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(54) COMPOSITIONS AND METHODS FOR TREATING PULMONARY HYPERTENSION

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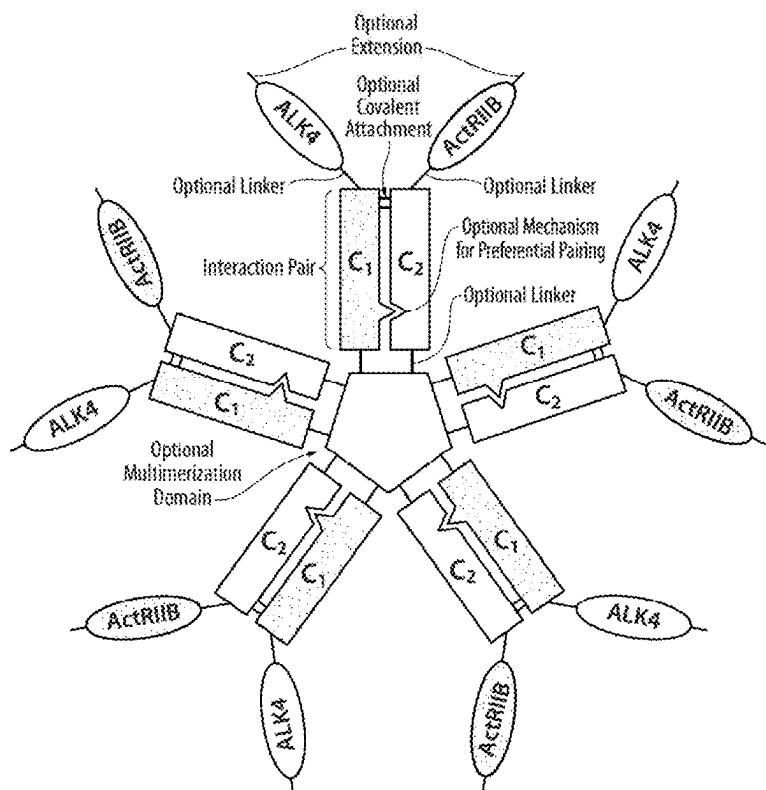
(52) U.S. Cl.

CPC *A61K 38/1875* (2013.01); *A61K 38/179* (2013.01); *A61K 38/1796* (2013.01); *A61K 38/45* (2013.01); *A61K 45/06* (2013.01); *A61P 7/00* (2018.01); *A61P 11/00* (2018.01); *C07K 14/71* (2013.01); *C07K 23/930* (2013.01)

ABSTRACT

In some aspects, the disclosure relates to GDF/BMP antagonists and methods of using GDF/BMP antagonists to treat, prevent, or reduce the progression rate and/or severity of pulmonary hypertension (PH), particularly treating, preventing or reducing the progression rate and/or severity of one or more PH-associated complications. The disclosure also provides methods of using a GDF/BMP antagonist to treat, prevent, or reduce the progression rate and/or severity of a variety of conditions including, but not limited to, pulmonary vascular remodeling, pulmonary fibrosis, and right ventricular hypertrophy. The disclosure further provides methods of using a GDF/BMP antagonist to reduce right ventricular systolic pressure in a subject in need thereof.

Specification includes a Sequence Listing.



ActRIIa ILGRSETQEC LFFNANWEKD RTNQTGVEPC YGDKDKRRH C FAIWKNISGS
ActRIIb GRGEAETREC IYYNANWELE RTNOSGLERC ECEQDKRLHC YASWRNSSGT

IEIVRKQGCWL DDINCYDRTD CVEKKDSPEV YFCCCEGNMC NEKFSYFP EM
IELVRKGWL DDFNICYDRQE CVATEENPQV YFCCCEGNPC NERFTHLPEA

EVTQPTSNSPV TPKPP
GGPEVTYEPP PTAPT

FIGURE 1

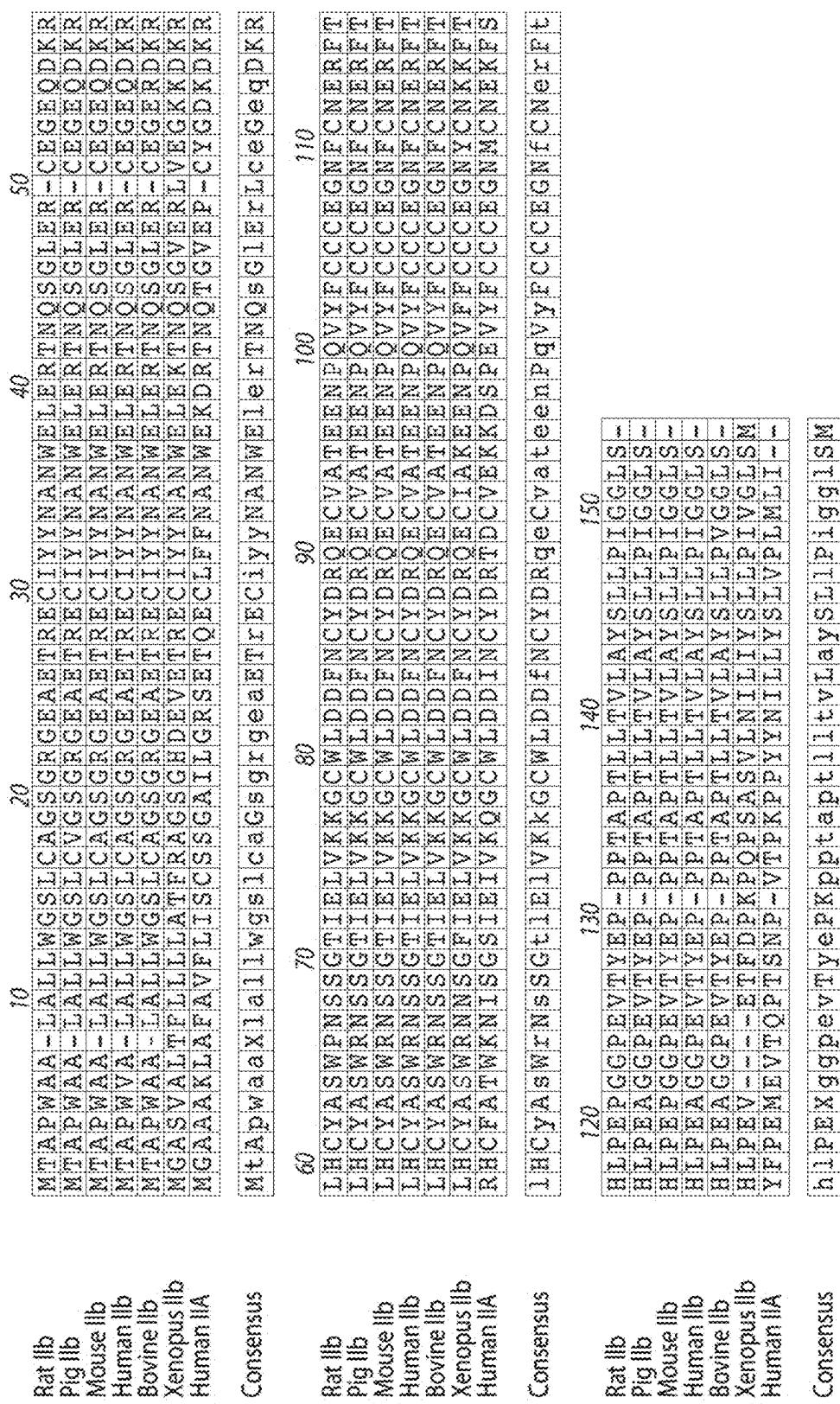


FIGURE 2

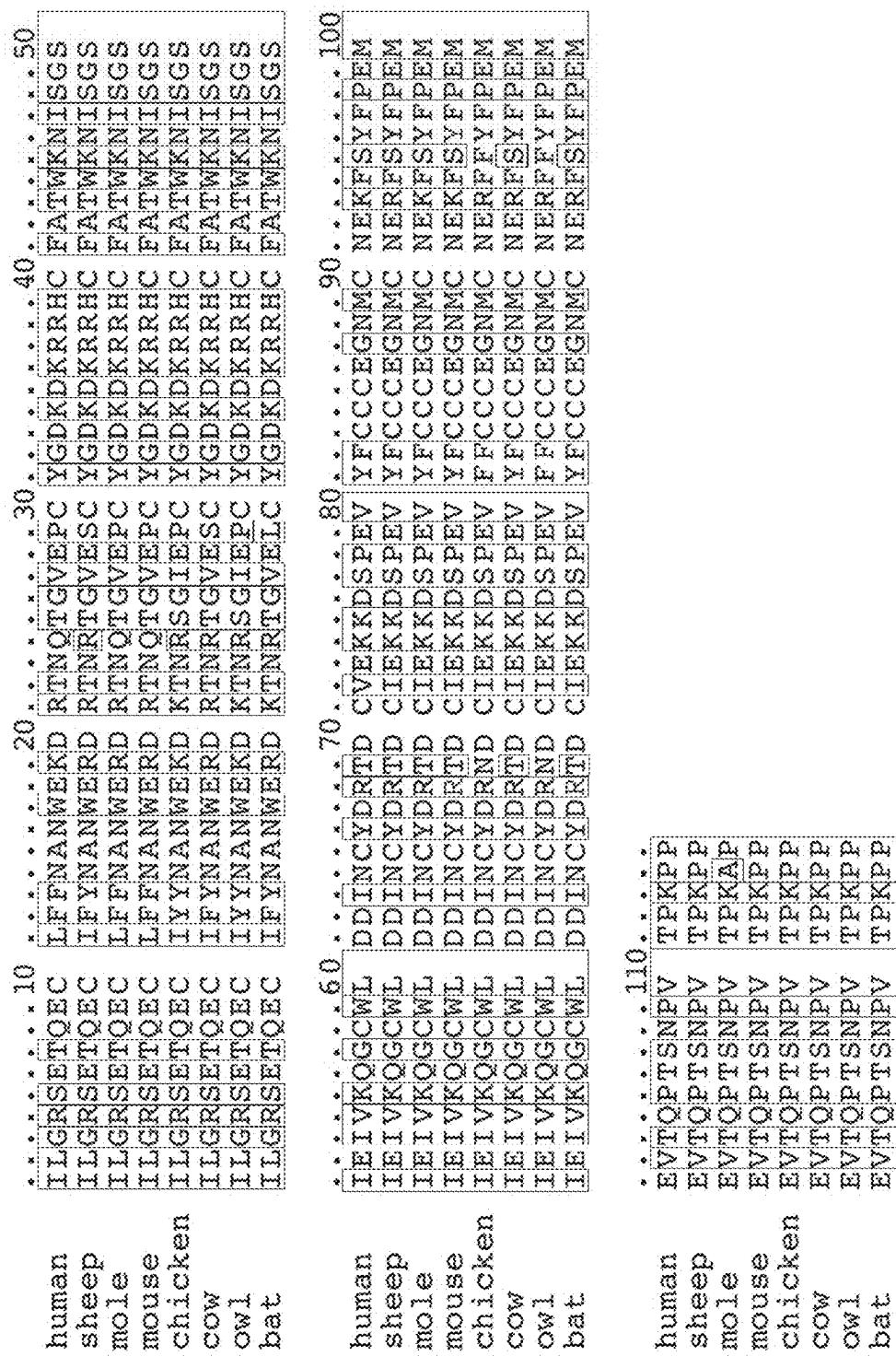


FIGURE 3

IgG1 -----THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF 53
IgG4 ---ESKYGPPCPSCPAPAEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSOEDPEVQF 57
IgG2 -----VECPPPAPPVAG-PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQF 51
IgG3 EPKSCDTPPPCPRCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQF 60

*** * *** . * *****;*****;*****;

IgG1 NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT 113
IgG4 NWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKT 117
IgG2 NWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKT 111
IgG3 KWyVDGVEVHNAKTKPREEQYNSTFRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT 120

;*****;***;*****;*****;***;*****;

IgG1 ISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGOPENNYKTTP 173
IgG4 ISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGOPENNYKTTP 177
IgG2 ISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGOPENNYKTTP 171
IgG3 ISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESSGOPENNYNTTP 180

;**;*****;*****;*****;*****;***

IgG1 PVLDSDGSSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK 225
IgG4 PVLDSDGSSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK 229
IgG2 PMLDSDGSSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK 223
IgG3 PMLDSDGSSFFLYSKLTVDKSRWQQGNIFSCSVMHEALHNRFQTQKSLSLSPGK 232

*;*****;*****;**;*****;*****;***** **

FIGURE 4

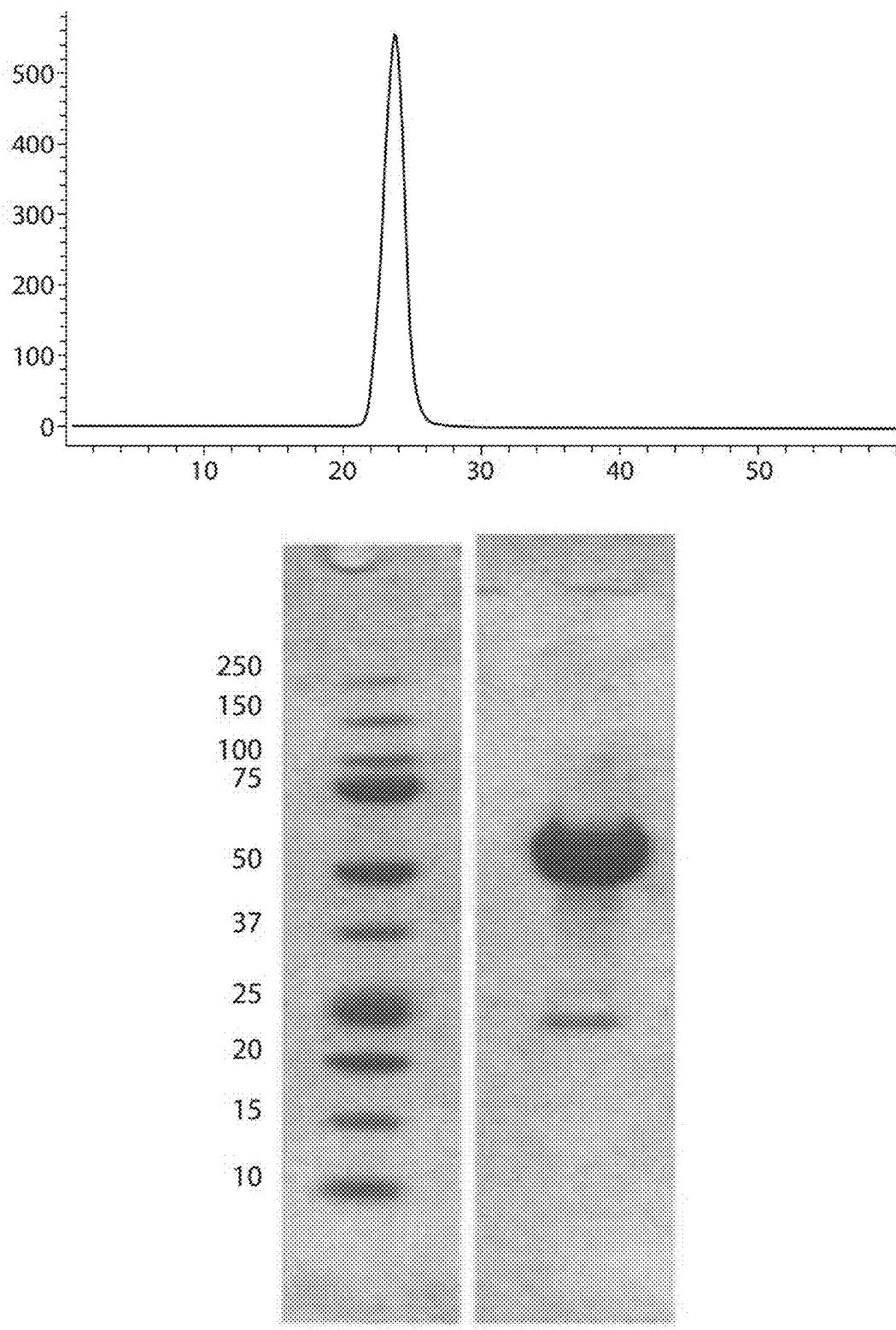


FIGURE 5

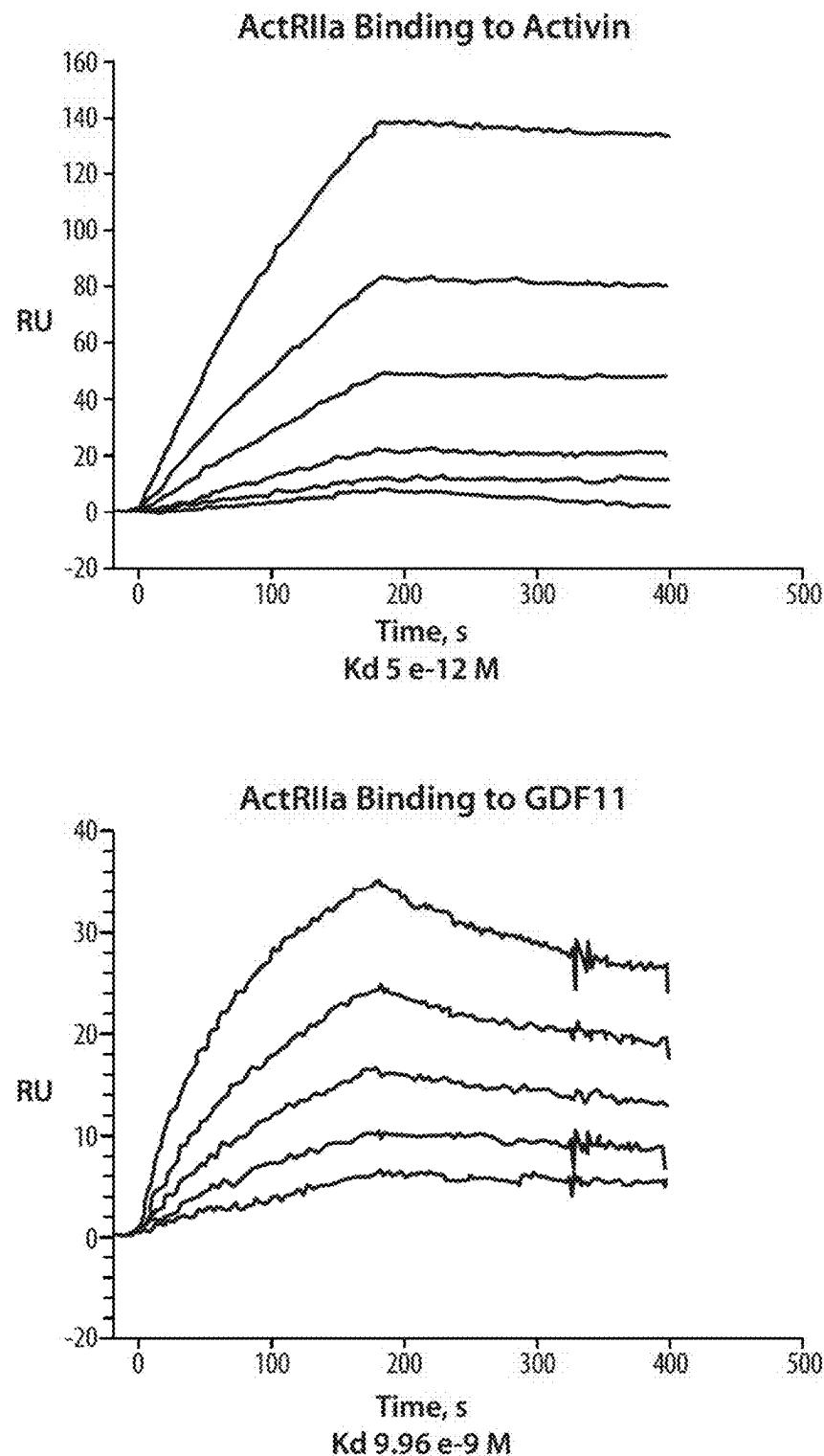


FIGURE 6

1 MDAMKRGGLCC VILLCGAVFV SPGAAETREC IYYNANWELE RTNQSGLERC
51 EGEQDKRLHC YASWRNNSGT IELVKKGCWL DDFNCYDRQE CVATEENPQV
101 YFCCCCEGNFC NERFTHLPEA GGPEVTYEPP PTGGGTHTCP PCPAPELLGG
151 PSVFLFPPKP KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA
201 KTKPREEQYN STYRVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTI
251 KAKGQPREPQ VYTLPPSREE MTKNQVSLTC LVKGFYPSDI AVEWESNGQP
301 ENNYKTTPPV LDSDGSFFLY SKLTVDKSRW QQGNVFSCSV MHEALHNHYT
351 QKSLSLSPGK (SEQ ID NO: 69)

FIGURE 7

1 ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
 TACCTACGTT ACTTCTCTCC CGAGACGACA CACGACGACG ACACACCTCG
 A E T R E C I Y Y
 51 AGTCTTCGTT TCGCCCCGGCG CGCGCTGAGAC ACGGGAGTGC ATCTACTACA
 TCAGAACCAA AGCGGGCCGC GGCGACTCTG TGCCCTCACG TAGATGATGT
 N A N W E L E R T N Q S G L E R C
 101 ACGCCAATG GGAGCTGGAG CGCACCAACC AGAGCGGCCCT GGACCGCTGC
 TGGGGTTGAC CCTCGACCTC CGCTGGTTGG TCTCGCCGGA CCTCGCGACG
 E G E O D K R L H C Y A S W R N S
 151 GAAGGGCGAGC AGGACAAGCG GCTGCACTGC TAGGCCTCCT GGCGCAACAG
 CTTCCGCTCG TCCTGTCGCG CGACGCTGACG ATGGGGAGGA CGCGGTTGTC
 S G T I E L V K X G C W L D D F
 201 CTCTGGCACC ATCGAGCTCG TGAAGAACGG CTGCTGGCTA GATGACTTCA
 GAGACCGTGG TAGCTCGAGC ACTTCTTCCC GACGACCGAT CTACTGAAGT
 N C Y D R Q E C V A T E E N P Q V
 251 ACTGCTACGA TAGGCAGGGAG TGTGTGGCCA CTGAGGAGAA CCCCCAGGTG
 TGACGATGCT ATCCGTCCTC ACACACCGGT GACTCCTCTT GGGGGTCCAC
 Y F C C C E G N F C N E R F T H L
 301 TACTTCTGCT GCTGTGAAGG CAACTTCTGC AACGAGCGCT TCACTCATTT
 ATGAAGACGA CGACACTTCC GTTGAAGACG TTGCTCGCGA AGTGAGTAAA
 P E A G G P E V T Y E P P P T
 351 GCCAGAGGCT GGGGGCCCGG AAGTCACGTA CGAGCCACCC CCGACAGGTG
 CGGTCTCCGA CCCCCGGGCC TTCAGTGCAT GCTCGGTGGG GGCTGTCAC
 GTGGAACTCA CACATGCCCA CGTGCCCCAG CACCTGAAC CCTGGGGGA
 401 CACCTTGAGT GTGTACGGGT GGCAACGGTC GTGCACTTGA GGACCCCCCT
 CCGTCAGTCT TCCTCTTCCC CCCAAAACCC AAGGACACCC TCATGATCTC
 GGCAGTCAGA AGGAGAACGG GGGTTTGAGG TTCCCTGTGGG AGTACTAGAG
 451 CCGGACCCCT GAGGTACACAT CGTGCGTGGT GGACGTGAGC CACGAAGACC
 GGCCTGGGA CTCCAGTGTG CCGACCCACCA CCTGCACTCG GTGCTTCTGG
 CTGAGGTCAA CTTCAACTGG TACGTGGAGC GCGTGGAGGT GCATAATGCC
 501 GACTCCAGTT CAAGTTGACC ATGCACCTGC CGCACCTCCA CGTATTACGG
 AAGACAAAGC CGCGGGAGGA CGAGTACAAC AGCACGTACC GTGTGGTCAG
 551 TTCTGTTCG GCGCCCTCCT CGTCATGTTG TCGTGCATGG CACACCAGTC
 CGTCCTCACCA GTCCTGCACC AGGACTGGCT GAATGGCAAG GAGTACAAGT
 GCAGGAGTGG CAGGACGTGG TCCTGACCGA CTTACCGTTC CTCATGTTCA
 GCAAGGTCTC CAACAAAGCC CTCCCAGCCC CCATCGAGAA AACCATCTCC
 601 CGTTCCAGAG GTTGTTCGG GAGGGTCGG GGTAGCTCTT TTGCTAGAGG
 AAAGCCAAAG GGCAGCCCCG AGAACCAACAG GTGTACACCC TGCCCCCATC
 TTTCGGTTTC CGTCGGGGC TCTTGGTGTG CACATGTGGG ACGGGGTAG

FIGURE 8A

801 CCGGGAGGAG ATGACCAAGA ACCAGGTCAG CCTGACCTGC CTGGTCAAAG
GCCCTCCTC TACTGGTTCT TGGTCCAGTC GGACTGGACG GACCAGTTTC

851 GCTTCTATCC CAGCGACATC GCCGTGGAGT GGGAGAGCAA TGGGCAGCCG
CGAAGATAGG GTCGCTGTAG CGGCACCTCA CCCTCTCGTT ACCCGTCGGC

901 GAGAACAACT ACAAGACCAC GCCTCCCGTG CTGGACTCCG AC GGCTCCTT
CTCTGTTGA TGTTCTGGTG CGGAGGGCAC GACCTGAGGC TGCCGAGGAA

951 CTTCTCTAT AGCAAGCTCA CCGTGGACAA GAGCAGGTGG CAGCAGGGGA
GAAGGAGATA TCGTTCGAGT GGCACCTGTT CTCGTCCACC GTCGTCCCCT

1001 ACGTCTTCTC ATGCTCCGTG ATGCATGAGG CTCTGCACAA CCACTACACG
TGCAGAAGAG TACGAGGCAC TACGTACTCC GAGACGTGTT GGTGATGTGC

1051 CAGAAGAGCC TCTCCCTGTC CCCGGTAAA TGA (SEQ ID NO: 70)
GTCTTCTCGG AGAGGGACAG GGGCCATTT ACT (SEQ ID NO: 71)

FIGURE 8B

1 ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
 TACCTACGTT ACTTCTCTCC CGAGACGACA CACGACGACG ACACACCTCG
 A E T R E C I Y Y
 51 AGTCTTCGTT TCGCCCCGGCG CGCGCGAAAC CGCGCGAATGT ATTATATTACA
 TCAGAACCAA AGCCGGCCGC GGCGGCTTTC GGCGCTTACA TAAATAATGT
 N A N W E L E R T N Q S G L E R C
 101 ATGCTTAATTG GGAACTCCCAA CGGACGAAACC AATCCGGGCT CGAACGGTGT
 TAGGATTAAC CCTTGAGCTT GCCTGCTTGG TTACGGCCGA GCTTGCCACA
 E G E Q D K R L H C Y A S W R N S
 151 GAGGGGGAAC AGGATAAACG CCTCCATTGC TATGCGTGT GGAGGAACTC
 CTCCCCCTTG TCCTATTGC GGAGGTAACG ATACGGCAGCA CCTCCTTGAG
 S G T I E L V K K G C W L D D F
 201 CTCCGGGACG ATTGAACCTGG TCAAGAAMGG GTGCTGGCTG GACGATTTC
 GAGGCCCTGC TAACTTGACC AGTTCTTTC CACGACCGAC CTGCTAAAGT
 N C Y D R Q E C V A T E E N P Q V
 251 ATTGTATGA CGGCCAGGAA TGTGTGGCGA CGGAAGAGAA TCCGCAGGTC
 TAACAATACT GCGGGTCTT ACACAGCGCT GGCTTCTCTT AGGCGTCCAG
 Y F C C C E G N F C N E R F T H L
 301 TATTTCTGTT GTTGCAGGG CAATTTCTGT AATGAACGGT TTACCCACCT
 ATAAAGACAA CAACGCTCCC CTTAAAGACA TTACTTGCCA AATGGGTGGA
 P E A G G P E V T Y E P P P T
 351 CGCCGAAGCC GCGGGGCCCG AGGTACACCTA TGAAACCCCG CCCACGGGTG
 GGGCTTCCG CGGCCCGGCC TCCACTGGAT ACTTGGGCC CGGTGGCAC
 GTGGAACTCA CACATGCCCA CGTGCCCCAG CACCTGAACCT CCTGGGGGGA
 401 CACCTTGAGT GTGTACGGGT GGCACGGTC GTGGACTTGA GGACCCCCCT
 CCGTCAGTCT TCCTCTCCC CCCAAAACCC AAGGACACCC TCATGATCTC
 451 GGCAGTCAGA AGGAGAAGGG GGGTTTGCG TTCCTGTGG AGTACTAGAG
 CGGGACCCCT GAGGTACACAT GCGTGGTGGT GGACGTGAGC CACGAAGACC
 501 GGCCTGGGA CTCCAGTGTG CCGACCCACCA CCTGCACACTCG GTGCTTCTGG
 CTGAGGTCAA GTTCAACTGG TACGTGGAGC GCGTGGAGGT GCATAATGCC
 551 GACTCCAGTT CAAGTTGACC ATGCACCTGC CGCACCTCCA CGTATTACGG
 AAGACAAAGG CGGGGAGGA CGAGTACAAC AGCACGTACC GTGTGGTCAG
 601 TTCTGTTTCG CGGCCCTCCT CGTCATGTG TCGTGCATGG CACACCAAGTC
 CGTCCTCACCG GTCTGCACC AGGACTGGCT GAATGGCAAG GAGTACAAGT
 651 GCAGGAGTGG CAGGACGTGG TCCTGACCGA CTTACCGTTC CTCATGTTCA
 GCAAGGTCTC CAACAAAGCC CTCCCAGCCC CCATCGAGAA AACCATCTCC
 701 CGTTCCAGAG GTTGTTCGG GAGGGTCGGGG GGTAGCTTT TTGGTAGAGG
 AAAGCCAAAG GGCAGCCCCG AGAACCAACAG GTGTACACCC TGCCCCCATC
 TTTCGGTTTC CGTCGGGGC TCTTGGTGTG CACATGTGGG ACGGGGTAG

FIGURE 9A

801 CCGGGAGGAG ATGACCAAGA ACCAGGTCAAG CCTGACCTGC CTGGTCAAAG
GCCCTCCTC TACTGGTTCT TGGTCCAGTC GGACTGGACG GACCAGTTTC

851 GCTTCTATCC CAGCGACATC GCCGTGGAGT GGGAGAGCAA TGGGCAGCCG
CGAAGATAGG GTGCGCTGTAG CGGCACCTCA CCCCTCTCGTT ACCCGTCGGC

901 GAGAACAACT ACAAGACCAC GCCTCCCGTG CTGGACTCCG ACGGCTCCTT
CTCTTGTGA TGTTCTGGTG CGGAGGGCAC GACCTGAGGC TGCCGAGGAA

951 CTTCCCTCAT AGCAAGCTCA CCGTGGACAA GAGCAGGTGG CAGCAGGGGA
GAAGGAGATA TCGTTCGAGT GGACCTGTGTT CTCGTCCACC GTCGTCCCCCT

1001 ACGTCTTCTC ATGCTCCGTG ATGCATGAGG CTCTGCACAA CCACTACACG
TGCAGAAGAG TACGAGGCAC TACGTACTCC GAGACGTGTT GGTGATGTGC

1051 CAGAAGAGCC TCTCCCTGTC CCCGGGTAAA TGA (SEQ ID NO: 72)
GTCTTCTCGG AGAGGGACAG GGGCCCATT ACT (SEQ ID NO: 73)

FIGURE 9B

1 MDAMKRLGLCC VLLLCGAVFV SPGASGRGEA ETRECIYYNA NWELERTNQS
51 GLERCEGEQD KRLHCYASWR NSSGTIELVK KGCGWDDDFNC YDRQECVATE
101 ENPQVYFCCC EGNFCNERFT HLPEAGGPEV TYEPPPTAFT GGGTHTCPPC
151 PAPELLGGPS VFLFPPPKPD TLMISRTPEV TCVVVDVSH DPEVKFNWYV
201 DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY KCKVSNKALP
251 APIEKTISKA KGQPREPQVY TLPPSREEMT KNQVSLTCLV KGFYPSDIAV
301 EWESNGOPEN NYKTTPPVLD SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH
351 EALHNHYTQK SLSLSPGK (SEQ ID NO: 74)

FIGURE 10

1 ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
TACCTACGTT ACTTCTCTCC CGAGACGACA CACGACGACG ACACACCTCG

51 AGTCTTCGTT TCGCCCCGGCG CCTCTGGGCG TGGGGAGGCT GAGACACGGG
TCAGAAAGCAA AGCGGGCCGC GGAGACCCGC ACCCCCTCCGA CTCTGTGCC

101 AGTGCATCTA CTACAACGCC AACTGGGAGC TGGAGCGCAC CAACCAGAGC
TCACGTAGAT GATGTTGCGG TTGACCCCTCG ACCTCGCGTG GTTGGTCTCG

151 GCCCTGGAGC GCTGCCGAAGG CGAGCAGGAC AAGCGGCTGC ACTGCTACGC
CCGGACCTCG CGACGCCCTCC GCTCGTCCTG TTCGCCGACG TGACGATGCG

201 CTCCCTGGCGC AACAGCTCTG GCACCATCGA GCTCGTGAAG AAGGGCTGCT
GAGGACCGCG TTGTCGAGAC CGTGGTAGCT CGAGCACTTC TTCCCGACGA

251 GGGATGATGA CTTCAACTGC TACCGATAAGC AGGAGTGTGT GGCCACTGAG
CCCTACTACT GAAGTTGACG ATGCTATCCG TCCTCACACACA CGGGTGACTC

301 GAGAACCCCC AGGTGTACTT CTGCTGCTGT GAAGGCAACT TCTGCAACGA
CTCTTGGGGG TCCACATGAA GACGACGACA CTTCCGTTGA AGACGPTGCT

351 GCGCTTCACT CATTGCCAG AGGCTGGGGG CCCGGAAGTC ACGTACGAGC
CGCGAAGTGA CTAAACCGTC TCCGACCCCCC GGGCCTTCAG TGCATGCTCG

401 CACCCCCGAC AGCCCCCACC CGTGGTGGAA CTCACACATG CCCACCGTGC
GTGGGGGCTG TCGGGGGTGG CCACCACTT GAGTGTGTAC GGGTGGCACG

451 CCAGCACCTG AACTCCTGGG GGGACCGTCA GTCTTCCTCT TCCCCCCAAA
GGTCGTGGAC TTGAGGACCC CCCTGGCAGT CAGAAGGAGA AGGGGGTTT

501 ACCCAAGGAC ACCCTCATGA TCTCCGGAC CCCTGAGGTC ACATGCGTGG
TGGGTTCTTG TGGGAGTACT AGAGGGCCTG GGGACTCCAG TGTACGCACC

551 TGGTGGACGT GAGCCACCAA GACCCTGAGG TCAAGTTCAA CTGGTACGTG
ACCACCTGCA CTCGGTCTT CTGGGACTCC AGTCAAGTT GACCATGCAC

601 GACGGCGTGG AGGTGCATAA TGCCAAGACA AACCCGGGG AGGAGCAGTA
CTGCCGCACC TCCACGTATT ACGGTTCTGT TTCCGGGCC CTCCTCGTCAT

651 CAACAGCACG TACCGTGTGG TCAGCGTCCT CACCGTCCTG CACCAGGACT
GTTGTCGTGC ATGGCACACC AGTCGCAGGA GTGGCAGGAC GTGGTCTG

701 GGCTGAATGG CAAGGAGTAC AAGTGCAGG TCTCCAACAA AGCCCTCCCA
CCGACTTACG GTTCCTCATG TTCACGTTCC AGAGGTTGTT TCGGGAGGGT

751 GCCCCCATCG AGAAAACCCT CTCCAAAGCC AAAGGGCAGC CCCGAGAAC
CGGGGGTAGC TCTTTGGTA GAGGTTTCGG TTTCCCGTGC GGGCTCTTGG

FIGURE 11A

801 ACAGGTGTAC ACCCTGCCCC CATCCGGGA GGAGATGACC AAGAACCAAGG
TGTCCACATG TGGGACGGGG GTAGGGCCCT CCTCTACTGG TTCTTGGTCC

851 TCAGCCTGAC CTGCCTGGTC AAAGGCTTCT ATCCCAGCGA CATGCCGTG
AGTCGGACTG GACGGACCAG TTTCCGAAGA TAGGGTCGCT GTAGCGGCAC

901 GAGTGGGAGA GCAATGGCA GCCGGAGAAC AACTACAAGA CCACGCCCTCC
CTCACCCCTCT CGTTACCCGT CGGCCTCTTG TTGATGTTCT GGTGCGGAGG

951 CCTGCTGGAC TCCGACGGCT CCTTCTTCCT CTATAGCAAG CTCACCGTGG
GCACGACCTG AGGCTGCCGA GGAAGAAGGA GATATCGTTC GAGTGGCACC

1001 ACAAGAGCAG GTGGCACCCAG GGGAACGTCT TCTCATGCTC CGTGATGCAT
TGTTCTCGTC CACCGTCGTC CCCTTGCAGA AGAGTACGAG GCACTACGTA

1051 GAGGCTCTGC ACAACCACCA CACGCAGAAC AGCCTCTCCC TGTCUUUUGGG
CTCCGAGACG TGTTGGTGAT GTGCCTCTTC TCGGAGAGGG ACAGGGGCC

1051 TAAATGA (SEQ ID NO: 75)
ATTTACT (SEQ ID NO: 76)

FIGURE 11B

1 MDAMKRLGCC VLLLCAVVFV SPGAAETREC IYYNANWELE RTNQSGLERC
51 EGEQDKRLHC YASWRNSSGT IELVKKGWCW DDFNCYDROE CVATEENPQV
101 YFCCCEGNPC NERFTHLPEA GGPEVTYEPP PTGGGTHTCP PCPAPELLGG
151 PSVFLFPPKP KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA
201 KTKPREEQYN STYRVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS
251 KAKGQPREPQ VYTLPPSREE MTKNQVSLTC LVKGFYPSDI AVEWESNGQP
301 ENNYKTTPPV LDSDGSFFLY SKLTVDKSRW QQGNVFSCSV MHEALHNHYT
351 QKSLSLSPGK (SEQ ID NO: 77)

FIGURE 12

1 ETRECIYYNA NWELERTNQS GLERCEGEQD KRLHCYASWR NSSGTIELVK
51 KGCWDDDFNC YDROECVATE ENPQVYPCCC EGNFCNERFT HLPEAGGPEV
101 TYEPPPTGGG THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPRVTCV
151 VVDVSHEDPE VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD
201 WLNGKEYKCK VSNKALPAPI EKTISKAKGQ PREPQVYTLR PSREEMTKNQ
251 VSLTCLVKGF YPSDIAVEWE SNGQPENNYK TPPPVLDSDG SFFLYSKLT
301 DKSRWQOQNV FSCSYVMHEAL HNHYTQKSLS LSPGK (SEQ ID NO: 78)

FIGURE 13

1 ETRECIYYNA NWELERTNQS GLERCEGEQD KRLHCYASWR NSSGTIELVK
51 KGCWDDDFNC YDRQECVATE ENPQVYFCCC EGNFCNERFT
101 HLPEAGGPEV TYEPPPT (SEQ ID NO: 79)

FIGURE 14

1 ATGGATGCAA TGAAGAGAGG CCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
TACCTACGTT ACTTCTCTCC CGAGACGACA CACGACGACG ACACACCTCG
 E T R E C I Y Y
 51 ACTCTTCGTT TCGCCCCGGCG CCGCTGAGAC ACGGGAGTGC ATCTACTACA
TCAGAAAGCAA AGCGGGCCGC GGCGACTCTG TGCCCTCACG TAGATGATGT
 N A N W E L E R T N Q S G L E R C
 101 ACGCCAAC TG GGAGCTGGAG CGCACCAACC AGAGCGGCCG GGAGCGCTGC
TGCCTGAC CCTCGACCTC GCGTGCTGG TCTCGCCCGA CCTCGCGACG
 E G E Q D K R L H C Y A S W R N S
 151 GAAGGGCGAGC AGGACAAGCG GCTGCACTGC TAGCCTCCT GGCGAACAG
CTTCCCGCTCG TCCTGTTCCG CGACGTGACG ATGCCGAGGA CGCGCTTGTG
 S G T I E L V K K G C W D D D F
 201 CTCTGGCACC ATCCGACTCG TGAAGAACGG CTGCTGGCAC GATCACTTCA
GAGACCGTGG TAGCTCGAGC ACTTCTTCCC GACGACCCCTG CTACTGAAGT
 N C Y D R Q E C V A T E E N P Q V
 251 ACTGCTACGA TAGGCAGGGAG TGTGTGGCCA CTGAGGGAGAA CCCCCAGGTG
TGACGATGCT ATCCGTCCTC ACACACCCGT GACTCCTCTT GGGGGTCCAC
 Y F C C C E G N F C N E R F T H L
 301 TACTTCTGCT GCTGTGAAGG CAACTTCTGC AACGAGCGCT TCACTCATT
ATGAAGACGA CGACACTTCC GTTGAAGACG TTGCTCGCGA AGTGAGTAAA
 P E A G G P E V T Y E P P P T
 351 GCCAGAGGCT GGGGGCCCGG AAGTCACGTA CGAGCCACCC CCGACAGGTG
CGGTCTCCGA CCCCCGGGCC TTCAGTGCAT GCTCGGTGGG GGCTGTCCAC
 GTGGAACTCA CACATGCCCA CCGTCCCCAG CACCTGAAC TCTGGGGGG
 401 CACCTTGACT GTGTACGGGT GGCACGGGTC GTGGACTTGA GGACCCCCCT
 CCGTCAGTCT TCCTCTTCCC CCCAAAACCC AAGGACACCC TCATGATCTC
 451 GGCAGTCAGA AGGAGAAGGG GGGTTTGCG TTCTGTGGG AGTACTAGAG
 CCGGACCCCT GAGGTACAT CGTGGTGGT GGACGTGAGC CACGAAGACC
 501 GGCCTGGGGGA CTCCAGTGTGTA CGCACCA ACC CCTGCACTCG GTGCTTCTGG
 CTGAGGTCAA GTTCAACTGG TACGTGGAGC CGGTGGAGGT GCATAATGCC
 551 GACTCCAGTT CAAGTTGACC CTGCACCTGC CGCACCTCCA CGTATTACGG

FIGURE 15A

601 AAGACAAAGC CGCGGGAGGA GCAGTACAAC ACCACGTACC GTGTGGTCAG
TTCTGTTTCG GCGCCCTCCT CGTCATGTTG TCGTGCATGG CACACCAAGTC

651 CGTCCTCACCC GTCCCTGCACC AGGACTGGCT GAATGGCAAG GAGTACAAGT
GCAGGAGTGG CAGGACGTGG TCCTGACCGA CTTACCGTTC CTCATGTTCA

701 GCAAGGTCTC CAACAAAGCC CTCCCAGCCC CCATCGAGAA AACCATCTCC
CGTTCCAGAG GTTGTTCGG GAGGGTCGGG GGTAGCTCTT TTGGTAGAGG

751 AAAGCCAAAG GGCAAGCCCCG AGAACACACAG GTGTACACCC TGCCCCCATC
TTTGGGTTTC CCGTCGGGGC TCTTGGTGTC CACATGTGGG ACGGGGGTAG

801 CCCGGACCGAC ATCACCAACA ACCAGCTCAG CCTGACCTGCC CTGGTCAAAC
GGCCCTCCTC TACTGGTTCT TGTTCCAGTC GGACTGGACG GACCAGTTTC

851 GCTTCTATCC CAGCGACATC GCCGTGGAGT GGGAGAGCAA TGGGCAGCCG
CGAAGATAAGG GTGGCTGTAG CGGCACCTCA CCCTCTCGTT ACCCGTCGGC

901 GAGAACAACT ACAAGACAC GCCTCCGTG CTGGACTCCG ACGGCTCCTT
CTCTTCTGTA TGTTCTGGTG CCGAGGGCAC GACCTGAGGC TGCCGAGGAA

951 CTTCTCTAT AGCAAGCTCA CCGTGGACAA GAGCAGGTGG CAGCAGGGGA
GAAGGAGATA TCGTTCGAGT GGCACCTGTT CTCGTCCACC GTCGTCCCC

1001 ACGTCTTCTC ATGCTCCGTG ATGCATGAGG CTCTGCACAA CCACTACACG
TGCAGAAGAG TACGAGGCAC TACGTACTCC GAGACGTGTT GGTGATGTGC

1051 CAGAAGAGCC TCTCCCTGTC CCCGGGTAAA TGA (SEQ ID NO: 80)
GTCTTCTCGG AGAGGGACAG GGGCCCATT ACT (SEQ ID NO: 81)

FIGURE 15B

1 ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
 TACCTACGTT ACTTCTCTCC CGAGACGACA CACGACGACG ACACACCTCG
 E T R E C I Y Y
 51 AGTCTTCGTT TCGCCCCGGCG CGGCCGAAC CGCGGAATGT ATTATTACCA
 TCAGAAGCAA AGCGGGCCGC GGCGGCTTTG GGCGCTTACA TAAATAATGT
 N A N N W E L E R T N Q S G L E R C
 101 ATGCTTAATTG CGAACACTCGAA CGCACCAACC AATCCGGGCT CGAACCGGTCT
 TACGATTAAC CCTTGAGCTT GCCTGCTTGG TTAGGCCCCGA CCTTGCCACA
 E G E Q D K R L H C Y A S W R N S
 151 GAGGGGGAAAC ACGATAAAACG CCTCCATGCC TATGCCCTCT CGACGAACTC
 CTCCCCCTTG TCCTATTGTC GGAGGTAACG ATACGCCAGCA CCTCCCTTGAG
 S G T I E L V K K G C W D D D F
 201 CTCCGGGACG ATTGAACCTGG TCAAGAAAGG GTGCTGGGAC GACGATTTCA
 GAGGCCCTGC TAACCTGACC ACTTCTTCC CACGACCCCTG CTGCTAAACT
 N C Y D R Q E C V A T E E N P Q V
 251 ATTGTTATGA CCGCCAGGAA TGTGTGGCGA CGGAAGAGAA TCCGCAGGTC
 TAACAATACT GGCGGTCCCT ACACAGGGCT GGCTTCTCTT AGGGTCCAG
 Y F C C C E G N F C N E R F T H L
 301 TATTCTGTT GTTGGAGGG GAATTCTGT AATGABCCTT TTACCCACCT
 ATAAGACAA CAACGCTCCC CTTAAAGACA TTACTTGCCA AATGGGTCCGA
 P E A G G P E V T Y E P P P T
 351 CGCCGAAGCC CGGGGGCCCG AGGTGACTTA TGAACCCCCCG CCCACCGGTG
 GGGCTTCGG CGGCCCGGGC TCCACTGGAT ACTTGGGGC GGGTGGCCAC
 GTGGAACCTCA CACATGCCCA CGTGCCCCAG CACCTGAACCT CCTGGGGGGA
 401 CACCTTGACT GTGTACGGGT CGCACGGTC GTGGACTTGA GGACCCCCCT
 CCGTCAGTCT CCCTCTTCCC CCCAAACCC AAGGACACCC TCATGATCTC
 451 GGCAGTCAGA AGGAGAAGGG GGGTTTGGG TTCCCTGTGGG AGTACTAGAG
 CCGGACCCCT GAGGTACAT CGGTGGTGGT GGACGTGAGC CACGAAGACC
 501 GGCCTGGGA CTCCAGTGTGTA CGCACCCACCA CCTGCACTCG GTGCTTCTGG
 CTGAGGTCAA GTTCAACTGG TACGTGGACG CGGTGGAGGT GCATAATGCC
 551 GACTCCAGTT CAAGTTGACC ATGCACCTGC CGCACCTCCA CGTATTACGG
 AACACAAAGC CGGGGAGGA CGACTACAAC ACCACGTACC GTGTGGTCAG
 601 TTCTGTTCG CGGCCCTCCT CGTCATGTTG TCGTGCATGG CACACCAGTC
 CGTCCTCACC GTCCTGCACC AGGACTGGCT GAATGGCAAG GAGTACAAGT
 651 GCAGGAGTGG CAGGACGTGG TCCCTGACCGA CTTACCGTTC CTCATGTTCA

FIGURE 16A

701 GCAAGGTCTC CAACAAAGCC CTCCCAGCCCC CCATCGAGAA AACCATCTCC
CGTTCCAGAG GTTGTTCGG GAGGGTCGGG GGTAGCTCTT TTGCTAGAGG

751 AAAGCCAAAG CGCAGCCCCG AGAACACAG GTGTACACCC TGCCCCCATC
TTTCGGTTTC CCGTCGGGGC TCTTGGTGTG CACATGTGGG ACCGGGGTAG

801 CCGGGAGGAG ATGACCAAGA ACCAGGTCAAG CCTGACCTGC CTGGTCAAAG
GGCCCTCCTC TACTGGTTCT TGGTCCAGTC GGACTGGACG GACCAGTTTC

851 GCTTCTATCC CAGCGACATC GCCGTGGAGT GGGAGAGCAA TGGCAGCCG
CGAACATAAGG GTGGCTGTAG CGGCACCTCA CCCTCTCGTT ACCCGTCGGC

901 GAGAACAACT ACAAGACCAC GCCTCCGTG CTGGACTCCG ACGGCTCCTT
CTCTTGTGA TGTTCTGGTG CGGAGGGCAC GACCTGAGGC TGCCGAGGAA

951 CTTCCTCTAT AGCAAGCTCA CCGTGGACAA GACCAGGTGG CAGCAGGGGA
GAAGGAGATA TCGTTCGAGT GGCACCTGTT CTCGTCCACC GTGGTCCCCT

1001 ACGTCTTCTC ATGCTCCGTG ATGCATGAGG CTCTGCACAA CCACTACACG
TGCAGAAGAG TACGAGGCAC TACGTACTCC GAGACGTGTT GGTGATGTGC

1051 CAGAAGAGCC TCTCCCTGTC CCCGGTAAA TGA (SEQ ID NO: 82)
GTCTTCTCGG AGAGGGACAG GGGCCCATTT ACT (SEQ ID NO: 83)

FIGURE 16B

GAAAC CCCCGAATGT ATTTATTACA ATGCCAATTG GGAACTCGAA CGGACGAACC
AATCCGGGCT CGAACCGGTGT GAGGGGGAAC AGGATAAAACG CCTCCATTGC TATGCCTCGT
GGAGGAACTC CTCGGGACG ATTGAAC ACTGG TCAAGAA AGG GTGCTGGGAC GACGATTTCA
ATTGTATGA CCGOCAGGAA TGTGTCGCGA CCGAAGAGAA TCCGCAGGTC TATTTCTGTT
GTTGCGAGGG GAATTCTGT AATGAACGGT TTAACCCACCT CCCCGAAGCC GGGGGGCCCG
AGGTGACCTA TGAACCCCCG CCCACCC (SEQ ID NO: 84)

FIGURE 17

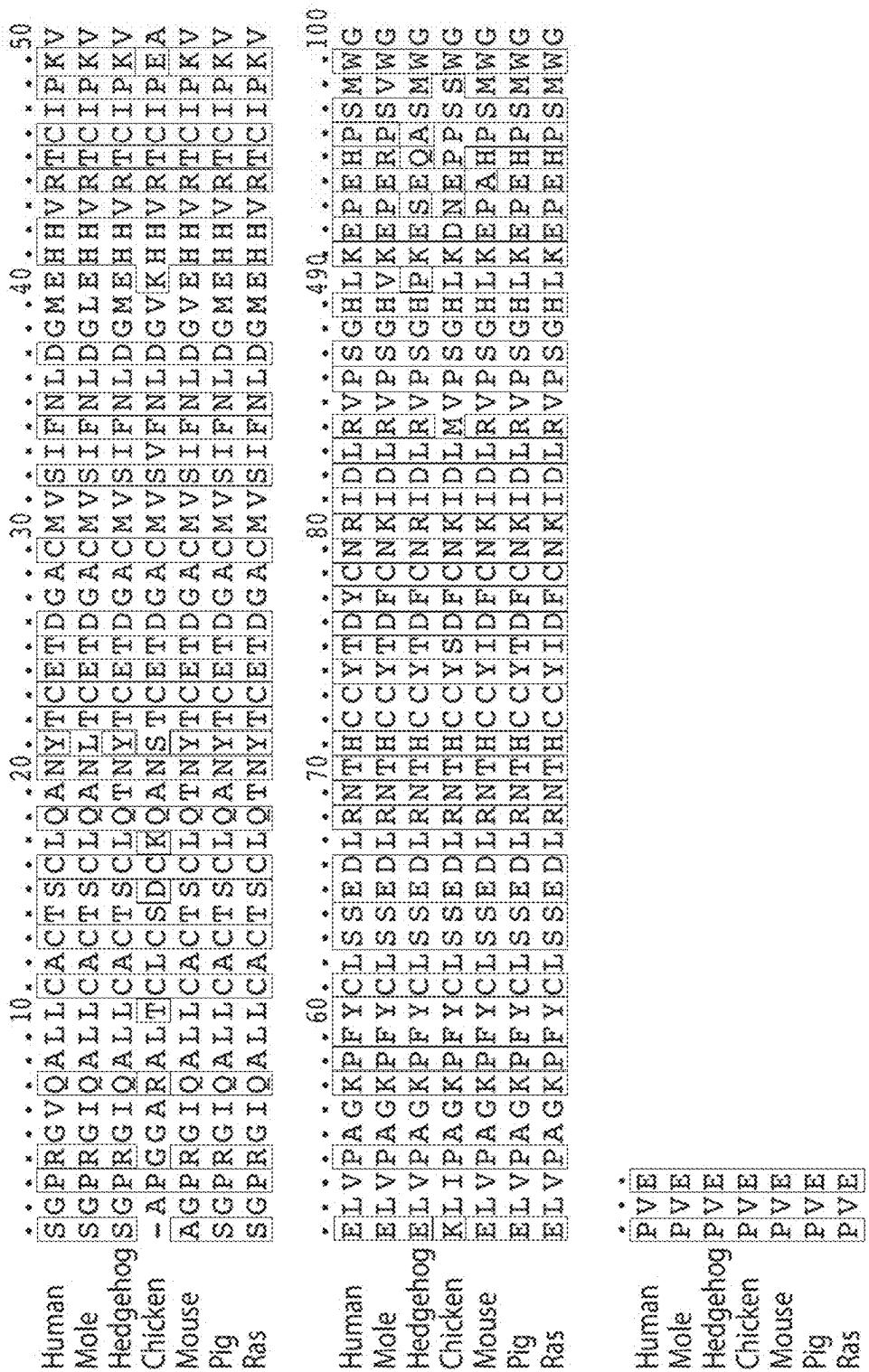


FIGURE 18

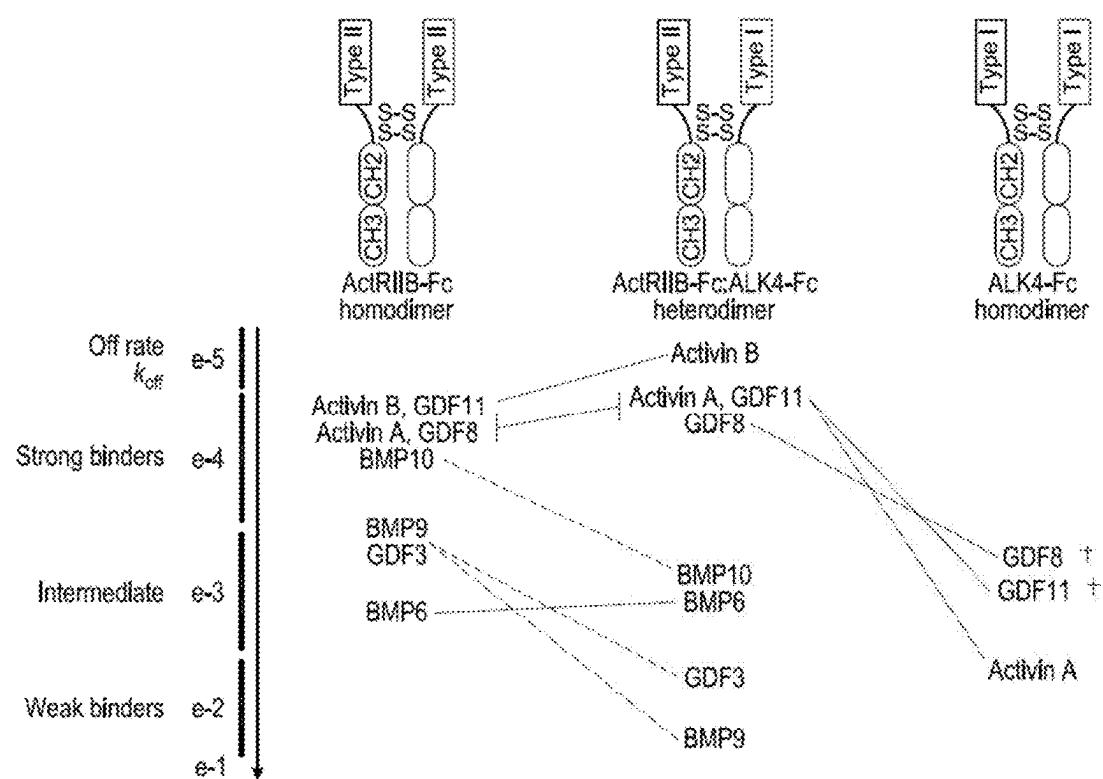


Figure 19

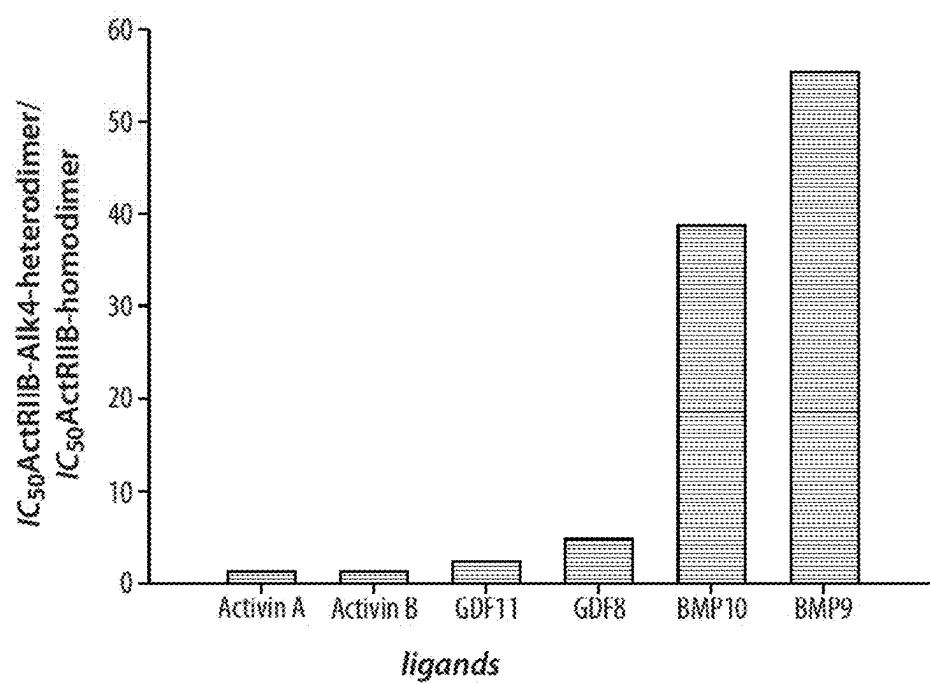


FIGURE 20

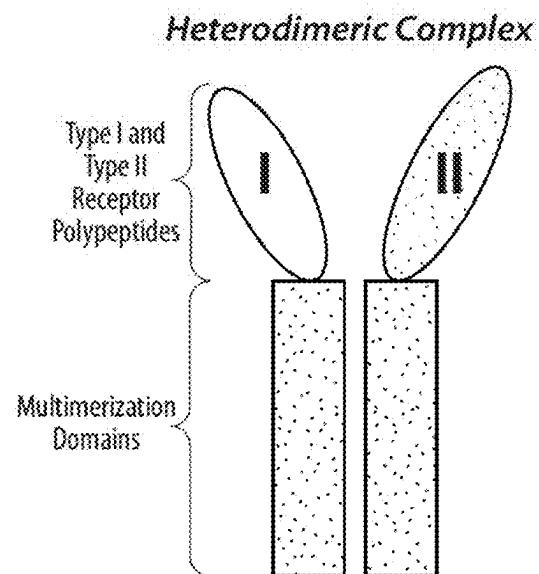


FIGURE 21A

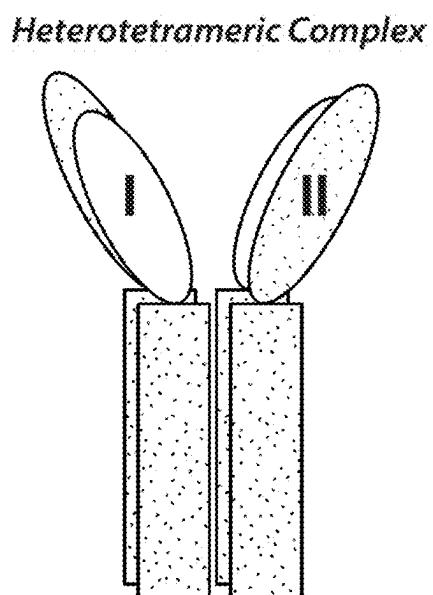


FIGURE 21B

Schematic Heteromeric Protein Complex

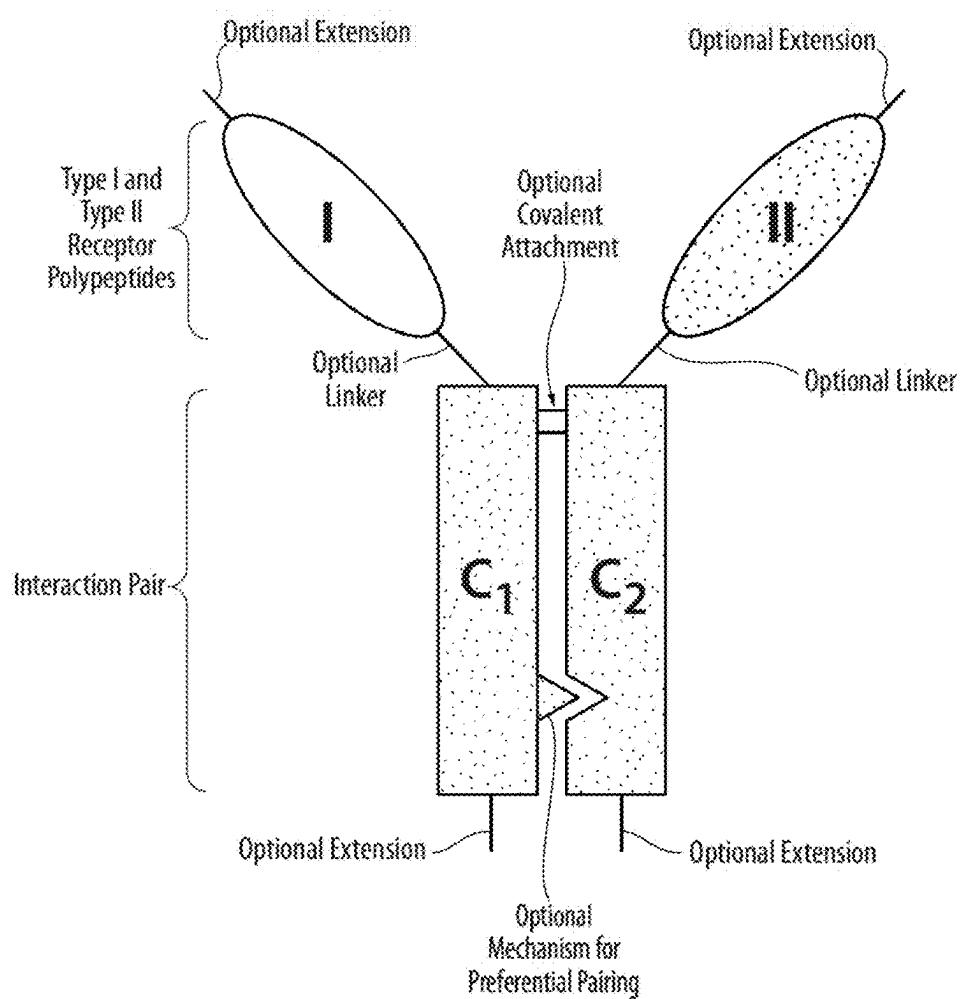


FIGURE 22

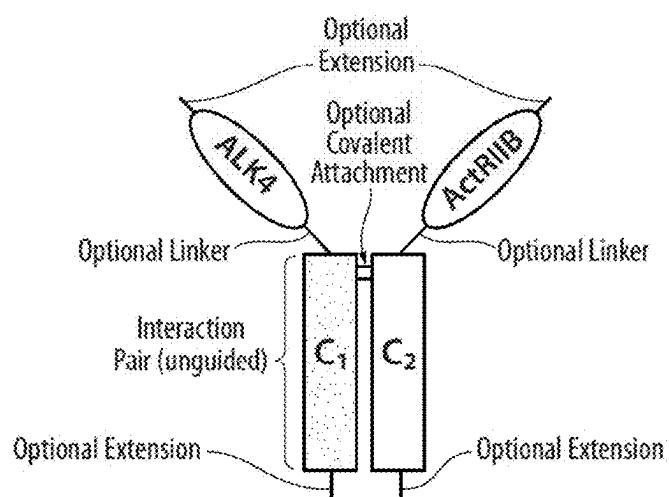


FIGURE 23A

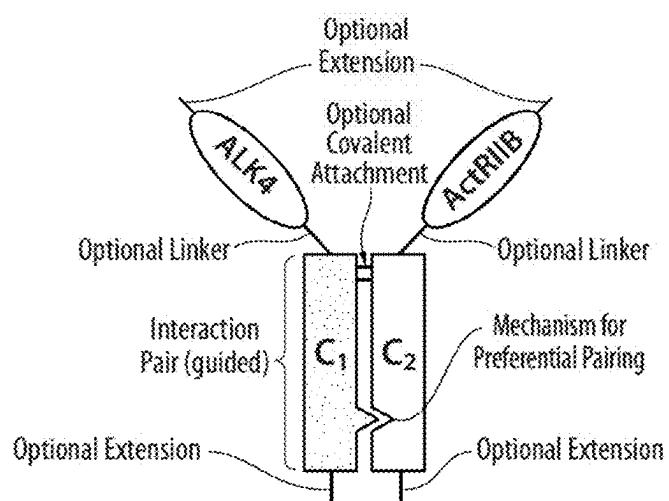


FIGURE 23B

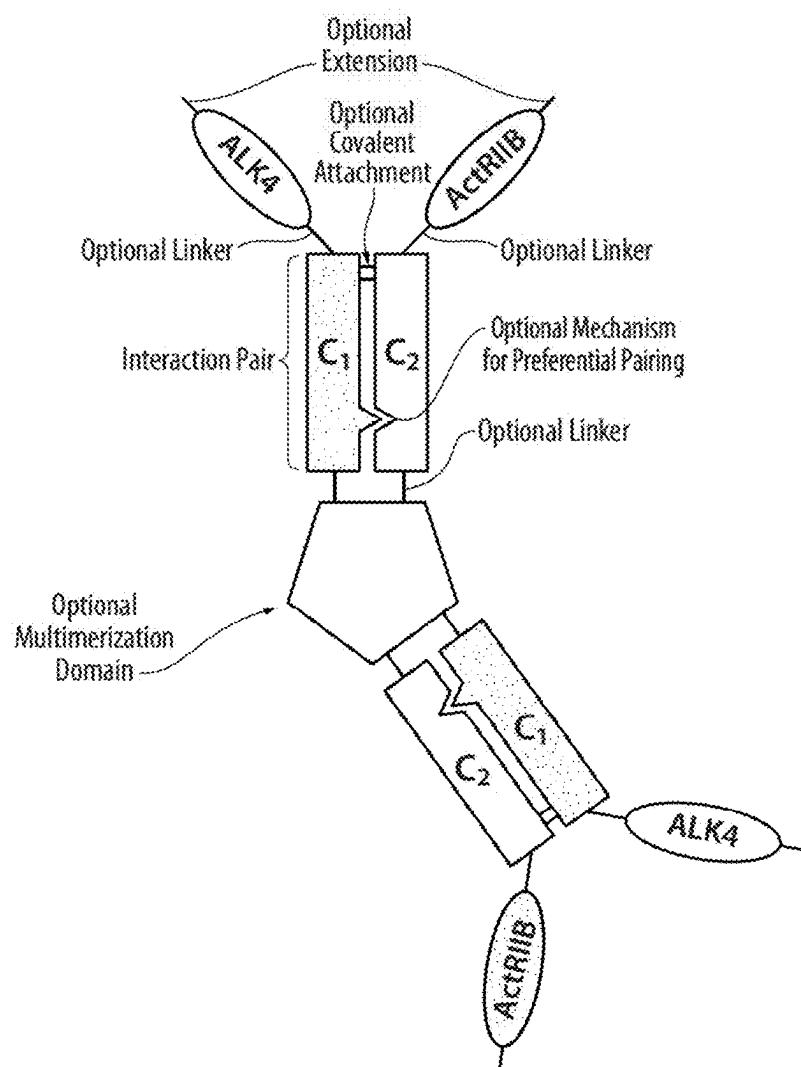


FIGURE 23C

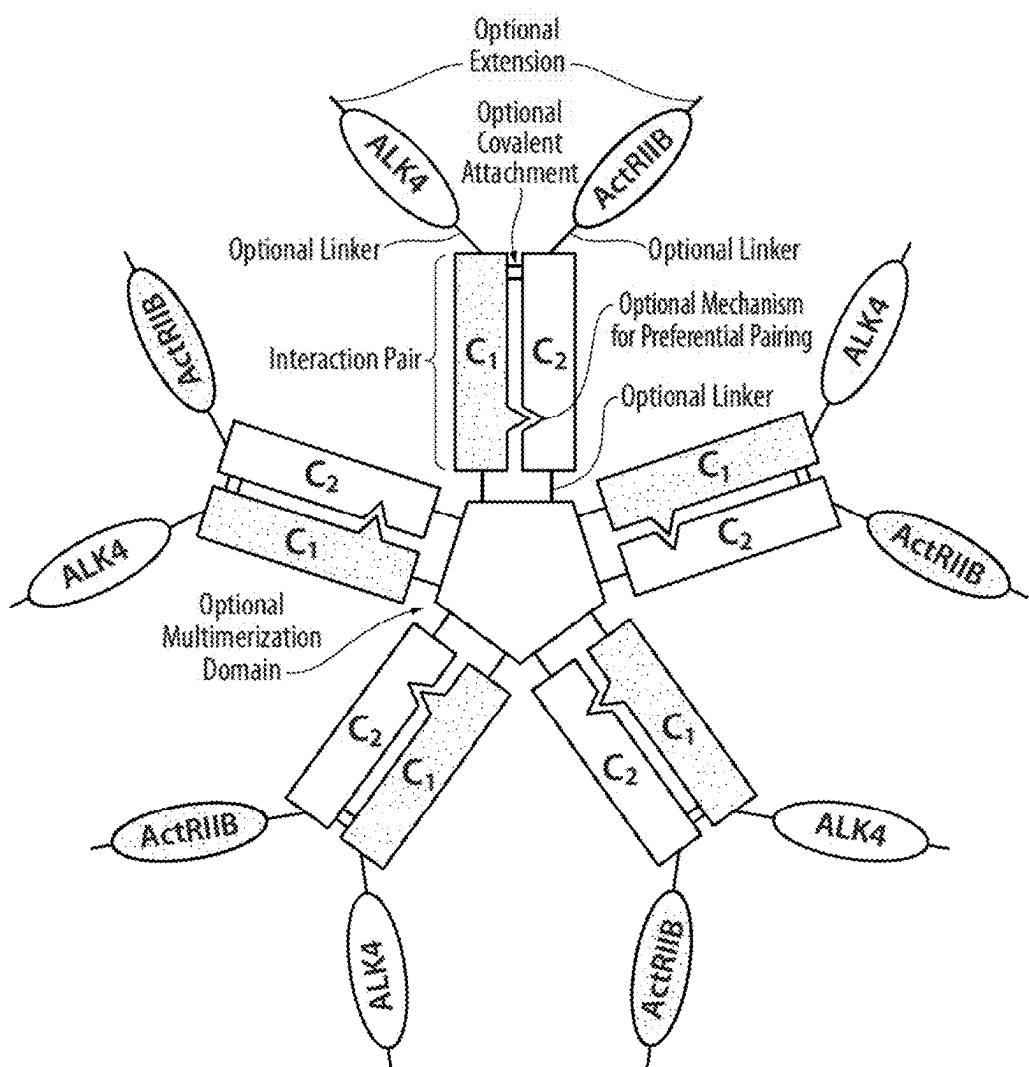


FIGURE 23D

COMPOSITIONS AND METHODS FOR TREATING PULMONARY HYPERTENSION

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation of U.S. application Ser. No. 17/960,911, filed Oct. 6, 2022 (now pending), which is a continuation of U.S. application Ser. No. 17/339,606, filed Jun. 4, 2021 (now U.S. Pat. No. 11,497,794), which is a continuation of U.S. application Ser. No. 17/002,542, filed Aug. 25, 2020 (now U.S. Pat. No. 11,065,303), which is a continuation of U.S. application Ser. No. 16/829,642, filed Mar. 25, 2020 (now U.S. Pat. No. 11,622,992), which is a continuation of U.S. application Ser. No. 15/945,565, filed Apr. 4, 2018 (now U.S. Pat. No. 10,695,405), which is a continuation of U.S. application Ser. No. 15/650,420, filed Jul. 14, 2017 (now U.S. Pat. No. 10,722,558), which claims the benefit of priority to U.S. provisional application Ser. No. 62/362,955, filed on Jul. 15, 2016 (now expired); 62/453,888, filed on Feb. 2, 2017 (now expired); and 62/510,403, filed on May 24, 2017 (now expired). The specifications of each of the foregoing applications are hereby incorporated by reference in their entirety.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted electronically in XML format and is hereby incorporated by reference in its entirety. Said XML copy, created on Mar. 26, 2025, is named 1848179-0002-114-112_SL.XML and is 182,884 bytes in size.

BACKGROUND OF THE INVENTION

[0003] Pulmonary hypertension (PH) is a disease characterized by high blood pressure in lung vasculature, including pulmonary arteries, pulmonary veins, and pulmonary capillaries. In general, PH is defined as a mean pulmonary arterial (PA) pressure ≥ 25 mm Hg at rest or ≥ 30 mm Hg with exercise [Hill et al., *Respiratory Care* 54(7):958-68 (2009)]. The main PH symptom is difficulty in breathing or shortness of breath, and other symptoms include fatigue, dizziness, fainting, peripheral edema (swelling in foot, legs or ankles), bluish lips and skin, chest pain, angina pectoris, light-headedness during exercise, non-productive cough, racing pulse and palpitations. PH can be a severe disease causing heart failure, which is one of the most common causes of death in people who have pulmonary hypertension. Postoperative pulmonary hypertension may complicate many types of surgeries or procedures, and present a challenge associated with a high mortality.

[0004] PH may be grouped based on different manifestations of the disease sharing similarities in pathophysiological mechanisms, clinical presentation, and therapeutic approaches [Simonneau et al., *JACC* 54(1):S44-54 (2009)]. Clinical classification of PH was first proposed in 1973, and a recent updated clinical classification was endorsed by the World Health Organization (WHO) in 2008. According to the updated PH clinical classification, there are five main groups of PH: pulmonary arterial hypertension (PAH), characterized by a PA wedge pressure >15 mm Hg; PH owing to a left heart disease (also known as pulmonary venous hypertension or congestive heart failure), characterized by a PA wedge pressure >15 mm Hg; PH owing to lung diseases and/or hypoxia; chronic thromboemboli PH; and PH with

unclear or multifactorial etiologies [Simonneau et al., *JACC* 54(1):S44-54 (2009); Hill et al., *Respiratory Care* 54(7):958-68 (2009)]. PAH is further classified into idiopathic PAH (IPAH), a sporadic disease in which there is neither a family history of PAH nor an identified risk factor; heritable PAH; PAH induced by drugs and toxins; PAH associated with connective tissue diseases, HIV infection, portal hypertension, congenital heart diseases, schistosomiasis, and chronic hemolytic anemia; and persistent PH of newborns [Simonneau et al., *JACC* 54(1):S44-54 (2009)]. Diagnosis of various types of PH requires a series of tests.

[0005] In general, PH treatment depends on the cause or classification of the PH. Where PH is caused by a known medicine or medical condition, it is known as a secondary PH, and its treatment is usually directed at the underlying disease. Treatment of pulmonary venous hypertension generally involves optimizing left ventricular function by administering diuretics, beta blockers, and ACE inhibitors, or repairing or replacing a mitral valve or aortic valve. PAH therapies include pulmonary vasodilators, digoxin, diuretics, anticoagulants, and oxygen therapy. Pulmonary vasodilators target different pathways, including prostacyclin pathway (e.g., prostacyclins, including intravenous epoprostenol, subcutaneous or intravenous treprostil, and inhaled iloprost), nitric oxide pathway (e.g., phosphodiesterase-5 inhibitors, including sildenafil and tadalafil), and endothelin-1 pathway (e.g., endothelin receptor antagonists, including oral bosentan and oral ambrisentan) [Humbert, M. *Am. J. Respir. Crit. Care Med.* 179:650-6 (2009); Hill et al., *Respiratory Care* 54(7):958-68 (2009)]. However, current therapies provide no cure for PH, and they do not directly treat the underlying vascular remodeling and muscularization of blood vessels observed in many PH patients.

[0006] Thus, there is a high, unmet need for effective therapies for treating pulmonary hypertension. Accordingly, it is an object of the present disclosure to provide methods for treating, preventing, or reducing the progression rate and/or severity of PH, particular treating, preventing or reducing the progression rate and/or severity of one or more PH-associated complications.

SUMMARY OF THE INVENTION

[0007] In part, the data presented herein demonstrates that GDF/BMP antagonists (inhibitors) can be used to treat pulmonary hypertension. For example, it was shown that a soluble ActRIIA polypeptide and an ALK4:ActRIIB heterodimer can be used, individually, to reduce blood pressure, cardiac hypertrophy, and lung weight in a monocrotaline-induced pulmonary arterial hypertension (PAH) model. Similar positive effects were observed for the ActRIIA polypeptide in the Sugen hypoxia PAH model. Histological analysis further revealed that the ActRIIA polypeptide had surprising and significant effects on decreasing vascular remodeling and muscularization of blood vessels in both the monocrotaline-induced and Sugen hypoxia models of PAH. Moreover, both the ActRIIA polypeptide and ALK4:ActRIIB heterodimer surprisingly had a greater effect on ameliorating various complications of PAH compared to sildenafil, which is a drug approved for the treatment of PAH. Thus, the disclosure establishes that antagonists of the ActRII (ActRIIA and ActRIIB) signaling pathways may be used to reduce the severity of pulmonary hypertension. While soluble ActRIIA polypeptides and ALK4:ActRIIB heteromultimers may affect pulmonary hypertension

through a mechanism other than ActRIIA/B ligand antagonists, the disclosure nonetheless demonstrates that desirable therapeutic agents may be selected on the basis of ActRII signaling antagonist activity. Therefore, in some embodiments, the disclosure provides methods for using various ActRII signaling antagonists for treating hypertension, particularly pulmonary hypertension, including, for example, antagonists that inhibit one or more ActRIIA/B ligands [e.g., activin (activin A, activin B, activin AB, activin C, activin AC, activin BC, activin E, activin AE, and/or activin BE), GDF8, GDF11, GDF3, BMP6, BMP15, and BMP10]; antagonists that inhibit of one or more type I and/or type II receptors (e.g., ActRIIA, ActRIIB, ALK4, ALK7, and ALK5); and antagonists that inhibit one or more downstream signaling components (e.g., Smad proteins such as Smads 2 and 3). As used herein, such signaling antagonists are collectively referred to as "GDF/BMP antagonists" or "GDF/BMP inhibitors". Accordingly, the disclosure provides, in part, GDF/BMP antagonist compositions and methods for treating pulmonary hypertension (e.g., PAH), particularly treating one or more complications of pulmonary hypertension (e.g., elevated blood pressure, cardiac hypertrophy, vascular remodeling, and muscularization of vessels). GDF/BMP antagonists to be used in accordance with the methods and uses of the disclosure include, for example, ligand traps (e.g., soluble ActRIIA polypeptides, ActRIIB polypeptides, ALK4:ActRIIB heterodimers, follistatin polypeptides, and FLRG polypeptides), antibody antagonists, small molecule antagonists, and nucleotide antagonists. Optionally, GDF/BMP antagonists may be used in combination with one or more supportive therapies and/or additional active agents for treating pulmonary hypertension.

