC, activin BC, activin AE, activin BE), GDF8 GDF3, GDF11, BMP10, nodal, and BMP5], one or more type I receptor and/or type II receptors (e.g., ActRIIB and/or ALK7), and/or one or more co-receptors. In some embodiments, a combination of antibodies that comprises a BMP6 antibody does not comprise a BMP9 antibody.

In certain aspects, an ALK7:ActRIIB antagonist antibody, or combination of antibodies, is an antibody that inhibits at least GDF3. Therefore, in some embodiments, an ALK7:ActRIIB antagonist antibody, or combination of antibodies, binds to at least GDF3. As used herein, a GDF3 antibody (or anti-GDF3 antibody) generally refers to an antibody that binds to GDF3 with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting GDF3. In certain embodiments, the extent of binding of a GDF3 antibody to an unrelated, non-GDF3 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 GDF3 as measured, for example, by a radioimmunoassay (RIA), Biacore, or other protein interaction or binding affinity assay. In certain embodiments, a GDF3 antibody binds to an epitope of GDF3 that is conserved among GDF3 from different species. In certain preferred embodiments, a GDF3 antibody binds to human GDF3. In some embodiments, a GDF3 antibody may inhibit GDF3 from binding to a type I and/or type II receptor (*e.g.*, ActRIIB and/or ALK7) and thus inhibit GDF3-mediated signaling (e.g., Smad signaling). In some embodiments, a GDF3 antibody may inhibit GDF3 from binding to a co-receptor and thus inhibit GDF3-mediated signaling (e.g., Smad signaling). In some embodiments, the disclosure relates to a multispecific antibody (e.g., bi-specific antibody), and uses thereof, that binds to GDF3 and further binds to, for example, one or more additional TGF-β superfamily ligands [*e.g.*, activin (e.g., activin

A, activin B, activin C, activin E, activin AB, activin AC, activin BC, activin AE, activin BE), GDF11, BMP10, BMP6, BMP5, and nodal , one or more type I receptor and/or type II receptors (e.g., ActRIIB and/or ALK7), and/or one or more co-receptors. In some embodiments, a multispecific antibody that binds to GDF3 does not bind or does not substantially bind to BMP9 (e.g., binds to BMP9 with a K_D of greater than 1 x 10^{-7} M or has relatively modest binding, e.g., about 1 x 10^{-8} M or about 1 x 10^{-9} M). In some embodiments, the disclosure relates to combinations of antibodies, and uses thereof, wherein the combination of antibodies comprises a GDF3 antibody and one or more additional antibodies that bind to, for example, one or more additional TGF-β superfamily ligand [*e.g.*, activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC, activin BC, activin AE, activin BE), GDF11, BMP10, BMP6, BMP5, and nodal , one or more type I receptor and/or type II receptors (e.g., ActRIIB and/or ALK7), and/or one or more co-receptors. In some embodiments, a combination of antibodies that comprises a GDF3 antibody does not comprise a BMP9 antibody.

In certain aspects, an ALK7:ActRIIB antagonist antibody, or combination of antibodies, is an antibody that inhibits at least BMP5. Therefore, in some embodiments, an ALK7:ActRIIB antagonist antibody, or combination of antibodies, binds to at least BMP5. As used herein, a BMP5 antibody (or anti-BMP5 antibody) generally refers to an antibody that binds to BMP5 with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting BMP5. In certain embodiments, the extent of binding of a BMP5 antibody to an unrelated, non-BMP5 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 BMP5 as measured, for example, by a radioimmunoassay (RIA), Biacore, or other protein interaction or binding affinity assay. In certain embodiments, a BMP5 antibody binds to an epitope of BMP5 that is conserved among BMP5 from different species. In certain preferred embodiments, an anti-BMP5 antibody binds to human BMP5. In some embodiments, a BMP5 antibody may inhibit BMP5 from binding to a type I and/or type II receptor (*e.g.*, ActRIIB and/or ALK7) and thus inhibit BMP5 -mediated signaling (e.g., Smad signaling). In some embodiments, a BMP5 antibody may inhibit BMP5 from binding to a co-receptor and thus inhibit BMP5-mediated signaling (e.g., Smad signaling). In some embodiments, the disclosure relates to a multispecific antibody (e.g., bi-specific antibody), and uses thereof, that binds to BMP5 and further binds to, for example, one or more additional TGF-β superfamily ligands [*e.g.*, activin (e.g., activin A, activin B, activin C, activin E, activin AB,

activin AC, activin BC, activin AE and activin BE), GDF8, BMP10, BMP6, GDF11, nodal, and GDF3], one or more type I receptor and/or type II receptors (e.g., ActRIIB and/or ALK7), and one or more co-receptors. In some embodiments, a multispecific antibody that binds to BMP5 does not bind or does not substantially bind to BMP9 (e.g., binds to BMP9 with a K_D of greater than 1 x 10^{-7} M or has relatively modest binding, e.g., about 1 x 10^{-8} M or about 1 x 10^{-9} M). In some embodiments, the disclosure relates to combinations of antibodies, and uses thereof, wherein the combination of antibodies comprises a BMP5 antibody and one or more additional antibodies that bind to, for example, one or more additional TGF-β superfamily ligand [e.g., activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC, activin BC, activin AE, and activin BE), GDF8 GDF3, BMP6, BMP10, nodal, and GDF11], type I receptor, and/or type II receptors (e.g., ActRIIB and/or ALK7), and/or co-receptor. In some embodiments, a combination of antibodies that comprises a BMP5 antibody does not comprise a BMP9 antibody.

In certain aspects, an ALK7:ActRIIB antagonist antibody, or combination of antibodies, is an antibody that inhibits at least BMP10. Therefore, in some embodiments, an ALK7:ActRIIB antagonist antibody, or combination of antibodies, binds to at least BMP10. As used herein, a BMP10 antibody (or anti-BMP10 antibody) generally refers to an antibody that can bind to BMP10 with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting BMP10. In certain embodiments, the extent of binding of a BMP10 antibody to an unrelated, non-BMP10 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 BMP10 as measured, for example, by a radioimmunoassay (RIA), Biacore, or other protein interaction or binding affinity assay. In certain embodiments, a BMP10 antibody binds to an epitope of BMP10 that is conserved among BMP10 from different species. In certain preferred embodiments, an anti-BMP10 antibody binds to human BMP10. In some embodiments, a BMP10 antibody may inhibit BMP10 from binding to a type I and/or type II receptor (*e.g.*, ActRIIB and/or ALK7) and thus inhibit BMP10 -mediated signaling (e.g., Smad signaling). In some embodiments, a BMP10 antibody may inhibit BMP10 from binding to a co-receptor and thus inhibit BMP10-mediated signaling (e.g., Smad signaling). In some embodiments, the disclosure relates to a multispecific antibody (e.g., bi-specific antibody), and uses thereof, that binds to BMP10 and further binds to, for example, one or more additional TGF-β superfamily ligands [*e.g.*, activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC, activin BC, activin AE and activin BE), GDF8, GDF11, BMP6, BMP5, nodal,

and GDF3], one or more type I receptor and/or type II receptors (e.g., ActRIIB and/or ALK7), and/or one or more co-receptors. In some embodiments, a multispecific antibody that binds to BMP10 does not bind or does not substantially bind to BMP9 (e.g., binds to BMP9 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 BMP10 antibody and one or more additional antibodies that bind to, for example, one or more additional TGF-β superfamily ligand [e.g., activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC, activin BC, activin AE and activin BE), GDF8 GDF3, BMP6, GDF11, nodal, and BMP5], one or more type I receptor and/or type II receptors (e.g., ActRIIB and/or ALK7), and/or one or more co-receptors. In some embodiments, a combination of antibodies that comprises a BMP10 antibody does not comprise a BMP9 antibody.

In certain aspects, an ALK7:ActRIIB antagonist antibody, or combination of antibodies, is an antibody that inhibits at least nodal. Therefore, in some embodiments, an ALK7:ActRIIB antagonist antibody, or combination of antibodies, binds to at least nodal. As used herein, a nodal antibody (or anti-nodal antibody) generally refers to an antibody that can bind to nodal with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting nodal. In certain embodiments, the extent of binding of a nodal antibody to an unrelated, non-nodal 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 nodal as measured, for example, by a radioimmunoassay (RIA), Biacore, or other protein interaction or binding affinity assay. In certain embodiments, a nodal antibody binds to an epitope of nodal that is conserved among nodal from different species. In certain preferred embodiments, an anti-nodal antibody binds to human nodal. In some embodiments, a nodal antibody may inhibit nodal from binding to a type I and/or type II receptor (*e.g.*, ActRIIB and/or ALK7) and thus inhibit nodal-mediated signaling (e.g., Smad signaling). In some embodiments, a nodal antibody may inhibit nodal from binding to a co-receptor and thus inhibit nodal-mediated signaling (e.g., Smad signaling). In some embodiments, the disclosure relates to a multispecific antibody (e.g., bi-specific antibody), and uses thereof, that binds to nodal and further binds to, for example, one or more additional TGF-β superfamily ligands [*e.g.*, activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC, activin BC, activin AE and activin BE), GDF8, GDF11, BMP6, BMP5, BMP10, and GDF3], one or more type I receptor and/or type II receptors (e.g., ActRIIB and/or ALK7), and/or one or more co-

receptors. In some embodiments, a multispecific antibody that binds to nodal does not bind or does not substantially bind to BMP9 (e.g., binds to BMP9 with a K_D of greater than 1 x 10^{-7} M or has relatively modest binding, e.g., about 1 x 10^{-8} M or about 1 x 10^{-9} M). In some embodiments, the disclosure relates to combinations of antibodies, and uses thereof, wherein the combination of antibodies comprises a nodal antibody and one or more additional antibodies that bind to, for example, one or more additional TGF-β superfamily ligand [e.g., activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC, activin BC, activin AE and activin BE), GDF8 GDF3, BMP6, GDF11, BMP10, and BMP5], one or more type I receptor and/or type II receptors (e.g., ActRIIB and/or ALK7), and/or one or more co-receptors. In some embodiments, a combination of antibodies that comprises a nodal antibody does not comprise a BMP9 antibody.

With respect to antibodies that bind to and antagonize ligands that bind to ALK7:ActRIIB, [e.g., activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC, activin BC, activin AE and activin BE), GDF8 GDF3, BMP6, GDF11, BMP10, and BMP5], it is contemplated that an antibody may be designed as a bispecific antibody comprising a first portion that binds to an epitope of such ligand, such that the first portion of the antibody competes for binding with a type I receptor and comprising a second portion that binds to an epitope of such ligand, such that the second portion of the antibody competes for binding with a type II receptor. In this manner, a bispecific antibody targeting a single ligand can be designed to mimic the dual type I-type II receptor binding blockade that may be conferred by an ALK7:ActRIIB heteromultimer. Similarly it is contemplated that the same effect could be achieved using a combination of two or more antibodies wherein at least a first antibody binds to an epitope of such ligand, such that the first antibody competes for binding with a type I receptor and at least a second antibody binds to an epitope of such ligand, such that the the second antibody competes for binding with a type II receptor.

In certain aspects, an ALK7:ActRIIB antagonist antibody, or combination of antibodies, is an antibody that inhibits at least ActRIIB. Therefore, in some embodiments, an ALK7:ActRIIB antagonist antibody, or combination of antibodies, binds to at least ActRIIB. As used herein, an ActRIIB antibody (anti-ActRIIB antibody) generally refers to an antibody that binds to ActRIIB with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting ActRIIB. In certain embodiments, the extent of binding of an anti- ActRIIB antibody to an unrelated, non-ActRIIB 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

ActRIIB as measured, for example, by a radioimmunoassay (RIA), Biacore, or other protein-protein interaction or binding affinity assay. In certain embodiments, an anti-ActRIIB antibody binds to an epitope of ActRIIB that is conserved among ActRIIB from different species. In certain preferred embodiments, an anti-ActRIIB antibody binds to human ActRIIB. In some embodiments, an anti-ActRIIB antibody may inhibit one or more TGF-β superfamily ligands [*e.g.*, GDF8, activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC, activin BC, activin AE and activin BE) GDF3, BMP6, BMP10, nodal and BMP9] from binding to ActRIIB and/or ALK7. In some embodiments, an anti-ActRIIB antibody is a multispecific antibody (e.g., bi-specific antibody) that binds to ActRIIB and one or more TGF-β superfamily ligands [*e.g.*, GDF11, GDF8, activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC) GDF3, BMP6, BMP10, nodal, and BMP9], type I receptor (e.g., ALK7), co-receptor, and/or an additional type II receptor. In some embodiments, the disclosure relates to combinations of antibodies, and uses thereof, wherein the combination of antibodies comprises an anti-ActRIIB antibody and one or more additional antibodies that bind to, for example, one or more TGF-β superfamily ligands [*e.g.*, GDF11, GDF8, activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC, activin BC, activin AE and activin BE) GDF3, BMP6, BMP10, nodal, and BMP9], co-receptors, type I receptors (e.g., ALK7), and/or additional type II receptors. It should be noted that ActRIIB has sequence similarity to ActRIIA and therefore antibodies that bind to ActRIIB, in some instances, may also bind to and/or inhibit ActRIIA.

In certain aspects, an ALK7:ActRIIB antagonist antibody, or combination of antibodies, is an antibody that inhibits at least ALK7. Therefore, in some embodiments, an ALK7:ActRIIB antagonist antibody, or combination of antibodies, binds to at least ALK7. As used herein, an ALK7 antibody (anti-ALK7 antibody) generally refers to an antibody that binds to ALK7 with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting ALK7. In certain embodiments, the extent of binding of an anti- ALK7 antibody to an unrelated, non-ALK7 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 ALK7 as measured, for example, by a radioimmunoassay (RIA), Biacore, or other protein-protein interaction or binding affinity assay. In certain embodiments, an anti-ALK7 antibody binds to an epitope of ALK7 that is conserved among ALK7 from different species. In certain preferred embodiments, an anti-ALK7 antibody binds to human ALK7. In some embodiments, an anti-ALK7 antibody may inhibit one or more TGF-β superfamily ligands

[*e.g.*, GDF11, GDF8, activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC, activin BC, activin AE and activin BE) GDF3, BMP5, BMP6, and BMP10] from binding to a type I receptor (e.g., ALK7), type II receptor (e.g., ActRIIB), or co-receptor. In some embodiments, an anti-ALK7 antibody is a multispecific antibody (e.g., bi-specific antibody) that binds to ALK7 and one or more TGF-β superfamily ligands [*e.g.*, activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC, activin BC, activin AE and activin BE), GDF11, GDF8, BMP10, BMP6, BMP5, nodal, and GDF3, type II receptors (e.g., ActRIIB), co-receptors, and/or an additional type I receptor. In some embodiments, the disclosure relates to combinations of antibodies, and uses thereof, wherein the combination of antibodies comprises an anti-ALK7 antibody and one or more additional antibodies that bind to, for example, one or more TGF-β superfamily ligands [*e.g.*, activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC, activin BC, activin AE and activin BE), GDF11, GDF8, BMP10, BMP6, BMP5, nodal, and GDF3] , co-receptors, an additional type I receptor, and/or type II receptors (e.g., ActRIIB).

In certain aspects, an ALK7:ActRIIB antagonist antibody, or combination of antibodies, is an antibody that inhibits at least Cripto or Cryptic. Therefore, in some embodiments, an ALK7:ActRIIB antagonist antibody, or combination of antibodies, binds to at least Cripto or Cryptic. As used herein, an Cripto antibody (anti-Cripto antibody) generally refers to an antibody that binds to Cripto with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting Cripto. In certain embodiments, the extent of binding of an anti-Cripto antibody to an unrelated, non-Cripto 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 Cripto as measured, for example, by a radioimmunoassay (RIA), Biacore, or other protein-protein interaction or binding affinity assay. In certain embodiments, an anti-Cripto antibody binds to an epitope of Cripto that is conserved among Cripto from different species. In certain preferred embodiments, an anti-Cripto antibody binds to human Cripto. As used herein, a Cryptic antibody (anti-Cryptic antibody) generally refers to an antibody that binds to Cryptic with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting Cryptic. In certain embodiments, the extent of binding of an anti-Cripto antibody to an unrelated, non-Cryptic 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 Cryptic as measured, for example, by a radioimmunoassay (RIA), Biacore, or other protein-protein interaction or binding affinity assay. In certain embodiments, an anti-

Cryptic antibody binds to an epitope of Cryptic that is conserved among Cryptic from different species. In certain preferred embodiments, an anti- Cryptic antibody binds to human Cryptic. In some embodiments, an anti-Cripto or Cryptic antibody may inhibit one or more TGF-β superfamily ligands [*e.g.*, activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC, activin BC, activin AE and activin BE), GDF11, GDF8, BMP10, BMP6, BMP5, nodal, and GDF3] from binding to Cripto or Cryptic, respectively, or to ActRIIB and/or ALK7. In some embodiments, an anti-Cripto or Cryptic antibody may inhibit nodal from binding to a type I and/or type II receptor. In some embodiments, an anti-Cripto or Cryptic antibody is a multispecific antibody (e.g., bi-specific antibody) that binds to Cripto or Cryptic and one or more TGF-β superfamily [*e.g.*, activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC, activin BC, activin AE and activin BE), GDF11, GDF8, BMP10, BMP6, BMP5, nodal, and GDF3] , type I receptor (e.g., ALK7), additional co-receptor, and/or type II receptor. In some embodiments an antibody may bind to both Cripto and Cryptic. In some embodiments, the disclosure relates to combinations of antibodies, and uses thereof, wherein the combination of antibodies comprises an anti-Cripto or Cryptic antibody and one or more additional antibodies that bind to, for example, one or more TGF-β superfamily ligands [*e.g.*, activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC, activin BC, activin AE and activin BE), GDF11, GDF8, BMP10, BMP6, BMP5, nodal, and GDF3], additional co-receptors, type I receptors (e.g., ALK7), and/or type II receptors.

As described herein, there are a variety of methods for generating heteromultimeric complexes. Such methods may be used to generate heteromultimer complexes comprising an antibody-binding domain (e.g., a complex of V_L and V_H chains) and one or more polypeptides selected from an ALK7 polypeptide, an ActRIIB polypeptide, an ALK7:ActRIIB heteromer, or an ALK7:ActRIIB single trap polypeptide. See Figure 10A, 10B and 12D. For example, in some embodiments, the disclosure provides protein complexes comprising a ligand-binding domain of an antibody that binds to an ALK7:ActRIIB-binding ligand [*e.g.*, activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC, activin BC, activin AE and activin BE), GDF11, GDF8, BMP10, BMP6, BMP5, nodal, and GDF3] which is covalently or non-covalently associated with an ALK7 polypeptide. In some embodiments, the disclosure provides protein complexes comprising a ligand-binding domain of an antibody that binds to an ALK7:ActRIIB-binding ligand [*e.g.*, activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC, activin BC, activin AE and activin BE),

GDF11, GDF8, BMP10, BMP6, BMP5, nodal, and GDF3] which is covalently or non-covalently associated with an ActRIIB polypeptide. In some embodiments, the disclosure provides protein complexes comprising a ligand-binding domain of an antibody that binds to an ALK7:ActRIIB-binding ligand [*e.g.*, activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC, activin BC, activin AE and activin BE), GDF11, GDF8, BMP10, BMP6, BMP5, nodal, and GDF3] which is covalently or non-covalently associated with an ALK7:ActRIIB single-chain ligand trap. In some embodiments, the disclosure provides protein complexes comprising a ligand-binding domain of an antibody that binds to an ALK7:ActRIIB-binding ligand [*e.g.*, activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC, activin BC, activin AE and activin BE), GDF11, GDF8, BMP10, BMP6, BMP5, nodal, and GDF3] which is covalently or non-covalently associated with ALK7:ActRIIB heteromultimer.

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 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 Pharmacology of Monoclonal Antibodies, vol. 113, Rosenburg 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]. 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 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]. 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, fluorescent compound, enzyme, or enzyme co-factor). In cetain preferred embodiments, the antibodies of the present disclosure are isolated antibodies. In certain preferred embodiments, the antibodies of the present disclosure are recombinant antibodies.

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, IgG_1, IgG_2, IgG_3, IgG_4, IgA_1, and IgA_2. 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, Biacore, radiolabeled antigen-binding assay (RIA), and ELISA. In some embodiments, antibodies of the present disclosure bind to their target antigens (*e.g.* ALK7, ActRIIB, activin, GDF11, GDF8, BMP10, BMP6, GDF3, nodal, and/or BMP5) with at least a K_D of $1x\ 10^{-7}$ or stronger, $1x10^{-8}$ or stronger, $1x10^{-9}$ or stronger, $1x10^{-10}$ or stronger, $1x10^{-11}$ or stronger, $1x10^{-12}$ or stronger, $1x10^{-13}$ or stronger, or $1x10^{-14}$ 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.*, ^{125}I-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 (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 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 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 μg/ml (about 0.2 μM) before injection at a flow rate of 5 μl/minute to achieve approximately 10 response units (RU) of coupled protein. Following the injection of antigen, 1 M ethanolamine is injected to block 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 at a flow rate of approximately 25 μl/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 sensorgrams. The equilibrium dissociation constant (K_D) is calculated as the ratio k_{off} / k_{on} [see, *e.g.*, Chen *et al.*, (1999) J. Mol. Biol. 293:865-881]. If the on-rate exceeds, for example, 10^6 M^{-1} 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 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 spectrophometer (Aviv Instruments) or a 8000-series SLM-AMINCO® spectrophotometer (ThermoSpectronic) with a stirred cuvette.

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 nucleic acid and amino acid sequences of human ActRIIB, activin (activin A, activin B, activin C, and activin E), GDF11, GDF8,

BMP10, BMP6, GDF3, nodal, and/or BMP5 are known in the art. In addition, numerous methods for generating antibodies are well known in the art, some of which are described herein. Therefore antibody antagonists for use in accordance with this disclosure may be routinely made by the skilled person in the art 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 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 (2008) Curr. Opin. Pharmacol. 5: 368-74 (2001) and Lonberg, Curr. Opin. Immunol. 20:450-459. For example, human antibodies may be prepared by administering an immunogen (*e.g.*, a GDF11 polypeptide, an activin B polypeptide, an ActRIIA polypeptide, or an ActRIIB 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. Biotech. 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-M 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. Techniques for selecting human antibodies from antibody libraries are known in the art and 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.*, ALK7, ActRIIB, activin, GDF11, GDF8, BMP10, BMP6, GDF3, and/or BMP5) without the requirement of constructing hybridomas. Alternatively, the naive repertoire can be cloned (*e.g.*, from human) to provide a

single source of antibodies to 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 unrearranged 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, 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) that have binding specificities for at least two different epitopes (*e.g.*, two, three, four, five, or six or more) on one or more (*e.g.*, two, three, four, five, six or more) antigens.

Techniques for making multispecific antibodies include, but are not limited to, recombinant co-expression of two immunoglobulin heavy-chain/light-chain pairs having different specificities [see, *e.g.*, Milstein and Cuello (1983) Nature 305: 537; International patent publication no. WO 93/08829; and Traunecker *et al.* (1991) EMBO J. 10: 3655, and U.S. Pat. No. 5,731,168 ("knob-in-hole" engineering)]. Multispecific antibodies may also be made by engineering electrostatic steering effects for making antibody Fc-heterodimeric molecules (see, *e.g.*, WO 2009/089004A1); cross-linking two or more antibodies or fragments [see, *e.g.*, U.S. Pat. No. 4,676,980; and Brennan *et al.* (1985) Science, 229: 81]; using leucine zippers to produce bispecific antibodies [see, *e.g.*, Kostelny *et al.* (1992) J. Immunol., 148(5):1547-1553]; using "diabody" technology for making bispecific antibody fragments [see, *e.g.*, Hollinger *et al.* (1993) Proc. Natl. Acad. Sci. USA, 90:6444-6448]; using single-chain Fv (sFv) dimers [see, *e.g.*, Gruber *et al.* (1994) J. Immunol., 152:5368]; and preparing trispecific antibodies (see, *e.g.*, Tutt *et al.* (1991) J. Immunol. 147: 60. Multispecific antibodies can be prepared as full-length antibodies or antibody fragments. 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, an antibody disclosed herein is a monoclonal antibody. Monoclonal antibody refers to an antibody obtained from a population of substantially 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 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 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.

For example, by using immunogens derived from activin, anti-protein/anti-peptide antisera or monoclonal antibodies can be made by standard protocols [see, *e.g.*, Antibodies: A Laboratory Manual ed. by Harlow and Lane (1988) Cold Spring Harbor Press: 1988]. A mammal, such as a mouse, hamster, or rabbit, can be immunized with an immunogenic form of the activin 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 portion of a activin 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 activin, antisera can be obtained and, if desired, polyclonal antibodies can be isolated from the serum. To produce monoclonal antibodies, antibody-producing cells (lymphocytes) can be harvested from an immunized animal and fused by standard somatic cell fusion procedures with immortalizing cells such as myeloma cells to yield hybridoma cells. Such techniques are well known in the art, and include, for example, the hybridoma technique [*see*, *e.g.*, Kohler 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 activin 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, 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 certain effector functions [*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 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. Natl. Acad. Sci. USA 83:7059-7063]; Hellstrom, I *et al.* (1985) Proc. Natl. Acad. Sci. USA 82:1499-1502; U.S. Pat. No. 5,821,337; Bruggemann, M. *et al.* (1987) J. Exp. Med. 166:1351-1361. Alternatively, non-radioactive assays 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. Natl. 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. 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) Intl. 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. 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 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 interactions 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 (e.g., and activin such as activin E and/or activin C binding).

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-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 is determined 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 the 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 nonproteinaceous 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, propropylene glycol homopolymers, prolypropylene 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 Molecule Antagonists

In other aspects, an ALK7:ActRIIB antagonist is a small molecule (ALK7:ActRIIB small molecule antagonist), or combination of small molecule antagonists. An ALK7:ActRIIB small molecule antagonist, or combination of small molecule antagonists, may inhibit, for example, one or more ALK7:ActRIIB-binding ligands, a type I receptor (e.g., ALK7), a type II receptor (e.g., ActRIIB), and/or co-receptor (e.g., Cripto or Cryptic). In some embodiments, ALK7:ActRIIB small molecule antagonist, or combination of small molecule antagonists, inhibits signaling mediated by one or more ALK7:ActRIIB-binding ligands, for example, as determined in a cell-based assay such as those described herein. As described herein, ALK7:ActRIIB small molecule antagonists may be used, alone or in combination with one or more supportive therapies or active agents, to treat a patient in need thereof (e.g., patients having kidney disease and/or a metabolic disorder).

In some embodiments, an ALK7:ActRIIB small molecule antagonist, or combination of small molecule antagonists, inhibits at least GDF11. In some embodiments, an ALK7:ActRIIB small molecule antagonist, or combination of small molecule antagonists, inhibits at least GDF8. In some embodiments, an ALK7:ActRIIB small molecule antagonist, or combination of small molecule antagonists, inhibits at least activin (activin A, activin B, activin C, activin E, activin AB, activin AC, activin BC, activin AE and/or activin BE). In some embodiments, an ALK7:ActRIIB small molecule antagonist, or combination of small molecule antagonists, inhibits at least GDF11, GDF8, and activin. In some embodiments, an ALK7:ActRIIB small molecule antagonist, or combination of small molecule antagonists, inhibits at least ALK7. In some embodiments, an ALK7:ActRIIB small molecule antagonist, or combination of small molecule antagonists, inhibits at least ActRIIB. In some embodiments, an ALK7:ActRIIB small molecule antagonist, or combination of small molecule antagonists, inhibits at least Cripto or Cryptic. In some embodiments, an ALK7:ActRIIB small molecule antagonist, or combination of small molecule antagonists, inhibits at least BMP6. In some embodiments, an ALK7:ActRIIB small molecule antagonist, or combination of small molecule antagonists, inhibits at least GDF3. In some embodiments, an ALK7:ActRIIB small molecule antagonist, or combination of small molecule antagonists,

inhibits at least BMP5. In some embodiments, an ALK7:ActRIIB small molecule antagonist, or combination of small molecule antagonists, inhibits at least BMP10. In some embodiments, an ALK7:ActRIIB small molecule antagonist, or combination of small molecule antagonists, inhibits at least nodal. In some embodiments, an ALK7:ActRIIB small molecule antagonist, or combination of small molecule antagonists, as disclosed herein does not inhibit or does not substantially inhibit BMP9.

ALK7:ActRIIB small molecule antagonists can be direct or indirect inhibitors. For example, an indirect small molecule antagonist, or combination of small molecule antagonists, may inhibit the expression (*e.g.*, transcription, translation, cellular secretion, or combinations thereof) of at least one or more TGF-β superfamily ligands [*e.g.*, activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC, activin B, activin BC, activin AE, or Activin BE), GDF11, BMP10, BMP9, BMP6, BMP5, GDF3, activin C, activin E, Activin AC, GDF8, BMP10, BMP6, BMP5, nodal, and GDF3, type I receptor (e.g., ALK7), type II receptors (e.g., ActRIIB), co-receptor (e.g., Cripto or Cryptic), and/or one or more downstream signaling components (e.g., Smads). Alternatively, a direct small molecule antagonist, or combination of small molecule antagonists, may directly bind to and inhibit, for example, one or more TGF-β superfamily ligands [*e.g.*, activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC, activin B, activin BC, activin AE, or Activin BE), GDF11, BMP10, BMP9, BMP6, BMP5, GDF3, activin C, activin E, activin AC, GDF8, BMP10, BMP6, BMP5, nodal, and GDF3, type I receptors (e.g., ALK7), type II receptors (e.g., ActRIIB), co-receptors (e.g., Cripto or Cryptic), and/or downstream signaling components (e.g., Smads). Combinations of one or more indirect and one or more direct ALK7:ActRIIB small molecule antagonists may be used in accordance with the methods disclosed herein.

Binding 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. These 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. Polynucleotide Antagonists

In other aspects, an ALK7:ActRIIB antagonist is a polynucleotide (ALK7:ActRIIB polynucleotide antagonist), or combination of polynucleotides. An ALK7:ActRIIB polynucleotide antagonist, or combination of polynucleotide antagonists, may inhibit, for example, one or more ALK7:ActRIIB-binding ligands, type I receptors (e.g., ALK7), type II receptors (e.g., ActRIIB), co-receptor (e.g., Cripto or Cryptic), and/or downstream signaling component (e.g., Smads). In some embodiments, ALK7:ActRIIB polynucleotide antagonist, or combination of polynucleotide antagonists, inhibits signaling mediated by one or more ALK7:ActRIIB-binding ligands, for example, as determined in a cell-based assay such as those described herein. As described herein, ALK7:ActRIIB polynucleotide antagonists may be used, alone or in combination with one or more supportive therapies or active agents, to treat a patient in need thereof (e.g., patients having kidney disease and/or a metabolic disorder).

In some embodiments, an ALK7:ActRIIB polynucleotide antagonists, or combination of polynucleotide antagonists, inhibits at least GDF11. In some embodiments, an ALK7:ActRIIB polynucleotide antagonist, or combination of polynucleotide antagonists, inhibits at least GDF8. In some embodiments, an ALK7:ActRIIB polynucleotide antagonist, or combination of polynucleotide antagonists, inhibits at least activin (activin A, activin B, activin C, activin E, activin AB, activin AC, activin AE, activin BC and/or activin BE). In some embodiments, an ALK7:ActRIIB polynucleotide antagonist, or combination of polynucleotide antagonists, inhibits at least GDF11, GDF8, and activin. In some

embodiments, an ALK7:ActRIIB polynucleotide antagonist, or combination of polynucleotide antagonists, inhibits at least ALK7. In some embodiments, an ALK7:ActRIIB polynucleotide antagonist, or combination of polynucleotide antagonists, inhibits at least ActRIIB. In some embodiments, an ALK7:ActRIIB polynucleotide antagonist, or combination of polynucleotide antagonists, inhibits at least Crypto or Cryptic. In some embodiments, an ALK7:ActRIIB polynucleotide antagonist, or combination of polynucleotide antagonists, inhibits at least ActRIIB. In some embodiments, an ALK7:ActIIB polynucleotide antagonist, or combination of polynucleotide antagonists, inhibits at least BMP6. In some embodiments, an ALK7:ActRIIB polynucleotide antagonist, or combination of polynucleotide antagonists, inhibits at least GDF3. In some embodiments, an ALK7:ActRIIB polynucleotide antagonist, or combination of polynucleotide antagonists, inhibits at least BMP10. In some embodiments, an ALK7:ActRIIB polynucleotide antagonist, or combination of polynucleotide antagonists, inhibits at least BMP5. In some embodiments, an ALK7:ActRIIB polynucleotide antagonist, or combination of polynucleotide antagonist, inhibits at least nodal. In some embodiments, an ALK7:ActRIIB polynucleotide antagonist, or combination of polynucleotide antagonists, as disclosed herein does not inhibit or does not substantially inhibit BMP9.

In some embodiments, the polynucleotide antagonists of the 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 GDF11, GDF8, activin (activin A, activin B, activin C, and activin E), BMP6, GDF3, BMP5, ALK7, ActRIIB, Cripto, Cryptic, Nodal, and BMP10 are known in the art. In addition, many different methods of generating polynucleotide antagonists are well known in the art. Therefore polynucleotide antagonists for use in accordance with this disclosure may be routinely made by the skilled person in the art based on the knowledge in the art and teachings provided herein.

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 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 gene disclosed herein. However, absolute complementarity, although preferred, is not required.

A sequence "complementary to at least a portion of an RNA," referred to herein, 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, 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 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'-non-translated, non-coding regions of a gene of the disclosure, could be used in an antisense approach to inhibit translation of an endogenous 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'-, 3'- or coding region of an mRNA of the disclosure, antisense 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 290:304-310], 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 (RNAi) molecules that target the expression of one or more of: GDF11, GDF8, activin (activin A, activin B, activin C, and activin E), BMP6, GDF3, BMP5, ALK7, ActRIIB, Cripto, Cryptic, nodal, and BMP10. 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 affect 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, 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. 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 nucleic acid ligands which bind with high affinity and specificity 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)].

F. Follistatin and FLRG Antagonists

It is known that members of the follistatin and FLRG group of proteins antagonize ligands that signal through the ALK7:ActRIIB pathway. Accordingly, in other aspects, an ALK7:ActRIIB antagonist is a follistatin or FLRG polypeptide, which may be used alone or in combination with one or more additional supportive therapies and/or active agents as disclosed herein to achieve a desired effect (e.g., treat patients having kidney disease and/or a metabolic disorder).

The term "follistatin polypeptide" includes polypeptides comprising any naturally occurring polypeptide of follistatin as well as any variants thereof (including mutants, fragments, fusions, and peptidomimetic forms) that retain a useful activity, and further includes any functional monomer or multimer of follistatin. In certain preferred embodiments, follistatin polypeptides of the disclosure bind to and/or inhibit activin and/or GDF8 activity. Variants of follistatin polypeptides that retain activin binding properties can be identified based on previous studies involving follistatin and activin interactions. For example, WO2008/030367 discloses specific follistatin domains ("FSDs") that are shown to be important for activin binding. As shown below in SEQ ID NOs: 90-94, the follistatin N-terminal domain ("FSND" SEQ ID NO: 92), FSD2 (SEQ ID NO: 94), and to a lesser extent

FSD1 (SEQ ID NO: 93) represent exemplary domains within follistatin that are important for activin binding. In addition, methods for making and testing libraries of polypeptides are described above in the context of ActRII polypeptides, and such methods also pertain to making and testing variants of follistatin. Follistatin polypeptides include polypeptides derived from the sequence of any known follistatin having a sequence at least about 80% identical to the sequence of a follistatin polypeptide, and optionally at least 85%, 90%, 95%, 96%, 97%, 98%, 99% or greater identity. Examples of follistatin polypeptides include the mature follistatin polypeptide or shorter isoforms or other variants of the human follistatin precursor polypeptide (SEQ ID NO: 90) as described, for example, in WO2005/025601.

The human follistatin precursor polypeptide isoform FST344 is as follows:

1 MVRARHQPGG LCLLLLLLCQ FMEDRSAQAG NCWLRQAKNG RCQVLYKTEL
51 SKEECCSTGR LSTSWTEEDV NDNTLFKWMI FNGGAPNCIP CKETCENVDC
101 GPGKKCRMNK KNKPRCVCAP DCSNITWKGP VCGLDGKTYR NECALLKARC
151 KEQPELEVQY QGRCKKTCRD VFCPGSSTCV VDQTNNAYCV TCNRICPEPA
201 SSEQYLCGND GVTYSSACHL RKATCLLGRS IGLAYEGKCI KAKSCEDIQC
251 TGGKKCLWDF KVGRGRCSLC DELCPDSKSD EPVCASDNAT YASECAMKEA
301 ACSSGVLLEV KHSGSCNSIS EDTEEEEEDE DQDYSFPISS ILEW

(SEQ ID NO: 90; NCBI Reference No. NP_037541.1)

The signal peptide is underlined; also underlined above are the last 27 residues which represent the C-terminal extension distinguishing this follistatin isoform from the shorter follistatin isoform FST317 shown below.

The human follistatin precursor polypeptide isoform FST317 is as follows:

1 MVRARHQPGG LCLLLLLLCQ FMEDRSAQAG NCWLRQAKNG RCQVLYKTEL
51 SKEECCSTGR LSTSWTEEDV NDNTLFKWMI FNGGAPNCIP CKETCENVDC
101 GPGKKCRMNK KNKPRCVCAP DCSNITWKGP VCGLDGKTYR NECALLKARC
151 KEQPELEVQY QGRCKKTCRD VFCPGSSTCV VDQTNNAYCV TCNRICPEPA
201 SSEQYLCGND GVTYSSACHL RKATCLLGRS IGLAYEGKCI KAKSCEDIQC
251 TGGKKCLWDF KVGRGRCSLC DELCPDSKSD EPVCASDNAT YASECAMKEA
301 ACSSGVLLEV KHSGSCN (SEQ ID NO: 91; NCBI Reference No. NP_006341.1)

The signal peptide is underlined.

The follistatin N-terminal domain (FSND) sequence is as follows:

GNCWLRQAKNGRCQVLYKTELSKEECCSTGRLSTSWTEEDVNDN TLFKWMIFNGGAPNCIPCK (SEQ ID NO: 92; FSND)

The FSD1 and FSD2 sequences are as follows:

ETCENVDCGPGKKCRMNKKNKPRCV (SEQ ID NO: 93; FSD1)

KTCRDVFCPGSSTCVVDQTNNAYCVT (SEQ ID NO: 94; FSD2)

In other aspects, an ALK7:ActRIIB antagonist is a follistatin-like related gene (FLRG), also known as follistatin-related protein 3 (FSTL3). The term "FLRG polypeptide" includes polypeptides comprising any naturally occurring polypeptide of FLRG as well as any variants thereof (including mutants, fragments, fusions, and peptidomimetic forms) that retain a useful activity. In certain embodiments, FLRG polypeptides of the disclosure bind to and/or inhibit activin activity, particularly activin A. Variants of FLRG polypeptides that retain activin binding properties can be identified using routine methods to assay FLRG and activin interactions (see, *e.g.*, US 6,537,966). In addition, methods for making and testing libraries of polypeptides are described above in the context of ActRII and ALK7 polypeptides and such methods also pertain to making and testing variants of FLRG. FLRG polypeptides include polypeptides derived from the sequence of any known FLRG having a sequence at least about 80% identical to the sequence of an FLRG polypeptide, and optionally at least 85%, 90%, 95%, 97%, 99% or greater identity.

The human FLRG precursor (follistatin-related protein 3 precursor) polypeptide is as follows:

1 MRPGAPGPLW PLPWGALAWA VGFVSSMGSG NPAPGGVCWL QQGQEATCSL

51 VLQTDVTRAE CCASGNIDTA WSNLTHPGNK INLLGFLGLV HCLPCKDSCD

101 GVECGPGKAC RMLGGRPRCE CAPDCSGLPA RLQVCGSDGA TYRDECELRA

151 ARCRGHPDLS VMYRGRCRKS CEHVVCPRPQ SCVVDQTGSA HCVVCRAAPC

201 PVPSSPGQEL CGNNNVTYIS SCHMRQATCF LGRSIGVRHA GSCAGTPEEP

251 PGGESAEEEE NFV (SEQ ID NO: 95; NCBI Reference No. NP_005851.1)

The signal peptide is underlined.

In certain embodiments, functional variants or modified forms of the follistatin polypeptides and FLRG polypeptides include fusion proteins having at least a portion of the follistatin polypeptide or FLRG polypeptide and one or more fusion domains, such as, for

example, domains that facilitate isolation, detection, stabilization or multimerization of the polypeptide. Suitable fusion domains are discussed in detail above with reference to the ActRII polypeptides. In some embodiment, an antagonist agent of the disclosure is a fusion protein comprising an activin-binding portion of a follistatin polypeptide fused to an Fc domain. In another embodiment, an antagonist agent of the disclosure is a fusion protein comprising an activin binding portion of an FLRG polypeptide fused to an Fc domain.

G. Lefty A and B

The Lefty A and B proteins are known to regulate Nodal and other proteins that signal through the ALK7:ActRIIB pathway. Accordingly, in other aspects, an ALK7:ActRIIB antagonist is a Lefty A or Lefty B polypeptide, which may be used alone or in combination with one or more additional supportive therapies and/or active agents as disclosed herein to achieve a desired effect (e.g., treat kidney disease and/or a metabolic condition or disorder).

The term "Lefty A polypeptide" includes polypeptides comprising any naturally occurring polypeptide of Lefty A as well as any variants thereof (including mutants, fragments, fusions, and peptidomimetic forms) that retain a useful activity, and further includes any functional monomer or multimer of Lefty A. In certain preferred embodiments, Lefty A polypeptides of the disclosure binds to and/or inhibit nodal activity. In addition, methods for making and testing libraries of polypeptides are described above in the context of ActRII and ALK7 polypeptides, and such methods also pertain to making and testing variants of Lefty A. Lefty A polypeptides include polypeptides derived from the sequence of any known Lefty A having a sequence at least about 80% identical to the sequence of a Lefty A polypeptide, and optionally at least 85%, 90%, 95%, 96%, 97%, 98%, 99% or greater identity. Examples of Lefty A polypeptides include the mature Lefty A polypeptide or shorter isoforms or other variants of the human Lefty A precursor polypeptide (SEQ ID NO: 96).

The human Lefty A precursor polypeptide is as follows:

```
  1 MWPLWLCWAL WVLPLAGPGA ALTEEQLLGS LLRQLQLSEV PVLDRADMEK LVIPAHVRAQ
 61 YVVLLRRSHG DRSRGKRFSQ SFREVAGRFL ASEASTHLLV FGMEQRLPPN SELVQAVLRL
121 FQEPVPKAAL HRHGRLSPRS AQARVTVEWL RVRDDGSNRT SLIDSRLVSV HESGWKAFDV
181 TEAVNFWQQL SRPRQPLLLQ VSVQREHLGP LASGAHKLVR FASQGAPAGL GEPQLELHTL
241 DLRDYGAQGD CDPEAPMTEG TRCCRQEMYI DLQGMKWAKN WVLEPPGFLA YECVGTCQQP
301 PEALAFNWPF LGPRQCIASE TASLPMIVSI KEGGRTRPQV VSLPNMRVQK CSCASDGALV
361 PRRLQP
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(SEQ ID NO: 96; GenBank Id: AAD48145.1)

The signal peptide is underlined.

The term "Lefty B polypeptide" includes polypeptides comprising any naturally occurring polypeptide of Lefty B as well as any variants thereof (including mutants, fragments, fusions, and peptidomimetic forms) that retain a useful activity, and further includes any functional monomer or multimer of Lefty B. In certain preferred embodiments, Lefty B polypeptides of the disclosure inhibit nodal activity. In addition, methods for making and testing libraries of polypeptides are described above in the context of ActRII and ALK7 polypeptides, and such methods also pertain to making and testing variants of Lefty B. Lefty B polypeptides include polypeptides derived from the sequence of any known Lefty B having a sequence at least about 80% identical to the sequence of a Lefty B polypeptide, and optionally at least 85%, 90%, 95%, 96%, 97%, 98%, 99% or greater identity. Examples of Lefty B polypeptides include the mature Lefty B polypeptide or shorter isoforms or other variants of the human Lefty B precursor polypeptide (SEQ ID NO: 97).

The human Lefty B precursor polypeptide is as follows:

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  1 MQPLWLCWAL WVLPLASPGA ALTGEQLLGS LLRQLQLKEV PTLDRADMEE LVIPTHVRAQ
 61 YVALLQRSHG DRSRGKRFSQ SFREVAGRFL ALEASTHLLV FGMEQRLPPN SELVQAVLRL
121 FQEPVPKAAL HRHGRLSPRS ARARVTVEWL RVRDDGSNRT SLIDSRLVSV HESGWKAFDV
181 TEAVNFWQQL SRPRQPLLLQ VSVQREHLGP LASGAHKLVR FASQGAPAGL GEPQLELHTL
241 DLGDYGAQGD CDPEAPMTEG TRCCRQEMYI DLQGMKWAEN WVLEPPGFLA YECVGTCRQP
301 PEALAFKWPF LGPRQCIASE TDSLPMIVSI KEGGRTRPQV VSLPNMRVQK CSCASDGALV
361 PRRLQP
```

(SEQ ID NO: 97; GenBank Id: AAD48144.1)

The signal peptide is underlined.

In certain embodiments, functional variants or modified forms of the Lefty A polypeptides and Lefty B polypeptides include fusion proteins having at least a portion of the Lefty A polypeptide or Lefty polypeptide and one or more fusion domains, such as, for example, domains that facilitate isolation, detection, stabilization or multimerization of the polypeptide. Suitable fusion domains are discussed in detail above with reference to the ActRII and ALK7 polypeptides. In some embodiment, an antagonist agent of the disclosure is a fusion protein comprising a nodal-binding portion of a Lefty A and/or Lefty B polypeptide fused to an Fc domain.

H. DAN-related Proteins

Members of the DAN family of proteins are known to regulate ligands that signal through the ALK7:ActRIIB pathway. Accordingly, in other aspects, an ALK7:ActRIIB antagonist is a DAN-related protein (e.g., Cerberus and Coco), which may be used alone or in

combination with one or more additional supportive therapies and/or active agents as disclosed herein to achieve a desired effect (e.g., treat patients having kidney disease and/or a metabolic disorder).

The term "Cerberus polypeptide" includes polypeptides comprising any naturally occurring polypeptide of Cerberus as well as any variants thereof (including mutants, fragments, fusions, and peptidomimetic forms) that retain a useful activity, and further includes any functional monomer or multimer of Cerberus. In certain preferred embodiments, Cerberus polypeptides of the disclosure bind to and/or inhibit nodal activity. In addition, methods for making and testing libraries of polypeptides are described above in the context of ActRII and ALK7 polypeptides, and such methods also pertain to making and testing variants of Cerberus. Cerberus polypeptides include polypeptides derived from the sequence of any known Cerberus having a sequence at least about 80% identical to the sequence of a Cerberus polypeptide, and optionally at least 85%, 90%, 95%, 96%, 97%, 98%, 99% or greater identity. Examples of Cerberus polypeptides include the mature Cerberus polypeptide or shorter isoforms or other variants of the human Cerberus precursor polypeptide (SEQ ID NO: 98).

The human Cerberus precursor polypeptide is as follows:

```
  1 MHLLLFQLLV LLPLGKTTRH QDGRQNQSSL SPVLLPRNQR ELPTGNHEEA EEKPDLFVAV
 61 PHLVATSPAG EGQRQREKML SRFGRFWKKP EREMHPSRDS DSEPFPPGTQ SLIQPIDGMK
121 MEKSPLREEA KKFWHHFMFR KTPASQGVIL PIKSHEVHWE TCRTVPFSQT ITHEGCEKVV
181 VQNNLCFGKC GSVHFPGAAQ HSHTSCSHCL PAKFTTMHLP LNCTELSSVI KVVMLVEECQ
241 CKVKTEHEDG HILHAGSQDS FIPGVSA
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(SEQ ID NO: 98; NCBI Reference: NP_005445.1)

The signal peptide is underlined.

The term "Coco polypeptide" includes polypeptides comprising any naturally occurring polypeptide of Coco as well as any variants thereof (including mutants, fragments, fusions, and peptidomimetic forms) that retain a useful activity, and further includes any functional monomer or multimer of Coco. In certain preferred embodiments, Coco polypeptides of the disclosure bind to and/or inhibit nodal activity. In addition, methods for making and testing libraries of polypeptides are described above in the context of ActRII and ALK7 polypeptides, and such methods also pertain to making and testing variants of Coco. Coco polypeptides include polypeptides derived from the sequence of any known Coco having a sequence at least about 80% identical to the sequence of a Coco polypeptide, and optionally at least 85%, 90%, 95%, 96%, 97%, 98%, 99% or greater identity. Examples of Coco polypeptides include the mature Coco polypeptide or shorter isoforms or other variants of the human Coco precursor polypeptide (SEQ ID NO: 99).

The human Coco precursor polypeptide is as follows:

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  1 MLLGQLSTLL CLLSGALPTG SGRPEPQSPR PQSWAAANQT WALGPGALPP LVPASALGSW
 61 KAFLGLQKAR QLGMGRLQRG QDEVAAVTLP LNPQEVIQGM CKAVPFVQVF SRPGCSAIRL
121 RNHLCFGHCS SLYIPGSDPT PLVLCNSCMP ARKRWAPVVL WCLTGSSASR RRVKISTMLI
181 EGCHCSPKA
```

(SEQ ID NO: 99; GenBank Id:)

The signal peptide is underlined.

In certain embodiments, functional variants or modified forms of the Cerberus polypeptides and Coco polypeptides include fusion proteins having at least a portion of the Cerberus polypeptide and/or Coco polypeptide and one or more fusion domains, such as, for example, domains that facilitate isolation, detection, stabilization or multimerization of the polypeptide. Suitable fusion domains are discussed in detail above with reference to the ActRII and ALK7 polypeptides. In some embodiment, an antagonist agent of the disclosure is a fusion protein comprising a nodal-binding portion of a Cerberus and/or Coco polypeptide fused to an Fc domain.

3. Screening Assays

In certain embodiments, ALK7:ActRIIB receptor heteromultimers (e.g., ALK7:ActRIIB heterodimers) may be used to generate and/or screen for ALK7:ActRIIB inhibitors, particularly inhibitors that interfere with ALK7-ligand and/or ALK7-Type II receptor (e.g., ActRIIB) interaction (e.g., anti-ALK7 antibodies).

As discussed herein, TGF-beta superfamily ligand signals are mediated by complexes of Type I and Type II kinase receptors, which phosphorylate and activate downstream Smad proteins upon ligand stimulation [see, e.g., Massague (2000) Nat. Rev. Mol. Cell. Biol. 1: 169-178]. While essential for signaling, Type I receptors generally have very weak affinity for TGF-beta superfamily ligands. Instead, Type II receptors generally bind to ligands with high affinity. This ligand binding promotes stable complex formation between Type I and II receptors, resulting in phosphorylation of type I receptors by Type II receptors and thus activation of Smad proteins. In view of the weaker affinity, it is generally difficult to observe ligand binding activity of Type I receptors in isolation.

Indeed, the examples herein demonstrate that an ActRIIB homodimer binds to many different ligands, many with high affinity. In contrast, an ALK7 homodimer did not display affinity for any of the ligands examined. As ligands do not detectably bind to ALK7, it is difficult to screen for/identify agents that can inhibit ALK7 activity. However, as demonstrated by the examples herein, the ligand-binding activity of ALK7 becomes measureable when paired with a type II receptor. Thus, ALK7:ActRIIB heteromultimers (e.g., heterodimers) are a useful tool for identifying ALK7 inhibitors using various screening assays such as described herein as well as those known in the art. Therefore, in some embodiments, the present disclosure relate to the use of ALK7:Type II receptor heteromultimers (e.g., ALK7:ActRIIB heteromultimers) to identify (screen for) ALK7 inhibitors.

For example, ALK7:Type II receptor heteromultimers (e.g., ALK7:ActRIIB heteromultimers) may be particular useful for generating/identifying antibodies that bind to ALK7, particularly antibodies that bind to and inhibit ALK7. While ALK7 polypeptides, including ALK7 homodimers as described herein, may be used to generate/identify antibodies that bind to ALK7, simple binding is not sufficient to demonstrate that a given ALK7 antibody can inhibit an ALK7 activity (e.g., interfere with ALK7-ligand or ALK7-Type II receptor interactions). As demonstrated herein, ALK7 polypeptides do not display ligand-binding activity in the absence of a Type II receptor, making it difficult to identify antibodies that can inhibit ALK7. ALK7:Type II receptor heteromultimers (e.g., ALK7:ActRIIB heteromultimers) solve this problem by providing a functional read out for ALK7 activity. Moreover, without wishing to be bound to any particular mechanisms of action, association with a Type II receptor (e.g., ActRIIB) may improve the ability to generate antibodies that inhibit ALK7 activity. For example, association with a Type II receptor may help to stabilize ALK7 in such a way as to optimize the processing and presentation of important ALK7 epitopes that mediate ligand-binding activities and thus improve the development of ALK7 inhibitory antibodies. Therefore, in some embodiments, the present disclosure relates to the use of ALK7:Type II heteromultimers (e.g., ALK7:ActRIIB heteromultimers) to generate antibodies that bind to and inhibit ALK7. Methods for generating and screening for antibodies are well known in the art and described herein, and ALK7-Type II heteromultimers (e.g., ALK7:ActRIIB heteromultimers) may be used in accordance with one or more of these methods to generate/identify antibodies that bind to and inhibit ALK7 activity.

In certain embodiments, ALK7:Type II receptor heterodimers (e.g., ALK7:ActRIIB heteromultimers such as heterodimers) may be used to identify activin C inhibitors, particularly inhibitors that bind to activin C and interfere with activin C-Type I/II receptor interaction.

Surprisingly, the data presented herein demonstrates that ALK7:ActRIIB heterodimers bind to activin C. Insight into this novel interaction provides an opportunity to develop/identify activin C inhibitors. In addition, given the structural similarity between activin C and activin E, it is expected that activin E also binds to ALK7:ActRIIB, and thus this novel interaction may be used to identify activin E antagonists as well. Heterodimeric ligands, such as activin AC, AE, BC and BE are also included in the reference to activin C or E, respectively in this section of the disclosure. Agents that affect both activin C and E are also contemplated. Various types of activin C or E antagonists are expected to be useful in accordance with the methods described herein including, for example, small molecules, antibodies, and inhibitor nucleic acids, particularly those that disrupt activin C (or E)-ALK7:ActRIIB interaction. An agent (e.g., an antibody or small molecule) that is specifically reactive with activin C or E and which either binds to activin C or E so as to compete with its binding to ALK7 and/or ActRIIB or otherwise inhibits activin C or E-mediated signaling may be used as an antagonist of activin C or E. Likewise, an agent that is specifically reactive with ALK7 and/or ActRIIB and which disrupts activin C or E binding may be used as an antagonist. In regard to antibodies, immunogens can be derived from an activin C or E polypeptide, ALK7 polypeptide, or ActRIIB polypeptide and used to generate antibodies using standard methods known in the art. See, e.g., Antibodies: A Laboratory Maneal ed. by Harlow and Lane, Cold Spring Harbor Press, 1988. Using various screening assays known in the art such as those described herein, ALK7:Type II receptor heterodimers (e.g., ALK7:ActRIIB heteromultimers such as heterodimers) may be used to identify activin C or E inhibitors.

In certain aspects, the present disclosure relates to the use of ALK7:ActRIIB heteromultimer complexes to identify compounds (agents) which are agonists or antagonists of TGFβ superfamily receptors. Compounds identified through this screening can be tested to assess their ability to modulate tissue growth, such as bone, cartilage, muscle, fat, and/or neurons, growth *in vivo* or *in vitro*. These compounds can be tested, for example, in animal models.

There are numerous approaches to screening for therapeutic agents for modulating tissue growth by targeting TGFβ superfamily ligand signaling (*e.g.*, SMAD signaling). In certain embodiments, high-throughput screening of compounds can be carried out to identify agents that perturb TGFβ superfamily receptor-mediated effects on a selected cell line. In certain embodiments, the assay is carried out to screen and identify compounds that specifically inhibit or reduce binding of an ALK7:ActRIIB heteromultimer to a binding partner (*e.g.*, activin, GDF11, GDF8, BMP6, GDF3, BMP5, nodal, and BMP10). Alternatively, the assay can be used to identify compounds that enhance binding of an ALK7:ActRIIB heteromultimer to a ligand. In a further embodiment, the compounds can be identified by their ability to interact with an ALK7:ActRIIB heteromultimer.

In some embodiments, the present disclosure relates to the use of an ALK7:ActRIIB heteromultimer and activin C or E polypeptides to identify compounds which are agonists or antagonists of the activin C (or E)-ALK7:ActRIIB signaling pathway. Compounds identified through this screening assay can be tested to assess their ability to modulate activin C or E 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 activin C or E and ALK7:ActRIIB heteromultimers. In certain embodiments, high-throughput screening of compounds can be carried out to identify agents that perturb activin C or E or ALK7:ActRIIB-mediated cell signaling. In certain embodiments, the assay is carried out to screen and identify compounds that specifically inhibit or reduce binding of an ALK7:ActRIIB heteromultimer to activin C or E. Alternatively, the assay can be used to identify compounds that enhance binding of an ALK7:ActRIIB heteromultimer to activin C or E. In a further embodiment, the compounds can be identified by their ability to interact with activin C or E or an ALK7:ActRIIB heteromultimer.

A variety of assay formats will suffice and, in light of the present disclosure, those not expressly described herein will nevertheless be comprehended by one of ordinary skill in the art. As described herein, the test compounds (agents) of the invention may be created by any combinatorial chemical method. Alternatively, the subject compounds may be naturally occurring biomolecules synthesized *in vivo* or *in vitro*. Compounds (agents) to be tested for their ability to act as modulators of tissue growth can be produced, for example, by bacteria, yeast, plants or other organisms (*e.g.*, natural products), produced chemically (*e.g.*, small molecules, including peptidomimetics), or produced recombinantly. Test compounds

contemplated by the present invention include non-peptidyl organic molecules, peptides, polypeptides, peptidomimetics, sugars, hormones, and nucleic acid molecules. In certain embodiments, the test agent is a small organic molecule having a molecular weight of less than about 2,000 Daltons.

The test compounds of the disclosure can be provided as single, discrete entities, or provided in libraries of greater complexity, such as made by combinatorial chemistry. These libraries can comprise, for example, alcohols, alkyl halides, amines, amides, esters, aldehydes, ethers and other classes of organic compounds. Presentation of test compounds to the test system can be in either an isolated form or as mixtures of compounds, especially in initial screening steps. Optionally, the compounds may be optionally derivatized with other compounds and have derivatizing groups that facilitate isolation of the compounds. Non-limiting examples of derivatizing groups include biotin, fluorescein, digoxygenin, green fluorescent protein, isotopes, polyhistidine, magnetic beads, glutathione S-transferase (GST), photoactivatible 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 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 an ALK7:ActRIIB heteromultimer complex and a binding partner (*e.g.*, activin C or E).

Merely to illustrate, in an exemplary screening assay of the present disclosure, the compound of interest is contacted with an isolated and purified ALK7:ActRIIB heteromultimer which is ordinarily capable of binding to activin C, as appropriate for the intention of the assay. To the mixture of the compound and ALK7:ActRIIB heteromultimer complex is then added to a composition containing activin C or E. Detection and quantification of ALK7:ActRIIB heteromultimer-activin C or E complexes provides a means for determining the compound's efficacy at inhibiting (or potentiating) complex formation between the ALK7:ActRIIB heteromultimer and activin C or E. 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 purified activin C or E is added to a composition containing the ALK7:ActRIIB heteromultimer, and the formation of ALK7:ActRIIB heteromultimer-activin C or E 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.

Binding of a ALK7:ActRIIB heteromultimer complex to another protein (e.g., activin C or E) 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 ^{3}H), fluorescently labeled (*e.g.*, FITC), or enzymatically labeled ALK7:ActRIIB heteromeric complex and/or a binding partner (e.g., activin C or E), by immunoassay, or by chromatographic detection.

In certain embodiments, the present disclosure 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 an ALK7:ActRIIB heteromultimer and a binding protein (e.g., activin C or E). 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 disclosure.

Moreover, the present disclosure contemplates the use of an interaction trap assay, also known as the "two-hybrid assay," for identifying agents that disrupt or potentiate interaction between an ALK7:ActRIIB heteromultimer and a binding partner (e.g., activin C or E). See, *e.g.*, 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 disclosure contemplates the use of reverse two-hybrid systems to identify compounds (*e.g.*, small molecules or peptides) that dissociate interactions between an ALK7:ActRIIB heteromultimer and a binding protein (e.g., activin C or E) [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 an ALK7:ActRIIB heteromultimer or activin C or E of the disclosure. The interaction between the compound and an ALK7:ActRIIB heteromultimer or activin C or E may be covalent or non-covalent. For example, such interaction can be identified at the protein level using *in vitro* biochemical methods, including photo-crosslinking, radiolabeled ligand binding, and affinity chromatography [Jakoby WB *et al.* (1974) Methods in Enzymology 46:1]. In certain cases, the compounds may be screened in a mechanism-based assay, such as an assay to detect compounds which bind to an ALK7:ActRIIB heteromultimer or activin C or E. This may include a solid-phase or fluid-phase binding event. Alternatively, the gene encoding an ALK7:ActRIIB heteromultimer or activin C or E 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 high-throughput screening or with individual members of the library. Other mechanism-based binding assays may be used; for example, binding assays which detect changes in free energy. Binding assays can be performed with the target fixed to a well, bead or chip or captured by an immobilized antibody or resolved by capillary electrophoresis. The bound compounds may be detected usually using colorimetric endpoints or fluorescence or surface plasmon resonance.

4. Exemplary Therapeutic Uses

In certain embodiments, an ALK7:ActRIIB antagonist, or combinations of such antagonists, of the present disclosure can be used to treat or prevent a disease or condition that is associated with abnormal activity of an ALK7:ActRIIB-binding ligand. These diseases, disorders, or conditions are generally referred to herein as "ALK7:ActRIIB-associated conditions" or "ALK7:ActRIIB-associated disorders." In certain embodiments, the present disclosure provides methods of treating or preventing an ALK7:ActRIIB-associated condition in an individual by administering to an individual in need thereof a therapeutically effective amount of an ALK7:ActRIIB antagonist (e.g., an ALK7:ActRIIB heteromultimer such as an ALK7:ActRIIB heterodimer), or combinations of such antagonists, as described herein. The terms "subject," an "individual," or a "patient" are interchangeable throughout the specification. Any of the ALK7:ActRIIB antagonists of the disclosure can

potentially be employed individually or in combination for therapeutic uses disclosed herein. These methods are particularly aimed at therapeutic and prophylactic treatments of mammals including, for example, rodents, primates, and humans.

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 term "treating" as used herein includes amelioration or elimination of the condition once it has been established. In either case, prevention or treatment may be discerned in the diagnosis provided by a physician or other health care provider and the intended result of administration of the therapeutic agent.

In general, treatment or prevention of a disease or condition as described in the present disclosure is achieved by administering an ALK7:ActRIIB antagonist, or combinations of such antagonists, of the present disclosure in an "effective amount". An effective amount of an agent refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic or prophylactic result. A "therapeutically effective amount" of an agent of the present disclosure may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the agent to elicit a desired response in the individual. A "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result.

The kidneys maintain many features of the blood, including volume, pH balance, electrolyte concentrations, and blood pressure, as well as bearing responsibility for toxin and waste filtration. These functions depend upon the intricate structure of the kidney nephrons, constant flow of blood through the various capillaries of the kidney, and the regulation of the kidney by signals from the rest of the body, including endocrine hormones. Problems with kidney function manifest by direct mechanisms (*e.g.* genetic defects, infection, or toxin exposure) and by indirect mechanisms progressively proceeding from long term stressors like hypertrophy and hyperfiltration (themselves often a result of more direct insults to kidney function). Due to the central role of the kidney in blood maintenance and waste secretion, kidney-associated disease manifestations are many and varied; they can be reviewed in Harrison's Principles of Internal Medicine, 18th edition, McGraw Hill, N.Y., Part 13, Chp 277-289.

As described herein, an ALK7:ActRIIB antagonist had various beneficial effects in a kidney disease model. In particular, treatment with an ALK7:ActRIIB heteromultimer reduced kidney tissue damage, inflammation, and fibrosis in subjects having unilateral ureteral obstruction. These data indicate that ALK7:ActRIIB antagonist may be used to treat or prevent kidney disease, particularly treating or preventing various complications (manifestations) of kidney disease including, for example, kidney tissue damage, inflammation, and/or fibrosis.

Therefore, methods of this invention can be applied to various kidney-associated diseases or conditions. As used herein, "kidney-associated disease or condition" can refer to any disease, disorder, or condition that affects the kidneys or the renal system. Examples of kidney-associated diseases or conditions include, but are not limited to, chronic kidney diseases (or failure), acute kidney diseases (or failure), primary kidney diseases, non-diabetic kidney diseases, glomerulonephritis, interstitial nephritis, diabetic kidney diseases, diabetic nephropathy, glomerulosclerosis, rapid progressive glomerulonephritis, renal fibrosis, Alport syndrome, IDDM nephritis, mesangial proliferative glomerulonephritis, membranoproliferative glomerulonephritis, crescentic glomerulonephritis, renal interstitial fibrosis, focal segmental glomerulosclerosis, membranous nephropathy, minimal change disease, pauci-immune rapid progressive glomerulonephritis, IgA nephropathy, polycystic kidney disease, Dent's disease, nephrocytinosis, Heymann nephritis, autosomal dominant (adult) polycystic kidney disease, autosomal recessive (childhood) polycystic kidney disease, acute kidney injury, nephrotic syndrome, renal ischemia, podocyte diseases or disorders, proteinuria, glomerular diseases, membranous glomerulonephritis, focal segmental glomerulonephritis, pre-eclampsia, eclampsia, kidney lesions, collagen vascular diseases, benign orthostatic (postural) proteinuria, IgM nephropathy, membranous nephropathy, sarcoidosis, diabetes mellitus, kidney damage due to drugs, Fabry's disease, aminoaciduria, Fanconi syndrome, hypertensive nephrosclerosis, interstitial nephritis, Sickle cell disease, hemoglobinuria, myoglobinuria, Wegener's Granulomatosis, Glycogen Storage Disease Type 1, chronic kidney disease, chronic renal failure, low Glomerular Filtration Rate (GFR), nephroangiosclerosis, lupus nephritis, ANCA-positive pauci-immune crescentic glomerulonephritis, chronic allograft nephropathy, nephrotoxicity, renal toxicity, kidney necrosis, kidney damage, glomerular and tubular injury, kidney dysfunction, nephritic syndrome, acute renal failure, chronic renal failure, proximal tubal dysfunction, acute kidney transplant rejection, chronic kidney transplant rejection, non-IgA mesangioproliferative

glomerulonephritis, postinfectious glomerulonephritis, vasculitides with renal involvement of any kind, any hereditary renal disease, any interstitial nephritis, renal transplant failure, kidney cancer, kidney disease associated with other conditions (e.g., hypertension, diabetes, and autoimmune disease), Dent's disease, nephrocytinosis, Heymann nephritis, a primary kidney disease, a collapsing glomerulopathy, a dense deposit disease, a cryoglobulinemia-associated glomerulonephritis, an Henoch-Schonlein disease, a postinfectious glomerulonephritis, a bacterial endocarditis, a microscopic polyangitis, a Churg-Strauss syndrome, an anti-GBM-antibody mediated glomerulonephritis, amyloidosis, a monoclonal immunoglobulin deposition disease, a fibrillary glomerulonephritis, an immunotactoid glomerulopathy, ischemic tubular injury, a medication-induced tubulo-interstitial nephritis, a toxic tubulo-interstitial nephritis, an infectious tubulo-interstitial nephritis, a bacterial pyelonephritis, a viral infectious tubulo-interstitial nephritis which results from a polyomavirus infection or an HIV infection, a metabolic-induced tubulo-interstitial disease, a mixed connective disease, a cast nephropathy, a crystal nephropathy which may results from urate or oxalate or drug-induced crystal deposition, an acute cellular tubulo-interstitial allograft rejection, a tumoral infiltrative disease which results from a lymphoma or a post-transplant lymphoproliferative disease, an obstructive disease of the kidney, vascular disease, a thrombotic microangiopathy, a nephroangiosclerosis, an atheroembolic disease, a mixed connective tissue disease, a polyarteritis nodosa, a calcineurin-inhibitor induced-vascular disease, an acute cellular vascular allograft rejection, an acute humoral allograft rejection, early renal function decline (ERFD), end stage renal disease (ESRD), renal vein thrombosis, acute tubular necrosis, acute interstitial nephritis, established chronic kidney disease, renal artery stenosis, ischemic nephropathy, uremia, drug and toxin-induced chronic tubulointerstitial nephritis, reflux nephropathy, kidney stones, Goodpasture's syndrome, normocytic normochromic anemia, renal anemia, diabetic chronic kidney disease, IgG4-related disease, von Hippel-Lindau syndrome, tuberous sclerosis, nephronophthisis, medullary cystic kidney disease, renal cell carcinoma, adenocarcinoma, nephroblastoma, lymphoma, leukemia, hyposialylation disorder, chronic cyclosporine nephropathy, renal reperfusion injury, renal dysplasia, azotemia, bilateral arterial occlusion, acute uric acid nephropathy, hypovolemia, acute bilateral obstructive uropathy, hypercalcemic nephropathy, hemolytic uremic syndrome, acute urinary retention, malignant nephrosclerosis, postpartum glomerulosclerosis, scleroderma, non-Goodpasture's anti-GBM disease, microscopic polyarteritis nodosa, allergic granulomatosis, acute radiation nephritis, post-streptococcal glomerulonephritis, Waldenstrom's macroglobulinemia, analgesic nephropathy, arteriovenous

fistula, arteriovenous graft, dialysis, ectopic kidney, medullary sponge kidney, renal osteodystrophy, solitary kidney, hydronephrosis, microalbuminuria, uremia, haematuria, hyperlipidemia, hypoalbuminaemia, lipiduria, acidosis, hyperkalemia, and edema.

In some embodiments, an ALK7:ActRIIB antagonist, or combinations of such antagonists, of the present disclosure (e.g., ALK7:ActRIIB heteromultimers such as an ALK7:ActRIIB heterodimer) may be used to treat or prevent chronic kidney disease, optionally in combination with one or more supportive therapies for treating chronic kidney disease. In some embodiments, an ALK7:ActRIIB antagonist, or combinations of such antagonists, of the present disclosure (e.g., ALK7:ActRIIB heteromultimers such as an ALK7:ActRIIB heterodimer) may be used to treat or prevent one or more complications (symptoms or manifestations) of chronic kidney disease (e.g., tissue damage, inflammation, and/or fibrosis), optionally in combination with one or more supportive therapies for treating chronic kidney disease. In some embodiments, an ALK7:ActRIIB antagonist, or combinations of such antagonists, of the present disclosure (e.g., ALK7:ActRIIB heteromultimers such as an ALK7:ActRIIB heterodimer) may be used to treat or prevent end-stage kidney failure, optionally in combination with one or more supportive therapies for treating end-stage kidney disease. Chronic kidney disease (CKD), also known as chronic renal disease, is a progressive loss in renal function over a period of months or years. The symptoms of worsening kidney function may include feeling generally unwell and experiencing a reduced appetite. Often, chronic kidney disease is diagnosed as a result of screening of people known to be at risk of kidney problems, such as those with high blood pressure or diabetes and those with a blood relative with CKD. This disease may also be identified when it leads to one of its recognized complications, such as cardiovascular disease, anemia, or pericarditis. Recent professional guidelines classify the severity of CKD in five stages, with stage 1 being the mildest and usually causing few symptoms and stage 5 being a severe illness with poor life expectancy if untreated. Stage 5 CKD is often called end-stage kidney disease, end-stage renal disease, or end-stage kidney failure, and is largely synonymous with the now outdated terms chronic renal failure or chronic kidney failure; and usually means the patient requires renal replacement therapy, which may involve a form of dialysis, but ideally constitutes a kidney transplant. CKD is initially without specific symptoms and is generally only detected as an increase in serum creatinine or protein in the urine. As the kidney function decreases, various symptoms may manifest as described below. Blood pressure may be increased due to fluid overload and production of vasoactive

hormones created by the kidney via the renin-angiotensin system, increasing one's risk of developing hypertension and/or suffering from congestive heart failure. Urea may accumulate, leading to azotemia and ultimately uremia (symptoms ranging from lethargy to pericarditis and encephalopathy). Due to its high systemic circulation, urea is excreted in eccrine sweat at high concentrations and crystallizes on skin as the sweat evaporates ("uremic frost"). Potassium may accumulate in the blood (hyperkalemia with a range of symptoms including malaise and potentially fatal cardiac arrhythmias). Hyperkalemia usually does not develop until the glomerular filtration rate falls to less than 20-25 ml/min/1.73 m2, at which point the kidneys have decreased ability to excrete potassium. Hyperkalemia in CKD can be exacerbated by acidemia (which leads to extracellular shift of potassium) and from lack of insulin. Erythropoietin synthesis may be decreased causing anemia. Fluid volume overload symptoms may occur, ranging from mild edema to life-threatening pulmonary edema. Hyperphosphatemia, due to reduced phosphate excretion, may occur generally following the decrease in glomerular filtration. Hyperphosphatemia is associated with increased cardiovascular risk, being a direct stimulus to vascular calcification. Hypocalcemia may manifest, which is generally caused by stimulation of fibroblast growth factor-23. Osteocytes are responsible for the increased production of FGF23, which is a potent inhibitor of the enzyme 1-alpha-hydroxylase (responsible for the conversion of 25-hydroxycholecalciferol into 1,25 dihydroxyvitamin D3). Later, this progresses to secondary hyperparathyroidism, renal osteodystrophy, and vascular calcification that further impairs cardiac function. Metabolic acidosis (due to accumulation of sulfates, phosphates, uric acid etc.) may occur and cause altered enzyme activity by excess acid acting on enzymes; and also increased excitability of cardiac and neuronal membranes by the promotion of hyperkalemia due to excess acid (acidemia). Acidosis is also due to decreased capacity to generate enough ammonia from the cells of the proximal tubule. Iron deficiency anemia, which increases in prevalence as kidney function decreases, is especially prevalent in those requiring haemodialysis. It is multifactoral in cause, but includes increased inflammation, reduction in erythropoietin, and hyperuricemia leading to bone marrow suppression. People with CKD suffer from accelerated atherosclerosis and are more likely to develop cardiovascular disease than the general population. Patients afflicted with CKD and cardiovascular disease tend to have significantly worse prognoses than those suffering only from the latter.

In another embodiment, an ALK7:ActRIIB antagonist (e.g., an ALK7:ActRIIB heterodimer), or combinations of such antagonists, may be used in patients with chronic

kidney disease mineral bone disorder (CKD-MBD), a broad syndrome of interrelated skeletal, cardiovascular, and mineral-metabolic disorders arising from kidney disease. CKD-MBD encompasses various skeletal pathologies often referred to as renal osteodystrophy (ROD), which is a preferred embodiment for treatment with, an ALK7:ActRIIB antagonist (e.g., an ALK7:ActRIIB heterodimer), or combinations of such antagonists. Depending on the relative contribution of different pathogenic factors, ROD is manifested as diverse pathologic patterns of bone remodeling (Hruska et al., 2008, Chronic kidney disease mineral bone disorder (CKD-MBD); in Rosen et al. (ed) Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism, 7th ed. American Society for Bone and Mineral Research, Washington D.C., pp 343-349). At one end of the spectrum is ROD with uremic osteodystrophy and low bone turnover, characterized by a low number of active remodeling sites, profoundly suppressed bone formation, and low bone resorption. At the other extreme is ROD with hyperparathyroidism, high bone turnover, and osteitis fibrosa. Given that an ALK7:ActRIIB antagonist (e.g., an ALK7:ActRIIB heterodimer), or combinations of such antagonists, may exert both anabolic and antiresorptive effects, these agents may be useful in patients across the ROD pathology spectrum.

In other embodiments, an ALK7:ActRIIB antagonist (e.g., an ALK7:ActRIIB heterodimer), or combinations of such antagonists, can be used for regulating body fat content in an animal and for treating or preventing conditions related thereto, and particularly, health-compromising conditions related thereto. According to the present invention, to regulate (control) body weight can refer to reducing or increasing body weight, reducing or increasing the rate of weight gain, or increasing or reducing the rate of weight loss, and also includes actively maintaining, or not significantly changing body weight (*e.g.*, against external or internal influences which may otherwise increase or decrease body weight). One embodiment of the present disclosure relates to regulating body weight by administering to an animal (*e.g.*, a human) in need thereof an ALK7:ActRIIB antagonist (e.g., an ALK7:ActRIIB heterodimer), or combinations of such antagonists. For example, in some embodiments, an ALK7:ActRIIB antagonist (e.g., an ALK7:ActRIIB heterodimer), or combinations of such antagonists, may be used to treat or prevent a disorder or condition slected from obesity (*e.g.,* abdominal obesity); overweight; insulin resistance; metabolic syndrome and other metabolic diseases or conditions; a lipid disorder such as, low HDL levels, high LDL levels, hyperlipidemia, hypertriglyceridemia or dyslipidemia; lipoprotein aberrations; decreased triglycerides; inflammation (*e.g.,* liver inflammation and/or

inflammation of adipose tissue), fatty liver disease; non-alcoholic fatty liver disease; hyperglycemia; impaired glucose tolerance (IGT); hyperinsulinemia; high cholesterol (*e.g.*, high LDL levels and hypercholesterolemia); cardiovascular disease such as, heart disease including coronary heart disease, congestive heart failure, stroke, peripheral vascular disease, atherosclerosis; arteriosclerosis, and hypertension; Syndrome X; vascular restenosis; neuropathy; retinopathy; neurodegenerative disease; endothelial dysfunction, respiratory dysfunction; pancreatitis; polycystic ovarian syndrome; elevated uric acid levels; haemochromatosis (iron overload); acanthosis nigricans (dark patches on the skin); or cancer (*e.g.*, ovarian, breast, endometrial, and colon cancer); or a another disorders/conditions associated with one or more of the above diseases or conditions. In some embodiments, the disease or condition treated using an ALK7:ActRIIB antagonist (e.g., an ALK7:ActRIIB heterodimer), or combinations of such antagonists, is associated with overweight (*e.g.*, BMI of $\geq$25 kg/m^2), or with too much body fat.

In one embodiment, the disclosure provides a method of reducing body weight comprising administering to a subject desiring to reduce body weight, or in need thereof, an effective amount of an ALK7:ActRIIB antagonist (e.g., an ALK7:ActRIIB heterodimer), or combinations of such antagonists. In some embodiments, the subject is overweight (*e.g.*, pre-obese). In some embodiments, the subject has a body mass index (BMI) of 25 kg/m^2 or greater. In further embodiments, the subject has a BMI of 25 kg/m^2 to 29.9 kg/m^2, 30 kg/m^2 to 39.9 kg/m^2, 25 kg/m^2 to 39.9 kg/m^2, or 25 kg/m^2 to 50 kg/m^2. In some embodiments, the subject is obese. In some embodiments, the subject has a BMI of 30 kg/m^2 or greater (*e.g.*, 30 to 39.9 kg/m^2 or 30 kg/m^2 to 50 kg/m^2). In some embodiments, the subject is morbidly obese. In some embodiments, the subject has a BMI of 40 kg/m^2 or greater. In further embodiments, the subject has a BMI of 40 kg/m^2 to 45 kg/m^2, or 40 kg/m^2 to 50 kg/m^2. In some embodiments, the subject has central obesity (*e.g.*, excess adiposity in the abdominal region, including belly fat and/or visceral fat). In some embodiments, the subject has a waist/hip circumference ratio (WHR) of 0.85 or greater. In some embodiments, the subject has peripheral obesity (*e.g.*, excess adiposity on the hips). In some embodiments, the subject has type 2 diabetes mellitus. The ALK7:ActRIIB antagonist, or combination of antagonists, may administered alone or as a combination therapy other type of supportive therapy. For example, in some embodiments, the supportive therapy is diet and/or exercise.

In one embodiment, the disclosure provides a method of reducing weight gain comprising administering to a subject desiring to reduce weight gain, or in need thereof, an

effective amount of an ALK7:ActRIIB antagonist (e.g., an ALK7:ActRIIB heterodimer), or combination of such antagonists. In some embodiments, the subject is overweight (*e.g.,* pre-obese). In some embodiments, the subject has a BMI of 25 kg/m^2 or greater. In further embodiments, the subject has a BMI of 25 kg/m^2 to 29.9 kg/m^2, 30 kg/m^2 to 39.9 kg/m^2, 25 kg/m^2 to 39.9 kg/m^2, or 25 kg/m^2 to 50 kg/m^2. In some embodiments, the subject is obese. In some embodiments, the subject has a BMI of 30 kg/m^2 or greater (*e.g.,* 30 to 39.9 kg/m^2 or 30 kg/m^2 to 50 kg/m^2). In some embodiments, the subject is morbidly obese. In some embodiments, the subject has a BMI of 40 kg/m^2 or greater. In further embodiments, the subject has a BMI of 40 kg/m^2 to 45 kg/m^2, or 40 kg/m^2 to 50 kg/m^2. In some embodiments, the subject has type 2 diabetes mellitus.

Also provided is a method of treating or preventing a disease or condition associated with excess body weight, comprising administering to a subject in need of treatment or prevention, an effective amount of an ALK7:ActRIIB antagonist (e.g., an ALK7:ActRIIB heterodimer), or combination of such antagonists. In one embodiment, the treated or prevented disease or condition is obesity. In one embodiment, the treated or prevented disease or condition is insulin resistance. In one embodiment, the treated or prevented disease or condition is a member selected from the group consisting of: dyslipidemia, hyperlipidemia (total cholesterol level >240 mg/dL), hypercholesterolemia (*e.g.,* total cholesterol level of >200 mg/dL, >220 mg/dL, >240 mg/dL, >250 mg/dL, or >275 mg/dL), low HDL serum level (*e.g.,* <40mg/dL, <45 mg/dL, or <50 mg/dL), high LDL serum level (*e.g.,* ≥ 100 mg/dL, ≥ 130 mg/dL, ≥ 160 mg/dL, or ≥ 190 mg/dL), and hypertriglyceridemia (*e.g.,* a fasting TG level of ≥ 150 mg/dL, ≥ 175 mg/dL, ≥ 200 mg/dL, ≥ 300 mg/dL, ≥ 400 mg/dL, or ≥ 499 mg/dL). In certain instances, the ALK7:ActRIIB antagonists treatment is an adjunct to diet and/or exercise.

In another embodiment the disclosure provides a method of reducing body weight in a subject who is overweight. The method includes administering to an overweight subject an effective amount of an ALK7:ActRIIB antagonist (e.g., an ALK7:ActRIIB heterodimer), or combination of such antagonists. In some embodiments, the subject has a body mass index (BMI) of 25 kg/m^2 or greater. In further embodiments, the subject has a BMI of 25 kg/m^2 to 29.9 kg/m^2, 30 kg/m^2 to 39.9 kg/m^2, 25 kg/m^2 to 39.9 kg/m^2, or 25 kg/m^2 to 50 kg/m^2, or 27 to 40 kg/m^2. In some embodiments, the subject is obese. In some embodiments, the subject has a BMI of 30 kg/m^2 or greater (*e.g.,* 30 to 39.9 kg/m^2 or 30 kg/m^2 to 50 kg/m^2). The ALK7-binding protein is administered alone or as a combination therapy. In some embodiments, the ALK7:ActRIIB antagonist treatment is an adjunct to diet and/or exercise.

In one embodiment the disclosure provides a method of reducing body weight in an obese subject. The method includes administering to the subject an effective amount of an ALK7:ActRIIB antagonist (e.g., an ALK7:ActRIIB heterodimer), or combination of such antagonists. In some embodiments, the subject has a BMI of 30 kg/m^2 or greater (*e.g.,* 30 to 39.9 kg/m^2 or 30 kg/m^2 to 50 kg/m^2. In some embodiments, the subject has a BMI of 40 kg/m^2 or greater. In some embodiments, the subject has central obesity (*e.g.,* excess adiposity in the abdominal region, including belly fat and/or visceral fat). In some embodiments, the subject has a waist/hip circumference ratio (WHR) of 0.85 or greater. In some embodiments, the subject has peripheral obesity (*e.g.,* excess adiposity on the hips). In some embodiments, the ALK7:ActRIIB antagonist treatment is an adjunct to diet and/or exercise.

In another embodiment, the disclosure provides a method of treating and/or ameliorating obesity or a disease or condition associated with obesity, comprising administering to an obese subject, an effective amount of an ALK7:ActRIIB antagonist (e.g., an ALK7:ActRIIB heterodimer), or combination of such antagonists. In some embodiments, the subject has a BMI of 30 kg/m^2 or greater. In further embodiments, the subject has a BMI of 30 to 39.9 kg/m^2 or 30 kg/m^2 to 50 kg/m^2. In some embodiments, the subject is morbidly obese. In some embodiments, the subject has a body BMI of 40 kg/m^2 or greater. In further embodiments, the subject has a BMI of 40 kg/m^2 to 45 kg/m^2, or 40 kg/m^2 to 50 kg/m^{2}In some embodiments, the subject has type 2 diabetes mellitus. In some embodiments, the subject has a BMI of 30 kg/m^2 or greater (*e.g.,* 30 to 39.9 kg/m^2). In some embodiments, the subject has a BMI of at least 40 kg/m^2. In some embodiments, the subject has central obesity (*e.g.,* excess adiposity in the abdominal region, including belly fat and/or visceral fat). In some embodiments, the subject has a waist/hip circumference ratio (WHR) of 0.85 or greater. In some embodiments, the subject has peripheral obesity (*e.g.,* excess adiposity on the hips). In some embodiments, the ALK7:ActRIIB antagonist treatment is an adjunct to diet and/or exercise.

Also provided is a method of treating or preventing a disease or condition associated with obesity, comprising administering to a subject in need of treatment or prevention, an effective amount of an ALK7:ActRIIB antagonist (e.g., an ALK7:ActRIIB heterodimer), or combination of such antagonists. In one embodiment, the treated or prevented disease or condition is a member selected from the group consisting of: dyslipidemia, hyperlipidemia (total cholesterol level >240 mg/dL), hypercholesterolemia (*e.g.,* total cholesterol level of >200 mg/dL, >220 mg/dL, >240 mg/dL, >250 mg/dL, or >275 mg/dL), low HDL serum level

(*e.g.*, <40mg/dL, <45 mg/dL, or <50 mg/dL), high LDL serum level (*e.g.*, ≥ 100 mg/dL, ≥ 130 mg/dL, ≥ 160 mg/dL, or ≥ 190 mg/dL), and hypertriglyceridemia (*e.g.*, a fasting TG level of ≥ 150 mg/dL, ≥ 175 mg/dL, ≥ 200 mg/dL, ≥ 300 mg/dL, ≥ 400 mg/dL, or ≥ 499 mg/dL). In one embodiment, the treated or prevented disease or condition is cardiovascular disease. In an additional embodiment, the treated or prevented disease or condition is hypertension (high blood pressure), myocardial infarction, peripheral artery disease, vasoregulatoin dysfunction, arteriosclerosis congestive heart failure, atherosclerosis, coronary heart disease, or microvascular disease. In one embodiment, the treated or prevented disease or condition is liver disease. In one embodiment, the treated or prevented liver disease or condition is NAFLD. In one embodiment, the liver disease is fatty liver. In one embodiment, the liver disease is NASH. In another embodiment, the treated or prevented disease or condition is a member selected from the group: steatohepatitis, steatosis, fibrosis, and/or cirrhosis. In certain instances, the ALK7:ActRIIB antagonist treatment is an adjunct to diet and/or exercise.

In another embodiment, the disclosure provides a method of treating, ameliorating, and/or preventing type 2 diabetes mellitus or a disease or condition associated with diabetes comprising administering to a subject having type 2 diabetes mellitus, or at risk of developing type 2 diabetes, an effective amount of an ALK7:ActRIIB antagonist (e.g., an ALK7:ActRIIB heterodimer), or combination of such antagonists. In some embodiments, the subject has a body mass index BMI of 30 kg/m^2 or greater (*e.g.*, 30 to 39.9 kg/m^2). In some embodiments, the subject has a BMI of at least 40 kg/m^2. In some embodiments, the subject has central obesity (*e.g.*, excess adiposity in the abdominal region, including belly fat and/or visceral fat). In some embodiments, the subject has a WHR of 0.85 or greater. In some embodiments, the subject has peripheral obesity (*e.g.*, excess adiposity on the hips). In some embodiments, the ALK7:ActRIIB antagonist treatment is an adjunct to diet and/or exercise.

Also provided is a method of treating, ameliorating or preventing a disease or condition associated with diabetes, comprising administering to a subject having diabetes, an effective amount of an ALK7:ActRIIB antagonist (e.g., an ALK7:ActRIIB heterodimer), or combination of such antagonists. In one embodiment, the treated or prevented disease or condition is a member selected from the group consisting of: dyslipidemia, hyperlipidemia (total cholesterol level >240 mg/dL), hypercholesterolemia (*e.g.*, total cholesterol level of >200 mg/dL, >220 mg/dL, >240 mg/dL, >250 mg/dL, or >275 mg/dL), low HDL serum level (*e.g.*, <40mg/dL, <45 mg/dL, or <50 mg/dL), high LDL serum level (*e.g.*, ≥ 100 mg/dL, ≥ 130 mg/dL, ≥ 160 mg/dL, or ≥ 190 mg/dL), and hypertriglyceridemia (*e.g.*, a fasting TG level

of ≥ 150 mg/dL, ≥ 175 mg/dL, ≥ 200 mg/dL, ≥ 300 mg/dL, ≥ 400 mg/dL, or ≥ 499 mg/dL). In one embodiment, the treated or prevented disease or condition is cardiovascular disease. In an additional embodiment, the treated or prevented disease or condition is hypertension (high blood pressure), myocardial infarction, peripheral artery disease, vasoregulatoin dysfunction, or arteriosclerosis. In one embodiment, the treated or prevented disease or condition is liver disease. In another embodiment, the treated or prevented disease or condition is a member selected from the group: fatty liver disease, steatohepatitis, steatosis, and/or cirrhosis. In one embodiment, the treated or prevented disease or condition is a member selected from the group consisting of: cataracts, obstructive sleep apnea, phlebitis, gout, osteoarthritis, gallbladder disease, and high cholesterol. In certain instances, the ALK7:ActRIIB antagonist treatment is an adjunct to diet and/or exercise.

The disclosure also provides a method for improving the blood-lipid profile in a subject, comprising administering to a subject in need of such treatment an effective amount of an ALK7:ActRIIB antagonist (e.g., an ALK7:ActRIIB heterodimer), or combination of such antagonists. In some embodiments, the disclosure provides a method for reducing levels of LDL cholesterol or increasing levels of HDL-cholesterol. In one embodiment, the subject has dyslipidemia. In another embodiment, the subject has elevated serum lipids (*e.g.,* cholesterol (hypercholesterolemia) and/or triglycerides (*e.g.,* hypertriglyceridemia). In one embodiment the subject has an LDL-C ≥ 100 mg/dL, ≥ 130 mg/dL, or ≥ 160 mg/dL). In one embodiment the subject has a TG ≥ 150 mg/dL, ≥ 160 mg/dL, ≥ 170 mg/dL). In one embodiment, the subject has elevated plasma insulin levels (hyperinsulinemia; e.g., fasting insulin level of >20 ug/ml can exceed 100). In some embodiments, the subject has type II diabetes.

According to one embodiment, the disclosure provides a method of treating or preventing a metabolic disease or disorder or a condition associated with a metabolic disease or disorder, comprising administering an ALK7:ActRIIB antagonist (e.g., an ALK7:ActRIIB heterodimer), or combination of such antagonists, to a subject in need thereof. In one embodiment, the treated metabolic disease, disorder, or condition is hyperglycemia (*e.g.,* > 130 mg/dL in the fasting state or following glucose administration during an oral glucose tolerance test). In one embodiment, the treated metabolic disease, disorder, or condition is a lipid metabolism disease, disorder, or condition. In one embodiment, the treated metabolic disease, disorder, or condition is dislipidemia. In a further embodiment, the lipid metabolism disease, disorder, or condition is a member selected from: low HDL levels, high LDL levels, high triglyceride levels, hyperlipidemia, and a lipoprotein aberration. In one embodiment, the

subject has a total cholesterol level of >200 mg/dL, >220 mg/dL, >240 mg/dL, >250 mg/dL, or >275 mg/dL. In one embodiment, the subject has a HDL serum level of <40mg/dL, <45 mg/dL, or <50 mg/dL). In one embodiment, the subject has a LDL serum level ≥ 100 mg/dL, ≥ 130 mg/dL, ≥ 160 mg/dL, or ≥ 190 mg/dL. In one embodiment, the subject has fasting TG level of ≥ 150 mg/dL, ≥ 175 mg/dL, ≥ 200 mg/dL, ≥ 300 mg/dL, ≥ 400 mg/dL, or ≥ 499 mg/dL. In one embodiment, the treated metabolic disease, disorder, or condition is a glucose metabolism disease, disorder, or condition. In a further embodiment, the glucose metabolism disease, disorder, or condition is a member selected from: glucose intolerance, insulin resistance, impaired glucose tolerance (IGT), impaired fasting glucose (IFG). In one embodiment, the treated metabolic disease, disorder, or condition is a member selected from the group consisting of: high uric acid levels, NAFLD, fatty liver, NASH, and polycystic ovarian syndrome. In one embodiment, the treated subject has hyperinsulinemia. In one embodiment, the treated subject is obese (*e.g.*, the subject has abdominal obesity). In another embodiment, the treated subject has type II diabetes.

Metabolic syndrome is a condition involving a set of disorders that enhances the risk of heart disease. The major components of metabolic syndrome are excess weight, the cardiovascular parameters (high blood pressure, dyslipidemia, high levels of triglycerides and/or low levels of HDL in the blood), atherosclerosis, diabetes, and/or insulin resistance. A subject having several of these components, *i.e.* metabolic syndrome, is highly prone to heart disease, though each component is a risk factor. The disclosure also provides a method for treating or preventing 1, 2, 3, or more of the above components of metabolic syndrome, comprising administering to a subject in need of treatment an effective amount of an ALK7:ActRIIB antagonist (e.g., an ALK7:ActRIIB heterodimer), or combination of such antagonists.

Additionally provided is a method of treating, preventing or ameliorating a cardiovascular disease or condition, comprising administering an ALK7:ActRIIB antagonist (e.g., an ALK7:ActRIIB heterodimer), or combination of such antagonists, to a subject in need thereof. In one embodiment, the treated, prevented, or ameliorated cardiovascular disease or condition is atherosclerosis. In one embodiment, the treated, prevented, or ameliorated cardiovascular disease or condition is hypertension (*e.g.*, blood pressure >130/80 mmHg or >140/90 mmHg, in a resting state. In one embodiment, the cardiovascular disease is atherosclerosis (coronary heart disease disease).

In one embodiment, the disclosure provides a method for treating and/or ameliorating an inflammatory liver disease or condition that comprises administering an ALK7:ActRIIB

antagonist (e.g., an ALK7:ActRIIB heterodimer), or combination of such antagonists, to a subject in need thereof. In one embodiment, the disease or condition is NAFLD. In a further embodiment, the disease or condition is fatty liver. In a further embodiment, the disease or condition is steatosis (*e.g.,* nonalcoholic steatohepatitis (NASH)). In a further embodiment, the disease or condition is alcoholic fatty liver disease.

This disclosure also provides a method of improving glycemic control, comprising administering to a subject in need of treatment an effective amount of an ALK7:ActRIIB antagonist (e.g., an ALK7:ActRIIB heterodimer). In one embodiment, the subject is administered has a fasting blood sugar level of >130, >135, >140, >145, or >150 mg/dL. In one embodiment, the subject is administered has a postprandial blood sugar level of >180, >185, >190, >195, or >200 mg/dL 2 hours after eating. In certain instances, the ALK7:ActRIIB antagonist treatment is an adjunct to diet and/or exercise. The administration can also reduce body weight or treat obesity. In certain instances, the subject has type 2 diabetes mellitus. In certain instances, the subject has a BMI of 27 to 40 kg/m2. In certain instances, the subject has a BMI of 30 to 39.9 kg/m2. In certain instances, the subject has a BMI of at least 40. In certain instances, the subject is overweight. In certain instances, the subject is obese. An improvement in glycemic control can be assessed using techniques known in the art such as a mixed-meal test.

The disclosure also provides compositions and methods for treating, preventing or ameliorating hyperglycemia or a condition associated with hyperglycemia in a subject comprising administering to a subject in need of such treatment an effective amount of an ALK7:ActRIIB antagonist (e.g., an ALK7:ActRIIB heterodimer). In one embodiment, the subject is administered has a fasting blood sugar level of >130, >135, >140, >145, or >150 mg/dL. In one embodiment, the subject is administered has a postprandial blood sugar level of >180, >185, >190, >195, or >200 mg/dL 2 hours after eating. In one embodiment, the result of the treatment, prevention or amelioration is a member selected from the group consisting of: a decrease in serum levels of glucose, a decrease in serum levels of triglycerides, a decrease in serum levels of insulin, and/or a decrease in serum levels of non-esterified fatty acids, as compared to serum levels in the subject prior to treatment. In one embodiment, the result of the treatment, prevention or amelioration is an increase in body temperature of about 0.4°C to 1°C as compared to body temperature of the subject prior to treatment. In some embodiments, the ALK7:ActRIIB treatment also reduces body weight of the subject.

In another embodiment, the disclosure provides a method of decreasing plasma insulin levels in a subject, comprising administering an effective amount of an ALK7:ActRIIB antagonist (e.g., an ALK7:ActRIIB heterodimer), to a subject in need of such treatment. In one embodiment, the subject has a fasting blood sugar level of >130, >135, >140, >145, or >150 mg/dL. In one embodiment, the subject has a postprandial blood sugar level of >180, >185, >190, >195, or >200 mg/dL 2 hours after eating. In one embodiment, the subject is overweight. In one embodiment, the subject is obese. In another embodiment, the subject has type 2 diabetes.

The disclosure also provides compositions and methods for treating, preventing or ameliorating hyperglycemia or a condition associated with hyperglycemia in a subject comprising administering to a subject in need of such treatment an effective amount of an ALK7:ActRIIB antagonist (e.g., an ALK7:ActRIIB heterodimer). In one embodiment, the subject has a fasting blood sugar level of >130, >135, >140, >145, or >150 mg/dL. In one embodiment, the subject has a postprandial blood sugar level of >180, >185, >190, >195, or >200 mg/dL 2 hours after eating. In one embodiment, the result of the treatment, prevention or amelioration is a member selected from the group consisting of: a decrease in serum levels of glucose, a decrease in serum levels of triglycerides, a decrease in serum levels of insulin, and/or a decrease in serum levels of non-esterified fatty acids, as compared to serum levels in the subject prior to treatment. In one embodiment, the result of the treatment, prevention or amelioration is an increase in body temperature of about 0.4°C to 1°C as compared to body temperature of the subject prior to treatment. In some embodiments, the ALK7:ActRIIB antagonist treatment also reduces body weight of the subject.

In another embodiment, the disclosure provides a method of decreasing plasma insulin levels in a subject, comprising administering an effective amount of an ALK7:ActRIIB antagonist (e.g., an ALK7:ActRIIB heterodimer), or combination of such antagonists, to a subject in need of such treatment. In one embodiment, the subject has a fasting blood sugar level of >130, >135, >140, >145, or >150 mg/dL. In one embodiment, the has a postprandial blood sugar level of >180, >185, >190, >195, or >200 mg/dL 2 hours after eating. In one embodiment, the subject is overweight. In one embodiment, the subject is obese. In another embodiment, the subject has type 2 diabetes.

In another embodiment, the disclosure provides a method of treating, preventing, or ameliorating liver disease in a subject, comprising administering an effective amount of an ALK7:ActRIIB antagonist (e.g., an ALK7:ActRIIB heterodimer), or combination of such antagonists, to a subject having a liver disease. In one embodiment, the subject has

inflammation of the liver. In one embodiment, the subject has NAFLD. In on embodiment the subject has fatty liver. In another embodiment, the subject has NASH. In on embodiment the subject has fatty liver. In another embodiment, the subject has alcoholic fatty liver disease. In one embodiment, the treated, prevented or ameliorated liver disease is fibrosis, scarring, cirrhosis, or liver failure. In another embodiment, the treated, prevented or ameliorated liver disease is liver cancer. In one embodiment, the subject is overweight. In another embodiment, the subject is obese. In another embodiment, the subject has type 2 diabetes.

In on embodiment, the disclosure provides a method of for increasing lipolysis in a cell (e.g., white or brown adipose cells or tissue) comprising administering effective amount of an ALK7:ActRIIB antagonist (e.g., an ALK7:ActRIIB heterodimer), or combination of such antagonists. In some embodiments the cell is contacted *in vitro*. In some embodiments the cell is contacted *in vivo*. In one embodiment, the method is carried out *in vivo*, for example, in a mammalian subject (*e.g.,* an animal model). In a further embodiment, the subject is a human. In some embodiments, the method leads to increased glycerol production. In further embodiments, the method leads to increased glycerol and/or free fatty acid in an adipocyte culture. In some embodiments, the method leads to decreased triglyceride (TG) content in the cell or tissue. In some embodiments, the method leads to a decreased plasma TG level in a subject.

In another embodiment, the disclosure provides a method of increasing adrenergic receptor-β (ADRB) signaling in a cell or tissue (e.g., white or brown adipose cells or tissue). The method comprises contacting a cell or tissue with an ALK7:ActRIIB antagonist (e.g., an ALK7:ActRIIB heterodimer), or combination of such antagonists, in an amount sufficient to increase ADRB signaling. In some embodiments the cell or tissue is contacted *in vitro*. In some embodiments the cell or tissue is contacted *in vivo*. In one embodiment, the method is carried out *in vivo*, for example, in a mammalian subject (*e.g.,* an animal model). In a further embodiment, the subject is a human. In some embodiments, the method leads to increased glycerol production. In further embodiments, the method leads to increased glycerol and/or free fatty acid in an adipocyte culture. In some embodiments, the method leads to decreased TG content in the cell or tissue. In some embodiments, the method leads to a decreased plasma TG level in a subject. In some embodiments, the method leads to an increased ADRB signaling in an adipocyte or adipose tissue during nutrient overload.

In another embodiment, the disclosure provides a method of decreasing peroxisome proliferator–activated receptor-gamma (PPAR gamma) signaling in a cell or tissue (e.g.,

white and/or brown adipose cell or tissue). The method includes contacting a cell or tissue with an ALK7:ActRIIB antagonist (e.g., an ALK7:ActRIIB heterodimer), or combination of such antagonists, in an amount effective to decrease PPAR gamma activity. In some embodiments the cells or tissue is contacted *in vitro*. In some embodiments the differentiated cells or tissue is contacted *in vivo*. In one embodiment, the method is carried out *in vivo*, for example, in a mammalian subject (*e.g.,* an animal model). In a further embodiment, the subject is a human. In some embodiments, the method leads to increased glycerol production. In further embodiments, the method leads to increased glycerol and/or free fatty acid in an adipocyte culture. In some embodiments, the method leads to decreased TG content in the cells or tissue. In some embodiments, the method leads to a decreased plasma TG level in a subject.

In another embodiment, the disclosure provides a method of decreasing insulin resistance in a cell or tissue (e.g., white and/or brown adipose cell or tissue). The method includes contacting a cell or tissue with an ALK7:ActRIIB antagonist (e.g., an ALK7:ActRIIB heterodimer), or combination of such antagonists, in an amount effective to reduce insulin resistance. In some embodiments the cell or tissue is contacted *in vitro*. In some embodiments the cell or tissue is contacted *in vivo*. In one embodiment, the method is carried out *in vivo*, for example, in a mammalian subject (*e.g.,* an animal model). In a further embodiment, the subject is a human.

In another embodiment, the disclosure provides a method of increasing the metabolic rate of a cell or tissue (e.g., white and/or brown adipose cell or tissue). The method includes contacting cell or tissue with an ALK7:ActRIIB antagonist (e.g., an ALK7:ActRIIB heterodimer), or combination of such antagonists, in an amount effective to increase metabolism of the cell or tissue. In some embodiments the cell or tissue is contacted *in vitro*. In some embodiments the cell or tissue is contacted *in vivo*. In one embodiment, the method is carried out *in vivo*, for example, in a mammalian subject (*e.g.,* an animal model). In a further embodiment, the subject is a human.

The disclosure provides methods that comprise administering a therapeutically effective amount of an ALK7:ActRIIB antagonist (e.g., an ALK7:ActRIIB heterodimer), or combination of such antagonists, alone or in combination with one or more additional therapies (*e.g.,* one or more additional therapeutic agents and/or supportive) to a subject having, or at risk for developing, an ALK7-mediated disease and/or condition such as, obesity (*e.g.,* abdominal obesity); overweight; insulin resistance; metabolic syndrome and other metabolic diseases or conditions; a lipid disorder such as, low HDL levels, high LDL

levels, hyperlipidemia, hypertriglyceridemia or dyslipidemia; lipoprotein aberrations; decreased triglycerides; fatty liver disease; non-alcoholic fatty liver disease; hyperglycemia; impaired glucose tolerance (IGT); hyperinsulinemia; high cholesterol (*e.g.,* high LDL levels and/or hypercholesterolemia); cardiovascular disease such as, heart disease including coronary heart disease, congestive heart failure, atherosclerosis; arteriosclerosis, and/or hypertension; Syndrome X; vascular restenosis; neuropathy; and/or other disorders/conditions associated with one or more of the above diseases or conditions, and/or with overweight (*e.g.*, BMI of $\geq$25 kg/m^2), or with too much body fat.

In additional embodiments, the disclosure provides methods of treating and/or ameliorating cancer or a condition associated with cancer, that comprises administering an ALK7:ActRIIB antagonist (e.g., an ALK7:ActRIIB heterodimer), or combination of such antagonists. In some embodiments, the subject has a cancer selected from the group consisting of melanoma, breast, colon, and endometrial, pancreatic, gastric, and uterine cancer. In some embodiments, the subject has myeloma (e.g., multiple myeloma, plasmacytoma, localized myeloma, and extramedullary myeloma). In some embodiments, the ALK7:ActRIIB antagonist is administered to treat or prevent lymphatic metastasis, bloodstream metastasis, tumor growth, or tumor invasion.

Fibrosis generally refers to an excessive deposition of both collagen fibers and extracellular matrix combined with a relative decrease of cell number in an organ or tissue. While this process is an important feature of natural wound healing following injury, fibrosis can lead to pathological damage in various tissue and organs including, for example, the lungs, kidneys, liver, bone, muscle, and skin. The role TGF-beta in fibrosis has been extensively study. However, other TGF-beta superfamily ligands have also been implicated in fibrosis including, for example, activins (e.g., activin A and activin B) and GDF8 [Hedger et al (2013) Cytokine and Growth Factor Reviews 24:285-295; Hardy et al. (2015) 93: 567-574; and Cantini et al. (2008) J Sex Med 5:1607-1622]. Therefore, in some embodiments, an ALK7:ActRIIB antagonist (e.g., an ALK7:ActRIIB heterodimer), or combinations of such antagonists, of the present disclosure can be used to treat fibrosis, particularly fibrosis-associated disorders and conditions. For example, an ALK7:ActRIIB antagonist (e.g., an ALK7:ActRIIB heterodimer), or combinations of such antagonists, may be used to treat or prevent one or more of: pulmonary fibrosis, hypersensitivity pneumonitis, idiopathic fibrosis, tuberculosis, pneumonia, cystic fibrosis, asthma, chronic obstructive pulmonary disease (COPD), emphysema, renal (kidney) fibrosis, renal (kidney) failure, chronic renal (kidney) disease, bone fibrosis, myelofibrosis, rheumatoid arthritis, systemic lupus erythematosus,

scleroderma, sarcoidosis, granulomatosis with polyangiitis, Peyronie's disease, liver fibrosis, Wilson's disease, glycogen storage diseases (particularly types III, IV, IX, and X), iron-overload, Gaucher disease, Zellweger syndrome, nonalcoholic and alcoholic steatohepatitis, biliary cirrhosis, sclerosing cholangitis, Budd-Chiari syndrome, surgery-associated fibrosis, Crohn's disease, Duputren's contracture, mediastinal fibrosis, nephrogeneic fibrosis, retroperitoneal fibrosis, atrial fibrosis, endomyocardial fibrosis, pancreatic fibrosis.

