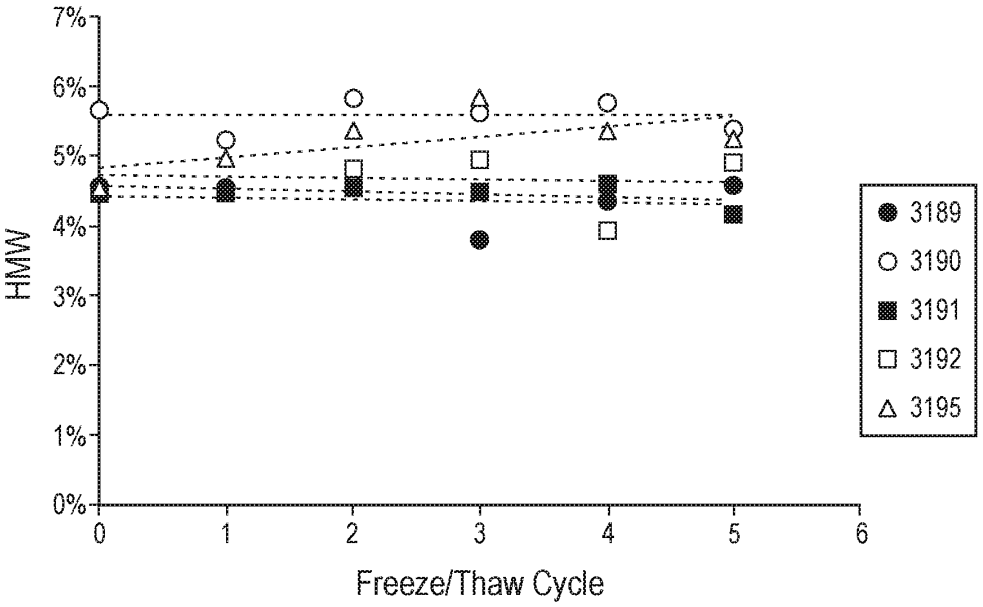

7%
6%
5%
4%
3%
2%
1%
0%
HMW
0
1
2
3
4
5
6
Freeze/Thaw Cycle
3189
3190
3191
3192
3195

FIG. 1

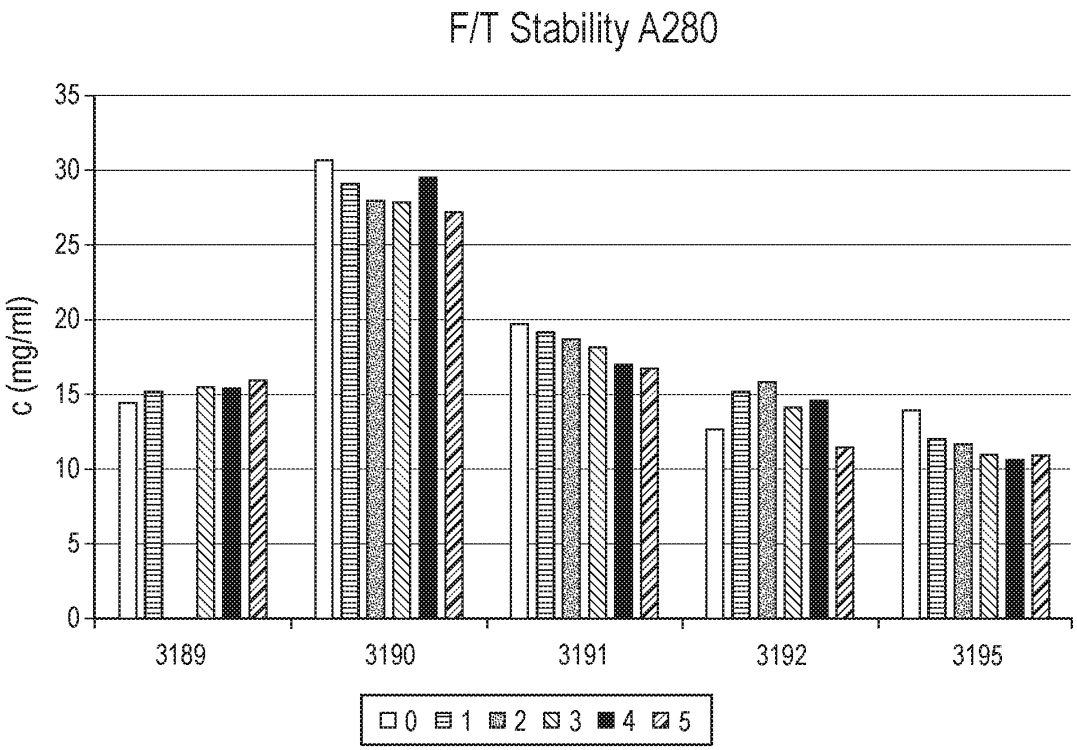

F/T Stability A280
35
30
25
20
15
10
5
0
c (mg/ml)
3189
3190
3191
3192
3195
0
1
2
3
4
5

FIG. 2A

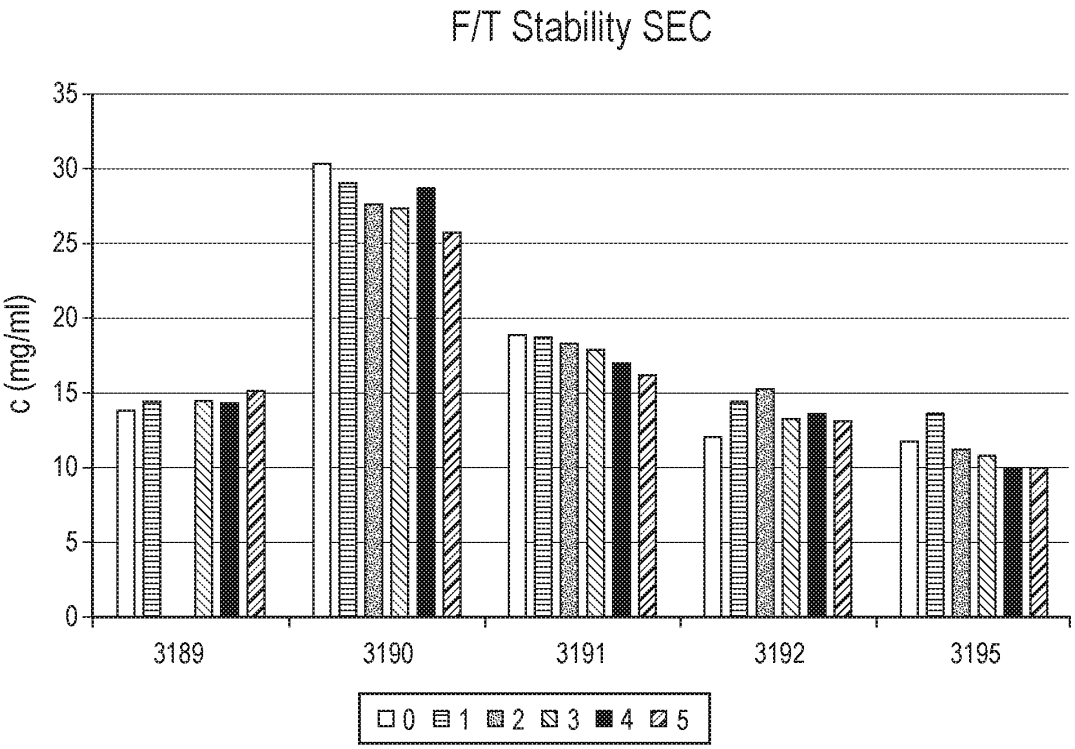

F/T Stability SEC
c (mg/ml)
35
30
25
20
15
10
5
0
3189
3190
3191
3192
3195
0 1 2 3 4 5

