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(54) Title: PACAP ANTIBODIES AND USES THEREOF

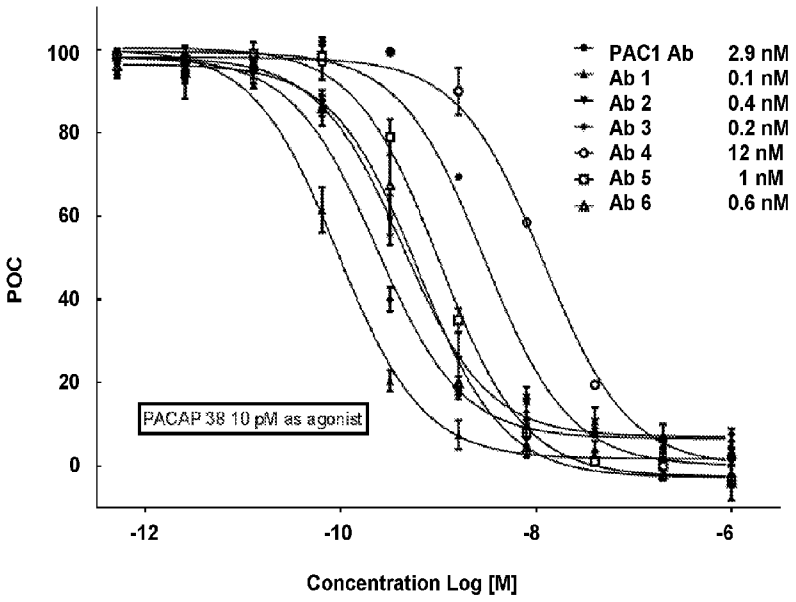


FIG. 1

(57) Abstract: The present invention relates to monoclonal antibodies that specifically bind to human pituitary adenylate cyclase activating polypeptide (PACAP) and pharmaceutical compositions comprising such antibodies. Methods of treating or preventing headache conditions, such as migraine and cluster headache, using the monoclonal antibodies are also described.

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# PACAP ANTIBODIES AND USES THEREOF

## CROSS-REFERENCE TO RELATED APPLICATIONS

**[0001]** This application claims the benefit of U.S. Provisional Application No. 62/267,822, filed December 15, 2015, which is hereby incorporated by reference in its entirety.

## DESCRIPTION OF THE TEXT FILE SUBMITTED ELECTRONICALLY

**[0002]** The present application contains a Sequence Listing, which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. The computer readable format copy of the Sequence Listing, which was created on December 15, 2016, is named A-2013-WO-PCT_ST25.txt and is 106 kilobytes in size.

## FIELD OF THE INVENTION

**[0003]** The present invention relates to the field of biopharmaceuticals. In particular, the invention relates to monoclonal antibodies that specifically bind to human pituitary adenylate cyclase-activating polypeptide (PACAP), pharmaceutical compositions comprising the monoclonal antibodies, and methods of producing and using such monoclonal antibodies.

## BACKGROUND OF THE INVENTION

**[0004]** Migraines are episodic headaches that can involve significant pain, are often accompanied by nausea, vomiting, and extreme sensitivity to light (photophobia) and sound (phonophobia), and are sometimes preceded by sensory warning symptoms or signs (auras). Migraine is a highly prevalent disease worldwide with approximately 12% of the European population, and 18% of women, 6% of men in the United States suffering from migraine attacks (Lipton *et al*, Neurology, Vol. 68:343-349, 2007; Lipton *et al*., Headache, Vol. 41:646-657, 2001). A study to assess the prevalence of migraine in the United States reported that nearly half the migraine patient population had three or more migraines per month (Lipton *et al*, Neurology, Vol. 68:343-349, 2007). Additionally, migraines are associated with a number of psychiatric and medical comorbidities such as depression and vascular disorders (Buse *et al*., J. Neurol. Neurosurg. Psychiatry, Vol. 81:428-432, 2010; Bigal *et al*., Neurology, Vol. 72:1864-1871, 2009). Most of the current migraine therapies are either not well tolerated or ineffective (Loder *et*

*al.*, Headache, Vol. 52:930-945, 2012; Lipton et al, 2001); thus, migraine remains an unmet medical need.

**[0005]** A major component of migraine pathogenesis involves the activation of the trigeminovascular system. The release of trigeminal and parasympathetic neurotransmitters from perivascular nerve fibers (Sánchez-del-Rio and Reuter, Curr. Opin. Neurol., Vol. 17(3):289-93, 2004) result in vasodilation of the cranial blood vessels and has been suggested to be associated with the onset of migraine headaches (Edvinsson, Cephalagia, Vol. 33(13): 1070-1072, 2013; Goadsby *et al.*, New Engl J Med., Vol. 364(4):257-270, 2002).

**[0006]** Pituitary adenylate cyclase-activating polypeptides (PACAP) are 38-amino acid (PACAP38), or 27-amino acid (PACAP27) peptides that were first isolated from an ovine hypothalamic extract on the basis of their ability to stimulate cAMP formation in anterior pituitary cells (Miyata *et al.*, Biochem Biophys Res Commun., Vol. 164:567-574, 1989; Miyata *et al.*, Biochem Biophys Res Commun., Vol.170:643-648, 1990). PACAP belongs to the VIP/secretin/glucagon superfamily. The sequence of PACAP 27 corresponds to the 27 N-terminal amino acids of PACAP 38 and shares 68% identity with vasoactive intestinal polypeptide (VIP) (Pantaloni *et al.*, J. Biol. Chem., Vol. 271: 22146-22151, 1996; Pisegna and Wank, Proc. Natl. Acad. Sci. USA, Vol. 90: 6345-49, 1993; Campbell and Scanes, Growth Regul., Vol. 2:175–191, 1992). The major form of PACAP peptide in the human body is PACAP38, and the pharmacology of PACAP 38 has not been shown to be different from the pharmacology of PACAP27. Three PACAP receptors have been reported: one receptor that binds PACAP with high affinity and has a much lower affinity for VIP (PAC1 receptor), and two receptors that recognize PACAP and VIP equally well (VPAC1 and VPAC2 receptors) (Vaudry *et al.*, Pharmacol Rev., Vol. 61:283-357, 2009).

**[0007]** Human experimental migraine models using PACAP as a challenge agent to induce migraine-like headaches support the approach for antagonism of the PACAP/PAC1 signaling pathway as a treatment for migraine prophylaxis. Infusion of PACAP38 caused headaches in healthy subjects and migraine-like headaches in migraine patients (Schytz *et al.*, Brain, Vol. 132:16-25, 2009). In addition, in the same model, VIP did not cause migraine-like headaches in migraine patients (Rahmann *et al.*, Cephalalgia, Vol. 28:226-236, 2008). The lack of migraine-like headache induction from VIP infusion suggests that PACAP38 peptide's effects are mediated through the PAC1 receptor, rather than VPAC1 or VPAC2 receptors, because VIP has

a much higher affinity at the latter two receptors. These data suggest that pharmacological agents that inhibit PACAP-activation of the PAC1 receptor have the potential to treat migraine.

## SUMMARY OF THE INVENTION

**[0008]** The present invention provides isolated antigen binding proteins that specifically bind to human PACAP. In certain embodiments, the isolated antigen binding proteins specifically bind to both human PACAP38 and human PACAP27. In other embodiments, the isolated antigen binding proteins specifically bind to human PACAP38, but not human PACAP27. The isolated antigen binding proteins can be used to inhibit, interfere with, or modulate the biological activity of PACAP, including inhibiting or reducing PACAP-induced activation of PAC1, VPAC1, and/or VPAC2 receptors, inhibiting or reducing vasodilation, and ameliorating or treating symptoms of migraine and other vascular headaches.

**[0009]** In one embodiment of the invention, the isolated antigen binding protein is an isolated monoclonal antibody or binding fragment thereof. The monoclonal antibody can be a chimeric antibody, humanized antibody, or fully human antibody. In some embodiments, the monoclonal antibody or binding fragment thereof specifically binds to a C-terminal domain of PACAP38, for example, at an epitope within amino acids 28 to 38 of SEQ ID NO: 1, at an epitope within amino acids 28 to 37 of SEQ ID NO: 1, or at an epitope within amino acids 34 to 38 of SEQ ID NO: 1. Such C-terminal binding monoclonal antibodies or binding fragments thereof may bind to a polypeptide consisting of the sequence of SEQ ID NO: 4 and/or a polypeptide consisting of the sequence of SEQ ID NO: 126. In certain embodiments, a C-terminal binding monoclonal antibody or binding fragment thereof may bind to a polypeptide consisting of the sequence of SEQ ID NO: 4 with a binding affinity at least 2-fold greater than that for a polypeptide consisting of the sequence of SEQ ID NO: 115. In other embodiments, the monoclonal antibody or binding fragment thereof specifically binds to an N-terminal domain of PACAP38, for example at an epitope within amino acids 1 to 13 of SEQ ID NO: 1. These N-terminal binding monoclonal antibodies or binding fragments thereof may bind a polypeptide consisting of the sequence of SEQ ID NO: 5. In certain embodiments, the monoclonal antibody or binding fragment thereof does not significantly bind to human VIP.

**[0010]** In certain embodiments, the antigen binding proteins of the invention (e.g. monoclonal antibodies and binding fragments thereof) are neutralizing antigen binding proteins. For instance,

the antigen binding proteins inhibit the binding of PACAP38 and/or PACAP27 to the human VPAC1, VPAC2, or PAC1 receptor and prevent or reduce receptor activation. In one embodiment, the antigen binding proteins inhibit PACAP38 binding to the PAC1 receptor and inhibit PACAP38-induced activation of the receptor. For instance, in some embodiments, the antigen binding proteins inhibit PACAP38-induced activation of human PAC1 receptor with an IC50 less than 5 nM as measured by a cell-based cAMP assay. In other embodiments, the antigen binding proteins inhibit PACAP38-induced activation of human PAC1 receptor with an IC50 less than 1 nM as measured by a cell-based cAMP assay. In certain embodiments, the antigen binding proteins of the invention inhibit PACAP38-induced activation of the human PAC1 receptor with an IC50 between about 100 pM and about 500 pM as measured by a cell-based cAMP assay. In some embodiments, the antigen binding proteins do not significantly inhibit the binding of PACAP27 to the PAC1 receptor or PACAP27-induced activation of the receptor.

**[0011]** The present invention also includes isolated polynucleotides and expression vectors encoding the anti-PACAP antigen binding proteins described herein as well as host cells, such as CHO cells, comprising the encoding polynucleotides and expression vectors.

**[0012]** In another embodiment, the present invention provides a method for producing the antigen binding proteins, including monoclonal antibodies and binding fragments thereof, described herein. In one embodiment, the method comprises culturing a host cell comprising an expression vector encoding the antigen binding protein under conditions that allow expression of the antigen binding protein, and recovering the antigen binding protein from the culture medium or host cell.

**[0013]** The antigen binding proteins described herein can be used in the manufacture of a pharmaceutical composition or medicament for the treatment or prevention of conditions associated with PACAP biological activity, such as headache, migraine, and chronic pain. Thus, the present invention also provides a pharmaceutical composition comprising an antigen binding protein and a pharmaceutically acceptable diluent, excipient or carrier. In some embodiments, the antigen binding protein specifically binds to a C-terminal domain of PACAP38, e.g. at an epitope within amino acids 28 to 38 of SEQ ID NO: 1, at an epitope within amino acids 28 to 37 of SEQ ID NO: 1, or at an epitope within amino acids 34 to 38 of SEQ ID NO: 1. In other embodiments, the antigen binding protein specifically binds to an N-terminal domain of

PACAP38, e.g., at an epitope within amino acids 1 to 13 of SEQ ID NO: 1. In still other embodiments, the antigen binding protein specifically binds to human PACAP38 at an epitope within amino acids 6 to 20 of SEQ ID NO: 1 or at an epitope within amino acids 14 to 27 of SEQ ID NO: 1.

**[0014]** In some embodiments, the present invention provides a method for treating or preventing a headache condition in a patient in need thereof comprising administering to the patient an effective amount of an antigen binding protein described herein. In certain embodiments, the antigen binding protein specifically binds to a C-terminal domain of PACAP38, e.g. at an epitope within amino acids 28 to 38 of SEQ ID NO: 1, at an epitope within amino acids 28 to 37 of SEQ ID NO: 1, or at an epitope within amino acids 34 to 38 of SEQ ID NO: 1. In other embodiments, the antigen binding protein specifically binds to an N-terminal domain of PACAP38, e.g., at an epitope within amino acids 1 to 13 of SEQ ID NO: 1. In still other embodiments, the antigen binding protein specifically binds to human PACAP38 at an epitope within amino acids 6 to 20 of SEQ ID NO: 1 or at an epitope within amino acids 14 to 27 of SEQ ID NO: 1. In some embodiments, the headache condition to be treated or prevented with the methods of the invention is migraine. The migraine can be episodic migraine or chronic migraine. In other embodiments, the headache condition to be treated or prevented with the methods of the invention is cluster headache. In particular embodiments, the methods provide prophylactic treatment for these conditions. The antigen binding protein may be administered to the patient by a parenteral route of administration, such as subcutaneous or intravenous administration.

**[0015]** The use of the anti-PACAP antigen binding proteins in any of the methods disclosed herein or for preparation of medicaments for administration according to any of the methods disclosed herein is specifically contemplated. For instance, the present invention includes an anti-PACAP antigen binding protein, such as anti-PACAP monoclonal antibody or binding fragment thereof, for use in a method for treating or preventing a headache condition in a patient in need thereof. The headache condition includes migraine (episodic and chronic migraine) and cluster headache.

**[0016]** The present invention also includes the use of an anti-PACAP antigen binding protein, such as anti-PACAP monoclonal antibody or binding fragment thereof, in the preparation of a

medicament for treating or preventing a headache condition in a patient in need thereof. The headache condition includes migraine (episodic and chronic migraine) and cluster headache.

## BRIEF DESCRIPTION OF THE DRAWINGS

**[0017] Figure 1** depicts dose-response curves for monoclonal anti-PACAP antibodies (Ab1, Ab2, Ab3, Ab4, Ab5, and Ab6) and a monoclonal anti-PAC1 receptor antibody (PAC1 Ab) for inhibition of PACAP38-induced activation of the human PAC1 receptor. The IC50 values for each of the antibodies are listed to the right of the antibody designation in the legend.

**[0018] Figures 2A and 2B** depict dose-response curves for monoclonal anti-PACAP antibodies (Ab1, Ab2, Ab3, Ab4, Ab5, and Ab6) and a monoclonal anti-PAC1 receptor antibody (PAC1 Ab) for inhibition of PACAP27-induced activation of the human PAC1 receptor. The IC50 values for each of the antibodies are listed to the right of the antibody designation in the legend.

**[0019] Figure 3A** is a bar graph showing the percent inhibition of monoclonal anti-PACAP antibody 1 (Ab1) binding to full-length PACAP38 by each of the peptide fragments consisting of the indicated amino acid residues of human PACAP38 (SEQ ID NO: 1).

**[0020] Figure 3B** is a bar graph showing the percent inhibition of monoclonal anti-PACAP antibody 2 (Ab2) binding to full-length PACAP38 by each of the peptide fragments consisting of the indicated amino acid residues of human PACAP38 (SEQ ID NO: 1).

**[0021] Figure 3C** is a bar graph showing the percent inhibition of monoclonal anti-PACAP antibody 3 (Ab3) binding to full-length PACAP38 by each of the peptide fragments consisting of the indicated amino acid residues of human PACAP38 (SEQ ID NO: 1).

**[0022] Figure 3D** is a bar graph showing the percent inhibition of monoclonal anti-PACAP antibody 4 (Ab4) binding to full-length PACAP38 by each of the peptide fragments consisting of the indicated amino acid residues of human PACAP38 (SEQ ID NO: 1).

**[0023] Figure 3E** is a bar graph showing the percent inhibition of monoclonal anti-PACAP antibody 5 (Ab5) binding to full-length PACAP38 by each of the peptide fragments consisting of the indicated amino acid residues of human PACAP38 (SEQ ID NO: 1).

**[0024] Figure 3F** is a bar graph showing the percent inhibition of monoclonal anti-PACAP antibody 6 (Ab6) binding to full-length PACAP38 by each of the peptide fragments consisting of the indicated amino acid residues of human PACAP38 (SEQ ID NO: 1).

## DETAILED DESCRIPTION

**[0025]** The present invention relates to isolated antigen binding proteins that specifically bind to human pituitary adenylate cyclase-activating polypeptide (PACAP). In humans, PACAP is produced from a 176 amino acid precursor protein (Genbank accession no. NP_001108.2) encoded by the *ADCYAP1* gene. There are two naturally-occurring isoforms of PACAP: a 38-amino acid peptide (PACAP38) and a 27-amino acid peptide (PACAP27), both of which are amidated at their carboxy termini (Vaudry *et al.*, Pharmacol. Rev., Vol. 52: 269-324, 2000). PACAP38 corresponds to amino acids 132-169 of the precursor protein and its sequence is HSDGIFTDSYSRYRKQMAVKKYLAAVLGKRYKQRVKNK (SEQ ID NO: 1). PACAP27 is an amino-terminal fragment of PACAP38 and corresponds to amino acids 132-158 of the precursor protein. The sequence of PACAP27 is HSDGIFTDSYSRYRKQMAVKKYLAAVL (SEQ ID NO: 2). Unless indicated otherwise by the context, the term "PACAP," as used herein, refers to both PACAP38 and PACAP27.

**[0026]** As used herein, the term "antigen binding protein" refers to a protein that specifically binds to one or more target antigens. An antigen binding protein can include an antibody and binding fragments thereof. An "antigen binding fragment," used interchangeably herein with "binding fragment" or "fragment," is a portion of an antibody that lacks at least some of the amino acids present in a full-length heavy chain and/or light chain, but which is still capable of specifically binding to an antigen. An antigen binding fragment includes, but is not limited to, a single-chain variable fragment (scFv), a nanobody (*e.g.* VH domain of camelid heavy chain antibodies; VHH fragment, *see* Cortez-Retamozo *et al.*, Cancer Research, Vol. 64:2853-57, 2004), a Fab fragment, a Fab' fragment, a $F(ab')_2$ fragment, a Fv fragment, a Fd fragment, and a complementarity determining region (CDR) fragment, and can be derived from any mammalian source, such as human, mouse, rat, rabbit, or camelid. Antigen binding fragments may compete for binding of a target antigen with an intact antibody and the fragments may be produced by the modification of intact antibodies (e.g. enzymatic or chemical cleavage) or synthesized *de novo* using recombinant DNA technologies or peptide synthesis.

**[0027]** An antigen binding protein can also include a protein comprising one or more antigen binding fragments incorporated into a single polypeptide chain or into multiple polypeptide chains. For instance, antigen binding proteins can include, but are not limited to, a diabody (*see, e.g.*, EP 404,097; WO 93/11161; and Hollinger *et al.*, Proc. Natl. Acad. Sci. USA, Vol. 90:6444-

6448, 1993); an intrabody; a domain antibody (single VL or VH domain or two or more VH domains joined by a peptide linker; *see Ward et al.*, Nature, Vol. 341:544-546, 1989); a maxibody (2 scFvs fused to Fc region, *see* Fredericks *et al.*, Protein Engineering, Design & Selection, Vol. 17:95-106, 2004 and Powers *et al.*, Journal of Immunological Methods, Vol. 251:123-135, 2001); a triabody; a tetrabody; a minibody (scFv fused to CH3 domain; *see* Olafsen *et al.*, Protein Eng Des Sel. , Vol.17:315-23, 2004); a peptibody (one or more peptides attached to an Fc region, *see* WO 00/24782); a linear antibody (a pair of tandem Fd segments (VH-CH1-VH-CH1) which, together with complementary light chain polypeptides, form a pair of antigen binding regions, *see* Zapata *et al.*, Protein Eng., Vol. 8:1057-1062, 1995); a small modular immunopharmaceutical (*see* U.S. Patent Publication No. 20030133939); and immunoglobulin fusion proteins (e.g. IgG-scFv, IgG-Fab, 2scFv-IgG, 4scFv-IgG, VH-IgG, IgG-VH, and Fab-scFv-Fc; *see, e.g.*, Spiess *et al.*, Mol. Immunol., Vol. 67(2 Pt A):95-106, 2015).

**[0028]** In certain embodiments of the invention, the antigen binding proteins specifically bind to both PACAP38 (SEQ ID NO: 1) and PACAP27 (SEQ ID NO: 2). An antigen binding protein "specifically binds" to a target antigen when it has a significantly higher binding affinity for, and consequently is capable of distinguishing, that antigen compared to its affinity for other unrelated proteins, under similar binding assay conditions. Antigen binding proteins that specifically bind an antigen may have an equilibrium dissociation constant ($K_D$) $\leq 1 \times 10^{-6}$ M. The antigen binding protein specifically binds antigen with "high affinity" when the $K_D$ is $\leq 1 \times 10^{-8}$ M. In one embodiment, the antigen binding proteins of the invention bind to human PACAP with a $K_D$ of $\leq 5 \times 10^{-7}$ M. In another embodiment, the antigen binding proteins of the invention bind to human PACAP with a $K_D$ of $\leq 1 \times 10^{-7}$ M. In yet another embodiment, the antigen binding proteins of the invention bind to human PACAP with a $K_D$ of $\leq 5 \times 10^{-8}$ M. In another embodiment, the antigen binding proteins of the invention bind to human PACAP with a $K_D$ of $\leq 1 \times 10^{-8}$ M. In certain embodiments, the antigen binding proteins of the invention bind to human PACAP with a $K_D$ of $\leq 5 \times 10^{-9}$ M. In other embodiments, the antigen binding proteins of the invention bind to human PACAP with a $K_D$ of $\leq 1 \times 10^{-9}$ M. In one particular embodiment, the antigen binding proteins of the invention bind to human PACAP with a $K_D$ of $\leq 5 \times 10^{-10}$ M. In another particular embodiment, the antigen binding proteins of the invention bind to PACAP with a $K_D$ of $\leq 1 \times 10^{-10}$ M.

**[0029]** Affinity is determined using a variety of techniques, an example of which is an affinity ELISA assay. In various embodiments, affinity is determined by a surface plasmon resonance assay (e.g., BIAcore®-based assay). Using this methodology, the association rate constant ($k_a$ in $M^{-1}s^{-1}$) and the dissociation rate constant ($k_d$ in $s^{-1}$) can be measured. The equilibrium dissociation constant ($K_D$ in M) can then be calculated from the ratio of the kinetic rate constants ($k_d/k_a$). In some embodiments, affinity is determined by a kinetic method, such as a Kinetic Exclusion Assay (KinExA) as described in Rathanaswami *et al*. Analytical Biochemistry, Vol. 373:52-60, 2008. Using a KinExA assay, the equilibrium dissociation constant ($K_D$ in M) and the association rate constant ($k_a$ in $M^{-1}s^{-1}$) can be measured. The dissociation rate constant ($k_d$ in $s^{-1}$) can be calculated from these values ($K_D$ x $k_a$). In other embodiments, affinity is determined by an equilibrium/solution method. In some embodiments, the antigen binding proteins described herein exhibit desirable characteristics such as binding avidity as measured by $k_d$ (dissociation rate constant) for human PACAP of about $10^{-2}$, $10^{-3}$, $10^{-4}$, $10^{-5}$, $10^{-6}$, $10^{-7}$, $10^{-8}$, $10^{-9}$, $10^{-10}$ $s^{-1}$ or lower (lower values indicating higher binding avidity), and/or binding affinity as measured by $K_D$ (equilibrium dissociation constant) for human PACAP of about $10^{-9}$, $10^{-10}$, $10^{-11}$, $10^{-12}$, $10^{-13}$, $10^{-14}$, $10^{-15}$, $10^{-16}$ M or lower (lower values indicating higher binding affinity).

**[0030]** Preferably, the antigen binding proteins do not significantly bind or cross react with other members of the VIP/secretin/glucagon superfamily, such as human VIP or human secretin. As used herein, an antigen binding protein does "not significantly bind" to a target antigen when it has a binding affinity for that antigen that is comparable to its affinity for other unrelated proteins, under similar binding assay conditions. Antigen binding proteins that do not significantly bind to a target antigen may also include those proteins that do not generate a statistically different signal than a negative control in an affinity assay, such as those described herein, for the target antigen. By way of example, an antigen binding protein, which produces a signal value in an ELISA- or a BIAcore®-based assay for determining binding to human PACAP that is not statistically different from the signal value produced with a negative control (e.g. buffer solution without antigen binding protein), would be considered to not significantly bind to human PACAP. Antigen binding proteins that do not significantly bind an antigen may have an equilibrium dissociation constant ($K_D$) for that antigen greater than 1 x $10^{-6}$ M, greater than 1 x $10^{-5}$ M, greater than 1 x $10^{-4}$ M, or greater than 1 x $10^{-3}$ M.

**[0031]** In some embodiments, the antigen binding proteins of the invention do not significantly bind to human VIP (HSDAVFTDNYTRLRKQMAVKKYLNSILN; SEQ ID NO: 3). Thus, in one embodiment, the antigen binding proteins do not significantly bind to a polypeptide consisting of the sequence of SEQ ID NO: 3. In related embodiments, the antigen binding proteins of the invention do not significantly bind to human secretin (HSDGTFTSELSRLREGARLQRLLQGLV; SEQ ID NO: 62). Accordingly, in another embodiment, the antigen binding proteins do not significantly bind to a polypeptide consisting of the sequence of SEQ ID NO: 62.

**[0032]** The antigen binding proteins may, in some embodiments, bind to a particular region or epitope of PACAP. As used herein, an "epitope" refers to any determinant capable of being specifically bound by an antibody or fragment thereof. An epitope can be contiguous or non-contiguous (e.g., (i) in a single-chain polypeptide, amino acid residues that are not contiguous to one another in the polypeptide sequence but that within in context of the molecule are bound by the antibody or functional fragment, or (ii) in a multimeric protein, e.g., comprising two or more individual components, amino acid residues present on two or more of the individual components, but that within the context of the multimeric protein are bound by the antibody or functional fragment). In some embodiments, the antigen binding proteins bind to PACAP at an epitope within an N-terminal domain (e.g. amino acids 1-13 of SEQ ID NO: 1). In related embodiments, the antigen binding proteins specifically bind to a polypeptide consisting of the sequence of SEQ ID NO: 5. As shown in the Examples herein, these N-terminal PACAP binding proteins specifically bind to both PACAP38 and PACAP27 and inhibit activation of the PAC1 receptor by both ligands.

**[0033]** In certain embodiments, the antigen binding proteins bind to PACAP at an epitope within a central region of the PACAP27 and PACAP38 proteins. For instance, in one embodiment, the antigen binding protein binds to PACAP at an epitope within amino acids 6 to 20 of SEQ ID NO: 1. In such an embodiment, the antigen binding protein specifically binds to a polypeptide consisting of the sequence of SEQ ID NO: 122. In another embodiment, the antigen binding protein binds to PACAP at an epitope within amino acids 14 to 27 of SEQ ID NO: 1. In these and other embodiments, the antigen binding protein specifically binds to a polypeptide consisting of the sequence of SEQ ID NO: 117 and a polypeptide consisting of the sequence of SEQ ID NO: 118, but does not significantly bind to a polypeptide consisting of the sequence of SEQ ID

NO: 116. In still another embodiment, the antigen binding protein specifically binds to a polypeptide consisting of the sequence of SEQ ID NO: 117 and a polypeptide consisting of the sequence of SEQ ID NO: 118, but does not significantly bind to a polypeptide consisting of the sequence of SEQ ID NO: 116 and a polypeptide consisting of the sequence of SEQ ID NO: 124.