[0008] In certain aspects, the disclosure relates to methods of treating pulmonary arterial hypertension comprising administering to a patient in need thereof an effective amount of an ActRIIA polypeptide. In some embodiments, the ActRIIA polypeptide comprises an amino acid sequence that is at least 70% (e.g., at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to an amino acid sequence that begins at any one of amino acids 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 of SEQ ID NO: 9 and ends at any one of amino acids 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, or 135 of SEQ ID NO: 9. In some embodiments, the ActRIIA polypeptide comprises an amino acid sequence that is at least 70% (e.g., at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to the amino acid sequence of SEQ ID NO: 10. In some embodiments, the ActRIIA polypeptide comprises an amino acid sequence that is at least 70% (e.g., at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to the amino acid sequence of SEQ ID NO: 11. In some embodiments, the ActRIIA polypeptide is a fusion protein comprising an ActRIIA domain and one or more polypeptide domains heterologous to ActRIIA. In some embodiments, the ActRIIA polypeptide is a fusion protein comprising an Fc domain of an immunoglobulin. In some embodiments, the Fc domain of the immunoglobulin is an Fc domain of an IgG1 immunoglobulin. In some embodiments, the ActRIIA fusion protein further comprises a linker domain positioned between the ActRIIA polypeptide domain and the one or

more heterologous domains (e.g., an Fc immunoglobulin domain). In some embodiments, the linker domain is selected from the group consisting of: TGGG (SEQ ID NO: 23), TGGGG (SEQ ID NO: 21), SGGGG (SEQ ID NO: 22), GGGGS (SEQ ID NO: 25), GGG (SEQ ID NO: 19), GGGG (SEQ ID NO: 20), and SGGG (SEQ ID NO: 24). In some embodiments, the ActRIIA polypeptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 32. In some embodiments, the ActRIIA polypeptide comprises the amino acid sequence of SEQ ID NO: 32. In some embodiments, the ActRIIA polypeptide consists of the amino acid sequence of SEQ ID NO: 32. In some embodiments, the ActRIIA polypeptide is part of a homodimer protein complex. In some embodiments, the ActRIIA polypeptide is glycosylated. In some embodiments, the ActRIIA polypeptide has a glycosylation pattern obtainable by expression in a Chinese hamster ovary cell. In some embodiments, administration of the ActRIIA polypeptide decreases pulmonary arterial pressure in the patient. In some embodiments, administration of the ActRIIA polypeptide decreases pulmonary arterial pressure in the patient by at least 10% (e.g., 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, or at least 80%). In some embodiments, administration of the ActRIIA polypeptide decreases ventricle hypertrophy in the patient. In some embodiments, administration of the ActRIIA polypeptide decreases ventricle hypertrophy in the patient by at least 10% (e.g., 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, or at least 80%). In some embodiments, administration of the ActRIIA polypeptide decreases smooth muscle hypertrophy in the patient. In some embodiments, administration of the ActRIIA polypeptide decreases smooth muscle hypertrophy in the patient by at least 10% (e.g., 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, or at least 80%). In some embodiments, administration of the ActRIIA polypeptide decreases pulmonary arteriole muscularity in the patient. In some embodiments, administration of the ActRIIA polypeptide decreases pulmonary arteriole muscularity in the patient by at least 10% (e.g., 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, or at least 80%). In some embodiments, administration of the ActRIIA polypeptide decreases pulmonary vascular resistance in the patient. In some embodiments, administration of the ActRIIA polypeptide decreases pulmonary vascular resistance in the patient by at least 10% (e.g., 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, or at least 80%). In some embodiments, administration of the ActRIIA polypeptide decreases pulmonary vascular resistance in the patient by at least 25-30%. In some embodiments, the patient has pulmonary arterial hypertension and has Functional Class II or Class III pulmonary hypertension in accordance with the World Health Organization's functional classification system for pulmonary hypertension. In some embodiments, the patient has pulmonary arterial hypertension that is classified as one or more subtypes selected from the group consisting of: idiopathic or heritable pulmonary arterial hypertension, drug- and/or toxin-induced pulmonary hypertension, pulmonary hypertension associated with connective tissue disease, and pulmonary hypertension associated with congenital systemic-to-pulmonary shunts at least 1 year following shunt

repair. In some embodiments, the patient has been treated with one or more vasodilators. In some embodiments, the patient has been treated with one or more agents selected from the group consisting of: phosphodiesterase type 5 inhibitors, soluble guanylate cyclase stimulators, prostacyclin receptor agonist, and endothelin receptor antagonists. In some embodiments, the one or more agents is selected from the group consisting of: bosentan, sildenafil, beraprost, macitentan, selexipag, epoprostenol, treprostинil, iloprost, ambrisentan, and tadalafil. In some embodiments, the method further comprises administration of one or more vasodilators. In some embodiments, the method further comprises administration of one or more agents selected from the group consisting of: phosphodiesterase type 5 inhibitors, soluble guanylate cyclase stimulators, prostacyclin receptor agonist, and endothelin receptor antagonists. In some embodiments, the one or more agents is selected from the group consisting of: bosentan, sildenafil, beraprost, macitentan, selexipag, epoprostenol, treprostинil, iloprost, ambrisentan, and tadalafil. In some embodiments, the patient has a 6-minute walk distance from 150 to 400 meters. In some embodiments, the method increases the patient's 6-minute walk distance by at least 10 meters (e.g., at least 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 125, 150, 175, 200, 250, 300, or more than 400 meters). In some embodiments, the patient has a hemoglobin level from >8 and <15 g/dl. In some embodiments, the method delays clinical worsening of pulmonary arterial hypertension. In some embodiments, the method delays clinical worsening of pulmonary hypertension in accordance with the World Health Organization's functional classification system for pulmonary hypertension. In some embodiments, the method reduces the risk of hospitalization for one or more complications associated with pulmonary arterial hypertension. In some embodiments, the ActRIIA polypeptides binds to one or more ligands selected from the group consisting of: activin A, activin B, GDF11, GDF8, BMP10, and BMP6.

[0009] In some embodiments, the present disclosure relates to methods of treating pulmonary hypertension comprising administering to a patient in need thereof an effective amount of a GDF/BMP antagonist, or combination of GDF/BMP antagonists. In certain aspects, the disclosure relates to methods of preventing pulmonary hypertension comprising administering to a patient in need thereof an effective amount of a GDF/BMP antagonist, or combination of GDF/BMP antagonists. In certain aspects, the disclosure relates to methods of reducing the progression rate of pulmonary hypertension comprising administering to a patient in need thereof an effective amount of a GDF/BMP antagonist, or combination of GDF/BMP antagonists. In some embodiments, the disclosure provides for a method of treating an interstitial lung disease, comprising administering to a patient in need thereof an effective amount of a GDF/BMP antagonist, wherein the GDF/BMP antagonist inhibits one or more of activin, GDF8, GDF11, GDF3, BMP6, BMP15, BMP10, ActRIIA, ActRIIB, ALK4, ALK5, and ALK7. In some embodiments, the disclosure provides for a method of treating, preventing, or reducing the progression rate and/or severity of one or more complications of an interstitial lung disease, comprising administering to a patient in need thereof an effective amount of a GDF/BMP antagonist, wherein the GDF/BMP antagonist inhibits one or more of activin, GDF8, GDF11, GDF3, BMP6, BMP15, BMP10, ActRIIA, ActRIIB, ALK4, ALK5, and ALK7. In some

embodiments, the interstitial lung disease is idiopathic pulmonary fibrosis. In certain aspects, the disclosure relates to methods of reducing the severity of pulmonary hypertension comprising administering to a patient in need thereof an effective amount of a GDF/BMP antagonist, or combination of GDF/BMP antagonists. In certain aspects, the disclosure relates to methods of treating one or more complications (e.g., smooth muscle and/or endothelial cell proliferation in the pulmonary artery, angiogenesis in the pulmonary artery, dyspnea, chest pain, pulmonary vascular remodeling, right ventricular hypertrophy, and pulmonary fibrosis) of pulmonary hypertension comprising administering to a patient in need thereof an effective amount of a GDF/BMP antagonist, or combination of GDF/BMP antagonists. In certain aspects, the disclosure relates to methods of preventing one or more complication of pulmonary hypertension (e.g., smooth muscle and/or endothelial cell proliferation in the pulmonary artery, dyspnea, chest pain, pulmonary vascular remodeling, right ventricular hypertrophy, and pulmonary fibrosis) comprising administering to a patient in need thereof an effective amount a GDF/BMP antagonist, or combination of GDF/BMP antagonists. In certain aspects, the disclosure relates to methods of reducing the progression rate of one or more complication of pulmonary hypertension (e.g., smooth muscle and/or endothelial cell proliferation in the pulmonary artery, angiogenesis in the pulmonary artery, dyspnea, chest pain, pulmonary vascular remodeling, right ventricular hypertrophy, and pulmonary fibrosis) comprising administering to a patient in need thereof an effective amount a GDF/BMP antagonist, or combination of GDF/BMP antagonists. In certain aspects, the disclosure relates to methods of reducing the severity of one or more complication of pulmonary hypertension (e.g., smooth muscle and/or endothelial cell proliferation in the pulmonary artery, angiogenesis in the pulmonary artery, dyspnea, chest pain, pulmonary vascular remodeling, right ventricular hypertrophy, and pulmonary fibrosis) comprising administering to a patient in need thereof an effective amount of a GDF/BMP antagonist, or combination of GDF/BMP antagonists. In certain preferred embodiments, methods described herein relate to a patient having pulmonary arterial hypertension. In some embodiments, methods described herein relate to a patient having a resting pulmonary arterial pressure (PAP) of at least 25 mm Hg (e.g., at least 25, 30, 35, 40, 45, or 50 mm Hg). In some embodiments, the methods described herein reduce PAP in a patient having pulmonary hypertension. For example, the method may reduce PAP by at least 3 mmHg (e.g., at least 3, 5, 7, 10, 12, 15, 20, or 25 mm Hg) in a patient having pulmonary hypertension. In some embodiments, the methods described herein reduce pulmonary vascular resistance in a patient having pulmonary hypertension. In some embodiments, the methods described herein increase pulmonary capillary wedge pressure in a patient having pulmonary hypertension. In some embodiments, the methods described herein increase left ventricular end-diastolic pressure in a patient having pulmonary hypertension. In some embodiments, the methods described herein increase exercise capacity (ability, tolerance) in a patient having pulmonary hypertension. For example, the method may increase 6-minute walk distance in a patient having pulmonary hypertension, optionally increasing 6-minute walk distance by at least 10 meters (e.g., at least 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 or more meters). In addition, the

method may reduce the patient's Borg dyspnea index (BDI), which optionally may be assessed after a 6-minute walk test. In some embodiments, the method reduces the patient's Borg dyspnea index (BDI) by at least 0.5 index points (e.g., at least 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, or 10 index points). In some embodiments, the methods described herein relate to a patient having Class I, Class II, Class III, or Class IV pulmonary hypertension as recognized by the World Health Organization. In some embodiments, the methods described herein relate to delaying clinical progression (worsening) of pulmonary hypertension (e.g., progression as measured by the World Health Organization standard). In some embodiments, the method prevents or delays pulmonary hypertension Class progression (e.g., prevents or delays progression from Class I to Class II, Class II to Class III, or Class III to Class IV pulmonary hypertension as recognized by the World Health Organization). In some embodiments, the method promotes or increases pulmonary hypertension Class regression (e.g., promotes or increases regression from Class IV to Class III, Class III to Class II, or Class II to Class I pulmonary hypertension as recognized by the World Health Organization). In some embodiments, the patient is further administered one or more supportive therapies or active agents for treating pulmonary hypertension in addition to the one or more GDF/BMP antagonist. For example, the patient also may be administered one or more supportive therapies or active agents selected from the group consisting of: prostacyclin and derivatives thereof (e.g., epoprostenol, treprostinal, and iloprost); prostacyclin receptor agonists (e.g., sel-exipag); endothelin receptor antagonists (e.g., thelin, ambrisentan, macitentan, and bosentan); calcium channel blockers (e.g., amlodipine, diltiazem, and nifedipine); anti-coagulants (e.g., warfarin); diuretics; oxygen therapy; atrial septostomy; pulmonary thromboendarterectomy; phosphodiesterase type 5 inhibitors (e.g., sildenafil and tadalafil); activators of soluble guanylate cyclase (e.g., cinaciguat and riociguat); ASK-1 inhibitors (e.g., CIIA; SCH79797; GS-4997; MSC2032964A; 3H-naphtho[1,2,3-de]quinoline-2,7-diones, NQDI-1; 2-thioxo-thiazolidines, 5-bromo-3-(4-oxo-2-thioxo-thiazolidine-5-ylidene)-1,3-dihydro-indol-2-one); NF-κB antagonists (e.g., dh404, CDDO-epoxide; 2,2-difluoropropionamide; C28 imidazole (CDDO-Im); 2-cyano-3,12-dioxoolean-1,9-dien-28-oic acid (CDDO); 3-Acetyloleanolic Acid; 3-Triflouroacetyloleanolic Acid; 28-Methyl-3-acetyloleanane; 28-Methyl-3-trifluoroacetyloleanane; 28-Methoxyoleanolic Acid; SZC014; SCZ015; SZC017; PEGylated derivatives of oleanolic acid; 3-O-(beta-D-glucopyranosyl) oleanolic acid; 3-O-[beta-D-glucopyranosyl-(1→3)-beta-D-glucopyranosyl]oleanolic acid; 3-O-[beta-D-glucopyranosyl-(1→2)-beta-D-glucopyranosyl]oleanolic acid; 3-O-[beta-D-glucopyranosyl-(1→3)-beta-D-glucopyranosyl]oleanolic acid 28-O-beta-D-glucopyranosyl ester; 3-O-[beta-D-glucopyranosyl-(1→2)-beta-D-glucopyranosyl]oleanolic acid 28-O-beta-D-glucopyranosyl ester; 3-O-[α-L-rhamnopyranosyl-(1→3)-beta-D-glucuronopyranosyl]oleanolic acid; 3-O-[alpha-L-rhamnopyranosyl-(1→3)-beta-D-glucuronopyranosyl]oleanolic acid 28-O-beta-D-glucopyranosyl ester; 28-O-β-D-glucopyranosyl-oleanolic acid; 3-O-β-D-glucopyranosyl (1→3)-β-D-glucopyranosiduronic acid (CS1); oleanolic acid 3-O-β-D-glucopyranosyl (1→3)-β-D-glucopyranosiduronic acid (CS2); methyl 3,11-dioxoolean-12-en-28-olate (DIOXOL); ZCVL₄-2; Benzyl 3-dehydr-oxy-1,2,5-oxadi-

azolo[3',4':2,3]oleanolate) lung and/or heart transplantation. In some embodiment, the patient may also be administered a BMP9 polypeptide. In some embodiments the BMP9 polypeptide is a mature BMP9 polypeptide. In some embodiments, the BMP9 polypeptide comprises a BMP9 prodomain polypeptide. In some embodiments, the BMP9 polypeptide is administered in a pharmaceutical preparation, which optionally may comprise a BMP9 prodomain polypeptide. In such BMP9 pharmaceutical preparations comprising a BMP9 prodomain polypeptide, the BMP9 polypeptide may be noncovalently associated with the BMP9 prodomain polypeptide. In some embodiments, BMP9 pharmaceutical preparations are substantially free, or does not comprise, of BMP9 prodomain polypeptide. In some embodiments, the patient may also be administered oleanolic acid or a derivative thereof.

[0010] In certain aspects, a GDF/BMP antagonist, or combination of antagonists, to be used in accordance with methods and uses described herein is an agent that inhibits at least GDF11 (e.g., a GDF11 antagonist). Effects on GDF11 inhibition may be determined, for example, using a cell-based assay including those described herein (e.g., a Smad signaling reporter assay). Therefore, in some embodiments, a GDF/BMP antagonist, or combination of antagonists, of the disclosure may bind to at least GDF11. Ligand binding activity may be determined, for example, using a binding affinity assay including those described herein. In some embodiments, a GDF/BMP antagonist, or combination of antagonists, of the disclosure binds to at least GDF11 with a K_D of at least 1×10^{-7} M (e.g., at least 1×10^{-8} M, at least 1×10^{-9} M, at least 1×10^{-10} M, at least 1×10^{-11} M, or at least 1×10^{-12} M). As described herein, various GDF/BMP antagonists that inhibit GDF11 can be used in accordance with the methods and uses described herein including, for example, ligand traps (e.g., ActRII polypeptides, GDF Traps, follistatin polypeptides, FLRG polypeptides, and ALK4:ActRIIB heteromultimers), antibodies, small molecules, nucleotide sequences, and combinations thereof. In certain embodiments, a GDF/BMP antagonist, or combination of antagonists, that inhibits GDF11 may further inhibit one or more of: activin (e.g., activin A, activin B, activin AB, activin C, activin AC, activin BC, activin E, activin AE, and/or activin BE), GDF8, GDF3, BMP6, BMP15, BMP10, ActRIIA, ActRIIB, ALK4, ALK5, ALK7, and one or more Smads (e.g., Smads 2 and 3).

[0011] In certain aspects, a GDF/BMP antagonist, or combination of antagonists, to be used in accordance with methods and uses described herein is an agent that inhibits at least GDF8 (e.g., a GDF8 antagonist). Effects on GDF8 inhibition may be determined, for example, using a cell-based assay including those described herein (e.g., a Smad signaling reporter assay). Therefore, in some embodiments, a GDF/BMP antagonist, or combination of antagonists, of the disclosure may bind to at least GDF8. Ligand binding activity may be determined, for example, using a binding affinity assay including those described herein. In some embodiments, a GDF/BMP antagonist, or combination of antagonists, of the disclosure binds to at least GDF8 with a K_D of at least 1×10^{-7} M (e.g., at least 1×10^{-8} M, at least 1×10^{-9} M, at least 1×10^{-10} M, at least 1×10^{-11} M, or at least 1×10^{-12} M). As described herein, various GDF/BMP antagonists that inhibit GDF8 can be used in accordance with the methods and uses described herein including, for example, ligand traps (e.g., ActRII polypeptides, GDF Traps, follista-

tin polypeptides, FLRG polypeptides, and ALK4:ActRIIB heteromultimers), antibodies, small molecules, nucleotide sequences, and combinations thereof. In certain embodiments, a GDF/BMP antagonist, or combination of antagonists, that inhibits GDF8 may further inhibit one or more of: activin (e.g., activin A, activin B, activin AB, activin C, activin AC, activin BC, activin E, activin AE, and/or activin BE), GDF11, GDF3, BMP6, BMP15, BMP10, ActRIIA, ActRIIB, ALK4, ALK5, ALK7, and one or more Smads (e.g., Smads 2 and 3).

[0012] In certain aspects, a GDF/BMP antagonist, or combination of antagonists, to be used in accordance with methods and uses described herein is an agent that inhibits at least GDF3 (e.g., a GDF3 antagonist). Effects on GDF3 inhibition may be determined, for example, using a cell-based assay including those described herein (e.g., a Smad signaling reporter assay). Therefore, in some embodiments, a GDF/BMP antagonist, or combination of antagonists, of the disclosure may bind to at least GDF3. Ligand binding activity may be determined, for example, using a binding affinity assay including those described herein. In some embodiments, a GDF/BMP antagonist, or combination of antagonists, of the disclosure binds to at least GDF3 with a K_D of at least 1×10^{-7} M (e.g., at least 1×10^{-8} M, at least 1×10^{-9} M, at least 1×10^{-10} M, at least 1×10^{-11} M, or at least 1×10^{-12} M). As described herein, various GDF/BMP antagonists that inhibit GDF3 can be used in accordance with the methods and uses described herein including, for example, ligand traps (e.g., ActRII polypeptides, GDF Traps, follistatin polypeptides, FLRG polypeptides, and ALK4:ActRIIB heteromultimers), antibodies, small molecules, nucleotide sequences, and combinations thereof. In certain embodiments, a GDF/BMP antagonist, or combination of antagonists, that inhibits GDF3 may further inhibit one or more of: activin (e.g., activin A, activin B, activin AB, activin C, activin AC, activin BC, activin E, activin AE, and/or activin BE), GDF8, GDF11, BMP6, BMP15, BMP10, ActRIIA, ActRIIB, ALK4, ALK5, ALK7, and one or more Smads (e.g., Smads 2 and 3).

[0013] In certain aspects, a GDF/BMP antagonist, or combination of antagonists, to be used in accordance with methods and uses described herein is an agent that inhibits at least BMP6 (e.g., a BMP6 antagonist). Effects on BMP6 inhibition may be determined, for example, using a cell-based assay including those described herein (e.g., a Smad signaling reporter assay). Therefore, in some embodiments, a GDF/BMP antagonist, or combination of antagonists, of the disclosure may bind to at least BMP6. Ligand binding activity may be determined, for example, using a binding affinity assay including those described herein. In some embodiments, a GDF/BMP antagonist, or combination of antagonists, of the disclosure binds to at least BMP6 with a K_D of at least 1×10^{-7} M (e.g., at least 1×10^{-8} M, at least 1×10^{-9} M, at least 1×10^{-10} M, at least 1×10^{-11} M, or at least 1×10^{-12} M). As described herein, various GDF/BMP antagonists that inhibit BMP6 can be used in accordance with the methods and uses described herein including, for example, ligand traps (e.g., ActRII polypeptides, GDF Traps, follistatin polypeptides, FLRG polypeptides, and ALK4:ActRIIB heteromultimers), antibodies, small molecules, nucleotide sequences, and combinations thereof. In certain embodiments, a GDF/BMP antagonist, or combination of antagonists, that inhibits BMP6 may further inhibit one or more of: activin (e.g., activin A, activin B, activin AB, activin C, activin AC, activin BC, activin E, activin AE, and/or activin BE), GDF8, GDF3, GDF11, BMP6, BMP15, ActRIIA, ActRIIB, ALK4, ALK5, ALK7, and one or more Smads (e.g., Smads 2 and 3).

activin AC, activin BC, activin E, activin AE, and/or activin BE), GDF8, GDF3, GDF11, BMP15, BMP10, ActRIIA, ActRIIB, ALK4, ALK5, ALK7, and one or more Smads (e.g., Smads 2 and 3).

[0014] In certain aspects, a GDF/BMP antagonist, or combination of antagonists, to be used in accordance with methods and uses described herein is an agent that inhibits at least BMP15 (e.g., a BMP15 antagonist). Effects on BMP15 inhibition may be determined, for example, using a cell-based assay including those described herein (e.g., a Smad signalling reporter assay). Therefore, in some embodiments, a GDF/BMP antagonist, or combination of antagonists, of the disclosure may bind to at least BMP15. Ligand binding activity may be determined, for example, using a binding affinity assay including those described herein. In some embodiments, a GDF/BMP antagonist, or combination of antagonists, of the disclosure binds to at least BMP15 with a K_D of at least 1×10^{-7} M (e.g., at least 1×10^{-8} M, at least 1×10^{-9} M, at least 1×10^{-10} M, at least 1×10^{-11} M, or at least 1×10^{-12} M). As described herein, various GDF/BMP antagonists that inhibit BMP15 can be used in accordance with the methods and uses described herein including, for example, ligand traps (e.g., ActRII polypeptides, GDF Traps, follistatin polypeptides, FLRG polypeptides, and ALK4:ActRIIB heteromultimers), antibodies, small molecules, nucleotide sequences, and combinations thereof. In certain embodiments, a GDF/BMP antagonist, or combination of antagonists, that inhibits BMP15 may further inhibit one or more of: activin (e.g., activin A, activin B, activin AB, activin C, activin AC, activin BC, activin E, activin AE, and/or activin BE), GDF8, GDF3, GDF11, BMP6, BMP10, ActRIIA, ActRIIB, ALK4, ALK5, ALK7, and one or more Smads (e.g., Smads 2 and 3).

[0015] In certain aspects, a GDF/BMP antagonist, or combination of antagonists, to be used in accordance with methods and uses described herein is an agent that inhibits at least BMP10 (e.g., a BMP10 antagonist). Effects on BMP10 inhibition may be determined, for example, using a cell-based assay including those described herein (e.g., a Smad signalling reporter assay). Therefore, in some embodiments, a GDF/BMP antagonist, or combination of antagonists, of the disclosure may bind to at least BMP10. Ligand binding activity may be determined, for example, using a binding affinity assay including those described herein. In some embodiments, a GDF/BMP antagonist, or combination of antagonists, of the disclosure binds to at least BMP10 with a K_D of at least 1×10^{-7} M (e.g., at least 1×10^{-8} M, at least 1×10^{-9} M, at least 1×10^{-10} M, at least 1×10^{-11} M, or at least 1×10^{-12} M). As described herein, various GDF/BMP antagonists that inhibit BMP10 can be used in accordance with the methods and uses described herein including, for example, ligand traps (e.g., ActRII polypeptides, GDF Traps, follistatin polypeptides, and FLRG polypeptides, and ALK4:ActRIIB heteromultimers FLRG polypeptides), antibodies, small molecules, nucleotide sequences, and combinations thereof. In certain embodiments, a GDF/BMP antagonist, or combination of antagonists, that inhibits BMP10 may further inhibit one or more of: activin (e.g., activin A, activin B, activin AB, activin C, activin AC, activin BC, activin E, activin AE, and/or activin BE), GDF8, GDF3, GDF11, BMP6, BMP15, ActRIIA, ActRIIB, ALK4, ALK5, ALK7, and one or more Smads (e.g., Smads 2 and 3).

[0016] In certain aspects, a GDF/BMP antagonist, or combination of antagonists, to be used in accordance with

methods and uses described herein is an agent that inhibits at least activin (e.g., activin A, activin B, activin AB, activin C, activin AC, activin BC, activin E, activin AE, and/or activin BE) (e.g., an activin antagonist). Effects on activin inhibition may be determined, for example, using a cell-based assay including those described herein (e.g., a Smad signaling reporter assay). Therefore, in some embodiments, a GDF/BMP antagonist, or combination of antagonists, of the disclosure may bind to at least activin. Ligand binding activity may be determined, for example, using a binding affinity assay including those described herein. In some embodiments, a GDF/BMP antagonist, or combination of antagonists, of the disclosure binds to at least activin with a K_D of at least 1×10^{-7} M (e.g., at least 1×10^{-8} M, at least 1×10^{-9} M, at least 1×10^{-10} M, at least 1×10^{-11} M, or at least 1×10^{-12} M). As described herein, various GDF/BMP antagonists that inhibit activin can be used in accordance with the methods and uses described herein including, for example, ligand traps (e.g., ActRII polypeptides, GDF Traps, follistatin polypeptides, FLRG polypeptides, and ALK4:ActRIIB heteromultimers), antibodies, small molecules, nucleotide sequences, and combinations thereof. In certain embodiments, a GDF/BMP antagonist, or combination of antagonists, that inhibits activin may further inhibit one or more of: BMP15, GDF8, GDF3, GDF11, BMP6, BMP10, ActRIIA, ActRIIB, ALK4, ALK5, ALK7, and one or more Smads (e.g., Smads 2 and 3). In certain preferred embodiments, a GDF/BMP antagonist, or combination of antagonists, to be used in accordance with methods and uses described herein is an agent that inhibits at least activin B. In some embodiments, a GDF/BMP antagonist, or combination of antagonists, to be used in accordance with methods and uses described herein does not substantially bind to activin A (e.g., binds to activin A with a K_D higher than 1×10^{-7} M or has relatively modest binding, e.g., about 1×10^{-8} M or about 1×10^{-9} M) and/or inhibit activin A activity. In certain preferred embodiments, a GDF/BMP antagonist, or combination of antagonists, to be used in accordance with methods and uses described herein is an agent that inhibits at least activin B but does not substantially bind to activin A (e.g., binds to activin A with a K_D higher than 1×10^{-7} M or has relatively modest binding, e.g., about 1×10^{-8} M or about 1×10^{-9} M) and/or inhibit activin A activity.

[0017] In certain aspects, a GDF/BMP antagonist, or combination of antagonists, to be used in accordance with methods and uses described herein is an agent that inhibits at least ActRII (e.g., ActRIIA and/or ActRIIB) (e.g., an ActRII antagonist). Effects on ActRII inhibition may be determined, for example, using a cell-based assay including those described herein (e.g., a Smad signaling reporter assay). Therefore, in some embodiments, a GDF/BMP antagonist, or combination of antagonists, of the disclosure may bind to at least ActRII. Ligand binding activity may be determined, for example, using a binding affinity assay including those described herein. In some embodiments, a GDF/BMP antagonist, or combination of antagonists, of the disclosure binds to at least ActRII with a K_D of at least 1×10^{-7} M (e.g., at least 1×10^{-8} M, at least 1×10^{-9} M, at least 1×10^{-10} M, at least 1×10^{-11} M, or at least 1×10^{-12} M). As described herein, various GDF/BMP antagonists that inhibit ActRII can be used in accordance with the methods and uses described herein including, for example, ligand traps (e.g., ActRII polypeptides, GDF Traps, follistatin polypeptides, FLRG polypeptides, and ALK4:ActRIIB heteromultimers), antibodies, small molecules, nucleotide sequences, and combinations thereof. In certain embodiments, a GDF/BMP antagonist, or combination of antagonists, that inhibits ActRII may further inhibit one or more of: activin (e.g., activin A, activin B, activin AB, activin C, activin AC, activin BC, activin E, activin AE, and/or activin BE), GDF8, GDF3, GDF11, BMP6, BMP15, BMP10, ALK4, ALK5, ALK7, and one or more Smads (e.g., Smads 2 and 3).

antibodies, small molecules, nucleotide sequences, and combinations thereof. In certain embodiments, a GDF/BMP antagonist, or combination of antagonists, that inhibits ActRII may further inhibit one or more of: activin (e.g., activin A, activin B, activin AB, activin C, activin AC, activin BC, activin E, activin AE, and/or activin BE), GDF8, GDF3, GDF11, BMP6, BMP15, BMP10, ALK4, ALK5, ALK7, and one or more Smads (e.g., Smads 2 and 3).

[0018] In certain aspects, a GDF/BMP antagonist, or combination of antagonists, to be used in accordance with methods and uses described herein is an agent that inhibits at least ALK4 (e.g., an ALK4 antagonist). Effects on ALK4 inhibition may be determined, for example, using a cell-based assay including those described herein (e.g., a Smad signaling reporter assay). Therefore, in some embodiments, a GDF/BMP antagonist, or combination of antagonists, of the disclosure may bind to at least ALK4. Ligand binding activity may be determined, for example, using a binding affinity assay including those described herein. In some embodiments, a GDF/BMP antagonist, or combination of antagonists, of the disclosure binds to at least ALK4 with a K_D of at least 1×10^{-7} M (e.g., at least 1×10^{-8} M, at least 1×10^{-9} M, at least 1×10^{-10} M, at least 1×10^{-11} M, or at least 1×10^{-12} M). As described herein, various GDF/BMP antagonists that inhibit ALK4 can be used in accordance with the methods and uses described herein including, for example, ligand traps (e.g., ActRII polypeptides, GDF Traps, follistatin polypeptides, FLRG polypeptides, and ALK4:ActRIIB heteromultimers), antibodies, small molecules, nucleotide sequences, and combinations thereof. In certain embodiments, a GDF/BMP antagonist, or combination of antagonists, that inhibits ALK4 may further inhibit one or more of: activin (e.g., activin A, activin B, activin AB, activin C, activin AC, activin BC, activin E, activin AE, and/or activin BE), GDF8, GDF3, GDF11, BMP6, BMP15, BMP10, ALK4, ALK5, ALK7, and one or more Smads (e.g., Smads 2 and 3).

[0019] In certain aspects, a GDF/BMP antagonist, or combination of antagonists, to be used in accordance with methods and uses described herein is an agent that inhibits at least ALK5 (e.g., an ALK5 antagonist). Effects on ALK5 inhibition may be determined, for example, using a cell-based assay including those described herein (e.g., a Smad signaling reporter assay). Therefore, in some embodiments, a GDF/BMP antagonist, or combination of antagonist, of the disclosure may bind to at least ALK5. Ligand binding activity may be determined, for example, using a binding affinity assay including those described herein. In some embodiments, a GDF/BMP antagonist, or combination of antagonists, of the disclosure binds to at least ALK5 with a K_D of at least 1×10^{-7} M (e.g., at least 1×10^{-8} M, at least 1×10^{-9} M, at least 1×10^{-10} M, at least 1×10^{-11} M, or at least 1×10^{-12} M). As described herein, various GDF/BMP antagonists that inhibit ALK5 can be used in accordance with the methods and uses described herein including, for example, ligand traps (e.g., ActRII polypeptides, GDF Traps, follistatin polypeptides, FLRG polypeptides, and ALK4:ActRIIB heteromultimers), antibodies, small molecules, nucleotide sequences, and combinations thereof. In certain embodiments, a GDF/BMP antagonist, or combination of antagonists, that inhibits ALK5 may further inhibit one or more of: activin (e.g., activin A, activin B, activin AB, activin C, activin AC, activin BC, activin E, activin AE, and/or activin BE), GDF8, GDF3, GDF11, BMP6, BMP15, BMP10, ALK4, ALK5, ALK7, and one or more Smads (e.g., Smads 2 and 3).

BE), GDF8, GDF3, GDF11, BMP6, BMP15, BMP10, ActRIIA, ActRIIB, ALK7, ALK4, and one or more Smads (e.g., Smads 2 and 3)

[0020] In certain aspects, a GDF/BMP antagonist, or combination of antagonists, to be used in accordance with methods and uses described herein is an agent that inhibits at least ALK7 (e.g., an ALK7 antagonist). Effects on ALK7 inhibition may be determined, for example, using a cell-based assay including those described herein (e.g., a Smad signaling reporter assay). Therefore, in some embodiments, a GDF/BMP antagonist, or combination of antagonists, of the disclosure may bind to at least ALK7. Ligand binding activity may be determined, for example, using a binding affinity assay including those described herein. In some embodiments, a GDF/BMP antagonist, or combination of antagonists, of the disclosure binds to at least ALK7 with a K_D of at least 1×10^{-7} M (e.g., at least 1×10^{-8} M, at least 1×10^{-9} M, at least 1×10^{-10} M, at least 1×10^{-11} M, or at least 1×10^{-12} M). As described herein, various GDF/BMP antagonists that inhibit ALK7 can be used in accordance with the methods and uses described herein including, for example, ligand traps (e.g., ActRII polypeptides, GDF Traps, follistatin polypeptides, FLRG polypeptides, and ALK4:ActRIIB heteromultimers), antibodies, small molecules, nucleotide sequences, and combinations thereof. In certain embodiments, a GDF/BMP antagonist, or combination of antagonists, that inhibits ALK7 may further inhibit one or more of: activin (e.g., activin A, activin B, activin AB, activin C, activin AC, activin BC, activin E, activin AE, and/or activin BE), GDF8, GDF3, GDF11, BMP6, BMP15, BMP10, ActRIIA, ActRIIB, ALK5, ALK4, and one or more Smads (e.g., Smads 2 and 3).

[0021] In certain aspects, a GDF/BMP antagonist, or combination of antagonists, to be used in accordance with methods and uses described herein is an agent that inhibits at least one or more Smad proteins (e.g., Smads 2 and 3). Effects on Smad inhibition may be determined, for example, using a cell-based assay including those described herein (e.g., a Smad signaling reporter assay). Therefore, in some embodiments, a GDF/BMP antagonist, or combination of antagonists, of the disclosure may bind to at least one or more one or more Smad proteins (e.g., Smads 2 and 3). Ligand binding activity may be determined, for example, using a binding affinity assay including those described herein. In some embodiments, a GDF/BMP antagonist, or combination of antagonists, of the disclosure binds to at least one or more Smad proteins (e.g., Smads 2 and 3) with a K_D of at least 1×10^{-7} M (e.g., at least 1×10^{-8} M, at least 1×10^{-9} M, at least 1×10^{-10} M, at least 1×10^{-11} M, or at least 1×10^{-12} M). As described herein, various GDF/BMP antagonists that inhibit one or more Smad proteins (e.g., Smads 2 and 3) can be used in accordance with the methods and uses described herein including, for example, ligand traps (e.g., ActRII polypeptides, GDF Traps, follistatin polypeptides, FLRG polypeptides, and ALK4:ActRIIB heteromultimers), antibodies, small molecules, nucleotide sequences, and combinations thereof. In certain embodiments, a GDF/BMP antagonist, or combination of antagonists, that inhibits one or more Smad proteins (e.g., Smads 2 and 3) may further inhibit one or more of: activin (e.g., activin A, activin B, activin AB, activin C, activin AC, activin BC, activin E, activin AE, and/or activin BE), GDF8, GDF3, GDF11, BMP6, BMP15, BMP10, ActRIIA, ActRIIB, ALK5, and ALK4.

[0022] In certain aspects, a GDF/BMP antagonist to be used in accordance with methods and uses described herein is an ActRII polypeptide. The term "ActRII polypeptide" collectively refers to naturally occurring ActRIIA and ActRIIB polypeptides as well as truncations and variants thereof such as those described herein (e.g., GDF trap polypeptides). Preferably ActRII polypeptides comprise, consist essentially of, or consist of a ligand-binding domain of an ActRII polypeptide or modified (variant) form thereof. For example, in some embodiments, an ActRIIA polypeptide comprises, consists essentially of, or consists of an ActRIIA ligand-binding domain of an ActRIIA polypeptide, for example, a portion of the ActRIIA extracellular domain. Similarly, an ActRIIB polypeptide may comprise, consist essentially of, or consist of an ActRIIB ligand-binding domain of an ActRIIB polypeptide, for example, a portion of the ActRIIB extracellular domain. Preferably, ActRII polypeptides to be used in accordance with the methods described herein are soluble polypeptides.

[0023] In certain aspects, the disclosure relates compositions comprising an ActRIIA polypeptide and uses thereof. For example, in some embodiments, an ActRIIA polypeptide of the disclosure comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence of amino acids 30-110 of SEQ ID NO: 9. In some embodiments, an ActRIIA polypeptides of the disclosure comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a portion of ActRIIA beginning at a residue corresponding to any one of amino acids 21-30 (e.g., beginning at any one of amino acids 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) of SEQ ID NO: 9 and ending at a position corresponding to any one amino acids 110-135 (e.g., ending at any one of amino acids 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, or 135) of SEQ ID NO: 9. In other embodiments, an ActRIIA polypeptide may comprise an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 9. In other embodiments, an ActRIIA polypeptide may comprise of an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 10. In even other embodiments, an ActRIIA polypeptide may comprise of an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 11. In still other embodiments, an ActRIIA polypeptide may comprise an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 32. In still even other embodiments, an ActRIIA polypeptide may comprise, consist essentially of, or consist of an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 36. In still even other embodiments, an ActRIIA polypeptide may comprise, consist essentially of, or consist of an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 37.

94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 39.

[0024] In other aspects, the disclosure relates compositions comprising an ActRIIB polypeptide and uses thereof. For example, in some embodiments, an ActRIIB polypeptide of the disclosure comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence of amino acids 29-109 of SEQ ID NO: 1. In some embodiments, an ActRIIB polypeptide may comprise an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence of amino acids 29-109 of SEQ ID NO: 1, wherein the ActRIIB polypeptide comprises an acidic amino acid [naturally occurring (E or D) or artificial acidic amino acid] at position 79 with respect to SEQ ID NO: 1. In other embodiments, an ActRIIB polypeptide may comprise an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence of amino acids 25-131 of SEQ ID NO: 1. In some embodiments, an ActRIIB polypeptide may comprise an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence of amino acids 25-131 of SEQ ID NO: 1, wherein the ActRIIB polypeptide comprises an acidic amino acid at position 79 with respect to SEQ ID NO: 1. In some embodiments, an ActRIIB polypeptide may comprise an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a sequence starting at a residue corresponding to any one of amino acids 20, 21, 22, 23, 24, 25, 26, 27, 28, or 29 of SEQ ID NO: 1 and ending at a residue corresponding to any one of amino acids 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, or 134 of SEQ ID NO: 1. In other embodiments, an ActRIIB polypeptide may comprise an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a sequence starting at a residue corresponding to any one of amino acids 20, 21, 22, 23, 24, 25, 26, 27, 28, or 29 of SEQ ID NO: 1 and ending at a residue corresponding to any one of amino acids 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, or 134 of SEQ ID NO: 1, wherein the ActRIIB polypeptide comprises an acidic amino acid at position 79 with respect to SEQ ID NO: 1. In other embodiments, an ActRIIB polypeptide may comprise an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 1. In some embodiments, an ActRIIB polypeptide may comprise an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 1, wherein the ActRIIB polypeptide comprises an acidic amino acid at position 79 with respect to SEQ ID NO: 1. In even other embodiments, an ActRIIB polypeptide may comprise an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 2. In other embodiments, an ActRIIB polypeptide may comprise an amino acid sequence that is at least 70%,

comprise an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 46, wherein the ActRIIB polypeptide comprises an acidic amino acid at position 79 with respect to SEQ ID NO: 1. In still even other embodiments, an ActRIIB polypeptide may comprise an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 47. In some embodiments, an ActRIIB polypeptide may comprise an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 47, wherein the ActRIIB polypeptide comprises an acidic amino acid at position 79 with respect to SEQ ID NO: 1. In still even other embodiments, an ActRIIB polypeptide may comprise an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 48. In some embodiments, an ActRIIB polypeptide may comprise an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 48, wherein the ActRIIB polypeptide comprises an acidic amino acid at position 79 with respect to SEQ ID NO: 1. In still even other embodiments, an ActRIIB polypeptide may comprise an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 69. In still even other embodiments, an ActRIIB polypeptide may comprise an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 74. In some embodiments, an ActRIIB polypeptide may comprise an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 74, wherein the ActRIIB polypeptide comprises an acidic amino acid at position 79 with respect to SEQ ID NO: 1. In still even other embodiments, an ActRIIB polypeptide may comprise an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 77. In some embodiments, an ActRIIB polypeptide may comprise an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 77, wherein the ActRIIB polypeptide comprises an acidic amino acid at position 79 with respect to SEQ ID NO: 1. In still even other embodiments, an ActRIIB polypeptide may comprise an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 78. In some embodiments, an ActRIIB polypeptide may comprise an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 78, wherein the ActRIIB polypeptide comprises an acidic amino acid at position 79 with respect to SEQ ID NO: 1. In still even other embodiments, an ActRIIB polypeptide may comprise an amino acid sequence that is at

least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 79. In some embodiments, an ActRIIB polypeptide may comprise an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 79, wherein the ActRIIB polypeptide comprises an acidic amino acid at position 79 with respect to SEQ ID NO: 1. In certain embodiments, ActRIIB polypeptides to be used in accordance with the methods and uses described herein do not comprise an acidic amino acid at the position corresponding to L79 of SEQ ID NO: 1.

[0025] As described herein, ActRII polypeptides, ALK4 polypeptides and variants thereof (GDF traps) may be homomultimers, for example, homodimer, homotrimers, homotetramers, homopentamers, and higher order homomultimer complexes. In certain preferred embodiments, ActRII polypeptides and variants thereof are homodimers. In certain embodiments, ActRII polypeptide dimers described herein comprise a first ActRII polypeptide covalently, or non-covalently, associated with a second ActRII polypeptide wherein the first polypeptide comprises an ActRII domain and an amino acid sequence of a first member (or second member) of an interaction pair (e.g., a constant domain of an immunoglobulin) and the second polypeptide comprises an ActRII polypeptide and an amino acid sequence of a second member (or first member) of the interaction pair.

[0026] In certain aspects, a GDF/BMP antagonist to be used in accordance with methods and uses described herein is an ALK4:ActRIIB heterotrimer. As described herein, it has been discovered that an ALK4:ActRIIB heterodimer protein complex has a different ligand-binding profile/selectivity compared to corresponding ActRIIB and ALK4 homodimers. In particular, ALK4:ActRIIB heterodimer displays enhanced binding to activin B compared to either homodimer, retains strong binding to activin A, GDF8, and GDF11 as observed with ActRIIB homodimer, and exhibits substantially reduced binding to BMP9, BMP10, and GDF3. In particular, BMP9 displays low to no observable affinity for ALK4:ActRIIB heterodimer, whereas this ligand binds strongly to ActRIIB homodimer. Like ActRIIB homodimer, ALK4:ActRIIB heterodimer retains intermediate-level binding to BMP6. See FIG. 19. These results therefore demonstrate that ALK4:ActRIIB heterodimers are a more selective antagonists (inhibitors) of activin A, activin B, GDF8, and GDF11 compared to ActRIIB homodimers. Accordingly, an ALK4:ActRIIB heterodimer will be more useful than an ActRIIB homodimer in certain applications where such selective antagonism is advantageous. Examples include therapeutic applications where it is desirable to retain antagonism of one or more of activin (e.g., activin A, activin B, activin AB, activin AC), GDF8, and GDF11 but minimize antagonism of one or more of BMP9, BMP10, and GDF3. Moreover, an ALK4:ActRIIB heterodimer has been shown treat PAH in patient. While not wishing to be bound to a particular mechanisms of action, it is expected that ALK4:ActRIIB heteromultimers, as well as variants thereof, that bind to at least one or more of activin (e.g., activin A, activin B, activin AB, and activin AC), GDF8, and/or GDF11 will be useful agents for promoting beneficial effects in PAH patients.

[0027] Therefore, the present disclosure provides heteromultimer complexes (heteromultimers) comprising at least one ALK4 polypeptide and at least one ActRIIB polypeptide (ALK4:ActRIIB heteromultimers) as well as uses thereof. Preferably, ALK4 polypeptides comprise a ligand-binding domain of an ALK4 receptor, for example, a portion of the ALK4 extracellular domain. Similarly, ActRIIB polypeptides generally comprise a ligand-binding domain of an ActRIIB receptor, for example, a portion of the ActRIIB extracellular domain. Preferably, such ALK4 and ActRIIB polypeptides, as well as resultant heteromultimers thereof, are soluble.

[0028] In certain aspects, an ALK4:ActRIIB heteromultimer comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 34-101 of SEQ ID NO: 100. In other embodiments, ALK4:ActRIIB heteromultimers comprises an ALK4 amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 101. In other embodiments, ALK4:ActRIIB heteromultimers comprises an ALK4 amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 105. In other embodiments, ALK4:ActRIIB heteromultimers comprises an ALK4 amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 122. In other embodiments, ALK4:ActRIIB heteromultimers comprise an ALK4 amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 124. In other embodiments, ALK4:ActRIIB heteromultimers comprise an ALK4 amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 116. In still other embodiments, ALK4:ActRIIB heteromultimers comprises an ALK4 amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 117. In other embodiments, ALK4:ActRIIB heteromultimers comprise an ALK4 amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 111. In still other embodiments, ALK4:ActRIIB heteromultimers comprises an ALK4 amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 113.

[0029] In certain aspects, an ALK4:ActRIIB heteromultimer comprises an ActRIIB amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 29-109 of SEQ ID NO: 1. In other embodiments, ALK4:ActRIIB heteromultimers comprises an ActRIIB amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 2. In other embodiments, ALK4:ActRIIB heteromultimers comprise an ActRIIB amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 10.

91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 3. In other embodiments, ALK4:ActRIIB heteromultimers comprise an ActRIIB amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 5. In other embodiments, ALK4:ActRIIB heteromultimers comprises an ActRIIB amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 6. In other embodiments, ALK4:ActRIIB heteromultimers comprise an ActRIIB amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 118. In still even other embodiments, ALK4:ActRIIB heteromultimers comprises an ActRIIB amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 120. In other embodiments, ALK4:ActRIIB heteromultimers comprise an ActRIIB amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 114. In other embodiments, ALK4:ActRIIB heteromultimers may comprise an ActRIIB amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 115. In other embodiments, ALK4:ActRIIB heteromultimers comprise an ActRIIB amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 108. In other embodiments, ALK4:ActRIIB heteromultimers may comprise an ActRIIB amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 110. In certain preferred embodiments, ALK4:ActRIIB heteromultimers do not comprise an ActRIIB polypeptide comprising an acidic amino acid (e.g., an E or D) at the position corresponding to L79 of SEQ ID NO: 1.

[0030] As described herein, ALK4:ActRIIB heteromultimer structures include, for example, heterodimers, heterotrimers, heterotetramers, heteropentamers, and higher order heteromultimer complexes. See, e.g., FIGS. 21-23. In certain preferred embodiments, ALK4:ActRIIB heteromultimers are heterodimers. In certain aspects, ALK4 and/or ActRIIB polypeptides may be fusion proteins.

[0031] In certain aspects, ActRII polypeptides, ALK4 polypeptides, including variants thereof (e.g., GDF traps), may be fusion proteins. For example, in some embodiments, an ActRII (or ALK4) polypeptide may be a fusion protein comprising an ActRII (or ALK4) polypeptide domain and one or more heterologous (non-ActRII) polypeptide domains. In some embodiments, an ActRII (or ALK4) polypeptide may be a fusion protein that has, as one domain, an amino acid sequence derived from an ActRII (or ALK4) polypeptide (e.g., a ligand-binding domain of an ActRII (or ALK4) receptor or a variant thereof) and one or more heterologous domains that provide a desirable property, such as improved pharmacokinetics, easier purification, targeting to particular tissues, etc. For example, a domain of a fusion protein may enhance one or more of in vivo stability, in vivo half-life, uptake/administration, tissue localization or distri-

bution, formation of protein complexes, multimerization of the fusion protein, and/or purification. Optionally, an ActRII (or ALK4) polypeptide domain of a fusion protein is connected directly (fused) to one or more heterologous polypeptide domains or an intervening sequence, such as a linker, may be positioned between the amino acid sequence of the ActRII (or ALK4) polypeptide and the amino acid sequence of the one or more heterologous domains. In certain embodiments, an ActRII (or ALK4) fusion protein comprises a relatively unstructured linker positioned between the heterologous domain and the ActRII (or ALK4) domain. This unstructured linker may correspond to the roughly 15 amino acid unstructured region at the C-terminal end of the extracellular domain of ActRII (or ALK4), or it may be an artificial sequence of between 3 and 15, 20, 30, 50 or more amino acids that are relatively free of secondary structure. A linker may be rich in glycine and/or proline residues and may, for example, contain repeating sequences of threonine-serine and glycines. Examples of linkers include, but are not limited to, the sequences TGGG (SEQ ID NO: 23), SGGG (SEQ ID NO: 24), TGGGG (SEQ ID NO: 21), SGGGG (SEQ ID NO: 22), GGGGS (SEQ ID NO: 25), GGGG (SEQ ID NO: 20), and GGG (SEQ ID NO: 19). In some embodiments, ActRII (or ALK4) fusion proteins may comprise a constant domain of an immunoglobulin, including, for example, the Fc portion of an immunoglobulin. For example, an amino acid sequence that is derived from an Fc domain of an IgG (IgG1, IgG2, IgG3, or IgG4), IgA (IgA1 or IgA2), IgE, or IgM immunoglobulin. For example, an Fc portion of an immunoglobulin domain may comprise, consist essentially of, or consist of an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to any one of SEQ ID NOS: 14-18. Such immunoglobulin domains may comprise one or more amino acid modifications (e.g., deletions, additions, and/or substitutions) that confer an altered Fc activity, e.g., decrease of one or more Fc effector functions. In some embodiment, an ActRII (or ALK4) fusion protein comprises an amino acid sequence as set forth in the formula A-B-C. For example, the B portion is an N- and C-terminally truncated ActRII (or ALK4) polypeptide, e.g., as described herein. The A and C portions may be independently zero, one, or more than one amino acids, and both A and C portions are heterologous to B. The A and/or C portions may be attached to the B portion via a linker sequence. In certain embodiments, an ActRII (or ALK4) fusion protein comprises a leader sequence. The leader sequence may be a native ActRII (or ALK4) leader sequence or a heterologous leader sequence. In certain embodiments, the leader sequence is a tissue plasminogen activator (TPA) leader sequence (e.g., SEQ ID NO: 34).

[0032] An ActRII polypeptide or ALK4 polypeptide, including variants thereof, may comprise a purification subsequence, such as an epitope tag, a FLAG tag, a polyhistidine sequence, and a GST fusion. Optionally, an ActRII polypeptide or ALK4 polypeptide comprises one or more modified amino acid residues selected from: a glycosylated amino acid, a PEGylated amino acid, a farnesylated amino acid, an acetylated amino acid, a biotinylated amino acid, and/or an amino acid conjugated to a lipid moiety. ActRII polypeptides and ALK4 polypeptides may comprise at least one N-linked sugar, and may include two, three or more N-linked sugars. Such polypeptides may also comprise O-linked sugars. In general, it is preferable that ActRII and

ALK4 polypeptides be expressed in a mammalian cell line that mediates suitably natural glycosylation of the polypeptide so as to diminish the likelihood of an unfavorable immune response in a patient. ActRII and ALK4 polypeptides may be produced in a variety of cell lines that glycosylate the protein in a manner that is suitable for patient use, including engineered insect or yeast cells, and mammalian cells such as COS cells, CHO cells, HEK cells and NSO cells. In some embodiments, an ActRII or ALK4 polypeptide is glycosylated and has a glycosylation pattern obtainable from a Chinese hamster ovary cell line. In some embodiments, ActRII or ALK4 polypeptides of the disclosure exhibit a serum half-life of at least 4, 6, 12, 24, 36, 48, or 72 hours in a mammal (e.g., a mouse or a human). Optionally, ActRII or ALK4 polypeptides may exhibit a serum half-life of at least 6, 8, 10, 12, 14, 20, 25, or 30 days in a mammal (e.g., a mouse or a human).

[0033] In certain aspects, the disclosure provides pharmaceutical preparations comprising one or more GDF/BMP antagonists of the present disclosure and a pharmaceutically acceptable carrier. A pharmaceutical preparation may also comprise one or more additional active agents such as a compound that is used to treat pulmonary hypertension, particularly treating or preventing one or more complications of pulmonary hypertension (e.g., smooth muscle and/or endothelial cell proliferation in the pulmonary artery, angiogenesis in the pulmonary artery, dyspnea, chest pain, pulmonary vascular remodeling, right ventricular hypertrophy, and pulmonary fibrosis) including, for example, vasodilators such as prostacyclin, epoprostenol, and sildenafil; endothelin receptor antagonists such as bosentan; calcium channel blockers such as amlodipine, diltiazem, and nifedipine; anticoagulants such as warfarin; diuretics; BMP9 polypeptides; BMP10 polypeptides; bardoxolone methyl; and oleanolic acid. In general pharmaceutical preparation will preferably be pyrogen-free (meaning pyrogen free to the extent required by regulations governing the quality of products for therapeutic use).

[0034] In certain instances, when administering an GDF/BMP antagonist, or combination of antagonists, of the disclosure to disorders or conditions described herein, it may be desirable to monitor the effects on red blood cells during administration of the GDF/BMP antagonist, or to determine or adjust the dosing of the GDF/BMP antagonist, in order to reduce undesired effects on red blood cells. For example, increases in red blood cell levels, hemoglobin levels, or hematocrit levels may cause undesirable increases in blood pressure.

[0035] In certain aspects, a GDF/BMP antagonist to be used in accordance with methods and uses of the disclosure is an antibody, or combination of antibodies. In some embodiments, the antibody binds to at least ActRII (ActRIIA and/or ActRIIB). In certain embodiments, an antibody that binds to ActRII inhibits ActRII signaling, optionally as measured in a cell-based assay such as those described herein. In certain embodiments, an antibody that binds to ActRII inhibits one or more GDF/BMP ligands, type I receptors, or co-receptors from binding to ActRII. In certain embodiments an antibody that binds to ActRII inhibits one or more GDF/BMP ligands from binding to ActRII selected from the group consisting of: activin (e.g., activin A, activin B, activin C, activin AB, activin AC, activin BC, activin E, activin AE, and activin BE), GDF8, GDF11, BMP6, BMP15, BMP10, and GDF3. In some embodiments, the

antibody binds to at least ALK4. In certain embodiments, an antibody that binds to ALK4 inhibits ALK4 signaling, optionally as measured in a cell-based assay such as those described herein. In certain embodiments, an antibody that binds to ALK4 inhibits one or more GDF/BMP ligands, type II receptors, or co-receptors from binding to ALK4. In certain embodiments an antibody that binds to ALK4 inhibits one or more GDF/BMP ligands from binding to ALK4 selected from the group consisting of: activin (e.g., activin A, activin B, activin C, activin AB, activin AC, activin BC, activin E, activin AE, and activin BE), GDF8, GDF11, BMP6, BMP15, BMP10, and GDF3. In some embodiments, the antibody binds to at least ALK5. In certain embodiments, an antibody that binds to ALK5 inhibits ALK5 signaling, optionally as measured in a cell-based assay such as those described herein. In certain embodiments, an antibody that binds to ALK5 inhibits one or more GDF/BMP ligands, type II receptors, or co-receptors from binding to ALK5. In certain embodiments an antibody that binds to ALK5 inhibits one or more GDF/BMP ligands from binding to ALK5 selected from the group consisting of: activin (e.g., activin A, activin B, activin C, activin AB, activin AC, activin BC, activin E, activin AE, and activin BE), GDF8, GDF11, BMP6, BMP15, BMP10, and GDF3. In some embodiments, the antibody binds to at least ALK7. In certain embodiments, an antibody that binds to ALK7 inhibits ALK7 signaling, optionally as measured in a cell-based assay such as those described herein. In certain embodiments, an antibody that binds to ALK7 inhibits one or more GDF/BMP ligands, type II receptors, or co-receptors from binding to ALK7. In certain embodiments an antibody that binds to ALK7 inhibits one or more GDF/BMP ligands from binding to ALK7 selected from the group consisting of: activin (e.g., activin A, activin B, activin C, activin AB, activin AC, activin BC, activin E, activin AE, and activin BE), GDF8, GDF11, BMP6, BMP15, BMP10, and GDF3. In some embodiments, the antibody binds to at least GDF11. In certain embodiments, an antibody that binds to GDF11 inhibits ActRII signaling, optionally as measured in a cell-based assay such as those described herein. In certain embodiments, an antibody that binds to GDF11 inhibits GDF11-ActRII binding and/or GDF11-ALK binding (e.g., GDF11-ALK4, GDF11-ALK5, and/or GDF11-ALK7 binding). In some embodiments, the antibody binds to at least GDF8. In certain embodiments, an antibody that binds to GDF8 inhibits ActRII signaling, optionally as measured in a cell-based assay such as those described herein. In certain embodiments, an antibody that binds to GDF8 inhibits GDF8-ActRII binding and/or GDF8-ALK binding (e.g., GDF8-ALK4, GDF8-ALK5, and/or GDF8-ALK7 binding). In some embodiments, the antibody binds to at least BMP6. In certain embodiments, an antibody that binds to BMP6 inhibits ActRII signaling, optionally as measured in a cell-based assay such as those described herein. In certain embodiments, an antibody that binds to BMP6 inhibits BMP6-ActRII binding and/or BMP6-ALK binding (e.g., BMP6-ALK4, BMP6-ALK5, and/or BMP6-ALK7 binding). In some embodiments, the antibody binds to at least BMP15. In certain embodiments, an antibody that binds to BMP15 inhibits ActRII signaling, optionally as measured in a cell-based assay such as those described herein. In certain embodiments, an antibody that binds to BMP15 inhibits BMP15-ActRII binding and/or BMP15-ALK binding (e.g., BMP15-ALK4, BMP15-ALK5, and/or BMP15-ALK7 bind-

ing). In some embodiments, the antibody binds to at least GDF3. In certain embodiments, an antibody that binds to GDF3 inhibits ActRII signaling, optionally as measured in a cell-based assay such as those described herein. In certain embodiments, an antibody that binds to GDF3 inhibits GDF3-ActRII binding and/or GDF3-ALK binding (e.g., GDF3-ALK4, GDF3-ALK5, and/or GDF3-ALK7 binding). In some embodiments, the antibody binds to at least BMP10. In certain embodiments, an antibody that binds to BMP10 inhibits ActRII signaling, optionally as measured in a cell-based assay such as those described herein. In certain embodiments, an antibody that binds to BMP10 inhibits BMP10-ActRII binding and/or BMP10-ALK binding (e.g., BMP10-ALK4, BMP10-ALK5, and/or BMP10-ALK7 binding). In some embodiments, the antibody binds to activin (e.g. activin A, activin B, activin C, activin AB, activin AC, activin BC, activin E, activin AE, and activin BE). In certain embodiments, an antibody that binds to activin (e.g. activin A, activin B, activin C, activin AB, activin AC, activin BC, activin E, activin AE, and activin BE) inhibits ActRII signaling, optionally as measured in a cell-based assay such as those described herein. In certain embodiments, an antibody that binds to activin (e.g. activin A, activin B, activin C, activin AB, activin AC, activin BC, activin E, activin AE, and activin BE) inhibits activin-ActRII binding and/or activin-ALK binding (e.g., activin-ALK4, activin-ALK5, and/or activin-ALK7 binding). In some embodiments, the antibody binds to activin B. In certain embodiments, an antibody that binds to activin B inhibits ActRII signaling, optionally as measured in a cell-based assay such as those described herein. In certain embodiments, an antibody that binds to activin B inhibits activin B-ActRII binding and/or activin B-ALK binding (e.g., activin B-ALK4, activin B-ALK5, and/or activin B-ALK7 binding). In some embodiments, the antibody is a multispecific antibody, or combination of multispecific antibodies that binds to one or more of ActRIIB, ActRIIA, ALK4, ALK5, ALK7, GDF11, GDF8, activin, BMP6, GDF3, BMP10, and BMP15. In certain aspects the multispecific antibody, or a combination of multispecific antibodies, inhibits signaling in a cell-based assay of one or more of ActRIIB, GDF11, GDF8, activin, BMP6, GDF3, BMP10 and BMP15. In some embodiments, antibody is a chimeric antibody, a humanized antibody, or a human antibody. In some embodiments, the antibody is a single-chain antibody, an F(ab')₂ fragment, a single-chain diabody, a tandem single-chain Fv fragment, a tandem single-chain diabody, a fusion protein comprising a single-chain diabody and at least a portion of an immunoglobulin heavy-chain constant region.

[0036] In certain aspects, the GDF/BMP antagonist is a small molecule inhibitor or combination of small molecule inhibitors. In some embodiments, the small molecule inhibitor is an inhibitor of at least ActRII (e.g., ActRIIA and/or ActRIIB). In some embodiments, the small molecule inhibitor is an inhibitor of at least ALK4. In some embodiments, the small molecule inhibitor is an inhibitor of at least ALK5. In some embodiments, the small molecule inhibitor is an inhibitor of at least ALK7. In some embodiments, the small molecule inhibitor is an inhibitor of at least GDF11. In some embodiments, the small molecule inhibitor is an inhibitor of at least GDF8. In some embodiments, the small molecule inhibitor is an inhibitor of at least BMP6. In some embodiments, the small molecule inhibitor is an inhibitor of at least BMP15. In some embodiments, the small molecule inhibitor

is an inhibitor of at least BMP10. In some embodiments, the small molecule inhibitor is an inhibitor of at least GDF3. In some embodiments, the small molecule inhibitor is an inhibitor of at least activin (e.g. activin A, activin B, activin C, activin AB, activin AC, activin BC, activin E, activin AE, and activin BE). In some embodiments, the small molecule inhibitor is an inhibitor of at least activin B. In some embodiments, the small molecule inhibitor is an inhibitor of at least one or more Smad proteins (e.g., Smads 2 and 3).

[0037] In certain aspects, the GDF/BMP antagonist is a nucleic acid inhibitor or combination of nucleic acid inhibitors. In some embodiments, the nucleic acid inhibitor is an inhibitor of at least ActRII (e.g., ActRIIA and/or ActRIIB). In some embodiments, the nucleic acid inhibitor is an inhibitor of at least ALK4. In some embodiments, the nucleic acid inhibitor is an inhibitor of at least ALK5. In some embodiments, the nucleic acid inhibitor is an inhibitor of at least ALK7. In some embodiments, the nucleic acid inhibitor is an inhibitor of at least GDF11. In some embodiments, the nucleic acid inhibitor is an inhibitor of at least GDF8. In some embodiments, the nucleic acid inhibitor is an inhibitor of at least BMP6. In some embodiments, the nucleic acid inhibitor is an inhibitor of at least BMP15. In some embodiments, the nucleic acid inhibitor is an inhibitor of at least BMP10. In some embodiments, the nucleic acid inhibitor is an inhibitor of at least GDF3. In some embodiments, the nucleic acid inhibitor is an inhibitor of at least activin (e.g. activin A, activin B, activin C, activin AB, activin AC, activin BC, activin E, activin AE, and activin BE). In some embodiments, the nucleic acid inhibitor is an inhibitor of at least activin B. In some embodiments, the nucleic acid inhibitor is an inhibitor of at least one or more Smads (e.g., Smads 2 and 3).

[0038] In certain aspects, the GDF/BMP antagonist is a follistatin polypeptide. In some embodiments, the follistatin polypeptide comprises an amino acid sequence that is at least 70%, 75% 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 26. In some embodiments, the follistatin polypeptide comprises an amino acid sequence that is at least 70%, 75% 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 27. In some embodiments, the follistatin polypeptide comprises an amino acid sequence that is at least 70%, 75% 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 28. In some embodiments, the follistatin polypeptide comprises an amino acid sequence that is at least 70%, 75% 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 29. In some embodiments, the follistatin polypeptide comprises an amino acid sequence that is at least 70%, 75% 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 30.

[0039] In certain aspects, the GDF/BMP antagonist is a FLRG polypeptide. In some embodiments, the FLRG polypeptide comprises an amino acid sequence that is at least 70%, 75% 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 31.

BRIEF DESCRIPTION OF THE DRAWINGS

[0040] FIG. 1 shows an alignment of extracellular domains of human ActRIIB (SEQ ID NO: 2) and human ActRIIA (SEQ ID NO: 10) with the residues that are deduced herein, based on composite analysis of multiple ActRIIB and ActRIIA crystal structures, to directly contact ligand indicated with boxes.

[0041] FIG. 2 shows a multiple sequence alignment of various vertebrate ActRIIB proteins (SEQ ID NOs: 53-58) and human ActRIIA (SEQ ID NO: 59) as well as a consensus ActRII sequence derived from the alignment (SEQ ID NO: 60).

[0042] FIG. 3 shows a multiple sequence alignment of various vertebrate ActRIIA proteins and human ActRIIA (SEQ ID NOs: 61-68).

[0043] FIG. 4 shows multiple sequence alignment of Fc domains from human IgG isotypes using Clustal 2.1. Hinge regions are indicated by dotted underline. Double underline indicates examples of positions engineered in IgG1 (SEQ ID NO: 133) Fc to promote asymmetric chain pairing and the corresponding positions with respect to other isotypes IgG2 (SEQ ID NO: 135), IgG3 (SEQ ID NO: 136) and IgG4 (SEQ ID NO: 134).

[0044] FIG. 5 shows the purification of ActRIIA-hFc expressed in CHO cells. The protein purifies as a single, well-defined peak as visualized by sizing column (top panel) and Coomassie stained SDS-PAGE (bottom panel) (left lane: molecular weight standards; right lane: ActRIIA-hFc).

[0045] FIG. 6 shows the binding of ActRIIA-hFc to activin (top panel) and GDF-11 (bottom panel), as measured by Biacore assay.

[0046] FIG. 7 shows the full, unprocessed amino acid sequence for ActRIIB(25-131)-hFc (SEQ ID NO: 69). The TPA leader (residues 1-22) and double-truncated ActRIIB extracellular domain (residues 24-131, using numbering based on the native sequence in SEQ ID NO: 1) are each underlined. Highlighted is the glutamate revealed by sequencing to be the N-terminal amino acid of the mature fusion protein, which is at position 25 relative to SEQ ID NO: 1.

[0047] FIGS. 8A and 8B show a nucleotide sequence encoding ActRIIB(25-131)-hFc (the coding strand is shown at top, SEQ ID NO: 70, and the complement shown at bottom 3'-5', SEQ ID NO: 71). Sequences encoding the TPA leader (nucleotides 1-66) and ActRIIB extracellular domain (nucleotides 73-396) are underlined. The corresponding amino acid sequence for ActRIIB(25-131) (SEQ ID NO: 138) is also shown.

[0048] FIGS. 9A and 9B show an alternative nucleotide sequence encoding ActRIIB(25-131)-hFc (the coding strand is shown at top, SEQ ID NO: 72, and the complement shown at bottom 3'-5', SEQ ID NO: 73). This sequence confers a greater level of protein expression in initial transformants, making cell line development a more rapid process. Sequences encoding the TPA leader (nucleotides 1-66) and ActRIIB extracellular domain (nucleotides 73-396) are underlined, and substitutions in the wild type nucleotide sequence of the ECD (see FIG. 8) are highlighted. The corresponding amino acid sequence for ActRIIB(25-131) (SEQ ID NO: 138) is also shown.

[0049] FIG. 10 shows the full amino acid sequence for the GDF trap ActRIIB(L79D 20-134)-hFc (SEQ ID NO: 74), including the TPA leader sequence (double underline), ActRIIB extracellular domain (residues 20-134 in SEQ ID

NO: 1; single underline), and hFc domain. The aspartate substituted at position 79 in the native sequence is double underlined and highlighted, as is the glycine revealed by sequencing to be the N-terminal residue in the mature fusion protein.

[0050] FIGS. 11A and 11B shows a nucleotide sequence encoding ActRIIB(L79D 20-134)-hFc. SEQ ID NO: 75 corresponds to the sense strand, and SEQ ID NO: 76 corresponds to the antisense strand. The TPA leader (nucleotides 1-66) is double underlined, and the ActRIIB extracellular domain (nucleotides 76-420) is single underlined.

[0051] FIG. 12 shows the full amino acid sequence for the truncated GDF trap ActRIIB(L79D 25-131)-hFc (SEQ ID NO: 77), including the TPA leader (double underline), truncated ActRIIB extracellular domain (residues 25-131 in SEQ ID NO: 1; single underline), and hFc domain. The aspartate substituted at position 79 in the native sequence is double underlined and highlighted, as is the glutamate revealed by sequencing to be the N-terminal residue in the mature fusion protein.

[0052] FIG. 13 shows the amino acid sequence for the truncated GDF trap ActRIIB(L79D 25-131)-hFc without a leader (SEQ ID NO: 78). The truncated ActRIIB extracellular domain (residues 25-131 in SEQ ID NO: 1) is underlined. The aspartate substituted at position 79 in the native sequence is double underlined and highlighted, as is the glutamate revealed by sequencing to be the N-terminal residue in the mature fusion protein.

[0053] FIG. 14 shows the amino acid sequence for the truncated GDF trap ActRIIB(L79D 25-131) without the leader, hFc domain, and linker (SEQ ID NO: 79). The aspartate substituted at position 79 in the native sequence is underlined and highlighted, as is the glutamate revealed by sequencing to be the N-terminal residue in the mature fusion protein.

[0054] FIGS. 15A and 15B shows a nucleotide sequence encoding ActRIIB(L79D 25-131)-hFc. SEQ ID NO: 80 corresponds to the sense strand, and SEQ ID NO: 81 corresponds to the antisense strand. The TPA leader (nucleotides 1-66) is double underlined, and the truncated ActRIIB extracellular domain (nucleotides 76-396) is single underlined. The amino acid sequence for the ActRIIB extracellular domain (SEQ ID NO: 79) is also shown.

[0055] FIGS. 16A and 16B shows an alternative nucleotide sequence encoding ActRIIB(L79D 25-131)-hFc. SEQ ID NO: 82 corresponds to the sense strand, and SEQ ID NO: 83 corresponds to the antisense strand. The TPA leader (nucleotides 1-66) is double underlined, the truncated ActRIIB extracellular domain (nucleotides 76-396) is underlined, and substitutions in the wild-type nucleotide sequence of the extracellular domain are double underlined and highlighted (compare with SEQ ID NO: 81, FIG. 15). The amino acid sequence for the ActRIIB extracellular domain (SEQ ID NO: 79) is also shown.

[0056] FIG. 17 shows nucleotides 76-396 (SEQ ID NO: 84) of the alternative nucleotide sequence shown in FIG. 16 (SEQ ID NO: 82). The same nucleotide substitutions indicated in FIG. 16 are also underlined and highlighted here. SEQ ID NO: 84 encodes only the truncated ActRIIB extracellular domain (corresponding to residues 25-131 in SEQ ID NO: 1) with a L79D substitution, e.g., ActRIIB(L79D 25-131).

[0057] FIG. 18 shows a multiple sequence alignment of various vertebrate ALK4 proteins and human ALK4 (SEQ ID NOs: 126-132).

[0058] FIG. 19 shows comparative ligand binding data for an ALK4-Fc:ActRIIB-Fc heterodimeric protein complex compared to ActRIIB-Fc homodimer and ALK4-Fc homodimer. For each protein complex, ligands are ranked by k_{off} , a kinetic constant that correlates well with ligand signaling inhibition, and listed in descending order of binding affinity (ligands bound most tightly are listed at the top). At left, yellow, red, green, and blue lines indicate magnitude of the off-rate constant. Solid black lines indicate ligands whose binding to heterodimer is enhanced or unchanged compared with homodimer, whereas dashed red lines indicate substantially reduced binding compared with homodimer. As shown, the ALK4-Fc:ActRIIB-Fc heterodimer displays enhanced binding to activin B compared with either homodimer, retains strong binding to activin A, GDF8, and GDF11 as observed with ActRIIB-Fc homodimer, and exhibits substantially reduced binding to BMP9, BMP10, and GDF3. Like ActRIIB-Fc homodimer, the heterodimer retains intermediate-level binding to BMP6.

[0059] FIG. 20 shows comparative ALK4-Fc:ActRIIB-Fc heterodimer/ActRIIB-Fc:ActRIIB-Fc homodimer IC₅₀ data as determined by an A-204 Reporter Gene Assay as described herein. ALK4-Fc:ActRIIB-Fc heterodimer inhibits activin A, activin B, GDF8, and GDF11 signaling pathways similarly to the ActRIIB-Fc:ActRIIB-Fc homodimer. However, ALK4-Fc:ActRIIB-Fc heterodimer inhibition of BMP9 and BMP10 signaling pathways is significantly reduced compared to the ActRIIB-Fc:ActRIIB-Fc homodimer. These data demonstrate that ALK4:ActRIIB heterodimers are more selective antagonists of activin A, activin B, GDF8, and GDF11 compared to corresponding ActRIIB:ActRIIB homodimers.

[0060] FIGS. 21A and 21B show two schematic examples of heteromeric protein complexes comprising type I receptor and type II receptor polypeptides. FIG. 21A depicts a heterodimeric protein complex comprising one type I receptor fusion polypeptide and one type II receptor fusion polypeptide, which can be assembled covalently or noncovalently via a multimerization domain contained within each polypeptide chain. Two assembled multimerization domains constitute an interaction pair, which can be either guided or unguided. FIG. 21B depicts a heterotetrameric protein complex comprising two heterodimeric complexes as depicted in FIG. 21A. Complexes of higher order can be envisioned.

[0061] FIG. 22 shows a schematic example of a heteromeric protein complex comprising a type I receptor polypeptide (indicated as "I") (e.g. a polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 97%, 98%, 99% or 100% identical to an extracellular domain of an ALK4 protein from humans or other species such as those described herein) and a type II receptor polypeptide (indicated as "II") (e.g. a polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 97%, 98%, 99% or 100% identical to an extracellular domain of an ActRIIB protein from humans or other species as such as those described herein). In the illustrated embodiments, the type I receptor polypeptide is part of a fusion polypeptide that comprises a first member of an interaction pair ("C₁"), and the type II receptor polypeptide is part of a fusion polypeptide that comprises a second member of an interaction pair ("C₂"). In each fusion polypeptide, a linker

may be positioned between the type I or type II receptor polypeptide and the corresponding member of the interaction pair. The first and second members of the interaction pair may be a guided (asymmetric) pair, meaning that the members of the pair associate preferentially with each other rather than self-associate, or the interaction pair may be unguided, meaning that the members of the pair may associate with each other or self-associate without substantial preference and may have the same or different amino acid sequences. Traditional Fc fusion proteins and antibodies are examples of unguided interaction pairs, whereas a variety of engineered Fc domains have been designed as guided (asymmetric) interaction pairs [e.g., Spiess et al (2015) Molecular Immunology 67(2A): 95-106].

[0062] FIGS. 23A-23D show schematic examples of heteromeric protein complexes comprising an ALK4 polypeptide (e.g. a polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 97%, 98%, 99% or 100% identical to an extracellular domain of an ALK4 protein from humans or other species such as those described herein) and an ActRIIB polypeptide (e.g. a polypeptide that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 97%, 98%, 99% or 100% identical to an extracellular domain of an ActRIIB protein from humans or other species such as those described herein). In the illustrated embodiments, the ALK4 polypeptide is part of a fusion polypeptide that comprises a first member of an interaction pair ("C₁"), and the ActRIIB polypeptide is part of a fusion polypeptide that comprises a second member of an interaction pair ("C₂"). Suitable interaction pairs included, for example, heavy chain and/or light chain immunoglobulin interaction pairs, truncations, and variants thereof such as those described herein [e.g., Spiess et al (2015) Molecular Immunology 67(2A): 95-106]. In each fusion polypeptide, a linker may be positioned between the ALK4 or ActRIIB polypeptide and the corresponding member of the interaction pair. The first and second members of the interaction pair may be unguided, meaning that the members of the pair may associate with each other or self-associate without substantial preference, and they may have the same or different amino acid sequences. See FIG. 23A. Alternatively, the interaction pair may be a guided (asymmetric) pair, meaning that the members of the pair associate preferentially with each other rather than self-associate. See FIG. 23B. Complexes of higher order can be envisioned. See FIGS. 23C and 23D.

DETAILED DESCRIPTION OF THE INVENTION

1. Overview

[0063] The TGF- β superfamily is comprised of over 30 secreted factors including TGF-betas, activins, nodals, bone morphogenetic proteins (BMPs), growth and differentiation factors (GDFs), and anti-Mullerian hormone (AMH) [Weiss et al. (2013) Developmental Biology, 2(1): 47-63]. Members of the superfamily, which are found in both vertebrates and invertebrates, are ubiquitously expressed in diverse tissues and function during the earliest stages of development throughout the lifetime of an animal. Indeed, TGF- β superfamily proteins are key mediators of stem cell self-renewal, gastrulation, differentiation, organ morphogenesis, and adult tissue homeostasis. Consistent with this ubiquitous activity, aberrant TGF-beta superfamily signaling is associated with

a wide range of human pathologies including, for example, autoimmune disease, cardiovascular disease, fibrotic disease, and cancer.

[0064] Ligands of the TGF-beta superfamily share the same dimeric structure in which the central 3½ turn helix of one monomer packs against the concave surface formed by the beta-strands of the other monomer. The majority of TGF-beta family members are further stabilized by an intermolecular disulfide bond. This disulfide bonds traverses through a ring formed by two other disulfide bonds generating what has been termed a 'cysteine knot' motif [Lin et al. (2006) Reproduction 132: 179-190; and Hinck et al. (2012) FEBS Letters 586: 1860-1870].

[0065] TGF-beta superfamily signaling is mediated by heteromeric complexes of type I and type II serine/threonine kinase receptors, which phosphorylate and activate downstream SMAD proteins (e.g., SMAD proteins 1, 2, 3, 5, and 8) upon ligand stimulation [Massague (2000) Nat. Rev. Mol. Cell Biol. 1:169-178]. These type I and type II receptors are transmembrane proteins, composed of a ligand-binding extracellular domain with cysteine-rich region, a transmembrane domain, and a cytoplasmic domain with predicted serine/threonine kinase specificity. In general, type I receptors mediate intracellular signaling while the type II receptors are required for binding TGF-beta superfamily ligands. Type I and II receptors form a stable complex after ligand binding, resulting in phosphorylation of type I receptors by type II receptors.

[0066] The TGF-beta family can be divided into two phylogenetic branches based on the type I receptors they bind and the Smad proteins they activate. One is the more recently evolved branch, which includes, e.g., the TGF-betas, activins, GDF8, GDF9, GDF11, BMP3 and nodal, which signal through type I receptors that activate Smads 2 and 3 [Hinck (2012) FEBS Letters 586:1860-1870]. The other branch comprises the more distantly related proteins of the superfamily and includes, e.g., BMP2, BMP4, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF1, GDF5, GDF6, and GDF7, which signal through Smads 1, 5, and 8.