FIG. 2B

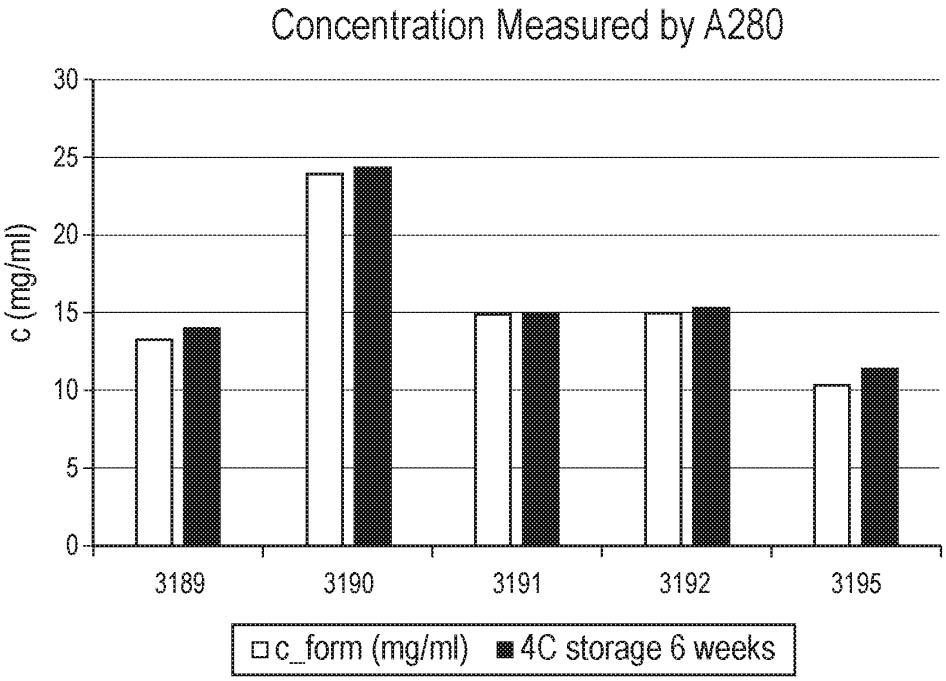

Concentration Measured by A280
c (mg/ml)
30
25
20
15
10
5
0
3189
3190
3191
3192
3195
c_form (mg/ml)
4C storage 6 weeks

FIG. 3

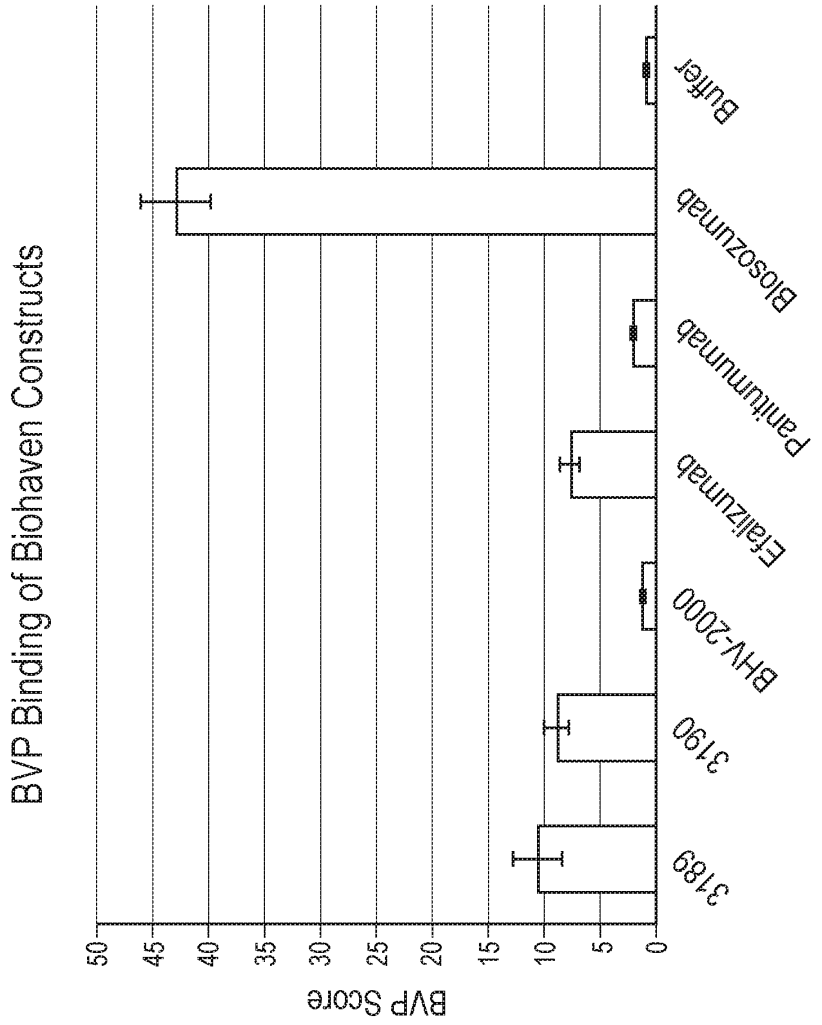

BVP Binding of Biohaven Constructs
BVP Score
0
5
10
15
20
25
30
35
40
45
50
3189
3190
BHV-2000
Ealizumab
Panlitumumab
Biosozumab
Buffer