In part, the present disclosure relate to the surprising discovery that ALK7:ActRIIB heteromultimers can bind to activin C. Therefore, ALK7:ActRIIB heteromultimers represent a new class of activin C inhibitors that can be used to treat or prevent activin C-related disorders or conditions. In some embodiments, ALK7:ActRIIB heteromultimers may be used to inhibit activin C activity in a patient in need thereof. Overexpression of activin C has been implicated in disease and disorders associated with liver, testis, and prostate, particular with cancer in such tissues. See, e.g., Gold et al. Am J Pathol (2009) 174(1): 184-195. . In some embodiments, ALK7:ActRIIB heteromultimers may be used to increase male fertility, increase sperm production, increase seminiferous tubule volume, decrease liver inflammation, treat liver (hepatic) cancer, treat testicular cancer, treat prostate cancer, decrease prostate inflammation, and/or treat prostate hypertrophy.

In some embodiments, ALK7:ActRIIB-associated conditions include neuromuscular disorders (*e.g.*, muscular dystrophy and muscle atrophy), congestive obstructive pulmonary disease (and muscle wasting associated with COPD), muscle wasting syndrome, sarcopenia, cachexia, adipose tissue disorders (*e.g.*, obesity), type 2 diabetes (NIDDM, adult-onset diabetes), and bone degenerative disease (*e.g.*, osteoporosis). Other exemplary ALK7:ActRIIB-associated conditions include musculodegenerative and neuromuscular disorders, tissue repair (*e.g.*, wound healing), neurodegenerative diseases (*e.g.*, amyotrophic lateral sclerosis), and immunologic disorders (*e.g.*, disorders related to abnormal proliferation or function of lymphocytes).

In certain embodiments, an ALK7:ActRIIB antagonist (e.g., an ALK7:ActRIIB heterodimer), or combinations of such antagonists, of the disclosure may be used as part of a treatment for a muscular dystrophy. The term "muscular dystrophy" refers to a group of degenerative muscle diseases characterized by gradual weakening and deterioration of skeletal muscles and sometimes the heart and respiratory muscles. Muscular dystrophies are genetic disorders characterized by progressive muscle wasting and weakness that begin with microscopic changes in the muscle. As muscles degenerate over time, the person's muscle

strength declines. Exemplary muscular dystrophies that can be treated with a regimen including the subject TGF-beta superfamily heteromultimer complexes include: Duchenne muscular dystrophy (DMD), Becker muscular dystrophy (BMD), Emery-Dreifuss muscular dystrophy (EDMD), limb-girdle muscular dystrophy (LGMD), facioscapulohumeral muscular dystrophy (FSH or FSHD) (also known as Landouzy-Dejerine), myotonic dystrophy (MMD; also known as Steinert's Disease), oculopharyngeal muscular dystrophy (OPMD), distal muscular dystrophy (DD), congenital muscular dystrophy (CMD).

Duchenne muscular dystrophy (DMD) was first described by the French neurologist Guillaume Benjamin Amand Duchenne in the 1860s. Becker muscular dystrophy (BMD) is named after the German doctor Peter Emil Becker, who first described this variant of DMD in the 1950s. DMD is one of the most frequent inherited diseases in males, affecting one in 3,500 boys. DMD occurs when the dystrophin gene, located on the short arm of the X chromosome, is defective. Since males only carry one copy of the X chromosome, they only have one copy of the dystrophin gene. Without the dystrophin protein, muscle is easily damaged during cycles of contraction and relaxation. While early in the disease muscle compensates by regeneration, later on muscle progenitor cells cannot keep up with the ongoing damage and healthy muscle is replaced by non-functional fibro-fatty tissue.

BMD results from different mutations in the dystrophin gene. BMD patients have some dystrophin, but it is either of insufficient quantity or poor quality. The presence of some dystrophin protects the muscles of patients with BMD from degenerating as severely or as quickly as those of patients with DMD.

Studies in animals indicate that inhibition of the GDF8 signaling pathway may effectively treat various aspects of disease in DMD and BMD patients (Bogdanovich et al., 2002, Nature 420:418-421; Pistilli et al., 2011, Am J Pathol 178:1287-1297). Thus, ALK7:ActRIIB antagonists of the disclosure may act as GDF8 inhibitors (antagonists), and constitute an alternative means of blocking signaling by GDF8 and/or related TGFβ superfamily ligands *in vivo* in DMD and BMD patients.

Similarly, ALK7:ActRIIB antagonists of the disclosure may provide an effective means to increase muscle mass in other disease conditions that are in need of muscle growth. For example, amyotrophic lateral sclerosis (ALS), also called Lou Gehrig's disease or motor neuron disease, is a chronic, progressive, and incurable CNS disorder that attacks motor neurons, which are components of the central nervous system required for initiation of

skeletal muscle contraction. In ALS, motor neurons deteriorate and eventually die, and though a person's brain normally remains fully functioning and alert, initiation of muscle contraction is blocked at the spinal level. Individuals who develop ALS are typically between 40 and 70 years old, and the first motor neurons to degenerate are those innervating the arms or legs. Patients with ALS may have trouble walking, may drop things, fall, slur their speech, and laugh or cry uncontrollably. As the disease progresses, muscles in the limbs begin to atrophy from disuse. Muscle weakness becomes debilitating, and patients eventually require a wheel chair or become confined to bed. Most ALS patients die from respiratory failure or from complications of ventilator assistance like pneumonia 3-5 years from disease onset.

Promotion of increased muscle mass by ALK7:ActRIIB antagonists might also benefit those suffering from muscle wasting diseases. Gonzalez-Cadavid *et al.* (supra) reported that GDF8 expression correlates inversely with fat-free mass in humans and that increased expression of the GDF8 gene is associated with weight loss in men with AIDS wasting syndrome. By inhibiting the function of GDF8 in AIDS patients, at least certain symptoms of AIDS may be alleviated, if not completely eliminated, thus significantly improving quality of life in AIDS patients.

Since loss of GDF8 function is also associated with fat loss without diminution of nutrient intake (Zimmers et al., supra; McPherron and Lee, supra), the subject ALK7:ActRIIB antagonists may further be used as a therapeutic agent for slowing or preventing the development of obesity and type 2 diabetes.

Cancer anorexia-cachexia syndrome is among the most debilitating and life-threatening aspects of cancer. This syndrome is a common feature of many types of cancer – present in approximately 80% of cancer patients at death – and is responsible not only for a poor quality of life and poor response to chemotherapy but also a shorter survival time than is found in patients with comparable tumors but without weight loss. Cachexia is typically suspected in patients with cancer if an involuntary weight loss of greater than five percent of premorbid weight occurs within a six-month period. Associated with anorexia, wasting of fat and muscle tissue, and psychological distress, cachexia arises from a complex interaction between the cancer and the host. Cancer cachexia affects cytokine production, release of lipid-mobilizing and proteolysis-inducing factors, and alterations in intermediary metabolism. Although anorexia is common, a decreased food intake alone is unable to account for the changes in body composition seen in cancer patients, and increasing nutrient intake is unable

to reverse the wasting syndrome. Currently, there is no treatment to control or reverse the cachexic process. Since systemic overexpression of GDF8 in adult mice was found to induce profound muscle and fat loss analogous to that seen in human cachexia syndromes (Zimmers et al., supra), the subject ALK7:ActRIIB antagonists may be beneficially used to prevent, treat, or alleviate the symptoms of the cachexia syndrome, where muscle growth is desired. An example of a heteromeric complex useful for preventing, treating, or alleviating muscle loss as described above is an ALK7:ActRIIB heterodimer.

In certain embodiments, an ALK7:ActRIIB antagonist (e.g., an ALK7:ActRIIB heterodimer), or combinations of such antagonists, of the present disclosure may be used in methods of inducing bone and/or cartilage formation, preventing bone loss, increasing bone mineralization, preventing the demineralization of bone, and/or increasing bone density. ALK7:ActRIIB antagonists may be useful in patients who are diagnosed with subclinical low bone density, as a protective measure against the development of osteoporosis.

In some embodiments, an ALK7:ActRIIB antagonist (e.g., an ALK7:ActRIIB heterodimer), or combinations of such antagonists, of the present disclosure may find medical utility in the healing of bone fractures and cartilage defects in humans and other animals. The subject methods and compositions may also have prophylactic use in closed as well as open fracture reduction and also in the improved fixation of artificial joints. De novo bone formation induced by an osteogenic agent is useful for repair of craniofacial defects that are congenital, trauma-induced, or caused by oncologic resection, and is also useful in cosmetic plastic surgery. Further, methods and compositions of the invention may be used in the treatment of periodontal disease and in other tooth repair processes. In certain cases, an ALK7:ActRIIB antagonist (e.g., an ALK7:ActRIIB heterodimer), or combinations of such antagonists, may provide an environment to attract bone-forming cells, stimulate growth of bone-forming cells, or induce differentiation of progenitors of bone-forming cells. An ALK7:ActRIIB antagonist (e.g., an ALK7:ActrIIB heterodimer), or combinations of such antagonists, of the disclosure may also be useful in the treatment of osteoporosis. Further, ALK7:ActRIIB antagonists may be used in repair of cartilage defects and prevention/reversal of osteoarthritis. Examples of heteromeric complexes useful for inducing bone formation, preventing bone loss, increasing bone mineralization, preventing the demineralization of bone, and/or increasing bone density as described herein are ALK7:ActRIIB heterodimers.

Rosen et al. (ed) Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism, 7th ed. American Society for Bone and Mineral Research, Washington D.C.

(incorporated herein by reference) provides an extensive discussion of bone disorders that may be subject to treatment with an ALK7:ActRIIB antagonist, or with combinations of such antagonists. A partial listing is provided herein. Methods and compositions of the invention can be applied to conditions characterized by or causing bone loss, such as osteoporosis (including secondary osteoporosis), hyperparathyroidism, chronic kidney disease mineral bone disorder, sex hormone deprivation or ablation (e.g. androgen and/or estrogen), glucocorticoid treatment, rheumatoid arthritis, severe burns, hyperparathyroidism, hypercalcemia, hypocalcemia, hypophosphatemia, osteomalacia (including tumor-induced osteomalacia), hyperphosphatemia, vitamin D deficiency, hyperparathyroidism (including familial hyperparathyroidism) and pseudohypoparathyroidism, tumor metastases to bone, bone loss as a consequence of a tumor or chemotherapy, tumors of the bone and bone marrow (e.g., multiple myeloma), ischemic bone disorders, periodontal disease and oral bone loss, Cushing's disease, Paget's disease, thyrotoxicosis, chronic diarrheal state or malabsorption, renal tubular acidosis, or anorexia nervosa. Methods and compositions of the invention may also be applied to conditions characterized by a failure of bone formation or healing, including non-union fractures, fractures that are otherwise slow to heal, fetal and neonatal bone dysplasias (e.g., hypocalcemia, hypercalcemia, calcium receptor defects and vitamin D deficiency), osteonecrosis (including osteonecrosis of the jaw) and osteogenesis imperfecta. Additionally, the anabolic effects will cause such antagonists to diminish bone pain associated with bone damage or erosion. As a consequence of the anti-resorptive effects, such antagonists may be useful to treat disorders of abnormal bone formation, such as osteoblastic tumor metastases (e.g., associated with primary prostate or breast cancer), osteogenic osteosarcoma, osteopetrosis, progressive diaphyseal dysplasia, endosteal hyperostosis, osteopoikilosis, and melorheostosis. Other disorders that may be treated include fibrous dysplasia and chondrodysplasias.

In another specific embodiment, the disclosure provides a therapeutic method and composition for repairing fractures and other conditions related to cartilage and/or bone defects or periodontal diseases. The invention further provides therapeutic methods and compositions for wound healing and tissue repair. The types of wounds include, but are not limited to, burns, incisions and ulcers. See, *e.g.*, PCT Publication No. WO 84/01106. Such compositions comprise a therapeutically effective amount of at least one of the ALK7:ActRIIB antagonists of the disclosure in admixture with a pharmaceutically acceptable vehicle, carrier, or matrix.

In some embodiments, an ALK7:ActRIIB antagonist (e.g., an ALK7:ActRIIB heterodimer), or combinations of such antagonists, of the disclosure can be applied to conditions causing bone loss such as osteoporosis, hyperparathyroidism, Cushing's disease, thyrotoxicosis, chronic diarrheal state or malabsorption, renal tubular acidosis, or anorexia nervosa. It is commonly appreciated that being female, having a low body weight, and leading a sedentary lifestyle are risk factors for osteoporosis (loss of bone mineral density, leading to fracture risk). However, osteoporosis can also result from the long-term use of certain medications. Osteoporosis resulting from drugs or another medical condition is known as secondary osteoporosis. In Cushing's disease, the excess amount of cortisol produced by the body results in osteoporosis and fractures. The most common medications associated with secondary osteoporosis are the corticosteroids, a class of drugs that act like cortisol, a hormone produced naturally by the adrenal glands. Although adequate levels of thyroid hormones are needed for the development of the skeleton, excess thyroid hormone can decrease bone mass over time. Antacids that contain aluminum can lead to bone loss when taken in high doses by people with kidney problems, particularly those undergoing dialysis. Other medications that can cause secondary osteoporosis include phenytoin (Dilantin) and barbiturates that are used to prevent seizures; methotrexate (Rheumatrex, Immunex, Folex PFS), a drug for some forms of arthritis, cancer, and immune disorders; cyclosporine (Sandimmune, Neoral), a drug used to treat some autoimmune diseases and to suppress the immune system in organ transplant patients; luteinizing hormone-releasing hormone agonists (Lupron, Zoladex), used to treat prostate cancer and endometriosis; heparin (Calciparine, Liquaemin), an anticlotting medication; and cholestyramine (Questran) and colestipol (Colestid), used to treat high cholesterol. Bone loss resulting from cancer therapy is widely recognized and termed cancer therapy-induced bone loss (CTIBL). Bone metastases can create cavities in the bone that may be corrected by treatment with An ALK7:ActRIIB antagonist (e.g., an ALK7:ActRIIB heterodimer). Bone loss can also be caused by gum disease, a chronic infection in which bacteria located in gum recesses produce toxins and harmful enzymes.

In a further embodiment, the present disclosure provides methods and therapeutic agents for treating diseases or disorders associated with abnormal or unwanted bone growth. For example, patients with the congenital disorder fibrodysplasia ossificans progressiva (FOP) are afflicted by progressive ectopic bone growth in soft tissues spontaneously or in response to tissue trauma, with a major impact on quality of life. Additionally, abnormal bone growth

can occur after hip replacement surgery and thus ruin the surgical outcome. This is a more common example of pathological bone growth and a situation in which the subject methods and compositions may be therapeutically useful. The same methods and compositions may also be useful for treating other forms of abnormal bone growth (e.g., pathological growth of bone following trauma, burns or spinal cord injury), and for treating or preventing the undesirable conditions associated with the abnormal bone growth seen in connection with metastatic prostate cancer or osteosarcoma.

In certain embodiments, an ALK7:ActRIIB antagonist (e.g., an ALK7:ActRIIB heterodimer), or combinations of such antagonists, of the disclosure may be used to promote bone formation in patients with cancer. Patients having certain tumors (e.g. prostate, breast, multiple myeloma or any tumor causing hyperparathyroidism) are at high risk for bone loss due to tumor-induced bone loss, bone metastases, and therapeutic agents. Such patients may be treated with a TGF-beta superfamily heteromultimer complex, or a combination of complexes, even in the absence of evidence of bone loss or bone metastases. Patients may also be monitored for evidence of bone loss or bone metastases, and may be treated with an ALK7:ActRIIB antagonist in the event that indicators suggest an increased risk. Generally, DEXA scans are employed to assess changes in bone density, while indicators of bone remodeling may be used to assess the likelihood of bone metastases. Serum markers may be monitored. Bone specific alkaline phosphatase (BSAP) is an enzyme that is present in osteoblasts. Blood levels of BSAP are increased in patients with bone metastasis and other conditions that result in increased bone remodeling. Osteocalcin and procollagen peptides are also associated with bone formation and bone metastases. Increases in BSAP have been detected in patients with bone metastasis caused by prostate cancer, and to a lesser degree, in bone metastases from breast cancer. BMP7 levels are high in prostate cancer that has metastasized to bone, but not in bone metastases due to bladder, skin, liver, or lung cancer. Type I carboxy-terminal telopeptide (ICTP) is a crosslink found in collagen that is formed during to the resorption of bone. Since bone is constantly being broken down and reformed, ICTP will be found throughout the body. However, at the site of bone metastasis, the level will be significantly higher than in an area of normal bone. ICTP has been found in high levels in bone metastasis due to prostate, lung, and breast cancer. Another collagen crosslink, Type I N-terminal telopeptide (NTx), is produced along with ICTP during bone turnover. The amount of NTx is increased in bone metastasis caused by many different types of cancer including lung, prostate, and breast cancer. Also, the levels of NTx increase with the

progression of the bone metastasis. Therefore, this marker can be used to both detect metastasis as well as measure the extent of the disease. Other markers of resorption include pyridinoline and deoxypyridinoline. Any increase in resorption markers or markers of bone metastases indicate the need for therapy with an ALK7:ActRIIB antagonist in a patient.

An ALK7:ActRIIB antagonist (e.g., an ALK7:ActRIIB heterodimer), or combinations of such antagonists, of the disclosure may be conjointly administered with other bone-active pharmaceutical agents. Conjoint administration may be accomplished by administration of a single co-formulation, by simultaneous administration, or by administration at separate times. ALK7:ActRIIB antagonists may be particularly advantageous if administered with other bone-active agents. A patient may benefit from conjointly receiving an ALK7:ActRIIB antagonist complex and taking calcium supplements, vitamin D, appropriate exercise and/or, in some cases, other medication. Examples of other medications incude, bisphosphonates (alendronate, ibandronate and risedronate), calcitonin, estrogens, parathyroid hormone and raloxifene. The bisphosphonates (alendronate, ibandronate and risedronate), calcitonin, estrogens and raloxifene affect the bone remodeling cycle and are classified as anti-resorptive medications. Bone remodeling consists of two distinct stages: bone resorption and bone formation. Anti-resorptive medications slow or stop the bone-resorbing portion of the bone-remodeling cycle but do not slow the bone-forming portion of the cycle. As a result, new formation continues at a greater rate than bone resorption, and bone density may increase over time. Teriparatide, a form of parathyroid hormone, increases the rate of bone formation in the bone remodeling cycle. Alendronate is approved for both the prevention (5 mg per day or 35 mg once a week) and treatment (10 mg per day or 70 mg once a week) of postmenopausal osteoporosis. Alendronate reduces bone loss, increases bone density and reduces the risk of spine, wrist and hip fractures. Alendronate also is approved for treatment of glucocorticoid-induced osteoporosis in men and women as a result of long-term use of these medications (i.e., prednisone and cortisone) and for the treatment of osteoporosis in men. Alendronate plus vitamin D is approved for the treatment of osteoporosis in postmenopausal women (70 mg once a week plus vitamin D), and for treatment to improve bone mass in men with osteoporosis. Ibandronate is approved for the prevention and treatment of postmenopausal osteoporosis. Taken as a once-a-month pill (150 mg), ibandronate should be taken on the same day each month. Ibandronate reduces bone loss, increases bone density and reduces the risk of spine fractures. Risedronate is approved for the prevention and treatment of postmenopausal osteoporosis. Taken daily (5 mg dose) or

weekly (35 mg dose or 35 mg dose with calcium), risedronate slows bone loss, increases bone density and reduces the risk of spine and non-spine fractures. Risedronate also is approved for use by men and women to prevent and/or treat glucocorticoid-induced osteoporosis that results from long-term use of these medications (i.e., prednisone or cortisone). Calcitonin is a naturally occurring hormone involved in calcium regulation and bone metabolism. In women who are more than 5 years beyond menopause, calcitonin slows bone loss, increases spinal bone density, and may relieve the pain associated with bone fractures. Calcitonin reduces the risk of spinal fractures. Calcitonin is available as an injection (50-100 IU daily) or nasal spray (200 IU daily).

A patient may also benefit from conjointly receiving an ALK7:ActRIIB antagonist, or combinations of such antagonists, and additional bone-active medications. Estrogen therapy (ET)/hormone therapy (HT) is approved for the prevention of osteoporosis. ET has been shown to reduce bone loss, increase bone density in both the spine and hip, and reduce the risk of hip and spinal fractures in postmenopausal women. ET is administered most commonly in the form of a pill or skin patch that delivers a low dose of approximately 0.3 mg daily or a standard dose of approximately 0.625 mg daily and is effective even when started after age 70. When estrogen is taken alone, it can increase a woman's risk of developing cancer of the uterine lining (endometrial cancer). To eliminate this risk, healthcare providers prescribe the hormone progestin in combination with estrogen (hormone replacement therapy or HT) for those women who have an intact uterus. ET/HT relieves menopause symptoms and has been shown to have a beneficial effect on bone health. Side effects may include vaginal bleeding, breast tenderness, mood disturbances and gallbladder disease. Raloxifene, 60 mg a day, is approved for the prevention and treatment of postmenopausal osteoporosis. It is from a class of drugs called Selective Estrogen Receptor Modulators (SERMs) that have been developed to provide the beneficial effects of estrogens without their potential disadvantages. Raloxifene increases bone mass and reduces the risk of spine fractures. Data are not yet available to demonstrate that raloxifene can reduce the risk of hip and other non-spine fractures. Teriparatide, a form of parathyroid hormone, is approved for the treatment of osteoporosis in postmenopausal women and men who are at high risk for a fracture. This medication stimulates new bone formation and significantly increases bone mineral density. In postmenopausal women, fracture reduction was noted in the spine, hip, foot, ribs and wrist. In men, fracture reduction was noted in the spine, but there were insufficient data to evaluate

fracture reduction at other sites. Teriparatide is self-administered as a daily injection for up to 24 months.

As used herein, "in combination with", "combinations of", or "conjoint administration" refers to any form of administration such that additional therapies (e.g., second, third, fourth, etc.) are still effective in the body (e.g., multiple compounds are simultaneously effective in the patient, which may include synergistic effects of those 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 ALK7:ActRIIB 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.

5. Pharmaceutical Compositions

In certain aspects, ALK7:ActRIIB antagonists (e.g., ALK7:ActRIIB heteromultimers), or combinations of such antagonists, of the present disclosure can be administered alone or as a component of a pharmaceutical formulation (also referred to as a therapeutic composition or pharmaceutical composition). A pharmaceutical formation refers to a preparation which is in such form as to permit the biological activity of an active ingredient (*e.g.*, an agent of the present disclosure) contained therein to be effective and which contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered. The subject compounds may be formulated for administration in any convenient way for use in human or veterinary medicine. For example, one or more agents of the present disclosure may be formulated with a pharmaceutically acceptable carrier. A pharmaceutically acceptable carrier refers to an ingredient in a pharmaceutical formulation, other than an active ingredient, which is generally nontoxic to a subject. A pharmaceutically acceptable carrier includes, but is not limited to, a buffer, excipient, stabilizer, and/or preservative. In general, pharmaceutical formulations for use in the present disclosure are in

a pyrogen-free, physiologically-acceptable form when administered to a subject. Therapeutically useful agents other than those described herein, which may optionally be included in the formulation as described above, may be administered in combination with the subject agents in the methods of the present disclosure.

In certain embodiments, compositions will be administered parenterally [*e.g.*, by intravenous (I.V.) injection, intraarterial injection, intraosseous injection, intramuscular injection, intrathecal injection, subcutaneous injection, or intradermal injection]. Pharmaceutical compositions suitable for parenteral administration may comprise one or more agents of the disclosure 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. Injectable solutions or dispersions may contain antioxidants, buffers, bacteriostats, suspending agents, thickening agents, or solutes which render the formulation isotonic with the blood of the intended recipient. Examples of suitable aqueous and nonaqueous carriers which may be employed in the pharmaceutical formulations of the present disclosure include water, ethanol, polyols (*e.g.*, glycerol, propylene glycol, polyethylene glycol, *etc.*), vegetable oils (*e.g.*, olive oil), injectable organic esters (*e.g.*, ethyl oleate), and suitable mixtures thereof. Proper fluidity can be maintained, for example, by the use of coating materials (*e.g.*, lecithin), by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

In some embodiments, a therapeutic method of the present disclosure includes administering the pharmaceutical composition systemically, or locally, from an implant or device. Further, the pharmaceutical composition may be encapsulated or injected in a form for delivery to a target tissue site (*e.g.*, bone marrow or muscle). In certain embodiments, compositions of the present disclosure may include a matrix capable of delivering one or more of the agents of the present disclosure to a target tissue site (*e.g.*, bone marrow or muscle), 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 one or more agents of the present disclosure. Such matrices may be formed of materials presently in use for other implanted medical applications.

In some embodiments, pharmaceutical compositions will be administered to the eye including, *e.g.*, by topical administration, intraocular (e.g., intravitreal) injection, or by

implant or device. An intravitreal injection can be injected, for example, through the pars plana, 3 mm to 4 mm posterior to the limbus. Pharmaceutical compositions for administration to the eye may formulated in a variety of ways including, for example, eye drops, ophthalmic solutions, ophthalmic suspensions, ophthalmic emulsions, intravitreal injections, sub-Tenon injections, ophthalmic biodrodible implant, and non-bioeordible ophthalmic inserts or depots.

The choice of matrix material may be based on one or more of: 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, tricalciumphosphate, hydroxyapatite, polylactic acid, and polyanhydrides. Other potential materials are biodegradable and biologically well-defined including, for example, bone or dermal collagen. Further matrices are comprised of pure proteins or extracellular matrix components. Other potential matrices are non-biodegradable and chemically defined including, for example, sintered hydroxyapatite, bioglass, aluminates, or other ceramics. Matrices may be comprised of combinations of any of the above mentioned types of material including, for example, polylactic acid and hydroxyapatite or collagen and tricalciumphosphate. The bioceramics may be altered in composition (*e.g.*, calcium-aluminate-phosphate) and processing to alter one or more of pore size, particle size, particle shape, and biodegradability.

In certain embodiments, pharmaceutical compositions of present disclosure can be administered topically. "Topical application" or "topically" means contact of the pharmaceutical composition with body surfaces including, for example, the skin, wound sites, and mucous membranes. The topical pharmaceutical compositions can have various application forms and typically comprises a drug-containing layer, which is adapted to be placed near to or in direct contact with the tissue upon topically administering the composition. Pharmaceutical compositions suitable for topical administration may comprise one or more one or more TGFβ superfamily type I and/or type II receptor polypeptide complexes of the disclosure in combination formulated as a liquid, a gel, a cream, a lotion, an ointment, a foam, a paste, a putty, a semi-solid, or a solid. Compositions in the liquid, gel, cream, lotion, ointment, foam, paste, or putty form can be applied by spreading, spraying, smearing, dabbing or rolling the composition on the target tissue. The compositions also may be impregnated into sterile dressings, transdermal patches, plasters, and bandages. Compositions of the putty, semi-solid or solid forms may be deformable. They may be

elastic or non-elastic (*e.g.,* flexible or rigid). In certain aspects, the composition forms part of a composite and can include fibers, particulates, or multiple layers with the same or different compositions.

Topical compositions in the liquid form may include pharmaceutically acceptable solutions, emulsions, microemulsions, and suspensions. In addition to the active ingredient(s), the liquid dosage form may contain an inert diluent commonly used in the art including, for example, water or other solvent, a solubilizing agent and/or emulsifier [*e.g.*, ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, or 1,3-butylene glycol, an oil (*e.g.*, cottonseed, groundnut, corn, germ, olive, castor, and sesame oil), glycerol, tetrahydrofuryl alcohol, a polyethylene glycol, a fatty acid ester of sorbitan, and mixtures thereof].

Topical gel, cream, lotion, ointment, semi-solid or solid compositions may include one or more thickening agents, such as a polysaccharide, synthetic polymer or protein-based polymer. In one embodiment of the invention, the gelling agent herein is one that is suitably nontoxic and gives the desired viscosity. The thickening agents may include polymers, copolymers, and monomers of: vinylpyrrolidones, methacrylamides, acrylamides N-vinylimidazoles, carboxy vinyls, vinyl esters, vinyl ethers, silicones, polyethyleneoxides, polyethyleneglycols, vinylalcohols, sodium acrylates, acrylates, maleic acids, NN-dimethylacrylamides, diacetone acrylamides, acrylamides, acryloyl morpholine, pluronic, collagens, polyacrylamides, polyacrylates, polyvinyl alcohols, polyvinylenes, polyvinyl silicates, polyacrylates substituted with a sugar (e.g., sucrose, glucose, glucosamines, galactose, trehalose, mannose, or lactose), acylamidopropane sulfonic acids, tetramethoxyorthosilicates, methyltrimethoxyorthosilicates, tetraalkoxyorthosilicates, trialkoxyorthosilicates, glycols, propylene glycol, glycerine, polysaccharides, alginates, dextrans, cyclodextrin, celluloses, modified celluloses, oxidized celluloses, chitosans, chitins, guars, carrageenans, hyaluronic acids, inulin, starches, modified starches, agarose, methylcelluloses, plant gums, hylaronans, hydrogels, gelatins, glycosaminoglycans, carboxymethyl celluloses, hydroxyethyl celluloses, hydroxy propyl methyl celluloses, pectins, low-methoxy pectins, cross-linked dextrans, starch-acrylonitrile graft copolymers, starch sodium polyacrylate, hydroxyethyl methacrylates, hydroxyl ethyl acrylates, polyvinylene, polyethylvinylethers, polymethyl methacrylates, polystyrenes, polyurethanes, polyalkanoates, polylactic acids, polylactates, poly(3-hydroxybutyrate), sulfonated hydrogels, AMPS (2-acrylamido-2-methyl-1-propanesulfonic acid), SEM (sulfoethylmethacrylate), SPM

(sulfopropyl methacrylate), SPA (sulfopropyl acrylate), N,N-dimethyl-N-methacryloxyethyl-N-(3-sulfopropyl)ammonium betaine, methacryllic acid amidopropyl-dimethyl ammonium sulfobetaine, SPI (itaconic acid-bis(1-propyl sulfonizacid-3) ester di-potassium salt), itaconic acids, AMBC (3-acrylamido-3-methylbutanoic acid), beta-carboxyethyl acrylate (acrylic acid dimers), and maleic anhydride-methylvinyl ether polymers, derivatives thereof, salts thereof, acids thereof, and combinations thereof. In certain embodiments, pharmaceutical compositions of present disclosure can be administered orally, for example, in the form of capsules, cachets, pills, tablets, lozenges (using a flavored basis such as sucrose and acacia or tragacanth), powders, granules, a solution or a suspension in an aqueous or non-aqueous liquid, an oil-in-water or water-in-oil liquid emulsion, or an elixir or syrup, or pastille (using an inert base, such as gelatin and glycerin, or sucrose and acacia), and/or a mouth wash, each containing a predetermined amount of a compound of the present disclosure and optionally one or more other active ingredients. A compound of the present disclosure and optionally one or more other active ingredients may also be administered as a bolus, electuary, or paste.

In solid dosage forms for oral administration (*e.g.*, capsules, tablets, pills, dragees, powders, and granules), one or more compounds of the present disclosure may be mixed with one or more pharmaceutically acceptable carriers including, for example, sodium citrate, dicalcium phosphate, a filler or extender (*e.g.*, a starch, lactose, sucrose, glucose, mannitol, and silicic acid), a binder (*e.g.* carboxymethylcellulose, an alginate, gelatin, polyvinyl pyrrolidone, sucrose, and acacia), a humectant (*e.g.*, glycerol), a disintegrating agent (*e.g.*, agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, a silicate, and sodium carbonate), a solution retarding agent (*e.g.* paraffin), an absorption accelerator (*e.g.* a quaternary ammonium compound), a wetting agent (*e.g.*, cetyl alcohol and glycerol monostearate), an absorbent (*e.g.*, kaolin and bentonite clay), a lubricant (*e.g.*, a talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate), a coloring agent, and mixtures thereof. In the case of capsules, tablets, and pills, the pharmaceutical formulation (composition) may also comprise a buffering agent. Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using one or more excipients including, e.g., lactose or a milk sugar as well as a high molecular-weight polyethylene glycol.

Liquid dosage forms for oral administration of the pharmaceutical composition may include pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups, and elixirs. In addition to the active ingredient(s), the liquid dosage form may contain

an inert diluent commonly used in the art including, for example, water or other solvent, a solubilizing agent and/or emulsifier [*e.g.*, ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, or 1,3-butylene glycol, an oil (*e.g.*, cottonseed, groundnut, corn, germ, olive, castor, and sesame oil), glycerol, tetrahydrofuryl alcohol, a polyethylene glycol, a fatty acid ester of sorbitan, and mixtures thereof]. Besides inert diluents, the oral formulation can also include an adjuvant including, for example, a wetting agent, an emulsifying and suspending agent, a sweetening agent, a flavoring agent, a coloring agent, a perfuming agent, a preservative agent, and combinations thereof.

Suspensions, in addition to the active compounds, may contain suspending agents including, for example, an ethoxylated isostearyl alcohol, polyoxyethylene sorbitol, a sorbitan ester, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar, tragacanth, and combinations thereof.

Prevention of the action and/or growth of microorganisms may be ensured by the inclusion of various antibacterial and antifungal agents including, for example, paraben, chlorobutanol, and phenol sorbic acid.

In certain embodiments, it may be desirable to include an isotonic agent including, for example, a sugar or sodium chloride into the compositions. In addition, prolonged absorption of an injectable pharmaceutical form may be brought about by the inclusion of an agent that delay absorption including, for example, 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 one or more of the agents of the present disclosure. In the case of ALK7:ActRIIB antagonists that promote red blood cell formation, various factors may include, but are not limited to, the patient's red blood cell count, hemoglobin level, the desired target red blood cell count, the patient's age, the patient's sex, the patient's diet, the severity of any disease that may be contributing to a depressed red blood cell level, the time of administration, and other clinical factors. The addition of other known active agents to the final composition may also affect the dosage. Progress can be monitored by periodic assessment of one or more of red blood cell levels, hemoglobin levels, reticulocyte levels, and other indicators of the hematopoietic process.

In certain embodiments, the present disclosure also provides gene therapy for the *in vivo* production of one or more of the agents of the present disclosure. Such therapy would

achieve its therapeutic effect by introduction of the agent sequences into cells or tissues having one or more of the disorders as listed above. Delivery of the agent sequences can be achieved, for example, by using a recombinant expression vector such as a chimeric virus or a colloidal dispersion system. Preferred therapeutic delivery of one or more of agent sequences of the disclosure 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 an RNA virus (*e.g.*, a retrovirus). The retroviral vector may be 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 one or more of the agents of the present disclosure.

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 one or more of the agents of the present disclosure is a colloidal dispersion system. Colloidal dispersion systems include, for example, macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. In certain embodiments, the preferred colloidal system of this disclosure 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 [Fraley, et al. (1981) Trends Biochem. Sci., 6:77]. Methods for efficient gene transfer using a liposome vehicle are known in the art [Mannino, *et al.* (1988) Biotechniques, 6:682, 1988].

The composition of the liposome is usually a combination of phospholipids, which may include a steroid (*e.g.*cholesterol). The physical characteristics of liposomes depend on pH, ionic strength, and the presence of divalent cations. Other phospholipids or other lipids may also be used including, for example a phosphatidyl compound (*e.g.*, phosphatidylglycerol, phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine, a sphingolipid, a cerebroside, and a ganglioside), 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.

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.

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.

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 and embodiments of the present invention, and are not intended to limit the invention.

Example 1. Generation of an ActRIIB-Fc:ALK7-Fc heterodimer

Applicants constructed a soluble ActRIIB-Fc:ALK7-Fc heteromeric complex comprising the extracellular domains of human ActRIIB and human ALK7, which are each fused to an Fc domain with a linker positioned between the extracellular domain and the Fc domain. The individual constructs are referred to as ActRIIB-Fc and ALK7-Fc, respectively.

A methodology for promoting formation of ActRIIB-Fc:ALK7-Fc heteromeric complexes, as opposed to the ActRIIB-Fc or ALK7-Fc homodimeric complexes, is to

introduce alterations in the amino acid sequence of the Fc domains to guide the formation of asymmetric heteromeric complexes. Many different approaches to making asymmetric interaction pairs using Fc domains are described in this disclosure.

In one approach, illustrated in the ActRIIB-Fc and ALK7-Fc polypeptide sequences disclosed below, respectively, one Fc domain is altered to introduce cationic amino acids at the interaction face, while the other Fc domain is altered to introduce anionic amino acids at the interaction face. The ActRIIB-Fc fusion polypeptide and ALK7-Fc fusion polypeptide each employ the tissue plasminogen activator (TPA) leader: MDAMKRGLCCVLLLCGAVFVSP (SEQ ID NO: 70).

The ActRIIB-Fc polypeptide sequence (SEQ ID NO: 71) is shown below:

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  1   MDAMKRGLCC VLLLCGAVFV SPGASGRGEA ETRECIYYNA NWELERTNQS
 51   GLERCEGEQD KRLHCYASWR NSSGTIELVK KGCWLDDFNC YDRQECVATE
101   ENPQVYFCCC EGNFCNERFT HLPEAGGPEV TYEPPPTAPT GGGTHTCPPC
151   PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV
201   DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY KCKVSNKALP
251   APIEKTISKA KGQPREPQVY TLPPSRKEMT KNQVSLTCLV KGFYPSDIAV
301   EWESNGQPEN NYKTTPPVLK SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH
351   EALHNHYTQK SLSLSPGK          (SEQ ID NO: 71)
```

The leader (signal) sequence and linker are underlined. To promote formation of the ActRIIB-Fc:ALK7-Fc heterodimer rather than either of the possible homodimeric complexes, two amino acid substitutions (replacing acidic amino acids with lysine) can be introduced into the Fc domain of the ActRIIB fusion protein as indicated by double underline above. The amino acid sequence of SEQ ID NO: 71 may optionally be provided with lysine (K) removed from the C-terminus.

This ActRIIB-Fc fusion protein is encoded by the following nucleic acid sequence (SEQ ID NO: 72):

```
  1   ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
 51   AGTCTTCGTT TCGCCCGGCG CCTCTGGGCG TGGGGAGGCT GAGACACGGG
101   AGTGCATCTA CTACAACGCC AACTGGGAGC TGGAGCGCAC CAACCAGAGC
151   GGCCTGGAGC GCTGCGAAGG CGAGCAGGAC AAGCGGCTGC ACTGCTACGC
201   CTCCTGGCGC AACAGCTCTG GCACCATCGA GCTCGTGAAG AAGGGCTGCT
251   GGCTAGATGA CTTCAACTGC TACGATAGGC AGGAGTGTGT GGCCACTGAG
301   GAGAACCCCC AGGTGTACTT CTGCTGCTGT GAAGGCAACT TCTGCAACGA
351   GCGCTTCACT CATTTGCCAG AGGCTGGGGG CCCGGAAGTC ACGTACGAGC
401   CACCCCCGAC AGCCCCCACC GGTGGTGGAA CTCACACATG CCCACCGTGC
451   CCAGCACCTG AACTCCTGGG GGGACCGTCA GTCTTCCTCT TCCCCCCAAA
501   ACCCAAGGAC ACCCTCATGA TCTCCCGGAC CCCTGAGGTC ACATGCGTGG
551   TGGTGGACGT GAGCCACGAA GACCCTGAGG TCAAGTTCAA CTGGTACGTG
601   GACGGCGTGG AGGTGCATAA TGCCAAGACA AAGCCGCGGG AGGAGCAGTA
651   CAACAGCACG TACCGTGTGG TCAGCGTCCT CACCGTCCTG CACCAGGACT
701   GGCTGAATGG CAAGGAGTAC AAGTGCAAGG TCTCCAACAA AGCCCTCCCA
```

```
 751  GCCCCCATCG AGAAAACCAT CTCCAAAGCC AAAGGGCAGC CCCGAGAACC
 801  ACAGGTGTAC ACCCTGCCCC CATCCCGGAA GGAGATGACC AAGAACCAGG
 851  TCAGCCTGAC CTGCCTGGTC AAAGGCTTCT ATCCCAGCGA CATCGCCGTG
 901  GAGTGGGAGA GCAATGGGCA GCCGGAGAAC AACTACAAGA CCACGCCTCC
 951  CGTGCTGAAG TCCGACGGCT CCTTCTTCCT CTATAGCAAG CTCACCGTGG
1001  ACAAGAGCAG GTGGCAGCAG GGGAACGTCT TCTCATGCTC CGTGATGCAT
1051  GAGGCTCTGC ACAACCACTA CACGCAGAAG AGCCTCTCCC TGTCTCCGGG
1101  TAAA          (SEQ ID NO: 72)
```

The mature ActRIIB-Fc fusion polypeptide (SEQ ID NO: 73) is as follows, and may optionally be provided with lysine removed from the C-terminus.

```
   1  GRGEAETREC IYYNANWELE RTNQSGLERC EGEQDKRLHC YASWRNSSGT
  51  IELVKKGCWL DDFNCYDRQE CVATEENPQV YFCCCEGNFC NERFTHLPEA
 101  GGPEVTYEPP PTAPTGGGTH TCPPCPAPEL LGGPSVFLFP PKPKDTLMIS
 151  RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV HNAKTKPREE QYNSTYRVVS
 201  VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQPR EPQVYTLPPS
 251  RKEMTKNQVS LTCLVKGFYP SDIAVEWESN GQPENNYKTT PPVLKSDGSF
 301  FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLS PGK
```

(SEQ ID NO: 73)

The complementary form of ALK7-Fc fusion protein (SEQ ID NO: 74) is as follows:

```
   1  MDAMKRGLCC VLLLCGAVFV SPGAGLKCVC LLCDSSNFTC QTEGACWASV
  51  MLTNGKEQVI KSCVSLPELN AQVFCHSSNN VTKTECCFTD FCNNITLHLP
 101  TASPNAPKLG PMETGGGTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR
 151  TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV
 201  LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSR
 251  EEMTKNQVSL TCLVKGFYPS DIAVEWESNG QPENNYDTTP PVLDSDGSFF
 301  LYSDLTVDKS RWQQGNVFSC SVMHEALHNH YTQKSLSLSP G
          (SEQ ID NO: 74)
```

The signal sequence and linker sequence are underlined. To promote formation of the ActRIIB-Fc:ALK7-Fc heterodimer rather than either of the possible homodimeric complexes, two amino acid substitutions (replacing lysines with aspartic acids) can be introduced into the Fc domain of the fusion protein as indicated by double underline above. The amino acid sequence of SEQ ID NO: 74 may optionally be provided with a lysine added at the C-terminus.

This ALK7-Fc fusion protein is encoded by the following nucleic acid (SEQ ID NO: 75):

```
   1  ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
  51  AGTCTTCGTT TCGCCCGGCG CCGGACTGAA GTGTGTATGT CTTTTGTGTG
 101  ATTCTTCAAA CTTTACCTGC CAAACAGAAG GAGCATGTTG GGCATCAGTC
 151  ATGCTAACCA ATGGAAAAGA GCAGGTGATC AAATCCTGTG TCTCCCTTCC
 201  AGAACTGAAT GCTCAAGTCT TCTGTCATAG TTCCAACAAT GTTACCAAAA
 251  CCGAATGCTG CTTCACAGAT TTTTGCAACA ACATAACACT GCACCTTCCA
 301  ACAGCATCAC CAAATGCCCC AAAACTTGGA CCCATGGAGA CCGGTGGTGG
 351  AACTCACACA TGCCCACCGT GCCCAGCACC TGAACTCCTG GGGGGACCGT
 401  CAGTCTTCCT CTTCCCCCCA AAACCCAAGG ACACCCTCAT GATCTCCCGG
```

```
 451  ACCCCTGAGG TCACATGCGT GGTGGTGGAC GTGAGCCACG AAGACCCTGA
 501  GGTCAAGTTC AACTGGTACG TGGACGGCGT GGAGGTGCAT AATGCCAAGA
 551  CAAAGCCGCG GGAGGAGCAG TACAACAGCA CGTACCGTGT GGTCAGCGTC
 601  CTCACCGTCC TGCACCAGGA CTGGCTGAAT GGCAAGGAGT ACAAGTGCAA
 651  GGTCTCCAAC AAAGCCCTCC CAGCCCCCAT CGAGAAAACC ATCTCCAAAG
 701  CCAAAGGGCA GCCCCGAGAA CCACAGGTGT ACACCCTGCC CCCATCCCGG
 751  GAGGAGATGA CCAAGAACCA GGTCAGCCTG ACCTGCCTGG TCAAAGGCTT
 801  CTATCCCAGC GACATCGCCG TGGAGTGGGA GAGCAATGGG CAGCCGGAGA
 851  ACAACTACGA CACCACGCCT CCCGTGCTGG ACTCCGACGG CTCCTTCTTC
 901  CTCTATAGCG ACCTCACCGT GGACAAGAGC AGGTGGCAGC AGGGGAACGT
 951  CTTCTCATGC TCCGTGATGC ATGAGGCTCT GCACAACCAC TACACGCAGA
1001  AGAGCCTCTC CCTGTCTCCG GGT      (SEQ ID NO: 75)
```

The mature ALK7-Fc fusion protein sequence (SEQ ID NO: 76) is expected to be as follows and may optionally be provided with a lysine added at the C-terminus.

```
  1   GLKCVCLLCD SSNFTCQTEG ACWASVMLTN GKEQVIKSCV SLPELNAQVF
 51   CHSSNNVTKT ECCFTDFCNN ITLHLPTASP NAPKLGPMET GGGTHTCPPC
101   PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV
151   DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY KCKVSNKALP
201   APIEKTISKA KGQPREPQVY TLPPSREEMT KNQVSLTCLV KGFYPSDIAV
251   EWESNGQPEN NYDTTPPVLD SDGSFFLYSD LTVDKSRWQQ GNVFSCSVMH
301   EALHNHYTQK SLSLSPG        (SEQ ID NO: 76)
```

The ActRIIB-Fc and ALK7-Fc fusion proteins of SEQ ID NO: 71 and SEQ ID NO: 74, respectively, may be co-expressed and purified from a CHO cell line to give rise to a heteromeric complex comprising ActRIIB-Fc:ALK7-Fc.

In another approach to promote the formation of heteromultimer complexes using asymmetric Fc fusion proteins, the Fc domains are altered to introduce complementary hydrophobic interactions and an additional intermolecular disulfide bond as illustrated in the ActRIIB-Fc and ALK7-Fc polypeptide sequences of disclosed below.

The ActRIIB-Fc polypeptide sequence (SEQ ID NO: 77) is shown below:

```
  1   MDAMKRGLCC VLLLCGAVFV SPGASGRGEA ETRECIYYNA NWELERTNQS
 51   GLERCEGEQD KRLHCYASWR NSSGTIELVK KGCWLDDFNC YDRQECVATE
101   ENPQVYFCCC EGNFCNERFT HLPEAGGPEV TYEPPPTAPT GGGTHTCPPC
151   PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV
201   DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY KCKVSNKALP
251   APIEKTISKA KGQPREPQVY TLPPCREEMT KNQVSLWCLV KGFYPSDIAV
301   EWESNGQPEN NYKTTPPVLD SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH
351   EALHNHYTQK SLSLSPGK         (SEQ ID NO: 77)
```

The leader sequence and linker are underlined. To promote formation of the ActRIIB-Fc:ALK7-Fc heterodimer rather than either of the possible homodimeric complexes, two amino acid substitutions (replacing a serine with a cysteine and a threonine with a trytophan) can be introduced into the Fc domain of the fusion protein as indicated by double

underline above. The amino acid sequence of SEQ ID NO: 77 may optionally be provided with lysine removed from the C-terminus.

The mature ActRIIB-Fc fusion polypeptide (SEQ ID NO: 78) is as follows and may optionally be provided with lysine removed from the C-terminus.

```
  1  GRGEAETREC IYYNANWELE RTNQSGLERC EGEQDKRLHC YASWRNSSGT
 51  IELVKKGCWL DDFNCYDRQE CVATEENPQV YFCCCEGNFC NERFTHLPEA
101  GGPEVTYEPP PTAPTGGGTH TCPPCPAPEL LGGPSVFLFP PKPKDTLMIS
151  RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV HNAKTKPREE QYNSTYRVVS
201  VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQPR EPQVYTLPPC
251  REEMTKNQVS LWCLVKGFYP SDIAVEWESN GQPENNYKTT PPVLDSDGSF
301  FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLS PGK
        (SEQ ID NO: 78)
```

The complementary form of ALK7-Fc fusion polypeptide (SEQ ID NO: 79) is as follows:

```
  1  MDAMKRGLCC VLLLCGAVFV SPGAGLKCVC LLCDSSNFTC QTEGACWASV
 51  MLTNGKEQVI KSCVSLPELN AQVFCHSSNN VTKTECCFTD FCNNITLHLP
101  TASPNAPKLG PMETGGGTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR
151  TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV
201  LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVCTLPPSR
251  EEMTKNQVSL SCAVKGFYPS DIAVEWESNG QPENNYKTTP PVLDSDGSFF
301  LVSKLTVDKS RWQQGNVFSC SVMHEALHNH YTQKSLSLSP GK
     (SEQ ID NO: 79)
```

The leader sequence and linker sequence are underlined. To guide heterodimer formation with the ActRIIB-Fc fusion polypeptide of SEQ ID NOs 76 and 78 above, four amino acid substitutions can be introduced into the Fc domain of the ALK7 fusion polypeptide as indicated by double underline above. The amino acid sequence of SEQ ID NO: 79 may optionally be provided with the lysine removed from the C-terminus.

The mature ALK7-Fc fusion protein sequence (SEQ ID NO: 80) is expected to be as follows and may optionally be provided with the lysine removed from the C-terminus.

```
  1  GLKCVCLLCD SSNFTCQTEG ACWASVMLTN GKEQVIKSCV SLPELNAQVF
 51  CHSSNNVTKT ECCFTDFCNN ITLHLPTASP NAPKLGPMET GGGTHTCPPC
101  PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV
151  DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY KCKVSNKALP
201  APIEKTISKA KGQPREPQVC TLPPSREEMT KNQVSLSCAV KGFYPSDIAV
251  EWESNGQPEN NYKTTPPVLD SDGSFFLVSK LTVDKSRWQQ GNVFSCSVMH
301  EALHNHYTQK SLSLSPGK       (SEQ ID NO: 80)
```

The ActRIIB-Fc and ALK7-Fc proteins of SEQ ID NO: 77 and SEQ ID NO: 79, respectively, may be co-expressed and purified from a CHO cell line, to give rise to a heteromeric complex comprising ActRIIB-Fc:ALK7-Fc.

Purification of various ActRIIB-Fc:ALK7-Fc complexes could 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.

Example 2. Ligand binding profile of ActRIIB-Fc:ALK7-Fc heterodimer compared to ActRIIB-Fc homodimer and ALK7-Fc homodimer

A Biacore™-based binding assay was used to compare ligand binding selectivity of the ActRIIB-Fc:ALK7-Fc heterodimeric complex described above with that of ActRIIB-Fc and ALK7-Fc homodimeric complexes. The ActRIIB-Fc:ALK7-Fc heterodimer, ActRIIB-Fc homodimer, and ALK7-Fc homodimer were independently captured onto the system using an anti-Fc antibody. Ligands were injected and allowed to flow over the captured receptor protein. Results are summarized in the table below, in which ligand off-rates (k_d) most indicative of effective ligand traps are denoted by gray shading.

Ligand binding profile of ActRIIB-Fc:ALK7-Fc heterodimer compared to ActRIIB-Fc homodimer and ALK7-Fc homodimer									
Ligand	ActRIIB-Fc homodimer			ALK7-Fc homodimer			ActRIIB-Fc:ALK7-Fc heterodimer		
	k_a (1/Ms)	k_d (1/s)	K_D (pM)	k_a (1/Ms)	k_d (1/s)	K_D (pM)	k_a (1/Ms)	k_d (1/s)	K_D (pM)
activin A	1.3×10^7	1.4×10^{-4}	11	No binding			4.4×10^7	1.9×10^{-3}	43
activin B	1.5×10^7	1.6×10^{-4}	8	No binding			1.2×10^7	2.0×10^{-4}	17
activin C	No binding			No binding			3.5×10^5	2.4×10^{-3}	6900
activin AC	2.0×10^7	3.1×10^{-3}	160	No binding			2.6×10^6	5.7×10^{-4}	220
BMP5	2.6×10^7	7.5×10^{-2}	2900	No binding			1.5×10^5	8.5×10^{-3}	57000
BMP6	2.4×10^7	3.9×10^{-3}	160	No binding			1.2×10^6	6.3×10^{-3}	5300
BMP9	1.2×10^8	1.2×10^{-3}	10	No binding			Transient*		>1400

BMP10	5.9 x10^6	1.5 x10^{-4}	25	No binding	1.5 x10^7	2.8 x10^{-3}	190
GDF3	1.4 x10^6	2.2 x10^{-3}	1500	No binding	2.3 x10^6	1.0 x10^{-2}	4500
GDF8	3.5 x10^6	2.4 x10^{-4}	69	No binding	3.7 x10^6	1.0 x10^{-3}	270
GDF11	9.6 x10^7	1.5 x10^{-4}	2	No binding	9.5 x10^7	7.5 x 10^{-4}	8
* Indeterminate due to transient nature of interaction --- Not tested							

These comparative binding data demonstrate that the ActRIIB-Fc:ALK7-Fc heterodimer has an altered binding profile/selectivity relative to either the ActRIIB-Fc homodimer or ALK7-Fc homodimer. Interestingly, four of the five ligands with the strongest binding to ActRIIB-Fc homodimer (activin A, BMP10, GDF8, and GDF11) exhibit reduced binding to the ActRIIB-Fc:ALK7-Fc heterodimer, the exception being activin B which retains tight binding to the heterodimer. Similarly, three of the four ligands with intermediate binding to ActRIIB-Fc homodimer (GDF3, BMP6, and particularly BMP9) exhibit reduced binding to the ActRIIB-Fc:ALK7-Fc heterodimer, whereas binding to activin AC is increased to become the second strongest ligand interaction with the heterodimer overall. Finally, activin C and BMP5 unexpectedly bind the ActRIIB-Fc:ALK7 heterodimer with intermediate strength despite no binding (activin C) or weak binding (BMP5) to ActRIIB-Fc homodimer. The net result is that the ActRIIB-Fc:ALK7-Fc heterodimer possesses a ligand-binding profile distinctly different from that of either ActRIIB-Fc homodimer or ALK7-Fc homodimer, which binds none of the foregoing ligands. See Figure 6.

These results therefore demonstrate that the ActRIIB-Fc:ALK7-Fc heterodimer is a more selective antagonist of activin B and activin AC compared to ActRIIB-Fc homodimer. Moreover, ActRIIB-Fc:ALK7-Fc heterodimer exhibits the unusual property of robust binding to activin C. Accordingly, an ActRIIB-Fc:ALK7-Fc heterodimer will be more useful than an ActRIIB-Fc homodimer in certain applications where such selective antagonism is advantageous. Examples include therapeutic applications where it is desirable to retain antagonism of activin B or activin AC but decrease antagonism of one or more of activin A, GDF3, GDF8, GDF11, BMP9, or BMP10. Also included are therapeutic, diagnostic, or analytic applications in which it is desirable to antagonize activin C or, based on the similarity between activin C and activin E, activin E.

Example 3. ALK7:ActRIIB heteromultimer treatment suppresses kidney fibrosis and inflammation and reduces kidney injury.

The effects of the ALK7-Fc:ActRIIB-Fc heterodimer described in Example 2 on kidney disease was assessed in a mouse unilateral ureteral obstruction model. See, e.g., Klahr and Morrissey (2002) Am J Physiol Renal Physiol 283: F861-F875.

Twenty-four C57BL/6 male mice 12 weeks of age underwent left unilateral ureteral ligation twice at the level of the lower pole of kidney. After 3 days, eight mice were euthanized and kidneys from individual animals were harvested to assess kidney injury. The remaining mice were randomized into two groups: i) eight mice were injected subcutaneously with the ALK7-Fc:ActRIIB-Fc heterodimer at a dose of 10mg/kg at day 3, day 7, day 10, and day 14 after surgery and a ii) eight mice were injected subcutaneously with vehicle control, phosphate buffered saline (PBS), at day 3, day 7, day 10, and day 14 after surgery. Both groups were sacrificed at day 17 in accordance with the relevant Animal Care Guidelines. Half kidneys from individual animals were collected for histology analysis (H&E, and Masson's Trichrome stain), from both the UUO kidney and contralateral kidney, and 1/4 kidneys were used for RNA extraction (RNeasy Midi Kit, Qiagen, IL).

Gene expression analysis on UUO kidney samples was performed to assess levels of various genes. QRT-PCR was performed on a CFX Connect™ Real-time PCR detection system (Bio-Rad, CA) to evaluate the expression of various fibrotic genes (Col1a1, Col3a1, Fibronectin, PAI-1, CTGF, and a-SMA), inflammatory genes (Tnfa, and MCP1), cytokines (TGFβ1, TGFβ2, TGFβ3, and activin A), kidney injury genes (NGAL), Hypoxia-inducible factor 1-alpha (HIF1a), and activin A receptor (ActRIIA). See Figure 13. Treatment of mice with ALK7-Fc:ActRIIB-Fc heterodimer significantly suppressed the expression of fibrotic and inflammatory genes, inhibited the upregulation of TGFβ 1/2/3, activin A, and ActRIIa, and reduced kidney injury. Histology data confirmed that ALK7-Fc:ActRIIB-Fc heterodimer treatment significantly inhibited kidney fibrosis and reduced kidney injury in the UUO model.

Together, these data demonstrate that ALK7:ActRIIB heteromultimer treatment suppresses kidney fibrosis and inflammation and reduces kidney injury. Moreover, these data indicate that other ALK7:ActRIIB antagonists may be useful in the treatment or preventing of kidney disease including, for example, antagonists of ALK7 and/or ActRIIB-binding ligands (e.g., ligand antibodies and other ligand traps such as follistatin, Cerberus and Lefty), antagonists of ALK7 and/or ActRIIB receptors, antagonists of ALK7 and/or ActRIIB

downstream signaling mediators (e.g., Smads), and antagonists of TGFβ superfamily co-receptors (e.g., antagonists of Crypto or Cryptic).

Example 4. ALK7:ActRIIB heteromultimer increases lipolysis in adipocytes.

Lipolysis is the hydrolysis of triglycerides within the cell into glycerol and free fatty acids. The glycerol and free fatty acids are then released into the bloodstream or culture media. While lipolysis occurs in essentially all cells, it is most abundant in white and brown adipocytes. ALK7 signaling is thought to suppress lipolysis and to consequently lead to fat accumulation in adipocytes and adipose tissue. Accordingly, the effects of the ALK7-Fc:ActRIIB-Fc heterodimer described in Example 2 on lipolysis in adipoctyes was assessed.

Specifically, 3T3-L1 cells (supplied by ATCC; ATCC® CL-173™) were grown in Dulbecco's Modified Eagle Medium (ATCC; ATCC® 30-2002™) containing 10% Bovine Serum (Life Technologies; 16170-060) until reaching confluency. To induce differentiation, at 2 days post-confluency medium was replaced by fresh Dulbecco's Modified Eagle Medium (ATCC; ATCC® 30-2002™) containing 10% fetal Bovine serum (Life Technologies; 10082147), dexamethasone (Sigma, D8893), IBMX (Sigma, I7018) and insulin (Sigma, I0516) for 2 weeks. Accumulation of lipid droplets on the cells, as determined by microscopy, was used to confirm a complete differentiation into mature adipocyte cells. Adipocytes were treated overnight with vehicle (PBS), activin B (50 ng/ml) or co-treated with activin B (50 ng/ml) and ALK7-Fc:ActRIIB-Fc heterodimer (5 μg/ml). Cells were washed two times with PBS and incubated with lipolysis assay buffer (supplied by Abcam; ab185433). Lipolysis assay buffer was collected after 3 hours and glycerol levels were measured according to manufacturer's instruction (Abcam; ab185433). It was determined that the ALK7-Fc:ActRIIB-Fc heterodimer significantly increased lipolysis activity by 44.36% in this cell-based assay.

Accordingly, these data demonstrate that ALK7-Fc:ActRIIB-Fc heterodimer can be used to antagonize ALK7-mediated suppression of lipolysis and thereby increase fatty acid breakdown in adipocytes. Moreover, these data indicate that ALK7-Fc:ActRIIB-Fc heterodimers as well as other ALK7:ActRIIB antagonists, such as those disclosed herein, may be used to treat a variety of disorder or conditions associated with low lipolysis activity and/or excessive fatty acid accumulation in cells, particularly adipocytes, including for

example, obesity, diabetes, insulin resistance; metabolic syndrome, fatty liver disease and other metabolic diseases or conditions, particularly those associated with excess fat levels.

We claim:

1. A recombinant heteromultimer comprising an ALK7 polypeptide and an ActRIIB polypeptide.

2. The heteromultimer of claim 1, wherein the ALK7 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a polypeptide that:

a) begins at any one of amino acids of 21, 22, 23, 24, 25, 26, 27, or 28 SEQ ID NO: 9, and

b) ends at any one of amino acids 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, or 113 of SEQ ID NO: 9.

3. The heteromultimer of claim 1, wherein the ALK7 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 28-92 of SEQ ID NO: 9.

4. The heteromultimer of claim 1, wherein the ALK7 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 10.

5. The heteromultimer of claim 1, wherein the ALK7 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 20.

6. The heteromultimer of claim 1, wherein the ALK7 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 39.

7. The heteromultimer of claim 1, wherein the ALK7 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 43 or 46.

8. The heteromultimer of any one of claims 1-7, wherein the ActRIIB polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%,

89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a polypeptide that:

a) begins at any one of amino acids of 20, 21, 22, 23, 24, 25, 26, 27, 28, or 29 SEQ ID NO: 1, and

b) ends at any one of amino acids 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, or 134 of SEQ ID NO: 1.

9. The heteromultimer of any one of claims 1-7, wherein the ActRIIB polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 29-109 of SEQ ID NO: 1.

10. The heteromultimer of any one of claims 1-7, wherein the ActRIIB polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 25-131 of SEQ ID NO: 1.

11. The heteromultimer of any one of claims 8-10, wherein the ActRIIB polypeptide does not comprise an acidic amino acid at the position corresponding to L79 of SEQ ID NO: 1.

12. The heteromultimer of any one of claims 1-11, wherein the ActRIIB polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 2.

13. The heteromultimer of any one of claims 1-11, wherein the ActRIIB polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 3.

14. The heteromultimer of any one of claims 1-11, wherein the ActRIIB polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 5.

15. The heteromultimer of any one of claims 1-11, wherein the ActRIIB polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 6.

16. The heteromultimer of any one of claims 1-15, wherein the ALK7 polypeptide is a fusion protein further comprises a heterologous domain.

17. The heteromultimer of any one of claims 1-16, wherein the ActRIIB polypeptide is a fusion protein further comprising a heterologous domain.

18. The heteromultimer of claim 16, wherein the ALK7 polypeptide comprises a first or second member of an interaction pair.

19. The heteromultimer of claim 17, wherein the ActRIIB polypeptide comprises a first or second member of an interaction pair.

20. The heteromultimer of any one of claims 16-19, wherein the heterologous domain comprises an Fc immunoglobulin domain.

21. The heteromultimer of claim 20, wherein the Fc immunoglobulin domain comprises one or more amino acid modifications that promotes heterodimer formation.

22. The heteromultimer of claim 20 or 21, wherein the immunoglobulin Fc domain comprises one or more amino acid modifications that inhibit homodimer formation.

23. The heteromultimer of any one of 20-22, wherein the heterologous domain comprises an Fc immunoglobulin domain from an IgG immunoglobulin.

24. The heteromultimer of claim 23, wherein the IgG immunoglobulin is selected from the group consisting of: an IgG1, IgG2, and IgG3, or IgG4.

25. The heteromultimer of claim 23, wherein the Fc immunoglobulin comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 23.

26. The heteromultimer of claim 25, wherein the Fc immunoglobulin comprises a positively charged amino acid at the positions corresponding to residues 134 and 177 of SEQ ID NO: 23.

27. The heteromultimer of claim 26, wherein the Fc immunoglobulin comprises a K at positions 134 and 177 of SEQ ID NO: 23.

28. The heteromultimer of any one of claims 25-27, wherein the Fc immunoglobulin does not comprise a positively charged amino acid at the position corresponding to residue 225 of SEQ ID NO: 23.

29. The heteromultimer of claim 28, wherein the Fc immunoglobulin does not comprises a K at position 225 of SEQ ID NO: 23.

30. The heteromultimer of claim 23, wherein the Fc immunoglobulin comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 24.

31. The heteromultimer of claim 30, wherein the Fc immunoglobulin comprises a negatively charged amino acid at the positions corresponding to residues 170 and 187 of SEQ ID NO: 24.

32. The heteromultimer of claim 31, wherein the Fc immunoglobulin comprises a D at positions 170 and 187 of SEQ ID NO: 24.

33. The heteromultimer of any one of claims 30-32, wherein the Fc immunoglobulin comprises a positively charged amino acid at the position corresponding to residue 225 of SEQ ID NO: 24.

34. The heteromultimer of claim 33, wherein the Fc immunoglobulin comprises a K at position 225 of SEQ ID NO: 24.

35. The heteromultimer of claim 23, wherein the Fc immunoglobulin comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 25.

36. The heteromultimer of claim 23, wherein the Fc immunoglobulin comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 26.

37. The heteromultimer of claim 23, wherein the Fc immunoglobulin comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 27.

38. The heteromultimer of claim 37, wherein the Fc immunoglobulin comprises a C at the position corresponding to residue 132 of SEQ ID NO: 27, and wherein the Fc immunoglobulin further comprises a W at the position corresponding to residue 144 of SEQ ID NO: 27.

39. The heteromultimer of claim 37 or 38, wherein the Fc immunoglobulin does not comprises a positively charged amino acid at the position corresponding to residue 225 of SEQ ID NO: 27.

40. The heteromultimer of claim 39, wherein the Fc immunoglobulin does not comprises a K at position 225 of SEQ ID NO: 27.

41. The heteromultimer of claim 23, wherein the Fc immunoglobulin comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 28.

42. The heteromultimer of claim 41, wherein the Fc immunoglobulin comprises a C at the position corresponding to residue 127 of SEQ ID NO: 28, wherein the Fc immunoglobulin further comprises a S at the position corresponding to residue 144 of SEQ ID NO: 28, wherein the Fc immunoglobulin further comprises a A at the position corresponding to residue 146 of SEQ ID NO: 28, and wherein the Fc immunoglobulin further comprises a V at the position corresponding to residue 185 of SEQ ID NO: 28.

43. The heteromultimer of any one of claim 41 or 42, wherein the Fc immunoglobulin does not comprises a positively charged amino acid at the position corresponding to residue 225 of SEQ ID NO: 28.

44. The heteromultimer of claim 43, wherein the Fc immunoglobulin not comprises a K at position 225 of SEQ ID NO: 28.

45. The heteromultimer of claim 23, wherein the Fc immunoglobulin comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 29.

46. The heteromultimer of claim 23, wherein the Fc immunoglobulin comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 30.

47. The heteromultimer of claim 23, wherein the Fc immunoglobulin comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 31.

48. The heteromultimer of claim 23, wherein the Fc immunoglobulin comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 32.

49. The heteromultimer of claim 23, wherein the Fc immunoglobulin comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 33.

50. The heteromultimer of claim 23, wherein the Fc immunoglobulin comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 34.

51. The heteromultimer of claim 23, wherein the Fc immunoglobulin comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 35.

52. The heteromultimer of claim 23, wherein the Fc immunoglobulin comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 36.

53. The heteromultimer of claim 23, wherein the Fc immunoglobulin comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 37.

54. The heteromultimer of claim 23, wherein:

a) the ALK7 polypeptide is a fusion protein further comprising a constant region of an immunoglobulin comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 23; and

b) the ActRIIB polypeptide is a fusion protein further comprising a constant region of an immunoglobulin comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 24.

55. The heteromultimer of claim 23, wherein:

a) the ALK7 polypeptide is a fusion protein further comprising a constant region of an immunoglobulin comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 24; and

b) the ActRIIB polypeptide is a fusion protein further comprising a constant region of an immunoglobulin comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 23.

56. The heteromultimer of claim 54 or 55, wherein the constant region comprises a positively charged amino acid at the positions corresponding to residues 134 and 177 of SEQ ID NO: 23.

57. The heteromultimer of claim 56, wherein the constant region comprises a K at positions 134 and 177 of SEQ ID NO: 23.

58. The heteromultimer of any one of claims 54-57, wherein the constant region does not comprise a positively charged amino acid at the position corresponding to residue 225 of SEQ ID NO: 23.

59. The heteromultimer of claim 58, wherein the constant region does not comprises a K at position 225 of SEQ ID NO: 23.

60. The heteromultimer of any one of claims 54-59, wherein the constant region comprises a negatively charged amino acid at the positions corresponding to residues 170 and 187 of SEQ ID NO: 24.

61. The heteromultimer of claim 60, wherein the constant region comprises a D at positions 170 and 187 of SEQ ID NO: 24.

62. The heteromultimer of any one of claims 54-61, wherein the constant region comprises a positively charged amino acid at the position corresponding to residue 225 of SEQ ID NO: 24.

63. The heteromultimer of claim 62, wherein the constant region comprises a K at position 225 of SEQ ID NO: 24.

64. The heteromultimer of claim 23, wherein:

a) the ALK7 polypeptide is a fusion protein further comprising a constant region of an immunoglobulin comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 27; and

b) the ActRIIB polypeptide is a fusion protein further comprising a constant region of an immunoglobulin comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 28.

65. The heteromultimer of claim 23, wherein:

a) the ALK7 polypeptide is a fusion protein further comprising a constant region of an immunoglobulin comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 28; and

b) the ActRIIB polypeptide is a fusion protein further comprising a constant region of an immunoglobulin comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 27.

66. The heteromultimer of claim 64 or 65, wherein the constant region comprises a C at the position corresponding to residue 132 of SEQ ID NO: 27, and wherein the constant region further comprises a W at the position corresponding to residue 144 of SEQ ID NO: 27.

67. The heteromultimer of any one of claims 64-66, wherein the constant region does not comprises a positively charged amino acid at the position corresponding to residue 225 of SEQ ID NO: 27.

68. The heteromultimer of claim 67, wherein the constant region does not comprises a K at position 225 of SEQ ID NO: 27.

69. The heteromultimer of any one of claims 64-68, wherein the constant region comprises a C at the position corresponding to residue 127 of SEQ ID NO: 28, wherein the constant region further comprises a S at the position corresponding to residue 144 of SEQ ID NO: 28, wherein the constant region further comprises a A at the position corresponding to residue 146 of SEQ ID NO: 28, and wherein the constant region further comprises a V at the position corresponding to residue 185 of SEQ ID NO: 28.

70. The heteromultimer of any one of claims 64-69, wherein the constant region does not comprises a positively charged amino acid at the position corresponding to residue 225 of SEQ ID NO: 28.

71. The heteromultimer of claim 70, wherein the constant region does not comprises a K at position 225 of SEQ ID NO: 28.

72. The heteromultimer of any one of claims 16-71, wherein the fusion protein further comprises a linker domain positioned between the ALK7 domain and the heterologous domain.

73. The heteromultimer of any one of claims 16-72, wherein the fusion protein further comprises a linker domain positioned between the ActRIIB domain and the heterologous domain.

74. The heteromultimer of claims 72 or 73, wherein the linker domain is selected from the group consisting of: TGGG (SEQ ID NO: 17), TGGGG (SEQ ID NO: 15), SGGGG (SEQ ID NO: 16), GGGGS (SEQ ID NO: 58), GGG (SEQ ID NO: 13), GGGG (SEQ ID NO: 14), and SGGG (SEQ ID NO: 18).

75. The heteromultimer of any one of claims 1-74, wherein the ALK7 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 74.

76. The heteromultimer of any one of claims 1-74, wherein the ALK7 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 76.

77. The heteromultimer of any one of claims 1-74, wherein the ActRIIB polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to 71.

78. The heteromultimer of any one of claims 1-74, wherein the ActRIIB polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to 73.

79. The heteromultimer of any one of claims 1-74, wherein the heteromultimer comprises:

a) an ALK7 polypeptide comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 76, and

b) an ActRIIB polypeptide comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 73.

80. The heteromultimer of any one of claims 1-74, wherein the ALK7 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to 79.

81. The heteromultimer of any one of claims 1-74, wherein the ALK7 polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to 80.

82. The heteromultimer of any one of claims 1-74, wherein the ActRIIB polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to 77.

83. The heteromultimer of any one of claims 1-74, wherein the ActRIIB polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to 78.

84. The heteromultimer of any one of claims 1-74, wherein the heteromultimer comprises:

a) an ALK7 polypeptide comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to 80, and

b) an ActRIIB polypeptide comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to 78.

85. The heteromultimer of any one of claims 1-84, wherein the ALK7 polypeptide comprises one or modified amino acid residues selected from the group consisting of: 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.

86. The heteromultimer of any one of claims 1-85, wherein the ActRIIB polypeptide comprises one or modified amino acid residues selected from the group consisting of: 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.

87. The heteromultimer of any one of claims 1-86, wherein the ALK7 polypeptide further comprises one or more purification sequences selected from the group consisting of: an epitope tag, a FLAG tag, a polyhistidne sequence, and a GST fusion.

88. The heteromultimer of any one of claims 1-87, wherein the ActRIIB polypeptide further comprises one or more purification sequences selected from the group consisting of: an epitope tag, a FLAG tag, a polyhistidne sequence, and a GST fusion.

89. The heteromultimer of any one of claims 1-88, wherein the ALK7 polypeptide is glycosylated and has a glycosylation pattern obtainable from a Chinese hamster ovary cell line.

90. The heteromultimer of any one of claims 1-89, wherein the ActRIIB polypeptide is glycosylated and has a glycosylation pattern obtainable from a Chinese hamster ovary cell line.

91. The heteromultimer of any one of claims 1-90, wherein the heteromultimer binds to activin.

92. The heteromultimer of any one of claims 1-91, wherein the heteromultimer binds to activin A.

93. The heteromultimer of any one of claims 1-92, wherein the heteromultimer binds to activin B.

94. The heteromultimer of any one of claims 1-93, wherein the heteromultimer binds to activin C an/or activin E.

95. The heteromultimer of any one of claims 1-94, wherein the heteromultimer binds to activin AB.

96. The heteromultimer of any one of claims 1-95, wherein the heteromultimer binds to activin AC, BC, AE, and/or BE.

97. The heteromultimer of any one of claims 1-96, wherein the heteromultimer binds to GDF8.

98. The heteromultimer of any one of claims 1-97, wherein the heteromultimer binds to GDF11.

99. The heteromultimer of any one of claims 1-98, wherein the heteromultimer binds to BMP6.

100. The heteromultimer of any one of claims 1-99, wherein the heteromultimer binds to nodal.

101. The heteromultimer of any one of claims 1-100, wherein the heteromultimer binds to BMP5.

102. The heteromultimer of any one of claims 1-101, wherein the heteromultimer binds to GDF3.

103. The heteromultimer of any one of claims 1-101, wherein the heteromultimer binds to BMP10.

104. The heteromultimer of any one of claims 1-103, wherein the heteromultimer binds to BMP10 with weaker affinity compared to a corresponding ActRIIB homomultimer.

105. The heteromultimer of any one of claims 1-104, wherein the heteromultimer binds to BMP9 with weaker affinity compared to a corresponding ActRIIB homomultimer.

106. The heteromultimer of any one of claims 1-105, wherein the heteromultimer binds to GDF3 with weaker affinity compared to a corresponding ActRIIB homomultimer.

107. The heteromultimer of any one of claims 1-106, wherein the heteromultimer binds to BMP6 with weaker affinity compared to a corresponding ActRIIB homomultimer.

108. The heteromultimer of any one of claims 1-107, wherein the heteromultimer binds to GDF8 with weaker affinity compared to a corresponding ActRIIB homomultimer.

109. The heteromultimer of any one of claims 1-108, wherein the heteromultimer binds to GDF11 with weaker affinity compared to a corresponding ActRIIB homomultimer.

110. The heteromultimer of any one of claims 1-109, wherein the heteromultimer binds to activin C with stronger affinity compared to a corresponding ActRIIB homomultimer.

111. The heteromultimer of any one of claims 1-110, wherein the heteromultimer binds to activin AC with stronger affinity compared to a corresponding ActRIIB homomultimer.

112. The heteromultimer of any one of claims 1-111, wherein the heteromultimer binds to BMP5 with stronger affinity compared to a corresponding ActRIIB homomultimer.

113. The heteromultimer of any one of claims 1-112, wherein the heteromultimer inhibits activin signaling in a cell-based assay.

114. The heteromultimer of any one of claims 1-113, wherein the heteromultimer inhibits activin A signaling in a cell-based assay.

115. The heteromultimer of any one of claims 1-114, wherein the homoromultimer inhibits activin B signaling in a cell-based assay.

116. The heteromultimer of any one of claims 1-115, wherein the homoromultimer inhibits activin C and/or Esignaling in a cell-based assay.

117. The heteromultimer of any one of claims 1-116, wherein the heteromultimer inhibits activin AC, BC, AE, and/or BE signaling in a cell-based assay.

118. The heteromultimer of any one of claims 1-117, wherein the heteromultimer inhibits GDF11 signaling in a cell-based assay.

119. The heteromultimer of any one of claims 1-118, wherein the heteromultimer inhibits GDF8 signaling in a cell-based assay.

120. The heteromultimer of any one of claims 1-119, wherein the heteromultimer inhibits BMP10 signaling in a cell-based assay.

121. The heteromultimer of any one of claims 1-120, wherein the heteromultimer inhibits BMP6 signaling in a cell-based assay.

122. The heteromultimer of any one of claims 1-121, wherein the heteromultimer inhibits BMP5 signaling in a cell-based assay.

123. The heteromultimer of any one of claims 1-122, wherein the heteromultimer inhibits GDF3 and/or nodal signaling in a cell-based assay.

124. The heteromultimer of any one of claims 1-123, wherein the heteromultimer is a weaker inhibitor of BMP9 signaling in a cell-based assay compared to a corresponding ActRIIB homomultimer.

125. The heteromultimer of any one of claims 1-124, wherein the heteromultimer is a weaker inhibitor of BMP10 signaling in a cell-based assay compared to a corresponding ActRIIB homomultimer.

126. The heteromultimer of any one of claims 1-125, wherein the heteromultimer is a weaker inhibitor of BMP6 signaling in a cell-based assay compared to a corresponding ActRIIB homomultimer.

127. The heteromultimer of any one of claims 1-126, wherein the heteromultimer is a weaker inhibitor of GDF3 signaling in a cell-based assay compared to a corresponding ActRIIB homomultimer.

128. The heteromultimer of any one of claims 1-127, wherein the heteromultimer is a weaker inhibitor of GDF11 signaling in a cell-based assay compared to a corresponding ActRIIB homomultimer.

129. The heteromultimer of any one of claims 1-128, wherein the heteromultimer is a weaker inhibitor of GDF8 signaling in a cell-based assay compared to a corresponding ActRIIB homomultimer.

130. The heteromultimer of any one of claims 1-129, wherein the heteromultimer is a weaker inhibitor of activin A signaling in a cell-based assay compared to a corresponding ActRIIB homomultimer.

131. The heteromultimer of any one of claims 1-129, wherein the heteromultimer is a weaker inhibitor of activin A signaling in a cell-based assay compared to a corresponding ActRIIB homomultimer.

132. The heteromultimer of any one of claims 1-130, wherein the heteromultimer is a stronger inhibitor of activin C signaling in a cell-based assay compared to a corresponding ActRIIB homomultimer.

133. The heteromultimer of any one of claims 1-132, wherein the heteromultimer is a stronger inhibitor of activin AC signaling in a cell-based assay compared to a corresponding ActRIIB homomultimer.

134. The heteromultimer of any one of claims 1-133, wherein the heteromultimer is a stronger inhibitor of BMP5 signaling in a cell-based assay compared to a corresponding ActRIIB homomultimer.

135. The heteromultimer of any one of claims 1-134, wherein the heteromultimer is an ALK7:ActRIIB heterodimer.

136. A pharmaceutical preparation comprising the heteromultimer of any one of claims 1-135.

137. The pharmaceutical preparation of claim 136, wherein the pharmaceutical preparation is substantially pyrogen-free.

138. The pharmaceutical preparation of claim 136 or 137 wherein the pharmaceutical preparation further comprises an additional active agent.

139. The pharmaceutical preparation of any one of claims 136-138 wherein the pharmaceutical preparation comprises less than about 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or less than about 1% ALK7 homomultimers.

140. The pharmaceutical preparation of claim 136-139, wherein the pharmaceutical preparation comprises less than about 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or less than about 1% ActRIIB homomultimers.

141. An isolated nucleic acid sequence comprising a coding sequence for the ALK7 polypeptide of any one of claims 1-135.

142. The isolated nucleic acid sequence of claim 141, wherein the nucleic acid comprises, consists essentially of, or consists of a nucleotide sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 12, 22, 41, 45 or 75.

143. An isolated nucleic acid sequence comprising a coding sequence for the ActRIIB polypeptide of any one of claims 1-134.

144. The isolated nucleic acid sequence of claim 143, wherein the nucleic acid comprises a nucleotide sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 8 or 72.

145. An isolated nucleic acid sequence comprising a coding sequence for the ALK7 polypeptide of any one of claims 1-135 and a coding sequence for the ActRIIB polypeptide of any one of claims 1-135.

146. The isolated nucleic acid sequence of claim 145, wherein the nucleic acid:

a) comprises a nucleotide sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 12, 22, 41, 45 or 75, and

b) comprises, consists essentially of, or consists of a nucleotide sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 8 or 72.

147. A recombinant polynucleotide comprising a promoter sequence operably linked to the coding sequence of the ALK7 polypeptide claim 141 or 142.

148. A recombinant polynucleotide comprising a promoter sequence operably linked to the coding sequence of the ActRIIB polypeptide claim 143 or 144.

149. A recombinant polynucleotide comprising a promoter sequence operably linked to the coding sequence of the ALK7 polypeptide of claim 141 or 142 and the coding sequence of the ActRIIB polypeptide of claim 143 or 144.

150. A vector comprising the recombinant polynucleotide of any one of claims 145-149.

151. A cell comprising the recombinant polynucleotide any one of claims 145-149 or the vector of claim 150.

152. The cell of claim 149, wherein the cell is a CHO cell.

153. A method of making a heteromultimer comprising an ALK7 polypeptide and an ActRIIB polypeptide comprising:

a) culturing a cell under conditions suitable for expression of an ALK7 polypeptide and an ActRIIB polypeptide, wherein the cell comprises the recombinant polynucleotides of claims 147 and 148; and

b) recovering the heteromultimer so expressed.

154. A method of making a heteromultimer comprising an ALK7 polypeptide and an ActRIIB polypeptide comprising:

a) culturing a cell under conditions suitable for expression of an ALK7 polypeptide and an ActRIIB polypeptide, wherein the cell comprises the recombinant polynucleotide of claims 149; and

b) recovering the heteromultimer so expressed.

155. A method of making a heteromultimer comprising an ALK7 polypeptide and an ActRIIB polypeptide comprising:

a) culturing a first cell under conditions suitable for expression of an ALK7 polypeptide, wherein the first cell comprises the recombinant polynucleotide of claim 147;

b) recovering the ALK7 polypeptide so expressed;

c) culturing a second cell under conditions suitable for expression of an ActRIIB polypeptide, wherein the second cell comprises the recombinant polynucleotide of claim 148;

d) recovering the ActRIIB polypeptide so expressed;

e) combining the recovered ALK7 polypeptide and the recovered ActRIIB polypeptide under conditions suitable for ALK7:ActRIIB heteromultimer formation; and

f) recovering the ALK7:ActRIIB heteromultimer.

156. The method of any one of claims 153-155, wherein the ALK7 polypeptide is expressed using a TPA leader sequence.

157. The method of any one of claims 153-156, wherein the ActRIIB polypeptide is expressed using a TPA leader sequence.

158. The method of claim 156 or 157, wherein the TPA leader comprises, consists essentially of, or consists of SEQ ID NO: 70.

159. The method of any one of claims 153-158, wherein the cell is a CHO cell.

160. The method of any one of claims 153-159, wherein the heteromultimer is a heterodimer.

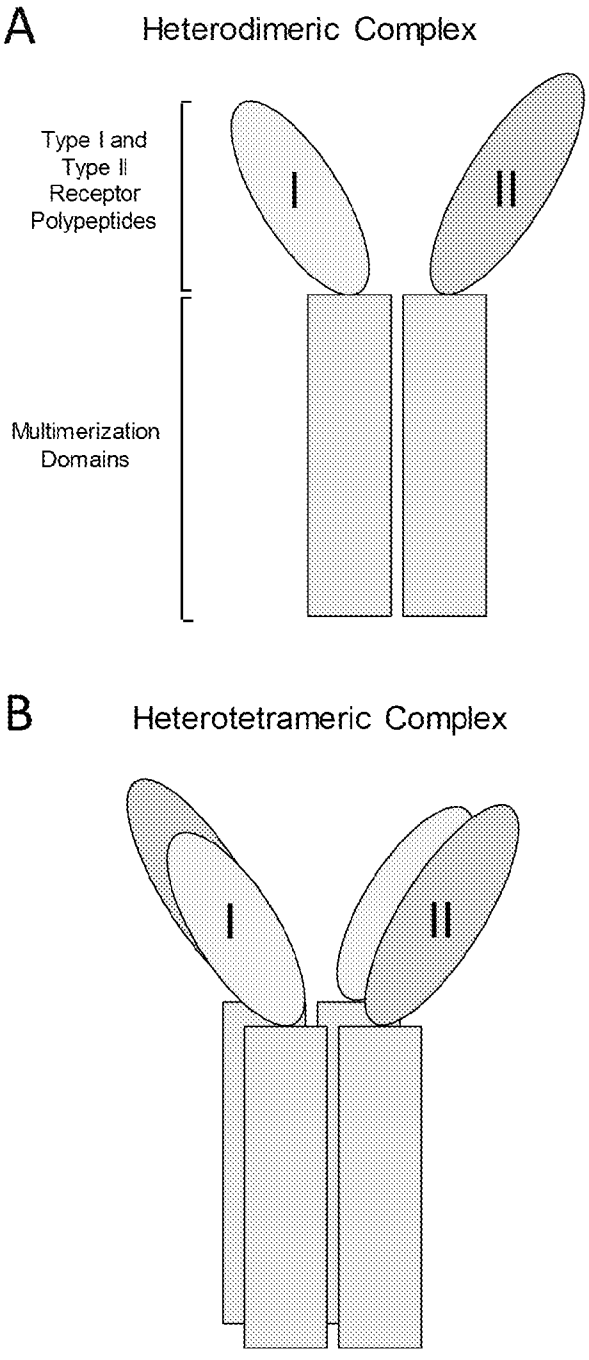
A
Heterodimeric Complex
Type I and
Type II
Receptor
Polypeptides
I
II
Multimerization
Domains
B
Heterotetrameric Complex
I
II

FIGURE 1

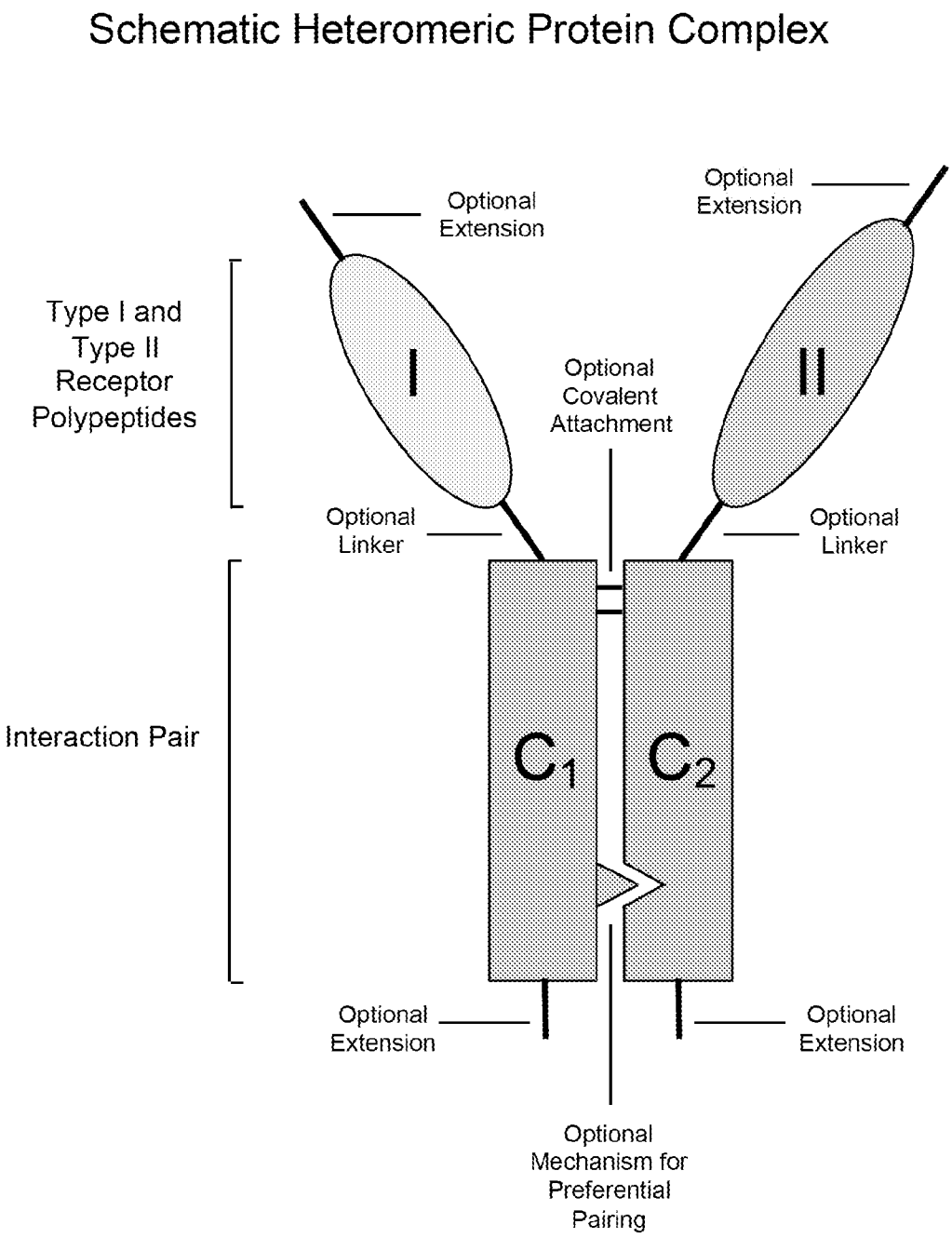
Schematic Heteromeric Protein Complex
Type I and Type II Receptor Polypeptides
Interaction Pair
Optional Extension
Optional Extension
I
II
Optional Covalent Attachment
Optional Linker
Optional Linker
C1
C2
Optional Extension
Optional Extension
Optional Mechanism for Preferential Pairing

FIGURE 2

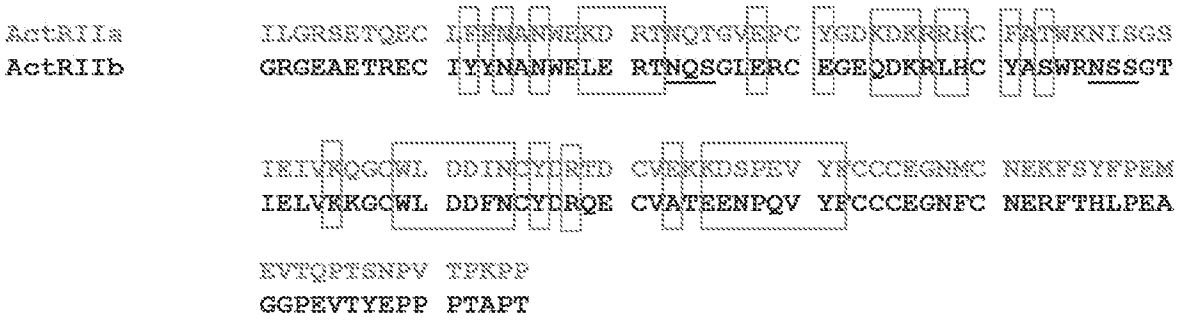
ActRIIa ILGRSETQEC [illegible] RTNQTGVEPC YGDKDKRRHC FPTWRNISGS
ActRIIb GRGEAETREC IYYNANWELE RTNQSGLERC EGEQDKRLHC YASWRNSSGT
[illegible] CVEKKDSPEV YFCCCEGNMC NEKFSYFPEM
IELVEKGCWL DDFNCYDRQE CVATEENPQV YFCCCEGNFC NERFTHLPEA
[illegible]
GGPEVTYEPP PTAPT

FIGURE 3

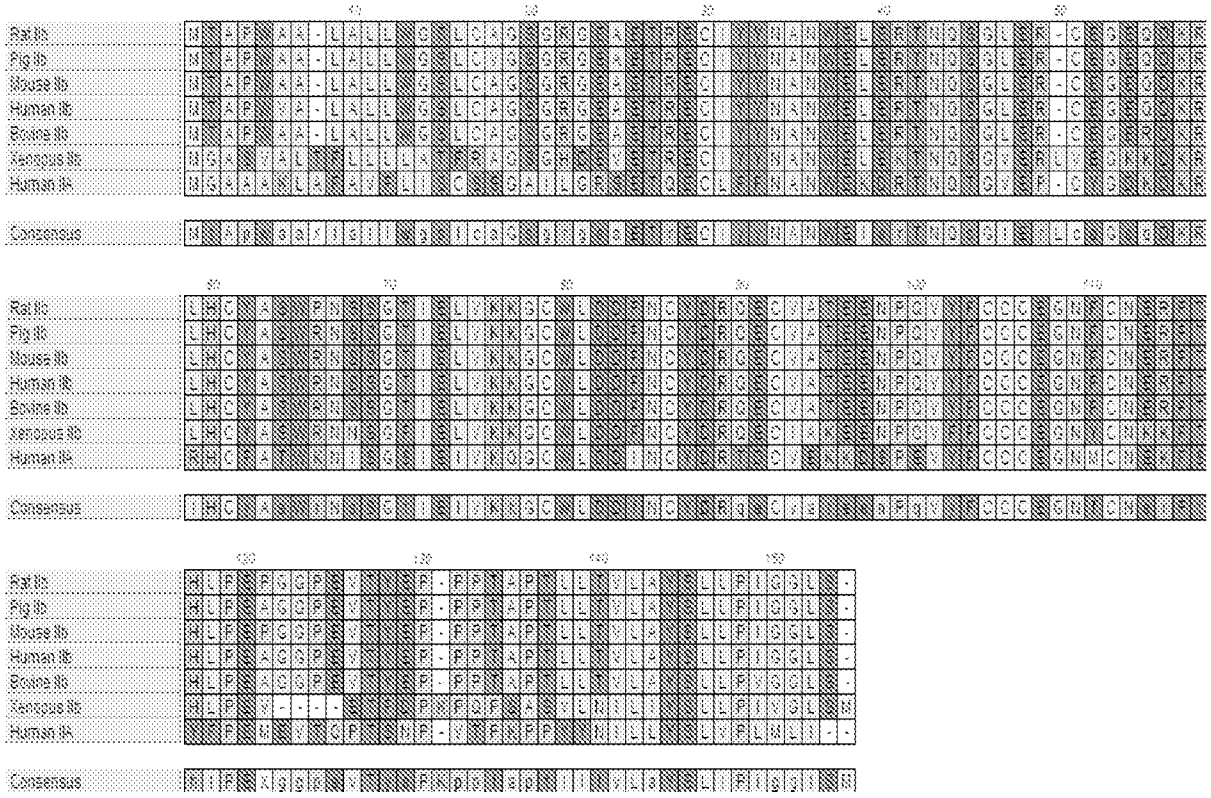
Rat Ib
Pig Ib
Mouse Ib
Human Ib
Bovine Ib
Xenopus Ib
Human IA
Consensus

FIGURE 4

```
IgG1    -------THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF 53
IgG4    ---ESKYGPPCPSCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQF 57
IgG2    --------VECPPCPAPPVAG-PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQF 51
IgG3    EPKSCDTPPPCPRCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQF 60
                 ** **** . * ********************************:******:*

IgG1    NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT 113
IgG4    NWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKT 117
IgG2    NWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKT 111
IgG3    KWYVDGVEVHNAKTKPREEQYNSTFRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT 120
        :*********************:***:********:******************.**:.****

IgG1    ISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP 173
IgG4    ISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP 177
IgG2    ISKTKGQPPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP 171
IgG3    ISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTP 180
        ***:****************:******************************.*******:***

