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(54) **ADMINISTRATION OF AN ANTI-ACTIVIN-A COMPOUND TO A SUBJECT**

(71) Applicants: **AMGEN INC.**, Thousand Oaks, CA (US); **Atara Biotherapeutics, Inc.**, South San Francisco, CA (US)

(72) Inventors: **Huiquan Han**, Thousand Oaks, CA (US); **Christopher Michael Haqq**, Newbury Park, CA (US); **Isaac Ciechanover**, Burlingame, CA (US); **Xiaolan Zhou**, Newbury Park, CA (US); **John Zhao-Nian Lu**, Alhambra, CA (US)

(73) Assignees: **AMGEN INC.**, Thousand Oaks, CA (US); **Atara Biotherapeutics, Inc.**, South San Francisco, CA (US)

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*Primary Examiner* — Gyan Chandra

(74) *Attorney, Agent, or Firm* — Lisa E. Alexander

(57) **ABSTRACT**

The present invention relates to methods of treating ovarian cancer in a subject by administering to the subject by evaluating the subject's expression levels of specific biomarkers or angiogenic an anti-activin-A compound, such as an anti-activin-A antibody or an activin-A-binding receptor. In some embodiments, at least two compounds are administered to the subject, where the first compound is an anti-activin A compound, and the second compound is a chemotherapeutic compound, for example capecitabine. The invention further relates to methods of identifying subjects for treatment factors.

**3 Claims, 49 Drawing Sheets**

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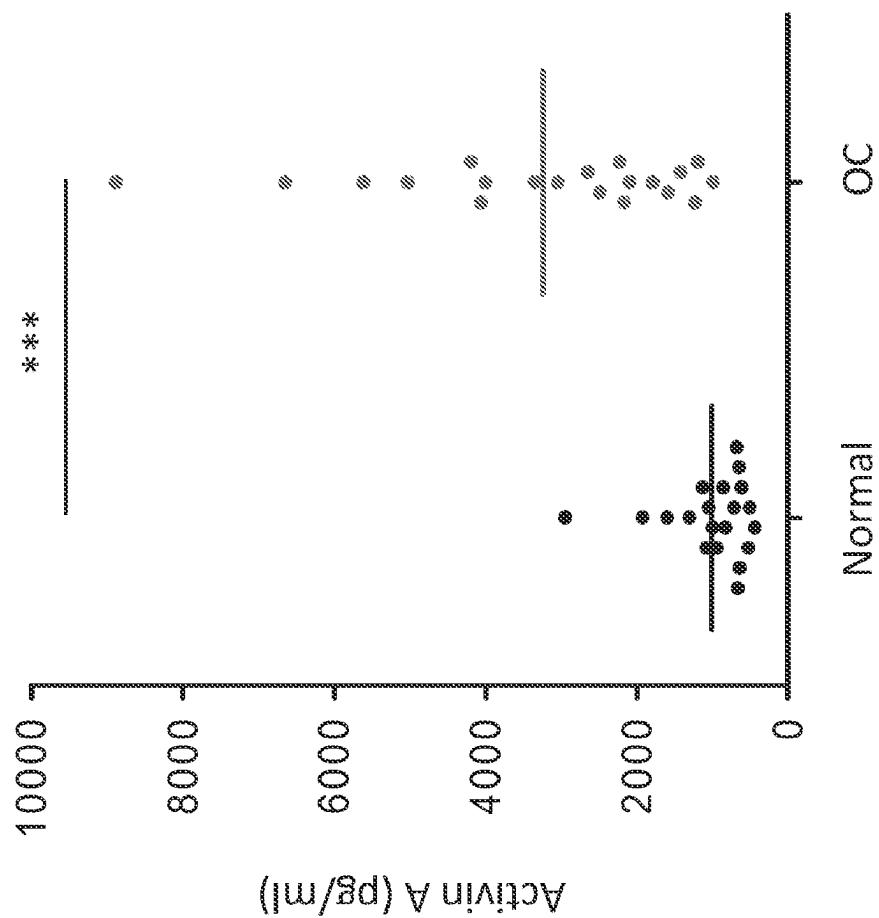


FIG. 1

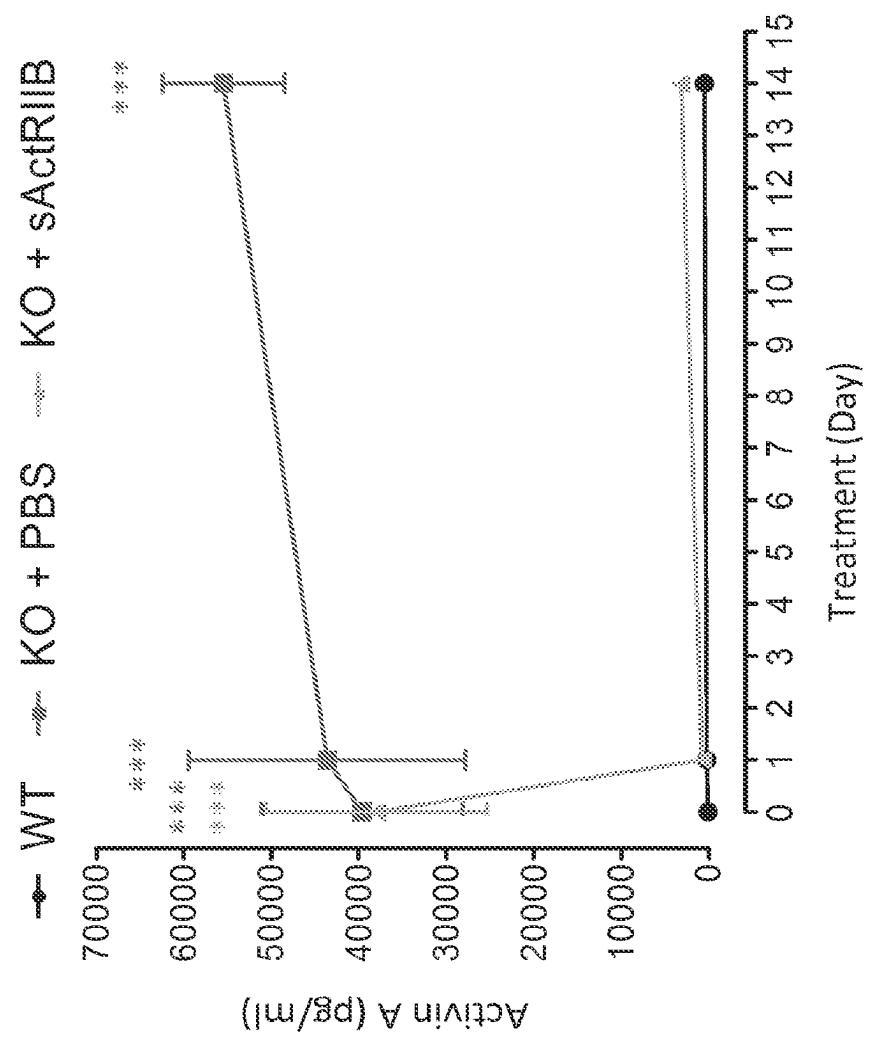


FIG. 2A

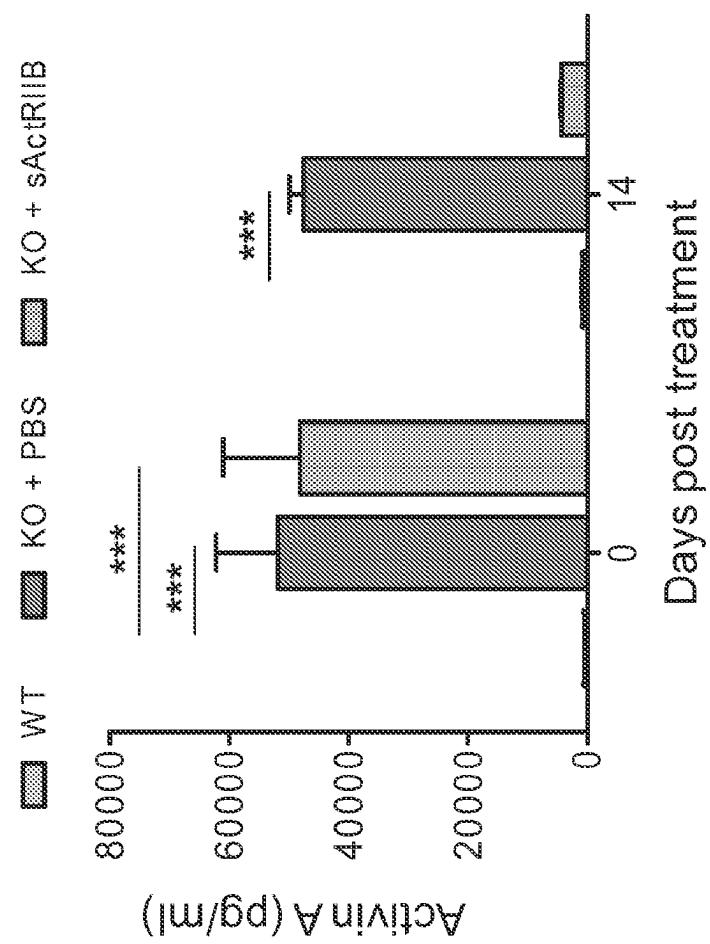


FIG. 2B

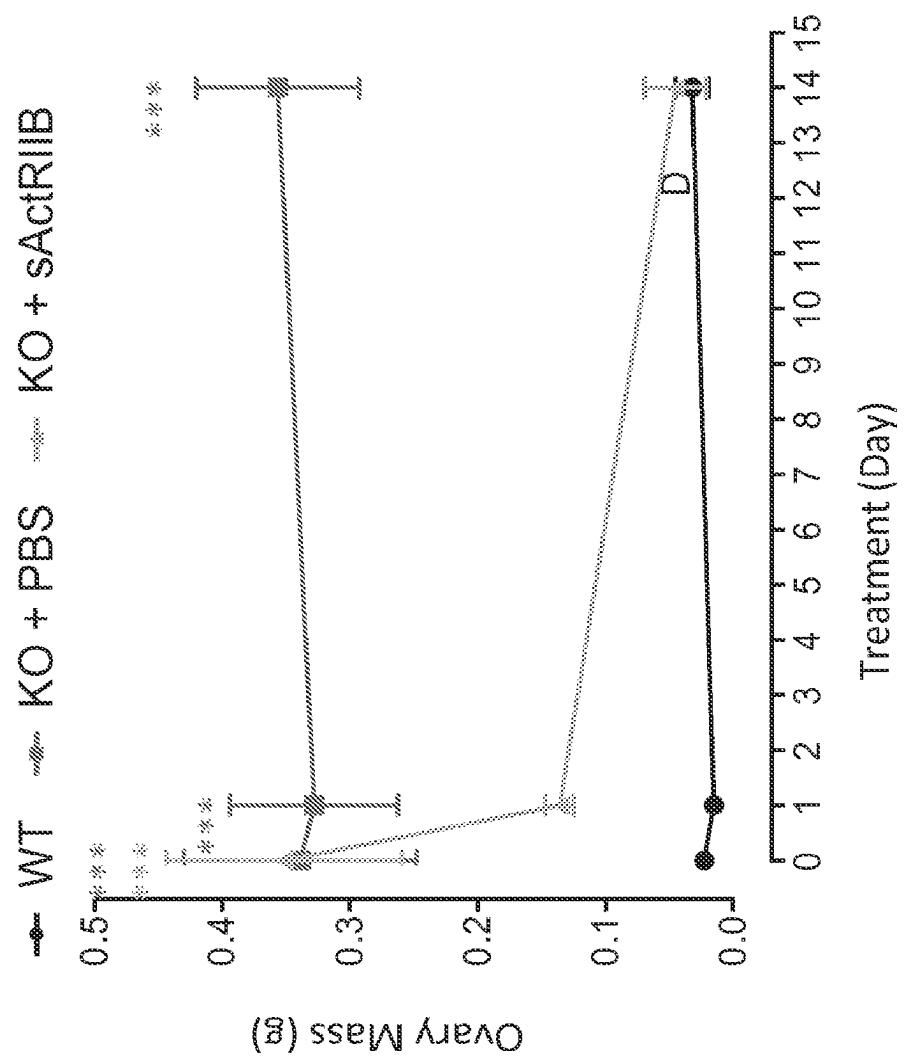


FIG. 3A

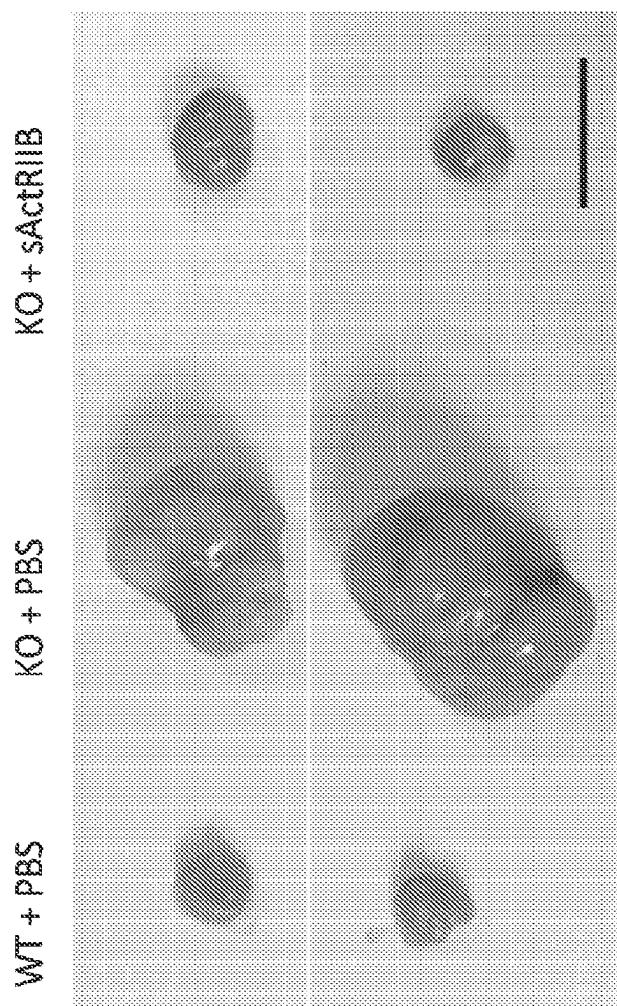


FIG. 3B

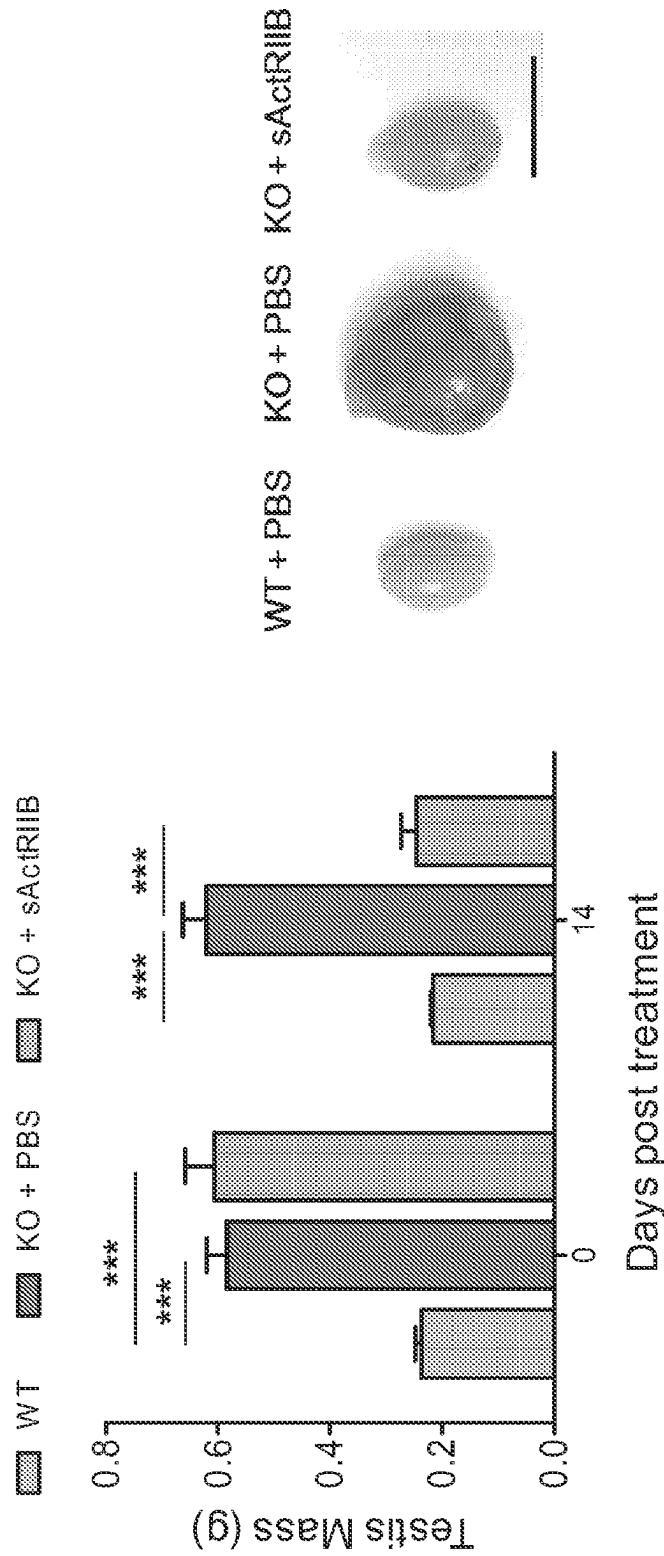


FIG. 4A

FIG. 4B

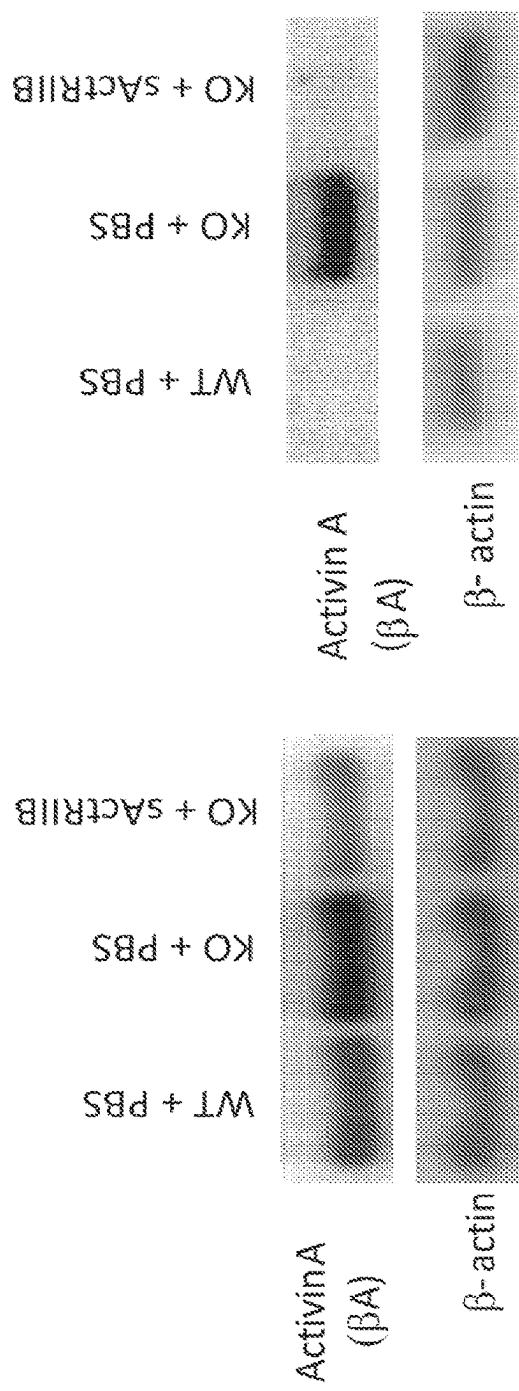


FIG. 5A

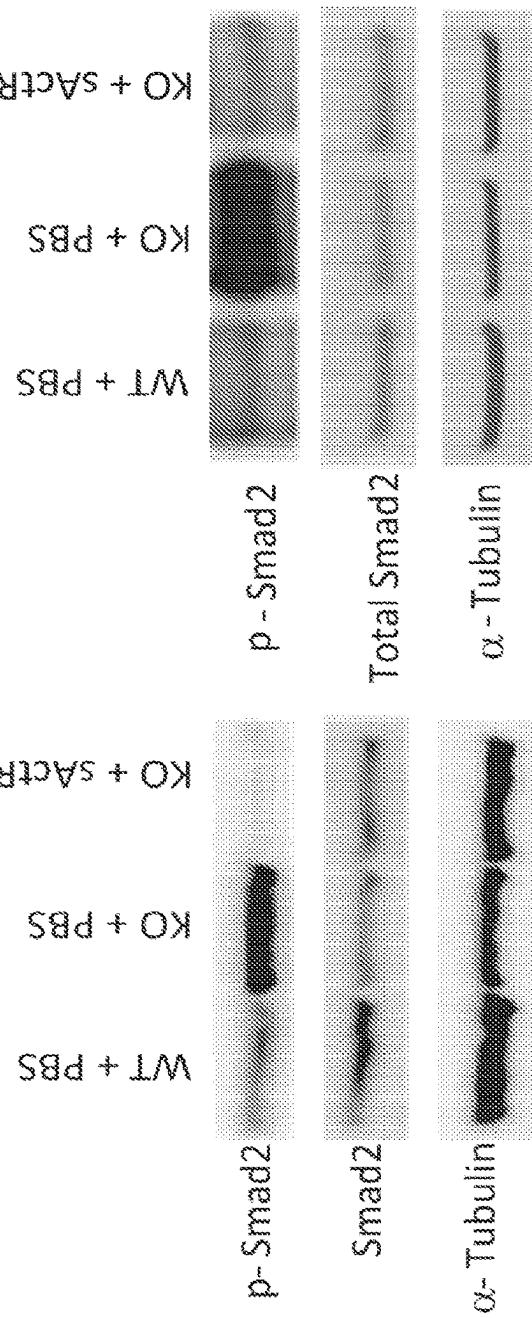


FIG. 5B

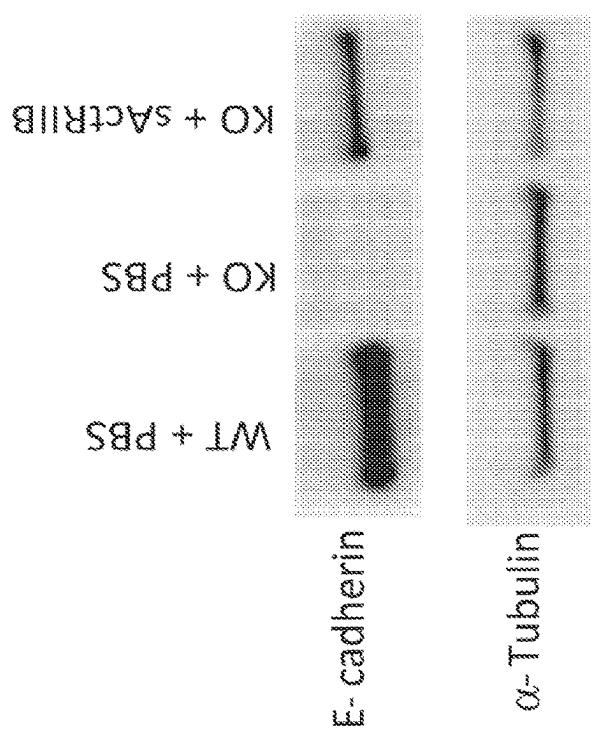


FIG. 6A

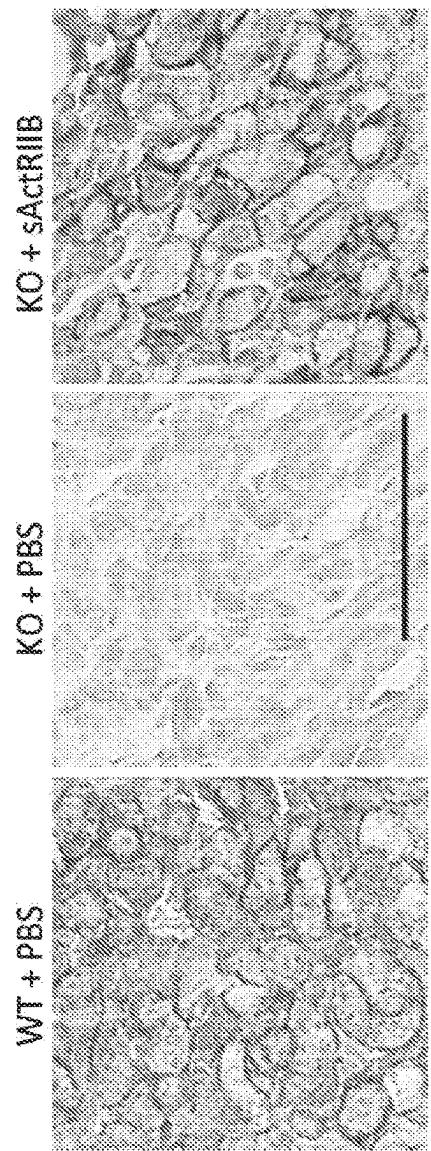


FIG. 6B

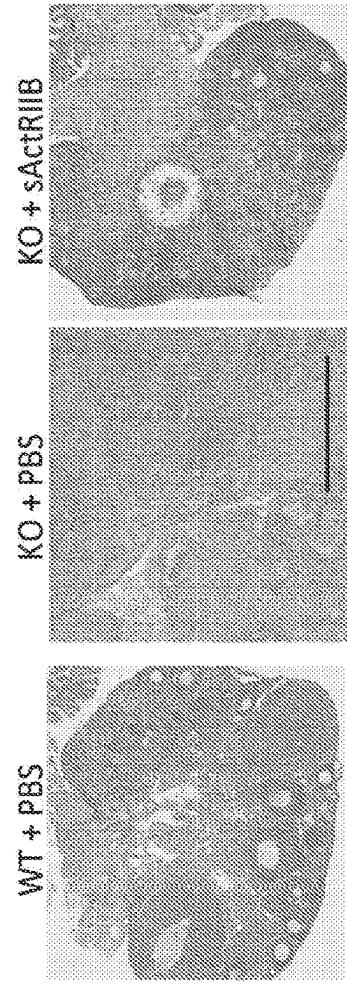


FIG. 7A

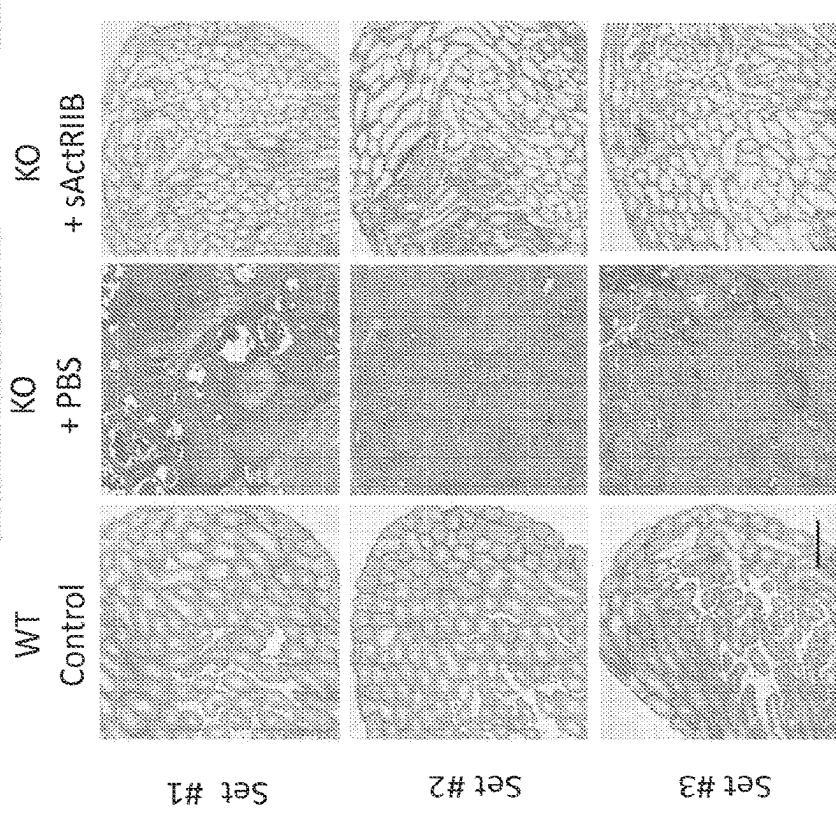


FIG. 7B

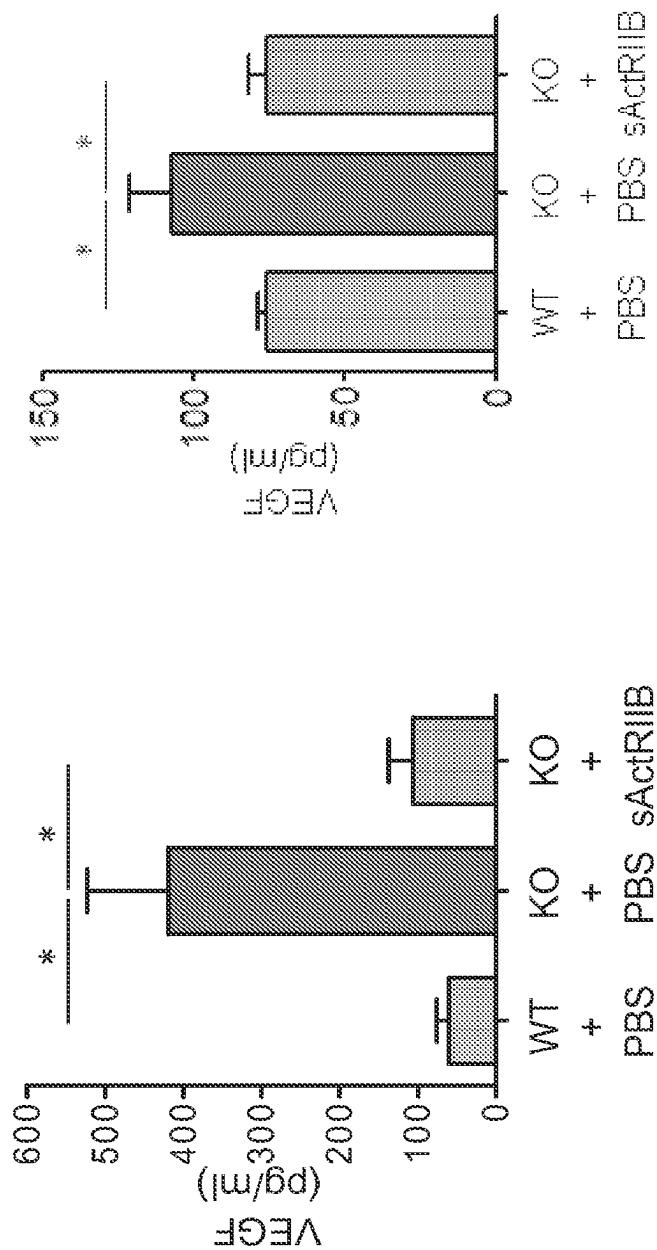
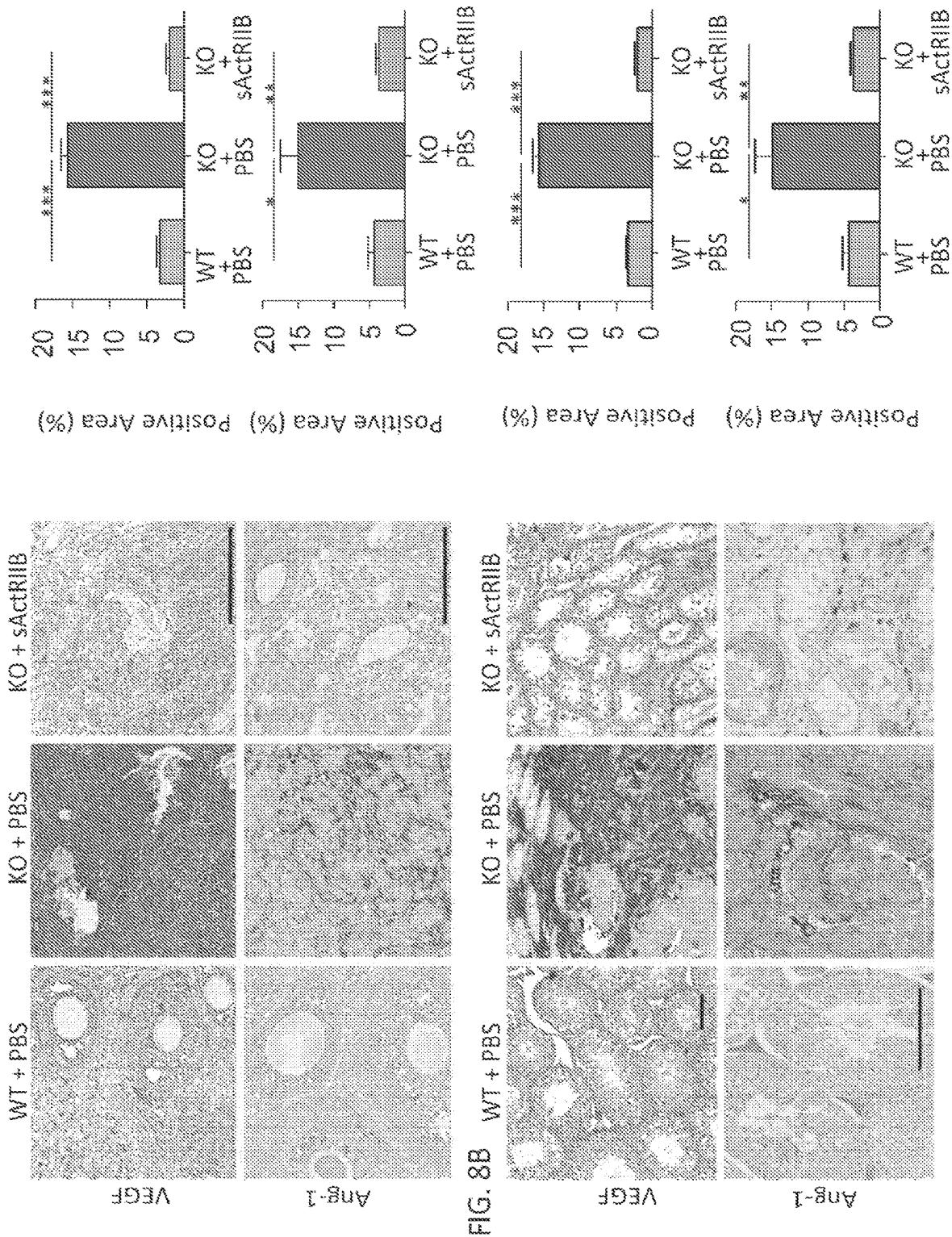


FIG. 8A



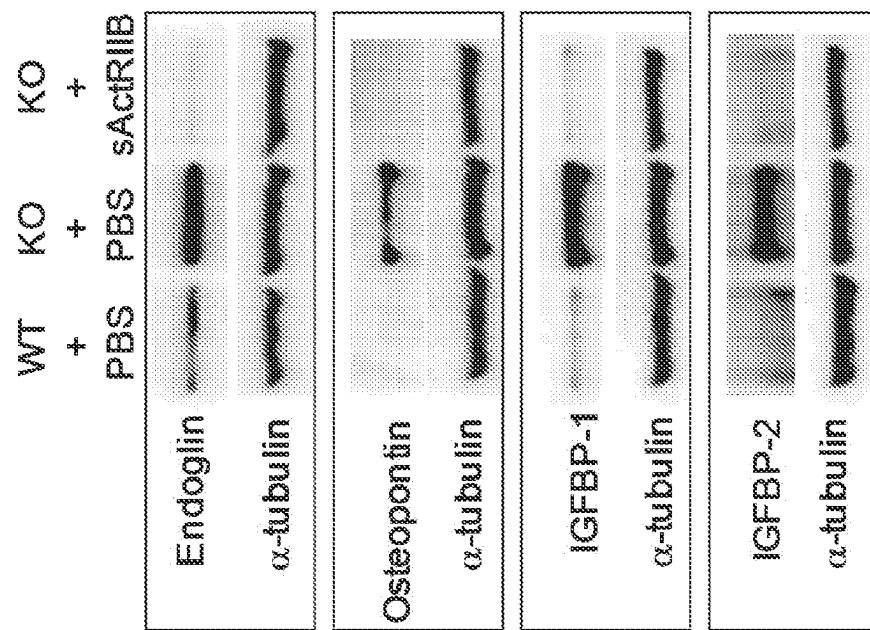


FIG. 8D

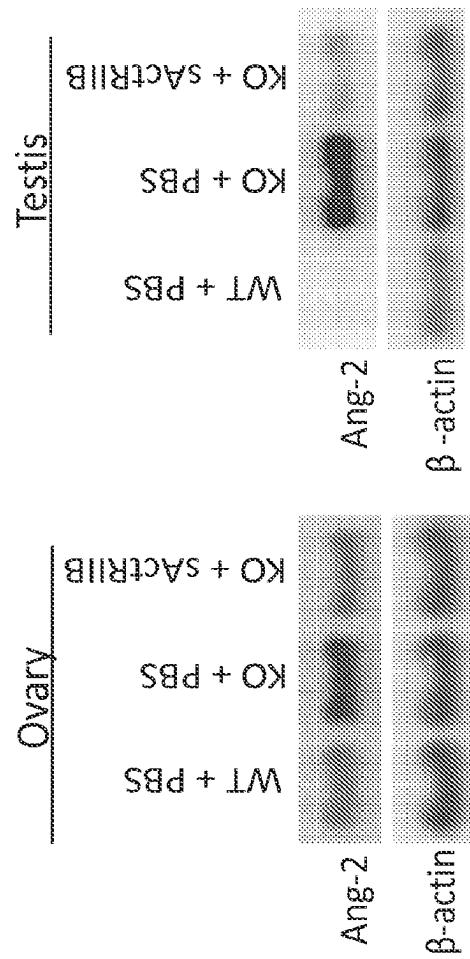


FIG. 8C

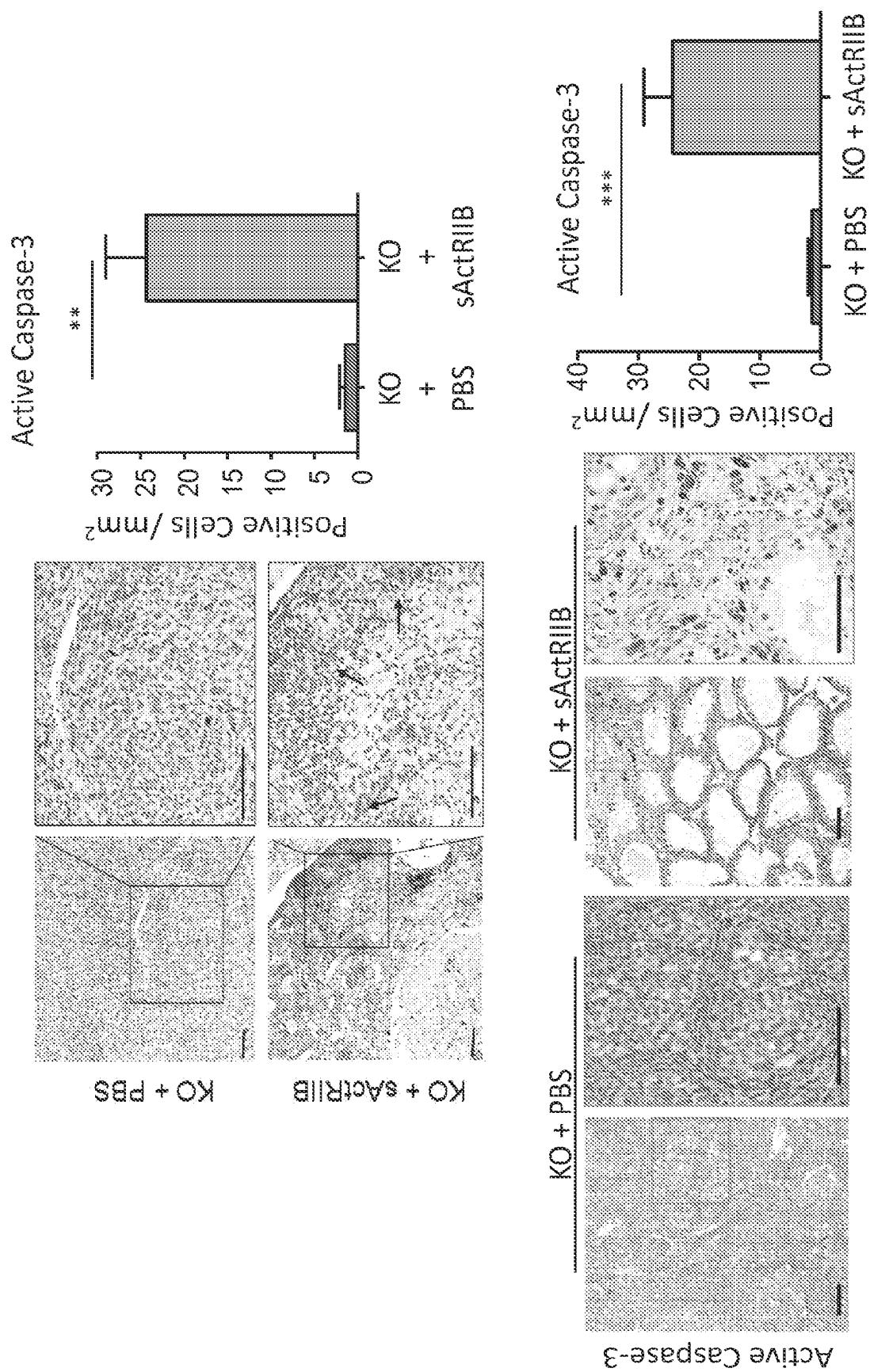


FIG. 9

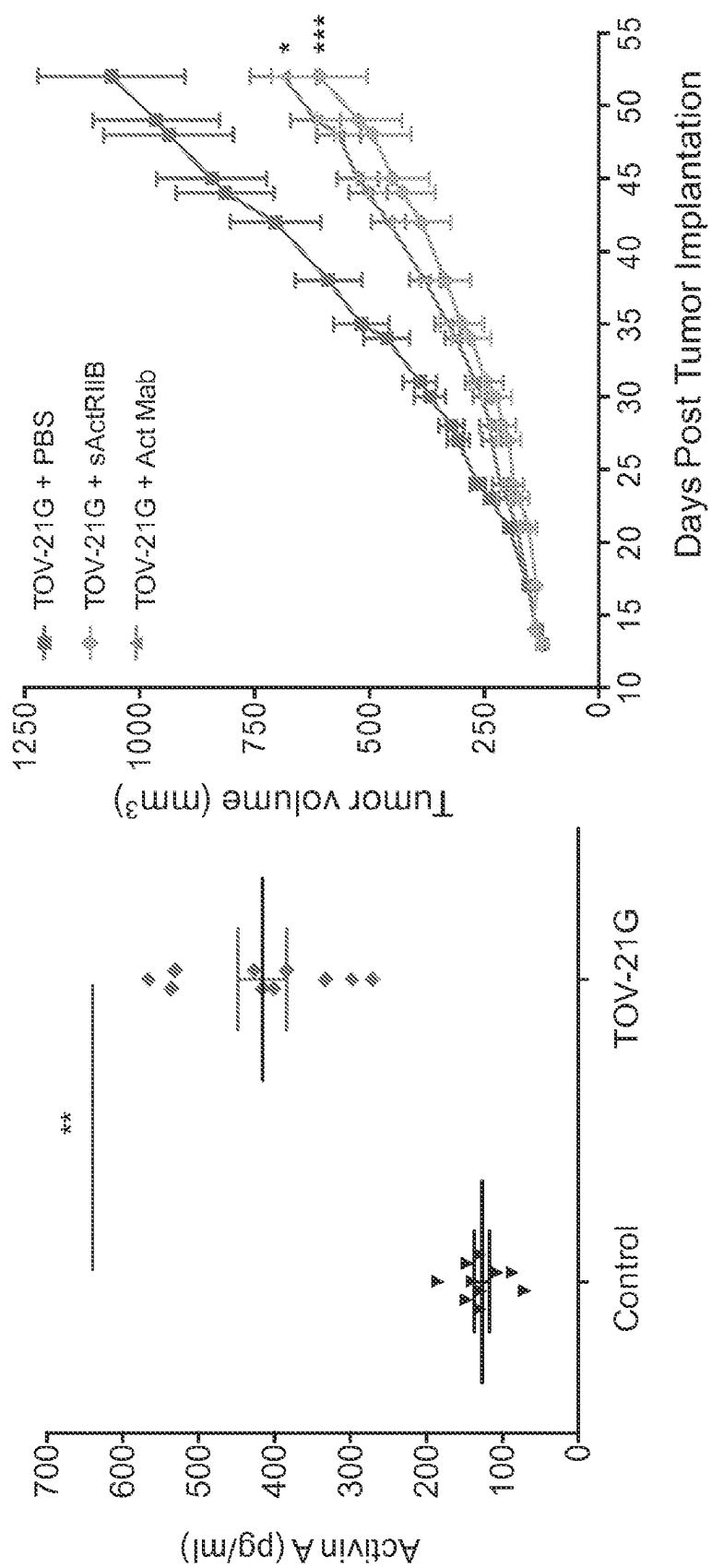


FIG. 10A

FIG. 10B

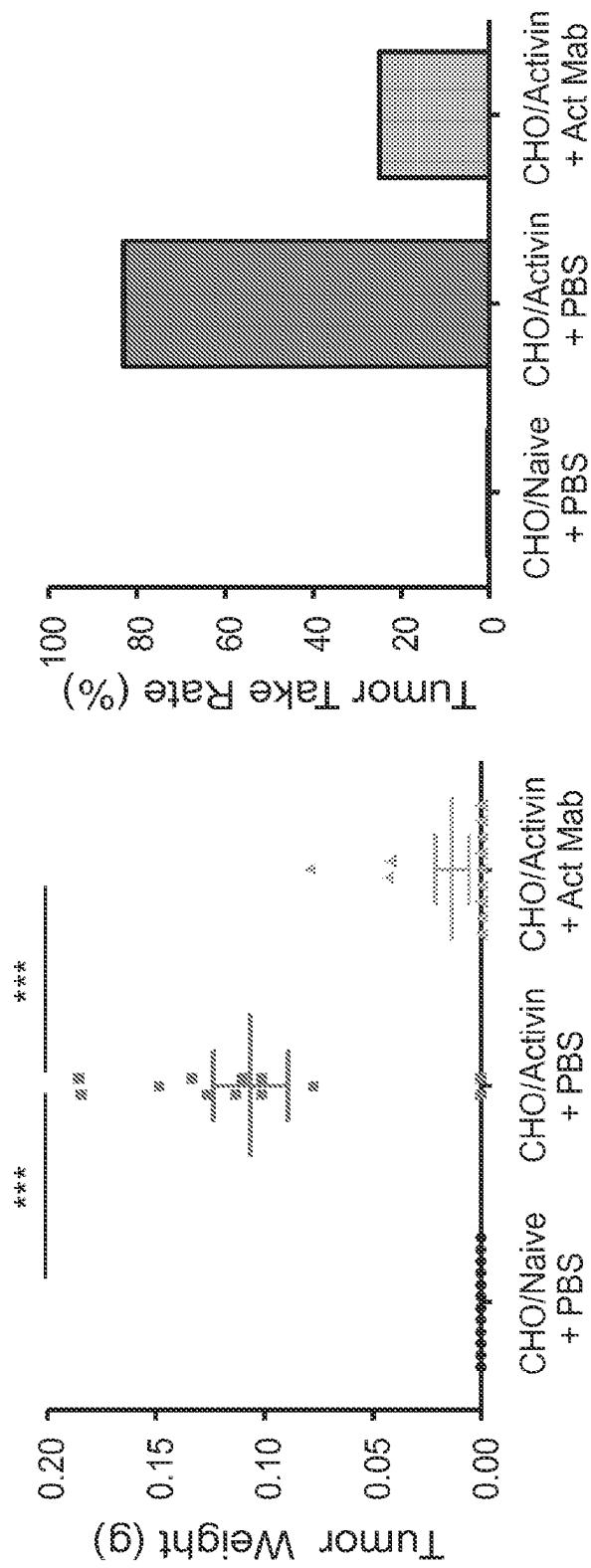


FIG. 11

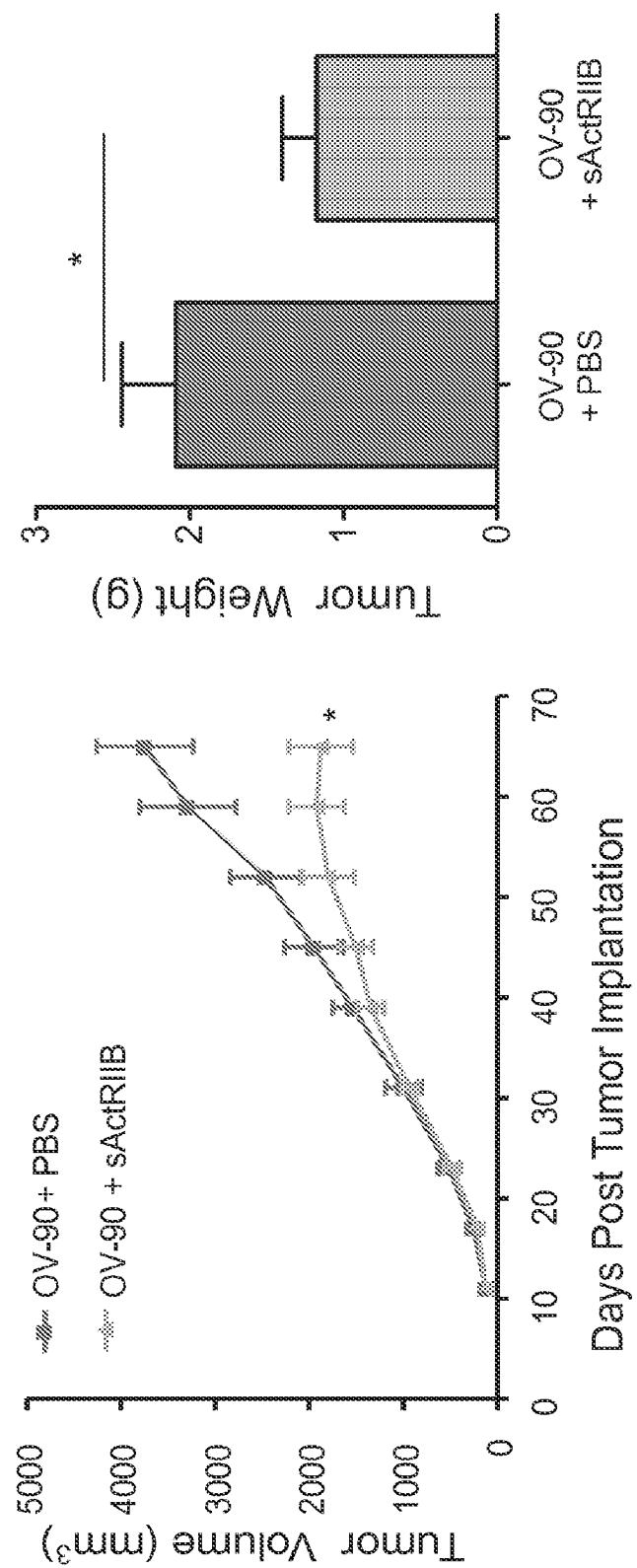


FIG. 12

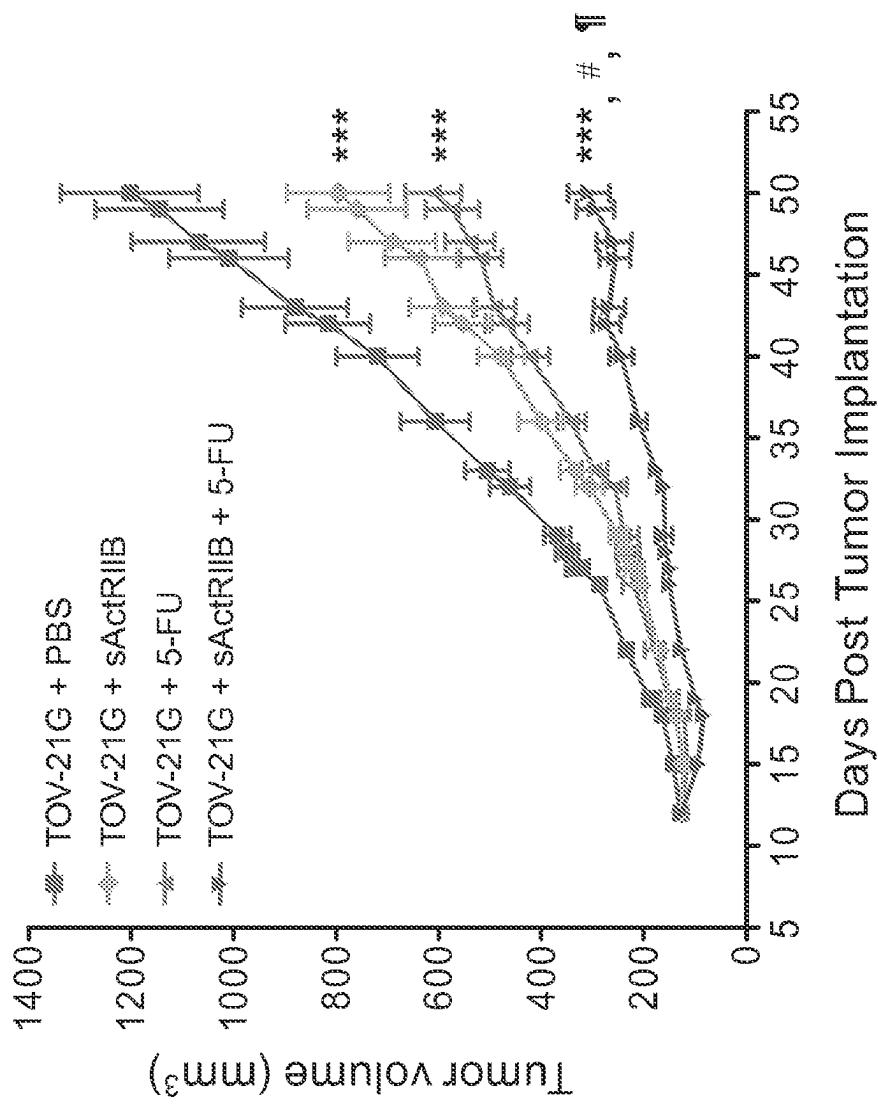


FIG. 13

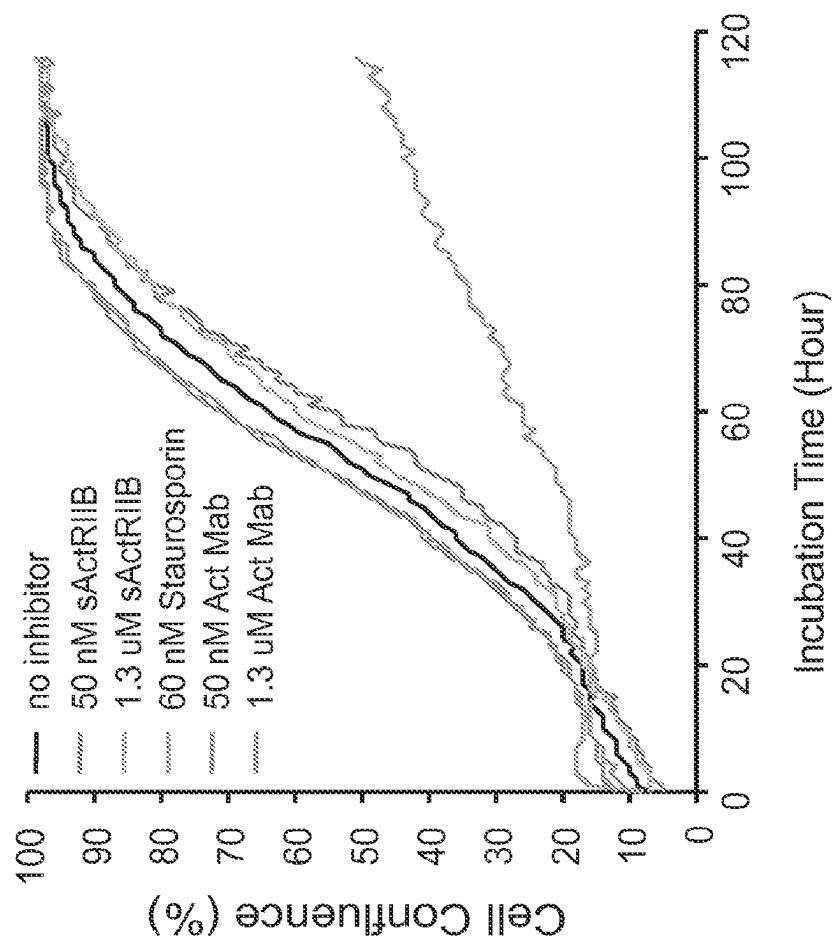


FIG. 14

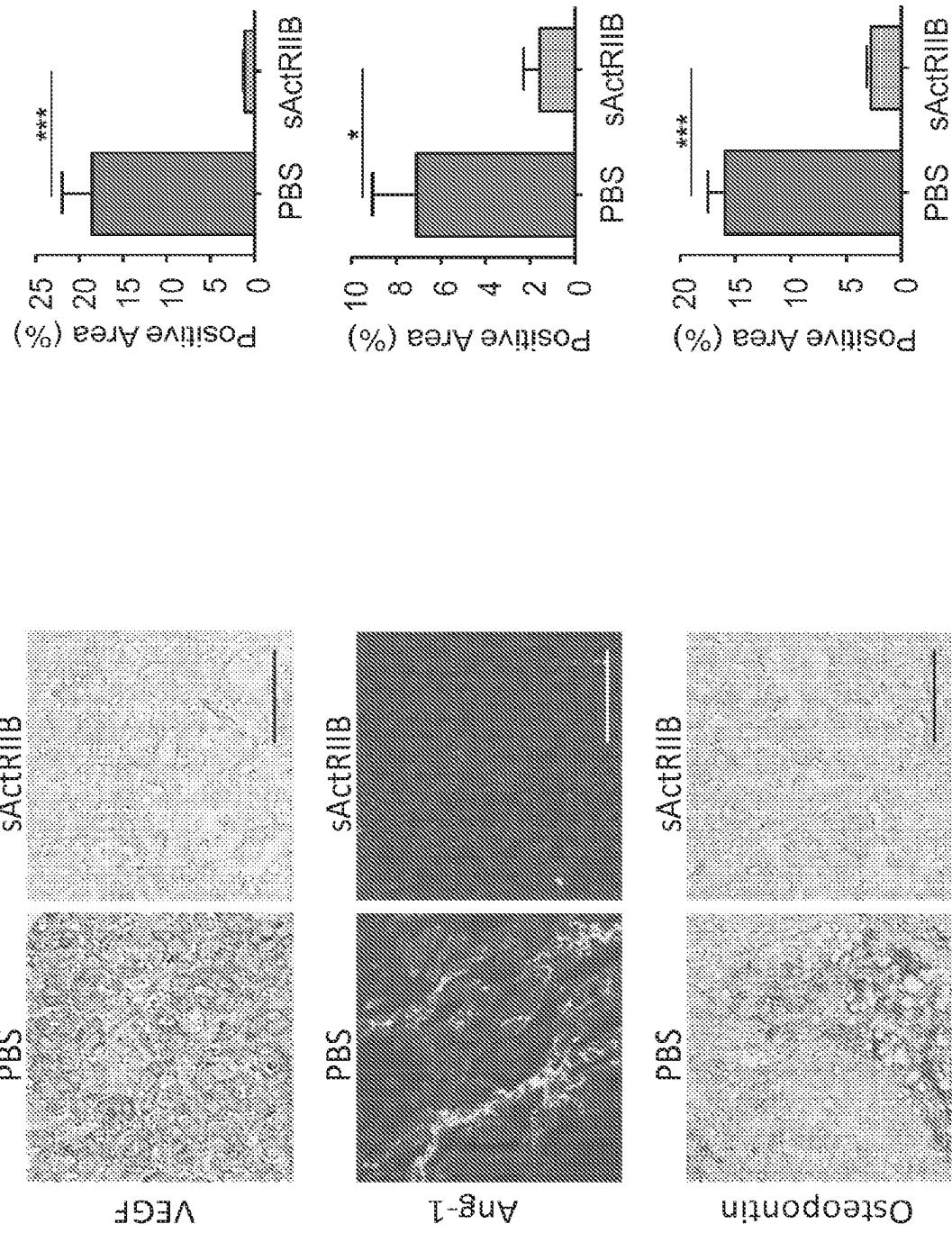


FIG. 15A

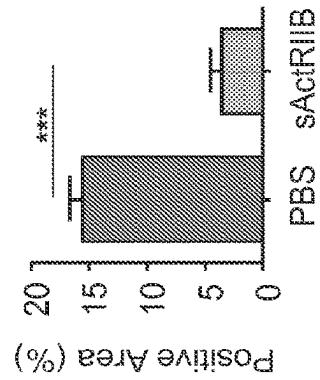


FIG. 15B

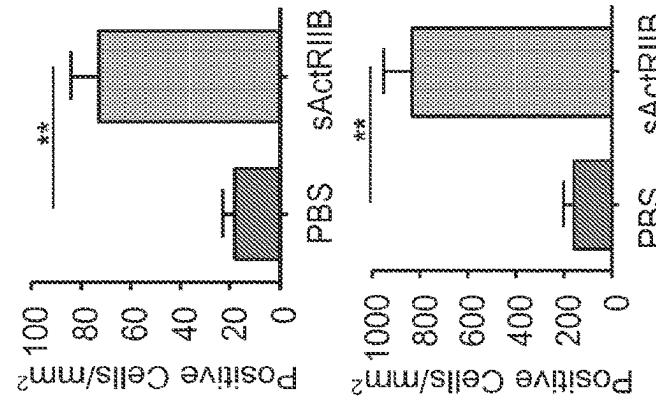
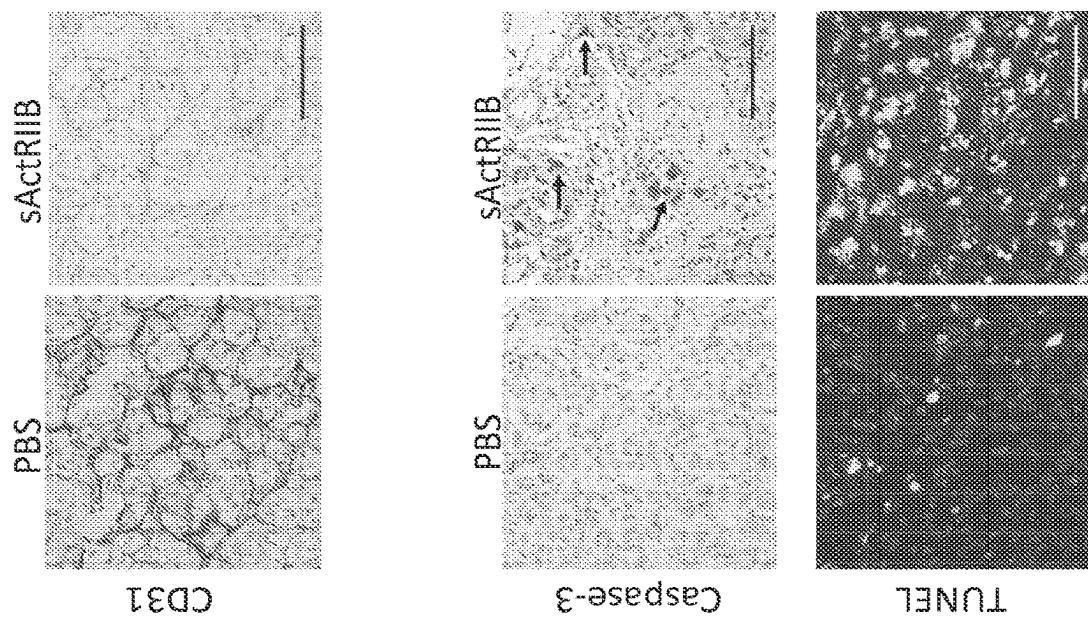


FIG. 15C



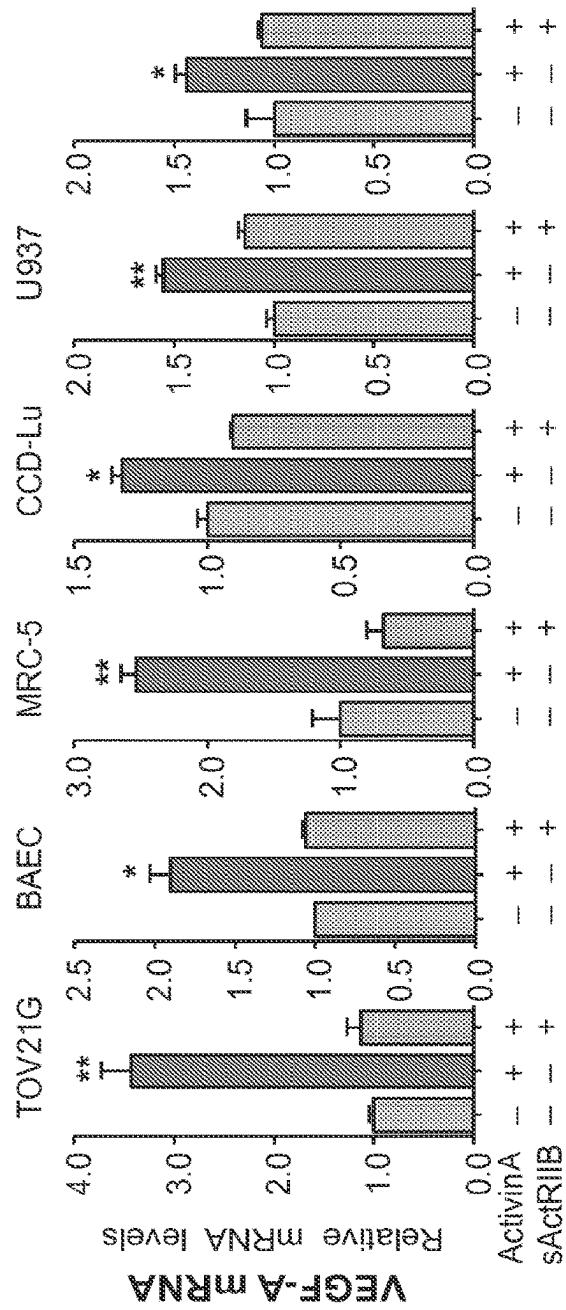


FIG. 16A

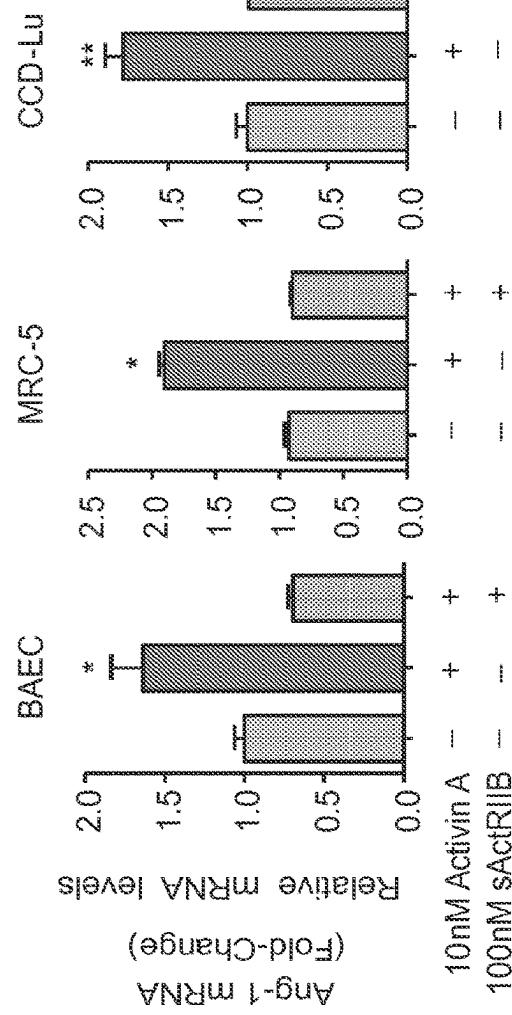


FIG. 16B

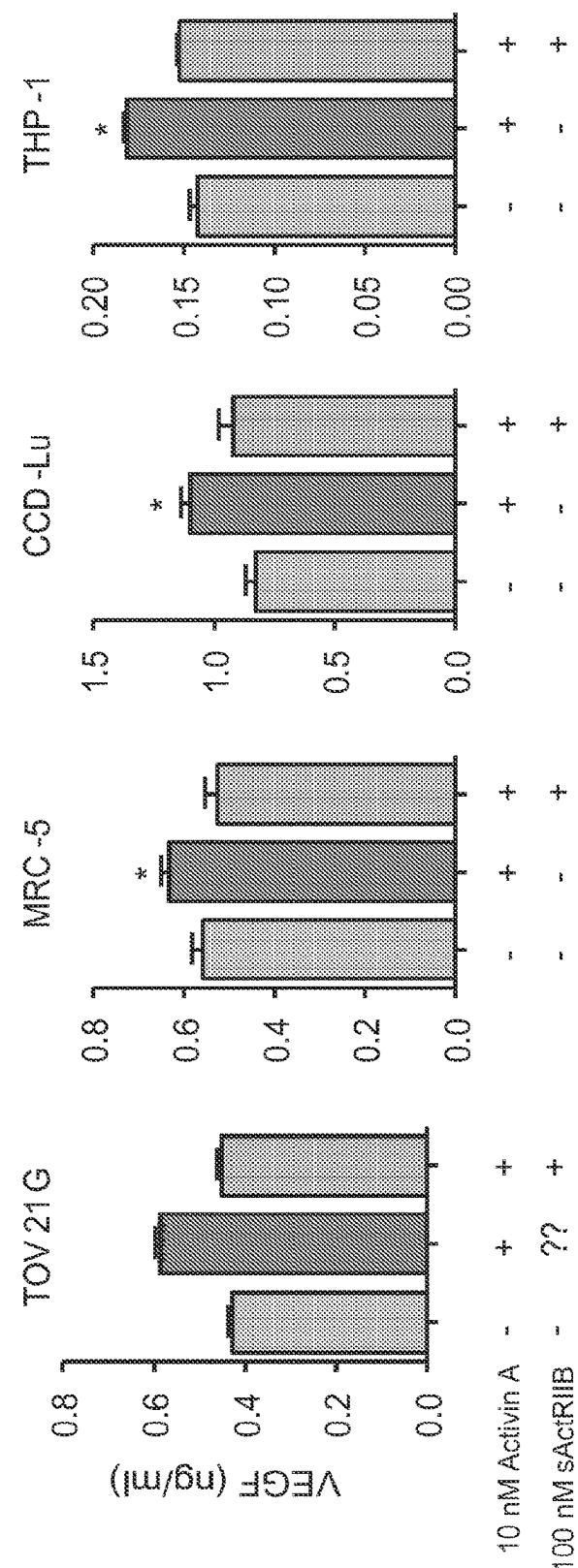


FIG. 17A

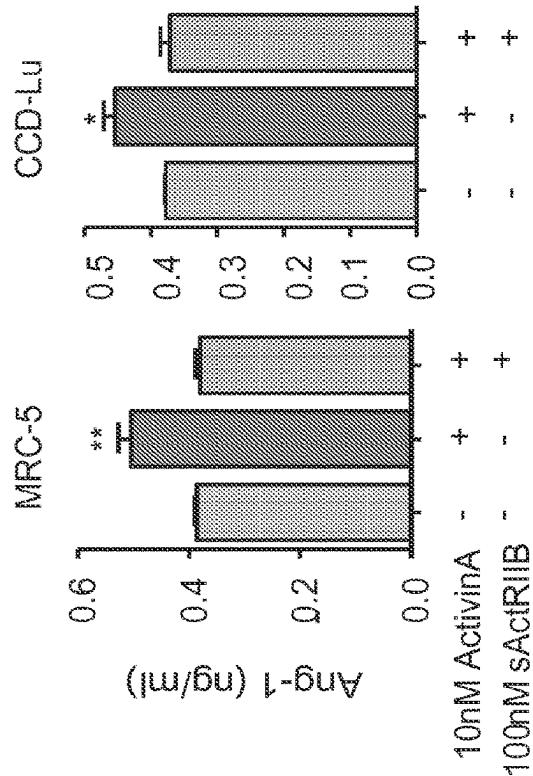


FIG. 17B

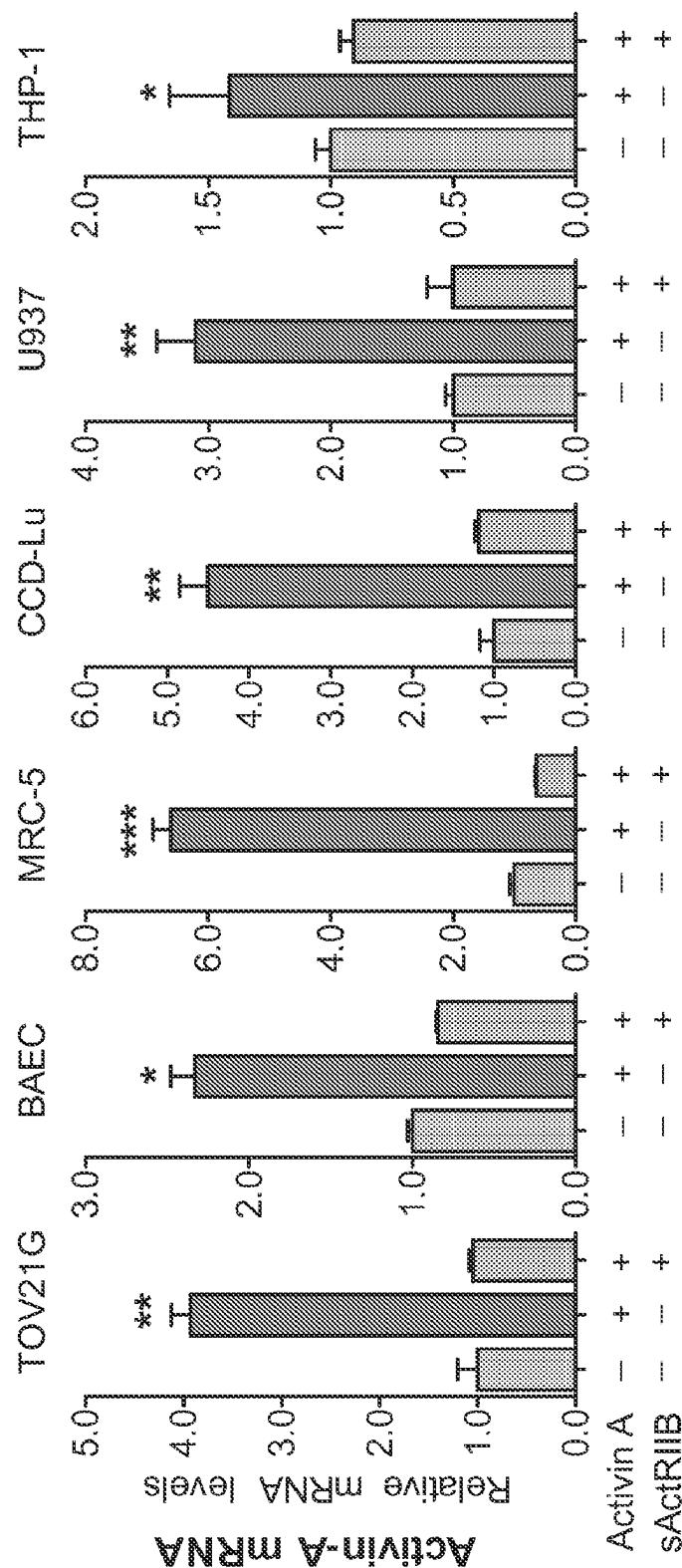


FIG. 18

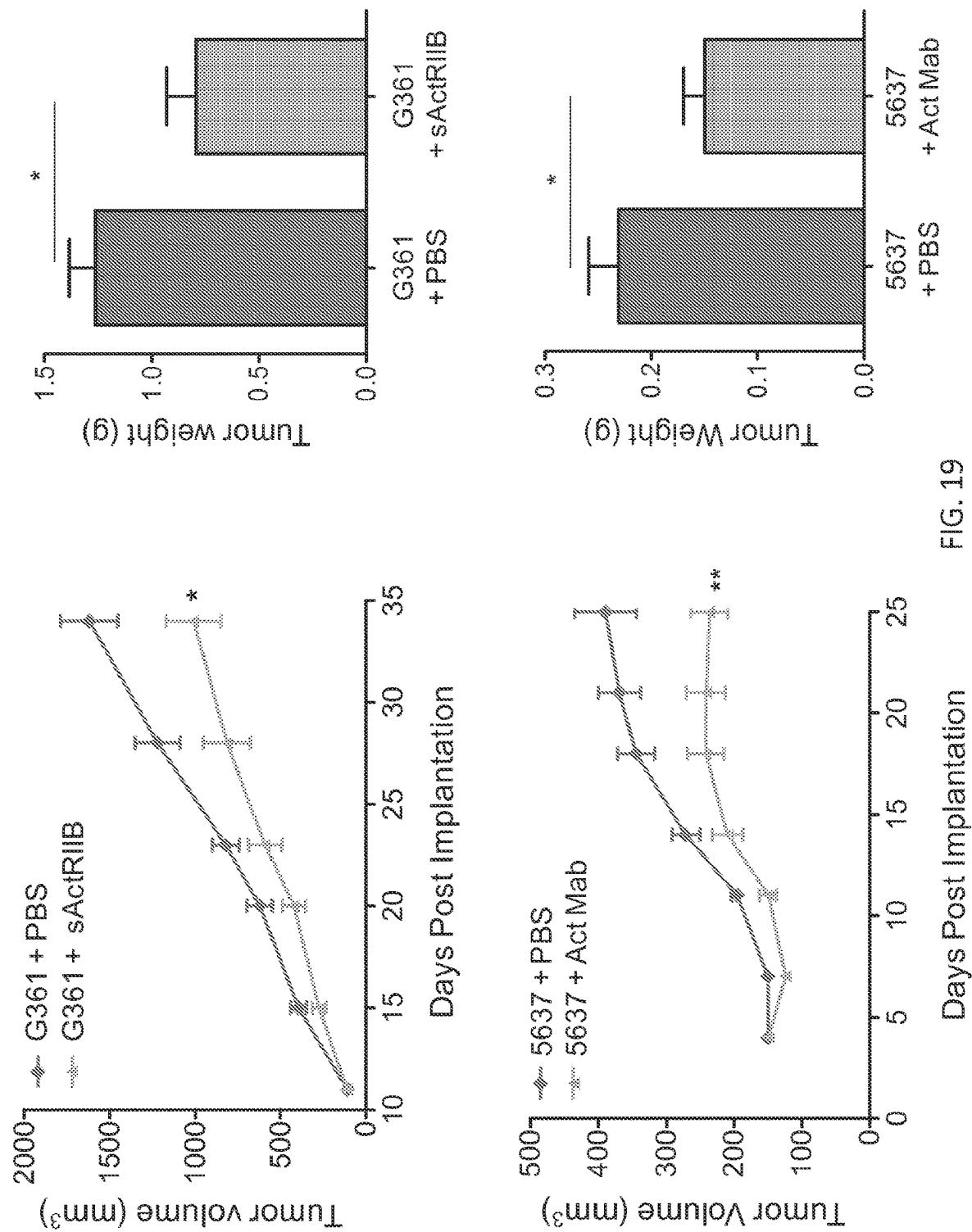


FIG. 19

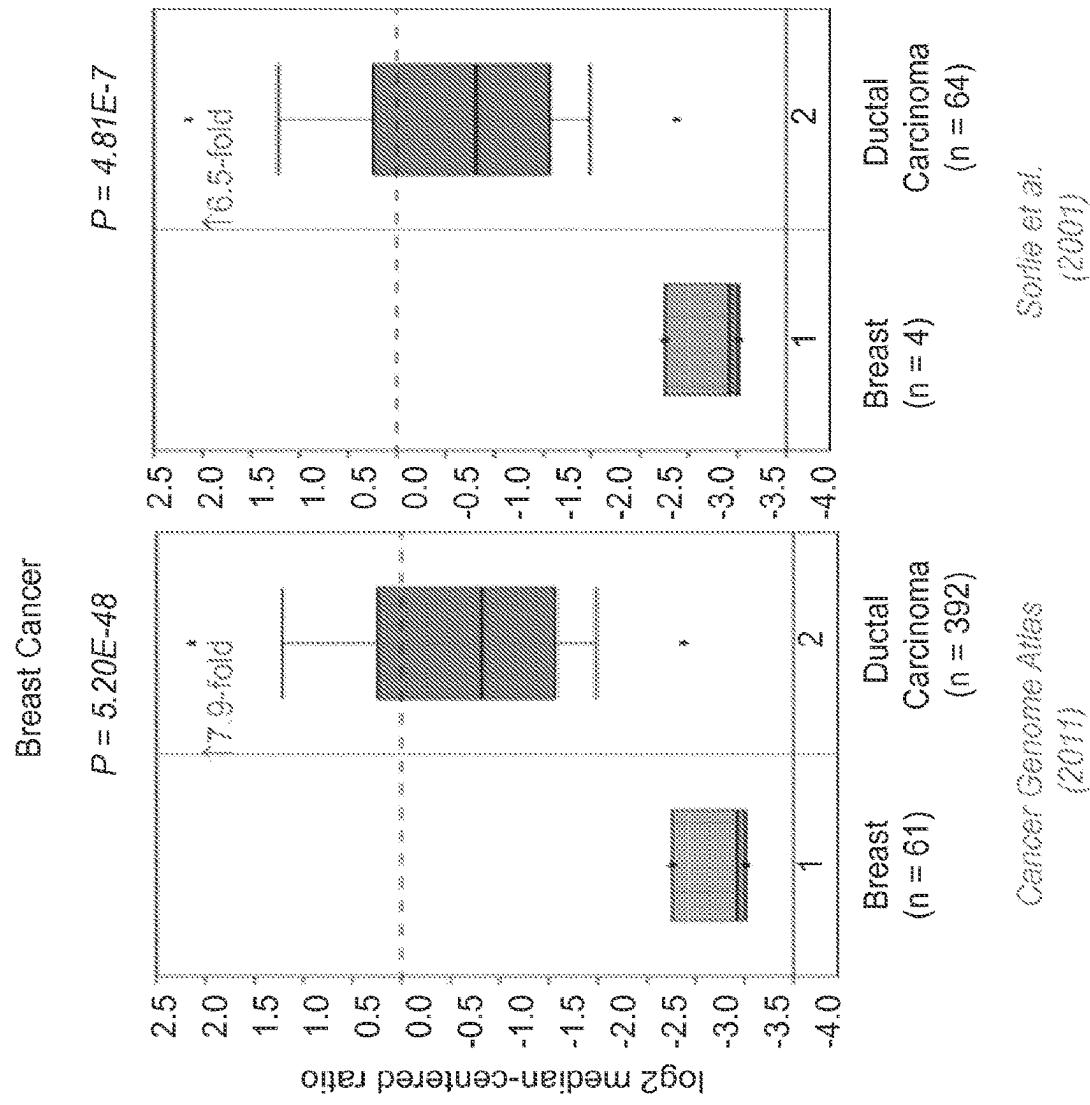
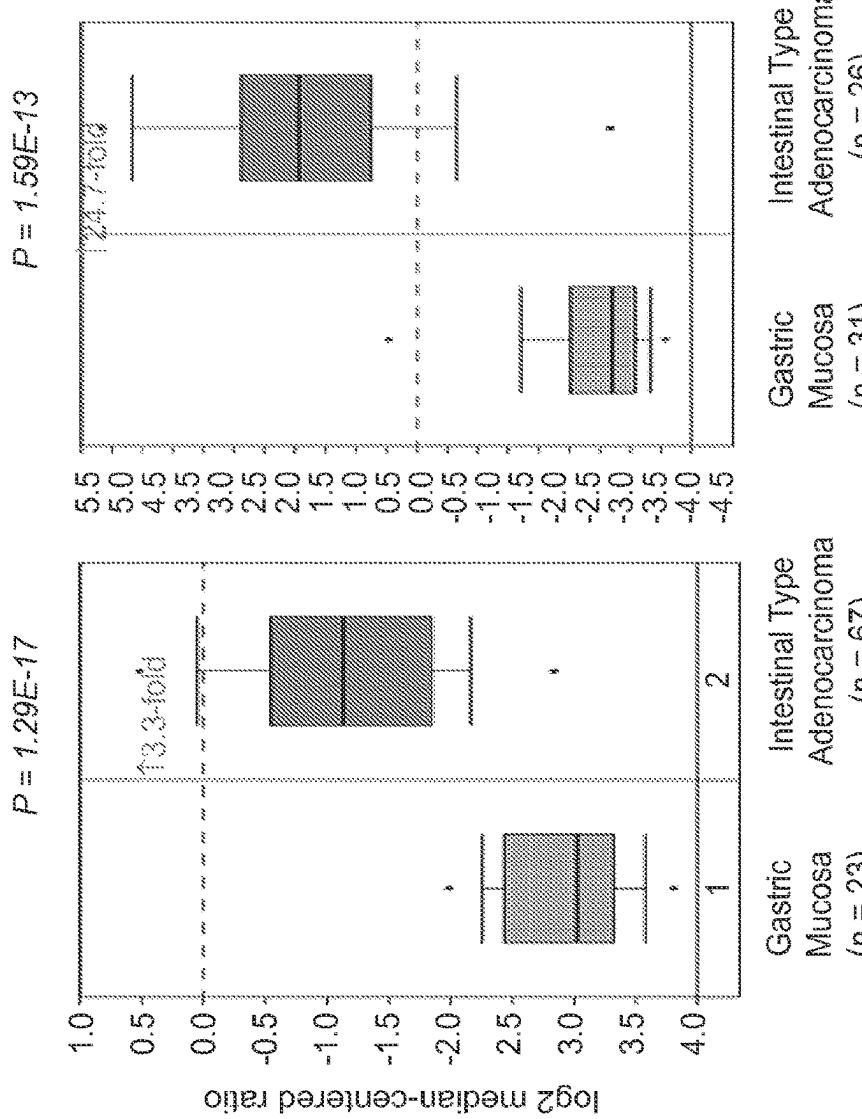


FIG. 20

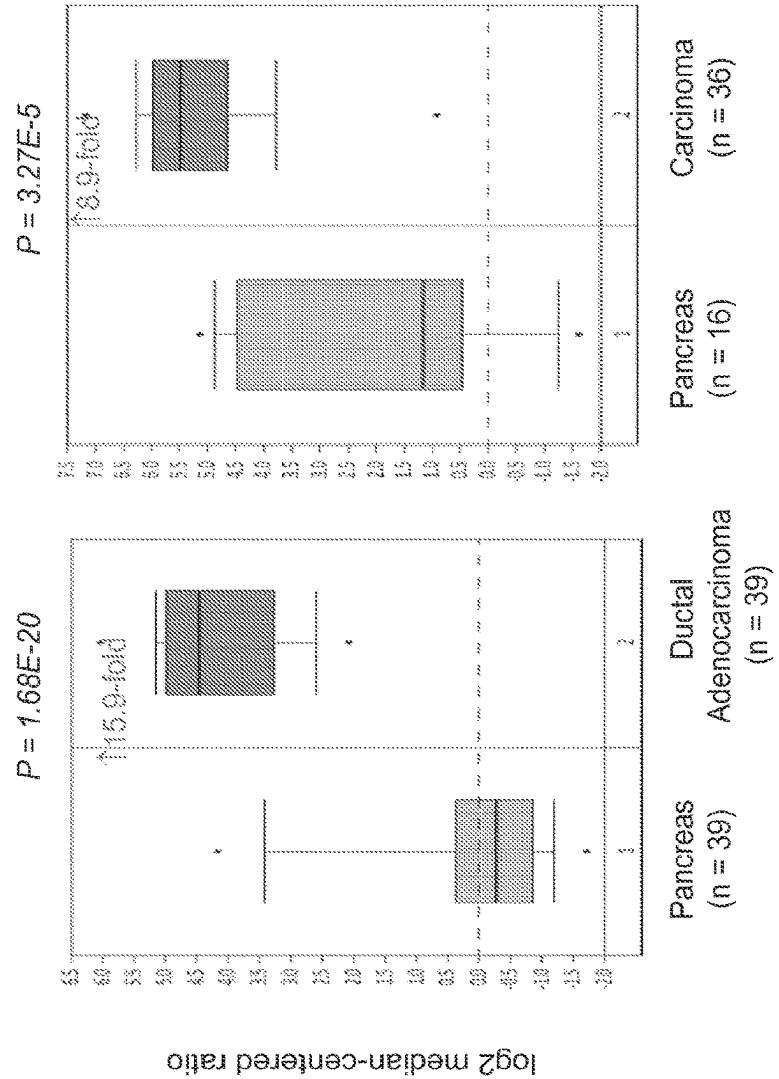
## Gastric Cancer



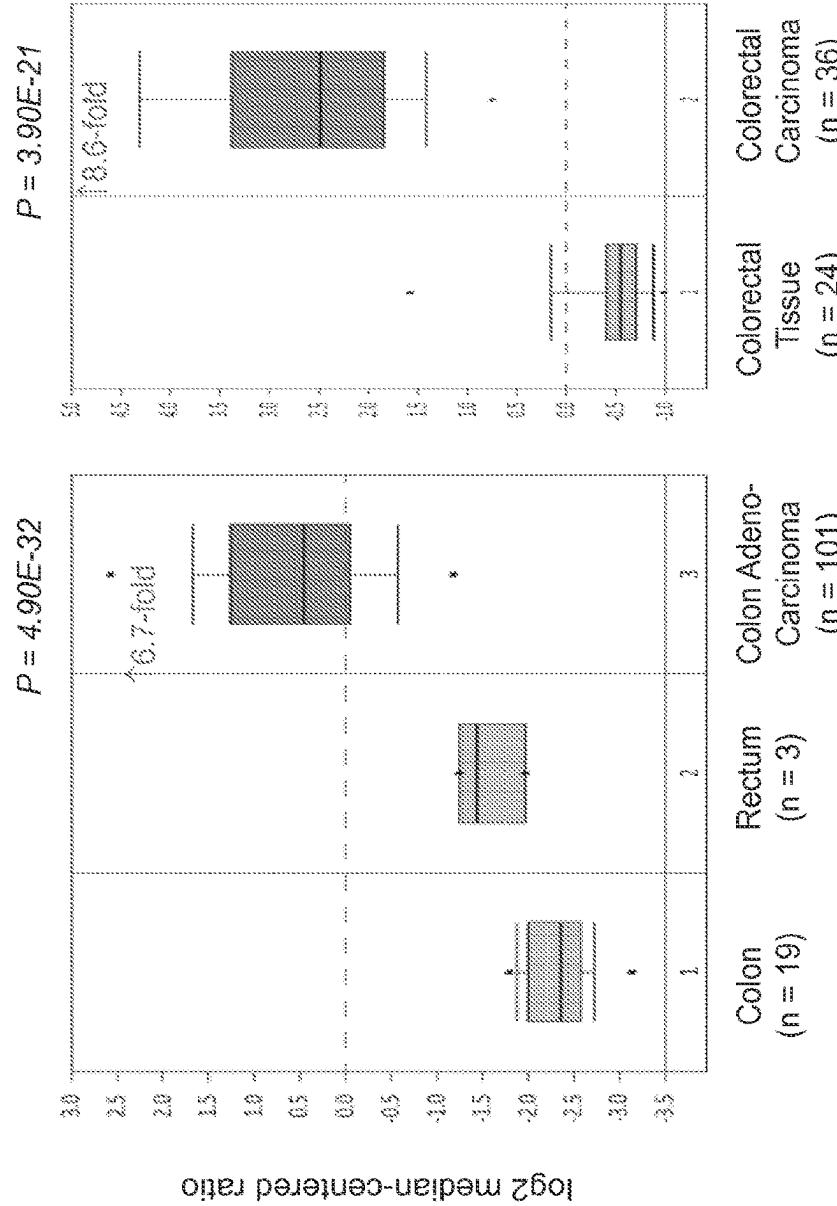
DEMICO et al.  
(2010)  
Chen et al.  
(2003)

FIG. 20 (Cont.)