FIG. 4

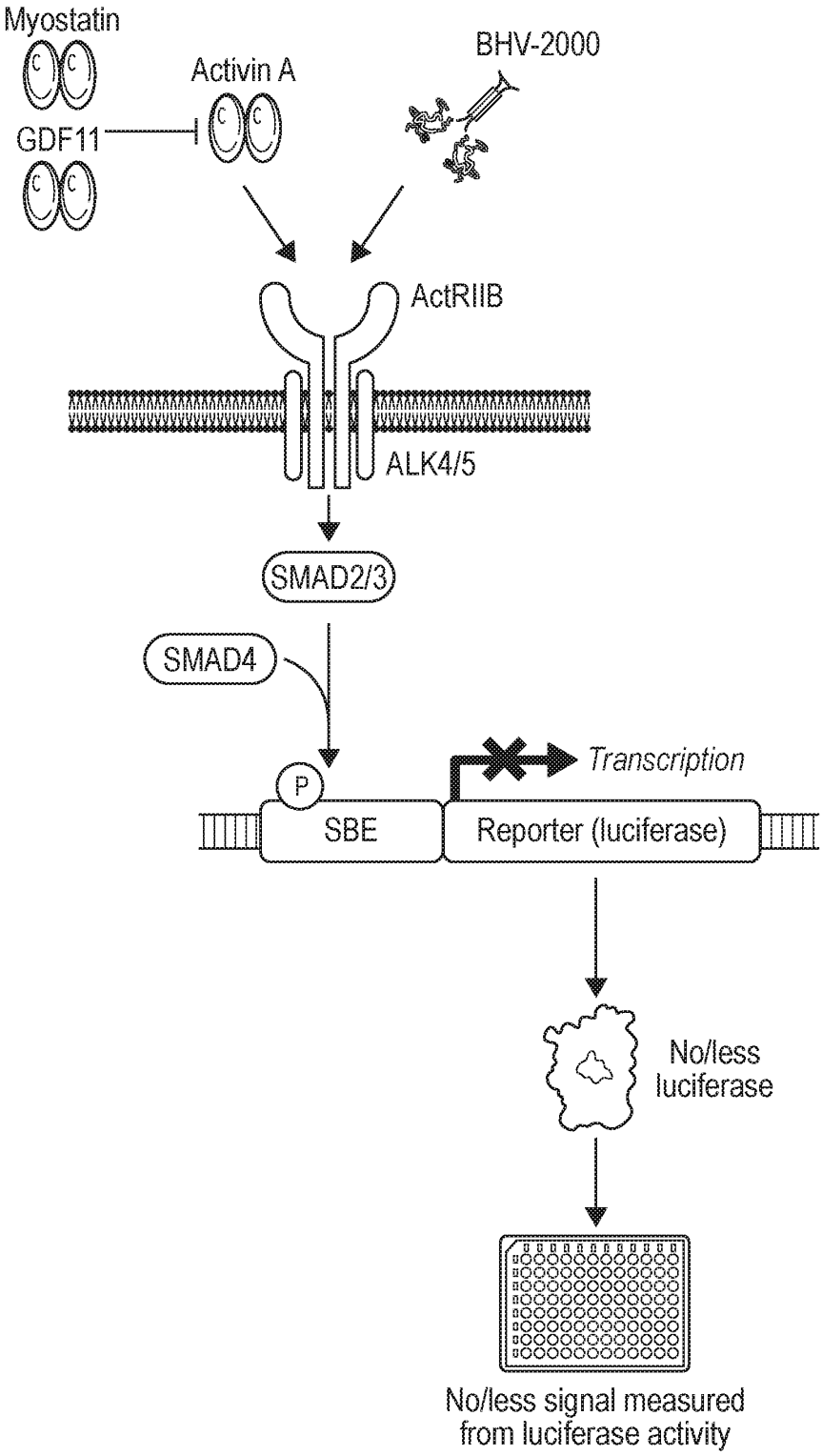

Myostatin
Activin A
BHV-2000
GDF11
ActRIIB
ALK4/5
SMAD2/3
SMAD4
P
Transcription
SBE
Reporter (luciferase)
No/less luciferase
No/less signal measured from luciferase activity

FIG. 5

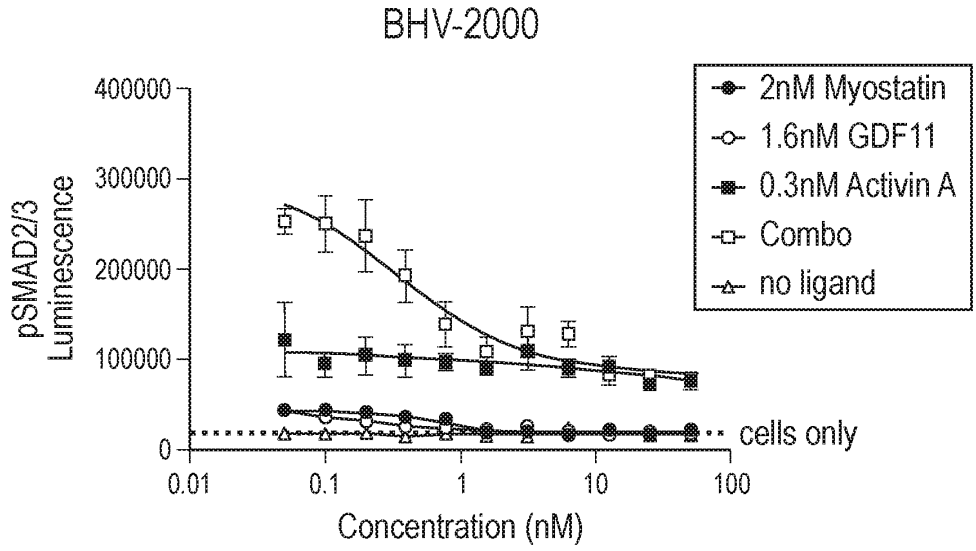

BHV-2000
pSMAD2/3 Luminescence
400000
300000
200000
100000
0
0.01
0.1
1
10
100
Concentration (nM)
2nM Myostatin
1.6nM GDF11
0.3nM Activin A
Combo
no ligand
cells only

FIG. 6A

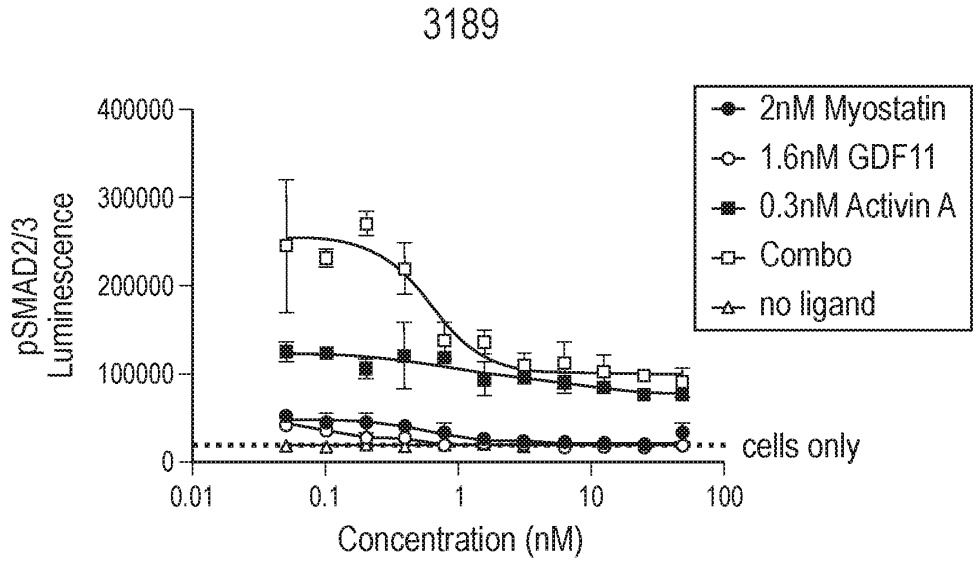

3189
pSMAD2/3 Luminescence
400000
300000
200000
100000
0
0.01
0.1
1
10
100
Concentration (nM)
2nM Myostatin
1.6nM GDF11
0.3nM Activin A
Combo
no ligand
cells only

FIG. 6B

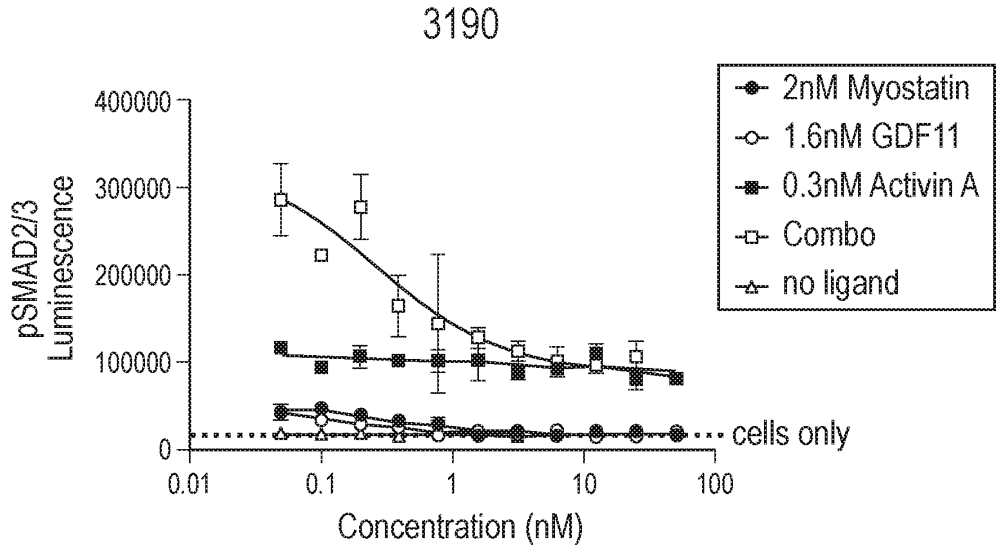

3190
pSMAD2/3 Luminescence
400000
300000
200000
100000
0
0.01
0.1
1
10
100
Concentration (nM)
2nM Myostatin
1.6nM GDF11
0.3nM Activin A
Combo
no ligand
cells only