**[0034]** In other embodiments, the antigen binding proteins bind to a particular region or epitope of PACAP such that PACAP38 is preferentially bound from PACAP27. For instance, in some embodiments, the antigen binding proteins bind to PACAP38 at an epitope within a C-terminal domain (e.g. amino acids 28-38 of SEQ ID NO: 1). In such embodiments, the antigen binding proteins may specifically bind to a polypeptide consisting of the sequence of SEQ ID NO: 4. In one embodiment, the antigen binding proteins bind to an epitope within residues 28 to 37 of PACAP38 (SEQ ID NO: 1). In another embodiment, the antigen binding proteins bind to an epitope within residues 34 to 38 of PACAP38 (SEQ ID NO: 1). In these and other embodiments, the antigen binding proteins specifically bind to PACAP38, but do not significantly bind to PACAP27. Thus, in certain embodiments, these antigen binding proteins do not significantly bind to a polypeptide consisting of the sequence of SEQ ID NO: 2. In related embodiments, the antigen binding proteins specifically bind to a polypeptide consisting of the sequence of SEQ ID NO: 4, but do not significantly bind to a polypeptide consisting of the sequence of SEQ ID NO: 5. In other embodiments, the antigen binding proteins specifically bind to a polypeptide consisting of the sequence of SEQ ID NO: 4 and/or a polypeptide consisting of the sequence of SEQ ID NO: 126. In certain embodiments, the antigen binding proteins bind to a polypeptide consisting of the sequence of SEQ ID NO: 4 with a binding affinity at least 2-fold greater than that for a polypeptide consisting of the sequence of SEQ ID NO: 115 as measured by a surface plasmon resonance assay (e.g., BIAcore®-based assay) as described herein. In other embodiments, the antigen binding proteins bind to a polypeptide consisting of the sequence of SEQ ID NO: 4 with a binding affinity at least 5-fold greater than that for a polypeptide consisting of the sequence of SEQ ID NO: 115 as measured by a surface plasmon resonance assay. In still other embodiments, the antigen binding proteins bind to a polypeptide consisting of the sequence of SEQ ID NO: 4 with a binding affinity at least 10-fold greater than that for a polypeptide consisting of the sequence of SEQ ID NO: 115 as measured by a surface plasmon resonance assay.

**[0035]** As further described in the Examples, these C-terminal PACAP binding proteins specifically bind to PACAP38 and potently inhibit its activation of the PAC1 receptor, but do not significantly bind PACAP27 or inhibit PACAP27's ability to activate the PAC1 receptor. The C-terminal PACAP binding proteins are more potent inhibitors of PACAP38-activation of the PAC1 receptor than the PACAP binding proteins binding to the N-terminus or central region of PACAP. *See* Example 2. This result is unexpected in view of the reports in the literature that the N-terminal domain of PACAP38 is essential for PAC1 receptor activation, whereas the C-terminal domain has no significant impact on the ability of PACAP38 to activate the PAC1 receptor. *See, e.g.*, Bourgault *et al.*, J. Med. Chem., Vol. 52: 3308-3316, 2009.

**[0036]** The antigen binding proteins of the invention may inhibit, interfere with, or modulate one or more biological activities of the human PAC1, VPAC1, and/or VPAC2 receptors. Biological activities of these receptors include, but are not limited to, induction of PACAP-mediated receptor signal transduction pathways, induction of vasodilation, and inhibition of vasoconstriction. In some embodiments, the antigen binding proteins of the invention inhibit binding of PACAP to the human PAC1, VPAC1, and/or VPAC2 receptors. "Inhibition of binding" occurs when an excess of antigen binding proteins reduces the quantity of human PAC1, VPAC1, and/or VPAC2 receptors bound to PACAP, or vice versa, for example, by at least about 40%, about 50%, about 60%, about 70%, about 80%, about 85%, about 90%, about 95%, about 97%, about 99% or more, for example by measuring binding in an *in vitro* competitive binding assay. In one embodiment, the antigen binding proteins inhibit the binding of PACAP38 and/or PACAP27 to the human PAC1 receptor. In another embodiment, the antigen binding proteins inhibit the binding of PACAP38 to the human PAC1 receptor, but do not significantly inhibit the binding of PACAP27 to the human PAC1 receptor.

**[0037]** In other embodiments, the antigen binding proteins of the invention inhibit PACAP-induced activation of the human PAC1, VPAC1, and/or VPAC2 receptors. Various assays for assessing activation of PAC1, VPAC1, and/or VPAC2 receptors are known in the art and include cell-based assays measuring ligand-induced calcium mobilization and cAMP production. An exemplary cell-based cAMP assay is described in Example 2. Other suitable PAC1, VPAC1, and VPAC2 receptor activation assays are described in Dickson *et al.*, Ann. N. Y. Acad. Sci., Vol. 1070:239-42, 2006; Bourgault *et al.*, J. Med. Chem., Vol. 52: 3308-3316, 2009; and U.S.

Patent Publication No. 2011/0229423, all of which are hereby incorporated by reference in their entireties.

**[0038]** The inhibitory activity of the antigen binding proteins on PAC1, VPAC1, and VPAC2 receptor activation can be quantitated by calculating an IC50 in any functional assay for these receptors, such as those described above. An "IC50" is the dose/concentration required to achieve 50% inhibition of a biological or biochemical function. With radioactive ligands, IC50 is the concentration of a competing ligand that displaces 50% of the specific binding of the radioligand. The IC50 of any particular substance or antagonist can be determined by constructing a dose-response curve and examining the effect of different concentrations of the drug or antagonist on reversing agonist activity in a particular functional assay. IC50 values can be calculated for a given antagonist or drug by determining the concentration needed to inhibit half of the maximum biological response of the agonist. Thus, the IC50 value for any PACAP antigen binding protein of the invention can be calculated by determining the concentration of the antigen binding protein needed to inhibit half of the maximum biological response of the PACAP ligand (PACAP-27 or PACAP-38) in activating the human PAC1, VPAC1, or VPAC2 receptor in any functional assay, such as the cAMP assay described in the Examples. A PACAP antigen binding protein that inhibits PACAP-induced activation of a receptor is understood to be a neutralizing antigen binding protein.

**[0039]** In certain embodiments, the antigen binding proteins of the invention inhibit PACAP38- or PACAP27-induced activation of the human PAC1 receptor. For instance, the antigen binding proteins may inhibit PACAP-induced activation of the human PAC1 receptor with an IC50 less than about 50 nM, less than about 40 nM, less than about 30 nM, less than about 25 nM, less than about 20 nM, less than about 15 nM, less than about 10 nM, less than about 5 nM, less than about 3 nM, or less than about 1 nM as measured by a cell-based calcium mobilization assay or cAMP assay. In one particular embodiment, the antigen binding proteins of the invention inhibit PACAP38-induced activation of the human PAC1 receptor with an IC50 less than about 5 nM as measured by a cell-based cAMP assay. In another particular embodiment, the antigen binding proteins of the invention inhibit PACAP38-induced activation of the human PAC1 receptor with an IC50 less than about 1 nM as measured by a cell-based cAMP assay. In still another particular embodiment, the antigen binding proteins of the invention inhibit PACAP38-induced activation of the human PAC1 receptor with an IC50 less than about 500 pM (e.g., less than

about 400 pM, less than about 300 pM, less than about 200 pM, or less than about 100 pM), as measured by a cell-based cAMP assay. In some embodiments, the antigen binding proteins of the invention inhibit PACAP38-induced activation of the human PAC1 receptor with an IC50 between about 0.1 nM and about 1 nM as measured by a cell-based cAMP assay. In other embodiments, the antigen binding proteins of the invention inhibit PACAP38-induced activation of the human PAC1 receptor with an IC50 between about 100 pM and about 500 pM as measured by a cell-based cAMP assay. Thus, in certain embodiments, the PACAP antigen binding proteins of the invention are neutralizing antigen binding proteins of PACAP38.

**[0040]** In some embodiments, the antigen binding proteins inhibit PACAP38-induced activation of the human PAC1, VPAC1, or VPAC2 receptor, but do not significantly inhibit PACAP27-induced activation of these receptors. As used herein, an antigen binding protein would "not significantly inhibit" the activation of a receptor or binding of a ligand to its receptor if there is no statistical difference between ligand-induced receptor activation or ligand binding to the receptor in the presence or absence of the antigen binding protein. For example, if the amount of cAMP production induced by PACAP in cells expressing human PAC1 receptor in the presence of an antigen binding protein is not statistically different than the amount produced in the absence of the antigen binding protein, then the antigen binding protein would be considered to not significantly inhibit PACAP-induced activation of the human PAC1 receptor. Similarly, if the amount of PACAP bound to the human PAC1 receptor in the presence of excess antigen binding protein is not statistically different than the amount of PACAP bound to the receptor in the absence of the antigen binding protein, then the antigen binding protein would be considered to not significantly inhibit the binding of PACAP to the human PAC1 receptor. In certain embodiments, the antigen binding proteins of the invention inhibit PACAP38-induced activation of the human PAC1 receptor, but do not significantly inhibit PACAP27-induced activation of the human PAC1 receptor. Thus, in these embodiments, the antigen binding proteins are neutralizing binding proteins of PACAP38, but not PACAP27. In such embodiments, the antigen binding proteins may bind to PACAP38 at an epitope within the C-terminal domain (e.g., within amino acids 28-38 of SEQ ID NO: 1).

**[0041]** The antigen binding proteins of the invention may comprise one or more complementarity determining regions (CDR) from the light and heavy chain variable regions of antibodies that specifically bind to human PACAP as described herein. The term "CDR" refers

to the complementarity determining region (also termed "minimal recognition units" or "hypervariable region") within antibody variable sequences. There are three heavy chain variable region CDRs (CDRH1, CDRH2 and CDRH3) and three light chain variable region CDRs (CDRL1, CDRL2 and CDRL3). The term "CDR region" as used herein refers to a group of three CDRs that occur in a single variable region (i.e. the three light chain CDRs or the three heavy chain CDRs). The CDRs in each of the two chains typically are aligned by the framework regions (FRs) to form a structure that binds specifically with a specific epitope or domain on the target protein (*e.g.*, human PACAP). From N-terminus to C-terminus, naturally-occurring light and heavy chain variable regions both typically conform with the following order of these elements: FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4. A numbering system has been devised for assigning numbers to amino acids that occupy positions in each of these domains. This numbering system is defined in Kabat Sequences of Proteins of Immunological Interest (1987 and 1991, NIH, Bethesda, MD), or Chothia & Lesk, 1987, *J. Mol. Biol.* 196:901-917; Chothia *et al.*, 1989, Nature 342:878-883. Complementarity determining regions (CDRs) and framework regions (FR) of a given antibody may be identified using this system. In some embodiments, the anti-PACAP antibody or binding fragment thereof comprises at least one heavy chain variable region comprising a CDRH1, CDRH2, and CDRH3 and at least one light chain variable region comprising a CDRL1, CDRL2, and CDRL3. Specific light and heavy chain CDRs are listed in Table 1.

**Table 1. Exemplary Light and Heavy Chain CDR Amino Acid Sequences**

| Antibody No. | Designation | Amino Acid Sequence | SEQ ID NO: |
|---|---|---|---|
| | | ***Light Chain CDRs*** | |
| mAb1; mAb3 | CDRL1-1 | RASEDIESFLA | 30 |
| mAb2 | CDRL1-2 | QASESIDSDLS | 31 |
| mAb4 | CDRL1-3 | QASQSIRNELS | 32 |
| mAb5 | CDRL1-4 | QSSESVYNNNYLS | 33 |
| mAb6 | CDRL1-5 | QASQSVYNNKNLA | 34 |
| mAb1; mAb3 | CDRL2-1 | RTSTLES | 35 |
| mAb2 | CDRL2-2 | RTFTLES | 36 |
| mAb4 | CDRL2-3 | KASTLAS | 37 |

| Antibody No. | Designation | Amino Acid Sequence | SEQ ID NO: |
|---|---|---|---|
| mAb5 | CDRL2-4 | GASTLAS | 38 |
| mAb6 | CDRL2-5 | FSSTLAS | 39 |
| mAb1; mAb3 | CDRL3-1 | QCTDGSSSSSSYGWDA | 40 |
| mAb2 | CDRL3-2 | QCTDGSSISGSYGWDA | 41 |
| mAb4 | CDRL3-3 | QNNYGTRRNNYVFP | 42 |
| mAb5 | CDRL3-4 | LGDYIIIENI | 43 |
| mAb6 | CDRL3-5 | LGEFGSIWA | 44 |
| ***Heavy Chain CDRs*** | | | |
| mAb1 | CDRH1-1 | NDYMC | 45 |
| mAb2 | CDRH1-2 | SSSYMC | 46 |
| mAb3 | CDRH1-3 | SNYYMC | 47 |
| mAb4 | CDRH1-4 | SYAMI | 48 |
| mAb5 | CDRH1-5 | TYNMC | 49 |
| mAb6 | CDRH1-6 | DNYLC | 50 |
| mAb1; mAb3 | CDRH2-1 | CIFTGSSGSTYYASWAKG | 51 |
| mAb2 | CDRH2-2 | CIFTGSSGNTYYATWAQG | 52 |
| mAb4 | CDRH2-3 | FIDAGDGNTYYASWAKG | 53 |
| mAb5 | CDRH2-4 | FINSDDSAYYASWAKG | 54 |
| mAb6 | CDRH2-5 | CIGIVLRSTGATYYASWAEG | 55 |
| mAb1 | CDRH3-1 | DRAISVLGYFYAAYFFDF | 56 |
| mAb2 | CDRH3-2 | DRAYFLVGYFYATYYFDL | 57 |
| mAb3 | CDRH3-3 | DRALSVVGYFYAAYYFDF | 58 |
| mAb4 | CDRH3-4 | GDPGWSNGFAL | 59 |
| mAb5 | CDRH3-5 | YDWDYYYSRLDL | 60 |
| mAb6 | CDRH3-6 | DLGYGGPL | 61 |

**[0042]** The anti-PACAP antigen binding proteins of the invention may comprise one or more of the light chain CDRs (i.e. CDRLs) and/or heavy chain CDRs (i.e. CDRHs) presented in Table 1. For instance, in certain embodiments, the anti-PACAP antigen binding proteins comprise one or more light chain CDRs selected from (i) a CDRL1 selected from SEQ ID NOs: 30 to 34, (ii) a CDRL2 selected from SEQ ID NOs: 35 to 39, and (iii) a CDRL3 selected from SEQ ID NOs: 40

to 44, and (iv) a CDRL of (i), (ii) and (iii) that contains one or more, e.g., one, two, three, four or more amino acid substitutions (e.g., conservative amino acid substitutions), deletions or insertions of no more than five, four, three, two, or one amino acids. In these and other embodiments, the anti-PACAP antigen binding proteins comprise one or more heavy chain CDRs selected from (i) a CDRH1 selected from SEQ ID NOs: 45 to 50, (ii) a CDRH2 selected from SEQ ID NOs: 51 to 55, and (iii) a CDRH3 selected from SEQ ID NOs: 56 to 61, and (iv) a CDRH of (i), (ii) and (iii) that contains one or more, e.g., one, two, three, four or more amino acid substitutions (e.g., conservative amino acid substitutions), deletions or insertions of no more than five, four, three, two, or one amino acids amino acids.

**[0043]** In certain embodiments, the anti-PACAP antigen binding proteins may comprise 1, 2, 3, 4, 5, or 6 variant forms of the CDRs listed in Table 1, each having at least 80%, 85%, 90% or 95% sequence identity to a CDR sequence listed in Table 1. In some embodiments, the anti-PACAP antigen binding proteins include 1, 2, 3, 4, 5, or 6 of the CDRs listed in Table 1, each differing by no more than 1, 2, 3, 4 or 5 amino acids from the CDRs listed in this table.

**[0044]** In particular embodiments, the anti-PACAP antigen binding proteins of the invention comprise a light chain variable region comprising a CDRL1, a CDRL2, and a CDRL3, wherein: (a) CDRL1, CDRL2, and CDRL3 have the sequence of SEQ ID NOs: 30, 35, and 40, respectively; (b) CDRL1, CDRL2, and CDRL3 have the sequence of SEQ ID NOs: 31, 36, and 41, respectively; (c) CDRL1, CDRL2, and CDRL3 have the sequence of SEQ ID NOs: 30, 35, and 40, respectively; (d) CDRL1, CDRL2, and CDRL3 have the sequence of SEQ ID NOs: 32, 37, and 42, respectively; (e) CDRL1, CDRL2, and CDRL3 have the sequence of SEQ ID NOs: 33, 38, and 43, respectively; or (f) CDRL1, CDRL2, and CDRL3 have the sequence of SEQ ID NOs: 34, 39, and 44, respectively.

**[0045]** In other particular embodiments, the anti-PACAP antigen binding proteins of the invention comprise a heavy chain variable region comprising a CDRH1, a CDRH2, and a CDRH3, wherein: (a) CDRH1, CDRH2, and CDRH3 have the sequence of SEQ ID NOs: 45, 51, and 56, respectively; (b) CDRH1, CDRH2, and CDRH3 have the sequence of SEQ ID NOs: 46, 52, and 57, respectively; (c) CDRH1, CDRH2, and CDRH3 have the sequence of SEQ ID NOs: 47, 51, and 58, respectively; (d) CDRH1, CDRH2, and CDRH3 have the sequence of SEQ ID NOs: 48, 53, and 59, respectively; (e) CDRH1, CDRH2, and CDRH3 have the sequence of SEQ

ID NOs: 49, 54, and 60, respectively; or (f) CDRH1, CDRH2, and CDRH3 have the sequence of SEQ ID NOs: 50, 55, and 61, respectively.

**[0046]** In certain embodiments, the anti-PACAP antigen binding proteins of the invention comprise a light chain variable region comprising a CDRL1, a CDRL2, and a CDRL3 and a heavy chain variable region comprising a CDRH1, a CDRH2, and a CDRH3, wherein:

(a) CDRL1, CDRL2, and CDRL3 have the sequence of SEQ ID NOs: 30, 35, and 40, respectively, and CDRH1, CDRH2, and CDRH3 have the sequence of SEQ ID NOs: 45, 51, and 56, respectively;

(b) CDRL1, CDRL2, and CDRL3 have the sequence of SEQ ID NOs: 31, 36, and 41, respectively, and CDRH1, CDRH2, and CDRH3 have the sequence of SEQ ID NOs: 46, 52, and 57, respectively;

(c) CDRL1, CDRL2, and CDRL3 have the sequence of SEQ ID NOs: 30, 35, and 40, respectively, and CDRH1, CDRH2, and CDRH3 have the sequence of SEQ ID NOs: 47, 51, and 58, respectively;

(d) CDRL1, CDRL2, and CDRL3 have the sequence of SEQ ID NOs: 32, 37, and 42, respectively, and CDRH1, CDRH2, and CDRH3 have the sequence of SEQ ID NOs: 48, 53, and 59, respectively;

(e) CDRL1, CDRL2, and CDRL3 have the sequence of SEQ ID NOs: 33, 38, and 43, respectively, and CDRH1, CDRH2, and CDRH3 have the sequence of SEQ ID NOs: 49, 54, and 60, respectively; or

(f) CDRL1, CDRL2, and CDRL3 have the sequence of SEQ ID NOs: 34, 39, and 44, respectively, and CDRH1, CDRH2, and CDRH3 have the sequence of SEQ ID NOs: 50, 55, and 61, respectively.

**[0047]** In some embodiments, the anti-PACAP antigen binding proteins specifically bind to PACAP38 at an epitope in the C-terminal domain (e.g., within amino acids 28-38 of SEQ ID NO: 1). Such C-terminal anti-PACAP antigen binding proteins of the invention may comprise a light chain variable region comprising a CDRL1, a CDRL2, and a CDRL3, wherein CDRL1 has the sequence of SEQ ID NO: 30 or 31, CDRL2 has the sequence of SEQ ID NO: 35 or 36, and CDRL3 has the sequence of SEQ ID NO: 40 or 41. In related embodiments, the C-terminal anti-PACAP antigen binding proteins may comprise a heavy chain variable region comprising a CDRH1, a CDRH2, and a CDRH3, wherein CDRH1 has a sequence selected from SEQ ID NOs:

45 to 47, CDRH2 has the sequence of SEQ ID NO: 51 or 52, and CDRH3 has a sequence selected from SEQ ID NOs: 56 to 58. In one embodiment, a C-terminal anti-PACAP antigen binding protein comprises a light chain variable region comprising a CDRL1, a CDRL2, and a CDRL3 and a heavy chain variable region comprising a CDRH1, a CDRH2, and a CDRH3, wherein:

(a) CDRL1, CDRL2, and CDRL3 have the sequence of SEQ ID NOs: 30, 35, and 40, respectively, and CDRH1, CDRH2, and CDRH3 have the sequence of SEQ ID NOs: 45, 51, and 56, respectively;

(b) CDRL1, CDRL2, and CDRL3 have the sequence of SEQ ID NOs: 31, 36, and 41, respectively, and CDRH1, CDRH2, and CDRH3 have the sequence of SEQ ID NOs: 46, 52, and 57, respectively; or

(c) CDRL1, CDRL2, and CDRL3 have the sequence of SEQ ID NOs: 30, 35, and 40, respectively, and CDRH1, CDRH2, and CDRH3 have the sequence of SEQ ID NOs: 47, 51, and 58, respectively.

**[0048]** In some embodiments, the anti-PACAP antigen binding proteins specifically bind to PACAP38 at an epitope in the N-terminal domain (e.g., within amino acids 1-13 of SEQ ID NO: 1). Such N-terminal anti-PACAP antigen binding proteins of the invention may comprise a light chain variable region comprising a CDRL1, a CDRL2, and a CDRL3, wherein CDRL1 has the sequence of SEQ ID NOs: 32 to 34, CDRL2 has the sequence of SEQ ID NOs: 37 to 39, and CDRL3 has the sequence of SEQ ID NOs: 42 to 44. In related embodiments, the N-terminal anti-PACAP antigen binding proteins may comprise a heavy chain variable region comprising a CDRH1, a CDRH2, and a CDRH3, wherein CDRH1 has a sequence selected from SEQ ID NOs: 48 to 50, CDRH2 has the sequence of SEQ ID NOs: 53 to 55, and CDRH3 has a sequence selected from SEQ ID NOs: 59 to 61. In one embodiment, an N-terminal anti-PACAP antigen binding protein comprises a light chain variable region comprising a CDRL1, a CDRL2, and a CDRL3 and a heavy chain variable region comprising a CDRH1, a CDRH2, and a CDRH3, wherein:

(a) CDRL1, CDRL2, and CDRL3 have the sequence of SEQ ID NOs: 32, 37, and 42, respectively, and CDRH1, CDRH2, and CDRH3 have the sequence of SEQ ID NOs: 48, 53, and 59, respectively;

(b) CDRL1, CDRL2, and CDRL3 have the sequence of SEQ ID NOs: 33, 38, and 43, respectively, and CDRH1, CDRH2, and CDRH3 have the sequence of SEQ ID NOs: 49, 54, and 60, respectively; or

(c) CDRL1, CDRL2, and CDRL3 have the sequence of SEQ ID NOs: 34, 39, and 44, respectively, and CDRH1, CDRH2, and CDRH3 have the sequence of SEQ ID NOs: 50, 55, and 61, respectively.

**[0049]** In particular embodiments, the antigen binding proteins of the invention comprise an immunoglobulin heavy chain variable region (VH) and an immunoglobulin light chain variable region (VL) from an antibody that specifically binds to human PACAP, such as the antibodies described herein. The "variable region," used interchangeably herein with "variable domain" (variable region of a light chain (VL), variable region of a heavy chain (VH)), refers to the region in each of the light and heavy immunoglobulin chains which is involved directly in binding the antibody to the antigen. As discussed above, the regions of variable light and heavy chains have the same general structure and each region comprises four framework (FR) regions, the sequences of which are widely conserved, connected by three CDRs. The framework regions adopt a beta-sheet conformation and the CDRs may form loops connecting the beta-sheet structure. The CDRs in each chain are held in their three-dimensional structure by the framework regions and form, together with the CDRs from the other chain, the antigen binding site.

**[0050]** Thus, in some embodiments, the anti-PACAP antigen binding proteins of the invention may comprise a light chain variable region selected from LV-01, LV-02, LV-03, LV-04, LV-05, or LV-06, and/or a heavy chain variable region selected from HV-01, HV-02, HV-03, HV-04, HV-05, or HV-06, as shown in Table 2 below, and binding fragments, derivatives, muteins and variants of these light chain and heavy chain variable regions.

**Table 2. Exemplary Light and Heavy Chain Variable Region Amino Acid Sequences**

| Antibody No. | Designation | Amino Acid Sequence | SEQ ID NO: |
|---|---|---|---|
| *Light Chain Variable Regions* | | | |

| Antibody No. | Designation | Amino Acid Sequence | SEQ ID NO: |
|---|---|---|---|
| mAb1 | LV-01 | ADVVMTQTPSPVSAAVGGTVTINCRASEDIESFLA WYQQKPGQPPKLLISRTSTLESGVSSRFKGSGSGTE FILTISDLECADAATYYCQCTDGSSSSSSYGWDAF GGGTEVVVKG | 18 |
| mAb2 | LV-02 | ADVVMTQTPSPVSAQVGGTVTINCQASESIDSDLS WYQQKPGQPPKLLIYRTFTLESGVPSRFKGSGSGT DYTLTISDLECADAAIYYCQCTDGSSISGSYGWDA FGGGTEVVVKG | 20 |
| mAb3 | LV-03 | ADVVMTQTPSPVSAAVGGTVTINCRASEDIESFLA WYQQKPGQPPKLLMSRTSTLESGVPSRFKGSGSGT EFTLTISDLECADAATYYCQCTDGSSSSSSYGWDA FGGGTEVVVKG | 22 |
| mAb4 | LV-04 | ADIVMTQTPASVSGAVGGTVTIKCQASQSIRNELS WYQQKPGQPPKLLIYKASTLASGVPSRFSGSGFGT EFTLTISGVQCDDAATYYCQNNYGTRRNNYVFPF GGGTEVVVKG | 24 |
| mAb5 | LV-05 | ADIVLTQTPASVSAAVGGTVSISCQSSESVYNNNY LSWFQQKPGQPPKLLIYGASTLASGVPSRFEGSGS GTQFTLTISDVQCDDAATYYCLGDYIIIENIFGGGT EVVVKG | 26 |
| mAb6 | LV-06 | AQVLTQTPASVSAAVGGTVTINCQASQSVYNNKN LAWYQQKPGQPPKLLIYFSSTLASGVPSRFRGSGS GTQFTLTISGVQCGDAATYYCLGEFGSIWAFGGGT EVVVKG | 28 |
| | | ***Heavy Chain Variable Regions*** | |
| mAb1 | HV-01 | QSLEESGGDLVKPGASLTLTCKASGIAFSNDYMC WVRQAPGKGLEWIACIFTGSSGSTYYASWAKGRF TISKTSSTTVTLQMTSLTAADTATYFCARDRAISVL GYFYAAYFFDFWGPGTLVTVSS | 19 |
| mAb2 | HV-02 | QSLEESGGGLVQPEGSLTLTCTASGFSFNSSSYMC WVRQAPGKGLEWIGCIFTGSSGNTYYATWAQGRF TISKTSTTVTLEMTSLTAADTATYFCARDRAYFLV GYFYATYYFDLWGPGTLVTVSS | 21 |
| mAb3 | HV-03 | QSLEESGGGLVQPGASLTLTCTASGFSFSSNYYMC WVRQAPGKGLEWIACIFTGSSGSTYYASWAKGRF TISKTSSTTVTLHVTSLTAADTATYFCARDRALSV VGYFYAAYYFDFWGPGTLVTVSS | 23 |

| Antibody No. | Designation | Amino Acid Sequence | SEQ ID NO: |
|---|---|---|---|
| mAb4 | HV-04 | QSVEESGGRLVTPGTPLTLTCTASGIDLSSYAMIW VRQAPGEGLEYIGFIDAGDGNTYYASWAKGRFTIS KTSTTVDLKITSPTTEDTATYFCARGDPGWSNGFA LWGQGTLVTVSS | 25 |
| mAb5 | HV-05 | QSVEESGGRLVTPGTPLTLTCTVSGFSLSTYNMCW VRQAPGKGLEWVGFINSDDSAYYASWAKGRFTFS KTSTTVDLKIASPTTEDTATYFCARYDWDYYYSRL DLWGPGTLVTVSS | 27 |
| mAb6 | HV-06 | QLEESGGGLVQPGASLTLTCTTSGFSLSDNYLCWV RQAPGRGLEWVACIGIVLRSTGATYYASWAEGRF TISKTSPTTVTLEMTSLTAADTATYFCALDLGYGG PLWGPGTLVTVSS | 29 |

**[0051]** Each of the light chain variable regions listed in Table 2 may be combined with any of the heavy chain variable regions shown in Table 2 to form an anti-PACAP binding domain of the antigen binding proteins of the invention. Examples of such combinations include, but are not limited to: LV-01 and HV-01; LV-02 and HV-02; LV-03 and HV-03; LV-04 and HV-04; LV-05 and HV-05; LV-06 and HV-06; LV-01 and HV-02; and LV-02 and HV-01. In certain embodiments, the anti-PACAP antigen binding proteins of the invention comprise: (a) LV-01 (SEQ ID NO: 18) and HV-01 (SEQ ID NO: 19); (b) LV-02 (SEQ ID NO: 20) and HV-02 (SEQ ID NO: 21); (c) LV-03 (SEQ ID NO: 22) and HV-03 (SEQ ID NO: 23); (d) LV-04 (SEQ ID NO: 24) and HV-04 (SEQ ID NO: 25); (e) LV-05 (SEQ ID NO: 26) and HV-05 (SEQ ID NO: 27); or (f) LV-06 (SEQ ID NO: 28) and HV-06 (SEQ ID NO: 29). In some embodiments, the anti-PACAP antigen binding proteins of the invention comprise: (a) LV-01 (SEQ ID NO: 18) and HV-01 (SEQ ID NO: 19); (b) LV-02 (SEQ ID NO: 20) and HV-02 (SEQ ID NO: 21); or (c) LV-03 (SEQ ID NO: 22) and HV-03 (SEQ ID NO: 23). In such embodiments, the anti-PACAP antigen binding proteins bind to an epitope within a C-terminal domain of PACAP38, such as an epitope within amino acids 28 to 38 of SEQ ID NO: 1.

