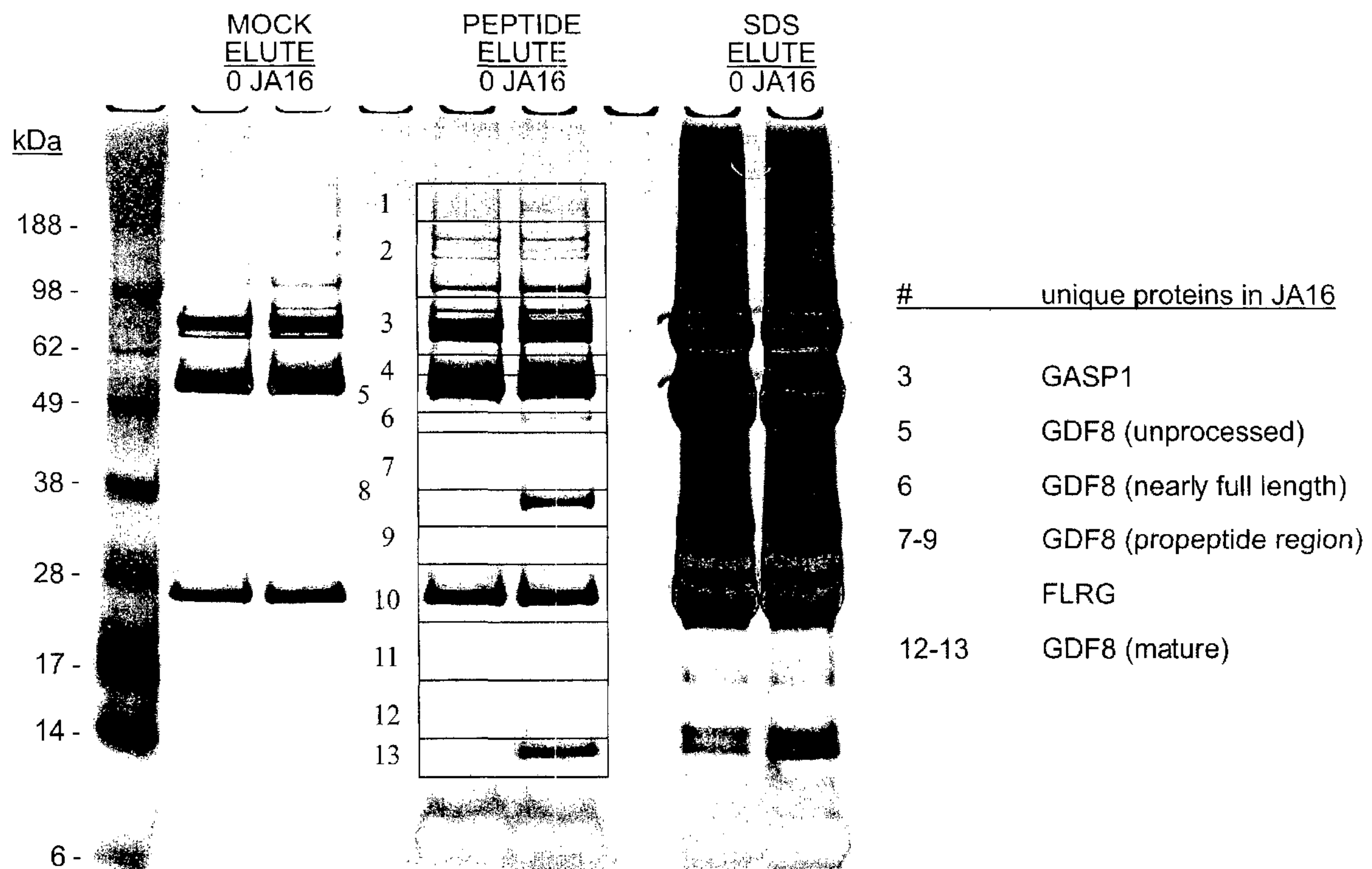




(86) Date de dépôt PCT/PCT Filing Date: 2003/02/21
(87) Date publication PCT/PCT Publication Date: 2003/09/04
(85) Entrée phase nationale/National Entry: 2004/08/17
(86) N° demande PCT/PCT Application No.: US 2003/005150
(87) N° publication PCT/PCT Publication No.: 2003/072714
(30) Priorités/Priorities: 2002/02/21 (60/357,846) US;
2002/12/20 (60/434,645) US

(51) Cl.Int.⁷/Int.Cl.⁷ A61K 38/17, A61K 31/7088, A61K 48/00,
A61P 21/00, C12P 21/02, C12N 15/63
(71) Demandeur/Applicant:
WYETH, US
(72) Inventeurs/Inventors:
HILL, JENNIFER J., US;
WOLFMAN, NEIL M., US
(74) Agent: SMART & BIGGAR

(54) Titre : DOMAINE FOLLISTATINE CONTENANT DES PROTEINES
(54) Title: FOLLISTATIN DOMAIN CONTAINING PROTEINS



(57) Abrégé/Abstract:

The present invention relates to the use of proteins comprising at least one follistatin domain to modulate the level or activity of growth and differentiation factor-8 (GDF-8). More particularly, the invention relates to the use of proteins comprising at least one follistatin domain, excluding follistatin itself, for treating disorders that are related to modulation of the level or activity of GDF-8. The invention is useful for treating muscular diseases and disorders, particularly those in which an increase in muscle tissue would be therapeutically beneficial. The invention is also useful for treating diseases and disorders related to metabolism, adipose tissue, and bone degeneration.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
4 September 2003 (04.09.2003)

PCT

(10) International Publication Number
WO 03/072714 A2

(51) International Patent Classification⁷: C12N

(21) International Application Number: PCT/US03/05150

(22) International Filing Date: 21 February 2003 (21.02.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/357,846 21 February 2002 (21.02.2002) US
60/434,645 20 December 2002 (20.12.2002) US

(71) Applicant (for all designated States except US): **WYETH**
[US/US]; Five Giralda Farms, Madison, NJ 07940 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **HILL, Jennifer, J.**
[US/—]; Apt. 16, 157R Summer Street, Somerville, MA
02143 (US). **WOLFMAN, Neil, M.** [US/US]; 5 Phillips
Lane, Dover, MA 02030 (US).

(74) Agent: **GARRETT, Arthur, S.**; Finnegan, Henderson,
Farabow, Garrett & Dunner, L.L.P., 1300 I Street NW,
Washington, DC 20005-3315 (US).

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU,
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU,
CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,
GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC,
LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW,
MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE,
SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ,
VC, VN, YU, ZA, ZM, ZW.

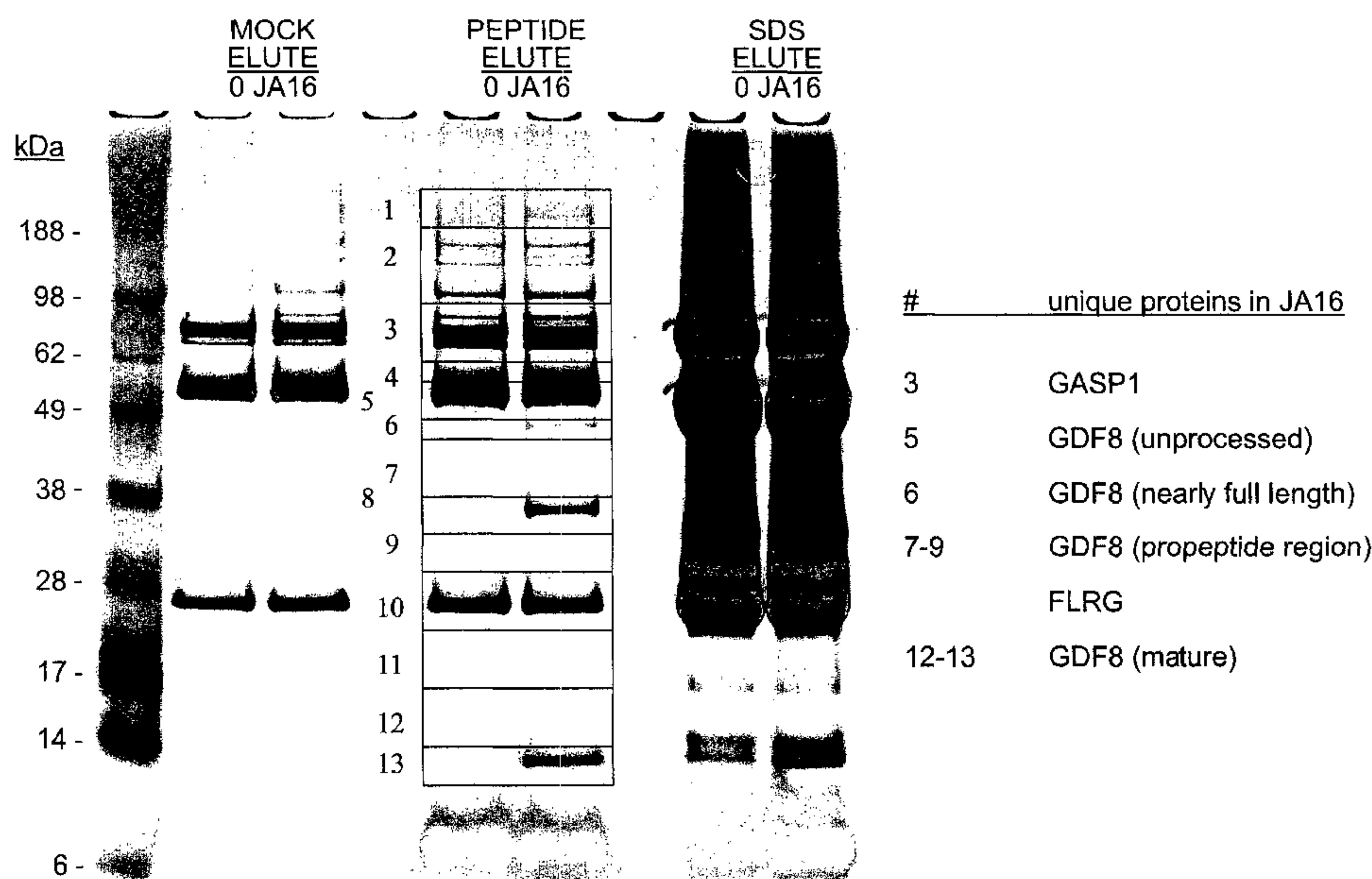
(84) Designated States (*regional*): ARIPO patent (GH, GM,
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW),
Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),
European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE,
ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, SE, SI,
SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN,
GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— without international search report and to be republished
upon receipt of that report

[Continued on next page]

(54) Title: FOLLISTATIN DOMAIN CONTAINING PROTEINS



(57) Abstract: The present invention relates to the use of proteins comprising at least one follistatin domain to modulate the level or activity of growth and differentiation factor-8 (GDF-8). More particularly, the invention relates to the use of proteins comprising at least one follistatin domain, excluding follistatin itself, for treating disorders that are related to modulation of the level or activity of GDF-8. The invention is useful for treating muscular diseases and disorders, particularly those in which an increase in muscle tissue would be therapeutically beneficial. The invention is also useful for treating diseases and disorders related to metabolism, adipose tissue, and bone degeneration.

WO 03/072714 A2

WO 03/072714 A2



For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

FOLLISTATIN DOMAIN CONTAINING PROTEINS

[001] This application claims the benefit of U.S. Provisional Application No. 60/357,846, filed February 21, 2002, and U.S. Provisional Application No. 60/434,645, filed December 20, 2002.

Field of the Invention

[002] The present invention relates to the use of proteins comprising at least one follistatin domain to modulate the level or activity of growth and differentiation factor-8 (GDF-8). More particularly, the invention relates to the use of proteins comprising at least one follistatin domain, excluding follistatin itself, for treating disorders that are related to modulation of the level or activity of GDF-8. The invention is useful for treating muscular diseases and disorders, particularly those in which an increase in muscle tissue would be therapeutically beneficial. The invention is also useful for treating diseases and disorders related to metabolism, adipose tissue, and bone degeneration.

Background of the Invention

[003] Growth and differentiation factor-8 (GDF-8), also known as myostatin, is a member of the transforming growth factor-beta (TGF- β) superfamily of structurally related growth factors, all of which possess important physiological growth-regulatory and morphogenetic properties (Kingsley *et al.* (1994) *Genes Dev.*, 8: 133-46; Hoodless *et al.* (1998) *Curr. Topics Microbiol. Immunol.*, 228: 235-72). GDF-8 is a negative regulator of skeletal muscle mass, and there is considerable interest in identifying factors which regulate its biological activity. For example, GDF-8 is highly expressed in the developing and adult skeletal muscle. The GDF-8 null mutation in transgenic mice is characterized by a marked hypertrophy and hyperplasia of the skeletal muscle (McPherron *et al.* (1997) *Nature*, 387: 83-90). Similar increases in skeletal muscle mass are evident in naturally occurring mutations of GDF-8 in cattle (Ashmore *et al.* (1974) *Growth*, 38: 501-507; Swatland and Kieffer (1994) *J. Anim. Sci.*, 38: 752-757; McPherron and Lee (1997) *Proc. Nat. Acad. Sci. U.S.A.*, 94: 12457-12461; and Kambadur *et al.* (1997) *Genome Res.*, 7: 910-915). Recent studies have also shown that muscle wasting associated with HIV-infection in humans is accompanied by increases

in GDF-8 protein expression (Gonzalez-Cadavid *et al.* (1998) *Proc. Natl. Acad. Sci. U.S.A.*, 95: 14938-43). In addition, GDF-8 can modulate the production of muscle-specific enzymes (e.g., creatine kinase) and modulate myoblast cell proliferation (WO 00/43781).

[004] A number of human and animal disorders are associated with loss of or functionally impaired muscle tissue. To date, very few reliable or effective therapies exist for these disorders. However, the terrible symptoms associated with these disorders may be substantially reduced by employing therapies that increase the amount of muscle tissue in patients suffering from the disorders. While not curing the conditions, such therapies would significantly improve the quality of life for these patients and could ameliorate some of the effects of these diseases. Thus, there is a need in the art to identify new therapies that may contribute to an overall increase in muscle tissue in patients suffering from these disorders.

[005] In addition to its growth-regulatory and morphogenetic properties in skeletal muscle, GDF-8 may also be involved in a number of other physiological processes (e.g., glucose homeostasis), as well as abnormal conditions, such as in the development of type 2 diabetes and adipose tissue disorders, such as obesity. For example, GDF-8 modulates preadipocyte differentiation to adipocytes (Kim *et al.* (2001) *B.B.R.C.* 281: 902-906). Thus, modulation of GDF-8 may be useful for treating these diseases, as well.

[006] The GDF-8 protein is synthesized as a precursor protein consisting of an amino-terminal propeptide and a carboxy-terminal mature domain (McPherron and Lee, (1997) *Proc. Nat. Acad. Sci. U.S.A.*, 94: 12457-12461). Before cleavage, the precursor GDF-8 protein forms a homodimer. The amino-terminal propeptide is then cleaved from the mature domain. The cleaved propeptide may remain noncovalently bound to the mature domain dimer, inactivating its biological activity (Miyazono *et al.* (1988) *J. Biol. Chem.*, 263: 6407-6415; Wakefield *et al.* (1988) *J. Biol. Chem.*, 263: 7646-7654; and Brown *et al.* (1990) *Growth Factors*, 3: 35-43). It is believed that two GDF-8 propeptides bind to the GDF-8 mature dimer (Thies *et al.* (2001) *Growth*

Factors, 18: 251-259). Due to this inactivating property, the propeptide is known as the "latency-associated peptide" (LAP), and the complex of mature domain and propeptide is commonly referred to as the "small latent complex" (Gentry and Nash (1990) *Biochemistry*, 29:6851-6857; Derynck *et al.* (1995) *Nature*, 316:701-705; and Massague (1990) *Ann. Rev. Cell Biol.*, 12: 597-641). Other proteins are also known to bind to GDF-8 or structurally related proteins and inhibit their biological activity. Such inhibitory proteins include follistatin (Gamer *et al.* (1999) *Dev. Biol.*, 208: 222-232). The mature domain of GDF-8 is believed to be active as a homodimer when the propeptide is removed.

[007] Clearly, GDF-8 is involved in the regulation of many critical biological processes. Due to its key function in these processes, GDF-8 may be a desirable target for therapeutic intervention. In particular, therapeutic agents that inhibit the activity of GDF-8 may be used to treat human or animal disorders in which an increase in muscle tissue would be therapeutically beneficial.

[008] Known proteins comprising at least one follistatin domain play roles in many biological processes, particularly in the regulation of TGF- β superfamily signaling and the regulation of extracellular matrix-mediated processes such as cell adhesion. Follistatin, follistatin related gene (FLRG, FSRP), and follistatin-related protein (FRP) have all been linked to TGF- β signaling, either through transcriptional regulation by TGF- β (Bartholin *et al.* (2001) *Oncogene*, 20: 5409-5419; Shibamura *et al.* (1993) *Eur. J. Biochem.* 217: 13-19) or by their ability to antagonize TGF- β signaling pathways (Phillips and de Kretser (1998) *Front. Neuroendocrin.*, 19: 287-322; Tsuchida *et al.* (2000) *J. Biol. Chem.*, 275: 40788-40796; Patel *et al.* (1996) *Dev. Biol.*, 178: 327-342; Amthor *et al.* (1996) *Dev. Biol.*, 178: 343-362). Protein names in parentheses are alternative names.

[009] Insulin growth factor binding protein 7 (IGFBP7, mac25), which comprise at least one follistatin domain, binds to insulin and blocks subsequent interaction with the insulin receptor. In addition, IGFBP7 has

been shown to bind to activin, a TGF- β family member (Kato (2000) *Mol. Med.*, 6: 126-135).

[010] Agrins and agrin related proteins contain upwards of nine follistatin domains and are secreted from nerve cells to promote the aggregation of acetylcholine receptors and other molecules involved in the formation of synapses. It has been suggested that the follistatin domains may serve to localize growth factors to the synapse (Patthy *et al.* (1993) *Trends Neurosci.*, 16: 76-81).

[011] Osteonectin (SPARC, BM40) and hevin (SC1, mast9, QR1) are closely related proteins that interact with extracellular matrix proteins and regulate cell growth and adhesion (Motamed (1999) *Int. J. Biochem. Cell. Biol.*, 31: 1363-1366; Girard and Springer (1996) *J. Biol. Chem.*, 271: 4511-4517). These proteins comprise at least one follistatin domain.

[012] Other follistatin domain proteins have been described or uncovered from the NCBI database (National Center for Biotechnology Information, Bethesda, Maryland, USA), however their functions are presently unknown. These proteins include U19878 (G01639, very similar to tomoregulin-1), T46914, human GASP1 (GDF-associated serum protein 1; described herein; Figure 7), human GASP2 (WFIKKN; Trexler *et al.* (2001) *Proc. Natl. Acad. Sci. U.S.A.*, 98: 3705-3709; Figure 9), and the proteoglycan family of testican (SPOCK) proteins (Alliel *et al.* (1993) *Eur. J. Biochem.*, 214: 347-350). Amino acid and nucleotide sequences for mouse GASP1 (Figure 6) and mouse GASP2 (Figure 8) were also determined from the Celera database (Rockville, MD). As described herein, the nucleotide sequence of cloned mouse GASP1 matched the predicted Celera sequence, with the exception of some base substitutions in wobble codons that did not change the predicted amino acid sequence (see Figure 13).

Summary of the Invention

[013] Accordingly, the invention relates to proteins, other than follistatin, comprising a unique structural feature, namely, the presence of at least one follistatin domain. Follistatin itself is not encompassed by the invention. The proteins comprising at least one follistatin domain are

specifically reactive with a mature GDF-8 protein or a fragment thereof, whether the GDF-8 protein is in monomeric form, a dimeric active form, or complexed in the GDF-8 latent complex. Proteins comprising at least one follistatin domain may bind to an epitope on the mature GDF-8 protein that results in a reduction in one or more of the biological activities associated with GDF-8, relative to a mature GDF-8 protein that is not bound by the same protein.

[014] The present invention provides methods for modulating the effects of GDF-8 on cells. Such methods comprise administering an effective amount of a protein comprising at least one follistatin domain. The present invention also encompasses methods for expressing a protein in a cell by administering a DNA molecule encoding a protein comprising at least one follistatin domain.

[015] According to the invention, proteins comprising at least one follistatin domain may be administered to a patient, in a therapeutically effective dose, to treat or prevent medical conditions in which an increase in muscle tissue would be therapeutically beneficial. Embodiments include treatment of diseases, disorders, and injuries involving cells and tissue that are associated with the production, metabolism, or activity of GDF-8.

[016] Proteins comprising at least one follistatin domain may be prepared in a pharmaceutical preparation. The pharmaceutical preparation may contain other components that aid in the binding of the mature GDF-8 protein or fragments thereof, whether it is in monomeric form, dimeric active form, or complexed in the GDF-8 latent complex.

[017] In addition, proteins comprising at least one follistatin domain may be used as a diagnostic tool to quantitatively or qualitatively detect mature GDF-8 protein or fragments thereof, whether it is in monomeric form, dimeric active form, or complexed in the GDF-8 latent complex. For example, proteins comprising at least one follistatin domain may be used to detect the presence, absence, or amount of GDF-8 protein in a cell, bodily fluid, tissue, or organism. The presence or amount of mature GDF-8 protein detected may be correlated with one or more of the medical conditions listed herein.

[018] Proteins comprising at least one follistatin domain may be provided in a diagnostic kit to detect mature GDF-8 protein or fragments thereof, whether it is in monomeric form, dimeric active form, or complexed in the GDF-8 latent complex, and help correlate the results with one or more of the medical conditions described herein. Such a kit may comprise at least one protein comprising at least one follistatin domain, whether it is labeled or unlabeled, and at least one agent that bind to this proteins, such as a labeled antibody. The kit may also include the appropriate biological standards and control samples to which one could compare the results of the experimental detection. It may also include buffers or washing solutions and instructions for using the kit. Structural components may be included on which one may carry out the experiment, such as sticks, beads, papers, columns, vials, or gels.

Brief Description of the Figures

[019] Figure 1 shows antibody purification of the GDF-8 complex from wild-type mouse serum. A silver stained reducing gel shows proteins purified from wild type mouse serum using the JA16 monoclonal antibody covalently coupled to agarose beads. A control purification (0) with mock-coupled beads was performed in parallel. Subsequent elutions with buffer (mock elute), a competing peptide, and SDS sample buffer revealed two visible protein bands which were specifically eluted with peptide from the JA16-conjugated beads (indicated by arrows).

[020] Figure 2 shows the identification of mature and unprocessed GDF-8 in affinity purified samples from normal mouse serum. Figure 2A shows a representative MS/MS spectrum of a GDF-8 derived peptide (SEQ ID NO:19) identified from the 12 kDa band visible in the affinity purified sample. Both N-terminal fragment ions (b ions) and C-terminal fragment ions (y ions) are visible. Notably, the most intense y fragment ions result from fragmentation before the proline residue, a common characteristic of proline containing peptides. Figure 2B shows a western blot probed with a polyclonal antibody that recognizes the mature region of GDF-8, confirming the presence of GDF-8 in the affinity purified samples. Both the mature and unprocessed forms of GDF-8 are visible.

[021] Figure 3 shows the GDF-8 propeptide and follistatin-like related gene (FLRG) bind to circulating GDF-8 isolated from normal mouse serum. Representative MS/MS spectra from GDF-8 propeptide (SEQ ID NO:23) (Figure 3A) and FLRG (SEQ ID NO:30) (Figure 3C) derived peptides identified in the 36 kDa band are shown. Figure 3B shows a western blot of affinity purified GDF-8 complex probed with a polyclonal antibody that specifically recognizes the propeptide region of GDF-8, confirming the mass spectrometric identification of this protein in the GDF-8 complex. Both the clipped propeptide and unprocessed GDF-8 are visible -- at longer exposures, unprocessed GDF-8 can also be seen in the SDS eluted sample. Figure 3D shows a western blot of affinity purified GDF-8 complex probed with a monoclonal antibody to FLRG.

[022] Figure 4 shows results from a thorough analysis of a large scale GDF-8 purification that identified GDF-8 propeptide, FLRG, and a novel protein as the major GDF-8 binding proteins in serum. A silver stained gel was dissected into 13 slices from the peptide eluted sample of both negative control and JA16 immunoprecipitates. The proteins in each slice were digested with trypsin and identified using nanoflow-LC-MS/MS and database searching. Proteins unique to the JA16 sample included only unprocessed and mature GDF-8, GDF-8 propeptide, FLRG, and a novel multidomain protease inhibitor (GDF-associated serum protein 1, GASP1). These proteins were identified from the noted regions of the gel.

[023] Figure 5 shows that a novel multidomain protease inhibitor, GASP1, is bound to GDF-8 in serum. Figures 5A (peptide assigned SEQ ID NO:31) and 5B (peptide assigned SEQ ID NO:33) show representative MS/MS spectra from two GASP1 peptides, identified in band 3 of the silver stained gel of Figure 4.

[024] Figure 6A shows the predicted nucleotide sequence to mouse GASP1. Figure 6B shows a predicted alternative nucleotide sequence to mouse GASP1. Figure 6C shows the predicted amino acid sequence encoded by the nucleotide sequences shown in Figures 6A and 6B. The protein sequences encoded by the two nucleotide sequences are identical

because the nucleotide differences are all in wobble codon positions. The follistatin domain is shown in bold and underlined.

[025] Figure 7A shows the predicted nucleotide sequence of human GASP1. Figure 7B shows the corresponding predicted amino acid sequence. The follistatin domain is shown in bold and underlined. Figure 7C shows the predicted nucleotide sequence of human GASP1 using an alternative start site. Figure 7D shows the corresponding predicted amino acid sequence. The follistatin domain is shown in bold and underlined. The end of the sequence is denoted by the asterisk.

[026] Figure 8A shows the predicted nucleotide sequence to mouse GASP2, while Figure 8B shows the corresponding predicted amino acid sequence. The follistatin domain is shown in bold and underlined.

[027] Figure 9A shows the predicted nucleotide sequence to human GASP2, while Figure 9B shows the corresponding predicted amino acid sequence. The follistatin domain is shown in bold and underlined.

[028] Figure 10 shows that mouse GASP1 is expressed in many adult tissues and during development. The figure shows tissue expression profiles of mouse GASP1. A 551 bp fragment of GASP1 was amplified from normalized first-strand cDNA panels from Clontech (Palo Alto, CA). A portion of glyceraldehyde-3-phosphate dehydrogenase (G3PDH) was amplified as a control. G3PDH expression is known to be high in skeletal muscle and low in testis. The cDNA panels were normalized against β -actin, phospholipase A2, and ribosomal protein S29, in addition to G3PDH.

[029] Figure 11 shows proteins isolated from human serum. Proteins from a JA16 immunoprecipitate or a control sample (0) were eluted in a mock PBS elution, a competing peptide elution, or a SDS elution. The proteins in the indicated regions of the gel were digested with trypsin and analyzed by LS-MS/MS and database searching. The proteins present in the JA16 sample but not in the control sample were mature GDF-8 (band 16), GDF-8 propeptide and FLRG (band 11), and human GASP1 (band 4). Figure 11B shows a western blot of an identical JA16 immunoprecipitate probed with an

antibody that recognizes mature GDF-8. Bands corresponding to mature and unprocessed GDF-8 isolated from human serum are visible.

[030] Figure 12 shows representative mass spectra of a peptide derived from GDF-8 and associated proteins isolated from bands 4, 11, and 16 (Figure 11). The peptide sequence and N-terminal (b ions) and C-terminal (y ions) are shown. A complete listing of identified peptides is provided in Table 1. Spectra are shown from a GASP1 peptide (SEQ ID NO:44) (Figure 12A), a FLRG peptide (SEQ ID NO:41) (Figure 12B), a GDF-8 propeptide peptide (SEQ ID NO:24) (Figure 12C), and a mature GDF-8 peptide (SEQ ID NO:13) (Figure 12D).

[031] Figure 13 shows the nucleotide (SEQ ID NO:48) and amino acid (SEQ ID NO:49) sequences of cloned mouse GASP1. The peptides identified by mass spectrometry in JA16 affinity-purified samples are underlined. The end of the sequence is denoted by the asterisk.

[032] Figure 14A shows the domain structure of GASP1. GASP1 has a signal sequence/cleavage site after amino acid 29. In addition, GASP1 contains two Kunitz/BPTI serine protease inhibitor domains, a follistatin domain (including a Kazal serine protease inhibitor motif) and a netrin domain, which may inhibit metalloproteases. Figure 14B shows the phylogenetic tree of GASP1 and GASP2 predicted from the mouse and human genomic sequences. Mouse and human GASP1 are 90% identical. GASP1 and GASP2 are 54% identical.

[033] Figure 15 shows that recombinantly-produced GASP1 binds separately to both GDF-8 and GDF-8 propeptide. (A) JA16 was used to immunoprecipitate GDF-8 from mock- or GASP1-V5-His transfected COS cell conditioned media supplemented with recombinant purified GDF-8 and/or propeptide. Western blots with anti-V5 (top panel), anti-GDF-8 (middle panel), or anti-propeptide polyclonal antibodies were used to determine whether these proteins were present in the immunoprecipitate. (B) Recombinantly-produced GASP1 protein was immunoprecipitated by anti-V5 tag antibodies from mock- or GASP1-V5-His conditioned media supplemented

with recombinant purified GDF-8 and/or propeptide. The immunoprecipitate was analyzed by western blotting as in (A).

[034] Figure 16 shows that GASP1 inhibits the biological activity of GDF-8 and the highly related BMP-11, but not activin or TGF- β . Various dilutions of conditioned media from mock (open circles) or GASP1-V5-His (filled squares) transfectants were incubated with (A) 10 ng/ml GDF-8, (B) 10 ng/ml BMP-11, (C) 10 ng/ml activin, or (D) 0.5 ng/ml TGF- β . These samples were then subjected to a luciferase reporter activity assay in A204 (A-C) or RD (D) cells to determine the activity of the added growth factors. Luciferase activity is shown in relative luciferase units. The activity resulting from each of the growth factors alone is shown by the filled diamonds and short dashed line. Without addition of any growth factor, the background activity in the assay is low, as shown by the long dashed line with no symbols.

[035] Figure 17 shows the potency of GASP1 inhibition of GDF-8. Purified GASP1 was tested for its ability to inhibit 20 ng/ml of myostatin in the (CAGA)₁₂ (SEQ ID NO:53) luciferase reporter assay in RD cells (filled squares). The activity resulting from GDF-8 alone is shown by the filled diamonds and short dashed line. The activity present when no growth factors are added is shown by the long dashed line.

Definitions

[036] The term “**folistatin domain**” refers to an amino acid domain or a nucleotide domain encoding for an amino acid domain, characterized by cysteine rich repeats. A folistatin domain typically encompasses a 65-90 amino acid span and contains 10 conserved cysteine residues and a region similar to Kazal serine protease inhibitor domains. In general, the loop regions between the cysteine residues exhibit sequence variability in folistatin domains, but some conservation is evident. The loop between the fourth and fifth cysteines is usually small, containing only 1 or 2 amino acids. The amino acids in the loop between the seventh and eighth cysteines are generally the most highly conserved containing a consensus sequence of (G,A)-(S,N)-(S,N,T)-(D,N)-(G,N) followed by a (T,S)-Y motif. The region between the ninth

and tenth cysteines generally contains a motif containing two hydrophobic residues (specifically V, I, or L) separated by another amino acid.

[037] The term “**protein comprising at least one follistatin domain**” refers to proteins comprising at least one, but possibly more than one follistatin domain. The term also refers to any variants of such proteins (including fragments; proteins with substitution, addition or deletion mutations; and fusion proteins) that maintain the known biological activities associated with the native proteins, especially those pertaining to GDF-8 binding activity, including sequences that have been modified with conservative or non-conservative changes to the amino acid sequence. These proteins may be derived from any source, natural or synthetic. The protein may be human or derived from animal sources, including bovine, chicken, murine, rat, porcine, ovine, turkey, baboon, and fish. Follistatin itself is not encompassed by the invention.

[038] The terms “**GDF-8**” or “**GDF-8 protein**” refer to a specific growth and differentiation factor. The terms include the full length unprocessed precursor form of the protein, as well as the mature and propeptide forms resulting from post-translational cleavage. The terms also refer to any fragments of GDF-8 that maintain the known biological activities associated with the protein, including sequences that have been modified with conservative or non-conservative changes to the amino acid sequence. These GDF-8 molecules may be derived from any source, natural or synthetic. The protein may be human or derived from animal sources, including bovine, chicken, murine, rat, porcine, ovine, turkey, baboon, and fish. Various GDF-8 molecules have been described in McPherron *et al.* (1997) *Proc. Natl. Acad. Sci. USA*, 94: 12457-12461.

[039] “**Mature GDF-8**” refers to the protein that is cleaved from the carboxy-terminal domain of the GDF-8 precursor protein. The mature GDF-8 may be present as a monomer, homodimer, or in a GDF-8 latent complex. Depending on the *in vivo* or *in vitro* conditions, mature GDF-8 may establish an equilibrium between any or all of these different forms. It is believed to be

biologically active as homodimer. In its biologically active form, the mature GDF-8 is also referred to as “**active GDF-8.**”

[040] “**GDF-8 propeptide**” refers to the polypeptide that is cleaved from the amino-terminal domain of the GDF-8 precursor protein. The GDF-8 propeptide is capable of binding to the propeptide binding domain on the mature GDF-8.

[041] “**GDF-8 latent complex**” refers to the complex of proteins formed between the mature GDF-8 homodimer and the GDF-8 propeptide. It is believed that two GDF-8 propeptides associate with the two molecules of mature GDF-8 in the homodimer to form an inactive tetrameric complex. The latent complex may include other GDF inhibitors in place of or in addition to one or more of the GDF-8 propeptides.

[042] The phrase “**GDF-8 activity**” refers to one or more of physiologically growth-regulatory or morphogenetic activities associated with active GDF-8 protein. For example, active GDF-8 is a negative regulator of skeletal muscle. Active GDF-8 can also modulate the production of muscle-specific enzymes (*e.g.*, creatine kinase), stimulate myoblast cell proliferation, and modulate preadipocyte differentiation to adipocytes. GDF-8 is also believed to increase sensitivity to insulin and glucose uptake in peripheral tissues, particularly in skeletal muscle or adipocytes. Accordingly, GDF-8 biological activities include but are not limited to inhibition of muscle formation, inhibition of muscle cell growth, inhibition of muscle development, decrease in muscle mass, regulation of muscle-specific enzymes, inhibition of myoblast cell proliferation, modulation of preadipocyte differentiation to adipocytes, increasing sensitivity to insulin, regulations of glucose uptake, glucose hemostasis, and modulate neuronal cell development and maintenance.

[043] The terms “**isolated**” or “**purified**” refer to a molecule that is substantially free of its natural environment. For instance, an isolated protein is substantially free of cellular material or other contaminating proteins from the cell or tissue source from which it is derived. The phrase “**substantially free of cellular material**” refers to preparations where the isolated protein is

at least 70% to 80% (w/w) pure, at least 80%-89% (w/w) pure, at least 90-95% pure, or at least 96%, 97%, 98%, 99% or 100% (w/w) pure.

[044] The term “**LC-MS/MS**” refers to liquid chromatography in line with a mass spectrometer programmed to isolate a molecular ion of particular mass/charge ratio, fragment this ion, and record the mass/charge ratio of the fragment ions. When analyzing peptide samples this technique allows upstream separation of complex samples through liquid chromatography, followed by the recording of fragment ion masses and subsequent determination of the peptide sequence.

[045] The term “**MS/MS**” refers to the process of using a mass spectrometer to isolate a molecular ion of a particular mass/charge ratio, fragment this ion, and record the mass/charge ratio of the resulting fragment ions. The fragment ions provide information about the sequence of a peptide.

[046] The term “**treating**” or “**treatment**” refers to both therapeutic treatment and prophylactic or preventative measures. Those in need of treatment may include individuals already having a particular medical disorder as well as those who may ultimately acquire the disorder (*i.e.*, those needing preventative measures). The term treatment includes both measures that address the underlying cause of a disorder and measures that reduce symptoms of a medical disorder without necessarily affecting its cause. Thus, improvement of quality of life and amelioration of symptoms are considered treatment, as are measures that counteract the cause of a disorder.

