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(54) **METHODS AND COMPOSITIONS FOR TREATING MYELOFIBROSIS**

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CPC ***A61K 38/179*** (2013.01); ***A61K 31/519*** (2013.01)

(57) **ABSTRACT**

In part, the present disclosure relates methods for treating, preventing, or reducing the progression rate and/or severity of myelofibrosis or one or more complications of myelofibrosis (extramedullary hematopoiesis, splenomegaly, anemia, and fibrosis). In certain aspects, the disclosure provides ActRIIB antagonists for use in treating, preventing, or reducing the progression rate and/or severity of one or more complications associated with Janus kinase inhibitor therapy in a patient (e.g., anemia).

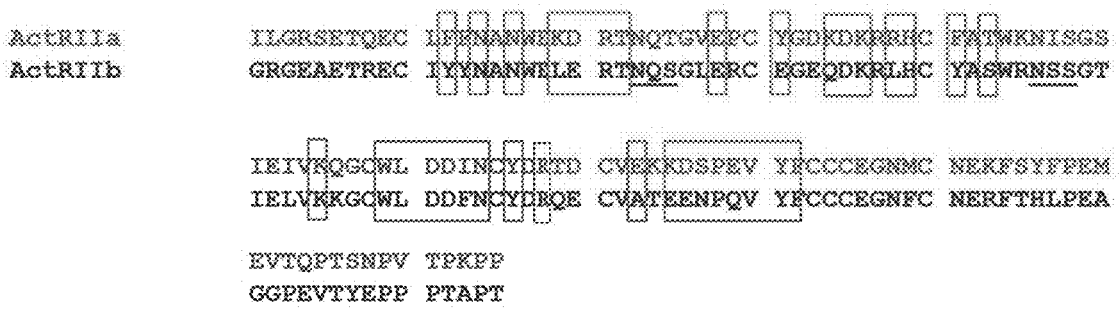
ActRIIa
ActRIIb
ILGRSETQEC
GRGEAETREC
IEIVKQGCWL DDINCYDRTD
IELVKKGCWL DDFNCYDRQE
EVTQPTSNPV TPKPP
GGPEVTYEPP PTAPT

FIGURE 1

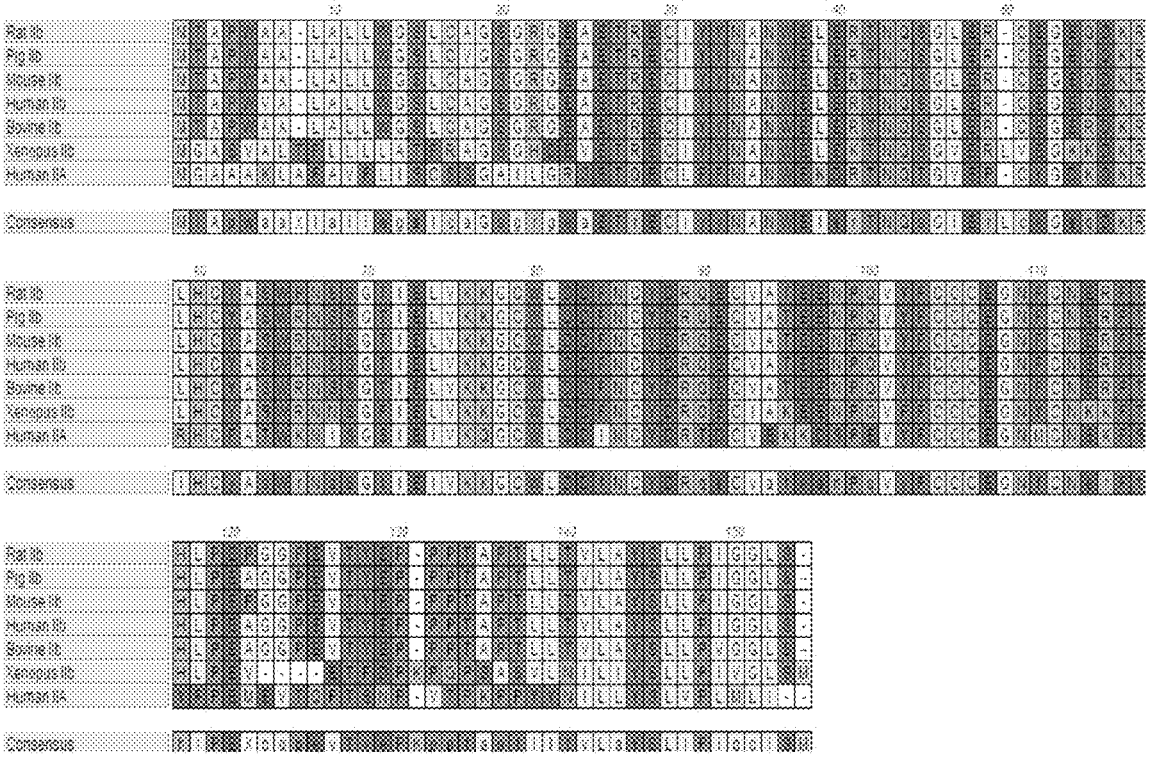

FIGURE 2

```
  1  MDAMKRGLCC VLLLCGAVFV SPGASQRGEA ETRECIYYNA NWELERTNQS
 51  GLERCEGEQD KRLHCYASWR NSSGTIELVK KGCWLDDFNC YDRQECVATE
101  ENPQVYFCCC EGNFCNERFT HLPEAGGPEV TYEPPPTAPT GGGTHTCPPC
151  PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV
201  DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY KCKVSNKALP
251  APIEKTISKA KGQPREPQVY TLPPSREEMT KNQVSLTCLV KGFYPSDIAV
301  EWESNGQPEN NYKTTPPVLD SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH
351  EALHNHYTQK SLSLSPGK (SEQ ID NO:45)
```

FIGURE 3

```
  1  ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
     TACCTACGTT ACTTCTCTCC CGAGACGACA CACGACGACG ACACACCTCG

 51  AGTCTTCGTT TCGCCCGGCG CCTCTGGGCG TGGGGAGGCT GAGACACGGG
     TCAGAAGCAA AGCGGGCCGC GGAGACCCGC ACCCCTCCGA CTCTGTGCCC

101  AGTGCATCTA CTACAACGCC AACTGGGAGC TGGAGCGCAC CAACCAGAGC
     TCACGTAGAT GATGTTGCGG TTGACCCTCG ACCTCGCGTG GTTGGTCTCG

151  GGCCTGGAGC GCTGCGAAGG CGAGCAGGAC AAGCGGCTGC ACTGCTACGC
     CCGGACCTCG CGACGCTTCC GCTCGTCCTG TTCGCCGACG TGACGATGCG

201  CTCCTGGCGC AACAGCTCTG GCACCATCGA GCTCGTGAAG AAGGGCTGCT
     GAGGACCGCG TTGTCGAGAC CGTGGTAGCT CGAGCACTTC TTCCCGACGA

251  GGGATGATGA CTTCAACTGC TACGATAGGC AGGAGTGTGT GGCCACTGAG
     CCCTACTACT GAAGTTGACG ATGCTATCCG TCCTCACACA CCGGTGACTC

301  GAGAACCCCC AGGTGTACTT CTGCTGCTGT GAAGGCAACT TCTGCAACGA
     CTCTTGGGGG TCCACATGAA GACGACGACA CTTCCGTTGA AGACGTTGCT

351  GCGCTTCACT CATTTGCCAG AGGCTGGGGG CCCGGAAGTC ACGTACGAGC
     CGCGAAGTGA GTAAACGGTC TCCGACCCCC GGGCCTTCAG TGCATGCTCG

401  CACCCCCGAC AGCCCCCACC GGTGGTGGAA CTCACACATG CCCACCGTGC
     GTGGGGGCTG TCGGGGGTGG CCACCACCTT GAGTGTGTAC GGGTGGCACG

451  CCAGCACCTG AACTCCTGGG GGGACCGTCA GTCTTCCTCT TCCCCCCAAA
     GGTCGTGGAC TTGAGGACCC CCCTGGCAGT CAGAAGGAGA AGGGGGGTTT

501  ACCCAAGGAC ACCCTCATGA TCTCCCGGAC CCCTGAGGTC ACATGCGTGG
     TGGGTTCCTG TGGGAGTACT AGAGGGCCTG GGGACTCCAG TGTACGCACC

551  TGGTGGACGT GAGCCACGAA GACCCTGAGG TCAAGTTCAA CTGGTACGTG
     ACCACCTGCA CTCGGTGCTT CTGGGACTCC AGTTCAAGTT GACCATGCAC

601  GACGGCGTGG AGGTGCATAA TGCCAAGACA AAGCCGCGGG AGGAGCAGTA
     CTGCCGCACC TCCACGTATT ACGGTTCTGT TTCGGCGCCC TCCTCGTCAT

651  CAACAGCACG TACCGTGTGG TCAGCGTCCT CACCGTCCTG CACCAGGACT
     GTTGTCGTGC ATGGCACACC AGTCGCAGGA GTGGCAGGAC GTGGTCCTGA

701  GGCTGAATGG CAAGGAGTAC AAGTGCAAGG TCTCCAACAA AGCCCTCCCA
     CCGACTTACC GTTCCTCATG TTCACGTTCC AGAGGTTGTT TCGGGAGGGT

751  GCCCCCATCG AGAAAACCAT CTCCAAAGCC AAAGGGCAGC CCCGAGAACC
     CGGGGGTAGC TCTTTTGGTA GAGGTTTCGG TTTCCCGTCG GGGCTCTTGG
```

FIGURE 4A

```
 801   ACAGGTGTAC ACCCTGCCCC CATCCCGGGA GGAGATGACC AAGAACCAGG
       TGTCCACATG TGGGACGGGG GTAGGGCCCT CCTCTACTGG TTCTTGGTCC

 851   TCAGCCTGAC CTGCCTGGTC AAAGGCTTCT ATCCCAGCGA CATCGCCGTG
       AGTCGGACTG GACGGACCAG TTTCCGAAGA TAGGGTCGCT GTAGCGGCAC

 901   GAGTGGGAGA GCAATGGGCA GCCGGAGAAC AACTACAAGA CCACGCCTCC
       CTCACCCTCT CGTTACCCGT CGGCCTCTTG TTGATGTTCT GGTGCGGAGG

 951   CGTGCTGGAC TCCGACGGCT CCTTCTTCCT CTATAGCAAG CTCACCGTGG
       GCACGACCTG AGGCTGCCGA GGAAGAAGGA GATATCGTTC GAGTGGCACC

1001   ACAAGAGCAG GTGGCAGCAG GGGAACGTCT TCTCATGCTC CGTGATGCAT
       TGTTCTCGTC CACCGTCGTC CCCTTGCAGA AGAGTACGAG GCACTACGTA

1051   GAGGCTCTGC ACAACCACTA CACGCAGAAG AGCCTCTCCC TGTCCCCGGG
       CTCCGAGACG TGTTGGTGAT GTGCGTCTTC TCGGAGAGGG ACAGGGGCCC

1101    TAAATGA (SEQ ID NO:48)
        ATTTACT (SEQ ID NO:49)
```

FIGURE 4B

```
  1  MDAMKRGLCC VLLLCGAVFV SPGAA▒TREC IYYNANWELE RTNQSGLERC
 51  EGEQDKRLHC YASWRNSSGT IELVKKGCW▒ DDFNCYDRQE CVATEENPQV
101  YFCCCEGNFC NERFTHLPEA GGPEVTYEPP PTGGGTHTCP PCPAPELLGG
151  PSVFLFPPKP KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA
201  KTKPREEQYN STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS
251  KAKGQPREPQ VYTLPPSREE MTKNQVSLTC LVKGFYPSDI AVEWESNGQP
301  ENNYKTTPPV LDSDGSFFLY SKLTVDKSRW QQGNVFSCSV MHEALHNHYT
351  QKSLSLSPGK (SEQ ID NO: 50)
```

FIGURE 5

```
   1  ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
      TACCTACGTT ACTTCTCTCC CGAGACGACA CACGACGACG ACACACCTCG

                                        E  T   R  E  C   I  Y  Y
  51  AGTCTTCGTT TCGCCCGGCG CCGCTGAGAC ACGGGAGTGC ATCTACTACA
      TCAGAAGCAA AGCGGGCCGC GGCGACTCTG TGCCCTCACG TAGATGATGT

      N  A  N  W   E  L  E   R  T  N   Q  S  G  L   E  R  C
 101  ACGCCAACTG GGAGCTGGAG CGCACCAACC AGAGCGGCCT GGAGCGCTGC
      TGCGGTTGAC CCTCGACCTC GCGTGGTTGG TCTCGCCGGA CCTCGCGACG

       E  G  E   Q  D  K  R   L  E  C   Y  A  S   W  R  N  S
 151  GAAGGCGAGC AGGACAAGCG GCTGCACTGC TACGCCTCCT GGCGCAACAG
      CTTCCGCTCG TCCTGTTCGC CGACGTGACG ATGCGGAGGA CCGCGTTGTC

        S  G  T   I  E  L   V  K  K  G   C  W  D   D  D  F
 201  CTCTGGCACC ATCGAGCTCG TGAAGAAGGG CTGCTGGGAC GATGACTTCA
      GAGACCGTGG TAGCTCGAGC ACTTCTTCCC GACGACCCTG CTACTGAAGT

      N  C  Y  D   R  Q  E   C  V  A   T  E  E  N   P  Q  V
 251  ACTGCTACGA TAGGCAGGAG 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 GGCTGTCCAC

 401  GTGGAACTCA CACATGCCCA CCGTGCCCAG CACCTGAACT CCTGGGGGGA
      CACCTTGAGT GTGTACGGGT GGCACGGGTC GTGGACTTGA GGACCCCCCT

 451  CCGTCAGTCT TCCTCTTCCC CCCAAAACCC AAGGACACCC TCATGATCTC
      GGCAGTCAGA AGGAGAAGGG GGGTTTTGGG TTCCTGTGGG AGTACTAGAG

 501  CCGGACCCCT GAGGTCACAT GCGTGGTGGT GGACGTGAGC CACGAAGACC
      GGCCTGGGGA CTCCAGTGTA CGCACCACCA CCTGCACTCG GTGCTTCTGG

 551  CTGAGGTCAA GTTCAACTGG TACGTGGACG GCGTGGAGGT GCATAATGCC
      GACTCCAGTT CAAGTTGACC ATGCACCTGC CGCACCTCCA CGTATTACGG
```

FIGURE 6A

```
 601  AAGACAAAGC CGCGGGAGGA GCAGTACAAC AGCACGTACC GTGTGGTCAG
      TTCTGTTTCG GCGCCCTCCT CGTCATGTTG TCGTGCATGG CACACCAGTC

 651  CGTCCTCACC GTCCTGCACC AGGACTGGCT GAATGGCAAG GAGTACAAGT
      GCAGGAGTGG CAGGACGTGG TCCTGACCGA CTTACCGTTC CTCATGTTCA

 701  GCAAGGTCTC CAACAAAGCC CTCCCAGCCC CCATCGAGAA AACCATCTCC
      CGTTCCAGAG GTTGTTTCGG GAGGGTCGGG GGTAGCTCTT TTGGTAGAGG

 751  AAAGCCAAAG GGCAGCCCCG AGAACCACAG GTGTACACCC TGCCCCCATC
      TTTCGGTTTC CCGTCGGGGC TCTTGGTGTC CACATGTGGG ACGGGGGTAG


 801  CCGGGAGGAG ATGACCAAGA ACCAGGTCAG CCTGACCTGC CTGGTCAAAG
      GGCCCTCCTC TACTGGTTCT TGGTCCAGTC GGACTGGACG GACCAGTTTC

 851  GCTTCTATCC CAGCGACATC GCCGTGGAGT GGGAGAGCAA TGGGCAGCCG
      CGAAGATAGG GTCGCTGTAG CGGCACCTCA CCCTCTCGTT ACCCGTCGGC

 901  GAGAACAACT ACAAGACCAC GCCTCCCGTG CTGGACTCCG ACGGCTCCTT
      CTCTTGTTGA TGTTCTGGTG CGGAGGGCAC GACCTGAGGC TGCCGAGGAA

 951  CTTCCTCTAT AGCAAGCTCA CCGTGGACAA GAGCAGGTGG CAGCAGGGGA
      GAAGGAGATA TCGTTCGAGT GGCACCTGTT CTCGTCCACC GTCGTCCCCT

1001  ACGTCTTCTC ATGCTCCGTG ATGCATGAGG CTCTGCACAA CCACTACACG
      TGCAGAAGAG TACGAGGCAC TACGTACTCC GAGACGTGTT GGTGATGTGC

1051   CAGAAGAGCC TCTCCCTGTC CCCGGGTAAA TGA (SEQ ID NO: 51)
       GTCTTCTCGG AGAGGGACAG GGGCCCATTT ACT (SEQ ID NO: 52)
```

FIGURE 6B

```
  1  [illegible]TRECIYYNA NWELERTNQS GLERCEGEQD KRLHCYASWR NSSGTIELVK
 51  KGCW[illegible]DDFNC YDRQECVATE ENPQVYFCCC EGNFCNERFT HLPEAGGPEV
101  TYEPPPTGGG THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV
151  VVDVSHEDPE VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD
201  WLNGKEYKCK VSNKALPAPI EKTISKAKGQ PREPQVYTLP PSREEMTKNQ
251  VSLTCLVKGF YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSKLTV
301  DKSRWQQGNV FSCSVMHEAL HNHYTQKSLS LSPGK (SEQ ID NO: 53)
```

FIGURE 7

```
  1   TRECIYYNA NWELERTNQS GLERCEGEQD KRLHCYASWR NSSGTIELVK
 51   KGCWDDFNC  YDRQECVATE  ENPQVYFCCC  EGNFCNERFT
101   HLPEAGGPEV TYEPPPT  (SEQ ID NO: 54)
```

FIGURE 8

```
   1    ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
        TACCTACGTT ACTTCTCTCC CGAGACGACA CACGACGACG ACACACCTCG

                                          E  T   R  E  C   I  Y  Y
  51    AGTCTTCGTT TCGCCCGGCG CCGCCGAAAC GCGCGAATGT ATTTATTACA
        TCAGAAGCAA AGCGGGCCGC GGCGGCTTTG GGCGCTTACA TAAATAATGT

        N  A  N  W   E  L  E   R  T  N   Q  S  G  L   E  R  C
 101    AAGCTAATTG GGAACTCGAA CGCACGAACC AATCCGGCCT CGACGCTGT
        TACGATTAAC CCTTGAGCTT GCCTGCTTGG TTAGGCCCGA GCTTGCCACA

         E  G  E   Q  D  K  R   L  H  C   Y  A  S   W  R  N  S
 151    GAGGGCGAAC AGGATAAACG CCTCCATTGC TATGCGTCGT GGAGCAACTC
        CTCCCCCTTG TCCTATTTGC GGAGGTAACG ATACGCAGCA CCTCCTTGAG

          S  G  T   I  E  L   V  K  K  G   C  W  D   D  D  F
 201    CTCCGGGACG ATTGAACTGG TCAAGAAAGG CTGCTGGGAC GAGGATTTCA
        GAGGCCCTGC TAACTTGACC AGTTCTTTCC CACGACCCTG CTGCTAAAGT

        N  C  Y  D   R  Q  E   C  V  A   T  E  E  N   P  Q  V
 251    ATTGTTATGA CCGCCAGGAA TGTGTCGCGA CCGAAGAGAA TCCCAGGTC
        TAACAATACT GGCGGTCCTT ACACAGCGCT GGCTTCTCTT AGGCGTCCAG

         Y  F  C   C  C  E  G   N  F  C   N  E  R   F  T  H  L
 301    TATTTCTGTT GTTGCGAGGG CAATTCTGT AATGAACGGT TTACCACACGT
        ATAAAGACAA CAACGCTCCC CTTAAAGACA TTACTTGCCA AATGGGTGGA

          P  E  A   G  G  P   E  V  T  Y   E  P  P   P  T
 351    CCCGAAGCC GGCGGCCCG AGGTCACTA CGACCCCCC CCCACCGGTG
        GGGGCTTCGG CCGCCCGGGC TCCACTGGAT ACTTGGGGGC GGGTGGCCAC

 401    GTGGAACTCA CACATGCCCA CCGTGCCCAG CACCTGAACT CCTGGGGGGA
        CACCTTGAGT GTGTACGGGT GGCACGGGTC GTGGACTTGA GGACCCCCCT

 451    CCGTCAGTCT TCCTCTTCCC CCCAAAACCC AAGGACACCC TCATGATCTC
        GGCAGTCAGA AGGAGAAGGG GGGTTTTGGG TTCCTGTGGG AGTACTAGAG

 501    CCGGACCCCT GAGGTCACAT GCGTGGTGGT GGACGTGAGC CACGAAGACC
        GGCCTGGGGA CTCCAGTGTA CGCACCACCA CCTGCACTCG GTGCTTCTGG

 551    CTGAGGTCAA GTTCAACTGG TACGTGGACG GCGTGGAGGT GCATAATGCC
        GACTCCAGTT CAAGTTGACC ATGCACCTGC CGCACCTCCA CGTATTACGG

 601    AAGACAAAGC CGCGGGAGGA GCAGTACAAC AGCACGTACC GTGTGGTCAG
        TTCTGTTTCG GCGCCCTCCT CGTCATGTTG TCGTGCATGG CACACCAGTC

 651    CGTCCTCACC GTCCTGCACC AGGACTGGCT GAATGGCAAG GAGTACAAGT
        GCAGGAGTGG CAGGACGTGG TCCTGACCGA CTTACCGTTC CTCATGTTCA
```

FIGURE 9A

```
 701    GCAAGGTCTC CAACAAAGCC CTCCCAGCCC CCATCGAGAA AACCATCTCC
        CGTTCCAGAG GTTGTTTCGG GAGGGTCGGG GGTAGCTCTT TTGGTAGAGG

 751    AAAGCCAAAG GGCAGCCCCG AGAACCACAG GTGTACACCC TGCCCCCATC
        TTTCGGTTTC CCGTCGGGGC TCTTGGTGTC CACATGTGGG ACGGGGGTAG

 801    CCGGGAGGAG ATGACCAAGA ACCAGGTCAG CCTGACCTGC CTGGTCAAAG
        GGCCCTCCTC TACTGGTTCT TGGTCCAGTC GGACTGGACG GACCAGTTTC

 851    GCTTCTATCC CAGCGACATC GCCGTGGAGT GGGAGAGCAA TGGGCAGCCG
        CGAAGATAGG GTCGCTGTAG CGGCACCTCA CCCTCTCGTT ACCCGTCGGC

 901    GAGAACAACT ACAAGACCAC GCCTCCCGTG CTGGACTCCG ACGGCTCCTT
        CTCTTGTTGA TGTTCTGGTG CGGAGGGCAC GACCTGAGGC TGCCGAGGAA

 951    CTTCCTCTAT AGCAAGCTCA CCGTGGACAA GAGCAGGTGG CAGCAGGGGA
        GAAGGAGATA TCGTTCGAGT GGCACCTGTT CTCGTCCACC GTCGTCCCCT

1001    ACGTCTTCTC ATGCTCCGTG ATGCATGAGG CTCTGCACAA CCACTACACG
        TGCAGAAGAG TACGAGGCAC TACGTACTCC GAGACGTGTT GGTGATGTGC

1051    CAGAAGAGCC TCTCCCTGTC CCCGGGTAAA TGA (SEQ ID NO: 55)
        GTCTTCTCGG AGAGGGACAG GGGCCCATTT ACT (SEQ ID NO: 56)
```

FIGURE 9B

GAAAC CCGCGAATGT ATTTATTACA ATGCTAATTG GGAACTCGAA CGCACGAACC

AATTCGGGCT CGAACGGTGT GAGGGGGAAC AGGATAAACG CCTCCATTGC TATGCGTCGT

GGAGCAACTC CTCCGGGACG ATTGAACTGG TCAAGAAAGG GTGCTGGGAC GACGATTTCA

ATTGTTATGA CCGGCAGGAA TGTGTCGCGA CCGAAGAGAA TCCGCAGGTC TATTTCTGTT

GTTGCGAGGG CAATTTCTGT AATGAACGGT TTACCCACCT CCCCGAAGCC GGCGGCCCGG

AGGTCACCTA TGAACCCCCG CCCACC (SEQ ID NO: 57)

FIGURE 10

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

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

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

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

FIGURE 11

```
  1  MDAMKRGLCC VLLLCGAVFV SPGAA▓TREC IYYNANWELE RTNQSGLERC
 51  EGEQDKRLHC YASWRNSSGT IELVKKGCWL DDFNCYDRQE CVATEENPQV
101  YFCCCEGNFC NERFTHLPEA GGPEVTYEPP PTGGGTHTCP PCPAPELLGG
151  PSVFLFPPKP KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA
201  KTKPREEQYN STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS
251  KAKGQPREPQ VYTLPPSREE MTKNQVSLTC LVKGFYPSDI AVEWESNGQP
301  ENNYKTTPPV LDSDGSFFLY SKLTVDKSRW QQGNVFSCSV MHEALHNHYT
351  QKSLSLSPGK (SEQ ID NO: 58)
```

FIGURE 12

```
                                                A  E  T   R  E  C   I  Y  Y
  1  ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
     TACCTACGTT ACTTCTCTCC CGAGACGACA CACGACGACG ACACACCTCG

                                                A  E  T   R  E  C   I  Y  Y
 51  AGTCTTCGTT TCGCCCGGCG CCGCTGAGAC ACGGGAGTGC ATCTACTACA
     TCAGAAGCAA AGCGGGCCGC GGCGACTCTG TGCCCTCACG TAGATGATGT

     N  A  N  W   E  L  E   R  T  N   Q  S  G  L   E  R  C
101  ACGCCAACTG GGAGCTGGAG CGCACCAACC AGAGCGGCCT GGAGCGCTGC
     TGCGGTTGAC CCTCGACCTC GCGTGGTTGG TCTCGCCGGA CCTCGCGACG

      E  G  E   Q  D  K  R   L  H  C   Y  A  S   W  R  N  S
151  GAAGGCGAGC AGGACAAGCG GCTGCACTGC TACGCCTCCT GGCGCAACAG
     CTTCCGCTCG TCCTGTTCGC CGACGTGACG ATGCGGAGGA CCGCGTTGTC

       S  G  T   I  E  L   V  K  K  G   C  W  L   D  D  F
201  CTCTGGCACC ATCGAGCTCG TGAAGAAGGG 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 TAGGCAGGAG TGTGTGGCCA CTGAGGAGAA CCCCCAGGTG
     TGACGATGCT ATCCGTCCTC ACACACCGGT GACTCCTCTT GGGGGTCCAC

      Y  F  C   C  C  E  G   W  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 GGCTGTCCAC

401  GTGGAACTCA CACATGCCCA CCGTGCCCAG CACCTGAACT CCTGGGGGGA
     CACCTTGAGT GTGTACGGGT GGCACGGGTC GTGGACTTGA GGACCCCCCT

451  CCGTCAGTCT TCCTCTTCCC CCCAAAACCC AAGGACACCC TCATGATCTC
     GGCAGTCAGA AGGAGAAGGG GGGTTTTGGG TTCCTGTGGG AGTACTAGAG

501  CCGGACCCCT GAGGTCACAT GCGTGGTGGT GGACGTGAGC CACGAAGACC
     GGCCTGGGGA CTCCAGTGTA CGCACCACCA CCTGCACTCG GTGCTTCTGG

551  CTGAGGTCAA GTTCAACTGG TACGTGGACG GCGTGGAGGT GCATAATGCC
     GACTCCAGTT CAAGTTGACC ATGCACCTGC CGCACCTCCA CGTATTACGG

601  AAGACAAAGC CGCGGGAGGA GCAGTACAAC AGCACGTACC GTGTGGTCAG
     TTCTGTTTCG GCGCCCTCCT CGTCATGTTG TCGTGCATGG CACACCAGTC

651  CGTCCTCACC GTCCTGCACC AGGACTGGCT GAATGGCAAG GAGTACAAGT
     GCAGGAGTGG CAGGACGTGG TCCTGACCGA CTTACCGTTC CTCATGTTCA

701  GCAAGGTCTC CAACAAAGCC CTCCCAGCCC CCATCGAGAA AACCATCTCC
     CGTTCCAGAG GTTGTTTCGG GAGGGTCGGG GGTAGCTCTT TTGGTAGAGG

751  AAAGCCAAAG GGCAGCCCCG AGAACCACAG GTGTACACCC TGCCCCCATC
     TTTCGGTTTC CCGTCGGGGC TCTTGGTGTC CACATGTGGG ACGGGGGTAG
```

FIGURE 13A

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 801   CCGGGAGGAG ATGACCAAGA ACCAGGTCAG CCTGACCTGC CTGGTCAAAG
       GGCCCTCCTC TACTGGTTCT TGGTCCAGTC GGACTGGACG GACCAGTTTC

 851   GCTTCTATCC CAGCGACATC GCCGTGGAGT GGGAGAGCAA TGGGCAGCCG
       CGAAGATAGG GTCGCTGTAG CGGCACCTCA CCCTCTCGTT ACCCGTCGGC

 901   GAGAACAACT ACAAGACCAC GCCTCCCGTG CTGGACTCCG ACGGCTCCTT
       CTCTTGTTGA TGTTCTGGTG CGGAGGGCAC GACCTGAGGC TGCCGAGGAA

 951   CTTCCTCTAT AGCAAGCTCA CCGTGGACAA GAGCAGGTGG CAGCAGGGGA
       GAAGGAGATA TCGTTCGAGT GGCACCTGTT CTCGTCCACC GTCGTCCCCT

1001   ACGTCTTCTC ATGCTCCGTG ATGCATGAGG CTCTGCACAA CCACTACACG
       TGCAGAAGAG TACGAGGCAC TACGTACTCC GAGACGTGTT GGTGATGTGC

1051   CAGAAGAGCC TCTCCCTGTC CCCGGGTAAA TGA (SEQ ID NO: 59)
       GTCTTCTCGG AGAGGGACAG GGGCCCATTT ACT (SEQ ID NO: 60)
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FIGURE 13B

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                                                A  E  T   R  E  C   I  Y  Y
1     ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
      TACCTACGTT ACTTCTCTCC CGAGACGACA CACGACGACG ACACACCTCG

                                                A  E  T   R  E  C   I  Y  Y
51    AGTCTTCGTT TCGCCCGGCG CCGCCGAAAC CCGCGATGC ATTTATTACA
      TCAGAAGCAA AGCGGGCCGC GGCGGCTTTG GGCGCTTACA TAAATAATGT

      N  A  N  W   E  L  E   R  T  N   Q  S  G  L   E  R  C
101   AAGCTAATTG GGACCTCGAA CGCACGAACC AATCCGGGCT CGAACGGTGC
      TACGATTAAC CCTTGAGCTT GCCTGCTTGG TTAGGCCCGA GCTTGCCACA

       E  G  E   Q  D  K  R   L  H  C   Y  A  S   W  R  N  S
151   GAAGGCGAAC AGGATAAACG CCTCCATTGC TATGCGTCGT GGAGCAACTC
      CTCCCCCTTG TCCTATTTGC GGAGGTAACG ATACGCAGCA CCTCCTTGAG

         S  G  T   I  E  L   V  K  K  G   C  W  L   D  D  F
201   CTCCGGCACG ATTGAACTGG TCAAGAAAGG GTGCTGGCTG GACGATTTCA
      GAGGCCCTGC TAACTTGACC AGTTCTTTCC CACGACCGAC CTGCTAAAGT

      N  C  Y  D   R  Q  E   C  V  A   T  E  E  N   P  Q  V
251   ATTGTTATGA CCGCCAGGAA TGTGTCGCGA CCGAAGAGAA TCCCCAGGTC
      TAACAATACT GGCGGTCCTT ACACAGCGCT GGCTTCTCTT AGGCGTCCAG

       Y  F  C   C  C  E  G   N  F  C   N  E  R   F  T  H  L
301   TATTTCTGTT GTTGCGAGGG CAATTTCTGC AATGAACGGT TTACCCACCT
      ATAAAGACAA CAACGCTCCC CTTAAAGACA TTACTTGCCA AATGGGTGGA

         P  E  A   G  G  P   E  V  T  Y   E  P  P   P  T
351   CCCCGAAGCC GGCGGCCCCG AGGTGACCTA TGAACCCCCC CCCACCGGTG
      GGGGCTTCGG CCGCCCGGGC TCCACTGGAT ACTTGGGGGC GGGTGGCCAC

401   GTGGAACTCA CACATGCCCA CCGTGCCCAG CACCTGAACT CCTGGGGGGA
      CACCTTGAGT GTGTACGGGT GGCACGGGTC GTGGACTTGA GGACCCCCCT

451   CCGTCAGTCT TCCTCTTCCC CCCAAAACCC AAGGACACCC TCATGATCTC
      GGCAGTCAGA AGGAGAAGGG GGGTTTTGGG TTCCTGTGGG AGTACTAGAG

501   CCGGACCCCT GAGGTCACAT GCGTGGTGGT GGACGTGAGC CACGAAGACC
      GGCCTGGGGA CTCCAGTGTA CGCACCACCA CCTGCACTCG GTGCTTCTGG

551   CTGAGGTCAA GTTCAACTGG TACGTGGACG GCGTGGAGGT GCATAATGCC
      GACTCCAGTT CAAGTTGACC ATGCACCTGC CGCACCTCCA CGTATTACGG

601   AAGACAAAGC CGCGGGAGGA GCAGTACAAC AGCACGTACC GTGTGGTCAG
      TTCTGTTTCG GCGCCCTCCT CGTCATGTTG TCGTGCATGG CACACCAGTC

651   CGTCCTCACC GTCCTGCACC AGGACTGGCT GAATGGCAAG GAGTACAAGT
      GCAGGAGTGG CAGGACGTGG TCCTGACCGA CTTACCGTTC CTCATGTTCA

701   GCAAGGTCTC CAACAAAGCC CTCCCAGCCC CCATCGAGAA AACCATCTCC
      CGTTCCAGAG GTTGTTTCGG GAGGGTCGGG GGTAGCTCTT TTGGTAGAGG

751   AAAGCCAAAG GGCAGCCCCG AGAACCACAG GTGTACACCC TGCCCCCATC
      TTTCGGTTTC CCGTCGGGGC TCTTGGTGTC CACATGTGGG ACGGGGGTAG
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FIGURE 14A

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 801    CCGGGAGGAG ATGACCAAGA ACCAGGTCAG CCTGACCTGC CTGGTCAAAG
        GGCCCTCCTC TACTGGTTCT TGGTCCAGTC GGACTGGACG GACCAGTTTC

 851    GCTTCTATCC CAGCGACATC GCCGTGGAGT GGGAGAGCAA TGGGCAGCCG
        CGAAGATAGG GTCGCTGTAG CGGCACCTCA CCCTCTCGTT ACCCGTCGGC

 901    GAGAACAACT ACAAGACCAC GCCTCCCGTG CTGGACTCCG ACGGCTCCTT
        CTCTTGTTGA TGTTCTGGTG CGGAGGGCAC GACCTGAGGC TGCCGAGGAA

 951    CTTCCTCTAT AGCAAGCTCA CCGTGGACAA GAGCAGGTGG CAGCAGGGGA
        GAAGGAGATA TCGTTCGAGT GGCACCTGTT CTCGTCCACC GTCGTCCCCT

1001    ACGTCTTCTC ATGCTCCGTG ATGCATGAGG CTCTGCACAA CCACTACACG
        TGCAGAAGAG TACGAGGCAC TACGTACTCC GAGACGTGTT GGTGATGTGC

1051    CAGAAGAGCC TCTCCCTGTC CCCGGGTAAA TGA (SEQ ID NO: 61)
        GTCTTCTCGG AGAGGGACAG GGGCCCATTT ACT (SEQ ID NO: 62)
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FIGURE 14B

METHODS AND COMPOSITIONS FOR TREATING MYELOFIBROSIS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority to U.S. provisional application Ser. No. 62/367,289, filed on Jul. 27, 2016. The disclosure of the foregoing application is hereby incorporated by reference in its entirety.

BACKGROUND OF THE INVENTION

[0002] Myelofibrosis is a rare disease mainly affecting people of older age. Myelofibrosis is a BCR-ABL1-negative myeloproliferative neoplasm that presents de novo (primary) or may be preceded by polycythemia vera (post-polycythemia vera) or essential thrombocythemia (post-essential thrombocythemia). Clinical features include progressive anemia, marked splenomegaly, fibrosis (e.g., bone marrow fibrosis), constitutional symptoms (e.g., fatigue, night sweats, bone pain, pruritus, and cough), and weight loss [Tefferi A (2000) N Engl J Med 342:1255-1265]. Median survival ranges from less than 2 years to over 15 years based on currently identified prognostic factors. Mutations involving JAK2, MPL, TET2, ASXL1, IDH1/IDH2, CBL, IKZF1, LNK, and EZH2 have been described in patients with myelofibrosis [James C et al. (2005) Nature 434:1144-1148, 2005; Scott L M et al. (2007) N Engl J Med 356:459-468, 2007; Pikman Y et al. (2006) PLoS Med 3:e270; Delhommeau F et al. (2009) N Engl J Med 360: 2289-2301; Carbuccia N et al. (2009) Leukemia 23:2183-2186; Green A et al. (2010) N Engl J Med 362:369-370; Tefferi A et al. (2010) Leukemia 24:1302-1309; Grand F H et al. (2009) Blood 113:6182-6192; Jager R et al. (2010) Leukemia 24:1290-1298; Oh S T et al. (2010) Blood 116: 988-992; and Ernst T et al., Nat Genet. 42:722-726]. Some mutations occur at high frequency in myelofibrosis (e.g. JAK2 mutations in about 50% patients), and either directly (e.g. JAK2 or MPL mutations) or indirectly (e.g. LNK or CBL mutations) induce JAK-STAT hyperactivation.

[0003] The only cure of myelofibrosis is bone marrow transplantation. However, treatment-related mortality is high, and only a minority of patients qualify for transplantation. Many of other currently available treatments are not effective in reversing the process of myelofibrosis, be it primary or secondary disease. Myelofibrosis treatments include, for example, cyto-reductive therapy (e.g., treatment with hydroxyurea); treatment of anemia with androgens and/or erythropoietin; and splenectomy. These therapies have not demonstrated improvement in survival and are largely seen as palliative [Cervantes F., Myelofibrosis: Biology and treatment options, European Journal of Haematology, 2007, 79 (suppl. 68) 13-17]. More recently, JAK inhibitors have been used to treat myelofibrosis. JAK inhibitors appear to be useful for reducing splenomegaly in myelofibrosis patients, but their effects on the disease are otherwise largely palliative [Gupta et al. (2012) *Blood* 120:1367-1379]. In particular, JAK inhibitors have little to no effect on many manifestations (complications) of the disease including, for example, cytopenia, transfusion dependence, accelerated or blast phase disease, and fibrosis. Moreover, JAK inhibitors have been shown to induce, or worsen, thrombocytopenia, anemia, and neutropenia in some patients.

[0004] Thus, there is a high, unmet need for effective therapies for treating myelofibrosis. Accordingly, it is an object of the present disclosure to provide methods for treating or preventing the myelofibrosis, particularly treating or preventing one or more complication of myelofibrosis.

SUMMARY OF THE INVENTION

[0005] In part, the present disclosure relates to the discovery that an ActRIIB antagonist (inhibitor) can be used to treat myelofibrosis, particularly ameliorating various complications of the disease including, for example, splenomegaly, extramedullary hematopoiesis, and fibrosis. In particular, the data presented herein show that a GDF trap polypeptide decrease splenomegaly, extramedullary hematopoiesis, and fibrosis in a JAK2V617F model of myelofibrosis. Accordingly, in certain aspects, the disclosure relates to compositions and methods for treating myelofibrosis, particularly treating or preventing one or more complications of myelofibrosis (e.g., splenomegaly, extramedullary hematopoiesis, anemia, and fibrosis), by administering to a patient in need thereof an effective amount of one or more ActRIIB antagonists, optionally in combination of one or more other supportive therapies or active agents for treating myelofibrosis. While GDF trap polypeptides may affect myelofibrosis through a mechanism other than ActRIIB antagonism [e.g., inhibition of one or more of GDF11, GDF8, activin B, BMP6, GDF3, and BMP10 may be an indicator of the tendency of an agent to inhibit the activities of a spectrum of additional agents, including, perhaps, other members of the TGF-beta superfamily, and such collective inhibition may lead to the desired effect on, for example, myelofibrosis], the disclosure nonetheless demonstrates that desirable therapeutic agents may be selected on the basis of ActRIIB antagonism. Therefore, while not wishing to be bound to a particular mechanism of action, it is expected that other ActRIIB antagonists [e.g., antagonists of the ActRIIB receptor, antagonists of one or more ActRIIB ligand (e.g., GDF11, GDF8, activin B, BMP6, GDF3, and BMP10), antagonists of one or more type I receptor (e.g., ALK4, ALK5, and/or ALK7), antagonists of one or more co-receptor, and/or antagonists of one or more ActRIIB downstream signaling components (e.g., Smads)], or combination of such antagonists] will useful in the treatment of myelofibrosis, particularly in treating or preventing one or more myelofibrosis complications (e.g., splenomegaly, extramedullary hematopoiesis, anemia, and fibrosis). Such agents are collectively referred to herein as "ActRIIB antagonists" or "ActRIIB inhibitors".