[0067] Activins are members of the TGF-beta superfamily and were initially discovered as regulators of secretion of follicle-stimulating hormone, but subsequently various reproductive and non-reproductive roles have been characterized. There are three principal activin forms (A, B, and AB) that are homo/heterodimers of two closely related β subunits ($\beta_A\beta_A$, $\beta_B\beta_B$, and $\beta_A\beta_B$, respectively). The human genome also encodes an activin C and an activin E, which are primarily expressed in the liver, and heterodimeric forms containing β_C or β_E are also known. In the TGF-beta superfamily, activins are unique and multifunctional factors that can stimulate hormone production in ovarian and placental cells, support neuronal cell survival, influence cell-cycle progress positively or negatively depending on cell type, and induce mesodermal differentiation at least in amphibian embryos [DePaolo et al. (1991) Proc Soc Ep Biol Med. 198:500-512; Dyson et al. (1997) Curr Biol. 7:81-84; and Woodruff (1998) Biochem Pharmacol. 55:953-963]. In several tissues, activin signaling is antagonized by its related heterodimer, inhibin. For example, in the regulation of follicle-stimulating hormone (FSH) secretion from the pituitary, activin promotes FSH synthesis and secretion, while inhibin reduces FSH synthesis and secretion. Other proteins that may regulate activin bioactivity and/or bind to activin

include follistatin (FS), follistatin-related protein (FSRP, also known as FLRG or FSTL3), and α_2 -macroglobulin.

[0068] As described herein, agents that bind to "activin A" are agents that specifically bind to the β_A subunit, whether in the context of an isolated β_A subunit or as a dimeric complex (e.g., a $\beta_A\beta_A$ homodimer or a $\beta_A\beta_B$ heterodimer). In the case of a heterodimer complex (e.g., a $\beta_A\beta_B$ heterodimer), agents that bind to "activin A" are specific for epitopes present within the β_A subunit, but do not bind to epitopes present within the non- β_A subunit of the complex (e.g., the β_B subunit of the complex). Similarly, agents disclosed herein that antagonize (inhibit) "activin A" are agents that inhibit one or more activities as mediated by a β_A subunit, whether in the context of an isolated β_A subunit or as a dimeric complex (e.g., a $\beta_A\beta_A$ homodimer or a $\beta_A\beta_B$ heterodimer). In the case of $\beta_A\beta_B$ heterodimers, agents that inhibit "activin A" are agents that specifically inhibit one or more activities of the β_A subunit, but do not inhibit the activity of the non- β_A subunit of the complex (e.g., the RB subunit of the complex). This principle applies also to agents that bind to and/or inhibit "activin B", "activin C", and "activin E". Agents disclosed herein that antagonize "activin AB" are agents that inhibit one or more activities as mediated by the β_A subunit and one or more activities as mediated by the RB subunit.

[0069] The BMPs and GDFs together form a family of cysteine-knot cytokines sharing the characteristic fold of the TGF-beta superfamily [Rider et al. (2010) Biochem J., 429(1):1-12]. This family includes, for example, BMP2, BMP4, BMP6, BMP7, BMP2a, BMP3, BMP3b (also known as GDF10), BMP4, BMP5, BMP6, BMP7, BMP8, BMP8a, BMP8b, BMP9 (also known as GDF2), BMP10, BMP11 (also known as GDF11), BMP12 (also known as GDF7), BMP13 (also known as GDF6), BMP14 (also known as GDF5), BMP15, GDF1, GDF3 (also known as VGR2), GDF8 (also known as myostatin), GDF9, GDF15, and decapentaplegic. Besides the ability to induce bone formation, which gave the BMPs their name, the BMP/GDFs display morphogenetic activities in the development of a wide range of tissues. BMP/GDF homo- and hetero-dimers interact with combinations of type I and type II receptor dimers to produce multiple possible signaling complexes, leading to the activation of one of two competing sets of SMAD transcription factors. BMP/GDFs have highly specific and localized functions. These are regulated in a number of ways, including the developmental restriction of BMP/GDF expression and through the secretion of several specific BMP antagonist proteins that bind with high affinity to the cytokines. Curiously, a number of these antagonists resemble TGF-beta superfamily ligands.

[0070] Growth and differentiation factor-8 (GDF8) is also known as myostatin. GDF8 is a negative regulator of skeletal muscle mass and is highly expressed in developing and adult skeletal muscle. The GDF8 null mutation in transgenic mice is characterized by a marked hypertrophy and hyperplasia of skeletal muscle [McPherron et al. Nature (1997) 387:83-90]. Similar increases in skeletal muscle mass are evident in naturally occurring mutations of GDF8 in cattle and, strikingly, in humans [Ashmore et al. (1974) Growth, 38:501-507; Swatland and Kieffer, J. Anim. Sci. (1994) 38:752-757; McPherron and Lee, Proc. Natl. Acad. Sci. USA (1997) 94:12457-12461; Kambadur et al. Genome Res. (1997) 7:910-915; and Schuelke et al. (2004) N Engl J Med, 350:2682-8]. Studies have also shown that muscle wasting associated with HIV-infection in humans is accompanied by

increases in GDF8 protein expression [Gonzalez-Cadavid et al., PNAS (1998) 95:14938-43]. In addition, GDF8 can modulate the production of muscle-specific enzymes (e.g., creatine kinase) and modulate myoblast cell proliferation [International Patent Application Publication No. WO 00/43781]. The GDF8 propeptide can noncovalently bind to the mature GDF8 domain dimer, inactivating its biological activity [Miyazono et al. (1988) J. Biol. Chem., 263: 6407-6415; Wakefield et al. (1988) J. Biol. Chem., 263; 7646-7654; and Brown et al. (1990) Growth Factors, 3: 35-43]. Other proteins which bind to GDF8 or structurally related proteins and inhibit their biological activity include follistatin, and potentially, follistatin-related proteins [Gamer et al. (1999) Dev. Biol., 208: 222-232].

[0071] GDF11, also known as BMP11, is a secreted protein that is expressed in the tail bud, limb bud, maxillary and mandibular arches, and dorsal root ganglia during mouse development [McPherron et al. (1999) Nat. Genet., 22: 260-264; and Nakashima et al. (1999) Mech. Dev., 80: 185-189]. GDF11 plays a unique role in patterning both mesodermal and neural tissues [Gamer et al. (1999) Dev Biol., 208:222-32]. GDF11 was shown to be a negative regulator of chondrogenesis and myogenesis in developing chick limb [Gamer et al. (2001) Dev Biol., 229:407-20]. The expression of GDF11 in muscle also suggests its role in regulating muscle growth in a similar way to GDF8. In addition, the expression of GDF11 in brain suggests that GDF11 may also possess activities that relate to the function of the nervous system. Interestingly, GDF11 was found to inhibit neurogenesis in the olfactory epithelium [Wu et al. (2003) Neuron., 37:197-207]. Hence, GDF11 may have in vitro and in vivo applications in the treatment of diseases such as muscle diseases and neurodegenerative diseases (e.g., amyotrophic lateral sclerosis).

[0072] As demonstrated herein, a soluble ActRIIA polypeptide and ALK4:ActRIIB heterodimer, which both bind to various ActRIIA and ActRIIB-interacting ligands, is effective in decreasing blood pressure and cardiac hypertrophy in a PAH model. While not wishing to be bound to any particular mechanism, it is expected that the effects of these agents is caused primarily by an ActRIIA/B signaling antagonist effect. Regardless of the mechanism, it is apparent from the data presented herein that ActRIIA/B signaling antagonists (GDF/BMP antagonists) do decrease blood pressure, decrease cardiac hypertrophy, and have other positivity effects in treating pulmonary hypertension. It should be noted that blood pressure and hypertrophy are dynamic, with changes depending on a balance of factors that increase blood pressure and hypertrophy and factors that decrease blood pressure and hypertrophy. Blood pressure and cardiac hypertrophy can be decreased by increasing factors that reduce blood pressure and cardiac hypertrophy, decreasing factors that promote elevated blood pressure and cardiac hypertrophy, or both. The terms decreasing blood pressure or decreasing cardiac hypertrophy refer to the observable physical changes in blood pressure and cardiac tissue and are intended to be neutral as to the mechanism by which the changes occur.

[0073] The rat models for PAH that were used in the studies described herein are considered to be predicated of efficacy in humans, and therefore, this disclosure provides methods for using ActRIIA polypeptides, ALK4:ActRIIB heteromultimers, and other GDF/BMP antagonists to treat pulmonary hypertension (e.g., PAH), particularly treating,

preventing, or reducing the severity or duration of one or more complications of pulmonary hypertension, in humans. As disclosed herein, the term GDF/BMP antagonists refers a variety of agents that may be used to antagonize ActRIIA/B signaling including, for example, antagonists that inhibit one or more ActRIIA/B ligands [e.g., activin (activin A, activin B, activin AB, activin C, activin AC, activin BC, activin E, activin AE, and/or activin BE), GDF8, GDF11, GDF3, BMP6, BMP15, BMP10]; antagonists that inhibit one or more type I and/or type II receptors (e.g., ActRIIA, ActRIIB, ALK4, ALK7, and ALK5); and antagonists that inhibit one or more downstream signaling components (e.g., Smad proteins such as Smads 2 and 3). GDF/BMP antagonists to be used in accordance with the methods and uses of the disclosure include a variety of forms, for example, ligand traps (e.g., soluble ActRIIA polypeptides, ActRIIB polypeptides, ALK4:ActRIIB heterodimers, follistatin polypeptides, and FLRG polypeptides), antibody antagonists (e.g., antibodies that inhibit one or more of activin, GDF8, GDF11, GDF3, BMP6, BMP15, BMP10, ActRIIA, ActRIIB, ALK4, ALK7, and ALK5), small molecule antagonists [e.g., small molecules that inhibit one or more of activin, GDF8, GDF11, GDF3, BMP6, BMP15, BMP10, ActRIIA, ActRIIB, ALK4, ALK7, ALK5, and one or more Smad proteins (e.g., Smads 2 and 3)], and nucleotide antagonists [e.g., nucleotide sequences that inhibit one or more of activin, GDF8, GDF11, GDF3, BMP6, BMP15, BMP10, ActRIIA, ActRIIB, ALK4, ALK7, ALK5, and one or more Smad proteins (e.g., Smads 2 and 3)].

[0074] The terms used in this specification generally have their ordinary meanings in the art, within the context of this disclosure and in the specific context where each term is used. Certain terms are discussed below or elsewhere in the specification to provide additional guidance to the practitioner in describing the compositions and methods of the disclosure and how to make and use them. The scope or meaning of any use of a term will be apparent from the specific context in which it is used.

[0075] "Homologous," in all its grammatical forms and spelling variations, refers to the relationship between two proteins that possess a "common evolutionary origin," including proteins from superfamilies in the same species of organism, as well as homologous proteins from different species of organism. Such proteins (and their encoding nucleic acids) have sequence homology, as reflected by their sequence similarity, whether in terms of percent identity or by the presence of specific residues or motifs and conserved positions. However, in common usage and in the instant application, the term "homologous," when modified with an adverb such as "highly," may refer to sequence similarity and may or may not relate to a common evolutionary origin.

[0076] The term "sequence similarity," in all its grammatical forms, refers to the degree of identity or correspondence between nucleic acid or amino acid sequences that may or may not share a common evolutionary origin.

[0077] "Percent (%) sequence identity" with respect to a reference polypeptide (or nucleotide) sequence is defined as the percentage of amino acid residues (or nucleic acids) in a candidate sequence that are identical to the amino acid residues (or nucleic acids) in the reference polypeptide (nucleotide) sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum

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

[0078] "Agonize", in all its grammatical forms, refers to the process of activating a protein and/or gene (e.g., by activating or amplifying that protein's gene expression or by inducing an inactive protein to enter an active state) or increasing a protein's and/or gene's activity.

[0079] "Antagonize", in all its grammatical forms, refers to the process of inhibiting a protein and/or gene (e.g., by inhibiting or decreasing that protein's gene expression or by inducing an active protein to enter an inactive state) or decreasing a protein's and/or gene's activity.

[0080] The terms "about" and "approximately" as used in connection with a numerical value throughout the specification and the claims denotes an interval of accuracy, familiar and acceptable to a person skilled in the art. In general, such interval of accuracy is +10%. Alternatively, and particularly in biological systems, the terms "about" and "approximately" may mean values that are within an order of magnitude, preferably <5-fold and more preferably <2-fold of a given value.

[0081] Numeric ranges disclosed herein are inclusive of the numbers defining the ranges.

[0082] The terms "a" and "an" include plural referents unless the context in which the term is used clearly dictates otherwise. The terms "a" (or "an"), as well as the terms "one or more," and "at least one" can be used interchangeably herein. Furthermore, "and/or" where used herein is to be taken as specific disclosure of each of the two or more specified features or components with or without the other. Thus, the term "and/or" as used in a phrase such as "A and/or B" herein is intended to include "A and B," "A or B," "A" (alone), and "B" (alone). Likewise, the term "and/or" as used in a phrase such as "A, B, and/or C" is intended to encompass each of the following aspects: A, B, and C; A, B, or C; A or C; A or B; B or C; A and C; A and B; B and C; A (alone); B (alone); and C (alone).

[0083] Throughout this specification, the word “comprise” or variations such as “comprises” or “comprising” will be understood to imply the inclusion of a stated integer or groups of integers but not the exclusion of any other integer or group of integers.

2. ActRII Polypeptides, ALK4 Polypeptides,
ALK4:ActRIIB Heteromultimers, and Variants
Thereof

[0084] In certain aspects, the disclosure relates ActRII polypeptides and uses thereof (e.g., of treating, preventing, or reducing the progression rate and/or severity of pulmonary hypertension or one or more complications of pulmonary hypertension) and/or an interstitial lung disease (e.g., idiopathic pulmonary fibrosis). As used herein, the term “ActRII” refers to the family of type II activin receptors. This family includes activin receptor type IIA (ActRIIA) and activin receptor type IIB (ActRIIB).

[0085] As used herein, the term “ActRIIB” refers to a family of activin receptor type IIB (ActRIIB) proteins from any species and variants derived from such ActRIIB proteins by mutagenesis or other modification. Reference to ActRIIB herein is understood to be a reference to any one of the currently identified forms. Members of the ActRIIB family are generally transmembrane proteins, composed of a ligand-binding extracellular domain comprising a cysteine-rich region, a transmembrane domain, and a cytoplasmic domain with predicted serine/threonine kinase activity.

[0086] The term “ActRIIB polypeptide” includes polypeptides comprising any naturally occurring polypeptide of an ActRIIB family member as well as any variants thereof (including mutants, fragments, fusions, and peptidomimetic forms) that retain a useful activity. Examples of such variant ActRIIB polypeptides are provided throughout the present disclosure as well as in International Patent Application Publication Nos. WO 2006/012627, WO 2008/097541, WO 2010/151426, and WO 2011/020045, which are incorporated herein by reference in their entirety. Numbering of amino acids for all ActRIIB-related polypeptides described herein is based on the numbering of the human ActRIIB precursor protein sequence provided below (SEQ ID NO: 1), unless specifically designated otherwise.

[0087] The human ActRIIB precursor protein sequence is as follow:

```
(SEQ ID NO: 1)
1 MTAPWVALAL LWGSLCAGSG RGEAETRECI YYNNANWELE TQSGLERCE
51 GEQDKRLHCY ASWRNNSGGT I ELVKKGCWLDF FN CYDRQEC VATEENPQVY
101 FCCCEGNFCN ERFTHLPEAG GPEVTYEPPE TAPTLTVLA YSLLPIGGLS
151 LIVLLAFWMY RHRKPPYGHV DIHEDPGPPP PSLVGLKPL QLLEIKAGR
201 FGCVWKAQLM NDFVAVKIFP LQDKQSQSE REIFSTPGMK HENLLQFIAA
251 EKRGSNLEVE LWLITAFHDK GSLTDYLKGN II TWNELCHV AETMSRGLSY
301 LHEDVPWC RG EGHKPSIAHR DF KSKNVLLK SDLTAVLADF GLAVRFEPKG
351 PPGDTHGQVG TRRYMAPEVL EGAINFQRDA FLRIDMYAMG LVLWELVSR
401 KAADGPVDEY MLPFEEEIGQ HPSLEELQE VVHKKMRPTI KDHWLKH PGL
451 AQLCVTIEEC WDHDAAEARLS AGCVEERVSL IRRSVNGTTS DCLVSLVTSV
501 TNVDLPPKES SI
```

[0088] The signal peptide is indicated with a single underline; the extracellular domain is indicated in bold font; and the potential, endogenous N-linked glycosylation sites are indicated with a double underline.

[0089] The processed (mature) extracellular ActRIIB polypeptide sequence is as follows:

```
(SEQ ID NO: 2)
GRGEAETRECI YYNNANWELE RTNQSGLERCE GEQDKRLHCY ASWRNNSGT
IELVKKGCWLDDFN CYDRQEC VATEENPQVY FCCCEGNFCN ERFTHLPEA
GGPEVTYEPPTAPT.
```

[0090] In some embodiments, the protein may be produced with an “SGR . . .” sequence at the N-terminus. The C-terminal “tail” of the extracellular domain is indicated by a single underline. The sequence with the “tail” deleted (a Δ15 sequence) is as follows:

```
(SEQ ID NO: 3)
GRGEAETRECI YYNNANWELE RTNQSGLERCE GEQDKRLHCY ASWRNNSGT
IELVKKGCWLDDFN CYDRQEC VATEENPQVY FCCCEGNFCN ERFTHLPEA
.
```

[0091] A form of ActRIIB with an alanine at position 64 of SEQ ID NO: 1 (A64) is also reported in the literature. See, e.g., Hilden et al. (1994) Blood, 83(8): 2163-2170. Applicants have ascertained that an ActRIIB-Fc fusion protein comprising an extracellular domain of ActRIIB with the A64 substitution has a relatively low affinity for activin and GDF11. By contrast, the same ActRIIB-Fc fusion protein with an arginine at position 64 (R64) has an affinity for activin and GDF11 in the low nanomolar to high picomolar range. Therefore, sequences with an R64 are used as the “wild-type” reference sequence for human ActRIIB in this disclosure. The form of ActRIIB with an alanine at position 64 is as follows:

(SEQ ID NO: 4)

```

1 MTAPWVALAL LWGSLCAGSG RGEAETRECI YYNANWELER TNQSGLERCE
51 GEQDKRLHCY ASWANSSTI ELVKKGCWLD DFNCYDRQEC VATEENPQVY
101 FCCCEGNFCN ERFTHLPEAG GPEVTVYEPPP TAPTLITVLA YSLLPIGGLS
151 LIVLLAFWMMY RHRKPPYGHV DIHEDPGPPP PSPLVGLKPL QLLEIKARGR
201 FGCVWKQALM NDFVAVKIFP LQDKQSWQSE REIFSTPGMK HENLLQFIAA
251 EKRGSNLEVE LWLITAFHDK GSLTDYLKGN IITWNELCHV AETMSRGLSY
301 LHEDVPWCRCG EGHKPSIAHR DFKSKNVLLK SDLTAVLADF GLAVRPEPGK
351 PPGDTHGQVG TRRYMAPEVL EGAINFQRDA FLRIDMYAMG LVLWELVSR
401 KAADGPVDEY MLPFEEEIGQ HPSLEELQEV VVHKKMRPTI KDHWLKHPGL
451 AQLCVTIEEC WDHDAAEARLS AGCVEERVSL IRRSNGTTT DCLVSLVTSV
501 TNVDLPPKES SI

```

[0092] The signal peptide is indicated by single underline and the extracellular domain is indicated by bold font.

[0093] The processed (mature) extracellular ActRIIB polypeptide sequence of the alternative A64 form is as follows:

(SEQ ID NO: 5)

```

GRGEAETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWANSST
IELVKKGCWLDDFN CYDRQECVATEENPQVYFCCCEGNFCNERFTHLPEA
GGPEVTVYEPPP TAPT

```

[0094] In some embodiments, the protein may be produced with an “SGR . . . ” sequence at the N-terminus. The

C-terminal “tail” of the extracellular domain is indicated by single underline. The sequence with the “tail” deleted (a Δ15 sequence) is as follows:

(SEQ ID NO: 6)

```

GRGEAETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWANSST
IELVKKGCWLDDFN CYDRQECVATEENPQVYFCCCEGNFCNERFTHLPEA

```

[0095] A nucleic acid sequence encoding the human ActRIIB precursor protein is shown below (SEQ ID NO: 7), representing nucleotides 25-1560 of Genbank Reference Sequence NM_001106.3, which encode amino acids 1-513 of the ActRIIB precursor. The sequence as shown provides an arginine at position 64 and may be modified to provide an alanine instead. The signal sequence is underlined.

(SEQ ID NO: 7)

```

1 ATGACGGCGC CCTGGGTGGC CCTCGCCCTC CTCTGGGAT CGCTGTGCGC
51 CGGCTCTGGG CGTGGGGAGG CTGAGACACG GGAGTGCATC TACTACAACG
101 CCAACTGGGA GCTGGAGCGC ACCAACAGA GCGGCCTGGA GCGCTGCGAA
151 GGCAGAGCAGG ACAAGCGGCT GCACTGCTAC GCCTCCTGGC GCAACAGCTC
201 TGGCACCATC GAGCTCGTGA AGAAGGGCTG CTGGCTAGAT GACTTCAACT
251 GCTACGATAG GCAGGAGTGT GTGGCCACTG AGGAGAACCC CCAGGTGTAC
301 TTCTGCTGCT GTGAAGGCAA CTTCTGCAAC GAACGCTTCA CTCATTGCC
351 AGAGGGCTGGG GGCCCGGAAG TCACGTACGA GCCACCCCCG ACAGCCCCCA
401 CCCTGCTCAC GGTGCTGGCC TACTCACTGC TGCCCATCGG GGGCCTTCC
451 CTCATCGTCC TGCTGGCCTT TTGGATGTAC CGGCATCGCA AGCCCCCTA
501 CGGTCACTGTG GACATCCATG AGGACCCTGG GCCTCCACCA CCATCCCCCTC
551 TGGTGGGCCTT GAAGCCACTG CAGCTGCTGG AGATCAAGGC TCGGGGGCGC
601 TTTGGCTGTG TCTGGAAGGC CCAGCTCATG AATGACTTTG TAGCTGTCAA
651 GATCTTCCCA CTCCAGGACA AGCAGTCGTG GCAGAGTGA CGGGAGATCT
701 TCAGCACACC TGGCATGAAG CACGAGAACCC TGCTACAGTT CATTGCTGCC
751 GAGAAGCGAG GCTCCAACCT CGAAGTAGAG CTGTGGCTCA TCACGGCCTT

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-continued

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801 CCATGACAAG GGCTCCCTCA CGGATTACCT CAAGGGGAAC ATCATCACAT
851 GGAACGAACT GTGTCATGTA GCAGAGACGA TGTACGAGG CCTCTCATAC
901 CTGCATGAGG ATGTGCCCTG GTGCCGTGGC GAGGGCCACA AGCCGTCTAT
951 TGCCCACAGG GACTTTAAAA GATAAGAATGT ATTGCTGAAG AGCGACCTCA
1001 CAGCCGTGCT GGCTGACTTT GGCTTGGCTG TTCGATTGAA GCCAGGGAAA
1051 CCTCCAGGGG ACACCCACGG ACAGGTAGGC ACGAGACGGT ACATGGCTCC
1101 TGAGGTGCTC GAGGGAGCCA TCAACTCCA GAGAGATGCC TTCCCTGCGCA
1151 TTGACATGTA TGCCATGGGG TTGGTGCTGT GGGAGCTTGT GTCTCGCTGC
1201 AAGGCTGCAG ACGGACCCGT GGATGAGTAC ATGCTGCCCT TTGAGGAAGA
1251 GATTGCCAG CACCCCTCGT TGGAGGAGCT GCAGGAGGTG GTGGTGACAA
1301 AGAAGATGAG GCCCACCATT AAAGATCACT GGTTGAAACA CCCGGGCCTG
1351 GCCCAGCTTT GTGTGACCAT CGAGGGAGTGC TGGGACCATG ATGCAGAGGC
1401 TCGCTGTCC GCGGGCTGTG TGGAGGAGCG GGTGTCCCTG ATTGGAGGT
1451 CGGTCAACGG CACTACCTCG GACTGTCTCG TTTCCTGGT GACCTCTGTC
1501 ACCAATGTGG ACCTGCCCTC TAAAGAGTCA AGCATC

```

[0096] A nucleic acid sequence encoding processed extracellular human ActRIIB polypeptide is as follows (SEQ ID NO: 8). The sequence as shown provides an arginine at position 64, and may be modified to provide an alanine instead.

that are important for normal ActRIIB-ligand binding activities as well as to predict amino acid positions that are likely to be tolerant to substitution without significantly altering normal ActRIIB-ligand binding activities. Therefore, an active, human ActRIIB variant polypeptide useful in accor-

(SEQ ID NO: 8)

```

1 GGGCGTGGGG AGGCTGAGAC ACGGGAGTGC ATCTACTACA ACGCCAAGT
51 GGAGCTGGAG CGCACCAACC AGAGCGGCCT GGAGCGCTGC GAAGGGCGAGC
101 AGGACAAGCG GCTGCACTGC TACGCCCTCCT GGCACAAAGCT CTCTGGCACC
151 ATCGAGCTCG TGAAGAAGGG CTGCTGGCTA GATGACTTCA ACTGCTACGA
201 TAGGCAGGAG TGTGTGGCCA CTGAGGAGAA CCCCCAGGTG TACTTCTGCT
251 GCTGTGAAGG CAACTTCTGC AACGAACGCT TCACTCATTT GCCAGAGGCT
301 GGGGGCCCGG AAGTCACGTGTA CGAGCCACCC CCGACAGGCC CCACC

```

[0097] An alignment of the amino acid sequences of human ActRIIB extracellular domain and human ActRIIA extracellular domain are illustrated in FIG. 1. This alignment indicates amino acid residues within both receptors that are believed to directly contact ActRII ligands. For example, the composite ActRII structures indicated that the ActRIIB-ligand binding pocket is defined, in part, by residues Y31, N33, N35, L38 through T41, E47, E50, Q53 through K55, L57, H58, Y60, S62, K74, W78 through N83, Y85, R87, A92, and E94 through F101. At these positions, it is expected that conservative mutations will be tolerated.

[0098] In addition, ActRIIB is well-conserved among vertebrates, with large stretches of the extracellular domain completely conserved. For example, FIG. 2 depicts a multi-sequence alignment of a human ActRIIB extracellular domain compared to various ActRIIB orthologs. Many of the ligands that bind to ActRIIB are also highly conserved. Accordingly, from these alignments, it is possible to predict key amino acid positions within the ligand-binding domain

dance with the presently disclosed methods may include one or more amino acids at corresponding positions from the sequence of another vertebrate ActRIIB, or may include a residue that is similar to that in the human or other vertebrate sequences. Without meaning to be limiting, the following examples illustrate this approach to defining an active ActRIIB variant. L46 in the human extracellular domain (SEQ ID NO: 2) is a valine in *Xenopus* ActRIIB (SEQ ID NO: 58), and so this position may be altered, and optionally may be altered to another hydrophobic residue, such as V, I or F, or a non-polar residue such as A. E52 in the human extracellular domain is a K in *Xenopus*, indicating that this site may be tolerant of a wide variety of changes, including polar residues, such as E, D, K, R, H, S, T, P, G, Y and probably A. T93 in the human extracellular domain is a K in *Xenopus*, indicating that a wide structural variation is tolerated at this position, with polar residues favored, such as S, K, R, E, D, H, G, P, G and Y. F108 in the human extracellular domain is a Y in *Xenopus*, and therefore Y or

other hydrophobic group, such as I, V or L should be tolerated. E111 in the human extracellular domain is K in *Xenopus*, indicating that charged residues will be tolerated at this position, including D, R, K and H, as well as Q and N. R112 in the human extracellular domain is K in *Xenopus*, indicating that basic residues are tolerated at this position, including R and H. A at position 119 in the human extracellular domain is relatively poorly conserved, and appears as P in rodents and V in *Xenopus*, thus essentially any amino acid should be tolerated at this position.

[0099] Moreover, ActRII proteins have been characterized in the art in terms of structural and functional characteristics, particularly with respect to ligand binding [Attisano et al. (1992) Cell 68(1):97-108; Greenwald et al. (1999) Nature Structural Biology 6(1): 18-22; Allendorph et al. (2006) PNAS 103(20): 7643-7648; Thompson et al. (2003) The EMBO Journal 22(7): 1555-1566; as well as U.S. Pat. Nos. 7,709,605, 7,612,041, and 7,842,663]. In addition to the teachings herein, these references provide amply guidance for how to generate ActRIIB variants that retain one or more normal activities (e.g., ligand-binding activity).

[0100] For example, a defining structural motif known as a three-finger toxin fold is important for ligand binding by type I and type II receptors and is formed by conserved cysteine residues located at varying positions within the extracellular domain of each monomeric receptor [Greenwald et al. (1999) Nat Struct Biol 6:18-22; and Hinck (2012) FEBS Lett 586:1860-1870]. Accordingly, the core ligand-binding domains of human ActRIIB, as demarcated by the outermost of these conserved cysteines, corresponds to positions 29-109 of SEQ ID NO: 1 (ActRIIB precursor). The structurally less-ordered amino acids flanking these cysteine-demarcated core sequences can be truncated by 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, or 28 residues at the N-terminus and/or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, or 22 residues at the C-terminus without necessarily altering ligand binding. Exemplary ActRIIB extracellular domains for N-terminal and/or C-terminal truncation include SEQ ID NOs: 2, 3, 5, and 6.

[0101] Attisano et al. showed that a deletion of the proline knot at the C-terminus of the extracellular domain of ActRIIB reduced the affinity of the receptor for activin. An ActRIIB-Fc fusion protein containing amino acids 20-119 of present SEQ ID NO: 1, "ActRIIB(20-119)-Fc", has reduced binding to GDF11 and activin relative to an ActRIIB(20-134)-Fc, which includes the proline knot region and the complete juxtamembrane domain (see, e.g., U.S. Pat. No. 7,842,663). However, an ActRIIB(20-129)-Fc protein retains similar, but somewhat reduced activity, relative to the wild-type, even though the proline knot region is disrupted.

[0102] Thus, ActRIIB extracellular domains that stop at amino acid 134, 133, 132, 131, 130 and 129 (with respect to SEQ ID NO: 1) are all expected to be active, but constructs stopping at 134 or 133 may be most active. Similarly, mutations at any of residues 129-134 (with respect to SEQ ID NO: 1) are not expected to alter ligand-binding affinity by large margins. In support of this, it is known in the art that mutations of P129 and P130 (with respect to SEQ ID NO: 1) do not substantially decrease ligand binding. Therefore, an ActRIIB polypeptide of the present disclosure may end as early as amino acid 109 (the final cysteine), however, forms ending at or between 109 and 119 (e.g., 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, or 119) are expected to have

reduced ligand binding. Amino acid 119 (with respect to present SEQ ID NO: 1) is poorly conserved and so is readily altered or truncated. ActRIIB polypeptides ending at 128 (with respect to SEQ ID NO: 1) or later should retain ligand-binding activity. ActRIIB polypeptides ending at or between 119 and 127 (e.g., 119, 120, 121, 122, 123, 124, 125, 126, or 127), with respect to SEQ ID NO: 1, will have an intermediate binding ability. Any of these forms may be desirable to use, depending on the clinical or experimental setting.

[0103] At the N-terminus of ActRIIB, it is expected that a protein beginning at amino acid 29 or before (with respect to SEQ ID NO: 1) will retain ligand-binding activity. Amino acid 29 represents the initial cysteine. An alanine-to-asparagine mutation at position 24 (with respect to SEQ ID NO: 1) introduces an N-linked glycosylation sequence without substantially affecting ligand binding [U.S. Pat. No. 7,842,663]. This confirms that mutations in the region between the signal cleavage peptide and the cysteine cross-linked region, corresponding to amino acids 20-29, are well tolerated. In particular, ActRIIB polypeptides beginning at position 20, 21, 22, 23, and 24 (with respect to SEQ ID NO: 1) should retain general ligand-binding activity, and ActRIIB polypeptides beginning at positions 25, 26, 27, 28, and 29 (with respect to SEQ ID NO: 1) are also expected to retain ligand-binding activity. It has been demonstrated, e.g., U.S. Pat. No. 7,842,663, that, surprisingly, an ActRIIB construct beginning at 22, 23, 24, or 25 will have the most activity.

[0104] Taken together, a general formula for an active portion (e.g., ligand-binding portion) of ActRIIB comprises amino acids 29-109 of SEQ ID NO: 1. Therefore ActRIIB polypeptides may, for example, comprise, consists essentially of, or consists of an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a portion of ActRIIB beginning at a residue corresponding to any one of amino acids 20-29 (e.g., beginning at any one of amino acids 20, 21, 22, 23, 24, 25, 26, 27, 28, or 29) of SEQ ID NO: 1 and ending at a position corresponding to any one amino acids 109-134 (e.g., ending at any one of amino acids 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, or 134) of SEQ ID NO: 1. Other examples include polypeptides that begin at a position from 20-29 (e.g., any one of positions 20, 21, 22, 23, 24, 25, 26, 27, 28, or 29) or 21-29 (e.g., any one of positions 21, 22, 23, 24, 25, 26, 27, 28, or 29) of SEQ ID NO: 1 and end at a position from 119-134 (e.g., any one of positions 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, or 134), 119-133 (e.g., any one of positions 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, or 133), 129-134 (e.g., any one of positions 129, 130, 131, 132, 133, or 134), or 129-133 (e.g., any one of positions 129, 130, 131, 132, or 133) of SEQ ID NO: 1. Other examples include constructs that begin at a position from 20-24 (e.g., any one of positions 20, 21, 22, 23, or 24), 21-24 (e.g., any one of positions 21, 22, 23, or 24), or 22-25 (e.g., any one of positions 22, 23, or 25) of SEQ ID NO: 1 and end at a position from 109-134 (e.g., any one of positions 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, or 134), 119-134 (e.g., any one of positions 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, or 134) or 129-134 (e.g., any one of positions 129, 130, 131, 132, 133, or 134) or 129-133 (e.g., any one of positions 129, 130, 131, 132, or 133) of SEQ ID NO: 1. Other examples include constructs that begin at a position from 20-24 (e.g., any one of positions 20, 21, 22, 23, or 24), 21-24 (e.g., any one of positions 21, 22, 23, or 24), or 22-25 (e.g., any one of positions 22, 23, or 25) of SEQ ID NO: 1 and end at a position from 109-134 (e.g., any one of positions 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, or 134), 119-134 (e.g., any one of positions 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, or 134) or 129-134 (e.g., any one of positions 129, 130, 131, 132, 133, or 134) or 129-133 (e.g., any one of positions 129, 130, 131, 132, or 133) of SEQ ID NO: 1.

132, 133, or 134) of SEQ ID NO: 1. Variants within these ranges are also contemplated, particularly those comprising, consisting essentially of, or consisting of an amino acid sequence that has at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity to the corresponding portion of SEQ ID NO: 1.

[0105] The variations described herein may be combined in various ways. In some embodiments, ActRIIB variants comprise no more than 1, 2, 5, 6, 7, 8, 9, 10 or 15 conservative amino acid changes in the ligand-binding pocket, optionally zero, one or more non-conservative alterations at positions 40, 53, 55, 74, 79 and/or 82 in the ligand-binding pocket. Sites outside the binding pocket, at which variability may be particularly well tolerated, include the amino and carboxy termini of the extracellular domain (as noted above), and positions 42-46 and 65-73 (with respect to SEQ ID NO: 1). An asparagine-to-alanine alteration at position 65 (N65A) does not appear to decrease ligand binding in the R64 background [U.S. Pat. No. 7,842,663]. This change probably eliminates glycosylation at N65 in the A64 background, thus demonstrating that a significant change in this region is likely to be tolerated. While an R64A change is poorly tolerated, R64K is well-tolerated, and thus another basic residue, such as H may be tolerated at position 64 [U.S. Pat. No. 7,842,663]. Additionally, the results of the mutagenesis program described in the art indicate that there are amino acid positions in ActRIIB that are often beneficial to conserve. With respect to SEQ ID NO: 1, these include position 80 (acidic or hydrophobic amino acid), position 78 (hydrophobic, and particularly tryptophan), position 37 (acidic, and particularly aspartic or glutamic acid), position 56 (basic amino acid), position 60 (hydrophobic amino acid, particularly phenylalanine or tyrosine). Thus, the disclosure provides a framework of amino acids that may be conserved in ActRIIB polypeptides. Other positions that may be desirable to conserve are as follows: position 52 (acidic amino acid), position 55 (basic amino acid), position 81 (acidic), 98 (polar or charged, particularly E, D, R or K), all with respect to SEQ ID NO: 1.

[0106] It has been previously demonstrated that the addition of a further N-linked glycosylation site (N-X-S/T) into the ActRIIB extracellular domain is well-tolerated (see, e.g., U.S. Pat. No. 7,842,663). Therefore, N-X-S/T sequences may be generally introduced at positions outside the ligand binding pocket defined in FIG. 1 in ActRIIB polypeptide of the present disclosure. Particularly suitable sites for the introduction of non-endogenous N-X-S/T sequences include amino acids 20-29, 20-24, 22-25, 109-134, 120-134 or 129-134 (with respect to SEQ ID NO: 1). N-X-S/T sequences may also be introduced into the linker between the ActRIIB sequence and an Fc domain or other fusion component as well as optionally into the fusion component itself. Such a site may be introduced with minimal effort by introducing an N in the correct position with respect to a pre-existing S or T, or by introducing an S or T at a position corresponding to a pre-existing N. Thus, desirable altera-

tions that would create an N-linked glycosylation site are: A24N, R64N, S67N (possibly combined with an N65A alteration), E105N, R112N, G120N, E123N, P129N, A132N, R12S and R112T (with respect to SEQ ID NO: 1). Any S that is predicted to be glycosylated may be altered to a T without creating an immunogenic site, because of the protection afforded by the glycosylation. Likewise, any T that is predicted to be glycosylated may be altered to an S. Thus the alterations S67T and S44T (with respect to SEQ ID NO: 1) are contemplated. Likewise, in an A24N variant, an S26T alteration may be used. Accordingly, an ActRIIB polypeptide of the present disclosure may be a variant having one or more additional, non-endogenous N-linked glycosylation consensus sequences as described above.

[0107] In certain embodiments, the disclosure relates to GDF/BMP antagonists (inhibitors) that comprise a ActRIIB polypeptide, which includes fragments, functional variants, and modified forms thereof as well as uses thereof (e.g., treating or preventing PH or one or more PH-associated complication). Preferably, ActRIIB polypeptides are soluble (e.g., comprise an extracellular domain of ActRIIB). In some embodiments, ActRIIB polypeptides antagonize activity (e.g., Smad signaling) of one or more GDF/BMP ligands [e.g., GDF11, GDF8, activin (activin A, activin B, activin AB, activin C, activin E) BMP6, GDF3, BMP15, and BMP10]. Therefore, in some embodiments, ActRIIB polypeptides bind to one or more GDF/BMP ligands [e.g., GDF11, GDF8, activin (activin A, activin B, activin AB, activin C, activin E) BMP6, GDF3, BMP15, and BMP10]. In some embodiments, ActRIIB polypeptides of the disclosure comprise, consist essentially of, or consist of an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a portion of ActRIIB beginning at a residue corresponding to amino acids 20-29 (e.g., beginning at any one of amino acids 20, 21, 22, 23, 24, 25, 26, 27, 28, or 29) of SEQ ID NO: 1 and ending at a position corresponding to amino acids 109-134 (e.g., ending at any one of amino acids 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, or 134) of SEQ ID NO: 1. In some embodiments, ActRIIB polypeptides comprise, consist, or consist essentially of an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical amino acids 29-109 of SEQ ID NO: 1, wherein the position corresponding to L79 of SEQ ID NO: 1 is an acidic amino acid (naturally occurring acidic amino acids D and E or an artificial acidic amino acid). In certain embodiments, ActRIIB polypeptides of the disclosure comprise, consist, or consist essentially of an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical amino acids 25-131 of SEQ ID NO: 1. In certain embodiments, ActRIIB polypeptides of the disclosure comprise, consist, or consist essentially of an amino acid sequence that is at least 70%, 75%,

80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical amino acids 25-131 of SEQ ID NO: 1, wherein the position corresponding to L79 of SEQ ID NO: 1 is an acidic amino acid. In some embodiments, ActRIIB polypeptide of disclosure comprise, consist, or consist essentially of an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 40, 42, 45, 46, 47, 48, 69, 74, 77, 78, 79, 108, 110, 114, 115, 118, and 120. In some embodiments, ActRIIB polypeptide of disclosure comprise, consist, or consist essentially of an amino acid

forms) that retain a useful activity. Examples of such variant ActRIIA polypeptides are provided throughout the present disclosure as well as in International Patent Application Publication Nos. WO 2006/012627 and WO 2007/062188, which are incorporated herein by reference in their entirety. Numbering of amino acids for all ActRIIA-related polypeptides described herein is based on the numbering of the human ActRIIA precursor protein sequence provided below (SEQ ID NO: 9), unless specifically designated otherwise.

[0110] The canonical human ActRIIA precursor protein sequence is as follows:

(SEQ ID NO: 9)

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1 MGAAAKLAFVFLISCSSGA ILGRSETQEC LFFNANWEKD RTNOTGVEPC
51 YGDKDKRRHC FATWKNISGS IEIVKQGCWL DDINCYDRTD CVEKEEDSPEV
101 YFCCCEGNMC NEKFSYFPEM EVTQPTSNPVP TPKPPYYNIL LYSLVPLMLI
151 AGIVICAFWV YRHHKMYAPP VLVPQTQDPGP PPPSPLLGLK PLQLLEVKAR
201 GRFGCVWKAQ LLNEYVAVKI FPIQDKQSWQ NEYEVYSLPG MKHENILQFI
251 GAEKRGTTSVD VDLWLITAFH EKGSLSDFLK ANVVSWNELC HIAETMARGL
301 AYLHEDIPGL KDGHKPAISH RDIKSKNVLL KNNLTACIAD FGLALKFEAG
351 KSAGDTHGQV GTRRYMAPEV LEGAINFQRD AFLRIDMYAM GLVLWELASR
401 CTAADGPVDE YMLPFEEEIG QHPSLEDMQE VVHKKRPV LRDYWQKHAG
451 MAMLCTIEE CWDHDAEARL SAGCVGERIT QMQLTNIIT TEDIVTVVTM
501 VTNVDFPPKE SSL

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sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 40, 42, 45, 46, 47, 48, 69, 74, 77, 78, 79, 108, 110, 114, 115, 118, and 120, wherein the position corresponding to L79 of SEQ ID NO: 1 is an acidic amino acid. In some embodiments, ActRIIB polypeptides of the disclosure comprise, consist, or consist essentially of, at least one ActRIIB polypeptide wherein the position corresponding to L79 of SEQ ID NO: 1 is not an acidic amino acid (i.e., is not naturally occurring acid amino acids D or E or an artificial acidic amino acid residue).

[0108] In certain embodiments, the present disclosure relates to ActRIIA polypeptides. As used herein, the term “ActRIIA” refers to a family of activin receptor type IIA (ActRIIA) proteins from any species and variants derived from such ActRIIA proteins by mutagenesis or other modification. Reference to ActRIIA herein is understood to be a reference to any one of the currently identified forms. Members of the ActRIIA family are generally transmembrane proteins, composed of a ligand-binding extracellular domain comprising a cysteine-rich region, a transmembrane domain, and a cytoplasmic domain with predicted serine/threonine kinase activity.

[0109] The term “ActRIIA polypeptide” includes polypeptides comprising any naturally occurring polypeptide of an ActRIIA family member as well as any variants thereof (including mutants, fragments, fusions, and peptidomimetic

[0111] The signal peptide is indicated by a single underline; the extracellular domain is indicated in bold font; and the potential, endogenous N-linked glycosylation sites are indicated by a double underline.

[0112] The processed (mature) extracellular human ActRIIA polypeptide sequence is as follows:

(SEQ ID NO: 10)

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ILGRSETQECLFFNANWEKDRTNQTGVEPCYGDKDKRRHCFFATWKNISGS
IEIVKQGCWLDDINCYDRTDCVEKKDSPEVYFCCCEGNMCNEKFSYFPEM
EVTQPTSNPVTPKPP

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[0113] The C-terminal “tail” of the extracellular domain is indicated by single underline. The sequence with the “tail” deleted (a Δ15 sequence) is as follows:

(SEQ ID NO: 11)

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ILGRSETQECLFFNANWEKDRTNQTGVEPCYGDKDKRRHCFFATWKNISGS
IEIVKQGCWLDDINCYDRTDCVEKKDSPEVYFCCCEGNMCNEKFSYFPEM

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[0114] The nucleic acid sequence encoding human ActRIIA precursor protein is shown below (SEQ ID NO: 12), as follows nucleotides 159-1700 of Genbank Reference Sequence NM_001616.4. The signal sequence is underlined.

(SEQ ID NO: 12)

1 ATGGGAGCTG CTGCAAAGTT GGCCTTGCC GTCTTCTTA TCTCCTGTC
 51 TTCAGGTGCT ATACTGGTA GATCAGAAC TCAGGAGTGT CTTTCTTTA
 101 ATGCTAATTG GGAAAAAGAC AGAACCAATC AACTGGTGT TGAACCGTGT
 151 TATGGTGACA AAGATAAACG CGGGCATTGT TTTGCTACCT GGAAGAAATAT
 201 TTCTGGTTC ATTGAAATAG TGAAAACAAGG TTGTTGGCTG GATGATATCA
 251 ACTGCTATGA CAGGACTGAT TGTGTAGAAA AAAAGACAG CCCTGAAGTA
 301 TATTTTGTT GCTGTGAGGG CAATATGTGT AATGAAAAGT TTTCTTATT
 351 TCCGGAGATG GAAGTCACAC AGCCCACCTC AAATCCAGTT ACACCTAAC
 401 CACCCATTAA CAACATCTG CTCTATTCCCT TGGTGCCACT TATGTTAATT
 451 GCGGGGATTG TCATTTGTGC ATTGTTGGTG TACAGGCATC ACAAGATGGC
 501 CTACCCTCCT GTACTGTTC CAACTCAAGA CCCAGGACCA CCCCCCACCTT
 551 CTCCATTACT AGGTTTGAAA CCACTGCAGT TATTAGAAGT GAAAGCAAGG
 601 GGAAGATTG GTTGTGTCG GAAAGCCCAG TTGCTTAACG AATATGTGGC
 651 TGTCAAAATA TTCCAATAC AGGACAAACA GTCATGGCAA AATGAATACG
 701 AAGTCTACAG TTGCCTGGA ATGAAGCATG AGAACATATT ACAGTTCAATT
 751 GGTGCAGAAA AACGAGGCAC CAGTGTGAT GTGGATCTTT GGCTGATCAC
 801 AGCATTTCAT GAAAAGGGTT CACTATCAGA CTTTCTTAAG GCTAATGTGG
 851 TCTCTGGAA TGAACGTGT CATATTGCAG AAACCATGGC TAGAGGATTG
 901 GCATATTTAC ATGAGGATAT ACCTGGCTA AAAGATGGCC ACAAACCTGC
 951 CATATCTCAC AGGGACATCA AAAGTAAAAA TGTGCTGTTG AAAAACAAAC
 1001 TGACAGCTTG CATTGCTGAC TTTGGGTTGG CCTTAAATT TGAGGCTGGC
 1051 AAGTCTGCAG GCGATACCCA TGGACAGGTT GGTACCCGGGA GGTACATGGC
 1101 TCCAGAGGTA TTAGAGGGTG CTATAAACTT CCAAAGGGAT GCATTTTGA
 1151 GGATAGATAT GTATGCCATG GGATTAGTCC TATGGGAACG GGCTTCTCGC
 1201 TGTACTGCTG CAGATGGACC TGTAGATGAA TACATGTTGC CATTGAGGA
 1251 GGAAATTGGC CAGCATCCAT CTCTTGAAGA CATGCAGGAA GTTGTGTC
 1301 ATAAAAAAA GAGGCCTGTT TTAAGAGATT ATTGGCAGAA ACATGCTGGA
 1351 ATGGCAATGC TCTGTGAAAC CATTGAAGAA TGTGGGATC ACGACGCAGA
 1401 AGCCAGGTTA TCAGCTGGAT GTGTAGGTGA AAGAATTACC CAGATGCAGA
 1451 GACTAACAAA TATTATTACC ACAGAGGACA TTGTAACAGT GGTACACAATG
 1501 GTGACAAATG TTGACTTTCC TCCCAAAGAA TCTAGTCTA

[0115] The nucleic acid sequence encoding processed soluble (extracellular) human ActRIIA polypeptide is as follows:

(SEQ ID NO: 13)

1 ATACTGGTA GATCAGAAC TCAGGAGTGT CTTTCTTTA ATGCTAATTG
 51 GGAAAAAGAC AGAACCAATC AACTGGTGT TGAACCGTGT TATGGTGACA
 101 AAGATAAACG CGGGCATTGT TTTGCTACCT GGAAGAAATAT TTCTGGTTC
 151 ATTGAAATAG TGAAAACAAGG TTGTTGGCTG GATGATATCA ACTGCTATGA

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201 CAGGACTGAT TGTGTAGAAA AAAAAGACAG CCCTGAAGTA TATTTTTGTT
251 GCTGTGAGGG CAATATGTGT AATGAAAAGT TTCTCTTATT TCCGGAGATG
301 GAAGTCACAC AGCCCACCTTC AAATCCAGTT ACACCTAACGC CACCC

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[0116] ActRIIA is well-conserved among vertebrates, with large stretches of the extracellular domain completely conserved. For example, FIG. 3 depicts a multi-sequence alignment of a human ActRIIA extracellular domain compared to various ActRIIA orthologs. Many of the ligands that bind to ActRIIA are also highly conserved. Accordingly, from these alignments, it is possible to predict key amino acid positions within the ligand-binding domain that are important for normal ActRIIA-ligand binding activities as well as to predict amino acid positions that are likely to be tolerant to substitution without significantly altering normal ActRIIA-ligand binding activities. Therefore, an active, human ActRIIA variant polypeptide useful in accordance with the presently disclosed methods may include one or more amino acids at corresponding positions from the sequence of another vertebrate ActRIIA, or may include a residue that is similar to that in the human or other vertebrate sequences.

[0117] Without meaning to be limiting, the following examples illustrate this approach to defining an active ActRIIA variant. As illustrated in FIG. 3, F13 in the human extracellular domain is Y in *Ovis aries* (SEQ ID NO: 62), *Gallus gallus* (SEQ ID NO: 65), *Bos Taurus* (SEQ ID NO: 66), *Tyto alba* (SEQ ID NO: 67), and *Myotis davidii* (SEQ ID NO: 68) ActRIIA, indicating that aromatic residues are tolerated at this position, including F, W, and Y. Q24 in the human extracellular domain is R in *Bos Taurus* ActRIIA, indicating that charged residues will be tolerated at this position, including D, R, K, H, and E. S95 in the human extracellular domain is F in *Gallus gallus* and *Tyto alba* ActRIIA, indicating that this site may be tolerant of a wide variety of changes, including polar residues, such as E, D, K, R, H, S, T, P, G, Y, and probably hydrophobic residue such as L, I, or F. E52 in the human extracellular domain is D in *Ovis aries* ActRIIA, indicating that acidic residues are tolerated at this position, including D and E. P29 in the human extracellular domain is relatively poorly conserved, appearing as S in *Ovis aries* ActRIIA and L in *Myotis davidii* ActRIIA, thus essentially any amino acid should be tolerated at this position.

[0118] Moreover, as discussed above, ActRII proteins have been characterized in the art in terms of structural/functional characteristics, particularly with respect to ligand binding [Attisano et al. (1992) Cell 68(1):97-108; Greenwald et al. (1999) Nature Structural Biology 6(1): 18-22; Allendorph et al. (2006) PNAS 103(20): 7643-7648; Thompson et al. (2003) The EMBO Journal 22(7): 1555-1566; as well as U.S. Pat. Nos. 7,709,605, 7,612,041, and 7,842,663]. In addition to the teachings herein, these references provide ample guidance for how to generate ActRII variants that retain one or more desired activities (e.g., ligand-binding activity).

[0119] For example, a defining structural motif known as a three-finger toxin fold is important for ligand binding by type I and type II receptors and is formed by conserved cysteine residues located at varying positions within the extracellular domain of each monomeric receptor [Greenwald et al. (1999) Nat Struct Biol 6:18-22; and Hinck (2012)

FEBS Lett 586:1860-1870]. Accordingly, the core ligand-binding domains of human ActRIIA, as demarcated by the outermost of these conserved cysteines, corresponds to positions 30-110 of SEQ ID NO: 9 (ActRIIA precursor). Therefore, the structurally less-ordered amino acids flanking these cysteine-demarcated core sequences can be truncated by about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, or 29 residues at the N-terminus and by about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 residues at the C-terminus without necessarily altering ligand binding. Exemplary ActRIIA extracellular domains truncations include SEQ ID NOs: 10 and 11.

[0120] Accordingly, a general formula for an active portion (e.g., ligand binding) of ActRIIA is a polypeptide that comprises, consists essentially of, or consists of amino acids 30-110 of SEQ ID NO: 9. Therefore ActRIIA polypeptides may, for example, comprise, consists essentially of, or consists of an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a portion of ActRIIA beginning at a residue corresponding to any one of amino acids 21-30 (e.g., beginning at any one of amino acids 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) of SEQ ID NO: 9 and ending at a position corresponding to any one amino acids 110-135 (e.g., ending at any one of amino acids 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, or 135) of SEQ ID NO: 9. Other examples include constructs that begin at a position selected from 21-30 (e.g., beginning at any one of amino acids 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30), 22-30 (e.g., beginning at any one of amino acids 22, 23, 24, 25, 26, 27, 28, 29, or 30), 23-30 (e.g., beginning at any one of amino acids 23, 24, 25, 26, 27, 28, 29, or 30), 24-30 (e.g., beginning at any one of amino acids 24, 25, 26, 27, 28, 29, or 30) of SEQ ID NO: 9, and end at a position selected from 111-135 (e.g., ending at any one of amino acids 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134 or 135), 112-135 (e.g., ending at any one of amino acids 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134 or 135), 113-135 (e.g., ending at any one of amino acids 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134 or 135), 120-135 (e.g., ending at any one of amino acids 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134 or 135), 130-135 (e.g., ending at any one of amino acids 130, 131, 132, 133, 134 or 135), 111-134 (e.g., ending at any one of amino acids 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, or 135), 111-133 (e.g., ending at any one of amino acids 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, or 133), 111-132 (e.g., ending at any one of amino acids 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, or 132),

or 111-131 (e.g., ending at any one of amino acids 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, or 131) of SEQ ID NO: 9. Variants within these ranges are also contemplated, particularly those comprising, consisting essentially of, or consisting of an amino acid sequence that has at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity to the corresponding portion of SEQ ID NO: 9. Thus, in some embodiments, an ActRIIA polypeptide may comprise, consists essentially of, or consist of a polypeptide that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 30-110 of SEQ ID NO: 9. Optionally, ActRIIA polypeptides comprise a polypeptide that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 30-110 of SEQ ID NO: 9, and comprising no more than 1, 2, 5, 10 or 15 conservative amino acid changes in the ligand-binding pocket.

[0121] In certain embodiments, the disclosure relates to GDF/BMP antagonists (inhibitors) that comprise an ActRIIA polypeptide, which includes fragments, functional variants, and modified forms thereof as well as uses thereof (e.g., increasing an immune response in a patient in need thereof and treating cancer). Preferably, ActRIIA polypeptides are soluble (e.g., an extracellular domain of ActRIIA). In some embodiments, ActRIIA polypeptides inhibit (e.g., Smad signaling) of one or more GDF/BMP ligands [e.g., GDF11, GDF8, activin (activin A, activin B, activin AB, activin C, activin E) BMP6, GDF3, BMP15, and/or BMP10]. In some embodiments, ActRIIA polypeptides bind to one or more GDF/BMP ligands [e.g., GDF11, GDF8, activin (activin A, activin B, activin AB, activin C, activin E) BMP6, GDF3, BMP15, and/or BMP10]. In some embodiments, ActRIIA polypeptide of the disclosure comprise, consist essentially of, or consist of an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a portion of ActRIIA beginning at a residue corresponding to amino acids 21-30 (e.g., beginning at any one of amino acids 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) of SEQ ID NO: 9 and ending at a position corresponding to any one amino acids 110-135 (e.g., ending at any one of amino acids 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134 or 135) of SEQ ID NO: 9. In some embodiments, ActRIIA polypeptides comprise, consist, or consist essentially of an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical amino acids 30-110 of SEQ ID NO: 9. In certain embodiments, ActRIIA polypeptides comprise, consist, or consist essentially of an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical amino acids 21-135 of SEQ ID NO: 9. In some embodiments, ActRIIA polypeptides comprise, consist, or consist essentially of an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NOS: 9, 10, 11, 32, 36, and 39.

[0122] In certain aspects, the present disclosure relates to GDF trap polypeptides (also referred to as "GDF traps"). In some embodiments, GDF traps of the present disclosure are variant ActRII polypeptides (e.g., ActRIIA and ActRIIB polypeptides) that comprise one or more mutations (e.g., amino acid additions, deletions, substitutions, and combinations thereof) in the extracellular domain (also referred to as the ligand-binding domain) of an ActRII polypeptide (e.g., a "wild-type" or unmodified ActRII polypeptide) such that the variant ActRII polypeptide has one or more altered ligand-binding activities than the corresponding wild-type ActRII polypeptide. In preferred embodiments, GDF trap polypeptides of the present disclosure retain at least one similar activity as a corresponding wild-type ActRII polypeptide. For example, preferable GDF traps bind to and inhibit (e.g. antagonize) the function of GDF11 and/or GDF8. In some embodiments, GDF traps of the present disclosure further bind to and inhibit one or more of ligand of the GDF/BMP. Accordingly, the present disclosure provides GDF trap polypeptides that have an altered binding specificity for one or more ActRII ligands.

[0123] To illustrate, one or more mutations may be selected that increase the selectivity of the altered ligand-binding domain for GDF11 and/or GDF8 over one or more ActRII-binding ligands such as activins (activin A, activin B, activin AB, activin C, and/or activin E), particularly activin A. Optionally, the altered ligand-binding domain has a ratio of K_d for activin binding to K_d for GDF11 and/or GDF8 binding that is at least 2-, 5-, 10-, 20-, 50-, 100- or even 1000-fold greater relative to the ratio for the wild-type ligand-binding domain. Optionally, the altered ligand-binding domain has a ratio of IC_{50} for inhibiting activin to IC_{50} for inhibiting GDF11 and/or GDF8 that is at least 2-, 5-, 10-, 20-, 50-, 100- or even 1000-fold greater relative to the wild-type ligand-binding domain. Optionally, the altered ligand-binding domain inhibits GDF11 and/or GDF8 with an IC_{50} at least 2-, 5-, 10-, 20-, 50-, 100- or even 1000-times less than the IC_{50} for inhibiting activin.

[0124] Amino acid residues of the ActRIIB proteins (e.g., E39, K55, Y60, K74, W78, L79, D80, and F101 with respect to SEQ ID NO: 1) are in the ActRIIB ligand-binding pocket and help mediated binding to its ligands including, for example, activin A, GDF11, and GDF8. Thus the present disclosure provides GDF trap polypeptides comprising an altered-ligand binding domain (e.g., a GDF8/GDF11-binding domain) of an ActRIIB receptor which comprises one or more mutations at those amino acid residues.

[0125] As a specific example, the positively-charged amino acid residue Asp (D80) of the ligand-binding domain of ActRIIB can be mutated to a different amino acid residue to produce a GDF trap polypeptide that preferentially binds to GDF8, but not activin. Preferably, the D80 residue with respect to SEQ ID NO: 1 is changed to an amino acid residue selected from the group consisting of: an uncharged amino acid residue, a negative amino acid residue, and a hydrophobic amino acid residue. As a further specific example, the hydrophobic residue L79 of SEQ ID NO: 1 can be altered to confer altered activin-GDF11/GDF8 binding properties. For example, an L79P substitution reduces GDF11 binding to a greater extent than activin binding. In contrast, replacement of L79 with an acidic amino acid [an aspartic acid or glutamic acid; an L79D or an L79E substitution] greatly reduces activin A binding affinity while retaining GDF11 binding affinity. In exemplary embodiments, the methods

described herein utilize a GDF trap polypeptide which is a variant ActRIIB polypeptide comprising an acidic amino acid (e.g., D or E) at the position corresponding to position 79 of SEQ ID NO: 1, optionally in combination with one or more additional amino acid substitutions, additions, or deletions.

[0126] In certain aspects, the disclosure relates ALK4 polypeptides and uses thereof. As used herein, the term "ALK4" refers to a family of activin receptor-like kinase-4 proteins from any species and variants derived from such ALK4 proteins by mutagenesis or other modification. Reference to ALK4 herein is understood to be a reference to any one of the currently identified forms. Members of the ALK4 family are generally transmembrane proteins, composed of a ligand-binding extracellular domain with a cysteine-rich region, a transmembrane domain, and a cytoplasmic domain with predicted serine/threonine kinase activity.

[0127] The term "ALK4 polypeptide" includes polypeptides comprising any naturally occurring polypeptide of an ALK4 family member as well as any variants thereof (including mutants, fragments, fusions, and peptidomimetic forms) that retain a useful activity. Numbering of amino acids for all ALK4-related polypeptides described herein is based on the numbering of the human ALK4 precursor protein sequence below (SEQ ID NO: 100), unless specifically designated otherwise.

[0128] A human ALK4 precursor protein sequence (NCBI Ref Seq NP_004293) is as follows:

```
(SEQ ID NO: 100)
1 MAESAGASSF FPLVVLLLAG SGGSGPRGVQ ALLCACTSCL QANYTCETDG ACMVSIFNLD
61 GMEHHVRCI PKVELVPAGK PFYCLSSEDL RNTHCCYTDY CNRIDLRVPS GHKEPEHPS
121 MWGPVELVGI IAGPVFLFL IIIIVFLVIN YHQRVYHNRO RLDMEDPSCE MCLSKDKTLQ
181 DLVYDLSTSG SGSGPLFLVQ RTVARTIVLQ EIIGKGFRGE VWRGRWRGGD VAVKIFSSRE
241 ERSWFREAEI YQTVMRLHEN ILGFIAADNK DNGTWTQLWL VSDYHEHGSL FDYLNRYTVT
301 IEGMIKLALS AASGLAHLHM EIVGTQGKPG IAHRDLKSKN ILVKKNGMCA IADLGLAVRH
361 DAVTDIDIA PNQRVGTKRY MAPEVLDADI NMKHFDSEFKC ADIYALGLVY WEIARRCNSG
421 GVHEEYQLPY YDLVPSDPSI EEMRKVVCDQ KLRPNIPNWQ QSYEARVMG KMMRECWYAN
481 GAARLTALRI KKTLSQLSVQ EDVVI
```

[0129] The signal peptide is indicated by a single underline and the extracellular domain is indicated in bold font.

[0130] A processed extracellular human ALK4 polypeptide sequence is as follows:

```
(SEQ ID NO: 101)
SGPRGVQALLCACTSCLQANYTCETDGACMVSIFNLDMEEHHVRCI PKV
ELVPAKPFYCLSSEDLRNTHCCYTDYCNRIDLRVPSGHKEPEHPSMWG
PVE
```

[0131] A nucleic acid sequence encoding the ALK4 precursor protein is shown below (SEQ ID NO: 102), corre-

sponding to nucleotides 78-1592 of Genbank Reference Sequence NM_004302.4. The signal sequence is underlined and the extracellular domain is indicated in bold font.

```
(SEQ ID NO: 102)
ATGGCGGAGTCGGCCGGAGCCTCCTCCTTCCCCCTTGTGCTCC
TGCTCGCCGGCAGCGCGGGTCCGGGCCCCGGGGTCCAGGCTCTGCT
GTGTGCGTGCACCAGCTGCCCTCAGGCCAACTACACGTGTGAGACAGAT
GGGGCCTGCATGGTTCCATTTCATCTGGATGGAGTGGACACCATG
TGCGCACCTGCATCCCCAAAGTGGAGCTGGTCCCTGCCGGGAAGCCCTT
CTACTGCCCTGAGCTCGGAGGACCTGCCAACCCACTGCTGCTACACT
GACTACTGCAACAGGATCGACTTGAGGGTGCCAGTGGTCACCTCAAGG
AGCCTGAGCACCCGTCATGTGGGGCCGGTGGAGCTGGTAGGCATCAT
CGCCGGCCGGTGTCTCCTGTTCTCATCATCATCATTGTTTCTT
GTCATTAACATCATCAGCGTGTCTATCACAAACGCCAGAGACTGGACA
TGGAAAGATCCCTCATGTGAGATGTGTCTCTCAAAGACAAGACGCTCCA
GGATCTGTCTACGATCTCCACCTCAGGGTCTGGCTCAGGGTTACCC
CTCTTGTCAGCGCACAGTGGCCGAACCATGTTTACAAGAGATTA
TTGGCAAGGTCGGTTGGGAAGTATGGCGGGCCGCTGGAGGGTGG
TGATGTGGCTGTGAAAATATTCTCTCGTGAAGAACGGCTTGGTTC
AGGAAAGCAGAGATATACCAGACGGTATGCTGCGCCATGAAAACATCC
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TTGGATTATTGCTGCTGACAATAAGATAATGGCACCTGGACACAGCT
GTGGCTTGTCTGACTATCATGAGCACGGTCCCTGTTGATTATCTG
AACCGGTACACAGTGACAATTGAGGGATGATTAAGCTGGCTTGTCTG
CTGCTAGTGGCTGGCACACCTGCACATGGAGATCGTGGCACCCAGG
GAAGCCTGGAATTGCTATCGAGACTAAAGTCAAAGAACATTCTGGTG
AAGAAAATGGCATGTGTGCCATAGCAGACCTGGCTGGCTGTCCGTC
ATGATGCAGTCAGTGCACACCATTGACATTGCCCGAATCAGAGGGTGGG
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-continued

```
GACCAAACGATACTGGCCCTGAAGTACTTGATGAAACCATAATATG  
AAACACTTTGACTCCTTAAATGTGCTGATATTGCCCCCTGGGCTTG  
TATATTGGGAGATTGCTCGAAGATGCAATTCTGGAGGAGTCCATGAAGA  
ATATCAGCTGCCATATTACGACTTAGTGCCCTGACCCCTCATTGAG  
GAAATGCGAAAGGTTGTATGTGATCAGAACGCTGCGTCCCACATCCCCA  
ACTGGTGGCAGAGTTATGAGGCACTGCGGGTGTGGGAAGATGATGCG  
AGAGTGTGGTATGCCAACGGCGCAGCCCGCTGACGGCCCTGCGCATC  
AAGAAGACCCCTCAGCTCAGCGTCAGGAAGACGTGAAGATC
```

[0132] A nucleic acid sequence encoding the extracellular ALK4 polypeptide is as follows:

```
(SEQ ID NO: 103)  
TCCGGGGCCCGGGGGTCCAGGCTCTGCTGTGCGTGCACCAGCTGCC  
TCCAGGCCAACTACACGTGTGAGACAGATGGGGCCTGCATGGTTCCAT  
TTTCAATCTGGATGGGATGGAGCACCATGTGCGCACCTGCATCCCCAA  
GTGGAGCTGGTCCCTGCCGGGAAGCCCTACTGCGCTGAGCTCGGAG  
ACCTGCGCAACACCCACTGCTGCTACACTGACTACTGCAACAGGATCGA  
CTTGAGGGTGCCTGAGCTGACCTCAAGGAGCCTGAGCACCCGTCCATG  
TGGGGCCCGGTGGAG
```

[0133] An alternative isoform of human ALK4 precursor protein sequence, isoform B (NCBI Ref Seq NP_064732.3), is as follows:

```
(SEQ ID NO: 104)  
1 MVSIFNLDGM EHHVRTCIPK VELVPAGKPF YCLSSEDLRN THCCYTDYCN RIDLRVPSGH  
61 LKEPEHPSMW GPVELVGIIA GPVFLFLII IIVFLVINYH QRVYHNRQRL DMEDPSCEMC  
121 LSKDKTLQDL VYDLSLSTSGSG SGLPLFVQRT VARTIVLQEI IGKGRFGEW RGRWRGGDVA  
181 VKIFSSREER SWFREAEIYQ TVMLRHENIL GFIAADNKDN GTWTQLWLVS DYHEHGSLFD  
241 YLNRYTVTIE GMIKLALSAA SGLAHLHMEI VGTQGKPGIA HRDLKSKNIL VKKNGMCAIA  
301 DLGLAVRHDA VTDTIDIAPN QRVGTKRYMA PEVLDETINM KHFDSFKCAD IYALGLVYWE  
361 IARRCNSSGV HEEYQLPYDD LVPSDPSIEE MRKVVCDQKL RPNIIPNWWQS YEALRVMGKM  
421 MRECWYANGA ARLTALRIKK TLSQLSVQED VKI
```

[0134] The extracellular domain is indicated in bold font.

[0135] A processed extracellular ALK4 polypeptide sequence is as follows:

```
(SEQ ID NO: 105)  
1 MVSIFNLDGM EHHVRTCIPK VELVPAGKPF YCLSSEDLRN THCCYTDYCN RIDLRVPSGH  
61 LKEPEHPSMW GPVE
```

[0136] A nucleic acid sequence encoding the ALK4 precursor protein (isoform B) is shown below (SEQ ID NO: 106), corresponding to nucleotides 186-1547 of Genbank Reference Sequence NM_020327.3. The nucleotides encoding the extracellular domain are indicated in bold font.

```
(SEQ ID NO: 106)  
1 ATGGTTTCCA TTTTCAATCT GGATGGGATG GAGCACCATG TGCGCACCTG  
51 CATCCCCAAA GTGGAGCTGG TCCCTGCCGG GAAGCCCTTC TACTGCCCTGA  
101 GCTCGGAGGA CCTGCGAAC ACCCACTGCT GCTACACTGA CTACTGCAAC  
151 AGGATGCACT TGAGGGTGC CAGTGGTCAC CTCAAGGAGC CTGAGCACCC  
201 GTCCATGTGG GCCCGGGTGG AGCTGGTAGG CATCATGCC GGCCGGTGT  
251 TCCCTCTGTT CCTCATCATC ATCATTGTTT TCCTTGTCA TAACTATCAT  
301 CAGCGTGTCT ATCACAAACCG CCAGAGACTG GACATGGAAG ATCCCTCATG  
351 TGAGATGTGT CTCTCCAAAG ACAAGACGCT CCAGGATCTT GTCTACGATC
```

- continued

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401 TCTCCACCTC AGGGTCTGGC TCAGGGTTAC CCCTCTTGT CCAGCGCACA
451 GTGGCCCGAA CCATCGTTT ACAAGAGATT ATTGGCAAGG GTCGGTTGG
501 GGAAGTATGG CGGGGCCGCT GGAGGGGTGG TGATGTGGCT GTGAAAATAT
551 TCTCTTCTCG TGAAGAACCG TCTTGGTTCA GGGAAAGCAGA GATATACCA
601 ACGGTCCATGC TGCGCCATGA AAACATCCTT GGATTTATTG CTGCTGACAA
651 TAAAGATAAT GGCACCTGGA CACAGCTGTG GCTTGTCT GACTATCATG
701 AGCACGGGTC CCTGGTTGAT TATCTGAACC GGTACACAGT GACAATTGAG
751 GGGATGATTA AGCTGGCCTT GTCTGCTGCT AGTGGGCTGG CACACCTGCA
801 CATGGAGATC GTGGGCACCC AAGGGAAGCC TCCAATTGCT CATCGAGACT
851 TAAAGTCAAA GAACATTCTG GTGAAGAAAA ATGGCATGTG TGCCATAGCA
901 GACCTGGGCC TGGCTGTCCG TCATGATGCA GTCACTGACA CCATTGACAT
951 TGCCCCGAAT CAGAGGGTGG GGACCAAAACG ATACATGGCC CCTGAAGTAC
1001 TTGATGAAAC CATTAATATG AAACACTTTG ACTCCTTTAA ATGTGCTGAT
1051 ATTTATGCC CCGGGCTTGT ATATTGGGAG ATTGCTCGAA GATGCAATTG
1101 TGGAGGAGTC CATGAAGAAAT ATCAGCTGCC ATATTACGAC TTAGTGCCTC
1151 CTGACCCCTTC CATTGAGGAA ATGGCAAAGG TTGTATGTGA TCAGAAAGCTG
1201 CGTCCCAACA TCCCCAAGTG GTGGCAGAGT TATGAGGCAC TGCGGGTGT
1251 GGGGAAGATG ATGCGAGAGT GTTGGTATGC CAACGGCGCA GCCCGCCTGA
1301 CGGCCCTCGC CATCAAGAAG ACCCTCTCCC AGCTCAGCGT GCAGGAAGAC
1351 GTGAAGATCT AA

```

[0137] A nucleic acid sequence encoding the extracellular ALK4 polypeptide (isoform B) is as follows:

(SEQ ID NO: 107)

```

1 ATGGTTCCA TTTCAATCT GGATGGGATG GAGCACCATG TGCGCACCTG
51 CATCCCCAAA GTGGAGCTGG TCCCTGCCGG GAAGCCCTTC TACTGCCCTGA
101 GCTCGGAGGA CCTGCGCAAC ACCCACTGCT GCTACACTGA CTACTGCAAC
151 AGGATCGACT TGAGGGTGCC CAGTGGTCAC CTCAAGGAGC CTGAGCACCC
201 GTCCATGTGG GGCCCGGTGG AGCTGGTAGG

```

[0138] ALK4 is well-conserved among vertebrates, with large stretches of the extracellular domain completely conserved. For example, FIG. 18 depicts a multi-sequence alignment of a human ALK4 extracellular domain compared to various ALK4 orthologs. Many of the ligands that bind to ALK4 are also highly conserved. Accordingly, from these alignments, it is possible to predict key amino acid positions within the ligand-binding domain that are important for normal ALK4-ligand binding activities as well as to predict amino acid positions that are likely to be tolerant to substitution without significantly altering normal ALK4-ligand binding activities. Therefore, an active, human ALK4 variant polypeptide useful in accordance with the presently disclosed methods may include one or more amino acids at corresponding positions from the sequence of another vertebrate ALK4, or may include a residue that is similar to that in the human or other vertebrate sequences.

[0139] Without meaning to be limiting, the following examples illustrate this approach to defining an active ALK4

variant. As illustrated in FIG. 18, V6 in the human ALK4 extracellular domain (SEQ ID NO: 126) is isoleucine in *Mus musculus* ALK4 (SEQ ID NO: 130), and so the position may be altered, and optionally may be altered to another hydrophobic residue such as L, I, or F, or a non-polar residue such as A, as is observed in *Gallus gallus* ALK4 (SEQ ID NO: 129). E40 in the human extracellular domain is K in *Gallus gallus* ALK4, indicating that this site may be tolerant of a wide variety of changes, including polar residues, such as E, D, K, R, H, S, T, P, G, Y, and probably a non-polar residue such as A. S15 in the human extracellular domain is D in *Gallus gallus* ALK4, indicating that a wide structural variation is tolerated at this position, with polar residues favored, such as S, T, R, E, K, H, G, P, G and Y. E40 in the human extracellular domain is K in *Gallus gallus* ALK4, indicating that charged residues will be tolerated at this position, including D, R, K, H, as well as Q and N. R80 in the human

extracellular domain is K in *Condylura cristata* ALK4 (SEQ ID NO: 127), indicating that basic residues are tolerated at this position, including R, K, and H. Y77 in the human extracellular domain is F in *Sus scrofa* ALK4 (SEQ ID NO: 131), indicating that aromatic residues are tolerated at this position, including F, W, and Y. P93 in the human extracellular domain is relatively poorly conserved, appearing as S in *Erinaceus europaeus* ALK4 (SEQ ID NO: 128) and N in *Gallus gallus* ALK4, thus essentially any amino acid should be tolerated at this position.

[0140] Moreover, ALK4 proteins have been characterized in the art in terms of structural and functional characteristics, particularly with respect to ligand binding [e.g., Harrison et al. (2003) J Biol Chem 278(23):21129-21135; Romano et al. (2012) J Mol Model 18(8):3617-3625; and Calvanese et al. (2009) 15(3):175-183]. In addition to the teachings herein, these references provide ample guidance for how to generate ALK4 variants that retain one or more normal activities (e.g., ligand-binding activity).

[0141] For example, a defining structural motif known as a three-finger toxin fold is important for ligand binding by type I and type II receptors and is formed by conserved cysteine residues located at varying positions within the extracellular domain of each monomeric receptor [Greenwald et al. (1999) Nat Struct Biol 6:18-22; and Hinck (2012) FEBS Lett 586:1860-1870]. Accordingly, the core ligand-binding domains of human ALK4, as demarcated by the outermost of these conserved cysteines, corresponds to positions 34-101 of SEQ ID NO: 100 (ALK4 precursor). The structurally less-ordered amino acids flanking these cysteine-demarcated core sequences can be truncated by 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33 residues at the N-terminus and/or by 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 residues at the C-terminus without necessarily altering ligand binding. Exemplary ALK4 extracellular domains for N-terminal and/or C-terminal truncation include SEQ ID NOs: 101 and 105.

[0142] Accordingly, a general formula for an active portion (e.g., a ligand-binding portion) of ALK4 comprises amino acids 34-101 with respect to SEQ ID NO: 100. Therefore ALK4 polypeptides may, for example, comprise, consists essentially of, or consists of an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a portion of ALK4 beginning at a residue corresponding to any one of amino acids 24-34 (e.g., beginning at any one of amino acids 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, or 34) of SEQ ID NO: 100 and ending at a position corresponding to any one amino acids 101-126 (e.g., ending at any one of amino acids 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, or 126) of SEQ ID NO: 100. Other examples include constructs that begin at a position from 24-34 (e.g., any one of positions 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, or 34), 25-34 (e.g., any one of positions 25, 26, 27, 28, 29, 30, 31, 32, 33, or 34), or 26-34 (e.g., any one of positions 26, 27, 28, 29, 30, 31, 32, 33, or 34) of SEQ ID NO: 100 and end at a position from 101-126 (e.g., any one of positions 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, or 126), 102-126 (e.g., any one of positions 102, 103, 104, 105, 106, 107, 108, 109, 110, 111,

112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, or 126), 101-125 (e.g., any one of positions 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124), 101-124 (e.g., any one of positions 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, or 121), 101-121 (e.g., any one of positions 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, or 124), 101-120 (e.g., any one of positions 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, or 121), 101-126 (e.g., any one of positions 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, or 125), 101-125 (e.g., any one of positions 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, or 125), 101-124 (e.g., any one of positions 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, or 125), 101-123 (e.g., any one of positions 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, or 125), 101-122 (e.g., any one of positions 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, or 125), 101-121 (e.g., any one of positions 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, or 125), 101-120 (e.g., any one of positions 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, or 125), 101-119 (e.g., any one of positions 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, or 125), 101-118 (e.g., any one of positions 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, or 125), 101-117 (e.g., any one of positions 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, or 125), 101-116 (e.g., any one of positions 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, or 125), 101-115 (e.g., any one of positions 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, or 125), 101-114 (e.g., any one of positions 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, or 125), 101-113 (e.g., any one of positions 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, or 125), 101-112 (e.g., any one of positions 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, or 125), 101-111 (e.g., any one of positions 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, or 125), 101-110 (e.g., any one of positions 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, or 125), 101-109 (e.g., any one of positions 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, or 125), 101-108 (e.g., any one of positions 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, or 125), 101-107 (e.g., any one of positions 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, or 125), 101-106 (e.g., any one of positions 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, or 125), 101-105 (e.g., any one of positions 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, or 125), 101-104 (e.g., any one of positions 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, or 125), 101-103 (e.g., any one of positions 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, or 125), 101-102 (e.g., any one of positions 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, or 125), 101-101 (e.g., any one of positions 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, or 125).

[0143] The variations described herein may be combined in various ways. In some embodiments, ALK4 variants comprise no more than 1, 2, 5, 6, 7, 8, 9, 10 or 15 conservative amino acid changes in the ligand-binding pocket. Sites outside the binding pocket, at which variability may be particularly well tolerated, include the amino and carboxy termini of the extracellular domain (as noted above).