[047] The term “**medical disorder**” refers to disorders of muscle, bone, or glucose homeostasis, and include disorders associated with GDF-8 and/or other members of the TGF- β superfamily (e.g., BMP-11). Examples of such disorders include, but are not limited to, metabolic diseases and disorders such as insulin-dependent (type 1) diabetes mellitus, noninsulin-dependent (type 2) diabetes mellitus, hyperglycemia, impaired glucose tolerance, metabolic syndrome (e.g., syndrome X), and insulin resistance induced by trauma (e.g., burns or nitrogen imbalance), and adipose tissue disorders (e.g., obesity); muscle and neuromuscular disorders such as muscular dystrophy (including but not limited to severe or benign X-linked

muscular dystrophy, limb-girdle dystrophy, facioscapulohumeral dystrophy, myotonic dystrophy, distal muscular dystrophy, progressive dystrophic ophthalmoplegia, oculopharyngeal dystrophy, Duchenne's muscular dystrophy, and Fakuyama-type congenital muscular dystrophy); amyotrophic lateral sclerosis (ALS); muscle atrophy; organ atrophy; frailty; carpal tunnel syndrome; congestive obstructive pulmonary disease; congenital myopathy; myotonia congenital; familial periodic paralysis; paroxysmal myoglobinuria; myasthenia gravis; Eaton-Lambert syndrome; secondary myasthenia; denervation atrophy; paroxymal muscle atrophy; and sarcopenia, cachexia and other muscle wasting syndromes. Other examples include osteoporosis, especially in the elderly and/or postmenopausal women; glucocorticoid-induced osteoporosis; osteopenia; osteoarthritis; osteoporosis-related fractures; and traumatic or chronic injury to muscle tissue. Yet further examples include low bone mass due to chronic glucocorticoid therapy, premature gonadal failure, androgen suppression, vitamin D deficiency, secondary hyperparathyroidism, nutritional deficiencies, and anorexia nervosa.

[048] The term “**increase in mass**” refers to the presence of a greater amount of muscle after treatment with proteins comprising at least one follistatin domain relative to the amount of muscle mass present before the treatment.

[049] The term “**therapeutic benefit**” refers to an improvement of symptoms of a disorder, a slowing of the progression of a disorder, or a cessation in the progression of a disorder. The therapeutic benefit is determined by comparing an aspect of a disorder, such as the amount of muscle mass, before and after at least one protein comprising at last one follistatin domain is administered.

[050] The term “**modulating**” refers to varying a property of a protein by increasing, decreasing, or inhibiting the activity, behavior, or amount of the protein. For example, proteins comprising at least one follistatin domain may modulate GDF-8 by inhibiting its activity.

[051] The term “**stabilizing modification**” is any modification known in the art or set forth herein capable of stabilizing a protein, enhancing the in vitro half life of a protein, enhancing circulatory half life of a protein and/or reducing proteolytic degradation of a protein. Such stabilizing modifications include but are not limited to fusion proteins (including, for example, fusion proteins comprising a protein comprising at least one follistatin domain and a second protein), modification of a glycosylation site (including, for example, addition of a glycosylation site to a protein comprising at least one follistatin domain), and modification of carbohydrate moiety (including, for example, removal of carbohydrate moieties from a protein comprising at least one follistatin domain). In the case of a stabilizing modification which comprises a fusion protein (e.g., such that a second protein is fused to a protein comprising at least one follistatin domain), the second protein may be referred to as a “**stabilizer portion**” or “**stabilizer protein**.” For example, a protein a human protein comprising at least one follistatin domain may be fused with an IgG molecule, wherein IgG acts as the stabilizer protein or stabilizer portion. As used herein, in addition to referring to a second protein of a fusion protein, a “**stabilizer portion**” also includes nonproteinaceous modifications such as a carbohydrate moiety, or nonproteinaceous polymer.

[052] The term “**Fc region of an IgG molecule**” refers to the Fc domain of an immunoglobulin of the isotype IgG, as is well known to those skilled in the art. The Fc region of an IgG molecule is that portion of IgG molecule (IgG1, IgG2, IgG3, and IgG4) that is responsible for increasing the in vivo serum half-life of the IgG molecule.

[053] “**In vitro half life**” refers to the stability of a protein measured outside the context of a living organism. Assays to measure in vitro half life are well known in the art and include but are not limited to SDS-PAGE, ELISA, cell-based assays, pulse-chase, western blotting, northern blotting, etc. These and other useful assays are well known in the art.

[054] “**In vivo half life**” refers to the stability of a protein in an organism. In vivo half life may be measured by a number of methods known

in the art including but not limited to in vivo serum half life, circulatory half life, and assays set forth in the examples herein.

[055] **"In vivo serum half life"** refers to the half-life of a protein circulating in the blood of an organism. Methods known in the art may be used to measure in vivo serum half life. For example, radioactive protein can be administered to an animal and the amount of labeled protein in the serum can be monitored over time.

[056] To assist in the identification of the sequences listed in the specification and figures, the following table is provided, which lists the SEQ ID NO, the figure location, and a description of the sequence.

SEQ ID NO:	REFERENCE	DESCRIPTION
1	Figure 6A	predicted mouse GASP1 nucleotide sequence
2	Figure 6B	predicted mouse GASP1 alternative nucleotide sequence
3	Figure 6C	predicted mouse GASP1 amino acid sequence encoded by both SEQ ID NOS:1 and 2
4	Figure 7A	predicted human GASP1 nucleotide sequence
5	Figure 7B	predicted human GASP1 amino acid sequence encoded by SEQ ID NO:4
6	Figure 7C	predicted human GASP1 nucleotide sequence, alternative start site
7	Figure 7D	predicted human GASP1 amino acid sequence, alternative start site encoded by SEQ ID NO:6
8	Figure 8A	predicted mouse GASP2 nucleotide sequence
9	Figure 8B	predicted mouse GASP2 amino acid sequence encoded by SEQ ID NO:8
10	Figure 9A	predicted human GASP2 nucleotide sequence
11	Figure 9B	predicted human GASP2 amino acid sequence encoded by SEQ ID NO:10
12	Example 2	competing peptide
13-20	Table 1, Examples 5, 6	mouse GDF-8 peptides
21-27	Table 1, Examples 5, 6	mouse GDF-8 propeptide peptides
28-30	Table 1, Example 5	mouse FLRG peptides
31-35	Table 1, Examples 5, 7	mouse GASP1 peptides
36-37	Table 1, Example 8	human GDF-8 peptides
38-39	Table 1, Example 8	human GDF-8 propeptide peptides
40-42	Table 1, Example 8	human FLRG peptides
43-45	Table 1, Example 8	human GASP1 peptides
46	Example 7	forward primer
47	Example 7	reverse primer
48	Figure 13	cloned mouse GASP1 nucleotide sequence
49	Figure 13	cloned mouse GASP1 amino acid sequence encoded by SEQ ID NO:48
50	Example 9	forward primer
51	Example 9	reverse primer
52	Example 9	illustrative N-terminal peptide sequence
53	Example 11	synthetic oligonucleotide

Detailed Description of the Invention**Proteins Comprising At Least One Follistatin Domain**

[057] The present invention relates to proteins, other than follistatin, having a unique structural feature, namely, that they comprise at least one follistatin domain. Follistatin itself is not encompassed by the invention. It is believed that proteins containing at least one follistatin domain will bind and inhibit GDF-8. Examples of proteins having at least one follistatin domain include, but are not limited to follistatin-like related gene (FLRG), FRP (flik, tsc 36), agrins, osteonectin (SPARC, BM40), hevin (SC1, mast9, QR1), IGFBP7 (mac25), and U19878. GASP1, comprising the nucleotide and amino acid sequences provided in Figures 6 and 7, and GASP2, comprising the nucleotide and amino acid sequences provided in Figures 8 and 9, are other examples of proteins comprising at least one follistatin domain.

[058] A follistatin domain, as stated above, is defined as an amino acid domain or a nucleotide domain encoding for an amino acid domain, characterized by cysteine rich repeats. A follistatin domain typically encompasses a 65-90 amino acid span and contains 10 conserved cysteine residues and a region similar to Kazal serine protease inhibitor domains. In general, the loop regions between the cysteine residues exhibit sequence variability in follistatin domains, but some conservation is evident. The loop between the fourth and fifth cysteines is usually small, containing only 1 or 2 amino acids. The amino acids in the loop between the seventh and eighth cysteines are generally the most highly conserved containing a consensus sequence of (G,A)-(S,N)-(S,N,T)-(D,N)-(G,N) followed by a (T,S)-Y motif. The region between the ninth and tenth cysteines generally contains a motif containing two hydrophobic residues (specifically V, I, or L) separated by another amino acid.

[059] Proteins comprising at least one follistatin domain, which may bind GDF-8, may be isolated using a variety of methods. For example, one may use affinity purification using GDF-8, as exemplified in the present invention. In addition, one may use a low stringency screening of a cDNA

library, or use degenerate PCR techniques using a probe directed toward a follistatin domain. As more genomic data becomes available, similarity searching using a number of sequence profiling and analysis programs, such as MotifSearch (Genetics Computer Group, Madison, WI), ProfileSearch (GCG), and BLAST (NCBI) could be used to find novel proteins containing significant homology with known follistatin domains.

[060] One of skill in the art will recognize that both GDF-8 or proteins comprising at least one follistatin domain may contain any number of conservative changes to their respective amino acid sequences without altering their biological properties. Such conservative amino acid modifications are based on the relative similarity of the amino acid side-chain substituents, for example, their hydrophobicity, hydrophilicity, charge, size, and the like. Exemplary conservative substitutions which take various of the foregoing characteristics into consideration are well known to those of skill in the art and include: arginine and lysine; glutamate and aspartate; serine and threonine; glutamine and asparagine; and valine, leucine, and isoleucine. Furthermore, proteins comprising at least one follistatin domain may be used to generate functional fragments comprising at least one follistatin domain. It is expected that such fragments would bind and inhibit GDF-8. In an embodiment of the invention, proteins comprising at least one follistatin domain specifically bind to mature GDF-8 or a fragment thereof, whether it is in monomeric form, active dimer form, or complexed in a GDF-8 latent complex, with an affinity of between 0.001 and 100 nM, or between 0.01 and 10 nM, or between 0.1 and 1 nM.

Nucleotide and Protein Sequences

[061] While not always necessary, if desired, one of ordinary skill in the art may determine the amino acid or nucleic acid sequences of a novel proteins comprising at least one follistatin domain. For example, the present invention provides the amino acid and nucleotide sequences for GASP1 and GASP2, as shown in Figures 6-9.

[062] The present invention also include variants, homologues, and fragments of such nucleic and amino acid sequences. For example, the nucleic or amino acid sequence may comprise a sequence at least 70% to 79% identical to the nucleic or amino acid sequence of the native protein, or at least 80% to 89% identical, or at least 90% to 95% identical, or at least 96% to 100% identical. One of skill in the art will recognize that the region that binds GDF-8 can tolerate less sequence variation than the other portions of the protein not involved in binding. Thus, these non-binding regions of the protein may contain substantial variations without significantly altering the binding properties of the protein. However, one of skill in the art will also recognize that many changes can be made to specifically increase the affinity of the protein for its target. Such affinity-increasing changes are typically determined empirically by altering the protein, which may be in the binding region, and testing the ability to bind GDF-8 or the strength of the binding. All such alterations, whether within or outside the binding region, are included in the scope of the present invention.

[063] Relative sequence similarity or identity may be determined using the "Best Fit" or "Gap" programs of the Sequence Analysis Software Package™ (Version 10; Genetics Computer Group, Inc., University of Wisconsin Biotechnology Center, Madison, WI). "Gap" utilizes the algorithm of Needleman and Wunsch (Needleman and Wunsch, 1970) to find the alignment of two sequences that maximizes the number of matches and minimizes the number of gaps. "BestFit" performs an optimal alignment of the best segment of similarity between two sequences. Optimal alignments are found by inserting gaps to maximize the number of matches using the local homology algorithm of Smith and Waterman (Smith and Waterman, 1981; Smith, *et al.*, 1983).

[064] The Sequence Analysis Software Package described above contains a number of other useful sequence analysis tools for identifying homologues of the presently disclosed nucleotide and amino acid sequences. For example, the "BLAST" program (Altschul, *et al.*, 1990) searches for sequences similar to a query sequence (either peptide or nucleic acid) in a

specified database (e.g., sequence databases maintained at the NCBI; “FastA” (Lipman and Pearson, 1985; see *also* Pearson and Lipman, 1988; Pearson, *et al.*, 1990) performs a Pearson and Lipman search for similarity between a query sequence and a group of sequences of the same type (nucleic acid or protein); “TfastA” performs a Pearson and Lipman search for similarity between a protein query sequence and any group of nucleotide sequences (it translates the nucleotide sequences in all six reading frames before performing the comparison); “FastX” performs a Pearson and Lipman search for similarity between a nucleotide query sequence and a group of protein sequences, taking frameshifts into account. “TfastX” performs a Pearson and Lipman search for similarity between a protein query sequence and any group of nucleotide sequences, taking frameshifts into account (it translates both strands of the nucleic sequence before performing the comparison).

Modified Proteins

[065] The invention encompasses fragments of proteins comprising at least one follistatin domain. Such fragments will likely include all or a part of the follistatin domain. Fragments may include all, a part, or none of the sequences between the follistatin domain and the N-terminus and/or between the follistatin domain and the C-terminus.

[066] It is understood by one of ordinary skill in the art that certain amino acids may be substituted for other amino acids in a protein structure without adversely affecting the activity of the protein, e.g., binding characteristics of a protein comprising at least one follistatin domain. It is thus contemplated by the inventors that various changes may be made in the amino acid sequences of proteins comprising at least one follistatin domain, or DNA sequences encoding the proteins, without appreciable loss of their biological utility or activity. Such changes may include deletions, insertions, truncations, substitutions, fusions, shuffling of motif sequences, and the like.

[067] In making such changes, the hydropathic index of amino acids may be considered. The importance of the hydropathic amino acid index in

conferring interactive biological function on a protein is generally understood in the art (Kyte and Doolittle (1982) *J. Mol. Biol.*, 157: 105-132). It is accepted that the relative hydropathic character of the amino acid contributes to the secondary structure of the resultant protein, which in turn defines the interaction of the protein with other molecules, for example, enzymes, substrates, receptors, DNA, antibodies, antigens, and the like.

[068] Each amino acid has been assigned a hydropathic index on the basis of its hydrophobicity and charge characteristics (Kyte and Doolittle, 1982); these are isoleucine (+4.5), valine (+4.2), leucine (+3.8), phenylalanine (+2.8), cysteine/cystine (+2.5), methionine (+1.9), alanine (+1.8), glycine (-0.4), threonine (-0.7), serine (-0.8), tryptophan (-0.9), tyrosine (-1.3), proline (-1.6), histidine (-3.2), glutamate (-3.5), glutamine (-3.5), aspartate (-3.5), asparagine (-3.5), lysine (-3.9), and arginine (-4.5). In making such changes, the substitution of amino acids whose hydropathic indices may be within ± 2 , within ± 1 , and within ± 0.5 .

[069] It is also understood in the art that the substitution of like amino acids can be made effectively on the basis of hydrophilicity. U.S. Patent 4,554,101 states that the greatest local average hydrophilicity of a protein, as governed by the hydrophilicity of its adjacent amino acids, correlates with a biological property of the protein.

[070] As detailed in U.S. Patent 4,554,101, the following hydrophilicity values have been assigned to amino acid residues: arginine (+3.0), lysine (+3.0), aspartate (+3.0 $\pm$ 1), glutamate (+3.0 $\pm$ 1), serine (+0.3), asparagine (+0.2), glutamine (+0.2), glycine (0), threonine (-0.4), proline (-0.5 $\pm$ 1), alanine (-0.5), histidine (-0.5), cysteine (-1.0), methionine (-1.3), valine (-1.5), leucine (-1.8), isoleucine (-1.8), tyrosine (-2.3), phenylalanine (-2.5), and tryptophan (-3.4). In making such changes, the substitution of amino acids whose hydrophilicity values may be within ± 2 , within ± 1 , and within ± 0.5 .

[071] The modifications may be conservative such that the structure or biological function of the protein is not affected by the change. Such conservative amino acid modifications are based on the relative similarity of

the amino acid side-chain substituents, for example, their hydrophobicity, hydrophilicity, charge, size, and the like. Exemplary conservative substitutions which take various of the foregoing characteristics into consideration are well known to those of skill in the art and include: arginine and lysine; glutamate and aspartate; serine and threonine; glutamine and asparagine; and valine, leucine, and isoleucine. The amino acid sequence of proteins comprising at least one follistatin domain may be modified to have any number of conservative changes, so long as the binding of the protein to its target antigen is not adversely affected. Such changes may be introduced inside or outside of the binding portion of the protein comprising at least one follistatin domain. For example, changes introduced inside of the antigen binding portion of the protein may be designed to increase the affinity of the protein for its target.

Stabilizing Modification

[072] Stabilizing modifications are capable of stabilizing a protein, enhancing the *in vitro* and/or *in vivo* half life of a protein, enhancing circulatory half life of a protein and/or reducing proteolytic degradation of a protein. Such stabilizing modifications include but are not limited to fusion proteins, modification of a glycosylation site, and modification of carbohydrate moiety. A stabilizer protein may be any protein which enhances the overall stability of the modified GDF propeptide. As will be recognized by one of ordinary skill in the art, such fusion protein may optionally comprise a linker peptide between the propeptide portion and the stabilizing portion. As is well known in the art, fusion proteins are prepared such that the second protein is fused in frame with the first protein such that the resulting translated protein comprises both the first and second proteins. For example, in the present invention, a fusion protein may be prepared such that a protein comprising at least one follistatin domain is fused to a second protein (e.g. a stabilizer protein portion.) Such fusion protein is prepared such that the resulting translated protein contains both the propeptide portion and the stabilizer portion.

[073] Proteins comprising at least one follistatin domain can be glycosylated or linked to albumin or a nonproteinaceous polymer. For instance, proteins comprising at least one follistatin domain may be linked to one of a variety of nonproteinaceous polymers, e.g., polyethylene glycol, polypropylene glycol, or polyoxyalkylenes, in the manner set forth in U.S. Patent Numbers 4,640,835; 4,496,689; 4,301,144; 4,670,417; 4,791,192 or 4,179,337. Proteins are chemically modified by covalent conjugation to a polymer to increase their circulating half-life, for example. Polymers, and methods to attach them to peptides, are also shown in U.S. Pat. Nos. 4,766,106; 4,179,337; 4,495,285; and 4,609,546.

[074] Proteins comprising at least one follistatin domain may be pegylated. Pegylation is a process whereby polyethylene glycol (PEG) is attached to a protein in order to extend the half-life of the protein in the body. Pegylation of proteins comprising at least one follistatin domain may decrease the dose or frequency of administration of the proteins needed for an optimal inhibition of GDF-8. Reviews of the technique are provided in Bhadra *et al.* (2002) *Pharmazie*, 57: 5-29, and in Harris *et al.* (2001) *Clin. Pharmacokinet.*, 40: 539-551.

[075] Proteins comprising at least one follistatin domain can be linked to an Fc region of an IgG molecule. Proteins comprising at least one follistatin domain may be fused adjacent to the Fc region of the IgG molecule, or attached to the Fc region of the IgG molecule via a linker peptide. Use of such linker peptides is well known in the protein biochemistry art. The Fc region may be derived from IgG1 or IgG4, for example.

[076] Proteins comprising at least one follistatin domain may be modified to have an altered glycosylation pattern (*i.e.*, altered from the original or native glycosylation pattern). As used herein, "altered" means having one or more carbohydrate moieties deleted, and/or having one or more glycosylation sites added to the original protein.

[077] Glycosylation of proteins is typically either N-linked or O-linked. N-linked refers to the attachment of the carbohydrate moiety to the side chain of an asparagine residue. The tripeptide sequences asparagine-X-serine and

asparagine-X-threonine, where X is any amino acid except proline, are the recognition sequences for enzymatic attachment of the carbohydrate moiety to the asparagine side chain. Thus, the presence of either of these tripeptide sequences in a polypeptide creates a potential glycosylation site. O-linked glycosylation refers to the attachment of one of the sugars N-aceylgalactosamine, galactose, or xylose to a hydroxyamino acid, most commonly serine or threonine, although 5-hydroxyproline or 5-hydroxylysine may also be used.

[078] Addition of glycosylation sites to proteins comprising at least one follistatin domain is conveniently accomplished by altering the amino acid sequence such that it contains one or more of the above-described tripeptide sequences (for N-linked glycosylation sites). The alteration may also be made by the addition of, or substitution by, one or more serine or threonine residues to the sequence of the original protein (for O-linked glycosylation sites). For ease, the protein amino acid sequence may be altered through changes at the DNA level.

[079] Another means of increasing the number of carbohydrate moieties on proteins is by chemical or enzymatic coupling of glycosides to the amino acid residues of the protein. These procedures are advantageous in that they do not require production of the GDF peptide inhibitor in a host cell that has glycosylation capabilities for N- or O-linked glycosylation. Depending on the coupling mode used, the sugars may be attached to (a) arginine and histidine, (b) free carboxyl groups, (c) free sulfhydryl groups such as those of cysteine, (d) free hydroxyl groups such as those of serine, threonine, or hydroxyproline, (e) aromatic residues such as those of phenylalanine, tyrosine, or tryptophan, or (f) the amide group of glutamine. These methods are described in WO 87/05330, and in Aplin and Wriston (1981) *CRC Crit. Rev. Biochem.*, 22: 259-306.

[080] Removal of any carbohydrate moieties present on proteins comprising at least one follistatin domain may be accomplished chemically or enzymatically. Chemical deglycosylation requires exposure of the protein to trifluoromethanesulfonic acid, or an equivalent compound. This treatment

results in the cleavage of most or all sugars except the linking sugar (N-acetylglucosamine or N-acetylgalactosamine), while leaving the amino acid sequence intact.

[081] Chemical deglycosylation is described by Hakimuddin *et al.* (1987) *Arch. Biochem. Biophys.*, 259: 52; and Edge *et al.* (1981) *Anal. Biochem.*, 118: 131. Enzymatic cleavage of carbohydrate moieties on GDF peptide inhibitors can be achieved by the use of a variety of endo- and exo-glycosidases as described by Thotakura *et al.* (1987) *Meth. Enzymol.*, 138: 350.

[082] Proteins comprising at least one follistatin domain may be linked to the protein albumin or a derivative of albumin. Methods for linking proteins and polypeptides to albumin or albumin derivatives are well known in the art. See, for example, U.S. Patent No. 5,116,944.

Pharmaceutical Compositions

[083] The present invention provides compositions containing proteins comprising at least one follistatin domain. Such compositions may be suitable for pharmaceutical use and administration to patients. The compositions typically contain one or more proteins comprising at least one follistatin domain and a pharmaceutically acceptable excipient. As used herein, the phrase "pharmaceutically acceptable excipient" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, that are compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is well known in the art. The compositions may also contain other active compounds providing supplemental, additional, or enhanced therapeutic functions. The pharmaceutical compositions may also be included in a container, pack, or dispenser together with instructions for administration.

[084] A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Methods to accomplish the administration are known to those of ordinary skill in the art.

The administration may, for example, be intravenous, intramuscular, or subcutaneous.

[085] Solutions or suspensions used for subcutaneous application typically include one or more of the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetra acetic acid; buffers such as acetates, citrates or phosphates; and agents for the adjustment of tonicity such as sodium chloride or dextrose. The pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. Such preparations may be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

[086] Pharmaceutical compositions suitable for injection include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, NJ) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, one may include isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, sodium chloride in the composition. Prolonged absorption of

the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

[087] In one embodiment, proteins comprising at least one follistatin domain are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Ind. Liposomal suspensions containing proteins comprising at least one follistatin domain can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

[088] Therapeutically useful agents, such as growth factors (e.g., BMPs, TGF- β , FGF, IGF), cytokines (e.g., interleukins and CDFs), antibiotics, and any other therapeutic agent beneficial for the condition being treated may optionally be included in or administered simultaneously or sequentially with, proteins comprising at least one follistatin domain.

[089] It is especially advantageous to formulate compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of individuals.

Treatment Indications

[090] Proteins comprising at least one follistatin domain are useful to prevent, diagnose, or treat various medical disorders in humans or animals. Accordingly, the present invention provides a method for treating diseases and disorders related to muscle cells and tissue, by administering to a subject a composition comprising at least one protein comprising at least one follistatin domain in an amount sufficient to ameliorate the symptoms of the disease. Such disorders include muscular dystrophies, including, but not limited to severe or benign X-linked muscular dystrophy, limb-girdle dystrophy, facioscapulohumeral dystrophy, myotonic dystrophy, distal muscular dystrophy, progressive dystrophic ophthalmoplegia, oculopharyngeal dystrophy, Duchenne's muscular dystrophy, and Fukuyama-type congenital muscular dystrophy); amyotrophic lateral sclerosis (ALS); muscle atrophy; organ atrophy; frailty; carpal tunnel syndrome; congestive obstructive pulmonary disease; congenital myopathy; myotonia congenital; familial periodic paralysis; paroxysmal myoglobinuria; myasthenia gravis; Eaton-Lambert syndrome; secondary myasthenia; denervation atrophy; paroxymal muscle atrophy; and sarcopenia, cachexia and other muscle wasting syndromes. The invention also relates to traumatic or chronic injury to muscle tissue.

[091] In addition to providing therapy for muscle diseases and disorders, the present invention also provides for methods for preventing or treating metabolic diseases or disorders resulting from abnormal glucose homeostasis. Such diseases or disorders include metabolic diseases and disorders (such as insulin-dependent (type 1) diabetes mellitus, noninsulin-dependent (type 2) diabetes mellitus), hyperglycemia, impaired glucose tolerance, metabolic syndrome (e.g., syndrome X), obesity and insulin resistance induced by trauma (e.g., burns or nitrogen imbalance), adipose tissue disorders (such as obesity), or bone degenerative diseases (such as osteoporosis, especially in the elderly and/or postmenopausal women; glucocorticoid-induced osteoporosis; osteopenia; osteoarthritis; and

osteoporosis-related fractures). Yet further examples include low bone mass due to chronic glucocorticoid therapy, premature gonadal failure, androgen suppression, vitamin D deficiency, secondary hyperparathyroidism, nutritional deficiencies, and anorexia nervosa.

[092] Normal glucose homeostasis requires the finely tuned orchestration of insulin secretion by pancreatic beta cells in response to subtle changes in blood glucose levels. One of the fundamental actions of insulin is to stimulate uptake of glucose from the blood into tissues, especially muscle and fat.

[093] Accordingly, the present invention provides a method for treating diabetes mellitus and related disorders, such as obesity or hyperglycemia, by administering to a subject a composition comprising at least one protein comprising at least one follistatin domain in an amount sufficient to ameliorate the symptoms of the disease. Type 2 or noninsulin-dependent diabetes mellitus (NIDDM), in particular, is characterized by a triad of (1) resistance to insulin action on glucose uptake in peripheral tissues, especially skeletal muscle and adipocytes, (2) impaired insulin action to inhibit hepatic glucose production, and (3) dysregulated insulin secretion (DeFronzo (1997) *Diabetes Rev.* 5: 177-269). Therefore, subjects suffering from type 2 diabetes can be treated according to the present invention by administration of protein comprising at least one follistatin domain, which increases sensitivity to insulin and glucose uptake by cells.

[094] Similarly, other diseases and metabolic disorders characterized by insulin dysfunction (e.g., resistance, inactivity, or deficiency) and/or insufficient glucose transport into cells also can be treated according to the present invention by administration of a protein comprising at least one follistatin domain, which increases sensitivity to insulin and glucose uptake by cells.

Methods of Treatment Using Proteins

[095] Proteins comprising at least one follistatin domain may be used to inhibit or reduce one or more activities associated with the GDF-8 protein (whether in monomeric form, dimeric active form, or complexed in a GDF-8

latent complex), relative to a GDF-8 protein not bound by the same protein. In an embodiment, the activity of the mature GDF-8 protein, when bound by a protein comprising at least one follistatin domain, is inhibited at least 50%, or at least 60, 62, 64, 66, 68, 70, 72, 72, 76, 78, 80, 82, 84, 86, or 88%, or at least 90, 91, 92, 93, or 94%, or at least 95% to 100% relative to a mature GDF-8 protein that is not bound by a protein having a follistatin domain.

[096] Pharmaceutical preparations comprising proteins comprising at least one follistatin domain are administered in therapeutically effective amounts. As used herein, an "effective amount" of the protein is a dosage which is sufficient to reduce the activity of GDF-8 to achieve a desired biological outcome. The desired biological outcome may be any therapeutic benefit including an increase in muscle mass, an increase in muscle strength, improved metabolism, decreased adiposity, or improved glucose homeostasis. Such improvements may be measured by a variety of methods including those that measure lean and fat body mass (such as dual x-ray scanning analysis), muscle strength, serum lipids, serum leptin, serum glucose, glycated hemoglobin, glucose tolerance, and improvement in the secondary complication of diabetes.

[097] Generally, a therapeutically effective amount may vary with the subject's age, condition, and sex, as well as the severity of the medical condition in the subject. The dosage may be determined by a physician and adjusted, as necessary, to suit observed effects of the treatment. Appropriate dosages for administering at least one protein comprising at least one follistatin domain may range from 5 mg to 100 mg, from 15 mg to 85 mg, from 30 mg to 70 mg, or from 40 mg to 60 mg. Proteins can be administered in one dose, or at intervals such as once daily, once weekly, and once monthly. Dosage schedules can be adjusted depending on the affinity of the protein for GDF-8, the half life of the protein, or the severity of the patient's condition. Generally, the compositions are administered as a bolus dose, to maximize the circulating levels of proteins comprising at least one follistatin domain for the greatest length of time after the dose. Continuous infusion may also be used after the bolus dose.

[098] Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the LD₅₀ (the dose lethal to 50% of the population) and the ED₅₀ (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD₅₀/ED₅₀. Proteins comprising at least one follistatin domain which exhibit large therapeutic indices may be used.

[099] Data obtained from the cell culture assays and animal studies can be used in evaluating a range of dosage for use in humans. The dosage of such compounds may lie within a range of circulating concentrations that include the ED₅₀ with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any protein comprising at least one follistatin domain used in the present invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC₅₀ (i.e., the concentration of the test protein which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Levels in plasma may be measured, for example, by high performance liquid chromatography. The effects of any particular dosage can be monitored by a suitable bioassay. Examples of suitable bioassays include GDF-8 protein/receptor binding assays, creatine kinase assays, assays based on glucose uptake in adipocytes, and immunological assays.

Methods of Administering DNA

[0100] The present invention also provides gene therapy for the in vivo production of proteins comprising at least one follistatin domain. Such therapy would achieve its therapeutic effect by introduction of the polynucleotide sequences into cells or tissues having the disorders as listed herein.

[0101] Delivery of polynucleotide sequences of proteins comprising at least one follistatin domain can be achieved using a recombinant expression

vector such as a chimeric virus or a colloidal dispersion system. Target liposomes may be used for therapeutic delivery of the polynucleotide sequences. Various viral vectors which can be utilized for gene therapy include adenovirus, herpes virus, vaccinia, or an RNA virus such as a retrovirus. The retroviral vector may be a derivative of a murine or avian retrovirus. Examples of retroviral vectors in which a single foreign gene can be inserted include, but are not limited to: Moloney murine leukemia virus (MoMuLV), Harvey murine sarcoma virus (HaMuSV), murine mammary tumor virus (MuMTV), and Rous sarcoma virus (RSV). A number of additional retroviral vectors can incorporate multiple genes. All of these vectors can transfer or incorporate a gene for a selectable marker so that transduced cells can be identified and generated. By inserting a GDF propeptide polynucleotide sequence of interest into the viral vector, along with another gene which encodes the ligand for a receptor on a specific target cell, for example, the vector is now target specific.

[0102] Retroviral vectors can be made target specific by attaching, for example, a sugar, a glycolipid, or a protein. Targeting may be accomplished by using an antibody. Those of skill in the art will recognize that specific polynucleotide sequences can be inserted into the retroviral genome or attached to a viral envelope to allow target specific delivery of the retroviral vector containing the polynucleotide of proteins comprising at least one follistatin domain. In one embodiment, the vector is targeted to muscle cells or muscle tissue.

[0103] Since recombinant retroviruses are defective, they require helper cell lines that contain plasmids encoding all of the structural genes of the retrovirus under the control of regulatory sequences within the LTR. These plasmids are missing a nucleotide sequence which enables the packaging mechanism to recognize an RNA transcript for encapsidation. Helper cell lines which have deletions of the packaging signal include, but are not limited to PSI.2, PA317 and PA12, for example. These cell lines produce empty virions, since no genome is packaged. If a retroviral vector is introduced into such cells in which the packaging signal is intact, but the

structural genes are replaced by other genes of interest, the vector can be packaged and vector virion produced.

[0104] Alternatively, other tissue culture cells can be directly transfected with plasmids encoding the retroviral structural genes gag, pol and env, by conventional calcium phosphate transfection. These cells are then transfected with the vector plasmid containing the genes of interest. The resulting cells release the retroviral vector into the culture medium.

[0105] Another targeted delivery system for a polynucleotide or a protein comprising at least one follistatin domain is a colloidal dispersion system. Colloidal dispersion systems include macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. Liposomes are artificial membrane vesicles which are useful as delivery vehicles in vitro and in vivo. RNA, DNA and intact virions can be encapsulated within the aqueous interior and be delivered to cells in a biologically active form (see, for example, Fraley, *et al.* (1981) *Trends Biochem. Sci.*, 6: 77). Methods for efficient gene transfer using a liposome vehicle, are known in the art (see, for example, Mannino, *et al.* (1988) *Biotechniques*, 6: 682). The composition of the liposome is usually a combination of phospholipids, usually in combination with steroids, especially cholesterol. Other phospholipids or other lipids may also be used. The physical characteristics of liposomes depend on pH, ionic strength, and the presence of divalent cations.

[0106] Examples of lipids useful in liposome production include phosphatidyl compounds, such as phosphatidylglycerol, phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine, sphingolipids, cerebrosides, and gangliosides. Illustrative phospholipids include egg phosphatidylcholine, dipalmitoylphosphatidylcholine and distearoylphosphatidylcholine. The targeting of liposomes is also possible based on, for example, organ-specificity, cell-specificity, and organelle-specificity and is known in the art.

[0107] There is a wide range of methods which can be used to deliver the cells expressing proteins comprising at least one follistatin domain to a site for use in modulating a GDF-8 response. In one embodiment of the

invention, the cells expressing follistatin protein can be delivered by direct application, for example, direct injection of a sample of such cells into the site of tissue damage. These cells can be purified. The such cells can be delivered in a medium or matrix which partially impedes their mobility so as to localize the cells to a site of injury. Such a medium or matrix could be semi-solid, such as a paste or gel, including a gel-like polymer. Alternatively, the medium or matrix could be in the form of a solid, a porous solid which will allow the migration of cells into the solid matrix, and hold them there while allowing proliferation of the cells.

Methods of Detection and Isolation of GDF-8

[0108] Proteins comprising at least one follistatin domain may be used to detect the presence or level of GDF-8, *in vivo* or *in vitro*. By correlating the presence or level of these proteins with a medical condition, one of skill in the art can diagnose the associated medical condition. The medical conditions that may be diagnosed by the proteins comprising at least one follistatin domain are set forth herein.

[0109] Such detection methods are well known in the art and include ELISA, radioimmunoassay, immunoblot, western blot, immunofluorescence, immuno-precipitation, and other comparable techniques. Proteins comprising at least one follistatin domain may further be provided in a diagnostic kit that incorporates one or more of these techniques to detect GDF-8. Such a kit may contain other components, packaging, instructions, or other material to aid the detection of the protein and use of the kit.

[0110] Where proteins comprising at least one follistatin domain are intended for diagnostic purposes, it may be desirable to modify them, for example with a ligand group (such as biotin) or a detectable marker group (such as a fluorescent group, a radioisotope or an enzyme). If desired, the proteins may be labeled using conventional techniques. Suitable labels include fluorophores, chromophores, radioactive atoms, electron-dense reagents, enzymes, and ligands having specific binding partners. Enzymes are typically detected by their activity. For example, horseradish peroxidase

is usually detected by its ability to convert 3,3',5,5'-tetramethylbenzidine (TMB) to a blue pigment, quantifiable with a spectrophotometer. Other suitable binding partners include biotin and avidin or streptavidin, IgG and protein A, and the numerous receptor-ligand couples known in the art. Other permutations and possibilities will be readily apparent to those of ordinary skill in the art, and are considered as equivalents within the scope of the instant invention.

[0111] Proteins comprising at least one follistatin domain or fragments thereof may also be useful for isolating GDF-8 in a purification process. In one type of process, proteins may be immobilized, for example, through incorporation into a column or resin. The proteins are used to bind GDF-8, and then subject to conditions which result in the release of the bound GDF-8. Such processes may be used for the commercial production of GDF-8.

[0112] The following examples provide embodiments of the invention. One of ordinary skill in the art will recognize the numerous modifications and variations that may be performed without altering the spirit or scope of the present invention. Such modifications and variations are believed to be encompassed within the scope of the invention. The examples do not in any way limit the invention. It is understood that all of the numbers in the specification and claims are modified by the term about, as small changes in dosages, for example, would be considered to be within the scope of the invention.

EXAMPLES

Example 1: Purification of JA16-Conjugated Beads

[0113] N-hydroxysuccinimidyl-activated beads (4% beaded agarose, Sigma H-8635, St Louis MO) were washed in MilliQ-H₂O and incubated for 4 hours at 4°C with the anti-GDF-8 JA16 monoclonal antibody (3-4 µg/µl in 100 mM MOPS, pH 7.5) at a ratio to allow a final concentration of 10 mg JA16/ml resin. Beads were washed extensively with 100 mM MOPS pH 7.5 and phosphate-buffered saline (PBS) (Ausubel *et al*, (1999) *Current Protocols in*

Molecular Biology, John Wiley & Sons) and stored at 4°C in PBS until use. Control beads were prepared identically without JA16 antibody.