[0006] Accordingly, in certain aspects, the disclosure relates to methods for treating myelofibrosis, comprising administering to a patient in need thereof an effective amount of an ActRIIB antagonist. In some embodiments, the disclosure relates to methods for treating one or more complications of myelofibrosis, comprising administering to a patient in need thereof an effective amount of an ActRIIB antagonist. In certain aspects, the disclosure relates to methods of preventing myelofibrosis, comprising administering to a patient in need thereof an effective amount of an ActRIIB antagonist. In some embodiments, the disclosure relates to methods of preventing one or more complications of myelofibrosis, comprising administering to a patient in need thereof an effective amount of an ActRIIB antagonist. In certain aspects, the disclosure relates to reducing the progression rate of myelofibrosis, comprising administering to a patient in need thereof an effective amount of an ActRIIB antagonist. In some embodiments, the disclosure relates to reducing the progression rate of one or more complications of myelofibrosis, comprising administering to a patient in need thereof an effective amount of an ActRIIB antagonist. In certain aspects, the disclosure relates to meth-

ods of reducing severity of myelofibrosis, comprising administering to a patient in need thereof an effective amount of an ActRIIB antagonist. In some embodiments, the disclosure relates to methods of reducing severity of one or more complications of myelofibrosis, comprising administering to a patient in need thereof an effective amount of an ActRIIB antagonist. In certain aspects, the disclosure relates to methods of treating, preventing, or reducing the progression rate and/or severity of myelofibrosis or one or more complications of myelofibrosis, wherein the patient has primary myelofibrosis. In certain aspects, the disclosure relates to methods of treating, preventing, or reducing the progression rate and/or severity of myelofibrosis or one or more complications of myelofibrosis, wherein the patient has post-polycythemia vera myelofibrosis. In certain aspects, the disclosure relates to methods of treating, preventing, or reducing the progression rate and/or severity of myelofibrosis or one or more complications of myelofibrosis, wherein the patient has post-essential thrombocythemia myelofibrosis. In certain aspects, the disclosure relates to methods of treating, preventing, or reducing the progression rate and/or severity of myelofibrosis or one or more complications of myelofibrosis, wherein the patient has low risk myelofibrosis according to the International Prognostic Scoring System (IPSS). In certain aspects, the disclosure relates to methods of treating, preventing, or reducing the progression rate and/or severity of myelofibrosis or one or more complications of myelofibrosis, wherein the patient has intermediate-1 risk myelofibrosis according to the IPSS. In certain aspects, the disclosure relates to methods of treating, preventing, or reducing the progression rate and/or severity of myelofibrosis or one or more complications of myelofibrosis, wherein the patient has intermediate-2 risk myelofibrosis according to the IPSS. In certain aspects, the disclosure relates to methods of treating, preventing, or reducing the progression rate and/or severity of myelofibrosis or one or more complications of myelofibrosis, wherein the patient has high-risk myelofibrosis risk myelofibrosis according to the IPSS. In certain aspects, the disclosure relates to methods of treating, preventing, or reducing the progression rate and/or severity of myelofibrosis or one or more complications of myelofibrosis, wherein the patient has low risk myelofibrosis according to the dynamic IPSS (DIPSS). In certain aspects, the disclosure relates to methods of treating, preventing, or reducing the progression rate and/or severity of myelofibrosis or one or more complications of myelofibrosis, wherein the patient has intermediate-1 risk myelofibrosis according to the DIPSS. In certain aspects, the disclosure relates to methods of treating, preventing, or reducing the progression rate and/or severity of myelofibrosis or one or more complications of myelofibrosis, wherein the patient has intermediate-2 risk myelofibrosis according to the DIPSS. In certain aspects, the disclosure relates to methods of treating, preventing, or reducing the progression rate and/or severity of myelofibrosis or one or more complications of myelofibrosis, wherein the patient has high-risk myelofibrosis risk myelofibrosis according to the DIPSS. In certain aspects, the disclosure relates to methods of treating, preventing, or reducing the progression rate and/or severity of myelofibrosis or one or more complications of myelofibrosis, wherein the patient has low risk myelofibrosis according to the DIPSS-plus. In certain aspects, the disclosure relates to methods of treating, preventing, or reducing the progression rate and/or severity of myelofibrosis or one or more complications of myelofibrosis, wherein the patient has intermediate-1 risk myelofibrosis according to the DIPSS-plus. In certain aspects, the disclosure relates to methods of treating, preventing, or reducing the progression rate and/or severity of myelofibrosis or one or more complications of myelofibrosis, wherein the patient has intermediate-2 risk myelofibrosis according to the DIPSS-plus. In certain aspects, the disclosure relates to methods of treating, preventing, or reducing the progression rate and/or severity of myelofibrosis or one or more complications of myelofibrosis, wherein the patient has high-risk myelofibrosis risk myelofibrosis according to the DIPSS-plus. In certain aspects, an ActRIIB antagonists may be used to prevent or delay risk progression of myelofibrosis in accordance with any of the recognized risk stratification models for myelofibrosis (e.g., IPSS, DIPPS, and DIPPS-plus). For example, in some embodiments, an ActRIIB antagonist may be used to prevent or delay myelofibrosis risk progression from low risk to intermediate-1 risk in accordance with IPSS, DIPPS, or DIPPS-plus. In other embodiments, an ActRIIB antagonist may be used to prevent or delay myelofibrosis risk progression from intermediate-1 risk to intermediate-2 risk in accordance with IPSS, DIPPS, or DIPPS-plus. In still other embodiments, an ActRIIB antagonist may be used to prevent or delay myelofibrosis risk progression from intermediate-2 risk to high risk in accordance with IPSS, DIPPS, or DIPPS-plus. In certain aspects, an ActRIIB antagonists may be used to promote or increase myelofibrosis risk regression in accordance with any of the recognized risk stratification models for myelofibrosis (e.g., IPSS, DIPPS, and DIPPS-plus). For example, in some embodiments, an ActRIIB antagonist may be used to promote or increase myelofibrosis risk regression from high risk to intermediate-2 risk in accordance with IPSS, DIPPS, or DIPPS-plus. In other embodiments, an ActRIIB antagonist may be used to promote or increase myelofibrosis risk regression from intermediate-2 risk to intermediate-1 risk in accordance with IPSS, DIPPS, or DIPPS-plus. In still other embodiments, an ActRIIB antagonist may be used to promote or increase myelofibrosis risk regression from intermediate-1 risk to low risk in accordance with IPSS, DIPPS, or DIPPS-plus. In certain aspects, the disclosure relates to methods of using ActRIIB antagonists to treat, prevent, or reduce the progression rate and/or severity of myelofibrosis or one or more complications of myelofibrosis wherein the patient comprises one or more gene mutations associated with myelofibrosis. For example, in some embodiments, an ActRIIB antagonist may be used to treat, prevent, or reduce the progression rate and/or severity of myelofibrosis or one or more complications of myelofibrosis wherein the myelofibrosis is associated with one or more gene mutations selected from the group consisting of: nullizygosity for JAK2 46/1 haplotype, JAK2V617F, IDH1, IDH2, EZH2, SRSF2, ASXL1, JAK1, JAK2, JAK3, TYK2, MPL, CALR, CALR+ASXL1−, CALR−ASKL1+, CALR+ASKL1+, CALR−ASKL1−, TET2, THPO, and LNK. In some embodiments, an ActRIIB antagonist may be used to treat, prevent, or reduce the progression rate and/or severity of myelofibrosis or one or more complications of myelofibrosis wherein the myelofibrosis is associated with one or more gene mutations in a Janus kinase (JAK) (e.g., JAK1, JAK2, and/or JAK3). In some embodiments, an ActRIIB antagonist may be used to treat, prevent, or reduce the progression rate and/or severity of myelofibrosis or one or more complications of myelofibrosis wherein the myelofibrosis is associated with one or more gene mutations in JAK2. In some embodiments, an ActRIIB antagonist may be used to treat, prevent, or reduce the progression rate and/or severity of myelofibrosis or one or more complications of myelofibrosis wherein the myelofibrosis is associated with a

JAK2V617F mutation. In certain aspects, the disclosure relates to methods of using an ActRIIB antagonist to treat, prevent, or reduce the progression rate and/or severity of myelofibrosis or one or more complications of myelofibrosis wherein the myelofibrosis is associated with one or more elevated serum markers selected from the group consisting of: increased serum IL-8 levels, increased serum IL-2R levels, and increased serum free light chain levels. In certain aspects, the disclosure relates to methods of using an ActRIIB antagonist to treat, prevent, or reduce the progression rate and/or severity of myelofibrosis or one or more complications of myelofibrosis wherein the patient has been treated with a Janus kinase inhibitor (e.g., ruxolitinib, fedratinib (SAR302503), monoelotinib (CYT387), pacritinib, lestaurtinib, AZD-1480, BMS-911543, NS-018, LY2784544, SEP-701, XL019, and AT-9283). In some embodiments, an ActRIIB antagonist may be used to treat, prevent, or reduce the progression rate and/or severity of myelofibrosis or one or more complications of myelofibrosis wherein the patient is intolerant of a Janus kinase inhibitor. In some embodiments, an ActRIIB antagonist may be used to treat, prevent, or reduce the progression rate and/or severity of myelofibrosis or one or more complications of myelofibrosis wherein the patient has an inadequate response to a Janus kinase inhibitor. In certain aspects, the disclosure relates to methods of using an ActRIIB antagonist to treat, prevent, or reduce the progression rate and/or severity of myelofibrosis or one or more complications of myelofibrosis wherein the patient has been treated with hydroxyurea. In some embodiments, an ActRIIB antagonist may be used to treat, prevent, or reduce the progression rate and/or severity of myelofibrosis or one or more complications of myelofibrosis wherein the patient is intolerant of hydroxyurea. In some embodiments, an ActRIIB antagonist may be used to treat, prevent, or reduce the progression rate and/or severity of myelofibrosis or one or more complications of myelofibrosis wherein the patient has an inadequate response to hydroxyurea.

[0007] As described herein myelofibrosis is a clonal neoplastic disorder of hematopoiesis that is associated with various clinical complications that may manifest during disease progression in a patient. The examples of the disclosure demonstrate that an ActRIIB antagonist may be used to mitigate a number of these clinical complications, indicating that an ActRIIB antagonist may be used to more broadly treat various complications myelofibrosis as opposed to many of the current myelofibrosis therapies, which only treat one or a limited number of complications of the disease. Therefore, in some embodiments, an ActRIIB antagonist may be used to treat, prevent, or reduce the progression rate and/or severity of ineffective hematopoiesis in a patient with myelofibrosis. In certain aspects, an ActRIIB antagonist may be used to treat, prevent, or reduce the progression rate and/or severity of extramedullary hematopoiesis in a patient with myelofibrosis. For example, an ActRIIB antagonist may be used to treat, prevent, or reduce the progression rate and/or severity of extramedullary hematopoiesis in the spleen (splenic extramedullary hematopoiesis) in a patient with myelofibrosis. In other embodiments, an ActRIIB antagonist may be used to treat, prevent, or reduce the progression rate and/or severity of extramedullary hematopoiesis in the liver (hepatic extramedullary hematopoiesis) in a patient with myelofibrosis. In even other embodiments, an ActRIIB antagonist may be used to treat, prevent, or reduce the progression rate and/or severity of extramedullary hematopoiesis in the lung (pulmonary extramedullary hematopoiesis) in a patient with myelofibrosis. In still other embodiments, an ActRIIB antagonist may be used to treat, prevent, or reduce the progression rate and/or severity of extramedullary hematopoiesis in the lymph nodes (lymphatic extramedullary hematopoiesis) in a patient with myelofibrosis. In certain aspects, an ActRIIB antagonist may be used to treat, prevent, or reduce the progression rate and/or severity of inflammation and/or enlargement (size) of an organ or tissue in a myelofibrosis patient. In some embodiments, an ActRIIB antagonist may be used to treat, prevent, or reduce the progression rate and/or severity of inflammation and/or enlargement (size) in the spleen of a patient with myelofibrosis. In some embodiments, an ActRIIB antagonist may be used to treat, prevent, or reduce the progression rate and/or severity of inflammation and/or enlargement (size) in the liver of a patient with myelofibrosis. In some embodiments, an ActRIIB antagonist may be used to treat, prevent, or reduce the progression rate and/or severity of inflammation and/or enlargement (size) in the lung(s) of a patient with myelofibrosis. In some embodiments, an ActRIIB antagonist may be used to treat, prevent, or reduce the progression rate and/or severity of inflammation and/or enlargement (size) in the lymph node(s) of a patient with myelofibrosis. In some embodiments, an ActRIIB antagonist may be used to treat, prevent, or reduce the progression rate and/or severity of splenomegaly in a patient with myelofibrosis. In some embodiments, an ActRIIB antagonist may be used to treat, prevent, or reduce the progression rate and/or severity of hepatomegaly in a patient with myelofibrosis. In certain aspects, an ActRIIB antagonist may be used to treat, prevent, or reduce the progression rate and/or severity of fibrosis in a patient with myelofibrosis. In some embodiments, an ActRIIB antagonist may be used to treat, prevent, or reduce the progression rate and/or severity of bone marrow fibrosis in a patient with myelofibrosis. In some embodiments, an ActRIIB antagonist may be used to treat, prevent, or reduce the progression rate and/or severity of spleen fibrosis in a patient with myelofibrosis. In some embodiments, an ActRIIB antagonist may be used to treat, prevent, or reduce the progression rate and/or severity of liver fibrosis in a patient with myelofibrosis. In some embodiments, an ActRIIB antagonist may be used to treat, prevent, or reduce the progression rate and/or severity of lung fibrosis in a patient with myelofibrosis. In some embodiments, an ActRIIB antagonist may be used to treat, prevent, or reduce the progression rate and/or severity of lymph node fibrosis in a patient with myelofibrosis. In certain aspects, an ActRIIB antagonist may be used to treat, prevent, or reduce the progression rate and/or severity of osteosclerosis in a patient with myelofibrosis. In certain aspects, an ActRIIB antagonist may be used to treat, prevent, or reduce the progression rate and/or severity of osteomyelofibrosis. In certain aspects, an ActRIIB antagonist may be used to treat, prevent, or reduce the progression rate and/or severity of one or more blood-related complications of myelofibrosis. In some embodiments, an ActRIIB antagonist may be used to treat, prevent, or reduce the progression rate and/or severity of anemia in a patient with myelofibrosis. In some embodiments, an ActRIIB antagonist may be used to treat, prevent, or reduce the progression rate and/or severity of thrombocytopenia in a patient with myelofibrosis. In some embodiments, an ActRIIB antagonist may be used to treat, prevent, or reduce the progression rate and/or severity of pancytopenia in a patient with myelofibrosis. In some embodiments, an ActRIIB antagonist may be used to treat, prevent, or reduce the progression rate and/or severity of poikilocytosis in a patient with myelofibrosis. In some embodiments, an

ActRIIB antagonist may be used to treat, prevent, or reduce the progression rate and/or severity of bleeding in a patient with myelofibrosis. In certain aspects, an ActRIIB antagonist may be used to treat, prevent, or reduce the progression rate and/or severity of one or more constitutional symptoms of myelofibrosis (e.g., fatigue, pruritus, weight loss, night sweats, fever, abdominal pain or discomfort, paresthesia, and early satiety). In certain aspects, an ActRIIB antagonist may be used to treat, prevent, or reduce the progression rate and/or severity of pain in a tissue and/or organ in a patient with myelofibrosis. In some embodiments, an ActRIIB antagonist may be used to treat, prevent, or reduce the progression rate and/or severity of bone pain in a patient with myelofibrosis. In some embodiments, an ActRIIB antagonist may be used to treat, prevent, or reduce the progression rate and/or severity of arthralgia in a patient with myelofibrosis. In some embodiments, an ActRIIB antagonist may be used to treat, prevent, or reduce the progression rate and/or severity of myalgia in a patient with myelofibrosis. In certain aspects, an ActRIIB antagonist may be used to treat, prevent, or reduce the progression rate and/or severity of cachexia in a patient with myelofibrosis. In certain aspects, disclosure relates to increasing red blood cell levels in a myelofibrosis patient by administering an effective amount of an ActRIIB antagonist. In certain aspects, disclosure relates to increasing hemoglobin levels in a myelofibrosis patient by administering an effective amount of an ActRIIB antagonist. In certain aspects, a myelofibrosis patient to be treated in accordance with the methods described herein has anemia. In some embodiments, ActRIIB antagonist may be used to treat, prevent, or reduce the progression rate and/or severity of anemia in a patient with myelofibrosis. In certain aspects, the disclosure relate to methods using an ActRIIB antagonists to treat, prevent, or reduce the progression rate and/or severity of myelofibrosis or a complication of myelofibrosis in patient that has been administered one or more blood cell transfusions (whole or red blood cell transfusions). In some embodiments, the disclosure relate to methods using an ActRIIB antagonists to treat, prevent, or reduce the progression rate and/or severity of myelofibrosis or a complication of myelofibrosis in patient that is blood cell transfusion-dependent. In certain aspects, an ActRIIB antagonist may be used to decrease blood cell transfusion burden in a patient with myelofibrosis. For example, an ActRIIB antagonist may be used to decrease blood cell transfusion by greater than about 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% for 4 to 8 weeks relative to the equal time prior to the start of the ActRIIB antagonist treatment. In some embodiments, an ActRIIB antagonist may be used to decrease blood cell transfusion by greater than about 50% for 4 to 8 weeks relative to the equal time prior to the start of the ActRIIB antagonist treatment in a patient with myelofibrosis. In certain aspects, an ActRIIB antagonist may be used to decrease iron overload in a patient with myelofibrosis. For example, an ActRIIB antagonist may be used to decrease iron overload in an organ or tissue in a patient with myelofibrosis. In some embodiments, an ActRIIB antagonist may be used to decrease iron overload in the spleen of a patient with myelofibrosis. In some embodiments, an ActRIIB antagonist may be used to decrease iron overload in the liver of a patient with myelofibrosis. In some embodiments, an ActRIIB antagonist may be used to decrease iron overload in the heart of a patient with myelofibrosis.

[0008] In any of the methods described herein, a myelofibrosis patient may further be administered one or more additional active agents and/or supportive therapies (in addition to administration of one or more ActRIIB antagonists) for treating, preventing, or reducing, the progression rate and/or severity of myelofibrosis or one or more complications of myelofibrosis. For example, in some embodiments, a patient may be further administered one or more supportive therapies or active agents is selected from the group consisting of: blood transfusion (whole blood or red blood cell transfusion), iron chelators (e.g., deferoxamine, deferiprone and deferasirox), corticosteroids, prednisone, ESAs (e.g., erythropoietin, epoetin alfa, epoetin beta, darbepoetin alfa, and methoxy polyethylene glycol-epoetin beta), androgens, danazol, thalidomide, lenalidomide, a cytoreductive agent, hydroxyurea, busulfan, melphalm, cladribine, splenectomy, radiotherapy, aspirin, pomalidonmide, Janus kinase inhibitors, mTOR inhibitors (e.g., rapamycin, sirolimus, deforolimus, everolimus, temsirolimus, NVP-BEZ235, BGT226, SF1126, PK1-587, INK128, AZD8055, and AZD2014), and histone deacetylase inhibitors (e.g., givinostat, panobinostat, and pracinostat). In certain aspects, the disclosure relates to methods for treating, preventing, or reducing the progression rate and/or severity of myelofibrosis or one or more complications of myelofibrosis, comprising administering to a patient in need thereof: a) a Janus kinase inhibitor; and b) an ActRIIB antagonists, wherein the Janus kinase inhibitor and ActRIIB antagonist are administered in an effective amount. In some embodiments, an ActRIIB antagonist is administered prior to treatment with the Janus kinase inhibitor. In other embodiments, an ActRIIB antagonist is administered after treatment with the Janus kinase inhibitor. In even other embodiments, an ActRIIB antagonist is administered concurrently with the Janus kinase inhibitor. Janus kinase inhibitors to be used in accordance with the methods described herein may be an agent that inhibits one or more Janus kinases selected from the group consisting of: JAK1, JAK2, and JAK3. For example, a Janus kinase inhibitor may be an agent that inhibits signaling of one or more of JAK1, JAK2, and JAK3 in a cell-based assay. In some embodiments, a Janus kinase inhibitor to be used in accordance with the methods described herein is selected from the group consisting of: ruxolitinib, fedratinib (SAR302503), monoelotinib (CYT387), pacritinib, lestaurtinib, AZD-1480, BMS-911543, NS-018, LY2784544, SEP-701, XL019, and AT-9283. In some preferred embodiments, a Janus kinase inhibitor to be used in accordance with the methods described herein is ruxolitinib.

[0009] Janus kinase inhibitors (e.g., ruxolitinib) have been approved for treatment of a variety of disorders including, for example, myelofibrosis. In addition, there are a number of other clinical investigations ongoing to determine the efficacy of Janus kinase inhibitors to treat various other diseases. A common adverse side-effect of Janus kinase inhibitor therapy is anemia. While blood cell transfusion and EPO receptor activator therapy may be used treated anemia in patients treated with a Janus kinase inhibitor, these anemia therapies also are associated with adverse effects in patients (e.g., promoting or increasing iron overload, inadequate response to EPO, and EPO intolerance). Therefore, there is a need in the art for alternative methods of increasing red blood cell/hemoglobin levels and treating anemia in patients treated with a Janus kinase inhibitor. In part, the present disclosure relates to the discovery that an ActRIIB antagonist (inhibitor) can be used to increase red blood cell and hemoglobin levels in patients treated with a Janus kinase inhibitor. Accordingly, in certain aspects, the disclosure relates to compositions and methods for increasing red blood cell/hemoglobin levels and treating or preventing anemia in

a patient treated with a Janus kinase inhibitor by administering to a patient in need thereof an effective amount of one or more ActRIIB antagonists, optionally in combination of one or more other supportive therapies or active agents for treating anemia. While GDF trap polypeptides may affect red blood cell and/or hemoglobin levels through a mechanism other than ActRIIB antagonism [e.g., inhibition of one or more of GDF11, GDF8, activin B, BMP6, GDF3, and BMP10 may be an indicator of the tendency of an agent to inhibit the activities of a spectrum of additional agents, including, perhaps, other members of the TGF-beta superfamily, and such collective inhibition may lead to the desired effect on, for example, red blood cell levels and/or hemoglobin levels in patients treated with a Janus kinase inhibitor], the disclosure nonetheless demonstrates that desirable therapeutic agents may be selected on the basis of ActRIIB antagonism. Therefore, while not wishing to be bound to a particular mechanism of action, it is expected that other ActRIIB antagonists [e.g., antagonists of the ActRIIB receptor, antagonists of one or more ActRIIB ligand (e.g., GDF11, GDF8, activin B, BMP6, GDF3, and BMP10), antagonists of one or more type I receptor (e.g., ALK4, ALK5, and/or ALK7), antagonists of one or more co-receptor, and/or antagonists of one or more ActRIIB downstream signaling components (e.g., Smads)], or combination of such antagonists] will useful in the treatment of patients treated with a Janus kinase, particularly in treating or preventing one or more complications associated with Janus kinase therapy (e.g., anemia, thrombocytopenia, and/or neutropenia). Such agents are collectively referred to herein as "ActRIIB antagonists" or "ActRIIB inhibitors".

[0010] In certain aspects, the disclosure relate to methods for increasing red blood cell levels and/or hemoglobin levels in a patient treated with a Janus kinase inhibitor by administering to a patient in need thereof an effective amount of an ActRIIB antagonist. In some embodiments, ActRIIB antagonists may be used to treat or prevent anemia in a patient treated with a Janus kinase inhibitor. In some embodiments, a patient treated with a Janus kinase inhibitor may have been administered one or more blood cell transfusions prior to the start of ActRIIB antagonist treatment. In some embodiments, a patient treated with a Janus kinase inhibitor is blood cell transfusion-dependent. In certain aspects, the disclosure relate to methods of using an ActRIIB antagonist to decrease blood cell transfusion burden in a patient treated with a Janus kinase inhibitor. For example, an ActRIIB antagonist may be used to decrease blood cell transfusion by greater than about 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% for 4 to 8 weeks relative to the equal time prior to the start of the ActRIIB antagonists treatment in a patient treated with a Janus kinase inhibitor. In some embodiments, an ActRIIB antagonist may be used to decrease blood cell transfusion by greater than about 50% for 4 to 8 weeks relative to the equal time prior to the start of the ActRIIB antagonists treatment in a patient treated with a Janus kinase inhibitor. In certain aspects, the disclosure relates to methods of using an ActRIIB antagonist to decrease iron overload in a patient treated with a Janus kinase inhibitor. In some embodiment, ActRIIB antagonists may be used to decrease iron content in the liver of a patient treated with a Janus kinase inhibitor. In some embodiment, ActRIIB antagonists may be used to decrease iron content in the spleen of a patient treated with a Janus kinase inhibitor. In some embodiment, ActRIIB antagonists may be used to decrease iron content in the heart of a patient treated with a Janus kinase inhibitor. In some embodiments, the ActRIIB antagonist is administered prior to treatment with the Janus kinase inhibitor. In other embodiments, the ActRIIB antagonist is administered after treatment with the Janus kinase inhibitor. In still other embodiments, the ActRIIB antagonist is administered concurrently with the Janus kinase inhibitor. In certain aspects, a patient treated with a Janus kinase inhibitor has been treated with an agent that inhibitor inhibits one or more of Janus kinases selected from the group consisting of: JAK1, JAK2, and JAK3. In some embodiments, the Janus kinase inhibitor inhibits signaling of one or more of JAK1, JAK2, and JAK3 in a cell-based assay. For example, a patient may be treated with one or more Janus kinase inhibitors selected from the group consisting of: ruxolitinib, fedratinib (SAR302503), monoelotinib (CYT387), pacritinib, lestaurtinib, AZD-1480, BMS-911543, NS-018, LY2784544, SEP-701, XL019, and AT-9283. In some embodiments, a patient may be treated with ruxolitinib.

[0011] In certain aspects, an ActRIIB 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, an ActRIIB 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, an ActRIIB 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 ActRIIB antagonists that inhibit GDF11 can be used in accordance with the methods and uses described herein including, for example, ligand traps (e.g., ActRIIB polypeptides, GDF Traps, follistatin polypeptides, and FLRG polypeptides), antibodies, small molecules, nucleotide sequences, and combinations thereof. In certain embodiments, an ActRIIB 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, BMP10, ActRIIB, ALK4, ALK5, and ALK7.

[0012] In certain aspects, an ActRIIB 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, an ActRIIB 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, an ActRIIB 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 ActRIIB antagonists that inhibit GDF8 can be used in accordance with the methods and uses described herein including, for example, ligand traps (e.g., ActRIIB polypeptides, GDF Traps, follistatin polypeptides, and FLRG polypeptides), antibodies, small molecules, nucleotide sequences, and combinations thereof. In certain embodiments, an ActRIIB 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, BMP10, ActRIIB, ALK4, ALK5, and ALK7.

[0013] In certain aspects, an ActRIIB 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, an ActRIIB 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, an ActRIIB 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 ActRIIB antagonists that inhibit GDF3 can be used in accordance with the methods and uses described herein including, for example, ligand traps (e.g., ActRIIB polypeptides, GDF Traps, follistatin polypeptides, and FLRG polypeptides), antibodies, small molecules, nucleotide sequences, and combinations thereof. In certain embodiments, an ActRIIB 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, BMP10, ActRIIB, ALK4, ALK5, and ALK7.

[0014] In certain aspects, an ActRIIB 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, an ActRIIB 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, an ActRIIB 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 ActRIIB antagonists that inhibit BMP6 can be used in accordance with the methods and uses described herein including, for example, ligand traps (e.g., ActRIIB polypeptides, GDF Traps, follistatin polypeptides, and FLRG polypeptides), antibodies, small molecules, nucleotide sequences, and combinations thereof. In certain embodiments, an ActRIIB 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, BMP10, ActRIIB, ALK4, ALK5, and ALK7.

[0015] In certain aspects, an ActRIIB 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 signaling reporter assay). Therefore, in some embodiments, an ActRIIB 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, an ActRIIB 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 ActRIIB antagonists that inhibit BMP10 can be used in accordance with the methods and uses described herein including, for example, ligand traps (e.g., ActRIIB polypeptides, GDF Traps, follistatin polypeptides, and FLRG polypeptides), antibodies, small molecules, nucleotide sequences, and combinations thereof. In certain embodiments, an ActRIIB 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, ActRIIB, ALK4, ALK5, and ALK7.

[0016] In certain aspects, an ActRIIB 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, an ActRIIB 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, an ActRIIB 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 ActRIIB antagonists that inhibit activin can be used in accordance with the methods and uses described herein including, for example, ligand traps (e.g., ActRIIB polypeptides, GDF Traps, follistatin polypeptides, and FLRG polypeptides), antibodies, small molecules, nucleotide sequences, and combinations thereof. In certain embodiments, an ActRIIB antagonist, or combination of antagonists, that inhibits activin may further inhibit one or more of: GDF8, GDF3, GDF11, BMP6, BMP10, ActRIIB, ALK4, ALK5, and ALK7. In certain preferred embodiments, an ActRIIB 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, an ActRIIB 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, an ActRIIB 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, an ActRIIB antagonist, or combination of antagonists, to be used in accordance with methods and uses described herein is an agent that inhibits at least ActRIIB (e.g., an ActRIIB antagonist). Effects on ActRIIB 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, an ActRIIB antagonist, or combination of antagonists, of the disclosure may bind to at least ActRIIB Ligand binding activity may be determined, for example, using a binding affinity assay including those described herein. In some embodiments, an ActRIIB antagonist, or combination of antagonists, of the disclosure binds to at least ActRIIB 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 ActRIIB antagonists that inhibit ActRIIB can be used in accordance with the methods and uses described herein including, for example, ligand traps (e.g., ActRIIB polypeptides, GDF Traps, follistatin polypeptides, and FLRG polypeptides), antibodies, small molecules, nucleotide sequences, and combinations thereof. In certain embodiments, an ActRIIB antagonist, or combination of antagonists, that inhibits ActRIIB 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, ALK4, ALK5, and ALK7.

[0018] In certain aspects, an ActRIIB 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, an ActRIIB 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, an ActRIIB 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 ActRIIB antagonists that inhibit ALK4 can be used in accordance with the methods and uses described herein including, for example, ligand traps (e.g., ActRIIB polypeptides, GDF Traps, follistatin polypeptides, and FLRG polypeptides), antibodies, small molecules, nucleotide sequences, and combinations thereof. In certain embodiments, an ActRIIB 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, BMP10, ActRIIB, ALK5, and ALK7.

[0019] In certain aspects, an ActRIIB 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, an ActRIIB antagonists, 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, an ActRIIB 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 ActRIIB antagonists that inhibit ALK5 can be used in accordance with the methods and uses described herein including, for example, ligand traps (e.g., ActRIIB polypeptides, GDF Traps, follistatin polypeptides, and FLRG polypeptides), antibodies, small molecules, nucleotide sequences, and combinations thereof. In certain embodiments, an ActRIIB 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, BMP10, ActRIIB, ALK4, and ALK7.

[0020] In certain aspects, an ActRIIB 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, an ActRIIB 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, an ActRIIB 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 ActRIIB antagonists that inhibit ALK7 can be used in accordance with the methods and uses described herein including, for example, ligand traps (e.g., ActRIIB polypeptides, GDF Traps, follistatin polypeptides, and FLRG polypeptides), antibodies, small molecules, nucleotide sequences, and combinations thereof. In certain embodiments, an ActRIIB 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, BMP10, ActRIIB, ALK5, and ALK4.

[0021] In part, the disclosure relates to ActRIIB antagonists that are ActRIIB polypeptides. The term "ActRIIB polypeptide" collectively refers to naturally occurring ActRIIB polypeptides as well as truncations and variants thereof such as those described herein (e.g., GDF trap polypeptides). Preferably ActRIIB polypeptides comprise, consist essentially of, or consist of a ligand-binding domain of an ActRIIB polypeptide or modified (variant) form thereof. For example, in some embodiments, an ActRIIB polypeptide comprises, consists essentially of, or consists of an ActRIIB ligand-binding domain of an ActRIIB polypeptide, for example, a portion of the ActRIIB extracellular domain. Preferably, ActRIIB polypeptides to be used in accordance with the methods described herein are soluble polypeptides.

[0022] In certain 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 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%, 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, wherein the ActRIIB polypeptide comprises an acidic amino acid at position 79 with respect to SEQ ID NO: 1. In still 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: 3. In other, 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: 3, 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: 4. 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: 4, wherein the ActRIIB polypeptide comprises an acidic amino acid at position 79 with respect to SEQ ID NO: 4. 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: 5. 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: 5, wherein the ActRIIB polypeptide comprises an acidic amino acid at position 79 with respect to SEQ ID NO: 4. 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: 6. 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: 6, wherein the ActRIIB polypeptide comprises an acidic amino acid at position 79 with respect to SEQ ID NO: 4. 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: 24. 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: 25. 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: 28. 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: 29. 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: 29, 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: 30. 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: 30, 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: 31. 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: 31, 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: 45. 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: 45, 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: 50. 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: 50, 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: 53. 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: 53, 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: 54. 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: 54, 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: 58. 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.

[0023] As described herein, ActRIIB polypeptides and variants thereof (GDF traps) may be homomultimers, for example, homodimer, homotrimers, homotetramers, homopentamers, and higher order homomultimer complexes. In certain preferred embodiments, ActRIIB polypeptides and variants thereof are homodimers. In certain embodiments, ActRII polypeptide dimers described herein comprise an first ActRIIB polypeptide covalently, or non-covalently, associated with an second ActRIIB polypeptide wherein the first polypeptide comprises an ActRIIB 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 ActRIIB polypeptide and an amino acid sequence of a second member (or first member) of the interaction pair.

[0024] In certain aspects, ActRIIB polypeptides, including variants thereof (e.g., GDF traps), may be fusion proteins. For example, in some embodiments, an ActRIIB polypeptide may be a fusion protein comprising an ActRIIB polypeptide domain and one or more heterologous (non-ActRIIB) polypeptide domains. In some embodiments, an ActRIIB polypeptide may be a fusion protein that has, as one domain, an amino acid sequence derived from an ActRIIB polypeptide (e.g., a ligand-binding domain of an ActRIIB 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 distribution, formation of protein complexes, multimerization of the fusion protein, and/or purification. Optionally, an ActRIIB 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 ActRIIB polypeptide and the amino acid sequence of the one or more heterologous domains. In certain embodiments, an ActRIIB fusion protein comprises a relatively unstructured linker positioned between the heterologous domain and the ActRIIB domain. This unstructured linker may correspond to the roughly 15 amino acid unstructured region at the C-terminal end of the extracellular domain of ActRIIB (the "tail"), 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 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: 18), SGGG (SEQ ID NO: 19), TGGGG (SEQ ID NO: 16), SGGGG (SEQ ID NO: 17), GGGGS (SEQ ID NO: 20), GGGG (SEQ ID NO: 15), and GGG (SEQ ID NO: 14). In some embodiments, ActRIIB 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, am 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: 9-13. 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 ActRIIB 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 ActRIIB polypeptide 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 ActRIIB fusion protein comprises a leader sequence. The leader sequence may be a native ActRIIB leader sequence or a heterologous leader sequence. In certain embodiments, the leader sequence is a tissue plasminogen activator (TPA) leader sequence.

[0025] An ActRIIB polypeptide, including variants thereof (e.g., GDF traps), may comprise a purification subsequence, such as an epitope tag, a FLAG tag, a polyhistidine sequence, and a GST fusion. Optionally, an ActRIIB 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. ActRIIB polypeptides may comprise at least one N-linked sugar, and may include two, three or more N-linked sugars. Such polypeptides may also comprise O-linked sugars. In general, it is preferable that ActRIIB 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. ActRIIB polypeptides may be produced in a variety of cell lines that glycosylate the protein in a manner that is suitable for patient use, including engineered insect or yeast cells, and mammalian cells such as COS cells, CHO cells, HEK cells and NSO cells. In some embodiments, an ActRIIB polypeptide is glycosylated and has a glycosylation pattern obtainable from a Chinese hamster ovary cell line. In some embodiments, ActRIIB 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, ActRIIB 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).

[0026] In certain aspects, the disclosure provides pharmaceutical preparations comprising one or more ActRIIB 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 myelofibrosis, particularly treating or preventing one or more complications of myelofibrosis (e.g., splenomegaly, extramedullary hematopoiesis, anemia, and fibrosis), and/or a patient treated with a Janus kinase inhibitor. 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).

[0027] In certain instances, when administering an ActRIIB 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 ActRIIB antagonist, or to determine or adjust the dosing of the ActRIIB 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.

[0028] In certain aspects, the ActRIIB antagonist is an antibody, or combination of antibodies. In some embodiments, the antibody binds to at least ActRIIB In certain embodiments an antibody that binds to ActRIIB inhibits ActRIIB signaling, optionally as measured in a cell-based assay such as those described herein. In certain embodiments, an antibody that binds to ActRIIB inhibits one or more TGF-beta superfamily ligands, TGF-beta superfamily type I receptors, or TGF-beta superfamily co-receptors from binding to ActRIIB In certain embodiments an antibody that binds to ActRIIB inhibits one or more TGF-beta superfamily ligands from binding to ActRIIB 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, GDF3, BMP6, BMP10, BMP9, and BMP5. In some embodiments, an antibody binds to at least GDF11. In certain embodiments, an antibody that binds to GDF11 inhibits ActRIIB 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-ActRIIB binding. In some embodiments, an antibody binds to at least GDF8. In certain embodiments, an antibody that binds to GDF8 inhibits ActRIIB 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-ActRIIB binding. In some embodiments, an antibody binds to at least BMP6. In certain embodiments, an antibody that binds to BMP6 inhibits ActRIIB 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-ActRIIB binding. In some embodiments, an antibody binds to BMP10. In certain embodiments, an antibody that binds to at least BMP10 inhibits ActRIIB 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-ActRIIB binding. In some embodiments, the antibody binds to at least GDF3. In certain embodiments, an antibody that binds to GDF3 inhibits ActRIIB 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-ActRIIB binding. In some embodiments, the antibody binds to 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 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 ActRIIB 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-ActRIIB binding. In some embodiments, the antibody binds to activin B. In certain embodiments, an antibody that binds to activin B inhibits ActRIIB 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-ActRIIB binding. In some embodiments, the antibody is a multispecific antibody, or combination of multispecific antibodies that binds to one or more of ActRIIB, GDF11, GDF8, activin A, activin B, BMP6, and BMP10. In some embodiments, an 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 ActRIIB ligands, type II receptors, or co-receptors from binding to ALK4. In certain embodiments an antibody that binds to ALK4 inhibits one or more ActRIIB 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, 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 ActRIIB ligands, type II receptors, or co-receptors from binding to ALK5. In certain embodiments an antibody that binds to ALK5 inhibits one or more ActRIIB 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, 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 ActRIIB ligands, type II receptors, or co-receptors from binding to ALK7. In certain embodiments an antibody that binds to ALK7 inhibits one or more ActRIIB 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, BMP10, and GDF3. In some embodiments, the antibody binds to at least GDF11. 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 A, activin B, GDF3, BMP6, and BMP10. 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 or a fusion protein comprising a single-chain diabody and at least a portion of an immunoglobulin heavy-chain constant region.

[0029] In certain aspects, the ActRIIB 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 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 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.

[0030] In certain aspects, the ActRIIB 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 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 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.

[0031] In certain aspects, the ActRIIB 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: 63. 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: 64. 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: 65. 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: 66. 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: 67.

[0032] In certain aspects, the ActRIIB 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: 68.

BRIEF DESCRIPTION OF THE DRAWINGS

[0033] The patent or application file contains at least one drawing executed in color.

[0034] FIG. **1** shows an alignment of extracellular domains of human ActRIIA (SEQ ID NO: 36) and human ActRIIB (SEQ ID NO: 2) with the residues that are deduced herein, based on composite analysis of multiple ActRIIB and ActRIIA crystal structures, to directly contact ligand indicated with boxes.

[0035] FIG. **2** shows a multiple sequence alignment of various vertebrate ActRIIB proteins and human ActRIIA (SEQ ID NOs: 37-43) as well as a consensus ActRII sequence derived from the alignment (SEQ ID NO: 44).

[0036] FIG. **3** shows the full amino acid sequence for the GDF trap ActRIIB(L79D 20-134)-hFc (SEQ ID NO: 45), 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.

[0037] FIGS. **4**A and **4**B show a nucleotide sequence encoding ActRIIB(L79D 20-134)-hFc. SEQ ID NO: 48 corresponds to the sense strand, and SEQ ID NO: 49 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.