[0144] In certain embodiments, the disclosure relates to BMP/GDF antagonists that are heteromultimers comprising at least one ALK4 polypeptide, which includes fragments, functional variants, and modified forms thereof as well as uses thereof (e.g., treating, preventing, or reducing the severity of PAH or one or more complications of PAH). Preferably, ALK4 polypeptides are soluble (e.g., an extracellular domain of ALK4). In some embodiments, heteromultimers comprising an ALK4 polypeptide inhibit (e.g., Smad signaling) of one or more TGF β superfamily ligands [e.g., GDF11, GDF8, activin (activin A, activin B, activin AB, activin C, activin E) BMP6, GDF3, BMP10, and/or BMP9]. In some embodiments, heteromultimers comprising an ALK4 polypeptide bind to one or more TGF β superfamily ligands [e.g., GDF11, GDF8, activin (activin A, activin B, activin AB, activin C, activin E) BMP6, GDF3, BMP10, and/or BMP9]. In some embodiments, heteromultimers comprise at least one ALK4 polypeptide that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 97%, 98%, 99%, 100% identical to amino acids 34-101 with respect to SEQ ID NO: 100. In some embodiments, heteromultimers comprise at least one ALK4 polypeptide that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 97%, 98%, 99%, 100% identical to amino acids 34-101 with respect to SEQ ID NO: 100. In some embodiments, heteromultimers comprise at least one ALK4 polypeptide that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 97%, 98%, 99%, 100% identical to the amino acid sequence of SEQ ID NO: 100, 101, 104, 105, 111, 116, 117, 122, and 124. In some embodiments, heteromultimer comprise at least one ALK4 polypeptide that consist or consist essentially of at least one ALK4 polypeptide that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%,

95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 100, 101, 104, 105, 111, 113, 116, 117, 122, and 124.

[0145] In certain aspects, the present disclosure relates to heteromultimer complexes comprising one or more ALK4 receptor polypeptides (e.g., SEQ ID Nos: 100, 101, 104, 105, 111, 113, 116, 117, 122, and variants thereof) and one or more ActRIIB receptor polypeptides (e.g., SEQ ID NOS: 1, 2, 3, 4, 5, 6, 58, 59, 60, 63, 64, 65, 66, 68, 69, 70, 71, 73, 77, 78, 108, 110, 114, 115, 118, and 120 and land variants thereof), which are generally referred to herein as “ALK4:ActRIIB heteromultimer complexes” or “ALK4:ActRIIB heteromultimers”, including uses thereof (e.g., increasing an immune response in a patient in need thereof and treating cancer). Preferably, ALK4:ActRIIB heteromultimers are soluble [e.g., a heteromultimer complex comprises a soluble portion (domain) of an ALK4 receptor and a soluble portion (domain) of an ActRIIB receptor]. In general, the extracellular domains of ALK4 and ActRIIB correspond to soluble portion of these receptors. Therefore, in some embodiments, ALK4:ActRIIB heteromultimers comprise an extracellular domain of an ALK4 receptor and an extracellular domain of an ActRIIB receptor. In some embodiments, ALK4:ActRIIB heteromultimers inhibit (e.g., Smad signaling) of one or more TGF β superfamily ligands [e.g., GDF11, GDF8, activin (activin A, activin B, activin AB, activin C, activin E) BMP6, GDF3, BMP10, and/or BMP9]. In some embodiments, ALK4:ActRIIB heteromultimers bind to one or more TGF β superfamily ligands [e.g., GDF11, GDF8, activin (activin A, activin B, activin AB, activin C, activin E) BMP6, GDF3, BMP10, and/or BMP9]. In some embodiments, ALK4:ActRIIB heteromultimers comprise at least one ALK4 polypeptide that comprises, consists essentially of, or consists of a sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94% 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 100, 101, 104, 105, 111, 113, 116, 117, 122, and 124. In some embodiments, ALK4:ActRIIB heteromultimer complexes of the disclosure comprise at least one ALK4 polypeptide that comprises, consists essentially of, consists of a sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94% 95%, 97%, 98%, 99%, or 100% identical to a portion of ALK4 beginning at a residue corresponding to any one of amino acids 24-34, 25-34, or 26-34 of SEQ ID NO: 100 and ending at a position from 101-126, 102-126, 101-125, 101-124, 101-121, 111-126, 111-125, 111-124, 121-126, 121-125, 121-124, or 124-126 of SEQ ID NO: 100. In some embodiments, ALK4:ActRIIB heteromultimers comprise at least one ALK4 polypeptide that comprises, consists essentially of, consists of a sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94% 95%, 97%, 98%, 99%, or 100% identical to amino acids 34-101 with respect to SEQ ID NO: 100. In some embodiments, ALK4:ActRIIB heteromultimers comprise at least one ActRIIB polypeptide that comprises, consists essentially of, consists of a sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94% 95%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NOS: 1, 2, 3, 4, 5, 6, 58, 59, 60, 63, 64, 65, 66, 68, 69, 70, 71, 73, 77, 78, 108, 110, 114, 115, 118, and 120. In some embodiments, ALK4:ActRIIB heteromultimer complexes of the disclosure comprise at least one ActRIIB polypeptide

that comprises, consists essentially of, consists of a sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94% 95%, 97%, 98%, 99%, or 100% identical to a portion of ActRIIB beginning at a residue corresponding to any one of amino acids 20-29, 20-24, 21-24, 22-25, or 21-29 and end at a position from 109-134, 119-134, 119-133, 129-134, or 129-133 of SEQ ID NO: 1. In some embodiments, ALK4:ActRIIB heteromultimers comprise at least one ActRIIB polypeptide that comprises, consists essentially of, consists of a sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94% 95%, 97%, 98%, 99%, or 100% identical to amino acids 29-109 of SEQ ID NO: 1. In some embodiments, ALK4:ActRIIB heteromultimers comprise at least one ActRIIB polypeptide that comprises, consists essentially of, consists of a sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94% 95%, 97%, 98%, 99%, or 100% identical to amino acids 25-131 of SEQ ID NO: 1. In certain embodiments, ALK4:ActRIIB heteromultimer complexes of the disclosure comprise at least one ActRIIB polypeptide wherein the position corresponding to L79 of SEQ ID NO: 1 is not an acidic amino acid (i.e., not naturally occurring D or E amino acid residues or an artificial acidic amino acid residue). ALK4:ActRIIB heteromultimers of the disclosure include, e.g., heterodimers, heterotrimers, heterotetramers and further higher order oligomeric structures. See, e.g., FIGS. 21-23. In certain preferred embodiments, heteromultimer complexes of the disclosure are ALK4:ActRIIB heterodimers.

[0146] In some embodiments, the present disclosure contemplates making functional variants by modifying the structure of an ActRII and/or ALK4 polypeptide for such purposes as enhancing therapeutic efficacy or stability (e.g., shelf-life and resistance to proteolytic degradation *in vivo*). Variants can be produced by amino acid substitution, deletion, addition, or combinations thereof. For instance, it is reasonable to expect that an isolated replacement of a leucine with an isoleucine or valine, an aspartate with a glutamate, a threonine with a serine, or a similar replacement of an amino acid with a structurally related amino acid (e.g., conservative mutations) will not have a major effect on the biological activity of the resulting molecule. Conservative replacements are those that take place within a family of amino acids that are related in their side chains. Whether a change in the amino acid sequence of a polypeptide of the disclosure results in a functional homolog can be readily determined by assessing the ability of the variant polypeptide to produce a response in cells in a fashion similar to the wild-type polypeptide, or to bind to one or more TGF- β ligands including, for example, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF- β 1, TGF- β 2, TGF- β 3, activin A, activin B, activin C, activin E, activin AB, activin AC, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty.

[0147] In certain embodiments, the present disclosure contemplates specific mutations of an ActRII and/or ALK4 polypeptide so as to alter the glycosylation of the polypeptide. Such mutations may be selected so as to introduce or eliminate one or more glycosylation sites, such as O-linked or N-linked glycosylation sites. Asparagine-linked glycosylation recognition sites generally comprise a tripeptide

sequence, asparagine-X-threonine or asparagine-X-serine (where "X" is any amino acid) which is specifically recognized by appropriate cellular glycosylation enzymes. The alteration may also be made by the addition of, or substitution by, one or more serine or threonine residues to the sequence of the polypeptide (for O-linked glycosylation sites). A variety of amino acid substitutions or deletions at one or both of the first or third amino acid positions of a glycosylation recognition site (and/or amino acid deletion at the second position) results in non-glycosylation at the modified tripeptide sequence. Another means of increasing the number of carbohydrate moieties on a polypeptide is by chemical or enzymatic coupling of glycosides to the polypeptide. Depending on the coupling mode used, the sugar(s) may be attached to (a) arginine and histidine; (b) free carboxyl groups; (c) free sulfhydryl groups such as those of cysteine; (d) free hydroxyl groups such as those of serine, threonine, or hydroxyproline; (e) aromatic residues such as those of phenylalanine, tyrosine, or tryptophan; or (f) the amide group of glutamine. Removal of one or more carbohydrate moieties present on a polypeptide may be accomplished chemically and/or enzymatically. Chemical deglycosylation may involve, for example, exposure of a polypeptide to the compound trifluoromethanesulfonic acid, or an equivalent compound. This treatment results in the cleavage of most or all sugars except the linking sugar (N-acetylglucosamine or N-acetylgalactosamine), while leaving the amino acid sequence intact. Enzymatic cleavage of carbohydrate moieties on polypeptides can be achieved by the use of a variety of endo- and exo-glycosidases as described by Thotakura et al. [Meth. Enzymol. (1987) 138:350]. The sequence of a polypeptide may be adjusted, as appropriate, depending on the type of expression system used, as mammalian, yeast, insect, and plant cells may all introduce differing glycosylation patterns that can be affected by the amino acid sequence of the peptide. In general, polypeptides of the present disclosure for use in humans may be expressed in a mammalian cell line that provides proper glycosylation, such as HEK293 or CHO cell lines, although other mammalian expression cell lines are expected to be useful as well.

[0148] The present disclosure further contemplates a method of generating mutants, particularly sets of combinatorial mutants of an ActRII and/or ALK4 polypeptide as well as truncation mutants. Pools of combinatorial mutants are especially useful for identifying functionally active (e.g., GDF/BMP ligand binding) ActRII sequences. The purpose of screening such combinatorial libraries may be to generate, for example, polypeptides variants which have altered properties, such as altered pharmacokinetic or altered ligand binding. A variety of screening assays are provided below, and such assays may be used to evaluate variants. For example, ActRII and/or ALK4 variants, and heteromultimers comprising the same, may be screened for ability to bind to one or more GDF/BMP ligands (e.g., BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF- β 1, TGF- β 2, TGF- β 3, activin A, activin B, activin C, activin E, activin AB, activin AC, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty), to prevent binding of a GDF/BMP ligand to an

ActRII and/or ALK4 polypeptide, as well as heteromultimers thereof, and/or to interfere with signaling caused by an GDF/BMP ligand.

[0149] The activity of ActRII polypeptides, ALK4 polypeptides, and ALK4:ActRIIB heterodimers may also be tested in a cell-based or in vivo assay. For example, the effect of an ActRII polypeptide, ALK4 polypeptide, or ALK4:ActRIIB heterodimer on the expression of genes involved in PH pathogenesis assessed. This may, as needed, be performed in the presence of one or more recombinant ligand proteins (e.g., BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF- β 1, TGF- β 2, TGF- β 3, activin A, activin B, activin C, activin E, activin AB, activin AC, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty), and cells may be transfected so as to produce an ActRII polypeptide, ALK4 polypeptide, or ALK4:ActRIIB heterodimer, and optionally, an GDF/BMP ligand. Likewise, an ActRII polypeptide, ALK4 polypeptide, or ALK4:ActRIIB heterodimer may be administered to a mouse or other animal and effects on PH pathogenesis may be assessed using art-recognized methods. Similarly, the activity of an ActRII polypeptide, ALK4 polypeptide, or ALK4:ActRIIB heterodimer or variant thereof may be tested in blood cell precursor cells for any effect on growth of these cells, for example, by the assays as described herein and those of common knowledge in the art. A SMAD-responsive reporter gene may be used in such cell lines to monitor effects on downstream signaling.

[0150] Combinatorial-derived variants can be generated which have increased selectivity or generally increased potency relative to a reference ActRII polypeptide, ALK4 polypeptide, or ALK4:ActRIIB heterodimer. Such variants, when expressed from recombinant DNA constructs, can be used in gene therapy protocols. Likewise, mutagenesis can give rise to variants which have intracellular half-lives dramatically different than the corresponding unmodified ActRII polypeptide, ALK4 polypeptide, or ALK4:ActRIIB heterodimer. For example, the altered protein can be rendered either more stable or less stable to proteolytic degradation or other cellular processes which result in destruction, or otherwise inactivation, of an unmodified polypeptide. Such variants, and the genes which encode them, can be utilized to alter polypeptide complex levels by modulating the half-life of the polypeptide. For instance, a short half-life can give rise to more transient biological effects and, when part of an inducible expression system, can allow tighter control of recombinant polypeptide complex levels within the cell. In an Fc fusion protein, mutations may be made in the linker (if any) and/or the Fc portion to alter the half-life of the ActRII polypeptide, ALK4 polypeptide, or ALK4:ActRIIB heterodimer.

[0151] A combinatorial library may be produced by way of a degenerate library of genes encoding a library of polypeptides which each include at least a portion of potential ActRII polypeptide, ALK4 polypeptide, or ALK4:ActRIIB heterodimer sequences. For instance, a mixture of synthetic oligonucleotides can be enzymatically ligated into gene sequences such that the degenerate set of potential ActRII and/or ALK4 encoding nucleotide sequences are expressible as individual polypeptides, or alternatively, as a set of larger fusion proteins (e.g., for phage display).

[0152] There are many ways by which the library of potential homologs can be generated from a degenerate oligonucleotide sequence. Chemical synthesis of a degenerate gene sequence can be carried out in an automatic DNA synthesizer, and the synthetic genes can then be ligated into an appropriate vector for expression. The synthesis of degenerate oligonucleotides is well known in the art [Narang, SA (1983) Tetrahedron 39:3; Itakura et al. (1981) Recombinant DNA, Proc. 3rd Cleveland Sympos. Macromolecules, ed. AG Walton, Amsterdam: Elsevier pp273-289; Itakura et al. (1984) Annu. Rev. Biochem. 53:323; Itakura et al. (1984) Science 198:1056; and Ike et al. (1983) Nucleic Acid Res. 11:477]. Such techniques have been employed in the directed evolution of other proteins [Scott et al., (1990) Science 249:386-390; Roberts et al. (1992) PNAS USA 89:2429-2433; Devlin et al. (1990) Science 249: 404-406; Cwirla et al., (1990) PNAS USA 87: 6378-6382; as well as U.S. Pat. Nos. 5,223,409, 5,198,346, and 5,096,815].

[0153] Alternatively, other forms of mutagenesis can be utilized to generate a combinatorial library. For example, ActRII polypeptides, ALK4 polypeptides, and ALK4:ActRIIB heterodimers of the disclosure can be generated and isolated from a library by screening using, for example, alanine scanning mutagenesis [Ruf et al. (1994) Biochemistry 33:1565-1572; Wang et al. (1994) J. Biol. Chem. 269:3095-3099; Balint et al. (1993) Gene 137:109-118; Grodberg et al. (1993) Eur. J. Biochem. 218:597-601; Nagashima et al. (1993) J. Biol. Chem. 268:2888-2892; Lowman et al. (1991) Biochemistry 30:10832-10838; and Cunningham et al. (1989) Science 244:1081-1085], by linker scanning mutagenesis [Gustin et al. (1993) Virology 193:653-660; and Brown et al. (1992) Mol. Cell Biol. 12:2644-2652; McKnight et al. (1982) Science 232:316], by saturation mutagenesis [Meyers et al., (1986) Science 232: 613]; by PCR mutagenesis [Leung et al. (1989) Method Cell Mol Biol 1:11-19]; or by random mutagenesis, including chemical mutagenesis [Miller et al. (1992) A Short Course in Bacterial Genetics, CSHL Press, Cold Spring Harbor, NY; and Greener et al. (1994) Strategies in Mol Biol 7:32-34]. Linker scanning mutagenesis, particularly in a combinatorial setting, is an attractive method for identifying truncated (bioactive) forms of ActRII polypeptides, ALK4 polypeptides, or ALK4:ActRIIB heterodimers.

[0154] A wide range of techniques are known in the art for screening gene products of combinatorial libraries made by point mutations and truncations, and, for that matter, for screening cDNA libraries for gene products having a certain property. Such techniques will be generally adaptable for rapid screening of the gene libraries generated by the combinatorial mutagenesis of ActRII polypeptides. The most widely used techniques for screening large gene libraries typically comprise cloning the gene library into replicable expression vectors, transforming appropriate cells with the resulting library of vectors, and expressing the combinatorial genes under conditions in which detection of a desired activity facilitates relatively easy isolation of the vector encoding the gene whose product was detected. Preferred assays include ligand (e.g., BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF- β 1, TGF- β 2, TGF- β 3, activin A, activin B, activin C, activin E, activin AB, activin AC, nodal, glial cell-derived neurotrophic factor (GDNF), neuritin, artemin, persephin, MIS, and Lefty).

artemin, persephin, MIS, and Lefty) binding assays and/or ligand-mediated cell signaling assays.

[0155] As will be recognized by one of skill in the art, most of the described mutations, variants or modifications described herein may be made at the nucleic acid level or, in some cases, by post-translational modification or chemical synthesis. Such techniques are well known in the art and some of which are described herein. In part, the present disclosure identifies functionally active portions (fragments) and variants of ActRII polypeptides, ALK4 polypeptides, or ALK4:ActRIIB heterodimesr that can be used as guidance for generating and using other variant ActRII polypeptides within the scope of the inventions described herein.

[0156] In certain embodiments, functionally active fragments of ActRII polypeptides, ALK4 polypeptides, and ALK4:ActRIIB heterodimesr of the present disclosure can be obtained by screening polypeptides recombinantly produced from the corresponding fragment of the nucleic acid encoding an ActRII and/or ALK4 polypeptides. In addition, fragments can be chemically synthesized using techniques known in the art such as conventional Merrifield solid phase f-Moc or t-Boc chemistry. The fragments can be produced (recombinantly or by chemical synthesis) and tested to identify those peptidyl fragments that can function as antagonists (inhibitors) of ActRII and/or ALK4 receptors and/or one or more ligands (e.g., BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF- β 1, TGF- β 2, TGF- β 3, activin A, activin B, activin C, activin E, activin AB, activin AC, nodal, glial cell-derived neurotrophic factor (GDNF), neuritin, artemin, persephin, MIS, and Lefty).

[0157] In certain embodiments, ActRII polypeptide, ALK4 polypeptide, and/or ALK4:ActRIIB heterodimer of the present disclosure may further comprise post-translational modifications in addition to any that are naturally present in the ActRII polypeptide, ALK4 polypeptide, or ALK4:ActRIIB heterodimer. Such modifications include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation, and acylation. As a result, the ActRII polypeptide, ALK4 polypeptide, or ALK4:ActRIIB heterodimer may contain non-amino acid elements, such as polyethylene glycols, lipids, polysaccharide or monosaccharide, and phosphates. Effects of such non-amino acid elements on the functionality of a ligand trap polypeptide may be tested as described herein for other ActRII, ALK4, and ALK4:ActRIIB variants. When a polypeptide of the disclosure is produced in cells by cleaving a nascent form of the polypeptide, post-translational processing may also be important for correct folding and/or function of the protein. Different cells (e.g., CHO, HeLa, MDCK, 293, W138, NIH-3T3 or HEK293) have specific cellular machinery and characteristic mechanisms for such post-translational activities and may be chosen to ensure the correct modification and processing of the ActRII polypeptides.

[0158] In certain aspects, ActRII and ALK4 polypeptides of the present disclosure include fusion proteins having at least a portion (domain) of an ActRII or ALK4 polypeptide

and one or more heterologous portions (domains). Well-known examples of such fusion domains include, but are not limited to, polyhistidine, Glu-Glu, glutathione S-transferase (GST), thioredoxin, protein A, protein G, an immunoglobulin heavy-chain constant region (Fc), maltose binding protein (MBP), or human serum albumin. A fusion domain may be selected so as to confer a desired property. For example, some fusion domains are particularly useful for isolation of the fusion proteins by affinity chromatography. For the purpose of affinity purification, relevant matrices for affinity chromatography, such as glutathione-, amylase-, and nickel- or cobalt-conjugated resins are used. Many of such matrices are available in "kit" form, such as the Pharmacia GST purification system and the QIAexpress™ system (Qiagen) useful with (HIS_n) (SEQ ID NO: 137) fusion partners. As another example, a fusion domain may be selected so as to facilitate detection of the ActRII or ALK4 polypeptide. Examples of such detection domains include the various fluorescent proteins (e.g., GFP) as well as "epitope tags," which are usually short peptide sequences for which a specific antibody is available. Well-known epitope tags for which specific monoclonal antibodies are readily available include FLAG, influenza virus haemagglutinin (HA), and c-myc tags. In some cases, the fusion domains have a protease cleavage site, such as for Factor Xa or thrombin, which allows the relevant protease to partially digest the fusion proteins and thereby liberate the recombinant proteins therefrom. The liberated proteins can then be isolated from the fusion domain by subsequent chromatographic separation. Other types of fusion domains that may be selected

sylation site (including, for example, addition of a glycosylation site to a polypeptide of the disclosure), and modifications of carbohydrate moiety (including, for example, removal of carbohydrate moieties from a polypeptide of the disclosure). As used herein, the term "stabilizer domain" not only refers to a fusion domain (e.g., an immunoglobulin Fc domain) as in the case of fusion proteins, but also includes nonproteinaceous modifications such as a carbohydrate moiety, or nonproteinaceous moiety, such as polyethylene glycol. In certain preferred embodiments, an ActRII polypeptide (or ALK4 polypeptide) is fused with a heterologous domain that stabilizes the polypeptide (a "stabilizer" domain), preferably a heterologous domain that increases stability of the polypeptide in vivo. Fusions with a constant domain of an immunoglobulin (e.g., a Fc domain) are known to confer desirable pharmacokinetic properties on a wide range of proteins. Likewise, fusions to human serum albumin can confer desirable properties.

[0160] An example of a native amino acid sequence that may be used for the Fc portion of human IgG1 (G1Fc) is shown below (SEQ ID NO: 14). Dotted underline indicates the hinge region, and solid underline indicates positions with naturally occurring variants. In part, the disclosure provides polypeptides comprising, consisting essential of, or consisting of amino acid sequences with 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 14. Naturally occurring variants in G1Fc would include E134D and M136L according to the numbering system used in SEQ ID NO: 14 (see Uniprot P01857).

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1 THTCPPCPAP ELLGGPSVPL FPPPKPKDTLM ISRTPEVTCV VVDVSHEDPE
51 VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK
101 VSNKALPAPI EKTISKAKGQ PREPQVYTLR PSREEMTKNQ VSLTCLVKGF
151 YPSDIAVEWE SNGQPENNYK TPPPVLDSDG SFFLYSKLTW DKSRWQQGNV
201 FSCSVMHEAL HNHYTQKSLS LSPGK      (SEQ ID NO: 14)

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include multimerizing (e.g., dimerizing, tetramerizing) domains and functional domains (that confer an additional biological function) including, for example constant domains from immunoglobulins (e.g., Fc domains).

[0159] In certain aspects, ActRII and ALK4 polypeptides of the present disclosure contain one or more modifications that are capable of "stabilizing" the polypeptides. By "stabilizing" is meant anything that increases the in vitro half-life, serum half-life, regardless of whether this is because of decreased destruction, decreased clearance by the kidney, or other pharmacokinetic effect of the agent. For example, such modifications enhance the shelf-life of the polypeptides, enhance circulatory half-life of the polypeptides, and/or reduce proteolytic degradation of the polypeptides. Such stabilizing modifications include, but are not limited to, fusion proteins (including, for example, fusion proteins comprising an ActRII polypeptide (or ALK4 polypeptide) domain and a stabilizer domain), modifications of a glyco-

[0161] Optionally, the IgG1 Fc domain has one or more mutations at residues such as Asp-265, lysine 322, and Asn-434. In certain cases, the mutant IgG1 Fc domain having one or more of these mutations (e.g., Asp-265 mutation) has reduced ability of binding to the Fcγ receptor relative to a wild-type Fc domain. In other cases, the mutant Fc domain having one or more of these mutations (e.g., Asn-434 mutation) has increased ability of binding to the MHC class I-related Fc-receptor (FcRN) relative to a wild-type IgG1 Fc domain.

[0162] An example of a native amino acid sequence that may be used for the Fc portion of human IgG2 (G2Fc) is shown below (SEQ ID NO: 15). Dotted underline indicates the hinge region and double underline indicates positions where there are data base conflicts in the sequence (according to UniProt P01859). In part, the disclosure provides polypeptides comprising, consisting essential of, or consisting of amino acid sequences with 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 15.

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1 VECPPCPAPP VAGPSVFLFP PKPKDTLMIS RTPEVTCVVV DVSHEDPEVQ
51 FNWYVDGVEV HNAKTKPREE QFNSTFRVVS VLTVHQDWL NGKEYKCKVS
101 NKGLPAPIEK TISKTKGQPR EPQVYTLPPS REEMTKNQVS LTCLVKGFYP
151 SDIAVEWESN QOPENNYKTT PPMLSDGSF FLYSKLTVDK SRWQGNVFS
201 CSVMHEALHN HYTQKSLSLSPGK (SEQ ID NO: 15)

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[0163] Two examples of amino acid sequences that may be used for the Fc portion of human IgG3 (G3Fc) are shown below. The hinge region in G3Fc can be up to four times as long as in other Fc chains and contains three identical 15-residue segments preceded by a similar 17-residue segment. The first G3Fc sequence shown below (SEQ ID NO: 16) contains a short hinge region consisting of a single 15-residue segment, whereas the second G3Fc sequence (SEQ ID NO: 17) contains a full-length hinge region. In each case, dotted underline indicates the hinge region, and solid underline indicates positions with naturally occurring variants according to UniProt P01859. In part, the disclosure provides polypeptides comprising, consisting essential of, or consisting of amino acid sequences with 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NOs: 16 and 17.

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1 EPKSCDTPPP CPRCPAPELL GGPSVFLFPP KPKDTLMSR TPEVTCVVVD
51 VSHEDPEVQF KWYVDGVEVH NAKTKPREEQ YNNSTFRVVSV LTVLHQDWLNL
101 GKEYKCKVSN KALPAPIEKT ISKTKGQPRE PQVYTLPPSR EEMTKNQVSL
151 TCLVKGFYPS DIAVEWESSG QOPENNYNTTP PMLSDGSFF LYSKLTVDKS
201 RWQQGNIFSC SVMHEALHNR FTQKSLSLSP GK (SEQ ID NO: 16)
1 ELKTPLGDT HTCPRCPEPK SCDTPPPCPR CPEPKSCDTP PPCPRCPEPK
51 SCDTPPPCPR CPAELLGGP SVFLFPPPKPK DTLMSRTPE VTCVVVDVSH
101 EDPEVQFKWY VDGVEVHNAK TKPREEQYNS TFRVSVLTV LHQDWLNGKE
151 YKCKVSNKAL PAPIEKTISK TKGQPREPQV YTLPPSREEM TKNQVSLTCL
201 VKGFYPSDIA VEWESSQPE NNYNTTPPML DSDGSFFLYS KLTVDKSRWQ
251 QGNIFSCSVM HEALHNRFTQ KSLSLSPGK (SEQ ID NO: 17)

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[0164] Naturally occurring variants in G3Fc (for example, see Uniprot P01860) include E68Q, P76L, E79Q, Y81F, D97N, N100D, T124A, S169N, S169del, F221Y when converted to the numbering system used in SEQ ID NO: 16, and the present disclosure provides fusion proteins comprising G3Fc domains containing one or more of these variations. In addition, the human immunoglobulin IgG3 gene (IGHG3) shows a structural polymorphism characterized by different hinge lengths [see Uniprot P01859]. Specifically, variant WIS is lacking most of the V region and all of the CH1 region. It has an extra interchain disulfide bond at position 7 in addition to the 11 normally present in the hinge region. Variant ZUC lacks most of the V region, all of the

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

[0165] An example of a native amino acid sequence that may be used for the Fc portion of human IgG4 (G4Fc) is shown below (SEQ ID NO: 18). Dotted underline indicates the hinge region. In part, the disclosure provides polypeptides comprising, consisting essential of, or consisting of amino acid sequences with 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 18.

1 ESKYGP_CPAPEFFLGGP SVFLFPPKPK DTLMISRTPE VTCVVVDVSQ
 51 EDPEVQFKWY VDGVEVHNAK TKPREEQFNS TYRVSVLTV LHQDWLNGKE
 101 YKCKVSNKAL PAPIEKTISK TKGQP_CPQV YTLPPSREEM TKNQVSLTCL
 151 VKGFYPSDIA VEWESNGQPE NNYKTPPPV_C LSDGSFFLYS RLTVDKSRWQ
 201 EGNVFSCSVM HEALHNHYTQ KSLSLSLGK (SEQ ID NO: 18)

[0166] A variety of engineered mutations in the Fc domain are presented herein with respect to the G1Fc sequence (SEQ ID NO: 14), and analogous mutations in G2Fc, G3Fc, and G4Fc can be derived from their alignment with G1Fc in FIG. 4. Due to unequal hinge lengths, analogous Fc positions based on isotype alignment (FIG. 4) possess different amino acid numbers in SEQ ID NOs: 14, 15, 16, 17, and 18. It can also be appreciated that a given amino acid position in an immunoglobulin sequence consisting of hinge, C_H2, and C_H3 regions (e.g., SEQ TD NOs: 14, 15, 16, 17, and 18) will be identified by a different number than the same position when numbering encompasses the entire IgG1 heavy-chain constant domain (consisting of the C_H1, hinge, C_H2, and C_H3 regions) as in the Uniprot database. For example, correspondence between selected C_H3 positions in a human G1Fc sequence (SEQ TD NO: 14), the human IgG1 heavy chain constant domain (Uniprot P01857), and the human IgG1 heavy chain is as follows.

Correspondence of C _H 3 Positions in Different Numbering Systems		
G1Fc (Numbering begins at first threonine in hinge region)	IgG1 heavy chain constant domain (Numbering begins at C _H 1)	IgG1 heavy chain (EU numbering scheme of Kabat et al., 1991*)
Y127	Y232	Y349
S132	S237	S354
E134	E239	E356
T144	T249	T366
L146	L251	L368
K170	K275	K392
D177	D282	D399
Y185	Y290	Y407
K187	K292	K409

*Kabat et al. (eds) 1991; pp. 688-696 in *Sequences of Proteins of Immunological Interest*, 5th ed., Vol. 1, NIH, Bethesda, MD.

[0167] In certain aspects, the polypeptides disclosed herein may form protein complexes comprising at least one ALK4 polypeptide associated, covalently or non-covalently, with at least one ActRIIB polypeptide. Preferably, polypeptides disclosed herein form heterodimeric complexes, although higher order heteromultimeric complexes (heteromultimers) are also included such as, but not limited to, heterotrimers, heterotetramers, and further oligomeric structures (see, e.g., FIG. 21-23). In some embodiments, ALK4 and/or ActRIIB polypeptides comprise at least one multimerization domain. As disclosed herein, the term "multimerization domain" refers to an amino acid or sequence of amino acids that promote covalent or non-covalent interaction between at least a first polypeptide and at least a second polypeptide. Polypeptides disclosed herein may be joined covalently or non-covalently to a multimerization domain. Preferably, a multimerization domain promotes interaction between a first polypeptide (e.g., an ALK4 polypeptide) and

a second polypeptide (e.g., an ActRIIB polypeptide) to promote heteromultimer formation (e.g., heterodimer formation), and optionally hinders or otherwise disfavors homomultimer formation (e.g., homodimer formation), thereby increasing the yield of desired heteromultimer (see, e.g., FIG. 22).

[0168] Many methods known in the art can be used to generate ALK4:ActRIIB heteromultimers. For example, non-naturally occurring disulfide bonds may be constructed by replacing on a first polypeptide (e.g., an ALK4 polypeptide) a naturally occurring amino acid with a free thiol-containing residue, such as cysteine, such that the free thiol interacts with another free thiol-containing residue on a second polypeptide (e.g., an ActRIIB polypeptide) such that a disulfide bond is formed between the first and second polypeptides. Additional examples of interactions to promote heteromultimer formation include, but are not limited to, ionic interactions such as described in Kjaergaard et al., WO2007147901; electrostatic steering effects such as described in Kannan et al., U.S. Pat. No. 8,592,562; coiled-coil interactions such as described in Christensen et al., U.S. 20120302737; leucine zippers such as described in Pack & Plueckthun, (1992) Biochemistry 31: 1579-1584; and helix-turn-helix motifs such as described in Pack et al., (1993) Bio/Technology 11: 1271-1277. Linkage of the various segments may be obtained via, e.g., covalent binding such as by chemical cross-linking, peptide linkers, disulfide bridges, etc., or affinity interactions such as by avidin-biotin or leucine zipper technology.

[0169] In certain aspects, a multimerization domain may comprise one component of an interaction pair. In some embodiments, the polypeptides disclosed herein may form protein complexes comprising a first polypeptide covalently or non-covalently associated with a second polypeptide, wherein the first polypeptide comprises the amino acid sequence of an ALK4 polypeptide and the amino acid sequence of a first member of an interaction pair; and the second polypeptide comprises the amino acid sequence of an ActRIIB polypeptide and the amino acid sequence of a second member of an interaction pair. The interaction pair may be any two polypeptide sequences that interact to form a complex, particularly a heterodimeric complex although operative embodiments may also employ an interaction pair that can form a homodimeric complex. One member of the interaction pair may be fused to an ALK4 or ActRIIB polypeptide as described herein, including for example, a polypeptide sequence comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence of any one of SEQ ID NOs: 2, 3, 5, 6, 101, and 103. An interaction pair may be selected to confer an improved property/activity such as increased serum half-

life, or to act as an adaptor on to which another moiety is attached to provide an improved property/activity. For example, a polyethylene glycol moiety may be attached to one or both components of an interaction pair to provide an improved property/activity such as improved serum half-life.

[0170] The first and second members of the interaction pair may be an asymmetric pair, meaning that the members of the pair preferentially associate with each other rather than self-associate. Accordingly, first and second members of an asymmetric interaction pair may associate to form a heterodimeric complex (see, e.g., FIG. 22). Alternatively, the interaction pair may be unguided, meaning that the members of the pair may associate with each other or self-associate without substantial preference and thus may have the same or different amino acid sequences. Accordingly, first and second members of an unguided interaction pair may associate to form a homodimer complex or a heterodimeric complex. Optionally, the first member of the interaction pair (e.g., an asymmetric pair or an unguided interaction pair) associates covalently with the second member of the interaction pair. Optionally, the first member of the interaction pair (e.g., an asymmetric pair or an unguided interaction pair) associates non-covalently with the second member of the interaction pair.

[0171] As specific examples, the present disclosure provides fusion proteins comprising ALK4 or ActRIIB fused to a polypeptide comprising a constant domain of an immunoglobulin, such as a CH1, CH2, or CH3 domain derived from human IgG1, IgG2, IgG3, and/or IgG4 that has been modified to promote heteromultimer formation. A problem that arises in large-scale production of asymmetric immunoglobulin-based proteins from a single cell line is known as the “chain association issue”. As confronted prominently in the production of bispecific antibodies, the chain association issue concerns the challenge of efficiently producing a desired multi-chain protein from among the multiple combinations that inherently result when different heavy chains and/or light chains are produced in a single cell line [Klein et al (2012) mAbs 4:653-663]. This problem is most acute when two different heavy chains and two different light chains are produced in the same cell, in which case there are a total of 16 possible chain combinations (although some of these are identical) when only one is typically desired. Nevertheless, the same principle accounts for diminished yield of a desired multi-chain fusion protein that incorporates only two different (asymmetric) heavy chains.

[0172] Various methods are known in the art that increase desired pairing of Fc-containing fusion polypeptide chains in a single cell line to produce a preferred asymmetric fusion protein at acceptable yields [Klein et al (2012) mAbs 4:653-663; and Spiess et al (2015) Molecular Immunology 67(2A): 95-106]. Methods to obtain desired pairing of Fc-containing chains include, but are not limited to, charge-based pairing (electrostatic steering), “knobs-into-holes” steric pairing, SEEDbody pairing, and leucine zipper-based pairing [Ridgway et al (1996) Protein Eng 9:617-621; Merchant et al (1998) Nat Biotech 16:677-681; Davis et al (2010) Protein Eng Des Sel 23:195-202; Gunasekaran et al (2010); 285:19637-19646; Wranik et al (2012) J Biol Chem 287:43331-43339; U.S. Pat. No. 5,932,448; WO 1993/011162; WO 2009/089004, and WO 2011/034605]. As

described herein, these methods may be used to generate ALK4-Fc:ActRIIB-Fc heteromultimer complexes. See, e.g., FIG. 23.

[0173] ALK4:ActRIIB heteromultimers and method of making such heteromultimers have been previously disclosed. See, for example, WO 2016/164497, the entire teachings of which are incorporated by reference herein.

[0174] It is understood that different elements of the fusion proteins (e.g., immunoglobulin Fc fusion proteins) may be arranged in any manner that is consistent with desired functionality. For example, an ActRII polypeptide (or ALK4 polypeptide) domain may be placed C-terminal to a heterologous domain, or alternatively, a heterologous domain may be placed C-terminal to an ActRII polypeptide (or ALK4 polypeptide) domain. The ActRII polypeptide (or ALK4 polypeptide) domain and the heterologous domain need not be adjacent in a fusion protein, and additional domains or amino acid sequences may be included C- or N-terminal to either domain or between the domains.

[0175] For example, an ActRII (or ALK4) receptor fusion protein may comprise an amino acid sequence as set forth in the formula A-B-C. The B portion corresponds to an ActRII (or ALK4) polypeptide domain. The A and C portions may be independently zero, one, or more than one amino acid, and both the A and C portions when present are heterologous to B. The A and/or C portions may be attached to the B portion via a linker sequence. A linker may be rich in glycine (e.g., 2-10, 2-5, 2-4, 2-3 glycine residues) or glycine and proline residues and may, for example, contain a single sequence of threonine-serine and glycines or repeating sequences of threonine-serine and/or glycines, e.g., GGG (SEQ ID NO: 19), GGGG (SEQ ID NO: 20), TGGGG (SEQ ID NO: 21), SGGGG (SEQ ID NO: 22), TGGG (SEQ ID NO: 23), SGGG (SEQ ID NO: 24), or GGGGS (SEQ ID NO: 25) singlets, or repeats. In certain embodiments, an ActRII (or ALK4) fusion protein comprises an amino acid sequence as set forth in the formula A-B-C, wherein A is a leader (signal) sequence, B consists of an ActRII (or ALK4) polypeptide domain, and C is a polypeptide portion that enhances one or more of in vivo stability, in vivo half-life, uptake/administration, tissue localization or distribution, formation of protein complexes, and/or purification. In certain embodiments, an ActRII (or ALK4) fusion protein comprises an amino acid sequence as set forth in the formula A-B-C, wherein A is a TPA leader sequence, B consists of an ActRII (or ALK4) receptor polypeptide domain, and C is an immunoglobulin Fc domain. Preferred fusion proteins comprise the amino acid sequence set forth in any one of SEQ ID NOs: 32, 36, 39, 40, 42, 45, 46, 48, 69, 74, 77, 78, 108, 110, 111, 113, 114, 115, 116, 117, 118, 120, 122, and 124.

[0176] In preferred embodiments, ActRII polypeptides, ALK4 polypeptides, and ALK4:ActRIIB heteromultimers to be used in accordance with the methods described herein are isolated polypeptides. As used herein, an isolated protein or polypeptide is one which has been separated from a component of its natural environment. In some embodiments, a polypeptide of the disclosure is purified to greater than 95%, 96%, 97%, 98%, or 99% purity as determined by, for example, electrophoretic (e.g., SDS-PAGE, isoelectric focusing (IEF), capillary electrophoresis) or chromatographic (e.g., ion exchange or reverse phase HPLC). Methods for assessment of purity are well known in the art [see, e.g., Flatman et al., (2007) J. Chromatogr. B 848:79-87]. In some embodiments, ActRII polypeptides, ALK4 polypep-

tides, and ALK4:ActRIIB heteromultimers to be used in accordance with the methods described herein are recombinant polypeptides.

[0177] ActRII polypeptides, ALK4 polypeptides, and ALK4:ActRIIB heteromultimers of the disclosure can be produced by a variety of art-known techniques. For example, polypeptides of the disclosure can be synthesized using standard protein chemistry techniques such as those described in Bodansky, M. *Principles of Peptide Synthesis*, Springer Verlag, Berlin (1993) and Grant G. A. (ed.), *Synthetic Peptides: A User's Guide*, W. H. Freeman and Company, New York (1992). In addition, automated peptide synthesizers are commercially available (e.g., Advanced ChemTech Model 396; Milligen/Bioscience 9600). Alternatively, the polypeptides of the disclosure, including fragments or variants thereof, may be recombinantly produced using various expression systems [e.g., *E. coli*, Chinese Hamster Ovary (CHO) cells, COS cells, baculovirus] as is well known in the art. In a further embodiment, the modified or unmodified polypeptides of the disclosure may be produced by digestion of recombinantly produced full-length ActRII polypeptides by using, for example, a protease, e.g., trypsin, thermolysin, chymotrypsin, pepsin, or paired basic amino acid converting enzyme (PACE). Computer analysis (using commercially available software, e.g., MacVector, Omega, PCGene, Molecular Simulation, Inc.) can be used to identify proteolytic cleavage sites. Alternatively, such polypeptides may be produced from recombinantly generated full-length ActRII or ALK4 polypeptides using chemical cleavage (e.g., cyanogen bromide, hydroxylamine, etc.).

3. Nucleic Acids Encoding ActRII and ALK4 Polypeptides and Variants Thereof

[0178] In certain embodiments, the present disclosure provides isolated and/or recombinant nucleic acids encoding ActRII and/or ALK4 polypeptides (including fragments, functional variants, and fusion proteins thereof). For example, SEQ ID NO: 7 encodes a naturally occurring human ActRIIB precursor polypeptide (the R64 variant described above), while SEQ ID NO: 8 encodes the processed extracellular domain of ActRIIB (the R64 variant described above). The subject nucleic acids may be single-stranded or double-stranded. Such nucleic acids may be DNA or RNA molecules. These nucleic acids may be used, for example, in methods for making ActRII-based ligand trap polypeptides as described herein.

[0179] As used herein, isolated nucleic acid(s) refers to a nucleic acid molecule that has been separated from a component of its natural environment. An isolated nucleic acid includes a nucleic acid molecule contained in cells that ordinarily contain the nucleic acid molecule, but the nucleic acid molecule is present extrachromosomally or at a chromosomal location that is different from its natural chromosomal location.

[0180] In certain embodiments, nucleic acids encoding ActRII or ALK4 polypeptides of the disclosure are understood to include nucleic acids that are variants of any one of SEQ ID NOs: 7, 8, 12, 13, 37, 43, 49, 70, 71, 72, 73, 75, 76, 80, 81, 82, 83, 84, 102, 103, 106, 107, 109, 112, 119, 121, 123, and 135. Variant nucleotide sequences include sequences that differ by one or more nucleotide substitutions, additions, or deletions including allelic variants, and therefore, will include coding sequence that differ from the nucleotide sequence designated in any one of SEQ ID NOs:

7, 8, 12, 13, 37, 43, 49, 70, 71, 72, 73, 75, 76, 80, 81, 82, 83, 84, 102, 103, 106, 107, 109, 112, 119, 121, 123, and 135.

[0181] In certain embodiments, ActRII or ALK4 polypeptides of the disclosure are encoded by isolated and/or recombinant nucleic acid sequences that are at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94% 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID NOs: 7, 8, 12, 13, 37, 43, 49, 70, 71, 72, 73, 75, 76, 80, 81, 82, 83, 84, 102, 103, 106, 107, 109, 112, 119, 121, 123, and 135. One of ordinary skill in the art will appreciate that nucleic acid sequences that are at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94% 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequences complementary to SEQ ID NOs: 7, 8, 12, 13, 37, 43, 49, 70, 71, 72, 73, 75, 76, 80, 81, 82, 83, 84, 102, 103, 106, 107, 109, 112, 119, 121, 123, and 135, and variants thereof, are also within the scope of the present disclosure. In further embodiments, the nucleic acid sequences of the disclosure can be isolated, recombinant, and/or fused with a heterologous nucleotide sequence, or in a DNA library.

[0182] In other embodiments, nucleic acids of the present disclosure also include nucleotide sequences that hybridize under highly stringent conditions to the nucleotide sequence designated in SEQ ID NOs: 7, 8, 12, 13, 37, 43, 49, 70, 71, 72, 73, 75, 76, 80, 81, 82, 83, 84, 102, 103, 106, 107, 109, 112, 119, 121, 123, and 135, complement sequences of SEQ ID NOs: 7, 8, 12, 13, 37, 43, 49, 70, 71, 72, 73, 75, 76, 80, 81, 82, 83, 84, 102, 103, 106, 107, 109, 112, 119, 121, 123, and 135, or fragments thereof. As discussed above, one of ordinary skill in the art will understand readily that appropriate stringency conditions which promote DNA hybridization can be varied. 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×sodium chloride/sodium citrate (SSC) at about 45° C., followed by a wash of 2.0×SSC at 50° C. For example, the salt concentration in the wash step can be selected from a low stringency of about 2.0×SSC at 50° C. to a high stringency of about 0.2×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×SSC at room temperature followed by a wash at 2×SSC at room temperature.

[0183] Isolated nucleic acids which differ from the nucleic acids as set forth in SEQ ID NOs: 7, 8, 12, 13, 37, 43, 49, 70, 71, 72, 73, 75, 76, 80, 81, 82, 83, 84, 102, 103, 106, 107, 109, 112, 119, 121, 123, and 135 to degeneracy in the genetic code are also within the scope of the disclosure. For example, a number of amino acids are designated by more than one triplet. Codons that specify the same amino acid, or synonyms (for example, CAU and CAC are synonyms for histidine) may result in "silent" mutations which do not affect the amino acid sequence of the protein. However, it is expected that DNA sequence polymorphisms that do lead to changes in the amino acid sequences of the subject proteins will exist among mammalian cells. One skilled in the art will appreciate that these variations in one or more nucleotides (up to about 3-5% of the nucleotides) of the nucleic acids encoding a particular protein may exist among individuals of

a given species due to natural allelic variation. Any and all such nucleotide variations and resulting amino acid polymorphisms are within the scope of this disclosure.

[0184] In certain 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 nucleotide sequences will generally be appropriate to the host cell used for expression. Numerous types of appropriate expression vectors and suitable regulatory sequences are known in the art and can be used in a variety of host cells. Typically, one or more regulatory nucleotide sequences may include, but are not limited to, promoter sequences, leader or signal sequences, ribosomal binding sites, transcriptional start and termination sequences, translational start and termination sequences, and enhancer or activator sequences. Constitutive or inducible promoters as known in the art are contemplated by the disclosure. The promoters may be either naturally occurring promoters, or hybrid promoters that combine elements of more than one promoter. An expression construct may be present in a cell on an episome, such as a plasmid, or the expression construct may be inserted in a chromosome. In some embodiments, the expression vector contains a selectable marker gene to allow the selection of transformed host cells. Selectable marker genes are well known in the art and can vary with the host cell used.

[0185] In certain aspects, the subject nucleic acid disclosed herein is provided in an expression vector comprising a nucleotide sequence encoding an ActRII and/or ALK4 polypeptide and operably linked to at least one regulatory sequence. Regulatory sequences are art-recognized and are selected to direct expression of the ActRII and/or ALK4 polypeptide. Accordingly, the term regulatory sequence includes promoters, enhancers, and other expression control elements. Exemplary regulatory sequences are described in Goeddel; Gene Expression Technology: Methods in Enzymology, Academic Press, San Diego, CA (1990). For instance, any of a wide variety of expression control sequences that control the expression of a DNA sequence when operatively linked to it may be used in these vectors to express DNA sequences encoding an ActRII and/or ALK4 polypeptide. Such useful expression control sequences, include, for example, the early and late promoters of SV40, tet promoter, adenovirus or cytomegalovirus immediate early promoter, RSV promoters, the lac system, the trp system, the TAC or TRC system, T7 promoter whose expression is directed by T7 RNA polymerase, the major operator and promoter regions of phage lambda, the control regions for fd coat protein, the promoter for 3-phosphoglycerate kinase or other glycolytic enzymes, the promoters of acid phosphatase, e.g., Pho5, the promoters of the yeast α -mating factors, the polyhedron promoter of the baculovirus system and other sequences known to control the expression of genes of prokaryotic or eukaryotic cells or their viruses, and various combinations thereof. It should be understood that the design of the expression vector may depend on such factors as the choice of the host cell to be transformed and/or the type of protein desired to be expressed. Moreover, the vector's copy number, the ability to control that copy number and the expression of any other protein encoded by the vector, such as antibiotic markers, should also be considered.

[0186] 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 ActRII and/or ALK4 polypeptide include plasmids and other vectors. For instance, suitable vectors include plasmids of the following types: pBR322-derived plasmids, pEMBL-derived plasmids, pEX-derived plasmids, pBTac-derived plasmids and pUC-derived plasmids for expression in prokaryotic cells, such as *E. coli*.

[0187] 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, e.g., Molecular Cloning A Laboratory Manual, 3rd Ed., ed. by Sambrook, Fritsch and Maniatis (Cold Spring Harbor Laboratory Press, 2001). In some instances, it may be desirable to express the recombinant polypeptides by the use of a baculovirus expression system. Examples of such baculovirus expression systems include pVL-derived vectors (such as pVL1392, pVL1393 and pVL941), pAcUW-derived vectors (such as pAcUW1), and pBlueBac-derived vectors (such as the β -gal containing pBlueBac III).

[0188] In a preferred embodiment, a vector will be designed for production of the subject ActRII and/or ALK4 polypeptides in CHO cells, such as a Pcmv-Script vector (Stratagene, La Jolla, Calif), pcDNA4 vectors (Invitrogen, Carlsbad, Calif.) and pCI-neo vectors (Promega, Madison, Wisc.). As will be apparent, the subject gene constructs can be used to cause expression of the subject ActRII polypeptides in cells propagated in culture, e.g., to produce proteins, including fusion proteins or variant proteins, for purification.

[0189] This disclosure also pertains to a host cell transfected with a recombinant gene including a coding sequence for one or more of the subject ActRII and/or ALK4 polypeptides. The host cell may be any prokaryotic or eukaryotic cell. For example, an ActRII and/or ALK4 polypeptide of the disclosure may be expressed in bacterial cells such as *E. coli*, insect cells (e.g., using a baculovirus expression system), yeast, or mammalian cells [e.g. a Chinese hamster ovary (CHO) cell line]. Other suitable host cells are known to those skilled in the art.

[0190] Accordingly, the present disclosure further pertains to methods of producing the subject ActRII and/or ALK4 polypeptides. For example, a host cell transfected with an expression vector encoding an ActRII and/or ALK4 polypeptide can be cultured under appropriate conditions to

allow expression of the ActRII and/or ALK4 polypeptide to occur. The polypeptide may be secreted and isolated from a mixture of cells and medium containing the polypeptide. Alternatively, the ActRII and/or ALK4 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. The subject polypeptides can be isolated from cell culture medium, host cells, or both, using techniques known in the art for purifying proteins, including ion-exchange chromatography, gel filtration chromatography, ultrafiltration, electrophoresis, immunoaffinity purification with antibodies specific for particular epitopes of the ActRII and/or ALK4 polypeptides, and affinity purification with an agent that binds to a domain fused to the ActRII polypeptide (e.g., a protein A column may be used to purify an ActRII-Fc and/or ALK4-Fc fusion proteins). In some embodiments, the ActRII and/or ALK4 polypeptide is a fusion protein containing a domain which facilitates its purification.

[0191] In some embodiments, purification is achieved by a series of column chromatography steps, including, for example, three or more of the following, in any order: protein A chromatography, Q sepharose chromatography, phenylsepharose chromatography, size exclusion chromatography, and cation exchange chromatography. The purification could be completed with viral filtration and buffer exchange. An ActRII and/or ALK4 protein may be purified to a purity of >90%, >95%, >96%, >98%, or >99% as determined by size exclusion chromatography and >90%, >95%, >96%, >98%, or >99% as determined by SDS PAGE. The target level of purity should be one that is sufficient to achieve desirable results in mammalian systems, particularly non-human primates, rodents (mice), and humans.

[0192] In another embodiment, a fusion gene coding for a purification leader sequence, such as a poly-(His)/enterokinase cleavage site sequence at the N-terminus of the desired portion of the recombinant ActRII and/or ALK4 polypeptide, can allow purification of the expressed fusion protein by affinity chromatography using a Ni²⁺ metal resin. The purification leader sequence can then be subsequently removed by treatment with enterokinase to provide the purified ActRII and/or ALK4 polypeptide. See, e.g., Hochuli et al. (1987) *J. Chromatography* 411:177; and Janknecht et al. (1991) *PNAS USA* 88:8972.

[0193] Techniques for making fusion genes are well known. Essentially, the joining of various DNA fragments coding for different polypeptide sequences is performed in accordance with conventional techniques, employing blunted or stagger-ended termini for ligation, restriction enzyme digestion to provide for appropriate termini, filling-in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and enzymatic ligation. In another embodiment, the fusion gene can be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor primers which give rise to complementary overhangs between two consecutive gene fragments which can subsequently be annealed to generate a chimeric gene sequence. See, e.g., Current Protocols in Molecular Biology, eds. Ausubel et al., John Wiley & Sons: 1992.

4. Antibody Antagonists

[0194] In certain aspects, a GDF/BMP antagonist to be used in accordance with the methods and uses disclosed herein is an antibody (GDF/BMP antagonist antibody), or combination of antibodies. A GDF/BMP antagonist antibody, or combination of antibodies, may bind to, for example, one or more ActRII ligands (e.g., activin, GDF8, GDF11, BMP6, BMP15, BMP10, and/or GDF3), ActRII receptor (ActRIIA and/or ActRIIB), type I receptor (ALK4, ALK5, and/or ALK7) and/or co-receptor. As described herein, GDF/BMP antagonist antibodies may be used, alone or in combination with one or more supportive therapies or active agents, to treat, prevent, or reduce the progression rate and/or severity of pulmonary hypertension (PH), particularly treating, preventing or reducing the progression rate and/or severity of one or more PH-associated complications.

[0195] In certain aspects, a GDF/BMP antagonist antibody, or combination of antibodies, is an antibody that inhibits at least activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC, activin BC, activin AE, and/or activin BE). Therefore, in some embodiments, a GDF/BMP antagonist antibody, or combination of antibodies, binds to at least activin. As used herein, an activin antibody (or anti-activin antibody) generally refers to an antibody that binds to activin with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting activin. In certain embodiments, the extent of binding of an activin antibody to an unrelated, non-activin protein is less than about 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or less than about 1% of the binding of the antibody to activin as measured, for example, by a radioimmunoassay (RIA), Biacore, or other protein interaction or binding affinity assay. In certain embodiments, an activin antibody binds to an epitope of activin that is conserved among activin from different species. In certain preferred embodiments, an anti-activin antibody binds to human activin. In some embodiments, an activin antibody may inhibit activin from binding to a type I and/or type II receptor (e.g., ActRIIA, ActRIIB, ALK4, ALK5, and/or ALK7) and thus inhibit activin-mediated signaling (e.g., Smad signaling). In some embodiments, an activin antibody may inhibit activin from binding to an ActRII co-receptor and thus inhibit activin-mediated signaling (e.g., Smad signaling). It should be noted that activin A has similar sequence homology to activin B and therefore antibodies that bind to activin A, in some instances, may also bind to and/or inhibit activin B, which also applies to anti-activin B antibodies. In some embodiments, the disclosure relates to a multispecific antibody (e.g., bi-specific antibody), and uses thereof, that binds to activin and further binds to, for example, one or more additional GDF/BMP ligands [e.g., GDF11, GDF8, GDF3, BMP15, BMP10, and BMP6], one or more type I receptor and/or type II receptors (e.g., ActRIIA, ActRIIB, ALK4, ALK5, and/or ALK7), and/or one or more co-receptors. In some embodiments, a multispecific antibody that binds to activin does not bind or does not substantially bind to BMP9 (e.g., binds to BMP9 with a K_D of greater than 1×10⁻⁷ M or has relatively modest binding, e.g., about 1×10⁻⁸ M or about 1×10⁻⁹ M). In some embodiments, a multispecific antibody that binds to activin does not bind or does not substantially bind to activin A (e.g., binds to activin A with a K_D of greater than 1×10⁻⁷ M or has relatively modest binding, e.g., about 1×10⁻⁸ M or about 1×10⁻⁹ M). In some embodiments, the disclosure relates to combinations of antibodies, and uses

thereof, wherein the combination of antibodies comprises an activin antibody and one or more additional antibodies that bind to, for example, one or more additional GDF/BMP superfamily ligands [e.g., GDF8, GDF11, GDF3, BMP6, and BMP15], one or more type I receptor and/or type II receptors (e.g., ActRIIA, ActRIIB, ALK4, ALK5, and/or ALK7), and/or one or more co-receptors. In some embodiments, a combination of antibodies that comprises an activin antibody does not comprise a BMP9 antibody. In some embodiments, a combination of antibodies that comprises an activin antibody does not comprise an activin A antibody.

[0196] In certain aspects, a GDF/BMP antagonist antibody, or combination of antibodies, is an antibody that inhibits at least activin B. Therefore, in some embodiments, a GDF/BMP antagonist antibody, or combination of antibodies, binds to at least activin B. As used herein, an activin B antibody (or anti-activin B antibody) generally refers to an antibody that binds to activin B with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting activin B. In certain embodiments, the extent of binding of an activin B antibody to an unrelated, non-activin B protein is less than about 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or less than about 1% of the binding of the antibody to activin as measured, for example, by a radioimmunoassay (RIA), Biacore, or other protein interaction or binding affinity assay. In certain embodiments, an activin B antibody binds to an epitope of activin B that is conserved among activin B from different species. In certain preferred embodiments, an anti-activin B antibody binds to human activin B. In some embodiments, an activin B antibody may inhibit activin B from binding to a type I and/or type II receptor (e.g., ActRIIA, ActRIIB, ALK4, ALK5, and/or ALK7) and thus inhibit activin B-mediated signaling (e.g., Smad signaling). In some embodiments, an activin B antibody may inhibit activin B from binding to a co-receptor and thus inhibit activin B-mediated signaling (e.g., Smad signaling). It should be noted that activin B has similar sequence homology to activin A and therefore antibodies that bind to activin B, in some instances, may also bind to and/or inhibit activin A. In some embodiments, the disclosure relates to a multispecific antibody (e.g., bi-specific antibody), and uses thereof, that binds to activin B and further binds to, for example, one or more additional GDF/BMP ligands [e.g., GDF11, GDF8, GDF3, BMP15, BMP10, and BMP6], one or more type I receptor and/or type II receptors (e.g., ActRIIA, ActRIIB, ALK4, ALK5, and/or ALK7), and/or one or more co-receptors. In some embodiments, a multispecific antibody that binds to activin B does not bind or does not substantially bind to BMP9 (e.g., binds to BMP9 with a K_D of greater than 1×10^{-7} M or has relatively modest binding, e.g., about 1×10^{-8} M or about 1×10^{-9} M). In some embodiments, a multispecific antibody that binds to activin B does not bind or does not substantially bind to activin A (e.g., binds to activin A with a K_D of greater than 1×10^{-7} M or has relatively modest binding, e.g., about 1×10^{-8} M or about 1×10^{-9} M). In some embodiments, the disclosure relates to combinations of antibodies, and uses thereof, wherein the combination of antibodies comprises an activin B antibody and one or more additional antibodies that bind to, for example, one or more additional GDF/BMP ligands [e.g., GDF8, GDF11, GDF3, BMP6, BMP10, and BMP15], one or more type I receptor and/or type II receptors (e.g., ActRIIA, ActRIIB, ALK4, ALK5, and/or ALK7), and/or one or more co-receptors. In some embodiments,

combination of antibodies that comprises an activin B antibody does not comprise a BMP9 antibody. In some embodiments, a combination of antibodies that comprises an activin B antibody does not comprise an activin A antibody.

[0197] In certain aspects, a GDF/BMP antagonist antibody, or combination of antibodies, is an antibody that inhibits at least GDF8. Therefore, in some embodiments, a GDF/BMP antagonist antibody, or combination of antibodies, binds to at least GDF8. As used herein, a GDF8 antibody (or anti-GDF8 antibody) generally refers to an antibody that binds to GDF8 with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting GDF8. In certain embodiments, the extent of binding of a GDF8 antibody to an unrelated, non-GDF8 protein is less than about 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or less than about 1% of the binding of the antibody to GDF8 as measured, for example, by a radioimmunoassay (RIA), Biacore, or other protein interaction or binding affinity assay. In certain embodiments, a GDF8 antibody binds to an epitope of GDF8 that is conserved among GDF8 from different species. In certain preferred embodiments, an anti-GDF8 antibody binds to human GDF8. In some embodiments, a GDF8 antibody may inhibit GDF8 from binding to a type I and/or type II receptor (e.g., ActRIIA, ActRIIB, ALK4, ALK5, and/or ALK7) and thus inhibit GDF8-mediated signaling (e.g., Smad signaling). In some embodiments, a GDF8 antibody may inhibit GDF8 from binding to a co-receptor and thus inhibit GDF8-mediated signaling (e.g., Smad signaling). It should be noted that GDF8 has high sequence homology to GDF11 and therefore antibodies that bind to GDF8, in some instances, may also bind to and/or inhibit GDF11. In some embodiments, the disclosure relates to a multispecific antibody (e.g., bi-specific antibody), and uses thereof, that binds to GDF8 and further binds to, for example, one or more additional GDF/BMP ligands [e.g., activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC, activin BC, activin AE, activin BE), GDF11, GDF3, BMP15, BMP10, and BMP6], one or more type I receptor and/or type II receptors (e.g., ActRIIA, ActRIIB, ALK4, ALK5, and/or ALK7), and/or one or more co-receptors. In some embodiments, a multispecific antibody that binds to GDF8 does not bind or does not substantially bind to BMP9 (e.g., binds to BMP9 with a K_D of greater than 1×10^{-7} M or has relatively modest binding, e.g., about 1×10^{-8} M or about 1×10^{-9} M). In some embodiments, a multispecific antibody that binds to GDF8 does not bind or does not substantially bind to activin A (e.g., binds to activin A with a K_D of greater than 1×10^{-7} M or has relatively modest binding, e.g., about 1×10^{-8} M or about 1×10^{-9} M). In some embodiments, the disclosure relates to combinations of antibodies, and uses thereof, wherein the combination of antibodies comprises a GDF8 antibody and one or more additional antibodies that bind to, for example, one or more additional GDF/BMP ligands [e.g., activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC, activin BC, activin AE, activin BE), GDF11, GDF3, BMP6, BMP10, and BMP15], one or more type I receptor and/or type II receptors (e.g., ActRIIA, ActRIIB, ALK4, ALK5, and/or ALK7), and/or one or more co-receptors. In some embodiments, a combination of antibodies that comprises a GDF8 antibody does not comprise a BMP9 antibody. In some embodiments, a combination of antibodies that comprises a GDF8 antibody does not comprise an activin A antibody.

[0198] In certain aspects, a GDF/BMP antagonist antibody, or combination of antibodies, is an antibody that inhibits at least GDF11. Therefore, in some embodiments, a GDF/BMP antagonist antibody, or combination of antibodies, binds to at least GDF11. As used herein, a GDF11 antibody (or anti-GDF11 antibody) generally refers to an antibody that binds to GDF11 with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting GDF11. In certain embodiments, the extent of binding of a GDF11 antibody to an unrelated, non-GDF11 protein is less than about 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or less than about 1% of the binding of the antibody to GDF11 as measured, for example, by a radioimmunoassay (RIA), Biacore, or other protein interaction or binding affinity assay. In certain embodiments, a GDF11 antibody binds to an epitope of GDF11 that is conserved among GDF11 from different species. In certain preferred embodiments, an anti-GDF11 antibody binds to human GDF11. In some embodiments, a GDF11 antibody may inhibit GDF11 from binding to a type I and/or type II receptor (e.g., ActRIIA, ActRIIB, ALK4, ALK5, and/or ALK7) and thus inhibit GDF11-mediated signaling (e.g., Smad signaling). In some embodiments, a GDF11 antibody may inhibit GDF11 from binding to a co-receptor and thus inhibit GDF11-mediated signaling (e.g., Smad signaling). It should be noted that GDF11 has high sequence homology to GDF8 and therefore antibodies that bind to GDF11, in some instances, may also bind to and/or inhibit GDF8. In some embodiments, the disclosure relates to a multispecific antibody (e.g., bi-specific antibody), and uses thereof, that binds to GDF11 and further binds to, for example, one or more additional GDF/BMP ligands [e.g., activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC, activin BC, activin AE, activin BE), GDF8, GDF3, BMP15, BMP10, and BMP6], one or more type I receptor and/or type II receptors (e.g., ActRIIA, ActRIIB, ALK4, ALK5, and/or ALK7), and/or one or more co-receptors. In some embodiments, a multispecific antibody that binds to GDF11 does not bind or does not substantially bind to BMP9 (e.g., binds to BMP9 with a K_D of greater than 1×10^{-7} M or has relatively modest binding, e.g., about 1×10^{-8} M or about 1×10^{-9} M). In some embodiments, a multispecific antibody that binds to GDF11 does not bind or does not substantially bind to activin A (e.g., binds to activin A with a K_D of greater than 1×10^{-7} M or has relatively modest binding, e.g., about 1×10^{-8} M or about 1×10^{-9} M). In some embodiments, the disclosure relates to combinations of antibodies, and uses thereof, wherein the combination of antibodies comprises a GDF11 antibody and one or more additional antibodies that bind to, for example, one or more additional GDF/BMP ligands [e.g., activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC, activin BC, activin AE, activin BE), GDF8, GDF3, BMP6, BMP10, and BMP15], one or more type I receptor and/or type II receptors (e.g., ActRIIA, ActRIIB, ALK4, ALK5, and/or ALK7), and/or one or more co-receptors. In some embodiments, a combination of antibodies that comprises a GDF11 antibody does not comprise a BMP9 antibody. In some embodiments, a combination of antibodies that comprises a GDF11 antibody does not comprise an activin A antibody.

[0199] In certain aspects, a GDF/BMP antagonist antibody, or combination of antibodies, is an antibody that inhibits at least BMP6. Therefore, in some embodiments, a GDF/BMP antagonist antibody, or combination of antibod-

ies, binds to at least BMP6. As used herein, a BMP6 antibody (or anti-BMP6 antibody) generally refers to an antibody that can bind to BMP6 with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting BMP6. In certain embodiments, the extent of binding of a BMP6 antibody to an unrelated, non-BMP6 protein is less than about 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or less than about 1% of the binding of the antibody to BMP6 as measured, for example, by a radioimmunoassay (RIA), Biacore, or other protein interaction or binding affinity assay. In certain embodiments, a BMP6 antibody binds to an epitope of BMP6 that is conserved among BMP6 from different species. In certain preferred embodiments, an anti-BMP6 antibody binds to human BMP6. In some embodiments, a BMP6 antibody may inhibit BMP6 from binding to a type I and/or type II receptor (e.g., ActRIIA, ActRIIB, ALK4, ALK5, and/or ALK7) and thus inhibit BMP6-mediated signaling (e.g., Smad signaling). In some embodiments, a BMP6 antibody may inhibit BMP6 from binding to a co-receptor and thus inhibit BMP6-mediated signaling (e.g., Smad signaling). In some embodiments, the disclosure relates to a multispecific antibody (e.g., bi-specific antibody), and uses thereof, that binds to BMP6 and further binds to, for example, one or more additional GDF/BMP ligands [e.g., activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC, activin BC, activin AE, activin BE), GDF8, GDF3, BMP15, BMP10, and GDF11], one or more type I receptor and/or type II receptors (e.g., ActRIIA, ActRIIB, ALK4, ALK5, and/or ALK7), and/or one or more co-receptors. In some embodiments, a multispecific antibody that binds to BMP6 does not bind or does not substantially bind to BMP9 (e.g., binds to BMP9 with a K_D of greater than 1×10^{-7} M or has relatively modest binding, e.g., about 1×10^{-8} M or about 1×10^{-9} M). In some embodiments, a multispecific antibody that binds to BMP6 does not bind or does not substantially bind to activin A (e.g., binds to activin A with a K_D of greater than 1×10^{-7} M or has relatively modest binding, e.g., about 1×10^{-8} M or about 1×10^{-9} M). In some embodiments, the disclosure relates to combinations of antibodies, and uses thereof, wherein the combination of antibodies comprises a BMP6 antibody and one or more additional antibodies that bind to, for example, one or more additional GDF/BMP ligands [e.g., activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC, activin BC, activin AE, activin BE), GDF8, GDF11, GDF3, BMP10, and BMP15], one or more type I receptor and/or type II receptors (e.g., ActRIIA, ActRIIB, ALK4, ALK5, and/or ALK7), and/or one or more co-receptors. In some embodiments, a combination of antibodies that comprises a BMP6 antibody does not comprise a BMP9 antibody. In some embodiments, a combination of antibodies that comprises a BMP6 antibody does not comprise an activin A antibody.

[0200] In certain aspects, a GDF/BMP antagonist antibody, or combination of antibodies, is an antibody that inhibits at least GDF3. Therefore, in some embodiments, a GDF/BMP antagonist antibody, or combination of antibodies, binds to at least GDF3. As used herein, a GDF3 antibody (or anti-GDF3 antibody) generally refers to an antibody that can bind to GDF3 with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting GDF3. In certain embodiments, the extent of binding of a GDF3 antibody to an unrelated, non-GDF3 protein is less than about 10%, 9%, 8%, 7%, 6%, 5%, 4%,

3%, 2%, or less than about 1% of the binding of the antibody to GDF3 as measured, for example, by a radioimmunoassay (RIA), Biacore, or other protein interaction or binding affinity assay. In certain embodiments, a GDF3 antibody binds to an epitope of GDF3 that is conserved among GDF3 from different species. In certain preferred embodiments, an anti-GDF3 antibody binds to human GDF3. In some embodiments, a GDF3 antibody may inhibit GDF3 from binding to a type I and/or type II receptor (e.g., ActRIIA, ActRIIB, ALK4, ALK5, and/or ALK7) and thus inhibit GDF3-mediated signaling (e.g., Smad signaling). In some embodiments, a GDF3 antibody may inhibit GDF3 from binding to a co-receptor and thus inhibit GDF3-mediated signaling (e.g., Smad signaling). In some embodiments, the disclosure relates to a multispecific antibody (e.g., bi-specific antibody), and uses thereof, that binds to GDF3 and further binds to, for example, one or more additional GDF/BMP ligands [e.g., activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC, activin BC, activin AE and activin BE), GDF8, GDF11, GDF3, BMP10, and BMP6], one or more type I receptor and/or type II receptors (e.g., ActRIIA, ActRIIB, ALK4, ALK5, and/or ALK7), and/or one or more co-receptors. In some embodiments, a multispecific antibody that binds to GDF3 does not bind or does not substantially bind to BMP9 (e.g., binds to BMP9 with a K_D of greater than 1×10^{-7} M or has relatively modest binding, e.g., about 1×10^{-8} M or about 1×10^{-9} M). In some embodiments, a multispecific antibody that binds to GDF3 does not bind or does not substantially bind to activin A (e.g., binds to activin A with a K_D of greater than 1×10^{-7} M or has relatively modest binding, e.g., about 1×10^{-8} M or about 1×10^{-9} M). In some embodiments, the disclosure relates to combinations of antibodies, and uses thereof, wherein the combination of antibodies comprises a GDF3 antibody and one or more additional antibodies that bind to, for example, one or more additional GDF/BMP ligands [e.g., activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC, activin BC, activin AE, activin BE), GDF8, GDF11, BMP6, BMP10, and BMP15], one or more type I receptor and/or type II receptors (e.g., ActRIIA, ActRIIB, ALK4, ALK5, and/or ALK7), and/or one or more co-receptors. In some embodiments, a combination of antibodies that comprises a GDF3 antibody does not comprise an activin A antibody.

[0201] In certain aspects, a GDF/BMP antagonist antibody, or combination of antibodies, is an antibody that inhibits at least BMP15. Therefore, in some embodiments, a GDF/BMP antagonist antibody, or combination of antibodies, binds to at least BMP15. As used herein, a BMP15 antibody (or anti-BMP15 antibody) generally refers to an antibody that can bind to BMP15 with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting BMP15. In certain embodiments, the extent of binding of a BMP15 antibody to an unrelated, non-BMP15 protein is less than about 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or less than about 1% of the binding of the antibody to BMP15 as measured, for example, by a radioimmunoassay (RIA), Biacore, or other protein interaction or binding affinity assay. In certain embodiments, a BMP15 antibody binds to an epitope of BMP15 that is conserved among BMP15 from different species. In certain preferred embodiments, an anti-BMP15 antibody binds to

human BMP15. In some embodiments, a BMP15 antibody may inhibit BMP15 from binding to a type I and/or type II receptor (e.g., ActRIIA, ActRIIB, ALK4, ALK5, and/or ALK7) and thus inhibit BMP15-mediated signaling (e.g., Smad signaling). In some embodiments, a BMP15 antibody may inhibit BMP15 from binding to a co-receptor and thus inhibit BMP15-mediated signaling (e.g., Smad signaling). In some embodiments, the disclosure relates to a multispecific antibody (e.g., bi-specific antibody), and uses thereof, that binds to BMP15 and further binds to, for example, one or more additional GDF/BMP ligands [e.g., activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC, activin BC, activin AE and activin BE), GDF8, GDF11, GDF3, BMP10, and BMP6], one or more type I receptor and/or type II receptors (e.g., ActRIIA, ActRIIB, ALK4, ALK5, and/or ALK7), and/or one or more co-receptors. In some embodiments, a multispecific antibody that binds to BMP15 does not bind or does not substantially bind to BMP9 (e.g., binds to BMP9 with a K_D of greater than 1×10^{-7} M or has relatively modest binding, e.g., about 1×10^{-8} M or about 1×10^{-9} M). In some embodiments, a multispecific antibody that binds to BMP15 does not bind or does not substantially bind to activin A (e.g., binds to activin A with a K_D of greater than 1×10^{-7} M or has relatively modest binding, e.g., about 1×10^{-8} M or about 1×10^{-9} M). In some embodiments, the disclosure relates to combinations of antibodies, and uses thereof, wherein the combination of antibodies comprises a BMP15 antibody and one or more additional antibodies that bind to, for example, one or more additional GDF/BMP ligands [e.g., activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC, activin BC, activin AE and activin BE), GDF8, GDF3 BMP6, BMP10, and GDF11], one or more type I receptor and/or type II receptors (e.g., ActRIIA, ActRIIB, ALK4, ALK5, and/or ALK7), and/or one or more co-receptors. In some embodiments, a combination of antibodies that comprises a BMP15 antibody does not comprise a BMP9 antibody. In some embodiments, a combination of antibodies that comprises a BMP15 antibody does not comprise an activin A antibody.

[0202] In certain aspects, a GDF/BMP antagonist antibody, or combination of antibodies, is an antibody that inhibits at least BMP10. Therefore, in some embodiments, a GDF/BMP antagonist antibody, or combination of antibodies, binds to at least BMP10. As used herein, a BMP10 antibody (or anti-BMP10 antibody) generally refers to an antibody that can bind to BMP10 with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting BMP10. In certain embodiments, the extent of binding of a BMP10 antibody to an unrelated, non-BMP10 protein is less than about 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or less than about 1% of the binding of the antibody to BMP10 as measured, for example, by a radioimmunoassay (RIA), Biacore, or other protein interaction or binding affinity assay. In certain embodiments, a BMP10 antibody binds to an epitope of BMP10 that is conserved among BMP10 from different species. In certain preferred embodiments, an anti-BMP10 antibody binds to human BMP10. In some embodiments, a BMP10 antibody may inhibit BMP10 from binding to a type I and/or type II receptor (e.g., ActRIIA, ActRIIB, ALK4, ALK5, and/or ALK7) and thus inhibit BMP10-mediated signaling (e.g., Smad signaling). In some embodiments, a BMP10 antibody may inhibit BMP10 from binding to a co-receptor and thus

inhibit BMP10-mediated signaling (e.g., Smad signaling). In some embodiments, the disclosure relates to a multispecific antibody (e.g., bi-specific antibody), and uses thereof, that binds to BMP10 and further binds to, for example, one or more additional GDF/BMP ligands [e.g., activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC, activin BC, activin AE and activin BE), GDF8, GDF11, GDF3, and BMP6], one or more type I receptor and/or type II receptors (e.g., ActRIIA, ActRIIB, ALK4, ALK5, and/or ALK7), and/or one or more co-receptors. In some embodiments, a multispecific antibody that binds to BMP10 does not bind or does not substantially bind to BMP9 (e.g., binds to BMP9 with a K_D of greater than 1×10^{-7} M or has relatively modest binding, e.g., about 1×10^{-8} M or about 1×10^{-9} M). In some embodiments, a multispecific antibody that binds to BMP10 does not bind or does not substantially bind to activin A (e.g., binds to activin A with a K_D of greater than 1×10^{-7} M or has relatively modest binding, e.g., about 1×10^{-8} M or about 1×10^{-9} M). In some embodiments, the disclosure relates to combinations of antibodies, and uses thereof, wherein the combination of antibodies comprises a BMP10 antibody and one or more additional antibodies that bind to, for example, one or more additional GDF/BMP ligands [e.g., activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC, activin BC, activin AE and activin BE), GDF8, GDF3, BMP6, BMP10, and GDF11], one or more type I receptor and/or type II receptors (e.g., ActRIIA, ActRIIB, ALK4, ALK5, and/or ALK7), and/or one or more co-receptors. In some embodiments, a combination of antibodies that comprises a BMP10 antibody does not comprise a BMP9 antibody. In some embodiments, a combination of antibodies that comprises a BMP10 antibody does not comprise an activin A antibody.

[0203] In certain aspects, a GDF/BMP antagonist antibody, or combination of antibodies, is an antibody that inhibits at least ActRIIB. Therefore, in some embodiments, a GDF/BMP antagonist antibody, or combination of antibodies, binds to at least ActRIIB. As used herein, an ActRIIB antibody (anti-ActRIIB antibody) generally refers to an antibody that binds to ActRIIB with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting ActRIIB. In certain embodiments, the extent of binding of an anti-ActRIIB antibody to an unrelated, non-ActRIIB protein is less than about 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or less than about 1% of the binding of the antibody to ActRIIB as measured, for example, by a radioimmunoassay (RIA), Biacore, or other protein-protein interaction or binding affinity assay. In certain embodiments, an anti-ActRIIB antibody binds to an epitope of ActRIIB that is conserved among ActRIIB from different species. In certain preferred embodiments, an anti-ActRIIB antibody binds to human ActRIIB. In some embodiments, an anti-ActRIIB antibody may inhibit one or more GDF/BMP ligands [e.g., GDF8, activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC, activin BC, activin AE and activin BE) GDF11, BMP6, GDF3, BMP10, and BMP15] from binding to ActRIIB. In some embodiments, an anti-ActRIIB antibody is a multi-specific antibody (e.g., bi-specific antibody) that binds to ActRIIB and one or more GDF/BMP ligands [e.g., GDF11, GDF8, activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC) GDF3, BMP6, and BMP10], type I receptor (e.g., ALK4, ALK5, and/or ALK7), co-receptor, and/or an additional type II receptor (e.g., ActRIIA). In some

embodiments, the disclosure relates to combinations of antibodies, and uses thereof, wherein the combination of antibodies comprises an anti-ActRIIB antibody and one or more additional antibodies that bind to, for example, one or more GDF/BMP ligands [e.g., GDF11, GDF8, activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC, activin BC, activin AE and activin BE) BMP6, GDF3, and BMP10], co-receptors, type I receptors (e.g., ALK4, ALK5, and/or ALK7), and/or additional type II receptors (e.g., ActRIIA). It should be noted that ActRIIB has sequence similarity to ActRIIA and therefore antibodies that bind to ActRIIB, in some instances, may also bind to and/or inhibit ActRIIA.