## Pancreatic Cancer



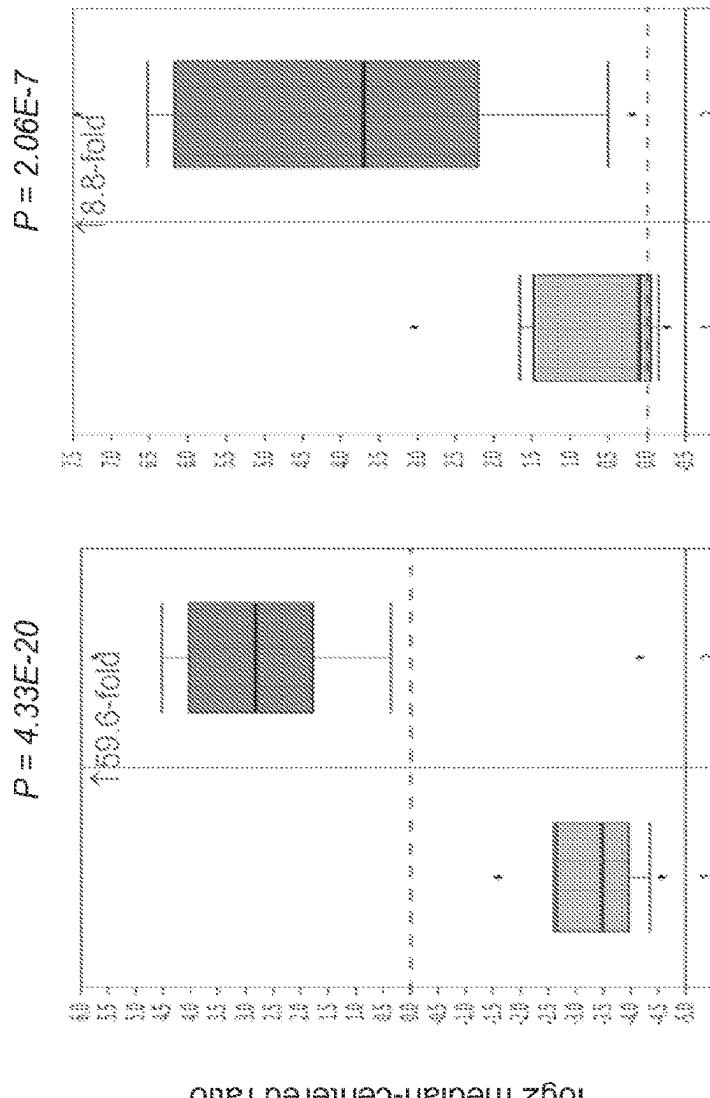
## Colorectal Cancer



Cancer Genome Atlas (2011)  
Skuzopecka et al. (2010)

FIG. 20 (Cont.)

## Head and Neck Cancer



*Zhinos et al.*  
(2004)

*Yeo et al.*  
(2008)

FIG. 20 (Cont.)

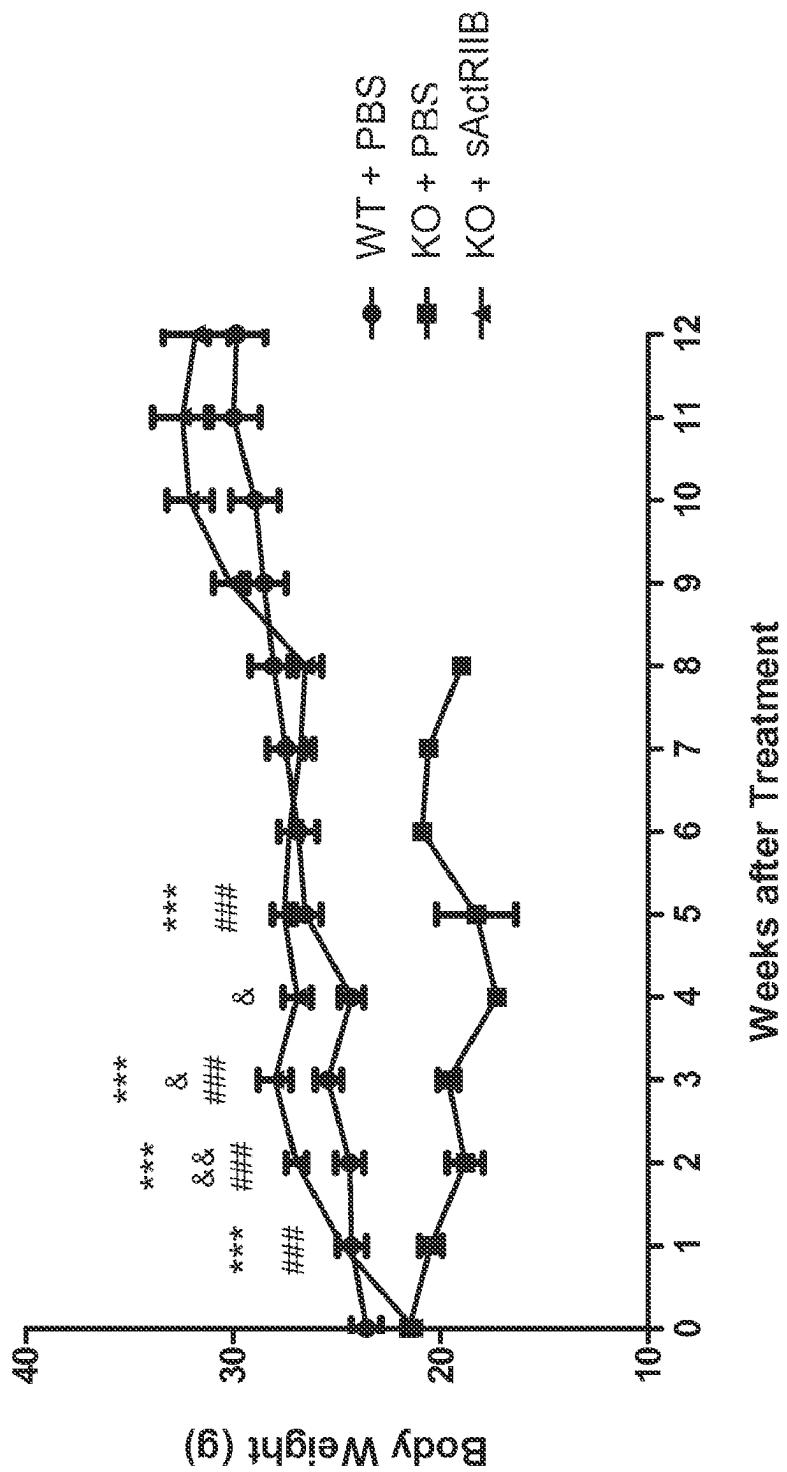


FIG. 21

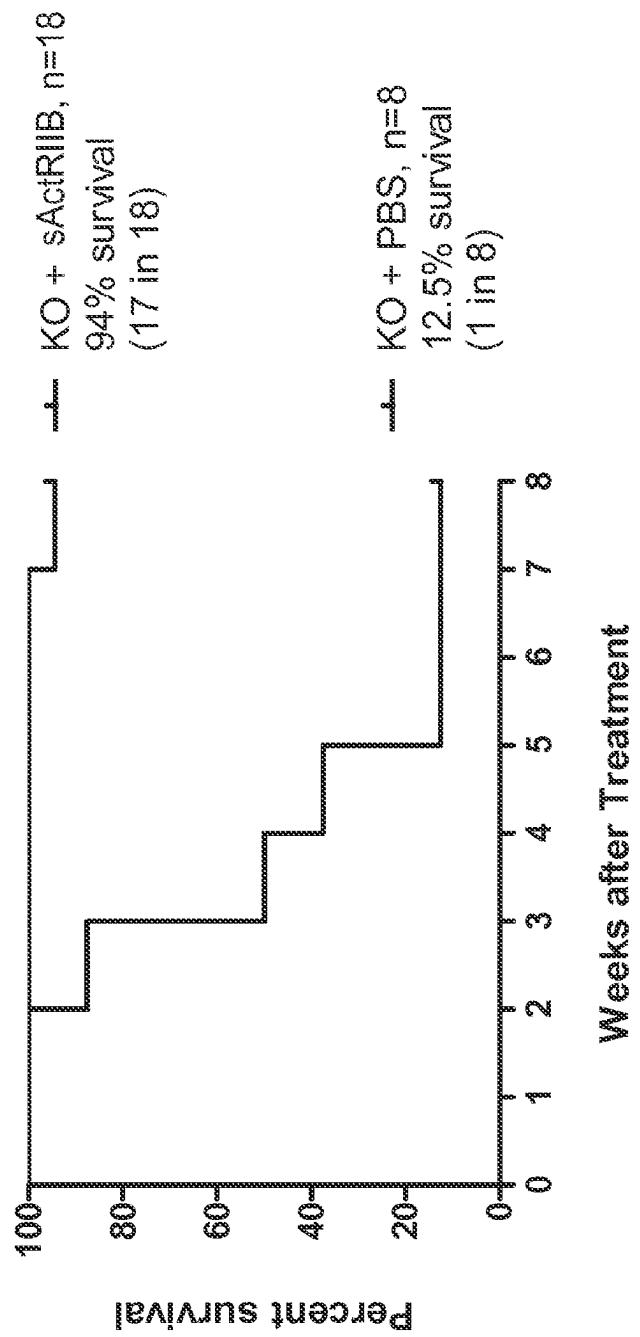


FIG. 22

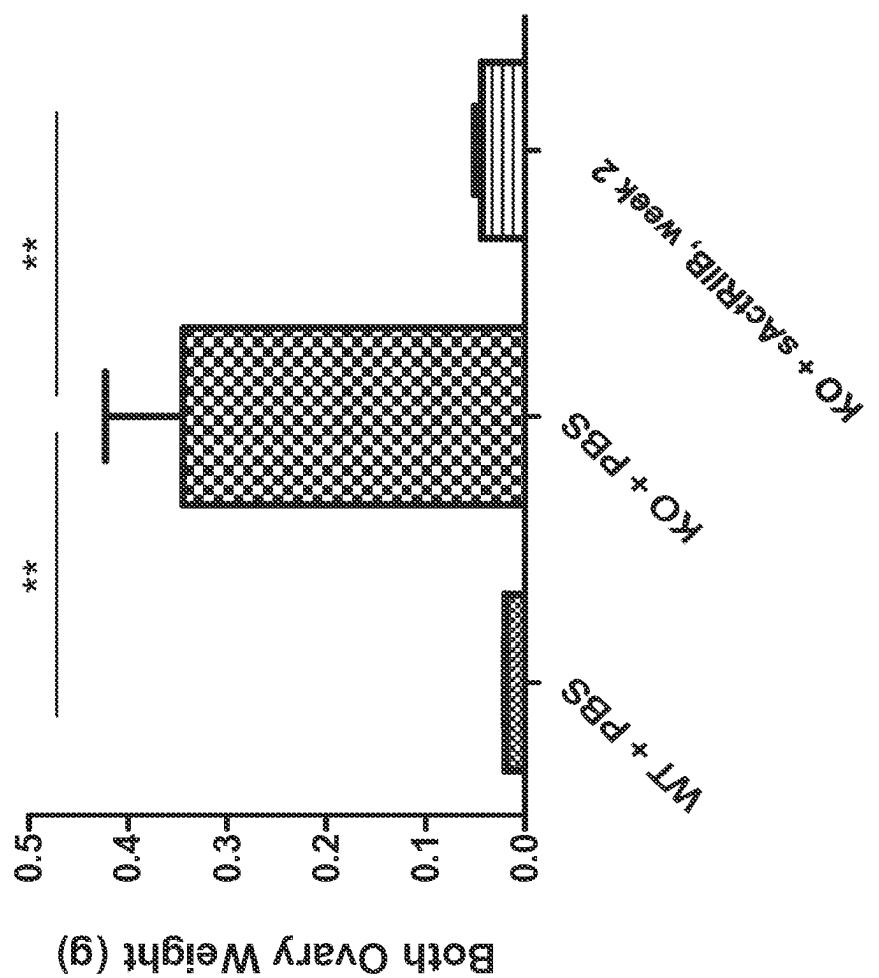


FIG. 23

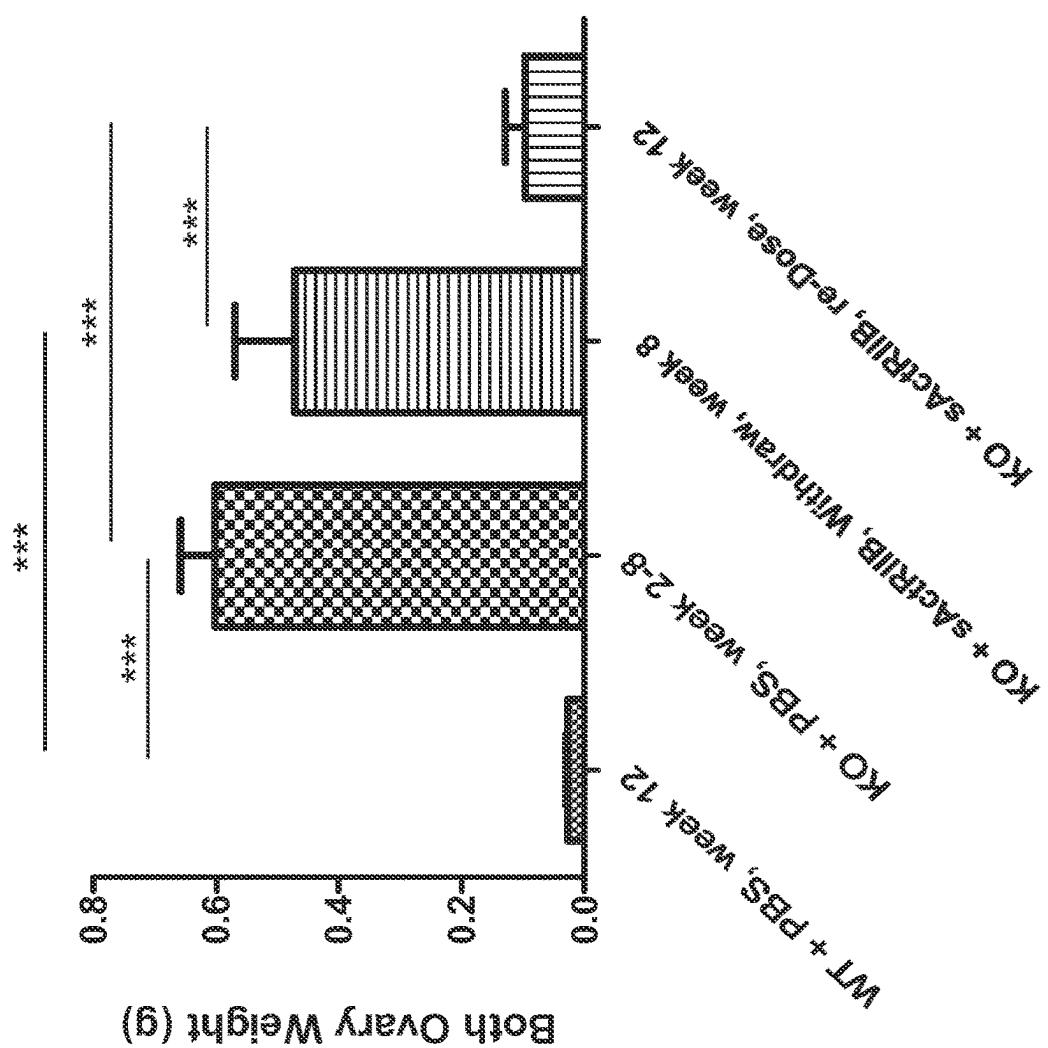


FIG. 24

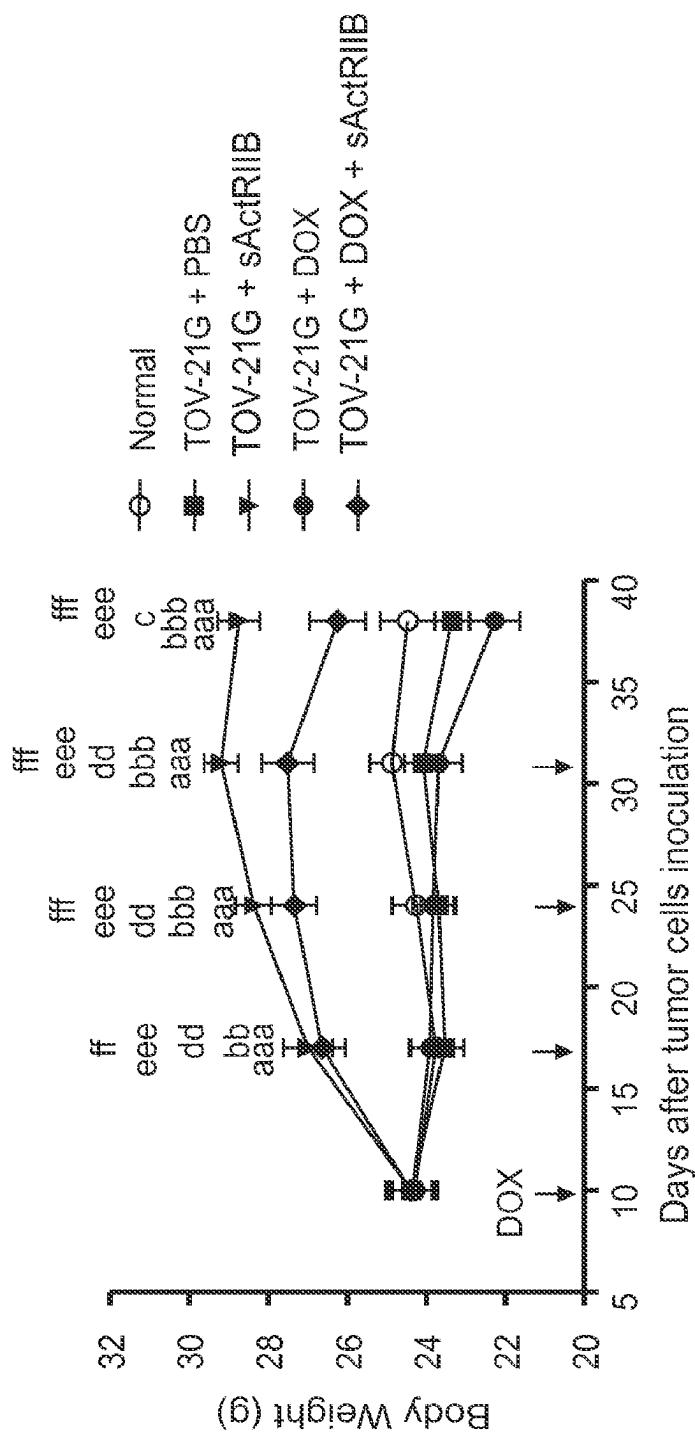


FIG. 25

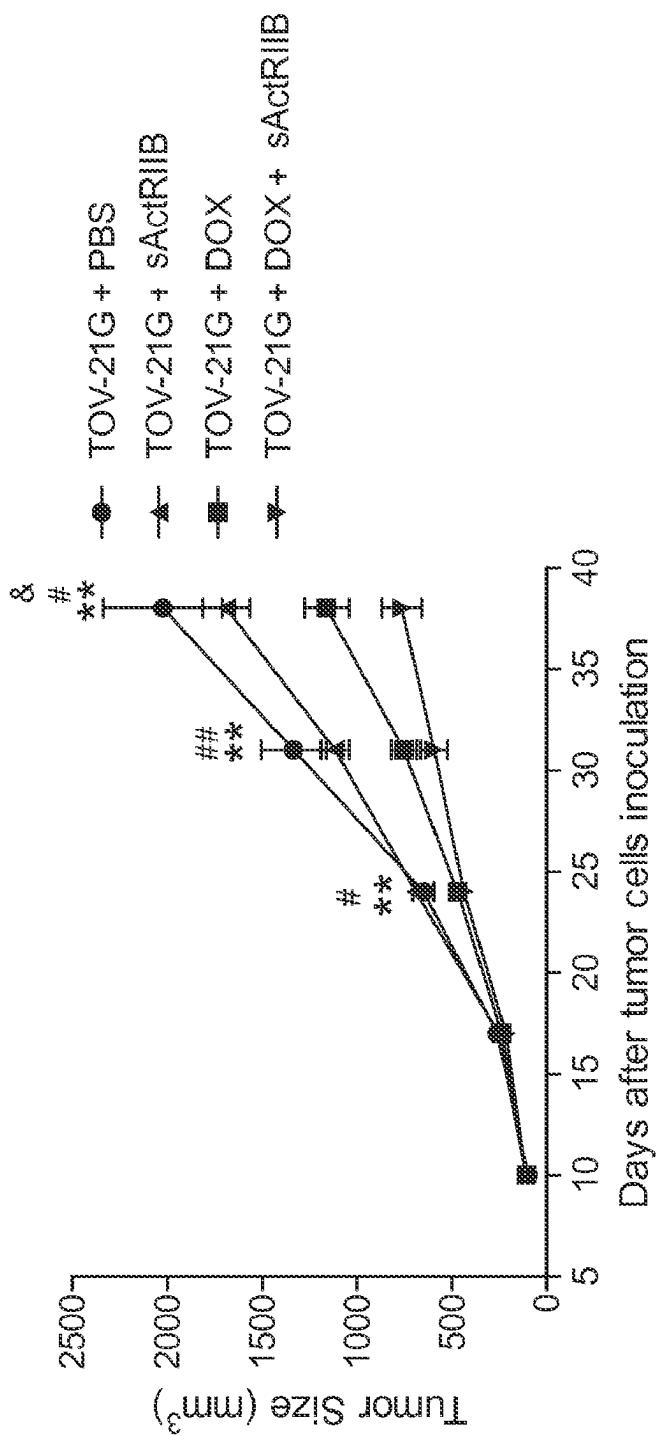


FIG. 26

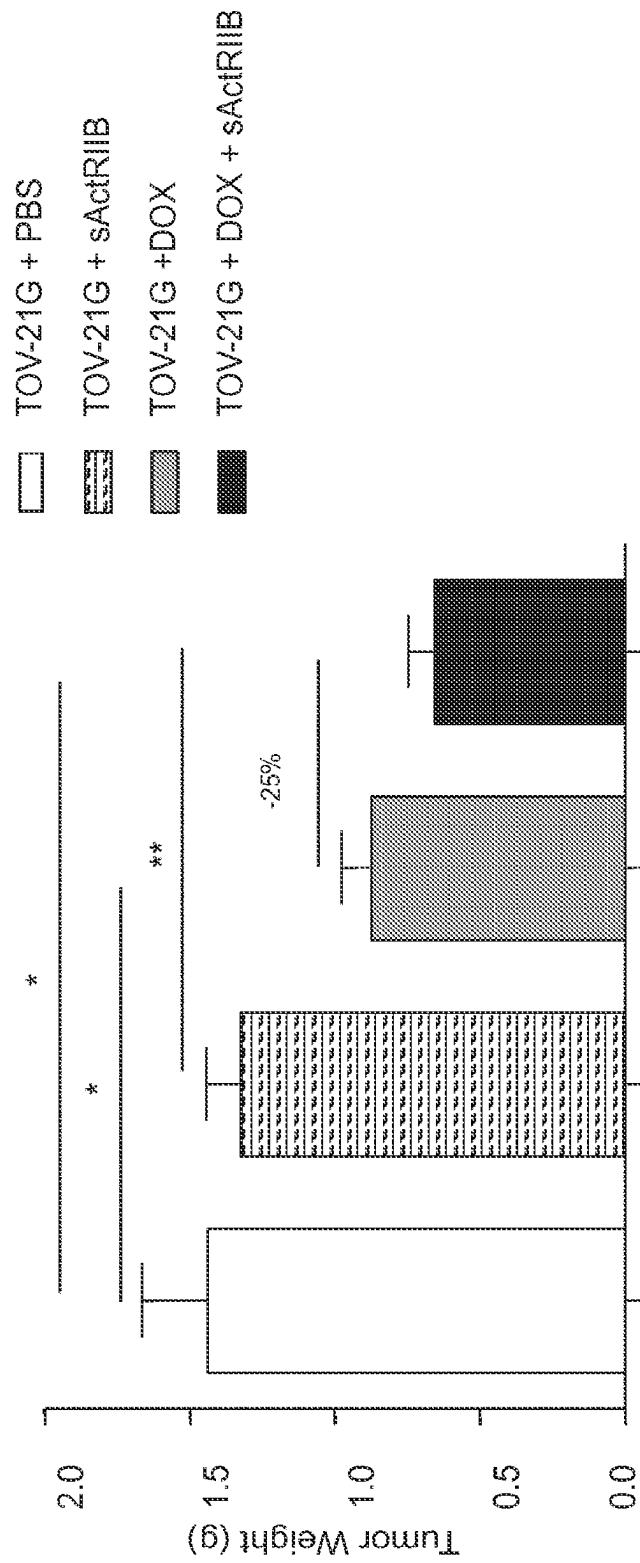
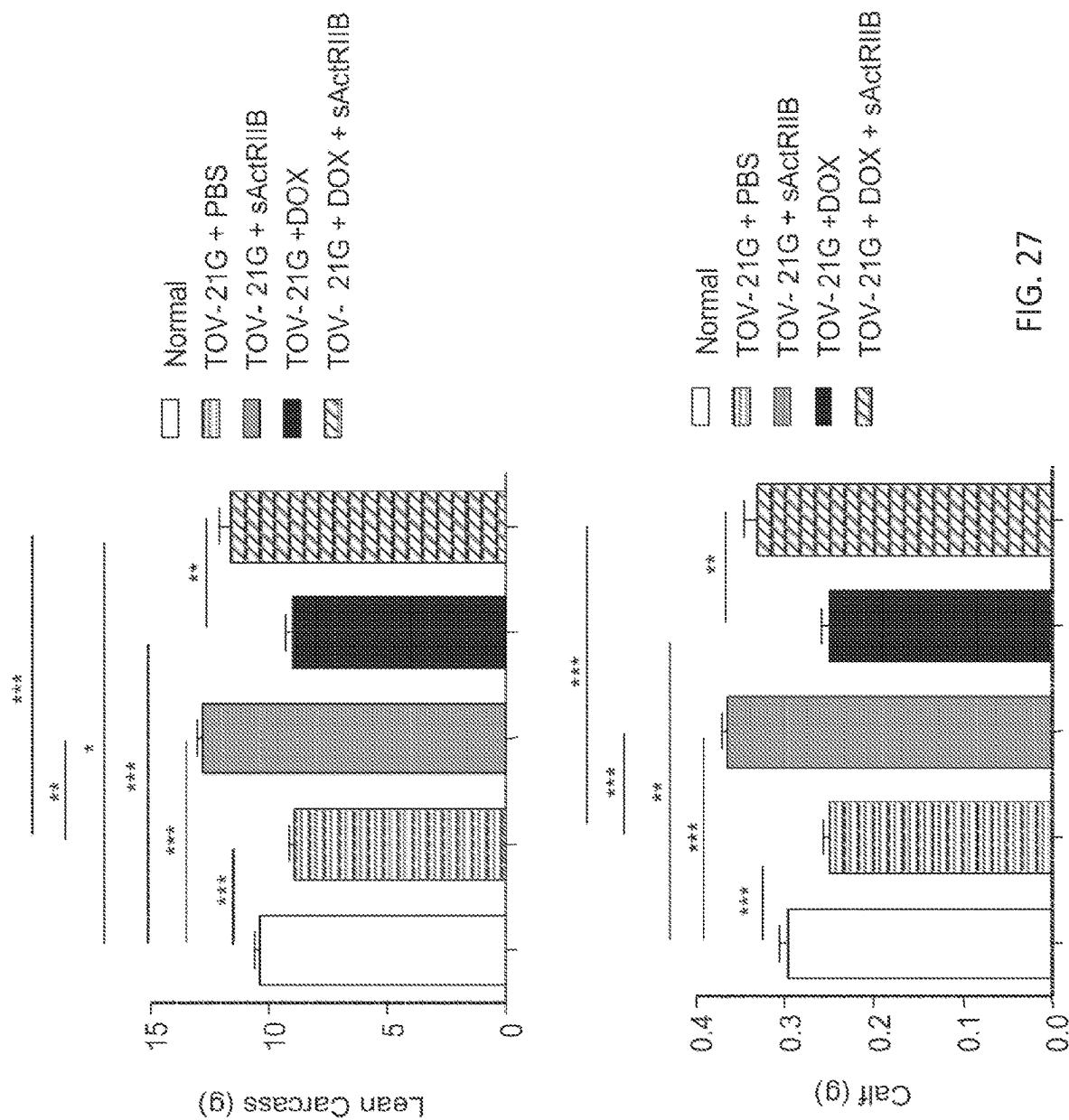


FIG. 26 (Cont.)



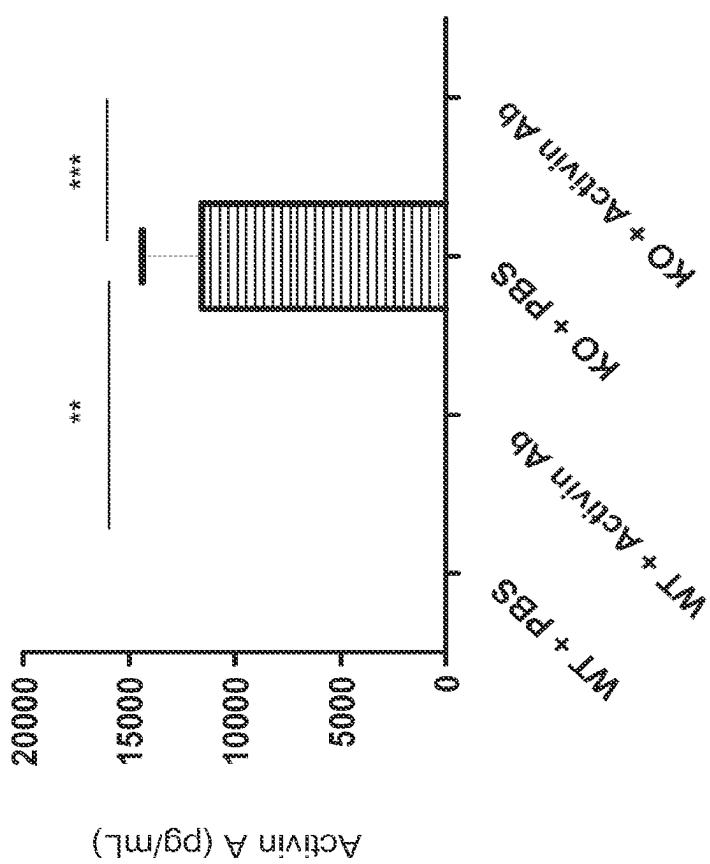


FIG. 28

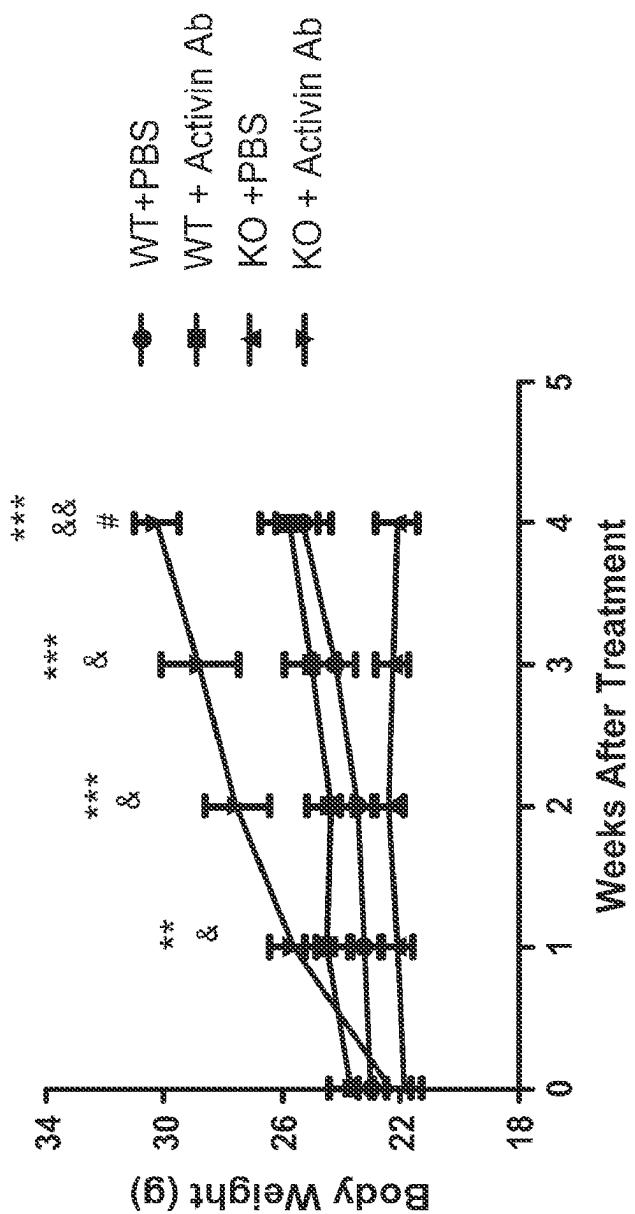


FIG. 29

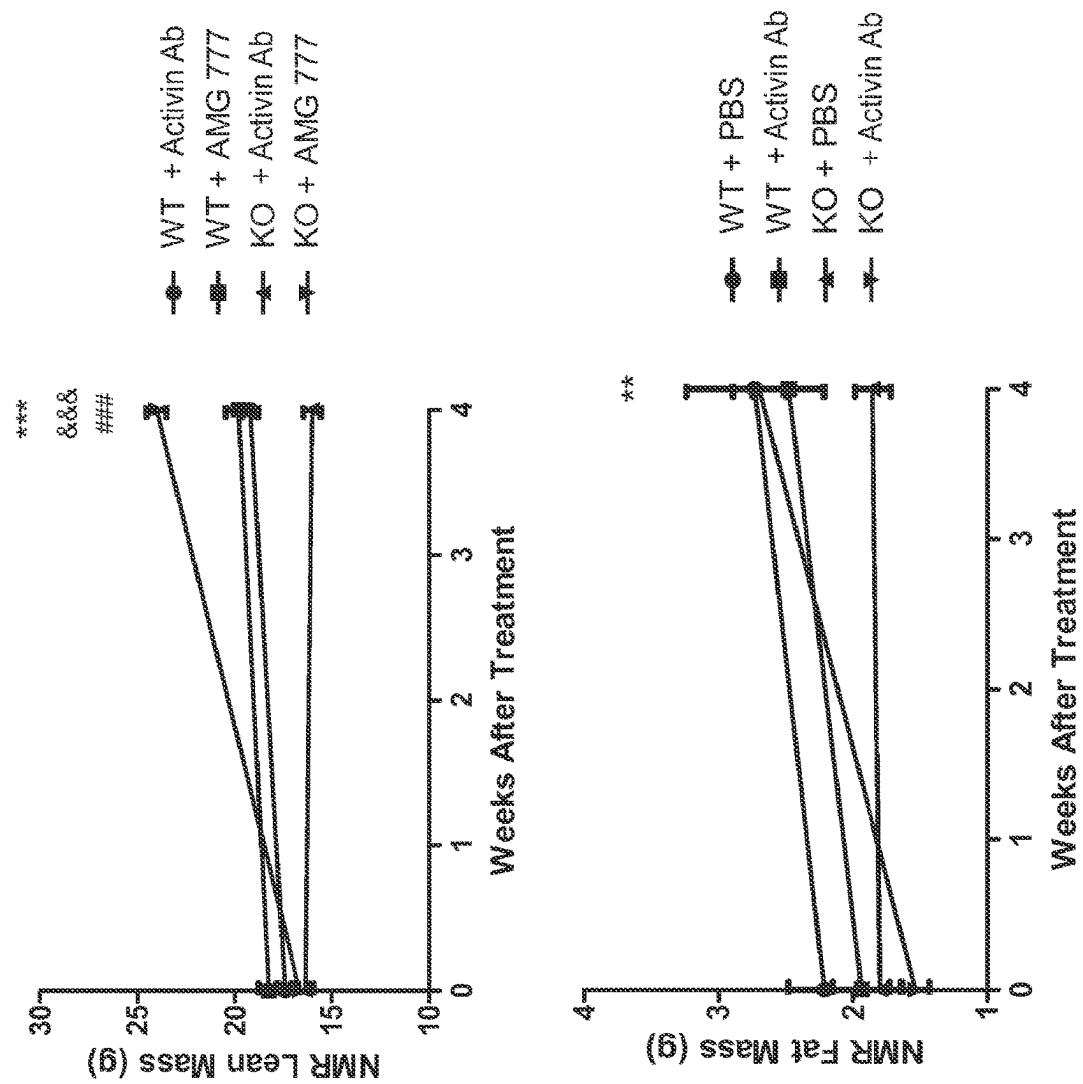


FIG. 30

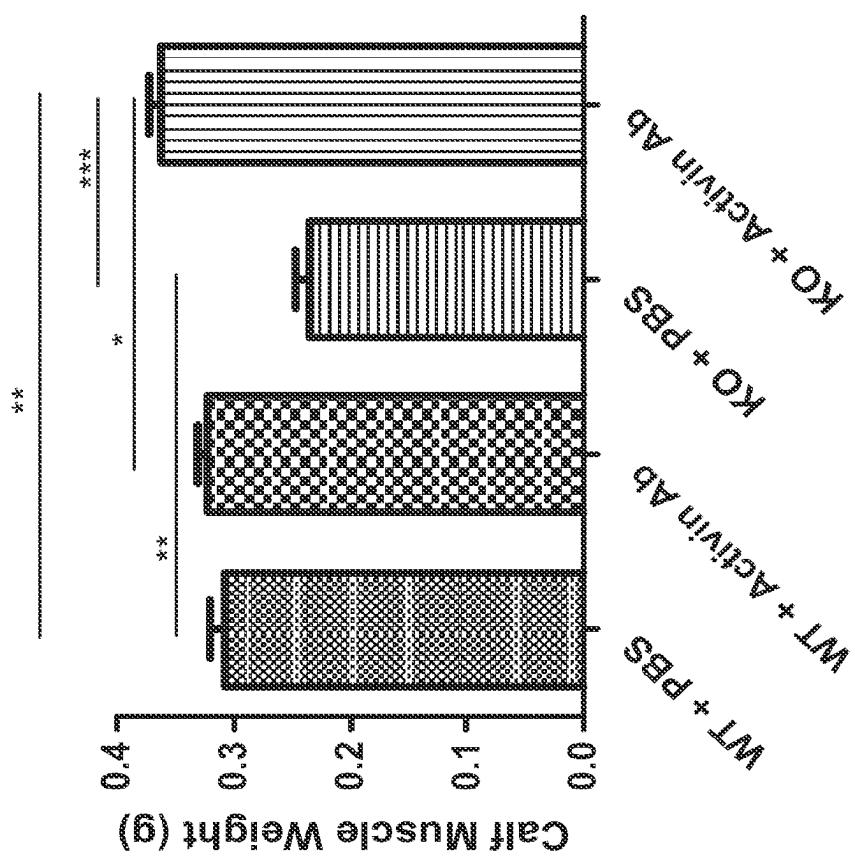


FIG. 31

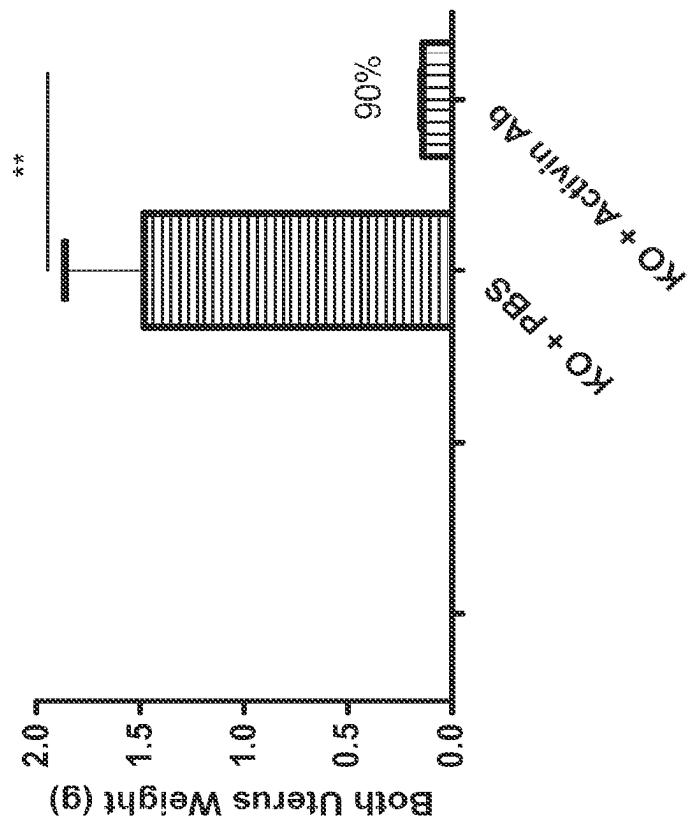


FIG. 32B

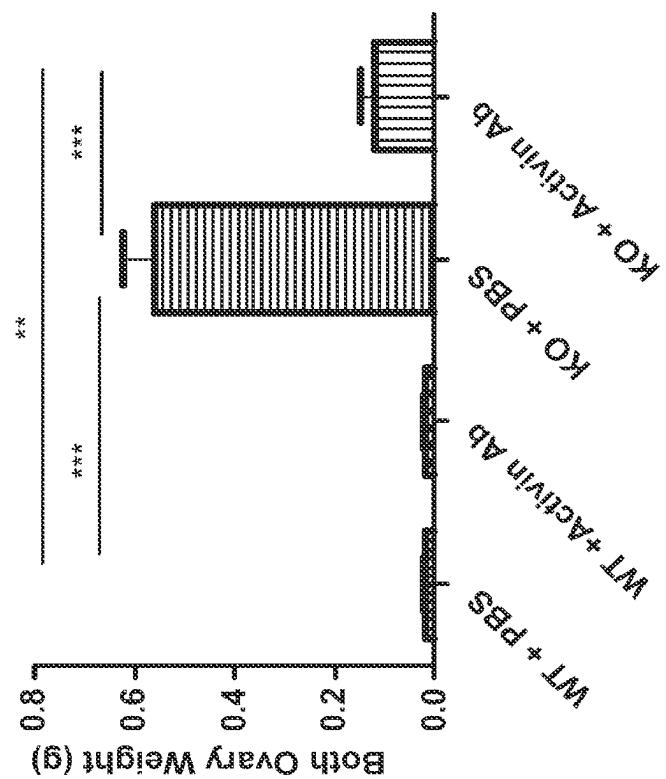


FIG. 32A

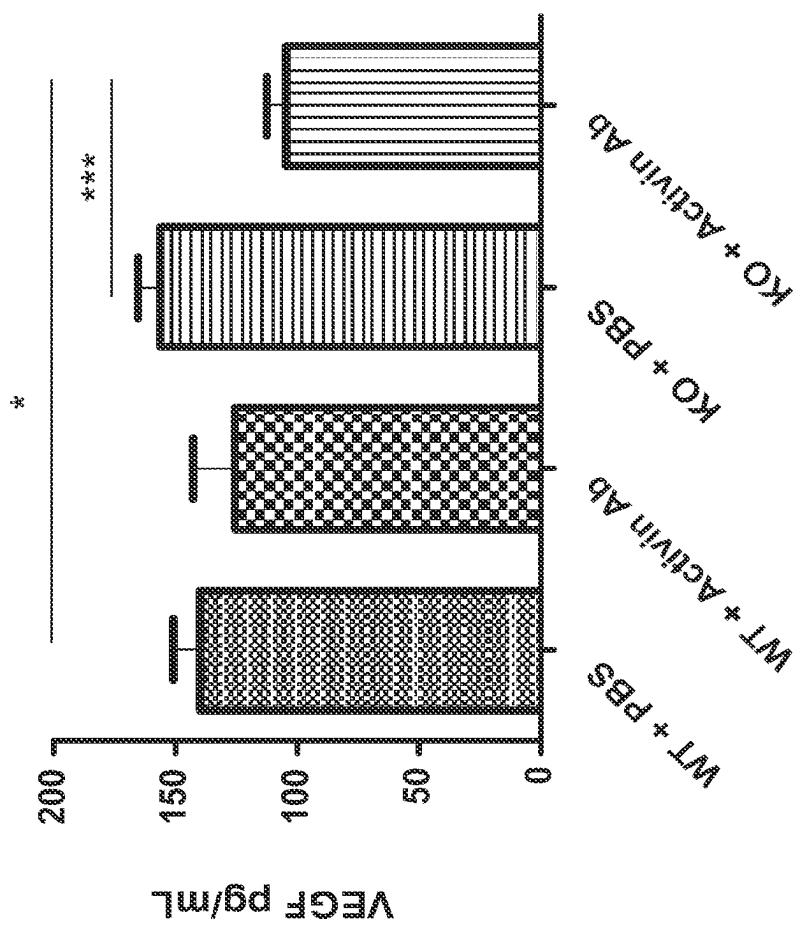


FIG. 33

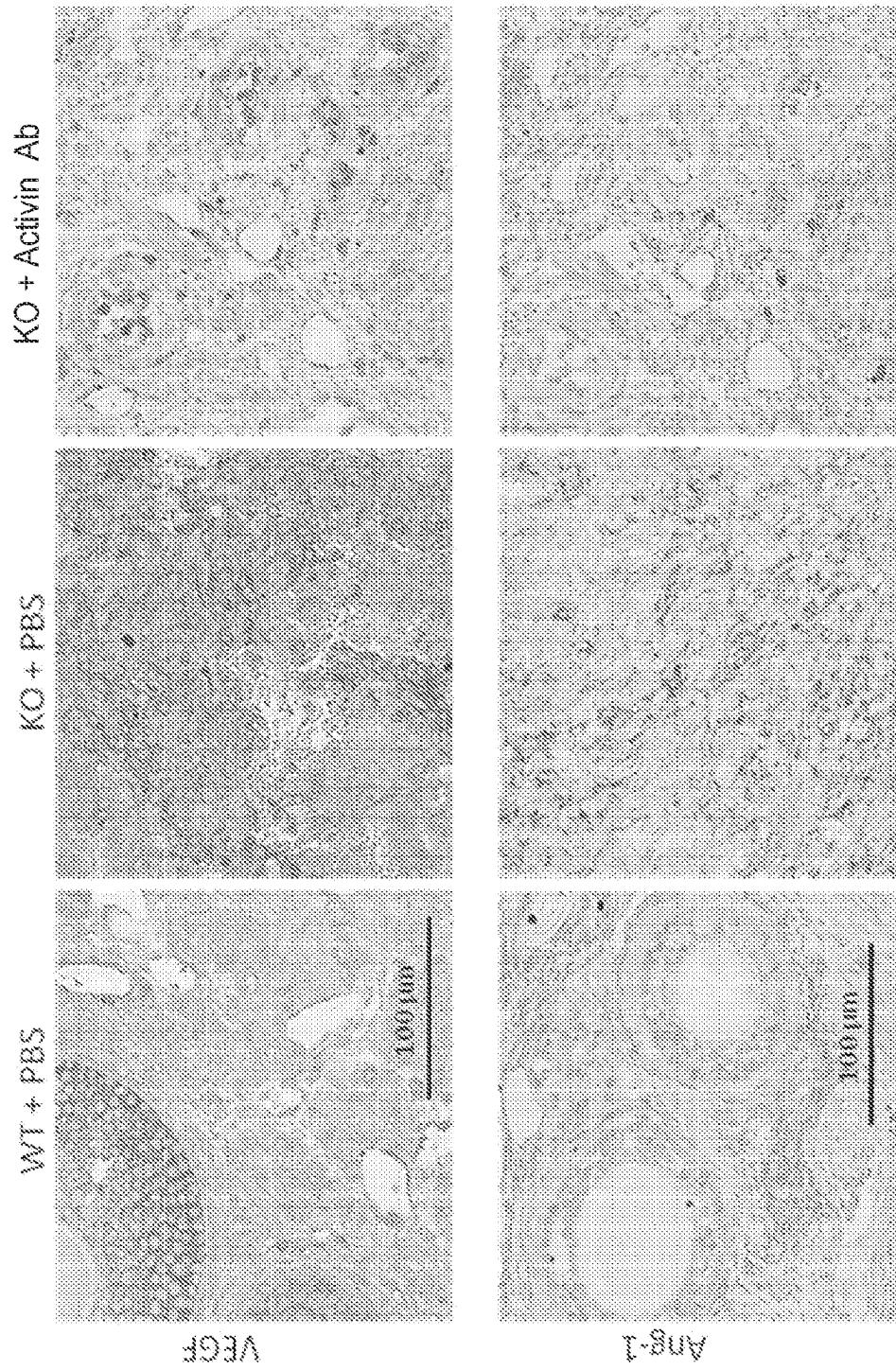


FIG. 34

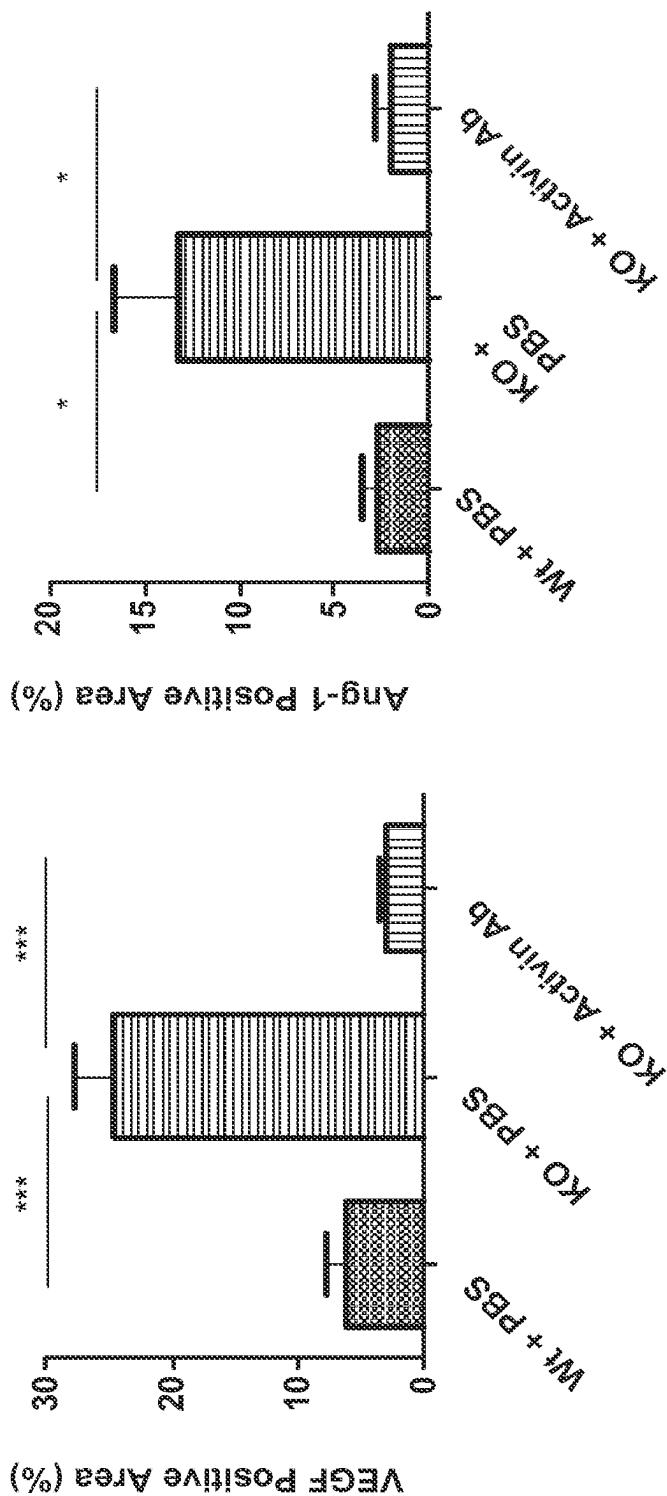


FIG. 34 (Cont.)

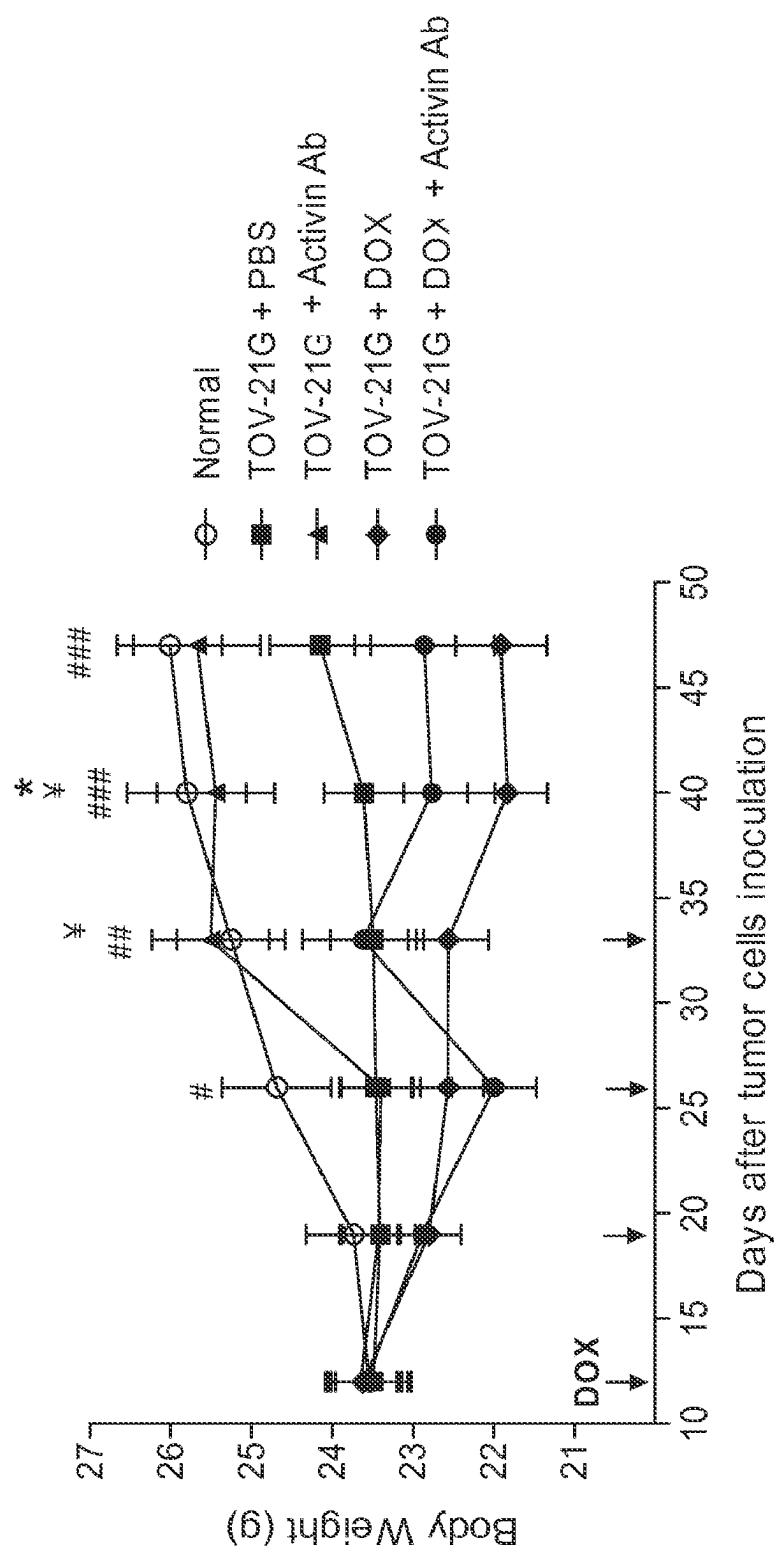


FIG. 35

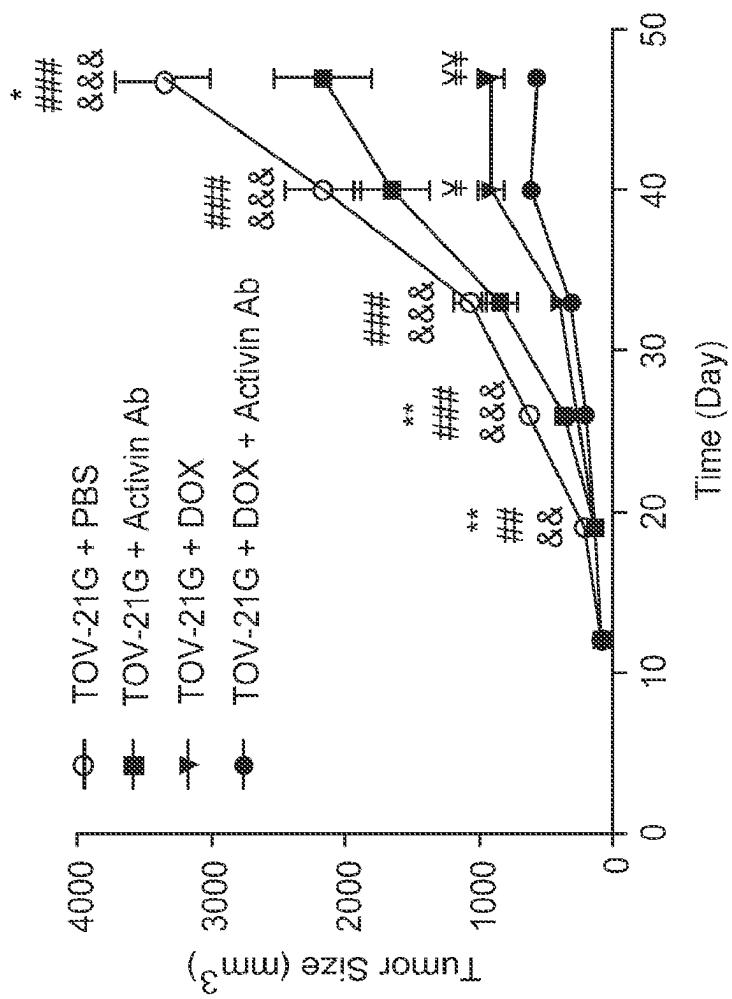


FIG. 36

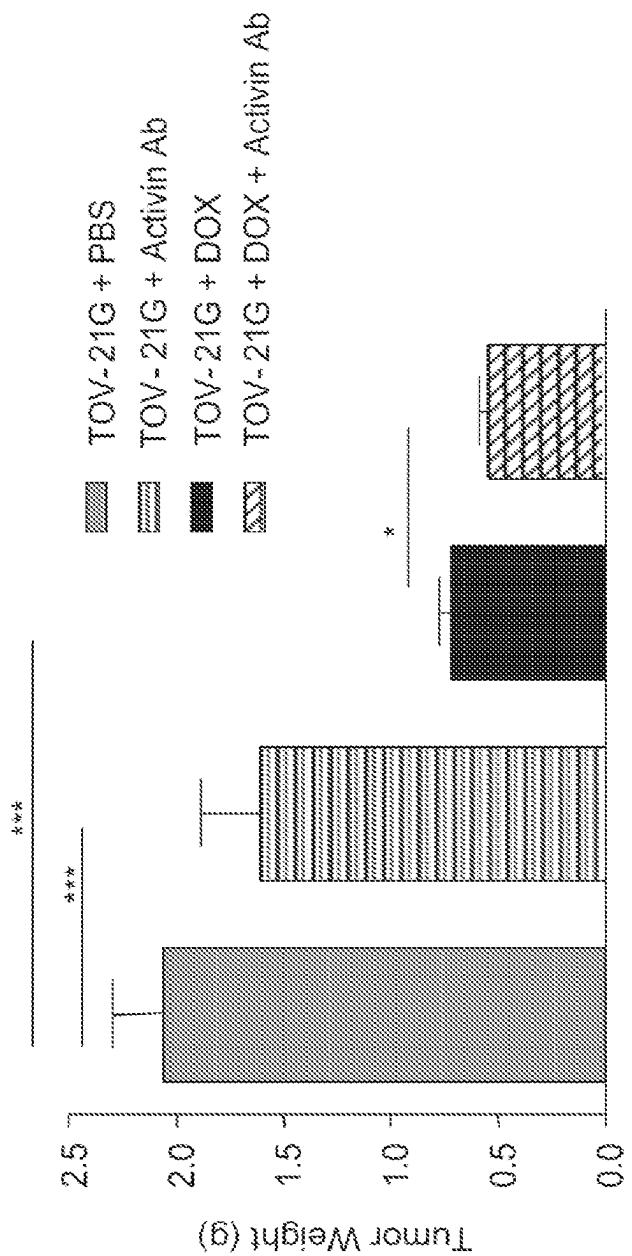


FIG. 37

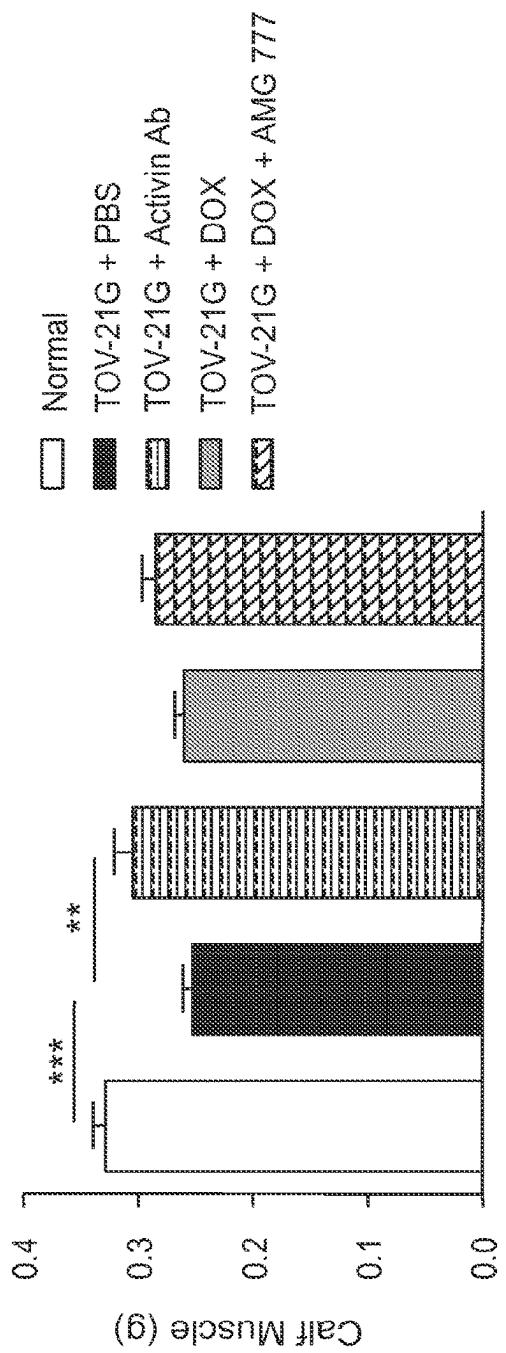
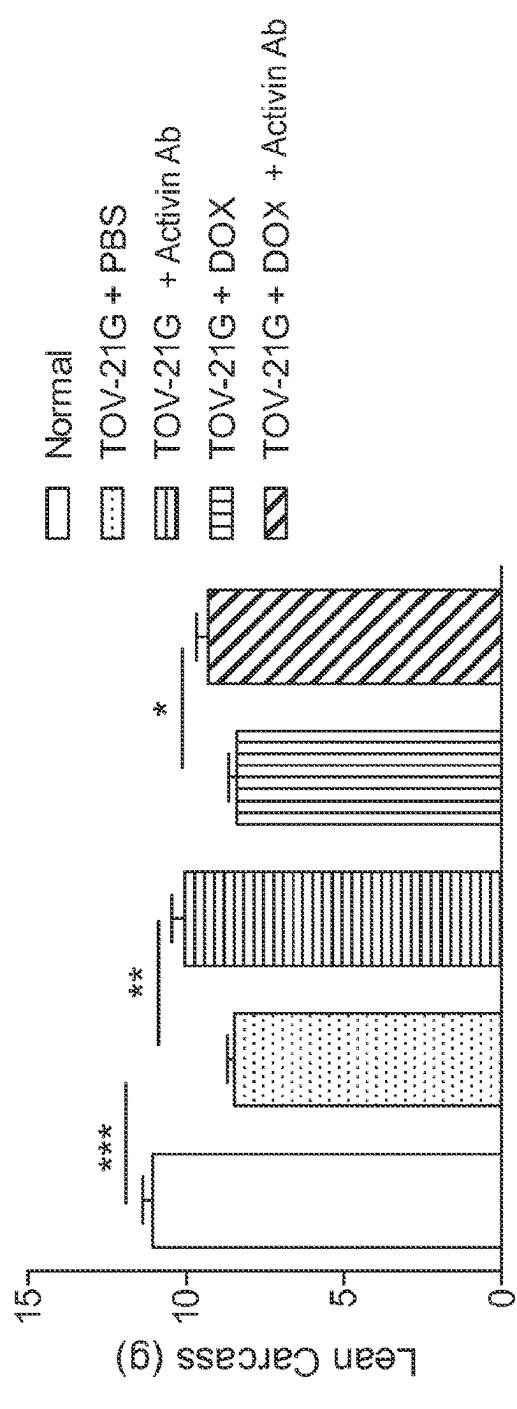


FIG. 38

## ADMINISTRATION OF AN ANTI-ACTIVIN-A COMPOUND TO A SUBJECT

### CROSS REFERENCE TO RELATED APPLICATIONS

This application is a continuation application of U.S. patent application Ser. No. 16/714,407, filed Dec. 13, 2019; which is a divisional application of U.S. patent application Ser. No. 14/764,288, filed Jul. 29, 2015; which is a national stage application under 35 U.S.C. § 371 of International Application No. PCT/US2014/014490, having an international filing date of Feb. 3, 2014; which claims the benefit of U.S. Provisional Application No. 61/759,961, filed Feb. 1, 2013, and U.S. Provisional Application No. 61/815,220, filed Apr. 23, 2013, all of which are incorporated by reference herein in their entireties.

This application is further related to: U.S. application Ser. No. 12/626,375, filed Nov. 25, 2009; U.S. application Ser. No. 13/080,515, filed Apr. 5, 2011; U.S. application Ser. No. 13/329,897, filed Dec. 19, 2011; U.S. application Ser. No. 13/550,447, filed Jul. 16, 2012; U.S. Pat. No. 8,309,082, filed Sep. 7, 2007; U.S. Pat. No. 7,947,646, filed Mar. 5, 2008; PCT Application No. WO 2008/031061, filed Sep. 7, 2007; PCT Application No. WO 2008/109167, filed Mar. 6, 2008; and PCT Application No. WO 2010/062383, filed Nov. 25, 2009, all herein incorporated in their entirety by reference.

### REFERENCE TO A SEQUENCE LISTING

This application includes a Sequence Listing submitted electronically as an .xml file. The sequence listing is incorporated by reference. The SEQ ID NO identifiers shown in the sequence listing should be disregarded. Said .xml copy, created on Dec. 22, 2022, is named A-1798-US07-CNT\_Seqlisting.xml and is 363,275 bytes in size.

### BACKGROUND

Activin-A is a member of the TGF- $\beta$  family that was originally identified in gonadal fluids. It plays an important role in regulating the menstrual cycle by controlling Follicle Stimulating Hormone (FSH) release from the pituitary gland. Activin-A is also known to serve diverse other functions such as in cell growth and differentiation, immune responses, and wound healing.

Ovarian cancer is the deadliest of all gynecologic cancers. In the United States, approximately one in every 60 women develops ovarian cancer, and more than 25,000 new cases are diagnosed each year. Less than 25% of ovarian cancer cases are diagnosed before cancer has spread beyond the ovary, and the chance of five-year survival for late stage ovarian cancer is less than 30%.

### SUMMARY

Disclosed herein are methods for treating ovarian cancer in a subject by administering anti-activin-A compounds to the subject, including anti-activin-A antibodies and/or activin receptors. Also disclosed are methods of identifying subjects for treatment of ovarian cancer by evaluating levels of specific proteins in a subject.

In one embodiment, the method comprises administering a therapeutically effective amount of an anti-activin-A com-

pound to a subject. In another embodiment, the anti-activin-A compound is formulated with a pharmaceutically-acceptable carrier.

In a further embodiment, the anti-activin-A compound comprises:

(a) a light chain CDR3 comprising a sequence selected from the group consisting of

i. a light chain CDR3 sequence that differs by no more than a total of two amino acid additions, substitutions, and/or deletions from a CDR3 sequence selected from the group consisting of the light chain CDR3 sequences disclosed herein, and;

ii.

(SEQ ID NO: 132)

$X_{73}QX_{74}X_{75}X_{76}X_{77}X_{78}X_{79}X_{80};$

iii.

(SEQ ID NO: 131)

$LQHNX_{81}YX_{82}X_{83}T;$   
and

iv.

(SEQ ID NO: 248)

$QAWDX_{84}STX_{85}X_{86};$

wherein  $X_{73}$  is a methionine residue, a glutamine residue, or an arginine residue,  $X_{74}$  is an alanine residue, a tyrosine residue, a glutamine residue, or a serine residue,  $X_{75}$  is a leucine residue, a tyrosine residue, or an asparagine residue,  $X_{76}$  is a glutamine residue, a serine residue, or a threonine residue,  $X_{77}$  is a threonine residue, a tyrosine residue, or an isoleucine residue,  $X_{78}$  is a proline residue or a serine residue,  $X_{79}$  is a cysteine residue, a tryptophan residue, a leucine residue, or a proline residue,  $X_{80}$  is a serine residue or a threonine residue,  $X_{81}$  is a threonine residue or a serine residue,  $X_{82}$  is a proline residue or a threonine residue,  $X_{83}$  is a phenylalanine residue or a tryptophan residue,  $X_{84}$  is an arginine residue or a serine residue,  $X_{85}$  is a valine residue or an alanine residue, and  $X_{86}$  is a valine residue or no residue, and said anti-activin-A compound binds specifically to human activin-A; or

(b) a heavy chain CDR3 comprising a sequence selected from the group consisting of:

i. a heavy chain CDR3 sequence that differs by no more than a total of three amino acid additions, substitutions, and/or deletions from a CDR3 sequence selected from the group consisting of the heavy chain CDR3 sequences disclosed herein;

ii.

(SEQ ID NO: 187)

$X_{87}X_{88}X_{89}X_{90}X_{91}X_{92}X_{93}X_{94}FDY;$

iii.

(SEQ ID NO: 188)

$X_{95}X_{96}X_{97}Y X_{98} D X_{99} X_{100} GWX_{101}X_{102}X_{103};$   
and

iv.

(SEQ ID NO: 249)

$X_{104}X_{105}X_{106}X_{107}X_{108}X_{109}YX_{110}X_{111}X_{112}X_{113}X_{114}X_{115}$

$X_{116}X_{117}X_{118};$

wherein  $X_{87}$  is a valine residue or no residue,  $X_{88}$  is a glutamine residue or no residue,  $X_{89}$  is an aspartate residue, a tryptophan residue, or no residue,  $X_{90}$  is a serine residue, a leucine residue, or no residue,  $X_{91}$  is

an isoleucine residue, a glutamate residue, or a glutamine residue, X<sub>92</sub> is an alanine residue, a leucine residue, or a glycine residue, X<sub>93</sub> is an alanine residue or a leucine residue, X<sub>94</sub> is a proline residue, a tyrosine residue, or a glycine residue, X<sub>95</sub> is an aspartate residue or no residue, X<sub>96</sub> is a glutamine residue or no residue, X<sub>97</sub> is an aspartate residue or an alanine residue, X<sub>98</sub> is a tyrosine residue or a glycine residue, X<sub>99</sub> is a serine residue or a tyrosine residue, X<sub>100</sub> is a serine residue or an arginine residue, X<sub>101</sub> is a phenylalanine residue or no residue, X<sub>102</sub> is a glycine residue or an aspartate residue, X<sub>103</sub> is a histidine residue or a proline residue, X<sub>104</sub> is a glycine residue or no residue X<sub>105</sub> is a serine residue, a glutamate residue, or no residue X<sub>106</sub> is an arginine residue, a serine residue, or no residue, X<sub>107</sub> is an aspartate residue, an asparagine residue, a serine residue, or a glutamine residue X<sub>108</sub> is a serine residue, an arginine residue, or a tryptophan residue, X<sub>109</sub> is a glycine residue, an aspartate residue, an asparagine residue, a tyrosine residue, or a leucine residue, X<sub>110</sub> is a serine residue, a glycine residue, an aspartate residue, or no residue, X<sub>111</sub> is a serine residue, a valine residue, an asparagine residue, or a tyrosine residue, X<sub>112</sub> is a serine residue, an asparagine residue, a tyrosine residue, or a histidine residue X<sub>113</sub> is a tryptophan residue, a tyrosine residue, or a glutamine residue, X<sub>114</sub> is a histidine residue, an aspartate residue, a tyrosine residue, or no residue, X<sub>115</sub> is a phenylalanine residue, an alanine residue, or a glycine residue, X<sub>116</sub> is an aspartate residue, a phenylalanine residue, a leucine residue, or a methionine residue, X<sub>117</sub> is a tyrosine residue, or an aspartate residue, X<sub>118</sub> is an isoleucine residue, a valine residue, or no residue, and said anti-activin-A compound binds specifically to human activin-A; or

(c) the light chain CDR3 sequence of (a) and the heavy chain CDR3 sequence of (b), and said anti-activin-A compound binds specifically to human activin-A.

In another embodiment, the anti-activin-A compound comprises:

- (a) a light chain variable domain comprising: i. a light chain CDR1 sequence disclosed herein; ii. a light chain CDR2 sequence disclosed herein; and iii. a light chain CDR3 sequence disclosed herein; or
- (b) a heavy chain variable domain comprising: i. a heavy chain CDR1 sequence disclosed herein; ii. a heavy chain CDR2 sequence disclosed herein; and iii. a heavy chain CDR3 sequence disclosed herein; or
- (c) the light chain variable domain of (a) and the heavy chain variable domain of (b).