Example 2: Affinity Purification

[0114] A total of 40 µl of packed JA16-conjugated or control beads were incubated with 15 ml normal Balb/C mouse serum (Golden West Biologicals, Temecula CA) or 30 ml pooled normal human serum (ICN Biomedical, Aurora OH) for 3 hours at 4°C. Beads were washed twice in ~10 ml of cold 1% Triton X-100/PBS, twice in ~10 ml of cold 0.1% Triton X-100/PBS, and twice in ~1 ml of cold PBS. Proteins were eluted from the beads in three subsequent steps. First, the beads were treated to a 'mock elution', where 100 µl of PBS was added to the beads and incubated at 4°C for 30 minutes. The supernatant was collected and combined with 30 µl 4x LDS sample buffer (Invitrogen, Carlsbad CA). Second, the beads were subject to a 'peptide elution', 100 µl of 1 µg/µl competing peptide (sequence: DFGLDSDEHSTESRSSRYPLTVDFEAFGWD-COOH (SEQ ID NO:12)) in PBS was added to the beads and again incubated at 4°C for 30 minutes. The supernatant was collected as before. Third, the beads were treated with an 'SDS elution' technique, where 30 µl of 4x LDS buffer (Invitrogen) and 100 µl of PBS was added to the beads and heated to 80°C for 10 minutes before transferring the supernatant to a fresh tube.

[0115] A silver stained gel of the proteins released in each of the elution steps is shown in Figure 1. Two protein bands in the silver-stained gel shown in Figure 1 of approximately 12 and 36 kDa were specifically eluted from JA16-conjugated beads, but not from unconjugated control beads.

Example 3: Mass Spectrometry

[0116] Samples were reduced with NuPage 10x reducing agent (Invitrogen) for 10 minutes at 80°C and alkylated with 110 µM iodoacetamide for 30 minutes at 22°C in the dark. Samples were run immediately on 10% NuPage Bis-Tris gels in an MES buffer system according to manufacturer's recommendations (Invitrogen) and silver stained using a gluteraldehyde-free

system (Shevchenko, *et al.*, (1996) *Anal. Chem.*, 68: 850-858). Bands were excised and digested with Sequencing Grade Modified Trypsin (Promega, Madison WI) in an Abimed Digest Pro (Langenfeld, Germany) or ProGest Investigator (Genomics Solutions, Ann Arbor MI). The volume of digested samples was reduced by evaporation and supplemented with 1% acetic acid to a final volume of ~20 μ l. Samples (5-10 μ l) were loaded onto a 10 cm x 75 μ m inner diameter C₁₈ reverse phase column packed in a Picofrit needle (New Objectives, Woburn MA). MS/MS data was collected using an LCQ Deca or LCQ Deca XP (Finnigan, San Jose CA) mass spectrometer and searched against the NCBI non-redundant database using the Sequest program (Finnigan). Unless otherwise noted, all peptide sequences listed in this paper corresponded to MS/MS spectra that were deemed high quality by manual inspection and produced X_{corr} scores > 2.5 in the Sequest scoring system.

Example 4: Western Blots

[0117] Proteins were transferred to a 0.45 μ m nitrocellulose membrane (Invitrogen) and blocked with blocking buffer (5% non-fat dry milk in Tris-buffered saline (TBS: 10 mM Tris-Cl, pH 7.5, 150 mM NaCl)) at 4°C overnight. Blots were then probed with primary antibody diluted 1:1000 in blocking buffer for 1-3 hours at room temperature, washed 5x with TBS, probed with horseradish peroxidase-conjugated secondary antibody in blocking buffer for 1-3 hours at room temperature, and washed as before. Signals were detected by autoradiography using the West Pico Substrate (Pierce).

Example 5: Isolation of GDF-8

[0118] An experiment using the methods described in the previous Examples resulted in the isolation of GDF-8. Since GDF-8 in its reduced form is 12 kDa, we speculated that the protein in the lower band from the silver-stained gel shown in Figure 1 was mature GDF-8. To confirm this hypothesis, we excised this band, digested it with trypsin, and obtained MS/MS spectra of the resulting peptides by LC-MS/MS. MS/MS spectra corresponding to six

tryptic peptides confirmed that mature GDF-8 was isolated from this region of the gel, as shown in Figure 2A and Table 1.

[0119] Table 1 lists peptides derived from GDF-8 (SEQ ID NO:13-20), GDF-8 propeptide (SEQ ID NO:21-27), FLRG (SEQ ID NO:28-30), and GASP1 (SEQ ID NO:31-35) that were found in JA16 immunoprecipitates from mouse and human serum. The immediately preceding amino acid in the protein sequence is shown in parentheses for each peptide and the charge state of the peptide (z) and the Sequest program correlation coefficient (X_{corr} , a measure of confidence) are listed. The sequence listing numbers in the table refer only to the isolated peptides and their sequences. The preceding amino acids in parentheses are not included in the peptides, but are provided only for reference. All spectra were confirmed by manual inspection.

[0120] Interestingly, the western blot also contained a band corresponding to unprocessed full-length GDF-8 (43 kDa), implying that some portion of this molecule is secreted into serum without undergoing proteolytic processing (Figure 2B). The presence of unprocessed GDF-8 was confirmed by mass spectrometry (data not shown). Thus, the affinity purification method effectively isolated GDF-8 from normal mouse serum.

[0121] Although the JA16 antibody recognizes both GDF-8 and the highly related protein BMP/GDF-11, we saw no evidence of BMP-11 peptides in our affinity purified samples by mass spectrometry.

Table 1: Peptides Identified in JA16 Immunoprecipitates

	mouse serum	z	X _{corr}
GDF-8 (mature)	(K) ANYCSGECFVFLQK (SEQ ID NO:13)	3+	4.63
	(K) MSPINMLYFNGK (SEQ ID NO:14)	2+	3.81
	(R) DFGLDCDEHSTESR (SEQ ID NO:15)	2+	3.47
	(K) ANYCSGECFVFLQK (SEQ ID NO:16)	2+	3.31
	(K) M*SPINMLYFNGK (SEQ ID NO:17)	3+	2.95
	(R) YPLTVDFEAFGWDWIIAPK (SEQ ID NO:18)	2+	2.86
	(K) M*SPINM*LYFNGK (SEQ ID NO:19)	2+	2.51
	(R) GSAGPCCTPTK (SEQ ID NO:20)	2+	2.43
GDF-8 (propeptide)	(K) LDM*SPGTGIWQSIDVK (SEQ ID NO:21)	2+	3.82
	(K) ALDENGHDLAVTFPGPGEDGLNPFLEVK (SEQ ID NO: 22)	3+	3.17
	(K) LDMSPGTGIWQSIDVK (SEQ ID NO:23)	2+	2.98
	(R) ELIDQYDVQR (SEQ ID NO:24)	2+	2.97
	(K) TPTTVFVQILR (SEQ ID NO:25)	2+	2.91
	(K) AQLWIYLRPVK (SEQ ID NO:26)	2+	2.77
	(K) EGLCNACAWR (SEQ ID NO:27)	2+	2.75
follistatin-like	(R) PQSCLVDQTGSAHCVVCR (SEQ ID NO:28)	3+	3.34
related gene (FLRG)	(K) DSCDGVECGPGK (SEQ ID NO:29)	2+	2.99
	(K) SCAQVVCPR (SEQ ID NO:30)	2+	2.59
novel multidomain protease inhibitor (GASP1)	(R) ECETDQECETYEK (SEQ ID NO:31)	2+	2.98
	(R) ADFPLSVVR (SEQ ID NO:32)	2+	2.56
	(R) EACEESCPFPR (SEQ ID NO:33)	2+	2.95
	(R) SDFVILGR (SEQ ID NO:34)	2+	2.73
	(R) VSELTEEQDSGR (SEQ ID NO:35)	2+	3.88

M* = oxidized methionine

	human serum	z	X _{corr}
GDF-8 (mature)	(K) ANYCSGECEFLVFLQK (SEQ ID NO:36)	2+	4.21
	(R) DFGLDCDEHSTESR (SEQ ID NO:37)	3+	2.08
GDF-8 (propeptide)	(K) ALDENGHDLAVTFPGPGEDGLNPFLEVK (SEQ ID NO:38)	3+	3.71
	(R) ELIDQYDVQR (SEQ ID NO:39)	2+	3.01
follistatin-like	(R) PQSCVVDQTGSAHCVVCR (SEQ ID NO:40)	3+	3.37
related gene (FLRG)	(R) CECAPDCSGLPAR (SEQ ID NO:41)	2+	3.21
	(R) LQVCGSDGATYR (SEQ ID NO:42)	2+	3.06
multidomain protease inhibitor (GASP1)	(R) VSELTEEPDSGR (SEQ ID NO:43)	2+	2.44
	(R) CYMDAEACSK (SEQ ID NO:44)	2+	2.69
	(K) GITLAVVTCR (SEQ ID NO:45)	2+	2.42

Example 6: Isolation of Proteins Bound to GDF-8

[0122] Once it was confirmed that the affinity purification technique could successfully isolate GDF-8 from normal mouse serum, we proceeded to identify proteins that bind to GDF-8 under native conditions. The 36 kDa band on the silver-stained gel shown in Figure 1 was analyzed as described above. Mass spectrometry identified two proteins in this region of the gel that were specific to the JA16-immunopurified sample. These were determined to be the GDF-8 propeptide and follistatin-like related gene (FLRG). The peptides identified from each of these proteins are shown in Table 1 (SEQ ID NO:13-27). High quality MS/MS spectra were found for six unique peptides from GDF-8 propeptide and three unique peptides from FLRG; representative peptides are shown in Figures 3A and 3C. Furthermore, the presence of both of these proteins was confirmed by western blotting with polyclonal antibodies specific to GDF-8 propeptide and FLRG respectively (Figures 3B and 3D). Thus, circulating GDF-8 appears to bind to the GDF-8 propeptide and to FLRG *in vivo*.

Example 7: Isolation of Novel Proteins that Bind GDF-8

[0123] To characterize the major components of the circulating GDF-8 complex *in vivo*, native GDF-8 and its associated proteins from wild-type mouse serum were isolated by affinity purification with an agarose-conjugated anti-GDF-8 monoclonal antibody, JA16. JA16-bound proteins were subjected to subsequent elution steps with PBS buffer alone (mock elution), a peptide that could compete with GDF-8 for JA16 binding, and SDS detergent. These samples were concentrated, run on a one-dimensional SDS-PAGE gel, and visualized by silver stain (Figure 4). Two bands unique to the JA16 purified samples are visible—a 12 kDa band identified as GDF-8, and a 36 kDa band containing both GDF-8 propeptide and FLRG.

[0124] In order to determine if one could identify other proteins that were bound to GDF-8 *in vivo*, we scaled up the purification approximately five-fold and used mass spectrometry to search for proteins that were present in the JA16 immunocomplex, but not in the negative control. To achieve this goal, we excised regions of the silver stained gel corresponding to molecular weights between 10 and 200 kDa into 13 gel slices, as shown in Figure 4. Each of these slices was subjected to in-gel trypsin digestion and LC-MS/MS. Comparison of the resulting MS/MS spectra to the non-redundant NCBI database of known proteins did not reveal any additional proteins specific to the JA16 immunoprecipitate, although the proteins previously described (mature GDF-8, GDF-8 propeptide, unprocessed GDF-8, and FLRG) were all identified in these samples (Figure 4). Background proteins that were found both in the JA16 immunocomplex and in the negative control sample included abundant serum proteins, such as albumin, immunoglobulins, and complement proteins. There was no evidence of other TGF- β superfamily members, including the highly related protein BMP-11/GDF-11, in the JA16 samples. Thus, the JA16 antibody specifically purified GDF-8 in these experiments.

[0125] Interestingly, we found no evidence of follistatin in our GDF-8 immunocomplexes, despite the fact that JA16 is capable of

immunoprecipitating a GDF-8/follistatin complex *in vitro* (data not shown). Follistatin has been shown to inhibit GDF-8 activity by antagonizing the association of GDF-8 with the ActRIIB receptor (Lee and McPherron (2001) *Proc. Natl. Acad. Sci. U.S.A.*, 98: 9306-9311). Our results suggest that follistatin does not play a major role in the regulation of the activity of the circulating GDF-8 complex under normal conditions.

[0126] Since the identification of proteins by this MS/MS procedure is dependent on the content of the database being searched, we further analyzed the data from Figure 4 by comparing the MS/MS spectra collected from the 13 samples to a database of proteins predicted from the Celera mouse genomic sequence. This analysis identified an additional protein specific to the JA16-purified sample, and is hereby referred to as GDF-associated serum protein 1 (GASP1). Since the initial identification of this protein, this sequence has been added to the NCBI nr database by the public genome sequencing effort under the accession number gi|20914039.

[0127] Five peptides corresponding to the sequence of GASP1 were identified on the basis of high-quality MS/MS spectra (Table 1 (SEQ ID NO:31-35); Figure 5A and B). The spectra corresponding to GASP1 peptides were found in band 3, which contains 70-80 kDa proteins. However, a specific band corresponding to this protein was not visible, probably due to the abundance of background immunoglobulins and albumin at this area (see Figure 4). Sequest X_{corr} scores above 2.3 are generally considered significant for 2^+ ions. Fortuitously, one of the peptides identified in our experiments (sequence = ECETDQECETYK (SEQ ID NO:31)) spans the junction between the two exons that code for this protein, verifying the accuracy of Celera's gene prediction algorithm in this instance.

[0128] The sequences of the GASP1 transcript and protein were predicted prior to the actual cloning of GASP1 (Figure 6). GASP1 was predicted to be a 571 amino acid protein with a predicted molecular mass of 63 kDa. It has a putative signal sequence/cleavage site at its N-terminus and two possible sites for N-glycosylation at amino acids 314 and 514. Analysis of the GASP1 protein sequence by Pfam and BLAST (according to the

techniques in Altschul *et al.* (1990) *J. Mol. Biol.*, 215: 403-410; Bateman *et al.* (2002) *Nucleic Acids Res.*, 30: 276-280) revealed that GASP1 contains many conserved domains, including a WAP domain, a follistatin/Kazal domain, an immunoglobulin domain, two tandem Kunitz domains, and a netrin domain (Figure 14A). WAP domains, originally identified in whey acidic protein, contain 8 cysteines that form a four-disulfide core and are often found in proteins with anti-protease activity (Hennighausen and Sippel (1982) *Nucleic Acids Res.*, 10: 2677-2684; Seemuller *et al.* (1986) *FEBS Lett.*, 199: 43-48). It is believed that the follistatin domain mediates the interaction between GDF-8 and GASP1. The C-terminal region of follistatin domains contains a similarity to Kazal serine protease inhibitor domains. In the case of GASP1, this region is even more closely related to Kazal domains than in follistatin or FLRG, suggesting the possibility that this region may have an additional protease inhibitor function. Kunitz domains, originally identified in bovine pancreatic trypsin inhibitor, also inhibit serine proteases, thus establishing a likely role for GASP1 in the regulation of this class of proteins. Furthermore, netrin domains have been implicated in the inhibition of metalloproteases (Banyai and Patthy, 1999; Mott *et al.*, 2000). Thus, based on the presence of these conserved regions, GASP1 is likely to inhibit the activity of proteases, perhaps regulating GDF-8 processing or activation of the latent GDF-8 complex.

[0129] BLAST searches against the mouse Celera transcript database revealed a protein that has >50% identity with GASP1, referred to here as GASP2. GASP2 contains the same domain structure as GASP1, suggesting that these proteins define a two member family of multivalent protease inhibitors (Figure 14B). Interestingly, only peptides corresponding to GASP1, not GASP2, were found in our JA16 purified samples. This result suggests that GASP1 and GASP2 likely have different biological specificity. Both GASP1 and GASP2 are conserved in humans (>90% identity with mouse). The sequence for human GASP1 is now available in the NCBI nr database under the accession number gi|18652308. Although, the concentration of GDF-8 in human serum is considerably lower than that found in mouse serum

(Hill et al. (2002) *J. Biol. Chem.*, 277: 40735-40741), the sensitivity of mass spectrometric analysis of proteins allowed us to identify 3 peptides corresponding to the human homolog of GASP1 from JA16 immunoprecipitations from human serum (Table 1). None of these peptides were found in the corresponding negative control. Again, there was no evidence of human GASP2 in these experiments. Thus, the interaction between GASP1 and GDF-8 is conserved between mouse and human.

[0130] GDF-8 is produced nearly exclusively in skeletal muscle. In order to determine the tissue distribution of GASP1 mRNA, a 551 bp fragment of GASP1 was amplified from first-strand cDNA produced from a variety of mouse tissues and staged embryos (Figure 10). A mouse GASP1 fragment was amplified from normalized mouse first-strand cDNA panels (Clontech, Palo Alto CA) using the Advantage cDNA PCR kit (Clontech) according to the manufacturer's recommendations (forward primer: 5' TTGGCCACTGCCACCACAATCTCAACCACTT 3' (SEQ ID NO:46); reverse primer: 5' TCTCAGCATGGCCATGCCGCCGTCGA 3' (SEQ ID NO:47)). GASP1 appears to be fairly widely-expressed, with particularly high expression in skeletal muscle and heart. Significant expression is also seen in brain, lung, and testis. In contrast, liver and kidney express relatively low levels of GASP1 mRNA. Developmentally, the level of GASP1 mRNA remains fairly constant, perhaps increasing slightly between day 7 and day 11 of mouse embryogenesis.

Example 8: GDF-8 in Human and Mouse Serum

[0131] The concentration of GDF-8 in human serum is considerably lower than that found in mouse serum. Since GDF-8 has potential as a therapeutic target, it was a goal to determine the composition of the circulating GDF-8 complex in humans. This knowledge would determine the validity of the mouse model and potentially identify alternative therapeutic targets. Thus, the JA16-based affinity purification of GDF-8 was repeated using human serum. Due to the lower level of GDF-8 in human serum compared with mouse, no bands corresponding to mature GDF-8 and GDF-8

propeptide/FLRG were visualized (Figure 11A). However, western blotting with a polyclonal antibody that recognizes the mature region of GDF-8 revealed the presence of mature and unprocessed GDF-8 in the JA16-purified samples (Figure 11B).

[0132] We took advantage of the high sensitivity of mass spectrometry to identify proteins that co-purified with mature GDF8. The lanes corresponding to peptide eluted samples from both negative control and JA16-conjugated beads were sliced into 16 pieces. These gel slices were subjected to in-gel trypsin digestion, nanoflow LC-MS/MS, and analysis with Sequest as before.

[0133] Interestingly, the only proteins that were identified specifically in the JA16 samples and not the negative control were mature GDF-8, GDF-8 propeptide, human FLRG, and the human homolog of GASP1. The peptides found from each of these proteins are listed in Table 1 (SEQ ID NO:36-45) and representative MS/MS spectra are shown in Figure 12. Thus the *in vivo* GDF-8 complex appears to be conserved between mouse and human.

Example 9: Cloning and Characterization of Mouse GASP1

[0134] After identifying the predicted GASP1 sequence, it was a goal to determine the actual sequence of mouse GASP1. Based on the Celera predicted sequence, the GASP1 coding sequence was amplified from mouse heart QUICKCLONE cDNA (Clontech) by PCR with PfuTurbo polymerase (Stratagene) using the following primers (fp: 5' CACCATGTGTGCCCCAGGGTATCATCGGTTCTGG 3' (SEQ ID NO:50); rp: 5' TTGCAAGCCCAGGAAGTCCTTGAGGAC 3' (SEQ ID NO:51)). The PCR product from this reaction ran as a single major band of approximately 1700 base pairs on a 1% agarose gel. The amplified DNA was then cloned into the TOPO sites of the pcDNA3.1D/V5-His-TOPO vector (Invitrogen) so as to include an in-frame C-terminal V5-His tag according to manufacturers' recommendations. The full-length cDNA insert was sequenced on both strands. The nucleotide sequence of the mouse GASP1 clone is shown in Figure 13. This clone matched the predicted Celera sequence, with the

exception of some base substitutions in wobble codons that did not change the predicted amino acid sequence (i.e., 288C:G; 294G:A; 615G:A; 738A:G; 768C:T; 1407A:G; 1419A:G; and 1584C:G, where the first base at the indicated position is that reported by Celera and the second base is that obtained from sequencing of the clone; see Figure 6A and B).

[0135] To determine the N-terminal processing of the GASP1 protein, we transfected COS1 cells with a mammalian expression vector encoding mouse GASP1 cloned with a C-terminal V5-His tag (GASP1-V5-His). Serum-free conditioned media was harvested 48 hours later and analyzed by western blot analysis with an anti-V5 polyclonal antibody (Sigma). More specifically, conditioned media was collected 48 hours after transfection of COS1 cells with GASP1-V5-His/pcDNA3.1D-V5-His-TOPO or empty vector using the FuGENE 6 reagent (Roche) in serum-free Dulbecco's modified Eagle's medium.

[0136] A single band, running at approximately 80 kDa was seen, confirming that GASP1 is secreted into the conditioned media (data not shown). Approximately 10 ml of this conditioned media was run over a His-affinity column and further purified by reverse phase chromatography. This purification scheme yielded a band the expected size of full-length GASP1 on a Coomassie stained SDS-PAGE gel. Edman sequencing of this band determined an N-terminal sequence of L-P-P-I-R-Y-S-H-A-G-I (SEQ ID NO:52). Thus, amino acids 1-29 of GASP1 constitute the signal sequence that is removed during processing and secretion.

Example 10: Binding of Recombinantly-Produced GASP1 to GDF-8 Propeptide and Mature GDF-8

[0137] Next, it was determined that recombinantly-produced GASP1 had the same binding pattern to GDF-8 as GASP1 isolated from mouse serum. For immunoprecipitations with recombinant proteins, 400 µl conditioned media from vector- or GASP1-transfected cells was combined with 1.2 µg of recombinant purified GDF-8 and/or GDF-8 propeptide protein (Thies *et al.*, 2001). JA16 (10 µl packed volume) or anti-V5 (30 µl) conjugated

agarose beads were incubated with the supplemented conditioned media for two hours at 4°C and washed twice in cold 1% Triton in phosphate-buffered saline (PBS) and twice in PBS. Beads were resuspended in 50 µl 1x LDS buffer with DTT. Western blots were performed as previously described (Hill *et al.*, 2002).

[0138] To confirm and further characterize the interaction between GDF-8 and GASP1, we incubated purified recombinant GDF-8 and purified recombinant GDF-8 propeptide with conditioned media from COS1 cells transfected with either a vector control or GASP1-V5-His. We then immunoprecipitated GDF-8 with JA16-conjugated agarose beads and looked for co-purification of GASP1 and GDF-8 propeptide using western blots (Figure 15A). Both GASP1 (lane 3) and GDF-8 propeptide (lane 1) co-immunoprecipitated with GDF-8, proving that GDF-8 can interact with both of these proteins. Neither GASP1 nor propeptide were detected in JA16 immunoprecipitates in the absence of GDF-8 (lane 4), eliminating the possibility of non-specific binding in these experiments. When all three proteins were present, both GASP1 and GDF-8 propeptide were pulled down with GDF-8, suggesting the possibility that these proteins may form a tertiary complex (lane 5). However, this experiment does not eliminate the possibility that GASP1 and propeptide are bound to the same epitope on separate GDF-8 molecules.

[0139] To further confirm the interaction between GASP1 and GDF-8, we performed the reverse immunoprecipitation by pulling down GASP1 from conditioned media supplemented with GDF-8 and/or GDF-8 propeptide recombinant protein. To achieve this, we used an agarose-conjugated monoclonal antibody directed against the V5 epitope of the C-terminal V5-His tag on GASP1. As expected, GDF-8 co-immunoprecipitated with GASP1 (Figure 15B, lanes 3 and 5), further confirming a direct interaction between these proteins. Surprisingly, GDF-8 propeptide also co-purified with GASP1, even in the absence of GDF-8 (lane 4), suggesting that GDF-8 propeptide can bind directly to GASP1. Thus, GASP1 binds to both GDF-8 and GDF-8 propeptide independently. This is in contrast to FLRG, another follistatin-

domain protein, that binds exclusively to mature GDF-8 (Hill et al. (2002) *J. Biol. Chem.*, 277: 40735-40741). Addition of both GDF-8 and propeptide consistently showed less propeptide binding to GASP1 than when propeptide was added alone. This observation suggests that GASP1 may not bind to the GDF-8 small latent complex.

Example 11: GASP1-Mediated Inhibition of GDF-8 and BMP-11, But Not Activin or TGF- β 1, Activity

[0140] A luciferase reporter construct, pGL3-(CAGA)₁₂ (SEQ ID NO:53) (Dennler et al. (1998) *EMBO J.*, 17: 3091-3100) was transiently transfected into A204 or RD rhabdomyosarcoma cells. Dilutions of conditioned media from vector or GASP1 transfected cells were incubated for 30 minutes at 37 °C with 10 ng/ml GDF-8, 10 ng/ml BMP-11, 10 ng/ml rh activin A (R&D Systems), or 0.5 ng/ml rh TGF- β 1 (R&D Systems). Luciferase activity was measured according to Thies et al. (2001) *Growth Factors*, 18: 251-259 and Zimmers et al. (2002) *Science*, 296: 1486-1488. In this assay, A204 cells respond to GDF-8, BMP-11, and activin, but do not respond well to TGF- β 1. RD cells respond to both GDF-8 and TGF- β 1. Thus, we used A204 cells to test for the ability of GASP1 to inhibit GDF-8, BMP-11, and activin and RD cells to monitor the activity of TGF- β and GDF-8. Results for GDF-8 are shown from A204 cells, but were similar in RD cells. A standard curve measuring the concentration dependence of the luciferase activity induced by each of these growth factors was generated for each experiment (data not shown). The growth factor concentrations used fall in the linear region of this curve such that small changes in concentration result in measurable changes in luciferase activity.

[0141] Two follistatin-domain proteins, follistatin and FLRG inhibit GDF-8 activity in a (CAGA)₁₂ (SEQ ID NO:53) luciferase transcriptional reporter assay, but also inhibit the biological activity of the related proteins, activin and BMP-11. The ability of GASP1 to inhibit GDF-8, BMP-11, activin, and TGF- β 1 activity in the (CAGA)₁₂ (SEQ ID NO:53) reporter assay was also tested.

[0142] Various dilutions of conditioned media from COS cells transfected with V5-His tagged GASP1 or a vector control were incubated with purified recombinant GDF-8 (10 ng/ml), BMP-11 (10 ng/ml), activin (10 ng/ml), or TGF- β 1 (0.5 ng/ml) and assayed for growth factor activity in rhabdomyosarcoma cells expressing the (CAGA)₁₂ (SEQ ID NO:53) reporter construct. GASP1 potently inhibited GDF-8 activity in a concentration dependent manner (Figure 16A). GASP1 similarly inhibited the activity of BMP-11 in this assay (Figure 16B), as might be expected since mature GDF-8 and BMP-11 are highly conserved and differ by only 11 amino acids. Surprisingly, GASP1 did not inhibit the activity of activin or TGF- β 1 (Figure 16C and D), suggesting a very high level of specificity, which is not demonstrated by follistatin itself. Thus, GASP1 exhibits specificity in its inhibition of GDF-8 and BMP-11.

[0143] The affinity of GASP1 for GDF-8 was evaluated by determining the IC₅₀ for inhibition of GDF-8 in the reporter gene assay. GASP1-V5-His protein was purified from conditioned media on a cobalt affinity column and eluted as described above. Fractions containing GASP1 were further purified by size exclusion chromatography in PBS using a BioSepS3000 column (Phenomenex). As shown in Figure 17, GASP1 inhibited GDF-8 with an IC₅₀ of approximately 3 nM.

Example 12: Treatment of Muscle Disorders

[0144] GASP1 may be administered to patients suffering from a disease or disorder related to the functioning of GDF-8 according to Table 2. Patients take the composition one time or at intervals, such as once daily, and the symptoms of their disease or disorder improve. For example, symptoms related to a muscle disorder are improved, as measured by muscle mass, muscle activity, and or muscle tone. This shows that the composition of the invention is useful for the treatment of diseases or disorders related to the functioning of GDF-8, such as muscle disorders.

Table 2: Administration of GASP1

Patient	Disease	Route of Administration	Dosage	Dosage Frequency	Predicted Results
1	muscular dystrophy	subcutaneous	25 mg	once daily	increase in muscle mass and improvement in muscle activity
2	"	"	50 mg	"	"
3	"	"	50 mg	once weekly	"
4	"	"	50 mg	once monthly	"
5	"	intramuscular	25 mg	once daily	"
6	"	"	50 mg	"	"
7	"	"	50 mg	once weekly	"
8	"	"	50 mg	once monthly	"
9	"	intravenous	25 mg	once daily	"
10	"	"	50 mg	"	"
11	"	"	50 mg	once weekly	"
12	"	"	50 mg	once monthly	"
13	diabetes	subcutaneous	50 mg	once daily	improvement in the management of blood sugar levels
14	"	"	50 mg	once weekly	"
15	"	intramuscular	50 mg	"	"
16	"	intravenous	50 mg	"	"
17	obesity	subcutaneous	50 mg	once daily	weight loss and increase in muscle mass
18	"	intramuscular	50 mg	once weekly	"
19	"	intravenous	50 mg	"	"

[0145] The entire contents of all references, patents and published patent applications cited throughout this application are herein incorporated by reference. The foregoing detailed description has been given for illustration purposes only. A wide range of changes and modifications can be made to the embodiments described above. It should therefore be understood that it is the following claims, including all equivalents, are intended to define the scope of the invention.

SEQUENCE LISTING

<110> HILL, JENNIFER J.
WOLFMAN, NEIL M.

<120> FOLLISTATIN DOMAIN CONTAINING PROTEINS

<130> 08702.0014-00

<140>

<141>

<150> 60/357,846

<151> 2002-02-21

<150> 60/434,645

<151> 2002-12-20

<160> 53

<170> PatentIn Ver. 2.1

<210> 1

<211> 1716

<212> DNA

<213> Mus sp.

<400> 1

```

atgtgtgccc cagggtatca tcggttctgg tttcactggg ggctgctggt gctgctgctc 60
ctcgaggctc cccttcgagg cctagcactg ccacccatcc gatactccca tgcggggcatc 120
tgccccaacg acatgaaccc caacctctgg gtggatgccc agagcacctg caagcgagag 180
tgtgaaacag accaggaatg tgagacctat gagaaatgct gcccgaatgt gtgtgggacc 240
aagagctgtg tggcagcccg ctacatggat gtgaaaggga agaagggccc tgtggggcatg 300
cccaaggagg ccacatgtga ccatttcatg tgcctgcagc agggctctga gtgtgacatc 360
tgggacggcc agcccggtgtg taagtgcaaa gatcgctgtg agaaggagcc cagcttcacc 420
tgtgcctctg atggccttac ctactacaac cgttgcttca tggacgccga agcctgctcc 480
aagggcatca cactgtctgt ggtcacctgt cgttatcact tcacctggcc taacaccagc 540
cctccaccgc ctgagaccac ggtgcatccc accaccgcct ctccggagac tctcgggctg 600
gacatggcag ccccggccct gctcaaccac cctgtccatc agtcagtcac cgtgggtgag 660
actgtgagtt tcctctgtga cgtggtaggc cggcctcggc cagagctcac ttgggagaaa 720
cagctggagg accgagaaaa tgttgtcatg aggcccaacc acgtgcgcgg taatgtggtg 780
gtcactaaca ttgcccagct ggtcatctac aacgtccagc cccaggatgc tggcatatac 840
acctgtacag ctcgaaatgt cgctggtgtc ctgagggctg acttcccgtt gtcggtggtc 900
aggggtggtc aggccagggc cacttcagag agcagtctca atggcacagc ttttccagca 960
acagagtgcc tgaagccccc agacagttag gactgtggag aggagcagac acgctggcac 1020
ttcgacgcc aggctaacaa ctgcctcact ttcacctttg gccactgcc aacacatctc 1080
aaccactttg agacctacga ggcctgtatg ctggcttgta tgagtgggcc attggccacc 1140
tgcagcctgc ctgccctgca agggccttgc aaagcttatg tcccacgctg ggcctacaac 1200
agccagacag gcctatgcc gtccttcgtc tatggcggct gtgagggcaa cggtaacaac 1260
tttgaaagcc gtgaggcttg tgaggagtgc tgtcccttcc cgaggggtaa ccagcactgc 1320
cgggcctgca agccccggca aaaacttgtt accagcttct gtcggagtga ctttgtcatc 1380
ctgggcaggg tctctgagct gaccgaagag caagactcag gccgtgccct ggtgaccgtg 1440
gatgaggtct taaaagatga gaagatgggc ctcaagtttc tgggccggga gcctctggaa 1500
gtcaccctgc ttcattgtaga ctggacctgt ccttgcccca acgtgacagt ggggtgagaca 1560
ccactcatca tcatggggga ggtcgacggc ggcattggca tgctgagacc cgatagcttt 1620
gtgggggcat cgagcacacg gcgggtcagg aagctccgtg aggtcatgta caagaaaacc 1680
tgtgacgtcc tcaaggactt cctgggcttg caatga 1716

```

<210> 2
 <211> 1716
 <212> DNA
 <213> Mus sp.

<400> 2
 atgtgtgccc cagggatatca tcggttcttg tttcactggg ggctgctggt gctgctgctc 60
 ctcgaggctc cccttcgagg cctagcactg ccacccatcc gatactccca tgcgggcatc 120
 tgccccaacg acatgaaccc caacctctgg gtggatgccc agagcacctg caagcgagag 180
 tgtgaaacag accaggaatg tgagacctat gagaaatgct gcccgaatgt gtgtgggacc 240
 aagagctgtg tggcagcccg ctacatggat gtgaaaggga agaagggggc tgtaggcatg 300
 cccaaggagg ccacatgtga ccatttcacg tgccctgcagc agggctctga gtgtgacatc 360
 tgggacggcc agcccgtgtg taagtgc aaa gatcgctgtg agaaggagcc cagcttcacc 420
 tgtgcctctg atggccttac ctactacaac cgttgcttca tggacgccga agcctgctcc 480
 aagggcatca cactgtctgt ggtcacctgt cgttatcact tcacctggcc taacaccagc 540
 cctccaccgc ctgagaccac ggtgcatccc accaccgcct ctccggagac tctcgggctg 600
 gacatggcag ccccagccct gctcaaccac cctgtccatc agtcagtcac cgtgggtgag 660
 actgtgagtt tctctgtga cgtggtaggc cggcctcggc cagagctcac ttgggagaaa 720
 cagctggagg accgagagaa tgttgtcatg aggcccaacc acgtgcgtgg taatgtggtg 780
 gtcactaaca ttgcccagct ggtcatctac aacgtccagc cccaggatgc tggcatatac 840
 acctgtacag ctcgaaatgt cgctgggtgc ctgagggctg acttcccgtt gtcgggtggc 900
 aggggtggtc agggcagggc cacttcagag agcagtctca atggcacagc ttttccagca 960
 acagagtgcc tgaagccccc agacagtga gactgtggag aggagcagac acgctggcac 1020
 ttcgacgcc aggctaacaa ctgcctcact ttcaccttg gccactgcc caacaatctc 1080
 aaccactttg agacctacga ggcctgtatg ctggcttgta tgagtgggccc attggccacc 1140
 tgcagcctgc ctgccctgca agggccttgc aaagcttatg tcccacgctg ggcctacaac 1200
 agccagacag gcctatgcc gtccttcgctc tatggcggct gtgagggcaa cggtaacaac 1260
 tttgaaagcc gtgaggcttg tgaggagtcg tgctccctcc cgaggggtaa ccagcactgc 1320
 cgggcctgca agccccggca aaaacttgtt accagcttct gtcggagtga ctttgtcatc 1380
 ctgggcaggg tctctgagct gaccgaggag caagactcgg gccgtgccct ggtgaccgtg 1440
 gatgaggtct taaaagatga gaagatgggc ctcaagtttc tgggcccggga gcctctggaa 1500
 gtcaccctgc ttcattgtaga ctggacctgt ccttgcccca acgtgacagt ggggtgagaca 1560
 ccactcatca tcatggggga ggtggacggc ggcattggca tgctgagacc cgatagcttt 1620
 gtgggggcat cgagcacacg gcgggtcagg aagctccgtg aggtcatgta caagaaaacc 1680
 tgtgacgtcc tcaaggactt cctgggcttg caatga 1716

<210> 3
 <211> 571
 <212> PRT
 <213> Mus sp.