**[0052]** In some embodiments, the anti-PACAP antigen binding proteins comprise a light chain variable region comprising a sequence of contiguous amino acids that differs from the sequence of a light chain variable region in Table 2, i.e. a VL selected from LV-01, LV-02, LV-03, LV-04, LV-05, or LV-06, at only 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 amino acid residues,

wherein each such sequence difference is independently either a deletion, insertion or substitution of one amino acid, with the deletions, insertions and/or substitutions resulting in no more than 15 amino acid changes relative to the foregoing variable domain sequences. The light chain variable region in some anti-PACAP antigen binding proteins comprises a sequence of amino acids that has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97% or at least 99% sequence identity to the amino acid sequences of SEQ ID NOs: 18, 20, 22, 24, 26, or 28 (i.e. the light chain variable regions in Table 2).

**[0053]** In these and other embodiments, the anti-PACAP antigen binding proteins comprise a heavy chain variable region comprising a sequence of contiguous amino acids that differs from the sequence of a heavy chain variable region in Table 2, i.e., a VH selected from HV-01, HV-02, HV-03, HV-04, HV-05, or HV-06, at only 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 amino acid residues, wherein each such sequence difference is independently either a deletion, insertion or substitution of one amino acid, with the deletions, insertions and/or substitutions resulting in no more than 15 amino acid changes relative to the foregoing variable domain sequences. The heavy chain variable region in some anti-PACAP antigen binding proteins comprises a sequence of amino acids that has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97% or at least 99% sequence identity to the amino acid sequences of SEQ ID NOs: 19, 21, 23, 25, 27, or 29 (i.e. the heavy chain variable regions in Table 2).

**[0054]** The term "identity," as used herein, refers to a relationship between the sequences of two or more polypeptide molecules or two or more nucleic acid molecules, as determined by aligning and comparing the sequences. "Percent identity," as used herein, means the percent of identical residues between the amino acids or nucleotides in the compared molecules and is calculated based on the size of the smallest of the molecules being compared. For these calculations, gaps in alignments (if any) must be addressed by a particular mathematical model or computer program (i.e., an "algorithm"). Methods that can be used to calculate the identity of the aligned nucleic acids or polypeptides include those described in Computational Molecular Biology, (Lesk, A. M., ed.), 1988, New York: Oxford University Press; Biocomputing Informatics and Genome Projects, (Smith, D. W., ed.), 1993, New York: Academic Press; Computer Analysis of Sequence Data, Part I, (Griffin, A. M., and Griffin, H. G., eds.), 1994, New Jersey: Humana Press; von Heinje, G., 1987, Sequence Analysis in Molecular Biology, New York: Academic

Press; Sequence Analysis Primer, (Gribskov, M. and Devereux, J., eds.), 1991, New York: M. Stockton Press; and Carillo et al., 1988, SIAM J. Applied Math. 48:1073. For example, sequence identity can be determined by standard methods that are commonly used to compare the similarity in position of the amino acids of two polypeptides. Using a computer program such as BLAST or FASTA, two polypeptide or two polynucleotide sequences are aligned for optimal matching of their respective residues (either along the full length of one or both sequences, or along a pre-determined portion of one or both sequences). The programs provide a default opening penalty and a default gap penalty, and a scoring matrix such as PAM 250 (a standard scoring matrix; see Dayhoff et al., in Atlas of Protein Sequence and Structure, vol. 5, supp. 3 (1978)) can be used in conjunction with the computer program. For example, the percent identity can then be calculated as: the total number of identical matches multiplied by 100 and then divided by the sum of the length of the longer sequence within the matched span and the number of gaps introduced into the longer sequences in order to align the two sequences. In calculating percent identity, the sequences being compared are aligned in a way that gives the largest match between the sequences.

**[0055]** The GCG program package is a computer program that can be used to determine percent identity, which package includes GAP (Devereux et al., 1984, Nucl. Acid Res. 12:387; Genetics Computer Group, University of Wisconsin, Madison, WI). The computer algorithm GAP is used to align the two polypeptides or two polynucleotides for which the percent sequence identity is to be determined. The sequences are aligned for optimal matching of their respective amino acid or nucleotide (the "matched span", as determined by the algorithm). A gap opening penalty (which is calculated as 3x the average diagonal, wherein the "average diagonal" is the average of the diagonal of the comparison matrix being used; the "diagonal" is the score or number assigned to each perfect amino acid match by the particular comparison matrix) and a gap extension penalty (which is usually 1/10 times the gap opening penalty), as well as a comparison matrix such as PAM 250 or BLOSUM 62 are used in conjunction with the algorithm. In certain embodiments, a standard comparison matrix (see, Dayhoff et al., 1978, Atlas of Protein Sequence and Structure 5:345-352 for the PAM 250 comparison matrix; Henikoff et al., 1992, Proc. Natl. Acad. Sci. U.S.A. 89:10915-10919 for the BLOSUM 62 comparison matrix) is also used by the algorithm.

**[0056]** Recommended parameters for determining percent identity for polypeptides or nucleotide sequences using the GAP program include the following:

Algorithm: Needleman et al., 1970, J. Mol. Biol. 48:443-453;

Comparison matrix: BLOSUM 62 from Henikoff et al., 1992, *supra*;

Gap Penalty: 12 (but with no penalty for end gaps)

Gap Length Penalty: 4

Threshold of Similarity: 0

**[0057]** Certain alignment schemes for aligning two amino acid sequences may result in matching of only a short region of the two sequences, and this small aligned region may have very high sequence identity even though there is no significant relationship between the two full-length sequences. Accordingly, the selected alignment method (GAP program) can be adjusted if so desired to result in an alignment that spans at least 50 contiguous amino acids of the target polypeptide.

**[0058]** In certain embodiments, the antigen binding proteins of the invention are antibodies or binding fragments thereof. As used herein, the term "antibody" refers to a tetrameric immunoglobulin protein comprising two light chain polypeptides (about 25 kDa each) and two heavy chain polypeptides (about 50-70 kDa each). The term "light chain" or "immunoglobulin light chain" refers to a polypeptide comprising, from amino terminus to carboxyl terminus, a single immunoglobulin light chain variable region (VL) and a single immunoglobulin light chain constant domain (CL). The immunoglobulin light chain constant domain (CL) can be a human kappa (κ) or human lambda (λ) constant domain. The term "heavy chain" or "immunoglobulin heavy chain" refers to a polypeptide comprising, from amino terminus to carboxyl terminus, a single immunoglobulin heavy chain variable region (VH), an immunoglobulin heavy chain constant domain 1 (CH1), an immunoglobulin hinge region, an immunoglobulin heavy chain constant domain 2 (CH2), an immunoglobulin heavy chain constant domain 3 (CH3), and optionally an immunoglobulin heavy chain constant domain 4 (CH4). Heavy chains are classified as mu (μ), delta (Δ), gamma (γ), alpha (α), and epsilon (ε), and define the antibody's isotype as IgM, IgD, IgG, IgA, and IgE, respectively. The IgG-class and IgA-class antibodies are further divided into subclasses, namely, IgG1, IgG2, IgG3, and IgG4, and IgA1 and IgA2, respectively. The heavy chains in IgG, IgA, and IgD antibodies have three domains (CH1, CH2, and CH3), whereas the heavy chains in IgM and IgE antibodies have four domains (CH1, CH2, CH3, and CH4). The immunoglobulin heavy chain constant domains can be from any immunoglobulin isotype, including subtypes. The antibody chains are linked together via inter-

polypeptide disulfide bonds between the CL domain and the CH1 domain (i.e. between the light and heavy chain) and between the hinge regions of the antibody heavy chains.

**[0059]** The anti-PACAP antibodies of the invention can comprise any immunoglobulin constant region. The term "constant region" as used herein refers to all domains of an antibody other than the variable region. The constant region is not involved directly in binding of an antigen, but exhibits various effector functions. As described above, antibodies are divided into particular isotypes (IgA, IgD, IgE, IgG, and IgM) and subtypes (IgG1, IgG2, IgG3, IgG4, IgA1 IgA2) depending on the amino acid sequence of the constant region of their heavy chains. The light chain constant region can be, for example, a kappa- or lambda-type light chain constant region, e.g., a human kappa- or lambda-type light chain constant region, which are found in all five antibody isotypes. Examples of human immunoglobulin light chain constant region sequences are shown in the following table.

**Table 3. Exemplary Human Immunoglobulin Light Chain Constant Regions**

| Designation | SEQ ID NO: | CL Domain Amino Acid Sequence |
|---|---|---|
| CL-1 | 63 | GQPKANPTVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKA DGSPVKAGVETTKPSKQSNNKYAASSYLSLTPEQWKSHRSYSC QVTHEGSTVEKTVAPTECS |
| CL-2 | 64 | GQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKA DSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQWKSHRSYSCQ VTHEGSTVEKTVAPTECS |
| CL-3 | 65 | GQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKA DSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQWKSHKSYSCQ VTHEGSTVEKTVAPTECS |
| CL-7 | 66 | GQPKAAPSVTLFPPSSEELQANKATLVCLVSDFYPGAVTVAWK ADGSPVKVGVETTKPSKQSNNKYAASSYLSLTPEQWKSHRSYS CRVTHEGSTVEKTVAPAECS |

**[0060]** The heavy chain constant region of the anti-PACAP antibodies of the invention can be, for example, an alpha-, delta-, epsilon-, gamma-, or mu-type heavy chain constant region, e.g., a human alpha-, delta-, epsilon-, gamma-, or mu-type heavy chain constant region. In some embodiments, the anti-PACAP antibodies comprise a heavy chain constant region from an IgG1, IgG2, IgG3, or IgG4 immunoglobulin. In one embodiment, the anti-PACAP antibody comprises a heavy chain constant region from a human IgG1 immunoglobulin. In another embodiment, the

anti-PACAP antibody comprises a heavy chain constant region from a human IgG2 immunoglobulin. Examples of human IgG1 and IgG2 heavy chain constant region sequences are shown below in Table 4.

**Table 4. Exemplary Human Immunoglobulin Heavy Chain Constant Regions**

| Ig isotype | SEQ ID NO: | Heavy Chain Constant Region Amino Acid Sequence |
|---|---|---|
| Human IgG1z | 67 | ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT SGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKV DKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVV SVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY TLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP VLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL SLSPGK |
| Human IgG1za | 68 | ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT SGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKV DKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVV SVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY TLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP VLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL SLSPGK |
| Human IgG1f | 69 | ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT SGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKV DKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVV SVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY TLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP VLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL SLSPGK |
| Human IgG1fa | 70 | ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT SGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKV DKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVV SVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY TLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP VLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL SLSPGK |
| Human IgG2 | 71 | ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALT SGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKV DKTVERKCCVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLT |

| Ig isotype | SEQ ID NO: | Heavy Chain Constant Region Amino Acid Sequence |
|---|---|---|
| | | VVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPP SREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLD SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP GK |

**[0061]** Each of the variable regions disclosed in Table 2 may be attached to the above light and heavy chain constant regions to form complete antibody light and heavy chains, respectively. Further, each of the so generated heavy and light chain sequences may be combined to form a complete antibody structure. It should be understood that the heavy chain and light chain variable regions provided herein can also be attached to other constant domains having different sequences than the exemplary sequences listed above.

**[0062]** Specific examples of full-length light and heavy chains of exemplary anti-PACAP antibodies of the invention and their corresponding amino acid sequences are summarized in Table 5.

**Table 5. Exemplary Antibody Light and Heavy Chain Amino Acid Sequences**

| Antibody No. | Designation | Amino Acid Sequence | SEQ ID NO: |
|---|---|---|---|
| | | ***Light Chains*** | |
| mAb1 | LC-01 | ADVVMTQTPSPVSAAVGGTVTINCRASEDIESFLA WYQQKPGQPPKLLISRTSTLESGVSSRFKGSGSGTE FILTISDLECADAATYYCQCTDGSSSSSSYGWDAF GGGTEVVVKGDPVAPTVLLFPPSSDEVATGTVTIV CVANKYFPDVTVTWEVDGTTQTTGIENSKTPQNS ADCTYNLSSTLTLTSTQYNSHKEYTCKVTQGTTSV VQSFSRKNC | 6 |
| mAb2 | LC-02 | ADVVMTQTPSPVSAQVGGTVTINCQASESIDSDLS WYQQKPGQPPKLLIYRTFTLESGVPSRFKGSGSGT DYTLTISDLECADAAIYYCQCTDGSSISGSYGWDA FGGGTEVVVKGDPVAPTVLLFPPSSDEVATGTVTI VCVANKYFPDVTVTWEVDGTTQTTGIENSKTPQN SADCTYNLSSTLTLTSTQYNSHKEYTCKVTQGTTS VVQSFSRKNC | 8 |

| Antibody No. | Designation | Amino Acid Sequence | SEQ ID NO: |
|---|---|---|---|
| mAb3 | LC-03 | ADVVMTQTPSPVSAAVGGTVTINCRASEDIESFLA WYQQKPGQPPKLLMSRTSTLESGVPSRFKGSGSGT EFTLTISDLECADAATYYCQCTDGSSSSSSYGWDA FGGGTEVVVKGDPVAPTVLLFPPSSDEVATGTVTI VCVANKYFPDVTVTWEVDGTTQTTGIENSKTPQN SADCTYNLSSTLTLTSTQYNSHKEYTCKVTQGTTS VVQSFSRKNC | 10 |
| mAb4 | LC-04 | ADIVMTQTPASVSGAVGGTVTIKCQASQSIRNELS WYQQKPGQPPKLLIYKASTLASGVPSRFSGSGFGT EFTLTISGVQCDDAATYYCQNNYGTRRNNYVFPF GGGTEVVVKGDPVAPTVLLFPPSSDEVATGTVTIV CVANKYFPDVTVTWEVDGTTQTTGIENSKTPQNS ADCTYNLSSTLTLTSTQYNSHKEYTCKVTQGTTSV VQSFSRKNC | 12 |
| mAb5 | LC-05 | ADIVLTQTPASVSAAVGGTVSISCQSSESVYNNNY LSWFQQKPGQPPKLLIYGASTLASGVPSRFEGSGS GTQFTLTISDVQCDDAATYYCLGDYIIIENIFGGGT EVVVKGDPVAPTVLLFPPSSDEVATGTVTIVCVAN KYFPDVTVTWEVDGTTQTTGIENSKTPQNSADCT YNLSSTLTLTSTQYNSHKEYTCKVTQGTTSVVQSF SRKNC | 14 |
| mAb6 | LC-06 | AQVLTQTPASVSAAVGGTVTINCQASQSVYNNKN LAWYQQKPGQPPKLLIYFSSTLASGVPSRFRGSGS GTQFTLTISGVQCGDAATYYCLGEFGSIWAFGGGT EVVVKGDPVAPTVLLFPPSSDEVATGTVTIVCVAN KYFPDVTVTWEVDGTTQTTGIENSKTPQNSADCT YNLSSTLTLTSTQYNSHKEYTCKVTQGTTSVVQSF SRKNC | 16 |
| ***Heavy Chains*** | | | |

| Antibody No. | Designation | Amino Acid Sequence | SEQ ID NO: |
|---|---|---|---|
| mAb1 | HC-01 | QSLEESGGDLVKPGASLTLTCKASGIAFSNDYMC<br>WVRQAPGKGLEWIACIFTGSSGSTYYASWAKGRF<br>TISKTSSTTVTLQMTSLTAADTATYFCARDRAISVL<br>GYFYAAYFFDFWGPGTLVTVSSGQPKAPSVFPLAP<br>CCGDTPSSTVTLGCLVKGYLPEPVTVTWNSGTLTN<br>GVRTFPSVRQSSGLYSLSSVVSVTSSSQPVTCNVA<br>HPATNTKVDKTVAPSTCSKPTCPPPELLGGP<br>SVFIFPPKPKDTLMISRTPEVTCVVVDVSQDDPEVQ<br>FTWYINNEQVRTARPPLREQQFNSTIRVVSTLPIAH<br>QDWLRGKEFKCKVHNKALPAPIEKTISKARGQPLE<br>PKVYTMGPPREELSSRSVSLTCMINGFYPSDISVEW<br>EKNGKAEDNYKTTPAVLDSDGSYFLYSKLSVPTSE<br>WQRGDVFTCSVMHEALHNHYTQKSISRSPGK | 7 |
| mAb2 | HC-02 | QSLEESGGGLVQPEGSLTLTCTASGFSFNSSSYMC<br>WVRQAPGKGLEWIGCIFTGSSGNTYYATWAQGRF<br>TISKTSTTVTLEMTSLTAADTATYFCARDRAYFLV<br>GYFYATYYFDLWGPGTLVTVSSGQPKAPSVFPLAP<br>CCGDTPSSTVTLGCLVKGYLPEPVTVTWNSGTLTN<br>GVRTFPSVRQSSGLYSLSSVVSVTSSSQPVTCNVA<br>HPATNTKVDKTVAPSTCSKPTCPPPELLGGPSVFIF<br>PPKPKDTLMISRTPEVTCVVVDVSQDDPEVQFTW<br>YINNEQVRTARPPLREQQFNSTIRVVSTLPIAHQD<br>WLRGKEFKCKVHNKALPAPIEKTISKARGQPLEPK<br>VYTMGPPREELSSRSVSLTCMINGFYPSDISVEWE<br>KNGKAEDNYKTTPAVLDSDGSYFLYSKLSVPTSE<br>WQRGDVFTCSVMHEALHNHYTQKSISRSPGK | 9 |
| mAb3 | HC-03 | QSLEESGGGLVQPGASLTLTCTASGFSFSSNYYMC<br>WVRQAPGKGLEWIACIFTGSSGSTYYASWAKGRF<br>TISKTSSTTVTLHVTSLTAADTATYFCARDRALSV<br>VGYFYAAYYFDFWGPGTLVTVSSGQPKAPSVFPL<br>APCCGDTPSSTVTLGCLVKGYLPEPVTVTWNSGTL<br>TNGVRTFPSVRQSSGLYSLSSVVSVTSSSQPVTCNV<br>AHPATNTKVDKTVAPSTCSKPTCPPPELLGG<br>PSVFIFPPKPKDTLMISRTPEVTCVVVDVSQDDPEV<br>QFTWYINNEQVRTARPPLREQQFNSTIRVVSTLPIA<br>HQDWLRGKEFKCKVHNKALPAPIEKTISKARGQP<br>LEPKVYTMGPPREELSSRSVSLTCMINGFYPSDISV<br>EWEKNGKAEDNYKTTPAVLDSDGSYFLYSKLSVP<br>TSEWQRGDVFTCSVMHEALHNHYTQKSISRSPGK | 11 |

| Antibody No. | Designation | Amino Acid Sequence | SEQ ID NO: |
|---|---|---|---|
| mAb4 | HC-04 | QSVEESGGRLVTPGTPLTLTCTASGIDLSSYAMIW VRQAPGEGLEYIGFIDAGDGNTYYASWAKGRFTIS KTSTTVDLKITSPTTEDTATYFCARGDPGWSNGFA LWGQGTLVTVSSGQPKAPSVFPLAPCCGDTPSSTV TLGCLVKGYLPEPVTVTWNSGTLTNGVRTFPSVR QSSGLYSLSSVVSVTSSSQPVTCNVAHPATNTKVD KTVAPSTCSKPTCPPPELLGGPSVFIFPPKPKDTLMI SRTPEVTCVVVDVSQDDPEVQFTWYINNEQVRTA RPPLREQQFNSTIRVVSTLPIAHQDWLRGKEFKCK VHNKALPAPIEKTISKARGQPLEPKVYTMGPPREE LSSRSVSLTCMINGFYPSDISVEWEKNGKAEDNYK TTPAVLDSDGSYFLYSKLSVPTSEWQRGDVFTCSV MHEALHNHYTQKSISRSPGK | 13 |
| mAb5 | HC-05 | QSVEESGGRLVTPGTPLTLTCTVSGFSLSTYNMCW VRQAPGKGLEWVGFINSDDSAYYASWAKGRFTFS KTSTTVDLKIASPTTEDTATYFCARYDWDYYYSRL DLWGPGTLVTVSSGQPKAPSVFPLAPCCGDTPSST VTLGCLVKGYLPEPVTVTWNSGTLTNGVRTFPSV RQSSGLYSLSSVVSVTSSSQPVTCNVAHPATNTKV DKTVAPSTCSKPTCPPPELLGGPSVFIFPPKPKDTL MISRTPEVTCVVVDVSQDDPEVQFTWYINNEQVR TARPPLREQQFNSTIRVVSTLPIAHQDWLRGKEFK CKVHNKALPAPIEKTISKARGQPLEPKVYTMGPPR EELSSRSVSLTCMINGFYPSDISVEWEKNGKAEDN YKTTPAVLDSDGSYFLYSKLSVPTSEWQRGDVFTC SVMHEALHNHYTQKSISRSPGK | 15 |
| mAb6 | HC-06 | QLEESGGGLVQPGASLTLTCTTSGFSLSDNYLCWV RQAPGRGLEWVACIGIVLRSTGATYYASWAEGRF TISKTSPTTVTLEMTSLTAADTATYFCALDLGYGG PLWGPGTLVTVSSGQPKAPSVFPLAPCCGDTPSST VTLGCLVKGYLPEPVTVTWNSGTLTNGVRTFPSV RQSSGLYSLSSVVSVTSSSQPVTCNVAHPATNTKV DKTVAPSTCSKPTCPPPELLGGPSVFIFPPKPKDTL MISRTPEVTCVVVDVSQDDPEVQFTWYINNEQVR TARPPLREQQFNSTIRVVSTLPIAHQDWLRGKEFK CKVHNKALPAPIEKTISKARGQPLEPKVYTMGPPR EELSSRSVSLTCMINGFYPSDISVEWEKNGKAEDN YKTTPAVLDSDGSYFLYSKLSVPTSEWQRGDVFTC SVMHEALHNHYTQKSISRSPGK | 17 |

**[0063]** Each of the exemplary light chains (LC-01, LC-02, LC-03 etc.) listed in Table 5 can be combined with any of the exemplary heavy chains in Table 5 to form an anti-PACAP antibody. Examples of such combinations include LC-01 combined with any of HC-01 through HC-06; LC-02 combined with any HC-01 through HC-06; LC-03 combined with any of HC-01 through HC-06, and so on. In some embodiments, the anti-PACAP antibodies include at least one heavy chain and one light chain from those listed in Table 5. In some embodiments, the anti-PACAP antibodies comprise two different heavy chains and two different light chains listed in Table 5. In other embodiments, the anti-PACAP antibodies contain two identical light chains and two identical heavy chains. As an example, an anti-PACAP antibody may include two HC-01 heavy chains and two LC-01 light chains, or two HC-02 heavy chains and two LC-02 light chains, or two HC-03 heavy chains and two LC-03 light chains and other similar combinations of pairs of light chains and pairs of heavy chains as listed in Table 5. In one embodiment, an anti-PACAP antibody of the invention comprises a light chain comprising a sequence selected from SEQ ID NOs: 6, 8, 10, 12, 14, or 16, and a heavy chain comprising a sequence selected from SEQ ID NOs: 7, 9, 11, 13, 15, or 17.

**[0064]** Variants of the anti-PACAP antibodies disclosed herein are also contemplated. For instance, variants of the antibodies can be formed by combination of heavy and light chains that each have at least 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% sequence identity to the amino acid sequences of the heavy and light chains listed in Table 5. In some instances, such antibodies include at least one heavy chain and one light chain, whereas in other instances the variant forms contain two identical light chains and two identical heavy chains.

**[0065]** In some embodiments, the anti-PACAP antibody comprises:

(a) a light chain comprising the sequence of SEQ ID NO: 6 and a heavy chain comprising the sequence of SEQ ID NO: 7;

(b) a light chain comprising the sequence of SEQ ID NO: 8 and a heavy chain comprising the sequence of SEQ ID NO: 9;

(c) a light chain comprising the sequence of SEQ ID NO: 10 and a heavy chain comprising the sequence of SEQ ID NO: 11;

(d) a light chain comprising the sequence of SEQ ID NO: 12 and a heavy chain comprising the sequence of SEQ ID NO: 13;

(e) a light chain comprising the sequence of SEQ ID NO: 14 and a heavy chain comprising the sequence of SEQ ID NO: 15; or

(f) a light chain comprising the sequence of SEQ ID NO: 16 and a heavy chain comprising the sequence of SEQ ID NO: 17.

**[0066]** The anti-PACAP antibodies of the invention can be monoclonal antibodies, polyclonal antibodies, recombinant antibodies, human antibodies, humanized antibodies, chimeric antibodies, or multispecific antibodies. In certain embodiments, the anti-PACAP antibody is a monoclonal antibody. In such embodiments, the anti-PACAP antibody may be a humanized antibody or a chimeric antibody having a human immunoglobulin constant domain. In these and other embodiments, the anti-PACAP antibody is an IgG1, IgG2, IgG3, or IgG4 antibody. Thus, the anti-PACAP antibody may, in some embodiments, have a human IgG1, IgG2, IgG3, or IgG4 constant domain. In one embodiment, the anti-PACAP antibody is a monoclonal IgG1 antibody. In another embodiment, the anti-PACAP antibody is a monoclonal IgG2 antibody.

**[0067]** The term "monoclonal antibody" (or "mAb") as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against an individual antigenic site or epitope, in contrast to polyclonal antibody preparations that typically include different antibodies directed against different epitopes. Monoclonal antibodies may be produced using any technique known in the art, e.g., by immortalizing spleen cells harvested from the transgenic animal after completion of the immunization schedule. The spleen cells can be immortalized using any technique known in the art, e.g., by fusing them with myeloma cells to produce hybridomas. Myeloma cells for use in hybridoma-producing fusion procedures preferably are non-antibody-producing, have high fusion efficiency, and enzyme deficiencies that render them incapable of growing in certain selective media which support the growth of only the desired fused cells (hybridomas). Examples of suitable cell lines for use in mouse fusions include Sp-20, P3-X63/Ag8, P3-X63-Ag8.653, NS1/1.Ag 4 1, Sp210-Ag14, FO, NSO/U, MPC-11, MPC11-X45-GTG 1.7 and S194/5XXO Bul; examples of cell lines used in rat fusions include R210.RCY3, Y3-Ag 1.2.3, IR983F and 4B210. Other cell lines useful for cell fusions are U-266, GM1500-GRG2, LICR-LON-HMy2 and UC729-6.

