NOVEL HYBRID ACTRIIB LIGAND TRAP PROTEINS FOR TREATING MUSCLE WASTING DISEASES

Cross-Reference to Related Applications

[001] This application claims benefit of U.S. Provisional Application No. 62/150,994, filed on April 22, 2015, and U.S. Provisional Application No. 62/262,356, filed on December 2, 2015, each incorporated in its entirety by reference herein.

Background Art

[002] Muscle wasting refers to the progressive loss of muscle mass and/or to the progressive weakening and degeneration of muscles, including skeletal or voluntary muscles, cardiac muscles controlling the heart (cardiomyopathies), and smooth muscles. Chronic muscle wasting is a condition (i.e., persisting over a long period of time) characterized by progressive loss of muscle mass, as well as muscle weakening and degeneration. The loss of muscle mass occurs when the rate of muscle protein degradation exceeds muscle protein synthesis.

[003] Muscle wasting is a debilitating and life-threatening disease state, which has been associated with the development of a number of chronic, neurological, genetic, inflammatory, fibrotic or infectious pathologies, including, e.g, muscular dystrophies, amyotrophic lateral sclerosis, myositis, denervation muscle atrophies, anorexia-cachexia syndrome, cancers, rheumatoid arthritis, osteoarthritis, diabetes, sarcopenic obesity, age-related sarcopenia, androgen deprivation, corticosteroid myopathy, inflammatory bowel disease, liver cirrhosis, chronic obstructive pulmonary disease, pulmonary fibrosis, chronic renal disease, trauma, cardiomyopathy, chronic heart failure and HIV infection. Other conditions said to cause muscle wasting include chronic lower back pain, advanced age, damage to central nervous system, peripheral nerve injury, chemical injury, extended burns, hip/knee replacement, disuse atrophy, exposure to microgravity, and long term hospitalization.

[004] Activin IIA receptor (ActRIIA) and Activin IIB receptor (ActRIIB) are type II receptors for a subset of TGF-β family members, including, e.g., activin A, myostatin (also

known as GDF-8), growth differentiation factor-11 (GDF-11), and various other bone morphogenetic proteins (BMPs) such as BMP-3, BMP-6, BMP-9 (also known as GDF-2) and BMP-10. The binding of these TGF-β family ligands to ActRIIA and/or ActRIIB can regulate cell differentiation, apoptosis, protein synthesis and degradation, mineralization, hematopoiesis, angiogenesis, steroid synthesis, adhesion, migration, extracellular matrix production and fibrogenesis. The specific response depends upon the types and levels of the TGF-β ligands and receptors as well as the cellular state and environment. Altered expression of these ligands is known to be associated with a variety of diseases and disorders. For instance, elevated serum activin A levels have been shown in disease states of muscle atrophy, cancer, chronic heart failure, chronic kidney disease, inflammation, fibrosis, anemia, bone loss and aging, and are believed to contribute to disease pathogenesis and progression.

[005] ActRIIA and ActRIIB have been identified as the type II receptors for activins, including activin A, activin B and activin AB. ActRIIB is a high affinity receptor for myostatin, a key negative regulator of muscle growth, and thus plays central role in controlling muscle mass.

[006] Various ActRIIA-Fc and ActRIIB-Fc fusion proteins have been, or are currently being clinically evaluated. An ActRIIA-Fc fusion protein (Acceleron Pharma's ACE-011) has been clinically evaluated (Ph1, Ph2a) in patients with osteolytic lesions of multiple myeloma and osteoporosis. Unfortunately, while appearing effective, the clinical potential of ACE-011 for treating osteoporosis was hampered by safety concerns (high RBC growth). ACE-011 is currently being evaluated for chemotherapy-induced anemia (CIA) in patients with metastatic non-small cell lung cancer (NSCLC) (Ph2/3) (ClinicalTrials.gov). An ActRIIB-Fc fusion protein (Acceleron Pharma's ACE-031) has been clinically evaluated (Ph1, Ph2) for therapeutic efficacy in Duchenne muscle dystrophy (DMD). ACE-031 demonstrated a significant muscle gain which was more pronounced than that caused by a myostatin-selective inhibitor, such as myostatin antibody, peptibody or propeptide. Unfortunately, in spite of the promising efficacy in muscle growth, ACE-031's clinical potential has been hampered by the adverse event in nose and gum bleeding seen in DMD patients, thus there was a clinical hold put on ACE-031 (Smith R.C. and Lin B.K., Curr Opin Support Palliat Care. 7(4): 352–360, 2013). An ActRIIB-Fc fusion protein (Acceleron Pharma's ACE-083) is presently being clinically evaluated in Ph1 in healthy subjects using local delivery via intramuscular injection (ClinicalTrials.gov) and Atara Biotherapeutic's

ActRIIB-Fc fusion protein (STM 434) is set to be evaluated (Ph1 recruitment in progress) in patients with ovarian cancer or other advanced solid tumors (ClinicalTrials.gov).

[007] Despite these advancements, there clearly still exists a critical need to provide novel therapeutics, which are both highly effective and safe, for the treatment of muscle wasting diseases.

Disclosure of the Disclosure

[008] In one aspect, the present disclosure provides isolated novel hybrid ActRIIB ligand trap proteins comprising novel hybrid soluble ActRIIB-ECD polypeptides which retain myostatin- and activin A-neutralizing activities, but demonstrate dramatically reduced BMP9-neutralization. In various embodiments, the hybrid ActRIIB ligand trap proteins comprise a hybrid soluble ActRIIB-ECD polypeptide having the amino acid sequence of SEQ ID NO: 1 wherein at least one of amino acid residues R3, I6, Y7, Y8, L14, E15, S20, L22, R24, E26, E28, Q29, L33, L48, Y36, S38, R40, S42, T45, K51, F58, Q64, E65, A68, T69, E70, E71, N72, Q74, F84, R88, T90, H91, L92, E94, A95, G96, G97, P98, E99, V100, Y102, E103, P105, P106, T107, A108, or T110 is substituted with another amino acid, and wherein the hybrid ActRIIB-ECD polypeptide is capable of binding myostatin and activin A, but demonstrates a decreased binding affinity for BMP9 relative to a wild-type ActRIIB-ECD polypeptide. In various embodiments, the hybrid ActRIIB ligand trap proteins comprise a hybrid soluble ActRIIB-ECD polypeptide having the amino acid sequence of SEQ ID NO: 1 wherein at least two of amino acid residues R3, I6, Y7, Y8, L14, E15, S20, L22, R24, E26, E28, Q29, L33, L48, Y36, S38, R40, S42, T45, K51, F58, Q64, E65, A68, T69, E70, E71, N72, Q74, F84, R88, T90, H91, L92, E94, A95, G96, G97, P98, E99, V100, Y102, E103, P105, P106, T107, A108, or T110 is substituted with another amino acid, and wherein the hybrid ActRIIB-ECD polypeptide is capable of binding myostatin and activin A, but demonstrates a decreased binding affinity for BMP9 relative to a wild-type ActRIIB-ECD polypeptide. In various embodiments, the hybrid ActRIIB ligand trap proteins comprise a hybrid soluble ActRIIB-ECD polypeptide having the amino acid sequence of SEQ ID NO: 1 wherein at least three of amino acid residues R3, I6, Y7, Y8, L14, E15, S20, L22, R24, E26, E28, Q29, L33, L48, Y36, S38, R40, S42, T45, K51, F58,

Q64, E65, A68, T69, E70, E71, N72, Q74, F84, R88, T90, H91, L92, E94, A95, G96, G97, P98, E99, V100, Y102, E103, P105, P106, T107, A108, or T110 is substituted with another amino acid, and wherein the hybrid ActRIIB-ECD polypeptide is capable of binding myostatin and activin A, but demonstrates a decreased binding affinity for BMP9 relative to a wild-type ActRIIB-ECD polypeptide. In various embodiments, the hybrid ActRIIB ligand trap proteins comprise a hybrid soluble ActRIIB-ECD polypeptide having the amino acid sequence of SEQ ID NO: 1 wherein at least four of amino acid residues R3, I6, Y7, Y8, L14, E15, S20, L22, R24, E26, E28, Q29, L33, L48, Y36, S38, R40, S42, T45, K51, F58, Q64, E65, A68, T69, E70, E71, N72, Q74, F84, R88, T90, H91, L92, E94, A95, G96, G97, P98, E99, V100, Y102, E103, P105, P106, T107, A108, or T110 is substituted with another amino acid, and wherein the hybrid ActRIIB-ECD polypeptide is capable of binding myostatin and activin A, but demonstrates a decreased binding affinity for BMP9 relative to a wild-type ActRIIB-ECD polypeptide. In various embodiments, the hybrid ActRIIB ligand trap proteins comprise a hybrid soluble ActRIIB-ECD polypeptide having the amino acid sequence of SEQ ID NO: 1 wherein at least five of amino acid residues R3, I6, Y7, Y8, L14, E15, S20, L22, R24, E26, E28, Q29, L33, L48, Y36, S38, R40, S42, T45, K51, F58, Q64, E65, A68, T69, E70, E71, N72, Q74, F84, R88, T90, H91, L92, E94, A95, G96, G97, P98, E99, V100, Y102, E103, P105, P106, T107, A108, or T110 is substituted with another amino acid, and wherein the hybrid ActRIIB-ECD polypeptide is capable of binding myostatin and activin A, but demonstrates a decreased binding affinity for BMP9 relative to a wild-type ActRIIB-ECD polypeptide. In various embodiments, the hybrid ActRIIB ligand trap proteins comprise a hybrid soluble ActRIIB-ECD polypeptide having the amino acid sequence of SEQ ID NO: 1 wherein at least six of amino acid residues R3, I6, Y7, Y8, L14, E15, S20, L22, R24, E26, E28, Q29, L33, L48, Y36, S38, R40, S42, T45, K51, F58, Q64, E65, A68, T69, E70, E71, N72, Q74, F84, R88, T90, H91, L92, E94, A95, G96, G97, P98, E99, V100, Y102, E103, P105, P106, T107, A108, or T110 is substituted with another amino acid, and wherein the hybrid ActRIIB-ECD polypeptide is capable of binding myostatin and activin A, but demonstrates a decreased binding affinity for BMP9 relative to a wild-type ActRIIB-ECD polypeptide. In various embodiments, the hybrid ActRIIB ligand trap proteins comprise a hybrid soluble ActRIIB-ECD polypeptide having the amino acid sequence of SEQ ID NO: 1 wherein at least seven of amino acid residues R3, I6, Y7, Y8, L14, E15, S20, L22, R24,

E26, E28, Q29, L33, L48, Y36, S38, R40, S42, T45, K51, F58, Q64, E65, A68, T69, E70, E71, N72, Q74, F84, R88, T90, H91, L92, E94, A95, G96, G97, P98, E99, V100, Y102, E103, P105, P106, T107, A108, or T110 is substituted with another amino acid, and wherein the hybrid ActRIIB-ECD polypeptide is capable of binding myostatin and activin A, but demonstrates a decreased binding affinity for BMP9 relative to a wild-type ActRIIB-ECD polypeptide. In various embodiments, the hybrid ActRIIB ligand trap proteins comprise a hybrid soluble ActRIIB-ECD polypeptide having the amino acid sequence of SEQ ID NO: 1 wherein at least eight of amino acid residues R3, I6, Y7, Y8, L14, E15, S20, L22, R24, E26, E28, Q29, L33, L48, Y36, S38, R40, S42, T45, K51, F58, Q64, E65, A68, T69, E70, E71, N72, Q74, F84, R88, T90, H91, L92, E94, A95, G96, G97, P98, E99, V100, Y102, E103, P105, P106, T107, A108, or T110 is substituted with another amino acid, and wherein the hybrid ActRIIB-ECD polypeptide is capable of binding myostatin and activin A, but demonstrates a decreased binding affinity for BMP9 relative to a wild-type ActRIIB-ECD polypeptide. In various embodiments, the hybrid ActRIIB ligand trap proteins comprise a hybrid soluble ActRIIB-ECD polypeptide having the amino acid sequence of SEQ ID NO: 1 wherein at least nine of amino acid residues R3, I6, Y7, Y8, L14, E15, S20, L22, R24, E26, E28, Q29, L33, L48, Y36, S38, R40, S42, T45, K51, F58, Q64, E65, A68, T69, E70, E71, N72, Q74, F84, R88, T90, H91, L92, E94, A95, G96, G97, P98, E99, V100, Y102, E103, P105, P106, T107, A108, or T110 is substituted with another amino acid, and wherein the hybrid ActRIIB-ECD polypeptide is capable of binding myostatin and activin A, but demonstrates a decreased binding affinity for BMP9 relative to a wild-type ActRIIB-ECD polypeptide. In various embodiments, the hybrid ActRIIB ligand trap proteins comprise a hybrid soluble ActRIIB-ECD polypeptide having the amino acid sequence of SEQ ID NO: 1 wherein at least ten of amino acid residues R3, I6, Y7, Y8, L14, E15, S20, L22, R24, E26, E28, Q29, L33, L48, Y36, S38, R40, S42, T45, K51, F58, Q64, E65, A68, T69, E70, E71, N72, Q74, F84, R88, T90, H91, L92, E94, A95, G96, G97, P98, E99, V100, Y102, E103, P105, P106, T107, A108, or T110 is substituted with another amino acid, and wherein the hybrid ActRIIB-ECD polypeptide is capable of binding myostatin and activin A, but demonstrates a decreased binding affinity for BMP9 relative to a wild-type ActRIIB-ECD polypeptide. In various embodiments, the hybrid ActRIIB ligand trap proteins comprise a hybrid soluble ActRIIB-ECD polypeptide having the amino acid sequence of SEQ ID NO: 1 wherein at least fifteen of amino

acid residues R3, I6, Y7, Y8, L14, E15, S20, L22, R24, E26, E28, Q29, L33, L48, Y36, S38, R40, S42, T45, K51, F58, Q64, E65, A68, T69, E70, E71, N72, Q74, F84, R88, T90, H91, L92, E94, A95, G96, G97, P98, E99, V100, Y102, E103, P105, P106, T107, A108, or T110 is substituted with another amino acid, and wherein the hybrid ActRIIB-ECD polypeptide is capable of binding myostatin and activin A, but demonstrates a decreased binding affinity for BMP9 relative to a wild-type ActRIIB-ECD polypeptide. In various embodiments, the hybrid ActRIIB ligand trap proteins comprise a hybrid soluble ActRIIB-ECD polypeptide having the amino acid sequence of SEQ ID NO: 1 wherein at least twenty of amino acid residues R3, I6, Y7, Y8, L14, E15, S20, L22, R24, E26, E28, Q29, L33, L48, Y36, S38, R40, S42, T45, K51, F58, Q64, E65, A68, T69, E70, E71, N72, Q74, F84, R88, T90, H91, L92, E94, A95, G96, G97, P98, E99, V100, Y102, E103, P105, P106, T107, A108, or T110 is substituted with another amino acid, and wherein the hybrid ActRIIB-ECD polypeptide is capable of binding myostatin and activin A, but demonstrates a decreased binding affinity for BMP9 relative to a wild-type ActRIIB-ECD polypeptide. In various embodiments, the hybrid ActRIIB ligand trap proteins comprise a hybrid soluble ActRIIB-ECD polypeptide having the amino acid sequence of SEQ ID NO: 1 wherein at least twenty-five of amino acid residues R3, I6, Y7, Y8, L14, E15, S20, L22, R24, E26, E28, Q29, L33, L48, Y36, S38, R40, S42, T45, K51, F58, Q64, E65, A68, T69, E70, E71, N72, Q74, F84, R88, T90, H91, L92, E94, A95, G96, G97, P98, E99, V100, Y102, E103, P105, P106, T107, A108, or T110 is substituted with another amino acid, and wherein the hybrid ActRIIB-ECD polypeptide is capable of binding myostatin and activin A, but demonstrates a decreased binding affinity for BMP9 relative to a wild-type ActRIIB-ECD polypeptide. In various embodiments, the hybrid ActRIIB ligand trap proteins comprise a hybrid soluble ActRIIB-ECD polypeptide having the amino acid sequence of SEQ ID NO: 1 wherein at least thirty of amino acid residues R3, I6, Y7, Y8, L14, E15, S20, L22, R24, E26, E28, Q29, L33, L48, Y36, S38, R40, S42, T45, K51, F58, Q64, E65, A68, T69, E70, E71, N72, Q74, F84, R88, T90, H91, L92, E94, A95, G96, G97, P98, E99, V100, Y102, E103, P105, P106, T107, A108, or T110 is substituted with another amino acid, and wherein the hybrid ActRIIB-ECD polypeptide is capable of binding myostatin and activin A, but demonstrates a decreased binding affinity for BMP9 relative to a wild-type ActRIIB-ECD polypeptide.

[009] In various embodiments, the hybrid ActRIIB ligand trap proteins comprise hybrid soluble ActRIIB-ECD polypeptides having the amino acid sequence set forth in SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, or SEQ ID NO: 37, wherein the hybrid ActRIIB-ECD polypeptide is capable of binding myostatin and activin A, but demonstrates a decreased binding affinity for BMP9 relative to a wild-type ActRIIB-ECD polypeptide. In various embodiments, the hybrid soluble ActRIIB polypeptides are hybrid soluble ActRIIB polypeptides having an amino acid sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to an amino acid sequence selected from SEQ ID NOs: 3-37, wherein the hybrid ActRIIB-ECD polypeptide is capable of binding myostatin and activin A, but demonstrates a decreased binding affinity for BMP9 relative to a wild-type ActRIIB-ECD polypeptide.

[010] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence selected from the group consisting of SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, SEQ ID NO: 73, SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 80, SEQ ID NO: 81, SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85, SEQ ID NO: 86, SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94, SEQ ID NO: 95, SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99, SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 110, SEQ ID NO: 111, SEQ ID NO: 112, SEQ ID NO: 113, SEQ ID NO: 114, SEQ ID NO: 115, SEQ ID NO: 116, or SEQ ID NO: 117, wherein the hybrid ActRIIB-ECD polypeptide is capable of binding myostatin and activin A, but demonstrates a

decreased binding affinity for BMP9 relative to a wild-type ActRIIB-ECD polypeptide. In various embodiments, the hybrid soluble ActRIIB polypeptides are hybrid soluble ActRIIB polypeptides having an amino acid sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to an amino acid sequence selected from SEQ ID NOs: 51-117, wherein the hybrid ActRIIB-ECD polypeptide is capable of binding myostatin and activin A, but demonstrates a decreased binding affinity for BMP9 relative to a wild-type ActRIIB-ECD polypeptide.

[011] In another aspect, the hybrid ActRIIB ligand trap proteins of the present disclosure comprise a hybrid soluble ActRIIB-ECD polypeptide and at least one heterologous protein, wherein the hybrid ActRIIB ligand trap is capable of binding myostatin and activin A, but demonstrates a decreased binding affinity for BMP9 relative to a wild-type ActRIIB-ECD polypeptide. In various embodiments, the heterologous protein is an Fc domain. In various embodiments, the Fc domain is a human IgG Fc domain. In various embodiments, the Fc domain is derived from the human IgG1 heavy chain constant domain sequence set forth in SEQ ID NO: 38. In various embodiments, the Fc domain is an Fc domain having the amino acid sequence set forth in SEQ ID NO: 39. In various embodiments, the Fc domain is derived from the human IgG2 heavy chain constant domain sequence set forth in SEQ ID NO: 40. In various embodiments, the Fc domain is an Fc domain having the amino acid sequence set forth in SEQ ID NO: 41. In various embodiments, the Fc domain is derived from the human IgG4 heavy chain constant domain sequence set forth in SEQ ID NO: 42. In various embodiments, the Fc domain is an Fc domain having the amino acid sequence set forth in SEQ ID NO: 43.

[012] In various embodiments, the heterologous protein is attached to the hybrid soluble ActRIIB-ECD polypeptide by a linker and/or a hinge linker peptide. The linker or hinge linker may be an artificial sequence of between 5, 10, 15, 20, 30, 40 or more amino acids that are relatively free of secondary structure. . In various embodiments, the linker is rich in G/S content (e.g., at least about 60%, 70%, 80%, 90%, or more of the amino acids in the linker are G or S. In various embodiments, the linker has a (GGGGS (SEQ ID NO: 44))$_n$ motif, wherein n = 1-6. In various embodiments, a linker having the amino acid sequence set forth in SEQ ID NO: 44 is used with a hinge linker having the amino acid sequence set forth in SEQ ID NO: 118 to link a human IgG4 Fc (SEQ ID NO: 43) to a hybrid soluble ActRIIB-ECD polypeptide (e.g., any one of SEQ ID NOs: 3-37 or 51-117) of the present disclosure.

[013] In another aspect, the present disclosure provides isolated nucleic acid molecules comprising a polynucleotide encoding a hybrid soluble ActRIIB-ECD polypeptide of the present disclosure. In various embodiments, the polynucleotides encodes one of the polypeptide sequences set forth in SEQ ID NOs: 3-37 or 51-117, wherein the hybrid ActRIIB-ECD polypeptide is capable of binding myostatin and activin A, but demonstrates a decreased binding affinity for BMP9 relative to a wild-type ActRIIB-ECD polypeptide. In various embodiments, the polynucleotides encode a polypeptide having an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to any one of the polypeptides sequences set forth in SEQ ID NOs: 3-37 or 51-117, wherein the hybrid ActRIIB-ECD polypeptide is capable of binding myostatin and activin A, but demonstrates a decreased binding affinity for BMP9 relative to a wild-type ActRIIB-ECD polypeptide. In various embodiments, the polynucleotides encode a polypeptide having at least 90% identity to any one of the polypeptides sequences set forth in SEQ ID NOs: 3-37 or 51-117, wherein the hybrid ActRIIB-ECD polypeptide is capable of binding myostatin and activin A, but demonstrates a decreased binding affinity for BMP9 relative to a wild-type ActRIIB-ECD polypeptide. In various embodiments, the polynucleotides encode a polypeptide having an amino acid sequence at least 95% identity to any one of the polypeptides sequences set forth in SEQ ID NOs: 3-37 or 51-117, wherein the hybrid ActRIIB-ECD polypeptide is capable of binding myostatin and activin A, but demonstrates a decreased binding affinity for BMP9 relative to a wild-type ActRIIB-ECD polypeptide.

[014] In various embodiments, the isolated nucleic acid molecules comprise the polynucleotides described herein, and further comprise a polynucleotide encoding at least one heterologous protein described herein. In various embodiments, the nucleic acid molecules further comprise polynucleotides encoding the linkers or hinge linkers described herein.

[015] In another aspect, the present disclosure provides vectors comprising the nucleic acids described herein. In various embodiments, the vector is an expression vector. In another aspect, the present disclosure provides isolated cells comprising the nucleic acids of the disclosure. In various embodiments, the cell is a host cell comprising the expression vector of the disclosure. In another aspect, methods of making the hybrid ActRIIB ligand trap proteins are

provided by culturing the host cells under conditions promoting expression of the proteins or polypeptides.

[016] In another aspect, the present disclosure provides a pharmaceutical composition comprising the isolated hybrid soluble ActRIIB polypeptides or hybrid ActRIIB ligand trap proteins in admixture with a pharmaceutically acceptable carrier.

[017] In another aspect, the present disclosure provides a method of treating or preventing a muscle wasting disease in a subject suffering from such a disorder by administering an effective amount of a pharmaceutical composition containing a hybrid ActRIIB ligand trap of the present disclosure to the subject. The muscle wasting disease or conditions include, but is not limited to, the following: muscular dystrophies (such as DMD, Becker MD, Limb-Girdle MD, Myotonic MD and FSHD), myositis (such as dermatomyositis, inclusion-body myositis, juvenile forms of myositis, polymyositis), myopathies (including inherited myopathy and acquired myopathy, such as diabetic myopathy or drug-induced myopathy), motoneuron diseases (such as Lou Gehrig's Disease or amyotrophic lateral sclerosis), myasthenia gravis, neurodegenerative diseases (such as Parkinson's disease, Huntington's disease and Alzheimer's disease), muscle wasting associated with cancers (such as pancreatic cancer, lung cancer, gastric cancer, ovarian cancer, colorectal cancer, melanoma leukemia, lung cancer, prostate cancer, brain cancer, bladder cancer, and head-neck cancer), muscle wasting associated with chronic heart failure (CHF), chronic kidney disease (CKD), liver failure, diabetes, chronic obstructive pulmonary disease (COPD), emphysema, cystic fibrosis, rheumatoid arthritis, osteoarthritis, liver fibrosis, cirrhosis, trauma (such as burns or motorcycle accident), bone fracture, organ transplantation (such as heart, lung, liver or kidney transplantation), ICU critical care, denervation (such as stoke or spinal cord injury), androgen-deprivation therapy, corticosteroid therapy, infections (such as AIDS or tuberculosis), prolonged bed rest, sarcopenic obesity, and age-associated sarcopenia.

[018] Also provided herein are methods for treating cardiovascular disease in a subject, comprising administering a therapeutically effective amount of the pharmaceutical compositions of the invention to a subject in need thereof. In one embodiment, the subject is a human subject. In various embodiments, the cardiovascular disease is selected from heart failure, cardiac atrophy, cardiac fibrosis, pulmonary hypertension, myocarditis, coronary artery

disease, myocardial infarction, cardiac arrhythmias, heart valve disease, cardiomyopathy, pericardial disease, aorta disease and Marfan syndrome.