In another embodiment, the anti-activin-A compound comprises:

- (a) a light chain variable domain sequence selected from the group consisting of: i. a sequence of amino acids at least 80% identical to a light chain variable domain sequence of L1-L14 of a light chain variable domain sequence disclosed herein; ii. a sequence of amino acids encoded by a polynucleotide sequence that is at least 80% identical to a polynucleotide sequence encoding a light chain variable domain sequence of L1-L14 of a light chain variable domain sequence disclosed herein; and iii. a sequence of amino acids encoded by a polynucleotide sequence that hybridizes under moderately stringent conditions to the complement of a polynucleotide consisting of a light chain variable domain sequence of L1-L14 of a light chain variable domain sequence disclosed herein; or

(b) a heavy chain variable domain sequence selected from the group consisting of: i. a sequence of amino acids at least 80% identical to a heavy chain variable domain sequence of H1-H14 of a heavy chain variable domain sequence disclosed herein; ii. a sequence of amino acids encoded by a polynucleotide sequence that is at least 80% identical to a polynucleotide sequence encoding a heavy chain variable domain sequence of H1-H14 of a heavy chain variable domain sequence disclosed herein; and iii. a sequence of amino acids encoded by a polynucleotide sequence that hybridizes under moderately stringent conditions to the complement of a polynucleotide consisting of a heavy chain variable domain sequence of H1-H14 of a heavy chain variable domain sequence disclosed herein; or

(c) the light chain variable domain of (a) and the heavy chain variable domain of (b); wherein said antigen binding protein binds to human activin-A.

In another embodiment, the anti-activin-A compound comprises:

- (a) a light chain variable domain sequence selected from the group consisting of L1-L14 of a light chain variable domain sequence disclosed herein; or
- (b) a heavy chain variable domain sequence selected from the group consisting of H1-H14 of a heavy chain variable domain sequence disclosed herein; or
- (c) the light chain variable domain of (a) and the heavy chain variable domain of (b).

In another embodiment, the anti-activin-A compound comprises a stabilized activin IIB receptor polypeptide (svActRIIB), wherein said polypeptide is selected from the group consisting of:

- (a) a polypeptide comprising a variant of the sequence set forth in SEQ ID NO: 2, wherein said variant sequence comprises an amino acid substitution at position 28, and an amino acid substitution at position 44, wherein the substitution at position 28 is selected from the group consisting of W and Y, and the substitution at position 44 is T;
- (b) a polypeptide comprising a variant of the sequence set forth in amino acids 19 through 134 of SEQ ID NO: 2, wherein said variant sequence comprises an amino acid substitution at position 28, and an amino acid substitution at position 44, wherein the substitution at position 28 is selected from the group consisting of W and Y, and the substitution at position 44 is T;
- (c) a polypeptide comprising a variant of the sequence set forth in amino acids 23 through 134 of SEQ ID NO: 2, wherein said variant sequence comprises an amino acid substitution at position 28, and an amino acid substitution at position 44, wherein the substitution at position 28 is selected from the group consisting of W and Y, and the substitution at position 44 is T;
- (d) a polypeptide comprising a variant of the sequence set forth in amino acids 25 through 134 of SEQ ID NO: 2, wherein said variant sequence comprises an amino acid substitution at position 28, and an amino acid substitution at position 44, wherein the substitution at position 28 is selected from the group consisting of W and Y, and the substitution at position 44 is T; and
- (e) a polypeptide having at least 80% sequence identity to any one of (a) through (d), wherein the sequence comprises an amino acid substitution at position 28, and an amino acid substitution at position 44, wherein the substitution at position 28 is selected from the group consisting of W and Y, and the substitution at

position 44 is T, wherein the polypeptide is capable of binding myostatin, activin-A, or GDF-11.

In another embodiment, the anti-activin-A compound comprises a stabilized activin IIB receptor polypeptide (svActRIIB), wherein said polypeptide is selected from the group consisting of:

- (a) a polypeptide consisting of the sequence set forth in the group consisting of SEQ ID NO: 4, 6, 12 and 14;
- (b) a polypeptide having at least 90% sequence identity to (a), wherein the polypeptide has a W or a Y at position 28 and a T at position 44, wherein the polypeptide is capable of binding myostatin, activin-A, or GDF-11, and
- (c) a polypeptide having at least 95% sequence identity to (a), wherein the polypeptide has a W or a Y at position 28 and a T at position 44, wherein the polypeptide is capable of binding myostatin, activin-A, or GDF-11.

In a further embodiment, the polypeptide is operably linked to at least one heterologous polypeptide. In another embodiment, the polypeptide comprises an alanine residue at position 64. In another embodiment, the heterologous polypeptide comprises an IgG Fc domain. In another embodiment, the heterologous polypeptide is operably linked to the anti-activin-A compound by a linker sequence. In a further embodiment, the linker is selected from the group consisting of: SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 49 and SEQ ID NO: 50.

In another embodiment, the anti-activin-A compound comprises a polypeptide selected from the group consisting of:

- (a) a polypeptide consisting of the sequence set forth in the group consisting of SEQ ID NO: 8, 10, 16 and 18;
- (b) a polypeptide having at least 90% sequence identity to (a), wherein the polypeptide has a W or a Y at position 28 and a T at position 44, wherein the polypeptide is capable of binding myostatin, activin-A, or GDF-11, and
- (c) a polypeptide having at least 95% sequence identity to (a), wherein the polypeptide has a W or a Y at position 28 and a T at position 44, wherein the polypeptide is capable of binding myostatin, activin-A, or GDF-11.

In some embodiments, a method of treating ovarian cancer (including serous ovarian cancer and clear cell ovarian cancer) in a subject comprises administering a therapeutically effective amount of at least two compounds: a first compound and a second compound, wherein the first compound is an anti-activin-A compound, and wherein the second compound is a chemotherapeutic compound. For example, the chemotherapeutic compound can be capecitabine, or a doxorubicin lipid complex. In a further embodiment, one or more of the at least two compounds is formulated with a pharmaceutically-acceptable carrier. In another embodiment, the second compound is administered after the first compound is administered. In another embodiment, the first compound is administered after the second compound is administered. In another embodiment, the first compound and the second compound are administered simultaneously.

In a further embodiment, the subject is identified by detecting elevated levels of biomarker CA-125 and/or activin-A in the subject compared to a control. In another embodiment, the subject is identified by a method comprising: evaluating the subject's expression levels of biomarker CA-125 and/or activin-A; comparing the subject's expression levels of biomarker CA-125 and/or activin-A to expression levels of biomarker CA-125 and/or activin-A in a

negative control sample; and determining that the expression levels of biomarker CA-125 and/or activin-A factors in the subject exceed the expression levels of biomarker CA-125 and/or activin-A in the negative control sample. In another embodiment, the subject is identified by a method comprising: evaluating the subject's expression levels of biomarker CA-125 and/or activin-A; comparing the subject's expression levels of biomarker CA-125 and/or activin-A to expression levels of biomarker CA-125 and/or activin-A in a positive control sample; and determining that the expression levels of biomarker CA-125 and/or activin-A factors in the subject meet or exceed the expression levels of biomarker CA-125 and/or activin-A in the positive control sample.

In another embodiment, the subject is identified by detecting elevated levels of activin-A, VEGF, and/or Ang-1 factors in the subject compared to a control. In another embodiment, the subject is identified by a method comprising: evaluating the subject's expression levels of activin-A, VEGF, and/or Ang-1 factors; comparing the subject's expression levels of activin-A, VEGF, and/or Ang-1 factors to expression levels of activin-A, VEGF, and/or Ang-1 factors in a negative control sample; and determining that the expression levels of activin-A, VEGF, and/or Ang-1 factors in the subject exceed the expression levels of activin-A, VEGF, and/or Ang-1 factors in the negative control sample. In another embodiment, the subject is identified by a method comprising: evaluating the subject's expression levels of activin-A, VEGF, and/or Ang-1 factors; comparing the subject's expression levels of activin-A, VEGF, and/or Ang-1 factors to expression levels of activin-A, VEGF, and/or Ang-1 factors in a positive control sample; and determining that the expression levels of activin-A, VEGF, and/or Ang-1 factors in the subject meet or exceed the expression levels of activin-A, VEGF, and/or Ang-1 factors in the positive control sample.

In another embodiment, the anti-activin-A compound is administered to a subject subcutaneously, intravenously, or intraperitoneally. In a further embodiment, the anti-activin-A compound is administered to a subject once a week at a dosage of at least 0.5 mg/kg. In another embodiment, the capecitabine is administered to a subject subcutaneously, intravenously, intraperitoneally. In a further embodiment, the capecitabine is administered to a subject orally. In a further embodiment, the capecitabine is administered to a subject twice daily for two weeks at a dosage of 1250 mg/m<sup>2</sup>. In some embodiments, there is a one week rest period after the capecitabine is administered for two weeks. In another embodiment, the doxorubicin lipid complex is administered to a subject subcutaneously, intravenously, or intraperitoneally. In another embodiment, the doxorubicin lipid complex is administered to a subject once every four weeks at a dosage of 40 mg/m<sup>2</sup>IV.

#### BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

These and other features, aspects, and advantages of the present invention will become better understood with regard to the following description, and accompanying drawings, where:

FIG. 1 shows activin-A levels in ovarian cancer subjects (OC) and normal control subjects. \*\*\*p<0.001; student's t-test; n=20.

FIG. 2A is a graph showing the effects of sActRIIB treatment on serum activin-A levels in inh-KO mice over time. \*\*\*p<0.001 vs. WT; student's t-test; n=6-12.

FIG. 2B is a bar graph showing the effects of sActRIIB treatment on serum activin-A levels in inh-KO mice over time. In each group of 3 bars, the left bar is wild-type, the middle bar is KO plus PBS, and the right bar is KO plus sActRIIB.

FIG. 3A is a graph showing the effects of sActRIIB treatment on the ovarian tumor mass in inh-KO mice over time. \*\*\*p<0.001 vs. WT. Student's t-test; n=6-12.

FIG. 3B is a representative gross morphology depicting advanced ovarian tumors in inh-KO mice after sActRIIB treatment. Scale bar=5 mm.

FIG. 4A is a graph showing the effects of sActRIIB treatment on the testicular tumor mass in inh-KO mice over time. In each group of 3 bars, the left bar is wild-type, the middle bar is KO plus PBS, and the right bar is KO plus sActRIIB.

FIG. 4B is a representative gross morphology depicting advanced testicular tumors in inh-KO mice after sActRIIB treatment. Scale bar=10 mm.

FIG. 5A shows two Northern blot analyses of activin-A mRNA in the ovarian tumors of inh-KO mice after sActRIIB treatment. n=10

FIG. 5B shows two Western blot analyses of p-Smad2 signaling in the ovarian tumors of inh-KO mice after sActRIIB treatment. n=10

FIG. 6A shows a Western blot analysis of E-cadherin protein in the ovarian tumors of inh-KO mice after sActRIIB treatment. n=10

FIG. 6B shows an immunohistochemical staining of E-cadherin in ovarian sections in inh-KO mice after sActRIIB treatment, where E-cadherin is stained in gray and cell nuclei are counterstained in red. Scale bar=50 µm.

FIG. 7A shows representative H & E microscopic images of ovarian sections in inh-KO mice after sActRIIB treatment. Scale bar=500 µm.

FIG. 7B shows representative H & E microscopic images of testicular tissue sections in inh-KO mice after sActRIIB treatment. Scale bar=500 µm.

FIG. 8A shows two graphs depicting the effects of sActRIIB treatment on serum VEGF in inh-KO mice. \*p<0.05; student's t-test; n=10.

FIG. 8B shows representative images of immunostaining depicting the effects of sActRIIB treatment on VEGF and Ang-1 immunoreactivities in ovarian (top) and testicular (bottom) tumor sections in inh-KO mice. Scale bar=100 µm. \*\*p<0.01; student's t-test. The bar graphs show the quantitative analyses of the VEGF and Ang-1 immunoreactivities.

FIG. 8C shows Northern blot analyses of Ang-2 mRNA expression levels in the ovarian or testicular tumors of inh-KO mice after sActRIIB treatment.

FIG. 8D shows Western blot analyses of endoglin, osteopontin, IGFBP-1 and IGFBP-2 proteins in the ovarian tumors of inh-KO mice after sActRIIB treatment.

FIG. 9 shows representative images of immunostaining depicting the effects of sActRIIB treatment on caspase-3 activation in the ovarian (top) and testicular (bottom) tumors of inh-KO mice. Arrows point to active caspase-3. The bar graphs show the quantitative analyses of the active caspase-3. n=10. \*p<0.05, \*\*p<0.01; student's t-test.

FIG. 10A is a graph showing serum activin-A levels in nude mice after subcutaneous TOV-21G implantation. \*\*p<0.01; student's t-test; n=10.

FIG. 10B is a graph showing the changes in TOV-21G tumor volumes after treatment with sActRIIB or activin-A antibody; \*p<0.05, \*\*\*p<0.001 vs. PBS; n=12.

FIG. 11 shows two graphs depicting either the tumor weight or tumor take rate (defined by the percentage of mice

with visually identifiable tumors in the quadriceps on day 21 post-implantation) after activin-A antibody treatment of CD1 nude mice implanted with naïve or activin-A-transfected CHO cells. \*\*\*p<0.001; one-way ANOVA; n=12.

FIG. 12 shows two graphs depicting either tumor volume or tumor weight after sActRIIB treatment of CD1 nude mice implanted with activin-A-transfected OV-90 cells. \*p<0.05; n=12-13.

FIG. 13 is a graph showing tumor volumes after treatment with sActRIIB and 5-fluorouracil in nude mice implanted with TOV-21G cells. \*\*\*p<0.001 vs. PBS; #p<0.05 vs. 5-FU; ¶p<0.01 vs. sActRIIB; n=12.

FIG. 14 is a graph showing the effects of sActRIIB and activin-A antibody on TOV-21G cell growth.

FIG. 15A shows representative images of immunostaining depicting the effects of sActRIIB treatment on VEGF, Ang-1, and osteopontin in TOV-21G xenograft tumors in mice. The bar graphs show the quantitative analyses of the VEGF, Ang-1, and osteopontin immunoreactivities. \*p<0.05; \*\*p<0.01; \*\*\*p<0.001.

FIG. 15B shows representative images of immunostaining depicting the effects of sActRIIB treatment on CD31 in TOV-21G xenograft tumors in mice. The bar graph shows the quantitative analysis of CD31 immunoreactivity. \*p<0.05; \*\*p<0.01; \*\*\*p<0.001.

FIG. 15C shows representative images of immunostaining depicting the effects of sActRIIB treatment on caspase-3 activation and cell apoptosis in TOV-21G xenograft tumors in mice. The bar graphs show the quantitative analysis of caspase-3 immunoreactivity and immunoreactivity changes due to apoptosis. \*p<0.05; \*\*p<0.01; \*\*\*p<0.001.

FIG. 16A shows graphs of VEGF-A mRNA expression levels in TOV-21G, BAEC, MRC-5, CCD-Lu, and U937 cell cultures after treatment with recombinant activin-A and sActRIIB. \*p<0.05; \*\*p<0.01; \*\*\*p<0.001; student's t-test; n=3.

FIG. 16B shows graphs of Ang-1 mRNA expression levels in BAEC, MRC-5, and CCD-Lu cell cultures after treatment with recombinant activin-A and sActRIIB. \*p<0.05; \*\*p<0.01; \*\*\*p<0.001; student's t-test; n=3.

FIG. 17A shows graphs of VEGF levels in TOV-21G, MRC-5, CCD-Lu, and THP-1 cell cultures after treatment with recombinant activin-A and sActRIIB. \*p<0.05; \*\*p<0.01; \*\*\*p<0.001; student's t-test; n=3.

FIG. 17B shows graphs of Ang-1 levels in MRC-5 and CCD-Lu cell cultures after treatment with recombinant activin-A and sActRIIB. \*p<0.05; \*\*p<0.01; \*\*\*p<0.001; student's t-test; n=3.

FIG. 18 shows graphs of activin-A mRNA expression levels in TOV-21G, BAEC, MRC-5, CCD-Lu, U937, and THP-1 cell cultures after treatment with recombinant activin-A and sActRIIB.

FIG. 19 shows the effects of sActRIIB treatment on the growth of human G361 melanoma xenografts in nude mice, and the effects of activin-A antibody on the growth of 5637 bladder carcinoma xenografts in nude mice. \*p<0.05; \*\*p<0.01.

FIG. 20 shows levels of activin-A transcripts in various cell types, based on analysis of the Oncomine microarray databases.

FIG. 21 shows the effect of sActRIIB single dose, withdrawal, and re-dose on body weight in female Inha KO mice and wild-type littermate control mice. Body weights for female Inha KO mice were plotted as the mean±SEM; \*\*\*p<0.001 for Inha KO groups treated with sActRIIB vs.

PBS.  $\&$  p<0.05 and  $\&\&$  p<0.01 for sActRIIB treated Inh $\alpha$  KO group vs. WT group.  $^{###}$  p<0.001 for PBS-treated Inh $\alpha$  KO group vs. WT group.

FIG. 22 shows a graph of percent survival in female Inh $\alpha$  KO mice and wild-type littermate control mice resulting from a single dose of sActRIIB, where chi square=23.72, P value <0.0001, and the survival curves are significantly different. Survival data were analyzed by chi-square t-test using GraphPad Prism 5.0 Software. p<0.0001: sActRIIB vs. PBS, n=8 to 18.

FIG. 23 shows a bar graph depicting ovarian tumor weights in female Inh $\alpha$  KO mice and wild-type littermate control mice at week 2 after a single dose of sActRIIB. Data were plotted as mean $\pm$ SEM; \*\*p<0.01. Standard 2-tailed Student's t-test (MS Excel 5.0), n=8.

FIG. 24 shows a bar graph depicting ovary tumor weights in female Inh $\alpha$  KO mice and wild-type littermate control mice at week 8 after a single dose of sActRIIB and at week 12 after re-administration (at week 8) of a single dose of sActRIIB. Data were plotted as mean $\pm$ SEM; \*\*\*p<0.001.

FIG. 25 shows a graph of the effect of sActRIIB in combination with doxorubicin on body weight in TOV-21G tumor-bearing mice. Body weight was recorded longitudinally. Data were plotted as mean $\pm$ SEM, n=10 to 18/group. Statistical significance is represented by  $^{aaa}$ p<0.001: TOV-21G+sActRIIB vs. TOV-21G+PBS;  $^{bb}$ p<0.01,  $^{bbb}$ p<0.001: TOV-21G+sActRIIB vs. Normal+PBS;  $^o$ p<0.05 TOV-21G+DOX vs. Normal+PBS;  $^{dd}$ p<0.01: TOV-21G+DOX+sActRIIB vs. Normal+PBS;  $^{eee}$ p<0.001: TOV-21G+DOX+sActRIIB vs. TOV-21G+PBS;  $^f$ p<0.01,  $^{ff}$ p<0.001 TOV-21G+DOX+sActRIIB vs. TOV-21G+DOX+PBS.

FIG. 26 shows the effects of sActRIIB in combination with doxorubicin on tumor size (top) and weight (bottom) in TOV-21G tumor-bearing mice. Tumor size (upper panel) and tumor weight (lower panel) were plotted as the mean $\pm$ standard error of the mean (SEM); n=10 to 18/group. Statistical significance is represented by \*p<0.05, \*\*p<0.01: TOV-21G+DOX+sActRIIB vs. TOV-21G+PBS;  $^p$ p<0.05;  $^{##}$ p<0.01: TOV-21G+DOX vs. TOV-21G+PBS; &p<0.05: TOV-21G+DOX+sActRIIB vs. TOV-21G+DOX.

FIG. 27 shows bar graphs illustrating the effect of sActRIIB in combination with doxorubicin on muscle mass in TOV-21G tumor-bearing mice. Lean carcass and calf muscle weights were determined at terminal necropsy. Data were plotted as mean $\pm$ SEM; n=10 to 18/group. Statistical significance is represented by \*p<0.05; \*\*p<0.01; \*\*\*p<0.001.

FIG. 28 shows a bar graph illustrating the effect of activin-A antibody on serum activin A levels in female Inh $\alpha$  KO mice and wild-type littermate control mice. Measurements of free activin A level in female Inh $\alpha$  KO mouse groups were plotted as the mean $\pm$ SEM; \*\*\*p<0.001 and \*\*p<0.01.

FIG. 29 shows the effect of activin-A antibody treatment on body weight in female Inh $\alpha$  KO mice and wild-type littermate control mice. Measurements of body weight in female Inh $\alpha$  KO mice were plotted as the mean $\pm$ SEM; \*\*p<0.01 and \*\*\*p<0.001 for Inh $\alpha$  KO groups treated with activin-A antibody vs PBS.  $\&$  p<0.05 and  $\&\&$  p<0.01 for activin-A antibody treated Inh $\alpha$  KO group vs PBS treated WT group.  $^{\#}$ p<0.05 for PBS treated Inh $\alpha$  KO group vs PBS treated WT group.

FIG. 30 shows the effect of activin-A antibody treatment on lean body mass and fat mass in female Inh $\alpha$  KO mice and wild-type littermate control mice. Measurements of lean mass (upper panel) and fat mass (lower panel) in female Inh $\alpha$  KO mouse were plotted as the mean $\pm$ SEM; \*\*\*p<0.001 and \*\*p<0.01 for Inh $\alpha$  KO groups treated with

activin-A antibody vs PBS.  $\&\&\&$ p<0.001 for activin-A antibody treated Inh $\alpha$  KO group vs PBS treated WT group.  $^{###}$ p<0.001 for PBS treated Inh $\alpha$  KO group vs PBS treated WT group.

5 FIG. 31 shows a bar graph illustrating the effect of activin-A antibody treatment on calf muscle weight in female Inh $\alpha$  KO mice and wild-type littermate control mice. Calf muscle weights were plotted as mean $\pm$ SEM; \*\*\*p<0.001, \*\*p<0.01, \*p<0.05.

10 FIG. 32A shows the effect of activin-A antibody treatment on ovary weight in female Inh $\alpha$  KO mice and wild-type littermate control mice. Data was plotted as mean $\pm$ SEM; \*\*\*p<0.001, \*\*p<0.01.

15 FIG. 32B shows the effect of activin-A antibody treatment on uterus weight in female Inh $\alpha$  KO mice and wild-type littermate control mice. Data was plotted as mean $\pm$ SEM; \*\*\*p<0.001, \*\*p<0.01.

20 FIG. 33 shows a bar graph illustrating the effect of activin-A antibody treatment on serum VEGF levels in female Inh $\alpha$  KO mice and wild-type littermate control mice. Measurements of serum VEGF level were plotted as the mean $\pm$ SEM; \*\*\*p<0.001, \*p<0.05.

25 FIG. 34 shows the effect of activin-A antibody treatment on VEGF and angiopoietin-1 protein expression levels in ovarian tumor tissues of Inh $\alpha$  KO mice and wild-type littermate control mice. Upper panel: Representative images of VEGF and Ang-1 immunostaining (grayish blue) on ovarian tissue sections. Nuclei were counter stained with Fast Red. Bar graphs: VEGF and Ang-1 immunoreactivities in ovarian sections from 3 animals per group were quantified by imaging with Metamorph software and plotted as the mean $\pm$ SEM. \*\*\*p<0.001 and \*p<0.05

30 FIG. 35 shows the effect of activin-A antibody in combination with doxorubicin on body weight in TOV-21G tumor-bearing mice. Body weight was recorded longitudinally. Arrows point to timings of doxorubicin dosing. Data were plotted as mean $\pm$ SEM; n=8-14 per group. Standard 2-tailed Student's t-test (MS Excel 5.0) was used to analyze the differences between groups. Statistical significance is represented by \*: p<0.05: Normal vs. TOV-21G+PBS; #: p<0.05, ##: p<0.01, ###: p<0.001: Normal vs. TOV-21G+DOX; ^: p<0.05: TOV-21G+PBS vs. TOV-21G+Activin Ab.

35 FIG. 36 shows the effect of activin-A antibody in combination with doxorubicin on tumor size in TOV-21G tumor-bearing mice. Measurements of tumor size were plotted as the mean $\pm$ standard error of the mean (SEM); n=8-14 per group. Standard 2-tailed Student's t-test (MS Excel 5.0) was used to analyze the differences between groups. Statistical significance is represented by \*p<0.05, \*\*p<0.01: TOV-21G+Activin Ab vs. TOV-21G+PBS,  $^{##}$ p<0.01;  $^{###}$ : p<0.001: TOV-21G+DOX vs. TOV-21G+PBS;  $\&\&$ p<0.01;  $\&\&\&$ p<0.001: TOV-21G+DOX+Activin Ab vs. TOV-21G+PBS;  $^{\gamma}$ p<0.05;  $^{yy}$ p<0.01: TOV-21G+DOX+Activin Ab vs. TOV-21G+DOX.

45 FIG. 37 shows the effect of activin-A antibody in combination with doxorubicin on tumor weight in TOV-21G tumor-bearing mice. Measurements of tumor weight were plotted as the mean $\pm$ standard error of the mean (SEM); n=8-14 per group. Standard 2-tailed Student's t-test (MS Excel 5.0) was used to analyze the differences between groups. Statistical significance is represented by \*: p<0.05; \*\*: p<0.01; \*\*\*: p<0.001.

50 FIG. 38 shows the effect of activin-A antibody in combination with doxorubicin on muscle mass in TOV-21G tumor-bearing mice. Lean carcass and calf muscle weights were determined at terminal necropsy procedures. Data were plotted as mean $\pm$ SEM; n=8-14 per group. Standard 2-tailed

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Student's t-test (MS Excel 5.0) was used to analyze the differences between groups. Statistical significance is represented by \*: p<0.05; \*\*: p<0.01; \*\*\*: p<0.001.

## DETAILED DESCRIPTION

The present invention relates to the effects of blocking activin-A. Blocking activin-A in vivo reduces several tumor angiogenesis factors and prevents tumor neovascularization, thereby inducing tumor apoptosis. In some aspects, the invention provides methods for identifying ovarian cancer in a subject by evaluating the subject's expression levels of various factors. In some aspects, the invention also provides methods of treating ovarian cancer, including serous ovarian cancer, by administering anti-activin-A compounds, including anti-activin-A antibodies and activin receptors, to a subject. In some aspects, the invention further provides methods of treating ovarian cancer, including serous ovarian cancer, clear cell ovarian cancer, Granulosa cell ovarian cancer, Leydig cell tumors, and sex cord stromal testicular tumors, by administering at least an anti-activin-A compound and a chemotherapeutic compound to a subject.

The details of one or more embodiments are set forth in the description below. Other features, objects, and advantages will be apparent from the description and the drawings, and from the claims.

Activin-A is the homodimer of the polypeptide chains  $\beta$ A (see GenBank Accession No: NM\_002192). Activins A, B, and AB are the homodimers and heterodimer respectively of two polypeptide chains,  $\beta$ A and  $\beta$ B. The term "activin" refers to activin-A, -B, and -AB, as well as variants and species homologs of that protein.

The present invention provides compositions, kits, and methods relating to molecules that bind to activin-A, including molecules and antigen-binding proteins that agonize or antagonize activin-A, such as activin IIB receptor polypeptides (svActRIIB), svActRIIB fragments, svActRIIB derivatives, anti-activin-A antibodies, antibody fragments, and antibody derivatives, e.g., antagonistic anti-activin-A antibodies, antibody fragments, or antibody derivatives. Also provided are compositions, kits, and methods relating to molecules that specifically bind to a portion of activin-A, such as amino acids R13-Y39, or amino acids V82-N107 of activin-A. Such molecules can include antibodies, antibody fragments, and antibody derivatives. Also provided are nucleic acids, and derivatives and fragments thereof, comprising a sequence of nucleotides that encodes all or a portion of a polypeptide that binds to activin-A, e.g., a nucleic acid encoding all or part of an activin IIB receptor, svActRIIB fragment, svActRIIB derivative, anti-activin-A antibody, antibody fragment, antibody variant, or antibody derivative, plasmids and vectors comprising such nucleic acids, and cells or cell lines comprising such nucleic acids and/or vectors and plasmids. The provided methods include, for example, methods of making, identifying, or isolating molecules that bind to activin-A, such as activin IIB receptors, anti-activin-A antibodies, methods of determining whether a molecule binds to activin-A, methods of making compositions, such as pharmaceutical compositions, comprising a molecule that binds to activin-A, and methods for administering a molecule that binds activin-A to a subject, for example, methods for treating a condition mediated by activin-A, and for modulating a biological activity of activin-A in vivo or in vitro.

The present invention relates to regions of the human activin-A that contain cysteine knot domains recognized by antibodies that also bind to full-length activin-A, and/or a

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region of activin-A that overlaps or encompasses a cysteine knot region of activin-A, and methods of making and using these cysteine knot domains. The invention also provides antigen binding agents, including antibodies, that specifically bind to activin-A or portions of activin-A, and methods for using such binding agents. The binding agents are useful to block or impair binding of human activin-A to one or more ligand.

Activins can interact with two structurally related classes 10 of serine/threonine kinase receptors (type I and type II). Inhibin antagonizes activin by binding to the proteoglycan, betaglycan, and forming a stable complex with and thereby sequestering type II activin receptors while excluding type I receptors. Two major forms of activin exist: activin-A is a 15 homodimer of  $\beta_A$ -subunits and activin B is a homodimer of  $\beta_B$ -subunits. (Vale, et al., *Recent Prog Horm Res* V. 44: 1-34, 1988). Heterodimers of an  $\alpha$ -subunit that is dissimilar to either  $\beta$ -subunit results in the functional antagonist inhibin.

The literature has shown that activin-A is overexpressed 20 and/or localized in cancer tissues. For example, high levels of serum activin-A were found in women with endometrial and cervical carcinoma (Petruglia, F. et al., *Jour. Clin. Endocrin. Metab.* 83:1194-1200, 1998). Activin-A was overexpressed in stage IV colorectal cancer (Wildi, S. et al., *Gut* 49:409-417, 2001). A role of activin-A in ovarian cancer was reported (Steller, M. D. et al., *Mol. Cancer Res.* 3:50-61, 2005).

The literature has also implicated activin-A in renal disease. (Yamashita, S. et al. *J. Am. Soc. Nephrol.* 15:91-101, 2004.) Serum immunoreactive activin-A levels in normal subjects and patients with disease were reported by Harada, K. et al. in *J. Clin. Endocrin. and Metab.* 81:2125-2130, 1996. Activin-A is a potent activator of renal interstitial fibroblasts (Harada, K. et al., *J. Am. Soc. Nephrol.* 15:91-101, 2004). Glomerular activin-A overexpression is linked to fibrosis in anti-Thy 1 glomerulonephritis (Gaedke, J. et al., *Neph. Dial. Transpl.* 20:319-328, 2005).

Serum activin-A levels in heart failure patients increased according to disease severity (Yndestad et al., *Circulation* 109:1379-1385, 2004). In a rat model of heart failure, serum activin-A elevated immediately after myocardial infarct and persisted for six months, and activin-A immunostaining was localized solely to cardiomyocytes (Yndestad et al., 2004). Elevated levels of activin-A were reported in heart failure (Yndestad, A. et al., *Circulation* 109:1379-1385, 2004).

Unless otherwise defined herein, scientific and technical terms used in connection with the present invention shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. Generally, nomenclatures used in connection with, and techniques of, cell and tissue culture, molecular biology, immunology, microbiology, genetics and protein and nucleic acid chemistry and hybridization described herein are those well known and commonly used in the art. The methods and techniques of the present invention are generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification unless otherwise indicated. See, e.g., Sambrook et al. Molecular Cloning: A Laboratory Manual, 2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989) and Ausubel et al., Current Protocols in Molecular Biology, Greene Publishing Associates (1992), and Harlow and Lane Antibodies: A Laboratory Manual Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1990), which are

incorporated herein by reference. Enzymatic reactions and purification techniques are performed according to manufacturer's specifications, as commonly accomplished in the art or as described herein. The terminology used in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well known and commonly used in the art. Standard techniques can be used for chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, and delivery, and treatment of patients.

The following terms, unless otherwise indicated, shall be understood to have the following meanings:

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

The terms "anti-activin-A compound", "activin-A inhibitor" and "activin-A antagonist" are used interchangeably. Each is a molecule that detectably inhibits at least one function of activin-A. Conversely, an "activin-A agonist" is a molecule that detectably increases at least one function of activin-A. The inhibition caused by an activin-A inhibitor need not be complete so long as it is detectable using an assay. Any assay of a function of activin-A can be used, examples of which are provided herein. Examples of functions of activin-A that can be inhibited by an activin-A inhibitor, or increased by an activin-A agonist, include binding to activin-A. Examples of types of activin-A inhibitors and activin-A agonists include, but are not limited to, activin-A binding polypeptides such as antigen binding proteins (e.g., activin-A inhibiting antigen binding proteins), activin IIB receptors (svActRIIB), svActRIIB fragments, svActRIIB derivatives, antibodies, antibody fragments, and antibody derivatives.

The terms "peptide," "polypeptide" and "protein" each refers to a molecule comprising two or more amino acid residues joined to each other by peptide bonds. These terms encompass, e.g., native and artificial proteins, protein fragments and polypeptide analogs (such as muteins, variants, and fusion proteins) of a protein sequence as well as post-translationally, or otherwise covalently or non-covalently, modified proteins. A peptide, polypeptide, or protein may be monomeric or polymeric. Polynucleotide and polypeptide sequences are indicated using standard one- or three-letter abbreviations. Unless otherwise indicated, polypeptide sequences have their amino termini at the left and their carboxy termini at the right, and single-stranded nucleic acid sequences, and the top strand of double-stranded nucleic acid sequences, have their 5' termini at the

left and their 3' termini at the right. A particular polypeptide or polynucleotide sequence also can be described by explaining how it differs from a reference sequence. Unless otherwise indicated, it is understood that polynucleotide and polypeptide sequences include each nucleic acid or amino acid listed, respectively, as well as the intervening nucleic acids or amino acids. For example, the polypeptide sequence R13-Y39 sets forth a polypeptide sequence that includes the amino acids R13, and Y39, as well as the amino acids found between R13 and Y39 in the polypeptide sequence. Correspondingly, the polynucleotide sequence C1-T5 sets forth a polynucleotide sequence that includes nucleic acids C1, and T5, as well as nucleic acids at positions 2, 3, and 4 of the sequence. Accordingly, designations of SEQ ID NO: 1-5 likewise designates the inclusive group of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, and SEQ ID NO: 5. Finally, amino acid groupings are also intended to be inclusive, unless otherwise designated. For example, the phrase "amino acids 1-5 of SEQ ID NO: 28" includes amino acids at positions 1, 2, 3, 4, and 5 of SEQ ID NO: 28.

Polypeptides of the invention include polypeptides that have been modified in any way and for any reason, for example, to: (1) reduce susceptibility to proteolysis, (2) reduce susceptibility to oxidation, (3) alter binding affinity for forming protein complexes, (4) alter binding affinities, and (4) confer or modify other physicochemical or functional properties. Analogs include muteins of a polypeptide. For example, single or multiple amino acid substitutions (e.g., conservative amino acid substitutions) may be made in the naturally occurring sequence (e.g., in the portion of the polypeptide outside the domain(s) forming intermolecular contacts). A "conservative amino acid substitution" is one that does not substantially change the structural characteristics of the parent sequence (e.g., a replacement amino acid should not tend to break a helix that occurs in the parent sequence, or disrupt other types of secondary structure that characterize the parent sequence or are necessary for its functionality). Examples of art-recognized polypeptide secondary and tertiary structures are described in Proteins, Structures and Molecular Principles (Creighton, Ed., W. H. Freeman and Company, New York (1984)); Introduction to Protein Structure (C. Branden and J. Tooze, eds., Garland Publishing, New York, N.Y. (1991)); and Thornton et al. Nature 354:105 (1991), which are each incorporated herein by reference.

The term "polypeptide fragment" as used herein refers to a polypeptide that has an amino-terminal and/or carboxy-terminal deletion as compared to a corresponding full-length protein. Fragments can be, for example, at least 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 20, 50, 70, 80, 90, 100, 150 or 200 amino acids in length. Fragments can also be, for example, at most 1,000, 750, 500, 250, 200, 175, 150, 125, 100, 90, 80, 70, 60, 50, 40, 30, 20, 15, 14, 13, 12, 11, or 10 amino acids in length. A fragment can further comprise, at either or both of its ends, one or more additional amino acids, for example, a sequence of amino acids from a different naturally-occurring protein (e.g., an Fc or leucine zipper domain) or an artificial amino acid sequence (e.g., an artificial linker sequence).

A "variant" of a polypeptide (e.g., an antibody) comprises an amino acid sequence wherein one or more amino acid residues are inserted into, deleted from and/or substituted into the amino acid sequence relative to the native polypeptide sequence, and retains essentially the same biological activity as the native polypeptide. The biological activity of the polypeptide can be measured using standard techniques in the art (for example, if the variant is an antibody, its

activity may be tested by binding assays, as described herein). Variants of the invention include fragments, analogs, recombinant polypeptides, synthetic polypeptides, and/or fusion proteins. A "derivative" of a polypeptide is a polypeptide (e.g., an antibody) that has been chemically modified, e.g., via conjugation to another chemical moiety such as, for example, polyethylene glycol, albumin (e.g., human serum albumin), phosphorylation, and glycosylation. Unless otherwise indicated, the term "antibody" includes, in addition to antibodies comprising two full-length heavy chains and two full-length light chains, derivatives, variants, fragments, and muteins thereof, examples of which are described below.

The terms "polynucleotide," "oligonucleotide" and "nucleic acid" are used interchangeably throughout and include DNA molecules (e.g., cDNA or genomic DNA), RNA molecules (e.g., mRNA), analogs of the DNA or RNA generated using nucleotide analogs (e.g., peptide nucleic acids and non-naturally occurring nucleotide analogs), and hybrids thereof. The nucleic acid molecule can be single-stranded or double-stranded. In one embodiment, the nucleic acid molecules of the invention comprise a contiguous open reading frame encoding an antibody, or a fragment, derivative, mutein, or variant thereof, of the invention.

The term percent "identity," in the context of two or more nucleic acid or polypeptide sequences, refer to two or more sequences or subsequences that have a specified percentage of nucleotides or amino acid residues that are the same, when compared and aligned for maximum correspondence, determined by comparing the sequences using the GAP computer program (a part of the GCG Wisconsin Package, version 10.3 (Accelrys, San Diego, CA)) using its default parameters. Depending on the application, the percent "identity" can exist over a region of the sequence being compared, e.g., over a functional domain, or, alternatively, exist over the full length of the two sequences to be compared.

For sequence comparison, typically one sequence acts as a reference sequence to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are input into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. The sequence comparison algorithm then calculates the percent sequence identity for the test sequence(s) relative to the reference sequence, based on the designated program parameters.

Two single-stranded polynucleotides are "the complement" of each other if their sequences can be aligned in an anti-parallel orientation such that every nucleotide in one polynucleotide is opposite its complementary nucleotide in the other polynucleotide, without the introduction of gaps, and without unpaired nucleotides at the 5' or the 3' end of either sequence. A polynucleotide is "complementary" to another polynucleotide if the two polynucleotides can hybridize to one another under moderately stringent conditions. Thus, a polynucleotide can be complementary to another polynucleotide without being its complement.

A "vector" is a nucleic acid that can be used to introduce another nucleic acid linked to it into a cell. One type of vector is a "plasmid," which refers to a linear or circular double stranded DNA molecule into which additional nucleic acid segments can be ligated. Another type of vector is a viral vector (e.g., replication defective retroviruses, adenoviruses and adeno-associated viruses), wherein additional DNA segments can be introduced into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors comprising a bacterial origin of replication

and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. An "expression vector" is a type of vector that can direct the expression of a chosen polynucleotide.

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

A "host cell" is a cell that can be used to express a nucleic acid, e.g., a nucleic acid of the invention. A host cell can be a prokaryote, for example, *E. coli*, or it can be a eukaryote, for example, a single-celled eukaryote (e.g., a yeast or other fungus), a plant cell (e.g., a tobacco or tomato plant cell), an animal cell (e.g., a human cell, a monkey cell, a hamster cell, a rat cell, a mouse cell, or an insect cell) or a hybridoma. Examples of host cells include CS-9 cells, the COS-7 line of monkey kidney cells (ATCC CRL 1651) (see Gluzman et al., 1981, *Cell* 23:175), L cells, C127 cells, 3T3 cells (ATCC CCL 163), Chinese hamster ovary (CHO) cells or their derivatives such as Veggie CHO and related cell lines which grow in serum-free media (see Rasmussen et al., 1998, *Cytotechnology* 28:31), HeLa cells, BHK (ATCC CRL 10) cell lines, the CV1/EBNA cell line derived from the African green monkey kidney cell line CV1 (ATCC CCL 70) (see McMahan et al., 1991, *EMBO J.* 10:2821), human embryonic kidney cells such as 293, 293 EBNA or MSR 293, human epidermal A431 cells, human Colo205 cells, other transformed primate cell lines, normal diploid cells, cell strains derived from in vitro culture of primary tissue, primary explants, HL-60, U937, HaK or Jurkat cells. Typically, a host cell is a cultured cell that can be transformed or transfected with a polypeptide-encoding nucleic acid, which can then be expressed in the host cell. The phrase "recombinant host cell" can be used to denote a host cell that has been transformed or transfected with a nucleic acid to be expressed. A host cell also can be a cell that comprises the nucleic acid but does not express it at a desired level unless a regulatory sequence is introduced into the host cell such that it becomes operably linked with the nucleic acid. It is understood that the term host cell refers not only to the particular subject cell but to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to, e.g., mutation or environmental influence, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

#### Nucleic Acids

In one aspect, the present invention provides isolated nucleic acid molecules. The nucleic acids comprise, for example, polynucleotides that encode all or part of an antigen binding protein, for example, one or both chains of an antibody of the invention, or a fragment, derivative,

mutein, or variant thereof, polynucleotides sufficient for use as hybridization probes, PCR primers or sequencing primers for identifying, analyzing, mutating or amplifying a polynucleotide encoding a polypeptide, anti-sense nucleic acids for inhibiting expression of a polynucleotide, and complementary sequences of the foregoing. The nucleic acids can be any length. They can be, for example, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 75, 100, 125, 150, 175, 200, 250, 300, 350, 400, 450, 500, 750, 1,000, 1,500, 3,000, 5,000 or more nucleotides in length, and/or can comprise one or more additional sequences, for example, regulatory sequences, and/or be part of a larger nucleic acid, for example, a vector. The nucleic acids can be single-stranded or double-stranded and can comprise RNA and/or DNA nucleotides, and artificial variants thereof (e.g., peptide nucleic acids).

Nucleic acids encoding antibody polypeptides (e.g., heavy or light chain, variable domain only, or full length) can be isolated from B-cells of mice that have been immunized with activin-A. The nucleic acid can be isolated by conventional procedures such as polymerase chain reaction (PCR).

Nucleic acid sequences encoding the variable regions of the heavy and light chain variable regions are shown herein. The skilled artisan will appreciate that, due to the degeneracy of the genetic code, each of the polypeptide sequences disclosed herein is encoded by a large number of other nucleic acid sequences. The present invention provides each degenerate nucleotide sequence encoding each antigen binding protein of the invention.

The invention further provides nucleic acids that hybridize to other nucleic acids (e.g., nucleic acids comprising a nucleotide sequence of any of A1-A14) under particular hybridization conditions. Methods for hybridizing nucleic acids are well-known in the art. See, e.g., *Curr. Prot. in Mol. Biol.*, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. As defined herein, a moderately stringent hybridization condition uses a prewashing solution containing 5× sodium chloride/sodium citrate (SSC), 0.5% SDS, 1.0 mM EDTA (pH 8.0), hybridization buffer of about 50% formamide, 6×SSC, and a hybridization temperature of 55° C. (or other similar hybridization solutions, such as one containing about 50% formamide, with a hybridization temperature of 42° C.), and washing conditions of 60° C., in 0.5×SSC, 0.1% SDS. A stringent hybridization condition hybridizes in 6×SSC at 45° C., followed by one or more washes in 0.1×SSC, 0.2% SDS at 68° C. Furthermore, one of skill in the art can manipulate the hybridization and/or washing conditions to increase or decrease the stringency of hybridization such that nucleic acids comprising nucleotide sequences that are at least 65, 70, 75, 80, 85, 90, 95, 98, or 99% identical to each other typically remain hybridized to each other. The basic parameters affecting the choice of hybridization conditions and guidance for devising suitable conditions are set forth by, for example, Sambrook, Fritsch, and Maniatis (1989, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., chapters 9 and 11; and *Curr. Prot. in Mol. Biol.* 1995, Ausubel et al., eds., John Wiley & Sons, Inc., sections 2.10 and 6.3-6.4), and can be readily determined by those having ordinary skill in the art based on, for example, the length and/or base composition of the DNA.

Changes can be introduced by mutation into a nucleic acid, thereby leading to changes in the amino acid sequence of a polypeptide (e.g., an antigen binding protein) that it encodes. Mutations can be introduced using any technique known in the art. In one embodiment, one or more particular amino acid residues are changed using, for example,

site-directed mutagenesis protocol. In another embodiment, one or more randomly selected residues are changed using, for example, a random mutagenesis protocol. However it is made, a mutant polypeptide can be expressed and screened for a desired property (e.g., binding to activin-A).

Mutations can be introduced into a nucleic acid without significantly altering the biological activity of a polypeptide that it encodes. For example, one can make nucleotide substitutions leading to amino acid substitutions at non-<sup>10</sup> essential amino acid residues. In one embodiment, a nucleotide sequence provided herein for A1-A14, or a desired fragment, variant, or derivative thereof, is mutated such that it encodes an amino acid sequence comprising one or more deletions or substitutions of amino acid residues that are shown herein for A1-A14 to be residues where two or more sequences differ. As described herein *inter alia*, A1-A14 refers to 14 sequences, A1, and A14, as well as the 12 intervening amino acid residues. In another embodiment, the mutagenesis inserts an amino acid adjacent to one or more amino acid residues shown herein for A1-A14 to be residues where two or more sequences differ. Alternatively, one or more mutations can be introduced into a nucleic acid that selectively change the biological activity (e.g., binding of activin-A) of a polypeptide that it encodes. For example, the mutation can quantitatively or qualitatively change the biological activity. Examples of quantitative changes include increasing, reducing or eliminating the activity. Examples of qualitative changes include changing the antigen specificity of an antigen binding protein.

In another aspect, the present invention provides nucleic acid molecules that are suitable for use as primers or hybridization probes for the detection of nucleic acid sequences of the invention. A nucleic acid molecule of the invention can comprise only a portion of a nucleic acid sequence encoding a full-length polypeptide of the invention, for example, a fragment that can be used as a probe or primer or a fragment encoding an active portion (e.g., an activin-A binding portion) of a polypeptide of the invention.

Probes based on the sequence of a nucleic acid of the invention can be used to detect the nucleic acid or similar nucleic acids, for example, transcripts encoding a polypeptide of the invention. The probe can comprise a label group, e.g., a radioisotope, a fluorescent compound, an enzyme, or an enzyme co-factor. Such probes can be used to identify a cell that expresses the polypeptide.

#### Expression Vectors

The present invention provides vectors comprising a nucleic acid encoding a polypeptide of the invention or a portion thereof. Examples of vectors include, but are not limited to, plasmids, viral vectors, non-episomal mammalian vectors and expression vectors, for example, recombinant expression vectors.

In another aspect of the present invention, expression vectors containing the nucleic acid molecules and polynucleotides of the present invention are also provided, and host cells transformed with such vectors, and methods of producing the polypeptides are also provided. The term "expression vector" refers to a plasmid, phage, virus or vector for expressing a polypeptide from a polynucleotide sequence. Vectors for the expression of the polypeptides contain at a minimum sequences required for vector propagation and for expression of the cloned insert. An expression vector comprises a transcriptional unit comprising an assembly of (1) a genetic element or elements having a regulatory role in gene expression, for example, promoters or enhancers, (2) a sequence that encodes polypeptides and proteins to be transcribed into mRNA and translated into protein, and

(3) appropriate transcription initiation and termination sequences. These sequences may further include a selection marker. Vectors suitable for expression in host cells are readily available and the nucleic acid molecules are inserted into the vectors using standard recombinant DNA techniques. Such vectors can include promoters which function in specific tissues, and viral vectors for the expression of polypeptides in targeted human or animal cells. For example, an expression vector suitable for expression of svActRIIB is the pDSRa, (described in WO 90/14363, herein incorporated by reference) and its derivatives, containing svActRIIB polynucleotides, as well as any additional suitable vectors known in the art.

The recombinant expression vectors of the invention can comprise a nucleic acid of the invention in a form suitable for expression of the nucleic acid in a host cell. The recombinant expression vectors include one or more regulatory sequences, selected on the basis of the host cells to be used for expression, which is operably linked to the nucleic acid sequence to be expressed. Regulatory sequences include those that direct constitutive expression of a nucleotide sequence in many types of host cells (e.g., SV40 early gene enhancer, Rous sarcoma virus promoter and cytomegalovirus promoter), those that direct expression of the nucleotide sequence only in certain host cells (e.g., tissue-specific regulatory sequences, see Voss et al., 1986, *Trends Biochem. Sci.* 11:287, Maniatis et al., 1987, *Science* 236:1237, incorporated by reference herein in their entireties), and those that direct inducible expression of a nucleotide sequence in response to particular treatment or condition (e.g., the metallothionein promoter in mammalian cells and the tet-responsive and/or streptomycin responsive promoter in both prokaryotic and eukaryotic systems (see id.). It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, etc. The expression vectors of the invention can be introduced into host cells to thereby produce proteins or peptides, including fusion proteins or peptides, encoded by nucleic acids as described herein.

The invention further provides methods of making polypeptides. A variety of other expression/host systems may be utilized. Vector DNA can be introduced into prokaryotic or eukaryotic systems via conventional transformation or transfection techniques. These systems include but are not limited to microorganisms such as bacteria (for example, *E. coli*) transformed with recombinant bacteriophage, plasmid or cosmid DNA expression vectors; yeast transformed with yeast expression vectors; insect cell systems infected with virus expression vectors (e.g., baculovirus); plant cell systems transfected with virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with bacterial expression vectors (e.g., Ti or pBR322 plasmid); or animal cell systems. Mammalian cells useful in recombinant protein production include but are not limited to VERO cells, HeLa cells, Chinese hamster ovary (CHO) cell lines, or their derivatives such as Veggie CHO and related cell lines which grow in serum-free media (see Rasmussen et al., 1998, *Cytotechnology* 28:31) or CHO strain DX-B11, which is deficient in DHFR (see Urlaub et al., 1980, *Proc. Natl. Acad. Sci. USA* 77:4216-20) COS cells such as the COS-7 line of monkey kidney cells (ATCC CRL 1651) (see Gluzman et al., 1981, *Cell* 23:175), W138, BHK, HepG2, 3T3 (ATCC CCL 163), RIN, MDCK, A549, PC12, K562, L cells, C127 cells, BHK (ATCC CRL 10) cell lines, the CV1/EBNA cell line derived from the African green monkey kidney cell line CV1 (ATCC CCL 70) (see McMa-

han et al., 1991, *EMBO J.* 10:2821), human embryonic kidney cells such as 293, 293 EBNA or MSR 293, human epidermal A431 cells, human Colo205 cells, other transformed primate cell lines, normal diploid cells, cell strains derived from in vitro culture of primary tissue, primary explants, HL-60, U937, HaK or Jurkat cells. Mammalian expression allows for the production of secreted or soluble polypeptides which may be recovered from the growth medium.

For stable transfection of mammalian cells, it is known that, depending upon the expression vector and transfection technique used, only a small fraction of cells may integrate the foreign DNA into their genome. In order to identify and select these integrants, a gene that encodes a selectable marker (e.g., for resistance to antibiotics) is generally introduced into the host cells along with the gene of interest. Once such cells are transformed with vectors that contain selectable markers as well as the desired expression cassette, the cells can be allowed to grow in an enriched media before they are switched to selective media, for example. The selectable marker is designed to allow growth and recovery of cells that successfully express the introduced sequences. Resistant clumps of stably transformed cells can be proliferated using tissue culture techniques appropriate to the cell line employed. An overview of expression of recombinant proteins is found in *Methods of Enzymology*, v. 185, Goeddel, D. V., ed., Academic Press (1990). Preferred selectable markers include those which confer resistance to drugs, such as G418, hygromycin and methotrexate. Cells stably transfected with the introduced nucleic acid can be identified by drug selection (e.g., cells that have incorporated the selectable marker gene will survive, while the other cells die), among other methods.

In some cases, such as in expression using prokaryotic systems, the expressed polypeptides of this invention may need to be "refolded" and oxidized into a proper tertiary structure and disulfide linkages generated in order to be biologically active. Refolding can be accomplished using a number of procedures well known in the art. Such methods include, for example, exposing the solubilized polypeptide to a pH usually above 7 in the presence of a chaotropic agent. The selection of chaotrope is similar to the choices used for inclusion body solubilization; however a chaotrope is typically used at a lower concentration. Exemplary chaotropic agents are guanidine and urea. In most cases, the refolding/oxidation solution will also contain a reducing agent plus its oxidized form in a specific ratio to generate a particular redox potential which allows for disulfide shuffling to occur for the formation of cysteine bridges. Some commonly used redox couples include cysteine/cystamine, glutathione/dithiobisGSH, cupric chloride, dithiothreitol DTT/dithiane DTT, and 2-mercaptoethanol (bME)/dithiobME. In many instances, a co-solvent may be used to increase the efficiency of the refolding. Commonly used cosolvents include glycerol, polyethylene glycol of various molecular weights, and arginine.

In addition, the polypeptides can be synthesized in solution or on a solid support in accordance with conventional techniques. Various automatic synthesizers are commercially available and can be used in accordance with known protocols. See, for example, Stewart and Young, *Solid Phase Peptide Synthesis*, 2d. Ed., Pierce Chemical Co. (1984); Tam et al., *J Am Chem Soc*, 105:6442, (1983); Merrifield, *Science* 232:341-347 (1986); Barany and Merrifield, *The Peptides*, Gross and Meienhofer, eds, Academic Press, New York, 1-284; Barany et al., *Int J Pep Protein Res*, 30:705-739 (1987).

The polypeptides and proteins of the present invention can be purified according to protein purification techniques are well known to those of skill in the art. These techniques involve, at one level, the crude fractionation of the proteinaceous and non-proteinaceous fractions. Having separated the peptide polypeptides from other proteins, the peptide or polypeptide of interest can be further purified using chromatographic and electrophoretic techniques to achieve partial or complete purification (or purification to homogeneity). The term "purified polypeptide" as used herein, is intended to refer to a composition, isolatable from other components, wherein the polypeptide is purified to any degree relative to its naturally-obtainable state. A purified polypeptide therefore also refers to a polypeptide that is free from the environment in which it may naturally occur. Generally, "purified" will refer to a polypeptide composition that has been subjected to fractionation to remove various other components, and which composition substantially retains its expressed biological activity. Where the term "substantially purified" is used, this designation will refer to a peptide or polypeptide composition in which the polypeptide or peptide forms the major component of the composition, such as constituting about 50%, about 60%, about 70%, about 80%, about 85%, or about 90% or more of the proteins in the composition.

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

Various methods for quantifying the degree of purification of polypeptide will be known to those of skill in the art in light of the present disclosure. These include, for example, determining the specific binding activity of an active fraction, or assessing the amount of peptide or polypeptide within a fraction by SDS/PAGE analysis. A preferred method for assessing the purity of a polypeptide fraction is to calculate the binding activity of the fraction, to compare it to the binding activity of the initial extract, and to thus calculate the degree of purification, herein assessed by a "fold purification number." The actual units used to represent the amount of binding activity will, of course, be dependent upon the particular assay technique chosen to follow the purification and whether or not the polypeptide or peptide exhibits a detectable binding activity.

#### Anti-Activin-A Antibody

Activin-A can be purified from host cells that have been transfected by a gene encoding activin-A by elution of filtered supernatant of host cell culture fluid using a Heparin HP column, using a salt gradient.

The term "antibody" refers to an intact immunoglobulin, or a binding fragment thereof. An antibody may comprise a complete antibody molecule (including polyclonal, monoclonal, chimeric, humanized, or human versions having full length heavy and/or light chains), or comprise an antigen binding fragment thereof. Antibody fragments include F(ab')<sub>2</sub>, Fab, Fab', Fv, Fc, and Fd fragments, and can be

incorporated into single domain antibodies, single-chain antibodies, maxibodies, minibodies, intrabodies, diabodies, triabodies, tetrabodies, v-NAR and bis-scFv (See e.g., Hollinger and Hudson, 2005, *Nature Biotech.*, 23, 9, 1126-1136).

A Fab fragment is a monovalent fragment having the V<sub>L</sub>, V<sub>H</sub>, C<sub>L</sub> and C<sub>H1</sub> domains; a F(ab')<sub>2</sub> fragment is a bivalent fragment having two Fab fragments linked by a disulfide bridge at the hinge region; a Fd fragment has the V<sub>H</sub> and C<sub>H1</sub> domains; an Fv fragment has the V<sub>L</sub> and V<sub>H</sub> domains of a single arm of an antibody; and a dAb fragment has a V<sub>H</sub> domain, a V<sub>L</sub> domain, or an antigen-binding fragment of a V<sub>H</sub> or V<sub>L</sub> domain (U.S. Pat. Nos. 6,846,634, 6,696,245, US App. Pub. No. 05/0202512, 04/0202995, 04/0038291, 04/0009507, 03/0039958, Ward et al., *Nature* 341:544-546, 1989).

Polynucleotide and polypeptide sequences of particular light and heavy chain variable domains are described below. Antibodies comprising a light chain and heavy chain are designated by combining the name of the light chain and the name of the heavy chain variable domains. For example, "L4H7," indicates an antibody comprising the light chain variable domain of L4 and the heavy chain variable domain of H7.

Kappa light chain constant sequences are shown in SEQ ID NO: 84, 100 and 108, and heavy chain constant sequence are shown in SEQ ID NOs: 214, 215 and 221. Polynucleotides encoding these sequences are shown in, for the light chains, respectively, SEQ ID NOs: 222, 223 and 239, and for the heavy chains, respectively, SEQ ID NOs: 240, 241, and 242. Thus, in addition to the variable sequences as disclosed herein, an antibody can comprise one or both of SEQ ID NOs: 84 and 214; or SEQ ID NOs: 215 and 223; or SEQ ID NOs: 108 and 221. These sequences are illustrated in the table below:

SEQ ID NO	Sequence
SEQ ID NO: 84	Gly Gln Pro Lys Ala Ala Pro Ser Val Thr Leu Phe Pro Pro Ser Ser Glu Glu Leu Gln Ala Asn Lys Ala Thr Leu Val Cys Leu Ile Ser Asp Phe Tyr Pro Gly Ala Val Thr Val Ala Trp Lys Ala Asp Ser Ser Pro Val Lys Ala Gly Val Glu Thr Thr Thr Pro Ser Lys Gln Ser Asn Asn Lys Tyr Ala Ala Ser Ser Tyr Leu Ser Leu Thr Pro Glu Gln Trp Lys Ser His Arg Ser Tyr Ser Cys Gln Val Thr His Glu Gly Ser Thr Val Glu Lys Thr Val Ala Pro Thr Glu Cys Ser
SEQ ID NO: 100	Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
SEQ ID NO: 108	Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys

-continued

SEQ ID NO	Sequence
SEQ ID NO: 214	Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Val Val Thr Val Pro Ser Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys Thr Val Glu Arg Lys Cys Cys Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Val Val Ser Val Leu Thr Val Val His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
SEQ ID NO: 215	Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys Thr Val Glu Arg Lys Cys Cys Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Val Val Ser Val Leu Thr Val Val His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
SEQ ID NO: 221	Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr

-continued

SEQ ID NO	Sequence
5	Val Pro Ser Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys Thr Val Glu Arg Lys Cys Cys Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Val Val Ser Val Leu Thr Val Val His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
10	
15	
20	
25	
30	ggtcagccca aggctgcccc ctcggtaact ctgtttccgc cctccctcta ggagcttcaa gccaacaagg ccacacttgt gtgtctcata agtacttct acccgggagc cgtgacagtg gcctggaagg cagatagcag ccccgtaag gcccggatgg agaccacac accctccaa caaagcaaca acaagtacgc ggccacgc tatctgagcc tgacgcctga gcagtggaaag tcccacagaa gctacagctg ccaggtcactg catgaaggaa gcacctgtga gaagacagtg gcccctacag aatgttca
35	cgaaactgtgg ctgcaccatc tgcatttcata ttcccccatt ctgatgagca gttgaaatct ggaactgcct ctgttgcgtg cctgtctaat aacttctatc ccagagggc caaagtacag tggaaagggtt ataacgcctt ccaatcggtt aactccagg agagtgtcac agagcaggac agcaaggaca gcacccatc cctcagcagc accctgacgc tgagcaaaagc agactacag aaacacaaaat tctacgcctg cgaagtacacc catcaggggcc tgagctcgcc cgtcacaaaa agttcaaca aaaaaaaa
40	
45	
50	cgaaactgtgg ctgcaccatc tgcatttcata ttcccccatt ctgatgagca gttgaaatct ggaactgcct ctgttgcgtg cctgtctaat aacttctatc ccagagggc caaagtacag tggaaagggtt ataacgcctt ccaatcggtt aactccagg agagtgtcac agagcaggac agcaaggaca gcacccatc cctcagcagc accctgacgc tgagcaaaagc agactacag aaacacaaaat tctacgcctg cgaagtacacc catcaggggcc tgagctcgcc cgtcacaaaa agttcaaca aaaaaaaa
55	gcctccacca agggccatc ggtttcccc ctggccct gtccaggag caccccgag agcacagccg ccctggctg cctggtaag gactactcc cccaacccgt gacgggttgtcg tggaaactcg gctgtctgac cagccggctg cacacccctc cagctgtctt acgtctctt ccgttccatc cgttccatc cgtggtaacc gttccatc cgttccatc cgttccatc tacacccgtca acgttagatca caagccaggc aacaccaagg tggacaaggc agttccatc aatgttgcgtt cggatgtccc acgttccatc cgttccatc cgttccatc cgttccatc cttccatc cttccatc cttccatc cttccatc ggaccctgtt ggttccatc cttccatc cttccatc cgttccatc cttccatc cttccatc cttccatc cgttccatc cttccatc cttccatc cttccatc gtggagggtc ataatgcacca gacaaaggcc cggggaggaggc agttcaacac agttccatc cgttccatc cttccatc tggatgtccc gacttccatc acggccatc gtacaatgtcc aagggttccatc aacaaaggcc cccagcccc atcgagaaaa ccatctccaa aaccaaaaggc cagcccccgg aaccacagg gtacacccctg ccccccattcc gggaggat gaccaagaac cgggttccatc tgacttccatc ggttccatc ttctccatc cgttccatc cttccatc cttccatc cttccatc
60	
65	

-continued

SEQ ID NO	Sequence
	gaacaactac aagaccacac ctccccatgt ggactccgaa ggctcttct tcctctacag caagctcacc gtggacaaga gcagggtggca gcaggggaaac gtcttcat gtcccgat gcatgaggct ctgcacaacc actacacgca gaagagcctc tccctgtctc cggtaaa
SEQ ID NO: 241	gcctccacca agggccatc ggtttcccc ctggccctc gtccaggag caccctcgag agcacagcgcc ctctggctg cctggtaag gactacttc cccaacccgt gacgggtgtcg tggaaactcg ggcgtctgac cagcggcggtg cacacccccc cagctgtctc acagtctca gggacttact ccctcagcag cgttgtgacc gtgccttca gcaacttcgg caccggac tacacctgca acgttagatca caagcccgaa aacaccaagg tggacaagac agttggccgaa aaatgttg tccgaggacc accgtgcccc gcaaccatcg tggcaggacc gtcaacttc ctttccccca caaaacccaa ggacaccctc atgatctcc ggaccctgt ggtcacgtgc gtgggtgtgg acgtgagcca cgaagacccc gagggttcactaactgtta cgtggacggc gtggagggtc ataatgccaa gacaaaggcca cggggaggac agttcaacac cacttccgt gtggtcacgc ttctcaccgt tggcaccag gactggctga acggcaaggaa gtacaagggtc aaatgttcca acaaaggctt cccaggcccccc atcgagaaaa ccatctccaa aaccaagggg cagccccggaa aaccacagg gtacaccctg cccccatccc gggaggagat gaccaagaa caggtcagcc tgactgtctc ggtcaaaaggc ttcttacccca gacgacatcgc cgtggagttg gaggacaaatg ggcggccgg gaacaactac aagaccacac ctccccatgt ggactccgaa ggctcttct tcctctacag caagctcacc gtggacaaga gcagggtggca gcaggggaaac gtcttcat gtcccgat gcatgaggct ctgcacaacc actacacgca gaagagcctc tccctgtctc cggtaaa
SEQ ID NO: 242	gcctccacca agggccatc ggtttcccc ctggccctc gtccaggag caccctcgag agcacagcgcc ctctggctg cctggtaag gactacttc cccaacccgt gacgggtgtcg tggaaactcg ggcgtctgac cagcggcggtg cacacccccc cagctgtctc acagtctca gggacttact ccctcagcag cgttgtgacc gtgccttca gcaacttcgg caccggac tacacctgca acgttagatca caagcccgaa aacaccaagg tggacaagac agttggccgaa aaatgttg tccgaggacc accgtgcccc gcaaccatcg tggcaggacc gtcaacttc ctttccccca caaaacccaa ggacaccctc atgatctcc ggaccctgt ggtcacgtgc gtgggtgtgg acgtgagcca cgaagacccc gagggttcactaactgtta cgtggacggc gtggagggtc ataatgccaa gacaaaggcca cggggaggac agttcaacac cacttccgt gtggtcacgc ttctcaccgt tggcaccag gactggctga acggcaaggaa gtacaagggtc aaatgttcca acaaaggctt cccaggcccccc atcgagaaaa ccatctccaa aaccaagggg cagccccggaa aaccacagg gtacaccctg cccccatccc gggaggagat gaccaagaa caggtcagcc tgactgtctc ggtcaaaaggc ttcttacccca gacgacatcgc cgtggagttg gaggacaaatg ggcggccgg gaacaactac aagaccacac ctcc

In other embodiments, an antibody may comprise a specific heavy or light chain, while the complementary light or heavy chain variable domain remains unspecified. In particular, certain embodiments herein include antibodies that bind a specific antigen (such as activin-A) by way of a specific light or heavy chain, such that the complementary heavy or light chain may be promiscuous, or even irrelevant, but may be determined by, for example, screening combinatorial libraries. Portolano et al., *J Immunol.* V. 150 (3), pp. 880-887 (1993); Clackson et al., *Nature* v. 352 pp. 624-628 (1991).

Naturally occurring immunoglobulin chains exhibit the same general structure of relatively conserved framework regions (FR) joined by three hypervariable regions, also called complementarity determining regions or CDRs. From N-terminus to C-terminus, both light and heavy chains comprise the domains FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4. The assignment of amino acids to each domain is

in accordance with the definitions of Kabat et al. in *Sequences of Proteins of Immunological Interest*, 5<sup>th</sup> Ed., US Dept. of Health and Human Services, PHS, NIH, NIH Publication no. 91-3242, 1991.

- 5 The term "human antibody," also referred to as "fully human antibody," includes all antibodies that have one or more variable and constant regions derived from human immunoglobulin sequences. In one embodiment, all of the variable and constant domains are derived from human immunoglobulin sequences (a fully human antibody). These antibodies may be prepared in a variety of ways, examples of which are described below, including through the immunization with an antigen of interest of a mouse that is genetically modified to express antibodies derived from human heavy and/or light chain-encoding genes.
- A humanized antibody has a sequence that differs from the sequence of an antibody derived from a non-human species by one or more amino acid substitutions, deletions, and/or additions, such that the humanized antibody is less likely to induce an immune response, and/or induces a less severe immune response, as compared to the non-human species antibody, when it is administered to a human subject. In one embodiment, certain amino acids in the framework and 10 constant domains of the heavy and/or light chains of the non-human species antibody are mutated to produce the humanized antibody. In another embodiment, the constant domain(s) from a human antibody are fused to the variable domain(s) of a non-human species. In another embodiment, 15 one or more amino acid residues in one or more CDR sequences of a non-human antibody are changed to reduce the likely immunogenicity of the non-human antibody when it is administered to a human subject, wherein the changed amino acid residues either are not critical for immunospecific binding of the antibody to its antigen, or the changes to the amino acid sequence that are made are conservative changes, such that the binding of the humanized antibody to the antigen is not significantly worse than the binding of the 20 non-human antibody to the antigen. Examples of how to make humanized antibodies may be found in U.S. Pat. Nos. 6,054,297, 5,886,152 and 5,877,293.

The term "chimeric antibody" refers to an antibody that contains one or more regions from one antibody and one or more regions from one or more other antibodies. In one embodiment, one or more of the CDRs are derived from a human anti-activin-A antibody. In another embodiment, all of the CDRs are derived from a human anti-activin-A antibody. In another embodiment, the CDRs from more than one human anti-activin-A antibodies are mixed and matched in a chimeric antibody. For instance, a chimeric antibody may comprise a CDR1 from the light chain of a first human anti-activin-A antibody, a CDR2 and a CDR3 from the light chain of a second human anti-activin-A antibody, and the CDRs from the heavy chain from a third anti-activin-A antibody. Further, the framework regions may be derived from one of the same anti-activin-A antibodies, from one or more different antibodies, such as a human antibody, or from a humanized antibody. In one example of a chimeric antibody, a portion of the heavy and/or light chain is identical 35 with, homologous to, or derived from an antibody from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is/are identical with, homologous to, or derived from an antibody (-ies) from another species or belonging to another antibody class or subclass. Also included are fragments of such antibodies that exhibit the desired biological activity (i.e., the ability to specifically bind activin-A).

Fragments or analogs of antibodies can be readily prepared by those of ordinary skill in the art following the teachings of this specification and using techniques well-known in the art. Preferred amino- and carboxy-termini of fragments or analogs occur near boundaries of functional domains. Structural and functional domains can be identified by comparison of the nucleotide and/or amino acid sequence data to public or proprietary sequence databases. Computerized comparison methods can be used to identify sequence motifs or predicted protein conformation domains that occur in other proteins of known structure and/or function. Methods to identify protein sequences that fold into a known three-dimensional structure are known. See, e.g., Bowie et al., 1991, *Science* 253:164.

Antigen binding fragments derived from an antibody can be obtained, for example, by proteolytic hydrolysis of the antibody, for example, pepsin or papain digestion of whole antibodies according to conventional methods. By way of example, antibody fragments can be produced by enzymatic cleavage of antibodies with pepsin to provide a 5S fragment termed F(ab')<sub>2</sub>. This fragment can be further cleaved using a thiol reducing agent to produce 3.5S Fab' monovalent fragments. Optionally, the cleavage reaction can be performed using a blocking group for the sulphydryl groups that result from cleavage of disulfide linkages. As an alternative, an enzymatic cleavage using papain produces two monovalent Fab fragments and an Fc fragment directly. These methods are described, for example, by Goldenberg, U.S. Pat. No. 4,331,647, Nisonoff et al., *Arch. Biochem. Biophys.* 89:230, 1960; Porter, *Biochem. J.* 73:119, 1959; Edelman et al., in *Methods in Enzymology* 1:422 (Academic Press 1967); and by Andrews, S. M. and Titus, J. A. in *Current Protocols in Immunology* (Coligan J. E., et al., eds), John Wiley & Sons, New York (2003), pages 2.8.1-2.8.10 and 2.10A.1-2.10A.5. Other methods for cleaving antibodies, such as separating heavy chains to form monovalent light-heavy chain fragments (Fd), further cleaving of fragments, or other enzymatic, chemical, or genetic techniques may also be used, so long as the fragments bind to the antigen that is recognized by the intact antibody.

An antibody fragment may also be any synthetic or genetically engineered protein. For example, antibody fragments include isolated fragments consisting of the light chain variable region, "Fv" fragments consisting of the variable regions of the heavy and light chains, recombinant single chain polypeptide molecules in which light and heavy variable regions are connected by a peptide linker (scFv proteins).

Another form of an antibody fragment is a peptide comprising one or more complementarity determining regions (CDRs) of an antibody. CDRs (also termed "minimal recognition units", or "hypervariable region") can be incorporated into a molecule either covalently or noncovalently to make it an antigen binding protein. CDRs can be obtained by constructing polynucleotides that encode the CDR of interest. Such polynucleotides are prepared, for example, by using the polymerase chain reaction to synthesize the variable region using mRNA of antibody-producing cells as a template (see, for example, Larrick et al., *Methods: A Companion to Methods in Enzymology* 2:106, 1991; Courtenay-Luck, "Genetic Manipulation of Monoclonal Antibodies," in *Monoclonal Antibodies: Production, Engineering and Clinical Application*, Ritter et al. (eds.), page 166 (Cambridge University Press 1995); and Ward et al., "Genetic Manipulation and Expression of Antibodies," in *Monoclonal Antibodies: Principles and Applications*, Birch et al., (eds.), page 137 (Wiley-Liss, Inc. 1995)).

Thus, in one embodiment, the binding agent comprises at least one CDR as described herein. The binding agent may comprise at least two, three, four, five or six CDR's as described herein. The binding agent further may comprise at least one variable region domain of an antibody described herein. The variable region domain may be of any size or amino acid composition and will generally comprise at least one CDR sequence responsible for binding to human activin-A, for example CDR-H1, CDR-H2, CDR-H3 and/or the light chain CDRs specifically described herein and which is adjacent to or in frame with one or more framework sequences. In general terms, the variable (V) region domain may be any suitable arrangement of immunoglobulin heavy (V<sub>H</sub>) and/or light (V<sub>L</sub>) chain variable domains. Thus, for example, the V region domain may be monomeric and be a V<sub>H</sub> or V<sub>L</sub> domain, which is capable of independently binding human activin-A with an affinity at least equal to 1×10<sup>-7</sup> M or less as described below. Alternatively, the V region domain may be dimeric and contain V<sub>H</sub>V<sub>H</sub>, V<sub>H</sub>V<sub>L</sub>, or V<sub>L</sub>V<sub>L</sub> dimers. The V region dimer comprises at least one V<sub>H</sub> and at least one V<sub>L</sub> chain that may be non-covalently associated (hereinafter referred to as Fv). If desired, the chains may be covalently coupled either directly, for example via a disulfide bond between the two variable domains, or through a linker, for example a peptide linker, to form a single chain Fv (scFv).

The variable region domain may be any naturally occurring variable domain or an engineered version thereof. By engineered version is meant a variable region domain that has been created using recombinant DNA engineering techniques. Such engineered versions include those created, for example, from a specific antibody variable region by insertions, deletions, or changes in or to the amino acid sequences of the specific antibody. Particular examples include engineered variable region domains containing at least one CDR and optionally one or more framework amino acids from a first antibody and the remainder of the variable region domain from a second antibody.

The variable region domain may be covalently attached at 40 a C-terminal amino acid to at least one other antibody domain or a fragment thereof. Thus, for example, a VH domain that is present in the variable region domain may be linked to an immunoglobulin CH1 domain, or a fragment thereof. Similarly a V<sub>L</sub> domain may be linked to a Ck domain or a fragment thereof. In this way, for example, the antibody may be a Fab fragment wherein the antigen binding domain contains associated V<sub>H</sub> and V<sub>L</sub> domains covalently linked at their C-termini to a CH1 and Ck domain, respectively. The CH1 domain may be extended with further amino acids, for example to provide a hinge region or a portion of a hinge region domain as found in a Fab' fragment, or to provide further domains, such as antibody CH2 and CH3 domains.

As described herein, antibodies comprise at least one of 55 these CDRs. For example, one or more CDR may be incorporated into known antibody framework regions (IgG1, IgG2, etc.), or conjugated to a suitable vehicle to enhance the half-life thereof. Suitable vehicles include, but are not limited to Fc, polyethylene glycol (PEG), albumin, transferrin, and the like. These and other suitable vehicles are known in the art. Such conjugated CDR peptides may be in monomeric, dimeric, tetrameric, or other form. In one embodiment, one or more water-soluble polymer is bonded at one or more specific position, for example at the amino terminus, of a binding agent.

Antigen specific (i.e. activin-A specific) antibodies can be produced by methods known in the art by using a specific

VL or VH domain to screen a library of the complementary variable domain. Such methods of producing antibodies are known in the art. For example, antibody fragments fused to another protein, such as a minor coat protein, can be used to enrich phage with antigen. Then, using a random combinatorial library of rearranged heavy (VH) and light (VL) chains from mice immune to the antigen (e.g. activin-A), diverse libraries of antibody fragments are displayed on the surface of the phage. These libraries can be screened for complementary variable domains, and the domains purified by, for example, affinity column. See Clackson et al., *Nature*, V. 352 pp. 624-628 (1991).

In another example, individual VL or VH chains from an antibody (i.e. activin-A antibody) can be used to search for other VH or VL chains that could form antigen-binding fragments (or Fab), with the same specificity. Thus, random combinations of VH and VL chain Ig genes can be expressed as antigen-binding fragments in a bacteriophage library (such as fd or lambda phage). For instance, a combinatorial library may be generated by utilizing the parent VL or VH chain library combined with antigen-binding specific VL or VH chain libraries, respectively. The combinatorial libraries may then be screened by conventional techniques, for example by using radioactively labeled probe (such as radioactively labeled activin-A). See, for example, Portolano et al., *J. Immunol.* V. 150 (3) pp. 880-887 (1993).

A "CDR grafted antibody" is an antibody comprising one or more CDRs derived from an antibody of a particular species or isotype and the framework of another antibody of the same or different species or isotype.

A "multi-specific antibody" is an antibody that recognizes more than one epitope on one or more antigens. A subclass of this type of antibody is a "bi-specific antibody" which recognizes two distinct epitopes on the same or different antigens.

An "antigen binding domain," "antigen binding region," or "antigen binding site" is a portion of an antigen binding protein that contains amino acid residues (or other moieties) that interact with an antigen and contribute to the antigen binding protein's specificity and affinity for the antigen. For an antibody that specifically binds to its antigen, this will include at least part of at least one of its CDR domains.

An "epitope" is the portion of a molecule that is bound by an antigen binding protein (e.g., by an antibody). An epitope can comprise non-contiguous portions of the molecule (e.g., in a polypeptide, amino acid residues that are not contiguous in the polypeptide's primary sequence but that, in the context of the polypeptide's tertiary and quaternary structure, are near enough to each other to be bound by an antigen binding protein), and includes the end sequence amino acids listed. For example the polypeptide sequence R13-Y39 includes amino acids R13, and Y39, as well as the amino acids found between R13 and Y39 in the sequence. In embodiments in which the epitope comprises non-contiguous portions of a molecule, the sequences will be noted accordingly.

#### Antigen Binding Proteins

In one aspect, the present invention provides antigen binding proteins (e.g., antibodies, antibody fragments, antibody derivatives, antibody muteins, and antibody variants), that bind to activin-A, e.g., human activin-A.

An "antigen binding protein" is a protein comprising a portion that binds to an antigen and, optionally, a scaffold or framework portion that allows the antigen binding portion to adopt a conformation that promotes binding of the antigen binding protein to the antigen. Examples of antigen binding proteins include antibodies, antibody fragments (e.g., an

antigen binding portion of an antibody), antibody derivatives, and antibody analogs. The antigen binding protein can comprise, for example, an alternative protein scaffold or artificial scaffold with grafted CDRs or CDR derivatives. Such scaffolds include, but are not limited to, antibody-derived scaffolds comprising mutations introduced to, for example, stabilize the three-dimensional structure of the antigen binding protein as well as wholly synthetic scaffolds comprising, for example, a biocompatible polymer. See, for example, Korndorfer et al., 2003, *Proteins: Structure, Function, and Bioinformatics*, Volume 53, Issue 1:121-129; Roque et al., 2004, *Biotechnol. Prog.* 20:639-654. In addition, peptide antibody mimetics ("PAMs") can be used, as well as scaffolds based on antibody mimetics utilizing fibronectin components as a scaffold.

An antigen binding protein can have, for example, the structure of a naturally occurring immunoglobulin. An "immunoglobulin" is a tetrameric molecule. In a naturally occurring immunoglobulin, each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kDa) and one "heavy" chain (about 50-70 kDa). The amino-terminal portion of each chain includes a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The carboxy-terminal portion of each chain defines a constant region primarily responsible for effector function. Human light chains are classified as kappa and lambda light chains. Heavy chains are classified as mu, delta, gamma, alpha, or epsilon, and define the antibody's isotype as IgM, IgD, IgG, IgA, and IgE, respectively. Within light and heavy chains, the variable and constant regions are joined by a "J" region of about 12 or more amino acids, with the heavy chain also including a "D" region of about 10 more amino acids. See generally, Fundamental Immunology Ch. 7 (Paul, W., ed., 2nd ed. Raven Press, N.Y. (1989)) (incorporated by reference in its entirety for all purposes). The variable regions of each light/heavy chain pair form the antibody binding site such that an intact immunoglobulin has two binding sites.

Antigen binding proteins in accordance with the present invention include antigen binding proteins that inhibit a biological activity of activin-A. For example, antigen binding proteins may attenuate cachexia, and this activity can be present when the antigen binding protein is fully human, such as a fully human antibody.

Different antigen binding proteins may bind to different domains or cysteine knot domains of activin-A or act by different mechanisms of action. Examples include but are not limited to antigen binding proteins that specifically bind one or more particular cysteine knot domains, or regions interspersed between disulfide bonds, including regions spanning from about amino acids 4-12, amino acids 11-81, amino acids 11-33, amino acids 13-39, amino acids 40-113, amino acids 44-115, amino acids 81-111, and/or amino acids 82-107 of the following sequence: tcctatggagg tgactcagggc accctcatg tccgtgtccc caggacagac agccagcatc acctgtctcg gagataaatt ggggataaa tatgttgtt ggtatcagca gaagccaggc cagtcctcg tgcgtgtcat ctataaatg agcaaggcggc cctcaggat cccgtgatc ttctctggc ccaactctgg aaacacagcc actctgtcc tcagcggac ccaggatcgatgatggatcgatctttacttacttgc tcaaggcgtgg gacagcagca ctgcgttatt cggcggaggaccgaactgaa cccgtcc (SEQ ID NO: 267)). As indicated herein inter alia, the domain region are designated such as to be inclusive of the group, unless otherwise indicated. For example, amino acids 4-12 refers to nine amino acids: amino acids at positions 4, and 12, as well as the seven intervening amino acids in the sequence. Other examples include antigen binding proteins that inhibit binding of activin-A to its receptor. An antigen

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binding protein need not completely inhibit an activin-A-induced activity to find use in the present invention; rather, antigen binding proteins that reduce a particular activity of activin-A are contemplated for use as well. (Discussions herein of particular mechanisms of action for activin-A-binding antigen binding proteins in treating particular diseases are illustrative only, and the methods presented herein are not bound thereby.)

In another aspect, the present invention provides antigen binding proteins that comprise a light chain variable region selected from the group consisting of A1-A14 or a heavy chain variable region selected from the group consisting of A1-A14, and fragments, derivatives, muteins, and variants thereof. Such an antigen binding protein can be denoted using the nomenclature "LxHy", wherein "x" corresponds to the number of the light chain variable region and "y" corresponds to the number of the heavy chain variable region as they are labeled in the sequences below. That is to say, for example, that "A1HC" denotes the heavy chain variable region of antibody A1; "A1LC" denotes the light chain variable region of antibody A1, and so forth. More generally speaking, "L2H1" refers to an antigen binding protein with a light chain variable region comprising the amino acid sequence of L2 and a heavy chain variable region comprising the amino acid sequence of H1. For clarity, all ranges denoted by at least two members of a group include all members of the group between and including the end range members. Thus, the group range A1-A14, includes all members between A1 and A14, as well as members A1 and A14 themselves. The group range A4-A6 includes members A4, A5, and A6, etc.