FIG. 6C

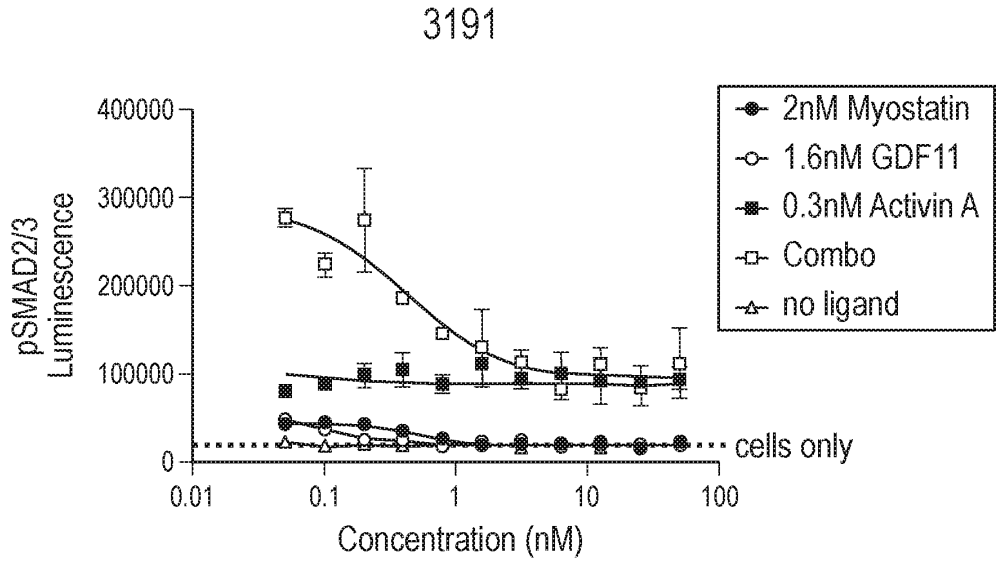

3191
pSMAD2/3 Luminescence
400000
300000
200000
100000
0
0.01
0.1
1
10
100
Concentration (nM)
2nM Myostatin
1.6nM GDF11
0.3nM Activin A
Combo
no ligand
cells only

FIG. 6D

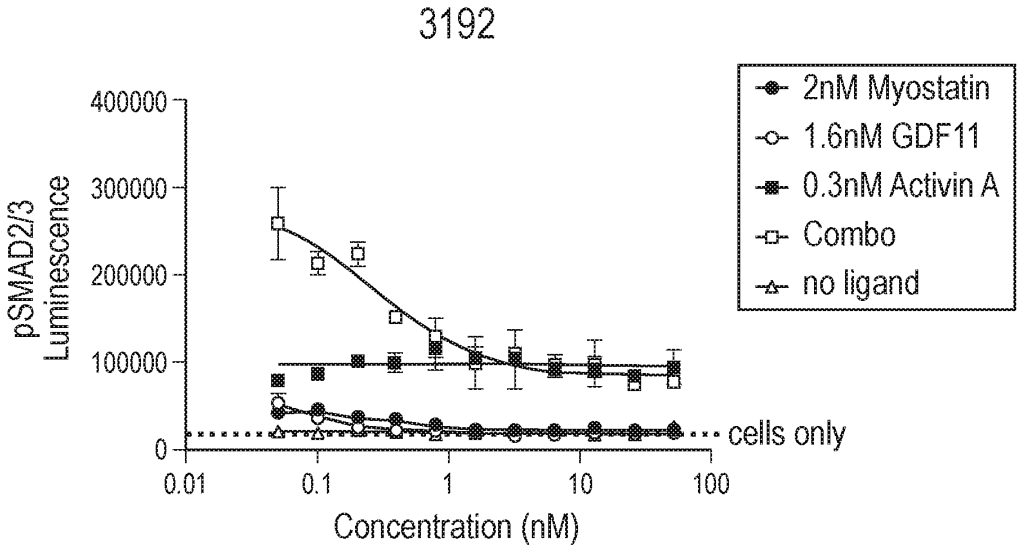

3192
pSMAD2/3 Luminescence
400000
300000
200000
100000
0
0.01
0.1
1
10
100
Concentration (nM)
cells only
2nM Myostatin
1.6nM GDF11
0.3nM Activin A
Combo
no ligand

FIG. 6E

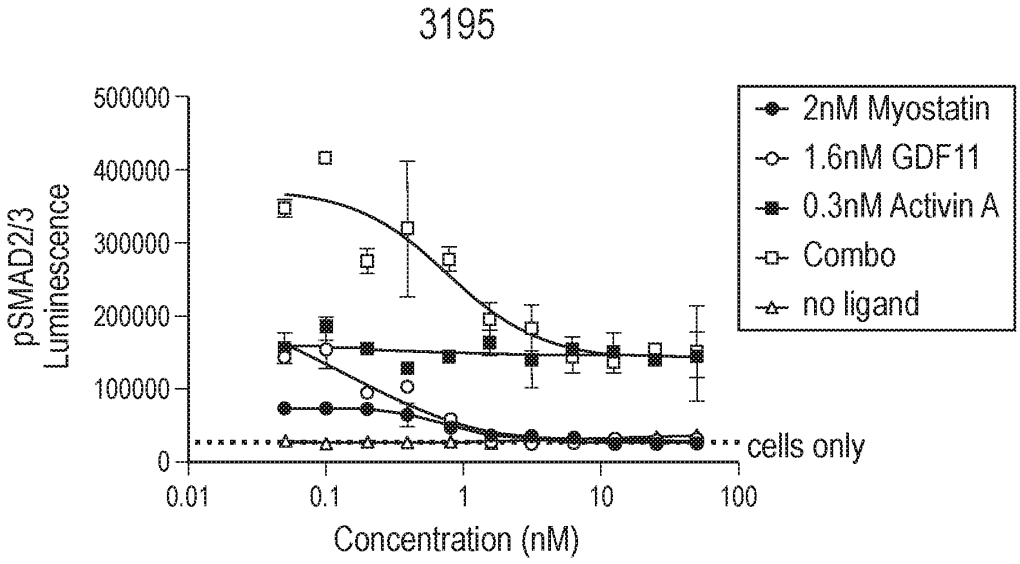

3195
pSMAD2/3 Luminescence
500000
400000
300000
200000
100000
0
0.01
0.1
1
10
100
Concentration (nM)
cells only
2nM Myostatin
1.6nM GDF11
0.3nM Activin A
Combo
no ligand