[0038] FIG. **5** shows the full amino acid sequence for the truncated GDF trap ActRIIB(L79D 25-131)-hFc (SEQ ID NO: 50), 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.

[0039] FIGS. **6**A and **6**B show a nucleotide sequence encoding ActRIIB(L79D 25-131)-hFc. SEQ ID NO: 51 corresponds to the sense strand, and SEQ ID NO: 52 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 (residues 25-131 in SEQ ID NO: 1) is also shown.

[0040] FIG. **7** shows the amino acid sequence for the truncated GDF trap ActRIIB(L79D 25-131)-hFc without a leader (SEQ ID NO: 53). 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.

[0041] FIG. **8** shows the amino acid sequence for the truncated GDF trap ActRIIB(L79D 25-131) without the leader, hFc domain, and linker (SEQ ID NO: 54). 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.

[0042] FIGS. **9**A and **9**B shows an alternative nucleotide sequence encoding ActRIIB(L79D 25-131)-hFc. SEQ ID NO: 55 corresponds to the sense strand, and SEQ ID NO: 56 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: 51, FIGS. **6**A and **6**B). The amino acid sequence for the ActRIIB extracellular domain (residues 25-131 in SEQ ID NO: 1) is also shown.

[0043] FIG. **10** shows nucleotides 76-396 (SEQ ID NO: 57) of the alternative nucleotide sequence shown in FIGS. **9**A and **9**B (SEQ ID NO: 55). The same nucleotide substitutions indicated in FIGS. **9**A and **9**B are also underlined and highlighted here. SEQ ID NO: 57 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).

[0044] FIG. **11** shows multiple sequence alignment of Fc domains from human IgG isotypes using Clustal 2.1. Hinge regions are indicated by dotted underline.

[0045] FIG. **12** shows the full, unprocessed amino acid sequence for ActRIIB(25-131)-hFc (SEQ ID NO: 58). 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.

[0046] FIGS. **13**A and **13**B show a nucleotide sequence encoding ActRIIB(25-131)-hFc (the coding strand is shown at top, SEQ ID NO: 59, and the complement shown at bottom 3'-5', SEQ ID NO: 60). 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) is also shown.

[0047] FIGS. **14**A and **14**B show an alternative nucleotide sequence encoding ActRIIB(25-131)-hFc (the coding strand is shown at top, SEQ ID NO: 61, and the complement shown at bottom 3'-5', SEQ ID NO: 62). 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 FIGS. **13**A and **13**B) are highlighted. The corresponding amino acid sequence for ActRIIB(25-131) is also shown.

DETAILED DESCRIPTION OF THE INVENTION

1. Overview

[0048] The transforming growth factor-beta (TGF-beta) superfamily contains a variety of growth factors that share common sequence elements and structural motifs. These proteins are known to exert biological effects on a large variety of cell types in both vertebrates and invertebrates. Members of the superfamily perform important functions during embryonic development in pattern formation and tissue specification and can influence a variety of differentiation processes, including adipogenesis, myogenesis, chondrogenesis, cardiogenesis, hematopoiesis, neurogenesis, and epithelial cell differentiation. By manipulating the activity of a member of the TGF-beta family, it is often possible to cause significant physiological changes in an organism. For example, the Piedmontese and Belgian Blue cattle breeds carry a loss-of-function mutation in the GDF8 (also called myostatin) gene that causes a marked increase in muscle mass [see, e.g., Grobet et al. (1997) Nat Genet. 17(1):71-4]. Furthermore, in humans, inactive alleles of GDF8 are associated with increased muscle mass and, reportedly, exceptional strength [see, e.g., Schuelke et al. (2004) N Engl J Med, 350:2682-8].

[0049] TGF-β signals are 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 [see, e.g., Massagué (2000) Nat. Rev. Mol. Cell Biol. 1:169-178]. These type I and type II receptors are transmembrane proteins, composed of a ligand-binding extracellular domain with cysteine-rich region, a transmembrane domain, and a cytoplasmic domain with predicted serine/threonine specificity. Type I receptors are essential for signaling. Type II receptors are required for binding ligands and for activation of type I receptors. Type I and II activin receptors form a stable complex after ligand binding, resulting in phosphorylation of type I receptors by type II receptors.

[0050] Two related type II receptors (ActRII), ActRIIA and ActRIIB, have been identified as the type II receptors for activins [see, e.g., Mathews and Vale (1991) Cell 65:973-982; and Attisano et al. (1992) Cell 68: 97-108]. Besides activins, ActRIIA and ActRIIB can biochemically interact with several other TGF-β family proteins including, for example, BMP6, BMP7, Nodal, GDF8, and GDF11 [see, e.g., Yamashita et al. (1995) J. Cell Biol. 130:217-226; Lee and McPherron (2001) Proc. Natl. Acad. Sci. USA 98:9306-9311; Yeo and Whitman (2001) Mol. Cell 7: 949-957; and Oh et al. (2002) Genes Dev. 16:2749-54]. ALK4 is the primary type I receptor for activins, particularly for activin A, and ALK-7 may serve as a receptor for other activins as well, particularly for activin B.

[0051] Activins are dimeric polypeptide growth factors that belong to the TGF-beta superfamily. 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.

[0052] 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]. Moreover, erythroid differentiation factor (EDF) isolated from the stimulated human monocytic leukemic cells was found to be identical to activin A [Murata et al. (1988) PNAS, 85:2434]. It has been suggested that activin A promotes erythropoiesis in the bone marrow. In several tissues, activin signaling is antagonized by its related heterodimer, inhibin. For example, during the release of follicle-stimulating hormone (FSH) from the pituitary, activin promotes FSH secretion and synthesis, while inhibin prevents FSH secretion and synthesis. 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.

[0053] 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 PA 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 PA subunit, whether in the context of an isolated PA subunit or as a dimeric complex (e.g., a PAPA homodimer or a PAN heterodimer). In the case of $\beta_A\beta_B$ heterodimers, agents that inhibit "activin A" are agents that specifically inhibit one or more activities of the β_A subunit, but do not inhibit the activity of the non-β_A subunit of the complex (e.g., the β_B subunit of the complex). This principle applies also to agents that bind to and/or inhibit "activin B", "activin C", and "activin E". Agents disclosed herein that antagonize "activin AB" are agents that inhibit one or more activities as mediated by the β_A subunit and one or more activities as mediated by the β_B subunit.

[0054] Growth and differentiation factor-8 (GDF8) is also known as myostatin. GDF8 is a negative regulator of skeletal muscle mass. GDF8 is highly expressed in the developing and adult skeletal muscle. The GDF8 null mutation in transgenic mice is characterized by a marked hypertrophy and hyperplasia of the 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 [see, e.g., Ashmore et al. (1974) Growth, 38:501-507; Swatland and Kieffer (1994) J. Anim. Sci. 38:752-757; McPherron and Lee (1997) Proc. Natl. Acad. Sci. USA 94:12457-12461; and Kambadur et al. (1997) Genome Res. 7:910-915] and, strikingly, in humans [see, e.g., 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 [see, e.g., Gonzalez-Cadavid et al. (1998) PNAS 95:14938-43]. In addition, GDF8 can modulate the production of muscle-specific enzymes (e.g., creatine kinase) and modulate myoblast cell proliferation [see, e.g. international patent application publication no. WO 00/43781]. The GDF8 propeptide can noncovalently bind to the mature GDF8 domain dimer, inactivating its biological activity [see, e.g., 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 [see, e.g., Gamer et al. (1999) Dev. Biol., 208: 222-232].

[0055] Growth and differentiation factor-11 (GDF11), also known as BMP11, is a secreted protein [McPherron et al. (1999) Nat. Genet. 22: 260-264]. GDF11 is expressed in the tail bud, limb bud, maxillary and mandibular arches, and dorsal root ganglia during mouse development [see, e.g., Nakashima et al. (1999) Mech. Dev. 80: 185-189]. GDF11 plays a unique role in patterning both mesodermal and neural tissues [see, e.g., 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 [see, e.g., 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 [see, e.g., Wu et al. (2003) Neuron. 37:197-207].

[0056] In part, the present disclosure relates to the discovery that an ActRIIB antagonist (inhibitor) can be used to treat myelofibrosis patients, particularly ameliorating various complications of the disease including, for example, splenomegaly, extramedullary hematopoiesis, and fibrosis. In particular, the data presented herein show that a GDF trap polypeptide decrease splenomegaly, extramedullary hematopoiesis, and fibrosis in a JAK2V617F model of myelofibrosis. Accordingly, in certain aspects, the discloses relates to compositions and method for treating myelofibrosis, particularly treating or preventing one or more complications of myelofibrosis (splenomegaly, extramedullary hematopoiesis, anemia, and fibrosis), by administering to a patient in need thereof an effective amount of one or more ActRIIB antagonists, optionally in combination of one or more other supportive therapies or active agents for treating myelofibrosis.

[0057] The terms used in this specification generally have their ordinary meanings in the art, within the context of this invention and in the specific context where each term is used. Certain terms are discussed below or elsewhere in the specification, to provide additional guidance to the practitioner in describing the compositions and methods of the invention and how to make and use them. The scope or meaning of any use of a term will be apparent from the specific context in which the term is used.

[0058] "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.

[0059] 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.

[0060] "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.

[0061] "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.

[0062] "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.

[0063] 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.

[0064] Numeric ranges disclosed herein are inclusive of the numbers defining the ranges.

[0065] 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).

[0066] Throughout this specification, the word "comprise" or variations such as "comprises" or "comprising" will be understood to imply the inclusion of a stated integer or groups of integers but not the exclusion of any other integer or group of integers.

2. ActRIIB Antagonists

[0067] In part, the present disclosure relates to the discovery that an ActRIIB antagonist (inhibitor) can be used to treat myelofibrosis patients, particularly ameliorating various complications of the disease including, for example, splenomegaly, extramedullary hematopoiesis, and fibrosis. In particular, the data presented herein show that a GDF trap polypeptide decreases splenomegaly, extramedullary hematopoiesis, and fibrosis in a JAK2V617F model of myelofibrosis. While soluble GDF trap polypeptides may affect myelofibrosis through a mechanism other than ActRIIB antagonism [e.g., inhibition of one or more of GDF11, GDF8, activin B, BMP6, GDF3, and BMP10 may be an indicator of the tendency of an agent to inhibit the activities of a spectrum of additional agents, including, perhaps, other members of the TGF-beta superfamily, and such collective inhibition may lead to the desired effect on, for example, myelofibrosis], the disclosure nonetheless demonstrates that desirable therapeutic agents may be selected on the basis of ActRIIB antagonism. Therefore, while not wishing to be bound to a particular mechanism of action, it is expected that other ActRIIB antagonists [e.g., antagonists of the ActRIIB receptor, antagonists of one or more ActRIIB-binding ligand (e.g., GDF11, GDF8, activin, BMP6, GDF3, and BMP10) antagonists of one or more type I receptor (e.g., ALK4, ALK5, and/or ALK7), antagonists of one or more co-receptor, antagonists of one or more ActRIIB downstream signaling components (e.g., Smads), or combination of such antagonists] will useful in the treatment of myelofibrosis, particularly in the treatment or preventing of various myelofibrosis-associated complications (e.g., splenomegaly, extramedullary hematopoiesis, and fibrosis). Such agents are collectively referred to herein as "ActRIIB antagonists" or "ActRIIB inhibitors".

[0068] A. ActRIIB Polypeptides and Variants Thereof

[0069] In certain aspects, the present disclosure relates to ActRIIB polypeptides and variants thereof (e.g., GDF traps). In particular, the disclosure provides methods of using ActRIIB polypeptides, alone or in combination with one or more additional supportive therapies, to treat myelofibrosis, particularly treating or preventing one or more complications of myelofibrosis (e.g., splenomegaly, extramedullary hematopoiesis, anemia, and fibrosis), and/or a patient treated with a Janus kinase inhibitor. 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.

[0070] 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 No. WO 2006/012627 and WO 2008/097541, which are incorporated herein by reference in its 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.

[0071] The human ActRIIB precursor protein sequence is as follows:

(SEQ ID NO: 1)

1 MTAPWVALAL LWGSLCAGSG RGEAETRECI YYNANWELER TNQSGLERCE

51 GEQDKRLHCY ASWRNSSGTI ELVKKGCWLD DFNCYDRQEC VATEENPQVY

-continued

```
101 FCCCEGNFCN ERFTHLPEAG GPEVTYEPPP TAPTLLTVLA YSLLPIGGLS

151 LIVLLAFWMY RHRKPPYGHV DIHEDPGPPP PSPLVGLKPL QLLEIKARGR

201 FGCVWKAQLM NDFVAVKIFP LQDKQSWQSE REIFSTPGMK HENLLQFIAA

251 EKRGSNLEVE LWLITAFHDK GSLTDYLKGN IITWNELCHV AETMSRGLSY

301 LHEDVPWCRG EGHKPSIAHR DFKSKNVLLK SDLTAVLADF GLAVRFEPGK

351 PPGDTHGQVG TRRYMAPEVL EGAINFQRDA FLRIDMYAMG LVLWELVSRC

401 KAADGPVDEY MLPFEEEIGQ HPSLEELQEV VVHKKMRPTI KDHWLKHPGL

451 AQLCVTIEEC WDHDAEARLS AGCVEERVSL IRRSVNGTTS DCLVSLVTSV

501 TNVDLPPKES SI
```

[0072] 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.

[0073] The processed (mature) extracellular ActRIIB polypeptide sequence is as follows:

(SEQ ID NO: 2)

```
GRGEAETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWRNSSGT

IELVKKGCWLDDFNCYDRQECVATEENPQVYFCCCEGNFCNERFTHLPEA

GGPEVTYEPPPTAPT.
```

[0074] 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)

```
GRGEAETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWRNSSGT

IELVKKGCWLDDFNCYDRQECVATEENPQVYFCCCEGNFCNERFTHL

PEA.
```

[0075] A form of ActRIIB with an alanine at position 64 of SEQ ID NO: 1 (A64) is also reported in the literature [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.

[0076] 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 ASWANSSGTI ELVKKGCWLD DFNCYDRQEC VATEENPQVY

101 FCCCEGNFCN ERFTHLPEAG GPEVTYEPPP TAPTLLTVLA YSLLPIGGLS

151 LIVLLAFWMY RHRKPPYGHV DIHEDPGPPP PSPLVGLKPL QLLEIKARGR

201 FGCVWKAQLM NDFVAVKIFP LQDKQSWQSE REIFSTPGMK HENLLQFIAA

251 EKRGSNLEVE LWLITAFHDK GSLTDYLKGN IITWNELCHV AETMSRGLSY

301 LHEDVPWCRG EGHKPSIAHR DFKSKNVLLK SDLTAVLADF GLAVRFEPGK

351 PPGDTHGQVG TRRYMAPEVL EGAINFQRDA FLRIDMYAMG LVLWELVSRC

401 KAADGPVDEY MLPFEEEIGQ HPSLEELQEV VVHKKMRPTI KDHWLKHPGL

451 AQLCVTIEEC WDHDAEARLS AGCVEERVSL IRRSVNGTTS DCLVSLVTSV

501 TNVDLPPKES SI
```

[0077] The signal peptide is indicated by a single underline and the extracellular domain is indicated by bold font.

[0078] The processed (mature) extracellular ActRIIB polypeptide sequence of the alternative A64 form is as follows:

(SEQ ID NO: 5)

GRGEAETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWANSSGT

IELVKKGCWLDDFNCYDRQECVATEENPQVYFCCCEGNFCNERFTHLPEA

GGPEVTYEPPPTAPT

[0079] 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)

GRGEAETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWANSSGT

IELVKKGCWLDDFNCYDRQECVATEENPQVYFCCCEGNFCNERFTHLPEA

[0080] A nucleic acid sequence encoding human ActRIIB precursor protein is shown below (SEQ ID NO: 7), consisting of 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 CTCTGGGGAT CGCTGTGCGC
  51 CGGCTCTGGG CGTGGGGAGG CTGAGACACG GGAGTGCATC TACTACAACG
 101 CCAACTGGGA GCTGGAGCGC ACCAACCAGA GCGGCCTGGA GCGCTGCGAA
 151 GGCGAGCAGG ACAAGCGGCT GCACTGCTAC GCCTCCTGGC GCAACAGCTC
 201 TGGCACCATC GAGCTCGTGA AGAAGGGCTG CTGGCTAGAT GACTTCAACT
 251 GCTACGATAG GCAGGAGTGT GTGGCCACTG AGGAGAACCC CCAGGTGTAC
 301 TTCTGCTGCT GTGAAGGCAA CTTCTGCAAC GAACGCTTCA CTCATTTGCC
 351 AGAGGCTGGG GGCCCGGAAG TCACGTACGA GCCACCCCCG ACAGCCCCCA
 401 CCCTGCTCAC GGTGCTGGCC TACTCACTGC TGCCCATCGG GGGCCTTTCC
 451 CTCATCGTCC TGCTGGCCTT TTGGATGTAC CGGCATCGCA AGCCCCCCTA
 501 CGGTCATGTG GACATCCATG AGGACCCTGG GCCTCCACCA CCATCCCCTC
 551 TGGTGGGCCT GAAGCCACTG CAGCTGCTGG AGATCAAGGC TCGGGGGCGC
 601 TTTGGCTGTG TCTGGAAGGC CCAGCTCATG AATGACTTTG TAGCTGTCAA
 651 GATCTTCCCA CTCCAGGACA AGCAGTCGTG GCAGAGTGAA CGGGAGATCT
 701 TCAGCACACC TGGCATGAAG CACGAGAACC TGCTACAGTT CATTGCTGCC
 751 GAGAAGCGAG GCTCCAACCT CGAAGTAGAG CTGTGGCTCA TCACGGCCTT
 801 CCATGACAAG GGCTCCCTCA CGGATTACCT CAAGGGGAAC ATCATCACAT
 851 GGAACGAACT GTGTCATGTA GCAGAGACGA TGTCACGAGG CCTCTCATAC
 901 CTGCATGAGG ATGTGCCCTG GTGCCGTGGC GAGGGCCACA AGCCGTCTAT
 951 TGCCCACAGG GACTTTAAAA GTAAGAATGT ATTGCTGAAG AGCGACCTCA
1001 CAGCCGTGCT GGCTGACTTT GGCTTGGCTG TTCGATTTGA GCCAGGGAAA
1051 CCTCCAGGGG ACACCCACGG ACAGGTAGGC ACGAGACGGT ACATGGCTCC
1101 TGAGGTGCTC GAGGGAGCCA TCAACTTCCA GAGAGATGCC TTCCTGCGCA
1151 TTGACATGTA TGCCATGGGG TTGGTGCTGT GGGAGCTTGT GTCTCGCTGC
1201 AAGGCTGCAG ACGGACCCGT GGATGAGTAC ATGCTGCCCT TTGAGGAAGA
1251 GATTGGCCAG CACCCTTCGT TGGAGGAGCT GCAGGAGGTG GTGGTGCACA
1301 AGAAGATGAG GCCCACCATT AAAGATCACT GGTTGAAACA CCCGGGCCTG
1351 GCCCAGCTTT GTGTGACCAT CGAGGAGTGC TGGGACCATG ATGCAGAGGC
1401 TCGCTTGTCC GCGGGCTGTG TGGAGGAGCG GGTGTCCCTG ATTCGGAGGT
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-continued

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1451 CGGTCAACGG CACTACCTCG GACTGTCTCG TTTCCCTGGT GACCTCTGTC

1501 ACCAATGTGG ACCTGCCCCC TAAAGAGTCA AGCATC
```

[0081] A nucleic acid sequence encoding the processed extracellular human ActRIIB polypeptide is as follows (SEQ ID NO: 8):

(SEQ ID NO: 8)

```
  1 GGGCGTGGGG AGGCTGAGAC ACGGGAGTGC ATCTACTACA ACGCCAACTG

 51 GGAGCTGGAG CGCACCAACC AGAGCGGCCT GGAGCGCTGC GAAGGCGAGC

101 AGGACAAGCG GCTGCACTGC TACGCCTCCT GGCGCAACAG CTCTGGCACC

151 ATCGAGCTCG TGAAGAAGGG CTGCTGGCTA GATGACTTCA ACTGCTACGA

201 TAGGCAGGAG TGTGTGGCCA CTGAGGAGAA CCCCCAGGTG TACTTCTGCT

251 GCTGTGAAGG CAACTTCTGC AACGAACGCT TCACTCATTT GCCAGAGGCT

301 GGGGGCCCGG AAGTCACGTA CGAGCCACCC CCGACAGCCC CCACC
```

The sequence as shown provides an arginine at position 64, and may be modified to provide an alanine instead.

[0082] 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.

[0083] In addition, ActRIIB is generally 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 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 accordance with the presently disclosed methods may include one or more amino acids at corresponding positions from the sequence of another vertebrate ActRIIB, or may include a residue that is similar to that in the human or other vertebrate sequences.

[0084] 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: 42), 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 (SEQ ID NOs: 37 and 39) and V in *Xenopus*, thus essentially any amino acid should be tolerated at this position.

[0085] Moreover, 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 amply guidance for how to generate ActRII variants that retain one or more desired activities (e.g., ligand-binding activity).

[0086] For example, a defining structural motif known as a three-finger toxin fold is important for ligand binding by type I and type II receptors and is formed by conserved cysteine residues located at varying positions within the extracellular domain of each monomeric receptor [Greenwald et al. (1999) Nat Struct Biol 6:18-22; and Hinck (2012) FEBS Lett 586:1860-1870]. Accordingly, the core ligand-binding domains of human ActRIIB, as demarcated by the outermost of these conserved cysteines, corresponds to positions 29-109 of SEQ ID NO: 1 (ActRIIB precursor). Thus, the structurally less-ordered amino acids flanking these cysteine-demarcated core sequences can be truncated by about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, or 28 residues at the N-terminus and/or by about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 residues a the C-terminus without necessarily altering ligand binding. Exemplary ActRIIB extracellular domains for N-terminal and/or C-terminal truncation include SEQ ID NOs: 2, 3, 5, and 6.

[0087] 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. 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.

[0088] 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-biding activity, and ActRIIB polypeptides beginning at positions 25, 26, 27, 28, and 29 (with respect to SEQ ID NO: 1) are also expected to retain ligand-biding activity. It has been demonstrated, e.g., U.S. Pat. No. 7,842,663, that, surprisingly, an ActRIIB construct beginning at 22, 23, 24, or 25 will have the most activity.

[0089] 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) and end at a position from 119-134 (e.g., any one of positions 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, or 134), 119-133 (e.g., any one of positions 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, or 133), 129-134 (e.g., any one of positions 129, 130, 131, 132, 133, or 134), or 129-133 (e.g., any one of positions 129, 130, 131, 132, or 133) of SEQ ID NO: 1. Other examples include constructs that begin at a position from 20-24 (e.g., any one of positions 20, 21, 22, 23, or 24), 21-24 (e.g., any one of positions 21, 22, 23, or 24), or 22-25 (e.g., any one of positions 22, 22, 23, or 25) and end at a position from 109-134 (e.g., any one of positions 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, or 134), 119-134 (e.g., any one of positions 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, or 134) or 129-134 (e.g., any one of positions 129, 130, 131, 132, 133, or 134) of SEQ ID NO: 1. Variants within these ranges are also contemplated, particularly those having at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity to the corresponding portion of SEQ ID NO: 1.

[0090] The variations described herein may be combined in various ways. In some embodiments, ActRIIB variants comprise no more than 1, 2, 5, 6, 7, 8, 9, 10 or 15 conservative amino acid changes in the ligand-binding pocket, and zero, one or more non-conservative alterations at positions 40, 53, 55, 74, 79 and/or 82 in the ligand-binding pocket. Sites outside the binding pocket, at which variability may be particularly well tolerated, include the amino and carboxy termini of the extracellular domain (as noted above), and positions 42-46 and 65-73 (with respect to SEQ ID NO: 1). An asparagine-to-alanine alteration at position 65 (N65A) actually improves ligand binding in the A64 background, and is thus expected to have no detrimental effect on ligand binding in the R64 background [U.S. 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.

[0091] 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. 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 alterations that would create an N-linked glycosylation site are: A24N, R64N, S67N (possibly combined with an N65A alteration), E105N, R112N, G120N, E123N, P129N, A132N, R112S 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.

[0092] In certain embodiments, the disclosure relates to ActRIIB antagonists that comprise at least one ActRIIB polypeptide, which includes fragments, functional variants, and modified forms thereof. Preferably, ActRIIB polypeptides for use in accordance with the disclosure are soluble (e.g., an extracellular domain of ActRIIB) In some embodiments, ActRIIB polypeptides for use in accordance with the disclosure inhibit (antagonize) activity (e.g., Smad signaling) of one or more TGF-beta superfamily ligands [e.g., GDF11, GDF8, activin (activin A, activin B, activin AB, activin C, activin E) BMP6, GDF3, and/or BMP10. In some embodiments, ActRIIB polypeptides for use in accordance with the disclosure bind to one or more TGF-beta 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, ActRIIB 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 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 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 29-109 of SEQ ID NO: 1. In some 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 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 preferred 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 preferred 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, 24, 25, 28, 29, 30, 31, 33, 34, 35, 45, 50, 53, 54, and 58. 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, 24, 25, 28, 29, 30, 31, 33, 34, 35, 45, 50, 53, 54, and 58, 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 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 a naturally occurring acid amino acids D or E or an artificial acidic amino acid residue).

[0093] 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 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 ActRIIB polypeptide (e.g., a "wild-type" or unmodified ActRII polypeptide) such that the variant ActRIIB polypeptide has one or more altered ligand-binding activities than the corresponding wild-type ActRIIB polypeptide. In preferred embodiments, GDF trap polypeptides of the present disclosure retain at least one similar activity as a corresponding wild-type ActRIIB 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 TGF-beta superfamily. Accordingly, the present disclosure provides GDF trap polypeptides that have an altered binding specificity for one or more ActRIIB ligands.

[0094] 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 ActRIIB-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 (e.g., activin A).

[0095] In certain preferred embodiments, GDF traps of the present disclosure are designed to preferentially bind to GDF11 and/or GDF8 (also known as myostatin). Optionally, GDF11 and/or GDF8-binding traps may further bind to activin B. Optionally, GDF11 and/or GDF8-binding traps may further bind to BMP6. Optionally, GDF11 and/or GDF8-binding traps may further bind to BMP10. Optionally, GDF11 and/or GDF8-binding traps may further bind to activin B and BMP6. In certain embodiments, GDF traps of the present disclosure have diminished binding affinity for activins (e.g., activin A, activin A/B, activin B, activin C, activin E), e.g., in comparison to a wild-type ActRIIB polypeptide. In certain preferred embodiments, a GDF trap polypeptide of the present disclosure has diminished binding affinity for activin A.

[0096] Amino acid residues of the ActRIIB proteins (e.g., E39, K55, Y60, K74, W78, L79, D80, and F101) 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.

[0097] 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.

[0098] In some embodiments, the present disclosure contemplates making functional variants by modifying the structure of an ActRIIB 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-beta ligands including, for example, BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty.

[0099] In certain embodiments, the present disclosure contemplates specific mutations of an ActRIIB polypeptide so as to alter the glycosylation of the polypeptide. Such mutations may be selected so as to introduce or eliminate one or more glycosylation sites, such as O-linked or N-linked glycosylation sites. Asparagine-linked glycosylation recognition sites generally comprise a tripeptide sequence, asparagine-X-threonine or asparagine-X-serine (where "X" is any amino acid) which is specifically recognized by appropriate cellular glycosylation enzymes. The alteration may also be made by the addition of, or substitution by, one or more serine or threonine residues to the sequence of the polypeptide (for O-linked glycosylation sites). A variety of amino acid substitutions or deletions at one or both of the first or third amino acid positions of a glycosylation recognition site (and/or amino acid deletion at the second position) results in non-glycosylation at the modified tripeptide sequence. Another means of increasing the number of carbohydrate moieties on a polypeptide is by chemical or enzymatic coupling of glycosides to the polypeptide. Depending on the coupling mode used, the sugar(s) may be attached to (a) arginine and histidine; (b) free carboxyl groups; (c) free sulfhydryl groups such as those of cysteine; (d) free hydroxyl groups such as those of serine, threonine, or hydroxyproline; (e) aromatic residues such as those of phenylalanine, tyrosine, or tryptophan; or (f) the amide group of glutamine. Removal of one or more carbohydrate moieties present on a polypeptide may be accomplished chemically and/or enzymatically. Chemical deglycosylation may involve, for example, exposure of a

polypeptide to the compound trifluoromethanesulfonic acid, or an equivalent compound. This treatment results in the cleavage of most or all sugars except the linking sugar (N-acetylglucosamine or N-acetylgalactosamine), while leaving the amino acid sequence intact. Enzymatic cleavage of carbohydrate moieties on polypeptides can be achieved by the use of a variety of endo- and exo-glycosidases as described by Thotakura et al. [Meth. Enzymol. (1987) 138:350]. The sequence of a polypeptide may be adjusted, as appropriate, depending on the type of expression system used, as mammalian, yeast, insect, and plant cells may all introduce differing glycosylation patterns that can be affected by the amino acid sequence of the peptide. In general, 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.

[0100] The present disclosure further contemplates a method of generating mutants, particularly sets of combinatorial mutants of an ActRIIB polypeptide as well as truncation mutants. Pools of combinatorial mutants are especially useful for identifying functionally active (e.g., TGF-beta superfamily ligand binding) ActRIIB sequences. The purpose of screening such combinatorial libraries may be to generate, for example, polypeptides variants which have altered properties, such as altered pharmacokinetic or altered ligand binding. A variety of screening assays are provided below, and such assays may be used to evaluate variants. For example, ActRIIB variants may be screened for ability to bind to one or more TGF-beta superfamily 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-β1, TGF-β2, TGF-β3, activin A, activin B, activin AB, activin AC, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty), to prevent binding of a TGF-beta superfamily ligand to a TGF-beta superfamily receptor, and/or to interfere with signaling caused by an TGF-beta superfamily ligand.

[0101] The activity of ActRIIB polypeptides may also be tested in a cell-based or in vivo assay. For example, the effect of an ActRIIB polypeptide on the expression of genes involved in myelofibrosis acuity may be assessed. This may, as needed, be performed in the presence of one or more recombinant ActRII 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-β1, TGF-β2, TGF-β3, 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 ActRIIB polypeptide, and optionally, an ActRIIB ligand. Likewise, an ActRIIB polypeptide may be administered to a mouse or other animal and effects on myelofibrosis may be assessed using art-recognized methods. Similarly, the activity of an ActRIIB polypeptide 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.

[0102] Combinatorial-derived variants can be generated which have increased selectivity or generally increased potency relative to a reference ActRIIB polypeptide. 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 ActRIIB polypeptide. 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 ActRIIB polypeptide.

[0103] 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 ActRIIB sequences. For instance, a mixture of synthetic oligonucleotides can be enzymatically ligated into gene sequences such that the degenerate set of potential ActRIIB encoding nucleotide sequences are expressible as individual polypeptides, or alternatively, as a set of larger fusion proteins (e.g., for phage display).

[0104] 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].

[0105] Alternatively, other forms of mutagenesis can be utilized to generate a combinatorial library. For example, ActRIIB polypeptides 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, N.Y.; 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 ActRIIB polypeptides.

[0106] 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 ActRIIB 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 TGF-beta ligand (e.g., BMP2, BMP2/7, BMP3, BMP4, BMP4/7, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP9, BMP10, GDF3, GDF5, GDF6/BMP13, GDF7, GDF8, GDF9b/BMP15, GDF11/BMP11, GDF15/MIC1, TGF-β1, TGF-β2, TGF-β3, activin A, activin B, activin C, activin E, activin AB, activin AC, nodal, glial cell-derived neurotrophic factor (GDNF), neurturin, artemin, persephin, MIS, and Lefty) binding assays and/or TGF-beta ligand-mediated cell signaling assays.

[0107] 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 ActRIIB polypeptides that can be used as guidance for generating and using other variant ActRIIB polypeptides within the scope of the inventions described herein.

[0108] In certain embodiments, functionally active fragments of ActRIIB polypeptides of the present disclosure can be obtained by screening polypeptides recombinantly produced from the corresponding fragment of the nucleic acid encoding an ActRIIB polypeptide (e.g., SEQ ID NOs: 7, 8, 26, 32, 48, 49, 51, 52, 55, 56, 57, 59, 60, 61, and 62). 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 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-β1, TGF-β2, TGF-β3, 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). In certain embodiments, ActRIIB polypeptides of the present disclosure may further comprise post-translational modifications in addition to any that are naturally present in the ActRIIB polypeptide. Such modifications include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation, and acylation. As a result, the ActRIIB polypeptide 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 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, WI38, NIH-3T3 or HEK293) have specific cellular machinery and characteristic mechanisms for such post-translational activities and may be chosen to ensure the correct modification and processing of the ActRII polypeptides.

[0109] In certain aspects, ActRIIB polypeptides of the present disclosure include fusion proteins having at least a portion (domain) of an ActRIIB 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_6) fusion partners. As another example, a fusion domain may be selected so as to facilitate detection of the ActRIIB 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 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).

[0110] In certain aspects, ActRIIB 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 ActRIIB polypeptide domain and a stabilizer domain), modifications of a glycosylation site (including, for example, addition of a glycosylation site to a polypeptide of the disclosure), and modifications of carbohydrate moiety (including, for example, removal of carbohydrate moieties from a polypeptide of the disclosure). As used herein, the term "stabilizer domain" not only refers to a fusion domain (e.g., an immunoglobulin Fc domain) as in the case of fusion proteins, but also includes nonproteinaceous modifications such as a carbohydrate moiety, or nonproteinaceous moiety, such as polyethylene glycol. In certain preferred embodiments, an ActRIIB 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.

[0111] 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: 9). 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: 9. Naturally occurring variants in G1Fc would include E134D and M136L according to the numbering system used in SEQ ID NO: 9 (see Uniprot P01857).

(SEQ ID NO: 9)

```
  1 THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE
 51 VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK
101 VSNKALPAPI EKTISKAKGQ PREPQVYTLP PSREEMTKNQ VSLTCLVKGF
151 YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSKLTV DKSRWQQGNV
201 FSCSVMHEAL HNHYTQKSLS LSPGK
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[0112] 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 Fey 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 MEW class I-related Fc-receptor (FeRN) relative to a wild-type IgG1 Fc domain.

[0113] 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: 10). 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: 10.

(SEQ ID NO: 10)

```
  1 VECPPCPAPP VAGPSVFLFP PKPKDTLMIS RTPEVTCVVV DVSHEDPEVQ
 51 FNWYVDGVEV HNAKTKPREE QFNSTFRVVS VLTVVHQDWL NGKEYKCKVS
101 NKGLPAPIEK TISKTKGQPR EPQVYTLPPS REEMTKNQVS LTCLVKGFYP
151 SDIAVEWESN GQPENNYKTT PPMLDSDGSF FLYSKLTVDK SRWQQGNVFS
201 CSVMHEALHN HYTQKSLSLS PGK
```

[0114] 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: 11) contains a short hinge region consisting of a single 15-residue segment, whereas the second G3Fc sequence (SEQ ID NO: 12) 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: 11 and 12.

(SEQ ID NO: 11)

```
  1 EPKSCDTPPP CPRCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD

 51 VSHEDPEVQF KWYVDGVEVH NAKTKPREEQ YNSTFRVVSV LTVLHQDWLN

101 GKEYKCKVSN KALPAPIEKT ISKTKGQPRE PQVYTLPPSR EEMTKNQVSL

151 TCLVKGFYPS DIAVEWESSG QPENNYNTTP PMLDSDGSFF LYSKLTVDKS

201 RWQQGNIFSC SVMHEALHNR FTQKSLSLSP GK
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(SEQ ID NO: 12)

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  1 ELKTPLGDTT HTCPRCPEPK SCDTPPPCPR CPEPKSCDTP PPCPRCPEPK

 51 SCDTPPPCPR CPAPELLGGP SVFLFPPKPK DTLMISRTPE VTCVVVDVSH

101 EDPEVQFKWY VDGVEVHNAK TKPREEQYNS TFRVVSVLTV LHQDWLNGKE

151 YKCKVSNKAL PAPIEKTISK TKGQPREPQV YTLPPSREEM TKNQVSLTCL

201 VKGFYPSDIA VEWESSGQPE NNYNTTPPML DSDGSFFLYS KLTVDKSRWQ

251 QGNIFSCSVM HEALHNRFTQ KSLSLSPGK
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[0115] 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: 11, 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 OMNI 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.

[0116] 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: 13). 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: 13.