Also shown below are the locations of the CDRs (underlined) that create part of the antigen-binding site, while the Framework Regions (FRs) are the intervening segments of these variable domain sequences. In both light chain variable regions and heavy chain variable regions there are three CDRs (CDR 1-3) and four FRs (FR 1-4). The CDR regions of each light and heavy chain also are grouped by antibody type (A1, A2, A3, etc.). Antigen binding proteins of the invention include, for example, antigen binding proteins having a combination of light chain and heavy chain variable domains selected from the group of combinations consisting of L1H1 (antibody A1), L2H2 (antibody A2), L3H3 (antibody A3), L4H4 (antibody A4), L5H5 (antibody A5), L6H6 (antibody A6), L7H7 (antibody A7), L8H8 (antibody A8), L9H9 (antibody A9), L10H10 (antibody A10), L11H11 (antibody A11), L12H12 (antibody A12), L13H13 (antibody A13), and L14H14 (antibody A14).

Antibodies A1-A14 heavy and light chain variable region polynucleotides (also referred to herein as H1-H14 and L1-L14).

A1 HC

(SEQ ID NO: 268)

```
CAGGTTCAGCTGGTGCAGTCTGGAGCTGAGGTGAAGAACGCTGGGCCCTC
AGTGAAGGTCTCTGCAAGGCTCTGGTTACACCTTACCGATTATGGTC
TCAGCTGGGTGCACAGGCCCTGGACAAGGGCTTGAGTGGATGGATGG
ATCATCCCTAACATGGTAACACAAACTCTGCACAGAAACTCCAGGGCAG
AGTCACCATGACCACAGACACATCCACGAGCACAGCCTACATGGAGCTGA
GGAGCCTGAGATCTGACGACACGGCGTGTATTCTGTGCGAGAGACAGG
GACTACGGTCAATTATGATGCTTTGATATCTGGGCCAAGGGACAAT
GGTCACCGTCTTCA
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(SEQ ID NO: 267)

TCCTATGAGGTGACTCAGGCACCCCTCAGTGTCCGTGCCCCAGGACAGAC

5 AGCCAGCATCACCTGCTGGAGATAAATTGGGGATAAAATATGCTTGTGGTATCAGCAGAACGCCAGGCCAGTCCCCTGTGCTGGTCATCTATCAAGATAGCAAGGGCCCTCAGGGATCCCTGAGCGATTCTCTGGCTCCAACTCTGG

10 AAACACAGCCACTCTGACCATCAGCGGGACCCAGGCTATGGATGAGGCTG

ACTATTACTGTCAGGCGTGGGACAGCAGCACTGCGTATTCGGCGGAGGG

ACCAAGGTGACCGTCTCA

15 A2 HC (SEQ ID NO: 269)

CAGGTGCAGCTGGTGGAGTCTGGGGAGGCGTCAGCCTGGAGGTCCCTGAGACTCTCTGTCAGCGCTCTGATTACCTTCAGTAGTTACGGCA20 TGCACTGGTCCGCCAGGCTCCAGGAAGGGCTGGAGTGGTGGCAGTT

ATATGGTATGATGGAAGTAATAAATACCATGCAGACTCCGTGAAGGGCCG

ATTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTGCAAGTGA

25 ACAGCCTGAGAGCGAGGGACACGGCTGTGATTACTGTGTGAGAAGTCGG

AACTGGAACTACGACAACTACTACGGTCTGGACGTCTGGGGCAAGG

GACCACGGTCACCGTCTCCTCAG

30 A2 LC (SEQ ID NO: 270)

GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGA

CAGAGTCACCATCATTGCCGGCAAGTCAGGGCATTAGAAATAATTAGGCTGGTATCAGCAGAAACCAGGAAAGCCCTAAGCGCTGATTTATGCT35 GCATCCAGTTGCAAAGTGGGTCCCATCAAGGTTCAGCGCAGTGGATCTGGGACAGAATTCACTCTCACAATCAGCAGTCTGCAGCCTGAAGATTTACAACTTATTACTGTCTACAGCATAATAGTTACCCGTGACGTTCGCCAA40 GGGACCAAGGTGAAATCAAA

A3 HC (SEQ ID NO: 271)

GAGGTGCAGTTGGTGGAGTCTGGGGAGGCTGGTCCAGCCTGGGGGTC

45 CCTGAGACTCTCTGTGCAGCCTCTGGATTACCTTAGTTAGTTATGGATGAGCTGGTCCGCCAGGCTCCAGGGAAAGGGCTGGAGTGCCTGGCCAA

ATAAAGCAAGATGGAAGTGAGGAATACTATGTGACTCTGTGAAGGGCCG

50 ATTCACCATCTCCAGAGACAACGCCAAGAATTCACTGTATCTGCAATGAACAGCCTGAGAGCCGAGGAACACGGCTGTGATTACTGTGCGAGAGGGTAGCAGCAGCTGGTACTACAAACTACGGTATGGACGTCTGGGGCAAGGGAC

55 CACGGTCACCGTCTCCTCA

A3 LC (SEQ ID NO: 272)

GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGA

60 CAGAGTCACCATCATTGCCGGCAAGTCAGGGCATTAGAAATGATTAGGCTGGTATCAGCAGAAACCAGGAAAGCCCTAAGCGCTGATCTATGCTGCATCCAGTTGCAAAGTGGGTCCCATCAAGGTTCAGCGCAGTGGATC65 TGGGACAGAATTCACTCTCACAATCAACGCGCTGAGCCTGAAGATTTG

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- continued  
 CAACTTATTACTGTCGACAGCAAAATACTTACCCGCTCACTTCGGCGGA  
GGGACCAAGGTGGAGATCAAA

A4 HC

(SEQ ID NO: 273)  
 CAGGTGCAGCTGGTGCAGTCTGGGCTGAGGTGAAGAAGCCTGGGCCTC

AGTGAAGGTCTCTGCAAGGCTCTGGATACACCTCACCGGCTACTATA

TCCACTGGGTGCGACAGGCCCTGGACAAGGGCTTGAGTGGATGGATGG

ATCAACCTAACAGTGGTGGCACAAACTATGCACAGAAGTTCAGGGCAG

GGTCACCATGACGGGACACGGTCCATCAGCACAGCCTACATGGACTGA

GCAGGCTGAGATCTGACGACACGGCCGTGTATTCTGTGCGAGAGATTCG

GGGTATAGCAGCAGCTGGCACTTGACTACTGGGCCAGGGAACCCGGT

CACCGTCTCCCTA

A4 LC

(SEQ ID NO: 274)  
 GATATTGTGATGACTCAGTCTCCACTCTCCCTGCCGTCCCCCTGGAGA

GCGGCCCTCCATCTCCTGCAGGTCTAGTCAGAGCCTCTGCATAGTACTG

GATACAACATTGGATTGGTACCTGCAGAACGCCAGGGCAGTCTCACAG

CTCCTGATCTATTGGTTCTTCGGGCCTCCGGGCTCCGTACAGGTT

CAGTGGCAGTGGTCAGGCACAGATTTACACTGAAAATCAGCAGAGTGG

AGGCTGAGGATGTTGGGTTTAACTGCATGCAAGCTCTCCAAACTCCG

TGCAGTTTGGGAGGGGACCAAGCTGGAGATCAAG

A5 HC

(SEQ ID NO: 66)  
 CAGGTGCAGCTCAGGAGTCGGGCCAGGACTGGTGAAGCCTCGGAGAC

CCTGTCCTCACCTGCACTGTCTCTGGTGGCTCATCAATAGTTCTACT

GGAGCTGGATCCGGCAGCCCCAGGAAGGGACTGGAGTGGATTGGGTAT

ATCTATTACAGTGGGAGCACCACATACAATCCCTCCCTCAAGAGTCGAGT

CACCATATCAGTAGACAGTCCAAGAACCCAGTCTCCCTGAAGCTGAGCT

CTGTGACCGCTCGGGACACGGCCGTGTATTACTGTGCGAGAGACAGTATA

GCAGCCCCCTTGTACTCTGGGCCAGGGAACCCCTGGTCACCGTCTCC

AGCTTCCACCAAGGGCCATCCGTCTTCCCCCTGGCGCCCTGCTCCAGGA

GCACCTCCGAGAGCACAGCCGCCCCTGGGCTGCGTGTCAAGGACTACTTC

CCCGAACCGGTGACGGTGTGGAACTCATGCGCCCT

A5 LC

(SEQ ID NO: 65)  
 GACATCGTGATGACCCAGTCTCCAGACTCCCTGGCTGTCTCGGCGA

GGGGCCACCATCACCTGCAAGTCCAGCCAGAGTATTATACAGTCCA

ACAATAAGAAGTATCTAGTTGGTACCAAGCAGAACCCAGGACAGCCTCCT

AAGCTGATCATTACTGGACATCTATGCGGAATCGGGGCTCTGACCG

ATTCAGTGGCAGCGGGCTGGGACAGATTTCACTCTCACCATCAACAGCC

TGCAGGGCTGAAGATGTGGCAGTTTATTACTGTCAGAATATTATAGTACT

CCGTGGACGTTGGCCAAGGGACCAAGGTGGAAATCAA

A6 HC

(SEQ ID NO: 82)  
 CAGGTGCAGCTACAGCAGTGGGGCGCAGGACTGGTGAAGCCTCGGAGAC

CCTGTCCTCACCTGCGCTGTATGGTGGCTCTTCAGTGCCTAACT

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- continued

GGAGCTGGATCCGCCAGCCCCAGGAAGGGACTGGAGTGGATTGGGAA

ATCAATCATAGTGGAGGCACCAACTACAACCCGTCCCTCAAGAGTCAGT

5 GACCATATCAGTAGACAGTCCAAGAACCCAGTTCTCCCTGAAGCTGAGCT

CTGTGACCGCCGCGACACGGCTGTGTATTACTGTGCGAGAGTACAGTGG

10 CTCGAACTGGCTACTTGACTACTGGGCCAGGGAACCCCTGGTCACCGT

CTCCTCA

A6 LC

(SEQ ID NO: 81)

GACATCCAGATGACCCAGTCTCCATCTCCCTGTCTGCATCTGTAGGAGA

15 CAGAGTCACCATCACTGCGGGCAAGTCAGAGCATTAGCAACTATTAA

ATTGGTATCAGCAGAGACCAGGGAAAGCCCTAAGCTCTGATCTATGCT

ACATCCAGTTGCAAAGTGTGGTCCATCAAGGTTAGTCAGTGGCAGTGGATC

20 TGGGACAGATTTCACTCTCACCATCAGCAGTCTGCAACCTGAAGATTG

TAAGTTACTACTGTCAACAGAGTTACAGTATTTCGCCACTTCGGCCG

GGGACCAAGGTGGAGAACAAA

A7 HC

(SEQ ID NO: 98)

CAGGTGCAGCTGGTGGACTCTGGGGAGGGCTGGTCCAGCCTGGGAGGTC

CCTGAGACTCTCTGTGCGAGCGTCTGGATTACCTTCATTAGCTATGGCA

30 TGCACTGGGTCCGCCAGGCCTCCAGGCAAGGGCTGGAGTGGGTGGCAGTT

ATCTGGTATGAGAAGTACTGAATACTATGCAGACTCCGTGAAGGGCCG

ATTACCCATCTCAGAGACAATTCCAAGAACACGCTGTATCTGCAAATGA

35 ACAGCCTGAGAGCCGAGGACACGGCTGTATTACTGTGCGAGAGAGG

CAGTGGCTCTACCACTACGGTATGGACGTCTGGGCCAGGGACCACGGT

CACCGTCTCCCTCA

A7 LC

(SEQ ID NO: 97)

GACATCCAGATGACCCAGTCTCCATCTCCCTGTCTGCATCTGTAGGAGA

CAGAGTCACCATCACTGCGGGCAGGTCAAGGCATTAGAAATGATTAG

TCTGGTATCAGCAGAAACCAGGGAAAGCCCTAAGGCCGTATCTATGCT

45 GCATCCAGTTGCAAAGTGGGTCCATCAAGGTTAGCAGCCGAGTGGATC

TGGGACAGAATTCACTCACAATCAGCAGCCTGCAAGCCTGAAGATTG

CAACTTATTACTGTCTACAAACATAATACTTACCCATTCACTTCGGCC

50 GGGACCAAGGTGGATATCAA

A8 HC

(SEQ ID NO: 114)

CAGGTGCAGCTGCAGGAGTCGGGCCAGGACTGGTGAAGCCCTCGGAGAC

55 CCTGTCCTCACCTGCACTGTCTCTGGTGGCTCCATCAATAGTTCTACT

GGAGCTGGATCCGGCAGCCCCAGGAAGGGACTGGAGTGGATTGGGTAT

ATCTATTACAGTGGGAGCACCACATACAATCCCTCCCTCAAGAGGCGAGT

60 CACCATATCAGTAGACAGTCCAAGAACCCAGTTCTCCCTGAAGCTGAGCT

CTGTGACCGCTCGGGACACGGCCGTGTATTACTGTGCGAGAGACAGTATA

GCAGCCCCCTTGTACTCTGGGCCAGGGAACCCCTGGTCACCGTCTCC

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A8 LC  
 (SEQ ID NO: 113)  
 GACATCGTGATGACCCAGTCTCCAGACTCCCTGGCTGTCTGGCGA  
 GAGGGCACCACCATCACTGCAAGTCCAGGCCAGAGTATTTATACAGCTCCA  
ACAATAAGAAGTATCTAGTTGGTACCCAGCAGAAACCAGGACAGCCTCCT  
AAAGTTGATCATTACTGGACATCTATGCCGAATCCGGGTCCTGACCG  
ATTCAGTGGCAGCGGTCTGGACAGATTTACTCTCACCATCAGCAGCC  
TGCAGGCTGAAGATGTGGCAGTTTACTGTCAGCAATATTATAGTACT

A9 HC  
 (SEQ ID NO: 130)  
 CAGGTGCAGCTGGTGGAGTCTGGGGAGGCGTGGTCCAGCCTGGAGGTC  
CTTGAGACTCTCTGTGAGCCTCTGGATTACCCAGTGTAGTTACGGCA

TGCACTGGTCCGCCAGGCTCCAGGAAGGGCTGGAGTGGTGGCAGTT  
ATATGGTATGAGAAGTAATAAACCATGCAGACTCCGTGAAGGGCCG  
ATTCACCATCTCAGAGACAATCCAAGAACACGCTGTATCTGCAAGTGA  
ACAGCCTGAGAGCCGAGGACACGGCTGTATTACTGTGTGAGAAGTCGG  
AACTGGAACTACGACAACTACTACGGTCTGGACGTCTGGGCAAGG

GACCACGGTCACCGTCTCCTCA

A9 LC  
 (SEQ ID NO: 129)  
 GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGAGGAGA  
CAGAGTCACCATCATTGCCGGCAAGTCAGGCATTAGAAAATTTAG

GCTGGTATCAGCAGAAACCAGGGAAAGCCCCTAAGCGCCTGATTTATGCT  
GCATCCAGTTGCAAAGTGGGTCCCATCAAGGTTAGCAGCCAGTGGATC

TGGGACAGAATTCACTTCACCATCAGCAGCCTGAGCCTGAAGATTTA  
CAACTTATTACTGTCTACAGCATATAAGTTACCCGTGGACGTCTGGCAA

GGGACCAAGGTGAAATCAA

A10 HC  
 (SEQ ID NO: 146)  
 GAGGTGCAGCTGGTGCAGTCTGGAGCAGAGGTGAAAAAGCCGGGAGTC  
TCTGAAGATCTCTGTCAGGGTCTGGATACAGCTTACAGCTACTGGA

TCCGGCTGGTGCCCAGATGCCGGAAAGGCCCTGGAGTGGATGGGATC  
ATCTATCTGGTACTCTGATACCAGATACAGCCGCTTCAAGGCCA

GGTCACCATCTAGCCGACAAGTCCATCAGCACCGCCTACCTGCAGTGGA  
GCAGCCTGAAGGCCTCGGACACCGCCATGTATT  
ACTGTGCGAGACAAGGACTGGGTTGACTACTGGGCCAGGGAAACCTG

GTCACCGTCTCCTCA

A10 LC  
 (SEQ ID NO: 145)  
 TCCTATGAGCTACTCAGCCACCCCTCAGTGTCCGTGCCCCAGGACAGAC  
AGCCAGCATCACCTGCTGGAGAAAATGGGGAGAGAAATATGCTTGT  
GGTATCAGCAGAAAGCCAGGCCAGTCCCCCTGTGCTGGTCATCTATCAAGAT  
ACCAAGGCCCTCCGGATCCGTGAGCATCTGGTGAGTCCATTCTGG  
GAACACAGCCACTCTGACCCAGGCTTATGGATGAGGCTG

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ACTATTATTGTCAGGCCGTGGACAGGAGCACTGTATTCGCCGGAGGGACC

AAGCTGACCGTCCTA

5 A11 HC  
 (SEQ ID NO: 162)  
 CAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTTACAGAC

CCTGTCCCTCACCTGCACTGTCTGTGGCTCCATCAGCAGTGGGT

10 ACTACTGGAGCGTGGATCGCCAGCACCCCAGGGAGGGCCTGGAGTGGATT

GGGTACATCTCTTACAGGTGGAGCACCCACTACAAACCCGTCCCTCAAGAG

TCGAGTTACCAATATCAAGGTTAAGACCCAGGTTTCTCCCTGAAG

15 TGAACTCTGTGACTGCCCGGAACGCCCGGTTATTACTGTGCCGCGGT

TACGGTACTATCGCGGTGGATCCCTGGGGCCAGGGAACCCTGG

CACCGTCTCCTCA

A11 LC  
 (SEQ ID NO: 161)  
 TCCTATGAGCTACTCAGCCACCCCTCAGTGTCCGTGTCCCCAGGGACAGAC

AGCCAGCATCACCTGCTGGAGATAAATTGGGGATAAATTGGTTCT

GGTATCAGCGTGAAGCCAGGGCCAGGTCCCCCTGTGGCTGTGGCTATCAAGAT

25 AACAAGGGCCCTCAGGGATTCCCTGTAGCGATTCCTGGCTCCAACTCTGG

GAACACGCCCACCTGTGACCATCAGCGGGACCAGGGCTATGGATGCGGCT

ACTTTTTACTGTCAGGGTGGGAAGGCAGCACTGTGGTATCGCGGGAGGG

30 ACCAAGGTACCCGTCTCA

A12 HL  
 (SEQ ID NO: 178)  
 CAGGTGCAGCTGGTGGAGTCTGGGGAGGCGTGGTCCAGCCTGGAGGTC  

35 CCTGAGACTCTCTGTGTAGCGGTCTGGATTCACCCTTCAGTGCCTATGGCA

TGCACTGGGTCCCCAGGGCTCCAGGCAAGGGGCTGGAGGTGGGTGGAGGT

ATATGGTATGTGAAGTAAAAATAACATATGCAGACCCGTGTTATACGGTTGGACGTCTGGGGCAAG

40 ATTCCATCTCCAGAGACAAATCCAAGAACCGCTGTGTTATACGGTTGGACGTCTGGGGCAAG

ACAGCCTGAAGGGCCGAGGACACGGCTGTGTTATACGGTTGGACGTCTGGGGCAAG

45 GACCACGGTCACCGTCTCCCA

A12 LC  
 (SEQ ID NO: 177)  
 GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGAGGAGA

50 CAGAGTCACCCATCATTGCGGGAAGTCAGGGCATTAGAAATGATTTAGG

GTGGTATCAGCAGAAACCCAGGGAAAGCCCTTAAGCCCTGTATCTATGCT

GCATCCAGTTTCGAAAAGGGGTCCCCATCAAGGTTCAGCCGCAGGTGGATC

55 TGGGACAGAATTCACTTCACCAATCAGCAGCCGTGCAGCCGTGAAGATGTGT

CAACTTTTTATGTCTACAGCATAATAGTTTAACGTGGACGTCTGGCCAA

GGGACCAAGGTGAAATCAA

60 A13 HC  
 (SEQ ID NO: 194)  
 CAGGTTCAGCTGGGTGCAGCTGGAGGTGAAGAGCCGTGGGGCT

AGTGAAGGTCTCTGTCAAGGGCTCTGTGGTTACCCTTACCAGTGTACGGCTGGAGGGATGGATGG

65 TCAGCTGGGTGCGAAGGGCCCTGGACAGGGCTGGACAGGGCTGGAGGGATGGATGG

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ATCAGCGCTTACAATGGTAACACAAACTATCACAGAACAGTCCAGGGCAG  
AGTCACCATGACCACAGACACATCAACGACCACAGCCTACATGGAGCTGA  
GGAGCCTGAGATCTGACGACACGGCCGTGTATTACTGTGCGAGAGATCAA  
GATTACTATGATAGTAGTGTTGGGCCACTGGGCCAGGGAACCTGGT  
CACCGTCTCCTCA  
A13 LC  
(SEQ ID NO: 193)  
TCCTATGAGCTGACTCAGCCACCCCTCAGTGTCCGTGTCCCCAGGACAGAC  
AGCCAGCATCACCTGCTCTGGAGATAAATTGGGGATAAAATGTTGTT  
GGTATCAGCAGAACGCCAGGCCAGTCCCCCTGAACCTGGTCATCTATCTAGAT  
ACAAGCGGCCCTCAGGGATCCCTGAGCGATTCTCTGGCTCCAACCTCTGG  
GAACACAGCCACTCTGACCATCAGCGGGACCCAGGCTATGGATGAGGCTG  
ACTATTACTGT~~CAGGC~~TGGGACAGCAGCACGGTATT~~CGCG~~GAGGGACC  
AAACTGACCGTCTG  
A14 HC  
(SEQ ID NO: 210)  
CAGGTT~~CAG~~TGGTGCAATCTGGAGCTGAGGTGAAGAACGCTGGGCCTC  
AGTGAAGGTCTCTGCAAGACTCTGGTACACCTTACCA~~G~~CTATGGTA  
TCAGCTGGGTGCGACAGGCCCTGGACAAGGGCTTGAGTGGATGGATGG  
ATCAGCCCTTACAATGGTAACACAAACTATGCACAGAACAGTCCAGGGCAG  
AGTCACCACGACACAGACAAATCCACGAGCACAGCCTACATGGAGCTGA  
GGAGCCTCGCATCTGAGCACAGGCCGTGTATTACTGTGCGAGAGATCAA  
GATTACTATGATAGTAGTGTTGGGCCACTGGGCCAGGGAACCTGGT  
CACCGTCTCCTCG  
A14 LC  
(SEQ ID NO: 209)  
TCCTATGAGCTGACTCAGCCACCCCTCAGTGTCCGTGTCCCCAGGACAGAC  
AGCCTCCATCACCTGCTCTGGAGATAAATTGGGGATAAAATGTTCT  
GGTATCAGCAGAACGCCAGGCCAGTCCCCCTGTGCTGGCTTCTATCATGAT  
ACCAAGCGGCCCTCAGGGATCCCTGAGCGATTCTCTGGCTCCAACCTCTGG  
GAACACAGCCACTCTGACCATCAGCGGGACCCAGGCTATGGATGAGGCTG  
ACTATCACTGT~~CAGGC~~TGGGACAGCAGCACGGTCTCGCGGAGGGACC  
AAGCTGACCGTCTAC

Antibodies A1-A14 amino acid sequences, light chain variable regions. CDR regions are underlined; the intervening segments or regions are referred to as framework (FR) herein.

A1

(SEQ ID NO: 275)

SYEV~~T~~QAPS~~S~~VSPGQTASITCSGD~~K~~LGD~~K~~YACWYQQKPGQSPV~~L~~VIY~~Q~~  
DSKRPSGI~~P~~ERFSGSNSGNTATLTISGTQAMDEADYYC~~Q~~A~~W~~DS~~S~~STAVFG  
GGTKLTVL

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A2  
(SEQ ID NO: 276)  
DIQMTQSPSSLSASVGDRV~~T~~ITCRASQIRNNNLGWYQQKPGKAPKRLIY  
5 AASSLQSGVPSRFSGSGSGTEFTLT~~I~~SSLQPEDFTYYCLQHNSYPWT~~F~~  
GGT~~K~~VEIK  
A3  
(SEQ ID NO: 277)  
10 DIQMTQSPSSLSASVGDRV~~T~~ITCRASQIRNDLGWYQQKPGKAPKRLIY  
AASSLQSGVPSRFSGSGSGTEFTLT~~I~~SSLQPEDFATYYC~~R~~QONTYPL~~F~~  
GGGT~~K~~VEIK  
15 A4  
(SEQ ID NO: 57)  
DIVMTQSPSLP~~V~~TPGE~~P~~AS~~I~~CRSSQSLLH~~S~~TGYNLDWYLQKPGQSP  
QLLIY~~L~~GSFRASGV~~P~~DRFS~~G~~SG~~G~~T~~D~~PTLK~~I~~RVEAE~~D~~VGVYYCM~~A~~LO  
20 TPCSFGQGT~~K~~LEIK  
A5  
(SEQ ID NO: 73)  
DIVMTQSPDSLAVSLGERATITCKSSQSILYSSNNKKYLWYQQKPGQP  
PKLIIY~~W~~TSMRESGV~~P~~DRFS~~G~~SG~~G~~T~~D~~FTLT~~I~~SSLQAEDVAVYYC~~Q~~YY  
25 STPWT~~F~~QGQ~~K~~VEIK  
A6  
(SEQ ID NO: 89)  
DIQMTQSPSSLSASVGDRV~~T~~ITCRASQSI~~S~~NYLNWYQQRPGKAPKLLIY  
30 ATSSLQSGVPSRFSGSGSGTDFTLT~~I~~SSLQPEDFVSY~~C~~QOSYSIS~~P~~TF  
GGGT~~K~~VENK  
A7  
(SEQ ID NO: 105)  
35 DIQMTQSPSSLSASVGDRV~~T~~ITCRAGQGIRNDLGWYQQKPGKAPKRLIY  
AASSLQSGVPSRFSGSGSGTEFTLT~~I~~SSLQPEDFATYYCLQHNTYP~~F~~  
GP~~G~~TKVDIK  
40 A8  
(SEQ ID NO: 121)  
DIVMTQSPDSLAVSLGERATITCKSSQSILYSSNNKKYLWYQQKPGQP  
PKLIIY~~W~~TSMRESGV~~P~~DRFS~~G~~SG~~G~~T~~D~~FTLT~~I~~SSLQAEDVAVYYC~~Q~~YY  
45 STPWT~~F~~QGQ~~K~~VEIK  
A9  
(SEQ ID NO: 137)  
50 DIQMTQSPSSLSASVGDRV~~T~~ITCRASQIRNNNLGWYQQKPGKAPKRLIY  
AASSLQSGVPSRFSGSGSGTEFTLT~~I~~SSLQPEDFTYYCLQHNSYPWT~~F~~  
GGT~~K~~VEIK  
A10  
(SEQ ID NO: 153)  
55 SYELTQPPSVSPGQTASITCSGEK~~W~~GEKYACWYQQKPGQSPV~~L~~VIY~~Q~~  
DTKRP~~S~~GI~~P~~ERFSGSISGNTATLTISGTQAMDEADYYC~~Q~~A~~W~~DRSTV~~F~~GG  
GTKLTVL  
60 A11  
(SEQ ID NO: 169)  
56 SYELTQPPSVSPGQTASITCSGD~~K~~LGD~~K~~F~~A~~FWYQLKPGQSPV~~L~~VIY~~Q~~  
DNKRP~~S~~GI~~P~~ERFSGSNSGNTATLTISGTQAMDAADF~~Y~~C~~Q~~A~~W~~DS~~S~~STVV~~F~~GG  
65 GGTKLTVL

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A12 (SEQ ID NO: 185)  
DIQMTQSPSSLSASVGDRVTITCRASQGIRNDLGWYQQKPGKAPKRLIY

AASSLQSGVPSRFSGSGSGTEFTLTISLQPEDCATYYCLQHNSYTWTF

GQGTVKEIK

A13

(SEQ ID NO: 201) SYELTQPPSVSPGQTASITCSGDKLGDKYCVWYQQKPGQSPELVIYL

DNKRPSGIPERFSGSNSGNTATLTISGTQAMDEADYYHQCAWDSSTVFGG

GTKLTVL

A14

(SEQ ID NO: 217) SYELTQPPSVSPGQTASITCSGDKLGDKYAFWYQQKPGQSPVLVFH

DTKRPSGIPERFSGSNSGNTATLTISGTQAMDEADYYHQCAWDSSTVFGG

GTKLTVL

Antibodies A1-A14, amino acid sequences of heavy chain variable regions. CDR regions are shaded and underlined; the other regions are referred to as framework (FR) herein.

A1

(SEQ ID NO: 278) QVQLVQSGAEVKKPGASVKVSCASGYTFTSYGLSWRQAPGGLEWMG  
WIIPYNGNTNSAQKLQGRVTMTTDTSTSTAYMELRSLRSDDTAVYFCAR  
RDYGVNYDAFDIWGQGTMVTVSS

A2

(SEQ ID NO: 279) QVQLVESGGVVQPGRSLRLSCAAASGFTFSSYGMHWVRQAPGKGLEWVA  
VIWDGSNKYHADSVKGRFTISRDNSKNTLLYQVNSLRAEDTAVYYCAR  
SRNWNYDNYYGLDVWQGTTVSS

A3

(SEQ ID NO: 280) QVQLVQSGAEVKKPGASVKVSCASGYTFTSYWMSWRQAPGGLEWMG  
WISAYNGNTNYAQKFQGRVTMTTDTSTTTAYMELRSLRSDDTAVYYCAR  
GSSWYYYGMDVWQGTTVSS

A4

(SEQ ID NO: 58) QVQLVQSGAEVKKPGASVKVSCASGYTFTGYYYHWVRQAPGGLEWMG  
WINPNSGGTNYAQKFQGRVTMTRTDTSSTAYMELRSLRSDDTAVYPCAR  
DSGYSSWHFDYWGQGTLVSS

A5

(SEQ ID NO: 74) QVQLQESGPGLVKPSETLSLTCTVSGGSINSFYWWIRQPPGKGLEWIG  
YYYSGTNYNPSLKSRVTISVDTSKQFLSLKSSVTAADTAVYYCAR  
SIAAPFDYWGQGTLVSS

A6

(SEQ ID NO: 90) QVQLQQWGAGLLKPSETLSLTCAVYGGSFSAYYWWWIRQPPGKGLEWIG  
EINHSGGTNYNPSLKSRVTISVDTSKQFLSLKSSVTAADTAVYYCARV  
QWLELAYFDYWGQGTLVSS

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A7 (SEQ ID NO: 106)  
QVQLVDSGGVVQPGRSLRLSCAAASGFTFSYGMHWVRQAPGKGLEWIG

VIWDGSTEYYADSVKGRFTISRDNSKNTLYQVNSLRAEDTAVYYCAR

ERWLYHYGMDWQGTTVSS

A8

(SEQ ID NO: 122) QVOLQESGPGLVKPSETLSLTCTVSGGSINSFYWWIRQPPGKGLEWIG  
YYYSGTNYNPSLKSRVTISVDTSKQFLSLKSSVTAADTAVYYCAR  
SIAAPFDYWGQGTLVSS

A9

(SEQ ID NO: 138) QVQLVESGGVVQPGRSLRLSCAAASGFTFSSYGMHWVRQAPGKGLEWIG  
VIWDGSNKYHADSVKGRFTISRDNSKNTLLYQVNSLRAEDTAVYYCAR

20 SRNWNYDNYYGLDVWQGTTVSS

A10

(SEQ ID NO: 154) EVQLVQSGAEVKKPGESLKISCQGSYFTSYWIGWVRQMPGKGLEWIG  
IIPGDSDTRYSPSFQQGTTISADKSISTAYQWLSLKASDTAVYYCAR  
QGLGFDYWGQGTLVSS

A11

(SEQ ID NO: 170) QVQLQESGPGLVKPSQTLSLTCTVSGGSISSGGYYWSWIRQHPGKGLEWIG  
IGYSGTYYNPSLKSRVTISVDTSKQFLSLKLNSVTADTAVYYCAR  
RAYGDYRWFDPWQGTLVSS

A12

(SEQ ID NO: 186) QVQLVESGGVVQPGRSLRLSCVASGFTFTSYWIGWVRQAPGKGLEWIG  
VIWDGSNKYYADSVKGRFTISRDNSKNTLLYQVNSLRAEDTAVYYCAR  
SRNWNYDSYYQYGLDVWQGTTVSS

40 A13

(SEQ ID NO: 202) QVQLVQSGAEVKKPGASVKVSCASGYTFTSYGISWVRQAPGQGLERMG  
WISAYNGNTNYAQKFQGRVTMTTDTSTTAYMELRSLRSDDTAVYYCAR  
DQDYYDSSGWGHWQGTLVTSS

A14

(SEQ ID NO: 218) QVQLVQSGAEVKKPGASVKVSCASGYTFTSYGISWVRQAPGQGLERMG  
WISPYNGNTNYAQKFQGRVTMTTDKSTSTAYMELRSLRSDDTAVYYCAR  
DQDYYDSSGWDPWQGTLVTSS

55 TABLE 1

Light chain CDR1 consensus sequences for Antibodies A1-A14.

Light Chain	CDR1 Sequence
60 L4	R S S Q S L L H S T G Y N - Y L D
65 L5, L8	K S S Q S I L Y S S N N K K Y L V
CONSENSUS:	X <sub>1</sub> S S Q S X <sub>2</sub> L X <sub>3</sub> S X <sub>4</sub> X <sub>5</sub> X <sub>6</sub> X <sub>7</sub> X <sub>8</sub> Y L X <sub>9</sub> (SEQ ID NOS 253, 75 and 115, respectively, in order of appearance)

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TABLE 1-continued

Light chain CDR1 consensus sequences for Antibodies A1-A14.	
Light Chain	CDR1 Sequence
L2, L9	R A S Q G I R N N L G
L3, L12	R A S Q G I R N D L G
L6	R A S Q S I S N Y L N
L7	R A G Q G I R N D L V
CONSENSUS:	R A X <sub>10</sub> Q X <sub>11</sub> X <sub>12</sub> N X <sub>13</sub> L X <sub>14</sub> (SEQ ID NOS 281-282, 91, 107 and 116, respectively, in order of appearance)
L1	S G D K L G D K Y A C
L10	S G E K W G E K Y A C
L11	S G D K L G D K F A F
L13	S G D K L G D K Y V C
L14	S G D K L G D K Y A F
CONSENSUS:	S G X <sub>15</sub> K X <sub>16</sub> G X <sub>17</sub> K X <sub>18</sub> X <sub>19</sub> X <sub>20</sub> (SEQ ID NOS 59, 155, 171, 203, 219 and 123, respectively, in order of appearance)

X<sub>1</sub> is an arginine residue or a lysine residue,  
X<sub>2</sub> is a leucine residue or a isoleucine residue,  
X<sub>3</sub> is a histidine residue or a tyrosine residue,  
X<sub>4</sub> is a threonine residue or a serine residue,  
X<sub>5</sub> is a glycine residue or an asparagine residue,  
X<sub>6</sub> is a tyrosine residue or an asparagine residue,  
X<sub>7</sub> is an asparagine residue or a lysine residue,  
X<sub>8</sub> is a lysine residue or no residue,  
X<sub>9</sub> is an aspartate residue or a valine residue  
X<sub>10</sub> is a serine residue or a glycine residue,  
X<sub>11</sub> is a serine residue or a glycine residue,  
X<sub>12</sub> is a serine residue or an arginine residue,  
X<sub>13</sub> is a tyrosine residue, an aspartate residue, or an asparagine residue  
X<sub>14</sub> is an aspartate residue, a valine residue, or a glycine residue  
X<sub>15</sub> is a glutamate residue or an aspartate residue,  
X<sub>16</sub> is a tryptophan residue or a leucine residue,  
X<sub>17</sub> is a glutamate residue or an aspartate residue,  
X<sub>18</sub> is a tyrosine residue or a phenylalanine residue,  
X<sub>19</sub> is an alanine residue or a valine residue,  
X<sub>20</sub> is a cysteine residue or a phenylalanine residue

TABLE 2

Light chain CDR2 consensus sequences for Antibodies A1-A14.	
Light Chain	CDR2 Sequence
L2	A T S S L Q S
L3, L6, L7, L9, L12	A A S S L Q S
L5, L8	W T S M R E S
L4	L G S F R A S
CONSENSUS:	X <sub>40</sub> X <sub>41</sub> SX <sub>42</sub> X <sub>43</sub> X <sub>44</sub> S (SEQ ID NOS 92, 283, 76, 254 and 124, respectively, in order of appearance)

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TABLE 2-continued

Light chain CDR2 consensus sequences for Antibodies A1-A14.	
Light Chain	CDR2 Sequence
L10	Q D T K R P S
L11	Q D N K R P S
L1	Q D S K R P S
L13	L D N K R P S
L14	H D T K R P S
CONSENSUS:	X <sub>45</sub> D X <sub>46</sub> K R P S (SEQ ID NOS 156, 172, 60, 204, 220 and 128, respectively, in order of appearance)

X<sub>40</sub> is an alanine residue, a tryptophan residue, or a leucine residue,  
X<sub>41</sub> is a threonine residue, an alanine residue, or a glycine residue,  
X<sub>42</sub> is a serine residue, a methionine residue, or a phenylalanine residue,  
X<sub>43</sub> is a leucine residue or an arginine residue,  
X<sub>44</sub> is a glutamine residue, a glutamate residue, or an alanine residue  
X<sub>45</sub> is a glutamine residue, a leucine residue, or a histidine residue,  
X<sub>46</sub> is a threonine residue, an asparagine residue, or a serine residue

35

TABLE 3

Light chain CDR3 consensus sequences for Antibodies A1-A14.	
Light Chain	CDR3 Sequence
L1	Q A W D S S T A V
L10	Q A W D R S T - V
L11	Q A W D S S T V V
L13, L14	Q A W D S S T V -
L2	L Q H N S Y P W T
L7	L Q H N T Y P F T
L9	L Q H N S Y P W T
L12	L Q H N S Y T W T
CONSENSUS:	L Q H N X <sub>81</sub> Y X <sub>82</sub> X <sub>83</sub> T (SEQ ID NOS 61, 157, 173, 205, 141, 109, 141, 189 and 131, respectively, in order of appearance)
L3	R Q Q N T Y P L T
L4	M Q A L Q T P C S
L5	Q Q Y Y S T P W T
L6	Q Q S Y S I S P T
L8	Q Q Y Y S T P W T

TABLE 3-continued

Light chain CDR3 consensus sequences for Antibodies A1-A14.	
Light Chain	CDR3 Sequence
CONSENSUS:	X <sub>73</sub> QX <sub>74</sub> X <sub>75</sub> X <sub>76</sub> X <sub>77</sub> X <sub>78</sub> X <sub>79</sub> X <sub>80</sub> (SEQ ID NOS 284, 255, 77, 93, 125 and 132, respectively, in order of appearance)
	5
	X <sub>73</sub> is a methionine residue, a glutamine residue, or an arginine residue, X <sub>74</sub> is an alanine residue, a tyrosine residue, a glutamine residue, or a serine residue, X <sub>75</sub> is a leucine residue, a tyrosine residue, or an asparagine residue, X <sub>76</sub> is a glutamine residue, a serine residue, or a threonine residue, X <sub>77</sub> is a threonine residue, a tyrosine residue, or an isoleucine residue, X <sub>78</sub> is a proline residue or a serine residue, X <sub>79</sub> is a cysteine residue, a tryptophan residue, a leucine residue, or a proline residue, X <sub>80</sub> is a serine residue or a threonine residue
	10
	X <sub>81</sub> is a threonine residue or a serine residue, X <sub>82</sub> is a proline residue or a threonine residue, X <sub>83</sub> is a phenylalanine residue or a tryptophan residue
	15
	20

TABLE 4

Heavy chain CDR1 consensus sequences for Antibodies A1-A14.	
Heavy Chain	CDR1 Sequence
H5	G G S I N S - - F Y W S
H6	G G S F S A - - Y Y W S
H8	G G S I N S - - F Y W S
H11	G G S I S S G G Y Y W S
CONSENSUS:	G G SX <sub>21</sub> X <sub>22</sub> X <sub>23</sub> X <sub>24</sub> X <sub>25</sub> X <sub>26</sub> YW S (SEQ ID NOS 126, 94, 126, 174 and 252, respectively, in order of appearance)
H7	G F T F I S Y G M H
H4	G Y T F T G Y Y I H
H2, H9	G F T F S S Y G M H
H10	G Y S F T S Y W I G
CONSENSUS:	G X <sub>27</sub> X <sub>28</sub> FX <sub>29</sub> X <sub>30</sub> YX <sub>31</sub> X <sub>32</sub> X <sub>33</sub> (SEQ ID NOS 110, 256, 285, 158 and 257, respectively, in order of appearance)
H13	G Y T F T S Y G L S
H12	G F T F S A Y G M H
H3	G F T F S S Y W M S
H1, H14	G Y T F T S Y G I S

TABLE 4-continued

Heavy chain CDR1 consensus sequences for Antibodies A1-A14.

Heavy Chain	CDR1 Sequence
CONSENSUS:	GX <sub>34</sub> TFX <sub>35</sub> X <sub>36</sub> YX <sub>37</sub> X <sub>38</sub> X <sub>39</sub> (SEQ ID NOS 62, 190, 286, 206 and 140, respectively, in order of appearance)
X <sub>21</sub>	is an isoleucine residue or a phenylalanine residue
X <sub>22</sub>	is an asparagine residue or a serine residue
X <sub>23</sub>	is a serine residue or an alanine residue
X <sub>24</sub>	is a glycine residue or no residue
X <sub>25</sub>	is a glycine residue or no residue
X <sub>26</sub>	is a phenylalanine residue or a tyrosine residue
X <sub>27</sub>	is a tyrosine residue or a phenylalanine residue,
X <sub>28</sub>	is a threonine residue or a serine residue,
X <sub>29</sub>	is a threonine residue, a serine residue, or an isoleucine residue,
X <sub>30</sub>	is a glycine residue or a serine residue,
X <sub>31</sub>	is a tyrosine residue, a glycine residue, or a tryptophan residue,
X <sub>32</sub>	is an isoleucine residue or a methionine residue,
X <sub>33</sub>	is a histidine residue or a glycine residue
X <sub>34</sub>	is a tyrosine residue or a phenylalanine residue,
X <sub>35</sub>	is a threonine residue or a serine residue,
X <sub>36</sub>	is a serine residue or an alanine residue,
X <sub>37</sub>	is a glycine residue or a tryptophan residue,
X <sub>38</sub>	is a leucine residue, a methionine residue, or an isoleucine residue,
X <sub>39</sub>	is a serine residue or a histidine residue

25

TABLE 5

Heavy chain CDR2 consensus sequences for Antibodies A1-A14.

Heavy Chain	CDR2 Sequence
H11	Y I S Y S G S T Y Y N P S L K S
H5	Y I Y Y S G S T N Y N P S L K S
H6	E I N H S G G T N Y N P S L K S
H8	Y I Y Y S G S T N Y N P S L K R
CONSENSUS:	X <sub>47</sub> I X <sub>48</sub> X <sub>49</sub> S G X <sub>50</sub> T X <sub>51</sub> Y N P S L K X <sub>52</sub> (SEQ ID NOS 175, 79, 95, 127 and 142, respectively, in order of appearance)
H2, H9	V I W Y D G S N K Y H A D S V K G
H12	V I W Y D G S N K Y Y A D S V K G
H3	N I K Q D G S E E Y Y V D S V K G
H7	V I W Y D G S T E Y Y A D S V K G
CONSENSUS:	X <sub>53</sub> I X <sub>54</sub> X <sub>55</sub> D G S X <sub>56</sub> X <sub>57</sub> Y X <sub>58</sub> X <sub>59</sub> D S V K G (SEQ ID NOS 143, 191, 287, 111 and 179, respectively, in order of appearance)
H4	W I N P N S G G T N Y A Q K F Q G
H1	W I I P Y N G N T N S A Q K L Q G
H13	W I S A Y N G N T N Y A Q K F Q G
H14	W I S P Y N G N T N Y A Q K F Q G
H10	I I Y P G D S D T R Y S P S F Q G

TABLE 5-continued

Heavy chain CDR2 consensus sequences for Antibodies A1-A14.

Heavy Chain	CDR2 Sequence
CONSENSUS:	X <sub>60</sub> I X <sub>61</sub> X <sub>62</sub> X <sub>63</sub> X <sub>64</sub> X <sub>65</sub> X <sub>66</sub> T X <sub>67</sub> X <sub>68</sub> X <sub>69</sub> X <sub>70</sub> X <sub>71</sub> X <sub>72</sub> Q G (SEQ ID NOS 258, 63, 207, 259, 159 and 180, respectively, in order of appearance)
X <sub>47</sub>	is a tyrosine residue or a glutamate residue,
X <sub>48</sub>	is a serine residue, a tyrosine residue, or an asparagine residue,
X <sub>49</sub>	is a tyrosine residue or a histidine residue
X <sub>50</sub>	is a serine residue or a glycine residue,
X <sub>51</sub>	is a tyrosine residue or an asparagine residue,
X <sub>52</sub>	is a serine residue or an arginine residue
X <sub>53</sub>	is an asparagine residue or a valine residue,
X <sub>54</sub>	is a tryptophan residue or a lysine residue,
X <sub>55</sub>	is a tyrosine residue or a glutamine residue,
X <sub>56</sub>	is an asparagine residue, a glutamate residue, or a serine residue,
X <sub>57</sub>	is a lysine residue or a glutamate residue,
X <sub>58</sub>	is a histidine residue or a tyrosine residue,
X <sub>59</sub>	is an alanine residue or a valine residue
X <sub>60</sub>	is a tryptophan residue or an isoleucine residue,
X <sub>61</sub>	is an asparagine residue, an isoleucine residue, a serine residue, or a tyrosine residue,
X <sub>62</sub>	is a proline residue or an alanine residue,
X <sub>63</sub>	is an asparagine residue, a tyrosine residue, or a glycine residue,
X <sub>64</sub>	is a serine residue, an asparagine residue, or an aspartate residue,
X <sub>65</sub>	is a glycine residue or a serine residue,
X <sub>66</sub>	is a glycine residue, an asparagine residue, or an aspartate residue,
X <sub>67</sub>	is an asparagine residue or an arginine residue,
X <sub>68</sub>	is a tyrosine residue or a serine residue,
X <sub>69</sub>	is an alanine residue or a serine residue
X <sub>70</sub>	is a glutamine residue or a proline residue,
X <sub>71</sub>	is a lysine residue or a serine residue,
X <sub>72</sub>	is a phenylalanine residue or a leucine residue

TABLE 6

Heavy chain CDR3 consensus sequences for Antibodies A1-A14.

Heavy Chain	CDR3 Sequence
H5, H8	- - D S I A A P F D Y
H6	V Q W L E L A Y F D Y
H10	- - - - Q G L G F D Y
CONSENSUS:	X <sub>87</sub> X <sub>88</sub> X <sub>89</sub> X <sub>90</sub> X <sub>91</sub> X <sub>92</sub> X <sub>93</sub> X <sub>94</sub> FDY (SEQ ID NOS 80, 96, 160 and 187, respectively, in order of appearance)
H13	D Q D Y Y D S S G W - G H
H14	D Q D Y Y D S S G W - D P
H11	- - A Y G D Y R G W F D P
CONSENSUS:	X <sub>95</sub> X <sub>96</sub> X <sub>97</sub> Y X <sub>98</sub> D X <sub>99</sub> X <sub>100</sub> G W X <sub>101</sub> X <sub>102</sub> X <sub>103</sub> (SEQ ID NOS 208, 224, 176 and 188, respectively, in order of appearance)
H4	- - - D S G Y S S S W H F D Y -
H1	- - - D R D Y G V N Y D A F D I
H2	- S R N W N Y D N Y Y Y G L D V
H12	- S R N W N Y D S Y Q Y G L D V
H9	- S R N W N Y D N Y Y Y G L D V
H3	G S S S W Y Y - Y N G M D V -
H7	- E R Q W L Y - - H Y G M D V

TABLE 6-continued

#### Heavy chain CDR3 consensus sequences for Antibodies A1-A14.

**Heavy Chain CDR3 Sequence**

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**CONSENSUS:** X<sub>104</sub>X<sub>105</sub>X<sub>106</sub>X<sub>107</sub>X<sub>108</sub>X<sub>109</sub>YX<sub>110</sub>X<sub>111</sub>X<sub>112</sub>X<sub>113</sub>X<sub>114</sub>X<sub>115</sub>  
X<sub>116</sub>X<sub>117</sub>X<sub>118</sub>  
(SEQ ID NOS 260, 64, 144, 192, 144, 261, 112 and  
249, respectively, in order of appearance)

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X<sub>87</sub> is a valine residue or no residue,  
X<sub>88</sub> is a glutamine residue or no residue,  
X<sub>89</sub> is an aspartate residue, a tryptophan residue, or no residue,  
X<sub>90</sub> is a serine residue, a leucine residue, or no residue,  
X<sub>91</sub> is an isoleucine residue, a glutamate residue, or a glutamine residue,  
X<sub>92</sub> is an alanine residue, a leucine residue, or a glycine residue,  
X<sub>93</sub> is an alanine residue or a leucine residue,  
X<sub>94</sub> is a proline residue, a tyrosine residue, or a glycine residue  
X<sub>95</sub> is an aspartate residue or no residue,  
X<sub>96</sub> is a glutamine residue or no residue,  
X<sub>97</sub> is an aspartate residue or an alanine residue,  
X<sub>98</sub> is a tyrosine residue or a glycine residue,  
X<sub>99</sub> is a serine residue or a tyrosine residue,  
X<sub>100</sub> is a serine residue or an arginine residue,  
X<sub>101</sub> is a phenylalanine residue or no residue,  
X<sub>102</sub> is a glycine residue or an aspartate residue,  
X<sub>103</sub> is a histidine residue or a proline residue  
X<sub>104</sub> is a glycine residue or no residue  
X<sub>105</sub> is a serine residue, a glutamate residue, or no residue  
X<sub>106</sub> is an arginine residue, a serine residue, or no residue,  
X<sub>107</sub> is an aspartate residue, an asparagine residue, a serine residue, or a glutamine residue  
X<sub>108</sub> is a serine residue, an arginine residue, or a tryptophan residue,  
X<sub>109</sub> is a glycine residue, an aspartate residue, an asparagine residue, a tyrosine residue, or a leucine residue,  
X<sub>110</sub> is a serine residue, a glycine residue, an aspartate residue, or no residue,  
X<sub>111</sub> is a serine residue, a valine residue, an asparagine residue, or a tyrosine residue,  
X<sub>112</sub> is a serine residue, an asparagine residue, a tyrosine residue, or a histidine residue  
X<sub>113</sub> is a tryptophan residue, a tyrosine residue, or a glutamine residue, X<sub>114</sub> is a histidine residue, an aspartate residue, a tyrosine residue, or no residue,  
X<sub>115</sub> is a phenylalanine residue, an alanine residue, or a glycine residue,  
X<sub>116</sub> an aspartate residue, a phenylalanine residue, a leucine residue, or a methionine residue  
X<sub>117</sub> a tyrosine residue, or an aspartate residue,  
X<sub>118</sub> is an isoleucine residue, a valine residue, or no residue

In one embodiment, the present invention provides an antigen binding protein comprising a light chain variable domain comprising a sequence of amino acids that differs from the sequence of a light chain variable domain selected from the group consisting of L1 through L14 only at 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 residues, wherein each such sequence difference is independently either a deletion, insertion, or substitution of one amino acid residue. In another embodiment, the light-chain variable domain comprises a sequence of amino acids that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, or 99% identical to the sequence of a light chain variable domain selected from the group consisting of L1-L14. In another embodiment, the light chain variable domain comprises a sequence of amino acids that is encoded by a nucleotide sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, or 99% identical to a nucleotide sequence that encodes a light chain variable domain selected from the group consisting of L1-L14 (which includes L1, L2, L3, L4, L5, L6, L7, L8, L9, L10, L11, L12, L13, and L14). In another embodiment, the light chain variable domain comprises a sequence of amino acids that is encoded by a polynucleotide that hybridizes under moderately stringent conditions to the complement of a polynucleotide that encodes a light chain variable domain selected from the group consisting of L1-L14. In another embodiment, the light chain variable domain comprises a sequence of amino acids that is encoded by a polynucleotide that hybridizes under moderately stringent conditions to the complement of a polynucleotide that encodes a light chain

variable domain selected from the group consisting of L1-L14. In another embodiment, the light chain variable domain comprises a sequence of amino acids that is encoded by a polynucleotide that hybridizes under moderately stringent conditions to a complement of a light chain polynucleotide of L1-L14.

45 In another embodiment, the present invention provides an antigen binding protein comprising a heavy chain variable domain comprising a sequence of amino acids that differs from the sequence of a heavy chain variable domain selected from the group consisting of H1-H14 only at 15, 14, 13, 12,  
50 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 residue(s), wherein each such sequence difference is independently either a deletion, insertion, or substitution of one amino acid residue. In another embodiment, the heavy chain variable domain comprises a sequence of amino acids that is at least 70%, 75%, 80%,  
55 85%, 90%, 95%, 97%, or 99% identical to the sequence of a heavy chain variable domain selected from the group consisting of H1-H14. In another embodiment, the heavy chain variable domain comprises a sequence of amino acids that is encoded by a nucleotide sequence that is at least 70%,  
60 75%, 80%, 85%, 90%, 95%, 97%, or 99% identical to a nucleotide sequence that encodes a heavy chain variable domain selected from the group consisting of H1-H14. In another embodiment, the heavy chain variable domain comprises a sequence of amino acids that is encoded by a  
65 polynucleotide that hybridizes under moderately stringent conditions to the complement of a polynucleotide that encodes a heavy chain variable domain selected from the

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group consisting of H1-H14. In another embodiment, the heavy chain variable domain comprises a sequence of amino acids that is encoded by a polynucleotide that hybridizes under moderately stringent conditions to the complement of a polynucleotide that encodes a heavy chain variable domain selected from the group consisting of H1-H14. In another embodiment, the heavy chain variable domain comprises a sequence of amino acids that is encoded by a polynucleotide that hybridizes under moderately stringent conditions to a complement of a heavy chain polynucleotide disclosed herein.

Particular embodiments of antigen binding proteins of the present invention comprise one or more amino acid sequences that are identical to the amino acid sequences of one or more of the CDRs and/or FRs referenced herein. In one embodiment, the antigen binding protein comprises a light chain CDR1 sequence illustrated above. In another embodiment, the antigen binding protein comprises a light chain CDR2 sequence illustrated above. In another embodiment, the antigen binding protein comprises a light chain CDR3 sequence illustrated above. In another embodiment, the antigen binding protein comprises a heavy chain CDR1 sequence illustrated above. In another embodiment, the antigen binding protein comprises a heavy chain CDR2 sequence illustrated above. In another embodiment, the antigen binding protein comprises a heavy chain CDR3 sequence illustrated above. In another embodiment, the antigen binding protein comprises a light chain FR1 sequence illustrated above. In another embodiment, the antigen binding protein comprises a light chain FR2 sequence illustrated above. In another embodiment, the antigen binding protein comprises a light chain FR3 sequence illustrated above. In another embodiment, the

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antigen binding protein comprises a light chain FR4 sequence illustrated above. In another embodiment, the antigen binding protein comprises a heavy chain FR1 sequence illustrated above. In another embodiment, the antigen binding protein comprises a heavy chain FR2 sequence illustrated above. In another embodiment, the antigen binding protein comprises a heavy chain FR3 sequence illustrated above. In another embodiment, the antigen binding protein comprises a heavy chain FR4 sequence illustrated above.

In one embodiment, the present invention provides an antigen binding protein that comprises one or more CDR sequences that differ from a CDR sequence shown above by no more than 5, 4, 3, 2, or 1 amino acid residues.

In another embodiment, at least one of the antigen binding protein's CDR3 sequences is a CDR3 sequence from A1-A14, as shown in Table 7 or Table 8. In another embodiment, the antigen binding protein's light chain CDR3 sequence is a light chain CDR3 sequence from A1-A14 as shown in Table 7 and the antigen binding protein's heavy chain CDR3 sequence is a heavy chain sequence from A1-A14 as shown in Table 8. In another embodiment, the antigen binding protein comprises 1, 2, 3, 4, or 5 CDR sequence(s) that each independently differs by 6, 5, 4, 3, 2, 1, or 0 single amino acid additions, substitutions, and/or deletions from a CDR sequence of A1-A14, and the antigen binding protein further comprises 1, 2, 3, 4, or 5 CDR sequence(s) that each independently differs by 6, 5, 4, 3, 2, 1, or 0 single amino acid additions, substitutions, and/or deletions from a CDR sequence.

The light chain CDRs of antibodies A1-A14 are shown below in Table 7, and the heavy chain CDRs of antibodies A1-A14 are shown below in Table 8.

TABLE 7

Antibody	Light chain CDRs		
	CDR1	CDR2	CDR3
A1	SGDKLGDKYAC (SEQ ID NO: 59)	QDSKRPS (SEQ ID NO: 60)	QAWDSSTAV (SEQ ID NO: 61)
A2	RASQGIRNNNLG (SEQ ID NO: 281)	AASSLQS (SEQ ID NO: 283)	LQHNSYPWT (SEQ ID NO: 141)
A3	RASQGIRNDLG (SEQ ID NO: 282)	AASSLQS (SEQ ID NO: 283)	RQQNTYPLT (SEQ ID NO: 284)
A4	RSSQSLLHSTGYNYLD (SEQ ID NO: 253)	LGSFRAS (SEQ ID NO: 254)	MQALQTPCS (SEQ ID NO: 255)
A5	KSSQSILYSSNNKKYLV (SEQ ID NO: 75)	WTSMRES (SEQ ID NO: 76)	QQYYSTPWT (SEQ ID NO: 77)
A6	RASQSIISNYLN (SEQ ID NO: 91)	ATSSLQS (SEQ ID NO: 92)	QQSYSISPT (SEQ ID NO: 93)
A7	RAGQGIRNDLV (SEQ ID NO: 107)	AASSLQS (SEQ ID NO: 283)	LQHNTYPFT (SEQ ID NO: 109)
A8	KSSQSILYSSNNKKYLV (SEQ ID NO: 75)	WTSMRES (SEQ ID NO: 76)	QQYYSTPWT (SEQ ID NO: 77)
A9	RASQGIRNNNLG (SEQ ID NO: 281)	AASSLQS (SEQ ID NO: 283)	LQHNSYPWT (SEQ ID NO: 141)
A10	SGEKWGEKYAC (SEQ ID NO: 155)	QDTKRPS (SEQ ID NO: 156)	QAWDRSTV (SEQ ID NO: 157)
A11	SGDKLGDKFAF (SEQ ID NO: 171)	QDNKRPS (SEQ ID NO: 172)	QAWDSSTVV (SEQ ID NO: 173)

TABLE 7-continued

Light chain CDRs			
Antibody	CDR1	CDR2	CDR3
A12	RASQGIRNDLG (SEQ ID NO: 282)	AASSLQS (SEQ ID NO: 283)	LQHNSYTWT (SEQ ID NO: 189)
A13	SGDKLGDKYVC (SEQ ID NO: 203)	LDNKRPS (SEQ ID NO: 204)	QAWDSSTV (SEQ ID NO: 205)
A14	SGDKLGDKYAF (SEQ ID NO: 219)	HDTKRPS (SEQ ID NO: 220)	QAWDSSTV (SEQ ID NO: 205)

TABLE 8

Heavy chain CDRs			
Antibody	CDR1	CDR2	CDR3
A1	GYTFTSYGLS (SEQ ID NO: 62)	WIIPYNGNTNSAQKLQG (SEQ ID NO: 63)	DRDYGVNYDAFDI (SEQ ID NO: 64)
A2	GPTFSSYGMH (SEQ ID NO: 285)	VIWYDGNSNKYHADSVKG (SEQ ID NO: 143)	SRNWNYDNEYGLDV (SEQ ID NO: 144)
A3	GPTFSSYWMS (SEQ ID NO: 286)	NIKQDGSEEEYVDSVKG (SEQ ID NO: 287)	GSSSWYYYNYGMDV (SEQ ID NO: 288)
A4	GYTFTGYYIH (SEQ ID NO: 256)	WINPNSSGGTNYAQKFQG (SEQ ID NO: 258)	DSGYSSWHFDY (SEQ ID NO: 260)
A5	GGSINSFYWS (SEQ ID NO: 78)	YIYYSGSTYNPNSLKS (SEQ ID NO: 79)	DSIAAPFDY (SEQ ID NO: 80)
A6	GGSFSAYWWS (SEQ ID NO: 94)	EINHSGGTNYNPSLKS (SEQ ID NO: 95)	VQWLELAYFDY (SEQ ID NO: 96)
A7	GPTFISYGMH (SEQ ID NO: 110)	VIWYDGSTEYYADSVKG (SEQ ID NO: 111)	ERQWLYHYGMDV (SEQ ID NO: 112)
A8	GGSINSFYWS (SEQ ID NO: 126)	YIYYSGSTYNPNSLKR (SEQ ID NO: 127)	DSIAAPFDY (SEQ ID NO: 80)
A9	GPTFSSYGMH (SEQ ID NO: 285)	VIWYDGNSNKYHADSVKG (SEQ ID NO: 143)	SRNWNYDNEYGLDV (SEQ ID NO: 144)
A10	GYSFTSYWIG (SEQ ID NO: 158)	IIYPGDSDTRYSPSFQG (SEQ ID NO: 159)	QGLGF DY (SEQ ID NO: 160)
A11	GGSISSSGGYYWS (SEQ ID NO: 174)	YISYSGSTYYNPSLKS (SEQ ID NO: 175)	AYGDYRGWFDP (SEQ ID NO: 176)
A12	GPTFSAYGMH (SEQ ID NO: 190)	VIWYDGNSNKYHADSVKG (SEQ ID NO: 191)	SRNWNYDSYQYGLDV (SEQ ID NO: 192)
A13	GYTFTSYGIS (SEQ ID NO: 206)	WISPYNGNTNYAQKFQG (SEQ ID NO: 207)	DQDYDSSGWGH (SEQ ID NO: 208)
A14	GYTFTSYGIS (SEQ ID NO: 206)	WISPYNGNTNYAQKFQG (SEQ ID NO: 259)	DQDYDSSGWDP (SEQ ID NO: 224)

The nucleotide sequences of A1-A14, or the amino acid sequences of A1-A14, can be altered, for example, by random mutagenesis or by site-directed mutagenesis (e.g., oligonucleotide-directed site-specific mutagenesis) to create an altered polynucleotide comprising one or more particular nucleotide substitutions, deletions, or insertions as compared to the non-mutated polynucleotide. Examples of techniques for making such alterations are described in Walder et al., 1986, Gene 42:133; Bauer et al. 1985, Gene 37:73; Craik, BioTechniques, January 1985, 12-19; Smith et al., 1981, *Genetic Engineering: Principles and Methods*, Ple-

num Press; and U.S. Pat. Nos. 4,518,584 and 4,737,462. These and other methods can be used to make, for example, derivatives of anti-activin-A antibodies that have a desired property, for example, increased affinity, avidity, or specificity for activin-A, increased activity or stability in vivo or in vitro, or reduced in vivo side-effects as compared to the underivatized antibody.

Other derivatives of anti-activin-A antibodies within the scope of this invention include covalent or aggregative conjugates of anti-activin-A antibodies, or fragments thereof, with other proteins or polypeptides, such as by

expression of recombinant fusion proteins comprising heterologous polypeptides fused to the N-terminus or C-terminus of an anti-activin-A antibody polypeptide. For example, the conjugated peptide may be a heterologous signal (or leader) polypeptide, e.g., the yeast alpha-factor leader, or a peptide such as an epitope tag. Antigen binding protein-containing fusion proteins can comprise peptides added to facilitate purification or identification of antigen binding protein (e.g., poly-His). An antigen binding protein also can be linked to the FLAG peptide Asp-Tyr-Lys-Asp-Asp-Asp-Lys (DYKDDDDK) (SEQ ID NO: 226) as described in Hopp et al., *Bio Technology* 6:1204, 1988, and U.S. Pat. No. 5,011,912. The FLAG peptide is highly antigenic and provides an epitope reversibly bound by a specific monoclonal antibody (mAb), enabling rapid assay and facile purification of expressed recombinant protein. Reagents useful for preparing fusion proteins in which the FLAG peptide is fused to a given polypeptide are commercially available (Sigma, St. Louis, MO).

The term "Fc polypeptide" as used herein includes native and mutein forms of polypeptides derived from the Fc region of an antibody. Truncated forms of such polypeptides containing the hinge region that promotes dimerization also are included. Fusion proteins comprising Fc moieties (and oligomers formed therefrom) offer the advantage of facile purification by affinity chromatography over Protein A or Protein G columns.

One suitable Fc polypeptide, described in PCT application WO 93/10151 (hereby incorporated by reference), is a single chain polypeptide extending from the N-terminal hinge region to the native C-terminus of the Fc region of a human IgG1 antibody. Another useful Fc polypeptide is the Fc mutein described in U.S. Pat. No. 5,457,035 and in Baum et al., 1994, *EMBO J.* 13:3992-4001. The amino acid sequence of this mutein is identical to that of the native Fc sequence presented in WO 93/10151, except that amino acid 19 has been changed from Leu to Ala, amino acid 20 has been changed from Leu to Glu, and amino acid 22 has been changed from Gly to Ala. The mutein exhibits reduced affinity for Fc receptors.

In other embodiments, the variable portion of the heavy and/or light chains of an anti-activin-A antibody may be substituted for the variable portion of an antibody heavy and/or light chain.

Oligomers that contain one or more antigen binding proteins may be employed as activin-A antagonists. Oligomers may be in the form of covalently-linked or non-covalently-linked dimers, trimers, or higher oligomers. Oligomers comprising two or more antigen binding protein are contemplated for use, with one example being a homodimer. Other oligomers include heterodimers, homotrimers, heterotrimers, homotetramers, heterotetramers, etc.

One embodiment is directed to oligomers comprising multiple antigen binding proteins joined via covalent or non-covalent interactions between peptide moieties fused to the antigen binding proteins. Such peptides may be peptide linkers (spacers), or peptides that have the property of promoting oligomerization. Leucine zippers and certain polypeptides derived from antibodies are among the peptides that can promote oligomerization of antigen binding proteins attached thereto, as described in more detail below.

In particular embodiments, the oligomers comprise from two to four antigen binding proteins. The antigen binding proteins of the oligomer may be in any form, such as any of the forms described above, e.g., variants or fragments. Preferably, the oligomers comprise antigen binding proteins that have activin-A binding activity.

In one embodiment, an oligomer is prepared using polypeptides derived from immunoglobulins. Preparation of fusion proteins comprising certain heterologous polypeptides fused to various portions of antibody-derived polypeptides (including the Fc domain) has been described, e.g., by Ashkenazi et al., 1991, *PNAS USA* 88:10535; Byrn et al., 1990, *Nature* 344:677; and Hollenbaugh et al., 1992 *Curr. Protos in Immunol.*, Suppl. 4, pages 10.19.1-10.19.11.

One embodiment of the present invention is directed to a dimer comprising two fusion proteins created by fusing an activin-A binding fragment of an anti-activin-A antibody to the Fc region of an antibody. The dimer can be made by, for example, inserting a gene fusion encoding the fusion protein into an appropriate expression vector, expressing the gene fusion in host cells transformed with the recombinant expression vector, and allowing the expressed fusion protein to assemble much like antibody molecules, whereupon interchain disulfide bonds form between the Fc moieties to yield the dimer.

Alternatively, the oligomer is a fusion protein comprising multiple antigen binding proteins, with or without peptide linkers (spacer peptides). Among the suitable peptide linkers are those described in U.S. Pat. Nos. 4,751,180 and 4,935,233.

Another method for preparing oligomeric antigen binding proteins involves use of a leucine zipper. Leucine zipper domains are peptides that promote oligomerization of the proteins in which they are found. Leucine zippers were originally identified in several DNA-binding proteins (Landschulz et al., 1988, *Science* 240:1759), and have since been found in a variety of different proteins. Among the known leucine zippers are naturally occurring peptides and derivatives thereof that dimerize or trimerize. Examples of leucine zipper domains suitable for producing soluble oligomeric proteins are described in PCT application WO 94/10308, and the leucine zipper derived from lung surfactant protein D (SPD) described in Hoppe et al., 1994, *FEBS Letters* 344:191, hereby incorporated by reference. The use of a modified leucine zipper that allows for stable trimerization of a heterologous protein fused thereto is described in Fanslow et al., 1994, *Semin. Immunol.* 6:267-78. In one approach, recombinant fusion proteins comprising an anti-activin-A antibody fragment or derivative fused to a leucine zipper peptide are expressed in suitable host cells, and the soluble oligomeric anti-activin-A antibody fragments or derivatives that form are recovered from the culture supernatant.

In one aspect, the present invention provides antigen binding proteins that interfere with the binding of activin-A to an activin-A receptor. Such antigen binding proteins can be made against activin-A, or a fragment, variant or derivative thereof, and screened in conventional assays for the ability to interfere with binding of activin-A to activin-A receptor. Examples of suitable assays are assays that test the antigen binding proteins for the ability to inhibit binding of activin-A to cells expressing activin-A receptor, or that test antigen binding proteins for the ability to reduce a biological or cellular response that results from the binding of activin-A to cell surface activin-A receptors. For example, antibodies can be screened according to their ability to bind to immobilized antibody surfaces (activin-A and/or activin B). Antigen binding proteins that block the binding of activin-A to an activin-A receptor can be employed in treating any activin-A-related condition, including but not limited to cachexia. In an embodiment, a human anti-

activin-A monoclonal antibody generated by procedures involving immunization of transgenic mice is employed in treating such conditions.

Antigen-binding fragments of antigen binding proteins of the invention can be produced by conventional techniques. Examples of such fragments include, but are not limited to, Fab and F(ab')<sub>2</sub> fragments. Antibody fragments and derivatives produced by genetic engineering techniques also are contemplated.

Additional embodiments include chimeric antibodies, e.g., humanized versions of non-human (e.g., murine) monoclonal antibodies. Such humanized antibodies may be prepared by known techniques, and offer the advantage of reduced immunogenicity when the antibodies are administered to humans. In one embodiment, a humanized monoclonal antibody comprises the variable domain of a murine antibody (or all or part of the antigen binding site thereof) and a constant domain derived from a human antibody. Alternatively, a humanized antibody fragment may comprise the antigen binding site of a murine monoclonal antibody and a variable domain fragment (lacking the antigen-binding site) derived from a human antibody. Procedures for the production of chimeric and further engineered monoclonal antibodies include those described in Riechmann et al., 1988, *Nature* 332:323, Liu et al., 1987, *Proc. Nat. Acad. Sci. USA* 84:3439, Larrick et al., 1989, *Bio/Technology* 7:934, and Winter et al., 1993, *TIPS* 14:139. In one embodiment, the chimeric antibody is a CDR grafted antibody. Techniques for humanizing antibodies are discussed in, e.g., U.S. Pat. Nos. 5,869,619, 5,225,539, 5,821,337, 5,859,205, 6,881,557, Padlan et al., 1995, *FASEB J.* 9:133-39, and Tamura et al., 2000, *J. Immunol.* 164:1432-41.

Procedures have been developed for generating human or partially human antibodies in non-human animals. For example, mice in which one or more endogenous immunoglobulin genes have been inactivated by various means have been prepared. Human immunoglobulin genes have been introduced into the mice to replace the inactivated mouse genes. Antibodies produced in the animal incorporate human immunoglobulin polypeptide chains encoded by the human genetic material introduced into the animal. In one embodiment, a non-human animal, such as a transgenic mouse, is immunized with an activin-A polypeptide, such that antibodies directed against the activin-A polypeptide are generated in the animal.

One example of a suitable immunogen is a soluble human activin-A, such as a polypeptide comprising the extracellular domain of the protein having the following sequence: Gly Leu Glu Cys Asp Gly Lys Val Asn Ile Cys Cys Lys Lys Gln Phe Phe Val Ser Phe Lys Asp Ile Gly Trp Asn Asp Trp Ile Ile Ala Pro Ser Gly Tyr His Ala Asn Tyr Cys Glu Gly Glu Cys Pro Ser His Ile Ala Gly Thr Ser Gly Ser Ser Leu Ser Phe His Ser Thr Val Ile Asn His Tyr Arg Met Arg Gly His Ser Pro Phe Ala Asn Leu Lys Ser Cys Cys Val Pro Thr Lys Leu Arg Pro Met Ser Met Leu Tyr Tyr Asp Asp Gly Gln Asn Ile Ile Lys Lys Asp Ile Gln Asn Met Ile Val Glu Glu Cys Gly Cys Ser (SEQ ID NO: 225)), or other immunogenic fragment of the protein. Examples of techniques for production and use of transgenic animals for the production of human or partially human antibodies are described in U.S. Pat. Nos. 5,814,318, 5,569,825, and 5,545,806, Davis et al., 2003, Production of human antibodies from transgenic mice in Lo, ed. *Antibody Engineering: Methods and Protocols*, Humana Press, NJ:191-200, Kellermann et al., 2002, *Curr Opin Biotechnol.* 13:593-97, Russel et al., 2000, *Infect Immun.* 68:1820-26, Gallo et al., 2000, *Eur J Immun.* 30:534-40, Davis et al., 1999, *Cancer Metastasis Rev.* 18:421-25, Green, 1999, J

*Immunol Methods.* 231:11-23, Jakobovits, 1998, *Advanced Drug Delivery Reviews* 31:33-42, Green et al., 1998, *J Exp Med.* 188:483-95, Jakobovits A, 1998, *Exp. Opin. Invest. Drugs.* 7:607-14, Tsuda et al., 1997, *Genomics.* 42:413-21, Mendez et al., 1997, *Nat Genet.* 15:146-56, Jakobovits, 1994, *Curr Biol.* 4:761-63, Arbones et al., 1994, *Immunity.* 1:247-60, Green et al., 1994, *Nat Genet.* 7:13-21, Jakobovits et al., 1993, *Nature.* 362:255-58, Jakobovits et al., 1993, *Proc Natl Acad Sci USA.* 90:2551-55, Chen, J., M. Trounstein, F. W. Alt, F. Young, C. Kurahara, J. Loring, D. Huszar, *Inter'l Immunol.* 5 (1993): 647-656, Choi et al., 1993, *Nature Genetics* 4: 117-23, Fishwild et al., 1996, *Nature Biotech.* 14: 845-51, Harding et al., 1995, Annals of the New York Academy of Sciences, Lonberg et al., 1994, *Nature* 368: 856-59, Lonberg, 1994, Transgenic Approaches to Human Monoclonal Antibodies in Handbook of Experimental Pharmacology 113: 49-101, Lonberg et al., 1995, Internal Review of Immunology 13: 65-93, Neuberger, 1996, *Nature Biotechnology* 14: 826, Taylor et al., 1992, *Nucleic Acids Res.* 20: 6287-95, Taylor et al., 1994, *Inter'l Immunol.* 6: 579-91, Tomizuka et al., 1997, *Nature Genetics* 16: 133-43, Tomizuka et al., 2000, *Proc. Nat'l Acad. Sci. USA* 97: 722-27, Tuailion et al., 1993, *Proc. Nat'l Acad. Sci. USA* 90: 3720-24, and Tuailion et al., 1994, *J Immunol.* 152: 2912-20.

In another aspect, the present invention provides monoclonal antibodies that bind to activin-A. Monoclonal antibodies of the invention may be generated using a variety of known techniques. In general, monoclonal antibodies that bind to specific antigens may be obtained by methods known to those skilled in the art (see, for example, Kohler et al., *Nature* 256:495, 1975; Coligan et al. (eds.), *Current Protocols in Immunology*, 1:2.5.12.6.7 (John Wiley & Sons 1991); U.S. Pat. Nos. RE 32,011, 4,902,614, 4,543,439, and 4,411, 993; *Monoclonal Antibodies, Hybridomas: A New Dimension in Biological Analyses*, Plenum Press, Kennett, McKeam, and Bechtol (eds.) (1980); and *Antibodies: A Laboratory Manual*, Harlow and Lane (eds.), Cold Spring Harbor Laboratory Press (1988); Picksley et al., "Production of monoclonal antibodies against proteins expressed in *E. coli*," in *DNA Cloning 2: Expression Systems*, 2nd Edition, Glover et al. (eds.), page 93 (Oxford University Press 1995)). Antibody fragments may be derived therefrom using any suitable standard technique such as proteolytic digestion, or optionally, by proteolytic digestion (for example, using papain or pepsin) followed by mild reduction of disulfide bonds and alkylation. Alternatively, such fragments may also be generated by recombinant genetic engineering techniques as described herein.

Monoclonal antibodies can be obtained by injecting an animal, for example, a rat, hamster, a rabbit, or preferably a mouse, including for example a transgenic or a knock-out, as known in the art, with an immunogen comprising human activin-A (cagggtcage tgcaggagtc gggcccaagga ctggtaaagc cttcgagac cctgtccctc acctgtcaactg tctctggtagt ctccatcaat agtttcattt ggagctggat ccggcagccc ccagggaaagg gactggagtg gat-tgggtat atcttattaca gtggggagac caactacaat ccctccctca agagtcgagt caccatataca gtagacacgt ccaagaccca gttctccctg aagctcgatct ctgtggccgc tgccggacacg gcccgttattt actgtgcgag agacagttata gcacccccct ttgactactg gggccaggaa accctggtaa cccgttcctc agettcacac aaggcccat cccgttcctc cctggccccc tgctccaggaa gcacccctcgaa gacccacggccgcctgggtt gcctggtaa agactacttc cccgaacccgg tgacgggttc tgccgtactca tgccgcct (SEQ ID NO: 66)), or a fragment thereof, according to methods known in the art and described herein. The presence of specific antibody production may be monitored after the initial injection and/or after a booster injection by obtaining a serum sample and detecting the presence of an antibody

that binds to human activin-A or peptide using any one of several immunodetection methods known in the art and described herein. From animals producing the desired antibodies, lymphoid cells, most commonly cells from the spleen or lymph node, are removed to obtain B-lymphocytes. The B lymphocytes are then fused with a drug-sensitized myeloma cell fusion partner, preferably one that is syngeneic with the immunized animal and that optionally has other desirable properties (e.g., inability to express endogenous Ig gene products, e.g., P3X63-Ag 8.653 (ATCC No. CRL 1580); NSO, SP20) to produce hybridomas, which are immortal eukaryotic cell lines.

The lymphoid (e.g., spleen) cells and the myeloma cells may be combined for a few minutes with a membrane fusion-promoting agent, such as polyethylene glycol or a nonionic detergent, and then plated at low density on a selective medium that supports the growth of hybridoma cells but not unfused myeloma cells. A preferred selection media is HAT (hypoxanthine, aminopterin, thymidine). After a sufficient time, usually about one to two weeks, colonies of cells are observed. Single colonies are isolated, and antibodies produced by the cells may be tested for binding activity to human activin-A, using any one of a variety of immunoassays known in the art and described herein. The hybridomas are cloned (e.g., by limited dilution cloning or by soft agar plaque isolation) and positive clones that produce an antibody specific to activin-A are selected and cultured. The monoclonal antibodies from the hybridoma cultures may be isolated from the supernatants of hybridoma cultures.

An alternative method for production of a murine monoclonal antibody is to inject the hybridoma cells into the peritoneal cavity of a syngeneic mouse, for example, a mouse that has been treated (e.g., pristane-primed) to promote formation of ascites fluid containing the monoclonal antibody. Monoclonal antibodies can be isolated and purified by a variety of well-established techniques. Such isolation techniques include affinity chromatography with Protein-A Sepharose, size-exclusion chromatography, and ion-exchange chromatography (see, for example, Coligan at pages 2.7.1-2.7.12 and pages 2.9.1-2.9.3; Baines et al., "Purification of Immunoglobulin G (IgG)," in *Methods in Molecular Biology*, Vol. 10, pages 79-104 (The Humana Press, Inc. 1992)). Monoclonal antibodies may be purified by affinity chromatography using an appropriate ligand selected based on particular properties of the antibody (e.g., heavy or light chain isotype, binding specificity, etc.). Examples of a suitable ligand, immobilized on a solid support, include Protein A, Protein G, an anticonstant region (light chain or heavy chain) antibody, an anti-idiotype antibody, and a TGF-beta binding protein, or fragment or variant thereof.

Monoclonal antibodies may be produced using any technique known in the art, e.g., by immortalizing spleen cells harvested from the transgenic animal after completion of the immunization schedule. The spleen cells can be immortalized using any technique known in the art, e.g., by fusing them with myeloma cells to produce hybridomas. Hybridoma cell lines are identified that produce an antibody that binds an activin-A polypeptide. Such hybridoma cell lines, and anti-activin-A monoclonal antibodies produced by them, are encompassed by the present invention. Myeloma cells for use in hybridoma-producing fusion procedures preferably are non-antibody-producing, have high fusion efficiency, and enzyme deficiencies that render them incapable of growing in certain selective media which support the growth of only the desired fused cells (hybridomas).

Examples of suitable cell lines for use in mouse fusions include Sp-20, P3-X63/Ag8, P3-X63-Ag8.653, NS1/1.Ag 41, Sp210-Agl4, FO, NSO/U, MPC-11, MPC11-X45-GTG 1.7 and S194/5XX0 Bul; examples of cell lines used in rat fusions include R210.RCY3, Y3-Ag 1.2.3, IR983F and 4B210. Other cell lines useful for cell fusions are U-266, GM1500-GRG2, LICR-LON-HMy2 and UC729-6. Hybridomas or mAbs may be further screened to identify mAbs with particular properties, such as the ability to block an activin-A-induced activity.

An antibody of the present invention may also be a fully human monoclonal antibody. An isolated fully human antibody is provided that specifically binds to the cysteine knot region (amino acids C11-S33 and/or amino acids C81-E111) 15 of activin-A, wherein the antigen binding protein possesses at least one in vivo biological activity of a human anti-activin-A antibody. The biological activity may be attenuation of cachexia, for example cachexia in colon cancer, such as in a mouse model of colon cancer described herein. The 20 cachexia amenable to such treatment is associated with loss of body weight, loss of muscle mass, and/or loss of fat mass. The cachexia may be associated with rheumatoid arthritis, such as in a collagen-induced animal model of rheumatoid arthritis. Treatment with a fully human antibody described 25 herein ameliorates the loss of body weight, the loss of muscle mass, and/or the loss of fat mass in vivo in a collagen-induced animal model of rheumatoid arthritis. A fully human antibody described herein ameliorates the loss of body weight in a AAV-activin-A transfected animal model. A fully human antibody described herein, that 30 specifically binds to the cysteine knot region (amino acids C11-S33 and/or amino acids C81-E111) of activin-A, inhibits the binding of activin-A to activin-A receptor in vitro. A fully human isolated antibody that specifically binds to the 35 cysteine knot region (amino acids C11-S33 and/or amino acids C81-E111) of activin-A, inhibits the binding of activin-A to activin-A receptor in vivo.

Fully human monoclonal antibodies may be generated by any number of techniques with which those having ordinary skill in the art will be familiar. Such methods include, but are not limited to, Epstein Barr Virus (EBV) transformation of human peripheral blood cells (e.g., containing B lymphocytes), in vitro immunization of human B-cells, fusion of spleen cells from immunized transgenic mice carrying inserted human immunoglobulin genes, isolation from human immunoglobulin V region phage libraries, or other procedures as known in the art and based on the disclosure herein. For example, fully human monoclonal antibodies may be obtained from transgenic mice that have been engineered to produce specific human antibodies in response to antigenic challenge. Methods for obtaining fully human antibodies from transgenic mice are described, for example, by Green et al., *Nature Genet.* 7:13, 1994; Lonberg et al., *Nature* 368:856, 1994; Taylor et al., *Int. Immun.* 6:579, 55 1994; U.S. Pat. No. 5,877,397; Bruggemann et al., 1997 *Curr. Opin. Biotechnol.* 8:455-58; Jakobovits et al., 1995 *Ann. N. Y. Acad. Sci.* 764:525-35. In this technique, elements of the human heavy and light chain locus are introduced into strains of mice derived from embryonic stem cell lines that 60 contain targeted disruptions of the endogenous heavy chain and light chain loci (see also Bruggemann et al., *Curr. Opin. Biotechnol.* 8:455-58 (1997)). For example, human immunoglobulin transgenes may be mini-gene constructs, or transloci on yeast artificial chromosomes, which undergo 65 B-cell-specific DNA rearrangement and hypermutation in the mouse lymphoid tissue. Fully human monoclonal antibodies may be obtained by immunizing the transgenic mice,

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which may then produce human antibodies specific for activin-A. Lymphoid cells of the immunized transgenic mice can be used to produce human antibody-secreting hybridomas according to the methods described herein. Polyclonal sera containing fully human antibodies may also be obtained from the blood of the immunized animals.

Another method for generating human antibodies of the invention includes immortalizing human peripheral blood cells by EBV transformation. See, e.g., U.S. Pat. No. 4,464,456. Such an immortalized B-cell line (or lymphoblastoid cell line) producing a monoclonal antibody that specifically binds to activin-A can be identified by immunodetection methods as provided herein, for example, an ELISA, and then isolated by standard cloning techniques. The stability of the lymphoblastoid cell line producing an anti-activin-A antibody may be improved by fusing the transformed cell line with a murine myeloma to produce a mouse-human hybrid cell line according to methods known in the art (see, e.g., Glasky et al., *Hybridoma* 8:377-89 (1989)). Still another method to generate human monoclonal antibodies is in vitro immunization, which includes priming human splenic B-cells with human activin-A, followed by fusion of primed B-cells with a heterohybrid fusion partner. See, e.g., Boerner et al., 1991 *J. Immunol.* 147:86-95.

In certain embodiments, a B-cell that is producing an anti-human activin-A antibody is selected and the light chain and heavy chain variable regions are cloned from the B-cell according to molecular biology techniques known in the art (WO 92/02551; U.S. Pat. No. 5,627,052; Babcock et al., *Proc. Natl. Acad. Sci. USA* 93:7843-48 (1996)) and described herein. B-cells from an immunized animal may be isolated from the spleen, lymph node, or peripheral blood sample by selecting a cell that is producing an antibody that specifically binds to activin-A. B-cells may also be isolated from humans, for example, from a peripheral blood sample. Methods for detecting single B-cells that are producing an antibody with the desired specificity are well known in the art, for example, by plaque formation, fluorescence-activated cell sorting, in vitro stimulation followed by detection of specific antibody, and the like. Methods for selection of specific antibody-producing B-cells include, for example, preparing a single cell suspension of B-cells in soft agar that contains human activin-A. Binding of the specific antibody produced by the B-cell to the antigen results in the formation of a complex, which may be visible as an immunoprecipitate. After the B-cells producing the desired antibody are selected, the specific antibody genes may be cloned by isolating and amplifying DNA or mRNA according to methods known in the art and described herein.

An additional method for obtaining antibodies of the invention is by phage display. See, e.g., Winter et al., 1994 *Annu. Rev. Immunol.* 12:433-55; Burton et al., 1994 *Adv. Immunol.* 57:191-280. Human or murine immunoglobulin variable region gene combinatorial libraries may be created in phage vectors that can be screened to select Ig fragments (Fab, Fv, sFv, or multimers thereof) that bind specifically to TGF-beta binding protein or variant or fragment thereof. See, e.g., U.S. Pat. No. 5,223,409; Huse et al., 1989 *Science* 246:1275-81; Sastry et al., *Proc. Natl. Acad. Sci. USA* 86:5728-32 (1989); Alting-Mees et al., *Strategies in Molecular Biology* 3:1-9 (1990); Kang et al., 1991 *Proc. Natl. Acad. Sci. USA* 88:4363-66; Hoogenboom et al., 1992 *J. Molec. Biol.* 227:381-388; Schlebusch et al., 1997 *Hybridoma* 16:47-52 and references cited therein. For example, a library containing a plurality of polynucleotide sequences encoding Ig variable region fragments may be inserted into the genome of a filamentous bacteriophage, such as M13 or a

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variant thereof, in frame with the sequence encoding a phage coat protein. A fusion protein may be a fusion of the coat protein with the light chain variable region domain and/or with the heavy chain variable region domain. According to certain embodiments, immunoglobulin Fab fragments may also be displayed on a phage particle (see, e.g., U.S. Pat. No. 5,698,426).

Heavy and light chain immunoglobulin cDNA expression libraries may also be prepared in lambda phage, for example, using  $\lambda$ ImmunoZap™ (H) and  $\lambda$ ImmunoZap™ (L) vectors (Stratagene, La Jolla, California). Briefly, mRNA is isolated from a B-cell population, and used to create heavy and light chain immunoglobulin cDNA expression libraries in the  $\lambda$ ImmunoZap(H) and  $\lambda$ ImmunoZap(L) vectors. These vectors may be screened individually or co-expressed to form Fab fragments or antibodies (see Huse et al., *supra*; see also Sastry et al., *supra*). Positive plaques may subsequently be converted to a non-lytic plasmid that allows high level expression of monoclonal antibody fragments from *E. coli*.

In one embodiment, in a hybridoma the variable regions of a gene expressing a monoclonal antibody of interest are amplified using nucleotide primers. These primers may be synthesized by one of ordinary skill in the art, or may be purchased from commercially available sources. (See, e.g., Stratagene (La Jolla, California), which sells primers for mouse and human variable regions including, among others, primers for  $V_{Ha}$ ,  $V_{Hb}$ ,  $V_{Hc}$ ,  $V_{Hd}$ ,  $C_{H1}$ ,  $V_L$  and  $C_L$  regions.) These primers may be used to amplify heavy or light chain variable regions, which may then be inserted into vectors such as ImmunoZAP™ H or ImmunoZAP™ L (Stratagene), respectively. These vectors may then be introduced into *E. coli*, yeast, or mammalian-based systems for expression. Large amounts of a single-chain protein containing a fusion of the  $V_H$  and  $V_L$  domains may be produced using these methods (see Bird et al., *Science* 242:423-426, 1988).

Once cells producing antibodies according to the invention have been obtained using any of the above-described immunization and other techniques, the specific antibody genes may be cloned by isolating and amplifying DNA or mRNA therefrom according to standard procedures as described herein. The antibodies produced therefrom may be sequenced and the CDRs identified and the DNA coding for the CDRs may be manipulated as described previously to generate other antibodies according to the invention.

Activin-A binding agents of the present invention preferably modulate activin-A function in the cell-based assay described herein and/or the in vivo assay described herein and/or bind to one or more of the cysteine knot domains described herein and/or cross-block the binding of one of the antibodies described in this application and/or are cross-blocked from binding activin-A by one of the antibodies described in this application. Accordingly such binding agents can be identified using the assays described herein.

In certain embodiments, antibodies are generated by first identifying antibodies that bind to one or more of the cysteine knot domains provided herein and/or neutralize in the cell-based and/or in vivo assays described herein and/or cross-block the antibodies described in this application and/or are cross-blocked from binding activin-A by one of the antibodies described in this application. The CDR regions from these antibodies are then used to insert into appropriate biocompatible frameworks to generate activin-A binding agents. The non-CDR portion of the binding agent may be composed of amino acids, or may be a non-protein molecule. The assays described herein allow the characteriza-

tion of binding agents. Preferably the binding agents of the present invention are antibodies as defined herein.

Other antibodies according to the invention may be obtained by conventional immunization and cell fusion procedures as described herein and known in the art.

Molecular evolution of the complementarity determining regions (CDRs) in the center of the antibody binding site also has been used to isolate antibodies with increased affinity, for example, antibodies having increased affinity for c-erbB-2, as described by Schier et al., 1996, *J Mol. Biol.* 263:551. Accordingly, such techniques are useful in preparing antibodies to activin-A. Antigen binding proteins directed against an activin-A can be used, for example, in assays to detect the presence of activin-A polypeptides, either *in vitro* or *in vivo*. The antigen binding proteins also may be employed in purifying activin-A proteins by immunoaffinity chromatography.

Antigen binding proteins (e.g., antibodies, antibody fragments, and antibody derivatives) of the invention can comprise any constant region known in the art. The light chain constant region can be, for example, a kappa- or lambda-type light chain constant region, e.g., a human kappa- or lambda-type light chain constant region. The heavy chain constant region can be, for example, an alpha-, delta-, epsilon-, gamma-, or mu-type heavy chain constant regions, e.g., a human alpha-, delta-, epsilon-, gamma-, or mu-type heavy chain constant region. In one embodiment, the light or heavy chain constant region is a fragment, derivative, variant, or mutein of a naturally occurring constant region.

Techniques are known for deriving an antibody of a different subclass or isotype from an antibody of interest, i.e., subclass switching. Thus, IgG antibodies may be derived from an IgM antibody, for example, and vice versa. Such techniques allow the preparation of new antibodies that possess the antigen-binding properties of a given antibody (the parent antibody), but also exhibit biological properties associated with an antibody isotype or subclass different from that of the parent antibody. Recombinant DNA techniques may be employed. Cloned DNA encoding particular antibody polypeptides may be employed in such procedures, e.g., DNA encoding the constant domain of an antibody of the desired isotype. See also Lantto et al., 2002, *Methods Mol. Biol.* 178:303-16.

In one embodiment, an antigen binding protein of the invention comprises the IgG1 heavy chain domain of any of A1-A14 (H1-H14) or a fragment of the IgG1 heavy chain domain of any of A1-A14 (H1-H14). In another embodiment, an antigen binding protein of the invention comprises the kappa light chain constant chain region of A1-A14 (L1-L14), or a fragment of the kappa light chain constant region of A1-A14 (L1-L14). In another embodiment, an antigen binding protein of the invention comprises both the IgG1 heavy chain domain, or a fragment thereof, of A1-A14 (L1-L14) and the kappa light chain domain, or a fragment thereof, of A1-A14 (L1-L14).

Accordingly, the antigen binding proteins of the present invention include those comprising, for example, the variable domain combinations L1H1, L2H2, L3H3, L4H4, L5H5, L6H6, L7H7, L8H8, L9H9, L10H10, L11H11, L12H12, L13H13, and L14H14, having a desired isotype (for example, IgA, IgG1, IgG2, IgG3, IgG4, IgM, IgE, and IgD) as well as Fab or F(ab')<sub>2</sub> fragments thereof. Moreover, if an IgG4 is desired, it may also be desired to introduce a point mutation (CPSCP→CPPCP) in the hinge region as described in Bloom et al., 1997, *Protein Science* 6:407, incorporated by reference herein) to alleviate a tendency to

form intra-H chain disulfide bonds that can lead to heterogeneity in the IgG4 antibodies.

In one embodiment, the antigen binding protein has a K<sub>off</sub> of 1×10<sup>-4</sup> s<sup>-1</sup> or lower. In another embodiment, the K<sub>off</sub> is 5×10<sup>-5</sup> s<sup>-1</sup> or lower. In another embodiment, the K<sub>off</sub> is substantially the same as an antibody having a combination of light chain and heavy chain variable domain sequences selected from the group of combinations consisting of L1H1, L2H2, L3H3, L4H4, L5H5, L6H6, L7H7, L8H8, L9H9, L10H10, L11H11, L12H12, L13H13, and L14H14. In another embodiment, the antigen binding protein binds to activin-A with substantially the same K<sub>off</sub> as an antibody that comprises one or more CDRs from an antibody having a combination of light chain and heavy chain variable domain sequences selected from the group of combinations consisting of L1H1, L2H2, L3H3, L4H4, L5H5, L6H6, L7H7, L8H8, L9H9, L10H10, L11H11, L12H12, L13H13, and L14H14. In another embodiment, the antigen binding protein binds to activin-A with substantially the same K<sub>off</sub> as an antibody that comprises one of the amino acid sequences illustrated above. In another embodiment, the antigen binding protein binds to activin-A with substantially the same K<sub>off</sub> as an antibody that comprises one or more CDRs from an antibody that comprises one of the amino acid sequences illustrated above.

Although human, partially human, or humanized antibodies will be suitable for many applications, particularly those involving administration of the antibody to a human subject, other types of antigen binding proteins will be suitable for certain applications. The non-human antibodies of the invention can be, for example, derived from any antibody-producing animal, such as mouse, rat, rabbit, goat, donkey, or non-human primate (such as monkey (e.g., cynomolgous or rhesus monkey) or ape (e.g., chimpanzee)). Non-human antibodies of the invention can be used, for example, in *in vitro* and cell-culture based applications, or any other application where an immune response to the antibody of the invention does not occur, is insignificant, can be prevented, is not a concern, or is desired. In one embodiment, a non-human antibody of the invention is administered to a non-human subject. In another embodiment, the non-human antibody does not elicit an immune response in the non-human subject. In another embodiment, the non-human antibody is from the same species as the non-human subject, e.g., a mouse antibody of the invention is administered to a mouse. An antibody from a particular species can be made by, for example, immunizing an animal of that species with the desired immunogen (e.g., a soluble activin-A polypeptide) or using an artificial system for generating antibodies of that species (e.g., a bacterial or phage display-based system for generating antibodies of a particular species), or by converting an antibody from one species into an antibody from another species by replacing, e.g., the constant region of the antibody with a constant region from the other species, or by replacing one or more amino acid residues of the antibody so that it more closely resembles the sequence of an antibody from the other species. In one embodiment, the antibody is a chimeric antibody comprising amino acid sequences derived from antibodies from two or more different species.

Antigen binding proteins may be prepared, and screened for desired properties, by any of a number of conventional techniques. Certain of the techniques involve isolating a nucleic acid encoding a polypeptide chain (or portion thereof) of an antigen binding protein of interest (e.g., an anti-activin-A antibody), and manipulating the nucleic acid through recombinant DNA technology. The nucleic acid

may be fused to another nucleic acid of interest, or altered (e.g., by mutagenesis or other conventional techniques) to add, delete, or substitute one or more amino acid residues, for example. Furthermore, the antigen binding proteins may be purified from cells that naturally express them (e.g., an antibody can be purified from a hybridoma that produces it), or produced in recombinant expression systems, using any technique known in the art. See, for example, *Monoclonal Antibodies, Hybridomas: A New Dimension in Biological Analyses*, Kennet et al. (eds.), Plenum Press, New York (1980); and *Antibodies: A Laboratory Manual*, Harlow and Land (eds.), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, (1988).