[0204] In certain aspects, a GDF/BMP antagonist antibody, or combination of antibodies, is an antibody that inhibits at least ActRIIA. Therefore, in some embodiments, a GDF/BMP antagonist antibody, or combination of antibodies, binds to at least ActRIIA. As used herein, an ActRIIA antibody (anti-ActRIIA antibody) generally refers to an antibody that binds to ActRIIA with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting ActRIIA. In certain embodiments, the extent of binding of an anti-ActRIIA antibody to an unrelated, non-ActRIIA protein is less than about 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or less than about 1% of the binding of the antibody to ActRIIA as measured, for example, by a radioimmunoassay (RIA), Biacore, or other protein-protein interaction or binding affinity assay. In certain embodiments, an anti-ActRIIA antibody binds to an epitope of ActRIIA that is conserved among ActRIIA from different species. In certain preferred embodiments, an anti-ActRIIA antibody binds to human ActRIIA. In some embodiments, an anti-ActRIIA antibody may inhibit one or more GDF/BMP ligands [e.g., GDF8, activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC, activin BC, activin AE and activin BE) GDF11, BMP6, GDF3, BMP10, and BMP15] from binding to ActRIIA. In some embodiments, an anti-ActRIIA antibody is a multi-specific antibody (e.g., bi-specific antibody) that binds to ActRIIA and one or more GDF/BMP ligands [e.g., GDF11, GDF8, activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC) GDF3, BMP6, and BMP10], type I receptor (e.g., ALK4, ALK5, and/or ALK7), co-receptor, and/or an additional type II receptor (e.g., ActRIIB). In some embodiments, the disclosure relates to combinations of antibodies, and uses thereof, wherein the combination of antibodies comprises an anti-ActRIIA antibody and one or more additional antibodies that bind to, for example, one or more GDF/BMP ligands [e.g., GDF11, GDF8, activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC, activin BC, activin AE and activin BE) BMP6, and BMP10], co-receptors, type I receptors (e.g., ALK4, ALK5, and/or ALK7), and/or additional type II receptors (e.g., ActRIIB). It should be noted that ActRIIA has sequence similarity to ActRIIB and therefore antibodies that bind to ActRIIA, in some instances, may also bind to and/or inhibit ActRIIB.

[0205] In certain aspects, a GDF/BMP antagonist antibody, or combination of antibodies, is an antibody that inhibits at least ALK4. Therefore, in some embodiments, a GDF/BMP antagonist antibody, or combination of antibodies, binds to at least ALK4. As used herein, an ALK4 antibody (anti-ALK4 antibody) generally refers to an antibody that binds to ALK4 with sufficient affinity such that the

antibody is useful as a diagnostic and/or therapeutic agent in targeting ALK4. In certain embodiments, the extent of binding of an anti-ALK4 antibody to an unrelated, non-ALK4 protein is less than about 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or less than about 1% of the binding of the antibody to ALK4 as measured, for example, by a radioimmunoassay (RIA), Biacore, or other protein-protein interaction or binding affinity assay. In certain embodiments, an anti-ALK4 antibody binds to an epitope of ALK4 that is conserved among ALK4 from different species. In certain preferred embodiments, an anti-ALK4 antibody binds to human ALK4. In some embodiments, an anti-ALK4 antibody may inhibit one or more GDF/BMP ligands [e.g., GDF8, activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC, activin BC, activin AE and activin BE) GDF11, BMP6, GDF3, BMP10, and BMP15] from binding to ALK4. In some embodiments, an anti-ALK4 antibody is a multispecific antibody (e.g., bi-specific antibody) that binds to ALK4 and one or more GDF/BMP ligands [e.g., GDF11, GDF8, activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC) GDF3, BMP6, and BMP10], type II receptor (e.g., ActRIIA and/or ActRIIB), co-receptor, and/or an additional type I receptor (e.g., ALK5 and/or ALK7). In some embodiments, the disclosure relates to combinations of antibodies, and uses thereof, wherein the combination of antibodies comprises an anti-ALK4 antibody and one or more additional antibodies that bind to, for example, one or more GDF/BMP ligands [e.g., GDF11, GDF8, activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC, activin BC, activin AE and activin BE) BMP6, and BMP10], co-receptors, type II receptors (e.g., ActRIIA and/or ActRIIB), and/or additional type I receptors (e.g., ALK5 and/or ALK7).

[0206] In certain aspects, a GDF/BMP antagonist antibody, or combination of antibodies, is an antibody that inhibits at least ALK5. Therefore, in some embodiments, a GDF/BMP antagonist antibody, or combination of antibodies, binds to at least ALK5. As used herein, an ALK5 antibody (anti-ALK5 antibody) generally refers to an antibody that binds to ALK5 with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting ALK5. In certain embodiments, the extent of binding of an anti-ALK5 antibody to an unrelated, non-ALK5 protein is less than about 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or less than about 1% of the binding of the antibody to ALK5 as measured, for example, by a radioimmunoassay (RIA), Biacore, or other protein-protein interaction or binding affinity assay. In certain embodiments, an anti-ALK5 antibody binds to an epitope of ALK5 that is conserved among ALK5 from different species. In certain preferred embodiments, an anti-ALK5 antibody binds to human ALK5. In some embodiments, an anti-ALK5 antibody may inhibit one or more GDF/BMP ligands [e.g., GDF8, activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC, activin BC, activin AE and activin BE) GDF11, BMP6, GDF3, BMP10, and BMP15] from binding to ALK5. In some embodiments, an anti-ALK5 antibody is a multispecific antibody (e.g., bi-specific antibody) that binds to ALK5 and one or more GDF/BMP ligands [e.g., GDF11, GDF8, activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC) GDF3, BMP6, and BMP10], type II receptor (e.g., ActRIIA and/or ActRIIB), co-receptor, and/or an additional type I receptor (e.g., ALK4 and/or ALK5). In some embodiments, the disclosure relates to combinations of antibodies, and uses thereof, wherein the combination of antibodies comprises an anti-ALK7 antibody and one or more additional antibodies that bind to, for example, one or more GDF/BMP ligands [e.g., GDF11, GDF8, activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC, activin BC, activin AE and activin BE) BMP6, and BMP10], co-receptors, type II receptors (e.g., ActRIIA and/or ActRIIB), and/or additional type I receptors (e.g., ALK4 and/or ALK7).

disclosure relates to combinations of antibodies, and uses thereof, wherein the combination of antibodies comprises an anti-ALK5 antibody and one or more additional antibodies that bind to, for example, one or more GDF/BMP ligands [e.g., GDF11, GDF8, activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC, activin BC, activin AE and activin BE) BMP6, and BMP10], co-receptors, type II receptors (e.g., ActRIIA and/or ActRIIB), and/or additional type I receptors (e.g., ALK4 and/or ALK7).

[0207] In certain aspects, a GDF/BMP antagonist antibody, or combination of antibodies, is an antibody that inhibits at least ALK7. Therefore, in some embodiments, a GDF/BMP antagonist antibody, or combination of antibodies, binds to at least ALK7. As used herein, an ALK7 antibody (anti-ALK7 antibody) generally refers to an antibody that binds to ALK7 with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting ALK7. In certain embodiments, the extent of binding of an anti-ALK7 antibody to an unrelated, non-ALK7 protein is less than about 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or less than about 1% of the binding of the antibody to ALK7 as measured, for example, by a radioimmunoassay (RIA), Biacore, or other protein-protein interaction or binding affinity assay. In certain embodiments, an anti-ALK7 antibody binds to an epitope of ALK7 that is conserved among ALK7 from different species. In certain preferred embodiments, an anti-ALK7 antibody binds to human ALK7. In some embodiments, an anti-ALK7 antibody may inhibit one or more GDF/BMP ligands [e.g., GDF8, activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC, activin BC, activin AE and activin BE) GDF11, BMP6, GDF3, BMP10, and BMP15] from binding to ALK7. In some embodiments, an anti-ALK7 antibody is a multispecific antibody (e.g., bi-specific antibody) that binds to ALK7 and one or more GDF/BMP ligands [e.g., GDF11, GDF8, activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC) GDF3, BMP6, and BMP10], type II receptor (e.g., ActRIIA and/or ActRIIB), co-receptor, and/or an additional type I receptor (e.g., ALK4 and/or ALK5). In some embodiments, the disclosure relates to combinations of antibodies, and uses thereof, wherein the combination of antibodies comprises an anti-ALK7 antibody and one or more additional antibodies that bind to, for example, one or more GDF/BMP ligands [e.g., GDF11, GDF8, activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC, activin BC, activin AE and activin BE) BMP6, and BMP10], co-receptors, type II receptors (e.g., ActRIIA and/or ActRIIB), and/or additional type I receptors (e.g., ALK4 and/or ALK5).

[0208] The term antibody is used herein in the broadest sense and encompasses various antibody structures, including but not limited to monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies), and antibody fragments so long as they exhibit the desired antigen-binding activity. An antibody fragment refers to a molecule other than an intact antibody that comprises a portion of an intact antibody that binds the antigen to which the intact antibody binds. Examples of antibody fragments include, but are not limited to, Fv, Fab, Fab', Fab'-SH, F(ab')₂; diabodies; linear antibodies; single-chain antibody molecules (e.g., scFv); and multispecific antibodies formed from antibody fragments [see, e.g., Hudson et al. (2003) Nat. Med. 9:129-134; Plückthun, in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosen-

burg and Moore eds., (Springer-Verlag, New York), pp. 269-315 (1994); WO 93/16185; and U.S. Pat. Nos. 5,571,894; 5,587,458; and 5,869,046]. Diabodies are antibody fragments with two antigen-binding sites that may be bivalent or bispecific [see, e.g., EP 404,097; WO 1993/01161; Hudson et al. (2003) Nat. Med. 9:129-134 (2003); and Hollinger et al. (1993) Proc. Natl. Acad. Sci. USA 90: 6444-6448]. Triabodies and tetrabodies are also described in Hudson et al. (2003) Nat. Med. 9:129-134. Single-domain antibodies are antibody fragments comprising all or a portion of the heavy-chain variable domain or all or a portion of the light-chain variable domain of an antibody. In certain embodiments, a single-domain antibody is a human single-domain antibody [see, e.g., U.S. Pat. No. 6,248,516]. Antibodies disclosed herein may be polyclonal antibodies or monoclonal antibodies. In certain embodiments, the antibodies of the present disclosure comprise a label attached thereto and able to be detected (e.g., the label can be a radioisotope, fluorescent compound, enzyme, or enzyme co-factor). In certain preferred embodiments, the antibodies of the present disclosure are isolated antibodies. In certain preferred embodiments, the antibodies of the present disclosure are recombinant antibodies.

[0209] The antibodies herein may be of any class. The class of an antibody refers to the type of constant domain or constant region possessed by its heavy chain. There are five major classes of antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), for example, IgG₁, IgG₂, IgG₃, IgG₄, IgA₁, and IgA₂. The heavy chain constant domains that correspond to the different classes of immunoglobulins are called alpha, delta, epsilon, gamma, and mu.

[0210] In general, an antibody for use in the methods disclosed herein specifically binds to its target antigen, preferably with high binding affinity. Affinity may be expressed as a K_D value and reflects the intrinsic binding affinity (e.g., with minimized avidity effects). Typically, binding affinity is measured in vitro, whether in a cell-free or cell-associated setting. Any of a number of assays known in the art, including those disclosed herein, can be used to obtain binding affinity measurements including, for example, Biacore, radiolabeled antigen-binding assay (RIA), and ELISA. In some embodiments, antibodies of the present disclosure bind to their target antigens (e.g. ActRIIB, ActRIIA, ALK4, ALK5, ALK7, activin, GDF11, GDF8, GDF3, BMP15, BMP10, and/or BMP6) with at least a K_D of 1×10^{-7} or stronger, 1×10^{-8} or stronger, 1×10^{-9} or stronger, 1×10^{-10} or stronger, 1×10^{-11} or stronger, 1×10^{-12} or stronger, 1×10^{-13} or stronger, or 1×10^{-14} or stronger.

[0211] In certain embodiments, K_D is measured by RIA performed with the Fab version of an antibody of interest and its target antigen as described by the following assay. Solution binding affinity of Fabs for the antigen is measured by equilibrating Fab with a minimal concentration of radiolabeled antigen (e.g., ¹²⁵I-labeled) in the presence of a titration series of unlabeled antigen, then capturing bound antigen with an anti-Fab antibody-coated plate [see, e.g., Chen et al. (1999) J. Mol. Biol. 293:865-881]. To establish conditions for the assay, multi-well plates (e.g., MICROTITER® from Thermo Scientific) are coated (e.g., overnight) with a capturing anti-Fab antibody (e.g., from Cappel Labs) and subsequently blocked with bovine serum albumin, preferably at room temperature (approximately 23° C.). In a non-adsorbent plate, radiolabeled antigen are mixed with

serial dilutions of a Fab of interest [e.g., consistent with assessment of the anti-VEGF antibody, Fab-12, in Presta et al., (1997) Cancer Res. 57:4593-4599]. The Fab of interest is then incubated, preferably overnight but the incubation may continue for a longer period (e.g., about 65 hours) to ensure that equilibrium is reached. Thereafter, the mixtures are transferred to the capture plate for incubation, preferably at room temperature for about one hour. The solution is then removed and the plate is washed times several times, preferably with polysorbate 20 and PBS mixture. When the plates have dried, scintillant (e.g., MICROSCINT® from Packard) is added, and the plates are counted on a gamma counter (e.g., TOPCOUNT® from Packard).

[0212] According to another embodiment, K_D is measured using surface plasmon resonance assays using, for example a BIACORE® 2000 or a BIACORE® 3000 (BIAcore, Inc., Piscataway, N.J.) with immobilized antigen CM5 chips at about 10 response units (RU). Briefly, carboxymethylated dextran biosensor chips (CM5, BIACORE, Inc.) are activated with N-ethyl-N'-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) according to the supplier's instructions. For example, an antigen can be diluted with 10 mM sodium acetate, pH 4.8, to 5 µg/ml (about 0.2 µM) before injection at a flow rate of 5 µl/minute to achieve approximately 10 response units (RU) of coupled protein. Following the injection of antigen, 1 M ethanolamine is injected to block unreacted groups. For kinetics measurements, two-fold serial dilutions of Fab (0.78 nM to 500 nM) are injected in PBS with 0.05% polysorbate 20 (TWEEN-20®) surfactant (PBST) at a flow rate of approximately 25 µl/min. Association rates (k_{on}) and dissociation rates (k_{off}) are calculated using, for example, a simple one-to-one Langmuir binding model (BIACORE® Evaluation Software version 3.2) by simultaneously fitting the association and dissociation sensorgrams. The equilibrium dissociation constant (K_D) is calculated as the ratio k_{off}/k_{on} [see, e.g., Chen et al., (1999) J. Mol. Biol. 293:865-881]. If the on-rate exceeds, for example, $10^6 \text{ M}^{-1} \text{ s}^{-1}$ by the surface plasmon resonance assay above, then the on-rate can be determined by using a fluorescent quenching technique that measures the increase or decrease in fluorescence emission intensity (e.g., excitation=295 nm; emission=340 nm, 16 nm band-pass) of a 20 nM anti-antigen antibody (Fab form) in PBS in the presence of increasing concentrations of antigen as measured in a spectrometer, such as a stop-flow equipped spectrophotometer (Aviv Instruments) or a 8000-series SLM-AMINCO® spectrophotometer (ThermoSpectronic) with a stirred cuvette.

[0213] Antibody fragments can be made by various techniques, including but not limited to proteolytic digestion of an intact antibody as well as production by recombinant host cells (e.g., *E. coli* or phage), as described herein. The nucleic acid and amino acid sequences of human ActRIIA, ActRIIB, ALK4, ALK5, ALK7, activin (activin A, activin B, activin C, and activin E), GDF11, GDF8, BMP15, GDF3, BMP10, and BMP6, are known in the art. In addition, numerous methods for generating antibodies are well known in the art, some of which are described herein. Therefore antibody antagonists for use in accordance with this disclosure may be routinely made by the skilled person in the art based on the knowledge in the art and teachings provided herein.

[0214] In certain embodiments, an antibody provided herein is a chimeric antibody. A chimeric antibody refers to an antibody in which a portion of the heavy and/or light

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

[0215] In certain embodiments, a chimeric antibody provided herein is a humanized antibody. A humanized antibody refers to a chimeric antibody comprising amino acid residues from non-human hypervariable regions (HVRs) and amino acid residues from human framework regions (FRs). In certain embodiments, a humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the HVRs (e.g., CDRs) correspond to those of a non-human antibody, and all or substantially all of the FRs correspond to those of a human antibody. A humanized antibody optionally may comprise at least a portion of an antibody constant region derived from a human antibody. A “humanized form” of an antibody, e.g., a non-human antibody, refers to an antibody that has undergone humanization. Humanized antibodies and methods of making them are reviewed, for example, in Almagro and Fransson (2008) *Front. Biosci.* 13:1619-1633 and are further described, for example, in Riechmann et al., (1988) *Nature* 332:323-329; Queen et al. (1989) *Proc. Nat'l Acad. Sci. USA* 86:10029-10033; U.S. Pat. Nos. 5,821,337; 7,527,791; 6,982,321; and U.S. Pat. No. 7,087,409; Kashmiri et al., (2005) *Methods* 36:25-34 [describing SDR (a-CDR) grafting]; Padlan, *Mol. Immunol.* (1991) 28:489-498 (describing “resurfacing”); Dall'Acqua et al. (2005) *Methods* 36:43-60 (describing “FR shuffling”); Osbourn et al. (2005) *Methods* 36:61-68; and Klimka et al. *Br. J. Cancer* (2000) 83:252-260 (describing the “guided selection” approach to FR shuffling). Human framework regions that may be used for humanization include but are not limited to: framework regions selected using the “best-fit” method [see, e.g., Sims et al. (1993) *J. Immunol.* 151:2296]; framework regions derived from the consensus sequence of human antibodies of a particular subgroup of light or heavy chain variable regions [see, e.g., Carter et al. (1992) *Proc. Natl. Acad. Sci. USA*, 89:4285; and Presta et al. (1993) *J. Immunol.*, 151:2623]; human mature (somatically mutated) framework regions or human germline framework regions [see, e.g., Almagro and Fransson (2008) *Front. Biosci.* 13:1619-1633]; and framework regions derived from screening FR libraries [see, e.g., Baca et al., (1997) *J. Biol. Chem.* 272:10678-10684; and Rosok et al., (1996) *J. Biol. Chem.* 271:22611-22618].

[0216] In certain embodiments, an antibody provided herein is a human antibody. Human antibodies can be produced using various techniques known in the art. Human antibodies are described generally in van Dijk and van de Winkel (2008) *Curr. Opin. Pharmacol.* 5: 368-74 (2001) and Lonberg, *Curr. Opin. Immunol.* 20:450-459. For example, human antibodies may be prepared by administering an immunogen (e.g., a GDF11 polypeptide, an activin B polypeptide, an ActRIIA polypeptide, or an ActRIIB polypep-

tide) to a transgenic animal that has been modified to produce intact human antibodies or intact antibodies with human variable regions in response to antigenic challenge. Such animals typically contain all or a portion of the human immunoglobulin loci, which replace the endogenous immunoglobulin loci, or which are present extrachromosomally or integrated randomly into the animal's chromosomes. In such transgenic animals, the endogenous immunoglobulin loci have generally been inactivated. For a review of methods for obtaining human antibodies from transgenic animals see, for example, Lonberg (2005) *Nat. Biotech.* 23:1117-1125; U.S. Pat. Nos. 6,075,181 and 6,150,584 (describing XENOMOUSE™ technology); U.S. Pat. No. 5,770,429 (describing HuMab® technology); U.S. Pat. No. 7,041,870 (describing K-M MOUSE® technology); and U.S. Patent Application Publication No. 2007/0061900 (describing VelociMouse® technology). Human variable regions from intact antibodies generated by such animals may be further modified, for example, by combining with a different human constant region.

[0217] Human antibodies provided herein can also be made by hybridoma-based methods. Human myeloma and mouse-human heteromyeloma cell lines for the production of human monoclonal antibodies have been described [see, e.g., Kozbor *J. Immunol.*, (1984) 133: 3001; Brodeur et al. (1987) *Monoclonal Antibody Production Techniques and Applications*, pp. 51-63, Marcel Dekker, Inc., New York; and Boerner et al. (1991) *J. Immunol.*, 147: 86]. Human antibodies generated via human B-cell hybridoma technology are also described in Li et al., (2006) *Proc. Natl. Acad. Sci. USA*, 103:3557-3562. Additional methods include those described, for example, in U.S. Pat. No. 7,189,826 (describing production of monoclonal human IgM antibodies from hybridoma cell lines) and Ni, Xiandai Mianyixue (2006) 26(4):265-268 (2006) (describing human-human hybridomas). Human hybridoma technology (Trioma technology) is also described in Vollmers and Brandlein (2005) *Histol. Histopathol.*, 20(3):927-937 (2005) and Vollmers and Brandlein (2005) *Methods Find Exp. Clin. Pharmacol.*, 27(3):185-91. Human antibodies provided herein may also be generated by isolating Fv clone variable-domain sequences selected from human-derived phage display libraries. Such variable-domain sequences may then be combined with a desired human constant domain. Techniques for selecting human antibodies from antibody libraries are known in the art and described herein.

[0218] For example, antibodies of the present disclosure may be isolated by screening combinatorial libraries for antibodies with the desired activity or activities. A variety of methods are known in the art for generating phage display libraries and screening such libraries for antibodies possessing the desired binding characteristics. Such methods are reviewed, for example, in Hoogenboom et al. (2001) in *Methods in Molecular Biology* 178:1-37, O'Brien et al., ed., Human Press, Totowa, N.J. and further described, for example, in the McCafferty et al. (1991) *Nature* 348:552-554; Clackson et al., (1991) *Nature* 352: 624-628; Marks et al. (1992) *J. Mol. Biol.* 222:581-597; Marks and Bradbury (2003) in *Methods in Molecular Biology* 248:161-175, Lo, ed., Human Press, Totowa, N.J.; Sidhu et al. (2004) *J. Mol. Biol.* 338(2):299-310; Lee et al. (2004) *J. Mol. Biol.* 340 (5):1073-1093; Fellouse (2004) *Proc. Natl. Acad. Sci. USA* 101(34):12467-12472; and Lee et al. (2004) *J. Immunol. Methods* 284(1-2): 119-132.

[0219] In certain phage display methods, repertoires of VH and VL genes are separately cloned by polymerase chain reaction (PCR) and recombined randomly in phage libraries, which can then be screened for antigen-binding phage as described in Winter et al. (1994) *Ann. Rev. Immunol.*, 12: 433-455. Phage typically display antibody fragments, either as single-chain Fv (scFv) fragments or as Fab fragments. Libraries from immunized sources provide high-affinity antibodies to the immunogen (e.g., ActRIIA, ActRIIB, activin, GDF11, GDF8, BMP15, GDF3, or BMP6) without the requirement of constructing hybridomas. Alternatively, the naive repertoire can be cloned (e.g., from human) to provide a single source of antibodies to a wide range of non-self and also self-antigens without any immunization as described by Griffiths et al. (1993) *EMBO J.*, 12: 725-734. Finally, naive libraries can also be made synthetically by cloning unarranged V-gene segments from stem cells, and using PCR primers containing random sequence to encode the highly variable CDR3 regions and to accomplish rearrangement in vitro, as described by Hoogenboom and Winter (1992) *J. Mol. Biol.*, 227: 381-388. Patent publications describing human antibody phage libraries include, for example: U.S. Pat. No. 5,750,373, and U.S. Patent Publication Nos. 2005/0079574, 2005/0119455, 2005/0266000, 2007/0117126, 2007/0160598, 2007/0237764, 2007/0292936, and 2009/0002360.

[0220] In certain embodiments, an antibody provided herein is a multispecific antibody, for example, a bispecific antibody. Multispecific antibodies (typically monoclonal antibodies) that have binding specificities for at least two different epitopes (e.g., two, three, four, five, or six or more) on one or more (e.g., two, three, four, five, six or more) antigens.

[0221] Techniques for making multispecific antibodies include, but are not limited to, recombinant co-expression of two immunoglobulin heavy-chain/light-chain pairs having different specificities [see, e.g., Milstein and Cuello (1983) *Nature* 305: 537; International patent publication no. WO 93/08829; and Traunecker et al. (1991) *EMBO J.* 10: 3655, and U.S. Pat. No. 5,731,168 ("knob-in-hole" engineering)]. Multispecific antibodies may also be made by engineering electrostatic steering effects for making antibody Fc-heterodimeric molecules (see, e.g., WO 2009/089004A1); cross-linking two or more antibodies or fragments [see, e.g., U.S. Pat. No. 4,676,980; and Brennan et al. (1985) *Science*, 229: 81]; using leucine zippers to produce bispecific antibodies [see, e.g., Kostelny et al. (1992) *J. Immunol.*, 148 (5):1547-1553]; using "diabody" technology for making bispecific antibody fragments [see, e.g., Hollinger et al. (1993) *Proc. Natl. Acad. Sci. USA*, 90:6444-6448]; using single-chain Fv (sFv) dimers [see, e.g., Gruber et al. (1994) *J. Immunol.*, 152:5368]; and preparing trispecific antibodies (see, e.g., Tutt et al. (1991) *J. Immunol.* 147: 60. Multispecific antibodies can be prepared as full-length antibodies or antibody fragments. Engineered antibodies with three or more functional antigen-binding sites, including "Octopus antibodies," are also included herein [see, e.g., US 2006/0025576A1].

[0222] In certain embodiments, an antibody disclosed herein is a monoclonal antibody. Monoclonal antibody refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical and/or bind the same epitope, except for possible variant antibodies, e.g.,

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

[0223] For example, by using immunogens derived from activin, anti-protein/anti-peptide antisera or monoclonal antibodies can be made by standard protocols [see, e.g., *Antibodies: A Laboratory Manual* ed. by Harlow and Lane (1988) Cold Spring Harbor Press: 1988]. A mammal, such as a mouse, hamster, or rabbit, can be immunized with an immunogenic form of the activin polypeptide, an antigenic fragment which is capable of eliciting an antibody response, or a fusion protein. Techniques for conferring immunogenicity on a protein or peptide include conjugation to carriers or other techniques well known in the art. An immunogenic portion of a activin polypeptide can be administered in the presence of adjuvant. The progress of immunization can be monitored by detection of antibody titers in plasma or serum. Standard ELISA or other immunoassays can be used with the immunogen as antigen to assess the levels of antibody production and/or level of binding affinity.

[0224] Following immunization of an animal with an antigenic preparation of activin, antisera can be obtained and, if desired, polyclonal antibodies can be isolated from the serum. To produce monoclonal antibodies, antibody-producing cells (lymphocytes) can be harvested from an immunized animal and fused by standard somatic cell fusion procedures with immortalizing cells such as myeloma cells to yield hybridoma cells. Such techniques are well known in the art, and include, for example, the hybridoma technique [see, e.g., Kohler and Milstein (1975) *Nature*, 256: 495-497], the human B cell hybridoma technique [see, e.g., Kozbar et al. (1983) *Immunology Today*, 4:72], and the EBV-hybridoma technique to produce human monoclonal antibodies [Cole et al. (1985) *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc. pp. 77-96]. Hybridoma cells can be screened immunochemically for production of antibodies specifically reactive with a activin polypeptide, and monoclonal antibodies isolated from a culture comprising such hybridoma cells.

[0225] In certain embodiments, one or more amino acid modifications may be introduced into the Fc region of an antibody provided herein thereby generating an Fc region variant. The Fc region variant may comprise a human Fc region sequence (e.g., a human IgG1, IgG2, IgG3 or IgG4 Fc region) comprising an amino acid modification (e.g., a substitution, deletion, and/or addition) at one or more amino acid positions.

[0226] For example, the present disclosure contemplates an antibody variant that possesses some but not all effector functions, which make it a desirable candidate for applications in which the half-life of the antibody in vivo is important yet certain effector functions [e.g., complement-dependent cytotoxicity (CDC) and antibody-dependent cellular cytotoxicity (ADCC)] are unnecessary or deleterious. In vitro and/or in vivo cytotoxicity assays can be conducted to confirm the reduction/depletion of CDC and/or ADCC activities. For example, Fc receptor (FcR) binding assays can be conducted to ensure that the antibody lacks FcγR binding (hence likely lacking ADCC activity), but retains FcRn binding ability. The primary cells for mediating ADCC, NK cells, express FcγRIII only, whereas monocytes express FcγRI, FcγRII and FcγRIII. FcR expression on hematopoietic cells is summarized in, for example, Ravetch and Kinet (1991) *Annu. Rev. Immunol.* 9:457-492. Non-limiting examples of in vitro assays to assess ADCC activity of a molecule of interest are described in U.S. Pat. No. 5,500,362; Hellstrom, I. et al. (1986) *Proc. Natl. Acad. Sci. USA* 83:7059-7063; Hellstrom, I. et al. (1985) *Proc. Natl. Acad. Sci. USA* 82:1499-1502; U.S. Pat. No. 5,821,337; Bruggemann, M. et al. (1987) *J. Exp. Med.* 166:1351-1361. Alternatively, non-radioactive assays methods may be employed (e.g., ACTITM, non-radioactive cytotoxicity assay for flow cytometry; CellTechnology, Inc. Mountain View, Calif.; and Cytotoxic 96® non-radioactive cytotoxicity assay, Promega, Madison, Wis.). Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and natural killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed in vivo, for example, in an animal model such as that disclosed in Clynes et al. (1998) *Proc. Natl. Acad. Sci. USA* 95:652-656. C1q binding assays may also be carried out to confirm that the antibody is unable to bind C1q and hence lacks CDC activity [see, e.g., C1q and C3c binding ELISA in WO 2006/029879 and WO 2005/100402]. To assess complement activation, a CDC assay may be performed [see, e.g., Gazzano-Santoro et al. (1996) *J. Immunol. Methods* 202:163; Cragg, M. S. et al. (2003) *Blood* 101:1045-1052; and Cragg, M. S. and M. J. Glennie (2004) *Blood* 103:2738-2743]. FcRn binding and in vivo clearance/half-life determinations can also be performed using methods known in the art [see, e.g., Petkova, S. B. et al. (2006) *Intl. Immunol.* 18(12):1759-1769]. Antibodies of the present disclosure with reduced effector function include those with substitution of one or more of Fc region residues 238, 265, 269, 270, 297, 327 and 329 (U.S. Pat. No. 6,737,056). Such Fc mutants include Fc mutants with substitutions at two or more of amino acid positions 265, 269, 270, 297 and 327, including the so-called “DANA” Fc mutant with substitution of residues 265 and 297 to alanine (U.S. Pat. No. 7,332,581).

[0227] In certain embodiments, it may be desirable to create cysteine engineered antibodies, e.g., “thioMAbs,” in which one or more residues of an antibody are substituted with cysteine residues. In particular embodiments, the substituted residues occur at accessible sites of the antibody. By substituting those residues with cysteine, reactive thiol groups are thereby positioned at accessible sites of the antibody and may be used to conjugate the antibody to other moieties, such as drug moieties or linker-drug moieties, to create an immunoconjugate, as described further herein. In certain embodiments, any one or more of the following residues may be substituted with cysteine: V205 (Kabat

numbering) of the light chain; A118 (EU numbering) of the heavy chain; and S400 (EU numbering) of the heavy chain Fc region. Cysteine engineered antibodies may be generated as described, for example, in U.S. Pat. No. 7,521,541.

[0228] In addition, the techniques used to screen antibodies in order to identify a desirable antibody may influence the properties of the antibody obtained. For example, if an antibody is to be used for binding an antigen in solution, it may be desirable to test solution binding. A variety of different techniques are available for testing interactions between antibodies and antigens to identify particularly desirable antibodies. Such techniques include ELISAs, surface plasmon resonance binding assays (e.g., the Biacore binding assay, Biacore AB, Uppsala, Sweden), sandwich assays (e.g., the paramagnetic bead system of IGEN International, Inc., Gaithersburg, Maryland), western blots, immunoprecipitation assays, and immunohistochemistry.

[0229] In certain embodiments, amino acid sequence variants of the antibodies and/or the binding polypeptides provided herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibody and/or binding polypeptide. Amino acid sequence variants of an antibody and/or binding polypeptides may be prepared by introducing appropriate modifications into the nucleotide sequence encoding the antibody and/or binding polypeptide, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of residues within the amino acid sequences of the antibody and/or binding polypeptide. Any combination of deletion, insertion, and substitution can be made to arrive at the final construct, provided that the final construct possesses the desired characteristics, e.g., target-binding (e.g., and activin such as activin E and/or activin C binding).

[0230] Alterations (e.g., substitutions) may be made in HVRs, for example, to improve antibody affinity. Such alterations may be made in HVR “hotspots,” i.e., residues encoded by codons that undergo mutation at high frequency during the somatic maturation process [see, e.g., Chowdhury (2008) *Methods Mol. Biol.* 207:179-196 (2008)], and/or SDRs (α-CDRs), with the resulting variant VH or VL being tested for binding affinity. Affinity maturation by constructing and reselecting from secondary libraries has been described in the art [see, e.g., Hoogenboom et al., in *Methods in Molecular Biology* 178:1-37, O’Brien et al., ed., Human Press, Totowa, N.J., (2001)]. In some embodiments of affinity maturation, diversity is introduced into the variable genes chosen for maturation by any of a variety of methods (e.g., error-prone PCR, chain shuffling, or oligonucleotide-directed mutagenesis). A secondary library is then created. The library is then screened to identify any antibody variants with the desired affinity. Another method to introduce diversity involves HVR-directed approaches, in which several HVR residues (e.g., 4-6 residues at a time) are randomized. HVR residues involved in antigen binding may be specifically identified, e.g., using alanine scanning mutagenesis or modeling. CDR-H3 and CDR-L3 in particular are often targeted.

[0231] In certain embodiments, substitutions, insertions, or deletions may occur within one or more HVRs so long as such alterations do not substantially reduce the ability of the antibody to bind to the antigen. For example, conservative alterations (e.g., conservative substitutions as provided herein) that do not substantially reduce binding affinity may

be made in HVRs. Such alterations may be outside of HVR "hotspots" or SDRs. In certain embodiments of the variant VH and VL sequences provided above, each HVR either is unaltered, or contains no more than one, two or three amino acid substitutions.

[0232] A useful method for identification of residues or regions of the antibody and/or the binding polypeptide that may be targeted for mutagenesis is called "alanine scanning mutagenesis" as described by Cunningham and Wells (1989) *Science*, 244:1081-1085. In this method, a residue or group of target residues (e.g., charged residues such as Arg, Asp, His, Lys, and Glu) are identified and replaced by a neutral or negatively charged amino acid (e.g., alanine or polyalanine) to determine whether the interaction of the antibody-antigen is affected. Further substitutions may be introduced at the amino acid locations demonstrating functional sensitivity to the initial substitutions. Alternatively, or additionally, a crystal structure of an antigen-antibody complex is determined to identify contact points between the antibody and antigen. Such contact residues and neighboring residues may be targeted or eliminated as candidates for substitution. Variants may be screened to determine whether they contain the desired properties.

[0233] Amino acid sequence insertions include amino-and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an antibody with an N-terminal methionyl residue. Other insertional variants of the antibody molecule include the fusion of the N- or C-terminus of the antibody to an enzyme (e.g., for ADEPT) or a polypeptide which increases the serum half-life of the antibody.

[0234] In certain embodiments, an antibody and/or binding polypeptide provided herein may be further modified to contain additional nonproteinaceous moieties that are known in the art and readily available. The moieties suitable for derivatization of the antibody and/or binding polypeptide include but are not limited to water soluble polymers. Non-limiting examples of water soluble polymers include, but are not limited to, polyethylene glycol (PEG), copolymers of ethylene glycol/propylene glycol, carboxymethyl-cellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone, poly-1,3-dioxolane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, polyaminoacids (either homopolymers or random copolymers), and dextran or poly(n-vinyl pyrrolidone)polyethylene glycol, propylene glycol homopolymers, prolypropylene oxide/ethylene oxide copolymers, polyoxyethylated polyols (e.g., glycerol), polyvinyl alcohol, and mixtures thereof. Polyethylene glycol propionaldehyde may have advantages in manufacturing due to its stability in water. The polymer may be of any molecular weight, and may be branched or unbranched. The number of polymers attached to the antibody and/or binding polypeptide may vary, and if more than one polymer are attached, they can be the same or different molecules. In general, the number and/or type of polymers used for derivatization can be determined based on considerations including, but not limited to, the particular properties or functions of the antibody and/or binding polypeptide to be improved, whether the antibody derivative and/or binding polypeptide derivative will be used in a therapy under defined conditions.

5. Small Molecule Antagonists

[0235] In other aspects, a GDF/BMP antagonist to be used in accordance with the methods and uses described herein is a small molecule (GDF/BMP small molecule antagonist), or combination of small molecule antagonists. A GDF/BMP small molecule antagonist, or combination of small molecule antagonists, may inhibit, for example, one or more GDF/BMP ligands (e.g., activin, GDF11, GDF8, GDF3, BMP6, BMP10, and/or BMP15), a type I receptor (e.g., ALK4, ALK5, and/or ALK7), a type II receptor (e.g., ActRIIB and/or ActRIIA), a co-receptor, and/or one or more signaling factors (e.g. Smad proteins such as Smads 2 and 3). In some embodiments, a GDF/BMP small molecule antagonist, or combination of small molecule antagonists, inhibits signaling mediated by one or more GDF/BMP ligands, for example, as determined in a cell-based assay such as those described herein. As described herein, GDF/BMP small molecule antagonists may be used, alone or in combination with one or more supportive therapies or active agents, to treat, prevent, or reduce the progression rate and/or severity of pulmonary hypertension (PH), particularly treating, preventing or reducing the progression rate and/or severity of one or more PH-associated complications.

[0236] In some embodiments, a GDF/BMP small molecule antagonist, or combination of small molecule antagonists, inhibits at least GDF11, optionally further inhibiting one or more of GDF8, activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC, activin BC, activin AE and/or activin BE), GDF3, BMP6, BMP10, ActRIIA, ActRIIB, ALK4, ALK5, ALK7, and one or more Smad proteins (e.g., Smads 2 and 3). In some embodiments, a GDF/BMP small molecule antagonist, or combination of small molecule antagonists, inhibits at least GDF8, optionally further inhibiting one or more of GDF11, activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC, activin BC, activin AE and/or activin BE), GDF3, BMP6, BMP10, ActRIIA, ActRIIB, ALK4, ALK5, ALK7, and one or more Smad proteins (e.g., Smads 2 and 3). In some embodiments, a GDF/BMP small molecule antagonist, or combination of small molecule antagonists, inhibits at least activin (activin A, activin B, activin C, activin E, activin AB, activin AC, activin BC, activin AE and/or activin BE), optionally further inhibiting one or more of GDF8, GDF11, GDF3, BMP6, BMP10, ActRIIA, ActRIIB, ALK4, ALK5, ALK7, and one or more Smad proteins (e.g., Smads 2 and 3). In some embodiments, a GDF/BMP small molecule antagonist, or combination of small molecule antagonists, inhibits at least activin B, optionally further inhibiting one or more of GDF8, GDF11, GDF3, BMP6, BMP10, ActRIIA, ActRIIB, ALK4, ALK5, ALK7, and one or more Smad proteins (e.g., Smads 2 and 3). In some embodiments, a GDF/BMP small molecule antagonist, or combination of small molecule antagonists, inhibits at least activin B, optionally further inhibiting one or more of GDF8, GDF11, GDF3, BMP6, BMP10, ActRIIA, ActRIIB, ALK4, ALK5, ALK7, and one or more Smad proteins (e.g., Smads 2 and 3). In some embodiments, a GDF/BMP small molecule antagonist, or combination of small molecule antagonists, inhibits at least activin B, optionally further inhibiting one or more of GDF8, GDF11, GDF3, BMP6, BMP10, ActRIIA, ActRIIB, ALK4, ALK5, ALK7, and one or more Smad proteins (e.g., Smads 2 and 3). In some embodiments, a GDF/BMP small molecule antagonist, or combination of small molecule antagonists, inhibits at least BMP6, optionally further inhibiting one or more of GDF8, activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC, activin BC, activin AE and/or activin BE), GDF3, GDF11, BMP10, ActRIIA, ActRIIB, ALK4, ALK5, ALK7, and one or more Smad proteins (e.g., Smads 2 and 3). In some embodiments, a GDF/BMP small molecule antagonist, or combination of small molecule antagonists, inhibits at least BMP15, optionally further inhibiting one or more of GDF8, activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC, activin BC, activin AE and/or activin BE), GDF3, BMP6, GDF11, BMP10, ActRIIA,

ActRIIB, ALK4, ALK5, ALK7, and one or more Smad proteins (e.g., Smads 2 and 3). In some embodiments, a GDF/BMP small molecule antagonist, or combination of small molecule antagonists, inhibits at least GDF3, optionally further inhibiting one or more of GDF8, activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC, activin BC, activin AE and/or activin BE), BMP15, BMP6, GDF11, BMP10, ActRIIA, ActRIIB, ALK4, ALK5, ALK7, and one or more Smad proteins (e.g., Smads 2 and 3). In some embodiments, a GDF/BMP small molecule antagonist, or combination of small molecule antagonists, inhibits at least BMP10, optionally further inhibiting one or more of GDF8, activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC, activin BC, activin AE and/or activin BE), BMP15, BMP6, GDF11, GDF3, ActRIIA, ActRIIB, ALK4, ALK5, ALK7, and one or more Smad proteins (e.g., Smads 2 and 3). In some embodiments, a GDF/BMP small molecule antagonist, or combination of small molecule antagonists, inhibits at least ActRIIB, optionally further inhibiting one or more of GDF8, activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC, activin BC, activin AE and/or activin BE), BMP15, BMP6, GDF11, GDF3, BMP10, ActRIIB, ALK4, ALK5, ALK7, and one or more Smad proteins (e.g., Smads 2 and 3). In some embodiments, a GDF/BMP small molecule antagonist, or combination of small molecule antagonists, inhibits at least ALK4, optionally further inhibiting one or more of GDF8, activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC, activin BC, activin AE and/or activin BE), BMP15, BMP6, GDF11, GDF3, ActRIIA, ActRIIB, ALK4, ALK5, ALK7, and one or more Smad proteins (e.g., Smads 2 and 3). In some embodiments, a GDF/BMP small molecule antagonist, or combination of small molecule antagonists, inhibits at least ALK5, optionally further inhibiting one or more of GDF8, activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC, activin BC, activin AE and/or activin BE), BMP15, BMP6, GDF11, GDF3, ActRIIA, ActRIIB, ALK4, BMP10, ALK5, ALK7, and one or more Smad proteins (e.g., Smads 2 and 3). In some embodiments, a GDF/BMP small molecule antagonist, or combination of small molecule antagonists, inhibits at least ALK7, optionally further inhibiting one or more of GDF8, activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC, activin BC, activin AE and/or activin BE), BMP15, BMP6, GDF11, GDF3, ActRIIA, ActRIIB, ALK4, ALK5, ALK7, and one or more Smad proteins (e.g., Smads 2 and 3). In some embodiments, a GDF/BMP small molecule antagonist, or combination of small molecule antagonists, as disclosed herein does not inhibit or does not substantially inhibit BMP9. In some embodiments, a GDF/BMP small molecule antagonist, or combination of small molecule antagonists, as disclosed herein does not inhibit or does not substantially inhibit activin A.

[0237] GDF/BMP small molecule antagonists can be direct or indirect inhibitors. For example, an indirect small molecule antagonist, or combination of small molecule

antagonists, may inhibit the expression (e.g., transcription, translation, cellular secretion, or combinations thereof) of at least one or more GDF/BMP ligands [e.g., activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC, activin B, activin BC, activin AE, or activin BE), GDF11, BMP15, BMP6, GDF3, BMP10, and/or GDF8], type I receptor (e.g., ALK4, ALK5, and/or ALK7), type II receptors (e.g., ActRIIA and/or ActRIIB), co-receptor, and/or one or more downstream signaling components (e.g., Smads). Alternatively, a direct small molecule antagonist, or combination of small molecule antagonists, may directly bind to and inhibit, for example, one or more one or more GDF/BMP ligands [e.g., activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC, activin B, activin BC, activin AE, or activin BE), GDF11, BMP15, BMP6, GDF3, BMP10, and/or GDF8], type I receptor (e.g., ALK4, ALK5 and/or ALK7), type II receptors (e.g., ActRIIA and/or ActRIIB), co-receptor, and/or one or more downstream signaling components (e.g., Smads). Combinations of one or more indirect and one or more direct GDF/BMP small molecule antagonists may be used in accordance with the methods disclosed herein.

[0238] Binding small-molecule antagonists of the present disclosure may be identified and chemically synthesized using known methodology (see, e.g., PCT Publication Nos. WO 00/00823 and WO 00/39585). In general, small molecule antagonists of the disclosure are usually less than about 2000 daltons in size, alternatively less than about 1500, 750, 500, 250 or 200 daltons in size, wherein such organic small molecules that are capable of binding, preferably specifically, to a polypeptide as described herein. These small molecule antagonists may be identified without undue experimentation using well-known techniques. In this regard, it is noted that techniques for screening organic small-molecule libraries for molecules that are capable of binding to a polypeptide target are well known in the art (see, e.g., international patent publication Nos. WO00/00823 and WO00/39585).

[0239] Binding organic small molecules of the present disclosure may be, for example, aldehydes, ketones, oximes, hydrazones, semicarbazones, carbazides, primary amines, secondary amines, tertiary amines, N-substituted hydrazines, hydrazides, alcohols, ethers, thiols, thioethers, disulfides, carboxylic acids, esters, amides, ureas, carbamates, carbonates, ketals, thioketals, acetals, thioacetals, aryl halides, aryl sulfonates, alkyl halides, alkyl sulfonates, aromatic compounds, heterocyclic compounds, anilines, alkynes, alkynes, diols, amino alcohols, oxazolidines, oxazolines, thiazolidines, thiazolines, enamines, sulfonamides, epoxides, aziridines, isocyanates, sulfonyl chlorides, diazo compounds, and acid chlorides.

6. Polynucleotide Antagonists

[0240] In other aspects, a GDF/BMP antagonist to be used in accordance with the methods and uses disclosed herein is a polynucleotide (GDF/BMP polynucleotide antagonist), or combination of polynucleotides. A GDF/BMP polynucleotide antagonist, or combination of polynucleotide antagonists, may inhibit, for example, one or more GDF/BMP ligands (e.g., activin, GDF11, GDF8, GDF3, BMP6, BMP10, and/or BMP15), type I receptors (e.g., ALK4, ALK5, and/or ALK7), type II receptors (e.g., ActRIIA and/or ActRIIB), co-receptor, and/or downstream signaling component (e.g., Smads). In some embodiments, a GDF/

BMP polynucleotide antagonist, or combination of polynucleotide antagonists, inhibits signaling mediated by one or more GDF/BMP ligands, for example, as determined in a cell-based assay such as those described herein. As described herein, GDF/BMP polynucleotide antagonists may be used, alone or in combination with one or more supportive therapies or active agents, to treat, prevent, or reduce the progression rate and/or severity of pulmonary hypertension (PH), particularly treating, preventing or reducing the progression rate and/or severity of one or more PH-associated complications.

[0241] In some embodiments, a GDF/BMP polynucleotide antagonist, or combination of polynucleotide antagonists, inhibits at least GDF11, optionally further inhibiting one or more of GDF8, activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC, activin BC, activin AE and/or activin BE), GDF3, BMP6, BMP15, BMP10, ActRIIA, ActRIIB, ALK4, ALK5, ALK7, and one or more Smad proteins (e.g., Smads 2 and 3). In some embodiments, a GDF/BMP polynucleotide antagonist, or combination of polynucleotide antagonists, inhibits at least GDF8, optionally further inhibiting one or more of GDF11, activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC, activin BC, activin AE and/or activin BE), GDF3, BMP6, BMP15, BMP10, ActRIIA, ActRIIB, ALK4, ALK5, ALK7, and one or more Smad proteins (e.g., Smads 2 and 3). In some embodiments, a GDF/BMP polynucleotide antagonist, or combination of polynucleotide antagonists, inhibits at least activin (activin A, activin B, activin C, activin E, activin AB, activin AC, activin BC, activin AE and/or activin BE), optionally further inhibiting one or more of GDF8, GDF11, GDF3, BMP6, BMP15, BMP10, ActRIIA, ActRIIB, ALK4, ALK5, ALK7, and one or more Smad proteins (e.g., Smads 2 and 3). In some embodiments, a GDF/BMP polynucleotide antagonist, or combination of polynucleotide antagonists, inhibits at least activin B, optionally further inhibiting one or more of GDF8, GDF11, GDF3, BMP6, BMP15, BMP10, ActRIIA, ActRIIB, ALK4, ALK5, ALK7, and one or more Smad proteins (e.g., Smads 2 and 3). In some embodiments, a GDF/BMP polynucleotide antagonist, or combination of polynucleotide antagonists, inhibits at least BMP6, optionally further inhibiting one or more of GDF8, activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC, activin BC, activin AE and/or activin BE), GDF3, GDF11, BMP15, BMP10, ActRIIA, ActRIIB, ALK4, ALK5, ALK7, and one or more Smad proteins (e.g., Smads 2 and 3). In some embodiments, a GDF/BMP polynucleotide antagonist, or combination of polynucleotide antagonists, inhibits at least BMP15, optionally further inhibiting one or more of GDF8, activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC, activin BC, activin AE and/or activin BE), GDF3, BMP6, GDF11, BMP15, BMP10, ActRIIA, ActRIIB, ALK4, ALK5, ALK7, and one or more Smad proteins (e.g., Smads 2 and 3). In some embodiments, a GDF/BMP polynucleotide antagonist, or combination of polynucleotide antagonists, inhibits at least GDF3, optionally further inhibiting one or more of GDF8, activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC, activin BC, activin AE and/or activin BE), BMP15, BMP6, GDF11, BMP15, BMP10, ActRIIA, ActRIIB, ALK4, ALK5, ALK7, and one or more Smad proteins (e.g., Smads 2 and 3). In some embodiments, a GDF/BMP polynucleotide antagonist, or combination of polynucleotide antagonists, inhibits at least GDF11, GDF8, activin (activin A, activin B, activin C, and activin E), BMP6, GDF3, ActRIIA, ActRIIB, BMP10, ALK4, ALK5, ALK7, BMP15, and Smad proteins are known in the art. In addition, many different methods of generating polynucleotide antagonists are well known in the art. Therefore polynucleotide antagonists for use in accordance with this disclosure may be routinely made by the skilled person in the art based on the knowledge in the art and teachings provided herein.

ally further inhibiting one or more of GDF8, activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC, activin BC, activin AE and/or activin BE), BMP15, BMP6, GDF11, GDF3, ActRIIA, ActRIIB, ALK4, ALK5, ALK7, and one or more Smad proteins (e.g., Smads 2 and 3). In some embodiments, a GDF/BMP polynucleotide antagonist, or combination of polynucleotide antagonists, inhibits at least ActRIIA, optionally further inhibiting one or more of GDF8, activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC, activin BC, activin AE and/or activin BE), BMP15, BMP6, GDF11, GDF3, BMP10, ActRIIB, ALK4, ALK5, ALK7, and one or more Smad proteins (e.g., Smads 2 and 3). In some embodiments, a GDF/BMP polynucleotide antagonist, or combination of polynucleotide antagonists, inhibits at least ActRIIB, optionally further inhibiting one or more of GDF8, activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC, activin BC, activin AE and/or activin BE), BMP15, BMP6, GDF11, GDF3, ActRIIA, ActRIIB, ALK4, ALK5, ALK7, and one or more Smad proteins (e.g., Smads 2 and 3). In some embodiments, a GDF/BMP polynucleotide antagonist, or combination of polynucleotide antagonists, inhibits at least ALK4, optionally further inhibiting one or more of GDF8, activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC, activin BC, activin AE and/or activin BE), BMP15, BMP6, GDF11, GDF3, ActRIIA, ActRIIB, BMP10, ALK4, ALK5, ALK7, and one or more Smad proteins (e.g., Smads 2 and 3). In some embodiments, a GDF/BMP polynucleotide antagonist, or combination of polynucleotide antagonists, inhibits at least ALK5, optionally further inhibiting one or more of GDF8, activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC, activin BC, activin AE and/or activin BE), BMP15, BMP6, GDF11, GDF3, ActRIIA, ActRIIB, BMP10, ALK4, ALK5, ALK7, and one or more Smad proteins (e.g., Smads 2 and 3). In some embodiments, a GDF/BMP polynucleotide antagonist, or combination of polynucleotide antagonists, inhibits at least ALK5, optionally further inhibiting one or more of GDF8, activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC, activin BC, activin AE and/or activin BE), BMP15, BMP6, GDF11, GDF3, ActRIIA, ActRIIB, ALK4, ALK5, BMP10, and one or more Smad proteins (e.g., Smads 2 and 3). In some embodiments, a GDF/BMP polynucleotide antagonist, or combination of polynucleotide antagonists, inhibits at least ALK7, optionally further inhibiting one or more of GDF8, activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC, activin BC, activin AE and/or activin BE), BMP15, BMP6, GDF11, GDF3, ActRIIA, ActRIIB, ALK4, ALK5, BMP10, and one or more Smad proteins (e.g., Smads 2 and 3). In some embodiments, a GDF/BMP polynucleotide antagonist, or combination of polynucleotide antagonists, inhibits at least ALK7, optionally further inhibiting one or more of GDF8, activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC, activin BC, activin AE and/or activin BE), BMP15, BMP6, GDF11, GDF3, ActRIIA, ActRIIB, ALK4, ALK5, BMP10, and one or more Smad proteins (e.g., Smads 2 and 3). In some embodiments, a GDF/BMP polynucleotide antagonist, or combination of polynucleotide antagonists, as disclosed herein does not inhibit or does not substantially inhibit BMP9. In some embodiments, a GDF/BMP polynucleotide antagonist, or combination of polynucleotide antagonists, as disclosed herein does not inhibit or does not substantially inhibit activin A.

[0242] In some embodiments, the polynucleotide antagonists of the disclosure may be an antisense nucleic acid, an RNAi molecule [e.g., small interfering RNA (siRNA), small-hairpin RNA (shRNA), microRNA (miRNA)], an aptamer and/or a ribozyme. The nucleic acid and amino acid sequences of human GDF11, GDF8, activin (activin A, activin B, activin C, and activin E), BMP6, GDF3, ActRIIA, ActRIIB, BMP10, ALK4, ALK5, ALK7, BMP15, and Smad proteins are known in the art. In addition, many different methods of generating polynucleotide antagonists are well known in the art. Therefore polynucleotide antagonists for use in accordance with this disclosure may be routinely made by the skilled person in the art based on the knowledge in the art and teachings provided herein.

[0243] Antisense technology can be used to control gene expression through antisense DNA or RNA, or through triple-helix formation. Antisense techniques are discussed, for example, in Okano (1991) *J. Neurochem.* 56:560; Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, Fla. (1988). Triple-helix formation is discussed in, for instance, Cooney et al. (1988) *Science* 241:456; and Dervan et al., (1991) *Science* 251: 1300. The methods are based on binding of a polynucleotide to a complementary DNA or RNA. In some embodiments, the antisense nucleic acids comprise a single-stranded RNA or DNA sequence that is complementary to at least a portion of an RNA transcript of a gene disclosed herein. However, absolute complementarity, although preferred, is not required.

[0244] A sequence "complementary to at least a portion of an RNA," referred to herein, means a sequence having sufficient complementarity to be able to hybridize with the RNA, forming a stable duplex; in the case of double-stranded antisense nucleic acids of a gene disclosed herein, a single strand of the duplex DNA may thus be tested, or triplex formation may be assayed. The ability to hybridize will depend on both the degree of complementarity and the length of the antisense nucleic acid. Generally, the larger the hybridizing nucleic acid, the more base mismatches with an RNA it may contain and still form a stable duplex (or triplex as the case may be). One skilled in the art can ascertain a tolerable degree of mismatch by use of standard procedures to determine the melting point of the hybridized complex.

[0245] Polynucleotides that are complementary to the 5' end of the message, for example, the 5'-untranslated sequence up to and including the AUG initiation codon, should work most efficiently at inhibiting translation. However, sequences complementary to the 3'-untranslated sequences of mRNAs have been shown to be effective at inhibiting translation of mRNAs as well [see, e.g., Wagner, R., (1994) *Nature* 372:333-335]. Thus, oligonucleotides complementary to either the 5'- or 3'-non-translated, non-coding regions of a gene of the disclosure, could be used in an antisense approach to inhibit translation of an endogenous mRNA. Polynucleotides complementary to the 5'-untranslated region of the mRNA should include the complement of the AUG start codon. Antisense polynucleotides complementary to mRNA coding regions are less efficient inhibitors of translation but could be used in accordance with the methods of the present disclosure. Whether designed to hybridize to the 5', 3'- or coding region of an mRNA of the disclosure, antisense nucleic acids should be at least six nucleotides in length, and are preferably oligonucleotides ranging from 6 to about 50 nucleotides in length. In specific aspects the oligonucleotide is at least 10 nucleotides, at least 17 nucleotides, at least 25 nucleotides or at least 50 nucleotides.

[0246] In one embodiment, the antisense nucleic acid of the present disclosure is produced intracellularly by transcription from an exogenous sequence. For example, a vector or a portion thereof is transcribed, producing an antisense nucleic acid (RNA) of a gene of the disclosure. Such a vector would contain a sequence encoding the desired antisense nucleic acid. Such a vector can remain episomal or become chromosomally integrated, as long as it can be transcribed to produce the desired antisense RNA. Such vectors can be constructed by recombinant DNA technology methods standard in the art. Vectors can be

plasmid, viral, or others known in the art, used for replication and expression in vertebrate cells. Expression of the sequence encoding desired genes of the instant disclosure, or fragments thereof, can be by any promoter known in the art to act in vertebrate, preferably human cells. Such promoters can be inducible or constitutive. Such promoters include, but are not limited to, the SV40 early promoter region [see, e.g., Benoist and Chambon (1981) *Nature* 290:304-310], the promoter contained in the 3' long-terminal repeat of Rous sarcoma virus [see, e.g., Yamamoto et al. (1980) *Cell* 22:787-797], the herpes thymidine promoter [see, e.g., Wagner et al. (1981) *Proc. Natl. Acad. Sci. U.S.A.* 78:1441-1445], and the regulatory sequences of the metallothionein gene [see, e.g., Brinster, et al. (1982) *Nature* 296:39-42].

[0247] In some embodiments, the polynucleotide antagonists are interfering RNA (RNAi) molecules that target the expression of one or more of: GDF11, GDF8, activin (activin A, activin B, activin C, and activin E), BMP6, GDF3, ActRIIA, ActRIIB, BMP10, ALK4, ALK5, ALK7, BMP15, and Smad proteins. RNAi refers to the expression of an RNA which interferes with the expression of the targeted mRNA. Specifically, RNAi silences a targeted gene via interacting with the specific mRNA through a siRNA (small interfering RNA). The ds RNA complex is then targeted for degradation by the cell. An siRNA molecule is a double-stranded RNA duplex of 10 to 50 nucleotides in length, which interferes with the expression of a target gene which is sufficiently complementary (e.g. at least 80% identity to the gene). In some embodiments, the siRNA molecule comprises a nucleotide sequence that is at least 85, 90, 95, 96, 97, 98, 99, or 100% identical to the nucleotide sequence of the target gene.

[0248] Additional RNAi molecules include short-hairpin RNA (shRNA); also short-interfering hairpin and microRNA (miRNA). The shRNA molecule contains sense and antisense sequences from a target gene connected by a loop. The shRNA is transported from the nucleus into the cytoplasm, and it is degraded along with the mRNA. Pol III or U6 promoters can be used to express RNAs for RNAi. Paddison et al. [Genes & Dev. (2002) 16:948-958, 2002] have used small RNA molecules folded into hairpins as a means to affect RNAi. Accordingly, such short-hairpin RNA (shRNA) molecules are also advantageously used in the methods described herein. The length of the stem and loop of functional shRNAs varies; stem lengths can range anywhere from about 25 to about 30 nt, and loop size can range between 4 to about 25 nt without affecting silencing activity. While not wishing to be bound by any particular theory, it is believed that these shRNAs resemble the double-stranded RNA (dsRNA) products of the DICER RNase and, in any event, have the same capacity for inhibiting expression of a specific gene. The shRNA can be expressed from a lentiviral vector. An miRNA is a single-stranded RNA of about 10 to 70 nucleotides in length that are initially transcribed as pre-miRNA characterized by a "stem-loop" structure, which are subsequently processed into mature miRNA after further processing through the RISC.

[0249] Molecules that mediate RNAi, including without limitation siRNA, can be produced *in vitro* by chemical synthesis (Hohjoh, FEBS Lett 521:195-199, 2002), hydrolysis of dsRNA (Yang et al., Proc Natl Acad Sci USA 99:9942-9947, 2002), by *in vitro* transcription with T7 RNA polymerase (Donzeet et al., Nucleic Acids Res 30:e46, 2002; Yu et al., Proc Natl Acad Sci USA 99:6047-6052, 2002), and

by hydrolysis of double-stranded RNA using a nuclease such as *E. coli* RNase III (Yang et al., Proc Natl Acad Sci USA 99:9942-9947, 2002).

[0250] According to another aspect, the disclosure provides polynucleotide antagonists including but not limited to, a decoy DNA, a double-stranded DNA, a single-stranded DNA, a complexed DNA, an encapsulated DNA, a viral DNA, a plasmid DNA, a naked RNA, an encapsulated RNA, a viral RNA, a double-stranded RNA, a molecule capable of generating RNA interference, or combinations thereof.

[0251] In some embodiments, the polynucleotide antagonists of the disclosure are aptamers. Aptamers are nucleic acid molecules, including double-stranded DNA and single-stranded RNA molecules, which bind to and form tertiary structures that specifically bind to a target molecule. The generation and therapeutic use of aptamers are well established in the art (see, e.g., U.S. Pat. No. 5,475,096). Additional information on aptamers can be found in U.S. Patent Application Publication No. 20060148748. Nucleic acid aptamers are selected using methods known in the art, for example via the Systematic Evolution of Ligands by Exponential Enrichment (SELEX) process. SELEX is a method for the in vitro evolution of nucleic acid molecules with highly specific binding to target molecules as described in, e.g., U.S. Pat. Nos. 5,475,096; 5,580,737; 5,567,588; 5,707,796; 5,763,177; 6,011,577; and 6,699,843. Another screening method to identify aptamers is described in U.S. Pat. No. 5,270,163. The SELEX process is based on the capacity of nucleic acids for forming a variety of two- and three-dimensional structures, as well as the chemical versatility available within the nucleotide monomers to act as ligands (form specific binding pairs) with virtually any chemical compound, whether monomeric or polymeric, including other nucleic acid molecules and polypeptides. Molecules of any size or composition can serve as targets. The SELEX method involves selection from a mixture of candidate oligonucleotides and step-wise iterations of binding, partitioning and amplification, using the same general selection scheme, to achieve desired binding affinity and selectivity. Starting from a mixture of nucleic acids, which can comprise a segment of randomized sequence, the SELEX method includes steps of contacting the mixture with the target under conditions favorable for binding; partitioning unbound nucleic acids from those nucleic acids which have bound specifically to target molecules; dissociating the nucleic acid-target complexes; amplifying the nucleic acids dissociated from the nucleic acid-target complexes to yield a ligand enriched mixture of nucleic acids. The steps of binding, partitioning, dissociating and amplifying are

repeated through as many cycles as desired to yield nucleic acid ligands which bind with high affinity and specificity to the target molecule.

[0252] Typically, such binding molecules are separately administered to the animal [see, e.g., O'Connor (1991) J. Neurochem. 56:560], but such binding molecules can also be expressed in vivo from polynucleotides taken up by a host cell and expressed in vivo [see, e.g., Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, Fla. (1988)].

7. Follistatin and FLRG Antagonists

[0253] In other aspects, a GDF/BMP antagonist is a follistatin or FLRG polypeptide. As described herein, follistatin and/or FLRG polypeptides may be used treat, prevent, or reduce the progression rate and/or severity of pulmonary hypertension (PH), particularly treating, preventing or reducing the progression rate and/or severity of one or more PH-associated complications.

[0254] The term “follistatin polypeptide” includes polypeptides comprising any naturally occurring polypeptide of follistatin as well as any variants thereof (including mutants, fragments, fusions, and peptidomimetic forms) that retain a useful activity, and further includes any functional monomer or multimer of follistatin. In certain preferred embodiments, follistatin polypeptides of the disclosure bind to and/or inhibit activin activity, particularly activin A. Variants of follistatin polypeptides that retain activin binding properties can be identified based on previous studies involving follistatin and activin interactions. For example, WO2008/030367 discloses specific follistatin domains (“FSDs”) that are shown to be important for activin binding. As shown below in SEQ ID NOS: 28-30, the follistatin N-terminal domain (“FSND” SEQ ID NO: 28), FSD2 (SEQ ID NO: 30), and to a lesser extent FSD1 (SEQ ID NO: 29) represent exemplary domains within follistatin that are important for activin binding. In addition, methods for making and testing libraries of polypeptides are described above in the context of ActRII polypeptides, and such methods also pertain to making and testing variants of follistatin. Follistatin polypeptides include polypeptides derived from the sequence of any known follistatin having a sequence at least about 80% identical to the sequence of a follistatin polypeptide, and optionally at least 85%, 90%, 95%, 96%, 97%, 98%, 99% or greater identity. Examples of follistatin polypeptides include the mature follistatin polypeptide or shorter isoforms or other variants of the human follistatin precursor polypeptide (SEQ ID NO: 26) as described, for example, in WO2005/025601.

[0255] The human follistatin precursor polypeptide isoform FST344 is as follows:

(SEQ ID NO: 26; NCBI Reference No. NP_037541.1)
 1 MVRARHQPGG LCLLLLLLQ FMEDRSAQAG NCWLRAKNG RCQVLYKTEL
 51 SKEECCSTGR LSTSWTEEDV NDNTLFKWMI FNNGAPNCIP CKETCENVDC
 101 GPGKKCRMNK KNKPRCVCAP DCSNITWKGP VCGLDGKTYR NECALLKARC
 151 KEQPELEVQY QGRCKKTCDR VFCPGSSTCV VDQTNNAYCV TCNRICPEPA
 201 SSEQYLCGND GVTYSSACHL RKATCLLGRS IGLAYEGKCI KAKSCEDIQC
 251 TGGKKCLWDF KVGRGRCSLC DELCPDSKSD EPVCASDNAT YASECAMKEA
 301 ACSSGVLLLEV KHSGSCNSIS EDTEEEEDE DQDYSFPPISS ILEW

[0256] The signal peptide is underlined; also underlined above are the last 27 residues which represent the C-terminal extension distinguishing this follistatin isoform from the shorter follistatin isoform FST317 shown below.

[0257] The human follistatin precursor polypeptide isoform FST317 is as follows:

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(SEQ ID NO: 27; NCBI Reference No. NP_006341.1)
1 MVRARHQPGG LCLLLLLLCQ FMEDRSAQAG NCWLRQAKNG RCQVLYKTEL
51 SKEECCSTGR LSTSWEEDV NDNTLFKWMI FNNGAPNCIP CKETCENVDC
101 GPGKKCRMNK KNKPRCVCAP DCSNITWKGP VCGLDGKTYR NECALLKARC
151 KEQPELEVQY QGRCKKTCDR VFCPGSSTCV VDQTNNAYCV TCNRICPEPA
201 SSEQYLCGND GVTYSSACHL RKATCLLGRS IGLAYEGKCI KAKSCEDIQC
251 TGGKKCLWDF KVGRGRCSLC DELCPDSKSD EPVCASDNAT YASECAMKEA
301 ACSSGVILLEV KHSGSCN
```

The signal peptide is underlined.

[0258] The follistatin N-terminal domain (FSND) sequence is as follows:

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(SEQ ID NO: 28; FSND)
GNCWLRQAKNGRCQVLYKTELSKEECCSTGRLSTSWEEDVNNDNTLFKW
MIFNGGAPNCIPCK
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methods for making and testing libraries of polypeptides are described above in the context of ActRII polypeptides and such methods also pertain to making and testing variants of FLRG. FLRG polypeptides include polypeptides derived from the sequence of any known FLRG having a sequence at least about 80% identical to the sequence of an FLRG polypeptide, and optionally at least 85%, 90%, 95%, 97%, 99% or greater identity.

[0261] The human FLRG precursor (follistatin-related protein 3 precursor) polypeptide is as follows:

```
(SEQ ID NO: 31; NCBI Reference No. NP_005851.1)
1 MRPGAPGPLW PLPWGALAWA VGFVSSMGSG NPAPGGVCWL QQQEAATCSL
51 VLQTDVTRAEC CASGNIDTA WSNLTHPGNK INLLGFLGLV HCLPCKDSCD
101 GVECGPGKAC RMLGGPRCE CAPDCSGLPA RLQVCGSDGA TYRDECCELRA
151 ARCRGHPDLS VMYRGRCRKS CEHVVCPRPQ SCVVDQTGSA HCVVCRAAPC
201 PVPSSPGQEL CGNNNNVTYIS SCHMRQATCF LGRSIGVRHA GSCAGTPEEP
251 PGGESAEEEE NFV
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[0259] The FSD1 and FSD2 sequences are as follows:

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(SEQ ID NO: 29; FSD1)
ETCENVDCGPGKKCRMNKKNKPRC
(SEQ ID NO: 30; FSD2)
KTCRDVFCPGSSTCVVDQTNNAYCV
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[0260] In other aspects, an agent for use in accordance with the methods disclosed herein is a follistatin-like related gene (FLRG), also known as follistatin-related protein 3 (FSTL3). The term "FLRG polypeptide" includes polypeptides comprising any naturally occurring polypeptide of FLRG as well as any variants thereof (including mutants, fragments, fusions, and peptidomimetic forms) that retain a useful activity. In certain preferred embodiments, FLRG polypeptides of the disclosure bind to and/or inhibit activin activity, particularly activin A. Variants of FLRG polypeptides that retain activin binding properties can be identified using routine methods to assay FLRG and activin interactions (see, e.g., U.S. Pat. No. 6,537,966). In addition,

The signal peptide is underlined.

[0262] In certain embodiments, functional variants or modified forms of the follistatin polypeptides and FLRG polypeptides include fusion proteins having at least a portion of the follistatin polypeptide or FLRG polypeptide and one or more fusion domains, such as, for example, domains that facilitate isolation, detection, stabilization or multimerization of the polypeptide. Suitable fusion domains are discussed in detail above with reference to the ActRII polypeptides. In some embodiment, an antagonist agent of the disclosure is a fusion protein comprising an activin-binding portion of a follistatin polypeptide fused to an Fc domain. In another embodiment, an antagonist agent of the disclosure is a fusion protein comprising an activin binding portion of an FLRG polypeptide fused to an Fc domain.

8. Screening Assays

[0263] In certain aspects, the present disclosure relates to the use of the subject GDF/BMP antagonists (e.g., ActRII polypeptides and variants thereof) to identify compounds

(agents) which may be used to treat, prevent, or reduce the progression rate and/or severity of pulmonary hypertension (PH), particularly treating, preventing or reducing the progression rate and/or severity of one or more PH-associated complications.

[0264] There are numerous approaches to screening for therapeutic agents for treating PH by targeting signaling (e.g., Smad signaling) of one or more GDF/BMP ligands. In certain embodiments, high-throughput screening of compounds can be carried out to identify agents that perturb GDF/BMP ligands-mediated effects on a selected cell line. In certain embodiments, the assay is carried out to screen and identify compounds that specifically inhibit or reduce binding of an GDF/BMP ligand (e.g., activin A, activin B, activin AB, activin C, GDF3, BMP6, GDF8, GDF15, GDF11 or BMP10) to its binding partner, such as an a type II receptor (e.g., ActRIIA and/or ActRIIB). Alternatively, the assay can be used to identify compounds that enhance binding of a GDF/BMP ligand to its binding partner such as a type II receptor. In a further embodiment, the compounds can be identified by their ability to interact with a type II receptor.

[0265] A variety of assay formats will suffice and, in light of the present disclosure, those not expressly described herein will nevertheless be comprehended by one of ordinary skill in the art. As described herein, the test compounds (agents) of the invention may be created by any combinatorial chemical method. Alternatively, the subject compounds may be naturally occurring biomolecules synthesized in vivo or in vitro. Compounds (agents) to be tested for their ability to act as modulators of tissue growth can be produced, for example, by bacteria, yeast, plants or other organisms (e.g., natural products), produced chemically (e.g., small molecules, including peptidomimetics), or produced recombinantly. Test compounds contemplated by the present invention include non-peptidyl organic molecules, peptides, polypeptides, peptidomimetics, sugars, hormones, and nucleic acid molecules. In certain embodiments, the test agent is a small organic molecule having a molecular weight of less than about 2,000 Daltons.

[0266] The test compounds of the disclosure can be provided as single, discrete entities, or provided in libraries of greater complexity, such as made by combinatorial chemistry. These libraries can comprise, for example, alcohols, alkyl halides, amines, amides, esters, aldehydes, ethers and other classes of organic compounds. Presentation of test compounds to the test system can be in either an isolated form or as mixtures of compounds, especially in initial screening steps. Optionally, the compounds may be optionally derivatized with other compounds and have derivatizing groups that facilitate isolation of the compounds. Non-limiting examples of derivatizing groups include biotin, fluorescein, digoxigenin, green fluorescent protein, isotopes, polyhistidine, magnetic beads, glutathione S-transferase (GST), photoactivatable crosslinkers or any combinations thereof.

[0267] In many drug-screening programs which test libraries of compounds and natural extracts, high-throughput assays are desirable in order to maximize the number of compounds surveyed in a given period of time. Assays which are performed in cell-free systems, such as may be derived with purified or semi-purified proteins, are often preferred as "primary" screens in that they can be generated to permit rapid development and relatively easy detection of

an alteration in a molecular target which is mediated by a test compound. Moreover, the effects of cellular toxicity or bioavailability of the test compound can be generally ignored in the in vitro system, the assay instead being focused primarily on the effect of the drug on the molecular target as may be manifest in an alteration of binding affinity between a GDF/BMP ligand (e.g., activin A, activin B, activin AB, activin C, GDF3, GDF15, GDF11, GDF3, BMP6, or BMP10) to its binding partner, such as an a type II receptor (e.g., ActRIIA and/or ActRIIB).

[0268] Merely to illustrate, in an exemplary screening assay of the present disclosure, the compound of interest is contacted with an isolated and purified ActRIIB polypeptide which is ordinarily capable of binding to an ActRIIB ligand, as appropriate for the intention of the assay. To the mixture of the compound and ActRIIB polypeptide is then added to a composition containing an ActRIIB ligand (e.g., GDF11). Detection and quantification of ActRIIB/ActRIIB-ligand complexes provides a means for determining the compound's efficacy at inhibiting (or potentiating) complex formation between the ActRIIB polypeptide and its binding protein. The efficacy of the compound can be assessed by generating dose-response curves from data obtained using various concentrations of the test compound. Moreover, a control assay can also be performed to provide a baseline for comparison. For example, in a control assay, isolated and purified ActRIIB ligand is added to a composition containing the ActRIIB polypeptide, and the formation of ActRIIB/ActRIIB ligand complex is quantitated in the absence of the test compound. It will be understood that, in general, the order in which the reactants may be admixed can be varied, and can be admixed simultaneously. Moreover, in place of purified proteins, cellular extracts and lysates may be used to render a suitable cell-free assay system.

[0269] Complex formation between GDF/BMP ligand and its binding protein may be detected by a variety of techniques. For instance, modulation of the formation of complexes can be quantitated using, for example, detectably labeled proteins such as radiolabeled (e.g., ³²P, ³⁵S, ¹⁴C or ³H), fluorescently labeled (e.g., FITC), or enzymatically labeled ActRIIB polypeptide and/or its binding protein, by immunoassay, or by chromatographic detection.

[0270] In certain embodiments, the present disclosure contemplates the use of fluorescence polarization assays and fluorescence resonance energy transfer (FRET) assays in measuring, either directly or indirectly, the degree of interaction between a GDF/BMP ligand and its binding protein. Further, other modes of detection, such as those based on optical waveguides (see, e.g., PCT Publication WO 96/26432 and U.S. Pat. No. 5,677,196), surface plasmon resonance (SPR), surface charge sensors, and surface force sensors, are compatible with many embodiments of the disclosure.

[0271] Moreover, the present disclosure contemplates the use of an interaction trap assay, also known as the "two-hybrid assay," for identifying agents that disrupt or potentiate interaction between a GDF/BMP ligand and its binding partner. See, e.g., U.S. Pat. No. 5,283,317; Zervos et al. (1993) Cell 72:223-232; Madura et al. (1993) J Biol Chem 268:12046-12054; Bartel et al. (1993) Biotechniques 14:920-924; and Iwabuchi et al. (1993) Oncogene 8:1693-1696). In a specific embodiment, the present disclosure contemplates the use of reverse two-hybrid systems to identify compounds (e.g., small molecules or peptides) that

dissociate interactions between a GDF/BMP ligand and its binding protein [see, e.g., Vidal and Legrain, (1999) Nucleic Acids Res 27:919-29; Vidal and Legrain, (1999) Trends Biotechnol 17:374-81; and U.S. Pat. Nos. 5,525,490; 5,955,280; and 5,965,368].

[0272] In certain embodiments, the subject compounds are identified by their ability to interact with a GDF/BMP ligand. The interaction between the compound and the GDF/BMP ligand may be covalent or non-covalent. For example, such interaction can be identified at the protein level using *in vitro* biochemical methods, including photo-crosslinking, radiolabeled ligand binding, and affinity chromatography [see, e.g., Jakoby W B et al. (1974) Methods in Enzymology 46:1]. In certain cases, the compounds may be screened in a mechanism-based assay, such as an assay to detect compounds which bind to a GDF/BMP ligand. This may include a solid-phase or fluid-phase binding event. Alternatively, the gene encoding GDF/BMP ligand can be transfected with a reporter system (e.g., β -galactosidase, luciferase, or green fluorescent protein) into a cell and screened against the library preferably by high-throughput screening or with individual members of the library. Other mechanism-based binding assays may be used; for example, binding assays which detect changes in free energy. Binding assays can be performed with the target fixed to a well, bead or chip or captured by an immobilized antibody or resolved by capillary electrophoresis. The bound compounds may be detected usually using colorimetric endpoints or fluorescence or surface plasmon resonance.