FIG. 6F

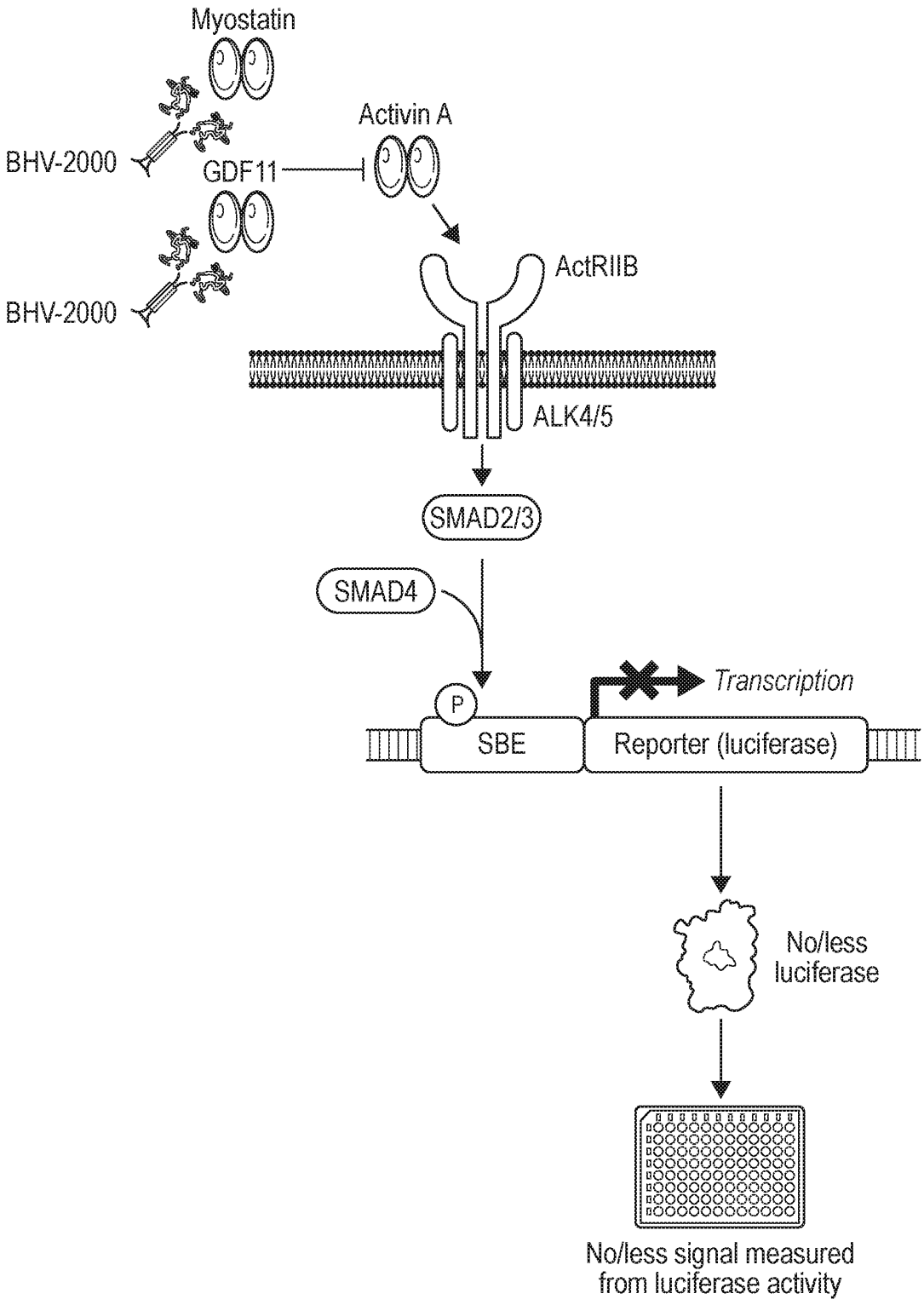

Myostatin
Activin A
BHV-2000
GDF11
BHV-2000
ActRIIB
ALK4/5
SMAD2/3
SMAD4
P
Transcription
SBE
Reporter (luciferase)
No/less luciferase
No/less signal measured from luciferase activity

FIG. 7

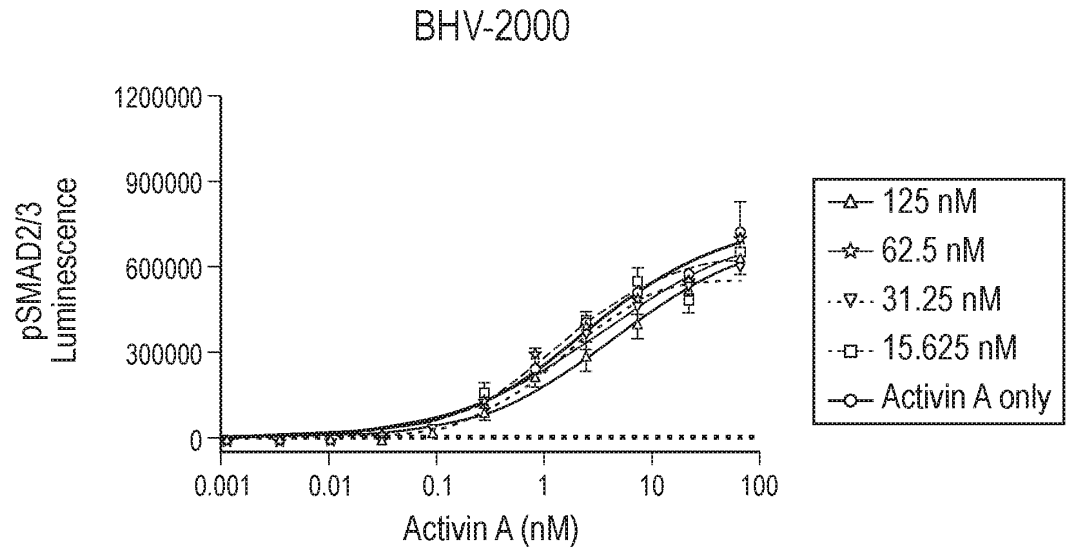

BHV-2000
pSMAD2/3 Luminescence
1200000
900000
600000
300000
0
0.001
0.01
0.1
1
10
100
Activin A (nM)
125 nM
62.5 nM
31.25 nM
15.625 nM
Activin A only

FIG. 8A

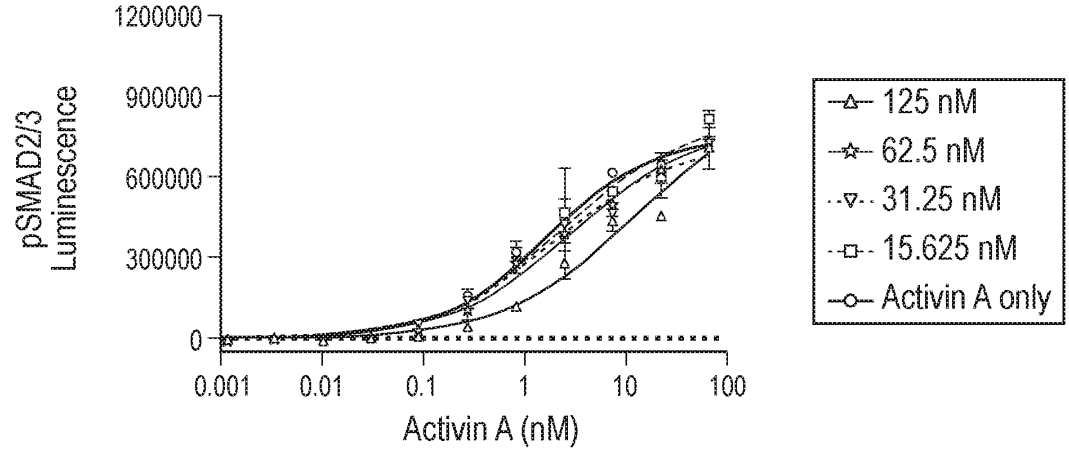

3189
pSMAD2/3 Luminescence
1200000
900000
600000
300000
0
0.001
0.01
0.1
1
10
100
Activin A (nM)
125 nM
62.5 nM
31.25 nM
15.625 nM
Activin A only