IgG1    PVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK 225
IgG4    PVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK 229
IgG2    PMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK 223
IgG3    PMLDSDGSFFLYSKLTVDKSRWQQGNIFSCSVMHEALHNRFTQKSLSLSPGK 232
        *:************:*********:**:*************::******** **
```

FIGURE 5

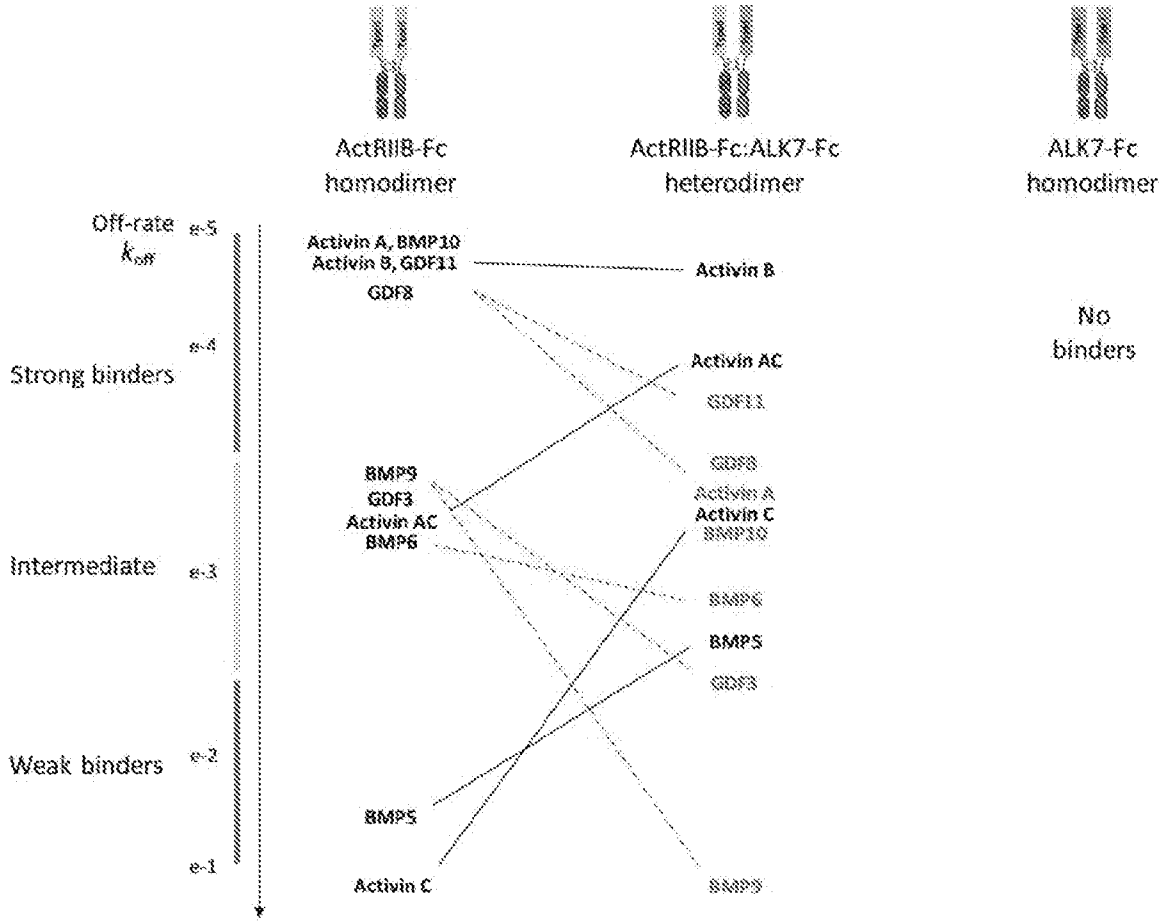
ActRIIB-Fc
homodimer
ActRIIB-Fc:ALK7-Fc
heterodimer
ALK7-Fc
homodimer
Off-rate
k_{off}
e-5
e-4
e-3
e-2
e-1
Strong binders
Intermediate
Weak binders
Activin A, BMP10
Activin B, GDF11
GDF8
BMP9
GDF3
Activin AC
BMP6
BMP5
Activin C
Activin B
Activin AC
GDF11
GDF8
Activin A
Activin C
BMP10
BMP6
BMP5
GDF3
BMP9
No
binders

Figure 6

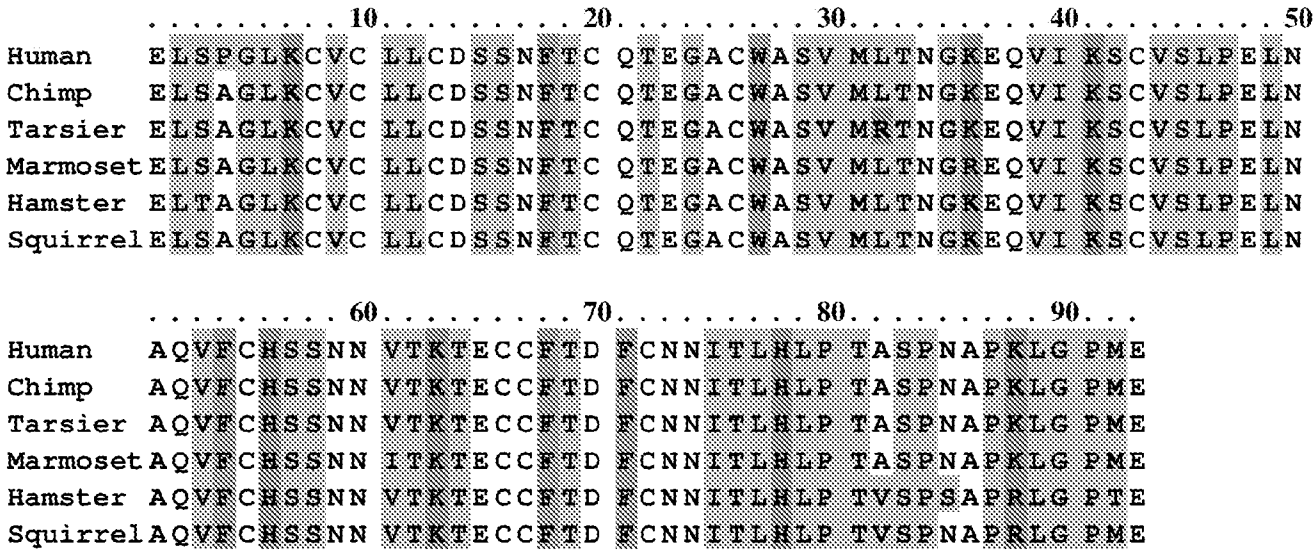
. . . . . 10 . . . . . 20 . . . . . 30 . . . . . 40 50
Human ELSPGLKCVC LLCDSSNFTC QTEGACWASV MLTNGKEQVI KSCVSLPELN
Chimp ELSAGLKCVC LLCDSSNFTC QTEGACWASV MLTNGKEQVI KSCVSLPELN
Tarsier ELSAGLKCVC LLCDSSNFTC QTEGACWASV MRTNGKEQVI KSCVSLPELN
Marmoset ELSAGLKCVC LLCDSSNFTC QTEGACWASV MLTNGREQVI KSCVSLPELN
Hamster ELTAGLKCVC LLCDSSNFTC QTEGACWASV MLTNGKEQVI KSCVSLPELN
Squirrel ELSAGLKCVC LLCDSSNFTC QTEGACWASV MLTNGKEQVI KSCVSLPELN
. . . . . 60 . . . . . 70 . . . . . 80 . . . . . 90 . . .
Human AQVFCHSSNN VTKTECCFTD FCNNITLHLP TASPNAPKLG PME
Chimp AQVFCHSSNN VTKTECCFTD FCNNITLHLP TASPNAPKLG PME
Tarsier AQVFCHSSNN VTKTECCFTD FCNNITLHLP TASPNAPKLG PME
Marmoset AQVFCHSSNN ITKTECCFTD FCNNITLHLP TASPNAPKLG PME
Hamster AQVFCHSSNN VTKTECCFTD FCNNITLHLP TVSPSAPRLG PTE
Squirrel AQVFCHSSNN VTKTECCFTD FCNNITLHLP TVSPNAPRLG PME

FIGURE 7

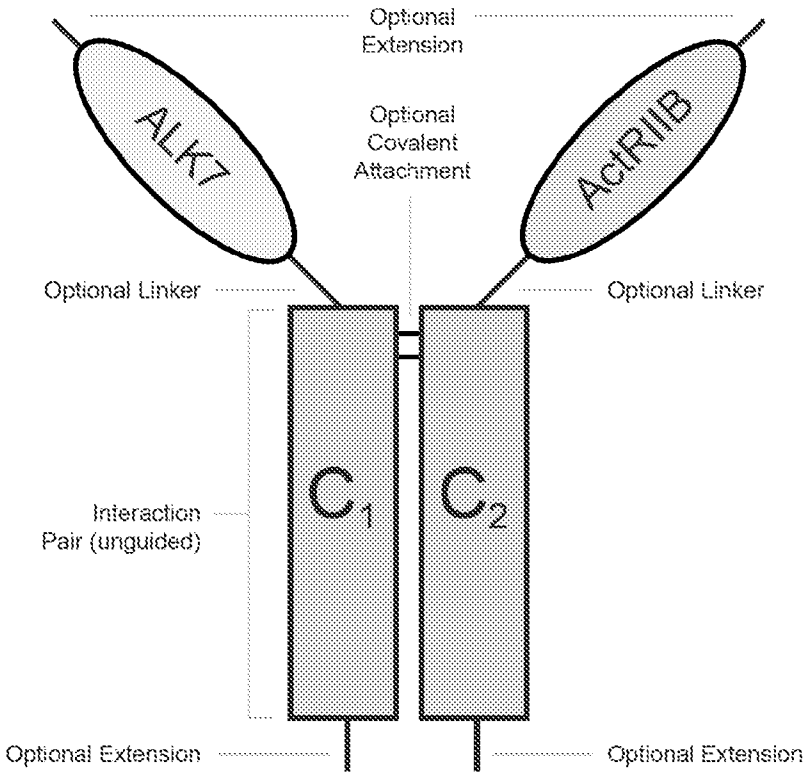
Optional
Extension
ALK7
Optional
Covalent
Attachment
ActRIIB
Optional Linker
Optional Linker
Interaction
Pair (unguided)
C1
C2
Optional Extension
Optional Extension

FIGURE 8A

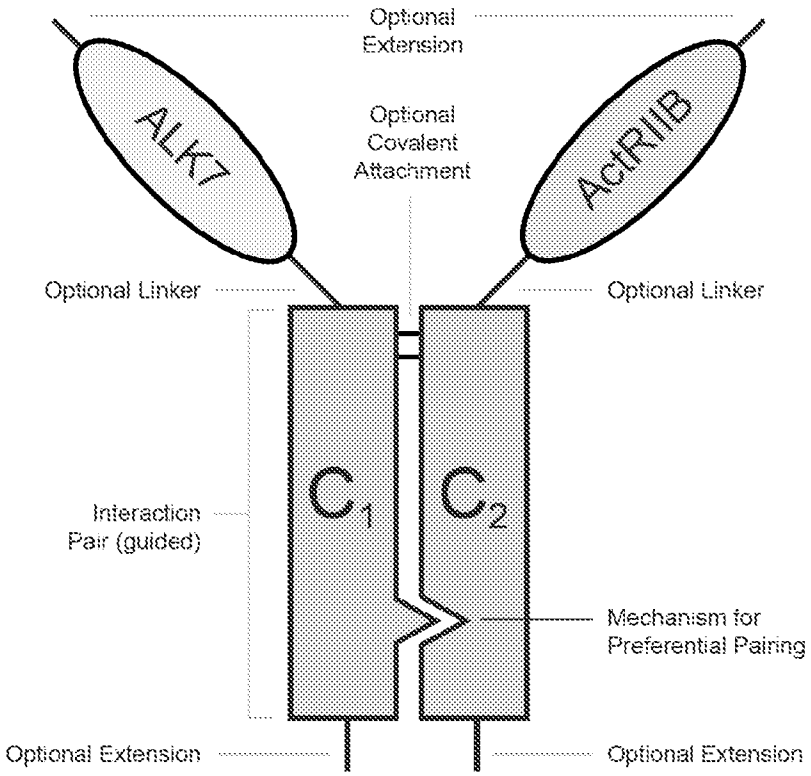
Optional
Extension
ALK7
Optional
Covalent
Attachment
ActRIIB
Optional Linker
Optional Linker
C1
C2
Interaction
Pair (guided)
Mechanism for
Preferential Pairing
Optional Extension
Optional Extension

FIGURE 8B

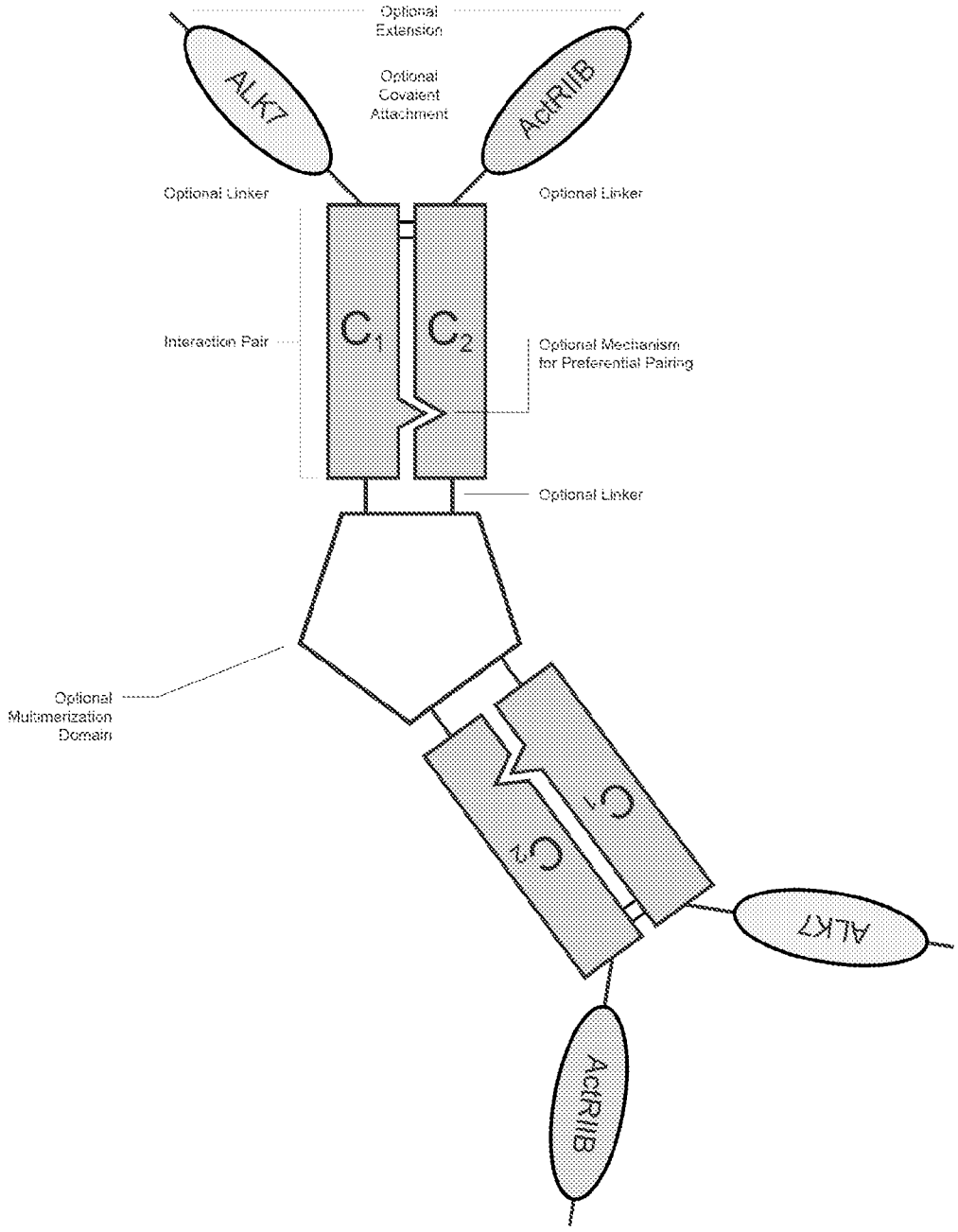
Optional Extension
Optional Covalent Attachment
ALK7
ActRIIB
Optional Linker
Optional Linker
Interaction Pair
C1
C2
Optional Mechanism for Preferential Pairing
Optional Linker
Optional Multimerization Domain
C1
C2
ALK7
ActRIIB

FIGURE 8C

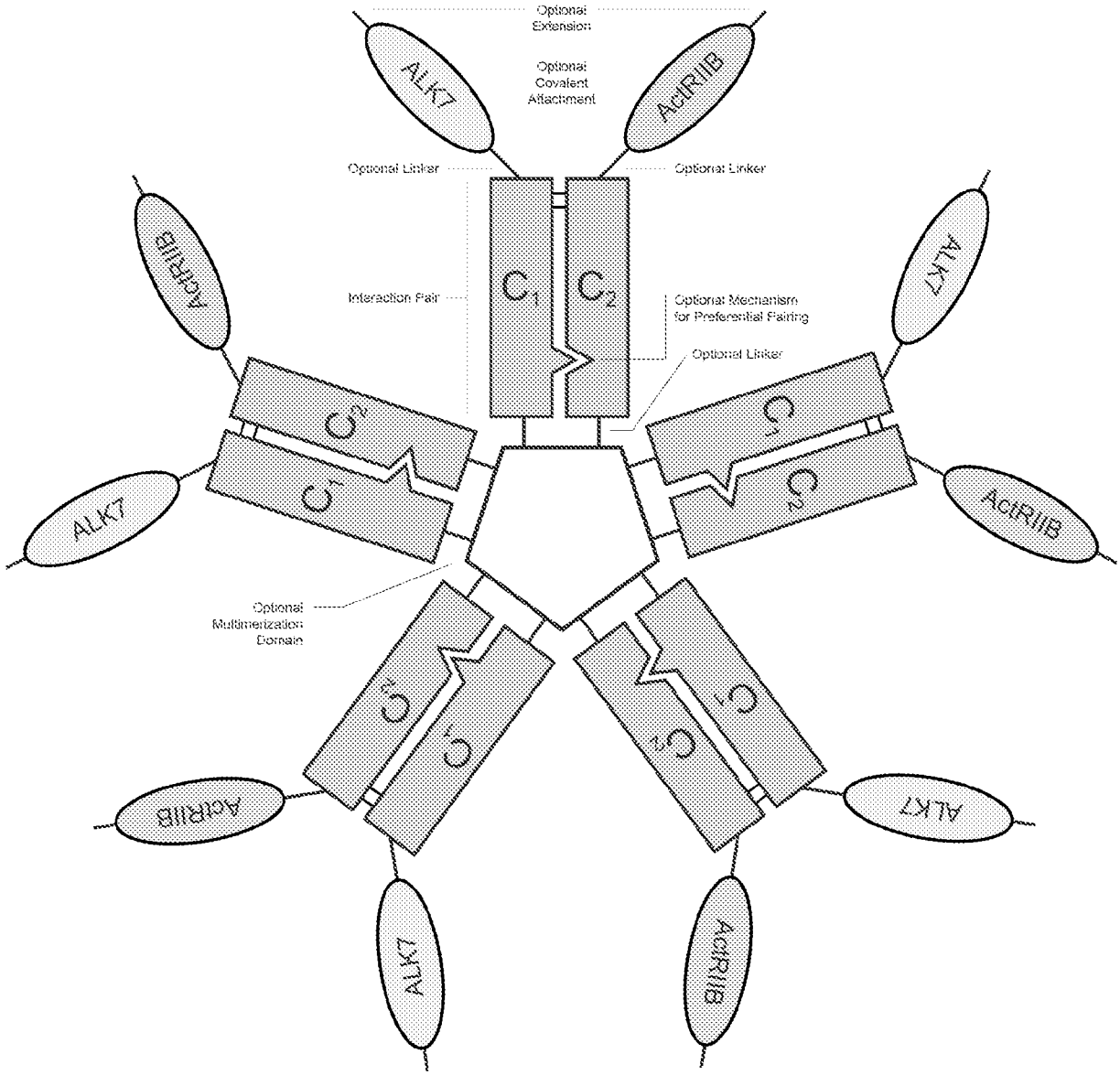
Optional Extension
Optional Covalent Attachment
ALK7
ActRIIB
Optional Linker
Optional Linker
Interaction Pair
Optional Mechanism for Preferential Pairing
Optional Linker
Optional Multimerization Domain

FIGURE 8D

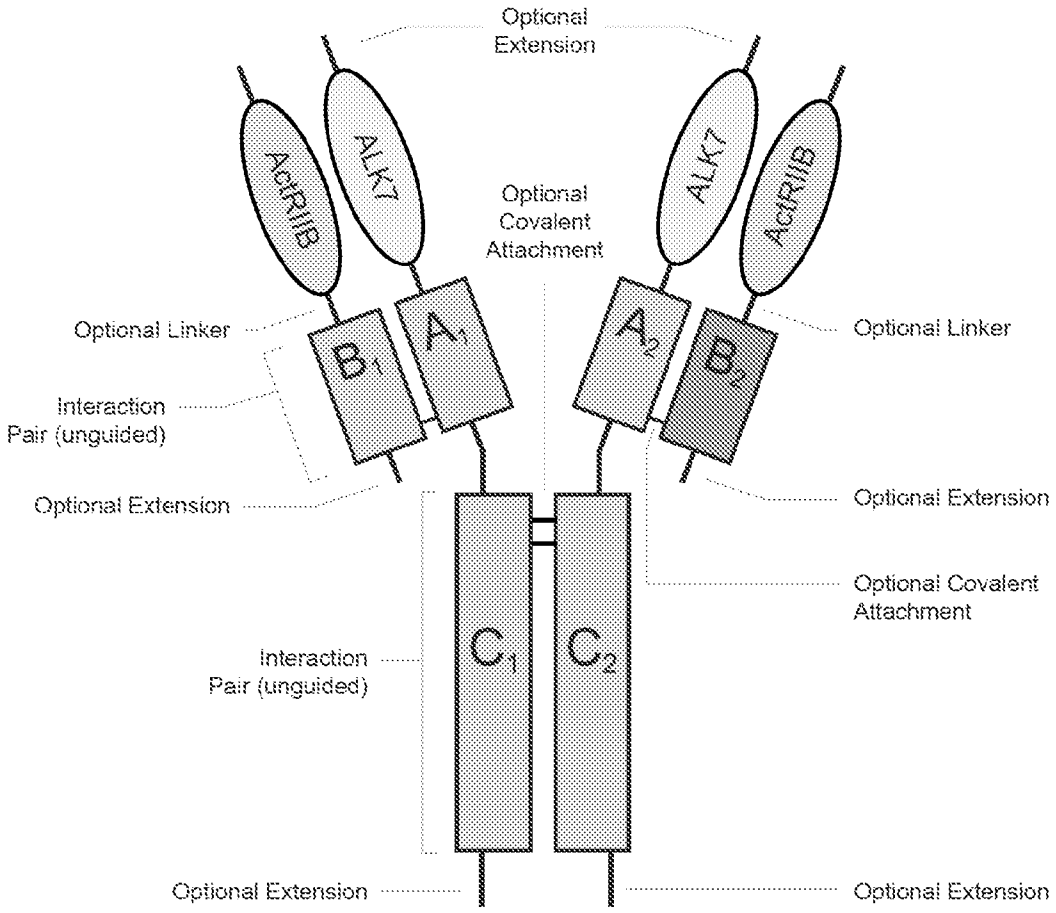
Optional Extension
ActRIIB
ALK7
Optional Covalent Attachment
ALK7
ActRIIB
Optional Linker
B1
A1
A2
B2
Optional Linker
Interaction Pair (unguided)
Optional Extension
Optional Extension
Optional Covalent Attachment
Interaction Pair (unguided)
C1
C2
Optional Extension
Optional Extension

FIGURE 9A

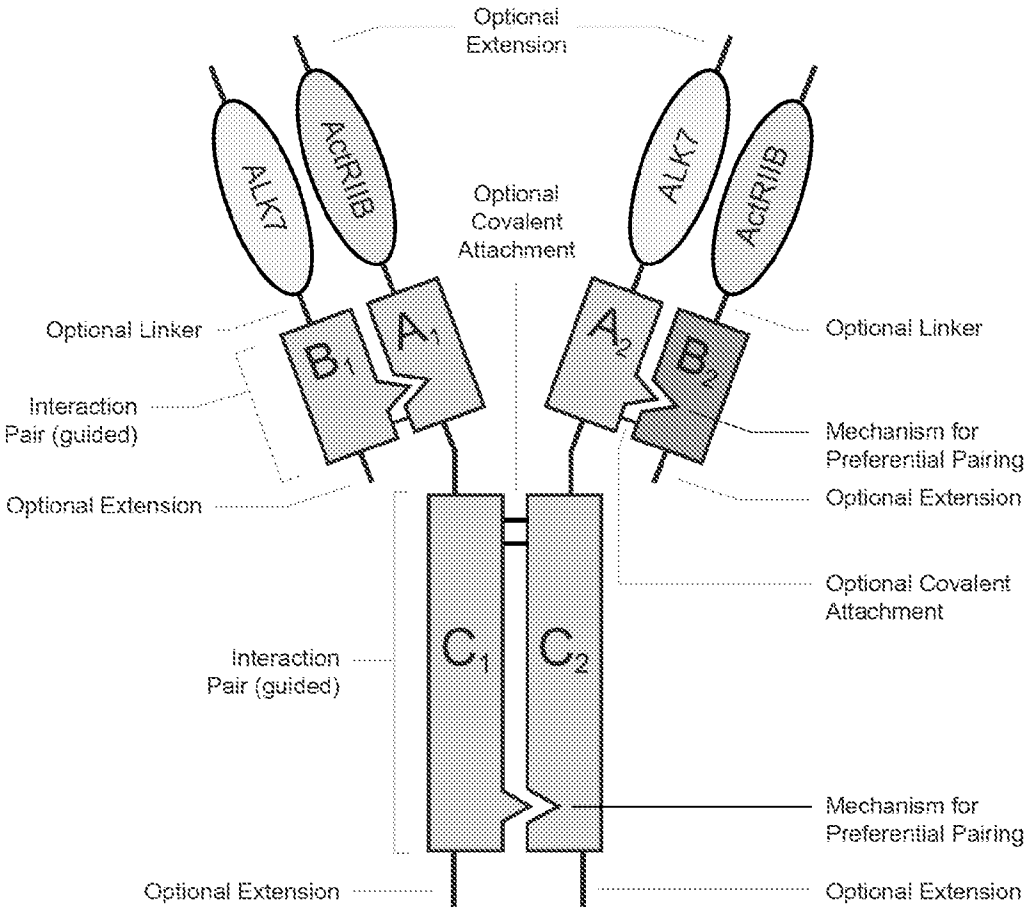
Optional Extension
ALK7
ActRIIB
Optional Covalent Attachment
ALK7
ActRIIB
Optional Linker
Interaction Pair (guided)
Optional Extension
B_1
A_1
A_2
B_2
Optional Linker
Mechanism for Preferential Pairing
Optional Extension
Optional Covalent Attachment
Interaction Pair (guided)
C_1
C_2
Mechanism for Preferential Pairing
Optional Extension
Optional Extension

FIGURE 9B

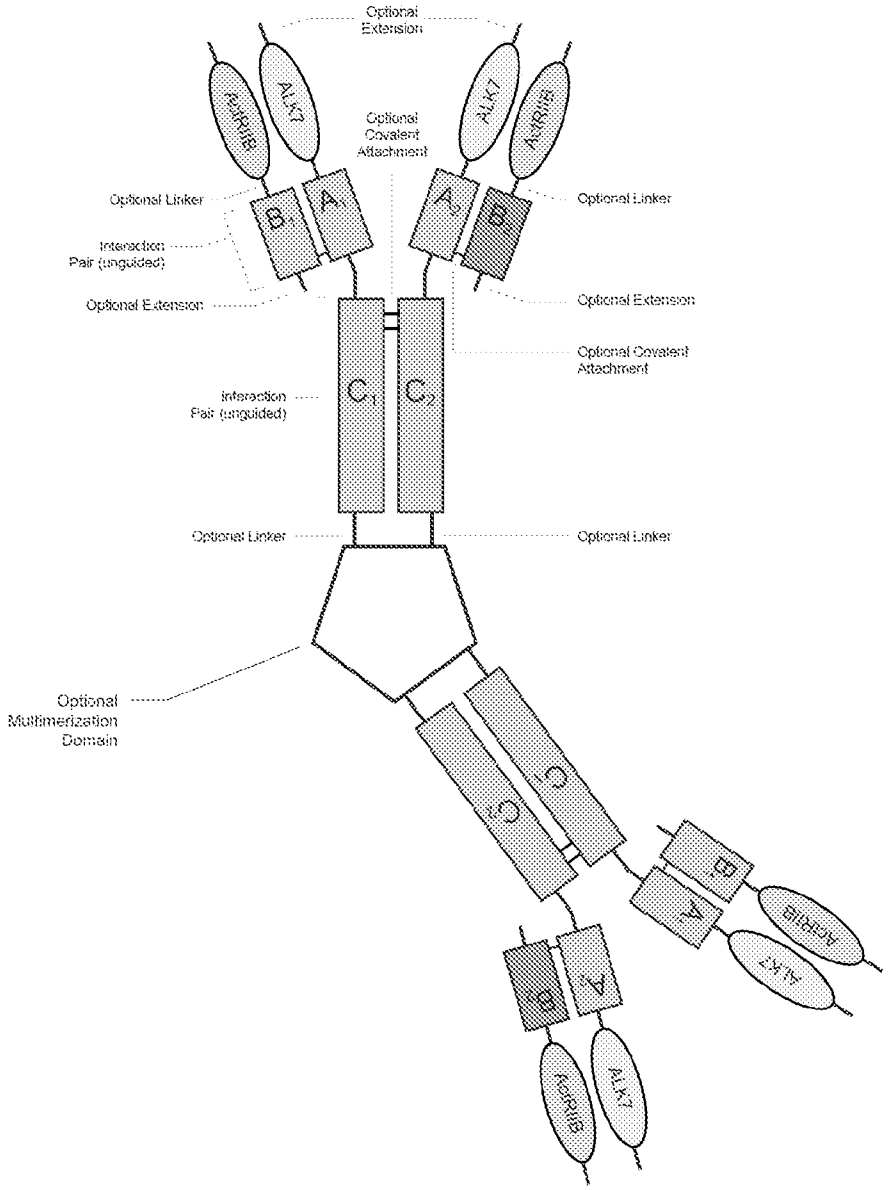
Optional Extension
ALK7
ActRIB
Optional Covalent Attachment
Optional Linker
Interaction Pair (unguided)
Optional Extension
Optional Linker
Optional Extension
Optional Covalent Attachment
Interaction Pair (unguided)
Optional Linker
Optional Linker
Optional Multimerization Domain

FIGURE 9C

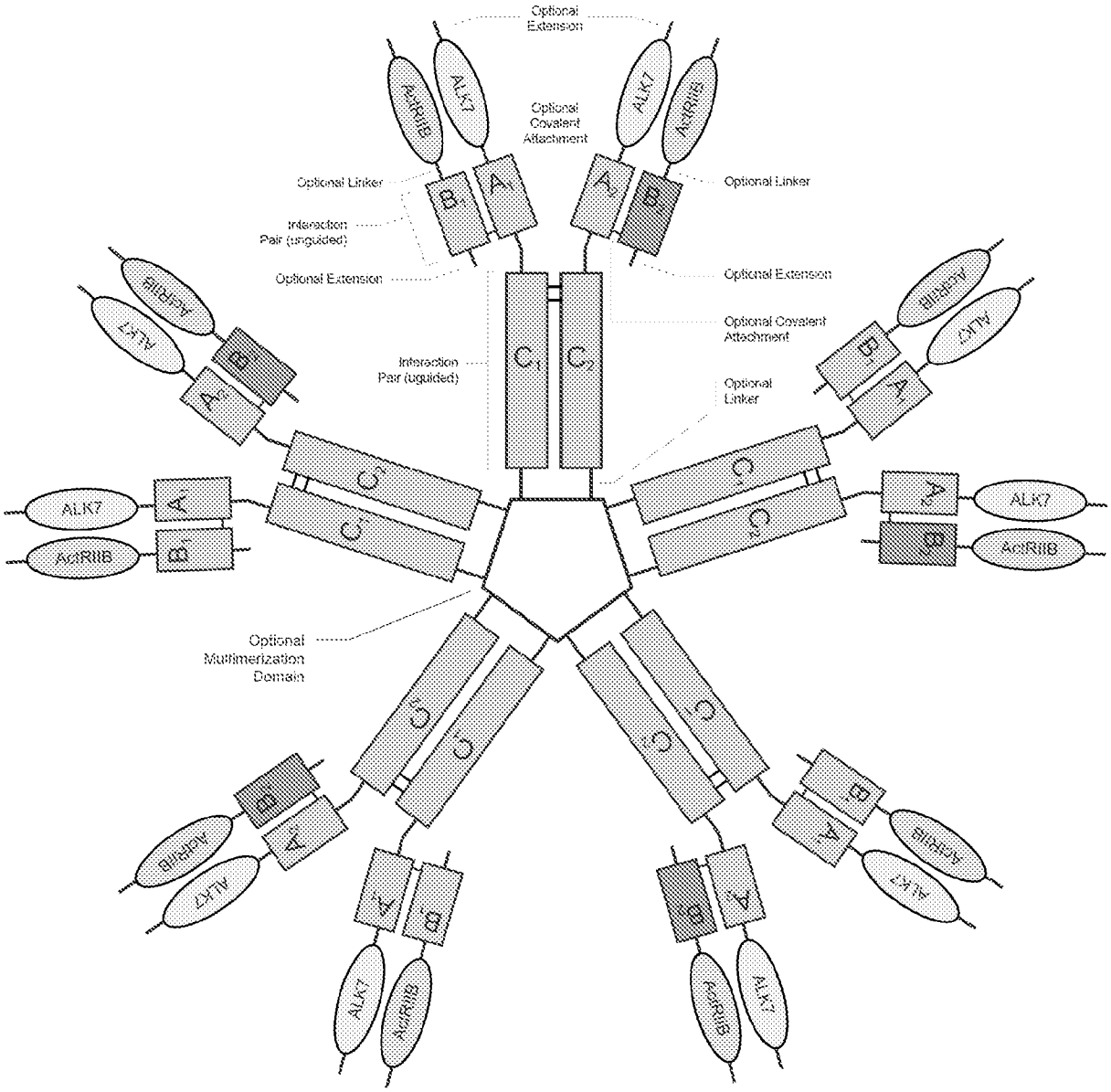
Optional Extension
Optional Covalent Attachment
Optional Linker
Interaction Pair (unguided)
Optional Extension
Interaction Pair (uguided)
Optional Covalent Attachment
Optional Linker
Optional Multimerization Domain
ALK7
ActRIIB

FIGURE 9D

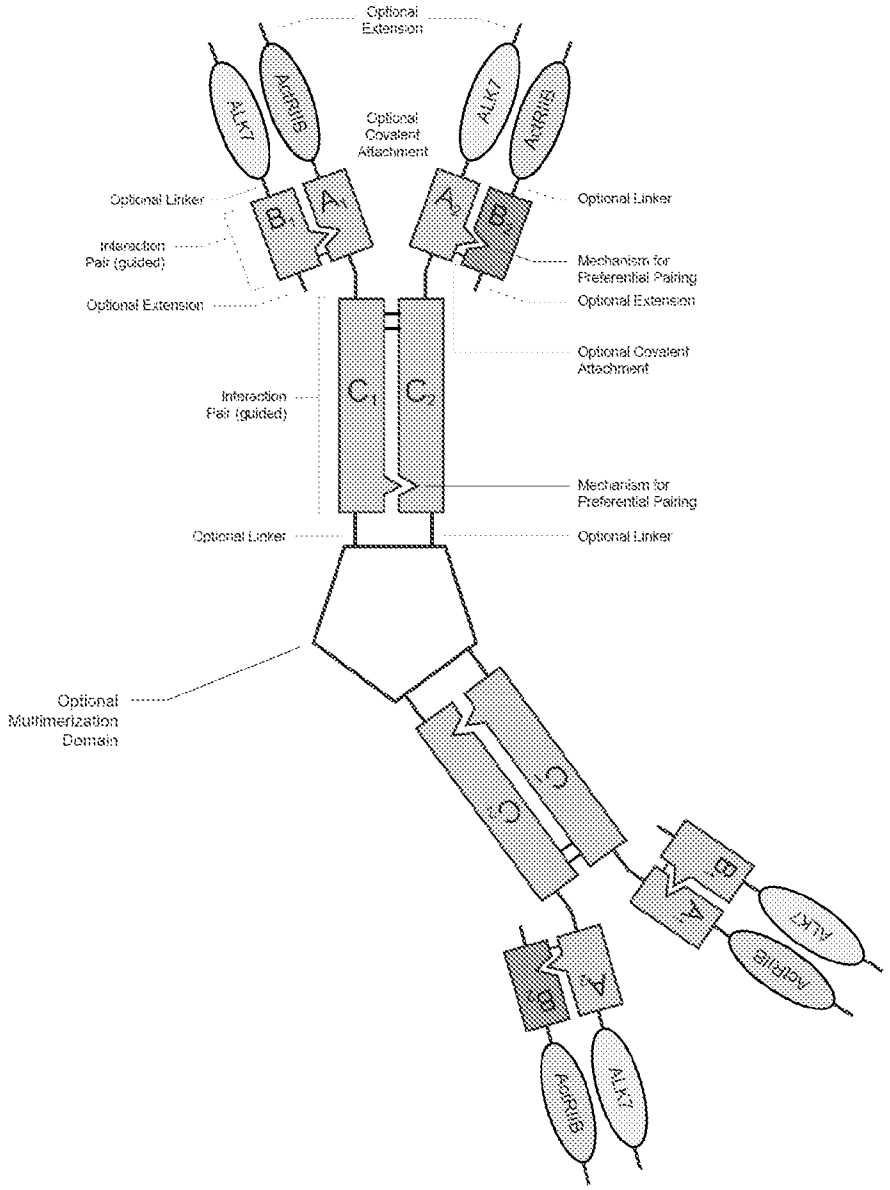
Optional Extension
ALK7
ActRIIB
Optional Covalent Attachment
Optional Linker
Interaction Pair (guided)
Optional Extension
Mechanism for Preferential Pairing
Optional Covalent Attachment
Interaction Pair (guided)
Mechanism for Preferential Pairing
Optional Linker
Optional Multimerization Domain

FIGURE 9E

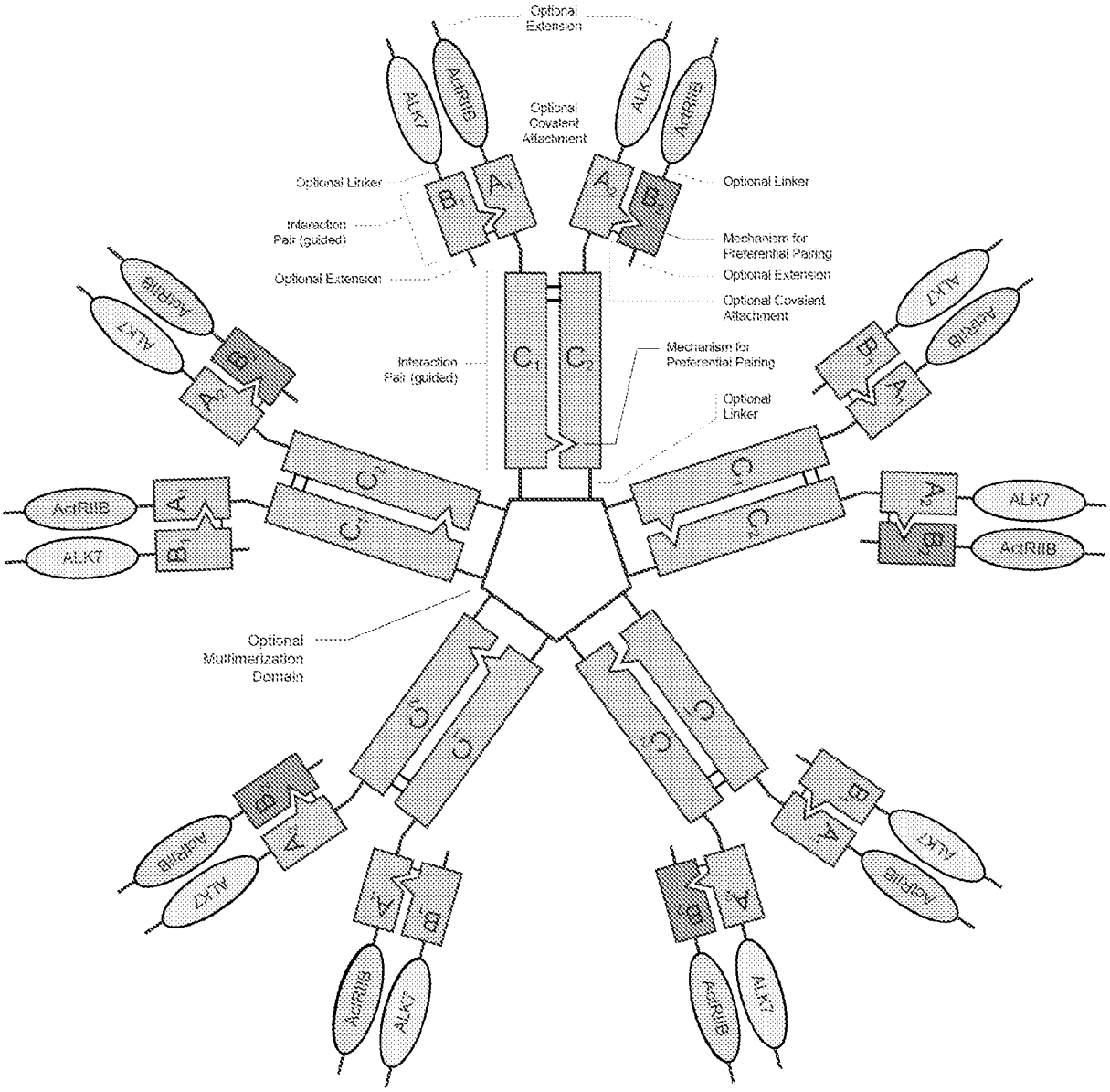
Optional Extension
Optional Covalent Attachment
Optional Linker
Interaction Pair (guided)
Optional Extension
Mechanism for Preferential Pairing
Optional Extension
Optional Covalent Attachment
Interaction Pair (guided)
Mechanism for Preferential Pairing
Optional Linker
Optional Multimerization Domain
ActRIIB
ALK7

FIGURE 9F

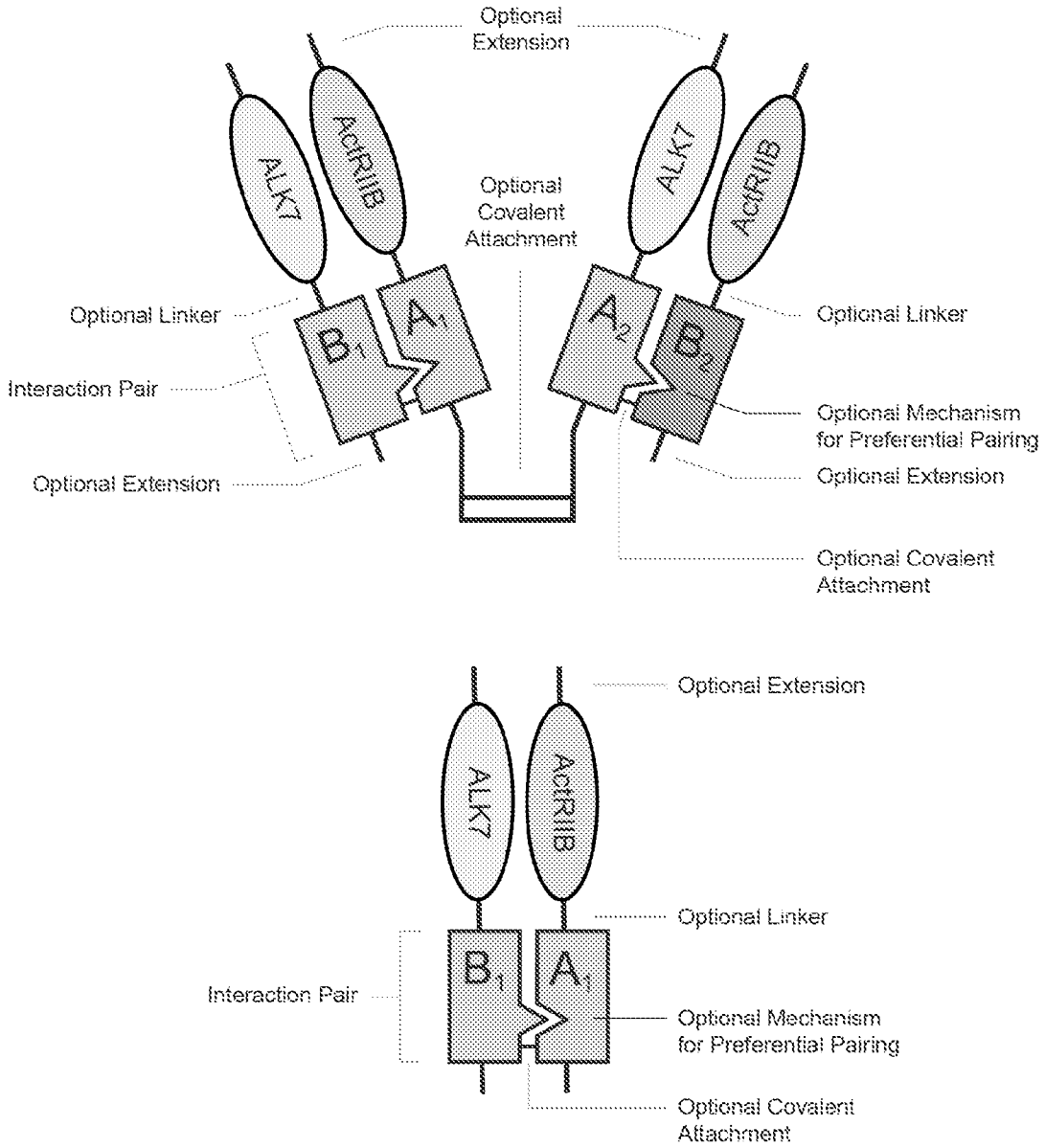
Optional Extension
ALK7
ActRIIB
Optional Covalent Attachment
ALK7
ActRIIB
Optional Linker
Interaction Pair
Optional Extension
Optional Linker
Optional Mechanism for Preferential Pairing
Optional Extension
Optional Covalent Attachment
Optional Extension
ALK7
ActRIIB
Optional Linker
Interaction Pair
Optional Mechanism for Preferential Pairing
Optional Covalent Attachment

FIGURE 9G

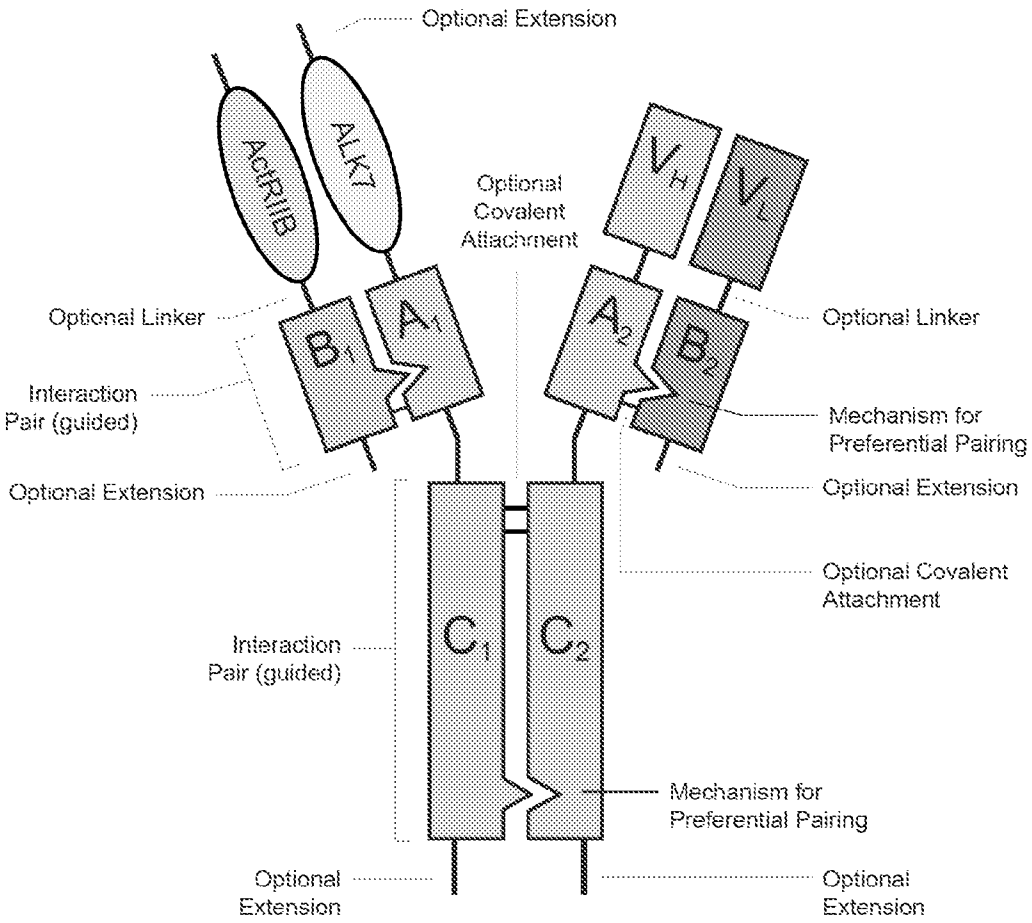
Optional Extension
ActRIIB
ALK7
Optional Covalent Attachment
V_H
V_L
Optional Linker
B_1
A_1
A_2
B_2
Optional Linker
Interaction Pair (guided)
Mechanism for Preferential Pairing
Optional Extension
Optional Extension
Optional Covalent Attachment
Interaction Pair (guided)
C_1
C_2
Mechanism for Preferential Pairing
Optional Extension
Optional Extension

FIGURE 10A

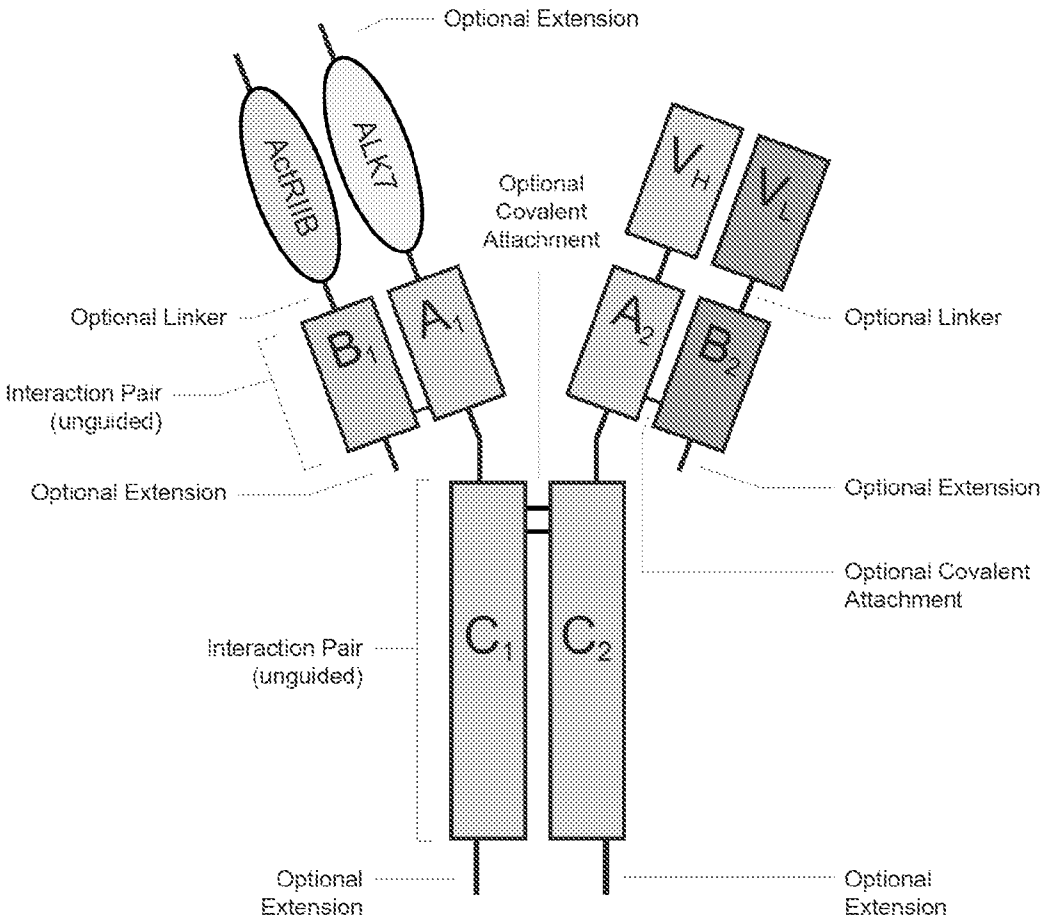
Optional Extension
ActRIIB
ALK7
Optional Covalent Attachment
V_H
V_L
Optional Linker
B_1
A_1
A_2
B_2
Optional Linker
Interaction Pair (unguided)
Optional Extension
Optional Extension
Optional Covalent Attachment
Interaction Pair (unguided)
C_1
C_2
Optional Extension
Optional Extension

FIGURE 10B

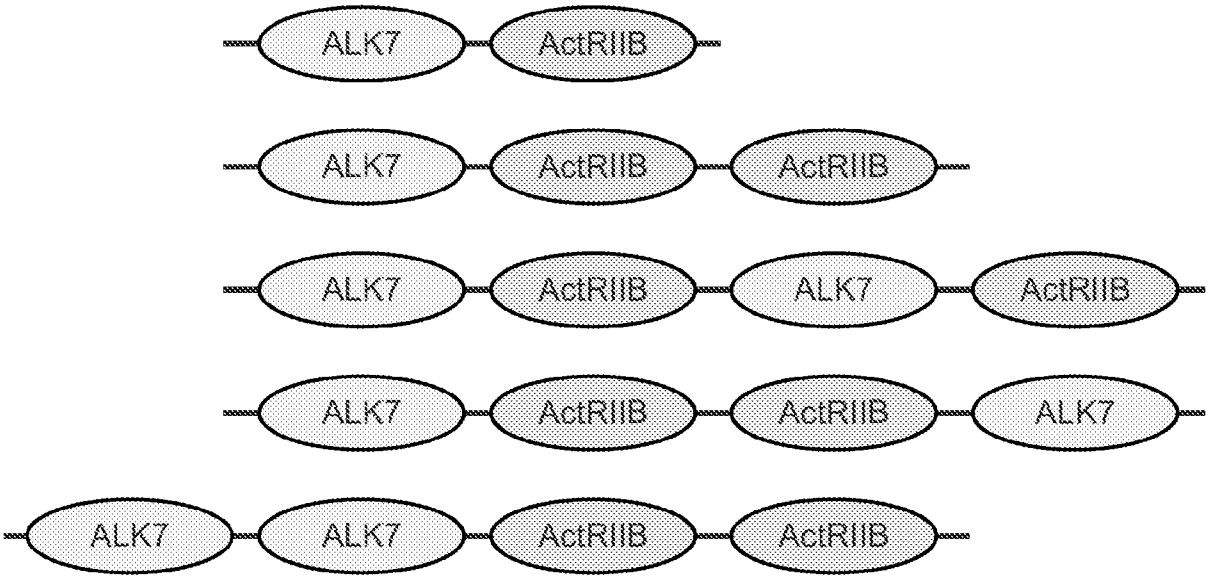
ALK7
ActRIIB
ALK7
ActRIIB
ActRIIB
ALK7
ActRIIB
ALK7
ActRIIB
ALK7
ActRIIB
ActRIIB
ALK7
ALK7
ALK7
ActRIIB
ActRIIB

FIGURE 11

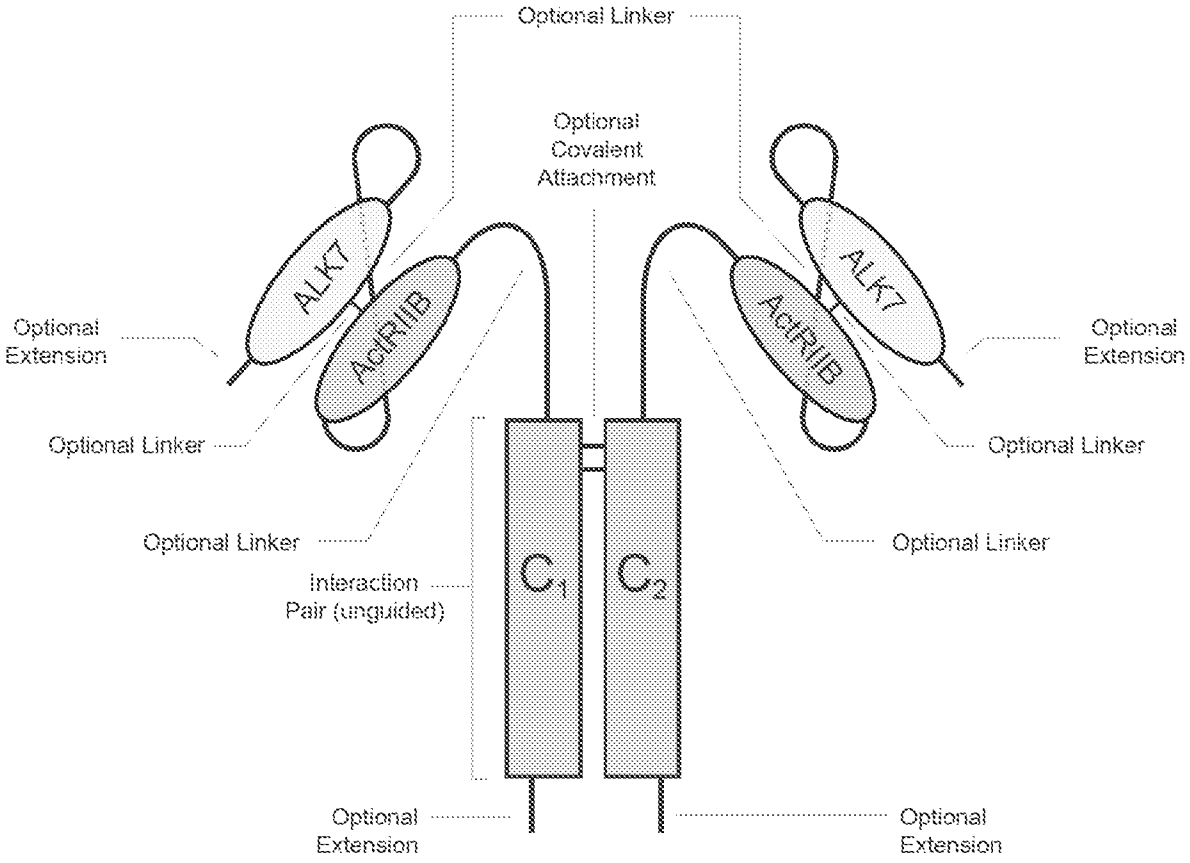
Optional Linker
Optional Covalent Attachment
ALK7
ActRIIB
ALK7
ActRIIB
Optional Extension
Optional Extension
Optional Linker
Optional Linker
Optional Linker
Optional Linker
Interaction Pair (unguided)
C1
C2
Optional Extension
Optional Extension

FIGURE 12A

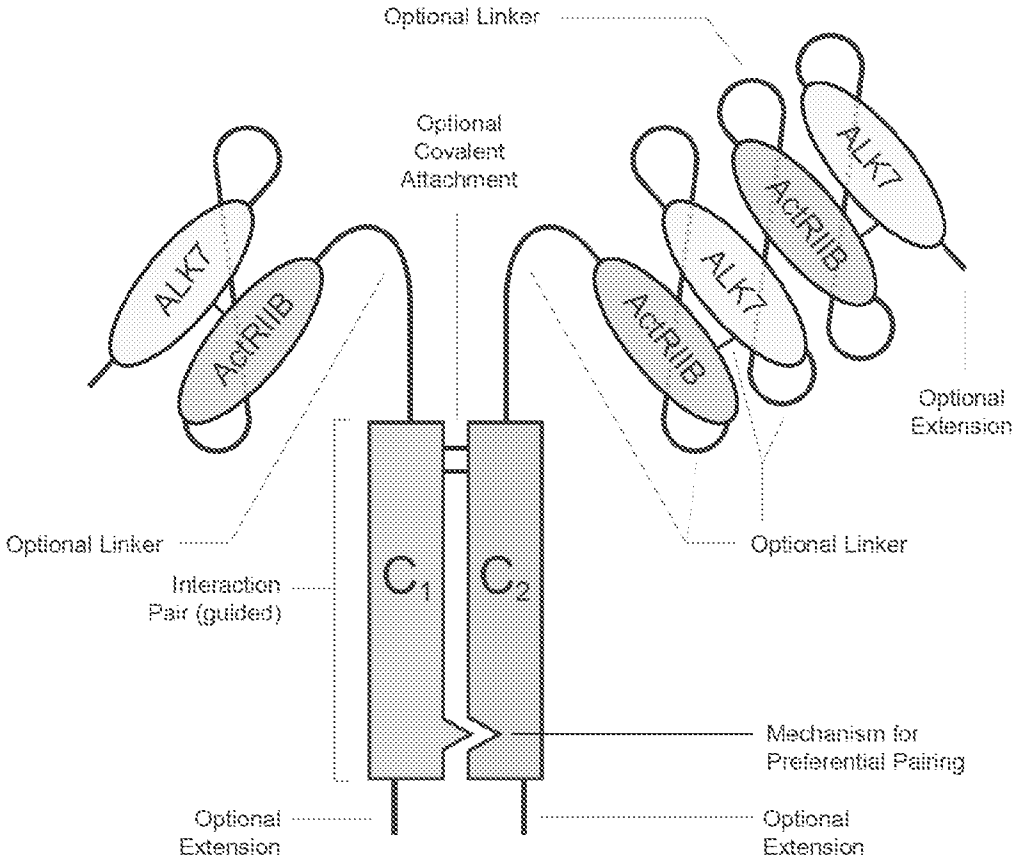
Optional Linker
Optional Covalent Attachment
ALK7
ActRIIB
ALK7
ActRIIB
ALK7
ActRIIB
Optional Extension
Optional Linker
Optional Linker
Interaction Pair (guided)
C1
C2
Mechanism for Preferential Pairing
Optional Extension
Optional Extension

FIGURE 12B

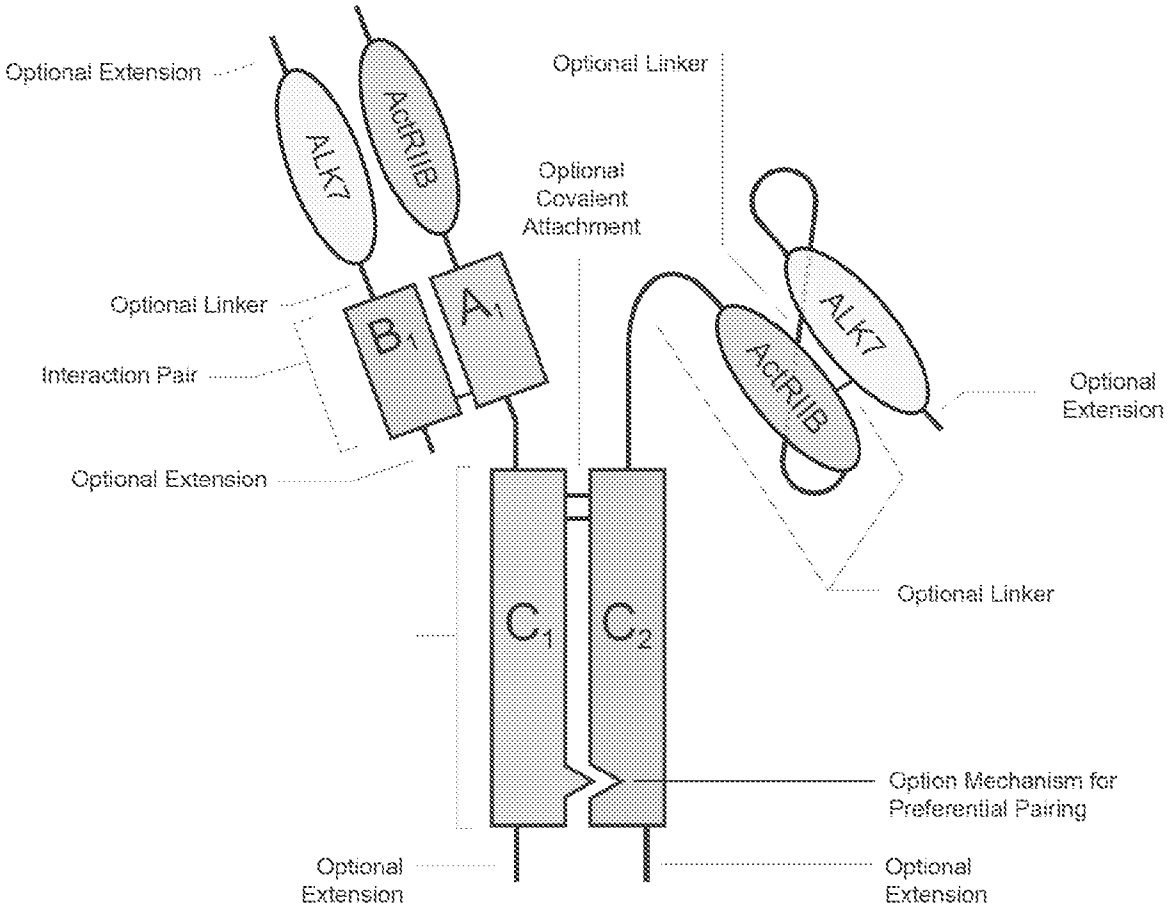
Optional Extension
ALK7
ActRIIB
Optional Linker
Optional Covalent Attachment
Optional Linker
Interaction Pair
B1
A1
ALK7
ActRIIB
Optional Extension
Optional Extension
C1
C2
Optional Linker
Option Mechanism for Preferential Pairing
Optional Extension
Optional Extension

FIGURE 12C

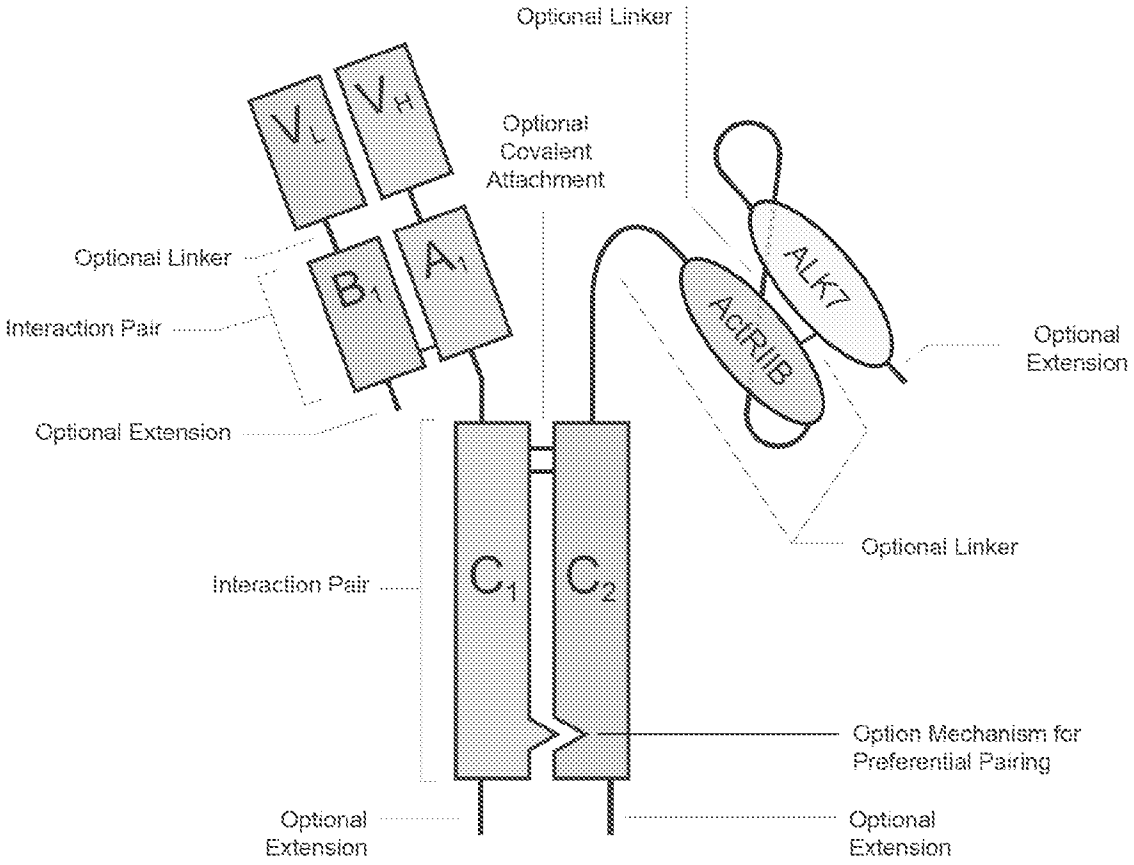
Optional Linker
V_L
V_H
Optional Covalent Attachment
Optional Linker
B_1
A_1
Interaction Pair
ALK7
ActRIIB
Optional Extension
Optional Extension
Optional Linker
Interaction Pair
C_1
C_2
Option Mechanism for Preferential Pairing
Optional Extension
Optional Extension

FIGURE 12D

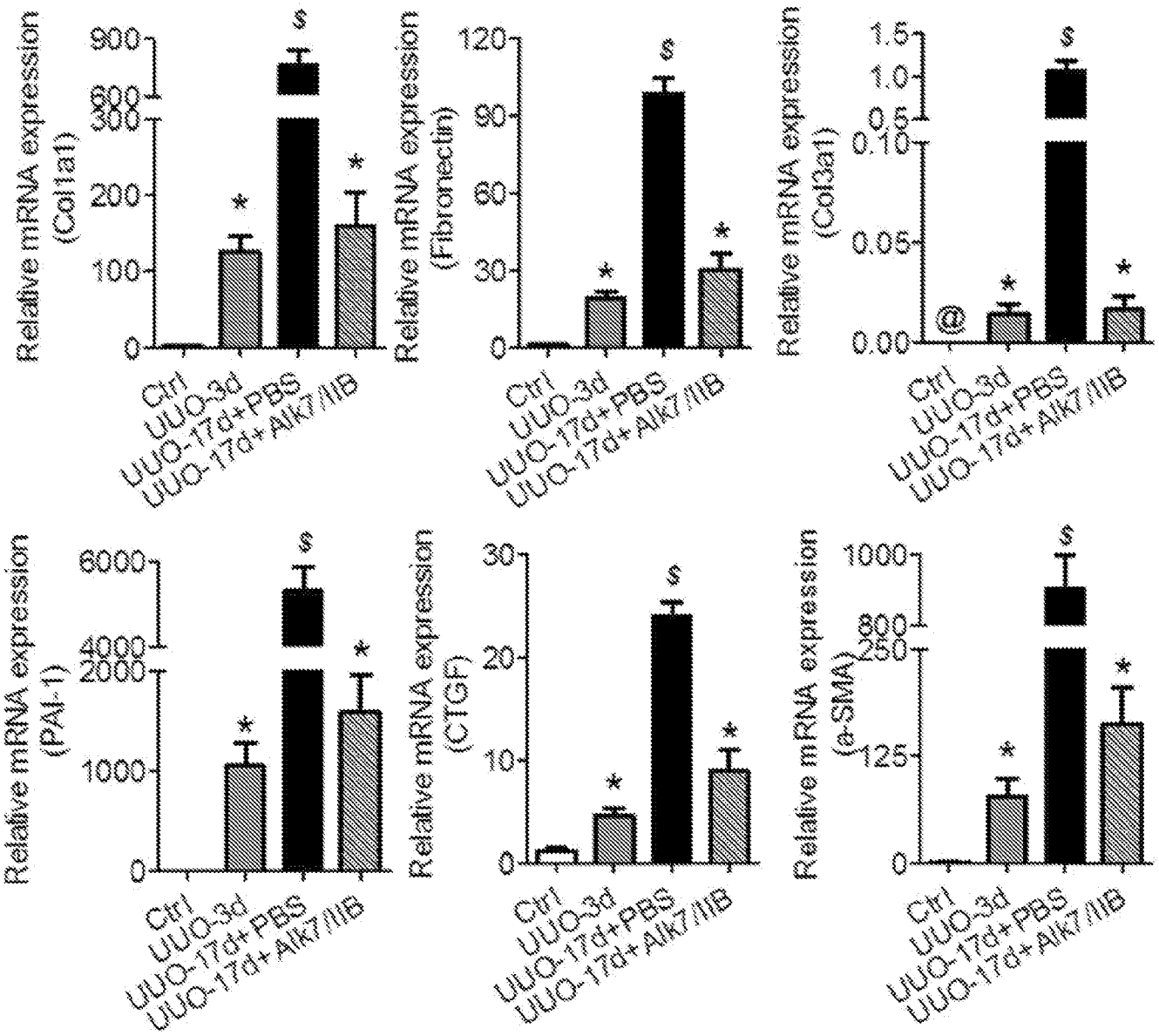
Relative mRNA expression (Col1a1)
900
800
300
200
100
0
$
*
*
Ctrl
UUO-3d
UUO-17d+PBS
UUO-17d+Alk7/IIB
Relative mRNA expression (Fibronectin)
120
90
60
30
0
$
*
*
Relative mRNA expression (Col3a1)
1.5
1.0
0.5
0.10
0.05
0.00
@
$
*
*
Relative mRNA expression (PAI-1)
6000
4000
2000
1000
0
$
*
*
Relative mRNA expression (CTGF)
30
20
10
0
$
*
*
Relative mRNA expression (α-SMA)
1000
800
250
125
0
$
*
*

Figure 13A

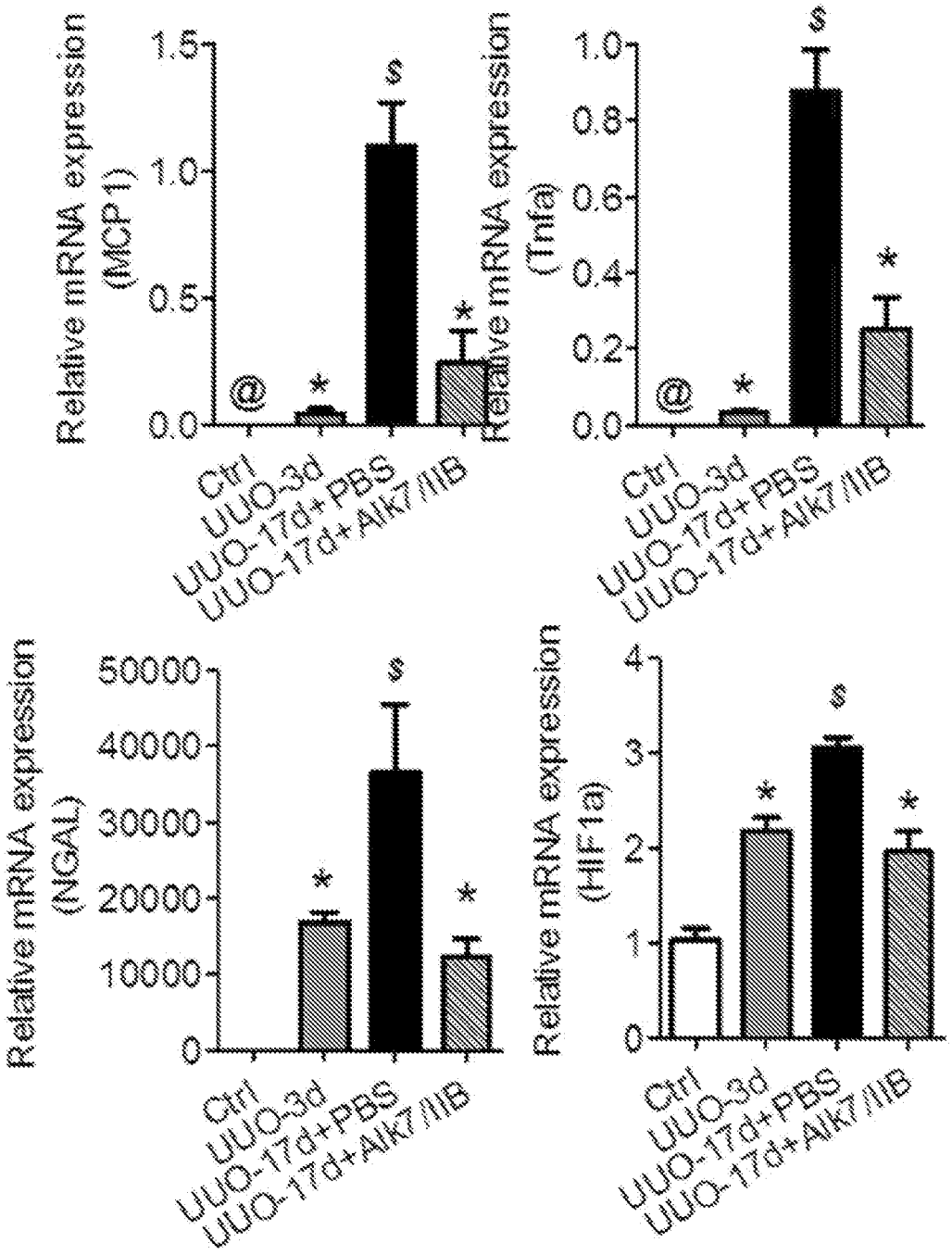
Relative mRNA expression (MCP1)
1.5
1.0
0.5
0.0
@
*
$
*
Ctrl
UUO-3d
UUO-17d+PBS
UUO-17d+AIK7/IIB
Relative mRNA expression (Tnfa)
1.0
0.8
0.6
0.4
0.2
0.0
$
*
Relative mRNA expression (NGAL)
50000
40000
30000
20000
10000
0
$
*
*
Relative mRNA expression (HIF1a)
4
3
2
1
0
$
*
*

Figure 13B

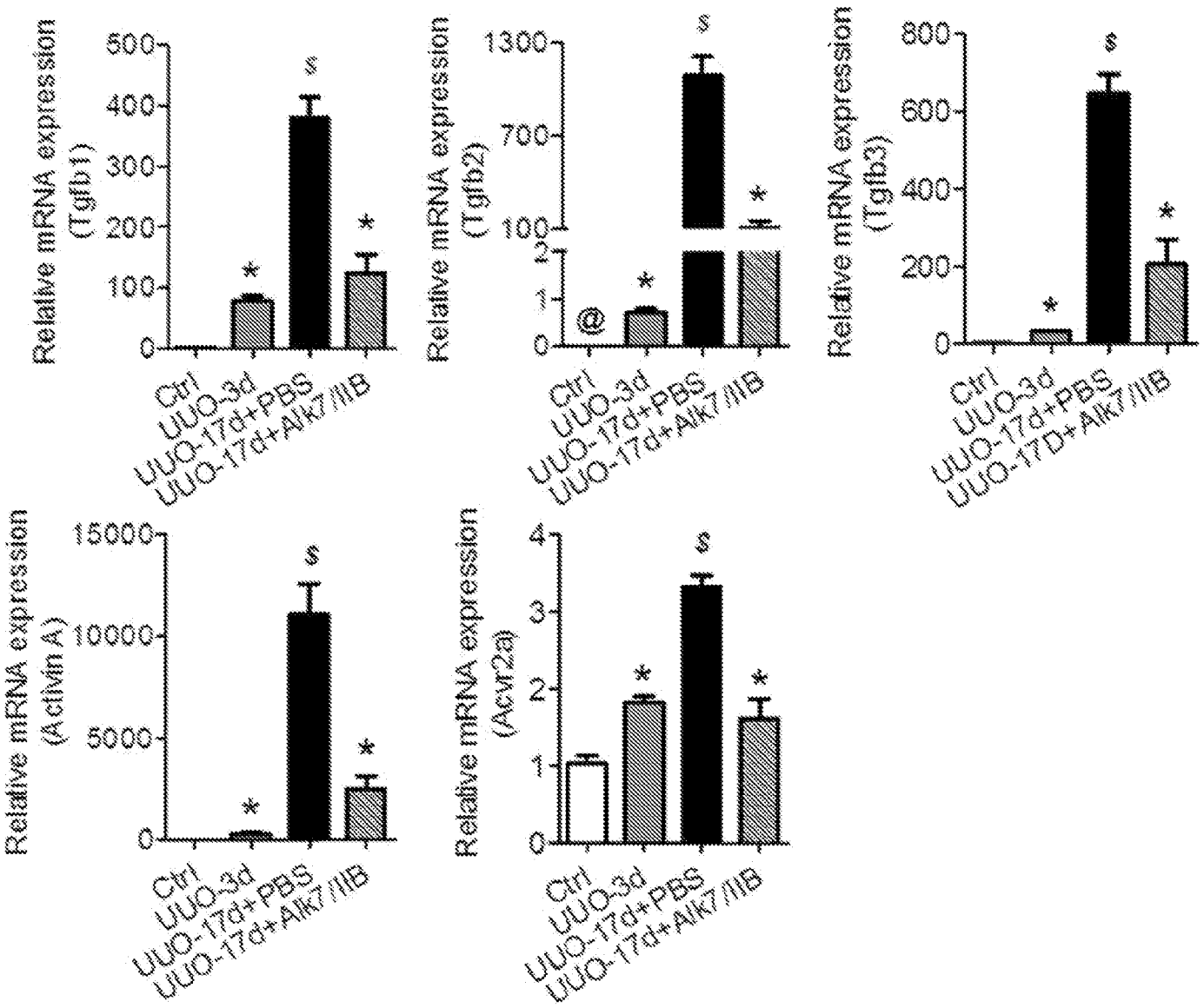
Relative mRNA expression (Tgfb1)
500
400
300
200
100
0
$
*
*
Ctrl
UUO-3d
UUO-17d+PBS
UUO-17d+Alk7/IIB
Relative mRNA expression (Tgfb2)
1300
700
100
3
1
0
@
$
*
*
Ctrl
UUO-3d
UUO-17d+PBS
UUO-17d+Alk7/IIB
Relative mRNA expression (Tgfb3)
800
600
400
200
0
$
*
*
Ctrl
UUO-3d
UUO-17d+PBS
UUO-17D+Alk7/IIB
Relative mRNA expression (Activin A)
15000
10000
5000
0
$
*
*
Ctrl
UUO-3d
UUO-17d+PBS
UUO-17d+Alk7/IIB
Relative mRNA expression (Acvr2a)
4
3
2
1
0
$
*
*
Ctrl
UUO-3d
UUO-17d+PBS
UUO-17d+Alk7/IIB

Figure 13C

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2016/026277

A. CLASSIFICATION OF SUBJECT MATTER

C07K 14/71 (2006.01) ***C07K 19/00 (2006.01)*** ***A61K 38/17 (2006.01)*** ***A61K 39/395 (2006.01)*** ***A61P 13/12 (2006.01)*** ***A61P 3/04 (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)

DATABASES: MEDLINE, EPODOC, WPIAP, CAPLUS, BIOSIS, EMBASE.

KEYWORDS: ALK 7, ACTRIIB, fusion, heterologous, multimer, heteromultimer, Fc, constant region, domain, immunoglobulin as well as synonyms and similar terms.

Applicant and/or Inventor searches of the patent and non-patent literature was performed using Patentscope (http://www.wipo.int/patentscope/en/), PubMed (http://www.ncbi.nlm.nih.gov/pubmed), and in internal databases provided by IP Australia.

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	

[X] Further documents are listed in the continuation of Box C [X] See patent family annex

* Special categories of cited documents:

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 June 2016	21 June 2016
Name and mailing address of the ISA/AU	**Authorised officer**
AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA Email address: pct@ipaustralia.gov.au	Monica Graham AUSTRALIAN PATENT OFFICE (ISO 9001 Quality Certified Service) Telephone No. 0262833179

INTERNATIONAL SEARCH REPORT C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		International application No. **PCT/US2016/026277**
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2008/097541 A2 (ACCELERON PHARMA INC.) 14 August 2008	
A	WO 2010/151426 A1 (ACCELERON PHARMA INC.) 29 December 2010	
A	WO 2013/063536 A1 (ACCELERON PHARMA INC.) 02 May 2013	
A	WO 2012/027065 A2 (CELGENE CORPORATION) 01 March 2012	
A	WO 2005/084699 A1 (ACCELERON PHARMA INC.) 15 September 2005	
A	US 2005/0186593 A1 (MATHEWS et al.) 25 August 2005	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2016/026277

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

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

   a. (means)

   ☐ on paper

   ☒ in electronic form

   b. (time)

   ☐ in the international application as filed

   ☐ together with the international application in electronic form

   ☒ subsequently to this Authority for the purposes of search

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

3. Additional comments:

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/US2016/026277**

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 2008/097541 A2	14 August 2008	WO 2008097541 A2	14 Aug 2008
		AR 065169 A1	20 May 2009
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Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/US2016/026277**

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

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/US2016/026277**

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

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/US2016/026277**

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**
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End of Annex

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

(19)中华人民共和国国家知识产权局

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2017.12.06

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A61P 3/04(2006.01)

权利要求书10页 说明书112页 附图24页

(54)发明名称

ALK7:ACTRIIB异多聚体和其用途

(57)摘要

在某些方面,本公开内容提供了可溶性异聚多肽复合物,其包含ALK7受体的细胞外结构域和ActRIIB的细胞外结构域。在某些方面,这些ALK7:ActRIIB异多聚体可用于改进有需要的患者的代谢参数。在某些方面,这些ALK7:ActRIIB异多聚体可用于在有需要的患者中治疗或预防一种或多种肾相关疾病或病况。

CN 107709358 A

1.重组异多聚体,其包含ALK7多肽和ActRIIB多肽。

2. 权利要求1的异多聚体,其中ALK7多肽包含与以下多肽具有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列:

a) 以SEQ ID NO: 9的氨基酸21、22、23、24、25、26、27或28的任一个开始,和

b) 以SEQ ID NO: 9的氨基酸92、93、94、95、96、97、98、99、100、101、102、103、104、105、106、107、108、109、110、111、112或113的任一个结束。

3. 权利要求1的异多聚体,其中ALK7多肽包含与SEQ ID NO: 9的氨基酸28-92具有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

4. 权利要求1的异多聚体,其中ALK7多肽包含与SEQ ID NO: 10具有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

5. 权利要求1的异多聚体,其中ALK7多肽包含与SEQ ID NO: 20具有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

6. 权利要求1的异多聚体,其中ALK7多肽包含与SEQ ID NO: 39具有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

7. 权利要求1的异多聚体,其中ALK7多肽包含与SEQ ID NO: 43或46具有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

8. 权利要求1-7中任一项的异多聚体,其中ActRIIB多肽包含与以下多肽具有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列:

a) 以SEQ ID NO: 1的氨基酸20、21、22、23、24、25、26、27、28或29的任一个开始,和

b) 以SEQ ID NO: 1的氨基酸109、110、111、112、113、114、115、116、117、118、119、120、121、122、123、124、125、126、127、128、129、130、131、132、133或134的任一个结束。

9. 权利要求1-7中任一项的异多聚体,其中ActRIIB多肽包含与SEQ ID NO: 1的氨基酸29-109具有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

10. 权利要求1-7中任一项的异多聚体,其中ActRIIB多肽包含与SEQ ID NO: 1的氨基酸25-131具有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

11. 权利要求8-10中任一项的异多聚体,其中ActRIIB多肽在对应于SEQ ID NO: 1的L79的位置处不包含酸性氨基酸。

12. 权利要求1-11中任一项的异多聚体,其中ActRIIB多肽包含与SEQ ID NO: 2具有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

13. 权利要求1-11中任一项的异多聚体，其中ActRIIB多肽包含与SEQ ID NO: 3具有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

14. 权利要求1-11中任一项的异多聚体，其中ActRIIB多肽包含与SEQ ID NO: 5具有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

15. 权利要求1-11中任一项的异多聚体，其中ActRIIB多肽包含与SEQ ID NO: 6具有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

16.权利要求1-15中任一项的异多聚体，其中ALK7多肽是还包含异源结构域的融合蛋白。

17.权利要求1-16中任一项的异多聚体，其中ActRIIB多肽是还包含异源结构域的融合蛋白。

18.权利要求16的异多聚体，其中ALK7多肽包含相互作用对的第一或第二成员。

19.权利要求17的异多聚体，其中ActRIIB多肽包含相互作用对的第一或第二成员。

20.权利要求16-19中任一项的异多聚体，其中异源结构域包含Fc免疫球蛋白结构域。

21.权利要求20的异多聚体，其中Fc免疫球蛋白结构域包含促进异二聚体形成的一种或多种氨基酸修饰。

22.权利要求20或21的异多聚体，其中免疫球蛋白Fc结构域包含抑制同二聚体形成的一种或多种氨基酸修饰。

23.权利要求20-22中任一项的异多聚体，其中异源结构域包含来自IgG免疫球蛋白的Fc免疫球蛋白结构域。

24.权利要求23的异多聚体，其中IgG免疫球蛋白选自:IgG1、IgG2和IgG3或IgG4。

25. 权利要求23的异多聚体，其中Fc免疫球蛋白包含与SEQ ID NO: 23具有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

26. 权利要求25的异多聚体，其中Fc免疫球蛋白在对应于SEQ ID NO: 23的残基134和177的位置处包含带正电荷的氨基酸。

27. 权利要求26的异多聚体，其中Fc免疫球蛋白在SEQ ID NO: 23的位置134和177处包含K。

28. 权利要求25-27中任一项的异多聚体，其中Fc免疫球蛋白在对应于SEQ ID NO: 23的残基225的位置处不包含带正电荷的氨基酸。

29. 权利要求28的异多聚体，其中Fc免疫球蛋白在SEQ ID NO: 23的位置225处不包含K。

30. 权利要求23的异多聚体，其中Fc免疫球蛋白包含与SEQ ID NO: 24具有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

31. 权利要求30的异多聚体，其中Fc免疫球蛋白在对应于SEQ ID NO: 24的残基170和187的位置处包含带负电荷的氨基酸。

32．权利要求31的异多聚体，其中Fc免疫球蛋白在SEQ ID NO：24的位置170和187处包含D。

33．权利要求30-32中任一项的异多聚体，其中Fc免疫球蛋白在对应于SEQ ID NO：24的残基225的位置处包含带正电荷的氨基酸。

34．权利要求33的异多聚体，其中Fc免疫球蛋白在SEQ ID NO：24的位置225处包含K。

35．权利要求23的异多聚体，其中Fc免疫球蛋白包含与SEQ ID NO：25具有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

36．权利要求23的异多聚体，其中Fc免疫球蛋白包含与SEQ ID NO：26具有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

37．权利要求23的异多聚体，其中Fc免疫球蛋白包含与SEQ ID NO：27具有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

38．权利要求37的异多聚体，其中Fc免疫球蛋白在对应于SEQ ID NO：27的残基132的位置处包含C，和其中Fc免疫球蛋白在对应于SEQ ID NO：27的残基144的位置处进一步包含W。

39．权利要求37或38的异多聚体，其中Fc免疫球蛋白在对应于SEQ ID NO：27的残基225的位置处不包含带正电荷的氨基酸。

40．权利要求39的异多聚体，其中Fc免疫球蛋白在SEQ ID NO：27的位置225处不包含K。

41．权利要求23的异多聚体，其中Fc免疫球蛋白包含与SEQ ID NO：28具有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

42．权利要求41的异多聚体，其中Fc免疫球蛋白在对应于SEQ ID NO：28的残基127的位置处包含C，其中Fc免疫球蛋白在对应于SEQ ID NO：28的残基144的位置处进一步包含S，其中Fc免疫球蛋白在对应于SEQ ID NO：28的残基146的位置处进一步包含A，和其中Fc免疫球蛋白在对应于SEQ ID NO：28的残基185的位置处进一步包含V。

43．权利要求41或42中任一项的异多聚体，其中Fc免疫球蛋白在对应于SEQ ID NO：28的残基225的位置处不包含带正电荷的氨基酸。

44．权利要求43的异多聚体，其中Fc免疫球蛋白在SEQ ID NO：28的位置225处不包含K。

45．权利要求23的异多聚体，其中Fc免疫球蛋白包含与SEQ ID NO：29具有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

46．权利要求23的异多聚体，其中Fc免疫球蛋白包含与SEQ ID NO：30具有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

47．权利要求23的异多聚体，其中Fc免疫球蛋白包含与SEQ ID NO：31具有至少70%、

75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

48. 权利要求23的异多聚体，其中Fc免疫球蛋白包含与SEQ ID NO: 32具有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

49. 权利要求23的异多聚体，其中Fc免疫球蛋白包含与SEQ ID NO: 33具有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

50. 权利要求23的异多聚体，其中Fc免疫球蛋白包含与SEQ ID NO: 34具有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

51. 权利要求23的异多聚体，其中Fc免疫球蛋白包含与SEQ ID NO: 35具有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

52. 权利要求23的异多聚体，其中Fc免疫球蛋白包含与SEQ ID NO: 36具有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

53. 权利要求23的异多聚体，其中Fc免疫球蛋白包含与SEQ ID NO: 37具有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

54. 权利要求23的异多聚体，其中：

a) ALK7多肽是还包含免疫球蛋白的恒定区的融合蛋白，所述免疫球蛋白的恒定区包含与SEQ ID NO: 23具有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列；和

b) ActRIIB多肽是还包含免疫球蛋白的恒定区的融合蛋白，所述免疫球蛋白的恒定区包含与SEQ ID NO: 24具有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

55. 权利要求23的异多聚体，其中：

a) ALK7多肽是还包含免疫球蛋白的恒定区的融合蛋白，所述免疫球蛋白的恒定区包含与SEQ ID NO: 24具有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列；和

b) ActRIIB多肽是还包含免疫球蛋白的恒定区的融合蛋白，所述免疫球蛋白的恒定区包含与SEQ ID NO: 23具有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

56. 权利要求54或55的异多聚体，其中恒定区在对应于SEQ ID NO: 23的残基134和177的位置处包含带正电荷的氨基酸。

57. 权利要求56的异多聚体，其中恒定区在SEQ ID NO: 23的位置134和177处包含K。

58. 权利要求54-57中任一项的异多聚体，其中恒定区在对应于SEQ ID NO: 23的残基225的位置处不包含带正电荷的氨基酸。

59. 权利要求58的异多聚体,其中恒定区在SEQ ID NO: 23的位置225处不包含K。

60. 权利要求54-59中任一项的异多聚体,其中恒定区在对应于SEQ ID NO: 24的残基170和187的位置处包含带负电荷的氨基酸。

61. 权利要求60的异多聚体,其中恒定区在SEQ ID NO: 24的位置170和187处包含D。

62. 权利要求54-61中任一项的异多聚体,其中恒定区在对应于SEQ ID NO: 24的残基225的位置处包含带正电荷的氨基酸。

63. 权利要求62的异多聚体,其中恒定区在SEQ ID NO: 24的位置225处包含K。

64. 权利要求23的异多聚体,其中:

a) ALK7多肽是还包含免疫球蛋白的恒定区的融合蛋白,所述恒定区包含与SEQ ID NO: 27具有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列;和

b) ActRIIB多肽是还包含免疫球蛋白的恒定区的融合蛋白,所述恒定区包含与SEQ ID NO: 28具有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

65. 权利要求23的异多聚体,其中:

a) ALK7多肽是还包含免疫球蛋白的恒定区的融合蛋白,所述恒定区包含与SEQ ID NO: 28具有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列;和

b) ActRIIB多肽是还包含免疫球蛋白的恒定区的融合蛋白,所述恒定区包含与SEQ ID NO: 27具有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

66. 权利要求64或65的异多聚体,其中恒定区在对应于SEQ ID NO: 27的残基132的位置处包含C,和其中恒定区在对应于SEQ ID NO: 27的残基144的位置处进一步包含W。

67. 权利要求64-66中任一项的异多聚体,其中恒定区在对应于SEQ ID NO: 27的残基225的位置处不包含带正电荷的氨基酸。

68. 权利要求67的异多聚体,其中恒定区在SEQ ID NO: 27的位置225处不包含K。

69. 权利要求64-68中任一项的异多聚体,其中恒定区在对应于SEQ ID NO: 28的残基127的位置处包含C,其中恒定区在对应于SEQ ID NO: 28的残基144的位置处进一步包含S,其中恒定区在对应于SEQ ID NO: 28的残基146的位置处进一步包含A,和其中恒定区在对应于SEQ ID NO: 28的残基185的位置处进一步包含V。

70. 权利要求64-69中任一项的异多聚体,其中恒定区在对应于SEQ ID NO: 28的残基225的位置处不包含带正电荷的氨基酸。

71. 权利要求70的异多聚体,其中恒定区在SEQ ID NO: 28的位置225处不包含K。

72.权利要求16-71中任一项的异多聚体,其中融合蛋白进一步包含位于ALK7结构域和异源结构域之间的接头结构域。

73.权利要求16-72中任一项的异多聚体,其中融合蛋白进一步包含位于ActRIIB结构域和异源结构域之间的接头结构域。

74. 权利要求72或73的异多聚体,其中接头结构域选自:TGGG (SEQ ID NO: 17)、TGGGG (SEQ ID NO: 15)、SGGGG (SEQ ID NO: 16)、GGGGS (SEQ ID NO: 58)、GGG (SEQ ID

NO: 13)、GGGG (SEQ ID NO: 14)和SGGG (SEQ ID NO: 18)。

75. 权利要求1-74中任一项的异多聚体，其中ALK7多肽包含与SEQ ID NO:74具有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

76. 权利要求1-74中任一项的异多聚体，其中ALK7多肽包含与SEQ ID NO:76具有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

77. 权利要求1-74中任一项的异多聚体，其中ActRIIB多肽包含与SEQ ID NO:71具有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

78. 权利要求1-74中任一项的异多聚体，其中ActRIIB多肽包含与SEQ ID NO:73具有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

79. 权利要求1-74中任一项的异多聚体，其中异多聚体包含：

ALK7多肽，其包含与SEQ ID NO:76具有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列，和

ActRIIB多肽，其包含与SEQ ID NO:73具有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

80.权利要求1-74中任一项的异多聚体，其中ALK7多肽包含与79具有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

81.权利要求1-74中任一项的异多聚体，其中ALK7多肽包含与80具有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

82.权利要求1-74中任一项的异多聚体，其中ActRIIB多肽包含与77具有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

83.权利要求1-74中任一项的异多聚体，其中ActRIIB多肽包含与78具有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

84. 权利要求1-74中任一项的异多聚体，其中异多聚体包含：

a) ALK7多肽，其包含与80具有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列，和

b) ActRIIB多肽，其包含与78具有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列。

85.权利要求1-84中任一项的异多聚体，其中ALK7多肽包含一个或多个修饰的氨基酸残基，其选自：糖基化氨基酸、PEG化氨基酸、法呢基化氨基酸、乙酰基化氨基酸、生物素化氨基酸和与脂质部分缀合的氨基酸。

86.权利要求1-85中任一项的异多聚体，其中ActRIIB多肽包含一个或多个修饰的氨基

酸残基，其选自：糖基化氨基酸、PEG化氨基酸、法呢基化氨基酸、乙酰基化氨基酸、生物素化氨基酸和与脂质部分缀合的氨基酸。

87.权利要求1-86中任一项的异多聚体，其中ALK7多肽进一步包含一种或多种纯化序列，其选自：表位标签、FLAG标签、多组氨酸序列和GST融合物。

88.权利要求1-87中任一项的异多聚体，其中ActRIIB多肽进一步包含一种或多种纯化序列，其选自：表位标签、FLAG标签、多组氨酸序列和GST融合物。

89.权利要求1-88中任一项的异多聚体，其中ALK7多肽被糖基化和具有可获自中国仓鼠卵巢细胞系的糖基化模式。

90.权利要求1-89中任一项的异多聚体，其中ActRIIB多肽被糖基化和具有可获自中国仓鼠卵巢细胞系的糖基化模式。

91.权利要求1-90中任一项的异多聚体，其中异多聚体结合活化素。

92.权利要求1-91中任一项的异多聚体，其中异多聚体结合活化素A。

93.权利要求1-92中任一项的异多聚体，其中异多聚体结合活化素B。

94.权利要求1-93中任一项的异多聚体，其中异多聚体结合活化素C和/或活化素E。

95.权利要求1-94中任一项的异多聚体，其中异多聚体结合活化素AB。

96.权利要求1-95中任一项的异多聚体，其中异多聚体结合活化素AC、BC、AE和/或BE。

97.权利要求1-96中任一项的异多聚体，其中异多聚体结合GDF8。

98.权利要求1-97中任一项的异多聚体，其中异多聚体结合GDF11。

99.权利要求1-98中任一项的异多聚体，其中异多聚体结合BMP6。

100.权利要求1-99中任一项的异多聚体，其中异多聚体结合nodal。

101.权利要求1-100中任一项的异多聚体，其中异多聚体结合BMP5。

102.权利要求1-101中任一项的异多聚体，其中异多聚体结合GDF3。

103.权利要求1-101中任一项的异多聚体，其中异多聚体结合BMP10。

104.权利要求1-103中任一项的异多聚体，其中异多聚体以比相应的ActRIIB同多聚体更弱的亲和力结合BMP10。

105.权利要求1-104中任一项的异多聚体，其中异多聚体以比相应的ActRIIB同多聚体更弱的亲和力结合BMP9。

106.权利要求1-105中任一项的异多聚体，其中异多聚体以比相应的ActRIIB同多聚体更弱的亲和力结合GDF3。

107.权利要求1-106中任一项的异多聚体，其中异多聚体以比相应的ActRIIB同多聚体更弱的亲和力结合BMP6。

108.权利要求1-107中任一项的异多聚体，其中异多聚体以比相应的ActRIIB同多聚体更弱的亲和力结合GDF8。

109.权利要求1-108中任一项的异多聚体，其中异多聚体以比相应的ActRIIB同多聚体更弱的亲和力结合GDF11。

110.权利要求1-109中任一项的异多聚体，其中异多聚体以比相应的ActRIIB同多聚体更强的亲和力结合活化素C。

111.权利要求1-110中任一项的异多聚体，其中异多聚体以比相应的ActRIIB同多聚体更强的亲和力结合活化素AC。

112.权利要求1-111中任一项的异多聚体,其中异多聚体以比相应的ActRIIB同多聚体更强的亲和力结合BMP5。

113.权利要求1-112中任一项的异多聚体,其中在基于细胞的测定法中异多聚体抑制活化素信号传导。

114.权利要求1-113中任一项的异多聚体,其中在基于细胞的测定法中异多聚体抑制活化素A信号传导。

115.权利要求1-114中任一项的异多聚体,其中在基于细胞的测定法中异多聚体抑制活化素B信号传导。

116.权利要求1-115中任一项的异多聚体,其中在基于细胞的测定法中异多聚体抑制活化素C和/或E信号传导。

117.权利要求1-116中任一项的异多聚体,其中在基于细胞的测定法中异多聚体抑制活化素AC、BC、AE和/或BE信号传导。

118.权利要求1-117中任一项的异多聚体,其中在基于细胞的测定法中异多聚体抑制GDF11信号传导。

119.权利要求1-118中任一项的异多聚体,其中在基于细胞的测定法中异多聚体抑制GDF8信号传导。

120.权利要求1-119中任一项的异多聚体,其中在基于细胞的测定法中异多聚体抑制BMP10信号传导。

121.权利要求1-120中任一项的异多聚体,其中在基于细胞的测定法中异多聚体抑制BMP6信号传导。

122.权利要求1-121中任一项的异多聚体,其中在基于细胞的测定法中异多聚体抑制BMP5信号传导。

123.权利要求1-122中任一项的异多聚体,其中在基于细胞的测定法中异多聚体抑制GDF3和/或nodal信号传导。

124.权利要求1-123中任一项的异多聚体,其中在基于细胞的测定法中异多聚体是比相应的ActRIIB同多聚体更弱的BMP9信号传导抑制剂。

125.权利要求1-124中任一项的异多聚体,其中在基于细胞的测定法中异多聚体是比相应的ActRIIB同多聚体更弱的BMP10信号传导抑制剂。

126.权利要求1-125中任一项的异多聚体,其中在基于细胞的测定法中异多聚体是比相应的ActRIIB同多聚体更弱的BMP6信号传导抑制剂。

127.权利要求1-126中任一项的异多聚体,其中在基于细胞的测定法中异多聚体是比相应的ActRIIB同多聚体更弱的GDF3信号传导抑制剂。

128.权利要求1-127中任一项的异多聚体,其中在基于细胞的测定法中异多聚体是比相应的ActRIIB同多聚体更弱的GDF11信号传导抑制剂。

129.权利要求1-128中任一项的异多聚体,其中在基于细胞的测定法中异多聚体是比相应的ActRIIB同多聚体更弱的GDF8信号传导抑制剂。

130.权利要求1-129中任一项的异多聚体,其中在基于细胞的测定法中异多聚体是比相应的ActRIIB同多聚体更弱的活化素A信号传导抑制剂。

131.权利要求1-129中任一项的异多聚体,其中在基于细胞的测定法中异多聚体是比

相应的ActRIIB同多聚体更弱的活化素A信号传导抑制剂。

132.权利要求1-130中任一项的异多聚体,其中在基于细胞的测定法中异多聚体是比相应的ActRIIB同多聚体更强的活化素C信号传导抑制剂。

133.权利要求1-132中任一项的异多聚体,其中在基于细胞的测定法中异多聚体是比相应的ActRIIB同多聚体更强的活化素AC信号传导抑制剂。

134.权利要求1-133中任一项的异多聚体,其中在基于细胞的测定法中异多聚体是比相应的ActRIIB同多聚体更强的BMP5信号传导抑制剂。

135.权利要求1-134中任一项的异多聚体,其中异多聚体是ALK7:ActRIIB异二聚体。

136.药物制剂,其包含权利要求1-135中任一项的异多聚体。

137.权利要求136的药物制剂,其中药物制剂是基本上无热原的。

138.权利要求136或137的药物制剂,其中药物制剂进一步包含另外的活性剂。

139. 权利要求136-138中任一项的药物制剂,其中药物制剂包含小于约10%、9%、8%、7%、6%、5%、4%、3%、2%或小于约1% ALK7同多聚体。

140. 权利要求136-139的药物制剂,其中药物制剂包含小于约10%、9%、8%、7%、6%、5%、4%、3%、2%或小于约1% ActRIIB同多聚体。

141.分离的核酸序列,其包含权利要求1-135中任一项的ALK7多肽的编码序列。

142. 权利要求141的分离的核酸序列,其中核酸包含与SEQ ID NO: 12、22、41、45或75具有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的核苷酸序列,基本上由其组成或由其组成。

143.分离的核酸序列,其包含权利要求1-134中任一项的ActRIIB多肽的编码序列。

144. 权利要求143的分离的核酸序列,其中核酸包含与SEQ ID NO: 8或72具有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的核苷酸序列。

145.分离的核酸序列,其包含权利要求1-135中任一项的ALK7多肽的编码序列和权利要求1-135中任一项的ActRIIB多肽的编码序列。

146. 权利要求145的分离的核酸序列,其中核酸:

a) 包含与SEQ ID NO: 12、22、41、45或75具有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的核苷酸序列,和

b) 包含与SEQ ID NO: 8或72具有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的核苷酸序列,基本上由其组成或由其组成。

147.重组多核苷酸,其包含可操作连接至权利要求141或142的ALK7多肽的编码序列的启动子序列。

148.重组多核苷酸,其包含可操作连接至权利要求143或144的ActRIIB多肽的编码序列的启动子序列。

149.重组多核苷酸,其包含可操作连接至权利要求141或142的ALK7多肽的编码序列和权利要求143或144的ActRIIB多肽的编码序列的启动子序列。

150.载体,其包含权利要求145-149中任一项的重组多核苷酸。

151.细胞,其包含权利要求145-149中任一项的重组多核苷酸或权利要求150的载体。

152.权利要求149的细胞,其中所述细胞是CHO细胞。

153. 制备包含ALK7多肽和ActRIIB多肽的异多聚体的方法,包括:

a) 在适合于表达ALK7多肽和ActRIIB多肽的条件下培养细胞,其中所述细胞包含权利要求147和148的重组多核苷酸;和

b) 回收如此表达的异多聚体。

154. 制备包含ALK7多肽和ActRIIB多肽的异多聚体的方法,包括:

a) 在适合于表达ALK7多肽和ActRIIB多肽的条件下培养细胞,其中所述细胞包含权利要求149的重组多核苷酸;和

b) 回收如此表达的异多聚体。

155.制备包含ALK7多肽和ActRIIB多肽的异多聚体的方法,包括:

a) 在适合于表达ALK7多肽的条件下培养第一细胞,其中第一细胞包含权利要求147的重组多核苷酸;

b) 回收如此表达的ALK7多肽;

c) 在适合于表达ActRIIB多肽的条件下培养第二细胞,其中第二细胞包含权利要求148的重组多核苷酸;

d) 回收如此表达的ActRIIB多肽;

e) 在适合于ALK7:ActRIIB异多聚体形成的条件下合并回收的ALK7多肽和回收的ActRIIB多肽;和

f) 回收ALK7:ActRIIB异多聚体。

156.权利要求153-155中任一项的方法,其中ALK7多肽使用TPA前导序列表达。

157.权利要求153-156中任一项的方法,其中ActRIIB多肽使用TPA前导序列表达。

158. 权利要求156或157方法,其中TPA前导序列包含SEQ ID NO: 70,基本上由其组成或由其组成。

159.权利要求153-158中任一项的方法,其中所述细胞是CHO细胞。

160.权利要求153-159中任一项的方法,其中所述异多聚体是异二聚体。

ALK7:ACTRIIB异多聚体和其用途

[0001] 相关申请的交叉引用

[0002] 本申请要求2015年4月6日提交的美国临时申请系列号 62/143,579的优先权的权益。前述申请的公开内容通过引用以其整体结合到本文中。

[0003] 发明背景

[0004] 转化生长因子-β(TGF-β)超家族包含各种生长因子,其享有共同的序列元件和结构基序。这些蛋白已知在脊椎动物和无脊椎动物二者中对多种细胞类型发挥生物学作用。超家族的成员在胚胎发育期间在模式形成和组织特化中执行重要的功能,和可影响各种分化过程,包括脂肪生成、肌生成、软骨发生、心脏发生、血细胞生成、神经发生和上皮细胞分化。该家族分成两个概括的系统发生进化枝:更近进化的超家族成员,其包括TGF-β、活化素和nodal,和更远相关的超家族蛋白的进化枝,其包括许多BMP和GDF[Hinck(2012)FEBS Letters 586:1860-1870]。TGF-β家族成员具有多样的,通常互补的生物学作用。通过操纵TGF-β家族的成员的活性,通常有可能引起生物体的显著生理学变化。例如,Piedmontese和Belgian Blue牛品种带有GDF8(亦称为肌抑素)基因的功能丢失突变,其导致肌肉质量显著增加[Grobet等 (1997)Nat Genet.、17(1):71-4]。此外,在人中,GDF8的失活的等位基因与肌肉质量增加有关,并据报道,与异常优越的力量有关 [Schuelke等(2004)N Engl J Med、350:2682-8]。

[0005] 纤维化、肌肉、骨、脂肪、红细胞和其它组织的变化可通过增强或抑制由TGF-β家族的配体介导的细胞内信号传导(例如,SMAD 1、 2、3、5和/或8)来实现。因此,对调节TGF-β超家族的各种配体的活性的试剂存在需要。

[0006] 发明简述

[0007] 如本文所述的,已发现ALK7:ActRIIB异二聚体蛋白复合物是 TGF-β超家族的配体的独特拮抗剂,与相应的ActRIIB和ALK7同二聚体比较,显示不同的配体-结合特征/选择性。特别地,与任一同二聚体比较,实例性ALK7:ActRIIB异二聚体显示与活化素AC、活化素C和BMP5的结合增强,保留如ActRIIB同二聚体所观察到的与活化素B的强结合,和显示与GDF11、GDF8、活化素A、BMP10、BMP6、 GDF3和BMP9的结合减少。特别地,ALK7:ActRIIB异二聚体显示低至无可观察到的对BMP9的亲和力,但该配体与ActRIIB同二聚体强结合。参见图6。这些结果因此证实与ActRIIB同二聚体比较, ALK7:ActRIIB异二聚体是TGF-β超家族的某些配体的更具选择性的拮抗剂(抑制剂)。因此,在其中这样的选择性拮抗作用是有利的某些应用中,ALK7:ActRIIB异二聚体比ActRIIB同二聚体更有用。实例包括治疗应用,其中需要拮抗活化素B、活化素AC、活化素C和BMP5 的一种或多种,伴随对活化素A、BMP10、BMP6、GDF3和BMP9 的一种或多种的拮抗作用减少。此外,ALK7:ActRIIB异二聚体显示在肾疾病的小鼠模型中具有治疗作用以及对脂肪细胞的有益分解代谢作用。因此,尽管不希望受限于特定的作用机制,预期ALK7:ActRIIB 异多聚体以及其变体,其结合/抑制活化素(例如,活化素A、活化素B、活化素AB、活化素C、活化素E、活化素AC、活化素AE、活化素 BC和活化素BE)、GDF8、GDF11、BMP10、BMP6、BMP5、GDF3 和/或nodal的至少一种或多种,是用于治疗肾疾病、特别是肾疾病的某些病症例如炎症和纤维化和/或促进对脂肪组织的有益分解代谢作

用的有用试剂。此外，预期模拟ALK7:ActRIIB异二聚体的结合/抑制性质的其它拮抗剂(抑制剂)或拮抗剂的组合以及直接或间接拮抗 ALK7和/或ActRIIB受体的试剂、直接或间接拮抗ALK7和/或 ActRIIB-结合配体的试剂、直接或间接拮抗下游信号传导介质(例如，Smads)的试剂和/或直接或间接拮抗TGFβ超家族共受体(例如，Cripto 或Cryptic)的试剂将具有类似的生物学作用。这些拮抗模拟物在文本中统称为“ALK7:ActRIIB拮抗剂”或“ALK7:ActRIIB抑制剂”。

[0008] 因此，本公开内容部分地提供了异多聚体复合物(异多聚体)，其包含至少一个ALK7多肽和至少一个ActRIIB多肽(ALK7:ActRIIB异多聚体)。优选地，ALK7多肽包含ALK7受体的配体-结合结构域，例如，ALK7细胞外结构域的一部分。类似地，ActRIIB多肽通常包含ActRIIB受体的配体-结合结构域，例如，ActRIIB细胞外结构域的一部分。优选地，这样的ALK7和ActRIIB多肽以及其得到的异多聚体是可溶性的。

[0009] 在某些方面，ALK7:ActRIIB异多聚体包含ALK7氨基酸序列，其与以SEQ ID NO:9的氨基酸21-28的任一个(例如，氨基酸21、22、 23、24、25、26、27或28)开始和以SEQ ID NO:9的氨基酸92-113 的任一个(例如，氨基酸92、93、94、95、96、97、98、99、100、101、 102、103、104、105、106、107、108、109、110、111、112或113) 结束的多肽具有至少70％同一性。例如，ALK7:ActRIIB异多聚体可包含与SEQ ID NO:9的氨基酸28-92具有至少70％、75％、80％、85％、 86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、 97％、98％、99％或100％同一性的氨基酸序列。在其它实施方案中， ALK7:ActRIIB异多聚体可包含ALK7氨基酸序列，其与SEQ ID NO： 9的氨基酸21-113具有至少70％、75％、80％、85％、86％、87％、88％、 89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性。在其它实施方案中，ALK7:ActRIIB异多聚体可包含 ALK7氨基酸序列，其与SEQ ID NO:10具有至少70％、75％、80％、 85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性。在仍其它实施方案中， ALK7:ActRIIB异多聚体可包含ALK7氨基酸序列，其与SEQ ID NO： 20具有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、 91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性。在甚至其它的实施方案中，ALK7:ActRIIB异多聚体可包含ALK7 氨基酸序列，其与SEQ ID NO:39具有至少70％、75％、80％、85％、 86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、 97％、98％、99％或100％同一性。在另外的实施方案中，ALK7:ActRIIB 异多聚体可包含ALK7氨基酸序列，其与SEQ ID NO:43具有至少70％、 75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、 94％、95％、96％、97％、98％、99％或100％同一性。在另外的实施方案中，ALK7:ActRIIB异多聚体可包含ALK7氨基酸序列，其与SEQ ID NO:46具有至少70％、75％、80％、85％、86％、87％、88％、89％、 90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性。

[0010] 在某些方面，ALK7:ActRIIB异多聚体包含ActRIIB氨基酸序列，其与以SEQ ID NO:1的氨基酸20-29的任一个开始(例如，20、21、 22、23、24、25、26、27、28或29)和以SEQ ID NO:1的氨基酸109-134 的任一个结束(109、110、111、112、113、114、115、116、117、118、119、120、121、122、123、124、125、126、127、128、129、130、 131、132、133或134)的多肽具有至少70％同一性。例如，ALK7:ActRIIB 异多聚体可包含与SEQ ID NO:1的氨基酸29-109具有至少70％、75％、 80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、

95％、96％、97％、98％、99％或100％同一性的氨基酸序列。在进一步的实施方案中，ALK7:ActRIIB异多聚体可包含与氨基酸25-131具有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、 92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的ActRIIB氨基酸序列。在其它实施方案中，ALK7:ActRIIB异多聚体可包含与SEQ ID NO:2具有至少70％、75％、80％、85％、86％、87％、 88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、 99％或100％同一性的ActRIIB氨基酸序列。在仍其它实施方案中，ALK7:ActRIIB异多聚体可包含与SEQ ID NO:3具有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、 95％、96％、97％、98％、99％或100％同一性的ActRIIB氨基酸序列。在甚至其它的实施方案中，ALK7:ActRIIB异多聚体可包含与SEQ ID NO:5具有至少70％、75％、80％、85％、86％、87％、88％、89％、 90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的ActRIIB氨基酸序列。在仍甚至其它的实施方案中， ALK7:ActRIIB异多聚体可包含与SEQ ID NO:6具有至少70％、75％、 80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、 95％、96％、97％、98％、99％或100％同一性的ActRIIB氨基酸序列。在某些优选的实施方案中，ALK7:ActRIIB异多聚体不包含在对应于 SEQ ID NO:1的L79的位置处包含酸性氨基酸(例如，天然存在的氨基酸E或D或人工酸性氨基酸)的ActRIIB多肽。

[0011] 关于ALK7:ActRIIB异多聚体，还考虑了本文所述的ALK7和 ActRIIB多肽的各种组合。例如，在某些方面，ALK7:ActRIIB异多聚体可包含a)多肽，包含与SEQ ID NO:9的氨基酸28-92具有至少70％、 75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的ALK7氨基酸序列，基本上由其组成或由其组成；和b)多肽，包含与SEQ ID NO: 1的氨基酸29-109具有至少70％、75％、80％、85％、86％、87％、88％、 89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的ActRIIB氨基酸序列，或基本上由其组成或由其组成；基本上由其组成或由其组成。在进一步的方面，ALK7:ActRIIB 异多聚体可包含a)多肽，包含与SEQ ID NO:9的氨基酸21-113具有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、 92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的 ALK7氨基酸序列，基本上由其组成或由其组成；和b)多肽，包含与 SEQ ID NO:1的氨基酸25-131具有至少70％、75％、80％、85％、86％、 87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的ActRIIB氨基酸序列，或基本上由其组成或由其组成；基本上由其组成或由其组成。在其它方面，ALK7:ActRIIB 异多聚体可包含a)多肽，包含与SEQ ID NO:10具有至少70％、75％、 80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、 95％、96％、97％、98％、99％或100％同一性的ALK7氨基酸序列，基本上由其组成或由其组成；和b)多肽，包含与SEQ ID NO:2具有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、 92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的 ActRIIB氨基酸序列，或基本上由其组成或由其组成；基本上由其组成或由其组成。在其它方面，ALK7:ActRIIB异多聚体可包含a)多肽，包含与SEQ ID NO:20具有至少70％、75％、80％、85％、86％、87％、 88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、 99％或100％同一性的ALK7氨基酸序列，基本上由其组成或由其组成；和b)多肽，包含与SEQ ID NO:2具有至少70％、75％、80％、 85％、86％、87％、88％、89％、90％、91％、92％、93％、

94％、95％、96％、97％、98％、99％或100％同一性的ActRIIB氨基酸序列，或基本上由其组成或由其组成；基本上由其组成或由其组成。在其它方面，ALK7:ActRIIB异多聚体可包含a)多肽，包含与SEQ ID NO:39具有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的ALK7氨基酸序列，基本上由其组成或由其组成；和b)多肽，包含与SEQ ID NO:2具有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的ActRIIB氨基酸序列，或基本上由其组成或由其组成；基本上由其组成或由其组成。在其它方面，ALK7:ActRIIB异多聚体可包含a)多肽，包含SEQ ID NO:43具有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的ALK7氨基酸序列与，基本上由其组成或由其组成；和b)多肽，包含与SEQ ID NO:2具有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的ActRIIB氨基酸序列，或基本上由其组成或由其组成；基本上由其组成或由其组成。在其它方面，ALK7:ActRIIB异多聚体可包含a)多肽，包含与SEQ ID NO:46具有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的ALK7氨基酸序列，基本上由其组成或由其组成；和b)多肽，包含与SEQ ID NO:2具有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的ActRIIB氨基酸序列，或基本上由其组成或由其组成；基本上由其组成或由其组成。在甚至其它方面，ALK7:ActRIIB异多聚体可包含a)多肽，包含与SEQ ID NO:10具有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的ALK7氨基酸序列，基本上由其组成或由其组成；和b)多肽，包含与SEQ ID NO:3具有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的ActRIIB氨基酸序列，或基本上由其组成或由其组成；基本上由其组成或由其组成。在甚至其它方面，ALK7:ActRIIB异多聚体可包含a)多肽，包含与SEQ ID NO:20具有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的ALK7氨基酸序列，基本上由其组成或由其组成；和b)多肽，包含与SEQ ID NO:3具有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的ActRIIB氨基酸序列，或基本上由其组成或由其组成；基本上由其组成或由其组成。在甚至其它方面，ALK7:ActRIIB异多聚体可包含a)多肽，包含与SEQ ID NO:39具有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的ALK7氨基酸序列，基本上由其组成或由其组成；和b)多肽，包含与SEQ ID NO:3具有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的ActRIIB氨基酸序列，或基本上由其组成或由其组成；基本上由其组成或由其组成。在甚至其它方面，ALK7:ActRIIB异多聚体可包含a)多肽，包含与SEQ ID NO:43 具有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的ALK7氨基酸序列，基本上由其组成或由其组成；和b)多肽，包含与SEQ ID NO:3具有至少70％、75％、80％、

85％、86％、87％、 88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、 99％或100％同一性的ActRIIB氨基酸序列，或基本上由其组成或由其组成；基本上由其组成或由其组成。在其它方面，ALK7:ActRIIB异多聚体可包含a)多肽，包含与SEQ ID NO:46具有至少70％、75％、 80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、 95％、96％、97％、98％、99％或100％同一性的ALK7氨基酸序列，基本上由其组成或由其组成；和b)多肽，包含与SEQ ID NO:3具有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、 92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的 ActRIIB氨基酸序列，或基本上由其组成或由其组成；基本上由其组成或由其组成。在仍其它方面，ALK7:ActRIIB异多聚体可包含a)多肽，包含与SEQ ID NO:10具有至少70％、75％、80％、85％、86％、 87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、 98％、99％或100％同一性的ALK7氨基酸序列，基本上由其组成或由其组成；和b)多肽，包含与SEQ ID NO:5具有至少70％、75％、80％、 85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的ActRIIB氨基酸序列，或基本上由其组成或由其组成；基本上由其组成或由其组成。在仍甚至其它方面，ALK7:ActRIIB异多聚体可包含a)多肽，包含与SEQ ID NO： 10具有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、 91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的ALK7氨基酸序列，基本上由其组成或由其组成；和b)多肽，包含与SEQ ID NO:6具有至少70％、75％、80％、85％、86％、87％、 88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、 99％或100％同一性的ActRIIB氨基酸序列，或基本上由其组成或由其组成；基本上由其组成或由其组成。

[0012] 如本文所述的，ALK7:ActRIIB异多聚体结构包括例如，异二聚体、异三聚体、异四聚体、异五聚体和更高级异多聚体复合物。参见例如，图1、2和8-10。在某些优选的实施方案中，ALK7:ActRIIB异多聚体是异二聚体。

[0013] 在某些方面，ALK7和/或ActRIIB多肽可以是融合蛋白。例如，在一些实施方案中，ALK7多肽可以是包含ALK7多肽结构域和一个或多个异源的(非-ALK7)多肽结构域的融合蛋白。类似地，在一些实施方案中，ActRIIB多肽可以是包含ActRIIB多肽结构域和一个或多个异源的(非-ActRIIB)多肽结构域的融合蛋白。任选地，ALK7多肽直接连接(融合)至一个或多个异源结构域，或间插序列，例如接头可位于ALK7多肽和一个或多个异源结构域的氨基酸序列之间。类似地， ActRIIB多肽可直接连接(融合)至一个或多个异源结构域，或间插序列，例如接头可位于ActRIIB多肽和一个或多个异源结构域的氨基酸序列之间。接头可在ActRIIB或ALK7的细胞外结构域的C-末端(“尾”)处对应于大约15个氨基酸非结构化区域，或其可以是相对不含二级结构的5和15、20、30、50、100或更多个氨基酸之间的人工序列。接头可以富含甘氨酸和脯氨酸残基，和可例如包含苏氨酸/丝氨酸和甘氨酸的重复序列。接头的实例包括但不限于，序列TGGG(SEQ ID NO:17)、SGGG(SEQ ID NO:18)、TGGGG(SEQ ID NO:15)、SGGGG(SEQ ID NO:16)、GGGGS(SEQ ID NO:58)、GGGG(SEQ ID NO:14)和GGG (SEQ ID NO:13)。在一些实施方案中，一个或多个异源结构域为ALK7 和/或ActRIIB融合蛋白提供需要的性质，包括例如，改进的药代动力学、更易纯化、靶向特定组织等。例如，融合蛋白的异源结构域可增强体内稳定性、体内半寿期、摄取/给药、组织定位或分布、蛋白复合物的形成、融合蛋白的多聚化和/或纯化中的一种或多种。ALK7或 ActRIIB融合蛋白可包括免疫球

蛋白Fc结构域(野生型或突变体)或血清白蛋白。在一些实施方案中,ALK7和/或ActRIIB多肽可包含纯化子序列,例如表位标签、FLAG标签、多组氨酸序列和GST融合物。

[0014] 在某些实施方案中,本文所述的ALK7:ActRIIB异多聚体包含与 ActRIIB多肽共价或非共价缔合的ALK7多肽,其中ALK7多肽包含 ALK7结构域和相互作用对的第一成员(或第二成员)的氨基酸序列,和ActRIIB多肽包含ActRIIB多肽和相互作用对的第二成员(或第一成员)的氨基酸序列。本文所述的相互作用对经设计以促进二聚化或形成更高级多聚体。参见例如,图1、2和8-10。在一些实施方案中,相互作用对可以是相互作用以形成复合物、特别是异二聚体复合物的任何两个多肽序列,尽管有效的实施方案也可使用形成同二聚体序列的相互作用对。相互作用对的第一和第二成员可以是不对称的对,意思是该对的成员优先彼此缔合,而非自缔合(即,引导的相互作用对)。因此,不对称的相互作用对的第一和第二成员可缔合形成异二聚体复合物。或者,相互作用对可以是非引导的,意思是该对的成员可彼此缔合或自缔合,没有实质的偏好,因此可具有相同或不同的氨基酸序列。因此,非引导的相互作用对的第一和第二成员可缔合形成同二聚体复合物或异二聚体复合物。任选地,相互作用对的第一成员(例如,不对称的对或非引导的相互作用对)与相互作用对的第二成员共价缔合。任选地,相互作用对的第一成员(例如,不对称的对或非引导的相互作用对)与相互作用对的第二成员非共价缔合。任选地,相互作用对(例如,不对称的对或非引导的相互作用对)的第一成员通过共价和非共价机制与相互作用对的第二成员缔合。

[0015] 在一些实施方案中,ALK7多肽是包含免疫球蛋白的Fc结构域的融合蛋白。类似地,在一些实施方案中,ActRIIB多肽是包含免疫球蛋白的Fc结构域的融合蛋白。传统的Fc融合蛋白和抗体是非引导的相互作用对的实例,尽管各种工程改造的Fc结构域已被设计为不对称的相互作用对[Spiess等(2015)Molecular Immunology 67(2A):95-106]。因此,本文所述的相互作用对的第一成员和/或第二成员可包含免疫球蛋白的恒定结构域,包括例如,免疫球蛋白的Fc部分。例如,相互作用对的第一成员可包含源自IgG(IgG1、IgG2、IgG3或IgG4)、IgA(IgA1或IgA2)、IgE或IgM免疫球蛋白的Fc结构域的氨基酸序列。这样的免疫球蛋白结构域可包含一种或多种氨基酸修饰(例如,缺失、添加和/或置换),其促进ALK7:ActRIIB异多聚体形成。例如,相互作用对的第一成员可包含与SEQ ID NOs:23-37的任一个具有至少 70%、75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、 97%、98%、99%或100%同一性的氨基酸序列,基本上由其组成,或由其组成。类似地,相互作用对的第二成员可包含源自IgG(IgG1、IgG2、 IgG3或IgG4)、IgA(IgA1或IgA2)、IgE或IgM的Fc结构域的氨基酸序列。这样的免疫球蛋白结构域可包含一种或多种氨基酸修饰(例如,缺失、添加和/或置换),其促进ALK7:ActRIIB异多聚体形成。例如,相互作用对的第二成员可包含与SEQ ID NOs:23-37的任一个具有至少70%、75%、80%、85%、90%、91%、92%、93%、94%、95%、 96%、97%、98%、99%或100%同一性的氨基酸序列,基本上由其组成,或由其组成。在一些实施方案中,相互作用对的第一成员和第二成员包含源自相同的免疫球蛋白类型和亚型的Fc结构域。在其它实施方案中,相互作用对的第一成员和第二成员包含源自不同的免疫球蛋白类型和亚型的Fc结构域。

[0016] 在一些实施方案中,ALK7:ActRIIB异二聚体包含i)ALK7多肽,其包含与SEQ ID NO:76具有至少70%、75%、80%、85%、86%、87%、 88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、 99%或100%同一性的氨基酸序列,基本上由其组成或由其

组成，和ii) ActRIIB多肽，其包含与SEQ ID NO:73具有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列，基本上由其组成或由其组成。在其它实施方案中，ALK7:ActRIIB异二聚体包含i) ALK7多肽，其包含与SEQ ID NO:80具有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列，基本上由其组成或由其组成，和ii)ActRIIB多肽，其包含与SEQ ID NO:78具有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列，基本上由其组成或由其组成。

[0017] 任选地，相互作用对的第一成员和/或第二成员(例如，不对称的对或非引导的相互作用对)包含免疫球蛋白的修饰的恒定结构域，包括例如，免疫球蛋白的修饰的Fc部分。例如，本公开内容的蛋白复合物可包含IgG的第一修饰Fc部分，其包含与选自SEQ ID NOs:23-37 的氨基酸序列具有至少80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％或99％同一性的氨基酸序列，基本上由其组成或由其组成；和IgG的第二修饰Fc部分，其可以与IgG的第一修饰的Fc部分的氨基酸序列相同或不同，包含与选自SEQ ID NOs:23-37 的氨基酸序列具有至少80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％或99％同一性的氨基酸序列，基本上由其组成或由其组成。在一些实施方案中，ALK7:ActRIIB异多聚体包含，基本上由其组成或由其组成:a)包含免疫球蛋白结构域的ALK7(或ActRIIB)融合蛋白，所述免疫球蛋白结构域包含与SEQ ID NO:23具有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列，任选地其中在对应于SEQ ID NO:23的残基134和177的位置处免疫球蛋白结构域包含带正电荷的氨基酸(例如，K、R或H)，和进一步任选地其中在对应于SEQ ID NO:23的残基225的位置处免疫球蛋白结构域不包含带正电荷的氨基酸(例如，K、R或H)，和b)包含免疫球蛋白结构域的ActRIIB(或ALK7)融合蛋白，所述免疫球蛋白结构域包含与SEQ ID NO:24具有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列，基本上由其组成或由其组成，任选地其中在对应于SEQ ID NO:24的残基170和187的位置处免疫球蛋白结构域包含带负电荷的(例如，D或E)氨基酸，和进一步任选地其中在对应于SEQ ID NO:24的残基225的位置处免疫球蛋白结构域包含带正电荷的氨基酸(例如，K、R或H)。在其它实施方案中， ALK7:ActRIIB异多聚体包含，基本上由其组成或由其组成:a)包含免疫球蛋白结构域的ALK7(或AetRIIB)融合蛋白，所述免疫球蛋白结构域包含与SEQ ID NO:27具有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列，任选地其中免疫球蛋白结构域包含在对应于SEQ ID NO:27的残基132的位置处的C和在对应于SEQ ID NO:27的残基144的位置处的W，和进一步任选地其中在对应于SEQ ID NO:27的残基225的位置处免疫球蛋白结构域不包含带正电荷的氨基酸(例如，K、R或H)，和b)包含免疫球蛋白结构域的ActRIIB(或ALK7)融合蛋白，所述免疫球蛋白结构域包含与SEQ ID NO:28具有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列，基本上由其组成或由其组成，任选地其中免疫球蛋白结构域包含在对应于SEQ ID NO:28的残基144的位置处的S、在对应于 SEQ ID NO:28的残基146的位

置处的A和在对应于SEQ ID NO:28 的残基185的位置处的V,和进一步任选地其中在对应于SEQ ID NO：28的残基225的位置处免疫球蛋白结构域不包含带正电荷的氨基酸 (例如,K、R或H)。

[0018] 任选地,ALK7和/或ActRIIB多肽包含一种或多种修饰的氨基酸残基,其选自:糖基化氨基酸、PEG化氨基酸、法呢基化氨基酸、乙酰基化氨基酸、生物素化氨基酸、与脂质部分缀合的氨基酸和与有机衍生剂缀合的氨基酸。ALK7和/或ActRIIB多肽可包含至少一种N-连接的糖,和可包括两种、三种或更多种N-连接的糖。这样的多肽还可包含O-连接的糖。ALK7和/或ActRIIB多肽可在以适合于患者使用的方式将蛋白糖基化的各种细胞系中产生,包括工程改造的昆虫或酵母细胞,和哺乳动物细胞例如COS细胞、CHO细胞、HEK细胞和 NSO细胞。在一些实施方案中ALK7和/或ActRIIB多肽被糖基化和具有可获自中国仓鼠卵巢细胞系的糖基化模式。优选地本公开内容的 ALK7:ActRIIB异多聚体复合物在哺乳动物(例如,小鼠或人)中显示至少4、6、12、24、36、48或72小时的血清半寿期。任选地,ALK7:ActRIIB 异多聚体在哺乳动物(例如,小鼠或人)中可显示至少6、8、10、12、 14、20、25或30天的血清半寿期。

[0019] 在某些方面,本公开内容的ALK7:ActRIIB异多聚体结合一种或多种TGF-β超家族配体。任选地,ALK7:ActRIIB异多聚体以小于或等于10^{-8}、10^{-9}、10^{-10}、10^{-11}或10^{-12}M的K_D结合一种或多种这些配体。例如,在一些实施方案中,ALK7:ActRIIB异多聚体结合活化素。在一些实施方案中,ALK7:ActRIIB异多聚体结合活化素A。在一些实施方案中,ALK7:ActRIIB异多聚体结合活化素B。在一些实施方案中,

[0020] ALK7:ActRIIB异多聚体结合活化素C。在一些实施方案中,

[0021] ALK7:ActRIIB异多聚体结合活化素AB。在一些实施方案中,

[0022] ALK7:ActRIIB异多聚体结合活化素AC。在一些实施方案中,

[0023] ALK7:ActRIIB异多聚体结合活化素BC。在一些实施方案中,

[0024] ALK7:ActRIIB异多聚体结合活化素E。在一些实施方案中,

[0025] ALK7:ActRIIB异多聚体结合活化素AE。在一些实施方案中,

[0026] ALK7:ActRIIB异多聚体结合活化素BE。在一些实施方案中,

[0027] ALK7:ActRIIB异多聚体结合GDF11。在一些实施方案中,

[0028] ALK7:ActRIIB异多聚体结合GDF8。在一些实施方案中,

[0029] ALK7:ActRIIB异多聚体结合BMP6。在一些实施方案中,

[0030] ALK7:ActRIIB异多聚体结合BMP10。在一些实施方案中,

[0031] ALK7:ActRIIB异多聚体结合BMP5。在一些实施方案中,

[0032] ALK7:ActRIIB异多聚体结合GDF3。在一些实施方案中,

[0033] ALK7:ActRIIB异多聚体结合nodal。在一些实施方案中,

[0034] ALK7:ActRIIB异多聚体不结合或基本上不结合BMP9。在一些实施方案中,ALK7:ActRIIB异多聚体以比相应的ActRIIB同多聚体更弱的亲和力结合BMP10。在一些实施方案中,ALK7:ActRIIB异多聚体以比相应的ActRIIB同多聚体更弱的亲和力结合BMP9。在一些实施方案中,ALK7:ActRIIB异多聚体以比相应的ActRIIB同多聚体更弱的亲和力结合GDF3。在一些实施方案中,ALK7:ActRIIB异多聚体以比相应的ActRIIB同多聚体更弱的亲和力结合BMP6。在一些实施方案中,ALK7:ActRIIB异多聚体以比相应的ActRIIB同多聚体更弱的亲和

力结合GDF8。在一些实施方案中,ALK7:ActRIIB异多聚体以比相应的ActRIIB同多聚体更弱的亲和力结合GDF11。在一些实施方案中, ALK7:ActRIIB异多聚体以比相应的ActRIIB同多聚体更强的亲和力结合活化素C。在一些实施方案中,ALK7:ActRIIB异多聚体以比相应的ActRIIB同多聚体更强的亲和力结合活化素AC。在一些实施方案中,ALK7:ActRIIB异多聚体以比相应的ActRIIB同多聚体更强的亲和力结合BMP5。

[0035] 一般而言,本公开内容的ALK7:ActRIIB异多聚体拮抗(抑制)至少一种TGF-β超家族配体的一种或多种活性和这样的活性改变可使用本领域已知的各种测定法测量,包括例如,基于细胞的测定法,例如本文所述的那些。在某些方面,在例如,基于细胞的测定法中

[0036] ALK7:ActRIIB异多聚体可用于抑制由一种或多种TGFβ超家族配体介导的信号传导(例如,Smad 2/3和/或Smad 1/5/8信号传导)。例如,在一些实施方案中,在基于细胞的测定法中ALK7:ActRIIB异多聚体抑制活化素信号传导。在一些实施方案中,在基于细胞的测定法中 ALK7:ActRIIB异多聚体抑制活化素A信号传导。在一些实施方案中,在基于细胞的测定法中ALK7:ActRIIB异多聚体抑制活化素B信号传导。在一些实施方案中,在基于细胞的测定法中ALK7:ActRIIB异多聚体抑制活化素C信号传导。在一些实施方案中,在基于细胞的测定法中ALK7:ActRIIB异多聚体抑制活化素AB信号传导。在一些实施方案中,在基于细胞的测定法中ALK7:ActRIIB异多聚体抑制活化素 AC信号传导。在一些实施方案中,在基于细胞的测定法中

[0037] ALK7:ActRIIB异多聚体抑制活化素BC信号传导。在一些实施方案中,在基于细胞的测定法中ALK7:ActRIIB异多聚体抑制活化素E信号传导。在一些实施方案中,在基于细胞的测定法中ALK7:ActRIIB异多聚体抑制活化素AE信号传导。在一些实施方案中,在基于细胞的测定法中ALK7:ActRIIB异多聚体抑制活化素BE信号传导。在一些实施方案中,在基于细胞的测定法中ALK7:ActRIIB异多聚体抑制 GDF11信号传导。在一些实施方案中,在基于细胞的测定法中

[0038] ALK7:ActRIIB异多聚体抑制GDF8信号传导。在一些实施方案中,在基于细胞的测定法中ALK7:ActRIIB异多聚体抑制BMP6信号传导。在一些实施方案中,在基于细胞的测定法中ALK7:ActRIIB异多聚体抑制BMP10信号传导。在一些实施方案中,在基于细胞的测定法中

[0039] ALK7:ActRIIB异多聚体抑制BMP5信号传导。在一些实施方案中,在基于细胞的测定法中ALK7:ActRIIB异多聚体可抑制GDF3信号传导。在一些实施方案中,在基于细胞的测定法中ALK7:ActRIIB异多聚体可抑制nodal信号传导。在一些实施方案中,在基于细胞的测定法中ALK7:ActRIIB异多聚体不抑制或基本上不抑制BMP9信号传导。在一些实施方案中,在基于细胞的测定法中ALK7:ActRIIB异多聚体是比相应的ActRIIB同多聚体更弱的BMP9信号传导抑制剂。在一些实施方案中,在基于细胞的测定法中ALK7:ActRIIB异多聚体是比相应的ActRIiB同多聚体更弱的BMP10信号传导抑制剂。在一些实施方案中,在基于细胞的测定法中ALK7:ActRIIB异多聚体是比相应的 ActRIIB同多聚体更弱的BMP6信号传导抑制剂。在一些实施方案中,在基于细胞的测定法中ALK7:ActRIIB异多聚体是比相应的ActRIIB 同多聚体更弱的GDF3信号传导抑制剂。在一些实施方案中,在基于细胞的测定法中ALK7:ActRIIB异多聚体是比相应的ActRIIB同多聚体更弱的GDF11信号传导抑制剂。在一些实施方案中,在基于细胞的测定法中ALK7:ActRIIB异多聚体是比相应的ActRIIB同多聚体更弱

的GDF8信号传导抑制剂。在一些实施方案中，在基于细胞的测定法中ALK7:ActRIIB异多聚体是比相应的ActRIIB同多聚体更弱的活化素A信号传导抑制剂。在一些实施方案中，在基于细胞的测定法中 ALK7:ActRIIB异多聚体是比相应的ActRIIB同多聚体更强的活化素C信号传导抑制剂。在一些实施方案中，在基于细胞的测定法中

[0040] ALK7:ActRIIB异多聚体是比相应的ActRIIB同多聚体更强的活化素 AC信号传导抑制剂。在一些实施方案中，在基于细胞的测定法中 ALK7:ActRIIB异多聚体是比相应的ActRIIB同多聚体更强的BMP5 信号传导抑制剂。

[0041] 本文所述的任何ALK7:ActRIIB异多聚体以及ALK7:ActRIIB拮抗剂可配制为药物制剂(组合物)。在一些实施方案中，药物制剂包含药学上可接受的载体。药物制剂优选地是无热原的(意思是无热原至管理治疗用产品质量的法规需要的程度)。药物制剂还可包括一种或多种另外的化合物，例如用于治疗本文所述的病症/病况的化合物。一般而言，ALK7:ActRIIB异多聚体药物制剂基本上不含ALK7和/或ActRIIB 同多聚体。例如，在一些实施方案中，ALK7:ActRIIB异多聚体药物制剂包含小于约10％、9％、8％、7％、6％、5％、4％、3％、2％或小于约1％ALK7同多聚体。在一些实施方案中，ALK7:ActRIIB异多聚体药物制剂包含小于约10％、9％、8％、7％、6％、5％、4％、3％、2％或小于约1％ActRIIB同多聚体。

[0042] 在某些方面，本公开内容提供了编码本文所述的ALK7或ActRIIB 多肽的核酸。例如，ActRIIB核酸可包含与SEQ ID NO:7的73-396 的序列或在严格性条件下与SEQ ID NO:7的核苷酸73-396的互补序列杂交的序列具有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的核酸，基本上由其组成或由其组成。这样的核酸可以是包含SEQ ID NOs:8或72的序列的核酸。在一些实施方案中， ActRIIB核酸包含与SEQ ID Nos:7、8和72的任一个具有至少70％、 75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、 94％、95％、96％、97％、98％、99％或100％同一性的核苷酸序列，基本上由其组成或由其组成。类似地，ALK7核酸可包含与SEQ ID NO： 11的61-339的序列或在严格性条件下与SEQ ID NO:11的核苷酸 61-339的互补序列杂交的序列具有至少70％、75％、80％、85％、86％、 87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、 98％、99％或100％同一性的核酸，基本上由其组成或由其组成。这样的ALK7核酸可以是包含SEQ ID NOs:12、22、41、45或75的序列的核酸。在一些实施方案中，ALK7核酸包含与SEQ ID NOs:11、12、 22、41、45或75的任一个具有至少70％、75％、80％、85％、86％、 87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、 98％、99％或100％同一性的核苷酸序列，基本上由其组成或由其组成。

[0043] 在某些方面，本公开内容提供了核酸序列，其包含ALK7多肽的编码序列和ActRIIB多肽的编码序列。例如，在一些实施方案中，本公开内容的核酸a)包含与SEQ ID NOs:11、12、21、22、40、41、44、 45或75的任一个具有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的核苷酸序列，基本上由其组成或由其组成，和b)包含与SEQ ID Nos:7、8和72的任一个具有至少70％、75％、80％、85％、 86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、 97％、98％、99％或100％同一性的核苷酸序列，基本上由其组成或由其组成。优选地，ALK7和/或ActRIIB核酸是分离和/或重组核酸。本文公开的核酸可与启动子可操作连接以表达。本公开内容进一步提供了包含这样的ALK7和/或ActRIIB多核苷酸的载体，以及包含这样的

ALK7和/或ActRIIB多核苷酸以及包含这样的ALK7和/或ActRIIB多核苷酸的载体的细胞(例如,CHO细胞),优选地自人或其它脊椎动物物种分离的细胞。

[0044] 在某些方面,ALK7多肽和/或ActRIIB多肽可在哺乳动物细胞系中表达,任选地介导ActRIIB或ALK7蛋白的合适天然糖基化以减少患者的不利免疫反应的可能性(包括兽医患者的可能性)的细胞系。已成功地使用人和CHO细胞系和预期也可使用其它常用的哺乳动物表达载体。因此本公开内容提供了包含本文公开的任何核酸的培养的细胞。这样的细胞可以是哺乳动物细胞,包括CHO细胞、NSO细胞、HEK细胞和COS细胞。其它细胞可根据预期患者的物种进行选择。其它细胞在本文公开。培养的细胞应理解为是指在实验室或其它人造条件下(例如,冷冻或在培养基中)保持和不是活生物体的一部分的细胞。

[0045] 在某些方面,本公开内容提供了制备本文所述的任何ALK7和 ActRIIB多肽以及包含这样的多肽的ALK7:ActRIIB异多聚体复合物的方法。这样的方法可包括在合适的细胞(例如,CHO细胞或COS细胞)中表达本文公开的任何核酸。例如,在一些实施方案中制备包含ALK7多肽和ActRIIB多肽的异多聚体的方法包括:a)在适合于表达 ALK7多肽和ActRIIB多肽的条件下培养细胞,其中细胞包含ALK7 多核苷酸和ActRIIB多核苷酸;和b)回收如此表达的异多聚体。或者,制备包含ALK7多肽和ActRIIB多肽的异多聚体的方法可包括: a)在适合于表达ALK7多肽的条件下培养第一细胞,其中第一细胞包含ALK7多核苷酸;b)回收如此表达的ALK7多肽;c)在适合于表达ActRIIB多肽的条件下培养第二细胞,其中第二细胞包含ActRIIB 多核苷酸;d)回收如此表达的ActRIIB多肽;e)在适合于

[0046] ALK7:ActRIIB异多聚体形成的条件下合并回收的ALK7多肽和回收的ActRIIB多肽;和f)回收ALK7:ActRIIB异多聚体。在某些实施方案中,ALK7和/或ActRIIB多肽使用TPA前导序列(例如,SEQ ID NO: 70)表达。在某些实施方案中,ALK7和/或ActRIIB多肽在CHO细胞中表达。本文所述的ALK7和ActRIIB多肽以及它们的蛋白复合物可使用用于从细胞培养物获得蛋白的任何众所周知的技术作为粗制、部分纯化或高度纯化的级分回收。一般而言,这样的方法产生基本上不含ALK7和/或ActRIIB同多聚体的ALK7:ActRIIB复合物。例如,在一些实施方案中,产生ALK7:ActRIIB异多聚体的方法产生小于约 10%、9%、8%、7%、6%、5%、4%、3%、2%或小于约1%ALK7同多聚体。在一些实施方案中,产生ALK7:ActRIIB异多聚体的方法产生小于约10%、9%、8%、7%、6%、5%、4%、3%、2%或小于约1% ActRIIB同多聚体。在一些实施方案中,产生ALK7:ActRIIB异多聚体的方法产生小于约10%、9%、8%、7%、6%、5%、4%、3%、2%或小于约1%ALK7同多聚体和在一些实施方案中,产生 ALK7:ActRIIB异多聚体的方法产生小于约10%、9%、8%、7%、6%、 5%、4%、3%、2%或小于约1%ActRIIB同多聚体。

[0047] 本公开内容进一步提供了用于治疗或预防各种活化素C-相关疾病和病况的方法和ALK7:ActRIIB异多聚体。例如,在一些实施方案中,ALK7:ActRIIB异多聚体可用于在有需要的患者中增加生育力、增加精子产生、增加生精小管容量、减少前列腺炎症、减少肝脏炎症。在一些实施方案中,ALK7:ActRIIB异多聚体可用于治疗或预防肝癌、睾丸癌或前列腺癌。在一些实施方案中,ALK7:ActRIIB异多聚体可用于减少有需要的患者的活化素C的水平(例如,血清水平)。

[0048] 本公开内容进一步提供了用于治疗或预防与例如,肾、脂肪、纤维化和其它组织有关的各种ALK7:ActRIIB-相关疾病和病况的方法和 ALK7:ActRIIB拮抗剂(例如,ALK7:

ActRIIB异多聚体)。这样的疾病或病况包括例如,慢性肾疾病或衰竭、急性肾疾病或衰竭、具有1期肾疾病的患者、具有2期肾疾病的患者、具有3期肾疾病的患者、具有4期肾疾病的患者、具有5期肾疾病的患者、非糖尿病性肾疾病、肾小球肾炎、间质性肾炎、糖尿病性肾疾病、糖尿病性肾病、肾小球硬化症、快速进行性肾小球肾炎、肾纤维化、奥尔波特综合征、IDDM肾炎、肾小球系膜增生性肾小球肾炎、膜增生性肾小球肾炎、新月体肾小球肾炎、肾间质性纤维化、局灶性节段性肾小球硬化症、膜性肾病、微小病变疾病、微量免疫快速进行性肾小球肾炎、IgA肾病、多囊性肾疾病、Dent病、肾胱氨酸病、海曼肾炎、常染色体显性(成人) 多囊性肾疾病、常染色体隐性(儿童)多囊性肾疾病、急性肾损伤、肾病综合征、肾缺血、足细胞疾病或病症,蛋白尿、肾小球疾病、膜性肾小球肾炎、局灶性节段性肾小球肾炎、先兆子痫、子痫、肾损伤、胶原血管病、良性体位性(姿势)蛋白尿、IgM肾病、膜性肾病、结节病、糖尿病、由于药物导致的肾损伤、法布里病、氨基酸尿症、范科尼综合征、高血压性肾硬化、间质性肾炎、镰状细胞疾病、血红蛋白尿、肌红蛋白尿、韦格纳肉芽肿病、糖原贮积病1型、慢性肾疾病、慢性肾衰竭、低肾小球滤过率(GFR)、肾血管硬化、狼疮肾炎、ANCA-阳性微量免疫新月体肾小球肾炎、慢性同种异体移植物肾病、肾毒性、肾中毒、肾坏死、肾损伤、肾小球和管损伤、肾功能障碍、肾炎综合征、急性肾衰竭、慢性肾衰竭、近端小管功能障碍、急性肾移植排斥、慢性肾移植排斥、非-IgA肾小球系膜增生性肾小球肾炎、传染后肾小球肾炎、伴有任何类型的肾累及的血管炎、任何遗传性肾疾病、任何间质性肾炎、肾移植衰竭、肾癌、与其它病况(例如,高血压、糖尿病和自身免疫疾病)有关的肾疾病、Dent病、肾胱氨酸病、海曼肾炎、原发性肾疾病、坍缩性肾小球病、致密沉积物病、冷球蛋白血症相关的肾小球肾炎、Henoch-Schonlein病、传染后肾小球肾炎、细菌心内膜炎、微观多脉管炎、丘-施二氏综合征、抗-GBM-抗体介导的肾小球肾炎、淀粉样变性病、单克隆免疫球蛋白沉积病、原纤维肾小球肾炎、免疫触须样肾小球病、缺血性肾小管损伤、药物-诱导的肾小管-间质性肾炎、毒性肾小管-间质性肾炎、感染性肾小管-间质性肾炎、细菌肾盂肾炎、自多瘤病毒感染或HIV感染产生的病毒感染性肾小管-间质性肾炎、代谢-诱导的肾小管-间质性疾病、混合结缔组织病、管型肾病、可自尿酸或草酸或药物-诱导的晶体沉积产生的晶体肾病、急性细胞肾小管-间质性同种异体移植物排斥、自淋巴瘤或移植后淋巴增生性疾病产生的肿瘤侵润疾病、肾的阻塞性疾病、血管疾病、血栓形成性微血管病、肾血管硬化、动脉粥样化栓塞疾病、混合结缔组织病、结节性多动脉炎、神经钙蛋白-抑制剂诱导的血管疾病、急性细胞血管同种异体移植物排斥、急性体液同种异体移植物排斥、早期肾功能减退(ERFD)、晚期肾疾病(ESRD)、肾静脉血栓症、急性肾小管坏死、肾阻塞、急性间质性肾炎、确立的慢性肾疾病、肾动脉狭窄、缺血性肾病、尿毒症、药物和毒素-诱导的慢性肾小管间质性肾炎、逆流性肾病、肾结石、古德帕斯丘综合征、正细胞性正色素性贫血、肾贫血、糖尿病性慢性肾疾病、IgG4-相关疾病、冯希-林二氏综合征、结节性硬化症、肾消耗病、髓状囊性肾疾病、肾细胞癌、腺癌、肾胚细胞瘤、淋巴瘤、白血病、低唾液酸化病症、慢性环孢霉素肾病、肾再灌注损伤、肾发育不良、氮质血症、双侧动脉闭塞、急性尿酸肾病、血容量不足、急性双侧梗阻性尿路病、血钙过高肾病、溶血性尿毒症综合征、急性尿潴留、恶性肾硬化、产后肾小球硬化症、硬皮病、非-古德帕斯丘抗 -GBM病、微观结节性多动脉炎、过敏性肉芽肿病、急性放射性肾炎、链球菌感染后肾小球肾炎、瓦尔登斯特伦氏巨球蛋白血症、镇痛剂肾病、动静脉瘘、动静脉移植、透析、异位肾、髓质海绵肾、肾性骨营养不良、孤立肾、肾积水、微白蛋白尿、尿毒症、血尿、高脂血症、低白

蛋白血症、脂肪尿、酸中毒和血钾过多。在一些实施方案中，本公开内容进一步提供了用于延迟或预防从1期至2期肾疾病、2期至3期肾疾病、3期至4期肾疾病或4期至5期肾疾病进展的方法和 ALK7:ActRIIB拮抗剂(例如，ALK7:ActRIIB异多聚体)。在一些实施方案中，本公开内容进一步提供了用于预防或减少肾炎症的方法和 ALK7:ActRIIB拮抗剂(例如，ALK7:ActRIIB异多聚体)。在一些实施方案中，本公开内容进一步提供了用于预防或减少肾损伤的方法和 ALK7:ActRIIB拮抗剂(例如，ALK7:ActRIIB异多聚体)。在一些实施方案中，本公开内容进一步提供了用于预防或减少肾纤维化的方法和 ALK7:ActRIIB拮抗剂(例如，ALK7:ActRIIB异多聚体)。

[0049] 在一些实施方案中，本公开内容进一步提供了在有需要的受试者中用于脂解活性的方法和ALK7:ActRIIB拮抗剂(例如，ALK7:ActRIIB 异多聚体)。在一些实施方案中，本公开内容进一步提供了在有需要的受试者中用于降低体脂肪含量或减少体脂肪含量的增加速率的方法和ALK7:ActRIIB拮抗剂(例如，ALK7:ActRIIB异多聚体)。在一些实施方案中，本公开内容进一步提供了在受试者中用于治疗与不需要的体重增加有关的病症或病况的方法和ALK7:ActRIIB拮抗剂(例如， ALK7:ActRIIB异多聚体)。这样的病症包括例如，肥胖(例如，腹部肥胖)；超重；胰岛素抵抗；代谢综合征和其它代谢疾病或病况；脂质病症例如，低HDL水平、高LDL水平、高脂血症、高甘油三酯血症或血脂障碍；脂蛋白异常；甘油三酯减少；脂肪性肝病；非-酒精性脂肪性肝病；高血糖症；葡萄糖耐量受损(IGT)；高胰岛素血症；高胆固醇 (例如，高LDL水平和/或高胆固醇血症)；心血管疾病例如，心脏病包括冠心病、充血性心力衰竭、动脉粥样硬化；动脉硬化和/或高血压； X综合征；血管再狭窄；神经病；和/或与一种或多种上述疾病或病况和/或超重(例如，BMI≥25kg/m^2)或太多体脂肪有关的其它病症/病况。在一些实施方案中，本公开内容提供了在受试者中减少体脂肪含量或减少体脂肪含量的增加速率的方法，包括给予有需要的受试者有效量的ALK7:ActRIIB拮抗剂或ALK7:ActRIIB拮抗剂的组合。在一些实施方案中，本公开内容提供了在受试者中治疗与不需要的体重增加有关的病症的方法，包括给予有需要的受试者有效量的ALK7:ActRIIB 拮抗剂或ALK7:ActRIIB拮抗剂组合。在一些实施方案中，在受试者中与不需要的体重增加有关的病症选自：肥胖、非-胰岛素依赖性糖尿病(NIDDM)、心血管疾病、高血压、骨关节炎、中风、呼吸问题和胆囊疾病。在一些实施方案中，本公开内容提供了在患者中减少胆固醇和/或甘油三酯的方法，包括给予有需要的患者有效量的 ALK7:ActRIIB拮抗剂或ALK7:ActRIIB拮抗剂的组合。

[0050] 在一些实施方案中，本公开内容进一步提供了用于治疗和/或改善癌症或与癌症有关的病况的方法和ALK7:ActRIIB拮抗剂(例如， ALK7:ActRIIB异多聚体)。在一些实施方案中，受试者具有选自黑素瘤、乳腺癌、结肠癌和子宫内膜癌、胰腺癌、胃癌和子宫癌的癌症。在一些实施方案中，受试者具有骨髓瘤(例如，多发性骨髓瘤、浆细胞瘤、局部骨髓瘤和髓外骨髓瘤)。在一些实施方案中，ALK7:ActRIIB 拮抗剂经给予以治疗或预防淋巴转移、血流转移、肿瘤生长或肿瘤侵袭。

[0051] 在一些实施方案中，本公开内容进一步提供了在有需要的患者中用于治疗或预防纤维化或与纤维化有关的病症或病况的方法和ALK7:ActRIIB拮抗剂(例如，ALK7:ActRIIB异多聚体)。这样的病症或病况包括例如，肺纤维化、超敏感性肺炎、特发性纤维化、肺结核、肺炎、囊性纤维化、哮喘、慢性阻塞性肺病(COPD)、肺气肿、肾(肾脏)纤维化、肾(肾脏)衰竭、

慢性肾(肾脏)疾病、骨纤维化、骨髓纤维化、类风湿性关节炎、系统性红斑狼疮、硬皮病、结节病、伴有多脉管炎的肉芽肿病、佩伦涅氏病、肝脏纤维化、威尔逊氏病、糖原贮积病(特别是III、IV、IX和X型)、铁-过载、戈谢病、泽韦格综合征、非酒精性和酒精性脂肪性肝炎、胆汁性肝硬化、硬化性胆管炎、巴-希二氏综合征、手术相关的纤维化、克罗恩病、掌挛缩、纵隔纤维化、肾原性纤维化、腹膜后纤维化、心房纤维化、心内膜心肌纤维化、胰脏纤维化。

[0052] 附图简述

[0053] 图1A和1B显示了包含I型受体和II型受体多肽的异聚蛋白复合物的两个示意性实例。图1A描述包含一个I型受体融合多肽和一个 II型受体融合多肽的异二聚体蛋白复合物,它们可通过各多肽链内包含的多聚化结构域共价或非共价装配。两个装配的多聚化结构域构成相互作用对,其可以是引导的或非引导的。图1B描述包含图1A描述的两个异二聚体复合物的异四聚体蛋白复合物。可预期更高级的复合物。

[0054] 图2显示了包含I型受体多肽(显示为“I”)(例如,与来自人或其它物种例如本文所述的那些的ALK7蛋白的细胞外结构域,例如,SEQ ID Nos:9、10、19、20、38、39、42、43、46、74、76、79和80具有至少70%、75%、80%、85%、90%、91%、92%、93%、94%、95%、 97%、98%、99%或100%同一性的多肽)和II型受体多肽(显示为“II”) (例如,与来自人或其它物种例如本文所述的那些的ActRIIB蛋白的细胞外结构域,例如,SEQ ID Nos:1、2、3、4、5、6、71、73、77和 78具有至少70%、75%、80%、85%、90%、91%、92%、93%、94%、 95%、97%、98%、99%或100%同一性的多肽)的异聚蛋白复合物的示意性实例。在该说明性的实施方案中,I型受体多肽是包含相互作用对的第一成员(“C_1”)的融合多肽的一部分,和II型受体多肽是包含相互作用对的第二成员(“C_2”)的融合多肽的一部分。在各融合多肽中,接头可位于I型或II型受体多肽和相互作用对的相应成员之间。相互作用对的第一和第二成员可以是引导的(不对称的)对,意思是该对的成员优先彼此缔合,而非自缔合,或相互作用对可以是非引导的,意思是该对的成员可彼此缔合或自缔合,没有实质的偏好,和可具有相同的或不同的氨基酸序列。传统的Fc融合蛋白和抗体是非引导的相互作用对的实例,而各种工程改造的Fc结构域已被设计为引导的(不对称的)相互作用对[例如,Spiess等(2015) Molecular Immunology 67(2A):95-106]。

[0055] 图3显示人ActRIIA(SEQ ID NO:49和人ActRIIB(SEQ ID NO:2) 的细胞外结构域的比对,其中根据多个ActRIIB和ActRIIA晶体结构的复合分析,本文推论直接接触配体的残基用框盒表示。

[0056] 图4显示不含其细胞内结构域的各种脊椎动物ActRIIB前体蛋白 (SEQ ID NOs:50-55)、不含其细胞内结构域的人ActRIIA前体蛋白 (SEQ ID NO:56)和共有序列ActRII前体蛋白(SEQ ID NO:57)的多个序列比对。

[0057] 图5显示使用Clustal 2.1,人IgG同种型的Fc结构域的多个序列比对。铰链区用虚下划线表示。双下划线表示在IgG1 Fc中经工程改造以促进不对称的链配对的位置和关于其它同种型IgG2、IgG3和 IgG4的相应位置的实例。

[0058] 图6显示ALK7-Fc:ActRIIB-Fc异二聚体蛋白复合物与ActRIIB-Fc 同二聚体和ALK7-Fc同二聚体比较的比较性配体结合数据。对于各蛋白复合物,配体根据k_{off}(与配体信号传导抑制良好相关的动力学常数) 排列,和以结合亲和力递减顺序列出(最紧密结合的配体列于最上方)。在左侧,黄色、红色、绿色和蓝色线表示离解速率常数的幅度。黑色实线

表示与同二聚体比较，与异二聚体的结合增强或未改变的配体，而红色虚线表示与同二聚体比较，结合显著减少。如所示的，与 ActRIIB-Fc同二聚体强结合的5种配体中的4种(活化素A、BMP10、GDF8和GDF11)显示与ActRIIB-Fc:ALK7-Fc异二聚体结合减少，除了活化素B，其保留与异二聚体的紧密结合。类似地，与ActRIIB-Fc 同二聚体中等结合的4种配体中的3种(GDF3、BMP6和特别是BMP9) 显示与ActRIIB-Fc:ALK7-Fc异二聚体结合减少，而与活化素AC的结合增加，成为总体上与异二聚体第二强的配体相互作用。最后，活化素C和BMP5出人意料地以中等强度结合ActRIIB-Fc:ALK7异二聚体，但与ActRIIB-Fc同二聚体没有结合(活化素C)或弱结合(BMP5)。未测试配体与ALK7-Fc同二聚体的结合。

[0059] 图7显示源自各个脊椎动物物种的ALK7细胞外结构域的多个序列比对(SEQ ID NOs:59-64)。

[0060] 图8A-8D显示包含ALK7多肽(例如，与来自人或其它物种例如本文所述的那些的ALK7蛋白的细胞外结构域、例如SEQ ID Nos:9、10、19、20、38、39、42、43、46、74、76、79和80具有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、97％、98％、99％或100％同一性的多肽)和ActRIIB多肽(例如，与来自人或其它物种例如本文所述的那些的ActRIIB蛋白的细胞外结构域，例如，SEQ ID Nos:1、2、3、4、5、6、71、73、77和78具有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、97％、98％、99％或100％同一性的多肽)的异聚蛋白复合物的示意性实例。在该说明性的实施方案中，ALK7多肽是包含相互作用对的第一成员(“C_1”)的融合多肽的一部分，和ActRIIB多肽是包含相互作用对的第二成员(“C_2”)的融合多肽的一部分。合适的相互作用对包括例如，重链和/或轻链免疫球蛋白相互作用对、其截短和变体，例如本文所述的那些[例如，Spiess等(2015) Molecular Immunology 67(2A):95-106]。在各融合多肽中，接头可位于 ALK7或ActRIIB多肽和相互作用对的相应成员之间。相互作用对的第一和第二成员可以是非引导的，意味着该对的成员可彼此缔合或自缔合，没有实质偏好，和它们可具有相同的或不同的氨基酸序列。参见图8A。或者，相互作用对可以是引导的(不对称的)对，意味着该对的成员优先彼此缔合而不是自缔合。参见图8B。可预期更高级的复合物。参见图8C和8D。

[0061] 图9A-9G显示包含两个ALK7多肽(例如，与来自人或其它物种例如本文所述的那些的ALK7蛋白的细胞外结构域，例如，SEQ ID Nos：9、10、19、20、38、39、42、43、46、74、76、79和80独立地具有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、97％、98％、99％或100％同一性的多肽)和两个ActRIIB多肽(例如，与来自人或其它物种例如本文所述的那些的ActRIIB蛋白的细胞外结构域，例如，SEQ ID Nos:1、2、3、4、5、6、71、73、77和78独立地具有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、97％、98％、99％或100％同一性的两个多肽)的异聚蛋白复合物的示意性实例。

[0062] 在该说明性的实施方案9A中，第一ALK7多肽(从左至右)是包含相互作用对的第一成员(“C_1”)和进一步包含另外的相互作用对的第一成员(“A_1”)的融合多肽的一部分；和第二ALK7多肽是包含相互作用对的第二成员(“C_2”)和进一步包含相互作用对的第一成员(“A_2”)的融合多肽的一部分。第一ActRIIB多肽(从左至右)是包含相互作用对的第二成员(“B_1”)的融合多肽的一部分；和第二ActRIIB多肽是包含相互作用对的第二成员(“B_2”)的融合多肽的一部分。A_1和A_2可以是相同的或不同的；B_1和B_2可以是相同的或不同的，和C_1和C_2可以是相同的或不同的。在各融合多肽中，接头可位于ALK7或ActRIIB多肽和相互作用对的相

应成员之间以及相互作用对之间。图9A是非引导的相互作用对的缔合的实例，意味着该对的成员可彼此缔合或自缔合，没有实质偏好，和可具有相同的或不同的氨基酸序列。

[0063] 在该说明性的实施方案9B中，第一ActRIIB多肽(从左至右)是包含相互作用对的第一成员(“C_1”)和进一步包含另外的相互作用对的第一成员(“A_1”)的融合多肽的一部分；和第二ActRIIB多肽是包含相互作用对的第二成员(“B_2”)的融合多肽的一部分。第一ALK7多肽(从左至右)是包含相互作用对的第二成员(“B_1”)的融合多肽的一部分；和第二ALK7多肽是包含相互作用对的第二成员(“C_2”)和进一步包含相互作用对的第一成员(“A_2”)的融合多肽的一部分。在各融合多肽中，接头可位于ALK7或ActRIIB多肽和相互作用对的相应成员之间以及相互作用对之间。图9B是引导的(不对称的)相互作用对的缔合的实例，意味着该对的成员优先彼此缔合而不是自缔合。

[0064] 合适的相互作用对包括例如，重链和/或轻链免疫球蛋白相互作用对、其截短和变体，如本文所述的[例如，Spiess等(2015)Molecular Immunology 67(2A):95-106]。可预期更高级的复合物。参见图9C-9F。使用类似的方法，特别是使用轻链和/或重链免疫球蛋白、其截短或变体的那些，相互作用对可用于产生ALK7:ActRIIB异二聚体，其类似于抗体Fab和$F(ab')_2$复合物[例如，Spiess等(2015)Molecular Immunology 67(2A):95-106]。参见图9G。

[0065] 图10A和10B显示包含ALK7多肽(例如，与来自人或本文所述的其它物种的ALK7蛋白的细胞外结构域，例如，SEQ ID Nos:9、10、19、20、38、39、42、43、46、74、76、79和80具有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、97％、98％、99％或100％同一性的多肽)、ActRIIB多肽(例如，与来自人或其它物种例如本文所述的那些的ActRIIB蛋白的细胞外结构域，例如，SEQ ID Nos：1、2、3、4、5、6、71、73、77和78具有至少70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、97％、98％、99％或100％同一性的多肽)和抗体的配体-结合结构域(例如，源自结合一种或多种 ALK7:ActRIIB-结合配体的抗体的配体-结合结构域)的异聚蛋白复合物的示意性实例。在该说明性的实施方案中，ALK7多肽是包含相互作用对的第一成员(“C_1”)和进一步包含另外的相互作用对的第一成员(“A_1”)的融合多肽的一部分。ActRIIB多肽是包含相互作用对的第二成员(“B_1”)的融合多肽的一部分。可变重链(V_H)多肽是包含相互作用对的第二成员(“C_2”)和进一步包含相互作用对的第一成员(“A_2”)的融合多肽的一部分。可变重链(V_L)多肽是包含相互作用对的第二成员(“B_2”) 的融合多肽的一部分。在各融合多肽中，接头可位于ALK7或ActRIIB 多肽和相互作用对的相应成员之间、相互作用对之间和V_H和V_L多肽和相互作用对的成员之间。A_1和A_2可以是相同的或不同的；B_1和B_2可以是相同的或不同的和C_1和C_2可以是相同的或不同的。合适的相互作用对包括例如，恒定重链和/或轻链免疫球蛋白相互作用对、其截短和变体，如本文所述的[例如，Spiess等(2015)Molecular Immunology 67(2A):95-106]。图10A是引导的(不对称的)相互作用对的缔合的实例，意味着该对的成员优先彼此缔合而不是自缔合。图10B是非引导的相互作用对的缔合的实例，意味着该对的成员可彼此缔合或自缔合，没有实质偏好和可具有相同的或不同的氨基酸序列。

[0066] 这样的抗体-ALK7:ActRIIB复合物可用于其中需要进一步结合/ 拮抗并非ALK7:ActRIIB配体的试剂的情况。或者，这样的抗体 -ALK7:ActRIIB复合物可用于其中需要进一步增强ALK7:ActRIIB配体结合/拮抗作用的情况。例如，如本文的实施例所证实的， ALK7:

ActRIIB异二聚体对数种配体具有高亲和力,包括例如,活化素B和活化素AC。此外,ALK7:ActRIIB异二聚体结合GDF3,但亲和力较弱。在其中除了一种或多种高亲和力-结合配体(例如,活化素B 和活化素AC)之外还需要拮抗GDF3活性的某些情况下,GDF3可被排除与ALK7:ActRIIB异二聚体的结合。在这些情况下,除了一种或多种较高亲和力ALK7:ActRIIB结合-配体(例如,活化素B和活化素 AC)之外,添加抗-GDF3抗体的结合结构域至ALK7:ActRIIB异多聚体复合物还将改进蛋白复合物拮抗GDF3的能力。

[0067] 图11显示ALK7:ActRIIB单-陷阱(trap)多肽的示意性实例。ALK7:ActRIIB单-陷阱多肽可包含具有相同的或不同的序列的多个 ALK7结构域(例如,1、2、3、4、5、6、7、8、9、10或更多个结构域),和具有相同的或不同的序列的多个ActRIIB结构域(例如,1、2、 3、4、5、6、7、8、9、10或更多个结构域)。这些ALK7和ActRIIB 结构域可以任何顺序排列和在一个或多个ALK7和ActRIIB结构域之间可包含一个或多个接头结构域位置。这样的配体陷阱(ligand trap)可用作治疗或预防本文所述的疾病或病症的治疗剂。

[0068] 图12A-12D显示包含至少一个ALK7:ActRIIB单链陷阱多肽的多聚体蛋白复合物的示意性实例。在该说明性的实施方案12A和12B中,第一ALK7:ActRIIB单链陷阱多肽(从左至右)是包含相互作用对的第一成员(“C_1”)的融合多肽的一部分;和第二ALK7:ActRIIB单链陷阱多肽是包含相互作用对的第二成员(“C_2”)的融合多肽的一部分。C_1和C_2可以是相同的或不同的。第一和第二ALK7:ActRIIB单链陷阱多肽可以是相同的或不同的。在各融合多肽中,接头可位于ALK7:ActRIIB 单链陷阱多肽和相互作用对的相应成员之间。合适的相互作用对包括例如,重链和/或轻链免疫球蛋白相互作用对、其截短和变体,如本文所述的[例如,Spiess等(2015)Molecular Immunology 67(2A):95-106]。图12A是非引导的相互作用对的缔合的实例,意味着该对的成员可彼此缔合或自缔合,没有实质偏好和可具有相同的或不同的氨基酸序列。图12B是引导的(不对称的)相互作用对的缔合的实例,意味着该对的成员优先彼此缔合而不是自缔合。可预期更高级的复合物。此外,这样的ALK7:ActRIIB单链陷阱多肽可类似地与一种或多种ALK7多肽和/或一种或多种ActRIIB多肽共价或非共价缔合。参见图12C。此外,这样的ALK7:ActRIIB单链陷阱多肽可类似地与抗体的一种或多种配体-结合结构域(例如,结合一种或多种ALK7:ActRIIB结合配体的抗体的配体-结合结构域)共价或非共价缔合。参见图12D。

[0069] 图13A-13C显示来自经过单侧输尿管梗阻(UUO)的小鼠肾的纤维化基因(Colla1、Col3a1、纤连蛋白、PAI-1、CTGF和a-SMA)、炎性基因(Tnfa和MCP1)、细胞因子基因(Tgfb1、Tgfb2、Tgfb3和活化素 A)、肾损伤基因(NGAL)、缺氧-可诱导因子1-α(HIFla)和活化素A受体(Acvr2A)的基因表达谱。来自对侧的、非-手术肾的样品用作对照 (Ctrl)。在手术后3天和17天获得基因表达谱。在手术后第3、7、10 和14天小鼠被给予PBS或ALK7-Fc:ActRIIB-Fc同二聚体。使用单向 ANOVA接着Tukey分析进行统计学。(*)表示在给予 ALK7-Fc:ActRIIB-Fc同二聚体的小鼠中i)在3天时对照样品与UUO 肾比较或ii)在17天时对照样品与UUO肾比较的统计学差异。($)表示在17天时在仅给予PBS的小鼠中UUO肾与在17天时在给予 ALK7-Fc:ActRIIB-Fc同二聚体的小鼠中UUO肾比较的统计学差异。(@)表示未检出转录物。

[0070] 发明详述

[0071] 1.综述

[0072] 部分地,本公开内容涉及包含TGFβ超家族I型受体多肽和TGFβ超家族II型受体多

肽的异多聚体、其用途和制备这样的异多聚体的方法。参见例如，图1和2。在某些优选的实施方案中，异多聚体包含 TGFβ超家族I型受体多肽的细胞外结构域和TGFβ超家族II型受体多肽的细胞外结构域。特别地，本公开内容提供了包含ALK7多肽和 ActRIIB多肽的异多聚体。优选地，ALK7多肽包含ALK7受体的配体-结合结构域和ActRIIB多肽包含ActRIIB受体的配体-结合结构域。在某些优选的实施方案中，与同多聚体的相应样品比较， ALK7:ActRIIB异多聚体(例如，与ActRIIB:ActRIIB同二聚体或 ALK7:ALK7同二聚体比较，ALK7:ActRIIB异二聚体)具有改变的 TGFβ超家族配体结合特征/特异性。

[0073] TGF-β超家族包含超过30种分泌的因子，包括TGF-β、活化素、 nodal、骨形态发生蛋白(BMP)、生长和分化因子(GDF)和抗苗勒管激素(AMH)[Weiss等(2013)Developmental Biology，2(1)：47-63]。存在于脊椎动物和无脊椎动物二者中的该超家族的成员，在各种组织中广泛表达和在整个动物寿命的最早发育阶段期间发挥功能。事实上， TGF-β超家族蛋白是干细胞自我更新、原肠胚形成、分化、器官形态发生和成年组织体内稳态的重要介质。与这种广泛的活性一致，异常的TGF-β超家族信号传导与广泛的人病理学有关，包括例如，自身免疫疾病、心血管疾病、纤维化疾病和癌症。

[0074] TGF-β超家族的配体共有相同的二聚体结构，其中一个单体的中心3-1/2转角螺旋紧靠另一单体的β-链形成的凹面堆积。大部分TGF-β家族成员通过分子间二硫键进一步稳定。该二硫键穿过由两个其它二硫键形成的环，产生所谓的“半胱氨酸结”基序[Lin等(2006) Reproduction 132:179-190和Hinck等(2012)FEBS Letters 586： 1860-1870]。

[0075] TGF-β超家族信号传导由I型和II型丝氨酸/苏氨酸激酶受体的异聚复合物介导，在配体刺激后其磷酸化和活化下游SMAD蛋白(例如，SMAD蛋白1、2、3、5和8)[Massagué(2000)Nat.Rev.Mol.Cell Biol. 1:169-178]。这些I型和II型受体是跨膜蛋白，由具有富含半胱氨酸的区域的配体-结合细胞外结构域、跨膜结构域和具有预期的丝氨酸/苏氨酸激酶特异性的胞质结构域构成。一般而言，I型受体介导细胞内信号传导，而II型受体是结合TGF-β超家族配体所需要的。在配体结合后I型和II受体形成稳定的复合物，导致I型受体被II型受体磷酸化。

[0076] 基于它们结合的I型受体和它们活化的Smad蛋白，TGF-β家族可分为两个系统发生分支。一个是较近进化的分支，其包括例如， TGF-β、活化素、GDF8、GDF9、GDF11、BMP3和nodal，其通过活化Smads 2和3的I型受体发出信号[Hinck(2012)FEBS Letters 586:1860-1870]。另一分支包括较远相关的超家族蛋白，和包括例如， BMP2、BMP4、BMP5、BMP6、BMP7、BMP8a、BMP8b、BMP9、 BMP10、GDF1、GDF5、GDF6和GDF7，其通过Smads 1、5和8发出信号。

[0077] TGF-β同种型是TGF-β超家族的基础性成员，其中在哺乳动物中存在3个已知同种型，称为TGF-β1、TGF-β2和TGF-β3。成熟的生物活性TGF-β配体作为同二聚体起作用，和主要通过I型受体ALK5发出信号，但还已发现在内皮细胞中通过ALK1另外发出信号[Goumans 等(2003)Mol Cell 12(4)：817-828]。TGF-β1是最丰富和遍在表达的同种型。已知TGF-β1在伤口愈合申具有重要的作用，和表达组成型活性的TGF-β1转基因的小鼠发生纤维化[Clouthier等，(1997)J Clin. Invest.100(11)：2697-2713]。TGF-β1还参与T细胞活化和T调节细胞的维持[Li等，(2006)Immunity 25(3)：455-471]。TGF-β2表达首先描述于人成胶质细胞瘤细胞中，和在胚胎神经系统的神经元和星形胶质细胞中发生。已知TGF-β2抑制T淋巴细胞的白细胞介素-2-依赖性生长。 TGF-β3最初从人横纹肌肉瘤细胞系分离，后来在肺

腺癌和肾癌细胞系中发现。已知TGF-β3对于腭和肺形态发生是重要的[Kubiczkova等，(2012)Journal of Translational Medicine 10:183]。

[0078] 活化素是TGF-β超家族的成员，并且最初作为促卵泡激素分泌的调节物发现，但随后表征了各种生殖和非-生殖作用。有三种主要的活化素形式(A、B和AB)，其是两个紧密相关的β亚基的同/异二聚体(分别为$\beta_A\beta_A$、$\beta_B\beta_B$和$\beta_A\beta_B$)。人基因组还编码活化素C和活化素E，其主要在肝脏中表达，和包含β_C或β_E的异二聚体形式也是已知的。在 TGF-β超家族中，活化素是独特和多功能的因子，其可在卵巢和胎盘细胞中刺激激素产生，支持神经元细胞存活，根据细胞类型正面或负面影响细胞周期进展，和至少在两栖动物胚胎中诱导中胚层分化[DePaolo等(1991)Proc Soc Ep Biol Med.198:500-512;Dyson等(1997) Curr Biol.7:81-84;和Woodruff(1998)Biochem Pharmacol.55:953-963]。在数种组织中，活化素信号传导被其相关的异二聚体抑制素拮抗。例如，在从脑垂体的促卵泡激素(FSH)分泌的调节中，活化素促进FSH 合成和分泌，而抑制素减少FSH合成和分泌。可调节活化素生物活性和/或结合活化素的其它蛋白包括卵泡抑素(FS)、卵泡抑素-相关蛋白 (FSRP，亦称为FLRG或FSTL3)和α_2-巨球蛋白。

[0079] 本文所述的结合“活化素A”的试剂是特异性结合β_A亚基的试剂，无论是在分离的β_A亚基的情况下还是作为二聚体复合物(例如，$\beta_A\beta_A$同二聚体或$\beta_A\beta_B$异二聚体)。在异二聚体复合物(例如，$\beta_A\beta_B$异二聚体) 的情况下，结合“活化素A”的试剂对β_A亚基内存在的表位是特异性的，但不结合复合物的非-β_A亚基(例如，复合物的β_B亚基)内存在的表位。类似地，本文公开的拮抗(抑制)“活化素A”的试剂是抑制由β_A亚基介导的一种或多种活性的试剂，无论是在分离的β_A亚基的情况下还是作为二聚体复合物(例如，$\beta_A\beta_A$同二聚体或$\beta_A\beta_B$异二聚体)。在$\beta_A\beta_B$异二聚体的情况下，抑制“活化素A”的试剂是特异性抑制β_A亚基的一种或多种活性，但不抑制复合物的非-β_A亚基(例如，复合物的β_B亚基)的活性的试剂。该原理也适用于结合和/或抑制“活化素B”、“活化素C”和“活化素E”的试剂。本文公开的拮抗“活化素AB”的试剂是抑制由β_A亚基介导的一种或多种活性和由β_B亚基介导的一种或多种活性的试剂。相同的原理也适用于结合和/或抑制“活化素AC”、“活化素AE”、“活化素BC”或“活化素BE”的试剂。

[0080] Nodal蛋白在中胚层和内胚层诱导和形成，以及中轴结构例如心脏和胃在早期胚胎发生中的随后组建中具有功能。已经证实，在发育的脊椎动物胚胎中背侧组织主要促进脊索和脊索前板的中轴结构，同时它募集周围的细胞形成非-中轴胚胎结构。Nodal显示通过I型和II 型受体二者，和称为SMAD蛋白的细胞内效应物发出信号。研究支持 ActRIIA和ActRIIB用作Nodal的II型受体的观点[Sakuma等(2002) Genes Cells.2002,7:401-12]。表明Nodal配体与它们的辅因子(例如， Crypto或Cryptic)相互作用，以活化活化素I型和II型受体，其将 SMAD2磷酸化。Nodal蛋白参与对早期脊椎动物胚胎关键的许多事件，包括中胚层形成、前部图式发育和左-右轴特化。实验证据已证实nodal 信号传导活化pAR3-Lux，一种萤光素酶报告物，其之前显示对活化素和TGF-β特异性响应。然而，nodal不能诱导pTlx2-Lux，一种对骨形态发生蛋白特异性响应的报告物。最近的结果提供了直接的生物化学证据，即Nodal信号传导由SMAD2和SMAD3介导，其也介导TGF-β和活化素的信号传导。进一步的证据表明，细胞外蛋白Cripto或Cryptic 对于nodal信号传导是需要的，使得它与活化素或TGF-β信号传导不同。

[0081] BMP和GDF一起形成半胱氨酸-结细胞因子的家族，其共有 TGF-β超家族的特征性

折叠[Rider等(2010)Biochem J.,429(1):1-12]。该家族包括例如,BMP2、BMP4、BMP6、BMP7、BMP2a、BMP3、 BMP3b(亦称为GDF10)、BMP4、BMP5、BMP6、BMP7、BMP8、 BMP8a、BMP8b、BMP9(亦称为GDF2)、BMP10、BMP11(亦称为 GDF11)、BMP12(亦称为GDF7)、BMP13(亦称为GDF6)、BMP14(亦称为GDF5)、BMP15、GDF1、GDF3(亦称为VGR2)、GDF8(亦称为肌抑素)、GDF9、GDF15和decapentaplegic。除了诱导骨形成的能力(这给予BMP其名称)之外,BMP/GDF还显示在各种组织的发育中的形态发生活性。BMP/GDF同和异二聚体与I型和II型受体二聚体的组合相互作用,以产生多种可能的信号传导复合物,导致两个竞争性的 SMAD转录因子组之一的活化。BMP/GDF具有高度特异性和局部的功能。这些以许多方式受到调节,包括BMP/GDF表达的发育限制,和通过数种特定的BMP拮抗剂蛋白的分泌,所述蛋白以高亲和力结合所述细胞因子。奇特的是,这些拮抗剂中许多类似于TGF-β超家族配体。

[0082] 生长和分化因子-8(GDF8)亦称为肌抑素。GDF8是骨骼肌肉质量的负调节剂,和在发育和成年骨骼肌肉中高度表达。在转基因小鼠中 GDF8无效突变的特征为骨骼肌肉的显著肥大和增生[McPherron等, Nature(1997)387:83-90]。在牛中以及出人意料地在人中,在天然存在的GDF8突变中骨骼肌肉质量的类似增加是明显的[Ashmore等(1974) Growth,38:501-507;Swatland和Kieffer,J.Anim.Sci.(1994) 38:752-757;McPherron和Lee,Proc.Natl.Acad.Sci.USA(1997) 94:12457-12461;Kambadur等,Genome Res.(1997)7:910-915;和 Schuelke等(2004)N EnglJ Med,350:2682-8]。研究已经表明,在人中与HIV-感染有关的肌肉消瘦伴随有GDF8蛋白表达的增加 [Gonzalez-Cadavid等,PNAS(1998)95:14938-43]。此外,GDF8可调节肌肉-特异性的酶(例如,肌酸激酶)的产生和调节成肌细胞细胞增殖 [国际专利申请公布号WO 00/43781]。GDF8前肽可非共价结合成熟的 GDF8结构域二聚体,灭活其生物学活性[Miyazono等(1988)J.Biol. Chem.,263:6407-6415;Wakefield等(1988)J.Biol.Chem.,263; 7646-7654;和Brown等(1990)Growth Factors,3:35-43]。结合GDF8 或结构相关蛋白和抑制它们的生物学活性的其它蛋白包括卵泡抑素,和可能地,卵泡抑素-相关蛋白[Gamer等(1999)Dev.Biol.,208: 222-232]。

[0083] GDF11,亦称为BMP11,是一种在小鼠发育期间在尾芽、肢芽、上颌和下颌弓和背根神经节中表达的分泌蛋白[McPherron等(1999) Nat.Genet.,22:260-264;和Nakashima等(1999)Mech.Dev.,80: 185-189]。GDF11在中胚层和神经组织二者的图式发育中起独特的作用[Gamer等(1999)Dev Biol.,208:222-32]。GDF11已表明是在鸡翼发育中软骨发生和肌生成的负调节剂[Gamer等(2001)Dev Biol., 229:407-20]。GDF11在肌肉中的表达也表明其以类似于GDF8的方式在调节肌肉生长中的作用。此外,GDF11在脑中的表达表明GDF11 还可具有与神经系统的功能有关的活性。感兴趣的是,发现GDF11 在嗅上皮中抑制神经发生[Wu等(2003)Neuron.,37:197-207]。因此, GDF11的抑制剂可具有在治疗疾病例如肌肉疾病和神经变性疾病(例如,肌萎缩侧索硬化)中的体外和体内应用。

[0084] BMP7,亦称为成骨蛋白-1(OP-1),众所周知诱导软骨和骨形成。此外,BMP7调节各种生理学过程。例如,BMP7可以是负责上皮骨发生的现象的骨诱导性因子。还发现,BMP7在钙调节和骨体内稳态中起作用。同活化素一样,BMP7结合II型受体,ActRIIA和ActRIIB。然而,BMP7和活化素募集不同的I型受体至异聚受体复合物。观察到的主要BMP7I型受体是ALK2,而活化素排他地结合ALK4 (ActRIIB)。BMP7和活化素引起不同的生物学反应和活化不同的 SMAD途径[Macias-Silva等(1998)J Biol Chem.273:25628-36]。

[0085] 如本文所述的，比较性结合数据证实与相应的ActRIIB或ALK7 同二聚体比较，ALK7:ActRIIB异二聚体具有改变的结合特征(配体选择性)。特别地，ALK7:ActRIIB异二聚体显示与任一同二聚体比较，与活化素C、活化素AC和BMP5结合增强和保留如ActRIIB同二聚体所观察到的与活化素B强结合。然而，ALK7:ActRIIB异二聚体显示与ActRIIB同二聚体比较，与GDF11、GDF8、活化素A、BMP10、 BMP6、GDF3和BMP9的结合减少。特别地，BMP9显示低或没有可观察到的对ALK7:ActRIIB异二聚体的亲和力，但该配体紧密结合 ActRIIB同二聚体。

[0086] 这些结果因此证实与ActRIIB同二聚体比较，ALK7:ActRIIB异二聚体是活化素B、活化素C、活化素AC和BMP5的更具选择性的拮抗剂。另外，鉴于与活化素B和C的结合，预期这样的异二聚体将紧密结合活化素BC。另外，鉴于在活化素C和E之间的结构类似性，预期这样的异二聚体将结合活化素E、活化素AE和活化素BE。因此，在其中这样的选择性拮抗作用是有利的某些应用中ALK7:ActRIIB异二聚体可能比ActRIIB同二聚体更有用。实例包括治疗应用，其中需要保留活化素B、活化素C、活化素AC、活化素BC、活化素E、活化素AE、活化素BE和BMP5的一种或多种的拮抗作用，但使活化素A、BMP9、BMP10、GDF3和BMP6的一种或多种的拮抗作用最小化。

[0087] 此外，本文所述的ALK7:ActRIIB异二聚体发挥对脂肪组织的有益分解代谢作用。另外，本文所述的ALK7:ActRIIB异二聚体显示在慢性肾疾病模型中具有有效的保护作用，效果包括抑制纤维化和炎症。然而，不像ActRIIB同二聚体，ActRIIB:ALK7异二聚体仅显示与BMP9 的低亲和力或短暂结合，因此将具有很少至没有对由BMP9介导的过程例如血管发生的同时抑制。这种新的选择性可用于例如，治疗需要对脂肪的抑制作用或对肾的保护作用，但不需要血管发生改变的患者。

[0088] 在本公开内容的上下文中和在使用每个术语的特定语境中，本说明书中使用的术语通常具有其在本领域中的普通含义。下文或说明书他处论述了某些术语，以在描述本公开内容的组合物和方法和如何制备和使用它们方面，向实施者提供另外的指导。术语的任何使用的范围或含义将根据使用其的具体上下文而显而易见。

[0089] 术语“异聚体”或“异多聚体”是至少包含第一多肽链和第二多肽链的复合物，其中在氨基酸序列方面第二多肽链与第一多肽链差别至少一个氨基酸残基。异聚体可包含由第一和第二多肽链形成的“异二聚体”，或可形成更高级结构，其中还存在除了第一和第二多肽链之外的一个或多个多肽链。异多聚体的实例性结构包括异二聚体、异三聚体、异四聚体和其它寡聚体结构。异二聚体在本文中称为X:Y或等同地 X-Y，其中X表示第一多肽链和Y表示第二多肽链。更高级异聚体和寡聚体结构在本文中以相应的方式命名。在某些实施方案中，异多聚体是重组的(例如，一种或多种多肽组分可以是重组蛋白)、分离的和/ 或纯化的。

[0090] “同源的”，以其所有的语法形式和拼写变化，是指两种蛋白之间的关系，所述蛋白具有“共同的进化来源”，包括来自相同的物种的生物体的超家族的蛋白，以及来自不同物种的生物体的同源蛋白。这样的蛋白(和它们的编码核酸)具有序列同源性，如通过它们的序列相似性所反映的，无论是在同一性百分比方面，还是通过特定残基或基序和保守位置的存在。然而，在通常使用中和在本申请中，术语“同源的”当被副词例如“高度地”修饰时，可以指序列相似性，和可能或可能不涉及共同的进化来源。

[0091] 术语“序列相似性”,以其所有的语法形式,是指核酸或氨基酸序列之间同一性或一致性的程度,所述序列可能或可能不享有共同的进化来源。

[0092] 相对于参比多肽(或核苷酸)序列的"百分比(%)序列同一性"定义为在比对序列和如果需要,引入空位以实现最大百分比序列同一性,和不考虑任何保守置换作为序列同一性的一部分后,在候选序列中与在参比多肽(核苷酸)序列中氨基酸残基(或核酸)相同的氨基酸残基(或核酸)的百分数。为确定百分比氨基酸序列同一性的目的的比对可以在本领域的技能范围内的各种方式实现,例如,使用公开可得的计算机软件,例如BLAST、BLAST-2、ALIGN或Megalign(DNASTAR)软件。本领域技术人员可确定合适的参数以比对序列,包括在所比较的序列的整个长度上实现最大比对所需的任何算法。为本文目的,然而,%氨基酸(核酸)序列同一性值使用序列比较计算机程序ALIGN-2产生。ALIGN-2序列比较计算机程序由Genentech,Inc.设计,和源代码已使用用户文档提交至U.S.Copyright Office,Washington D.C.,20559,其中它注册为U.S.Copyright Registration No.TXU510087。ALIGN-2 程序可公开获自Genentech,Inc.,South San Francisco,Calif.,或可从源代码编译。ALIGN-2程序应经编译在UNIX操作系统上使用,包括digital UNIX V4.0D。所有的序列比较参数通过ALIGN-2程序设定,并且不改变。

[0093] “激动”,以其所有语法形式,是指激活蛋白和/或基因(例如,通过激活或放大蛋白的基因表达或通过诱导失活的蛋白进入活性状态) 或增加蛋白和/或基因的活性的过程。

[0094] “拮抗”,以其所有语法形式,是指抑制蛋白和/或基因(例如,通过抑制或降低蛋白的基因表达或通过诱导活性蛋白进入失活状态)或降低蛋白和/或基因的活性的过程。

[0095] 如本文所用的,除非另外说明,“基本上不结合X”意指试剂对“X”具有大于约10^{-7}、10^{-6}、10^{-5}、10^{-4}或更大的K_D(例如,通过用于检测 K_D的测定法,不可检测的结合),其中“X”是指定的试剂,例如蛋白或核酸。

[0096] 说明书和权利要求书全文中结合数值使用的术语"约"和"大约"表示准确度的区间,其为本领域技术人员熟悉和可接受的。一般而言,这样的准确度区间是±10%。或者和特别是在生物学系统中,术语"约"和"大约"可意指在给定值的一个数量级内的值,优选地≤5倍,和更优选地≤2倍。

[0097] 本文公开的数值范围包括定义该范围的数字。

[0098] 术语"一个"和"一种"包括复数指代,除非使用该术语的上下文明确地另外指明。术语"一个"(或"一种")以及术语"一种或多种"和"至少一个"在本文中可互换使用。此外,本文使用的"和/或"应视为明确公开了两个或更多个指定的特征或组分的每一个,含或不含其它特征或组分。因此,在本文的词语例如"A和/或B"中使用的术语“和/或"意图包括"A和B"、"A或B"、"A"(单独)和"B"(单独)。同样地,在词语例如"A、B和/或C"中使用的术语"和/或"意图包括以下方面的每一个:A、B和C;A、B或C;A或C;A或B;B或C;A和C;A和B;B 和C;A(单独);B(单独);和C(单独)。

[0099] 在整个说明书中,词语“包含”或变型例如“包括”或“含有”应理解为暗示包括所述的整体或整体组,但不排除任何其它整体或整体组。

[0100] 2.ActRIIB:ALK7拮抗剂

[0101] 如本文所述的,已发现与相应的ActRIIB和ALK7同二聚体比较, ALK7:ActRIIB异二聚体具有独特的配体-结合特征/选择性。引人注意的是,与ActRIIB同二聚体最强结合的

5种配体中的4种(活化素A、BMP10、GDF8和GDF11)显示与ALK7:ActRIIB异二聚体的结合减少,除了活化素B,其保留与异二聚体的紧密结合。类似地,与ActRIIB 同二聚体中等结合的4种配体中的3种(GDF3、BMP6和特别是BMP9) 显示与ALK7:ActRIIB异二聚体结合减少,而与活化素AC结合增加,总体上成为与异二聚体的第二强配体相互作用。最后,活化素C和 BMP5出人意料地以中等强度结合ALK7:ActRIIB异二聚体,但与 ActRIIB同二聚体无结合(活化素C)或弱结合(BMP5)。鉴于与活化素B 和活化素C的结合,预期ALK7:ActRIIB异二聚体紧密结合活化素BC。净结果是ALK7:ActRIIB异二聚体具有明显不同于不结合任何前述配体的ActRIIB同二聚体或ALK7同二聚体的配体-结合特征。

[0102] 这些结果因此表明与ActRIIB同多聚体比较,ALK7:ActRIIB异多聚体是活化素B、活化素AC和活化素BC的更具选择性是拮抗剂。此外,ALK7:ActRIIB异二聚体显示稳健结合活化素C的不寻常的性质。因此,在其中这样的选择性拮抗作用是有利的某些应用中 ALK7:ActRIIB异多聚体比ActRIIB同多聚体更有用。

[0103] 此外,ALK7:ActRIIB异多聚体令人惊讶地有效改善肾疾病的各种并发症(例如,治疗或预防肾损伤、炎症和损伤)以及对脂肪细胞发挥有益的合成代谢作用。尽管ALK7:ActRIIB异多聚体可通过并非配体抑制的机制发挥生物学作用[例如,抑制活化素B、活化素AC、活化素C、GDF11、GDF8、活化素A、BMP10、BMP6、BMP5、nodal 和GDF3的一种或多种可以是试剂抑制各种另外的试剂(可能包括 TGF-β超家族的其它成员)的活性的趋势的指示物,和这样的集体抑制可对例如,肾疾病或代谢病症产生需要的效果],但其它类型的TGF-β超家族配体拮抗剂以及I型和II型受体拮抗剂(例如,天然配体陷阱,例如卵泡抑素和Lefty,抗体、抑制性核酸和抑制性小分子)或这样的拮抗剂的组合预期可根据本文所述的方法使用,特别是模拟 ALK7:ActRIIB异二聚体的结合/抑制性质的那些以及直接或间接拮抗 ALK7和/或ActRIIB受体的试剂、直接或间接拮抗ALK7和/或 ActRIIB-结合配体的试剂、直接或间接拮抗下游信号传导介质(例如, Smads)的试剂和/或直接或间接拮抗TGFβ超家族共受体(例如,Cripto 或Cryptic)的试剂将具有类似的生物学作用。总之,ALK7:ActRIIB异多聚体和这些可选的拮抗剂在本文被称为"ALK7:ActRIIB拮抗剂"或"ALK7:ActRIIB抑制剂"。

[0104] A.ALK7:ActRIIB异多聚体

[0105] 在某些方面,本公开内容涉及包含一种或多种ALK7受体多肽(例如,SEQ ID NOs:9、10、19、20、38、39、42、43、46、74、76、79 和80)和一种或多种ActRIIB受体多肽(例如,SEQ ID NOs:1、2、3、 4、5、6、71、73、77和78)的异多聚体,其在本文通常称为"ALK7:ActRIIB 异多聚体复合物"或"ALK7:ActRIIB异多聚体"。优选地,本公开内容的ALK7:ActRIIB异多聚体是可溶性的,例如,异多聚体可包含ALK7 受体的可溶性部分(结构域)和ActRIIB受体的可溶性部分(结构域)。一般而言,ALK7和ActRIIB的细胞外结构域对应于这些受体的可溶性部分。因此,在一些实施方案中,本公开内容的异多聚体包含ALK7 受体的细胞外结构域和ActRIIB受体的细胞外结构域。ALK7和 ActRIIB受体的实例性细胞外结构域在本文公开,和这些序列以及其片段、功能变体和修饰形式可根据本公开内容的发明使用(例如, ALK7:ActRIIB异多聚体和其用途)。本公开内容的ALK7:ActRIIB异多聚体包括例如,异二聚体、异三聚体、异四聚体和更高级寡聚体结构。参见例如,图1、2和8-10。在某些优选的实施方案中,本公开内容的异多聚体是ALK7:ActRIIB异二聚体。

[0106] 优选地,本公开内容的ALK7:ActRIIB异多聚体结合一种或多种 TGF-β超家族配

体。在一些实施方案中，ALK7:ActRIIB异多聚体结合活化素(例如，活化素A、活化素B、活化素C、活化素E、活化素 AB、活化素AC、活化素AE、活化素BC和活化素BE)、GDF8和 GDF11的一种或多种。任选地，在一些实施方案中，ALK7:ActRIIB 异多聚体进一步结合BMP6。任选地，在一些实施方案中，

[0107] ALK7:ActRIIB异多聚体进一步结合BMP10。任选地，在一些实施方案中，ALK7:ActRIIB异多聚体进一步结合BMP5。任选地，在一些实施方案中，ALK7:ActRIIB异多聚体进一步结合GDF3。任选地，在一些实施方案中，ALK7:ActRIIB异多聚体进一步结合nodal。任选地，在一些实施方案中，ALK7:ActRIIB异多聚体进一步结合BMP10。任选地，在一些实施方案中，ALK7:ActRIIB异多聚体不结合或基本上不结合BMP9。在某些优选的实施方案中，ALK7:ActRIIB异多聚体以比相应的ActRIIB同多聚体更弱的亲和力结合GDF11、GDF8、活化素A、BMP10、BMP6、GDF3和BMP9的一种或多种。在其它优选的实施方案中，ALK7:ActRIIB异多聚体以比相应的ActRIIB同多聚体更强的亲和力结合活化素C、活化素AC、活化素BC和BMP5的一种或多种。任选地，ALK7:ActRIIB异多聚体进一步结合BMP2、 BMP2/7、BMP3、BMP4、BMP4/7、BMP7、BMP8a、BMP8b、GDF5、 GDF6/BMP13、GDF7、GDF9b/BMP15、GDF15/MIC1、TGF-β1、TGF-β2、TGF-β3、神经胶质细胞衍生的神经营养因子(GDNF)、neurturin、artemin、 persephin、MIS和Lefty的一种或多种。

[0108] TGF-β超家族配体-结合的ALK7:ActRIIB异多聚体可用于抑制 (拮抗)由一种或多种TGFβ超家族配体介导的信号传导(例如，Smad 2/3 和/或Smad 1/5/8信号传导)。特别地，在例如，基于细胞的测定法例如本文所述的测定法中本公开内容的ALK7:ActRIIB异多聚体可用于抑制通过一种或多种TGFβ超家族配体的信号传导。例如，在基于细胞的测定法中ALK7:ActRIIB异多聚体抑制由例如，活化素(例如，活化素A、活化素B、活化素C、活化素E、活化素AB、活化素AC、活化素BC、活化素AE和活化素BE)、GDF11、GDF8、BMP10、BMP6、 BMP5、nodal和GDF3的一种或多种介导的信号传导。任选地，在一些实施方案中，在基于细胞的测定法中ALK7:ActRIIB异多聚体可进一步抑制BMP6信号传导。任选地，在一些实施方案中，在基于细胞的测定法中ALK7:ActRIIB异多聚体可进一步抑制GDF3信号传导。任选地，在一些实施方案中，在基于细胞的测定法中ALK7:ActRIIB 异多聚体可进一步抑制nodal信号传导。任选地，在一些实施方案中，在基于细胞的测定法中ALK7:ActRIIB异多聚体可进一步抑制BMP5 信号传导。任选地，在一些实施方案中，在基于细胞的测定法中 ALK7:ActRIIB异多聚体可进一步抑制BMP10信号传导。任选地，在一些实施方案中，在基于细胞的测定法中ALK7:ActRIIB异多聚体不抑制或基本上不抑制由BMP9介导的信号传导。在某些优选的实施方案中，在基于细胞的测定法中ALK7:ActRIIB异多聚体具有比相应的 ActRIIB同多聚体更弱的对由GDF11、GDF8、活化素A、BMP10、 BMP6、GDF3和BMP9的一种或多种介导的信号传导的抑制作用。在其它优选的实施方案中，在基于细胞的测定法中ALK7:ActRIIB异多聚体具有比相应的ActRIIB同多聚体更强的对由活化素AC、活化素BC、活化素C和BMP5的一种或多种介导的信号传导的抑制作用。任选地，在基于细胞的测定法中ALK7:ActRIIB异多聚体可进一步抑制通过BMP2、BMP2/7、BMP3、BMP4、BMP4/7、BMP7、BMP8a、 BMP8b、GDF5、GDF6/BMP13、GDF7、GDF9b/BMP15、GDF15/MIC1、 TGF-β1、TGF-β2、TGF-β3、神经胶质细胞衍生的神经营养因子(GDNF)、 neurturin、artemin、persephin、MIS和Lefty的一种或多种的信号传导。

[0109] 如本文所用的，术语“ActRIIB”是指来自任何物种的活化素受体II 型B(ActRIIB)

蛋白家族，和通过诱变或其它修饰源自这样的ActRIIB 蛋白的变体。本文提及ActRIIB应理解为提及任何一个目前鉴定的形式。ActRIIB家族的成员通常是跨膜蛋白，由包含富含半胱氨酸的区域的配体-结合细胞外结构域、跨膜结构域和具有预期的丝氨酸/苏氨酸激酶活性的胞质结构域构成。

[0110] 术语“ActRIIB多肽”包括包含ActRIIB家族成员的任何天然存在的多肽以及其保留有用活性的任何变体(包括突变体、片段、融合物和拟肽形式)的多肽。这样的变体ActRIIB多肽的实例在本公开内容全文以及国际专利申请公布号WO 2006/012627、WO 2008/097541和WO 2010/151426中提供，其通过引用以其整体结合到本文中。本文所述的所有ActRIIB-相关多肽的氨基酸编号基于下文提供的人ActRIIB前体蛋白序列的编号(SEQ ID NO:1)，除非另外特别指出。

[0111] 人ActRIIB前体蛋白序列如下：