<400> 3
 Met Cys Ala Pro Gly Tyr His Arg Phe Trp Phe His Trp Gly Leu Leu
 1 5 10 15
 Leu Leu Leu Leu Leu Glu Ala Pro Leu Arg Gly Leu Ala Leu Pro Pro
 20 25 30
 Ile Arg Tyr Ser His Ala Gly Ile Cys Pro Asn Asp Met Asn Pro Asn
 35 40 45
 Leu Trp Val Asp Ala Gln Ser Thr Cys Lys Arg Glu Cys Glu Thr Asp
 50 55 60
 Gln Glu Cys Glu Thr Tyr Glu Lys Cys Cys Pro Asn Val Cys Gly Thr
 65 70 75 80

Lys Ser Cys Val Ala Ala Arg Tyr Met Asp Val Lys Gly Lys Lys Gly
 85 90 95
 Pro Val Gly Met Pro Lys Glu Ala Thr Cys Asp His Phe Met Cys Leu
 100 105 110
 Gln Gln Gly Ser Glu Cys Asp Ile Trp Asp Gly Gln Pro Val Cys Lys
 115 120 125
 Cys Lys Asp Arg Cys Glu Lys Glu Pro Ser Phe Thr Cys Ala Ser Asp
 130 135 140
 Gly Leu Thr Tyr Tyr Asn Arg Cys Phe Met Asp Ala Glu Ala Cys Ser
 145 150 155 160
 Lys Gly Ile Thr Leu Ser Val Val Thr Cys Arg Tyr His Phe Thr Trp
 165 170 175
 Pro Asn Thr Ser Pro Pro Pro Pro Glu Thr Thr Val His Pro Thr Thr
 180 185 190
 Ala Ser Pro Glu Thr Leu Gly Leu Asp Met Ala Ala Pro Ala Leu Leu
 195 200 205
 Asn His Pro Val His Gln Ser Val Thr Val Gly Glu Thr Val Ser Phe
 210 215 220
 Leu Cys Asp Val Val Gly Arg Pro Arg Pro Glu Leu Thr Trp Glu Lys
 225 230 235 240
 Gln Leu Glu Asp Arg Glu Asn Val Val Met Arg Pro Asn His Val Arg
 245 250 255
 Gly Asn Val Val Val Thr Asn Ile Ala Gln Leu Val Ile Tyr Asn Val
 260 265 270
 Gln Pro Gln Asp Ala Gly Ile Tyr Thr Cys Thr Ala Arg Asn Val Ala
 275 280 285
 Gly Val Leu Arg Ala Asp Phe Pro Leu Ser Val Val Arg Gly Gly Gln
 290 295 300
 Ala Arg Ala Thr Ser Glu Ser Ser Leu Asn Gly Thr Ala Phe Pro Ala
 305 310 315 320
 Thr Glu Cys Leu Lys Pro Pro Asp Ser Glu Asp Cys Gly Glu Glu Gln
 325 330 335
 Thr Arg Trp His Phe Asp Ala Gln Ala Asn Asn Cys Leu Thr Phe Thr
 340 345 350
 Phe Gly His Cys His His Asn Leu Asn His Phe Glu Thr Tyr Glu Ala
 355 360 365
 Cys Met Leu Ala Cys Met Ser Gly Pro Leu Ala Thr Cys Ser Leu Pro
 370 375 380

4

Ala Leu Gln Gly Pro Cys Lys Ala Tyr Val Pro Arg Trp Ala Tyr Asn
385 390 395 400

Ser Gln Thr Gly Leu Cys Gln Ser Phe Val Tyr Gly Gly Cys Glu Gly
405 410 415

Asn Gly Asn Asn Phe Glu Ser Arg Glu Ala Cys Glu Glu Ser Cys Pro
420 425 430

Phe Pro Arg Gly Asn Gln His Cys Arg Ala Cys Lys Pro Arg Gln Lys
435 440 445

Leu Val Thr Ser Phe Cys Arg Ser Asp Phe Val Ile Leu Gly Arg Val
450 455 460

Ser Glu Leu Thr Glu Glu Gln Asp Ser Gly Arg Ala Leu Val Thr Val
465 470 475 480

Asp Glu Val Leu Lys Asp Glu Lys Met Gly Leu Lys Phe Leu Gly Arg
485 490 495

Glu Pro Leu Glu Val Thr Leu Leu His Val Asp Trp Thr Cys Pro Cys
500 505 510

Pro Asn Val Thr Val Gly Glu Thr Pro Leu Ile Ile Met Gly Glu Val
515 520 525

Asp Gly Gly Met Ala Met Leu Arg Pro Asp Ser Phe Val Gly Ala Ser
530 535 540

Ser Thr Arg Arg Val Arg Lys Leu Arg Glu Val Met Tyr Lys Lys Thr
545 550 555 560

Cys Asp Val Leu Lys Asp Phe Leu Gly Leu Gln
565 570

<210> 4

<211> 1923

<212> DNA

<213> Homo sapiens

<220>

<221> modified_base

<222> (732)

<223> a, t, c or g

<400> 4

atgaatccca acctctgggt ggacgcacag agcacctgca ggcgaggagtg tgagacggac 60
caggagtgtg agatggacca ggtgagtggg atccagaagc cacagtgtga ggcagaccag 120
gtgaatgggg tccagaagcc gcaatgtgag atggaccaga agtgggagtg tgagggttgac 180
cagggtgagt ggggtccagaa gccggtgtgt gaggcggacc aggtgagtgg ggtccagaag 240
ccacagtgtg agatggacca ggtgagtggg atccagaagc tggagtgtga ggcggaccag 300
aagtgggagt atgaggtgga ccaggtgagt ggggtccaga agccacagtg tgagatggac 360
cagggtgagt ggatccagaa gctggagtgt gaggcggacc aggagtgtga gacctatgag 420
aagtgctgcc ccaacgtatg tgggaccaag agctgcgtgg cggcccgccta catggacgtg 480
aaaggaaga agggcccagt gggcatgccc aaggaggcca catgtgacca cttcatgtgt 540
ctgcagcagg gctctgagtg tgacatctgg gatggccagc ccgtgtgtaa gtgcaaagac 600

5

```

cgctgtgaga aggagcccag ctttacctgc gcctcggacg gcctcaccta ctataaccgc 660
tgctacatgg atgccgaggg ctgctccaaa ggcacacacac tggccgttgt aacctgccgc 720
tatcacttca cntggcccaa caccagcccc ccaccacctg agaccaccat gcaccccacc 780
acagcctccc cagagacccc tgagctggac atggcggccc ctgcgctgct caacaaccct 840
gtgcaccagt cggtcaccat gggtagagac gtgagcttcc tctgtgatgt ggtgggcccg 900
ccccggcctg agatcacctg ggagaagcag ttggaggatc gggagaatgt ggtcatgcgg 960
cccaaccatg tgcgtggcaa cgtgggtggtc accaacattg cccagctggg catctataac 1020
gcccagctgc aggatgctgg gatctacacc tgcacggccc ggaacgtggc tggggtcctg 1080
agggctgatt tcccgtgtgc ggtgggtcagg ggtcatcagg ctgcagccac ctgagagagc 1140
agccccaatg gcacggcttt cccggcggcc gagtgccctga agccccaga cagtaggagc 1200
tgtggcgaag agcagacccg ctggcacttc gatgcccagg ccaacaactg cctgaccttc 1260
accttcggcc actgccaccg taacctcaac cactttgaga cctatgaggc ctgcatgctg 1320
gcctgcatga gcgggcccgt ggccgcgtgc agcctgcccc cctgcaggg gccctgcaaa 1380
gcctacgcgc ctgcgtgggc ttacaacagc cagacgggcc agtgccagtc ctttgtctat 1440
ggtaggtgctg agggcaatgg caacaacttt gagagccgtg aggcctgtga ggagtcgtgc 1500
cccttccccca gggggaacca gcgctgtcgg gcctgcaagc ctgcgcagaa gctcgttacc 1560
agcttctgtc gcagcgactt tgtcatcctg ggccgagctc ctgagctgac cgaggagcct 1620
gactcgggcc gcgccctggg gactgtggat gaggtcctaa aggatgagaa aatgggcctc 1680
aagttcctgg gccaggagcc attggaggtc actctgcttc acgtggactg ggcacgcccc 1740
tgccccaacg tgaccgtgag cgagatgccg ctcatcatca tgggggaggt ggacggcggc 1800
atggccatgc tgcgccccga tagctttgtg ggcgcacatga gtgcccgcgc ggtcaggaag 1860
cttcgtgagg tcatgcacaa gaagacctgt gacgtcctca aggagtttct tggcttgcac 1920
tga

```

<210> 5

<211> 640

<212> PRT

<213> Homo sapiens

<400> 5

```

Met Asn Pro Asn Leu Trp Val Asp Ala Gln Ser Thr Cys Arg Arg Glu
 1             5             10             15

Cys Glu Thr Asp Gln Glu Cys Glu Met Asp Gln Val Ser Gly Ile Gln
          20             25             30

Lys Pro Gln Cys Glu Ala Asp Gln Val Asn Gly Val Gln Lys Pro Gln
          35             40             45

Cys Glu Met Asp Gln Lys Trp Glu Cys Glu Val Asp Gln Val Ser Gly
          50             55             60

Val Gln Lys Pro Val Cys Glu Ala Asp Gln Val Ser Gly Val Gln Lys
          65             70             75             80

Pro Gln Cys Glu Met Asp Gln Val Ser Gly Ile Gln Lys Leu Glu Cys
          85             90             95

Glu Ala Asp Gln Lys Trp Glu Tyr Glu Val Asp Gln Val Ser Gly Val
          100            105            110

Gln Lys Pro Gln Cys Glu Met Asp Gln Val Ser Gly Ile Gln Lys Leu
          115            120            125

Glu Cys Glu Ala Asp Gln Glu Cys Glu Thr Tyr Glu Lys Cys Cys Pro
          130            135            140

```

6

Asn Val Cys Gly Thr Lys Ser Cys Val Ala Ala Arg Tyr Met Asp Val	
145	155 160
Lys Gly Lys Lys Gly Pro Val Gly Met Pro Lys Glu Ala Thr Cys Asp	
	165 170 175
His Phe Met Cys Leu Gln Gln Gly Ser Glu Cys Asp Ile Trp Asp Gly	
	180 185 190
Gln Pro Val Cys Lys Cys Lys Asp Arg Cys Glu Lys Glu Pro Ser Phe	
	195 200 205
Thr Cys Ala Ser Asp Gly Leu Thr Tyr Tyr Asn Arg Cys Tyr Met Asp	
	210 215 220
Ala Glu Ala Cys Ser Lys Gly Ile Thr Leu Ala Val Val Thr Cys Arg	
	225 230 235 240
Tyr His Phe Thr Trp Pro Asn Thr Ser Pro Pro Pro Pro Glu Thr Thr	
	245 250 255
Met His Pro Thr Thr Ala Ser Pro Glu Thr Pro Glu Leu Asp Met Ala	
	260 265 270
Ala Pro Ala Leu Leu Asn Asn Pro Val His Gln Ser Val Thr Met Gly	
	275 280 285
Glu Thr Val Ser Phe Leu Cys Asp Val Val Gly Arg Pro Arg Pro Glu	
	290 295 300
Ile Thr Trp Glu Lys Gln Leu Glu Asp Arg Glu Asn Val Val Met Arg	
	305 310 315 320
Pro Asn His Val Arg Gly Asn Val Val Val Thr Asn Ile Ala Gln Leu	
	325 330 335
Val Ile Tyr Asn Ala Gln Leu Gln Asp Ala Gly Ile Tyr Thr Cys Thr	
	340 345 350
Ala Arg Asn Val Ala Gly Val Leu Arg Ala Asp Phe Pro Leu Ser Val	
	355 360 365
Val Arg Gly His Gln Ala Ala Ala Thr Ser Glu Ser Ser Pro Asn Gly	
	370 375 380
Thr Ala Phe Pro Ala Ala Glu Cys Leu Lys Pro Pro Asp Ser Glu Asp	
	385 390 395 400
Cys Gly Glu Glu Gln Thr Arg Trp His Phe Asp Ala Gln Ala Asn Asn	
	405 410 415
Cys Leu Thr Phe Thr Phe Gly His Cys His Arg Asn Leu Asn His Phe	
	420 425 430
Glu Thr Tyr Glu Ala Cys Met Leu Ala Cys Met Ser Gly Pro Leu Ala	
	435 440 445

7

Ala Cys Ser Leu Pro Ala Leu Gln Gly Pro Cys Lys Ala Tyr Ala Pro
 450 455 460

Arg Trp Ala Tyr Asn Ser Gln Thr Gly Gln Cys Gln Ser Phe Val Tyr
 465 470 475 480

Gly Gly Cys Glu Gly Asn Gly Asn Asn Phe Glu Ser Arg Glu Ala Cys
 485 490 495

Glu Glu Ser Cys Pro Phe Pro Arg Gly Asn Gln Arg Cys Arg Ala Cys
 500 505 510

Lys Pro Arg Gln Lys Leu Val Thr Ser Phe Cys Arg Ser Asp Phe Val
 515 520 525

Ile Leu Gly Arg Val Ser Glu Leu Thr Glu Glu Pro Asp Ser Gly Arg
 530 535 540

Ala Leu Val Thr Val Asp Glu Val Leu Lys Asp Glu Lys Met Gly Leu
 545 550 555 560

Lys Phe Leu Gly Gln Glu Pro Leu Glu Val Thr Leu Leu His Val Asp
 565 570 575

Trp Ala Cys Pro Cys Pro Asn Val Thr Val Ser Glu Met Pro Leu Ile
 580 585 590

Ile Met Gly Glu Val Asp Gly Gly Met Ala Met Leu Arg Pro Asp Ser
 595 600 605

Phe Val Gly Ala Ser Ser Ala Arg Arg Val Arg Lys Leu Arg Glu Val
 610 615 620

Met His Lys Lys Thr Cys Asp Val Leu Lys Glu Phe Leu Gly Leu His
 625 630 635 640

<210> 6

<211> 1731

<212> DNA

<213> Homo sapiens

<400> 6

atgtgggccc caaggtgtcg ccggttctgg tctcgctggg agcaggtggc agcgctgctg 60
 ctgctgctgc tactgctcgg ggtgcccccg cgaagcctgg cgctgccgcc catccgctat 120
 tcccacgccg gcatctgccc caacgacatg aatcccaacc tctgggtgga cgcacagagc 180
 acctgcaggc gggagtgtga gacggaccag gagtgtgaga cctatgagaa gtgctgcccc 240
 aacgtatgtg ggaccaagag ctgcgtggcg gcccgctaca tggacgtgaa agggaagaag 300
 ggcccagtgg gcatgccc aa ggaggccaca tgtgaccact tcatgtgtct gcagcagggc 360
 tctgagtgtg acatctggga tggccagccc gtgtgtaagt gcaaagaccg ctgtgagaag 420
 gagcccagct ttacctgcgc ctcggacggc ctcacctact ataaccgctg ctacatggat 480
 gccgaggcct gctccaaagg catcacactg gccgttgtaa cctgccgcta tcacttcacc 540
 tggcccaaca ccagcccccc accacctgag accaccatgc accccaccac agcctcccca 600
 gagacccttg agctggacat ggcgccccct gcgctgctca acaaccctgt gcaccagtgc 660
 gtcaccatgg gtgagacagt gagtttcttc tgtgatgtgg tgggcccggc ccggcctgag 720
 atcacctggg agaagcagtt ggaggatcgg gagaatgtgg tcatgcggcc caaccatgtg 780
 cgtggcaacg tgggtggtcac caacattgcc cagctgggtca tctataacgc ccagctgcag 840
 gatgctggga tctacacctg cacggccccg aacgtggctg gggtcctgag ggctgatttc 900

```

ccgctgtcgg tggtcagggg tcatcaggct gcagccacct cagagagcag cccaatggc 960
acggctttcc cggcggccga gtgcctgaag ccccagaca gtgaggactg tggcgaagag 1020
cagacccgct ggcacttcga tgcccaggcc aacaactgcc tgaccttcac ctcggccac 1080
tgccaccgta acctcaacca ctttgagacc tatgaggcct gcatgctggc ctgcatgagc 1140
gggcccgtgg ccgcgtgcag cctgcccgcc ctgcaggggc cctgcaaagc ctacgcgcct 1200
cgctgggctt acaacagcca gacgggccag tgccagtcct ttgtctatgg tggctgcgag 1260
ggcaatggca acaactttga gagccgtgag gcctgtgagg agtcgtgccc cttccccagg 1320
gggaaccagc gctgtcgggc ctgcaagcct cggcagaagc tcgttaccag cttctgtcgc 1380
agcgactttg tcatcctggg ccgagtctct gagctgaccg aggagcctga ctcggggccgc 1440
gccctggtga ctgtggatga ggtcctaaag gatgagaaaa tgggcctcaa gttcctgggc 1500
caggagccat tggaggtcac tctgcttcac gtggactggg catgcccctg cccaacgtg 1560
accgtgagcg agatgccgct catcatcatg ggggaggtgg acggcggcat ggccatgctg 1620
cgccccgata gctttgtggg cgcctcgagt gcccgccggg tcaggaagct tcgtgaggtc 1680
atgcacaaga agacctgtga cgtcctcaag gagtttcttg gcttgcactg a 1731

```

<210> 7

<211> 576

<212> PRT

<213> Homo sapiens

<400> 7

```

Met Trp Ala Pro Arg Cys Arg Arg Phe Trp Ser Arg Trp Glu Gln Val
  1                      5                      10                      15

```

```

Ala Ala Leu Leu Leu Leu Leu Leu Leu Gly Val Pro Pro Arg Ser
                20                      25                      30

```

```

Leu Ala Leu Pro Pro Ile Arg Tyr Ser His Ala Gly Ile Cys Pro Asn
    35                      40                      45

```

```

Asp Met Asn Pro Asn Leu Trp Val Asp Ala Gln Ser Thr Cys Arg Arg
    50                      55                      60

```

```

Glu Cys Glu Thr Asp Gln Glu Cys Glu Thr Tyr Glu Lys Cys Cys Pro
    65                      70                      75                      80

```

```

Asn Val Cys Gly Thr Lys Ser Cys Val Ala Ala Arg Tyr Met Asp Val
                85                      90                      95

```

```

Lys Gly Lys Lys Gly Pro Val Gly Met Pro Lys Glu Ala Thr Cys Asp
    100                      105                      110

```

```

His Phe Met Cys Leu Gln Gln Gly Ser Glu Cys Asp Ile Trp Asp Gly
    115                      120                      125

```

```

Gln Pro Val Cys Lys Cys Lys Asp Arg Cys Glu Lys Glu Pro Ser Phe
    130                      135                      140

```

```

Thr Cys Ala Ser Asp Gly Leu Thr Tyr Tyr Asn Arg Cys Tyr Met Asp
    145                      150                      155                      160

```

```

Ala Glu Ala Cys Ser Lys Gly Ile Thr Leu Ala Val Val Thr Cys Arg
                165                      170                      175

```

```

Tyr His Phe Thr Trp Pro Asn Thr Ser Pro Pro Pro Pro Glu Thr Thr
    180                      185                      190

```

Met His Pro Thr Thr Ala Ser Pro Glu Thr Pro Glu Leu Asp Met Ala
 195 200 205
 Ala Pro Ala Leu Leu Asn Asn Pro Val His Gln Ser Val Thr Met Gly
 210 215 220
 Glu Thr Val Ser Phe Leu Cys Asp Val Val Gly Arg Pro Arg Pro Glu
 225 230 235 240
 Ile Thr Trp Glu Lys Gln Leu Glu Asp Arg Glu Asn Val Val Met Arg
 245 250 255
 Pro Asn His Val Arg Gly Asn Val Val Val Thr Asn Ile Ala Gln Leu
 260 265 270
 Val Ile Tyr Asn Ala Gln Leu Gln Asp Ala Gly Ile Tyr Thr Cys Thr
 275 280 285
 Ala Arg Asn Val Ala Gly Val Leu Arg Ala Asp Phe Pro Leu Ser Val
 290 295 300
 Val Arg Gly His Gln Ala Ala Ala Thr Ser Glu Ser Ser Pro Asn Gly
 305 310 315 320
 Thr Ala Phe Pro Ala Ala Glu Cys Leu Lys Pro Pro Asp Ser Glu Asp
 325 330 335
 Cys Gly Glu Glu Gln Thr Arg Trp His Phe Asp Ala Gln Ala Asn Asn
 340 345 350
 Cys Leu Thr Phe Thr Phe Gly His Cys His Arg Asn Leu Asn His Phe
 355 360 365
 Glu Thr Tyr Glu Ala Cys Met Leu Ala Cys Met Ser Gly Pro Leu Ala
 370 375 380
 Ala Cys Ser Leu Pro Ala Leu Gln Gly Pro Cys Lys Ala Tyr Ala Pro
 385 390 395 400
 Arg Trp Ala Tyr Asn Ser Gln Thr Gly Gln Cys Gln Ser Phe Val Tyr
 405 410 415
 Gly Gly Cys Glu Gly Asn Gly Asn Asn Phe Glu Ser Arg Glu Ala Cys
 420 425 430
 Glu Glu Ser Cys Pro Phe Pro Arg Gly Asn Gln Arg Cys Arg Ala Cys
 435 440 445
 Lys Pro Arg Gln Lys Leu Val Thr Ser Phe Cys Arg Ser Asp Phe Val
 450 455 460
 Ile Leu Gly Arg Val Ser Glu Leu Thr Glu Glu Pro Asp Ser Gly Arg
 465 470 475 480
 Ala Leu Val Thr Val Asp Glu Val Leu Lys Asp Glu Lys Met Gly Leu
 485 490 495

10

Lys Phe Leu Gly Gln Glu Pro Leu Glu Val Thr Leu Leu His Val Asp
500 505 510

Trp Ala Cys Pro Cys Pro Asn Val Thr Val Ser Glu Met Pro Leu Ile
515 520 525

Ile Met Gly Glu Val Asp Gly Gly Met Ala Met Leu Arg Pro Asp Ser
530 535 540

Phe Val Gly Ala Ser Ser Ala Arg Arg Val Arg Lys Leu Arg Glu Val
545 550 555 560

Met His Lys Lys Thr Cys Asp Val Leu Lys Glu Phe Leu Gly Leu His
565 570 575

<210> 8

<211> 1659

<212> DNA

<213> Mus sp.

<400> 8

```

atgcctgccc cacagccatt cctgcctctg ctctttgtct tcgtgctcat ccatctgacc 60
tcggagacca acctgctgcc agatcccgga agccatcctg gcatgtgccc caacgagctc 120
agccccacc tgtgggtcga cgcccagagc acctgtgagc gtgagtgtac cggggaccag 180
gactgtgcgg catccgagaa gtgctgcacc aatgtgtgtg ggctgcagag ctgcgtggct 240
gcccgtttc ccagtgggtg cccagctgta cctgagacag cagcctcctg tgaaggcttc 300
caatgcccac aacaggggtc tgactgtgac atctgggatg ggcagccagt ttgtcgctgc 360
cgtgaccgct gtgaaaaaga acccagcttc acatgtgctt ctgatggcct tacctattac 420
aaccgctgct acatggacgc agaagcctgc ctgcggggtc tccacctgca cgttgtaccc 480
tgtaagcaca ttctcagttg gccgcccagc agcccgggac cacccgagac cactgctcgc 540
ccaaccctg gggctgctcc catgccacct gccctgtaca acagcccctc accacaggca 600
gtgcatgttg gggggacagc cagcctccac tgtgatgtta gtggccgtcc accacctgct 660
gtgacctggg agaagcagag ccatcagcgg gagaacctga tcatgcgccc tgaccaaagt 720
tatggcaacg tggttgtcac cagtatcgga cagctagtc tctacaatgc tcagttggag 780
gatgcgggcc tgtatacctg cactgcacga aacgctgccg gcctgctgcg ggccgacttt 840
cccccttccg ttttacagcg ggcaactact caggacaggg acccaggtat cccagccttg 900
gctgagtgcc aggccgacac acaagcctgt gttggggcac ctactcccca tcatgtcctt 960
tggcgctttg acccacagag aggcagctgc atgacattcc cagccctcag atgtgatggg 1020
gctgcccggg gctttgagac ctatgaggca tgccagcagg cctgtgttcg tggccccggg 1080
gatgtctgtg cactgcctgc agttcagggg ccctgccagg gctgggagcc acgctggggc 1140
tacagcccac tgctacagca gtgccacccc tttgtataca gtggctgtga aggaaacagc 1200
aataactttg agaccgggga gagctgtgag gatgcttgcc ctgtaccacg cacaccacc 1260
tgtcgtgcct gccgcctcaa gagcaagctg gctctgagct tgtgccgcag tgactttgcc 1320
atcgtgggga gactcacaga ggtcctggag gagcccagag ctgcaggcgg catagctcgt 1380
gtggccttgg atgatgtgct aaaggacgac aagatgggcc tcaagttctt gggcaccaaa 1440
tacctggagg tgacattgag tggcatggac tgggcctgcc catgccccaa cgtgacagct 1500
gtcgaatggc cactgggtcat catgggtgag gttcgtgaag gtgtggctgt gttggacgcc 1560
aacagctatg tccgtgctgc cagcgagaag cgagtcaaga agattgtgga actgctcgag 1620
aagaaggctt gtgaactgct caaccgcttc caagactag 1659

```

<210> 9

<211> 552

<212> PRT

<213> Mus sp.

11

<400> 9

```

Met Pro Ala Pro Gln Pro Phe Leu Pro Leu Leu Phe Val Phe Val Leu
 1           5           10           15

Ile His Leu Thr Ser Glu Thr Asn Leu Leu Pro Asp Pro Gly Ser His
          20           25           30

Pro Gly Met Cys Pro Asn Glu Leu Ser Pro His Leu Trp Val Asp Ala
      35           40           45

Gln Ser Thr Cys Glu Arg Glu Cys Thr Gly Asp Gln Asp Cys Ala Ala
 50           55           60

Ser Glu Lys Cys Cys Thr Asn Val Cys Gly Leu Gln Ser Cys Val Ala
 65           70           75           80

Ala Arg Phe Pro Ser Gly Gly Pro Ala Val Pro Glu Thr Ala Ala Ser
          85           90           95

Cys Glu Gly Phe Gln Cys Pro Gln Gln Gly Ser Asp Cys Asp Ile Trp
          100           105           110

Asp Gly Gln Pro Val Cys Arg Cys Arg Asp Arg Cys Glu Lys Glu Pro
      115           120           125

Ser Phe Thr Cys Ala Ser Asp Gly Leu Thr Tyr Tyr Asn Arg Cys Tyr
      130           135           140

Met Asp Ala Glu Ala Cys Leu Arg Gly Leu His Leu His Val Val Pro
      145           150           155           160

Cys Lys His Ile Leu Ser Trp Pro Pro Ser Ser Pro Gly Pro Pro Glu
          165           170           175

Thr Thr Ala Arg Pro Thr Pro Gly Ala Ala Pro Met Pro Pro Ala Leu
          180           185           190

Tyr Asn Ser Pro Ser Pro Gln Ala Val His Val Gly Gly Thr Ala Ser
      195           200           205

Leu His Cys Asp Val Ser Gly Arg Pro Pro Pro Ala Val Thr Trp Glu
      210           215           220

Lys Gln Ser His Gln Arg Glu Asn Leu Ile Met Arg Pro Asp Gln Met
      225           230           235           240

Tyr Gly Asn Val Val Val Thr Ser Ile Gly Gln Leu Val Leu Tyr Asn
          245           250           255

Ala Gln Leu Glu Asp Ala Gly Leu Tyr Thr Cys Thr Ala Arg Asn Ala
          260           265           270

Ala Gly Leu Leu Arg Ala Asp Phe Pro Leu Ser Val Leu Gln Arg Ala
          275           280           285

Thr Thr Gln Asp Arg Asp Pro Gly Ile Pro Ala Leu Ala Glu Cys Gln
      290           295           300

```

12

Ala Asp Thr Gln Ala Cys Val Gly Pro Pro Thr Pro His His Val Leu
 305 310 315 320

Trp Arg Phe Asp Pro Gln Arg Gly Ser Cys Met Thr Phe Pro Ala Leu
 325 330 335

Arg Cys Asp Gly Ala Ala Arg Gly Phe Glu Thr Tyr Glu Ala Cys Gln
 340 345 350

Gln Ala Cys Val Arg Gly Pro Gly Asp Val Cys Ala Leu Pro Ala Val
 355 360 365

Gln Gly Pro Cys Gln Gly Trp Glu Pro Arg Trp Ala Tyr Ser Pro Leu
 370 375 380

Leu Gln Gln Cys His Pro Phe Val Tyr Ser Gly Cys Glu Gly Asn Ser
 385 390 395 400

Asn Asn Phe Glu Thr Arg Glu Ser Cys Glu Asp Ala Cys Pro Val Pro
 405 410 415

Arg Thr Pro Pro Cys Arg Ala Cys Arg Leu Lys Ser Lys Leu Ala Leu
 420 425 430

Ser Leu Cys Arg Ser Asp Phe Ala Ile Val Gly Arg Leu Thr Glu Val
 435 440 445

Leu Glu Glu Pro Glu Ala Ala Gly Gly Ile Ala Arg Val Ala Leu Asp
 450 455 460

Asp Val Leu Lys Asp Asp Lys Met Gly Leu Lys Phe Leu Gly Thr Lys
 465 470 475 480

Tyr Leu Glu Val Thr Leu Ser Gly Met Asp Trp Ala Cys Pro Cys Pro
 485 490 495

Asn Val Thr Ala Val Asp Gly Pro Leu Val Ile Met Gly Glu Val Arg
 500 505 510

Glu Gly Val Ala Val Leu Asp Ala Asn Ser Tyr Val Arg Ala Ala Ser
 515 520 525

Glu Lys Arg Val Lys Lys Ile Val Glu Leu Leu Glu Lys Lys Ala Cys
 530 535 540

Glu Leu Leu Asn Arg Phe Gln Asp
 545 550

<210> 10

<211> 1695

<212> DNA

<213> Homo sapiens

<400> 10

atgcccgccc tacgtccact cctgccgctc ctgctcctcc tccggctgac ctcgggggct 60
 ggcttgctgc cagggctggg gagccacccg ggcgtgtgcc ccaaccagct cagccccaac 120
 ctgtgggtgg acgcccagag cacctgtgag cgcgagtgtg gcagggacca ggactgtgcg 180

13

```

gctgctgaga agtgctgcat caacgtgtgt ggactgcaca gctgcggtggc agcacgcttc 240
cccggcagcc cagctgcgcc gacgacagcg gcctcctgcg agggcctttgt gtgcccacag 300
cagggctcgg actgcgacat ctgggacggg cagcccgtgt gccgctgccg cgaccgctgt 360
gagaaggagc ccagcttcac ctgcgcctcg gacggcctca cctactacaa ccgctgctat 420
atggacgccg aggcctgcct gcggggcctg cacctccaca tcgtgccctg caagcacgtg 480
ctcagctggc cgcccagcag cccggggccg ccggagacca ctgcccggcc cacacctggg 540
gccgcgcccc tgctcctgc cctgtacagc agccctccc cacaggcggt gcaggttggg 600
ggtagcgcca gcctccactg cgacgtcagc ggccgcccgc cgcctgctgt gacctgggag 660
aagcagagtc accagcgaga gaacctgatc atgcgccctg atcagatgta tggcaacgtg 720
gtggtcacca gcatcgggca gctggtgctc tacaacgcgc ggcccgaaga cgccggcctg 780
tacacctgca ccgcgcgcaa cgctgctggg ctgctgcggg ctgacttccc actctctgtg 840
gtccagcgag agccggccag ggacgcagcc cccagcatcc cagccccggc cgagtgcctg 900
ccggatgtgc aggcctgcac gggccccact tccccacacc ttgtcctctg gcactacgac 960
ccgcagcggg gcggctgcat gaccttcccg gcccggtggc gtgatggggc ggcccgcggc 1020
tttgagacct acgaggcatg ccagcaggcc tgtgcccgcg gccccggcga cgcctgcgtg 1080
ctgcctgccg tgcagggccc ctgccggggc tgggagccgc gctgggccta cagcccgctg 1140
ctgcagcagt gccatccctt cgtgtacggt ggctgcgagg gcaacggcaa caacttccac 1200
agccgcgaga gctgcgagga tgctgcccc gtgccgcgca caccgcccctg ccgcgcctgc 1260
cgcctccgga gcaagctggc gctgagcctg tgccgcagcg acttcgccat cgtggggcgg 1320
ctcacggagg tgctggagga gcccgaggcc gccggcggca tcgcccgcg ggcgctcgag 1380
gacgtgctca aggatgacaa gatgggcctc aagttcttgg gcaccaagta cctggagggtg 1440
acgctgagtg gcatggactg ggcctgcccc tgccccaaca tgacggcggg cgacgggccc 1500
ctggtcatca tgggtgaggt gcgcgatggc gtggccgtgc tggacgccgg cagctacgtc 1560
cgcgccgcca gcgagaagcg cgtcaagaag atcttgagc tgctggagaa gcaggcctgc 1620
gagctgctca accgcttcca ggactagccc ccgcaggggc ctgcgccacc ccgtcctggt 1680
gaataaacgc actcc

```

<210> 11

<211> 548

<212> PRT

<213> Homo sapiens

<400> 11

```

Met Pro Ala Leu Arg Pro Leu Leu Pro Leu Leu Leu Leu Arg Leu
  1                      5                      10                     15

Thr Ser Gly Ala Gly Leu Leu Pro Gly Leu Gly Ser His Pro Gly Val
      20                      25                      30

Cys Pro Asn Gln Leu Ser Pro Asn Leu Trp Val Asp Ala Gln Ser Thr
      35                      40                      45

Cys Glu Arg Glu Cys Ser Arg Asp Gln Asp Cys Ala Ala Ala Glu Lys
      50                      55                      60

Cys Cys Ile Asn Val Cys Gly Leu His Ser Cys Val Ala Ala Arg Phe
      65                      70                      75                      80

Pro Gly Ser Pro Ala Ala Pro Thr Thr Ala Ala Ser Cys Glu Gly Phe
      85                      90                      95

Val Cys Pro Gln Gln Gly Ser Asp Cys Asp Ile Trp Asp Gly Gln Pro
      100                     105                     110

Val Cys Arg Cys Arg Asp Arg Cys Glu Lys Glu Pro Ser Phe Thr Cys
      115                     120                     125

```

14

Ala Ser Asp Gly Leu Thr Tyr Tyr Asn Arg Cys Tyr Met Asp Ala Glu
 130 135 140

Ala Cys Leu Arg Gly Leu His Leu His Ile Val Pro Cys Lys His Val
 145 150 155 160

Leu Ser Trp Pro Pro Ser Ser Pro Gly Pro Pro Glu Thr Thr Ala Arg
 165 170 175

Pro Thr Pro Gly Ala Ala Pro Val Pro Pro Ala Leu Tyr Ser Ser Pro
 180 185 190

Ser Pro Gln Ala Val Gln Val Gly Gly Thr Ala Ser Leu His Cys Asp
 195 200 205

Val Ser Gly Arg Pro Pro Pro Ala Val Thr Trp Glu Lys Gln Ser His
 210 215 220

Gln Arg Glu Asn Leu Ile Met Arg Pro Asp Gln Met Tyr Gly Asn Val
 225 230 235 240

Val Val Thr Ser Ile Gly Gln Leu Val Leu Tyr Asn Ala Arg Pro Glu
 245 250 255

Asp Ala Gly Leu Tyr Thr Cys Thr Ala Arg Asn Ala Ala Gly Leu Leu
 260 265 270

Arg Ala Asp Phe Pro Leu Ser Val Val Gln Arg Glu Pro Ala Arg Asp
 275 280 285

Ala Ala Pro Ser Ile Pro Ala Pro Ala Glu Cys Leu Pro Asp Val Gln
 290 295 300

Ala Cys Thr Gly Pro Thr Ser Pro His Leu Val Leu Trp His Tyr Asp
 305 310 315 320

Pro Gln Arg Gly Gly Cys Met Thr Phe Pro Ala Arg Gly Cys Asp Gly
 325 330 335

Ala Ala Arg Gly Phe Glu Thr Tyr Glu Ala Cys Gln Gln Ala Cys Ala
 340 345 350

Arg Gly Pro Gly Asp Ala Cys Val Leu Pro Ala Val Gln Gly Pro Cys
 355 360 365

Arg Gly Trp Glu Pro Arg Trp Ala Tyr Ser Pro Leu Leu Gln Gln Cys
 370 375 380

His Pro Phe Val Tyr Gly Gly Cys Glu Gly Asn Gly Asn Asn Phe His
 385 390 395 400

Ser Arg Glu Ser Cys Glu Asp Ala Cys Pro Val Pro Arg Thr Pro Pro
 405 410 415

Cys Arg Ala Cys Arg Leu Arg Ser Lys Leu Ala Leu Ser Leu Cys Arg
 420 425 430

15

Ser Asp Phe Ala Ile Val Gly Arg Leu Thr Glu Val Leu Glu Glu Pro
 435 440 445
 Glu Ala Ala Gly Gly Ile Ala Arg Val Ala Leu Glu Asp Val Leu Lys
 450 455 460
 Asp Asp Lys Met Gly Leu Lys Phe Leu Gly Thr Lys Tyr Leu Glu Val
 465 470 475 480
 Thr Leu Ser Gly Met Asp Trp Ala Cys Pro Cys Pro Asn Met Thr Ala
 485 490 495
 Gly Asp Gly Pro Leu Val Ile Met Gly Glu Val Arg Asp Gly Val Ala
 500 505 510
 Val Leu Asp Ala Gly Ser Tyr Val Arg Ala Ala Ser Glu Lys Arg Val
 515 520 525
 Lys Lys Ile Leu Glu Leu Leu Glu Lys Gln Ala Cys Glu Leu Leu Asn
 530 535 540
 Arg Phe Gln Asp
 545

<210> 12
 <211> 30
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Illustrative
 competing peptide

<400> 12
 Asp Phe Gly Leu Asp Ser Asp Glu His Ser Thr Glu Ser Arg Ser Ser
 1 5 10 15
 Arg Tyr Pro Leu Thr Val Asp Phe Glu Ala Phe Gly Trp Asp
 20 25 30

<210> 13
 <211> 15
 <212> PRT
 <213> Mus sp.