(SEQ ID NO: 13)

```
  1 ESKYGPPCPS CPAPEFLGGP SVFLFPPKPK DTLMISRTPE VTCVVVDVSQ

 51 EDPEVQFNWY VDGVEVHNAK TKPREEQFNS TYRVVSVLTV LHQDWLNGKE

101 YKCKVSNKGL PSSIEKTISK AKGQPREPQV YTLPPSQEEM TKNQVSLTCL

151 VKGFYPSDIA VEWESNGQPE NNYKTTPPVL DSDGSFFLYS RLTVDKSRWQ

201 EGNVFSCSVM HEALHNHYTQ KSLSLSLGK
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[0117] A variety of engineered mutations in the Fc domain are presented herein with respect to the G1Fc sequence (SEQ ID NO: 9), and analogous mutations in G2Fc, G3Fc, and G4Fc can be derived from their alignment with G1Fc in FIG. **11**. Due to unequal hinge lengths, analogous Fc positions based on isotype alignment (FIG. **11**) possess different amino acid numbers in SEQ ID NOs: 9, 10, 11, 12, and 13. It can also be appreciated that a given amino acid position in an immunoglobulin sequence consisting of hinge, C_H2, and C_H3 regions (e.g., SEQ ID NOs: 9, 10, 11, 12, and 13) will be identified by a different number than the same position when numbering encompasses the entire IgG1 heavy-chain constant domain (consisting of the C_H1, hinge, C_H2, and C_H3 regions) as in the Uniprot database. For example, correspondence between selected C_H3 positions in a human G1Fc sequence (SEQ ID NO: 9), the human IgG1 heavy chain constant domain (Uniprot P01857), and the human IgG1 heavy chain is as follows.

Correspondence of C_H3 Positions in Different Numbering Systems

G1Fc (Numbering begins at first threonine in hinge region)	IgG1 heavy chain constant domain (Numbering begins at C_H1)	IgG1 heavy chain (EU numbering scheme of Kabat etal., 1991*)
Y127	Y232	Y349
S132	S237	S354

-continued

Correspondence of C_H3 Positions in Different Numbering Systems

G1Fc (Numbering begins at first threonine in hinge region)	IgG1 heavy chain constant domain (Numbering begins at C_H1)	IgG1 heavy chain (EU numbering scheme of Kabat etal., 1991*)
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.

[0118] 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 ActRIIB polypeptide domain may be placed C-terminal to a heterologous domain, or alternatively, a heterologous domain may be placed C-terminal to an ActRIIB polypeptide domain. The ActRIIB polypeptide domain and the heterologous domain need not be adjacent in a fusion protein, and additional domains or amino acid sequences may be included C- or N-terminal to either domain or between the domains.

[0119] For example, an ActRIIB receptor fusion protein may comprise an amino acid sequence as set forth in the formula A-B-C. The B portion corresponds to an ActRIIB polypeptide domain. The A and C portions may be independently zero, one, or more than one amino acid, and both the A and C portions when present are heterologous to B. The A and/or C portions may be attached to the B portion via a linker sequence. A linker may be rich in glycine (e.g., 2-10, 2-5, 2-4, 2-3 glycine residues) or glycine and proline residues and may, for example, contain a single sequence of threonine/serine and glycines or repeating sequences of threonine/serine and/or glycines, e.g., GGG (SEQ ID NO: 14), GGGG (SEQ ID NO: 15), TGGGG (SEQ ID NO: 16), SGGGG(SEQ ID NO: 17), TGGG(SEQ ID NO: 18), SGGG (SEQ ID NO: 19), or GGGGS (SEQ ID NO: 20) singlets, or repeats. In certain embodiments, an ActRIIB fusion protein comprises an amino acid sequence as set forth in the formula A-B-C, wherein A is a leader (signal) sequence, B consists of an ActRIIB polypeptide domain, and C is a polypeptide portion that enhances one or more of in vivo stability, in vivo half-life, uptake/administration, tissue localization or distribution, formation of protein complexes, and/or purification. In certain embodiments, an ActRIIB fusion protein comprises an amino acid sequence as set forth in the formula A-B-C, wherein A is a TPA leader sequence, B consists of an ActRIIB receptor polypeptide domain, and C is an immunoglobulin Fc domain. Preferred fusion proteins comprise the amino acid sequence set forth in any one of SEQ ID NOs: 24, 25, 28, 29, 31, 33, 34, 45, 50, 53, and 58.

[0120] In preferred embodiments, ActRIIB polypeptides 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 antibody purity are well known in the art [see, e.g., Flatman et al., (2007) J. Chromatogr. B 848:79-87]. In some embodiments, ActRIIB polypeptides to be used in accordance with the methods described herein are recombinant polypeptides.

[0121] ActRIIB polypeptides 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/Biosearch 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 ActRIIB polypeptides by using, for example, a protease, e.g., trypsin, thermolysin, chymotrypsin, pepsin, or paired basic amino acid converting enzyme (PACE). Computer analysis (using commercially available software, e.g., MacVector, Omega, PCGene, Molecular Simulation, Inc.) can be used to identify proteolytic cleavage sites. Alternatively, such polypeptides may be produced from recombinantly generated full-length ActRIIB polypeptides using chemical cleavage (e.g., cyanogen bromide, hydroxylamine, etc.).

[0122] B. Nucleic Acids Encoding ActRIIB Polypeptides

[0123] In certain embodiments, the present disclosure provides isolated and/or recombinant nucleic acids encoding the ActRIIB polypeptides (including fragments, functional variants (e.g., GDF traps), 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.

[0124] 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.

[0125] In certain embodiments, nucleic acids encoding ActRIIB polypeptides of the disclosure are understood to include nucleic acids that are variants of any one of SEQ ID NOs: 7, 8, 26, 32, 48, 49, 51, 52, 55, 56, 57, 59, 60, 61, and 62. 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, 26, 32, 48, 49, 51, 52, 55, 56, 57, 59, 60, 61, and 62.

[0126] In certain embodiments, ActRIIB 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, 26, 32, 48, 49, 51, 52, 55, 56, 57, 59, 60, 61, and 62. 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, 26, 32, 48, 49, 51, 52, 55, 56, 57, 59, 60, 61, and 62, 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.

[0127] 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, 26, 32, 48, 49, 51, 52, 55, 56, 57, 59, 60, 61, and 62, complement sequences of SEQ ID NOs: 7, 8, 26, 32, 48, 49, 51, 52, 55, 56, 57, 59, 60, 61, and 62, 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.

[0128] Isolated nucleic acids which differ from the nucleic acids as set forth in SEQ ID NOs: 7, 8, 26, 32, 48, 49, 51, 52, 55, 56, 57, 59, 60, 61, and 62 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.

[0129] 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.

[0130] In certain aspects, the subject nucleic acid disclosed herein is provided in an expression vector comprising a nucleotide sequence encoding an ActRIIB polypeptide and operably linked to at least one regulatory sequence. Regulatory sequences are art-recognized and are selected to direct expression of the ActRIIB polypeptide. Accordingly, the term regulatory sequence includes promoters, enhancers, and other expression control elements. Exemplary regulatory sequences are described in Goeddel; *Gene Expression Technology: Methods in Enzymology,* Academic Press, San Diego, Calif. (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 ActRIIB 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.

[0131] A recombinant nucleic acid of the present disclosure can be produced by ligating the cloned gene, or a portion thereof, into a vector suitable for expression in either prokaryotic cells, eukaryotic cells (yeast, avian, insect or mammalian), or both. Expression vehicles for production of a recombinant ActRIIB polypeptide include plasmids and other vectors. For instance, suitable vectors include plasmids of the 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.*

[0132] 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).

[0133] In a preferred embodiment, a vector will be designed for production of the subject ActRIIB polypeptides in CHO cells, such as a Pcmv-Script vector (Stratagene, La Jolla, Calif.), pcDNA4 vectors (Invitrogen, Carlsbad, Calif.) and pCI-neo vectors (Promega, Madison, Wis.). 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.

[0134] This disclosure also pertains to a host cell transfected with a recombinant gene including a coding sequence for one or more of the subject ActRIIB polypeptides. The host cell may be any prokaryotic or eukaryotic cell. For example, an ActRIIB 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.

[0135] Accordingly, the present disclosure further pertains to methods of producing the subject ActRIIB polypeptides. For example, a host cell transfected with an expression vector encoding an ActRIIB polypeptide can be cultured under appropriate conditions to allow expression of the ActRIIB polypeptide to occur. The polypeptide may be secreted and isolated from a mixture of cells and medium containing the polypeptide. Alternatively, the ActRIIB 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 ActRIIB polypeptides, and affinity purification with an agent that binds to a domain fused to the ActRIIB polypeptide (e.g., a protein A column may be used to purify an ActRIIB-Fc fusion protein). In some embodiments, the ActRIIB polypeptide is a fusion protein containing a domain which facilitates its purification.

[0136] 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 ActRIIB 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.

[0137] 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 ActRIIB polypeptide, can allow purification of the expressed fusion protein by affinity chromatography using a Ni^{2+} metal resin. The purification leader sequence can then be subsequently removed by treatment with enterokinase to provide the purified ActRIIB polypeptide. See, e.g., Hochuli et al. (1987) *J Chromatography* 411:177; and Janknecht et al. (1991) *PNAS USA* 88:8972.

[0138] Techniques for making fusion genes are well known. Essentially, the joining of various DNA fragments coding for different polypeptide sequences is performed in accordance with conventional techniques, employing blunt-ended or stagger-ended termini for ligation, restriction enzyme digestion to provide for appropriate termini, filling-in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and enzymatic ligation. In another embodiment, the fusion gene can be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor primers which give rise to complementary overhangs between two consecutive gene fragments which can subsequently be annealed to generate a chimeric gene sequence. See, e.g., Current Protocols in Molecular Biology, eds. Ausubel et al., John Wiley & Sons: 1992.

[0139] C. Antibody Antagonists

[0140] In certain aspects, an ActRIIB antagonist to be used in accordance with the method and uses disclosed herein is an antibody (ActRIIB antagonist antibody), or combination of antibodies. An ActRIIB antagonist antibody, or combination of antibodies, may bind to, for example, one or more ActRIIB-binding ligands (e.g., activin, GDF11, GDF8, GDF3, BMP10, and BMP6), ActRIIB receptor, type I receptor (e.g., ALK4, ALK5, and/or ALK7), and/or ActRIIB co-receptor. As described herein, ActRIIB antagonist antibodies may be used alone or in combination with one or

more supportive therapies or active agents, to treat myelofibrosis, particularly treating or preventing one or more complications of myelofibrosis (e.g., splenomegaly, extramedullary hematopoiesis, anemia, and fibrosis), and/or a patient treated with a Janus kinase inhibitor.

[0141] In certain aspects, a ActRIIB 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 ActRIIB 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 (MA), 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., 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 ActRIIB 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 ActRIIB ligands [e.g., GDF11, GDF8, GDF3, BMP10, and BMP6], one or more type I receptor and/or type II receptors (e.g., 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 ActRIIB superfamily ligands [e.g., GDF8, GDF11, GDF3, and BMP6], one or more type I receptor and/or type II receptors (e.g., 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.

[0142] In certain aspects, an ActRIIB antagonist antibody, or combination of antibodies, is an antibody that inhibits at least activin B. Therefore, in some embodiments, an ActRIIB 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 (MA), 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., 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 ActRIIB ligands [e.g., GDF11, GDF8, GDF3, BMP10, and BMP6], one or more type I receptor and/or type II receptors (e.g., 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 ActRIIB ligands [e.g., GDF8, GDF11, GDF3, BMP6, and BMP10], one or more type I receptor and/or type II receptors (e.g., ActRIIB, ALK4, ALK5, and/or ALK7), and/or one or more co-receptors. In some embodiments, a 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.

[0143] In certain aspects, an ActRIIB antagonist antibody, or combination of antibodies, is an antibody that inhibits at least GDF8. Therefore, in some embodiments, an ActRIIB antagonist antibody, or combination of antibodies, binds to at least GDF8. As used herein, a GDF8 antibody (or anti-GDF8 antibody) generally refers to an antibody that binds to GDF8 with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting GDF8. In certain embodiments, the extent of binding of a GDF8 antibody to an unrelated, non-GDF8 protein is less than about 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or less than

about 1% of the binding of the antibody to GDF8 as measured, for example, by a radioimmunoassay (MA), Biacore, or other protein interaction or binding affinity assay. In certain embodiments, a GDF8 antibody binds to an epitope of GDF8 that is conserved among GDF8 from different species. In certain preferred embodiments, an anti-GDF8 antibody binds to human GDF8. In some embodiments, a GDF8 antibody may inhibit GDF8 from binding to a type I and/or type II receptor (e.g., ActRIIB, 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 ActRIIB 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, BMP10, and BMP6], one or more type I receptor and/or type II receptors (e.g., 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 ActRIIB 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., 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.

[0144] In certain aspects, an ActRIIB antagonist antibody, or combination of antibodies, is an antibody that inhibits at least GDF11. Therefore, in some embodiments, an ActRIIB antagonist antibody, or combination of antibodies, binds to at least GDF11. As used herein, a GDF11 antibody (or anti-GDF11 antibody) generally refers to an antibody that binds to GDF11 with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting GDF11. In certain embodiments, the extent of binding of a GDF11 antibody to an unrelated, non-GDF11 protein is less than about 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or less than about 1% of the binding of the antibody to GDF11 as measured, for example, by a radioimmunoassay (MA), Biacore, or other protein interaction or binding affinity assay. In certain embodiments, a GDF11 antibody binds to an epitope of GDF11 that is conserved among GDF11 from different species. In certain preferred embodiments, an anti-GDF11 antibody binds to human GDF11. In some embodiments, a GDF11 antibody may inhibit GDF11 from binding to a type I and/or type II receptor (e.g., ActRIIB, 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 ActRIIB 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, BMP10, and BMP6], one or more type I receptor and/or type II receptors (e.g., 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 ActRIIB 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, and BMP10], one or more type I receptor and/or type II receptors (e.g., 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.

[0145] In certain aspects, an ActRIIB antagonist antibody, or combination of antibodies, is an antibody that inhibits at least BMP6. Therefore, in some embodiments, an ActRIIB antagonist antibody, or combination of antibodies, binds to at least BMP6. As used herein, a BMP6 antibody (or anti-BMP6 antibody) generally refers to an antibody that can bind to BMP6 with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting BMP6. In certain embodiments, the extent of binding of a BMP6 antibody to an unrelated, non-BMP6 protein is less than about 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or less than about 1% of the binding of the antibody to BMP6 as measured, for example, by a radioimmunoassay (MA), Biacore, or other protein interaction or binding affinity assay. In certain embodiments, a BMP6 antibody binds to an epitope of BMP6 that is conserved among BMP6 from different species. In certain preferred embodiments, an anti-BMP6 antibody binds to human BMP6. In some embodiments, a BMP6 antibody may inhibit BMP6 from binding to a type I and/or type II receptor (e.g., ActRIIB, 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 ActRIIB 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, BMP10, and GDF11], one or more type I receptor and/or type II receptors (e.g., 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 ActRIIB 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, and BMP10], one or more type I receptor and/or type II receptors (e.g., 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.

[0146] In certain aspects, an ActRIIB antagonist antibody, or combination of antibodies, is an antibody that inhibits at least GDF3. Therefore, in some embodiments, an ActRIIB antagonist antibody, or combination of antibodies, binds to at least GDF3. As used herein, a GDF3 antibody (or anti-GDF3antibody) 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 (MA), 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., 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 ActRIIB 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, BMP6, BMP10, and GDF11], one or more type I receptor and/or type II receptors (e.g., 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 ActRIIB 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, and BMP10], one or more type I receptor and/or type II receptors (e.g., 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 a BMP9 antibody. In some embodiments, a combination of antibodies that comprises a GDF3 antibody does not comprise an activin A antibody.

[0147] In certain aspects, an ActRIIB antagonist antibody, or combination of antibodies, is an antibody that inhibits at least BMP10. Therefore, in some embodiments, an ActRIIB antagonist antibody, or combination of antibodies, binds to at least BMP10. As used herein, a BMP10 antibody (or anti-BMP10 antibody) generally refers to an antibody that can bind to BMP10 with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting BMP10. In certain embodiments, the extent of binding of a BMP10 antibody to an unrelated, non-BMP10 protein is less than about 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or less than about 1% of the binding of the antibody to BMP10 as measured, for example, by a radioimmunoassay (MA), Biacore, or other protein interaction or binding affinity assay. In certain embodiments, a BMP10 antibody binds to an epitope of BMP10 that is conserved among BMP10 from different species. In certain preferred embodiments, an anti-BMP10 antibody binds to human BMP10. In some embodiments, a BMP10 antibody may inhibit BMP10 from binding to a type I and/or type II receptor (e.g., ActRIIB, 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 ActRIIB 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., 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 ActRIIB 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., 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.

[0148] In certain aspects, an ActRIIB antagonist antibody, or combination of antibodies, is an antibody that inhibits at least ActRIIB Therefore, in some embodiments, an ActRIIB antagonist antibody, or combination of antibodies, binds to at least ActRIIB As used herein, an ActRIIB antibody (anti-ActRIIB antibody) generally refers to an antibody that binds to ActRIIB with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting ActRIIB In certain embodiments, the extent of binding of an anti-ActRIIB antibody to an unrelated, non-ActRIIB protein is less than about 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or less than about 1% of the binding of the antibody to ActRIIB as measured, for example, by a radioimmunoassay (MA), 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 ActRIIB 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, and BMP10] from binding to ActRIIB In some embodiments, an anti-ActRIIB antibody is a multispecific antibody (e.g., bi-specific antibody) that binds to ActRIIB and one or more ActRIIB 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 ActRIIB 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.

[0149] In certain aspects, an ActRIIB antagonist antibody, or combination of antibodies, is an antibody that inhibits at least ALK4. Therefore, in some embodiments, an ActRIIB 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 (MA), 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 ActRIIB 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, and BMP10] 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., 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 ActRIIB 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., ActRIIB), and/or additional type I receptors (e.g., ALK5 and/or ALK7).

[0150] In certain aspects, an ActRIIB antagonist antibody, or combination of antibodies, is an antibody that inhibits at least ALK5. Therefore, in some embodiments, an ActRIIB 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 (MA), 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 ActRIIB 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, and BMP10] 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 ActRIIB 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., ActRIIB), co-receptor, and/or an additional type I receptor (e.g., ALK4 and/or ALK7). In some embodiments, the

disclosure relates to combinations of antibodies, and uses thereof, wherein the combination of antibodies comprises an anti-ALK5 antibody and one or more additional antibodies that bind to, for example, one or more ActRIIB 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., ActRIIB), and/or additional type I receptors (e.g., ALK4 and/or ALK7).

[0151] In certain aspects, an ActRIIB antagonist antibody, or combination of antibodies, is an antibody that inhibits at least ALK7. Therefore, in some embodiments, an ActRIIB antagonist antibody, or combination of antibodies, binds to at least ALK7. As used herein, an ALK7 antibody (anti-ALK7 antibody) generally refers to an antibody that binds to ALK7 with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting ALK7. In certain embodiments, the extent of binding of an anti-ALK7 antibody to an unrelated, non-ALK7 protein is less than about 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or less than about 1% of the binding of the antibody to ALK7 as measured, for example, by a radioimmunoassay (MA), 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 ActRIIB 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, and BMP10] from binding to ALK7. In some embodiments, an anti-ALK7 antibody is a multispecific antibody (e.g., bispecific antibody) that binds to ALK7 and one or more ActRIIB 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., 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 ActRIIB 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., ActRIIB), and/or additional type I receptors (e.g., ALK4 and/or ALK5).

[0152] The term antibody is used herein in the broadest sense and encompasses various antibody structures, including but not limited to monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies), and antibody fragments so long as they exhibit the desired antigen-binding activity. An antibody fragment refers to a molecule other than an intact antibody that comprises a portion of an intact antibody that binds the antigen to which the intact antibody binds. Examples of antibody fragments include, but are not limited to, Fv, Fab, Fab', Fab'-SH, $F(ab')_2$; diabodies; linear antibodies; single-chain antibody molecules (e.g., scFv); and multispecific antibodies formed from antibody fragments [see, e.g., Hudson et al. (2003) Nat. Med. 9:129-134; Plückthun, in The Pharmacology of Monoclonal Antibodies, vol. 113, Rosenburg and Moore eds., (Springer-Verlag, New York), pp. 269-315 (1994); WO 93/16185; and U.S. Pat. Nos. 5,571,894; 5,587,458; and 5,869,046]. Diabodies are antibody fragments with two antigen-binding sites that may be bivalent or bispecific [see, e.g., EP 404,097; WO 1993/01161; Hudson et al. (2003) Nat. Med. 9:129-134 (2003); and Hollinger et al. (1993) Proc. Natl. Acad. Sci. USA 90: 6444-6448]. Triabodies and tetrabodies are also described in Hudson et al. (2003) Nat. Med. 9:129-134. Single-domain antibodies are antibody fragments comprising all or a portion of the heavy-chain variable domain or all or a portion of the light-chain variable domain of an antibody. In certain embodiments, a single-domain antibody is a human single-domain antibody [see, e.g., U.S. Pat. No. 6,248,516]. Antibodies disclosed herein may be polyclonal antibodies or monoclonal antibodies. In certain embodiments, the antibodies of the present disclosure comprise a label attached thereto and able to be detected (e.g., the label can be a radioisotope, fluorescent compound, enzyme, or enzyme co-factor). In 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.

[0153] The antibodies herein may be of any class. The class of an antibody refers to the type of constant domain or constant region possessed by its heavy chain. There are five major classes of antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), for example, IgG_1, IgG_2, IgG_3, IgG_4, IgA_1, and IgA_2. The heavy chain constant domains that correspond to the different classes of immunoglobulins are called alpha, delta, epsilon, gamma, and mu.

[0154] 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. ALK4, ALK5, ALK7, ActRIIB, GDF3, activin, GDF11, GDF8, 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.

[0155] In certain embodiments, K_D is measured by RIA performed with the Fab version of an antibody of interest and its target antigen as described by the following assay. Solution binding affinity of Fabs for the antigen is measured by equilibrating Fab with a minimal concentration of radiolabeled antigen (e.g., ^{125}I-labeled) in the presence of a titration series of unlabeled antigen, then capturing bound antigen with an anti-Fab antibody-coated plate [see, e.g., Chen et al. (1999) J. Mol. Biol. 293:865-881]. To establish conditions for the assay, multi-well plates (e.g., MICROTITER® from Thermo Scientific) are coated (e.g., overnight) with a capturing anti-Fab antibody (e.g., from Cappel Labs) and subsequently blocked with bovine serum albumin, preferably at room temperature (approximately 23° C.). In a non-adsorbent plate, radiolabeled antigen are mixed with serial dilutions of a Fab of interest [e.g., consistent with

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

[0156] 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 CMS chips at about 10 response units (RU). Briefly, carboxymethylated dextran biosensor chips (CMS, BIACORE, Inc.) are activated with N-ethyl-N'-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NETS) 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, $10^6 M^{-1}$ s^{-1} by the surface plasmon resonance assay above, then the on-rate can be determined by using a fluorescent quenching technique that measures the increase or decrease in fluorescence emission intensity (e.g., excitation=295 nm; emission=340 nm, 16 nm band-pass) of a 20 nM anti-antigen antibody (Fab form) in PBS in the presence of increasing concentrations of antigen as measured in a spectrometer, such as a stop-flow equipped spectrophometer (Aviv Instruments) or a 8000-series SLM-AMINCO® spectrophotometer (ThermoSpectronic) with a stirred cuvette.

[0157] 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 ALK4, ALK5, ALK7, ActRIIB, activin (activin A, activin B, activin C, and activin E), GDF11, GDF8, BMP10, GDF3, 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.

[0158] 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.

[0159] In certain embodiments, a chimeric antibody provided herein is a humanized antibody. A humanized antibody refers to a chimeric antibody comprising amino acid residues from non-human hypervariable regions (HVRs) and amino acid residues from human framework regions (FRs). In certain embodiments, a humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the HVRs (e.g., CDRs) correspond to those of a non-human antibody, and all or substantially all of the FRs correspond to those of a human antibody. A humanized antibody optionally may comprise at least a portion of an antibody constant region derived from a human antibody. A "humanized form" of an antibody, e.g., a non-human antibody, refers to an antibody that has undergone humanization. Humanized antibodies and methods of making them are reviewed, for example, in Almagro and Fransson (2008) Front. Biosci. 13:1619-1633 and are further described, for example, in Riechmann et al., (1988) Nature 332:323-329; Queen et al. (1989) Proc. Nat'l Acad. Sci. USA 86:10029-10033; U.S. Pat. Nos. 5,821,337; 7,527,791; 6,982,321; and 7,087,409; Kashmiri et al., (2005) Methods 36:25-34 [describing SDR (a-CDR) grafting]; Padlan, Mol. Immunol. (1991) 28:489-498 (describing "resurfacing"); Dall'Acqua et al. (2005) Methods 36:43-60 (describing "FR shuffling"); Osbourn et al. (2005) Methods 36:61-68; and Klimka et al. Br. J. Cancer (2000) 83:252-260 (describing the "guided selection" approach to FR shuffling). Human framework regions that may be used for humanization include but are not limited to: framework regions selected using the "best-fit" method [see, e.g., Sims et al. (1993) J. Immunol. 151:2296]; framework regions derived from the consensus sequence of human antibodies of a particular subgroup of light or heavy chain variable regions [see, e.g., Carter et al. (1992) Proc. Natl. Acad. Sci. USA, 89:4285; and Presta et al. (1993) J. Immunol., 151:2623]; human mature (somatically mutated) framework regions or human germline framework regions [see, e.g., Almagro and Fransson (2008) Front. Biosci. 13:1619-1633]; and framework regions derived from screening FR libraries [see, e.g., Baca et al., (1997) J. Biol. Chem. 272:10678-10684; and Rosok et al., (1996) J. Biol. Chem. 271:22611-22618].

[0160] In certain embodiments, an antibody provided herein is a human antibody. Human antibodies can be produced using various techniques known in the art. Human antibodies are described generally in van Dijk and van de Winkel (2008) Curr. Opin. Pharmacol. 5: 368-74 (2001) and Lonberg, Curr. Opin. Immunol. 20:450-459. For example, human antibodies may be prepared by administering an immunogen (e.g., a GDF11 polypeptide, an activin B polypeptide, an ActRIIA polypeptide, or an ActRIIB polypeptide) to a transgenic animal that has been modified to

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

[0161] 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.

[0162] 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.

[0163] 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., ALK4, ALK5, ALK7, ActRIIB, activin, GDF11, GDF8, GDF3, BMP10, 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 unrearranged V-gene segments from stem cells, and using PCR primers containing random sequence to encode the highly variable CDR3 regions and to accomplish rearrangement in vitro, as described by Hoogenboom and Winter (1992) J. Mol. Biol., 227: 381-388. Patent publications describing human antibody phage libraries include, for example: U.S. Pat. No. 5,750,373, and U.S. Patent Publication Nos. 2005/0079574, 2005/0119455, 2005/0266000, 2007/0117126, 2007/0160598, 2007/0237764, 2007/0292936, and 2009/0002360.

[0164] 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.

[0165] 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].

[0166] 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.

[0167] 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 an 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.

[0168] 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.

[0169] 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.

[0170] For example, the present disclosure contemplates an antibody variant that possesses some but not all effector functions, which make it a desirable candidate for applications in which the half-life of the antibody in vivo is important yet certain effector functions [e.g., complement-dependent cytotoxicity (CDC) and antibody-dependent cellular cytotoxicity (ADCC)] are unnecessary or deleterious. In vitro and/or in vivo cytotoxicity assays can be conducted to confirm the reduction/depletion of CDC and/or ADCC activities. For example, Fc receptor (FcR) binding assays can be conducted to ensure that the antibody lacks FcγR binding (hence likely lacking ADCC activity), but retains FcRn binding ability. The primary cells for mediating ADCC, NK cells, express FcγRIII only, whereas monocytes express FcγRI, FcγRII and FcγRIII FcR expression on hematopoietic cells is summarized in, for example, Ravetch and Kinet (1991) Annu. Rev. Immunol. 9:457-492. Non-limiting examples of in vitro assays to assess ADCC activity of a molecule of interest are described in U.S. Pat. No. 5,500,362; Hellstrom, I. et al. (1986) Proc. Natl. Acad. Sci. USA 83:7059-7063]; Hellstrom, I et al. (1985) Proc. Natl. Acad. Sci. USA 82:1499-1502; U.S. Pat. No. 5,821,337; Bruggemann, M. et al. (1987) J. Exp. Med. 166:1351-1361. Alternatively, non-radioactive assays methods may be employed (e.g., ACTI™, non-radioactive cytotoxicity assay for flow cytometry; CellTechnology, Inc. Mountain View, Calif.; and CytoTox 96° non-radioactive cytotoxicity assay, Promega, Madison, Wis.). Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and natural killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed in vivo, for example, in an animal model such as that disclosed in Clynes et al. (1998) Proc. Natl. Acad. Sci. USA 95:652-656. C1q binding assays may also be carried out to confirm that the antibody is unable to bind C1q and hence lacks CDC activity [see, e.g., C1q and C3c binding ELISA in WO 2006/029879 and WO 2005/100402]. To assess complement activation, a CDC assay may be performed [see, e.g, Gazzano-Santoro et al. (1996) J. Immunol. Methods 202:163; Cragg, M. S. et al. (2003) Blood 101:1045-1052; and Cragg, M. S, and M. J. Glennie (2004) Blood 103:2738-2743]. FcRn binding and in vivo clearance/half-life determinations can also be performed using methods known in the art [see, e.g., Petkova, S. B. et al. (2006) Intl. Immunol. 18(12):1759-1769]. Antibodies of the present disclosure with reduced effector function include those with substitution of one or more of Fc region residues 238, 265, 269, 270, 297, 327 and 329 (U.S. Pat. No. 6,737,056). Such Fc mutants include Fc mutants with substitutions at two or more of amino acid positions 265, 269, 270, 297 and 327, including the so-called "DANA" Fc mutant with substitution of residues 265 and 297 to alanine (U.S. Pat. No. 7,332,581).

[0171] 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.

[0172] 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, Md.), western blots, immunoprecipitation assays, and immunohistochemistry.

[0173] 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).

[0174] Alterations (e.g., substitutions) may be made in HVRs, for example, to improve antibody affinity. Such alterations may be made in HVR "hotspots," i.e., residues encoded by codons that undergo mutation at high frequency during the somatic maturation process [see, e.g., Chowdhury (2008) Methods Mol. Biol. 207:179-196 (2008)], and/or SDRs (a-CDRs), with the resulting variant VH or VL being tested for binding affinity. Affinity maturation by constructing and reselecting from secondary libraries has been described in the art [see, e.g., Hoogenboom et al., in Methods in Molecular Biology 178:1-37, O'Brien et al., ed., Human Press, Totowa, N.J., (2001). In some embodiments of affinity maturation, diversity is introduced into the variable genes chosen for maturation by any of a variety of methods (e.g., error-prone PCR, chain shuffling, or oligonucleotide-directed mutagenesis). A secondary library is then created. The library is then screened to identify any antibody variants with the desired affinity. Another method to introduce diversity involves HVR-directed approaches, in which several HVR residues (e.g., 4-6 residues at a time) are randomized. HVR residues involved in antigen binding may be specifically identified, e.g., using alanine scanning mutagenesis or modeling. CDR-H3 and CDR-L3 in particular are often targeted.

[0175] 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.

[0176] 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.

[0177] 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.

[0178] In certain embodiments, an antibody and/or binding polypeptide provided herein may be further modified to contain additional nonproteinaceous moieties that are known in the art and readily available. The moieties suitable for derivatization of the antibody and/or binding polypeptide include but are not limited to water soluble polymers. Non-limiting examples of water soluble polymers include, but are not limited to, polyethylene glycol (PEG), copolymers of ethylene glycol/propylene glycol, carboxymethylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone, poly-1,3-dioxolane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, polyaminoacids (either homopolymers or random copolymers), and dextran or poly(n-vinyl pyrrolidone)polyethylene glycol, propropylene glycol homopolymers, prolypropylene oxide/ethylene oxide 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.

[0179] D. Small Molecule Antagonists

[0180] In other aspects, an ActRIIB antagonist to be used in accordance with the methods and uses described herein is a small molecule (ActRIIB antagonists small molecule), or combination of small molecule antagonists. An ActRIIB antagonist small molecule, or combination of small molecule antagonists, may inhibit, for example, one or more ActRIIB ligands (e.g., activin, GDF11, GDF8, GDF3, BMP6, and/or BMP10), a type I receptor (e.g., ALK4, ALK5, and/or ALK7), a type II receptor (e.g., ActRIIB), and/or a co-receptor. In some embodiments, an ActRIIB antagonist small molecule, or combination of small molecule antagonists, inhibits signaling mediated by one or more ActRIIB ligands, for example, as determined in a cell-based assay such as those described herein. As described herein, ActRIIB antagonist small molecule 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 myelofibrosis particularly treating, preventing or reducing the progression rate and/or severity of one or more myelofibrosis-associated complications (e.g., splenomegaly, extramedullary hematopoiesis, anemia and fibrosis and/or treat a patient treated with a Janus kinase inhibitor.

[0181] In some embodiments, an ActRIIB antagonist small molecule, 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, ActRIIB, ALK4, ALK5, and ALK7. In some embodiments, an ActRIIB antagonist small molecule, 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, ActRIIB, ALK4, ALK5, and ALK7. In some embodiments, an ActRIIB antagonist small molecule, 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, ActRIIB, ALK4, ALK5, and ALK7. In some embodiments, an ActRIIB antagonist small molecule, or combination of small molecule antagonists, inhibits at least activin B, optionally further inhibiting one or more of GDF8, GDF11, GDF3, BMP6, BMP10, ActRIIB, ALK4, ALK5, and ALK7. In some embodiments, an ActRIIB antagonist small molecule, 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, ActRIIB, ALK4, ALK5, and ALK7. In some embodiments, an ActRIIB antagonist small molecule, 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, ActRIIB, ALK4, ALK5, and ALK7. In some embodiments, an ActRIIB antagonist small molecule, 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, ActRIIB, ALK4, ALK5, and ALK7. In some embodiments, an ActRIIB antagonist small molecule, 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, ALK4, ALK5, and ALK7. In some embodiments an ActRIIB antagonist small molecule, 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, ActRIIB, BMP10, ALK5, and ALK7. In some embodiments, an ActRIIB antagonist small molecule, 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, ActRIIB, ALK4, BMP10, and ALK7. In some embodiments, an ActRIIB antagonist small molecule, 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, ActRIIB, ALK4, ALK5, and BMP10. In some embodiments, an ActRIIB antagonist small molecule, or combination of small molecule antagonists, as disclosed herein does not inhibit or does not substantially inhibit BMP9. In some embodiments, an ActRIIB antagonist small molecule, or combination of small molecule antagonists, as disclosed herein does not inhibit or does not substantially inhibit activin A.

[0182] ActRIIB antagonist small molecules 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 ActRIIB ligands [e.g., activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC, activin B, activin BC, activin AE, or activin BE), GDF11, BMP10, BMP9, BMP6, BMP5, GDF3, and/or GDF8], type I receptor (e.g., ALK4, ALK5, and/or ALK7), type II receptors (e.g., ActRIIB), co-receptor, and/or one or more downstream ActRIIB 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 ActRIIB ligands [e.g., activin (e.g., activin A, activin B, activin C, activin E, activin AB, activin AC, activin B, activin BC, activin AE, or activin BE), GDF11, BMP10, BMP9, BMP6, BMP5, GDF3, and/or GDF8], type I receptor (e.g., ALK4, ALK5 and/or ALK7), type II receptors (e.g., ActRIIB), co-receptor, and/or one or more downstream ActRIIB signaling components (e.g., Smads). Combinations of one or more indirect and one or more direct ActRIIB antagonists small molecule may be used in accordance with the methods disclosed herein.

[0183] 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).

[0184] Binding organic small molecules of the present disclosure may be, for example, aldehydes, ketones, oximes, hydrazones, semicarbazones, carbazides, primary amines, secondary amines, tertiary amines, N-substituted hydrazines, hydrazides, alcohols, ethers, thiols, thioethers, disulfides, carboxylic acids, esters, amides, ureas, carbamates, carbonates, ketals, thioketals, acetals, thioacetals, aryl halides, aryl sulfonates, alkyl halides, alkyl sulfonates, aromatic compounds, heterocyclic compounds, anilines, alkenes, alkynes, diols, amino alcohols, oxazolidines, oxazolines, thiazolidines, thiazolines, enamines, sulfonamides, epoxides, aziridines, isocyanates, sulfonyl chlorides, diazo compounds, and acid chlorides.

[0185] E. Polynucleotide Antagonists

[0186] In other aspects, an ActRIIB antagonist to be used in accordance with the methods and uses disclosed herein is a polynucleotide (ActRIIB antagonist polynucleotide), or combination of polynucleotides. An ActRIIB antagonist polynucleotide, or combination of polynucleotide antagonists, may inhibit, for example, one or more ActRIIB ligands (e.g., activin, GDF11, GDF8, GDF3, BMP6, and/or BMP10,), type I receptors (e.g., ALK4, ALK5, and/or ALK7), type II receptors (e.g., ActRIIB), co-receptor, and/or downstream signaling component (e.g., Smads). In some embodiments, an ActRIIB antagonist polynucleotide, or combination of polynucleotide antagonists, inhibits signaling mediated by one or more ActRIIB ligands, for example, as determined in a cell-based assay such as those described herein. As described herein, ActRIIB antagonist polynucleotides 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 myelofibrosis, particularly treating, preventing or reducing the progression rate and/or severity of one or more myelofibrosis-associated complications (e.g., splenomegaly, extramedullary hematopoiesis, anemia, and fibrosis) and/or treat a patient treated with a Janus kinase inhibitor.

[0187] In some embodiments, an ActRIIB antagonist polynucleotide, 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, BMP10, ActRIIB, ALK4, ALK5, and ALK7. In some embodiments, an ActRIIB antagonist polynucleotide, 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, BMP10, ActRIIB, ALK4, ALK5, and ALK7. In some embodiments, an ActRIIB antagonist polynucleotide, 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, BMP10, ActRIIB, ALK4, ALK5, and ALK7. In some embodiments, an ActRIIB antagonist polynucleotide, or combination of polynucleotide antagonists, inhibits at least activin B, optionally further inhibiting one or more of GDF8, GDF11, GDF3, BMP6, BMP10, ActRIIB, ALK4, ALK5, and ALK7. In some embodiments, an ActRIIB antagonist polynucleotide, 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, BMP10, ActRIIB, ALK4, ALK5, and ALK7. In some embodiments, an ActRIIB antagonist polynucleotide, 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), BMP6, GDF11, BMP10, ActRIIB, ALK4, ALK5, and ALK7. In some embodiments, an ActRIIB antagonist polynucleotide, or combination of polynucleotide 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), BMP6, GDF11, GDF3, ActRIIB, ALK4, ALK5, and ALK7. In some embodiments, an ActRIIB antagonist polynucleotide, 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), BMP6, GDF11, GDF3, BMP10, ALK4, ALK5, and ALK7. In some embodiments, a GDF/BMP polynucleotide antagonist an ActRIIB antagonist polynucleotide, 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), BMP6, GDF11, GDF3, ActRIIB, BMP10, ALK5, and ALK7. In some embodiments, an ActRIIB antagonist polynucleotide, 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), BMP6, GDF11, GDF3, ActRIIB, ALK4, BMP10, and ALK7. In some embodiments, an ActRIIB antagonist polynucleotide, 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), BMP6, GDF11, GDF3, ActRIIB, ALK4, ALK5, and BMP10. In some embodiments, an ActRIIB antagonist polynucleotide, or combination of polynucleotide antagonists, as disclosed herein does not inhibit or does not substantially inhibit BMP9. In some embodiments, an ActRIIB antagonist polynucleotide, or combination of polynucleotide antagonists, as disclosed herein does not inhibit or does not substantially inhibit activin A.

[0188] 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, ActRIIB, ALK4, ALK5, ALK7, and BMP10 are known in the art. In addition, many different methods of generating polynucleotide antagonists are well known in the art. Therefore polynucleotide antagonists for use in accordance with this disclosure may be routinely made by the skilled person in the art based on the knowledge in the art and teachings provided herein.

[0189] 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.

[0190] 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.

[0191] 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.

[0192] 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].

[0193] 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, ActRIIB, GDF3, ALK4, ALK5, ALK7, and BMP10. RNAi refers to the expression of an RNA which interferes with the expression of the targeted mRNA. Specifically, RNAi silences a targeted gene via interacting with the specific mRNA through a siRNA (small interfering RNA). The ds RNA complex is then targeted for degradation by the cell. An siRNA molecule is a double-stranded RNA duplex of 10 to 50 nucleotides in length, which interferes with the expression of a target gene which is sufficiently complementary (e.g. at least 80% identity to the gene). In some embodiments, the siRNA molecule comprises a nucleotide sequence that is at least 85, 90, 95, 96, 97, 98, 99, or 100% identical to the nucleotide sequence of the target gene.

[0194] Additional RNAi molecules include short-hairpin RNA (shRNA); also short-interfering hairpin and micro-RNA (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.

[0195] 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).

[0196] 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.

[0197] 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.

[0198] 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)].

[0199] F. Follistatin and FLRG Antagonists

[0200] In other aspects, an ActRIIB antagonist is a follistatin or FLRG polypeptide. As described herein, follistatin and/or FLRG polypeptides 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 myelofibrosis, particularly treating, preventing or reducing the progression rate and/or severity of one or more myelofibrosis-associated complications (e.g., splenomegaly, extramedullary hematopoiesis, anemia, and fibrosis) and/or treat a patient treated with a Janus kinase inhibitor.

[0201] 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: 65-67, the follistatin N-terminal domain ("FSND" SEQ ID NO: 65), FSD2 (SEQ ID NO: 67), and to a lesser extent FSD1 (SEQ ID NO: 66) 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: 63) as described, for example, in WO2005/025601.

[0202] The human follistatin precursor polypeptide isoform FST344 is as follows:

(SEQ ID NO: 63; NCBI Reference No. NP_037541.1)

1 MVRARHQPGG LCLLLLLLCQ FMEDRSAQAG NCWLRQAKNG RCQVLYKTEL

51 SKEECCSTGR LSTSWTEEDV NDNTLFKWMI FNGGAPNCIP CKETCENVDC

-continued

```
101 GPGKKCRMNK KNKPRCVCAP DCSNITWKGP VCGLDGKTYR NECALLKARC

151 KEQPELEVQY QGRCKKTCRD VFCPGSSTCV VDQTNNAYCV TCNRICPEPA

201 SSEQYLCGND GVTYSSACHL RKATCLLGRS IGLAYEGKCI KAKSCEDIQC

251 TGGKKCLWDF KVGRGRCSLC DELCPDSKSD EPVCASDNAT YASECAMKEA

301 ACSSGVLLEV KHSGSCNSIS EDTEEEEEDE DQDYSFPISS ILEW
```

[0203] 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.

[0204] The human follistatin precursor polypeptide isoform FST317 is as follows:

(SEQ ID NO: 64; NCBI Reference No. NP_006341.1)

```
  1 MVRARHQPGG LCLLLLLLCQ FMEDRSAQAG NCWLRQAKNG RCQVLYKTEL

 51 SKEECCSTGR LSTSWTEEDV NDNTLFKWMI FNGGAPNCIP CKETCENVDC

101 GPGKKCRMNK KNKPRCVCAP DCSNITWKGP VCGLDGKTYR NECALLKARC

151 KEQPELEVQY QGRCKKTCRD VFCPGSSTCV VDQTNNAYCV TCNRICPEPA

201 SSEQYLCGND GVTYSSACHL RKATCLLGRS IGLAYEGKCI KAKSCEDIQC

251 TGGKKCLWDF KVGRGRCSLC DELCPDSKSD EPVCASDNAT YASECAMKEA

301 ACSSGVLLEV KHSGSCN
```

The signal peptide is underlined.

[0205] The follistatin N-terminal domain (FSND) sequence is as follows:

(SEQ ID NO: 65; FSND)

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GNCWLRQAKNGRCQVLYKTELSKEECCSTGRLSTSWTEEDVNDNTLF

KWMIFNGGAPNCIPCK
```

[0206] The FSD1 and FSD2 sequences are as follows:

(SEQ ID NO: 66; FSD1)

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ETCENVDCGPGKKCRMNKKNKPRCV
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(SEQ ID NO: 67; FSD2)

```
KTCRDVFCPGSSTCVVDQTNNAYCVT
```

[0207] 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, 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.

[0208] The human FLRG precursor (follistatin-related protein 3 precursor) polypeptide is as follows:

```
  1MRPGAPGPLW PLPWGALAWA VGFVSSMGSG NPAPGGVCWL QQGQEATCSL

 51VLQTDVTRAE CCASGNIDTA WSNLTHPGNK INLLGFLGLV HCLPCKDSCD

101GVECGPGKAC RMLGGRPRCE CAPDCSGLPA RLQVCGSDGA TYRDECELRA

151ARCRGHPDLS VMYRGRCRKS CEHVVCPRPQ SCVVDQTGSA HCVVCRAAPC

201PVPSSPGQEL CGNNNVTYIS SCHMRQATCF LGRSIGVRHA GSCAGTPEEP

251PGGESAEEEE NFV (SEQ ID NO: 68; NCBI Reference No. NP_005851.1)
```

The signal peptide is underlined.