```
  1  MTAPWVALAL LWGSLCAGSG RGEAETRECI YYNANWELER TNQSGLERCE
 51  GEQDKRLHCY ASWRNSSGTI ELVKKGCWLD DFNCYDRQEC VATEENPQVY
101  FCCCEGNFCN ERFTHLPEAG GPEVTYEPPP TAPTLLTVLA YSLLPIGGLS
151  LIVLLAFWMY RHRKPPYGHV DIHEDPGPPP PSPLVGLKPL QLLEIKARGR
201  FGCVWKAQLM NDFVAVKIFP LQDKQSWQSE REIFSTPGMK HENLLQFIAA
```

[0112]

```
251  EKRGSNLEVE LWLITAFHDK GSLTDYLKGN IITWNELCHV AETMSRGLSY
301  LHEDVPWCRG EGHKPSIAHR DFKSKNVLLK SDLTAVLADF GLAVRFEPGK
351  PPGDTHGQVG TRRYMAPEVL EGAINFQRDA FLRIDMYAMG LVLWELVSRC
401  KAADGPVDEY MLPFEEEIGQ HPSLEELQEV VVHKKMRPTI KDHWLKHPGL
451  AQLCVTIEEC WDHDAEARLS AGCVEERVSL IRRSVNGTTS DCLVSLVTSV
501  TNVDLPPKES SI  (SEQ ID NO: 1)
```

[0113] 信号肽用单下划线表示；细胞外结构域以粗体表示；和潜在的内源N-连接的糖基化位点用双下划线表示。

[0114] 加工(成熟)的细胞外ActRIIB多肽序列如下：

[0115] GRGEAETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWRNSSGTIELVKKGCWLDDFNCYDRQECVATEENPQVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPT(SEQ ID NO:2)

[0116] 在一些实施方案中，所述蛋白可以“SGR...”序列在N-末端而产生。细胞外结构域的C-末端“尾”通过单下划线表示。“尾”缺失的序列(Δ15 序列)如下：

[0117] GRGEAETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWRNSSGTIELVKKGCWLDDFNCYDRQECVATEENPQVYFCCCEGNFCNERFTHLPEA(SEQ ID NO:3)

[0118] SEQ ID NO:1的位置64为丙氨酸(A64)的ActRIIB形式也在文献中报道。参见例如，Hilden等(1994)Blood、83(8):2163-2170。申请人已经确定，包含具有A64置换的ActRIIB细胞外结构域的ActRIIB-Fc 融合蛋白具有对活化素和GDF11相对低的亲和力。相比之下，位置 64为精氨酸(R64)的相同的ActRIIB-Fc融合蛋白具有对活化素和 GDF11的低纳摩尔至高皮摩尔范围的亲和力。因此，在本公开内容中，具有R64的序列用作人ActRIIB的“野生型”参比序列。

[0119] 位置64为丙氨酸的ActRIIB形式如下：

1 MTAPWVALAL LWGSLCAGSG **RGEAETRECI YYNANWELER TNQSGLERCE**

51 GEQDKRLHCY ASWANSSGTI ELVKKGCWLD DFNCYDRQEC VATEENPQVY

101 FCCCEGNFCN ERFTHLPEAG GPEVTYEPPP TAPTLLTVLA YSLLPIGGLS

151 LIVLLAFWMY RHRKPPYGHV DIHEDPGPPP PSPLVGLKPL QLLEIKARGR

201 FGCVWKAQLM NDFVAVKIFP LQDKQSWQSE REIFSTPGMK HENLLQFIAA

[0120] 251 EKRGSNLEVE LWLITAFHDK GSLTDYLKGN IITWNELCHV AETMSRGLSY

301 LHEDVPWCRG EGHKPSIAHR DFKSKNVLLK SDLTAVLADF GLAVRFEPGK

351 PPGDTHGQVG TRRYMAPEVL EGAINFQRDA FLRIDMYAMG LVLWELVSRC

401 KAADGPVDEY MLPFEEEIGQ HPSLEELQEV VVHKKMRPTI KDHWLKHPGL

451 AQLCVTIEEC WDHDAEARLS AGCVEERVSL IRRSVNGTTS DCLVSLVTSV

501 TNVDLPPKES SI (SEQ ID NO: 4)

[0121] 信号肽通过单下划线表示和细胞外结构域通过粗体表示。

[0122] 可选的A64形式的加工(成熟)的细胞外ActRIIB多肽序列如下：

[0123] GRGEAETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWANSSGTIELVKKGCWLDDFNCYDRQECVATEENPQVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPT(SEQ ID NO:5)

[0124] 在一些实施方案中,所述蛋白可以“SGR...”序列在N-末端而产生。细胞外结构域的C-末端“尾”通过单下划线表示。“尾”缺失的序列(Δ15 序列)如下：

[0125] GRGEAETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWANSSGTIELVKKGCWLDDFNCYDRQECVATEENPQVYFCCCEGNFCNERFTHLPEA(SEQ ID NO:6)

[0126] 编码人ActRIIB前体蛋白的核酸序列在下文显示(SEQ ID NO:7),代表Genbank参比序列NM_001106.3的核苷酸25-1560,这些核苷酸编码ActRIIB前体的氨基酸1-513。所示的序列提供了位置64的精氨酸,和可经修饰以代替提供丙氨酸。信号序列用下划线表示。

```
   1  ATGACGGCGC CCTGGGTGGC CCTCGCCCTC CTCTGGGGAT CGCTGTGCGC
  51  CGGCTCTGGG CGTGGGGAGG CTGAGACACG GGAGTGCATC TACTACAACG
 101  CCAACTGGGA GCTGGAGCGC ACCAACCAGA GCGGCCTGGA GCGCTGCGAA
 151  GGCGAGCAGG ACAAGCGGCT GCACTGCTAC GCCTCCTGGC GCAACAGCTC
 201  TGGCACCATC GAGCTCGTGA AGAAGGGCTG CTGGCTAGAT GACTTCAACT
 251  GCTACGATAG GCAGGAGTGT GTGGCCACTG AGGAGAACCC CCAGGTGTAC
 301  TTCTGCTGCT GTGAAGGCAA CTTCTGCAAC GAACGCTTCA CTCATTTGCC
 351  AGAGGCTGGG GGCCCGGAAG TCACGTACGA GCCACCCCCG ACAGCCCCCA
 401  CCCTGCTCAC GGTGCTGGCC TACTCACTGC TGCCCATCGG GGGCCTTTCC
 451  CTCATCGTCC TGCTGGCCTT TTGGATGTAC CGGCATCGCA AGCCCCCCTA
 501  CGGTCATGTG GACATCCATG AGGACCCTGG GCCTCCACCA CCATCCCCTC
 551  TGGTGGGCCT GAAGCCACTG CAGCTGCTGG AGATCAAGGC TCGGGGGCGC
 601  TTTGGCTGTG TCTGGAAGGC CCAGCTCATG AATGACTTTGTAGCTGTCAA
 651  GATCTTCCCA CTCCAGGACA AGCAGTCGTG GCAGAGTGAA CGGGAGATCT
 701  TCAGCACACC TGGCATGAAG CACGAGAACC TGCTACAGTT CATTGCTGCC
 751  GAGAAGCGAG GCTCCAACCT CGAAGTAGAG CTGTGGCTCA TCACGGCCTT
 801  CCATGACAAG GGCTCCCTCA CGGATTACCT CAAGGGGAAC ATCATCACAT
 851  GGAACGAACT GTGTCATGTA GCAGAGACGA TGTCACGAGG CCTCTCATAC
 901  CTGCATGAGG ATGTGCCCTG GTGCCGTGGC GAGGGCCACA AGCCGTCTAT
 951  TGCCCACAGG GACTTTAAAA GTAAGAATGT ATTGCTGAAG AGCGACCTCA
1001  CAGCCGTGCT GGCTGACTTT GGCTTGGCTG TTCGATTTGA GCCAGGGAAA
1051  CCTCCAGGGG ACACCCACGG ACAGGTAGGC ACGAGACGGT ACATGGCTCC
1101  TGAGGTGCTC GAGGGAGCCA TCAACTTCCA GAGAGATGCC TTCCTGCGCA
1151  TTGACATGTA TGCCATGGGG TTGGTGCTGT GGGAGCTTGT GTCTCGCTGC
1201  AAGGCTGCAG ACGGACCCGT GGATGAGTAC ATGCTGCCCT TTGAGGAAGA
1251  GATTGGCCAG CACCCTTCGT TGGAGGAGCT GCAGGAGGTG GTGGTGCACA
1301  AGAAGATGAG GCCCACCATT AAAGATCACT GGTTGAAACA CCCGGGCCTG
1351  GCCCAGCTTT GTGTGACCAT CGAGGAGTGC TGGGACCATG ATGCAGAGGC
1401  TCGCTTGTCC GCGGGCTGTG TGGAGGAGCG GGTGTCCCTG ATTCGGAGGT
1451  CGGTCAACGG CACTACCTCG GACTGTCTCG TTTCCCTGGT GACCTCTGTC
1501  ACCAATGTGG ACCTGCCCCC TAAAGAGTCA AGCATC (SEQ ID NO: 7)
```

[0127]

[0128] 编码加工的细胞外人ActRIIB多肽的核酸序列如下所示(SEQ ID NO:8)。所示的序列提供了位置64的精氨酸,和可经修饰以代替提供丙氨酸。