<400> 13
 Ala Asn Tyr Cys Ser Gly Glu Cys Glu Phe Val Phe Leu Gln Lys
 1 5 10 15

<210> 14
 <211> 12
 <212> PRT
 <213> Mus sp.

16

<400> 14

Met	Ser	Pro	Ile	Asn	Met	Leu	Tyr	Phe	Asn	Gly	Lys
1				5					10		

<210> 15

<211> 14

<212> PRT

<213> Mus sp.

<400> 15

Asp	Phe	Gly	Leu	Asp	Cys	Asp	Glu	His	Ser	Thr	Glu	Ser	Arg
1				5					10				

<210> 16

<211> 15

<212> PRT

<213> Mus sp.

<400> 16

Ala	Asn	Tyr	Cys	Ser	Gly	Glu	Cys	Glu	Phe	Val	Phe	Leu	Gln	Lys
1				5					10					15

<210> 17

<211> 12

<212> PRT

<213> Mus sp.

<400> 17

Met	Ser	Pro	Ile	Asn	Met	Leu	Tyr	Phe	Asn	Gly	Lys
1				5					10		

<210> 18

<211> 19

<212> PRT

<213> Mus sp.

<400> 18

Tyr	Pro	Leu	Thr	Val	Asp	Phe	Glu	Ala	Phe	Gly	Trp	Asp	Trp	Ile	Ile
1				5					10					15	

Ala Pro Lys

<210> 19

<211> 12

<212> PRT

<213> Mus sp.

<400> 19

Met	Ser	Pro	Ile	Asn	Met	Leu	Tyr	Phe	Asn	Gly	Lys
1				5					10		

17

<210> 20
<211> 11
<212> PRT
<213> Mus sp.

<400> 20
Gly Ser Ala Gly Pro Cys Cys Thr Pro Thr Lys
1 5 10

<210> 21
<211> 16
<212> PRT
<213> Mus sp.

<400> 21
Leu Asp Met Ser Pro Gly Thr Gly Ile Trp Gln Ser Ile Asp Val Lys
1 5 10 15

<210> 22
<211> 28
<212> PRT
<213> Mus sp.

<400> 22
Ala Leu Asp Glu Asn Gly His Asp Leu Ala Val Thr Phe Pro Gly Pro
1 5 10 15

Gly Glu Asp Gly Leu Asn Pro Phe Leu Glu Val Lys
20 25

<210> 23
<211> 16
<212> PRT
<213> Mus sp.

<400> 23
Leu Asp Met Ser Pro Gly Thr Gly Ile Trp Gln Ser Ile Asp Val Lys
1 5 10 15

<210> 24
<211> 10
<212> PRT
<213> Mus sp.

<400> 24
Glu Leu Ile Asp Gln Tyr Asp Val Gln Arg
1 5 10

<210> 25
<211> 11
<212> PRT
<213> Mus sp.

WO 03/072714

PCT/US03/05150

18

<400> 25

Thr	Pro	Thr	Thr	Val	Phe	Val	Gln	Ile	Leu	Arg
1				5					10	

<210> 26

<211> 11

<212> PRT

<213> Mus sp.

<400> 26

Ala	Gln	Leu	Trp	Ile	Tyr	Leu	Arg	Pro	Val	Lys
1				5					10	

<210> 27

<211> 10

<212> PRT

<213> Mus sp.

<400> 27

Glu	Gly	Leu	Cys	Asn	Ala	Cys	Ala	Trp	Arg
1				5					10

<210> 28

<211> 18

<212> PRT

<213> Mus sp.

<400> 28

Pro	Gln	Ser	Cys	Leu	Val	Asp	Gln	Thr	Gly	Ser	Ala	His	Cys	Val	Val
1				5					10					15	

Cys Arg

<210> 29

<211> 12

<212> PRT

<213> Mus sp.

<400> 29

Asp	Ser	Cys	Asp	Gly	Val	Glu	Cys	Gly	Pro	Gly	Lys
1				5					10		

<210> 30

<211> 9

<212> PRT

<213> Mus sp.

<400> 30

Ser	Cys	Ala	Gln	Val	Val	Cys	Pro	Arg
1				5				

19

<210> 31
<211> 13
<212> PRT
<213> Mus sp.

<400> 31
Glu Cys Glu Thr Asp Gln Glu Cys Glu Thr Tyr Glu Lys
1 5 10

<210> 32
<211> 9
<212> PRT
<213> Mus sp.

<400> 32
Ala Asp Phe Pro Leu Ser Val Val Arg
1 5

<210> 33
<211> 11
<212> PRT
<213> Mus sp.

<400> 33
Glu Ala Cys Glu Glu Ser Cys Pro Phe Pro Arg
1 5 10

<210> 34
<211> 8
<212> PRT
<213> Mus sp.

<400> 34
Ser Asp Phe Val Ile Leu Gly Arg
1 5

<210> 35
<211> 12
<212> PRT
<213> Mus sp.

<400> 35
Val Ser Glu Leu Thr Glu Glu Gln Asp Ser Gly Arg
1 5 10

<210> 36
<211> 15
<212> PRT
<213> Homo sapiens

<400> 36
Ala Asn Tyr Cys Ser Gly Glu Cys Glu Phe Val Phe Leu Gln Lys
1 5 10 15

20

<210> 37
<211> 14
<212> PRT
<213> Homo sapiens

<400> 37
Asp Phe Gly Leu Asp Cys Asp Glu His Ser Thr Glu Ser Arg
1 5 10

<210> 38
<211> 28
<212> PRT
<213> Homo sapiens

<400> 38
Ala Leu Asp Glu Asn Gly His Asp Leu Ala Val Thr Phe Pro Gly Pro
1 5 10 15

Gly Glu Asp Gly Leu Asn Pro Phe Leu Glu Val Lys
20 25

<210> 39
<211> 10
<212> PRT
<213> Homo sapiens

<400> 39
Glu Leu Ile Asp Gln Tyr Asp Val Gln Arg
1 5 10

<210> 40
<211> 18
<212> PRT
<213> Homo sapiens

<400> 40
Pro Gln Ser Cys Val Val Asp Gln Thr Gly Ser Ala His Cys Val Val
1 5 10 15

Cys Arg

<210> 41
<211> 13
<212> PRT
<213> Homo sapiens

<400> 41
Cys Glu Cys Ala Pro Asp Cys Ser Gly Leu Pro Ala Arg
1 5 10

21

<210> 42
<211> 12
<212> PRT
<213> Homo sapiens

<400> 42
Leu Gln Val Cys Gly Ser Asp Gly Ala Thr Tyr Arg
1 5 10

<210> 43
<211> 12
<212> PRT
<213> Homo sapiens

<400> 43
Val Ser Glu Leu Thr Glu Glu Pro Asp Ser Gly Arg
1 5 10

<210> 44
<211> 10
<212> PRT
<213> Homo sapiens

<400> 44
Cys Tyr Met Asp Ala Glu Ala Cys Ser Lys
1 5 10

<210> 45
<211> 10
<212> PRT
<213> Homo sapiens

<400> 45
Gly Ile Thr Leu Ala Val Val Thr Cys Arg
1 5 10

<210> 46
<211> 31
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 46
ttggccactg ccaccacaat ctcaaccact t

31

<210> 47
<211> 26
<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Primer

<400> 47

tctcagcatg gccatgccgc cgtcga

26

<210> 48

<211> 1716

<212> DNA

<213> Mus sp.

<220>

<221> CDS

<222> (1)..(1713)

<400> 48

atg tgt gcc cca ggg tat cat cgg ttc tgg ttt cac tgg ggg ctg ctg	48
Met Cys Ala Pro Gly Tyr His Arg Phe Trp Phe His Trp Gly Leu Leu	
1 5 10 15	
ttg ctg ctg ctc ctc gag gct ccc ctt cga ggc cta gca ctg cca ccc	96
Leu Leu Leu Leu Leu Glu Ala Pro Leu Arg Gly Leu Ala Leu Pro Pro	
20 25 30	
atc cga tac tcc cat gcg ggc atc tgc ccc aac gac atg aac ccc aac	144
Ile Arg Tyr Ser His Ala Gly Ile Cys Pro Asn Asp Met Asn Pro Asn	
35 40 45	
ctc tgg gtg gat gcc cag agc acc tgc aag cga gag tgt gaa aca gac	192
Leu Trp Val Asp Ala Gln Ser Thr Cys Lys Arg Glu Cys Glu Thr Asp	
50 55 60	
cag gaa tgt gag acc tat gag aaa tgc tgc ccc aat gtg tgt ggg acc	240
Gln Glu Cys Glu Thr Tyr Glu Lys Cys Cys Pro Asn Val Cys Gly Thr	
65 70 75 80	
aag agc tgt gtg gca gcc cgc tac atg gat gtg aaa ggg aag aag ggg	288
Lys Ser Cys Val Ala Ala Arg Tyr Met Asp Val Lys Gly Lys Lys Gly	
85 90 95	
cct gta ggc atg ccc aag gag gcc aca tgt gac cat ttc atg tgc ctg	336
Pro Val Gly Met Pro Lys Glu Ala Thr Cys Asp His Phe Met Cys Leu	
100 105 110	
cag cag ggc tct gag tgt gac atc tgg gac ggc cag ccc gtg tgt aag	384
Gln Gln Gly Ser Glu Cys Asp Ile Trp Asp Gly Gln Pro Val Cys Lys	
115 120 125	
tgc aaa gat cgc tgt gag aag gag ccc agc ttc acc tgt gcc tct gat	432
Cys Lys Asp Arg Cys Glu Lys Glu Pro Ser Phe Thr Cys Ala Ser Asp	
130 135 140	
ggc ctt acc tac tac aac cgt tgc ttc atg gac gcc gaa gcc tgc tcc	480
Gly Leu Thr Tyr Tyr Asn Arg Cys Phe Met Asp Ala Glu Ala Cys Ser	
145 150 155 160	

23

aag ggc atc aca ctg tct gtg gtc acc tgt cgt tat cac ttc acc tgg	528
Lys Gly Ile Thr Leu Ser Val Val Thr Cys Arg Tyr His Phe Thr Trp	
165 170 175	
cct aac acc agc cct cca ccg cct gag acc acg gtg cat ccc acc acc	576
Pro Asn Thr Ser Pro Pro Pro Pro Glu Thr Thr Val His Pro Thr Thr	
180 185 190	
gcc tct ccg gag act ctc ggg ctg gac atg gca gcc cca gcc ctg ctc	624
Ala Ser Pro Glu Thr Leu Gly Leu Asp Met Ala Ala Pro Ala Leu Leu	
195 200 205	
aac cac cct gtc cat cag tca gtc acc gtg ggt gag act gtg agt ttc	672
Asn His Pro Val His Gln Ser Val Thr Val Gly Glu Thr Val Ser Phe	
210 215 220	
ctc tgt gac gtg gta ggc cgg cct cgg cca gag ctc act tgg gag aaa	720
Leu Cys Asp Val Val Gly Arg Pro Arg Pro Glu Leu Thr Trp Glu Lys	
225 230 235 240	
cag ctg gag gac cga gag aat gtt gtc atg agg ccc aac cac gtg cgt	768
Gln Leu Glu Asp Arg Glu Asn Val Val Met Arg Pro Asn His Val Arg	
245 250 255	
ggt aat gtg gtg gtc act aac att gcc cag ctg gtc atc tac aac gtc	816
Gly Asn Val Val Val Thr Asn Ile Ala Gln Leu Val Ile Tyr Asn Val	
260 265 270	
cag ccc cag gat gct ggc ata tac acc tgt aca gct cga aat gtc gct	864
Gln Pro Gln Asp Ala Gly Ile Tyr Thr Cys Thr Ala Arg Asn Val Ala	
275 280 285	
ggt gtc ctg agg gct gac ttc ccg ttg tcg gtg gtc agg ggt ggt cag	912
Gly Val Leu Arg Ala Asp Phe Pro Leu Ser Val Val Arg Gly Gly Gln	
290 295 300	
gcc agg gcc act tca gag agc agt ctc aat ggc aca gct ttt cca gca	960
Ala Arg Ala Thr Ser Glu Ser Ser Leu Asn Gly Thr Ala Phe Pro Ala	
305 310 315 320	
aca gag tgc ctg aag ccc cca gac agt gag gac tgt gga gag gag cag	1008
Thr Glu Cys Leu Lys Pro Pro Asp Ser Glu Asp Cys Gly Glu Glu Gln	
325 330 335	
aca cgc tgg cac ttc gac gcc cag gct aac aac tgc ctc act ttc acc	1056
Thr Arg Trp His Phe Asp Ala Gln Ala Asn Asn Cys Leu Thr Phe Thr	
340 345 350	
ttt ggc cac tgc cac cac aat ctc aac cac ttt gag acc tac gag gcc	1104
Phe Gly His Cys His His Asn Leu Asn His Phe Glu Thr Tyr Glu Ala	
355 360 365	
tgt atg ctg gct tgt atg agt ggg cca ttg gcc acc tgc agc ctg cct	1152
Cys Met Leu Ala Cys Met Ser Gly Pro Leu Ala Thr Cys Ser Leu Pro	
370 375 380	

24

gcc ctg caa ggg cct tgc aaa gct tat gtc cca cgc tgg gcc tac aac 1200
 Ala Leu Gln Gly Pro Cys Lys Ala Tyr Val Pro Arg Trp Ala Tyr Asn
 385 390 395 400
 agc cag aca ggc cta tgc cag tcc ttc gtc tat ggc ggc tgt gag ggc 1248
 Ser Gln Thr Gly Leu Cys Gln Ser Phe Val Tyr Gly Gly Cys Glu Gly
 405 410 415
 aac ggt aac aac ttt gaa agc cgt gag gct tgt gag gag tcg tgt ccc 1296
 Asn Gly Asn Asn Phe Glu Ser Arg Glu Ala Cys Glu Glu Ser Cys Pro
 420 425 430
 ttc ccg agg ggt aac cag cac tgc cgg gcc tgc aag ccc cgg caa aaa 1344
 Phe Pro Arg Gly Asn Gln His Cys Arg Ala Cys Lys Pro Arg Gln Lys
 435 440 445
 ctt gtt acc agc ttc tgt cgg agt gac ttt gtc atc ctg ggc agg gtc 1392
 Leu Val Thr Ser Phe Cys Arg Ser Asp Phe Val Ile Leu Gly Arg Val
 450 455 460
 tct gag ctg acc gag gag caa gac tcg ggc cgt gcc ctg gtg acc gtg 1440
 Ser Glu Leu Thr Glu Glu Gln Asp Ser Gly Arg Ala Leu Val Thr Val
 465 470 475 480
 gat gag gtc tta aaa gat gag aag atg ggc ctc aag ttt ctg ggc cgg 1488
 Asp Glu Val Leu Lys Asp Glu Lys Met Gly Leu Lys Phe Leu Gly Arg
 485 490 495
 gag cct ctg gaa gtc acc ctg ctt cat gta gac tgg acc tgt cct tgc 1536
 Glu Pro Leu Glu Val Thr Leu Leu His Val Asp Trp Thr Cys Pro Cys
 500 505 510
 ccc aac gtg aca gtg ggt gag aca cca ctc atc atc atg ggg gag gtg 1584
 Pro Asn Val Thr Val Gly Glu Thr Pro Leu Ile Ile Met Gly Glu Val
 515 520 525
 gac ggc ggc atg gcc atg ctg aga ccc gat agc ttt gtg ggg gca tcg 1632
 Asp Gly Gly Met Ala Met Leu Arg Pro Asp Ser Phe Val Gly Ala Ser
 530 535 540
 agc aca cgg cgg gtc agg aag ctc cgt gag gtc atg tac aag aaa acc 1680
 Ser Thr Arg Arg Val Arg Lys Leu Arg Glu Val Met Tyr Lys Lys Thr
 545 550 555 560
 tgt gac gtc ctc aag gac ttc ctg ggc ttg caa tga 1716
 Cys Asp Val Leu Lys Asp Phe Leu Gly Leu Gln
 565 570

<210> 49

<211> 571

<212> PRT

<213> Mus sp.

<400> 49

Met Cys Ala Pro Gly Tyr His Arg Phe Trp Phe His Trp Gly Leu Leu
 1 5 10 15

25

Leu Leu Leu Leu Leu Glu Ala Pro Leu Arg Gly Leu Ala Leu Pro Pro
 20 25 30

Ile Arg Tyr Ser His Ala Gly Ile Cys Pro Asn Asp Met Asn Pro Asn
 35 40 45

Leu Trp Val Asp Ala Gln Ser Thr Cys Lys Arg Glu Cys Glu Thr Asp
 50 55 60

Gln Glu Cys Glu Thr Tyr Glu Lys Cys Cys Pro Asn Val Cys Gly Thr
 65 70 75 80

Lys Ser Cys Val Ala Ala Arg Tyr Met Asp Val Lys Gly Lys Lys Gly
 85 90 95

Pro Val Gly Met Pro Lys Glu Ala Thr Cys Asp His Phe Met Cys Leu
 100 105 110

Gln Gln Gly Ser Glu Cys Asp Ile Trp Asp Gly Gln Pro Val Cys Lys
 115 120 125

Cys Lys Asp Arg Cys Glu Lys Glu Pro Ser Phe Thr Cys Ala Ser Asp
 130 135 140

Gly Leu Thr Tyr Tyr Asn Arg Cys Phe Met Asp Ala Glu Ala Cys Ser
 145 150 155 160

Lys Gly Ile Thr Leu Ser Val Val Thr Cys Arg Tyr His Phe Thr Trp
 165 170 175

Pro Asn Thr Ser Pro Pro Pro Pro Glu Thr Thr Val His Pro Thr Thr
 180 185 190

Ala Ser Pro Glu Thr Leu Gly Leu Asp Met Ala Ala Pro Ala Leu Leu
 195 200 205

Asn His Pro Val His Gln Ser Val Thr Val Gly Glu Thr Val Ser Phe
 210 215 220

Leu Cys Asp Val Val Gly Arg Pro Arg Pro Glu Leu Thr Trp Glu Lys
 225 230 235 240

Gln Leu Glu Asp Arg Glu Asn Val Val Met Arg Pro Asn His Val Arg
 245 250 255

Gly Asn Val Val Val Thr Asn Ile Ala Gln Leu Val Ile Tyr Asn Val
 260 265 270

Gln Pro Gln Asp Ala Gly Ile Tyr Thr Cys Thr Ala Arg Asn Val Ala
 275 280 285

Gly Val Leu Arg Ala Asp Phe Pro Leu Ser Val Val Arg Gly Gly Gln
 290 295 300

Ala Arg Ala Thr Ser Glu Ser Ser Leu Asn Gly Thr Ala Phe Pro Ala
 305 310 315 320

26

Thr Glu Cys Leu Lys Pro Pro Asp Ser Glu Asp Cys Gly Glu Glu Gln
 325 330 335
 Thr Arg Trp His Phe Asp Ala Gln Ala Asn Asn Cys Leu Thr Phe Thr
 340 345 350
 Phe Gly His Cys His His Asn Leu Asn His Phe Glu Thr Tyr Glu Ala
 355 360 365
 Cys Met Leu Ala Cys Met Ser Gly Pro Leu Ala Thr Cys Ser Leu Pro
 370 375 380
 Ala Leu Gln Gly Pro Cys Lys Ala Tyr Val Pro Arg Trp Ala Tyr Asn
 385 390 395 400
 Ser Gln Thr Gly Leu Cys Gln Ser Phe Val Tyr Gly Gly Cys Glu Gly
 405 410 415
 Asn Gly Asn Asn Phe Glu Ser Arg Glu Ala Cys Glu Glu Ser Cys Pro
 420 425 430
 Phe Pro Arg Gly Asn Gln His Cys Arg Ala Cys Lys Pro Arg Gln Lys
 435 440 445
 Leu Val Thr Ser Phe Cys Arg Ser Asp Phe Val Ile Leu Gly Arg Val
 450 455 460
 Ser Glu Leu Thr Glu Glu Gln Asp Ser Gly Arg Ala Leu Val Thr Val
 465 470 475 480
 Asp Glu Val Leu Lys Asp Glu Lys Met Gly Leu Lys Phe Leu Gly Arg
 485 490 495
 Glu Pro Leu Glu Val Thr Leu Leu His Val Asp Trp Thr Cys Pro Cys
 500 505 510
 Pro Asn Val Thr Val Gly Glu Thr Pro Leu Ile Ile Met Gly Glu Val
 515 520 525
 Asp Gly Gly Met Ala Met Leu Arg Pro Asp Ser Phe Val Gly Ala Ser
 530 535 540
 Ser Thr Arg Arg Val Arg Lys Leu Arg Glu Val Met Tyr Lys Lys Thr
 545 550 555 560
 Cys Asp Val Leu Lys Asp Phe Leu Gly Leu Gln
 565 570

<210> 50

<211> 34

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Primer

27

<400> 50
caccatgtgt gccccagggt atcatcggtt ctgg

34

<210> 51
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 51
ttgcaagccc aggaagtcct tgaggac

27

<210> 52
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Illustrative
N-terminal peptide sequence

<400> 52
Leu Pro Pro Ile Arg Tyr Ser His Ala Gly Ile
1 5 10

<210> 53
<211> 48
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 53
cagacagaca gacagacaga cagacagaca gacagacaga cagacaga

48

We claim:

1. A pharmaceutical composition comprising:
 - i) at least one protein comprising at least one follistatin domain, wherein the protein is not follistatin, and
 - ii) at least one pharmaceutically acceptable carrier.
2. The composition of claim 1, wherein the protein is chosen from FLRG, FRP, agrins, osteonectin, hevin, IGFBP7, U19878 and GASP2.
3. The composition of claim 1, wherein the protein has a stabilizing modification.
4. The composition of claim 3, wherein the modification is a fusion to the Fc region of an IgG molecule.
5. The composition of claim 4, wherein the IgG molecule is IgG1 or IgG4, or derivatives thereof.
6. The composition of claim 5, wherein the IgG molecule is IgG1 or a derivative thereof.
7. The composition of claim 4, wherein the IgG molecule is fused to the protein comprising at least one follistatin domain by a linker peptide.
8. The composition of claim 3, wherein the modification comprises an altered glycosylation site.
9. The composition of claim 3, wherein the modification comprises at least one carbohydrate moiety.
10. The composition of claim 3, wherein the modification comprises albumin or an albumin derivative.

11. The composition of claim 3, wherein the modification comprises a nonproteinaceous polymer.
12. The composition of claim 3, wherein the modification comprises pegylation.
13. A diagnostic kit comprising at least one protein comprising at least one follistatin domain, wherein the protein is not follistatin and at least one other kit component chosen from:
 - i) at least one agent that binds the protein;
 - ii) at least one buffer and/or solution; and
 - iii) at least one structural component.
14. The kit of claim 13, wherein the protein is chosen from FLRG, FRP, agrins, osteonectin, hevin, IGFBP7, U19878 and GASP2.
15. A recombinant cell comprising a nucleic acid encoding a protein comprising at least one follistatin domain, wherein the protein is not follistatin.
16. The recombinant cell of claim 15, wherein the protein has a stabilizing modification.
17. The recombinant cell of claim 15 or 16, wherein the protein is chosen from FLRG, FRP, agrins, osteonectin, hevin, IGFBP7, U19878 and GASP2.
18. A method of modulating GDF-8 comprising administering at least one protein comprising at least one follistatin domain, wherein the protein is not follistatin, and allowing the protein to interact with GDF-8.

19. A method of treating a patient suffering from a medical disorder, comprising administering a therapeutically effective dose of at least one protein comprising at least one follistatin domain, wherein the protein is not follistatin, and allowing the protein to interact with GDF-8.
20. A method of treating a patient suffering from a medical disorder, comprising administering a nucleic acid encoding a protein comprising at least one follistatin domain, wherein the protein is not follistatin, allowing the nucleic acid to be translated into a protein, and allowing the translated protein to interact with GDF-8.
21. A method of expressing a nucleic acid, comprising
 - i) administering a nucleic acid encoding a protein comprising at least one follistatin domain, wherein the protein is not follistatin, to a cell,
 - ii) allowing the nucleic acid to enter the cell, and
 - iii) allowing the cell to express the protein.
22. The method of claim 18, 19, 20, or 21, wherein the protein is chosen from FLRG, FRP, agrins, osteonectin, hevin, IGFBP7, U19878 and GASP2.
23. The method of claim 18, 19, 20, or 21, wherein the protein has a stabilizing modification.
24. The method of claim 23, wherein the modification is a fusion to the Fc region of an IgG molecule.
25. The method of claim 24, wherein the IgG molecule is IgG1 or IgG4, or derivatives thereof.

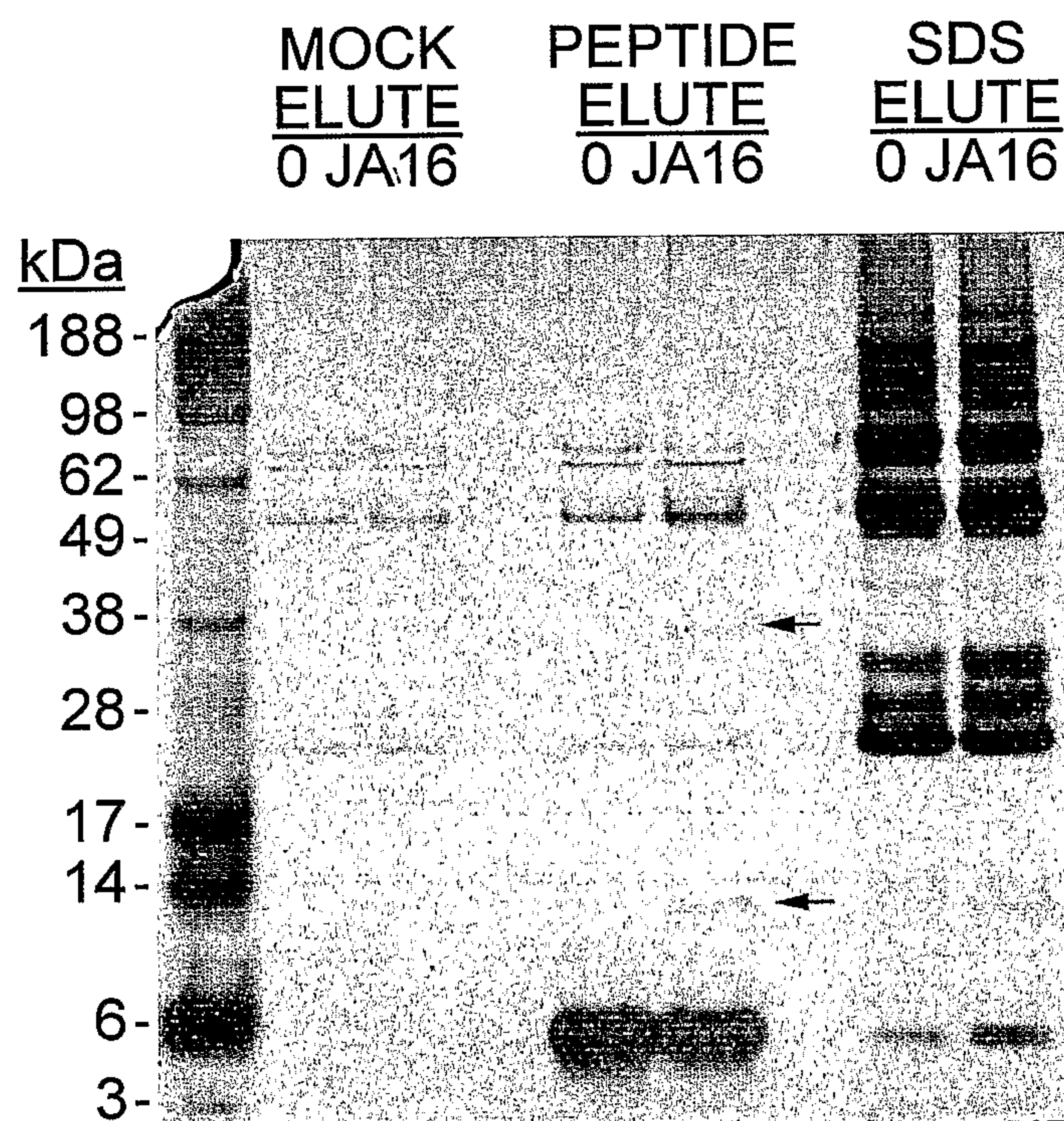
26. The method of claim 25, wherein the IgG molecule is IgG1 or a derivative thereof.
27. The method of claim 24, wherein the IgG molecule is fused to the protein comprising at least one follistatin domain by a linker peptide.
28. The method of claim 23, wherein the modification comprises an altered glycosylation site.
29. The method of claim 23, wherein the modification comprises at least one carbohydrate moiety.
30. The method of claim 23, wherein the modification comprises albumin or an albumin derivative.
31. The method of claim 23, wherein the modification comprises a nonproteinaceous polymer.
32. The method of claim 23, wherein the modification comprises pegylation.
33. The method of claim 19, wherein the patient would therapeutically benefit from an increase in mass or quantity of muscle tissue.
34. The method of claim 19, wherein the disorder is a muscular disorder.
35. The method of claim 34, wherein the muscular disorder is muscular dystrophy.
36. The method of claim 35, wherein the muscular dystrophy is chosen from severe or benign X-linked muscular dystrophy, limb-girdle dystrophy, facioscapulohumeral dystrophy, myotonic dystrophy, distal muscular dystrophy, progressive dystrophic ophthalmoplegia,

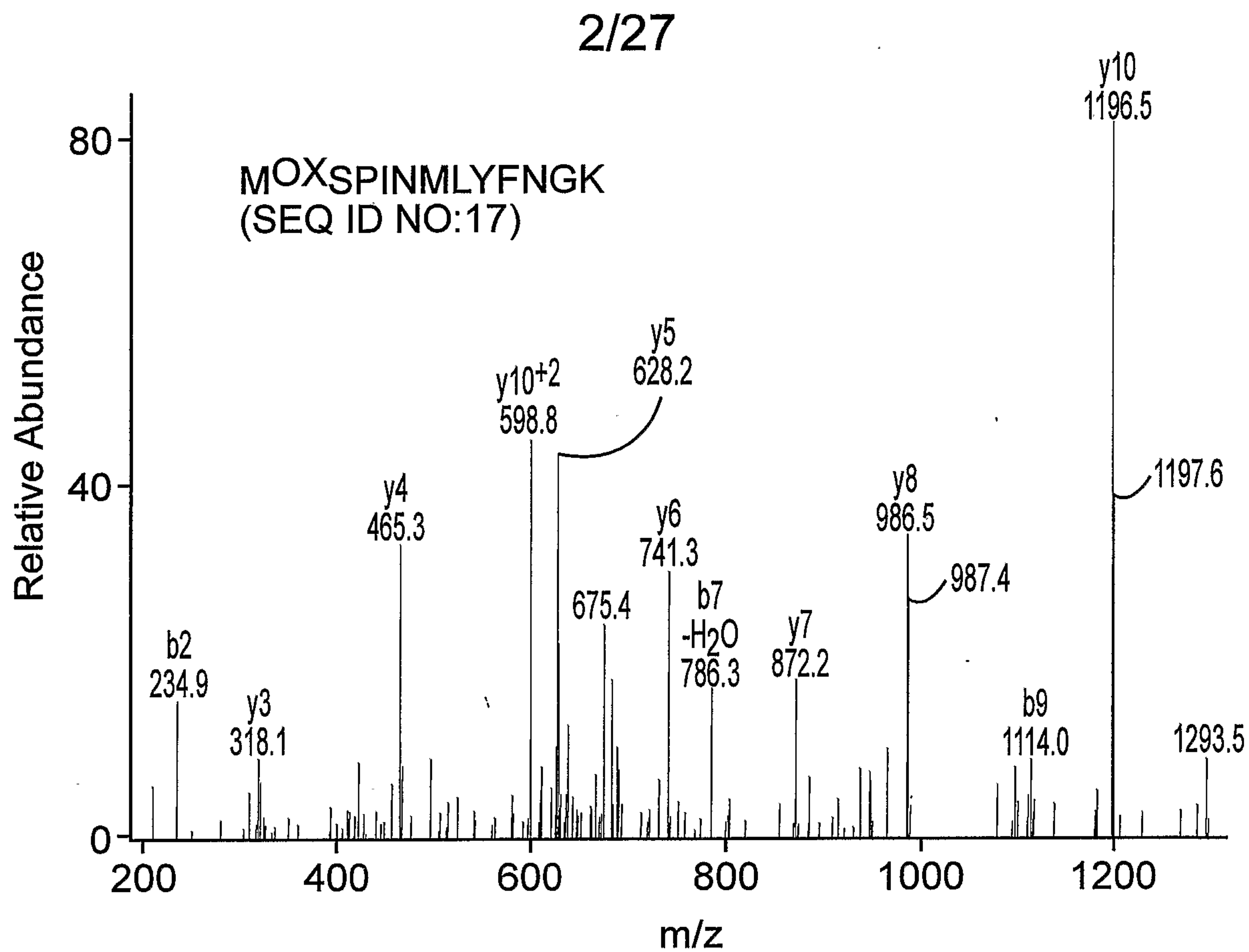
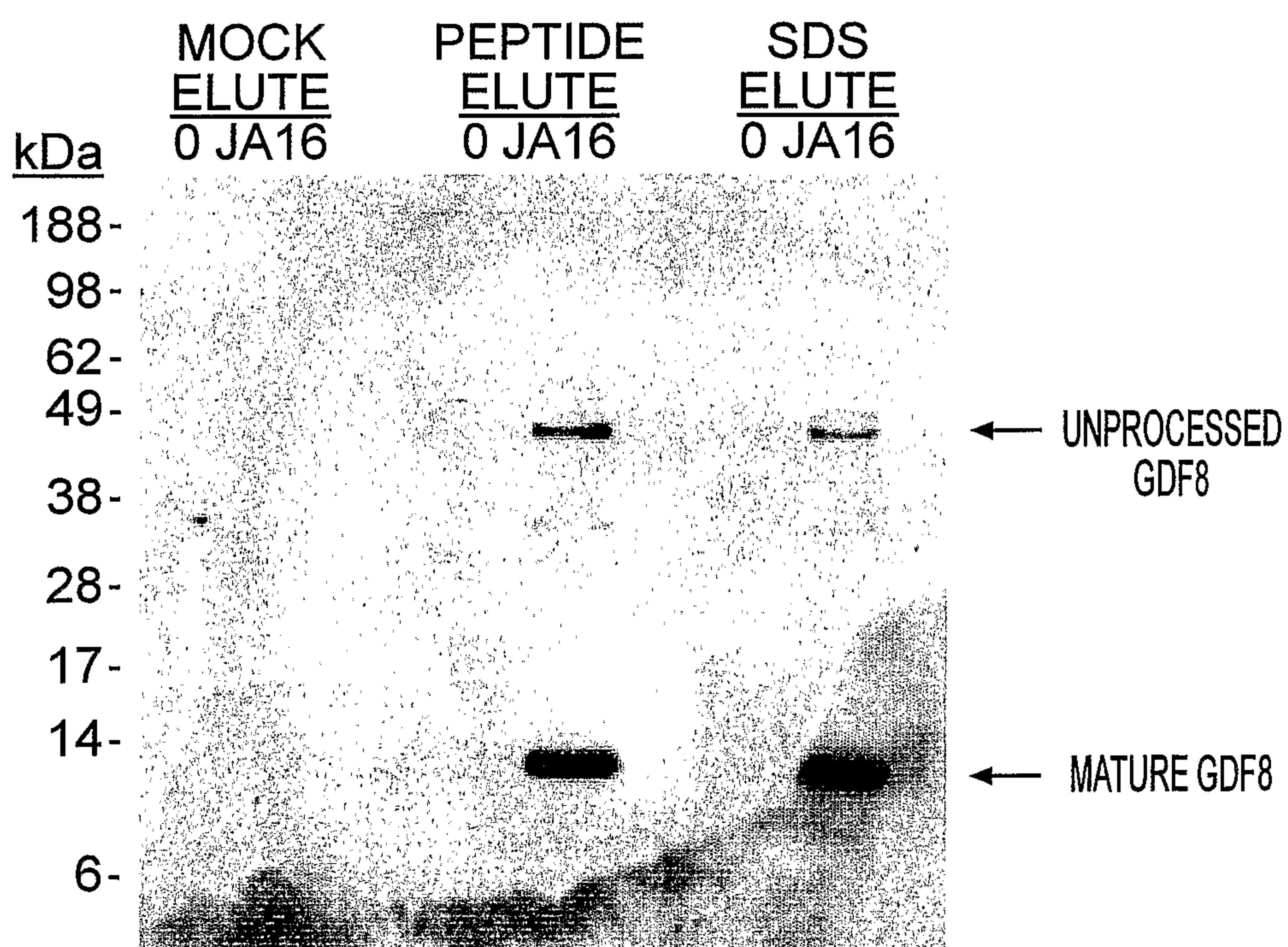
oculopharyngeal dystrophy, and Fakuyama-type congenital muscular dystrophy.