[019] Also provided herein are methods for treating metabolic disorder in a subject, comprising administering a therapeutically effective amount of the pharmaceutical compositions of the invention to a subject in need thereof. In one embodiment, the subject is a human subject. In various embodiments, the metabolic disease is selected from obesity, dyslipidemia, sarcopenic obesity, non-alcoholic fatty liver disease such as non-alcoholic steatohepatitis, alcoholic fatty liver disease, insulin resistance, diabetes and metabolic syndrome, as well as diabetic myopathy, diabetic nephropathy, diabetic neuropathy, diabetic retinopathy, and hemochromatosis.

[020] Also provided herein are methods for treating cancer or cancer metastasis in a subject, comprising administering a therapeutically effective amount of the pharmaceutical compositions of the invention to a subject in need thereof. In one embodiment, the subject is a human subject. In various embodiments, the cancer is selected from pancreatic cancer, gastric cancer, ovarian cancer, colorectal cancer, melanoma, leukemia, myelodysplastic syndrome, lung cancer, prostate cancer, brain cancer, bladder cancer, head-neck cancer, or rhabdomyosarcoma.

[021] Also provided herein are methods for treating autoimmune disease in a subject, comprising administering a therapeutically effective amount of the pharmaceutical compositions of the invention to a subject in need thereof. In one embodiment, the subject is a human subject. In various embodiments, the disease is selected from autoimmune disorders including multiple sclerosis, systemic sclerosis, diabetes (type-1), glomerulonephritis, myasthenia gravis, psoriasis, systemic lupus erythematosus, polymyositis, Crohn's disease, ulcerative colitis, and primary biliary cirrhosis.

[022] Also provided herein are methods for treating arthritis in a subject, comprising administering a therapeutically effective amount of the pharmaceutical compositions of the invention to a subject in need thereof. In one embodiment, the subject is a human subject. In various embodiments, the arthritis is selected from rheumatoid arthritis, or osteoarthritis.

[023] Also provided herein are methods for treating anorexia in a subject, comprising administering a therapeutically effective amount of the pharmaceutical compositions of the

invention to a subject in need thereof. In one embodiment, the subject is a human subject. In various embodiments, the anorexia is selected from anorexia nervosa and anorexia-cachexia syndrome.

[024] Also provided herein are methods for treating liver disease in a subject, comprising administering a therapeutically effective amount of the pharmaceutical compositions of the invention to a subject in need thereof. In one embodiment, the subject is a human subject. In various embodiments, the liver disease is selected from fatty liver including non-alcoholic steatohepatitis, liver fibrosis or cirrhosis, liver failure, autoimmune hepatitis, and hepatocellular carcinoma.

[025] Also provided herein are methods for organ or tissue transplantation in a subject, comprising administering a therapeutically effective amount of the pharmaceutical compositions of the invention to a subject in need thereof. In one embodiment, the subject is a human subject. In various embodiments, the transplantation is selected from organ transplantations of the heart, kidneys, liver, lungs, pancreas, intestine and thymus or from tissues transplantations of the bones, tendons, cornea, skin, heart valves, nerves and veins.

[026] Also provided herein are methods for treating anemia in a subject, comprising administering a therapeutically effective amount of the pharmaceutical compositions of the invention to a subject in need thereof. In one embodiment, the subject is a human subject. In various embodiments, the anemia is selected from various anemia disorders including iron deficiency anemia, iron overload, thalassemia, hemolytic anemia, sickle cell anemia, pernicious anemia, fanconi anemia and aplastic anemia (such as cancer-associated anemia and chemotherapy-induced anemia).

[027] Also provided herein are methods for treating fibrosis in a subject, comprising administering a therapeutically effective amount of the pharmaceutical compositions of the invention to a subject in need thereof. In one embodiment, the subject is a human subject. In various embodiments, the fibrosis is selected from interstitial lung disease, idiotypic pulmonary fibrosis, cystic fibrosis, liver fibrosis, cirrhosis, cardiac fibrosis, renal fibrosis, myelofibrosis, idiopathic retroperitoneal fibrosis, nephrogenic fibrosing dermopathy, inflammatory bowel disease, keloid, scleroderma or arthrofibrosis.

[028] Also provided herein are methods of treating pain in a subject, comprising administering a therapeutically effective amount of the pharmaceutical compositions of the invention to a subject in need thereof. In one embodiment, the subject is a human subject. In various embodiments, the pain is selected from neuropathic pain, somatic pain, visceral pain, inflammatory pain, cancer pain, back pain, or joint pain.

[029] Also provided herein are methods of treating bone disease in a subject, comprising administering a therapeutically effective amount of the pharmaceutical compositions of the invention to a subject in need thereof. In one embodiment, the subject is a human subject. In various embodiments, the bone disease is selected from osteoporosis, osteomalacia, osteogenesis imperfecta, fibrodysplasia ossificans progressiva, corticosteroid-induced bone loss, bone fracture, or bone metastasis.

[030] Also provided herein are methods of treating aging condition in a subject, comprising administering a therapeutically effective amount of the pharmaceutical compositions of the invention to a subject in need thereof. In one embodiment, the subject is a human subject. In various embodiments, the aging condition is selected from frailty of the elderly, age-related sarcopenia, or osteoarthritis.

[031] Also provided herein are methods of inducing stem cell growth for tissue repair or organ regeneration in a subject, comprising administering a therapeutically effective amount of the pharmaceutical compositions of the invention to a subject in need thereof. In one embodiment, the subject is a human subject. In various embodiments, the stem cell is selected from muscle stem (satellite) cell, cardiac stem cell, bone marrow-derived mesynchymal stem cell and pluripotent stem cell.

[032] In another aspect, the disclosure provides uses of the hybrid ActRIIB ligand trap proteins for making a medicament for the treatment of any disorder or condition as described herein.

Brief Description of the Figures

[033] FIG. 1 depicts the two exemplary molecular configurations for the hybrid ActRIIB ligand trap proteins of the present disclosure.

[034] FIG. 2 shows line graphs depicting the results of the cell-based assays used to evaluate the myostatin-neutralizing (left panels), activin A-neutralizing (middle panels), and BMP9-neutralizing (right panels) abilities for the hybrid ActRIIB ligand trap protein having the amino acid sequence of AG-0003 (SEQ ID NO: 5) and the hybrid ActRIIB ligand trap protein having the amino acid sequence of AG-0005 (SEQ ID NO: 7), in comparison to those of the wild-type ActRIIB-Fc protein as a benchmark (WT).

[035] FIG. 3 shows line graphs depicting the results of the cell-based neutralizing activities on myostatin (top left), activin A (top right), activin B (bottom left) and BMP9 (bottom right) for the hybrid ActRIIB ligand trap protein having the amino acid sequence of AG-0014 (SEQ ID NO: 16) and the hybrid ActRIIB ligand trap protein having the amino acid sequence of AG-0027 (SEQ ID NO: 29) in comparison to those of the wild-type ActRIIB-Fc protein as a benchmark (WT). Myostatin-, activin A- and activin B-neutralizing activities were examined by using C2C12-CAGA-luc reporter assay and BMP9-neutralizing activities were analyzed by using C2C12-BRE-luc reporter assay as described in the Examples.

[036] FIG. 4 shows line graphs depicting the effects on body weight in 9-week-old male C57Bl/6 mice subcutaneously injected with PBS (Vehicle), wild-type ActRIIB-Fc (WT), AG-0014 (SEQ ID NO: 16) and AG-0027 (SEQ ID NO: 29), respectively, at the dosage of 10 mg/kg, once per week. Body weights were recorded at day 0, day 5, day 12 and day 18. n=6/8 per group. Excel Student T-TEST was performed for statistical analysis. ***: $P<0.001$ vs. Vehicle group.

[037] FIG. 5 is a bar graph depicting the effects on muscle mass in 9-week-old male C57Bl/6 mice subcutaneously injected with PBS (Vehicle), wild-type ActRIIB-Fc (WT), AG-0014 (SEQ ID NO: 16) and AG-0027 (SEQ ID NO: 29), respectively, at the dosage of 10 mg/kg, once per week (n=6/8 per group). Individual calf muscles from each animal were dissected and weighed during terminal necropsy. Statistical analysis was performed by Excel Student T-TEST. ***: $P<0.001$ vs. Vehicle group.

[038] FIG. 6 shows Evans blue vascular permeability test images of mouse abdominal cavity. Representative images of surgically exposed abdominal cavity of each group are shown as labeled in the figure. 8-week-old male BalbC mice were treated with PBS (Vehicle), wild-type ActRIIB-Fc (WT), AG-0014 (SEQ ID NO: 16) and AG-0027 (SEQ ID NO: 29), respectively, at the dosage of 10 mg/kg, once per week. Two weeks after treatment, 200 µl of Evans blue dye

(0.5% in PBS, pH7.2) was injected into each group of animals (n=4) via the tail vein. Necropsy was performed at 90 min after Evans blue dye injection.

[039] FIG. 7 shows Evans blue vascular permeability test images of mouse testis. Representative images of the dissected testis organ from each group are shown as labeled in the figure. 8-week-old male BalbC mice were treated with PBS (Vehicle), wild-type ActRIIB-Fc (WT), AG-0014 (SEQ ID NO: 16) and AG-0027 (SEQ ID NO: 29), respectively, at 10 mg/kg, once per week. Two weeks after treatment, 200 µl of Evans blue dye (0.5% in PBS, pH7.2) was injected into each group of animals (n=4) via the tail vein. Necropsy was performed at 90 min post Evans blue dye injection.

[040] FIG. 8 shows Evans blue vascular permeability test images of mouse lung. Representative images of the dissected lung tissues from each group are shown as labeled in the figure. 8-week-old male BalbC mice were treated with PBS (Vehicle), wild-type ActRIIB-Fc (WT), AG-0014 (SEQ ID NO: 16) and AG-0027 (SEQ ID NO: 29), respectively, at 10 mg/kg, once per week. Two weeks after treatment, 200 µl of Evans blue dye (0.5% in PBS, pH7.2) was injected into each group of animals (n=4) via the tail vein. Necropsy was performed at 90 min after Evans blue dye injection.

[041] FIG. 9 shows bar graphs depicting the amounts of extravasated Evans blue dye per mg of wet lung tissue (left panel) and testis tissue (right panel) in different treatment groups as labeled in the figure. 8-week-old male BalbC mice were treated with PBS (Vehicle), the wild-type ActRIIB-Fc protein (WT), AG-0014 (SEQ ID NO: 16) and AG-0027 (SEQ ID NO: 29), respectively, at 10 mg/kg, once per week. Two weeks after treatment, 200 µl of Evans blue dye (0.5% in PBS, pH7.2) was injected into each group of animals (n=4) via the tail vein. Necropsy was performed at 90 min after Evans blue dye injection to collect testis and lung tissues. The tissues were weighed and then placed individually into vials containing formamide to extract the Evans blue dye. After incubation at 55°C for 24 hours, the samples were centrifuge and the absorbance of the aqueous phase of each sample was measured at the wavelength of 610 nm using a spectrophotometer. Statistical analysis was performed by using Excel Student T-TEST. *: $P<0.05$.

[042] FIG. 10 shows line graphs depicting the results of the cell-based neutralizing activities on BMP9 for a number of examplary hybrid ActRIIB ligand trap proteins including AG-

0014 (SEQ ID NO: 16), AG-0023 (SEQ ID NO: 25), AG-0024 (SEQ ID NO: 26), AG-0025 (SEQ ID NO: 27), AG0027 (SEQ ID NO: 29), AG-0028 (SEQ ID NO: 30), AG-0029 (SEQ ID NO: 31) and AG-0035 (SEQ ID NO: 37) in comparison to those of the wild-type ActRIIB-Fc protein as a benchmark (Wild type). BMP9-neutralizing activities were analyzed by using C2C12-BRE-luc reporter assay.

[043] FIG. 11 illustrates the differences in myostatin-neutralizing IC_{50} values between several exemplary hybrid ActRIIB ligand trap proteins relative to that of the WT ActRIIB-Fc protein. Myostatin-neutralizing activities of the individual proteins were examined using C2C12-CAGA-luc reporter cultures and the IC_{50} values were calculated by using Prism software. The graph shows the percentage difference in myostatin-neutralizing IC_{50} value of each of the examplary hybrid ActRIIB ligand trap proteins, including AG-0014 (SEQ ID NO: 16), AG-0023 (SEQ ID NO: 25), AG-0024 (SEQ ID NO: 26), AG0027 (SEQ ID NO: 29), AG-0028 (SEQ ID NO: 30) and AG-0029 (SEQ ID NO: 31), compared to that of the wild-type ActRIIB-Fc.

[044] FIG. 12 shows the results of ELISA analysis on BMP9 binding of two exemplary hybrid ActRIIB ligand trap proteins AG-0014 (SEQ ID NO: 16) and AG-0027 (SEQ ID NO: 29), respectively, in comparison to wild-type ActRIIB-Fc as well as to wild-type ActRIIA-Fc at increasing concentrations. Automated ELISA was performed using KinEXA instrument (Sapidyne Instruments). 20 µg/ml of BMP9 was coupled to NHS-Activated Sepharose 4 Fast Flow beads (GE Healthcare) using the experimental procedure recommended by Sapidyne Instruments. WT ActRIIB-Fc, WT ActRIIA-Fc and each hybrid ActRIIB ligand trap protein were tested for BMP9 binding at 100 pM, 1 nM and 10 nM concentrations as shown in the figure. The wild-type and hybrid proteins were captured on the BMP9-coated beads and detected by Alexa Fluor 647-labeled goat anti-human-Fc antibody (Jackson ImmunoResearch Laboratories, Inc.). The BMP9 binding signals were recorded with KinExA Pro software (Sapidyne Instruments).

Mode(s) for Carrying out the Disclosure

[045] The present disclosure provides novel isolated hybrid ActRIIB ligand trap proteins genetically engineered as fusion proteins which function as a multi-cytokine antagonist designed to selectively block the actions of multiple cachectic (atrophy-inducing) cytokines

without affecting the signaling of non-muscle related cytokines. In various embodiments, the hybrid ActRIIB ligand trap proteins comprise isolated hybrid soluble ActRIIB-ECD polypeptides which are capable of binding myostatin and activin A, but demonstrate a decreased binding affinity for BMP9 (i.e., retain myostatin- and activin A-neutralizing activities, but demonstrate dramatically reduced BMP9-neutralization) relative to a wild-type ActRIIB-ECD polypeptide. The present disclosure is based in part on the inventors' unique insight that a hybrid ActRIIB ligand trap engineered to exhibit significantly reduced binding to BMP9 (therefore having reduced BMP9-neutralization), while retaining its strong neutralizing activities against myostatin and activin, would provide myostatin inhibitors which are safer and more effective molecules than the currently available myostatin inhibitors. Specifically, the inventors postulated that a hybrid soluble ActRIIB polypeptide engineered to selectively replace amino acids residues within the ActRIIB extracellular domain (ECD) with corresponding amino acid residues from the ActRIIA ECD, could provide novel hybrid soluble ActRIIB polypeptides which preferentially neutralize myostatin and activin A (the key negative regulators of muscle growth) over BMP9. BMP9 plays an important role in a number of physiological processes (see, e.g, Tillet E, et al., Front Genet. 8;5:456, 2015) and BMP9 signaling has been shown to be essential in maintaining normal blood vasculature/permeability (see e.g., David L., et al., Circ Res. 25;102(8):914-22, 2008). It is thus postulated that subjects treated with the novel hybrid ActRIIB ligand trap proteins described herein may avoid the nose and gum bleeding side effects observed in subjects treated with the existing ActRIIB-Fc molecules which bind and neutralize BMP9 strongly. The therapeutic advantages provided by these novel hybrid ActRIIB ligand trap proteins offer next-generation therapeutics that are safe and effective for reversal of severe muscle loss and cachexia and for the treatment of a wide range of chronic catabolic diseases that involve muscle atrophy, bone loss, inflammation, and fibrosis.

Definitions

[046] The terms "polypeptide", "peptide" and "protein" are used interchangeably herein to refer to a polymer of amino acid residues. In various embodiments, "peptides", "polypeptides", and "proteins" are chains of amino acids whose alpha carbons are linked

through peptide bonds. The terminal amino acid at one end of the chain (amino terminal) therefore has a free amino group, while the terminal amino acid at the other end of the chain (carboxy terminal) has a free carboxyl group. As used herein, the term "amino terminus" (abbreviated N-terminus) refers to the free α-amino group on an amino acid at the amino terminal of a peptide or to the α-amino group (imino group when participating in a peptide bond) of an amino acid at any other location within the peptide. Similarly, the term "carboxy terminus" refers to the free carboxyl group on the carboxy terminus of a peptide or the carboxyl group of an amino acid at any other location within the peptide. Peptides also include essentially any polyamino acid including, but not limited to, peptide mimetics such as amino acids joined by an ether as opposed to an amide bond

[047] Polypeptides of the disclosure include polypeptides that have been modified in any way and for any reason, for example, to: (1) reduce susceptibility to proteolysis, (2) reduce susceptibility to oxidation, (3) alter binding affinity for forming protein complexes, (4) alter binding affinities, and (5) confer or modify other physicochemical or functional properties. For example, single or multiple amino acid substitutions (e.g., conservative amino acid substitutions) may be made in the naturally occurring sequence (e.g., in the portion of the polypeptide outside the domain(s) forming intermolecular contacts). A "conservative amino acid substitution" refers to the substitution in a polypeptide of an amino acid with a functionally similar amino acid. The following six groups each contain amino acids that are conservative substitutions for one another:

1) Alanine (A), Serine (S), and Threonine (T)
2) Aspartic acid (D) and Glutamic acid (E)
3) Asparagine (N) and Glutamine (Q)
4) Arginine (R) and Lysine (K)
5) Isoleucine (I), Leucine (L), Methionine (M), and Valine (V)
6) Phenylalanine (F), Tyrosine (Y), and Tryptophan (W)

[048] A “non-conservative amino acid substitution” refers to the substitution of a member of one of these classes for a member from another class. In making such changes,

according to various embodiments, the hydropathic index of amino acids may be considered. Each amino acid has been assigned a hydropathic index on the basis of its hydrophobicity and charge characteristics. They are: isoleucine (+4.5); valine (+4.2); leucine (+3.8); phenylalanine (+2.8); cysteine/cystine (+2.5); methionine (+1.9); alanine (+1.8); glycine (-0.4); threonine (-0.7); serine (-0.8); tryptophan (-0.9); tyrosine (-1.3); proline (-1.6); histidine (-3.2); glutamate (-3.5); glutamine (-3.5); aspartate (-3.5); asparagine (-3.5); lysine (-3.9); and arginine (-4.5).

[049] The importance of the hydropathic amino acid index in conferring interactive biological function on a protein is understood in the art (see, for example, Kyte et al., 1982, J. Mol. Biol. 157:105-131). It is known that certain amino acids may be substituted for other amino acids having a similar hydropathic index or score and still retain a similar biological activity. In making changes based upon the hydropathic index, in various embodiments, the substitution of amino acids whose hydropathic indices are within $\pm$ 2 is included. In various embodiments, those that are within $\pm$ 1 are included, and in various embodiments, those within $\pm$ 0.5 are included.

[050] It is also understood in the art that the substitution of like amino acids can be made effectively on the basis of hydrophilicity, particularly where the biologically functional protein or peptide thereby created is intended for use in immunological embodiments, as disclosed herein. In various embodiments, the greatest local average hydrophilicity of a protein, as governed by the hydrophilicity of its adjacent amino acids, correlates with its immunogenicity and antigenicity, i.e., with a biological property of the protein.

[051] The following hydrophilicity values have been assigned to these amino acid residues: arginine (+3.0); lysine (+3.0); aspartate (+3.0.+-.1); glutamate (+3.0.+-.1); serine (+0.3); asparagine (+0.2); glutamine (+0.2); glycine (0); threonine (-0.4); proline (-0.5.+-.1); alanine (-0.5); histidine (-0.5); cysteine (-1.0); methionine (-1.3); valine (-1.5); leucine (-1.8); isoleucine (-1.8); tyrosine (-2.3); phenylalanine (-2.5) and tryptophan (-3.4). In making changes based upon similar hydrophilicity values, in various embodiments, the substitution of amino acids whose hydrophilicity values are within $\pm$ 2 is included, in various embodiments, those that are within $\pm$ 1 are included, and in various embodiments, those within $\pm$ 0.5 are included.

[052] Exemplary amino acid substitutions are set forth in Table 1.

Table 1

Original Residues	Exemplary Substitutions	Preferred Substitutions
Ala	Val, Leu, Ile	Val
Arg	Lys, Gln, Asn	Lys
Asn	Gln	
Asp	Glu	
Cys	Ser, Ala	Ser
Gln	Asn	Asn
Glu	Asp	Asp
Gly	Pro, Ala	Ala
His	Asn, Gln, Lys, Arg	Arg
Ile	Leu, Val, Met, Ala, Phe, Norleucine	Leu
Leu	Norleucine, Ile, Val, Met, Ala, Phe	Ile
Lys	Arg, 1,4 Diamino-butyric Acid, Gln, Asn	Arg
Met	Leu, Phe, Ile	Leu
Phe	Leu, Val, Ile, Ala, Tyr	Leu
Pro	Ala	Gly
Ser	Thr, Ala, Cys	Thr
Thr	Ser	
Trp	Tyr, Phe	Tyr
Tyr	Trp, Phe, Thr, Ser	Phe
Val	Ile, Met, Leu, Phe, Ala, Norleucine	Leu

[053] A skilled artisan will be able to determine suitable variants of polypeptides as set forth herein using well-known techniques. In various embodiments, one skilled in the art may

identify suitable areas of the molecule that may be changed without destroying activity by targeting regions not believed to be important for activity. In other embodiments, the skilled artisan can identify residues and portions of the molecules that are conserved among similar polypeptides. In further embodiments, even areas that may be important for biological activity or for structure may be subject to conservative amino acid substitutions without destroying the biological activity or without adversely affecting the polypeptide structure.

[054] Additionally, one skilled in the art can review structure-function studies identifying residues in similar polypeptides that are important for activity or structure. In view of such a comparison, the skilled artisan can predict the importance of amino acid residues in a polypeptide that correspond to amino acid residues important for activity or structure in similar polypeptides. One skilled in the art may opt for chemically similar amino acid substitutions for such predicted important amino acid residues.

[055] One skilled in the art can also analyze the three-dimensional structure and amino acid sequence in relation to that structure in similar polypeptides. In view of such information, one skilled in the art may predict the alignment of amino acid residues of a polypeptide with respect to its three-dimensional structure. In various embodiments, one skilled in the art may choose to not make radical changes to amino acid residues predicted to be on the surface of the polypeptide, since such residues may be involved in important interactions with other molecules. Moreover, one skilled in the art may generate test variants containing a single amino acid substitution at each desired amino acid residue. The variants can then be screened using activity assays known to those skilled in the art. Such variants could be used to gather information about suitable variants. For example, if one discovered that a change to a particular amino acid residue resulted in destroyed, undesirably reduced, or unsuitable activity, variants with such a change can be avoided. In other words, based on information gathered from such routine experiments, one skilled in the art can readily determine the amino acids where further substitutions should be avoided either alone or in combination with other mutations.

[056] The term "polypeptide fragment" and “truncated polypeptide” as used herein refers to a polypeptide that has an amino-terminal and/or carboxy-terminal deletion as compared to a corresponding full-length protein. In certain embodiments, fragments can be, *e.g.,* at least 5, at least 10, at least 25, at least 50, at least 100, at least 150, at least 200, at

least 250, at least 300, at least 350, at least 400, at least 450, at least 500, at least 600, at least 700, at least 800, at least 900 or at least 1000 amino acids in length. In certain embodiments, fragments can also be, *e.g.,* at most 1000, at most 900, at most 800, at most 700, at most 600, at most 500, at most 450, at most 400, at most 350, at most 300, at most 250, at most 200, at most 150, at most 100, at most 50, at most 25, at most 10, or at most 5 amino acids in length. A fragment can further comprise, at either or both of its ends, one or more additional amino acids, for example, a sequence of amino acids from a different naturally-occurring protein (*e.g.,* an Fc or leucine zipper domain) or an artificial amino acid sequence (*e.g.*, an artificial linker sequence).

[057] The terms "polypeptide variant", “hybrid polypeptide” and “polypeptide mutant” as used herein refers to a polypeptide that comprises an amino acid sequence wherein one or more amino acid residues are inserted into, deleted from and/or substituted into the amino acid sequence relative to another polypeptide sequence. In certain embodiments, the number of amino acid residues to be inserted, deleted, or substituted can be, *e.g.,* at least 1, at least 2, at least 3, at least 4, at least 5, at least 10, at least 25, at least 50, at least 75, at least 100, at least 125, at least 150, at least 175, at least 200, at least 225, at least 250, at least 275, at least 300, at least 350, at least 400, at least 450 or at least 500 amino acids in length. Hybrids of the present disclosure include fusion proteins.

[058] A "derivative" of a polypeptide is a polypeptide that has been chemically modified, *e.g.,* conjugation to another chemical moiety such as, for example, polyethylene glycol, albumin (*e.g.,* human serum albumin), phosphorylation, and glycosylation.