Any expression system known in the art can be used to make the recombinant polypeptides of the invention. Expression systems are detailed comprehensively above. In general, host cells are transformed with a recombinant expression vector that comprises DNA encoding a desired polypeptide. Among the host cells that may be employed are prokaryotes, yeast or higher eukaryotic cells. Prokaryotes include gram negative or gram positive organisms, for example *E. coli* or Bacilli. Higher eukaryotic cells include insect cells and established cell lines of mammalian origin. Examples of suitable mammalian host cell lines include the COS-7 line of monkey kidney cells (ATCC CRL 1651) (Gluzman et al., 1981, Cell 23:175), L cells, 293 cells, C127 cells, 3T3 cells (ATCC CCL 163), Chinese hamster ovary (CHO) cells, HeLa cells, BHK (ATCC CRL 10) cell lines, and the CVI/EBNA cell line derived from the African green monkey kidney cell line CVI (ATCC CCL 70) as described by McMahan et al., 1991, EMBO J. 10: 2821. Appropriate cloning and expression vectors for use with bacterial, fungal, yeast, and mammalian cellular hosts are described by Pouwels et al. (*Cloning Vectors: A Laboratory Manual*, Elsevier, New York, 1985).

The transformed cells can be cultured under conditions that promote expression of the polypeptide, and the polypeptide recovered by conventional protein purification procedures (as defined above). One such purification procedure includes the use of affinity chromatography, e.g., over a matrix having all or a portion (e.g., the extracellular domain) of activin-A bound thereto. Polypeptides contemplated for use herein include substantially homogeneous recombinant mammalian anti-activin-A antibody polypeptides substantially free of contaminating endogenous materials.

In one aspect, the present invention provides antigen-binding fragments of an anti-activin-A antibody of the invention. Such fragments can consist entirely of antibody-derived sequences or can comprise additional sequences. Examples of antigen-binding fragments include Fab, F(ab')<sup>2</sup>, single chain antibodies, diabodies, triabodies, tetrabodies, and domain antibodies. Other examples are provided in Lunde et al., 2002, Biochem. Soc. Trans. 30:500-06.

Single chain antibodies (scFv) may be formed by linking heavy and light chain variable domain (Fv region) fragments via an amino acid bridge (short peptide linker, e.g., a synthetic sequence of amino acid residues), resulting in a single polypeptide chain. Such single-chain Fvs (scFvs) have been prepared by fusing DNA encoding a peptide linker between DNAs encoding the two variable domain polypeptides (V<sub>L</sub> and V<sub>H</sub>). The resulting polypeptides can fold back on themselves to form antigen-binding monomers, or they can form multimers (e.g., dimers, trimers, or tetramers), depending on the length of a flexible linker between the two variable domains (Kortt et al., 1997, Prot. Eng. 10:423; Kortt et al., 2001, Biomol. Eng. 18:95-108, Bird et al., 1988, Science 242:423-26 and Huston et al., 1988, Proc. Natl.

Acad. Sci. USA 85:5879-83). By combining different V<sub>L</sub> and V<sub>H</sub>-comprising polypeptides, one can form multimeric scFvs that bind to different epitopes (Kriangkum et al., 2001, Biomol. Eng. 18:31-40). Techniques developed for the production of single chain antibodies include those described in U.S. Pat. No. 4,946,778; Bird, 1988, Science 242:423; Huston et al., 1988, Proc. Natl. Acad. Sci. USA 85:5879; Ward et al., 1989, Nature 334:544, de Graaf et al., 2002, Methods Mol. Biol. 178:379-87. Single chain antibodies derived from antibodies provided herein include, but are not limited to, scFvs comprising the variable domain combinations L1H1, L2H2, L3H3, L4H4, L5H5, L6H6, L7H7, L8H8, L9H9, L10H10, L11H11, L12H12, L13H13, and L14H14 are encompassed by the present invention.

Diabodies are bivalent antibodies comprising two polypeptide chains, wherein each polypeptide chain comprises V<sub>H</sub> and V<sub>L</sub> domains joined by a linker that is too short to allow for pairing between two domains on the same chain, thus allowing each domain to pair with a complementary domain on another polypeptide chain (see, e.g., Holliger et al., 1993, Proc. Natl. Acad. Sci. USA 90:6444-48, and Poljak et al., 1994, Structure 2:1121-23). If the two polypeptide chains of a diabody are identical, then a diabody resulting from their pairing will have two identical antigen binding sites. Polypeptide chains having different sequences can be used to make a diabody with two different antigen binding sites. Similarly, tribodies and tetrabodies are antibodies comprising three and four polypeptide chains, respectively, and forming three and four antigen binding sites, respectively, which can be the same or different.

Antibody polypeptides are also disclosed in U.S. Pat. No. 6,703,199, including fibronectin polypeptide monobodies. Other antibody polypeptides are disclosed in U.S. Patent Publication 2005/0238646, which are single-chain polypeptides.

In certain preferred embodiments, an antibody comprises one or more water soluble polymer attachments, including, but not limited to, polyethylene glycol, polyoxyethylene glycol, or polypropylene glycol. See, e.g., U.S. Pat. Nos. 4,640,835, 4,496,689, 4,301,144, 4,670,417, 4,791,192 and 4,179,337. In certain embodiments, a derivative binding agent comprises one or more of monomethoxy-polyethylene glycol, dextran, cellulose, or other carbohydrate based polymers, poly-(N-vinyl pyrrolidone)-polyethylene glycol, propylene glycol homopolymers, a polypropylene oxide/ethylene oxide co-polymer, polyoxyethylated polyols (e.g., glycerol) and polyvinyl alcohol, as well as mixtures of such polymers. In certain embodiments, one or more water-soluble polymer is randomly attached to one or more side chains. In certain embodiments, PEG can act to improve the therapeutic capacity for a binding agent, such as an antibody. Certain such methods are discussed, for example, in U.S. Pat. No. 6,133,426, which is hereby incorporated by reference for any purpose.

It will be appreciated that an antibody of the present invention may have at least one amino acid substitution, providing that the antibody retains binding specificity. Therefore, modifications to the antibody structures are encompassed within the scope of the invention. These may include amino acid substitutions, which may be conservative or non-conservative, that do not destroy the activin-A binding capability of an antibody. Conservative amino acid substitutions may encompass non-naturally occurring amino acid residues, which are typically incorporated by chemical peptide synthesis rather than by synthesis in biological systems. These include peptidomimetics and other reversed or inverted forms of amino acid moieties. A conservative

amino acid substitution may also involve a substitution of a native amino acid residue with a normative residue such that there is little or no effect on the polarity or charge of the amino acid residue at that position.

Non-conservative substitutions may involve the exchange of a member of one class of amino acids or amino acid mimetics for a member from another class with different physical properties (e.g. size, polarity, hydrophobicity, charge). Such substituted residues may be introduced into regions of the human antibody that are homologous with non-human antibodies, or into the non-homologous regions of the molecule.

Moreover, one skilled in the art may generate test variants containing a single amino acid substitution at each desired amino acid residue. The variants can then be screened using activity assays known to those skilled in the art. Such variants could be used to gather information about suitable variants. For example, if one discovered that a change to a particular amino acid residue resulted in destroyed, undesirably reduced, or unsuitable activity, variants with such a change may be avoided. In other words, based on information gathered from such routine experiments, one skilled in the art can readily determine the amino acids where further substitutions should be avoided either alone or in combination with other mutations.

A skilled artisan will be able to determine suitable variants of the polypeptide as set forth herein using well-known techniques. In certain embodiments, one skilled in the art may identify suitable areas of the molecule that may be changed without destroying activity by targeting regions not believed to be important for activity. In certain embodiments, one can identify residues and portions of the molecules that are conserved among similar polypeptides. In certain embodiments, even areas that may be important for biological activity or for structure may be subject to conservative amino acid substitutions without destroying the biological activity or without adversely affecting the polypeptide structure.

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

One skilled in the art can also analyze the three-dimensional structure and amino acid sequence in relation to that structure in similar polypeptides. In view of such information, one skilled in the art may predict the alignment of amino acid residues of an antibody with respect to its three dimensional structure. In certain embodiments, one skilled in the art may choose not to make radical changes to amino acid residues predicted to be on the surface of the protein, since such residues may be involved in important interactions with other molecules.

A number of scientific publications have been devoted to the prediction of secondary structure. See Moult J., *Curr. Op. in Biotech.*, 7(4):422-427 (1996), Chou et al., *Biochem.*, 13(2):222-245 (1974); Chou et al., *Biochem.*, 113(2):211-222 (1974); Chou et al., *Adv. Enzymol. Relat. Areas Mol. Biol.*, 47:45-148 (1978); Chou et al., *Ann. Rev. Biochem.*, 47:251-276 and Chou et al., *Biophys. J.*, 26:367-384 (1979). Moreover, computer programs are currently available to assist with predicting secondary structure. One method of predicting secondary structure is based upon homology

modeling. For example, two polypeptides or proteins which have a sequence identity of greater than 30%, or similarity greater than 40% often have similar structural topologies. The recent growth of the protein structural database (PDB) has provided enhanced predictability of secondary structure, including the potential number of folds within a polypeptide's or protein's structure. See Holm et al., *Nucl. Acid. Res.*, 27(1):244-247 (1999). It has been suggested (Brenner et al., *Curr. Op. Struct. Biol.*, 7(3):369-376 (1997)) that there are a limited number of folds in a given polypeptide or protein and that once a critical number of structures have been resolved, structural prediction will become dramatically more accurate.

Additional methods of predicting secondary structure include "threading" (Jones, D., *Curr. Opin. Struct. Biol.*, 7(3):377-87 (1997); Sippl et al., *Structure*, 4(1):15-19 (1996)), "profile analysis" (Bowie et al., *Science*, 253:164-170 (1991); Gribskov et al., *Meth. Enzym.*, 183:146-159 (1990); Gribskov et al., *Proc. Nat. Acad. Sci.*, 84(13):4355-4358 (1987)), and "evolutionary linkage" (See Holm, supra (1999), and Brenner, supra (1997)).

In certain embodiments, variants of antibodies include glycosylation variants wherein the number and/or type of glycosylation site has been altered compared to the amino acid sequences of a parent polypeptide. In certain embodiments, variants comprise a greater or a lesser number of N-linked glycosylation sites than the native protein. An N-linked glycosylation site is characterized by the sequence: Asn-X-Ser or Asn-X-Thr, wherein the amino acid residue designated as X may be any amino acid residue except proline. The substitution of amino acid residues to create this sequence provides a potential new site for the addition of an N-linked carbohydrate chain. Alternatively, substitutions which eliminate this sequence will remove an existing N-linked carbohydrate chain. Also provided is a rearrangement of N-linked carbohydrate chains wherein one or more N-linked glycosylation sites (typically those that are naturally occurring) are eliminated and one or more new N-linked sites are created. Additional preferred antibody variants include cysteine variants wherein one or more cysteine residues are deleted from or substituted for another amino acid (e.g., serine) as compared to the parent amino acid sequence. Cysteine variants may be useful when antibodies must be refolded into a biologically active conformation such as after the isolation of insoluble inclusion bodies. Cysteine variants generally have fewer cysteine residues than the native protein, and typically have an even number to minimize interactions resulting from unpaired cysteines.

Desired amino acid substitutions (whether conservative or non-conservative) can be determined by those skilled in the art at the time such substitutions are desired. In certain embodiments, amino acid substitutions can be used to identify important residues of antibodies to activin-A, or to increase or decrease the affinity of the antibodies to activin-A described herein.

According to certain embodiments, preferred amino acid substitutions are those which: (1) reduce susceptibility to proteolysis, (2) reduce susceptibility to oxidation, (3) alter binding affinity for forming protein complexes, (4) alter binding affinities, and/or (4) confer or modify other physicochemical or functional properties on such polypeptides. According to certain embodiments, single or multiple amino acid substitutions (in certain embodiments, conservative amino acid substitutions) may be made in the naturally-occurring sequence (in certain embodiments, in the portion of the polypeptide outside the domain(s) forming intermo-

lecular contacts). In certain embodiments, a conservative amino acid substitution typically may not substantially change the structural characteristics of the parent sequence (e.g., a replacement amino acid should not tend to break a helix that occurs in the parent sequence, or disrupt other types of secondary structure that characterizes the parent sequence). Examples of art-recognized polypeptide secondary and tertiary structures are described in Proteins, Structures and Molecular Principles (Creighton, Ed., W. H. Freeman and Company, New York (1984)); Introduction to Protein Structure (C. Branden and J. Tooze, eds., Garland Publishing, New York, N.Y. (1991)); and Thornton et al. *Nature* 354:105 (1991), which are each incorporated herein by reference.

In certain embodiments, antibodies of the invention may be chemically bonded with polymers, lipids, or other moieties.

The binding agents may comprise at least one of the CDRs described herein incorporated into a biocompatible framework structure. In one example, the biocompatible framework structure comprises a polypeptide or portion thereof that is sufficient to form a conformationally stable structural support, or framework, or scaffold, which is able to display one or more sequences of amino acids that bind to an antigen (e.g., CDRs, a variable region, etc.) in a localized surface region. Such structures can be a naturally occurring polypeptide or polypeptide "fold" (a structural motif), or can have one or more modifications, such as additions, deletions or substitutions of amino acids, relative to a naturally occurring polypeptide or fold. These scaffolds can be derived from a polypeptide of any species (or of more than one species), such as a human, other mammal, other vertebrate, invertebrate, plant, bacteria or virus.

Typically the biocompatible framework structures are based on protein scaffolds or skeletons other than immunoglobulin domains. For example, those based on fibronectin, ankyrin, lipocalin, neocarzinostatin, cytochrome b, CP1 zinc finger, PST1, coiled coil, LACI-D1, Z domain and tandem-mist domains may be used (See e.g., Nygren and Uhlen, 1997, *Curr. Opin. in Struct. Biol.*, 7, 463-469).

It will be appreciated that the antibodies of the invention include the humanized antibodies described herein. Humanized antibodies such as those described herein can be produced using techniques known to those skilled in the art (Zhang, W., et al., *Molecular Immunology*. 42(12):1445-1451, 2005; Hwang W. et al., *Methods*. 36(1):35-42, 2005; Dall'Acqua W F, et al., *Methods* 36(1):43-60, 2005; and Clark, M., *Immunology Today*. 21(8):397-402, 2000).

Additionally, one skilled in the art will recognize that suitable binding agents include portions of these antibodies, such as one or more of CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2 and CDR-L3 as specifically disclosed herein. At least one of the regions of CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2 and CDR-L3 may have at least one amino acid substitution, provided that the antibody retains the binding specificity of the non-substituted CDR. The non-CDR portion of the antibody may be a non-protein molecule, wherein the binding agent cross-blocks the binding of an antibody disclosed herein to activin-A and/or neutralizes activin-A. The non-CDR portion of the antibody may be a non-protein molecule in which the antibody exhibits a similar binding pattern to human activin-A peptides in a competition binding assay as that exhibited by at least one of antibodies A1-A14, and/or neutralizes activin-A. The non-CDR portion of the antibody may be composed of amino acids, wherein the antibody is a recombinant binding protein or a synthetic peptide, and the recombinant binding

protein cross-blocks the binding of an antibody disclosed herein to activin-A and/or neutralizes activin-A. The non-CDR portion of the antibody may be composed of amino acids, wherein the antibody is a recombinant antibody, and the recombinant antibody exhibits a similar binding pattern to human activin-A peptides in the human activin-A peptide epitope competition binding assay (described hereinbelow) as that exhibited by at least one of the antibodies A1-A14, and/or neutralizes activin-A.

Where an antibody comprises one or more of CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2 and CDR-L3 as described above, it may be obtained by expression from a host cell containing DNA coding for these sequences. A DNA coding for each CDR sequence may be determined on the basis of the amino acid sequence of the CDR and synthesized together with any desired antibody variable region framework and constant region DNA sequences using oligonucleotide synthesis techniques, site-directed mutagenesis and polymerase chain reaction (PCR) techniques as appropriate. DNA coding for variable region frameworks and constant regions is widely available to those skilled in the art from genetic sequences databases such as GenBank®.

Once synthesized, the DNA encoding an antibody of the invention or fragment thereof may be propagated and expressed according to any of a variety of well-known procedures for nucleic acid excision, ligation, transformation, and transfection using any number of known expression vectors. Thus, in certain embodiments expression of an antibody fragment may be preferred in a prokaryotic host, such as *Escherichia coli* (see, e.g., Pluckthun et al., 1989 *Methods Enzymol.* 178:497-515). In certain other embodiments, expression of the antibody or a fragment thereof may be preferred in a eukaryotic host cell, including yeast (e.g., *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, and *Pichia pastoris*), animal cells (including mammalian cells) or plant cells. Examples of suitable animal cells include, but are not limited to, myeloma (such as a mouse NSO line), COS, CHO, or hybridoma cells. Examples of plant cells include tobacco, corn, soybean, and rice cells.

One or more replicable expression vectors containing DNA encoding an antibody variable and/or constant region may be prepared and used to transform an appropriate cell line, for example, a non-producing myeloma cell line, such as a mouse NSO line or a bacteria, such as *E. coli*, in which production of the antibody will occur. In order to obtain efficient transcription and translation, the DNA sequence in each vector should include appropriate regulatory sequences, particularly a promoter and leader sequence operatively linked to the variable domain sequence. Particular methods for producing antibodies in this way are generally well-known and routinely used. For example, basic molecular biology procedures are described by Maniatis et al. (*Molecular Cloning, A Laboratory Manual*, 2nd ed., Cold Spring Harbor Laboratory, New York, 1989; see also Maniatis et al., 3rd ed., Cold Spring Harbor Laboratory, New York, (2001)). DNA sequencing can be performed as described in Sanger et al. (PNAS 74:5463, (1977)) and the Amersham International plc sequencing handbook, and site directed mutagenesis can be carried out according to methods known in the art (Kramer et al., *Nucleic Acids Res.* 12:9441, (1984); Kunkel Proc. Natl. Acad. Sci. USA 82:488-92 (1985); Kunkel et al., *Methods in Enzymol.* 154:367-82 (1987); the Anglian Biotechnology Ltd. handbook). Additionally, numerous publications describe techniques suitable for the preparation of antibodies by manipulation of DNA, creation of expression vectors, and transformation and culture of

appropriate cells (Mountain A and Adair, J R in *Biotechnology and Genetic Engineering Reviews* (ed. Tombs, M P, 10, Chapter 1, 1992, Intercept, Andover, UK); "Current Protocols in Molecular Biology", 1999, F. M. Ausubel (ed.), Wiley Interscience, New York).

Where it is desired to improve the affinity of antibodies according to the invention containing one or more of the above-mentioned CDRs can be obtained by a number of affinity maturation protocols including maintaining the CDRs (Yang et al., *J. Mol. Biol.*, 254, 392-403, 1995), chain shuffling (Marks et al., *Bio/Technology*, 10, 779-783, 1992), use of mutation strains of *E. coli*. (Low et al., *J. Mol. Biol.*, 250, 350-368, 1996), DNA shuffling (Patten et al., *Curr. Opin. Biotechnol.*, 8, 724-733, 1997), phage display (Thompson et al., *J. Mol. Biol.*, 256, 7-88, 1996) and sexual PCR (Crameri, et al., *Nature*, 391, 288-291, 1998). All of these methods of affinity maturation are discussed by Vaughan et al. (*Nature Biotech.*, 16, 535-539, 1998).

It will be understood by one skilled in the art that some proteins, such as antibodies, may undergo a variety of posttranslational modifications. The type and extent of these modifications often depends on the host cell line used to express the protein as well as the culture conditions. Such modifications may include variations in glycosylation, methionine oxidation, diketopiperazine formation, aspartate isomerization and asparagine deamidation. A frequent modification is the loss of a carboxy-terminal basic residue (such as lysine or arginine) due to the action of carboxypeptidases (as described in Harris, R. J. *Journal of Chromatography* 705:129-134, 1995).

#### svActRIIB: Activin IIB Receptor

The present invention discloses an isolated protein comprising a stabilized human activin IIB receptor (svActRIIB) polypeptide. The protein and polypeptide of the invention are characterized by their ability to bind to at least one of three TGF- $\beta$  proteins, myostatin (GDF-8), activin-A, or GDF-11, to inhibit the activities of at least one of these proteins, and to have improved manufacturability properties compared with other ActRIIB soluble receptors. The stabilized human activin IIB receptor polypeptide is characterized by amino acid substitutions at both positions E28 and S44 with reference to the extracellular domain of ActRIIB, as set forth in the following sequence: Met Thr Ala Pro Trp Val Ala Leu Ala Leu Leu Trp Gly Ser Leu Cys Ala Gly Ser Gly Arg Gly Glu Ala Glu Thr Arg Glu Cys Ile Tyr Tyr Asn Ala Asn Trp Glu Leu Glu Arg Thr Asn Gln Ser Gly Leu Glu Arg Cys Glu Gly Glu Gln Asp Lys Arg Leu His Cys Tyr Ala Ser Trp Arg Asn Ser Ser Gly Thr Ile Glu Leu Val Lys Lys Gly Cys Trp Leu Asp Asp Phe Asn Cys Tyr Asp Arg Gln Glu Cys Val Ala Thr Glu Glu Asn Pro Gln Val Tyr Phe Cys Cys Cys Glu Gly Asn Phe Cys Asn Glu Arg Phe Thr His Leu Pro Glu Ala Gly Gly Pro Glu Val Thr Tyr Glu Pro Pro Pro Thr Ala Pro Thr (SEQ ID NO:2). In one embodiment, a stabilized human activin IIB receptor polypeptide can have a further substitution of alanine at position 64 with respect to the above sequence.

"TGF- $\beta$  family members" or "TGF- $\beta$  proteins" refers to the structurally related growth factors of the transforming growth factor family including activins, and growth and differentiation factor (GDF) proteins (Kingsley et al. *Genes Dev.* 8: 133-146 (1994), McPherron et al., Growth factors and cytokines in health and disease, Vol. 1B, D. LeRoith and C. Bondy, ed., JAI Press Inc., Greenwich, Conn, USA: pp 357-393).

GDF-8, also referred to as myostatin, is a negative regulator of skeletal muscle tissue (McPherron et al. *PNAS USA* 94:12457-12461 (1997)). Myostatin is synthesized as an

inactive protein approximately 375 amino acids in length, having GenBank Accession No: AAB86694 for human. The precursor protein is activated by proteolytic cleavage at a tetrabasic processing site to produce an N-terminal inactive prodomain and an approximately 109 amino acid C-terminal protein which dimerizes to form a homodimer of about 25 kDa. This homodimer is the mature, biologically active protein (Zimmers et al., *Science* 296, 1486 (2002)).

A "prodomain" or "propeptide" is the inactive N-terminal protein which is cleaved off to release the active C-terminal protein. As used herein the term "myostatin" or "mature myostatin" refers to the mature, biologically active C-terminal polypeptide, in monomer, dimer or other form, as well as biologically active fragments or related polypeptides including allelic variants, splice variants, and fusion peptides and polypeptides. The mature myostatin has been reported to have 100% sequence identity among many species including human, mouse, chicken, porcine, turkey, and rat (Lee et al., *PNAS* 98, 9306 (2001)).

GDF-11 refers to the BMP (bone morphogenic protein) having Swissprot accession number 095390, as well as variants and species homologs of that protein. GDF-11 is involved in the regulation of anterior/posterior patterning of the axial skeleton (McPherron et al., *Nature Genet.* 22 (93): 260-264 (1999); Gamer et al., *Dev. Biol.* 208 (1), 222-232 (1999)) but postnatal functions are unknown.

#### Receptor Polypeptides

An activin type II B receptor (ActRIIB) can be a human activin receptor having accession number NP\_001097 or a variant thereof, such as that having the arginine at position 64 substituted with alanine. The term soluble ActRIIB (wild type) refers to the extracellular domain of ActRIIB, amino acids 1 to 134 (with signal sequence), or amino acids 19 through 134 of SEQ ID NO: 2 (without signal sequence).

The present invention provides an isolated protein comprising a stabilized ActRIIB receptor polypeptide (referred herein as "svActRIIB polypeptide"). A "svActRIIB protein" is a protein comprising a stabilized ActRIIB polypeptide. The term "isolated" refers to a protein or polypeptide molecule purified to some degree from endogenous material. These polypeptides and proteins are characterized as having the ability to bind and inhibit the activity of any one of activin-A, myostatin, or GDF-11, in addition to having improved manufacturability characteristics.

The stabilized ActRIIB polypeptide is characterized by having an amino acid substitution at both position 28 and 44 with respect to SEQ ID NO: 2. For consistency, the amino acid positions on the stabilized ActRIIB polypeptides and proteins are always referred to with respect to the positions in SEQ ID NO: 2, regardless of whether the polypeptide is mature or truncated. As used herein, the term "mature" refers to a polypeptide or peptide without its signal sequence. As used herein, the term "truncated" refers to polypeptides having N terminal amino acids or C terminal amino acids removed.

In one embodiment, the isolated stabilized activin IIB receptor polypeptide (svActRIIB) has the polypeptide sequence set forth in SEQ ID NO: 2, except for a single amino acid substitution at position 28, and a single amino acid substitution at position 44, wherein the substitution at position 28 is selected from W or Y, and the substitution at position 44 is T. In another embodiment, the polypeptide has the sequence set forth in amino acids 19 through 134 of SEQ ID NO: 2, except for a single amino acid substitution at position 28, and a single amino acid substitution at position 44, wherein the substitution at position 28 is selected from W or Y, and the substitution at position 44 is T. In another

embodiment, the polypeptide has the sequence set forth in amino acids 23 through 134 of SEQ ID NO: 2, except for a single amino acid substitution at position 28, and a single amino acid substitution at position 44, wherein the substitution at position 28 is selected from W or Y, and the substitution at position 44 is T. In another embodiment, the polypeptide has the sequence set forth in amino acids 25 through 134 of SEQ ID NO: 2, except for a single amino acid substitution at position 28, and a single amino acid substitution at position 44, wherein the substitution at position 28 is selected from W or Y, and the substitution at position 44 is T. In another embodiment, the polypeptide has an amino acid sequence with at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to any one of the polypeptides above, wherein the polypeptide has single amino acid substitution at position 28, and a single amino acid substitution at position 44, wherein the substitution at position 28 is selected from W or Y, and the substitution at position 44 is T, and wherein the polypeptide is capable of binding myostatin, activin-A, or GDF-11.

In one embodiment, the svActRIIB polypeptide includes a signal sequence, for example, SEQ ID NO: 4, 8, 12, and 16 (see below for sequences). However, various signal peptides can be used in the preparation of the polypeptides of the instant application. The signal peptides can have the sequence set forth in amino acids 1 to 19 of SEQ ID NO: 4, for example, or the signal sequences set forth in SEQ ID NO: 31 and 32. Any other signal peptides useful for expressing svActRIIB polypeptides may be used. In other embodiments, the signal sequence is removed, leaving the mature peptide. Examples of svActRIIB polypeptides lacking a signal sequence includes, for example, SEQ ID NO: 6, 10, 14 and 18.

In one embodiment, the protein comprises a stabilized activin IIB receptor polypeptide, wherein the polypeptide is selected from the group consisting of polypeptides having the sequence set forth in the group consisting of SEQ ID NO: 4, 6, 12 and 14. These polypeptides represent amino acids 25 to 134 of SEQ ID NO: 2, wherein the polypeptide has single amino acid substitution at position 28, and a single amino acid substitution at position 44, wherein the substitution at position 28 is selected from W or Y, and the substitution at position 44 is T, and wherein the polypeptide is capable of binding myostatin, activin-A, or GDF-11, with and without a signal sequence different from that shown in SEQ ID NO: 2. In another embodiment the protein comprises a polypeptide having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 4, 6, 12 or 14, wherein the polypeptide has a W or Y at position 28 and a T at position 44, and wherein the polypeptide is capable of binding myostatin, activin-A, or GDF-11. In one embodiment, the substitution at position 28 is W and the substitution at position 44 is T, wherein the polypeptide is capable of binding myostatin, activin-A or GDF-11.

In a further embodiment the svActRIIB protein further comprises a heterologous protein. In one embodiment, the heterologous protein is an Fc domain. In a further embodiment, the Fc domain is a human IgG Fc domain. In one embodiment, the protein comprises a polypeptide having the sequence set forth in the group consisting of SEQ ID NO: 8, 10, 16 and 18. In another embodiment, the protein comprises a polypeptide having at least 80%, 85%, 90%, 95%, 96%,

97%, 98%, or 99% sequence identity to SEQ ID NO: 8, 10, 16 or 18, wherein the polypeptide has a W or Y at position 28 and a T at position 44, and wherein the polypeptide is capable of binding myostatin, activin-A, or GDF-11. In one embodiment, the substitution at position 28 is W and the substitution at position 44 is T, wherein the polypeptide is capable of binding myostatin, activin-A or GDF-11.

In a further embodiment, the protein comprises the any one of the polypeptides described above, wherein the amino acid residue at position 64 is alanine.

In another embodiment, the term svActRIIB polypeptide and protein encompasses proteins comprising fragments of SEQ ID NO: 2, 4, 6, 12 and 14, including N and C terminal truncations, wherein position 28 is W or Y, and position 44 is T, and wherein the polypeptide is capable of binding myostatin, activin-A or GDF-11.

The term "derivative" of the svActRIIB polypeptide refers to the attachment of at least one additional chemical moiety, or at least one additional polypeptide to form 20 covalent or aggregate conjugates such as glycosyl groups, lipids, acetyl groups, or C-terminal or N-terminal fusion polypeptides, conjugation to PEG molecules, and other modifications which are described more fully below. Stabilized ActRIIB receptor polypeptides can also include additional modifications and derivatives, including modifications to the C and N termini which arise from processing due to expression in various cell types such as mammalian cells, *E. coli*, yeasts and other recombinant host cells.

The svActRIIB proteins of the present invention may 30 further comprise heterologous polypeptides attached to the svActRIIB polypeptide either directly or through a linker sequence to form a fusion protein. As used herein the term "fusion protein" refers to a protein having a heterologous polypeptide attached via recombinant DNA techniques. Heterologous polypeptides include but are not limited to Fc 35 polypeptides, his tags, and leucine zipper domains to promote oligomerization and further stabilization of the stabilized ActRIIB polypeptides as described in, for example, WO 00/29581, which is herein incorporated by reference. In 40 one embodiment, the heterologous polypeptide is an Fc polypeptide or domain. In one embodiment, the Fc domain is selected from a human IgG1 Fc (SEQ ID NO: 23), modified IgG1 Fc (SEQ ID NO: 47), IgG2 Fc (SEQ ID NO: 22), and IgG4 Fc (SEQ ID NO: 24) domain. The svActRIIB 45 protein can further comprise all or a portion of the hinge sequence of the IgG1 (SEQ ID NO: 29), IgG2 (SEQ ID NO: 28), or IgG4 (SEQ ID NO: 30). Exemplary svActRIIB polypeptides are selected from polypeptides consisting of the sequences as set forth in SEQ ID NO: 8, 10, 16 and 18, 50 as well as those polypeptides having substantial similarity to these sequences, wherein the substitutions at positions 28 and 44 are retained. As used herein, "substantial similarity" refers to sequences that are at least 80% identical, 85% identical, 90% identical, 95% identical, 96% identical, 97% identical, 98% identical, 99% identical to any of SEQ ID NO: 8, 10, 16, and 18, wherein the polypeptides retain W or Y at position 28 and T at position 44, and wherein the polypeptide is capable of binding myostatin, activin-A or GDF-11. In one embodiment, the substitution at position 28 55 is W and the substitution at position 44 is T, wherein the polypeptide is capable of binding myostatin, activin-A or GDF-11.

The svActRIIB polypeptide can optionally further comprise a "linker" sequence. Linkers serve primarily as a spacer between a polypeptide and a second heterologous polypeptide or other type of fusion or between two or more stabilized ActRIIB polypeptides. In one embodiment, the

linker is made up of amino acids linked together by peptide bonds, preferably from 1 to 20 amino acids linked by peptide bonds, wherein the amino acids are selected from the 20 naturally occurring amino acids. One or more of these amino acids may be glycosylated, as is understood by those of skill in the art. In one embodiment, the 1 to 20 amino acids may be selected from glycine, alanine, proline, asparagine, glutamine, and lysine. In one embodiment, a linker is made up of a majority of amino acids that are sterically unhindered, such as glycine and alanine. Exemplary linkers are polyglycines (particularly (Gly)<sub>5</sub> (SEQ ID NO: 289), (Gly)<sub>8</sub> (SEQ ID NO: 290), poly(Gly-Ala), and polyalanines. One exemplary suitable linker as shown in the Examples below is (Gly)<sub>4</sub>Ser (SEQ ID NO: 25). In a further embodiment, svActRIIB can comprise a "hinge linker", that is a linker sequence provided adjacent to a hinge region or a partial hinge region of an IgG, as exemplified in SEQ ID NO: 27. Hinge sequences include IgG2Fc (SEQ ID NO: 28), IgG1Fc (SEQ ID NO: 29), and IgG4Fc (SEQ ID NO: 30).

Hinge linker sequences may also be designed to improve manufacturability and stability of the svActRIIB-Fc proteins. In one embodiment, the hinge linkers of SEQ ID NO: 27, 38, 40, 42, 44, 45, and 46 are designed to improve manufacturability with the IgG2 Fc (SEQ ID NO: 22) when attached to svActRIIB polypeptides. In one embodiment, the hinge linker sequences is designed to improve manufacturability when attaching svActRIIB polypeptides to a human IgG1 Fc (SEQ ID NO: 23) or a modified human IgG1 Fc (SEQ ID NO: 47), for example, the hinge linkers having SEQ ID NO: 48, SEQ ID NO: 49 and SEQ ID NO: 50.

Linkers may also be non-peptide linkers. For example, alkyl linkers such as —NH—(CH<sub>2</sub>)<sub>s</sub>—C(O)—, wherein s=2-20 can be used. These alkyl linkers may further be substituted by any non-sterically hindering group such as lower alkyl (e.g., C<sub>1</sub>-C<sub>6</sub>) lower acyl, halogen (e.g., Cl, Br), CN, NH<sub>2</sub>, phenyl, etc.

The svActRIIB polypeptides disclosed herein can also be attached to a non-polypeptide molecule for the purpose of conferring desired properties such as reducing degradation and/or increasing half-life, reducing toxicity, reducing immunogenicity, and/or increasing the biological activity of the svActRIIB polypeptides. Exemplary molecules include but are not limited to linear polymers such as polyethylene glycol (PEG), polylysine, a dextran; a lipid; a cholesterol group (such as a steroid); a carbohydrate, or an oligosaccharide molecule.

The svActRIIB proteins and polypeptides have improved manufacturability properties when compared to other ActRIIB soluble polypeptides. As used herein, the term "manufacturability" refers to the stability of a particular protein during recombinant expression and purification of that protein. Manufacturability is believed to be due to the intrinsic properties of the molecule under conditions of expression and purification.

Activities of the svActRIIB polypeptides include, but are not limited to, the ability to bind to myostatin or activin-A or GDF-11, and the ability to inhibit or neutralize an activity of myostatin or activin-A or GDF-11. As used herein, the term "capable of binding" to myostatin, activin-A, or GDF-11 refers to binding measured by methods known in the art. In vitro inhibition of myostatin, activin-A, or GDF-11 can be measured using, for example, the pMARE C2C12 cell-based assay. In vivo activity, is demonstrated by increased lean muscle mass in mouse models. In vivo activities of the svActRIIB polypeptides and proteins include but are not limited to increasing body weight, increasing lean muscle mass, and increasing the ratio of lean muscle to fat mass.

Therapeutic activities further include reducing or preventing cachexia caused by certain types of tumors, preventing the growth of certain types of tumors, and increasing survival of certain animal models. Further discussion of the svActRIIB protein and polypeptide activities is provided below.

In another aspect, the present invention provides an isolated nucleic acid molecule comprising a polynucleotide encoding an svActRIIB polypeptide of the present invention. As used herein the term "isolated" refers to nucleic acid molecules purified to some degree from endogenous material.

In one embodiment, the polynucleotide encodes a polypeptide having the sequence set forth in SEQ ID NO: 2, except for a single amino acid substitution at position 28, and a single amino acid substitution at position 44, wherein the substitution at position 28 is selected from W or Y, and the substitution at position 44 is T. In another embodiment, the polynucleotide encodes a polypeptide having the sequence set forth in amino acids 19 through 134 of SEQ ID NO: 2, except for a single amino acid substitution at position 28, and a single amino acid substitution at position 44, wherein the substitution at position 28 is selected from W or Y, and the substitution at position 44 is T. In another embodiment, the polynucleotide encodes a polypeptide having the sequence set forth in amino acids 23 through 134 of SEQ ID NO: 2, except for a single amino acid substitution at position 28, and a single amino acid substitution at position 44, wherein the substitution at position 28 is selected from W or Y, and the substitution at position 44 is T. In another embodiment, the polynucleotide encodes a polypeptide having the sequence set forth in amino acids 25 through 134 of SEQ ID NO: 2, except for a single amino acid substitution at position 28, and a single amino acid substitution at position 44, wherein the substitution at position 28 is selected from W or Y, and the substitution at position 44 is T. In another embodiment, the polynucleotide encodes a polypeptide having an amino acid sequence at least 80%, 85%, 90%, 95%, 98% or 99% identity to any one of the polypeptides above, wherein the polypeptide has a single amino acid substitution at position 28, and a single amino acid substitution at position 44, wherein the substitution at position 28 is selected from W or Y, and the substitution at position 44 is T, and wherein the polypeptide is capable of binding myostatin, activin-A, or GDF-11. In one embodiment, the polynucleotide of the above embodiments encodes a polypeptide wherein the substitution at position 28 is W and the substitution at position 44 is T.

In one embodiment, the isolated nucleic acid molecule of the present invention comprises a polynucleotide encoding a polypeptide having the sequence set forth in the group consisting of SEQ ID NO: 4, 6, 12, and 14. In another embodiment, the nucleic acid comprises a polynucleotide encoding a polypeptide having at least 80%, 90%, 95%, 96%, 97%, 98%, 99% sequence identity to SEQ ID NO: 4, 6, 12 or 14, wherein the polypeptide has a W or Y at position 28 and a T at position 44, and wherein the polypeptide is capable of binding activin-A, GDF-11, or myostatin. In one embodiment, the polynucleotide of the above embodiments encodes a polypeptide wherein the substitution at position 28 is W and the substitution at position 44 is T, and wherein the polypeptide is capable of binding activin-A, GDF-11 or myostatin.

In another embodiment, the isolated nucleic acid molecule further comprises a polynucleotide encoding at least one heterologous protein. In one embodiment, the heterologous protein is an Fc domain, in a further embodiment, the Fc domain is a human IgG Fc domain. In another embodiment,

ment, the nucleic acid molecule further comprises polynucleotides encoding the linkers and hinge linkers set forth in SEQ ID NO: 25, 27, 38, 40, 42, 44, 45, 46, 48, 49 or 50. In a further embodiment, such polynucleotides have sequences selected from the group consisting of SEQ ID NO: 26, 37, 39, 41, and 43.

In one embodiment, the nucleic acid molecule comprises a polynucleotide encoding a polypeptide consisting of the sequence set forth in the group consisting of SEQ ID NO: 8, 10, 16 and 18. In another embodiment, the nucleic acid comprises a polynucleotide encoding a polypeptide having at least 80%, 90%, 95%, 96%, 97%, 98%, 99% sequence identity to the group consisting of SEQ ID NO: 8, 10, 16 and 18, wherein the polypeptide has a W or Y at position 28 and a T at position 44, and wherein the polypeptide is capable of binding activin-A, GDF-11, or myostatin. In one embodiment, the polynucleotide of the above embodiments encodes a polypeptide wherein the substitution at position 28 is W and the substitution at position 44 is T, and wherein the polypeptide is capable of binding myostatin, activin-A or GDF-11.

In one embodiment, the isolated nucleic acid molecule comprises a polynucleotide having the sequence selected from the group consisting of SEQ ID NO: 3, 5, 11 or 13, or its complement. In another embodiment, the isolated nucleic acid molecule comprises a polynucleotide having the sequence selected from the group consisting of the sequence SEQ ID NO: 7, 9, 15 and 17, or its complement. In a further embodiment the isolated nucleic acid molecule hybridizes under stringent or moderate conditions with SEQ ID NO: 3, 5, 7, 9, 11, 13, 15 or 17 wherein the encoded polypeptide is substantially similar to SEQ ID NO: 4, 6, 8, 10, 12, 14, 16,

or 18, wherein the polypeptide comprises an amino acid sequence having W or Y at position 28, and T at position 44, and wherein the encoded polypeptide is capable of binding or inhibiting activin-A, myostatin or GDF-11.

Nucleic acid molecules of the invention include DNA in both single-stranded and double-stranded form, as well as the RNA complement thereof. DNA includes, for example, cDNA, genomic DNA, synthetic DNA, DNA amplified by PCR, and combinations thereof. Genomic DNA may be isolated by conventional techniques, such as by using the DNA of SEQ ID NO: 3, 5, 11 or 13, or a suitable fragment thereof, as a probe. Genomic DNA encoding ActRIIB polypeptides is obtained from genomic libraries which are available for a number of species. Synthetic DNA is available from chemical synthesis of overlapping oligonucleotide fragments followed by assembly of the fragments to reconstitute part or all of the coding regions and flanking sequences. RNA may be obtained from prokaryotic expression vectors which direct high-level synthesis of mRNA, such as vectors using T7 promoters and RNA polymerase. cDNA is obtained from libraries prepared from mRNA isolated from various tissues that express ActRIIB. The DNA molecules of the invention include full length genes as well as polynucleotides and fragments thereof. The full length gene may also include sequences encoding the N-terminal signal sequence.

The invention further provides the nucleic acid molecule described above, wherein the polynucleotide is operably linked to a transcriptional or translational regulatory sequence.

#### Exemplary Polynucleotide and Polypeptide Sequences

svActRIIB without signal sequence  
(SEQ ID NO: 2)

Met Thr Ala Pro Trp Val Ala Leu Ala Leu Trp Gly Ser Leu Cys Ala Gly Ser  
Gly Arg Gly Glu Ala Glu Thr Arg Glu Cys Ile Tyr Tyr Asn Ala Asn Trp Glu Leu Glu Arg  
Thr Asn Gln Ser Gly Leu Glu Arg Cys Glu Gly Glu Gln Asp Lys Arg Leu His Cys Tyr Ala  
Ser Trp Arg Asn Ser Ser Gly Thr Ile Glu Leu Val Lys Lys Gly Cys Trp Leu Asp Asp Phe  
Asn Cys Tyr Asp Arg Gln Glu Cys Val Ala Thr Glu Glu Asn Pro Gln Val Tyr Phe Cys Cys  
Cys Glu Gly Asn Phe Cys Asn Glu Arg Phe Thr His Leu Pro Glu Ala Gly Gly Pro Glu Val  
Thr Tyr Glu Pro Pro Pro Thr Ala Pro Thr

svActRIIB (E28W, S44T) with signal sequence  
(SEQ ID NO: 3)

atggagtttggctgagctgggtttcctcggtcttttaagagggtccagtgtgagacacggtggtcatctactacaac  
gccaactggagctggagcgcaccaaccagaccggcctggagcgctgcgaaggcgagcaggacaagcggtctgcactgtca  
tcctggcgcaacagctctggcaccatcgagctcgtgaagaaggctgtctgttagatgactcaactgtca  
tgtgtggccactgaggagaacccccagggtacttctgtctgtgagggcaattctgcaacgagcgcttcactcatttgc  
gaggctggggcccgaaactcacgtacgaggccaccccgacagccccac

svActRIIB (E28W, S44T) with signal sequence  
(SEQ ID NO: 4)

mefglswvflvallrgvqce trwciyynanwelrtntqtlcercegeqdkrhcyaswrnssgtielvkkgcwlddfn

cydrqecvateenpqvyfcccegnfcnerfthlpeaggpevtyeppptapt

svActRIIB (E28W, S44T) without signal sequence

(SEQ ID NO: 5)

gagacacggtggtgcataactacaacgcactggagctggagcgcaccaaccagaccggcctggagcgctgcgaa

ggcgagcaggacaagcggtgcactgtacgcctctggcaacagctctggaccatcgagctcgtgaagaaggcc

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tgctggctagatgacttcaactgctacgataggcaggagtgtgtggccactgaggagaaccccaggtgtacttctgc  
 tgctgtagggaacttctgcaacgcgcgttcaactcattgccagaggctggggcccgaaagtacgtacgagcca  
 cccccgacagccccacc

svActRIIB (E28W, S44T) without signal sequence  
 (SEQ ID NO: 6)  
 etrwciiyynanwelertnqtglercegeqdkr1hcyaswrnssgtielvkkcwlddfnchydrqecvateen

pqvyfcccegnfcnerfthlpeaggpevtyeppptapt

svActRIIB-Fc (E28W, S44T) polynucleotide sequence with signal sequence  
 (SEQ ID NO: 7)  
 atggagttgggctgagctgggttttctcgctgtcttttaagagggtgtccagtgtgagacacgggtggcatc

tactacaacgccaactgggagctggagcgcaccaaccagaccggctggagcgtgcgaaggcgagcaggacaag  
 cggctgactgtacgcctctggcgacacagctctggcaccatcgagctcgtaagaagggtgtggcttagat  
 gacttcaactgtacgataggcaggagtgtgtggcaactgaggagaaccccccagggttacttctgtgtgag  
 ggcaacttctgcaacgagcgttcactcattccagggctggggccggaaagtacgtacgagccaccccg  
 acagccccacggagggggaggatctgtcagtgccacccgtgcccagcacccatgtggcaggaccgtcagtc  
 ttccctttccccaaaaccaaggacaccctcatgtatctccggacccttggatcactgtgcgtgtggac  
 gtgagccacgaagaccccgaggtccagttcaactggtacggtggacggcgtggagggtgcataatgcaagacaag  
 ccacgggaggaggcgttcaacacgacgttccgtgtggatcagctccgttgcaccaggactggctgaac  
 ggcaaggagtacaagtgcaggtctcaacaaaggctccagccccatcgagaaaaccatctccaaaaccaaa  
 gggcagccccgagaaccacagggttacaccctggccatccggaggatgccaagaaccaggctcagctg  
 acctgcctggtaaaggcttatccagcgttgcacccatccggaggatggcaatggcagccgagaacaac  
 tacaagaccacacctccatgtggactccgacgggttgcacagcaagctcaccgtggacaagagc  
 aggtggcagcaggaaacgttctcatgtccgtgtggatcgtggaggcttgcacaaccactacacgcagaagagc  
 ctccctgtctccggtaaa

svActRIIB-Fc (E28W, S44T) polypeptide sequence with signal sequence  
 (SEQ ID NO: 8)  
 mefglswwflvallrgvqcetrwciiyynanwelertnqtglercegeqdkr1hcyaswrnssgtielvkkcwld  
 dfnchydrqecvateenpqvyfcccegnfcnerfthlpeaggpevtyeppptaptgggsvvecpcpappvagsv  
 f1fppkpkdtlmisrtpevtvvvdvshedpevqfnwyvdgvvhnaktpreeqfnstfrvsvltvhqdwln  
 gkeyckvsnkglpapiektsktkgpypqvtlppsreemtknqvslltclvkgfypsdiavewesngqpenn  
 ykttppmldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqksllspgk

svActRIIB-Fc (E28W, S44T) polynucleotide sequence without signal sequence  
 (SEQ ID NO: 9)  
 gagacacgggtggcatctactacaacgccaactggagctggagcgcaccaaccagaccggctggagcgct  
 gcgaaggcgagcaggacaaggccgtcactgtacgcctctggcgcaacagctcgaccatcgagtcgt  
 gaagaaggctgtggcttagatgacttcaactgtacgataggcaggagtgtgtggccactgaggagaacccc  
 cagggtgtacttctgtgtggcaacttctgcaacgcgcgttcaactcattgccagggctggggcc  
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 accccctgaggtacgtacgccgtggacgtgagccacgaagaccccgagggtccagttcaactggtacg  
 acggcgtggagggtgcataatgccaagacaaaggccacggaggagcgttcaacagcacgttccgtgtggc  
 cgttccatccgtgtgcaccaggactggctgaacggcaaggatcactgtcaagggtctccaaacaaaggc  
 ccagccccatcgagaaaaccatctccaaaaccaaggccagccccgagaaccacagggttacaccctg  
 catcccgaggagatgaccaagaaccaggtacgctgacctgcgttcaaggcttcatccagcgacat

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cgccgtggagtgaggagcaatggcagccgagaacaactacaagaccacacctccatgtggactccgac  
 ggctcttcttccttacagcaagctaccgtggacaagagcagggtggcagcagggaaacgtttctcatgct  
 ccgtatgtcatgaggctctgcacaaccacacgcagaagagcgcctccgtccggtaaa  
 svActRIIB-Fc (E28W, S44T), polypeptide sequence without signal sequence (SEQ ID NO: 10)  
 etrwciyynanwelertnqtglercegeqdkrhcyaswrnssgtielvkkgcwlddfnchydrqecvateenp  
 qvyfcccegnfcnerfthlpeaggpevtyeppptaptgggsvccppcpappavagsvflfpkpkdtmlisr  
 tpevtcvvvvdvshedpevqfnwyvdgvevhnaktkpreeqfnstfrvvsvltvvhqdwlngkeykckvsnkg1  
 papiektisktkggpypqvtlppreemtnknqvsltclvkgyfypsdiavewesngqpennyktppldsd  
 gsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslslspgk  
 svActRIIB (E28Y, S44T) with signal sequence (SEQ ID NO: 11)  
 atggagttggctgagctgggtttccctgtgtcttttaagagggtgtccagtgtgagacacggtactgcac  
 tactacaacgccaactggagctggagcgcaccaaccagaccggctggagcgtgcgaaggcagcaggacaag  
 cggctgactgtacgcctctggcgcaacagctctggcaccatcgagctcgtgaagaaggctgtggctagat  
 gacttcaactgtacgataggcaggagtgtgtggccactgaggagaacccccagggttacttctgtctgtgag  
 ggcacactctgcaacgagcgcctcactcattgccagaggctggggccggaaagtcaacgtacgagccaccc  
 acagccccccacc  
 svActRIIB (E28Y, S44T) with signal sequence (SEQ ID NO: 12)  
 mefglswvflvalrgvqcetrciyynanwelertnqtglercegeqdkrhcyaswrnssgtielvkkgcwl  
 ddfnchydrqecvateenpqvyfcccegnfcnerfthlpeaggpevtyeppptapt  
 svActRIIB (E28Y, S44T) without signal sequence (SEQ ID NO: 13)  
 gagacacggtaactgcacactacaacgccaactggagctggagcgcaccaaccagaccggctggagcgt  
 gcaaggcggcaggacaagccgtcaactgtacgcctctggcgcaacagctctggcaccatcgagctcgt  
 gaagaaggctgtggtagatgacttcaactgtacgataggcaggagtgtgtggccactgaggagaacccc  
 cagggttacttctgtgtgagggcaacttctgcaacgagcgcctcactcattgccagaggctggggcc  
 cggaaagtcaacgtacgagccaccccgacagccccacc  
 svActRIIB (E28Y, S44T) without signal sequence (SEQ ID NO: 14)  
 etryciyynanwelertnqtglercegeqdkrhcyaswrnssgtielvkkgcwlddfnchydrqecvateenpqyfc  
 ccegnfcnerfthlpeaggpevtyeppptapt  
 svActRIIB-Fc (E28Y, S44T) polynucleotide sequence with signal sequence (SEQ ID NO: 15)  
 atggagttggctgagctgggtttccctgtgtcttttaagagggtgtccagtgtgagacacggtactgcac  
 tacaacgccaactggagctggagcgcaccaaccagaccggctggagcgtgcgaaggcagcaggacaacggctg  
 cactgctacgcctctggcgcaacagctctggcaccatcgagctcgtgaagaaggctgtggctagatgacttcaac  
 tgctacgataggcaggagtgtgtggccactgaggagaacccccagggttacttctgtgtgtggggcaacttgc  
 aacgagcgcctcactcattgccagaggctggggccggaaagtcaacgtacgagccaccccgacagccccacc  
 gggggaggatctgtcgagtggccacccgtggccacccgtggcaggacccgtcgttcccttccccaaaa  
 cccaaaggacacccctcatgtctccggacccctgaggtcacgtgcgtggggacgtgaggccacgaagacccgag  
 gtccagttcaactgggtacgtggacggcgtggaggtgcataatgcaagacaaggccacggggaggcagttcaac  
 acgttccgtgtggcgtcgactccgtggcaccaggactggctgaacggcaaggagtacaagtgcacggacttcc  
 aacaaaggccctccagccccatcgagaaaaccatctccaaaccaaggccagccccgagaaccacagggttacacc  
 ctgccccatccggggaggagatgaccaagaaccaggtaagctgacctgcctggtaaaggcttctatccagcgac  
 atgcggcgtggagtgggagagcaatggcagccggagaacaactacaagaccacacccatgtggactccgacggc

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tccttcttcctacagcaagtcaccgtggacaagagcaggcggcagcagggaaacgtcttcatgctccgtatg  
 catgaggctctgcacaaccactacacgcagaagagcctctccctgtctccggtaaa  
 svActRIIB-Fc (E28Y, S44T) polypeptide sequence with signal sequence  
 (SEQ ID NO: 16)  
 mefglswvflvalrrgvqcetrciyynanwelertnqtglercegeqdkr1hcyaswrnssgtielvkkgcwlddfn  
 cydrqecvateenpqvyyfcccegnfcnerfthlpeaggpevtyleppptaptgggsveccccpappvagsvflfppk  
 pkdtlmisrtpevtcvvvvdvshedpevqfnwyvdgvevhnaktkpreeqfnstfrvvsvltvvhqdwlngkeykckvs  
 nkglpapipektisktkgqpqrepqvytlppsreemtnqvslltclvkgfypsdiavewesngqpennykttppmlsdg  
 sfflyskltvdksrwqqgnvfscvmhealhnhytqkslslspgk  
 svActRIIB-Fc (E28Y, S44T) polynucleotide sequence without signal sequence  
 (SEQ ID NO: 17)  
 gagacacacggtaactgcatactacaacgcactggagctggagcgcaccaaccagaccggcctggagcgtgc  
 gaaggcgagcaggacaagcggtgcactgctacgcctctggcaacagctctggcaccatcgagctgtgaag  
 aaggcgtctggctagatgactcaactgctacatgaggcaggagtgtgtggccactgaggagaacccccaggatg  
 tacttcgtctgtgaggcaacttgcacgcgttcactcattgcagaggctggggccggaaagtc  
 acgtacgagccaccccccacagccccccacggaggggaggatctgtcgagtgcaccctggccaggcaccac  
 gtggcaggaccgtcagtcttccttccccaaaacccaaggacaccctcatgatctccggaccctgaggtc  
 acgtgcgtgggtggcgtgaggcaactacaacgcgttcactggtaactggacggcgtggaggatg  
 cataatgccaagacaagccacgggaggagcagttcaacagcacgttccgtgttgtcagegtctcacccgttg  
 caccaggactggctgaacggcaaggatgactggcaggatcacaaggatcacaacaaaggccctccagccccatcgagaaa  
 accatctccaaaaccaaagggcagccccgagaaccacaggatgactccctgcggatccggaggatgacc  
 aagaaccaggcgtaccgtgaccctggtcaaggatctatccagcgacatcgccgtggaggatgaggcaat  
 gggcagccggagaacaactacaagaccacacccatgtgactccgcggccttcttctacagcaag  
 ctcaccgtggacaagaggcagggtggcagggaaacgttcttcatgctccgtatgcatgaggctctgcacaac  
 cactacacgcagaagaggcctccctgtctccggtaaa  
 svActRIIB-Fc (E28Y, S44T) polypeptide sequence without signal sequence  
 (SEQ ID NO: 18)  
 etryciyynanwelertnqtglercegeqdkr1hcyaswrnssgtielvkkgcwlddfnycdrqecvateenp  
 qvyfcccegnfcnerfthlpeaggpevtyleppptaptgggsveccccpappvagsvflfppkpkdtlmisr  
 tpevtcvvvvdvshedpevqfnwyvdgvevhnaktkpreeqfnstfrvvsvltvvhqdwlngkeykckvsnkgl  
 papiektisktkgqpqrepqvytlppsreemtnqvslltclvkgfypsdiavewesngqpennykttppmlsd  
 gsfflyskltvdksrwqqgnvfscvmhealhnhytqkslslspgk  
 (SEQ ID NO: 19)  
 Glu Thr Arg Trp Cys Ile Tyr Tyr Asn Ala Asn Trp Glu Leu Glu Arg Thr Asn Gln  
 Ser Gly Leu Glu Arg Cys Glu Gly Glu Gln Asp Lys Arg Leu His Cys Tyr Ala Ser Trp Arg  
 Asn Ser Ser Gly Thr Ile Glu Leu Val Lys Lys Gly Cys Trp Leu Asp Asp Phe Asn Cys Tyr  
 Asp Arg Gln Glu Cys Val Ala Thr Glu Glu Asn Pro Gln Val Tyr Phe Cys Cys Glu Gly  
 Asn Phe Cys Asn Glu Arg Phe Thr His Leu Pro Glu Ala Gly Gly Pro Glu Val Thr Tyr Glu  
 Pro Pro Pro Thr Ala Pro Thr  
 (SEQ ID NO: 22)  
 Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr  
 Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro  
 Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg  
 Glu Glu Gln Phe Asn Ser Thr Phe Arg Val Val Ser Val Leu Thr Val Val His Gln Asp Trp

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Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu Lys  
 Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg  
 Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser  
 Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro  
 Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg  
 Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr  
 Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys

(SEQ ID NO: 23)

Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp  
 Ile Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp  
 Pro Glu Val Lys Phe Asn Trp Tyr Val Gly Gly Val Glu Val His Asn Ala Lys Thr Lys Pro  
 Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp  
 Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu  
 Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser  
 Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro  
 Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro  
 Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser  
 Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr  
 Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys

(SEQ ID NO: 24)

Ala Pro Glu Phe Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp  
 Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp  
 Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro  
 Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp  
 Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu  
 Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser  
 Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser  
 Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro  
 Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg  
 Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr  
 Gln Lys Ser Leu Ser Leu Ser Leu Gly Lys

Linker

(SEQ ID NO: 25)

Gly Gly Gly Ser

Hinge Linker

(SEQ ID NO: 26)

gga ggg gga gga tct gtc gag tgc cca ccg tgc cca

Hinge Linker

(SEQ ID NO: 27)

Gly Gly Gly Ser Val Glu Cys Pro Pro Cys Pro

(SEQ ID NO: 28)

Glu Arg Lys Cys Cys Val Glu Cys Pro Pro Cys Pro

(SEQ ID NO: 29)

Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro

(SEQ ID NO: 30)

Glu Ser Lys Thr Gly Pro Pro Cys Pro Ser Cys Pro

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**87****88**

-continued

(SEQ ID NO: 31)

Met Thr Ala Pro Trp Val Ala Leu Ala Leu Leu Trp Gly Ser Leu Trp Pro Gly

(SEQ ID NO: 32)

Met Thr Ala Pro Trp Val Ala Leu Ala Leu Leu Trp Gly Ser Leu Cys Ala Gly

Hinge Linker

(SEQ ID NO: 37)

gga ggg gga gga tct gag cgc aaa tgt tgt gtc gag tgc cca ccg tgc

Hinge Linker

(SEQ ID NO: 38)

Gly Gly Gly Ser Glu Arg Lys Cys Cys Val Glu Cys Pro Pro Cys

Hinge Linker

(SEQ ID NO: 39)

gga ggg gga gga tct ggt gga ggt ggt tca ggt cca ccg tgc

Hinge Linker

(SEQ ID NO: 40)

Gly Gly Gly Ser Gly Gly Ser Gly Pro Pro Cys

(SEQ ID NO: 41)

gga ggg gga gga tct ggt gga ggt ggt tca ggt cca ccg gga

(SEQ ID NO: 42)

Gly Gly Gly Ser Gly Gly Ser Gly Pro Pro Gly

Hinge Linker

(SEQ ID NO: 43)

gga ggg gga gga tct gag cgc aaa tgt cca cct tgt gtc gag tgc cca ccg tgc

Hinge Linker

(SEQ ID NO: 44)

Gly Gly Gly Ser Glu Arg Lys Cys Pro Pro Cys Val Glu Cys Pro Pro Cys

Hinge Linker

(SEQ ID NO: 45)

Gly Pro Ala Ser Gly Gly Pro Ala Ser Gly Pro Pro Cys Pro

Hinge Linker

(SEQ ID NO: 46)

Gly Pro Ala Ser Gly Gly Pro Ala Ser Gly Cys Pro Pro Cys Val Glu Cys Pro Pro

Cys Pro

(SEQ ID NO: 47)

Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp

Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp

Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro

Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp

Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu

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

Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser

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

Val Leu Asp Ser Asp Gly Ser Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr

Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys

Hinge Linker

(SEQ ID NO: 48)

Gly Gly Gly Ser Val Asp Lys Thr His Thr Cys Pro Pro Cys Pro

Hinge Linker

(SEQ ID NO: 49)

Gly Gly Gly Ser Val Asp Lys Thr His Thr Gly Pro Pro Cys Pro

(SEQ ID NO: 50)

Gly Gly Gly Ser Gly Gly Ser Val Asp Lys Thr His Thr Gly Pro Pro

Cys Pro

Stabilized activin type IIB polypeptides bind to ligands that activate muscle-degradation cascades. svActRIIB polypeptides capable of binding and inhibiting the activity of the ligands activin-A, myostatin, and/or GDF-11, and have the ability to treat diseases that involve muscle atrophy, as well as the treatment of certain cancers, and other diseases.

Pharmaceutical Compositions and Methods for Treatment

Methods of Treatment

In one aspect, the present invention provides methods of treating a subject. The method can, for example, have a generally beneficial effect on the subject's health, e.g., it can increase the subject's expected longevity. Alternatively, the method can, for example, treat, prevent, cure, relieve, or ameliorate ("treat") a disease, disorder, condition, or illness ("a condition"). Among the conditions to be treated in accordance with the present invention are conditions characterized by inappropriate expression or activity of activin-A. In some such conditions, the expression or activity level is too high, and the treatment comprises administering an activin-A antagonist as described herein. As used herein the term "subject" refers to any animal, such as mammals including humans.

One example of a type of condition that can be treated using the methods and compositions of the present invention is a condition that involves cell growth, for example, a cancerous condition which is accompanied by cachexia. Thus, in one embodiment, the present invention provides compositions and methods for treating a cancerous condition. In particular, the cancerous condition is a gonadal cancer, including tumors of the ovary and testis. (Fujii, Y. et al., *Am. J. Phys. Endocrin. Metab.*, 286:E927-E931, 2004; Reis, F. M. et al., *J Clin. Endocrin.* 87:2277-2282, 2005.) Activin-A is known for its action in stimulating FSH biosynthesis and secretion in the pituitary gland, and has a physiological role in the regulation of gonadal function. Activin-A has been associated with many types of human cancers and in particular with tumors of the reproductive system. Specifically, overexpression or deregulation of activin-A has been implicated in ovarian cancer, (Menon U, et al., *BJOG: An International Journal of Obstetrics & Gynaecology*; 107(9):1069-74, 2000. Choi K C, et al., *Molecular & Cellular Endocrinology*. 174(1-2):99-110, 2001; Zheng W, et al., *American Journal of Reproductive Immunology*. 44(2):104-13, 2000; Lambert-Messerlian G M, et al., *Gynecologic Oncology*. 74(1):93-7, 1999; Steller M D, et al., *Molecular Cancer Research: MCR*. 3(1):50-61, 2005; Corbellis L, et al., *Journal of the Society for Gynecologic Investigation*. 11(4):203-6, 2004; Welt C K, et al., *Journal of Clinical Endocrinology & Metabolism*. 82(11):3720-7, 1997; and Harada K., et al., *Journal of Clinical Endocrinology & Metabolism*. 81(6):2125-30, 1996, endometrial adenocarcinoma Otani, T, et a., *Gynecologic Oncology*. 83(1):31-8, 2001; Tanaka T, et al., *International Journal of Oncology*. 23(3):657-63, 2003 and prostate cancer (Thomas T Z, et al., *Journal of Clinical Endocrinology & Metabolism*. 82(11):3851-8, 1997; Zhang, Z, et al., *Biochemical & Biophysical Research Communications*. 234(2):362-5, 1997; and Risbridger G P, et al., *Molecular & Cellular Endocrinology*. 180(1-2):149-53, 2001).

The cancerous condition can be any cancerous condition that can be treated using the compositions comprised herein, for example, anti-activin-A compounds such as activin IIB receptor polypeptides (svActRIIB), and activin-A antigen

binding proteins such as anti-activin-A antibodies, antibody fragments, or antibody derivatives. Examples of cancerous conditions include, for example, acute lymphoblastic leukemia, adrenocortical carcinoma, AIDS-related cancers, AIDS-related lymphoma, anal cancer, childhood cerebellar astrocytoma, childhood cerebral astrocytoma, basal cell carcinoma, extrahepatic bile duct cancer, bladder cancer, osteosarcoma/malignant fibrous histiocytoma bone cancer, brain tumors (e.g., brain stem glioma, cerebellar astrocytoma, cerebral astrocytoma/malignant glioma, ependymoma, medulloblastoma, supratentorial primitive neuroectodermal tumors, visual pathway and hypothalamic glioma), breast cancer, bronchial adenomas/carcinoids, Burkitt's Lymphoma, carcinoid tumor, gastrointestinal carcinoid tumor, carcinoma of unknown primary, primary central nervous system, cerebellar astrocytoma, cerebral astrocytoma/malignant glioma, cervical cancer, childhood cancers, chronic lymphocytic leukemia, chronic myelogenous leukemia, chronic myeloproliferative disorders, colon cancer, colorectal cancer, cutaneous t-cell lymphoma, endometrial cancer, ependymoma, esophageal cancer, ewing's family of tumors, extracranial germ cell tumor, extragonadal germ cell tumor, extrahepatic bile duct cancer, intraocular melanoma eye cancer, retinoblastoma eye cancer, gallbladder cancer, gastric (stomach) cancer, gastrointestinal carcinoid tumor, germ cell tumors (e.g., extracranial, extragonadal, and ovarian, gestational trophoblastic tumor, glioma (e.g., adult, childhood brain stem, childhood cerebral astrocytoma, childhood visual pathway and hypothalamic), hairy cell leukemia, head and neck cancer, hepatocellular (liver) cancer, Hodgkin's lymphoma, hypopharyngeal cancer, hypothalamic and visual pathway glioma, intraocular melanoma, islet cell carcinoma (endocrine pancreas), Kaposi's Sarcoma, kidney (renal cell) cancer, laryngeal cancer, leukemia (e.g., acute lymphoblastic, acute myeloid, chronic lymphocytic, chronic myelogenous, and hairy cell), lip and oral cavity cancer, liver cancer, non-small cell lung cancer, small cell lung cancer, lymphoma (e.g., AIDS-related, Burkitt's, cutaneous t-cell, Hodgkin's, non-Hodgkin's, and primary central nervous system), Waldenstrom's Macroglobulinemia, malignant fibrous histiocytoma of bone/osteosarcoma, medulloblastoma, melanoma, intraocular (eye) melanoma, Merkel cell carcinoma, mesothelioma, metastatic squamous neck cancer with occult primary, multiple endocrine neoplasia syndrome, multiple myeloma/plasma cell neoplasm, mycosis fungoides, myelodysplastic syndromes, myelodysplastic/myeloproliferative diseases, myelogenous leukemia, chronic myeloid leukemia, multiple myeloma, chronic myeloproliferative disorders, nasal cavity and paranasal sinus cancer, nasopharyngeal cancer, neuroblastoma, oral cancer, oropharyngeal cancer, osteosarcoma/malignant fibrous histiocytoma of bone, ovarian cancer, ovarian epithelial cancer, ovarian germ cell tumor, ovarian low malignant potential tumor, pancreatic cancer, islet cell pancreatic cancer, paranasal sinus and nasal cavity cancer, parathyroid cancer, penile cancer, pheochromocytoma, pineoblastoma, pituitary tumor, plasma cell neoplasm/multiple myeloma, pleuropulmonary blastoma, primary central nervous system lymphoma, prostate cancer, rectal cancer, renal cell (kidney) cancer, renal pelvis and ureter transitional cell cancer, rhabdomyosarcoma, salivary gland cancer, soft tissue sarcoma, uterine sarcoma, Sezary syndrome, non-melanoma skin cancer, merkel cell skin carcinoma, small intestine cancer, soft tissue sarcoma, squamous cell carcinoma, cutaneous t-cell lymphoma, testicular cancer, thymoma, thymic carcinoma, thyroid cancer, gestational trophoblastic tumor, carcinoma of unknown primary site,

cancer of unknown primary site, urethral cancer, endometrial uterine cancer, uterine sarcoma, vaginal cancer, visual pathway and hypothalamic glioma, vulvar cancer, Waldenstrom's Macroglobulinemia, and Wilms' Tumor.

Certain methods provided herein comprise administering an activin-A binding protein to a subject, thereby reducing an activin-A-induced biological response that plays a role in a particular condition. In particular embodiments, methods of the invention involve contacting endogenous activin-A with an activin-A binding protein, e.g., via administration to a subject or in an ex vivo procedure.

The term "treatment" encompasses alleviation or prevention of at least one symptom or other aspect of a disorder, or reduction of disease severity, and the like. In addition, "treatment" further relates to administering a therapeutic agent described herein for preventing or alleviating at least one symptom or other aspect of a disorder in a subject in need thereof. An antigen binding protein need not affect a complete cure, or eradicate every symptom or manifestation of a disease, to constitute a viable therapeutic agent. As is recognized in the pertinent field, drugs employed as therapeutic agents may reduce the severity of a given disease state, but need not abolish every manifestation of the disease to be regarded as useful therapeutic agents. Similarly, a prophylactically administered treatment need not be completely effective in preventing the onset of a condition in order to constitute a viable prophylactic agent. Simply reducing the impact of a disease (for example, by reducing the number or severity of its symptoms, or by increasing the effectiveness of another treatment, or by producing another beneficial effect), or reducing the likelihood that the disease will occur or worsen in a subject, is sufficient. One embodiment of the invention is directed to a method comprising administering to a patient an activin-A antagonist in an amount and for a time sufficient to induce a sustained improvement over baseline of an indicator that reflects the severity of the particular disorder.

Use of antigen binding proteins in ex vivo procedures also is contemplated. For example, a patient's blood or other bodily fluid may be contacted with a protein that binds full-length activin-A, one or more activin-A isoform, or other partial length activin-A ex vivo. The antigen binding protein may be bound to a suitable insoluble matrix or solid support material.

#### Identifying a Subject for Treatment

A subject's levels of biomarker CA-125 and/or activin-A can be monitored to identify a subject in need of treatment for ovarian cancer, including serous ovarian cancer (ovarian neoplasms, including surface epithelial-stromal tumors). For example, levels of biomarker CA-125 and/or activin-A can be detected in the subject and compared to a control. First, the subject's expression levels of CA-125 and/or activin A are evaluated. Next, the subject's expression levels of CA-125 and/or activin-A are compared to expression levels in a negative control sample or a positive control sample. If the expression levels of CA-125 and/or activin-A in the subject exceed the expression levels in the negative control sample, or if the expression levels meet or exceed the expression levels in the positive control sample, the subject is identified as one needing ovarian cancer treatment. In some aspects, if the expression levels exceed the expression levels of the subject taken at a previous time, in particular when the tumor was in its early stages, the subject can be identified as one needing ovarian cancer treatment. Known techniques can be employed for measuring CA-125 and/or activin-A levels, e.g., in a subject's serum. CA-125 and/or

activin-A levels in blood samples can be measured using any suitable technique, for example, ELISA or RT-PCR.

A subject's levels of activin-A, VEGF, and/or Ang-1 factors can be monitored to identify a subject in need of treatment for ovarian cancer, including clear cell ovarian cancer (epithelial ovarian neoplasm arising from embryonic mesonephros), Granulosa cell ovarian cancer (neoplasms from sex-cord stromal cells), Leydig cell tumors (testicular tumor derived from Leydig cells), and sex cord stromal testicular tumors (derived from testicular and ovarian stroma). Levels of activin-A, VEGF, and/or Ang-1 factors can be detected in the subject and compared to a control. First, the subject's expression levels of activin-A, VEGF, and/or Ang-1 are evaluated. Next, the subject's expression levels of activin-A, VEGF, and/or Ang-1 are compared to expression levels in a negative control sample or a positive control sample. If the expression levels of activin-A, VEGF, and/or Ang-1 in the subject exceed the expression levels in the negative control sample, or if the expression levels meet or exceed the expression levels of the respective factors in the positive control sample, the subject is identified as one needing ovarian cancer treatment. In one embodiment, if activin-A levels in a subject are three times the activin-A levels in the average person of the same age, or if activin-A levels in a subject exceed 3200 pg/mL, it can predict that the particular subject should begin receiving treatment. Known techniques can be employed for measuring activin-A, VEGF, and/or Ang-1 levels, e.g., in a subject's serum. Activin-A, VEGF, and/or Ang-1 levels in blood samples can be measured using any suitable technique, for example, ELISA.

In some embodiments, the subject has a mutated activin gene or a mutated activin counter regulator gene, such as inhibin. In further embodiments, the mutation is an Asn386Ser mutation in the Beta-A-subunit of inhibin or activin proteins (GenBank Accession Number: NM\_002192.2; MIM #147290), an Arg60Leu mutation of the alpha prodomain of inhibin or activin proteins, (GenBank Accession Number: NM\_002191.3), or a Gly280Glu mutation of the alpha prodomain of inhibin or activin proteins (GenBank Accession Number: NM\_002192.2) (see Tournier et al., *Hum. Mutat.* 0:1-4, 2013).

#### Compositions

Pharmaceutical compositions containing the proteins and polypeptides of the present invention are also provided. Such compositions comprise a therapeutically or prophylactically effective amount of the polypeptide or protein in admixture with pharmaceutically acceptable materials, and physiologically acceptable formulation materials. The pharmaceutical composition may contain formulation materials for modifying, maintaining or preserving, for example, the pH, osmolarity, viscosity, clarity, color, isotonicity, odor, sterility, stability, rate of dissolution or release, adsorption or penetration of the composition.

Suitable formulation materials include, but are not limited to, amino acids (such as glycine, glutamine, asparagine, arginine or lysine); antimicrobials; antioxidants (such as ascorbic acid, sodium sulfite or sodium hydrogen-sulfite); buffers (such as borate, bicarbonate, Tris-HCl, citrates, phosphates, other organic acids); bulking agents (such as mannitol or glycine), chelating agents (such as ethylenediamine tetraacetic acid (EDTA)); complexing agents (such as caffeine, polyvinylpyrrolidone, beta-cyclodextrin or hydroxy-propyl-beta-cyclodextrin); fillers; monosaccharides; disaccharides and other carbohydrates (such as glucose, mannose, or dextrans); proteins (such as serum albumin, gelatin or immunoglobulins); coloring; flavoring and diluting agents;

emulsifying agents; hydrophilic polymers (such as polyvinylpyrrolidone); low molecular weight polypeptides; salt-forming counterions (such as sodium); preservatives (such as benzalkonium chloride, benzoic acid, salicylic acid, thimerosal, phenethyl alcohol, methylparaben, propylparaben, chlorhexidine, sorbic acid or hydrogen peroxide); solvents (such as glycerin, propylene glycol or polyethylene glycol); sugar alcohols (such as mannitol or sorbitol); suspending agents; surfactants or wetting agents (such as pluronics, PEG, sorbitan esters, polysorbates such as polysorbate 20, polysorbate 80, triton, tromethamine, lecithin, cholesterol, tyloxapal); stability enhancing agents (sucrose or sorbitol); tonicity enhancing agents (such as alkali metal halides (preferably sodium or potassium chloride, mannitol or sorbitol); delivery vehicles; diluents; excipients and/or pharmaceutical adjuvants. Neutral buffered saline or saline mixed with conspecific serum albumin are examples of appropriate diluents. In accordance with appropriate industry standards, preservatives such as benzyl alcohol may also be added. The composition may be formulated as a lyophilizate using appropriate excipient solutions (e.g., sucrose) as diluents. Suitable components are nontoxic to recipients at the dosages and concentrations employed. Further examples of components that may be employed in pharmaceutical formulations are presented in Remington's Pharmaceutical Sciences, 16<sup>th</sup> Ed. (1980) and 20<sup>th</sup> Ed. (2000), Mack Publishing Company, Easton, PA

Optionally, the composition additionally comprises one or more physiologically active agents, for example, a second activin-A receptor-inhibiting substance, an anti-angiogenic substance, a chemotherapeutic substance (such as capecitabine, 5-fluorouracil, or doxorubicin), an analgesic substance, etc., non-exclusive examples of which are provided herein. In various particular embodiments, the composition comprises one, two, three, four, five, or six physiologically active agents in addition to an activin-A-binding protein.

In another embodiment of the invention, the compositions disclosed herein may be formulated in a neutral or salt form. Illustrative pharmaceutically-acceptable salts include the acid addition salts (formed with the free amino groups of the protein) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, histidine, procaine and the like. Upon formulation, solutions will be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically effective.

The carriers can further comprise any and all solvents, dispersion media, vehicles, coatings, diluents, antibacterial and antifungal agents, isotonic and absorption delaying agents, buffers, carrier solutions, suspensions, colloids, and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, its use in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions. The phrase "pharmaceutically-acceptable" refers to molecular entities and compositions that do not produce an allergic or similar untoward reaction when administered to a human.

The optimal pharmaceutical composition will be determined by one skilled in the art depending upon, for example, the intended route of administration, delivery format, and

desired dosage. See for example, Remington's Pharmaceutical Sciences, supra. Such compositions may influence the physical state, stability, rate of in vivo release, and rate of in vivo clearance of the polypeptide. For example, suitable compositions may be water for injection, physiological saline solution for parenteral administration.

#### Administration of Treatment

The formulations can be delivered in a variety of methods, for example, subcutaneously, intravenously, intraperitoneally, orally, or by inhalation therapy. Such approaches are well known to the skilled artisan, some of which are further described, for example, in U.S. Pat. Nos. 5,543,158; 5,641,515 and 5,399,363. When parenteral administration is contemplated, the therapeutic compositions for use in this invention may be in the form of a pyrogen-free, parenterally acceptable aqueous solution comprising the desired polypeptide in a pharmaceutically acceptable vehicle. A particularly suitable vehicle for parenteral injection is sterile distilled water in which a polypeptide is formulated as a sterile, isotonic solution, properly preserved. Yet another preparation can involve the formulation of the desired molecule with an agent, such as injectable microspheres, bio-erodible particles, polymeric compounds (polylactic acid, polyglycolic acid), beads, or liposomes, that provides for the controlled or sustained release of the product which may then be delivered via a depot injection. Hyaluronic acid may also be used, and this may have the effect of promoting sustained duration in the circulation. Other suitable means for the introduction of the desired molecule include implantable drug delivery devices.

In another aspect, pharmaceutical formulations suitable for injectable administration may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks' solution, Ringer's solution, or physiologically buffered saline. Aqueous injection suspensions may contain substances that increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils, such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate, triglycerides, or liposomes. Non-lipid polycationic amino polymers may also be used for delivery. Optionally, the suspension may also contain suitable stabilizers or agents to increase the solubility of the compounds and allow for the preparation of highly concentrated solutions. In another embodiment, a pharmaceutical composition may be formulated for inhalation. Inhalation solutions may also be formulated with a propellant for aerosol delivery. In yet another embodiment, solutions may be nebulized. Pulmonary administration is further described in PCT Application No. PCT/US94/001875, which describes pulmonary delivery of chemically modified proteins.