37. The method of claim 34, wherein the disorder is chosen from congenital myopathy, myotonia congenital, familial periodic paralysis, paroxysmal myoglobinuria, myasthenia gravis, Eaton-Lambert syndrome, secondary myasthenia, denervation atrophy, paroxymal muscle atrophy, muscle wasting syndrome, sarcopenia, and cachexia.
38. The method of claim 34, wherein the disorder is a muscular disorder chosen from a traumatic injury to muscle tissue and a chronic injury to muscle tissue.
39. The method of claim 19, wherein the disorder is a metabolic disease or disorder.
40. The method of claim 39, wherein the disorder is type 2 diabetes, noninsulin-dependent diabetes mellitus, hyperglycemia, or obesity.
41. The method of claim 19, wherein the disorder is an adipose tissue disorder such as obesity.
42. The method of claim 19, wherein the disorder is a bone degenerative disease such as osteoporosis.
43. The method of claim 19, wherein the protein is administered at one time, or at daily, weekly, or monthly intervals.
44. The method of claim 19, wherein the protein is administered at a dose of from 5 mg to 100 mg.
45. The method of claim 19, wherein the protein is administered at a dose of from 15 mg to 85 mg.

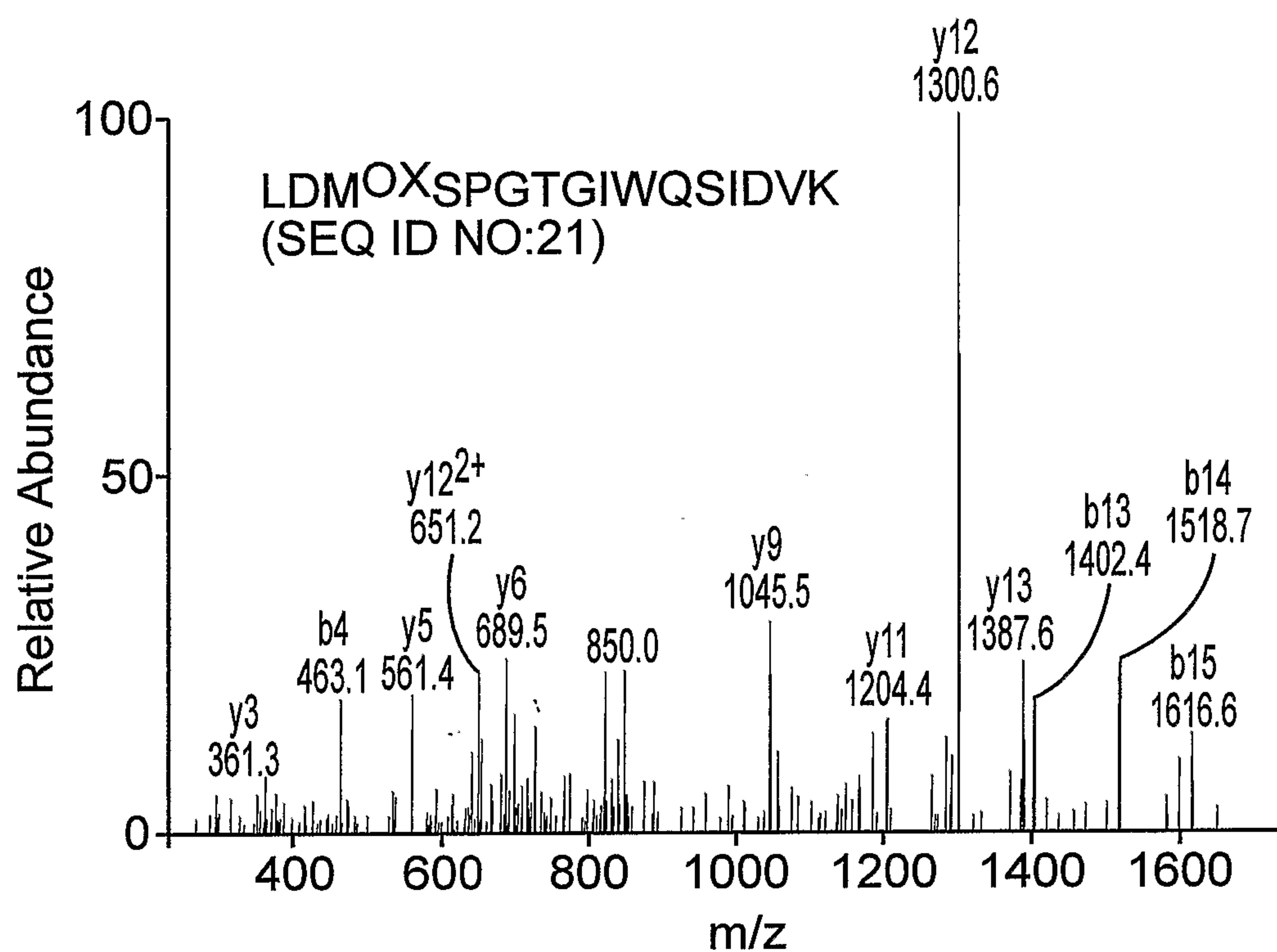
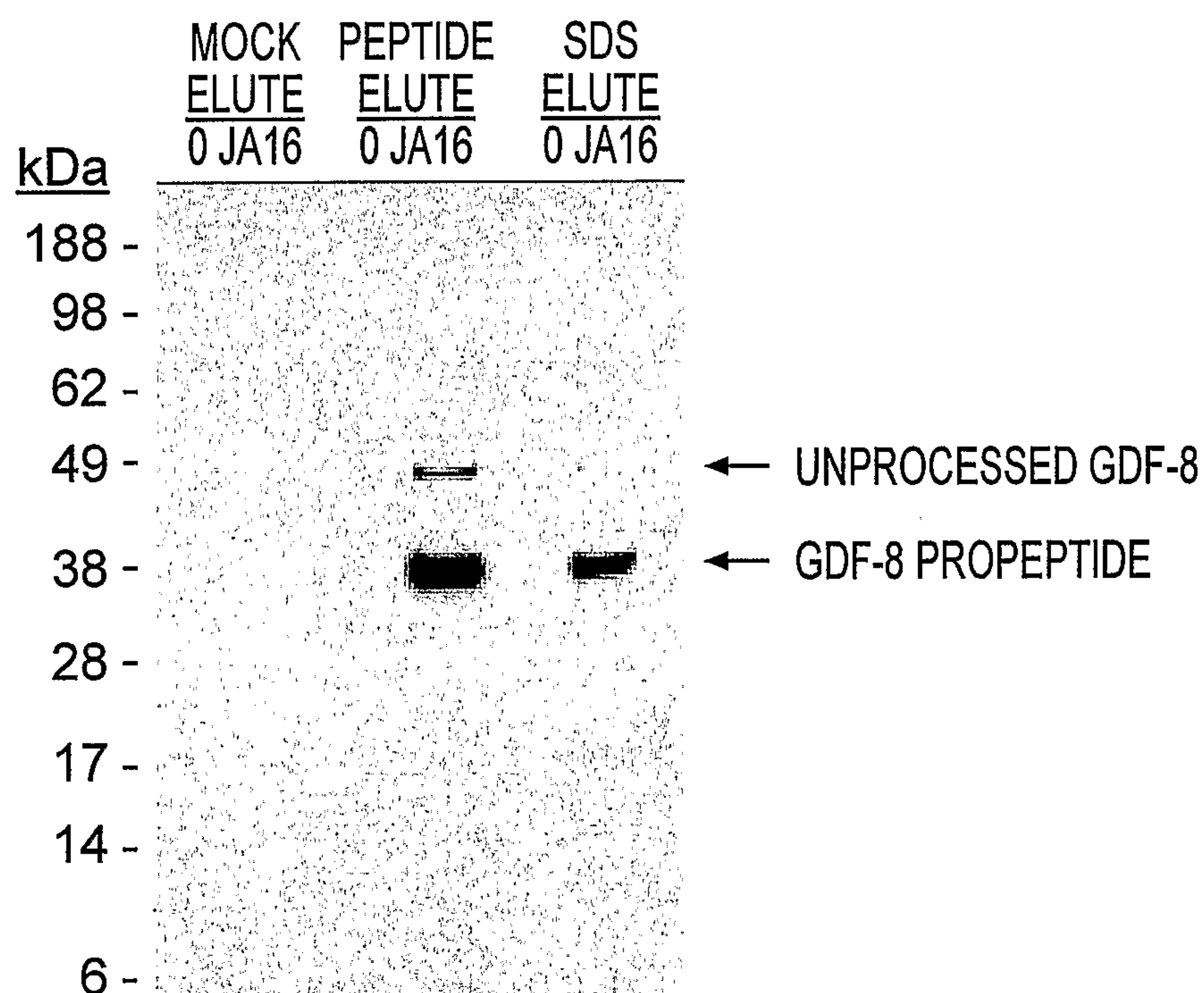
46. The method of claim 19, wherein the protein is administered at a dose of from 3 mg to 70 mg.
47. The method of claim 19, wherein the protein is administered at a dose of from 40 mg to 60 mg.

1/27

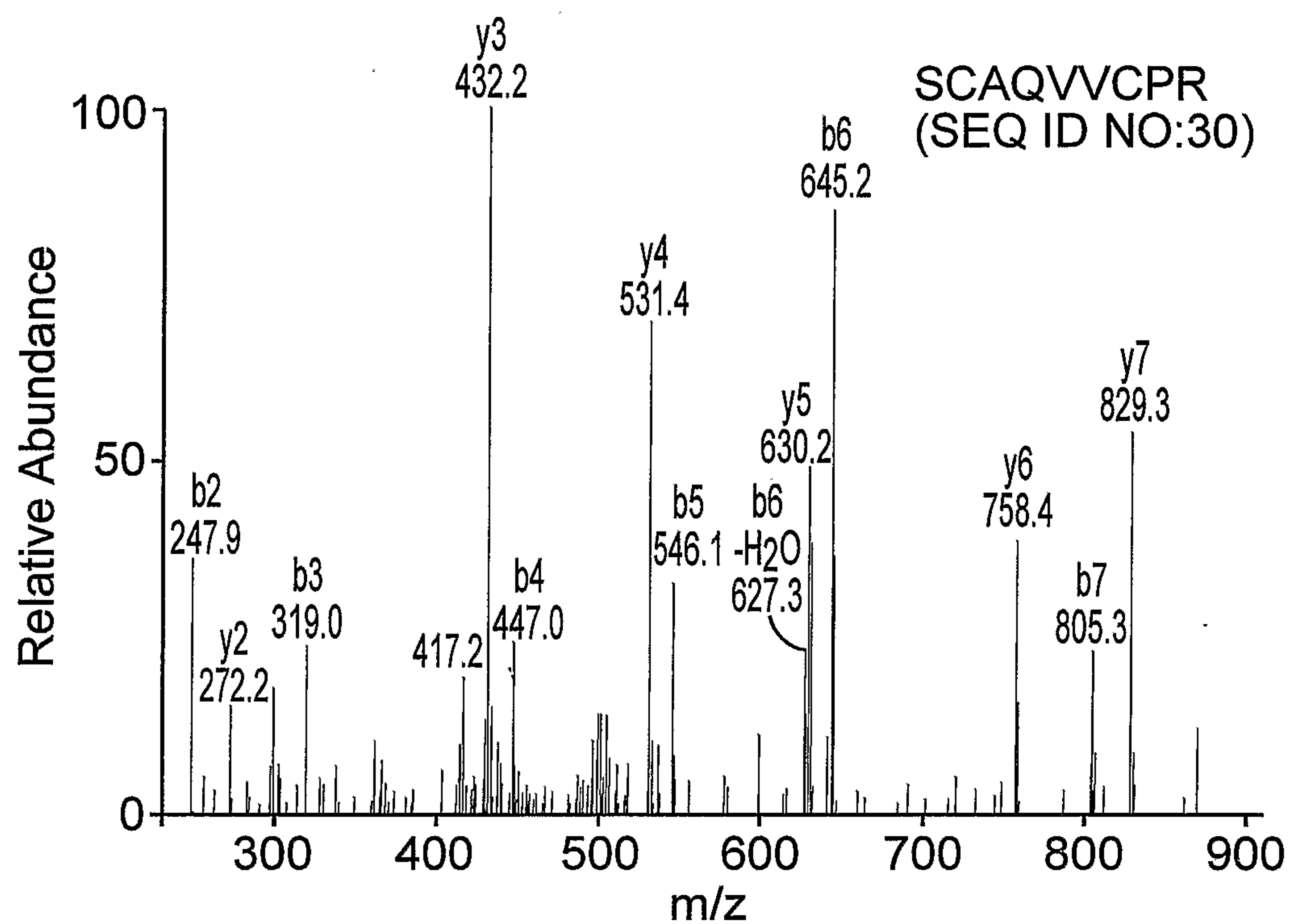
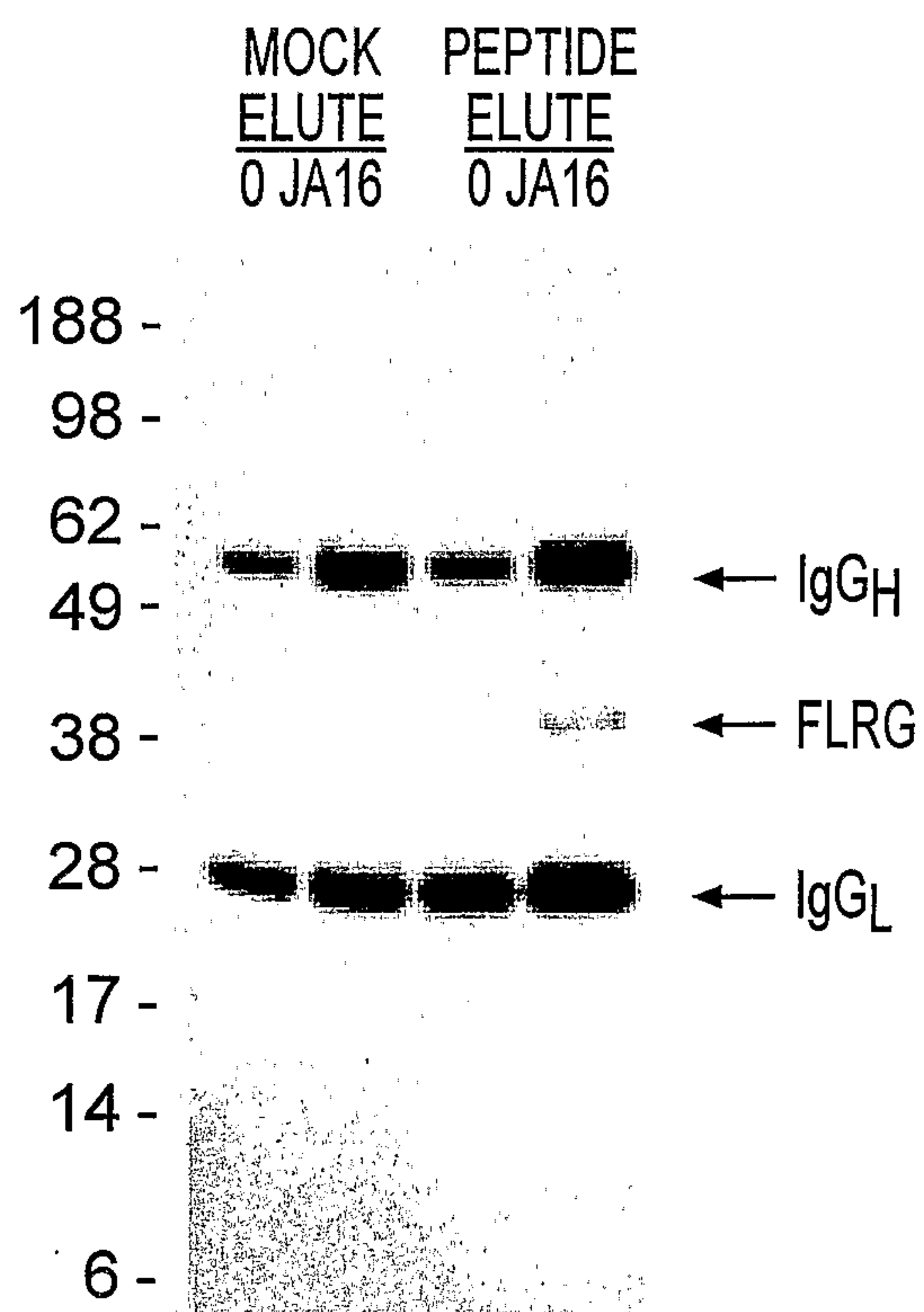
**FIG. 1**

**FIG. 2A****FIG. 2B**

3/27

**FIG. 3A****FIG. 3B**

4/27

**FIG. 3C****FIG. 3D**

5/27

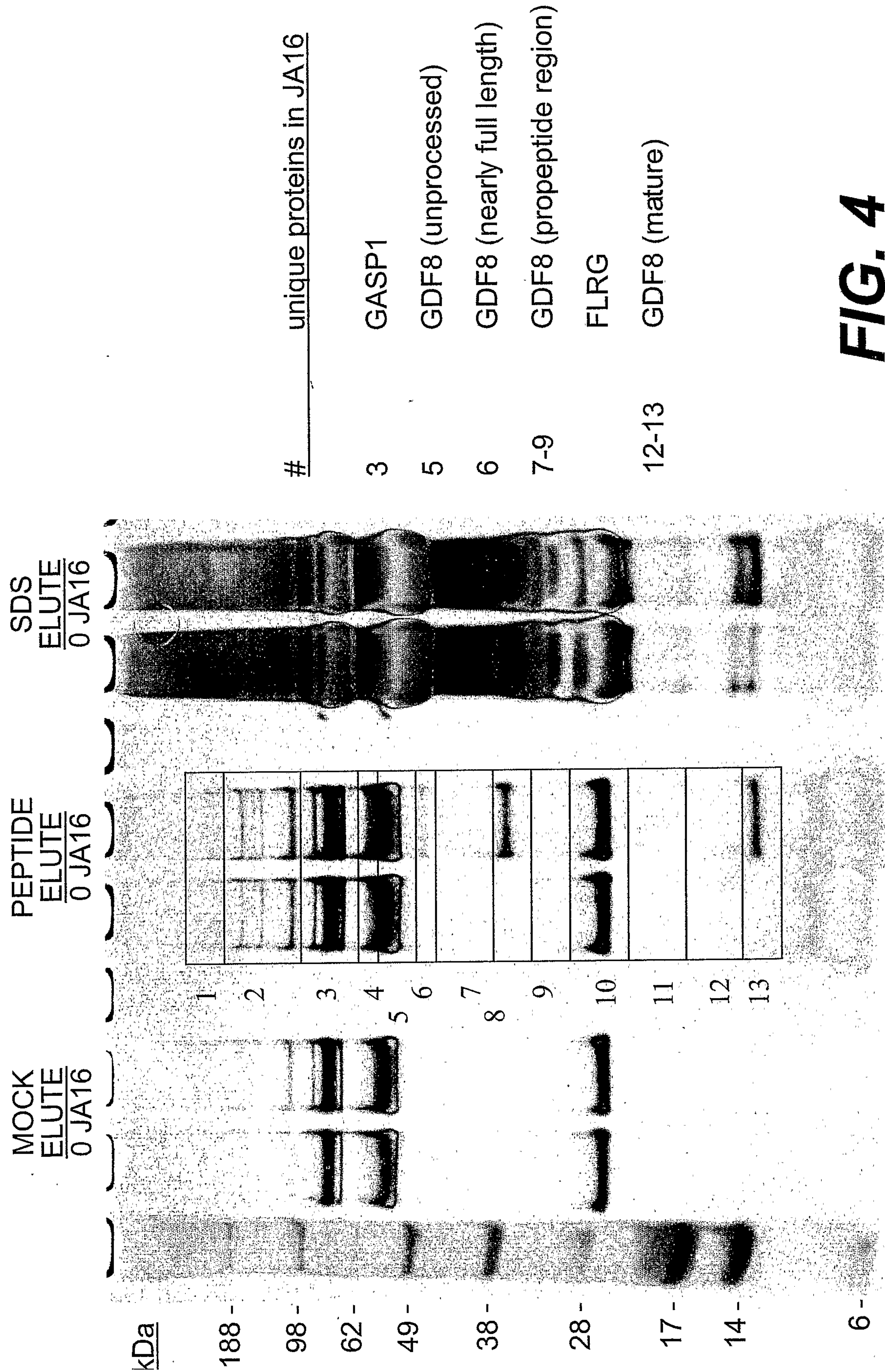
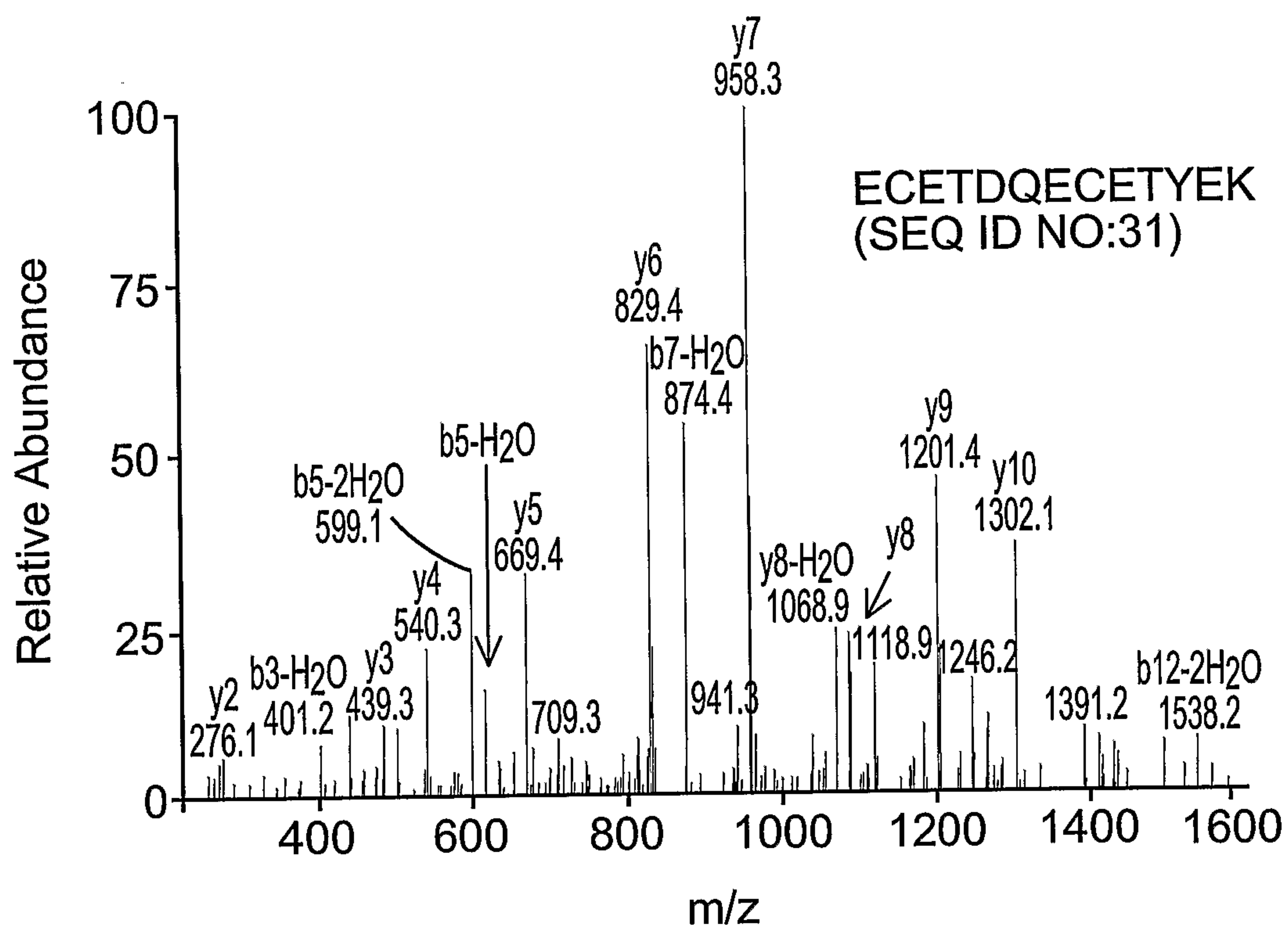
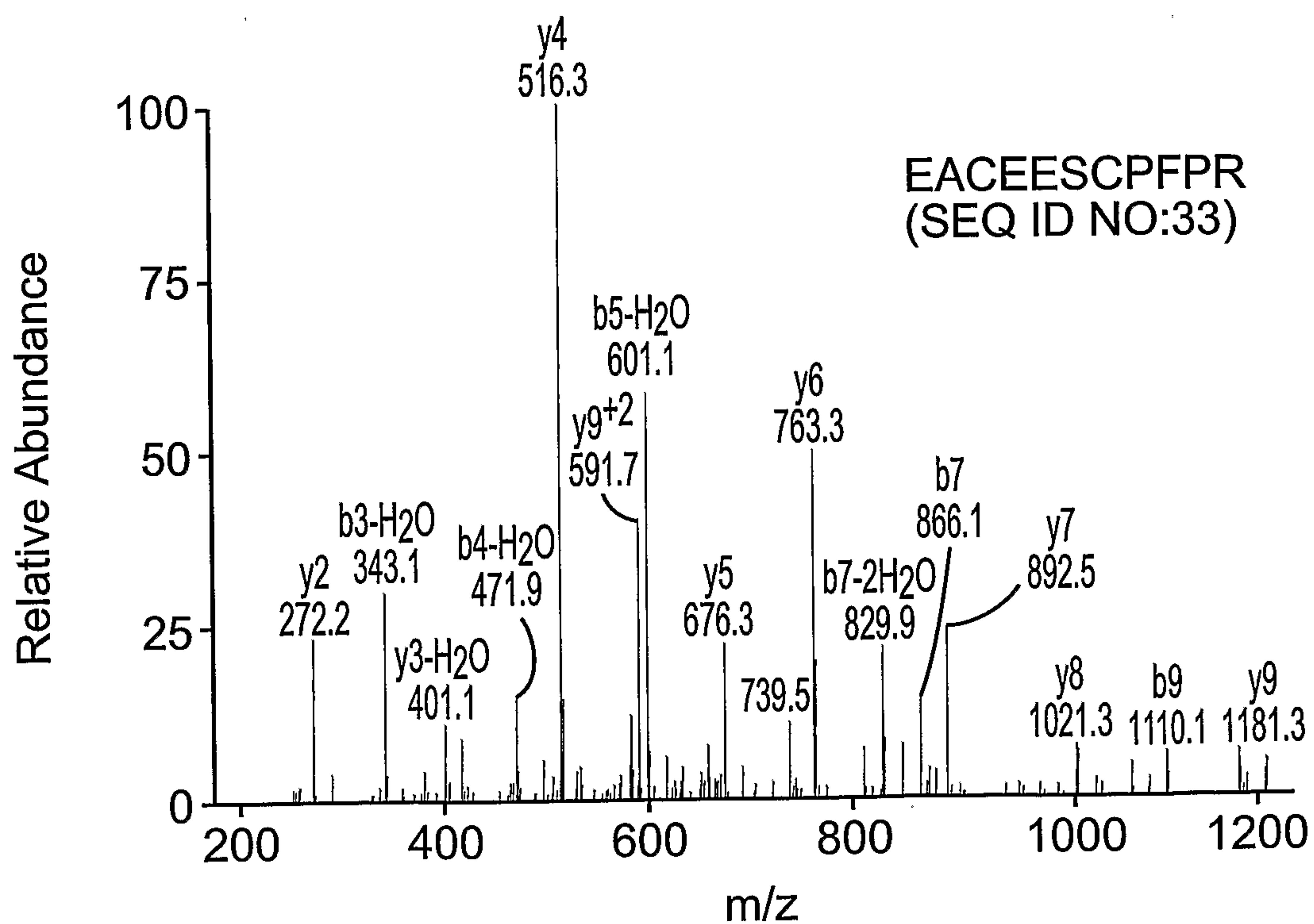


FIG. 4

6/27

**FIG. 5A****FIG. 5B**

7/27

PREDICTED MOUSE GASP1 NUCLEOTIDE SEQUENCE
SEQ ID NO:1

```
1 atgtgtgccc cagggatatca tcggttctggt tttcactggg ggctgctggt gctgctgctc
61 ctcgaggctc cccttcgagg cctagcactg ccacccatcc gatactccca tgcgggcatc
121 tgccccaacg acatgaaccc caacctctgg gtggatgccc agagcacctg caagcgagag
181 tgtgaaacag accaggaatg tgagacctat gagaaatgct gcccgaatgt gtgtgggacc
241 aagagctgtg tggcagcccg ctacatggat gtgaaaggga agaaggggccc tgtgggcatg
301 cccaaggagg ccacatgtga ccatttcatg tgcctgcagc agggctctga gtgtgacatc
361 tgggacggcc agcccgtgtg taagtgcaaa gatcgctgtg agaaggagcc cagcttcacc
421 tgtgcctctg atggccttac ctactacaac cgttgcttca tggacgccga agcctgctcc
481 aagggcatac cactgtctgt ggtcacctgt cgttatcact tcacctggcc taacaccagc
541 cctccaccgc ctgagaccac ggtgcatccc accaccgctt ctccggagac tctcgggctg
601 gacatggcag ccccgccctt gctcaaccac cctgtccatc agtcagtcac cgtgggtgag
661 actgtgagtt tcctctgtga cgtggtaggc cggcctcggc cagagctcac ttgggagaaa
721 cagctggagg accgagaaaa tgttgtcatg agggccaacc acgtgcgcgg taatgtggtg
781 gtcactaaca ttgcccagct ggtcatctac aacgtccagc cccaggatgc tggcatatac
841 acctgtacag ctcgaaatgt cgctggtgtc ctgagggctg acttcccgtt gtcggtggtc
901 aggggtggtc aggccagggc cacttcagag agcagtctca atggcacagc ttttccagca
961 acagagtgcc tgaagcccc agacagtgag gactgtggag aggagcagac acgctggcac
1021 ttcgacgccc aggctaacaa ctgcctcact ttcacctttg gccactgcca ccacaatctc
1081 aaccactttg agacctacga ggcctgtatg ctggcttgta tgagtgggcc attggccacc
1141 tgcagcctgc ctgccctgca agggccttgc aaagcttatg tcccacgctg ggcctacaac
1201 agccagacag gcctatgcca gtccttcgtc tatggcggct gtgagggcaa cggtaacaac
1261 tttgaaagcc gtgaggcttg tgaggagtcg tgtcccttcc cgaggggtaa ccagcactgc
1321 cgggcctgca agccccggca aaaacttggt accagcttct gtcggagtga ctttgtcatc
1381 ctgggcaggg tctctgagct gaccgaagag caagactcag gccgtgccct ggtgaccgtg
1441 gatgaggtct taaaagatga gaagatgggc ctcaagtttc tgggccggga gcctctggaa
1501 gtcaccctgc ttcattgtaga ctggacctgt ccttgcccca acgtgacagt gggtagagaa
1561 ccactcatca tcatggggga ggtcgacggc ggcattggca tgctgagacc cgatagcttt
1621 gtgggggcat cgagcacacg gcgggtcagg aagctccgtg aggtcatgta caagaaaacc
1681 tgtgacgtcc tcaaggactt cctgggcttg caatga
```

FIG. 6A

8/27

PREDICTED MOUSE GASP1 ALTERNATIVE NUCLEOTIDE SEQUENCE
SEQ ID NO:2

```
1  atgtgtgccc cagggatatca tcggttctgg tttcactggg ggctgctgtt
51  gctgctgctc ctcgaggctc cccttcgagg cctagcactg ccacccatcc
101 gatactccca tgcgggcatc tgccccaacg acatgaaccc caacctctgg
151 gtggatgccc agagcacctg caagcgagag tgtgaaacag accaggaatg
201 tgagacctat gagaaatgct gcccgaatgt gtgtgggacc aagagctgtg
251 tggcagcccg ctacatggat gtgaaaggga agaagggggc tgtaggcatg
301 cccaaggagg ccacatgtga ccatttcatg tgcctgcagc agggctctga
351 gtgtgacatc tgggacggcc agcccgtgtg taagtgcaaa gatcgctgtg
401 agaaggagcc cagcttcacc tgtgcctctg atggccttac ctactacaac
451 cgttgcttca tggacgccga agcctgctcc aagggcatac cactgtctgt
501 ggtcacctgt cgttatcact tcacctggcc taacaccagc cctccaccgc
551 ctgagaccac ggtgcatccc accaccgcct ctccggagac tctcgggctg
601 gacatggcag ccccagccct gctcaaccac cctgtccatc agtcagtcac
651 cgtgggtgag actgtgagtt tcctctgtga cgtggtaggc cggcctcggc
701 cagagctcac ttgggagaaa cagctggagg accgagagaa tgttgatcatg
751 aggcccaacc acgtgcgtgg taatgtgggtg gtcactaaca ttgcccagct
801 ggatcatctac aacgtccagc cccaggatgc tggcatatac acctgtacag
851 ctcgaaatgt cgctgggtgtc ctgaggggctg acttcccgtt gtcgggtggtc
901 aggggtggtc aggccagggc cacttcagag agcagtctca atggcacagc
951 ttttccagca acagagtgcc tgaagcccc agacagtgag gactgtggag
1001 aggagcagac acgctggcac ttcgacggcc aggctaacaa ctgcctcact
1051 ttcacctttg gccactgcca ccacaatctc aaccactttg agacctacga
1101 ggcctgtatg ctggccttgta tgagtgggccc attggccacc tgcagcctgc
1151 ctgccctgca agggccttgc aaagcttatg tcccacgctg ggcctacaac
1201 agccagacag gcctatgcca gtccttcgtc tatggcggct gtgagggcaa
1251 cggtaacaac tttgaaagcc gtgaggcttg tgaggagtcg tgtcccttcc
1301 cgaggggtaa ccagcactgc cgggcctgca agccccggca aaaacttggt
1351 accagcttct gtcggagtga ctttgtcatc ctgggcaggg tctctgagct
1401 gaccgaggag caagactcgg gccgtgccct ggtgaccgtg gatgaggtct
1451 taaaagatga gaagatgggc ctcaagtttc tgggccggga gcctctggaa
1501 gtcaccctgc ttcattgtaga ctggacctgt ccttgcccca acgtgacagt
1551 gggtagagaca ccactcatca tcatggggga ggtggacggc ggcattggca
1601 tgctgagacc cgatagcttt gtggggggcat cgagcacacg gcgggtcagg
1651 aagctccgtg aggtcatgta caagaaaacc tgtgacgtcc tcaaggactt
1701 cctgggcttg caatga
```

FIG. 6B

9/27

PREDICTED MOUSE GASP1 AMINO ACID SEQUENCE
SEQ ID NO:3

1 MCAPGYHREWF FHWGLLLLLL LEAPLRGLAL PPIRYSHAGI CPNDMNPNLW VDAQSTCKRE
61 CETDQECETY EKCCPNVCGT KSCVAARYMD VKGKKGPVGM PKEAT**CDHFM** **CLOOGSECDI**
121 **WDGQPVCKCK** **DRCEKEPSFT** **CASDGLTYYN** **RCFMDAEACS** **KGITLSVVT** RYHFTWPNTS
181 PPPPETTVHP TTASPETLGL DMAAPALLNH PVHQSVTVGE TVSFLCDVVG RPRPELTWEK
241 QLEDRENVVM RPNHVRGNV VTNIAQLVIY NVQPQDAGIY TCTARNVAGV LRADFPLSVV
301 RGGQARATSE SSLNGTAFPA TECLKPPDSE DCGEEQTRWH FDAQANNCLT FTFGHCHHNL
361 NHFETYEACM LACMSGPLAT CSLPALQGPC KAYVPRWAYN SQTGLCQSFV YGGCEGNGNN
421 FESREACEES CPFPRGNQHC RACKPRQKLV TSFCRSDFVI LGRVSELTEE QDSGRALVTV
481 DEVLKDEKMG LKFLGREPLE VTLLHVDWTC PCPNVTVGET PLIIMGEVDG GMAMLRPDSF
541 VGASSTRVR KLREVMYKKT CDVLKDFLGL Q