[059] The term "% sequence identity" is used interchangeably herein with the term "% identity" and refers to the level of amino acid sequence identity between two or more peptide sequences or the level of nucleotide sequence identity between two or more nucleotide sequences, when aligned using a sequence alignment program. For example, as used herein, 80% identity means the same thing as 80% sequence identity determined by a defined algorithm, and means that a given sequence is at least 80% identical to another length of another sequence. In certain embodiments, the % identity is selected from, *e.g.,* at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% or more sequence identity to a given sequence. In certain embodiments, the %

identity is in the range of, e.g., about 60% to about 70%, about 70% to about 80%, about 80% to about 85%, about 85% to about 90%, about 90% to about 95%, or about 95% to about 99%.

[060] The term "% sequence homology" is used interchangeably herein with the term "% homology" and refers to the level of amino acid sequence homology between two or more peptide sequences or the level of nucleotide sequence homology between two or more nucleotide sequences, when aligned using a sequence alignment program. For example, as used herein, 80% homology means the same thing as 80% sequence homology determined by a defined algorithm, and accordingly a homologue of a given sequence has greater than 80% sequence homology over a length of the given sequence. In certain embodiments, the % homology is selected from, *e.g.,* at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% or more sequence homology to a given sequence. In certain embodiments, the % homology is in the range of, e.g., about 60% to about 70%, about 70% to about 80%, about 80% to about 85%, about 85% to about 90%, about 90% to about 95%, or about 95% to about 99%.

[061] Exemplary computer programs which can be used to determine identity between two sequences include, but are not limited to, the suite of BLAST programs, e.g., BLASTN, BLASTX, and TBLASTX, BLASTP and TBLASTN, publicly available on the Internet at the NCBI website. See also Altschul et al., J. Mol. Biol. 215:403-10, 1990 (with special reference to the published default setting, i.e., parameters w=4, t=17) and Altschul et al., Nucleic Acids Res., 25:3389-3402, 1997. Sequence searches are typically carried out using the BLASTP program when evaluating a given amino acid sequence relative to amino acid sequences in the GenBank Protein Sequences and other public databases. The BLASTX program is preferred for searching nucleic acid sequences that have been translated in all reading frames against amino acid sequences in the GenBank Protein Sequences and other public databases. Both BLASTP and BLASTX are run using default parameters of an open gap penalty of 11.0, and an extended gap penalty of 1.0, and utilize the BLOSUM-62 matrix. See Id.

[062] In addition to calculating percent sequence identity, the BLAST algorithm also performs a statistical analysis of the similarity between two sequences (see, e.g., Karlin & Altschul, Proc. Nat'l. Acad. Sci. USA, 90:5873-5787, 1993). One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of

the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is considered similar to a reference sequence if the smallest sum probability in a comparison of the test nucleic acid to the reference nucleic acid is, *e.g.,* less than about 0.1, less than about 0.01, or less than about 0.001.

[063] The term "heterologous" as used herein refers to a composition or state that is not native or naturally found, for example, that may be achieved by replacing an existing natural composition or state with one that is derived from another source. Similarly the expression of a protein in an organism other than the organism in which that protein is naturally expressed constitutes a heterologous expression system and a heterologous protein.

[064] The term "antibody" as used herein refers to a protein comprising one or more polypeptides substantially or partially encoded by immunoglobulin genes or fragments of immunoglobulin genes and having specificity to a tumor antigen or specificity to a molecule overexpressed in a pathological state. The recognized immunoglobulin genes include the kappa, lambda, alpha, gamma, delta, epsilon and mu constant region genes, as well as subtypes of these genes and myriad of immunoglobulin variable region genes. Light chains (LC) are classified as either kappa or lambda. Heavy chains (HC) are classified as gamma, mu, alpha, delta, or epsilon, which in turn define the immunoglobulin classes, IgG, IgM, IgA, IgD and IgE, respectively. A typical immunoglobulin (e.g., antibody) structural unit comprises a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kD) and one "heavy" chain (about 50-70 kD). The N-terminus of each chain defines a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition.

[065] The term "Fc region" as used herein defines the C-terminal region of an immunoglobulin heavy chain, which may be generated by papain digestion of an intact antibody. The Fc region may be a native sequence Fc region or a variant Fc region. The Fc region of an immunoglobulin generally comprises two constant domains, a C_{H2} domain and a C_{H3} domain, and optionally comprises a C_{H4} domain. The Fc portion of an antibody mediates several important effector functions e.g. cytokine induction, ADCC, phagocytosis, complement dependent cytotoxicity (CDC) and half-life/clearance rate of antibody and antigen-antibody complexes (e.g., the neonatal FcR (FcRn) binds to the Fc region of IgG at acidic pH in the

endosome and protects IgG from degradation, thereby contributing to the long serum half-life of IgG). Replacements of amino acid residues in the Fc portion to alter antibody effector function are known in the art (see, e.g., Winter et al., U.S. Patent No. 5,648,260 and 5,624,821).

[066] "Polynucleotide" refers to a polymer composed of nucleotide units. Polynucleotides include naturally occurring nucleic acids, such as deoxyribonucleic acid ("DNA") and ribonucleic acid ("RNA") as well as nucleic acid analogs. Nucleic acid analogs include those which include non-naturally occurring bases, nucleotides that engage in linkages with other nucleotides other than the naturally occurring phosphodiester bond or which include bases attached through linkages other than phosphodiester bonds. Thus, nucleotide analogs include, for example and without limitation, phosphorothioates, phosphorodithioates, phosphorotriesters, phosphoramidates, boranophosphates, methylphosphonates, chiral-methyl phosphonates, 2-O-methyl ribonucleotides, peptide-nucleic acids (PNAs), and the like. Such polynucleotides can be synthesized, for example, using an automated DNA synthesizer. The term "nucleic acid" typically refers to large polynucleotides. The term "oligonucleotide" typically refers to short polynucleotides, generally no greater than about 50 nucleotides. It will be understood that when a nucleotide sequence is represented by a DNA sequence (i.e., A, T, G, C), this also includes an RNA sequence (i.e., A, U, G, C) in which "U" replaces "T."

[067] Conventional notation is used herein to describe polynucleotide sequences: the left-hand end of a single-stranded polynucleotide sequence is the 5'-end; the left-hand direction of a double-stranded polynucleotide sequence is referred to as the 5'-direction. The direction of 5' to 3' addition of nucleotides to nascent RNA transcripts is referred to as the transcription direction. The DNA strand having the same sequence as an mRNA is referred to as the "coding strand"; sequences on the DNA strand having the same sequence as an mRNA transcribed from that DNA and which are located 5' to the 5'-end of the RNA transcript are referred to as "upstream sequences"; sequences on the DNA strand having the same sequence as the RNA and which are 3' to the 3' end of the coding RNA transcript are referred to as "downstream sequences."

[068] "Complementary" refers to the topological compatibility or matching together of interacting surfaces of two polynucleotides. Thus, the two molecules can be described as complementary, and furthermore, the contact surface characteristics are complementary to

each other. A first polynucleotide is complementary to a second polynucleotide if the nucleotide sequence of the first polynucleotide is substantially identical to the nucleotide sequence of the polynucleotide binding partner of the second polynucleotide, or if the first polynucleotide can hybridize to the second polynucleotide under stringent hybridization conditions.

[069] "Hybridizing specifically to" or "specific hybridization" or "selectively hybridize to", refers to the binding, duplexing, or hybridizing of a nucleic acid molecule preferentially to a particular nucleotide sequence under stringent conditions when that sequence is present in a complex mixture (e.g., total cellular) DNA or RNA. The term "stringent conditions" refers to conditions under which a probe will hybridize preferentially to its target subsequence, and to a lesser extent to, or not at all to, other sequences. "Stringent hybridization" and "stringent hybridization wash conditions" in the context of nucleic acid hybridization experiments such as Southern and northern hybridizations are sequence-dependent, and are different under different environmental parameters. An extensive guide to the hybridization of nucleic acids can be found in Tijssen, 1993, Laboratory Techniques in Biochemistry and Molecular Biology--Hybridization with Nucleic Acid Probes, part I, chapter 2, "Overview of principles of hybridization and the strategy of nucleic acid probe assays", Elsevier, N.Y.; Sambrook et al., 2001, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, 3.sup.rd ed., NY; and Ausubel et al., eds., Current Edition, Current Protocols in Molecular Biology, Greene Publishing Associates and Wiley Interscience, NY.

[070] Generally, highly stringent hybridization and wash conditions are selected to be about 5℃ lower than the thermal melting point (Tm) for the specific sequence at a defined ionic strength and pH. The Tm is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. Very stringent conditions are selected to be equal to the Tm for a particular probe. An example of stringent hybridization conditions for hybridization of complementary nucleic acids which have more than about 100 complementary residues on a filter in a Southern or northern blot is 50% formalin with 1 mg of heparin at 42℃, with the hybridization being carried out overnight. An example of highly stringent wash conditions is 0.15 M NaCl at 72℃ for about 15 minutes. An example of stringent wash conditions is a 0.2 x SSC wash at 65℃ for 15 minutes. See Sambrook et al. for a description of SSC buffer. A high stringency wash can be preceded by a low stringency wash to

remove background probe signal. An exemplary medium stringency wash for a duplex of, e.g., more than about 100 nucleotides, is 1 x SSC at 45°C for 15 minutes. An exemplary low stringency wash for a duplex of, e.g., more than about 100 nucleotides, is 4-6 x SSC at 40°C for 15 minutes. In general, a signal to noise ratio of 2 x (or higher) than that observed for an unrelated probe in the particular hybridization assay indicates detection of a specific hybridization.

[071] "Primer" refers to a polynucleotide that is capable of specifically hybridizing to a designated polynucleotide template and providing a point of initiation for synthesis of a complementary polynucleotide. Such synthesis occurs when the polynucleotide primer is placed under conditions in which synthesis is induced, i.e., in the presence of nucleotides, a complementary polynucleotide template, and an agent for polymerization such as DNA polymerase. A primer is typically single-stranded, but may be double-stranded. Primers are typically deoxyribonucleic acids, but a wide variety of synthetic and naturally occurring primers are useful for many applications. A primer is complementary to the template to which it is designed to hybridize to serve as a site for the initiation of synthesis, but need not reflect the exact sequence of the template. In such a case, specific hybridization of the primer to the template depends on the stringency of the hybridization conditions. Primers can be labeled with, e.g., chromogenic, radioactive, or fluorescent moieties and used as detectable moieties.

[072] "Probe," when used in reference to a polynucleotide, refers to a polynucleotide that is capable of specifically hybridizing to a designated sequence of another polynucleotide. A probe specifically hybridizes to a target complementary polynucleotide, but need not reflect the exact complementary sequence of the template. In such a case, specific hybridization of the probe to the target depends on the stringency of the hybridization conditions. Probes can be labeled with, e.g., chromogenic, radioactive, or fluorescent moieties and used as detectable moieties. In instances where a probe provides a point of initiation for synthesis of a complementary polynucleotide, a probe can also be a primer.

[073] A "vector" is a polynucleotide that can be used to introduce another nucleic acid linked to it into a cell. One type of vector is a "plasmid," which refers to a linear or circular double stranded DNA molecule into which additional nucleic acid segments can be ligated. Another type of vector is a viral vector (e.g., replication defective retroviruses, adenoviruses and

adeno-associated viruses), wherein additional DNA segments can be introduced into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors comprising a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. An "expression vector" is a type of vector that can direct the expression of a chosen polynucleotide.

[074] A "regulatory sequence" is a nucleic acid that affects the expression (e.g., the level, timing, or location of expression) of a nucleic acid to which it is operably linked. The regulatory sequence can, for example, exert its effects directly on the regulated nucleic acid, or through the action of one or more other molecules (e.g., polypeptides that bind to the regulatory sequence and/or the nucleic acid). Examples of regulatory sequences include promoters, enhancers and other expression control elements (e.g., polyadenylation signals). Further examples of regulatory sequences are described in, for example, Goeddel, 1990, Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, Calif. and Baron et al., 1995, Nucleic Acids Res. 23:3605-06. A nucleotide sequence is "operably linked" to a regulatory sequence if the regulatory sequence affects the expression (e.g., the level, timing, or location of expression) of the nucleotide sequence.

[075] A "host cell" is a cell that can be used to express a polynucleotide of the disclosure. A host cell can be a prokaryote, for example, *E. coli*, or it can be a eukaryote, for example, a single-celled eukaryote (e.g., a yeast or other fungus), a plant cell (e.g., a tobacco or tomato plant cell), an animal cell (e.g., a human cell, a monkey cell, a hamster cell, a rat cell, a mouse cell, or an insect cell) or a hybridoma. Typically, a host cell is a cultured cell that can be transformed or transfected with a polypeptide-encoding nucleic acid, which can then be expressed in the host cell. The phrase "recombinant host cell" can be used to denote a host cell that has been transformed or transfected with a nucleic acid to be expressed. A host cell also can be a cell that comprises the nucleic acid but does not express it at a desired level unless a regulatory sequence is introduced into the host cell such that it becomes operably linked with the nucleic acid. It is understood that the term host cell refers not only to the particular subject cell but also to the progeny or potential progeny of such a cell. Because

certain modifications may occur in succeeding generations due to, e.g., mutation or environmental influence, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

[076] The term "isolated molecule" (where the molecule is, for example, a polypeptide or a polynucleotide) is a molecule that by virtue of its origin or source of derivation (1) is not associated with naturally associated components that accompany it in its native state, (2) is substantially free of other molecules from the same species (3) is expressed by a cell from a different species, or (4) does not occur in nature. Thus, a molecule that is chemically synthesized, or expressed in a cellular system different from the cell from which it naturally originates, will be "isolated" from its naturally associated components. A molecule also may be rendered substantially free of naturally associated components by isolation, using purification techniques well known in the art. Molecule purity or homogeneity may be assayed by a number of means well known in the art. For example, the purity of a polypeptide sample may be assayed using polyacrylamide gel electrophoresis and staining of the gel to visualize the polypeptide using techniques well known in the art. For certain purposes, higher resolution may be provided by using HPLC or other means well known in the art for purification.

[077] A protein or polypeptide is "substantially pure," "substantially homogeneous," or "substantially purified" when at least about 60% to 75% of a sample exhibits a single species of polypeptide. The polypeptide or protein may be monomeric or multimeric. A substantially pure polypeptide or protein will typically comprise about 50%, 60%, 70%, 80% or 90% W/W of a protein sample, more usually about 95%, and preferably will be over 99% pure. Protein purity or homogeneity may be indicated by a number of means well known in the art, such as polyacrylamide gel electrophoresis of a protein sample, followed by visualizing a single polypeptide band upon staining the gel with a stain well known in the art. For certain purposes, higher resolution may be provided by using HPLC or other means well known in the art for purification.

[078] "Linker" refers to a molecule that joins two other molecules, either covalently, or through ionic, van der Waals or hydrogen bonds, e.g., a nucleic acid molecule that hybridizes to one complementary sequence at the 5' end and to another complementary sequence at the 3' end, thus joining two non-complementary sequences. A "cleavable linker" refers to a linker that

can be degraded or otherwise severed to separate the two components connected by the cleavable linker. Cleavable linkers are generally cleaved by enzymes, typically peptidases, proteases, nucleases, lipases, and the like. Cleavable linkers may also be cleaved by environmental cues, such as, for example, changes in temperature, pH, salt concentration, etc.

[079] The terms "label" or "labeled" as used herein refers to incorporation of another molecule in the antibody. In one embodiment, the label is a detectable marker, e.g., incorporation of a radiolabeled amino acid or attachment to a polypeptide of biotinyl moieties that can be detected by marked avidin (e.g., streptavidin containing a fluorescent marker or enzymatic activity that can be detected by optical or calorimetric methods). In another embodiment, the label or marker can be therapeutic, e.g., a drug conjugate or toxin. Various methods of labeling polypeptides and glycoproteins are known in the art and may be used. Examples of labels for polypeptides include, but are not limited to, the following: radioisotopes or radionuclides (e.g., ^{3}H, ^{14}C, ^{15}N, ^{35}S, ^{90}Y, ^{99}Tc, ^{111}In, ^{125}I, ^{131}I), fluorescent labels (e.g., FITC, rhodamine, lanthanide phosphors), enzymatic labels (e.g., horseradish peroxidase, β-galactosidase, luciferase, alkaline phosphatase), chemiluminescent markers, biotinyl groups, predetermined polypeptide epitopes recognized by a secondary reporter (e.g., leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, epitope tags), magnetic agents, such as gadolinium chelates, toxins such as pertussis toxin, taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicine, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. In some embodiments, labels are attached by spacer arms of various lengths to reduce potential steric hindrance.

[080] "Pharmaceutical composition" refers to a composition suitable for pharmaceutical use in an animal. A pharmaceutical composition comprises a pharmacologically effective amount of an active agent and a pharmaceutically acceptable carrier. "Pharmacologically effective amount" refers to that amount of an agent effective to produce the intended pharmacological result. "Pharmaceutically acceptable carrier" refers to any of the standard pharmaceutical carriers, vehicles, buffers, and excipients, such as a phosphate buffered saline

solution, 5% aqueous solution of dextrose, and emulsions, such as an oil/water or water/oil emulsion, and various types of wetting agents and/or adjuvants. Suitable pharmaceutical carriers and formulations are described in Remington's Pharmaceutical Sciences, 21st Ed. 2005, Mack Publishing Co, Easton. A "pharmaceutically acceptable salt" is a salt that can be formulated into a compound for pharmaceutical use including, e.g., metal salts (sodium, potassium, magnesium, calcium, etc.) and salts of ammonia or organic amines.

[081] The terms "treat", "treating" and "treatment" refer to a method of alleviating or abrogating a biological disorder and/or at least one of its attendant symptoms. As used herein, to "alleviate" a disease, disorder or condition means reducing the severity and/or occurrence frequency of the symptoms of the disease, disorder, or condition. Further, references herein to "treatment" include references to curative, palliative and prophylactic treatment.

[082] It is understood that aspect and embodiments of the disclosure described herein include “consisting” and/or “consisting essentially of” aspects and embodiments.

[083] Reference to "about" a value or parameter herein includes (and describes) variations that are directed to that value or parameter per se. For example, description referring to "about X" includes description of "X".

[084] As used herein and in the appended claims, the singular forms "a," "or," and "the" include plural referents unless the context clearly dictates otherwise. It is understood that aspects and variations of the disclosure described herein include "consisting" and/or "consisting essentially of" aspects and variations.

Activin Receptor Polypeptides

[085] As used herein, the term activin type II B receptors (ActRIIB) refers to the human activin receptors having accession number NP_ 001097.2 (SEQ ID NO: 45 herein), and variants thereof. The term “wild-type ActRIIB-ECD” refers to the extracellular domain of ActRIIB, amino acids 1 to 134 (with signal sequence), or amino acids 19 through 134 of SEQ ID NO: 45 (without signal sequence) (referred to herein as SEQ ID NO: 46). The term activin type II A receptors (ActRIIA) refers to the human activin receptors having accession number UniProtKB/Swiss-Prot P27037.1 (SEQ ID NO: 47 herein), and variants thereof. The term “wild-type ActRIIA-ECD”

refers to the extracellular domain of ActRIIA, amino acids 1 to 135 (with signal sequence), or amino acids 20 through 135 of SEQ ID NO: 46 (without signal sequence) (referred to herein as SEQ ID NO: 48).

Soluble Hybrid ActRIIB Polypeptides

[086] The present disclosure provides novel hybrid soluble ActRIIB-ECD polypeptides that are derived from wild-type ActRIIB-ECD and wild-type ActRIIA-ECD. The hybrid soluble ActRIIB polypeptides are specifically engineered by replacing one or more amino acids of a truncated wild-type ActRIIB-ECD with the amino acids from a truncated wild-type ActRIIA-ECD at corresponding positions based on sequence alignment between the two truncated ActRII ECD domains at the amino acid level. The one or more amino acid replacements are specifically selected for purposes of providing hybrid soluble ActRIIB-ECD polypeptides which demonstrate a marked reduction of BMP9-neutralization as compared to wild-type ActRIIB-ECD polypeptide, while fully retaining strong myostatin- and activin A-neutralization.

[087] In various embodiments, the truncated extracellular domain of ActRIIB used to prepare the novel hybrid soluble ActRIIB-ECD polypeptides has the 110 amino acid sequence set forth in SEQ ID NO: 1:

ETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWRNSSGTIELVKKGC
WLDDFNCYDRQECVATEENPQVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPP
TAPT (SEQ ID NO: 1)

[088] In various embodiments, the truncated extracellular domain of ActRIIA used to prepare the novel hybrid soluble ActRIIB-ECD polypeptides has the 110 amino acid sequence set forth in SEQ ID NO: 2:

ETQECLFFNANWEKDRTNQTGVEPCYGDKDKRRHCFATWKNISGSIEIVKQGCW
LDDINCYDRTDCVEKKDSPEVYFCCCEGNMCNEKFSYFPEMEVTQPTSNPVTPKPP
(SEQ ID NO: 2)

[089] In various embodiments, the hybrid ActRIIB ligand trap proteins comprise a hybrid soluble ActRIIB-ECD polypeptide having the amino acid sequence of SEQ ID NO: 1 wherein at least one of amino acid residues R3, I6, Y7, Y8, L14, E15, S20, L22, R24, E26, E28, Q29, L33, L48, Y36, S38, R40, S42, T45, K51, F58, Q64, E65, A68, T69, E70, E71, N72, Q74, F84, R88, T90, H91, L92, E94, A95, G96, G97, P98, E99, V100, Y102, E103, P105, P106, T107, A108, or T110 is substituted with the amino acid at the corresponding position of wild-type ActRIIA-ECD sequence (SEQ ID NO: 2), and wherein the hybrid soluble ActRIIB-ECD polypeptide is capable of binding myostatin and activin A, but demonstrates a decreased binding affinity for BMP9 relative to a wild-type ActRIIB-ECD polypeptide.

[090] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 3, wherein amino acid residues E26, E28, Q29, L33, F58, Q64, E65, A68, T69, E70, E71, N72, and Q74 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[091] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 4, wherein amino acid residues E26, E28, Q29, L33, Q64, E65, A68, T69, E70, E71, N72, and Q74 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[092] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 5, wherein amino acid residues F58, Q64, E65, A68, T69, E70, E71, N72, and Q74 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[093] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 6, wherein amino acid residues F58, Q64, E65, A68, T69, E70, E71, and N72 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[094] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 7, wherein amino acid residues Q64, E65, A68, T69, E70, E71, and N72 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[095] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 8, wherein amino acid residues Q64, E65, A68, T69, E70, E71, N72, and Q74 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[096] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 9, wherein amino acid residues A68, T69, E70, E71, N72 and Q74 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[097] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 10, wherein amino acid residues A68, T69, E70, E71, and N72 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[098] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 11, wherein amino acid residues F58, A68, T69, E70, E71, N72, and Q74 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[099] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 12, wherein amino acid residues Q64, E65, A68, T69, E70, E71, N72, Q74, and F84 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0100] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 13, wherein amino acid residues A68, T69, E70, E71, N72, Q74, and F84 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0101] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 14, wherein amino acid residues R3, L14, E15, S20, L22, R24, E26, E28, Q29, and L33 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0102] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 15, wherein amino acid residues R3, L14, E15, S20,

L22, and R24 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0103] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 16, wherein amino acid residues E26, E28, Q29, and L33 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0104] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 17, wherein amino acid residues L14, E15, S20, L22, and R24 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0105] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 18, wherein amino acid residues R3, L14, E15, S20, L22, and R24 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0106] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 19, wherein amino acid residues R3, L14, E15, and S20 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0107] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 20, wherein amino acid residues R3, L14, and E15 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0108] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 21, wherein amino acid residues L14 and E15 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0109] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 22, wherein amino acid residue R3 of SEQ ID NO: 1 has been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0110] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 23, wherein amino acid residues Y36, S38, and K51 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0111] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 24, wherein amino acid residues E26, E28, Q29, L33, and F58 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0112] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 25, wherein amino acid residue E70 of SEQ ID NO: 1 has been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0113] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 26, wherein amino acid residue F58 of SEQ ID NO: 1 has been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0114] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 27, wherein amino acid residues F58 and E70 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0115] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 28, wherein amino acid residues E28, Q29, F58, and E70 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0116] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 29, wherein amino acid residues E28, F58, and E70 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0117] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 30, wherein amino acid residues E28 and E70 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0118] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 31, wherein amino acid residue E28 of SEQ ID NO: 1 has been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0119] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 32, wherein amino acid residues E26, E28, Q29, L33, A68, T69, E70, E71, N72, and Q74 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0120] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 33, wherein amino acid residues Y7, Y8, L14, E15, S20, L22, and R24 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0121] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 34, wherein amino acid residues Y36, S38, R40, S42, T45, and K51 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0122] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 35, wherein amino acid residues Q64 and E65 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0123] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 36, wherein amino acid residue F84 of SEQ ID NO: 1 have been replaced by the amino acid residue in the corresponding position of SEQ ID NO: 2.