In one embodiment, for parenteral administration in an aqueous solution, the solution should be suitably buffered if necessary and the liquid diluent first rendered isotonic with sufficient saline or glucose. These particular aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous and intraperitoneal administration. In this connection, a sterile aqueous medium that can be employed will be known to those of skill in the art in light of the present disclosure. For example, one dosage may be dissolved in 1 ml of isotonic NaCl solution and either added to 1000 ml of hypodermoclysis fluid or injected at the proposed site of infusion, (see for example, *Remington's Pharmaceutical Sciences*, 15th ed., pp. 1035-1038 and 1570-1580). Some variation in dosage will necessarily occur depending on the

condition of the subject being treated. Moreover, for human administration, preparations will of course preferably meet sterility, pyrogenicity, and the general safety and purity standards as required by FDA Office of Biologics standards.

It is also contemplated that certain formulations may be administered orally. In one embodiment of the present invention, molecules that are administered in this fashion can be formulated with or without those carriers customarily used in the compounding of solid dosage forms such as tablets and capsules. For example, a capsule may be designed to release the active portion of the formulation at the point in the gastrointestinal tract when bioavailability is maximized and pre-systemic degradation is minimized. Additional agents can be included to facilitate absorption of the therapeutic molecule. Diluents, flavorings, low melting point waxes, vegetable oils, lubricants, suspending agents, tablet disintegrating agents, and binders may also be employed. Pharmaceutical compositions for oral administration can also be formulated using pharmaceutically acceptable carriers well known in the art in dosages suitable for oral administration. Such carriers enable the pharmaceutical compositions to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for ingestion by the patient.

Pharmaceutical preparations for oral use can be obtained through combining active compounds with solid excipient and processing the resultant mixture of granules (optionally, after grinding) to obtain tablets or dragee cores. Suitable auxiliaries can be added, if desired. Suitable excipients include carbohydrate or protein fillers, such as sugars, including lactose, sucrose, mannitol, and sorbitol; starch from corn, wheat, rice, potato, or other plants; cellulose, such as methyl cellulose, hydroxypropylmethyl-cellulose, or sodium carboxymethylcellulose; gums, including arabic and tragacanth; and proteins, such as gelatin and collagen. If desired, disintegrating or solubilizing agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, and alginic acid or a salt thereof, such as sodium alginate.

Dragee cores may be used in conjunction with suitable coatings, such as concentrated sugar solutions, which may also contain gum arabic, talc, polyvinylpyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for product identification or to characterize the quantity of active compound, i.e., dosage.

Pharmaceutical preparations that can be used orally also include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a coating, such as glycerol or sorbitol. Push-fit capsules can contain active ingredients mixed with fillers or binders, such as lactose or starches, lubricants, such as talc or magnesium stearate, and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid, or liquid polyethylene glycol with or without stabilizers.

Additional pharmaceutical compositions will be evident to those skilled in the art, including formulations involving polypeptides in sustained- or controlled-delivery formulations. Techniques for formulating a variety of other sustained- or controlled-delivery means, such as liposome carriers, bio-erodible microparticles or porous beads and depot injections, are also known to those skilled in the art. See for example, PCT/US93/00829 that describes controlled release of porous polymeric microparticles for the delivery of pharmaceutical compositions. Additional examples of sustained-release preparations include semipermeable polymer

matrices in the form of shaped articles, e.g. films, or microcapsules. Sustained release matrices may include polyesters, hydrogels, polylactides (U.S. Pat. No. 3,773,919, EP 58,481), copolymers of L-glutamic acid and gamma ethyl-L-glutamate (Sidman et al., *Biopolymers*, 22:547-556 (1983), poly(2-hydroxyethyl-methacrylate) (Langer et al., *J. Biomed. Mater. Res.*, 15:167-277, (1981); Langer et al., *Chem. Tech.*, 12:98-105(1982)), ethylene vinyl acetate (Langer et al., *supra*) or poly-D(-)-3-hydroxybutyric acid (EP 133,988). Sustained-release compositions also include liposomes, which can be prepared by any of several methods known in the art. See e.g., Eppstein et al., *PNAS (USA)*, 82:3688 (1985); EP 36,676; EP 88,046; EP 143,949.

In certain embodiments, liposomes, nanocapsules, 15 microparticles, lipid particles, vesicles, and the like, are used for the introduction of the compositions of the present invention into suitable host cells/organisms. In particular, the compositions of the present invention may be formulated for delivery either encapsulated in a lipid particle, a liposome, a vesicle, a nanosphere, or a nanoparticle or the like. 20 Alternatively, compositions of the present invention can be bound, either covalently or non-covalently, to the surface of such carrier vehicles.

The formation and use of liposome and liposome-like 25 preparations as potential drug carriers is generally known to those of skill in the art (see for example, Lasic, *Trends Biotechnol.* 16(7):307-21, 1998; Takakura, *Nippon Rinsho* 56(3):691-95, 1998; Chandran et al., *Indian J Exp. Biol.* 35(8):801-09, 1997; Margalit, *Crit. Rev. Ther. Drug Carrier Syst.* 12(2-3):233-61, 1995; U.S. Pat. Nos. 5,567,434; 5,552, 30 157; 5,565,213; 5,738,868 and 5,795,587, each specifically incorporated herein by reference in its entirety). The use of liposomes does not appear to be associated with autoimmune responses or unacceptable toxicity after systemic delivery. In certain embodiments, liposomes are formed from phospholipids that are dispersed in an aqueous medium and spontaneously form multilamellar concentric bilayer vesicles (also termed multilamellar vesicles (MLVs)).

Alternatively, in other embodiments, the invention provides 40 for pharmaceutically-acceptable nanocapsule formulations of the compositions of the present invention. Nanocapsules can generally entrap compounds in a stable and reproducible way (see, for example, Quintanar-Guerrero et al., *Drug Dev. Ind. Pharm.* 24(12):1113-28, 1998). To avoid 45 side effects due to intracellular polymeric overloading, such ultrafine particles (sized around 0.1p m) may be designed using polymers able to be degraded in vivo. Such particles can be made as described, for example, by Couvreur et al., *Crit. Rev. Ther. Drug Carrier Syst.* 5(1):1-20, 1988; zur 50 Muhlen et al., *Eur. J. Pharm. Biopharm.* 45(2):149-55, 1998; Zambaux et al., *J. Controlled Release* 50(1-3):31-40, 1998; and U.S. Pat. No. 5,145,684.

The pharmaceutical composition to be used for in vivo 55 administration typically must be sterile. This may be accomplished by filtration through sterile filtration membranes. Where the composition is lyophilized, sterilization using this method may be conducted either prior to or following lyophilization and reconstitution. The composition for parenteral administration may be stored in lyophilized form or in solution. In addition, parenteral compositions generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

Once the pharmaceutical composition has been formulated, it may be stored in sterile vials as a solution, suspension, gel, emulsion, solid, or a dehydrated or lyophilized powder. Such formulations may be stored either in a ready-

to-use form or in a form (e.g., lyophilized) requiring reconstitution prior to administration.

In a specific embodiment, the present invention is directed to kits for producing a single-dose administration unit. The kits may each contain both a first container having a dried protein and a second container having an aqueous formulation. Also included within the scope of this invention are kits containing single and multi-chambered pre-filled syringes (e.g., liquid syringes and lyosyringes).

In addition, pharmaceutical compositions of the present invention may be placed within containers, along with packaging material that provides instructions regarding the use of such pharmaceutical compositions. Generally, such instructions will include a tangible expression describing the reagent concentration, as well as within certain embodiments, relative amounts of excipient ingredients or diluents (e.g., water, saline or PBS) that may be necessary to reconstitute the pharmaceutical composition.

The invention also provides a diagnostic kit comprising at least one anti-activin-A binding agent according to the present invention. The binding agent may be an antibody. In addition, such a kit may optionally comprise one or more of the following: (1) instructions for using the one or more binding agent(s) for screening, diagnosis, prognosis, therapeutic monitoring or any combination of these applications; (2) a labeled binding partner to the anti-activin-A binding agent(s); (3) a solid phase (such as a reagent strip) upon which the anti-activin-A binding agent(s) is immobilized; and (4) a label or insert indicating regulatory approval for screening, diagnostic, prognostic or therapeutic use or any combination thereof. If no labeled binding partner to the binding agent(s) is provided, the binding agent(s) itself can be labeled with one or more of a detectable marker(s), e.g. a chemiluminescent, enzymatic, fluorescent, or radioactive moiety.

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

Dosages and the frequency of administration may vary according to such factors as the route of administration, the particular proteins employed, the nature and severity of the disease to be treated, whether the condition is acute or chronic, and the size and general condition of the subject.

Appropriate dosages can be determined by procedures known in the pertinent art, e.g. in clinical trials that may involve dose escalation studies.

A polypeptide or protein of the invention may be administered, for example, once or more than once, e.g., at regular intervals over a period of time. In particular embodiments, a protein is administered over a period of at least a month or more, e.g., for one, two, or three months or even indefinitely. For treating chronic conditions, long-term treatment is generally most effective. However, for treating acute conditions, administration for shorter periods, e.g. from one to six weeks, may be sufficient. In general, the protein is administered until the patient manifests a medically relevant degree of improvement over baseline for the chosen indicator or indicators.

Particular embodiments of the present invention involve administering a protein at a dosage of from about 1 ng of protein per kg of subject's weight per day ("1 ng/kg/day") to about 10 mg/kg/day, more preferably from about 500 ng/kg/day to about 5 mg/kg/day, and most preferably from about 5 µg/kg/day to about 2 mg/kg/day, to a subject. In additional embodiments, a protein is administered to adults one time per week, two times per week, or three or more times per week, to treat an activin-A mediated disease, condition or disorder, e.g., a medical disorder disclosed herein. If injected, the effective amount of protein per adult dose may range from 1-20 mg/m<sup>2</sup>, and preferably is about 5-12 mg/m<sup>2</sup>. Alternatively, a flat dose may be administered; the amount may range from 5-100 mg/dose. One range for a flat dose is about 20-30 mg per dose. In one embodiment of the invention, a flat dose of 25 mg/dose is repeatedly administered by injection. If a route of administration other than injection is used, the dose is appropriately adjusted in accordance with standard medical practices. One example of a therapeutic regimen involves injecting a dose of about 20-30 mg of protein one to three times per week over a period of at least three weeks, though treatment for longer periods may be necessary to induce the desired degree of improvement. For pediatric subjects (age 4-17), one exemplary suitable regimen involves the subcutaneous injection of 0.4 mg/kg, up to a maximum dose of 25 mg of protein administered two or three times per week.

Particular embodiments of the methods provided herein involve subcutaneous injection of from 0.5 mg to 10 mg, preferably from 3 to 5 mg, of a protein, once or twice per week. Another embodiment is directed to pulmonary administration (e.g., by nebulizer) of 3 or more mg of protein once a week.

Examples of therapeutic regimens provided herein comprise subcutaneous injection of a protein once a week, at a dose of 1.5 to 3 mg, to treat a condition in which activin-A signaling plays a role. Examples of such conditions are provided herein and include, for example, cachexia, cancer, rheumatoid arthritis, and all conditions in which loss of body weight, body mass, body fat, or inability to maintain body weight, body mass, body fat, play a role. Weekly administration of protein is continued until a desired result is achieved, e.g., the subject's symptoms subside. Treatment may resume as needed, or, alternatively, maintenance doses may be administered.

Other examples of therapeutic regimens provided herein comprise subcutaneous or intravenous administration of a dose of 0.5, 1, 3, 5, 6, 7, 8, 9, 10, 11, 12, 15, or 20 milligrams of an activin-A inhibitor of the present invention per kilogram body mass of the subject (mg/kg). The dose can be administered once to the subject, or more than once at a certain interval, for example, once a day, three times a week,

twice a week, once a week, three times a month, twice a month, once a month, once every two months, once every three months, once every six months, or once a year. The duration of the treatment, and any changes to the dose and/or frequency of treatment, can be altered or varied during the course of treatment in order to meet the particular needs of the subject.

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

In another embodiment, a protein is administered to the subject in an amount and for a time sufficient to induce an improvement, preferably a sustained improvement, in at least one indicator that reflects the severity of the disorder that is being treated. Various indicators that reflect the extent of the subject's illness, disease or condition may be assessed for determining whether the amount and time of the treatment is sufficient. Such indicators include, for example, clinically recognized indicators of disease severity, symptoms, or manifestations of the disorder in question. In one embodiment, an improvement is considered to be sustained if the subject exhibits the improvement on at least two occasions separated by two to four weeks. The degree of improvement generally is determined by a physician, who may make this determination based on signs, symptoms, biopsies, or other test results, and who may also employ questionnaires that are administered to the subject, such as quality-of-life questionnaires developed for a given disease.

A subject's levels of activin-A may be monitored before, during and/or after treatment with a protein, to detect changes, if any, in their levels. For some disorders, the incidence of elevated activin-A levels may vary according to such factors as the stage of the disease or the particular form of the disease. Known techniques may be employed for measuring activin-A levels, e.g., in a subject's serum. Activin-A levels in blood samples may be measured using any suitable technique, for example, ELISA. In one embodiment, if activin-A levels in a subject are three times the activin-A levels in the average person of the same age, or if activin-A levels in a subject exceed 3200 pg/mL, it indicates that the particular subject should begin receiving treatment. In a further embodiment, activin-A levels can be monitored to determine whether treatment should continue. For example, if activin-A levels in a subject have declined from a baseline level after a certain period of treatment, it indicates that the particular subject is benefitting from the treatment and should continue to receive treatment.

In some cases, the polypeptides of the present invention can be delivered by implanting certain cells that have been genetically engineered, using methods such as those described herein, to express and secrete the polypeptide.

Such cells may be animal or human cells, and may be autologous, heterologous, or xenogeneic. Optionally, the cells may be immortalized. In order to decrease the chance of an immunological response, the cells may be encapsulated to avoid infiltration of surrounding tissues. The encapsulation materials are typically biocompatible, semi-permeable polymeric enclosures or membranes that allow the release of the polypeptide product(s) but prevent the destruction of the cells by the patient's immune system or by other detrimental factors from the surrounding tissues.

Gene therapy *in vivo* is also envisioned wherein a nucleic acid molecule encoding a polypeptide of the present invention, or a derivative of a polypeptide of the present invention is introduced directly into the subject. For example, a nucleic acid sequence encoding a polypeptide of the present invention is introduced into target cells via local injection of a nucleic acid construct with or without an appropriate delivery vector, such as an adeno-associated virus vector. Alternative viral vectors include, but are not limited to, retroviruses, adenovirus, herpes simplex, virus and papilloma virus vectors. Physical transfer of the virus vector may be achieved *in vivo* by local injection of the desired nucleic acid construct or other appropriate delivery vector containing the desired nucleic acid sequence, liposome-mediated transfer, direct injection (naked DNA), or microparticle bombardment (gene-gun).

#### Combination Therapy

The compositions of the present disclosure may be used alone or in combination with other therapeutic agents to enhance their therapeutic effects or decrease potential side effects. Particular embodiments of methods and compositions of the invention involve the use of an antigen binding protein and one or more additional activin-A antagonists, for example, two or more antigen binding proteins of the invention, or an antigen binding protein of the invention and one or more other activin-A antagonists. In further embodiments, antigen binding protein are administered alone or in combination with other agents useful for treating the condition with which the patient is afflicted. Examples of such agents include both proteinaceous and non-proteinaceous drugs. When multiple therapeutics are co-administered, dosages may be adjusted accordingly, as is recognized in the pertinent art. "Co-administration" and combination therapy are not limited to simultaneous administration, but also include treatment regimens in which a protein is administered at least once during a course of treatment that involves administering at least one other therapeutic agent to the patient.

Examples of other agents that may be co-administered with a protein are other proteins or therapeutic polypeptides that are chosen according to the particular condition to be treated. Alternatively, non-proteinaceous drugs that are useful in treating one of the particular conditions discussed above may be co-administered with an activin-A antagonist.

In one embodiment, a combination therapy achieves synergy or an additive effect by, for example, attacking multiple sites or molecular targets in a tumor. Types of combination therapies that can be used in connection with the present invention include inhibiting or activating (as appropriate) multiple nodes in a single disease-related pathway, multiple pathways in a target cell, and multiple cell types within a target tissue (e.g., within a tumor). For example, an activin-A inhibitor of the present invention can be combined with a treatment that promotes apoptosis or inhibits angiogenesis. In another embodiment, a targeted agent, that, when used by itself, fails to elicit a therapeutically desired effect, could be used to, for example, sensitize

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cancer cells or augment treatment effect of other agents. In another embodiment, an activin-A inhibitor according to the invention is used in combination with a cytotoxic drug or other targeted agent that induces apoptosis. In another embodiment, an activin-A inhibitor is used in combination with one or more agents that inhibit different targets that are involved in cell survival (e.g., PKB, mTOR), different receptor tyrosine kinases (e.g., ErbB1, ErbB2, c-Met, c-kit), or different cell types (e.g., KDR inhibitors, c-fms). In another embodiment, an activin-A inhibitor of the invention is added to the existing standard of care for a particular condition. In another embodiment, the combination therapy comprises treating a subject with an activin-A inhibiting proteins and anti-cancer treatments (such as surgery, ultrasound, radiotherapy, chemotherapy, or treatment with another anti-cancer agent).

Where a method of combination therapy comprises administering more than one treatment to a subject, it is to be understood that the order, timing, number, concentration, and volume of the administrations is limited only by the medical requirements and limitations of the treatment, i.e., two treatments can be administered to the subject, e.g., simultaneously, consecutively, alternately, or according to any other regimen. Examples of agents that can be administered in combination with the activin-A antagonists described herein include, but are not limited to, capecitabine, 5-fluorouracil, doxorubicin, taxol, taxotere, CPT-11, neutrophil-boosting agents, irinotecan, SN-38, gemcitabine, herstatin, or an activin-A-binding herstatin derivative (as described, for example, in U.S. Pat. App. No. 05/0272637), AVASTIN® (Genentech, South San Francisco, CA), HERCEPTIN® (Genentech), RITUXAN® (Genentech), ARIMIDEX® (AstraZeneca, Wilmington, DE), IRESSA® (AstraZeneca), BEXXAR® (Corixa, Seattle, WA), ZEVALIN® (Biogen Idec, Cambridge, MA), ERBITUX® (Imclone Systems Inc., New York, N.Y.), GEMZAR® (Eli Lilly and Co., Indianapolis, IN), CAMPTOSAR® (Pfizer, New York, N.Y.), GLEEVEC® (Novartis), SU-11248 (Pfizer), BMS-354825 (Bristol-Myers Squibb), panitumumab (Abgenix, Fremont, CA/Amgen Inc., Thousand Oaks, CA), and denosumab (Amgen Inc., Thousand Oaks, CA).

In one embodiment, both an anti-activin-A compound and capecitabine are administered to a subject. The capecitabine, or XEODAR® (Roche) (which is converted in the body to 5-fluorouracil), can be administered orally to a subject at 1250 mg/m<sup>2</sup> twice a day for two weeks, followed by a one week rest period. The capecitabine can also be administered at a different dosage and schedule. In another embodiment, both an anti-activin-A compound and a doxorubicin lipid complex are administered to a subject. The doxorubicin lipid complex, or DOXIL® (Janssen Biotech, Inc.), can be administered to a subject at 40 mg/m<sup>2</sup>IV once every four weeks. The doxorubicin lipid complex can also be administered as a different dosage and schedule.

The development of suitable dosing and treatment regimens for using the particular compositions described herein in a variety of treatment regimens, including e.g., subcutaneous, oral, parenteral, intravenous, intranasal, and intramuscular administration and formulation, is well known in the art, and is described above.

#### Antibody Treatment

Therapeutic antibodies may be used that specifically bind to intact activin-A, in which sequences in the region of approximately C11-S33 (first loop) and approximately C81-E111 (second loop) retain the conformation of native activin-A.

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An oligopeptide or polypeptide is within the scope of the invention if it has an amino acid sequence that is at least 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to least one of the CDR's of antibodies A1-A14; and/or to a CDR of a activin-A binding agent that cross-blocks the binding of at least one of antibodies A1-A14 to activin-A, and/or is cross-blocked from binding to activin-A by at least one of antibodies A1-A14; and/or to a CDR of a activin-A binding agent wherein the binding agent can block the binding of activin-A to activin-A receptor.

Activin-A binding agent polypeptides and antibodies are within the scope of the invention if they have amino acid sequences that are at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to a variable region of at least one of antibodies A1-A14, and cross-block the binding of at least one of antibodies A1-A14 to activin-A, and/or are cross-blocked from binding to activin-A by at least one of antibodies A1-A14; and/or can block the inhibitory effect of activin-A on an activin-A receptor.

Antibodies according to the invention may have a binding affinity for human activin-A of less than or equal to  $1 \times 10^{-7}$  M, less than or equal to  $1 \times 10^{-8}$  M, less than or equal to  $1 \times 10^{-9}$  M, less than or equal to  $1 \times 10^{-10}$  M, less than or equal to  $1 \times 10^{-11}$  M, or less than or equal to  $1 \times 10^{-12}$  M.

The affinity of an antibody or binding partner, as well as the extent to which an antibody inhibits binding, can be determined by one of ordinary skill in the art using conventional techniques, for example those described by Scatchard et al. (*Ann. N. Y. Acad. Sci.* 51:660-672 (1949)) or by surface plasmon resonance (SPR; BIACore, Biosensor, Piscataway, NJ). For surface plasmon resonance, target molecules are immobilized on a solid phase and exposed to ligands in a mobile phase running along a flow cell. If ligand binding to the immobilized target occurs, the local refractive index changes, leading to a change in SPR angle, which can be monitored in real time by detecting changes in the intensity of the reflected light. The rates of change of the SPR signal can be analyzed to yield apparent rate constants for the association and dissociation phases of the binding reaction. The ratio of these values gives the apparent equilibrium constant (affinity) (see, e.g., Wolff et al., *Cancer Res.* 53:2560-65 (1993)).

An antibody according to the present invention may belong to any immunoglobulin class, for example IgG, IgE, IgM, IgD, or IgA. It may be obtained from or derived from an animal, for example, fowl (e.g., chicken) and mammals, which includes but is not limited to a mouse, rat, hamster, rabbit, or other rodent, cow, horse, sheep, goat, camel, human, or other primate. The antibody may be an internalizing antibody. Production of antibodies is disclosed generally in U.S. Patent Publication No. 2004/0146888 A1.

In the methods described above to generate antibodies according to the invention, including the manipulation of the specific A1-A14 CDRs into new frameworks and/or constant regions, appropriate assays are available to select the desired antibodies (i.e. assays for determining binding affinity to activin-A; cross-blocking assays; BIACore-based competition binding assay;" in vivo assays). svActRIIB Treatment

The present invention provides methods and pharmaceutical compositions for reducing or neutralizing the amount or activity of myostatin, activin-A, or GDF-11 in vivo and in vitro. svActRIIB polypeptides have a high binding affinity for myostatin, activin-A, and GDF-11, and are capable of

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reducing and inhibiting the biological activities of at least one of myostatin, activin-A and GDF-11.

In one aspect, the present invention provides methods and reagents for treating myostatin-related and/or activin-A related disorders in a subject in need of such a treatment by administering an effective dosage of an svActRIIB composition to the subject.

The compositions of the present invention are useful for increasing lean muscle mass in a subject. The compositions may also be useful to increase lean muscle mass in proportion to fat mass, and thus decrease fat mass as percentage of body weight in a subject. Example 3 demonstrates that the svActRIIB polypeptides and proteins of the invention can increase lean muscle mass in animals.

The disorders that can be treated by an svActRIIB composition include but are not limited to various forms of muscle wasting, as well as metabolic disorders such as diabetes and related disorders, and bone degenerative diseases such as osteoporosis.

Muscle wasting disorders also include dystrophies such as Duchenne's muscular dystrophy, progressive muscular dystrophy, Becker's type muscular dystrophy, Dejerine-Landouzy muscular dystrophy, Erb's muscular dystrophy, and infantile neuroaxonal muscular dystrophy. Additional muscle wasting disorders arise from chronic diseases or disorders such as amyotrophic lateral sclerosis, congestive obstructive pulmonary disease, cancer, AIDS, renal failure, organ atrophy, androgen deprivation, and rheumatoid arthritis.

Over-expression of myostatin and/or activin may contribute to cachexia, a severe muscle wasting syndrome. Cachexia results from cancers, and also arises due to rheumatoid arthritis, diabetic nephropathy, renal failure, chemotherapy, injury due to burns, as well as other causes. In another example, serum and intramuscular concentrations of myostatin-immunoreactive protein was found to be increased in men exhibiting AIDS-related muscle wasting and was inversely related to fat-free mass (Gonzalez-Cadavid et al., PNAS USA 95: 14938-14943 (1998)). Myostatin levels have also been shown to increase in response to burns injuries, resulting in a catabolic muscle effect (Lang et al., FASEB J 15, 1807-1809 (2001)). Additional conditions resulting in muscle wasting may arise from inactivity due to disability such as confinement in a wheelchair, prolonged bed rest due to stroke, illness, spinal chord injury, bone fracture or trauma, and muscular atrophy in a microgravity environment (space flight). For example, plasma myostatin immunoreactive protein was found to increase after prolonged bed rest (Zachwieja et al. J Gravit Physiol. 6(2):11 (1999)). It was also found that the muscles of rats exposed to a microgravity environment during a space shuttle flight expressed an increased amount of myostatin compared with the muscles of rats which were not exposed (Lalani et al., J. Endocrin 167 (3):417-28 (2000)).

In addition, age-related increases in fat to muscle ratios, and age-related muscular atrophy appear to be related to myostatin. For example, the average serum myostatin-immunoreactive protein increased with age in groups of young (19-35 yr. old), middle-aged (36-75 yr. old), and elderly (76-92 yr old) men and women, while the average muscle mass and fat-free mass declined with age in these groups (Yarasheski et al. J Nutr Aging 6(5):343-8 (2002)). In addition, myostatin has now been found to be expressed at low levels in heart muscle and expression is upregulated in cardiomyocytes after infarct (Sharma et al., J Cell Physiol.

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180 (1):1-9 (1999)). Therefore, reducing myostatin levels in the heart muscle may improve recovery of heart muscle after infarct.

Myostatin also appears to influence metabolic disorders including type 2 diabetes, noninsulin-dependent diabetes mellitus, hyperglycemia, and obesity. For example, lack of myostatin has been shown to improve the obese and diabetic phenotypes of two mouse models (Yen et al. *FASEB J* 8:479 (1994)). The svActRIIB polypeptides of the present disclosure are suitable for treating such metabolic disorders. Therefore, administering the compositions of the present invention will improve diabetes, obesity, and hyperglycemic conditions in suitable subjects. In addition, compositions containing the svActRIIB polypeptides may decrease food intake in obese individuals.

Administering the stabilized ActRIIB polypeptides of the present invention may improve bone strength and reduce osteoporosis and other degenerative bone diseases. It has been found, for example, that myostatin-deficient mice showed increased mineral content and density of the mouse humerus and increased mineral content of both trabecular and cortical bone at the regions where the muscles attach, as well as increased muscle mass (Hamrick et al. *Calcif Tissue Int* 71(1):63-8 (2002)). In addition, the svActRIIB compositions of the present invention can be used to treat the effects of androgen deprivation in cases such as androgen deprivation therapy used for the treatment of prostate cancer, for example.

The present invention also provides methods and compositions for increasing muscle mass in food animals by administering an effective dosage of the svActRIIB proteins to the animal. Since the mature C-terminal myostatin polypeptide is similar or identical in all species tested, svActRIIB polypeptides would be expected to be effective for increasing lean muscle mass and reducing fat in any agriculturally important species including cattle, chicken, turkeys, and pigs.

The svActRIIB polypeptides and compositions of the present invention also antagonize the activity of activin-A, as shown in the in vitro assays below. Activin-A is known to be expressed in certain types of cancers, particularly gonadal tumors such as ovarian carcinomas, and to cause severe cachexia. (Ciprano et al. *Endocrinol* 141 (7):2319-27 (2000), Shou et al., *Endocrinol* 138 (11):5000-5 (1997); Coerver et al. *Mol Endocrinol* 10(5):534-43 (1996); Ito et al. *British J Cancer* 82(8):1415-20 (2000), Lambert-Messerlian, et al. *Gynecologic Oncology* 74:93-7 (1999)). Therefore, the compositions of the present disclosure may be used to treat conditions related to activin-A overexpression, as well as myostatin expression, such as cachexia from certain cancers and the treatment of certain gonadal type tumors.

In addition, the svActRIIB polypeptides of the present invention are useful for detecting and quantitating myostatin, activin-A, or GDF-11 in any number of assays. In general, the stabilized ActRIIB polypeptides of the present invention are useful as capture agents to bind and immobilize myostatin, activin-A, or GDF-11 in a variety of assays, similar to those described, for example, in Asai, ed., *Methods in Cell Biology*, 37, *Antibodies in Cell Biology*, Academic Press, Inc., New York (1993). The polypeptides may be labeled in some manner or may react with a third molecule such as an antibody which is labeled to enable myostatin to be detected and quantitated. For example, a polypeptide or a third molecule can be modified with a detectable moiety, such as biotin, which can then be bound by a fourth molecule, such as enzyme-labeled streptavidin,

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or other proteins. (Akerstrom, *J Immunol* 135:2589 (1985); Chaubert, *Mod Pathol* 10:585 (1997)).

## EXAMPLES

Below are examples of specific embodiments for carrying out the present invention. The examples are offered for illustrative purposes only, and are not intended to limit the scope of the present invention in any way. Efforts have been made to ensure accuracy with respect to numbers used (e.g., amounts, temperatures, etc.), but some experimental error and deviation should, of course, be allowed for.

The practice of the present invention will employ, unless otherwise indicated, conventional methods of protein chemistry, biochemistry, recombinant DNA techniques and pharmacology, within the skill of the art. Such techniques are explained fully in the literature. See, e.g., T. E. Creighton, *Proteins: Structures and Molecular Properties* (W.H. Freeman and Company, 1993); A. L. Lehninger, *Biochemistry* (Worth Publishers, Inc., current addition); Sambrook, et al., *Molecular Cloning: A Laboratory Manual* (2nd Edition, 1989); *Methods In Enzymology* (S. Colowick and N. Kaplan

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eds., Academic Press, Inc.); *Remington's Pharmaceutical Sciences*, 18th Edition (Easton, Pennsylvania: Mack Publishing Company, 1990); Carey and Sundberg *Advanced Organic Chemistry* 3<sup>rd</sup> Ed. (Plenum Press) Vols A and B(1992).

Methods  
Materials

sActRIIB (soluble ActRIIB-Fc) expression construct was engineered by subcloning a cDNA fragment corresponding to the extracellular domain of human activin type-2B receptor (aa7-100) into an IgG2 Fc fusion split vector. The construct was transfected into CHO cells and the recombinant sActRIIB was purified from culture medium using a mAb Select SuRe affinity column (GE) followed by Fractogel chromatography (EMD Chemicals).

Activin-A antibody (fully human monoclonal antibody against activin-A) was generated using XenoMouse technology (Amgen Inc). Recombinant activin-A was produced using mammalian expression system (Amgen Inc).

The sequences of the sActRIIB peptide and the Activin-A antibody used below are shown in the tables below.

TABLE 9

sActRIIB sequences				
	ActRIIB Peptide	Linker	IgG2 Fc Domain	Full Length
sActRIIB	ETRWCIIYNNANWE LERTTNQ <u>S</u> GLERCE GEQDKRLHCYASW RNSSGTIELVKKG CWLDDFNCYDRQE CVATEENPQVYFC CCEGNFCNCFRTH LPEAGGPEVTYEP PPTAPT (SEQ ID NO: 19)	GGGGSV ECPVCP (SEQ ID NO: 27) WYVDGVEVHNAKTKP REEQFNSTFRVVSVL TVVHQDWLNKEYKC KVSNKGLPAPIEKTI SKTKQCPREPQVYTL PPSREEMTNQVSLT CLVKGFYPSDIAVEW ESNGQPENNYKTPPP MLDSDGSSFFLYSKLT VDKSRWQQGNVFSCS VMHEALHNHYTQKSL SLSPGK (SEQ ID NO: 22)	APPVAGPSVPLFP PKDTLMISRTPEVTC VVVDVSHEDPEVQFN WYVDPVAKTLP REEQFNSTFRVVSVL TVVHQDWLNKEYKC KVSNKGLPAPIEKTI SKTKQCPREPQVYTL PPSREEMTNQVSLT CLVKGFYPSDIAVEW ESNGQPENNYKTPPP MLDSDGSSFFLYSKLT VDKSRWQQGNVFSCS VMHEALHNHYTQKSL SLSPGK (SEQ ID NO: 21)	ETRWCIIYNNANWE <u>LERTTNQ</u> <del>S</del> GLERCE EQDKRLHCYASWRNSSGTITLVKKGCW LDDFNCYDRQECVATEENPQVYFCCCE GNFCNCFRTHLPFAGGPEVTYBPPPTA PTGGGSVECPCCPAPPVAGPSVFLFP PKPKDTLMISRTPEVTCVVVDVSHEDP TFRVVSVLTVVHQDWLNKEYKCKVSN KGLPAPIEKTIKTKGQPREPQVYTL PSREEMTNQVSLTCLVKGFYPSDIAV EWESNGQPENNYKTPPMLDSDGSFFL YSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSLSPGK (SEQ ID NO: 21)

TABLE 10

Activin-A light and heavy chain variable domain sequences		
	Light Chain Variable Domain	Heavy Chain Variable Domain
Activin A Antibody	SYEVQTQAPSIVSVPQQTASITCSGD KLGDKYACWYQQKPGQSPVVLVIYQD SKRPSGIPERFSGSNNSGNTATLTIS GTQAMDEADYYCQAWDSSTAVFGGG TKLTVL (SEQ ID NO: 275)	QVOLVQSGAEVKPGASVKVSCKASGYTF TSYGLSWRQAPGQGLEWMGWIIYPNGNT NSAQKLQGRVTMTTDSTSTAYMELRSLR SDDTAVYFCARDRDYGVNYDAFDIWQGQT MVTVSS (SEQ ID NO: 278)

TABLE 11

Activin-A light and heavy chain constant domain sequences		
	Light Chain Constant Domain	Heavy Chain Constant Domain
Activin A Antibody	Gly Gln Pro Lys Ala Ala Pro Ser Val Thr Leu Phe Pro Pro Ser Ser Glu Glu Leu Gln Ala Asn Lys Ala	Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly

TABLE 11-continued

Activin-A light and heavy chain constant domain sequences	
Light Chain Constant Domain	Heavy Chain Constant Domain
<p>Thr Leu Val Cys Leu Ile Ser Asp  Phe Tyr Pro Gly Ala Val Thr Val  Ala Trp Lys Ala Asp Ser Ser Pro  Val Lys Ala Gly Val Glu Thr Thr  Thr Pro Ser Lys Gln Ser Asn Asn  Lys Tyr Ala Ala Ser Ser Tyr Leu Ser  Leu Thr Pro Glu Gln Trp Lys Ser  His Arg Ser Tyr Ser Cys Gln Val  Thr His Glu Gly Ser Thr Val Glu  Lys Thr Val Ala Pro Thr Glu Cys  Ser (SEQ ID NO: 84)</p>	<p>Cys Leu Val Lys Asp Tyr Phe Pro  Glu Pro Val Thr Val Ser Trp Asn Ser  Gly Ala Leu Thr Ser Gly Val His  Thr Phe Pro Ala Val Leu Gln Ser Ser  Gly Leu Tyr Ser Leu Ser Ser Val Val  Thr Val Pro Ser Ser Asn Phe Gly Thr  Gln Thr Tyr Thr Cys Asn Val Asp  His Lys Pro Ser Asn Thr Lys Val  Asp Lys Thr Val Glu Arg Lys Cys  Cys Val Glu Cys Pro Pro Cys Pro  Ala Pro Pro Val Ala Gly Pro Ser Val  Phe Leu Phe Pro Pro Lys Pro Lys  Asp Thr Leu Met Ile Ser Arg Thr Pro  Glu Val Thr Cys Val Val Val Asp  Val Ser His Glu Asp Pro Glu Val  Gln Phe Asn Trp Tyr Val Asp Gly  Val Glu Val His Asn Ala Lys Thr  Lys Pro Arg Glu Glu Gln Phe Asn  Ser Thr Phe Arg Val Val Ser Val  Leu Thr Val Val His Gln Asp Trp  Leu Asn Gly Lys Glu Tyr Lys Cys  Lys Val Ser Asn Lys Gly Leu Pro  Ala Pro Ile Glu Lys Thr Ile Ser Lys  Thr Lys Gly Gln Pro Arg Glu Pro  Gln Val Tyr Thr Leu Pro Pro Ser  Arg Glu Glu Met Thr Lys Asn Gln  Val Ser Leu Thr Cys Leu Val Lys  Gly Phe Tyr Pro Ser Asp Ile Ala Val  Glu Trp Glu Ser Asn Gly Gln Pro  Glu Asn Asn Tyr Lys Thr Thr Pro  Pro Met Leu Asp Ser Asp Gly Ser  Phe Phe Leu Tyr Ser Lys Leu Thr  Val Asp Lys Ser Arg Trp Gln Gln  Gly Asn Val Phe Ser Cys Ser Val  Met His Glu Ala Leu His Asn His  Tyr Thr Gln Lys Ser Leu Ser Leu Ser  Pro Gly Lys (SEQ ID NO: 214)</p>

#### Mouse Models

Ethics committee approval. All mouse experiments were performed with the approval of Institutional Animal Care and Use Committee and are in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

Inh-KO mice. 12-week-old female and 8-week-old male inh-KO mice with fully established ovarian or testicular tumors received a single injection of PBS or sActRIIB (30 mg/kg, SC). As a control, age-matched wild-type littermates received a single injection of PBS. Ovarian and testicular organ weights were determined by necropsy 14 days after the injection.

TOV-21G xenograft.  $5 \times 10^6$  TOV-21G ovarian cancer cells were implanted subcutaneously into individual female athymic nu/nu mice (Harlan). Treatment was initiated at day 12 after tumor implantation, when the average tumor volume reached approximately 150 mm<sup>3</sup>. The mice received PBS, sActRIIB (30 mg/kg, SC, 1x/week) or activin-A antibody (30 mg/kg, SC, 2x/week). In a separate chemotherapy combination experiment, the mice were treated with PBS, sActRIIB (10 mg/kg, SC, 1x/week), 5-FU (50 mg/kg, IP, 3 cycles, 4 daily injections per cycle) or sActRIIB and 5-FU combination at the same doses above.

CHO xenograft.  $3 \times 10^6$  naïve or activin-A-transfected CHO cells were implanted intramuscularly into the right quadriceps in individual female CD1 nude mice (Harlan). The mice received PBS or activin-A antibody (20 mg/kg, 1x/week, SC) at the time of implantation.

OV-90 xenograft.  $3 \times 10^6$  OV-90 ovarian cancer cells transfected with activin-A were implanted SC in individual

female CD1 nude mice (CRL). The mice were treated with PBS or sActRIIB (20 mg/kg, SC, 1x/week) beginning at day 40 11 post tumor implantation, when the average tumor volume had reached approximately 150 mm<sup>3</sup>.

G361 and 5637 xenografts.  $5 \times 10^6$  G361 melanoma cells and 5637 bladder carcinoma cells, respectively, were inoculated SC into individual athymic nu/nu mice (Harlan Inc). 45 Treatment was initiated 4 days after 5637 implantation and 14 days after G361 implantation, when the tumor volumes reached 130 mm<sup>3</sup>–150 mm<sup>3</sup>. 5637-implanted mice received PBS or activin-A antibody (10 mg/kg, SC, 2x/week). G361-implanted mice received PBS or sActRIIB (20 mg/kg, SC, 1x/week).

#### Tumor Size and Weight

For all xenograft experiments, the tumor sizes were measured longitudinally by using an electronic caliper. Immediately prior to the 1<sup>st</sup> dose, the tumor-bearing mice were randomized to ensure even distribution in tumor sizes across different groups. Tumor volumes (mm<sup>3</sup>) were calculated as tumor length (mm)×tumor width (mm)×tumor height (mm). Tumor weights were determined by necropsy. 55 Cell Cultures

Primary BAEC cultures (Lanza) were grown at 37° C. in 5% CO<sub>2</sub> in DMEM with 10% fetal bovine serum (Invitrogen). TOV-21G cells (ATCC) were cultured in a 1:1 mixture of MCDB 105 medium (Sigma, M6395) and Medium 199 65 (Invitrogen) containing 15% fetal bovine serum. MRC-5 and CCD-Lu cells (ATCC) were cultured in MEM (Invitrogen), supplemented with 10% FBS. U937 and THP-1 cells

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(ATCC) were grown in RPMI 1640 medium (Invitrogen) containing 10% FBS and L-glutamine.

#### In Vitro Proliferation Assay

In vitro growth rates of TOV-21G cancer cells were analyzed by using a real-time live cell imaging system (IncuCyte) following the manufacturer's recommended protocol.

#### Real-Time RT-PCR

Total RNA was isolated from cell cultures using the RNeasy mini RNA kit (QIAGEN). 25 ng of total RNA was subjected to one-step quantitative RT-PCR analysis using the TaqMan one-Step RT-PCR Master Mix Reagents and the Prism 7900HT Detection System (Applied Biosystems). GAPDH was used to normalize gene expression levels. All primers used for real-time PCR analyses except the human  $\beta$ A primer set were obtained from Applied Biosystems. The catalog numbers for the specific primers used in the current studies are as follows:

Bovine primers: VEGF (Bt03213282), Ang-1 (Bt03249559); Activin ( $\beta$ A) (Bt03259358), GAPDH (Bt03210913); Human Primers: VEGF (Hs00900054), Ang-1 (Hs00375822), GAPDH (Hs02758991). The human  $\beta$ A primer sequences used are as follows: 5'-GAA AAG GAG CAG TCG CAC AGA-3' (SEQ ID NO: 291), 5'-C TTC TGG TGG GAG TAG CGG-3' (SEQ ID NO: 292), and TaqMan probe ATG CTG CAG GCC CGG CAG TC (SEQ ID NO: 293).

#### Northern Blot

Total RNA was isolated from individual tissue samples after homogenization in Trizol (Invitrogen). A pool of 10  $\mu$ g RNA for each group containing equal amounts of total RNA isolated from individual animals was subjected to Northern blot analysis. The northern probes used for  $\beta$ A and Ang-2 were generated by using RT-PCR (Phusion, Biolabs).  $\beta$ A primer set: 5'-CCC TTG CTT TGG CTG AGA GGA-3' (SEQ ID NO: 294) and 5'-TC ACA GGT CGT CGT AGG TCG-3' (SEQ ID NO: 295); Ang-2 primer set: 5'-TGT GCC GGG GAG AAG AG (SEQ ID NO: 296) and 5'-TAC AGT AGT GGG TTG AGG TTC-3' (SEQ ID NO: 297). To normalize the expression, northern blot membranes were re-probed with  $\beta$ -actin.

#### Western Blot

Protein extracts were prepared from cell cultures or tissues in T-PER tissue protein extraction reagent (Pierce) containing a mixture of protease inhibitors (Roche). A pool of 50  $\mu$ g total protein for each group containing equal amounts of protein extract isolated from individual animals was separated by NuPAGE 4-12% Bis-Tris gel (Invitrogen) and transferred to PVDF. The membranes were probed with primary antibodies against total Smad2, p-Smad2 or E-cadherin (1:1000; Cell Signaling), endoglin, osteopontin (1:500; R & D Systems), IGFBP-1, IGFBP-2 (1:500; Abcam) followed by HRP-conjugated secondary antibody (1:2000; Cell Signaling). The membranes were stripped and re-probed with antibody against  $\alpha$ -tubulin (1:1000; Cell Signaling).

#### Activin-A ELISA

All serum samples from ovarian cancer patients and healthy subjects were collected under informed consent and were purchased from Bioreclamation, Inc. The serum samples were diluted in buffer (DY995, R & D Systems) and pretreated overnight at 4° C. with 4 M urea (Sigma) to dissociate any protein bound to activin-A. The samples were then transferred to 96 well plates pre-coated with an activin-A monoclonal antibody. After 2 hr incubation at room temperature and a washing step (0.05% Tween 20 in DPBS), a biotin-labeled activin-A monoclonal antibody was

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added for 1 hr at room temperature. The plates were then washed and incubated with Streptavidin-Horseradish Peroxidase (Amersham) for 1 h at room temperature. Following a washing step, tetramethylbenzidine (KPL) substrate was added to the wells for 10 minutes at room temperature. An acidic stopping solution (KPL) was added and the degree of enzymatic turnover of the substrate was determined by wavelength absorbance measurement at 450 nm. The absorbance measured is directly proportional to the concentration of activin-A present. A standard curve of absorbance versus activin-A concentration was used to determine the amount of Activin-A in the test sample. Serum activin-A levels in inh-KO mice were measured by using ELISA.

#### VEGF and Ang-1 ELISA

The serum VEGF levels in inh-KO mice were measured by using immunoassay kit (R & D Systems), and the levels of human VEGF and Ang-1 in cell line culture medium were quantified using ELISA kits purchased from Invitrogen (VEGF) and R & D Systems (Ang-1), by following the manufacturers' recommended protocols.

#### Histology and Light Microscopy

25 Testes and ovaries from inh-KO mice were fixed with Zinc-formalin. Tissue sections were subjected to H & E staining and then examined with a Nikon Eclipse 90i microscope.

#### Immunohistochemistry

30 Zinc-formalin fixed paraffin tumor tissue sections of 4  $\mu$ m in thickness were prepared. The sections were subjected to antigen retrieval by microwave 3 min in Unmask solution (Vector H-3300) followed by incubation in 10  $\mu$ g/ml Proteinase-K for 20 min and in 1% H<sub>2</sub>O<sub>2</sub> in dH<sub>2</sub>O for 10 min at room temperature. The sections were further incubated in 0.1% Tween-20 in PBS for 3 min to permeabilize the cell membrane and in goat serum for 30 min to block non-specific binding. The sections were then incubated at room temperature with specific primary antibody for 3 hours followed by incubation in biotinylated or fluorescently labeled secondary antibody. Substrate developed in Vector SG kit (SK-4700) or DAB and nuclear-counterstained in Vector Fast Red (H-3403) or in hematoxylin. The immunostained tissue sections were analyzed and photographed using a Nikon Eclipse 90i microscope equipped with DS-Ri1 camera. The primary antibodies used and their dilutions are as follows: VEGF (BD Pharmingen 550549) 1:20 or VEGF (Abcam ab46154) 1:100, active caspase-3 (Abcam ab32042) 1:50, Ang-1 (Abcam ab8451) 1:500, osteopontin (Abcam ab8448) 1:200, CD-31 (Abcam ab56299) 1:100, E-cadherin (Abcam ab76319) 1:80. For immunofluorescence staining, FITC-conjugated secondary antibody (Invitrogen) was added at 1:50 dilution in PBS and incubated for 30 min. Cell nuclei were counterstained with Vectashield PE (Vector).

#### TUNEL Assay

Cell apoptosis in TOV-21G tumors was analyzed by measuring the amounts of fragmented DNA in the tumor sections using the DeadEnd Fluorometric TUNEL System following the manufacturer's recommended protocols (Promega, G3250). The fluorescein-12-dUTP-labeled DNA was visualized by Nikon fluorescence microscopy.

#### Statistics

Groups of tissue samples were compared using Student's t-test. Longitudinal measurements were analyzed by repeat measures ANOVA. P values <0.05 were considered significant.

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**Example 1: Activin Blockade Causes Regression of Advanced Ovarian and TESTICULAR Tumors in Inhibin-Deficient Mice**

**Activin-A Measurements in Inh-KO Mice**

Serum activin-A levels were measured in patients with ovarian cancer and in healthy controls. As shown in FIG. 1, circulating activin-A levels were significantly higher in ovarian cancer patients.

Next, to understand the mechanism by which activin-A influences tumor growth, effects were analyzed of activin blockade on further growth of gonadal tumors that had been fully established in the inhibin- $\alpha$  KO mice (a model of activin deregulation, spontaneous tumor formation and cancer cachexia) (referred to below as inh-KO mice). Activin signaling was interrupted after the gonadal tumors had developed to an advanced stage to better evaluate the therapeutic potential of activin-Antagonism.

Measurements of tumor weights as a function of age in inh-KO mice indicated that by 12 weeks in females and 8 weeks in males, the ovarian and testicular tumors had been fully established. A single dose of the activin-Antagonist sActRIIB was administered to 12-week-old-female and 8-week-old male inh-KO mice and the resulting alterations in activin-A levels and ovarian and testicular tumor sizes were examined. As expected, there was a marked increase in serum activin-A levels in these inh-KO mice with established gonadal tumors (FIG. 2A and FIG. 2B). However, within one day after administration, sActRIIB reduced the elevated activin-A in the inh-KO mice to normal control levels seen in the wild-type (WT) mice, and this activin-A neutralizing effect persisted throughout the 14-day study period. Unexpectedly, necropsy analysis revealed that upon activin neutralization by the sActRIIB treatment, the very large ovarian tumor masses in the inh-KO mice regressed rapidly to the sizes seen in the WT control mice (FIG. 3A and FIG. 3B). Similarly, in the male inh-KO mice treated with sActRIIB, there was a dramatic regression of testicular tumor masses to the WT control levels (FIG. 4A and FIG. 4B). Thus, sActRIIB rapidly and completely eradicated the ovarian and testicular tumor masses that had been fully established in the inh-KO mice.

**Northern Blot Analysis**

Next, activin-A ( $\beta$ A) mRNA expression in the tumors was examined by Northern blot analysis. The levels of  $\beta$ A transcripts in the tumors were much greater than in WT controls, but this increase was completely blocked by the sActRIIB treatment (FIG. 5A). This finding suggests the existence of a novel feed-forward loop within the tumors by which activin-A upregulates its own expression (see below). Activin-A-induced Smad2 signaling was also markedly increased in the tumors above levels in the WT controls, as shown by Western blot assay of the amounts of phospho-Smad2. Furthermore, sActRIIB treatment eliminated this increase in phospho-Smad2 in the tumor tissues (FIG. 5B). Thus, sActRIIB prevented both the upregulation of activin-A mRNA and the activation of Smad2 signaling in the ovarian and testicular tumors.

**Western Blot Analysis**

To verify that the marked decreases in ovarian tumor size in response to sActRIIB treatment indeed reflected tumor regression, Western blot analysis was used to examine the expression in the tumors of E-cadherin, a cell adhesion protein that is critical in maintaining normal differentiation of the ovary. Remarkably, no E-cadherin protein could be detected in the ovarian tumors from the untreated inh-KO mice, but the single injection of sActRIIB dramatically

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restored the lost E-cadherin (FIG. 6A). These observations were corroborated by immunostaining. Although no immunoreactivity for E-cadherin was detected in the sections of the ovarian tumors in untreated inh-KO mice, the treatment with sActRIIB led to the reappearance of distinctive E-cadherin immunoreactivity in the ovarian sections (FIG. 6B). Thus, the increased activin signaling down-regulates E-cadherin in the ovary. The reversal of this down-regulation is noteworthy because the loss of E-cadherin has been implicated in ovarian cancer progression.

**Light Microscopy Analysis**

The morphological changes in the ovarian and testicular tumors were examined by light microscopy. In the untreated female inh-KO mice, the greatly enlarged ovaries were predominantly filled with solid tumor mass and many hemorrhagic lesions with virtually no recognizable follicles remaining. By contrast, in the sActRIIB-treated female inh-KO mice, the ovaries were normal in size and contained many recognizable follicles, minimal tumor cell invasion and few hemorrhagic lesions (FIG. 7A). In the untreated male inh-KO mice, the normal structures in the testes were displaced by massive, undifferentiated solid tumor mass, and no seminiferous tubules were evident. By contrast, in the sActRIIB-treated male inh-KO mice, the testes were normal in size and filled with seminiferous tubules, although the number of spermatogonia was less than normal and a few small areas still contained tumor cells (FIG. 7B). These histological findings imply that sActRIIB treatment not only caused regression of the gonadal tumors, but also promoted normal tissue differentiation. Thus, the shrinkage of tumors upon sActRIIB treatment (FIG. 3A, FIG. 3B, FIG. 4A, and FIG. 4B) is not simply an involution of mass, but represents a reversal to a differentiated phenotype.

**Example 2: Activin Blockade Abolishes Angiogenesis Factor Induction and Causes Caspase-3 Activation in Gonadal Tumors**

The profound tumor suppression seen upon activin neutralization makes it likely that tumor-derived activin-A stimulates tumor progression by inducing known tumorigenesis-related factors. To test this possibility, angiogenic factors VEGF and angiopoietins that play well-established roles in tumor angiogenesis and tumorigenesis were analyzed. ELISA revealed that the inh-KO mice with advanced ovarian and testicular tumors had greatly increased levels of VEGF in their circulation. A single dose of sActRIIB rapidly lowered the elevated VEGF to WT control levels (FIG. 8A). Furthermore, both VEGF and Ang-1 immunoreactivities were dramatically increased in sections of the ovarian and testicular tumors; however, sActRIIB treatment completely abolished the VEGF and Ang-1 inductions in the tumors (FIG. 8B, top and bottom respectively). In addition, Northern blot analysis revealed that Ang-2 mRNA was expressed at high levels in the ovarian and testicular tumors, while sActRIIB treatment inhibited its overexpression (FIG. 8C). Furthermore, Western blot analyses revealed that several other factors known to be involved in ovarian tumor angiogenesis and growth, including endoglin, osteopontin, IGFBP-1, and IGFBP-2, were markedly upregulated in the ovarian tumors, but the inductions of these tumorigenesis-related proteins were abolished completely by sActRIIB administration (FIG. 8D).

Next, immunostaining was used to analyze the activity of apoptotic enzyme caspase-3 in tumor tissue sections. No active caspase-3 was detected in the ovarian or testicular tumor sections from the untreated inh-KO mice; however, in

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sActRIIB-treated inh-KO mice, strong immunostaining of active caspase-3 was found in the ovarian and testicular tissue sections at the regions where residual tumor cells were clustered (FIG. 9), indicating activation of tumor apoptosis. These results show that elevated activin-A in the tumors drives the overproduction of multiple tumor angiogenesis- and tumorigenesis-related factors and accordingly, blocking tumor-derived activin-A causes the deprivation of these factors, which in turn induces caspase-3 activation and apoptosis in the tumor cells, leading to tumor suppression.

**Example 3: Activin-Antagonist Inhibits In Vivo Growth of Human Ovarian Cancer Xenografts with Additive Effects with Chemotherapy**

To further determine whether activin-antagonism can suppress growth of tumors that secrete activin-A, the in vivo growth of multiple xenograft tumors in nude mice was analyzed. The analysis heavily focused on the growth in vivo of TOV-21G xenograft, a human epithelial ovarian cancer model, because in cultures, these cancer cells secrete a high amount of activin-A. Subcutaneous implantation of TOV-21G in nude mice resulted in a sharp rise in serum activin-A (FIG. 10A). We administered sActRIIB or activin-A antibody to TOV-21G-implanted mice after the tumors had established. Both activin-A antagonists significantly inhibited the growth of the TOV-21G ovarian cancer xenografts (FIG. 10B).

To further evaluate the functional relevance of elevated activin-A to ovarian tumor growth, two additional ovarian tumor xenografts were analyzed, including the Chinese hamster ovary (CHO) and the human ovarian cancer OV-90 xenografts. After implantation into the quadriceps, naïve CHO cells failed to form detectable tumors. However, when the CHO cells were transfected with activin-A, they became highly capable of forming tumors in the nude mice. Moreover, activin-Antagonist treatment greatly reduced the rate of tumor formation by the activin-A transfected CHO cells (FIG. 11). Furthermore, activin blockade markedly inhibited the growth of activin-A overexpressing OV-90 xenografts in nude mice (FIG. 12). These observations provide additional evidence that the elevated activin-A is an important stimulus of tumor growth.

These findings suggested that activin-Antagonism might be a valuable therapy in ovarian cancer treatment. The effects of sActRIIB on the growth of TOV-21G xenografts receiving 5-Fluorouracil (5-FU) chemotherapy was examined. When sActRIIB treatment or 5-FU was administered alone to TOV-21G xenograft-bearing mice, each decreased the rate of tumor growth significantly (FIG. 13), but when sActRIIB and 5-FU were injected together, an even greater effect on tumor growth inhibition was observed (FIG. 13). Thus, sActRIIB and 5-FU clearly show additive effects in tumor suppression.

In another experiment, athymic nude mice received TOV-21G xenografts in the abdominal flank. After 14 days, subcutaneous hu-sActRIIB-Fc was administered weekly alone or in combination with 5-FU.

52 days after tumor cell injection, hu-sActRIIB-Fc treatment resulted in 43% ( $p<0.0001$ ) tumor growth reduction, versus the vehicle-treated tumor-bearing group tested using ANOVA. 5-FU monotherapy resulted in 47% ( $p<0.0001$ ) tumor growth reduction, and the combination of hu-sActRIIB-Fc and 5-FU together resulted in 73% ( $p<0.0001$ ) tumor growth reduction. During the course of this experiment, the body weight of the mice receiving hu-sActRIIB-Fc increased by 26%, while the body weight of the mice

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receiving hu-sActRIIB-Fc and 5-FU increased by 22%, while control tumor-bearing mice receiving vehicle exhibited a 10% body weight loss.

Next, the effects of activin-A antagonists on the growth of TOV-21G in cell cultures was examined. Surprisingly, increasing concentrations of sActRIIB or activin-A antibody were found to have no direct effect on TOV-21G cell proliferation in vitro (FIG. 14). Thus, the tumor-suppressive effect of the activin-Antagonists in TOV-21G xenograft mice must have been achieved through an indirect mechanism in vivo.

**Example 4: Blocking Activin-A Prevents Angiogenesis and Induces Apoptosis in Human Ovarian Cancer Xenografts**

Because activin-A induced overexpression of several angiogenic factors in the tumors in inh-KO mice, the influence of blockade of activin-A on angiogenesis in TOV-21G tumor xenografts in vivo was analyzed. Examination of the TOV-21G tumor sections revealed strong immunostaining for VEGF and Ang-1 in the untreated sections, but virtually none in the sActRIIB-treated sections (FIG. 15A). Similar results were found for immunostaining of osteopontin, a secreted protein involved in tumor angiogenesis and cancer progression, in the tumor sections (FIG. 15A). Immunostaining of CD31, a marker for newly formed microvessels, further demonstrated the existence of neo-microvasculature in the untreated tumor sections and the lack of such new microvessels in sections of the sActRIIB-treated tumors (FIG. 15B). These results indicate that sActRIIB treatment suppressed multiple angiogenesis factors and prevented neovascularization in the TOV-21G tumors. To assess the possible impact of this angiogenesis deprivation on tumor apoptosis, active caspase-3 immunostaining and TUNEL assays were performed on the tumor sections. As shown in FIG. 15C, sActRIIB treatment led to profound increases in active caspase-3 and DNA fragmentation in the treated tumors. Therefore, consistent with those on gonadal tumors in the inh-KO mice, these findings from the TOV-21G ovarian cancer xenografts further demonstrate a major role of activin-A in tumor angiogenesis and growth.

**Example 5: Activin-A Stimulates Angiogenic Factor Overproduction in Cancer and Stromal Cells**

In addition to cancer cells, the tumor microenvironment contains the neighboring stromal, endothelial and infiltrating immune cells. There is growing evidence that the complex interplay between the cancer and non-cancer cells in the tumor is critical in determining the tumor's malignant state and progression. To understand the cellular mechanisms by which activin-A regulates tumor growth, the effect of activin-A on the expression of angiogenesis factors was examined in four different cell types found in tumors—cancer cells, fibroblasts, endothelial cells, and monocytes. Specifically, cultures of TOV-21G cancer cells, BAEC endothelial cells, MRC-5 or CCD-Lu fibroblasts, and U937 monocytic cells were each treated with recombinant activin-A and the expression of VEGF and Ang-1 were analyzed by real-time PCR. Activin-A treatment caused marked increases in the levels of VEGF transcripts in all these cultures (FIG. 16A) and also of Ang-1 mRNA in BAEC, MRC-5 and CCD-Lu cultures (FIG. 16B). Accordingly, the activin-Antagonist sActRIIB prevented this induction of VEGF and Ang-1 by recombinant activin-A (FIG. 16A and FIG. 16B). Moreover, ELISA revealed that

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activin-A treatment increased the release of VEGF by the TOV21G, MRC-5, CCD-Lu and TPH-1 cells (FIG. 17A) and of Ang-1 by MRC-5 and CCD-Lu cells (FIG. 17B) into the culture medium, while sActRIIB blocked completely this activin-A-induced release of angiogenic factors (FIG. 17A and FIG. 17B). Thus, activin-A is able to upregulate the transcription and secretion of angiogenesis factors in various cell types that reside in the tumor microenvironment. In addition, the effects of exposure to activin-A were examined, particularly to determine whether the exposure could induce endogenous expression of activin-A ( $\beta$ A) mRNA in these cell lines. Remarkably, addition of recombinant activin-A to the TOV21G, BAEC, MRC-5, CCD-Lu, U937 and THP-1 cultures markedly upregulated  $\beta$ A expression in all these cells (FIG. 18), and this induction could be blocked completely by sActRIIB. Thus, activin-A production can amplify its own expression in cancer cells and also in endothelial cells, fibroblasts and monocytes. These findings demonstrate a novel feed-forward angiogenic mechanism, in which cancer cell-derived activin-A via autocrine and paracrine actions triggers increasingly higher activin-A overexpression in multiple cell types, leading to enhanced production of VEGF and Ang-1 in the tumor microenvironment.

**Example 6: Activin Blockade Inhibits Growth of Human Melanoma and Bladder Carcinoma Xenografts**

To learn whether activin-A may also contribute to pathogenesis of non-ovarian cancers, the *in vivo* growth of two other cancer types, the G361 human melanoma and 5637 human bladder carcinoma were examined, because they were shown to release activin-A when cultured *in vitro*. Nude mice were implanted with G361 and 5637 xenografts and after the tumors were established, the implanted mice were treated with sActRIIB or activin-A antibody. As shown in FIG. 19, activin-A blockade significantly decreased the growth rates and sizes of both these non-ovarian xenografts. This inhibition raises the possibility that activin-A may influence the progression of various malignancies.

**Example 7: Activin-A Transcripts are Highly Elevated in Many Human Cancers**

There is increasing evidence for elevated activin-A in multiple kinds of cancer. To further validate activin-A overexpression in human cancers, the Oncomine microarray databases were used to search for activin-A ( $\beta$ A) expression levels. As shown in FIG. 20, in a wide variety of human cancer types examined, including breast, gastric, pancreatic, colorectal, and head and neck cancers, the levels of  $\beta$ A

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transcript were elevated in the cancerous tissues compared to the respective control tissues.

**Example 8: Effects of Withdrawal and Re-Administration of sActRIIB on Ovarian Tumor Growth and Cachexia in Female Inhibin- $\alpha$  Knockout Mice**

The objectives of this study were to examine the long term pharmacological effects of sActRIIB withdrawal and re-administration on body weight, tumor mass and survival in Inh $\alpha$  KO (inhibin- $\alpha$ -deficient knock-out) mice with established ovarian tumors. Inh $\alpha$  KO mice (Matzuk et al, 1992) were licensed from Dr. Martin M. Matzuk (Baylor College of Medicine, Houston, TX). Mice were maintained on a mixed C57BL6/129S6/SvEv genetic background and the colonies were bred at Charles River Laboratories, Inc. (Wilmington, MA). Genotyping of Inh $\alpha$  KO mice was conducted by PCR using genomic tail DNA and performed by Genetically Engineered Models and Services (Charles River Laboratories, Inc. Wilmington, MA).

Eleven-to-fourteen-week-old female Inh $\alpha$  KO mice with body weight from 19.41 to 26.82 grams were subcutaneously (SC) injected with either 30 mg/kg sActRIIB or PBS. Age-matched WT littermate control mice were injected with PBS and served as baseline controls. The study ended by necropsy 2 weeks after injections. Two cohorts of mice were used in the study. Eleven-to-thirteen-week-old female Inh $\alpha$  KO mice with body weight from 17.7 to 27.4 grams were SC injected with either 30 mg/kg sAcLRIIB or PBS. Age-matched WT littermate control mice were injected with PBS and served as baseline controls. The withdrawal lasted for 8 weeks. At the end of the withdrawal, the sActRIIB treated mice were divided by balanced body weight into 2 groups (Group 3 and Group 4).

Group 2 and Group 3 were euthanized to examine the ovarian tumors. Group 4 received another dose of 30 mg/kg sActRIIB and the mice were euthanized together with the WT mice 4 weeks later (total 12 weeks). Mouse body weights were recorded once per week up to 12 weeks. For necropsy, mice were euthanized in a CO<sub>2</sub> chamber. Normal ovaries in WT mice and ovarian tumors in Inh $\alpha$  KO mice were collected and weighed. All results were expressed as the mean $\pm$ standard error of the mean (SEM). Statistical significance of difference between groups was analyzed using Student's 2-tailed t-test on MS Excel 5.0 software. The Chi-Square test (GraphPad Software Inc, San Diego, CA) was used to examine the differences in animal survival time. Statistical significance between groups is represented by p<0.05 values.

TABLE 12

sActRIIB Single Dosing for Two Weeks, Study Schedule							
Group No.	Treatment	n	Route	Dose (mg/kg)	Conc. (mg/mL)	Volume (mL)	VOLUME Dosing Schedule
1 (WT)	PBS	8	SC	—	0	0.1	Single Dose Week 0
2 (Inh $\alpha$ KO)	PBS	8	SC	—	0	0.1	Single Dose Week 0
3 (Inh $\alpha$ KO-wk2)	sActRIIB	8	SC	30	9	0.1	Single Dose Week 0

TABLE 13

sActRIIB Withdrawal and Re-administration Schedule							
Group No.	Treatment	n	Route	Dose (mg/kg)	Conc. (mg/mL)	Volume (mL)	Dosing Schedule
1 (WT)	PBS	11	SC	—	0	0.1	Single Dose Week 0 and Week 8
2 (Inha KO)	PBS	8	SC	—	0	0.1	Single Dose Week 0
3 (Inha KO-wk8)	sActRIIB	7	SC	30	9	0.1	Single Dose Week 0
4 (Inha KO-wk12)	sActRIIB	11	SC	30	9	0.1	Single Dose Week 0 and Week 8

A single dose of sActRIIB (30 mg/kg, SC) resulted in sustained weight gain in Inha KO mice that exceeded the level of WT littermate control during the first 4 weeks. Thereafter and up to 8 weeks after the initial dose, the body weight of the sActRIIB-treated Inha KO mice was similar to the WT control, while the average body weight of PBS-treated KO mice was significantly below WT control throughout the study period. At week 8, eleven of the sActRIIB-treated Inha KO mice were given another single dose of sActRIIB. The re-administration of sActRIIB stimulated further weight gain in Inha KO mice during the proceeding 4-week period (up to week 12) (FIG. 21).

The Inha KO mice treated with PBS developed ovarian tumors and displayed dramatic muscle and organ wasting around 15 weeks of age. When the lethal conditions occurred in the mice, they were euthanized by CO<sub>2</sub> inhalation. There was one WT mouse found dead in cage (DIC) with no clear explanation as to the cause of death. There was also one Inha KO mouse treated with sActRIIB found DIC at week 7 post the initial dose. The Inha KO mice were analyzed for survival rates during the 8-week period after a single dose of sActRIIB. At week 8, survival rate of sActRIIB-treated Inha KO mice was 94% (17 in 18 survival) compared to 12.5% (1 in 8 survival) in the PBS-treated Inha KO group (FIG. 22).

Two weeks after a single dose of sActRIIB, the average tumor weight of KO mice was reduced to the WT control level. During the same period the PBS-treated Inha KO mice developed large ovarian tumors (FIG. 23). At 8 weeks after withdrawal from the initial single dose, the ovarian tumor weight in sActRIIB-treated Inha KO mice had grown in size similar to that of the PBS-treated Inha KO mice, suggesting a regrowth of the ovarian tumors during the compound withdrawal period. Re-administration of sActRIIB was given to 11 of the withdrawal Inha KO mice at week 8. Data on ovarian tumor mass analyzed at week 12 (4 weeks after re-administration) indicate that the re-administration of sActRIIB after 8 weeks withdrawal effectively reduced the tumor mass (FIG. 24).

15      The present study demonstrates that sActRIIB is effective in reversing cancer cachexia and suppressing ovarian tumor growth in female Inha KO mice. A single dose of sActRIIB has a long-lasting effect on body weight gain in the Inha KO mice. The data indicates that sActRIIB treatment significantly suppressed ovarian tumor growth in the Inha KO mice. The ovarian tumor mass in the Inha KO mice regressed to WT control level after 2 weeks of a single dose treatment with sActRIIB. After 8 weeks of withdrawal from 20 the initial dose of sActRIIB, the weight of the ovarian tumors in Inha KO mice was nearly the same as that seen in the PBS-treated group. However, re-administration of sActRIIB effectively regressed the ovarian tumor mass to the size of the WT control group. These data indicate that 25 intermittent administration of sActRIIB given at a prolonged interval of 8 weeks is highly effective in preventing weight loss, suppressing ovarian tumor growth, and prolonging survival in female Inha KO mice.

35      Example 9: Effects of sActRIIB in Combination with Doxorubicin on Tumor Growth, Body Weight and Muscle Mass in TOV-21G Ovarian Carcinoma-Implanted Nude Female Mice

40      The objective of the present study was to examine the effect of pharmacological administration of sActRIIB, doxorubicin (dox), and sActRIIB plus doxorubicin, respectively, on body weight, tumor growth and muscle mass in nude mice implanted with TOV-21G ovarian xenograft tumors. 45      Eight-week-old female Athymic nude mice were SC injected with 0.2 mL of 5×10<sup>6</sup> TOV-21G cells into the left site of the lower flank of the mice. After 10 days of tumor implantation, the mice were divided into 4 groups by body weight and tumor size and then treated with vehicle, sActRIIB, doxorubicin or the combination of sActRIIB and doxorubicin. In addition, a group of non-tumor bearing mice was used as normal control and received PBS. The dosing and treatment schedule are indicated in the table below:

TABLE 14

Dosing and treatment schedule							
Group	n	Test Article	Dose mg/kg	Conc. mg/mL	Volume mL/20 g	Route	Regimen
Normal	10	PBS	NA	NA	NA	SC	1 x/week
TOV-21G + PBS	18	PBS	NA	NA	NA	SC	1 x/week
TOV-21G + sActRIIB	14	sActRIIB	10 mg/kg	1	0.2	SC	1 x/week

TABLE 14-continued

Dosing and treatment schedule							
Group	n	Test Article	Dose mg/kg	Conc. mg/mL	Volume mL/20 g	Route	Regimen
TOV-21G + DOX	14	DOX	2 mg/kg	0.2	0.2	IP	1 x/week 4 consecutive IP injections
TOV-21G + sActRIIB + DOX		sActRIIB	10 mg/kg	1	0.2	SC	1 x/week
TOV-21G + sActRIIB + DOX	14	DOX	2 mg/kg	0.2	0.2	IP	1 x/week 4 consecutive IP injections

Mice were weighed weekly. Body weight data were recorded longitudinally. Tumor size was measured longitudinally by using an electronic caliper. The following formula was used to calculate actual tumor volume (Tomayko M and Reynolds C, 1989): (Volume of a rectangular solid tumor: Tumor volume ( $\text{mm}^3$ )=length (mm)×width (mm)×height (mm) of tumor). At the end of the study, mice in all groups were subjected to terminal necropsy and lean carcass weight (excluding skin, adipose tissue, internal organs, and head) was determined by using standard anatomical dissection procedures. The calf muscles from left and right sides of each mouse were excised and weighed. All results were expressed as the mean±SEM (standard error of the mean). For statistical analysis, a standard 2-tailed t-test was used in conjunction with the MS Excel 5.0 software to determine the statistical differences. Any p value less than 0.05 was considered to be statistically significant.

As shown in FIG. 25, TOV-21G tumor-bearing mice showed a loss in body weight compared with non-tumor-bearing normal control mice. sActRIIB administration in TOV-21G tumor-bearing mice effectively prevented the weight loss. Doxorubicin treatment resulted in a further decline (non-statistically significant) in body weight in the TOV-21G implanted mice; however, the decrease was significant when compared to normal control mice at day 38. sActRIIB administered in combination with doxorubicin effectively mitigated the weight loss as seen in doxorubicin treated TOV-21G implanted mice.

Tumor size of each individual mouse was measured every week throughout the 4-week study period. Tumor weights were recorded via terminal necropsy procedures at week 4. As shown in FIG. 26, DOX significantly reduced tumor size and tumor weight in TOV-21G tumor bearing mice compared with vehicle-treated TOV-21G group. sActRIIB in combination with DOX treatment further inhibited the tumor growth and reduced the tumor size compared with vehicle-treated TOV-21G tumor bearing mice. At day 38, sActRIIB in combination with DOX treatment significantly reduced the tumor size compared to DOX-treated TOV-21G tumor bearing mice, the tumor weight was reduced 25%. In addition, sActRIIB and doxorubicin had additive effects on tumor suppression. Thus, sActRIIB and doxorubicin were

each capable of inhibiting TOV-21G tumor growth in nude mice and when combined, they led to greater inhibition of TOV-21G tumor growth.

Mouse lean carcass weight and calf muscle weight were determined via necropsy procedures at the end of the 4-week experiment. As shown in FIG. 27, TOV-21G tumor-bearing mice showed significant decreases in lean carcass weight and calf muscle mass compared with normal controls; however, administration of sActRIIB prevented the loss in lean carcass weight and calf muscle mass in TOV-21G-implanted nude mice. Doxorubicin had no effect on the loss of lean carcass weight and calf muscle mass in TOV-21G xenograft mice; however, combination treatment with sActRIIB significantly prevented the loss in lean carcass weight and calf muscle mass in TOV-21G-implanted nude mice. Thus, sActRIIB administered alone or in combination with doxorubicin was capable of preventing muscle loss in TOV-21G tumor-bearing mice.

sActRIIB administered alone or in combination with doxorubicin inhibited TOV-21G xenograft tumor growth and attenuated muscle wasting in the tumor-bearing nude mice. Moreover, sActRIIB and doxorubicin appeared to have additive effects on suppression of TOV-21G xenograft tumor growth in nude mice.

#### Example 10: Effects of Activin a Blockade with Activin-A Antibody on Body Weight, Muscle Mass, Lean Body and Fat Mass, Organ Weights, Ovarian Tumor Growth and Tumor Angiogenesis Factor Expression in Female Inhibin- $\alpha$ Knockout Mice

The objectives of this study were to examine the pharmacological effects of activin A antibody on circulating activin A level, body weight, lean body and fat mass, muscle and organ weights, and ovarian tumor weight, as well as tumor angiogenic factor (VEGF and Ang-1) expression levels in ovarian tissues, in Inh $\alpha$  KO mice with established ovarian tumors and cachexia.

Inh $\alpha$  KO mice were licensed from Dr. Martin M. Matzuk (Baylor College of Medicine, Houston, TX). Mice were maintained on a mixed C57BL6/129S6/SvEv genetic background and the colonies were bred at Charles River Laboratories, Inc. (Wilmington, MA). Genotyping of Inh $\alpha$  KO mice was conducted by PCR using genomic tail DNA and performed by Genetically Engineered Models and Services (Charles River Laboratories, Inc. Wilmington, MA).

Weekly Injection for 4 Week Experiment

Group No.	Treatment	n	Route	Dose (mg/kg)	Conc. (mg/mL)	Volume (mL)	Dosing Schedule
1 (WT)	PBS	6	SC	—	0	0.1	1 x/week
2 (WT)	Activin A Ab	5	SC	20	6	0.1	1 x/week
3 (Inhα KO)	PBS	9	SC	—	0	0.1	1 x/week
4 (Inhα KO)	Activin A Ab	9	SC	20	6	0.1	1 x/week

Eleven-week-old female Inhα KO mice were subcutaneously (SC) injected with either 20 mg/kg activin-A antibody or PBS (vehicle control). The activin-A antibody used was the same as described in the Materials section above. Age-matched WT littermate control mice were injected with 20 mg/kg activin-A antibody or PBS (served as baseline controls). The weekly injections lasted for 4 weeks. At the end of the 4-week study, terminal blood samples were drawn by cardiac puncture and serum was stored at -80° C. for activin A analysis. MSD Standard plates were used to detect free activin A levels according to the protocol provided by the manufacturer. Serum collected at necropsy was used in the assay. VEGF Immunoassay kit was used to detect VEGF (vascular endothelial growth factor) levels by following the protocol provided.