[0209] 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 ActRIIB 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.

3. Screening Assays

[0210] In certain aspects, the present disclosure relates to the use of the subject ActRIIB polypeptides and variants thereof (e.g., GDF8 traps) to identify compounds (agents) which are agonist or antagonists of ActRIIB polypeptides. Compounds identified through this screening can be tested to assess their ability to treat myelofibrosis, for example, in animal models.

[0211] There are numerous approaches to screening for therapeutic agents for treating myelofibrosis by targeting ActRIIB signaling (e.g., Smad signaling). In certain embodiments, high-throughput screening of compounds can be carried out to identify agents that perturb ActRIIB-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 ActRIIB polypeptide to its binding partner, such as an ActRIIB ligand (e.g., activin A, activin B, activin AB, activin C, GDF8, GDF3, GDF11 or BMP10). Alternatively, the assay can be used to identify compounds that enhance binding of an ActRII polypeptide to its binding partner such as an ActRIIB ligand. In a further embodiment, the compounds can be identified by their ability to interact with an ActRIIB polypeptide.

[0212] 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.

[0213] The test compounds of the disclosure can be provided as single, discrete entities, or provided in libraries of greater complexity, such as made by combinatorial chemistry. These libraries can comprise, for example, alcohols, alkyl halides, amines, amides, esters, aldehydes, ethers and other classes of organic compounds. Presentation of test compounds to the test system can be in either an isolated form or as mixtures of compounds, especially in initial screening steps. Optionally, the compounds may be optionally derivatized with other compounds and have derivatizing groups that facilitate isolation of the compounds. Non-limiting examples of derivatizing groups include biotin, fluorescein, digoxygenin, green fluorescent protein, isotopes, polyhistidine, magnetic beads, glutathione S-transferase (GST), photoactivatible crosslinkers or any combinations thereof.

[0214] In many drug-screening programs which test libraries of compounds and natural extracts, high-throughput assays are desirable in order to maximize the number of compounds surveyed in a given period of time. Assays which are performed in cell-free systems, such as may be derived with purified or semi-purified proteins, are often preferred as "primary" screens in that they can be generated to permit rapid development and relatively easy detection of an alteration in a molecular target which is mediated by a test compound. Moreover, the effects of cellular toxicity or bioavailability of the test compound can be generally ignored in the in vitro system, the assay instead being focused primarily on the effect of the drug on the molecular target as may be manifest in an alteration of binding affinity between an ActRIIB polypeptide and its binding partner (e.g., an ActRIIB ligand).

[0215] 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.

[0216] Complex formation between an ActRIIB polypeptide 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., ^{32}P, ^{35}S, ^{14}C or ^{3}H), fluorescently labeled (e.g., FITC), or enzymatically labeled ActRIIB polypeptide and/or its binding protein, by immunoassay, or by chromatographic detection.

[0217] In certain embodiments, the present disclosure contemplates the use of fluorescence polarization assays and fluorescence resonance energy transfer (FRET) assays in measuring, either directly or indirectly, the degree of interaction between an ActRIIB polypeptide 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.

[0218] Moreover, the present disclosure contemplates the use of an interaction trap assay, also known as the "two-hybrid assay," for identifying agents that disrupt or potentiate interaction between an ActRIIB polypeptide 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 an ActRII polypeptide or GDF trap 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].

[0219] In certain embodiments, the subject compounds are identified by their ability to interact with an ActRIIB polypeptide. The interaction between the compound and the ActRIIB polypeptide may be covalent or non-covalent. For example, such interaction can be identified at the protein level using in vitro biochemical methods, including photocrosslinking, 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 an ActRIIB polypeptide. This may include a solid-phase or fluid-phase binding event. Alternatively, the gene encoding an ActRIIB polypeptide can be transfected with a reporter system (e.g., β-galactosidase, luciferase, or green fluorescent protein) into a cell and screened against the library preferably by high-throughput screening or with individual members of the library. Other mechanism-based binding assays may be used; for example, binding assays which detect changes in free energy. Binding assays can be performed with the target fixed to a well, bead or chip or captured by an immobilized antibody or resolved by capillary electrophoresis. The bound compounds may be detected usually using colorimetric endpoints or fluorescence or surface plasmon resonance.

4. Exemplary Therapeutic Uses

[0220] As described in the examples of the disclosure, it has been discovered that an ActRIIB antagonist (inhibitor) can be used to treat myelofibrosis patients, particularly ameliorating various complications of the disease including, for example, splenomegaly, extramedullary hematopoiesis, and fibrosis. In particular, the data presented herein show that a GDF trap polypeptide decrease splenomegaly, extramedullary hematopoiesis, and fibrosis in a JAK2V617F model of myelofibrosis. Accordingly, in certain aspects, the discloses relates to compositions and method for treating myelofibrosis, particularly treating or preventing one or more complications of myelofibrosis (splenomegaly, extramedullary hematopoiesis, anemia, and fibrosis), by administering to a patient in need thereof an effective amount of one or more ActRIIB antagonists, optionally in combination of one or more other supportive therapies or active agents for treating myelofibrosis.

[0221] As used herein, a therapeutic that "prevents" a disorder or condition refers to a compound that, in a statistical sample, reduces the occurrence of the disorder or condition in the treated sample relative to an untreated control sample, or delays the onset or reduces the severity of one or more symptoms of the disorder or condition relative to the untreated control sample. The term "treating" as used herein includes amelioration or elimination of the condition once it has been established. In either case, prevention or treatment may be discerned in the diagnosis provided by a physician or other health care provider and the intended result of administration of the therapeutic agent.

[0222] In general, treatment or prevention of a disease or condition as described in the present disclosure is achieved by administering an ActRIIB 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.

[0223] 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).

[0224] Myelofibrosis is a clonal neoplastic disorder of hematopoiesis, generally characterized by progressive bone marrow fibrosis resulting in increasingly ineffective hematopoiesis, extramedullary hematopoiesis, a variety of inflammatory complications, and shortened survival [Mascarenhas et al. (2012) *Curr Med Chem* 19:4399-4413; and Vannucchi et al. (2011) *Hematol Am Soc Hematol Educ Prog* 2011:222-230]. It is one of the myeloproliferative disorders of the bone marrow in which excess cells are produced. Production of cytokines such fibroblast growth factor by the abnormal hematopoietic cell clone leads to replacement of the hematopoietic tissue of the bone marrow by connective tissue via collagen fibrosis. The decrease in hematopoietic tissue impairs the patient's ability to generate new blood cells, resulting in progressive pancytopenia, a shortage of all blood types. However, the proliferation and fibroblasts and deposition of collagen is a secondary phenomenon, and the fibroblasts themselves are not part of the abnormal cell clone. As a result of progressive scarring, or fibrosis, of the bone marrow, patients develop extramedullary hematopoiesis as the haemopoetic cells are forced to migrate to other areas, particularly the liver and spleen. This causes an enlargement of these organs. In the liver, the condition is call hepatomegaly. Enlargement of the spleen is called splenomegaly, which also contributes pancytopenia, particularly thrombocytopenia and anemia. There are also reports of extramedullary hematopoiesis occurring in the lungs and lymph nodes. Another complication of extramedullary hematopoiesis is poikilocytosis, of the presence of abnormally shaped red blood cells. Common clinical manifestations of myelofibrosis include progressive hepatosplenomegaly, abnormal blood counts, and debilitating symptoms such as fatigue, weight loss, night sweats, fever, pruritus,

bone pain, early satiety, abdominal pain or discomfort, arthralgias, myalgias, parasthesias, cachexia, splenic infarct and bleeding. Until recently, the only treatment with a clearly demonstrated impact on disease progression has been allogeneic hematopoietic stem cell transplantation alloHSCT, but treatment-related mortality is high and only a minority of patients qualify for this intensive therapy [Gupta et al. (2012) *Blood* 120: 1367-1379].

[0225] In certain aspects, ActRIIB 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 myelofibrosis (e.g., primary myelofibrosis, post-polycythemia vera myelofibrosis, and post-essential thrombocythemia myelofibrosis). In particular, ActRIIB 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 one or more complications of myelofibrosis including, for example, ineffective hematopoiesis, anemia, inflammation, fibrosis (e.g., bone marrow fibrosis, spleen fibrosis, and liver fibrosis), pancytopenia, thrombocytopenia, extramedullary hematopoiesis (e.g., splenic extramedullary hematopoiesis, hepatic extramedullary hematopoiesis, pulmonary extramedullary hematopoiesis, and lymphatic extramedullary hematopoiesis), hepatomegaly, splenomegaly, osteosclerosis, osteomyelofibrosis, poikilocytosis, fatigue, weight loss, night sweats, fever, pruritus, bone pain, early satiety, abdominal pain or discomfort, arthralgias, myalgias, parasthesias, cachexia, splenic infarct, and bleeding.

[0226] Current diagnosis of primary myelofibrosis (PMF) is based on World Health Organization (WHO)-criteria and involves a composite assessment of clinical and laboratory features [Tefferi A et al. (2007) Blood. 110:1092-1097]. There are three WHO diagnostic primary criteria: 1) megakaryocyte proliferation and atypia (small to large megakaryocytes with aberrant nuclear/cytoplasmic ratio and hyperchromatic and irregularly folded nuclei and dense clustering) accompanied by either reticulin and/or collagen fibrosis or, in the absence of reticulin fibrosis, the megakaryocyte changes must be accompanied by increased marrow cellularity, granulocytic proliferation, and often decreased erythropoiesis (i.e., pre-fibrotic primary myelofibrosis), 2) not meeting WHO criteria for chronic myelogenous leukemia, polycythemia vera, myelodysplastic syndrome, or other myeloid neoplasm, and 3) demonstration of JAK2V617F or other clonal marker or no evidence of reactive bone marrow fibrosis. In addition, there are four WHO diagnostic minor criteria: 1) leukoerythroblastosis, 2) increased serum LDH levels, 3) anemia, and 4) palpable splenomegaly. Peripheral blood leukoerythroblastosis (i.e., presence of nucleated red cells, immature granulocytes, and dacryocytes) is a typical but not invariable feature of PMF; prefibrotic PMF might not display overt leukoerythroblastosis [Kvasnicka et al. (2010) Am J Hematol. 85:62-69]. Bone marrow fibrosis in PMF is usually associated with JAK2V617F or mutant CALR, or MPL, trisomy 9, or del(13q) [Hussein et al. (2009) Eur J Haematol. 82:329-338]. The presence of these genetic markers, therefore, strongly supports a diagnosis of PMF, in the presence of a myeloid neoplasm associated with bone marrow fibrosis. In certain aspects, the disclosure relates to methods and uses of ActRIIB antagonists to treat, prevent, or reduce the progression rate and/or severity of primary myelofibrosis, particularly treating, preventing, or reducing the progression rate and/or severity of one or more complications of primary myelofibrosis.

[0227] Current diagnosis of post-polycythemia vera myelofibrosis (post-PV MF) and post-essential thrombocythemia myelofibrosis (post-ET MF) are based on the criteria published by the International Working Group for MPN Research and Treatment (IWG-MRT) [Barosi G et al. (2008) Leukemia. 22:437-438]. There are two IWG-MRT primary criteria for post-PV MF: 1) documentation of previous diagnosis of polycythemia vera as defined by the WHO criteria, and 2) bone marrow fibrosis grade 2-3 (on 0-3 scale) or grade 3-4 (on 0-4 scale). Grade 2-3 according to the European classification: diffuse, often coarse fiber network with no evidence of collagenization (negative trichrome stain) or diffuse, coarse fiber network with areas of collagenization (positive trichrome stain) [Thiele et al. (2005) Haematologica. 90:1128-1132]. Grade 3-4 according to the standard classification: diffuse and dense increase in reticulin with extensive intersections, occasionally with only focal bundles of collagen and/or focal osteosclerosis or diffuse and dense increase in reticulin with extensive intersections with coarse bundles of collagen, often associated with significant osteosclerosis [Manoharan et al. (1979) Br J Haematol 43:185-190]. In addition, there are four IWG-MRT diagnostic secondary criteria, of which two must be detected in a patient along with the IWG-MRT primary criteria for a post-PV MF diagnosis: 1) anemia or sustained loss of requirement for phlebotomy in the absence of cytoreductive therapy, 2) a leukoerythroblastic peripheral blood picture, 3) increasing splenomegaly defined as either an increase in palpable splenomegaly of ≥5 cm or the appearance of a newly palpable splenomegaly, 4) development of ≥1 of three constitutional symptoms: >10% weight loss in six months, night sweats, unexplained fever. There are two IWG-MRT primary criteria for post-ET MF: 1) documentation of a previous diagnosis of polycythemia vera as defined by the WHO criteria, 2) bone marrow fibrosis grade 2-3 (on 0-3 scale) or grade 3-4 (on 0-4 scale). In addition, there are five IWG-MRT diagnostic secondary criteria, of which two must be detected in a patient along with the IWG-MRT primary criteria for a post-ET MF diagnosis: 1) anemia and a ≥2 g/dL decrease from baseline hemoglobin levels, 2) a leukoerythroblastic peripheral blood picture, 3) increasing splenomegaly defined as either an increase in palpable splenomegaly of ≥5 cm or the appearance of a newly palpable splenomegaly, 4) increased lactate dehydrogenase, and 5) development of ≥1 of three constitutional symptoms: >10% weight loss in six months, night sweats, unexplained fever. In certain aspects, the disclosure relates to methods and uses of ActRIIB antagonists to treat, prevent, or reduce the progression rate and/or severity of post-polycythemia vera myelofibrosis, particularly treating, preventing, or reducing the progression rate and/or severity of one or more complications of post-polycythemia vera myelofibrosis. In certain aspects, the disclosure relates to methods and uses of ActRIIB antagonists to treat, prevent, or reduce the progression rate and/or severity of post-essential thrombocythemia myelofibrosis, particularly treating, preventing, or reducing the progression rate and/or severity of one or more complications of post-essential thrombocythemia myelofibrosis.

[0228] Robust prognostic modeling in myelofibrosis started with the development of the International Prognostic

Scoring System (IPSS) in 2009 [Cervantes F et al. (2009) Blood 113:2895-2901]. The IPSS for myelofibrosis is applicable to patients being evaluated at time of initial diagnosis and uses five independent predictors of inferior survival: age >65 years, hemoglobin <10 g/dL, leukocyte count >25×109/L, circulating blasts ≥1%, and presence of constitutional symptoms. The presence of 0, 1, 2, and ≥3 adverse factors defines low, intermediate-1, intermediate-2, and high-risk disease, respectively. The corresponding median survivals were 11.3, 7.9, 4, and 2.3 years, respectively. In certain aspects, the disclosure relates to methods and uses of ActRIIB antagonists to treat, prevent, or reduce the progression rate and/or severity of myelofibrosis in a patient that has low, intermediate-1, intermediate-2, or high-risk myelofibrosis according to the IPSS. In some embodiments, the disclosure relates to methods and uses of ActRIIB antagonists to prevent or delay myelofibrosis risk progression according to the IPSS (e.g., prevents or delays risk progression from low to intermediate-1 risk, intermediate-1 to intermediate-2 risk, and intermediate-2 to high risk according to the IPSS). In some embodiments, the disclosure relates to methods and uses of ActRIIB antagonists to promote or increase myelofibrosis risk regression according to the IPSS (e.g., promotes or increase regression from high to intermediate-2 risk, intermediate-2 to intermediate-1 risk, and intermediate-1 to low risk according to the IPSS).

[0229] The IWG-MRT subsequently developed a dynamic prognostic model (dynamic international prognostic scoring system [DIPSS]) that uses the same prognostic variables used in IPSS but can be applied at any time during the disease course [Passamonti F et al. (2010) Blood. 115:1703-1708]. DIPSS assigns two, instead of one, adverse points for hemoglobin <10 g/dL and risk categorization is accordingly modified: low (0 adverse points), intermediate-1 (1 or 2 points), intermediate-2 (3 or 4 points), and high (5 or 6 points). The corresponding median survivals were not reached, 14.2, 4, and 1.5 years. In certain aspects, the disclosure relates to methods and uses of ActRIIB antagonists to treat, prevent, or reduce the progression rate and/or severity of myelofibrosis in a patient that has low, intermediate-1, intermediate-2, or high-risk myelofibrosis according to the DIPSS. In some embodiments, the disclosure relates to methods and uses of ActRIIB antagonists to prevent or delay myelofibrosis risk progression according to the DIPSS (e.g., prevents or delays risk progression from low to intermediate-1 risk, intermediate-1 to intermediate-2 risk, and intermediate-2 to high risk according to the DIPSS). In some embodiments, the disclosure relates to methods and uses of ActRIIB antagonists to promote or increase myelofibrosis risk regression according to the DIPSS (e.g., promotes or increase regression from high to intermediate-2 risk, intermediate-2 to intermediate-1 risk, and intermediate-1 to low risk according to the DIPSS).

[0230] IPSS- and DIPSS-independent risk factors for survival in myelofibrosis were subsequently identified and included unfavorable karyotype (i.e., complex karyotype or sole or two abnormalities that include +8, −7/7q−, i(17q), inv(3), −5/5q−, 12p−, or 11q23 rearrangement) [Hussein et al. (2010) Blood. 115:496-499], red cell transfusion need [Tefferi et al. (2009) Am J Hematol. 85:14-17], and platelet count <100×109/L [Patnaik et al. (2010) Eur J Haematol. 84:105-108]. Accordingly, DIPSS was modified into DIPSS-plus by incorporating these three additional DIPSS-independent risk factors: platelet count <100×109/L, red cell transfusion need, and unfavorable karyotype. The four DIPSS-plus risk categories based on the aforementioned eight risk factors are low (no risk factors), intermediate-1 (one risk factor), intermediate-2 (two or three risk factors), and high (four or more risk factors) with respective median survivals of 15.4, 6.5, 2.9, and 1.3 years. In certain aspects, the disclosure relates to methods and uses of ActRIIB antagonists to treat, prevent, or reduce the progression rate and/or severity of myelofibrosis in a patient that has low, intermediate-1, intermediate-2, or high-risk myelofibrosis according to the DIPSS-plus. In some embodiments, the disclosure relates to methods and uses of ActRIIB antagonists to prevent or delay myelofibrosis risk progression according to the DIPSS-plus (e.g., prevents or delays risk progression from low to intermediate-1 risk, intermediate-1 to intermediate-2 risk, and intermediate-2 to high risk according to the DIPSS-plus). In some embodiments, the disclosure relates to methods and uses of ActRIIB antagonists to promote or increase myelofibrosis risk regression according to the DIPSS-plus (e.g., promotes or increase regression from high to intermediate-2 risk, intermediate-2 to intermediate-1 risk, and intermediate-1 to low risk according to the DIPSS-plus).

[0231] Since the publication of DIPSS-plus, several studies that suggest additional prognostic information have been published. For example, a >80% 2-year mortality in myelofibrosis was predicted by monosomal karyotype, inv(3)/i(17q) abnormalities, or any two of circulating blasts >9%, leukocytes ≥40×109/L or other unfavorable karyotype [Tefferi et al. (2011) Blood. 118:4595-4598.]. Similarly, inferior survival in myelofibrosis has been associated with nullizygosity for JAK2 46/1 haplotype, low JAK2V617F allele burden, or presence of IDH, EZH2, SRSF2, or ASXL1 mutations [Tefferi, Ayalew (2014) Am. J. Hematol. 89:916-925]. In contrast, the presence or absence of JAK2V617F, MPL, or TET2 mutations did not appear to affect survival. Survival in myelofibrosis was also affected by increased serum IL-8 and IL-2R levels as well as serum free light chain levels, both independent of DIPSS-plus. Most recently, Tefferi et al. studied 254 patients with myelofibrosis and reported mutational frequencies of 58% for JAK2, 25% CALR, 8% MPL, and 9% wild-type for all three mutations (i.e., triple-negative) [Tefferi et al. (2014) Leukemia. prepublished as DOI 10.1038/leu.2014.3]. CALR mutational frequency in JAK2/MPL-unmutated cases was 74%. CALR mutations were associated with younger age, higher platelet count, and lower DIPSS-plus score. CALR-mutated patients were also less likely to be anemic, require transfusions, or display leukocytosis. Spliceosome mutations were infrequent in CALR-mutated patients. In a subsequent international study of 570 patients, the authors reported the longest survival in CALR+ASXL1− patients (median 10.4 years) and shortest in CALR−ASXL1+ patients (median 2.3 years) [Tefferi et al. (2014) Leukemia. prepublished as DOI 10.1038/leu.2014.57]. CALR+ ASXL1+ and CALR−ASXL1− patients had similar survival and were grouped together in an intermediate risk category (median survival 5.8 years). As is becoming evident for overall survival, leukemia-free survival is also significantly compromised in patients carrying certain mutations including IDH and SRSF2 [Tefferi et al. (2012) Leukemia. 26:475-480; Lasho et al. (2012) Blood. 120:4168-4171]. In addition, mutations in LNK and THPO have also been associated with myelofibrosis.

[0232] The discovery of a Janus kinase 2 (JAK2) gain-of-function mutation, JAK2V617F, has led to significant improvements in the understanding of the biology underlying myelofibrosis, as well as the development of ruxolitinib, a JAK2 inhibitor which is the first drug approved by the FDA for the treatment of myelofibrosis [Baxter et al. (2005) *Lancet* 365:1054-1061; James C. et al. (2005) *Nature* 434: 1144-1148; Kralovics et al. (2005) *N Engl J Med.* 352:1779-1790; and Levine et al. (2005) Cancer Cell 7:387-397]. The Janus kinase family of receptor tyrosine kinases includes four different proteins (JAK1, JAK2, JAK3 and TYK2), and this family proteins is known to play a crucial role in myeloid and lymphoid cell growth and development. In particular, they mediate intracellular interactions from cytokine receptors, resulting in activation of signal transducer activator of transcription (STAT) factors and downstream promotion of genes that regulate cellular proliferation and differentiation [Quintas-Cardama et al. (2011) *Nat Rev Drug Discov* 10:127-140]. The JAK217617F mutation results in constitutive activation of JAK2 and thus promotes myeloid cell proliferation and differentiation. Other Janus kinase inhibitors undergoing clinical trials include, for example, fedratinib (SAR302503), monoelotinib (CYT387), pacritinib, lestaurtinib, AZD-1480, BMS-911543, NS-018, LY2784544, SEP-701, XL019, and AT-9283.

[0233] In certain aspects, the disclosure relates to methods and uses of ActRIIB antagonists to treat, prevent, or reduce the progression rate and/or severity of myelofibrosis in a patient that has one or more of: monosomal karyotype, inv(3)/i(17q) abnormalities, circulating blasts >9% and/or leukocytes ≥40×109/L, nullizygosity for JAK2 46/1 haplotype, JAK2V617F mutation, IDH1 mutation, IDH2 mutation, EZH2 mutation, SRSF2 mutation, ASXL1 mutation, increased serum IL-8 levels, increased serum IL-2R levels, increased free light chain levels, JAK1 mutation, JAK2 mutation, JAK3 mutation, TYK2 mutation, MPL mutation, CALR mutation, CALR+ASXL1−, CALR−ASKL1+, CALR+ASKL1+, CALR−ASKL1−, TET2 mutation, THPO mutation, and LNK mutation.

[0234] The management of anemia can be one of the most challenging aspects of treating patients with myelofibrosis [Tefferi A. (2011) *Blood* 117(13):3949-3504; Barosi et al. (2011) *Expert Opin Pharmacother* 12(10):1597-1611]. Blood transfusion (whole blood or red blood cell transfusion) is a standard therapy for symptomatically anemic myelofibrosis patients. In addition to transfusion, there are a variety of conventional agents used to treat anemia in these patients. For example, erythropoiesis-stimulating agents [e.g., ESAs such as erythropoietin (EPO) and derivatives thereof], androgens (e.g., testosterone enanthate and fluoxymesterone), prednisone, danazol, thalidomide, prednisone, and lenalidomide are commonly used to treat anemia in myelofibrosis patients. In general, ESAs are used in patients with moderate, non-transfusion-dependent anemia and low serum erythropoietin levels. Response rates vary from 20-60% with no clear support for darbepoetin-alpha versus conventional recombinant erythropoietin. ESAs responses are usually short-lived (around 1 year). If ESAs do not work or have poor efficacy, danazol or androgen preparations are typically used to treat anemic patients with a response rate around 20%. Low-dose thalidomide in association with tapering prednisone has produced responses in anemia in approximately 20-40% of patients [Thapaliya et al. (2011) *Am J Hematol* 86(1):86-98]. However, thalidomide treatment is often poorly tolerated with peripheral neuropathies, constipation, and somnolence leading to discontinuation of the drug in most patients. In myelofibrosis patients with del(5q31)-associated anemia, lenalidomide is the recommended first line therapy because significant improvement, with resolution of anemia and occasionally evidenced of molecular remission, has been reported [Tefferi et al. (2007) *Leukemia* 21(8):1827-1828]. In certain aspects, the disclosure relates to methods and uses of ActRIIB antagonists to treat, prevent, or reduce the progression rate and/or severity of myelofibrosis in a patient that has anemia. In some embodiments, the disclosure relates to methods and uses of ActRIIB antagonists to treat, prevent, or reduce the progression rate and/or severity of anemia in a myelofibrosis patient. In some embodiments, the disclosure relates to a method of treating, preventing, or reducing the progression rate and/or severity myelofibrosis in a patient in need thereof of comprises administration of one or more ActRIIB antagonists conjointly with one or more additional active agents selected from the group consisting of: an erythropoiesis-stimulating agent [e.g., ESAs such as erythropoietin (EPO) and derivatives thereof], androgen (e.g., testosterone enanthate and fluoxymesterone), prednisone, danazol, thalidomide, prednisone, and lenalidomide. In some embodiments, the disclosure relates to a method of treating, preventing, or reducing the progression rate and/or severity anemia in a myelofibrosis patient in need thereof of comprises administration of one or more ActRIIB antagonists conjointly with one or more additional active agents selected from the group consisting of: an erythropoiesis-stimulating agent [e.g., ESAs such as erythropoietin (EPO) and derivatives thereof], androgen (e.g., testosterone enanthate and fluoxymesterone), prednisone, danazol, thalidomide, prednisone, and lenalidomide. In some embodiments, the disclosure relates a method of treating, preventing, or reducing the progression rate and/or severity anemia in a myelofibrosis patient in need thereof of comprises administration of one or more ActRIIB antagonists conjointly with a blood transfusion (whole blood or red blood cell transfusion).

[0235] When monitoring hemoglobin and/or hematocrit levels in humans, a level of less than normal for the appropriate age and gender category may be indicative of anemia, although individual variations are taken into account. For example, a hemoglobin level from 10-12.5 g/dl, and typically about 11.0 g/dl is considered to be within the normal range in healthy adults, although, in terms of therapy, a lower target level may cause fewer cardiovascular side effects. See, e.g., Jacobs et al. (2000) Nephrol Dial Transplant 15, 15-19. Alternatively, hematocrit levels (percentage of the volume of a blood sample occupied by the cells) can be used as a measure of anemia. Hematocrit levels for healthy individuals range from about 41-51% for adult males and from 35-45% for adult females. In certain embodiments, a patient may be treated with a dosing regimen intended to restore the patient to a target level of red blood cells, hemoglobin, and/or hematocrit or allow the reduction or elimination of red blood cell transfusions (reduce transfusion burden) while maintaining an acceptable level of red blood cells, hemoglobin and/or hematocrit. As hemoglobin and hematocrit levels vary from person to person, optimally, the target hemoglobin and/or hematocrit level can be individualized for each patient.

[0236] In patients who receive frequent transfusions of whole blood or red blood cells, normal mechanisms of iron

homeostasis can be overwhelmed, eventually leading to toxic and potentially fatal accumulation of iron in vital tissues such as heart, liver, and endocrine glands. Regular red blood cell transfusions require exposure to various donor units of blood and hence a higher risk of alloimmunization. Difficulties with vascular access, availability of and compliance with iron chelation, and high cost are some of the reasons why it can be beneficial to limit the number of red blood cell transfusions.

[0237] In certain aspects, one or more ActRIIB antagonists, optionally combined with an EPO receptor activator, may be used in combination with one or more iron-chelating molecules to promote iron excretion in the urine and/or stool and thereby prevent or reverse tissue iron overload in myelofibrosis patients. Effective iron-chelating agents should be able to selectively bind and neutralize ferric iron, the oxidized form of non-transferrin bound iron which likely accounts for most iron toxicity through catalytic production of hydroxyl radicals and oxidation products [see, e.g., Esposito et al. (2003) Blood 102:2670-2677]. These agents are structurally diverse, but all possess oxygen or nitrogen donor atoms able to form neutralizing octahedral coordination complexes with individual iron atoms in stoichiometries of 1:1 (hexadentate agents), 2:1 (tridentate), or 3:1 (bidentate) [Kalinowski et al. (2005) Pharmacol Rev 57:547-583]. In general, effective iron-chelating agents also are relatively low molecular weight (e.g., less than 700 daltons), with solubility in both water and lipids to enable access to affected tissues. Specific examples of iron-chelating molecules include deferoxamine, a hexadentate agent of bacterial origin requiring daily parenteral administration, and the orally active synthetic agents deferiprone (bidentate) and deferasirox (tridentate). Combination therapy consisting of same-day administration of two iron-chelating agents shows promise in patients unresponsive to chelation monotherapy and also in overcoming issues of poor patient compliance with dereroxamine alone [Cao et al. (2011) Pediatr Rep 3(2):e17; and Galanello et al. (2010) Ann NY Acad Sci 1202:79-86].

[0238] One or more ActRIIB antagonists of the disclosure may be used in combination with an EPO receptor activator to achieve an increase in red blood cells, particularly at lower dose ranges. This may be beneficial in reducing the known off-target effects and risks associated with high doses of EPO receptor activators. The primary adverse effects of ESAs include, for example, an excessive increase in the hematocrit or hemoglobin levels and polycythemia. Elevated hematocrit levels can lead to hypertension (more particularly aggravation of hypertension). Other adverse effects of ESAs which have been reported, some of which relate to hypertension, are headaches, influenza-like syndrome, obstruction of shunts, myocardial infarctions and cerebral convulsions due to thrombosis, hypertensive encephalopathy, and red cell blood cell aplasia. See, e.g., Singibarti (1994) J. Clin Investig 72(suppl 6), S36-S43; Horl et al. (2000) Nephrol Dial Transplant 15(suppl 4), 51-56; Delanty et al. (1997) Neurology 49, 686-689; and Bunn (2002) N Engl J Med 346(7), 522-523). In certain embodiments, the present disclosure provides methods of treating or preventing anemia in a myelofibrosis patient by administering to the patient a therapeutically effective amount of one or more ActRIIB antagonists and a EPO receptor activator. In certain embodiments, an ActRIIB antagonists of the disclosure may be used in combination with EPO receptor activators to reduce the required dose of these activators in patients that are susceptible to adverse effects of ESAs. These methods may be used for therapeutic and prophylactic treatments of a patient.

[0239] Provided that ActRIIB antagonists of the present disclosure act by a different mechanism that ESAs, these antagonists may be useful for increasing red blood cell and hemoglobin levels in patients that do not respond well to ESAs or other EPO receptor activators. For example, an ActRIIB antagonist of the present disclosure may be beneficial for a patient in which administration of a normal to increased (>300 IU/kg/week) dose of ESA does not result in the increase of hemoglobin level up to the target level. An inadequate response to ESAs can be either constitutive (observed upon the first treatment with ESA) or acquired (observed upon repeated treatment with ESA).

[0240] Cytoreductive agents have been the treatment of choice for most patients with symptomatic splenomegaly. Hydroxycarbamide (hydroxyurea, HC) is the most commonly used cytoreductive agent, which usually produces modest responses at higher doses. However, HC can often exacerbate cytopenias and therefore is often not well tolerated. Reductions in spleen size from 25%-50% have been reported in up to 35% of the patients treated with HC [Martinez-Trillos et al. (2010) *Ann Hematol.* 89(12):1233-1237]. In patients who do not respond to HC, busulfan or melphalan may be used, especially in older patients, since there is evidence that these agents can increase the frequency of leukemic transformation. Spleen responses with low-dose thalidomide are low (<20%). However, lenalidomide has been shown to result in a 33% response rate in a study that included some patients who had failed prior thalidomide therapy. In cases of massive refractory splenomegaly, monthly intravenous cladribine courses have produced responses up to 50%, with severe but reversible cytopenias being the primary toxicity [Faoro et al. (2005) *Eur Haematol* 74(2):117-120]. Ruxolitinib has proven superior to HC in recent studies and thus is becoming first line agent to control symptomatic or progressive splenomegaly. Unfortunately, a common side effect of ruxolitinib is the development, or worsening, of anemia in myelofibrosis patients. Therefore, while JAK inhibitors may be useful to treat splenomegaly, they may actually worsen other complications of myelofibrosis, particularly anemia and anemia-associated disorders.

[0241] In addition to JAK2 inhibition, several other treatment strategies are under investigation for the treatment of myeloproliferative disorders, including immunomodulating drugs (e.g., pomalidomide), inhibitors of the mammalian target of the mTOR pathway (e.g., rapamycin, sirolimus, deforolimus, everolimus, temsirolimus, NVP-BEZ235, BGT226, SF1126, PK1-587, INK128, AZD8055, and AZD2014), and epigenetic factors modulators (e.g., histone deacetylases inhibitors such as givinostat (ITF2357), panobinostat (LBH589) and pracinostat) [Mascarenhas et al. (2013) Haematologica 98(10): 1499-1509].

[0242] The present disclosure further contemplates the use of ActRIIB antagonist in combination with one or more other therapeutic modalities in the treatment of patients as described herein. For example, ActRIIB antagonists can be administered in combination with cytotoxins, immunosuppressive agents, radiotoxic agents, and/or therapeutic antibodies. Particular co-therapeutics contemplated by the present invention include, but are not limited to, steroids (e.g.,

corticosteroids, such as Prednisone), immune-suppressing and/or anti-inflammatory agents (e.g., gamma-interferon, cyclophosphamide, azathioprine, methotrexate, penicillamine, cyclosporine, colchicine, antithymocyte globulin, mycophenolate mofetil, and hydroxychloroquine), cytotoxic drugs, calcium channel blockers (e.g., nifedipine), angiotensin converting enzyme inhibitors (ACE) inhibitors, paraaminobenzoic acid (PABA), dimethyl sulfoxide, transforming growth factor beta (TGFβ) inhibitors, interleukin-5 (IL-5) inhibitors, and pan caspase inhibitors. Additional agents that may be used in combination with ActRIIB antagonist include, but are not limited to, lectins (as described in, for example, U.S. Pat. No. 7,026,283, the entire contents of which is incorporated herein by reference), as well as the anti-fibrotic agents described by Wynn et al (2007, J Clin Invest 117:524-529). For example, additional anti-fibrotic agents and therapies include, but are not limited to, various anti-inflammatory/immunosuppressive/cytotoxic drugs (including colchicine, azathioprine, cyclophosphamide, prednisone, thalidomide, pentoxifylline and theophylline), TGFβ signaling modifiers (including relaxin, SMAD7, HGF, and BMP7, as well as TGFβ1, TβRI, EGR-I, and CTGF inhibitors), cytokine and cytokine receptor antagonists (inhibitors of IL-1β, IL-5, IL-6, IL-13, IL-21, IL-4R, IL-13Rαl, GM-CSF, TNF-α, oncostatin M, W1SP-I, and PDGFs), cytokines and chemokincs (IFN-γ, IFN-α/β, IL-12, IL-10, HGF, CXCL10, and CXCL11), chemokine antagonists (inhibitors of CXCL1, CXCL2, CXCL12, CCL2, CCL3, CCL6, CCL17, and CCL18), chemokine receptor antagonists (inhibitors of CCR2, CCR3, CCR5, CCR7, CXCR2, and CXCR4), TLR antagonists (inhibitors of TLR3, TLR4, and TLR9), angiogenesis antagonists (VEGF-specific antibodies and adenosine deaminase replacement therapy), antihypertensive drugs (beta blockers and inhibitors of ANG 11, ACE, and aldosterone), vasoactive substances (ET-1 receptor antagonists and bosetan), inhibitors of the enzymes that synthesize and process collagen (inhibitors of prolyl hydroxylase), B cell antagonists (rituximab), integrin/adhesion molecule antagonists (molecules that block αlβl and αvβ6 integrins, as well as inhibitors of integrin-linked kinase, and antibodies specific for ICAM-I and VCAM-I), proapoptotic drugs that target myofibroblasts, MMP inhibitors (inhibitors of MMP2, MMP9, and MMP12), and TIMP inhibitors (antibodies specific for TIMP-1).

[0243] In certain embodiments, an ActRIIB antagonists of the disclosure can be used alone to treat a patient in need thereof. Alternatively, the ActRIIB antagonists may be used in combination with conventional therapeutic approaches directed to treatment or prevention of proliferative disorders described herein, including, for example, surgery (e.g., splenectomy), cytotoxic agents, radiological treatments involving irradiation or administration of radioactive substances, chemotherapeutic agents, anti-hormonal agents, growth inhibitory agents, anti-neoplastic compositions, and treatment with anti-cancer agents listed herein and known in the art, or combinations thereof.

[0244] In general, cytotoxic agent refers to a substance that inhibits or prevents the function of cells and/or causes destruction of cells. The term is intended to include radioactive isotopes (e.g., At^{211}, I^{131}, I^{125}, Y^{90}, Re^{186}, Re^{188}, Sm^{153}, Bi^{212}, P^{32} and radioactive isotopes of Lu), chemotherapeutic agents e.g. methotrexate, adriamicin, *vinca* alkaloids (vincristine, vinblastine, etoposide), doxorubicin, melphalan, mitomycin C, chlorambucil, daunorubicin or other intercalating agents, enzymes and fragments thereof such as nucleolytic enzymes, antibiotics, and toxins such as small molecule toxins or enzymatically active toxins of bacterial, fungal, plant or animal origin, including fragments and/or variants thereof, and the various antitumor or anticancer agents disclosed below. Other cytotoxic agents are described below. A tumoricidal agent causes destruction of tumor cells.

[0245] In general, a chemotherapeutic agent is a chemical compound useful in the treatment of cancer. Examples of chemotherapeutic agents include alkylating agents such as thiotepa and CYTOXAN® cyclosphosphamide; alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, trietylenephosphoramide, triethiylenethiophosphoramide and trimethylolomelamine; acetogenins (especially bullatacin and bullatacinone); delta-9-tetrahydrocannabinol (dronabinol, MARINOL®); beta-lapachone; lapachol; colchicines; betulinic acid; a camptothecin (including the synthetic analogue topotecan (HYCAMTIN®), CPT-11 (irinotecan, CAMPTOSAR®), acetylcamptothecin, scopolectin, and 9-aminocamptothecin); bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); podophyllotoxin; podophyllinic acid; teniposide; cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogues, KW-2189 and CB1-TM1); eleutherobin; pancratistatin; a sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlornaphazine, cyclophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and ranimnustine; antibiotics such as the enediyne antibiotics (e.g., calicheamicin, especially calicheamicin gammall and calicheamicin omegall (see, e.g., Agnew, Chem Intl. Ed. Engl., 33: 183-186 (1994)); dynemicin, including dynemicin A; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antiobiotic chromophores), aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabicin, carminomycin, carzinophilin, chromomycinis, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, ADRIAMYCIN® doxorubicin (including morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin and deoxydoxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins such as mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, porfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as folinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amsacrine; bestrabucil; bisantrene; edatrax-

ate; demecolcine; diaziquone; elfornithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidamine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidanmol; nitraerine; pentostatin; phenamet; pirarubicin; losoxantrone; 2-ethylhydrazide; procarbazine; PSK® polysaccharide complex (JHS Natural Products, Eugene, Oreg.); razoxane; rhizoxin; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2"-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine (ELDISINE®, FILDESIN®); dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); thiotepa; taxoids, e.g., TAXOL® paclitaxel (Bristol-Myers Squibb Oncology, Princeton, N.J.), ABRAXANE™ Cremophor-free, albumin-engineered nanoparticle formulation of paclitaxel (American Pharmaceutical Partners, Schaumberg, Ill.), and TAXOTERE® doxetaxel (Rhone-Poulenc Rorer, Antony, France); chlorambucil; gemcitabine (GEMZAR®); 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblastine (VELBAN®); platinum; etoposide (VP-16); ifosfamide; mitoxantrone; vincristine (ONCOVIN®); oxaliplatin; leucovovin; vinorelbine (NAVELBINE®); novantrone; edatrexate; daunomycin; aminopterin; ibandronate; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMYELOFIBROSIS O); retinoids such as retinoic acid; capecitabine (XELODA®); pharmaceutically acceptable salts, acids or derivatives of any of the above; as well as combinations of two or more of the above such as CHOP, an abbreviation for a combined therapy of cyclophosphamide, doxorubicin, vincristine, and prednisolone, and FOLFOX, an abbreviation for a treatment regimen with oxaliplatin (ELOXATIN™) combined with 5-FU and leucovovin.