[0124] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 37, wherein amino acid residues E28 and F58 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0125] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 51, wherein amino acid residues R3, I6, Y7, Y8, L14, E15, L22, R24, E26, E28, Q29, L33 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0126] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 52, wherein amino acid residues R3, I6, Y7, Y8, L14, E15, L22, R24 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0127] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 53, wherein amino acid residues I6, Y7, Y8, L14, E15, L22, R24 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0128] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 54, wherein amino acid residues I6, Y7, Y8, L14, E15, L22, R24, E26 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0129] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 55, wherein amino acid residues I6, Y7, Y8, L14, E15, L22, R24, E26, E28, Q29, L33 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0130] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 56, wherein amino acid residues I6, Y7, Y8, L14, E15, L22, R24, E26, E28, Q29, L33, Y36, S38, R40, S42, T45, L48, K51 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0131] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 57, wherein amino acid residues I6, Y7, Y8, L14, E15, L22, R24, E26, E28, Q29, L33, Y36, S38, R40, S42, T45, L48, K51, F58 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0132] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 58, wherein amino acid residues I6, Y7, Y8, L14, E15, L22, R24, E26, E28, Q29, L33, Y36, S38, R40, S42, T45, L48, K51, F58, Q64, E65, A68, T69, E70, E71, N72, Q74 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0133] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 59, wherein amino acid residues R3, E26, E28, Q29, L33, Y36, S38, R40, S42, T45, L48, K51, F58 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0134] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 60, wherein amino acid residues E26, E28, Q29, L33, Y36, S38, R40, S42, T45, L48, K51, F58, Q64, E65, A68, T69, E70, E71, N72, Q74 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0135] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 61, wherein amino acid residues E26, E28, Q29, L33, Y36, S38, R40, S42, T45, L48, K51, F58, Q64, E65, A68, T69, E70, E71, N72, Q74, F84 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0136] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 62, wherein amino acid residues Y36, S38, R40, S42, T45, L48, K51, F58, Q64, E65, A68, T69, E70, E71, N72, Q74 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0137] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 63, wherein amino acid residues Y36, S38, R40, S42, T45, L48, K51, F58, Q64, E65 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0138] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 64, wherein amino acid residues Y36, S38, R40, S42, T45, L48, K51, Q64, E65 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0139] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 65, wherein amino acid residues Y36, S38, R40, S42, T45, L48, K51 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0140] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 66, wherein amino acid residues R3, E26, E28, Q29, L33, F58, Q64, E65, A68, T69, E70, E71, N72, Q74 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0141] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 67, wherein amino acid residues R3, E26, E28, Q29, L33, F58, Q64, E65, A68, T69, E70, E71, N72, Q74, F84 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0142] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 68, wherein amino acid residues R3, E26, E28, Q29, L33, Y36, S38, R40, S42, T45, L48, K51, F58, Q64, E65, A68, T69, E70, E71, N72, Q74, F84 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0143] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 69, wherein amino acid residues R3, E26, E28, Q29, L33, Y36, S38, R40, S42, T45, L48, K51, F58, Q64, E65, A68, T69, E70, E71, N72, Q74 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0144] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 70, wherein amino acid residues I6, Y7, Y8, L14, E15, L22, R24, Y36, S38, R40, S42, T45, L48, K51, F58, Q64, E65, A68, T69, E70, E71, N72, Q74 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0145] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 71, wherein amino acid residues I6, Y7, Y8, L14, E15, L22, R24, F58, Q64, E65, A68, T69, E70, E71, N72, Q74 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0146] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 72, wherein amino acid residues I6, Y7, Y8, L14, E15,

L22, R24, E26, E28, Q29, L33, F58, Q64, E65, A68, T69, E70, E71, N72, Q74 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0147] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 73, wherein amino acid residues E26, E28, Q29, L33, Q64, E65 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0148] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 74, wherein amino acid residues E26, E28, Q29, L33, K51, Q64, E65 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0149] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 75, wherein amino acid residues E26, E28, Q29, L33, L48, Q64, E65 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0150] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 76, wherein amino acid residues E26, E28, Q29, L33, T45, Q64, E65 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0151] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 77, wherein amino acid residues E26, E28, Q29, L33, T45, L48, Q64, E65 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0152] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 78, wherein amino acid residues E26, E28, Q29, L33, T45, L48, K51, Q64, E65 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0153] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 79, wherein amino acid residues Q64, E65, F84 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0154] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 80, wherein amino acid residues R88, T90, H91, L92, A95, G96, G97, P98, E99, V100, Y102, E103, P105, P106, T107, A108, T110 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0155] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 81, wherein amino acid residues R88, T90, H91, L92, E94, A95, G96, G97, P98, E99, V100, Y102, E103, P105, P106, T107, A108, T110 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0156] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 82, wherein amino acid residues E26, E28, Q29, L33, F58, Q64, E65, A68, T69, E70, E71, N72, Q74, R88, T90, H91, L92, A95, G96, G97, P98, E99, V100, Y102, E103, P105, P106, T107, A108, T110 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0157] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 83, wherein amino acid residues E26, E28, Q29, L33, Q64, E65, A68, T69, E70, E71, N72, Q74, R88, T90, H91, L92, E94, A95, G96, G97, P98, E99, V100, Y102, E103, P105, P106, T107, A108, T110 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0158] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 84, wherein amino acid residues E26, E28, Q29, L33, R88, T90, H91, L92, E94, A95, G96, G97, P98, E99, V100, Y102, E103, P105, P106, T107, A108, T110 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0159] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 85, wherein amino acid residues E26, E28, Q29, L33, K51, R88, T90, H91, L92, E94, A95, G96, G97, P98, E99, V100, Y102, E103, P105, P106, T107, A108, T110 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0160] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 86, wherein amino acid residues E26, E28, Q29, L33, L48, R88, T90, H91, L92, E94, A95, G96, G97, P98, E99, V100, Y102, E103, P105, P106, T107, A108, T110 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0161] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 87, wherein amino acid residues E26, E28, Q29, L33, T45, R88, T90, H91, L92, E94, A95, G96, G97, P98, E99, V100, Y102, E103, P105, P106, T107, A108, T110 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0162] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 88, wherein amino acid residues T45, R88, T90, H91, L92, E94, A95, G96, G97, P98, E99, V100, Y102, E103, P105, P106, T107, A108, T110 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0163] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 89, wherein amino acid residues L48, R88, T90, H91, L92, E94, A95, G96, G97, P98, E99, V100, Y102, E103, P105, P106, T107, A108, T110 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0164] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 90, wherein amino acid residues K51, R88, T90, H91, L92, E94, A95, G96, G97, P98, E99, V100, Y102, E103, P105, P106, T107, A108, T110 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0165] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 91, wherein amino acid residues A68, R88, T90, H91, L92, E94, A95, G96, G97, P98, E99, V100, Y102, E103, P105, P106, T107, A108, T110 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0166] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 92, wherein amino acid residues A68, R88, T90, H91, L92, E94, A95, G96, G97, P98, E99, V100, Y102, E103, P105, P106, T107, A108, T110 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0167] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 93, wherein amino acid residues E70, R88, T90, H91, L92, E94, A95, G96, G97, P98, E99, V100, Y102, E103, P105, P106, T107, A108, T110 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0168] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 94, wherein amino acid residues E71, R88, T90, H91, L92, E94, A95, G96, G97, P98, E99, V100, Y102, E103, P105, P106, T107, A108, T110 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0169] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 95, wherein amino acid residues N72, R88, T90, H91, L92, E94, A95, G96, G97, P98, E99, V100, Y102, E103, P105, P106, T107, A108, T110 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0170] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 96, wherein amino acid residues Q74, R88, T90, H91, L92, E94, A95, G96, G97, P98, E99, V100, Y102, E103, P105, P106, T107, A108, T110 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0171] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 97, wherein amino acid residues E28, Q29, A68, R88, T90, H91, L92, E94, A95, G96, G97, P98, E99, V100, Y102, E103, P105, P106, T107, A108, T110 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0172] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 98, wherein amino acid residues Q29, T69, R88, T90, H91, L92, E94, A95, G96, G97, P98, E99, V100, Y102, E103, P105, P106, T107, A108, T110 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0173] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 99, wherein amino acid residues E28, E70, R88, T90, H91, L92, E94, A95, G96, G97, P98, E99, V100, Y102, E103, P105, P106, T107, A108, T110 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0174] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 100, wherein amino acid residues E28, Q29, K51, T69, E70, R88, T90, H91, L92, E94, A95, G96, G97, P98, E99, V100, Y102, E103, P105, P106, T107, A108, T110 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0175] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 101, wherein amino acid residues E28, Q29, L48, K51, T69E, E70, R88, T90, H91, L92, E94, A95, G96, G97, P98, E99, V100, Y102, E103, P105, P106, T107, A108, T110 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0176] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 102, wherein amino acid residues E26, E28, T45, L48, K51, T69, E70, R88, T90, H91, L92, E94, A95, G96, G97, P98, E99, V100, Y102, E103, P105, P106, T107, A108, T110 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0177] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 103, wherein amino acid residues Q29, L48, E70, R88, T90, H91, L92, E94, A95, G96, G97, P98, E99, V100, Y102, E103, P105, P106, T107, A108, T110 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0178] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 104, wherein amino acid residues E26, E28, L33, Q70, R88, T90, H91, L92, E94, A95, G96, G97, P98, E99, V100, Y102, E103, P105, P106, T107, A108, T110 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0179] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 105, wherein amino acid residues L33, R88, T90, H91, L92, E94, A95, G96, G97, P98, E99, V100, Y102, E103, P105, P106, T107, A108, T110 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0180] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 106, wherein amino acid residues E26, T45, L48, Q64, E65, R88, T90, H91, L92, E94, A95, G96, G97, P98, E99, V100, Y102, E103, P105, P106, T107, A108, T110 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0181] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 107, wherein amino acid residues L33, T45, T69, R88, T90, H91, L92, E94, A95, G96, G97, P98, E99, V100, Y102, E103, P105, P106, T107, A108, T110 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0182] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 108, wherein amino acid residues L33, L48, T69, R88, T90, H91, L92, E94, A95, G96, G97, P98, E99, V100, Y102, E103, P105, P106, T107, A108, T110 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0183] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 109, wherein amino acid residues L33, T45, L48, E70, R88, T90, H91, L92, A95, G96, G97, P98, E99, V100, Y102, E103, P105, P106, T107, A108, T110 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0184] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 110, wherein amino acid residues E28, L48, E70, R88, T90, H91, L92, E94, A95, G96, G97, P98, E99, V100, Y102, E103, P105, P106, T107, A108, T110 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0185] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 111, wherein amino acid residues E28, T45, E70, R88, T90, H91, L92, E94, A95, G96, G97, P98, E99, V100, Y102, E103, P105, P106, T107, A108, T110 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0186] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 112, wherein amino acid residues E28, E70, R88, T90, H91, L92, E94, A95, G96, G97, P98, E99, V100, Y102, E103, P105, P106, T107, A108, T110 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0187] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 113, wherein amino acid residues L48, E70, R88, T90, H91, L92, E94, A95, G96, G97, P98, E99, V100, Y102, E103, P105, P106, T107, A108, T110 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0188] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 114, wherein amino acid residues E70, R88, T90, H91, L92, E94, A95, G96, G97, P98, E99, V100, Y102, E103, P105, P106, T107, A108, T110 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0189] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 115, wherein amino acid residues E28, L48, T79, E70, R88, T90, H91, L92, E94, A95, G96, G97, P98, E99, V100, Y102, E103, P105, P106, T107, A108, T110 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0190] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 116, wherein amino acid residues R3, I6, Y7, Y8, L14, E15, S20, L22, R24, E26, E28, Q29, L33, Y36, S38, R40, S42, T45, L48, K51, F58, Q64, E65, A68, T69, E71, N72, Q74, F84 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

[0191] In various embodiments, the hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 117, wherein amino acid residues E26, E28, Q29, L33, F56, E68 of SEQ ID NO: 1 have been replaced by the amino acid residues in the corresponding positions of SEQ ID NO: 2.

Heterologous Proteins – Fc Domains

[0192] In another aspect, the hybrid ActRII ligand traps comprise a hybrid soluble ActRIIB-ECD polypeptide and at least one heterologous protein attached to the ActRIIB-ECD polypeptide either directly or through a linker sequence to form a fusion protein referred to herein as hybrid ActRIIB ligand trap. As used herein the term "fusion protein" refers to a protein having a heterologous polypeptide attached via recombinant DNA techniques. In various embodiments, the heterologous protein is selected from, but not limited to, a polyhistidine tag, a Glu-Glu, a glutathione S transferase (GST), a thioredoxin, a protein A, a protein G, a fluorescent protein, a maltose binding protein (MBP), a human serum albumin or an Fc polypeptide or Fc domain. In various embodiments, the Fc domain is a human IgG Fc domain. In various embodiments, the Fc domain is derived from the human IgG1 heavy chain constant domain sequence set forth in SEQ ID NO: 38. In various embodiments, the Fc domain is an Fc domain having the amino acid sequence set forth in SEQ ID NO: 39. In various embodiments, the Fc domain is derived from the human IgG2 heavy chain constant domain sequence set forth in SEQ ID NO: 40. In various embodiments, the Fc domain is an Fc domain having the amino acid sequence set forth in SEQ ID NO: 41. In various embodiments, the Fc domain is derived from the human IgG4 heavy chain constant domain sequence set forth in SEQ ID NO: 42. In various embodiments, the Fc domain is an Fc domain having the amino acid sequence set forth in SEQ ID NO: 43.

Linkers

[0193] The hybrid ActRIIB hybrid traps can optionally further comprise a "linker" or "hinge linker" sequence. Linkers serve primarily as a spacer between a hybrid soluble ActRIIB-ECD polypeptide a heterologous protein or other type of fusion or between two or more hybrid soluble ActRIIB-ECD polypeptides. In various embodiments, the heterologous protein is attached to the hybrid soluble ActRIIB-ECD polypeptide by a linker or a hinge linker peptide. The linker and/or hinge linker may be an artificial sequence of between 5, 10, 15, 20, 30, 40 or more amino acids that are relatively free of secondary structure. In various embodiments, the linkers comprise amino acids selected from glycine, alanine, proline, asparagine, glutamine, and lysine. In various embodiments, a linker is made up of a majority of amino acids that are sterically unhindered, such as glycine and alanine, and are polyglycines (particularly $(Gly)_5$, $(Gly)_8$, poly(Gly-Ala), and polyalanines. In various embodiments, the linker is rich in G/S content (e.g., at least about 60%, 70%, 80%, 90%, or more of the amino acids in the linker are G or S. In various embodiemnts, the linker has a $(GGGGS$ (SEQ ID NO: 44$))_n$ motif, wherein n = 1-6. Such linkers and hinge linkers have been described extensively in art (see, e.g., US 8,410,043 (Sun et al), incorporated by reference herein for the purposes of teaching such linkers). In various embodiments, a linker having the amino acid sequence set forth in SEQ ID NO: 44 and a hinge linker having the amino acid sequence set forth in SEQ ID NO: 118 is used to link a human IgG1 Fc (SEQ ID NO: 39) or a human IgG4 Fc (SEQ ID NO: 43) to a hybrid soluble ActRIIB-ECD polypeptide of the present disclosure.

[0194] Linkers may also be non-peptide linkers. For example, alkyl linkers such as --NH--$(CH_2)_s$-C(O)--, wherein s = 2-20 can be used. These alkyl linkers may further be substituted by any non-sterically hindering group such as lower alkyl (e.g., C_1-C_6) lower acyl, halogen (e.g., Cl, Br), CN, NH_2, phenyl, etc.

Molecular Configurations for the Hybrid ActRIIB ligand trap proteins

[0195] It is understood that the different elements of the hybrid ActRIIB ligand trap may be arranged in any manner that is consistent with the desired functionality. For example, a heterologous protein may be placed C-terminal to a hybrid soluble ActRIIB-ECD polypeptide, or alternatively the hybrid soluble ActRIIB-ECD polypeptide may be placed C-terminal to a heterologous domain. The hybrid soluble ActRII-ECD polypeptide domain and the heterologous domain need not be adjacent, and additional domains or amino acid sequences may be included C- or N-terminal to either domain or between the domains (i.e. include a linker described herein). Exemplary molecular configurations for the novel ActRIIB ligand traps are depicted in FIG. 1

[0196] An exemplary configuration of a synthetic DNA cassette encoding a hybrid ActRIIB ligand trap can be generally described as comprising the following elements: 1) a signal peptide (or leader sequence) placed at the N-terminus, which can be either the native signal peptide of ActRIIB (e.g., SEQ ID NO: 49) or any surrogate signal peptide capable of mediating the processing and secretion of secreted proteins (e.g., by using the human immunoglobulin light chain leader sequence (SEQ ID NO: 50) as a surrogate signal peptide, efficient secretion of hybrid ActRIIB ligand trap proteins in CHO cells can be achieved); 2) a hybrid soluble ActRIIB-ECD polypeptide sequence (e.g., any one of SEQ ID NOs: 3-37 or 51-117) fused to the signal peptide sequence; 3) a peptide linker sequence (e.g., SEQ ID NO: 44) and hinge linker sequence (SEQ ID NO: 118), and 4) an Fc domain (e.g., SEQ ID NOs: 39, 41 or 43) fused to the hybrid soluble ActRIIB-ECD polypeptide sequence by the peptide/hinge linker.

[0197] Examples of various embodiments of the present disclosure include, but are not limited to, the hybrid ActRIIB ligand trap proteins described in Table 2.

Table 2

Hybrid Soluble ActRIIB-ECD polypeptide (SEQ ID NO)	**Linker and Hinge Linker (SEQ ID NO)**	**Heterologous Protein (SEQ ID NO)**	**Leader Sequence (SEQ ID NO)**
SEQ ID NO: 3	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 4	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 5	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 6	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50

SEQ ID NO: 7	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 8	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 9	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 10	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 11	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 12	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 13	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 14	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 15	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 16	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 17	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 18	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 19	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 20	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 21	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 22	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 23	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 24	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 25	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 26	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 27	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 28	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 29	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 30	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 31	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 32	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 33	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 34	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 35	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 36	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 37	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 51	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 52	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 53	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 54	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 55	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 56	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 57	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50

SEQ ID NO: 58	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 59	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 60	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 61	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 62	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 63	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 64	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 65	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 66	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 67	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 68	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 69	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 70	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 71	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 72	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 73	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 74	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 75	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 76	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 77	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 78	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 79	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 80	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 81	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 82	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 83	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 84	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 85	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 86	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 87	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 88	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 89	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 90	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 91	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 92	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 93	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 94	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 95	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50

SEQ ID NO: 96	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 97	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 98	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 99	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 100	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 101	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 102	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 103	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 104	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 105	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 106	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 107	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 108	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 109	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 110	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 111	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 112	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 113	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 114	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 115	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 116	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50
SEQ ID NO: 117	SEQ ID NOs: 44 and 118	SEQ ID NO: 39 or 41 or 43	SEQ ID NO: 49 or 50

Polynucleotides

[0198] In another aspect, the present disclosure provides isolated nucleic acid molecules comprising a polynucleotide encoding a hybrid soluble ActRIIB-ECD polypeptide of the present disclosure. The subject nucleic acids may be single-stranded or double stranded. Such nucleic acids may be DNA or RNA molecules. DNA includes, for example, cDNA, genomic DNA, synthetic DNA, DNA amplified by PCR, and combinations thereof. Genomic DNA encoding ActRIIB polypeptides is obtained from genomic libraries which are available for a number of species. Synthetic DNA is available from chemical synthesis of overlapping oligonucleotide fragments followed by assembly of the fragments to reconstitute part or all of the coding regions and flanking sequences. RNA may be obtained from prokaryotic expression vectors which direct high-level synthesis of mRNA, such as vectors using T7 promoters and

RNA polymerase. cDNA is obtained from libraries prepared from mRNA isolated from various tissues that express ActRIIB. The DNA molecules of the disclosure include full-length genes as well as polynucleotides and fragments thereof. The full-length gene may also include sequences encoding the N-terminal signal sequence.

[0199] Such nucleic acids may be used, for example, in methods for making the novel hybrid soluble ActRIIB-ECD polypeptides. In various embodiments, the polynucleotides encodes any one of the polypeptide sequences set forth in SEQ ID NOs: 3-37 or 51-117, wherein the hybrid ActRIIB-ECD polypeptide is capable of binding myostatin and activin A, but demonstrates a decreased binding affinity for BMP9 relative to a wild-type ActRIIB-ECD polypeptide. In various embodiments, the polynucleotides encode a polypeptide having an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to any one of the polypeptides sequences set forth in SEQ ID NOs: 3-37 or 51-117, wherein the hybrid ActRIIB-ECD polypeptide is capable of binding myostatin and activin A, but demonstrates a decreased binding affinity for BMP9 relative to a wild-type ActRIIB-ECD polypeptide. In various embodiments, the polynucleotides encode a polypeptide having at least 90% identity to any one of the polypeptides sequences set forth in SEQ ID NOs: 3-37 or 51-117, wherein the hybrid ActRIIB-ECD polypeptide is capable of binding myostatin and activin A, but demonstrates a decreased binding affinity for BMP9 relative to a wild-type ActRIIB-ECD polypeptide. In various embodiments, the polynucleotides encode a polypeptide having an amino acid sequence at least 95% identity to any one of the polypeptides sequences set forth in SEQ ID NOs: 3-37 or 51-117, wherein the hybrid ActRIIB-ECD polypeptide is capable of binding myostatin and activin A, but demonstrates a decreased binding affinity for BMP9 relative to a wild-type ActRIIB-ECD polypeptide. In various embodiments, the nucleic acid sequences of the present disclosure can be isolated, recombinant, and/or fused with a heterologous nucleotide sequence, or in a DNA library.

[0200] In various embodiments, the present disclosure provides nucleic acid molecules which hybridize under stringent or moderate conditions with the polypeptide-encoding regions of the polynucleotides described herein, wherein the encoded polypeptide comprises an amino acid sequence as set forth in SEQ ID NOs: 3-37 or 51-117 and wherein the encoded polypeptide is capable of binding myostatin and activin A, but demonstrates a decreased

binding affinity for BMP9 relative to a wild-type ActRIIB-ECD polypeptide. 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.

[0201] In various embodiments, the isolated nucleic acid molecules comprise the polynucleotides described herein, and further comprise a polynucleotide encoding at least one heterologous protein described herein. In various embodiments, the nucleic acid molecules further comprise polynucleotides encoding the linkers or hinge linkers described herein.

[0202] In various embodiments, the recombinant nucleic acids of the present disclosure may be operably linked to one or more regulatory nucleotide sequences in an expression construct. Regulatory sequences are art-recognized and are selected to direct expression of the hybrid soluble ActRIIB-ECD 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, Calif. (1990). 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 present 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 various 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.

[0203] In another aspect of the present disclosure, the subject nucleic acid is provided in an expression vector comprising a nucleotide sequence encoding a hybrid soluble ActRIIB-ECD polypeptide and operably linked to at least one regulatory sequence. The term "expression vector" refers to a plasmid, phage, virus or vector for expressing a polypeptide from a polynucleotide sequence. Vectors suitable for expression in host cells are readily available and the nucleic acid molecules are inserted into the vectors using standard recombinant DNA techniques. Such vectors can include 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 hybrid soluble ActRIIB-ECD polypeptide. Such useful expression control sequences, include, for example, the early and late promoters of SV40, tet promoter, adenovirus or cytomegalovirus immediate early promoter, RSV promoters, the lac system, the trp system, the TAC or TRC system, T7 promoter whose expression is directed by T7 RNA polymerase, the major operator and promoter regions of phage lambda , the control regions for fd coat protein, the promoter for 3-phosphoglycerate kinase or other glycolytic enzymes, the promoters of acid phosphatase, e.g., PhoS, the promoters of the yeast a-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. An exemplary expression vector suitable for expression of vActRIIB is the pDSRa, (described in WO 90/14363, herein incorporated by reference) and its derivatives, containing vActRIIB polynucleotides, as well as any additional suitable vectors known in the art or described below.

[0204] 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 ActRIIB polypeptide include plasmids and other vectors. For instance, suitable vectors include plasmids of the types: pBR322-derived plasmids, pEMBL-derived plasmids, pEX-derived plasmids, pBTac-derived plasmids and pUC-derived plasmids for expression in prokaryotic cells, such as *E. coli*.

[0205] Some mammalian expression vectors contain both prokaryotic sequences to facilitate the propagation of the vector in bacteria, and one or more eukaryotic transcription units that are expressed in eukaryotic cells. The pcDNAI/amp, pcDNAI/neo, pRc/CMV, pSV2gpt, pSV2neo, pSV2-dhfr, pTk2, pRSVneo, pMSG, pSVT7, pko-neo and pHyg derived vectors are examples of mammalian expression vectors suitable for transfection of eukaryotic cells. Some of these vectors are modified with sequences from bacterial plasmids, such as pBR322, to facilitate replication and drug resistance selection in both prokaryotic and eukaryotic cells. Alternatively, derivatives of viruses such as the bovine papilloma virus (BPV-1), or Epstein-Barr virus (pHEBo, pREP-derived and p205) can be used for transient expression of proteins in eukaryotic cells. Examples of other viral (including retroviral) expression systems can be found below in the description of gene therapy delivery systems. The various methods employed in the preparation of the plasmids and in transformation of host organisms are well known in the art. For other suitable expression systems for both prokaryotic and eukaryotic cells, as well as general recombinant procedures, see Molecular Cloning A Laboratory Manual, 2nd Ed., ed. by Sambrook, Fritsch and Maniatis (Cold Spring Harbor Laboratory Press, 1989) Chapters 16 and 17. 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 B-gal containing pBlueBac III).