9. Therapeutic Uses

[0273] In part, the present disclosure relates to methods of treating pulmonary hypertension (e.g., pulmonary arterial hypertension) comprising administering to a patient in need thereof an effective amount of a GDF/BMP antagonist (e.g., an antagonist of one or more of activin, GDF8, GDF11, GDF3, BMP6, BMP15, BMP10, ActRIIA, ActRIIB, ALK4, ALK5, ALK7, and one or more Smad proteins). In some embodiments, the disclosure contemplates methods of treating one or more complications of pulmonary hypertension (e.g., smooth muscle and/or endothelial cell proliferation in the pulmonary artery, angiogenesis in the pulmonary artery, dyspnea, chest pain, pulmonary vascular remodeling, right ventricular hypertrophy, and pulmonary fibrosis) comprising administering to a patient in need thereof an effective amount of a GDF/BMP antagonist. In some embodiments, the disclosure contemplates methods of preventing one or more complications of pulmonary hypertension comprising administering to a patient in need thereof an effective amount of a GDF/BMP antagonist. In some embodiments, the disclosure contemplates methods of reducing the progression rate of pulmonary hypertension comprising administering to a patient in need thereof an effective amount of a GDF/BMP antagonist. In some embodiments, the disclosure contemplates methods of reducing the progression rate of one or more complications of pulmonary hypertension comprising administering to a patient in need thereof an effective amount of a GDF/BMP antagonist. In some embodiments, the disclosure contemplates methods of reducing the severity of pulmonary hypertension comprising administering to a patient in need thereof an effective amount of a GDF/BMP antagonist. In some embodiments, the disclosure contemplates methods of reducing the severity of one or more complications of pulmonary hypertension comprising

administering to a patient in need thereof an effective amount of a GDF/BMP antagonist. Optionally, methods disclosed herein for treating, preventing, or reducing the progression rate and/or severity of pulmonary hypertension, particularly treating, preventing, or reducing the progression rate and/or severity of one or more complications of pulmonary hypertension, may further comprise administering to the patient one or more supportive therapies or additional active agents for treating pulmonary hypertension. For example, the patient also may be administered one or more supportive therapies or active agents selected from the group consisting of: prostacyclin and derivatives thereof (e.g., epoprostenol, treprostinil, and iloprost); prostacyclin receptor agonists (e.g., selexipag); endothelin receptor antagonists (e.g., thelin, ambrisentan, macitentan, and bosentan); calcium channel blockers (e.g., amlodipine, diltiazem, and nifedipine); anticoagulants (e.g., warfarin); diuretics; oxygen therapy; atrial septostomy; pulmonary thromboendarterectomy; phosphodiesterase type 5 inhibitors (e.g., sildenafil and tadalafil); activators of soluble guanylate cyclase (e.g., cinaciguat and riociguat); ASK-1 inhibitors (e.g., CIIA; SCH79797; GS-4997; MSC2032964A; 3H-naphtho[1,2,3-de]quinaline-2,7-diones; NQDI-1; 2-thioxo-thiazolidines, 5-bromo-3-(4-oxo-2-thioxo-thiazolidine-5-ylidene)-1,3-dihydro-indol-2-one); NF- κ B antagonists (e.g., dh404, CDDO-epoxide; 2,2-difluoropropionamide; C28 imidazole (CDDO-Im); 2-cyano-3,12-dioxolean-1,9-dien-28-oic acid (CDDO); 3-Acetyloleanolic Acid; 3-Triflouroacetyloleanolic Acid; 28-Methyl-3-acetyloleanane; 28-Methyl-3-trifluoroacetyloleanane; 28-Methyloxyoleanolic Acid; SZC014; SCZ015; SZC017; PEGylated derivatives of oleanolic acid; 3-O-(β -D-glucopyranosyl) oleanolic acid; 3-O-[β -D-glucopyranosyl-(1 \rightarrow 3)- β -D-glucopyranosyl]oleanolic acid; 3-O-[β -D-glucopyranosyl-(1 \rightarrow 2)- β -D-glucopyranosyl]oleanolic acid; 3-O-[β -D-glucopyranosyl-(1 \rightarrow 3)- β -D-glucopyranosyl]oleanolic acid 28-O- β -D-glucopyranosyl ester; 3-O-[β -D-glucopyranosyl-(1 \rightarrow 2)- β -D-glucopyranosyl]oleanolic acid 28-O- β -D-glucopyranosyl ester; 3-O-[α -L-rhamnopyranosyl-(1 \rightarrow 3)- β -D-glucuronopyranosyl]oleanolic acid; 3-O-[α -L-rhamnopyranosyl-(1 \rightarrow 3)- β -D-glucuronopyranosyl]OLEANOLIC ACID; 3-O-[β -D-glucopyranosyl-(1 \rightarrow 3)- β -D-glucopyranosyl ester; 28-O- β -D-glucopyranosyl ester; 28-O- β -D-glucopyranosyl-oleanolic acid; 3-O- β -D-glucopyranosyl(1 \rightarrow 3)- β -D-glucopyranosiduronic acid (CS1); oleanolic acid 3-O- β -D-glucopyranosyl(1 \rightarrow 3)- β -D-glucopyranosiduronic acid (CS2); methyl 3,11-dioxolean-12-en-28-olate (DIOXOL); ZCVI₄-2; Benzyl 3-dehydr-oxy-1,2,5-oxadiazolo[3',4':2,3]oleanolate) lung and/or heart transplantation. In some embodiment, the patient may also be administered a BMP9 polypeptide. In some embodiments the BMP9 polypeptide is a mature BMP9 polypeptide. In some embodiments, the BMP9 polypeptide comprises a BMP9 prodomain polypeptide. In some embodiments, the BMP9 polypeptide is administered in a pharmaceutical preparation, which optionally may comprise a BMP9 prodomain polypeptide. In such BMP9 pharmaceutical preparations comprising a BMP9 prodomain polypeptide, the BMP9 polypeptide may be noncovalently associated with the BMP9 prodomain polypeptide. In some embodiments, BMP9 pharmaceutical preparations are substantially free, or does not comprise, of BMP9 prodomain polypeptide. BMP9 polypeptides (mature and pro-polypeptides), BMP9 prodomain polypeptides, pharmaceutical compositions comprising the same as well as method of generative such polypeptides and

pharmaceutical compositions are described in, for example, WO 2013/152213, which is incorporated by reference herein in its entirety. As used herein, a therapeutic that "prevents" a disorder or condition refers to a compound that, in a statistical sample, reduces the occurrence of the disorder or condition in the treated sample relative to an untreated control sample, or delays the onset or reduces the severity of one or more symptoms of the disorder or condition relative to the untreated control sample.

[0274] In some embodiments, the present disclosure relates to methods of treating an interstitial lung disease (e.g., idiopathic pulmonary fibrosis) comprising administering to a patient in need thereof an effective amount of any of the GDF/BMP antagonists disclosed herein (e.g., an antagonist of one or more of activin, GDF8, GDF11, GDF3, BMP6, BMP15, BMP10, ActRIIA, ActRIIB, ALK4, ALK5, ALK7, and one or more Smad proteins). In some embodiments, the interstitial lung disease is pulmonary fibrosis. In some embodiments, the interstitial lung disease is caused by any one of the following: silicosis, asbestosis, berylliosis, hypersensitivity pneumonitis, drug use (e.g., antibiotics, chemotherapeutic drugs, antiarrhythmic agents, statins), systemic sclerosis, polymyositis, dermatomyositis, systemic lupus erythematosus, rheumatoid arthritis, an infection (e.g., atypical pneumonia, *pneumocystis* pneumonia, tuberculosis, *Chlamydia trachomatis*, and/or respiratory syncytial virus), lymphangitic carcinomatosis, cigarette smoking, or developmental disorders. In some embodiments, the interstitial lung disease is idiopathic (e.g., sarcoidosis, idiopathic pulmonary fibrosis, Hamman-Rich syndrome, and/or antisynthetase syndrome). In particular embodiments, the interstitial lung disease is idiopathic pulmonary fibrosis. In some embodiments, the treatment for idiopathic pulmonary fibrosis is administered in combination with an additional therapeutic agent. In some embodiments, the additional therapeutic agent is selected from the group consisting of: pirfenidone, N-acetylcysteine, prednisone, azathioprine, mirtedanib, derivatives thereof and combinations thereof.

[0275] The term "treating" as used herein includes amelioration or elimination of the condition once it has been established. In either case, prevention or treatment may be discerned in the diagnosis provided by a physician or other health care provider and the intended result of administration of the therapeutic agent.

[0276] In general, treatment or prevention of a disease or condition as described in the present disclosure is achieved by administering a GDF/BMP antagonist in an effective amount. An effective amount of an agent refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic or prophylactic result. A therapeutically effective amount of an agent of the present disclosure may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the agent to elicit a desired response in the individual. A prophylactically effective amount refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result.

[0277] The terms "subject," an "individual," or a "patient" are interchangeable throughout the specification and generally refer to mammals. Mammals include, but are not limited to, domesticated animals (e.g., cows, sheep, cats, dogs, and horses), primates (e.g., humans and non-human primates such as monkeys), rabbits, and rodents (e.g., mice and rats).

[0278] Pulmonary hypertension (PH) has been previously classified as primary (idiopathic) or secondary. Recently, the World Health Organization (WHO) has classified pulmonary hypertension into five groups: Group 1: pulmonary arterial hypertension (PAH); Group 2: pulmonary hypertension with left heart disease; Group 3: pulmonary hypertension with lung disease and/or hypoxemia; Group 4: pulmonary hypertension due to chronic thrombotic and/or embolic disease; and Group 5: miscellaneous conditions (e.g., sarcoidosis, histiocytosis X, lymphangiomatosis and compression of pulmonary vessels). See, for example, Rubin (2004) Chest 126:7-10.

[0279] In certain aspects, the disclosure relates to methods of treating, preventing, or reducing the progression rate and/or severity of pulmonary hypertension (e.g., treating, preventing, or reducing the progression rate and/or severity of one or more complications of pulmonary hypertension) comprising administering to a patient in need thereof an effective amount of a GDF/BMP antagonist (e.g., an antagonist of one or more of activin, GDF8, GDF11, GDF3, BMP6, BMP15, BMP10, ActRIIA, ActRIIB, ALK4, ALK5, ALK7, and one or more Smad proteins). In some embodiments, the method relates to pulmonary hypertension patients that have pulmonary arterial hypertension. In some embodiments, the method relates to pulmonary hypertension patients that have pulmonary hypertension with left heart disease. In some embodiments, the method relates to pulmonary hypertension patients that have lung disease and/or hypoxemia. In some embodiments, the method relates to pulmonary hypertension patients that have chronic thrombotic and/or embolic disease. In some embodiments, the method relates to pulmonary hypertension patients that have sarcoidosis, histiocytosis X, or lymphangiomatosis and compression of pulmonary vessels.

[0280] Pulmonary arterial hypertension is a serious, progressive and life-threatening disease of the pulmonary vasculature, characterized by profound vasoconstriction and an abnormal proliferation of smooth muscle cells in the walls of the pulmonary arteries. Severe constriction of the blood vessels in the lungs leads to very high pulmonary arterial pressures. These high pressures make it difficult for the heart to pump blood through the lungs to be oxygenated. Patients with PAH suffer from extreme shortness of breath as the heart struggles to pump against these high pressures. Patients with PAH typically develop significant increases in pulmonary vascular resistance (PVR) and sustained elevations in pulmonary artery pressure (PAP), which ultimately lead to right ventricular failure and death. Patients diagnosed with PAH have a poor prognosis and equally compromised quality of life, with a mean life expectancy of 2 to 5 years from the time of diagnosis if untreated.

[0281] A variety of factors contribute to the pathogenesis of pulmonary hypertension including proliferation of pulmonary cells which can contribute to vascular remodeling (i.e., hyperplasia). For example, pulmonary vascular remodeling occurs primarily by proliferation of arterial endothelial cells and smooth muscle cells of patients with pulmonary hypertension. Overexpression of various cytokines is believed to promote pulmonary hypertension. Further, it has been found that pulmonary hypertension may rise from the hyperproliferation of pulmonary arterial smooth cells and pulmonary endothelial cells. Still further, advanced PAH may be characterized by muscularization of distal pulmonary arterioles, concentric intimal thickening, and obstruc-

tion of the vascular lumen by proliferating endothelial cells. Pietra et al., *J. Am. Coll. Cardiol.*, 43:255-325 (2004).

[0282] In certain aspects, the disclosure relates to methods of treating, preventing, or reducing the progression rate and/or severity of pulmonary hypertension (e.g., treating, preventing, or reducing the progression rate and/or severity of one or more complications of pulmonary hypertension) comprising administering to a patient in need thereof an effective amount of a GDF/BMP antagonist (e.g., an antagonist of one or more of activin, GDF8, GDF11, GDF3, BMP6, BMP15, BMP10, ActRIIA, ActRIIB, ALK4, ALK5, ALK7, and one or more Smad proteins), wherein the patient has resting pulmonary arterial pressure (PAP) of at least 25 mm Hg (e.g., 25, 30, 35, 40, 45, or 50 mm Hg). In some embodiments, the method relates to patients having a resting PAP of at least 25 mm Hg. In some embodiments, the method relates to patients having a resting PAP of at least 30 mm Hg. In some embodiments, the method relates to patients having a resting PAP of at least 35 mm Hg. In some embodiments, the method relates to patients having a resting PAP of at least 40 mm Hg. In some embodiments, the method relates to patients having a resting PAP of at least 45 mm Hg. In some embodiments, the method relates to patients having a resting PAP of at least 50 mm Hg.

[0283] In some embodiments, the disclosure relates to methods of adjusting one or more hemodynamic parameters in the PH patient toward a more normal level (e.g., normal as compared to healthy people of similar age and sex), comprising administering to a patient in need thereof an effective amount of a GDF/BMP antagonist (e.g., an antagonist of one or more of activin, GDF8, GDF11, GDF3, BMP6, BMP15, BMP10, ActRIIA, ActRIIB, ALK4, ALK5, ALK7, and one or more Smad proteins). In some embodiments, the method relates to reducing PAP. In some embodiments, the method relates to reducing the patient's PAP by at least 3 mmHg. In certain embodiments, the method relates to reducing the patient's PAP by at least 5 mmHg. In certain embodiments, the method relates to reducing the patient's PAP by at least 7 mmHg. In certain embodiments, the method relates to reducing the patient's PAP by at least 10 mmHg. In certain embodiments, the method relates to reducing the patient's PAP by at least 12 mmHg. In certain embodiments, the method relates to reducing the patient's PAP by at least 15 mmHg. In certain embodiments, the method relates to reducing the patient's PAP by at least 20 mmHg. In certain embodiments, the method relates to reducing the patient's PAP by at least 25 mmHg. In some embodiments, the method relates to reducing pulmonary vascular resistance (PVR). In some embodiments, the method relate to increasing pulmonary capillary wedge pressure (PCWP). In some embodiments, the method relate to increasing left ventricular end-diastolic pressure (LVEDP).

[0284] In certain aspects, the disclosure relates to methods of treating, preventing, or reducing the progression rate and/or severity of one or more complications of pulmonary hypertension comprising administering to a patient in need thereof an effective amount of a GDF/BMP antagonist (e.g., an antagonist of one or more of activin, GDF8, GDF11, GDF3, BMP6, BMP15, BMP10, ActRIIA, ActRIIB, ALK4, ALK5, ALK7, and one or more Smad proteins). In some embodiments, the method relates to treating, preventing, or reducing the progression rate and/or severity of cell proliferation in the pulmonary artery of a pulmonary hypertension

patient. In some embodiments, the method relates to treating, preventing, or reducing the progression rate and/or severity of smooth muscle and/or endothelial cells proliferation in the pulmonary artery of a pulmonary hypertension patient. In some embodiments, the method relates to treating, preventing, or reducing the progression rate and/or severity of angiogenesis in the pulmonary artery of a pulmonary hypertension patient. In some embodiments, the method relates to increasing physical activity of a patient having pulmonary hypertension. In some embodiments, the method relates to treating, preventing, or reducing the progression rate and/or severity of dyspnea in a pulmonary hypertension patient. In some embodiments, the method relates to treating, preventing, or reducing the progression rate and/or severity of chest pain in a pulmonary hypertension patient. In some embodiments, the method relates to treating, preventing, or reducing the progression rate and/or severity of fatigue in a pulmonary hypertension patient. In some embodiments, the method relates to treating, preventing, or reducing the progression rate and/or severity of pulmonary fibrosis in a pulmonary hypertension patient. In some embodiments, the method relates to treating, preventing, or reducing the progression rate and/or severity of fibrosis in a pulmonary hypertension patient. In some embodiments, the method relates to treating, preventing, or reducing the progression rate and/or severity of pulmonary vascular remodeling in a pulmonary hypertension patient. In some embodiments, the method relates to treating, preventing, or reducing the progression rate and/or severity of right ventricular hypertrophy in a pulmonary hypertension patient.

[0285] In certain aspects, the disclosure relates to methods of increasing exercise capacity in a patient having pulmonary hypertension comprising administering to a patient in need thereof an effective amount of a GDF/BMP antagonist (e.g., an antagonist of one or more of activin, GDF8, GDF11, GDF3, BMP6, BMP15, BMP10, ActRIIA, ActRIIB, ALK4, ALK5, ALK7, and one or more Smad proteins). Any suitable measure of exercise capacity can be used. For example, exercise capacity in a 6-minute walk test (6MWT), which measures how far the subject can walk in 6 minutes, i.e., the 6-minute walk distance (6MWD), is frequently used to assess pulmonary hypertension severity and disease progression. The Borg dyspnea index (BDI) is a numerical scale for assessing perceived dyspnea (breathing discomfort). It measures the degree of breathlessness, for example, after completion of the 6MWT, where a BDI of 0 indicates no breathlessness and 10 indicates maximum breathlessness. In some embodiments, the method relates to increasing 6MWD by at least 10 meters in the patient having pulmonary hypertension. In some embodiments, the method relates to increasing 6MWD by at least 20 meters in the patient having pulmonary hypertension. In some embodiments, the method relates to increasing 6MWD by at least 30 meters in the patient having pulmonary hypertension. In some embodiments, the method relates to increasing 6MWD by at least 40 meters in the patient having pulmonary hypertension. In some embodiments, the method relates to increasing 6MWD by at least 50 meters in the patient having pulmonary hypertension. In some embodiments, the method relates to increasing 6MWD by at least 60 meters in the patient having pulmonary hypertension. In some embodiments, the method relates to increasing 6MWD by at least 70 meters in the patient having pulmonary hypertension. In

some embodiments, the method relates to increasing 6MWD by at least 80 meters in the patient having pulmonary hypertension. In some embodiments, the method relates to increasing 6MWD by at least 90 meters in the patient having pulmonary hypertension. In some embodiments, the method relates to increasing 6MWD by at least 100 meters in the patient having pulmonary hypertension. In some embodiments, the method relate to lowering BDI by at least 0.5 index points in the patient having pulmonary hypertension. In some embodiments, the method relate to lowering BDI by at least 1 index points in the patient having pulmonary hypertension. In some embodiments, the method relate to lowering BDI by at least 1.5 index points in the patient having pulmonary hypertension. In some embodiments, the method relate to lowering BDI by at least 2 index points in the patient having pulmonary hypertension. In some embodiments, the method relate to lowering BDI by at least 2.5 index points in the patient having pulmonary hypertension. In some embodiments, the method relate to lowering BDI by at least 3 index points in the patient having pulmonary hypertension. In some embodiments, the method relate to lowering BDI by at least 3.5 index points in the patient having pulmonary hypertension. In some embodiments, the method relate to lowering BDI by at least 4 index points in the patient having pulmonary hypertension. In some embodiments, the method relate to lowering BDI by at least 4.5 index points in the patient having pulmonary hypertension. In some embodiments, the method relate to lowering BDI by at least 5 index points in the patient having pulmonary hypertension. In some embodiments, the method relate to lowering BDI by at least 5.5 index points in the patient having pulmonary hypertension. In some embodiments, the method relate to lowering BDI by at least 6 index points in the patient having pulmonary hypertension. In some embodiments, the method relate to lowering BDI by at least 6.5 index points in the patient having pulmonary hypertension. In some embodiments, the method relate to lowering BDI by at least 7 index points in the patient having pulmonary hypertension. In some embodiments, the method relate to lowering BDI by at least 7.5 index points in the patient having pulmonary hypertension. In some embodiments, the method relate to lowering BDI by at least 8 index points in the patient having pulmonary hypertension. In some embodiments, the method relate to lowering BDI by at least 8.5 index points in the patient having pulmonary hypertension. In some embodiments, the method relate to lowering BDI by at least 9 index points in the patient having pulmonary hypertension. In some embodiments, the method relate to lowering BDI by at least 9.5 index points in the patient having pulmonary hypertension. In some embodiments, the method relate to lowering BDI by at least 3 index points in the patient having pulmonary hypertension. In some embodiments, the method relate to lowering BDI by 10 index points in the patient having pulmonary hypertension. Pulmonary hypertension at baseline can be mild, moderate or severe, as measured for example by World Health Organization (WHO) functional class, which is a measure of disease severity in patients with pulmonary hypertension. The WHO functional classification is an adaptation of the New York Heart Association (NYHA) system and is routinely used to qualitatively assess activity tolerance, for example in monitoring disease progression and response to treatment (Rubin (2004) Chest 126:7-10). Four functional classes are recognized in the WHO system: Class I: pulmo-

nary hypertension without resulting limitation of physical activity; ordinary physical activity does not cause undue dyspnea or fatigue, chest pain or near syncope; Class II: pulmonary hypertension resulting in slight limitation of physical activity; patient comfortable at rest; ordinary physical activity causes undue dyspnea or fatigue, chest pain or near syncope; Class III: pulmonary hypertension resulting in marked limitation of physical activity; patient comfortable at rest; less than ordinary activity causes undue dyspnea or fatigue, chest pain or near syncope; Class IV: pulmonary hypertension resulting in inability to carry out any physical activity without symptoms; patient manifests signs of right-heart failure; dyspnea and/or fatigue may be present even at rest; discomfort is increased by any physical activity.

[0286] In certain aspects, the disclosure relates to methods of treating, preventing, or reducing the progression rate and/or severity of pulmonary hypertension (e.g., treating, preventing, or reducing the progression rate and/or severity of one or more complications of pulmonary hypertension) comprising administering to a patient in need thereof an effective amount of a GDF/BMP antagonist (e.g., an antagonist of one or more of activin, GDF8, GDF11, GDF3, BMP6, BMP15, BMP10, ActRIIA, ActRIIB, ALK4, ALK5, ALK7, and one or more Smad proteins), wherein the patient has Class I, Class II, Class III, or Class IV pulmonary hypertension as recognized by the WHO. In some embodiments, the method relates to a patient that has Class I pulmonary hypertension as recognized by the WHO. In some embodiments, the method relates to a patient that has Class II pulmonary hypertension as recognized by the WHO. In some embodiments, the method relates to preventing or delaying patient progression from Class I pulmonary hypertension to Class II pulmonary hypertension as recognized by the WHO. In some embodiments, the method relates to promoting or increasing patient regression from Class II pulmonary hypertension to Class I pulmonary hypertension as recognized by the WHO. In some embodiments, the method relates to a patient that has Class III pulmonary hypertension as recognized by the WHO. In some embodiments, the method relates to preventing or delaying patient progression from Class II pulmonary hypertension to Class III pulmonary hypertension as recognized by the WHO. In some embodiments, the method relates to promoting or increasing patient regression from Class III pulmonary hypertension to Class II pulmonary hypertension as recognized by the WHO. In some embodiments, the method relates to a patient that has Class IV pulmonary hypertension as recognized by the WHO. In some embodiments, the method relates to preventing or delaying patient progression from Class III pulmonary hypertension to Class IV pulmonary hypertension as recognized by the WHO. In some embodiments, the method relates to promoting or increasing patient regression from Class IV pulmonary hypertension to Class III pulmonary hypertension as recognized by the WHO. In some embodiments, the method relates to promoting or increasing patient regression from Class IV pulmonary hypertension to Class II pulmonary hypertension as recognized by the WHO. In some embodiments, the method relates to promoting or increasing patient regression from Class IV pulmonary hypertension to Class II pulmonary hypertension as recognized by the WHO. In some embodiments, the method relates to promoting or increasing patient regression from Class IV pulmonary hypertension to Class II pulmonary hypertension as recognized by the WHO. In some embodiments, the method relates to promoting or increasing patient regression from Class IV pulmonary hypertension to Class II pulmonary hypertension as recognized by the WHO.

increasing patient regression from Class IV pulmonary hypertension to Class I pulmonary hypertension as recognized by the WHO.

[0287] There is no known cure for pulmonary hypertension; current methods of treatment focus on prolonging patient lifespan and enhancing patient quality of life. Current methods of treatment of pulmonary hypertension include administration of: vasodilators such as prostacyclin, epoprostenol, and sildenafil; endothelin receptor antagonists such as bosentan; calcium channel blockers such as amlodipine, diltiazem, and nifedipine; anticoagulants such as warfarin; and diuretics. Treatment of pulmonary hypertension has also been carried out using oxygen therapy, atrial septostomy, pulmonary thromboendarterectomy, and lung and/or heart transplantation. Each of these methods, however, suffers from one or multiple drawbacks which may include lack of effectiveness, serious side effects, low patient compliance, and high cost. In certain aspects, the method relate to treating, preventing, or reducing the progression rate and/or severity of pulmonary hypertension (e.g., treating, preventing, or reducing the progression rate and/or severity of one or more complications of pulmonary hypertension) comprising administering to a patient in need thereof an effective amount of a GDF/BMP antagonist (e.g., an antagonist of one or more of activin, GDF8, GDF11, GDF3, BMP6, BMP15, BMP10, ActRIIA, ActRIIB, ALK4, ALK5, ALK7, and one or more Smad proteins) in combination (e.g., administered at the same time or different times, but generally in such a manner as to achieve overlapping pharmacological/physiological effects) with one or more additional active agents and/or supportive therapies for treating pulmonary hypertension (e.g., vasodilators such as prostacyclin, epoprostenol, and sildenafil; endothelin receptor antagonists such as bosentan; calcium channel blockers such as amlodipine, diltiazem, and nifedipine; anticoagulants such as warfarin; diuretics; oxygen therapy; atrial septostomy; pulmonary thromboendarterectomy; and lung and/or heart transplantation); BMP9 polypeptides; BMP10 polypeptides; bardoxolone methyl or a derivative thereof, olecanolic acid or derivative thereof.

[0288] In certain embodiments, the present disclosure provides methods for managing a patient that has been treated with, or is a candidate to be treated with, one or more one or more GDF/BMP antagonists of the disclosure (e.g., ligand traps such as ActRIIA polypeptides, ActRIIB polypeptides, and GDF Trap polypeptides) by measuring one or more hematologic parameters in the patient. The hematologic parameters may be used to evaluate appropriate dosing for a patient who is a candidate to be treated with the antagonist of the present disclosure, to monitor the hematologic parameters during treatment, to evaluate whether to adjust the dosage during treatment with one or more antagonist of the disclosure, and/or to evaluate an appropriate maintenance dose of one or more antagonists of the disclosure. If one or more of the hematologic parameters are outside the normal level, dosing with one or more GDF/BMP antagonists may be reduced, delayed or terminated.

[0289] Hematologic parameters that may be measured in accordance with the methods provided herein include, for example, red blood cell levels, blood pressure, iron stores, and other agents found in bodily fluids that correlate with increased red blood cell levels, using art recognized methods. Such parameters may be determined using a blood

sample from a patient. Increases in red blood cell levels, hemoglobin levels, and/or hematocrit levels may cause increases in blood pressure.

[0290] In one embodiment, if one or more hematologic parameters are outside the normal range or on the high side of normal in a patient who is a candidate to be treated with one or more GDF/BMP antagonists, then onset of administration of the one or more antagonists of the disclosure may be delayed until the hematologic parameters have returned to a normal or acceptable level either naturally or via therapeutic intervention. For example, if a candidate patient is hypertensive or pre-hypertensive, then the patient may be treated with a blood pressure lowering agent in order to reduce the patient's blood pressure. Any blood pressure lowering agent appropriate for the individual patient's condition may be used including, for example, diuretics, adrenergic inhibitors (including alpha blockers and beta blockers), vasodilators, calcium channel blockers, angiotensin-converting enzyme (ACE) inhibitors, or angiotensin II receptor blockers. Blood pressure may alternatively be treated using a diet and exercise regimen. Similarly, if a candidate patient has iron stores that are lower than normal, or on the low side of normal, then the patient may be treated with an appropriate regimen of diet and/or iron supplements until the patient's iron stores have returned to a normal or acceptable level. For patients having higher than normal red blood cell levels and/or hemoglobin levels, then administration of the one or more antagonists of the disclosure may be delayed until the levels have returned to a normal or acceptable level.

[0291] In certain embodiments, if one or more hematologic parameters are outside the normal range or on the high side of normal in a patient who is a candidate to be treated with one or more GDF/BMP antagonists, then the onset of administration may not be delayed. However, the dosage amount or frequency of dosing of the one or more antagonists of the disclosure may be set at an amount that would reduce the risk of an unacceptable increase in the hematologic parameters arising upon administration of the one or more antagonists of the disclosure. Alternatively, a therapeutic regimen may be developed for the patient that combines one or more GDF/BMP antagonists with a therapeutic agent that addresses the undesirable level of the hematologic parameter. For example, if the patient has elevated blood pressure, then a therapeutic regimen may be designed involving administration of one or more GDF/BMP antagonist agents and a blood pressure lowering agent. For a patient having lower than desired iron stores, a therapeutic regimen may be developed involving one or more GDF/BMP antagonists of the disclosure and iron supplementation.

[0292] In one embodiment, baseline parameter(s) for one or more hematologic parameters may be established for a patient who is a candidate to be treated with one or more GDF/BMP antagonists of the disclosure and an appropriate dosing regimen established for that patient based on the baseline value(s). Alternatively, established baseline parameters based on a patient's medical history could be used to inform an appropriate antagonist dosing regimen for a patient. For example, if a healthy patient has an established baseline blood pressure reading that is above the defined normal range it may not be necessary to bring the patient's blood pressure into the range that is considered normal for the general population prior to treatment with the one or more antagonist of the disclosure. A patient's baseline values for one or more hematologic parameters prior to

treatment with one or more GDF/BMP antagonists of the disclosure may also be used as the relevant comparative values for monitoring any changes to the hematologic parameters during treatment with the one or more antagonists of the disclosure.

[0293] In certain embodiments, one or more hematologic parameters are measured in patients who are being treated with one or more GDF/BMP antagonists. The hematologic parameters may be used to monitor the patient during treatment and permit adjustment or termination of the dosing with the one or more antagonists of the disclosure or additional dosing with another therapeutic agent. For example, if administration of one or more GDF/BMP antagonists results in an increase in blood pressure, red blood cell level, or hemoglobin level, or a reduction in iron stores, then the dose of the one or more antagonists of the disclosure may be reduced in amount or frequency in order to decrease the effects of the one or more antagonists of the disclosure on the one or more hematologic parameters. If administration of one or more GDF/BMP antagonists results in a change in one or more hematologic parameters that is adverse to the patient, then the dosing of the one or more antagonists of the disclosure may be terminated either temporarily, until the hematologic parameter(s) return to an acceptable level, or permanently. Similarly, if one or more hematologic parameters are not brought within an acceptable range after reducing the dose or frequency of administration of the one or more antagonists of the disclosure, then the dosing may be terminated. As an alternative, or in addition to, reducing or terminating the dosing with the one or more antagonists of the disclosure, the patient may be dosed with an additional therapeutic agent that addresses the undesirable level in the hematologic parameter(s), such as, for example, a blood pressure lowering agent or an iron supplement. For example, if a patient being treated with one or more GDF/BMP antagonists has elevated blood pressure, then dosing with the one or more antagonists of the disclosure may continue at the same level and a blood-pressure-lowering agent is added to the treatment regimen, dosing with the one or more antagonist of the disclosure may be reduced (e.g., in amount and/or frequency) and a blood-pressure-lowering agent is added to the treatment regimen, or dosing with the one or more antagonist of the disclosure may be terminated and the patient may be treated with a blood-pressure-lowering agent.

10. Pharmaceutical Compositions

[0294] The therapeutic agents described herein (e.g., GDF/BMP antagonists) may be formulated into pharmaceutical compositions. Pharmaceutical compositions for use in accordance with the present disclosure may be formulated in conventional manner using one or more physiologically acceptable carriers or excipients. Such formulations will generally be substantially pyrogen-free, in compliance with most regulatory requirements.

[0295] In certain embodiments, the therapeutic methods of the disclosure include administering the composition systemically, or locally as an implant or device. When administered, the therapeutic composition for use in this disclosure is in a substantially pyrogen-free, or pyrogen-free, physiologically acceptable form. Therapeutically useful agents other than the GDF/BMP antagonists which may also optionally be included in the composition as described

above, may be administered simultaneously or sequentially with the subject compounds in the methods disclosed herein.

[0296] Typically, protein therapeutic agents disclosed herein will be administered parentally, and particularly intravenously or subcutaneously. Pharmaceutical compositions suitable for parenteral administration may comprise one or more GDF/BMP antagonist in combination with one or more pharmaceutically acceptable sterile isotonic aqueous or non-aqueous solutions, dispersions, suspensions or emulsions, or sterile powders which may be reconstituted into sterile injectable solutions or dispersions just prior to use, which may contain antioxidants, buffers, bacteriostats, solutes which render the formulation isotonic with the blood of the intended recipient or suspending or thickening agents. Examples of suitable aqueous and nonaqueous carriers which may be employed in the pharmaceutical compositions of the disclosure include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

[0297] The compositions and formulations may, if desired, be presented in a pack or dispenser device which may contain one or more unit dosage forms containing the active ingredient. The pack may for example comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration

[0298] Further, the composition may be encapsulated or injected in a form for delivery to a target tissue site. In certain embodiments, compositions of the present invention may include a matrix capable of delivering one or more therapeutic compounds (e.g., GDF/BMP antagonists) to a target tissue site, providing a structure for the developing tissue and optimally capable of being resorbed into the body. For example, the matrix may provide slow release of the GDF/BMP antagonist. Such matrices may be formed of materials presently in use for other implanted medical applications.

[0299] The choice of matrix material is based on biocompatibility, biodegradability, mechanical properties, cosmetic appearance and interface properties. The particular application of the subject compositions will define the appropriate formulation. Potential matrices for the compositions may be biodegradable and chemically defined calcium sulfate, tricalcium phosphate, hydroxyapatite, polylactic acid and polyanhydrides. Other potential materials are biodegradable and biologically well defined, such as bone or dermal collagen. Further matrices are comprised of pure proteins or extracellular matrix components. Other potential matrices are non-biodegradable and chemically defined, such as sintered hydroxyapatite, bioglass, aluminates, or other ceramics. Matrices may be comprised of combinations of any of the above mentioned types of material, such as polylactic acid and hydroxyapatite or collagen and tricalcium phosphate. The bioceramics may be altered in composition, such as in calcium-aluminate-phosphate and processing to alter pore size, particle size, particle shape, and biodegradability.

[0300] In certain embodiments, methods of the invention can be administered for orally, e.g., in the form of capsules, cachets, pills, tablets, lozenges (using a flavored basis,

usually sucrose and acacia or tragacanth), powders, granules, or as a solution or a suspension in an aqueous or non-aqueous liquid, or as an oil-in-water or water-in-oil liquid emulsion, or as an elixir or syrup, or as pastilles (using an inert base, such as gelatin and glycerin, or sucrose and acacia) and/or as mouth washes and the like, each containing a predetermined amount of an agent as an active ingredient. An agent may also be administered as a bolus, electuary or paste.

[0301] In solid dosage forms for oral administration (capsules, tablets, pills, dragees, powders, granules, and the like), one or more therapeutic compounds of the present invention may be mixed with one or more pharmaceutically acceptable carriers, such as sodium citrate 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 as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof, and (10) coloring agents. In the case of capsules, tablets and pills, the pharmaceutical compositions may also comprise buffering agents. Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugars, as well as high molecular weight polyethylene glycols and the like.

[0302] 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.

[0303] Suspensions, in addition to the active compounds, may contain suspending agents such as ethoxylated isostearyl alcohols, polyoxyethylene sorbitol, and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth, and mixtures thereof.

[0304] The compositions of the invention may also contain adjuvants, such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of the action of microorganisms may be ensured by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It may also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like into the compositions.

In addition, prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents which delay absorption, such as aluminum monostearate and gelatin.

[0305] It is understood that the dosage regimen will be determined by the attending physician considering various factors which modify the action of the subject compounds of the disclosure (e.g., GDF/BMP antagonists). The various factors include, but are not limited to, the patient's age, sex, and diet, the severity disease, time of administration, and other clinical factors. Optionally, the dosage may vary with the type of matrix used in the reconstitution and the types of compounds in the composition. The addition of other known growth factors to the final composition, may also affect the dosage. Progress can be monitored by periodic assessment of bone growth and/or repair, for example, X-rays (including DEXA), histomorphometric determinations, and tetracycline labeling.

[0306] In certain embodiments, the present invention also provides gene therapy for the in vivo production of GDF/BMP antagonists. Such therapy would achieve its therapeutic effect by introduction of the GDF/BMP antagonist polynucleotide sequences into cells or tissues having the disorders as listed above. Delivery of GDF/BMP antagonist polynucleotide sequences can be achieved using a recombinant expression vector such as a chimeric virus or a colloidal dispersion system. Preferred for therapeutic delivery of GDF/BMP antagonist polynucleotide sequences is the use of targeted liposomes.

[0307] Various viral vectors which can be utilized for gene therapy as taught herein include adenovirus, herpes virus, vaccinia, or, preferably, an RNA virus such as a retrovirus. Preferably, the retroviral vector is a derivative of a murine or avian retrovirus. Examples of retroviral vectors in which a single foreign gene can be inserted include, but are not limited to: Moloney murine leukemia virus (MoMuLV), Harvey murine sarcoma virus (HaMuSV), murine mammary tumor virus (MuMTV), and Rous Sarcoma Virus (RSV). A number of additional retroviral vectors can incorporate multiple genes. All of these vectors can transfer or incorporate a gene for a selectable marker so that transduced cells can be identified and generated. Retroviral vectors can be made target-specific by attaching, for example, a sugar, a glycolipid, or a protein. Preferred targeting is accomplished by using an antibody. Those of skill in the art will recognize that specific polynucleotide sequences can be inserted into the retroviral genome or attached to a viral envelope to allow target specific delivery of the retroviral vector containing the GDF/BMP antagonist. In a preferred embodiment, the vector is targeted to bone or cartilage.

[0308] Alternatively, tissue culture cells can be directly transfected with plasmids encoding the retroviral structural genes gag, pol and env, by conventional calcium phosphate transfection. These cells are then transfected with the vector plasmid containing the genes of interest. The resulting cells release the retroviral vector into the culture medium.

[0309] Another targeted delivery system for GDF/BMP antagonist polynucleotides is a colloidal dispersion system. Colloidal dispersion systems include macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. The preferred colloidal system of this invention is a liposome. Liposomes are artificial membrane vesicles which are useful as delivery vehicles in vitro

and in vivo. RNA, DNA and intact virions can be encapsulated within the aqueous interior and be delivered to cells in a biologically active form (see e.g., Fraley, et al., Trends Biochem. Sci., 6:77, 1981). Methods for efficient gene transfer using a liposome vehicle, are known in the art, see e.g., Mannino, et al., Biotechniques, 6:682, 1988. The composition of the liposome is usually a combination of phospholipids, usually in combination with steroids, especially cholesterol. Other phospholipids or other lipids may also be used. The physical characteristics of liposomes depend on pH, ionic strength, and the presence of divalent cations.

[0310] 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.

[0311] The disclosure provides formulations that may be varied to include acids and bases to adjust the pH; and buffering agents to keep the pH within a narrow range.

EXEMPLIFICATION

[0312] The invention now being generally described, it will be more readily understood by reference to the following examples, which are included merely for purposes of illustration of certain embodiments of the present invention, and are not intended to limit the invention.

Example 1: ActRIIA-Fc Fusion Proteins

[0313] A soluble ActRIIA fusion protein was constructed that has the extracellular domain of human ActRIIA fused to a human or mouse Fc domain with a minimal linker in between. The constructs are referred to as ActRIIA-hFc and ActRIIA-mFc, respectively.

[0314] ActRIIA-hFc is shown below as purified from CHO cell lines (SEQ ID NO: 32):

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IILRSETQECLFFNANWEKDRTNQTGVEPCYGDKDKRHCFATWKNISGS
IEIVKQGCWLDDINCYDRTDCVEKKDSPEVYFCCCEGNMCNEKFYSFPEM
EVTQPTSNPVTPKPPTGGGTHTCPPCPAPELLGGPSVFLFPFPKDKTLMI
SRTPEVTCVVVDVSHEDEPVKFVNWYVGVEVHNAKTKPREEQYNSTYRVV
SVLTVLHQDWLNKEYKCKVSNKALPVPIEKTIKAKGQPREPQVYTLPP
SREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGS
FFLYSKLTVDKSRWQQGNVFSCVMHEALHNHYTOKSLSLSPKG

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[0315] The ActRIIA-hFc and ActRIIA-mFc proteins were expressed in CHO cell lines. Three different leader sequences were considered:

(i) Honey bee mellitin (HBML) :
(SEQ ID NO: 33)
MKFLVNVALVFMVYIISIYA

(ii) Tissue plasminogen activator (TPA) :
(SEQ ID NO: 34)
MDAMKRLCCVLLCGAVFVSP

-continued

(iii) Native:

(SEQ ID NO: 35)

MGAAAKLAFAVFLISCGSSGA.

[0316] The selected form employs the TPA leader and has the following unprocessed amino acid sequence:

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(SEQ ID NO: 36)
MDAMKRLCCVLLCGAVFVSPGAII LGRSETQECLFFNANWEKDRTNQT
GVEPCYGDKDKRHCFATWKNISGSIEIVKQGCWLDDINCYDRTDCVEKK
DSPEVYFCCCEGNMCNEKFYSFPEMЕVTQPTSНPVTPKPPTGGGTHTCPP
CPAPELLGGPSVFLFPFPKDKTLMISRTPEVTCVVVDVSHEDEPVKFVNWY
VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNKEYKCKVSNKAL
PVPIEKTIKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIA
VEWESNGQPENNYKTPPVLDSDGSFPLYSKLTVDKSRWQQGNVFSCVM
HEALHNHYTOKSLSLSPKG

```

[0317] This polypeptide is encoded by the following nucleic acid sequence:

```

(SEQ ID NO: 37)
ATGGATGCAATGAAGAGAGGGCTCTGCTGTGCTGCTGTGTTGGAGC
AGTCTTCGTTTCGCCCGCGCCGTATACTGGTAGATCAGAAACTCAGG
AGTGTCTTTTTTAATGCTAATTGGAAAAAGACAGAACCAATCAAATCG
GTGTTGAACCGTGTATGGTGACAAGATAACGGCGCATTGTTTGCT
ACCTGGAAGAATATTCTGGTTCATTGAATAGTGAACAAAGGTTGTGG
CTGGATGATATCAACTGCTATGACAGGACTGATTGTGAGAAAAAAAAGA
CAGCCCTGAAGTATATTCTGGTGTGAGGGCAATATGTGTAATGAAA
AGTTTCTTATTTCCGGAGATGGAAGTCACACAGCCCACCTCAAATCCA
GTTACACCTAACGCCACCCACCGGTGGAACTCACACATGCCACCGTG
CCCATGAACTCCTGGGGGACCGTCAGTCCTCTCCCCCAA
AACCCAAGGACACCCCTCATGATCTCCGGACCCCTGAGGTACATGCGTG
GTGGTGGACGTGAGCCACGAAGACCTGAGGTCAAGTTCACTGGTACGT
GGACGGCGTGGAGGTGCAATGCCAACAGCCGCGGGAGGAGCAGT
ACAAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCTGCACCAAGAC
TGGCTGAATGGCAAGGGAGTACAAGTGAAGGTCTCCAACAAAGCCCTCCC
AGTCCCCATCGAGAAAACCATCTCAAAGCCAAAGGGCAGCCCCGAGAAC
CACAGGTGTACACCTGCCCATCCGGAGGAGATGACCAAGAACAG
GTCAGCCTGACCTGCCTGGTCAAAGGTTCTATCCAGCGACATGCCGT
GGAGTGGGAGAGCAATGGGAGCCGAGAACAAACTACAAGACCAAGCCTC
CCGTGCTGGACTCCGACGGCTCTTCTCTATAGCAAGCTCACCGTG
GACAAGAGCAGGTGGCAGCAGGGAAACGTCTCTCATGCTCCGTGATGCA
TGAGGCTCTGCACAACCAACTACACGCGAGAACAGGCCTCCCTGTCTCCGG
GTAAATGAGAATTC

```

[0318] Both ActRIIA-hFc and ActRIIA-mFc were remarkably amenable to recombinant expression. As shown in FIG. 5, the protein was purified as a single, well-defined peak of protein. N-terminal sequencing revealed a single sequence of -ILGRSETQE (SEQ ID NO: 38). Purification could be achieved by a series of column chromatography steps, including, for example, three or more of the following, in any order: protein A chromatography, Q sepharose chromatography, phenylsepharose chromatography, size exclusion chromatography, and cation exchange chromatography. The purification could be completed with viral filtration and buffer exchange. The ActRIIA-hFc protein was purified to a purity of >98% as determined by size exclusion chromatography and >95% as determined by SDS PAGE.

[0319] ActRIIA-hFc and ActRIIA-mFc showed a high affinity for ligands. GDF11 or activin A were immobilized on a Biacore™ CM5 chip using standard amine-coupling procedure. ActRIIA-hFc and ActRIIA-mFc proteins were loaded onto the system, and binding was measured. ActRIIA-hFc bound to activin with a dissociation constant (K_D) of 5×10^{-12} and bound to GDF11 with a K_D of 9.96×10^{-9} . See FIG. 6. Using a similar binding assay, ActRIIA-hFc was determined to have high to moderate affinity for other TGF-beta superfamily ligands including, for example, activin B, GDF8, BMP6, and BMP10. ActRIIA-mFc behaved similarly.

[0320] The ActRIIA-hFc was very stable in pharmacokinetic studies. Rats were dosed with 1 mg/kg, 3 mg/kg, or 10 mg/kg of ActRIIA-hFc protein, and plasma levels of the protein were measured at 24, 48, 72, 144 and 168 hours. In a separate study, rats were dosed at 1 mg/kg, 10 mg/kg, or 30 mg/kg. In rats, ActRIIA-hFc had an 11-14 day serum half-life, and circulating levels of the drug were quite high after two weeks (11 g/ml, 110 g/ml, or 304 g/ml for initial administrations of 1 mg/kg, 10 mg/kg, or 30 mg/kg, respectively.) In cynomolgus monkeys, the plasma half-life was substantially greater than 14 days, and circulating levels of the drug were 25 g/ml, 304 g/ml, or 1440 g/ml for initial administrations of 1 mg/kg, 10 mg/kg, or 30 mg/kg, respectively.

Example 2: Characterization of an ActRIIA-hFc Protein

[0321] ActRIIA-hFc fusion protein was expressed in stably transfected CHO-DUKX B11 cells from a pAID4 vector (SV40 ori/enhancer, CMV promoter), using a tissue plasminogen leader sequence of SEQ ID NO: 34. The protein, purified as described above in Example 1, had a sequence of SEQ ID NO: 32. The Fc portion is a human IgG1 Fc sequence, as shown in SEQ ID NO: 32. Protein analysis reveals that the ActRIIA-hFc fusion protein is formed as a homodimer with disulfide bonding.

[0322] The CHO-cell-expressed material has a higher affinity for activin B ligand than that reported for an ActRIIa-hFc fusion protein expressed in human 293 cells [see, del Re et al. (2004) J Biol Chem. 279(51):53126-53135]. Additionally, the use of the TPA leader sequence provided greater production than other leader sequences and, unlike ActRIIA-Fc expressed with a native leader, provided a highly pure N-terminal sequence. Use of the native leader sequence resulted in two major species of ActRIIA-Fc, each having a different N-terminal sequence.

Example 3: Alternative ActRIIA-Fc Proteins

[0323] A variety of ActRIIA variants that may be used according to the methods described herein are described in the International Patent Application published as WO2006/012627 (see e.g., pp. 55-58), incorporated herein by reference in its entirety. An alternative construct may have a deletion of the C-terminal tail (the final 15 amino acids of the extracellular domain of ActRIIA. The sequence for such a construct is presented below (Fc portion underlined) (SEQ ID NO: 39):

```
ILGRSETQECLFFNANWEKDRTNQTVGVEPCYGDKDRRHCFATWKNI SG
SIEIVKGQCWLDINDCYDRTDCVEKKDSPEVYFCCCEGNMCNEKF SYFP
EMTGGGTHTCPCCPAPELLGGPSVFLFPPPKDKTLMSRTPEVTCVVVD
VSHEDPEVKFNWYVDGVEVHNNAKTKPREEQYNSTYRVVSVLTVLHQDWL
NGKEYKCKVSNKALPVPTEKTISKAKGQPREPQVYTLPPSREEMTKNQV
SLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLT
DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPKG
```

Example 4: Generation of ActRIIB-Fc Fusion Proteins

[0324] Applicants constructed a soluble ActRIIB fusion protein that has the extracellular domain of human ActRIIB fused to a human or mouse Fc domain with a minimal linker in between. The constructs are referred to as ActRIIB-hFc and ActRIIB-mFc, respectively.

[0325] ActRIIB-hFc is shown below as purified from CHO cell lines (SEQ ID NO: 40):

```
GRGEAETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWRNSSG
TIELVKKGWLDNFNCYDRQECVATEENPQVYFCCCEGNFCNERFTHL P
EAGGPEVTYEPPPTAPGGGTHTCPCCPAPELLGGPSVFLFPPPKDKTL
MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNNAKTKPREEQYNSTY
RVSVLTVLHQDWLNGKEYKCKVSNKALPVPTEKTISKAKGQPREPQVY
TLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPV L
DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPKG
```

[0326] The ActRIIB-hFc and ActRIIB-mFc proteins were expressed in CHO cell lines. Three different leader sequences were considered: (i) Honey bee mellitin (HBML), ii) Tissue plasminogen activator (TPA), and (iii) Native: MGAAAKLAFAVFLISCSSGA (SEQ ID NO: 41).

[0327] The selected form employs the TPA leader and has the following unprocessed amino acid sequence (SEQ ID NO: 42):

```
MDAMKRGGLCCVLLLCGAVFVSPGASGRGEAETRECIYYNANWELE RTNQ
SGLERCEGEQDKRLHCYASWRNSSGTIELVKKGWLDNFNCYDRQECVA
TEENPQVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPGGGTHTC
PPCPAPELLGGPSVFLFPPPKDKTLMSRTPEVTCVVVDVSHEDPEVKF
```

- continued

NWYVDGVEVHNAAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVS
NKALPVPIEKTIKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFY
PSDIAVEWESNGQPENNYKTPPVLDGSFFLYSKLTVDKSRWQQGNV
FSCSVMHEALHNHYTQKSLSLSPKG

[0328] This polypeptide is encoded by the following nucleic acid sequence (SEQ ID NO: 43):

```
A TGGATGCAAT GAAGAGAGGG CTCTGCTGTG TGCTGCTGCT GTGTGGAGCA
GTCTTCGTTT CGCCCGGCAGC CTCTGGCGT GGGGAGGCTG AGACACGGGA
GTGCATCTAC TACAACGCCA ACTGGGAGCT GGAGCGCAC ACCAGAGCG
GCCTGGAGCG CTGCGAAGGC GAGCAGGACA AGCGGCTGCA CTGCTACGCC
TCCTGGCGCA ACAGCTCTGG CACCATCGAG CTCGTGAAGA AGGGCTGCTG
GCTAGATGAC TTCAACTGCT ACGATAGGCA GGAGTGTGTG GCCACTGAGG
AGAACCCCCA GGTGTACTTC TGCTGCTGTG AAGGCAACTT CTGCAACGAG
CGCTTCACTC ATTTGCCAGA GGCTGGGGGC CCGGAAGTCA CGTACGAGCC
ACCCCCGACA GCCCCCACCG GTGGTGGAAAC TCACACATGC CCACCGTGCC
CAGCACCTGA ACTCCTGGGG GGACCGTCAG TCTTCCTCTT CCCCCAAAAA
CCAAGGACA CCCTCATGAT CTCCCGGACC CCTGAGGTCA CATGCGTGGT
GGTGGACGTG AGCCACGAAG ACCCTGAGGT CAAGTTCAAC TGGTACGTGG
ACGGCGTGG A GGTGCATAAT GCCAAGACAA AGCCGCGGGG GGAGCAGTAC
AACAGCACGT ACCGTGTGGT CAGCGTCCTC ACCGTCCCTGC ACCAGGACTG
GCTGAATGGC AAGGAGTACA AGTGCAAGGT CTCCAACAAA CCCCTCCCAG
TCCCCATCGA GAAAACCATC TCCAAAGCCA AAGGGCAGCC CCGAGAACCA
CAGGTGTACA CCCTGCCCCC ATCCCGGGAG GAGATGACCA AGAACCGAGGT
CAGCCTGACC TGCCTGGTCA AAGGCTTCTA TCCCAGCGAC ATCGCCGTGG
AGTGGGAGAG CAATGGGCAG CCGGAGAACAA ACTACAAGAC CACGCCCTCCC
GTGCTGGACT CCGACGGCTC CTTCTCCCTC TATAGCAAGC TCACCGTGG
CAAGAGCAGG TGGCAGCAGG GGAACGTCTT CTCATGCTCC GTGATGCATG
AGGCTCTGCA CAACCAACTAC ACGCAGAAGA GCCTCTCCCT GTCTCCGGGT
AAATGA
```

[0329] N-terminal sequencing of the CHO-cell-produced material revealed a major sequence of -GRGEAE (SEQ ID NO: 44). Notably, other constructs reported in the literature begin with an -SGR . . . sequence.

[0330] Purification could be achieved by a series of column chromatography steps, including, for example, three or more of the following, in any order: protein A chromatography, Q sepharose chromatography, phenylsepharose chromatography, size exclusion chromatography, and cation exchange chromatography. The purification could be completed with viral filtration and buffer exchange.

[0331] ActRIIB-Fc fusion proteins were also expressed in HEK293 cells and COS cells. Although material from all cell lines and reasonable culture conditions provided protein with muscle-building activity *in vivo*, variability in potency was observed perhaps relating to cell line selection and/or culture conditions.

[0332] Applicants generated a series of mutations in the extracellular domain of ActRIIB and produced these mutant proteins as soluble fusion proteins between extracellular ActRIIB and an Fc domain. The background ActRIIB-Fc fusion has the sequence of SEQ ID NO: 40.

[0333] Various mutations, including N- and C-terminal truncations, were introduced into the background ActRIIB-Fc protein. Based on the data presented herein, it is expected that these constructs, if expressed with a TPA leader, will

lack the N-terminal serine. Mutations were generated in ActRIIB extracellular domain by PCR mutagenesis. After PCR, fragments were purified through a Qiagen column, digested with SfoI and AgeI and gel purified. These fragments were ligated into expression vector pAID4 (see WO2006/012627) such that upon ligation it created fusion chimera with human IgG1. Upon transformation into *E. coli* DH5 alpha, colonies were picked and DNAs were isolated. For murine constructs (mFc), a murine IgG2a was substituted for the human IgG1. Sequences of all mutants were verified. All of the mutants were produced in HEK293T cells by transient transfection. In summary, in a 500 ml spinner, HEK293T cells were set up at 6×10^5 cells/ml in Freestyle (Invitrogen) media in 250 ml volume and grown overnight. Next day, these cells were treated with DNA:PEI (1:1) complex at 0.5 ug/ml final DNA concentration. After 4 hrs,

250 ml media was added and cells were grown for 7 days. Conditioned media was harvested by spinning down the cells and concentrated.

[0334] Mutants were purified using a variety of techniques, including, for example, a protein A column, and eluted with low pH (3.0) glycine buffer. After neutralization, these were dialyzed against PBS.

[0335] Mutants were also produced in CHO cells by similar methodology. Mutants were tested in binding assays and/or bioassays described in WO 2008/097541 and WO 2006/012627 incorporated by reference herein. In some instances, assays were performed with conditioned medium rather than purified proteins. Additional variations of ActRIIB are described in U.S. Pat. No. 7,842,663.

[0336] Applicant generated an ActRIIB(25-131)-hFc fusion protein, which comprises the human ActRIIB extracellular domain with N-terminal and C-terminal truncations (residues 25-131 of the native protein SEQ ID NO: 1) fused N-terminally with a TPA leader sequence substituted for the native ActRIIB leader and C-terminally with a human Fc domain via a minimal linker (three glycine residues) (FIG. 7). A nucleotide sequence encoding this fusion protein is shown in FIG. 8. Applicants modified the codons and found a variant nucleic acid encoding the ActRIIB(25-131)-hFc protein that provided substantial improvement in the expression levels of initial transformants (FIG. 9).

[0337] The mature protein has an amino acid sequence as follows (N-terminus confirmed by N-terminal sequencing) (SEQ ID NO: 45):

```

ETRECIYYNA NWELERTNQS GLERCEGEQD KRLHCYASWR NSSGTIELVK
_____
KGCWLDDFNC YDRQECVATE ENPQVYFCCC EGNFCNERFT HLPEAGGPBV
_____
TYEPPPTGGG THTCPPCPAP ELLGGPSVFL FPPPKPKDTLM ISRTPEVTCV
_____
VVVDVSHEDPE VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD
_____
WLNGKEYKCK VSNKALPAPI EKTISKAKGQ PREPQVYTLPL PSREEMTKNQ
_____
VSLTCLVKGF YPSDIAVEWE SNGQPENNYK TPPVLDSDG SFFLYSKLT
_____
DKSRWQQGNV PSCSVMHEAL HNHYTQKSLS LSPGK

```

[0338] The expressed molecule was purified using a series of column chromatography steps, including for example, three or more of the following, in any order: Protein A chromatography, Q sepharose chromatography, phenylsepharose chromatography, size exclusion chromatography and cation exchange chromatography. The purification could be completed with viral filtration and buffer exchange.

[0339] Affinities of several ligands for ActRIIB(25-131)-hFc and its full-length counterpart ActRIIB(20-134)-hFc were evaluated in vitro with a Biacore™ instrument, and the results are summarized in the table below. Kd values were obtained by steady-state affinity fit due to very rapid association and dissociation of the complex, which prevented accurate determination of k_{on} and k_{off} . ActRIIB(25-131)-hFc bound, for example, activin A, activin B, and GDF11 with high affinity.

Ligand Affinities of ActRIIB-hFc Forms:

Fusion Construct	Activin A (e-11)	Activin B (e-11)	GDF11 (e-11)
ActRIIB(20-134)-hFc	1.6	1.2	3.6
ActRIIB(25-131)-hFc	1.8	1.2	3.1

Example 5: Generation of a GDF Trap

[0340] A GDF trap was constructed as follows. A polypeptide having a modified extracellular domain of ActRIIB (amino acids 20-134 of SEQ ID NO: 1 with an L79D substitution) with greatly reduced activin A binding relative to GDF11 and/or myostatin (as a consequence of a leucine-to-aspartate substitution at position 79 in SEQ ID NO: 1) was fused to a human or mouse Fc domain with a minimal linker in between. The constructs are referred to as ActRIIB(L79D 20-134)-hFc and ActRIIB(L79D 20-134)-mFc, respectively. Alternative forms with a glutamate rather than an aspartate at position 79 performed similarly (L79E). Alternative forms with an alanine rather than a valine at position 226 with respect to SEQ ID NO: 64, below were also generated and performed equivalently in all respects tested. The aspartate at position 79 (relative to SEQ ID NO: 1) is indicated with double underlining below. The valine at position 226 relative to SEQ ID NO: 64 is also indicated by double underlining below.

[0341] The GDF trap ActRIIB(L79D 20-134)-hFc is shown below as purified from CHO cell lines (SEQ ID NO: 46).

```

GRGEAETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWRNSSG
TIELVKKGCWDDDFNCYDRQECVATEENPQVYFCCCEGNFCNERFTHLP
EAGGPBVTYEPPPTAPTGGGTHTCCPCPAELLGGPSVFLFPPPKPKDTLM
ISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQNSTYRV
RVVSVLTVLHQDWLNGKEYKCKVSNKALPVPIEKTISKAKGQPREPQYV
TLPPSREEMTKQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVL
DSDGSFFLYSKLTVDKSRWQQGNVFCSSVMHEALHNYTQKSLSLSPGK

```

[0342] The ActRIIB-derived portion of the GDF trap has an amino acid sequence set forth below (SEQ ID NO: 47),

and that portion could be used as a monomer or as a non-Fc fusion protein as a monomer, dimer, or greater-order complex.

(SEQ ID NO: 47)
GRGEAETRECIYYNANWELERTNQSGLERCEGQDKRLHCYASWRNSSG
TIELVKKGCWDDDFNCYDRQECVATEENPQVYFCCCEGNFCNERFTHLP
EAGGPEVTVYEPPPTAPT

[0343] The GDF trap protein was expressed in CHO cell lines. Three different leader sequences were considered:

[0344] (i) Honey bee melittin (HBML), (ii) Tissue plasminogen activator (TPA), and (iii) Native.

[0345] The selected form employs the TPA leader and has the following unprocessed amino acid sequence:

(SEQ ID NO: 48)
MDAMKRLCCVLLLCGAVFVSPGASGRGEAETRECIYYNANWELERTNQ
SGLERCEGEQDKRLHCYASWRNSSGTIELVKKGCWDDDFNCYDRQECVA
TEENPQVYFCCCEGNFCNERFTHLPEAGGPEVTVYEPPPTAPTGGGTHTC
PPCPAPEELLGGPSVFLPPPKDTLMSRTPEVTCVVVDVSHEDPEVKF
NWYVDGVEVHNAKTPREEQYNNSTYRVSVLTVLHQDWLNGKEYKCKVS
NKALPAPIEKTISAKGQPREPVYTLPPSREEMTKNQVSLTCLVKGFY
PSDIAVEWESNGQOPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNV
FSCSVMHEALHNHYTQKSLSLSPGK

[0346] This polypeptide is encoded by the following nucleic acid sequence (SEQ ID NO: 49):

```

A TGGATGCAAT GAAGAGAGGG CTCTGCTGTG TGCTGCTGCT GTGTGGAGCA
GTCTTCGTTT CGCCCGGCAGC CTCTGGCGT GGGGAGGCTG AGACACGGGA
GTGCATCTAC TACAACGCCA ACTGGGAGCT GGAGCGCAC ACCAGAGCG
GCCTGGAGCG CTGCGAAGGC GAGCAGGACA AGCGGCTGCA CTGCTACGCC
TCCTGGCGCA ACAGCTCTGG CACCATCGAG CTCGTGAAGA AGGGCTGCTG
GGACGATGAC TTCAACTGCT ACGATAGGCA GGAGTGTGTG GCCACTGAGG
AGAACCCCCCA GGTGTACTTC TGCTGCTGTG AAGGCAACTT CTGCAACGAG
CGCTTCACTC ATTTGCCAGA GGCTGGGGGC CGGAAAGTCA CGTACGAGCC
ACCCCCGACA GCCCCCACCG GTGGTGAAC TCACACATGC CCACCGTGCC
CAGCACCTGA ACTCCTGGGG GGACCGTCAG TCTTCCTCTT CCCCCAAAAA
CCCAAGGACA CCCTCATGAT CTCCCGGACC CCTGAGGTCA CATGCGTGGT
GGTGGACGTG AGCCACGAAG ACCCTGAGGT CAAGTTCAAC TGGTACGTGG
ACGGCGTGA GGTGCATAAT GCCAAGACAA AGCCGCGGGA GGAGCAGTAC
AACAGCACGT ACCGTGTGGT CAGCGTCCTC ACCGTCTGC ACCAGGACTG
GCTGAATGGC AAGGAGTACA AGTGAAGGT CTCCAACAAA GCCCTCCCAG
TCCCCATCGA GAAAACCATC TCAAAGCCA AAGGGCAGCC CCGAGAACCA
CAGGTGTACA CCCTGGGGCC ATCCGGGGAG GAGATGACCA AGAACCGAGGT
CAGCCTGACC TGCCTGGTCA AAGGCTTCTA TCCCAGCGAC ATCGCCGTGG
AGTGGGAGAG CAATGGGCAG CGGGAGAACAA ACTACAAGAC CACGCCCTCCC
GTGCTGGACT CCGACGGCTC CTTCTTCCTC TATAGCAAGC TCACCGTGAG
CAAGAGCAGG TGGCAGCAGG GGAACGTCTT CTCATGCTCC GTGATGCATG
AGGCTCTGCA CAACCACTAC ACGCAGAAGA GCCTCTCCCT GTCTCCGGGT
AAATGA

```

[0347] Purification could be achieved by a series of column chromatography steps, including, for example, three or more of the following, in any order: protein A chromatography, Q sepharose chromatography, phenylsepharose chromatography, size exclusion chromatography, and cation exchange chromatography. The purification could be completed with viral filtration and buffer exchange. In an example of a purification scheme, the cell culture medium is passed over a protein A column, washed in 150 mM Tris/NaCl (pH 8.0), then washed in 50 mM Tris/NaCl (pH 8.0) and eluted with 0.1 M glycine, pH 3.0. The low pH eluate is kept at room temperature for 30 minutes as a viral clearance step. The eluate is then neutralized and passed over a Q-sepharose ion-exchange column and washed in 50 mM Tris pH 8.0, 50 mM NaCl, and eluted in 50 mM Tris pH 8.0, with an NaCl concentration between 150 mM and 300 mM. The eluate is then changed into 50 mM Tris pH 8.0, 1.1 M ammonium sulfate and passed over a phenyl sepharose column, washed, and eluted in 50 mM Tris pH 8.0 with ammonium sulfate between 150 and 300 mM. The eluate is dialyzed and filtered for use.

[0348] Additional GDF traps (ActRIIB-Fc fusion proteins modified so as to reduce the ratio of activin A binding relative to myostatin or GDF11 binding) are described in WO 2008/097541 and WO 2006/012627, incorporated by reference herein.

Example 6: Bioassay for GDF11- and Activin-Mediated Signaling

[0349] An A-204 reporter gene assay was used to evaluate the effects of ActRIIB-Fc proteins and GDF traps on signaling by GDF-11 and activin A. Cell line: human rhabdomyosarcoma (derived from muscle). Reporter vector: pGL3(CAGA)12 (described in Dennler et al, 1998, EMBO 17: 3091-3100). The CAGA12 motif is present in TGF-beta responsive genes (e.g., PAI-1 gene), so this vector is of general use for factors signaling through SMAD2 and 3.

[0350] Day 1: Split A-204 cells into 48-well plate.

[0351] Day 2: A-204 cells transfected with 10 ug pGL3(CAGA)12 or pGL3(CAGA)12(10 ug)+pRLCMV (1 µg) and Fugene.

[0352] Day 3: Add factors (diluted into medium+0.1% BSA). Inhibitors need to be preincubated with factors for 1 hr before adding to cells. Six hrs later, cells were rinsed with PBS and lysed.

[0353] This is followed by a luciferase assay. In the absence of any inhibitors, activin A showed 10-fold stimulation of reporter gene expression and an ED₅₀~2 ng/ml. GDF-11: 16 fold stimulation, ED₅₀: ~1.5 ng/ml.

[0354] ActRIIB(20-134) is a potent inhibitor of, for example, activin A, GDF-8, and GDF-11 activity in this assay. As described below, ActRIIB variants were also tested in this assay.

Example 7: ActRIIB-Fc Variants, Cell-Based Activity

[0355] Activity of ActRIIB-Fc proteins and GDF traps was tested in a cell-based assay as described above. Results are summarized in the table below. Some variants were tested in different C-terminal truncation constructs. As discussed above, truncations of five or fifteen amino acids caused reduction in activity. The GDF traps (L79D and

L79E variants) showed substantial loss of activin A inhibition while retaining almost wild-type inhibition of GDF11. Soluble ActRIIB-Fc binding to GDF11 and Activin A:

ActRIIB-Fc Variations	Portion of ActRIIB (corresponds to amino acids of SEQ ID NO: 1)	GDF11 Inhibition Activity	Activin Inhibition Activity
R64	20-134	+++ (approx. 10^{-8} M K _D)	+++ (approx. 10^{-8} M K _D)
A64	20-134	+ (approx. 10^{-6} M K _D)	+ (approx. 10^{-6} M K _D)
R64	20-129	+++	+++
R64 K74A	20-134	++++	++++
R64 A24N	20-134	+++	+++
R64 A24N	20-119	++	++
R64 A24N K74A	20-119	+	+
R64 L79P	20-134	+	+
R64 L79P K74A	20-134	+	+
R64 L79D	20-134	+++	+
R64 L79E	20-134	+++	+
R64K	20-134	+++	+++
R64K	20-129	+++	+++
R64 P129S	20-134	+++	+++
P130A			
R64N	20-134	+	+

+ Poor activity (roughly 1×10^{-6} K_D)

++ Moderate activity (roughly 1×10^{-7} K_D)

+++ Good (wild-type) activity (roughly 1×10^{-8} K_D)

++++ Greater than wild-type activity

[0356] The A24N variant has activity in the cell-based assay (above) and that is equivalent to the wild-type molecule. The A24N variant, and any of the other variants tested above, may be combined with the GDF trap molecules, such as the L79D or L79E variants.

Example 8: GDF11 and Activin A Binding

[0357] Binding of certain ActRIIB-Fc proteins and GDF traps to ligands was tested in a Biacore assay.

[0358] The ActRIIB-Fc variants or wild-type protein were captured onto the system using an anti-hFc antibody. Ligands were injected and flowed over the captured receptor proteins. Results are summarized in the tables below.

Ligand-Binding Specificity IIB Variants.

Protein	Kon (1/Ms)	Koff (1/s)	KD (M)
	GDF11		
ActRIIB(20-134)-hFc	1.34e-6	1.13e-4	8.42e-11
ActRIIB(A24N 20-134)-hFc	1.21e-6	6.35e-5	5.19e-11
ActRIIB(L79D 20-134)-hFc	6.7e-5	4.39e-4	6.55e-10
ActRIIB(L79E 20-134)-hFc	3.8e-5	2.74e-4	7.16e-10
ActRIIB(R64K 20-134)-hFc	6.77e-5	2.41e-5	3.56e-11
GDF8			
ActRIIB(20-134)-hFc	3.69e-5	3.45e-5	9.35e-11
ActRIIB(A24N 20-134)-hFc			
ActRIIB(L79D 20-134)-hFc	3.85e-5	8.3e-4	2.15e-9
ActRIIB(L79E 20-134)-hFc	3.74e-5	9e-4	2.41e-9
ActRIIB(R64K 20-134)-hFc	2.25e-5	4.71e-5	2.1e-10
ActRIIB(R64K 20-129)-hFc	9.74e-4	2.09e-4	2.15e-9
ActRIIB(P129S, P130R 20-134)-hFc	1.08e-5	1.8e-4	1.67e-9
ActRIIB(K74A 20-134)-hFc	2.8e-5	2.03e-5	7.18e-11

-continued

Protein	Kon (1/Ms)	Koff (1/s)	KD (M)
	Activin A		
ActRIIB(20-134)-hFc	5.94e6	1.59e-4	2.68e-11
ActRIIB(A24N 20-134)-hFc	3.34e6	3.46e-4	1.04e-10
ActRIIB(L79D 20-134)-hFc			Low binding
ActRIIB(L79E 20-134)-hFc			Low binding
ActRIIB(R64K 20-134)-hFc	6.82e6	3.25e-4	4.76e-11
ActRIIB(R64K 20-129)-hFc	7.46e6	6.28e-4	8.41e-11
ActRIIB(P129S, P130R 20-134)-hFc	5.02e6	4.17e-4	8.31e-11

[0359] These data obtained in a cell-free assay confirm the cell-based assay data, demonstrating that the A24N variant retains ligand-binding activity that is similar to that of the ActRIIB(20-134)-hFc molecule and that the L79D or L79E molecule retains myostatin and GDF11 binding but shows markedly decreased (non-quantifiable) binding to activin A. **[0360]** Other variants have been generated and tested, as reported in WO2006/012627 (incorporated herein by reference in its entirety). See, e.g., pp. 59-60, using ligands coupled to the device and flowing receptor over the coupled ligands. Notably, K74Y, K74F, K74I (and presumably other hydrophobic substitutions at K74, such as K74L), and D80I, cause a decrease in the ratio of activin A (ActA) binding to GDF11 binding, relative to the wild-type K74 molecule. A table of data with respect to these variants is reproduced below:

Soluble ActRIIB-Fc Variants Binding to GDF11 and Activin a (Biacore™ Assay)

ActRIIB	ActA	GDF11
WT (64A)	KD = 1.8e-7M (+)	KD = 2.6e-7M (+)
WT (64R)	na	KD = 8.6e-8M (+++)
+15tail	KD ~2.6e-8M (+++)	KD = 1.9e-8M (++++)
E37A	*	*
R40A	-	-
D54A	-	*
K55A	++	*
R56A	*	*
K74A	KD = 4.35e-9 M +++++	KD = 5.3e-9M +++++
K74Y	*	--
K74F	*	--
K74I	*	--
W78A	*	*
L79A	+	*
D80K	*	*
D80R	*	*
D80A	*	*
D80F	*	*
D80G	*	*
D80M	*	*
D80N	*	*
D80I	*	--
F82A	++	-

* No observed binding

-- <1/5 WT binding

- ~1/2 WT binding

+ WT

++ <2x increased binding

+++ ~5x increased binding

++++ ~10x increased binding

+++++ ~40x increased binding

Example 9: Generation of a GDF Trap with Truncated ActRIIB Extracellular Domain

[0361] A GDF trap referred to as ActRIIB(L79D 20-134)-hFc was generated by N-terminal fusion of TPA leader to the ActRIIB extracellular domain (residues 20-134 in SEQ ID NO: 1) containing a leucine-to-aspartate substitution (at residue 79 in SEQ ID NO: 1) and C-terminal fusion of human Fc domain with minimal linker (three glycine residues) (FIG. 10; SEQ ID NO: 74). A nucleotide sequence corresponding to this fusion protein is shown in FIG. 11 (SEQ ID NO: 75, sense strand; and SEQ ID NO: 76, antisense strand).

[0362] A GDF trap with truncated ActRIIB extracellular domain, referred to as ActRIIB(L79D 25-131)-hFc, was generated by N-terminal fusion of TPA leader to truncated extracellular domain (residues 25-131 in SEQ ID NO: 1) containing a leucine-to-aspartate substitution (at residue 79 in SEQ ID NO: 1) and C-terminal fusion of human Fc domain with minimal linker (three glycine residues) (FIG. 12, SEQ ID NO: 77). The sequence of the cell purified form of ActRIIB(L79D 25-131)-hFc is presented in FIG. 13 (SEQ ID NO: 78). One nucleotide sequence encoding this fusion protein is shown in FIG. 15 (SEQ ID NO: 80) along with its complementary sequence (SEQ ID NO: 81), and an alternative nucleotide sequence encoding exactly the same fusion protein is shown in FIG. 16 (SEQ ID NO: 82) and its complementary sequence (SEQ ID NO: 83).

Example 10: Selective Ligand Binding by GDF Trap with Double-Truncated ActRIIB Extracellular Domain

[0363] The affinity of GDF traps and other ActRIIB-hFc proteins for several ligands was evaluated in vitro with a Biacore™ instrument. Results are summarized in the table below. Kd values were obtained by steady-state affinity fit due to the very rapid association and dissociation of the complex, which prevented accurate determination of k_{on} and k_{off} .

Ligand Selectivity of ActRIIB-hFc Variants:

Fusion Construct	Activin A (Kd e-11)	Activin B (Kd e-11)	GDF11 (Kd e-11)
ActRIIB(L79 20-134)-hFc	1.6	1.2	3.6
ActRIIB(L79D 20-134)-hFc	1350.0	78.8	12.3
ActRIIB(L79 25-131)-hFc	1.8	1.2	3.1
ActRIIB(L79D 25-131)-hFc	2290.0	62.1	7.4

[0364] The GDF trap with a truncated extracellular domain, ActRIIB(L79D 25-131)-hFc, equaled or surpassed the ligand selectivity displayed by the longer variant, ActRIIB(L79D 20-134)-hFc, with pronounced loss of activin A binding, partial loss of activin B binding, and nearly full retention of GDF11 binding compared to ActRIIB-hFc counterparts lacking the L79D substitution. Note that truncation alone (without L79D substitution) did

not alter selectivity among the ligands displayed here [compare ActRIIB(L79 25-131)-hFc with ActRIIB(L79 20-134)-hFc]. ActRIIB(L79D 25-131)-hFc also retains strong to intermediate binding to the Smad 2/3 signaling ligand GDF8 and the Smad 1/5/8 ligands BMP6 and BMP10.

Example 11: GDF Trap Derived from ActRIIB5

[0365] Others have reported an alternate, soluble form of ActRIIB (designated ActRIIB5), in which exon 4, including the ActRIIB transmembrane domain, has been replaced by a different C-terminal sequence (see, e.g., WO 2007/053775).

[0366] The sequence of native human ActRIIB5 without its leader is as follows:

```
(SEQ ID NO: 50)
GRGEAETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWRNSSG
TIELVKKGCWLDDFNCYDRQECVATEENPQVYFCCCEGNFCNERFTHLP
EAGGPEGPWASTTIPSGGPEATAAGDQGSGALWLCLEGAHE
```

[0367] An leucine-to-aspartate substitution, or other acidic substitutions, may be performed at native position 79 (underlined) as described to construct the variant ActRIIB5 (L79D), which has the following sequence:

```
(SEQ ID NO: 51)
GRGEAETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWRNSSG
TIELVKKGCWDDDNCYDRQECVATEENPQVYFCCCEGNFCNERFTHLP
EAGGPEGPWASTTIPSGGPEATAAGDQGSGALWLCLEGAHE
```

[0368] This variant may be connected to human Fc (double underline) with a TGGG linker (SEQ ID NO: 23) (single underline) to generate a human ActRIIB5(L79D)-hFc fusion protein with the following sequence:

```
(SEQ ID NO: 52)
GRGEAETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWRNSSG
TIELVKKGCWDDDNCYDRQECVATEENPQVYFCCCEGNFCNERFTHLP
```

- continued

```
EAGGPEGPWASTTIPSGGPEATAAGDQGSGALWLCLEGAHETGGG
HTCPPCPAPELLGGPSVFLFPPPKPDKTLMSRTPEVTCVVVDVSHEDP
EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY
KCKVSNKALPAPIEKTISKAKGQPREQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTPPVLDGSFFLYSKLTVDKS
RWOOGNVFSCSVHMEALHNHYTQKSLSPGK
```

[0369] This construct may be expressed in CHO cells.

Example 12: Generation of an ALK4:ActRIIB Heterodimer

[0370] An ALK4-Fc:ActRIIB-Fc heteromeric complex was constructed comprising the extracellular domains of human ActRIIB and human ALK4, which are each separately fused to an Fc domain with a linker positioned between the extracellular domain and the Fc domain. The individual constructs are referred to as ActRIIB-Fc fusion polypeptide and ALK4-Fc fusion polypeptide, respectively, and the sequences for each are provided below.

[0371] A methodology for promoting formation of ALK4-Fc:ActRIIB-Fc heteromeric complexes, as opposed to ActRIIB-Fc or ALK4-Fc homodimeric complexes, is to introduce alterations in the amino acid sequence of the Fc domains to guide the formation of asymmetric heteromeric complexes. Many different approaches to making asymmetric interaction pairs using Fc domains are described in this disclosure.

[0372] In one approach, illustrated in the ActRIIB-Fc and ALK4-Fc polypeptide sequences of SEQ ID NOS: 108 and 110 and SEQ ID Nos: 111 and 113, respectively, one Fc domain is altered to introduce cationic amino acids at the interaction face, while the other Fc domain is altered to introduce anionic amino acids at the interaction face. ActRIIB-Fc fusion polypeptide and ALK4-Fc fusion polypeptide each employ the tissue plasminogen activator (TPA) leader. The ActRIIB-Fc polypeptide sequence (SEQ ID NO: 108) is shown below:

(SEQ ID NO: 108)

```
1 MDAMKRLCC VLLLCGAVFV SPGASGRGEA ETRECIYYNA NWELERTNQS
51 GLERCEGQD KRLHCYASWR NSSGTIELVK KGCWDDFNC YDRQECVATE
101 ENPQVYFCCC EGNFCNERFT HLPEAGGPEV TYEPPPTAPT GGGTHTCPCP
151 PAPELGGPS VFLFPPKPD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV
201 DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY KCKVSNKALP
251 APIEKTISKA KGQPREQVY TLPPSRKEMT KNQVSLTCLV KGFYPSDIA
301 EWESNGQPEN NYKTPPVLK SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH
351 EALHNHYTQK SLSLSPGK
```

[0373] The leader (signal) sequence and linker are underlined. To promote formation of ALK4-Fc:ActRIIB-Fc heterodimer rather than either of the possible homodimeric complexes, two amino acid substitutions (replacing acidic amino acids with lysine) can be introduced into the Fc domain of the ActRIIB fusion protein as indicated by double

underline above. The amino acid sequence of SEQ ID NO: 108 may optionally be provided with lysine (K) removed from the C-terminus.

[0374] This ActRIIB-Fc fusion protein is encoded by the following nucleic acid sequence (SEQ ID NO: 109):

(SEQ ID NO: 109)

```

1 ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
51 AGTCTTCGTT TCGCCCGGCG CCTCTGGCG TGAGGAGGCT GAGACACGGG
101 AGTGCATCTA CTACAACGCC AACTGGGAGC TGGAGCGCAC CAACCAAGAGC
151 GGCTGGAGC GCTGCGAAGG CGAGCAGGAC AAGCGGCTGC ACTGCTACGC
201 CTCTGGCGC AACAGCTCTG GCACCATCGA GCTCGTGAAG AAGGGCTGCT
251 GGCTAGATGA CTTCAACTGC TACGATAGGC AGGAGTGTGT GGCCACTGAG
301 GAGAACCCCC AGGTGTACTT CTGCTGCTGT GAAGGCAACT TCTGCAACGA
351 GCGCTTCACT CATTGCCAG AGGCTGGGG CCCGGAAGTC ACGTACGAGC
401 CACCCCCGAC AGCCCCCACC GGTGGTGGAA CTCACACATG CCCACCGTGC
451 CCAGCACCTG AACTCCTGGG GGGACCGTCA GTCTTCCTCT TCCCCCCAAA
501 ACCCAAGGAC ACCCTCATGA TCTCCCGAC CCCTGAGGTC ACATGCGTGG
551 TGGTGGACGT GAGCCACGAA GACCCCTGAGG TCAAGTTCAA CTGGTACGTG
601 GACGGCGTGG AGGTGCATAA TGCCAAGACA AAGCCGCGGG AGGAGCAGTA
651 CAACAGCACG TACCGTGTGG TCAGCGTCCT CACCGTCCTG CACCAGGACT
701 GGCTGAATGG CAAGGGAGTAC AAGTGCAAGG TCTCCAACAA AGCCCTCCCA
751 GCCCCCCATCG AGAAAACCAT CTCCAAAGCC AAAGGGCAGC CCCGAGAAC
801 ACAGGGTGTAC ACCCTGCCCT CATCCCGAA GGAGATGACC AAGAACCGAG
851 TCAGCCTGAC CTGCCCTGGTC AAAGGCTTCT ATCCCAGCGA CATCGCCGTG
901 GAGTGGGAGA GCAATGGGCA GCCGGAGAAC AACTACAAGA CCACGCCTCC
951 CGTGCTGAAG TCCGACGGCT CCTTCTTCCT CTATAGCAAG CTCACCGTGG
1001 ACAAGAGCAG GTGGCAGCAG GGGAACGTCT TCTCATGCTC CGTGATGCAT
1051 GAGGCTCTGC ACAACCACTA CACGCAGAAG AGCCTCTCCC TGTCTCCGGG
1101 TAAA

```

[0375] A mature ActRIIB-Fc fusion polypeptide (SEQ ID NO: 110) is as follows, and may optionally be provided with lysine (K) removed from the C-terminus.

(SEQ ID NO: 110)

```

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

```

[0376] A complementary form of ALK4-Fc fusion poly-peptide (SEQ ID NO: 111) is as follows:

```
(SEQ ID NO: 111)
1 MDAMKRLGCC VLLLCGAVFV SPGASGPRGV QALLCACTSC LQANYTCETD
51 GACMVSIFNL DGMEHHVRTC IPKVELVPAG KPFYCLSSED LRNTHCCYTD
101 YCNRIDLRPV SGHLKEPEHP SMWGPVETGG GTHTCPCPCA PELLGGPSVF
151 LFPPPKPKDTL MISRTPEVTC VVVDVSHEPD EVKFNWyVDG VEVHNAAKTP
201 REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC KVSNKALPAP IEKTISKAKG
251 QPREPQVYTL PPSREEMTKN QVSLTCLVKG FYPSDIAVEW ESNQQPENNY
301 DTTPPVLDSD GSFFLYSDLT VDKSRWQQGN VFSCSVMHEA LHNHYTQKSL
351 SLSPG
```

[0377] The leader sequence and linker are underlined. To guide heterodimer formation with the ActRIIB-Fc fusion polypeptide of SEQ ID NOs: 108 and 110 above, two amino acid substitutions (replacing lysines with aspartic acids) can be introduced into the Fc domain of the ALK4-Fc fusion

polypeptide as indicated by double underline above. The amino acid sequence of SEQ ID NO: 111 may optionally be provided with lysine (K) added at the C-terminus.

[0378] This ALK4-Fc fusion protein is encoded by the following nucleic acid (SEQ ID NO: 112):

```
(SEQ ID NO: 112)
1 ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
51 AGTCTTCGTT TCGCCCCGGCG CCTCCGGGCC CCGGGGGGTC CAGGCTCTGC
101 TGTGTGCGTG CACCAGCTGC CTCCAGGCC ACTACACGTG TGAGACAGAT
151 GGGGCCTGCA TGGTTTCCAT TTTCAATCTG GATGGGATGG AGCACCATGT
201 GCGCACCTGC ATCCCCAAAG TGGAGCTGGT CCCTGCCGGG AAGCCCTTCT
251 ACTGCCTGAG CTCGGAGGAC CTGCGCAACA CCCACTGCTG CTACACTGAC
301 TACTGCAACA GGATCGACTT GAGGGTGCC AGTGGTCACC TCAAGGAGCC
351 TGAGCACCCG TCCATGTGGG GCCCCGTGGA GACCGGGTGGT GGAAACTCACA
401 CATGCCACC GTGCCAGCA CCTGAACTCC TGGGGGGACC GTCAGTCCTTC
451 CTCTTCCCCC CAAAACCAA GGACACCCTC ATGATCTCCC GGACCCCTGA
501 GGTACATGC GTGGTGGTGG ACGTGAGGCC CGAACACCCT GAGGTCAAGT
551 TCAACTGGTA CGTGGACGGC GTGGAGGTGC ATAATGCCA GACAAGCCG
601 CGGGAGGAGC AGTACAACAG CACGTACCGT GTGGTCAGCG TCCTCACCGT
651 CCTGCACCAG GACTGGCTGA ATGGCAAGGA GTACAAGTG AAGGTCTCCA
701 ACAAAAGCCCT CCCAGCCCCC ATCGAGAAAA CCATCTCAA AGCCAAAGGG
751 CAGCCCCGAG AACCAACAGGT GTACACCCTG CCCCCATCCC GGGAGGAGAT
801 GACCAAGAAC CAGGTCAGCC TGACCTGCCT GGTCAAAGGC TTCTATCCCA
851 GCGACATCGC CGTGGAGTGG GAGAGCAATG GGCAGCCGGA GAACAACTTAC
901 GACACCACGC CTCCCGTGT GGACTCCGAC GGCTCCTTCT TCCTCTATAG
951 CGACCTCACC GTGGACAAGA GCAGGGTGGCA GCAGGGGAAC GTCTTCTCAT
1001 GCTCCGTGAT GCATGAGGCT CTGCACAACC ACTACACGCCA GAAGAGCCTC
1051 TCCCTGTCTC CGGGT
```

[0379] A mature ALK4-Fc fusion protein sequence (SEQ ID NO: 113) is as follows and may optionally be provided with lysine (K) added at the C-terminus.