**[0068]** In some instances, a hybridoma cell line is produced by immunizing an animal (e.g., a rabbit, rat, mouse, or a transgenic animal having human immunoglobulin sequences) with a PACAP immunogen; harvesting spleen cells from the immunized animal; fusing the harvested spleen cells to a myeloma cell line, thereby generating hybridoma cells; establishing hybridoma cell lines from the hybridoma cells, and identifying a hybridoma cell line that produces an antibody that binds to PACAP. Another useful method for producing monoclonal antibodies is the SLAM method described in Example 1 herein and in Babcook *et al.*, Proc. Natl. Acad. Sci. USA, Vol. 93: 7843-7848, 1996, which is hereby incorporated by reference in its entirety.

**[0069]** Monoclonal antibodies secreted by a hybridoma cell line can be purified using any technique known in the art, such as protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography. Hybridoma supernatants or mAbs may be further screened to identify mAbs with particular properties, such as the ability to bind PACAP38, PACAP27, or regions thereof (e.g. C- or N-terminal domains); ability to block or interfere with the binding of the PACAP ligand to its receptors, or the ability to functionally block PACAP-induced activation of the VPAC1, VPAC2, or PAC1 receptors, e.g., using a cAMP assay as described herein.

**[0070]** In some embodiments, the anti-PACAP antibodies of the invention are chimeric or humanized antibodies based upon the CDR and variable region sequences of the antibodies described herein. A chimeric antibody is an antibody composed of protein segments from different antibodies that are covalently joined to produce functional immunoglobulin light or heavy chains or binding fragments thereof. Generally, a portion of the heavy chain and/or light chain is identical with or homologous to a corresponding sequence in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is/are identical with or homologous to a corresponding sequence in antibodies derived from another species or belonging to another antibody class or subclass. For methods relating to chimeric antibodies, see, for example, United States Patent No. 4,816,567 and Morrison *et al.*, 1985, Proc. Natl. Acad. Sci. USA 81:6851-6855, both of which are hereby incorporated by reference in their entireties.

**[0071]** Generally, the goal of making a chimeric antibody is to create a chimera in which the number of amino acids from the intended species is maximized. One example is the "CDR-grafted" antibody, in which the antibody comprises one or more CDRs from a particular species

or belonging to a particular antibody class or subclass, while the remainder of the antibody chain(s) is/are identical with or homologous to a corresponding sequence in antibodies derived from another species or belonging to another antibody class or subclass. CDR grafting is described, for example, in United States Patent No. 6,180,370, No. 5,693,762, No. 5,693,761, No. 5,585,089, and No. 5,530,101. For use in humans, the variable region or selected CDRs from a rodent or rabbit antibody often are grafted into a human antibody, replacing the naturally-occurring variable regions or CDRs of the human antibody.

**[0072]** For instance, in some embodiments, a chimeric antibody of the invention comprises a light chain variable region and a heavy chain variable region selected from those listed in Table 2 fused to a human light chain constant region, such as those listed in Table 3, and/or a human heavy chain constant region, such as those listed in Table 4. In one embodiment, the chimeric antibody specifically binds to a C-terminal domain of PACAP38 (e.g. within amino acids 28-38 of SEQ ID NO: 1; within amino acids 28-37 of SEQ ID NO: 1; or within amino acids 34-38 of SEQ ID NO: 1) and comprises a light chain variable region comprising a sequence selected from SEQ ID NOs: 18, 20, and 22; a heavy chain variable region comprising a sequence selected from SEQ ID NOs: 19, 21, and 23; and a human constant region. In another embodiment, the chimeric antibody specifically binds to an N-terminal domain or central domain of PACAP38 (e.g. within amino acids 1-13 of SEQ ID NO: 1; within amino acids 6-20 of SEQ ID NO: 1; or within amino acids 14-27 of SEQ ID NO: 1) and comprises a light chain variable region comprising a sequence selected from SEQ ID NOs: 24, 26, and 28; a heavy chain variable region comprising a sequence selected from SEQ ID NOs: 25, 27, and 29; and a human constant region. The human light chain constant region can be from a human lambda or kappa light chain, and the human heavy chain constant region can be from an IgG (e.g. IgG1, IgG2, IgG3, or IgG4) constant region. In certain embodiments, the chimeric antibodies comprise a human IgG1, IgG2, IgG3, or IgG4 Fc region. As used herein, the term "Fc region" refers to the C-terminal region of an immunoglobulin heavy chain which may be generated by papain digestion of an intact antibody. The Fc region of an immunoglobulin generally comprises two constant domains, a CH2 domain and a CH3 domain, and optionally comprises a CH4 domain. In certain embodiments, the Fc region is an Fc region from an IgG1, IgG2, IgG3, or IgG4 immunoglobulin. In some embodiments, the Fc region comprises CH2 and CH3 domains from a human IgG1 or human IgG2 immunoglobulin. The Fc region may retain effector function, such as C1q binding,

complement dependent cytotoxicity (CDC), Fc receptor binding, antibody-dependent cell-mediated cytotoxicity (ADCC), and phagocytosis. In other embodiments, the Fc region may be modified to reduce or eliminate effector function as described in further detail herein.

**[0073]** One useful type of chimeric antibody is a "humanized" antibody. Generally, a humanized antibody is produced from a monoclonal antibody raised initially in a non-human animal, such as a rodent or rabbit. Certain amino acid residues in this monoclonal antibody, typically from non-antigen recognizing portions of the antibody, are modified to be homologous to corresponding residues in a human antibody of corresponding isotype. Humanization can be performed, for example, using various methods by substituting at least a portion of a rodent or rabbit variable region for the corresponding regions of a human antibody (see, e.g., United States Patent No. 5,585,089, and No. 5,693,762; Jones *et al.*, 1986, Nature 321:522-525; Riechmann *et al.*, 1988, Nature 332:323-27; and Verhoeyen *et al.*, 1988, Science 239:1534-1536).

**[0074]** In one aspect, the CDRs of the light and heavy chain variable regions of the antibodies provided herein (see, Table 1) are grafted to framework regions (FRs) from antibodies from the same, or a different, phylogenetic species. For example, the CDRs of the heavy and light chain variable regions listed in Table 1 can be grafted to consensus human FRs. To create consensus human FRs, FRs from several human heavy chain or light chain amino acid sequences may be aligned to identify a consensus amino acid sequence. Alternatively, the grafted variable regions from the one heavy or light chain may be used with a constant region that is different from the constant region of that particular heavy or light chain as disclosed herein. In other embodiments, the grafted variable regions are part of a single chain Fv antibody.

**[0075]** In some embodiments, a humanized antibody of the invention specifically binds to a C-terminal domain of PACAP38 (e.g. within amino acids 28-38 of SEQ ID NO: 1; within amino acids 28-37 of SEQ ID NO: 1; or within amino acids 34-38 of SEQ ID NO: 1). Such C-terminal-binding humanized antibodies may comprise a CDRL1 of SEQ ID NO: 30 or 31, a CDRL2 of SEQ ID NO: 35 or 36, a CDRL3 of SEQ ID NO: 40 or 41, a CDRH1 selected from SEQ ID NOs: 45-47, a CDRH2 of SEQ ID NO: 51 or 52, a CDRH3 selected from SEQ ID NOs: 56-58, human framework regions, and a human constant region. In another embodiment, the humanized antibody specifically binds to an N-terminal domain or central domain of PACAP38 (e.g. within amino acids 1-13 of SEQ ID NO: 1; within amino acids 6-20 of SEQ ID NO: 1; or within amino acids 14-27 of SEQ ID NO: 1). Such N-terminal- or central domain-binding humanized

antibodies may comprise a CDRL1 selected from SEQ ID NOs: 32-34, a CDRL2 selected from SEQ ID NOs: 37-39, a CDRL3 selected from SEQ ID NOs: 42-44, a CDRH1 selected from SEQ ID NOs: 48-50, a CDRH2 selected from SEQ ID NO: 53-55, a CDRH3 selected from SEQ ID NOs: 59-61, human framework regions, and a human constant region. In certain embodiments, the humanized antibodies comprise a human IgG1, IgG2, IgG3, or IgG4 Fc region.

**[0076]** Fully human antibodies that specifically bind to human PACAP can be generated using the immunogens or fragments thereof described herein, such as polypeptides consisting of the sequences of SEQ ID NOs: 1, 2, 4, and 5. A "fully human antibody" is an antibody that comprises variable and constant regions derived from or indicative of human germ line immunoglobulin sequences. One specific means provided for implementing the production of fully human antibodies is the "humanization" of the mouse humoral immune system. Introduction of human immunoglobulin (Ig) loci into mice in which the endogenous Ig genes have been inactivated is one means of producing fully human monoclonal antibodies (mAbs) in mouse, an animal that can be immunized with any desirable antigen. Using fully human antibodies can minimize the immunogenic and allergic responses that can sometimes be caused by administering mouse or mouse-derived mAbs to humans as therapeutic agents.

**[0077]** Fully human antibodies can be produced by immunizing transgenic animals (usually mice) that are capable of producing a repertoire of human antibodies in the absence of endogenous immunoglobulin production. Antigens for this purpose typically have six or more contiguous amino acids, and optionally are conjugated to a carrier, such as a hapten. *See, e.g.,* Jakobovits *et al.*, 1993, *Proc. Natl. Acad. Sci. USA* 90:2551-2555; Jakobovits *et al.*, 1993, Nature 362:255-258; and Bruggermann *et al.*, 1993, Year in Immunol. 7:33. In one example of such a method, transgenic animals are produced by incapacitating the endogenous mouse immunoglobulin loci encoding the mouse heavy and light immunoglobulin chains therein, and inserting into the mouse genome large fragments of human genome DNA containing loci that encode human heavy and light chain proteins. Partially modified animals, which have less than the full complement of human immunoglobulin loci, are then cross-bred to obtain an animal having all of the desired immune system modifications. When administered an immunogen, these transgenic animals produce antibodies that are immunospecific for the immunogen but have human rather than murine amino acid sequences, including the variable regions. For further details of such methods, *see*, for example, WO96/33735 and WO94/02602. Additional methods

relating to transgenic mice for making human antibodies are described in United States Patent No. 5,545,807; No. 6,713,610; No. 6,673,986; No. 6,162,963; No. 5,939,598; No. 5,545,807; No. 6,300,129; No. 6,255,458; No. 5,877,397; No. 5,874,299 and No. 5,545,806; in PCT publications WO91/10741, WO90/04036, WO 94/02602, WO 96/30498, WO 98/24893 and in EP 546073B1 and EP 546073A1.

**[0078]** The transgenic mice described above, referred to herein as "HuMab" mice, contain a human immunoglobulin gene minilocus that encodes unrearranged human heavy (mu and gamma) and kappa light chain immunoglobulin sequences, together with targeted mutations that inactivate the endogenous mu and kappa chain loci (Lonberg *et al.*, 1994, Nature 368:856-859). Accordingly, the mice exhibit reduced expression of mouse IgM and kappa proteins and in response to immunization, the introduced human heavy and light chain transgenes undergo class switching and somatic mutation to generate high affinity human IgG kappa monoclonal antibodies (Lonberg *et al.*, *supra.*; Lonberg and Huszar, 1995, Intern. Rev. Immunol. 13: 65-93; Harding and Lonberg, 1995, Ann. N.Y Acad. Sci. 764:536-546). The preparation of HuMab mice is described in detail in Taylor *et al.*, 1992, Nucleic Acids Research 20:6287-6295; Chen *et al.*, 1993, International Immunology 5:647-656; Tuaillon *et al.*, 1994, J. Immunol. 152:2912-2920; Lonberg *et al.*, 1994, Nature 368:856-859; Lonberg, 1994, Handbook of Exp. Pharmacology 113:49-101; Taylor *et al.*, 1994, International Immunology 6:579-591; Lonberg and Huszar, 1995, Intern. Rev. Immunol. 13:65-93; Harding and Lonberg, 1995, Ann. N.Y Acad. Sci. 764:536-546; Fishwild *et al.*, 1996, Nature Biotechnology 14:845-851; the foregoing references are hereby incorporated by reference in their entireties for all purposes. *See*, further United States Patent No. 5,545,806; No. 5,569,825; No. 5,625,126; No. 5,633,425; No. 5,789,650; No. 5,877,397; No. 5,661,016; No. 5,814,318; No. 5,874,299; and No. 5,770,429; as well as United States Patent No. 5,545,807; International Publication Nos. WO 93/1227; WO 92/22646; and WO 92/03918, the disclosures of all of which are hereby incorporated by reference in their entireties for all purposes. Technologies utilized for producing human antibodies in these transgenic mice are disclosed also in WO 98/24893, and Mendez *et al.*, 1997, Nature Genetics 15:146-156, which are hereby incorporated by reference. For example, the HCo7 and HCo12 transgenic mice strains can be used to generate fully human anti-PACAP antibodies.

**[0079]** Human-derived antibodies can also be generated using phage display techniques. Phage display is described in e.g., Dower *et al.*, WO 91/17271, McCafferty *et al.*, WO 92/01047, and Caton and Koprowski, Proc. Natl. Acad. Sci. USA, 87:6450-6454 (1990), each of which is incorporated herein by reference in its entirety. The antibodies produced by phage technology are usually produced as antigen binding fragments, e.g. Fv or Fab fragments, in bacteria and thus lack effector functions. Effector functions can be introduced by one of two strategies: The fragments can be engineered either into complete antibodies for expression in mammalian cells, or into bispecific antibody fragments with a second binding site capable of triggering an effector function, if desired. Typically, the Fd fragment (VH-CH1) and light chain (VL-CL) of antibodies are separately cloned by PCR and recombined randomly in combinatorial phage display libraries, which can then be selected for binding to a particular antigen. The antibody fragments are expressed on the phage surface, and selection of Fv or Fab (and therefore the phage containing the DNA encoding the antibody fragment) by antigen binding is accomplished through several rounds of antigen binding and re-amplification, a procedure termed panning. Antibody fragments specific for the antigen are enriched and finally isolated. Phage display techniques can also be used in an approach for the humanization of rodent monoclonal antibodies, called "guided selection" (*see* Jespers, L. S., et al., Bio/Technology 12, 899-903 (1994)). For this, the Fd fragment of the mouse monoclonal antibody can be displayed in combination with a human light chain library, and the resulting hybrid Fab library may then be selected with antigen. The mouse Fd fragment thereby provides a template to guide the selection. Subsequently, the selected human light chains are combined with a human Fd fragment library. Selection of the resulting library yields entirely human Fab.

**[0080]** In certain embodiments, the anti-PACAP antigen binding proteins of the invention (e.g. monoclonal antibodies or binding fragments thereof) compete for binding to human PACAP38 (SEQ ID NO: 1) with a reference antibody, such as one or more of the anti-PACAP antibodies described herein. The term "compete" refers to the ability of an antibody or other antigen binding protein to interfere with the binding of other antibodies or binding fragments to a target (e.g. human PACAP38). The extent to which an antibody or binding fragment is able to interfere with the binding of another antibody or binding fragment to a target (e.g. human PACAP38), and therefore whether it can be said to compete, can be determined using competition binding assays. Numerous types of competitive binding assays can be used, including for example: solid phase

direct or indirect radioimmunoassay (RIA), solid phase direct or indirect enzyme immunoassay (EIA), sandwich competition assay (*see, e.g.*, Stahli *et al.*, 1983, Methods in Enzymology 9:242-253); solid phase direct biotin-avidin EIA (*see, e.g.*, Kirkland *et al.*, 1986, J. Immunol. 137:3614-3619); solid phase direct-labeled assay, solid phase direct-labeled sandwich assay (*see, e.g.*, Harlow and Lane, 1988, Antibodies, A Laboratory Manual, Cold Spring Harbor Press); solid phase direct label RIA using I-125 label (*see, e.g.*, Morel *et al.*, 1988, Molec. Immunol. 25:7-15); solid phase direct biotin-avidin EIA (*see, e.g.*, Cheung, *et al.*, 1990, Virology 176:546-552); and direct labeled RIA (Moldenhauer *et al.*, 1990, Scand. J. Immunol. 32:77-82). Typically, a competitive binding assay involves the use of purified antigen bound to a solid surface or cells bearing the antigen, an unlabeled test antibody or other antigen binding protein, and a labeled reference antibody or other antigen binding protein. Competitive inhibition is measured by determining the amount of label bound to the solid surface or cells in the presence of the test antibody or other antigen binding protein. Usually the test antibody or other antigen binding protein is present in excess. Antibodies or other antigen binding proteins identified by competition assay (i.e. competing antibodies and antigen binding proteins) include antibodies and antigen binding proteins binding to the same epitope as the reference antibody or antigen binding protein. Usually, when a competing antibody or other antigen binding protein is present in excess, it will inhibit specific binding of a reference antibody or other antigen binding protein to a target antigen by at least 40%, 45%, 50%, 55%, 60%, 65%, 70% or 75%. In some instances, binding of the reference antibody or other antigen binding protein is inhibited by at least 80%, 85%, 90%, 95%, or 97% or more. In some embodiments, a competing antigen binding protein (e.g. antibody or binding fragment thereof) reduces human PACAP38 binding of a reference antibody between about 40% and about 100%, such as about 60% and about 100%, specifically between about 70% and about 100%, and more specifically between about 80% and about 100%.

**[0081]** A particularly suitable quantitative assay for detecting competitive binding uses a Biacore machine which measures the extent of interactions using surface plasmon resonance technology. An exemplary Biacore-based competitive binding assay involves the immobilization of a reference antibody to a sensor chip. The target antigen is then contacted with the sensor chip where the target antigen is captured by the immobilized reference antibody. Test antibodies are then injected over the captured target antigen. If the injected test antibody recognizes a distinct epitope from that recognized by the immobilized antibody, then a second binding event is

observed and the test antibody would be considered not to compete for binding to the target antigen with the reference antibody.

**[0082]** In one embodiment, an antigen binding protein of the invention competes with a reference antibody for binding to human PACAP38, wherein the reference antibody comprises: (a) a light chain variable region comprising the sequence of SEQ ID NO: 18 and a heavy chain variable region comprising the sequence of SEQ ID NO: 19; (b) a light chain variable region comprising the sequence of SEQ ID NO: 20 and a heavy chain variable region comprising the sequence of SEQ ID NO: 21; or (c) a light chain variable region comprising the sequence of SEQ ID NO: 22 and a heavy chain variable region comprising the sequence of SEQ ID NO: 23. In some embodiments, the reference antibody comprises: (a) a light chain comprising the sequence of SEQ ID NO: 6 and a heavy chain comprising the sequence of SEQ ID NO: 7; (b) a light chain comprising the sequence of SEQ ID NO: 8 and a heavy chain comprising the sequence of SEQ ID NO: 9; or (c) a light chain comprising the sequence of SEQ ID NO: 10 and a heavy chain comprising the sequence of SEQ ID NO: 11. As shown by the Examples herein, reference antibodies defined by these sequences (e.g. mAb1, mAb2, and mAb3) have been found to bind to an epitope within the C-terminal domain of PACAP38, e.g. within amino acids 28-38 of SEQ ID NO: 1. Thus, antigen binding proteins that compete with these reference antibodies would also bind to a similar epitope within the C-terminal domain of PACAP38.

**[0083]** In another embodiment, an antigen binding protein of the invention competes with a reference antibody for binding to human PACAP38, wherein the reference antibody comprises: (a) a light chain variable region comprising the sequence of SEQ ID NO: 24 and a heavy chain variable region comprising the sequence of SEQ ID NO: 25; (b) a light chain variable region comprising the sequence of SEQ ID NO: 26 and a heavy chain variable region comprising the sequence of SEQ ID NO: 27; or (c) a light chain variable region comprising the sequence of SEQ ID NO: 28 and a heavy chain variable region comprising the sequence of SEQ ID NO: 29. In some embodiments, the reference antibody comprises: (a) a light chain comprising the sequence of SEQ ID NO: 12 and a heavy chain comprising the sequence of SEQ ID NO: 13; (b) a light chain comprising the sequence of SEQ ID NO: 14 and a heavy chain comprising the sequence of SEQ ID NO: 15; or (c) a light chain comprising the sequence of SEQ ID NO: 16 and a heavy chain comprising the sequence of SEQ ID NO: 17. As shown by the Examples herein, reference antibodies defined by these sequences (e.g. mAb4, mAb5, and mAb6) have been found to bind to

an epitope within the N-terminal domain or central domain of PACAP38, e.g. within amino acids 1-13 of SEQ ID NO: 1; within amino acids 6-20 of SEQ ID NO: 1; or within amino acids 14-27 of SEQ ID NO: 1. Accordingly, antigen binding proteins that compete with these reference antibodies would also bind to a similar epitope within the N-terminal or central domain of PACAP38.

**[0084]** The heavy chain constant regions or the Fc regions of the antigen binding proteins (e.g. monoclonal antibodies) described herein may comprise one or more amino acid substitutions that affect the glycosylation and/or effector function of the antigen binding protein. One of the functions of the Fc region of an immunoglobulin is to communicate to the immune system when the immunoglobulin binds its target. This is commonly referred to as "effector function." Communication leads to antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), and/or complement dependent cytotoxicity (CDC). ADCC and ADCP are mediated through the binding of the Fc region to Fc receptors on the surface of cells of the immune system. CDC is mediated through the binding of the Fc with proteins of the complement system, e.g., C1q. In some embodiments, the antigen binding proteins, e.g. monoclonal antibodies, of the invention comprise one or more amino acid substitutions in the constant region to enhance effector function, including ADCC activity, CDC activity, ADCP activity, and/or the clearance or half-life of the antigen binding protein. Exemplary amino acid substitutions (EU numbering) that can enhance effector function include, but are not limited to, E233L, L234I, L234Y, L235S, G236A, S239D, F243L, F243V, P247I, D280H, K290S, K290E, K290N, K290Y, R292P, E294L, Y296W, S298A, S298D, S298V, S298G, S298T, T299A, Y300L, V305I, Q311M, K326A, K326E, K326W, A330S, A330L, A330M, A330F, I332E, D333A, E333S, E333A, K334A, K334V, A339D, A339Q, P396L, or combinations of any of the foregoing.

**[0085]** In other embodiments, the antigen binding proteins (e.g. monoclonal antibodies) of the invention comprise one or more amino acid substitutions in the constant region to reduce effector function. Exemplary amino acid substitutions (EU numbering) that can reduce effector function include, but are not limited to, C220S, C226S, C229S, E233P, L234A, L234V, V234A, L234F, L235A, L235E, G237A, P238S, S267E, H268Q, N297A, N297G, V309L, E318A, L328F, A330S, A331S, P331S or combinations of any of the foregoing.

**[0086]** Glycosylation can contribute to the effector function of antibodies, particularly IgG1 antibodies. Thus, in some embodiments, the antigen binding proteins of the invention may comprise one or more amino acid substitutions that affect the level or type of glycosylation of the binding proteins. Glycosylation of polypeptides 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 tri-peptide 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 tri-peptide sequences in a polypeptide creates a potential glycosylation site. O-linked glycosylation refers to the attachment of one of the sugars N-acetylgalactosamine, galactose, or xylose, to a hydroxyamino acid, most commonly serine or threonine, although 5-hydroxyproline or 5-hydroxylysine may also be used.

**[0087]** In certain embodiments, glycosylation of the antigen binding proteins described herein is increased by adding one or more glycosylation sites, e.g., to the Fc region of the binding protein. Addition of glycosylation sites to the antigen binding protein can be conveniently accomplished by altering the amino acid sequence such that it contains one or more of the above-described tri-peptide 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 starting sequence (for O-linked glycosylation sites). For ease, the antigen binding protein amino acid sequence may be altered through changes at the DNA level, particularly by mutating the DNA encoding the target polypeptide at preselected bases such that codons are generated that will translate into the desired amino acids.

**[0088]** The invention also encompasses production of antigen binding protein molecules with altered carbohydrate structure resulting in altered effector activity, including antigen binding proteins with absent or reduced fucosylation that exhibit improved ADCC activity. Various methods are known in the art to reduce or eliminate fucosylation. For example, ADCC effector activity is mediated by binding of the antibody molecule to the FcγRIII receptor, which has been shown to be dependent on the carbohydrate structure of the N-linked glycosylation at the N297 residue of the CH2 domain. Non-fucosylated antibodies bind this receptor with increased affinity and trigger FcγRIII-mediated effector functions more efficiently than native, fucosylated antibodies. For example, recombinant production of non-fucosylated antibody in CHO cells in

which the alpha-1,6-fucosyl transferase enzyme has been knocked out results in antibody with 100-fold increased ADCC activity (*see* Yamane-Ohnuki *et al.*, Biotechnol Bioeng. 87(5):614-22, 2004). Similar effects can be accomplished through decreasing the activity of alpha-1,6-fucosyl transferase enzyme or other enzymes in the fucosylation pathway, e.g., through siRNA or antisense RNA treatment, engineering cell lines to knockout the enzyme(s), or culturing with selective glycosylation inhibitors (*see* Rothman *et al.*, Mol Immunol. 26(12):1113-23, 1989). Some host cell strains, e.g. Lec13 or rat hybridoma YB2/0 cell line naturally produce antibodies with lower fucosylation levels (*see* Shields *et al.*, J Biol Chem. 277(30):26733-40, 2002 and Shinkawa *et al.*, J Biol Chem. 278(5):3466-73, 2003). An increase in the level of bisected carbohydrate, e.g. through recombinantly producing antibody in cells that overexpress GnTIII enzyme, has also been determined to increase ADCC activity (*see* Umana *et al.*, Nat Biotechnol. 17(2):176-80, 1999).

**[0089]** In other embodiments, glycosylation of the antigen binding proteins described herein is decreased or eliminated by removing one or more glycosylation sites, e.g., from the Fc region of the binding protein. Amino acid substitutions that eliminate or alter N-linked glycosylation sites can reduce or eliminate N-linked glycosylation of the antigen binding protein. In certain embodiments, the antigen binding proteins described herein comprise a mutation at position N297 (EU numbering), such as N297Q, N297A, or N297G. In one particular embodiment, the antigen binding proteins of the invention comprise an Fc region from a human IgG1 antibody with a N297G mutation. To improve the stability of molecules comprising a N297 mutation, the Fc region of the molecules may be further engineered. For instance, in some embodiments, one or more amino acids in the Fc region are substituted with cysteine to promote disulfide bond formation in the dimeric state. Residues corresponding to V259, A287, R292, V302, L306, V323, or I332 (EU numbering) of an IgG1 Fc region may thus be substituted with cysteine. Preferably, specific pairs of residues are substituted with cysteine such that they preferentially form a disulfide bond with each other, thus limiting or preventing disulfide bond scrambling. Preferred pairs include, but are not limited to, A287C and L306C, V259C and L306C, R292C and V302C, and V323C and I332C. In particular embodiments, the antigen binding proteins described herein comprise an Fc region from a human IgG1 antibody with mutations R292C and V302C. In such embodiments, the Fc region may also comprise a N297G mutation.

**[0090]** Modifications of the antigen binding proteins of the invention to increase serum half-life also may desirable, for example, by incorporation of or addition of a salvage receptor binding epitope (e.g., by mutation of the appropriate region or by incorporating the epitope into a peptide tag that is then fused to the antigen binding protein at either end or in the middle, e.g., by DNA or peptide synthesis; *see, e.g.*, WO96/32478) or adding molecules such as PEG or other water soluble polymers, including polysaccharide polymers. The salvage receptor binding epitope preferably constitutes a region wherein any one or more amino acid residues from one or two loops of an Fc region are transferred to an analogous position in the antigen binding protein. Even more preferably, three or more residues from one or two loops of the Fc region are transferred. Still more preferred, the epitope is taken from the CH2 domain of the Fc region (e.g., an IgG Fc region) and transferred to the CH1, CH3, or VH region, or more than one such region, of the antigen binding protein. Alternatively, the epitope is taken from the CH2 domain of the Fc region and transferred to the CL region or VL region, or both, of the antigen binding protein. *See* International applications WO 97/34631 and WO 96/32478 for a description of Fc variants and their interaction with the salvage receptor.