[0206] In various embodiments, a vector will be designed for production of the subject hybrid soluble ActRIIB-ECD 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, Wis.). As will be apparent, the subject gene constructs can be used to cause expression of the subject hybrid soluble ActRIIB-ECD polypeptides in cells propagated in culture, e.g., to produce proteins, including fusion proteins or variant proteins, for purification.

[0207] This present disclosure also pertains to a host cell transfected with a recombinant gene including a nucleotide sequence coding an amino acid sequence (e.g., SEQ ID NOs: 3-37 or 51-117) for one or more of the subject hybrid soluble ActRIIB-ECD polypeptide. The host cell may be any prokaryotic or eukaryotic cell. For example, a hybrid soluble ActRIIB-ECD polypeptide of the present disclosure may be expressed in bacterial cells such as *E. coli*, insect cells (e.g., using a baculovirus expression system), yeast, or mammalian cells. Other suitable host cells are known to those skilled in the art.

[0208] Accordingly, the present disclosure further pertains to methods of producing the subject hybrid soluble ActRIIB-ECD polypeptides. For example, a host cell transfected with an expression vector encoding a hybrid soluble ActRIIB-ECD polypeptide can be cultured under appropriate conditions to allow expression of the hybrid soluble ActRIIB-ECD polypeptide to occur. The hybrid soluble ActRIIB-ECD polypeptide may be secreted and isolated from a mixture of cells and medium containing the hybrid soluble ActRIIB-ECD polypeptide. Alternatively, the hybrid soluble ActRIIB-ECD polypeptide may be retained cytoplasmically or in a membrane fraction and the cells harvested, lysed and the protein isolated. A cell culture includes host cells, media and other byproducts. Suitable media for cell culture are well known in the art.

[0209] The polypeptides and proteins of the present disclosure can be purified according to protein purification techniques are well known to those of skill in the art. These techniques involve, at one level, the crude fractionation of the proteinaceous and non-proteinaceous fractions. Having separated the peptide polypeptides from other proteins, the peptide or polypeptide of interest can be further purified using chromatographic and electrophoretic techniques to achieve partial or complete purification (or purification to homogeneity). The term "isolated polypeptide" or "purified polypeptide" as used herein, is intended to refer to a composition, isolatable from other components, wherein the polypeptide is purified to any degree relative to its naturally-obtainable state. A purified polypeptide therefore also refers to a polypeptide that is free from the environment in which it may naturally occur. Generally, "purified" will refer to a polypeptide composition that has been subjected to fractionation to remove various other components, and which composition substantially retains its expressed biological activity. Where the term "substantially purified" is used, this designation

will refer to a peptide or polypeptide composition in which the polypeptide or peptide forms the major component of the composition, such as constituting about 50%, about 60%, about 70%, about 80%, about 85%, or about 90% or more of the proteins in the composition.

[0210] Various techniques suitable for use in purification will be well known to those of skill in the art. These include, for example, precipitation with ammonium sulphate, PEG, antibodies (immunoprecipitation) and the like or by heat denaturation, followed by centrifugation; chromatography such as affinity chromatography (Protein-A columns), ion exchange, gel filtration, reverse phase, hydroxylapatite, hydrophobic interaction chromatography; isoelectric focusing; gel electrophoresis; and combinations of these techniques. As is generally known in the art, it is believed that the order of conducting the various purification steps may be changed, or that certain steps may be omitted, and still result in a suitable method for the preparation of a substantially purified polypeptide.

Pharmaceutical Compositions

[0211] In another aspect, the present disclosure provides a pharmaceutical composition comprising the isolated hybrid soluble ActRIIB polypeptides or hybrid ActRIIB ligand trap proteins in admixture with a pharmaceutically acceptable carrier. Such pharmaceutically acceptable carriers are well known and understood by those of ordinary skill and have been extensively described (see, e.g., Remington's Pharmaceutical Sciences, 18th Edition, A. R. Gennaro, ed., Mack Publishing Company, 1990). The pharmaceutically acceptable carriers may be included for purposes of modifying, maintaining or preserving, for example, the pH, osmolarity, viscosity, clarity, color, isotonicity, odor, sterility, stability, rate of dissolution or release, adsorption or penetration of the composition. Such pharmaceutical compositions may influence the physical state, stability, rate of *in vivo* release, and rate of *in vivo* clearance of the polypeptide. Suitable pharmaceutically acceptable carriers include, but are not limited to, amino acids (such as glycine, glutamine, asparagine, arginine or lysine); antimicrobials; antioxidants (such as ascorbic acid, sodium sulfite or sodium hydrogen-sulfite); buffers (such as borate, bicarbonate, Tris-HCl, citrates, phosphates, other organic acids); bulking agents (such as mannitol or glycine), chelating agents (such as ethylenediamine tetraacetic acid (EDTA));

complexing agents (such as caffeine, polyvinylpyrrolidone, beta-cyclodextrin or hydroxypropyl-beta-cyclodextrin); fillers; monosaccharides; disaccharides and other carbohydrates (such as glucose, mannose, or dextrins); proteins (such as serum albumin, gelatin or immunoglobulins); coloring; flavoring and diluting agents; emulsifying agents; hydrophilic polymers (such as polyvinylpyrrolidone); low molecular weight polypeptides; salt-forming counter ions (such as sodium); preservatives (such as benzalkonium chloride, benzoic acid, salicylic acid, thimerosal, phenethyl alcohol, methylparaben, propylparaben, chlorhexidine, sorbic acid or hydrogen peroxide); solvents (such as glycerin, propylene glycol or polyethylene glycol); sugar alcohols (such as mannitol or sorbitol); suspending agents; surfactants or wetting agents (such as pluronics, PEG, sorbitan esters, polysorbates such as polysorbate 20, polysorbate 80, triton, tromethamine, lecithin, cholesterol, tyloxapal); stability enhancing agents (sucrose or sorbitol); tonicity enhancing agents (such as alkali metal halides (preferably sodium or potassium chloride, mannitol sorbitol); delivery vehicles; diluents; excipients and/or pharmaceutical adjuvants.

[0212] The primary vehicle or carrier in a pharmaceutical composition may be either aqueous or non-aqueous in nature. For example, a suitable vehicle or carrier may be water for injection, physiological saline solution or artificial cerebrospinal fluid, possibly supplemented with other materials common in compositions for parenteral administration. Neutral buffered saline or saline mixed with serum albumin are further exemplary vehicles. Other exemplary pharmaceutical compositions comprise Tris buffer of about pH 7.0-8.5, or acetate buffer of about pH 4.0-5.5, which may further include sorbitol or a suitable substitute thereof. In one embodiment of the present disclosure, compositions may be prepared for storage by mixing the selected composition having the desired degree of purity with optional formulation agents (Remington's Pharmaceutical Sciences, supra) in the form of a lyophilized cake or an aqueous solution. Further, the therapeutic composition may be formulated as a lyophilizate using appropriate excipients such as sucrose. The optimal pharmaceutical composition will be determined by one of ordinary skill in the art depending upon, for example, the intended route of administration, delivery format, and desired dosage.

[0213] When parenteral administration is contemplated, the therapeutic pharmaceutical compositions may be in the form of a pyrogen-free, parenterally acceptable aqueous solution

comprising the desired ActRIIB polypeptide in a pharmaceutically acceptable vehicle. A particularly suitable vehicle for parenteral injection is sterile distilled water in which a polypeptide is formulated as a sterile, isotonic solution, properly preserved. In various embodiments, pharmaceutical formulations suitable for injectable administration may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks' solution, Ringer's solution, or physiologically buffered saline. Aqueous injection suspensions may contain substances that increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Optionally, the suspension may also contain suitable stabilizers or agents to increase the solubility of the compounds and allow for the preparation of highly concentrated solutions.

[0214] In various embodiments, the therapeutic pharmaceutical compositions may be formulated for targeted delivery using a colloidal dispersion system. Colloidal dispersion systems include macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. Examples of lipids useful in liposome production include phosphatidyl compounds, such as phosphatidylglycerol, phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine, sphingolipids, cerebrosides, and gangliosides. Illustrative phospholipids include egg phosphatidylcholine, dipalmitoylphosphatidylcholine, and distearoylphosphatidylcholine. The targeting of liposomes is also possible based on, for example, organ-specificity, cell-specificity, and organelle-specificity and is known in the art.

[0215] In various embodiments, oral administration of the pharmaceutical compositions is contemplated. Pharmaceutical compositions that are administered in this fashion can be formulated with or without those carriers customarily used in the compounding of solid dosage forms such as tablets and capsules. In solid dosage forms for oral administration (capsules, tablets, pills, dragees, powders, granules, and the like), one or more therapeutic compounds of the present disclosure may be mixed with one or more pharmaceutically acceptable carriers, such as sodium citrate or dicalcium phosphate, and/or any of the following: (1) fillers or extenders, such as starches, lactose, sucrose, glucose, mannitol, and/or silicic acid; (2) binders, such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinyl pyrrolidone, sucrose,

and/or acacia; (3) humectants, such as glycerol; (4) disintegrating agents, such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate; (5) solution retarding agents, such as paraffin; (6) absorption accelerators, such as quaternary ammonium compounds; (7) wetting agents, such as, for example, cetyl alcohol and glycerol monostearate; (8) absorbents, such as kaolin and bentonite clay; (9) lubricants, such a talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof; and (10) coloring agents. In the case of capsules, tablets and pills, the pharmaceutical compositions may also comprise buffering agents. Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugars, as well as high molecular weight polyethylene glycols and the like. Liquid dosage forms for oral administration include pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups, and elixirs. In addition to the active ingredient, the liquid dosage forms may contain inert diluents commonly used in the art, such as water or other solvents, solubilizing agents and emulsifiers, such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor, and sesame oils), glycerol, tetrahydrofuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof Besides inert diluents, the oral compositions can also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, coloring, perfuming, and preservative agents.

[0216] In various embodiments, topical administration of the pharmaceutical compositions, either to skin or to mucosal membranes, is contemplated. The topical formulations may further include one or more of the wide variety of agents known to be effective as skin or stratum corneum penetration enhancers. Examples of these are 2-pyrrolidone, N-methyl-2-pyrrolidone, dimethylacetamide, dimethylformamide, propylene glycol, methyl or isopropyl alcohol, dimethyl sulfoxide, and azone. Additional agents may further be included to make the formulation cosmetically acceptable. Examples of these are fats, waxes, oils, dyes, fragrances, preservatives, stabilizers, and surface active agents. Keratolytic agents such as those known in the art may also be included. Examples are salicylic acid and sulfur. Dosage forms for the topical or transdermal administration include powders, sprays, ointments, pastes,

creams, lotions, gels, solutions, patches, and inhalants. The active compound may be mixed under sterile conditions with a pharmaceutically acceptable carrier, and with any preservatives, buffers, or propellants which may be required. The ointments, pastes, creams and gels may contain, in addition to a subject compound of the disclosure (e.g., a hybrid ActRIIB ligand trap), excipients, such as animal and vegetable fats, oils, waxes, paraffins, starch, tragacanth, cellulose derivatives, polyethylene glycols, silicones, bentonites, silicic acid, talc and zinc oxide, or mixtures thereof.

[0217] Additional pharmaceutical compositions contemplated for use herein include formulations involving polypeptides in sustained- or controlled-delivery formulations. Techniques for formulating a variety of other sustained- or controlled-delivery means, such as liposome carriers, bio-erodible microparticles or porous beads and depot injections, are also known to those skilled in the art.

[0218] An effective amount of a pharmaceutical composition to be employed therapeutically will depend, for example, upon the therapeutic context and objectives. One skilled in the art will appreciate that the appropriate dosage levels for treatment will thus vary depending, in part, upon the molecule delivered, the indication for which the polypeptide is being used, the route of administration, and the size (body weight, body surface or organ size) and condition (the age and general health) of the patient. Accordingly, the clinician may titer the dosage and modify the route of administration to obtain the optimal therapeutic effect. A typical dosage may range from about 0.1 mg/kg to up to about 100 mg/kg or more, depending on the factors mentioned above. Polypeptide compositions may be preferably injected or administered intravenously. Long-acting pharmaceutical compositions may be administered every three to four days, every week, or biweekly depending on the half-life and clearance rate of the particular formulation. The frequency of dosing will depend upon the pharmacokinetic parameters of the polypeptide in the formulation used. Typically, a composition is administered until a dosage is reached that achieves the desired effect. The composition may therefore be administered as a single dose, or as multiple doses (at the same or different concentrations/dosages) over time, or as a continuous infusion. Further refinement of the appropriate dosage is routinely made. Appropriate dosages may be ascertained through use of appropriate dose-response data.

[0219] The route of administration of the pharmaceutical composition is in accord with known methods, e.g. orally, through injection by intravenous, intraperitoneal, intracerebral (intra-parenchymal), intracerebroventricular, intramuscular, intra-ocular, intraarterial, intraportal, intralesional routes, intramedullary, intrathecal, intraventricular, transdermal, subcutaneous, or intraperitoneal; as well as intranasal, enteral, topical, sublingual, urethral, vaginal, or rectal means, by sustained release systems or by implantation devices. Where desired, the compositions may be administered by bolus injection or continuously by infusion, or by implantation device. Alternatively or additionally, the composition may be administered locally via implantation of a membrane, sponge, or another appropriate material on to which the desired molecule has been absorbed or encapsulated. Where an implantation device is used, the device may be implanted into any suitable tissue or organ, and delivery of the desired molecule may be via diffusion, timed-release bolus, or continuous administration.

Therapeutic Uses

[0220] In another aspect, the present disclosure provides a method for treating myostatin-related or activin A-related disorders in a subject, comprising administering to said subject a therapeutically effective amount (either as monotherapy or in a combination therapy regimen) of a hybrid ActRIIB ligand trap of the present disclosure in pharmaceutically acceptable carrier. Importantly, the pharmaceutical compositions of the present disclosure can be used to increase lean muscle mass as a percentage of body weight and decrease fat mass as percentage of body weight, while avoiding safety concerns reported for existing ActRIIB-Fc fusion protein-based therapeutics.

[0221] The present disclosure provides a method for treating muscle wasting disease in a subject, comprising administering to said subject a therapeutically effective amount (either as monotherapy or in a combination therapy regimen) of a hybrid ActRIIB ligand trap of the present disclosure in pharmaceutically acceptable carrier, wherein such administration attenuates the loss of muscle mass and/or loss of muscle function. Specifically, a hybrid ActRIIB ligand trap of the present disclosure is useful in treating various muscle diseases, including, but not limited to, muscular dystrophies (such as DMD, Becker MD, Limb-Girdle MD, Myotonic MD and FSHD),

myositis, myopathies (including inherited myopathy and acquired myopathy), motoneuron diseases (such as Lou Gehrig's Disease or ALS), neurodegenerative diseases (such as Parkinson's disease, Huntington's disease and Alzheimer's disease), muscle wasting associated with cancers (such as pancreatic cancer, lung cancer, gastric cancer, ovarian cancer, colorectal cancer, melanoma leukemia, lung cancer, prostate cancer, brain cancer, bladder cancer, and head-neck cancer), muscle wasting associated with chronic heart failure, chronic kidney disease (CKD), diabetes, chronic obstructive pulmonary disease (COPD), infections (such as AIDS, tuberculosis, and sepsis), rheumatoid arthritis, trauma (such as burns or motorcycle accident), ICU critical care, denervation (such as stoke or spinal cord injury), prolonged bed rest, sarcopenic obesity, and age-associated sarcopenia.

[0222] The present disclosure provides for a method of treating cardiovascular disease in a subject, comprising administering to said subject a therapeutically effective amount (either as monotherapy or in a combination therapy regimen) of a hybrid ActRIIB ligand trap of the present disclosure in pharmaceutically acceptable carrier, wherein such administration attenuates the loss of muscle mass and/or loss of muscle function. Specifically, a hybrid ActRIIB ligand trap of the present disclosure is useful in treating heart failure, cardiac atrophy, hypertension, myocarditis, coronary artery disease, myocardial infarction, cardiac arrhythmias, heart valve disease, cardiomyopathy, pericardial disease, aorta disease and Marfan syndrome.

[0223] The present disclosure provides for a method of treating cardiac dysfunction or heart failure in a subject comprising administering an effective amount of a hybrid ActRIIB ligand trap into the subject. The modulation may improve cardiac function of said subject by at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95%. The improvement of cardiac function can be evaluated by echocardiography to measure 1) cardiac pump functions focusing on the ejected blood volume and the efficiency of ejection and 2) myocardial functions focusing on the strength of myocardial contraction.

[0224] The present disclosure provides for methods for treating metabolic disorders in a subject, comprising administering to said subject a therapeutically effective amount (either as monotherapy or in a combination therapy regimen) of a hybrid ActRIIB ligand trap of the present

disclosure in pharmaceutically acceptable carrier. Specifically, a hybrid ActRIIB ligand trap of the present disclosure is useful in treating a metabolic disease selected from obesity, dyslipidemia, diabetes, insulin resistance, sarcopenic obesity, steatosis, and metabolic syndrome, as well as diabetic myopathy, nephropathy, neuropathy, retinopathy, bone loss, impaired glucose tolerance, hyperglycemia, and androgen deprivation.

[0225] The present disclosure provides for a method of treating cancer cells in a subject, comprising administering to said subject a therapeutically effective amount (either as monotherapy or in a combination therapy regimen) of a hybrid ActRIIB ligand trap of the present disclosure in pharmaceutically acceptable carrier, wherein such administration inhibits the growth and/or proliferation of a cancer cell. Specifically, a hybrid ActRIIB ligand trap of the present disclosure is useful in treating disorders characterized as cancer. Such disorders include, but are not limited to solid tumors, such as cancers of the breast, respiratory tract, brain, reproductive organs, digestive tract, urinary tract, eye, liver, skin, head and neck, thyroid, parathyroid and their distant metastases, lymphomas, sarcomas, multiple myeloma and leukemia. Examples of breast cancer include, but are not limited to invasive ductal carcinoma, invasive lobular carcinoma, ductal carcinoma in situ, and lobular carcinoma in situ. Examples of cancers of the respiratory tract include, but are not limited to small-cell and non-small-cell lung carcinoma, as well as bronchial adenoma and pleuropulmonary blastoma. Examples of brain cancers include, but are not limited to brain stem and hypophthalmic glioma, cerebellar and cerebral astrocytoma, medulloblastoma, ependymoma, as well as neuroectodermal and pineal tumor. Tumors of the male reproductive organs include, but are not limited to prostate and testicular cancer. Tumors of the female reproductive organs include, but are not limited to endometrial, cervical, ovarian, vaginal, and vulvar cancer, as well as sarcoma of the uterus. Tumors of the digestive tract include, but are not limited to anal, colon, colorectal, esophageal, gallbladder, gastric, pancreatic, rectal, small-intestine, and salivary gland cancers. Tumors of the urinary tract include, but are not limited to bladder, penile, kidney, renal pelvis, ureter, and urethral cancers. Eye cancers include, but are not limited to intraocular melanoma and retinoblastoma. Examples of liver cancers include, but are not limited to hepatocellular carcinoma (liver cell carcinomas with or without fibrolamellar variant), cholangiocarcinoma (intrahepatic bile duct carcinoma), and mixed hepatocellular cholangiocarcinoma. Skin cancers

include, but are not limited to squamous cell carcinoma, Kaposi's sarcoma, malignant melanoma, Merkel cell skin cancer, and non-melanoma skin cancer. Head-and-neck cancers include, but are not limited to nasopharyngeal cancer, and lip and oral cavity cancer. Lymphomas include, but are not limited to AIDS-related lymphoma, non-Hodgkin's lymphoma, cutaneous T-cell lymphoma, Hodgkin's disease, and lymphoma of the central nervous system. Sarcomas include, but are not limited to sarcoma of the soft tissue, osteosarcoma, malignant fibrous histiocytoma, lymphosarcoma, and rhabdomyosarcoma. Leukemias include, but are not limited to acute myeloid leukemia, acute lymphoblastic leukemia, chronic lymphocytic leukemia, chronic myelogenous leukemia, and hairy cell leukemia. In certain embodiments, the cancer will be a cancer with high expression of TGF-β family member, such as activin A, myostatin, TGF-β and GDF15, e.g., pancreatic cancer, gastric cancer, ovarian cancer, colorectal cancer, melanoma leukemia, lung cancer, prostate cancer, brain cancer, bladder cancer, and head-neck cancer.

[0226] The present disclosure provides for a method of treating chronic kidney disease (CKD) in a subject, comprising administering to said subject a therapeutically effective amount (either as monotherapy or in a combination therapy regimen) of a hybrid ActRIIB ligand trap of the present disclosure in pharmaceutically acceptable carrier, wherein such administration attenuates the loss of muscle mass and/or loss of muscle function or inhibits kidney fibrosis. Specifically, a hybrid ActRIIB ligand trap of the present disclosure is useful in treating CKD including renal failure, interstitial fibrosis, and kidney dialysis as well as protein energy wasting (PEW) associated with CKD. The modulation may improve CKD or PEW of said subject by at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95%. The improvement of renal function can be evaluated by measuring protein/creatinine ratio (PCR) in the urine and glomerular filtration rate (GFR). Improvement of PEW can be evaluated by measuring serum levels of albumin and inflammatory cytokines, rate of protein synthesis and degradation, body mass, muscle mass, physical activity and nutritional outcomes.

[0227] The present disclosure provides for methods for treating autoimmune disease in a subject, comprising administering to said subject a therapeutically effective amount (either as

monotherapy or in a combination therapy regimen) of a hybrid ActRIIB ligand trap of the present disclosure in pharmaceutically acceptable carrier. Specifically, a hybrid ActRIIB ligand trap of the present disclosure is useful in treating an autoimmune disorder selected from multiple sclerosis, diabetes (type-1), glomerulonephritis, myasthenia gravis, psoriasis, systemic sclerosis and systemic lupus erythematosus, polymyositis and primary biliary cirrhosis.

[0228] The present disclosure provides for methods for treating arthritis in a subject, comprising administering to said subject a therapeutically effective amount (either as monotherapy or in a combination therapy regimen) of a hybrid ActRIIB ligand trap of the present disclosure in pharmaceutically acceptable carrier. Specifically, a hybrid ActRIIB ligand trap of the present disclosure is useful in treating an arthritis selected from rheumatoid arthritis and osteoarthritis.

[0229] The present disclosure provides for methods for treating anorexia in a subject, comprising administering to said subject a therapeutically effective amount (either as monotherapy or in a combination therapy regimen) of a hybrid ActRIIB ligand trap of the present disclosure in pharmaceutically acceptable carrier. Specifically, a hybrid ActRIIB ligand trap of the present disclosure is useful in treating an anorexia selected from anorexia nervosa and anorexia-cachexia syndrome.

[0230] The present disclosure provides for methods for treating liver disease in a subject, comprising administering to said subject a therapeutically effective amount (either as monotherapy or in a combination therapy regimen) of a hybrid ActRIIB ligand trap of the present disclosure in pharmaceutically acceptable carrier. Specifically, a hybrid ActRIIB ligand trap of the present disclosure is useful in treating a liver disease selected from non-alcoholic fatty liver disease, non-alcoholic steatohepatitis, alcoholic fatty liver disease, liver cirrhosis, liver failure, autoimmune hepatitis and hepatocellular carcinoma.

[0231] The present disclosure provides for methods for organ or tissue transplantation in a subject, comprising administering to said subject a therapeutically effective amount (either as monotherapy or in a combination therapy regimen) of a hybrid ActRIIB ligand trap of the present disclosure in pharmaceutically acceptable carrier. Specifically, a hybrid ActRIIB ligand trap of the present disclosure is useful in treating a transplantation selected from organ transplantations

of the heart, kidneys, liver, lungs, pancreas, intestine and thymus or from tissues transplantations of the bones, tendons, cornea, skin, heart valves, nerves and veins.

[0232] The present disclosure provides for methods for treating anemia in a subject, comprising administering to said subject a therapeutically effective amount (either as monotherapy or in a combination therapy regimen) of a hybrid ActRIIB ligand trap of the present disclosure in pharmaceutically acceptable carrier. In various embodiments, the anemia is selected from various anemia disorders including cancer-associated anemia, chemotherapy-induced anemia, chronic kidney disease-associated anemia, iron-deficiency anemia, thalassemia, sickle cell disease, aplastic anemia and myelodysplastic syndromes.

[0233] The present disclosure provides methods for treating fibrosis in a subject, comprising administering a therapeutically effective amount of the pharmaceutical compositions of the invention to a subject in need thereof. In one embodiment, the subject is a human subject. In various embodiments, the fibrosis is selected from pulmonary fibrosis (such as idiopathic pulmonary fibrosis and cystic fibrosis), liver fibrosis (such as non-alcoholic steatohepatitis and liver cirrhosis), cardiac fibrosis (such as myocardial infarction, diastolic dysfunction or cardiac valve disease), renal fibrosis (such as interstitial fibrosis), myelofibrosis, idiopathic retroperitoneal fibrosis, nephrogenic fibrosing dermopathy, Crohn's Disease, keloid, scleroderma, systemic sclerosis, and arthrofibrosis.