FIG. 8B

3190

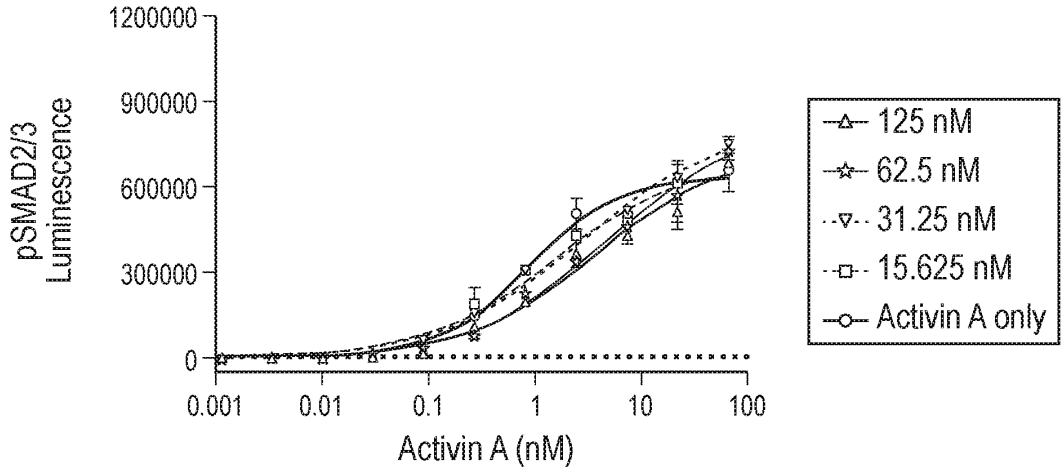


FIG. 8C

3195

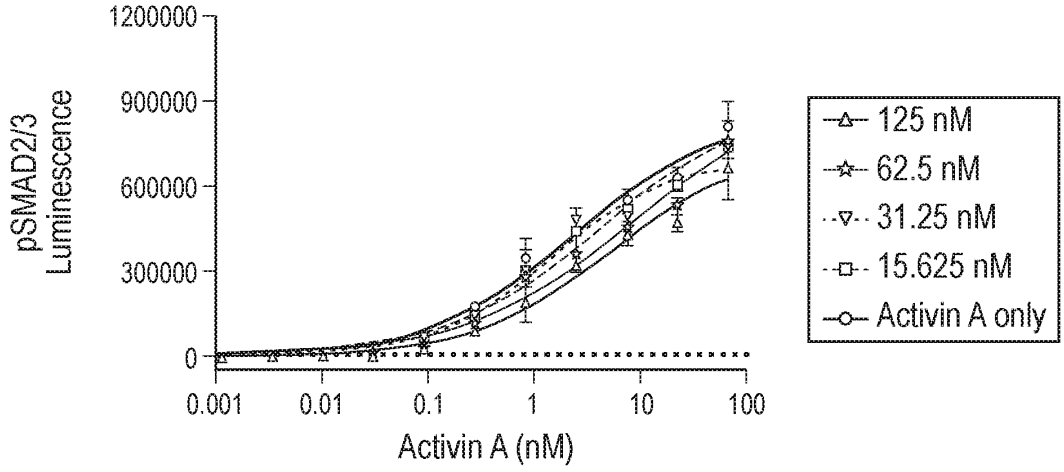


FIG. 8D

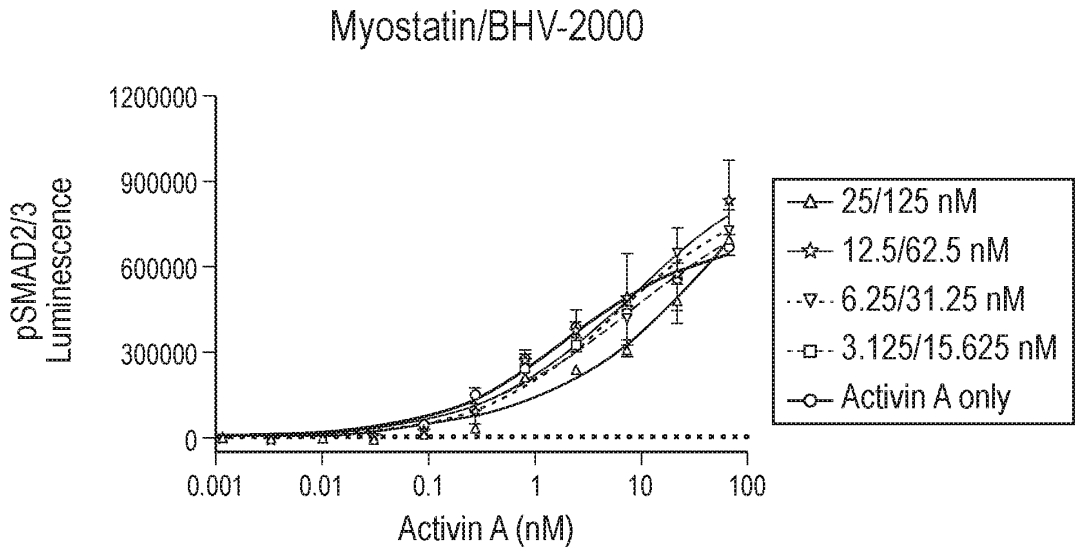

Myostatin/BHV-2000
pSMAD2/3 Luminescence
1200000
900000
600000
300000
0
0.001
0.01
0.1
1
10
100
Activin A (nM)
25/125 nM
12.5/62.5 nM
6.25/31.25 nM
3.125/15.625 nM
Activin A only

FIG. 8E

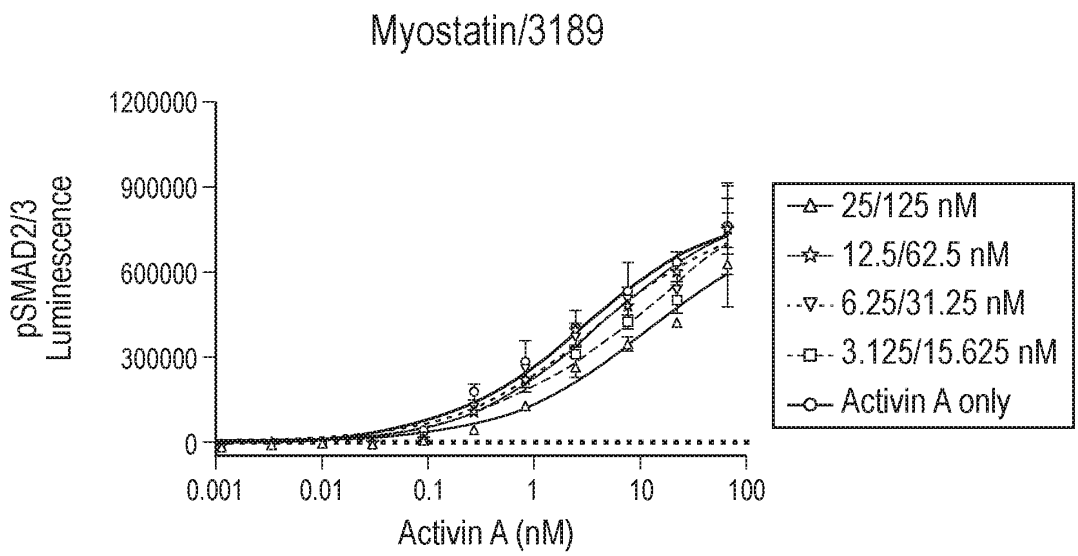

Myostatin/3189
pSMAD2/3 Luminescence
1200000
900000
600000
300000
0
0.001
0.01
0.1
1
10
100
Activin A (nM)
25/125 nM
12.5/62.5 nM
6.25/31.25 nM
3.125/15.625 nM
Activin A only

FIG. 8F

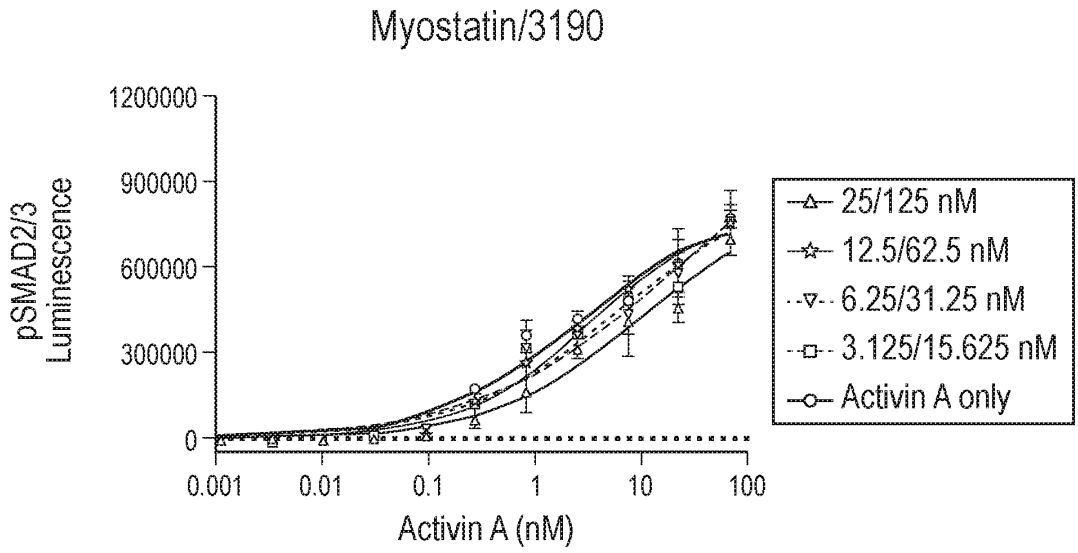

Myostatin/3190
pSMAD2/3 Luminescence
1200000
900000
600000
300000
0
0.001
0.01
0.1
1
10
100
Activin A (nM)
25/125 nM
12.5/62.5 nM
6.25/31.25 nM
3.125/15.625 nM
Activin A only