```
   1  GGGCGTGGGG AGGCTGAGAC ACGGGAGTGC ATCTACTACA ACGCCAACTG
  51  GGAGCTGGAG CGCACCAACC AGAGCGGCCT GGAGCGCTGC GAAGGCGAGC
 101  AGGACAAGCG GCTGCACTGC TACGCCTCCT GGCGCAACAG CTCTGGCACC
 151  ATCGAGCTCG TGAAGAAGGG CTGCTGGCTA GATGACTTCA ACTGCTACGA
 201  TAGGCAGGAG TGTGTGGCCA CTGAGGAGAA CCCCCAGGTG TACTTCTGCT
 251  GCTGTGAAGG CAACTTCTGC AACGAACGCT TCACTCATTT GCCAGAGGCT
 301  GGGGGCCCGG AAGTCACGTA CGAGCCACCC CCGACAGCCC CCACC
(SEQ ID NO: 8)
```

[0129]

[0130]

[0131] 人ActRIIB细胞外结构域和人ActRIIA细胞外结构域的氨基酸序列比对示于图3。该比对显示两种受体内被认为直接接触ActRII配体的氨基酸残基。例如,该复合ActRII结

构表示ActRIIB-配体结合袋部分地被残基Y31、N33、N35、L38至T41、E47、E50、Q53至K55、L57、H58、Y60、S62、K74、W78至N83、Y85、R87、A92和E94 至F101定义。在这些位置，预期保守突变将是耐受的。

[0132] 此外，ActRIIB在脊椎动物中是充分保守的，其中大段的细胞外结构域完全保守。例如，图4描述人ActRIIB细胞外结构域与各种 ActRIIB直向同源物比较的多-序列比对。许多结合ActRIIB的配体也是高度保守的。因此，根据这些比对，可能预测在配体-结合结构域内对正常ActRIIB-配体结合活性是重要的关键氨基酸位置以及预测可能耐受置换而不显著改变正常ActRIIB-配体结合活性的氨基酸位置。因此，根据本发明公开的方法使用的活性的人ActRIIB变体多肽可在相应位置处包括来自另一脊椎动物ActRIIB的序列的一个或多个氨基酸，或可包括类似于人或其它脊椎动物序列的残基。不意图限制，以下实例说明了这种定义活性ActRIIB变体的方法。人细胞外结构域(SEQ ID NO:53)中的L46在非洲蟾蜍中ActRIIB(SEQ ID NO:55)是缬氨酸，因此该位置可被改变和任选地可被改变为另一疏水残基，例如V、I或F 或非极性残基例如A。在人细胞外结构域中的E52在非洲蟾蜍中是K，表明该位点可耐受各种变化，包括极性残基例如E、D、K、R、H、S、 T、P、G、Y和可能A。在人细胞外结构域中的T93在非洲蟾蜍中是 K，表明在在该位置处广泛的结构变异是耐受的，其中极性残基是有利的，例如S、K、R、E、D、H、G、P、G和Y。在人细胞外结构域中的F108在非洲蟾蜍中是Y，因此Y或其它疏水基团，例如I、V 或L应是耐受的。在人细胞外结构域中的E111在非洲蟾蜍中是K，表明带电荷残基在该位置处是耐受的，包括D、R、K和H，以及Q 和N。在人细胞外结构域中的R112在非洲蟾蜍中是K，表明碱性残基在该位置处是耐受的，包括R和H。人细胞外结构域的位置119处的A相对地保守性差，在啮齿动物中作为P和在非洲蟾蜍中作为V 出现，因此基本上任何氨基酸在该位置处应该是耐受的。

[0133] 此外，在结构和功能特征方面，特别是关于配体结合，ActRII蛋白已在本领域中被表征[Attisano等(1992)Cell 68(1):97-108;Greenwald 等(1999)Nature Structural Biology 6(1):18-22;Allendorph等(2006) PNAS 103(20:7643-7648;Thompson等(2003) The EMBO Journal 22(7): 1555-1566;以及美国专利号:7,709,605、7,612,041和7,842,663]。除了本文的教导之外，这些参考资料对于如何产生保留一种或多种正常活性(例如，配体-结合活性)的ActRIIB变体也提供了详细指导。

[0134] 例如，称为三指毒素折叠的定义的结构基序对于通过I型和II型受体的配体结合是重要的，和通过位于各单体受体的细胞外结构域内的不同位置的保守半胱氨酸残基形成[Greenwald等(1999)Nat Struct Biol 6:18-22;和Hinck(2012)FEBS Lett 586:1860-1870]。因此，由这些保守半胱氨酸的最外端所划分的人ActRIIB的核心配体-结合结构域，对应于SEQ ID NO:1(ActRIIB前体)的位置29-109。因此，侧连这些半胱氨酸-划分的核心序列的结构更加无序的氨基酸可在N-末端截短约1、2、3、4、5、6、7、8、9、10、11、12、13、14、15、16、17、 18、19、20、21、22、23、24、25、26、27或28个残基和/或在C- 末端截短约1、2、3、4、5、6、7、8、9、10、11、12、13、14、15、 16、17、18、19、20、21、22、23、24或25个残基，而不必然改变配体结合。对于N-末端和/或C-末端截短的实例性的ActRIIB细胞外结构域包括SEQ ID NOs:2、3、5和6。

[0135] Attisano等表明ActRIIB的细胞外结构域的C-末端的脯氨酸结的缺失减少受体对活化素的亲和力。相对于包含脯氨酸结区域和完整的近膜结构域的ActRIIB(20-134)-Fc，

包含本发明的SEQ ID NO:1的氨基酸20-119的ActRIIB-Fc融合蛋白,“ActRIIB(20-119)-Fc”,具有减少的对DF11和活化素的结合(参见例如,美国专利号7,842,663)。然而,相对于野生型,ActRIIB(20-129)-Fc蛋白保留类似的但略微减少的活性,即使脯氨酸结区域被破坏。

[0136] 因此,在氨基酸134、133、132、131、130和129处终止(相对于 SEQ ID NO:1)的ActRIIB细胞外结构域全部预期是有活性的,但在 134或133处终止的构建体可最具有活性。类似地,残基129-134的任一个处的突变(相对于SEQ ID NO:1)在很大程度上不预期改变配体 -结合亲和力。为支持此观点,本领域已知P129和P130的突变(相对于SEQ ID NO:1)基本上不降低配体结合。因此,本公开内容的ActRIIB 多肽可早在氨基酸109(最后一个半胱氨酸)终止,然而,在109和119 或其之间(例如,109、110、111、112、113、114、115、116、117、 118或119)终止的形式预期具有减少的配体结合。氨基酸119(相对于本发明的SEQ ID NO:1)保守性差,因此容易改变或截短。在128(相对于SEQ ID NO:1)或更后终止的ActRIIB多肽应保留配体-结合活性。相对于SEQ ID NO:1在119和127或其之间(例如,119、120、121、122、123、124、125、126或127)终止的ActRIIB多肽将具有中等结合能力。任何这些形式可能对于根据临床或实验背景的应用是理想的。

[0137] 在ActRIIB的N-末端,预期在氨基酸29或之前(相对于SEQ ID NO:1)开始的蛋白将保留配体-结合活性。氨基酸29表示最初的半胱氨酸。位置24(相对于SEQ ID NO:1)的丙氨酸至天冬酰胺突变引入 N-连接的糖基化序列,基本上不影响配体结合[美国专利号7,842,663]。这证实了信号裂解肽和半胱氨酸交联区之间的区域(对应于氨基酸 20-29)中的突变是良好耐受的。特别地,在位置20、21、22、23和24 (相对于SEQ ID NO:1)开始的ActRIIB多肽应保留大致的配体-结合活性,和在位置25、26、27、28和29(相对于SEQ ID NO:1)开始的ActRIIB 多肽也被预期保留配体-结合活性。已经证实,例如,美国专利号 7,842,663,令人惊讶地,在22、23、24或25开始的ActRIIB构建体将具有最大活性。

[0138] 总之,ActRIIB的活性部分(例如,配体-结合部分)的通式包含SEQ ID NO:1的氨基酸29-109。因此,ActRIIB多肽可例如,包含与开始于对应于SEQ ID NO:1的氨基酸20-29的任何一个的残基(例如,开始于氨基酸20、21、22、23、24、25、26、27、28或29的任何一个) 和结束于对应于SEQ ID NO:1的氨基酸109-134的任何一个的位置 (例如,结束于氨基酸109、110、111、112、113、114、115、116、 117、118、119、120、121、122、123、124、125、126、127、128、129、130、131、132、133或134的任何一个)的ActRIIB的部分具有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、 92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列,基本上由其组成,或由其组成。其它实例包括开始于SEQ ID NO:1的20-29(例如,位置20、21、22、23、24、25、26、27、28 或29的任何一个)或21-29(例如,位置21、22、23、24、25、26、27、 28或29的任何一个)的位置和结束于SEQ ID NO:1的119-134(例如,位置119、120、121、122、123、124、125、126、127、128、129、 130、131、132、133或134的任何一个)、119-133(例如,位置119、 120、121、122、123、124、125、126、127、128、129、130、131、 132或133的任何一个)、129-134(例如,位置129、130、131、132、 133或134的任何一个)或129-133(例如,位置129、130、131、132 或133的任何一个)的位置的多肽。其它实例包括开始于SEQ ID NO:1 的20-24(例如,位置20、21、22、23或24的任何一个)、21-24(例如,位置21、22、23或24的任何一个)或22-25(例如,位置22、22、23 或25的任何一个)的位置和结

束于SEQ ID NO:1的109-134(例如,位置109、110、111、112、113、114、115、116、117、118、119、120、 121、122、123、124、125、126、127、128、129、130、131、132、 133或134的任何一个)、119-134(例如,位置119、120、121、122、 123、124、125、126、127、128、129、130、131、132、133或134 的任何一个)或129-134(例如,位置129、130、131、132、133或134 的任何一个)的位置的构建体。还考虑了这些范围内的变体,特别是与SEQ ID NO:1的相应部分具有至少70%、75%、80%、85%、86%、 87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、 98%、99%或100%同一性的那些。

[0139] 本文所述的变化可以各种方式组合。在一些实施方案中,ActRIIB 变体包含在配体-结合袋中不超过1、2、5、6、7、8、9、10或15个保守氨基酸变化,和在配体-结合袋的位置40、53、55、74、79和/ 或82处的0、1或多个非保守改变。结合袋外的位点,其中变异性可特别良好耐受,包括细胞外结构域(如上所述)的氨基和羧基端和位置42-46和65-73(相对于SEQ ID NO:1)。位置65的天冬酰胺至丙氨酸的改变(N65A)实际上改进A64背景的配体结合,和因此预期对R64 背景的配体结合没有有害影响[美国专利号7,842,663]。这种改变可能消除A64背景中N65的糖基化,因此证实该区域中显著改变可能是耐受的。尽管R64A变化耐受性差,但R64K是良好耐受的,因此另一碱性残基例如H可在位置64耐受[美国专利号7,842,663]。另外,本领域描述的诱变程序的结果表明,ActRIIB中的氨基酸位置通常对于保守是有益的。相对于SEQ ID NO:1,这些包括位置80(酸性或疏水氨基酸)、位置78(疏水的,特别是色氨酸)、位置37(酸性的,特别是天冬氨酸或谷氨酸)、位置56(碱性氨基酸)、位置60(疏水氨基酸,特别是苯丙氨酸或酪氨酸)。因此,本公开内容提供了在ActRIIB多肽中可能是保守的氨基酸框架。可能需要保守的其它位置如下:位置52(酸性氨基酸)、位置55(碱性氨基酸)、位置81(酸性)、98(极性或带电荷,特别是E、D、R或K),全部相对于SEQ ID NO:1。

[0140] 在某些实施方案中,本公开内容涉及包含至少一种ActRIIB多肽的异多聚体,所述多肽包括其片段、功能变体和修饰形式。优选地,根据本公开内容使用的ActRIIB多肽是可溶性的(例如,ActRIIB的细胞外结构域)。在其它优选的实施方案中,根据本公开内容使用的 ActRIIB多肽结合一种或多种TGF-β超家族配体。因此,在一些实施方案中,根据本公开内容使用的ActRIIB多肽抑制(拮抗)一种或多种TGF-β超家族配体的活性(例如,抑制Smad信号传导)。在一些实施方案中,本公开内容的异多聚体包含至少一种ActRIIB多肽,其包含与开始于对应于SEQ ID NO:1的氨基酸20-29的残基(例如,开始于氨基酸20、21、22、23、24、25、26、27、28或29的任何一个)和结束于对应于SEQ ID NO:1的氨基酸109-134的位置(例如,结束于氨基酸109、110、111、112、113、114、115、116、117、118、119、120、 121、122、123、124、125、126、127、128、129、130、131、132、 133或134的任何一个)的ActRIIB的部分具有至少70%、75%、80%、 85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列,基本上由其组成,或由其组成。在某些优选的实施方案中,本公开内容的异多聚体包含至少一种ActRIIB多肽,其包含与SEQ ID NO:1的氨基酸29-109 具有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%同一性的氨基酸序列,由其组成,或基本上由其组成。在其它优选的实施方案中,本公开内容的异多聚体包含至少一种ActRIIB多肽,其包含与SEQ ID NO:1的氨基酸25-131具有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、 97%、98%、99%或100%

同一性的氨基酸序列，由其组成，或基本上由其组成。在一些实施方案中，本公开内容的异多聚体包含至少一种 ActRIIB多肽，其与SEQ ID NOs:1、2、3、4、5、6、71、73、77和78的任一个的氨基酸序列具有至少70％、75％、80％、85％、86％、 87％、88％、89％、90％、91％、92％、93％、94％、95％、97％、98％、 99％或100％同一性。在某些实施方案中，本公开内容的异多聚体包含至少一种ActRIIB多肽，其中对应于SEQ ID NO:1的L79的氨基酸位置不是酸性氨基酸(即，不是天然存在的D或E氨基酸残基或人工酸性氨基酸)。

[0141] 在某些方面，本公开内容涉及包含ALK7多肽的蛋白复合物。如本文所用的，术语“ALK7”是指来自任何物种的活化素受体-样激酶-7 蛋白家族和通过诱变或其它修饰而源自这样的ALK7蛋白的变体。提及ALK7在文本中应理解为提及任何一种当前鉴定的形式。ALK7家族的成员通常是跨膜蛋白，由具有富含半胱氨酸的区域的配体-结合细胞外结构域、跨膜结构域和具有预期的丝氨酸/苏氨酸激酶活性的胞质结构域构成。

[0142] 术语“ALK7多肽”包括包含ALK7家族成员的任何天然存在的多肽以及其保留有用活性的任何变体(包括突变体、片段、融合物和拟肽形式)的多肽。本文所述的所有ALK7-相关多肽的氨基酸编号基于下文人ALK7前体蛋白序列(SEQ ID NO:9)的编号，除非另外特别指出。

[0143] 存在各种天然存在的人ALK7同种型。标准的人ALK7同种型1 前体蛋白的序列(NCBI Ref Seq NP_660302.2)如下：

1 MTRALCSALR QALLLLAAAA **ELSPGLKCVC LLCDSSNFTC QTEGACWASV MLTNGKEQVI**

61 **KSCVSLPELN AQVFCHSSNN VTKTECCFTD FCNNITLHLP TASPNAPKLG PME**LAIIITV

121 PVCLLSIAAM LTVWACQGRQ CSYRKKKRPN VEEPLSECNL VNAGKTLKDL IYDVTASGSG

181 SGLPLLVQRT IARTIVLQEI VGKGRFGEVW HGRWCGEDVA VKIFSSRDER SWFREAEIYQ

[0144] 241 TVMLRHENIL GFIAADNKDN GTWTQLWLVS EYHEQGSLYD YLNRNIVTVA GMIKLALSIA

301 SGLAHLHMEI VGTQGKPAIA HRDIKSKNIL VKKCETCAIA DLGLAVKHDS ILNTIDIPQN

361 PKVGTKRYMA PEMLDDTMNV NIFESFKRAD IYSVGLVYWE IARRCSVGGI VEEYQLPYYD

421 MVPSDPSIEE MRKVVCDQKF RPSIPNQWQS CEALRVMGRI MRECWYANGA ARLTALRIKK

481 TISQLCVKED CKA (SEQ ID NO: 9)

[0145] 信号肽由单下划线表示和细胞外结构域以粗体表示。

[0146] 加工的细胞外ALK7同种型1多肽序列如下：

[0147] ELSPGLKCVCLLCDSSNFTCQTEGACWASVMLTNGKEQVIKSCVSLPELNAQVFCHSSNN VTKTECCFTDFCNNITLHLPTASPNAPKLGPME(SEQ ID NO:10)

[0148] 编码人ALK7同种型1前体蛋白的核酸序列在下文显示(SEQ ID NO:11)，对应于Genbank参比序列NM_145259.2的核苷酸244-1722。信号序列用下划线表示和细胞外结构域以粗体表示。

[0149]

ATGACCCGGGCGCTCTGCTCAGCGCTCCGCCAGGCTCTCCTGCTGCTCGCAGCGGCCGCC
GAGCTCTCGCCAGGACTGAAGTGTGTATGTCTTTTGTGTGATTCTTCAAACTTTACCTGCCAA
ACAGAAGGAGCATGTTGGGCATCAGTCATGCTAACCAATGGAAAAGAGCAGGTGATCAAATCC
TGTGTCTCCCTTCCAGAACTGAATGCTCAAGTCTTCTGTCATAGTTCCAACAATGTTACCAAA
ACCGAATGCTGCTTCACAGATTTTTGCAACAACATAACACTGCACCTTCCAACAGCATCACCA
AATGCCCCAAAACTTGGACCCATGGAGCTGGCCATCATTATTACTGTGCCTGTTTGCCTCCTG
TCCATAGCTGCGATGCTGACAGTATGGGCATGCCAGGGTCGACAGTGCTCCTACAGGAAGAAA
AAGAGACCAAATGTGGAGGAACCACTCTCTGAGTGCAATCTGGTAAATGCTGGAAAAACTCTG
AAAGATCTGATTTATGATGTGACCGCCTCTGGATCTGGCTCTGGTCTACCTCTGTTGGTTCAA
AGGACAATTGCAAGGACGATTGTGCTTCAGGAAATAGTAGGAAAAGGTAGATTTGGTGAGGTG
TGGCATGGAAGATGGTGTGGGGAAGATGTGGCTGTGAAAATATTCTCCTCCAGAGATGAAAGA
TCTTGGTTTCGTGAGGCAGAAATTTACCAGACGGTCATGCTGCGACATGAAAACATCCTTGGT
TTCATTGCTGCTGACAACAAAGATAATGGAACTTGGACTCAACTTTGGCTGGTATCTGAATAT
CATGAACAGGGCTCCTTATATGACTATTTGAATAGAAATATAGTGACCGTGGCTGGAATGATC
AAGCTGGCGCTCTCAATTGCTAGTGGTCTGGCACACCTTCATATGGAGATTGTTGGTACACAA
GGTAAACCTGCTATTGCTCATCGAGACATAAAATCAAAGAATATCTTAGTGAAAAAGTGTGAA
ACTTGTGCCATAGCGGACTTAGGGTTGGCTGTGAAGCATGATTCAATACTGAACACTATCGAC
ATACCTCAGAATCCTAAAGTGGGAACCAAGAGGTATATGGCTCCTGAAATGCTTGATGATACA
ATGAATGTGAATATCTTTGAGTCCTTCAAACGAGCTGACATCTATTCTGTTGGTCTGGTTTAC
TGGGAAATAGCCCGGAGGTGTTCAGTCGGAGGAATTGTTGAGGAGTACCAATTGCCTTATTAT
GACATGGTGCCTTCAGATCCCTCGATAGAGGAAATGAGAAAGGTTGTTTGTGACCAGAAGTTT
CGACCAAGTATCCCAAACCAGTGGCAAAGTTGTGAAGCACTCCGAGTCATGGGGAGAATAATG
CGTGAGTGTTGGTATGCCAACGGAGCGGCCCGCCTAACTGCTCTTCGTATTAAGAAGACTATA
TCTCAACTTTGTGTCAAAGAAGACTGCAAAGCC (SEQ ID NO: 11)

[0150] 编码加工的细胞外ALK7多肽(同种型1)的核酸序列如下：

[0151] GAGCTCTCGCCAGGACTGAAGTGTGTATGTCTTTTGTGTGATTCTTCAAACTTTACCTGC
CAAACAGAAGGAGCATGTTGGGCATCAGTCATGCTAACCAATGGAAAAGAGCAGGTGATCAAA
TCCTGTGTCTCCCTTCCAGAACTGAATGCTCAAGTCTTCTGTCATAGTTCCAACAATGTTACC
AAAACCGAATGCTGCTTCACAGATTTTTGCAACAACATAACACTGCACCTTCCAACAGCATCA
CCAAATGCCCCAAAACTTGGACCCATGGAG(SEQ ID NO:12)

[0152] 人ALK7的可选同种型,同种型2的氨基酸序列(NCBI Ref Seq NP_001104501.1)以其加工形式显示如下(SEQ ID NO:19),其中细胞外结构域以粗体表示。

[0153] 1 **MLTNGKEQVI KSCVSLPELN AQVFCHSSNN VTKTECCFTD FCNNITLHLP TASPNAPKLG**

61 **PME**LAIIITV PVCLLSIAAM LTVWACQGRQ CSYRKKKRPN VEEPLSECNL VNAGKTLKDL

121 IYDVTASGSG SGLPLLVQRT IARTIVLQEI VGKGRFGEVW HGRWCGEDVA VKIFSSRDER

181 SWFREAEIYQ TVMLRHENIL GFIAADNKDN GTWTQLWLVS EYHEQGSLYD YLNRNIVTVA

241 GMIKLALSIA SGLAHLHMEI VGTQGKPAIA HRDIKSKNIL VKKCETCAIA

[0154] DLGLAVKHDS

301 ILNTIDIPQN PKVGTKRYMA PEMLDDTMNV NIFESFKRAD IYSVGLVYWE IARRCSVGGI

361 VEEYQLPYYD MVPSDPSIEE MRKVVCDQKF RPSIPNQWQS CEALRVMGRI MRECWYANGA

421 ARLTALRIKK TISQLCVKED CKA (SEQ ID NO: 19)

[0155] 细胞外ALK7多肽(同种型2)的氨基酸序列如下：

[0156] MLTNGKEQVIKSCVSLPELNAQVFCHSSNNVTKTECCFTDFC NNITLHLPTASPNAPKLGPME(SEQ ID NO:20).

[0157] 编码加工的ALK7多肽(同种型2)的核酸序列在下文显示(SEQ ID NO:21),对应于NCBI参比序列NM_001111031.1的核苷酸279-1607。细胞外结构域以粗体表示。

[0158]

ATGCTAACCAATGGAAAAGAGCAGGTGATCAAATCCTGTGTCTCCCTTCCAGAACTGAAT GCTCAAGTCTTCTGTCATAGTTCCAACAATGTTACCAAAACCGAATGCTGCTTCACAGATTTT TGCAACAACATAACACTGCACCTTCCAACAGCATCACCAAATGCCCCAAAACTTGGACCCATG GAGCTGGCCATCATTATTACTGTGCCTGTTTGCCTCCTGTCCATAGCTGCGATGCTGACAGTA TGGGCATGCCAGGGTCGACAGTGCTCCTACAGGAAGAAAAAGAGACCAAATGTGGAGGAACCA CTCTCTGAGTGCAATCTGGTAAATGCTGGAAAAACTCTGAAAGATCTGATTTATGATGTGACC GCCTCTGGATCTGGCTCTGGTCTACCTCTGTTGGTTCAAAGGACAATTGCAAGGACGATTGTG CTTCAGGAAATAGTAGGAAAAGGTAGATTTGGTGAGGTGTGGCATGGAAGATGGTGTGGGGAA GATGTGGCTGTGAAAATATTCTCCTCCAGAGATGAAAGATCTTGGTTTCGTGAGGCAGAAATT TACCAGACGGTCATGCTGCGACATGAAAACATCCTTGGTTTCATTGCTGCTGACAACAAAGAT AATGGAACTTGGACTCAACTTTGGCTGGTATCTGAATATCATGAACAGGGCTCCTTATATGAC TATTTGAATAGAAATATAGTGACCGTGGCTGGAATGATCAAGCTGGCGCTCTCAATTGCTAGT GGTCTGGCACACCTTCATATGGAGATTGTTGGTACACAAGGTAAACCTGCTATTGCTCATCGA GACATAAAATCAAAGAATATCTTAGTGAAAAAGTGTGAAACTTGTGCCATAGCGGACTTAGGG TTGGCTGTGAAGCATGATTCAATACTGAACACTATCGACATACCTCAGAATCCTAAAGTGGGA ACCAAGAGGTATATGGCTCCTGAAATGCTTGATGATACAATGAATGTGAATATCTTTGAGTCC TTCAAACGAGCTGACATCTATTCTGTTGGTCTGGTTTACTGGGAAATAGCCCGGAGGTGTTCA GTCGGAGGAATTGTTGAGGAGTACCAATTGCCTTATTATGACATGGTGCCTTCAGATCCCTCG ATAGAGGAAATGAGAAAGGTTGTTTGTGACCAGAAGTTTCGACCAAGTATCCCAAACCAGTGG CAAAGTTGTGAAGCACTCCGAGTCATGGGGAGAATAATGCGTGAGTGTTGGTATGCCAACGGA GCGGCCCGCCTAACTGCTCTTCGTATTAAGAAGACTATATCTCAACTTTGTGTCAAAGAAGAC TGCAAAGCC (SEQ ID NO: 21)

[0159] 编码细胞外ALK7多肽(同种型2)的核酸序列如下(SEQ ID NO：22)：

[0160] ATGCTAACCAATGGAAAAGAGCAGGTGATCAAATCCTGTGTCTCCCTTCCAGAACTGAAT GCTCAAGTCTTCTGTCATAGTTCCAACAATGTTACCAAAACCGAATGCTGCTTCACAGATTTT

TGCAACAACATAACACTGCACCTTCCAACAGCATCACCAAATGCCCCAAAACTTGGACCCATG GAG(SEQ ID NO:22)

[0161] 可选的人ALK7前体蛋白,同种型3的氨基酸序列(NCBI Ref Seq NP_001104502.1)显示如下(SEQ ID NO:38),其中信号肽由单下划线表示。

[0162]

1 MTRALCSALR QALLLLAAAA ELSPGLKCVC LLCDSSNFTC QTEGACWASV MLTNGKEQVI

61 KSCVSLPELN AQVFCHSSNN VTKTECCFTD FCNNITLHLP TGLPLLVQRT IARTIVLQEI

121 VGKGRFGEVW HGRWCGEDVA VKIFSSRDER SWFREAEIYQ TVMLRHENIL GFIAADNKDN

181 GTWTQLWLVS EYHEQGSLYD YLNRNIVTVA GMIKLALSIA SGLAHLHMEI VGTQGKPAIA

241 HRDIKSKNIL VKKCETCAIA DLGLAVKHDS ILNTIDIPQN PKVGTKRYMA PEMLDDTMNV

301 NIFESFKRAD IYSVGLVYWE IARRCSVGGI VEEYQLPYYD MVPSDPSIEE MRKVVCDQKF

361 RPSIPNQWQS CEALRVMGRI MRECWYANGA ARLTALRIKK TISQLCVKED CKA

(SEQ ID NO: 38)

[0163] 加工的ALK7多肽(同种型3)的氨基酸序列如下(SEQ ID NO:39)。该同种型缺少跨膜结构域,因此提出以其整体是可溶性是(Roberts等, 2003,Biol Reprod 68:1719-1726)。SEQ ID NO:39的N-末端变体如下所述预测。

[0164]

1 ELSPGLKCVC LLCDSSNFTC QTEGACWASV MLTNGKEQVI KSCVSLPELN AQVFCHSSNN

61 VTKTECCFTD FCNNITLHLP TGLPLLVQRT IARTIVLQEI VGKGRFGEVW HGRWCGEDVA

121 VKIFSSRDER SWFREAEIYQ TVMLRHENIL GFIAADNKDN GTWTQLWLVS EYHEQGSLYD

181 YLNRNIVTVA GMIKLALSIA SGLAHLHMEI VGTQGKPAIA HRDIKSKNIL VKKCETCAIA

241 DLGLAVKHDS ILNTIDIPQN PKVGTKRYMA PEMLDDTMNV NIFESFKRAD IYSVGLVYWE

301 IARRCSVGGI VEEYQLPYYD MVPSDPSIEE MRKVVCDQKF RPSIPNQWQS

[0165] CEALRVMGRI

361 MRECWYANGA ARLTALRIKK TISQLCVKED CKA (SEQ ID NO: 39)

[0166] 编码未加工的ALK7多肽前体蛋白(同种型3)的核酸序列在下文显示(SEQ ID NO:40),对应于NCBI参比序列NM_001111032.1的核苷酸244-1482。信号序列由实下划线表示。

[0167] ATGACCCGGGCGCTCTGCTCAGCGCTCCGCCAGGCTCTCCTGCTGCTCGCAGCGGCCGCC GAGCTCTCGCCAGGACTGAAGTGTGTATGTCTTTTGTGTGATTCTTCAAACTTTACCTGCCAA ACAGAAGGAGCATGTTGGGCATCAGTCATGCTAACCAATGGAAAAGAGCAGGTGATCAAATCC TGTGTCTCCCTTCCAGAACTGAATGCTCAAGTCTTCTGTCATAGTTCCAACAATGTTACCAAA ACCGAATGCTGCTTCACAGATTTTTGCAACAACATAACACTGCACCTTCCAACAGGTCTACCT

CTGTTGGTTCAAAGGACAATTGCAAGGACGATTGTGCTTCAGGAAATAGTAGGAAAAGGTAGA
TTTGGTGAGGTGTGGCATGGAAGATGGTGTGGGGAAGATGTGGCTGTGAAAATATTCTCCTCC
AGAGATGAAAGATCTTGGTTTCGTGAGGCAGAAATTTACCAGACGGTCATGCTGCGACATGAA
AACATCCTTGGTTTCATTGCTGCTGACAACAAAGATAATGGAACTTGGACTCAACTTTGGCTG
GTATCTGAATATCATGAACAGGGCTCCTTATATGACTATTTGAATAGAAATATAGTGACCGTG
GCTGGAATGATCAAGCTGGCGCTCTCAATTGCTAGTGGTCTGGCACACCTTCATATGGAGATT
GTTGGTACACAAGGTAAACCTGCTATTGCTCATCGAGACATAAAATCAAAGAATATCTTAGTG
AAAAAGTGTGAAACTTGTGCCATAGCGGACTTAGGGTTGGCTGTGAAGCATGATTCAATACTG
AACACTATCGACATACCTCAGAATCCTAAAGTGGGAACCAAGAGGTATATGGCTCCTGAAATG
CTTGATGATACAATGAATGTGAATATCTTTGAGTCCTTCAAACGAGCTGACATCTATTCTGTT
GGTCTGGTTTACTGGGAAATAGCCCGGAGGTGTTCAGTCGGAGGAATTGTTGAGGAGTACCAA
TTGCCTTATTATGACATGGTGCCTTCAGATCCCTCGATAGAGGAAATGAGAAAGGTTGTTTGT
GACCAGAAGTTTCGACCAAGTATCCCAAACCAGTGGCAAAGTTGTGAAGCACTCCGAGTCATG
GGGAGAATAATGCGTGAGTGTTGGTATGCCAACGGAGCGGCCCGCCTAACTGCTCTTCGTATT
AAGAAGACTATATCTCAACTTTGTGTCAAAGAAGACTGCAAAGCC(SEQ ID NO:40)

[0168] 编码加工的ALK7多肽(同种型3)的核酸序列如下(SEQ ID NO：41)：

[0169] GAGCTCTCGCCAGGACTGAAGTGTGTATGTCTTTTGTGTGATTCTTCAAACTTTACCTGC
CAAACAGAAGGAGCATGTTGGGCATCAGTCATGCTAACCAATGGAAAAGAGCAGGTGATCAAA
TCCTGTGTCTCCCTTCCAGAACTGAATGCTCAAGTCTTCTGTCATAGTTCCAACAATGTTACC
AAAACCGAATGCTGCTTCACAGATTTTTGCAACAACATAACACTGCACCTTCCAACAGGTCTA
CCTCTGTTGGTTCAAAGGACAATTGCAAGGACGATTGTGCTTCAGGAAATAGTAGGAAAAGGT
AGATTTGGTGAGGTGTGGCATGGAAGATGGTGTGGGGAAGATGTGGCTGTGAAAATATTCTCC
TCCAGAGATGAAAGATCTTGGTTTCGTGAGGCAGAAATTTACCAGACGGTCATGCTGCGACAT
GAAAACATCCTTGGTTTCATTGCTGCTGACAACAAAGATAATGGAACTTGGACTCAACTTTGG
CTGGTATCTGAATATCATGAACAGGGCTCCTTATATGACTATTTGAATAGAAATATAGTGACC
GTGGCTGGAATGATCAAGCTGGCGCTCTCAATTGCTAGTGGTCTGGCACACCTTCATATGGAG
ATTGTTGGTACACAAGGTAAACCTGCTATTGCTCATCGAGACATAAAATCAAAGAATATCTTA
GTGAAAAAGTGTGAAACTTGTGCCATAGCGGACTTAGGGTTGGCTGTGAAGCATGATTCAATA
CTGAACACTATCGACATACCTCAGAATCCTAAAGTGGGAACCAAGAGGTATATGGCTCCTGAA
ATGCTTGATGATACAATGAATGTGAATATCTTTGAGTCCTTCAAACGAGCTGACATCTATTCT
GTTGGTCTGGTTTACTGGGAAATAGCCCGGAGGTGTTCAGTCGGAGGAATTGTTGAGGAGTAC
CAATTGCCTTATTATGACATGGTGCCTTCAGATCCCTCGATAGAGGAAATGAGAAAGGTTGTT
TGTGACCAGAAGTTTCGACCAAGTATCCCAAACCAGTGGCAAAGTTGTGAAGCACTCCGAGTC
ATGGGGAGAATAATGCGTGAGTGTTGGTATGCCAACGGAGCGGCCCGCCTAACTGCTCTTCGT
ATTAAGAAGACTATATCTCAACTTTGTGTCAAAGAAGACTGCAAAGCC(SEQ ID NO:308)

[0170] 可选的人ALK7前体蛋白,同种型4的氨基酸序列(NCBI RefSeq NP_001104503.1)显示如下(SEQ ID NO:42),其中信号肽由单下划线表示。

[0171]

1 MTRALCSALR QALLLLAAAA ELSPGLKCVC LLCDSSNFTC QTEGACWASV MLTNGKEQVI

61 KSCVSLPELN AQVFCHSSNN VTKTECCFTD FCNNITLHLP TDNGTWTQLW LVSEYHEQGS

121 LYDYLNRNIV TVAGMIKLAL SIASGLAHLH MEIVGTQGKP AIAHRDIKSK NILVKKCETC

181 AIADLGLAVK HDSILNTIDI PQNPKVGTKR YMAPEMLDDT MNVNIFESFK RADIYSVGLV

241 YWEIARRCSV GGIVEEYQLP YYDMVPSDPS IEEMRKVVCD QKFRPSIPNQ WQSCEALRVM

301 GRIMRECWYA NGAARLTALR IKKTISQLCV KEDCKA (SEQ ID NO: 42)

[0172] 加工的ALK7多肽(同种型4)的氨基酸序列如下(SEQ ID NO:43)。像ALK7同种型3，同种型4缺少跨膜结构域，因此提出以其整体是可溶性的(Roberts等，2003，BiolReprod 68:1719-1726)。SEQ ID NO：43的N-末端变体如下所述预测。

1 ELSPGLKCVC LLCDSSNFTC QTEGACWASV MLTNGKEQVI KSCVSLPELN AQVFCHSSNN

61 VTKTECCFTD FCNNITLHLP TDNGTWTQLW LVSEYHEQGS LYDYLNRNIV TVAGMIKLAL

121 SIASGLAHLH MEIVGTQGKP AIAHRDIKSK NILVKKCETC AIADLGLAVK

[0173] HDSILNTIDI

181 PQNPKVGTKR YMAPEMLDDT MNVNIFESFK RADIYSVGLV YWEIARRCSV GGIVEEYQLP

240 YYDMVPSDPS IEEMRKVVCD QKFRPSIPNQ WQSCEALRVM GRIMRECWYA NGAARLTALR

301 IKKTISQLCV KEDCKA (SEQ ID NO: 43)

[0174] 编码未加工的ALK7多肽前体蛋白(同种型4)的核酸序列在下文显示(SEQ ID NO：44)，对应于NCBI参比序列NM_001111033.1的核苷酸244-1244。信号序列由实下划线表示。

[0175] ATGACCCGGGCGCTCTGCTCAGCGCTCCGCCAGGCTCTCCTGCTGCTCGCAGCGGCCGCC
GAGCTCTCGCCAGGACTGAAGTGTGTATGTCTTTTGTGTGATTCTTCAAACTTTACCTGCCAA
ACAGAAGGAGCATGTTGGGCATCAGTCATGCTAACCAATGGAAAAGAGCAGGTGATCAAATCC
TGTGTCTCCCTTCCAGAACTGAATGCTCAAGTCTTCTGTCATAGTTCCAACAATGTTACCAAA
ACCGAATGCTGCTTCACAGATTTTTGCAACAACATAACACTGCACCTTCCAACAGATAATGGA
ACTTGGACTCAACTTTGGCTGGTATCTGAATATCATGAACAGGGCTCCTTATATGACTATTTG
AATAGAAATATAGTGACCGTGGCTGGAATGATCAAGCTGGCGCTCTCAATTGCTAGTGGTCTG
GCACACCTTCATATGGAGATTGTTGGTACACAAGGTAAACCTGCTATTGCTCATCGAGACATA
AAATCAAAGAATATCTTAGTGAAAAAGTGTGAAACTTGTGCCATAGCGGACTTAGGGTTGGCT
GTGAAGCATGATTCAATACTGAACACTATCGACATACCTCAGAATCCTAAAGTGGGAACCAAG
AGGTATATGGCTCCTGAAATGCTTGATGATACAATGAATGTGAATATCTTTGAGTCCTTCAAA
CGAGCTGACATCTATTCTGTTGGTCTGGTTTACTGGGAAATAGCCCGGAGGTGTTCAGTCGGA
GGAATTGTTGAGGAGTACCAATTGCCTTATTATGACATGGTGCCTTCAGATCCCTCGATAGAG

GAAATGAGAAAGGTTGTTTGTGACCAGAAGTTTCGACCAAGTATCCCAAACCAGTGGCAAAGT
TGTGAAGCACTCCGAGTCATGGGGAGAATAATGCGTGAGTGTTGGTATGCCAACGGAGCGGCC
CGCCTAACTGCTCTTCGTATTAAGAAGACTATATCTCAACTTTGTGTCAAAGAAGACTGCAAA GCCTAA(SEQ ID NO:44)

[0176] 编码加工的ALK7多肽(同种型4)的核酸序列如下(SEQ ID NO：45)：

[0177] GAGCTCTCGCCAGGACTGAAGTGTGTATGTCTTTTGTGTGATTCTTCAAACTTTACCTGC
CAAACAGAAGGAGCATGTTGGGCATCAGTCATGCTAACCAATGGAAAAGAGCAGGTGATCAAA
TCCTGTGTCTCCCTTCCAGAACTGAATGCTCAAGTCTTCTGTCATAGTTCCAACAATGTTACC
AAAACCGAATGCTGCTTCACAGATTTTTGCAACAACATAACACTGCACCTTCCAACAGATAAT
GGAACTTGGACTCAACTTTGGCTGGTATCTGAATATCATGAACAGGGCTCCTTATATGACTAT
TTGAATAGAAATATAGTGACCGTGGCTGGAATGATCAAGCTGGCGCTCTCAATTGCTAGTGGT
CTGGCACACCTTCATATGGAGATTGTTGGTACACAAGGTAAACCTGCTATTGCTCATCGAGAC
ATAAAATCAAAGAATATCTTAGTGAAAAAGTGTGAAACTTGTGCCATAGCGGACTTAGGGTTG
GCTGTGAAGCATGATTCAATACTGAACACTATCGACATACCTCAGAATCCTAAAGTGGGAACC
AAGAGGTATATGGCTCCTGAAATGCTTGATGATACAATGAATGTGAATATCTTTGAGTCCTTC
AAACGAGCTGACATCTATTCTGTTGGTCTGGTTTACTGGGAAATAGCCCGGAGGTGTTCAGTC
GGAGGAATTGTTGAGGAGTACCAATTGCCTTATTATGACATGGTGCCTTCAGATCCCTCGATA
GAGGAAATGAGAAAGGTTGTTTGTGACCAGAAGTTTCGACCAAGTATCCCAAACCAGTGGCAA
AGTTGTGAAGCACTCCGAGTCATGGGGAGAATAATGCGTGAGTGTTGGTATGCCAACGGAGCG
GCCCGCCTAACTGCTCTTCGTATTAAGAAGACTATATCTCAACTTTGTGTCAAAGAAGACTGC AAAGCCTAA(SEQ ID NO:45)

[0178] 基于在大鼠中全长ALK7(同种型1)的信号序列(见NCBI参比序列NP_620790.1)和在人和大鼠ALK7之间高程度的序列同一性,预测加工形式的人ALK7同种型1如下(SEQ ID NO:46)。

1 LKCVCLLCDS SNFTCQTEGA CWASVMLTNG KEQVIKSCVS LPELNAQVFC

[0179] HSSNNVTKTE

61 CCFTDFCNNI TLHLPTASPN APKLGPME (SEQ ID NO: 46)

[0180] 预测了加工的ALK7同种型1的活性变体,其中SEQ ID NO:10 在N-末端被截短1、2、3、4、5、6或7个氨基酸和SEQ ID NO:46 在N-末端被截短1或2个氨基酸。与SEQ ID NO:46一致,进一步预期亮氨酸是加工形式的人ALK7同种型3(SEQ ID NO:39)和人ALK7 同种型4(SEQ ID NO:43)的N-末端氨基酸。

[0181] 在某些实施方案中,本公开内容涉及包含至少一种ALK7多肽的异多聚体,所述多肽包括其片段、功能变体和修饰形式。优选地,根据本公开内容的发明(例如,包含ALK7多肽的异多聚体和其用途)使用的ALK7多肽是可溶性的(例如,ALK7的细胞外结构域)。在其它优选的实施方案中,根据本公开内容使用的ALK7多肽结合一种或多种TGF-β超家族配体。因此,在一些优选的实施方案中,根据本公开内容使用的ALK7多肽抑制(拮抗)一种或多种TGF-β超家族配体的活性(例如,Smad 2/3和/或Smad 1/5/8信号传导的诱导)。在一些实施方案中,本公开内容的异多聚体包含至少一种ALK7多肽,其与SEQ ID NO:9、10、19、20、38、39、42、43、46、74、75、79或80的氨基酸序列具有至少70％、75％、80％、85％、86％、87％、

88％、89％、 90％、91％、92％、93％、94％、95％、97％、98％或99％同一性。在一些实施方案中，本公开内容的异多聚体由至少一种ALK7多肽组成或基本上由其组成，所述至少一种ALK7多肽与SEQ ID NO:9、10、 19、20、38、39、42、43、46、74、75、79和80的氨基酸序列具有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、 92％、93％、94％、95％、97％、98％或99％同一性。

[0182] ALK7在脊椎动物中是充分保守的，其中一大段细胞外结构域完全保守。例如，图7描述了与各种ALK7直向同源物相比，人ALK7 细胞外结构域的多序列比对。因此，根据这些比对，可能预测配体- 结合结构域内对于正常的ALK7-配体结合活性是重要的关键氨基酸位置以及预测可能耐受置换而不显著改变正常的ALK7-配体结合活性的氨基酸位置。因此，可根据本文公开的方法使用的活性的人ALK7 变体多肽可包括来自另一脊椎动物ALK7的序列的相应位置的一种或多种氨基酸，或可包括类似于人或其它脊椎动物序列的残基。不意图限制，以下实例说明了这种定义活性ALK7变体的方法。人ALK7细胞外结构域(SEQ ID NO:59)中的V61在狨猴(Callithrix jacchus)ALK7 (SEQ ID NO:62)中是异亮氨酸，因此该位置可改变，和任选地可改变为另一疏水残基例如L、I或F或非极性残基例如A。人细胞外结构域中的L32在Tarsius syrichta(SEQ ID NO:63)ALK7中是R，表明该位点可耐受各种变化，包括极性残基例如E、D、K、R、H、S、T、P、 G、Y和可能非极性残基例如A。人细胞外结构域中的K37在Pan troglodytes ALK7(SEQ ID NO:60)中是R，表明碱性残基在该位置是耐受的，包括R、K和H。人细胞外结构域中的P4是相对保守差的，在Pan troglodytes ALK7中作为A出现，由此表明各种氨基酸在该位置可耐受。

[0183] 此外，关于结构和功能特征，ALK7蛋白已在本领域中表征[例如， Romano等(2012) Journal of Molecular Modeling 18(8):3617-3625]。例如，称为三指毒素折叠的定义结构基序对于通过I型和II型受体的配体结合是重要的和通过位于各单体受体的细胞外结构域内的不同位置的保守半胱氨酸残基形成[Greenwald等(1999)Nat Struct Biol 6:18-22;和Hinck(2012)FEBS Lett 586:1860-1870]。因此，由这些保守半胱氨酸的最外端所划界的人ALK7的核心配体-结合结构域，对应于 SEQ ID NO:9的位置28-92。这些半胱氨酸-划界的核心序列侧翼的结构更加无序的氨基酸可在N-末端截短1、2、3、4、5、6、7、8、9、 10、11、12、13、14、15、16、17、18、19、20、21、22、23、24、 25、26或27个残基和在C-末端截短1、2、3、4、5、6、7、8、9、10、11、12、13、14、15、16、17、18、19、20或21个残基，而不必然改变配体结合。N-末端和/或C-末端截短的实例性的ALK7细胞外结构域包括SEQ ID NOs:10、20、39和43。

[0184] 因此，ALK7的活性部分(例如，配体-结合部分)的通式包含氨基酸28-92。因此ALK7多肽可例如，包含与开始于对应于SEQ ID NO： 9的氨基酸20-28的任何一个(例如，开始于氨基酸20、21、22、23、 24、25、26、27或28的任何一个)的残基和结束于对应于SEQ ID NO： 9的氨基酸92-113的任何一个(例如，结束于氨基酸92、93、94、95、 96、97、98、99、100、101、102、103、104、105、106、107、108、 109、110、111、112或113的任何一个)的位置的ALK7的部分具有至少70％、75％、80％、85％、86％、87％、88％、89％、90％、91％、 92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列，基本上由其组成或由其组成。

[0185] 其它实例包括开始于SEQ ID NO:9的21-28(例如，位置21、22、 23、24、25、26、27或28的任何一个)、24-28(例如，位置24、25、 26、27或28的任何一个)或25-28(例如，位置25、26、27或28的任何一个)的位置和结束于SEQ ID NO:9的93-112(例如，位置93、94、 95、96、

97、98、99、100、101、102、103、104、105、106、107、 108、109、110、111或112的任何一个)、93-110(例如,位置93、94、 95、96、97、98、99、100、101、102、103、104、105、106、107、 108、109或110的任何一个)、93-100(例如,位置93、94、95、96、 97、98、99或100的任何一个)或93-95(例如,位置93、94或95的任何一个)的位置的构建体。也考虑了在这些范围内的变体,特别是与 SEQ ID NO:9的相应部分具有至少70%、75%、80%、85%、86%、 87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、 98%、99%或100%同一性的那些。

[0186] 本文所述的变化可以各种方式组合。在一些实施方案中,ALK7 变体在配体-结合袋中包含不超过1、2、5、6、7、8、9、10或15个保守氨基酸变化。结合袋外的位点,其中变异性可特别良好耐受,包括细胞外结构域的氨基和羧基端(如上所述)。

[0187] 在某些方面,本公开内容涉及包含一种或多种ALK7受体多肽(例如,SEQ ID Nos:9、10、19、20、38、39、42、43、46、74、76、79 和80)和一种或多种ActRIIB受体多肽(例如,SEQ ID NOs:1、2、3、 4、5、6、71、73、77和78)的异多聚体,其在本文通常称为“ALK7:ActRIIB 异多聚体复合物”或“ALK7:ActRIIB异多聚体”。优选地,

[0188] ALK7:ActRIIB异多聚体是可溶性的,例如,异多聚体包含ALK7受体的可溶性部分(结构域)和ActRIIB受体的可溶性部分(结构域)。一般而言,ALK7和ActRIIB的细胞外结构域对应于这些受体的可溶性部分。因此,在一些实施方案中,异多聚体包含ALK7受体的细胞外结构域和ActRIIB受体的细胞外结构域。ALK7和ActRIIB受体的实例性的细胞外结构域在本文公开和这样的序列以及其片段、功能变体和修饰形式可根据本公开内容的发明(例如,ALK7:ActRIIB异多聚体组合物和其用途)使用。在一些实施方案中,ALK7:ActRIIB异多聚体包含至少一种ALK7多肽,其包含与SEQ ID NO:9、10、19、20、38、 39、42、43、46、74、76、79和80的氨基酸序列具有至少70%、75%、 80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、 95%、97%、98%、99%或100%同一性的序列,基本上由其组成或由其组成。在一些实施方案中,ALK7:ActRIIB异多聚体包含至少一种 ALK7多肽,其包含与开始于对应于SEQ ID NO:9的氨基酸21-28、 22-28、23-28、24-28、25-28、26-28或27-28的任何一个的残基和结束于SEQ ID NO:9的93-112、93、110、93-100、93-95的位置的ALK7 的部分具有至少70%、75%、80%、85%、86%、87%、88%、89%、 90%、91%、92%、93%、94%、95%、97%、98%、99%或100%同一性的序列,基本上由其组成或由其组成。在一些实施方案中,ALK7:ActRIIB异多聚体包含至少一种ALK7多肽,其包含与SEQ ID NO:9的氨基酸28-92具有至少70%、75%、80%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、97%、98%、99%或100%同一性的序列,基本上由其组成或由其组成。在一些实施方案中,ALK7-ActRIIB异多聚体包含至少一种ActRIIB多肽,其包含与SEQ ID NOs:1、2、3、4、5、6、71、73、77和78的任何一个的氨基酸序列具有至少70%、75%、80%、85%、86%、87%、88%、89%、 90%、91%、92%、93%、94%、95%、97%、98%、99%或100%同一性的序列,基本上由其组成或由其组成。在一些实施方案中, ALK7:ActRIIB异多聚体包含至少一种ActRIIB多肽,其包含与开始于对应于氨基酸20-29、20-24、21-24、22-25或21-29的任何一个的残基和结束于SEQ ID NO:1的109-134、119-134、119-133、129-134 或129-133的位置的ActRIIB的部分具有至少70%、75%、80%、85%、 86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、97%、 98%、99%或100%同一性的序列,基本上由其组成或由其组成。在一些实施方案中,ALK7:ActRIIB异多聚体包含至少一种ActRIIB多肽,其包含与SEQ ID NO:1的

氨基酸29-109具有至少70％、75％、80％、 85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、 97％、98％、99％或100％同一性的序列，基本上由其组成或由其组成。在一些实施方案中，ALK7:ActRIIB异多聚体包含至少一种ActRIIB 多肽，其包含与SEQ ID NO:1的氨基酸25-125具有至少70％、75％、 80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、 95％、97％、98％、99％或100％同一性的序列，基本上由其组成或由其组成。在某些优选的实施方案中，ALK7:ActRIIB异多聚体包含至少一种ActRIIB多肽，其中对应于SEQ ID NO:1的L79的位置不是酸性氨基酸(即，不是天然存在的D或E氨基酸或人工酸性残基)。

[0189] ALK7:ActRIIB异多聚体包括例如，异二聚体、异三聚体、异四聚体和进一步更高级寡聚体结构。参见例如，图1、2和8-10。在某些优选的实施方案中，本公开内容的异多聚体是ALK7:ActRIIB异二聚体。

[0190] 在一些实施方案中，本公开内容考虑通过修饰ALK7多肽和/或ActRIIB多肽的结构来制备功能变体。变体可通过氨基酸置换、缺失、添加或其组合产生。例如，合理的是预期单独置换亮氨酸为异亮氨酸或缬氨酸、天冬氨酸为谷氨酸、苏氨酸为丝氨酸或类似置换氨基酸为结构相关的氨基酸(例如，保守突变)对得到的分子的生物学活性不将具有显著影响。保守置换是在其侧链上相关的氨基酸家族内发生的那些。本公开内容的多肽的氨基酸序列的变化是否产生功能同源物可通过评价变体多肽在细胞中以类似于野生型多肽的方式产生反应，或结合一种或多种TGF-β超家族配体，包括例如BMP2、BMP2/7、BMP3、 BMP4、BMP4/7、BMP5、BMP6、BMP7、BMP8a、BMP8b、BMP9、 BMP10、GDF3、GDF5、GDF6/BMP13、GDF7、GDF8、GDF9b/BMP15、 GDF11/BMP11、GDF15/MIC1、TGF-β1、TGF-β2、TGF-β3、活化素 A、活化素B、活化素C、活化素E、活化素AB、活化素AC、活化素AE、活化素BC、活化素BE、nodal、神经胶质细胞衍生的神经营养因子(GDNF)、neurturin、artemin、persephin、MIS和Lefty的能力，容易地确定。

[0191] 在一些实施方案中，本公开内容考虑了为了例如增强治疗功效或稳定性(例如，保存期限和抵抗体内蛋白水解降解)等目的，通过修饰 ALK7多肽和/或ActRIIB多肽的结构来制备功能变体。

[0192] 在一些实施方案中，本公开内容考虑了ALK7多肽和/或ActRIIB 多肽的特定突变以改变多肽的糖基化。这样的突变可经选择，以引入或消除一个或多个糖基化位点，例如O-连接或N-连接的糖基化位点。天冬酰胺-连接的糖基化识别位点通常包含三肽序列，天冬酰胺-X-苏氨酸或天冬酰胺-X-丝氨酸(其中“X”是任何氨基酸)，其被合适的细胞糖基化酶特异性识别。还可通过添加一个或多个丝氨酸或苏氨酸残基至多肽的序列或通过一个或多个丝氨酸或苏氨酸残基置换多肽的序列进行改变(对于O-连接的糖基化位点)。糖基化识别位点的第一或第三氨基酸位置的一个或两个处的各种氨基酸置换或缺失(和/或第二位置处的氨基酸缺失)，导致在修饰的三肽序列处非-糖基化。增加多肽上的糖部分的数量的另一方法是通过糖苷化学或酶偶联至多肽。根据使用的偶联模式，糖可连接至(a)精氨酸和组氨酸；(b)游离的羧基； (c)游离的巯基，例如半胱氨酸的那些；(d)游离的羟基，例如丝氨酸、苏氨酸或羟基脯氨酸的那些；(e)芳香族残基，例如苯丙氨酸、酪氨酸或色氨酸的那些；或(f)谷氨酰胺的酰胺基。除去多肽上存在的一个或多个糖部分可经化学和/或酶促实现。化学去糖基化可包括例如，将多肽暴露于化合物三氟甲磺酸或等效的化合物。这种处理导致大部分或所有的糖裂解，除了连接糖(N-乙酰基葡糖胺或N-乙酰基半乳糖胺)之外，同时保

留氨基酸序列完整。多肽上糖部分的酶裂解可通过使用各种内切和外切糖苷酶实现，如Thotakura等[Meth.Enzymol.(1987) 138:350]所述。在合适时，多肽的序列可根据使用的表达系统的类型调整，因为哺乳动物、酵母、昆虫和植物细胞可全都引入可受所述肽的氨基酸序列影响的不同糖基化模式。一般而言，在人中使用的本公开内容的异聚复合物可在提供合适糖基化的哺乳动物细胞系中表达，例如HEK293或CHO细胞系，尽管其它哺乳动物表达细胞系预期也可使用。

[0193] 本公开内容还考虑了产生ALK7和/或ActRIIB多肽的突变体、特别是组合突变体组，以及截短突变体的方法。组合突变体的库可特别用于鉴定功能活性的(例如，TGF-β超家族配体结合)ALK7和/或 ActRIIB序列。筛选这样的组合文库的目的可以是产生例如，具有改变的性质、例如改变的药代动力学或改变的配体结合的多肽变体。各种筛选测定法在下文提供和这样的测定法可用于评价变体。例如，可筛选ALK7:ActRIIB复合物变体结合一种或多种TGF-β超家族配体以阻止TGF-β超家族配体与TGF-β超家族受体结合和/或干扰由TGF-β超家族配体引起的信号传导的能力。

[0194] ALK7:ActRIIB异多聚体的活性可例如，在基于细胞的测定法或体内测定法中测试。例如，可评价ALK7:ActRIIB异多聚体对参与脂肪代谢、肾损伤、肾炎症和/或肾纤维化的基因表达或蛋白活性的影响。按需要，这可在一种或多种重组TGF-β超家族配体蛋白的存在下进行，和细胞可经转染，以产生ALK7:ActRIIB异多聚体和任选地，TGF-β超家族配体。同样地，ALK7:ActRIIB异多聚体可给予小鼠或其它动物，可使用公认的方法评价一种或多种度量例如脂肪代谢或肾损伤、炎症、纤维化和/或功能。类似地，ALK7:ActRIIB异多聚体或其变体的活性可在例如，脂肪细胞和/或肾小球细胞中测试对这些细胞的生长的任何影响，例如，通过本文所述的测定法和本领域众所周知的那些。SMAD-响应报告基因可在这样的细胞系中用于监测对下游信号传导的影响。

[0195] 可产生组合来源的变体，相对于参比ALK7:ActRIIB异多聚体，其具有增加的选择性或普遍增加的效力。这样的变体，当从重组DNA 构建体表达时，可用于基因治疗方案。同样地，诱变可产生变体，其具有显著不同于相应的未修饰的ALK7:ActRIIB异多聚体的细胞内半寿期。例如，改变的蛋白可被赋予对蛋白水解降解或导致未修饰多肽的破坏或以其它方式失活的其它细胞过程更稳定或更不稳定。这样的变体和编码它们的基因，可通过调节多肽的半寿期，用于改变多肽复合物水平。例如，短的半寿期可产生更短暂的生物学作用，并且当诱导型表达系统的一部分时，可允许更紧密控制细胞内的重组多肽复合物水平。在Fc融合蛋白中，可在接头(如果有的话)和/或Fc部分中进行突变以改变ALK7:ActRIIB异多聚体的一种或多种活性，包括例如免疫原性、半寿期和溶解度。

[0196] 组合文库可通过编码多肽文库的简并基因文库产生，所述多肽各自至少包括潜在的ALK7和/或ActRIIB序列的一部分。例如，合成的寡核苷酸的混合物可经酶促连接至基因序列，使得潜在的ALK7和/ 或ActRIIB编码核苷酸序列的简并组可作为各个多肽表达，或者，作为较大的融合蛋白组表达(例如，对于噬菌体展示)。

[0197] 存在许多可自简并寡核苷酸序列产生潜在的同源物的文库的方法。简并基因序列的化学合成可在自动DNA合成仪中进行，和合成的基因然后可连接至合适的载体用于表达。简并寡核苷酸的合成是本领域众所周知的[Narang,SA(1983)Tetrahedron 39:3;Itakura等(1981) Recombinant DNA,Proc.3rd Cleveland Sympos.Macromolecules,ed. AG

Walton,Amsterdam:Elsevier pp273-289;Itakura等(1984)Annu.Rev. Biochem.53:323;Itakufa等(1984)Science 198:1056;和Ike等(1983) Nucleic Acid Res.11:477]。这样的技术已经用于其它蛋白的定向进化 [Scott等,(1990)Science 249:386-390;Roberts等(1992)PNAS USA 89:2429-2433;Devlin等(1990)Science 249:404-406;Cwirla等,(1990)PNAS USA 87:6378-6382;以及美国专利号:5,223,409、5,198,346和 5,096,815]。

[0198] 或者,其它形式的诱变可用于产生组合文库。例如,ALK7:ActRIIB 异多聚体可通过筛选,使用例如,丙氨酸扫描诱变[Ruf等(1994) Biochemistry 33:1565-1572;Wang等(1994)J.Biol.Chem. 269:3095-3099;Balint等(1993)Gene 137:109-118;Grodberg等(1993) Eur.J.Biochem.218:597-601;Nagashima等(1993)J.Biol.Chem. 268:2888-2892;Lowman等(1991)Biochemistry 30:10832-10838;和 Cunningham等(1989)Science 244:1081-1085],通过接头扫描诱变 [Gustin等(1993)Virology 193:653-660;和Brown等(1992)Mol.Cell Biol.12:2644-2652;McKnight等(1982)Science 232:316],通过饱和诱变[Meyers等,(1986)Science 232:613];通过PCR诱变[Leung等(1989) Method Cell Mol Biol 1:11-19];或通过随机诱变,包括化学诱变[Miller 等(1992)A Short Course in Bacterial Genetics,CSHL Press,Cold Spring Harbor,NY;和Greener等(1994)Strategies in Mol Biol 7:32-34],从文库产生和分离。接头扫描诱变,特别是在组合的背景下,是一种用于鉴定截短(生物活性)形式的ALK7和/或ActRIIB多肽的有吸引力的方法。

[0199] 本领域已知用于筛选通过点突变和截短制备的组合文库的基因产物的各种技术,和在这方面,用于筛选cDNA文库的具有一定性质的基因产物的各种技术。这样的技术一般可适用于通过ALK7:ActRIIB 异多聚体的组合诱变产生的基因文库的快速筛选。用于筛选大基因文库的最广泛使用的技术通常包括克隆基因文库至可复制表达载体,用得到的载体文库转化合适的细胞,和在其中所需活性的检测促进相对容易的分离编码其产物被检测的基因的载体的条件下表达组合基因。优选的测定法包括TGF-β超家族配体(例如,BMP2、BMP2/7、BMP3、 BMP4、BMP4/7、BMP5、BMP6、BMP7、BMP8a、BMP8b、BMP9、 BMP10、GDF3、GDF5、GDF6/BMP13、GDF7、GDF8、GDF9b/BMP15、 GDF11/BMP11、GDF15/MIC1、TGF-β1、TGF-β2、TGF-β3、活化素 A、活化素B、活化素C、活化素E、活化素AB、活化素AC、活化素BC、活化素AE、活化素BE、nodal、神经胶质细胞衍生的神经营养因子(GDNF)、neurturin、artemin、persephin、MIS和Lefty)结合测定法和/或TGF-β超家族配体-介导细胞信号传导测定法。

[0200] 在某些实施方案中,除了在ALK7和/或ActRIIB多肽中天然存在的任何修饰之外,ALK7:ActRIIB异多聚体可进一步包含翻译后修饰。这样的修饰包括但不限于,乙酰基化、羧基化、糖基化、磷酸化、脂质化和酰基化。结果,ALK7:ActRIIB异多聚体可包含非-氨基酸元件,例如聚乙二醇、脂质、多糖或单糖和磷酸酯。这样的非-氨基酸元件对异多聚体复合物的功能性的影响可按本文对于其它异多聚体变体所述的进行测试。当本公开内容的多肽在细胞中通过裂解新生形式的多肽产生时,翻译后加工也可能对于蛋白的正确折叠和/或功能是重要的。不同的细胞(例如,CHO、HeLa、MDCK、293、WI38、NIH-3T3或 HEK293)具有用于这样的翻译后活性的特定细胞机器和特征性机制,和可经选择以确保ALK7和/或ActRIIB多肽以及包含它们的异多聚体的正确修饰和加工。

[0201] 在某些优选的实施方案中,本文所述的异多聚体包含与至少一种 ActRIIB多肽共

价或非共价缔合的至少一种ALK7多肽。优选地，本文公开的多肽形成异二聚体复合物，尽管还包括更高级的异多聚体复合物，例如，但不限于，异三聚体、异四聚体和另外的寡聚体结构(参见例如，图1、2和8-10)。在一些实施方案中，ALK7和/或ActRIIB 多肽包含至少一个多聚化结构域。如本文公开的，术语“多聚化结构域”是指促进至少第一多肽和至少第二多肽之间的共价或非共价相互作用的氨基酸或氨基酸序列。本文公开的多肽可共价或非共价连接至多聚化结构域。优选地，多聚化结构域促进在第一多肽(例如，ALK7多肽)和第二多肽(例如，ActRIIB多肽)之间的相互作用以促进异多聚体形成(例如，异二聚体形成)，和任选地妨碍或以其它方式不利于同多聚体形成(例如，同二聚体形成)，从而增加所需的异多聚体的收率(参见例如，图2)。

[0202] 本领域已知的许多方法可用于产生ALK7:ActRIIB异多聚体。例如，非天然存在的二硫键可通过替换第一多肽(例如，ALK7多肽)上的天然存在的氨基酸为游离的含硫醇残基，例如半胱氨酸构建，使得游离的硫醇与第二多肽(例如，ActRIIB多肽)上的另一游离的含硫醇残基相互作用，以致在第一和第二多肽之间形成二硫键。促进异多聚体形成的相互作用的另外的实例包括但不限于，离子相互作用，例如描述于Kjaergaard等，WO2007147901;静电导引作用，例如描述于 Kannan等，U.S.8,592,562;卷曲螺旋相互作用，例如描述于Christensen 等，U.S.20120302737;亮氨酸拉链，例如描述于Pack&Plueckthun，(1992)Biochemistry 31:1579-1584;和螺旋-转角-螺旋基序，例如描述于Pack等，(1993)Bio/Technology 11:1271-1277。各种区段的连接可通过例如共价结合，例如通过化学交联、肽接头、二硫桥等或亲和相互作用，例如通过亲和素-生物素或亮氨酸拉链技术获得。

[0203] 在某些方面，多聚化结构域可包含相互作用对的一个组分。在一些实施方案中，本文公开的多肽可形成蛋白复合物，其包含与第二多肽共价或非共价缔合的第一多肽，其中第一多肽包含ALK7多肽的氨基酸序列和相互作用对的第一成员的氨基酸序列;和第二多肽包含 ActRIIB多肽的氨基酸序列和相互作用对的第二成员的氨基酸序列。相互作用对可以是任何两个多肽序列，其相互作用以形成复合物、特别是异二聚体复合物，尽管有效的实施方案也可使用可形成同二聚体复合物的相互作用对。相互作用对的一个成员可融合至本文所述的 ALK7或ActRIIB多肽，包括例如，包含与SEQ ID NOs:2、3、5、6、 10、20、39、43和46的任何一个的序列具有至少70％、75％、80％、 85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、 96％、97％、98％、99％或100％同一性的氨基酸序列的多肽序列，基本上由其组成或由其组成。相互作用对可经选择以赋予改进的性质/ 活性，例如增加的血清半寿期，或作为衔接子起作用，另一部分与衔接子连接以提供改进的性质/活性。例如，聚乙二醇部分可连接至相互作用对的一个或两个组分以提供改进的性质/活性，例如改进的血清半寿期。

[0204] 相互作用对的第一和第二成员可以是不对称的对，意味着该对的成员优先彼此缔合，而非自缔合。因此，不对称的相互作用对的第一和第二成员可缔合形成异二聚体复合物(参见例如，图2)。或者，相互作用对可以是非引导的，意味着该对的成员可彼此缔合或自缔合，没有实质的偏好，因此可具有相同的或不同的氨基酸序列。因此，非引导的相互作用对的第一和第二成员可缔合形成同二聚体复合物或异二聚体复合物。任选地，相互作用对(例如，不对称的对或非引导的相互作用对)的第一成员与相互作用对的第二成员共价缔合。任

选地，相互作用对(例如，不对称的对或非引导的相互作用对)的第一成员与相互作用对的第二成员非共价缔合。

[0205] 作为特定的实例，本公开内容提供了融合蛋白，其包含融合至包含免疫球蛋白的恒定结构域例如免疫球蛋白的CH1、CH2或CH3结构域或Fc结构域的多肽的ALK7或ActRIIB。本文提供了源自人IgG1、IgG2、IgG3和IgG4的Fc结构域。已知减少CDC或ADCC活性的其它突变，总的来说，任何这些变体包括在本公开内容内，和可用作本公开内容的异多聚体复合物的有利组分。任选地，SEQ ID NO:31的IgG1 Fc结构域在残基例如Asp-265、Lys-322和Asn-434处具有一个或多个突变(根据相应的全长IgG1编号)。在某些情况下，相对于野生型Fc结构域，具有一个或多个这些突变(例如，Asp-265突变)的突变体Fc结构域具有减少的结合Fcγ受体的能力。在其它情况下，相对于野生型Fc结构域，具有一个或多个这些突变(例如，Asn-434突变)的突变体Fc结构域具有增加的结合MHC I类相关Fc-受体(FcRN)的能力。

[0206] 可用于人IgG1的Fc部分(G1Fc)的天然氨基酸序列的实例在下文显示 (SEQ ID NO:31)。虚下划线表示铰链区，和实下划线表示具有天然存在的变体的位置。部分地，本公开内容提供了包含与SEQ ID NO:31具有70％、 75％、80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％同一性的氨基酸序列的多肽，基本上由其组成或由其组成。根据SEQ ID NO:31使用的编号系统，G1Fc 的天然存在的变体包括E134D和M136L(参见Uniprot P01857)。

```
   1  THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE
  51  VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK
101  VSNKALPAPI EKTISKAKGQ PREPQVYTLP PSREEMTKNQ VSLTCLVKGF
151  YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSKLTV DKSRWQQGNV
201  FSCSVMHEAL HNHYTQKSLS LSPGK      (SEQ ID NO: 31)
```

[0207]

[0208] 可用于人IgG2的Fc部分(G2Fc)的天然氨基酸序列的实例在下文显示(SEQ ID NO:32)。虚下划线表示铰链区和双下划线表示其中在序列 (根据UniProt P01859)中存在数据库冲突的位置。部分地，本公开内容提供了包含与SEQ ID NO:32具有70％、75％、80％、85％、86％、87％、 88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、 99％或100％同一性的氨基酸序列的多肽，基本上由其组成或由其组成。

```
  1  VECPPCPAPP VAGPSVFLFP PKPKDTLMIS RTPEVTCVVV DVSHEDPEVQ
 51  FNWYVDGVEV HNAKTKPREE QFNSTFRVVS VLTVVHQDWL NGKEYKCKVS
101  NKGLPAPIEK TISKTKGQPR EPQVYTLPPS REEMTKNQVS LTCLVKGFYP
151  SDIAVEWESN GQPENNYKTT PPMLDSDGSF FLYSKLTVDK SRWQQGNVFS
201  CSVMHEALHN HYTQKSLSLS PGK       (SEQ ID NO: 32)
```

[0209]

[0210] 可用于人IgG3的Fc部分(G3Fc)的氨基酸序列的两个实例在下文显示。G3Fc的铰链区可达到其它Fc链的4倍长，和包含三个相同的15- 残基区段，前面是类似的17-残基区段。在下文显示的第一G3Fc序列(SEQ ID NO:33)包含由单一15-残基区段组成的短的铰链区，而第二G3Fc序列(SEQ ID NO:34)包含全长铰链区。在各情况下，虚下划线表示铰链区，和实下划线表示根据UniProt P01859具有天然存在的变体的位置。部分地，本公开内容提供了包含与SEQ ID NO:33和34具有70％、75％、80％、 85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、 96％、97％、98％、99％或100％同一性的氨基酸序列的多肽，基本上由其组成或由其组成。

```
  1  EPKSCDTPPP CPRCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD
 51  VSHEDPEVQF KWYVDGVEVH NAKTKPREEQ YNSTFRVVSV LTVLHQDWLN
101  GKEYKCKVSN KALPAPIEKT ISKTKGQPRE PQVYTLPPSR EEMTKNQVSL
151  TCLVKGFYPS DIAVEWESSG QPENNYNTTP PMLDSDGSFF LYSKLTVDKS
201  RWQQGNIFSC SVMHEALHNR FTQKSLSLSP GK    (SEQ ID NO: 33)
```

[0211]

```
  1  ELKTPLGDTT HTCPRCPEPK SCDTPPPCPR CPEPKSCDTP PPCPRCPEPK
 51  SCDTPPPCPR CPAPELLGGP SVFLFPPKPK DTLMISRTPE VTCVVVDVSH
101  EDPEVQFKWY VDGVEVHNAK TKPREEQYNS TFRVVSVLTV LHQDWLNGKE
151  YKCKVSNKAL PAPIEKTISK TKGQPREPQV YTLPPSREEM TKNQVSLTCL
201  VKGFYPSDIA VEWESSGQPE NNYNTTPPML DSDGSFFLYS KLTVDKSRWQ
251  QGNIFSCSVM HEALHNRFTQ KSLSLSPGK       (SEQ ID NO: 34)
```

[0212] 当转化为SEQ ID NO:33中使用的编号系统时,G3Fc(例如,参见Uniprot P01860)的天然存在的变体包括E68Q、P76L、E79Q、 Y81F、D97N、N100D、T124A、S169N、S169del、F221Y,和本公开内容提供了包含G3Fc结构域的融合蛋白,所述结构域包含一个或多个这些变化。此外,人免疫球蛋白IgG3基因(IGHG3)显示特征为不同的铰链长度的结构多态性[参见Uniprot P01859]。特别地,变体WIS 缺少大部分V区和所有的CH1区。除了铰链区的位置11正常存在的链间二硫键之外,在位置7处具有额外的链间二硫键。变体ZUC缺少大部分V区、所有的CH1区和部分的铰链。变体OMM可表示等位基因形式或另一γ链亚类。本公开内容提供了包含G3Fc结构域的另外的融合蛋白,所述结构域包含一种或多种这些变体。

[0213] 可用于人IgG4的Fc部分(G4Fc)的天然氨基酸序列的实例在下文显示(SEQ ID NO:35)。虚下划线表示铰链区。部分地,本公开内容提供了包含与SEQ ID NO:35具有70%、75%、80%、85%、86%、87%、88%、 89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或 100%同一性的氨基酸序列的多肽,基本上由其组成或由其组成。

```
  1  ESKYGPPCPS CPAPEFLGGP SVFLFPPKPK DTLMISRTPE VTCVVVDVSQ
 51  EDPEVQFNWY VDGVEVHNAK TKPREEQFNS TYRVVSVLTV LHQDWLNGKE
101  YKCKVSNKGL PSSIEKTISK AKGQPREPQV YTLPPSQEEM TKNQVSLTCL
151  VKGFYPSDIA VEWESNGQPE NNYKTTPPVL DSDGSFFLYS RLTVDKSRWQ
201  EGNVFSCSVM HEALHNHYTQ KSLSLSLGK      (SEQ ID NO: 35)
```

[0214]

[0215] 对于G1Fc序列(SEQ ID NO:31),本文提供了Fc结构域的各种工程改造的突变,和G2Fc、G3Fc和G4Fc中的类似突变可源自在图5 中它们与G1Fc的比对。由于不等的铰链长度,基于同种型比对(图5) 的类似的Fc位置在SEQ ID NOs:31、32、33、34和35中具有不同的氨基酸编号。还可理解,在由铰链、C_H2和C_H3区组成的免疫球蛋白序列(例如,SEQ ID NOs:31、32、33、34和35)中给定的氨基酸位置将通过与当编号包括Uniprot数据库中整个IgG1重链恒定结构域(由 $C_{II}1$、铰链、$C_{II}2$和$C_{II}3$区组成)时的相同位置不同的编号来鉴定。例如,在人G1Fc序列(SEQ ID NO:31)、人IgG1重链恒定结构域(Uniprot P01857)和人IgG1重链中选择的C_H3位置之间的对应关系如下。

[0216]

不同的编号系统中的 C_H3 位置的对应关系		
G1Fc (编号开始于铰链区的第一个苏氨酸)	IgG1 重链 恒定结构域 (编号开始于 C_H1)	IgG1 重链 (Kabat 等，1991*的 EU 编号方案)
Y127	Y232	Y349
S132	S237	S354
E134	E239	E356
T144	T249	T366
L146	L251	L368
K170	K275	K392
D177	D282	D399

[0217]

Y185	Y290	Y407
K187	K292	K409
* Kabat 等(编辑) 1991; *Sequences of Proteins of Immunological Interest*, 5th ed., Vol. 1, NIH, Bethesda, MD 的 pp. 688-696		

[0218] 从单一细胞系大规模生产不对称的基于免疫球蛋白的蛋白产生的问题被称为“链缔合问题”。如在产生双特异性抗体中所主要面临的，链缔合问题涉及从当不同的重链和/或轻链在单一细胞系中产生时固有产生的多个组合中有效产生需要的多链蛋白的挑战[Klein等(2012) mAbs 4:653-663]。当两种不同的重链和两种不同的轻链在相同的细胞中产生时，该问题是极其明显的，在该情况下存在总共16种可能的链组合(尽管这些中的一些是相同的)，此时仅一种可能通常是需要的。然而，相同的原理说明了掺有仅两种不同的(不对称的)重链的所需多链融合蛋白的降低的收率。

[0219] 在单一细胞系中增加含Fc融合多肽链的所需配对以可接受的收率产生优选的不对称的融合蛋白的各种方法是本领域已知的[Klein等 (2012)mAbs 4:653-663;和Spiess等(2015)Molecular Immunology 67(2A):95-106]。获得需要的含Fc链的配对的方法包括但不限于，基于电荷的配对(静电导引)、“结进孔(knobs-into-holes)”空间配对、SEEDbody配对和基于亮氨酸拉链的配对[Ridgway等(1996)Protein Eng 9:617-621;Merchant等(1998)Nat Biotech 16:677-681;Davis等 (2010)Protein Eng Des Sel 23:195-202;Gunasekaran等(2010); 285:19637-19646;Wranik等(2012)J Biol Chem 287:43331-43339; US5932448;WO 1993/011162;WO 2009/089004和WO 2011/034605]。如本文所述的，这些方法可用于产生ALK7-Fc:ActRIIB-Fc异多聚体复合物，参见图8-10。

[0220] 例如，可促进特定多肽之间的相互作用的一种方法是通过改造结节进洞(protuberance-into-cavity)(结进孔(knobs-into-holes))互补区，例如描述于Arathoon等,U.S.7,183,076和Carter等,U.S.5,731,168。“结节”通过用较大的侧链(例如，酪氨酸或色氨酸)替换来自第一多肽(例如，第一相互作用对)的界面的小的氨基酸侧链构建。与结节相同或类似大小的互补“洞”在第二多肽(例如，第二相互作用对)的界面上任选地通过用较

小的氨基酸侧链(例如,丙氨酸或苏氨酸)替换大的氨基酸侧链产生。在合适定位和尺寸的结节或洞存在于第一或第二多肽的界面处时,只需要在邻近界面处各自改造相应的洞或结节。

[0221] 在中性pH(7.0)时,天冬氨酸和谷氨酸带负电荷,和赖氨酸、精氨酸和组氨酸带正电荷。这些带电荷残基可用于促进异二聚体形成,同时妨碍同二聚体形成。吸引相互作用在相反电荷之间发生,和排斥相互作用在相同电荷之间发生。部分地,通过进行带电荷界面残基的定点诱变,本文公开的蛋白复合物利用吸引相互作用以促进异多聚体形成(例如,异二聚体形成),和任选地利用排斥相互作用以妨碍同二聚体形成(例如,同二聚体形成)。

[0222] 例如,IgG1 CH3结构域界面包含四个独特的带电荷残基对,其参与结构域-结构域相互作用:Asp356-Lys439'、Glu357-Lys370'、Lys392-Asp399'和Asp399-Lys409'[第二链中的残基编号通过(')表示]。应注意,此处用于指定IgG1 CH3结构域中的残基的编号方案符合 Kabat的EU编号方案。由于CH3-CH3结构域相互作用中存在的2- 倍对称,每个独特的相互作用在结构中将出现两次(例如, Asp-399-Lys409'和Lys409-Asp399')。在野生型序列中,K409-D399'有利于异二聚体和同二聚体二者形成。第一链中改变电荷极性的单一突变(例如,K409E;正电荷至负电荷)导致对形成第一链同二聚体不利的相互作用。该不利的相互作用由于在相同电荷之间发生的排斥相互作用(负-负;K409E-D399'和D399-K409E')而产生。第二链中改变电荷极性的类似的突变(D399K';负电荷至正电荷)导致对于第二链同二聚体形成不利的相互作用(K409'-D399K'和D399K-K409')。但同时,这两个突变(K409E和D399K')导致对于异二聚体形成有利的相互作用(K409E-D399K'和D399-K409')。

[0223] 对异二聚体形成和同二聚体阻碍的静电导引作用可进一步通过另外的带电荷残基的突变来增强,所述残基可以与或可以不与第二链中的相反电荷残基,包括例如,Arg355和Lys360配对。下表列出了可使用的可能的电荷改变突变(单独或组合),以增强ALK7:ActRIIB 异多聚体形成。

[0224]

增强异二聚体形成的配对带电荷残基突变的实例			
第一链中的位置	第一链中的突变	第二链中的相互作用位置	第二链中相应的突变
Lys409	Asp 或 Glu	Asp399'	Lys、Arg 或 His
Lys392	Asp 或 Glu	Asp399'	Lys、Arg 或 His
Lys439	Asp 或 Glu	Asp356'	Lys、Arg 或 His
Lys370	Asp 或 Glu	Glu357'	Lys、Arg 或 His
Asp399	Lys、Arg 或 His	Lys409'	Asp 或 Glu
Asp399	Lys、Arg 或 His	Lys392'	Asp 或 Glu
Asp356	Lys、Arg 或 His	Lys439'	Asp 或 Glu
Glu357	Lys、Arg 或 His	Lys370'	Asp 或 Glu

[0225] 在一些实施方案中,构成本中请的融合蛋白中的CH3-CH3界面的一个或多个残基

被带电荷氨基酸替换，使得相互作用成为静电不利的。例如，界面中的带正电荷的氨基酸(例如，赖氨酸、精氨酸或组氨酸)被带负电荷的氨基酸(例如，天冬氨酸或谷氨酸)替换。或者，或与前述置换组合，界面中的带负电荷的氨基酸被带正电荷的氨基酸替换。在某些实施方案中，氨基酸被具有所需的电荷性质的非天然存在的氨基酸替换。应注意，将带负电荷残基(Asp或Glu)突变为His导致侧链体积增加，这可引起空间问题。此外，His质子供体-和受体-形式取决于局部环境。设计策略应考虑这些问题。因为界面残基在人和小鼠IgG 亚类中高度保守，本文公开的静电导引作用可适用于人和小鼠IgG1、IgG2、IgG3和IgG4。该策略还可扩张至改变CH3结构域界面处的不带电荷的残基为带电荷的残基。

[0226] 部分地，本公开内容提供了使用经工程改造以根据电荷配对(静电导引)互补的Fc序列，不对称的含Fc多肽链的所需配对。具有静电互补性的一对Fc序列之一可任意地融合至构建体的ALK7或ActRIIB 多肽，含或不含任选的接头，以产生ALK7:ActRIIB异多聚体。该单一链可在选择的细胞中同与第一Fc互补的Fc序列一起共表达，以帮助产生所需的多链构建体(例如，ALK7:ActRIIB异多聚体)。在基于静电导引的该实例中，SEQ ID NO:23[人G1Fc(E134K/D177K)]和SEQ ID NO:24[人G1Fc(K170D/K187D)]是互补Fc序列的实例，其中工程改造的氨基酸置换用双下划线表示，和构建体的TGF-β超家族I型或 II型受体多肽可融合至SEQ ID NO:23或SEQ ID NO:24，但不是二者。鉴于在天然hG1Fc、天然hG2Fc、天然hG3Fc和天然hG4Fc之间高程度的氨基酸序列同一性，可以理解，在hG2Fc、hG3Fc或hG4Fc 的相应位置处的氨基酸置换(参见图5)将产生互补Fc对，其可用于代替下文的互补hG1Fc对(SEQ ID NOs:23和24)。

[0227]

```
  1  THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE
 51  VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK
101  VSNKALPAPI EKTISKAKGQ PREPQVYTLP PSRKEMTKNQ VSLTCLVKGF
151  YPSDIAVEWE SNGQPENNYK TTPPVLKSDG SFFLYSKLTV DKSRWQQGNV
201  FSCSVMHEAL HNHYTQKSLS LSPGK      (SEQ ID NO: 23)

  1  THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE
 51  VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK
101  VSNKALPAPI EKTISKAKGQ PREPQVYTLP PSREEMTKNQ VSLTCLVKGF
151  YPSDIAVEWE SNGQPENNYD TTPPVLDSDG SFFLYSDLTV DKSRWQQGNV
201  FSCSVMHEAL HNHYTQKSLS LSPGK      (SEQ ID NO: 24)
```

[0228] 部分地，本公开内容提供了使用对于空间互补性经工程改造的Fc 序列，不对称的含Fc多肽链的所需配对。部分地，本公开内容提供了结进孔配对作为空间互补性的实例。具有空间互补性的一对Fc序列之一可任意地融合至构建体的ALK7或ActRIIB多肽，含或不含任选的接头，以产生ALK7:ActRIIB异多聚体。该单一链可在选择的细胞中同与第一Fc互补的Fc序列一起共-表达，以帮助产生所需的多链构建体。在基于结进孔配对的该实例中，SEQ ID NO:25[人G1Fc(T144Y)]和SEQ ID NO:26[人G1Fc(Y185T)]是互补Fc序列的实例，其中工程改造的氨基酸置换用双下划线表示和构建体的ALK7或 ActRIIB多肽可融合至SEQ ID NO:25或SEQ ID NO:26，但不是二者。鉴于在天然hG1Fc、天然hG2Fc、天然hG3Fc和天然hG4Fc之间高程度的氨基酸序列同一性，可以理解，在hG2Fc、hG3Fc或hG4Fc的相应位置处的氨基酸置换(参见图5)将产生互补Fc对，其可用于代替下文的互补hG1Fc对(SEQ ID NOs:25和26)。

[0229]

```
  1  THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE
 51  VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK
101  VSNKALPAPI EKTISKAKGQ PREPQVYTLP PSREEMTKNQ VSLYCLVKGF
151  YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSKLTV DKSRWQQGNV
201  FSCSVMHEAL HNHYTQKSLS LSPGK          (SEQ ID NO: 25)
  1  THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE
 51  VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK
101  VSNKALPAPI EKTISKAKGQ PREPQVYTLP PSREEMTKNQ VSLTCLVKGF
151  YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLTSKLTV DKSRWQQGNV
201  FSCSVMHEAL HNHYTQKSLS LSPGK          (SEQ ID NO: 26)
```

[0230] 基于结进孔配对结合工程改造的二硫键的Fc互补性的实例公开于 SEQ ID NO:27[hG1Fc(S132C/T144W)]和SEQ ID NO:28 [hG1Fc(Y127C/T144S/L146A/Y185V)]。这些序列中的工程改造的氨基酸置换用双下划线表示,和构建体的TGF-β超家族I型或II型多肽可融合至 SEQ ID NO:27或SEQ ID NO:28,但不是二者。鉴于在天然hG1Fc、天然 hG2Fc、天然hG3Fc和天然hG4Fc之间高程度的氨基酸序列同一性,可以理解,在hG2Fc、hG3Fc或hG4Fc的相应位置处的氨基酸置换(参见图5)将产生互补Fc对,其可用于代替下文的互补hG1Fc对(SEQ ID NOs:27和28)。

[0231]

```
  1  THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE
 51  VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK
101  VSNKALPAPI EKTISKAKGQ PREPQVYTLP PCREEMTKNQ VSLWCLVKGF
151  YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSKLTV DKSRWQQGNV
201  FSCSVMHEAL HNHYTQKSLS LSPGK          (SEQ ID NO: 27)
```

[0232]

```
  1  THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE
 51  VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK
101  VSNKALPAPI EKTISKAKGQ PREPQVCTLP PSREEMTKNQ VSLSCAVKGF
151  YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLVSKLTV DKSRWQQGNV
201  FSCSVMHEAL HNHYTQKSLS LSPGK          (SEQ ID NO: 28)
```

[0233] 部分地,使用经工程改造以产生人IgG和IgA C_H3结构域的互相交叉β-链区段的Fc序列,本公开内容提供了所需的不对称的含Fc多肽链的配对。这样的方法包括使用链交换工程改造结构域(SEED)C_H3异二聚体,其允许形成SEEDbody融合蛋白[Davis等(2010) Protein Eng Design Sel 23:195-202]。具有SEEDbody互补性的一对Fc序列之一可任意地融合至构建体的ALK7或ActRIIB,含或不含任选的接头,以产生ALK7或ActRIIB 融合多肽。该单一链可在选择的细胞中同与第一Fc互补的Fc序列一起共表达以有利于所需多链构建体的产生。在基于SEEDbody(Sb)配对的该实例中,SEQ ID NO:29[hG1Fc(Sb_{AG})]和SEQ ID NO:30[hG1Fc(Sb_{GA})]是互补IgG Fc序列的实例,其中来自IgA Fc的工程改造的氨基酸置换用双下划线表示,和构建体的ALK7或ActRIIB多肽可融合至SEQ ID NO:29或SEQ ID NO:30,但不是二者。鉴于在天然hG1Fc、天然hG2Fc、天然hG3Fc和天然hG4Fc之间高程度的氨基酸序列同一性,可以理解,在hG1Fc、hG2Fc、 hG3Fc或hG4Fc的相应位置处的氨基酸置换(见图5)将产生Fc单体,其可用于下文的互补IgG-IgA对(SEQ ID NOs:29和30)。

[0234]

```
  1  THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE
 51  VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK
101  VSNKALPAPI EKTISKAKGQ PFRPEVHLLP PSREEMTKNQ VSLTCLARGF
151  YPKDIAVEWE SNGQPENNYK TTPSRQEPSQ GTTTFAVTSK LTVDKSRWQQ
201  GNVFSCSVMH EALHNHYTQK TISLSPGK     (SEQ ID NO: 29)
```

```
  1  THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE
 51  VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK
101  VSNKALPAPI EKTISKAKGQ PREPQVYTLP PPSEELALNE LVTLTCLVKG
151  FYPSDIAVEW ESNGQELPRE KYLTWAPVLD SDGSFFLYSI LRVAAEDWKK
201  GDTFSCSVMH EALHNHYTQK SLDRSPGK     (SEQ ID NO: 30)
```

[0235] 部分地，本公开内容提供了所需的不对称的含Fc多肽链的配对，其中可裂解的亮氨酸拉链结构域连接至Fc C_H3结构域的C-末端。亮氨酸拉链的连接足以引起异二聚体抗体重链的优先装配[Wranik等 (2012)J Biol Chem 287:43331-43339]。如本文公开的，连接至亮氨酸拉链-形成链的一对Fc序列之一可任意地融合至构建体的ALK7或 ActRIIB多肽，含或不含任选的接头，以产生ALK7或ActRIIB融合多肽。该单一链可在选择的细胞中同连接至互补亮氨酸拉链-形成链的 Fc序列一起共表达，以利于所需多链构建体的产生。纯化后构建体用细菌内切蛋白酶Lys-C进行蛋白水解消化可释放亮氨酸拉链结构域，产生Fc构建体，其结构与天然Fc相同。在基于亮氨酸拉链配对的该实例中，SEQ ID NO:36[hG1Fc-Ap1(酸性)]和SEQ ID NO:37 [hG1Fc-Bp1(碱性)]是互补IgG Fc序列的实例，其中工程改造的互补亮氨酸拉链序列用下划线表示，和构建体的ALK7或ActRIIB多肽可融合至SEQ ID NO:36或SEQ ID NO:37，但不是二者。鉴于在天然 hG1Fc、天然hG2Fc、天然hG3Fc和天然hG4Fc之间高程度的氨基酸序列同一性，可以理解，连接至hG1Fc、hG2Fc、hG3Fc或hG4Fc的亮氨酸拉链-形成序列(含或不含任选的接头)(见图5)将产生Fc单体，其可用于下文的互补亮氨酸拉链-形成对(SEQ ID NOs:36和37)。

[0236]

```
  1  THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE
 51  VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK
101  VSNKALPAPI EKTISKAKGQ PREPQVYTLP PSREEMTKNQ VSLTCLVKGF
151  YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSKLTV DKSRWQQGNV
201  FSCSVMHEAL HNHYTQKSLS LSPGKGGSAQ LEKELQALEK ENAQLEWELQ
251  ALEKELAQGA T          (SEQ ID NO: 36)
```