[0234] The present disclosure provides methods of treating pain in a subject, comprising administering a therapeutically effective amount of the pharmaceutical compositions of the invention to a subject in need thereof. In one embodiment, the subject is a human subject. In various embodiments, the pain is selected from neuropathic pain, inflammatory pain, or cancer pain.

[0235] The present disclosure provides methods of treating bone disease in a subject, comprising administering a therapeutically effective amount of the pharmaceutical compositions of the invention to a subject in need thereof. In one embodiment, the subject is a human subject. In various embodiments, the bone disease is selected from osteomalacia, osteoporosis, osteogenesis imperfecta, fibrodysplasia ossificans progressive, corticosteroid-induced bone loss, bone fracture, and bone metastasis.

[0236] The present disclosure provides for a method of inhibiting loss of muscle mass and/or muscle function in a subject comprising administering an effective amount of a hybrid ActRIIB ligand trap into the subject. The modulation may attenuate the loss of the muscle mass and/or function of said subject by at least 5%, 10%, at least 25%, at least 50%, at least 75%, or at least 90%. The inhibition of loss of muscle mass and function can be evaluated by using imaging techniques and physical strength tests. Examples of imaging techniques for muscle mass evaluation include Dual-Energy X-Ray Absorptiometry (DEXA), Magnetic Resonance Imaging (MRI), and Computed Tomography (CT). Examples of muscle function tests include grip strength test, stair climbing test, short physical performance battery (SPPB) and 6-minute walk, as well as maximal inspiratory pressure (MIP) and maximal expiratory pressure (MEP) that are used to measure respiratory muscle strength.

[0237] "Therapeutically effective amount" or “therapeutically effective dose” refers to that amount of the therapeutic agent being administered which will relieve to some extent one or more of the symptoms of the disorder being treated.

[0238] A therapeutically effective dose can be estimated initially from cell culture assays by determining an IC_{50}. A dose can then be formulated in animal models to achieve a circulating plasma concentration range that includes the IC_{50} as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by HPLC. The exact composition, route of administration and dosage can be chosen by the individual physician in view of the subject's condition.

[0239] Dosage regimens can be adjusted to provide the optimum desired response (e.g., a therapeutic or prophylactic response). For example, a single bolus can be administered, several divided doses (multiple or repeat or maintenance) can be administered over time and the dose can be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier.

The specification for the dosage unit forms of the present disclosure will be dictated primarily by the unique characteristics of the antibody and the particular therapeutic or prophylactic effect to be achieved.

[0240] Thus, the skilled artisan would appreciate, based upon the disclosure provided herein, that the dose and dosing regimen is adjusted in accordance with methods well-known in the therapeutic arts. That is, the maximum tolerable dose can be readily established, and the effective amount providing a detectable therapeutic benefit to a subject may also be determined, as can the temporal requirements for administering each agent to provide a detectable therapeutic benefit to the subject. Accordingly, while certain dose and administration regimens are exemplified herein, these examples in no way limit the dose and administration regimen that may be provided to a subject in practicing the present disclosure.

[0241] It is to be noted that dosage values may vary with the type and severity of the condition to be alleviated, and may include single or multiple doses. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that dosage ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition. Further, the dosage regimen with the compositions of this disclosure may be based on a variety of factors, including the type of disease, the age, weight, sex, medical condition of the subject, the severity of the condition, the route of administration, and the particular antibody employed. Thus, the dosage regimen can vary widely, but can be determined routinely using standard methods. For example, doses may be adjusted based on pharmacokinetic or pharmacodynamic parameters, which may include clinical effects such as toxic effects and/or laboratory values. Thus, the present disclosure encompasses intra-subject dose-escalation as determined by the skilled artisan. Determining appropriate dosages and regimens are well-known in the relevant art and would be understood to be encompassed by the skilled artisan once provided the teachings disclosed herein.

[0242] An exemplary, non-limiting daily dosing range for a therapeutically or prophylactically effective amount of a hybrid ActRIIB ligand trap of the disclosure can be 0.001 to 100 mg/kg, 0.001 to 90 mg/kg, 0.001 to 80 mg/kg, 0.001 to 70 mg/kg, 0.001 to 60 mg/kg,

0.001 to 50 mg/kg, 0.001 to 40 mg/kg, 0.001 to 30 mg/kg, 0.001 to 20 mg/kg, 0.001 to 10 mg/kg, 0.001 to 5 mg/kg, 0.001 to 4 mg/kg, 0.001 to 3 mg/kg, 0.001 to 2 mg/kg, 0.001 to 1 mg/kg, 0.010 to 50 mg/kg, 0.010 to 40 mg/kg, 0.010 to 30 mg/kg, 0.010 to 20 mg/kg, 0.010 to 10 mg/kg, 0.010 to 5 mg/kg, 0.010 to 4 mg/kg, 0.010 to 3 mg/kg, 0.010 to 2 mg/kg, 0.010 to 1 mg/kg, 0.1 to 50 mg/kg, 0.1 to 40 mg/kg, 0.1 to 30 mg/kg, 0.1 to 20 mg/kg, 0.1 to 10 mg/kg, 0.1 to 5 mg/kg, 0.1 to 4 mg/kg, 0.1 to 3 mg/kg, 0.1 to 2 mg/kg, 0.1 to 1 mg/kg, 1 to 50 mg/kg, 1 to 40 mg/kg, 1 to 30 mg/kg, 1 to 20 mg/kg, 1 to 10 mg/kg, 1 to 5 mg/kg, 1 to 4 mg/kg, 1 to 3 mg/kg, 1 to 2 mg/kg, or 1 to 1 mg/kg body weight. It is to be noted that dosage values may vary with the type and severity of the conditions to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that dosage ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition.

[0243] In various embodiments, the total dose administered will achieve a plasma antibody concentration in the range of, e.g., about 1 to 1000 μg/ml, about 1 to 750 μg/ml, about 1 to 500 μg/ml, about 1 to 250 μg/ml, about 10 to 1000 μg/ml, about 10 to 750 μg/ml, about 10 to 500 μg/ml, about 10 to 250 μg/ml, about 20 to 1000 μg/ml, about 20 to 750 μg/ml, about 20 to 500 μg/ml, about 20 to 250 μg/ml, about 30 to 1000 μg/ml, about 30 to 750 μg/ml, about 30 to 500 μg/ml, about 30 to 250 μg/ml.

[0244] Toxicity and therapeutic index of the pharmaceutical compositions of the disclosure can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effective dose is the therapeutic index and it can be expressed as the ratio LD_{50}/ED_{50}. Compositions that exhibit large therapeutic indices are generally preferred.

[0245] The dosing frequency of the administration of the hybrid ActRIIB ligand trap pharmaceutical composition depends on the nature of the therapy and the particular disease being treated. The subject can be treated at regular intervals, such as weekly or monthly, until a desired therapeutic result is achieved. Exemplary dosing frequencies include, but are not limited to: once weekly without break; once weekly, every other week; once every 2 weeks; once every

3 weeks; weakly without break for 2 weeks, then monthly; weakly without break for 3 weeks, then monthly; monthly; once every other month; once every three months; once every four months; once every five months; or once every six months, or yearly.

Combination Therapy

[0246] As used herein, the terms "co-administration", "co-administered" and "in combination with", referring to the a hybrid ActRIIB ligand trap of the disclosure and one or more other therapeutic agents, is intended to mean, and does refer to and include the following: simultaneous administration of such combination of a hybrid ActRIIB ligand trap of the disclosure and therapeutic agent(s) to a subject in need of treatment, when such components are formulated together into a single dosage form which releases said components at substantially the same time to said subject; substantially simultaneous administration of such combination of a hybrid ActRIIB ligand trap of the disclosure and therapeutic agent(s) to a subject in need of treatment, when such components are formulated apart from each other into separate dosage forms which are taken at substantially the same time by said subject, whereupon said components are released at substantially the same time to said subject; sequential administration of such combination of a hybrid ActRIIB ligand trap of the disclosure and therapeutic agent(s) to a subject in need of treatment, when such components are formulated apart from each other into separate dosage forms which are taken at consecutive times by said subject with a significant time interval between each administration, whereupon said components are released at substantially different times to said subject; and sequential administration of such combination of a hybrid ActRIIB ligand trap of the disclosure and therapeutic agent(s) to a subject in need of treatment, when such components are formulated together into a single dosage form which releases said components in a controlled manner whereupon they are concurrently, consecutively, and/or overlappingly released at the same and/or different times to said subject, where each part may be administered by either the same or a different route.

[0247] In another aspect, the present disclosure relates to methods of treating muscle wasting diseases in a subject, comprising administration of a combination of a) a therapeutically effective amount of a hybrid ActRIIB ligand trap of the present disclosure; and b) a second

agent. This combination therapy may be particularly effective against a muscle wasting disease that is resistant or refractory to treatment using the second agent alone. In various embodiments, second agent is selected from growth hormone, ghrelin, IGF1, antagonists to inflammatory cytokines such as TNF-alpha and TNF-alpha, IL-6, IL-1 and their receptors, and other antagonists to myostatin and activin A and their receptors.

[0248] In various embodiments, the combination therapy comprises administering a hybrid ActRIIB ligand trap and the second agent composition simultaneously, either in the same pharmaceutical composition or in separate pharmaceutical composition. In various embodiments, a hybrid ActRIIB ligand trap composition and the second agent composition are administered sequentially, i.e., a hybrid ActRIIB ligand trap composition is administered either prior to or after the administration of the second agent composition.

[0249] In various embodiments, the administrations of a hybrid ActRIIB ligand trap composition and the second agent composition are concurrent, i.e., the administration period of a hybrid ActRIIB ligand trap composition and the second agent composition overlap with each other.

[0250] In various embodiments, the administrations of a hybrid ActRIIB ligand trap composition and the second agent composition are non-concurrent. For example, in various embodiments, the administration of a hybrid ActRIIB ligand trap composition is terminated before the second agent composition is administered. In various embodiments, the administration second agent composition is terminated before a hybrid ActRIIB ligand trap composition is administered.

[0251] The following examples are offered to more fully illustrate the disclosure, but are not construed as limiting the scope thereof.

Example 1

[0252] The polypeptides of the present disclosure can be prepared according to recombinant DNA techniques that are well known to those of skill in the art. In this example, the preparation of the hybrid soluble ActRIIB-ECD polypeptides is generally described.

[0253] Various hybrid ActRIIB-ECD polypeptides were designed by substituting multiple amino acid residues at selective positions within the human ActRIIB extracellular domain with amino acid residues derived from the human ActRIIA extracellular domain at corresponding positions based on sequence alignment at the amino acid level. DNA expression cassettes encoding the hybrid ActRIIB-ECD polypeptides were generated by using site-directed mutagenesis and subsequently engineered into Fc fusion protein constructs by placing in frame a cDNA fragment encoding human immunoglobulin light chain signal peptide at the 5' end and a DNA fragment encoding a peptide linker followed by human Fc at the 3' end.

Example 2

[0254] In this example, the preparation of the hybrid ActRIIB ligand trap proteins configured as depicted in FIG. 1 is generally described.

[0255] Synthetic DNA cassettes encoding various hybrid ActRIIB ligand trap proteins, each containing a signal peptide leader sequence (SEQ ID NO: 49 or 50), a hybrid soluble ActRIIB-ECD polypeptide from Example 1 (or a wild-type ActRIIB-ECD sequence), a peptide linker sequence (SEQ ID NO: 44), a hinge linker sequence (SEQ ID NO: 118) and an Fc domain sequence (SEQ ID NO: 39 or 41 or 43), are cloned into Freedom pCHO 1.0 and pcDNA3.1 expression vectors (Life Technologies).

[0256] For stable transfection, the pCHO 1.0 expression vectors encoding the various hybrid ActRIIB ligand trap proteins were each transfected in CHO-S cells using FreeStyle MAX Reagent (Life Technologies). 48 hours after transfection, the cells were grown in serum-free CD FortiCHO medium containing puromycin and methotrexate (MTX) selection for 3-7 weeks at 37°C in a shaker CO_2 incubator. The stable pool were generated until cells exceed 90% viability containing 30 M puromycin and 500 nM methotrexate. Stable clones were generated by dilution cloning following the manufacturer's recommended protocols (Life Technologies). For transient transfection, pcDNA3.1 expression plasmids encoding various hybrid ActRIIB ligand trap proteins were each transfected in Expi293 cells using ExpriFectamine293 transfection reagent (Life Technologies).

[0257] Following transfection, stably transfected CHO-S cells were grown in completed serum-free CD FortiCHO medium supplied with glucose at 37°C in a CO_2 shaker incubator for up to 14 days. The conditioned medium was collected for protein purification. Transiently transfected Expi293 cells were cultured in Expi293 expression medium at 37°C in a CO_2 shaker incubator for up to 7 days and the medium was collected for protein purification.

[0258] For purification, condition medium containing the hybrid ActRIIB ligand trap protein was purified via a Hitrap Protein A High Performance Column by using AKTA FPLC (GE Healthcare). The hybrid ActRIIB ligand trap proteins were eluted with acetic acid buffer (pH 3.6), neutralized with 1 M Tris-HCl (pH 8.0), and then subjected to buffer-exchange. Protein concentrations were determined by using a spectrophotometer (Beckman).

Example 3

[0259] In this example, the myostatin and BMP9 binding activities of seven of the hybrid ActRIIB ligand trap proteins is evaluated.

[0260] Myostatin and BMP9 binding activities of various hybrid ActRIIB ligand trap proteins were initially analyzed using Octect Red (ForteBio). Purified proteins or conditioned media were individually loaded to AHC biosensors with maximum loading. Following a baseline washing phase, the sensors were exposed to 10 nM myostatin or BMP9, respectively, for an association step followed by a dissociation step. All experiments were performed with shaking at 1,000 rpm. Binding activity was analyzed using ForteBio's software with KD being calculated using the ratio Kd/Ka.

Results

[0261] Hybrid ActRIIB ligand trap proteins were examined in comparison with the wild-type ActRIIB-ECD-Fc fusion protein for binding activities against myostatin and BMP9. The results indicate that the hybrid ActRIIB ligand trap proteins exhibit a marked reduction in binding affinity to BMP9 as compared to the wild-type ActRIIB-ECD-Fc fusion protein. A number of the hybrid ActRIIB ligand trap proteins showed dramatically decreased BMP9 binding affinities that

are more than 100-fold weaker than that of the wild-type ActRIIB-ECD-Fc protein and in the meantime, they retained a strong myostatin binding affinity that is similar to that of the wild-type ActRIIB-ECD-Fc protein. A summary of the preliminary binding data obtained by Octet Red analysis is shown in Table 3.

Table 3

ActRIIB-ECD-polypeptide	Myostatin Binding	BMP9 Binding
Wild-type	+++	+++
AG-0003 (SEQ ID NO: 5)	+++	++
AG-0005 (SEQ ID NO: 7)	+++	+
AG-0006 (SEQ ID NO: 8)	+++	+
AG-0007 (SEQ ID NO: 9)	+++	+
AG-0008 (SEQ ID NO: 10)	+++	++
AG-0011 (SEQ ID NO: 13)	+++	N.D.
AG-0027 (SEQ ID NO: 29)	+++	N.D.

+++ KD $<10^{-8}$ M
++ KD: 10^{-6} - 10^{-7} M
+ KD 10^{-4} - 10^{-6} M
N.D. No detectable binding

[0262] AG-0014 and AG-0027 were analyzed by kinetic exclusion assay (KinExA) (Sapidyne Instruments, Inc.). 20-30 µg/ml of myostatin, activin A or BMP-9 was separately coupled to NHS-Activated Sepharose 4 Fast Flow beads (GE Healthcare) using experimental procedures recommended by Sapidyne Instruments. The concentration for each hybrid ActRIIB ligand trap protein was held constant as the ligand was titrated in a 2.5-fold serial dilution. Solutions were allowed to reach equilibrium by incubation at room temperature up to 24 hours and subsequently passed through a flow cell pre-packed with ligand-coated Sepharose beads on a KinExA 3000 machine (Sapidyne Instruments). The free hybrid ActRIIB ligand trap proteins captured on the beads was detected by Alexa Fluor 647-labeled goat anti-human-Fc antibody (Jackson ImmunoResearch Laboratories, Inc.). The ligand binding affinity values (K_D) were calculated using the KinExA Pro software (Sapidyne Instruments).

Results

[0263] A summary of the preliminary binding data obtained by KinExA analysis is shown in Table 4. The data indicate that similar to wild-type ActRIIB-Fc, the two exemplary hybrid ActRIIB ligand trap proteins have high affinities for both myostatin and activin A at the single-digit pM range. However, the two hybrid ActRIIB ligand trap proteins show no detectable binding to BMP9, in contrast to the wild-type ActRIIB-Fc which displays a strong binding affinity to BMP9 at the single-digit pM range.

Table 4

Molecule	**Myostatin**	**Activin A**	**BMP9**
	K_D (pM)	K_D (pM)	K_D (pM)
WT ActRIIB-Fc	5.06 pM	1.38 pM	4.25 pM
AG-0014 (SEQ ID NO: 16)	8.75 pM	0.357 pM	No binding
AG-0027 (SEQ ID NO: 29)	7.87 pM	1.09 pM	No binding

Example 4

[0264] In this example, a myostatin/activin A signaling assay and a BMP9 signaling assay are described which were used to quantify the myostatin/activin A-blocking activities, and BMP9-blocking activity, respectively, of the hybrid ActRIIB ligand trap proteins.

[0265] To evaluate myostatin/activin A signaling, a reporter construct with 12 repeats of CAGA sequence (Dennler et al, EMBO 17: 3091-3100, 1998) was cloned into a pGL3-luc reporter vector (Promega). The engineered pGL3-$CAGA_{12}$-luc vector was stably transfected in C2C12 cells to generate a luciferase reporter cell line, C2C12-CAGA-luc, capable of sensing Smad3/4 signaling mediated by myostatin or activin A. To measure myostatin- and activin A-neutralizing activities, 4 nM of recombinant myostatin or activin A was preincubated with increasing concentrations of various hybrid ActRIIB ligand trap proteins as well as a wild-type

ActRIIB-ECD-Fc fusion protein (as a control) for 1 hour at room temperature. Subsequently, the reaction mixtures were added to the C2C12-CAGA-luc cell cultures. After incubation for 5 hours in CO_2 incubator at 37°C, the luciferase activities of the C2C12-CAGA-luc reporter cultures were measured by using LuminoSkan Ascent (Thermo Scientific).

[0266] BMP9 signaling was tested in C2C12 cells that had been stably transfected with a luciferase reporter containing BMP responsive element (BRE) that senses Smad1/5/8-signaling (Korchynski et.al, J. Biol. Chem. 277:4883-4891, 2002). Specifically, a two-repeat BMP-responsive element (Briter et at, PLoS One, 2012) was synthesized and cloned into the pGL3-luc vector (Promega). The pGL3-2XBRE-luc vector was then stably transfected into C2C12 cells. A stably transfected reporter cell line, C2C12-BRE-luc, was used to quantify the BMP9-mediated Smad1/5/8 signaling. To measure the BMP-neutralizing activity, 4 nM of BMP9 was preincubated with increasing concentrations of various hybrid ActRIIB ligand trap proteins as well as a wild-type ActRIIB-ECD-Fc fusion protein (as a control) for 1 hour at room temperature. The reaction mixtures were then added to the C2C12-BRE-luc cell cultures. After 5 hours of incubation at 37°C in a CO2 incubator, the luciferase activities of the C2C12 BRE-luc reporter cultures were measured by using Luminoskan Ascent (Thermo Scientific).

Results

[0267] The results revealed that in comparison to the wild-type ActRIIB-ECD-Fc fusion protein, two exemplary hybrid ActRIIB ligand trap proteins retained strong myostatin- and activin A-neutralizing activities but had marked reductions in BMP9-neutralizing activity (see Figure 2). Figure 2 shows the cell-based neutralizing activities against myostatin, activin A and BMP9 for two exemplary hybrid ActRIIB ligand trap proteins, AG-0003 (SEQ ID NO: 5) and AG-0005 (SEQ ID NO: 7), in comparison to those of the wild-type control ActRIIB-ECD-Fc fusion protein.

Example 5

[0268] In this Example, the myostatin/activin A signaling assay and BMP9 signaling assay described in Example 4 were used to quantify the myostatin/activin A-blocking activities, and BMP9-blocking activity, respectively, of the following hybrid ActRIIB ligand trap protein: AG-0003 (SEQ ID NO: 5), AG-0014 (SEQ ID NO: 16), AG-0023 (SEQ ID NO: 25), AG-0024 (SEQ ID NO: 26), AG-0025 (SEQ ID NO: 27), AG-0027 (SEQ ID NO: 29), AG-0028 (SEQ ID NO: 30), AG-0029 (SEQ ID NO: 31), and AG-0035 (SEQ ID NO: 37).

Results

[0269] The results revealed that in comparison to the wild-type ActRIIB-ECD-Fc fusion protein, several of these hybrid ActRIIB ligand trap proteins retained strong myostatin- and activin A-neutralizing activities but had marked reductions in BMP9-neutralizing activity. Figure 3 shows the cell-based neutralizing activities against myostatin, activin A and BMP9 for two exemplary hybrid ActRIIB ligand trap proteins, AG-0014 and AG-0027, in comparison to those of the wild-type control ActRIIB-ECD-Fc fusion protein.

[0270] And as shown in Tables 5 and 6, in comparison to the wild-type ActRIIB-ECD-Fc fusion protein (WT ActRIIB-Fc), various exemplary hybrid ActRIIB ligand trap proteins showed dramatically reduced BMP9-neutralizing activity in cell-based Smad1/5/8 BRE-luc reporter assay, while retaining strong neutralizing activities against myostatin, activin A and activin B in cell-based Smad2/3 CAGA-luc reporter assay. Table 5 shows the IC_{50} values on cell-based neutralization against myostatin, activin A, activin B and BMP9 of two exemplary hybrid ActRIIB ligand trap proteins AG-0014 (SEQ ID NO: 16) and AG-0027 (SEQ ID NO: 29), respectively, in comparison to those of WT ActRIIB-Fc. Table 6 outlines the BMP9- and myostatin-neutralizing activities of several exemplary hybrid ActRIIB ligand trap proteins in comparison to those of WT ActRIIB-Fc. Compared to WT ActRIIB-Fc, hybrid proteins AG-0003 (SEQ ID NO: 5), AG-0004 (SEQ ID NO: 6), AG-0005 (SEQ ID NO: 7), AG-0014 (SEQ ID NO: 16), AG-0023 (SEQ ID NO: 25), AG-0024 (SEQ ID NO: 26), AG-0025 (SEQ ID NO: 27), AG-0027 (SEQ ID NO: 29) and AG-0028 (SEQ ID NO: 30) showed dramatically reduced or virtually no BMP9-neutralizing activity (also see FIG. 10); AG-0003 (SEQ ID NO: 5), AG-0005 (SEQ ID NO: 7), AG-0014 (SEQ ID NO: 16) and AG-0027 (SEQ ID NO: 29) retained full myostatin-neutralizing activity, whereas AG-

0004 (SEQ ID NO: 6), AG-0023 (SEQ ID NO: 25), AG-0024 (SEQ ID NO: 26), AG-0025 (SEQ ID NO: 27) and AG-0028 (SEQ ID NO: 30) exhibited a loss in myostatin-neutralizing activity (also see Figure 11). Overall, these results demonstrate an ability of various hybrid ActRIIB ligand trap proteins to preferentially block myostatin/activin-mediated Smad2/3 signaling with minimal or no impact on BMP9-mediated Smad1/5/8 signaling.

Table 5

	Cell-Based IC_{50} (nM)			
	Against Myostatin	Against Activin A	Against Activin B	Against BMP9
WT ActRIIB-Fc (SEQ ID NO: 1)	1.24	1.27	1.04	3.40
AG-0014 (SEQ ID NO: 16)	0.95	1.15	2.10	N.D.
AG-0027 (SEQ ID NO: 29)	1.14	1.62	1.30	N.D.