[0246] Also are anti-hormonal agents that act to regulate, reduce, block, or inhibit the effects of hormones that can promote the growth of cancer, and are often in the form of systemic, or whole-body treatment. They may be hormones themselves. Examples include anti-estrogens and selective estrogen receptor modulators (SERMs), including, for example, tamoxifen (including NOLVADEX® tamoxifen), EVISTA® raloxifene, droloxifene, 4-hydroxytamoxifen, trioxifene, keoxifene, LY1 17018, onapristone, and FARESTON® toremifene; anti-progesterones; estrogen receptor down-regulators (ERDs); agents that function to suppress or shut down the ovaries, for example, luteinizing hormone-releasing hormone (LHRH) agonists such as LUPRON® and ELIGARD® leuprolide acetate, goserelin acetate, buserelin acetate and tripterelin; other anti-androgens such as flutamide, nilutamide and bicalutamide; and aromatase inhibitors that inhibit the enzyme aromatase, which regulates estrogen production in the adrenal glands, such as, for example, 4(5)-imidazoles, aminoglutethimide, MEGASE® megestrol acetate, AROMASIN® exemestane, formestane, fadrozole, RIVIS OR® vorozole, FEMARA® letrozole, and ARIMIDEX® anastrozole. In addition, such definition of chemotherapeutic agents includes bisphosphonates such as clodronate (for example, BONEFOS® or OSTAC®), DIDROC AL® etidronate, NE-58095, ZOMET A® zoledronic acid/zoledronate, FOSAMAX® alendronate, AREDIA® pamidronate, SKELID® tiludronate, or ACTONEL® risedronate; as well as troxacitabine (a 1,3-dioxolane nucleoside cytosine analog); antisense oligonucleotides, particularly those that inhibit expression of genes in signaling pathways implicated in aberrant cell proliferation, such as, for example, PKC-alpha, Raf, H-Ras, and epidermal growth factor receptor (EGF-R); vaccines such as THERATOPE® vaccine and gene therapy vaccines, for example, ALLOVECTIN® vaccine, LEUVECTIN® vaccine, and VAXID® vaccine; LURTOTECAN® topoisomerase 1 inhibitor; ABARELIX® rmRH; lapatinib ditosylate (an ErbB-2 and EGFR dual tyrosine kinase small-molecule inhibitor also known as GW572016); and pharmaceutically acceptable salts, acids or derivatives of any of the above.

[0247] A growth inhibitory agent generally refers to a compound or composition which inhibits growth of a cell either in vitro or in vivo. Thus, the growth inhibitory agent may be one which significantly reduces the percentage of cells in S phase. Examples of growth inhibitory agents include agents that block cell cycle progression (at a place other than S phase), such as agents that induce Gl arrest and M-phase arrest. Classical M-phase blockers include the vincas (vincristine and vinblastine), taxanes, and topoisomerase II inhibitors such as doxorubicin, epirubicin, daunorubicin, etoposide, and bleomycin. Those agents that arrest Gl also spill over into S-phase arrest, for example, DNA alkylating agents such as tamoxifen, prednisone, dacarbazine, mechlorethamine, cisplatin, methotrexate, 5-fluorouracil, and ara-C. Further information can be found in The Molecular Basis of Cancer, Mendelsohn and Israel, eds., Chapter 1, entitled "Cell cycle regulation, oncogenes, and antineoplastic drugs" by Murakami et al. (WB Saunders: Philadelphia, 1995), especially p. 13. The taxanes (paclitaxel and docetaxel) are anticancer drugs both derived from the yew tree. Docetaxel (TAXOTERE®, Rhone-Poulenc Rorer), derived from the European yew, is a semisynthetic analogue of paclitaxel (TAXOL®, Bristol-Myers Squibb). Paclitaxel and docetaxel promote the assembly of microtubules from tubulin dimers and stabilize microtubules by preventing depolymerization, which results in the inhibition of mitosis in cells.

[0248] An ActRIIB antagonist and an additional active agent (e.g., co-therapeutic agent) can be administered in the same formulation or separately. In the case of separate administration, the ActRIIB antagonist can be administered before, after, or concurrently with the additional active agent. One agent may precede or follow administration of the other agent by intervals ranging from minutes to weeks. In embodiments where two or more different kinds of therapeutic agents are applied separately to a subject, one would generally ensure that a significant period of time did not expire between the time of each delivery, such that these different kinds of agents would still be able to exert an advantageously combined effect on the target tissues or cells.

[0249] 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 ActRIIB antagonist agents of the disclosure 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 param-

eters are outside the normal level, dosing with one or more ActRIIB antagonists may be reduced, delayed or terminated.

[0250] 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.

[0251] 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 ActRIIB 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.

[0252] 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 ActRIIB 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 ActRIIB 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 ActRIIB antagonist agents of the 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 ActRIIB antagonists of the disclosure and iron supplementation.

[0253] 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 ActRIIB 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 ActRIIB 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.

[0254] In certain embodiments, one or more hematologic parameters are measured in patients who are being treated with a one or more ActRIIB 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 ActRIIB 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 ActRIIB 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 ActRIIB 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.

[0255] As used herein, "in combination with", "combinations of", or "conjoint" administration refers to any form of administration such that additional therapies (e.g., second, third, fourth, etc.) are still effective in the body (e.g., multiple compounds are simultaneously effective in the patient, which may include synergistic effects of those compounds). Effectiveness may not correlate to measurable concentration of the agent in blood, serum, or plasma. For example, the different therapeutic compounds can be administered either in the same formulation or in separate formulations, either concomitantly or sequentially, and on different schedules. Thus, an individual who receives such treatment

can benefit from a combined effect of different therapies. One or more ActRIIB antagonists, or combinations of such polypeptides, of the disclosure can be administered concurrently with, prior to, or subsequent to, one or more other additional agents or supportive therapies. In general, each therapeutic agent will be administered at a dose and/or on a time schedule determined for that particular agent. The particular combination to employ in a regimen will take into account compatibility of the antagonist of the present disclosure with the therapy and/or the desired.

5. Pharmaceutical Compositions

[0256] The therapeutic agents described herein (e.g., ActRIIB antagonist) 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.

[0257] In certain embodiments, the therapeutic method of the disclosure includes administering the composition systemically, or locally as an implant or device. When administered, the therapeutic composition for use in this disclosure is in a pyrogen-free, physiologically acceptable form. Therapeutically useful agents other than the ActRIIB signaling antagonists which may also optionally be included in the composition as described above, may be administered simultaneously or sequentially with the subject compounds (e.g., ActRIIB polypeptides) in the methods disclosed herein.

[0258] 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 ActRIIB antagonist in combination with one or more pharmaceutically acceptable sterile isotonic aqueous or nonaqueous solutions, dispersions, suspensions or emulsions, or sterile powders which may be reconstituted into sterile injectable solutions or dispersions just prior to use, which may contain antioxidants, buffers, bacteriostats, solutes which render the formulation isotonic with the blood of the intended recipient or suspending or thickening agents. Examples of suitable aqueous and nonaqueous carriers which may be employed in the pharmaceutical compositions of the disclosure include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

[0259] 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

[0260] 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., ActRIIB polypeptides) to a target tissue site, providing a structure for the developing tissue and optimally capable of being resorbed into the body. For example, the matrix may provide slow release of the ActRIIB antagonist. Such matrices may be formed of materials presently in use for other implanted medical applications.

[0261] 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.

[0262] 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.

[0263] 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 a talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof; and (10) coloring agents. In the case of capsules, tablets and pills, the pharmaceutical compositions may also comprise buffering agents. Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugars, as well as high molecular weight polyethylene glycols and the like.

[0264] 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.

[0265] 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.

[0266] 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.

[0267] It is understood that the dosage regimen will be determined by the attending physician considering various factors which modify the action of the subject compounds of the invention (e.g., TβRII polypeptides). The various factors include, but are not limited to, the patient's age, sex, and diet, the severity disease, time of administration, and other clinical factors. Optionally, the dosage may vary with the type of matrix used in the reconstitution and the types of compounds in the composition. The addition of other known growth factors to the final composition, may also affect the dosage. Progress can be monitored by periodic assessment of bone growth and/or repair, for example, X-rays (including DEXA), histomorphometric determinations, and tetracycline labeling.

[0268] In certain embodiments, the present invention also provides gene therapy for the in vivo production of ActRIIB antagonist. Such therapy would achieve its therapeutic effect by introduction of the ActRIIB polynucleotide sequences into cells or tissues having the disorders as listed above. Delivery of ActRIIB 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 ActRIIB polynucleotide sequences is the use of targeted liposomes.

[0269] 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 ActRIIB polynucleotide. In a preferred embodiment, the vector is targeted to bone or cartilage.

[0270] 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.

[0271] Another targeted delivery system for ActRIIB 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.

[0272] 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 di stearoylphosphatidylcholine. The targeting of liposomes is also possible based on, for example, organ-specificity, cell-specificity, and organelle-specificity and is known in the art.

[0273] 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

[0274] 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: Generation of ActRIIB-Fc Fusion Proteins

[0275] 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 (three glycine amino acids) in between. The constructs are referred to as ActRIIB-hFc and ActRIIB-Fc, respectively.

[0276] ActRIIB-hFc is shown below as purified from CHO cell lines (SEQ ID NO: 24):

```
GRGEAETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWRNSSGT
IELVKKGCWLDDENCYDRQECVATEENPQVYFCCCEGNECNERFTHLPEA
GGPEVTYEPPPTAPTGGGTHTCPPCPAPELLGGPSVFLEPPKPKDTLMIS
RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS
VLTVLHQDWLNGKEYKCKVSNKALPVPIEKTISKAKGQPREPQVYTLPPS
REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSF
FLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
```

[0277] The ActRIIB-hFc and ActRIIB-Fc proteins were expressed in CHO cell lines. Three different leader sequences were considered: (i) Honey bee mellitin (HBML):

```
                                        (SEQ ID NO: 21)
            MKFLVNVALVFMVVYISYIYA,
```

ii) Tissue plasminogen activator (TPA):

```
                                        (SEQ ID NO: 22)
          MDAMKRGLCCVLLLCGAVFVSP,
```

and (iii) Native:

```
                                        (SEQ ID NO: 23)
            MGAAAKLAFAVFLISCSSGA.
```

[0278] The selected form employs the TPA leader and has the following unprocessed amino acid sequence (SEQ ID NO: 25):

```
MDAMKRGLCCVLLLCGAVFVSPGASGRGEAETRECIYYNANWELERTNQS
GLERCEGEQDKRLHCYASWRNSSGTIELVKKGCWLDDFNCYDRQECVATE
ENPQVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPTGGGTHTCPPC
PAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV
DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
VPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAV
EWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMH
EALHNHYTQKSLSLSPGK
```

[0279] This polypeptide is encoded by the following nucleic acid sequence (SEQ ID NO: 26):

```
A TGGATGCAAT GAAGAGAGGG CTCTGCTGTG TGCTGCTGCT
GTGTGGAGCA GTCTTCGTTT CGCCCGGCGC CTCTGGGCGT
GGGGAGGCTG AGACACGGGA GTGCATCTAC TACAACGCCA
ACTGGGAGCT GGAGCGCACC AACCAGAGCG GCCTGGAGCG
```

-continued

```
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 GTGGTGGAAC TCACACATGC
CCACCGTGCC CAGCACCTGA ACTCCTGGGG GGACCGTCAG
TCTTCCTCTT CCCCCCAAAA CCCAAGGACA CCCTCATGAT
CTCCCGGACC CCTGAGGTCA CATGCGTGGT GGTGGACGTG
AGCCACGAAG ACCCTGAGGT CAAGTTCAAC TGGTACGTGG
ACGGCGTGGA GGTGCATAAT GCCAAGACAA AGCCGCGGGA
GGAGCAGTAC AACAGCACGT ACCGTGTGGT CAGCGTCCTC
ACCGTCCTGC ACCAGGACTG GCTGAATGGC AAGGAGTACA
AGTGCAAGGT CTCCAACAAA GCCCTCCCAG TCCCCATCGA
GAAAACCATC TCCAAAGCCA AAGGGCAGCC CCGAGAACCA
CAGGTGTACA CCCTGCCCCC ATCCCGGGAG GAGATGACCA
AGAACCAGGT CAGCCTGACC TGCCTGGTCA AAGGCTTCTA
TCCCAGCGAC ATCGCCGTGG AGTGGGAGAG CAATGGGCAG
CCGGAGAACA ACTACAAGAC CACGCCTCCC GTGCTGGACT
CCGACGGCTC CTTCTTCCTC TATAGCAAGC TCACCGTGGA
CAAGAGCAGG TGGCAGCAGG GGAACGTCTT CTCATGCTCC
GTGATGCATG AGGCTCTGCA CAACCACTAC ACGCAGAAGA
GCCTCTCCCT GTCTCCGGGT AAATGA
```

[0280] N-terminal sequencing of the CHO-cell-produced material revealed a major sequence of -GRGEAE (SEQ ID NO: 27). Notably, other constructs reported in the literature begin with an -SGR . . . sequence.

[0281] 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.

[0282] 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.

[0283] 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: 24.

[0284] 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.

[0285] 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.

[0286] 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.

[0287] 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. **12**). A nucleotide sequence encoding this fusion protein is shown in FIG. **13**. 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. **14**).

[0288] The mature protein has an amino acid sequence as follows (N-terminus confirmed by N-terminal sequencing) (SEQ ID NO: 28):

ETRECIYYNA NWELERTNQS GLERCEGEQD KRLHCYASWR

NSSGTIELVK KGCWLDDFNC YDRQECVATE ENPQVYFCCC

EGNFCNERFT HLPEAGGPEV TYEPPPTGGG THTCPPCPAP

ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE

VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD

WLNGKEYKCK VSNKALPAPI EKTISKAKGQ PREPQVYTLP

PSREEMTKNQ VSLTCLVKGF YPSDIAVEWE SNGQPENNYK

TTPPVLDSDG SFFLYSKLTV DKSRWQQGNV FSCSVMHEAL

HNHYTQKSLS LSPGK

Amino Acids 1-107 are Derived from ActRIIB

[0289] 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.

[0290] 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 activin A, activin B, and GDF11 with high affinity.

Ligand Affinities of ActRIIB-hFc Forms:

[0291]

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 2: Generation of a GDF Trap

[0292] Applicants constructed a GDF trap 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 (three glycine amino acids) in between. The constructs are referred to as ActRIIB(L79D 20-134)-hFc and ActRIIB(L79D 20-134)-Fc, 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: 44, below were also generated and performed equivalently in all respects tested. The aspartate at position 79 (relative to SEQ ID NO: 1, or position 60 relative to SEQ ID NO: 29) is indicated with double underlining below. The valine at position 226 relative to SEQ ID NO: 29 is also indicated by double underlining below.

[0293] The GDF trap ActRIIB(L79D 20-134)-hFc is shown below as purified from CHO cell lines (SEQ ID NO: 29).

GRGEAETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWRNSSGT

IELVKKGCWDDDFNCYDRQECVATEENPQVYFCCCEGNFCNERFTHLPEA

GGPEVTYEPPPTAPTGGGTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS

RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS

VLTVLHQDWLNGKEYKCKVSNKALPVPIEKTISKAKGQPREPQVYTLPPS

REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSF

FLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

[0294] The ActRIIB-derived portion of the GDF trap has an amino acid sequence set forth below (SEQ ID NO: 30), 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: 30)

GRGEAETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWRNSSGT

IELVKKGCWDDDFNCYDRQECVATEENPQVYFCCCEGNFCNERFTHLPEA

GGPEVTYEPPPTAPT

[0295] The GDF trap protein was expressed in CHO cell lines. Three different leader sequences were considered: (i) Honey Bee Melittin (HBML), (ii) Tissue Plasminogen Activator (TPA), and (iii) Native.

[0296] The selected form employs the TPA leader and has the following unprocessed amino acid sequence:

(SEQ ID NO: 31)

MDAMKRGLCCVLLLCGAVFVSPGASGRGEAETRECIYYNANWELERTNQS

GLERCEGEQDKRLHCYASWRNSSGTIELVKKGCWDDDFNCYDRQECVATE

ENPQVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPTGGGTHTCPPC

PAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV

DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP

APIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAV

EWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMH

EALHNHYTQKSLSLSPGK

[0297] This polypeptide is encoded by the following nucleic acid sequence (SEQ ID NO: 32):

A TGGATGCAAT GAAGAGAGGG CTCTGCTGTG TGCTGCTGCT

GTGTGGAGCA GTCTTCGTTT CGCCCGGCGC CTCTGGGCGT

GGGGAGGCTG AGACACGGGA GTGCATCTAC TACAACGCCA

ACTGGGAGCT GGAGCGCACC AACCAGAGCG GCCTGGAGCG

CTGCGAAGGC GAGCAGGACA AGCGGCTGCA CTGCTACGCC

TCCTGGCGCA ACAGCTCTGG CACCATCGAG CTCGTGAAGA

AGGGCTGCTG GGACGATGAC TTCAACTGCT ACGATAGGCA

GGAGTGTGTG GCCACTGAGG AGAACCCCCA GGTGTACTTC

TGCTGCTGTG AAGGCAACTT CTGCAACGAG CGCTTCACTC

ATTTGCCAGA GGCTGGGGGC CCGGAAGTCA CGTACGAGCC

ACCCCCGACA GCCCCCACCG GTGGTGGAAC TCACACATGC

CCACCGTGCC CAGCACCTGA ACTCCTGGGG GGACCGTCAG

TCTTCCTCTT CCCCCCAAAA CCCAAGGACA CCCTCATGAT

CTCCCGGACC CCTGAGGTCA CATGCGTGGT GGTGGACGTG

AGCCACGAAG ACCCTGAGGT CAAGTTCAAC TGGTACGTGG

ACGGCGTGGA GGTGCATAAT GCCAAGACAA AGCCGCGGGA

-continued

GGAGCAGTAC AACAGCACGT ACCGTGTGGT CAGCGTCCTC

ACCGTCCTGC ACCAGGACTG GCTGAATGGC AAGGAGTACA

AGTGCAAGGT CTCCAACAAA GCCCTCCCAG TCCCCATCGA

GAAAACCATC TCCAAAGCCA AAGGGCAGCC CCGAGAACCA

CAGGTGTACA CCCTGCCCCC ATCCCGGGAG GAGATGACCA

AGAACCAGGT CAGCCTGACC TGCCTGGTCA AAGGCTTCTA

TCCCAGCGAC ATCGCCGTGG AGTGGGAGAG CAATGGGCAG

CCGGAGAACA ACTACAAGAC CACGCCTCCC GTGCTGGACT

CCGACGGCTC CTTCTTCCTC TATAGCAAGC TCACCGTGGA

CAAGAGCAGG TGGCAGCAGG GGAACGTCTT CTCATGCTCC

GTGATGCATG AGGCTCTGCA CAACCACTAC ACGCAGAAGA

GCCTCTCCCT GTCTCCGGGT AAATGA

[0298] 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/NaC1 (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.

[0299] 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 3: Bioassay for GDF-11- and Activin-Mediated Signaling

[0300] 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.

[0301] Day 1: Split A-204 cells into 48-well plate.

[0302] Day 2: A-204 cells transfected with 10 ug pGL3 (CAGA)12 or pGL3(CAGA)12(10 ug)+pRLCMV (1 μg) and Fugene.

[0303] 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.

[0304] 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.

[0305] ActRIIB(20-134) is a potent inhibitor of activin A, GDF-8, and GDF-11 activity in this assay. As described below, ActRIIB variants were also tested in this assay.

Example 4: ActRIIB-Fc Variants, Cell-Based Activity

[0306] 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 GDF-11.

Soluble ActRIIB-Fc Binding to GDF11 and Activin A:

[0307]

ActRIIB-Fc Variations	Portion of ActRIIB (corresponds to amino acids of SEQ ID NO: 1)	GDF 11 Inhibition Activity	Activin Inhibition Activity
R64	20-134	+++ (approx. 10^{-8}M K_I)	+++ (approx. 10^{-8}M K_I)
A64	20-134	+ (approx. 10^{-6}M K_I)	+ (approx. 10^{-6}M K_I)
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 P130A	20-134	+++	+++
R64N	20-134	+	+

+ Poor activity (roughly 1×10^{-6} K_I)
++ Moderate activity (roughly 1×10^{-7} K_I)
+++ Good (wild-type) activity (roughly 1×10^{-8} K_I)
++++ Greater than wild-type activity

[0308] Several variants have been assessed for serum half-life in rats. ActRIIB(20-134)-Fc has a serum half-life of approximately 70 hours. ActRIIB(A24N 20-134)-Fc has a serum half-life of approximately 100-150 hours. Any of the variants tested above, may be combined with the GDF trap molecules.

Example 5: GDF-11 and Activin a Binding

[0309] Binding of certain ActRIIB-Fc proteins and GDF traps to ligands was tested in a Biacore™ assay.

[0310] 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.

[0311]

	GDF11		
Protein	Kon (1/Ms)	Koff (Vs)	KD (M)
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		
Protein	Kon (1/Ms)	Koff (Vs)	KD (M)
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
	Activin A		
Protein	Kon (1/Ms)	Koff (Vs)	KD (M)
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

[0312] 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.

[0313] 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, K741 (and presumably other hydrophobic substitutions at K74, such as K74L), and D801, 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)

[0314]

ActRIIB	ActA	GDF11
WT (64A)	KD = 1.8e-7M (+)	KD = 2.6e-7M (+)

-continued

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

* No observed binding
−− <1/5 WT binding
− ~WT binding
+ WT
++ <2x increased binding
+++ ~5x increased binding
++++ ~10x increased binding
+++++ ~40x increased binding

Example 6: Generation of a GDF Trap with Truncated ActRIIB Extracellular Domain

[0315] 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. **3**). A nucleotide sequence corresponding to this fusion protein is shown in FIG. **4**.

[0316] 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. **5**, SEQ ID NO: 50). One nucleotide sequence encoding this fusion protein is shown in FIG. **6** (SEQ ID NO: 51), and an alternative nucleotide sequence encoding exactly the same fusion protein is shown in FIG. **9** (SEQ ID NO: 55).

Example 7: Selective Ligand Binding by GDF Trap with Double-Truncated ActRIIB Extracellular Domain

[0317] 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_0 and k_{off}.

[0318] 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

[0319] 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 8: GDF Trap Derived from ActRIIB5

[0320] 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).

[0321] The sequence of native human ActRIIB5 without its leader is as follows:

(SEQ ID NO: 33)
GRGEAETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWRNSSGT

IELVKKGCWLDDFNCYDRQECVATEENPQVYFCCCEGNFCNERFTHLPEA

GGPEGPWASTTIPSGGPEATAAAGDQGSGALWLCLEGPAHE

[0322] 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: 34)
GRGEAETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWRNSSGT

IELVKKGCWDDDFNCYDRQECVATEENPQVYFCCCEGNFCNERFTHLPEA

GGPEGPWASTTIPSGGPEATAAAGDQGSGALWLCLEGPAHE

[0323] This variant may be connected to human Fc (double underline) with a TGGG linker (single underline) to generate a human ActRIIB5(L79D)-hFc fusion protein with the following sequence:

(SEQ ID NO: 35)
GRGEAETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWRNSSGT

IELVKKGCWDDDFNCYDRQECVATEENPQVYFCCCEGNFCNERFTHLPEA

-continued

GGPEGPWASTTIPSGGPEATAAAGDQGSGALWLCLEGPAHETGGGTHTCP

PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW

YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA

LPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDI

AVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV

MHEALHNHYTQKSLSLSPGK.

[0324] This construct may be expressed in CHO cells.

Example 9: Effect of a GDF Trap in in a JAK2V617F Animal Model

[0325] Transgenic JAK2V617F mutant mice [the A line as described in Xing et al. (2008) Blood 111: 5109-5117] were used to understand the effects of ActRIIB(L79D 25-131)-Fc on myelofibrosis.

[0326] To understand the onset and progression of myelofibrosis disease, the complete blood counts and degree of fibrosis in JAK2V617F mice was compared, at various ages, to data obtained from control animals (age-matched wild-type mice). Red blood cell (RBC) and platelet levels were elevated in JAK2V617F mice at all ages compared to wild-type, with a trend toward increased levels in mutant animals between 2 to 5 months followed by a progressive decrease between 8 to 12 months. Fibrosis was detectable in bone marrow of JAK2V617F mice starting around 5 months, which worsened with age. JAK2V617F mice also displayed splenomegaly around 3 to 4 months, which also worsened with age.

[0327] For the GDF trap study, treatment was initiated at 12 months of age, which corresponds to late stage myelofibrosis. Mice were placed into one of two groups: i) treatment of JAK2V617F mice with ActRIIB(L79D 25-131)-Fc on a dosing schedule of 10 mg/kg twice weekly; and ii) treatment of JAK2V617F mice with vehicle (TBS) twice weekly (i.e., control animals). Following 10 weeks, ActRIIB(L79D 25-131)-Fc treated animals displayed reduced spleen size (−12.5%) compared to control animals. Consistent with this observation, histopathology revealed a decrease of extramedullary hematopoiesis in the spleens of ActRIIB(L79D 25-131)-Fc treated mice compared to control animals. Histopathology also showed decreased bone marrow fibrosis in ActRIIB(L79D 25-131)-Fc treated mice compared to control animals.

[0328] Accordingly, treatment with a GDF trap is effective at ameliorating various complications of myelofibrosis in this JAK2V617F model, particularly in decreasing splenomegaly, extramedullary hematopoiesis, and fibrosis. Thus, these data indicate that ActRIIB antagonists may be used for treating myelofibrosis. For example, ActRIIB antagonists may be particularly useful in the treatment of various complications of myelofibrosis including, for example, decreasing splenomegaly, decreasing extramedullary hematopoiesis, increasing red blood cell levels, and/or decreasing fibrosis (e.g., bone marrow fibrosis).

Example 10: Effect of a GDF Trap in in Ruxolitinib Treated Animals

[0329] Ruxolitinib is a Janus kinase inhibitor that has been approved for the treatment of intermediate or high-risk myelofibrosis. In particular, ruxolitinib shows significant effects in reduction of spleen size and ameliorating symptoms associated with splenomegaly in myelofibrosis patients. However, a variety of hematological side effects have been observed in patients treated with ruxolitinib including, for example, anemia. Nine month old C57BL/6 mice were used to understand the effects of ActRIIB(L79D 25-131)-Fc treatment on various hematological parameters in ruxolitinib treated mice.

[0330] For this study, treatment was initiated at 6-7 months of age. Mice were placed into one of four groups: i) treatment with ActRIIB(L79D 25-131)-Fc on a dosing schedule of 10 mg/kg twice weekly; ii) treatment with ruxolitinib on a dosing schedule of 60 mg/kg twice daily; iii) treatment with ActRIIB(L79D 25-131)-Fc on a dosing schedule of 10 mg/kg twice weekly and ruxolitinib on a dosing schedule of 60 mg/kg twice daily; and iv) treatment with vehicle (TBS) twice weekly (i.e., control animals). Following four weeks of treatment, ActRIIB(L79D 25-131)-Fc mice were observed to have increased red blood cell (~15%) and hemoglobin (~13%) levels compared to control (TBS treated) mice, demonstrating ActRIIB(L79D 25-131)-Fc increases erythropoietic activity in C57BL/6 mice. In contrast, ruxolitinib treatment resulted in a decrease of red blood cell (~4%) and hemoglobin (~4%) levels compared to control animals. ActRIIB(L79D 25-131)-Fc and ruxolitinib conjointly treated mice displayed an increase in red blood cell (~8%) and hemoglobin (~5%) levels compared to control animals.

[0331] These data demonstrate that ActRIIB(L79D 25-131)-Fc can reverse ruxolitinib-induced anemia in normal, healthy mice. Therefore, the data suggest that ActRIIB antagonists may be useful in alleviating Janus kinase inhibitor induced-anemia in a variety of patient populations including, for example, myelofibrosis patients that have been or are undergoing treatment with one or more Janus kinase inhibitors. Accordingly, ActRIIB antagonists may be useful as part of a conjoint therapy with Janus kinase inhibitors to treat a variety of patient populations including, for example, myelofibrosis patients that have been or are undergoing treatment with one or more Janus kinase inhibitors, particularly those that present anemia.

INCORPORATION BY REFERENCE

[0332] 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.

[0333] 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.

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            20                  25                  30

Asn Ala Asn Trp Glu Leu Glu Arg Thr Asn Gln Ser Gly Leu Glu Arg
        35                  40                  45

Cys Glu Gly Glu Gln Asp Lys Arg Leu His Cys Tyr Ala Ser Trp Arg
    50                  55                  60

Asn Ser Ser Gly Thr Ile Glu Leu Val Lys Lys Gly Cys Trp Leu Asp
65                  70                  75                  80

Asp Phe Asn Cys Tyr Asp Arg Gln Glu Cys Val Ala Thr Glu Glu Asn
                85                  90                  95

Pro Gln Val Tyr Phe Cys Cys Cys Glu Gly Asn Phe Cys Asn Glu Arg
            100                 105                 110

Phe Thr His Leu Pro Glu Ala Gly Gly Pro Glu Val Thr Tyr Glu Pro
        115                 120                 125

Pro Pro Thr Ala Pro Thr Leu Leu Thr Val Leu Ala Tyr Ser Leu Leu
    130                 135                 140

Pro Ile Gly Gly Leu Ser Leu Ile Val Leu Leu Ala Phe Trp Met Tyr
145                 150                 155                 160

Arg His Arg Lys Pro Pro Tyr Gly His Val Asp Ile His Glu Asp Pro
                165                 170                 175

Gly Pro Pro Pro Pro Ser Pro Leu Val Gly Leu Lys Pro Leu Gln Leu
            180                 185                 190

Leu Glu Ile Lys Ala Arg Gly Arg Phe Gly Cys Val Trp Lys Ala Gln
        195                 200                 205

Leu Met Asn Asp Phe Val Ala Val Lys Ile Phe Pro Leu Gln Asp Lys
    210                 215                 220

Gln Ser Trp Gln Ser Glu Arg Glu Ile Phe Ser Thr Pro Gly Met Lys
225                 230                 235                 240

His Glu Asn Leu Leu Gln Phe Ile Ala Ala Glu Lys Arg Gly Ser Asn
                245                 250                 255

Leu Glu Val Glu Leu Trp Leu Ile Thr Ala Phe His Asp Lys Gly Ser
            260                 265                 270

Leu Thr Asp Tyr Leu Lys Gly Asn Ile Ile Thr Trp Asn Glu Leu Cys
        275                 280                 285

His Val Ala Glu Thr Met Ser Arg Gly Leu Ser Tyr Leu His Glu Asp
    290                 295                 300

Val Pro Trp Cys Arg Gly Glu Gly His Lys Pro Ser Ile Ala His Arg
305                 310                 315                 320

Asp Phe Lys Ser Lys Asn Val Leu Leu Lys Ser Asp Leu Thr Ala Val
                325                 330                 335

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    370                 375                 380

Asp Met Tyr Ala Met Gly Leu Val Leu Trp Glu Leu Val Ser Arg Cys
385                 390                 395                 400

Lys Ala Ala Asp Gly Pro Val Asp Glu Tyr Met Leu Pro Phe Glu Glu
                405                 410                 415

Glu Ile Gly Gln His Pro Ser Leu Glu Glu Leu Gln Glu Val Val Val
            420                 425                 430

His Lys Lys Met Arg Pro Thr Ile Lys Asp His Trp Leu Lys His Pro
        435                 440                 445

Gly Leu Ala Gln Leu Cys Val Thr Ile Glu Glu Cys Trp Asp His Asp
    450                 455                 460

Ala Glu Ala Arg Leu Ser Ala Gly Cys Val Glu Glu Arg Val Ser Leu
465                 470                 475                 480

Ile Arg Arg Ser Val Asn Gly Thr Thr Ser Asp Cys Leu Val Ser Leu
                485                 490                 495

Val Thr Ser Val Thr Asn Val Asp Leu Pro Pro Lys Glu Ser Ser Ile
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            20                  25                  30

Glu Gln Asp Lys Arg Leu His Cys Tyr Ala Ser Trp Arg Asn Ser Ser
        35                  40                  45

Gly Thr Ile Glu Leu Val Lys Lys Gly Cys Trp Leu Asp Asp Phe Asn
    50                  55                  60

Cys Tyr Asp Arg Gln Glu Cys Val Ala Thr Glu Glu Asn Pro Gln Val
65                  70                  75                  80

Tyr Phe Cys Cys Cys Glu Gly Asn Phe Cys Asn Glu Arg Phe Thr His
                85                  90                  95

Leu Pro Glu Ala Gly Gly Pro Glu Val Thr Tyr Glu Pro Pro Pro Thr
            100                 105                 110

Ala Pro Thr
        115


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            20                  25                  30
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Glu Gln Asp Lys Arg Leu His Cys Tyr Ala Ser Trp Arg Asn Ser Ser
        35                  40                  45

Gly Thr Ile Glu Leu Val Lys Lys Gly Cys Trp Leu Asp Asp Phe Asn
    50                  55                  60

Cys Tyr Asp Arg Gln Glu Cys Val Ala Thr Glu Glu Asn Pro Gln Val
65                  70                  75                  80

Tyr Phe Cys Cys Cys Glu Gly Asn Phe Cys Asn Glu Arg Phe Thr His
                85                  90                  95

Leu Pro Glu Ala
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            20                  25                  30

Asn Ala Asn Trp Glu Leu Glu Arg Thr Asn Gln Ser Gly Leu Glu Arg
        35                  40                  45

Cys Glu Gly Glu Gln Asp Lys Arg Leu His Cys Tyr Ala Ser Trp Ala
    50                  55                  60

Asn Ser Ser Gly Thr Ile Glu Leu Val Lys Lys Gly Cys Trp Leu Asp
65                  70                  75                  80

Asp Phe Asn Cys Tyr Asp Arg Gln Glu Cys Val Ala Thr Glu Glu Asn
                85                  90                  95

Pro Gln Val Tyr Phe Cys Cys Cys Glu Gly Asn Phe Cys Asn Glu Arg
            100                 105                 110

Phe Thr His Leu Pro Glu Ala Gly Gly Pro Glu Val Thr Tyr Glu Pro
        115                 120                 125

Pro Pro Thr Ala Pro Thr Leu Leu Thr Val Leu Ala Tyr Ser Leu Leu
    130                 135                 140

Pro Ile Gly Gly Leu Ser Leu Ile Val Leu Leu Ala Phe Trp Met Tyr
145                 150                 155                 160

Arg His Arg Lys Pro Pro Tyr Gly His Val Asp Ile His Glu Asp Pro
                165                 170                 175

Gly Pro Pro Pro Pro Ser Pro Leu Val Gly Leu Lys Pro Leu Gln Leu
            180                 185                 190

Leu Glu Ile Lys Ala Arg Gly Arg Phe Gly Cys Val Trp Lys Ala Gln
        195                 200                 205

Leu Met Asn Asp Phe Val Ala Val Lys Ile Phe Pro Leu Gln Asp Lys
    210                 215                 220

Gln Ser Trp Gln Ser Glu Arg Glu Ile Phe Ser Thr Pro Gly Met Lys
225                 230                 235                 240

His Glu Asn Leu Leu Gln Phe Ile Ala Ala Glu Lys Arg Gly Ser Asn
                245                 250                 255

Leu Glu Val Glu Leu Trp Leu Ile Thr Ala Phe His Asp Lys Gly Ser
            260                 265                 270

Leu Thr Asp Tyr Leu Lys Gly Asn Ile Ile Thr Trp Asn Glu Leu Cys
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305                 310                 315                 320

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Leu Ala Asp Phe Gly Leu Ala Val Arg Phe Glu Pro Gly Lys Pro Pro
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Gly Asp Thr His Gly Gln Val Gly Thr Arg Arg Tyr Met Ala Pro Glu
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Val Leu Glu Gly Ala Ile Asn Phe Gln Arg Asp Ala Phe Leu Arg Ile
    370                 375                 380

Asp Met Tyr Ala Met Gly Leu Val Leu Trp Glu Leu Val Ser Arg Cys
385                 390                 395                 400

Lys Ala Ala Asp Gly Pro Val Asp Glu Tyr Met Leu Pro Phe Glu Glu
                405                 410                 415

Glu Ile Gly Gln His Pro Ser Leu Glu Glu Leu Gln Glu Val Val Val
            420                 425                 430

His Lys Lys Met Arg Pro Thr Ile Lys Asp His Trp Leu Lys His Pro
        435                 440                 445

Gly Leu Ala Gln Leu Cys Val Thr Ile Glu Glu Cys Trp Asp His Asp
    450                 455                 460

Ala Glu Ala Arg Leu Ser Ala Gly Cys Val Glu Glu Arg Val Ser Leu
465                 470                 475                 480

Ile Arg Arg Ser Val Asn Gly Thr Thr Ser Asp Cys Leu Val Ser Leu
                485                 490                 495

Val Thr Ser Val Thr Asn Val Asp Leu Pro Pro Lys Glu Ser Ser Ile
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Glu Gln Asp Lys Arg Leu His Cys Tyr Ala Ser Trp Ala Asn Ser Ser
        35                  40                  45

Gly Thr Ile Glu Leu Val Lys Lys Gly Cys Trp Leu Asp Asp Phe Asn
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Cys Tyr Asp Arg Gln Glu Cys Val Ala Thr Glu Glu Asn Pro Gln Val
65                  70                  75                  80

Tyr Phe Cys Cys Cys Glu Gly Asn Phe Cys Asn Glu Arg Phe Thr His
                85                  90                  95

Leu Pro Glu Ala Gly Gly Pro Glu Val Thr Tyr Glu Pro Pro Pro Thr
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Gly Thr Ile Glu Leu Val Lys Lys Gly Cys Trp Leu Asp Asp Phe Asn
50 55 60

Cys Tyr Asp Arg Gln Glu Cys Val Ala Thr Glu Glu Asn Pro Gln Val
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Tyr Phe Cys Cys Cys Glu Gly Asn Phe Cys Asn Glu Arg Phe Thr His
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Leu Pro Glu Ala
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gatgacttca actgctacga taggcaggag tgtgtggcca ctgaggagaa cccccaggtg 240

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35 40 45

Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
50 55 60

Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
65 70 75 80

Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
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Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
100 105 110

Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
115 120 125

Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr
130 135 140

Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
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Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
165 170 175

Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
180 185 190

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```
Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
        195                 200                 205

Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
    210                 215                 220

Lys
225


<210> SEQ ID NO 10
<211> LENGTH: 223
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 10

Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val
1               5                   10                  15

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
            20                  25                  30

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
        35                  40                  45

Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
    50                  55                  60

Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Val Val Ser
65                  70                  75                  80

Val Leu Thr Val Val His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
                85                  90                  95

Cys Lys Val Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu Lys Thr Ile
            100                 105                 110

Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
        115                 120                 125

Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
    130                 135                 140

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
145                 150                 155                 160

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met Leu Asp Ser
                165                 170                 175

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
            180                 185                 190

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
        195                 200                 205

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
    210                 215                 220


<210> SEQ ID NO 11
<211> LENGTH: 232
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 11

Glu Pro Lys Ser Cys Asp Thr Pro Pro Pro Cys Pro Arg Cys Pro Ala
1               5                   10                  15

Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
            20                  25                  30

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
        35                  40                  45
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```
Val Asp Val Ser His Glu Asp Pro Glu Val Gln Phe Lys Trp Tyr Val
    50                  55                  60

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
65                  70                  75                  80

Tyr Asn Ser Thr Phe Arg Val Val Ser Val Leu Thr Val Leu His Gln
                85                  90                  95

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
            100                 105                 110

Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro
        115                 120                 125

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr
    130                 135                 140

Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
145                 150                 155                 160

Asp Ile Ala Val Glu Trp Glu Ser Ser Gly Gln Pro Glu Asn Asn Tyr
                165                 170                 175

Asn Thr Thr Pro Pro Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
            180                 185                 190

Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Ile Phe
        195                 200                 205

Ser Cys Ser Val Met His Glu Ala Leu His Asn Arg Phe Thr Gln Lys
    210                 215                 220

Ser Leu Ser Leu Ser Pro Gly Lys
225                 230


<210> SEQ ID NO 12
<211> LENGTH: 279
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 12

Glu Leu Lys Thr Pro Leu Gly Asp Thr Thr His Thr Cys Pro Arg Cys
1               5                   10                  15

Pro Glu Pro Lys Ser Cys Asp Thr Pro Pro Pro Cys Pro Arg Cys Pro
            20                  25                  30

Glu Pro Lys Ser Cys Asp Thr Pro Pro Pro Cys Pro Arg Cys Pro Glu
        35                  40                  45

Pro Lys Ser Cys Asp Thr Pro Pro Pro Cys Pro Arg Cys Pro Ala Pro
    50                  55                  60

Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
65                  70                  75                  80

Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
                85                  90                  95

Asp Val Ser His Glu Asp Pro Glu Val Gln Phe Lys Trp Tyr Val Asp
            100                 105                 110

Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr
        115                 120                 125

Asn Ser Thr Phe Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
    130                 135                 140

Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu
145                 150                 155                 160

Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg
                165                 170                 175
```

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```
Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys
            180                 185                 190

Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
        195                 200                 205

Ile Ala Val Glu Trp Glu Ser Ser Gly Gln Pro Glu Asn Asn Tyr Asn
    210                 215                 220

Thr Thr Pro Pro Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
225                 230                 235                 240

Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Ile Phe Ser
                245                 250                 255

Cys Ser Val Met His Glu Ala Leu His Asn Arg Phe Thr Gln Lys Ser
            260                 265                 270

Leu Ser Leu Ser Pro Gly Lys
        275


<210> SEQ ID NO 13
<211> LENGTH: 229
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 13

Glu Ser Lys Tyr Gly Pro Pro Cys Pro Ser Cys Pro Ala Pro Glu Phe
1               5                   10                  15

Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
            20                  25                  30

Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
        35                  40                  45

Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val
    50                  55                  60

Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser
65                  70                  75                  80

Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu
                85                  90                  95

Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser
            100                 105                 110

Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
        115                 120                 125

Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln
    130                 135                 140

Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
145                 150                 155                 160

Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
                165                 170                 175

Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu
            180                 185                 190

Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser Cys Ser
        195                 200                 205

Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
    210                 215                 220

Leu Ser Leu Gly Lys
225
```

-continued

<210> SEQ ID NO 14
<211> LENGTH: 3
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 14

Gly Gly Gly
1

<210> SEQ ID NO 15
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 15

Gly Gly Gly Gly
1

<210> SEQ ID NO 16
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 16

Thr Gly Gly Gly Gly
1 5

<210> SEQ ID NO 17
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 17

Ser Gly Gly Gly Gly
1 5

<210> SEQ ID NO 18
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 18

Thr Gly Gly Gly
1

<210> SEQ ID NO 19
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic

-continued

peptide

<400> SEQUENCE: 19

Ser Gly Gly Gly
1

<210> SEQ ID NO 20
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 20

Gly Gly Gly Gly Ser
1 5

<210> SEQ ID NO 21
<211> LENGTH: 21
<212> TYPE: PRT
<213> ORGANISM: Apis mellifera

<400> SEQUENCE: 21

Met Lys Phe Leu Val Asn Val Ala Leu Val Phe Met Val Val Tyr Ile
1 5 10 15

Ser Tyr Ile Tyr Ala
20

<210> SEQ ID NO 22
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Description of Unknown:
Tissue plasminogen activator (TPA) sequence

<400> SEQUENCE: 22

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly
1 5 10 15

Ala Val Phe Val Ser Pro
20

<210> SEQ ID NO 23
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Description of Unknown:
Native leader sequence

<400> SEQUENCE: 23

Met Gly Ala Ala Ala Lys Leu Ala Phe Ala Val Phe Leu Ile Ser Cys
1 5 10 15

Ser Ser Gly Ala
20

<210> SEQ ID NO 24
<211> LENGTH: 343
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic

-continued

polypeptide

<400> SEQUENCE: 24

Gly Arg Gly Glu Ala Glu Thr Arg Glu Cys Ile Tyr Tyr Asn Ala Asn
1 5 10 15

Trp Glu Leu Glu Arg Thr Asn Gln Ser Gly Leu Glu Arg Cys Glu Gly
20 25 30

Glu Gln Asp Lys Arg Leu His Cys Tyr Ala Ser Trp Arg Asn Ser Ser
35 40 45

Gly Thr Ile Glu Leu Val Lys Lys Gly Cys Trp Leu Asp Asp Phe Asn
50 55 60

Cys Tyr Asp Arg Gln Glu Cys Val Ala Thr Glu Glu Asn Pro Gln Val
65 70 75 80

Tyr Phe Cys Cys Cys Glu Gly Asn Phe Cys Asn Glu Arg Phe Thr His
85 90 95

Leu Pro Glu Ala Gly Gly Pro Glu Val Thr Tyr Glu Pro Pro Pro Thr
100 105 110

Ala Pro Thr Gly Gly Gly Thr His Thr Cys Pro Pro Cys Pro Ala Pro
115 120 125

Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
130 135 140

Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
145 150 155 160

Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp
165 170 175

Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr
180 185 190

Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
195 200 205

Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu
210 215 220

Pro Val Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
225 230 235 240

Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys
245 250 255

Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
260 265 270

Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
275 280 285

Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
290 295 300

Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser
305 310 315 320

Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
325 330 335

Leu Ser Leu Ser Pro Gly Lys
340

<210> SEQ ID NO 25
<211> LENGTH: 368
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

-continued

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 25

```
Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly
1               5                  10                  15

Ala Val Phe Val Ser Pro Gly Ala Ser Gly Arg Gly Glu Ala Glu Thr
            20                  25                  30

Arg Glu Cys Ile Tyr Tyr Asn Ala Asn Trp Glu Leu Glu Arg Thr Asn
        35                  40                  45

Gln Ser Gly Leu Glu Arg Cys Glu Gly Glu Gln Asp Lys Arg Leu His
    50                  55                  60

Cys Tyr Ala Ser Trp Arg Asn Ser Ser Gly Thr Ile Glu Leu Val Lys
65                  70                  75                  80

Lys Gly Cys Trp Leu Asp Asp Phe Asn Cys Tyr Asp Arg Gln Glu Cys
                85                  90                  95

Val Ala Thr Glu Glu Asn Pro Gln Val Tyr Phe Cys Cys Cys Glu Gly
            100                 105                 110

Asn Phe Cys Asn Glu Arg Phe Thr His Leu Pro Glu Ala Gly Gly Pro
        115                 120                 125

Glu Val Thr Tyr Glu Pro Pro Pro Thr Ala Pro Thr Gly Gly Gly Thr
    130                 135                 140

His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser
145                 150                 155                 160

Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg
                165                 170                 175

Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro
            180                 185                 190

Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala
        195                 200                 205

Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val
    210                 215                 220

Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr
225                 230                 235                 240

Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Val Pro Ile Glu Lys Thr
                245                 250                 255

Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu
            260                 265                 270

Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys
        275                 280                 285

Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser
    290                 295                 300

Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp
305                 310                 315                 320

Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser
                325                 330                 335

Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala
            340                 345                 350

Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
        355                 360                 365
```

<210> SEQ ID NO 26

-continued

<211> LENGTH: 1107
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polynucleotide

<400> SEQUENCE: 26

atggatgcaa tgaagagagg gctctgctgt gtgctgctgc tgtgtggagc agtcttcgtt 60

tcgcccggcg cctctgggcg tggggaggct gagacacggg agtgcatcta ctacaacgcc 120

aactgggagc tggagcgcac caaccagagc ggcctggagc gctgcgaagg cgagcaggac 180

aagcggctgc actgctacgc ctcctggcgc aacagctctg gcaccatcga gctcgtgaag 240

aagggctgct ggctagatga cttcaactgc tacgataggc aggagtgtgt ggccactgag 300

gagaaccccc aggtgtactt ctgctgctgt gaaggcaact tctgcaacga gcgcttcact 360

catttgccag aggctggggg cccggaagtc acgtacgagc cacccccgac agcccccacc 420

ggtggtggaa ctcacacatg cccaccgtgc ccagcacctg aactcctggg gggaccgtca 480

gtcttcctct tccccccaaa acccaaggac accctcatga tctcccggac ccctgaggtc 540

acatgcgtgg tggtggacgt gagccacgaa gaccctgagg tcaagttcaa ctggtacgtg 600

gacggcgtgg aggtgcataa tgccaagaca aagccgcggg aggagcagta caacagcacg 660

taccgtgtgg tcagcgtcct caccgtcctg caccaggact ggctgaatgg caaggagtac 720

aagtgcaagg tctccaacaa agccctccca gtccccatcg agaaaaccat ctccaaagcc 780

aaagggcagc cccgagaacc acaggtgtac accctgcccc catcccggga ggagatgacc 840

aagaaccagg tcagcctgac ctgcctggtc aaaggcttct atcccagcga catcgccgtg 900

gagtgggaga gcaatgggca gccggagaac aactacaaga ccacgcctcc cgtgctggac 960

tccgacggct ccttcttcct ctatagcaag ctcaccgtgg acaagagcag gtggcagcag 1020

gggaacgtct tctcatgctc cgtgatgcat gaggctctgc acaaccacta cacgcagaag 1080

agcctctccc tgtctccggg taaatga 1107

<210> SEQ ID NO 27
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 27

Gly Arg Gly Glu Ala Glu
1 5

<210> SEQ ID NO 28
<211> LENGTH: 335
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 28

Glu Thr Arg Glu Cys Ile Tyr Tyr Asn Ala Asn Trp Glu Leu Glu Arg
1 5 10 15

Thr Asn Gln Ser Gly Leu Glu Arg Cys Glu Gly Glu Gln Asp Lys Arg

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            20                  25                  30

Leu His Cys Tyr Ala Ser Trp Arg Asn Ser Ser Gly Thr Ile Glu Leu
        35                  40                  45

Val Lys Lys Gly Cys Trp Leu Asp Asp Phe Asn Cys Tyr Asp Arg Gln
    50                  55                  60

Glu Cys Val Ala Thr Glu Glu Asn Pro Gln Val Tyr Phe Cys Cys Cys
65                  70                  75                  80

Glu Gly Asn Phe Cys Asn Glu Arg Phe Thr His Leu Pro Glu Ala Gly
                85                  90                  95

Gly Pro Glu Val Thr Tyr Glu Pro Pro Pro Thr Gly Gly Gly Thr His
            100                 105                 110

Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val
        115                 120                 125

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
    130                 135                 140

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
145                 150                 155                 160

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
                165                 170                 175

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
            180                 185                 190

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
        195                 200                 205

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
    210                 215                 220

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
225                 230                 235                 240

Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
                245                 250                 255

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
            260                 265                 270

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
        275                 280                 285

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
    290                 295                 300

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
305                 310                 315                 320

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
                325                 330                 335


<210> SEQ ID NO 29
<211> LENGTH: 343
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      polypeptide

<400> SEQUENCE: 29

Gly Arg Gly Glu Ala Glu Thr Arg Glu Cys Ile Tyr Tyr Asn Ala Asn
1               5                   10                  15

Trp Glu Leu Glu Arg Thr Asn Gln Ser Gly Leu Glu Arg Cys Glu Gly
            20                  25                  30
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```
Glu Gln Asp Lys Arg Leu His Cys Tyr Ala Ser Trp Arg Asn Ser Ser
        35                  40                  45

Gly Thr Ile Glu Leu Val Lys Lys Gly Cys Trp Asp Asp Asp Phe Asn
    50                  55                  60

Cys Tyr Asp Arg Gln Glu Cys Val Ala Thr Glu Glu Asn Pro Gln Val
65                  70                  75                  80

Tyr Phe Cys Cys Cys Glu Gly Asn Phe Cys Asn Glu Arg Phe Thr His
                85                  90                  95

Leu Pro Glu Ala Gly Gly Pro Glu Val Thr Tyr Glu Pro Pro Pro Thr
            100                 105                 110

Ala Pro Thr Gly Gly Gly Thr His Thr Cys Pro Pro Cys Pro Ala Pro
        115                 120                 125

Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
    130                 135                 140

Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
145                 150                 155                 160

Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp
                165                 170                 175

Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr
            180                 185                 190

Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
        195                 200                 205

Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu
    210                 215                 220

Pro Val Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
225                 230                 235                 240

Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys
                245                 250                 255

Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
            260                 265                 270

Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
        275                 280                 285

Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
    290                 295                 300

Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser
305                 310                 315                 320

Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
                325                 330                 335

Leu Ser Leu Ser Pro Gly Lys
            340


<210> SEQ ID NO 30
<211> LENGTH: 115
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      polypeptide

<400> SEQUENCE: 30

Gly Arg Gly Glu Ala Glu Thr Arg Glu Cys Ile Tyr Tyr Asn Ala Asn
1               5                   10                  15

Trp Glu Leu Glu Arg Thr Asn Gln Ser Gly Leu Glu Arg Cys Glu Gly
            20                  25                  30
```

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Glu Gln Asp Lys Arg Leu His Cys Tyr Ala Ser Trp Arg Asn Ser Ser
35 40 45

Gly Thr Ile Glu Leu Val Lys Lys Gly Cys Trp Asp Asp Asp Phe Asn
50 55 60

Cys Tyr Asp Arg Gln Glu Cys Val Ala Thr Glu Glu Asn Pro Gln Val
65 70 75 80

Tyr Phe Cys Cys Cys Glu Gly Asn Phe Cys Asn Glu Arg Phe Thr His
85 90 95

Leu Pro Glu Ala Gly Gly Pro Glu Val Thr Tyr Glu Pro Pro Pro Thr
100 105 110

Ala Pro Thr
115

<210> SEQ ID NO 31
<211> LENGTH: 368
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 31

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly
1 5 10 15

Ala Val Phe Val Ser Pro Gly Ala Ser Gly Arg Gly Glu Ala Glu Thr
20 25 30

Arg Glu Cys Ile Tyr Tyr Asn Ala Asn Trp Glu Leu Glu Arg Thr Asn
35 40 45

Gln Ser Gly Leu Glu Arg Cys Glu Gly Glu Gln Asp Lys Arg Leu His
50 55 60

Cys Tyr Ala Ser Trp Arg Asn Ser Ser Gly Thr Ile Glu Leu Val Lys
65 70 75 80

Lys Gly Cys Trp Asp Asp Asp Phe Asn Cys Tyr Asp Arg Gln Glu Cys
85 90 95

Val Ala Thr Glu Glu Asn Pro Gln Val Tyr Phe Cys Cys Cys Glu Gly
100 105 110

Asn Phe Cys Asn Glu Arg Phe Thr His Leu Pro Glu Ala Gly Gly Pro
115 120 125

Glu Val Thr Tyr Glu Pro Pro Pro Thr Ala Pro Thr Gly Gly Gly Thr
130 135 140

His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser
145 150 155 160

Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg
165 170 175

Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro
180 185 190

Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala
195 200 205

Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val
210 215 220

Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr
225 230 235 240

Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr

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               245                 250                 255

Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu
            260                 265                 270

Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys
        275                 280                 285

Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser
    290                 295                 300

Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp
305                 310                 315                 320

Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser
                325                 330                 335

Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala
            340                 345                 350

Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
        355                 360                 365


<210> SEQ ID NO 32
<211> LENGTH: 1107
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      polynucleotide

<400> SEQUENCE: 32

atggatgcaa tgaagagagg gctctgctgt gtgctgctgc tgtgtggagc agtcttcgtt     60

tcgcccggcg cctctgggcg tggggaggct gagacacggg agtgcatcta ctacaacgcc    120

aactgggagc tggagcgcac caaccagagc ggcctggagc gctgcgaagg cgagcaggac    180

aagcggctgc actgctacgc ctcctggcgc aacagctctg gcaccatcga gctcgtgaag    240

aagggctgct gggacgatga cttcaactgc tacgataggc aggagtgtgt ggccactgag    300

gagaaccccc aggtgtactt ctgctgctgt gaaggcaact tctgcaacga gcgcttcact    360

catttgccag aggctggggg cccggaagtc acgtacgagc cacccccgac agcccccacc    420

ggtggtggaa ctcacacatg cccaccgtgc ccagcacctg aactcctggg gggaccgtca    480

gtcttcctct tccccccaaa acccaaggac accctcatga tctcccggac ccctgaggtc    540

acatgcgtgg tggtggacgt gagccacgaa gaccctgagg tcaagttcaa ctggtacgtg    600

gacggcgtgg aggtgcataa tgccaagaca aagccgcggg aggagcagta caacagcacg    660

taccgtgtgg tcagcgtcct caccgtcctg caccaggact ggctgaatgg caaggagtac    720

aagtgcaagg tctccaacaa agccctccca gtccccatcg agaaaaccat ctccaaagcc    780

aaagggcagc cccgagaacc acaggtgtac accctgcccc catcccggga ggagatgacc    840

aagaaccagg tcagcctgac ctgcctggtc aaaggcttct atcccagcga catcgccgtg    900

gagtgggaga gcaatgggca gccggagaac aactacaaga ccacgcctcc cgtgctggac    960

tccgacggct ccttcttcct ctatagcaag ctcaccgtgg acaagagcag gtggcagcag   1020

gggaacgtct tctcatgctc cgtgatgcat gaggctctgc acaaccacta cacgcagaag   1080

agcctctccc tgtctccggg taaatga                                       1107


<210> SEQ ID NO 33
<211> LENGTH: 141
<212> TYPE: PRT
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-continued

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 33

Gly Arg Gly Glu Ala Glu Thr Arg Glu Cys Ile Tyr Tyr Asn Ala Asn
1 5 10 15

Trp Glu Leu Glu Arg Thr Asn Gln Ser Gly Leu Glu Arg Cys Glu Gly
20 25 30

Glu Gln Asp Lys Arg Leu His Cys Tyr Ala Ser Trp Arg Asn Ser Ser
35 40 45

Gly Thr Ile Glu Leu Val Lys Lys Gly Cys Trp Leu Asp Asp Phe Asn
50 55 60

Cys Tyr Asp Arg Gln Glu Cys Val Ala Thr Glu Glu Asn Pro Gln Val
65 70 75 80

Tyr Phe Cys Cys Cys Glu Gly Asn Phe Cys Asn Glu Arg Phe Thr His
85 90 95

Leu Pro Glu Ala Gly Gly Pro Glu Gly Pro Trp Ala Ser Thr Thr Ile
100 105 110

Pro Ser Gly Gly Pro Glu Ala Thr Ala Ala Ala Gly Asp Gln Gly Ser
115 120 125

Gly Ala Leu Trp Leu Cys Leu Glu Gly Pro Ala His Glu
130 135 140

<210> SEQ ID NO 34
<211> LENGTH: 141
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 34

Gly Arg Gly Glu Ala Glu Thr Arg Glu Cys Ile Tyr Tyr Asn Ala Asn
1 5 10 15

Trp Glu Leu Glu Arg Thr Asn Gln Ser Gly Leu Glu Arg Cys Glu Gly
20 25 30

Glu Gln Asp Lys Arg Leu His Cys Tyr Ala Ser Trp Arg Asn Ser Ser
35 40 45

Gly Thr Ile Glu Leu Val Lys Lys Gly Cys Trp Asp Asp Asp Phe Asn
50 55 60

Cys Tyr Asp Arg Gln Glu Cys Val Ala Thr Glu Glu Asn Pro Gln Val
65 70 75 80

Tyr Phe Cys Cys Cys Glu Gly Asn Phe Cys Asn Glu Arg Phe Thr His
85 90 95

Leu Pro Glu Ala Gly Gly Pro Glu Gly Pro Trp Ala Ser Thr Thr Ile
100 105 110

Pro Ser Gly Gly Pro Glu Ala Thr Ala Ala Ala Gly Asp Gln Gly Ser
115 120 125

Gly Ala Leu Trp Leu Cys Leu Glu Gly Pro Ala His Glu
130 135 140

<210> SEQ ID NO 35
<211> LENGTH: 370
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic

-continued

polypeptide

<400> SEQUENCE: 35

```
Gly Arg Gly Glu Ala Glu Thr Arg Glu Cys Ile Tyr Tyr Asn Ala Asn
1               5                  10                  15

Trp Glu Leu Glu Arg Thr Asn Gln Ser Gly Leu Glu Arg Cys Glu Gly
            20                  25                  30

Glu Gln Asp Lys Arg Leu His Cys Tyr Ala Ser Trp Arg Asn Ser Ser
        35                  40                  45

Gly Thr Ile Glu Leu Val Lys Lys Gly Cys Trp Asp Asp Asp Phe Asn
    50                  55                  60

Cys Tyr Asp Arg Gln Glu Cys Val Ala Thr Glu Glu Asn Pro Gln Val
65                  70                  75                  80

Tyr Phe Cys Cys Cys Glu Gly Asn Phe Cys Asn Glu Arg Phe Thr His
                85                  90                  95

Leu Pro Glu Ala Gly Gly Pro Glu Gly Pro Trp Ala Ser Thr Thr Ile
            100                 105                 110

Pro Ser Gly Gly Pro Glu Ala Thr Ala Ala Ala Gly Asp Gln Gly Ser
        115                 120                 125

Gly Ala Leu Trp Leu Cys Leu Glu Gly Pro Ala His Glu Thr Gly Gly
    130                 135                 140

Gly Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
145                 150                 155                 160

Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
                165                 170                 175

Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
            180                 185                 190

Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
        195                 200                 205

Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
    210                 215                 220

Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
225                 230                 235                 240

Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
                245                 250                 255

Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
            260                 265                 270

Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu
        275                 280                 285

Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
    290                 295                 300

Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
305                 310                 315                 320

Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
                325                 330                 335

Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
            340                 345                 350

Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
        355                 360                 365

Gly Lys
    370
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-continued

<210> SEQ ID NO 36
<211> LENGTH: 115
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 36

Ile Leu Gly Arg Ser Glu Thr Gln Glu Cys Leu Phe Phe Asn Ala Asn
1 5 10 15

Trp Glu Lys Asp Arg Thr Asn Gln Thr Gly Val Glu Pro Cys Tyr Gly
20 25 30

Asp Lys Asp Lys Arg Arg His Cys Phe Ala Thr Trp Lys Asn Ile Ser
35 40 45

Gly Ser Ile Glu Ile Val Lys Gln Gly Cys Trp Leu Asp Asp Ile Asn
50 55 60

Cys Tyr Asp Arg Thr Asp Cys Val Glu Lys Lys Asp Ser Pro Glu Val
65 70 75 80

Tyr Phe Cys Cys Cys Glu Gly Asn Met Cys Asn Glu Lys Phe Ser Tyr
85 90 95

Phe Pro Glu Met Glu Val Thr Gln Pro Thr Ser Asn Pro Val Thr Pro
100 105 110

Lys Pro Pro
115

<210> SEQ ID NO 37
<211> LENGTH: 150
<212> TYPE: PRT
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 37

Met Thr Ala Pro Trp Ala Ala Leu Ala Leu Leu Trp Gly Ser Leu Cys
1 5 10 15

Ala Gly Ser Gly Arg Gly Glu Ala Glu Thr Arg Glu Cys Ile Tyr Tyr
20 25 30

Asn Ala Asn Trp Glu Leu Glu Arg Thr Asn Gln Ser Gly Leu Glu Arg
35 40 45

Cys Glu Gly Glu Gln Asp Lys Arg Leu His Cys Tyr Ala Ser Trp Pro
50 55 60

Asn Ser Ser Gly Thr Ile Glu Leu Val Lys Lys Gly Cys Trp Leu Asp
65 70 75 80

Asp Phe Asn Cys Tyr Asp Arg Gln Glu Cys Val Ala Thr Glu Glu Asn
85 90 95

Pro Gln Val Tyr Phe Cys Cys Cys Glu Gly Asn Phe Cys Asn Glu Arg
100 105 110

Phe Thr His Leu Pro Glu Pro Gly Gly Pro Glu Val Thr Tyr Glu Pro
115 120 125

Pro Pro Thr Ala Pro Thr Leu Leu Thr Val Leu Ala Tyr Ser Leu Leu
130 135 140

Pro Ile Gly Gly Leu Ser
145 150

<210> SEQ ID NO 38
<211> LENGTH: 150

-continued

<212> TYPE: PRT
<213> ORGANISM: Sus scrofa

<400> SEQUENCE: 38

Met Thr Ala Pro Trp Ala Ala Leu Ala Leu Leu Trp Gly Ser Leu Cys
1 5 10 15

Val Gly Ser Gly Arg Gly Glu Ala Glu Thr Arg Glu Cys Ile Tyr Tyr
20 25 30

Asn Ala Asn Trp Glu Leu Glu Arg Thr Asn Gln Ser Gly Leu Glu Arg
35 40 45

Cys Glu Gly Glu Gln Asp Lys Arg Leu His Cys Tyr Ala Ser Trp Arg
50 55 60

Asn Ser Ser Gly Thr Ile Glu Leu Val Lys Lys Gly Cys Trp Leu Asp
65 70 75 80

Asp Phe Asn Cys Tyr Asp Arg Gln Glu Cys Val Ala Thr Glu Glu Asn
85 90 95

Pro Gln Val Tyr Phe Cys Cys Cys Glu Gly Asn Phe Cys Asn Glu Arg
100 105 110

Phe Thr His Leu Pro Glu Ala Gly Gly Pro Glu Val Thr Tyr Glu Pro
115 120 125

Pro Pro Thr Ala Pro Thr Leu Leu Thr Val Leu Ala Tyr Ser Leu Leu
130 135 140

Pro Ile Gly Gly Leu Ser
145 150

<210> SEQ ID NO 39
<211> LENGTH: 150
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 39

Met Thr Ala Pro Trp Ala Ala Leu Ala Leu Leu Trp Gly Ser Leu Cys
1 5 10 15

Ala Gly Ser Gly Arg Gly Glu Ala Glu Thr Arg Glu Cys Ile Tyr Tyr
20 25 30

Asn Ala Asn Trp Glu Leu Glu Arg Thr Asn Gln Ser Gly Leu Glu Arg
35 40 45

Cys Glu Gly Glu Gln Asp Lys Arg Leu His Cys Tyr Ala Ser Trp Arg
50 55 60

Asn Ser Ser Gly Thr Ile Glu Leu Val Lys Lys Gly Cys Trp Leu Asp
65 70 75 80

Asp Phe Asn Cys Tyr Asp Arg Gln Glu Cys Val Ala Thr Glu Glu Asn
85 90 95

Pro Gln Val Tyr Phe Cys Cys Cys Glu Gly Asn Phe Cys Asn Glu Arg
100 105 110

Phe Thr His Leu Pro Glu Pro Gly Gly Pro Glu Val Thr Tyr Glu Pro
115 120 125

Pro Pro Thr Ala Pro Thr Leu Leu Thr Val Leu Ala Tyr Ser Leu Leu
130 135 140

Pro Ile Gly Gly Leu Ser
145 150

<210> SEQ ID NO 40
<211> LENGTH: 150

-continued

<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 40

Met Thr Ala Pro Trp Val Ala Leu Ala Leu Leu Trp Gly Ser Leu Cys
1 5 10 15

Ala Gly Ser Gly Arg Gly Glu Ala Glu Thr Arg Glu Cys Ile Tyr Tyr
20 25 30

Asn Ala Asn Trp Glu Leu Glu Arg Thr Asn Gln Ser Gly Leu Glu Arg
35 40 45

Cys Glu Gly Glu Gln Asp Lys Arg Leu His Cys Tyr Ala Ser Trp Arg
50 55 60

Asn Ser Ser Gly Thr Ile Glu Leu Val Lys Lys Gly Cys Trp Leu Asp
65 70 75 80

Asp Phe Asn Cys Tyr Asp Arg Gln Glu Cys Val Ala Thr Glu Glu Asn
85 90 95

Pro Gln Val Tyr Phe Cys Cys Cys Glu Gly Asn Phe Cys Asn Glu Arg
100 105 110

Phe Thr His Leu Pro Glu Ala Gly Gly Pro Glu Val Thr Tyr Glu Pro
115 120 125

Pro Pro Thr Ala Pro Thr Leu Leu Thr Val Leu Ala Tyr Ser Leu Leu
130 135 140

Pro Ile Gly Gly Leu Ser
145 150

<210> SEQ ID NO 41
<211> LENGTH: 150
<212> TYPE: PRT
<213> ORGANISM: Bos taurus

<400> SEQUENCE: 41

Met Thr Ala Pro Trp Ala Ala Leu Ala Leu Leu Trp Gly Ser Leu Cys
1 5 10 15

Ala Gly Ser Gly Arg Gly Glu Ala Glu Thr Arg Glu Cys Ile Tyr Tyr
20 25 30

Asn Ala Asn Trp Glu Leu Glu Arg Thr Asn Gln Ser Gly Leu Glu Arg
35 40 45

Cys Glu Gly Glu Arg Asp Lys Arg Leu His Cys Tyr Ala Ser Trp Arg
50 55 60

Asn Ser Ser Gly Thr Ile Glu Leu Val Lys Lys Gly Cys Trp Leu Asp
65 70 75 80

Asp Phe Asn Cys Tyr Asp Arg Gln Glu Cys Val Ala Thr Glu Glu Asn
85 90 95

Pro Gln Val Tyr Phe Cys Cys Cys Glu Gly Asn Phe Cys Asn Glu Arg
100 105 110

Phe Thr His Leu Pro Glu Ala Gly Gly Pro Glu Val Thr Tyr Glu Pro
115 120 125

Pro Pro Thr Ala Pro Thr Leu Leu Thr Val Leu Ala Tyr Ser Leu Leu
130 135 140

Pro Val Gly Gly Leu Ser
145 150

<210> SEQ ID NO 42
<211> LENGTH: 150

-continued

<212> TYPE: PRT
<213> ORGANISM: Xenopus sp.

<400> SEQUENCE: 42

Met Gly Ala Ser Val Ala Leu Thr Phe Leu Leu Leu Leu Ala Thr Phe
1 5 10 15

Arg Ala Gly Ser Gly His Asp Glu Val Glu Thr Arg Glu Cys Ile Tyr
20 25 30

Tyr Asn Ala Asn Trp Glu Leu Glu Lys Thr Asn Gln Ser Gly Val Glu
35 40 45

Arg Leu Val Glu Gly Lys Lys Asp Lys Arg Leu His Cys Tyr Ala Ser
50 55 60

Trp Arg Asn Asn Ser Gly Phe Ile Glu Leu Val Lys Lys Gly Cys Trp
65 70 75 80

Leu Asp Asp Phe Asn Cys Tyr Asp Arg Gln Glu Cys Ile Ala Lys Glu
85 90 95

Glu Asn Pro Gln Val Phe Phe Cys Cys Cys Glu Gly Asn Tyr Cys Asn
100 105 110

Lys Lys Phe Thr His Leu Pro Glu Val Glu Thr Phe Asp Pro Lys Pro
115 120 125

Gln Pro Ser Ala Ser Val Leu Asn Ile Leu Ile Tyr Ser Leu Leu Pro
130 135 140

Ile Val Gly Leu Ser Met
145 150

<210> SEQ ID NO 43
<211> LENGTH: 150
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 43

Met Gly Ala Ala Ala Lys Leu Ala Phe Ala Val Phe Leu Ile Ser Cys
1 5 10 15

Ser Ser Gly Ala Ile Leu Gly Arg Ser Glu Thr Gln Glu Cys Leu Phe
20 25 30

Phe Asn Ala Asn Trp Glu Lys Asp Arg Thr Asn Gln Thr Gly Val Glu
35 40 45

Pro Cys Tyr Gly Asp Lys Asp Lys Arg Arg His Cys Phe Ala Thr Trp
50 55 60

Lys Asn Ile Ser Gly Ser Ile Glu Ile Val Lys Gln Gly Cys Trp Leu
65 70 75 80

Asp Asp Ile Asn Cys Tyr Asp Arg Thr Asp Cys Val Glu Lys Lys Asp
85 90 95

Ser Pro Glu Val Tyr Phe Cys Cys Cys Glu Gly Asn Met Cys Asn Glu
100 105 110

Lys Phe Ser Tyr Phe Pro Glu Met Glu Val Thr Gln Pro Thr Ser Asn
115 120 125

Pro Val Thr Pro Lys Pro Pro Tyr Tyr Asn Ile Leu Leu Tyr Ser Leu
130 135 140

Val Pro Leu Met Leu Ile
145 150

<210> SEQ ID NO 44
<211> LENGTH: 154

-continued

<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic consensus sequence
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: Thr, Ala or absent
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (121)..(121)
<223> OTHER INFORMATION: Pro, Ala, Val or Met

<400> SEQUENCE: 44

Met Thr Ala Pro Trp Ala Ala Xaa Leu Ala Leu Leu Trp Gly Ser Leu
1 5 10 15

Cys Ala Gly Ser Gly Arg Gly Glu Ala Glu Thr Arg Glu Cys Ile Tyr
20 25 30

Tyr Asn Ala Asn Trp Glu Leu Glu Arg Thr Asn Gln Ser Gly Leu Glu
35 40 45

Arg Leu Cys Glu Gly Glu Gln Asp Lys Arg Leu His Cys Tyr Ala Ser
50 55 60

Trp Arg Asn Ser Ser Gly Thr Ile Glu Leu Val Lys Lys Gly Cys Trp
65 70 75 80

Leu Asp Asp Phe Asn Cys Tyr Asp Arg Gln Glu Cys Val Ala Thr Glu
85 90 95

Glu Asn Pro Gln Val Tyr Phe Cys Cys Cys Glu Gly Asn Phe Cys Asn
100 105 110

Glu Arg Phe Thr His Leu Pro Glu Xaa Gly Gly Pro Glu Val Thr Tyr
115 120 125

Glu Pro Lys Pro Pro Thr Ala Pro Thr Leu Leu Thr Val Leu Ala Tyr
130 135 140

Ser Leu Leu Pro Ile Gly Gly Leu Ser Met
145 150

<210> SEQ ID NO 45
<211> LENGTH: 368
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 45

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly
1 5 10 15

Ala Val Phe Val Ser Pro Gly Ala Ser Gly Arg Gly Glu Ala Glu Thr
20 25 30

Arg Glu Cys Ile Tyr Tyr Asn Ala Asn Trp Glu Leu Glu Arg Thr Asn
35 40 45

Gln Ser Gly Leu Glu Arg Cys Glu Gly Glu Gln Asp Lys Arg Leu His
50 55 60

Cys Tyr Ala Ser Trp Arg Asn Ser Ser Gly Thr Ile Glu Leu Val Lys
65 70 75 80

Lys Gly Cys Trp Asp Asp Asp Phe Asn Cys Tyr Asp Arg Gln Glu Cys
85 90 95

Val Ala Thr Glu Glu Asn Pro Gln Val Tyr Phe Cys Cys Cys Glu Gly

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            100                 105                 110

Asn Phe Cys Asn Glu Arg Phe Thr His Leu Pro Glu Ala Gly Gly Pro
        115                 120                 125

Glu Val Thr Tyr Glu Pro Pro Pro Thr Ala Pro Thr Gly Gly Gly Thr
    130                 135                 140

His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser
145                 150                 155                 160

Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg
                165                 170                 175

Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro
            180                 185                 190

Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala
        195                 200                 205

Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val
    210                 215                 220

Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr
225                 230                 235                 240

Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr
                245                 250                 255

Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu
            260                 265                 270

Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys
        275                 280                 285

Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser
    290                 295                 300

Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp
305                 310                 315                 320

Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser
                325                 330                 335

Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala
            340                 345                 350

Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
        355                 360                 365


<210> SEQ ID NO 46

<400> SEQUENCE: 46

000


<210> SEQ ID NO 47

<400> SEQUENCE: 47

000


<210> SEQ ID NO 48
<211> LENGTH: 1107
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      polynucleotide

<400> SEQUENCE: 48

atggatgcaa tgaagagagg gctctgctgt gtgctgctgc tgtgtggagc agtcttcgtt     60
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tcgcccggcg cctctgggcg tggggaggct gagacacggg agtgcatcta ctacaacgcc 120

aactgggagc tggagcgcac caaccagagc ggcctggagc gctgcgaagg cgagcaggac 180

aagcggctgc actgctacgc ctcctggcgc aacagctctg gcaccatcga gctcgtgaag 240

aagggctgct gggatgatga cttcaactgc tacgataggc aggagtgtgt ggccactgag 300

gagaaccccc aggtgtactt ctgctgctgt gaaggcaact tctgcaacga gcgcttcact 360

catttgccag aggctggggg cccggaagtc acgtacgagc cacccccgac agcccccacc 420

ggtggtggaa ctcacacatg cccaccgtgc ccagcacctg aactcctggg gggaccgtca 480

gtcttcctct tccccccaaa acccaaggac accctcatga tctcccggac ccctgaggtc 540

acatgcgtgg tggtggacgt gagccacgaa gaccctgagg tcaagttcaa ctggtacgtg 600

gacggcgtgg aggtgcataa tgccaagaca aagccgcggg aggagcagta caacagcacg 660

taccgtgtgg tcagcgtcct caccgtcctg caccaggact ggctgaatgg caaggagtac 720

aagtgcaagg tctccaacaa agccctccca gcccccatcg agaaaaccat ctccaaagcc 780

aaagggcagc cccgagaacc acaggtgtac accctgcccc catcccggga ggagatgacc 840

aagaaccagg tcagcctgac ctgcctggtc aaaggcttct atcccagcga catcgccgtg 900

gagtgggaga gcaatgggca gccggagaac aactacaaga ccacgcctcc cgtgctggac 960

tccgacggct ccttcttcct ctatagcaag ctcaccgtgg acaagagcag gtggcagcag 1020

gggaacgtct tctcatgctc cgtgatgcat gaggctctgc acaaccacta cacgcagaag 1080

agcctctccc tgtccccggg taaatga 1107

<210> SEQ ID NO 49
<211> LENGTH: 1107
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polynucleotide

<400> SEQUENCE: 49

tcatttaccc ggggacaggg agaggctctt ctgcgtgtag tggttgtgca gagcctcatg 60

catcacggag catgagaaga cgttcccctg ctgccacctg ctcttgtcca cggtgagctt 120

gctatagagg aagaaggagc cgtcggagtc cagcacggga ggcgtggtct tgtagttgtt 180

ctccggctgc ccattgctct cccactccac ggcgatgtcg ctgggataga agcctttgac 240

caggcaggtc aggctgacct ggttcttggt catctcctcc cgggatgggg gcagggtgta 300

cacctgtggt tctcggggct gccctttggc tttggagatg gttttctcga tgggggctgg 360

gagggctttg ttggagacct tgcacttgta ctccttgcca ttcagccagt cctggtgcag 420

gacggtgagg acgctgacca cacggtacgt gctgttgtac tgctcctccc gcggctttgt 480

cttggcatta tgcacctcca cgccgtccac gtaccagttg aacttgacct cagggtcttc 540

gtggctcacg tccaccacca cgcatgtgac ctcaggggtc cgggagatca tgagggtgtc 600

cttgggtttt gggggggaaga ggaagactga cggtcccccc aggagttcag gtgctgggca 660

cggtgggcat gtgtgagttc caccaccggt gggggctgtc gggggtggct cgtacgtgac 720

ttccgggccc ccagcctctg gcaaatgagt gaagcgctcg ttgcagaagt tgccttcaca 780

gcagcagaag tacacctggg ggttctcctc agtggccaca cactcctgcc tatcgtagca 840

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gttgaagtca tcatcccagc agcccttctt cacgagctcg atggtgccag agctgttgcg 900

ccaggaggcg tagcagtgca gccgcttgtc ctgctcgcct tcgcagcgct ccaggccgct 960

ctggttggtg cgctccagct cccagttggc gttgtagtag atgcactccc gtgtctcagc 1020

ctccccacgc ccagaggcgc cgggcgaaac gaagactgct ccacacagca gcagcacaca 1080

gcagagccct ctcttcattg catccat 1107

<210> SEQ ID NO 50
<211> LENGTH: 360
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 50

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly
1 5 10 15

Ala Val Phe Val Ser Pro Gly Ala Ala Glu Thr Arg Glu Cys Ile Tyr
20 25 30

Tyr Asn Ala Asn Trp Glu Leu Glu Arg Thr Asn Gln Ser Gly Leu Glu
35 40 45

Arg Cys Glu Gly Glu Gln Asp Lys Arg Leu His Cys Tyr Ala Ser Trp
50 55 60

Arg Asn Ser Ser Gly Thr Ile Glu Leu Val Lys Lys Gly Cys Trp Asp
65 70 75 80

Asp Asp Phe Asn Cys Tyr Asp Arg Gln Glu Cys Val Ala Thr Glu Glu
85 90 95

Asn Pro Gln Val Tyr Phe Cys Cys Cys Glu Gly Asn Phe Cys Asn Glu
100 105 110

Arg Phe Thr His Leu Pro Glu Ala Gly Gly Pro Glu Val Thr Tyr Glu
115 120 125

Pro Pro Pro Thr Gly Gly Gly Thr His Thr Cys Pro Pro Cys Pro Ala
130 135 140

Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
145 150 155 160

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
165 170 175

Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
180 185 190

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
195 200 205

Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
210 215 220

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
225 230 235 240

Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
245 250 255

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr
260 265 270

Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
275 280 285

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr

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```
    290                 295                 300

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
305                 310                 315                 320

Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
                325                 330                 335

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
            340                 345                 350