```
  1  THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE
 51  VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK
101  VSNKALPAPI EKTISKAKGQ PREPQVYTLP PSREEMTKNQ VSLTCLVKGF
151  YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSKLTV DKSRWQQGNV
201  FSCSVMHEAL HNHYTQKSLS LSPGKGGSAQ LKKKLQALKK KNAQLKWKLQ
251  ALKKKLAQGA T          (SEQ ID NO: 37)
```

[0237] 如上文所述的，在单一细胞系中增加含Fc融合多肽链的所需配对以可接受的收率产生优选的不对称的融合蛋白的各种方法是本领域已知的[Klein等(2012)mAbs 4:653-663;和Spiess等(2015)Molecular Immunology 67(2A):95-106]。此外，ALK7:ActRIIB异多聚体可使用包含ALK7或ActRIIB多肽的重链和轻链融合蛋白的组合产生。例如，在一些实施方案中，ALK7多肽可融合(含或不含接头结构域)至免疫球蛋白重链(IgG1、IgG2、IgG3、

IgG4、IgM、IgA1或IgA2)，其包含至少一部分C_H1结构域。类似地，ActRIIB多肽可融合(含或不含接头结构域)至免疫球蛋白轻链(κ或λ)，其包含至少一部分轻链恒定结构域(C_L)。在可选的实施方案中，ActRIIB多肽可融合(含或不含接头结构域)至免疫球蛋白重链(IgG1、IgG2、IgG3、IgG4、IgM、IgA1或IgA2)，其包含至少一部分C_H1结构域，和ALK7多肽可融合(含或不含接头结构域)至免疫球蛋白轻链(κ或λ)，其包含至少一部分轻链恒定结构域(C_L)。该设计利用重链与轻链异二聚化的天然能力。特别是，重链和轻链的异二聚化在C_H1与C_L之间发生，其通常通过经过二硫桥共价连接两个结构域来稳定。使用全长重链或包含铰链区的至少一部分重链的构建体可产生抗体-样分子，其包含两个“轻链”和两个“重链”。参见图9。该设计的潜在益处是其可更紧密模拟天然存在的ALK7-配体-ActRIIB复合物和可显示比相当的单异二聚体更高的配体亲和力。在一些实施方案中，该设计可通过掺入各种重链截短进行修改，包括例如，包含C_H1结构域和一些或全部铰链结构域的截短(产生$F(ab')_2$- 样分子)以及仅包含C_H1结构域或其片段的截短(产生Fab-样分子)。参见图9G。用于设计这样的异多聚体构建体的各种方法描述于US 2009/0010879、Klein等[(2012)mAbs 4:653-663]和Spiess等[(2015) Molecular Inmunology 67(2A):95-106]，其内容以其整体并入本文。

[0238] 在一些实施方案中，需要产生抗体-样ALK7:ActRIIB异二聚体，其包含至少一个分支的复合物，其包含ALK7-C_L:ActRIIB-C_H1异二聚体对；和至少第二分支，其包含ActRIIB-C_L:ALK7-C_H1异二聚体对。参见例如，图9B。这样的异二聚体复合物可例如，使用重链和轻链不对称配对技术的组合产生[Spiess等(2015)Molecular Immunology 67(2A):95-106]。例如，在CrossMab技术[Schaefer等(2011).Proc.Natl. Acad.Sci.U.S.A.108:11187-11192]中，轻链错配使用结构域交叉 (crossover)和使用结进孔异二聚化的重链克服[Merchant等(1998)Nat. Biotechnol.16:677-681]。对于结构域交叉，可变结构域或恒定结构域在轻链和重链之间交换以产生两个不对称的Fab臂，其驱动同源轻链配对，同时保留可变结构域的结构和功能完整性[Fenn等(2013)PLoS ONE 8:e61953]。用于克服轻链错配的可选的方法是设计具有正交Fab 界面的重链和轻链[Lewis(2014)Nat.Biotechnol.32:191-198]。这已通过计算建模[Das等(2008)Annu.Rev.Biochem.77:363-382]与X-射线晶体学的组合实现，以鉴定V_H/V_L和C_H1/C_L界面的突变。对于使用该方法产生的异二聚体，可能需要工程改造突变至V_H/V_L和C_H1/C_L界面二者以最小化重链/轻链错配。设计的正交Fab界面可结合重链异二聚化策略使用，以促进在单一宿主细胞中有效的IgG产生。静电导引也可用于产生正交Fab界面以促进这样的异二聚体的构建。肽接头可用于确保轻链和重链以称为”LUZ-Y”的形式同源配对[Wranik等(2012)J. Biol.Chem.287:43331-43339]，其中重链异二聚化使用亮氨酸拉链实现，其可随后通过蛋白质水解体外除去。

[0239] 或者，ALK7:ActRIIB异多聚体可包含本文所述的一种或多种单链配体陷阱，任选地其可与一种或多种ALK7或ActRIIB多肽以及另外的ALK7:ActRIIB单链配体陷阱共价或非共价缔合[US 2011/0236309和US2009/0010879]。参见图12。如本文所述的，单链配体陷阱不需要与任何多聚化结构域例如多价的盘绕线圈Fc结构域融合。一般而言，本公开内容的单链配体陷阱至少包含一个ALK7多肽结构域和一个ActRIIB多肽结构域。ALK7和ActRIIB多肽结构域，在本文通常称为结合结构域(BD)，任选地可通过接头区连接。

[0240] 例如，在一个方面，本公开内容提供了异多聚体，其包含具有以下结构的多肽：

[0241] $(<BD1>\text{-接头1})_k\text{-}[<BD2>\text{-接头2-}\{<BD3>\text{-接头3}\}_f]_n\text{-}(<BD4>)_m\text{-}(\text{接头4-}BD5>_d)_h$

[0242] 其中:n和h独立地大于或等于1;d、f、m和k独立地等于或大于0;BD1、BD2、BD3、BD4和BD5独立地是ALK7或ActRIIB多肽结构域,其中BD1、BD2、BD3和BD4的至少一个是ALK7多肽结构域和其中BD1、BD2、BD3和BD4的至少一个是ActRIIB多肽结构域,和接头1、接头2、接头3和接头4独立地大于或等于0。在一些实施方案中,ALK7:ActRIIB单链陷阱包含至少两种不同的ALK7 多肽。在一些实施方案中,ALK7:ActRIIB单链陷阱包含至少两种不同的ActRIIB多肽。在一些实施方案中,ALK7:ActRIIB单链陷阱包含至少两种不同的接头。根据d、f、h、k、m和n的选择的值,异多聚体结构可包含以各种组合的大量重复单元,或可以是相对简单的结构。

[0243] 在另一个方面,本公开内容提供了异多聚体,其包含具有以下结构的多肽:

[0244] <BD1>-接头1-<BD2>

[0245] 在又一方面,本公开内容提供了异多聚体,其包含具有以下结构的多肽:

[0246] <BD1>-(接头2-<BD2>)$_n$

[0247] 其中n大于或等于1。

[0248] 本发明的另一方面提供了异多聚体,其包含具有以下结构的多肽:

[0249] (<BD1>-接头1-<BD1>)$_f$-接头2-(<BD2>-接头3-<BD3>)$_g$

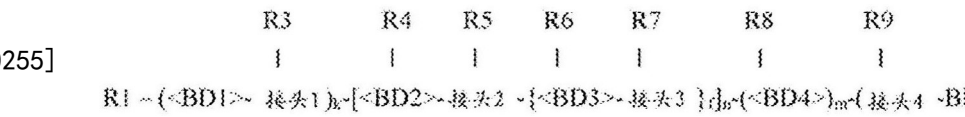
[0250] 其中f和g大于或等于1。

[0251] 在其中BD2和BD3相同和f和g是相同数字的实施方案中,这可产生围绕接头2的实质上镜像对称的结构,只是接头中存在差异。在其中BD2不同于BD3和/或其中f和g是不同数字的情况下,将产生不同的结构。根据本文的公开内容和本领域的知识选择合适的结合结构域、接头和重复频率在本领域普通技术人员的能力范围内。根据本公开内容的这样的单链配体陷阱的具体的非限制性实例在图11中图示提供。

[0252] 接头(1、2、3和4)可以是相同的或不同的。接头区提供了不同于 ALK7和ActRIIB的结构化配体-结合结构域的区段,因此可用于与附加分子(例如,可用于增加稳定性的分子,例如PEG化部分)缀合,无需化学修饰结合结构域。接头可包括非结构化的氨基酸序列,其可以与目的配体的受体或TGF-β超家族的另一受体的细胞外部分中的天然非结构化区域的序列相同,或源自其保守修饰。在其它情况下,这样的接头在组成和来源上可以是完全人工的,但包含经选择以提供非结构化的柔性接头的氨基酸,当紧密靠近目的配体时其具有遇到静电或空间位阻复杂化的低可能性。当允许位于接头的每个N-和C-端上的结合结构域结合它们在其天然配体上的天然结合位点时,接头长度被认为是可接受的,使得配体以比通过仅结合一个结合结构域更高的亲和力与如此结合的两个结合结构域结合。在一些实例中,天然或人工来源的接头的氨基酸残基数量经选择为等于或大于同时(桥接)结合至ALK7和/或ActRIIB配体上的两个结合位点的最小需要距离。例如并且不希望以任何方式受到限制,接头长度可以为约1-10个氨基酸、10-20个氨基酸、18-80个氨基酸、25-60个氨基酸、35-45个氨基酸或任何其它合适的长度。

[0253] 接头可经设计以促进多肽的纯化。选择的准确纯化方案将决定需要什么样的修饰,例如和不希望受到限制,考虑添加纯化“标签”例如 His标签;在其它实例中,接头可包括促进添加货物或附加分子的区域。当这样的添加影响接头的非结构化性质或引入潜在的静电或空间顾虑时,进行接头长度的合适增加以确保两个结合结构域能够结合它们各自在配体上的位点。根据本文的方法和教导,本领域技术人员可常规进行这样的决定。

[0254] 此外，本发明的设计允许连接其它货物分子(例如成像剂，例如荧光分子)、毒素等。例如和不希望以任何方式受到限制，单链多肽可经修饰以添加一种或多种货物和/或附加分子(在本文统称为R1、R2、R3、 R4等)：

[0255] 

[0256] 不限制可用的R取代基的普遍性，R1、R2、R3、R4、R5、R6、 R7、R8、R9可以存在或可以不存在；当存在时，它们可以是相同的或不同的和可独立地是以下一种或多种：用于靶向的融合蛋白，例如但不限于例如抗体片段(例如，单链Fv)和/或单结构域抗体(sdAb)；放射疗法和/或成像剂，例如但不限于放射性核素(例如，^{123}I、^{111}In、^{18}F、^{64}C、^{68}Y、^{124}I、^{131}I、^{90}Y、^{177}Lu、^{57}Cu、^{213}Bi、^{211}At)、荧光染料(例如， Alexa Fluor、Cy染料)和/或荧光蛋白标签(例如，GFP、DsRed)；用于化学疗法的细胞毒性剂，例如但不限于多柔比星、卡奇霉素、类美登素衍生物(例如，DM1、DM4)、毒素(例如，截短的假单胞菌内毒素A、白喉毒素)；基于纳米颗粒的载体，例如但不限于聚乙二醇(PEG)、缀合至药物的聚合物、纳米载体或成像剂(例如，聚合物N-(2-羟基丙基) 甲基丙烯酰胺(HPMA)、谷氨酸、PEG、葡聚糖)；药物(例如，但不限于多柔比星、喜树碱、紫杉醇、palatinate)；纳米载体，例如但不限于纳米外壳或脂质体；成像剂，例如但不限于超磁氧化铁(SPIO)；树枝状聚体；和/或用于配体纯化、浓缩或隔离的固体支持物(例如，纳米颗粒、惰性树脂、合适的二氧化硅支持物)。

[0257] 一般而言，不优选在所有可能的位置具有货物或附加分子，因为这可引起空间或静电复杂化。然而，按本文所教导的，添加货物或附加分子至任何给定的一个或多个位置对结构的影响可根据本文的公开内容通过对在结合结构域之间的接头建模和进行分子动力学模拟常规确定，以显著最小化分子力学能量和减少在接头和ALK7和 ActRIIB多肽之间的空间和静电不相容性。

[0258] 可能优选添加货物或附加分子至试剂的接头部分，而不是结合结构域，以减少干扰结合功能的可能性。然而，添加至结合结构域是可能的，在一些情况下可能是理想的，和这样的添加的影响可通过用按本文所述的提议的添加对结合剂和接头建模，常规地预先确定。

[0259] 缀合方法可使用市售的试剂盒进行，所述试剂盒能够通过常见的反应性基团，例如伯胺、琥珀酰亚胺(NHS)酯和巯基-反应性基团进行缀合。一些非限制性实例是：Alexa Fluor 488蛋白标记试剂盒 (Molecular Probes，Invitrogen detection technologies)和PEG化试剂盒 (Pierce Biotechnology Inc.)。

[0260] 在某些方面，ALK7:ActRIIB单链陷阱可与一种或多种ALK7或 ActRIIB多肽以及另外的ALK7:ActRIIB单链配体陷阱共价或非共价缔合，以形成更高级异多聚体，其可根据本文所述的方法使用。参见例如，图12。例如，ALK7:ActRIIB单链配体陷阱还可包含本文所述的多聚化结构域。在一些实施方案中，ALK7:ActRIIB单链配体陷阱包含Ig免疫球蛋白的恒定结构域。这样的免疫球蛋白恒定结构域可经选择以促进包含至少一个单链ALK7:ActRIIB陷阱的对称或不对称的复合物。

[0261] 在某些方面，ALK7:ActRIIB单链陷阱或这样的陷阱的组合可用作ALK7:ActRIIB拮抗剂以治疗或预防本文所述的ALK7:ActRIIB病症或疾病(例如，肾疾病和/或代谢病症或病

况)。

[0262] 应理解,融合蛋白(例如,免疫球蛋白Fc融合蛋白)的不同的元件可以与所需功能性一致的任何方式排列。例如,ALK7和/或ActRIIB 多肽结构域可位于异源结构域的C-末端,或者,异源结构域可位于 ALK7和/或ActRIIB多肽结构域的C-末端。ALK7和/或ActRIIB多肽结构域和异源结构域在融合蛋白中不需要相邻,和另外的结构域或氨基酸序列可包括在任一结构域的C-或N-末端或在结构域之间。

[0263] 例如,ALK7和/或ActRIIB受体融合蛋白可包含式A-B-C所示的氨基酸序列。B部分对应于ALK7或ActRIIB多肽结构域。A和C部分可独立地是零、一个或超过一个氨基酸,和A和C部分二者当存在时对B是异源的。A和/或C部分可通过接头序列连接至B部分。接头可以富含甘氨酸(例如,2-10、2-5、2-4、2-3个甘氨酸残基)或甘氨酸和脯氨酸残基,和可例如包含苏氨酸/丝氨酸和甘氨酸的单一序列,或苏氨酸/丝氨酸和/或甘氨酸的重复序列,例如,GGG(SEQ ID NO: 13)、GGGG(SEQ ID NO:14)、TGGGG(SEQ ID NO:15)、SGGGG(SEQ ID NO:16)、TGGG(SEQ ID NO:17)、SGGG(SEQ ID NO:18)或 GGGGS(SEQ ID NO:58)单一态或重复。在某些实施方案中,ALK7 和/或ActRIIB融合蛋白包含式A-B-C所示的氨基酸序列,其中A是前导(信号)序列,B由ALK7和/或ActRIIB多肽结构域组成,和C是增强体内稳定性、体内半寿期、摄取/给药、组织定位或分布、蛋白复合物形成和/或纯化的一项或多项的多肽部分。在某些实施方案中, ALK7和/或ActRIIB融合蛋白包含式A-B-C所示的氨基酸序列,其中 A是TPA前导序列,B是ALK7或ActRIIB受体多肽结构域组成,和 C是免疫球蛋白Fc结构域。优选的融合蛋白包含SEQ ID NOs:71、 73、74、76、77、78、79和80的任一个所示的氨基酸序列。

[0264] 在一些实施方案中,ALK7:ActRIIB异多聚体进一步包含一个或多个异源的部分(结构域)以赋予需要的性质。例如,一些融合结构域可特别用于通过亲和色谱分离融合蛋白。这样的融合结构域的众所周知的实例包括但不限于多组氨酸、Glu-Glu、谷胱甘肽S-转移酶(GST)、硫氧还蛋白、蛋白A、蛋白G、免疫球蛋白重链恒定区(Fc)、麦芽糖结合蛋白(MBP)或人血清白蛋白。为了亲和纯化的目的,使用亲和色谱的相关基质,例如谷胱甘肽-、淀粉酶-和镍-或钴-缀合的树脂。许多这样的基质可以“试剂盒”的形式获得,例如Pharmacia GST纯化系统和与(HIS_6)融合配偶体一起使用的QIAexpress™系统(Qiagen)。作为另一实例,融合结构域可经选择,以促进配体陷阱多肽的检测。这样的检测结构域的实例包括各种荧光蛋白(例如,GFP)以及“表位标签”,其通常是特异性抗体可利用的短的肽序列。特异性单克隆抗体可容易利用的众所周知的表位标签包括FLAG、流感病毒血球凝集素(HA)和 c-myc标签。在一些情况下,融合结构域具有蛋白酶裂解位点,例如对于因子Xa或凝血酶,其允许相关的蛋白酶部分地消化融合蛋白,从而从其中释放重组蛋白。释放的蛋白然后可通过随后的色谱分离,从融合结构域分离。

[0265] 在某些实施方案中,ALK7和/或ActRIIB多肽包含能够使多肽稳定的一种或多种修饰。例如,这样的修饰增强多肽的体外半寿期,增强多肽的循环半寿期和/或减少多肽的蛋白水解降解。这样的稳定修饰包括但不限于,融合蛋白(包括例如,包含ALK7和/或ActRIIB多肽结构域和稳定剂结构域的融合蛋白),糖基化位点的修饰(包括例如,添加糖基化位点至本公开内容的多肽)和糖部分的修饰(包括例如,从本公开内容的多肽除去糖部分)。如本文所用的,术语“稳定剂结构域”不仅是指在融合蛋白的情况下的融合结构域(例如,免疫球蛋白Fc结构域),而且包括非蛋白性修饰,例如糖部分或非蛋白性部分,例如聚乙二醇。

[0266] 在优选的实施方案中，根据本文所述的方法使用的ALK7:ActRIIB 异多聚体是分离的复合物。如本文所用的，分离的蛋白(或蛋白复合物) 或多肽(或多肽复合物)是已经与其天然环境的组分分离的蛋白或多肽。在一些实施方案中，本公开内容的异多聚体被纯化至大于95％、96％、 97％、98％或99％纯度，如通过例如，电泳(例如，SDS-PAGE、等电点聚焦(IEF)、毛细管电泳)或色谱(例如，离子交换或反相HPLC)所测定的。用于评估抗体纯度的方法是本领域众所周知的[Flatman等， (2007)J.Chromatogr.B 848:79-87]。在一些实施方案中，ALK7:ActRIIB 异多聚体制备物基本上不含ALK7和/或ActRIIB同多聚体。例如，在一些实施方案中，ALK7:ActRIIB异多聚体制备物包含小于约10％、 9％、8％、7％、6％、5％、4％、3％、2％或小于约1％ALK7同多聚体。在一些实施方案中，ALK7:ActRIIB异多聚体制备物包含小于约10％、 9％、8％、7％、6％、5％、4％、3％、2％或小于约1％ActRIIB同多聚体。在一些实施方案中，ALK7:ActRIIB异多聚体制备物包含小于约 10％、9％、8％、7％、6％、5％、4％、3％、2％或小于约1％ALK7同多聚体和小于约10％、9％、8％、7％、6％、5％、4％、3％、2％或小于约1％ActRIIB同多聚体。

[0267] 在某些实施方案中，ALK7和/或ActRIIB多肽以及包含它们的异多聚体可通过各种本领域已知的技术产生。例如，多肽可使用标准蛋白化学技术合成，例如描述于Bodansky，M.Principles of Peptide Synthesis，Springer Verlag，Berlin(1993)和Grant G.A.(ed.)，Synthetic Peptides:A User′s Guide，W.H.Freeman and Company，New York(1992) 的那些。此外，自动肽合成仪可市售获得(Advanced ChemTech Model 396；Milligen/Biosearch 9600)。或者，多肽和复合物，包括其片段或变体，可使用各种表达系统[大肠杆菌、中国仓鼠卵巢(CHO)细胞、COS 细胞、杆状病毒]经重组产生，如本领域众所周知的。在进一步的实施方案中，修饰或未修饰的多肽可通过使用例如，蛋白酶，例如，胰蛋白酶、嗜热菌蛋白酶、糜蛋白酶、胃蛋白酶或配对的碱性氨基酸转换酶(PACE)，消化重组产生的全长ALK7和/或ActRIIB多肽产生。计算机分析(使用市售可得的软件，例如，MacVector，Omega，PCGene， Molecular Simulation，Inc.)可用于鉴定蛋白水解裂解位点。

[0268] B.编码ALK7和/或ActRIIB多肽的核酸

[0269] 在某些实施方案中，本公开内容提供了编码本文公开的ALK7和 /或ActRIIB受体(包括其片段、功能变体和融合蛋白)的分离和/或重组的核酸。例如，SEQ ID NO:11编码天然存在的人ALK7前体多肽，而SEQ ID NO:12编码ALK7的成熟的细胞外结构域。本发明的核酸可以是单链的或双链的。这样的核酸可以是DNA或RNA分子。这些核酸可用于例如，制备本文描述的ALK7:ActRIIB异多聚体的方法。

[0270] 如本文所用的，分离的核酸是指已经与其天然环境的组分分开的核酸分子。分离的核酸包括在正常包含核酸分子的细胞中包含的核酸分子，但所述核酸分子存在于染色体外或不同于其天然的染色体位置的染色体位置。

[0271] 在某些实施方案中，编码本公开内容的ALK7和/或ActRIIB多肽的核酸应理解为包括SEQ ID NOs:7、8、11、12、21、22、40、41、 44、45、72或75的任何一个以及其变体。变体核苷酸序列包括差异一个或多个核苷酸置换、添加或缺失的序列，包括等位基因变体，因此，将包括与SEQ ID NOs:7、8、11、12、21、22、40、41、44、45、 72或75中任一个指定的核苷酸序列不同的编码序列。

[0272] 在某些实施方案中，本公开内容的TGFβ超家族ALK7和/或 ActRIIB多肽由分离或

重组核酸序列编码，其包含与SEQ ID NOs:7、8、11、12、21、22、40、41、44、45、72或75具有至少70％、75％、 80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、 95％、96％、97％、98％、99％或100％同一性的序列，基本上由其组成或由其组成。本领域普通技术人员将理解包含对于与SEQ ID NOs:7、 8、11、12、21、22、40、41、44、45、72或75具有至少70％、75％、 80％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、 95％、96％、97％、98％、99％或100％同一性的序列互补的序列，基本上由其组成或由其组成的核酸序列，也在本公开内容的范围内。在另外的实施方案中，本公开内容的核酸序列可分离的、重组的，和/ 或与异源的核苷酸序列融合或在DNA文库中。

[0273] 在其它实施方案中，本公开内容的核酸还包括在严格性条件下与 SEQ ID NOs:7、8、11、12、21、22、40、41、44、45、72或75指定的核苷酸序列、SEQ ID NOs:7、8、11、12、21、22、40、41、44、 45、72或75的互补序列或其片段杂交的核苷酸序列。本领域普通技术人员将容易理解，促进DNA杂交的合适的严格性条件可以改变。例如，可在6.0x氯化钠/柠檬酸钠(SSC)中在约45℃下进行杂交，接着在50℃下用2.0x SSC洗涤。例如，在洗涤步骤中盐浓度可选自在 50℃下约2.0x SSC的低严格性至在50℃下约0.2x SSC的高严格性。此外，在洗涤步骤中温度可从在室温约22℃下的低严格性条件增加至在约65℃下的高严格性条件。温度和盐二者可以改变，或温度或盐浓度可以保持恒定，而改变其它变量。在一个实施方案中，本公开内容提供了核酸，其在室温下6x SSC的低严格性条件下杂交，接着在室温下用2x SSC洗涤。

[0274] 由于遗传密码的简并性而不同于SEQ ID NOs:7、8、11、12、21、 22、40、41、44、45、72或75所示的核酸的分离的核酸也在本公开内容的范围内。例如，许多氨基酸由超过一个三联体指定。规定相同的氨基酸的密码子，或同物异名(例如，CAU和CAC是组氨酸的同物异名)可导致“沉默”突变，其不影响蛋白的氨基酸序列。然而，预期确实导致本发明的蛋白的氨基酸序列变化的DNA序列多态性将存在于哺乳动物细胞中。本领域技术人员将理解，由于天然等位基因变异，编码特定蛋白的核酸的一个或多个核苷酸(至多约3-5％的核苷酸)的这些变化可存在于给定物种的个体中。任何和所有这样的核苷酸变化和产生的氨基酸多态性在本公开内容的范围内。

[0275] 在某些实施方案中，本公开内容的重组核酸可以与在表达构建体中的一种或多种调节性核苷酸序列可操作连接。调节性核苷酸序列通常适合于用于表达的宿主细胞。对于各种宿主细胞，许多类型的合适表达载体和合适的调节性序列是本领域已知的。通常，所述一种或多种调节性核苷酸序列可包括但不限于，启动子序列、前导或信号序列、核糖体结合位点、转录起始和终止序列、翻译起始和终止序列、和增强子或激活物序列。本公开内容设想了本领域已知的组成型或诱导型启动子。启动子可以是天然存在的启动子，或合并了超过一个启动子的元件的杂合启动子。表达构建体可存在于细胞的附加体上，例如质粒，或表达构建体可插入染色体中。在一些实施方案中，表达载体包含可选择标记基因以允许选择转化的宿主细胞。可选择标记基因是本领域众所周知的和随使用的宿主细胞而改变。

[0276] 在某些方面，在表达载体中提供本发明的核酸，所述载体包含编码ALK7和/或ActRIIB多肽和可操作连接至至少一种调节性序列的核苷酸序列。调节性序列是公认的和经选择以指导ALK7和/或ActRIIB 多肽的表达。因此，术语调节性序列包括启动子、增强子和其它表达控制元件。实例性调节性序列描述于Goeddel;Gene Expression Technology:

Methods in Enzymology,Academic Press,San Diego,CA (1990)。例如,当与其可操作连接时控制DNA序列表达的各种表达控制序列的任何一种可用于这些载体以表达编码ALK7和/或ActRIIB多肽的DNA序列。这样的可用的表达控制序列包括例如,SV40的早期和晚期启动子、tet启动子、腺病毒或巨细胞病毒立即早期启动子、RSV 启动子、lac系统、trp系统、TAC或TRC系统、表达受T7RNA聚合酶指导的T7启动子、噬菌体λ的主要操纵基因和启动子区、fd外壳蛋白的控制区、3-磷酸甘油酸激酶或其它糖酵解的酶的启动子、酸性磷酸酶(例如,Pho5)的启动子、酵母α-交配因子的启动子、杆状病毒系统的多面体启动子和已知控制原核或真核细胞或它们的病毒的基因表达的其它序列,和其各种组合。应理解,表达载体的设计可取决于例如待转化的宿主细胞的选择和/或需要表达的蛋白类型等因素。此外,还应考虑载体的拷贝数、控制拷贝数的能力和由载体编码的任何其它蛋白的表达,例如抗生素标记。

[0277] 本公开内容的重组核酸可通过将克隆的基因或其部分连接至适合于在原核细胞、真核细胞(酵母、禽、昆虫或哺乳动物)或二者中表达的载体来产生。用于产生重组ALK7和/或ActRIIB多肽的表达载体包括质粒和其它载体。例如,合适的载体包括以下类型的质粒:pBR322-衍生的质粒、pEMBL-衍生的质粒、pEX-衍生的质粒、pBTac- 衍生的质粒和pUC-衍生的质粒,用于在原核细胞例如大肠杆菌中表达。

[0278] 一些哺乳动物表达载体同时包含促进载体在细菌中增殖的原核序列和在真核细胞中表达的一个或多个真核转录单元。pcDNAI/amp、 pcDNAI/neo、pRc/CMV、pSV2gpt、pSV2neo、pSV2-dhfr、pTk2、pRSVneo、 pMSG、pSVT7、pko-neo和pHyg衍生的载体是适合于转染真核细胞的哺乳动物表达载体的实例。这些载体的一些用来自细菌质粒、例如 pBR322的序列修饰,以促进在原核和真核细胞中复制和耐药性选择。或者,病毒例如牛乳头瘤病毒(BPV-1)或爱泼斯坦-巴尔病毒(pHEBo、 pREP-衍生的和p205)的衍生株可用于在真核细胞中蛋白瞬时表达。其它病毒(包括逆转录病毒)表达系统的实例可见于下文的基因疗法递送系统的描述。用于制备质粒和转化宿主生物体的各种方法是本领域众所周知的。对于原核和真核细胞二者的其它合适的表达系统,以及通用的重组程序[Molecular Cloning A Laboratory Manual,第3版, Sambrook、Fritsch和Maniatis编辑,Cold Spring Harbor Laboratory Press,2001]。在一些实例中,可能需要通过使用杆状病毒表达系统来表达重组多肽。这样的杆状病毒表达系统的实例包括pVL-衍生的载体(例如 pVL1392、pVL1393和pVL941)、pAcUW-衍生的载体(例如pAcUW1) 和pBlueBac-衍生的载体(例如包含β-gal的pBlueBac III)。

[0279] 在优选的实施方案中,载体经设计以在CHO细胞中产生本发明的ALK7和/或ActRIIB多肽,例如Pcmv-Script载体(Stratagene,La Jolla, Calif.)、pcDNA4载体(Invitrogen,Carlsbad,Calif.)和pCI-neo载体 (Promega,Madison,Wisc.)。如显而易见的,本发明的基因构建体可用于导致本发明的ALK7和/或ActRIIB多肽在培养物中增殖的细胞中表达,例如,以产生蛋白,包括融合蛋白或变体蛋白,用于纯化。

[0280] 本公开内容还涉及用重组基因转染的宿主细胞,所述基因包括一种或多种本发明的ALK7和/或ActRIIB多肽的编码序列。宿主细胞可以是任何原核或真核细胞。例如,ALK7和/或ActRIIB多肽可在细菌细胞例如大肠杆菌、昆虫细胞(例如,使用杆状病毒表达系统)、酵母或哺乳动物细胞[例如,中国仓鼠卵巢(CHO)细胞系]中表达。其它合适的宿主细胞是本领域技术人员已知的。

[0281] 因此,本公开内容还涉及产生本发明的ALK7和/或ActRIIB多肽的方法。例如,用编码ALK7和/或ActRIIB多肽的表达载体转染的宿主细胞可在合适的条件下培养,以允许表达ALK7和/或ActRIIB多肽发生。多肽可经分泌并从细胞和包含多肽的培养基的混合物分离。或者,ALK7和/或ActRIIB多肽可获自收获和裂解的细胞的胞质或膜部分分离。细胞培养物包括宿主细胞、培养基和其它副产物。用于细胞培养的合适培养基是本领域众所周知的。本发明的多肽可使用本领域已知用于纯化蛋白的技术从细胞培养基、宿主细胞或二者分离,所述技术包括离子交换色谱、凝胶过滤色谱、超滤、电泳、用对ALK7和 /或ActRIIB多肽的特定表位特异性的抗体的免疫亲和纯化、和用结合融合至ALK7和/或ActRIIB多肽的结构域的试剂的亲和纯化(例如,蛋白A柱可用于纯化ALK7-Fc和/或ActRIIB-Fc融合蛋白)。在一些实施方案中,ALK7和/或ActRIIB多肽是包含促进其纯化的结构域的融合蛋白。

[0282] 在一些实施方案中,纯化通过一系列柱色谱步骤实现,包括例如,以任何次序的以下的三种或更多种:蛋白A色谱、Q sepharose色谱、 phenylsepharose色谱、尺寸排阻色谱和阳离子交换色谱。纯化可以用病毒过滤和缓冲液交换完成。ALK7和/或ActRIIB多肽以及其融合蛋白和异聚复合物可纯化至>90%、>95%、>96%、>98%或>99%的纯度,如通过尺寸排阻色谱所测定的,和>90%、>95%、>96%、>98%或>99%的纯度,如通过SDS PAGE所测定的。目标水平的纯度应该是足以在哺乳动物系统、特别是非-人灵长类动物、啮齿动物(小鼠)和人中实现需要的结果的纯度。

[0283] 在另一实施方案中,编码在重组ALK7和/或ActRIIB多肽的所需部分的N-末端的纯化前导序列、例如聚-(His)/肠激酶裂解位点序列的融合基因,可允许通过亲和色谱使用Ni^{2+}金属树脂纯化表达的融合蛋白。然后纯化前导序列可随后通过用肠激酶处理除去,以提供纯化的 ALK7和/或ActRIIB多肽以及其异多聚体[Hochuli等(1987)J. Chromatography 411:177;和Janknecht等(1991)PNAS USA 88:8972]。

[0284] 用于制备融合基因的技术是众所周知的。基本上,编码不同多肽序列的各种DNA片段的连接根据常规技术进行,使用平端或交错端末端连接,限制性酶消化以提供合适的末端,合适时粘端填充,碱性磷酸酶处理以避免不需要的连接,和酶连接。在另一实施方案中,融合基因可通过常规技术合成,包括自动DNA合成仪。或者,基因片段的PCR扩增可使用锚定引物进行,其在两个相邻的基因片段之间产生互补的突出端,其可随后被退火以产生嵌合基因序列。参见例如, Current Protocols in Molecular Biology,Ausubel等编辑,John Wiley& Sons:1992。

[0285] C.抗体拮抗剂

[0286] 在某些方面,ALK7:ActRIIB拮抗剂是抗体(ALK7:ActRIIB拮抗剂抗体)或抗体的组合。ALK7:ActRIIB拮抗剂抗体或抗体的组合可结合例如,一种或多种ALK7配体、ActRIIB配体、ALK7:ActRIIB-结合配体、ALK7受体、ActRIIB受体和/或TGF-β超家族共受体(例如,Crypto 或Cryptic)。如本文所述的,ALK7:ActRIIB拮抗剂抗体可单独或与一种或多种支持性疗法或活性剂组合使用以治疗有需要的患者(例如,具有肾疾病和/或代谢病症或病况的患者)。

[0287] 在某些方面,ALK7:ActRIIB拮抗剂抗体或抗体的组合是抑制结合或可能结合ALK7:ActRIIB异多聚体的一种或多种配体的抗体,所述配体例如活化素B、GDF11、活化素A、BMP10、BMP6、BMP5、 GDF3、活化素C、活化素E、活化素AC、活化素BC、活化素AE、 nodal或活化

素BE。因此，在一些实施方案中，ALK7:ActRIIB拮抗剂抗体或抗体的组合结合至少一种这样的配体。例如，如本文所用的，活化素C抗体(或抗-活化素C抗体)通常是指可以足够的亲和力结合活化素C使得所述抗体可用作靶向活化素C的诊断剂和/或治疗剂的抗体。在某些实施方案中，活化素C抗体与不相关的非-活化素C蛋白的结合程度为小于所述抗体与活化素C的结合的约10％、9％、8％、 7％、6％、5％、4％、3％、2％或小于约1％，如通过例如放射免疫测定法(RIA)、Biacore或其它蛋白相互作用或结合亲和力测定法所测量的。在某些实施方案中，活化素C抗体结合在不同物种的活化素C中保守的活化素C的表位。在某些优选的实施方案中，抗-活化素C抗体结合人活化素C。在一些实施方案中，活化素C抗体可抑制活化素 C免于结合至I型和/或II型受体(例如，ActRIIB和/或ALK7)，因此抑制活化素C-介导的信号传导(例如，Smad信号传导)。在一些实施方案中，活化素C抗体可抑制活化素C免于结合共受体，因此抑制活化素C-介导的信号传导(例如，Smad信号传导)。应注意，活化素C与活化素A、B和E共有一些序列同源性，因此结合活化素C的抗体在一些情况下还可结合和/或抑制另一活化素。在一些实施方案中，本公开内容涉及多特异性抗体(例如，双特异性抗体)和其用途，其结合例如，活化素C和进一步结合至例如一种或多种另外的结合

[0288] ALK7:ActRIIB异多聚体的TGF-β超家族配体[例如，活化素(例如，活化素A、活化素B、活化素C、活化素E、活化素AB、活化素AC、活化素BC、活化素AE或活化素BE)、GDF11、GDF8、BMP10、BMP6、 BMP5、nodal和GDF3]、一种或多种I型受体和/或II型受体(例如， ActRIIB和/或ALK7)和/或一种或多种共受体。在一些实施方案中，结合活化素C的多特异性抗体不结合或基本不结合BMP9(例如，以大于1x 10^{-7}M的K_D结合BMP9或具有相对适度的结合，例如，约1x 10^{-8} M或约1x 10^{-9}M)。在一些实施方案中，本公开内容涉及抗体的组合和其用途，其中抗体的组合包含结合例如，两种或更多种结合

[0289] ALK7:ActRIIB异多聚体的TGF-β超家族配体[例如，活化素(例如，活化素A、活化素B、活化素C、活化素E、活化素AB、活化素AC、活化素BC、活化素AE或活化素BE)、GDF11、GDF8、BMP10、BMP6、 BMP5、nodal和GDF]、一种或多种I型受体和/或II型受体(例如， ActRIIB和/或ALK7)和/或一种或多种共受体的抗体的组合。在一些实施方案中，抗体的组合不包含BMP9抗体。

[0290] 在某些方面，ALK7:ActRIIB拮抗剂抗体或抗体的组合是至少抑制GDF8的抗体。因此，在一些实施方案中，ALK7:ActRIIB拮抗剂抗体或抗体的组合至少结合GDF8。如本文所用的，GDF8抗体(或抗 -GDF8抗体)通常是指以足够的亲和力结合GDF8使得所述抗体可用作靶向GDF8的诊断剂和/或治疗剂的抗体。在某些实施方案中，GDF8 抗体与不相关的非-GDF8蛋白的结合程度小于所述抗体与GDF8的结合的约10％、9％、8％、7％、6％、5％、4％、3％、2％或小于约1％，如通过例如放射免疫测定法(RIA)、Biacore或其它蛋白相互作用或结合亲和力测定法所测量的。在某些实施方案中，GDF8抗体结合在不同物种的GDF8中保守的GDF8的表位。在某些优选的实施方案中，抗-GDF8抗体结合人GDF8。在一些实施方案中，GDF8抗体可抑制 GDF8免于结合至I型和/或II型受体(例如，ActRIIB和/或ALK7)，因此抑制GDF8-介导的信号传导(例如，Smad信号传导)。在一些实施方案中，GDF8抗体可抑制GDF8免于结合共受体，因此抑制GDF8- 介导的信号传导(例如，Smad信号传导)。应注意，GDF8与GDF11 具有高度序列同源性，因此结合GDF8的抗体在一些情况下还可结合和/或抑制GDF11。在一些实施方案中，本公开内容涉及多特异性抗体(例如，双特异性抗体)和其用途，其结合GDF8和进一

步结合至例如一种或多种另外的TGF-β超家族配体[例如,活化素(例如,活化素 A、活化素B、活化素C、活化素E、活化素AB、活化素AC、活化素BC、活化素AE、活化素BE)、GDF11、BMP10、BMP6、BMP5、 nodal和GDF3]、一种或多种I型受体和/或II型受体(例如,ActRIIB 和/或ALK7)和/或一种或多种共受体。在一些实施方案中,结合GDF8 的多特异性抗体不结合或基本不结合BMP9(例如,以大于1x 10^{-7}M 的K_D结合BMP9或具有相对适度的结合,例如,约1x 10^{-8}M或约1 x 10^{-9}M)。在一些实施方案中,本公开内容涉及抗体的组合和其用途,其中抗体的组合包含GDF8抗体和一种或多种另外的结合例如,一种或多种另外的TGF-β超家族配体[例如,活化素(例如,活化素A、活化素B、活化素C、活化素E、活化素AB、活化素AC、活化素BC、活化素AE、活化素BE)、GDF11、GDF3、BMP6、BMP10、nodal和 BMP5]、一种或多种I型受体和/或II型受体(例如,ActRIIB和/或ALK7) 和/或一种或多种共受体的抗体。在一些实施方案中,包含GDF8抗体的抗体的组合不包含BMP9抗体。

[0291] 在某些方面,ALK7:ActRIIB拮抗剂抗体或抗体的组合是至少抑制GDF11的抗体。因此,在一些实施方案中,ALK7:ActRIIB拮抗剂抗体或抗体的组合至少结合GDF11。如本文所用的,GDF11抗体(或抗-GDF11抗体)通常是指以足够的亲和力结合GDF11使得所述抗体可用作靶向GDF11的诊断剂和/或治疗剂的抗体。在某些实施方案中, GDF11抗体与不相关的非-GDF11蛋白的结合程度小于所述抗体与 GDF11的结合的约10%、9%、8%、7%、6%、5%、4%、3%、2%或小于约1%,如通过例如放射免疫测定法(RIA)、Biacore或其它蛋白相互作用或结合亲和力测定法所测量的。在某些实施方案中,GDF11抗体结合在不同物种的GDF11中保守的GDF11的表位。在某些优选的实施方案中,抗-GDF11抗体结合人GDF11。在一些实施方案中, GDF11抗体可抑制GDF11免于结合至I型和/或II型受体(例如, ActRIIB和/或ALK7),因此抑制GDF11-介导的信号传导(例如,Smad 信号传导)。在一些实施方案中,GDF11抗体可抑制GDF11免于结合共受体,因此抑制GDF11-介导的信号传导(例如,Smad信号传导)。应注意,GDF11与GDF8具有高度序列同源性,因此结合GDF11的抗体在一些情况下还可结合和/或抑制GDF8。在一些实施方案中,本公开内容涉及多特异性抗体(例如,双特异性抗体)和其用途,其结合 GDF11和进一步结合至例如一种或多种另外的TGF-β超家族配体[例如,活化素(例如,活化素A、活化素B、活化素C、活化素E、活化素AB、活化素AC、活化素BC、活化素AE、活化素BE)、GDF11、 BMP10、BMP6、BMP5、nodal和GDF3]、一种或多种I型受体和/或II型受体(例如,ActRIIB和/或ALK7)和/或一种或多种共受体。在一些实施方案中,结合GDF11的多特异性抗体不结合或基本不结合 BMP9(例如,以大于1x 10^{-7}M的K_D结合BMP9或具有相对适度的结合,例如,约1x 10^{-8}M或约1x 10^{-9}M)。在一些实施方案中,本公开内容涉及抗体的组合和其用途,其中抗体的组合包含GDF11抗体和一种或多种另外的结合例如,一种或多种另外的TGF-β超家族配体 [例如,活化素(例如,活化素A、活化素B、活化素C、活化素E、活化素AB、活化素AC、活化素BC、活化素AE、活化素BE)、GDF8、 GDF3、BMP6、BMP10、nodal和BMP5]、一种或多种I型受体和/或 II型受体(例如,ActRIIB和/或ALK7)和/或一种或多种共受体的抗体。在一些实施方案中,包含GDF11抗体的抗体的组合不包含BMP9抗体。

[0292] 在某些方面,ALK7:ActRIIB拮抗剂抗体或抗体的组合是至少抑制活化素(活化素A、活化素B、活化素C、活化素E、活化素AB、活化素AC、活化素AE、活化素BC和/或活化素BE)的抗体。因此,在一些实施方案中,ALK7:ActRIIB拮抗剂抗体或抗体的组合至少结合活化素(活化素A、活化素B、活化素C、活化素E、活化素AB、活化素AC、活化素AE、活化素BC和/或活化

素BE)。如本文所用的,活化素抗体(或抗-活化素抗体)通常是指可以足够的亲和力结合一种形式的活化素使得所述抗体可用作靶向该形式的活化素的诊断剂和/ 或治疗剂的抗体。在某些实施方案中,活化素抗体与不相关的非-活化素蛋白的结合程度小于抗体与活化素的结合的约10%、9%、8%、7%、 6%、5%、4%、3%、2%或小于约1%,如通过例如放射免疫测定法(RIA)、 Biacore或其它蛋白相互作用或结合亲和力测定法所测量的。在某些实施方案中,活化素抗体结合在不同物种的活化素中保守的活化素的表位。在某些优选的实施方案中,抗-活化素抗体结合人活化素。在其它优选的实施方案中,活化素抗体可抑制活化素免于结合I型和/或II 型受体(例如,ActRIIB和/或ALK7),因此抑制活化素-介导的信号传导(例如,Smad信号传导)。在一些实施方案中,活化素抗体结合活化素B。在一些实施方案中,活化素抗体结合活化素A。在一些实施方案中,活化素抗体结合活化素A和活化素B。在一些实施方案中,活化素抗体结合活化素AB。在一些实施方案中,活化素抗体结合活化素C。在一些实施方案中,活化素抗体结合活化素E。在一些实施方案中,活化素抗体结合活化素A和活化素C。在一些实施方案中,活化素抗体结合活化素AC。在一些实施方案中,活化素抗体结合活化素A和活化素E。在一些实施方案中,活化素抗体结合活化素AE。在一些实施方案中,活化素抗体结合活化素B和活化素C。在一些实施方案中,活化素抗体结合活化素BC。在一些实施方案中,活化素抗体结合活化素B和活化素E。在一些实施方案中,活化素抗体结合活化素BE。在一些实施方案中,活化素抗体结合活化素A、活化素B 和活化素C。在一些实施方案中,活化素抗体结合活化素A、活化素B和活化素E。任选地,结合活化素A、活化素B和活化素C的一种或多种的活化素抗体可进一步结合活化素E。在一些实施方案中,本公开内容涉及多特异性抗体(例如,双特异性抗体)和其用途,其结合活化素和进一步结合至例如一种或多种另外的TGF-β超家族配体[例如,GDF11、GDF8、BMP10、BMP6、BMP5、nodal和GDF3]、一种或多种I型受体和/或II型受体(例如,ActRIIB和/或ALK7)和/或一种或多种共受体。在一些实施方案中,结合活化素的多特异性抗体不结合或基本不结合BMP9(例如,以大于1x 10^{-7}M的K_D结合BMP9或具有相对适度的结合,例如,约1x 10^{-8}M或约1x 10^{-9}M。在一些实施方案中,本公开内容涉及抗体的组合和其用途,其中抗体的组合包含活化素抗体和一种或多种另外的结合例如,一种或多种另外的 TGF-β超家族配体[例如,GDF11、GDF8GDF3、BMP6、BMP10、nodal和BMP5]、一种或多种I型受体和/或II型受体(例如,ActRIIB和/或 ALK7)和/或一种或多种共受体的抗体。在一些实施方案中,包含活化素抗体的抗体的组合不包含BMP9抗体。

[0293] 在某些方面,ALK7:ActRIIB拮抗剂抗体或抗体的组合是至少抑制BMP6的抗体。因此,在一些实施方案中,ALK7:ActRIIB拮抗剂抗体或抗体的组合至少结合BMP6。如本文所用的,BMP6抗体(或抗 -BMP6抗体)通常是指可以足够的亲和力结合BMP6使得所述抗体可用作靶向BMP6的诊断剂和/或治疗剂的抗体。在某些实施方案中, BMP6抗体与不相关的非-BMP6蛋白的结合程度小于抗体与BMP6的结合的约10%、9%、8%、7%、6%、5%、4%、3%、2%或小于约1%,如通过例如放射免疫测定法(RIA)、Biacore或其它蛋白相互作用或结合亲和力测定法所测量的。在某些实施方案中,BMP6抗体结合在不同物种的BMP6中保守的BMP6的表位。在某些优选的实施方案中,抗-BMP6抗体结合人BMP6。在一些实施方案中,BMP6抗体可抑制 BMP6免于结合至I型和/或II型受体(例如,ActRIIB和/或ALK7),因此抑制BMP6-介导的信号传导(例如,Smad信号传导)。在一些实施方案中,BMP6抗体可抑制BMP6免于结合共受体,因此抑制BMP6- 介导的信号传导(例如,Smad信号传导)。在一些实施方案中,本公开内

容涉及多特异性抗体(例如,双特异性抗体)和其用途,其结合BMP6 和进一步结合至例如一种或多种另外的TGF-β超家族配体[例如,活化素(例如,活化素A、活化素B、活化素C、活化素E、活化素AB、活化素AC、活化素BC、活化素AE、活化素BE)、GDF8、BMP10、 GDF11、BMP5、nodal和GDF3]、一种或多种I型受体和/或II型受体 (例如,ActRIIB和/或ALK7)和/或一种或多种共受体。在一些实施方案中,结合BMP6的多特异性抗体不结合或基本不结合BMP9(例如,以大于1x 10^{-7}M的K_D结合BMP9或具有相对适度的结合,例如,约 1x 10^{-8}M或约1x 10^{-9}M)。在一些实施方案中,本公开内容涉及抗体的组合和其用途,其中抗体的组合包含BMP6抗体和一种或多种另外的结合例如,一种或多种另外的TGF-β超家族配体[例如,活化素(例如,活化素A、活化素B、活化素C、活化素E、活化素AB、活化素 AC、活化素BC、活化素AE、活化素BE)、GDF8、GDF3、GDF11、 BMP10、nodal和BMP5]、一种或多种I型受体和/或II型受体(例如,ActRIIB和/或ALK7)和/或一种或多种共受体的抗体。在一些实施方案中,包含BMP6抗体的抗体的组合不包含BMP9抗体。

[0294] 在某些方面,ALK7:ActRIIB拮抗剂抗体或抗体的组合是至少抑制GDF3的抗体。因此,在一些实施方案中,ALK7:ActRIIB拮抗剂抗体或抗体的组合至少结合GDF3。如本文所用的,GDF3抗体(或抗 -GDF3抗体)通常是指以足够的亲和力结合GDF3使得所述抗体可用作靶向GDF3的诊断剂和/或治疗剂的抗体。在某些实施方案中,GDF3 抗体与不相关的非-GDF3蛋白的结合程度小于抗体与GDF3的结合的约10%、9%、8%、7%、6%、5%、4%、3%、2%或小于约1%,如通过例如放射免疫测定法(RIA)、Biacore或其它蛋白相互作用或结合亲和力测定法所测量的。在某些实施方案中,GDF3抗体结合在不同物种的GDF3中保守的GDF3的表位。在某些优选的实施方案中,GDF3 抗体结合人GDF3。在一些实施方案中,GDF3抗体可抑制GDF3免于结合至I型和/或II型受体(例如,ActRIIB和/或ALK7),因此抑制 GDF3-介导的信号传导(例如,Smad信号传导)。在一些实施方案中, GDF3抗体可抑制GDF3免于结合共受体,因此抑制GDF3-介导的信号传导(例如,Smad信号传导)。在一些实施方案中,本公开内容涉及多特异性抗体(例如,双特异性抗体)和其用途,其结合GDF3和进一步结合至例如一种或多种另外的TGF-β超家族配体[例如,活化素(例如,活化素A、活化素B、活化素C、活化素E、活化素AB、活化素 AC、活化素BC、活化素AE、活化素BE)、GDF11、BMP10、BMP6、 BMP5、和nodal、一种或多种I型受体和/或II型受体(例如,ActRIIB 和/或ALK7)和/或一种或多种共受体。在一些实施方案中,结合GDF3 的多特异性抗体不结合或基本不结合BMP9(例如,以大于1x 10^{-7}M 的K_D结合BMP9或具有相对适度的结合,例如,约1x 10^{-8}M或约1 x 10^{-9}M)。在一些实施方案中,本公开内容涉及抗体的组合和其用途,其中抗体的组合包含GDF3抗体和一种或多种另外的结合例如,一种或多种另外的TGF-β超家族配体[例如,活化素(例如,活化素A、活化素B、活化素C、活化素E、活化素AB、活化素AC、活化素BC、活化素AE、活化素BE)、GDF11、BMP10、BMP6、BMP5和nodal]、一种或多种I型受体和/或II型受体(例如,ActRIIB和/或ALK7)和/或一种或多种共受体的抗体。在一些实施方案中,包含GDF3抗体的抗体的组合不包含BMP9抗体。

[0295] 在某些方面,ALK7:ActRIIB拮抗剂抗体或抗体的组合是至少抑制BMP5的抗体。因此,在一些实施方案中,ALK7:ActRIIB拮抗剂抗体或抗体的组合至少结合BMP5。如本文所用的,BMP5抗体(或抗 -BMP5抗体)通常是指以足够的亲和力结合BMP5使得所述抗体可用作靶向BMP5的诊断剂和/或治疗剂的抗体。在某些实施方案中,BMP5 抗体与不相关的非-BMP5蛋

白的结合程度小于抗体与BMP5的结合的约10％、9％、8％、7％、6％、5％、4％、3％、2％或小于约1％，如通过例如放射免疫测定法(RIA)、Biacore或其它蛋白相互作用或结合亲和力测定法所测量的。在某些实施方案中，BMP5抗体结合在不同物种的BMP5中保守的BMP5的表位。在某些优选的实施方案中，抗 -BMP5抗体结合人BMP5。在一些实施方案中，BMP5抗体可抑制BMP5免于结合至I型和/或II型受体(例如，ActRIIB和/或ALK7)，因此抑制BMP5-介导的信号传导(例如，Smad信号传导)。在一些实施方案中，BMP5抗体可抑制BMP5免于结合共受体，因此抑制BMP5- 介导的信号传导(例如，Smad信号传导)。在一些实施方案中，本公开内容涉及多特异性抗体(例如，双特异性抗体)和其用途，其结合BMP5 和进一步结合至例如一种或多种另外的TGF-β超家族配体[例如，活化素(例如，活化素A、活化素B、活化素C、活化素E、活化素AB、活化素AC、活化素BC、活化素AE和活化素BE)、GDF8、BMP10、 BMP6、GDF11、nodal和GDF3]、一种或多种I型受体和/或II型受体 (例如，ActRIIB和/或ALK7)和一种或多种共受体。在一些实施方案中，结合BMP5的多特异性抗体不结合或基本不结合BMP9(例如，以大于1x 10^{-7}M的K_D结合BMP9或具有相对适度的结合，例如，约1x 10^{-8} M或约1x 10^{-9}M)。在一些实施方案中，本公开内容涉及抗体的组合和其用途，其中抗体的组合包含BMP5抗体和一种或多种另外的结合例如，一种或多种另外的TGF-β超家族配体[例如，活化素(例如，活化素A、活化素B、活化素C、活化素E、活化素AB、活化素AC、活化素BC、活化素AE和活化素BE)、GDF8GDF3、BMP6、BMP10、 nodal和GDF11]、I型受体和/或II型受体(例如，ActRIIB和/或ALK7) 和/或共受体的抗体。在一些实施方案中，包含BMP5抗体的抗体的组合不包含BMP9抗体。

[0296] 在某些方面，ALK7:ActRIIB拮抗剂抗体或抗体的组合是至少抑制BMP10的抗体。因此，在一些实施方案中，ALK7:ActRIIB拮抗剂抗体或抗体的组合至少结合BMP10。如本文所用的，BMP10抗体(或抗-BMP10抗体)通常是指可以足够的亲和力结合BMP10使得所述抗体可用作靶向BMP10的诊断剂和/或治疗剂的抗体。在某些实施方案中，BMP10抗体与不相关的非-BMP10蛋白的结合程度小于抗体与 BMP10的结合的约10％、9％、8％、7％、6％、5％、4％、3％、2％或小于约1％，如通过例如放射免疫测定法(RIA)、Biacore或其它蛋白相互作用或结合亲和力测定法所测量的。在某些实施方案中，BMP10 抗体结合在不同物种的BMP10中保守的BMP10的表位。在某些优选的实施方案中，抗-BMP10抗体结合人BMP10。在一些实施方案中， BMP10抗体可抑制BMP10免于结合至I型和/或II型受体(例如， ActRIIB和/或ALK7)，因此抑制BMP10-介导的信号传导(例如，Smad 信号传导)。在一些实施方案中，BMP10抗体可抑制BMP10免于结合共受体，因此抑制BMP10-介导的信号传导(例如，Smad信号传导)。在一些实施方案中，本公开内容涉及多特异性抗体(例如，双特异性抗体)和其用途，其结合BMP10和进一步结合至例如一种或多种另外的 TGF-β超家族配体[例如，活化素(例如，活化素A、活化素B、活化素C、活化素E、活化素AB、活化素AC、活化素BC、活化素AE 和活化素BE)、GDF8、GDF11、BMP6、BMP5、nodal和GDF3]、一种或多种I型受体和/或II型受体(例如，ActRIIB和/或ALK7)和/或一种或多种共受体。在一些实施方案中，结合BMP10的多特异性抗体不结合或基本不结合BMP9(例如，以大于1x 10^{-7}M的K_D结合BMP9 或具有相对适度的结合，例如，约1x 10^{-8}M或约1x 10^{-9}M)。在一些实施方案中，本公开内容涉及抗体的组合和其用途，其中抗体的组合包含BMP10抗体和一种或多种另外的结合例如，一种或多种另外的 TGF-β超家族配体[例如，活化素(例如，活化素A、活化素B、活化素C、活化素E、活化素AB、活化素AC、活化素

BC、活化素AE 和活化素BE)、GDF8、GDF3、BMP6、GDF11、nodal和BMP5]、一种或多种I型受体和/或II型受体(例如,ActRIIB和/或ALK7)和/或一种或多种共受体的抗体。在一些实施方案中,包含BMP10抗体的抗体的组合不包含BMP9抗体。

[0297] 在某些方面,ALK7:ActRIIB拮抗剂抗体或抗体的组合是至少抑制nodal的抗体。因此,在一些实施方案中,ALK7:ActRIIB拮抗剂抗体或抗体的组合至少结合nodal。如本文所用的,nodal抗体(或抗-nodal 抗体)通常是指可以足够的亲和力结合nodal使得所述抗体可用作靶向 nodal的诊断剂和/或治疗剂的抗体。在某些实施方案中,nodal抗体与不相关的非-nodal蛋白的结合程度小于抗体与nodal的结合的约10%、 9%、8%、7%、6%、5%、4%、3%、2%或小于约1%,如通过例如放射免疫测定法(RIA)、Biacore或其它蛋白相互作用或结合亲和力测定法所测量的。在某些实施方案中,nodal抗体结合在不同物种的nodal中保守的nodal的表位。在某些优选的实施方案中,抗-nodal抗体结合人nodal。在一些实施方案中,nodal抗体可抑制nodal免于结合至I 型和/或II型受体(例如,ActRIIB和/或ALK7),因此抑制nodal-介导的信号传导(例如,Smad信号传导)。在一些实施方案中,nodal抗体可抑制nodal免于结合共受体,因此抑制nodal-介导的信号传导(例如, Smad信号传导)。在一些实施方案中,本公开内容涉及多特异性抗体 (例如,双特异性抗体)和其用途,其结合nodal和进一步结合至例如一种或多种另外的TGF-β超家族配体[例如,活化素(例如,活化素A、活化素B、活化素C、活化素E、活化素AB、活化素AC、活化素BC、活化素AE和活化素BE)、GDF8、GDF11、BMP6、BMP5、BMP10 和GDF3]、一种或多种I型受体和/或II型受体(例如,ActRIIB和/或 ALK7)和/或一种或多种共受体。在一些实施方案中,结合nodal的多特异性抗体不结合或基本不结合BMP9(例如,以大于1x 10^{-7}M的 K_D结合BMP9或具有相对适度的结合,例如,约1x 10^{-8}M或约1x 10^{-9} M)。在一些实施方案中,本公开内容涉及抗体的组合和其用途,其中抗体的组合包含nodal抗体和一种或多种另外的结合例如,一种或多种另外的TGF-β超家族配体[例如,活化素(例如,活化素A、活化素 B、活化素C、活化素E、活化素AB、活化素AC、活化素BC、活化素AE和活化素BE)、GDF8GDF3、BMP6、GDF11、BMP10和BMP5]、一种或多种I型受体和/或II型受体(例如,ActRIIB和/或ALK7)和/或一种或多种共受体的抗体。在一些实施方案中,包含nodal抗体的抗体的组合不包含BMP9抗体。

[0298] 关于结合和拮抗结合ALK7:ActRIIB的配体[例如,活化素(例如,活化素A、活化素B、活化素C、活化素E、活化素AB、活化素AC、活化素BC、活化素AE和活化素BE)、GDF8、GDF3、BMP6、GDF11、 BMP10和BMP5]的抗体,考虑了抗体可设计为包含结合这样的配体的表位使得抗体的第一部分与I型受体竞争结合的第一部分和包含结合这样的配体的表位使得抗体的第二部分与II型受体竞争结合的第二部分的双特异性抗体。以此方式,靶向单一配体的双特异性抗体可经设计以模拟可由ALK7:ActRIIB异多聚体赋予的双重I型-II型受体结合阻断。类似地,考虑了使用两种或更多种抗体的组合可实现相同效果,其中至少第一抗体结合这样的配体的表位,使得第一抗体与I型受体竞争结合和至少第二抗体结合这样的配体的表位,使得第二抗体与II型受体竞争结合。

[0299] 在某些方面,ALK7:ActRIIB拮抗剂抗体或抗体的组合是至少抑制ActRIIB的抗体。因此,在一些实施方案中,ALK7:ActRIIB拮抗剂抗体或抗体的组合至少结合ActRIIB。如本文所用的,ActRIIB抗体 (抗-ActRIIB抗体)通常是指以足够的亲和力结合ActRIIB使得所述抗体可用作靶向ActRIIB的诊断剂和/或治疗剂的抗体。在某些实施方案中,抗-ActRIIB

抗体与不相关的非-ActRIIB蛋白的结合程度小于所述抗体与ActRIIB的结合的约10％、9％、8％、7％、6％、5％、4％、3％、 2％或小于约1％，如通过例如放射免疫测定法(RIA)、Biacore或其它蛋白-蛋白相互作用或结合亲和力测定法所测量的。在某些实施方案中，抗-ActRIIB抗体结合在不同物种的ActRIIB中保守的ActRIIB的表位。在某些优选的实施方案中，抗-ActRIIB抗体结合人ActRIIB。在一些实施方案中，抗-ActRIIB抗体可抑制一种或多种TGF-β超家族配体[例如，GDF8、活化素(例如，活化素A、活化素B、活化素C、活化素E、活化素AB、活化素AC、活化素BC、活化素AE和活化素BE)、GDF3、 BMP6、BMP10、nodal和BMP9]免于结合ActRIIB和/或ALK7。在一些实施方案中，抗-ActRIIB抗体是多特异性抗体(例如，双特异性抗体)，其结合ActRIIB和一种或多种TGF-β超家族配体[例如，GDF11、GDF8、活化素(例如，活化素A、活化素B、活化素C、活化素E、活化素 AB、活化素AC)、GDF3、BMP6、BMP10、nodal和BMP9]、I型受体(例如，ALK7)、共受体和/或另外的II型受体。在一些实施方案中，本公开内容涉及抗体的组合和其用途，其中抗体的组合包含抗 -ActRIIB抗体和一种或多种另外的结合例如，一种或多种TGF-β超家族配体[例如，GDF11、GDF8、活化素(例如，活化素A、活化素B、活化素C、活化素E、活化素AB、活化素AC、活化素BC、活化素 AE和活化素BE)、GDF3、BMP6、BMP10、nodal和BMP9]、共受体、 I型受体(例如，ALK7)和/或另外的II型受体的抗体。应注意，ActRIIB 与ActRIIA具有序列相似性，因此结合ActRIIB的抗体在一些情况下还可结合和/或抑制ActRIIA。

[0300] 在某些方面，ALK7:ActRIIB拮抗剂抗体或抗体的组合是至少抑制ALK7的抗体。因此，在一些实施方案中，ALK7:ActRIIB拮抗剂抗体或抗体的组合至少结合ALK7。如本文所用的，ALK7抗体(抗 -ALK7抗体)通常是指以足够的亲和力结合ALK7使得所述抗体可用作靶向ALK7的诊断剂和/或治疗剂的抗体。在某些实施方案中，抗 -ALK7抗体与不相关的非-ALK7蛋白的结合程度小于所述抗体与 ALK7的结合的约10％、9％、8％、7％、6％、5％、4％、3％、2％或小于约1％，如通过例如放射免疫测定法(RIA)、Biacore或其它蛋白- 蛋白相互作用或结合亲和力测定法所测量的。在某些实施方案中，抗 -ALK7抗体结合在不同物种的ALK7中保守的ALK7的表位。在某些优选的实施方案中，抗-ALK7抗体结合人ALK7。在一些实施方案中，抗-ALK7抗体可抑制一种或多种TGF-β超家族配体[例如，GDF11、 GDF8、活化素(例如，活化素A、活化素B、活化素C、活化素E、活化素AB、活化素AC、活化素BC、活化素AE和活化素BE)GDF3、 BMP5、BMP6和BMP10]免于结合I型受体(例如，ALK7)、II型受体 (例如，ActRIIB)或共受体。在一些实施方案中，抗-ALK7抗体是多特异性抗体(例如，双特异性抗体)，其结合ALK7和一种或多种TGF-β超家族配体[例如，活化素(例如，活化素A、活化素B、活化素C、活化素E、活化素AB、活化素AC、活化素BC、活化素AE和活化素BE)、GDF11、GDF8、BMP10、BMP6、BMP5、nodal和GDF3、 II型受体(例如，ActRIIB)、共受体和/或另外的I型受体。在一些实施方案中，本公开内容涉及抗体的组合和其用途，其中抗体的组合包含抗-ALK7抗体和一种或多种另外的结合例如，一种或多种TGF-β超家族配体[例如，活化素(例如，活化素A、活化素B、活化素C、活化素E、活化素AB、活化素AC、活化素BC、活化素AE和活化素BE)、 GDF11、GDF8、BMP10、BMP6、BMP5、nodal和GDF3]、共受体、另外的I型受体和/或II型受体(例如，ActRIIB)的抗体。

[0301] 在某些方面，ALK7:ActRIIB拮抗剂抗体或抗体的组合是至少抑制Cripto或Cryptic的抗体。因此，在一些实施方案中，ALK7:ActRIIB 拮抗剂抗体或抗体的组合至少结

合Cripto或Cryptic。如本文所用的，Cripto抗体(抗-Cripto抗体)通常是指以足够的亲和力结合Cripto使得所述抗体可用作靶向Cripto的诊断剂和/或治疗剂的抗体。在某些实施方案中，抗-Cripto抗体与不相关的非-Cripto蛋白的结合程度小于所述抗体与Cripto的结合的约10％、9％、8％、7％、6％、5％、4％、3％、 2％或小于约1％，如通过例如放射免疫测定法(RIA)、Biacore或其它蛋白-蛋白相互作用或结合亲和力测定法所测量的。在某些实施方案中，抗-Cripto抗体结合在不同物种的Cripto中保守的Cripto的表位。在某些优选的实施方案中，抗-Cripto抗体结合人Cripto。如本文所用的，Cryptic抗体(抗-Cryptic抗体)通常是指以足够的亲和力结合Cryptic使得所述抗体可用作靶向Cryptic的诊断剂和/或治疗剂的抗体。在某些实施方案中，抗-Cripto抗体与不相关的非-Cryptic蛋白的结合程度小于所述抗体与Cryptic的结合的约10％、9％、8％、7％、6％、5％、4％、 3％、2％或小于约1％，如通过例如放射免疫测定法(RIA)、Biacore或其它蛋白-蛋白相互作用或结合亲和力测定法所测量的。在某些实施方案中，抗-Cryptic抗体结合在不同物种的Cryptic中保守的Cryptic的表位。在某些优选的实施方案中，抗-Cryptic抗体结合人Cryptic。在一些实施方案中，抗-Cripto或Cryptic抗体可抑制一种或多种TGF-β超家族配体[例如，活化素(例如，活化素A、活化素B、活化素C、活化素E、活化素AB、活化素AC、活化素BC、活化素AE和活化素BE)、GDF11、GDF8、BMP10、BMP6、BMP5、nodal和GDF3] 免于分别结合Cripto或Cryptic或结合ActRIIB和/或ALK7。在一些实施方案中，抗-Cripto或Cryptic抗体可抑制nodal免于结合至I型和 /或II型受体。在一些实施方案中，抗-Cripto或Cryptic抗体是多特异性抗体(例如，双特异性抗体)，其结合Cripto或Cryptic和一种或多种 TGF-β超家族[例如，活化素(例如，活化素A、活化素B、活化素C、活化素E、活化素AB、活化素AC、活化素BC、活化素AE和活化素BE)、GDF11、GDF8、BMP10、BMP6、BMP5、nodal和GDF3]、 I型受体(例如，ALK7)、另外的共受体和/或II型受体。在一些实施方案中，抗体可结合Cripto和Cryptic二者。在一些实施方案中，本公开内容涉及抗体的组合和其用途，其中抗体的组合包含抗-Cripto或 Cryptic抗体和一种或多种另外的结合例如，一种或多种TGF-β超家族配体[例如，活化素(例如，活化素A、活化素B、活化素C、活化素E、活化素AB、活化素AC、活化素BC、活化素AE和活化素BE)、GDF11、GDF8、BMP10、BMP6、BMP5、nodal和GDF3]、另外的共受体、I型受体(例如，ALK7)和/或II型受体的抗体。

[0302] 如本文所述的，存在产生异多聚体复合物的各种方法。这样的方法可用于产生包含抗体-结合结构域(例如，V_L和V_H链的复合物)和一种或多种选自ALK7多肽、ActRIIB多肽、ALK7:ActRIIB异聚体或 ALK7:ActRIIB单一陷阱多肽的多肽的异多聚体复合物。参见图10A、10B和12D。例如，在一些实施方案中，本公开内容提供了蛋白复合物，其包含结合ALK7:ActRIIB-结合配体[例如，活化素(例如，活化素A、活化素B、活化素C、活化素E、活化素AB、活化素AC、活化素BC、活化素AE和活化素BE)、GDF11、GDF8、BMP10、BMP6、 BMP5、nodal和GDF3]的抗体的配体-结合结构域，其与ALK7多肽共价或非共价缔合。在一些实施方案中，本公开内容提供了蛋白复合物，其包含结合ALK7:ActRIIB-结合配体[例如，活化素(例如，活化素A、活化素B、活化素C、活化素E、活化素AB、活化素AC、活化素BC、活化素AE和活化素BE)、GDF11、GDF8、BMP10、BMP6、 BMP5、nodal和GDF3]的抗体的配体-结合结构域，其与ActRIIB多肽共价或非共价缔合。在一些实施方案中，本公开内容提供了蛋白复合物，其包含结合ALK7:ActRIIB-结合配体[例如，活化素(例如，活化素A、活化素B、活化素C、活化素E、活化素

AB、活化素AC、活化素BC、活化素AE和活化素BE)、GDF11、GDF8、BMP10、BMP6、 BMP5、nodal和GDF3]的抗体的配体-结合结构域,其与ALK7:ActRIIB 单链配体陷阱共价或非共价缔合。在一些实施方案中,本公开内容提供了蛋白复合物,其包含结合ALK7:ActRIIB-结合配体[例如,活化素 (例如,活化素A、活化素B、活化素C、活化素E、活化素AB、活化素AC、活化素BC、活化素AE和活化素BE)、GDF11、GDF8、 BMP10、BMP6、BMP5、nodal和GDF3]的抗体的配体-结合结构域,其与ALK7:ActRIIB异多聚体共价或非共价缔合。

[0303] 术语抗体在本文以最广含义使用,涵盖各种抗体结构,包括但不限于单克隆抗体、多克隆抗体、多特异性抗体(例如,双特异性抗体) 和抗体片段,只要它们显示需要的抗原-结合活性。抗体片段是指并非完整抗体的分子,其包含结合完整抗体所结合的抗原的完整抗体的一部分。抗体片段的实例包括但不限于Fv、Fab、Fab′、Fab′-SH、F(ab′)$_2$;双抗体;线性抗体;单链抗体分子(例如,scFv);和自抗体片段形成的多特异性抗体[参见例如,Hudson等(2003)Nat.Med.9:129-134;Plückthun,The Pharmacology of Monoclonal Antibody,vol.113, Rosenburg和Moore编辑,(Springer-Verlag,New York),pp.269-315 (1994);WO 93/16185;和美国专利号5,571,894;5,587,458;和5,869,046]。双抗体是具有两个抗原结合位点的抗体片段,其可以是二价的或双特异性的[参见例如,EP 404,097;WO 1993/01161;Hudson等(2003)Nat. Med.9:129-134(2003);和Hollinger等(1993)Proc.Natl.Acad.Sci.USA 90:6444-6448]。三抗体和四抗体也描述于Hudson等(2003)Nat.Med. 9:129-134。单-结构域抗体是包含抗体的所有或一部分重链可变结构域或所有或一部分轻链可变结构域的抗体片段。在某些实施方案中,单 -结构域抗体是人单-结构域抗体[参见例如,美国专利号6,248,516]。本文公开的抗体可以是多克隆抗体或单克隆抗体。在某些实施方案中,本公开内容的抗体包含与其连接和能够被检测的标记(例如,标记可以是放射性同位素、荧光化合物、酶或酶辅因子)。在某些优选的实施方案中,本公开内容的抗体是分离的抗体。在某些优选的实施方案中,本公开内容的抗体是重组抗体。

[0304] 本文的抗体可具有任何类型。抗体的类型是指其重链具有的恒定结构域或恒定区的类型。存在5个主要类型的抗体:IgA、IgD、IgE、 IgG和IgM,若干这些类型可进一步分为亚类(同种型),例如,IgG_1、 IgG_2、IgG_3、IgG_4、IgA_1和IgA_2。对应于不同类型的免疫球蛋白的重链恒定结构域被称为α、δ、ε、γ 和μ。

[0305] 一般而言,用于本文公开的方法的抗体特异性结合其靶标抗原,优选地具有高结合亲和力。亲和力可表示为K_D值,反映了固有结合亲和力(例如,具有最小的亲合力作用)。通常,结合亲和力经体外测量,无论是在无细胞还是细胞相关的条件下。本领域已知的许多测定法的任一种,包括本文公开的那些,可用于获得结合亲和力度量,包括例如,Biacore、放射性标记的抗原-结合测定法(RIA)和ELISA。在一些实施方案中,本公开内容的抗体以至少1x 10^{-7}或更强、1x10^{-8}或更强、1x10^{-9}或更强、1x10^{-10}或更强、1x10^{-11}或更强、1x10^{-12}或更强、1x10^{-13}或更强或1x10^{-14}或更强的K_D结合它们的靶标抗原(例如,ALK7、 ActRIIB、活化素、GDF11、GDF8、BMP10、BMP6、GDF3、nodal 和/或BMP5)。

[0306] 在某些实施方案中,K_D通过以Fab形式的目的抗体和其靶标抗原进行的RIA测量,如以下测定法所述的。Fab对抗原的溶液结合亲和力通过在滴定系列的未标记抗原的存在下用最小浓度的放射性标记的抗原(例如,^{125}I-标记)平衡Fab,然后用抗-Fab抗体-包被的板捕获结合的抗原来测量[参见例如,Chen等(1999)J.Mol.Biol.293:865-881]。为了建立

该测定的条件，多孔板(例如，MICROTITER®，来自Thermo Scientific)用捕获性抗-Fab抗体(例如，来自Cappel Labs)包被(例如，过夜)和随后用牛血清白蛋白封闭，优选地在室温下(大约23℃)。在非 -吸附板中，放射性标记的抗原与系列稀释的目的Fab混合[例如，与抗-VEGF抗体Fab-12的评价一致，Presta等，(1997)Cancer Res. 57:4593-4599]。然后将目的Fab孵育，优选地过夜，但孵育可持续更长时间(例如，约65小时)以确保达到平衡。之后，将混合物转移至捕获板以孵育，优选地在室温下约1小时。然后除去溶液，和将板洗涤数次，优选地用聚山梨醇酯20和PBS混合物。当板已经干燥时，加入闪烁剂(例如，MICROSCINT®，来自Packard)和将板在γ计数器(例如，TOPCOUNT®，来自Packard)上计数。

[0307] 根据另一实施方案，K_D使用表面等离子体共振测定法，使用例如BIACORE®2000或BIACORE®3000(BIAcore，Inc.，Piscataway，N.J.) 用约10个响应单位(RU)的固定抗原CM5芯片测量。简言之，羧基甲基化葡聚糖生物传感器芯片(CM5，BIACORE，Inc.)用N-乙基-N′-(3- 二甲基氨基丙基)-碳二亚胺盐酸盐(EDC)和N-羟基琥珀酰亚胺(NHS) 根据供应商的说明书活化。例如，抗原可用10mM乙酸钠pH 4.8稀释至5μg/ml(约0.2μM)，然后以5μl/分钟的流速注射以实现大约10 个响应单位(RU)的偶联蛋白。在注射抗原后，注射1M乙醇胺以封闭未反应的基团。对于动力学度量，以大约25μl/min的流速注射在含0.05％聚山梨醇酯20(TWEEN-20®)表面活性剂的PBS(PBST)中的2 倍系列稀释的Fab(0.78nM至500nM)。缔合速率(k_{on})和离解速率(k_{off}) 使用例如，简单的一对一Langmuir结合模型(BIACORE®Evaluation 软件版本3.2)，通过同时拟合缔合和离解传感图谱计算。平衡离解常数(K_D)计算为比率k_{off}/k_{on}[参见例如，Chen等，(1999)J.Mol.Biol. 293:865-881]。如果通过上述表面等离子体共振测定法，缔合速率超过例如，$10^6 M^{-1}s^{-1}$，则缔合速率可通过使用测量在增加浓度的抗原的存在下20nM抗-抗原抗体(Fab形式)/PBS的荧光发射强度的增加或减少(例如，激发＝295nm；发射＝340nm，16nm带通)的荧光淬灭技术测定，如在带有搅拌杯的分光计，例如停流装备的分光光度计(Aviv Instruments)或8000-系列SLM-AMINCO®分光光度计 (ThermoSpectronic)中测量的。

[0308] 抗体片段可通过各种技术制备，包括但不限于蛋白水解消化完整抗体以及通过重组宿主细胞(例如，大肠杆菌或噬菌体)产生，如本文所述的。人ActRIIB、活化素(活化素A、活化素B、活化素C和活化素E)、GDF11、GDF8、BMP10、BMP6、GDF3、nodal和/或BMP5 的核酸和氨基酸序列是本领域众所周知的。此外，许多产生抗体的方法是本领域众所周知的，其中一些在本文中描述。因此，根据本公开内容使用的抗体拮抗剂可由本领域技术人员，基于本领域的知识和本文提供的教导常规制备。

[0309] 在某些实施方案中，本文提供的抗体是嵌合抗体。嵌合抗体是指其中重链和/或轻链的一部分源自特定的来源或物种，而重链和/或轻链的剩余部分源自不同的来源或物种的抗体。某些嵌合抗体描述于例如，美国专利号4,816,567；和Morrison等，(1984)Proc.Natl.Acad.Sci. USA,81:6851-6855。在一些实施方案中，嵌合抗体包含非-人可变区(例如，源自小鼠、大鼠、仓鼠、兔或非-人灵长类例如猴的可变区)和人恒定区。在一些实施方案中，嵌合抗体是"类别转换"抗体，其中类型或亚类已从亲本抗体改变。一般而言，嵌合

抗体包括其抗原-结合片段。

[0310] 在某些实施方案中，本文提供的嵌合抗体是人源化抗体。人源化抗体是指包含来自非-人超变区(HVR)的氨基酸残基和来自人框架区 (FR)的氨基酸残基的嵌合抗体。在某些实施方案中，人源化抗体包含基本上所有的至少一个和通常两个可变结构域，其中所有或基本上所有的HVR(例如，CDR)对应于非-人抗体的那些，和所有或基本上所有的FR对应于人抗体的那些。人源化抗体任选地可至少包含源自人抗体的抗体恒定区的一部分。"人源化形式"的抗体，例如，非-人抗体，是指已经过人源化的抗体。人源化抗体和制备它们的方法综述于例如， Almagro和Fransson(2008)Front.Biosci.13:1619-1633和进一步描述于例如，Riechmann等，(1988)Nature 332:323-329;Queen等(1989)Proc. Nat'l Acad.Sci.USA 86:10029-10033;美国专利号5,821,337; 7,527,791;6,982,321;和7,087,409;Kashmiri等，(2005)Methods 36:25-34[描述了SDR(a-CDR)移植];Padlan,Mol.Immunol.(1991) 28:489-498(描述了"表面重建");Dall'Acqua等(2005)Methods 36:43-60(描述了"FR改组");Osbourn等(2005)Methods 36:61-68;和 Klimka等Br.J.Cancer(2000)83:252-260(描述了FR改组的"引导选择"方法)。可用于人源化的人框架区包括但不限于：使用"最佳拟合"方法选择的框架区[参见例如，Sims等(1993)J.Immunol.151:2296];源自特定亚组的轻链或重链可变区的人抗体的共有序列的框架区[参见例如，Carter等(1992)Proc.Natl.Acad.Sci.USA,89:4285;和Presta等 (1993)J.Immunol.,151:2623];人成熟的(体细胞成熟)框架区或人种系框架区[参见例如，Almagro和Fransson(2008)Front.Biosci. 13:1619-1633];和源自筛选FR文库的框架区[参见例如，Baca等，(1997)J.Biol.Chem.272:10678-10684;和Rosok等，(1996)J.Biol.Chem. 271:22611-22618]。

[0311] 在某些实施方案中，本文提供的抗体是人抗体。人抗体可使用本领域已知的各种技术产生。人抗体总体描述于van Dijk和van de Winkel(2008)Curr.Opin.Pharmacol.5:368-74(2001)和Lonberg,Curr. Opin.Immunol.20:450-459。例如，人抗体可通过给予免疫原(例如， GDF11多肽、活化素B多肽、ActRIIA多肽或ActRIIB多肽)至转基因动物来制备，所述转基因动物已经修饰以在响应抗原攻击时产生完整人抗体或具有人可变区的完整抗体。这样的动物通常包含所有或一部分的人免疫球蛋白基因座，其替换了内源免疫球蛋白基因座或其在染色体外存在或随机整合至动物的染色体。在这样的转基因动物中，内源免疫球蛋白基因座总体上已被失活。对于从转基因动物获得人抗体的方法的综述，参见例如，Lonberg(2005)Nat.Biotech.23:1117-1125;美国专利号6,075,181和6,150,584(描述了XENOMOUSE™技术);美国专利号5,770,429(描述了HuMab®技术);美国专利号7,041,870(描述了K-M MOUSE®技术);和美国专利申请公开号2007/0061900(描述了VelociMouse®技术)。来自由这样的动物产生的完整抗体的人可变区可被进一步修饰，例如，通过与不同的人恒定区组合。

[0312] 本文提供的人抗体还可通过基于杂交瘤的方法制备。用于产生人单克隆抗体的人骨髓瘤和小鼠-人异骨髓瘤细胞系已有描述[参见例如， Kozbor J.Immunol.,(1984)133:3001;Brodeur等(1987)Monoclonal Antibody Production Techniques and Applications,pp.51-63,Marcel Dekker,Inc.,New York;和Boerner等(1991)J.Immunol.,147:86]。通过人B-细胞杂交瘤技术产生的人抗体还描述于Li等，(2006)Proc.

Natl.Acad.Sci.USA,103:3557-3562。另外的方法包括描述于例如,美国专利号7,189,826(描述了从杂交瘤细胞系产生单克隆人IgM抗体) 和Ni,Xiandai Mianyixue(2006)26(4):265-268(2006)(描述了人-人杂交瘤)中的那些。人杂交瘤技术(Trioma技术)还描述于Vollmers和 Brandlein(2005)Histol.Histopathol.,20(3):927-937(2005)和Vollmers和Brandlein(2005)Methods Find Exp.Clin.Pharmacol.,27(3):185-91。本文提供的人抗体还可通过分离选自人-衍生的噬菌体展示文库的Fv 克隆可变-结构域序列产生。这样的可变-结构域序列然后可与需要的人恒定结构域组合。从抗体文库选择人抗体的技术是本领域已知的和在本文描述。

[0313] 例如,本公开内容的抗体可通过筛选组合文库的具有所需的一种或多种活性的抗体分离。产生噬菌体展示文库和筛选这样的文库的具有所需结合特征的抗体的各种方法是本领域已知的。这样的方法综述于例如,Hoogenboom等(2001)的Methods in Molecular Biology 178:1-37,O′Brien等编辑,Human Press,Totowa,N.J.和进一步描述于例如,McCafferty等(1991)Nature 348:552-554;Clackson等,(1991) Nature 352:624-628;Marks等(1992)J.Mol.Biol.222:581-597;Marks 和Bradbury(2003)的Methods in Molecular Biology 248:161-175,Lo编辑,Human Press,Totowa,N.J.;Sidhu等(2004)J.Mol.Biol. 338(2):299-310;Lee等(2004)J.Mol.Biol.340(5):1073-1093;Fellouse(2004)Proc.Natl.Acad.Sci.USA 101(34):12467-12472;和Lee等(2004)J.Immunol.Methods 284(1-2):119-132。

[0314] 在某些噬菌体展示方法中,VH和VL基因库分别通过聚合酶链反应(PCR)克隆和在噬菌体文库中随机重组,其然后可对于抗原-结合噬菌体进行筛选,如描述于Winter等(1994)Ann.Rev.Immunol.,12: 433-455。噬菌体通常作为单链Fv(scFv)片段或作为Fab片段展示抗体片段。来自免疫来源的文库提供对免疫原(例如,ALK7、ActRIIB、活化素、GDF11、GDF8、BMP10、BMP6、GDF3和/或BMP5)的高- 亲和力抗体,不需要构建杂交瘤。或者,天然库可被克隆(例如,从人) 以提供对各种非-自身以及自身抗原的单一来源的抗体,不需要任何免疫,如描述于Griffiths等(1993)EMBO J,12:725-734。最后,天然文库还可通过从干细胞克隆未重排V-基因区段和使用包含随机序列的PCR引物合成制备,以编码高度可变的CDR3区和实现体外重排,如描述于Hoogenboom和Winter(1992)J.Mol.Biol.,227:381-388。描述人抗体噬菌体文库的专利出版物包括例如:美国专利号5,750,373 和美国专利公开号2005/0079574、2005/0119455、2005/0266000、 2007/0117126、2007/0160598、2007/0237764、2007/0292936和2009/0002360。

[0315] 在某些实施方案中,本文提供的抗体是多特异性抗体,例如,双特异性抗体。多特异性抗体(通常单克隆抗体)具有对一种或多种(例如,两种、三种、四种、五种、六种或更多种)抗原上的至少两个不同的表位(例如,两个、三个、四个、五个或六个或更多个)的结合特异性。

[0316] 制备多特异性抗体的技术包括但不限于,具有不同的特异性的两个免疫球蛋白重链/轻链对的重组共表达[参见例如,Milstein和Cuello (1983)Nature 305:537;国际专利公开号WO 93/08829;和Traunecker 等(1991)EMBO J.10:3655和美国专利号5,731,168(″结进孔″工程改造)]。多特异性抗体还可通过用于制备抗体Fc-异二聚体分子的工程改造静电导引作用(参见例如,WO 2009/089004A1);交联两个或更多个抗体或片段[参见例如,美

国专利号4,676,980;和Brennan等(1985) Science,229:81];使用亮氨酸拉链以产生双特异性抗体[参见例如, Kostelny等(1992)J.Immunol.,148(5):1547-1553];使用"双抗体"技术以制备双特异性抗体片段[参见例如,Hollinger等(1993)Proc.Natl. Acad.Sci.USA,90:6444-6448];使用单链Fv(sFv)二聚体[参见例如, Gruber等(1994)J.Immunol.,152:5368];和制备三特异性抗体(参见例如,Tutt等(1991)J.Immunol.147:60)制备。多特异性抗体可作为全长抗体或抗体片段制备。具有三个或更多个功能抗原结合位点的工程改造的抗体,包括"章鱼抗体(Octopus antibodies)",也包括在本文中[参见例如,US 2006/0025576A1]。

[0317] 在某些实施方案中,本文公开的抗体是单克隆抗体。单克隆抗体是指获自一群基本上同质的抗体的抗体,即,包含该群的单个抗体是相同的和/或结合相同的表位,除了可能的变体抗体之外,例如,包含天然存在的突变或在产生单克隆抗体制备物期间产生,这样的变体通常以较少量存在。与通常包括针对不同表位的不同抗体的多克隆抗体制备物形成对比,单克隆抗体制备物的每个单克隆抗体针对抗原上的单一表位。因此,修饰语"单克隆"表示获自抗体的基本同质群的抗体的特性和不应解释为需要通过任何特定方法产生抗体。例如,根据本发明的方法使用的单克隆抗体可通过各种技术制备,包括但不限于杂交瘤方法、重组DNA方法、噬菌体展示方法和利用包含所有或部分的人免疫球蛋白基因座的转基因动物的方法,这样的方法和制备单克隆抗体的其它实例性方法在本文中描述。

[0318] 例如,通过使用源自活化素的免疫原,抗-蛋白/抗-肽抗血清或单克隆抗体可通过标准方案制备[参见例如,Antibodies:A Laboratory Manual,由Harlow和Lane编辑(1988)Cold Spring Harbor Press:1988]。哺乳动物,例如小鼠、仓鼠或兔,可用免疫原性形式的活化素多肽(一种能够引发抗体反应的抗原片段)或融合蛋白免疫。赋予蛋白或肽免疫原性的技术包括与载体缀合或本领域熟知的其它技术。活化素多肽的免疫原性部分可在佐剂的存在下给予。免疫进展可通过检测血浆或血清中的抗体滴度来监测。用作为抗原的免疫原,标准ELISA或其它免疫测定法可用于评价抗体产生水平和/或结合亲和力水平。

[0319] 用活化素的抗原制备物免疫动物后,可获得抗血清,并且如果需要,可从血清分离多克隆抗体。为了产生单克隆抗体,产生抗体的细胞(淋巴细胞)可自免疫的动物收获,和通过标准体细胞融合程序与永生细胞例如骨髓瘤细胞融合,得到杂交瘤细胞。这样的技术是本领域众所周知的和包括例如,杂交瘤技术[参见例如,Kohler和Milstein (1975)Nature,256:495-497]、人B细胞杂交瘤技术[参见例如,Kozbar 等(1983)Immunology Today,4:72]和产生人单克隆抗体的EBV-杂交瘤技术[Cole等(1985)Monoclonal Antibodies and Cancer Therapy,Alan R.Liss,Inc.pp.77-96]。杂交瘤细胞可经免疫化学筛选以产生与活化素多肽特异性反应的抗体和从包含这样的杂交瘤细胞的培养物分离单克隆抗体。

[0320] 在某些实施方案中,一个或多个氨基酸修饰可引入至本文提供的抗体的Fc区,从而产生Fc区变体。Fc区变体可包含在一个或多个氨基酸位置处包含氨基酸修饰(例如,置换、缺失和/或添加)的人Fc区序列(例如,人IgG1、IgG2、IgG3或IgG4Fc区)。

[0321] 例如,本公开内容考虑了具有一些但并非所有效应功能的抗体变体,对于其中抗体体内半寿期是重要的但某些效应功能[例如,补体依赖性细胞毒性(CDC)和抗体依赖性细胞毒性(ADCC)]是不需要的或有害的应用,这使得它成为理想的候选物。可进行体外和/或体内细胞毒性测定以证实CDC和/或ADCC活性的减少/耗尽。例如,可进行Fc 受体(FcR)结合

测定法以确保抗体缺少FcγR结合(因此可能缺少ADCC 活性),但保留FcRn结合能力。介导ADCC的原代细胞,NK细胞,仅表达FcγRIII,但单核细胞表达FcγRI、FcγRII和FcγRIII。生血细胞上FcR表达概述于例如,Ravetch和Kinet(1991)Annu.Rev.Immunol. 9:457-492。评价目的分子的ADCC活性的体外测定法的非限制性实例描述于美国专利号5,500,362;Hellstrom,I.等(1986)Proc.Natl.Acad. Sci.USA 83:7059-7063];Hellstrom,I等(1985)Proc.Natl.Acad.Sci. USA 82:1499-1502;美国专利号5,821,337;Bruggemann,M.等(1987) J.Exp.Med.166:1351-1361。或者,可使用非-放射性测定方法(例如, ACTI™,用于流式细胞术的非-放射性细胞毒性测定法; CellTechnology,Inc.Mountain View,Calif.;和CytoTox96®非-放射性细胞毒性测定法,Promega,Madison,Wis.)。可用于这样的测定法的效应细胞包括外周血单核细胞(PBMC)和天然杀伤(NK)细胞。或者,或另外,目的分子的ADCC活性可体内评价,例如,在动物模型中,例如公开于Clynes等(1998)Proc.Natl.Acad.Sci.USA 95:652-656。C1q 也可进行结合测定法以证实抗体不能结合C1q和因此缺少CDC活性 [参见例如,C1q和C3c结合ELISA,WO 2006/029879和WO 2005/100402]。为了评价补体活化,可进行CDC测定法[参见例如, Gazzano-Santoro等(1996)J.Immunol.Methods 202:163;Cragg,M.S. 等(2003)Blood 101:1045-1052;和Cragg,M.S和M.J.Glennie(2004) Blood 103:2738-2743]。FcRn结合和体内清除率/半寿期测定也可使用本领域已知的方法进行[参见例如,Petkova,S.B.等(2006)Intl. Immunol.18(12):1759-1769]。具有减少的效应功能的本公开内容的抗体包括其中Fc区残基238、265、269、270、297、327和329的一个或多个被置换的那些(美国专利号6,737,056)。这样的Fc突变体包括在氨基酸位置265、269、270、297和327的两个或更多个处具有置换的Fc突变体,包括残基265和297置换为丙氨酸的所谓的"DANA"Fc 突变体(美国专利号7,332,581)。

[0322] 在某些实施方案中,可能需要产生半胱氨酸工程改造的抗体,例如,"thioMAb",其中抗体的一个或多个残基被半胱氨酸残基置换。在具体的实施方案中,置换的残基出现在抗体的可接近位点。通过用半胱氨酸置换这些残基,反应性硫醇基团从而位于抗体的可接近位点,可用于使抗体与其它部分例如药物部分或接头-药物部分缀合,以产生免疫缀合物,如本文进一步描述的。在某些实施方案中,任何一种或多种以下残基可被半胱氨酸置换:轻链的V205(Kabat编号);重链的 A118(EU编号);和重链Fc区的S400(EU编号)。半胱氨酸工程改造的抗体可按例如美国专利号7,521,541中所述产生。

[0323] 此外,用于筛选抗体以鉴定需要的抗体的技术可影响获得的抗体的性质。例如,如果抗体用于在溶液中结合抗原,可能需要测试溶液结合。可获得各种不同的技术以测试抗体和抗原之间的相互作用,以鉴定特别需要的抗体。这样的技术包括ELISA、表面等离子体共振结合测定法(例如,Biacore结合测定法,Biacore AB,Uppsala,Sweden)、夹心测定法(例如,IGEN International,Inc.,Gaithersburg,Maryland 的顺磁珠系统)、蛋白质印迹、免疫沉淀测定法和免疫组织化学。

[0324] 在某些实施方案中,考虑了本文提供的抗体和/或结合多肽的氨基酸序列变体。例如,可能需要改进抗体和/或结合多肽的结合亲和力和 /或其它生物学性质。抗体和/或结合多肽的氨基酸序列变体可通过引入合适的修饰至编码抗体和/或结合多肽的核苷酸序列或通过肽合成来制备。这样的修饰包括例如,抗体和/或结合多肽的氨基酸序列内的残基的缺失和/或插入和/或置换。可进行缺失、插入和置换的任何组合以实现最终的构建体,只要

最终的构建体具有需要的特征，例如，靶标结合(例如，和活化素例如活化素E和/或活化素C结合)。

[0325] 可在HVR中进行改变(例如，置换)，例如以改进抗体亲和力。这样的改变可在HVR"热点"，即由在体细胞成熟过程中经过高频率突变的密码子编码的残基[参见例如，Chowdhury(2008)Methods Mol.Biol. 207:179-196(2008)]和/或SDRs(a-CDRs)中进行，其中测试得到的变体 VH或VL的结合亲和力。本领域已描述通过构建二级文库和从中再选择的亲和力成熟[参见例如，Hoogenboom等，Methods in Molecular Biology 178:1-37，O'Brien等编辑，Human Press，Totowa，N.J.，(2001)。在亲和力成熟的一些实施方案中，通过各种方法的任一种将多样性引入经选择用于成熟的可变基因中(例如，易错PCR、链改组或寡核苷酸-定点诱变)。然后产生二级文库。然后筛选该文库以鉴定具有所需亲和力的任何抗体变体。引入多样性的另一方法包括HVR-定向方法，其中数个HVR残基(例如，一次4-6残基)被随机化。参与抗原结合的 HVR残基可被特别鉴定，例如，使用丙氨酸扫描诱变或建模。特别是，经常靶向CDR-H3和CDR-L3。

[0326] 在某些实施方案中，置换、插入或缺失可在一个或多个HVR内发生，只要这样的改变基本上不减少抗体结合抗原的能力。例如，基本上不减少结合亲和力的保守改变(例如，本文提供的保守置换)可在 HVR中进行。这样的改变可在HVR"热点"或SDR外。在上述提供的变体VH和VL序列的某些实施方案中，每个HVR是未改变的，或包含不超过一个、两个或三个氨基酸置换。

[0327] 用于鉴定抗体和/或结合多肽的可靶向诱变的残基或区域的方法被称为"丙氨酸扫描诱变"，描述于Cunningham和Wells (1989)Science， 244:1081-1085。在该方法中，残基或一组靶残基(例如，带电荷残基，例如Arg、Asp、His、Lys和Glu)被鉴定和被中性或带负电荷的氨基酸(例如，丙氨酸或多聚丙氨酸)置换，以确定抗体-抗原相互作用是否受到影响。进一步的置换可在证实对初始置换功能敏感性的氨基酸位置引入。或者，或另外，抗原-抗体复合物的晶体结构经测定以鉴定抗体和抗原之间的接触点。这样的接触残基和邻近残基可被靶向或消除，作为置换候选者。变体可经筛选以确定它们是否包含需要的性质。

[0328] 氨基酸序列插入包括长度范围为1个残基至包含一百或更多个残基的多肽的氨基-和/或羧基-末端融合物，以及单个或多个氨基酸残基的序列内插入。末端插入的实例包括具有N-末端甲硫氨酰残基的抗体。抗体分子的其它插入变体包括抗体的N-或C-末端融合至酶(例如，对于ADEPT)或增加抗体的血清半寿期的多肽。

[0329] 在某些实施方案中，本文提供的抗体和/或结合多肽可进一步修饰以包含本领域已知的和容易获得的另外的非蛋白性部分。适合于衍生抗体和/或结合多肽的部分包括但不限于水可溶性聚合物。水可溶性聚合物的非限制性实例包括但不限于聚乙二醇(PEG)、乙二醇/丙二醇的共聚物、羧基甲基纤维素、葡聚糖、聚乙烯醇、聚乙烯基吡咯烷酮、聚-1,3-二氧杂环戊烷、聚-1,3,6-三氧杂环已烷、乙烯/马来酸酐共聚物、聚氨基酸(同聚物或随机共聚物)和葡聚糖或聚(n-乙烯基吡咯烷酮)聚乙二醇、丙二醇同聚物、环氧丙烷/环氧乙烷共聚物、聚氧乙基化多元醇(例如，甘油)、聚乙烯醇和其混合物。聚乙二醇丙醛由于其水稳定性可具有制造益处。聚合物可具有任何分子量和可以是分支的或未分支的。连接至抗体和/或结合多肽的聚合物的数量可改变，和如果连接超过一个聚合物，它们可以是相同的或不同的分子。一般而言，用于衍生的聚合物的数量和/或类型可根据以下考虑来确定，包括但

不限于待改进的抗体和/或结合多肽的特定性质或功能，抗体衍生物和/或结合多肽衍生物是否将在限定的条件下用于疗法。

[0330] D.小分子拮抗剂

[0331] 在其它方面，ALK7:ActRIIB拮抗剂是小分子(ALK7:ActRIIB小分子拮抗剂)或小分子拮抗剂的组合。ALK7:ActRIIB小分子拮抗剂或小分子拮抗剂的组合可抑制例如，一种或多种ALK7:ActRIIB-结合配体、I型受体(例如，ALK7)、II型受体(例如，ActRIIB)和/或共受体(例如，Cripto或Cryptic)。在一些实施方案中，ALK7:ActRIIB小分子拮抗剂或小分子拮抗剂的组合抑制由一种或多种ALK7:ActRIIB-结合配体介导的信号传导，例如，在基于细胞的测定法中例如本文所述的那些中所测定的。如本文所述的，ALK7:ActRIIB小分子拮抗剂可单独或与一种或多种支持性疗法或活性剂组合使用，以治疗有需要的患者(例如，具有肾疾病和/或代谢病症的患者)。

[0332] 在一些实施方案中，ALK7:ActRIIB小分子拮抗剂或小分子拮抗剂的组合至少抑制GDF11。在一些实施方案中，ALK7:ActRIIB小分子拮抗剂或小分子拮抗剂的组合至少抑制GDF8。在一些实施方案中，ALK7:ActRIIB小分子拮抗剂或小分子拮抗剂的组合至少抑制活化素 (活化素A、活化素B、活化素C、活化素E、活化素AB、活化素AC、活化素BC、活化素AE和/或活化素BE)。在一些实施方案中，

[0333] ALK7:ActRIIB小分子拮抗剂或小分子拮抗剂的组合至少抑制GDF11、GDF8和活化素。在一些实施方案中，ALK7:ActRIIB小分子拮抗剂或小分子拮抗剂的组合至少抑制ALK7。在一些实施方案中，

[0334] ALK7:ActRIIB小分子拮抗剂或小分子拮抗剂的组合至少抑制

[0335] ActRIIB。在一些实施方案中，ALK7:ActRIIB小分子拮抗剂或小分子拮抗剂的组合至少抑制Cripto或Cryptic。在一些实施方案中，

[0336] ALK7:ActRIIB小分子拮抗剂或小分子拮抗剂的组合至少抑制BMP6。在一些实施方案中，ALK7:ActRIIB小分子拮抗剂或小分子拮抗剂的组合至少抑制GDF3。在一些实施方案中，ALK7:ActRIIB小分子拮抗剂或小分子拮抗剂的组合至少抑制BMP5。在一些实施方案中，

[0337] ALK7:ActRIIB小分子拮抗剂或小分子拮抗剂的组合至少抑制BMP10。在一些实施方案中，ALK7:ActRIIB小分子拮抗剂或小分子拮抗剂的组合至少抑制nodal。在一些实施方案中，本文公开的ALK7:ActRIIB 小分子拮抗剂或小分子拮抗剂的组合不抑制或基本上不抑制BMP9。

[0338] ALK7:ActRIIB小分子拮抗剂可以是直接或间接抑制剂。例如，间接小分子拮抗剂或小分子拮抗剂的组合可抑制至少一种或多种TGF-β超家族配体[例如，活化素(例如，活化素A、活化素B、活化素C、活化素E、活化素AB、活化素AC、活化素B、活化素BC、活化素AE或活化素BE)、GDF11、BMP10、BMP9、BMP6、BMP5、 GDF3、活化素C、活化素E、活化素AC、GDF8、BMP10、BMP6、 BMP5、nodal和GDF3、I型受体(例如，ALK7)、II型受体(例如，ActRIIB)、共受体(例如，Cripto或Cryptic)和/或一种或多种下游信号传导组分(例如，Smads)的表达(例如，转录、翻译、细胞分泌或其组合)。或者，直接小分子拮抗剂或小分子拮抗剂的组合可直接结合和抑制例如，一种或多种TGF-β超家族配体[例如，活化素(例如，活化素A、活化素 B、活化素C、活化素E、活化素AB、活化素AC、活化素B、活化素BC、活化素AE或活化素BE)、GDF11、BMP10、BMP9、BMP6、 BMP5、GDF3、活化素C、活化素E、活化素AC、GDF8、BMP10、 BMP6、BMP5、nodal和

GDF3、I型受体(例如,ALK7)、II型受体(例如,ActRIIB)、共受体(例如,Cripto或Cryptic)和/或下游信号传导组分(例如,Smads)。一种或多种间接和一种或多种直接ALK7:ActRIIB小分子拮抗剂的组合可根据本文公开的方法使用。

[0339] 本公开内容的结合小分子拮抗剂可经鉴定和使用已知的方法化学合成(参见例如,PCT公开号WO 00/00823和WO 00/39585)。一般而言,本公开内容的小分子拮抗剂通常小于约2000道尔顿大小,或者小于约1500、750、500、250或200道尔顿大小,其中这样的有机小分子能够结合、优选地特异性结合本文所述的多肽。这些小分子拮抗剂可使用众所周知的技术鉴定而无需过度实验。在此方面,注意用于筛选有机小分子文库的能够结合多肽靶标的分子的技术是本领域众所周知的(参见例如,国际专利公开号WO00/00823和WO00/39585)。

[0340] 本公开内容的结合有机小分子可以是例如,醛、酮、肟、腙、缩氨基脲、碳二酰肼、伯胺、仲胺、叔胺、N-取代的肼、酰肼、醇、醚、硫醇、硫醚、二硫化物、羧酸、酯、酰胺、脲、氨基甲酸酯、碳酸酯、缩酮、硫代缩酮、缩醛、硫代缩醛、芳基卤化物、芳基磺酸酯、烷基卤化物、烷基磺酸酯、芳香族化合物、杂环化合物、苯胺、烯、炔、二醇、氨基醇、噁唑烷、噁唑啉、噻唑烷、噻唑啉、烯胺、磺酰胺、环氧化物、氮丙啶、异氰酸酯、磺酰氯、重氮基化合物和酰基氯。

[0341] E.多核苷酸拮抗剂

[0342] 在其它方面,ALK7:ActRIIB拮抗剂是多核苷酸(ALK7:ActRIIB多核苷酸拮抗剂)或多核苷酸的组合。ALK7:ActRIIB多核苷酸拮抗剂或多核苷酸拮抗剂的组合可抑制例如,一种或多种ALK7:ActRIIB-结合配体、I型受体(例如,ALK7)、II型受体(例如,ActRIIB)、共受体(例如,Cripto或Cryptic)和/或下游信号传导组分(例如,Smads)。在一些实施方案中,ALK7:ActRIIB多核苷酸拮抗剂或多核苷酸拮抗剂的组合抑制由一种或多种ALK7:ActRIIB-结合配体介导的信号传导,例如,在基于细胞的测定法中例如本文所述的那些中所测定的。如本文所述的,ALK7:ActRIIB多核苷酸拮抗剂可单独或与一种或多种支持性疗法或活性剂组合使用,以治疗有需要的患者(例如,具有肾疾病和/或代谢病症的患者)。

[0343] 在一些实施方案中,ALK7:ActRIIB多核苷酸拮抗剂或多核苷酸拮抗剂的组合至少抑制GDF11。在一些实施方案中,ALK7:ActRIIB 多核苷酸拮抗剂或多核苷酸拮抗剂的组合至少抑制GDF8。在一些实施方案中,ALK7:ActRIIB多核苷酸拮抗剂或多核苷酸拮抗剂的组合至少抑制活化素(活化素A、活化素B、活化素C、活化素E、活化素 AB、活化素AC、活化素AE、活化素BC和/或活化素BE)。在一些实施方案中,ALK7:ActRIIB多核苷酸拮抗剂或多核苷酸拮抗剂的组合至少抑制GDF11、GDF8和活化素。在一些实施方案中,

[0344] ALK7:ActRIIB多核苷酸拮抗剂或多核苷酸拮抗剂的组合至少抑制 ALK7。在一些实施方案中,ALK7:ActRIIB多核苷酸拮抗剂或多核苷酸拮抗剂的组合至少抑制ActRIIB。在一些实施方案中,ALK7:ActRIIB 多核苷酸拮抗剂或多核苷酸拮抗剂的组合至少抑制Crypto或Cryptic。在一些实施方案中,ALK7:ActRIIB多核苷酸拮抗剂或多核苷酸拮抗剂的组合至少抑制ActRIIB。在一些实施方案中,ALK7:ActIIB多核苷酸拮抗剂或多核苷酸拮抗剂的组合至少抑制BMP6。在一些实施方案中,ALK7:ActRIIB多核苷酸拮抗剂或多核苷酸拮抗剂的组合至少抑制GDF3。在一些实施方案中,ALK7:ActRIIB多核苷酸拮抗剂或多核苷酸拮抗剂的组合至少抑制BMP10。在一些实施方案中,

[0345] ALK7:ActRIIB多核苷酸拮抗剂或多核苷酸拮抗剂的组合至少抑制 BMP5。在一些

实施方案中，ALK7:ActRIIB多核苷酸拮抗剂或多核苷酸拮抗剂的组合至少抑制nodal。在一些实施方案中，本文公开的 ALK7:ActRIIB多核苷酸拮抗剂或多核苷酸拮抗剂的组合不抑制或基本上不抑制BMP9。

[0346] 在一些实施方案中，本公开内容的多核苷酸拮抗剂可以是反义核酸、RNAi分子[例如，小干扰RNA(siRNA)、小-发夹RNA (shRNA)、微RNA(miRNA)]、适体和/或核酶。人GDF11、GDF8、活化素(活化素A、活化素B、活化素C和活化素E)、BMP6、GDF3、 BMP5、ALK7、ActRIIB、Cripto、Cryptic、Nodal和BMP10的核酸和氨基酸序列是本领域已知的。此外，产生多核苷酸拮抗剂的许多不同的方法是本领域众所周知的。因此，根据本公开内容使用的多核苷酸拮抗剂可由本领域技术人员基于本领域的知识和本文提供的教导常规制备。

[0347] 反义技术可通过反义DNA或RNA或通过三股螺旋形成，用于控制基因表达。反义技术论述于例如，Okano(1991)J.Neurochem.56:560; Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression,CRC Press,Boca Raton,Fla.(1988)。三股螺旋形成论述于例如，Cooney等 (1988)Science 241:456;和Dervan等，(1991)Science 251:1300。该方法基于多核苷酸与互补DNA或RNA的结合。在一些实施方案中，反义核酸包含单链的RNA或DNA序列，其与本文公开的基因的RNA转录物的至少一部分互补。然而，绝对互补性尽管是优选的，但不是必需的。

[0348] 本文提及的"与RNA的至少一部分互补的"序列是指具有足够的互补性能够与RNA杂交，形成稳定的双链体的序列;在本文公开的基因的双链反义核酸的情况下，可因此测试双链体DNA的单链，或可测定三链体形成。杂交能力取决于互补性的程度和反义核酸的长度。通常，杂交核酸越长，其可包含越多的与RNA的碱基错配，和仍形成稳定的双链体(或根据情况，三链体)。本领域技术人员可通过使用确定杂交复合物的解链温度的标准程序来确定错配的耐受程度。

[0349] 与信使的5′端、例如直至和包括AUG起始密码子的5′-非翻译序列互补的多核苷酸应在抑制翻译中最有效地起作用。然而，与mRNA 的3′-非翻译序列互补的序列已表明也有效抑制mRNA的翻译[参见例如，Wagner,R.,(1994)Nature 372:333-335]。因此，与本公开内容的基因的5′-或3′-非翻译、非编码区互补的寡核苷酸可用于反义方法以抑制内源mRNA的翻译。与mRNA的5′-非翻译区互补的多核苷酸应包括AUG起始密码子的组分。与mRNA编码区互补的反义多核苷酸是较不有效的翻译抑制剂，但能够根据本公开内容的方法使用。无论设计为与本公开内容的mRNA的5′-、3′-或编码区杂交，反义核酸应为至少6个核苷酸长度和优选地范围为6-约50个核苷酸长度的寡核苷酸。在特别的方面，寡核苷酸为至少10个核苷酸、至少17个核苷酸、至少25个核苷酸或至少50个核苷酸。

[0350] 在一个实施方案中，本公开内容的反义核酸通过自异源序列转录在细胞内产生。例如，载体或其部分经转录，产生本公开内容的基因的反义核酸(RNA)。这样的载体包含编码需要的反义核酸的序列。这样的载体可保持为附加体或成为染色体整合的，只要它可被转录以产生需要的反义RNA。这样的载体可通过本领域标准的重组DNA技术方法构建。载体可以是质粒、病毒或本领域已知的其它载体，用于在脊椎动物细胞中复制和表达。编码本公开内容的需要的基因或其片段的序列的表达可通过本领域已知的用于脊椎动物、优选地人细胞的任何启动子进行。这样的启动子可以是诱导型或组成型的。这样的启动子包括但不限于SV40早期启动子区[参见例如，Benoist和Chambon (1981)Nature 290:304-310]、在劳

斯肉瘤病毒的3′长-末端重复区中包含的启动子[参见例如,Yamamoto等(1980)Cell 22:787-797]、疱疹胸腺嘧啶启动子[参见例如,Wagner等(1981)Proc.Natl.Acad.Sci.U.S.A.78:1441-1445]和金属硫蛋白基因的调节序列[参见例如,Brinster,等 (1982)Nature 296:39-42]。

[0351] 在一些实施方案中,多核苷酸拮抗剂是干扰RNA(RNAi)分子,其靶向以下一种或多种的表达:GDF11、GDF8、活化素(活化素A、活化素B、活化素C和活化素E)、BMP6、GDF3、BMP5、ALK7、 ActRIIB、Cripto、Cryptic、nodal和BMP10。RNAi是指干扰靶标 mRNA的表达的RNA的表达。特别地,RNAi通过与特定的mRNA 通过siRNA(小干扰RNA)相互作用来沉默靶标基因。ds RNA复合物然后被细胞靶向降解。siRNA分子是10-50个核苷酸长度的双链的 RNA双链体,其干扰足够互补的靶基因(例如,与该基因具有至少80%同一性)的表达。在一些实施方案中,siRNA分子包含与靶基因的核苷酸序列具有至少85、90、95、96、97、98、99或100%同一性的核苷酸序列。

[0352] 另外的RNAi分子包括短发夹RNA(shRNA);以及短干扰发夹和微RNA(miRNA)。shRNA分子包含通过环连接的来自靶基因的有义和反义序列。shRNA从细胞核转运至细胞质并且其与mRNA一起降解。对于RNAi,PolIII或U6启动子可用于表达RNA。Paddison等 [Genes&Dev.(2002)16:948-958,2002]已经使用折叠成发夹的小的 RNA分子作为实现RNAi的工具。因此,这样的短发夹RNA(shRNA) 分子也可有利地用于本文所述的方法。功能shRNA的茎和环的长度不同;茎长度范围可大概为约25-约30nt,和环大小范围可为4-约 25nt而不影响沉默活性。尽管不希望受任何特定理论的束缚,认为这些shRNA类似于DICER RNase的双链RNA(dsRNA)产物,并且在任何情况下具有相同的抑制特定基因表达的能力。shRNA可从慢病毒载体表达。miRNA是约10-70个核苷酸长度的单链RNA,其初始转录为特征为“茎-环”结构的前-miRNA,后者在通过RISC进一步加工后,随后被加工成成熟miRNA。

[0353] 介导RNAi的分子,包括但不限于siRNA,可通过化学合成(Hohjoh, FEBS Lett 521:195-199,2002)、dsRNA水解(Yang等,Proc Natl Acad Sci USA 99:9942-9947,2002),通过用T7RNA聚合酶体外转录 (Donzeet等,Nucleic Acids Res 30:e46,2002;Yu等,Proc Natl Acad Sci USA 99:6047-6052,2002)和使用核酸酶例如大肠杆菌RNase III通过双链RNA水解(Yang等,Proc Natl Acad Sci USA 99:9942-9947,2002) 体外产生。

[0354] 根据另一方面,本公开内容提供了多核苷酸拮抗剂,其包括但不限于诱饵DNA、双链DNA、单链DNA、复合DNA、包封DNA、病毒DNA、质粒DNA、裸RNA、包封RNA、病毒RNA、双链RNA、能够产生RNA干扰的分子或其组合。

[0355] 在一些实施方案中,本公开内容的多核苷酸拮抗剂是适体。适体是核酸分子,包括双链DNA和单链RNA分子,其结合和形成特异性结合靶分子的三级结构。适体的产生和治疗用途是本领域充分确立的 (参见例如,美国专利号5,475,096)。关于适体的另外信息可见于美国专利申请公开号20060148748。核酸适体使用本领域已知的方法选择,例如通过Systematic Evolution of Ligands by Exponential Enrichment (SELEX)方法。SELEX是具有与靶分子高度特异性结合的核酸分子的体外进化的方法,如描述于例如,美国专利号5,475,096;5,580,737; 5,567,588;5,707,796;5,763,177;6,011,577和6,699,843。鉴定适体的另一筛选方法描述于美国专利号5,270,163。SELEX方法基于核酸形成各种二维和三维结构的能力,以及核苷酸单体内作为配体的可用化学多功能性(与基本上任何化学化合

物形成特异性结合对，无论是单体或聚合物，包括其它核酸分子和多肽)。任何大小的分子或组分可用作靶标。SELEX方法包括从候选寡核苷酸的混合物选择，和结合、分隔和扩增的逐步重复，使用相同的通用选择方案，以实现需要的结合亲和力和选择性。从可包含随机化序列的区段的核酸的混合物开始，SELEX方法包括以下步骤：使混合物与靶标在有利于结合的条件下接触；分隔未结合的核酸与已经特异性结合至靶分子的那些核酸；离解核酸-靶标复合物；扩增从核酸-靶标复合物离解的核酸以得到富含配体的核酸混合物。结合、分隔、离解和扩增的步骤按需要重复许多循环，以得到以高亲和力和特异性结合靶标分子的核酸配体。

[0356] 通常，这样的结合分子单独给予动物[参见例如，O′Connor(1991)J.Neurochem.56:560]，但这样的结合分子也可自宿主细胞摄入的多核苷酸体内表达，和体内表达[参见例如，Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression,CRC Press,Boca Raton,Fla. (1988)]。

[0357] F.卵泡抑素和FLRG拮抗剂

[0358] 已知卵泡抑素和FLRG族蛋白的成员拮抗通过ALK7:ActRIIB途径发信号的配体。因此，在其它方面，ALK7:ActRIIB拮抗剂是卵泡抑素或FLRG多肽，其可单独或与本文公开的一种或多种另外的支持性疗法和/或活性剂组合使用，以实现需要的效果(例如，治疗具有肾疾病和/或代谢病症的患者)。

[0359] 术语"卵泡抑素多肽"包括包含卵泡抑素的任何天然存在的多肽以及其保留有用活性的任何变体(包括突变体、片段、融合物和拟肽形式) 的多肽，和进一步包括卵泡抑素的任何功能单体或多聚体。在某些优选的实施方案中，本公开内容的卵泡抑素多肽结合和/或抑制活化素和 /或GDF8活性。卵泡抑素多肽的保留活化素结合性质的变体可根据之前的涉及卵泡抑素和活化素相互作用的研究鉴定。例如，WO2008/030367公开了特定的卵泡抑素结构域("FSD")，其表明对于活化素结合是重要的。如在下文SEQ ID NOs:90-94中显示的，卵泡抑素 N-末端结构域("FSND"SEQ ID NO:92)、FSD2(SEQ ID NO:94)和较少程度上FSD1(SEQ ID NO:93)代表了卵泡抑素内对于活化素结合是重要的实例性结构域。此外，用于制备和测试多肽文库的方法在上文的 ActRII多肽的情况下进行了描述，和这样的方法还涉及制备和测试卵泡抑素变体。卵泡抑素多肽包括源自任何已知卵泡抑素的序列的多肽，其具有与卵泡抑素多肽的序列具有至少约80％同一性和任选地至少 85％、90％、95％、96％、97％、98％、99％或更大同一性的序列。卵泡抑素多肽的实例包括成熟的卵泡抑素多肽或人卵泡抑素前体多肽 (SEQ ID NO:90)的较短的同种型或其它变体，如描述于例如WO2005/025601。

[0360] 人卵泡抑素前体多肽同种型FST344如下：