N.D.: No detectable neutralizing activity

Table 6

	ActRIIB Extracellular Domain Mutation	BMP9-Neutralizing Activity	Myostatin-Neutralizing Activity
WT ActRIIB-Fc (SEQ ID NO: 1)	Wild-type	++++	++++
AG-0003 (SEQ ID NO: 5)	F58I+Q64T+E65D +A68E+T69K+E70K +E71D+N72S+Q74E	+/-	++++

AG-0004 (SEQ ID NO: 6)	F58I+Q64T+E65D +A68E+T69K+E70K +E71D+N72S	+/-	++
AG-0005 (SEQ ID NO: 7)	Q64T+E65D +A68E+T69K+E70K +E71D+N72S	-	++++
AG-0007 (SEQ ID NO: 9)	A68E+T69K+E70K +E71D+N72S+Q74E	+++	++
AG-0008 (SEQ ID NO: 10)	A68E+T69K+E70K +E71D+N72S	+++	+++
AG-0014 (SEQ ID NO: 16)	E26Y+E28D+Q29K +L33R	-/+	++++
AG-0027 (SEQ ID NO: 29)	E28D+F58I+E70K	-	++++
AG-0029 (SEQ ID NO: 31)	E28D	+++	++
AG-0024 (SEQ ID NO: 26)	F58I	+++	++++
AG-0023 (SEQ ID NO: 25)	E70K	-	+
AG-0028 (SEQ ID NO: 30)	E28D+E70K	-	+
AG-0025 (SEQ ID NO: 27)	F58I+E70K	-	+++
AG-0035 (SEQ ID NO: 37)	E28D+F58I	+++	+++

++++: Full neutralizing activity; +++: Partial neutralizing activity; ++: Weak neutralizing activity; +: Very weak neutralizing activity; +/-: Little or no neutralizing activity; -: No neutralizing activity

Example 6

[0271] In this Example, the effects on body weight and muscle mass in 9-week-old male C57Bl/6 mice subcutaneously injected with PBS (Vehicle), wild-type ActRIIB-Fc (WT), AG-0014 (SEQ ID NO: 16) and AG-0027 (SEQ ID NO: 29), respectively, at the dosage of 10 mg/kg, once per week were evaluated. Body weights were recorded at day 0, day 5, day 12 and day 18. n=6/8 per group. The values for body weight change are calculated as percentage of weight increase from the baseline at day 0. Individual calf muscles from each animal were dissected and weighed during terminal necropsy. The values are expressed as percent increase of the average calf muscle mass in each treatment group compared to that of the vehicle group. As depicted in Figure 4 and Table 7, administration of each of the two exemplary hybrid ligand trap proteins is capable of markedly increasing body weight gain in the mice, in a similar manner as wild type ActRIIB-Fc.

Table 7

Body Weight Increase from Baseline

Groups	Day 5	Day 12	Day 18
Vehicle	1.5 %	3.9 %	5.6 %
WT ActRIIB-Fc	9.4 %	20.1 %	25.8 %
AG-0014	9.0 %	16.8 %	25.2 %
AG-0027	7.1 %	18.1 %	24.3 %

[0272] As depicted in Figure 5 and Table 8, administration of the two exemplary hybrid ligand trap proteins AG-0014 (SEQ ID NO: 16) and AG-0027 (SEQ ID NO: 29), respectively, is capable of markedly increasing muscle mass in the mice in a similar manner as wild type ActRIIB-Fc.

Table 8

Muscle Mass Increase Compared to Vehicle

Groups	Increase in Calf Muscle Mass Compared to Vehicle
WT ActRIIB-Fc	31.3 %
AG-0014	30.0 %
AG-0027	30.7 %

Example 7

[0273] In this Example, the effects on mouse abdominal cavity, mouse testis, and mouse lung tissues in 8-week-old male BalbC mice treated with PBS (Vehicle), wild-type ActRIIB-Fc (WT), AG-0014 (SEQ ID NO: 16) and AG-0027 (SEQ ID NO: 29), respectively, at the dosage of 10 mg/kg, once per week, were evaluated. Two weeks after treatment, 200 µl of Evans blue dye (0.5% in PBS, pH7.2) was injected into each group of animals (n=4) via the tail vein. Necropsy was performed at 90 min after Evans blue dye injection. Representative images of surgically exposed abdominal cavity, dissected testis organ, and dissected lung tissue of each group are shown as labeled in Figures 6-8, respectively. Blue color indicates the leakage of blood vessel. Testis and lung tissues were weighed and then placed individually into vials containing formamide to extract the Evans blue dye. After incubation at 55°C for 24 hours, the samples were centrifuged. The absorbance of the aqueous phase of each sample was measured at the wavelength of 610 nm using a spectrophotometer. The amounts of extravasated Evans blue dye per mg of wet lung tissue (left panel) and testis tissue (right panel) in different treatment groups are shown in Figure 9.

[0274] Importantly, as depicted in Figures 6-9, administration of the two exemplary hybrid ligand trap proteins markedly decreases the level of blood vessel leakage as compared to wild type ActRIIB-Fc protein in all tissues evaluated in the treated animals.

Example 8

[0275] Automated ELISA analysis was performed to further characterize the BMP9 binding of hybrid ActRIIB ligand trap proteins at different concentrations in comparison to both wild-type ActRIIB ECD-Fc (WT ActRIIB-Fc) and wild-type ActRIIA ECD-Fc (WT ActRIIA-Fc). As shown in Figure 12, data reveal that the two exemplary hybrid proteins AG-0014 (SEQ ID NO: 16) and AG-0027 (SEQ ID NO: 29), respectively, exhibited greatly reduced BMP9 binding compared to either WT ActRIIB-Fc or WT ActRIIA-Fc. These data indicate that the hybrid ActRIIB ligand trap proteins have a remarkable BMP9 binding selectivity that differs from WT ActRIIB-Fc and WT ActRIIA-Fc.

[0276] This data demonstrates that the hybrid ActRIIB ligand trap proteins described herein potently bind and neutralize multiple atrophy-inducing cytokines. And, importantly, the hybrid ActRIIB ligand trap proteins have dramatically improved selectivity for muscle, i.e, while potently blocking the actions of muscle atrophy-inducing cytokines, they leave the signaling of non-muscle related cytokines intact, thus maintaining the normal physiological functioning of non-muscle cells. As stated above, BMP plays an important role in a number of physiological processes and BMP9 signaling has been shown to be essential in maintaining normal blood vasculature/permeability. By sparing BMP9 signaling and preferentially antagonizing myostatin and activin signaling, the hybrid ActRIIB ligand trap proteins of the present disclosure offer a more effective and safer treatment than existing soluble ActRIIB proteins, which potently neutralize BMP-9, i.e., by selectively targeting multiple atrophy-inducing cytokines in parallel and by avoiding interfering with the signaling of non-muscle related cytokines, these hybrid ActRIIB ligand trap proteins represent a class of clinical candidates armed with a superior muscle growth efficacy and an improved safety profile and thereby it offers the potential to become a best-in-class treatment for combating muscle wasting, cachexia and frailty. As such, these novel hybrid ActRIIB ligand trap proteins are believed to have a wide range of clinical indications, including age-related sarcopenia, cancer cachexia, muscle atrophy associated with chronic diseases (CHF, CKD, COPD, Diabetes, etc.), muscle atrophy due to disuse or denervation, drug-induced myopathy, various forms of myositis, neuromuscular diseases, and neurodegenerative diseases.

[0277] All of the articles and methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the articles and methods of this disclosure have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the articles and methods without departing from the spirit and scope of the disclosure. All such variations and equivalents apparent to those skilled in the art, whether now existing or later developed, are deemed to be within the spirit and scope of the disclosure as defined by the appended claims. All patents, patent applications, and publications mentioned in the specification are indicative of the levels of those of ordinary skill in the art to which the disclosure pertains. All patents, patent applications, and publications are herein incorporated by reference in their entirety for all purposes and to the same extent as if each individual publication was specifically and individually indicated to be incorporated by reference in its entirety for any and all purposes. The disclosure illustratively described herein suitably may be practiced in the absence of any element(s) not specifically disclosed herein. Thus, it should be understood that although the present disclosure has been specifically disclosed by preferred embodiments and optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this disclosure as defined by the appended claims.

Sequence Listings

The nucleic and amino acid sequences listed in the accompanying sequence listing are shown using standard letter abbreviations for nucleotide bases and three letter code for amino acids, as defined in 37 C.F.R. 1.822.

SEQ ID NO: 1 is the amino acid sequence of a truncated wild-type human ActRIIB-ECD polypeptide.

SEQ ID NO: 2 is the amino acid sequence of a truncated wild-type human ActRIIA-ECD polypeptide.

SEQ ID NOS: 3-37 are the amino acid sequences of various hybrid soluble ActRIIB-ECD polypeptides.

SEQ ID NO: 38 is the amino acid sequence of a human immunoglobulin gamma-1 (IgG1) heavy chain constant region

SEQ ID NO: 39 is the amino acid sequence of an IgG1 Fc Domain

SEQ ID NO: 40 is the amino acid sequence of a human immunoglobulin gamma-2 chain heavy constant region

SEQ ID NO: 41 is the amino acid sequence of an IgG2 Fc Domain

SEQ ID NO: 42 is the amino acid sequence of a human immunoglobulin gamma-4 chain heavy constant region

SEQ ID NO: 43 is the amino acid sequence of an IgG4 Fc Domain

SEQ ID NO: 44 is the amino acid sequence of peptide linker.

SEQ ID NO: 45 is the full length amino acid sequence of Human ActRIIB polypeptide

SEQ ID NO: 46 is the amino acid sequence of wild-type human ActRIIB extracellular domain (19-134 of SEQ ID NO: 45)

SEQ ID NO: 47 is the full length amino acid sequence of Human ActRIIA polypeptide

SEQ ID NO: 48 is the amino acid sequence of wild-type human ActRIIA extracellular domain (20-135 of SEQ ID NO: 47)

SEQ ID NO: 49 is the amino acid sequence of a ActRIIB native signal peptide

SEQ ID NO: 50 is the amino acid sequence of an Immunoglobulin light chain signal peptide.

SEQ ID NOS: 51-117 are the amino acid sequences of various hybrid soluble ActRIIB-ECD polypeptides.

SEQ ID NO: 118 is the amino acid sequence of peptide linker.

SEQUENCE LISTINGS

Truncated wild-type ActRIIB-ECD
ETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWRNSSGTIELVKKGCWLD
DFNCYDRQECVATEENPQVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPT
(SEQ ID NO: 1)

Truncated wild-type ActRIIA-ECD
ETQECLFFNANWEKDRTNQTGVEPCYGDKDKRRHCFATWKNISGSIEIVKQGCW
LDDINCYDRTDCVEKKDSPEVYFCCCEGNMCNEKFSYFPEMEVTQPTSNPVTPKPP
(SEQ ID NO: 2)

Hybrid hu-ActRIIB-ECD (AG-0001)
ETRECIYYNANWELERTNQSGLERCYGDKDKRRHCYASWRNSSGTIELVKKGCWLDDINCYD
RTDCVEKKDSPEVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPT (SEQ ID NO: 3)

Hybrid hu-ActRIIB-ECD (AG-0002)
ETRECIYYNANWELERTNQSGLERCYGDKDKRRHCYASWRNSSGTIELVKKGCWLDDFNCYD
RTDCVEKKDSPEVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPT (SEQ ID NO: 4)

Hybrid hu-ActRIIB-ECD (AG-0003)
ETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWRNSSGTIELVKKGCWLDDINCYD
RTDCVEKKDSPEVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPT (SEQ ID NO: 5)

Hybrid hu-ActRIIB-ECD (AG-0004)
ETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWRNSSGTIELVKKGCWLDDINCYD
RTDCVEKKDSPQVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPT (SEQ ID NO: 6)

Hybrid hu-ActRIIB-ECD (AG-0005)
ETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWRNSSGTIELVKKGCWLDDFNCYD
RTDCVEKKDSPQVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPT (SEQ ID NO: 7)

Hybrid hu-ActRIIB-ECD (AG-0006)
ETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWRNSSGTIELVKKGCWLDDFNCYD
RTDCVEKKDSPEVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPT (SEQ ID NO: 8)

Hybrid hu-ActRIIB-ECD (AG-0007)
ETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWRNSSGTIELVKKGCWLDDFNCYD
RQECVEKKDSPEVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPT (SEQ ID NO: 9)

Hybrid hu-ActRIIB-ECD (AG-0008)
ETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWRNSSGTIELVKKGCWLDDFNCYD
RQECVEKKDSPQVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPT (SEQ ID NO: 10)

Hybrid human ActRIIA-ECD (AG-0009)
ETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWRNSSGTIELVKKGCWLDDINCYD
RQECVEKKDSPEVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPT (SEQ ID NO: 11)

Hybrid hu-ActRIIB-ECD (AG-0010)
ETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWRNSSGTIELVKKGCWLDDFNCYD
RTDCVEKKDSPEVYFCCCEGNMCNERFTHLPEAGGPEVTYEPPPTAPT (SEQ ID NO: 12)

Hybrid hu-ActRIIB-ECD (AG-0011)
ETRECIYYNANWELERTNQSGLERCEGDQDKRLHCYASWRNSSGTIELVKKGCWLDDFNCYD
RQECVEKKDSPEVYFCCCEGNMCNERFTHLPEAGGPEVTYEPPPTAPT (SEQ ID NO: 13)

Hybrid hu-ActRIIB-ECD (AG-0012)
ETQECIYYNANWEKDRTNQTGVEPCYGDKDKRRHCYASWRNSSGTIELVKKGCWLDDFNCY
DRQECVATEENPQVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPT (SEQ ID NO: 14)

Hybrid hu-ActRIIB-ECD (AG-0013)
ETQECIYYNANWEKDRTNQTGVEPCEGDQDKRLHCYASWRNSSGTIELVKKGCWLDDFNCY
DRQECVATEENPQVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPT (SEQ ID NO: 15)

Hybrid hu-ActRIIB-ECD (AG-0014)
ETRECIYYNANWELERTNQSGLERCYGDKDKRRHCYASWRNSSGTIELVKKGCWLDDFNCYD
RQECVATEENPQVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPT (SEQ ID NO: 16)

Hybrid hu-ActRIIB-ECD (AG-0015)
ETRECIYYNANWEKDRTNQTGVEPCEGDQDKRLHCYASWRNSSGTIELVKKGCWLDDFNCY
DRQECVATEENPQVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPT (SEQ ID NO: 17)

Hybrid hu-ActRIIB-ECD (AG-0016)
ETQECIYYNANWEKDRTNQTGVEPCEGDQDKRLHCYASWRNSSGTIELVKKGCWLDDFNCY
DRQECVATEENPQVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPT (SEQ ID NO: 18)

Hybrid hu-ActRIIB-ECD (AG-0017)
ETQECIYYNANWEKDRTNQTGLERCEGEQDKRLHCYASWRNSSGTIELVKKGCWLDDFNCY
DRQECVATEENPQVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPT (SEQ ID NO: 19)

Hybrid hu-ActRIIB-ECD (AG-0018)
ETQECIYYNANWEKDRTNQSGLERCEGEQDKRLHCYASWRNSSGTIELVKKGCWLDDFNCY
DRQECVATEENPQVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPT (SEQ ID NO: 20)

Hybrid hu-ActRIIB-ECD (AG-0019)
ETRECIYYNANWEKDRTNQSGLERCEGEQDKRLHCYASWRNSSGTIELVKKGCWLDDFNCY
DRQECVATEENPQVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPT (SEQ ID NO: 21)

Hybrid hu-ActRIIB-ECD (AG-0020)
ETQECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWRNSSGTIELVKKGCWLDDFNCYD
RQECVATEENPQVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPT (SEQ ID NO: 22)

Hybrid hu-ActRIIB-ECD (AG-0021)
ETRECIYYNANWELERTNQSGLERCEGEQDKRLHCFATWRNSSGTIELVKQGCWLDDFNCYD
RQECVATEENPQVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPT (SEQ ID NO: 23)

Hybrid hu-ActRIIB-ECD (AG-0022)

ETRECIYYNANWELERTNQSGLERCYGDKDKRRHCYASWRNSSGTIELVKKGCWLDDINCYD
RQECVATEENPQVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPT (SEQ ID NO: 24)

Hybrid hu-ActRIIB-ECD (AG-0023)
ETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWRNSSGTIELVKKGCWLDDFNCYD
RQECVATKENPQVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPT (SEQ ID NO: 25)

Hybrid hu-ActRIIB-ECD (AG-0024)
ETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWRNSSGTIELVKKGCWLDDINCYD
RQECVATEENPQVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPT (SEQ ID NO: 26)

Hybrid hu-ActRIIB-ECD (AG-0025)
ETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWRNSSGTIELVKKGCWLDDINCYD
RQECVATKENPQVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPT (SEQ ID NO: 27)

Hybrid hu-ActRIIB-ECD (AG-0026)
ETRECIYYNANWELERTNQSGLERCEGDKDKRLHCYASWRNSSGTIELVKKGCWLDDINCYD
RQECVATKENPQVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPT (SEQ ID NO: 28)

Hybrid hu-ActRIIB-ECD (AG-0027)
ETRECIYYNANWELERTNQSGLERCEGDQDKRLHCYASWRNSSGTIELVKKGCWLDDINCYD
RQECVATKENPQVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPT (SEQ ID NO: 29)

Hybrid hu-ActRIIB-ECD (AG-0028)
ETRECIYYNANWELERTNQSGLERCEGDQDKRLHCYASWRNSSGTIELVKKGCWLDDFNCYD
RQECVATKENPQVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPT (SEQ ID NO: 30)

Hybrid hu-ActRIIB-ECD (AG-0029)
ETRECIYYNANWELERTNQSGLERCEGDQDKRLHCYASWRNSSGTIELVKKGCWLDDFNCYD
RQECVATEENPQVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPT (SEQ ID NO: 31)

Hybrid hu-ActRIIB-ECD (AG-0030)
ETRECIYYNANWELERTNQSGLERCYGDKDKRRHCYASWRNSSGTIELVKKGCWLDDINCYD
RQECVEKKDSPEVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPT (SEQ ID NO: 32)

Hybrid hu-ActRIIB-ECD (AG-0031)
ETRECIFFNANWEKDRTNQTGVEPCEGEQDKRLHCYASWRNSSGTIELVKKGCWLDDFNCYD
RQECVATEENPQVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPT (SEQ ID NO: 33)

Hybrid hu-ActRIIB-ECD (AG-0032)
ETRECIYYNANWELERTNQSGLERCEGEQDKRLHCFATWKNISGSIELVKQGCWLDDFNCYD
RQECVATEENPQVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPT (SEQ ID NO: 34)

Hybrid hu-ActRIIB-ECD (AG-0033)

ETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWRNSSGTIELVKKGCWLDDFNCYD
RTDCVATEENPQVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPT (SEQ ID NO: 35)

Hybrid hu-ActRIIB-ECD (AG-0034)
ETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWRNSSGTIELVKKGCWLDDFNCYD
RQECVATEENPQVYFCCCEGNMCNERFTHLPEAGGPEVTYEPPPTAPT (SEQ ID NO: 36)

Hybrid hu-ActRIIB-ECD (AG-0035)
ETRECIYYNANWELERTNQSGLERCEGDQDKRLHCYASWRNSSGTIELVKKGCWLDDINCYD
RQECVATEENPQVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPT (SEQ ID NO: 37)

Human immunoglobulin gamma-1 heavy chain constant region
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGG
PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN
STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKN
QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVF
SCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 38)

IgG1 Fc Domain
VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV
VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT
CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM
HEALHNHYTQKSLSLSPGK (SEQ ID NO: 39)

Human immunoglobulin gamma-2 heavy chain constant region
ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY
SLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPSVFLFPPKP
KDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVH
QDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKG
FYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH
NHYTQKSLSLSPGK (SEQ ID NO: 40)

IgG2 Fc Domain
VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRV
VSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSL
TCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFCSVM
HEALHNHYTQKSLSLSPGK (SEQ ID NO: 41)

Human immunoglobulin gamma-4 heavy chain constant region
ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY
SLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPSCPAPEFLGGPSVFLFPPK
PKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVL
HQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVK

GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHE
ALHNHYTQKSLSLSLGK (SEQ ID NO: 42)

IgG4 Fc Domain
APEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPRE
EQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQE
EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQ
EGNVFSCSVMHEALHNHYTQKSLSLSLGK (SEQ ID NO: 43)

Peptide Linker sequence
GGGGS (SEQ ID NO: 44)

Full Length Amino Acid Sequence of Human ActRIIB polypeptide
MTAPWVALALLWGSLCAGSGRGEAETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYA
SWRNSSGTIELVKKGCWLDDFNCYDRQECVATEENPQVYFCCCEGNFCNERFTHLPEAGGP
EVTYEPPPTAPTLLTVLAYSLLPIGGLSLIVLLAFWMYRHRKPPYGHVDIHEDPGPPPPSPLVGL
KPLQLLEIKARGRFGCVWKAQLMNDFVAVKIFPLQDKQSWQSEREIFSTPGMKHENLLQFIAAE
KRGSNLEVELWLITAFHDKGSLTDYLKGNIITWNELCHVAETMSRGLSYLHEDVPWCRGEGHK
PSIAHRDFKSKNVLLKSDLTAVLADFGLAVRFEPGKPPGDTHGQVGTRRYMAPEVLEGAINFQ
RDAFLRIDMYAMGLVLWELVSRCKAADGPVDEYMLPFEEEIGQHPSLEELQEVVVHKKMRPTI
KDHWLKHPGLAQLCVTIEECWDHDAEARLSAGCVEERVSLIRRSVNGTTSDCLVSLVTSVTNV
DLPPKESSI (SEQ ID NO: 45)

Wild-type human ActRIIB extracellular domain (19-134 of SEQ ID NO: 45)
SGRGEAETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWRNSSGTIELVKKGCWLD
DFNCYDRQECVATEENPQVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPT
(SEQ ID NO: 46)

Full Length Amino Acid Sequence of Human ActRIIA polypeptide
MGAATKLAFAVFLISCSSGAILGRSETQECIYYNANWEKDKTNRSGIEPCYGDKDKRRHCFAT
WKNISGSIEIVKQGCWLDDINCYDRNDCIEKKDSPEVFFCCCEGNMCNERFFYFPEMEVTQPT
SNPVTPKPPLFNTLLYSLVPIMGIAVIVLFSFWMYRHHKLAYPPVLVPTQDPGPPPPSPLMGLK
PLQLLEIKARGRFGCVWKAQLLNEYVAVKIFPIQDKQSWQNEYEIYSLPGMKHDNILQFIGAEKR
GTSIDVDLWLITAFHEKGSLTDFLKANVVSWNELCHIAQTMARGLAYLHEDIPGLKDGHKPAISH
RDIKSKNVLLKNNLTACIADFGLALKFEAGKSAGDTHGQVGTRRYMAPEVLEGAINFQRDAFLRI
DMYAMGLVLWELASRCTASDGPVDEYMLPFEEEIGQHPSLEDMQEVVVHKKKRPVLRECWQ
KHSGMAMLCETIEECWDHDAEARLSAGCVEERIIQMQKLTNIITTEDIVTVVTMVTNVDFPPKES
SL (SEQ ID NO: 47)

Wild-type human ActRIIA extracellular domain (20-135 of SEQ ID NO: 47)
AILGRSETQECLFFNANWEKDRTNQTGVEPCYGDKDKRRHCFATWKNISGSIEIVKQGCW
LDDINCYDRTDCVEKKDSPEVYFCCCEGNMCNEKFSYFPEMEVTQPTSNPVTPKPP
(SEQ ID NO: 48)

ActRIIB native signal peptide:

MTAPWVALALLWGSLCAG (SEQ ID NO: 49)

Immunoglobulin light chain signal peptide:
MDMRVPAQLLGLLLLWLRGARC (SEQ ID NO: 50)

Hybrid hu-ActRIIB-ECD
ETQECLFFNANWEKDRTNQSGVEPCYGDKDKRRHCYASWRNSSGTIELVKKGCWLDDFNCY
DRQECVATEENPQVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPT (SEQ ID NO: 51)

Hybrid hu-ActRIIB-ECD
ETQECLFFNANWEKDRTNQSGVEPCEGEQDKRLHCYASWRNSSGTIELVKKGCWLDDFNCY
DRQECVATEENPQVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPT (SEQ ID NO: 52)

Hybrid hu-ActRIIB-ECD
ETRECLFFNANWEKDRTNQSGVEPCEGEQDKRLHCYASWRNSSGTIELVKKGCWLDDFNCY
DRQECVATEENPQVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPT (SEQ ID NO: 53)

Hybrid hu-ActRIIB-ECD
ETQECLFFNANWEKDRTNQSGVEPCYGEQDKRLHCYASWRNSSGTIELVKKGCWLDDFNCY
DRQECVATEENPQVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPT (SEQ ID NO: 54)

Hybrid hu-ActRIIB-ECD
ETRECLFFNANWEKDRTNQSGVEPCYGDKDKRRHCYASWRNSSGTIELVKKGCWLDDFNCY
DRQECVATEENPQVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPT (SEQ ID NO: 55)

Hybrid hu-ActRIIB-ECD
ETRECLFFNANWEKDRTNQTGVEPCYGDKDKRRHCFATWKNISGSIEIVKQGCWLDDFNCYD
RQECVATEENPQVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPT (SEQ ID NO: 56)

Hybrid hu-ActRIIB-ECD
ETRECLFFNANWEKDRTNQTGVEPCYGDKDKRRHCFATWKNISGSIEIVKQGCWLDDINCYD
RQECVATEENPQVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPT (SEQ ID NO: 57)

Hybrid hu-ActRIIB-ECD
ETRECLFFNANWEKDRTNQTGVEPCYGDKDKRRHCFATWKNISGSIEIVKQGCWLDDINCYD
RTDCVEKKDSPEVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPT (SEQ ID NO: 58)

Hybrid hu-ActRIIB-ECD
ETQECIYYNANWELERTNQSGLERCYGDKDKRRHCFATWKNISGSIEIVKQGCWLDDINCYDR
QECVATEENPQVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPT (SEQ ID NO: 59)

Hybrid hu-ActRIIB-ECD
ETRECIYYNANWELERTNQSGLERCYGDKDKRRHCFATWKNISGSIEIVKQGCWLDDINCYDR
TDCVEKKDSPEVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPT (SEQ ID NO: 60)