**[0091]** The present invention includes one or more isolated polynucleotides or isolated nucleic acids encoding the antigen binding proteins, such as monoclonal antibodies, described herein. Nucleic acid molecules of the invention include DNA and RNA in both single-stranded and double-stranded form, as well as the corresponding complementary sequences. DNA includes, for example, cDNA, genomic DNA, chemically synthesized DNA, DNA amplified by PCR, and combinations thereof. The nucleic acid molecules of the invention include full-length genes or cDNA molecules as well as a combination of fragments thereof. The nucleic acids of the invention can be derived from human sources as well as non-human species.

**[0092]** Relevant amino acid sequences from an immunoglobulin or region thereof (e.g. variable region, Fc region, etc.) or polypeptide of interest may be determined by direct protein sequencing, and suitable encoding nucleotide sequences can be designed according to a universal codon table. Alternatively, genomic or cDNA encoding monoclonal antibodies or binding fragments thereof of the invention can be isolated and sequenced from cells producing such antibodies using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the monoclonal antibodies).

**[0093]** An "isolated nucleic acid," which is used interchangeably herein with "isolated polynucleotide," is a nucleic acid that has been separated from adjacent genetic sequences present in the genome of the organism from which the nucleic acid was isolated, in the case of nucleic acids isolated from naturally- occurring sources. In the case of nucleic acids synthesized enzymatically from a template or chemically, such as PCR products, cDNA molecules, or oligonucleotides for example, it is understood that the nucleic acids resulting from such processes are isolated nucleic acids. An isolated nucleic acid molecule refers to a nucleic acid molecule in the form of a separate fragment or as a component of a larger nucleic acid construct. In one preferred embodiment, the nucleic acids are substantially free from contaminating endogenous material. The nucleic acid molecule has preferably been derived from DNA or RNA isolated at least once in substantially pure form and in a quantity or concentration enabling identification, manipulation, and recovery of its component nucleotide sequences by standard biochemical methods (such as those outlined in Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1989)). Such sequences are preferably provided and/or constructed in the form of an open reading frame uninterrupted by internal non-translated sequences, or introns, that are typically present in eukaryotic genes. Sequences of non-translated DNA can be present 5' or 3' from an open reading frame, where the same do not interfere with manipulation or expression of the coding region. Unless specified otherwise, the left-hand end of any single-stranded polynucleotide sequence discussed herein is the 5' end; the left-hand direction of double-stranded polynucleotide sequences is referred to as the 5' direction. The direction of 5' to 3' production of nascent RNA transcripts is referred to as the transcription direction; sequence regions on the DNA strand having the same sequence as the RNA transcript that are 5' to the 5' end of the RNA transcript are referred to as "upstream sequences;" sequence regions on the DNA strand having the same sequence as the RNA transcript that are 3' to the 3' end of the RNA transcript are referred to as "downstream sequences."

**[0094]** The present invention also includes nucleic acids that hybridize under moderately stringent conditions, and more preferably highly stringent conditions, to nucleic acids encoding polypeptides as described herein. The basic parameters affecting the choice of hybridization conditions and guidance for devising suitable conditions are set forth by Sambrook,, Fritsch, and Maniatis (1989, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory

Press, Cold Spring Harbor, N.Y., chapters 9 and 11; and Current Protocols in Molecular Biology, 1995, Ausubel et al., eds., John Wiley & Sons, Inc., sections 2.10 and 6.3-6.4), and can be readily determined by those having ordinary skill in the art based on, for example, the length and/or base composition of the DNA. One way of achieving moderately stringent conditions involves the use of a prewashing solution containing 5 x SSC, 0.5% SDS, 1.0 mM EDTA (pH 8.0), hybridization buffer of about 50% formamide, 6 x SSC, and a hybridization temperature of about 55°C (or other similar hybridization solutions, such as one containing about 50% formamide, with a hybridization temperature of about 42°C), and washing conditions of about 60°C, in 0.5 x SSC, 0.1% SDS. Generally, highly stringent conditions are defined as hybridization conditions as above, but with washing at approximately 68°C, 0.2 x SSC, 0.1% SDS. SSPE (l x SSPE is 0.15M NaCl, 10 mM $NaH_2PO_4$, and 1.25 mM EDTA, pH 7.4) can be substituted for SSC (l x SSC is 0.15M NaCl and 15 mM sodium citrate) in the hybridization and wash buffers; washes are performed for 15 minutes after hybridization is complete. It should be understood that the wash temperature and wash salt concentration can be adjusted as necessary to achieve a desired degree of stringency by applying the basic principles that govern hybridization reactions and duplex stability, as known to those skilled in the art and described further below (*see, e.g.*, Sambrook *et al.*, 1989).

**[0095]** When hybridizing a nucleic acid to a target nucleic acid of unknown sequence, the hybrid length is assumed to be that of the hybridizing nucleic acid. When nucleic acids of known sequence are hybridized, the hybrid length can be determined by aligning the sequences of the nucleic acids and identifying the region or regions of optimal sequence complementarity. The hybridization temperature for hybrids anticipated to be less than 50 base pairs in length should be 5 to 10°C less than the melting temperature (Tm) of the hybrid, where Tm is determined according to the following equations. For hybrids less than 18 base pairs in length, Tm (°C) = 2(# of A + T bases) + 4(# of G + C bases). For hybrids above 18 base pairs in length, Tm (°C) = 81.5 + 16.6(log10 [Na+]) + 0.41(% G + C) - (600/N), where N is the number of bases in the hybrid, and [Na+] is the concentration of sodium ions in the hybridization buffer ([Na+] for l x SSC = 0.165M). Preferably, each such hybridizing nucleic acid has a length that is at least 15 nucleotides (or more preferably at least 18 nucleotides, or at least 20 nucleotides, or at least 25 nucleotides, or at least 30 nucleotides, or at least 40 nucleotides, or most preferably at least 50 nucleotides), or at least 25% (more preferably at least 50%, or at least 60%, or at least 70%, and

most preferably at least 80%) of the length of the nucleic acid of the present invention to which it hybridizes, and has at least 60% sequence identity (more preferably at least 70%, at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%, and most preferably at least 99.5%) with the nucleic acid of the present invention to which it hybridizes, where sequence identity is determined by comparing the sequences of the hybridizing nucleic acids when aligned so as to maximize overlap and identity while minimizing sequence gaps as described in more detail above.

**[0096]** Variants of the antigen binding proteins described herein can be prepared by site-specific mutagenesis of nucleotides in the DNA encoding the polypeptide, using cassette or PCR mutagenesis or other techniques well known in the art, to produce DNA encoding the variant, and thereafter expressing the recombinant DNA in cell culture as outlined herein. However, antigen binding proteins comprising variant CDRs having up to about 100-150 residues may be prepared by *in vitro* synthesis using established techniques. The variants typically exhibit the same qualitative biological activity as the naturally occurring analogue, e.g., binding to antigen. Such variants include, for example, deletions and/or insertions and/or substitutions of residues within the amino acid sequences of the antigen binding proteins. Any combination of deletion, insertion, and substitution is made to arrive at the final construct, provided that the final construct possesses the desired characteristics. The amino acid changes also may alter post-translational processes of the antigen binding protein, such as changing the number or position of glycosylation sites. In certain embodiments, antigen binding protein variants are prepared with the intent to modify those amino acid residues which are directly involved in epitope binding. In other embodiments, modification of residues which are not directly involved in epitope binding or residues not involved in epitope binding in any way, is desirable, for purposes discussed herein. Mutagenesis within any of the CDR regions and/or framework regions is contemplated. Covariance analysis techniques can be employed by the skilled artisan to design useful modifications in the amino acid sequence of the antigen binding protein. *See, e.g.*, Choulier, *et al.*, Proteins 41:475-484, 2000; Demarest *et al.*, J. Mol. Biol. 335:41-48, 2004; Hugo *et al.*, Protein Engineering 16(5):381-86, 2003; Aurora *et al.*, US Patent Publication No. 2008/0318207 A1; Glaser *et al.*, US Patent Publication No. 2009/0048122 A1; Urech *et al.*, WO 2008/110348

A1; Borras *et al.*, WO 2009/000099 A2. Such modifications determined by covariance analysis can improve potency, pharmacokinetic, pharmacodynamic, and/or manufacturability characteristics of an antigen binding protein.

**[0097]** Table 6 shows exemplary nucleic acid sequences encoding the light and heavy chain variable regions of anti-PACAP antibodies, and Table 7 lists exemplary nucleic acid sequences encoding the full-length light and heavy chains of the anti-PACAP antibodies. Polynucleotides encoding the anti-PACAP variable regions and full chains can be used to construct the antigen binding proteins described herein.

**Table 6. Exemplary Anti-PACAP Variable Region Nucleic Acid Sequences**

| Antibody ID | Designation | Nucleic Acid Sequence | SEQ ID NO: |
|---|---|---|---|
| | | *Light chain variable regions* | |
| mAb1 | LV-01 | GCCGATGTCGTGATGACCCAGACTCCATCCCCCGTGTCTGCAGCTG TGGGAGGCACAGTCACCATCAATTGCCGGGCCAGTGAGGACATTG AAAGCTTTTTAGCCTGGTATCAGCAGAAACCAGGGCAGCCTCCCA AGCTCCTGATCTCCAGGACATCCACTCTGGAATCTGGGGTCTCATC GCGGTTCAAAGGCAGTGGATCGGGGACAGAGTTCATTCTCACCAT CAGCGACCTGGAGTGTGCCGATGCTGCCACTTACTACTGTCAATGT ACTGATGGTAGTAGTAGTAGTAGTAGTTATGGTTGGGATGCTTTCG GCGGAGGGACCGAGGTGGTGGTCAAAGGT | 72 |
| mAb2 | LV-02 | GCCGATGTCGTGATGACCCAGACTCCATCCCCCGTGTCTGCGCAGG TGGGAGGCACAGTCACCATCAATTGCCAGGCCAGTGAGAGCATTG ATAGTGACTTATCCTGGTATCAACAGAAACCAGGGCAGCCTCCCA AGCTCCTGATCTACAGGACATTCACTCTGGAATCTGGGGTCCCATC GCGGTTCAAAGGCAGTGGATCTGGGACAGACTACACTCTCACCAT CAGCGACCTGGAGTGTGCCGATGCTGCCATTTACTACTGTCAATGC ACTGATGGTAGTAGTATTAGTGGTAGTTATGGTTGGGATGCTTTCG GCGGAGGGACCGAGGTGGTGGTCAAAGGT | 73 |
| mAb3 | LV-03 | GCCGATGTCGTGATGACCCAGACTCCATCCCCCGTGTCTGCAGCTG TGGGAGGCACAGTCACCATCAATTGCCGGGCCAGTGAGGACATTG AAAGCTTTTTAGCCTGGTATCAGCAGAAACCAGGGCAGCCTCCCA AGCTCCTGATGTCCAGGACATCCACTCTGGAATCTGGGGTCCCATC GCGGTTCAAAGGCAGTGGATCTGGGACAGAGTTCACTCTCACCAT CAGCGACCTGGAGTGTGCCGATGCTGCCACATATTACTGTCAATGT ACTGATGGTAGTAGTAGTAGTAGTAGTTATGGTTGGGATGCTTTCG GCGGAGGGACCGAGGTGGTGGTCAAAGGT | 74 |
| mAb4 | LV-04 | GCTGACATTGTGATGACCCAGACTCCAGCCTCCGTGTCTGGAGCTG TGGGAGGCACAGTCACCATCAAGTGCCAGGCCAGTCAGAGCATTA GGAATGAATTATCCTGGTATCAGCAGAAACCAGGGCAGCCTCCCA AGCTCCTGATCTACAAGGCATCCACTCTGGCATCTGGGGTCCCATC GCGGTTCAGTGGCAGTGGATTTGGGACAGAGTTCACTCTCACCATC AGCGGTGTGCAGTGTGATGATGCTGCCACTTACTACTGTCAAAAC AATTATGGTACTAGGCGTAATAATTATGTTTTTCCTTTCGGCGGAG GGACCGAGGTGGTGGTCAAAGGT | 75 |

| Antibody ID | Designation | Nucleic Acid Sequence | SEQ ID NO: |
|---|---|---|---|
| mAb5 | LV-05 | GCTGACATCGTGCTGACCCAGACTCCAGCCTCCGTGTCTGCAGCTG TGGGAGGCACAGTCAGCATCAGTTGCCAGTCCAGTGAAAGTGTTT ATAATAACAACTACTTATCCTGGTTTCAGCAGAAACCAGGGCAGC CTCCCAAGCTCCTGATCTATGGTGCATCCACTCTGGCATCTGGGGT CCCATCGCGGTTTGAAGGCAGTGGATCTGGGACACAGTTCACTCTC ACCATCAGCGACGTGCAGTGTGATGATGCTGCCACTTACTACTGTC TAGGCGATTATATTATTATTGAGAATATTTTCGGCGGAGGGACCGA GGTGGTGGTCAAAGGT | 76 |
| mAb6 | LV-06 | GCGCAAGTGCTGACCCAGACTCCAGCCTCCGTGTCTGCGGCTGTG GGAGGCACAGTCACCATCAATTGCCAGGCCAGTCAGAGTGTTTAT AACAACAAAAATTTAGCCTGGTATCAGCAGAAACCAGGGCAGCC TCCCAAGCTCCTGATCTATTTTTCATCCACTCTGGCATCTGGGGTCC CATCGCGGTTCAGAGGCAGTGGATCTGGGACACAGTTCACTCTCA CCATCAGTGGCGTGCAGTGTGGCGATGCTGCCACTTACTACTGTCT AGGCGAATTTGGTAGTATTTGGGCTTTCGGCGGAGGGACCGAGGT GGTGGTCAAAGGT | 77 |
| | | ***Heavy chain variable regions*** | |
| mAb1 | HV-01 | CAGTCGTTGGAGGAGTCCGGGGGAGACCTGGTCAAGCCTGGGG CATCTCTGACACTCACCTGCAAAGCCTCTGGAATCGCCTTCAGTAA CGACTACATGTGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGA GTGGATCGCATGTATTTTTACTGGTAGTAGTGGTAGTACTTACTAC GCGAGCTGGGCGAAAGGTCGATTCACCATCTCCAAAACCTCGTCG ACCACGGTGACTCTGCAAATGACCAGTCTGACAGCCGCGGACACG GCCACCTATTTCTGTGCGAGAGATAGGGCTATTTCTGTACTTGGTT ATTTCTATGCTGCATACTTCTTTGACTTCTGGGGCCCAGGCACCCT GGTCACCGTCTCCTCA | 78 |
| mAb2 | HV-02 | CAGTCGTTGGAGGAGTCCGGGGGAGGCCTGGTCCAGCCTGAGG GATCCCTGACACTCACCTGCACAGCTTCTGGATTCTCCTTCAATAG CAGCTCCTACATGTGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCT GGAGTGGATCGGATGCATTTTTACTGGTAGTAGTGGTAATACCTAC TACGCGACCTGGGCGCAAGGTCGATTCACCATCTCCAAGACCTCG ACCACGGTGACTCTGGAAATGACCAGTCTGACAGCCGCGGACACG GCCACCTATTTCTGTGCGAGAGATAGGGCTTATTTTTTGGTTGGTT ATTTCTATGCTACATATTATTTTGACTTATGGGGCCCAGGCACCCT GGTCACCGTCTCCTCA | 79 |
| mAb3 | HV-03 | CAGTCGTTGGAGGAGTCCGGGGGAGGCCTGGTCCAGCCTGGGG CATCTCTGACACTCACCTGTACAGCCTCTGGATTCTCCTTCAGTAG CAACTACTACATGTGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCT GGAGTGGATCGCATGCATTTTTACTGGTAGTAGTGGTAGCACTTAC TACGCGAGCTGGGCGAAAGGCCGATTCACCATCTCCAAAACCTCG TCGACCACGGTGACTCTGCACGTGACCAGTCTGACAGCCGCGGAC ACGGCCACCTATTTCTGTGCGAGAGATAGGGCTTTGTCTGTTGTTG GTTATTTCTATGCTGCATACTACTTTGACTTCTGGGGCCCAGGCAC CCTGGTCACCGTCTCCTCA | 80 |
| mAb4 | HV-04 | CAGTCGGTGGAGGAGTCCGGGGGTCGCCTGGTCACGCCTGGGACA CCCCTGACACTCACCTGCACAGCCTCTGGAATCGACCTCAGTAGCT ATGCAATGATCTGGGTCCGCCAGGCTCCAGGAGAGGGACTGGAAT ACATCGGATTCATTGATGCTGGTGATGGTAACACTTACTACGCGAG CTGGGCAAAAGGCCGATTCACCATCTCCAAAACCTCGACCACGGT GGATCTGAAAATCACCAGTCCGACAACCGAGGACACGGCCACCTA TTTCTGTGCCAGAGGAGATCCTGGTTGGAGTAATGGTTTTGCCTTG TGGGGCCAAGGCACCCTGGTCACCGTCTCCTCA | 81 |

| Antibody ID | Designation | Nucleic Acid Sequence | SEQ ID NO: |
|---|---|---|---|
| mAb5 | HV-05 | CAGTCGGTGGAGGAGTCCGGGGGTCGCCTGGTCACGCCTGGGACA CCCCTGACACTCACCTGCACAGTCTCTGGATTCTCCCTCAGTACCT ACAATATGTGCTGGGTCCGCCAGGCTCCAGGGAAGGGCTTGGAAT GGGTCGGTTTCATTAATTCTGATGATAGCGCGTACTACGCGAGCTG GGCGAAAGGCCGCTTCACCTTCTCCAAGACCTCGACCACGGTGGA TCTGAAAATCGCCAGTCCGACAACCGAGGACACGGCCACCTATTT CTGTGCCAGATATGATTGGGATTATTATTATAGTCGGTTGGATCTC TGGGGCCCGGGCACCCTGGTCACCGTCTCCTCA | 82 |
| mAb6 | HV-06 | CAGCTGGAGGAGTCCGGGGGAGGCCTGGTCCAGCCTGGGGCATCC CTGACACTCACCTGCACAACTTCTGGATTCTCTCTCAGTGATAATT ATTTGTGTTGGGTCCGCCAGGCTCCAGGGCGTGGGCTGGAGTGGG TCGCATGCATTGGAATTGTTCTTCGTAGTACTGGTGCCACTTACTA CGCGAGCTGGGCGGAAGGCCGATTCACCATCTCCAAAACCTCGCC GACCACGGTGACTCTGGAGATGACCAGTCTGACAGCCGCGGACAC GGCCACCTACTTCTGTGCGCTAGATCTCGGATATGGTGGTCCTTTG TGGGGCCCGGGCACCCTGGTCACCGTCTCCTCA | 83 |

**Table 7. Exemplary Anti-PACAP Light and Heavy Chain Nucleic Acid Sequences**

| Antibody ID | Designation | Nucleic Acid Sequence | SEQ ID NO: |
|---|---|---|---|
| | | ***Light chains*** | |
| mAb1 | LC-01 | GCCGATGTCGTGATGACCCAGACTCCATCCCCCGTGTCTGCAGCTG TGGGAGGCACAGTCACCATCAATTGCCGGGCCAGTGAGGACATTG AAAGCTTTTTAGCCTGGTATCAGCAGAAACCAGGGCAGCCTCCCA AGCTCCTGATCTCCAGGACATCCACTCTGGAATCTGGGGTCTCATC GCGGTTCAAAGGCAGTGGATCGGGGACAGAGTTCATTCTCACCAT CAGCGACCTGGAGTGTGCCGATGCTGCCACTTACTACTGTCAATGT ACTGATGGTAGTAGTAGTAGTAGTAGTTATGGTTGGGATGCTTTCG GCGGAGGGACCGAGGTGGTGGTCAAAGGTGATCCAGTTGCACCTA CTGTCCTCCTCTTCCCACCATCTAGCGATGAGGTGGCAACTGGAAC AGTCACCATCGTGTGTGTGGCGAATAAATACTTTCCCGATGTCACC GTCACCTGGGAGGTGGATGGCACCACCCAAACAACTGGCATCGAG AACAGTAAAACACCGCAGAATTCTGCAGATTGTACCTACAACCTC AGCAGCACTCTGACACTGACCAGCACACAGTACAACAGCCACAAA GAGTACACCTGCAAGGTGACCCAGGGCACGACCTCAGTCGTCCAG AGCTTCAGTAGGAAGAACTGT | 84 |
| mAb2 | LC-02 | GCCGATGTCGTGATGACCCAGACTCCATCCCCCGTGTCTGCGCAGG TGGGAGGCACAGTCACCATCAATTGCCAGGCCAGTGAGAGCATTG ATAGTGACTTATCCTGGTATCAACAGAAACCAGGGCAGCCTCCCA AGCTCCTGATCTACAGGACATTCACTCTGGAATCTGGGGTCCCATC GCGGTTCAAAGGCAGTGGATCTGGGACAGACTACACTCTCACCAT CAGCGACCTGGAGTGTGCCGATGCTGCCATTTACTACTGTCAATGC ACTGATGGTAGTAGTATTAGTGGTAGTTATGGTTGGGATGCTTTCG GCGGAGGGACCGAGGTGGTGGTCAAAGGTGATCCAGTTGCACCTA CTGTCCTCCTCTTCCCACCATCTAGCGATGAGGTGGCAACTGGAAC AGTCACCATCGTGTGTGTGGCGAATAAATACTTTCCCGATGTCACC GTCACCTGGGAGGTGGATGGCACCACCCAAACAACTGGCATCGAG AACAGTAAAACACCGCAGAATTCTGCAGATTGTACCTACAACCTC AGCAGCACTCTGACACTGACCAGCACACAGTACAACAGCCACAAA | 85 |

| Antibody ID | Designation | Nucleic Acid Sequence | SEQ ID NO: |
|---|---|---|---|
| | | GAGTACACCTGCAAGGTGACCCAGGGCACGACCTCAGTCGTCCAG AGCTTCAGTAGGAAGAACTGT | |
| mAb3 | LC-03 | GCCGATGTCGTGATGACCCAGACTCCATCCCCCGTGTCTGCAGCTG TGGGAGGCACAGTCACCATCAATTGCCGGGCCAGTGAGGACATTG AAAGCTTTTTAGCCTGGTATCAGCAGAAACCAGGGCAGCCTCCCA AGCTCCTGATGTCCAGGACATCCACTCTGGAATCTGGGGTCCCATC GCGGTTCAAAGGCAGTGGATCTGGGACAGAGTTCACTCTCACCAT CAGCGACCTGGAGTGTGCCGATGCTGCCACATATTACTGTCAATGT ACTGATGGTAGTAGTAGTAGTAGTAGTTATGGTTGGGATGCTTTCG GCGGAGGGACCGAGGTGGTGGTCAAAGGTGATCCAGTTGCACCTA CTGTCCTCCTCTTCCCACCATCTAGCGATGAGGTGGCAACTGGAAC AGTCACCATCGTGTGTGTGGCGAATAAATACTTTCCCGATGTCACC GTCACCTGGGAGGTGGATGGCACCACCCAAACAACTGGCATCGAG AACAGTAAAACACCGCAGAATTCTGCAGATTGTACCTACAACCTC AGCAGCACTCTGACACTGACCAGCACACAGTACAACAGCCACAAA GAGTACACCTGCAAGGTGACCCAGGGCACGACCTCAGTCGTCCAG AGCTTCAGTAGGAAGAACTGT | 86 |
| mAb4 | LC-04 | GCTGACATTGTGATGACCCAGACTCCAGCCTCCGTGTCTGGAGCTG TGGGAGGCACAGTCACCATCAAGTGCCAGGCCAGTCAGAGCATTA GGAATGAATTATCCTGGTATCAGCAGAAACCAGGGCAGCCTCCCA AGCTCCTGATCTACAAGGCATCCACTCTGGCATCTGGGGTCCCATC GCGGTTCAGTGGCAGTGGATTTGGGACAGAGTTCACTCTCACCATC AGCGGTGTGCAGTGTGATGATGCTGCCACTTACTACTGTCAAAAC AATTATGGTACTAGGCGTAATAATTATGTTTTTCCTTTCGGCGGAG GGACCGAGGTGGTGGTCAAAGGTGATCCAGTTGCACCTACTGTCC TCCTCTTCCCACCATCTAGCGATGAGGTGGCAACTGGAACAGTCAC CATCGTGTGTGTGGCGAATAAATACTTTCCCGATGTCACCGTCACC TGGGAGGTGGATGGCACCACCCAAACAACTGGCATCGAGAACAGT AAAACACCGCAGAATTCTGCAGATTGTACCTACAACCTCAGCAGC ACTCTGACACTGACCAGCACACAGTACAACAGCCACAAAGAGTAC ACCTGCAAGGTGACCCAGGGCACGACCTCAGTCGTCCAGAGCTTC AGTAGGAAGAACTGT | 87 |
| mAb5 | LC-05 | GCTGACATCGTGCTGACCCAGACTCCAGCCTCCGTGTCTGCAGCTG TGGGAGGCACAGTCAGCATCAGTTGCCAGTCCAGTGAAAGTGTTT ATAATAACAACTACTTATCCTGGTTTCAGCAGAAACCAGGGCAGC CTCCCAAGCTCCTGATCTATGGTGCATCCACTCTGGCATCTGGGGT CCCATCGCGGTTTGAAGGCAGTGGATCTGGGACACAGTTCACTCTC ACCATCAGCGACGTGCAGTGTGATGATGCTGCCACTTACTACTGTC TAGGCGATTATATTATTATTGAGAATATTTTCGGCGGAGGGACCGA GGTGGTGGTCAAAGGTGATCCAGTTGCACCTACTGTCCTCCTCTTC CCACCATCTAGCGATGAGGTGGCAACTGGAACAGTCACCATCGTG TGTGTGGCGAATAAATACTTTCCCGATGTCACCGTCACCTGGGAGG TGGATGGCACCACCCAAACAACTGGCATCGAGAACAGTAAAACAC CGCAGAATTCTGCAGATTGTACCTACAACCTCAGCAGCACTCTGAC ACTGACCAGCACACAGTACAACAGCCACAAAGAGTACACCTGCAA GGTGACCCAGGGCACGACCTCAGTCGTCCAGAGCTTCAGTAGGAA GAACTGT | 88 |
| mAb6 | LC-06 | GCGCAAGTGCTGACCCAGACTCCAGCCTCCGTGTCTGCGGCTGTG GGAGGCACAGTCACCATCAATTGCCAGGCCAGTCAGAGTGTTTAT AACAACAAAAATTTAGCCTGGTATCAGCAGAAACCAGGGCAGCC TCCCAAGCTCCTGATCTATTTTTCATCCACTCTGGCATCTGGGGTCC CATCGCGGTTCAGAGGCAGTGGATCTGGGACACAGTTCACTCTCA CCATCAGTGGCGTGCAGTGTGGCGATGCTGCCACTTACTACTGTCT | 89 |