FIG. 8G

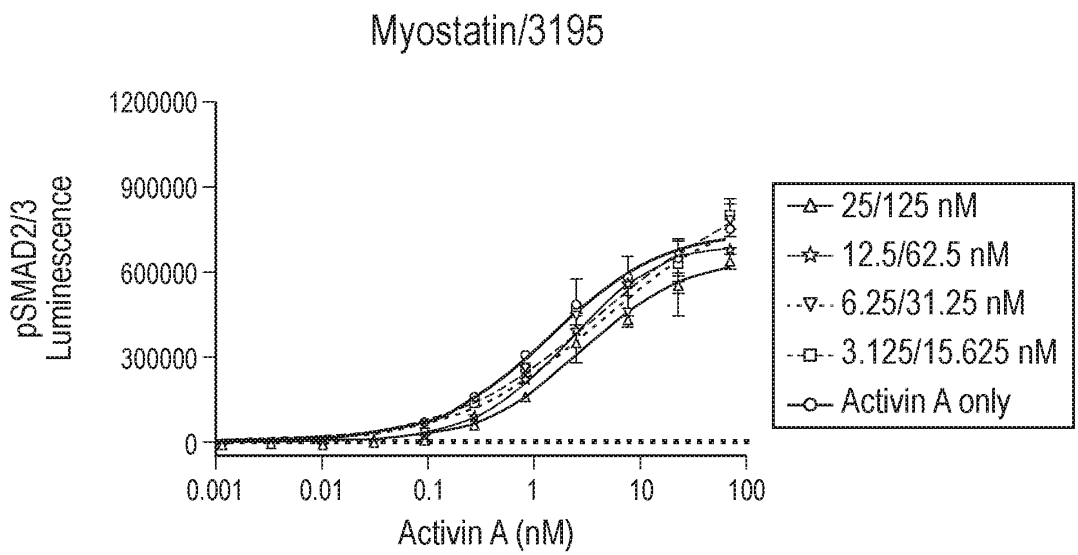

Myostatin/3195
pSMAD2/3 Luminescence
1200000
900000
600000
300000
0
0.001
0.01
0.1
1
10
100
Activin A (nM)
25/125 nM
12.5/62.5 nM
6.25/31.25 nM
3.125/15.625 nM
Activin A only

FIG. 8H

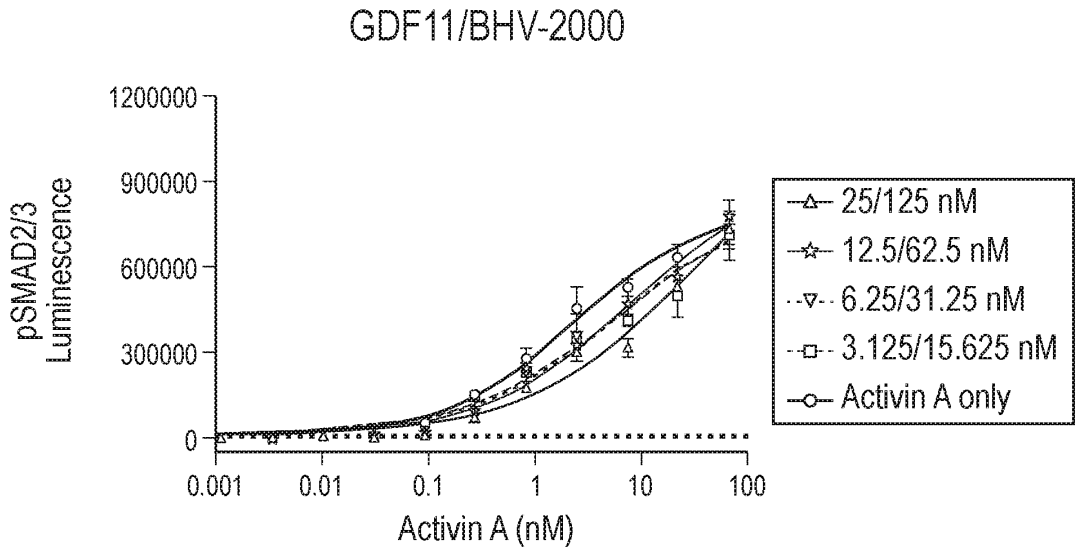

GDF11/BHV-2000
pSMAD2/3 Luminescence
1200000
900000
600000
300000
0
0.001
0.01
0.1
1
10
100
Activin A (nM)
25/125 nM
12.5/62.5 nM
6.25/31.25 nM
3.125/15.625 nM
Activin A only

FIG. 8I

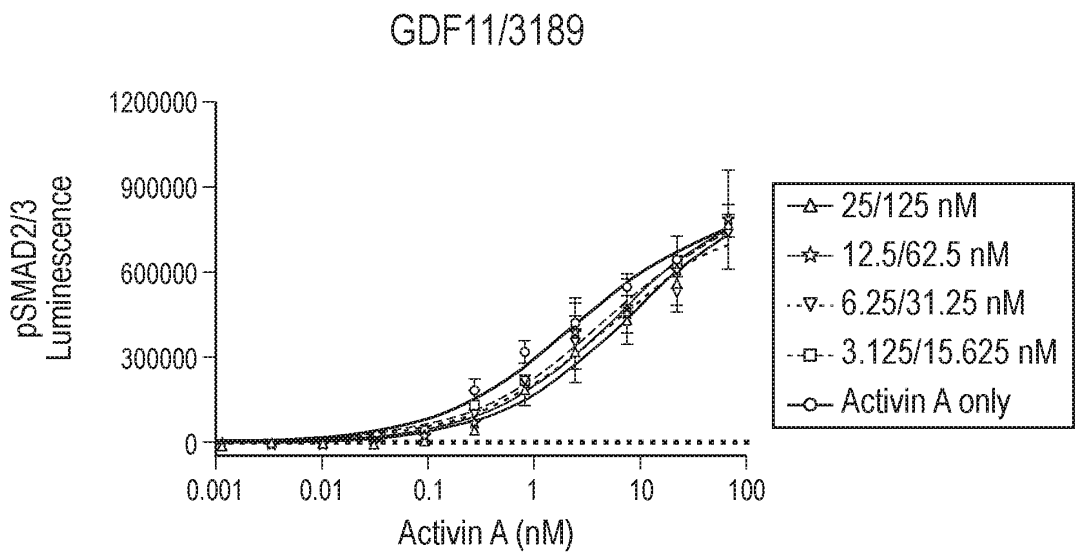

GDF11/3189
pSMAD2/3 Luminescence
1200000
900000
600000
300000
0
0.001
0.01
0.1
1
10
100
Activin A (nM)
25/125 nM
12.5/62.5 nM
6.25/31.25 nM
3.125/15.625 nM
Activin A only