```
  1 MVRARHQPGG LCLLLLLLCQ FMEDRSAQAG NCWLRQAKNG RCQVLYKTEL
 51   SKEECCSTGR LSTSWTEEDV NDNTLFKWMI FNGGAPNCIP CKETCENVDC
101   GPGKKCRMNK KNKPRCVCAP DCSNITWKGP VCGLDGKTYR NECALLKARC
151   KEQPELEVQY QGRCKKTCRD VFCPGSSTCV VDQTNNAYCV TCNRICPEPA
201   SSEQYLCGND GVTYSSACHL RKATCLLGRS IGLAYEGKCI KAKSCEDIQC
251   TGGKKCLWDF KVGRGRCSLC DELCPDSKSD EPVCASDNAT YASECAMKEA
301 ACSSGVLLEV KHSGSCNSIS EDTEEEEEDE DQDYSFPISS ILEW
```

[0361]

[0362] (SEQ ID NO:90;NCBI参考号NP_037541.1)

[0363] 信号肽用下划线表示;上文的下划线还表示最后27个残基,其代表了区分该卵泡抑素同种型与下文所示的较短的卵泡抑素同种型 FST317的C-末端延伸。

[0364] 人卵泡抑素前体多肽同种型FST317如下:

[0365] 1 MVRARHQPGGLCLLLLLLCQFMEDRSAQAG NCWLRQAKNG RCQVLYKTEL

[0366] 51 SKEECCSTGR LSTSWTEEDV NDNTLFKWMI FNGGAPNCIP CKETCENVDC

[0367] 101 GPGKKCRMNK KNKPRCVCAP DCSNITWKGP VCGLDGKTYR NECALLKARC

[0368] 151 KEQPELEVQY QGRCKKTCRD VFCPGSSTCV VDQTNNAYCV TCNRICPEPA

[0369] 201 SSEQYLCGND GVTYSSACHL RKATCLLGRS IGLAYEGKCI KAKSCEDIQC

[0370] 251 TGGKKCLWDF KVGRGRCSLC DELCPDSKSD EPVCASDNAT YASECAMKEA

[0371] 301 ACSSGVLLEV KHSGSCN(SEQ ID NO:91;NCBI参考号 NP_006341.1)

[0372] 信号肽用下划线表示。

[0373] 卵泡抑素N-末端结构域(FSND)序列如下:

[0374] GNCWLRQAKNGRCQVLYKTELSKEECCSTGRLSTS WTEEDVNDNTLFKWMIFNGGAPNCIPCK(SEQ ID NO:92; FSND)

[0375] FSD1和FSD2序列如下:

[0376] ETCENVDCGPGKKCRMNKKNKPRCV(SEQ ID NO: 93;FSD1)

[0377] KTCRDVFCPGSSTCVVDQTNNAYCVT(SEQ ID NO:94;FSD2)

[0378] 在其它方面,ALK7:ActRIIB拮抗剂是卵泡抑素-样相关基因 (FLRG),亦称为卵泡抑素-相关蛋白3(FSTL3)。术语"FLRG多肽"包括包含FLRG的任何天然存在的多肽以及其保留有用活性的任何变体(包括突变体、片段、融合物和拟肽形式)的多肽。在某些实施方案中,本公开内容的FLRG多肽结合和/或抑制活化素活性、特别是活化素A。FLRG多肽的保留活化素结合性质的变体可使用测定FLRG和活化素相互作用的常规方法鉴定(参见例如,US 6,537,966)。此外,用于制备和测试多肽文库的方法在上文的ActRII和ALK7多肽的背景下进行描述,和这样的方法还涉及制备和测试FLRG的变体。FLRG多肽包括源自任何已知FLRG的序列的多肽,其具有与FLRG多肽的序列具有至少约80%同一性和任选地至少85%、90%、95%、97%、99%或更大同一性的序列。

[0379] 人FLRG前体(卵泡抑素-相关蛋白3前体)多肽如下:

[0380] 1 MRPGAPGPLWPLPWGALAWAVGFVSSMGSG NPAPGGVCWL QQGQEATCSL

[0381] 51VLQTDVTRAE CCASGNIDTA WSNLTHPGNK INLLGFLGLV HCLPCKDSCD

[0382] 101 GVECGPGKAC RMLGGRPRCE CAPDCSGLPA RLQVCGSDGA TYRDECELRA

[0383] 151 ARCRGHPDLS VMYRGRCRKS CEHVVCPRPQ SCVVDQTGSA HCVVCRAAPC

[0384] 201 PVPSSPGQEL CGNNNVTYIS SCHMRQATCF LGRSIGVRHA GSCAGTPEEP

[0385] 251 PGGESAEEEE NFV(SEQ ID NO:95;NCBI参考号 NP_005851.1)

[0386] 信号肽用下划线表示。

[0387] 在某些实施方案中,卵泡抑素多肽和FLRG多肽的功能变体或修饰形式包括具有至少一部分的卵泡抑素多肽或FLRG多肽和一种或多种融合结构域、例如促进多肽的分离、检测、稳定或多聚化的结构域的融合蛋白。合适的融合结构域在上文参照ActRII多肽详细论述。在一些实施方案中,本公开内容的拮抗剂是包含融合至Fc结构域的卵泡抑素多肽的活化素-结合部分的融合蛋白。在另一实施方案中,本公开内容的拮抗剂是包含融合至Fc结构

域的FLRG多肽的活化素结合部分的融合蛋白。

[0388] G.Lefty A和B

[0389] Lefty A和B蛋白已知调节通过ALK7:ActRIIB途径发信号的Nodal 和其它蛋白。因此,在其它方面,ALK7:ActRIIB拮抗剂是Lefty A或 Lefty B多肽,其可单独或与一种或多种另外的本文公开的支持性疗法和/或活性剂组合使用,以实现需要的效果(例如,治疗肾疾病和/或代谢病况或病症)。

[0390] 术语"Lefty A多肽"包括包含任何天然存在的Lefty A多肽以及其保留有用活性的任何变体(包括突变体、片段、融合物和拟肽形式)的多肽,还包括Lefty A的任何功能单体或多聚体。在某些优选的实施方案中,本公开内容的Lefty A多肽结合和/或抑制nodal活性。此外,制备和测试多肽文库的方法在上文的ActRII和ALK7多肽的情况下进行描述,和这样的方法也涉及制备和测试Lefty A的变体。Lefty A多肽包括源自任何已知Lefty A的序列的多肽,其具有与Lefty A多肽的序列具有至少约80%同一性和任选地至少85%、90%、95%、96%、97%、98%、 99%或更大同一性的序列。Lefty A多肽的实例包括成熟的Lefty A多肽或较短的同种型,或人Lefty A前体多肽(SEQ ID NO:96)的其它变体。

[0391] 人Lefty A前体多肽如下:

[0392]
```
  1 MWPLWLCWAL WVLPLAGPGA ALTEEQLLGS LLRQLQLSEV PVLDRADMEK LVIPAHVRAQ
 61 YVVLLRRSHG DRSRGKRFSQ SFREVAGRFL ASEASTHLLV FGMEQRLPPN SELVQAVLRL
121 FQEPVPKAAL HRHGRLSPRS AQARVTVEWL RVRDDGSNRT SLIDSRLVSV HESGWKAFDV
181 TEAVNFWQQL SRPRQPLLLQ VSVQREHLGP LASGAHKLVR FASQGAPAGL GEPQLELHTL
241 DLRDYGAQGD CDPEAPMTEG TRCCRQEMYI DLQGMKWAKN WVLEPPGFLA YECVGTCQQP
301 PEALAFNWPF LGPRQCIASE TASLPMIVSI KEGGRTRPQV VSLPNMRVQK CSCASDGALV
361 PRRLQP
```

[0393] (SEQ ID NO:96;GenBank Id:AAD48145.1)

[0394] 信号肽用下划线表示。

[0395] 术语"Lefty B多肽"包括包含任何天然存在的Lefty B多肽以及其保留有用活性的任何变体(包括突变体、片段、融合物和拟肽形式)的多肽,还包括Lefty B的任何功能单体或多聚体。在某些优选的实施方案中,本公开内容的Lefty B多肽抑制nodal活性。此外,制备和测试多肽文库的方法在上文的ActRII和ALK7多肽的情况下进行描述,和这样的方法也涉及制备和测试Lefty B的变体。Lefty B多肽包括源自任何已知Lefty B的序列的多肽,其具有与Lefty B多肽的序列具有至少约80%同一性和任选地至少85%、90%、95%、96%、97%、98%、99%或更大同一性的序列。Lefty B多肽的实例包括成熟的Lefty B多肽或较短的同种型,或人Lefty B前体多肽(SEQ ID NO:97)的其它变体。

[0396] 人Lefty B前体多肽如下:

[0397]
```
  1 MQPLWLCWAL WVLPLASPGA ALTGEQLLGS LLRQLQLKEV PTLDRADMEE LVIPTHVRAQ
 61 YVALLQRSHG DRSRGKRFSQ SFREVAGRFL ALEASTHLLV FGMEQRLPPN SELVQAVLRL
121 FQEPVPKAAL HRHGRLSPRS ARARVTVEWL RVRDDGSNRT SLIDSRLVSV HESGWKAFDV
181 TEAVNFWQQL SRPRQPLLLQ VSVQREHLGP LASGAHKLVR FASQGAPAGL GEPQLELHTL
241 DLGDYGAQGD CDPEAPMTEG TRCCRQEMYI DLQGMKWAEN WVLEPPGFLA YECVGTCRQP
301 PEALAFKWPF LGPRQCIASE TDSLPMIVSI KEGGRTRPQV VSLPNMRVQK CSCASDGALV
361 PRRLQP
```

[0398] (SEQ ID NO:97;GenBank Id:AAD48144.1)

[0399] 信号肽用下划线表示。

[0400] 在某些实施方案中，Lefty A多肽和Lefty B多肽的功能变体或修饰形式包括具有至少一部分的Lefty A多肽或Lefty多肽和一种或多种融合结构域例如，促进多肽的分离、检测、稳定或多聚化的结构域的融合蛋白。合适的融合结构域在上文参考ActRII和ALK7多肽详细论述。在一些实施方案中，本公开内容的拮抗剂是包含融合至Fc结构域的Lefty A和/或Lefty B多肽的nodal-结合部分的融合蛋白。

[0401] H.DAN-相关蛋白

[0402] DAN蛋白家族的成员已知调节通过ALK7:ActRIIB途径发信号的配体。因此，在其它方面，ALK7:ActRIIB拮抗剂是DAN-相关蛋白(例如，Cerberus和Coco)，其可单独或与一种或多种另外的本文公开的支持性疗法和/或活性剂组合使用以实现需要的效果(例如，治疗具有肾疾病和/或代谢病症的患者)。

[0403] 术语"Cerberus多肽"包括包含任何天然存在的Cerberus多肽以及其保留有用活性的任何变体(包括突变体、片段、融合物和拟肽形式) 的多肽，还包括Cerberus的任何功能单体或多聚体。在某些优选的实施方案中，本公开内容的Cerberus多肽结合和/或抑制nodal活性。此外，制备和测试多肽文库的方法在上文的ActRII和ALK7多肽的情况下进行描述，和这样的方法也涉及制备和测试Cerberus的变体。Cerberus 多肽包括源自任何已知Cerberus的序列的多肽，其具有与Cerberus多肽的序列具有至少约80％同一性和任选地至少85％、90％、95％、96％、 97％、98％、99％或更大同一性的序列。Cerberus多肽的实例包括成熟的Cerberus多肽或较短的同种型，或人Cerberus前体多肽(SEQ ID NO： 98)的其它变体。

[0404] 人Cerberus前体多肽如下：

[0405]
```
  1 MHLLLFQLLV LLPLGKTTRH QDGRQNQSSL SPVLLPRNQR ELPTGNHEEA EEKPDLFVAV
 61 PHLVATSPAG EGQRQREKML SRFGRFWKKP EREMHPSRDS DSEPFPPGTQ SLIQPIDGMK
121 MEKSPLREEA KKFWHHFMFR KTPASQGVIL PIKSHEVHWE TCRTVPFSQT ITHEGCEKVV
181 VQNNLCFGKC GSVHFPGAAQ HSHTSCSHCL PAKFTTMHLP LNCTELSSVI KVVMLVEECQ
241 CKVKTEHEDG HILHAGSQDS FIPGVSA
```

[0406] (SEQ ID NO:98;NCBI Reference:NP_005445.1)

[0407] 信号肽由下划线表示。

[0408] 术语"Coco多肽"包括包含任何天然存在的Coco多肽以及其保留有用活性的任何变体(包括突变体、片段、融合物和拟肽形式)的多肽，还包括Coco的任何功能单体或多聚体。在某些优选的实施方案中，本公开内容的Coco多肽结合和/或抑制nodal活性。此外，制备和测试多肽文库的方法在上文的ActRII和ALK7多肽的情况下进行描述，和这样的方法也涉及制备和测试Coco的变体。Coco多肽包括源自任何已知 Coco的序列的多肽，其具有与Coco多肽的序列具有至少约80％同一性和任选地至少85％、90％、95％、96％、97％、98％、99％或更大同一性的序列。Coco多肽的实例包括成熟的Coco多肽或较短的同种型，或人Coco前体多肽(SEQ ID NO:99)的其它变体。

[0409] 人Coco前体多肽如下：

[0410]
```
  1 MLLGQLSTLL CLLSGALPTG SGRPEPQSPR PQSWAAANQT WALGPGALPP LVPASALGSW
 61 KAFLGLQKAR QLGMGRLQRG QDEVAAVTLP LNPQEVIQGM CKAVPFVQVF SRPGCSAIRL
121 RNHLCFGHCS SLYIPGSDPT PLVLCNSCMP ARKRWAPVVL WCLTGSSASR RRVKISTMLI
181 EGCHCSPKA
```

[0411] (SEQ ID NO:99;GenBank Id:)

[0412] 信号肽由下划线表示。

[0413] 在某些实施方案中,Cerberus多肽和Coco多肽的功能变体或修饰形式包括具有至少一部分的Cerberus多肽和/或Coco多肽和一种或多种融合结构域例如,促进多肽的分离、检测、稳定或多聚化的结构域的融合蛋白。合适的融合结构域在上文参考ActRII和ALK7多肽详细论述。在一些实施方案中,本公开内容的拮抗剂是包含融合至Fc 结构域的Cerberus和/或Coco多肽的nodal-结合部分的融合蛋白。

[0414] 3.筛选测定法

[0415] 在某些实施方案中,ALK7:ActRIIB受体异多聚体(例如, ALK7:ActRIIB异二聚体)可用于产生和/或筛选ALK7:ActRIIB抑制剂、特别是干扰ALK7-配体和/或ALK7-II型受体(例如,ActRIIB)相互作用的抑制剂(例如,抗-ALK7抗体)。

[0416] 如本文所述的,TGF-β超家族配体信号由I型和II型激酶受体的复合物介导,其在配体刺激时磷酸化和活化下游Smad蛋白[参见例如, Massague(2000)Nat.Rev.Mol.Cell.Biol.1:169-178]。尽管对于信号传导是必需的,I型受体通常对TGF-β超家族配体具有非常弱的亲和力。相反,II型受体通常以高亲和力结合配体。该配体结合促进在I 型和II受体之间稳定的复合物形成,导致通过II型受体的I型受体的磷酸化,因此活化Smad蛋白。鉴于较弱的亲和力,通常难以观察到孤立I型受体的配体结合活性。

[0417] 事实上,本文的实例证实了ActRIIB同二聚体结合许多不同的配体,许多具有高亲和力。相比之下,ALK7同二聚体不显示对任何检查的配体的亲和力。因为配体不能可检测地结合ALK7,难以筛选/鉴定可抑制ALK7活性的试剂。然而,如本文的实例所证实的,ALK7 的配体-结合活性当与II型受体配对时变得可测量。因此, ALK7:ActRIIB异多聚体(例如,异二聚体)是可使用各种筛选测定法例如本文所述的以及本领域已知的那些鉴定ALK7抑制剂的有用工具。因此,在一些实施方案中,本公开内容涉及ALK7:II型受体异多聚体 (例如,ALK7:ActRIIB异多聚体)鉴定(筛选)ALK7抑制剂的用途。

[0418] 例如,ALK7:II型受体异多聚体(例如,ALK7:ActRIIB异多聚体) 可特别用于产生/鉴定结合ALK7的抗体,特别是结合和抑制ALK7 的抗体。尽管本文所述的ALK7多肽,包括ALK7同二聚体,可用于产生/鉴定结合ALK7的抗体,简单结合不足以证实给定的ALK7抗体可抑制ALK7活性(例如,干扰ALK7-配体或ALK7-II型受体相互作用)。如本文所示的,在缺少II型受体的情况下ALK7多肽不显示配体-结合活性,使得难以鉴定可抑制ALK7的抗体。ALK7:II型受体异多聚体(例如,ALK7:ActRIIB异多聚体)通过提供ALK7活性的功能读出解决了该问题。此外,不希望限制于任何特定的作用机制,与II型受体(例如,ActRIIB)缔合可改进产生抑制ALK7活性的抗体的能力。例如,与II型受体缔合可有助于稳定ALK7,其方式为优化介导配体 -结合活性的重要ALK7表位的加工和呈递,因此改进ALK7抑制抗体的开发。因此,在一些实施方案中,本公开内容涉及ALK7:II型异多聚体(例如,ALK7:ActRIIB异多聚体)产生结合和抑制ALK7的抗体的用途。用于产生和筛选抗体的方法是本领域众所周知的和在本文中描述,和ALK7-II型异多聚体(例如,ALK7:ActRIIB异多聚体)可根据一种或多种这些方法使用,以产生/鉴定结合和抑制ALK7活性的抗体。

[0419] 在某些实施方案中,ALK7:II型受体异二聚体(例如, ALK7:ActRIIB异多聚体例如异二聚体)可用于鉴定活化素C抑制剂、特别是结合活化素C和干扰活化素C-I型/II受体相互作用的抑制剂。

[0420] 令人惊讶地，本文提供的数据证实了ALK7:ActRIIB异二聚体结合活化素C。对这种新的相互作用的洞察提供了开发/鉴定活化素C抑制剂的机会。此外，鉴于在活化素C和活化素E之间的结构相似性，预期活化素E也结合ALK7:ActRIIB，因此这种新的相互作用也可用于鉴定活化素E拮抗剂。在本部分的公开内容中在提及活化素C或E 时，还分别包括异二聚体配体例如活化素AC、AE、BC和BE。还考虑了影响活化素C和E二者的试剂。各种类型的活化素C或E拮抗剂预期可用于本文所述的方法，包括例如，小分子、抗体和抑制剂核酸，特别是破坏活化素C(或E)-ALK7:ActRIIB相互作用的那些。与活化素C或E特异性反应和结合活化素C或E以竞争结合ALK7和/ 或ActRIIB或以其它方式抑制活化素C或E-介导的信号传导的试剂 (例如，抗体或小分子)可用作活化素C或E的拮抗剂。同样地，与ALK7 和/或ActRIIB特异性反应和破坏活化素C或EO结合的试剂可用作拮抗剂。关于抗体，免疫原可源自活化素C或E多肽、ALK7多肽或 ActRIIB多肽和用于使用本领域已知的标准方法产生抗体。参见例如， Antibodies:A Laboratory Maneal，Harlow和Lane编辑，Cold Spring Harbor Press，1988。使用本领域已知的各种筛选测定法例如本文所述的那些，ALK7:II型受体异二聚体(例如，ALK7:ActRIIB异多聚体例如异二聚体)可用于鉴定活化素C或E抑制剂。

[0421] 在某些方面，本公开内容涉及ALK7:ActRIIB异多聚体复合物鉴定作为TGFβ超家族受体的激动剂或拮抗剂的化合物(试剂)的用途。通过该筛选鉴定的化合物可经测试以评价其体内或体外调节组织生长，例如骨、软骨、肌肉、脂肪和/或神经元生长的能力。这些化合物可例如，在动物模型中测试。

[0422] 存在许多筛选用于通过靶向TGFβ超家族配体信号传导(例如， SMAD信号传导)调节组织生长的治疗剂的方法。在某些实施方案中，化合物的高通量筛选可在选择的细胞系上进行以鉴定干扰TGFβ超家族受体-介导的作用的试剂。在某些实施方案中，该测定进行以筛选和鉴定特异性抑制或减少ALK7:ActRIIB异多聚体与结合配偶体(例如，活化素、GDF11、GDF8、BMP6、GDF3、BMP5、nodal和BMP10) 的结合的化合物。或者，该测定可用于鉴定增强ALK7:ActRIIB异多聚体与配体的结合的化合物。在进一步的实施方案中，化合物可通过其与ALK7:ActRIIB异多聚体相互作用的能力来鉴定。

[0423] 在一些实施方案中，本公开内容涉及ALK7:ActRIIB异多聚体和活化素C或E多肽鉴定作为活化素C(或E)-ALK7:ActRIIB信号传导途径的激动剂或拮抗剂的化合物的用途。通过该筛选测定法鉴定的化合物可经测试以评价其体外调节活化素C或E信号传导活性的能力。任选地，这些化合物可进一步在动物模型中测试以评价其体内调节组织生长的能力。存在许多筛选用于通过靶向活化素C或E和 ALK7:ActRIIB异多聚体调节组织生长的治疗剂的方法。在某些实施方案中，化合物的高通量筛选可进行以鉴定干扰活化素C或E或 ALK7:ActRIIB-介导细胞信号传导的试剂。在某些实施方案中，该测定进行以筛选和鉴定特异性抑制或减少ALK7:ActRIIB异多聚体与活化素C或E的结合的化合物。或者，该测定法可用于鉴定增强 ALK7:ActRIIB异多聚体与活化素C或E的结合的化合物。在进一步的实施方案中，化合物可通过其与活化素C或E或ALK7:ActRIIB异多聚体相互作用的能力来鉴定。

[0424] 各种测定法形式符合要求，并且根据本公开内容，本文未明确描述的那些将仍然被本领域普通技术人员所理解。如本文所述的，本发明的试验化合物(试剂)可通过任何组合化学方法产生。或者，本发明的化合物可以是体内或体外合成的天然存在的生物分子。待测试作为组织生长的调节剂的能力的化合物(试剂)可例如，通过细菌、酵母、植物或其它生

物体产生(例如,天然产物),通过化学产生(例如,小分子,包括拟肽)或通过重组产生。本发明考虑的试验化合物包括无肽基有机分子、肽、多肽、拟肽、糖、激素和核酸分子。在某些实施方案中,试验试剂是具有小于约2,000道尔顿的分子量的小的有机分子。

[0425] 本公开内容的试验化合物可作为单一的离散实体提供,或在较大复杂性的文库(例如通过组合化学制备)中提供。这些文库可包含,例如,醇、烷基卤化物、胺、酰胺、酯、醛、醚和其它类型的有机化合物。试验化合物至试验系统的呈递可以为分离的形式或作为化合物的混合物,尤其是在初始筛选步骤中。任选地,化合物可任选地用其它化合物衍生,和具有促进化合物的分离的衍生基团。衍生基团的非限制性实例包括生物素、荧光素、洋地黄毒苷、绿色荧光蛋白、同位素、多组氨酸、磁珠、谷胱甘肽S-转移酶(GST)、光可激活交联剂或其任何组合。

[0426] 在测试化合物的文库和天然提取物的许多药物-筛选程序中,需要高通量测定法以最大化在给定的时间内调查的化合物的数量。在无细胞系统中进行的测定法(例如可用纯化或半纯化的蛋白得到),通常优选作为“初级”筛选,因为它们可经产生以允许由试验化合物介导的分子靶标改变的快速发展和相对容易检测。此外,试验化合物的细胞毒性或生物利用率的影响在体外系统中一般可忽略,该测定法而是主要关注药物对分子靶标的影响,如可以在ALK7:ActRIIB异多聚体复合物和结合配偶体(例如,活化素C或E)之间的结合亲和力的改变所证明的。

[0427] 仅为说明,在本公开内容的实例性筛选测定法中,按对于测定法的目的合适的,将目的化合物与分离和纯化的通常能够结合活化素C 的ALK7:ActRIIB异多聚体接触。然后向化合物和ALK7:ActRIIB异多聚体复合物的混合物中加入包含活化素C或E的组合物。

[0428] ALK7:ActRIIB异多聚体-活化素C或E复合物的检测和定量提供了检测化合物抑制(或加强)ALK7:ActRIIB异多聚体和活化素C或E之间的复合物形成的功效的手段。化合物的功效可从使用各种浓度的试验化合物获得的数据通过产生剂量-反应曲线评价。此外,对照测定也可进行以提供基线用于比较。例如,在对照测定中,分离和纯化的活化素C或E被加入至包含ALK7:ActRIIB异多聚体的组合物和在缺少试验化合物的情况下定量ALK7:ActRIIB异多聚体-活化素C或E的形成。将理解,一般而言,其中反应物可混合的顺序可改变,和可同时混合。此外,代替纯化蛋白,细胞提取物和裂解物可用于提供合适的无细胞测定系统。

[0429] ALK7:ActRIIB异多聚体复合物与另一蛋白(例如,活化素C或E) 的结合可通过各种技术检测。例如,复合物形成的调节可使用例如,可检测标记的蛋白例如放射性标记的(例如,^{32}P、^{35}S、^{14}C或^{3}H)、荧光标记的(例如,FITC)或酶标记的ALK7:ActRIIB异聚复合物和/或结合配偶体(例如,活化素C或E),通过免疫测定法或通过色谱检测来量化。

[0430] 在某些实施方案中,本公开内容考虑了荧光极化测定法和荧光共振能量转移(FRET)测定法在直接或间接测量ALK7:ActRIIB异多聚体和结合蛋白(例如,活化素C或E)之间相互作用的程度中的用途。此外,其它检测模式,例如基于光波导的那些(PCT公布WO 96/26432 和美国专利号5,677,196)、表面等离子体共振(SPR)、表面电荷传感器和表面力传感器,与本公开内容的许多实施方案相容。

[0431] 此外,本公开内容考虑了相互作用陷阱测定法,亦称为“双杂交测定法”用于鉴定破坏或增强ALK7:ActRIIB异多聚体和结合配偶体(例如,活化素C或E)之间相互作用的试剂的用途。参见例如,美国专利号5,283,317;Zervos等(1993)Cell 72:223-232;Madura等

(1993)J Biol Chem 268:12046-12054;Bartel等(1993)Biotechniques 14:920-924;和Iwabuchi等(1993)Oncogene 8:1693-1696)。在特定的实施方案中,本公开内容考虑了反转双杂交系统鉴定离解ALK7:ActRIIB异多聚体和结合蛋白(例如,活化素C或E)之间相互作用的化合物(例如,小分子或肽)的用途[Vidal和Legrain,(1999)Nucleic Acids Res 27:919-29; Vidal和Legrain,(1999)Trends Biotechnol 17:374-81;和美国专利号 5,525,490;5,955,280;和5,965,368]。

[0432] 在某些实施方案中,本发明的化合物通过其与本公开内容的 ALK7:ActRIIB异多聚体或活化素C或E相互作用的能力来鉴定。在化合物和ALK7:ActRIIB异多聚体或活化素C或E之间的相互作用可以是共价或非共价的。例如,这样的相互作用可在蛋白水平上使用体外生物化学方法,包括光交联、放射性标记的配体结合和亲和色谱鉴定[Jakoby WB等(1974)Methods in Enzymology 46:1]。在某些情况下,化合物可在基于机制的测定法中筛选,例如检测结合ALK7:ActRIIB 异多聚体或活化素C或E的化合物的测定法。这可包括固相或液相结合事件。或者,编码ALK7:ActRIIB异多聚体或活化素C或E的基因可用报告系统(例如,β-半乳糖苷酶、萤光素酶或绿色荧光蛋白)转染至细胞和针对文库优选地通过高通量筛选或以文库的各个成员进行筛选。可使用其它基于机制的结合测定法;例如,检测自由能变化的结合测定法。结合测定法可用固定至孔、珠或芯片的,或通过固定的抗体捕获的,或通过毛细管电泳解析的靶标进行。结合的化合物可通常使用比色终点或荧光或表面等离子体共振检测。

[0433] 4.实例性治疗用途

[0434] 在某些实施方案中,本公开内容的ALK7:ActRIIB拮抗剂或这样的拮抗剂的组合可用于治疗或预防与ALK7:ActRIIB-结合配体的异常活性有关的疾病或病况。这些疾病、病症或病况在本文通常称为“ALK7:ActRIIB-相关病况”或“ALK7:ActRIIB-相关病症”。在某些实施方案中,本公开内容提供了通过给予有需要的个体治疗有效量的本文所述的ALK7:ActRIIB拮抗剂(例如,ALK7:ActRIIB异多聚体例如ALK7:ActRIIB异二聚体)或这样的拮抗剂的组合,治疗或预防个体的 ALK7:ActRIIB-相关病况的方法。术语"受试者"、"个体"或"患者"在说明书全文中可互换。本公开内容的任何ALK7:ActRIIB拮抗剂可潜在地单独或组合使用,用于本文公开的治疗用途。这些方法的目的特别在于治疗性和预防性治疗哺乳动物,包括例如,啮齿动物、灵长类动物和人。

[0435] 如本文所用的,“预防”病症或病况的治疗剂是指在统计学样品中,相对于未治疗的对照样品在治疗的样品中减少病症或病况的发生,或相对于未治疗的对照样品延迟病症或病况的一种或多种症状的发生或减少其严重性的化合物。如本文所用的术语“治疗”包括一旦已经确立,则改善或消除病况。在任一情况下,预防或治疗可在由医生或其它健康护理提供者提供的诊断和给予治疗剂的预期结果中辨别。

[0436] 一般而言,本公开内容中描述的疾病或病况的治疗或预防通过给予"有效量"的本公开内容的ALK7:ActRIIB拮抗剂或这样的拮抗剂的组合来实现。试剂的有效量是指在需要的剂量和时间下有效实现需要的治疗或预防结果的量。本公开内容的试剂的"治疗有效量"可根据因素例如个体的疾病状态、年龄、性别和体重,和试剂在个体中引发需要的反应的能力而改变。"预防有效量"是指在需要的剂量和时间下有效实现需要的预防结果的量。

[0437] 肾维持血液的许多特征,包括体积、pH平衡、电解质浓度和血压,以及负责毒素和

废物过滤。这些功能依赖于肾的肾单位的复杂结构、血液持续流过肾的各种毛细管和通过来自身体其余部分的信号调节肾，包括内分泌激素。肾功能问题表现为直接机制(例如，遗传缺陷、感染或毒素暴露)和自长期应激物例如肥大和超滤进行性发生的间接机制(本身通常是对肾功能的更直接损伤的结果)。由于肾在血液维持和废物分泌中的重要作用，肾-相关的疾病表现是许多和多变的；它们可综述于Harrison的Principles of Internal Medicine，第18版，McGraw Hill，N.Y.，Part 13，Chp 277-289。

[0438] 如本文所述的，ALK7:ActRIIB拮抗剂在肾疾病模型中具有各种有益作用。特别地，用ALK7:ActRIIB异多聚体治疗减少具有单侧输尿管梗阻的受试者的肾组织损伤、炎症和纤维化。这些数据表明 ALK7:ActRIIB拮抗剂可用于治疗或预防肾疾病、特别是治疗或预防肾疾病的各种并发症(表现)，包括例如，肾组织损伤、炎症和/或纤维化。

[0439] 因此，本发明的方法可应用于各种肾-相关疾病或病况。如本文所用的，"肾-相关的疾病或病况"可以指影响肾或肾脏系统的任何疾病、病症或病况。肾-相关疾病或病况的实例包括但不限于慢性肾疾病(或衰竭)、急性肾疾病(或衰竭)、原发性肾疾病、非糖尿病性肾疾病、肾小球肾炎、间质性肾炎、糖尿病性肾疾病、糖尿病性肾病、肾小球硬化症、快速进行性肾小球肾炎、肾纤维化、奥尔波特综合征、IDDM 肾炎、肾小球系膜增生性肾小球肾炎、膜增生性肾小球肾炎、新月体肾小球肾炎、肾间质性纤维化、局灶性节段性肾小球硬化症、膜性肾病、微小病变疾病、微量免疫快速进行性肾小球肾炎、IgA肾病、多囊性肾疾病、Dent病、肾胱氨酸病(nephrocytinosis)、海曼肾炎、常染色体显性(成人)多囊性肾疾病、常染色体隐性(儿童)多囊性肾疾病、急性肾损伤、肾病综合征、肾缺血、足细胞疾病或病症，蛋白尿、肾小球疾病、膜性肾小球肾炎、局灶性节段性肾小球肾炎、先兆子痫、子痫、肾损伤、胶原血管病、良性体位性(姿势)蛋白尿、IgM肾病、膜性肾病、结节病、糖尿病、由于药物导致的肾损伤、法布里病、氨基酸尿症、范科尼综合征、高血压性肾硬化、间质性肾炎、镰状细胞疾病、血红蛋白尿、肌红蛋白尿、韦格纳肉芽肿病、糖原贮积病1型、慢性肾疾病、慢性肾衰竭、低肾小球滤过率(GFR)、肾血管硬化、狼疮肾炎、ANCA-阳性微量免疫新月体肾小球肾炎、慢性同种异体移植物肾病、肾毒性、肾中毒、肾坏死、肾损伤、肾小球和管损伤、肾功能障碍、肾炎综合征、急性肾衰竭、慢性肾衰竭、近端小管功能障碍、急性肾移植排斥、慢性肾移植排斥、非-IgA肾小球系膜增生性肾小球肾炎、传染后肾小球肾炎、伴有任何类型的肾累及的血管炎、任何遗传性肾疾病、任何间质性肾炎、肾移植衰竭、肾癌、与其它病况有关的肾疾病(例如，高血压、糖尿病和自身免疫疾病)、Dent病、肾胱氨酸病(nephrocytinosis)、海曼肾炎、原发性肾疾病、坍缩性肾小球病、致密沉积物病、冷球蛋白血症相关的肾小球肾炎、Henoch-Schonlein病、传染后肾小球肾炎、细菌心内膜炎、微观多脉管炎、丘-施二氏综合征、抗-GBM-抗体介导的肾小球肾炎、淀粉样变性病、单克隆免疫球蛋白沉积病、原纤维肾小球肾炎、免疫触须样肾小球病、缺血性肾小管损伤、药物-诱导的肾小管-间质性肾炎、毒性肾小管-间质性肾炎、感染性肾小管-间质性肾炎、细菌肾盂肾炎、自多瘤病毒感染或HIV感染产生的病毒感染性肾小管-间质性肾炎、代谢-诱导的肾小管-间质性疾病、混合结缔组织病、管型肾病、可自尿酸或草酸或药物-诱导的晶体沉积产生的晶体肾病、急性细胞肾小管-间质性同种异体移植物排斥、自淋巴瘤或移植后淋巴增生性疾病产生的肿瘤侵润疾病、肾的阻塞性疾病、血管疾病、血栓形成性微血管病、肾血管硬化、动脉粥样化栓塞疾病、混合结缔组织病、结节性多动脉炎、神经钙蛋白-抑制剂诱导的血管疾病、急性细胞血管同种异体移

植物排斥、急性体液同种异体移植物排斥、早期肾功能减退(ERFD)、晚期肾疾病(ESRD)、肾静脉血栓症、急性肾小管坏死、急性间质性肾炎、确立的慢性肾疾病、肾动脉狭窄、缺血性肾病、尿毒症、药物和毒素-诱导的慢性肾小管间质性肾炎、逆流性肾病、肾结石、古德帕斯丘综合征、正细胞性正色素性贫血、肾贫血、糖尿病性慢性肾疾病、IgG4-相关疾病、冯希-林二氏综合征、结节性硬化症、肾消耗病、髓状囊性肾疾病、肾细胞癌、腺癌、肾胚细胞瘤、淋巴瘤、白血病、低唾液酸化病症、慢性环孢霉素肾病、肾再灌注损伤、肾发育不良、氮质血症、双侧动脉闭塞、急性尿酸肾病、血容量不足、急性双侧梗阻性尿路病、血钙过高肾病、溶血性尿毒症综合征、急性尿潴留、恶性肾硬化、产后肾小球硬化症、硬皮病、非-古德帕斯丘抗-GBM病、微观结节性多动脉炎、过敏性肉芽肿病、急性放射性肾炎、链球菌感染后肾小球肾炎、瓦尔登斯特伦氏巨球蛋白血症、镇痛剂肾病、动静脉瘘、动静脉移植、透析、异位肾、髓质海绵肾、肾性骨营养不良、孤立肾、肾积水、微白蛋白尿、尿毒症、血尿、高脂血症、低白蛋白血症、脂肪尿、酸中毒、血钾过多和水肿。

[0440] 在一些实施方案中,本公开内容的ALK7:ActRIIB拮抗剂或这样的拮抗剂的组合(例如,ALK7:ActRIIB异多聚体例如ALK7:ActRIIB 异二聚体)可用于治疗或预防慢性肾疾病,任选地与一种或多种治疗慢性肾疾病的支持性疗法组合。在一些实施方案中,本公开内容的 ALK7:ActRIIB拮抗剂或这样的拮抗剂的组合(例如,ALK7:ActRIIB异多聚体例如ALK7:ActRIIB异二聚体)可用于治疗或预防慢性肾疾病的一种或多种并发症(症状或表现)(例如,组织损伤、炎症和/或纤维化),任选地与一种或多种治疗慢性肾疾病的支持性疗法组合。在一些实施方案中,本公开内容的ALK7:ActRIIB拮抗剂或这样的拮抗剂的组合 (例如,ALK7:ActRIIB异多聚体例如ALK7:ActRIIB异二聚体)可用于治疗或预防晚期肾衰竭,任选地与一种或多种治疗晚期肾疾病的支持性疗法组合。慢性肾脏疾病(CKD),亦称为慢性肾疾病,是一种在数月或数年的时间内肾功能的进行性损失。恶化肾功能的症状可包括全身感觉不良和经历食欲减少。通常,慢性肾疾病作为筛选已知处于肾问题的风险的人群,例如具有高血压或糖尿病的那些和具有与CKD 相关的血液的那些的结果而被诊断。该疾病当其导致其公认的并发症之一(例如心血管疾病、贫血或心包炎)时也可被鉴定。最近的专业指南将CKD的严重性分类为5个阶段,其中阶段1是最轻的,通常引起很少症状;和阶段5是严重疾病,如果不治疗则具有差的预期寿命。阶段5的CKD通常被称为晚期肾疾病、晚期肾疾病或晚期肾衰竭,很大程度上与现在已过时的术语慢性肾衰竭或慢性肾衰竭同义;和通常意指患者需要肾置换疗法,这可包括一种形式的透析,但理想地是制定肾移植。CKD最初没有明确的症状,和通常仅检测为血清肌酸酐或尿蛋白增加。随着肾功能降低,各种症状可表现为下文所述。由于流体过载和通过肾素-增压素系统由肾产生的血管作用的激素的产生,血压可能增加,这增加了发生高血压和/或患有充血性心力衰竭的风险。尿素可积累,导致氮质血症和最终导致尿毒症(症状范围为昏睡至心包炎和脑病)。由于其高的系统循环,尿素在外分泌腺汗液中以高浓度排泄,和在皮肤上作为汗液蒸发物结晶(″尿毒症霜″)。钾可在血液中积累(血钾过多,症状范围包括不适和潜在地,致死性心律失常)。血钾过多通常不发生,直到肾小球滤过率下降至小于20-25ml/min/1.73m2,此时肾排泄钾的能力减少。CKD中血钾过多可被酸血症(其导致钾的细胞外迁移)和因为缺少胰岛素而恶化。红细胞生成素合成可减少,引起贫血。流体体积过载症状可发生,范围为轻度水肿至威胁生命的肺水肿。高磷酸盐血症,由于磷酸盐排泄减少导致,可一般在肾小球过滤减少后发生。高磷酸盐血症与

心血管风险增加有关，是血管钙化的直接刺激物。低钙血症可出现，其通常由成纤维细胞生长因子-23的刺激引起。骨细胞负责FGF23的产生增加，其是酶1-α-羟化酶(负责转化25-羟基胆钙化醇为1,25二羟基维生素D3)的有效的抑制剂。随后，这进展至继发性甲状旁腺功能亢进、肾性骨营养不良和血管钙化，其进一步损害心脏功能。代谢性酸中毒(由于硫酸盐、磷酸盐、尿酸等的累积)可发生，和通过对酶的过量酸作用导致酶活性改变；以及由于过量的酸(酸血症)导致通过促进血钾过多增加心脏和神经元膜的兴奋性。酸中毒还由于从近端小管的细胞产生足够的氨的能力减少导致。铁缺乏性贫血，其随肾功能降低而流行率增加，在需要血液透析的那些人中特别流行。其在原因上是多因素的，但包括炎症增加、红细胞生成素减少和导致骨髓抑制的高尿酸血症。具有CKD的人群患有加速的动脉粥样硬化和比普通人群更可能发生心血管疾病。患有CKD 和心血管疾病的患者趋于具有比仅患有后者的患者显著更差的预后。

[0441] 在另一实施方案中，ALK7:ActRIIB拮抗剂(例如，ALK7:ActrIIB 异二聚体)或这样的拮抗剂的组合可用于慢性肾病矿物骨病症 (CKD-MBD)的患者，其为一种自肾病产生的相关骨骼、心血管和矿物 -代谢病症的广泛综合征。CKD-MBD包括各种骨骼病理，通常称为肾性骨营养不良(ROD)，其是用ALK7:ActRIIB拮抗剂(例如， ALK7:ActRIIB异二聚体)或这样的拮抗剂的组合治疗的优选实施方案。根据不同的病原性因子的相对贡献，ROD表现为多样的骨重塑病理模式(Hruska等，2008，Chronic kidney disease mineral bone disorder (CKD-MBD)；Rosen等(编辑)Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism,7th ed.American Society for Bone and Mineral Research, Washington D.C.,pp 343-349)。范围的一端是具有尿毒症骨营养不良和低骨周转的ROD，特征为低数量的活性重塑部位，明显抑制的骨形成和低骨再吸收。另外一端是具有甲状旁腺功能亢进、高骨周转和纤维性骨炎的ROD。鉴于ALK7:ActRIIB拮抗剂 (例如，ALK7:ActRIIB异二聚体)或这样的拮抗剂的组合可发挥合成代谢和抗再吸收作用，这些试剂可用于在ROD病理范围内的患者。

[0442] 在其它实施方案中，ALK7:ActRIIB拮抗剂(例如，ALK7:ActRIIB 异二聚体)或这样的拮抗剂的组合可用于在动物中调节体脂肪含量和用于治疗或预防与其相关的病况，和特别是，与其相关的危及健康的病况。根据本发明，调节(控制)体重可以指减少或增加体重，减少或增加体重增加速率，或增加或减少体重减轻速率，和还包括有效维持或不显著改变体重(例如，对抗可以其它方式增加或减少体重的外部或内部影响)。本公开内容的一个实施方案涉及通过给予有需要的动物 (例如，人)ALK7:ActRIIB拮抗剂(例如，ALK7:ActRIIB异二聚体)或这样的拮抗剂的组合来调节体重。例如，在一些实施方案中，

[0443] ALK7:ActRIIB拮抗剂(例如，ALK7:ActRIIB异二聚体)或这样的拮抗剂的组合可用于治疗或预防选自以下的病症或病况：肥胖(例如，腹部肥胖)；超重；胰岛素抵抗；代谢综合征和其它代谢疾病或病况；脂质病症，例如，低HDL水平、高LDL水平、高脂血症、高甘油三酯血症或血脂障碍；脂蛋白异常；甘油三酯减少；炎症(例如，肝脏炎症和 /或脂肪组织的炎症)、脂肪性肝病；非-酒精性脂肪性肝病；高血糖症；葡萄糖耐量受损(IGT)；高胰岛素血症；高胆固醇(例如，高LDL水平和高胆固醇血症)；心血管疾病，例如，心脏病包括冠心病、充血性心力衰竭、中风、外周血管疾病、动脉粥样硬化；动脉硬化和高血压； X综合征；血管再狭窄；神经病；视网膜病；神经变性疾病；内皮功能障碍、呼吸功能障碍；胰腺炎；多囊性卵巢综

合征;尿酸水平升高;血色病(铁过载);黑棘皮病(皮肤上的深色斑);或癌症(例如,卵巢、乳腺、子宫内膜和结肠癌);或与一种或多种以上疾病或病况有关的另外的病症/病况。在一些实施方案中,使用ALK7:ActRIIB拮抗剂(例如, ALK7:ActRIIB异二聚体)或这样的拮抗剂的组合治疗的疾病或病况与超重(例如,BMI≥25kg/m^2)或太多的体脂肪有关。

[0444] 在一个实施方案中,本公开内容提供了减少体重的方法,包括给予需要减少体重或有需要的受试者有效量的ALK7:ActRIIB拮抗剂(例如,ALK7:ActRIIB异二聚体)或这样的拮抗剂的组合。在一些实施方案中,受试者是超重的(例如,前-肥胖)。在一些实施方案中,受试者具有25kg/m^2或更大的体重指数(BMI)。在另外的实施方案中,受试者具有25kg/m^2-29.9kg/m^2、30kg/m^2-39.9kg/m^2、25kg/m^2-39.9kg/m^2或25kg/m^2-50kg/m^2的BMI。在一些实施方案中,受试者是肥胖的。在一些实施方案中,受试者具有30kg/m^2或更大(例如,30-39.9kg/m^2或30kg/m^2-50kg/m^2)的BMI。在一些实施方案中,受试者是病态肥胖的。在一些实施方案中,受试者具有40kg/m^2或更大的BMI。在另外的实施方案中,受试者具有40kg/m^2-45kg/m^2或40kg/m^2-50kg/m^2的 BMI。在一些实施方案中,受试者具有向心性肥胖(例如,腹部区域脂肪过多,包括腹部脂肪和/或内脏脂肪)。在一些实施方案中,受试者具有0.85或更大的腰/髋周围比(WHR)。在一些实施方案中,受试者具有外周肥胖(例如,髋上脂肪过多)。在一些实施方案中,受试者具有2型糖尿病。ALK7:ActRIIB拮抗剂或拮抗剂的组合可单独或作为与其它类型的支持性疗法的组合疗法给予。例如,在一些实施方案中,支持性疗法是饮食和/或锻炼。

[0445] 在一个实施方案中,本公开内容提供了减少体重增加的方法,包括给予需要减少体重增加或有需要的受试者有效量的ALK7:ActRIIB 拮抗剂(例如,ALK7:ActRIIB异二聚体)或这样的拮抗剂的组合。在一些实施方案中,受试者是超重的(例如,前-肥胖)。在一些实施方案中,受试者具有25kg/m^2或更大的BMI。在另外的实施方案中,受试者具有25kg/m^2-29.9kg/m^2、30kg/m^2-39.9kg/m^2、25kg/m^2-39.9kg/m^2或 25kg/m^2-50kg/m^2的BMI。在一些实施方案中,受试者是肥胖的。在一些实施方案中,受试者具有30kg/m^2或更大(例如,30-39.9kg/m^2或30kg/m^2-50kg/m^2)的BMI。在一些实施方案中,受试者是病态肥胖的。在一些实施方案中,受试者具有40kg/m^2或更大的BMI。在另外的实施方案中,受试者具有40kg/m^2-45kg/m^2或40kg/m^2-50kg/m^2的 BMI。在一些实施方案中,受试者具有2型糖尿病。

[0446] 还提供了治疗或预防与过量体重有关的疾病或病况的方法,包括给予需要治疗或预防的受试者有效量的ALK7:ActRIIB拮抗剂(例如, ALK7:ActRIIB异二聚体)或这样的拮抗剂的组合。在一个实施方案中,治疗或预防的疾病或病况是肥胖。在一个实施方案中,治疗或预防的疾病或病况是胰岛素抵抗。在一个实施方案中,治疗或预防的疾病或病况是选自以下的成员:血脂障碍、高脂血症(总胆固醇水平>240 mg/dL)、高胆固醇血症(例如,总胆固醇水平>200mg/dL、>220mg/dL、>240mg/dL、>250mg/dL或>275mg/dL)、低HDL血清水平(例如,<40mg/dL、<45mg/dL或<50mg/dL)、高LDL血清水平(例如,≥100 mg/dL、≥130mg/dL、≥160mg/dL或≥190mg/dL)和高甘油三酯血症 (例如,空腹TG水平≥150mg/dL、≥175mg/dL、≥200mg/dL、≥300 mg/dL、≥400mg/dL或≥499mg/dL)。在某些情况下,ALK7:ActRIIB 拮抗剂治疗是对饮食和/或锻炼的辅助。

[0447] 在另一实施方案中本公开内容提供了减少超重受试者的体重的方法。该方法包括给予超重受试者有效量的ALK7:ActRIIB拮抗剂(例如,ALK7:ActRIIB异二聚体)或这样的拮

抗剂的组合。在一些实施方案中，受试者具有25kg/m^2或更大的体重指数(BMI)。在另外的实施方案中，受试者具有25kg/m^2-29.9kg/m^2、30kg/m^2-39.9kg/m^2、25 kg/m^2-39.9kg/m^2或25kg/m2-50kg/m^2或27-40kg/m^2的BMI。在一些实施方案中，受试者是肥胖的。在一些实施方案中，受试者具有30 kg/m^2或更大(例如，30-39.9kg/m^2或30kg/m^2-50kg/m^2)的BMI。ALK7 结合蛋白单独或作为组合疗法给予。在一些实施方案中，ALK7:ActRIIB拮抗剂治疗是对饮食和/或锻炼的辅助。

[0448] 在一个实施方案中本公开内容提供了减少肥胖受试者的体重的方法。该方法包括给予受试者有效量的ALK7:ActRIIB拮抗剂(例如，ALK7:ActRIIB异二聚体)或这样的拮抗剂的组合。在一些实施方案中，受试者具有30kg/m^2或更大(例如，30-39.9kg/m^2或30kg/m^2-50kg/m^2的BMI。在一些实施方案中，受试者具有40kg/m^2或更大的BMI。在一些实施方案中，受试者具有向心性肥胖(例如，腹部区域脂肪过多，包括腹部脂肪和/或内脏脂肪)。在一些实施方案中，受试者具有0.85 或更大的腰/髋周围比(WHR)。在一些实施方案中，受试者具有外周肥胖(例如，髋上脂肪过多)。在一些实施方案中，ALK7:ActRIIB拮抗剂治疗是对饮食和/或锻炼的辅助。

[0449] 在另一实施方案中，本公开内容提供了治疗和/或改善肥胖或与肥胖有关的疾病或病况的方法，包括给予肥胖受试者有效量的 ALK7:ActRIIB拮抗剂(例如，ALK7:ActRIIB异二聚体)或这样的拮抗剂的组合。在一些实施方案中，受试者具有30kg/m^2或更大的BMI。在另外的实施方案中，受试者具有30-39.9kg/m^2或30kg/m^2-50kg/m^2的BMI。在一些实施方案中，受试者是病态肥胖的。在一些实施方案中，受试者具有40kg/m^2或更大的身体BMI。在另外的实施方案中，受试者具有40kg/m^2-45kg/m^2或40kg/m^2-50kg/m^2的BMI。在一些实施方案中，受试者具有2型糖尿病。在一些实施方案中，受试者具有 30kg/m^2或更大(例如，30-39.9kg/m^2)的BMI。在一些实施方案中，受试者具有至少40kg/m^2的BMI。在一些实施方案中，受试者具有向心性肥胖(例如，腹部区域脂肪过多，包括腹部脂肪和/或内脏脂肪)。在一些实施方案中，受试者具有0.85或更大的腰/髋周围比(WHR)。在一些实施方案中，受试者具有外周肥胖(例如，髋上脂肪过多)。在一些实施方案中，ALK7:ActRIIB拮抗剂治疗是对饮食和/或锻炼的辅助。

[0450] 还提供了治疗或预防与肥胖有关的疾病或病况的方法，包括给予需要治疗或预防的受试者有效量的ALK7:ActRIIB拮抗剂(例如，ALK7:ActRIIB异二聚体)或这样的拮抗剂的组合。在一个实施方案中，治疗或预防的疾病或病况是选自以下的成员：血脂障碍、高脂血症(总胆固醇水平＞240mg/dL)、高胆固醇血症(例如，总胆固醇水平＞200 mg/dL、＞220mg/dL、＞240mg/dL、＞250mg/dL或＞275mg/dL)、低 HDL血清水平(例如，＜40mg/dL、＜45mg/dL或＜50mg/dL)、高LDL 血清水平(例如，≥100mg/dL、≥130mg/dL、≥160mg/dL或≥190mg/dL) 和高甘油三酯血症(例如，空腹TG水平≥150mg/dL、≥175mg/dL、≥ 200mg/dL、≥300mg/dL、≥400mg/dL或≥499mg/dL)。在一个实施方案中，治疗或预防的疾病或病况是心血管疾病。在另外的实施方案中，治疗或预防的疾病或病况是高血压(高的血压)、心肌梗塞、外周动脉疾病、血管调节功能障碍、动脉硬化、充血性心力衰竭、动脉粥样硬化、冠心病或微血管疾病。在一个实施方案中，治疗或预防的疾病或病况是肝脏疾病。在一个实施方案中，治疗或预防的肝脏疾病或病况是NAFLD。在一个实施方案中，肝脏疾病是脂肪肝。在一个实施方案中，肝脏疾病是NASH。在另一实施方案中，治疗或预防的疾病或病况是选自以下的

成员:脂肪性肝炎、脂肪变性、纤维化和/或硬化。在某些情况下,ALK7:ActRIIB拮抗剂治疗是对饮食和/或锻炼的辅助。

[0451] 在另一实施方案中,本公开内容提供了治疗、改善和/或预防2型糖尿病或与糖尿病有关的疾病或病况的方法,包括给予具有2型糖尿病或处于发生2型糖尿病的风险的受试者有效量的ALK7:ActRIIB拮抗剂(例如,ALK7:ActRIIB异二聚体)或这样的拮抗剂的组合。在一些实施方案中,受试者具有30kg/m^2或更大(例如,30-39.9kg/m^2)的体重指数BMI。在一些实施方案中,受试者具有至少40kg/m^2的BMI。在一些实施方案中,受试者具有向心性肥胖(例如,腹部区域脂肪过多,包括腹部脂肪和/或内脏脂肪)。在一些实施方案中,受试者具有0.85 或更大的WHR。在一些实施方案中,受试者具有外周肥胖(例如,髋上脂肪过多)。在一些实施方案中,ALK7:ActRIIB拮抗剂治疗是对饮食和/或锻炼的辅助。

[0452] 还提供了治疗、改善或预防与糖尿病有关的疾病或病况的方法,包括给予具有糖尿病的受试者有效量的ALK7:ActRIIB拮抗剂(例如, ALK7:ActRIIB异二聚体)或这样的拮抗剂的组合。在一个实施方案中,治疗或预防的疾病或病况是选自以下的成员:血脂障碍、高脂血症(总胆固醇水平＞240mg/dL)、高胆固醇血症(例如,总胆固醇水平＞200 mg/dL、＞220mg/dL、＞240mg/dL、＞250mg/dL或＞275mg/dL)、低 HDL血清水平(例如,＜40mg/dL、＜45mg/dL或＜50mg/dL)、高LDL 血清水平(例如,≥100mg/dL、≥130mg/dL、≥160mg/dL或≥190mg/dL) 和高甘油三酯血症(例如,空腹TG水平≥150mg/dL、≥175mg/dL、≥200mg/dL、≥300mg/dL、≥400mg/dL或≥499mg/dL)。在一个实施方案中,治疗或预防的疾病或病况是心血管疾病。在另外的实施方案中,治疗或预防的疾病或病况是高血压(高的血压)、心肌梗塞、外周动脉疾病、血管调节功能障碍或动脉硬化。在一个实施方案中,治疗或预防的疾病或病况是肝脏疾病。在另一实施方案中,治疗或预防的疾病或病况是选自以下的成员:脂肪性肝病、脂肪性肝炎、脂肪变性和/或硬化。在一个实施方案中,治疗或预防的疾病或病况是选自以下的成员:白内障、阻塞性睡眠呼吸暂停、静脉炎、痛风、骨关节炎、胆囊疾病和高胆固醇。在某些情况下,ALK7:ActRIIB拮抗剂治疗是对饮食和/或锻炼的辅助。

[0453] 本公开内容还提供了用于改善受试者的血脂概况的方法,包括给予需要这样的治疗的受试者有效量的ALK7:ActRIIB拮抗剂(例如, ALK7:ActRIIB异二聚体)或这样的拮抗剂的组合。在一些实施方案中,本公开内容提供了用于减少LDL胆固醇水平或增加HDL-胆固醇水平的方法。在一个实施方案中,受试者具有血脂障碍。在另一实施方案中,受试者具有升高的血清脂质(例如,胆固醇(高胆固醇血症)和/或甘油三酯(例如,高甘油三酯血症)。在一个实施方案中受试者具有≥100 mg/dL、≥130mg/dL或≥160mg/dL的LDL-C)。在一个实施方案中受试者具有≥150mg/dL、≥160mg/dL、≥170mg/dL的TG)。在一个实施方案中,受试者具有升高的血浆胰岛素水平(高胰岛素血症;例如,＞20ug/ml的空腹胰岛素水平可超过100)。在一些实施方案中,受试者具有II型糖尿病。

[0454] 根据一个实施方案,本公开内容提供了治疗或预防代谢疾病或病症或与代谢疾病或病症有关的病况的方法,包括给予有需要的受试者 ALK7:ActRIIB拮抗剂(例如,ALK7:ActRIIB异二聚体)或这样的拮抗剂的组合。在一个实施方案中,治疗的代谢疾病、病症或病况是高血糖症(例如,在空腹状态下或在口服葡萄糖耐受试验期间在葡萄糖给予后,＞130mg/dL)。在一个实施方案中,治疗的代谢疾病、病症或病况是脂质代谢疾病、病症或病况。在一个实施方案中,治疗的代谢疾病、病症或病况是血脂紊乱。在进一步的实施方案中,

脂质代谢疾病、病症或病况是选自以下的成员:低HDL水平、高LDL水平、高甘油三酯水平、高脂血症和脂蛋白畸变。在一个实施方案中,受试者具有>200mg/dL、>220mg/dL、>240mg/dL、>250mg/dL或>275mg/dL 的总胆固醇水平。在一个实施方案中,受试者具有<40mg/dL、<45 mg/dL或<50mg/dL的HDL血清水平)。在一个实施方案中,受试者具有≥100mg/dL、≥130mg/dL、≥160mg/dL或≥190mg/dL的LDL 血清水平。在一个实施方案中,受试者具有≥150mg/dL、≥175mg/dL、≥200mg/dL、≥300mg/dL、≥400mg/dL或≥499mg/dL的空腹TG水平。在一个实施方案中,治疗的代谢疾病、病症或病况是葡萄糖代谢疾病、病症或病况。在进一步的实施方案中,葡萄糖代谢疾病、病症或病况是选自以下的成员:葡萄糖不耐受、胰岛素抵抗、葡萄糖耐量受损(IGT)、空腹葡萄糖受损(IFG)。在一个实施方案中,治疗的代谢疾病、病症或病况是选自以下的成员:高尿酸水平、NAFLD、脂肪肝、 NASH和多囊性卵巢综合征。在一个实施方案中,治疗的受试者具有高胰岛素血症。在一个实施方案中,治疗的受试者是肥胖的(例如,受试者具有腹部肥胖)。在另一实施方案中,治疗的受试者具有II型糖尿病。

[0455] 代谢综合征是包括一组提高心脏病的风险的病症的病况。代谢综合征的主要成分是过高体重、心血管参数(高血压、血脂障碍、在血液中高水平的甘油三酯和/或低水平的HDL)、动脉粥样硬化、糖尿病和/ 或胰岛素抵抗。具有数个这些成分,即代谢综合征的受试者对心脏病高度易感,但每个成分都是风险因素。本公开内容还提供了治疗或预防1、2、3或更多种代谢综合征的上述成分的方法,包括给予需要治疗的受试者有效量的ALK7:ActRIIB拮抗剂(例如,ALK7:ActRIIB异二聚体)或这样的拮抗剂的组合。

[0456] 另外提供了治疗、预防或改善心血管疾病或病况的方法,包括给予有需要的受试者ALK7:ActRIIB拮抗剂(例如,ALK7:ActRIIB异二聚体)或这样的拮抗剂的组合。在一个实施方案中,治疗、预防或改善的心血管疾病或病况是动脉粥样硬化。在一个实施方案中,治疗、预防或改善的心血管疾病或病况是高血压(例如,在休息状态下血压>130/80mmHg或>140/90mmHg)。在一个实施方案中,心血管疾病是动脉粥样硬化(冠心病疾病)。

[0457] 在一个实施方案中,本公开内容提供了治疗和/或改善炎性肝脏疾病或病况的方法,包含给予有需要的受试者ALK7:ActRIIB拮抗剂(例如,ALK7:ActRIIB异二聚体)或这样的拮抗剂的组合。在一个实施方案中,疾病或病况是NAFLD。在进一步的实施方案中,疾病或病况是脂肪肝。在进一步的实施方案中,疾病或病况是脂肪变性(例如,非酒精性脂肪性肝炎(NASH))。在进一步的实施方案中,疾病或病况是酒精性脂肪性肝病。

[0458] 本公开内容还提供了改善血糖控制的方法,包括给予需要治疗的受试者有效量的ALK7:ActRIIB拮抗剂(例如,ALK7:ActRIIB异二聚体)。在一个实施方案中,给予的受试者具有>130、>135、>140、>145 或>150mg/dL的空腹血糖水平。在一个实施方案中,给予的受试者在进食后2小时具有>180、>185、>190、>195或>200mg/dL的餐后血糖水平。在某些情况下,ALK7:ActRIIB拮抗剂治疗是对饮食和/或锻炼的辅助。给予还可减少体重或治疗肥胖。在某些情况下,受试者具有2型糖尿病。在某些情况下,受试者具有27-40kg/m2的BMI。在某些情况下,受试者具有30-39.9kg/m2的BMI。在某些情况下,受试者具有至少40的BMI。在某些情况下,受试者是超重的。在某些情况下,受试者是肥胖的。血糖控制的改善可使用本领域已知的技术,例如混餐检验评价。

[0459] 本公开内容还提供了用于在受试者中治疗、预防或改善高血糖症或与高血糖症有关的病况的组合物和方法,包括给予需要这样的治疗的受试者有效量的ALK7:ActRIIB拮抗

剂(例如,ALK7:ActRIIB异二聚体)。在一个实施方案中,给予的受试者具有＞130、＞135、＞140、＞145或＞150mg/dL的空腹血糖水平。在一个实施方案中,给予的受试者在进食后2小时具有＞180、＞185、＞190、＞195或＞200mg/dL的餐后血糖水平。在一个实施方案中,治疗、预防或改善的结果是选自以下的成员:与治疗前受试者的血清水平比较,葡萄糖的血清水平减少、甘油三酯的血清水平减少、胰岛素的血清水平减少和/或非-酯化脂肪酸的血清水平减少。在一个实施方案中,治疗、预防或改善的结果是与治疗前受试者的身体温度比较,身体温度增加约0.4℃-1℃。在一些实施方案中,ALK7:ActRIIB治疗还减少受试者的体重。

[0460] 在另一实施方案中,本公开内容提供了降低受试者的血浆胰岛素水平的方法,包括给予需要这样的治疗的受试者有效量的 ALK7:ActRIIB拮抗剂(例如,ALK7:ActRIIB异二聚体)。在一个实施方案中,受试者具有＞130、＞135、＞140、＞145或＞150mg/dL的空腹血糖水平。在一个实施方案中,受试者在进食后2小时具有＞180、＞185、＞190、＞195或＞200mg/dL的餐后血糖水平。在一个实施方案中,受试者是超重的。在一个实施方案中,受试者是肥胖的。在另一实施方案中,受试者具有2型糖尿病。

[0461] 本公开内容还提供了用于在受试者中治疗、预防或改善高血糖症或与高血糖症有关的病况的组合物和方法,包括给予需要这样的治疗的受试者有效量的ALK7:ActRIIB拮抗剂(例如,ALK7:ActRIIB异二聚体)。在一个实施方案中,受试者具有＞130、＞135、＞140、＞145或＞150mg/dL的空腹血糖水平。在一个实施方案中,受试者在进食后2 小时具有＞180、＞185、＞190、＞195或＞200mg/dL的餐后血糖水平。在一个实施方案中,治疗、预防或改善的结果是选自以下的成员:与治疗前受试者的血清水平比较,葡萄糖的血清水平减少、甘油三酯的血清水平减少、胰岛素的血清水平减少和/或非-酯化脂肪酸的血清水平减少。在一个实施方案中,治疗、预防或改善的结果是与治疗前受试者的身体温度比较,身体温度增加约0.4℃-1℃。在一些实施方案中, ALK7:ActRIIB拮抗剂治疗还减少受试者的体重。

[0462] 在另一实施方案中,本公开内容提供了降低受试者的血浆胰岛素水平方法,包括给予需要这样的治疗的受试者有效量的ALK7:ActRIIB 拮抗剂(例如,ALK7:ActRIIB异二聚体)或这样的拮抗剂的组合。在一个实施方案中,受试者具有＞130、＞135、＞140、＞145或＞150mg/dL 的空腹血糖水平。在一个实施方案中,受试者在进食后2小时具有＞180、＞185、＞190、＞195或＞200mg/dL的餐后血糖水平。在一个实施方案中,受试者是超重的。在一个实施方案中,受试者是肥胖的。在另一实施方案中,受试者具有2型糖尿病。

[0463] 在另一实施方案中,本公开内容提供了治疗、预防或改善受试者的肝脏疾病的方法,包括给予具有肝脏疾病的受试者有效量的 ALK7:ActRIIB拮抗剂(例如,ALK7:ActRIIB异二聚体)或这样的拮抗剂的组合。在一个实施方案中,受试者具有肝脏的炎症。在一个实施方案中,受试者具有NAFLD。在一个实施方案中受试者具有脂肪肝。在另一实施方案中,受试者具有NASH。在一个实施方案中受试者具有脂肪肝。在另一实施方案中,受试者具有酒精性脂肪性肝病。在一个实施方案中,治疗、预防或改善的肝脏疾病是纤维化、瘢痕化、硬化或肝脏衰竭。在另一实施方案中,治疗、预防或改善的肝脏疾病是肝脏癌症。在一个实施方案中,受试者是超重的。在另一实施方案中,受试者是肥胖的。在另一实施方案中,受试者具有2型糖尿病。

[0464] 在一个实施方案中,本公开内容提供了在细胞(例如,白色或褐色脂肪细胞或组

织)中增加脂解的方法,包括给予有效量的 ALK7:ActRIIB拮抗剂(例如,ALK7:ActRIIB异二聚体)或这样的拮抗剂的组合。在一些实施方案中细胞经体外接触。在一些实施方案中细胞经体内接触。在一个实施方案中,方法体内进行,例如,在哺乳动物受试者(例如,动物模型)中。在进一步的实施方案中,受试者是人。在一些实施方案中,方法导致甘油产生增加。在另外的实施方案中,方法导致在脂肪细胞培养物中甘油和/或游离脂肪酸增加。在一些实施方案中,方法导致在细胞或组织中甘油三酯(TG)含量减少。在一些实施方案中,方法导致受试者的血浆TG水平减少。

[0465] 在另一实施方案中,本公开内容提供了增加细胞或组织(例如,白色或褐色脂肪细胞或组织)的肾上腺素能受体-β(ADRB)信号传导的方法。方法包括使细胞或组织与足以增加ADRB信号传导的量的 ALK7:ActRIIB拮抗剂(例如,ALK7:ActRIIB异二聚体)或这样的拮抗剂的组合接触。在一些实施方案中细胞或组织经体外接触。在一些实施方案中细胞或组织经体内接触。在一个实施方案中,方法在体内进行,例如,在哺乳动物受试者(例如,动物模型)中。在进一步的实施方案中,受试者是人。在一些实施方案中,方法导致甘油产生增加。在另外的实施方案中,方法导致在脂肪细胞培养物中甘油和/或游离脂肪酸增加。在一些实施方案中,方法导致在细胞或组织中TG含量减少。在一些实施方案中,方法导致受试者的血浆TG水平减少。在一些实施方案中,方法导致在营养物过载期间脂肪细胞或脂肪组织的 ADRB信号传导增加。

[0466] 在另一实施方案中,本公开内容提供了在细胞或组织(例如,白色和/或褐色脂肪细胞或组织)中减少过氧化物酶体增殖子-活化受体-γ (PPARγ)信号传导的方法。方法包括使细胞或组织与有效减少PPARγ活性的量的ALK7:ActRIIB拮抗剂(例如,ALK7:ActRIIB异二聚体)或这样的拮抗剂的组合接触。在一些实施方案中,细胞或组织经体外接触。在一些实施方案中,分化的细胞或组织经体内接触。在一个实施方案中,方法在体内进行,例如,在哺乳动物受试者(例如,动物模型) 中。在进一步的实施方案中,受试者是人。在一些实施方案中,方法导致甘油产生增加。在另外的实施方案中,方法导致脂肪细胞培养物中甘油和/或游离脂肪酸增加。在一些实施方案中,方法导致细胞或组织中TG含量减少。在一些实施方案中,方法导致受试者的血浆TG 水平减少。

[0467] 在另一实施方案中,本公开内容提供了降低细胞或组织(例如,白色和/或褐色脂肪细胞或组织)的胰岛素抵抗的方法。方法包括使细胞或组织与有效减少胰岛素抵抗的量的ALK7:ActRIIB拮抗剂(例如, ALK7:ActRIIB异二聚体)或这样的拮抗剂的组合接触。在一些实施方案中细胞或组织经体外接触。在一些实施方案中细胞或组织经体内接触。在一个实施方案中,方法在体内进行,例如,在哺乳动物受试者 (例如,动物模型)中。在进一步的实施方案中,受试者是人。

[0468] 在另一实施方案中,本公开内容提供了增加细胞或组织(例如,白色和/或褐色脂肪细胞或组织)的代谢速率的方法。方法包括使细胞或组织与有效增加细胞或组织的代谢的量的ALK7:ActRIIB拮抗剂(例如, ALK7:ActRIIB异二聚体)或这样的拮抗剂的组合接触。在一些实施方案中细胞或组织经体外接触。在一些实施方案中细胞或组织经体内接触。在一个实施方案中,方法在体内进行,例如,在哺乳动物受试者 (例如,动物模型)中。在进一步的实施方案中,受试者是人。

[0469] 本公开内容提供了方法,包括单独或与一种或多种另外的疗法(例如,一种或多种

另外的治疗剂和/或支持疗法)组合给予治疗有效量的 ALK7:ActRIIB拮抗剂(例如,ALK7:ActRIIB异二聚体)或这样的拮抗剂的组合至具有或有风险发生ALK7-介导疾病和/或病况的受试者,例如,肥胖(例如,腹部肥胖);超重;胰岛素抵抗;代谢综合征和其它代谢疾病或病况;脂质病症例如,低HDL水平、高LDL水平、高脂血症、高甘油三酯血症或血脂障碍;脂蛋白异常;甘油三酯减少;脂肪性肝病;非-酒精性脂肪性肝病;高血糖症;葡萄糖耐量受损(IGT);高胰岛素血症;高胆固醇(例如,高LDL水平和/或高胆固醇血症);心血管疾病例如,心脏病包括冠心病、充血性心力衰竭、动脉粥样硬化;动脉硬化和/或高血压;X综合征;血管再狭窄;神经病;和/或与一种或多种上述疾病或病况有关和/或与超重(例如,BMI of≥25kg/m^2) 有关或与太多体脂肪有关的其它病症/病况。

[0470] 在另外的实施方案中,本公开内容提供了治疗和/或改善癌症或与癌症有关的病况的方法,包括给予ALK7:ActRIIB拮抗剂(例如, ALK7:ActRIIB异二聚体)或这样的拮抗剂的组合。在一些实施方案中,受试者具有选自黑素瘤、乳腺癌、结肠癌和子宫内膜癌、胰腺癌、胃癌和子宫癌的癌症。在一些实施方案中,受试者具有骨髓瘤(例如,多发性骨髓瘤、浆细胞瘤、局部骨髓瘤和髓外骨髓瘤)。在一些实施方案中,ALK7:ActRIIB拮抗剂经给予以治疗或预防淋巴转移、血流转移、肿瘤生长或肿瘤侵袭。

[0471] 纤维化通常是指器官或组织中胶原纤维和细胞外基质二者过度沉积,以及细胞数量相对减少。尽管该过程是在损伤后自然伤口愈合的重要特征,但纤维化可导致各种组织和器官(包括例如,肺、肾、肝脏、骨、肌肉和皮肤)的病理性损伤。TGF-β在纤维化中的作用已被广泛研究。然而,其它TGF-β超家族配体也参与纤维化,包括例如,活化素(例如,活化素A和活化素B)和GDF8[Hedger等(2013)Cytokine and Growth Factor Reviews 24:285-295;Hardy等(2015)93:567-574;和 Cantini等(2008)J Sex Med 5:1607-1622]。因此,在一些实施方案中,本公开内容的ALK7:ActRIIB拮抗剂(例如,ALK7:ActRIIB异二聚体) 或这样的拮抗剂的组合可用于治疗纤维化、特别是纤维化-相关的病症和病况。例如,ALK7:ActRIIB拮抗剂(例如,ALK7:ActRIIB异二聚体) 或这样的拮抗剂的组合可用于治疗或预防以下一种或多种:肺纤维化、超敏感性肺炎、特发性纤维化、肺结核、肺炎、囊性纤维化、哮喘、慢性阻塞性肺病(COPD)、肺气肿、肾纤维化、肾衰竭、慢性肾疾病、骨纤维化、骨髓纤维化、类风湿性关节炎、系统性红斑狼疮、硬皮病、结节病、伴有多脉管炎的肉芽肿病、佩伦涅氏病、肝脏纤维化、威尔逊氏病、糖原贮积病(特别是III、IV、IX和X型)、铁-过载、戈谢病、泽韦格综合征、非酒精性和酒精性脂肪性肝炎、胆汁性肝硬化、硬化性胆管炎、巴-希二氏综合征、手术相关的纤维化、克罗恩病、掌挛缩、纵隔纤维化、肾原性纤维化、腹膜后纤维化、心房纤维化、心内膜心肌纤维化、胰脏纤维化。

[0472] 部分地,本公开内容涉及令人惊讶的发现,即ALK7:ActRIIB异多聚体可结合活化素C。因此,ALK7:ActRIIB异多聚体提供一类新的活化素C抑制剂,其可用于治疗或预防活化素C-相关的病症或病况。在一些实施方案中,ALK7:ActRIIB异多聚体可用于在有需要的患者中抑制活化素C活性。活化素C的过表达涉及与肝脏、睾丸和前列腺,特别是这样的组织的癌症有关的疾病和病症。参见例如,Gold等Am J Pathol(2009)174(1):184-195。在一些实施方案中,ALK7:ActRIIB异多聚体可用于增加雄性生育力,增加精子产生,增加生精小管容量,减少肝脏炎症,治疗肝脏(肝)癌,治疗睾丸癌,治疗前列腺癌,减少前列腺炎症和/或治疗前列腺肥大。

[0473] 在一些实施方案中,ALK7:ActRIIB-相关病况包括神经肌肉病症 (例如,肌营养不良和肌肉萎缩)、充血性阻塞性肺病(和与COPD有关的肌肉消瘦)、肌肉消瘦综合征、肌少症、恶病质、脂肪组织病症(例如,肥胖)、2型糖尿病(NIDDM、成年型糖尿病)和骨退化疾病(例如,骨质疏松症)。其它实例性ALK7:ActRIIB-相关病况包括肌肉退化和神经肌肉病症、组织修复(例如,伤口愈合)、神经变性疾病(例如,肌萎缩侧索硬化)和免疫学病症(例如,与淋巴细胞的异常增殖或功能有关的病症)。

[0474] 在某些实施方案中,本公开内容的ALK7:ActRIIB拮抗剂(例如, ALK7:ActRIIB异二聚体)或这样的拮抗剂的组合可用作肌营养不良的治疗的一部分。术语"肌营养不良"是指一组退化肌肉疾病,特征为逐渐虚弱,和骨骼肌肉,有时心脏和呼吸肌肉的退化。肌营养不良是遗传病症,特征为渐进性肌肉消瘦和虚弱,其以肌肉的微观变化开始。因为肌肉随时间退化,人的肌肉力量下降。可用包括本发明的TGF-β超家族异多聚体复合物的方案治疗的实例性肌营养不良包括:杜氏肌营养不良(DMD)、贝克肌营养不良(BMD)、埃-德二氏肌营养不良 (EDMD)、角膜缘带肌营养不良(LGMD)、面肩肱型肌营养不良(FSH 或FSHD)(亦称为Landouzy-Dejerine)、强直性肌营养不良(MMD;亦称为斯太纳特氏病)、眼咽肌营养不良(OPMD)、远端肌营养不良(DD)、先天性肌营养不良(CMD)。

[0475] 杜氏肌营养不良(DMD)在19世纪60年代首次由法国神经学家 Guillaume Benjamin Amand Duchenne描述。贝克肌营养不良(BMD)根据德国医生Peter Emil Becker命名,其在20世纪50年代首次描述了这种DMD变体。DMD是在男性中最频繁遗传的疾病之一,影响1/3,500的男孩。当位于X染色体的短臂上的肌养蛋白基因缺陷时,DMD发生。因为男性仅携带一个拷贝的X染色体,因此他们仅具有一个拷贝的肌养蛋白基因。没有肌养蛋白,在收缩和舒张循环期间肌肉容易受到损害。尽管在疾病早期肌肉通过再生得到补偿,但晚期肌肉祖细胞不能跟上进行中的损害和健康肌肉被无功能的纤维脂肪组织代替。

[0476] BMD由肌养蛋白基因的不同突变产生。BMD患者具有一些肌养蛋白,但其数量不足或质量差。一些肌养蛋白的存在保护BMD患者的肌肉免于与DMD患者一样严重或快速地退化。

[0477] 动物中的研究表明,抑制GDF8信号传导途径可有效地治疗DMD 和BMD患者的各种方面的疾病(Bogdanovich等,2002,Nature 420:418-421;Pistilli等,2011,Am J Pathol178:1287-1297)。因此,本公开内容的ALK7:ActRIIB拮抗剂可用作GDF8抑制剂(拮抗剂),和构成在DMD和BMD患者体内通过GDF8和/或相关的TGFβ超家族配体阻断信号传导的可选方式。

[0478] 类似地,本公开内容的ALK7:ActRIIB拮抗剂可提供在需要肌肉生长的其它疾病情况下增加肌肉质量的有效方式。例如,肌萎缩侧索硬化(ALS),亦称为Lou Gehrig病或运动神经元疾病,是一种慢性的渐进性和难治的CNS病症,其攻击作为起始骨骼肌肉收缩所需的中枢神经系统的组分的运动神经元。在ALS中,运动神经元退化和最终死亡,和虽然人脑正常保持完整功能和警觉,但肌肉收缩的起始在脊椎水平上被阻断。发生ALS的个体通常在40-70岁,和退化的第一运动神经元是神经支配臂或腿的那些。ALS患者可具有行走障碍,可掉东西,跌倒,口齿不清,和不受控制地笑或哭。随着疾病进展,四肢的肌肉开始从不使用到萎缩。肌肉虚弱变得衰弱,和患者最终需要轮椅或限制于床上。大多数ALS患者在疾病发作后3-5年死于呼吸衰竭或呼吸机辅助样肺炎的并发症。

[0479] 通过ALK7:ActRIIB拮抗剂促进肌肉质量增加还可能使患有肌肉消瘦疾病的那些患者获益。Gonzalez-Cadavid等(同上)报道了GDF8 表达与人中的无脂肪体重负相关,和GDF8基因的表达增加与AIDS 消瘦综合征的男性中体重减轻有关。通过抑制AIDS患者中的GDF8 功能,如果没有完全消除的话,AIDS的至少某些症状可得到减轻,因此显著改进AIDS患者的生活质量。

[0480] 因为GDF8功能丢失还伴随在没有减少营养物摄入的情况下脂肪减少(Zimmers等,同上;McPherron和Lee,同上),本发明的 ALK7:ActRIIB拮抗剂可进一步用作减慢或预防肥胖和2型糖尿病的发生的治疗剂。

[0481] 癌症食欲缺乏-恶病质综合征是癌症的最虚弱和威胁生命的方面。该综合征是许多类型的癌症的共同特征(存在于大约80%的死亡癌症患者中),并且不仅是生活质量差和对化学疗法反应差的原因,而且是比具有相当的肿瘤但没有体重减轻的患者更短存活时间的原因。如果在6个月时间内大于5%的发病前体重的无意体重减轻发生,则通常认为恶病质在癌症患者中。与食欲缺乏、脂肪和肌肉组织消瘦以及心理苦恼有关,恶病质从癌症和宿主之间的复杂相互作用产生。癌症恶病质影响细胞因子产生、脂质运动和蛋白水解-诱导因子的释放和中间代谢的改变。尽管食欲缺乏是常见的,但仅食物摄入降低不能够解释癌症患者中见到的身体组成的变化,和增加营养物摄入不能逆转消瘦综合征。目前,没有控制或逆转恶病质过程的治疗。因为发现在成年小鼠中GDF8的系统过量表达诱导显著的肌肉和脂肪损失,类似于人恶病质综合征中所见到的(Zimmers等,同上),因此本发明的 ALK7:ActRIIB拮抗剂可有利地用于预防、治疗或减轻其中需要肌肉生长的恶病质综合征的症状。上文所述的可用于预防、治疗或减轻肌肉损失的异聚复合物的实例是ALK7:ActRIIB异二聚体。

[0482] 在某些实施方案中,本公开内容的ALK7:ActRIIB拮抗剂(例如, ALK7:ActRIIB异二聚体)或这样的拮抗剂的组合可用于诱导骨和/或软骨形成,预防骨丢失,增加骨矿化,预防骨的去矿化和/或增加骨密度的方法。ALK7:ActRIIB拮抗剂可用于经诊断有亚临床低骨密度的患者,作为针对发生骨质疏松症的保护措施。

[0483] 在一些实施方案中,本公开内容的ALK7:ActRIIB拮抗剂(例如, ALK7:ActRIIB异二聚体)或这样的拮抗剂的组合可在人和其它动物中在骨折和软骨缺陷的治愈中具有医学功用。本发明的方法和组合物还可在关闭以及打开骨折复位术中以及在人工关节的改进固定中具有预防用途。由成骨剂诱导的从新骨形成可用于修复先天的、创伤-诱导的或由肿瘤切除引起的颅面缺损,和还可用于美容整形手术。此外,本发明的方法和组合物可用于治疗牙周疾病和其它牙齿修复过程。在某些情况下,ALK7:ActRIIB拮抗剂(例如,ALK7:ActRIIB异二聚体) 或这样的拮抗剂的组合可提供吸引骨-形成细胞、刺激骨-形成细胞的生长或诱导骨-形成细胞的祖细胞分化的环境。本公开内容的

[0484] ALK7:ActRIIB拮抗剂(例如,ALK7:ActRIIB异二聚体)或这样的拮抗剂的组合还可用于治疗骨质疏松症。此外,ALK7:ActRIIB拮抗剂可用于修复软骨缺陷和预防/逆转骨关节炎。本文所述的可用于诱导骨形成、预防骨丢失、增加骨矿化、预防骨去矿化和/或增加骨密度的异聚复合物的实例是ALK7:ActRIIB异二聚体。

[0485] Rosen等(编辑)Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism,第7版.American Society for Bone and Mineral Research,

Washington D.C.(通过引用并入本文)提供了可用 ALK7:ActRIIB拮抗剂或用这样的拮抗剂的组合治疗的骨病症的深入讨论。本文提供了部分列表。本发明的方法和组合物可应用于特征为或引起骨丢失的病况,例如骨质疏松症(包括继发性骨质疏松症)、甲状旁腺功能亢进、慢性肾病矿物骨病症、性激素丧失或消除(例如,雄激素和/或雌激素)、糖皮质激素治疗、类风湿性关节炎、重度烧伤、甲状旁腺功能亢进、高钙血症、低钙血症、低磷酸盐血症、骨软化症 (包括肿瘤-诱导的骨软化症)、高磷酸盐血症、维生素D缺乏、甲状旁腺功能亢进(包括家族性甲状旁腺功能亢进)和假性甲状旁腺功能减退症、肿瘤转移至骨、因为肿瘤或化学疗法导致的骨丢失、骨和骨髓的肿瘤(例如,多发性骨髓瘤)、缺血性骨病症、牙周疾病和口腔骨丢失、库兴氏病、佩吉特病、甲状腺毒症、慢性腹泻体质或吸收不良、肾小管性酸中毒或神经性厌食。本发明的方法和组合物还可应用于特征为不能骨形成或愈合的病况,包括非连接骨折、以其它方式减慢愈合的骨折、胎儿和新生儿骨发育异常(例如,低钙血症、高钙血症、钙受体缺陷和维生素D缺乏)、骨坏死(包括颚的骨坏死)和骨发生不全。另外,合成代谢作用将导致这样的拮抗剂减少与骨损坏或侵蚀有关的骨疼痛。作为抗-再吸收作用的结果,这样的拮抗剂可用于治疗异常骨形成的病症,例如成骨细胞肿瘤转移(例如,与原发性前列腺或乳腺癌有关)、成骨骨肉瘤、骨硬化症、进行性骨干发育异常、骨内膜骨质增生、骨斑症和肢骨纹状肥大。可治疗的其它病症包括纤维性结构不良和软骨发育异常。

[0486] 在另一具体的实施方案中,本公开内容提供了用于修复骨折和与软骨和/或骨缺陷有关的其它病况或牙周疾病的治疗方法和组合物。本发明进一步提供了用于伤口愈合和组织修复的治疗方法和组合物。伤口的类型包括但不限于,烧伤、切伤和溃疡。参见例如,PCT公布号 WO 84/01106。这样的组合物包含治疗有效量的至少一种本公开内容的ALK7:ActRIIB拮抗剂与药学上可接受的溶媒、载体或基质的混合物。

[0487] 在一些实施方案中,本公开内容的ALK7:ActRIIB拮抗剂(例如, ALK7:ActrIIB异二聚体)或这样的拮抗剂的组合可应用于引起骨丢失的病况,例如骨质疏松症、甲状旁腺功能亢进、库兴氏病、甲状腺毒症、慢性腹泻体质或吸收不良、肾小管性酸中毒或神经性厌食。通常应理解,作为女性,具有低体重和导致久坐生活方式是骨质疏松症的风险因素(骨矿物密度丢失,导致骨折风险)。然而,骨质疏松症也可自长期使用某些药物产生。自药物或另一医学病况产生的骨质疏松症被称为继发性骨质疏松症。在库兴氏病中,身体产生的过量皮质醇导致骨质疏松症和骨折。与继发性骨质疏松症有关的最常见的药物是皮质类固醇,一类像皮质醇(由肾上腺天然产生的激素)一样起作用的药物。尽管需要足够水平的甲状腺激素以发育骨骼,但过量的甲状腺激素可随时间减少骨量。包含铝的抗酸药当有肾问题的人、特别是经过透析的那些人以高剂量服用时,可导致骨丢失。可引起继发性骨质疏松症的其它药物包括用于预防癫痫的苯妥英(Dilantin)和巴比妥类;甲氨蝶呤(Rheumatrex,Immunex,Folex PFS),一些类型的关节炎、癌症和免疫病症的药物;环孢霉素(Sandimmune,Neoral),用于治疗一些自身免疫疾病和在器官移植患者中抑制免疫系统的药物;黄体化激素-释放激素激动剂(Lupron,Zoladex),用于治疗前列腺癌和子宫内膜异位症;肝素(Calciparine,Liquaemin),一种抗凝血药物;和考来烯胺(Questran)和考来替泊(Colestid),用于治疗高胆固醇。自癌症疗法产生的骨丢失是广泛公认的,和被称为癌症疗法-诱导的骨丢失(CTIBL)。骨转移可在骨中产生洞,其可通过用ALK7:ActRIIB拮抗剂(例如, ALK7:ActRIIB异二聚体)治疗来纠正。骨丢失也可由牙龈疾病引起,一种其中位于牙龈

隐窝中的细菌产生毒素和有害的酶的慢性感染。

[0488] 在进一步的实施方案中，本公开内容提供了用于治疗与异常或不需要的骨生长有关的疾病或病症的方法和治疗剂。例如，具有先天性病症进行性骨化性纤维发育不良(FOP)的患者受累于在软组织中自发地或响应于组织创伤的进行性异位骨生长，对生活质量具有重大影响。另外，异常骨生长可在髋置换手术后发生，因此破坏手术结果。这是较常见的病理性骨生长的实例，和其中本发明的方法和组合物可在治疗上有用的情况。所述方法和组合物也可用于治疗其它形式的异常骨生长(例如，创伤、烧伤或脊椎损伤后骨的病理性生长)和用于治疗或预防在转移性前列腺癌或骨肉瘤中所见到的与异常骨生长有关的不需要的病况。

[0489] 在某些实施方案中，本公开内容的ALK7:ActRIIB拮抗剂(例如，ALK7:ActRIIB异二聚体)或这样的拮抗剂的组合可用于在癌症患者中促进骨形成。因为肿瘤-诱导的骨丢失、骨转移和治疗剂，具有某些肿瘤(例如，前列腺、乳腺、多发性骨髓瘤或引起甲状旁腺功能亢进的任何肿瘤)的患者对于骨丢失是高风险的。这样的患者可用TGF-β超家族异多聚体复合物或复合物的组合治疗，甚至在不存在骨丢失或骨转移的证据时。还可监测患者的骨丢失或骨转移的证据，和在指示物表明风险增加的情况下，可用ALK7:ActRIIB拮抗剂治疗患者。一般而言，DEXA扫描用于评价骨密度的变化，而骨重塑的指示物可用于评价骨转移的可能性。可监测血清标记。骨特异性碱性磷酸酶(BSAP) 是存在于成骨细胞中的酶。BSAP的血液水平在具有骨转移和导致骨重塑增加的其它病况的患者中增加。骨钙素和前胶原肽也与骨形成和骨转移有关。在由前列腺癌引起的骨转移的患者中，和在较小程度上在来自乳腺癌的骨转移中，已检测到BSAP增加。BMP7水平在已转移至骨的前列腺癌中很高，但在由于膀胱、皮肤、肝脏或肺癌导致的骨转移中不高。I型羧基-末端尾肽(ICTP)是一种在骨的再吸收期间形成的胶原中存在的交联点。因为骨经常打破和再形成，ICTP存在于整个身体内。然而，在骨转移的部位，水平显著高于正常骨的区域。ICTP 以高水平存在于由于前列腺、肺和乳腺癌导致的骨转移中。另一胶原交联点，I型N-末端尾肽(NTx)，在骨周转期间与ICTP一起产生。NTx 的量在由许多不同类型的癌症引起的骨转移中增加，所述癌症包括肺、前列腺和乳腺癌。此外，NTx的水平随骨转移的进展而增加。因此，该标记可用于检测转移以及测量疾病的程度。再吸收的其它标记包括吡啶诺林和脱氧吡啶诺林。再吸收标记或骨转移标记的任何增加表明在患者中对用ALK7:ActRIIB拮抗剂的疗法的需要。

[0490] 本公开内容的ALK7:ActRIIB拮抗剂(例如，ALK7:ActrIIB异二聚体)或这样的拮抗剂的组合可与其它骨-活性药剂联合给予。联合给予可通过给予单一共制剂，通过同时给予，或通过以分开的时间给予来实现。如果与其它骨-活性剂一起给予，ALK7:ActRIIB拮抗剂可能是特别有利的。患者可获益于联合接受ALK7:ActRIIB拮抗剂复合物和服用钙补充剂、维生素D、合适的运动和/或，在一些情况下，其它药物。其它药物的实例包括，二膦酸盐(阿伦膦酸盐、伊班膦酸盐和利塞膦酸盐)、降钙素、雌激素、甲状旁腺激素和雷洛昔芬。二膦酸盐(阿伦膦酸盐、伊班膦酸盐和利塞膦酸盐)、降钙素、雌激素和雷洛昔芬影响骨重塑周期，和分类为抗-再吸收药物。骨重塑由两个不同阶段组成：骨再吸收和骨形成。抗-再吸收药物减慢或终止骨-重塑周期的骨-再吸收部分，但不减慢周期的骨-形成部分。结果是，新形成以比骨再吸收更大的速率持续，和骨密度可随时间而增加。特立帕肽，一种甲状旁腺激素形式，增加在骨重塑周期中骨形成的速率。阿伦膦酸盐经批准用于预防(5mg每天或35mg

每周一次)和治疗(10mg每天或70mg每周一次)绝经后骨质疏松症。阿伦膦酸盐减少骨丢失，增加骨密度和减少脊柱、腕和髋骨折的风险。阿伦膦酸盐还经批准用于治疗在男性和女性中由于长期使用这些药物(即，泼尼松和可的松)导致的糖皮质激素-诱导的骨质疏松症和用于治疗男性的骨质疏松症。阿伦膦酸盐加维生素D经批准用于治疗绝经后女性的骨质疏松症(70mg每周一次加维生素D)，和用于治疗以改进骨质疏松症的男性的骨量。伊班膦酸盐经批准用于预防和治疗绝经后骨质疏松症。作为每月一次丸剂(150mg) 服用，伊班膦酸盐应在每月的同一天服用。伊班膦酸盐减少骨丢失，增加骨密度和减少脊柱骨折的风险。利塞膦酸盐经批准用于预防和治疗绝经后骨质疏松症。每日(5mg剂量)或每周(35mg剂量或35mg剂量与钙)服用，利塞膦酸盐减慢骨丢失，增加骨密度和减少脊柱和非- 脊柱骨折的风险。利塞膦酸盐还经批准用于男性和女性使用以预防和 /或治疗自长期使用这些药物(即，泼尼松或可的松)产生的糖皮质激素- 诱导的骨质疏松症。降钙素是天然存在的激素，其参与钙调节和骨代谢。在绝经期后超过5年的女性中，降钙素减慢骨丢失，增加脊椎骨密度，和可减轻与骨折有关的疼痛。降钙素减少脊椎骨折的风险。降钙素可作为注射剂(50-100IU每日)或鼻喷雾剂(200IU每日)获得。

[0491] 患者还可获益于联合接受ALK7:ActRIIB拮抗剂或这样的拮抗剂的组合和另外的骨-活性药物。雌激素疗法(ET)/激素疗法(HT)经批准用于预防骨质疏松症。ET已表明减少骨丢失，增加在脊柱和髋二者中的骨密度和在绝经后女性中减少髋和脊椎骨折的风险。ET最常见以递送大约0.3mg每日的低剂量或大约0.625mg每日的标准剂量的丸剂或皮肤贴剂的形式给予，和甚至当70岁后开始也是有效的。当雌激素单独服用时，其可增加女性发生子宫衬里的癌症(子宫内膜癌症) 的风险。为了消除该风险，健康护理提供者为具有完整子宫的那些女性处方激素黄体酮与雌激素的组合(激素置换疗法或HT)。ET/HT减轻绝经期症状和已表明对骨健康具有有益作用。副作用可包括阴道出血、乳房触痛、情绪障碍和胆囊疾病。雷洛昔芬，60mg每天，经批准用于预防和治疗绝经后骨质疏松症。已开发一类称为选择性雌激素受体调节剂(SERM)的药物来提供雌激素的有益作用，而没有其潜在的缺点。雷洛昔芬增加骨量和减少脊柱骨折的风险。尚未获得数据来证实雷洛昔芬可减少髋和其它非-脊柱骨折的风险。特立帕肽，一种甲状旁腺激素形式，经批准用于在绝经后女性和具有骨折高风险的男性中治疗骨质疏松症。该药物刺激新骨形成和显著增加骨矿物密度。在绝经后女性中，在脊柱、髋、足、肋骨和腕中注意到骨折复位。在男性中，在脊柱中注意到骨折复位，但没有足够的数据评价在其它部位的骨折复位。特立帕肽作为每日注射剂经自我-给予至多24个月。

[0492] 如本文所用的，“与.....组合”、“.....的组合”或“联合给予”是指任何形式的给予，使得另外的疗法(例如第二、第三、第四等)在体内仍有效(例如，多种化合物在患者中同时有效，其可包括这些化合物的协同作用)。有效性可能不关联到试剂在血液、血清或血浆中可测量的浓度。例如，不同的治疗化合物可在相同的制剂中或在分开的制剂中同时或序贯地，和以不同的时间表给予。因此，接受这样的治疗的个体可获益于不同的疗法的组合效果。本公开内容的一种或多种 ALK7:ActRIIB拮抗剂可与一种或多种其它另外的试剂或支持性疗法同时、在其之前或随后给予。一般而言，各治疗剂以对该特定试剂所确定的剂量和/或时间表给予。在方案中使用的特定组合应考虑本公开内容的拮抗剂与疗法和/或需求的相容性。

[0493] 5.药物组合物

[0494] 在某些方面,本公开内容的ALK7:ActRIIB拮抗剂(例如, ALK7:ActRIIB异多聚体)或这样的拮抗剂的组合可单独或作为药物制剂(亦称为治疗组合物或药物组合物)的组分给予。药物制剂是指呈允许其中包含的活性成分(例如,本公开内容的试剂)的生物学活性是有效的形式和不包含对制剂所给予的受试者有不可接受的毒性的另外的组分的制剂。本发明的化合物可经配制以对人或兽医药物使用方便的任何方式给予。例如,本公开内容的一种或多种试剂可用药学上可接受的载体配制。药学上可接受的载体是指药物制剂中并非活性成分的成分,其通常对受试者是无毒的。药学上可接受的载体包括但不限于,缓冲剂、赋形剂、稳定剂和/或防腐剂。一般而言,本公开内容使用的药物制剂当给予受试者时是无热原、生理学可接受的形式。并非本文所述的那些的治疗上有用的试剂,其可任选地包括在上述制剂中,可在本公开内容的方法中与本发明的试剂组合给予。

[0495] 在某些实施方案中,组合物将胃肠外给予[例如,通过静脉内(I.V.) 注射、动脉内注射、骨内注射、肌内注射、鞘内注射、皮下注射或皮内注射]。适合于胃肠外给予的药物组合物可包含本公开内容的一种或多种试剂与一种或多种药学上可接受的无菌等渗水性或非水性溶液、分散液、混悬液或乳液、或可在临用前重构成无菌可注射溶液或分散液的无菌粉末的组合。可注射溶液或分散液可包含抗氧化剂、缓冲剂、抑菌剂、助悬剂、增稠剂或赋予制剂与预期受体的血液等渗的溶质。可用于本公开内容的药物制剂的合适的水性和非水性载体的实例包括水、乙醇、多元醇(例如,甘油、丙二醇、聚乙二醇等)、植物油(例如,橄榄油)、可注射有机酯(例如,油酸乙酯)和其合适的混合物。适当的流动性可例如,通过使用包衣材料(例如,卵磷脂),在分散液的情况下通过维持需要的粒度,和通过使用表面活性剂来维持。

[0496] 在一些实施方案中,本公开内容的治疗方法包括从植入物或装置,系统或局部给予药物组合物。此外,药物组合物可以递送至靶组织部位(例如,骨髓或肌肉)的形式被包封或注射。在某些实施方案中,本公开内容的组合物可包括能够递送本公开内容的一种或多种试剂至靶组织部位(例如,骨髓或肌肉),为发展中的组织提供结构,和最好能够再吸收至体内的基质。例如,基质可提供本公开内容的一种或多种试剂的减慢释放。这样的基质可由目前用于其它植入的医学应用的材料构成。

[0497] 在一些实施方案中,药物组合物将给予至眼,包括例如,通过局部给予、眼内(例如,玻璃体内)注射或通过植入物或装置。玻璃体内注射可通过例如,睫状环(缘后3mm-4mm)注射。给予至眼的药物组合物可以各种方式配制,包括例如,滴眼剂、眼用溶液剂、眼用混悬剂、眼用乳剂、玻璃体内注射剂、sub-Tenon注射剂、眼用生物可侵蚀植入物和非-生物可侵蚀眼用插入物或贮库。

[0498] 基质材料的选择可基于以下一项或多项进行:生物相容性、生物降解能力、机械性质、美容外观和界面性质。本发明的组合物的特定应用将定义合适的制剂。用于组合物的潜在基质可以是生物可降解的和化学上确定的硫酸钙、磷酸三钙、羟基磷灰石、聚乳酸和聚酐。其它潜在的材料是生物可降解的和生物学上充分确定的,包括例如,骨或皮肤胶原。其它基质包括纯的蛋白或细胞外基质组分。其它潜在的基质是非-生物可降解的和化学上确定的,包括例如,烧结的羟基磷灰石、生物玻璃、铝酸盐或其它陶瓷。基质可包含任何上述类型的材料的组合,包括例如,聚乳酸和羟基磷灰石或胶原和磷酸三钙。生物陶瓷可在组分中

改变(例如,钙-铝酸盐-磷酸盐)和经加工以改变孔径、粒度、颗粒形状和生物降解能力的一项或多项。

[0499] 在某些实施方案中,本公开内容的药物组合物可经局部给予。“局部应用”或“局部地”意指药物组合物与身体表面(包括例如,皮肤、伤口部位和粘膜)接触。局部药物组合物可具有各种应用形式,和通常包括含药物层,在局部给予组合物后其适合位于组织附近或与组织直接接触。适合于局部给予的药物组合物可包含组合配制为液体、凝胶、霜剂、洗剂、软膏剂、泡沫、糊剂、擦剂(putty)、半固体或固体的本公开内容的一种或多种TGFβ超家族I型和/或II型受体多肽复合物。呈液体、凝胶、霜剂、洗剂、软膏剂、泡沫、糊剂或擦剂形式的组合物可通过涂覆、喷雾、涂抹、轻拍或滚擦组合物至靶组织上来施用。组合物还可被浸渍至无菌敷料、透皮贴剂、石膏和绷带中。擦剂、半固体或固体形式的组合物可以是可变形的。它们可以是弹性的或非弹性的(例如,柔性或刚性)。在某些方面,组合物形成复合材料的一部分,和可包括纤维、颗粒或具有相同或不同的组分的多个层。

[0500] 呈液体形式的局部组合物可包括药学上可接受的溶液剂、乳剂、微乳剂和混悬剂。除了活性成分外,液体剂量形式可包含本领域常用的惰性稀释剂,包括例如,水或其它溶剂、增溶剂和/或乳化剂[例如,乙醇、异丙醇、碳酸乙酯、乙酸乙酯、苯甲醇、苯甲酸苯甲酯、丙二醇或1,3-丁二醇、油(例如,棉籽油、花生油、玉米油、胚芽油、橄榄油、蓖麻油和芝麻油)、甘油、四氢呋喃甲醇、聚乙二醇、去水山梨糖醇的脂肪酸酯和其混合物]。

[0501] 局部凝胶、霜剂、洗剂、软膏剂、半固体或固体组合物可包括一种或多种增稠剂,例如多糖、合成的聚合物或基于蛋白的聚合物。在本发明的一个实施方案中,在本文中胶凝剂是一种合适地是无毒的和产生需要的粘度的试剂。增稠剂可包括聚合物、共聚物和其单体:乙烯基吡咯烷酮、甲基丙烯酰胺、丙烯酰胺N-乙烯基咪唑、羧基乙烯基、乙烯基酯、乙烯基醚、硅酮、聚环氧乙烷、聚乙二醇、乙烯基醇、丙烯酸钠、丙烯酸酯、马来酸、NN-二甲基丙烯酰胺、双丙酮丙烯酰胺、丙烯酰胺、丙烯酰基吗啉、普卢兰尼克、胶原、聚丙烯酰胺、聚丙烯酸酯、聚乙烯基醇、聚乙烯、聚乙烯基硅酸酯、被糖(例如,蔗糖、葡萄糖、葡糖胺、半乳糖、海藻糖、甘露糖或乳糖)取代的聚丙烯酸酯、酰胺基丙烷磺酸、四甲氧基原硅酸酯、甲基三甲氧基原硅酸酯、四烷氧基原硅酸酯、三烷氧基原硅酸酯、二醇、丙二醇、甘油、多糖、藻酸盐、葡聚糖、环糊精、纤维素、改性纤维素、氧化纤维素、壳聚糖、甲壳质、瓜儿胶、角叉菜胶、透明质酸、菊糖、淀粉、改性淀粉、琼脂糖、甲基纤维素、植物树胶、hylaronans、水凝胶、明胶、氨基葡聚糖、羧基甲基纤维素、羟基乙基纤维素、羟基丙基甲基纤维素、果胶、低-甲氧基果胶、交联葡聚糖、淀粉-丙烯腈接枝共聚物、淀粉钠聚丙烯酸酯、羟基乙基甲基丙烯酸酯、羟基乙基丙烯酸酯、聚乙烯、聚乙基乙烯基醚、聚甲基甲基丙烯酸酯、聚苯乙烯、聚氨酯、聚烷酸酯、聚乳酸、聚乳酸酯、聚(3-羟基丁酸酯)、磺酸化水凝胶、AMPS(2-丙烯酰胺基-2-甲基-1-丙磺酸)、SEM(磺基乙基甲基丙烯酸酯)、SPM(磺基丙基甲基丙烯酸酯)、SPA(磺基丙基丙烯酸酯)、N,N-二甲基-N-甲基丙烯基氧基乙基-N-(3-磺基丙基)铵甜菜碱、甲基丙烯酸酰胺基丙基- 二甲基铵磺基甜菜碱、SPI(衣康酸-双(1-丙基磺酸-3)酯二钾盐)、衣康酸、AMBC(3-丙烯酰胺基-3-甲基丁酸)、β-羧基乙基丙烯酸酯(丙烯酸二聚体)和马来酸酐-甲基乙烯基醚聚合物、其衍生物、其盐、其酸和其组合。在某些实施方案中,本公开内容的药物组合物可口服给予,例如,以胶囊剂、扁囊剂、丸剂、片剂、锭剂(使用矫味基质,例如蔗糖和阿拉伯胶或西黄蓍胶)、粉剂、颗粒剂、在水性或非水性液体中溶液剂或混悬剂、水包油或油包水液体乳

剂、或酏剂或糖浆剂、或软锭剂(使用惰性基质,例如明胶和甘油,或蔗糖和阿拉伯胶)和/或口腔洗剂的形式,各自包含预定量的本公开内容的化合物和任选地一种或多种其它活性成分。本公开内容的化合物和任选地一种或多种其它活性成分还可作为巨丸剂、药糖剂或糊剂给予。

[0502] 在用于口腔给予的固体剂型(例如,胶囊剂、片剂、丸剂、糖锭剂、粉剂和颗粒剂)中,本公开内容的一种或多种化合物可与一种或多种药学上可接受的载体混合,其包括例如,柠檬酸钠、磷酸二钙、填充剂或膨胀剂(例如,淀粉、乳糖、蔗糖、葡萄糖、甘露糖和硅酸)、结合剂(例如,羧基甲基纤维素、藻酸盐、明胶、聚乙烯基吡咯烷酮、蔗糖和阿拉伯胶)、湿润剂(例如,甘油)、崩解剂(例如,琼脂、碳酸钙、马铃薯或木薯淀粉、藻酸、硅酸盐和碳酸钠)、溶解阻滞剂(例如,石蜡)、吸收加速剂(例如,季铵化合物)、浸湿剂(例如,鲸蜡醇和甘油单硬脂酸酯)、吸收剂(例如,高岭土和膨润土)、润滑剂(例如,滑石粉、硬脂酸钙、硬脂酸镁、固体聚乙二醇、月桂基硫酸钠)、着色剂和其混合物。在胶囊剂、片剂和丸剂的情况下,药物制剂(组合物)还可包含缓冲剂。类似类型的固体组合物也可用作软和硬填充明胶胶囊的填充物,使用一种或多种赋形剂包括例如,乳糖(lactose)或乳糖(milk sugar)以及高分子量聚乙二醇。

[0503] 用于口腔给予药物组合物的液体剂型可包括药学上可接受的乳剂、微乳剂、溶液剂、混悬剂、糖浆剂和酏剂。除了活性成分外,液体剂型可包含本领域常用的惰性稀释剂,包括例如,水或其它溶剂、增溶剂和/或乳化剂[例如,乙醇、异丙醇、碳酸乙酯、乙酸乙酯、苯甲醇、苯甲酸苯甲酯、丙二醇或1,3-丁二醇、油(例如,棉籽油、花生油、玉米油、胚芽油、橄榄油、蓖麻油和芝麻油)、甘油、四氢呋喃甲醇、聚乙二醇、去水山梨糖醇的脂肪酸酯和其混合物]。除了惰性稀释剂外,口服制剂还可包括助剂,包括例如,浸湿剂、乳化剂和助悬剂、甜味剂、矫味剂、着色剂、芳香剂、防腐剂和其组合。

[0504] 混悬剂,除了活性化合物外,还可包含助悬剂,包括例如,乙氧基化异硬脂醇、聚氧乙烯山梨醇、去水山梨糖醇酯、微晶纤维素、偏氢氧化铝、膨润土、琼脂、西黄蓍胶和其组合。

[0505] 微生物的作用和/或生长的预防可通过包括各种抗细菌剂和抗真菌剂来确保,其包括例如,对羟基苯甲酸酯、氯丁醇和苯酚山梨酸。

[0506] 在某些实施方案中,组合物中可能需要包括等渗剂,包括例如,糖或氯化钠。此外,可通过包括延迟吸收的试剂包括,例如,单硬脂酸铝和明胶导致可注射药物形式的延长吸收。

[0507] 应理解,剂量方案由主治医生考虑各种因素来确定,所述因素改变本公开内容的一种或多种试剂的作用。在促进红细胞形成的 ALK7:ActRIIB拮抗剂的情况下,各种因素可包括但不限于,患者的红细胞计数、血红蛋白水平、需要的目标红细胞计数、患者的年龄、患者的性别、患者的饮食、可促进红细胞水平降低的任何疾病的严重性、给予次数和其它临床因素。添加其它已知的活性剂至最终组合物中也可影响剂量。可通过定期评估红细胞水平、血红蛋白水平、网织红细胞水平和生血过程的其它指示物中的一项或多项监测进展。

[0508] 在某些实施方案中,本公开内容还提供了用于体内产生本公开内容的一种或多种试剂的基因疗法。这样的疗法通过引入试剂序列至具有上文列出的一种或多种病症的细胞或组织来实现其治疗效果。递送试剂序列可例如,通过使用重组表达载体例如嵌合病毒或胶体分散系统来实现。本公开内容的一种或多种试剂序列的优选的治疗递送使用靶向脂质

体进行。

[0509] 本文教导的可用于基因疗法的各种病毒载体包括腺病毒、疱疹病毒、牛痘或RNA病毒(例如,逆转录病毒)。逆转录病毒载体可以是鼠或禽逆转录病毒的衍生株。其中可插入单一外源基因的逆转录病毒载体的实例包括但不限于:莫洛尼鼠白血病病毒(MoMuLV)、哈维鼠肉瘤病毒(HaMuSV)、鼠乳腺肿瘤病毒(MuMTV)和劳斯肉瘤病毒(RSV)。许多另外的逆转录病毒载体可掺有多种基因。所有这些载体可传递或掺有用于可选择标记的基因,使得转导的细胞可鉴定和产生。通过连接例如,糖、糖脂或蛋白,可使得逆转录病毒载体为靶标特异性的。优选的靶向通过使用抗体实现。本领域技术人员将认识到,特定的多核苷酸序列可插入逆转录病毒基因组或连接至病毒被膜以允许包含本公开内容的一种或多种试剂的逆转录病毒载体的靶标特异性递送。

[0510] 或者,组织培养细胞可通过常规磷酸钙转染,用编码逆转录病毒结构基因(gag、pol和env)的质粒,直接转染。然后这些细胞用包含目的基因的载体质粒转染。得到的细胞释放逆转录病毒载体至培养基。

[0511] 用于本公开内容的一种或多种试剂的另一靶向递送系统是胶体分散系统。胶体分散系统包括例如,大分子复合物、纳米胶囊、微球、珠和基于脂质的系统,包括水包油乳剂、胶束、混合型胶束和脂质体。在某些实施方案中,本公开内容的优选的胶体系统是脂质体。脂质体是人造膜囊,其可体外和体内用作递送载体。RNA、DNA和完整的病毒体可包封在水性内心内和以生物活性形式递送至细胞[Fraley,等 (1981)Trends Biochem.Sci.,6:77]。使用脂质体载体的有效基因传递的方法是本领域已知的[Mannino,等(1988)Biotechniques,6:682,1988]。

[0512] 脂质体组分通常是磷脂的组合,其可包括甾族化合物(例如,胆固醇)。脂质体的物理特征取决于pH、离子强度和二价阳离子的存在。其它磷脂或其它脂质也可使用,包括例如磷脂酰基化合物(例如,磷脂酰基甘油、磷脂酰基胆碱、磷脂酰基丝氨酸、磷脂酰基乙醇胺、鞘脂、脑苷脂和神经节苷脂)、卵磷脂酰基胆碱、二棕榈酰基磷脂酰基胆碱和二硬脂酰基磷脂酰基胆碱。脂质体的靶向还可能基于例如,器官-特异性、细胞-特异性和细胞器-特异性,并且是本领域已知的。

[0513] 通过引用并入

[0514] 本文提及的所有出版物和专利通过引用以其整体结合到本文中,如同每个单独的出版物或专利特别和独立地指示通过引入并入。

[0515] 尽管已经论述了本发明的具体实施方案,但上述说明书是说明性的而非限制性的。在阅读本说明书和随后的权利要求书后,许多变化对本领域技术人员而言将变得显而易见。本发明的整个范围应通过权利要求书以及它们整个范围的等同方案和说明书以及这样的变化来确定。

[0516] 例证

[0517] 本发明已总体上进行了描述,参照以下实施例将更容易理解,实施例仅为说明本发明的某些实施方案的目的包括在内,和不意图限制本发明。

[0518] 实施例1.ActRIIB-Fc:ALK7-Fc异二聚体的产生

[0519] 申请人构建了可溶性ActRIIB-Fc:ALK7-Fc异聚复合物,其包含人 ActRIIB和人ALK7的细胞外结构域,其各自用位于细胞外结构域和 Fc结构域之间的接头融合至Fc结构

域。各个构建体分别被称为ActRIIB-Fc和ALK7-Fc。

[0520] 与ActRIIB-Fc或ALK7-Fc同二聚体复合物相反，促进形成 ActRIIB-Fc:ALK7-Fc异聚复合物的方法是引入Fc结构域的氨基酸序列的改变，以指导不对称的异聚复合物的形成。使用Fc结构域制备不对称的相互作用对的许多不同的方法描述于本公开内容。

[0521] 在一种方法中，以分别在下文公开的ActRIIB-Fc和ALK7-Fc多肽序列举例说明，一个Fc结构域经改变以在相互作用界面处引入阳离子氨基酸，而另一个Fc结构域经改变以在相互作用界面处引入阴离子氨基酸。ActRIIB-Fc融合多肽和ALK7-Fc融合多肽各自使用组织纤溶酶原激活物(TPA)前导序列:MDAMKRGLCCVLLLCGAVFVSP (SEQ ID NO:70)。

[0522] ActRIIB-Fc多肽序列(SEQ ID NO:71)显示如下：

[0523]
```
  1  MDAMKRGLCC VLLLCGAVFV SPGASGRGEA ETRECIYYNA NWELERTNQS
 51  GLERCEGEQD KRLHCYASWR NSSGTIELVK KGCWLDDFNC YDRQECVATE
101  ENPQVYFCCC EGNFCNERFT HLPEAGGPEV TYEPPPTAPT GGGTHTCPPC
151  PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV
201  DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY KCKVSNKALP
251  APIEKTISKA KGQPREPQVY TLPPSRKEMT KNQVSLTCLV KGFYPSDIAV
301  EWESNGQPEN NYKTTPPVLK SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH
351  EALHNHYTQK SLSLSPGK        (SEQ ID NO: 71)
```

[0524] 前导(信号)序列和接头用下划线表示。为了促进形成 ActRIIB-Fc:ALK7-Fc异二聚体而不是任一可能的同二聚体复合物，两个氨基酸置换(用赖氨酸替换酸性氨基酸)可被引入至ActRIIB融合蛋白的Fc结构域，如上文通过双下划线所示的。SEQ ID NO:71的氨基酸序列可任选地以从C-末端除去赖氨酸(K)来提供。

[0525] 该ActRIIB-Fc融合蛋白通过以下核酸序列(SEQ ID NO:72)编码：

[0526]
```
  1  ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
 51  AGTCTTCGTT TCGCCCGGCG CCTCTGGGCG TGGGGAGGCT GAGACACGGG
101  AGTGCATCTA CTACAACGCC AACTGGGAGC TGGAGCGCAC CAACCAGAGC
151  GGCCTGGAGC GCTGCGAAGG CGAGCAGGAC AAGCGGCTGC ACTGCTACGC
201  CTCCTGGCGC AACAGCTCTG GCACCATCGA GCTCGTGAAG AAGGGCTGCT
251  GGCTAGATGA CTTCAACTGC TACGATAGGC AGGAGTGTGT GGCCACTGAG
301  GAGAACCCCC AGGTGTACTT CTGCTGCTGT GAAGGCAACT TCTGCAACGA
```

[0527]

```
 351  GCGCTTCACT CATTTGCCAG AGGCTGGGGG CCCGGAAGTC ACGTACGAGC
 401  CACCCCCGAC AGCCCCCACC GGTGGTGGAA CTCACACATG CCCACCGTGC
 451  CCAGCACCTG AACTCCTGGG GGGACCGTCA GTCTTCCTCT TCCCCCCAAA
 501  ACCCAAGGAC ACCCTCATGA TCTCCCGGAC CCCTGAGGTC ACATGCGTGG
 551  TGGTGGACGT GAGCCACGAA GACCCTGAGG TCAAGTTCAA CTGGTACGTG
 601  GACGGCGTGG AGGTGCATAA TGCCAAGACA AAGCCGCGGG AGGAGCAGTA
 651  CAACAGCACG TACCGTGTGG TCAGCGTCCT CACCGTCCTG CACCAGGACT
 701  GGCTGAATGG CAAGGAGTAC AAGTGCAAGG TCTCCAACAA AGCCCTCCCA
 751  GCCCCCATCG AGAAAACCAT CTCCAAAGCC AAAGGGCAGC CCCGAGAACC
 801  ACAGGTGTAC ACCCTGCCCC CATCCCGGAA GGAGATGACC AAGAACCAGG
 851  TCAGCCTGAC CTGCCTGGTC AAAGGCTTCT ATCCCAGCGA CATCGCCGTG
 901  GAGTGGGAGA GCAATGGGCA GCCGGAGAAC AACTACAAGA CCACGCCTCC
 951  CGTGCTGAAG TCCGACGGCT CCTTCTTCCT CTATAGCAAG CTCACCGTGG
1001  ACAAGAGCAG GTGGCAGCAG GGGAACGTCT TCTCATGCTC CGTGATGCAT
1051  GAGGCTCTGC ACAACCACTA CACGCAGAAG AGCCTCTCCC TGTCTCCGGG
1101  TAAA          (SEQ ID NO: 72)
```

[0528] 成熟的ActRIIB-Fc融合多肽(SEQ ID NO:73)如下和可任选地以从C-末端除去赖氨酸提供。

[0529]

```
  1  GRGEAETREC IYYNANWELE RTNQSGLERC EGEQDKRLHC YASWRNSSGT
 51  IELVKKGCWL DDFNCYDRQE CVATEENPQV YFCCCEGNFC NERFTHLPEA
101  GGPEVTYEPP PTAPTGGGTH TCPPCPAPEL LGGPSVFLFP PKPKDTLMIS
151  RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV HNAKTKPREE QYNSTYRVVS
201  VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQPR EPQVYTLPPS
251  RKEMTKNQVS LTCLVKGFYP SDIAVEWESN GQPENNYKTT PPVLKSDGSF
301  FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLS PGK
    (SEQ ID NO: 73)
```

[0530] 互补形式的ALK7-Fc融合蛋白(SEQ ID NO:74)如下:

[0531]

```
  1  MDAMKRGLCC VLLLCGAVFV SPGAGLKCVC LLCDSSNFTC QTEGACWASV
 51  MLTNGKEQVI KSCVSLPELN AQVFCHSSNN VTKTECCFTD FCNNITLHLP
101  TASPNAPKLG PMETGGGTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR
151  TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV
201  LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSR
251  EEMTKNQVSL TCLVKGFYPS DIAVEWESNG QPENNYDTTP PVLDSDGSFF
301  LYSDLTVDKS RWQQGNVFSC SVMHEALHNH YTQKSLSLSP G
        (SEQ ID NO: 74)
```

[0532] 信号序列和接头序列用下划线表示。为了促进形成 ActRIIB-Fc:ALK7-Fc异二聚体而不是任一可能的同二聚体复合物,两个氨基酸置换(用天冬氨酸替换赖氨酸)可被引入至融合蛋白的Fc结构域,如上文通过双下划线所示的。SEQ ID NO:74的氨基酸序列可任选地以在C-末端添加赖氨酸来提供。

[0533] 该ALK7-Fc融合蛋白通过以下核酸(SEQ ID NO:75)编码:

```
   1  ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
  51  AGTCTTCGTT TCGCCCGGCG CCGGACTGAA GTGTGTATGT CTTTTGTGTG
 101  ATTCTTCAAA CTTTACCTGC CAAACAGAAG GAGCATGTTG GGCATCAGTC
 151  ATGCTAACCA ATGGAAAAGA GCAGGTGATC AAATCCTGTG TCTCCCTTCC
 201  AGAACTGAAT GCTCAAGTCT TCTGTCATAG TTCCAACAAT GTTACCAAAA
 251  CCGAATGCTG CTTCACAGAT TTTTGCAACA ACATAACACT GCACCTTCCA
 301  ACAGCATCAC CAAATGCCCC AAAACTTGGA CCCATGGAGA CCGGTGGTGG
 351  AACTCACACA TGCCCACCGT GCCCAGCACC TGAACTCCTG GGGGGACCGT
 401  CAGTCTTCCT CTTCCCCCCA AAACCCAAGG ACACCCTCAT GATCTCCCGG
 451  ACCCCTGAGG TCACATGCGT GGTGGTGGAC GTGAGCCACG AAGACCCTGA
[0534] 501  GGTCAAGTTC AACTGGTACG TGGACGGCGT GGAGGTGCAT AATGCCAAGA
 551  CAAAGCCGCG GGAGGAGCAG TACAACAGCA CGTACCGTGT GGTCAGCGTC
 601  CTCACCGTCC TGCACCAGGA CTGGCTGAAT GGCAAGGAGT ACAAGTGCAA
 651  GGTCTCCAAC AAAGCCCTCC CAGCCCCCAT CGAGAAAACC ATCTCCAAAG
 701  CCAAAGGGCA GCCCCGAGAA CCACAGGTGT ACACCCTGCC CCCATCCCGG
 751  GAGGAGATGA CCAAGAACCA GGTCAGCCTG ACCTGCCTGG TCAAAGGCTT
 801  CTATCCCAGC GACATCGCCG TGGAGTGGGA GAGCAATGGG CAGCCGGAGA
 851  ACAACTACGA CACCACGCCT CCCGTGCTGG ACTCCGACGG CTCCTTCTTC
 901  CTCTATAGCG ACCTCACCGT GGACAAGAGC AGGTGGCAGC AGGGGAACGT
 951  CTTCTCATGC TCCGTGATGC ATGAGGCTCT GCACAACCAC TACACGCAGA
1001  AGAGCCTCTC CCTGTCTCCG GGT      (SEQ ID NO: 75)
```

[0535] 成熟的ALK7-Fc融合蛋白序列(SEQ ID NO:76)预期如下和可任选地以在C-末端添加赖氨酸来提供。