FIG. 6C

10/27

PREDICTED HUMAN GASP1 NUCLEOTIDE SEQUENCE
SEQ ID NO:4

```
1 atgaatccca acctctgggt ggacgcacag agcacctgca ggcgggagtg tgagacggac
61 caggagtgtg agatggacca ggtgagtggg atccagaagc cacagtgtga ggcagaccag
121 gtgaatgggg tccagaagcc gcaatgtgag atggaccaga agtgggagtg tgaggttgac
181 caggtgagtg ggggccagaa gccggtgtgt gaggcggacc aggtgagtgg ggtccagaag
241 ccacagtgtg agatggacca ggtgagtggg atccagaagc tggagtgtga ggcggaccag
301 aagtgggagt atgaggtgga ccaggtgagt ggggtccaga agccacagtg tgagatggac
361 caggtgagtg ggatccagaa gctggagtgt gaggcggacc aggagtgtga gacctatgag
421 aagtgctgcc ccaacgtatg tgggaccaag agctgcgtgg cggcccgtta catggacgtg
481 aaagggaaga agggcccagt gggcatgccc aaggaggcca catgtgacca cttcatgtgt
541 ctgcagcagg gctctgagtg tgacatctgg gatggccagc ccgtgtgtaa gtgcaaagac
601 cgctgtgaga aggagcccag ctttacctgc gcctcggacg gcctcaccta ctataaccgc
661 tgctacatgg atgccgaggc ctgctccaaa ggcatacacac tggccgttgt aacctgccgc
721 tatcacttca cntggcccaa caccagcccc ccaccacctg agaccaccat gcaccccacc
781 acagcctccc cagagacccc tgagctggac atggcggccc ctgcgctgct caacaaccct
841 gtgcaccagt cggtcaccat gggtgagaca gtgagcttcc tctgtgatgt ggtgggcccg
901 ccccggcctg agatcacctg ggagaagcag ttggaggatc gggagaatgt ggtcatgcgg
961 cccaaccatg tgcgtggcaa cgtggtggtc accaacattg ccagctgggt catctataac
1021 gcccagctgc aggatgctgg gatctacacc tgcacggccc ggaacgtggc tggggtcctg
1081 agggctgatt tcccgtgtc ggtggtcagg ggtcatcagg ctgcagccac ctgagagagc
1141 agccccaatg gcacggcttt cccggcggcc gagtgcctga agccccaga cagtgaggac
1201 tgtggcgaag agcagacccg ctggcacttc gatgccagg ccaacaactg cctgaccttc
1261 accttcggcc actgccaccg taacctcaac cactttgaga cctatgaggc ctgcatgctg
1321 gcctgcatga gcgggccgct ggccgcgtgc agcctgcccg ccctgcaggg gccctgcaaa
1381 gcctacgcgc ctgcgtgggc ttacaacagc cagacgggcc agtgccagtc ctttgtctat
1441 ggtggctgcg agggcaatgg caacaacttt gagagccgtg aggcctgtga ggagtcgtgc
1501 cccttcccca gggggaacca gcgctgtcgg gcctgcaagc ctcggcagaa gctcgttacc
1561 agcttctgtc gcagcgactt tgtcatcctg ggccgagtct ctgagctgac cgaggagcct
1621 gactcgggcc gcgccctggt gactgtggat gaggtcctaa aggatgagaa aatgggcctc
1681 aagttcctgg gccaggagcc attggaggtc actctgcttc acgtggactg ggcattgccc
1741 tgccccaacg tgaccgtgag cgagatgccg ctcatcatca tgggggaggt ggacggcggc
1801 atggccatgc tgcgccccga tagctttgtg ggcgcatacga gtgcccggcg ggtcaggaag
1861 cttcgtgagg tcatgcacaa gaagacctgt gacgtcctca aggagtttct tggcttgac
1921 tga
```

FIG. 7A

11/27

PREDICTED AMINO ACID SEQUENCE OF HUMAN GASP1

SEQ ID NO:5

1	MNP	NLW	VDAQ	STCR	RECETD	QEC	EMDQVSG	IQKP	QCEADQ	VNGV	QKPQCE	MDQK	WECEVD
61	QVSG	VQKPVC	EADQ	VSGVQK	PQCE	MDQVSG	IQKL	ECEADQ	KWEY	EVDQVS	GVQK	PQCEMD	
121	QVSG	IQKLEC	EADQ	ECETYE	KCCP	NVCGTK	SCVA	ARYMDV	KGKK	GPVGMP	KEAT	<u>TCDFMC</u>	
181	<u>LQOGSECDIW</u>	<u>DGQPVCKCKD</u>	<u>RCEKEPSFTC</u>	<u>ASDGLTYYNR</u>	<u>CYMDAEACSK</u>	<u>GITLAVVTCR</u>							
241	YHFT	WPNTSP	PPP	ETTMHPT	TASP	ETPELD	MAAP	ALLNNP	VHQS	VTMGET	VSFL	CDVVGR	
301	PRPE	ITWEKQ	LED	RENVVMR	PNHV	RGNVVV	TNIA	QLVIYN	AQLQ	DAGIYT	CTAR	NVAGVL	
361	RADF	PLSVVR	GHQA	AATSES	SPNG	TAFPAA	ECLK	PPDSED	CGEE	QTRWHF	DAQA	NNCLTF	
421	TFGH	CHRNLN	HFET	YEACML	ACMS	GPLAAC	SLPA	LQGPKV	AYAP	RWAYNS	QTGQ	CQSFVY	
481	GGCE	GNGNNF	ESRE	ACEESC	PFPR	GNQR	CRACK	PRQKLV	SFCR	SDFVIL	GRVS	ELTEEP	
541	DSGR	ALVTVD	EVLK	DEKMGL	KFLG	QEPLEV	TLLH	VDWACP	CPNV	TVSEMP	LIIM	GEVDGG	
601	MAMLR	PDSFV	GASS	ARRVRK	LREVM	HKKTC	DVLK	EFLGLH					

FIG. 7B

12/27

PREDICTED NUCLEOTIDE SEQUENCE OF HUMAN GASP1 USING AN ALTERNATIVE START SITE

SEQ ID NO:6

```
1 atgtgggccc caaggtgtcg ccggttctgg tctcgtctgg agcaggtggc agcgtgctg
61 ctgctgctgc tactgctcgg ggtgcccccg cgaagcctgg cgctgccgcc catccgctat
121 tcccacgccg gcatctgccc caacgacatg aatcccaacc tctgggtgga cgcacagagc
181 acctgcaggc gggagtgtga gacggaccag gagtgtgaga cctatgagaa gtgctgcccc
241 aacgtatgtg ggaccaagag ctgctgtggc gcccgctaca tggacgtgaa agggaagaag
301 ggcccagtgg gcatgcccac ggaggccaca tgtgaccact tcatgtgtct gcagcagggc
361 tctgagtgtg acatctggga tggccagccc gtgtgtaagt gcaaagaccg ctgtgagaag
421 gagcccagct ttacctgcgc ctccggacgg ctcacctact ataaccgctg ctacatggat
481 gccgaggcct gctccaaagg catcacactg gccgttgtaa cctgccgcta tcaactcacc
541 tggcccaaca ccagccccc accacctgag accaccatgc accccaccac agcctcccca
601 gagaccctg agctggacat ggcgggccct gcgtgctca acaaccctgt gcaccagtgc
661 gtcaccatgg gtgagacagt gagtttcctc tgtgatgtgg tgggccggcc ccggcctgag
721 atcacctggg agaagcagtt ggaggatcgg gagaatgtgg tcatgcggcc caaccatgtg
781 cgtggcaacg tgggtggcac caacattgcc cagctggcca tctataacgc ccagctgcag
841 gatgctggga tctacacctg cacggcccgg aacgtggctg gggtcctgag ggctgatttc
901 ccgctgtcgg tggtcagggg tcatcaggct gcagccacct cagagagcag cccaatggc
961 acggctttcc cggcggccga gtgcctgaag ccccagaca gtgaggactg tggcgaagag
1021 cagaccgct ggcacttcga tgcccaggcc aacaactgcc tgacctcac cttcggccac
1081 tgccaccgta acctcaacca ctttgagacc tatgaggcct gcatgctggc ctgcatgagc
1141 gggccgctgg ccgctgagc cctgcccggc ctgcaggggc cctgcaaagc ctacgcgcct
1201 cgctgggctt acaacagcca gacggggcag tgccagtcct ttgtctatgg tggctgagag
1261 ggcaatggca acaactttga gagccgtgag gcctgtgagg agtcgtgccc cttccccagg
1321 gggaaccagc gctgtcgggc ctgcaagcct cggcagaagc tcgttaccag cttctgtcgc
1381 agcgactttg tcatcctggg ccgagtctct gagctgaccg aggagcctga ctcggggccg
1441 gccctggtga ctgtggatga ggtcctaaag gatgagaaaa tgggcctcaa gttcctgggc
1501 caggagccat tggaggtcac tctgcttcac gtggactggg catgcccctg cccaacgtg
1561 accgtgagcg agatgccgct catcatcatg ggggaggtgg acggcggcat ggccatgctg
1621 cgccccgata gctttgtggg cgcacgcagt gcccgccggg tcaggaagct tcgtgaggtc
1681 atgcacaaga agacctgtga cgtcctcaag gagtttcttg gcttgcaactg a
```

FIG. 7C

13/27

PREDICTED AMINO ACID SEQUENCE OF HUMAN GASP1 USING AN ALTERNATIVE START SITE

SEQ ID NO:7

1 MWAPRCRRFW SRWEQVAALL LLLLLLLGVPP RSLALPPIRY SHAGICPNDM NPNLWVDAQS
61 TCRRECETDQ ECETYEKCCP NVCGTKSCVA ARYMDVKGKK GPVGMPKEAT **CDHFMCLQQG**
121 **SECDIWDGQP** **VCKCKDRCEK** **EPSFTCASDG** **LYYNNRCYMD** **AEACSKGITL** **AVVTCRYHFT**
181 WPNTSPPPPE TTMHPTTASP ETPELDMAAP ALLNNPVHQS VTMGETVSFL CDVVGRPRPE
241 ITWEKQLEDR ENVVMRPNHV RGNVVVTNIA QLVIYNAQLQ DAGIYTCTAR NVAGVLRADF
301 PLSVVRGHQA AATSESSPNG TAFPAAECLK PPDSEDCGEE QTRWHFDAQA NNCLTFTFGH
361 CHRNLNHFET YEACMLACMS GPLAACSLPA LQGPCKAYAP RWAYNSQTGQ CQSFVYGGCE
421 GNGNNFESRE ACEESCPFPR GNQRCRACKP RQKLVTSTFCR SDFVILGRVS ELTEEPDSGR
481 ALVTVDEVLK DEKMGLKFLG QEPLEVTLLH VDWACPCPNV TVSEMPIIM GEVDGGMAML
541 RPDSFVGASS ARRVRKLREV MHKKTCDVLC EFLGLH*

FIG. 7D

14/27

PREDICTED MOUSE GASP2 NUCLEOTIDE SEQUENCE
SEQ ID NO:8

```
1 atgcctgccc cacagccatt cctgcctctg ctctttgtct tcgtgctcat ccatctgacc
61 tcggagacca acctgctgcc agatcccgga agccatcctg gcatgtgccc caacgagctc
121 agccccacc tgtgggtcga cgcccagagc acctgtgagc gtgagtgtac cggggaccag
181 gactgtgcgg catccgagaa gtgctgcacc aatgtgtgtg ggctgcagag ctgcgtggct
241 gcccgctttc ccagtgggtg cccagctgta cctgagacag cagcctcctg tgaaggcttc
301 caatgcccac aacaggggtc tgactgtgac atctgggatg ggcagccagt ttgtcgctgc
361 cgtgaccgct gtgaaaaaga acccagcttc acatgtgctt ctgatggcct tacctattac
421 aaccgctgct acatggacgc agaagcctgc ctgcggggtc tccacctgca cgttgtaccc
481 tgtaagcaca ttctcagttg gccgcccagc agcccgggac cacccgagac cactgctcgc
541 ccaaccctg gggctgctcc catgccacct gccctgtaca acagcccctc accacaggca
601 gtgcatgttg gggggacagc cagcctccac tgtgatgtta gtggccgtcc accacctgct
661 gtgacctggg agaagcagag ccatcagcgg gagaacctga tcatgcgccc tgaccaaagt
721 tatggcaacg tggttgtcac cagtatcgga cagctagtcc tctacaatgc tcagttggag
781 gatgcggggc tgtatacctg cactgcacga aacgctgccg gcctgctgcg ggccgacttt
841 cccctttccg ttttacagcg ggcaactact caggacaggg acccaggtat cccagccttg
901 gctgagtgcc aggccgacac acaagcctgt gttggggcac ctactcccca tcatgtcctt
961 tggcgctttg acccacagag aggcagctgc atgacattcc cagccctcag atgtgatggg
1021 gctgcccggg gctttgagac ctatgaggca tgccagcagg cctgtgttcg tggccccggg
1081 gatgtctgtg cactgcctgc agttcagggg ccctgccagg gctgggagcc acgctggggc
1141 tacagcccac tgctacagca gtgccacccc tttgtataca gtggctgtga aggaaacagc
1201 aataactttg agaccgggga gagctgtgag gatgcttgcc ctgtaccacg cacaccaccc
1261 tgtcgtgcct gccgcctcaa gagcaagctg gctctgagct tgtgccgcag tgactttgcc
1321 atcgtgggga gactcacaga ggtcctggag gagcccgagg ctgcaggcgg catagctcgt
1381 gtggccttgg atgatgtgct aaaggacgac aagatgggac tcaagttctt gggcaccaaa
1441 tacctggagg tgacattgag tggcatggac tgggcctgcc catgccccaa cgtgacagct
1501 gtcgatgggc cactgggtcat catgggtgag gttcgtgaag gtgtggctgt gttggacgcc
1561 aacagctatg tccgtgctgc cagcgagaag cgagtcaaga agattgtgga actgctcgag
1621 aagaaggctt gtgaactgct caaccgcttc caagactag
```

FIG. 8A

15/27

PREDICTED MOUSE GASP2 AMINO ACID SEQUENCE
SEQ ID NO:9

1 MPAPQPFLPL LFVFLIHLT SETNLLPDPG SHPGMCPNEL SPHLWVDAQS TCERECTGDQ
61 DCAASEKCCT NVCGLQSCVA ARFPSGGPAV PETAASCEGF QCPQQGSDCD IWDGQPVCR
121 RDRCEKEPSF TCASDGLTY NRCYMDAEAC LRGLHLHVVP CKHILSWPPS SPGPPETTAR
181 PTPGAAPMPP ALYNPSPQA VHVGGTASLH CDVSGRPPPA VTWEKQSHQR ENLIMRPDQM
241 YGNVVVTSIG QLVLYNAQLE DAGLYTCTAR NAAGLLRADF PLSVLQRATT QDRDPGIPAL
301 AECQADTQAC VGPPTPHHVL WRFPQQRGSC MTFPALRCDG AARGFETYEACQQACVRGPG
361 DVCALPAVQG PCQGWEPRAWA YSPLLQQCHP FVYSGCEGNS NNFETRESCE DACVPRTTP
421 CRACRLKSKL ALSLCRSDFA IVGRLTEVLE EPEAAGGIAR VALDDVLKDD KMGLKFLGTK
481 YLEVTLTGMD WACPCPNVTA VDGPLVIMGE VREGVAVLDA NSYVRAASEK RVKKIVELLE
541 KKACELLNRF QD

FIG. 8B

16/27

PREDICTED NUCLEOTIDE SEQUENCE OF HUMAN GASP2

SEQ ID NO:10

```
1 atgcccgcctt tacgtccact cctgcccgtc ctgctcctcc tccggctgac ctccgggggt
61 ggcttgctgc cagggtgtgg gagccacccg ggcgtgtgcc ccaaccagct cagccccaac
121 ctgtgggtgg acgcccagag cacctgtgag cgcgagtgtg gcagggacca ggactgtgct
181 gctgctgaga agtgctgcat caacgtgtgt ggactgcaca gctgctgtgc agcacgcttc
241 cccggcagcc cagctgcgcc gacgacagcg gcctcctgct agggctttgt gtgcccacag
301 cagggtctcg actgcgacat ctgggacggg cagcccgtgt gccgtgccg cgaccgctgt
361 gagaaggagc ccagcttcac ctgcgcctcg gacggcctca cctactacaa ccgctgctat
421 atggacgccg aggcctgcct gcggggcctg cacctccaca tcgtgccctg caagcacgtg
481 ctgagctggc cgcgcagcag cccggggccg ccggagacca ctgcccgcct cacacctggg
541 gccgcgcccg tgctcctgct cctgtacagc agcccctccc cacaggcggg gcagggtggg
601 ggtacggcca gcctccactg cgacgtcagc ggccgcccgc cgcctgctgt gacctgggag
661 aagcagagtc accagcgaga gaacctgac atgcgccctg atcagatgtg tggcaacgtg
721 gtggtcacca gcatcgggca gctggtgctc tacaacgcgc ggcccgaaga cgcgggcctg
781 tacacctgca ccgcgcgcaa cgctgctggg ctgctgcggg ctgacttccc actctctgtg
841 gtccagcgag agccggccag ggacgcagcc cccagcatcc cagccccggc cgagtgcctg
901 ccggatgtgc aggcctgcac gggccccact tccccacacc ttgtcctctg gcactacgac
961 ccgcagcggg gcggctgcat gaccttcccg gcccggtggg gtgatggggc ggcccgcggc
1021 tttgagacct acgaggcatg ccagcaggcc tgtgcccgcg gcccggcgga cgcctgctgt
1081 ctgcctgccg tgcaggggcc ctgccggggc tgggagccgc gctgggccta cagcccgctg
1141 ctgcagcagt gccatccctt cgtgtacggg ggctgcgagg gcaacggcaa caacttccac
1201 agccgcgaga gctgcgagga tgctgcccc gtgccgcgca caccgccctg ccgcgcctgc
1261 cgcctccgga gcaagctggc gctgagcctg tgccgcagcg acttcgcat cgtggggcgg
1321 ctcacggagg tgctggagga gcccgaggcc gccggcggca tcgcccgcgt ggcgctcgag
1381 gacgtgctca aggatgacaa gatgggcctc aagttcttgg gcaccaagta cctggagggtg
1441 acgctgagtg gcatggactg ggctgcccc tgccccaaca tgacggcggg cgacggggccg
1501 ctggtcatca tgggtgaggt gcgcgatggc gtggccgtgc tggacgccgg cagctacgtc
1561 cgcgccgcca gcgagaagcg cgtcaagaag atcttggagc tgctggagaa gcaggcctgc
1621 gagctgctca accgcttcca ggactagccc ccgcaggggc ctgcgccacc ccgtcctggt
1681 gaataaacgc actcc
```

FIG. 9A

17/27

PREDICTED AMINO ACID SEQUENCE OF HUMAN GASP-2

SEQ ID NO:11

1 MPALRPLLPL LLLLRLTSGA GLLPGLGSHP GVCPNQLSPN LWVDAQSTCE RECSRQDQCA
61 AAEKCCINVC GLHSCVAARF PGSPAAPTTA ASCEGFVCPQ QGSDCDIWDG QPVCRCRDRC
121 EKEPSFTCAS DGLTYYNRCY MDAEACLRGL HLHIVPCKHV LSWPPSSPGP PETTARPTPG
181 AAPVPPALYS SPSPQAVQVG GTASLHCDVS GRPPPAVTWE KQSHQRENLI MRPDQMYGNV
241 VVTSIGQLVL YNARPEDAGL YTCTARNAAG LLRADFPLSV VQREPARDAA PSIPAPAECL
301 PDVQACTGPT SPHLVLWHYD PQRGGCMTFP ARGCDGAARG FETYEACQQA CARGPGDACV
361 LPAVQGPCRG WEPRWAYSPL LQQCHPFVYG GCEGNGNNFH SRESCEDACP VPRTPPCRAC
421 RLRSKLALSL CRSDFAIVGR LTEVLEEPEA AGGIARVALE DVLKDDKMGL KFLGTKYLEV
481 TLSGMDWACP CPNMTAGDGP LVIMGEVRDG VAVLDAGSYV RAASEKRVKK ILELLEKQAC
541 ELLNRFQD

FIG. 9B

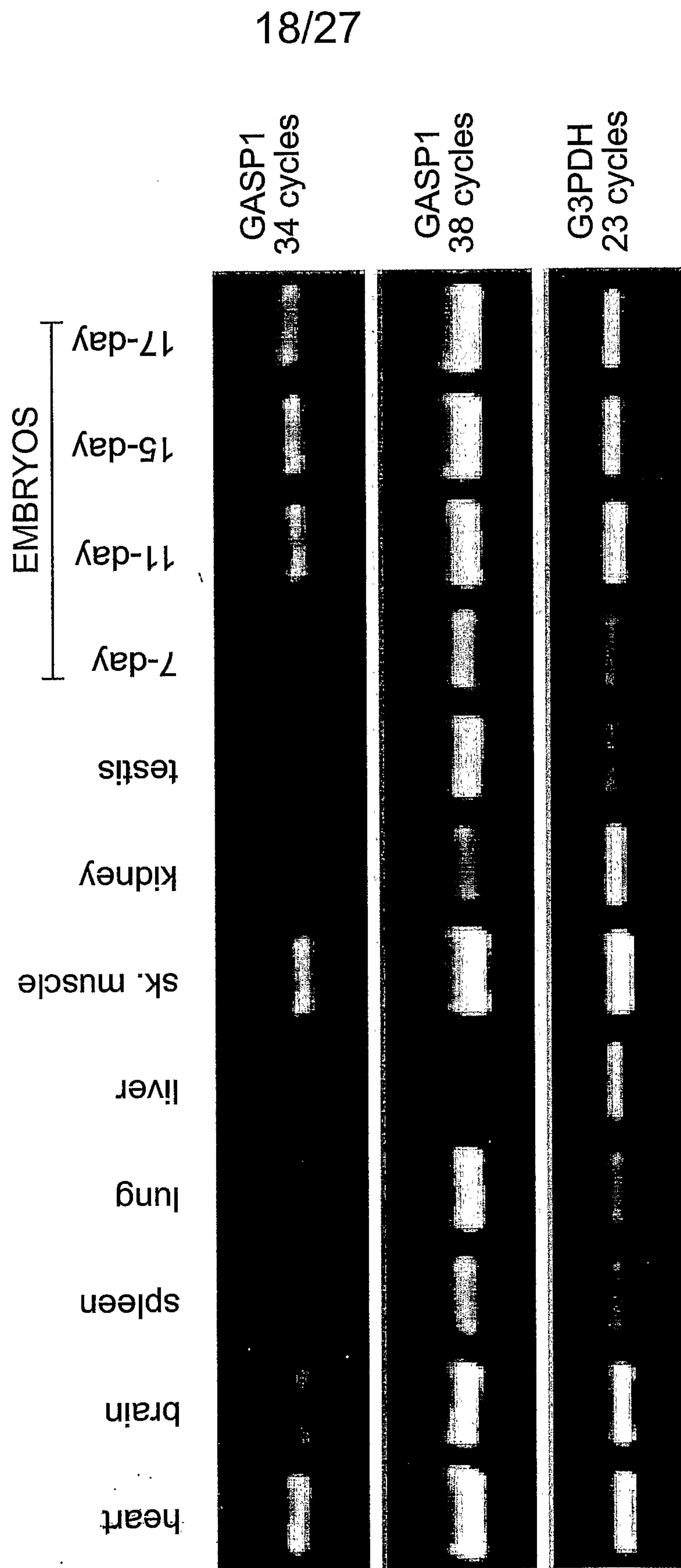
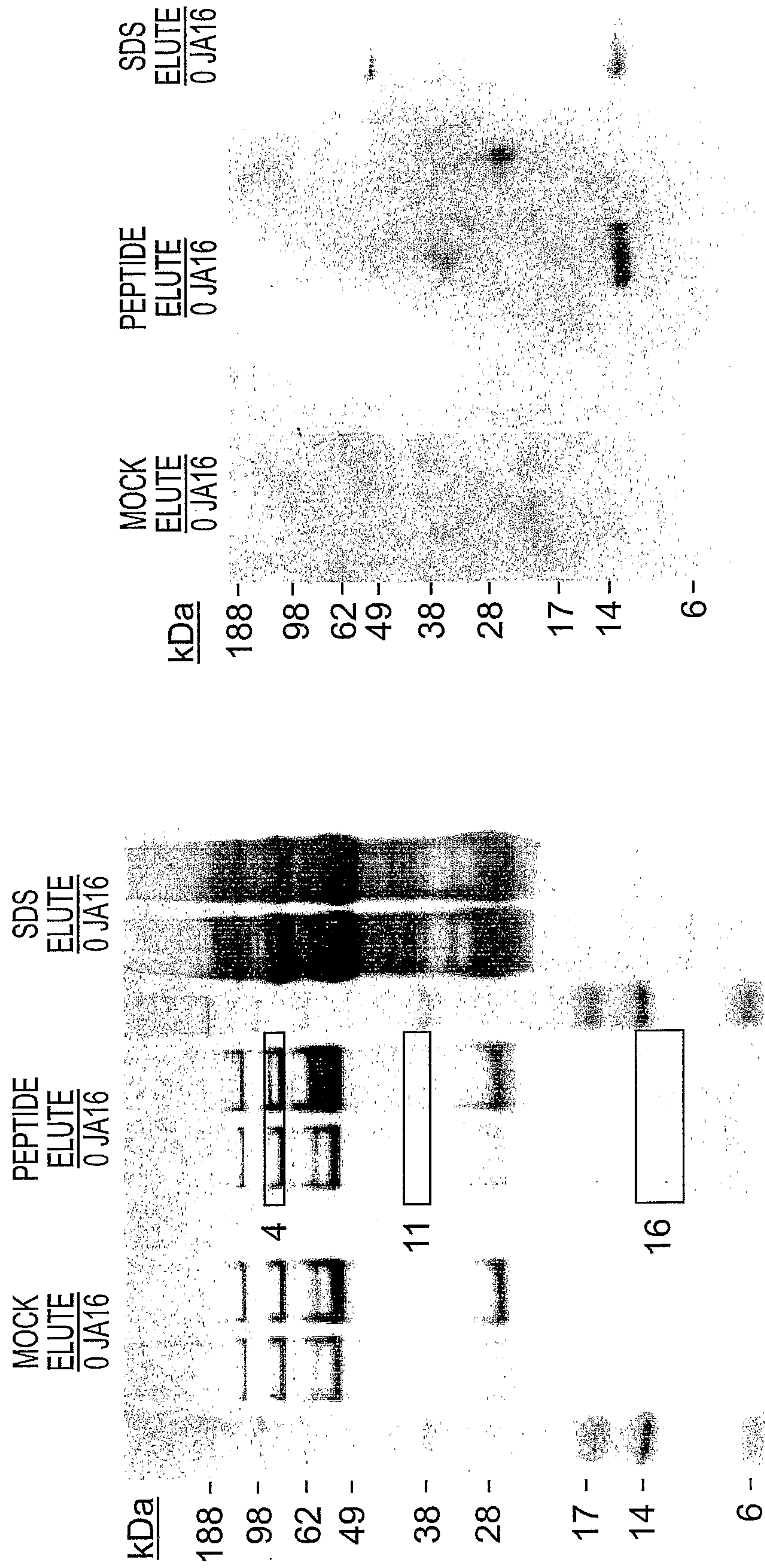
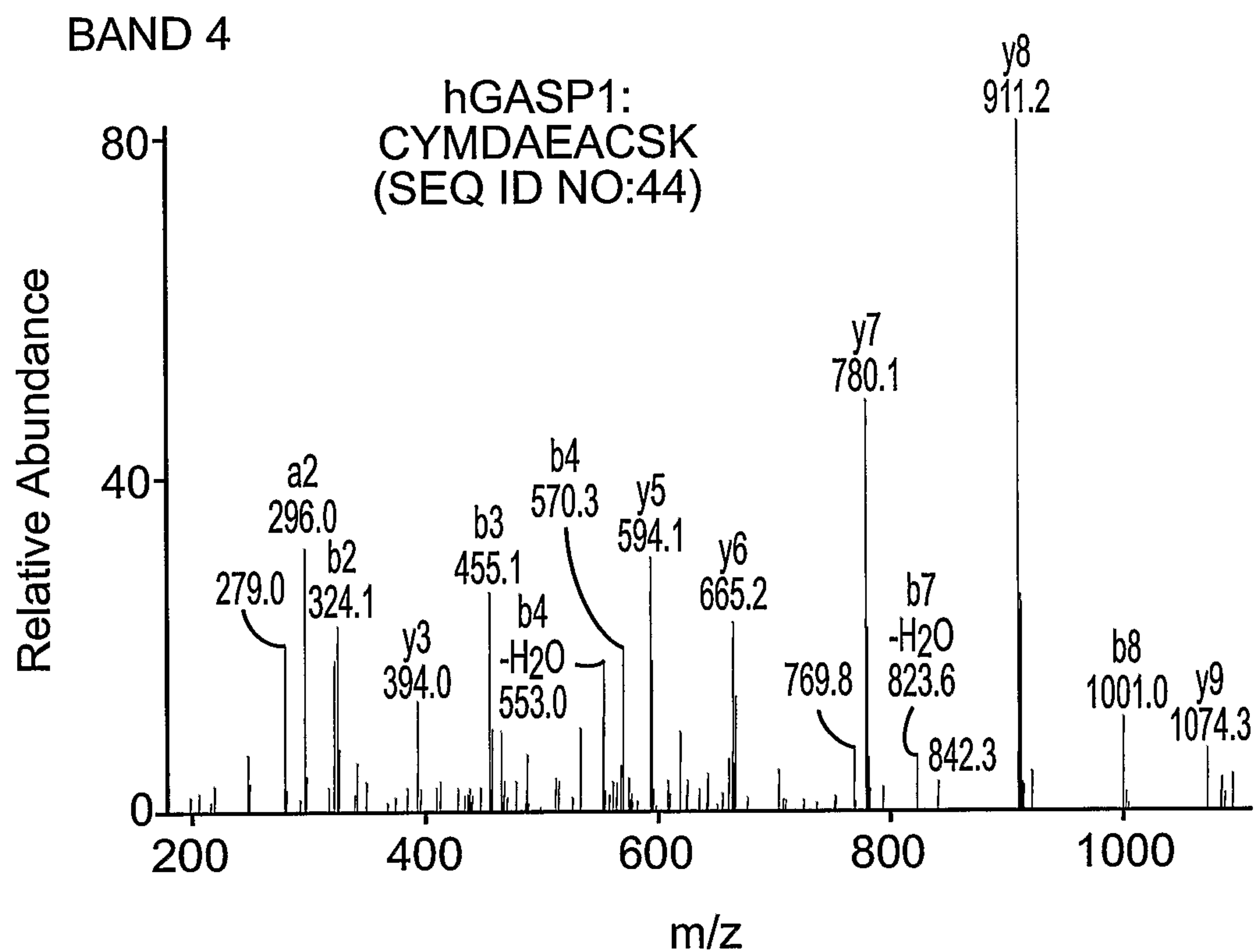
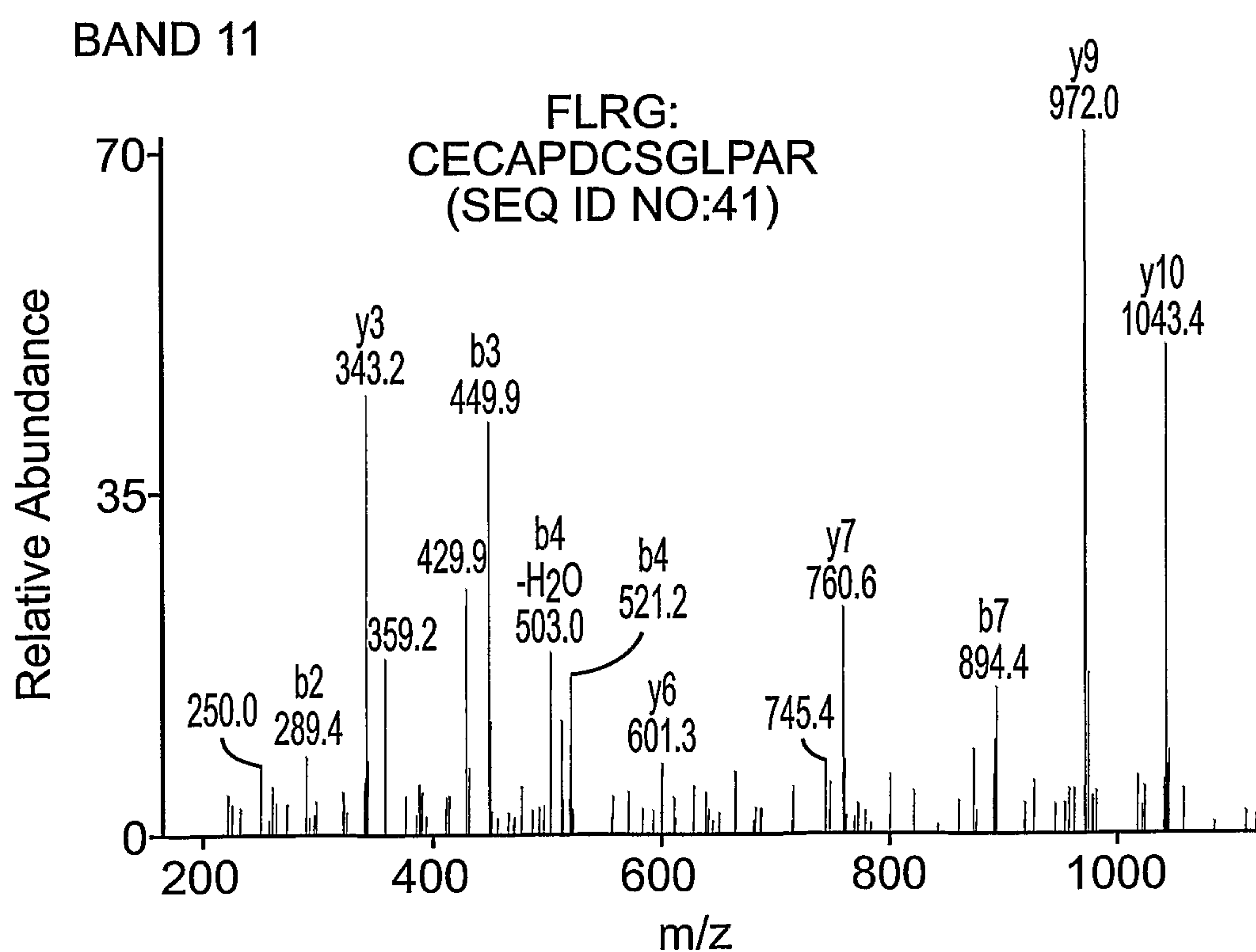