FIG. 8J

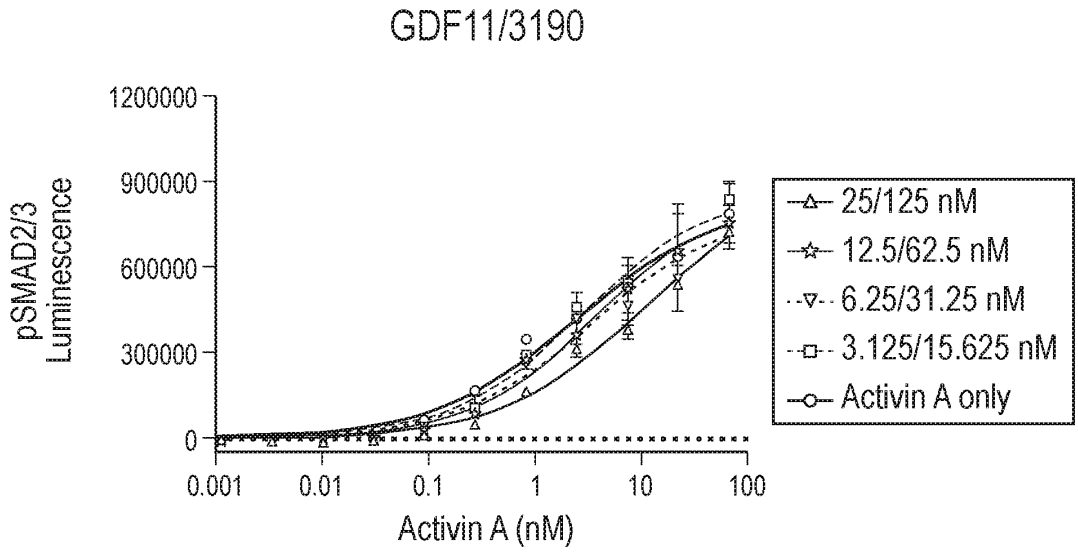

GDF11/3190
pSMAD2/3 Luminescence
1200000
900000
600000
300000
0
0.001
0.01
0.1
1
10
100
Activin A (nM)
25/125 nM
12.5/62.5 nM
6.25/31.25 nM
3.125/15.625 nM
Activin A only

FIG. 8K

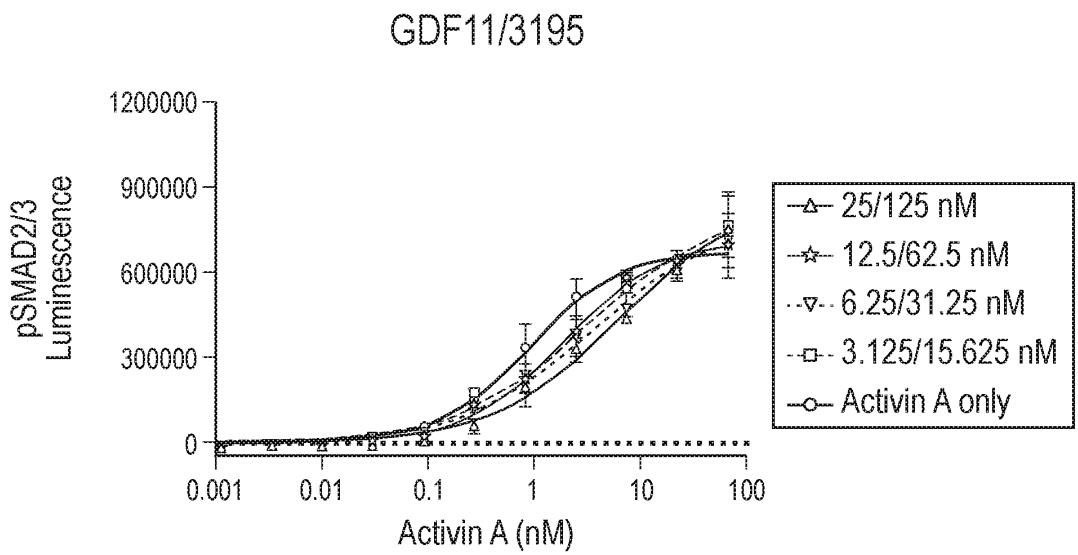

GDF11/3195
pSMAD2/3 Luminescence
1200000
900000
600000
300000
0
0.001
0.01
0.1
1
10
100
Activin A (nM)
25/125 nM
12.5/62.5 nM
6.25/31.25 nM
3.125/15.625 nM
Activin A only

FIG. 8L

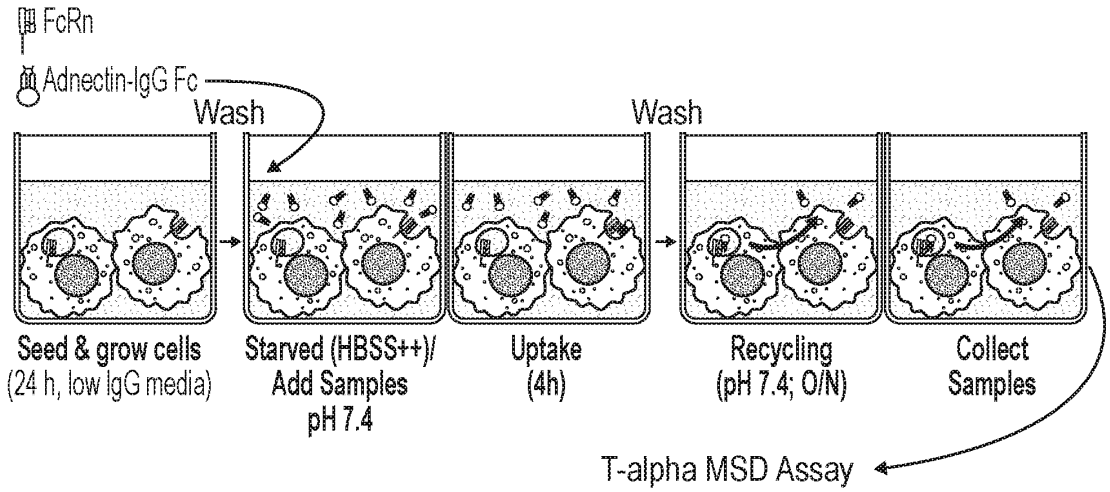

FcRn
Adnectin-IgG Fc
Wash
Wash
Seed & grow cells
(24 h, low IgG media)
Starved (HBSS++)/
Add Samples
pH 7.4
Uptake
(4h)
Recycling
(pH 7.4; O/N)
Collect
Samples
T-alpha MSD Assay

FIG. 9

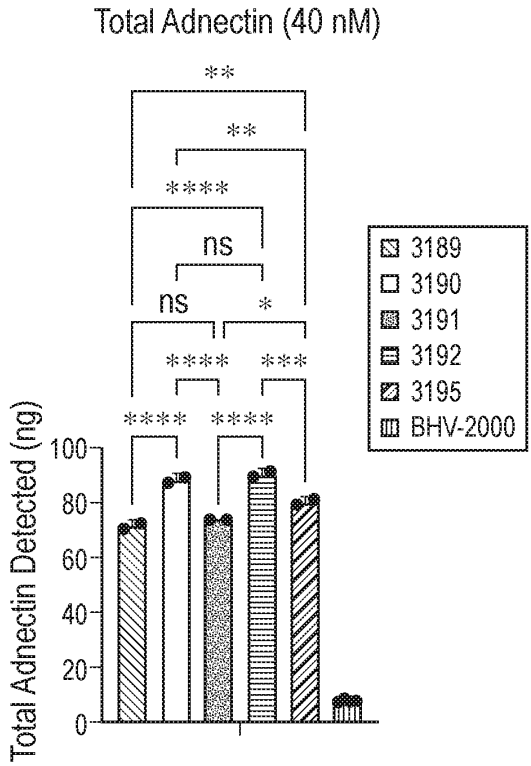

Total Adnectin (40 nM)
**
**
****
ns
ns
*
****
***
****
****
3189
3190
3191
3192
3195
BHV-2000
Total Adnectin Detected (ng)
100
80
60
40
20
0

FIG. 10