Ser Leu Ser Leu Ser Pro Gly Lys
        355                 360
```

<210> SEQ ID NO 51
<211> LENGTH: 1083
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polynucleotide

<400> SEQUENCE: 51

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atggatgcaa tgaagagagg gctctgctgt gtgctgctgc tgtgtggagc agtcttcgtt     60

tcgcccggcg ccgctgagac acgggagtgc atctactaca acgccaactg ggagctggag    120

cgcaccaacc agagcggcct ggagcgctgc gaaggcgagc aggacaagcg gctgcactgc    180

tacgcctcct ggcgcaacag ctctggcacc atcgagctcg tgaagaaggg ctgctgggac    240

gatgacttca actgctacga taggcaggag tgtgtggcca ctgaggagaa cccccaggtg    300

tacttctgct gctgtgaagg caacttctgc aacgagcgct tcactcattt gccagaggct    360

gggggcccgg aagtcacgta cgagccaccc ccgacaggtg gtggaactca cacatgccca    420

ccgtgcccag cacctgaact cctgggggga ccgtcagtct tcctcttccc cccaaaaccc    480

aaggacaccc tcatgatctc ccggacccct gaggtcacat gcgtggtggt ggacgtgagc    540

cacgaagacc ctgaggtcaa gttcaactgg tacgtggacg gcgtggaggt gcataatgcc    600

aagacaaagc cgcgggagga gcagtacaac agcacgtacc gtgtggtcag cgtcctcacc    660

gtcctgcacc aggactggct gaatggcaag gagtacaagt gcaaggtctc caacaaagcc    720

ctcccagccc ccatcgagaa aaccatctcc aaagccaaag ggcagccccg agaaccacag    780

gtgtacaccc tgcccccatc ccgggaggag atgaccaaga accaggtcag cctgacctgc    840

ctggtcaaag gcttctatcc cagcgacatc gccgtggagt gggagagcaa tgggcagccg    900

gagaacaact acaagaccac gcctcccgtg ctggactccg acggctcctt cttcctctat    960

agcaagctca ccgtggacaa gagcaggtgg cagcagggga acgtcttctc atgctccgtg   1020

atgcatgagg ctctgcacaa ccactacacg cagaagagcc tctccctgtc cccgggtaaa   1080

tga                                                                 1083
```

<210> SEQ ID NO 52
<211> LENGTH: 1083
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polynucleotide

<400> SEQUENCE: 52

```
tcatttaccc ggggacaggg agaggctctt ctgcgtgtag tggttgtgca gagcctcatg     60
```

-continued

catcacggag catgagaaga cgttcccctg ctgccacctg ctcttgtcca cggtgagctt 120

gctatagagg aagaaggagc cgtcggagtc cagcacggga ggcgtggtct tgtagttgtt 180

ctccggctgc ccattgctct cccactccac ggcgatgtcg ctgggataga agcctttgac 240

caggcaggtc aggctgacct ggttcttggt catctcctcc cgggatgggg gcagggtgta 300

cacctgtggt tctcggggct gccctttggc tttggagatg gttttctcga tgggggctgg 360

gagggctttg ttggagacct tgcacttgta ctccttgcca ttcagccagt cctggtgcag 420

gacggtgagg acgctgacca cacggtacgt gctgttgtac tgctcctccc gcggctttgt 480

cttggcatta tgcacctcca cgccgtccac gtaccagttg aacttgacct cagggtcttc 540

gtggctcacg tccaccacca cgcatgtgac ctcaggggtc cgggagatca tgagggtgtc 600

cttgggtttt gggggggaaga ggaagactga cggtcccccc aggagttcag gtgctgggca 660

cggtgggcat gtgtgagttc caccacctgt cgggggtggc tcgtacgtga cttccgggcc 720

cccagcctct ggcaaatgag tgaagcgctc gttgcagaag ttgccttcac agcagcagaa 780

gtacacctgg gggttctcct cagtggccac acactcctgc ctatcgtagc agttgaagtc 840

atcgtcccag cagcccttct tcacgagctc gatggtgcca gagctgttgc gccaggaggc 900

gtagcagtgc agccgcttgt cctgctcgcc ttcgcagcgc tccaggccgc tctggttggt 960

gcgctccagc tcccagttgg cgttgtagta gatgcactcc cgtgtctcag cggcgccggg 1020

cgaaacgaag actgctccac acagcagcag cacacagcag agccctctct tcattgcatc 1080

cat 1083

<210> SEQ ID NO 53
<211> LENGTH: 335
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 53

Glu Thr Arg Glu Cys Ile Tyr Tyr Asn Ala Asn Trp Glu Leu Glu Arg
1 5 10 15

Thr Asn Gln Ser Gly Leu Glu Arg Cys Glu Gly Glu Gln Asp Lys Arg
20 25 30

Leu His Cys Tyr Ala Ser Trp Arg Asn Ser Ser Gly Thr Ile Glu Leu
35 40 45

Val Lys Lys Gly Cys Trp Asp Asp Asp Phe Asn Cys Tyr Asp Arg Gln
50 55 60

Glu Cys Val Ala Thr Glu Glu Asn Pro Gln Val Tyr Phe Cys Cys Cys
65 70 75 80

Glu Gly Asn Phe Cys Asn Glu Arg Phe Thr His Leu Pro Glu Ala Gly
85 90 95

Gly Pro Glu Val Thr Tyr Glu Pro Pro Pro Thr Gly Gly Gly Thr His
100 105 110

Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val
115 120 125

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
130 135 140

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
145 150 155 160

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Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
                165                 170                 175

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
            180                 185                 190

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
        195                 200                 205

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
    210                 215                 220

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
225                 230                 235                 240

Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
                245                 250                 255

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
            260                 265                 270

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
        275                 280                 285

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
    290                 295                 300

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
305                 310                 315                 320

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
                325                 330                 335


<210> SEQ ID NO 54
<211> LENGTH: 107
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      polypeptide

<400> SEQUENCE: 54

Glu Thr Arg Glu Cys Ile Tyr Tyr Asn Ala Asn Trp Glu Leu Glu Arg
1               5                   10                  15

Thr Asn Gln Ser Gly Leu Glu Arg Cys Glu Gly Glu Gln Asp Lys Arg
            20                  25                  30

Leu His Cys Tyr Ala Ser Trp Arg Asn Ser Ser Gly Thr Ile Glu Leu
        35                  40                  45

Val Lys Lys Gly Cys Trp Asp Asp Asp Phe Asn Cys Tyr Asp Arg Gln
    50                  55                  60

Glu Cys Val Ala Thr Glu Glu Asn Pro Gln Val Tyr Phe Cys Cys Cys
65                  70                  75                  80

Glu Gly Asn Phe Cys Asn Glu Arg Phe Thr His Leu Pro Glu Ala Gly
                85                  90                  95

Gly Pro Glu Val Thr Tyr Glu Pro Pro Pro Thr
            100                 105


<210> SEQ ID NO 55
<211> LENGTH: 1083
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      polynucleotide

<400> SEQUENCE: 55
```

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atggatgcaa tgaagagagg gctctgctgt gtgctgctgc tgtgtggagc agtcttcgtt 60

tcgcccggcg ccgccgaaac ccgcgaatgt atttattaca atgctaattg ggaactcgaa 120

cggacgaacc aatccgggct cgaacggtgt gaggggggaac aggataaacg cctccattgc 180

tatgcgtcgt ggaggaactc ctccgggacg attgaactgg tcaagaaagg gtgctgggac 240

gacgatttca attgttatga ccgccaggaa tgtgtcgcga ccgaagagaa tccgcaggtc 300

tatttctgtt gttgcgaggg gaatttctgt aatgaacggt ttacccacct ccccgaagcc 360

ggcgggcccg aggtgaccta tgaacccccg cccaccggtg gtggaactca cacatgccca 420

ccgtgcccag cacctgaact cctgggggga ccgtcagtct tcctcttccc cccaaaaccc 480

aaggacaccc tcatgatctc ccggacccct gaggtcacat gcgtggtggt ggacgtgagc 540

cacgaagacc ctgaggtcaa gttcaactgg tacgtggacg gcgtggaggt gcataatgcc 600

aagacaaagc cgcgggagga gcagtacaac agcacgtacc gtgtggtcag cgtcctcacc 660

gtcctgcacc aggactggct gaatggcaag gagtacaagt gcaaggtctc caacaaagcc 720

ctcccagccc ccatcgagaa aaccatctcc aaagccaaag ggcagccccg agaaccacag 780

gtgtacaccc tgcccccatc ccgggaggag atgaccaaga accaggtcag cctgacctgc 840

ctggtcaaag gcttctatcc cagcgacatc gccgtggagt gggagagcaa tgggcagccg 900

gagaacaact acaagaccac gcctcccgtg ctggactccg acggctcctt cttcctctat 960

agcaagctca ccgtggacaa gagcaggtgg cagcagggga acgtcttctc atgctccgtg 1020

atgcatgagg ctctgcacaa ccactacacg cagaagagcc tctccctgtc cccgggtaaa 1080

tga 1083

<210> SEQ ID NO 56
<211> LENGTH: 1083
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polynucleotide

<400> SEQUENCE: 56

tcatttaccc ggggacaggg agaggctctt ctgcgtgtag tggttgtgca gagcctcatg 60

catcacggag catgagaaga cgttcccctg ctgccacctg ctcttgtcca cggtgagctt 120

gctatagagg aagaaggagc cgtcggagtc cagcacggga ggcgtggtct tgtagttgtt 180

ctccggctgc ccattgctct cccactccac ggcgatgtcg ctgggataga agcctttgac 240

caggcaggtc aggctgacct ggttcttggt catctcctcc cgggatgggg gcagggtgta 300

cacctgtggt tctcggggct gcccctttggc tttggagatg gttttctcga tgggggctgg 360

gagggctttg ttggagacct tgcacttgta ctccttgcca ttcagccagt cctggtgcag 420

gacggtgagg acgctgacca cacggtacgt gctgttgtac tgctcctccc gcggctttgt 480

cttggcatta tgcacctcca cgccgtccac gtaccagttg aacttgacct cagggtcttc 540

gtggctcacg tccaccacca cgcatgtgac ctcaggggtc cgggagatca tgagggtgtc 600

cttgggtttt gggggggaaga ggaagactga cggtcccccc aggagttcag gtgctgggca 660

cggtgggcat gtgtgagttc caccaccggt gggcgggggt tcataggtca cctcgggccc 720

gccggcttcg gggaggtggg taaaccgttc attacagaaa ttcccctcgc aacaacagaa 780

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atagacctgc ggattctctt cggtcgcgac acattcctgg cggtcataac aattgaaatc 840

gtcgtcccag cacccttttct tgaccagttc aatcgtcccg gaggagttcc tccacgacgc 900

atagcaatgg aggcgtttat cctgttcccc ctcacaccgt tcgagcccgg attggttcgt 960

ccgttcgagt tcccaattag cattgtaata aatacattcg cgggtttcgg cggcgccggg 1020

cgaaacgaag actgctccac acagcagcag cacacagcag agccctctct tcattgcatc 1080

cat 1083

<210> SEQ ID NO 57
<211> LENGTH: 321
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polynucleotide

<400> SEQUENCE: 57

gaaacccgcg aatgtattta ttacaatgct aattgggaac tcgaacggac gaaccaatcc 60

gggctcgaac ggtgtgaggg ggaacaggat aaacgcctcc attgctatgc gtcgtggagg 120

aactcctccg ggacgattga actggtcaag aaagggtgct gggacgacga tttcaattgt 180

tatgaccgcc aggaatgtgt cgcgaccgaa gagaatccgc aggtctattt ctgttgttgc 240

gaggggaatt tctgtaatga acggtttacc cacctccccg aagccggcgg gcccgaggtg 300

acctatgaac ccccgcccac c 321

<210> SEQ ID NO 58
<211> LENGTH: 360
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 58

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly
1 5 10 15

Ala Val Phe Val Ser Pro Gly Ala Ala Glu Thr Arg Glu Cys Ile Tyr
20 25 30

Tyr Asn Ala Asn Trp Glu Leu Glu Arg Thr Asn Gln Ser Gly Leu Glu
35 40 45

Arg Cys Glu Gly Glu Gln Asp Lys Arg Leu His Cys Tyr Ala Ser Trp
50 55 60

Arg Asn Ser Ser Gly Thr Ile Glu Leu Val Lys Lys Gly Cys Trp Leu
65 70 75 80

Asp Asp Phe Asn Cys Tyr Asp Arg Gln Glu Cys Val Ala Thr Glu Glu
85 90 95

Asn Pro Gln Val Tyr Phe Cys Cys Cys Glu Gly Asn Phe Cys Asn Glu
100 105 110

Arg Phe Thr His Leu Pro Glu Ala Gly Gly Pro Glu Val Thr Tyr Glu
115 120 125

Pro Pro Pro Thr Gly Gly Gly Thr His Thr Cys Pro Pro Cys Pro Ala
130 135 140

Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
145 150 155 160

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Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
                165                 170                 175

Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
            180                 185                 190

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
        195                 200                 205

Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
    210                 215                 220

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
225                 230                 235                 240

Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
                245                 250                 255

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr
            260                 265                 270

Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
        275                 280                 285

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
    290                 295                 300

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
305                 310                 315                 320

Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
                325                 330                 335

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
            340                 345                 350

Ser Leu Ser Leu Ser Pro Gly Lys
        355                 360


<210> SEQ ID NO 59
<211> LENGTH: 1083
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      polynucleotide

<400> SEQUENCE: 59

atggatgcaa tgaagagagg gctctgctgt gtgctgctgc tgtgtggagc agtcttcgtt     60

tcgcccggcg ccgctgagac acgggagtgc atctactaca acgccaactg ggagctggag    120

cgcaccaacc agagcggcct ggagcgctgc gaaggcgagc aggacaagcg gctgcactgc    180

tacgcctcct ggcgcaacag ctctggcacc atcgagctcg tgaagaaggg ctgctggcta    240

gatgacttca actgctacga taggcaggag tgtgtggcca ctgaggagaa cccccaggtg    300

tacttctgct gctgtgaagg caacttctgc aacgagcgct tcactcattt gccagaggct    360

gggggcccgg aagtcacgta cgagccaccc ccgacaggtg gtggaactca cacatgccca    420

ccgtgcccag cacctgaact cctgggggga ccgtcagtct tcctcttccc cccaaaaccc    480

aaggacaccc tcatgatctc ccggacccct gaggtcacat gcgtggtggt ggacgtgagc    540

cacgaagacc ctgaggtcaa gttcaactgg tacgtggacg gcgtggaggt gcataatgcc    600

aagacaaagc cgcgggagga gcagtacaac agcacgtacc gtgtggtcag cgtcctcacc    660

gtcctgcacc aggactggct gaatggcaag gagtacaagt gcaaggtctc caacaaagcc    720

ctcccagccc ccatcgagaa aaccatctcc aaagccaaag ggcagccccg agaaccacag    780
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gtgtacaccc tgcccccatc ccgggaggag atgaccaaga accaggtcag cctgacctgc 840

ctggtcaaag gcttctatcc cagcgacatc gccgtggagt gggagagcaa tgggcagccg 900

gagaacaact acaagaccac gcctcccgtg ctggactccg acggctcctt cttcctctat 960

agcaagctca ccgtggacaa gagcaggtgg cagcagggga acgtcttctc atgctccgtg 1020

atgcatgagg ctctgcacaa ccactacacg cagaagagcc tctccctgtc cccgggtaaa 1080

tga 1083

<210> SEQ ID NO 60
<211> LENGTH: 1083
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polynucleotide

<400> SEQUENCE: 60

tcatttaccc ggggacaggg agaggctctt ctgcgtgtag tggttgtgca gagcctcatg 60

catcacggag catgagaaga cgttcccctg ctgccacctg ctcttgtcca cggtgagctt 120

gctatagagg aagaaggagc cgtcggagtc cagcacggga ggcgtggtct tgtagttgtt 180

ctccggctgc ccattgctct cccactccac ggcgatgtcg ctgggataga agcctttgac 240

caggcaggtc aggctgacct ggttcttggt catctcctcc cgggatgggg gcagggtgta 300

cacctgtggt tctcggggct gccctttggc tttggagatg gttttctcga tgggggctgg 360

gagggctttg ttggagacct tgcacttgta ctccttgcca ttcagccagt cctggtgcag 420

gacggtgagg acgctgacca cacggtacgt gctgttgtac tgctcctccc gcggctttgt 480

cttggcatta tgcacctcca cgccgtccac gtaccagttg aacttgacct cagggtcttc 540

gtggctcacg tccaccacca cgcatgtgac ctcaggggtc cgggagatca tgagggtgtc 600

cttgggtttt gggggggaaga ggaagactga cggtcccccc aggagttcag gtgctgggca 660

cggtgggcat gtgtgagttc caccacctgt cgggggtggc tcgtacgtga cttccgggcc 720

cccagcctct ggcaaatgag tgaagcgctc gttgcagaag ttgccttcac agcagcagaa 780

gtacacctgg gggttctcct cagtggccac acactcctgc ctatcgtagc agttgaagtc 840

atctagccag cagcccttct tcacgagctc gatggtgcca gagctgttgc gccaggaggc 900

gtagcagtgc agccgcttgt cctgctcgcc ttcgcagcgc tccaggccgc tctggttggt 960

gcgctccagc tcccagttgg cgttgtagta gatgcactcc cgtgtctcag cggcgccggg 1020

cgaaacgaag actgctccac acagcagcag cacacagcag agccctctct tcattgcatc 1080

cat 1083

<210> SEQ ID NO 61
<211> LENGTH: 1083
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polynucleotide

<400> SEQUENCE: 61

atggatgcaa tgaagagagg gctctgctgt gtgctgctgc tgtgtggagc agtcttcgtt 60

tcgcccggcg ccgccgaaac ccgcgaatgt atttattaca atgctaattg ggaactcgaa 120

-continued

cggacgaacc aatccgggct cgaacggtgt gaggggggaac aggataaacg cctccattgc 180

tatgcgtcgt ggaggaactc ctccgggacg attgaactgg tcaagaaagg gtgctggctg 240

gacgatttca attgttatga ccgccaggaa tgtgtcgcga ccgaagagaa tccgcaggtc 300

tatttctgtt gttgcgaggg gaatttctgt aatgaacggt ttacccacct ccccgaagcc 360

ggcgggcccg aggtgaccta tgaacccccg cccaccggtg gtggaactca cacatgccca 420

ccgtgcccag cacctgaact cctgggggga ccgtcagtct tcctcttccc cccaaaaccc 480

aaggacaccc tcatgatctc ccggacccct gaggtcacat gcgtggtggt ggacgtgagc 540

cacgaagacc ctgaggtcaa gttcaactgg tacgtggacg gcgtggaggt gcataatgcc 600

aagacaaagc cgcgggagga gcagtacaac agcacgtacc gtgtggtcag cgtcctcacc 660

gtcctgcacc aggactggct gaatggcaag gagtacaagt gcaaggtctc caacaaagcc 720

ctcccagccc ccatcgagaa aaccatctcc aaagccaaag ggcagccccg agaaccacag 780

gtgtacaccc tgcccccatc ccgggaggag atgaccaaga accaggtcag cctgacctgc 840

ctggtcaaag gcttctatcc cagcgacatc gccgtggagt gggagagcaa tgggcagccg 900

gagaacaact acaagaccac gcctcccgtg ctggactccg acggctcctt cttcctctat 960

agcaagctca ccgtggacaa gagcaggtgg cagcagggga acgtcttctc atgctccgtg 1020

atgcatgagg ctctgcacaa ccactacacg cagaagagcc tctccctgtc cccgggtaaa 1080

tga 1083

<210> SEQ ID NO 62
<211> LENGTH: 1083
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polynucleotide

<400> SEQUENCE: 62

tcatttaccc ggggacaggg agaggctctt ctgcgtgtag tggttgtgca gagcctcatg 60

catcacggag catgagaaga cgttcccctg ctgccacctg ctcttgtcca cggtgagctt 120

gctatagagg aagaaggagc cgtcggagtc cagcacggga ggcgtggtct tgtagttgtt 180

ctccggctgc ccattgctct cccactccac ggcgatgtcg ctgggataga agcctttgac 240

caggcaggtc aggctgacct ggttcttggt catctcctcc cgggatgggg gcagggtgta 300

cacctgtggt tctcggggct gcccctttggc tttggagatg gttttctcga tgggggctgg 360

gagggctttg ttggagacct tgcacttgta ctccttgcca ttcagccagt cctggtgcag 420

gacggtgagg acgctgacca cacggtacgt gctgttgtac tgctcctccc gcggctttgt 480

cttggcatta tgcacctcca cgccgtccac gtaccagttg aacttgacct cagggtcttc 540

gtggctcacg tccaccacca cgcatgtgac ctcaggggtc cgggagatca tgagggtgtc 600

cttgggtttt gggggggaaga ggaagactga cggtcccccc aggagttcag gtgctgggca 660

cggtgggcat gtgtgagttc caccaccggt gggcgggggt tcataggtca cctcgggccc 720

gccggcttcg gggaggtggg taaaccgttc attacagaaa ttcccctcgc aacaacagaa 780

atagacctgc ggattctctt cggtcgcgac acattcctgg cggtcataac aattgaaatc 840

gtccagccag cacccttttct tgaccagttc aatcgtcccg gaggagttcc tccacgacgc 900

atagcaatgg aggcgtttat cctgttcccc ctcacaccgt tcgagcccgg attggttcgt 960

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ccgttcgagt tcccaattag cattgtaata aatacattcg cgggtttcgg cggcgccggg 1020

cgaaacgaag actgctccac acagcagcag cacacagcag agccctctct tcattgcatc 1080

cat 1083

<210> SEQ ID NO 63
<211> LENGTH: 344
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 63

Met Val Arg Ala Arg His Gln Pro Gly Gly Leu Cys Leu Leu Leu Leu
1 5 10 15

Leu Leu Cys Gln Phe Met Glu Asp Arg Ser Ala Gln Ala Gly Asn Cys
20 25 30

Trp Leu Arg Gln Ala Lys Asn Gly Arg Cys Gln Val Leu Tyr Lys Thr
35 40 45

Glu Leu Ser Lys Glu Glu Cys Cys Ser Thr Gly Arg Leu Ser Thr Ser
50 55 60

Trp Thr Glu Glu Asp Val Asn Asp Asn Thr Leu Phe Lys Trp Met Ile
65 70 75 80

Phe Asn Gly Gly Ala Pro Asn Cys Ile Pro Cys Lys Glu Thr Cys Glu
85 90 95

Asn Val Asp Cys Gly Pro Gly Lys Lys Cys Arg Met Asn Lys Lys Asn
100 105 110

Lys Pro Arg Cys Val Cys Ala Pro Asp Cys Ser Asn Ile Thr Trp Lys
115 120 125

Gly Pro Val Cys Gly Leu Asp Gly Lys Thr Tyr Arg Asn Glu Cys Ala
130 135 140

Leu Leu Lys Ala Arg Cys Lys Glu Gln Pro Glu Leu Glu Val Gln Tyr
145 150 155 160

Gln Gly Arg Cys Lys Lys Thr Cys Arg Asp Val Phe Cys Pro Gly Ser
165 170 175

Ser Thr Cys Val Val Asp Gln Thr Asn Asn Ala Tyr Cys Val Thr Cys
180 185 190

Asn Arg Ile Cys Pro Glu Pro Ala Ser Ser Glu Gln Tyr Leu Cys Gly
195 200 205

Asn Asp Gly Val Thr Tyr Ser Ser Ala Cys His Leu Arg Lys Ala Thr
210 215 220

Cys Leu Leu Gly Arg Ser Ile Gly Leu Ala Tyr Glu Gly Lys Cys Ile
225 230 235 240

Lys Ala Lys Ser Cys Glu Asp Ile Gln Cys Thr Gly Gly Lys Lys Cys
245 250 255

Leu Trp Asp Phe Lys Val Gly Arg Gly Arg Cys Ser Leu Cys Asp Glu
260 265 270

Leu Cys Pro Asp Ser Lys Ser Asp Glu Pro Val Cys Ala Ser Asp Asn
275 280 285

Ala Thr Tyr Ala Ser Glu Cys Ala Met Lys Glu Ala Ala Cys Ser Ser
290 295 300

Gly Val Leu Leu Glu Val Lys His Ser Gly Ser Cys Asn Ser Ile Ser
305 310 315 320

Glu Asp Thr Glu Glu Glu Glu Glu Asp Glu Asp Gln Asp Tyr Ser Phe

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                325                 330                 335

Pro Ile Ser Ser Ile Leu Glu Trp
            340


<210> SEQ ID NO 64
<211> LENGTH: 317
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 64

Met Val Arg Ala Arg His Gln Pro Gly Gly Leu Cys Leu Leu Leu Leu
1               5                   10                  15

Leu Leu Cys Gln Phe Met Glu Asp Arg Ser Ala Gln Ala Gly Asn Cys
            20                  25                  30

Trp Leu Arg Gln Ala Lys Asn Gly Arg Cys Gln Val Leu Tyr Lys Thr
        35                  40                  45

Glu Leu Ser Lys Glu Glu Cys Cys Ser Thr Gly Arg Leu Ser Thr Ser
    50                  55                  60

Trp Thr Glu Glu Asp Val Asn Asp Asn Thr Leu Phe Lys Trp Met Ile
65                  70                  75                  80

Phe Asn Gly Gly Ala Pro Asn Cys Ile Pro Cys Lys Glu Thr Cys Glu
                85                  90                  95

Asn Val Asp Cys Gly Pro Gly Lys Lys Cys Arg Met Asn Lys Lys Asn
            100                 105                 110

Lys Pro Arg Cys Val Cys Ala Pro Asp Cys Ser Asn Ile Thr Trp Lys
        115                 120                 125

Gly Pro Val Cys Gly Leu Asp Gly Lys Thr Tyr Arg Asn Glu Cys Ala
    130                 135                 140

Leu Leu Lys Ala Arg Cys Lys Glu Gln Pro Glu Leu Glu Val Gln Tyr
145                 150                 155                 160

Gln Gly Arg Cys Lys Lys Thr Cys Arg Asp Val Phe Cys Pro Gly Ser
                165                 170                 175

Ser Thr Cys Val Val Asp Gln Thr Asn Asn Ala Tyr Cys Val Thr Cys
            180                 185                 190

Asn Arg Ile Cys Pro Glu Pro Ala Ser Ser Glu Gln Tyr Leu Cys Gly
        195                 200                 205

Asn Asp Gly Val Thr Tyr Ser Ser Ala Cys His Leu Arg Lys Ala Thr
    210                 215                 220

Cys Leu Leu Gly Arg Ser Ile Gly Leu Ala Tyr Glu Gly Lys Cys Ile
225                 230                 235                 240

Lys Ala Lys Ser Cys Glu Asp Ile Gln Cys Thr Gly Gly Lys Lys Cys
                245                 250                 255

Leu Trp Asp Phe Lys Val Gly Arg Gly Arg Cys Ser Leu Cys Asp Glu
            260                 265                 270

Leu Cys Pro Asp Ser Lys Ser Asp Glu Pro Val Cys Ala Ser Asp Asn
        275                 280                 285

Ala Thr Tyr Ala Ser Glu Cys Ala Met Lys Glu Ala Ala Cys Ser Ser
    290                 295                 300

Gly Val Leu Leu Glu Val Lys His Ser Gly Ser Cys Asn
305                 310                 315


<210> SEQ ID NO 65
<211> LENGTH: 63
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-continued

<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 65

Gly Asn Cys Trp Leu Arg Gln Ala Lys Asn Gly Arg Cys Gln Val Leu
1 5 10 15

Tyr Lys Thr Glu Leu Ser Lys Glu Glu Cys Cys Ser Thr Gly Arg Leu
20 25 30

Ser Thr Ser Trp Thr Glu Glu Asp Val Asn Asp Asn Thr Leu Phe Lys
35 40 45

Trp Met Ile Phe Asn Gly Gly Ala Pro Asn Cys Ile Pro Cys Lys
50 55 60

<210> SEQ ID NO 66
<211> LENGTH: 25
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 66

Glu Thr Cys Glu Asn Val Asp Cys Gly Pro Gly Lys Lys Cys Arg Met
1 5 10 15

Asn Lys Lys Asn Lys Pro Arg Cys Val
20 25

<210> SEQ ID NO 67
<211> LENGTH: 26
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 67

Lys Thr Cys Arg Asp Val Phe Cys Pro Gly Ser Ser Thr Cys Val Val
1 5 10 15

Asp Gln Thr Asn Asn Ala Tyr Cys Val Thr
20 25

<210> SEQ ID NO 68
<211> LENGTH: 263
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 68

Met Arg Pro Gly Ala Pro Gly Pro Leu Trp Pro Leu Pro Trp Gly Ala
1 5 10 15

Leu Ala Trp Ala Val Gly Phe Val Ser Ser Met Gly Ser Gly Asn Pro
20 25 30

Ala Pro Gly Gly Val Cys Trp Leu Gln Gln Gly Gln Glu Ala Thr Cys
35 40 45

Ser Leu Val Leu Gln Thr Asp Val Thr Arg Ala Glu Cys Cys Ala Ser
50 55 60

Gly Asn Ile Asp Thr Ala Trp Ser Asn Leu Thr His Pro Gly Asn Lys
65 70 75 80

Ile Asn Leu Leu Gly Phe Leu Gly Leu Val His Cys Leu Pro Cys Lys
85 90 95

Asp Ser Cys Asp Gly Val Glu Cys Gly Pro Gly Lys Ala Cys Arg Met
100 105 110

Leu Gly Gly Arg Pro Arg Cys Glu Cys Ala Pro Asp Cys Ser Gly Leu
115 120 125

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Pro Ala Arg Leu Gln Val Cys Gly Ser Asp Gly Ala Thr Tyr Arg Asp
    130                 135                 140

Glu Cys Glu Leu Arg Ala Ala Arg Cys Arg Gly His Pro Asp Leu Ser
145                 150                 155                 160

Val Met Tyr Arg Gly Arg Cys Arg Lys Ser Cys Glu His Val Val Cys
                165                 170                 175

Pro Arg Pro Gln Ser Cys Val Val Asp Gln Thr Gly Ser Ala His Cys
            180                 185                 190

Val Val Cys Arg Ala Ala Pro Cys Pro Val Pro Ser Ser Pro Gly Gln
        195                 200                 205

Glu Leu Cys Gly Asn Asn Asn Val Thr Tyr Ile Ser Ser Cys His Met
    210                 215                 220

Arg Gln Ala Thr Cys Phe Leu Gly Arg Ser Ile Gly Val Arg His Ala
225                 230                 235                 240

Gly Ser Cys Ala Gly Thr Pro Glu Glu Pro Pro Gly Gly Glu Ser Ala
                245                 250                 255

Glu Glu Glu Glu Asn Phe Val
            260


<210> SEQ ID NO 69
<211> LENGTH: 225
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 69

Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro
1               5                   10                  15

Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
            20                  25                  30

Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
        35                  40                  45

Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
    50                  55                  60

Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
65                  70                  75                  80

Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
                85                  90                  95

Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
            100                 105                 110

Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
        115                 120                 125

Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr
    130                 135                 140

Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
145                 150                 155                 160

Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
                165                 170                 175

Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
            180                 185                 190

Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
        195                 200                 205

Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
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    210                 215                 220

Lys
225


<210> SEQ ID NO 70
<211> LENGTH: 229
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 70

Glu Ser Lys Tyr Gly Pro Pro Cys Pro Ser Cys Pro Ala Pro Glu Phe
1               5                   10                  15

Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
            20                  25                  30

Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
        35                  40                  45

Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val
    50                  55                  60

Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser
65                  70                  75                  80

Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu
                85                  90                  95

Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser
            100                 105                 110

Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
        115                 120                 125

Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln
    130                 135                 140

Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
145                 150                 155                 160

Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
                165                 170                 175

Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu
            180                 185                 190

Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser Cys Ser
        195                 200                 205

Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
    210                 215                 220

Leu Ser Leu Gly Lys
225


<210> SEQ ID NO 71
<211> LENGTH: 223
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 71

Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val
1               5                   10                  15

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
            20                  25                  30

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
        35                  40                  45

Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
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    50                  55                  60

Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Val Val Ser
65                  70                  75                  80

Val Leu Thr Val Val His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
                85                  90                  95

Cys Lys Val Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu Lys Thr Ile
            100                 105                 110

Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
        115                 120                 125

Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
    130                 135                 140

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
145                 150                 155                 160

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met Leu Asp Ser
                165                 170                 175

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
            180                 185                 190

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
        195                 200                 205

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
    210                 215                 220


<210> SEQ ID NO 72
<211> LENGTH: 232
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 72

Glu Pro Lys Ser Cys Asp Thr Pro Pro Pro Cys Pro Arg Cys Pro Ala
1               5                   10                  15

Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
            20                  25                  30

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
        35                  40                  45

Val Asp Val Ser His Glu Asp Pro Glu Val Gln Phe Lys Trp Tyr Val
    50                  55                  60

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
65                  70                  75                  80

Tyr Asn Ser Thr Phe Arg Val Val Ser Val Leu Thr Val Leu His Gln
                85                  90                  95

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
            100                 105                 110

Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro
        115                 120                 125

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr
    130                 135                 140

Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
145                 150                 155                 160

Asp Ile Ala Val Glu Trp Glu Ser Ser Gly Gln Pro Glu Asn Asn Tyr
                165                 170                 175

Asn Thr Thr Pro Pro Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
            180                 185                 190
```

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Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Ile Phe
        195                 200                 205

Ser Cys Ser Val Met His Glu Ala Leu His Asn Arg Phe Thr Gln Lys
    210                 215                 220

Ser Leu Ser Leu Ser Pro Gly Lys
225                 230


<210> SEQ ID NO 73
<211> LENGTH: 108
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      polypeptide

<400> SEQUENCE: 73

Ala Glu Thr Arg Glu Cys Ile Tyr Tyr Asn Ala Asn Trp Glu Leu Glu
1               5                   10                  15

Arg Thr Asn Gln Ser Gly Leu Glu Arg Cys Glu Gly Glu Gln Asp Lys
            20                  25                  30

Arg Leu His Cys Tyr Ala Ser Trp Arg Asn Ser Ser Gly Thr Ile Glu
        35                  40                  45

Leu Val Lys Lys Gly Cys Trp Leu Asp Asp Phe Asn Cys Tyr Asp Arg
    50                  55                  60

Gln Glu Cys Val Ala Thr Glu Glu Asn Pro Gln Val Tyr Phe Cys Cys
65                  70                  75                  80

Cys Glu Gly Asn Phe Cys Asn Glu Arg Phe Thr His Leu Pro Glu Ala
                85                  90                  95

Gly Gly Pro Glu Val Thr Tyr Glu Pro Pro Pro Thr
            100                 105


<210> SEQ ID NO 74
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      6xHis tag

<400> SEQUENCE: 74

His His His His His His
1               5
```

1-2. (canceled)

3. A method for treating myelofibrosis, comprising administering to a patient in need thereof: a) a Janus kinase inhibitor; and b) an ActRIIB polypeptide, wherein the Janus kinase inhibitor and ActRIIB polypeptide are administered in an effective amount, and wherein the ActRIIB 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 a sequence starting at a residue corresponding to any one of amino acids 20-29 of SEQ ID NO: 1 and ending at a residue corresponding to any one of amino acids 109-134 of SEQ ID NO: 1.

4. (canceled)

5. The method of claim **3**, wherein the method decreases one or more of: bone marrow fibrosis, spleen fibrosis, liver fibrosis, lung fibrosis, and lymph node fibrosis.

6-11. (canceled)

12. The method of claim **3**, wherein the method increases red blood cell levels in the patient.

13. The method of claim **3**, wherein the method increases hemoglobin levels in the patient.

14. The method of claim **3**, wherein the patient has anemia.

15. The method of claim **14**, wherein the method treats the anemia.

16. The method of claim **3**, wherein the patient has been administered one or more blood cell transfusions prior to the start of ActRIIB polypeptide treatment.

17. The method of claim **3**, wherein the patient is blood cell transfusion-dependent.

18. The method of claim **17**, wherein the method decreases blood cell transfusion burden.

19. The method of claim **18**, wherein the method decreases blood cell transfusion by greater than about 30%

for 4 to 8 weeks relative to the equal time prior to the start of the ActRIIB polypeptide treatment.

20-22. (canceled)

23. The method of claim **3** wherein the patient has primary myelofibrosis.

24. The method of claim **3** wherein the patient has post-polycythemia vera myelofibrosis.

25. The method of claim **3** wherein the patient has post-essential thrombocythemia myelofibrosis.

26-31. (canceled)

32. The method of claim **3**, wherein the myelofibrosis is associated with one or more mutations in JAK2.

33. The method of claim **32**, wherein the JAK2 mutation is JAK2V617F.

34. (canceled)

35. The method of claim **3**, wherein the patient has been treated with a Janus kinase inhibitor.

36. The method of claim **3**, wherein the patient is intolerant of or has an inadequate response to a Janus kinase inhibitor.

37-42. (canceled)

43. The method of claim **36**, wherein the Janus kinase inhibitor is selected from the group consisting of: ruxolitinib, fedratinib (SAR302503), monoelotinib (CYT387), pacritinib, lestaurtinib, AZD-1480, BMS-911543, NS-018, LY2784544, SEP-701, XL019, and AT-9283.

44. The method of claim **43**, wherein the Janus kinase inhibitor is ruxolitinib.

45-46. (canceled)

47. The method of claim **3**, wherein the patient is further administered hydroxyurea or has previously been treated with hydroxyurea.

48. The method of claim **3**, wherein the patient is intolerant of hydroxyurea or has an inadequate response to hydroxyurea.

49. A method for increasing red blood cell levels and/or hemoglobin levels in a patient treated with a Janus kinase inhibitor, comprising administering to a patient in need thereof an effective amount of an ActRIIB polypeptide, wherein the ActRIIB 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 a sequence starting at a residue corresponding to any one of amino acids 20-29 of SEQ ID NO: 1 and ending at a residue corresponding to any one of amino acids 109-134 of SEQ ID NO: 1.

50. The method of claim **49**, wherein the patient has anemia.

51. The method of claim **50**, wherein the method treats the anemia.

52-53. (canceled)

54. The method of claim **49**, wherein the patient has been administered one or more blood cell transfusions prior to the start of ActRIIB polypeptide treatment.

55. The method of claim **49**, wherein the patient is blood cell transfusion-dependent.

56. The method of claim **55**, wherein the method decreases blood cell transfusion burden.

57. The method of claim **56**, wherein the method decreases blood cell transfusion by greater than about 30% for 4 to 8 weeks relative to the equal time prior to the start of the ActRIIB polypeptide treatment.

58-65. (canceled)

66. The method of claim **49**, wherein the Janus kinase inhibitor is selected from the group consisting of: ruxolitinib, fedratinib (SAR302503), monoelotinib (CYT387), pacritinib, lestaurtinib, AZD-1480, BMS-911543, NS-018, LY2784544, SEP-701, XL019, and AT-9283.

67. The method of claim **66**, wherein the Janus kinase inhibitor is ruxolitinib.

68. (canceled)

69. The method of claim **3**, wherein the ActRIIB polypeptide is selected from the group consisting of:

a) a polypeptide comprising 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 amino acids 29-109 of SEQ ID NO: 1;

b) a polypeptide comprising 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 amino acids 25-131 of SEQ ID NO: 1;

c) a polypeptide comprising 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;

d) a polypeptide comprising 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: 3;

e) a polypeptide comprising 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: 4;

f) a polypeptide comprising 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: 5;

g) a polypeptide comprising 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: 6;

h) a polypeptide comprising 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; and

i) a polypeptide comprising 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: 54.

70. The method of claim **69**, wherein the polypeptide comprises an acidic amino acid at the amino acid position corresponding to position 79 of SEQ ID NO: 1.

71. The method of claim **70**, wherein the polypeptide comprises a D at the amino acid position corresponding to position 79 of SEQ ID NO: 1.

72. The method of claim **70**, wherein the polypeptide comprises a E at the amino acid position corresponding to position 79 of SEQ ID NO: 1.

73. The method of claim **69**, wherein the polypeptide is a fusion protein comprising an immunoglobulin Fc domain.

74. The method of claim **73**, wherein the immunoglobulin Fc domain is from an IgG1 Fc domain.

75. (canceled)

76. The method of claim **73**, wherein the fusion protein further comprises a linker domain positioned between the ActRIIB domain and the immunoglobulin Fc domain.

77. (canceled)

78. A method for treating myelofibrosis, comprising administering to a patient in need thereof: a Janus kinase inhibitor; and b) an ActRIIB polypeptide, wherein the Janus kinase inhibitor are administered in an effective amount, and wherein the polypeptide is an ActRIIB-Fc fusion protein comprising a polypeptide selected from:

a) a polypeptide comprising 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: 24;

b) a polypeptide comprising 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: 25;

c) a polypeptide comprising 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;

d) a polypeptide comprising 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;

e) a polypeptide comprising 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;

f) a polypeptide comprising 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: 45;

g) a polypeptide comprising 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: 50;

h) a polypeptide comprising 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: 53; and

i) a polypeptide comprising 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: 58.

79-91. (canceled)

92. A method of for treating myelofibrosis, comprising administering to a patient in need thereof: a Janus kinase inhibitor; and b) an ActRIIB antagonist, wherein the Janus kinase inhibitor are administered in an effective amount, and wherein the ActRIIB antagonist is:

i. an antibody or combination of antibodies

ii. a follistatin polypeptide

iii. a FLRG polypeptide.

93-114. (canceled)

115. The method of claim **3**, wherein the ActRIIB polypeptide comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 53.

116. The method of claim **3**, wherein the ActRIIB polypeptide comprises the amino acid sequence of SEQ ID NO: 53.

* * * * *