| Antibody ID | Designation | Nucleic Acid Sequence | SEQ ID NO: |
|---|---|---|---|
| | | AGGCGAATTTGGTAGTATTTGGGCTTTCGGCGGAGGGACCGAGGT<br>GGTGGTCAAAGGTGATCCAGTTGCACCTACTGTCCTCCTCTTCCCA<br>CCATCTAGCGATGAGGTGGCAACTGGAACAGTCACCATCGTGTGT<br>GTGGCGAATAAATACTTTCCCGATGTCACCGTCACCTGGGAGGTG<br>GATGGCACCACCCAAACAACTGGCATCGAGAACAGTAAAACACCG<br>CAGAATTCTGCAGATTGTACCTACAACCTCAGCAGCACTCTGACAC<br>TGACCAGCACACAGTACAACAGCCACAAAGAGTACACCTGCAAGG<br>TGACCCAGGGCACGACCTCAGTCGTCCAGAGCTTCAGTAGGAAGA<br>ACTGT | |
| | | ***Heavy chains*** | |
| mAb1 | HC-01 | CAGTCGTTGGAGGAGTCCGGGGGAGACCTGGTCAAGCCTGGGG<br>CATCTCTGACACTCACCTGCAAAGCCTCTGGAATCGCCTTCAGTAA<br>CGACTACATGTGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGA<br>GTGGATCGCATGTATTTTTACTGGTAGTAGTGGTAGTACTTACTAC<br>GCGAGCTGGGCGAAAGGTCGATTCACCATCTCCAAAACCTCGTCG<br>ACCACGGTGACTCTGCAAATGACCAGTCTGACAGCCGCGGACACG<br>GCCACCTATTTCTGTGCGAGAGATAGGGCTATTTCTGTACTTGGTT<br>ATTTCTATGCTGCATACTTCTTTGACTTCTGGGGCCCAGGCACCCT<br>GGTCACCGTCTCCTCAGGGCAACCTAAGGCTCCATCAGTCTTCCCA<br>CTGGCCCCCTGCTGCGGGGACACACCCAGCTCCACGGTGACCTTG<br>GGCTGCCTGGTCAAAGGCTACCTCCCGGAGCCAGTGACCGTGACC<br>TGGAACTCGGGCACCCTCACCAATGGGGTACGCACCTTCCCGTCC<br>GTCCGGCAGTCCTCAGGCCTCTACTCGCTGAGCAGCGTGGTGAGC<br>GTGACCTCAAGCAGCCAGCCCGTCACCTGCAACGTGGCCCACCCA<br>GCCACCAACACCAAAGTGGACAAGACCGTTGCGCCCTCGACATGC<br>AGCAAGCCCACGTGCCCACCCCCTGAACTCCTGGGGGGACCGTCT<br>GTCTTCATCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCAC<br>GCACCCCCGAGGTCACATGCGTGGTGGTGGACGTGAGCCAGGATG<br>ACCCCGAGGTGCAGTTCACATGGTACATAAACAACGAGCAGGTGC<br>GCACCGCCCGGCCGCCGCTACGGGAGCAGCAGTTCAACAGCACGA<br>TCCGCGTGGTCAGCACCCTCCCCATCGCGCACCAGGACTGGCTGA<br>GGGGCAAGGAGTTCAAGTGCAAAGTCCACAACAAGGCACTCCCG<br>GCCCCCATCGAGAAAACCATCTCCAAAGCCAGAGGGCAGCCCCTG<br>GAGCCGAAGGTCTACACCATGGGCCCTCCCCGGGAGGAGCTGAGC<br>AGCAGGTCGGTCAGCCTGACCTGCATGATCAACGGCTTCTACCCTT<br>CCGACATCTCGGTGGAGTGGGAGAAGAACGGGAAGGCAGAGGAC<br>AACTACAAGACCACGCCGGCCGTGCTGGACAGCGACGGCTCCTAC<br>TTCCTCTACAGCAAGCTCTCAGTGCCCACGAGTGAGTGGCAGCGG<br>GGCGACGTCTTCACCTGCTCCGTGATGCACGAGGCCTTGCACAACC<br>ACTACACGCAGAAGTCCATCTCCCGCTCTCCGGGTAAA | 90 |
| mAb2 | HC-02 | CAGTCGTTGGAGGAGTCCGGGGGAGGCCTGGTCCAGCCTGAGG<br>GATCCCTGACACTCACCTGCACAGCTTCTGGATTCTCCTTCAATAG<br>CAGCTCCTACATGTGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCT<br>GGAGTGGATCGGATGCATTTTTACTGGTAGTAGTGGTAATACCTAC<br>TACGCGACCTGGGCGCAAGGTCGATTCACCATCTCCAAGACCTCG<br>ACCACGGTGACTCTGGAAATGACCAGTCTGACAGCCGCGGACACG<br>GCCACCTATTTCTGTGCGAGAGATAGGGCTTATTTTTTGGTTGGTT<br>ATTTCTATGCTACATATTATTTTGACTTATGGGGCCCAGGCACCCT<br>GGTCACCGTCTCCTCAGGGCAACCTAAGGCTCCATCAGTCTTCCCA<br>CTGGCCCCCTGCTGCGGGGACACACCCTCTAGCACGGTGACCTTG<br>GGCTGCCTGGTCAAAGGCTACCTCCCGGAGCCAGTGACCGTGACC<br>TGGAACTCGGGCACCCTCACCAATGGGGTACGCACCTTCCCGTCC | 91 |

| Antibody ID | Designation | Nucleic Acid Sequence | SEQ ID NO: |
|---|---|---|---|
| | | GTCCGGCAGTCCTCAGGCCTCTACTCGCTGAGCAGCGTGGTGAGC<br>GTGACCTCAAGCAGCCAGCCCGTCACCTGCAACGTGGCCCACCCA<br>GCCACCAACACCAAAGTGGACAAGACCGTTGCGCCCTCGACATGC<br>AGCAAGCCCACGTGCCCACCCCCTGAACTCCTGGGGGGACCGTCT<br>GTCTTCATCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCAC<br>GCACCCCCGAGGTCACATGCGTGGTGGTGGACGTGAGCCAGGATG<br>ACCCCGAGGTGCAGTTCACATGGTACATAAACAACGAGCAGGTGC<br>GCACCGCCCGGCCGCCGCTACGGGAGCAGCAGTTCAACAGCACGA<br>TCCGCGTGGTCAGCACCCTCCCCATCGCGCACCAGGACTGGCTGA<br>GGGGCAAGGAGTTCAAGTGCAAAGTCCACAACAAGGCACTCCCG<br>GCCCCCATCGAGAAAACCATCTCCAAAGCCAGAGGGCAGCCCCTG<br>GAGCCGAAGGTCTACACCATGGGCCCTCCCCGGGAGGAGCTGAGC<br>AGCAGGTCGGTCAGCCTGACCTGCATGATCAACGGCTTCTACCCTT<br>CCGACATCTCGGTGGAGTGGGAGAAGAACGGGAAGGCAGAGGAC<br>AACTACAAGACCACGCCGGCCGTGCTGGACAGCGACGGCTCCTAC<br>TTCCTCTACAGCAAGCTCTCAGTGCCCACGAGTGAGTGGCAGCGG<br>GGCGACGTCTTCACCTGCTCCGTGATGCACGAGGCCTTGCACAACC<br>ACTACACGCAGAAGTCCATCTCCCGCTCTCCGGGTAAA | |
| mAb3 | HC-03 | CAGTCGTTGGAGGAGTCCGGGGGAGGCCTGGTCCAGCCTGGGG<br>CATCTCTGACACTCACCTGTACAGCCTCTGGATTCTCCTTCAGTAG<br>CAACTACTACATGTGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCT<br>GGAGTGGATCGCATGCATTTTTACTGGTAGTAGTGGTAGCACTTAC<br>TACGCGAGCTGGGCGAAAGGCCGATTCACCATCTCCAAAACCTCG<br>TCGACCACGGTGACTCTGCACGTGACCAGTCTGACAGCCGCGGAC<br>ACGGCCACCTATTTCTGTGCGAGAGATAGGGCTTTGTCTGTTGTTG<br>GTTATTTCTATGCTGCATACTACTTTGACTTCTGGGGCCCAGGCAC<br>CCTGGTCACCGTCTCCTCAGGGCAACCTAAGGCTCCATCAGTCTTC<br>CCACTGGCCCCCTGCTGCGGGGACACACCCTCTAGCACGGTGACC<br>TTGGGCTGCCTGGTCAAAGGCTACCTCCCGGAGCCAGTGACCGTG<br>ACCTGGAACTCGGGCACCCTCACCAATGGGGTACGCACCTTCCCG<br>TCCGTCCGGCAGTCCTCAGGCCTCTACTCGCTGAGCAGCGTGGTGA<br>GCGTGACCTCAAGCAGCCAGCCCGTCACCTGCAACGTGGCCCACC<br>CAGCCACCAACACCAAAGTGGACAAGACCGTTGCGCCCTCGACAT<br>GCAGCAAGCCCACGTGCCCACCCCCTGAACTCCTGGGGGGACCGT<br>CTGTCTTCATCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTC<br>ACGCACCCCCGAGGTCACATGCGTGGTGGTGGACGTGAGCCAGGA<br>TGACCCCGAGGTGCAGTTCACATGGTACATAAACAACGAGCAGGT<br>GCGCACCGCCCGGCCGCCGCTACGGGAGCAGCAGTTCAACAGCAC<br>GATCCGCGTGGTCAGCACCCTCCCCATCGCGCACCAGGACTGGCT<br>GAGGGGCAAGGAGTTCAAGTGCAAAGTCCACAACAAGGCACTCCC<br>GGCCCCCATCGAGAAAACCATCTCCAAAGCCAGAGGGCAGCCCCT<br>GGAGCCGAAGGTCTACACCATGGGCCCTCCCCGGGAGGAGCTGAG<br>CAGCAGGTCGGTCAGCCTGACCTGCATGATCAACGGCTTCTACCCT<br>TCCGACATCTCGGTGGAGTGGGAGAAGAACGGGAAGGCAGAGGA<br>CAACTACAAGACCACGCCGGCCGTGCTGGACAGCGACGGCTCCTA<br>CTTCCTCTACAGCAAGCTCTCAGTGCCCACGAGTGAGTGGCAGCG<br>GGGCGACGTCTTCACCTGCTCCGTGATGCACGAGGCCTTGCACAA<br>CCACTACACGCAGAAGTCCATCTCCCGCTCTCCGGGTAAA | 92 |
| mAb4 | HC-04 | CAGTCGGTGGAGGAGTCCGGGGGTCGCCTGGTCACGCCTGGGACA<br>CCCCTGACACTCACCTGCACAGCCTCTGGAATCGACCTCAGTAGCT<br>ATGCAATGATCTGGGTCCGCCAGGCTCCAGGAGAGGGACTGGAAT<br>ACATCGGATTCATTGATGCTGGTGATGGTAACACTTACTACGCGAG<br>CTGGGCAAAAGGCCGATTCACCATCTCCAAAACCTCGACCACGGT | 93 |

| Antibody ID | Designation | Nucleic Acid Sequence | SEQ ID NO: |
|---|---|---|---|
| | | GGATCTGAAAATCACCAGTCCGACAACCGAGGACACGGCCACCTA<br>TTTCTGTGCCAGAGGAGATCCTGGTTGGAGTAATGGTTTTGCCTTG<br>TGGGGCCAAGGCACCCTGGTCACCGTCTCCTCAGGGCAACCTAAG<br>GCTCCATCAGTCTTCCCACTGGCCCCCTGCTGCGGGGACACACCCT<br>CTAGCACGGTGACCTTGGGCTGCCTGGTCAAAGGCTACCTCCCGG<br>AGCCAGTGACCGTGACCTGGAACTCGGGCACCCTCACCAATGGGG<br>TACGCACCTTCCCGTCCGTCCGGCAGTCCTCAGGCCTCTACTCGCT<br>GAGCAGCGTGGTGAGCGTGACCTCAAGCAGCCAGCCCGTCACCTG<br>CAACGTGGCCCACCCAGCCACCAACACCAAAGTGGACAAGACCGT<br>TGCGCCCTCGACATGCAGCAAGCCCACGTGCCCACCCCCTGAACT<br>CCTGGGGGGACCGTCTGTCTTCATCTTCCCCCCAAAACCCAAGGAC<br>ACCCTCATGATCTCACGCACCCCCGAGGTCACATGCGTGGTGGTG<br>GACGTGAGCCAGGATGACCCCGAGGTGCAGTTCACATGGTACATA<br>AACAACGAGCAGGTGCGCACCGCCCGGCCGCCGCTACGGGAGCA<br>GCAGTTCAACAGCACGATCCGCGTGGTCAGCACCCTCCCCATCGC<br>GCACCAGGACTGGCTGAGGGGCAAGGAGTTCAAGTGCAAAGTCCA<br>CAACAAGGCACTCCCGGCCCCCATCGAGAAAACCATCTCCAAAGC<br>CAGAGGGCAGCCCCTGGAGCCGAAGGTCTACACCATGGGCCCTCC<br>CCGGGAGGAGCTGAGCAGCAGGTCGGTCAGCCTGACCTGCATGAT<br>CAACGGCTTCTACCCTTCCGACATCTCGGTGGAGTGGGAGAAGAA<br>CGGGAAGGCAGAGGACAACTACAAGACCACGCCGGCCGTGCTGG<br>ACAGCGACGGCTCCTACTTCCTCTACAGCAAGCTCTCAGTGCCCAC<br>GAGTGAGTGGCAGCGGGGCGACGTCTTCACCTGCTCCGTGATGCA<br>CGAGGCCTTGCACAACCACTACACGCAGAAGTCCATCTCCCGCTCT<br>CCGGGTAAA | |
| mAb5 | HC-05 | CAGTCGGTGGAGGAGTCCGGGGGTCGCCTGGTCACGCCTGGGACA<br>CCCCTGACACTCACCTGCACAGTCTCTGGATTCTCCCTCAGTACCT<br>ACAATATGTGCTGGGTCCGCCAGGCTCCAGGGAAGGGCTTGGAAT<br>GGGTCGGTTTCATTAATTCTGATGATAGCGCGTACTACGCGAGCTG<br>GGCGAAAGGCCGCTTCACCTTCTCCAAGACCTCGACCACGGTGGA<br>TCTGAAAATCGCCAGTCCGACAACCGAGGACACGGCCACCTATTT<br>CTGTGCCAGATATGATTGGGATTATTATTATAGTCGGTTGGATCTC<br>TGGGGCCCGGGCACCCTGGTCACCGTCTCCTCAGGGCAACCTAAG<br>GCTCCATCAGTCTTCCCACTGGCCCCCTGCTGCGGGGACACACCCT<br>CTAGCACGGTGACCTTGGGCTGCCTGGTCAAAGGCTACCTCCCGG<br>AGCCAGTGACCGTGACCTGGAACTCGGGCACCCTCACCAATGGGG<br>TACGCACCTTCCCGTCCGTCCGGCAGTCCTCAGGCCTCTACTCGCT<br>GAGCAGCGTGGTGAGCGTGACCTCAAGCAGCCAGCCCGTCACCTG<br>CAACGTGGCCCACCCAGCCACCAACACCAAAGTGGACAAGACCGT<br>TGCGCCCTCGACATGCAGCAAGCCCACGTGCCCACCCCCTGAACT<br>CCTGGGGGGACCGTCTGTCTTCATCTTCCCCCCAAAACCCAAGGAC<br>ACCCTCATGATCTCACGCACCCCCGAGGTCACATGCGTGGTGGTG<br>GACGTGAGCCAGGATGACCCCGAGGTGCAGTTCACATGGTACATA<br>AACAACGAGCAGGTGCGCACCGCCCGGCCGCCGCTACGGGAGCA<br>GCAGTTCAACAGCACGATCCGCGTGGTCAGCACCCTCCCCATCGC<br>GCACCAGGACTGGCTGAGGGGCAAGGAGTTCAAGTGCAAAGTCCA<br>CAACAAGGCACTCCCGGCCCCCATCGAGAAAACCATCTCCAAAGC<br>CAGAGGGCAGCCCCTGGAGCCGAAGGTCTACACCATGGGCCCTCC<br>CCGGGAGGAGCTGAGCAGCAGGTCGGTCAGCCTGACCTGCATGAT<br>CAACGGCTTCTACCCTTCCGACATCTCGGTGGAGTGGGAGAAGAA<br>CGGGAAGGCAGAGGACAACTACAAGACCACGCCGGCCGTGCTGG<br>ACAGCGACGGCTCCTACTTCCTCTACAGCAAGCTCTCAGTGCCCAC<br>GAGTGAGTGGCAGCGGGGCGACGTCTTCACCTGCTCCGTGATGCA | 94 |

| Antibody ID | Designation | Nucleic Acid Sequence | SEQ ID NO: |
|---|---|---|---|
| | | CGAGGCCTTGCACAACCACTACACGCAGAAGTCCATCTCCCGCTCT CCGGGTAAA | |
| mAb6 | HC-06 | CAGCTGGAGGAGTCCGGGGGAGGCCTGGTCCAGCCTGGGGCATCC CTGACACTCACCTGCACAACTTCTGGATTCTCTCTCAGTGATAATT ATTTGTGTTGGGTCCGCCAGGCTCCAGGGCGTGGGCTGGAGTGGG TCGCATGCATTGGAATTGTTCTTCGTAGTACTGGTGCCACTTACTA CGCGAGCTGGGCGGAAGGCCGATTCACCATCTCCAAAACCTCGCC GACCACGGTGACTCTGGAGATGACCAGTCTGACAGCCGCGGACAC GGCCACCTACTTCTGTGCGCTAGATCTCGGATATGGTGGTCCTTTG TGGGGCCCGGGCACCCTGGTCACCGTCTCCTCAGGGCAACCTAAG GCTCCATCAGTCTTCCCACTGGCCCCCTGCTGCGGGGACACACCCT CTAGCACGGTGACCTTGGGCTGCCTGGTCAAAGGCTACCTCCCGG AGCCAGTGACCGTGACCTGGAACTCGGGCACCCTCACCAATGGGG TACGCACCTTCCCGTCCGTCCGGCAGTCCTCAGGCCTCTACTCGCT GAGCAGCGTGGTGAGCGTGACCTCAAGCAGCCAGCCCGTCACCTG CAACGTGGCCCACCCAGCCACCAACACCAAAGTGGACAAGACCGT TGCGCCCTCGACATGCAGCAAGCCCACGTGCCCACCCCCTGAACT CCTGGGGGGACCGTCTGTCTTCATCTTCCCCCCAAAACCCAAGGAC ACCCTCATGATCTCACGCACCCCCGAGGTCACATGCGTGGTGGTG GACGTGAGCCAGGATGACCCCGAGGTGCAGTTCACATGGTACATA AACAACGAGCAGGTGCGCACCGCCCGGCCGCCGCTACGGGAGCA GCAGTTCAACAGCACGATCCGCGTGGTCAGCACCCTCCCCATCGC GCACCAGGACTGGCTGAGGGGCAAGGAGTTCAAGTGCAAAGTCCA CAACAAGGCACTCCCGGCCCCCATCGAGAAAACCATCTCCAAAGC CAGAGGGCAGCCCCTGGAGCCGAAGGTCTACACCATGGGCCCTCC CCGGGAGGAGCTGAGCAGCAGGTCGGTCAGCCTGACCTGCATGAT CAACGGCTTCTACCCTTCCGACATCTCGGTGGAGTGGGAGAAGAA CGGGAAGGCAGAGGACAACTACAAGACCACGCCGGCCGTGCTGG ACAGCGACGGCTCCTACTTCCTCTACAGCAAGCTCTCAGTGCCCAC GAGTGAGTGGCAGCGGGGCGACGTCTTCACCTGCTCCGTGATGCA CGAGGCCTTGCACAACCACTACACGCAGAAGTCCATCTCCCGCTCT CCGGGTAAA | 95 |

**[0098]** Isolated nucleic acids encoding the anti-PACAP binding domain of the antigen binding proteins of the invention may comprise a nucleotide sequence that is at least 80% identical, at least 90% identical, at least 95% identical, or at least 98% identical to any of the nucleotide sequences listed in Table 6. In some embodiments, an isolated nucleic acid encoding an anti-PACAP light chain variable region comprises a sequence that is at least 80% identical, at least 90% identical, at least 95% identical, or at least 98% identical to a sequence selected from SEQ ID NOs: 72 to 77. In certain embodiments, an isolated nucleic acid encoding an anti-PACAP light chain variable region comprises a sequence selected from SEQ ID NOs: 72 to 77. In related embodiments, an isolated nucleic acid encoding an anti-PACAP heavy chain variable region comprises a sequence that is at least 80% identical, at least 90% identical, at least 95% identical, or at least 98% identical to a sequence selected from SEQ ID NOs: 78 to 83. In other related

embodiments, an isolated nucleic acid encoding an anti-PACAP heavy chain variable region comprises a sequence selected from SEQ ID NOs: 78 to 83.

**[0099]** In embodiments in which the antigen binding protein of the invention is an antibody, the isolated nucleic acid encoding the antibody light chain and heavy chain may comprise a nucleotide sequence that is at least 60% identical, at least 70% identical, at least 80% identical, at least 90% identical, at least 95% identical, or at least 98% identical to any of the nucleotide sequences listed in Table 7. In certain embodiments, the isolated nucleic acid encoding a light chain of an anti-PACAP antibody of the invention comprises a sequence that is at least 60% identical, at least 70% identical, at least 80% identical, at least 90% identical, at least 95% identical, or at least 98% identical to a sequence selected from SEQ ID NOs: 84 to 89. In some embodiments, the isolated nucleic acid encoding a light chain of an anti-PACAP antibody comprises a sequence selected from SEQ ID NOs: 84 to 89. In these and other embodiments, the isolated nucleic acid encoding a heavy chain of an anti-PACAP antibody of the invention may comprise a nucleotide sequence that is at least 60% identical, at least 70% identical, at least 80% identical, at least 90% identical, at least 95% identical, or at least 98% identical to a sequence selected from SEQ ID NOs: 90 to 95. In certain embodiments, the isolated nucleic acid encoding a heavy chain of an anti-PACAP antibody of the invention comprises a sequence selected from SEQ ID NOs: 90 to 95.

**[0100]** The nucleic acid sequences provided in Tables 6 and 7 are exemplary only. As will be appreciated by those in the art, due to the degeneracy of the genetic code, an extremely large number of nucleic acids may be made, all of which encode the CDRs, variable regions, and heavy and light chains or other components of the antigen binding proteins described herein. Thus, having identified a particular amino acid sequence, those skilled in the art could make any number of different nucleic acids, by simply modifying the sequence of one or more codons in a way which does not change the amino acid sequence of the encoded protein.

**[0101]** The present invention also includes vectors comprising one or more nucleic acids encoding one or more components of the antigen binding proteins of the invention (e.g. variable regions, light chains, and heavy chains). The term "vector" refers to any molecule or entity (e.g., nucleic acid, plasmid, bacteriophage or virus) used to transfer protein coding information into a host cell. Examples of vectors include, but are not limited to, plasmids, viral vectors, non-episomal mammalian vectors and expression vectors, for example, recombinant expression

vectors. The term "expression vector" or "expression construct" as used herein refers to a recombinant DNA molecule containing a desired coding sequence and appropriate nucleic acid control sequences necessary for the expression of the operably linked coding sequence in a particular host cell. An expression vector can include, but is not limited to, sequences that affect or control transcription, translation, and, if introns are present, affect RNA splicing of a coding region operably linked thereto. Nucleic acid sequences necessary for expression in prokaryotes include a promoter, optionally an operator sequence, a ribosome binding site and possibly other sequences. Eukaryotic cells are known to utilize promoters, enhancers, and termination and polyadenylation signals. A secretory signal peptide sequence can also, optionally, be encoded by the expression vector, operably linked to the coding sequence of interest, so that the expressed polypeptide can be secreted by the recombinant host cell, for more facile isolation of the polypeptide of interest from the cell, if desired. For instance, in some embodiments, signal peptide sequences may be appended/fused to the amino terminus of any of the polypeptides sequences listed in Tables 2 and 5. In certain embodiments, a signal peptide having the amino acid sequence of MDMRVPAQLLGLLLLWLRGARC (SEQ ID NO: 96) is fused to the amino terminus of any of the polypeptide sequences in Tables 2 and 5. In other embodiments, a signal peptide having the amino acid sequence of MAWALLLLTLLTQGTGSWA (SEQ ID NO: 97) is fused to the amino terminus of any of the polypeptide sequences in Tables 2 and 5. In still other embodiments, a signal peptide having the amino acid sequence of MTCSPLLLTLLIHCTGSWA (SEQ ID NO: 98) is fused to the amino terminus of any of the polypeptide sequences in Tables 2 and 5. Other suitable signal peptide sequences that can be fused to the amino terminus of the polypeptide sequences described herein include: MEAPAQLLFLLLLWLPDTTG (SEQ ID NO: 99), MEWTWRVLFLVAAATGAHS (SEQ ID NO: 100), METPAQLLFLLLLWLPDTTG (SEQ ID NO: 101), METPAQLLFLLLLWLPDTTG (SEQ ID NO: 102), MKHLWFFLLLVAAPRWVLS (SEQ ID NO: 103), MEWSWVFLFFLSVTTGVHS (SEQ ID NO: 104), MDIRAPTQLLGLLLLWLPGAKC (SEQ ID NO: 105), MDIRAPTQLLGLLLLWLPGARC (SEQ ID NO: 106), MDTRAPTQLLGLLLLWLPGATF (SEQ ID NO: 107), MDTRAPTQLLGLLLLWLPGARC (SEQ ID NO: 108), METGLRWLLLVAVLKGVQC (SEQ ID NO: 109), METGLRWLLLVAVLKGVQCQE (SEQ ID NO: 110), and MDMRAPTQLLGLLLLWLPGARC (SEQ ID NO: 111). Other signal

peptides are known to those of skill in the art and may be fused to any of the polypeptide chains listed in Tables 2 and 5, for example, to facilitate or optimize expression in particular host cells.

**[0102]** Typically, expression vectors used in the host cells to produce the antigen binding proteins of the invention will contain sequences for plasmid maintenance and for cloning and expression of exogenous nucleotide sequences encoding the components of the antigen binding proteins. Such sequences, collectively referred to as "flanking sequences," in certain embodiments will typically include one or more of the following nucleotide sequences: a promoter, one or more enhancer sequences, an origin of replication, a transcriptional termination sequence, a complete intron sequence containing a donor and acceptor splice site, a sequence encoding a leader sequence for polypeptide secretion, a ribosome binding site, a polyadenylation sequence, a polylinker region for inserting the nucleic acid encoding the polypeptide to be expressed, and a selectable marker element. Each of these sequences is discussed below.

**[0103]** Optionally, the vector may contain a "tag"-encoding sequence, i.e., an oligonucleotide molecule located at the 5' or 3' end of the polypeptide coding sequence; the oligonucleotide tag sequence encodes polyHis (such as hexaHis), FLAG, HA (hemaglutinin influenza virus), myc, or another "tag" molecule for which commercially available antibodies exist. This tag is typically fused to the polypeptide upon expression of the polypeptide, and can serve as a means for affinity purification or detection of the polypeptide from the host cell. Affinity purification can be accomplished, for example, by column chromatography using antibodies against the tag as an affinity matrix. Optionally, the tag can subsequently be removed from the purified polypeptide by various means such as using certain peptidases for cleavage.

**[0104]** Flanking sequences may be homologous (i.e., from the same species and/or strain as the host cell), heterologous (i.e., from a species other than the host cell species or strain), hybrid (i.e., a combination of flanking sequences from more than one source), synthetic or native. As such, the source of a flanking sequence may be any prokaryotic or eukaryotic organism, any vertebrate or invertebrate organism, or any plant, provided that the flanking sequence is functional in, and can be activated by, the host cell machinery.

**[0105]** Flanking sequences useful in the vectors of this invention may be obtained by any of several methods well known in the art. Typically, flanking sequences useful herein will have been previously identified by mapping and/or by restriction endonuclease digestion and can thus be isolated from the proper tissue source using the appropriate restriction endonucleases. In some

cases, the full nucleotide sequence of a flanking sequence may be known. Here, the flanking sequence may be synthesized using routine methods for nucleic acid synthesis or cloning.

**[0106]** Whether all or only a portion of the flanking sequence is known, it may be obtained using polymerase chain reaction (PCR) and/or by screening a genomic library with a suitable probe such as an oligonucleotide and/or flanking sequence fragment from the same or another species. Where the flanking sequence is not known, a fragment of DNA containing a flanking sequence may be isolated from a larger piece of DNA that may contain, for example, a coding sequence or even another gene or genes. Isolation may be accomplished by restriction endonuclease digestion to produce the proper DNA fragment followed by isolation using agarose gel purification, Qiagen® column chromatography (Chatsworth, CA), or other methods known to the skilled artisan. The selection of suitable enzymes to accomplish this purpose will be readily apparent to one of ordinary skill in the art.