Mouse body weights were recorded once per week for 4 weeks. Body composition (lean mass and fat mass) was analyzed by nuclear magnetic resonance (NMR) imaging on week 0 and week 4 using the Mini Spec NMR imaging instrument (Bruker BioSpin GmbH, Rheinstetten, Germany) according to the protocol provided by the manufacturer. At the end of the 4-week study, all animals were euthanized in a CO<sub>2</sub> chamber and were subjected to terminal necropsy procedures. Immediately following euthanization, the calf muscle and ovary, as well as uterus in Inhα KO mice were excised and weighed.

Mouse ovaries and ovarian tumors were fixed in Zinc-formalin for paraffin blocks. Paraffin sections of 4 µm in thickness were used for IHC. Antigen retriever was by microwaving 3 min in Unmask Solution (Vector H-3300). None specific staining blocking was in CAS (Zymed Lab 00-8120) for 30 minutes at room temperature. Primary antibodies diluted in CAS are: rabbit anti Angiopoietin 1 (Abcam ab8451) 1:500; rabbit anti VEGF (Abcam ab46154) 1:150. Incubation was at room temperature for 3 hours. The secondary antibody was linked by Vector Elite rabbit IgG ABC kit (pk-6101). Vector SG kit (SK-4700) was used for the blue/gray stain with nuclear counterstained in Fast Red (Vector H-3403). All results were expressed as the mean±standard error of the mean (SEM). Statistical significance of difference between activin-A antibody-treated groups and PBS-treated groups was analyzed for all data, using Student's 2-tailed t-test. Statistical significance between groups is represented by p<0.05 values.

The serum activin A levels were significantly elevated in Inhα KO mice compared to the WT control groups. The injections of activin-A antibody completely eliminated the increase in serum activin A in Inhα KO mice after 4-weeks of treatment (FIG. 28). Administration of activin-A antibody in Inhα KO mice increased body weight significantly compared to the PBS-treated Inhα KO and WT littermates within 1 week of treatment. The significant body weight increase continued through week 4. During this 4-week period, the body weight of the Inhα KO mice treated with PBS

remained constant. In the WT littermate group, activin-A antibody had no effect on the body weight (FIG. 29).

As revealed by NMR imaging, administration of 20 mg/kg activin-A antibody in female Inhα KO mice led to the significant increase of lean body mass beyond that of the PBS-treated Inhα KO mice and WT littermates by week 4. Conversely, Inhα KO mice treated with PBS had significantly lower lean body mass compared to the WT mice and activin-A antibody-treated Inhα KO mice. In the WT littermate control groups, activin-A antibody had no effect on lean body mass. Activin-A antibody in Inhα KO mice increased fat mass to the levels of WT littermate control group by the end of the 4-week treatment period, and it was significantly higher than that of Inhα KO mice treated with PBS. In the WT littermate control groups, activin-A antibody had no significant effect on fat mass (FIG. 30).

The calf muscle mass was measured at the end of the study via terminal necropsy procedures. Activin-A antibody administration of Inhα KO mice resulted in significantly increased muscle mass compared to the PBS-treated Inhα KO mice and WT littermate control groups. Activin-A antibody had no significant effect on calf muscle mass in the WT littermate control groups (FIG. 31).

Ovaries and uterus (Inhα KO mice only) were examined at the end of the study via necropsy procedures. The data revealed that all the female Inhα KO mice developed large hemorrhagic ovarian tumors. Gross weights of the ovaries of Inhα KO mice were significantly higher than that of the WT littermate control group. Administration of activin-A antibody in Inhα KO mice led to a significantly reduced tumor sizes in comparison to the tumors in the Inhα KO mice treated with PBS. Furthermore, most of the uterus in the Inhα KO group with PBS were enlarged full of fluid. The activin-A antibody treatment significantly reduced the uterus weight by 90%. Activin-A antibody had no effect on ovaries weights in the WT littermate control groups (FIG. 32).

Serum VEGF ELISA revealed that the Inhα KO mice with advanced ovarian tumors had greatly increased levels of VEGF in their circulation (FIG. 33). Both VEGF and angiopoietins-1 (Ang-1) immunoreactivities were significantly increased in the sections of ovarian tumors from PBS-treated Inhα KO mice. Activin-A antibody treatment abolished the VEGF and Ang-1 inductions in the ovaries (FIG. 34).

The results from the present study indicate that weekly dose of 20 mg/kg activin-A antibody for 4 weeks reduced circulating activin levels, ameliorated cachexia, suppressed ovarian tumor growth and decreased the expression of tumor angiogenesis factors in female Inhα KO mice. Activin-A antibody administration significantly increased body weights and skeletal muscle mass, decreased ovarian tumor size, and abolished VEGF and Ang-1 overexpression in the ovaries in Inhα KO mice.

Example 11: Effects of Activin-A Antibody in Combination with Doxorubicin on Tumor Growth, Body Weight and Muscle Mass in Nude Female Mice Implanted with TOV-21G Ovarian Carcinoma

The objective of this study was to examine the effects of pharmacological administration of activin-A antibody, doxorubicin, and activin-A antibody plus doxorubicin, on body weight, tumor growth and muscle mass in nude mice implanted with TOV-21G ovarian xenograft tumors. The activin-A antibody used was the same as described in the Materials section above. Eight-week-old female Athymic nude mice were each injected with  $2.2 \times 10^6$  TOV-21G cells subcutaneously (SC) into the left site of the lower flank of the mice. On day 12 post tumor implantation, the mice were divided into 4 groups by body weight and tumor size and then treated with vehicle, activin-A antibody, doxorubicin, or the combination of activin-A antibody and doxorubicin. In addition, a group of non-tumor bearing mice was used as normal control and received PBS. The dosing and treatment schedule are indicated in the table below:

Group	n	Test Article	Dose mg/kg	Conc. mg/mL	Volume mL/20 g	Route	Regimen
Normal	8	PBS	NA	NA	NA	SC	1 x/week
TOV-21G + PBS	14	PBS	NA	NA	NA	SC	1 x/week
TOV-21G + Activin-A Ab	14	Activin-A Ab	20 mg/kg	2	0.2	SC	1 x/week
TOV-21G + DOX	14	DOX	4 mg/kg	0.4	0.2	IP	1 x/week 4 consecutive IP injections
TOV-21G + Activin-A Ab + DOX	14*	Activin-A Ab	20 mg/kg	2	0.2	SC	1 x/week
		DOX	4 mg/kg	0.4	0.2	IP	1 x/week 4 consecutive IP injections

\*Two mice in Group 5 were killed by other mice in the cage, they were multicaged (4 mice/cage).

Mice were weighed weekly. Body weight data were recorded longitudinally. Tumor size was measured longitudinally by using an electronic caliper (Fred V. Fowler Company, Inc.). The following formula was used to calculate actual tumor volume (Tomayko and Reynolds, 1989): Volume of a rectangular solid tumor: Tumor volume ( $\text{mm}^3$ ) = length (mm)  $\times$  width (mm)  $\times$  height (mm) of tumor. At the end of the study, mice in all groups were subjected to terminal necropsy and lean carcass weight (excluding skin, adipose tissue, internal organs, and head) was determined by using standard anatomical dissection procedures. The calf muscles from left and right sides of each mouse were excised and weighed. All results were expressed as the mean  $\pm$  SEM. For statistical analysis, a standard 2-tailed t-test was used to determine the statistical differences. Any p value less than 0.05 was considered to be statistically significant.

As shown in FIG. 35, TOV-21G tumor-bearing mice showed a significant loss in body weight compared with non-tumor-bearing normal control mice. Activin-A antibody administration prevented the weight loss in TOV-21G tumor-bearing mice. Doxorubicin treatment led to further decline (non-statistically significant) in body weight in TOV-21G implanted mice. Combination treatment with activin-A antibody and doxorubicin appeared to cause less weight loss (non-statistically significant) than doxorubicin treatment alone.

Tumor size of each individual mouse was measured every week throughout the 5-week study period. Tumor weights

were recorded via terminal necropsy procedures at week 5. As shown in FIG. 36 and FIG. 37, statistically significant decreases in the tumor size and tumor weight at day 47 were observed in activin-A antibody-treated TOV-21G-bearing mice versus the vehicle-treated TOV-21G-bearing mice. In addition, combination treatment with activin-A antibody and doxorubicin had an additive effect on tumor suppression. Thus, activin-A antibody or doxorubicin was each capable of inhibiting TOV-21G tumor growth in nude mice and when these two agents were combined, they led to greater inhibition of TOV-21G tumor growth.

Mouse lean carcass weight and calf muscle weight were determined via necropsy procedures at the end of the 5-week experiment. As shown in FIG. 38, TOV-21G tumor-bearing mice showed significant decreases in lean carcass weight and calf muscle mass compared with normal controls; however, administration of activin-A antibody prevented the loss in lean carcass weight and calf muscle mass in TOV-21G-implanted nude mice. Doxorubicin treatment had no effect on the loss of lean carcass weight and calf muscle mass in TOV-21G xenograft mice; however, combination

treatment with activin-A antibody attenuated the loss in lean carcass weight and calf muscle mass in TOV-21G-implanted nude mice. Thus, activin-A antibody administered alone or in combination with doxorubicin was capable of preventing muscle loss in TOV-21G tumor-bearing mice.

Activin-A antibody administered alone or in combination with doxorubicin inhibited TOV-21G xenograft tumor growth and also attenuated muscle wasting in the tumor-bearing nude mice. Moreover, activin-A antibody treatment appeared to have an additive effect with doxorubicin chemotherapy on suppression of in vivo growth of TOV-21G xenograft tumors in nude mice.

While the invention has been particularly shown and described with reference to a preferred embodiment and various alternate embodiments, it will be understood by persons skilled in the relevant art that various changes in form and details can be made therein without departing from the spirit and scope of the invention.

All references, issued patents and patent applications cited within the body of the instant specification are hereby incorporated by reference in their entirety, for all purposes.

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## SEQUENCE LISTING

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CDS                   1..1071

SEQUENCE: 7  
atggagttt ggctgagctg ggttttcctc gttgctttt taagagggtt ccagtgttag 60  
acacgggtgt gcatctacta caacgcacaa tggagctgg agcgccacaa ccagaccggc 120  
ctggagcgct gcgaaggcga gcaggacaag cggctgcact gctacgcctc ctggcgcaac 180  
agctctggca ccatecgact cgtgaagaag ggctgtggc tagatgacct caactgtcac 240  
gataggcagg agtgtgtggc cactgaggag aacccccagg tgtaacttctg ctgtgttag 300  
ggcaacttgc gcaacgagcg cttcaactat ttggccagagg ctggggggccc ggaagtcaacg 360  
tacgagccac ccccgacacg ccccccacggggggaggat ctgtcgatgc cccaccgtgc 420  
ccagcaccac ctgtggcagg accgtcgtgc ttctcttcc ccccaaaaaacc caaggacacc 480  
ctcatgtatct cccggacccc tgagggtc acgtgggggg tggacgttag ccacgaaagac 540  
cccgagggtcc agttcaactg gtacgtggac ggcgtggagg tgcataatgc caagacaaag 600  
ccacggggagg agcagtcaacg cggcacgtc cgtgtggtca ggcgtccac cgttgtgcac 660  
caggactggc tgaacggcaa ggagtgatcaag tgcaagggtct ccaacaaaagg cctcccaaggc 720  
cccatcgaga aaaccatctc caaaaacaaa ggccagcccc gagaaccaca ggtgtacacc 780  
ctggcccccattt cccggggaggat gatgaccaag aaccagggtca ggcgtggagg cctgtgtcaaa 840  
ggcttctatcc agcgacat cggccgtggag tgggagacca atgggcagcc ggagaacaaac 900  
tacaagacca cacctccat gctggactcc gacggcttca ctttcttca cagcaactc 960  
accgtggaca agacgagggtg gcaacgggg aacgttttcat gatgtccgt gatgtcatgag 1020  
gctctgcaca accactacac gcaagaagacg cttccctgt ctccgggtaa a 1071

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SEQ ID NO: 8                    moltype = AA length = 357  
 FEATURE                        Location/Qualifiers  
 source                        1..357  
 mol\_type = protein  
 organism = Homo sapiens

SEQUENCE: 8  
 MEFGLSWVFL VALLRGVQCE TRWCYIYNAN WELERTNQTG LERCEGEQDK RLHCYASWRN 60  
 SSGTIELVKK GCWLDDFN CY DRQECVATEE NPQVYFCCCE GNFCNERFTH LPEAGGPEVT 120  
 YEPPTAPTG PAPPVAGPSV FLFPKPKD LMISRTPEVT CVVVDVSHED 180  
 PEVQFNWYV DVEVHNNAKTK PREEQFNSTF RVVSVLTVHH QDWLNKEYK CKVSNKGLPA 240  
 PIEKTISKTK GQPREPQVYT LPPSREEMTK NQVSLTCLVK GFYPSDIAVE WESNGQPN 300  
 YKTTPPMLDS DGSSFLY SKL TVDKSRWQOQ NVFSCSVMHE ALHNHYTQKS LSLSPGK 357

SEQ ID NO: 9                    moltype = DNA length = 1014  
 FEATURE                        Location/Qualifiers  
 source                        1..1014  
 mol\_type = other DNA  
 organism = Homo sapiens

CDS                            1..1014

SEQUENCE: 9  
 gagacacggg ggtgcata cttacaacggc aactggggc tggagcgac caaccaggacc 60  
 ggcctggagg gtcgcaggc cgaggcaggac aagccggctgc actgtacgc ctccctggc 120  
 aacagctctg gcacccatcga gctctgtgg aagggtctgc ggcttagatga cttcaactgc 180  
 tacatgttgc aggagtgtgt ggccactgtgg gagaaccccc aggtgtactt ctgtgtgtgt 240  
 gaggggcaact tctgcacacgg cgcgttcaact catttgcacgg aggtgtgggg cccggaaatgc 300  
 acgtacggc caccggccggc agccccccacc ggaggggggat gatctgtcga gtggccaccgg 360  
 tgcccggc caccgttggc aggaccgtca gtcttcctct tccccccaaa acccaaggac 420  
 accctcatgta tctccggc ccctggggc acgtgcgtgg tggtgacgt gaggccacgaa 480  
 gacccggagg ttccatcaa ctggatcgac gacggcgatgg aggtgcataa tgccaagaca 540  
 aaggccacggg aggaggcgtt caacacggc ttccgttgc tcagcgtctt caccgttgc 600  
 caccaggact ggcttacacgg caaggaggatc aagtgcacgg tctccaaacc accggctccca 660  
 gccccatcg agaaaaaccat ctccaaaacc aaaggggcggc cccggaaacc acagggtgtac 720  
 accctggccatccatccgggaa ggagatggc aagaaccagg tcagcgttgc ctggctgtgc 780  
 aaaggcttccatccggcgtt gatggggaga gcaatggggca gccggggaaac 840  
 aactacaaga ccacacccatc catgtggac tccggacggc cttcttcctt ctacaccaag 900  
 ctcaccgtgg acaagacggcgtt gttggcggcggc gggacgttgc tctcatgtc cgtatgtcat 960  
 gaggtctgc acaaccacta cacggcggc aacccatccatc tttttccggg taaa 1014

SEQ ID NO: 10                    moltype = AA length = 338  
 FEATURE                        Location/Qualifiers  
 source                        1..338  
 mol\_type = protein  
 organism = Homo sapiens

SEQUENCE: 10  
 ETWRCYIYNNA WELERTNQTG GLERCEGEQDK KRLHCYASWR NSSGTIELVKK KGWLDDFN 60  
 YDRQECVATEE ENPQVYFCCCE EGNFCNERFTH LPEAGGPEVT TYEPPTAPT GGGGSVECPP 120  
 CPAPPVAGPSV VFLFPKPKD LMISRTPEVT CVVVDVSHED DEPVQFNWYV DGVEVHNNAKTK 180  
 KPREEQFNSTF PRVSVLTVV HQDWLNKEYK CKVSNKGLPA APIEKTISKTK KGQPREPQVY 240  
 TLPPSREEMTK KNQVSLTCLVK GFYPSDIAVE WESNGQPN NYKTTPPMLDS SDGSFFLYSK 300  
 LTVDKSRWQOQ GNFSCSVME EALHNHYTQKS SLSLSPGK 338

SEQ ID NO: 11                    moltype = DNA length = 387  
 FEATURE                        Location/Qualifiers  
 source                        1..387  
 mol\_type = other DNA  
 organism = Homo sapiens

CDS                            1..387

SEQUENCE: 11  
 atggagttt ggctgagctg gttttccctc gttgtctttt taagagggtt ccagtgttag 60  
 acacggtaact gcatctacta caacgcacac tggagctgg agcgccacca ccagccggc 120  
 ctggagccgtt gcaaggcga gcaggacaa cggctgcactt gtaacgcctc ctggccacac 180  
 agctctggca ccatacgatc cgttgcacgg aggtgtggc tagatgtactt caactgttac 240  
 gataggcagg agtgtgttgc cactggagg aaccccccagg tttttttttt ctgtgtgtgt 300  
 ggcaacttgc gcaacggcgtt cttttttttt ttggcggagg ctggggggcc ggaatgttac 360  
 tacggccaccc cccggccaccc ccccccaccc 387

SEQ ID NO: 12                    moltype = AA length = 129  
 FEATURE                        Location/Qualifiers  
 source                        1..129  
 mol\_type = protein  
 organism = Homo sapiens

SEQUENCE: 12  
 MEFGLSWVFL VALLRGVQCE TRYCIYNNAN WELERTNQTG LERCEGEQDK RLHCYASWRN 60  
 SSGTIELVKK GCWLDDFN CY DRQECVATEE NPQVYFCCCE GNFCNERFTH LPEAGGPEVT 120  
 YEPPTAPT 129

SEQ ID NO: 13                    moltype = DNA length = 330  
 FEATURE                        Location/Qualifiers

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source 1..330  
 mol\_type = other DNA  
 organism = Homo sapiens

CDS 1..330

SEQUENCE: 13  
 gagacacggg actgcatacta ctacaacgcc aactggggc tggagcgac caaccagacc 60  
 ggccctggagg gtcgcgaagg cgagcaggac aagcggtc actgcatacg ctcctggc 120  
 aacagctctg gcaccaatcga gctcgtaag aagggtgc ggcttagatga cttcaactgc 180  
 tacatggaggc aggagtgtgt ggccactgag gagaaccccc aggtgtactt ctgtgtgt 240  
 gaggggcaact tctgcacacg ggcgttact catttgcacg aggctggggg cccggaaatgc 300  
 acgtacgacg caccggcacc agccccccacc 330

SEQ ID NO: 14 moltype = AA length = 110  
 FEATURE Location/Qualifiers  
 source 1..110  
 mol\_type = protein  
 organism = Homo sapiens

SEQUENCE: 14  
 TRYCIYVNA NWELERTNQ GLERCEGEQD KRLHCYASWR NSSGTIELVK KGCWLDDFNC 60  
 YDRQECVATE ENPQVYFCCC EGNFCNERFT HLPEAGGPEV TYEPPPTAPT 110

SEQ ID NO: 15 moltype = DNA length = 1071  
 FEATURE Location/Qualifiers  
 source 1..1071  
 mol\_type = other DNA  
 organism = Homo sapiens

CDS 1..1071

SEQUENCE: 15  
 atggagttt ggctgagctg ggtttccctc gttgctttta taagggtgt ccagtgttag 60  
 acacggtaact gcatctacta caacggccaaact tggaggtgg agcgcaccaa ccagacccgc 120  
 ctggagggctc gcgaggacaag cggtgtcact gctacgcctc ctggcgcaac 180  
 agtctggca ccatecgagct cgttaaagaag gggtgtgtgc tagatgactt caactgtac 240  
 gataggcagg agtgtgtggc cactgaggag aacccccagg tttacttctg ctgtgtgt 300  
 ggcaacttctt gcaacggcgtt cttcaactcat ttggccagag ctggggggccc ggaagtcaag 360  
 tacgagggccccc cccggcggcggggggaggat ctgtcgaggc cccacccgtc 420  
 ccacggccacc ctgtggcagg accgtcgttc ttctcttcc ccccaaaaaacc caaggacacc 480  
 ctcatgtatctt cccggacccc tgagggtcactc tgctgtgtgg tggacgtgag ccacgaagac 540  
 cccggagggtc agtcaactg gtacgtggac gggtgtggagg tgcataatgc caagacaaag 600  
 ccacggggaggc agcgttcaaa cgcacgttc cgtgtgttca gcgtccctcac cgttgtgcac 660  
 caggactgtc tgaacggcaa gggttacaag tgcagggtt ccaacaaagg cctcccaagcc 720  
 cccatcgaga aaaccatctc caaaaacaaa gggcagcccc gagaaccaca ggtgtacacc 780  
 ctgccccccat cccggggaggat gatgtaccaag aaccagggttca gcctgtactc cctgttcaaa 840  
 ggcttctatcc cagcgttcaat cccggggaggat tggggagatca atggggcggcc ggagaacaaac 900  
 tacaagacca caccctccat gctggactcc gacggctctt tttctcttca cagaactgc 960  
 accgtggaca agagcagggtg gcaacgttctt catgtccctgt gatgtcatgag 1020  
 gtcctgtcaca accactacac gcagaagacg ctctccctgt ctccgggtaa a 1071

SEQ ID NO: 16 moltype = AA length = 357  
 FEATURE Location/Qualifiers  
 source 1..357  
 mol\_type = protein  
 organism = Homo sapiens

SEQUENCE: 16  
 MEGFLSWVFL VALLRGVQCE TRYCIYNNAN WELERTNQGL LERCEGEQDK RLHCYASWRN 60  
 SSGTIELVKK GCWLDDFN CY DRQECVATEE NPQVYFCCCE GNFCNERFTL PEAAGGPEVT 120  
 YEPPTAPTG GGGSVCECP PC PAPVAVGSPV FLPPPKPKDT LMISRTPEVT CVVVDVSHE 180  
 PEVQFNWYVD GVEVHNNAKTK PREEQFNSTF RVVSLTVVVH QDWLNGKEYK CKVSNKGLPA 240  
 PIKETKISKTK QOPREPQVYT LPPSREEMTK NQVSLTCLVKK GFYPSDIAVE WESNQOPENN 300  
 YKTTPPMLDS DGSFFLYSKL TVDKSRWQQG NVFSCSVMH E ALHNHYTOKS LSLSPGK 357

SEQ ID NO: 17 moltype = DNA length = 1014  
 FEATURE Location/Qualifiers  
 source 1..1014  
 mol\_type = other DNA  
 organism = Homo sapiens

CDS 1..1014

SEQUENCE: 17  
 gagacacggg actgcatacta ctacaacgcc aactggggc tggagcgac caaccagacc 60  
 ggccctggagg gtcgcgaagg cgagcaggac aagcggtc actgcatacg ctcctggc 120  
 aacagctctg gcaccaatcga gctcgtaag aagggtgc ggcttagatga cttcaactgc 180  
 tacatggaggc aggagtgtgt ggccactgag gagaaccccc aggtgtactt ctgtgtgt 240  
 gaggggcaact tctgcacacg ggcgttactt catttgcacg aggctggggg cccggaaatgc 300  
 acgtacgacg caccggcacc agccccccacc ggagggggggat gatgtcga gtggccaccg 360  
 tgccctgtgc acacgttcaat cccggggggat tttttttttt ccccccacca acccaaggac 420  
 accctcatga tctccggac ccctggggat acgtgtgtgg tgggtggactt gacccacggaa 480  
 gacccgggg tccaggtaaa ctggtaatgtt gacgggtgggg agggtgcataa tgccaaagaca 540  
 aacggccacggg aggaggatcaac gtcacgttcaat tccatgtgtgg tcagcgttctt caccgttg 600  
 caccaggact ggctgaacgg caaggactt aatgtcaagg tctccaaacaa aggctccca 660  
 gccccccatcg agaaaaccat ctccaaacacc aaaggccggc cccggagaacc acagggttac 720

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accctgcccc	catccggga	ggagatgacc	aagaaccagg	tcagcctgac	ctgcctgtc	780
aaaggctct	atcccagcg	catgccgtg	gagtggaga	gcaatggca	gcccggaaac	840
aactacaaga	ccacaccc	catgctggac	tccgacggct	ccttcttct	ctacagcaag	900
ctcaccgtgg	acaagagcag	gtggcagcag	gggaacgtct	tctcatgtc	cgtatgtcat	960
gaggctctgc	acaaccacta	cacgagaag	agcctctccc	tgtctccggg	taaa	1014

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SEQ ID NO: 18            moltype = AA length = 338  
 FEATURE                Location/Qualifiers  
 source                1..338  
 mol\_type = protein  
 organism = Homo sapiens

SEQUENCE: 18  
 ETRYCIYNA NWELERTNQT GLERCEGEQD KRLHYCAYASWR NSSGTIELVK KGCWLDDFNC 60  
 YDRQECVATE ENPQVYFCCC EGNFCNERFT HLPEAGGPEV TYEPPPTAPT GGGGSVECPP 120  
 CPAPPVAGPS VFLFPKPKD TLMISRTPEV TCVVVDVSHE DPEVQFNWYV DGVEVHNAKT 180  
 KPREEQFNST FRVSVSLTVV HQDWLNGKEY KCKVSNKGLP APIEKTIKT KGQPREPQVY 240  
 TLPSREEMT KNQVSLTCLV KGFYPSDIAV EWESNGOPEN NYKTPPMID SDGSFFLYSK 300  
 LTVDKSRWQQ GNVFSCSVMH EALHNHYTQK SLSLSPKG 338

SEQ ID NO: 19            moltype = AA length = 110  
 FEATURE                Location/Qualifiers  
 source                1..110  
 mol\_type = protein  
 organism = Homo sapiens

SEQUENCE: 19  
 ETRYCIYNA NWELERTNQS GLERCEGEQD KRLHYCAYASWR NSSGTIELVK KGCWLDDFNC 60  
 YDRQECVATE ENPQVYFCCC EGNFCNERFT HLPEAGGPEV TYEPPPTAPT 110

SEQ ID NO: 20            moltype = DNA length = 1014  
 FEATURE                Location/Qualifiers  
 source                1..1014  
 mol\_type = other DNA  
 organism = Homo sapiens  
 CDS                   1..1014

SEQUENCE: 20  
 gagacacacgg tttgtcatcta ctacaacggc aactgggagc tggagggcac caaccagac 60  
 ggcctggagc gctcgcaagg cgaggcggac aagcggctgc actgtacgc ctcctggcgc 120  
 aacagctcg gcaccatcgatcg gctcgtaag aagggtcgct ggcttagatga cttaactcg 180  
 tacatggc aggatgtgtg ggccactgag gagaacccccc aggtgtactt ctgtgtctgt 240  
 gagggcaact ttcgtcaacgat ggcgttact catttggccat aggtgtggggg cccggaaatc 300  
 acgtacgacg caccggccac agccccccacc ggaggaggag gatctgtcga gtgcccacccg 360  
 tgcccgacac caccatgtggc aggacgtca gctttctct tccccccaaa acccaaggac 420  
 accctctatcg ttcgtccggac ccctggatcg acgtgcgtgg ttgtggacgt gagccacgaa 480  
 gaccccgagg tccagttcaa ctgttgcgtg gacggcgttg aggtgtatcaa tgccaagac 540  
 aagccacggg aggacgtt caacagcagc ttccgtgtgg tcaagcgtctt caccgttgt 600  
 caccggact ggctgaaacgg caaggatcg aqgtgcaacaa tctccaaacaa aggccctccca 660  
 gccccatcg agaaaaatcg ctccaaaacc aaaggccggc cccggaaacc acagggtgtac 720  
 accctggccc catccggga ggatgtacc aqaaaccagg tcagcgttgc ctgcgtgtc 780  
 aaaggctct atcccgacgat catcgccgtg gatgtggaga gcaatggca gcccggaaac 840  
 aactacaaga ccacacccatcgatcgacatcgatggc ctttcttctt ctacagcaag 900  
 ctccacgtgg acaagagcag gtggcagcag gggaaacgtct ttcgtatgtc cgtatgtcat 960  
 gaggctctgc acaaccacta cacgagaag agcctctccc tgcgtccggg taaa 1014

SEQ ID NO: 21            moltype = AA length = 338  
 FEATURE                Location/Qualifiers  
 source                1..338  
 mol\_type = protein  
 organism = Homo sapiens

SEQUENCE: 21  
 ETRYCIYNA NWELERTNQS GLERCEGEQD KRLHYCAYASWR NSSGTIELVK KGCWLDDFNC 60  
 YDRQECVATE ENPQVYFCCC EGNFCNERFT HLPEAGGPEV TYEPPPTAPT GGGGSVECPP 120  
 CPAPPVAGPS VFLFPKPKD TLMISRTPEV TCVVVDVSHE DPEVQFNWYV DGVEVHNAKT 180  
 KPREEQFNST FRVSVSLTVV HQDWLNGKEY KCKVSNKGLP APIEKTIKT KGQPREPQVY 240  
 TLPSREEMT KNQVSLTCLV KGFYPSDIAV EWESNGOPEN NYKTPPMID SDGSFFLYSK 300  
 LTVDKSRWQQ GNVFSCSVMH EALHNHYTQK SLSLSPKG 338

SEQ ID NO: 22            moltype = AA length = 216  
 FEATURE                Location/Qualifiers  
 source                1..216  
 mol\_type = protein  
 organism = Homo sapiens

SEQUENCE: 22  
 APPVAGPSVF LFPPPKPKDTL MISRTPEVTC VVVDVSHEDP EVQFNWYVDG VEVHNAKTKP 60  
 REEQFNSTFR VVSVLTVVHQ DWLNGKEYKC KVSNKGLPAP IEKTIKTG QPREPVYTL 120  
 PPSREEMTKN QVSLTCLVKG FYPSDIAVEW ESNGOPENNY KTTPPMLSD GSFFLYSKLT 180  
 VDKSRWQQGN VFSCSVMHEA LHNHYTQKSL SLSLSPKG 216

SEQ ID NO: 23            moltype = AA length = 217  
 FEATURE                Location/Qualifiers

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source          1..217
               mol_type = protein
               organism = Homo sapiens
SEQUENCE: 23
APELLGGPSV FLFPPPKD1 LMISRTPEVT CVVVDVSHED PEVKFNWYVG GVEVHNAKTK 60
PREEQYNSTY RVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT 120
LPPSRDELTK NQVSLTCLVK GFYPSDIAVE WESNGQPEENN YKTPPVVLDS DGSFFLYSKL 180
TVDKSRWQOG NVFSCSVMHE ALHNHYTQKS LSLSPGK 217

SEQ ID NO: 24      moltype = AA length = 217
FEATURE
source          1..217
               mol_type = protein
               organism = Homo sapiens
SEQUENCE: 24
APEFLGGPSV FLFPPPKD1 LMISRTPEVT CVVVDVSHED PEVKFNWYVG GVEVHNAKTK 60
PREEQFNSTY RVSVLTVLH QDWLNGKEYK CKVSNKGLPS SIEKTISKAK GQPREPQVYT 120
LPPSQEEMTK NQVSLTCLVK GFYPSDIAVE WESNGQPEENN YKTPPVVLDS DGSFFLYSRL 180
TVDKSRWQEG NVFSCSVMHE ALHNHYTQKS LSLSLGK 217

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

SEQ ID NO: 26      moltype = DNA length = 36
FEATURE
misc_feature    1..36
               note = Description of Artificial Sequence: Synthetic hinge
               linker oligonucleotide
source          1..36
               mol_type = other DNA
               organism = synthetic construct
CDS             1..36
SEQUENCE: 26
ggagggggag gatctgtcga gtgcccaccc tgccca                                         36

SEQ ID NO: 27      moltype = AA length = 12
FEATURE
REGION          1..12
               note = Description of Artificial Sequence: Synthetic hinge
               linker peptide
source          1..12
               mol_type = protein
               organism = synthetic construct
SEQUENCE: 27
GGGSVECPP CP                                         12

SEQ ID NO: 28      moltype = AA length = 12
FEATURE
source          1..12
               mol_type = protein
               organism = Homo sapiens
SEQUENCE: 28
ERKCCVECPP CP                                         12

SEQ ID NO: 29      moltype = AA length = 15
FEATURE
source          1..15
               mol_type = protein
               organism = Homo sapiens
SEQUENCE: 29
EPKSCDKTHT CPPCP                                         15

SEQ ID NO: 30      moltype = AA length = 12
FEATURE
source          1..12
               mol_type = protein
               organism = Homo sapiens
SEQUENCE: 30
ESKTGPPCPS CP                                         12

SEQ ID NO: 31      moltype = AA length = 18

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FEATURE source	Location/Qualifiers 1..18 mol_type = protein organism = Homo sapiens	
SEQUENCE: 31 MTAPWVALAL LWGSLWPG		18
SEQ ID NO: 32 FEATURE source	moltype = AA length = 18 Location/Qualifiers 1..18 mol_type = protein organism = Homo sapiens	
SEQUENCE: 32 MTAPWVALAL LWGSLCAG		18
SEQ ID NO: 33 FEATURE source	moltype = AA length = 512 Location/Qualifiers 1..512 mol_type = protein organism = Homo sapiens	
SEQUENCE: 33 MTAPWVALAL LWGSLCAGSG RGEAETRECI YYNANWELE R TNQSGLERCE GEQDKRLHCY 60 ASWRNSSGTI ELVKKGCWLD DFNCYDRQEC VATEENPOVY FCCCEGNFCN ERFTHLPEAG 120 GPEVTYEPPP TAPTLTVLA YSLPIGGGLS LIVLLAFWMY RHRKPPYGHV DIHEDPGPPP 180 PSPLVGLKPL QLLEIKARGE FFGCVWKAQLM NDFVAVKIFP LQDKQSWSQE REIFSTPGMK 240 HENLLQFIAA EKRGSNLEVE LWLITAFHDK GSLTDYLKGK IIITWNELCHV AETMSRGLSY 300 LHEVDVPWRG EGHKPSIAHF DFKSKNVLLK SDLTAVLADF GLAVRFEPGK PPGDTHGQVG 360 TRRYMAPEVL EGAINFQRDA FLRIDMYAMG LVLWELVSRC KAADGPVDEY MLPFEEEIQ 420 HPSLEELQEV VVHKKMRPTI KDHWLKHPL AQLCVTIEEC WDHDAEARLS AGCVEERVSL 480 IRRSVNGTTS DCLVSLVTSV TNVDPKES SI 512		
SEQ ID NO: 34 FEATURE source	moltype = AA length = 426 Location/Qualifiers 1..426 mol_type = protein organism = Homo sapiens	
SEQUENCE: 34 MPLLWLRGFL LASCWIIVRS SPTPGSEGH S AAPDCPSCAL AALPKDV PNS QPEMVEAVKK 60 HILNMLHLKK RPDVTQPVPK AALLNAIRKL HVGKVGENGY VEIEDDIGRR AEMNLMEQT 120 SEIITFAESG TARKTLHFEI SKEGSDLSVV ERAEVWLFLK VPKANRTRTK VTIRLFQQQK 180 HPQGSLDTGE EAAEVGLKGE RSELLLSEKV VDARKSTWHV FPVSSSIQLR LDQGKSSL DV 240 RIACEQCQES GASLVLLGKK KKKEEEGEGK KKGGGEGGAG ADEEKEQS SHR PFLMLQARQS 300 EDPHPHRRRR GLECDGKVNI CCKKQFFV SF KDIGWNDWII APSGYHANYC EGECPSHIAG 360 TSGSSLSFH S TVINHYRMRG HSPFANLKSC CVPTKLRPM S MLYYDDQNI IKKDIONMIV 420 EECGCS 426		
SEQ ID NO: 35 FEATURE source	moltype = AA length = 375 Location/Qualifiers 1..375 mol_type = protein organism = Homo sapiens	
SEQUENCE: 35 MQKLQLCVYI YLFMLIVAGP VDLNENSEQK ENVEKEGLCN ACTWRQNTKS SRIEAIKIQI 60 LSKLRLETA P NISKDVIRQL LPKAPPLREL IDQYDVQRD SSDGSLEDDD YHATTEITIIT 120 MPTESDFLMO VDGKPCKCF F KFSKIQYNNK VVKAQLWIYL RPVETPTTVF VQILRLIKPM 180 KDGTTRYTGIR SLKLDMMNP GT GIWQSIDVKT V LQNLWKOPE SNLGIEIKAL DENGHD LAVT 240 FPGPGEDGLN PFLEVKTDT PKRSRDRFGL DCDEHSTESR CCRYPLTVDF EA FGWDWIIA 300 PKRYKANYCS GCECFVFLQK YPHTHLVHQ A N P R G S A G P C C T P T K M S P I N M LYFNGKEQII 360 YGKIPAMVVD RCGCS 375		
SEQ ID NO: 36 FEATURE source	moltype = AA length = 217 Location/Qualifiers 1..217 mol_type = protein organism = Homo sapiens	
SEQUENCE: 36 APELLGGPSV F LF PPPKPKD I LMISRTPEVT CVVVDVSHED PEVKFNWYVG GVEVHN A KTK 60 PREEQYNSTY RVVSVLT VLVH QD WLNGKEYN CKVSNKALPA PIEKTISKAK GQP REPQVYT 120 LP PSRDELTK N QV S L T C L V K GF YPS DIA V E WES NGQ PENN Y KTT PPV L DS DGS FFL Y SKL 180 TV DK SRW Q QG NV F SC S VM H E AL HN HY T Q K S L S L S P G K 217		
SEQ ID NO: 37 FEATURE misc_feature	moltype = DNA length = 48 Location/Qualifiers 1..48 note = Description of Artificial Sequence: Synthetic hinge linker oligonucleotide	
source	1..48 mol_type = other DNA organism = synthetic construct	

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CDS	1..48	
SEQUENCE: 37		
ggagggggag gatctgagcg caaatgttgt gtctgactgcc caccgtgc		48
SEQ ID NO: 38	moltype = AA length = 16	
FEATURE	Location/Qualifiers	
REGION	1..16	
	note = Description of Artificial Sequence: Synthetic hinge	
	linker peptide	
source	1..16	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 38		
GGGGSERKCC VECPPC		16
SEQ ID NO: 39	moltype = DNA length = 42	
FEATURE	Location/Qualifiers	
misc_feature	1..42	
	note = Description of Artificial Sequence: Synthetic hinge	
	linker oligonucleotide	
source	1..42	
	mol_type = other DNA	
	organism = synthetic construct	
CDS	1..42	
SEQUENCE: 39		
ggagggggag gatctggtgg aggtggttca ggtccacccgt gc		42
SEQ ID NO: 40	moltype = AA length = 14	
FEATURE	Location/Qualifiers	
REGION	1..14	
	note = Description of Artificial Sequence: Synthetic hinge	
	linker peptide	
source	1..14	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 40		
GGGGSGGGGS GPPC		14
SEQ ID NO: 41	moltype = DNA length = 42	
FEATURE	Location/Qualifiers	
misc_feature	1..42	
	note = Description of Artificial Sequence: Synthetic hinge	
	linker oligonucleotide	
source	1..42	
	mol_type = other DNA	
	organism = synthetic construct	
CDS	1..42	
SEQUENCE: 41		
ggagggggag gatctggtgg aggtggttca ggtccacccgg ga		42
SEQ ID NO: 42	moltype = AA length = 14	
FEATURE	Location/Qualifiers	
REGION	1..14	
	note = Description of Artificial Sequence: Synthetic hinge	
	linker peptide	
source	1..14	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 42		
GGGGSGGGGS GPPG		14
SEQ ID NO: 43	moltype = DNA length = 54	
FEATURE	Location/Qualifiers	
misc_feature	1..54	
	note = Description of Artificial Sequence: Synthetic hinge	
	linker oligonucleotide	
source	1..54	
	mol_type = other DNA	
	organism = synthetic construct	
CDS	1..54	
SEQUENCE: 43		
ggagggggag gatctgagcg caaatgtcca ccttgtgtcg agtgcaccgtc gtgc		54
SEQ ID NO: 44	moltype = AA length = 18	
FEATURE	Location/Qualifiers	
REGION	1..18	
	note = Description of Artificial Sequence: Synthetic hinge	
	linker peptide	
source	1..18	

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mol_type = protein
organism = synthetic construct

SEQUENCE: 44
GGGGSERKCP PCVECPPC                                         18

SEQ ID NO: 45      moltype = AA  length = 14
FEATURE
REGION          Location/Qualifiers
1..14           note = Description of Artificial Sequence: Synthetic hinge
                  linker peptide
source          1..14
mol_type = protein
organism = synthetic construct

SEQUENCE: 45
GPASGGPASG PPCP                                         14

SEQ ID NO: 46      moltype = AA  length = 21
FEATURE
REGION          Location/Qualifiers
1..21           note = Description of Artificial Sequence: Synthetic hinge
                  linker peptide
source          1..21
mol_type = protein
organism = synthetic construct

SEQUENCE: 46
GPASGGPASG CPPCVCPC P                                         21

SEQ ID NO: 47      moltype = AA  length = 217
FEATURE
source          Location/Qualifiers
1..217          note = Description of Artificial Sequence: Synthetic hinge
                  linker peptide
mol_type = protein
organism = Homo sapiens

SEQUENCE: 47
APELLGGPSV FLFPPPKPKDT LMISRTPEV CVVVDVSHED PEVKFNWYVD GVEVHNNAKTK 60
PREEQYNSTY RRVSVLTVLH QDWLNGKEVK CKVSNKALPA PIEKTISKAK GQPREPQVYT 120
LPPSREEMTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTPPVLDS DGSFFLYSKL 180
TVDKRSRWQOG NVFSCSVMHE ALHNHYTQKS LSLSPGK                                         217

SEQ ID NO: 48      moltype = AA  length = 16
FEATURE
REGION          Location/Qualifiers
1..16           note = Description of Artificial Sequence: Synthetic hinge
                  linker peptide
source          1..16
mol_type = protein
organism = synthetic construct

SEQUENCE: 48
GGGGSVDKTH TCPPCP                                         16

SEQ ID NO: 49      moltype = AA  length = 16
FEATURE
REGION          Location/Qualifiers
1..16           note = Description of Artificial Sequence: Synthetic hinge
                  linker peptide
source          1..16
mol_type = protein
organism = synthetic construct

SEQUENCE: 49
GGGGSVDKTH TGPPCP                                         16

SEQ ID NO: 50      moltype = AA  length = 21
FEATURE
REGION          Location/Qualifiers
1..21           note = Description of Artificial Sequence: Synthetic hinge
                  linker peptide
source          1..21
mol_type = protein
organism = synthetic construct

SEQUENCE: 50
GGGGSGGGGS VDKTHTGPPC P                                         21

SEQ ID NO: 51      moltype = DNA  length = 48
FEATURE
source          Location/Qualifiers
1..48           note = Description of Artificial Sequence: Synthetic hinge
                  linker peptide
mol_type = other DNA
organism = Homo sapiens

SEQUENCE: 51
aggtcttagtc agagcctcct gcatagtact ggataacaact atttggat                                         48

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SEQ ID NO: 52      moltype = DNA  length = 21
FEATURE
source           Location/Qualifiers
1..21
mol_type = other DNA
organism = Homo sapiens
SEQUENCE: 52
ttgggttctt ttcgggcctc c                                21

SEQ ID NO: 53      moltype = DNA  length = 26
FEATURE
source           Location/Qualifiers
1..26
mol_type = other DNA
organism = Homo sapiens
SEQUENCE: 53
atgcaagctc tccaaactcc gtgcag                                26

SEQ ID NO: 54      moltype = DNA  length = 30
FEATURE
source           Location/Qualifiers
1..30
mol_type = other DNA
organism = Homo sapiens
SEQUENCE: 54
ggatacacacct tcacccggcta ctatatccac                                30

SEQ ID NO: 55      moltype = DNA  length = 51
FEATURE
source           Location/Qualifiers
1..51
mol_type = other DNA
organism = Homo sapiens
SEQUENCE: 55
tggatcaacc ctaacagtgg tggcacaaaac tatgcacaga agtttcaggg c      51

SEQ ID NO: 56      moltype = DNA  length = 36
FEATURE
source           Location/Qualifiers
1..36
mol_type = other DNA
organism = Homo sapiens
SEQUENCE: 56
gattcggggt atagcagcag ctggcacttt gactac                                36

SEQ ID NO: 57      moltype = AA   length = 112
FEATURE
source           Location/Qualifiers
1..112
mol_type = protein
organism = Homo sapiens
SEQUENCE: 57
DIVMTQSPLS LPVTPGEPAS ISCRSSQSLL HSTGYNYLDW YLQKPGQSPQ LLIYLGSFRA 60
SGVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYCMQALQTP CSFGQQTKLE IK          112

SEQ ID NO: 58      moltype = AA   length = 121
FEATURE
source           Location/Qualifiers
1..121
mol_type = protein
organism = Homo sapiens
SEQUENCE: 58
QVOLVQSGAE VKKPGASVKV SCKASGYTFT GYYIHWVRQA PGQGLEWMGW INPNSSGGTNY 60
AQKFQGRVTM TRDTSISTAY MELSLRSDD TAVYFCARDS GYSSSSWHFDY WGQGTLVTVS 120
S                                         121

SEQ ID NO: 59      moltype = AA   length = 11
FEATURE
source           Location/Qualifiers
1..11
mol_type = protein
organism = Homo sapiens
SEQUENCE: 59
SGDKLGDKYA C                                11

SEQ ID NO: 60      moltype = AA   length = 7
FEATURE
source           Location/Qualifiers
1..7
mol_type = protein
organism = Homo sapiens
SEQUENCE: 60
QDSKRPS                                7

SEQ ID NO: 61      moltype = AA   length = 9
FEATURE
source           Location/Qualifiers
1..9
mol_type = protein

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SEQUENCE: 61	organism = Homo sapiens	
QAWDSSTAV		9
SEQ ID NO: 62	moltype = AA length = 10	
FEATURE	Location/Qualifiers	
source	1..10	
	mol_type = protein	
SEQUENCE: 62	organism = Homo sapiens	
GYTFTSYGLS		10
SEQ ID NO: 63	moltype = AA length = 17	
FEATURE	Location/Qualifiers	
source	1..17	
	mol_type = protein	
SEQUENCE: 63	organism = Homo sapiens	
WIPIPYNGNTN SAQKLQG		17
SEQ ID NO: 64	moltype = AA length = 13	
FEATURE	Location/Qualifiers	
source	1..13	
	mol_type = protein	
SEQUENCE: 64	organism = Homo sapiens	
DRDYGVNYDA PDI		13
SEQ ID NO: 65	moltype = DNA length = 339	
FEATURE	Location/Qualifiers	
source	1..339	
	mol_type = other DNA	
SEQUENCE: 65	organism = Homo sapiens	
gacatcgta tgacccagtc tccagactcc ctggctgtgt ctctggcga gagggccacc	60	
atcacctgc a gtcaggcca gatgttttac acaataaaga gtatcttagtt	120	
tggtaccaggc agaaaccaggc acagcctctt aagctgtatca ttacttgac atctatgcgg	180	
gaatccgggg tccctgaccg attcaatggc agcgggtctc ggacagattt cactctcacc	240	
atcaacagcc tgcaggctga agatgtggca gtttattact gtcagcaata ttatagta	300	
ccgtggacgt tcggccaaggc gaccaaggta gaaaatcaa	339	
SEQ ID NO: 66	moltype = DNA length = 488	
FEATURE	Location/Qualifiers	
source	1..488	
	mol_type = other DNA	
SEQUENCE: 66	organism = Homo sapiens	
caggtgcagc tgcaggaggc ggcccaggta ctggtaagc cttcgagac cctgtccctc	60	
acctgcactg tctctgtgg ctccatcaat agtttctact ggagctggat ccggcagccc	120	
ccaggaaagg gactggatc gatgggtat atcttattaca gtgggacac caactacaat	180	
cctccctca agagtgcgat caccatata ctagacacgtt ccaagacca gtttccctg	240	
aaactgtgact ctgtgaccgc tgccgacacg gccgtgtatt actgtgcgag agacagata	300	
gcagccccct ttgactactg gggccaggga accctggtaa cctgtccctc agcttccacc	360	
aaggggccat ccgttctccc cctggcgccc tgctccaggta gcacccctca gagcacagcc	420	
gccttggcgt gccttggtaa ggactacttc cccgaaccgg tgacgggttc gtggaaactca	480	
tgcccccct	488	
SEQ ID NO: 67	moltype = DNA length = 51	
FEATURE	Location/Qualifiers	
source	1..51	
	mol_type = other DNA	
SEQUENCE: 67	organism = Homo sapiens	
aagtccagcc agatgtttt atacagttcc aacaataaga agtatctagt t	51	
SEQ ID NO: 68	moltype = DNA length = 21	
FEATURE	Location/Qualifiers	
source	1..21	
	mol_type = other DNA	
SEQUENCE: 68	organism = Homo sapiens	
tggacatcta tgcgggaaatc c	21	
SEQ ID NO: 69	moltype = DNA length = 27	
FEATURE	Location/Qualifiers	
source	1..27	
	mol_type = other DNA	
SEQUENCE: 69	organism = Homo sapiens	

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cagcaatatt atagtactcc gtggacg	27
SEQ ID NO: 70 moltype = DNA length = 30	
FEATURE Location/Qualifiers	
source 1..30	
mol_type = other DNA	
organism = Homo sapiens	
SEQUENCE: 70	
ggtggctcca tcaatagttt ctactggagc	30
SEQ ID NO: 71 moltype = DNA length = 48	
FEATURE Location/Qualifiers	
source 1..48	
mol_type = other DNA	
organism = Homo sapiens	
SEQUENCE: 71	
tatatatctt acagtgaggag caccactac aatccctccc tcaagagt	48
SEQ ID NO: 72 moltype = DNA length = 27	
FEATURE Location/Qualifiers	
source 1..27	
mol_type = other DNA	
organism = Homo sapiens	
SEQUENCE: 72	
gacagtagatag cagccccctt tgactac	27
SEQ ID NO: 73 moltype = AA length = 113	
FEATURE Location/Qualifiers	
source 1..113	
mol_type = protein	
organism = Homo sapiens	
SEQUENCE: 73	
DIVMTQSPDS LAVSLGERAT ITCKSSQSIL YSSNNKKYLV WYQQKPGQPP KLIIYWTSMR 60	
ESGVPDRFSG SGSGTDFTLT INSLQAEDVA VYYCQQYYST PWTFGQGTKV EIK 113	
SEQ ID NO: 74 moltype = AA length = 117	
FEATURE Location/Qualifiers	
source 1..117	
mol_type = protein	
organism = Homo sapiens	
SEQUENCE: 74	
QVQLQESGPQ LVKPSETLSL TCTVSGGSIN SFYWSWIROP PGKGLEWIGY IYYSGSTYN 60	
PSLKSRTVIS VDTSKTQFSL KLSSVTAADT AVYYCARDSI AAPFDYWQGQ TLTVSS 117	
SEQ ID NO: 75 moltype = AA length = 17	
FEATURE Location/Qualifiers	
source 1..17	
mol_type = protein	
organism = Homo sapiens	
SEQUENCE: 75	
KSSQSILYSS NNKKYLV	17
SEQ ID NO: 76 moltype = AA length = 7	
FEATURE Location/Qualifiers	
source 1..7	
mol_type = protein	
organism = Homo sapiens	
SEQUENCE: 76	
WTSMRES	7
SEQ ID NO: 77 moltype = AA length = 9	
FEATURE Location/Qualifiers	
source 1..9	
mol_type = protein	
organism = Homo sapiens	
SEQUENCE: 77	
QQYYSTPWT	9
SEQ ID NO: 78 moltype = AA length = 10	
FEATURE Location/Qualifiers	
source 1..10	
mol_type = protein	
organism = Homo sapiens	
SEQUENCE: 78	
GGSINSFYWS	10
SEQ ID NO: 79 moltype = AA length = 16	
FEATURE Location/Qualifiers	
source 1..16	

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SEQUENCE: 79 YIYYSGSTNY NPSLK5	mol_type = protein organism = Homo sapiens	
		16
SEQ ID NO: 80 FEATURE source	moltype = AA length = 9 Location/Qualifiers 1..9	
	mol_type = protein organism = Homo sapiens	
SEQUENCE: 80 DSIAAPFDY		9
SEQ ID NO: 81 FEATURE source	moltype = DNA length = 321 Location/Qualifiers 1..321	
	mol_type = other DNA organism = Homo sapiens	
SEQUENCE: 81 gacatccaga tgacccagtc tccatcctcc ctgtctgcatt ctgttaggaga cagagtccacc atcaacttgc gggcaagtca gagcatttgc aactattaa attggatata gcagagacca gggaaagccc ctaaaggcttcatctatgtt acatcccgat tgcaaagggg ggtcccatca agggttcagtgc gagttggatc tggacatgtt tcactctca ccatcagcag tctgcaacct gaagatTTT taagttacta ctgtcaacag agttacatgtt ttgcgeccac tttcggccgc gggaccaagg tggagaacaa a	60 120 180 240 300 321	
SEQ ID NO: 82 FEATURE source	moltype = DNA length = 357 Location/Qualifiers 1..357	
	mol_type = other DNA organism = Homo sapiens	
SEQUENCE: 82 caggtgcgc tacagcagtg gggcgcaggaa ctgttgcaggc cttcggagac cctgtccctc acctgcgtc tctatgggg gtccttcgtt gtttactact ggagctggat ccggccggcc ccaggaaagg gactggaggat gatggggaa atcaatata gtggaggcac caactacaac ccgtccctca agatgcgatc cccatata ctagacatgtt ccaagaaacc gtttccctcg aagctgatctt ctgtgaccgc cgcggacacg gctgtgtatt actgtgcgag agtacatgtt ctcgaacttgc ctacttttgc ctactggggc cagggaaacc tggtaaccgtt ctcctca	60 120 180 240 300 357	
SEQ ID NO: 83 FEATURE source	moltype = DNA length = 33 Location/Qualifiers 1..33	
	mol_type = other DNA organism = Homo sapiens	
SEQUENCE: 83 cgggcaagtc agagcattag caactattaa aat		33
SEQ ID NO: 84 FEATURE source	moltype = AA length = 106 Location/Qualifiers 1..106	
	mol_type = protein organism = Homo sapiens	
SEQUENCE: 84 GQPKAAPSVT LFPPSSEELQ ANKATLVCLI SDFYPGAVTV AWKADSSPVK AGVETTPSK QSNNKYAASS YLSLTPEQWK SHRSYSCQVT HEGSTVEKTV APTECS		60 106
SEQ ID NO: 85 FEATURE source	moltype = DNA length = 27 Location/Qualifiers 1..27	
	mol_type = other DNA organism = Homo sapiens	
SEQUENCE: 85 caacagagtt acagtatttc gcccaact		27
SEQ ID NO: 86 FEATURE source	moltype = DNA length = 30 Location/Qualifiers 1..30	
	mol_type = other DNA organism = Homo sapiens	
SEQUENCE: 86 ggtgggtcct tcagtgctta ctactggagc		30
SEQ ID NO: 87 FEATURE source	moltype = DNA length = 48 Location/Qualifiers 1..48	
	mol_type = other DNA organism = Homo sapiens	
SEQUENCE: 87 gaaaatcaatc atagtgaggc caccaactac aaccctgtccc tcaagatgtt		48

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SEQ ID NO: 88      moltype = DNA length = 33
FEATURE
source          Location/Qualifiers
1..33
mol_type = other DNA
organism = Homo sapiens
SEQUENCE: 88
gtacagtggc tcgaactggc ctactttgac tac           33

SEQ ID NO: 89      moltype = AA length = 107
FEATURE
source          Location/Qualifiers
1..107
mol_type = protein
organism = Homo sapiens
SEQUENCE: 89
DIQMTQSPSS LSASVGDRVT ITCRASQSIIS NYLNWYQQRP GKAPKLLIYA TSSLQSGVPS 60
RFSGSGSGTD FTLTISSSLQP EDFVSYYCQQ SYSISPTFGG GTKVENK 107

SEQ ID NO: 90      moltype = AA length = 119
FEATURE
source          Location/Qualifiers
1..119
mol_type = protein
organism = Homo sapiens
SEQUENCE: 90
QVQLQQWGAG LLKPSETLSL TCAVYGGGSFS AYYWSWIRQP PGKGLEWIGE INHSGGTNYN 60
PSSLKSRVTIS VDTSKNQFSL KLSSVTAADT AVYYCARVQW LELAYFDYWG QGTLTVSS 119

SEQ ID NO: 91      moltype = AA length = 11
FEATURE
source          Location/Qualifiers
1..11
mol_type = protein
organism = Homo sapiens
SEQUENCE: 91
RASQSIISNYL N           11

SEQ ID NO: 92      moltype = AA length = 7
FEATURE
source          Location/Qualifiers
1..7
mol_type = protein
organism = Homo sapiens
SEQUENCE: 92
ATSSLQS           7

SEQ ID NO: 93      moltype = AA length = 9
FEATURE
source          Location/Qualifiers
1..9
mol_type = protein
organism = Homo sapiens
SEQUENCE: 93
QQSYSISPT           9

SEQ ID NO: 94      moltype = AA length = 10
FEATURE
source          Location/Qualifiers
1..10
mol_type = protein
organism = Homo sapiens
SEQUENCE: 94
GGSFSAYYWS           10

SEQ ID NO: 95      moltype = AA length = 16
FEATURE
source          Location/Qualifiers
1..16
mol_type = protein
organism = Homo sapiens
SEQUENCE: 95
EINHSGGTNY NPSLKS           16

SEQ ID NO: 96      moltype = AA length = 11
FEATURE
source          Location/Qualifiers
1..11
mol_type = protein
organism = Homo sapiens
SEQUENCE: 96
VQWLLELAYFD Y           11

SEQ ID NO: 97      moltype = DNA length = 321
FEATURE
source          Location/Qualifiers
1..321
mol_type = other DNA

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organism = Homo sapiens

SEQUENCE: 97  
gacatccaga tgacccagtc tccatcctcc ctgtctgcatt ctgttaggaga cagagtacc 60  
atcaacttgc gggcagggtca gggcattaga aatgatttag tctggatca gcagaaacca 120  
ggaaaagccc ctaagccgcct gatctatgtc gcatccagt tgcaaagtgg ggtcccatca 180  
aggttcagcg gcaatggatc tggacagaa ttcaactctca caatcagcag cctgcagcc 240  
gaagatttt caacttatta ctgtctacaa cataatactt acccattcac tttcggccct 300  
ggaccaaaag tggatatcaa a 321

SEQ ID NO: 98                    moltype = DNA length = 363  
FEATURE                          Location/Qualifiers  
source                          1..363  
mol\_type = other DNA  
organism = Homo sapiens

SEQUENCE: 98  
caggtgcagc tgggtggactc tgggggaggc gtgggtccagc ctggggaggc cctgagactc 60  
tctctgtcag cgtctggatt cacccattt agctatggca tgcactgggt ccggcaggct 120  
ccaggcaagg ggctggatgt ggtggcgtt atctggatgt atggaaatgtc tgaataactat 180  
gcagactccg tgaaggcccg attaccatc tccagagaca attccaaagaa cacgctgtat 240  
ctgcaaataatgc acagcctgag agccgaggac acggctgtgtt attactgtgc gagagagagg 300  
cagtggctctt accactacgg tatggacgtc tggggccaag ggaccacggt caccgtctcc 360  
tca 363

SEQ ID NO: 99                    moltype = DNA length = 33  
FEATURE                          Location/Qualifiers  
source                          1..33  
mol\_type = other DNA  
organism = Homo sapiens

SEQUENCE: 99  
cgggcaggc agggcattag aaatgattta gtc 33

SEQ ID NO: 100                 moltype = AA length = 107  
FEATURE                          Location/Qualifiers  
source                          1..107  
mol\_type = protein  
organism = Homo sapiens

SEQUENCE: 100  
RTVAAPSVFI FPPSDEQLKS GTASVVCLLN NFYPREAKVQ WKVDNALQSG NSQESVTEQD 60  
SKDSTYSLSS TLTLSKADYE KHKVYACEVT HQGLSSPVTK SFNRGEC 107

SEQ ID NO: 101                 moltype = DNA length = 27  
FEATURE                          Location/Qualifiers  
source                          1..27  
mol\_type = other DNA  
organism = Homo sapiens

SEQUENCE: 101  
ctacaacata atacttaccc attcaact 27

SEQ ID NO: 102                 moltype = DNA length = 30  
FEATURE                          Location/Qualifiers  
source                          1..30  
mol\_type = other DNA  
organism = Homo sapiens

SEQUENCE: 102  
ggattcacct tcattagcta tggcatgcac 30

SEQ ID NO: 103                 moltype = DNA length = 51  
FEATURE                          Location/Qualifiers  
source                          1..51  
mol\_type = other DNA  
organism = Homo sapiens

SEQUENCE: 103  
gttatcttgtt atgatggaaag tactgaataac tatgcagact ccgtgaaggg c 51

SEQ ID NO: 104                 moltype = DNA length = 36  
FEATURE                          Location/Qualifiers  
source                          1..36  
mol\_type = other DNA  
organism = Homo sapiens

SEQUENCE: 104  
gagaggcagt ggctctacca ctacggatgt gacgtc 36

SEQ ID NO: 105                 moltype = AA length = 107  
FEATURE                          Location/Qualifiers  
source                          1..107  
mol\_type = protein  
organism = Homo sapiens

SEQUENCE: 105  
DIQMTQSPSS LSASVGDRVT ITCRAGQGIR NDLVWYQQKP GKAPKRLIYA ASSLQSGVPS 60

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RFSGSGSGTE FTLTISSLQP EDFATYYCLQ HNTYPFTFGP GTKVDIK	107
SEQ ID NO: 106 moltype = AA length = 121	
FEATURE Location/Qualifiers	
source 1..121	
mol_type = protein	
organism = Homo sapiens	
SEQUENCE: 106	
QVQLVDSGGG VVQPGRLRL SCAASGFTPI SYGMHWRQA PGKGLEWVAV IWYDGSTEY 60	
ADSVKGRFTI SRDVKRNLTY LQMNSLRAED TAVYYCARER QWLYHYGM DV WGQGTTVTS 120	
S 121	
SEQ ID NO: 107 moltype = AA length = 11	
FEATURE Location/Qualifiers	
source 1..11	
mol_type = protein	
organism = Homo sapiens	
SEQUENCE: 107	
RAGQGIRNDL V	11
SEQ ID NO: 108 moltype = AA length = 107	
FEATURE Location/Qualifiers	
source 1..107	
mol_type = protein	
organism = Homo sapiens	
SEQUENCE: 108	
RTVAAPSVFI PPPSDEQLKS GTASVVCLLN NFYPREAKVQ WKVDNALQSG NSQESVTEQD 60	
SKDSTYSLSS TLTLSKADYE KHKVYACEVT HQGLSSPVTK SFNRGEC	107
SEQ ID NO: 109 moltype = AA length = 9	
FEATURE Location/Qualifiers	
source 1..9	
mol_type = protein	
organism = Homo sapiens	
SEQUENCE: 109	
LQHNTYPFT	9
SEQ ID NO: 110 moltype = AA length = 10	
FEATURE Location/Qualifiers	
source 1..10	
mol_type = protein	
organism = Homo sapiens	
SEQUENCE: 110	
GFTFISYGMH	10
SEQ ID NO: 111 moltype = AA length = 17	
FEATURE Location/Qualifiers	
source 1..17	
mol_type = protein	
organism = Homo sapiens	
SEQUENCE: 111	
VIWYDGSTEY YADSVKG	17
SEQ ID NO: 112 moltype = AA length = 12	
FEATURE Location/Qualifiers	
source 1..12	
mol_type = protein	
organism = Homo sapiens	
SEQUENCE: 112	
ERQWLYHYGM DV	12
SEQ ID NO: 113 moltype = DNA length = 339	
FEATURE Location/Qualifiers	
source 1..339	
mol_type = other DNA	
organism = Homo sapiens	
SEQUENCE: 113	
gacatcgtga tgaccaggc tccagactcc ctggctgtgt ctctggccga gaggcccacc 60	
atcacctgca agtccagcca gagttttta tacagctcca acaataaaggaa gtatcttagtt 120	
tggtaccaggc agaaaaccaggc acagoctctt aagttgtatcc ttactggac atctatgcgg 180	
gaatccgggg tccctgaccg attcagtggc agcgggtctg ggacagattt cactctcacc 240	
atcagcagcc tgcaggctga agatgtggca gtttattact gtcagcaata ttatagtact 300	
ccgtggacgt tcggccaagg gaccaagggtg gaaatcaa 339	
SEQ ID NO: 114 moltype = DNA length = 351	
FEATURE Location/Qualifiers	
source 1..351	
mol_type = other DNA	
organism = Homo sapiens	

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SEQUENCE: 114  
cagggtcgc tgcaggagtc gggcccagga ctggtaaagc cctcgagac cctgtccctc 60  
acctgactg tctctgggtt ctccatcaat agtttcat ggagctggat ccggcagccc 120  
ccaggaaagg gactgggtt gattgggtt atctattaca gtgggagcac caactacaat 180  
ccctccctca agaggcgagt caccatatacgttagaacatgtt ccaagaccca gttcccttg 240  
aagctgacgt ctgtgaccgc tgccggacacg gccgtgtatt actgtgcgag agacagtata 300  
gcagccccct ttgactacty gggccaggga accctggta ccgtctccct a 351

SEQ ID NO: 115 moltype = AA length = 17  
FEATURE Location/Qualifiers  
MOD\_RES 1  
note = Arg or Lys  
MOD\_RES 6  
note = Leu or Ile  
MOD\_RES 8  
note = His or Tyr  
MOD\_RES 10  
note = Thr or Ser  
MOD\_RES 11  
note = Gly or Asn  
MOD\_RES 12  
note = Tyr or Asn  
MOD\_RES 13  
note = Asn or Lys  
MOD\_RES 14  
note = May or may not be present  
MOD\_RES 17  
note = Asp or Val  
source 1..17  
mol\_type = protein  
organism = Homo sapiens

SEQUENCE: 115  
XSSQSXLXSX XXXKYLX 17

SEQ ID NO: 116 moltype = AA length = 11  
FEATURE Location/Qualifiers  
MOD\_RES 3  
note = Ser or Gly  
MOD\_RES 5  
note = Ser or Gly  
MOD\_RES 7  
note = Ser or Arg  
MOD\_RES 9  
note = Tyr, Asp or Asn  
MOD\_RES 11  
note = Asp, Val or Gly  
source 1..11  
mol\_type = protein  
organism = Homo sapiens

SEQUENCE: 116  
RAXQXIXNXL X 11

SEQ ID NO: 117 moltype = DNA length = 27  
FEATURE Location/Qualifiers  
source 1..27  
mol\_type = other DNA  
organism = Homo sapiens

SEQUENCE: 117  
cagcaattt atagtaatcc gtggacg 27

SEQ ID NO: 118 moltype = DNA length = 30  
FEATURE Location/Qualifiers  
source 1..30  
mol\_type = other DNA  
organism = Homo sapiens

SEQUENCE: 118  
ggtggtccca tcaatagttt ctactggagc 30

SEQ ID NO: 119 moltype = DNA length = 48  
FEATURE Location/Qualifiers  
source 1..48  
mol\_type = other DNA  
organism = Homo sapiens

SEQUENCE: 119  
tatatctatt acagtgggag caccaactac aatccctccc tcaagagg 48

SEQ ID NO: 120 moltype = DNA length = 27  
FEATURE Location/Qualifiers  
source 1..27

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mol_type = other DNA
organism = Homo sapiens
SEQUENCE: 120
gacagtatag cagccccctt tgactac                                27

SEQ ID NO: 121      moltype = AA length = 113
FEATURE          Location/Qualifiers
source           1..113
mol_type = protein
organism = Homo sapiens

SEQUENCE: 121
DIVMTQSPDS LAVSLGERAT ITCKSSQSIL YSSNNKKYLV WYQQKPGQPP KLIIYWTSMR 60
ESGVPDRFSG SGSQTDFTLT ISSLQAEDVA VYYCQQYYST PWTFGQGTKV EIK        113

SEQ ID NO: 122      moltype = AA length = 117
FEATURE          Location/Qualifiers
source           1..117
mol_type = protein
organism = Homo sapiens

SEQUENCE: 122
QVQLQESPGV LVKPSETLSL TCTVSGGSIN SFYWSWIRQP PGKGLEWIGY IYYSGSTYN 60
PSLKRRVTIS VDTSKTQFSL KLSSVTAADT AVYYCARDSI AAPFDYWQGQ TLTVSS        117

SEQ ID NO: 123      moltype = AA length = 11
FEATURE          Location/Qualifiers
MOD_RES          3
note = Glu or Asp
MOD_RES          5
note = Trp or Leu
MOD_RES          7
note = Glu or Asp
MOD_RES          9
note = Tyr or Phe
MOD_RES          10
note = Ala or Val
MOD_RES          11
note = Cys or Phe
source           1..11
mol_type = protein
organism = Homo sapiens

SEQUENCE: 123
SGXKXGXKXX X                                11

SEQ ID NO: 124      moltype = length =
SEQUENCE: 124
000

SEQ ID NO: 125      moltype = AA length = 9
FEATURE          Location/Qualifiers
source           1..9
mol_type = protein
organism = Homo sapiens

SEQUENCE: 125
QQYYSTPWT                                9

SEQ ID NO: 126      moltype = AA length = 10
FEATURE          Location/Qualifiers
source           1..10
mol_type = protein
organism = Homo sapiens

SEQUENCE: 126
GGSINSFYWS                                10

SEQ ID NO: 127      moltype = AA length = 16
FEATURE          Location/Qualifiers
source           1..16
mol_type = protein
organism = Homo sapiens

SEQUENCE: 127
YIYYSGSTNY NPSLKR                                16

SEQ ID NO: 128      moltype = AA length = 7
FEATURE          Location/Qualifiers
MOD_RES          1
note = Gln, Leu or His
MOD_RES          3
note = Thr, Asn or Ser
source           1..7
mol_type = protein

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organism = Homo sapiens
SEQUENCE: 128
XDXKRPS                                         7

SEQ ID NO: 129      moltype = DNA  length = 321
FEATURE          Location/Qualifiers
source           1..321
                 mol_type = other DNA
                 organism = Homo sapiens

SEQUENCE: 129
gacatccaga tgacctcagtc tccatcctcc ctgtctgcatt ctgttaggaga cagagtacc 60
atcaacttgc gggcaagtca gggcattaga aataatttag gctggatca gcagaaacca 120
ggaaaaggcc ctaagcgccct gatttatgtc gcatccagt tgcaaagtgg ggtccccatca 180
aggttcagcg cgactggatc tggcacaga ttcaacttcata caatcagcag cctgcagcct 240
gaagattttca caacttata ctgtctacag cataatagtt acccgtggac gttcggccaa 300
gggaccaagg tggaaatcaa a                                         321

SEQ ID NO: 130      moltype = DNA  length = 372
FEATURE          Location/Qualifiers
source           1..372
                 mol_type = other DNA
                 organism = Homo sapiens

SEQUENCE: 130
cagggtgcagc tgggtggatc tgggggaggc gtgggtccagc ctggggaggc cctgagactc 60
tcctgtcgag cgtctggatt cacccatgt agttacggca tgcactgggt ccggcaggct 120
ccaggcaagg ggctggatgt ggtggcaggat atatggatgt atggaaatggaa taaataccat 180
gcagactccg tgaaggggccg attaccatc tccagagaca attccaagaa cacgtgtat 240
ctgcaagtga acagcctgag agccgaggac acggctgtgtt attactgtgt gagaagtccg 300
aactgaaact acgacaacta ctactacgtt ctggacgtctt ggggccaagg gaccacggc 360
acgggtctca                                         372

SEQ ID NO: 131      moltype = AA   length = 9
FEATURE          Location/Qualifiers
MOD_RES          5
                 note = Thr or Ser
MOD_RES          7
                 note = Pro or Thr
MOD_RES          8
                 note = Phe or Trp
source           1..9
                 mol_type = protein
                 organism = Homo sapiens

SEQUENCE: 131
LQHNXYXXT                                         9

SEQ ID NO: 132      moltype =     length =
SEQUENCE: 132
000

SEQ ID NO: 133      moltype = DNA  length = 27
FEATURE          Location/Qualifiers
source           1..27
                 mol_type = other DNA
                 organism = Homo sapiens

SEQUENCE: 133
ctacagcata atagttaccc gtggacg                                         27

SEQ ID NO: 134      moltype = AA   length = 11
FEATURE          Location/Qualifiers
MOD_RES          4
                 note = Ile or Phe
MOD_RES          5
                 note = Asn or Ser
MOD_RES          6
                 note = Ser or Ala
MOD_RES          7
                 note = Gly or absent
MOD_RES          8
                 note = Gly or absent
MOD_RES          9
                 note = Phe or Tyr
source           1..11
                 mol_type = protein
                 organism = Homo sapiens

SEQUENCE: 134
GGSXXXXXXW                                         11

SEQ ID NO: 135      moltype = DNA  length = 51
FEATURE          Location/Qualifiers

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source          1..51
               mol_type = other DNA
               organism = Homo sapiens
SEQUENCE: 135
tttatatgtt atgatggaaag taataaatac catgcagact ccgtgaagg c      51

SEQ ID NO: 136      moltype = DNA length = 45
FEATURE          Location/Qualifiers
source           1..45
               mol_type = other DNA
               organism = Homo sapiens
SEQUENCE: 136
agtccgaaact ggaactacga caactactac tacggctcg acgtc      45

SEQ ID NO: 137      moltype = AA length = 107
FEATURE          Location/Qualifiers
source           1..107
               mol_type = protein
               organism = Homo sapiens
SEQUENCE: 137
DIQMTQSPSS LSASVGDRVT ITCRASQGIR NNLGWYQQKP GKAPKRLIYA ASSLQSGVPS 60
RFSGSGSGTE FTLTISSSLQP EDFTTYCQLQ HNSYPWTFGQ GTKVEIK      107

SEQ ID NO: 138      moltype = AA length = 124
FEATURE          Location/Qualifiers
source           1..124
               mol_type = protein
               organism = Homo sapiens
SEQUENCE: 138
QVQLVESGGG VVQPGRSRL SCAASGFTFS SYGMHWVRQA PGKGLEWVAV IWYDGSNKYH 60
ADSVVKGRFTI SRDNSKNLTY LQVNSLRAED TAVYYCVRSR NWNYDNYYYG LDVWGQGTTV 120
TVSS                           124

SEQ ID NO: 139      moltype = length =
SEQUENCE: 139
000

SEQ ID NO: 140      moltype = AA length = 10
FEATURE          Location/Qualifiers
MOD_RES          2
               note = Tyr or Phe
MOD_RES          5
               note = Thr or Ser
MOD_RES          6
               note = Ser or Ala
MOD_RES          8
               note = Gly or Trp
MOD_RES          9
               note = Leu, Met or Ile
MOD_RES          10
               note = Ser or His
source           1..10
               mol_type = protein
               organism = Homo sapiens
SEQUENCE: 140
GXTFXXX 10

SEQ ID NO: 141      moltype = AA length = 9
FEATURE          Location/Qualifiers
source           1..9
               mol_type = protein
               organism = Homo sapiens
SEQUENCE: 141
LQHNSYPWT      9

SEQ ID NO: 142      moltype = AA length = 16
FEATURE          Location/Qualifiers
MOD_RES          1
               note = Tyr or Glu
MOD_RES          3
               note = Ser, Tyr or Asn
MOD_RES          4
               note = Tyr or His
MOD_RES          7
               note = Ser or Gly
MOD_RES          9
               note = Tyr or Asn
MOD_RES          16
               note = Ser or Arg

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source	1..16	
	mol_type = protein	
	organism = Homo sapiens	
SEQUENCE: 142		
XIXXSGXTXY NPSLKX		16
SEQ ID NO: 143	moltype = AA length = 17	
FEATURE	Location/Qualifiers	
source	1..17	
	mol_type = protein	
	organism = Homo sapiens	
SEQUENCE: 143		
VIWYDGNSNKY HADSVKG		17
SEQ ID NO: 144	moltype = AA length = 15	
FEATURE	Location/Qualifiers	
source	1..15	
	mol_type = protein	
	organism = Homo sapiens	
SEQUENCE: 144		
SRNWNYDNYY YGLDV		15
SEQ ID NO: 145	moltype = DNA length = 315	
FEATURE	Location/Qualifiers	
source	1..315	
	mol_type = other DNA	
	organism = Homo sapiens	
SEQUENCE: 145		
tccatgagc tgactcagcc accctcagtg tccgtgtccc caggacagac agccagcatc	60	
acctgcctcg gagaaaaatg gggagagaaa tatgtttgtt ggtatcagca gaagccaggc	120	
cagtccccctg tgctggatcat ctatcaagat accaagcggc cctccggat ccctgagcga	180	
tttcctggctt ccattttctgg gaacacagcc actctgacca tcagcgggac ccaggctatg	240	
gatgagggtg actattatttgc tcaggcgtgg gacaggagca ctgtattcgg cggaggacc	300	
aagctgaccc tccta	315	
SEQ ID NO: 146	moltype = DNA length = 348	
FEATURE	Location/Qualifiers	
source	1..348	
	mol_type = other DNA	
	organism = Homo sapiens	
SEQUENCE: 146		
gagggtcagtc tggtgca gtcggacagag gtgaaaaaagc cggggggatc tctgaagatc	60	
tccctgtcagg gttctggata cagcttacc agctactggaa tcggctgggt gcgccagatg	120	
ccccggaaag gctgtggatg gatggggatc atctatctggatgtgactctga taccagatac	180	
agcccgctct tccaaggccca ggtcaccatc tcagccgacaa agtccatcag caccgcctac	240	
cttcaggcgtggaa gcagcctgaa ggcctcgac accggcatgtt attactgtgc gagacaagga	300	
ctgggggttgc actactggggcc ccaggaaacc ctggtcaccgc tctctca	348	
SEQ ID NO: 147	moltype = DNA length = 33	
FEATURE	Location/Qualifiers	
source	1..33	
	mol_type = other DNA	
	organism = Homo sapiens	
SEQUENCE: 147		
tctggagaaa aatggggaga gaaatatgtcttgt	33	
SEQ ID NO: 148	moltype = DNA length = 21	
FEATURE	Location/Qualifiers	
source	1..21	
	mol_type = other DNA	
	organism = Homo sapiens	
SEQUENCE: 148		
caagataccca agcggccctc c	21	
SEQ ID NO: 149	moltype = DNA length = 24	
FEATURE	Location/Qualifiers	
source	1..24	
	mol_type = other DNA	
	organism = Homo sapiens	
SEQUENCE: 149		
caggcgtggg acaggagcacatgt	24	
SEQ ID NO: 150	moltype = DNA length = 30	
FEATURE	Location/Qualifiers	
source	1..30	
	mol_type = other DNA	
	organism = Homo sapiens	
SEQUENCE: 150		
ggatacagct ttaccagcta ctggatcgcc	30	

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SEQ ID NO: 151      moltype = DNA length = 51
FEATURE
source
1..51
mol_type = other DNA
organism = Homo sapiens
SEQUENCE: 151
atcatctatc ctggtgactc tgataccaga tacagcccg c          51

SEQ ID NO: 152      moltype = DNA length = 21
FEATURE
source
1..21
mol_type = other DNA
organism = Homo sapiens
SEQUENCE: 152
caaggactgg ggtttgacta c                                21

SEQ ID NO: 153      moltype = AA length = 105
FEATURE
source
1..105
mol_type = protein
organism = Homo sapiens
SEQUENCE: 153
SYELTQPPSV SVSPGQTASI TCSGEKWGEK YACWYQQKPG QSPVLVIYQD TKRPSGIPER 60
FSGSISGNTA TLTISGTQAM DEADYYCQAW DRSTVFGGGT KLTVL          105

SEQ ID NO: 154      moltype = AA length = 116
FEATURE
source
1..116
mol_type = protein
organism = Homo sapiens
SEQUENCE: 154
EVQLVQSGAE VKKPGESLKI SCQGSGYSFT SYWIGWVRQM PGKGLEWMGI IYPGDSDTRY 60
SPSFQGQVTI SADKSISTAY LQWSSLKASD TAMYYCARQG LGFDYWGQGT LTVSS          116

SEQ ID NO: 155      moltype = AA length = 11
FEATURE
source
1..11
mol_type = protein
organism = Homo sapiens
SEQUENCE: 155
SGEKWGEKYA C                                11

SEQ ID NO: 156      moltype = AA length = 7
FEATURE
source
1..7
mol_type = protein
organism = Homo sapiens
SEQUENCE: 156
QDTKRPS                                         7

SEQ ID NO: 157      moltype = AA length = 8
FEATURE
source
1..8
mol_type = protein
organism = Homo sapiens
SEQUENCE: 157
QAWDRSTV                                         8

SEQ ID NO: 158      moltype = AA length = 10
FEATURE
source
1..10
mol_type = protein
organism = Homo sapiens
SEQUENCE: 158
GYSFTSYWIG                                         10

SEQ ID NO: 159      moltype = AA length = 17
FEATURE
source
1..17
mol_type = protein
organism = Homo sapiens
SEQUENCE: 159
IIYPGDSDTR YSPSFQG                                         17

SEQ ID NO: 160      moltype = AA length = 7
FEATURE
source
1..7
mol_type = protein

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organism = Homo sapiens  
 SEQUENCE: 160  
 QGLGF DY 7

SEQ ID NO: 161 moltype = DNA length = 318  
 FEATURE Location/Qualifiers  
 source 1..318  
 mol\_type = other DNA  
 organism = Homo sapiens

SEQUENCE: 161  
 tcctatgac tgactcagcc accctcagtgc tccgtgtccc caggacagac agccagcatc 60  
 acctgcctcg gagataaaa gggggataaa ttgttctt ggtatcagct gaagccaggc 120  
 cagtccccctg tgctggctat ctatcaagat aacaageggc cctcaggat ccctgagcga 180  
 ttctctggct ccaactctgg gaacacagcc actctgacca tcagcgggac ccaggctatg 240  
 gatgcggctg atctttactg tcaggcgtgg gacagcagca ctgtggatt cggcggaggg 300  
 accaagctga ccgtccta 318

SEQ ID NO: 162 moltype = DNA length = 363  
 FEATURE Location/Qualifiers  
 source 1..363  
 mol\_type = other DNA  
 organism = Homo sapiens

SEQUENCE: 162  
 caggtgcagc tgcaggaggc gggcccgaga ctgggtgaagc cttcacagac cctgtccctc 60  
 acctgcactg tctctggctt ctccatcago agtgtgggtt actactggag ctggatccgc 120  
 cagcaccctcg ggaaggccct ggagtggat ggttacatctt cttacagttt gggcacctac 180  
 tacaacccgtt ccctcaagag tcgagttacc atatcagttt acacgtttaa gaaccaggtc 240  
 tccctgaagc tgaactctgt gactgccgac gacacggccg tggattactg tgcgcgcgt 300  
 tacgggtact atcgcggctg ttgcaccccc tggggccagg gaaccttgtt caccgtctcc 360  
 tca 363

SEQ ID NO: 163 moltype = DNA length = 33  
 FEATURE Location/Qualifiers  
 source 1..33  
 mol\_type = other DNA  
 organism = Homo sapiens

SEQUENCE: 163  
 tctggagata aattggggga taaatttgc ttc 33

SEQ ID NO: 164 moltype = DNA length = 21  
 FEATURE Location/Qualifiers  
 source 1..21  
 mol\_type = other DNA  
 organism = Homo sapiens

SEQUENCE: 164  
 caagataaca agcggccctc a 21

SEQ ID NO: 165 moltype = DNA length = 27  
 FEATURE Location/Qualifiers  
 source 1..27  
 mol\_type = other DNA  
 organism = Homo sapiens

SEQUENCE: 165  
 caggcgtggg acagcagcac tgggtt 27

SEQ ID NO: 166 moltype = DNA length = 36  
 FEATURE Location/Qualifiers  
 source 1..36  
 mol\_type = other DNA  
 organism = Homo sapiens

SEQUENCE: 166  
 ggtggctcca tcagcagttgg tggttactac tggagc 36

SEQ ID NO: 167 moltype = DNA length = 48  
 FEATURE Location/Qualifiers  
 source 1..48  
 mol\_type = other DNA  
 organism = Homo sapiens

SEQUENCE: 167  
 tacatcttcc acagtgggag cacctactac aacccgtccc tcaagagt 48

SEQ ID NO: 168 moltype = DNA length = 33  
 FEATURE Location/Qualifiers  
 source 1..33  
 mol\_type = other DNA  
 organism = Homo sapiens

SEQUENCE: 168  
 gcttacggtg actatcgcgg ctggttcgac ccc 33

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SEQ ID NO: 169            moltype = AA length = 106  
 FEATURE                Location/Qualifiers  
 source                1..106  
 mol\_type = protein  
 organism = Homo sapiens

SEQUENCE: 169  
 SYELTQPPSV SVSPGQTASI TCSGDKLGDK FAFWYQLKPG QSPVLVIYQD NKRPSGIPER 60  
 FSGSNSGNTA TLTISGTQAM DAADFYCQAW DSSTVVFGGG TKLTVL 106

SEQ ID NO: 170            moltype = AA length = 121  
 FEATURE                Location/Qualifiers  
 source                1..121  
 mol\_type = protein  
 organism = Homo sapiens

SEQUENCE: 170  
 QVQLQESPGV LVKPSQTL SL TCTVSGGSIS SGYYYWSWIR QHPGKGLEWI GYISYSGSTY 60  
 YNPSLKSRTV ISVDTSKNQF SLKLNSVTAA DTAVYYCARA YGDYRGWFDP WGQGTLVTVS 120  
 S 121

SEQ ID NO: 171            moltype = AA length = 11  
 FEATURE                Location/Qualifiers  
 source                1..11  
 mol\_type = protein  
 organism = Homo sapiens

SEQUENCE: 171  
 SGDKLGDKFA F 11

SEQ ID NO: 172            moltype = AA length = 7  
 FEATURE                Location/Qualifiers  
 source                1..7  
 mol\_type = protein  
 organism = Homo sapiens

SEQUENCE: 172  
 QDNKRPS 7

SEQ ID NO: 173            moltype = AA length = 9  
 FEATURE                Location/Qualifiers  
 source                1..9  
 mol\_type = protein  
 organism = Homo sapiens

SEQUENCE: 173  
 QAWDSSTVV 9

SEQ ID NO: 174            moltype = AA length = 12  
 FEATURE                Location/Qualifiers  
 source                1..12  
 mol\_type = protein  
 organism = Homo sapiens

SEQUENCE: 174  
 GGSISSSGGYY WS 12

SEQ ID NO: 175            moltype = AA length = 16  
 FEATURE                Location/Qualifiers  
 source                1..16  
 mol\_type = protein  
 organism = Homo sapiens

SEQUENCE: 175  
 YISYSGSTYY NPSLKS 16

SEQ ID NO: 176            moltype = AA length = 11  
 FEATURE                Location/Qualifiers  
 source                1..11  
 mol\_type = protein  
 organism = Homo sapiens

SEQUENCE: 176  
 AYGDYRGWFD P 11

SEQ ID NO: 177            moltype = DNA length = 321  
 FEATURE                Location/Qualifiers  
 source                1..321  
 mol\_type = other DNA  
 organism = Homo sapiens

SEQUENCE: 177  
 gacatccaga tgaccaggc tccatcctcc ctgtctgcat ctgttaggaga cagagtccacc 60  
 atcaacttgcc gggcaagtca gggcattaga aatgatttag gctggatca gcagaaacca 120  
 gggaaagccc ctaagcgccct gatctatgcg gcatccagg tgcaaagtgg ggtccatca 180  
 aggttcagcg gcagtggatc tgggacacaa ttcaactctca caatcagcag cctgcaggct 240  
 gaagattgtc caacttatta ttgtctacag cataatagtt atacgtggac gttcggccaa 300  
 gggaccaagg tggaaatcaa a 321

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SEQ ID NO: 178      moltype = DNA length = 372
FEATURE          Location/Qualifiers
source           1..372
                  mol_type = other DNA
                  organism = Homo sapiens
SEQUENCE: 178
cagggtgcagc tgggtggagtccgggggaggtc gtgggtccagc ctggggagggtc cctgagactc 60
tcttgtgttag cgtagtggatt cacccatcggt gccttatggca tgcactgggt ccggccagggt 120
ccaggcaagg ggctggatgtt atatggatgtt atggaaagttaaataactat 180
gcagactccg tgaaggggccg attcatcato tccagagaca attccaagaa cacgctgtat 240
ctgtcaaatgtt acagcctgag agccgaggac acggctgtgtt attactgtgc gagaagtcgg 300
aacttggaaactt accgactccta ccaatacggt ttggacgtctt ggggccaagg gaccacggc 360
accgtctctt ca                                         372

SEQ ID NO: 179      moltype = AA length = 17
FEATURE          Location/Qualifiers
MOD_RES          1
                  note = Asn or Val
MOD_RES          3
                  note = Trp or Lys
MOD_RES          4
                  note = Tyr or Gln
MOD_RES          8
                  note = Asn, Glu or Ser
MOD_RES          9
                  note = Lys or Glu
MOD_RES          11
                  note = His or Tyr
MOD_RES          12
                  note = Ala or Val
source           1..17
                  mol_type = protein
                  organism = Homo sapiens
SEQUENCE: 179
XIXXDGSXXY XXDSVKG                                         17

SEQ ID NO: 180      moltype = AA length = 17
FEATURE          Location/Qualifiers
MOD_RES          1
                  note = Trp or Ile
MOD_RES          3
                  note = Asn, Ile, Ser or Tyr
MOD_RES          4
                  note = Pro or Ala
MOD_RES          5
                  note = Asn, Tyr or Gly
MOD_RES          6
                  note = Ser, Asn or Asp
MOD_RES          7
                  note = Gly or Ser
MOD_RES          8
                  note = Gly, Asn or Asp
MOD_RES          10
                  note = Asn or Arg
MOD_RES          11
                  note = Tyr or Ser
MOD_RES          12
                  note = Ala or Ser
MOD_RES          13
                  note = Gln or Pro
MOD_RES          14
                  note = Lys or Ser
MOD_RES          15
                  note = Phe or Leu
source           1..17
                  mol_type = protein
                  organism = Homo sapiens
SEQUENCE: 180
XIXXXXXXXTX XXXXXQG                                         17

SEQ ID NO: 181      moltype = DNA length = 27
FEATURE          Location/Qualifiers
source           1..27
                  mol_type = other DNA
                  organism = Homo sapiens
SEQUENCE: 181
ctacagcata atagttatac gtggacg                                         27

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SEQ ID NO: 182 moltype = DNA length = 30  
 FEATURE Location/Qualifiers  
 source 1..30  
 mol\_type = other DNA  
 organism = Homo sapiens

SEQUENCE: 182 ggattcacct tcagtgcccta tggcatgcac 30

SEQ ID NO: 183 moltype = DNA length = 51  
 FEATURE Location/Qualifiers  
 source 1..51  
 mol\_type = other DNA  
 organism = Homo sapiens

SEQUENCE: 183 gttatatgtt atgatggaag taataatac tatgcagact ccgtgaaggg c 51

SEQ ID NO: 184 moltype = DNA length = 45  
 FEATURE Location/Qualifiers  
 source 1..45  
 mol\_type = other DNA  
 organism = Homo sapiens

SEQUENCE: 184 agtcgaaact ggaactacga ctcctaccaa tacggtttg acgtc 45

SEQ ID NO: 185 moltype = AA length = 107  
 FEATURE Location/Qualifiers  
 source 1..107  
 mol\_type = protein  
 organism = Homo sapiens

SEQUENCE: 185 DIQMTQSPSS LSASVGDRVT ITCRASQGIR NDLGWYQQKP GKAPKRLIYA ASSLQSGVPS 60  
 RFSGSGSGTE FTLTISLQP EDCATYYCLQ HNSYTWTFGQ GTKVEIK 107

SEQ ID NO: 186 moltype = AA length = 124  
 FEATURE Location/Qualifiers  
 source 1..124  
 mol\_type = protein  
 organism = Homo sapiens

SEQUENCE: 186 QVQLVESGGG VVQPGRLSLRL SCVASGFTFS AYGMHWVRQA PGKGLEWVAW IWYDGSNKYY 60  
 ADSVKGRFII SRDNSKNLY LQMNSLRAED TAVYYCARSR NWNYDSYQYG LDVWGQGTTV 120  
 TVSS 124

SEQ ID NO: 187 moltype = AA length = 11  
 FEATURE Location/Qualifiers  
 MOD\_RES 1..2  
 note = May or may not be present  
 MOD\_RES 3  
 note = Asp, Trp or absent  
 MOD\_RES 4  
 note = Ser, Leu or absent  
 MOD\_RES 5  
 note = Ile, Glu or Gln  
 MOD\_RES 6  
 note = Ala, Leu or Gly  
 MOD\_RES 7  
 note = Ala or Leu  
 MOD\_RES 8  
 note = Pro, Tyr or Gly  
 source 1..11  
 mol\_type = protein  
 organism = Homo sapiens

SEQUENCE: 187 VQXXXXXXFD Y 11

SEQ ID NO: 188 moltype = AA length = 13  
 FEATURE Location/Qualifiers  
 MOD\_RES 1..2  
 note = May or may not be present  
 MOD\_RES 3  
 note = Asp or Ala  
 MOD\_RES 5  
 note = Tyr or Gly  
 MOD\_RES 7  
 note = Ser or Tyr  
 MOD\_RES 8  
 note = Ser or Arg  
 MOD\_RES 11  
 note = May or may not be present

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MOD_RES	12 note = Gly or Asp	
MOD_RES	13 note = His or Pro	
source	1..13 mol_type = protein organism = Homo sapiens	
SEQUENCE: 188	DQXYXDXXXGW FXX	13
SEQ ID NO: 189	moltype = AA length = 9	
FEATURE	Location/Qualifiers	
source	1..9 mol_type = protein organism = Homo sapiens	
SEQUENCE: 189	LQHNSYTWT	9
SEQ ID NO: 190	moltype = AA length = 10	
FEATURE	Location/Qualifiers	
source	1..10 mol_type = protein organism = Homo sapiens	
SEQUENCE: 190	GFTFSAYGMH	10
SEQ ID NO: 191	moltype = AA length = 17	
FEATURE	Location/Qualifiers	
source	1..17 mol_type = protein organism = Homo sapiens	
SEQUENCE: 191	VIWYDGDSNKY YADSVKG	17
SEQ ID NO: 192	moltype = AA length = 15	
FEATURE	Location/Qualifiers	
source	1..15 mol_type = protein organism = Homo sapiens	
SEQUENCE: 192	SRNWNYDSYQ YGLDV	15
SEQ ID NO: 193	moltype = DNA length = 315	
FEATURE	Location/Qualifiers	
source	1..315 mol_type = other DNA organism = Homo sapiens	
SEQUENCE: 193	tcctatgagc tgactcagcc accctcagtg tccgtgtccc caggacagac agccagcatc acctgcctg gagataaaatt gggggataaa tatgttttgtt ggttatcgaca gaagccaggc cagtccctgt aactggtcat ctatctatgaa aacaaggccg cctcaggat ccctgagcga tttcttgctt ccaactctgg gaacacagcc actctgacca tcagcgggac ccaggctatg gatgaggctg actattactg tcaggcgtgg gacagcaga cggatttcgg cggagggacc aaaactgaccg tcctg	60 120 180 240 300 315
SEQ ID NO: 194	moltype = DNA length = 363	
FEATURE	Location/Qualifiers	
source	1..363 mol_type = other DNA organism = Homo sapiens	
SEQUENCE: 194	cagtttcagc ttgttcagtc tggagctgag gtgttgcggc ctggggcctc agtgaaggc tcctgcagg ctctggta caccttacc agctatggta tcacgtgggt gcgcacaggcc cctggacaaagg ggcttcggat gatggatgg atcagcgtt cacaatgtt cacaactat gcacagaagt tccaggccggc agtccaccatg accacagacaca catcaacgac cacagctac atggagctga ggagccgtgg atctgcacgc acggccgtgtt attactgtgc gagagatcaa gattactatg atatgtatgg ttggggccac ttggggccagg gaacctgtt caccgtctcc tca	60 120 180 240 300 360 363
SEQ ID NO: 195	moltype = DNA length = 33	
FEATURE	Location/Qualifiers	
source	1..33 mol_type = other DNA organism = Homo sapiens	
SEQUENCE: 195	tctggagata aattggggga taaatatgtt tgt	33
SEQ ID NO: 196	moltype = DNA length = 21	
FEATURE	Location/Qualifiers	

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179

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source          1..21
               mol_type = other DNA
               organism = Homo sapiens
SEQUENCE: 196
ctagataaca agcgccctc a                                21

SEQ ID NO: 197      moltype = DNA  length = 24
FEATURE           Location/Qualifiers
source            1..24
               mol_type = other DNA
               organism = Homo sapiens
SEQUENCE: 197
caggcgtggg acagcagcac ggta                            24

SEQ ID NO: 198      moltype = DNA  length = 30
FEATURE           Location/Qualifiers
source            1..30
               mol_type = other DNA
               organism = Homo sapiens
SEQUENCE: 198
ggttacacct ttaccagcta tggtatcago                      30

SEQ ID NO: 199      moltype = DNA  length = 51
FEATURE           Location/Qualifiers
source            1..51
               mol_type = other DNA
               organism = Homo sapiens
SEQUENCE: 199
tggatcagcg cttacaatgg taacacaaac tatgcacaga agttccaggg c    51

SEQ ID NO: 200      moltype = DNA  length = 36
FEATURE           Location/Qualifiers
source            1..36
               mol_type = other DNA
               organism = Homo sapiens
SEQUENCE: 200
gatcaagatt actatgatag tagtggttgg ggccac                      36

SEQ ID NO: 201      moltype = AA   length = 105
FEATURE           Location/Qualifiers
source            1..105
               mol_type = protein
               organism = Homo sapiens
SEQUENCE: 201
SYELTQPPSV SVSPGQTASI TCSGDKLGDK YVCWYQQKPG QSPELVIYLD NKRPSGIPER 60
FSGSNNSGNTA TLTISGTQAM DEADYYCQAW DSSTVFGGGT KLTVL                         105

SEQ ID NO: 202      moltype = AA   length = 121
FEATURE           Location/Qualifiers
source            1..121
               mol_type = protein
               organism = Homo sapiens
SEQUENCE: 202
QVOLVQSGAE VKKPGASVKV SCKASGYTFT SYGISWVRQA PGQGLERMGW ISAYNGNTNY 60
AQKFQGRVTM TTDTSSTTAY MELRSLRSDD TAVYYCARDQ DYYDSSGWGH WGQGTLVTVS 120
S                                         121

SEQ ID NO: 203      moltype = AA   length = 11
FEATURE           Location/Qualifiers
source            1..11
               mol_type = protein
               organism = Homo sapiens
SEQUENCE: 203
SGDKLGDKYV C                                              11

SEQ ID NO: 204      moltype = AA   length = 7
FEATURE           Location/Qualifiers
source            1..7
               mol_type = protein
               organism = Homo sapiens
SEQUENCE: 204
LDNKRPS                                                 7

SEQ ID NO: 205      moltype = AA   length = 8
FEATURE           Location/Qualifiers
source            1..8
               mol_type = protein
               organism = Homo sapiens
SEQUENCE: 205

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QAWDSSTV

8

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SEQ ID NO: 206      moltype = AA  length = 10
FEATURE
source          Location/Qualifiers
1..10
mol_type = protein
organism = Homo sapiens
SEQUENCE: 206
GYTFTSYGIS

SEQ ID NO: 207      moltype = AA  length = 17
FEATURE
source          Location/Qualifiers
1..17
mol_type = protein
organism = Homo sapiens
SEQUENCE: 207
WISAYNGNTN YAQKFQG

SEQ ID NO: 208      moltype = AA  length = 12
FEATURE
source          Location/Qualifiers
1..12
mol_type = protein
organism = Homo sapiens
SEQUENCE: 208
DQDYDSSGW GH

SEQ ID NO: 209      moltype = DNA  length = 316
FEATURE
source          Location/Qualifiers
1..316
mol_type = other DNA
organism = Homo sapiens
SEQUENCE: 209
tccttatgagc tgactcagcc accctcagtg tccgtgtccc caggacagac agcctccatc 60
acctgcctg gagataaaatt gggggataaa tatgctttctt ggtatcagca gaagccaggc 120
cagtcccttg tgcgtggctt ctatcatg accaaggccg cctcaggat ccctgagcga 180
ttctctggct ccaactctgg gaacacagcc actctgacca tcagcgggac ccaggctatg 240
gatgaggctg actatcactg tcaggcgtgg gacagcaga cggcttcgg cggaggacc 300
aagctgacc tcctac 316

SEQ ID NO: 210      moltype = DNA  length = 363
FEATURE
source          Location/Qualifiers
1..363
mol_type = other DNA
organism = Homo sapiens
SEQUENCE: 210
caagttcagc ttgttcaatc tggactgag gtgtaaagaac ctggggcctc agtgaaggc 60
tccttcaaga ctcttgttaa caccatggatc agctatggt ctacgtgggt gcgacaggcc 120
cctggacaag ggcttgagtg gatggatgg atcagccctt acaatggtaa cacaactat 180
gcacagaagt tccaggccag agtcaccatg accacagaca aatccacgag cacagctac 240
atggagctga ggagccctgcg atctgacac acggccgtgtt attactgtgc gagagatcaa 300
gattactatg atatgtatgg ttgggacccc ttggggccagg gaaccctgtt caccgtctcc 360
tcg 363

SEQ ID NO: 211      moltype = DNA  length = 33
FEATURE
source          Location/Qualifiers
1..33
mol_type = other DNA
organism = Homo sapiens
SEQUENCE: 211
tctggagata aattggggga taaaatatgtc ttc 33

SEQ ID NO: 212      moltype = DNA  length = 21
FEATURE
source          Location/Qualifiers
1..21
mol_type = other DNA
organism = Homo sapiens
SEQUENCE: 212
catgataccca agcggccctc a 21

SEQ ID NO: 213      moltype = DNA  length = 360
FEATURE
misc_feature    Location/Qualifiers
1..360
note = Description of Artificial Sequence: Synthetic
       activin A/B chimera polynucleotide
source          1..360
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 213
ggtcttagagt gtgatggcaa ggtcaacatc tgctgttaaga aacagttttc tgcgtttc 60

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aaggacatcg gctggaatga ctggatcatt gctccctctg gctatcatgc caactactgc 120
gagggtgagt gcccggcca tatagcaggo acgtccgggt caagcttgc cttccactca 180
acagtcatca accactaccg catgcggggc catagccccct ttgccaacct caaatcatgc 240
tgttattccca ccaagctgag caccatgtcc atgttgact ttgatgtga gtacaacatc 300
gtcaaaaaggc acgtttccgaa catgtatcgta gaggagtgt ggtgctcatg agcggccgct 360
```