FIG. 10

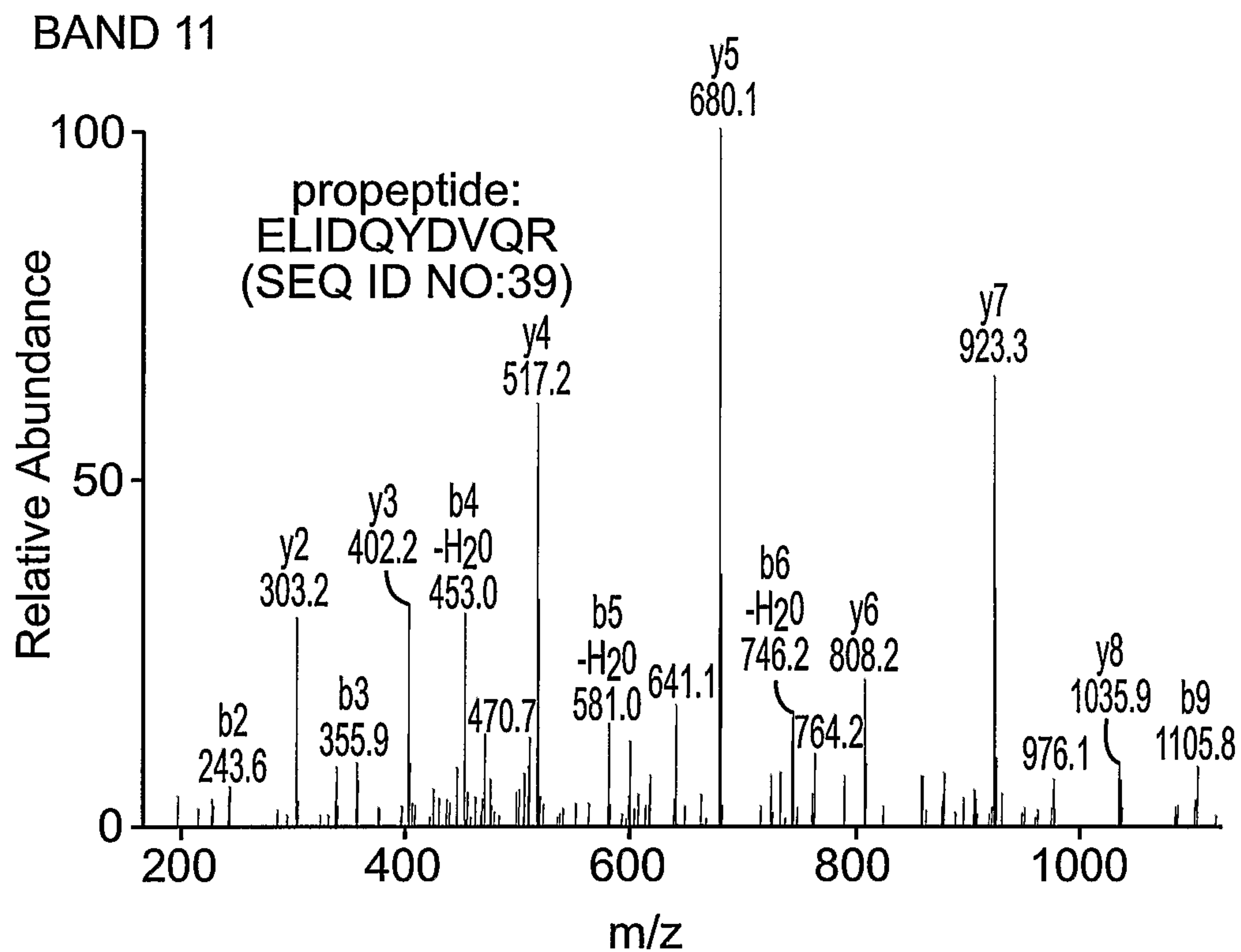
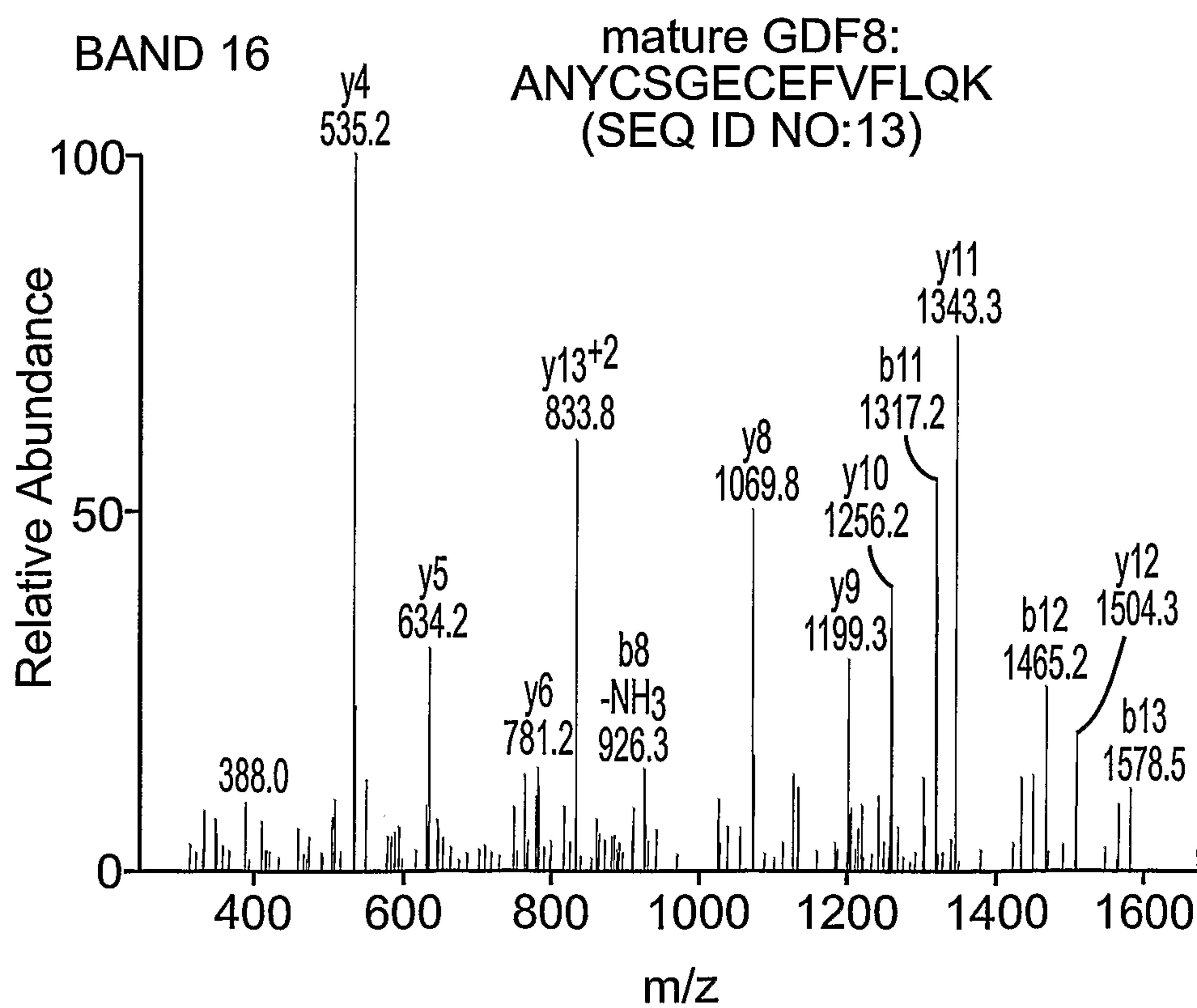
19/27

**FIG. 11A****FIG. 11B**

20/27

**FIG. 12A****FIG. 12B**

21/27

**FIG. 12C****FIG. 12D**

22/27

CLONED MOUSE GASP1 NUCLEOTIDE AND AMINO ACID SEQUENCES
SEQ ID NO: 48

M C A P G Y H R F W F H W G L L L L L L L E A P L
 1 ATGTGTGCCCCAGGG TATCATCGGTTCTGG TTTCAGTGGGGGCTG CTGTTGCTGCTGCTC CTCGAGGCTCCCCTT
 R G L A L P P I R Y S H A G I C P N D M N P N L W
 76 CGAGGCCTAGCACTG CCACCCATCCGATAC TCCCATGCGGGCATC TGCCCCAACGACATG AACCCCAACCTCTGG
 V D A Q S T C K R E C E T D O E C E T Y E K C C P
 151 GTGGATGCCAGAGC ACCTGCAAGCGAGAG TGTGAAACAGACCAG GAATGTGAGACCTAT GAGAAATGCTGCCCC
 N V C G T K S C V A A R Y M D V K G K K G P V G M
 226 AATGTGTGTGGGACC AAGAGCTGTGTGGCA GCCCGCTACATGGAT GTGAAAGGGAAGAAG GGGCCTGTAGGCATG
 P K E A T C D H F M C L Q Q G S E C D I W D G Q P
 301 CCCAAGGAGGCCACA TGTGACCATTTTCATG TGCCTGCAGCAGGGC TCTGAGTGTGACATC TGGGACGGCCAGCCC
 V C K C K D R C E K E P S F T C A S D G L T Y Y N
 376 GTGTGTAAGTGCAA GATCGCTGTGAGAAG GAGCCAGCTTCACC TGTGCCTCTGATGGC CTTACCTACTACAAC
 R C F M D A E A C S K G I T L S V V T C R Y H F T
 451 CGTTGCTTCATGGAC GCCGAAGCCTGCTCC AAGGGCATCACACTG TCTGTGGTCACCTGT CGTTATCACTTCACC
 W P N T S P P P P E T T V H P T T A S P E T L G L
 526 TGGCCTAACACCAGC CCTCCACCGCCTGAG ACCACGGTGCATCCC ACCACCGCCTCTCCG GAGACTCTCGGGCTG
 D M A A P A L L N H P V H Q S V T V G E T V S F L
 601 GACATGGCAGCCCCA GCCCTGCTCAACCAC CCTGTCCATCAGTCA GTCACCGTGGGTGAG ACTGTGAGTTTCCTC
 C D V V G R P R P E L T W E K Q L E D R E N V V M
 676 TGTGACGTGGTAGGC CGGCCTCGGCCAGAG CTCACCTGGGAGAAA CAGCTGGAGGACCGA GAGAATGTTGTCATG
 R P N H V R G N V V V T N I A Q L V I Y N V Q P Q
 751 AGGCCCAACCACGTG CGTGGTAATGTGGTG GTCATAACATTGCC CAGCTGGTCATCTAC AACGTCCAGCCCCAG
 D A G I Y T C T A R N V A G V L R A D F P L S V V
 826 GATGCTGGCATATAC ACCTGTACAGCTCGA AATGTCGCTGGTGTG CTGAGGGCTGACTTC CCGTTGTCGGTGGTC
 R G G Q A R A T S E S S L N G T A F P A T E C L K
 901 AGGGGTGGTCAGGCC AGGGCCACTTCAGAG AGCAGTCTCAATGGC ACAGCTTTTCCAGCA ACAGAGTGCCTGAAG
 P P D S E D C G E E Q T R W H F D A Q A N N C L T
 976 CCCCAGACAGTGAG GACTGTGGAGAGGAG CAGACACGCTGGCAC TTCGACGCCAGGCT ACAAAGTGCCTCACT
 F T F G H C H H N L N H F E T Y E A C M L A C M S
 1051 TTCACCTTTGGCCAC TGCCACCACAATCTC AACCCTTTGAGACC TACGAGGCCTGTATG CTGGCTTGTATGAGT
 G P L A T C S L P A L Q G P C K A Y V P R W A Y N
 1126 GGGCATTGGCCACC TGCAGCCTGCCTGCC CTGCAAGGGCCTTGC AAAGCTTATGTCCA CGCTGGGCCTACAAC
 S Q T G L C Q S F V Y G G C E G N G N N F E S R E
 1201 AGCCAGACAGGCCTA TGCCAGTCCTTCGTC TATGGCGGCTGTGAG GGCAACGGTAACAAC TTTGAAAGCCGTGAG
 A C E E S C P F P R G N Q H C R A C K P R Q K L V
 1276 GCTTGTGAGGAGTCG TGTCCCTTCCCGAGG GGTAACCAGCACTGC CGGGCCTGCAAGCCC CGGCAAAAACCTTGT
 T S F C R S D F V I L G R V S E L T E E Q D S G R
 1351 ACCAGCTTCTGTGCG AGTGACTTTGTCATC CTGGGCAGGGTCTCT GAGCTGACCGAGGAG CAAGACTCGGGCCGT
 A L V T V D E V L K D E K M G L K F L G R E P L E
 1426 GCCCTGGTGACCGTG GATGAGGTCTTAAAA GATGAGAAGATGGGC CTCAAGTTTCTGGGC CGGGAGCCTCTGGAA
 V T L L H V D W T C P C P N V T V G E T P L I I M
 1501 GTCACCCTGCTTCAT GTAGACTGGACCTGT CCTTGCCCCAACGTG ACAGTGGGTGAGACA CCACTCATCATCATG
 G E V D G G M A M L R P D S F V G A S S T R R V R
 1576 GGGGAGGTGGACGGC GGCATGGCCATGCTG AGACCCGATAGCTTT GTGGGGGCATCGAGC ACACGGCGGGTCAGG
 K L R E V M Y K K T C D V L K D F L G L Q *
 1651 AAGCTCCGTGAGGTC ATGTACAAGAAACC TGTGACGTCCTCAAG GACTTCCTGGGCTTG CAATGA

FIG. 13

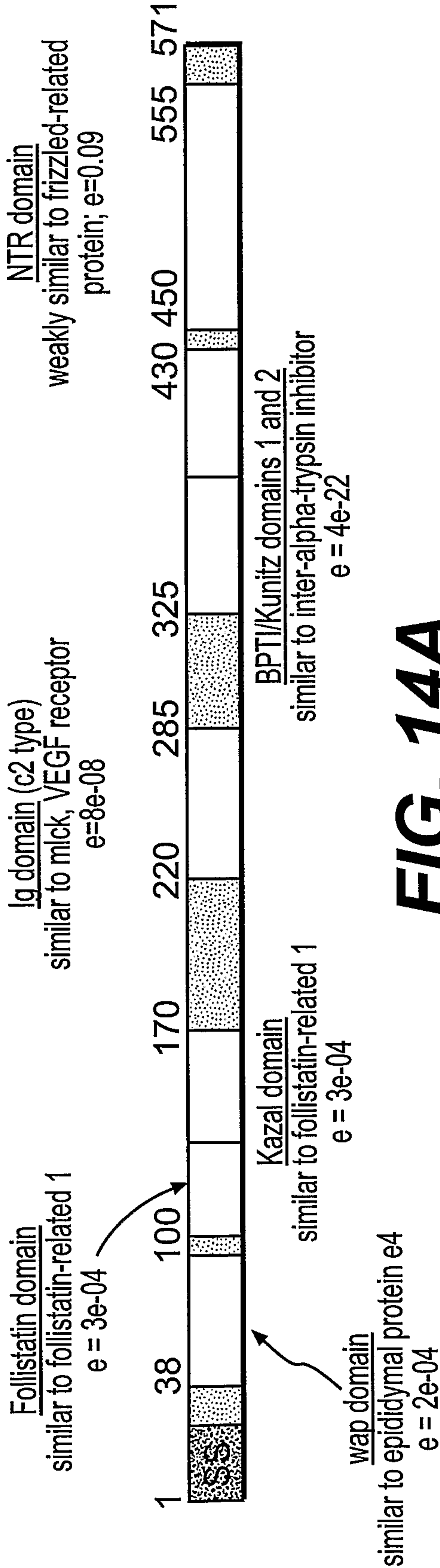


FIG. 14A

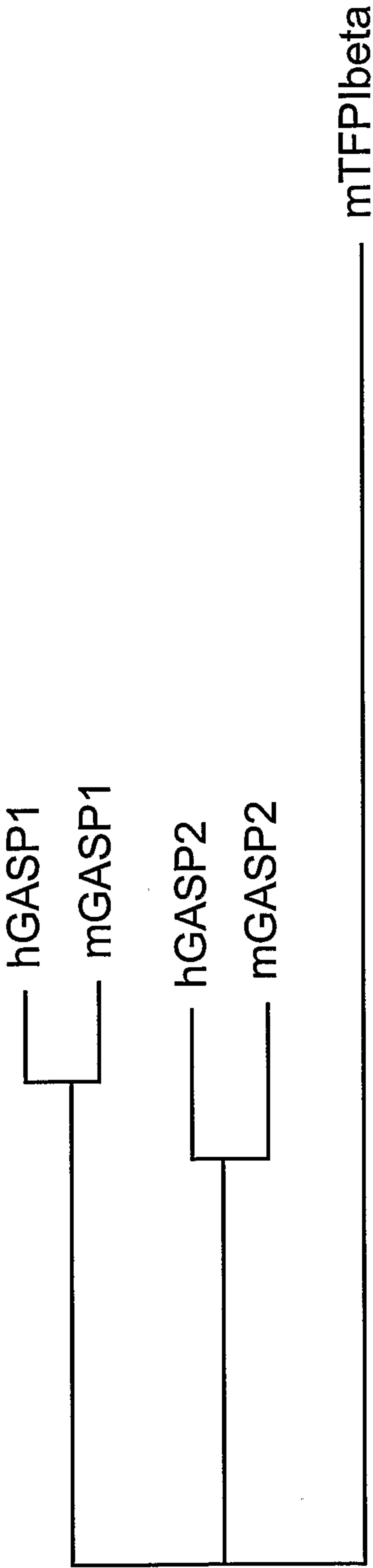


FIG. 14B

24/27

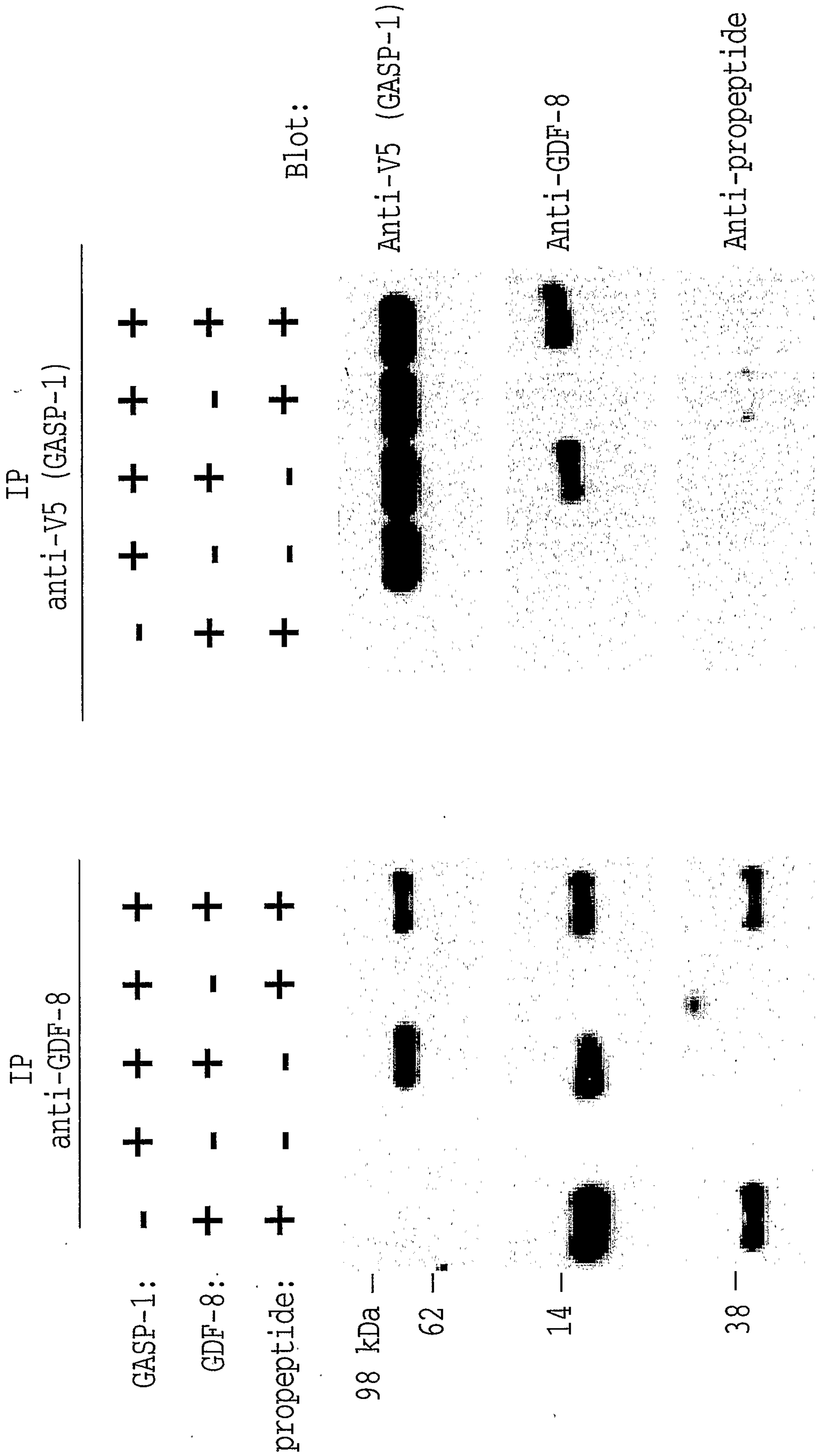
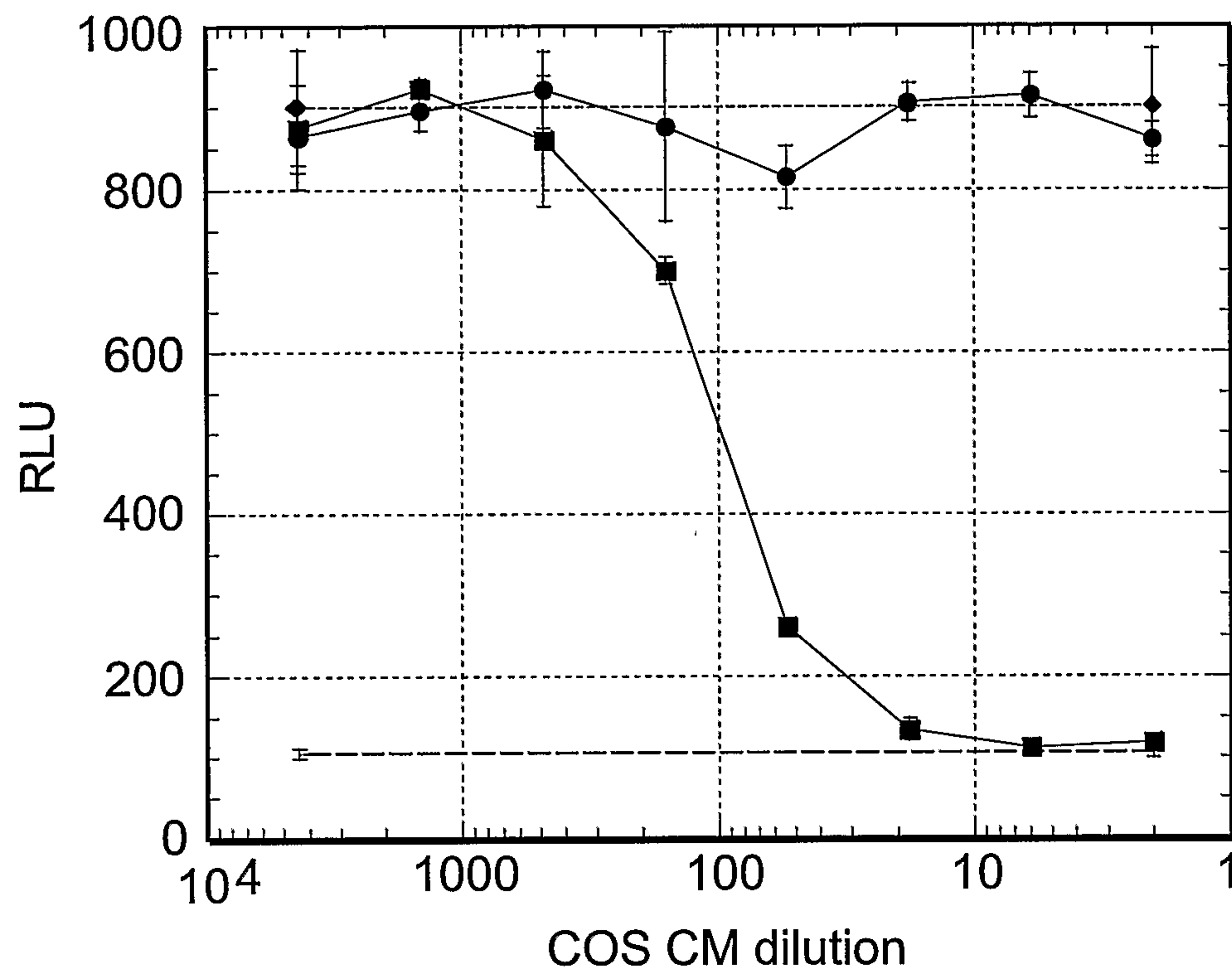
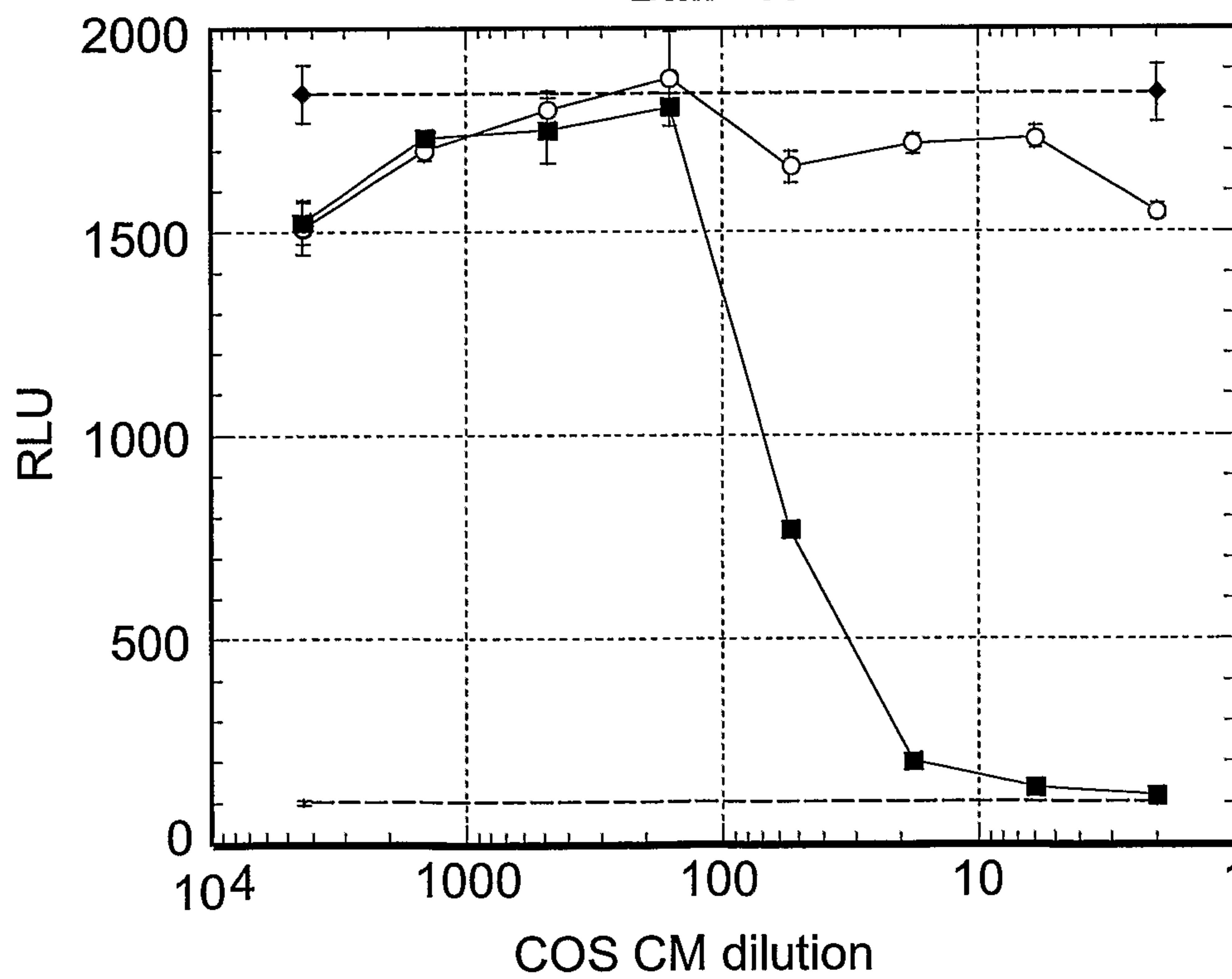
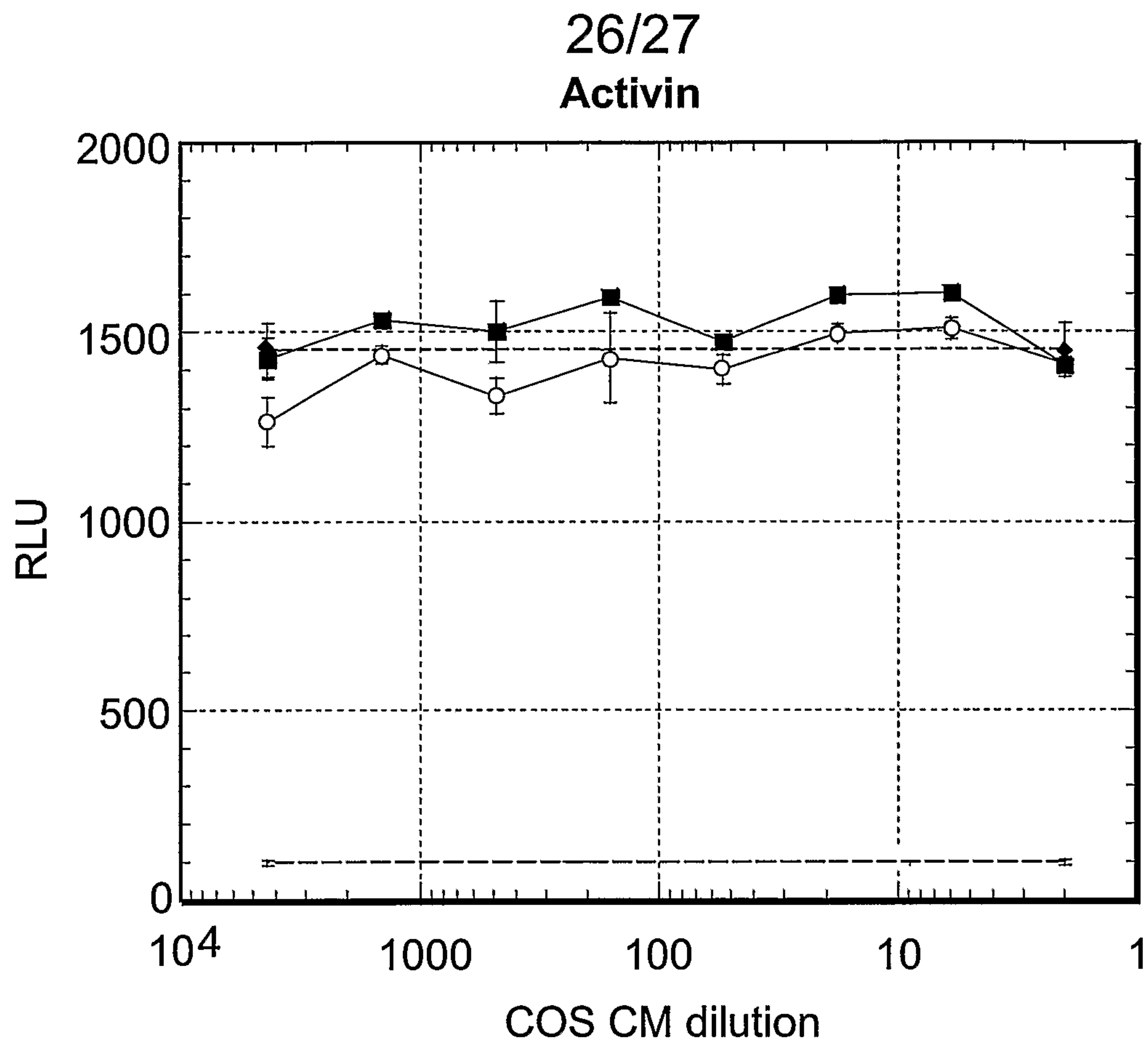
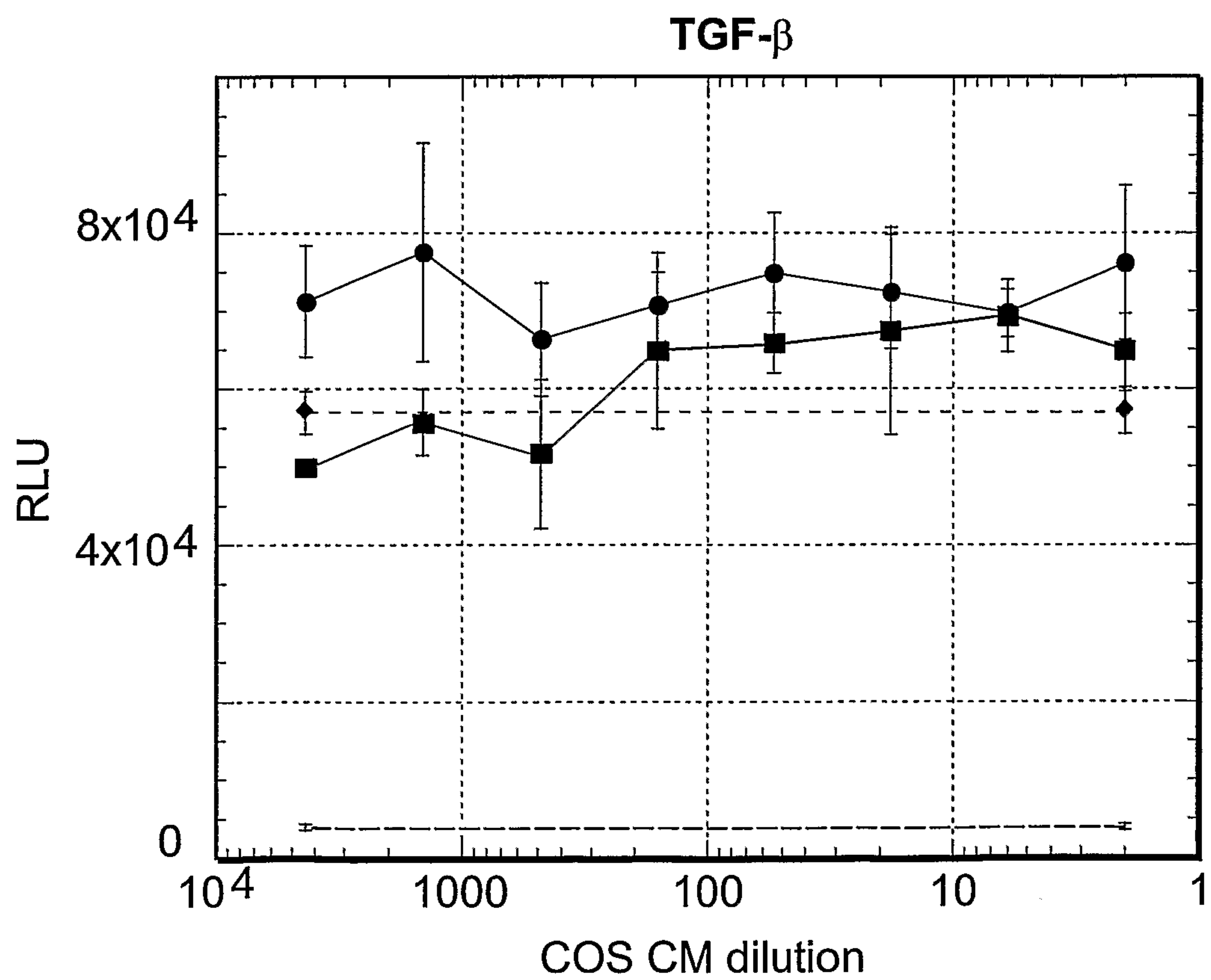


FIG. 15A

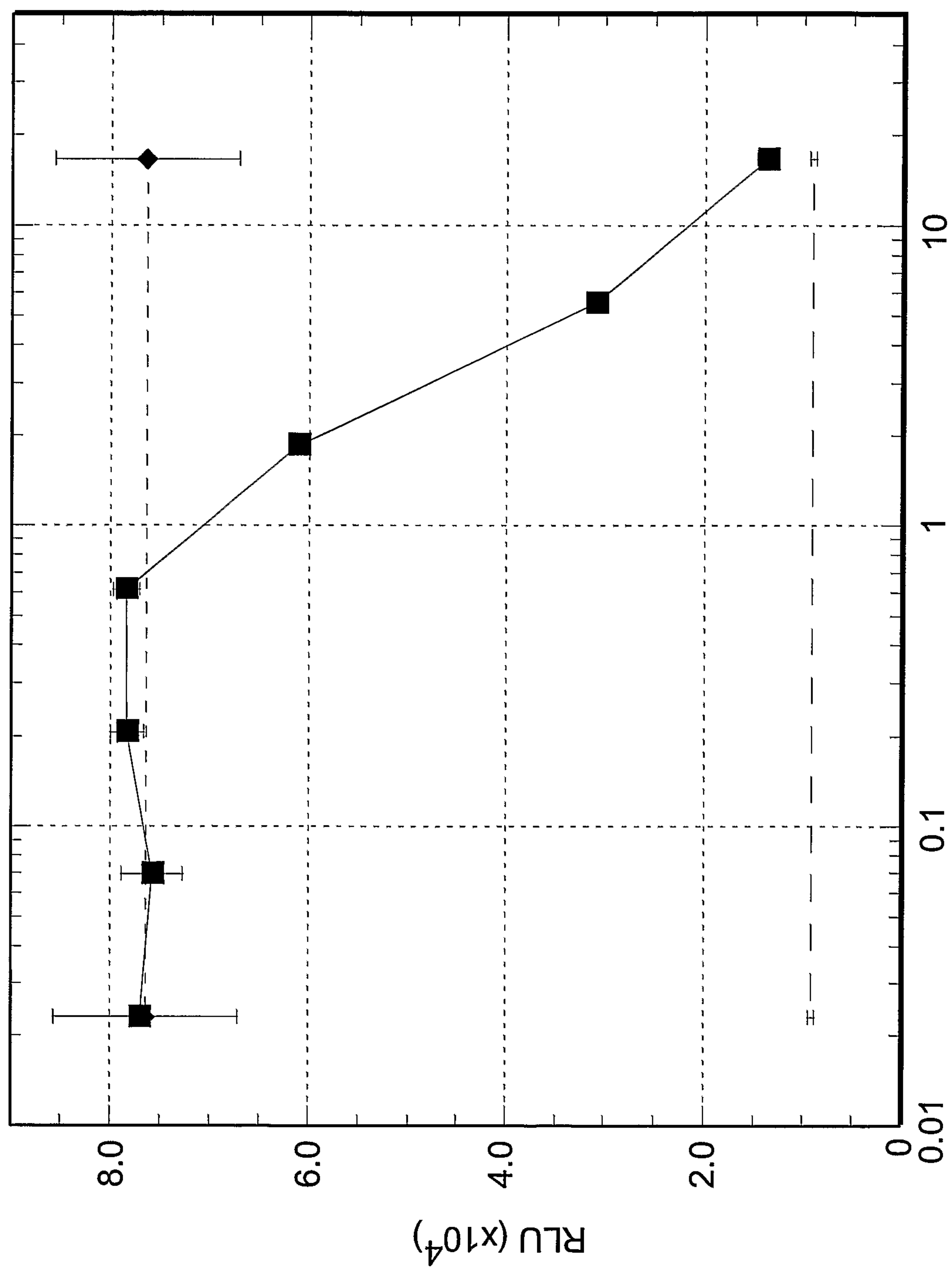
FIG. 15B

25/27

GDF-8**FIG. 16A****BMP-11****FIG. 16B**

**FIG. 16C****FIG. 16D**

27/27



GASP-1 (nM)

FIG. 17