```
   1  GLKCVCLLCD SSNFTCQTEG ACWASVMLTN GKEQVIKSCV SLPELNAQVF
  51  CHSSNNVTKT ECCFTDFCNN ITLHLPTASP NAPKLGPMET GGGTHTCPPC
 101  PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV
[0536] 151  DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY KCKVSNKALP
 201  APIEKTISKA KGQPREPQVY TLPPSREEMT KNQVSLTCLV KGFYPSDIAV
 251  EWESNGQPEN NYDTTPPVLD SDGSFFLYSD LTVDKSRWQQ GNVFSCSVMH
 301  EALHNHYTQK SLSLSPG       (SEQ ID NO: 76)
```

[0537] 分别为SEQ ID NO:71和SEQ ID NO:74的ActRIIB-Fc和 ALK7-Fc融合蛋白可自CHO细胞系共-表达和纯化,以产生包含 ActRIIB-Fc:ALK7-Fc的异聚复合物。

[0538] 在使用不对称的Fc融合蛋白促进异多聚体复合物形成的另一方法中,Fc结构域经改变以引入互补疏水相互作用和另外的分子间二硫键,如在下文公开的ActRIIB-Fc和ALK7-Fc多肽序列中举例说明的。

[0539] ActRIIB-Fc多肽序列(SEQ ID NO:77)如下显示:

[0540]
```
  1  MDAMKRGLCC VLLLCGAVFV SPGASGRGEA ETRECIYYNA NWELERTNQS
 51  GLERCEGEQD KRLHCYASWR NSSGTIELVK KGCWLDDFNC YDRQECVATE
101  ENPQVYFCCC EGNFCNERFT HLPEAGGPEV TYEPPPTAPT GGGTHTCPPC
151  PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV
201  DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY KCKVSNKALP
251  APIEKTISKA KGQPREPQVY TLPPCREEMT KNQVSLWCLV KGFYPSDIAV
301  EWESNGQPEN NYKTTPPVLD SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH
351  EALHNHYTQK SLSLSPGK        (SEQ ID NO: 77)
```

[0541] 前导序列和接头用下划线表示。为了促进形成 ActRIIB-Fc:ALK7-Fc异二聚体而不是任一可能的同二聚体复合物,两个氨基酸置换(用半胱氨酸替换丝氨酸和用色氨酸替换苏氨酸)可被引入至融合蛋白的Fc结构域,如上文通过双下划线所示的。SEQ ID NO:77的氨基酸序列可任选地以从C-末端除去赖氨酸提供。

[0542] 成熟的ActRIIB-Fc融合多肽(SEQ ID NO:78)如下和可任选地以从C-末端除去赖氨酸提供。

[0543]
```
  1  GRGEAETREC IYYNANWELE RTNQSGLERC EGEQDKRLHC YASWRNSSGT
 51  IELVKKGCWL DDFNCYDRQE CVATEENPQV YFCCCEGNFC NERFTHLPEA
101  GGPEVTYEPP PTAPTGGGTH TCPPCPAPEL LGGPSVFLFP PKPKDTLMIS
151  RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV HNAKTKPREE QYNSTYRVVS
201  VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQPR EPQVYTLPPC
251  REEMTKNQVS LWCLVKGFYP SDIAVEWESN GQPENNYKTT PPVLDSDGSF
301  FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLS PGK
       (SEQ ID NO: 78)
```

[0544] 互补形式的ALK7-Fc融合多肽(SEQ ID NO:79)如下:

[0545]
```
  1  MDAMKRGLCC VLLLCGAVFV SPGAGLKCVC LLCDSSNFTC QTEGACWASV
 51  MLTNGKEQVI KSCVSLPELN AQVFCHSSNN VTKTECCFTD FCNNITLHLP
101  TASPNAPKLG PMETGGGTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR
151  TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV
201  LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVCTLPPSR
251  EEMTKNQVSL SCAVKGFYPS DIAVEWESNG QPENNYKTTP PVLDSDGSFF
301  LVSKLTVDKS RWQQGNVFSC SVMHEALHNH YTQKSLSLSP GK
   (SEQ ID NO: 79)
```

[0546] 前导序列和接头序列用下划线表示。为了指导含上述SEQ ID NOs 76和78的ActRIIB-Fc融合多肽的异二聚体形成,四个氨基酸置换可被引入至ALK7融合多肽的Fc结构域,如上文通过双下划线所示的。SEQ ID NO:79的氨基酸序列可任选地以从C-末端除去赖氨酸提供。

[0547] 成熟的ALK7-Fc融合蛋白序列(SEQ ID NO:80)预期如下和可任选地以从C-末端除去赖氨酸提供。

[0548]

```
              1 GLKCVCLLCD SSNFTCQTEG ACWASVMLTN GKEQVIKSCV
SLPELNAQVF
       51  CHSSNNVTKT ECCFTDFCNN ITLHLPTASP NAPKLGPMET GGGTHTCPPC
      101  PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV
      151  DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY KCKVSNKALP
      201  APIEKTISKA KGQPREPQVC TLPPSREEMT KNQVSLSCAV KGFYPSDIAV
      251  EWESNGQPEN NYKTTPPVLD SDGSFFLVSK LTVDKSRWQQ GNVFSCSVMH
      301  EALHNHYTQK SLSLSPGK      (SEQ ID NO: 80)
```

[0549] 分别为SEQ ID NO:77和SEQ ID NO:79的ActRIIB-Fc和 ALK7-Fc蛋白可自CHO细胞系共-表达和纯化,以产生包含 ActRIIB-Fc:ALK7-Fc的异聚复合物。

[0550] 各种ActRIIB-Fc:ALK7-Fc复合物的纯化可通过一系列柱色谱步骤实现,包括例如,以任何次序的三个或更多个以下步骤:蛋白A色谱、Q sepharose色谱、phenylsepharose色谱、尺寸排阻色谱和阳离子交换色谱。纯化可用病毒过滤和缓冲液交换完成。

[0551] 实施例2.与ActRIIB-Fc同二聚体和ALK7-Fc同二聚体比较，ActRIIB-Fc:ALK7-Fc异二聚体的配体结合特征

[0552] 基于Biacore™-的结合测定法用于比较上述ActRIIB-Fc:ALK7-Fc 异二聚体复合物与ActRIIB-Fc和ALK7-Fc同二聚体复合物的配体结合选择性。使用抗-Fc抗体将ActRIIB-Fc:ALK7-Fc异二聚体、ActRIIB-Fc同二聚体和ALK7-Fc同二聚体独立地捕获到系统上。注入配体和允许流经捕获的受体蛋白。结果在下表中概述,其中表示最有效的配体陷阱的配体离解速率(k_d)通过灰色阴影表示。

[0553]

与 ActRIIB-Fc 同二聚体和 ALK7-Fc 同二聚体比较，ActRIIB-Fc:ALK7-Fc 异二聚体的配体结合特征			
配体	ActRIIB-Fc	ALK7-Fc	ActRIIB-Fc:ALK7-Fc 异二

[0554]

	同二聚体			同二聚体			聚体		
	k_a (1/Ms)	k_d (1/s)	K_D (pM)	k_a (1/Ms)	k_d (1/s)	K_D (pM)	k_a (1/Ms)	k_d (1/s)	K_D (pM)
活化素 A	1.3×10^7	1.4×10^{-4}	11	无结合			4.4×10^7	1.9×10^{-3}	43
活化素 B	1.5×10^7	1.6×10^{-4}	8	无结合			1.2×10^7	2.0×10^{-4}	17
活化素 C	无结合			无结合			3.5×10^5	2.4×10^{-3}	6900
活化素 AC	2.0×10^7	3.1×10^{-3}	160	无结合			2.6×10^6	5.7×10^{-4}	220
BMP5	2.6×10^7	7.5×10^{-2}	2900	无结合			1.5×10^5	8.5×10^{-3}	57000
BMP6	2.4×10^7	3.9×10^{-3}	160	无结合			1.2×10^6	6.3×10^{-3}	5300
BMP9	1.2×10^8	1.2×10^{-3}	10	无结合			短暂*		>1400
BMP10	5.9×10^6	1.5×10^{-4}	25	无结合			1.5×10^7	2.8×10^{-3}	190
GDF3	1.4×10^6	2.2×10^{-3}	1500	无结合			2.3×10^6	1.0×10^{-2}	4500
GDF8	3.5×10^6	2.4×10^{-4}	69	无结合			3.7×10^6	1.0×10^{-3}	270
GDF11	9.6×10^7	1.5×10^{-4}	2	无结合			9.5×10^7	7.5×10^{-4}	8
* 由于相互作用的短暂性质而不确定 --- 未测试									

[0555] 这些比较性结合数据证实，相对于ActRIIB-Fc同二聚体或 ALK7-Fc同二聚体，ActRIIB-Fc:ALK7-Fc异二聚体具有改变的结合特征/选择性。引人注意的是，具有与ActRIIB-Fc同二聚体最强结合的5 种配体中的4种(活化素A、BMP10、GDF8和GDF11)显示与ActRIIB-Fc:ALK7-Fc异二聚体的结合减少，除了活化素B，其保留与异二聚体紧密结合。类似地，具有与ActRIIB-Fc同二聚体中等结合的 4种配体中的3种(GDF3、BMP6和特别是BMP9)显示与ActRIIB-Fc:ALK7-Fc异二聚体结合减少，而与活化素AC结合增加，总体上成为与异二聚体的第二强配体相互作用。最后，活化素C和 BMP5出人意料地以中等强度结合ActRIIB-Fc:ALK7异二聚体，但与 ActRIIB-Fc同二聚体无结合(活化素C)或弱结合(BMP5)。净结果是 ActRIIB-Fc:ALK7-Fc异二聚体具有明显不同于不结合任何前述配体的ActRIIB-Fc同二聚体或ALK7-Fc同二聚体的配体-结合特征。参见图6。

[0556] 这些结果因此证实,与ActRIIB-Fc同二聚体比较, ActRIIB-Fc:ALK7-Fc异二聚体是活化素B和活化素AC的更有选择性的拮抗剂。此外,ActRIIB-Fc:ALK7-Fc异二聚体显示稳健结合活化素 C的不寻常的性质。因此,在其中这样的选择性拮抗作用是有利的某些应用中,ActRIIB-Fc:ALK7-Fc异二聚体比ActRIIB-Fc同二聚体更有用。实例包括治疗应用,其中需要保留活化素B或活化素AC的拮抗作用,但减少活化素A、GDF3、GDF8、GDF11、BMP9或BMP10的一种或多种的拮抗作用。还包括治疗、诊断或分析应用,其中需要拮抗活化素C,或基于活化素C和活化素E之间的相似性,拮抗活化素E。

[0557] 实施例3.ALK7:ActRIIB异多聚体治疗抑制肾纤维化和炎症和减少肾损伤

[0558] 描述于实施例2的ALK7-Fc:ActRIIB-Fc异二聚体对肾疾病的作用在小鼠单侧输尿管梗阻模型中进行评价。参见例如,Klahr和Morrissey (2002)Am J Physiol Renal Physiol 283:F861-F875。

[0559] 24只12周龄的C57BL/6雄性小鼠在肾的低极的水平上经过左单侧输尿管结扎两次。3天后,8只小鼠被安乐死,和收获各个动物的肾以评价肾损伤。余下的小鼠被随机分为两组:i)在手术后第3天、第7天、第10天和第14天向8只小鼠皮下注射10mg/kg剂量的ALK7-Fc:ActRIIB-Fc异二聚体,和ii)在手术后第3天、第7天、第 10天和第14天向8只小鼠皮下注射溶媒对照磷酸盐缓冲盐水(PBS)。在第17天根据相关的动物管理指南处死两组动物。收集各个动物的半肾用于组织学分析(H&E和马松三色染色),来自UUO肾和对侧肾二者,和1/4肾用于RNA提取(RNeasy Midi Kit,Qiagen,IL)。

[0560] 对UUO肾样品进行基因表达分析以评价各种基因的水平。QRT-PCR在CFX Connect™ Real-time PCR检测系统(Bio-Rad,CA) 上进行,以评价各种纤维化基因(Col1a1、Col3a1、纤连蛋白、PAI-1、 CTGF和a-SMA)、炎性基因(Tnfa和MCP1)、细胞因子(TGFβ1、TGFβ2、 TGFβ3和活化素A)、肾损伤基因(NGAL)、缺氧-可诱导因子1-α(HIF1a) 和活化素A受体(ActRIIA)的表达。参见图13。用ALK7-Fc:ActRIIB-Fc 异二聚体治疗小鼠显著抑制纤维化和炎性基因的表达,抑制TGFβ 1/2/3、活化素A和ActRIIa的上调和减少肾损伤。组织学数据证实 ALK7-Fc:ActRIIB-Fc异二聚体治疗在UUO模型中显著抑制肾纤维化和减少肾损伤。

[0561] 总之,这些数据证实ALK7:ActRIIB异多聚体治疗抑制肾纤维化和炎症和减少肾损伤。此外,这些数据表明其它ALK7:ActRIIB拮抗剂可用于治疗或预防肾疾病,包括例如,ALK7和/或ActRIIB-结合配体的拮抗剂(例如,配体抗体和其它配体陷阱,例如卵泡抑素、Cerberus 和Lefty)、ALK7和/或ActRIIB受体的拮抗剂、ALK7和/或ActRIIB 下游信号传导介质(例如,Smads)的拮抗剂和TGFβ超家族共受体的拮抗剂(例如,Crypto或Cryptic的拮抗剂)。

[0562] 实施例4.ALK7:ActRIIB异多聚体增加脂肪细胞中的脂解作用。

[0563] 脂解作用是细胞内的甘油三酯水解成甘油和游离脂肪酸。甘油和游离脂肪酸然后释放至血流或培养基。尽管脂解作用发生在基本上所有的细胞中,但在白色和褐色脂肪细胞中最丰富。ALK7信号传导被认为抑制脂解作用,因此导致在脂肪细胞和脂肪组织中脂肪累积。因此,评价了实施例2中描述的ALK7-Fc:ActRIIB-Fc异二聚体对脂肪细胞中的脂解作用的影响。

[0564] 特别地,3T3-L1细胞(由ATCC提供;ATCC® CL-173™)在包含 10%牛血清(Life

Technologies;16170-060)的Dulbecco改良Eagle培养基(ATCC;ATCC®30-2002™)中生长,直到达到汇合。为了诱导分化,在汇合后2天,培养基周包含10％胎牛血清(Life Technologies; 10082147)、地塞米松(Sigma,D8893)、IBMX(Sigma,I7018)和胰岛素(Sigma,I0516)的新鲜的Dulbecco改良Eagle培养基(ATCC;ATCC®30-2002™)替换,达2周。在细胞上脂质小滴的累积,如通过显微术检测的,用于证实完全分化成成熟的脂肪细胞。脂肪细胞用溶媒(PBS)、活化素B(50ng/ml)处理过夜,或用活化素B(50ng/ml)和 ALK7-Fc:ActRIIB-Fc异二聚体(5μg/ml)共处理。细胞用PBS洗涤两次和用脂解测定缓冲液(supplied by Abcam;ab185433)孵育。脂解测定缓冲液在3小时后收集,和甘油水平根据制造商的说明书(Abcam; ab185433)测量。确定在该基于细胞的测定法中ALK7-Fc:ActRIIB-Fc 异二聚体显著增加脂解活性达44.36％。

[0565] 因此,这些数据证实ALK7-Fc:ActRIIB-Fc异二聚体可用于拮抗 ALK7-介导的脂解抑制,从而增加脂肪细胞中的脂肪酸分解。此外,这些数据表明ALK7-Fc:ActRIIB-Fc异二聚体以及其它ALK7:ActRIIB 拮抗剂、例如本文公开的那些,可用于治疗与细胞、特别是脂肪细胞中的低脂解活性和/或过度脂肪酸累积有关的各种病症或病况,包括例如,肥胖、糖尿病、胰岛素抵抗;代谢综合征、脂肪性肝病和其它代谢疾病或病况,特别是与过量的脂肪水平有关的那些。

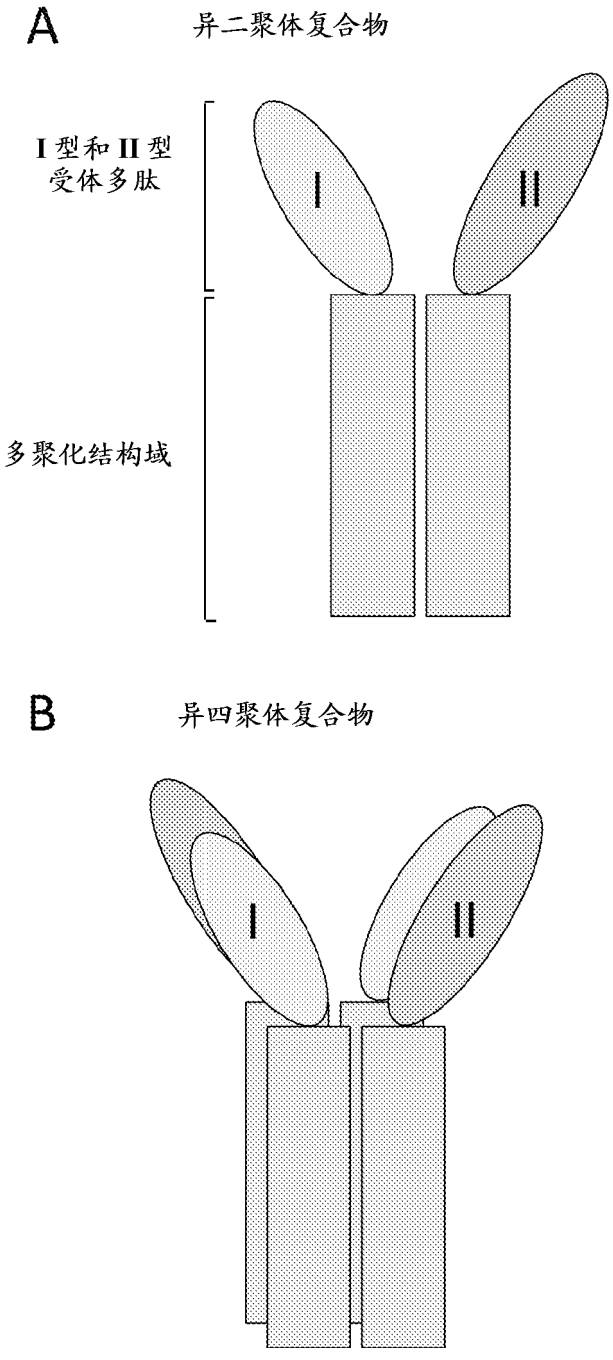


图 1

示意性的异聚蛋白复合物

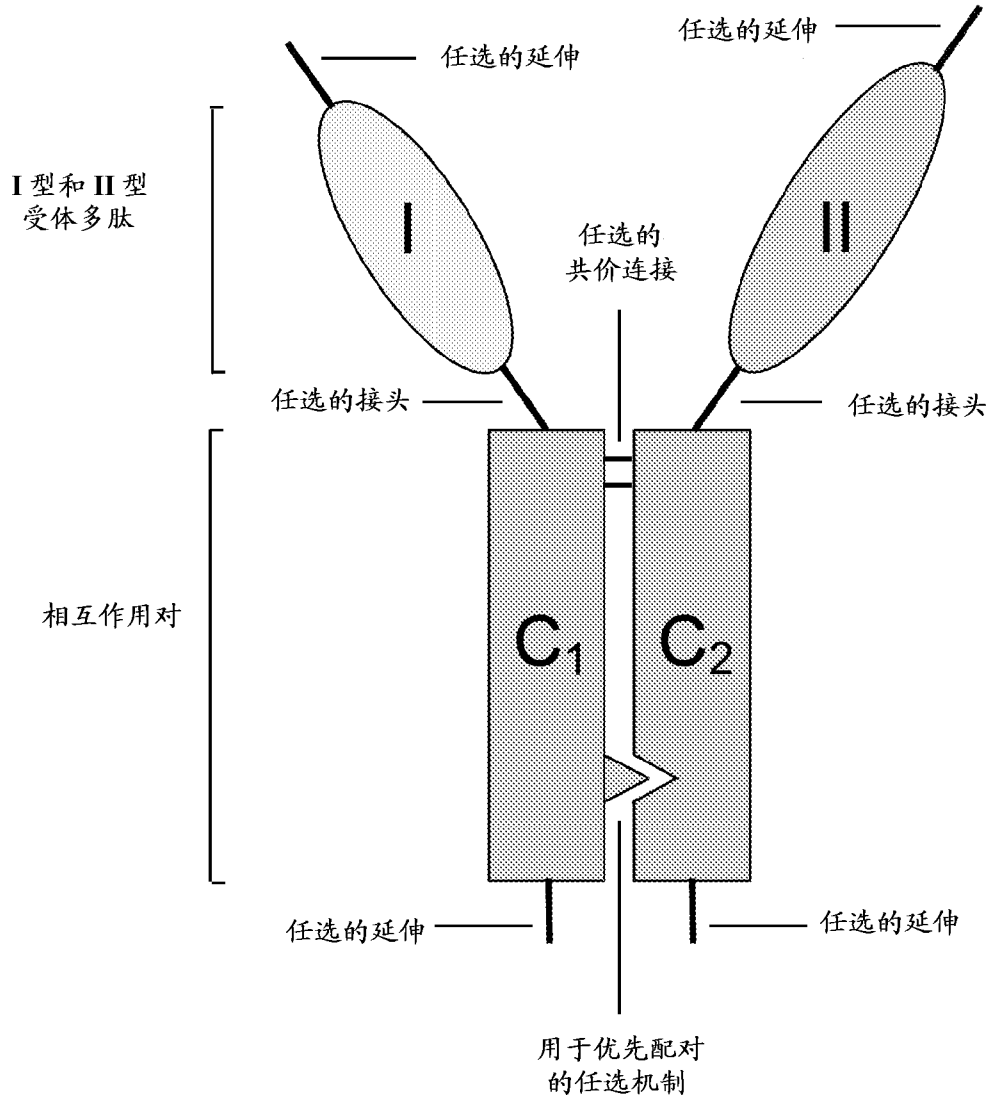


图 2

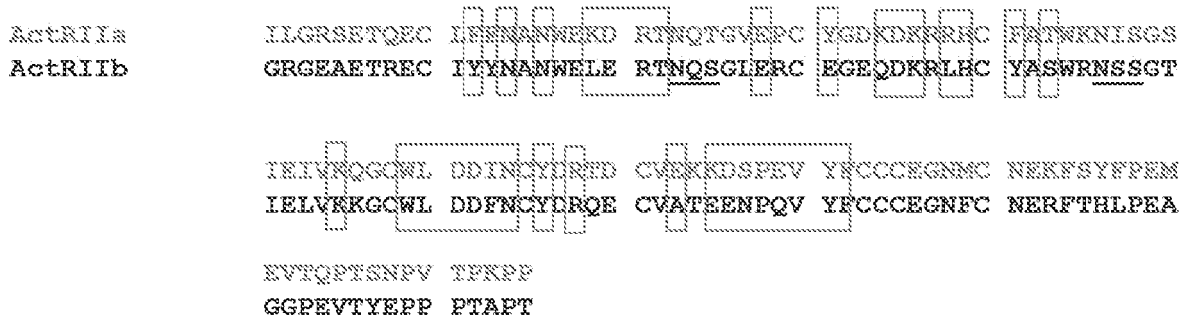


图 3

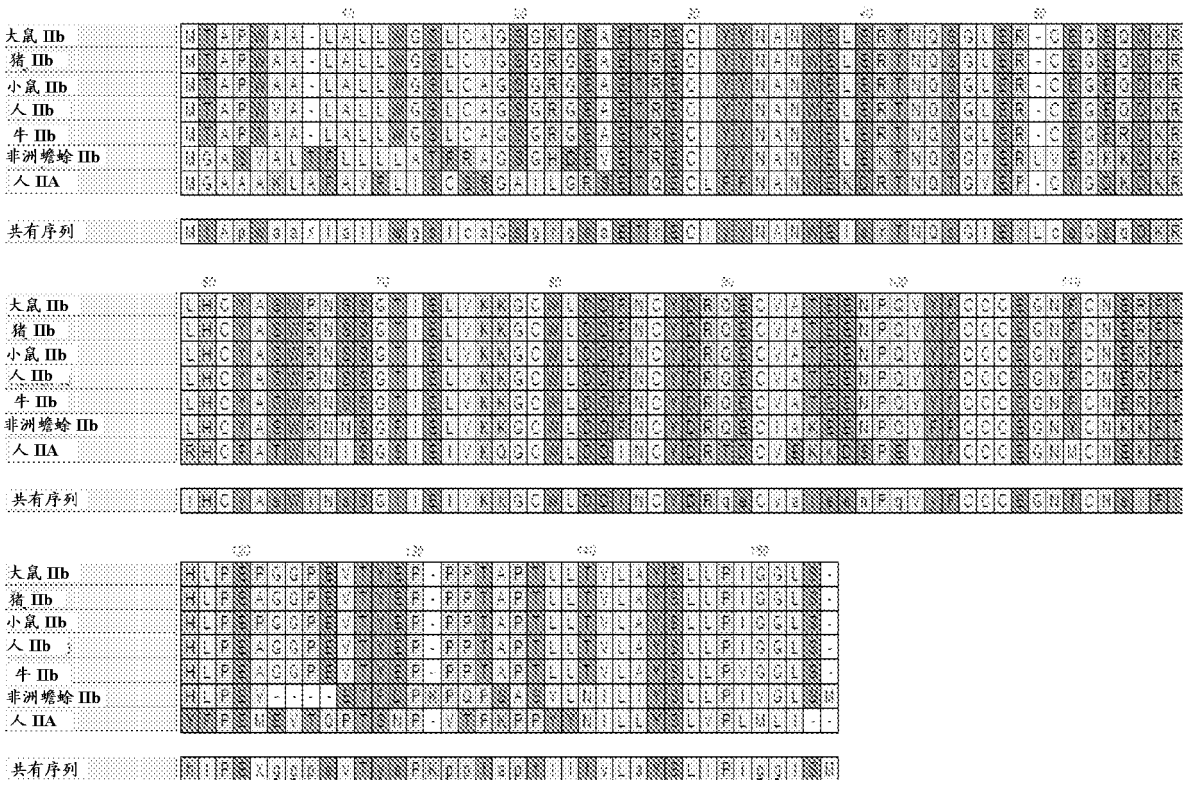


图 4

```
IgG1    -------THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF 53
IgG4    ---ESKYGPPCPSCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQF 57
IgG2    ---------VECPPCPAPPVAG-PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQF 51
IgG3    EPKSCDTPPPCPRCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQF 60
                  ** **** . * ********************************:*****:*

IgG1    NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT 113
IgG4    NWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKT 117
IgG2    NWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKT 111
IgG3    KWYVDGVEVHNAKTKPREEQYNSTFRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT 120
        :*********************:***:********:******************.**:.****

IgG1    ISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP 173
IgG4    ISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP 177
IgG2    ISKTKGQPPEPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP 171
IgG3    ISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTP 180
        ***:****************:*****************************.*******:***

IgG1    PVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK 225
IgG4    PVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK 229
IgG2    PMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK 223
IgG3    PMLDSDGSFFLYSKLTVDKSRWQQGNIFSCSVMHEALHNRFTQKSLSLSPGK 232
        *:***********:*********:**:*************::******** **
```

图 5

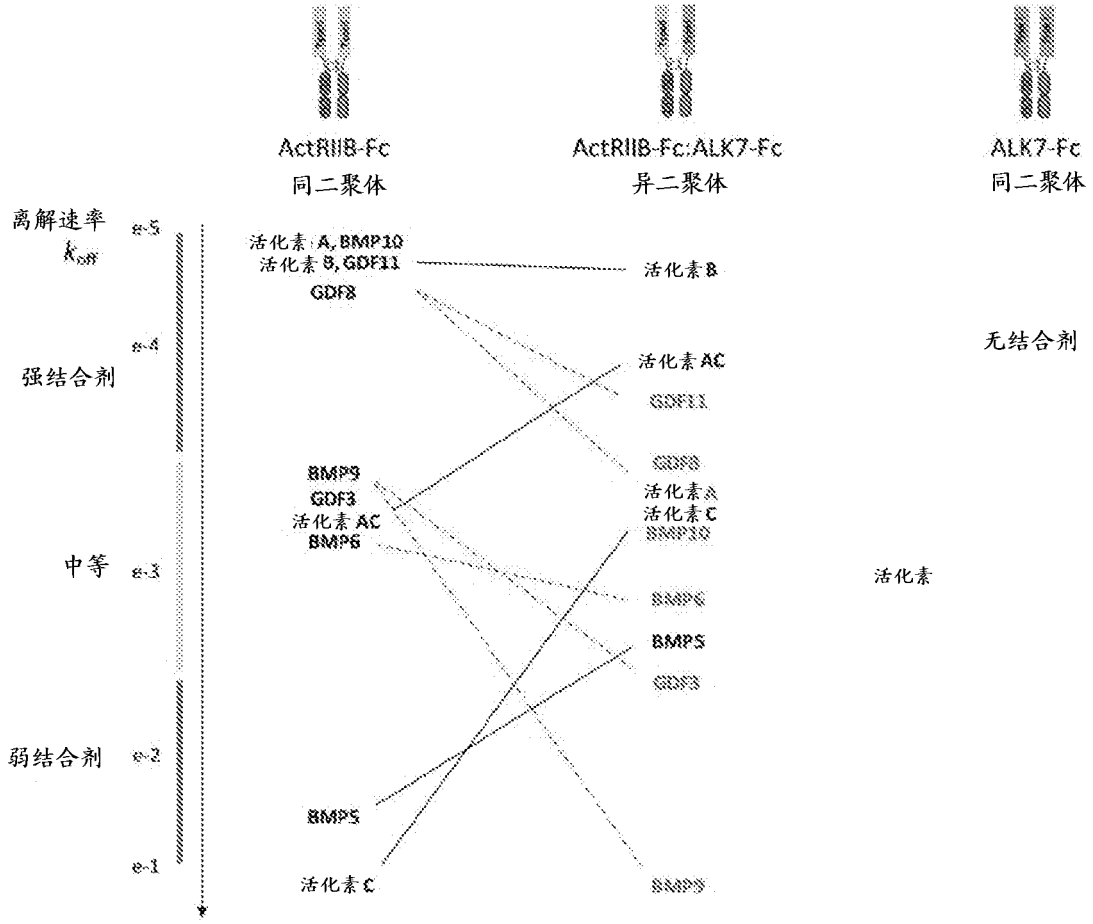

ActRIIB-Fc
同二聚体
ActRIIB-Fc:ALK7-Fc
异二聚体
ALK7-Fc
同二聚体
离解速率
k_{off}
e-5
e-4
e-3
e-2
e-1
强结合剂
中等
弱结合剂
活化素A,BMP10
活化素B,GDF11
GDF8
BMP9
GDF3
活化素AC
BMP6
BMP5
活化素C
活化素B
活化素AC
GDF11
GDF8
活化素A
活化素C
BMP10
BMP6
BMP5
GDF3
BMP9
无结合剂
活化素

图 6

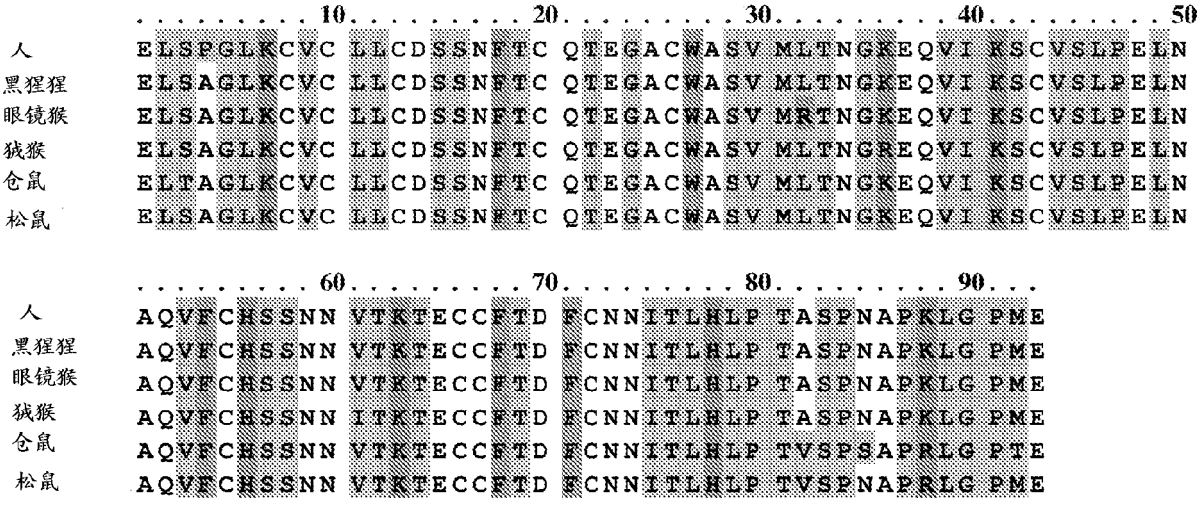

10 20 30 40 50
人 ELSPGLKCVC LLCDSSNFTC QTEGACWASV MLTNGKEQVI KSCVSLPELN
黑猩猩 ELSAGLKCVC LLCDSSNFTC QTEGACWASV MLTNGKEQVI KSCVSLPELN
眼镜猴 ELSAGLKCVC LLCDSSNFTC QTEGACWASV MRTNGKEQVI KSCVSLPELN
狨猴 ELSAGLKCVC LLCDSSNFTC QTEGACWASV MLTNGREQVI KSCVSLPELN
仓鼠 ELTAGLKCVC LLCDSSNFTC QTEGACWASV MLTNGKEQVI KSCVSLPELN
松鼠 ELSAGLKCVC LLCDSSNFTC QTEGACWASV MLTNGKEQVI KSCVSLPELN
60 70 80 90
人 AQVFCHSSNN VTKTECCFTD FCNNITLHLP TASPNAPKLG PME
黑猩猩 AQVFCHSSNN VTKTECCFTD FCNNITLHLP TASPNAPKLG PME
眼镜猴 AQVFCHSSNN VTKTECCFTD FCNNITLHLP TASPNAPKLG PME
狨猴 AQVFCHSSNN ITKTECCFTD FCNNITLHLP TASPNAPKLG PME
仓鼠 AQVFCHSSNN VTKTECCFTD FCNNITLHLP TVSPSAPRLG PTE
松鼠 AQVFCHSSNN VTKTECCFTD FCNNITLHLP TVSPNAPRLG PME

图 7

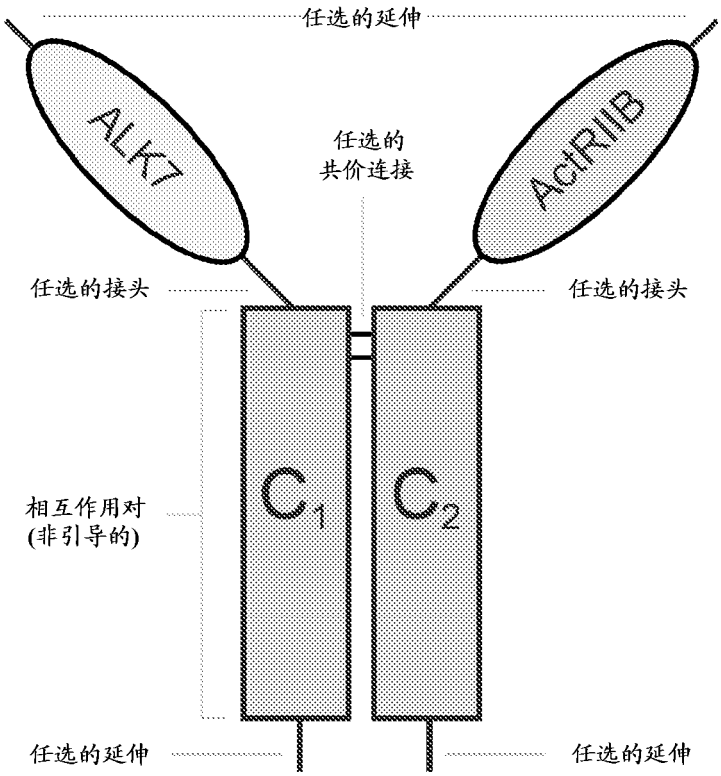

任选的延伸
ALK7
ActRIIB
任选的
共价连接
任选的接头
任选的接头
相互作用对
(非引导的)
C1
C2
任选的延伸
任选的延伸

图 8A

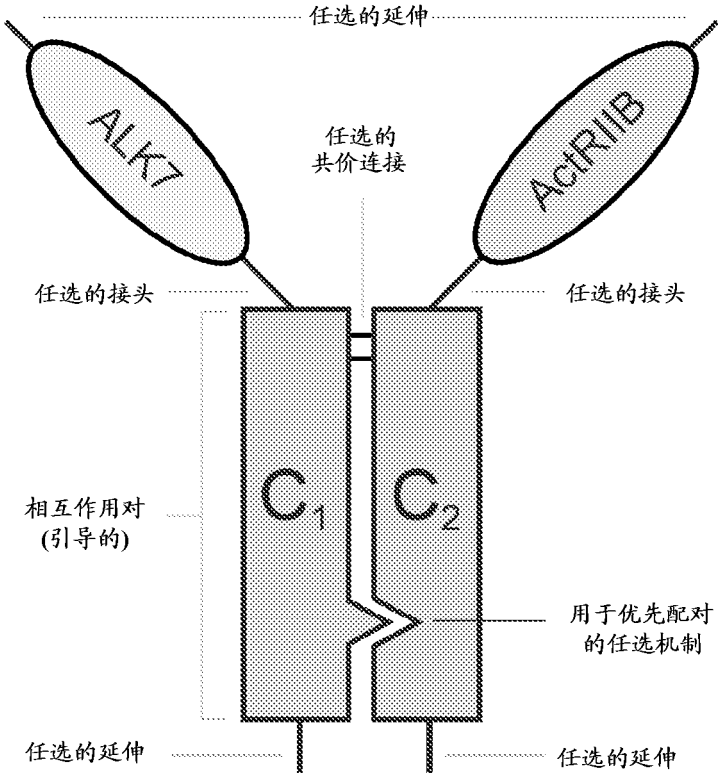

任选的延伸
ALK7
ActRIIB
任选的
共价连接
任选的接头
任选的接头
相互作用对
(引导的)
C1
C2
用于优先配对
的任选机制
任选的延伸
任选的延伸

图 8B

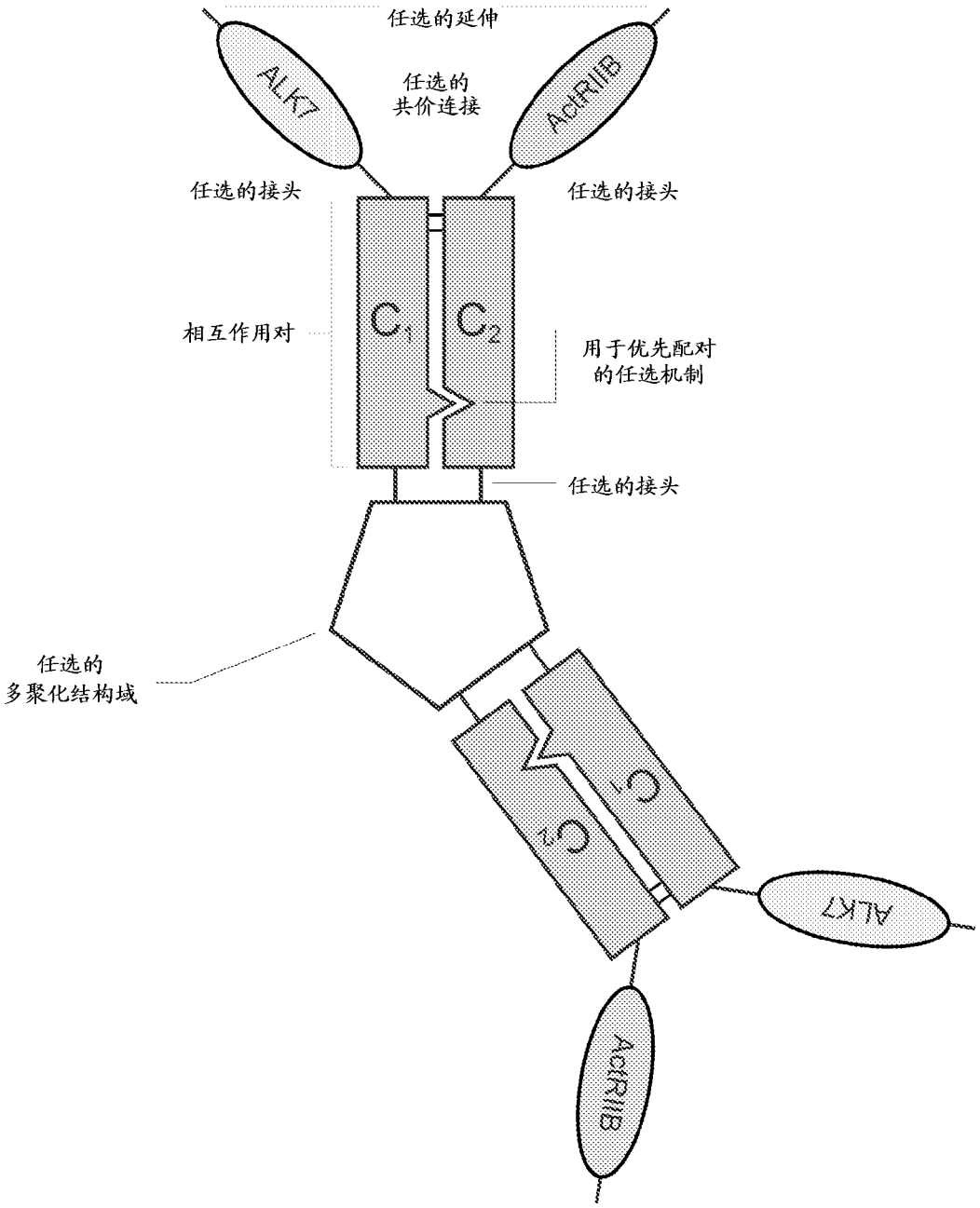

任选的延伸
ALK7
任选的
共价连接
ActRIIB
任选的接头
任选的接头
相互作用对
C1
C2
用于优先配对
的任选机制
任选的接头
任选的
多聚化结构域
C1
C2
ALK7
ActRIIB

图 8C

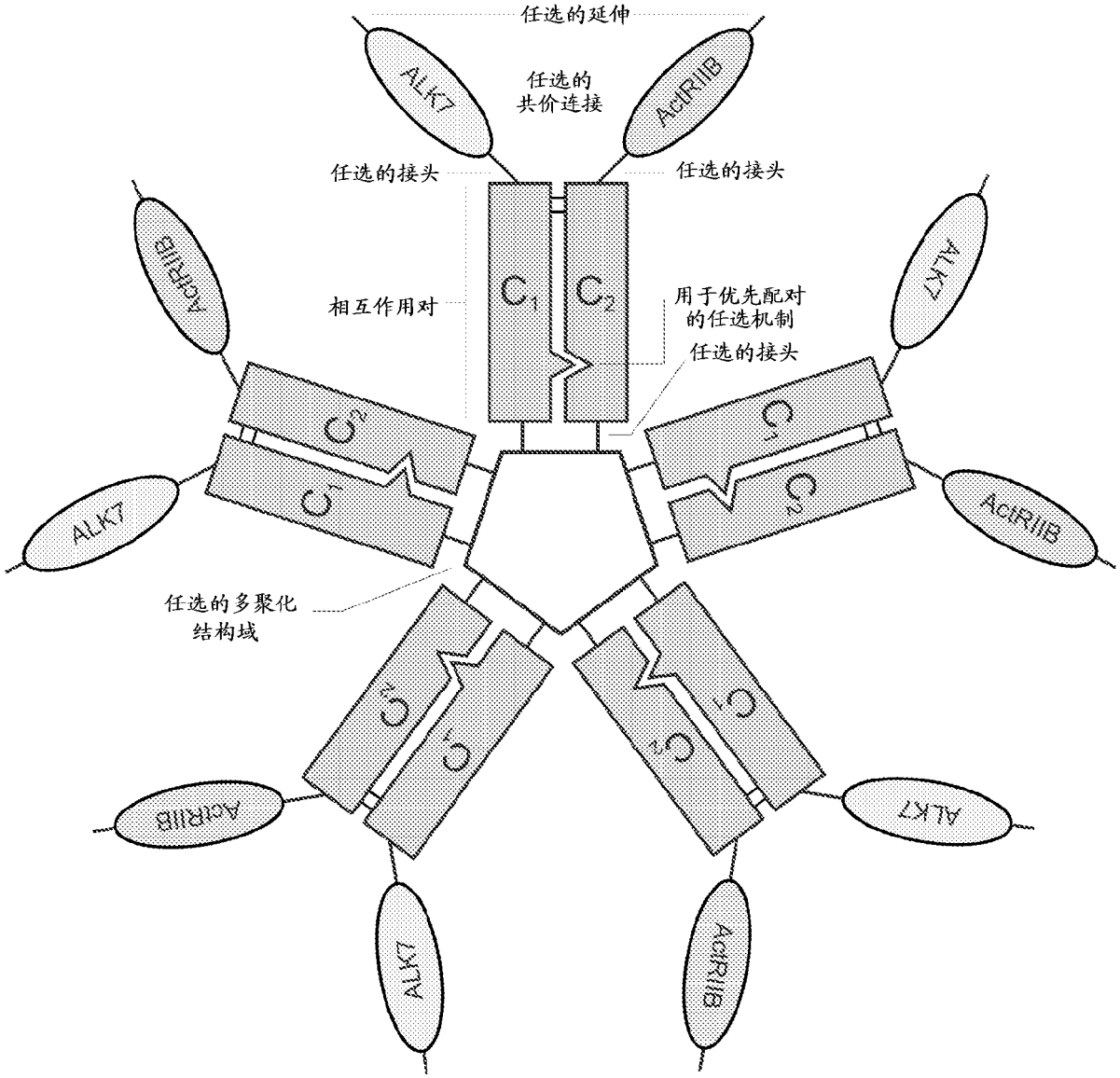

任选的延伸
ALK7
任选的
共价连接
ActRIIB
任选的接头
任选的接头
ACVR1B
相互作用对
C1
C2
用于优先配对
的任选机制
任选的接头
ALK7
C3'
C1'
C3
C1
ALK7
ActRIIB
任选的多聚化
结构域
C3''
C1''
C2''
C2
ACVR1B
ALK7
ALK7
ActRIIB

图 8D

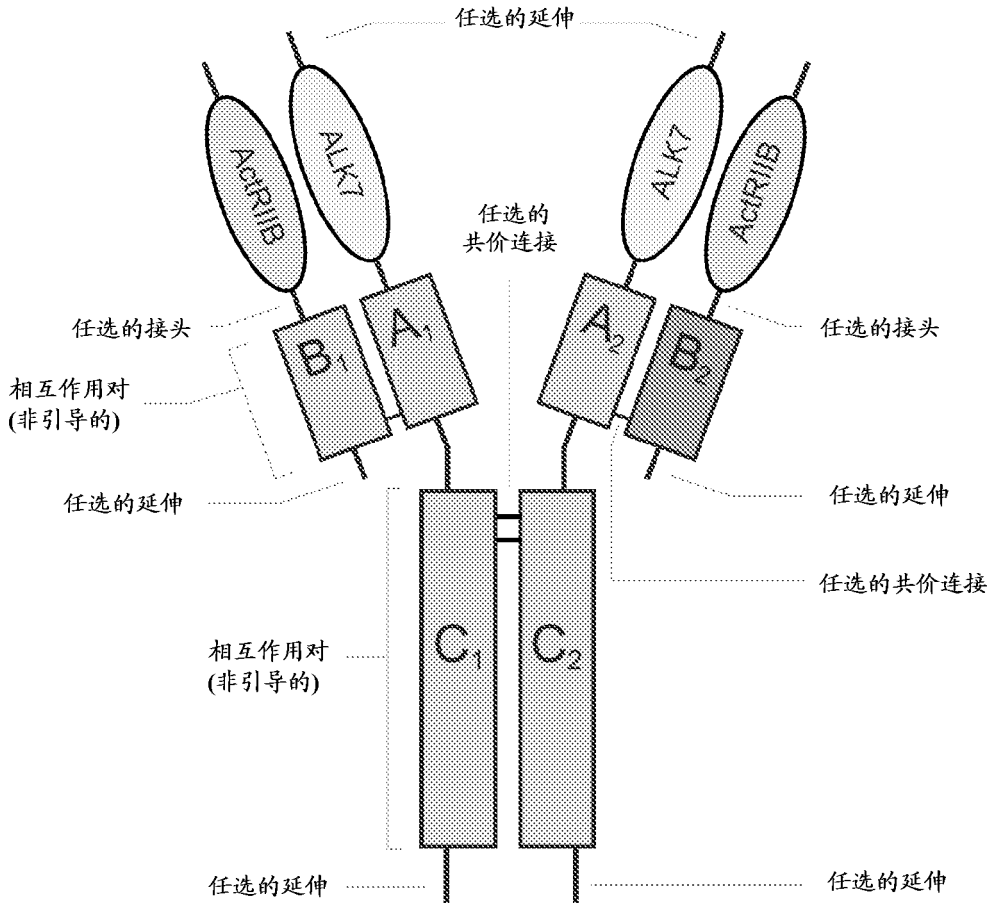

任选的延伸
ACVR1B
ALK7
任选的
共价连接
ALK7
ACVR1B
任选的接头
相互作用对
(非引导的)
任选的延伸
任选的接头
任选的延伸
任选的共价连接
相互作用对
(非引导的)
任选的延伸
任选的延伸

图 9A

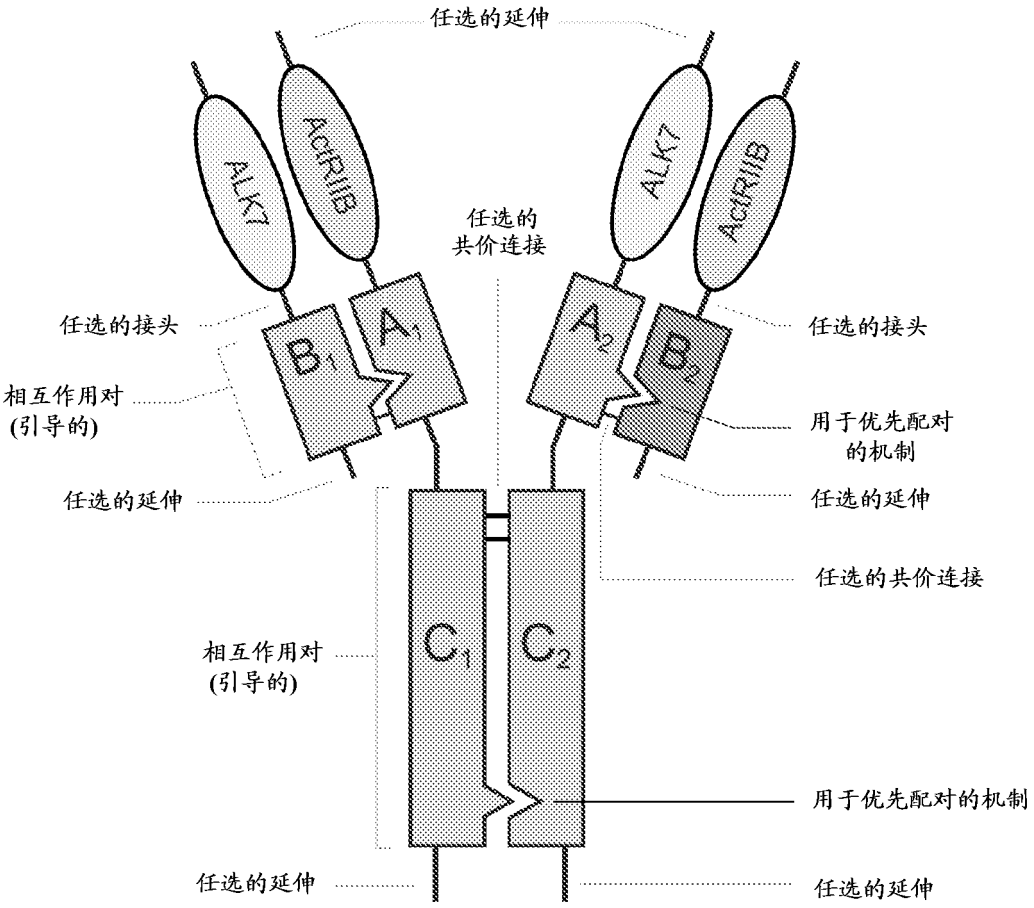

任选的延伸
ALK7
ActRIIB
任选的
共价连接
ALK7
ActRIIB
任选的接头
相互作用对
(引导的)
任选的延伸
任选的接头
用于优先配对
的机制
任选的延伸
任选的共价连接
相互作用对
(引导的)
用于优先配对的机制
任选的延伸
任选的延伸

图 9B

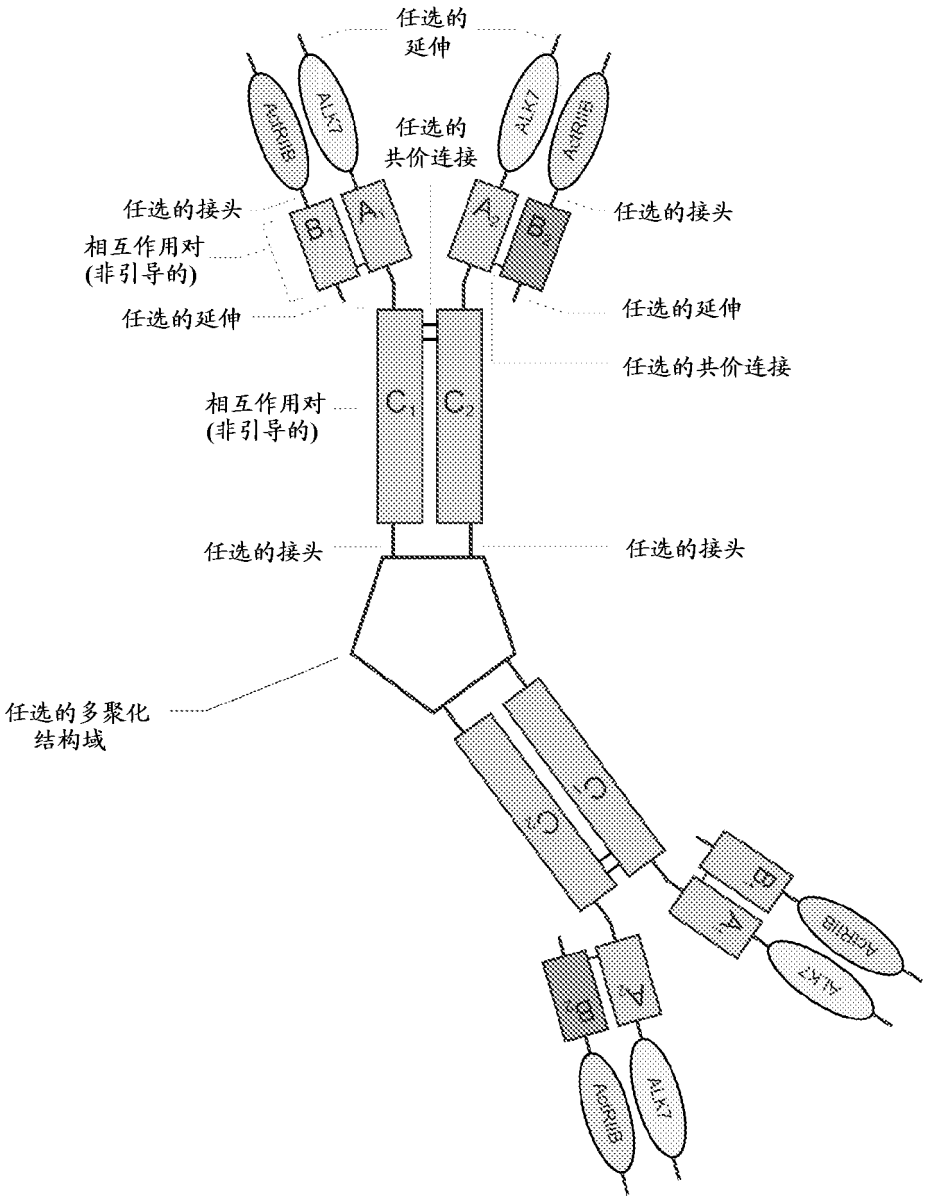

任选的
延伸
任选的
共价连接
任选的接头
相互作用对
(非引导的)
任选的延伸
任选的接头
任选的延伸
任选的共价连接
相互作用对
(非引导的)
任选的接头
任选的接头
任选的多聚化
结构域

图 9C

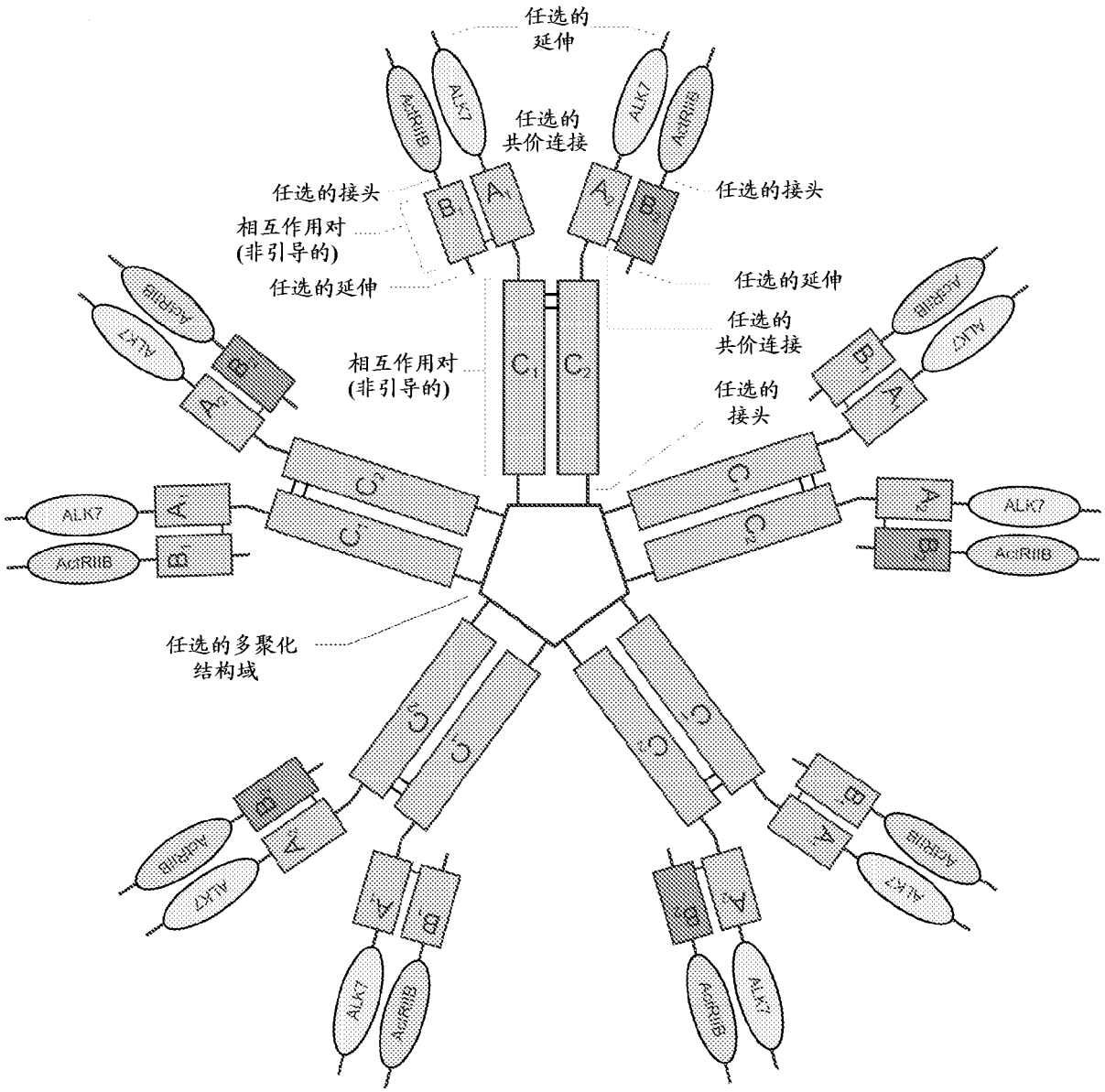

任选的
延伸
ALK7
ActRIIB
任选的
共价连接
任选的接头
相互作用对
(非引导的)
任选的延伸
任选的接头
任选的延伸
任选的
共价连接
相互作用对
(非引导的)
任选的
接头
任选的多聚化
结构域

图 9D

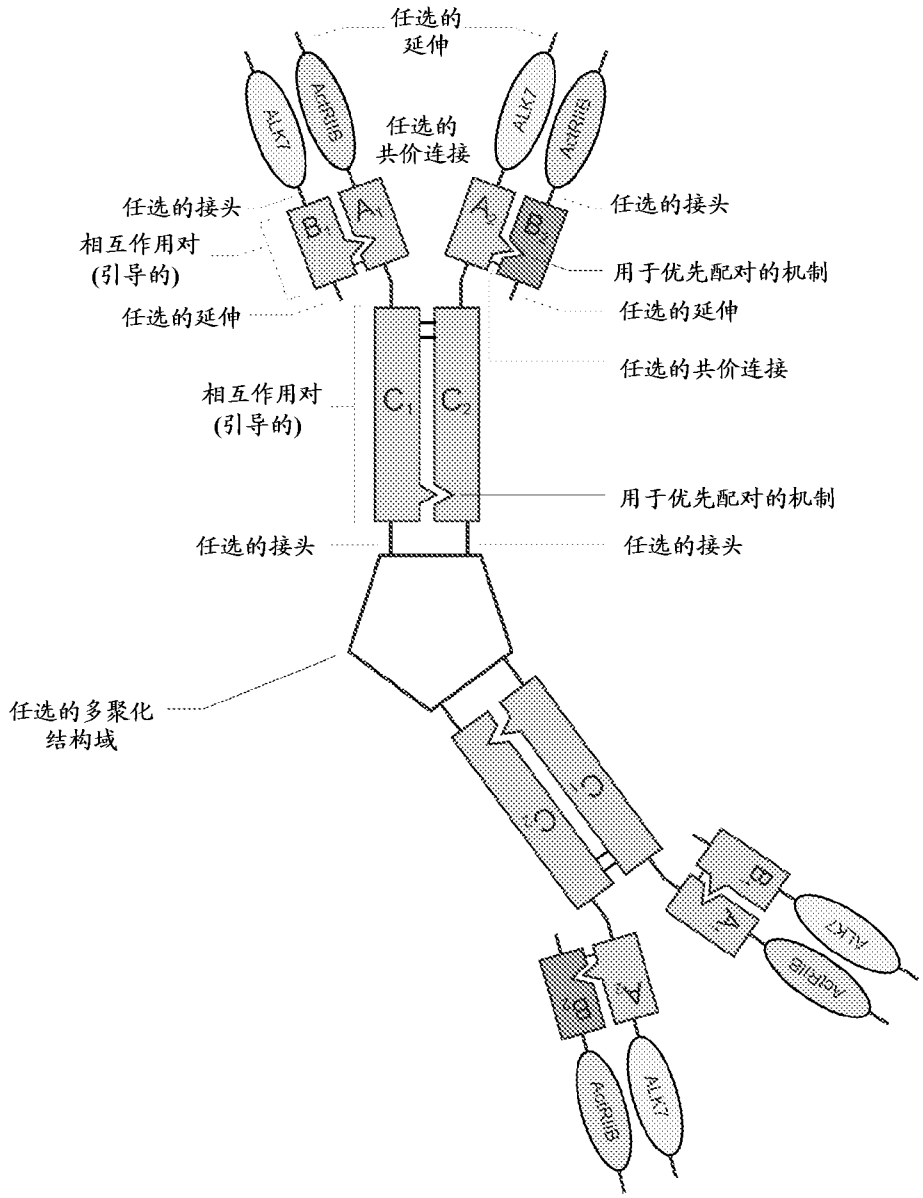

任选的
延伸
ALK7
ACVR1B
任选的
共价连接
ALK7
ACVR1B
任选的接头
相互作用对
(引导的)
任选的延伸
任选的接头
用于优先配对的机制
任选的延伸
任选的共价连接
相互作用对
(引导的)
用于优先配对的机制
任选的接头
任选的接头
任选的多聚化
结构域

图 9E

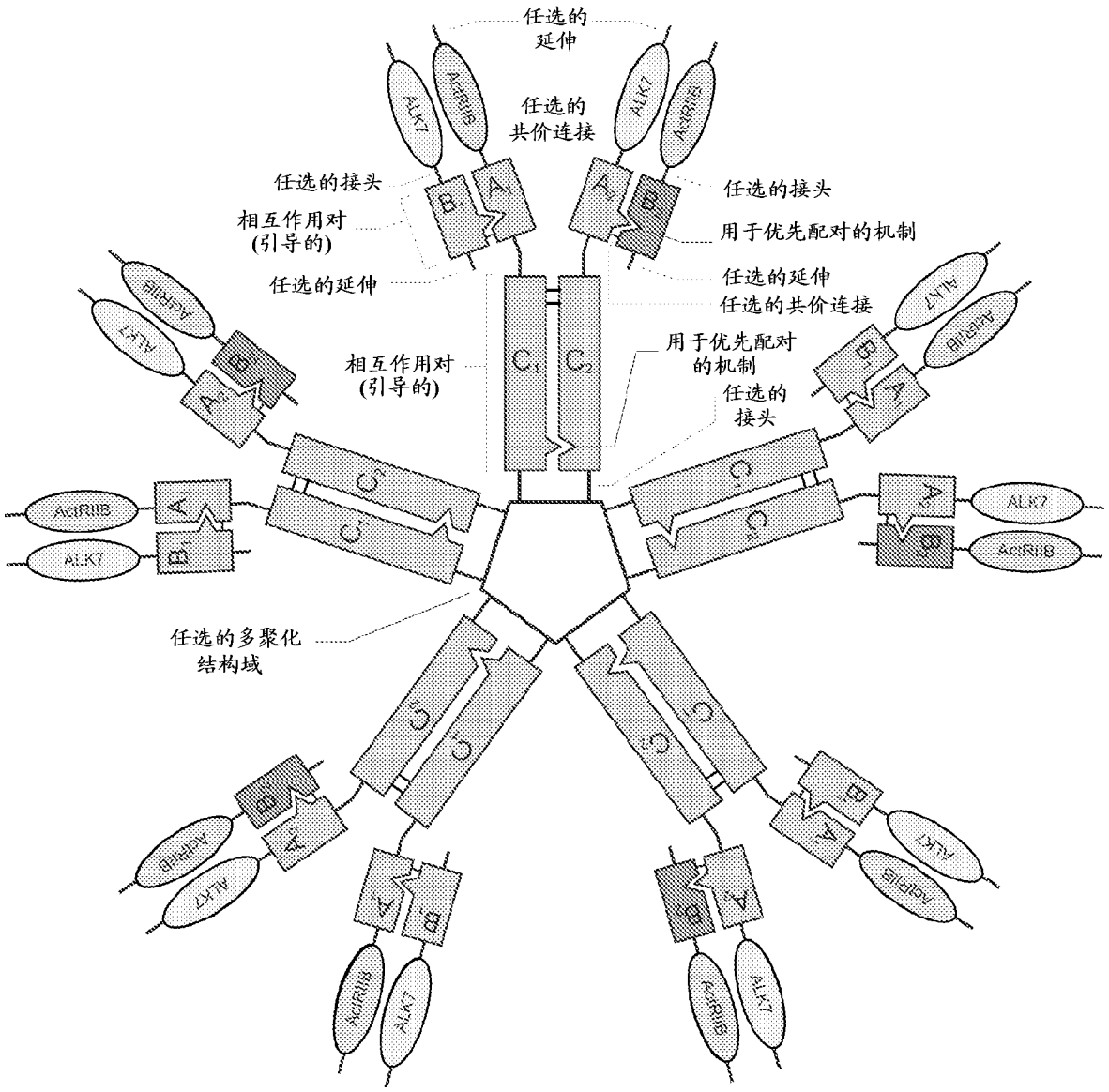

任选的
延伸
任选的
共价连接
任选的接头
相互作用对
(引导的)
任选的延伸
任选的接头
用于优先配对的机制
任选的延伸
任选的共价连接
用于优先配对
的机制
任选的
接头
相互作用对
(引导的)
任选的多聚化
结构域
ALK7
ActRIIB

图 9F

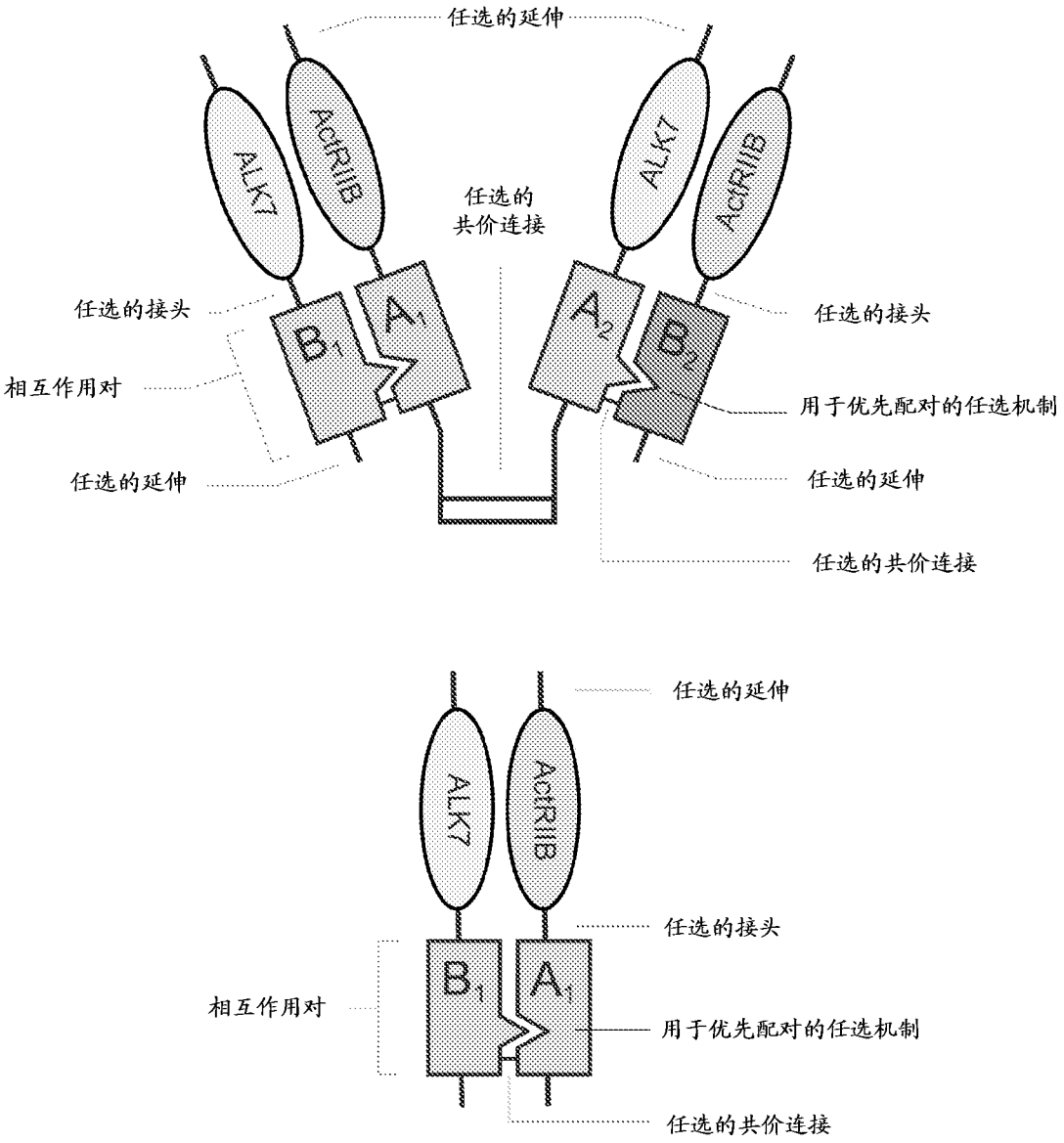

任选的延伸
ALK7
ActRIIB
任选的
共价连接
ALK7
ActRIIB
任选的接头
相互作用对
任选的延伸
用于优先配对的任选机制
任选的延伸
任选的共价连接
任选的延伸
ALK7
ActRIIB
任选的接头
相互作用对
用于优先配对的任选机制
任选的共价连接

图 9G

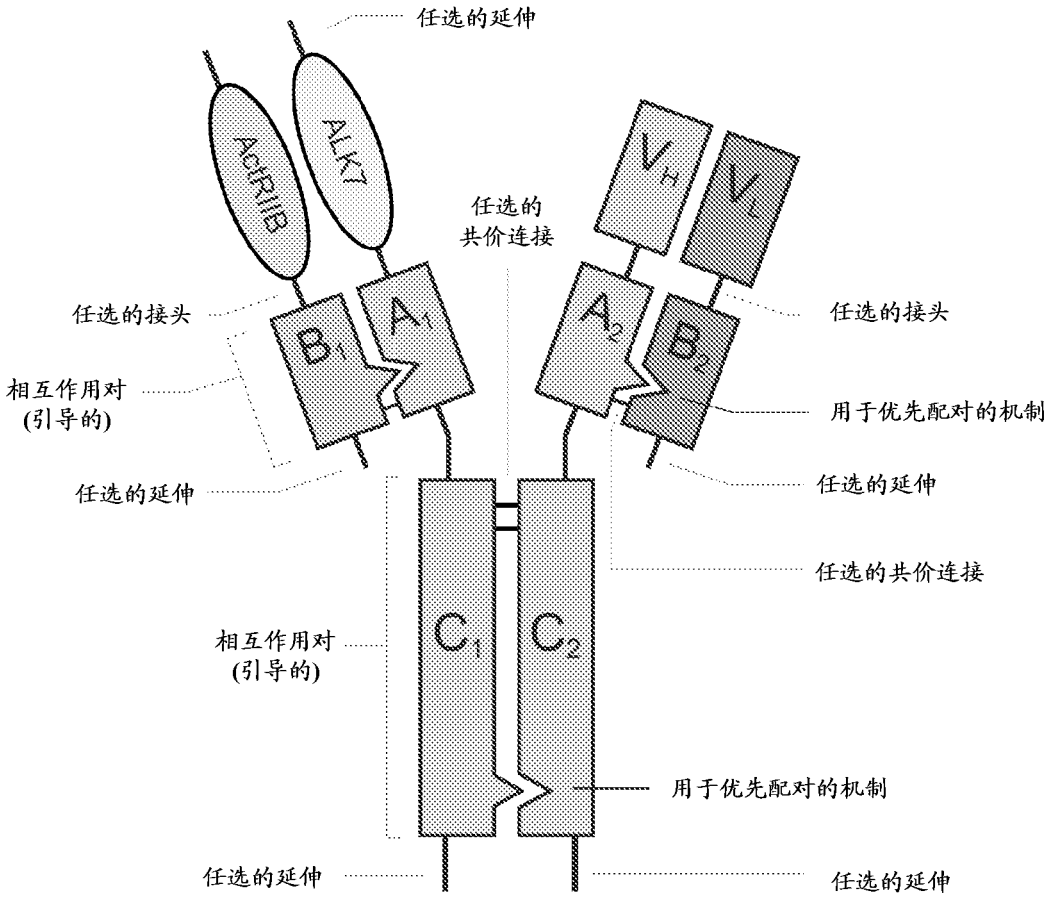

任选的延伸
ACVR1B
ALK7
任选的
共价连接
V_H
V_L
任选的接头
B_1
A_1
A_2
B_2
任选的接头
相互作用对
(引导的)
用于优先配对的机制
任选的延伸
任选的延伸
任选的共价连接
相互作用对
(引导的)
C_1
C_2
用于优先配对的机制
任选的延伸
任选的延伸

图 10A

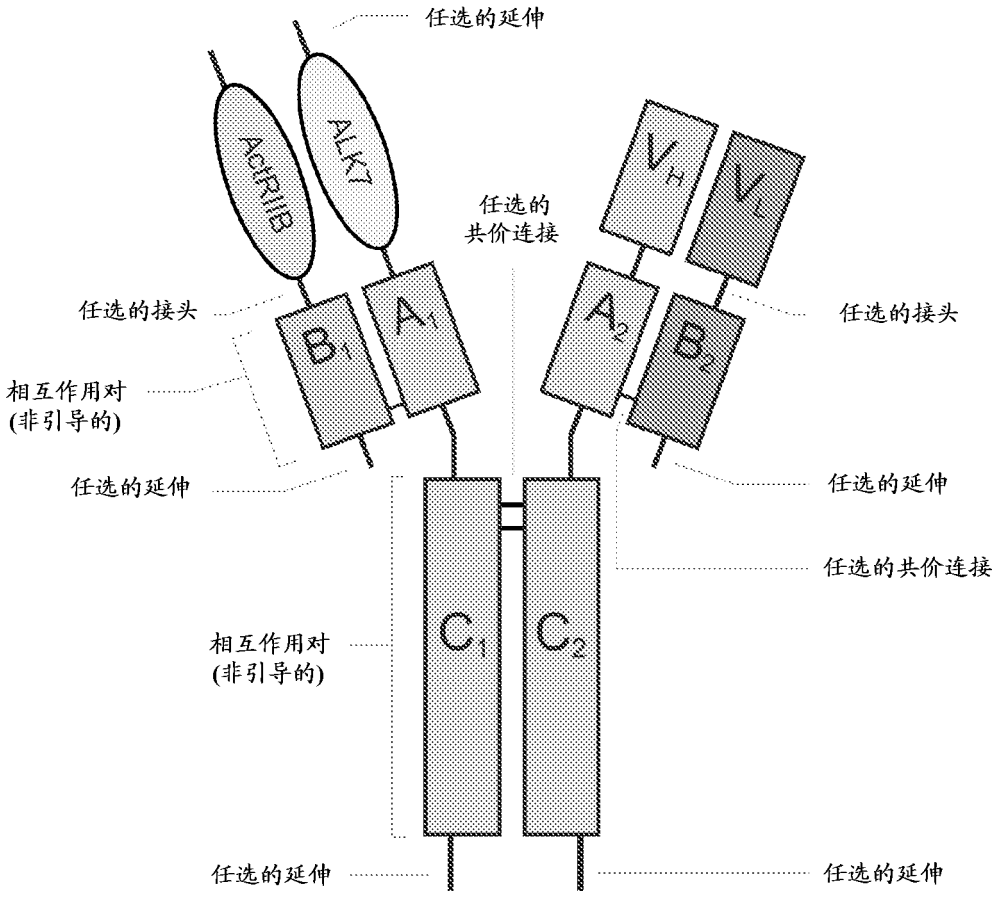

任选的延伸
ActRIIB
ALK7
V_H
V_L
任选的
共价连接
任选的接头
A_1
A_2
任选的接头
B_1
B_2
相互作用对
(非引导的)
任选的延伸
任选的延伸
任选的共价连接
相互作用对
(非引导的)
C_1
C_2
任选的延伸
任选的延伸

图 10B

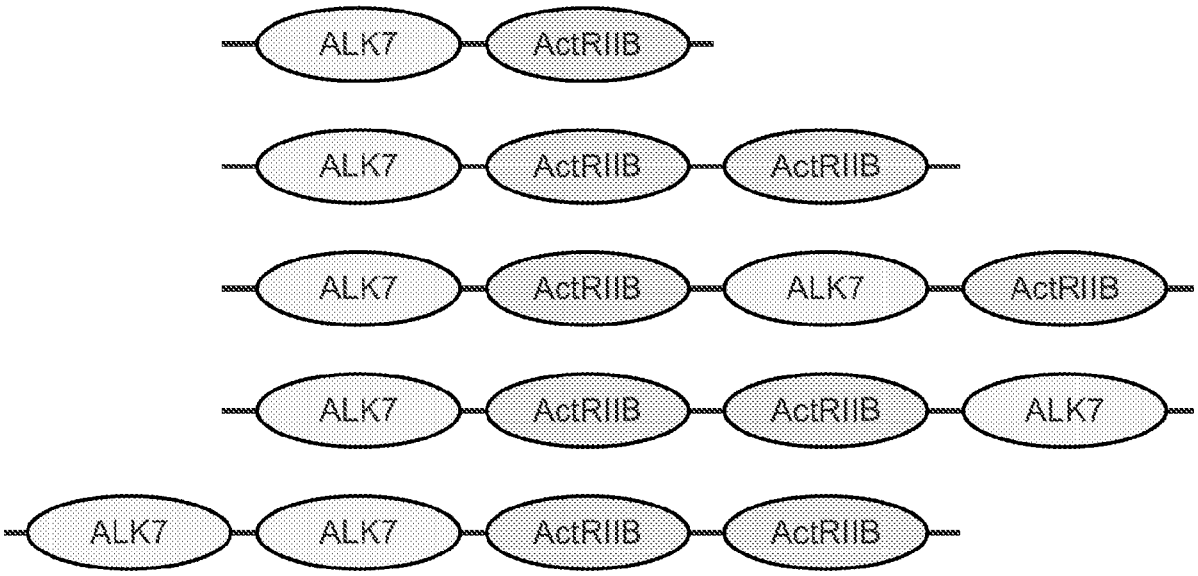

ALK7
ActRIIB
ALK7
ActRIIB
ActRIIB
ALK7
ActRIIB
ALK7
ActRIIB
ALK7
ActRIIB
ActRIIB
ALK7
ALK7
ALK7
ActRIIB
ActRIIB

图 11

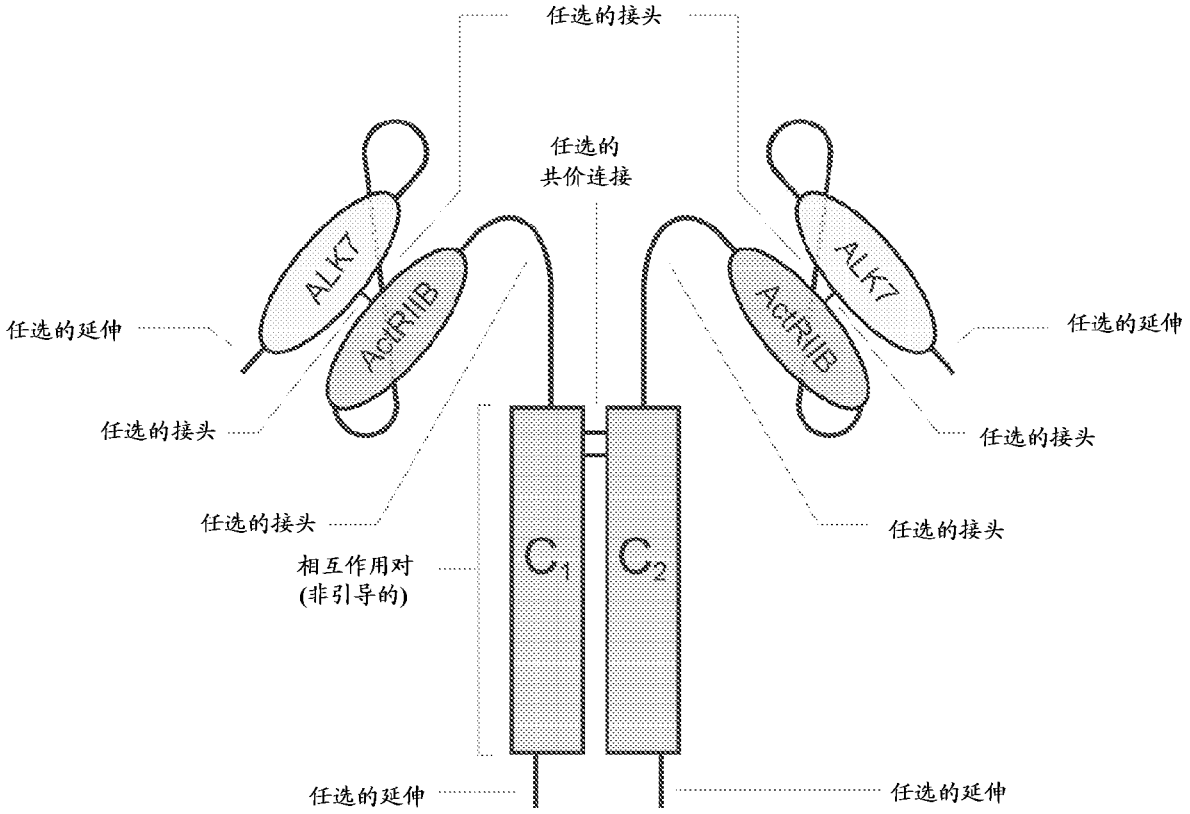

任选的接头
任选的
共价连接
ALK7
ActRIIB
ALK7
ActRIIB
任选的延伸
任选的延伸
任选的接头
任选的接头
任选的接头
任选的接头
相互作用对
(非引导的)
C1
C2
任选的延伸
任选的延伸

图 12A

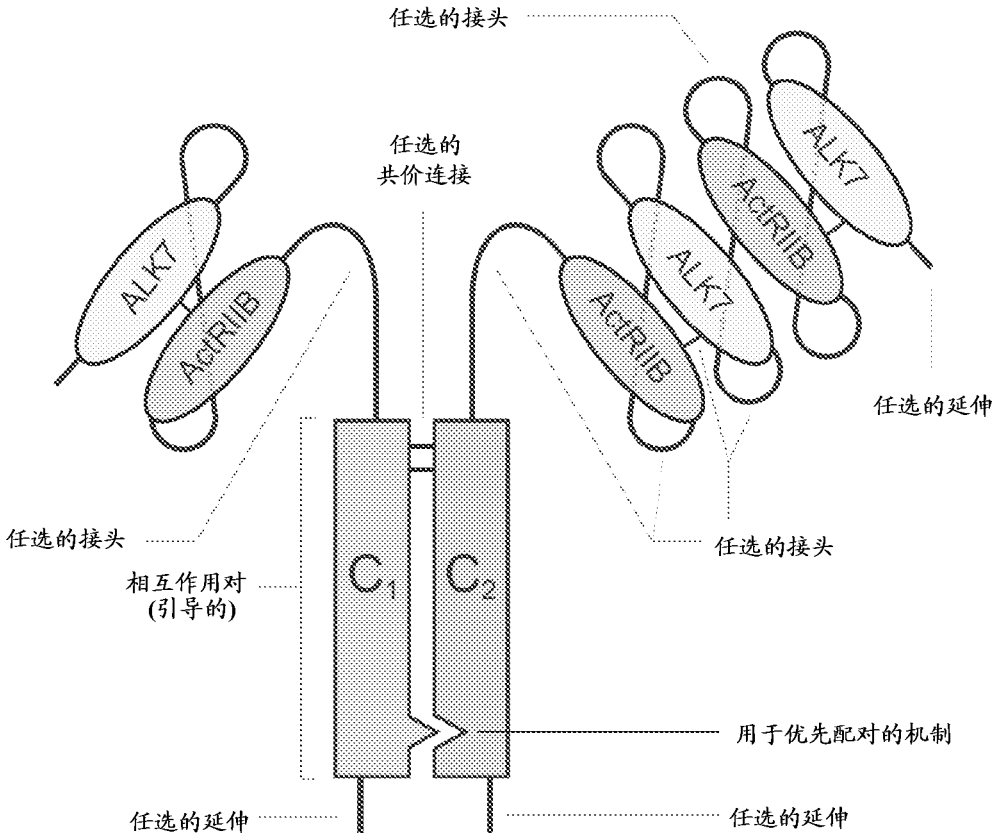

任选的接头
任选的
共价连接
ALK7
ActRIIB
ALK7
ActRIIB
ALK7
ActRIIB
任选的延伸
任选的接头
任选的接头
相互作用对
(引导的)
C1
C2
用于优先配对的机制
任选的延伸
任选的延伸

图 12B

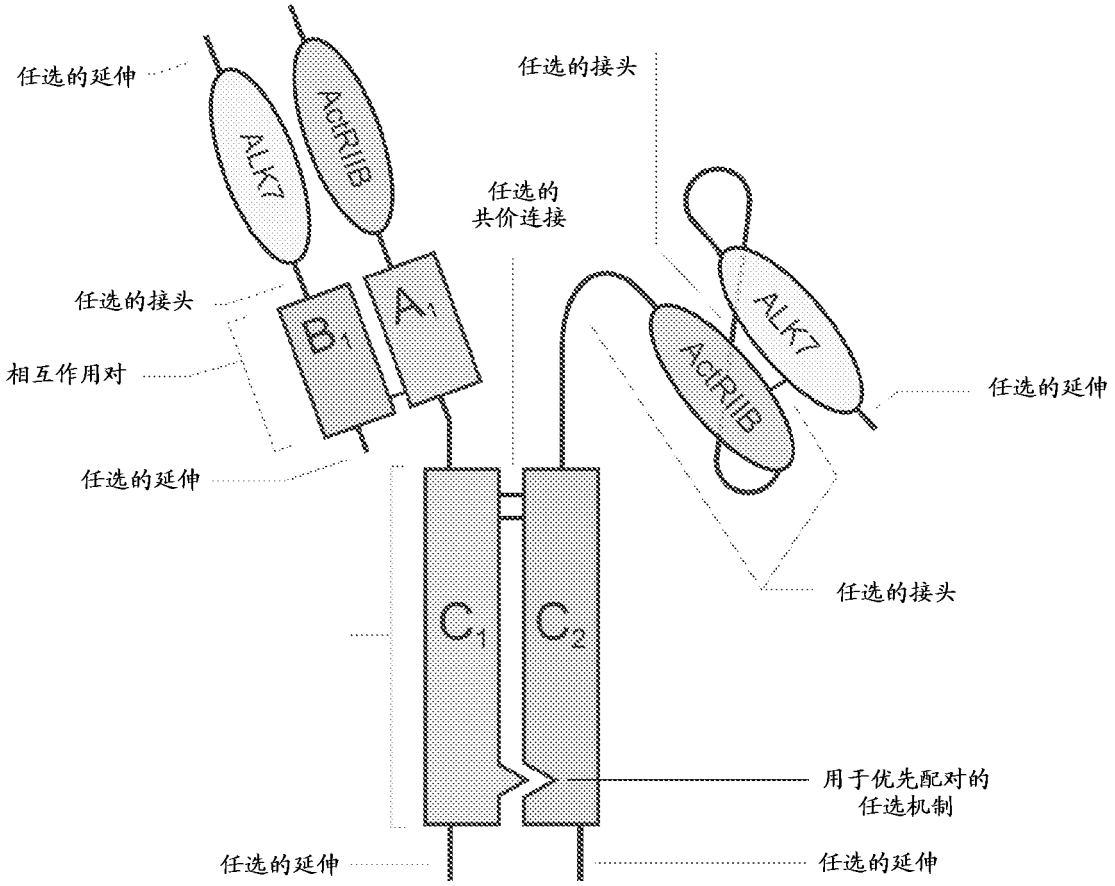

任选的延伸
ALK7
ActRIIB
任选的接头
任选的
共价连接
任选的接头
相互作用对
B1
A1
任选的延伸
ALK7
ActRIIB
任选的延伸
任选的接头
C1
C2
用于优先配对的
任选机制
任选的延伸
任选的延伸

图 12C

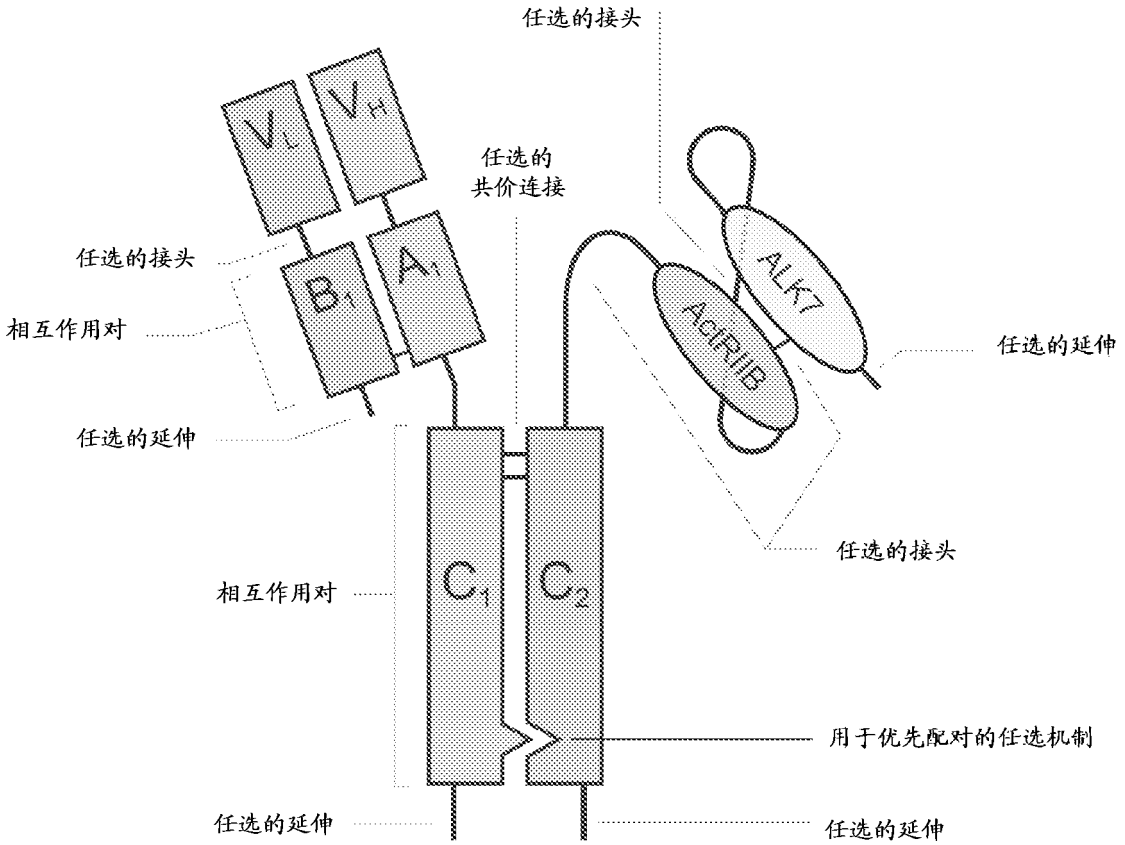

任选的接头
V_L
V_H
任选的
共价连接
任选的接头
B_1
A_1
相互作用对
ALK7
ACTRIB
任选的延伸
任选的延伸
任选的接头
相互作用对
C_1
C_2
用于优先配对的任选机制
任选的延伸
任选的延伸

图 12D

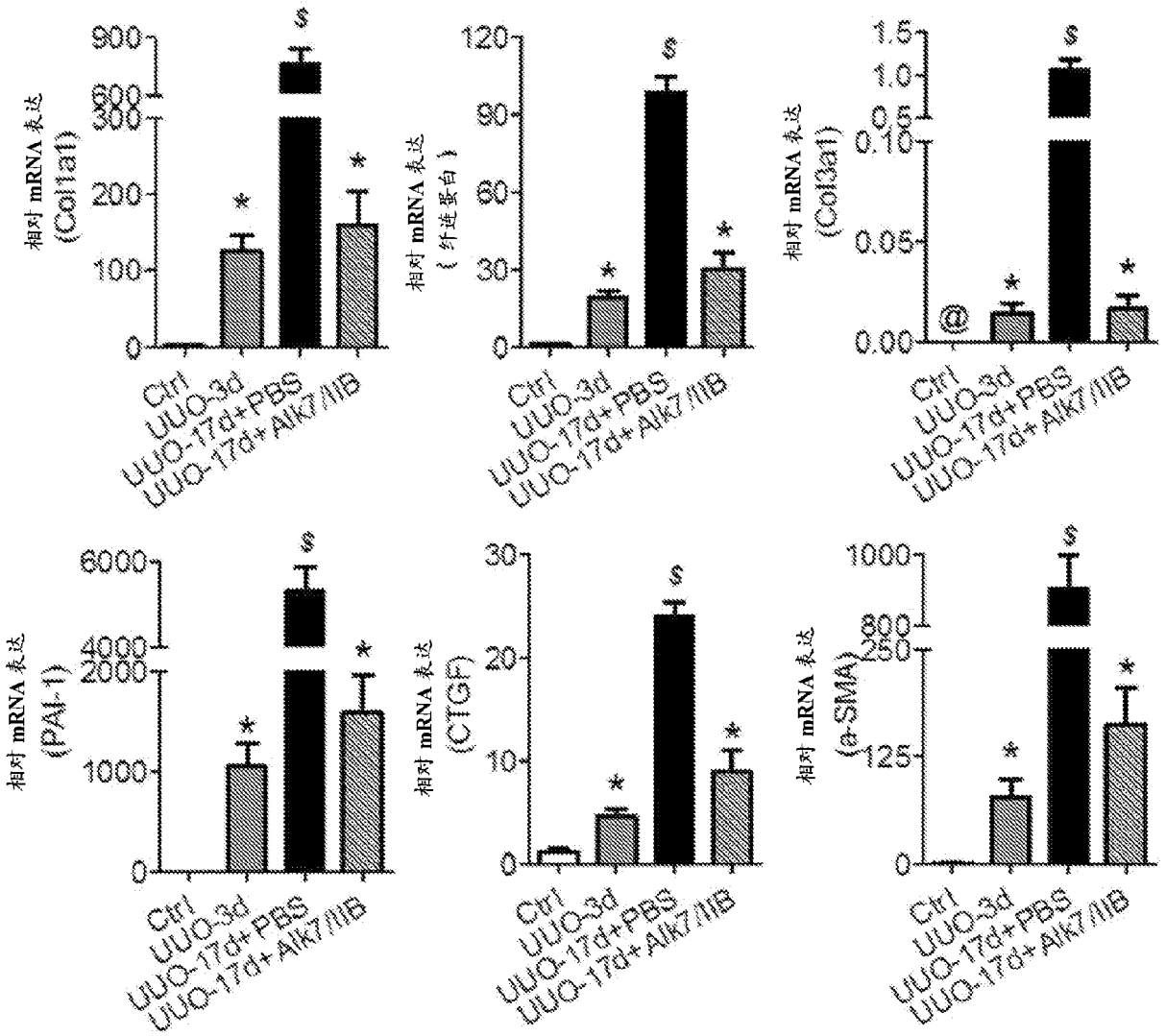

相对 mRNA 表达
(Col1a1)
900
800
300
200
100
0
*
$
*
Ctrl
UUO-3d
UUO-17d+PBS
UUO-17d+Alk7/IIB
相对 mRNA 表达
(纤连蛋白)
120
90
60
30
0
*
$
*
Ctrl
UUO-3d
UUO-17d+PBS
UUO-17d+Alk7/IIB
相对 mRNA 表达
(Col3a1)
1.5
1.0
0.5
0.10
0.05
0.00
@
*
$
*
Ctrl
UUO-3d
UUO-17d+PBS
UUO-17d+Alk7/IIB
相对 mRNA 表达
(PAI-1)
6000
4000
2000
1000
0
*
$
*
Ctrl
UUO-3d
UUO-17d+PBS
UUO-17d+Alk7/IIB
相对 mRNA 表达
(CTGP)
30
20
10
0
*
$
*
Ctrl
UUO-3d
UUO-17d+PBS
UUO-17d+Alk7/IIB
相对 mRNA 表达
(α-SMA)
1000
800
250
125
0
*
$
*
Ctrl
UUO-3d
UUO-17d+PBS
UUO-17d+Alk7/IIB

图 13A

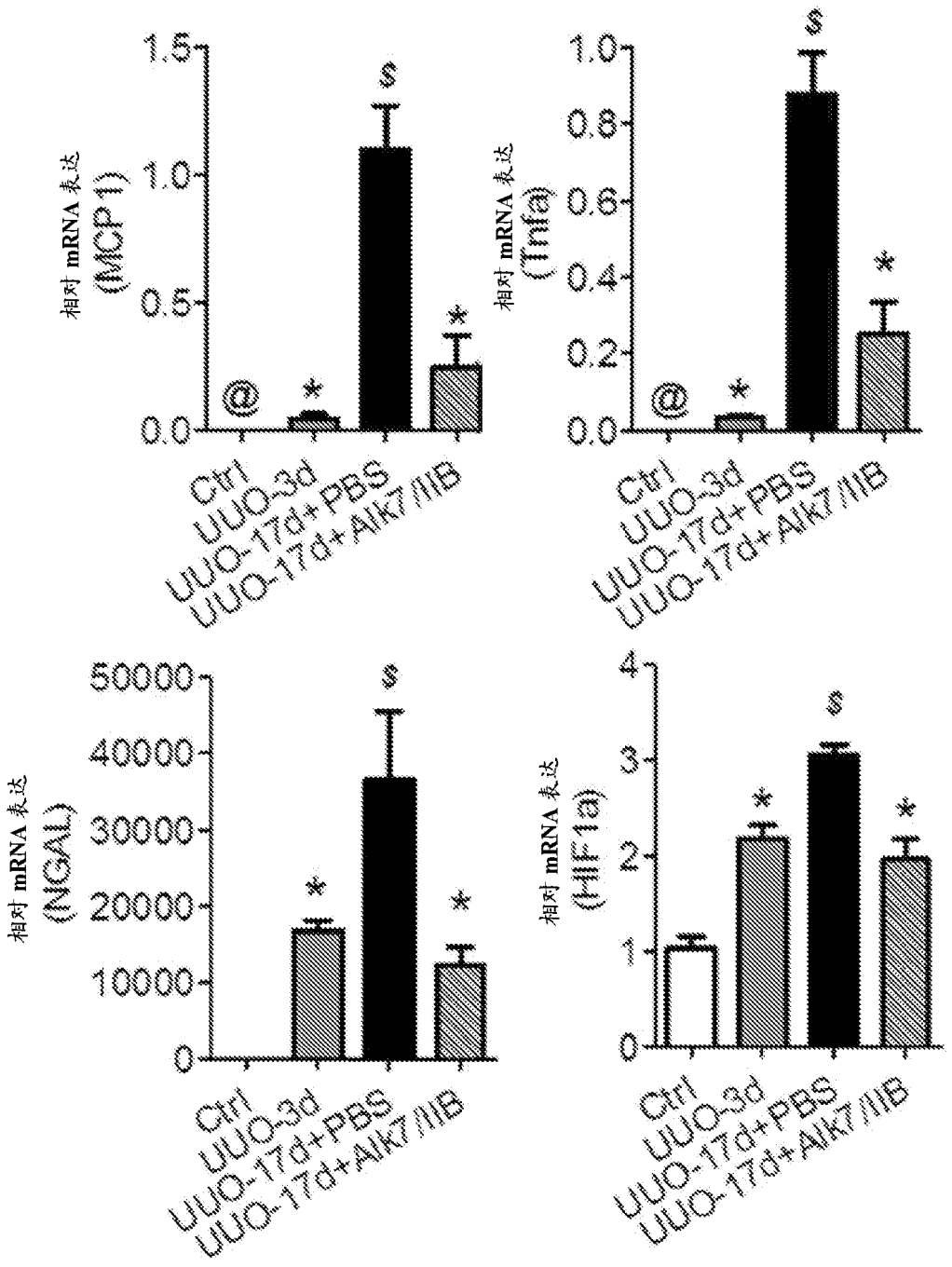

相对 mRNA 表达
(MCP1)
1.5
1.0
0.5
0.0
@
*
$
*
Ctrl
UUO-3d
UUO-17d+PBS
UUO-17d+ALK7/IIB
相对 mRNA 表达
(Tnfa)
1.0
0.8
0.6
0.4
0.2
0.0
相对 mRNA 表达
(NGAL)
50000
40000
30000
20000
10000
0
相对 mRNA 表达
(HIF1a)
4
3
2
1
0

图 13B

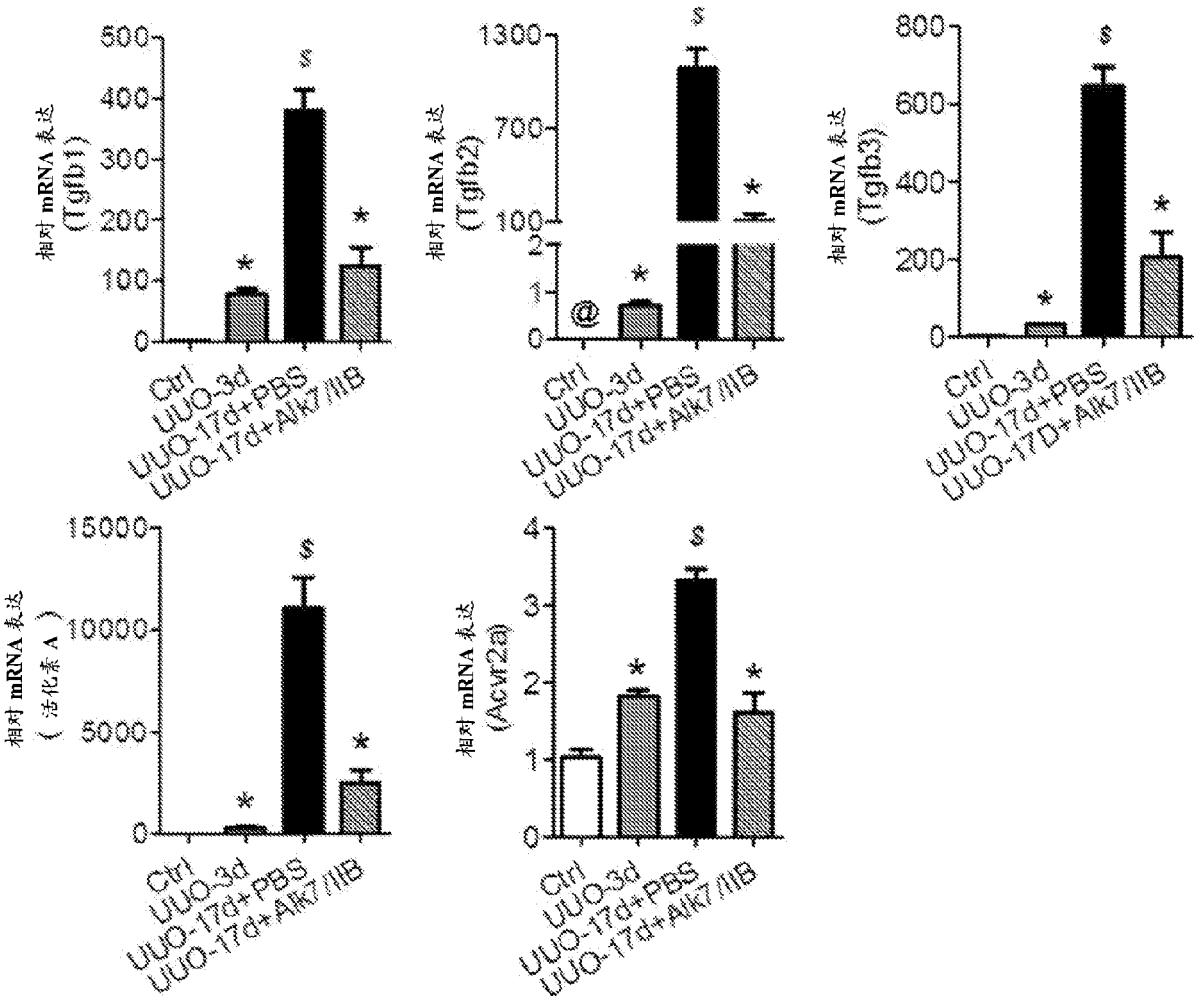

相对 mRNA 表达 (Tgfb1)
相对 mRNA 表达 (Tgfb2)
相对 mRNA 表达 (Tgfb3)
相对 mRNA 表达 (活化素 A)
相对 mRNA 表达 (Acvr2a)
Ctrl
UUO-3d
UUO-17d+PBS
UUO-17d+Alk7/IIB
*
$
@

图 13C