SEQ ID NO: 214            moltype = AA length = 326  
 FEATURE                Location/Qualifiers  
 source                1..326  
 mol\_type = protein  
 organism = Homo sapiens

SEQUENCE: 214  
 ASTKGPSVFP LAPCSRSTSE STAALGCLVK DYFPEPVTVS WNSGALTSGV HTFPAVLQSS 60
 GLYSLSSVVT VPSSNPGTQT YTCNDHKPS NTKVDKTVER KCCVECPSCP APPVAGPSVF 120
 LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVQFNWYVDG VEVHNAKTKP REEQFNSTFR 180
 VVSVLTVVHQ DWLNGKEYKC KVSNKGLPAP IEKTISKTKG QPREPQVYTL PPSREEMTKN 240
 QVSLTCLVKG FYPDSIAVEW ESNQOPENNY KTPPMMLSD GSFFFLYSKLT VDKSRWQQGN 300
 VFSCSVMHEA LHNHYTQKSL SLSPGK    326

SEQ ID NO: 215            moltype = AA length = 326  
 FEATURE                Location/Qualifiers  
 source                1..326  
 mol\_type = protein  
 organism = Homo sapiens

SEQUENCE: 215  
 ASTKGPSVFP LAPCSRSTSE STAALGCLVK DYFPEPVTVS WNSGALTSGV HTFPAVLQSS 60
 GLYSLSSVVT VPSSNPGTQT YTCNDHKPS NTKVDKTVER KCCVECPSCP APPVAGPSVF 120
 LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVQFNWYVDG VEVHNAKTKP REEQFNSTFR 180
 VVSVLTVVHQ DWLNGKEYKC KVSNKGLPAP IEKTISKTKG QPREPQVYTL PPSREEMTKN 240
 QVSLTCLVKG FYPDSIAVEW ESNQOPENNY KTPPMMLSD GSFFFLYSKLT VDKSRWQQGN 300
 VFSCSVMHEA LHNHYTQKSL SLSPGK    326

SEQ ID NO: 216            moltype = DNA length = 36  
 FEATURE                Location/Qualifiers  
 source                1..36  
 mol\_type = other DNA  
 organism = Homo sapiens

SEQUENCE: 216  
 gatcaagatt actatgatag tagtggttgg gacccc                                    36

SEQ ID NO: 217            moltype = AA length = 105  
 FEATURE                Location/Qualifiers  
 source                1..105  
 mol\_type = protein  
 organism = Homo sapiens

SEQUENCE: 217  
 SEYLTQPPSV SVSPGQTASI TCSGDKLGDK YAFWYQQKPG QSPVLVFYHD TKRPGSIPER 60
 FSGGSNSGNTA TLTISGTQAM DEADYHCQAW DSSTVFGGGT KLTVL                    105

SEQ ID NO: 218            moltype = AA length = 121  
 FEATURE                Location/Qualifiers  
 source                1..121  
 mol\_type = protein  
 organism = Homo sapiens

SEQUENCE: 218  
 QVOLVQSGAE VKKPGASVKV SCKTSGYTF SYGISWVRQA PGQGLEWMGW ISPYNGNTNY 60
 AQKFQGRVTM TTDKSTSTAY MELRSLRSDD TAVYYCARDQ DYYDSSGWDP WGQGTLVTVS 120
 S    121

SEQ ID NO: 219            moltype = AA length = 11  
 FEATURE                Location/Qualifiers  
 source                1..11  
 mol\_type = protein  
 organism = Homo sapiens

SEQUENCE: 219  
 SGDKLGDKYA F    11

SEQ ID NO: 220            moltype = AA length = 7  
 FEATURE                Location/Qualifiers  
 source                1..7  
 mol\_type = protein  
 organism = Homo sapiens

SEQUENCE: 220  
 HDTKRPS    7

SEQ ID NO: 221            moltype = AA length = 326  
 FEATURE                Location/Qualifiers  
 source                1..326  
 mol\_type = protein

-continued

organism = Homo sapiens

SEQUENCE: 221  
ASTKGPSVFP LAPCSRSTSE STAALGCLVK DYFPEPVTVS WNSGALTSGV HTFPAVLQSS 60  
GLYSLSSVVT VPSSNFGTQT YTCAVDHKPS NTKVDKTVER KCCVECPGP APPVAGPSVF 120  
LFPPKPKDTL MISRTPEVTC VVVDFSHEDP EVQFNWVYDG VEVHNAKTPR REEQFNSTFR 180  
VVSVLTVVHQ DWLNGKEYKC KVSNKGLPAP IEKTISKTKP QPREPQVYTL PPSREEMTKN 240  
QVSLTCLVKG FYPSPDIAVEV ESNQOPENNY KTTPPMLDSD GSFFFLYSKLT VDKSRWQQGN 300  
VFSCSVMHEA LHNHYTQKSL SLSPGK 326

SEQ ID NO: 222      moltype = DNA length = 318  
FEATURE  
source      1..318  
mol\_type = other DNA  
organism = Homo sapiens

SEQUENCE: 222  
ggtcagccca aggctgcccc ctcggtaact ctgttcccgcc ctcctctgaa ggagttcaa 60  
gccaacaagg ccacactgggt gtgttcatca atgtgacttct acccggggac cgtgacagt 120  
gccttggagg cagatagcgg cccctggtaag gcggggatgg agaccaccc accctccaaa 180  
caaaagcaaca acaagtagcgc ggcggcggc tatctggacg tgacgctgaa qcagtggaa 240  
tcccacagaa gctacagctg ccaggtcactg catgaaggaa gcaccgtgaa gaagacagt 300  
gcccctacag aatgttca 318

SEQ ID NO: 223      moltype = DNA length = 321  
FEATURE  
source      1..321  
mol\_type = other DNA  
organism = Homo sapiens

SEQUENCE: 223  
cgaactgtgg ctgcaccatc tgcgttccatc ttcccgccat ctgtatggca gttgaaatct 60  
ggaactgccc ctgttgtgtg cctgtgtaa aacttccatc ccagagggc caaagtacag 120  
tggaaaggatgg ataacgcctt ccaatccgggt aacttcccgag agatgtcac agagcggac 180  
agaaggacca gcacctacag cctcggcggc accctgacgc tgagcaaaagc agactacag 240  
aaacacaacaa tctacgcctg cgaagtcacc catcaggccc tgagctgcc cgtcacaag 300  
agttcaaca gggggagatgt 321

SEQ ID NO: 224      moltype = AA length = 12  
FEATURE  
source      1..12  
mol\_type = protein  
organism = Homo sapiens

SEQUENCE: 224  
DQDYYDSSGW DP 12

SEQ ID NO: 225      moltype = AA length = 116  
FEATURE  
source      1..116  
mol\_type = protein  
organism = Homo sapiens

SEQUENCE: 225  
GLECDGKVNI CCKKQFFFVFS KDIGWNDWII APSGYHANYC EGECPSHIAG TSGSSLFHS 60  
TVINHYRMRG HSPFANLKSC CVPTKLRPMS MLYYDDGQNI IKKDIQNMIV EECGCS 116

SEQ ID NO: 226      moltype = AA length = 8  
FEATURE  
source      1..8  
mol\_type = protein  
organism = Homo sapiens

SEQUENCE: 226  
DYKDDDDK 8

SEQ ID NO: 227      moltype = DNA length = 636  
FEATURE  
source      1..636  
mol\_type = other DNA  
organism = Homo sapiens

SEQUENCE: 227  
tcctatgggg tgacttcaggcc acccttcaggc tcgggttccc caggacagac agccagcatc 60  
acctgtctgg gagataatggggataaa tatgtttgtt ggtatcgca gaagccaggc 120  
cagtcccttg tgctggatcat ctatcaaatg agcaagcgcc cctcaggat ccctgaggcga 180  
ttctctggctt ccaactctgg aaacacagcc actctgacca tcagcgggac ccaggctatg 240  
gatgagggtgt actattactg tcaggcgtgg gacagcggca ctgcggattt cggcggagg 300  
accaaggctga ccgttcctagg tcagcccaag gctgccccctt cggtactt gttcccgccc 360  
tcctctgggg agcttcaagc caacaaggcc acactgggtt gtctcataag tgacttctac 420  
ccggggagccg tgacagttggc ctggaaaggca tagacgacggc cggtaaaggc gggaggatgg 480  
accaccacac cctccaaaca aagcaacaac aagtagcgggg ccagcggcta tctgagctg 540  
acgcctgagc agtggaaatgc ccacagaago tacagctgcc aggtcacgca tgaaggagc 600  
accgtggaga agacagttggc ccctacagaa tggtca 636

SEQ ID NO: 228      moltype = DNA length = 1344

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FEATURE source	Location/Qualifiers 1..1344 mol_type = other DNA organism = Homo sapiens
<b>SEQUENCE: 228</b>	
cagggttcagc tgggtcgagtc tggagcttagt gtgaagaagc ctggggccctc agtgaaggtc 60	
tccctgcgaaagg cttctggta caccttacc agttatggtc tcagctgggt gcgacaggcc 120	
cctggacaag ggcttgagtg gatgggatgg atcatccctt acaaattgtaa cacaactct 180	
gcacagaaac tccaggggcag agtccccatc accacagaca catccacggg cacagccctac 240	
atggagctga ggagcttgatc atctgcacgac acggccgtgt atttctgtgc gagagacagg 300	
gactacggtg tcaattatgt tgctttgtat atctggggcc aaggggaaat ggtcaccgtc 360	
tcttcagccct ccaccaagggg cccatcggtc ttccccctgg cgccctgtc caggaggcacc 420	
tcccgagagca cagccggccct gggtggctgt gtcaaggact acttccccca accgggtgacg 480	
gtgtcggtgg actcaggcgc tctggcaggc ggcgtgcacca cttcccccage tgcgttacag 540	
tccctcaggac tctactccct cagcagcggtc gtgaccgtgc cttccacaa cttccggcacc 600	
cagacctaca cctgcacacgt agatcacaag cccagcaaca ccaagggtgga caagacagtt 660	
gagcgcacaaat gtgtgtcgta gtgcggccacgg tgccctggc caccctgtggc aggaccgtca 720	
gtcttcctccaa acccaaggac accctcatgtatc tctccggac ccctggggc 780	
acgtcgctgg tgggtggacgt gacccacaa gacccggagg tccagtccaa ctggatcggt 840	
gacggcggtgg aggtgcataa tgccaagaca aagccacggg aggagcagtt caacacgca 900	
ttccctgtgg tcaagtcgtc caccgttgcg caccaggact ggctgaacggg caaggagttac 960	
aagtgcacccaaatcgg tctccaaacaa aggtttccca aggtttccatc agaaaaacccat ctccaaaacc 1020	
aaaggggcaggc cccggaaacc acagggtgtac accctgtcccc catccggga ggagatgacc 1080	
aagaaccaggc tcaagctgtac ctgcgtggc aaagggttctt accccacgca catcgccgt 1140	
gagtgggaga gcaatgggca gcccggagaa aactacaaga ccacacccatc catgtggac 1200	
tcggacggct cttcttcctt ctacgcacgg ctcaccgtgg acaagagcag gtggcagcag 1260	
ggggAACGCTC tttcatgtc cgtgtatc gagggtctgc acaaccacta caccggaa 1320	
agccctctccc tgcgttccggg taaa 1344	
SEQ ID NO: 229 moltype = DNA length = 642	
FEATURE source	Location/Qualifiers 1..642 mol_type = other DNA organism = Homo sapiens
<b>SEQUENCE: 229</b>	
gacatccaga tgaccccgatc tccatccctcc ctgtctgtat ctgttagggaa cagagtcaacc 60	
atcaacttgcg gggcaagtca gggcattaga aataatttag gctggatca gcagaaacca 120	
ggggaaagcccttcaagggccctt gattttatgt gcatcccgat tgcggaaatggg ggtcccatca 180	
aggttcagcg gcaatggatc tggggacagaa ttcaacttca caatcagcag tctgcggcc 240	
gaagatttta caacttattat ctgtctgtat cataatagt acccggtggac gttcgccaa 300	
ggggaccaagg tggaaatcaa acgaaactgtg gctgcaccaat ctgtcttcat cttccggcca 360	
tctgtatggc agttggaaatc tggggatccctt gctgtgtgtt gctgtgtggaa taacttctat 420	
cccgagaggcccaaaatcaca gttggaaatggg gataacggcc tcaatcgggg taactccca 480	
gagagtgtca cagacggacca cggacggac accctgtaca gcttcacggg caccctgtac 540	
ctgagcaagc cagactacga gaaacacaaaatc gtcgtacgtc gcaaggatcac ccattcaggc 600	
ctgagctcgc cccgttcaacaa gagttcaac agggggagatgt 642	
SEQ ID NO: 230 moltype = DNA length = 1350	
FEATURE source	Location/Qualifiers 1..1350 mol_type = other DNA organism = Homo sapiens
<b>SEQUENCE: 230</b>	
cagggtcgacg tgggtggggatc tggggggggc gtgggtccagc ctgggggggtc cctggggggc 60	
tccctgtcgag cgtctggatt cacccatcgat agttacggca tgcactgggt ccggccaggct 120	
ccaggcaagg ggctggggatc ggtggggatc atatggatcg atggaaatggaa taaataccat 180	
gcagactccg tgaaggcccg attcaccatc tccagagaca atttcaagaa caccgtgtat 240	
ctgcaagttca acaggctgtatc agccggggggc accggctgtgtt attactgtgtt gagaaggctgg 300	
aactggaaacttca accggacacta ctactacgtt ctggacgttccggggccaaagg gaccacgggtc 360	
accgtctctt cggccatccac caaggggccca tccgtgttcc ccttggccccc ctgtccagg 420	
agcacccctcg agaggccatccac ggcocctggc tccgtgttcc accggactactt cccggaaacc 480	
gtgtacgggtt ctggggggatc agggccgttcc accggccggc tgcacacccccc cccaggctgtc 540	
ctacatgttcc cggggggatc cttcccttgcg agcggtgtgtt gctgtgtgtt gctgtgtggaa 600	
ggcacccggatc cttccatgtc caacggatgtt gaccaacccca gcaacacccaa ggtggacaa 660	
acagttgtatc gcaaatgtt gtcgtatgtt ccaccgttcc cggccatccac tgggggggg 720	
ccgtcgttcc tcccttttttcc cccaaacccca aaggacacccatc tcatgtatcc cccggaccct 780	
ggggatcgatgtt ggtgtgtgtt gggtgtatcc gacggacccatc cccggccatcc cccgggggg 840	
tacgtggggatc ggtgtgtgtt gaccaatgtt gacggacccatc cccggccatcc cccgggggg 900	
agcacgttcc ggtgtgtgtt cttcccttgcg gacggatgtt gacggatgtt gacggatgtt 960	
gagttacaatgtt gcaagggttcc caacaaatggg ctccggccatcc cccggccatcc cccgggggg 1020	
aaaaccaaaatggcaggccatcc gacggatgtt gacggatgtt gacggatgtt gacggatgtt 1080	
atggggatcgatgtt ggtgtgtgtt gggtgtatcc gacggacccatc cccggccatcc cccgggggg 1140	
ggccgtggggatc gggggggatc tggggggggatc gacggatgtt gacggatgtt gacggatgtt 1200	
ctggacttccg acggccatccatc ttttttttttcc gacggatgtt gacggatgtt gacggatgtt 1260	
cacggggggatc acgtttttccatc atgtttttccatc atgtttttccatc gacggatgtt gacggatgtt 1320	
cagaagggatc ttttttttttccatc ttttttttttccatc ttttttttttccatc gacggatgtt gacggatgtt 1350	
SEQ ID NO: 231 moltype = DNA length = 642	
FEATURE	Location/Qualifiers

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source	1..642
	mol_type = other DNA
	organism = Homo sapiens
SEQUENCE: 231	
gacatccaga tgaccaggc tccatcttc ctgtctgc atctggaga cagagtacc 60	
atcaacttgc gggcaagtca gggcattaga aatgatttag gctggatca gcagaaacca 120	
gggaaagccc ctaagcgcc tgcattatgc gcatccagg tgcaaagggg ggtccatca 180	
aggttcagcg cgactggatc tggcagacaa ttcaactctca caatcagcag cctgcagcc 240	
gaagatttt caacttata ctgtcgacg caaaacttactt acccgctcac ttccggcga 300	
gggaccaagg tggagatcaa acgaatgtg gctgcacat ctgtctcat ctcccccca 360	
tctgtatgc agttaaatc tggactgc tctgttggtg gctgtgtaa taacttctat 420	
cccagagagg ccaaagtaca gtggaaagggt gataacgccc tccaaatcggg taactccag 480	
gagagtgtca cagacgaggc cagaacggac agcacatcaca gctcagcag caccctgacg 540	
ctgagcaaaag cagaactcga gaaacacaaa gcttaacgccc gctcagtcac ccatacgggc 600	
ctgagctcgc ccgtcacaaa gagttcaac agggagatgt 642	
SEQ ID NO: 232	moltype = DNA length = 1347
FEATURE	Location/Qualifiers
source	1..1347
	mol_type = other DNA
	organism = Homo sapiens
SEQUENCE: 232	
gagggtcagt tggggggggc ttgggtccagg ctgggggggc cctgagactc 60	
tcctgtcgag cctctggatt caccttagt agttattggg tgagctgggt ccgcaggct 120	
ccaggaaagg ggctggaggc cgtggccaa ataaaggcaag atggaaatgtg ggaataactat 180	
gtggactctg tgaaggccg attcaccat tccagagacaa acggccaaagggttcaactgtat 240	
ctgcaatgtc acagctcgatc acggaggac acggctgtgtt attactgtgc gagaggtagc 300	
agcagctgtt actactacaa ctacggatgc gacgtctggg gccaaggggac cacggtcacc 360	
gttccctcag ctccaccaa gggccatcg gtcttcccc tggcgcctg ctccaggagc 420	
acotcccgaga gacacggcggc cctgggtcg ctgtcaagg actacttccc cgaacgggtg 480	
acgggtgtcg tggactcagg cgctgtgacc acggcgtgc acacccccc agctgtctta 540	
cagtccctcg gactctactc cctcagcago gtgtgaccg tgccctccag caacttcggc 600	
acccagaccc acacccgtca cgtagatcac aagcccaagggt ggacaaagaca 660	
gttgaggegca aatgttggt cgtggccca cctggccagg caccacccgtt ggcaggaccc 720	
tcagtcttcc ttttccccc aaaaaaaagg gacacccctca tgatctccg gaccctcgag 780	
gtcacgtcg tgggtgtgca cgtgagccac gaagaccccg aggtccaggta caacttgtac 840	
gtggacggcg tggaggtgca taatgccaag acaaaggccac gggaggagca gttcaacagc 900	
acgttccctg tgggtcgatc cctcaccgtt gtgcacccagg actggctgaa cggcaaggag 960	
tacaagtgtca aggtctccaa ccaaggccctc ccaggccccca tcgagaaaac catctccaa 1020	
acccaaaggcc accccggaga accacagggt tacaccctgc ccccatcccg ggaggagatg 1080	
accaagaacc aggtcagcc gacctgcctg gtcaaaagggt tctaccccg cgacatcgcc 1140	
gttgaggtgg agagaatgg cgacggccgg aacaactaca agaccacacc tcccatgtg 1200	
gactcccgacg gtccttctt cctctacago aagtcacccg tggacaaggag caggtggcag 1260	
caaggaaacg tcttctcatg ctccgtatg catgggctc tgcacaacca ctacacgcg 1320	
aagagcctct ccctgtctcc gggtaaa 1347	
SEQ ID NO: 233	moltype = AA length = 212
FEATURE	Location/Qualifiers
source	1..212
	mol_type = protein
	organism = Homo sapiens
SEQUENCE: 233	
SYEVVTQAPSV SVSPGQTASI TCSGDGLDK YACWYQQKPG QSPVLVIYQD SKRPSGIPER 60	
FSGSNSNGNTA TLTIISGTQAM DEADYYCQAW DESTAVFGGG TKLTIVLGQPK AAPSVTLFPP 120	
SSEELQANKA TLVCLISDFY PGAVTVAKA DSSPVKAGVE TTTPSKQSNN KYAASSYLSL 180	
TPEQWKSHRS YSCQVTHEGS TVEKTVAPTE CS 212	
SEQ ID NO: 234	moltype = AA length = 448
FEATURE	Location/Qualifiers
source	1..448
	mol_type = protein
	organism = Homo sapiens
SEQUENCE: 234	
QVOLVQSGAE VKPGASVKV SCKASGYTFT SYGLSWVRQA PGQGLEWMGW IIPYNGNTNS 60	
AQKLQGRVTM TTDTSTSTAY MELRLSRSDD TAVYFCARDR DYGVNYDAFD IWGQGTMVTV 120	
SSASTKGPSV FPLAPCSRST SESTAALGCL VKYDVFPEVPT VSWNSGALTS GVHTPPAVLQ 180	
SSGGLYSLSV VTVPSNSFGT QTYYTCNDHK PSNTKVDKTY ERKCCVECPP CPAPPVAGPS 240	
VFLPPPKD TLMSIRTPEV CTVVVDSVHS DEPEVQFNWVY DGVEVHNAKT KPREEQFNST 300	
FRVVSVLTVV HQDWLNGKEY KCKVSNKGLP APIEKTISKT KGQPREEQVY TLPPSREEMT 360	
KNQVSLTCLV KGFYPSDIAV EWESNGQOPEN NYKTTPPMLD SDGSFFLYSK LTVDKSRWQQ 420	
GNVFSCSVMH EALHNHYTQK SLSLSPGK 448	
SEQ ID NO: 235	moltype = AA length = 214
FEATURE	Location/Qualifiers
source	1..214
	mol_type = protein
	organism = Homo sapiens
SEQUENCE: 235	
DIQMTQSPSS LSASVGDRVT ITCRASQGIR NNLGWWQQKP GKAPKRLIYA ASSLQSGVPS 60	

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RFSGSGSGTE FTLTISSLQP EDFFTYYCLQ HNSYPWTFGQ GTKVEIKRTV AAPSVFIFPP 120  
 SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSTLT 180  
 LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEc 214

SEQ ID NO: 236 moltype = AA length = 450  
 FEATURE Location/Qualifiers  
 source 1..450  
 mol\_type = protein  
 organism = Homo sapiens  
 SEQUENCE: 236  
 QVOLVESGGG VVQPGRSRLR SCAASGFTFS SYGMHWVRQA PGKGLEWVAV IWYDGSNKYH 60  
 ADSVKGRFTI SRDNSKNLTY LQVNSLRAED TAVYYCVRSR NWNYDNYYYG LDVWGQGTTV 120  
 TVSSASTKGP SVFPLAPCSR STSESTAALG CLVKDYFPPEP VTWSWNSGAL TSGVHTFPBV 180  
 LQSSGLYLS SVVTVPSSNR HKPSNTKVDK TVERKCCVEC PPCPAPPVAG 240  
 PSVFLFPKPK KDTLMISRTP EVTCVVVDVS HEDPEVQFNW YVDGVEVHNA KTKPREEQFN 300  
 STFRVSVLVT VVHQDWLNGK EYKCKVSNKG LPAPIEKTIS KTKGQPREPQ VYTLPPSREE 360  
 MTKNQVSLTC LVKGFYPSDI AVEWESNGQ ENNYKTTPPM LDSDGSFFLY SKLTVDKSRW 420  
 QQGNVFSCSV MHEALHNHYT QKSLSLSPGK 450

SEQ ID NO: 237 moltype = AA length = 214  
 FEATURE Location/Qualifiers  
 source 1..214  
 mol\_type = protein  
 organism = Homo sapiens  
 SEQUENCE: 237  
 DIQMTQSPSS LSASVGDRVT ITCRASQGIR NDLGWYQQKP GKAPKRRIYA ASSLQSGVPS 60  
 RFSGSGSGTE FTLTISSLQP EDFATYYCRQ QNTYPLTPGG GTKVEIKRTV AAPSVFIFPP 120  
 SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSTLT 180  
 LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEc 214

SEQ ID NO: 238 moltype = AA length = 449  
 FEATURE Location/Qualifiers  
 source 1..449  
 mol\_type = protein  
 organism = Homo sapiens  
 SEQUENCE: 238  
 EVQLVESGGG LVQPGGSLRL SCAASGFTFS SYWMSWVRQA PGKGLECVAN IKQDGSEEEY 60  
 VDSVKGRFTI SRDNNAKNSLY LQMNSLRAED TAVYYCARGS SSWWYYNYGM DVWGQGTTV 120  
 VSSASTKGPS VFPLAPCSR S TSESTAALGC LVKDYFPPEPV TVSWNSGALT SGVHTFPAVL 180  
 QSSGLYLS VVTVPPSNFG TQTYTCNVDH KPSNTKVDKT VERKCCVECP PCPAPPVAGP 240  
 SVPLFPKPKP DTLMISRTPE VTCVVVDVSH EDPEVQFNWY VDGVEVHNAK TKPREEQFN 300  
 TFRVSVLVT VHQDWLNGK YKCKVSNKG LPAPIEKTISK TKGQPREPQV YTLPPSREEM 360  
 TKNQVSLTCL VKGFYPSDIA WEVESNGQPE NNYKTTPPM LDSDGSFFLYS KLTVDKSRWQ 420  
 QGNVFSCSV MHEALHNHYTQ KSLSLSPGK 449

SEQ ID NO: 239 moltype = DNA length = 321  
 FEATURE Location/Qualifiers  
 source 1..321  
 mol\_type = other DNA  
 organism = Homo sapiens  
 SEQUENCE: 239  
 cgaactgtgg ctgcaccatc tgcatttcata ttcccgccat ctgatgagca gttgaatct 60  
 ggaactgcct ctgttgtgtg cctgtgaat aaccttctatc ccagagaggc caaagtacag 120  
 tggaaagggtgg ataacgcctt ccaatcggtt aactccccagg agagtgtcac agagcaggac 180  
 agcaaggatc acgcattcag cctcgcgc accctgacgc tgccaaaggc agactacag 240  
 aacacacaag tttacgcctg ccaaggatcacc catcaggccc tgagctggc cgtcacaaag 300  
 agttcaaca ggggagatgt t 321

SEQ ID NO: 240 moltype = DNA length = 978  
 FEATURE Location/Qualifiers  
 source 1..978  
 mol\_type = other DNA  
 organism = Homo sapiens  
 SEQUENCE: 240  
 gcctccacca agggcccatc ggtttcccc ctggcgccct gctccaggag cacctccgag 60  
 agcacagcg ggcttggtctg cctgtcaag gactacttcc cccgaaacgggt gacgggtgtcg 120  
 tggaaactcg ggcgttgcac cggcgccgtt cacacccctt cagctgtctt acagttccca 180  
 ggactctact ccctcagcag cgtgggtgacc gtgccttccca gcaacttcgg caccaggacc 240  
 tacacccgtca acgttagatca caagccccago aacccaagg tggacaaaggc agttgagcgc 300  
 aaatgttgg tcgagtggcc accgtggcca gcaccacgtt tggcaggacc gtcagtctt 360  
 ctcttccccca aaaaacccaa ggacacccctt atgatctttt ggaccccttga ggtcacgttc 420  
 gtgggtgggg acgtgagccca cgaagacccc gaggtccatgt tcaactgtta cgtggacggc 480  
 gtggagggatgc ataattggccaa gacaaaggcca cggggaggagc agttcaacacg caccgttccgt 540  
 gtggtcacgc ttcttaccgt tttgtcaccatggc gactgggttgc acggcaaggaa gtacaatgtc 600  
 aagggtctcca acaaaggccctt cccaggcccc atcgagaaaaa ccatctccaa aaccaaaagg 660  
 cagccccggg aaccacacggt gtacacccctt ccccccattttt gggaggagat gaccaagaac 720  
 caggtcagcc tgacccgtt ggtcaaaaggcc ttcttaccatggc gacccatccgc cgtggatgtt 780  
 gagagcaatg ggcagccggaa gaacaactaac aagaccacac cttccatgtt ggtactccgac 840  
 ggctcccttctt ttcttcttacag caagtcaccatggc gacccatccgc cgtggatgtt 900

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gtcttctcat gtcgggtat gcatgaggct ctgcacaacc actacacgca gaagagcctc 960
tccctgtctc cgggtaaa 978
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SEQ ID NO: 241 moltype = DNA length = 978  
 FEATURE Location/Qualifiers  
 source 1..978  
 mol\_type = other DNA  
 organism = Homo sapiens  
 SEQUENCE: 241  
 gcctccacca agggcccate ggtttcccc ctggcgccc gtcggaggag caccccgag 60
 agcacacggg ccctgggtg cctgtcaag gactacttc cggaaacggg gacgggttcg 120
 tggaaactcg ggcgtctgac cagggcggtg cacacccctc cagctgtct acagtctca 180
 ggactctact ccctcagcg cgtgtgtacc gtggccctcca gcaacttcgg caccaggacc 240
 tacacccgtca agcttagtca caagccaggc aacaccaaggc tggacaaggc agttgagcgc 300
 aaatgttg tccagtgccc accgtgccc gcaccaccc tggcaggacc gtcagtctc 360
 ctttttcccc caaaacccaa ggacacccctc atgatctccc ggacccctga ggtcacgtgc 420
 gtgggttgttgg acgtgaccca cgaagacccc gaggtccagg tcaactggta cgtggacggc 480
 gtggagggttca ataatggccaa gacaaggcca cgggaggaggc agttcaacag cacgttcgt 540
 gtggtcacgcttccatccacccgt tttgtccacca gactgggtca acggcaaggaa gtacaagtgc 600
 aagggtctcca acaaaggccct ccacccccc atcgagaaaa ccacccatccaa aaccaagg 660
 cagcccccgag aaccacagggt gtacacccctg ccccccattccc gggaggagat gaccaaggaaac 720
 cagggtcagcc tgacccctgccttccatccca ggcacatcgc cgtggagttgg 780
 gagagactatggcagccgaa aacaaactac aagaccacac ccctccatgtc ggactccgac 840
 ggctcccttc ttctctacag caagtcacc gtggacaaga gcaagggtggca gcaggggaaac 900
 gtcttctcat gtcgggtat gcatgaggct ctgcacaacc actacacgca gaagagcctc 960
 tccctgtctc cgggtaaa 978

SEQ ID NO: 242 moltype = DNA length = 824  
 FEATURE Location/Qualifiers  
 source 1..824  
 mol\_type = other DNA  
 organism = Homo sapiens  
 SEQUENCE: 242  
 gcctccacca agggcccate ggtttcccc ctggcgccc gtcggaggag caccccgag 60
 agcacacggg ccctgggtg cctgtcaag gactacttc cggaaacggg gacgggttcg 120
 tggaaactcg ggcgtctgac cagggcggtg cacacccctc cagctgtct acagtctca 180
 ggactctact ccctcagcg cgtgtgtacc gtggccctcca gcaacttcgg caccaggacc 240
 tacacccgtca agcttagtca caagccaggc aacaccaaggc tggacaaggc agttgagcgc 300
 aaatgttg tccagtgccc accgtgccc gcaccaccc tggcaggacc gtcagtctc 360
 ctttttcccc caaaacccaa ggacacccctc atgatctccc ggacccctga ggtcacgtgc 420
 gtgggttgttgg acgtgaccca cgaagacccc gaggtccagg tcaactggta cgtggacggc 480
 gtggagggttca ataatggccaa gacaaggcca cgggaggaggc agttcaacag cacgttcgt 540
 gtggtcacgcttccatccacccgt tttgtccacca gactgggtca acggcaaggaa gtacaagtgc 600
 aagggtctcca acaaaggccct ccacccccc atcgagaaaa ccacccatccaa aaccaagg 660
 cagcccccgag aaccacagggt gtacacccctg ccccccattccc gggaggagat gaccaaggaaac 720
 cagggtcagcc tgacccctgccttccatccca ggcacatcgc cgtggagttgg 780
 gagagactatggcagccgaa aacaaactac aagaccacac ccctccatgtc ggactccgac 840

SEQ ID NO: 243 moltype = AA length = 116  
 FEATURE Location/Qualifiers  
 REGION 1..116  
 note = Description of Artificial Sequence: Synthetic activin A/B chimera polypeptide  
 source 1..116  
 mol\_type = protein  
 organism = synthetic construct  
 SEQUENCE: 243  
 GLECDGKVNI CCRQQFFIDF RLIGWNDWII APTGYYGNYC EGECPHSIAG TSGSSLFHS 60
 TVINHYMRMG HSPFANLKSC CVPTKLRPMS MLYYDDGQNI IKKDIQNMIIV EECGCS 116

SEQ ID NO: 244 moltype = AA length = 116  
 FEATURE Location/Qualifiers  
 REGION 1..116  
 note = Description of Artificial Sequence: Synthetic activin A/B chimera polypeptide  
 source 1..116  
 mol\_type = protein  
 organism = synthetic construct  
 SEQUENCE: 244  
 GLECDGKVNI CCKKQFFVVF KDIGWNDWII APSGYHANYC EGECPHSIAG TSGSSLFHS 60
 TVINHYMRMG HSPFANLKSC CIPTKLSTMS MLYFDDEYNI VKRDVPNMIV EECGCS 116

SEQ ID NO: 245 moltype = DNA length = 31  
 FEATURE Location/Qualifiers  
 misc\_feature 1..31  
 note = Description of Artificial Sequence: Synthetic oligonucleotide  
 source 1..31  
 mol\_type = other DNA

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organism = synthetic construct
SEQUENCE: 245
ctcgaggatcg actagaccac catgcccttg c 31
SEQ ID NO: 246      moltype = DNA length = 28
FEATURE          Location/Qualifiers
misc_feature     1..28
                  note = Description of Artificial Sequence: Synthetic
                  oligonucleotide
source           1..28
                  mol_type = other DNA
                  organism = synthetic construct
SEQUENCE: 246
ccatcacact cttagaccccg ccgacgccc 28
SEQ ID NO: 247      moltype = DNA length = 360
FEATURE          Location/Qualifiers
misc_feature     1..360
                  note = Description of Artificial Sequence: Synthetic
                  activin A/B chimera polynucleotide
source           1..360
                  mol_type = other DNA
                  organism = synthetic construct
SEQUENCE: 247
gggtcttagagt gtgtatggcaa ggtaaacatc tgctgttaggc aacagtcttt tatcgatttc 60
aggcttcatcg gctggaaatga ctggatcatt gctcccaactg gtttatgg caactactgc 120
gagggttagt gccccagcca tatacgccgg acgtccgggtt caagcttgc cttccactca 180
acagtcatca accactaccg catggggggc catagccctt ttgccaacct caaatcatgc 240
tgtgtgccccca ccaagcttagt accccatgtcc atgttgtact atgtatggg tcaaaaatcc 300
ataaaaaagg acattcagaa catgtatcgat gaggagtgtt ggtgtctatg agcggccgtt 360
SEQ ID NO: 248      moltype = AA length = 9
FEATURE          Location/Qualifiers
REGION           1..9
                  note = Description of Artificial Sequence: Synthetic anti
                  activin A antibody peptide
MOD_RES          5
                  note = Arg or Ser
MOD_RES          8
                  note = Val or Ala
MOD_RES          9
                  note = May or may not be present
source           1..9
                  mol_type = protein
                  organism = synthetic construct
SEQUENCE: 248
QAWDXSTXV 9
SEQ ID NO: 249      moltype = length =
SEQUENCE: 249
000
SEQ ID NO: 250      moltype = AA length = 11
FEATURE          Location/Qualifiers
REGION           1..11
                  note = Description of Artificial Sequence: Synthetic anti
                  activin A antibody peptide
MOD_RES          3
                  note = Ser or Gly
MOD_RES          7
                  note = Ser or Arg
MOD_RES          9
                  note = Asp or Asn
MOD_RES          11
                  note = Val or Gly
source           1..11
                  mol_type = protein
                  organism = synthetic construct
SEQUENCE: 250
RAXQGIXNXL X 11
SEQ ID NO: 251      moltype = AA length = 12
FEATURE          Location/Qualifiers
REGION           1..12
                  note = Description of Artificial Sequence: Synthetic anti
                  activin A antibody peptide
source           1..12
                  mol_type = protein
                  organism = synthetic construct

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SEQUENCE: 251  
RASQSIISNYL NT

SEQ ID NO: 252 moltype = AA length = 12  
FEATURE Location/Qualifiers  
REGION 1..12  
note = Description of Artificial Sequence: Synthetic anti activin A antibody peptide

MOD\_RES 4  
note = Ile or Phe

MOD\_RES 5  
note = Asn or Ser

MOD\_RES 6  
note = Ser or Ala

MOD\_RES 7..8  
note = May or may not be present

MOD\_RES 9  
note = Phe or Tyr

source 1..12  
mol\_type = protein  
organism = synthetic construct

SEQUENCE: 252  
GGSXXXGGXY WS

12

SEQ ID NO: 253 moltype = AA length = 16  
FEATURE Location/Qualifiers  
REGION 1..16  
note = Description of Artificial Sequence: Synthetic anti activin A antibody peptide

source 1..16  
mol\_type = protein  
organism = synthetic construct

SEQUENCE: 253  
RSSQSLLHST GNYLD

16

SEQ ID NO: 254 moltype = AA length = 7  
FEATURE Location/Qualifiers  
REGION 1..7  
note = Description of Artificial Sequence: Synthetic anti activin A antibody peptide

source 1..7  
mol\_type = protein  
organism = synthetic construct

SEQUENCE: 254  
LGSFRAS

7

SEQ ID NO: 255 moltype = AA length = 9  
FEATURE Location/Qualifiers  
REGION 1..9  
note = Description of Artificial Sequence: Synthetic anti activin A antibody peptide

source 1..9  
mol\_type = protein  
organism = synthetic construct

SEQUENCE: 255  
MQALQTPCS

9

SEQ ID NO: 256 moltype = AA length = 10  
FEATURE Location/Qualifiers  
REGION 1..10  
note = Description of Artificial Sequence: Synthetic anti activin A antibody peptide

source 1..10  
mol\_type = protein  
organism = synthetic construct

SEQUENCE: 256  
GYTFTGYYIH

10

SEQ ID NO: 257 moltype = length =  
SEQUENCE: 257  
000

SEQ ID NO: 258 moltype = AA length = 17  
FEATURE Location/Qualifiers  
REGION 1..17  
note = Description of Artificial Sequence: Synthetic anti activin A antibody peptide

source 1..17  
mol\_type = protein

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	organism = synthetic construct	
SEQUENCE: 258 WINPNSGGTN YAQKFQG		17
SEQ ID NO: 259 FEATURE REGION	moltype = AA length = 17 Location/Qualifiers 1..17 note = Description of Artificial Sequence: Synthetic anti activin A antibody peptide source 1..17 mol_type = protein organism = synthetic construct	
SEQUENCE: 259 WISPYNGNTN YAQKFQG		17
SEQ ID NO: 260 FEATURE REGION	moltype = AA length = 12 Location/Qualifiers 1..12 note = Description of Artificial Sequence: Synthetic anti activin A antibody peptide source 1..12 mol_type = protein organism = synthetic construct	
SEQUENCE: 260 DSGYSSSWHF DY		12
SEQ ID NO: 261 FEATURE REGION	moltype = AA length = 13 Location/Qualifiers 1..13 note = Description of Artificial Sequence: Synthetic anti activin A antibody peptide source 1..13 mol_type = protein organism = synthetic construct	
SEQUENCE: 261 GSSSWYYYNG MDV		13
SEQ ID NO: 262 FEATURE REGION	moltype = AA length = 30 Location/Qualifiers 1..30 note = Description of Artificial Sequence: Synthetic polypeptide corresponding to amino acid residues 1-30 of activin A source 1..30 mol_type = protein organism = synthetic construct	
SEQUENCE: 262 GLECDGKVNI CKKQFFVVF KDIGWNDII		30
SEQ ID NO: 263 FEATURE REGION	moltype = AA length = 30 Location/Qualifiers 1..30 note = Description of Artificial Sequence: Synthetic polypeptide corresponding to amino acid residues 31-60 of activin A source 1..30 mol_type = protein organism = synthetic construct	
SEQUENCE: 263 APSGYHANYC EGECPSHIAG TSGSSLFHS		30
SEQ ID NO: 264 FEATURE REGION	moltype = AA length = 30 Location/Qualifiers 1..30 note = Description of Artificial Sequence: Synthetic polypeptide corresponding to amino acid residues 61-90 of activin A source 1..30 mol_type = protein organism = synthetic construct	
SEQUENCE: 264 TVINHYRMRG HSPFANLKSC CVPTKLRPMS		30
SEQ ID NO: 265 FEATURE REGION	moltype = AA length = 26 Location/Qualifiers 1..26 note = Description of Artificial Sequence: Synthetic peptide corresponding to amino acid residues 91-116 of activin A	

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source 1..26  
mol\_type = protein  
organism = synthetic construct

SEQUENCE: 265  
MLYYDDGQNI IKKDIQNMIV EECGCS 26

SEQ ID NO: 266 moltype = AA length = 116  
FEATURE Location/Qualifiers  
REGION 1..116  
note = Description of Artificial Sequence: Synthetic activin A 13/39B polypeptide

source 1..116  
mol\_type = protein  
organism = synthetic construct

SEQUENCE: 266  
GLECDGKVNI CCRQQFFIDF RLIGWNDWII APTGYYGNYC EGECPSHIAG TSGSSLSFHS 60  
TVINHYRMRG HSPFANLKSC CVPTKLRPMs MLYYDDGQNI IKKDIQNMIV EECGCS 116

SEQ ID NO: 267 moltype = DNA length = 318  
FEATURE Location/Qualifiers  
source 1..318  
mol\_type = other DNA  
organism = Homo sapiens

SEQUENCE: 267  
tccttatagg tgactcaggc acccttcagtg tccgtgtccc caggacagac agccagcatc 60  
acctgctatg gagataatt gggggataaa tatgcttggt ggtatcgaca gaagccaggc 120  
cagtccccctg tgctggatcat ctatcaagat agcaaggcgc cctcaggat ccctgaggca 180  
tttcctggct ccaactctgg aaacacagcc actctgacca tcagcgggac ccaggctatg 240  
gatgaggctg actattactg tcaggcgtgg gacagcgcga ctgcggattt cggcggaggg 300  
accaagctga cogtctta 318

SEQ ID NO: 268 moltype = DNA length = 366  
FEATURE Location/Qualifiers  
source 1..366  
mol\_type = other DNA  
organism = Homo sapiens

SEQUENCE: 268  
caagttcagc tgggtcagtc tggagctgag gtgaagaagc ctggggccctc agtgaaggtc 60  
tcctgcaagg cttctggatcac cacccttacc agttatggtc tcagctgggt gcgacaggcc 120  
cctggacaag ggcttgagtg gatgggatgg atcatccctt acaaattgtt cacaactct 180  
gcacagaaac tccaggccag agtcacatcg accacagaca catccacgag cacagctac 240  
atggagctga ggagccctgag atctgacac acggccgtgtt atttctgtgc gagagacagg 300  
gactacggtg tcaattatga tgcttttgcgat atctggggcc aaggacaat ggtcacccgtc 360  
tcttca 366

SEQ ID NO: 269 moltype = DNA length = 373  
FEATURE Location/Qualifiers  
source 1..373  
mol\_type = other DNA  
organism = Homo sapiens

SEQUENCE: 269  
caagttcagc tgggtggagtc tggggggggc gtgggtccagc ctggggaggc cctgagactc 60  
tcctgtcgag cgtctggattt cacccatgtt agttacggca tgcactgggt ccggcaggct 120  
ccaggcaagg ggctggatgtt ggtggcgtttt atatggatgtt atggaaatgtt taaaatccat 180  
gcagactccg tcaaggcccg attccatc tccagagaca attccaaagg cacgtgttat 240  
ctgtcaatgtt acagccgtgg acggcggggc acggccgtgtt attactgtgtt gagaaggccgg 300  
aactggaaactt acgacaacta ctactacggt ctggacgtctt gggggccaaagg gaccacggcc 360  
accgtctcc 373

SEQ ID NO: 270 moltype = DNA length = 321  
FEATURE Location/Qualifiers  
source 1..321  
mol\_type = other DNA  
organism = Homo sapiens

SEQUENCE: 270  
gacatccaga tgacccaggc tccatccctcc ctgtctgtcat ctgttaggaga cagagtacc 60  
atcaacttgcg gggcaatgtt gggcatttgcg aataatttgcg gctggatataca gcagaaacca 120  
ggggaaaggccc ttaagccctt gattttatgtt gcataccaggat tgcacaaatgtt ggtcccatca 180  
aggttcaatgcg gcaatggatc tggggacacaa ttcaatcttca caatcagcag tctgcaggct 240  
gaagatgttta caacttattt ctgttacatgc cataatgtt acccgtggac gttcggccaa 300  
gggaccaagg tggaaatcaa a 321

SEQ ID NO: 271 moltype = DNA length = 369  
FEATURE Location/Qualifiers  
source 1..369  
mol\_type = other DNA  
organism = Homo sapiens

SEQUENCE: 271  
gagggtcagtc tgggtggagtc tggggggggc ttgggtccagc ctgggggggtc cctgagactc 60

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tcctgtgcag cctctggatt caccttagt agttattgga tgagctgggt ccgccaggct 120  
 ccagggaaagg ggctggagtg cggtggccaaataaaggcaatggaaagtga ggaataactat 180  
 gtggactctgtgtaaaggcccttaccacatc tccagagaca acgccaagaa ttcaactgtat 240  
 ctgcaaatagaaacagctgag agccgaggac acggctgtt attactgtgc gagaggtac 300  
 acgagctgt actactacaa ctacggatg gacgtctggg gccaaggac cacggtcacc 360  
 gtctctca 369

SEQ ID NO: 272 moltype = DNA length = 321  
 FEATURE Location/Qualifiers  
 source 1..321  
 mol\_type = other DNA  
 organism = Homo sapiens

SEQUENCE: 272  
 gacatccaga tgaccaggc tccatccttc ctgtctgcattgttagaga cagagtacc 60  
 atcaacttgc gggcaagtca gggcattaga aatgatttag gctggatca gcagaaacca 120  
 gggaaagccc ctaagcgccctatctatgt gcatccagg ttgcaaaatgg ggtcccatca 180  
 aggttcaagcg gcaatggatc tggacacaaatccatca caatcagcag cctgcaggct 240  
 gaagattttcaacttattatgtcgacag caaaataactt acccgtcac tttcggcgga 300  
 gggaccaagg tggatcaa a 321

SEQ ID NO: 273 moltype = DNA length = 363  
 FEATURE Location/Qualifiers  
 source 1..363  
 mol\_type = other DNA  
 organism = Homo sapiens

SEQUENCE: 273  
 caggtgcagctgggtcagtc tggggcttagt gtgaagaagc ctggggccctc agtgaaggc 60  
 tcctgcaggcttctggata cacattcacc ggctactata tccactgggt ggcacaggcc 120  
 cctggacaag ggcttgatgtt gatggatgg atcaacccttta acagtgggtt cacaactat 180  
 gcacagaatgttcaaggcag ggtcaccatg accaggggacatgcacatcg cacaacctac 240  
 atggagatgtgacaggctgatctgacgc acggccgtgtt atttctgtgc gagagatcg 300  
 gggtatagca gcaatggca ctgtgactac tggggccagg gaaccctgtt caccgtctcc 360  
 tca 363

SEQ ID NO: 274 moltype = DNA length = 336  
 FEATURE Location/Qualifiers  
 source 1..336  
 mol\_type = other DNA  
 organism = Homo sapiens

SEQUENCE: 274  
 gatatttgtatgtactcgttc tccactcttc ctgccccgttcccccctggaga gcccggctcc 60  
 atcttcgtcagttctgttgc ggttactgttgc gggcccttcatatgttacttggatgtt 120  
 tacctgcaga agccaggcgtt gttccatcgatcttgcgttatttgggttcc ttttcgggccc 180  
 tccgggggtcc ctgacagggtt cttgtggatgtt gggtcaggccatgttttac actgaaatc 240  
 agcagatgttggaggctgatgttgc ttttttttttattactgtca tgcaagatcttccaaactccg 300  
 tgcgttttgcacaggggac caatgtggatca 336

SEQ ID NO: 275 moltype = AA length = 106  
 FEATURE Location/Qualifiers  
 source 1..106  
 mol\_type = protein  
 organism = Homo sapiens

SEQUENCE: 275  
 SYEVVTQAPSV SVSPGQTASI TCSGDKLGDK YACWYQQKPG QSPVLIYQD SKRPSGIPER 60  
 FSGSNNSGNTA TLTISGTQAM DEADYYCQAW DSSTAVFGGG TKLTVL 106

SEQ ID NO: 276 moltype = AA length = 107  
 FEATURE Location/Qualifiers  
 source 1..107  
 mol\_type = protein  
 organism = Homo sapiens

SEQUENCE: 276  
 DIQMTQSPSS LSASVGDRVT ITCRASQGIR NNLLGWYQQKP GKAPKRLIYA ASSLQSGVPS 60  
 RFSGSGSGTE FTLTISSSLQP EDFTTYYCLQ HNSYPWTFGQ GTKVEIK 107

SEQ ID NO: 277 moltype = AA length = 107  
 FEATURE Location/Qualifiers  
 source 1..107  
 mol\_type = protein  
 organism = Homo sapiens

SEQUENCE: 277  
 DIQMTQSPSS LSASVGDRVT ITCRASQGIR NDLGWYQQKP GKAPKRLIYA ASSLQSGVPS 60  
 RFSGSGSGTE FTLTISSSLQP EDFATYYCQD QNTYPLTFGG GTKVEIK 107

SEQ ID NO: 278 moltype = AA length = 122  
 FEATURE Location/Qualifiers  
 source 1..122  
 mol\_type = protein  
 organism = Homo sapiens

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SEQUENCE: 278  
 QVQLVQSGAE VKKPGASVKV SCKASGYTFT SYGLSWVRQA PGQGLEWMGW IIPYNGNTNS 60  
 AQKLQGRVTM TTDSTSTAY MELRSLRSDD TAVYFCARDR DYGVNYDAFD IWGQGTMVTV 120  
 SS 122

SEQ ID NO: 279 moltype = AA length = 124  
 FEATURE Location/Qualifiers  
 source 1..124  
 mol\_type = protein  
 organism = Homo sapiens

SEQUENCE: 279  
 QVQLVESGGG VVQPGRLRL SCAASGFTFS SYGMHWVRQA PGKGLEWVAV IWYDGSNKYH 60  
 ADSVKGRFTI SRDNKNTLY LQVNSLRAED TAVYYCVRSR NWNYDNYYYG LDVWGQGTTV 120  
 TVSS 124

SEQ ID NO: 280 moltype = AA length = 123  
 FEATURE Location/Qualifiers  
 source 1..123  
 mol\_type = protein  
 organism = Homo sapiens

SEQUENCE: 280  
 EVQLVESGGG LVQPGGSLRL SCAASGFTFS SYWMSWVRQA PGKGLECVAN IKQDGSEEEY 60  
 VDSVKGRFTI SRDNAKNSLY LQMNSLRAED TAVYYCARGS SSWYYYNYGM DVWGQGTTV 120  
 VSS 123

SEQ ID NO: 281 moltype = AA length = 11  
 FEATURE Location/Qualifiers  
 source 1..11  
 mol\_type = protein  
 organism = Homo sapiens

SEQUENCE: 281  
 RASQGIRNNL G 11

SEQ ID NO: 282 moltype = AA length = 11  
 FEATURE Location/Qualifiers  
 source 1..11  
 mol\_type = protein  
 organism = Homo sapiens

SEQUENCE: 282  
 RASQGIRNDL G 11

SEQ ID NO: 283 moltype = AA length = 7  
 FEATURE Location/Qualifiers  
 source 1..7  
 mol\_type = protein  
 organism = Homo sapiens

SEQUENCE: 283  
 AASSLQS 7

SEQ ID NO: 284 moltype = AA length = 9  
 FEATURE Location/Qualifiers  
 source 1..9  
 mol\_type = protein  
 organism = Homo sapiens

SEQUENCE: 284  
 RQQNTYPLT 9

SEQ ID NO: 285 moltype = AA length = 10  
 FEATURE Location/Qualifiers  
 source 1..10  
 mol\_type = protein  
 organism = Homo sapiens

SEQUENCE: 285  
 GFTFSSYGMH 10

SEQ ID NO: 286 moltype = AA length = 10  
 FEATURE Location/Qualifiers  
 source 1..10  
 mol\_type = protein  
 organism = Homo sapiens

SEQUENCE: 286  
 GFTFSSYWMS 10

SEQ ID NO: 287 moltype = AA length = 17  
 FEATURE Location/Qualifiers  
 source 1..17  
 mol\_type = protein  
 organism = Homo sapiens

SEQUENCE: 287

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NIKQDGSEYY YVDSVKKG	17
SEQ ID NO: 288	moltype = AA length = 14
FEATURE	Location/Qualifiers
source	1..14
	mol_type = protein
	organism = Homo sapiens
SEQUENCE: 288	
GSSSWYYYYN GMDV	14
SEQ ID NO: 289	moltype = AA length = 5
FEATURE	Location/Qualifiers
REGION	1..5
	note = Description of Artificial Sequence: Synthetic peptide
source	1..5
	mol_type = protein
	organism = synthetic construct
SEQUENCE: 289	
GGGGG	5
SEQ ID NO: 290	moltype = AA length = 8
FEATURE	Location/Qualifiers
REGION	1..8
	note = Description of Artificial Sequence: Synthetic peptide
source	1..8
	mol_type = protein
	organism = synthetic construct
SEQUENCE: 290	
GGGGGGGG	8
SEQ ID NO: 291	moltype = DNA length = 21
FEATURE	Location/Qualifiers
misc_feature	1..21
	note = Description of Artificial Sequence: Synthetic primer
source	1..21
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 291	
gaaaaggagc agtcgcacag a	21
SEQ ID NO: 292	moltype = DNA length = 19
FEATURE	Location/Qualifiers
misc_feature	1..19
	note = Description of Artificial Sequence: Synthetic primer
source	1..19
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 292	
tttctgggtgg gagtagcgcc	19
SEQ ID NO: 293	moltype = DNA length = 20
FEATURE	Location/Qualifiers
misc_feature	1..20
	note = Description of Artificial Sequence: Synthetic probe
source	1..20
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 293	
atgctgcagg cccggcagtc	20
SEQ ID NO: 294	moltype = DNA length = 21
FEATURE	Location/Qualifiers
misc_feature	1..21
	note = Description of Artificial Sequence: Synthetic primer
source	1..21
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 294	
cccttgctt ggctgagagg a	21
SEQ ID NO: 295	moltype = DNA length = 20
FEATURE	Location/Qualifiers
misc_feature	1..20
	note = Description of Artificial Sequence: Synthetic primer
source	1..20
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 295	
tcacaggatcg tcgttaggtcg	20

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SEQ ID NO: 296      moltype = DNA length = 17
FEATURE
misc_feature
source
SEQUENCE: 296      note = Description of Artificial Sequence: Synthetic primer
tgccgggg agaagag               1..17
                                         mol_type = other DNA
                                         organism = synthetic construct
                                         17

SEQ ID NO: 297      moltype = DNA length = 21
FEATURE
misc_feature
source
SEQUENCE: 297      note = Description of Artificial Sequence: Synthetic primer
tacagtagtg gggtgagggtt c               1..21
                                         mol_type = other DNA
                                         organism = synthetic construct
                                         21

SEQ ID NO: 298      moltype = DNA length = 33
FEATURE
source
SEQUENCE: 298      note = Description of Artificial Sequence: Homo sapiens
tctggagata aattggggga taaatatgtc tgt               1..33
                                         mol_type = other DNA
                                         organism = Homo sapiens
                                         33

SEQ ID NO: 299      moltype = DNA length = 21
FEATURE
source
SEQUENCE: 299      note = Description of Artificial Sequence: Homo sapiens
caagatagca agcgccctc a               1..21
                                         mol_type = other DNA
                                         organism = Homo sapiens
                                         21

SEQ ID NO: 300      moltype = DNA length = 27
FEATURE
source
SEQUENCE: 300      note = Description of Artificial Sequence: Homo sapiens
caggcgtggg acagcagcac tgccgta               1..27
                                         mol_type = other DNA
                                         organism = Homo sapiens
                                         27

SEQ ID NO: 301      moltype = DNA length = 30
FEATURE
source
SEQUENCE: 301      note = Description of Artificial Sequence: Homo sapiens
ggttacacct ttaccagttt tggtctcago               1..30
                                         mol_type = other DNA
                                         organism = Homo sapiens
                                         30

SEQ ID NO: 302      moltype = DNA length = 51
FEATURE
source
SEQUENCE: 302      note = Description of Artificial Sequence: Homo sapiens
tggatcatcc cttacaatgg taacacaaac tctgcacaga aactccaggg c               1..51
                                         mol_type = other DNA
                                         organism = Homo sapiens
                                         51

SEQ ID NO: 303      moltype = DNA length = 39
FEATURE
source
SEQUENCE: 303      note = Description of Artificial Sequence: Homo sapiens
gacagggact acgggtgtcaa ttatgtatc tttgatatac               1..39
                                         mol_type = other DNA
                                         organism = Homo sapiens
                                         39

SEQ ID NO: 304      moltype = DNA length = 33
FEATURE
source
SEQUENCE: 304      note = Description of Artificial Sequence: Homo sapiens
cgggcaagtc agggcattag aaataattt ggc               1..33
                                         mol_type = other DNA
                                         organism = Homo sapiens
                                         33

SEQ ID NO: 305      moltype = DNA length = 21
FEATURE

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source          1..21
               mol_type = other DNA
               organism = Homo sapiens
SEQUENCE: 305
gctgcatcca gtttgcaag t                               21

SEQ ID NO: 306      moltype = DNA  length = 30
FEATURE           Location/Qualifiers
source            1..30
               mol_type = other DNA
               organism = Homo sapiens
SEQUENCE: 306
ggattcacct tcagtagtta cggcatgcac                   30

SEQ ID NO: 307      moltype = DNA  length = 33
FEATURE           Location/Qualifiers
source            1..33
               mol_type = other DNA
               organism = Homo sapiens
SEQUENCE: 307
cgggcaagtc agggcattag aatgatttta ggc                  33

SEQ ID NO: 308      moltype = DNA  length = 21
FEATURE           Location/Qualifiers
source            1..21
               mol_type = other DNA
               organism = Homo sapiens
SEQUENCE: 308
gctgcatcca gtttgcaag t                               21

SEQ ID NO: 309      moltype = DNA  length = 27
FEATURE           Location/Qualifiers
source            1..27
               mol_type = other DNA
               organism = Homo sapiens
SEQUENCE: 309
cgacagcaaa atacttaccc gctcact                     27

SEQ ID NO: 310      moltype = DNA  length = 30
FEATURE           Location/Qualifiers
source            1..30
               mol_type = other DNA
               organism = Homo sapiens
SEQUENCE: 310
ggattcacct ttagtagtta ttggatgagc                   30

SEQ ID NO: 311      moltype = DNA  length = 51
FEATURE           Location/Qualifiers
source            1..51
               mol_type = other DNA
               organism = Homo sapiens
SEQUENCE: 311
aacataaaagc aagatggaag tgagaaatac tatgtggact ctgtgaaggg c   51

SEQ ID NO: 312      moltype = DNA  length = 42
FEATURE           Location/Qualifiers
source            1..42
               mol_type = other DNA
               organism = Homo sapiens
SEQUENCE: 312
ggttagcagca gctggtacta ctacaactac ggtatggacg tc   42

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The invention claimed is:

1. A method for treating serous ovarian cancer in a subject 55  
in need thereof comprising administering a therapeutically  
effective amount of an anti-activin-A antibody to the subject  
wherein the anti-activin-A antibody comprises:

(a) a light chain variable domain sequence comprising a  
sequence of amino acids of antibody A1 (SEQ ID NO:  
267);

(b) a heavy chain variable domain sequence a sequence of  
amino acids of a heavy chain antibody A1 (SEQ ID  
NO: 268).

2. The method of claim 1 wherein the patient is also  
treated with capecitabine.

3. The method of claim 1 wherein the patient is also treated  
with a doxorubicin liquid complex.

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