**[0107]** An origin of replication is typically a part of those prokaryotic expression vectors purchased commercially, and the origin aids in the amplification of the vector in a host cell. If the vector of choice does not contain an origin of replication site, one may be chemically synthesized based on a known sequence, and ligated into the vector. For example, the origin of replication from the plasmid pBR322 (New England Biolabs, Beverly, MA) is suitable for most gram-negative bacteria, and various viral origins (e.g., SV40, polyoma, adenovirus, vesicular stomatitus virus (VSV), or papillomaviruses such as HPV or BPV) are useful for cloning vectors in mammalian cells. Generally, the origin of replication component is not needed for mammalian expression vectors (for example, the SV40 origin is often used only because it also contains the virus early promoter).

**[0108]** A transcription termination sequence is typically located 3' to the end of a polypeptide coding region and serves to terminate transcription. Usually, a transcription termination sequence in prokaryotic cells is a G-C rich fragment followed by a poly-T sequence. While the sequence is easily cloned from a library or even purchased commercially as part of a vector, it can also be readily synthesized using known methods for nucleic acid synthesis.

**[0109]** A selectable marker gene encodes a protein necessary for the survival and growth of a host cell grown in a selective culture medium. Typical selection marker genes encode proteins that (a) confer resistance to antibiotics or other toxins, e.g., ampicillin, tetracycline, or kanamycin for prokaryotic host cells; (b) complement auxotrophic deficiencies of the cell; or (c)

supply critical nutrients not available from complex or defined media. Specific selectable markers are the kanamycin resistance gene, the ampicillin resistance gene, and the tetracycline resistance gene. Advantageously, a neomycin resistance gene may also be used for selection in both prokaryotic and eukaryotic host cells.

**[0110]** Other selectable genes may be used to amplify the gene that will be expressed. Amplification is the process wherein genes that are required for production of a protein critical for growth or cell survival are reiterated in tandem within the chromosomes of successive generations of recombinant cells. Examples of suitable selectable markers for mammalian cells include dihydrofolate reductase (DHFR) and promoterless thymidine kinase genes. Mammalian cell transformants are placed under selection pressure wherein only the transformants are uniquely adapted to survive by virtue of the selectable gene present in the vector. Selection pressure is imposed by culturing the transformed cells under conditions in which the concentration of selection agent in the medium is successively increased, thereby leading to the amplification of both the selectable gene and the DNA that encodes another gene, such as one or more components of the antigen binding proteins described herein. As a result, increased quantities of a polypeptide are synthesized from the amplified DNA.

**[0111]** A ribosome-binding site is usually necessary for translation initiation of mRNA and is characterized by a Shine-Dalgarno sequence (prokaryotes) or a Kozak sequence (eukaryotes). The element is typically located 3' to the promoter and 5' to the coding sequence of the polypeptide to be expressed. In certain embodiments, one or more coding regions may be operably linked to an internal ribosome binding site (IRES), allowing translation of two open reading frames from a single RNA transcript.

**[0112]** In some cases, such as where glycosylation is desired in a eukaryotic host cell expression system, one may manipulate the various pre- or prosequences to improve glycosylation or yield. For example, one may alter the peptidase cleavage site of a particular signal peptide, or add prosequences, which also may affect glycosylation. The final protein product may have, in the -1 position (relative to the first amino acid of the mature protein) one or more additional amino acids incident to expression, which may not have been totally removed. For example, the final protein product may have one or two amino acid residues found in the peptidase cleavage site, attached to the amino-terminus. Alternatively, use of some enzyme cleavage sites may result in a

slightly truncated form of the desired polypeptide, if the enzyme cuts at such area within the mature polypeptide.

**[0113]** Expression and cloning vectors of the invention will typically contain a promoter that is recognized by the host organism and operably linked to the molecule encoding the polypeptide. The term "operably linked" as used herein refers to the linkage of two or more nucleic acid sequences in such a manner that a nucleic acid molecule capable of directing the transcription of a given gene and/or the synthesis of a desired protein molecule is produced. For example, a control sequence in a vector that is "operably linked" to a protein coding sequence is ligated thereto so that expression of the protein coding sequence is achieved under conditions compatible with the transcriptional activity of the control sequences. More specifically, a promoter and/or enhancer sequence, including any combination of cis-acting transcriptional control elements is operably linked to a coding sequence if it stimulates or modulates the transcription of the coding sequence in an appropriate host cell or other expression system.

**[0114]** Promoters are non-transcribed sequences located upstream (i.e., 5') to the start codon of a structural gene (generally within about 100 to 1000 bp) that control transcription of the structural gene. Promoters are conventionally grouped into one of two classes: inducible promoters and constitutive promoters. Inducible promoters initiate increased levels of transcription from DNA under their control in response to some change in culture conditions, such as the presence or absence of a nutrient or a change in temperature. Constitutive promoters, on the other hand, uniformly transcribe a gene to which they are operably linked, that is, with little or no control over gene expression. A large number of promoters, recognized by a variety of potential host cells, are well known. A suitable promoter is operably linked to the DNA encoding e.g., heavy chain, light chain, or other component of the antigen binding proteins of the invention, by removing the promoter from the source DNA by restriction enzyme digestion and inserting the desired promoter sequence into the vector.

**[0115]** Suitable promoters for use with yeast hosts are also well known in the art. Yeast enhancers are advantageously used with yeast promoters. Suitable promoters for use with mammalian host cells are well known and include, but are not limited to, those obtained from the genomes of viruses such as polyoma virus, fowlpox virus, adenovirus (such as Adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, retroviruses, hepatitis-B virus and

most preferably Simian Virus 40 (SV40). Other suitable mammalian promoters include heterologous mammalian promoters, for example, heat-shock promoters and the actin promoter.

**[0116]** Additional promoters which may be of interest include, but are not limited to: SV40 early promoter (Benoist and Chambon, 1981, Nature 290:304-310); CMV promoter (Thornsen et al., 1984, Proc. Natl. Acad. U.S.A. 81:659-663); the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto et al., 1980, Cell 22:787-797); herpes thymidine kinase promoter (Wagner et al., 1981, Proc. Natl. Acad. Sci. U.S.A. 78: 1444-1445); promoter and regulatory sequences from the metallothionine gene Prinster et al., 1982, Nature 296:39-42); and prokaryotic promoters such as the beta-lactamase promoter (Villa-Kamaroff et al., 1978, Proc. Natl. Acad. Sci. U.S.A. 75:3727-3731); or the tac promoter (DeBoer et al., 1983, Proc. Natl. Acad. Sci. U.S.A. 80:21-25). Also of interest are the following animal transcriptional control regions, which exhibit tissue specificity and have been utilized in transgenic animals: the elastase I gene control region that is active in pancreatic acinar cells (Swift et al., 1984, Cell 38:639-646; Ornitz et al., 1986, Cold Spring Harbor Symp. Quant. Biol. 50:399-409; MacDonald, 1987, Hepatology 7:425-515); the insulin gene control region that is active in pancreatic beta cells (Hanahan, 1985, Nature 315: 115-122); the immunoglobulin gene control region that is active in lymphoid cells (Grosschedl et al., 1984, Cell 38:647-658; Adames et al., 1985, Nature 318:533-538; Alexander et al., 1987, Mol. Cell. Biol. 7: 1436-1444); the mouse mammary tumor virus control region that is active in testicular, breast, lymphoid and mast cells (Leder et al., 1986, Cell 45:485-495); the albumin gene control region that is active in liver (Pinkert et al., 1987, Genes and Devel. 1 :268-276); the alpha-feto-protein gene control region that is active in liver (Krumlauf et al., 1985, Mol. Cell. Biol. 5: 1639-1648; Hammer et al., 1987, Science 253:53-58); the alpha 1-antitrypsin gene control region that is active in liver (Kelsey et al., 1987, Genes and Devel. 1: 161-171); the beta-globin gene control region that is active in myeloid cells (Mogram et al, 1985, Nature 315:338-340; Kollias et al, 1986, Cell 46:89-94); the myelin basic protein gene control region that is active in oligodendrocyte cells in the brain (Readhead et al., 1987, Cell 48:703-712); the myosin light chain-2 gene control region that is active in skeletal muscle (Sani, 1985, Nature 314:283-286); and the gonadotropic releasing hormone gene control region that is active in the hypothalamus (Mason et al., 1986, Science 234: 1372-1378).

**[0117]** An enhancer sequence may be inserted into the vector to increase transcription of DNA encoding a component of the antigen binding proteins (e.g., light chain, heavy chain, or variable

regions) by higher eukaryotes. Enhancers are cis-acting elements of DNA, usually about 10-300 bp in length, that act on the promoter to increase transcription. Enhancers are relatively orientation and position independent, having been found at positions both 5' and 3' to the transcription unit. Several enhancer sequences available from mammalian genes are known (e.g., globin, elastase, albumin, alpha-feto-protein and insulin). Typically, however, an enhancer from a virus is used. The SV40 enhancer, the cytomegalovirus early promoter enhancer, the polyoma enhancer, and adenovirus enhancers known in the art are exemplary enhancing elements for the activation of eukaryotic promoters. While an enhancer may be positioned in the vector either 5' or 3' to a coding sequence, it is typically located at a site 5' from the promoter. A sequence encoding an appropriate native or heterologous signal sequence (leader sequence or signal peptide) can be incorporated into an expression vector, to promote extracellular secretion of the antigen binding protein. The choice of signal peptide or leader depends on the type of host cells in which the antibody binding protein is to be produced, and a heterologous signal sequence can replace the native signal sequence. Examples of signal peptides are described above. Other signal peptides that are functional in mammalian host cells include the signal sequence for interleukin-7 (IL-7) described in US Patent No. 4,965,195; the signal sequence for interleukin-2 receptor described in Cosman *et al.*,1984, Nature 312:768; the interleukin-4 receptor signal peptide described in EP Patent No. 0367 566; the type I interleukin-1 receptor signal peptide described in U.S. Patent No. 4,968,607; the type II interleukin-1 receptor signal peptide described in EP Patent No. 0 460 846.

**[0118]** The expression vectors that are provided may be constructed from a starting vector such as a commercially available vector. Such vectors may or may not contain all of the desired flanking sequences. Where one or more of the flanking sequences described herein are not already present in the vector, they may be individually obtained and ligated into the vector. Methods used for obtaining each of the flanking sequences are well known to one skilled in the art. The expression vectors can be introduced into host cells to thereby produce proteins, including fusion proteins, encoded by nucleic acids as described herein.

**[0119]** After the vector has been constructed and the one or more nucleic acid molecules encoding the components of the antigen binding proteins described herein has been inserted into the proper site(s) of the vector or vectors, the completed vector(s) may be inserted into a suitable host cell for amplification and/or polypeptide expression. Thus, the present invention

encompasses an isolated host cell comprising one or more expression vectors encoding the components of the antigen binding proteins. The term "host cell" as used herein refers to a cell that has been transformed, or is capable of being transformed, with a nucleic acid and thereby expresses a gene of interest. The term includes the progeny of the parent cell, whether or not the progeny is identical in morphology or in genetic make-up to the original parent cell, so long as the gene of interest is present. A host cell that comprises an isolated nucleic acid of the invention, preferably operably linked to at least one expression control sequence (e.g. promoter or enhancer), is a "recombinant host cell."

**[0120]** The transformation of an expression vector for an antigen binding protein into a selected host cell may be accomplished by well-known methods including transfection, infection, calcium phosphate co-precipitation, electroporation, microinjection, lipofection, DEAE-dextran mediated transfection, or other known techniques. The method selected will in part be a function of the type of host cell to be used. These methods and other suitable methods are well known to the skilled artisan, and are set forth, for example, in Sambrook *et al.*, 2001, *supra*.

**[0121]** A host cell, when cultured under appropriate conditions, synthesizes an antigen binding protein that can subsequently be collected from the culture medium (if the host cell secretes it into the medium) or directly from the host cell producing it (if it is not secreted). The selection of an appropriate host cell will depend upon various factors, such as desired expression levels, polypeptide modifications that are desirable or necessary for activity (such as glycosylation or phosphorylation) and ease of folding into a biologically active molecule.

**[0122]** Exemplary host cells include prokaryote, yeast, or higher eukaryote cells. Prokaryotic host cells include eubacteria, such as Gram-negative or Gram-positive organisms, for example, *Enterobacteriaceae* such as *Escherichia*, e.g., *E. coli*, *Enterobacter*, *Erwinia*, *Klebsiella*, *Proteus*, *Salmonella*, e.g., *Salmonella typhimurium*, *Serratia*, e.g., *Serratia marcescans*, and *Shigella*, as well as *Bacillus*, such as *B. subtilis* and *B. licheniformis*, *Pseudomonas*, and *Streptomyces*. Eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for recombinant polypeptides. *Saccharomyces cerevisiae*, or common baker's yeast, is the most commonly used among lower eukaryotic host microorganisms. However, a number of other genera, species, and strains are commonly available and useful herein, such as *Pichia*, e.g. *P. pastoris*, *Schizosaccharomyces pombe*; *Kluyveromyces*, *Yarrowia*; *Candida*; *Trichoderma reesia*; *Neurospora crassa*; *Schwanniomyces*, such as *Schwanniomyces*

*occidentalis*; and filamentous fungi, such as, e.g., *Neurospora*, *Penicillium*, *Tolypocladium*, and *Aspergillus* hosts such as *A. nidulans* and *A. niger*.

**[0123]** Host cells for the expression of glycosylated antigen binding proteins can be derived from multicellular organisms. Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains and variants and corresponding permissive insect host cells from hosts such as *Spodoptera frugiperda* (caterpillar), *Aedes aegypti* (mosquito), *Aedes albopictus* (mosquito), *Drosophila melanogaster* (fruitfly), and *Bombyx mori* have been identified. A variety of viral strains for transfection of such cells are publicly available, e.g., the L-1 variant of *Autographa californica* NPV and the Bm-5 strain of *Bombyx mori* NPV.

**[0124]** Vertebrate host cells are also suitable hosts, and recombinant production of antigen binding proteins from such cells has become routine procedure. Mammalian cell lines available as hosts for expression are well known in the art and include, but are not limited to, immortalized cell lines available from the American Type Culture Collection (ATCC), including but not limited to Chinese hamster ovary (CHO) cells, including CHOK1 cells (ATCC CCL61), DXB-11, DG-44, and Chinese hamster ovary cells/-DHFR (CHO, Urlaub *et al.*, Proc. Natl. Acad. Sci. USA 77: 4216, 1980); monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, (Graham *et al.*, J. Gen Virol. 36: 59, 1977); baby hamster kidney cells (BHK, ATCC CCL 10); mouse sertoli cells (TM4, Mather, Biol. Reprod. 23: 243-251, 1980); monkey kidney cells (CV1 ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL-1587); human cervical carcinoma cells (HELA, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human hepatoma cells (Hep G2, HB 8065); mouse mammary tumor (MMT 060562, ATCC CCL51); TRI cells (Mather *et al.*, Annals N.Y Acad. Sci. 383: 44-68, 1982); MRC 5 cells or FS4 cells; mammalian myeloma cells, and a number of other cell lines. In certain embodiments, cell lines may be selected through determining which cell lines have high expression levels and constitutively produce antigen binding proteins with PACAP binding properties. In another embodiment, a cell line from the B cell lineage that does not make its own antibody but has a capacity to make and secrete a heterologous antibody can be selected. CHO cells are preferred host cells in some embodiments for expressing the antigen binding proteins of the invention.

**[0125]** Host cells are transformed or transfected with the above-described nucleic acids or vectors for production of antigen binding proteins and are cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences. In addition, novel vectors and transfected cell lines with multiple copies of transcription units separated by a selective marker are particularly useful for the expression of antigen binding proteins. Thus, the present invention also provides a method for producing an antigen binding protein described herein, such as a monoclonal antibody or binding fragment thereof, comprising culturing a host cell comprising one or more expression vectors described herein in a culture medium under conditions permitting expression of the antigen binding protein encoded by the one or more expression vectors; and recovering the antigen binding protein from the culture medium or host cell.

**[0126]** The host cells used to produce the antigen binding proteins of the invention may be cultured in a variety of media. Commercially available media such as Ham's F10 (Sigma), Minimal Essential Medium (MEM, Sigma), RPMI-1640 (Sigma), and Dulbecco's Modified Eagle's Medium (DMEM, Sigma) are suitable for culturing the host cells. In addition, any of the media described in Ham *et al.*, Meth. Enz. 58: 44, 1979; Barnes *et al.*, Anal. Biochem. 102: 255, 1980; U.S. Patent Nos. 4,767,704; 4,657,866; 4,927,762; 4,560,655; or 5,122,469; WO90103430; WO 87/00195; or U.S. Patent Re. No. 30,985 may be used as culture media for the host cells. Any of these media may be supplemented as necessary with hormones and/or other growth factors (such as insulin, transferrin, or epidermal growth factor), salts (such as sodium chloride, calcium, magnesium, and phosphate), buffers (such as HEPES), nucleotides (such as adenosine and thymidine), antibiotics (such as Gentamycin™ drug), trace elements (defined as inorganic compounds usually present at final concentrations in the micromolar range), and glucose or an equivalent energy source. Any other necessary supplements may also be included at appropriate concentrations that would be known to those skilled in the art. The culture conditions, such as temperature, pH, and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinary skilled artisan.

**[0127]** Upon culturing the host cells, the antigen binding protein can be produced intracellularly, in the periplasmic space, or directly secreted into the medium. If the antigen binding protein is produced intracellularly, as a first step, the particulate debris, either host cells or lysed fragments, is removed, for example, by centrifugation or ultrafiltration. The antigen binding protein can be

purified using, for example, hydroxyapatite chromatography, cation or anion exchange chromatography, or preferably affinity chromatography, using the antigen(s) of interest or protein A or protein G as an affinity ligand. Protein A can be used to purify proteins that include polypeptides that are based on human γ1, γ2, or γ4 heavy chains (Lindmark *et al.*, J. Immunol. Meth. 62: 1-13, 1983). Protein G is recommended for all mouse isotypes and for human γ3 (Guss *et al.*, EMBO J. 5: 15671575, 1986). The matrix to which the affinity ligand is attached is most often agarose, but other matrices are available. Mechanically stable matrices such as controlled pore glass or poly(styrenedivinyl)benzene allow for faster flow rates and shorter processing times than can be achieved with agarose. Where the protein comprises a CH3 domain, the Bakerbond ABX™ resin (J. T. Baker, Phillipsburg, N.J.) is useful for purification. Other techniques for protein purification such as ethanol precipitation, Reverse Phase HPLC, chromatofocusing, SDS-PAGE, and ammonium sulfate precipitation are also possible depending on the particular antigen binding protein to be recovered.

**[0128]** In certain embodiments, the invention provides a composition (e.g. a pharmaceutical composition) comprising one or a plurality of the antigen binding proteins of the invention (e.g. monoclonal antibodies or binding fragments thereof) together with pharmaceutically acceptable diluents, carriers, excipients, solubilizers, emulsifiers, preservatives, and/or adjuvants. Pharmaceutical compositions of the invention include, but are not limited to, liquid, frozen, and lyophilized compositions. "Pharmaceutically-acceptable" refers to molecules, compounds, and compositions that are non-toxic to human recipients at the dosages and concentrations employed and/or do not produce allergic or adverse reactions when administered to humans. In some embodiments, the pharmaceutical composition may contain formulation materials for modifying, maintaining or preserving, for example, the pH, osmolarity, viscosity, clarity, color, isotonicity, odor, sterility, stability, rate of dissolution or release, adsorption or penetration of the composition. In such embodiments, suitable formulation materials include, but are not limited to, amino acids (such as glycine, glutamine, asparagine, arginine or lysine); antimicrobials; antioxidants (such as ascorbic acid, sodium sulfite or sodium hydrogen-sulfite); buffers (such as borate, bicarbonate, Tris-HCl, citrates, phosphates or other organic acids); bulking agents (such as mannitol or glycine); chelating agents (such as ethylenediamine tetraacetic acid (EDTA)); complexing agents (such as caffeine, polyvinylpyrrolidone, beta-cyclodextrin or hydroxypropyl-beta-cyclodextrin); fillers; monosaccharides; disaccharides; and other carbohydrates (such as

glucose, mannose or dextrins); proteins (such as serum albumin, gelatin or immunoglobulins); coloring, flavoring and diluting agents; emulsifying agents; hydrophilic polymers (such as polyvinylpyrrolidone); low molecular weight polypeptides; salt-forming counterions (such as sodium); preservatives (such as benzalkonium chloride, benzoic acid, salicylic acid, thimerosal, phenethyl alcohol, methylparaben, propylparaben, chlorhexidine, sorbic acid or hydrogen peroxide); solvents (such as glycerin, propylene glycol or polyethylene glycol); sugar alcohols (such as mannitol or sorbitol); suspending agents; surfactants or wetting agents (such as pluronics, PEG, sorbitan esters, polysorbates such as polysorbate 20, polysorbate 80, triton, tromethamine, lecithin, cholesterol, tyloxapal); stability enhancing agents (such as sucrose or sorbitol); tonicity enhancing agents (such as alkali metal halides, preferably sodium or potassium chloride, mannitol sorbitol); delivery vehicles; diluents; excipients and/or pharmaceutical adjuvants. Methods and suitable materials for formulating molecules for therapeutic use are known in the pharmaceutical arts, and are described, for example, in REMINGTON'S PHARMACEUTICAL SCIENCES, 18th Edition, (A.R. Genrmo, ed.), 1990, Mack Publishing Company.

**[0129]** In some embodiments, the pharmaceutical composition of the invention comprises a standard pharmaceutical carrier, such as a sterile phosphate buffered saline solution, bacteriostatic water, and the like. A variety of aqueous carriers may be used, e.g., water, buffered water, 0.4% saline, 0.3% glycine and the like, and may include other proteins for enhanced stability, such as albumin, lipoprotein, globulin, etc., subjected to mild chemical modifications or the like.

**[0130]** Exemplary concentrations of the antigen binding proteins in the formulation may range from about 0.1 mg/ml to about 200 mg/ml or from about 0.1 mg/mL to about 50 mg/mL, or from about 0.5 mg/mL to about 25 mg/mL, or alternatively from about 2 mg/mL to about 10 mg/mL. An aqueous formulation of the antigen binding protein may be prepared in a pH-buffered solution, for example, at pH ranging from about 4.5 to about 6.5, or from about 4.8 to about 5.5, or alternatively about 5.0. Examples of buffers that are suitable for a pH within this range include acetate (e.g. sodium acetate), succinate (such as sodium succinate), gluconate, histidine, citrate and other organic acid buffers. The buffer concentration can be from about 1 mM to about 200 mM, or from about 10 mM to about 60 mM, depending, for example, on the buffer and the desired isotonicity of the formulation.

**[0131]** A tonicity agent, which may also stabilize the antigen binding protein, may be included in the formulation. Exemplary tonicity agents include polyols, such as mannitol, sucrose or trehalose. Preferably the aqueous formulation is isotonic, although hypertonic or hypotonic solutions may be suitable. Exemplary concentrations of the polyol in the formulation may range from about 1% to about 15% w/v.

**[0132]** A surfactant may also be added to the antigen binding protein formulation to reduce aggregation of the formulated antigen binding protein and/or minimize the formation of particulates in the formulation and/or reduce adsorption. Exemplary surfactants include nonionic surfactants such as polysorbates (e.g. polysorbate 20 or polysorbate 80) or poloxamers (e.g. poloxamer 188). Exemplary concentrations of surfactant may range from about 0.001% to about 0.5%, or from about 0.005% to about 0.2%, or alternatively from about 0.004% to about 0.01% w/v.

**[0133]** In one embodiment, the formulation contains the above-identified agents (i.e. antigen binding protein, buffer, polyol and surfactant) and is essentially free of one or more preservatives, such as benzyl alcohol, phenol, m-cresol, chlorobutanol and benzethonium chloride. In another embodiment, a preservative may be included in the formulation, e.g., at concentrations ranging from about 0.1% to about 2%, or alternatively from about 0.5% to about 1%. One or more other pharmaceutically acceptable carriers, excipients or stabilizers such as those described in REMINGTON'S PHARMACEUTICAL SCIENCES, 18th Edition, (A.R. Genrmo, ed.), 1990, Mack Publishing Company, may be included in the formulation provided that they do not adversely affect the desired characteristics of the formulation.

**[0134]** Therapeutic formulations of the antigen binding protein are prepared for storage by mixing the antigen binding protein having the desired degree of purity with optional physiologically acceptable carriers, excipients or stabilizers (REMINGTON'S PHARMACEUTICAL SCIENCES, 18th Edition, (A.R. Genrmo, ed.), 1990, Mack Publishing Company), in the form of lyophilized formulations or aqueous solutions. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol;

cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, maltose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g., Zn-protein complexes); and/or non-ionic surfactants such as TWEEN™, PLURONICS™ or polyethylene glycol (PEG).

**[0135]** In one embodiment, a suitable formulation of the claimed invention contains an isotonic buffer such as a phosphate, acetate, or TRIS buffer in combination with a tonicity agent, such as a polyol, sorbitol, sucrose or sodium chloride, which tonicifies and stabilizes. One example of such a tonicity agent is 5% sorbitol or sucrose. In addition, the formulation could optionally include a surfactant at 0.01% to 0.02% wt/vol, for example, to prevent aggregation or improve stability. The pH of the formulation may range from 4.5-6.5 or 4.5 to 5.5. Other exemplary descriptions of pharmaceutical formulations for antigen binding proteins may be found in US Patent Publication No. 2003/0113316 and US Patent No. 6,171,586, each of which is hereby incorporated by reference in its entirety.

**[0136]** Suspensions and crystal forms of antigen binding proteins are also contemplated. Methods to make suspensions and crystal forms are known to one of skill in the art.

**[0137]** The formulations to be used for *in vivo* administration must be sterile. The compositions of the invention may be sterilized by conventional, well known sterilization techniques. For example, sterilization is readily accomplished by filtration through sterile filtration membranes. The resulting solutions may be packaged for use or filtered under aseptic conditions and lyophilized, the lyophilized preparation being combined with a sterile solution prior to administration.

**[0138]** The process of freeze-drying is often employed to stabilize polypeptides for long-term storage, particularly when the polypeptide is relatively unstable in liquid compositions. A lyophilization cycle is usually composed of three steps: freezing, primary drying, and secondary drying (*see* Williams and Polli, Journal of Parenteral Science and Technology, Volume 38, Number 2, pages 48-59, 1984). In the freezing step, the solution is cooled until it is adequately frozen. Bulk water in the solution forms ice at this stage. The ice sublimes in the primary drying

stage, which is conducted by reducing chamber pressure below the vapor pressure of the ice, using a vacuum. Finally, sorbed or bound water is removed at the secondary drying stage under reduced chamber pressure and an elevated shelf temperature. The process produces a material known as a lyophilized cake. Thereafter the cake can be reconstituted prior to use.

**[0139]** The standard reconstitution practice for lyophilized material is to add back a volume of pure water (typically equivalent to the volume removed during lyophilization), although dilute solutions of antibacterial agents are sometimes used in the production of pharmaceuticals for parenteral administration (*see* Chen, Drug Development and Industrial Pharmacy, Volume 18: 1311-1354, 1992).

**[0140]** Excipients have been noted in some cases to act as stabilizers for freeze-dried products (*see* Carpenter *et al.*, Volume 74: 225-239, 1991). For example, known excipients include polyols (including mannitol, sorbitol and glycerol); sugars (including glucose and sucrose); and amino acids (including alanine, glycine and glutamic acid).

**[0141]** In addition, polyols and sugars are also often used to protect polypeptides from freezing and drying-induced damage and to enhance the stability during storage in the dried state. In general, sugars, in particular disaccharides, are effective in both the freeze-drying process and during storage. Other classes of molecules, including mono- and di-saccharides and polymers such as PVP, have also been reported as stabilizers of lyophilized products.

**[0142]** For injection, the pharmaceutical formulation and/or medicament may be a powder suitable for reconstitution with an appropriate solution as described above. Examples of these include, but are not limited to, freeze dried, rotary dried or spray dried powders, amorphous powders, granules, precipitates, or particulates. For injection, the formulations may optionally contain stabilizers, pH modifiers, surfactants, bioavailability modifiers and combinations of these.