```
(SEQ ID NO: 113)
1 SGPRGVQALL CACTSCLQAN YTCETDGACM VSIFNLDGME HHVRTCIPKV
51 ELVPAGKPFY CLSSEDLRNT HCCYTDYCNR IDLRVPSGHL KEPEHPSMWG
101 PVETGGGTHT CPPCPAPELL GGPSPVFLFPP KPKDTLMISR TPEVTCVVVD
151 VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN
201 GKEYKCKVSN KALPAPIEK TISKAKGQPRT PQVYTLPPSR EEMTKNQVSL
251 TCLVKGFYPS DIAVEWESNG QPENNYDTTP PVLDSDGSFF LYSDLTVDKS
301 RWQQGNVFSC SVMHEALHNH YTQKSLSLSP G
```

[0380] The ActRIIB-Fc and ALK4-Fc proteins of SEQ ID NO: 110 and SEQ ID NO: 113, respectively, may be co-expressed and purified from a CHO cell line, to give rise to a heteromeric complex comprising ALK4-Fc:ActRIIB-Fc.

[0381] In another approach to promote the formation of heteromultimer complexes using asymmetric Fc fusion proteins the Fc domains are altered to introduce complementary

hydrophobic interactions and an additional intermolecular disulfide bond as illustrated in the ActRIIB-Fc and ALK4-Fc polypeptide sequences of SEQ ID NOS: 114 and 115 and SEQ ID Nos: 116 and 117, respectively. The ActRIIB-Fc fusion polypeptide and ALK4-Fc fusion polypeptide each employ the tissue plasminogen activator (TPA) leader.

[0382] The ActRIIB-Fc polypeptide sequence (SEQ ID NO: 114) is shown below:

```
(SEQ ID NO: 114)
1 MDAMKRLGCC VLLLCGAVFV SPGASGRGEA ETRECIYYNA NWELERTNQS
51 GLERCEGEQD KRLHCYASWR NSSGTIELVK KGCWLDDFNC YDRQECVATE
101 ENPQVYFCCC EGNFCNERFT HLPEAGGPEV TYEPPPTAPT GGGTHTCPPC
151 PAPELLGGPS VFLFPPPKPD TLMISRTPEV TCVVVDSHE DPEVKFNWYV
201 DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY KCKVSNKALP
251 APIEKTISKA KGQPPREPQVY TLPPCREEMT KNQVSLWCLV KGFPPSDIA
301 EWESNGQOPEN NYKTTPPVLD SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH
351 EALHNHYTQK SLSLSPGK
```

[0383] The leader (signal) sequence and linker are underlined. To promote formation of the ALK4-Fc:ActRIIB-Fc heterodimer rather than either of the possible homodimeric complexes, two amino acid substitutions (replacing a serine with a cysteine and a threonine with a tryptophan) can be introduced into the Fc domain of the fusion protein as indicated by double underline above. The amino acid sequence of SEQ ID NO: 114 may optionally be provided with lysine (K) removed from the C-terminus.

[0384] A mature ActRIIB-Fc fusion polypeptide is as follows:

```
(SEQ ID NO: 115)
1 GRGEAETREC IYYNANWELE RTNQSGLERC EGEQDKRLHC YASWRNSSGT
51 IELVKKGCWL DDFNCYDRQE CVATEENPQV YFCCCEGNFC NERFTHLPEA
101 GGPEVTYEPP PTAPTGGGTH TCPPCPAPEL LGGPSVFLFP PKPKDTLMIS
151 RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV HNAKTKPREE QYNSTYRVVS
201 VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQPRT EPQVYTLPPC
251 REEMTKNQVS LWCLVKGFYPS SDIAVEWESN QPENNYKTT PPVLDSDGSF
301 FLYSKLTVDK SRWQQGNVFS CSVHMEALHNHYTQK SLSLSPGK
```

[0385] A complementary form of ALK4-Fc fusion polypeptide (SEQ ID NO: 116) is as follows and may optionally be provided with lysine (K) removed from the C-terminus.

(SEQ ID NO: 116)

```

1 MDAMKRLGCC VLLLCGAVFV SPGASGPRGV QALLCACTSC LQANYTCETD
5 GACMVSIFNL DGMEHHVRTC IPKVELVPAG KPFYCLSSED LRNTHCCYTD
101 YCNRIDLRVP SGHLKEPEHP SMWGPVETGG GTHTCPPCPA PELLGGPSVF
151 LFPPPKPDL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG VEVHNAKTKP
201 REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC KVSNKALPAP IEKTISKAKG
251 QPREPQVCTLP PPSREEMTKN QVSLSCAVKG FYPPSDIAVEW ESNGQPENNY
301 KTPPPVLDSD GSFFLVSKLT VDKSRWQQGN VFSCSVMHEA LHNHYTQKSL
351 SLSPGK

```

[0386] The leader sequence and the linker are underlined. To guide heterodimer formation with the ActRIIB-Fc fusion polypeptide of SEQ ID NOs: 114 and 115 above, four amino acid substitutions can be introduced into the Fc domain of the ALK4 fusion polypeptide as indicated by double underline above. The amino acid sequence of SEQ ID NO: 116 may optionally be provided with lysine (K) removed from the C-terminus.

[0387] A mature ALK4-Fc fusion protein sequence is as follows and may optionally be provided with lysine (K) removed from the C-terminus.

(SEQ ID NO: 117)

```

1 SGPRGVQALL CACTSCLQAN YTCETDGACM VSIFNLDGME HHVRTCIPKV
5 ELVPAGKPFY CLSSEDLRNT HCCYTDYCNR IDLRVPSGHL KEPEHPSMWG
101 PVETGGGTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD
151 VSHEDPEVKF NWYVGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN
201 GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVCTLPPSR EEMTKNQVSL
251 SCAVKGFYPS DIAVEWESNG QPENNYKTTP PVLSDGSFF LVSKLTVDKS
301 RWQQGNVFSC SVMHEALHNH YTQKSLSLSP GK

```

[0388] ActRIIB-Fc and ALK4-Fc proteins of SEQ ID NO: 115 and SEQ ID NO: 117 respectively, may be co-expressed and purified from a CHO cell line, to give rise to a heteromeric complex comprising ALK4-Fc:ActRIIB-Fc.

[0389] Purification of various ALK4-Fc:ActRIIB-Fc complexes could be achieved by a series of column chromatography steps, including, for example, three or more of the following, in any order: protein A chromatography, Q sepharose chromatography, phenylsepharose chromatography, size exclusion chromatography, and cation exchange chromatography. The purification could be completed with viral filtration and buffer exchange.

[0390] In another approach to promote the formation of heteromultimer complexes using asymmetric Fc fusion proteins, the Fc domains are altered to introduce complementary hydrophobic interactions, an additional intermolecular disulfide bond, and electrostatic differences between the two Fc domains for facilitating purification based on net molecular charge, as illustrated in the ActRIIB-Fc and ALK4-Fc polypeptide sequences of SEQ ID NOs: 118-121 and 122-125, respectively. The ActRIIB-Fc fusion polypeptide and ALK4-Fc fusion polypeptide each employ the tissue plasminogen activator (TPA) leader.

[0391] The ActRIIB-Fc polypeptide sequence (SEQ ID NO: 118) is shown below:

(SEQ ID NO: 118)

```

1 MDAMKRLGCC VLLLCGAVFV SPGASGRGEA ETRECIYYNA NWELERTNQS
51 GLERCEGEQD KRLHCYASWR NSSGTIELVK KGCWLDDFNC YDRQECVATE
101 ENPQVYFCCC EGNFCNERFT HLPEAGGPEV TYEPPPTAPT GGGTHTCPPC

```

- continued

```

151 PAPELLGGPS VFLFPPPKPD TLMISRTPEV TCVVVDSHE DPEVKFNWYV
201 DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY KCKVSNKALP
251 APIEKTISKA KGQPREPQVY TLPPCREEMT ENQVSLWCLV KGFYPPSDIAV
301 EWESNGOPEN NYKTTPPVLD SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH
351 EALHNHYTQD SLSLSPG

```

[0392] The leader sequence and linker are underlined. To promote formation of the ALK4-Fc:ActRIIB-Fc heterodimer rather than either of the possible homodimeric complexes, two amino acid substitutions (replacing a serine with a cysteine and a threonine with a tryptophan) can be introduced into the Fc domain of the fusion protein as indicated by double underline above. To facilitate purification of the ALK4-Fc:ActRIIB-Fc heterodimer, two amino acid substi-

tutions (replacing lysines with acidic amino acids) can also be introduced into the Fc domain of the fusion protein as indicated by double underline above. The amino acid sequence of SEQ ID NO: 118 may optionally be provided with a lysine added at the C-terminus.

[0393] This ActRIIB-Fc fusion protein is encoded by the following nucleic acid (SEQ ID NO: 119):

```

(SEQ ID NO: 119)
1 ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
51 AGTCTTCGTT TCGCCCGGCC CCTCTGGCGC TGGGGAGGCT GAGACACGGG
101 AGTCATCTA CTACAACGCC AACTGGGAGC TGGAGCGCAC CAACCAGAGC
151 GGCCTGGAGC GCTGCGAAGG CGAGCAGGAC AAGCGGCTGC ACTGCTACGC
201 CTCCTGGCGC AACAGCTCTG GCACCATCGA GCTCGTGAAG AAGGGCTGCT
251 GGCTAGATGA CTTCAACTGC TACGATAGGC AGGAGTGTGT GGCCACTGAG
301 GAGAACCCCC AGGTGTACTT CTGCTGCTGT GAAGGCAACT TCTGAAACGA
351 GCGCTTCACT CATTGCCAG AGGCTGGGG CCCGGAAGTC ACGTACGAGC
401 CACCCCCGAC AGCCCCCACC GGTGGTGAA CTCACACATG CCCACCGTGC
451 CCAGCACCTG AACTCCTGGG GGGACCGTCA GTCTTCCTCT TCCCCCCCAAA
501 ACCCAAGGAC ACCCTCATGA TCTCCCGGC CCCTGAGGTC ACATGCGTGG
551 TGGTGGACGT GAGCCACGAA GACCCTGAGG TCAAGTTCAA CTGGTACGTG
601 GACGGCGTGG AGGTGCATAA TGCCAAGACA AAGCCGCGGG AGGAGCAGTA
651 CAACAGCACG TACCGTGTGG TCAGCGTCCT CACCGTCTG CACCAGGACT
701 GGCTGAATGG CAAGGAGTAC AAGTGCAGG TCTCCAACAA AGCCCTCCCA
751 GCCCCCATCG AGAAAACCAT CTCCAAAGGC AAAGGGCAGC CCCGAGAAC
801 ACAGGTGTAC ACCCTGCCCA CATGCCGGGA GGAGATGACC GAGAACCAGG
851 TCAGCCTGTG GTGCCGGTC AAAGGCTTCT ATCCCGCGA CATGCCGTGG
901 GAGTGGGAGA GCAATGGGCA GCCGGAGAAC AACTACAAGA CCACGCCCTCC
951 CGTGCTGGAC TCCGACGGCT CCTTCTTCCT CTATAGCAAG CTCACCGTGG
1001 ACAAGAGCAG GTGGCAGCAG GGGAACGTCT TCTCATGCTC CGTGATGCAT
1051 GAGGCTCTGC ACAACCACCA CACGCAGGAC AGCCTCTCCC TGTCTCCGGG
1101 T

```

[0394] The mature ActRIIB-Fc fusion polypeptide is as follows (SEQ ID NO: 120) and may optionally be provided with a lysine added to the C-terminus.

(SEQ ID NO: 120)

```

1 GRGEAETREC IYYNANWELE RTNQSGLERC EGEQDKRLHC YASWRNSSGT
51 IELVKKGCWL DDFNACYDRQE CVATEENPQV YFCCCEGNFC NERFTHLPEA
101 GGPEVTYEP P TAPPTGGTH TCPPCPAPEL LGGPSVFLFP PKPKDTLMIS
151 RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV HNAKTKPREE QYNSTYRVVS
201 VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQPR EPQVYTLPPC
251 REEMTENQVS LWCLVKGFYP SDIAVEWESN GQPENNYKTT PPVLDSDGSF
301 FLYSKLTVDK SRWQQGNVFS CSVMEHALHN HYTQDSLSSL PG

```

[0395] This ActRIIB-Fc fusion polypeptide is encoded by the following nucleic acid (SEQ ID NO: 121):

(SEQ ID NO: 121)

```

1 GGGCGTGGGG AGGCTGAGAC ACGGGAGTGC ATCTACTACA ACGCCAAC TG
51 GGAGCTGGAG CGCACCAACC AGAGCGGCCT GGAGCGCTGC GAAGGGCAGC
101 AGGACAAGCG GCTGCACTGC TACGCCCTCC GGCACAAACAG CTCTGGCAC
151 ATCGAGCTCG TGAAGAAGGG CTGCTGGCTA GATGACTTCA ACTGCTACGA
201 TAGGCAGGAG TGTGTGGCCA CTGAGGAGAA CCCCCAGGTG TACTTCTGCT
251 GCTGTGAAGG CAACTTCTGC AACGAGCGCT TCACTCATTT GCCAGAGGCT
301 GGGGGCCCGG AAGTCACGTA CGAGCACCCC CCGACAGCCC CCACCGGTGG
351 TGGAACTCAC ACATGCCAC CGTGCCCAGC ACCTGAACTC CTGGGGGGAC
401 CGTCAGTCTT CCTCTTCCCC CCAAAACCCA AGGACACCCCT CATGATCTCC
451 CGGACCCCTG AGGTCAACATG CGTGGTGGTG GACGTGAGCC ACGAAGACCC
501 TGAGGTCAAG TTCAACTGGT ACGTGGACGG CGTGGAGGTG CATAATGCCA
551 AGACAAAGCC GCGGGAGGAG CAGTACAACA GCACGTACCG TGTGGTCAGC
601 GTCTCACCG TCCTGCACCA GGACTGGCTG AATGGCAAGG AGTACAAGTG
651 CAAGGTCTCC AACAAAGCCC TCCCAAGCCCC CATCGAGAAA ACCATCTCCA
701 AAGCCAAAGG GCAGCCCCGA GAACCACAGG TGTACACCCCT GCCCCCATGC
751 CGGGAGGAGA TGACCGAGAA CCAGGTCAGC CTGTGGTGCG TGGTCAAAGG
801 CTTCTATCCC AGCGACATCG CCGTGGAGTG GGAGAGCAAT GGGCAGCCGG
851 AGAACAACTA CAAGACCACG CCTCCCGTGC TGGACTCCGA CGGCTCCTTC
901 TTCTCTCTATA GCAAGCTCAC CGTGGACAAG AGCAGGGTGGC AGCAGGGGAA
951 CGTCTCTCA TGCTCCGTGA TGCATGAGGC TCTGCACAAAC CACTACACGC
1001 AGGACAGCCT CTCCCTGTCT CCGGGT

```

[0396] The complementary form of ALK4-Fc fusion polypeptide (SEQ ID NO: 122) is as follows and may optionally be provided with lysine removed from the C-terminus.

(SEQ ID NO: 122)

```

1 MDAMKRGLCC VLLLCGAVFV SPGASGPRGV QALLCACTSC LQANYTCETD
51 GACMVSIFNL DGMEHHVRTE IPKVELVPAG KPFYCLSSED LRNTHCCYTD

```

- continued

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101 YCNRIDLRP SGHLKEPEHP SMWGPVETGG GTHTCPPCPA PELLGGPSVF
151 LFFPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG VEVHNAKTP
201 REEQYNSTYR VVSVLIVLHQ DWLNGKEYKC KVSNKALPAP IEKTISKAKG
251 QPREPQVCTL PPSREEMTKN QVSLSCAVKG FYPPSDIAVEW ESRGQPENNY
301 KTPPVLDSR GSFLVSKLT VDKSRWQQGN VFSCSVMHEA LHNHYTQKSL
351 SLSPGK

```

[0397] The leader sequence and the linker are underlined. To guide heterodimer formation with the ActRIIB-Fc fusion polypeptide of SEQ ID NOs: 118 and 120 above, four amino acid substitutions (replacing a tyrosine with a cysteine, a threonine with a serine, a leucine with an alanine, and a tyrosine with a valine) can be introduced into the Fc domain of the ALK4 fusion polypeptide as indicated by double underline above. To facilitate purification of the ALK4-Fc: ActRIIB-Fc heterodimer, two amino acid substitutions (re-

placing an asparagine with an arginine and an aspartate with an arginine) can also be introduced into the Fc domain of the ALK4-Fc fusion polypeptide as indicated by double underline above. The amino acid sequence of SEQ ID NO: 122 may optionally be provided with lysine removed from the C-terminus.

[0398] This ALK4-Fc fusion polypeptide is encoded by the following nucleic acid (SEQ ID NO: 123):

```

(SEQ ID NO: 123)
1 ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
51 AGTCTTCGTT TCGCCCGGCG CCTCCGGGCC CGGGGGGTC CAGGCTCTGC
101 TGTGTGGTG CACCAGCTGC CTCCAGGCCA ACTACACGTG TGAGACAGAT
151 GGGGCCTGCA TGGTTTCCAT TTTCAATCTG GATGGGATGG AGCACCATGT
201 CGGCACCTGC ATCCCCAAAG TGGAGCTGGT CCCTGCCGGG AAGCCCTCT
251 ACTGCCTGAG CTCGGAGGAC CTGCGAACCA CCCACTGCTG CTACACTGAC
301 TACTGCAACA GGATCGACTT GAGGGTGCCC AGTGGTCACC TCAAGGAGCC
351 TGAGCACCCG TCCATGTGGG GCCCGGTGG GACCGGTGGT GGAACTCACA
401 CATGCCACC GTGCCCAGCA CCTGAACTCC TGGGGGGACC GTCAGTCTT
451 CTCTTCCCCC CAAAACCCAA GGACACCCTC ATGATCTCCC GGACCCTGA
501 GGTCACATGC GTGGTGGTGG ACGTGAGGCC CGAAGACCCGTGGTCAAGT
551 TCAACTGGTA CGTGGACGGC GTGGAGGTGC ATAATGCCAA GACAAAGCCG
601 CGGGAGGAGC AGTACAAACAG CACGTACCGT GTGGTCAGCG TCCTCACCGT
651 CCTGCACCAG GACTGGCTGA ATGGCAAGGA GTACAAGTGC AAGGTCTCCA
701 ACAAAGGCCCT CCCAGCCCCC ATCGAGAAAA CCATCTCAA AGCCAAAGGG
751 CAGCCCCGAG AACCACAGGT GTGCACCCTG CCCCATCCC GGGAGGAGAT
801 GACCAAGAAC CAGGTCAGCC TGTCC CGC CGTCAAAGGC TTCTATCCCA
851 GCGACATCGC CGTGGAGTGG GAGAGCCGGC GAGCCGGGA GAACAAACTAC
901 AAGACCACGC CTCCCGTGCT GGACTCCCGC GGCTCCTTCT TCCTCGGAG
951 CAAGGCTCACC GTGGACAAGA GCAGGTGGCA GCAGGGGAAC GTCTTCTCAT
1001 GCTCCGTGAT GCATGAGGGCT CTGCACAAACC ACTACACGCA GAAGAGCCTC
1051 TCCCTGTCTC CGGGTAAA

```

[0399] The mature ALK4-Fc fusion polypeptide sequence is as follows (SEQ ID NO: 124) and may optionally be provided with lysine removed from the C-terminus.

```
(SEQ ID NO: 124)
1 SGPRGVQALL CACTSCLQAN YTCTDGACM VSIFNLDGME HHVRTCIPKV
51 ELVPAGKPFY CLSSEDLRNT HCCYTDYCNR IDLRVPSGHL KEPEHPSMWG
101 PVETGGHTH CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD
151 VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN
201 GKEYKCKVSN KALPAPIEKT ISKAKGQPREG PQVCTLPPSR EEMTKNQVSL
251 SCAVKGFYPS DIAVEWESRG QPENNYKTPP PVLDLRSGSFF LVSKLTVDKS
301 RWQQGNVFSC SVMHEALHNH YTQKSLSLSP GK
```

[0400] This ALK4-Fc fusion polypeptide is encoded by the following nucleic acid (SEQ ID NO: 125):

```
(SEQ ID NO: 125)
1 TCCGGGCCCG GGGGGTCCA GGCTCTGCTG TGTGCGTGCA CCAGCTGCCT
51 CCAGGCCAAC TACACGTGTG AGACAGATGG GGCCTGCATG GTTCCATT
101 TCAATCTGGA TGGGATGGAG CACCATGTGC GCACCTGCAT CCCAAAGTG
151 GAGCTGGTCC CTGCCGGGAA GCCCTTCTAC TGCCTGAGCT CGGAGGACCT
201 GCGCAACACC CACTGCTGCT ACACTGACTA CTGCAACAGG ATCGACTTGA
251 GGGTGCCAG TGGTCACCTC AAGGAGCCTG AGCACCCGTC CATGTGGGGC
301 CCGGTGGAGA CCGGTGGTGG AACTCACACA TGCCCACCGT GCCCAGCACC
351 TGAACCTCTG GGGGGACCGT CAGTCTTCCT CTTCCCCCA AAACCCAAGG
401 ACACCCCTCAT GATCTCCCGG ACCCCTGAGG TCACATGCGT GGTGGTGGAC
451 GTGAGCCACG AAGACCCCTGA GGTCAAGTTC AACTGGTAGC TGGACGGCGT
501 GGAGGTGCAT AATGCCAAGA CAAAGCCGCG GGAGGAGCAG TACAACAGCA
551 CGTACCGTGT GGTCAAGCGTC CTCACCGTCC TGCAACAGGA CTGGCTGAAT
601 GGCAAGGAGT ACAAGTGCAA GGTCTCCAAC AAAGCCCTCC CAGCCCCAT
651 CGAGAAAACCTCTCAAAG CCAAAAGGGCA GCCCCGAGAA CCACAGGTGT
701 GCACCCCTGCC CCCATCCCGG GAGGAGATGA CCAAGAACCA GGTCAAGCCTG
751 TCCTGCGCCG TCAAAGGCTT CTATCCCAGC GACATCGCCG TGGAGTGGGA
801 GAGCCGGCGG CAGCCGGAGA ACAACTACAA GACCACGCCT CCCGTGCTGG
851 ACTCCCGCGG CTCCTTCTTC CTCGTGAGCA AGCTCACCGT GGACAAGAGC
901 AGGTGGCAGC AGGGGAACGT CTTCTCATGC TCCGTGATGC ATGAGGCTCT
951 GCACAAACCAC TACACGCAGA AGAGCCTCTC CCTGTCTCCG GGTAAA
```

[0401] ActRIIB-Fc and ALK4-Fc proteins of SEQ ID NO: 120 and SEQ ID NO: 124, respectively, may be co-expressed and purified from a CHO cell line, to give rise to a heteromeric complex comprising ALK4-Fc:ActRIIB-Fc.

[0402] Purification of various ALK4-Fc:ActRIIB-Fc complexes could be achieved by a series of column chromatography steps, including, for example, three or more of the following, in any order: protein A chromatography, Q sep-

harose chromatography, phenylsepharose chromatography, size exclusion chromatography, cation exchange chromatography, epitope-based affinity chromatography (e.g., with an antibody or functionally equivalent ligand directed against an epitope on ALK4 or ActRIIB), and multimodal chromatography (e.g., with resin containing both electrostatic and hydrophobic ligands). The purification could be completed with viral filtration and buffer exchange.

Example 13. Ligand Binding Profile of
ALK4-Fc:ActRIIB-Fc Heterodimer Compared to
ActRIIB-Fc Homodimer and ALK4-Fc Homodimer

[0403] A Biacore™-based binding assay was used to compare ligand binding selectivity of the ALK4-Fc:ActRIIB-Fc heterodimeric complex described above with that of ActRIIB-Fc and ALK4-Fc homodimer complexes. The ALK4-Fc:ActRIIB-Fc heterodimer, ActRIIB-Fc homodimer, and ALK4-Fc homodimer were independently captured onto the system using an anti-Fc antibody. Ligands were injected and allowed to flow over the captured receptor protein. Results are summarized in the table below, in which ligand off-rates (k_d) most indicative of effective ligand traps are denoted in bold font.

Ligand binding profile of ALK4-Fc: ActRIIB-Fc heterodimer compared to ActRIIB-Fc homodimer and ALK4-Fc homodimer									
	ActRIIB-Fc homodimer			ALK4-Fc homodimer			ALK4-Fc: ActRIIB-Fc heterodimer		
Ligand	k_a (1/Ms)	k_d (1/s)	K_D (pM)	k_a (1/Ms)	k_d (1/s)	K_D (pM)	k_a (1/Ms)	k_d (1/s)	K_D (pM)
Activin A	1.2×10^7	2.3×10^{-4}	19	5.8×10^5	1.2×10^{-2}	20000	1.3×10^7	1.5×10^{-4}	12
Activin B	5.1×10^6	1.0×10^{-4}	20		No binding		7.1×10^6	4.0×10^{-5}	6
BMP6	3.2×10^7	6.8×10^{-3}	190		—		2.0×10^6	5.5×10^{-3}	2700
BMP9	1.4×10^7	1.1×10^{-3}	77		—		Transient*		3400
BMP10	2.3×10^7	2.6×10^{-4}	11		—		5.6×10^7	4.1×10^{-3}	74
GDF3	1.4×10^6	2.2×10^{-3}	1500		—		3.4×10^6	1.7×10^{-2}	4900
GDF8	8.3×10^5	2.3×10^{-4}	280	1.3×10^5	1.9×10^{-3}	15000 †	3.9×10^5	2.1×10^{-4}	550
GDF11	5.0×10^7	1.1×10^{-4}	2	5.0×10^6	4.8×10^{-3}	270 †	3.8×10^7	1.1×10^{-4}	3

*Indeterminate due to transient nature of interaction

†Very low signal

— Not tested

[0404] These comparative binding data demonstrate that ALK4-Fc:ActRIIB-Fc heterodimer has an altered binding profile/selectivity relative to either ActRIIB-Fc or ALK4-Fc homodimers. ALK4-Fc:ActRIIB-Fc heterodimer displays enhanced binding to activin B compared with either homodimer, retains strong binding to activin A, GDF8, and GDF11 as observed with ActRIIB-Fc homodimer, and exhibits substantially reduced binding to BMP9, BMP10, and GDF3. In particular, BMP9 displays low or no observable affinity for ALK4-Fc:ActRIIB-Fc heterodimer, whereas this ligand binds strongly to ActRIIB-Fc homodimer. Like the ActRIIB-Fc homodimer, the heterodimer retains intermediate-level binding to BMP6. See FIG. 19.

[0405] In addition, an A-204 Reporter Gene Assay was used to evaluate the effects of ALK4-Fc:ActRIIB-Fc heterodimer and ActRIIB-Fc:ActRIIB-Fc homodimer on signaling by activin A, activin B, GDF11, GDF8, BMP10, and BMP9. Cell line: Human Rhabdomyosarcoma (derived from muscle). Reporter vector: pGL3(CAGA)12 (as described in Dennler et al. 1998, EMBO 17: 3091-3100). The CAGA12 motif is present in TGFβ responsive genes (PAI-1 gene), so this vector is of general use for factors signaling through Smad2 and 3. An exemplary A-204 Reporter Gene Assay is outlined below.

[0406] Day 1: Split A-204 cells into 48-well plate.

[0407] Day 2: A-204 cells transfected with 10 ug pGL3(CAGA)12 or pGL3(CAGA)12(10 ug)+pRLCMV (1 ug) and Fugene.

[0408] Day 3: Add factors (diluted into medium+0.1% BSA). Inhibitors need to be pre-incubated with Factors for

about one hr before adding to cells. About six hrs later, cells are rinsed with PBS and then lysed.

[0409] Following the above steps, a Luciferase assay was performed.

[0410] Both the ALK4-Fc:ActRIIB-Fc heterodimer and ActRIIB-Fc:ActRIIB-Fc homodimer were determined to be potent inhibitors of activin A, activin B, GDF11, and GDF8 in this assay. In particular, as can be seen in the comparative homodimer/heterodimer IC₅₀ data illustrated in FIG. 19, ALK4-Fc:ActRIIB-Fc heterodimer inhibits activin A, activin B, GDF8, and GDF11 signaling pathways similarly to the ActRIIB-Fc:ActRIIB-Fc homodimer. However, ALK4-Fc:ActRIIB-Fc heterodimer inhibition of BMP9 and BMP10 signaling pathways is significantly reduced com-

pared to the ActRIIB-Fc:ActRIIB-Fc homodimer. This data is consistent with the above-discussed binding data in which it was observed that both the ALK4-Fc:ActRIIB-Fc heterodimer and ActRIIB-Fc:ActRIIB-Fc homodimer display strong binding to activin A, activin B, GDF8, and GDF11, but BMP10 and BMP9 have significantly reduced affinity for the ALK4-Fc:ActRIIB-Fc heterodimer compared to the ActRIIB-Fc:ActRIIB-Fc homodimer.

[0411] Together, these data therefore demonstrate that ALK4-Fc:ActRIIB-Fc heterodimer is a more selective antagonist of activin A, activin B, GDF8, and GDF11 compared to ActRIIB-Fc homodimer. Accordingly, an ALK4-Fc:ActRIIB-Fc heterodimer will be more useful than an ActRIIB-Fc homodimer in certain applications where such selective antagonism is advantageous. Examples include therapeutic applications where it is desirable to retain antagonism of one or more of activin A, activin B, activin AC, GDF8, and GDF11 but minimize antagonism of one or more of BMP9, BMP10, GDF3, and BMP6.

Example 14: Effects of an ActRII Polypeptide and
ALK4:ActRIIB Heterodimer on Pulmonary
Hypertension in a Monocrotaline Rat Model

[0412] The effects of an ActRIIA-mFc fusion protein (ActRIIA-mFc homodimer as described in Example 1), an ALK4-Fc:ActRIIB-Fc heterodimer (as described in Examples 12 and 13), and sildenafil (a phosphodiesterase-5 inhibitor approved for the treatment of PAH) were examined in a rat model of pulmonary arterial hypertension (PAH). In

this model, Sprague Dawley rats received a subcutaneous injection of monocrotaline (MCT) to induce PAH 24 hours prior to start of therapy.

[0413] Rats were separated into different treatment groups (10 mice per group): 1) treatment with MCT (60 mg/kg administered i.p. as a single dose at day 1 of study) and Tris buffered saline (i.p. as 1 ml/kg, every three days) (vehicle treatment group), 2) treatment with an ActRIIA-mFc polypeptide (10 mg/kg administered i.p. every three days) and MCT (60 mg/kg administered i.p. as a single dose at day 1 of study), 3) treatment with an ALK4-Fc:ActRIIB-Fc heterodimer (10 mg/kg administered i.p. every three days) and MCT (60 mg/kg administered i.p. as a single dose at day 1 of study), 4) treatment with sildenafil (30 mg/kg administered orally twice daily) and MCT (60 mg/kg administered i.p. as a single dose at day 1 of study), and 5) control rats (Tris buffered saline administered i.p. as 1 ml/kg, every three days). Rats were treated for 28 days. Body weights were recorded prior to first dose on Day 1 and then weekly throughout the study.

[0414] On day 28, rats were anesthetized by an intraperitoneal injection of ketamine/xylazine (80/10 mg/kg). An incision was made in the neck, and a jugular vein was isolated and ligated anteriorly. A fluid-filled pressure catheter was introduced into the right jugular vein to measure pulmonary artery pressure (PAP). Another incision was made in the inguinal region, and femoral artery was isolated and ligated anteriorly. A Millar pressure catheter was introduced into a femoral artery to measure systolic arterial pressure, diastolic pressure, and heart rate. Mean arterial pressure and right PAP were monitored using the Notocord HEM (Croissy sur Seine, France) v3.5 data capture system for approximately 5-10 minutes until stable measurements were obtained. During the measurements, rats were maintained at approximately 37° C. on a heating pad and body temperature was monitored throughout the procedure with a rectal temperature probe. At the conclusion of the procedure, rats were euthanized, and the hearts and lungs were removed. The entire heart was weighed. Next, the atria were removed and the left ventricle with septum (LV+S) was separated from the right ventricle (RV). The ventricles were weighed separately. Hypertrophy was assessed, in part, by calculating RV/LV+S. The lungs were also weighed.

[0415] Compared to control animals, monocrotaline treated rats (vehicle treatment group) were observed to have decreased body weight, elevated PAP, right heart hypertrophy, and increased lung weight, indicating establishment of PAH. Sildenafil treated rats did not have any improvement in body weight compared to monocrotaline treated rats. However, sildenafil treatment did reduce elevated PAP by 30%, decrease right heart hypertrophy by 18.5%, and decrease lung weight by 10% compared to monocrotaline treated rats. Surprisingly, both ALK4-Fc:ActRIIB-Fc and ActRIIA-mFc were found to have significantly greater effects in treating PAH in this model compared to sildenafil. For example, ALK4-Fc:ActRIIB-Fc treatment resulted in improvement in body weight (+5.1%), reduced elevated PAP by 44.6%, decreased right heart hypertrophy by 39.6%, and decreased lung weight by 19.0%. While ActRIIA-mFc treatment did not show improvement in body weight, it had significant effects in treating other complications of PAH. For example, ActRIIA-Fc treatment resulted in a reduction of elevated PAP by 68%, decreased right heart hypertrophy by 47.1%, and decreased lung weight by 18.4%.

[0416] Similar trends were observed on vessel muscularity based on histopathologic scoring. After staining tissue samples to detect α SMA/elastin, 100 pulmonary arterioles, between 10 m and 50 m in size, per animal were categorized as non-muscularized, partially muscularized, or completely muscularized. Pulmonary arterioles from vehicle treated rats were determined to be 62.3% completely muscularized, 36.4% partially muscularized, and 1.4% non-muscularized. Sildenafil treatment had only a modest effect on decreasing vessel muscularity (e.g., pulmonary arterioles being 57.9% completely muscularized, 41.6% partially muscularized, and 0.9% non-muscularized). In contrast, ActRIIA-mFc treatment resulted in significant decreases in vessel muscularity compared to sildenafil treated animals (e.g., pulmonary arterioles being 25.8% completely muscularized, 66.9% partially muscularized, and 7.3% non-muscularized compared to vehicle treated animals). Histopathological scoring of smooth muscle hypertrophy of pulmonary arterioles were also recorded as follows: 0 (normal), 1 (minimal), 2 (mild), 3 (moderate), or 4 (marked). Vehicle treated rats had an average smooth muscle hypertrophy of moderate to marked (3.8 score). Again, sildenafil treatment was observed to have a modest effect on hypertrophy with an average score of 3 (moderate). While ActRIIA-mFc treated animals were observed to have significant reduction in smooth muscle hypertrophy (average score of 1.6) compared to both vehicle and sildenafil treated animals. Overall, ActRIIA-mFc treatment significantly reduced vessel muscularity and hypertrophy in this PAH model.

[0417] Together, these data demonstrate that both ActRIIA-mFc and ALK4-Fc:ActRIIB-Fc are effective in ameliorate various complications of PAH in this monocrotaline-induced model. In particular, both ActRIIA-mFc and ALK4-Fc:ActRIIB-Fc had a greater effect in reducing artery pressure, right heart hypertrophy, and vascular muscularization than was observed for sildenafil, which is an approved drug for the treatment of PAH. Furthermore, the data indicate that other GDF/BMP antagonists, particularly ones having activities similar to ActRIIA-mFc and ALK4-Fc:ActRIIB-Fc, may be useful in the treatment of PAH, particularly in preventing or reducing the severity various complications of PAH.

Example 15: Effects of an ActRII Polypeptide and ALK4:ActRIIB Heterodimer on Pulmonary Hypertension in the Sugen Hypoxia Rat Model

[0418] The effects of an ActRIIA-mFc fusion protein (ActRIIA-mFc homodimer as described in Example 1 and sildenafil (a phosphodiesterase-5 inhibitor approved for the treatment of PAH) were further examined the Sugen Hypoxia model of PAH. In this model, rats receive daily doses of semaxanib and are placed in a low oxygen environment (approximately 13% oxygen) to induce PAH 24 hours prior to start of therapy.

[0419] Rats were separated into different treatment groups (10 mice per group): 1) treatment with semaxanib (200 mg/kg administered s.c. as a single dose daily)/hypoxia and Tris buffered saline (administered i.p. as 1 ml/kg, every three days) (vehicle treatment group), 2) treatment with an ActRIIA-mFc polypeptide (10 mg/kg administered i.p. every three days) and semaxanib (200 mg/kg administered s.c. as a single dose daily)/hypoxia, 3) treatment with sildenafil (30 mg/kg administered orally twice daily) and semaxanib (200 mg/kg administered s.c. as a single dose

daily)/hypoxia, and 4) control rats (Tris buffered saline administered i.p. as 1 ml/kg, every three days). Rats were treated for 28 days. Body weights were recorded prior to first dose on Day 1 and then weekly throughout the study.

[0420] On day 28, rats were anesthetized by an intraperitoneal injection of ketamine/xylazine (80/10 mg/kg). An incision was made in the neck, and a jugular vein was isolated and ligated anteriorly. A fluid-filled pressure catheter was introduced into the right jugular vein to measure pulmonary artery pressure (PAP). Another incision was made in the inguinal region, and femoral artery was isolated and ligated anteriorly. A Millar pressure catheter was introduced into a femoral artery to measure systolic arterial pressure, diastolic pressure, and heart rate. Mean arterial pressure and right PAP were monitored using the Notocord HEM (Croissy sur Seine, France) v3.5 data capture system for approximately 5-10 minutes until stable measurements were obtained. During the measurements, rats were maintained at approximately 37° C. on a heating pad and body temperature was monitored throughout the procedure with a rectal temperature probe. At the conclusion of the procedure, rats were euthanized, and the hearts and lungs were removed. The entire heart was weighed. Next, the atria were removed and the left ventricle with septum (LV+S) was separated from the right ventricle (RV). The ventricles were weighed separately. Hypertrophy was assessed, in part, by calculating RV/LV+S. The lungs were also weighed.

[0421] Compared to control animals, semaxanib/hypoxia treated rats (vehicle treatment group) were observed to have decreased body weight, elevated PAP, right heart hypertrophy, and increased lung weight, indicating establishment of PAH. Sildenafil treatment reduced mean pulmonary arterial pressure by 22.4% and decreased right heart hypertrophy by 10% compared to vehicle treated animals. Again, ActRIIA-mFc treatment was found have significantly greater effects in treating PAH in this model compared to sildenafil. For example, ActRIIA-mFc treatment resulted in a reduction of mean pulmonary arterial pressure by 51.3% and decreased right heart hypertrophy by 53.5% compared to vehicle treated animals.

[0422] Similar trends were observed on vessel muscularity based on histopathologic scoring. After staining tissue samples to detect α SMA/elastin, 100 pulmonary arterioles, between 10 m and 50 m in size, per animal were categorized as non-muscularized, partially muscularized, or completely muscularized. Pulmonary arterioles from vehicle treated rats were determined to be 72.5% completely muscularized,

27.4% partially muscularized, and 0.1% non-muscularized. Sildenafil treatment had only a modest effect on decreasing vessel muscularity (e.g., pulmonary arterioles being 67.4% completely muscularized, 31.6% partially muscularized, and 1.0% non-muscularized) compared to vehicle treated animals. In contrast, ActRIIA-mFc treatment resulted in significant decreases in vessel muscularity compared to sildenafil treated animals (e.g., pulmonary arterioles being 29.3% completely muscularized, 69.3% partially muscularized, and 1.4% non-muscularized compared to vehicle treated animals). Histopathological scoring of smooth muscle hypertrophy of pulmonary arterioles were also recorded as follows: 0 (normal), 1 (minimal), 2 (mild), 3 (moderate), or 4 (marked). Vehicle treated rats had an average smooth muscle hypertrophy of moderate to marked (3.6 score). Again, sildenafil treatment was observed to have a modest effect on hypertrophy with an average score of 3 (moderate). While ActRIIA-mFc treated animals were observed to have significant reduction in smooth muscle hypertrophy (average score of 1.4) compared to sildenafil treated animals. Overall, ActRIIA-mFc treatment significantly reduced vessel muscularity and hypertrophy in this PAH model.

[0423] Together, these data demonstrate that ActRIIA-mFc is effective in ameliorate various complications of PAH in the Sugen Hypoxia model. In particular, ActRIIA-mFc had a greater effect in reducing artery pressure, right heart hypertrophy, and vessel muscularization than was observed for sildenafil, which is an approved drug for the treatment of PAH. Furthermore, the data indicate that other GDF/BMP antagonists, particularly ones having activities similar to ActRIIA-mFc may be useful in the treatment of PAH, particularly in preventing or reducing the severity various complications of PAH.

INCORPORATION BY REFERENCE

[0424] All publications and patents mentioned herein are hereby incorporated by reference in their entirety as if each individual publication or patent was specifically and individually indicated to be incorporated by reference.

[0425] While specific embodiments of the subject matter have been discussed, the above specification is illustrative and not restrictive. Many variations will become apparent to those skilled in the art upon review of this specification and the claims below. The full scope of the invention should be determined by reference to the claims, along with their full scope of equivalents, and the specification, along with such variations.

SEQUENCE LISTING

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Sequence total quantity: 138
SEQ ID NO: 1      moltype = AA    length = 512
FEATURE          Location/Qualifiers
source           1..512
mol_type = protein
organism = Homo sapiens

SEQUENCE: 1
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ASWRNSSGTI ELVKKGWCWL DFNCYDRQEC VATEENPQVY FCCCEGNFCN ERFTHLPEAG 120
GPEVITYEPPV TAPTLTTLVLA YSLLPPIGLS LIVLЛАFWMY RHRKPYGHV DIHEDPGPPP 180
PSPLVGLKPL QLLEIKARGR FGCVWKAQLM NDFVAVKIFP LQDKQSWSQE REIFSTPGMK 240
HENLLQFIAA EKRGSNLEVE LWLITAFHDK GSLTDYLGKGN IITWNELCHV AETMSRGLSY 300
LHEDVPWCRG EGHKPSIAHR DFKSKNVLLK SDLTAVLADF GLAVRFEPGK PPGDTHGQVG 360
TRYMAPEVL EGAINFQRDA FLRIDMYAMG LVLWELVSRC KAADGPVDEY MLPFEEIEGQ 420
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	mol_type = protein
	organism = Homo sapiens
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DDFN CYDRQE CVATEENPQV YFCCCEGNFC NERFTHLPEA GGPEVTYEPPTA PTAPT	115
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FEATURE	Location/Qualifiers
source	1..100
	mol_type = protein
	organism = Homo sapiens
SEQUENCE: 3	
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DDFN CYDRQE CVATEENPQV YFCCCEGNFC NERFTHLPEA	100
SEQ ID NO: 4	moltype = AA length = 512
FEATURE	Location/Qualifiers
source	1..512
	mol_type = protein
	organism = Homo sapiens
SEQUENCE: 4	
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ASWANSSTGI ELVKKGCWLDF DFNCYDRQEC VATEENPQVY FCCCEGNFCN ERFTHLPEAG	120
GPEVTYEPPL TAPTLTVLA YSLLPIGGLS LIVLLAFWML RHRKPPYGHV DIHEDPGPPP	180
PSPLVGLKPL QLLEIKARGR FGCVWKAQLM NDFAVAKIFP LQDKQSWSQE REIFSTPGMK	240
HENLLQFIAA EKRGSNLEVE LWLITAFHDK GSLTDYLKGN IITWNELCHV AETMSRGLSY	300
LHEDVPVRG EGHKPNSIAHR DFKSKNVLKK SLDTAVLADF GLAVRFPGK PPGDTHGQVG	360
TRRYMAPEVL EGAINFQRDA FLRIDMYAMG LVLWELVSRKA ADGBVDEY MLPFEEIGQ	420
HPSLEELQEV VVHKMRPTI KDHWLKHPL AQLCVTIEEC WDHDAEARLS AGCVEERVSL	480
IRRSVNGTTS DCLVSLVTSV TNVDLPPKES SI	512
SEQ ID NO: 5	moltype = AA length = 115
FEATURE	Location/Qualifiers
source	1..115
	mol_type = protein
	organism = Homo sapiens
SEQUENCE: 5	
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DDFN CYDRQE CVATEENPQV YFCCCEGNFC NERFTHLPEA GGPEVTYEPPTA PTAPT	115
SEQ ID NO: 6	moltype = AA length = 100
FEATURE	Location/Qualifiers
source	1..100
	mol_type = protein
	organism = Homo sapiens
SEQUENCE: 6	
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DDFN CYDRQE CVATEENPQV YFCCCEGNFC NERFTHLPEA	100
SEQ ID NO: 7	moltype = DNA length = 1536
FEATURE	Location/Qualifiers
source	1..1536
	mol_type = other DNA
	organism = Homo sapiens
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ctccaggaca agcagtcgtg gcagatgtaa cggagatct tcagcacacc tggcatgaag	720
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tacgcctctt ggcgaacag ctctggcacc atcgagctcg tgaagaagggtt ctgctggcta	180
gatgacttca actgctacga tagggaggatgtgtggccca ctgaggagaa ccccccagggtt	240
tacttctgtt gctgtgaagg caacttctgc aacgaacgc tcaactcattt gccagaggctt	300
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	mol_type = protein
	organism = Homo sapiens
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FATWKNISGS IEIVKQGCWL DDINCYDRTD CVEKKDSPEV YFCCCEGNMC NEKFSYFPEM	120
EVTQPTSNPV TPKPPYYNIL LYSLVPLMLI AGIVICAFWV YRHHKMAYPV VLVPVQDPGP	180
PPPSPLLGLK PLQLLLEVKAAR GRFGCIVWKAQ LLNEYYVAVKI FPIQDKQSWSQ NEYEVYSLPG	240
MKHENILQFII GAEKRGTSVD VDLWLITAFH EKGSSLSDFLK ANVVSWNELC HIAETMARGL	300
AYLHEDIPGLK KDGHKPAISH RDIKSKNVLL KNNLTACIAD FGLALKFEAG KSAGDTHGQV	360
GTRRYMAPEV LEGAINFQRD AFLRIDMYAM GLVLWELASR CTAADGPVDE YMLPFEETIG	420
QHPSLEDQMEE VVVHKKKRPV LRDYWQKHAG MAML CETIEE CWDHDAAEURL SAGCVGERIT	480
QMQRLTNIIT TEDIVTVVMT VTNVDFPPKE SSL	513
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FEATURE	Location/Qualifiers
source	1..115
	mol_type = protein
	organism = Homo sapiens
SEQUENCE: 10	
ILGRSETQEC LFFNNANWEKD RTNQTGVEPC YGDKDKRHC FATWKNISGS IEIVKQGCWL	60
DDINCYDRTD CVEKKDSPEV YFCCCEGNMC NEKFSYFPEM EVTQPTSNPV TPKPP	115
 SEQ ID NO: 11	moltype = AA length = 100
FEATURE	Location/Qualifiers
source	1..100
	mol_type = protein
	organism = Homo sapiens
SEQUENCE: 11	
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DDINCYDRTD CVEKKDSPEV YFCCCEGNMC NEKFSYFPEM	100
 SEQ ID NO: 12	moltype = DNA length = 1539
FEATURE	Location/Qualifiers
source	1..1539
	mol_type = other DNA
	organism = Homo sapiens
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cagatgcaga gactaacaaa tattattacc acagaggaca ttgtaaacgt ggtcacaatg	1500
gtgacaaatg ttgacttcc tcccaagaa tctagtctc	1539
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mol_type = other DNA	
organism = Homo sapiens	
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agaaccaatc aaacttggtgt tgaaccgtgt ttttgtgaca aagataaaccg gcggcatgt	120
tttgctacct ggaagaatataat ttctgttcc attggaaatag tggaaacaagg ttgttgtgt	180
gatgatatca actgttatca caggactgtat ttttgtgtgt aatggaaaatg ttttttattt tccggagatg	240
tttttttgtt gttgtgaggaa caaatatgtgt aatggaaaatg ttttttattt tccggagatg	300
gaagtcaacac agccccacttc aaatocagtt acacctaagg caccc	345
 SEQ ID NO: 14 moltype = AA length = 225	
FEATURE Location/Qualifiers	
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mol_type = protein	
organism = Homo sapiens	
 SEQUENCE: 14	
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EVHNNAKTPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK VSNKALPAPI EKTISKAKGQ	120
PREPQVYTLPS PSREEMTKNQ VSLTCLVKGF YPSDIAVEWE SNGQPENNYK TPPVLDSDG	180
SFFLYSKLTV DKSRWQQGNV FSCSVMHEAL HNHYTQKSLS LSPKG	225
 SEQ ID NO: 15 moltype = AA length = 223	
FEATURE Location/Qualifiers	
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mol_type = protein	
organism = Homo sapiens	
 SEQUENCE: 15	
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HNNAKTPRQE QFNSTFRVVS VLTVVHQDWL NGKEYKCKVS NKGLPAPIEK TISKTKGQPR	120
EPQVYTLPPS REEMTKNQVS LTCLVKGFYPS SDIAVEWESN GQPENNYKTT PPMLDSDGSF	180
FLYSKLTVDK SRWQQGNVFS CSVMSHEALHN HYTQKSLSLS PGK	223
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mol_type = protein	
organism = Homo sapiens	
 SEQUENCE: 16	
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KWYVDGVEVH NAKTPRREEQ YNSTFRVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT	120
ISKTKGQPRE PQVYTLPPSR EEMTKNQVSL TCLVKGFYPS DIAVEWESSG QPENNYNTTP	180
PMULDSDGSFF LYSKLTVDKS RWQGNIFSC SVMHEALHN FTQKSLSLS GK	232
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FEATURE Location/Qualifiers	
source 1..279	
mol_type = protein	
organism = Homo sapiens	
 SEQUENCE: 17	
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CAPAEPLLGGP SVFLFPKPK DTLMISRTPE VTCVVVDVSH EDPEVQFKWY VDGVEVHNAAK	120
TKPREEQYNS TFRVSVLTV LHQDWLNGKE YKCKVSNKAL PAPIEKTISK TKGPREPQV	180
YTLPPSREEM TKNQVSLTCL VKGFYPSDIA VEWESSGPOE NNYNTTPM DSDGSFFLYS	240
KLTVDKSRWQ QGNIFSCSVM HEALHNRFQ KSLSLSPKG	279
 SEQ ID NO: 18 moltype = AA length = 229	
FEATURE Location/Qualifiers	
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mol_type = protein
organism = Homo sapiens
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ESKYGPPCPS CPAPEFLGGP SVFLFPPKPK DTLMISRTPE VTCVVVDVSQL EDPEVQFNWY 60
VDGVVEVHNAK TKPREEQFNS TYRVVSVLTV LHQDWLNGKE YKCKVSNKGL PSSIEKTISK 120
AKGQPREPQV YTLPPSQEEM TKNQVSLTCL VKGFYPSDIA VEWESNGQPE NNYKTPPVVL 180
DSDGSFFLYS RLTVDKSRWQ EGNVFSCSVM HEALHNHYTQ KSLSLSLGK 229

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SEQUENCE: 19
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SEQ ID NO: 20      moltype = AA length = 4
FEATURE
REGION
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note = Description of Artificial Sequence: Synthetic peptide
source
1..4
mol_type = protein
organism = synthetic construct
SEQUENCE: 20
GGGG
4

SEQ ID NO: 21      moltype = AA length = 5
FEATURE
REGION
1..5
note = Description of Artificial Sequence: Synthetic peptide
source
1..5
mol_type = protein
organism = synthetic construct
SEQUENCE: 21
TGGGG
5

SEQ ID NO: 22      moltype = AA length = 5
FEATURE
REGION
1..5
note = Description of Artificial Sequence: Synthetic peptide
source
1..5
mol_type = protein
organism = synthetic construct
SEQUENCE: 22
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5

SEQ ID NO: 23      moltype = AA length = 4
FEATURE
REGION
1..4
note = Description of Artificial Sequence: Synthetic peptide
source
1..4
mol_type = protein
organism = synthetic construct
SEQUENCE: 23
TGGG
4

SEQ ID NO: 24      moltype = AA length = 4
FEATURE
REGION
1..4
note = Description of Artificial Sequence: Synthetic peptide
source
1..4
mol_type = protein
organism = synthetic construct
SEQUENCE: 24
SGGG
4

SEQ ID NO: 25      moltype = AA length = 5
FEATURE
REGION
1..5
note = Description of Artificial Sequence: Synthetic peptide
source
1..5
mol_type = protein
organism = synthetic construct
SEQUENCE: 25
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5

SEQ ID NO: 26      moltype = AA length = 344
FEATURE
source
1..344
mol_type = protein

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SEQUENCE: 26          organism = Homo sapiens
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DCSNITWKGP VCGLDGKTYR NECALLKARC KEQPELEVQY QGRCKKTCDR VFCPGSSTCV 180
VDQTNNAYCV TCNRICPEPA SSEQYLCGND GVTYSSACHL RKATCLLGRS IGLAYEGKCI 240
KAKSCEDIQC TGGKKCLWDF KVGRGRCSLC DELCPDSKSD EPVCASDNAT YASECAMKEA 300
ACSSGVILLEV KHSGSCNSIS EDTEEEEEDE DQDYSFPISS ILEW 344

SEQ ID NO: 27          moltype = AA length = 317
FEATURE
source
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mol_type = protein
organism = Homo sapiens

SEQUENCE: 27
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DCSNITWKGP VCGLDGKTYR NECALLKARC KEQPELEVQY QGRCKKTCDR VFCPGSSTCV 180
VDQTNNAYCV TCNRICPEPA SSEQYLCGND GVTYSSACHL RKATCLLGRS IGLAYEGKCI 240
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ACSSGVILLEV KHSGSCN 317

SEQ ID NO: 28          moltype = AA length = 63
FEATURE
source
1..63
mol_type = protein
organism = Homo sapiens

SEQUENCE: 28
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PCK 63

SEQ ID NO: 29          moltype = AA length = 25
FEATURE
source
1..25
mol_type = protein
organism = Homo sapiens

SEQUENCE: 29
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SEQ ID NO: 30          moltype = AA length = 26
FEATURE
source
1..26
mol_type = protein
organism = Homo sapiens

SEQUENCE: 30
KTCRDVFCPG SSTCVVDQTN NAYCVT 26

SEQ ID NO: 31          moltype = AA length = 263
FEATURE
source
1..263
mol_type = protein
organism = Homo sapiens

SEQUENCE: 31
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CAPDCSGLPA RLQVGCGSDGA TYRDECELRA ARCRGHPDLS VMYRGRCRKS CEHVCPRPQ 180
SCVVDQGTSA HCVVCRRAAPC PVPSSPGQEL CGNNNVTYIS SCHMRQATCF LGRSIGVRHA 240
GSCAGTPEEP PGGESAEAAA NFV 263

SEQ ID NO: 32          moltype = AA length = 344
FEATURE
REGION
1..344
note = Description of Artificial Sequence: Synthetic
polypeptide
source
1..344
mol_type = protein
organism = synthetic construct

SEQUENCE: 32
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DDINCYDRTD CVEKKDSPEV YFCCCEGNMC NEKFSYFPREM EVTQPTSNPV TPKPPTGGGT 120
HTCPPCPAPE LLGGPSVFLP PPKPKDTLMF SRTPEVTCVV VDVSHEDPEV KFNWVVDGVE 180
VHNAKTKPRE EQYNSTYRVV SVLTVLHQDW LNGKEYKCKV SNKALPVPIE KTISKAKGQP 240
REQQVYTLPP SREEMTQNQV SLTCLVKGFY PSDIAVEWES NGQPENNYKT TPPVLSDG 300
FFLYSKLTVD KSRWQQGNVF SCSTMHEALH NHYTQKSLSL SPGK 344

SEQ ID NO: 33          moltype = AA length = 21

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	organism = Apis mellifera
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SEQ ID NO: 34	moltype = AA length = 22
FEATURE	Location/Qualifiers
REGION	1..22
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source	1..22
	mol_type = protein
	organism = unidentified
SEQUENCE: 34	
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SEQ ID NO: 35	moltype = AA length = 20
FEATURE	Location/Qualifiers
REGION	1..20
	note = Description of Unknown: Native leader sequence
source	1..20
	mol_type = protein
	organism = unidentified
SEQUENCE: 35	
MGAAKLAF A VPLISCSSGA	20
SEQ ID NO: 36	moltype = AA length = 369
FEATURE	Location/Qualifiers
REGION	1..369
	note = Description of Artificial Sequence: Synthetic polypeptide
source	1..369
	mol_type = protein
	organism = synthetic construct
SEQUENCE: 36	
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YFPEMEVTFQPS TSNPVTPKPP CGGGTHTCP CPAPELLGGP SVFLFPKP KPK DTLMISRTPE 180	
VTCVVVDVSH EDPEVKFNWY VDGVEVHNNAK TKPREEQVNS TYRVSVLTV LHQDWLNKE 240	
YCKKVSNSKAL PVPIEKTK AKGQPREFPV YTLPSSREEM TKNQVSLTCL VKGFYPSDIA 300	
VEWESNGQPE NNYKTTPPVL DSDGSFFLYS KLTVDKSRWQ QGNVFSCSVM HEALHNHYTQ 360	
KSLSLSPGK	369
SEQ ID NO: 37	moltype = DNA length = 1114
FEATURE	Location/Qualifiers
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	note = Description of Artificial Sequence: Synthetic polynucleotide
source	1..1114
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 37	
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attggggaaaa agacacaacc aatcaaactg gtgttgaaac gtgttatggt gacaagata 180	
aacggcgcga ttgtttgtct accttggaaat atatttctgg ttccattgaa tagtgaaca 240	
aggttgtgg ctggatgata tcaactgtca tgacaggatc gattgtgttag aaaaaaaaaaga 300	
cagccccgtggatgaaat tcttttttttctt gttgtgtga gggcatatggatgaaat agttttctta 360	
ttttccggatg atggaaagtca cacagccccat tccaaatcca gttacaccta agccaccac 420	
cgggtgtggaa actcacacat gcccacccgtg cccagcacccat gaactcctgg gggggccgtc 480	
agtcttcctc ttccccccaa aacccaagga caccctcatg atctcccgaa cccctgaggt 540	
cacatcgctg gtgggtggacg tgagccacca agacccctgacg gtcaagtca actgttacgt 600	
ggacggccgtg gagggtgcata atgccaacaa aaaggccgcgg gaggagcaatg acaacagcac 660	
gtacccgtgtg gtccacgttcc tcacccgttcc gcacccaggac tggcttaatg gcaaggagta 720	
caagtgcacat gtctccaaaca aagccctccc agtccccatc gagaaccaatccat tctccaaac 780	
caaaggggcag ccccgagaac cacagggtta caccctgtcc ccatcccgaa aggagatgac 840	
caagaacccat gtcacgttcc cctgtgttcc taaaaggcttc tatccctggcc acatccgcgt 900	
ggagtgccggatc agcaatgggc agccggagaa caactacaag accacgcctc ccgtgttcc 960	
ctccggacggc tcctttttcc tctatagca gtcacccgtg gacaagagca ggtggcagca 1020	
ggggaaacgtc ttctcatgtt ccgtgtatca tgaggctctg cacaaccact acacgcagaa 1080	
gagccttcctcc ctgtctccgg gtaaatgaga attc	1114
SEQ ID NO: 38	moltype = AA length = 9
FEATURE	Location/Qualifiers

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REGION          1..9
source          note = Description of Artificial Sequence: Synthetic peptide
                1..9
                mol_type = protein
                organism = synthetic construct
SEQUENCE: 38
ILGRSETQE          9

SEQ ID NO: 39      moltype = AA length = 329
FEATURE          Location/Qualifiers
REGION          1..329
source          note = Description of Artificial Sequence: Synthetic
                polypeptide
                1..329
                mol_type = protein
                organism = synthetic construct
SEQUENCE: 39
ILGRSETQEC LFFNANWEKD RTNQTGVEPC YGDSDKRRHC FATWKNISGS IEIVKQGCWL 60
DDINCYDRQE CVEKKDSPEV YFCCCEGNMC NEKFSYFPREM TGGGTHTCPP CPAPELLGGP 120
SVPFLFPKPK DTLMSRTPE VTCVVVDVSH EDPEVKFNWY VDGVEVHNAAK TKPREEQYNS 180
TYRVSVSLTV LHQDWLNGKE YKCKVSNKAL PVPPIEKTISK AKGQPREPQV YTLPPSREEM 240
TKNQVSLTCL VKGFYPSDIA VEWESNGQPE NNYKTTPPVL SDGSFFLYS KLTVDKSRWQ 300
QGNVFSCSVM HEALHNHYTQ KSLSLSPGK 329

SEQ ID NO: 40      moltype = AA length = 343
FEATURE          Location/Qualifiers
REGION          1..343
source          note = Description of Artificial Sequence: Synthetic
                polypeptide
                1..343
                mol_type = protein
                organism = synthetic construct
SEQUENCE: 40
GRGEAETREC IYYNANWELE RTNQSGLERC EGEQDKRLHC YASWRNSSGT IELVKKGCGWL 60
DDFNCYDRQE CVATEENPQV YFCCCEGNFC NERFTHLPEA GGPEVTVYEPPTA PTAPGGGTH 120
TCPCCPAPEL LGGPSVFLFP PKPKDTLMIS RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV 180
HNAKTKPREE QYNSTYRVVS VLTVLHQDWL NGKEYKCKVS NKALPVPIEK TISKAKGQPR 240
EPQVYTLPPS REEMTKNQVS LTCLVKGFYD SDIAVEWESN GQPENNYKTT PPVLDSDGSF 300
FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLSGK 343

SEQ ID NO: 41      moltype = AA length = 20
FEATURE          Location/Qualifiers
REGION          1..20
source          note = Description of Unknown: Native leader sequence
                1..20
                mol_type = protein
                organism = unidentified
SEQUENCE: 41
MGAAKLAF A VFLISCSSGA          20

SEQ ID NO: 42      moltype = AA length = 368
FEATURE          Location/Qualifiers
REGION          1..368
source          note = Description of Artificial Sequence: Synthetic
                polypeptide
                1..368
                mol_type = protein
                organism = synthetic construct
SEQUENCE: 42
MDAMKRLGCC VLLLCGAVFV SPGASGRGEA ETRECIYNYA NWELERTNQS GLERCEGEQD 60
KRLHCYASWR NSSGTIELVK KGCLWDDFN C YDRQECVATE ENPQVYFCCC EGNFCNERFT 120
HLPEAGGPVE TYEPPPTAPT GGGTHTCPCPAPELLGGP VFLFPKPKD TLMISRTPEV 180
TCVVVDVSHE DPEVKFNWYV DGVEVHNAAK KPREEQYNS YRVSVSLTVL HQDWLNGKEY 240
KCKVSNKALP VPIEKTISK AKGQPREPQVY TLPPSREEMT KNQVSLTCLV KGFYPSDIAV 300
EWESNGQOPEN NYKTTPPVL SDGSFFLYSK LTVDKSRWQO GNVFSCSVMH EALHNHYTQK 360
SLSLSPGK 368

SEQ ID NO: 43      moltype = DNA length = 1107
FEATURE          Location/Qualifiers
misc_feature    1..1107
source          note = Description of Artificial Sequence: Synthetic
                polynucleotide
                1..1107
                mol_type = other DNA
                organism = synthetic construct
SEQUENCE: 43

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atggatgcaa tgaagagagg gctctgtgt gtgtgtggac agtcttcgtt 60
tcgcggccg cctctggcg tggggaggt gagacacggc agtgcaccta ctacaacgcc 120
aactgggagc tggagcgcac caaccagago ggcttgaggc gtcgcgaagg cgagcaggac 180
aagcggtcgc actgtacgc ctctggcg aacagctctg gcacccatcga gtcgtgaaag 240
aagggtctgc ggcttagatga cttecaactgc tacgataggc aggagtgtgt ggccactgag 300
gagaacccccc aggtgtactt ctgtgtgtgt gaaggcaact tctgcaacga gcgttcaact 360
catttgcacag aggctggggg cccggaaatc acgtacgcgac cacecccgac agccccccacc 420
ggtgtgtggaa ctcacacatg cccacatcg ccagcacctg aactcttggg gggaccgtca 480
gtcttcctct tccccccaaa acceaaggac accctcatgta tctccggac ccctgagggtc 540
acatgcgtgg tgggtggacgt gaggccacaa gaccctgagg tcaagtccaa ctggtacgtg 600
gacggcgtgg aggtggacataa tgccaagaca aagccgcggg aggagcagta caacagcag 660
taccgtgtgg tcaagtcctt caccgttctg caccaggact ggctgtaaatgg caaggagttac 720
aagtgcacagg tctccaacaa agccctccca gtcccccattcg agaaaaaccat ctccaaagcc 780
aaaggccggc cccgagaacc acagggtgtac accctgcccc catcccgga ggagatgacc 840
aagaaccagg tcaagcgttgc ctgctgtgtc aaaggcttcat atccccaggca catgcccgtg 900
gagtgggaga gcaatggca gcccggaaac aactacaaga ccacgccttc cgtgtggac 960
tccgacggtc ctttcttcct ctatagcaag ctcaccgtgg acaagagcag gtggcagcag 1020
ggaaacgtct ttcatgtcgtc cggtatgtc gaggctgtc acaaccacta cacgcagaag 1080
agectctccc tggctccggg taaatga 1107

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SEQ ID NO: 44      moltype = AA length = 6
FEATURE
REGION          1..6
note = Description of Artificial Sequence: Synthetic peptide
source           1..6
mol_type = protein
organism = synthetic construct

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SEQUENCE: 44      GRGEAE
6
```

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SEQ ID NO: 45      moltype = AA length = 335
FEATURE
REGION          1..335
note = Description of Artificial Sequence: Synthetic
polypeptide
source           1..335
mol_type = protein
organism = synthetic construct

```

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SEQUENCE: 45      ETRECIYNYA NWELERTNQS GLERCEGEQD KRLHCYASWR NSSGTIELVK KGCWLDDFNC 60
YDRQECVATE ENPQVYFCCC EGNPCNERFT HLPEAGGPEV TYEPPPTGGG THTCPPCPAP 120
ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDE VKFNWYVDGV EVHNAKTAKPR 180
EQYQNSTYRV VSVLTVLHQWL WLNKEYKCK VSNKALPAPI EKTISKAKGQ PREPQVYTL 240
PSREEMTKNQ VSLTCLVKGF YPSDIAREWE SNGQPENNYK TPPVLDSDG SFFLYSKLT 300
DKSRWQQGNV FSCSVMEAL HNHYTQKSLS LSPKG 335

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SEQ ID NO: 46      moltype = AA length = 343
FEATURE
REGION          1..343
note = Description of Artificial Sequence: Synthetic
polypeptide
source           1..343
mol_type = protein
organism = synthetic construct

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SEQUENCE: 46      GRGEAETREC IYYNANWELE RTNQSGLERC EGEQDKRLHC YASWRNSSGT IELVKKGCWD 60
DDFNQCYDRQE CVATEENPQV YFCCEGPNFC NERFTHLPEA GGPEVTYEPP PTAPTGGGTH 120
TCPPCPAPEL LGGPSVFLFP PKPKDTLMIS RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV 180
HNAKTKPREE QYNSTYRVVS VLTVLHQDWL NGKEYKCKVS NKALPVPIEK TISKAKGQPR 240
EPQVYTLPPS REEMTKNQVS LTCLVKGFYP SDIAVEWESN GQPENNYKTT PPVLDSDGSF 300
FLYSKLTVDK SRWQQGNVFS CSVMEALHN HYTQKSLSLS PGK 343