Hybrid hu-ActRIIB-ECD
ETRECIYYNANWELERTNQSGLERCYGDKDKRRHCFATWKNISGSIEIVKQGCWLDDINCYDR
TDCVEKKDSPEVYFCCCEGNMCNERFTHLPEAGGPEVTYEPPPTAPT (SEQ ID NO: 61)

Hybrid hu-ActRIIB-ECD
ETRECIYYNANWELERTNQSGLERCEGEQDKRLHCFATWKNISGSIEIVKQGCWLDDINCYDR
TDCVEKKDSPEVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPT (SEQ ID NO: 62)

Hybrid hu-ActRIIB-ECD
ETRECIYYNANWELERTNQSGLERCEGEQDKRLHCFATWKNISGSIEIVKQGCWLDDINCYDR
TDCVATEENPQVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPT (SEQ ID NO: 63)

Hybrid hu-ActRIIB-ECD
ETRECIYYNANWELERTNQSGLERCEGEQDKRLHCFATWKNISGSIEIVKQGCWLDDFNCYDR
TDCVATEENPQVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPT (SEQ ID NO: 64)

Hybrid hu-ActRIIB-ECD
ETRECIYYNANWELERTNQSGLERCEGEQDKRLHCFATWKNISGSIEIVKQGCWLDDFNCYDR
QECVATEENPQVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPT (SEQ ID NO: 65)

Hybrid hu-ActRIIB-ECD
ETQECIYYNANWELERTNQSGLERCYGDKDKRRHCYASWRNSSGTIELVKKGCWLDDINCYD
RTDCVEKKDSPEVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPT (SEQ ID NO: 66)

Hybrid hu-ActRIIB-ECD
ETQECIYYNANWELERTNQSGLERCYGDKDKRRHCYASWRNSSGTIELVKKGCWLDDINCYD
RTDCVEKKDSPEVYFCCCEGNMCNERFTHLPEAGGPEVTYEPPPTAPT (SEQ ID NO: 67)

Hybrid hu-ActRIIB-ECD
ETQECIYYNANWELERTNQSGLERCYGDKDKRRHCFATWKNISGSIEIVKQGCWLDDINCYDR
TDCVEKKDSPEVYFCCCEGNMCNERFTHLPEAGGPEVTYEPPPTAPT (SEQ ID NO: 68)

Hybrid hu-ActRIIB-ECD
ETQECIYYNANWELERTNQSGLERCYGDKDKRRHCFATWKNISGSIEIVKQGCWLDDINCYDR
TDCVEKKDSPEVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPT (SEQ ID NO: 69)

Hybrid hu-ActRIIB-ECD
ETRECLFFNANWEKDRTNQTGVEPCEGEQDKRLHCFATWKNISGSIEIVKQGCWLDDINCYDR
TDCVEKKDSPEVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPT (SEQ ID NO: 70)

Hybrid hu-ActRIIB-ECD
ETRECLFFNANWEKDRTNQSGVEPCEGEQDKRLHCYASWRNSSGTIELVKKGCWLDDINCYD
RTDCVEKKDSPEVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPT (SEQ ID NO: 71)

Hybrid hu-ActRIIB-ECD
ETRECLFFNANWEKDRTNQSGVEPCYGDKDKRRHCYASWRNSSGTIELVKKGCWLDDINCY
DRTDCVEKKDSPEVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPT (SEQ ID NO: 72)

Hybrid hu-ActRIIB-ECD
ETRECIYYNANWELERTNQSGLERCYGDKDKRRHCYASWRNSSGTIELVKKGCWLDDFNCYD
RTDCVATEENPQVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPT (SEQ ID NO: 73)

Hybrid hu-ActRIIB-ECD
ETRECIYYNANWELERTNQSGLERCYGDKDKRRHCYASWRNSSGTIELVKQGCWLDDFNCY
DRTDCVATEENPQVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPT (SEQ ID NO: 74)

Hybrid hu-ActRIIB-ECD
ETRECIYYNANWELERTNQSGLERCYGDKDKRRHCYASWRNSSGTIEIVKKGCWLDDFNCYD
RTDCVATEENPQVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPT (SEQ ID NO: 75)

Hybrid hu-ActRIIB-ECD
ETRECIYYNANWELERTNQSGLERCYGDKDKRRHCYASWRNSSGSIELVKKGCWLDDFNCY
DRTDCVATEENPQVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPT (SEQ ID NO: 76)

Hybrid hu-ActRIIB-ECD
ETRECIYYNANWELERTNQSGLERCYGDKDKRRHCYASWRNSSGSIEIVKKGCWLDDFNCYD
RTDCVATEENPQVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPT (SEQ ID NO: 77)

Hybrid hu-ActRIIB-ECD
ETRECIYYNANWELERTNQSGLERCYGDKDKRRHCYASWRNSSGSIEIVKQGCWLDDFNCYD
RTDCVATEENPQVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPT (SEQ ID NO: 78)

Hybrid hu-ActRIIB-ECD
ETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWRNSSGTIELVKKGCWLDDFNCYD
RTDCVATEENPQVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPT (SEQ ID NO: 79)

Hybrid hu-ActRIIB-ECD
ETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWRNSSGTIELVKKGCWLDDFNCYD
RQECVATEENPQVYFCCCEGNFCNEKFSYFPEMEVTQPTSNPVTPKPP (SEQ ID NO: 80)

Hybrid hu-ActRIIB-ECD
ETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWRNSSGTIELVKKGCWLDDFNCYD
RQECVATEENPQVYFCCCEGNFCNEKFSYFPQMEVTQPTSNPVTPKPP (SEQ ID NO: 81)

Hybrid hu-ActRIIB-ECD
ETRECIYYNANWELERTNQSGLERCYGDKDKRRHCYASWRNSSGTIELVKKGCWLDDINCYD
RTDCVEKKDSPEVYFCCCEGNFCNEKFSYFPEMEVTQPTSNPVTPKPP (SEQ ID NO: 82)

Hybrid hu-ActRIIB-ECD
ETRECIYYNANWELERTNQSGLERCYGDKDKRRHCYASWRNSSGTIELVKKGCWLDDINCYD
RTDCVEKKDSPEVYFCCCEGNFCNEKFSYFPQMEVTQPTSNPVTPKPP (SEQ ID NO: 83)

Hybrid hu-ActRIIB-ECD
ETRECIYYNANWELERTNQSGLERCYGDKDKRRHCYASWRNSSGTIELVKKGCWLDDFNCYD
RQECVATEENPQVYFCCCEGNFCNEKFSYFPQMEVTQPTSNPVTPKPP (SEQ ID NO: 84)

Hybrid hu-ActRIIB-ECD
ETRECIYYNANWELERTNQSGLERCYGDKDKRRHCYASWRNSSGTIELVKQGCWLDDFNCY
DRQECVATEENPQVYFCCCEGNFCNEKFSYFPQMEVTQPTSNPVTPKPP (SEQ ID NO: 85)

Hybrid hu-ActRIIB-ECD
ETRECIYYNANWELERTNQSGLERCYGDKDKRRHCYASWRNSSGTIEIVKKGCWLDDFNCYD
RQECVATKENPQVYFCCCEGNFCNEKFSYFPQMEVTQPTSNPVTPKPP (SEQ ID NO: 86)

Hybrid hu-ActRIIB-ECD
ETRECIYYNANWELERTNQSGLERCYGDKDKRRHCYASWRNSSGSIELVKKGCWLDDFNCY
DRQECVATKENPQVYFCCCEGNFCNEKFSYFPQMEVTQPTSNPVTPKPP (SEQ ID NO: 87)

Hybrid hu-ActRIIB-ECD
ETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWRNSSGSIELVKKGCWLDDFNCYD
RQECVATEENPQVYFCCCEGNFCNEKFSYFPQMEVTQPTSNPVTPKPP (SEQ ID NO: 88)

Hybrid hu-ActRIIB-ECD
ETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWRNSSGTIEIVKKGCWLDDFNCYD
RQECVATEENPQVYFCCCEGNFCNEKFSYFPQMEVTQPTSNPVTPKPP (SEQ ID NO: 89)

Hybrid hu-ActRIIB-ECD
ETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWRNSSGTIELVKQGCWLDDFNCYD
RQECVATEENPQVYFCCCEGNFCNEKFSYFPQMEVTQPTSNPVTPKPP (SEQ ID NO: 90)

Hybrid hu-ActRIIB-ECD
ETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWRNSSGTIELVKKGCWLDDFNCYD
RQECVETEENPQVYFCCCEGNFCNEKFSYFPQMEVTQPTSNPVTPKPP (SEQ ID NO: 91)

Hybrid hu-ActRIIB-ECD
ETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWRNSSGTIELVKKGCWLDDFNCYD
RQECVAKEENPQVYFCCCEGNFCNEKFSYFPQMEVTQPTSNPVTPKPP (SEQ ID NO: 92)

Hybrid hu-ActRIIB-ECD
ETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWRNSSGTIELVKKGCWLDDFNCYD
RQECVATKENPQVYFCCCEGNFCNEKFSYFPQMEVTQPTSNPVTPKPP (SEQ ID NO: 93)

Hybrid hu-ActRIIB-ECD
ETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWRNSSGTIELVKKGCWLDDFNCYD
RQECVATEDNPQVYFCCCEGNFCNEKFSYFPQMEVTQPTSNPVTPKPP (SEQ ID NO: 94)

Hybrid hu-ActRIIB-ECD
ETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWRNSSGTIELVKKGCWLDDFNCYD
RQECVATEESPQVYFCCCEGNFCNEKFSYFPQMEVTQPTSNPVTPKPP (SEQ ID NO: 95)

Hybrid hu-ActRIIB-ECD
ETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWRNSSGTIELVKKGCWLDDFNCYD
RQECVATEENPEVYFCCCEGNFCNEKFSYFPQMEVTQPTSNPVTPKPP (SEQ ID NO: 96)

Hybrid hu-ActRIIB-ECD
ETRECIYYNANWELERTNQSGLERCEGDKDKRLHCYASWRNSSGTIELVKKGCWLDDFNCYD
RQECVETEENPQVYFCCCEGNFCNEKFSYFPQMEVTQPTSNPVTPKPP (SEQ ID NO: 97)

Hybrid hu-ActRIIB-ECD
ETRECIYYNANWELERTNQSGLERCEGEKDKRLHCYASWRNSSGTIELVKKGCWLDDFNCYD
RQECVAKEENPQVYFCCCEGNFCNEKFSYFPQMEVTQPTSNPVTPKPP (SEQ ID NO: 98)

Hybrid hu-ActRIIB-ECD
ETRECIYYNANWELERTNQSGLERCEGDQDKRLHCYASWRNSSGTIELVKKGCWLDDFNCYD
RQECVATKENPQVYFCCCEGNFCNEKFSYFPQMEVTQPTSNPVTPKPP (SEQ ID NO: 99)

Hybrid hu-ActRIIB-ECD
ETRECIYYNANWELERTNQSGLERCEGDKDKRLHCYASWRNSSGTIELVKQGCWLDDFNCYD
RQECVAKKENPQVYFCCCEGNFCNEKFSYFPQMEVTQPTSNPVTPKPP (SEQ ID NO: 100)

Hybrid hu-ActRIIB-ECD
ETRECIYYNANWELERTNQSGLERCEGDKDKRLHCYASWRNSSGTIEIVKQGCWLDDFNCYD
RQECVAEKENPQVYFCCCEGNFCNEKFSYFPQMEVTQPTSNPVTPKPP (SEQ ID NO: 101)

Hybrid hu-ActRIIB-ECD
ETRECIYYNANWELERTNQSGLERCYGDQDKRLHCYASWRNSSGSIEIVKQGCWLDDFNCYD
RQECVAKKENPQVYFCCCEGNFCNEKFSYFPQMEVTQPTSNPVTPKPP (SEQ ID NO: 102)

Hybrid hu-ActRIIB-ECD
ETRECIYYNANWELERTNQSGLERCEGEKDKRRHCYASWRNSSGTIEIVKKGCWLDDFNCYD
RQECVATKENPQVYFCCCEGNFCNEKFSYFPQMEVTQPTSNPVTPKPP (SEQ ID NO: 103)

Hybrid hu-ActRIIB-ECD
ETRECIYYNANWELERTNQSGLERCYGDQDKRRHCYASWRNSSGTIELVKKGCWLDDFNCY
DRQECVATEENPEVYFCCCEGNFCNEKFSYFPQMEVTQPTSNPVTPKPP (SEQ ID NO: 104)

Hybrid hu-ActRIIB-ECD
ETRECIYYNANWELERTNQSGLERCEGEQDKRRHCYASWRNSSGTIELVKKGCWLDDFNCY
DRQECVATEENPQVYFCCCEGNFCNEKFSYFPQMEVTQPTSNPVTPKPP (SEQ ID NO: 105)

Hybrid hu-ActRIIB-ECD
ETRECIYYNANWELERTNQSGLERCYGEQDKRLHCYASWRNSSGSIEIVKKGCWLDDFNCYD
RTDCVATEENPQVYFCCCEGNFCNEKFSYFPQMEVTQPTSNPVTPKPP (SEQ ID NO: 106)

Hybrid hu-ActRIIB-ECD
ETRECIYYNANWELERTNQSGLERCEGEQDKRRHCYASWRNSSGSIELVKKGCWLDDFNCY
DRQECVAKEENPQVYFCCCEGNFCNEKFSYFPQMEVTQPTSNPVTPKPP (SEQ ID NO: 107)

Hybrid hu-ActRIIB-ECD
ETRECIYYNANWELERTNQSGLERCEGEQDKRRHCYASWRNSSGTIEIVKKGCWLDDFNCYD
RQECVAKEENPQVYFCCCEGNFCNEKFSYFPQMEVTQPTSNPVTPKPP (SEQ ID NO: 108)

Hybrid hu-ActRIIB-ECD
ETRECIYYNANWELERTNQSGLERCEGEQDKRRHCYASWRNSSGSIEIVKKGCWLDDFNCYD
RQECVATKENPQVYFCCCEGNFCNEKFSYFPQMEVTQPTSNPVTPKPP (SEQ ID NO: 109)

Hybrid hu-ActRIIB-ECD
ETRECIYYNANWELERTNQSGLERCEGDQDKRLHCYASWRNSSGTIEIVKKGCWLDDFNCYD
RQECVATKENPQVYFCCCEGNFCNEKFSYFPQMEVTQPTSNPVTPKPP (SEQ ID NO: 110)

Hybrid hu-ActRIIB-ECD
ETRECIYYNANWELERTNQSGLERCEGDQDKRLHCYASWRNSSGSIELVKKGCWLDDFNCYD
RQECVATKENPQVYFCCCEGNFCNEKFSYFPQMEVTQPTSNPVTPKPP (SEQ ID NO: 111)

Hybrid hu-ActRIIB-ECD
ETRECIYYNANWELERTNQSGLERCEGDQDKRLHCYASWRNSSGTIELVKKGCWLDDFNCYD
RQECVATKENPQVYFCCCEGNFCNEKFSYFPQMEVTQPTSNPVTPKPP (SEQ ID NO: 112)

Hybrid hu-ActRIIB-ECD
ETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWRNSSGTIEIVKKGCWLDDFNCYD
RQECVATKENPQVYFCCCEGNFCNEKFSYFPQMEVTQPTSNPVTPKPP (SEQ ID NO: 113)

Hybrid hu-ActRIIB-ECD
ETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWRNSSGTIELVKKGCWLDDFNCYD
RQECVATKENPQVYFCCCEGNFCNEKFSYFPQMEVTQPTSNPVTPKPP (SEQ ID NO: 114)

Hybrid hu-ActRIIB-ECD
ETRECIYYNANWELERTNQSGLERCEGDQDKRLHCYASWRNSSGTIEIVKKGCWLDDFNCYD
RQECVAKKENPQVYFCCCEGNFCNEKFSYFPQMEVTQPTSNPVTPKPP (SEQ ID NO: 115)

Hybrid hu-ActRIIB-ECD
ETQECLFFNANWEKDRTNQTGVEPCYGDKDKRRHCFATWKNISGSIEIVKQGCWLDDINCYD
RTDCVEKKDSPEVYFCCCEGNMCNERFTHLPEAGGPEVTYEPPPTAPT (SEQ ID NO: 116)

Hybrid hu-ActRIIB-ECD
ETRECIYYNANWELERTNQSGLERCYGDKDKRRHCYASWRNSSGTIELVKKGCWLDDINCYD
RQECVATKENPQVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPT (SEQ ID NO: 117)

Peptide Linker sequence
ESKYGPPCPPCP (SEQ ID NO: 118)

What is claimed is:

1. An isolated protein comprising a hybrid soluble activin IIB receptor-extracellular domain (ActRIIB-ECD) polypeptide, wherein said hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 1, wherein at least one of amino acid residues R3, I6, Y7, Y8, L14, E15, S20, L22, R24, E26, E28, Q29, L33, L48, Y36, S38, R40, S42, T45, K51, F58, Q64, E65, A68, T69, E70, E71, N72, Q74, F84, R88, T90, H91, L92, E94, A95, G96, G97, P98, E99, V100, Y102, E103, P105, P106, T107, A108, or T110 of SEQ ID NO: 1 is substituted with another amino acid, and wherein said hybrid ActRIIB-ECD polypeptide is capable of binding myostatin and activin A, but demonstrates a decreased binding affinity for BMP9 relative to a wild-type ActRIIB-ECD polypeptide.

2. The isolated protein of claim 1, wherein at least one of amino acid residues R3, I6, Y7, Y8, L14, E15, S20, L22, R24, E26, E28, Q29, L33, L48, Y36, S38, R40, S42, T45, K51, F58, Q64, E65, A68, T69, E70, E71, N72, Q74, F84, R88, T90, H91, L92, E94, A95, G96, G97, P98, E99, V100, Y102, E103, P105, P106, T107, A108, or T110 of SEQ ID NO: 1 is substituted with the amino acid at the corresponding position of SEQ ID NO: 2, and wherein said hybrid ActRIIB-ECD polypeptide is capable of binding myostatin and activin A, but demonstrates a decreased binding affinity for BMP9 relative to a wild-type ActRIIB-ECD polypeptide.

3. The isolated protein of claim 1, wherein said hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence selected from the group consisting of: SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36 and SEQ ID NO: 37.

4. The isolated protein of claim 1, wherein said hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence selected from the group consisting of: SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO:

57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, SEQ ID NO: 73, SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 80, SEQ ID NO: 81, SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85, SEQ ID NO: 86, SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94, SEQ ID NO: 95, SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99, SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 110, SEQ ID NO: 111, SEQ ID NO: 112, SEQ ID NO: 113, SEQ ID NO: 114, SEQ ID NO: 115, SEQ ID NO: 116, and SEQ ID NO: 117.

5. A protein according to any of claims 1-4, wherein the hybrid soluble ActRIIB-ECD polypeptide is fused to at least one heterologous protein.

6. The protein of claim 5, wherein the heterologous protein comprises a constant domain of an immunoglobulin.

7. The protein of claim 6, wherein the heterologous protein comprises an Fc domain of an immunoglobulin.

8. The protein of claim 7, wherein the Fc domain is selected from the Fc domain of a human IgG1, the Fc domain of a human IgG2, or the Fc domain of a human IgG4.

9. The protein of claim 5, wherein the heterologous protein is fused to the hybrid soluble ActRIIB-ECD polypeptide by a linker sequence.

10. An isolated nucleic acid molecule comprising a polynucleotide encoding the polypeptide of any one of claims 1-9.

11. The isolated nucleic acid molecule of claim 10, wherein the polynucleotide further comprises polynucleotides encoding at least one heterologous protein.

12. A recombinant vector comprising the nucleic acid molecule of claim 11.

13. A host cell comprising the recombinant vector of claim 12.

14. The host cell of claim 13, wherein the host cell is a mammalian cell.

15. A method of producing a hybrid ActRIIB protein comprising culturing the host cell of claim 14 under conditions promoting the expression of the protein, and recovering the protein.

16. A pharmaceutical composition comprising a therapeutically effective amount of the protein of any one of claims 1-9 in admixture with a pharmaceutically acceptable carrier.

17. A method of treating myostatin-related or activin A-related disorders in a subject, comprising administering to said subject a therapeutically effective amount of the composition of claim 16 to the subject.

18. A method for treating muscle wasting disease in a subject, comprising administering a therapeutically effective amount of the composition of claim 16 to the subject.

19. The method of claim 18, wherein the muscle wasting disease is selected from the group consisting of muscular dystrophy, amyotrophic lateral sclerosis, myositis, ICU myopathy, drug-induced myopathy (e.g., corticosteroid myopathy and statin myopathy), androgen deprivation, congestive obstructive pulmonary disease, emphysema, cystic fibrosis, chronic heart failure, cardiac atrophy, cancer cachexia, renal failure, uremia, protein energy wasting, anorexia, malnutrition, sarcopenia, AIDS, sepsis, burn injury, diabetes, Huntington's disease, Parkinson's disease, Alzheimer's disease, carpal tunnel syndrome, and muscle wasting due to prolonged bed rest, spinal cord injury, stroke, bone fracture, aging and exposure to microgravity.

20. A method of treating cardiovascular disease in a subject, comprising administering to said subject, comprising administering a therapeutically effective amount of the composition of claim 16 to said subject.

21. The method of claim 20, wherein the cardiovascular disease is selected from the group consisting of heart failure, cardiac atrophy, hypertension, myocarditis, coronary artery disease, myocardial infarction, cardiac arrhythmias, heart valve disease, cardiomyopathy, pericardial disease, aorta disease, cardiac transplantation, and Marfan syndrome.

22. A method of treating metabolic disorders in a subject, comprising administering a therapeutically effective amount of the composition of claim 16 to said subject.

23. The method of claim 22, wherein the metabolic disorder is selected from selected from the group consisting of insulin resistance, diabetes, obesity, sarcopenic obesity, dyslipidemia and fatty liver disease.

24. A method of treating cancer cells in a subject, comprising administering a therapeutically effective amount of the composition of claim 16 to said subject.

25. The method of claim 24, wherein the cancer is selected from the group consisting of lung cancer, pancreatic cancer, prostate cancer, gastric cancer, esophageal cancer, colon cancer, breast cancer, head and neck cancer, ovarian cancer, testicular cancer, multiple myeloma, lymphoma, leukemia, myelodysplastic syndrome, or rhabdomyosarcoma and other cancer.

26. A method of treating renal disease in a subject, comprising administering a therapeutically effective amount of the composition of claim 16 to the subject.

27. The method of claim 26, wherein the renal disease is selected from the group consisting of chronic kidney disease, end-stage renal disease, uremia, protein energy wasting, and kidney transplantation.

28. A method of treating inflammatory/autoimmune disease in a subject, comprising administering a therapeutically effective amount of the composition of claim 16 to said subject.

29. The method of claim 28, wherein the inflammatory/autoimmune disease is selected from the group consisting of rheumatoid arthritis, inflammatory bowel disease, lupus, and multiple sclerosis.

30. A method of treating a fibrosis disease in a subject, comprising administering a therapeutically effective amount of the composition of claim 16 to the subject.

31. The method of claim 30, wherein the fibrosis disease is selected from from the group consisting of pulmonary fibrosis, liver cirrhosis, cardiac fibrosis, renal fibrosis, myelofibrosis, idiopathic retroperitoneal fibrosis, nephrogenic fibrosing dermopathy, Crohn's Disease, keloid, scleroderma, systemic sclerosis, and arthrofibrosis.

32. A method of treating anemia in a subject, comprising administering a therapeutically effective amount of the composition of claim 16 to the subject.

33. The method of claim 32, wherein the anemia is selected from selected from the group consisting of iron deficiency anemia, thalassemia, hemolytic anemia, sickle cell anemia, pernicious anemia, fanconi anemia and aplastic anemia (including cancer-associated anemia and chemotherapy-induced anemia).

34. A method of treating pain in a subject, comprising administering a therapeutically effective amount of the composition of claim 16 to the subject.

35. The method of claim 34, wherein the pain is selected from the group consisting of neuropathic pain, somatic pain, visceral pain, inflammatory pain, cancer pain, back pain and joint pain.

36. A method of treating aging condition in a subject, comprising administering a therapeutically effective amount of the composition of claim 16 to the subject.

37. The method of claim 36, wherein the aging condition is selected from the group consisting of frailty of the elderly, age-related sarcopenia, and osteoarthritis.

38. A method of inducing stem cell growth for tissue repair or organ regeneration in a subject, comprising administering a therapeutically effective amount of the composition of claim 16 to the subject.

39. The method of claim 38, wherein the stem cell is selected from the group consisting of muscle stem (satellite) cell, cardiac stem cell, bone marrow derived mesynchymal stem cell, and pluripotent stem cell.

40. A method of treating bone disorders in a subject, comprising administering a therapeutically effective amount of the composition of claim 16 to the subject.

41. The method of claim 40, wherein the bone disorder is selected from the group consisting of osteomalacia, osteoporosis, osteogenesis imperfecta, fibrodysplasia ossificans progressive, corticosteroid-induced bone loss, bone fracture, and bone metastasis.

42. A method of treating a muscle wasting or metabolic or fibrotic or inflammatory or activin related disorder in a subject, comprising administering the vector of claim 12 to said subject, wherein the vector is capable of directing expression of a hybrid ActRIIB protein in said subject.