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SEQ ID NO: 47      moltype = AA length = 115
FEATURE
REGION          1..115
note = Description of Artificial Sequence: Synthetic
polypeptide
source           1..115
mol_type = protein
organism = synthetic construct

```

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SEQUENCE: 47      GRGEAETREC IYYNANWELE RTNQSGLERC EGEQDKRLHC YASWRNSSGT IELVKKGCWD 60
DDFNQCYDRQE CVATEENPQV YFCCEGPNFC NERFTHLPEA GGPEVTYEPP PTAPT 115

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SEQ ID NO: 48      moltype = AA length = 368
FEATURE

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REGION          1..368
                note = Description of Artificial Sequence: Synthetic
                polypeptide
source          1..368
                mol_type = protein
                organism = synthetic construct
SEQUENCE: 48
MDAMKRLGCC VLLLCGAVFV SPGASGRGEA ETRECIYYNA NWELERTNQS GLERCEGEQD 60
KRLHCYASWR NSSGTIELVK KGCWDDDFNC YDRQECVATE ENPQVYFCCC EGNFCNERFT 120
HLPPEAGGPEV TYEPPPTAPT GGGHTTCPPC PAPELGGPS VFLFPKKPKD TLMISRTPEV 180
TCVVVDVSHE DGVEVHNNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY 240
KCKVSNKALP APIEKTSKA KGQPREPQVY TLPSREEMT KNQVSLTCLV KGFYPSDIAV 300
EWESNGQOPEN NYKTTPPVLD SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH EALHNHYTQK 360
SLSLSPGK               368

SEQ_ID NO: 49      moltype = DNA length = 1107
FEATURE          Location/Qualifiers
misc_feature     1..1107
                note = Description of Artificial Sequence: Synthetic
                polynucleotide
source          1..1107
                mol_type = other DNA
                organism = synthetic construct
SEQUENCE: 49
atggatgcaa tgaagagagg gctctgtgt gtgctgtgc tggatggcggc agtcttcgtt 60
tcgcggccgc ctctctggcg tggatggcggc gagacacggg agtgcacatca ctacaacggcc 120
aactggggac tcggagcgcac caaccagggc ggctctggcgc gtcgcggagg cgagcaggac 180
aagcggctgc acgtctacgc ctctctggcg aacagcttcg gcacccatcga gtcgcgttgc 240
aaggatgtgtt gggatgtttt ctgcgtgtgtt gaaggatgtt ttcgcgttgc ggcgttgc 300
gagaaccccccc aggtgttactt ctgcgtgtgtt gaaggatgtt ttcgcgttgc ggcgttgc 360
catatggccggc aggtgtgggg cccggaaatgc acgtacgcgc caccggggcggc aggggggggg 420
gttggatgggg ctcacatcgat cccaccgtgc ccacccatgc aactctggg gggaccgtca 480
gttccctctt tcccccggaaa acccaaggac accctcatgtt tctccggac ccctcgagggtc 540
acatgcgtgg tggatggacgt gagccacggaa gaccctgggg tcaagtccaa ctggatgttgc 600
gacggcggtgg aggtgttataa tgccaaatggc aaggatggggcggc agggatgttgc 660
taccgtgtgg ttcggatgttgc caccgttgc caccggatgttgc ggcgttgc 720
aagtgcgttgc ttcacatcgat cccaccgtgc ccacccatgc aactctggg gggaccgtca 780
aaaggccggc cccggaaatggc acaggatgttgc accctggccc catccggggcgggatgttgc 840
aaagggccggc ttcggatgttgc ctcggatgttgc aaaggatgttgc atccggatgttgc 900
gatgtgtgggg gcaatggggc gccggggatgttgc aactacaaatggc ccacggatgttgc 960
tccggatgttgc ctcggatgttgc ttcacatcgat cccaccgtgc ccacccatgc aactctggg 1020
ggggatgttgc ttcacatcgat ctcggatgttgc acaaccatgc aactacaaatggc 1080
agccgttcccgatccatcgat taaatgttgc 1107

SEQ_ID NO: 50      moltype = AA length = 141
FEATURE          Location/Qualifiers
source          1..141
                mol_type = protein
                organism = Homo sapiens
SEQUENCE: 50
GRGEAAETREC IYYNANWELE RTNQSGLERC EGEQDKRLHC YASWRNSSGT IELVKKGCWL 60
DDFN CYDRQE CVATEENPQV YFC CCEGNFC NERFTHLPEA GGPEGPWAST TIPSGGPEAT 120
AAAGDQGSGA LWLCLEGPAH E               141

SEQ_ID NO: 51      moltype = AA length = 141
FEATURE          Location/Qualifiers
REGION          1..141
                note = Description of Artificial Sequence: Synthetic
                polypeptide
source          1..141
                mol_type = protein
                organism = synthetic construct
SEQUENCE: 51
GRGEAAETREC IYYNANWELE RTNQSGLERC EGEQDKRLHC YASWRNSSGT IELVKKGCWD 60
DDFN CYDRQE CVATEENPQV YFC CCEGNFC NERFTHLPEA GGPEGPWAST TIPSGGPEAT 120
AAAGDQGSGA LWLCLEGPAH E               141

SEQ_ID NO: 52      moltype = AA length = 370
FEATURE          Location/Qualifiers
REGION          1..370
                note = Description of Artificial Sequence: Synthetic
                polypeptide
source          1..370
                mol_type = protein
                organism = synthetic construct
SEQUENCE: 52

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GRGEAETREC IYYNANWELE RTNQSGLERC EGEQDKRLHC YASWRNSSGT IELVKKGWCW	60
DFNCYDRQEC VATEENPQVY FCCCEGNFCN ERFTHLPEAG 120	
AAAGDQGSGA LWLCLEGPAH ETGGGHTTCP PCPAPELLGG PSVFLFPKPK KDTLMISRTP 180	
EVTCVVVDVS HEDPEVKFNV YVDGVEVHNA KTKPREEQYN STYRVVSVLT VLHQDWLNGK 240	
EYCKVSNKA LPAPIEKTI KAKGQPREPQ VYTLPPSREE MTKNQVSLTC LVKGFYPSDI 300	
AVEWESNGQP ENNYKTPPPV LDSDGSFFLY SKLTVDKSRW QQGNVFSCSV MHEALHNHYT 360	
QKSLSLSPGK	370
 SEQ ID NO: 53 moltype = AA length = 150	
FEATURE Location/Qualifiers	
source 1..150	
mol_type = protein	
organism = Rattus sp.	
 SEQUENCE: 53	
MTAPWAALAL LWGSLCAGSG RGEAETRECI YYNANWELEI TNQSGLERCE GEQDKRLHCY 60	
ASWRNSSGTI ELVKKGCWLD DFNCYDRQEC VATEENPQVY FCCCEGNFCN ERFTHLPEPG 120	
GPEVTVYEPPP TAPTLTTVLA YSLLPIGGLS	150
 SEQ ID NO: 54 moltype = AA length = 150	
FEATURE Location/Qualifiers	
source 1..150	
mol_type = protein	
organism = Sus scrofa	
 SEQUENCE: 54	
MTAPWAALAL LWGSLCAGSG RGEAETRECI YYNANWELEI TNQSGLERCE GEQDKRLHCY 60	
ASWRNSSGTI ELVKKGCWLD DFNCYDRQEC VATEENPQVY FCCCEGNFCN ERFTHLPEAG 120	
GPEVTVYEPPP TAPTLTTVLA YSLLPIGGLS	150
 SEQ ID NO: 55 moltype = AA length = 150	
FEATURE Location/Qualifiers	
source 1..150	
mol_type = protein	
organism = Mus musculus	
 SEQUENCE: 55	
MTAPWAALAL LWGSLCAGSG RGEAETRECI YYNANWELEI TNQSGLERCE GEQDKRLHCY 60	
ASWRNSSGTI ELVKKGCWLD DFNCYDRQEC VATEENPQVY FCCCEGNFCN ERFTHLPEPG 120	
GPEVTVYEPPP TAPTLTTVLA YSLLPIGGLS	150
 SEQ ID NO: 56 moltype = AA length = 150	
FEATURE Location/Qualifiers	
source 1..150	
mol_type = protein	
organism = Homo sapiens	
 SEQUENCE: 56	
MTAPWAALAL LWGSLCAGSG RGEAETRECI YYNANWELEI TNQSGLERCE GEQDKRLHCY 60	
ASWRNSSGTI ELVKKGCWLD DFNCYDRQEC VATEENPQVY FCCCEGNFCN ERFTHLPEAG 120	
GPEVTVYEPPP TAPTLTTVLA YSLLPIGGLS	150
 SEQ ID NO: 57 moltype = AA length = 150	
FEATURE Location/Qualifiers	
source 1..150	
mol_type = protein	
organism = Bos taurus	
 SEQUENCE: 57	
MTAPWAALAL LWGSLCAGSG RGEAETRECI YYNANWELEI TNQSGLERCE GERDKRLHCY 60	
ASWRNSSGTI ELVKKGCWLD DFNCYDRQEC VATEENPQVY FCCCEGNFCN ERFTHLPEAG 120	
GPEVTVYEPPP TAPTLTTVLA YSLLPVGGLS	150
 SEQ ID NO: 58 moltype = AA length = 150	
FEATURE Location/Qualifiers	
source 1..150	
mol_type = protein	
organism = Xenopus sp.	
 SEQUENCE: 58	
MGASVALTFL LLLATPRAGS GHDEVETREC IYYNANWELE KTNQSGVERL VEGKKDKRLH 60	
CYASWRNNSG FIELVKKGCW LDGFNCYDRQ ECIAKEENPQ VFFCCCEGNY CNKKPTHLP 120	
VETFDPKPQP SASVLNILY SLLPIVGLSM	150
 SEQ ID NO: 59 moltype = AA length = 150	
FEATURE Location/Qualifiers	
source 1..150	
mol_type = protein	
organism = Homo sapiens	
 SEQUENCE: 59	
MGAAAALKAFVFLISCSSGA ILGRSETQEC LFFPNANWEKD RTNQQTGVEPC YGDKDKRRHC 60	
FATWKNISGS IEIVKQGCWL DDINCYDRTD CVEKKDSPEV YFCCCEGNMC NEKFSYFPEM 120	

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EVTQPTSNPV	TPKPPYYNIL	LYSLVPLMLI	150
SEQ ID NO: 60	moltype = AA length = 154		
FEATURE	Location/Qualifiers		
REGION	1..154		
	note = Description of Artificial Sequence: Synthetic		
	consensus sequence		
VARIANT	8		
	note = Thr, Ala or absent		
VARIANT	121		
	note = Pro, Ala, Val or Met		
source	1..154		
	mol_type = protein		
	organism = synthetic construct		
SEQUENCE: 60			
MTAPWAAXLA LLWGLSLCAGS GRGEAETREC IYYNNANWELE RTNQSLERL CEGEODKRLH	60		
CYASWRNSSG TIELVKKGWC LDDFN CYDRQ ECVATEENPQ YFCCCEGNF CNERFTHLPE	120		
XGGPEVTVYEP KPPTAPTLT VLAYSLLPIG GLSM	154		
SEQ ID NO: 61	moltype = AA length = 115		
FEATURE	Location/Qualifiers		
source	1..115		
	mol_type = protein		
	organism = Homo sapiens		
SEQUENCE: 61			
ILGRSETQEC LFFNANWEKD RTNQTGVEPC YGDKD KRRHC FATWKNISGS IEIVKQGCWL	60		
DDINCYDRTD CVEKKDSPEV YFCCCEGNMC NEKFSYFPEM EVTQPTSNPV TPKPP	115		
SEQ ID NO: 62	moltype = AA length = 115		
FEATURE	Location/Qualifiers		
source	1..115		
	mol_type = protein		
	organism = Ovis aries		
SEQUENCE: 62			
ILGRSETQEC IFYNANWERD RTNRTGVES C YGDKD KRRHC FATWKNISGS IDIVKQGCWL	60		
DDINCYDRTD CIEKKDSPEV YFCCCEGNMC NERFSYFPEM EVTQPTSNPV TPKPP	115		
SEQ ID NO: 63	moltype = AA length = 115		
FEATURE	Location/Qualifiers		
source	1..115		
	mol_type = protein		
	organism = Condylura cristata		
SEQUENCE: 63			
ILGRSETQEC LFFNANWERD RTNQTGVEPC YGDKD KRRHC FATWKNISGS IEIVKQGCWL	60		
DDINCYDRTD CIEKKDSPEV YFCCCEGNMC NEKFSYFPEM EVTQPTSNPV TPKAP	115		
SEQ ID NO: 64	moltype = AA length = 115		
FEATURE	Location/Qualifiers		
source	1..115		
	mol_type = protein		
	organism = Mus musculus		
SEQUENCE: 64			
ILGRSETQEC LFFNANWERD RTNQTGVEPC YGDKD KRRHC FATWKNISGS IEIVKQGCWL	60		
DDINCYDRTD CIEKKDSPEV YFCCCEGNMC NEKFSYFPEM EVTQPTSNPV TPKPP	115		
SEQ ID NO: 65	moltype = AA length = 115		
FEATURE	Location/Qualifiers		
source	1..115		
	mol_type = protein		
	organism = Gallus gallus		
SEQUENCE: 65			
ILGRSETQEC IYYNNANWEKD KTNRSGIEPC YGDKD KRRHC FATWKNISGS IEIVKQGCWL	60		
DDINCYDRND CIEKKDSPEV FFCCCEGNMC NERFFYFPEM EVTQPTSNPV TPKPP	115		
SEQ ID NO: 66	moltype = AA length = 115		
FEATURE	Location/Qualifiers		
source	1..115		
	mol_type = protein		
	organism = Bos taurus		
SEQUENCE: 66			
ILGRSETQEC IFYNANWERD RTNRTGVES C YGDKD KRRHC FATWKNISGS IEIVKQGCWL	60		
DDINCYDRTD CIEKKDSPEV YFCCCEGNMC NERFSYFPEM EVTQPTSNPV TPKPP	115		
SEQ ID NO: 67	moltype = AA length = 115		
FEATURE	Location/Qualifiers		
source	1..115		

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mol_type = protein
organism = Tyto alba
SEQUENCE: 67
ILGRSETQEC IYYNANWEKD KTNRSGIEPC YGDKDKRRHC FATWKNISGS IEIVKQGCWL 60
DDINCYDRND CIEKKDSPEV FFCCCEGNMC NERFFYFPEM EVTQPTSNPV TPKPP 115

SEQ ID NO: 68      moltype = AA length = 115
FEATURE           Location/Qualifiers
source            1..115
mol_type = protein
organism = Myotis davidii
SEQUENCE: 68
ILGRSETQEC IFYNANWERD KTNRTGVELC YGDKDKRRHC FATWKNISGS IEIVKQGCWL 60
DDINCYDRTD CIEKKDSPEV YFCCEGNMC NERFSYFPEM EVTQPTSNPV TPKPP 115

SEQ ID NO: 69      moltype = AA length = 360
FEATURE           Location/Qualifiers
REGION            1..360
note = Description of Artificial Sequence: Synthetic
polypeptide
source            1..360
mol_type = protein
organism = synthetic construct
SEQUENCE: 69
MDAMKRLGCC VLLLCGAVFV SPGAAETREC IYYNANWELE RTNQSLERC EGEQDKRLHC 60
YASWRNSSGT IELVKKGWL DDFNCYDROE CVATEENPOV YFCCCEGNFC NERFTHLPEA 120
GGPEVTYEPPT PTVGGGHTCP PCPAPELLGG PSVFLFPPKP KDTLMISRTP EVTCVVVDVS 180
HEDPEVKPNW YVDGVEVHNA KTKPREEQVN STYRVVSVLT VLHQDWLNKG EYKCKVSNKA 240
LPAPIEKTTIS KAKGQPREPQ VYTLPPSREE MTKNQVSLTC LVKGFPYPSDI AVEWESNGQP 300
ENNYKTTTPV LDSDGSFFLY SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPGK 360

SEQ ID NO: 70      moltype = DNA length = 1083
FEATURE           Location/Qualifiers
misc_feature      1..1083
note = Description of Artificial Sequence: Synthetic
polynucleotide
source            1..1083
mol_type = other DNA
organism = synthetic construct
CDS               73..396
SEQUENCE: 70
atggatgcaa tgaagagagg gctctgctgt gtgctgctgc tgtgtggagc agtcttcgtt 60
tcggccggcc cccgctgagac acggggagtgc atctactaca acggccaactg ggagctggag 120
cgcaccaacc agagcggccct ggagcgtgc aaaggcgagc aggacaacgc gtcgactgc 180
taaggcctctt ggcgaacacag ctctggcaco atcgactctg tgaagaagggt ctgtggcta 240
gatgacttca actgtcacga taggcaggag tttgtggcca ctgaggagaa cccccaggtg 300
taacttctgtc gtgtggagg caacttctgc aacggaggct tcacttattt gccaagggtc 360
ggggggccgg aagtcaacgtt ccggacacccccc acggacagggt gtggaaactca cacatgcca 420
ccgtggcccaacg caccgtcaactt cctggggggc ccgtcgtctt tctcttccc cccaaaaccc 480
aaggacaccc tcatgtatctc cgggacccctt gaggtcacat gctgtgggtt ggacgtgagc 540
cacaagacc ttgggttcaaa gttcaactgg tacgtggacg gctgtggagggt gcataatgcc 600
aagacaacaaaccc cgccggggaggaa gcaatgtac accgtacgttccatgtgggttccatgtgg 660
gtcttgacc accgttgttcaatggcaatgg gatgttcaatgg gcaagggttccatgtgggttccatgtgg 720
ctcccaagccccc ccatcgagaa aaccatctcc aaagccaaag ggccggcccg agaaccacag 780
gtgtacaccctt tgcccccattt ccggggaggag atgaccaaggaa accagggttccatgtgggttccatgtgg 840
ctgggttcaatgg gtttcttccatgtgggttccatgtgggttccatgtgggttccatgtgg 900
gagaacaatcc acaagaccac ccctccctgtt ctggacttccatgtgggttccatgtgggttccatgtgg 960
agcaaggtca ccgtggacaa gaggcagggttccatgtgggttccatgtgggttccatgtgg 1020
atgcatgagg ctctgcacaa ccactacacg cagaagggccatgtgggttccatgtgggttccatgtgg 1080
tga                                         1083

SEQ ID NO: 71      moltype = DNA length = 1083
FEATURE           Location/Qualifiers
misc_feature      1..1083
note = Description of Artificial Sequence: Synthetic
polynucleotide
source            1..1083
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 71
tcatttaccc gggggacaggaggg agaggcttctt ctgcgtgttag tgggttgca gagcctcatg 60
catcacggag catgagaaga cgttccctgtt ctggcaccttgc ctcttgcacatggatgtt 120
gctatagagg aagaaggaggc cgtcgaggatc cagcacggggc ggcgtggatcttgc 180
ctccggctgc ccattgttccatgtgggttccatgtgggttccatgtgggttccatgtgg 240
caggcagggttccatgtgggttccatgtgggttccatgtgggttccatgtgggttccatgtgg 300
caccctgtgttccatgtgggttccatgtgggttccatgtgggttccatgtgggttccatgtgg 360

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-continued

gggggcttg ttggagacct	tgcacttgcata	ctccttgcac	ttcagccagt	cctgggtcag	420
gacgggtgagg	acgcgtgacca	cacggtaacgt	gctgttgta	tgctccccc	480
cttggcatta	tgcaccccca	cgccgtccat	gtaccatgg	aacttgacat	540
gtggctcacg	tccaccacca	cgcacgtac	ctcagggggtc	cgggagatca	600
cttgggttt	ggggggaaaga	ggaagactga	cggtcccccc	aggagttcag	660
cggtggccat	gtgtgagttc	caccacgtgt	cgggggtggc	tcgtacgtga	720
cccaagcctct	ggcaaatgag	tgaagcgctc	gttgcagaaag	ttgccttcac	780
gtacacccatgg	gggtttccct	cagtggccac	acactcttc	ctatcgtagc	840
atctagecag	oagcccttc	tcacgagtc	gatgggtccca	gagctgttgc	900
gtacgactgc	acgcgttgc	cctgtcgcc	ttcgcagcgc	tcaggccgc	960
gcgtccacgc	tcccaagtgg	cgttgtaga	gatgcaactc	cgtgtctcag	1020
cgaacacgaag	actgctccac	acagcagcag	cacacagcag	agccctctct	1080
cat				tcattgcac	1083

SEQ ID NO: 72	moltype = DNA	length = 1083			
FEATURE	Location/Qualifiers				
misc_feature	1..1083				
	note = Description of Artificial Sequence: Synthetic				
	polynucleotide				
source	1..1083				
	mol_type = other DNA				
	organism = synthetic construct				
CDS	73..396				
SEQUENCE: 72					
atggatgcaa tgaagagagg	gctctgtgt	gtgtgtggagc	agtcttcgtt	60	
tcgccccggc	ccggccaaac	ccgcaatgt	atttattaca	atgctaattg	120
cggacgaaac	aatccgggt	cgaacgggt	gagggggaaac	aggataaacg	180
tatgcgttgt	ggagggaaactc	tcggccggac	attgaacttg	tcaagaaaagg	240
gacgatttca	atgttatg	ccggcaggaa	tgtgtcgca	ccgaagagaa	300
tatccgtt	gttgcgaggg	gaatttctgt	aatgaacggt	ttacccactt	360
ggccggcccg	aggtgaccc	tgaacccccc	ccccgggtg	gtggaaactca	420
ccgtgcccc	cacccgtact	cctgggggg	ccgtcagtt	tctcttccc	480
aaggacaccc	tcatgtatc	ccggacccct	gagggtcacat	gcgtgttgt	540
cacgaagacc	ctgaggatca	gttcaactgg	tacgtggacg	gcgtggaggt	600
aagacaaagg	ccggggggaa	gcagtaacaa	agcacgtacc	gtgtgttcag	660
gtcctgcacc	aggactggc	aatgtggcag	gatgtacaatg	gcaagggttcc	720
ctcccaagccc	ccatcgagaa	aaatctcc	aaagccaaag	ggcagcccc	780
gtgtacaccc	tgccccatc	ccggggggag	atgaccaaga	accagggtcag	840
cttgtcaaa	gttttatcatc	cagggacatc	ccgggtggag	ggggagacaa	900
gagaacaacta	aaagacacc	gcctccctgt	ctggactcc	acggctctt	960
agcaaggtca	ccgtggacaa	gagggtgg	cacgggggg	acgttttcc	1020
atgcatgagg	ctctgcacaa	ccactacacg	cagaagacg	tctccctgtc	1080
tga					1083

SEQ ID NO: 73	moltype = DNA	length = 1083			
FEATURE	Location/Qualifiers				
misc_feature	1..1083				
	note = Description of Artificial Sequence: Synthetic				
	polynucleotide				
source	1..1083				
	mol_type = other DNA				
	organism = synthetic construct				
SEQUENCE: 73					
tcatttaccc	ggggacagg	agaggcttt	ctcggtgt	tgtgttgca	60
catcacggg	catgagaaga	cgttccctg	ctggcaccc	ctttgtcca	120
gtatagagg	agaaggaggc	cgtggaggt	cagcacggg	ggcgtgttt	180
ctccggctgc	ccatgtct	cccaatcc	ggcgatgtc	ctggataga	240
caggcagggtc	aggctgaccc	ggtttttgtt	catctctcc	cgggatgggg	300
cacctgttgt	tctgggggt	gcccccttgc	tttggatgt	ttgggggtgg	360
gagggttttgc	ttggggatct	tgcacttgc	cttccatgg	cctgtgttgc	420
gacgggtgg	aggctgtacca	cacggtaacgt	gtgtgttac	tgctccccc	480
cttggcattt	tgcacccca	cgccgtccac	gtaccatgg	aacttgaccc	540
gtggctcacg	tccaccacca	cgcacgtac	ctcagggttc	cgggagatca	600
cttgggtttt	ggggggaa	ggaagactca	cggtcccccc	aggagttcag	660
cggtggccat	gtgtgagttc	caccacgg	gggggggggt	tcatagggtca	720
gccggcttcg	gggggggggg	taaaacgttc	attacagaaa	ttcccttcgc	780
atagacctgc	ggattttctt	cggtgccac	acattctgg	cggtcataac	840
gtccagccag	cacccttct	tgaccatgtt	aatgtcccg	gagggttcc	900
atagactgg	aggcgtttat	cgcacccgt	tgcagcccg	attggttcgt	960
ccgttcaggt	tcccaattag	cattgtata	aatacattcg	cggtttccgg	1020
cgaacacgaag	actgctccac	acagcagcag	cacacagcag	agccctctct	1080
cat					1083

SEQ ID NO: 74	moltype = AA	length = 368
FEATURE	Location/Qualifiers	
REGION	1..368	

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note = Description of Artificial Sequence: Synthetic
      polypeptide
source 1..368
mol_type = protein
organism = synthetic construct

SEQUENCE: 74
MDAMKRLGLCC VLLLCGAVFV SPGASGRGEA ETRECIYNYNA NWELERTNQS GLERCEGEQD 60
KRLHCYASWR NSSGTIELVK KGCWDFFFNC YDRQECVATE ENPQVYFCCC EGNFCNERFT 120
HLPEAGGPDEV TYEPPPTAPT GGGTHTCPCP PAPELLGGPS VFLFPKPKD TLMISRTPEV 180
TCVVVDSHE DPEVKPENWYV DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNKEY 240
KCKVSNKALP APIEKRTISKA KGQPREPQVY TLPPSREEMT KNQVSLTCLV KGFYPSDIAV 300
EWESENOPEN NYKTPPVLD SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH EALHNHYTQK 360
SLSLSPGK 368

SEQ ID NO: 75      moltype = DNA length = 1107
FEATURE          Location/Qualifiers
misc_feature    1..1107
note = Description of Artificial Sequence: Synthetic
      polynucleotide
source 1..1107
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 75
atggatcaa tgaagagagg gctctgtgt gtgctgtgc tgggtggaggc agtcttcgtt 60
tcggcccgcg ctctctggcg tggggaggct gagacacggg agtgcatactt ctacaacgcc 120
aactgggagc tggagcgcac caaccaggaa ggcccgtggc gctgcgaagg cgagcaggac 180
aagcggctgc actgcatacg ctctctggcg aacagcttcg gcaccatcga gctctgtgg 240
aaggggctgtc tggatgtca cttaacttgc tacatggc aggagtgtt ggccacttgag 300
gagaacccccc aggtgtactt ctgtgtgtt gaaggcaact tctgcacca ggcgttcaact 360
catttggcag aggctggggg cccggaaatgc acgtacggc caccggggac agccccccacc 420
gtgtggggaa ctcacacatc cccacgttc ccacggcactt aactctggg gggaccgtca 480
gttcttcctt cccccccaaa acccaaggac accctcatgtt tctccggac ccctggggc 540
acatgcgtgg tgggtggacgt gagccacaa gaccctgagg tcaaggtaaa ctggtagctg 600
gacggcgctgg aggtgcataa tgccaagaca aagccgggg aggagcagta caacagcacg 660
taccgtgtgg tcacgcgttc caccgttgc caccaggact ggctgaatgg caaggatgtc 720
aagtgcacgg ttcaccaaaa agccctccca gccccccatgc agaaaaaccat ctccaaagcc 780
aaaggggcagc cccgagaacc acagggtgtac accctggccc catccggga ggagatgacc 840
aagaaccagg tcacgcgtac ctgcgtggc aaaggcttc atcccacgca catgcgttg 900
gatgtgggaga gcaatggggc gccggagaa aactacaaga ccacgcctcc cgtgtggac 960
tccgacggct ctttcttcctt ctatagcaag ctcacccgtgg acaagagcag gtggcagcag 1020
ggaaacgtct ttcatgttc cgtgtatgc gaggctctgc acaaccacta cacgcagaag 1080
agcctctccc tggccggggaa taaatgt 1107

SEQ ID NO: 76      moltype = DNA length = 1107
FEATURE          Location/Qualifiers
misc_feature    1..1107
note = Description of Artificial Sequence: Synthetic
      polynucleotide
source 1..1107
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 76
tcattttaccc gggggacaggg agaggcttt ctgcgtgtt ggggttgca gggctcatg 60
catcacggg catgagaaga cgttccctgt ctgcacactg ctcttgtcca cggtgagctt 120
gtctataggaa aagaaggaggc cgtcgaggatc cggcggggaa ggcgtgtgtt tggatgtt 180
ctccgggttc cccatgttc cccatccca gggatgttc cttggataga agcctttgac 240
caggcagggtc aggtgtaccc ggttttggt ctatcccttc cggggatgggg gcagggtgtt 300
cacctgtgtt ttcggggctt gcccggc ttggagatg gttttctca tgggggttgg 360
gagggttttgggatgtt ttcggatgtt ctccttgcca ttcacccgtt cctgggtcag 420
gacgggtgggg acgtgttgcac caccgttgc gtcgtgttgc tgcctctcc ggggttttgt 480
cttggccatca ttcacccatca cccgttccac gtaaccgtttt aactgttgcac cagggttttc 540
gtggctcacg tccacccatca cccgttccac gtaaccgtttt aactgttgcac cagggttttc 600
tttgggtttt gggggggaaa ggaagactga cggccccccc agggatgttgc gttgtggca 660
cggtggggatgtt ttcacccatca cccgttccac gtaaccgtttt aactgttgcac cagggttttc 720
tttggggggggcc cccgttccac gtaaccgtttt aactgttgcac cagggttttc 780
gcacggcggcc cccgttccac gtaaccgtttt aactgttgcac cagggttttc 840
gttggatgttca ttcacccatca cccgttccac gtaaccgtttt aactgttgcac cagggttttc 900
ccaggaggcg tagcgttgca gccgttgcct ctgcgtcgct tcgcagcgctt ccaggccgt 960
cttgggtggggcc cccgttccac gtaaccgtttt aactgttgcac cagggttttc 1020
cttggggggggcc cccgttccac gtaaccgtttt aactgttgcac cagggttttc 1080
gcacggcggcc cccgttccac gtaaccgtttt aactgttgcac cagggttttc 1107

SEQ ID NO: 77      moltype = AA length = 360
FEATURE          Location/Qualifiers
REGION          1..360
note = Description of Artificial Sequence: Synthetic

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source          polypeptide
               1..360
               mol_type = protein
               organism = synthetic construct

SEQUENCE: 77
MDAMKRLGCC VLLLCGAVFV SPGAETREC IYYNANWELE RTNQSGLERC EGEQDKRLHC 60
YASWRNSSGT IELVKKGCD DDFNCYDRQE CVATEENPQV YFCCCEGNFC NERFTHLPEA 120
GGPEVTYEPP PTGGGTHTCP PCPAPELLGG PSVFLFPKKP KDTLMISRTP EVTCVVVDVS 180
HEDPEVKFNW YVDGVEVHNA KTKPREEQYN STYRVSVLT VLHQDWLNKG EYKCKVSNKA 240
LPAPIEKTS KAKGQPREPQ VYTLPPSREE MTKNQVSLTC LVKGFYPSDI AVEWESNGQP 300
ENNYKTTPPV LDSDGSFFLY SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPKG 360

SEQ ID NO: 78      moltype = AA length = 335
FEATURE          Location/Qualifiers
REGION           1..335
note = Description of Artificial Sequence: Synthetic
               polypeptide
source          1..335
               mol_type = protein
               organism = synthetic construct

SEQUENCE: 78
ETRECIYNYA NWELERTNQS GLERCEGEQD KRLHCYASWR NSSGTIELVK KGCWDDDFNC 60
YDRQECVATE ENPQVYFCCC EGNFCNERFT HLPEAGGPEV TYEPPPTGGG THTCPPCPAP 120
ELLGGGPSVFL FPPPKPDLM ISRTPEVTCV VVDVSHEDPE VKFNWYVDGV EVHNAKTCKP 180
EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK VSNKALPAPI EKTISKAKGQ PREPQVYTL 240
PSREEMTKNQ VSLTCLVKGF YPSDIAVEWE SNGOPENNYK TPPVLDSDG SFFLYSKLT 300
DKSRWQQGNV FSCSVMHEAL HNHYTQKSLS LSPKG                         335

SEQ ID NO: 79      moltype = AA length = 107
FEATURE          Location/Qualifiers
REGION           1..107
note = Description of Artificial Sequence: Synthetic
               polypeptide
source          1..107
               mol_type = protein
               organism = synthetic construct

SEQUENCE: 79
ETRECIYNYA NWELERTNQS GLERCEGEQD KRLHCYASWR NSSGTIELVK KGCWDDDFNC 60
YDRQECVATE ENPQVYFCCC EGNFCNERFT HLPEAGGPEV TYEPPPTGGG THTCPPCPAP 107

SEQ ID NO: 80      moltype = DNA length = 1083
FEATURE          Location/Qualifiers
misc_feature     1..1083
note = Description of Artificial Sequence: Synthetic
               polynucleotide
source          1..1083
               mol_type = other DNA
               organism = synthetic construct
CDS              76..396

SEQUENCE: 80
atggatcaa tgaagagagg gctctgtgt gtgtgtgtgc tgggtggagc agtttcggt 60
tcgccccggcg ccgctgagac acgggagtgc atctactaca acggcaactg ggagctggag 120
ccgaccacaaac agagcggccct ggagcgcgtgc qaaggcgagc aggacaaggc gctgcactgc 180
tacgcccctt ggcgcacacag ctctggcacc atcgagctcg tgaagaaggg ctgctggac 240
gtatgacttca acgtctacga taggcaggag tgggtggcca ctgaggagaa cccccagggt 300
tacttctgtc gctgtgaagg caacttctgc aacgagcgcgt tcactcatt gccagaggct 360
ggggggccccc aagtacacgt cgagccaccc cccacagggtt gtggactca cacatgcccc 420
ccgtggcccaag caccgtaaact cctgggggaa cctgtcgtctt tcccttccc cccaaaaccc 480
aaggacaccc tcatgtatcc cccggacccctt gaggtcacat gggtgggtt ggacgtgagc 540
cacgaagacc ctgaggatcaa gttcaacttg tacgtggacg gggtggagggt gcataatgcc 600
aagacaaaacg cccggggaggaa gcaactacaa acgcacgtacc tggtggtcag cgtcttcacc 660
gtcctgtcacc aggactggct gaatggcaag gatgtacaatg gcaagggttc caacaaagcc 720
ctccccagccc ccacatcgagaa aaccatctcc aaagccaaag ggcagccccc agaaccacag 780
gtgtacaccc tgccccccatc cccggggaggat atgaccaaga accagggtcag cctgaccc 840
ctggtaaagg gtttctatcc cagcgacatc gccgtggaggat gggagagcaa tggggcaggcg 900
gagaacaacaat acaagaccac gcctccctgtc ctggactccg acggcttc tttcttcata 960
agcaagctca ccgtggacaa gaggcagggtgg cagcaggggaa acgttcttc atgctccgt 1020
atgcatgagg ctctgcacaa ccactacacg cagaagagcc tctccctgtc cccgggtaaa 1080
tga                                         1083

SEQ ID NO: 81      moltype = DNA length = 1083
FEATURE          Location/Qualifiers
misc_feature     1..1083
note = Description of Artificial Sequence: Synthetic
               polynucleotide
source          1..1083

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mol_type = other DNA
organism = synthetic construct

SEQUENCE: 81
tcatttaccc ggggacaggg agaggcttt ctgcgttag tgggttgca gagcctcatg 60
catcacggg catgagaaga cgttcccctg ctgccacctg ctcttgtcca cggtgagctt 120
gtatagagg aagaaggagc cgteggagtc cagcacggga ggcgtggct tggatgttt 180
ctccggctgc ccattgtct cccactccac ggogatgtcg ctggataga agccttgac 240
caggcaggtc aggctgaccc ggttttggt catctctcc cgggatgggg gcagggtgta 300
cacctgtggt tctcgggct gcccttggc tttggagatg gttttcgtga tggggctgg 360
gagggttggg ttggagaccc tgcaacttgc ctcccttgcca ttccagccagt cctgggtcag 420
gacgggtgagg acgctgacca caggttgcgt gttttgttgc tgcgttcc cgggtttgt 480
cttggcattt tgcacccatc cgcggcttccac gtaccatgtt aacttgcact cagggttcc 540
gtggctcactg tccaccacca cgcacgttgc ctcaagggttgc cgggagatca tgagggtgtc 600
cttgggtttt gggggaaaga ggaagactga cgggtcccccc aggagttcag gtgttggca 660
cggtgggttc tggtgatcc caccacatc cgggggttgc tgcgttgc tttccgggccc 720
ccacgttcc tggaaattag gtaaagcgctc gttgcagaag ttgccttccac agcagcaqaa 780
gtacacctgg ggggttccct cagtgccac acactctgc ctatcgtagc agttaagtc 840
atcgcccccc cagcccttcc tcacgaccc gatggtgcac gaggctgtc gccaggaggc 900
gtagcgtgc acggcgatgtt cctgtcgcc ttcaggccgc tttgggttgg 960
ggcgtccacg tcccaatgg cttgttagta gatgcacttcc cttgttccag cggccgggg 1020
cgaaaccaag actgctccac acagoagcag cacacagcag agccctctt tcattgcatac 1080
cat 1083

SEQ ID NO: 82      moltype = DNA length = 1083
FEATURE           Location/Qualifiers
misc_feature      1..1083
note = Description of Artificial Sequence: Synthetic
          polynucleotide
source            1..1083
mol_type = other DNA
organism = synthetic construct
CDS               76..396

SEQUENCE: 82
atggatgcaa tgaagagagg gctctgtgtgt gttgtggagc agtcttcgtt 60
tcggccggcc cggccggaaac ccgcaaatgtt atttattaca atgctaatttggaaactcgaa 120
cggacgaacc aatccggctc cgaacgggttggaggggaaac aggataaaacg cctccatgc 180
tatgcgtgtt ggaggaaactc ctccggggacg attgaacttgc tcaagaaagg gtgttggac 240
gacgatttca attgttatga ccggcaggaa tttgttgcgcgaa ccgaagagaaa tccgcaggc 300
tatttcgtt gttgtcgaggaa gatattctgtt aatggacccgtt ttacccactt ccccgaaagcc 360
ggcgccggccg aggttgcactt gtaaaaaacccccc cccacccgttgc tggaaactca cacaatgc 420
ccgtgcggccat caccgttactt cttggggggaa ccgttcgttcc ttctttccccc cccaaaccc 480
aaggacaccc tcatgtatcc ccggacccctt gaggttccatc gctgttgggtt ggacgtgagc 540
cacaaggaccc ctgggttcaatccatc gttgtggaggc gctcaatggc 600
aaggacaccc ccggggggggaa gcaatgttgc acgttgcacttcc 660
gttctgcacc accgttgcgtt gatggcaag gatgttgcactt gcaagggttcc caacaagcc 720
ctcccgccccc ccatcgagaa aaccatctcc aaagccaaag ggcaagccccc agaaccacag 780
gtgtacaccct tggcccccattt ccggggggggatgatggacca accgggttgc cttgttgc 840
ctgggttcaatccatc ccggacatc gccgtggaggatggggggccaa tggggcaggcc 900
gagaacaaact acaagaccac ccctccctgtt ctggactccctt acggcttccctt cttctctat 960
agcaagtc tccgtggacaa gagggttgc cttggggggaa acgttccatc atgttccgtt 1020
atgtatgggg ctctgcacaa ccactacacg cagaagaccc tttccctgttcccccgggtaaa 1080
tga 1083

SEQ ID NO: 83      moltype = DNA length = 1083
FEATURE           Location/Qualifiers
misc_feature      1..1083
note = Description of Artificial Sequence: Synthetic
          polynucleotide
source            1..1083
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 83
tcatttaccc ggggacaggg agaggcttt ctgcgttag tgggttgca gagcctcatg 60
catcacggg catgagaaga cgttcccctg ctgccacctg ctcttgtcca cggtgagctt 120
gtatagagg aagaaggagc cgteggagtc cagcacggga ggcgtggct tggatgttt 180
ctccggctgc ccattgtct cccactccac ggatgttgc ctggataga agccttgac 240
caggcaggtc aggctgaccc ggttttggt catctctcc cgggatgggg gcagggtgta 300
cacctgtggt tctcgggct gcccttggc tttggagatg gttttcgtga tggggctgg 360
gagggttggg ttggagaccc tgcaacttgc ctcccttgcca ttccagccagt cctgggtcag 420
gacgggtgagg acgctgacca caggttgcgt gttttgttgc tgcgttcc cgggtttgt 480
cttggcattt tgcacccatc cgcggcttccac gtaccatgtt aacttgcactt cagggttcc 540
gtggctcactg tccaccacca cgcacgttgc ctcaagggttgc cgggagatca tgagggtgtc 600
cttgggtttt gggggaaaga ggaagactga cgggtcccccc aggagttcag gtgttggca 660
ccgtggccat gtgtgatgtt caccacccgtt ggggggggggt tcataggtca cttccggggcc 720
ccggccgttc ggggggttggg taaaccgttcc attacagaaa ttccctcgc aacaacagaa 780
atagacgttc cggatttctt ccgttcgcac acatttccgtt ccgttataac aattgtaaatc 840

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gtcgtcccg cacccttct tgaccagttc aatcgcccc gaggagttcc tccacgacgc 900
atagcaatgg aggctttat cctgtcccc ctacacccgt tcgagcccc attggttcgt 960
ccgttcgagt tcccaattag cattgtataa aatacattcg cgggttcgg cggcgcggg 1020
cgaaacgaag actgctccac acagcagcag cacacagcag agccctctc tcattgcac 1080
cat                                              1083

SEQ ID NO: 84          moltype = DNA  length = 321
FEATURE
misc_feature           Location/Qualifiers
1..321
note = Description of Artificial Sequence: Synthetic
      polynucleotide
source                1..321
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 84
aaaccccg aatgtattt ttacaatgtc aattggaa tcgaacggac gaaccaatcc 60
gggctcgaac ggtgtgaggg ggaacaggat aaacgcctcc attgctatgc gtcgtggagg 120
aactccctcg ggacgattga actgtcaag aaagggtgtc gggacgacga ttcaattgt 180
tatgaccgccc aggaatgtgt cgcgaccgaa gagaatccgc aggtctatcc ctgttggtgc 240
gagggaattt tctgtatga acggtttacc cacctccccc aagccggccg gcccggagg 300
acatatgaaac ccccgccccac c                                              321

SEQ ID NO: 85          moltype = length =
SEQUENCE: 85
000

SEQ ID NO: 86          moltype = length =
SEQUENCE: 86
000

SEQ ID NO: 87          moltype = length =
SEQUENCE: 87
000

SEQ ID NO: 88          moltype = length =
SEQUENCE: 88
000

SEQ ID NO: 89          moltype = length =
SEQUENCE: 89
000

SEQ ID NO: 90          moltype = length =
SEQUENCE: 90
000

SEQ ID NO: 91          moltype = length =
SEQUENCE: 91
000

SEQ ID NO: 92          moltype = length =
SEQUENCE: 92
000

SEQ ID NO: 93          moltype = length =
SEQUENCE: 93
000

SEQ ID NO: 94          moltype = length =
SEQUENCE: 94
000

SEQ ID NO: 95          moltype = length =
SEQUENCE: 95
000

SEQ ID NO: 96          moltype = length =
SEQUENCE: 96
000

SEQ ID NO: 97          moltype = length =
SEQUENCE: 97
000

SEQ ID NO: 98          moltype = length =
SEQUENCE: 98

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SEQ ID NO: 99      moltype = length =
SEQUENCE: 99
000

SEQ ID NO: 100      moltype = AA length = 505
FEATURE
source
1..505
mol_type = protein
organism = Homo sapiens
SEQUENCE: 100
MAESAGASSF FPLVVLLAG SGSGSPRGVQ ALLCACTSCL QANYTCETDG ACMVSIFNLD 60
GMEEHHVRCTI PKVELVPAGK PFYCLSSEDL RNTHCCYTD CNRIDLRVPS GHLKEPEHPS 120
MWGPVELVGI IAGPVFLFL IIIIVFLVIN YHQRVYHNROQ RLDMEDPSCE MCLSKDKTLQ 180
DLVYDLSTSG SGSGLPLFVQ RTVARTIVLQ EIIKGKGRPGE VWRGRWRGGD VAVKIFSSRE 240
ERSWFREAEI YTQVMLRHEN ILGFIAADNK DNGTWTQLWL VSDYHEHGSL FDYLNRYTVT 300
IEGMIKLALS AASGLAHLHM EIVGATQGKPG IAHRDLKSNN ILVKKNMCA IADLGLAVRH 360
DAVDTDIDIA PNQRVGTKRY MAPEVLDETI NMKHFDSFKC ADIYALGLVY WEIARRCNSG 420
GVHEEYQLPY YDLVPSDPST EEMRKVVCDO KLRPNIPNWQ QSYEALRVMG KMMRECWYAN 480
GAARLTALRI KKTLSQLSVQ EDVKI 505

SEQ ID NO: 101      moltype = AA length = 103
FEATURE
source
1..103
mol_type = protein
organism = Homo sapiens
SEQUENCE: 101
SGPRGVQALL CACTSCLQAN YTCTDGACM VSIFNLDGMH HHVRTCIPKV ELVPAGKPFY 60
CLSSEDLRNT HCCYTDYCNR IDLRVPSGHL KEPEHPSMWG PVE 103

SEQ ID NO: 102      moltype = DNA length = 1515
FEATURE
source
1..1515
mol_type = other DNA
organism = Homo sapiens
SEQUENCE: 102
atggcgaggat cggccggagc ctcccttc ttcccccttg ttgtccctct gctcgccggc 60
agccggcggtt cggggccggg gggggccggc gctctgcgtt gtgcgtgcac cagctgcctc 120
caggcccaact acacgtgtga gacagatggg gcctgcattt caatctggat 180
gggatggagc accatgtgcg cacatgcac cccaaatgg agctggccc tgccggaaag 240
cccttctact gcctgagctc ggaggaccc tcgtgcata cactgactac 300
tgcacacggg tccgacttggat ggtggccatg ggtcacccatc aggagccatc gcacccgtcc 360
atgtggggcc cgggtggatc ggttaggcatt atgcggccgc cgggtgttct cctgttctc 420
atcatatca ttgttttctt tgcattaaatc tgcatttcgc gtgtctatca caacccgcag 480
agactggaca tggaaagatcc ctcatgttag atgtgtctc ccaaagacaa gacgtccag 540
gtatctgtct acatgttctc cacatcgatgg tctggctcgtt ggttaccctt ctttgtccag 600
cgacacgtgg cccgaacatc ctgttttacaa gagattatgg gcaagggtcg gtttggggaa 660
gtatgggggg gocgtggag ggggtggat gtggctgtga aaatatttctc ttctgtgaa 720
gaacggctt ggttcaggaa agcagagata taccagacgg tcatgtgcg ccataaaaaac 780
atccctggat tatttgtctc tgaaataaaa gataatggca cctggacaca gctgtggctt 840
gtttctgact atcatggaca cgggttctt tttgattatc tgaaccggata cacagtgaca 900
attggggggta tgatggatc ggcctgtct gtcgtctggatggcata cctgcacatg 960
gagatgtgg gcacccaagg gaagcttggaa attgtctatc gagacttaaa gtcacaaagac 1020
attctgtgtca agaaaaatgg catgtgtgcc atagcagacc tgggcctggc tgcgttgcatt 1080
gatgcagtca ctgacacatc tgatgttgc ccaatcaga ggggtggggac caaacgatc 1140
atggccctgt aagtacttgc tgaaaccatt aatatggaaatc actttgactc cttttatgt 1200
gtgtatattt atgcctcgg gttgttatat tgggagatgg ctgcgaatgt caattcttgg 1260
ggagtccatg aagaatatac gtcgtccat tacgacttag tggcccttgc cccttccatt 1320
gaggaaatgc gaaagggtgt atgtgtatc aagctgcgtc ccaacatccc caactgggtt 1380
caagatgtatc aggactgtgg ggtgtgggg aagatgtatc gagatgttgc gatgtggcc 1440
ggcgccggcc cctgtacatc aagaagaccc tctcccagct cagcgtgcag 1500
gaagacgtga agatc 1515

SEQ ID NO: 103      moltype = DNA length = 309
FEATURE
source
1..309
mol_type = other DNA
organism = Homo sapiens
SEQUENCE: 103
tccggggcccc ggggggttca ggctctgtgt tgcgtgcac ccagctgcct ccaggccaa 60
tacacgtgtg agacagatgg ggcctgcattt tcaatctggat tgggatggag 120
caccatgtgc gcacatgcac cccaaatgg gagctggcc ctgcccggaa gccccttctac 180
tgcctgactt gggaggaccct ggcacacacc cactgtgtctt acactgtacta ctgcaacagg 240
atcgacttgc ggtgtccatc tggtcaccc tggatgttgc aagggcctgc catgtggggc 300
ccgggtggag 309

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SEQ ID NO: 104      moltype = AA  length = 453
FEATURE
source          Location/Qualifiers
1..453
mol_type = protein
organism = Homo sapiens
SEQUENCE: 104
MVSIFNLDGM EHHVRTCIPK VELVPAGKPF YCLSSEDLRN THCCYTDYCN RIDLRVPSGH 60
LKEPEHPSMW GPVELVGIIA GPVFLLFLII IIVFLVINYH QRVYHNQRQL DMEDPSCEMC 120
LSKDKTLQDL VYDLSTSGSG SGLPLFVORT VARTIVLQEII IGKGRFGEVV RGRWRGGDVA 180
VKIFSSREERIYQ TVMLRERENL GFIAADNKDN GTWTQLWLVS DYHEHGSLFD 240
YLNRYTVTIE GMIKLALSAA SGLAHLHMEI VGTQGKPGIA HRDLKSKNIL VKKNGMCAIA 300
DLGLAVRHDA VTDTIDIANP QRVGTKRYMA PEVLDENIMM KHFDSFKCAD IYALGLVYWE 360
IARRCNSGGV HEHEYQLPYYD LVPSPSIEB MRKVVCQDKL RPNIPIWWQS YEALRVMGKM 420
MRECWYANGA ARLTALRIKK TLSQLSVQED VKI 453

SEQ ID NO: 105      moltype = AA  length = 74
FEATURE
source          Location/Qualifiers
1..74
mol_type = protein
organism = Homo sapiens
SEQUENCE: 105
MVSIFNLDGM EHHVRTCIPK VELVPAGKPF YCLSSEDLRN THCCYTDYCN RIDLRVPSGH 60
LKEPEHPSMW GPVE 74

SEQ ID NO: 106      moltype = DNA  length = 1362
FEATURE
source          Location/Qualifiers
1..1362
mol_type = other DNA
organism = Homo sapiens
SEQUENCE: 106
atggtttcca tttcaatct ggatggatg gaggcaccatg tgccgcacccatg catccccaaa 60
gtggagctgg tccctgcccgg gaagcccttc tactgcgtga gctcgaggaa cctgcgcac 120
acccactgtc gtcacactga ctactgcac acggatcgact tgagggtgcc cagtggcac 180
ctcaaggagc ctggacccggc gtccatgtgg ggcccggtgg agctggtagg catcatcgcc 240
ggcccggtgt tcctccgtt cctcatcatacattgttt tccttgcata taactatcat 300
cagcgtgtct atacaacccg ccagagactg gacatggaaat atccctcatg tgagatgtgt 360
ctctccaaag acaagacgtc ccaggatctt gtctacgcac tctccaccc acgggtctggc 420
tcagggttac ccctctttgc ccaggcgcaca gtggccggaa ccatcggtt acaagagat 480
attggcaagg gtccgttgg ggaagtgatgg cggggccgcgt ggagggttgg tgatgtggct 540
gtgaaaatat ttcttctcg tgaagaacggg tcttggatca gggaaacgaa gatataccag 600
acggtcatgc tgcgcattga aaacatcctt ggattttatg ctgtgcacaa taaagataat 660
ggacccatggc cacatgtgtc gcttttttgc gactatcatg acaacgggtc cctgtttgt 720
tatctgaaacc ggtacacagt gacaattgtgg gggatgatgg agetggccctt gtctgtgt 780
agtgggttgg cacacccgtca catggatgc gtggccaccc aaggaaaggcc tggatgtgt 840
catcgagact taaagtcaaa gaacattctg gtgaagaaaa atggcatgtg tgccatagca 900
gacccgtggcc tggctgtccg tcatgtatgc gtcaactgacca ccattgtacat tgcccccgaat 960
cagagggtgg gggccaaacg atacatggcc cctggaaatc ttgatgaaac cattaatatg 1020
aaacactttg atcccttaa atgtgtgtat attatgtcccg tcgggttgtt atattggag 1080
attgtctcgaa gatgcaattt tggaggatgtc catgaagaat atcagctgcc atattacac 1140
ttatgtccctt ctggcccttc cattggaggat atgcgaaagg ttgtatgtga tcagaagctg 1200
cgtcccaaca tcccccaactg gtggcagatgtatggatgc tggccgtat gggaaagatg 1260
atgcgagatgttggatgc caacggcgcacq gcccggctga cggccctggc catcaagaag 1320
accctctccc agctcagcgt gcaggaaagac gtgaagatct aa 1362

SEQ ID NO: 107      moltype = DNA  length = 230
FEATURE
source          Location/Qualifiers
1..230
mol_type = other DNA
organism = Homo sapiens
SEQUENCE: 107
atggtttcca tttcaatct ggatggatg gaggcaccatg tgccgcacccatg catccccaaa 60
gtggagctgg tccctgcccgg gaagcccttc tactgcgtga gctcgaggaa cctgcgcac 120
acccactgtc gtcacactga ctactgcac acggatcgact tgagggtgcc cagtggcac 180
ctcaaggagc ctggacccggc gtccatgtgg ggcccggtgg agctggtagg 230

SEQ ID NO: 108      moltype = AA  length = 368
FEATURE
REGION          Location/Qualifiers
1..368
note = Description of Artificial Sequence: Synthetic
polypeptide
source          mol_type = protein
organism = synthetic construct
SEQUENCE: 108
MDAMKRLGCC VLLLCGAVFV SPGASGRGEA ETRECIYYNA NWELERTNQS GLERCEGEQD 60

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KRLHCYASWR	NSSGTIELVK	KGCWLDDFNC	YDRQECVATE	ENPQVYFC	EGNFCNERFT	120
HLP EAGGPEV	TYEPPPTAPT	GGGHTTCPPC	PAPELLGGPS	VFLFPKPKD	TLMISRTPEV	180
TCVVVDVSHE	DPEVKFNWYV	DGVEVHNAKT	KPREEQYNST	YRVVSLTVL	HQDWLNGKEY	240
KCKVSNKALP	APIKTISKA	KQQPREPVY	TLPPSRKEMT	KNQVSLTCLV	KGFYPSDI	300
EWESNGQOPEN	NYKTPPPVVLK	SDGSFFLYSK	LTVDSRWRQQ	GNVFSCSVMH	EALHNHYTQK	360
SLSLSPGK						368

SEQ ID NO: 109	moltype = DNA	length = 1104
FEATURE	Location/Qualifiers	
misc_feature	1..1104	
	note = Description of Artificial Sequence: Synthetic	
	polynucleotide	
source	1..1104	
	mol_type = other DNA	
	organism = synthetic construct	
SEQUENCE: 109		
atggatgc aa tgaagagg ggtctgtgt gtgctgtgc tggatggc agtcttcgtt	60	
tcgcggccg cctctggccg tggatggc gagacacggg agtgcatact ctacaacgccc	120	
aactgggagc tggatggc acaccaggc ggcttgaggc gtcgcaagg cgaggcaggac	180	
aaggcggtgc atctgtacgc ctctggccg aacacgttcg gcacccatcga gtcgtggaa	240	
aagggtgtct ggcttagatga cttcaactgc tacgatggc aggagtgtgt ggccactgg	300	
gagaaccccc aggtgtactt ctgtgtgtgt gaaggcaact tctgcacca ggcgttca	360	
catttgcgcg aggctggggg cccggaaatc acgtacggc caccggccgac agccccccacc	420	
gttgcgtggaa ctcacatcgat ccacccatgc ccacccatcg aactctggg gggaccgtca	480	
gtttccctt tccccccaaa acccaaggac accctcatga tctccggac ccctgagggtc	540	
acatgcgtgg tggatggacgt gaggccacaa gaccctgagg tcaagtccaa ctggta	600	
gacggcgtgg aggtgtataa tgccaaaggc aaggccggg aggacggata caacacgca	660	
taccgcgtgg tcagcgtcc caccgcgttgc caccaggact ggctgtatgg caaggatgtac	720	
aagtgcacaa ttcacacaa agccctccca gccccatcg agaaaaacat ctccaaagcc	780	
aaaggggcgc cccggaaacc acagggtgtac accctgcccc catccggaa ggagatgacc	840	
aagaaccagg tcagcgtccgat ctgcgtggaa aagggttttct atccacgcg catgcgttg	900	
gagtgggaga gcaatggc gccggaaac aactacaaga ccacgcctcc cgtgtgtaa	960	
tccgacgcctt ctttcgttct ctatagcaag ctcaccgtgg acaagagcag gtggcagcag	1020	
gggaacgtct tctcatgtc cgtgtatgc gaggctgtc acaaccacta cacgcagaag	1080	
agccctcccc tggatccccc taaa	1104	
SEQ ID NO: 110	moltype = AA	length = 343
FEATURE	Location/Qualifiers	
REGION	1..343	
	note = Description of Artificial Sequence: Synthetic	
	polypeptide	
source	1..343	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 110		
GRGEAETREC IYYNANWELE RTNQSGLERC EGEQDKRLHC YASWRNSSGT IELVKKGCWL	60	
DDPNCYDRQE CVATEENPQV NERFTHLPEA GGPEVITYEPP PTAPITGGGTH	120	
TCPCCPAPEL LGGPSVFLFP PKPKDTLMIS RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV	180	
HNAKTKPREE QYNSTYRVVS VLTVLHQDWL NGKEYKCKVS NKLPAPIEK TISKAKGQPR	240	
EPQVYTLPPS RKEMTKNQVS LTCLVKGFYPS DIAVEWESN GQPENNYKTT PPVLKSDGSF	300	
FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSL PGK	343	
SEQ ID NO: 111	moltype = AA	length = 355
FEATURE	Location/Qualifiers	
REGION	1..355	
	note = Description of Artificial Sequence: Synthetic	
	polypeptide	
source	1..355	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 111		
MDAMKRLGCC VLLLCGAVFV SPGASGPRGV QALLCACTSC LQANYTCETD GACMVSIFNL	60	
DGMEEHHVRTC IPKVELVPAG KPFYCLSSED LRNTHCCYTD YCNRIDLRVP SGHLKEPEHP	120	
SMWGPVETGG GTHTCPPCPA PELLGGPSVF LFPPKPKDIL MISRTPEVTC VVVDVSHEDP	180	
EVKFNWYVDG VEVHNAKTKP REEYNSTYR VVSVLTVLHQ DWLNGKEYKC KVSNKALPAP	240	
IEKTISAKG QPREPOVYTL PPSREEMTKN QVSLTCLVKG FYPYPSDI	300	
DTTPPVLDSD GSFFFLYSDLT VDKSRWRQQGN VFSCSVMHEA LHNHYTQKSL SLSPG	355	
SEQ ID NO: 112	moltype = DNA	length = 1065
FEATURE	Location/Qualifiers	
misc_feature	1..1065	
	note = Description of Artificial Sequence: Synthetic	
	polynucleotide	
source	1..1065	
	mol_type = other DNA	
	organism = synthetic construct	

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SEQUENCE: 112

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atggatcaa tgaagagagg gctctgtgt gtgtgtgtgc tgggtggagc agtcttcgtt 60
tcggccggcg cttccgggccc cgggggggtc caggtctgc tgggtgtgc caccgtgc 120
cttcaggcoca actacacgtg tgagacatg ggggcctgc tgggttccat ttcaacttg 180
gatgggtatgg agcaccatgt ggcacactgc atccccaaag tggagctgtt ccctgcccgg 240
aaggcccttct actgccttagt ctggaggagc ctgcgcacaa cccactgtgc ctacactgac 300
tactgcaaca ggtatgcattt ggggtgtccc agtgggtcacc tcaaggagcc tgagcacccg 360
tccatgtggg gcccgggtgg gacccgtgtt ggaacttcaca catgccccccat gtgccccagca 420
cctgaactcc tggggggacc gtcatgtttc ctttttcccc caaaacccaa ggacaccctc 480
atgtatccccc ggaccatggc ggtcacatgc gtgggtgtgg acgtgagcc cgaqaccct 540
gagggtcaagt tcaactgttgc ctggggggccg gtgggggtggc ataatggccaa gacaaggccg 600
cggggaggagc agtacaacag cactgttccatgttgc tgggtcaggc tcttcacccgt cctgcacccag 660
gactggctga atggcaagga gtacaagtgc aagggtctcca acaaaggccct cccagcccc 720
atcgagaaaa ccatactccca agccaaaggcc cagccccccat gacccacgggt gtacaccctg 780
ccccccatccc gggaggagat gaccaagaac cagggtcagcc tgactgtgtt ggtcaaaaggc 840
tttataccca ggcacatcgc ctgggggtgg gaggacatgc ggcacccggaa gaaaactac 900
gacaccacgc ctccgggtgttgc ggactccgac gggtcccttct tcctctatag cgacccatcacc 960
gtggacaaga gaagggtggc gcaggggaaac gtcttctcat gtccgggtgtt gcatgaggct 1020
ctgcacaacc actacacgcga gaagagccctc tccctgttcc cgggt 1065

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SEQ ID NO: 113 moltype = AA length = 331
FEATURE Location/Qualifiers
REGION 1..331
 note = Description of Artificial Sequence: Synthetic polypeptide
source 1..331
 mol_type = protein
 organism = synthetic construct

SEQUENCE: 113

```

SGPRGVQALL CACTSCLQAN YTCETDGACM VSIFNLDGME HHVRTCIPKV ELVPAGKPFY 60
CLSSEDLRNT HCCYTDYCNR IDLRLVPSGHL KEPEHPSMWG PVETGGGTHT CPPCPAPELL 120
GGPSVFLFPP KPKDTLMISR TPEVTCVVVD VSHEDPEVFK NWYVDGVEVH NAKTKPREFQ 180
YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSR 240
EEMTKNQVSL TCLVKGFYPS DIAVEWESNG QPENNYDTPP PVLDSDGSFF LYSDLTVDKS 300
RWQQGNVFSC SVMHEALHNH YTQKSLSLSP G 331

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SEQ ID NO: 114 moltype = AA length = 368
FEATURE Location/Qualifiers
REGION 1..368
 note = Description of Artificial Sequence: Synthetic polypeptide
source 1..368
 mol_type = protein
 organism = synthetic construct

SEQUENCE: 114

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MDAMKRLGCC VLLLCGAVFV SPGASGRGEA ETRECIYVNA NWELERTNQS GLERCEGEQD 60
KRLHCYASWR NSSGTIELVK KGCVLDDDFNC YDRQECVATE ENPVQVYFCCC EGNFCNERFT 120
HLPFAGGPV TYEPPTTAPT GGGHTHTCPCP PAPELLGGPS VFLFPPPKFD TLMISRTPEV 180
TCVVVDVSHE DPEVKPNWYV DGVEVHNAAK TPREEQYNST YRVVSVLTVL HQDWLNGKEY 240
KCKVSNKALP APIEKTISKA KGQPREPQVY TLPPCREEMT KNQVSLWCLV KGFYPDSIAV 300
EWESNGOPEN NYKTPPVLD SDGSFFFLYSK LTVDKSRWQQ GNVFSCSVMH EALHNHYTQK 360
SLSLSPGK 368

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SEQ ID NO: 115 moltype = AA length = 343
FEATURE Location/Qualifiers
REGION 1..343
 note = Description of Artificial Sequence: Synthetic polypeptide
source 1..343
 mol_type = protein
 organism = synthetic construct

SEQUENCE: 115

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GRGEAAETREC IYYNANWELE RTNQSGGLERC EGEQDKRLHC YASWRNSSGT IELVKKGCWL 60
DDFNCDYDRQE CVATEENPQV YFCCCEGNFNC NERFTHLPEA GGPEVITYEPP PTAPITGGGTH 120
TCPPCPAPEL LGGPSVFLFPP PKPKDTLMIS RTPEVTCVVVD DVSHEDPEVFK FNWYVDGVEV 180
HNAKTKPREFQ QYNSTYRVVSV LTVLHQDWLN NGKEYKCKVNS NKALPAPIEKT TISKAKGQPR 240
EPQVYTLPPC REEMTKNQVS LWCLVKGFYPS SDIAVEWESN QPENNYKTT PPVLDSDGSF 300
FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLSPGK 343

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SEQ ID NO: 116 moltype = AA length = 356
FEATURE Location/Qualifiers
REGION 1..356
 note = Description of Artificial Sequence: Synthetic polypeptide
source 1..356
 mol_type = protein

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SEQUENCE: 116          organism = synthetic construct
MDAMKRLGCC VLLLCGAVFV SPGASGPRGV QALLCACTSC LQANYTCETD GACMVSIFNL 60
DGMEHHVRTC IPKVELVPAG KPFYCLLSSED LRNTHCCYTD YCNRIDLRVP SGHLKEPEHP 120
SMWGPVETGG GTHTCPPCPA PELLGGPSVF LFPPPKPKDTL MISRTPEVTC VVVDVSHEPD 180
EVKFNWVVDG VEVHNAKTKP REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC KVSNKALPAP 240
IEKTISKAKG QPREPVQVCTL PPSREEMTKN QVSLSCAVKG FYPSDIAVEW ESNGQPENNY 300
KTPPPVLDSD GSFFLVSKLT VDKSRWQQGN VFSCSVMHEA LHNHYTQKSL SLSPGK 356

SEQ ID NO: 117          moltype = AA length = 332
FEATURE
REGION
1..332
note = Description of Artificial Sequence: Synthetic
polypeptide
source
1..332
mol_type = protein
organism = synthetic construct

SEQUENCE: 117
SGPRGVQALL CACTSCLQAN YTCTEDGACM VSIFNLDGME HHVRTCIPKV ELVPAGKPFY 60
CLSEDLEDLRTN HCCYTDYCNR IDLRVPSGHL KEPEHPSMWG PVETGGGTHT CPPCPAPELL 120
GGPSVFLFPK KPDKTLMISR TPEVTCVVVD VSHEDPEVFK NWYVDGVEVH NAKTKPREEQ 180
YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVCTLPPSR 240
EEMTKNQVSL SCAVKGKFYPS DIAVEWESNG QPENNYKTTP PVLDSDGSFF LVSKLTVDKS 300
RWQQGNVFS SVMHEALHNH YTQKSLSLSP GK 332

SEQ ID NO: 118          moltype = AA length = 367
FEATURE
REGION
1..367
note = Description of Artificial Sequence: Synthetic
polypeptide
source
1..367
mol_type = protein
organism = synthetic construct

SEQUENCE: 118
MDAMKRLGCC VLLLCGAVFV SPGASGRGEA ETRECIYYNA NWELETNQS GLERCEGEQD 60
KRLHCYASWR NSSGTIELVK KGCLWDDDFNC YDRQECVATE ENPQVYFCCC EGNFCNERFT 120
HILPEAGGPVE TYEPPPTAPT GGGTHTCPCP PAPELGGPS VFLFPPKPKD TLMISRTPEV 180
TCVVVDVSHE DPEVKFNWYV DGVEVHNAKT KPREEQYNT YRVVSVLTVL HQDWLNGKEY 240
KCKVSVNALK APIEKTKA KGQPREPQVY TLPPCREEMT ENQVSLWCLV KGFYPSDIAV 300
EWESNGQOPEN NYKTPPVLD SDGSFFFLYSK LTVDKSRWQQ GNVFSCSVMH EALHNHYTQD 360
SLSLSPG 367

SEQ ID NO: 119          moltype = DNA length = 1101
FEATURE
misc_feature
1..1101
note = Description of Artificial Sequence: Synthetic
polynucleotide
source
1..1101
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 119
atggatgcaa tgaagagagg gctctgtgt gtgtgtggc agtcttcgtt 60
tcggcccgccg cctctggcg tggggaggct qagacacggg agtgcatactca ctacaacgcc 120
aactgggagc tggagcgcac caaccagago ggcctggcgc gctgcgaagg cgagcaggac 180
aaacggctgc actgtacgc ctccctggcg aacagcttcg gcaccatcga gctctgtgaag 240
aaggggctgc ggcttagatca cttcaactcg tacatagggc aggagtgtgt ggccactgag 300
gagaaccccc aggtgtactt ctgtgtgtg gaaggcaact tctgcacca ggcgttcaact 360
catttgcacag aggctggggg cccggaaatgc acgtacggc caccggccgac agcccccaacc 420
gggtgggtggaa ctcacacatg cccacacgtgc ccagcacctg aactctggg gggaccgtca 480
gtcttcctct cccccccaaa accccaaggac accctcatgatc tctccctggac cccttgagggtc 540
acatgcgtgg tggtggacgt gagccacgaa gacccctggg tcaaggtaaa ctggtagctg 600
gacggcgtgg aggtgcataaa tgccaagaca aagccgcggg aggagcagta caacagcactg 660
taccgtgtgg tcagcgtcct caccgtctg caccaggact ggctgaatgg caaggagttac 720
aagtgcacgg tctccaacaaa agccctccca gccccatcg agaaaaacccat ctccaaagcc 780
aaaggggcagc cccgagaacc acatgtccccc catgccccggaa ggagatgacc 840
gagaacccagg tcaagcgtgt gtgcctggc aaaggcttc atcccaacgca catgcctgt 900
gagtgggaga gcaatgggca gccggagaac aactacaaga ccacgcctcc cgtgtggac 960
tccgacggtc cttcttcct ctatagcaag ctcaccgtgg acaagagcag gtggcagcag 1020
ggaaacgtct tctcatgctc cgtgatgcat gaggctctgc acaaccacta cacgcaggac 1080
agectctccc tgtctccggg t 1101

SEQ ID NO: 120          moltype = AA length = 342
FEATURE
REGION
1..342
note = Description of Artificial Sequence: Synthetic
polypeptide

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source          1..342
               mol_type = protein
               organism = synthetic construct

SEQUENCE: 120
GRGEAETREC IYYNANWELE RTNQSGLERC EGEQDKRLHC YASWRNSSGT IELVKKGCWL 60
DFPNCYDRQE CVATEENPQV YFCCCEGNFC NERFTHLPEA GGPEVITYEPP PTAPTTGGGTH 120
TCPPCPAPEL LGGPSVFLFP PKPKDTLMIS RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV 180
HNAKTKPREE QYNSTYRVVS VLTVLHQDWL NGKEYKCKVS NKALPAIEK TISKAKGQPR 240
EPQVYTLPPC REEMTENQVS LWCLVKGFYP SDIAVEWESN GQPENNYKTT PPVLDSDGSF 300
FLYSKLTVDK SRWQQGNVFS CSVMEHEALHN HYTQDSLSSL PG 342

SEQ ID NO: 121      moltype = DNA length = 1026
FEATURE           Location/Qualifiers
misc_feature     1..1026
note = Description of Artificial Sequence: Synthetic
            polynucleotide
source            1..1026
               mol_type = other DNA
               organism = synthetic construct

SEQUENCE: 121
ggcgctgggg aggctgagac acggaggatgc atctactaca acggcaactg ggagctggag 60
cgccaccaacc agagcgcgcg ggacgcgtgc gaaggcgacg aggacaaacgc gctgcactgc 120
tacgcctctt ggcgcacacg ctctggcaco atcgagctcg tgaagaagggg ctgcggcta 180
gtatgacttca actgtacga taggcaggag tggtggccca ctgaggagaa cccccagggt 240
tacttctgtc gctgtgaagg caacctctgc aacgagcgct tcactcatt gccagaggt 300
ggggggccccc aagtacacgtc cgacgcaccc ccgacagcccc ccacccgggtgg tggactcac 360
acatgcaccc acgtgcacccg acctgcacto ctggggggac cgtcactttt ccttcctccc 420
ccaaaaaccca aggacacccct catgtatcc cggaccccttg aggtcacatg cgtgggtgt 480
gacgtgagcc accaaagaccc tgagggtcaag ttcaactgggt acgtggacgg cgtggagggt 540
cataatgcca agacaaaagcc gcgggaggag cagtacaaca gcacgtaccc tgggttcac 600
gtctctacccg tcttgacccca ggactggctg aatggcaagg agtacaatgg caagggttcc 660
aacaaaggccc tcccgcccccc catgcggaaa accatctcca aagccaaagg gcagccccga 720
gaaccacagg tggcacccct gcccccatgc cgggaggagaa tgaccgagaa ccagggtcagc 780
ctgtgggtcc tggtaaaagg cttctatccc agcgacatcg cctggggatgg ggagagcaat 840
gggcagccgg agaacaacta caagaccacg cttccctgtgc tggactccga cgggtcccttc 900
ttccctctata gcaagtcac cttggacaaag agcagggtggc agcaggggaa cgttttcttc 960
tgctccgtga tgcatacgaggc tctgcacaaad cactacacgc aggacacgtt ctccctgtct 1020
ccgggt 1026

SEQ ID NO: 122      moltype = AA length = 356
FEATURE           Location/Qualifiers
REGION            1..356
note = Description of Artificial Sequence: Synthetic
            polypeptide
source            1..356
               mol_type = protein
               organism = synthetic construct

SEQUENCE: 122
MDAMKRLGLCC VLLLCGAVFV SPGASGPRGV QALLCACTSC LQANYTCETD GACMVISIFNL 60
DGMEHHVRTC IPKVELVPAG KPFYCLSSED LRNTHCCYTD YCNRIDLRVP SGHLKEPEHP 120
SMWGPVETGG GTHTCPCPA PELLPGPSVF LFPPKPKDML MISRTPEVTC VVVDVSHEDP 180
EVKFNWYVNDG VEVHNAKTPK REEQYNSTYR VVSVLTVLHQ DWLNKEYKC KVSNKALPAP 240
IEKTISAKAG QPREPOVCTL PPSREEMTKN QVSLSCAVKG FYPSPDIAVEW ESRGOPENNY 300
KTTPPVLDLSR GSFFLVSKLT VDKSRWQQGN VFSCSVMHEA LHNHYTQKSL SLSPGK 356

SEQ ID NO: 123      moltype = DNA length = 1068
FEATURE           Location/Qualifiers
misc_feature     1..1068
note = Description of Artificial Sequence: Synthetic
            polynucleotide
source            1..1068
               mol_type = other DNA
               organism = synthetic construct

SEQUENCE: 123
atggatgcaa tgaagagagg gctctgtgt gtgtgtggc agtcttcgtt 60
tcggcccgcc cttccggcc cgggggggtc caggctctgc tgggtgcgtg caccagctgc 120
ctccaggccca actacacgtg tgagacagat ggggcctgc tgggttccat tttcaatctg 180
gatgggtatgg agcaccatgt gcgcacactgc atccccaaag tggagctgtt ccctggccgg 240
aaaccccttactgcgttag ctggaggacat ctggcgaacca cccactgcgtg ctacactgac 300
tactgcaaca ggtatcgactt gagggtggcc agtgggtcacc tcaaggaggcc tgagcaccgg 360
tccatgtggg gcccgggtgg gaccgggtgtt ggaactcaca catgccccacc gtgccccggca 420
cctgaactcc tggggggacc gtcaacttcc ctcttccccc caaaacccaa ggacacccctc 480
atgatctccc ggacccctga ggtcacatgc gtgggtgggg acgtgagccca cgaagacccct 540
gaggtcaagt tcaactggta cgtggacggc gtggagggtgc ataatgcacaa gacaaaggcc 600
cgggaggagc agtacaacacg cacgtaccgt gtggtcagcc tctcaccgt cctgcaccag 660
gactggctgaa atggcaagga gtacaagtgc aaggcttccca acaaaggccct cccagcccc 720

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atcgagaaaa	ccatctccaa	agccaaaggg	cagccccgag	aaccacaggt	gtgcacctg	780
cccccatccc	gggaggagat	gaccaagaac	caagtcagc	tgtcctcgcc	cgtcaaaggc	840
ttctatccca	gcccacatcgc	cgtggagtgg	gagagccgcg	ggcagecgga	gaacaactac	900
aagaccacgc	ctcccggtct	ggactcccg	ggctccctct	tcctcgtag	caagtcacc	960
gtggacaaga	gcaggtggca	gcaggggaaac	gtcttctcat	gtcccgtag	gcatgaggt	1020
ctgcacaacc	actacacgca	gaagagccctc	tccctgtctc	cgggtaaa		1068

SEQ ID NO: 124	moltype = AA	length = 332
FEATURE	Location/Qualifiers	
REGION	1..332	
source	note - Description of Artificial Sequence: Synthetic polypeptide	
SEQUENCE: 124	1..332	
SGPRGVQALL CACTSCLQAN	YTCTDGACM VSIFNLDGME HHVRTCIPKV ELVPAGKPFY	60
CLSSEDLRNT HCCYTDYCNR	IDLRVPSGHL KEPEHPSMWG PVETGGGTHT CPPCPAPELL	120
GGPSVFLFPV	TPEVTCVVVD VSHEDPEVKP NWYVGDVEVH NAKTPREEQ	180
YNSTYRVVSPV	LTVLHQDWLN GKEYCKVSN KALPAPIEKT ISKAKQPRE PQVCTLPPSR	240
EEMTKNQVSL	SCAVKGFYPS DIAVEWESRG QPENNYKTPP PVLDLRSFFF LVSKLTVDKS	300
RWQQGNVFSC	SVMHEALHNH YTQKSLSLSP GK	332

SEQ ID NO: 125	moltype = DNA	length = 996			
FEATURE	Location/Qualifiers				
misc_feature	1..996				
source	note - Description of Artificial Sequence: Synthetic polynucleotide				
SEQUENCE: 125	1..996				
tccggggccc	ggctctgctg	tgtgcgtgca	ccagctgcct	ccaggccaa	60
tacacgtgt	agacagatgg	ggcctgcatt	tcaatctgga	tggatggag	120
caccatgtgc	gcacccgtcat	ccccaaatgt	gagctggtcc	ctggcgaaa	180
tgcctgagct	cgaggagacct	gcgcacacc	cactgctgtc	actactgacta	240
atcgacttga	gggtgcccag	tggcaccc	aaggagcttg	agcacccgtc	300
ccgggtggaga	ccgggtgtgg	aactcacaca	tgcacccatgt	gcccagcacc	360
ggggggccgt	cagtcattcc	cttccccca	aaaccccaagg	acacccatgt	420
accctgtgg	tccatcgct	gggtggtag	gtgagccacg	aagacccatgt	480
aactgttacg	ttggacccgt	ggagggtcat	aatgccaaga	caaagccgcg	540
tacaacacgca	cgtagctgt	ggtcacgc	ctcaccgtcc	tgcaccagga	600
ggcaaggagt	acaatgtca	ggtcacccat	aaacccatcg	cgagaaaaacc	660
atctccaaag	ccaaaggcga	gccccggaa	ccacagggtt	gcacccgtcc	720
gaggagatgt	ccaagaacca	ggtcacgtt	tccgtgcggc	tcaaaaggctt	780
gacatcgccg	tggagtggga	gagccgggg	cagccggaga	acaactacaa	840
ccctgtggact	actcccgccg	ctccatctc	ctctgttagca	agtcacccgt	900
agttggccaggc	aggggaaacgt	cttctatgtc	tccgtgtatgc	atggggctt	960
tacacgcaga	agagcccttc	cctgtctccg	ggtaaa	gcacaaccac	996

SEQ ID NO: 126	moltype = AA	length = 103
FEATURE	Location/Qualifiers	
source	1..103	
SEQUENCE: 126	moltype = protein	
SGPRGVQALL CACTSCLQAN	YTCTDGACM VSIFNLDGME HHVRTCIPKV ELVPAGKPFY	60
CLSSEDLRNT HCCYTDYCNR	IDLRVPSGHL KEPEHPSMWG PVE	103

SEQ ID NO: 127	moltype = AA	length = 103
FEATURE	Location/Qualifiers	
source	1..103	
SEQUENCE: 127	moltype = protein	
SGPRGIQALL CACTSCLQAN	LTCETDGACM VSIFNLDGLE HHVRTCIPKV ELVPAGKPFY	60
CLSSEDLRNT HCCYTDFCNR	IDLRVPSGHV KEPERPSVWG PVE	103

SEQ ID NO: 128	moltype = AA	length = 103
FEATURE	Location/Qualifiers	
source	1..103	
SEQUENCE: 128	moltype = protein	
SGPRGIQALL CACTSCLQTN	YTCTDGACM VSIFNLDGME HHVRTCIPKV ELVPAGKPFY	60
CLSSEDLRNT HCCYTDFCNR	IDLRVPSGHP KESEQQASMWG PVE	103

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SEQ ID NO: 129      moltype = AA  length = 102
FEATURE          Location/Qualifiers
source           1..102
                  mol_type = protein
                  organism = Gallus gallus
SEQUENCE: 129
APGGARALTC LCSDCKQANS TCETDGACMV SVFNLDGVKH HVRCTIPEAK LIPAGKPFYC 60
LSSEDLRNTH CCYSDFCNKI DLMVPSGHLK DNEPPSSWGP VE               102

SEQ ID NO: 130      moltype = AA  length = 103
FEATURE          Location/Qualifiers
source           1..103
                  mol_type = protein
                  organism = Mus musculus
SEQUENCE: 130
SGPRGIQALL CACTSCLQTN YTCTDGACM VSIFNLDGVE HHVRTCIPKV ELVPAGKPFY 60
CLSSEDLRNT HCCYIDFCNK IDLRVPSGHL KEPEHPSMWG PVE               103

SEQ ID NO: 131      moltype = AA  length = 103
FEATURE          Location/Qualifiers
source           1..103
                  mol_type = protein
                  organism = Sus scrofa
SEQUENCE: 131
SGPRGIQALL CACTSCLQAN YTCTDGACM VSIFNLDGME HHVRTCIPKV ELVPAGKPFY 60
CLSSEDLRNT HCCYIDFCNK IDLRVPSGHL KEPEHPSMWG PVE               103

SEQ ID NO: 132      moltype = AA  length = 103
FEATURE          Location/Qualifiers
source           1..103
                  mol_type = protein
                  organism = Rattus norvegicus
SEQUENCE: 132
SGPRGIQALL CACTSCLQTN YTCTDGACM VSIFNLDGME HHVRTCIPKV ELVPAGKPFY 60
CLSSEDLRNT HCCYIDFCNK IDLRVPSGHL KEPEHPSMWG PVE               103

SEQ ID NO: 133      moltype = AA  length = 225
FEATURE          Location/Qualifiers
source           1..225
                  mol_type = protein
                  organism = Homo sapiens
SEQUENCE: 133
THCTPPCPAP ELLGGPSVFL FPPPKDFTL ISRTPEVTC VVDVSHEPDE VKFNWYVDGV 60
EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK VSNKALPAPI EKTISKAKGQ 120
PREPQVYTLPS PSREEMTKNQ VSLTCLVKGF YPSDIAVEWE SNGQPENNYK TPPVLDSDG 180
SFFLYSKLTV DKSRWQQGNV FSCSVMHEAL HNHYTQKSLS LSPKG               225

SEQ ID NO: 134      moltype = AA  length = 229
FEATURE          Location/Qualifiers
source           1..229
                  mol_type = protein
                  organism = Homo sapiens
SEQUENCE: 134
ESKYGPCCPS CPAPEFLGGP SVFLFPPKPK DTLMSRTPE VTCVVVDVSQ EDPEVQFNWY 60
VDGVEVHNK TKPREEQFNS YTRVSVLTV LHQDWLNGKE YKCKVSNKGL PSSIEKTISK 120
AKQOPREPVY YTLPPSQEEM TKNQVSLTCL VKGFYPSDIA VEWESENQPE NNYKTPPVLP 180
DSDGSSFLY RLTVDKSRWQ EGNVFSCSVM HEALHNHYTQKSLSLSLKG               229

SEQ ID NO: 135      moltype = AA  length = 223
FEATURE          Location/Qualifiers
source           1..223
                  mol_type = protein
                  organism = Homo sapiens
SEQUENCE: 135
VECPPCPAPP VAGPSVFLFP PKPKDTLMIS RTPEVTCVVY DVSHEDPEVQ FNWYVDGVEV 60
HNAKTKPREE QFNSTFRVVS VLTVVHQDWL NGKEYKCKVS NKGLPAPIEK TISKTKGQPR 120
EPQVYTLPS REEMTKNQVS LTCLVKGFYD SDIAVEWESN GOPENNYKTT PPMLDSDGSF 180
FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLS PGK               223

SEQ ID NO: 136      moltype = AA  length = 232
FEATURE          Location/Qualifiers
source           1..232
                  mol_type = protein
                  organism = Homo sapiens
SEQUENCE: 136

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EPKSCDTPPP CPRCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD VSHEDPEVQF 60
KWYVVDGVEVH NAKTKPREEQ YNSTFRVSV LTVLHQDWLN GKEYKCKVSN KALPAIEKT 120
ISKTKGQPRE PQVYTLPPSR EEMTKNQVSL TCLVKGFYPS DIAVEWESSG QPENNYNTTP 180
PMLDSDGGSFF LYSKLTVDKS RWQQGNIFSC SVMHEALHNR FTQKSLSLSP GK 232

SEQ ID NO: 137 moltype = AA length = 6
FEATURE Location/Qualifiers
REGION 1..6
note = Description of Artificial Sequence: Synthetic 6xHis
tag
source 1..6
mol_type = protein
organism = synthetic construct

SEQUENCE: 137
HHHHHHH

SEQ ID NO: 138 moltype = AA length = 108
FEATURE Location/Qualifiers
REGION 1..108
note = Description of Artificial Sequence: Synthetic
polypeptide
source 1..108
mol_type = protein
organism = synthetic construct

SEQUENCE: 138
AETRECIIYN ANWELEERTNQ SGLERCEGEQ DKRLHCYASW RNSSGTIELV KKGCWLDDFN 60
CYDRQECVAT EENPQVYFCC CEGNFCNERF THLPEAGGPE VTYEPPPT 108

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1-30. (canceled)

- 31.** A method of treating pulmonary hypertension with left heart disease, comprising administering to a patient in need thereof an effective amount of a fusion protein comprising:
- an ActRIIA polypeptide comprising the amino acid sequence of SEQ ID NO: 10;
 - an Fc domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO: 14; and
 - a linker.

32. The method of claim **31**, wherein the fusion protein comprises a linker positioned between the ActRII polypeptide and the Fc domain.

33. The method of claim **32**, wherein the Fc domain comprises an amino acid sequence that is at least 97% identical to SEQ ID NO: 14.

34. The method of claim **33**, wherein the Fc domain comprises an amino acid sequence that is at least 99% identical to SEQ ID NO: 14.

35. The method of claim **31**, wherein the linker comprises TGGG (SEQ ID NO: 23).

36. The method of claim **31**, comprising administering to a patient in need thereof an effective amount of a polypeptide comprising an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 32.

37. The method of claim **36**, wherein the polypeptide comprises an amino acid sequence that is at least 97% identical to the amino acid sequence of SEQ ID NO: 32.

38. The method of claim **36**, wherein the polypeptide comprises an amino acid sequence that is at least 99% identical to the amino acid sequence of SEQ ID NO: 32.

39. The method of claim **31**, wherein the patient has resting pulmonary arterial pressure (PAP) of at least 25 mm Hg.

40. The method of claim **31**, wherein the patient has Functional Class II or Class III pulmonary hypertension as recognized by the World Health Organization.

41. The method of claim **31**, wherein the method prevents or delays pulmonary hypertension Functional Class progression.

42. The method of claim **31**, wherein the polypeptide is part of a homodimer protein complex.

43. The method of claim **31**, wherein the polypeptide is glycosylated.

44. The method of claim **31**, wherein the polypeptide binds to one or more ligands selected from the group consisting of: activin A, activin B, and GDF11.

45. The method of claim **31**, comprising further administering to the patient an additional active agent and/or supportive therapy for treating pulmonary hypertension.

46. The method of claim **45**, wherein the additional active agent and/or supportive therapy is selected from the group consisting of: prostacyclin and derivatives thereof; prostacyclin receptor agonists; endothelin receptor; calcium channel blockers; anticoagulants; diuretics; oxygen therapy; atrial septostomy; pulmonary thromboendarterectomy; phosphodiesterase type 5 inhibitors; activators of soluble guanylate cyclase; ASK-1 inhibitors; NF-κB antagonists; lung and/or heart transplantation.

47. The method of claim **31**, wherein the patient has been treated with one or more vasodilators.

48. The method of claim **31**, wherein the patient has been treated with one or more agents selected from the group consisting of: phosphodiesterase type 5 inhibitors, soluble guanylate cyclase stimulators, prostacyclin receptor agonist, and endothelin receptor antagonists.

49. The method of claim **48**, wherein the one or more agents is selected from the group consisting of: bosentan, sildenafil, beraoprost, macitentan, seleipipag, epoprostenol, treprostinil, iloprost, ambrisentan, and tadalafil.

50. The method of claim **31**, wherein the method further comprises administration of one or more vasodilators.

51. The method of claim **31**, wherein the method further comprises administration of one or more agents selected from the group consisting of: phosphodiesterase type 5

inhibitors, soluble guanylate cyclase stimulators, prostacyclin receptor agonist, and endothelin receptor antagonists.

52. The method of claim **51**, wherein the one or more agents is selected from the group consisting of: bosentan, sildenafil, beraprost, macitentan, selexipag, epoprostenol, treprostinil, iloprost, ambrisentan, and tadalafil.

* * * * *