**[0143]** Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antigen binding protein, which matrices are in the form of shaped articles, e.g., films, or microcapsule. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Patent No. 3,773,919), copolymers of L-glutamic acid and y ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the Lupron

Depot™ (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid. While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release proteins for shorter time periods. When encapsulated polypeptides remain in the body for a long time, they may denature or aggregate as a result of exposure to moisture at 37°C, resulting in a loss of biological activity and possible changes in immunogenicity. Rational strategies can be devised for stabilization depending on the mechanism involved. For example, if the aggregation mechanism is discovered to be intermolecular S--S bond formation through thio-disulfide interchange, stabilization may be achieved by modifying sulfhydryl residues, lyophilizing from acidic solutions, controlling moisture content, using appropriate additives, and developing specific polymer matrix compositions.

**[0144]** The formulations of the invention may be designed to be short-acting, fast-releasing, long-acting, or sustained-releasing as described herein. Thus, the pharmaceutical formulations may also be formulated for controlled release or for slow release.

**[0145]** Specific dosages may be adjusted depending on conditions of disease, the age, body weight, general health conditions, sex, and diet of the subject, dose intervals, administration routes, excretion rate, and combinations of drugs. Any of the above dosage forms containing effective amounts are well within the bounds of routine experimentation and therefore, well within the scope of the instant invention.

**[0146]** The antigen binding protein is administered by any suitable means, including parenteral, subcutaneous, intraperitoneal, intrapulmonary, and intranasal, and, if desired for local treatment, intralesional administration. Parenteral administration includes intravenous, intraarterial, intraperitoneal, intramuscular, intradermal or subcutaneous administration. In addition, the antigen binding protein is suitably administered by pulse infusion, particularly with declining doses of the antigen binding protein. Preferably, the dosing is given by injections, most preferably intravenous or subcutaneous injections, depending in part on whether the administration is brief or chronic. Other administration methods are contemplated, including topical, particularly transdermal, transmucosal, rectal, oral or local administration e.g. through a catheter placed close to the desired site. Most preferably, the antigen binding protein of the invention is administered intravenously or subcutaneously in a physiological solution at a dose ranging between 0.01 mg/kg to 100 mg/kg at a frequency ranging from daily to weekly to

monthly (e.g. every day, every other day, every third day, or 2, 3, 4, 5, or 6 times per week), preferably a dose ranging from 0.1 to 45 mg/kg, 0.1 to 15 mg/kg or 0.1 to 10 mg/kg at a frequency of once per week, once every two weeks, or once a month.

**[0147]** The antigen binding proteins described herein (e.g. monoclonal antibodies and binding fragments thereof) are useful for treating or ameliorating a condition associated with the biological activity of PACAP in a patient in need thereof. As used herein, the term "treating" or "treatment" is an intervention performed with the intention of preventing the development or altering the pathology of a disorder. Accordingly, "treatment" refers to both therapeutic treatment and prophylactic or preventative measures. Those in need of treatment include those already diagnosed with or suffering from the disorder or condition as well as those in which the disorder or condition is to be prevented. "Treatment" includes any indicia of success in the amelioration of an injury, pathology or condition, including any objective or subjective parameter such as abatement, remission, diminishing of symptoms, or making the injury, pathology or condition more tolerable to the patient, slowing in the rate of degeneration or decline, making the final point of degeneration less debilitating, or improving a patient's physical or mental well-being. The treatment or amelioration of symptoms can be based on objective or subjective parameters, including the results of a physical examination, self-reporting by a patient, neuropsychiatric exams, and/or a psychiatric evaluation.

**[0148]** Accordingly, in some embodiments, the present invention provides a method for treating or preventing a condition associated with the biological activity of PACAP, such as activation of VPAC1, VPAC2, and/or PAC1 receptors, in a patient in need thereof, comprising administering to the patient an effective amount of an antigen binding protein described herein. In particular embodiments, the antigen binding protein is a monoclonal antibody or binding fragment thereof. The term "patient" includes human patients. PACAP biological activity has been implicated in various physiological processes, including cardiovascular function, metabolic and endocrine function, inflammation, stress response, and regulation of the autonomic nervous system, particularly the balance between the sympathetic and parasympathetic systems. *See, e.g.*, Tanida *et al.*, Regulatory Peptides, Vol. 161: 73-80, 2010; Moody *et al.*, Curr. Opin. Endocrinol. Diabetes Obes., Vol. 18: 61-67, 2011; and Hashimoto *et al.*, Current Pharmaceutical Design, Vol. 17: 985-989, 2011.

**[0149]** An "effective amount" is generally an amount sufficient to reduce the severity and/or frequency of symptoms, eliminate the symptoms and/or underlying cause, prevent the occurrence of symptoms and/or their underlying cause, and/or improve or remediate the damage that results from or is associated with a particular condition (e.g. chronic pain, headache or migraine). In some embodiments, the effective amount is a therapeutically effective amount or a prophylactically effective amount. A "therapeutically effective amount" is an amount sufficient to remedy a disease state (e.g., a headache, migraine, or chronic pain) or symptom(s), particularly a state or symptom(s) associated with the disease state, or otherwise prevent, hinder, retard or reverse the progression of the disease state or any other undesirable symptom associated with the disease in any way whatsoever (i.e. that provides "therapeutic efficacy"). A "prophylactically effective amount" is an amount of a pharmaceutical composition that, when administered to a subject, will have the intended prophylactic effect, e.g., preventing or delaying the onset (or reoccurrence) of the condition (e.g. headache or migraine), or reducing the likelihood of the onset (or reoccurrence) of the condition (e.g. headache, migraine, or headache symptoms). The full therapeutic or prophylactic effect does not necessarily occur by administration of one dose, and may occur only after administration of a series of doses. Thus, a therapeutically or prophylactically effective amount may be administered in one or more administrations.

**[0150]** In certain embodiments, the present invention provides a method for inhibiting activation of the PAC1 receptor in a patient having a headache condition comprising administering to the patient an effective amount of an antigen binding protein, such as a monoclonal antibody or binding fragment thereof, described herein. In some embodiments, the method may treat or prevent symptoms of the headache condition in the patient. Accordingly, the present invention also includes a method for treating or preventing a headache condition, particularly migraine headache, in a patient in need thereof comprising administering to the patient an effective amount of an antigen binding protein (e.g. monoclonal antibody or binding fragment thereof) described herein. In certain embodiments, the antigen binding protein administered to the patient specifically binds to the C-terminal domain of PACAP38, for example within amino acids 28 to 38 of SEQ ID NO: 1. In one embodiment, the antigen binding proteins bind to an epitope within residues 28 to 37 of PACAP38 (SEQ ID NO: 1). In another embodiment, the antigen binding proteins bind to an epitope within residues 34 to 38 of PACAP38 (SEQ ID NO: 1). As described

in Example 2 herein, it has been found that monoclonal antibodies that bind to this C-terminal region of PACAP38 are surprisingly potent inhibitors of PACAP38-induced PAC1 receptor activation (e.g. with IC50 values less than 500 pM as measured by a cell-based cAMP assay), and as such would be useful therapeutics for treating or preventing migraine headache, in particular, as explained in further detail below. In some embodiments, the antigen binding protein administered to the patient specifically binds to a polypeptide consisting of the sequence of SEQ ID NO: 4, but does not significantly bind to a polypeptide consisting of the sequence of SEQ ID NO: 5. In other embodiments, the antigen binding protein administered to the patient specifically binds to a polypeptide consisting of the sequence of SEQ ID NO: 4 and/or a polypeptide consisting of the sequence of SEQ ID NO: 126. In certain embodiments, the antigen binding protein administered to the patient binds to a polypeptide consisting of the sequence of SEQ ID NO: 4 with a binding affinity at least 2-fold greater than that for a polypeptide consisting of the sequence of SEQ ID NO: 115 as measured by a surface plasmon resonance assay. In other embodiments, the antigen binding protein administered to the patient binds to a polypeptide consisting of the sequence of SEQ ID NO: 4 with a binding affinity at least 5-fold greater than that for a polypeptide consisting of the sequence of SEQ ID NO: 115 as measured by a surface plasmon resonance assay. In still other embodiments, the antigen binding protein administered to the patient binds to a polypeptide consisting of the sequence of SEQ ID NO: 4 with a binding affinity at least 10-fold greater than that for a polypeptide consisting of the sequence of SEQ ID NO: 115 as measured by a surface plasmon resonance assay.

**[0151]** In some embodiments of the methods of the invention, the headache condition to be treated, prevented or ameliorated is migraine. Migraine headaches are recurrent headaches lasting about 4 to about 72 hours that are characterized by unilateral, pulsating, and/or moderate to severe pain and/or pain that is exacerbated by physical activity. Migraine headaches are often accompanied by nausea, vomiting, and/or sensitivity to light (photophobia), sound (phonophobia), or smell. In some patients, an aura precedes the onset of the migraine headache. The aura is typically a visual, sensory, language, or motor disturbance that signals the headache will soon occur. The methods described herein prevent, treat, or ameliorate one or more symptoms of migraine headaches with and without aura in human patients.

**[0152]** PACAP38, through activation of its receptors, induces vasodilation, particularly vasodilation of the dura vasculature (Schytz *et al.*, Neurotherapeutics, Vol. 7(2):191-196, 2010).

The PACAP38/PAC1 receptor signaling cascade, in particular, has been implicated in migraine pathophysiology (Amin *et al.*, Brain, Vol. 137: 779-794, 2014). Infusion of PACAP38, which has a higher affinity for the PAC1 receptor than the VPAC1 and VPAC2 receptors, causes migraine-like headache in migraine patients (Schytz *et al.*, Brain 132:16-25, 2009; Amin *et al.*, Brain, Vol. 137: 779-794, 2014). In addition, PACAP38 levels are elevated in cranial circulation in patients experiencing a migraine attack, and the PACAP38 levels are reduced following treatment of the migraine symptoms with triptans (Tuka *et al.*, Cephalalgia, Vol. 33, 1085-1095, 2013; Zagami *et al.*, Ann. Clin. Transl. Neurol., Vol. 1: 1036-1040, 2014). These reports suggest that endogenous release of PACAP38 is an important trigger of migraine headache and its effects are primarily mediated through activation of the PAC1 receptor.

**[0153]** In some embodiments, the patients to be treated according to the methods of the invention have, suffer from, or are diagnosed with episodic migraine. Episodic migraine is diagnosed when patients with a history of migraine (e.g. at least five lifetime attacks of migraine headache) have 14 or fewer migraine headache days per month. A "migraine headache day" includes any calendar day during which a patient experiences the onset, continuation, or recurrence of a "migraine headache" with or without aura lasting greater than 30 minutes. A "migraine headache" is a headache associated with nausea or vomiting or sensitivity to light or sound and/or a headache characterized by at least two of the following pain features: unilateral pain, throbbing pain, moderate to severe pain intensity, or pain exacerbated by physical activity. In certain embodiments, patients having, suffering from, or diagnosed with episodic migraine have at least four, but less than 15 migraine headache days per month on average. In related embodiments, patients having, suffering from, or diagnosed with episodic migraine have fewer than 15 headache days per month on average. As used herein, a "headache day" is any calendar day in which the patient experiences a migraine headache as defined herein or any headache that lasts greater than 30 minutes or requires acute headache treatment.

**[0154]** In certain embodiments, the patients to be treated according to the methods of the invention have, suffer from, or are diagnosed with chronic migraine. Chronic migraine is diagnosed when migraine patients (i.e. patients with at least five lifetime attacks of migraine headache) have 15 or more headache days per month and at least 8 of the headache days are migraine headache days. In some embodiments, patients having, suffering from, or diagnosed with chronic migraine have 15 or more migraine headache days per month on average. In certain

embodiments of the methods described herein, administration of an antigen binding protein of the invention prevents, reduces, or delays the progression of episodic migraine to chronic migraine in the patient.

**[0155]** In other embodiments, the present invention provides a method for treating or ameliorating cluster headache in a patient in need thereof comprising administering to the patient an effective amount of an antigen binding protein (e.g. monoclonal antibody or binding fragment thereof) described herein. Cluster headache is a condition that involves, as its most prominent feature, recurrent, severe headaches on one side of the head, typically around the eye (*see* Nesbitt *et al.*, BMJ, Vol. 344:e2407, 2012). Some doctors and scientists have described the pain resulting from cluster headaches as the most intense pain a human can endure — worse than giving birth, burns or broken bones. Cluster headaches often occur periodically: spontaneous remissions interrupt active periods of pain. Cluster headaches are often accompanied by cranial autonomic symptoms, such as tearing, nasal congestion, ptosis, pupil constriction, facial blushing, sweating, and swelling around the eye, often confined to the side of the head with the pain. The average age of onset of cluster headache is ~30-50 years. It is more prevalent in males with a male to female ratio of about 2.5:1 to about 3.5:1. Sphenopalatine ganglion (SPG) stimulation has been used for the treatment of cluster headache. A neurostimulation system, which delivers low-level (but high frequency, physiologic-blocking) electrical stimulation to the SPG, has demonstrated efficacy in relieving the acute debilitating pain of cluster headache in a recent clinical trial (*see* Schoenen J, *et al.*, Cephalalgia, Vol. 33(10):816-30, 2013). In view of this evidence and because PACAP is one of the major neurotransmitters in SPG, inhibition of PACAP signaling with an antigen binding protein described herein is expected to have efficacy in treating cluster headache in humans.

**[0156]** Other conditions associated with PACAP biological activity that may be treated according to the methods of the invention include, but are not limited to, inflammatory skin conditions, such as rosacea (*see* U.S. Patent Publication No. 20110229423), chronic pain syndromes, such as neuropathic pain (*see* Jongsma *et al.*, Neuroreport, Vol. 12: 2215-2219, 2001; Hashimoto *et al.*, Annals of the New York Academy of Sciences, Vol. 1070: 75-89, 2006), tension-type headaches, hemiplegic migraine, retinal migraine, anxiety disorders, such as posttraumatic stress disorder (*see* Hammack and May, Biol. Psychiatry, Vol.78(3):167-177, 2015), irritable bowel syndrome, and vasomotor symptoms (*e.g.* hot flashes, facial flushing,

sweating, and night sweats), such as those associated with menopause. In one embodiment, the condition is chronic pain. In another embodiment, the condition is neuropathic pain. In any of the methods described herein, the treatment can comprise prophylactic treatment. Prophylactic treatment refers to treatment designed to be taken before the onset of a condition or an attack (e.g. before a migraine attack or onset of a cluster headache episode) to reduce the frequency, severity, and/or length of the symptoms (e.g. migraine or cluster headaches) in the patient.

**[0157]** The antigen binding proteins of the invention are useful for detecting PACAP (i.e. PACAP38 and PACAP27) in biological samples and identification of cells or tissues that express the PACAP peptide. For instance, the antigen binding proteins can be used in diagnostic assays, *e.g.*, immunoassays to detect and/or quantify PACAP peptide expressed in a tissue or cell or presence in a bodily fluid, such as cerebrospinal fluid, blood, serum, or plasma. In some embodiments, the antigen binding proteins can be used to differentially detect and/or quantitate PACAP38 from PACAP27. For instance, N-terminal or central domain binding antibodies described herein (e.g. mAb4, mAb5, and mAb6) can bind to both PACAP38 and PACAP27, whereas C-terminal antibodies described herein (e.g. mAb1, mAb2, and mAb3) can only bind to PACAP38. Thus, N-terminal/central domain antigen binding proteins and C-terminal antigen binding proteins can be employed in immunoassays to detect PACAP38, PACAP27, or both in various tissues and bodily fluids. In addition, the antigen binding proteins described herein can be used to inhibit VPAC1, VPAC2, and/or PAC1 receptor from forming a complex with PACAP, thereby modulating the biological activity of these receptors in a cell or tissue. Such biological activities include vasodilation.

**[0158]** The antigen binding proteins described herein can be used for diagnostic purposes to detect, diagnose, or monitor diseases and/or conditions associated with PACAP38, including migraine, cluster headache, and anxiety disorders, such as posttraumatic stress disorder. Elevated levels of PACAP38 in blood have been associated with these conditions. *See, e.g.,* Ressler *et al.*, Nature, Vol. 470: 492-497, 2011; Tuka *et al.*, Cephalalgia, Vol. 33, 1085-1095, 2013; Zagami *et al.*, Ann. Clin. Transl. Neurol., Vol. 1: 1036-1040, 2014. Also provided are methods for the detection of the presence of PACAP in a sample using classical immunohistological methods known to those of skill in the art (e.g., Tijssen, 1993, Practice and Theory of Enzyme Immunoassays, Vol 15 (Eds R.H. Burdon and P.H. van Knippenberg, Elsevier, Amsterdam); Zola, 1987, Monoclonal Antibodies: A Manual of Techniques, pp. 147-

158 (CRC Press, Inc.); Jalkanen et al., 1985, J. Cell. Biol. 101:976-985; Jalkanen et al., 1987, J. Cell Biol. 105:3087-3096). Examples of methods useful in the detection of the presence of PACAP include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA), using the antigen binding proteins described herein. The detection of PACAP can be performed *in vivo* or *in vitro*.

**[0159]** For diagnostic applications, the antigen binding protein can be labeled with a detectable labeling group. Suitable labeling groups include, but are not limited to, the following: radioisotopes or radionuclides (e.g., $^{3}H$, $^{14}C$, $^{15}N$, $^{35}S$, $^{90}Y$, $^{99}Tc$, $^{111}In$, $^{125}I$, $^{131}I$), fluorescent groups (e.g., FITC, rhodamine, lanthanide phosphors), enzymatic groups (e.g., horseradish peroxidase, β-galactosidase, luciferase, alkaline phosphatase), chemiluminescent groups, biotinyl groups, or predetermined polypeptide epitopes recognized by a secondary reporter (e.g., leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, epitope tags). In some embodiments, the labeling group is coupled to the antigen binding protein via spacer arms of various lengths to reduce potential steric hindrance. Various methods for labeling proteins are known in the art and may be used.

**[0160]** In another embodiment, the antigen binding proteins described herein can be used to identify a cell or cells that express PACAP38 and/or PACAP27. In a specific embodiment, the antigen binding protein is labeled with a labeling group and the binding of the labeled antigen binding protein to PACAP38 and/or PACAP27 is detected. The antigen binding proteins, particularly the binding proteins that specifically bind to the C-terminus of PACAP38, can also be used in immunoprecipitation assays to separate PACAP38 from PACAP27 in biological samples. In a further specific embodiment, the binding of the antigen binding protein to PACAP is detected *in vivo*. In a further specific embodiment, the antigen binding protein is isolated and measured using techniques known in the art. *See*, for example, Harlow and Lane, 1988, Antibodies: A Laboratory Manual, New York: Cold Spring Harbor (ed. 1991 and periodic supplements); John E. Coligan, ed., 1993, Current Protocols In Immunology New York: John Wiley & Sons.

**[0161]** The following examples, including the experiments conducted and the results achieved, are provided for illustrative purposes only and are not to be construed as limiting the scope of the appended claims.

# EXAMPLES

## Example 1. Generation of Anti-PACAP Antibodies

**[0162]** Rabbits were immunized with human PACAP38 peptide (HSDGIFTDSYSRYRKQMAVKKYLAAVLGKRYKQRVKNK; SEQ ID NO: 1). Monoclonal antibodies were generated from the immunized rabbits using the Selected Lymphocyte Antibody Method (SLAM) as described in Babcook *et al.*, Proc. Natl. Acad. Sci. USA, Vol. 93: 7843-7848, 1996. Briefly, B cells were harvested from spleens from the immunized rabbits and expanded in culture. The supernatant from the expanded B-cell cultures was initially screened for binding to an amino terminal PACAP38 peptide (HSDGIFTDSYSRY; SEQ ID NO: 5) and a carboxy terminal PACAP38 peptide (GKRYKQRVKNK; SEQ ID NO: 4) using an ELISA assay. From the initial screen, 2,935 N-terminal and/or C-terminal binders were identified. The affinity of these antibodies for full-length human PACAP38 (SEQ ID NO: 1) was assessed by ELISA assay. The top 53 antibodies with the highest affinity to full-length human PACAP38 and that bound only the N-terminal peptide or the C-terminal peptide were screened for binding to vasoactive intestinal peptide (VIP; HSDAVFTDNYTRLRKQMAVKKYLNSILN; SEQ ID NO: 3) at 10-fold higher concentrations than that used for the full-length PACAP38 peptide to identify antibodies that did not cross-react with VIP. Data for the top 13 hits from the VIP counter-screen are shown in Table 8 below. Generally, these antibodies had high affinity to full-length PACAP38 and bound to either the N-terminal PACAP peptide (SEQ ID NO: 5) or C-terminal PACAP peptide (SEQ ID NO: 4), but did not significantly bind to VIP.

**Table 8. ELISA Assay Screening Data for Anti-PACAP Antibodies[1]**

| Antibody Designation | C-terminal Peptide 500 ng/mL | N-terminal Peptide 500 ng/mL | Full-length PACAP38 10 ng/mL | VIP 100 ng/mL |
|---|---|---|---|---|
| 1 (c20) | 5.33 | 0.05 | 4.09 | 0.08 |
| 2 (c81) | 3.74 | 0.06 | 3.39 | 0.09 |
| 3 (c89) | 3.59 | 0.06 | 3.17 | 0.07 |
| 7 (c8) | 0.59 | 0.07 | 3.93 | 0.08 |
| 8 (c15) | 0.41 | 0.07 | 3.38 | 0.07 |
| 9 (c64) | 0.69 | 0.07 | 3.61 | 0.07 |
| 4 (c47) | 0.05 | 3.68 | 2.09 | 0.24 |
| 5 (c71) | 0.05 | 1.34 | 3.21 | 0.12 |
| 6 (c55) | 0.05 | 4.24 | 3.55 | 0.95 |
| 10 | 0.05 | 1.02 | 2.06 | 0.06 |

| Antibody Designation | C-terminal Peptide 500 ng/mL | N-terminal Peptide 500 ng/mL | Full-length PACAP38 10 ng/mL | VIP 100 ng/mL |
|---|---|---|---|---|
| 11 | 0.06 | 1.72 | 1.70 | 0.13 |
| 12 (c26) | 0.08 | 0.38 | 6.00 | 0.20 |
| 13 | 0.07 | 0.31 | 4.19 | 1.62 |

[1]Optical Density values are provided; higher values indicate a higher level of binding

**[0163]** The top 13 hits from the supernatant screens were evaluated in the hemolytic plaque assay to identify and isolate single antibody forming cells (AFCs) as described in Babcook *et al.*, Proc. Natl. Acad. Sci. USA, Vol. 93: 7843-7848, 1996. The central AFC in each plaque was isolated, and mRNA was subsequently isolated from the single cell. The antibody sequences were amplified from the single cell mRNA using reverse-transcriptase PCR. Six of the monoclonal antibodies (3 C-terminal binders and 3 N-terminal binders) were cloned, recombinantly expressed, and purified. The amino acid sequences for each of the six antibodies (mAb1, mAb2, mAb3, mAb4, mAb5, and mAb6) are shown in Tables 1 (CDRs), 2 (variable regions), and 5 (full heavy and light chains).

**Example 2. Neutralizing Activity of Anti-PACAP Monoclonal Antibodies**

**[0164]** The recombinantly-produced, purified monoclonal antibodies (mAbs) were evaluated for binding to full-length human PACAP38 (SEQ ID NO: 1) and its isoform PACAP27 (HSDGIFTDSYSRYRKQMAVKKYLAAVL; SEQ ID NO: 2) using an ELISA assay. The ELISA assay used unlabeled mAbs 1 to 6 as capture reagents at the concentration of 13 nM. Analyte PACAP38 (SEQ ID NO: 1) and its isoform PACAP27 (SEQ ID NO: 2) were evaluated at two concentration levels (6.6 and 657 pM for PACAP38; 9.5 and 956 pM for PACAP27). Biotinylated mAbs 1 to 6 at the concentration of 5 nM were the secondary assay reagents. Neutravidin Horseradish Peroxidase (HRP) reacted with the biotinylated mAbs and produced colorimetric signals, optical density, in the presence of 3,3′,5,5′-Tetramethylbenzidine (TMB) substrate solution. The intensity of optical density corresponded to the analyte concentration. The results of this assay revealed that mAb1, mAb2, and mAb3 bound to PACAP38, but not PACAP27. MAb4, mAb5, and mAb6 bound to both PACAP38 and PACAP27. The results suggest that mAbs 1, 2, and 3 bind to PACAP38 at an epitope within amino acids 28 to 38 of

PACAP38, and the data are consistent with the data in Table 8 in Example 1 showing that these three mAbs bind to a C-terminal PACAP peptide, but not an N-terminal peptide.

**[0165]** Next, the functional activity of the six mAbs was assessed using a cell-based PAC1 receptor activity assay. Both PACAP38 and PACAP27 are agonists of the PAC1 receptor, activation of which results in an increase in intracellular cAMP. The assay employed a human neuroblastoma-derived cell line (SH-SY5Y; Biedler JL, *et al.*, Cancer Res. 38: 3751-3757, 1978) obtained from ATCC (ATCC Number CRL-2266; "CRL-2266 cells"). CRL-2266 cells express human PAC1 receptor (Monaghan *et al.*, J Neurochem. 104(1): 74–88, 2008). The LANCE Ultra cAMP assay kit (PerkinElmer, Boston, MA) was used to measure cAMP concentration.

**[0166]** On the day of the assay, the frozen CRL-2266 cells were thawed at 37°C and were washed once with assay buffer. 10 µL of cell suspension containing 2,000 cells was added into 96 half-area white plates. After adding 5µL of the anti-PACAP monoclonal antibody (10 point dose response curve: concentration range from 1 µM to 0.5 fM), the mixture was incubated for 30 min at room temperature. Then, 5µL of either human PACAP38 (10 pM final concentration) or human PACAP27 (10 pM final concentration) was added and the mixture was further incubated for 15 min at room temperature. After human PACAP38 or PACAP27 stimulation, 20 µL of detection mix was added and incubated for 45 minutes at room temperature. The plates were read on EnVision instrument (PerkinElmer, Boston, MA) at emission wavelength 665 nm. Data were processed and analyzed by Prizm (GraphPad Software Inc.).

**[0167]** **Figure 1** depicts the dose-response curve for each of the six mAbs for inhibiting PACAP38-induced activation of the PAC1 receptor. The IC50 values for each of the antibodies are shown to the right of the antibody designations in the legend. MAb1, mAb2, and mAb3, all of which bind to the C-terminus of PACAP38, potently inhibited PAC1 receptor activation by PACAP38 with IC50 values in the subnanomolar range. The C-terminal binders were more potent than the N-terminal binders (mAbs 4, 5, and 6). MAbs 1, 2, and 3 were about 10-fold more potent than a human antibody against the human PAC1 receptor (PAC1 Ab). Consistent with the data from the PACAP peptide binding experiments, mAbs 1, 2, and 3 did not inhibit PACAP27-induced activation of the PAC1 receptor. *See* **Figures 2A** and **2B**. Thus, antibodies that bind within amino acids 28 to 38 of PACAP38 exhibit ligand-specific inhibition of PAC1 receptor activity, whereas antibodies that bind to the N-terminus of PACAP38 inhibit PAC1 receptor activation induced by both ligands.

**[0168]** Previous structure-function studies report that the N-terminus of the PACAP peptide is critical for activation of the PAC1 receptor. For example, truncations of the peptide at the N-terminus result in loss of potency in PAC1 receptor-mediated adenylate cyclase activity and peptides lacking the first five N-terminal amino acids are converted into competitive antagonists (Robberecht *et al.*, Mol. Pharmacol., Vol. 42: 347-355, 1992). The conformation of the N-terminal domain comprised of the first seven amino acids of the PACAP peptide is essential for PAC1 receptor binding and subsequent activation of the receptor (Bourgault *et al.*, J. Med. Chem., Vol. 52: 3308-3316, 2009).

**[0169]** In contrast, truncations at the C-terminus of PACAP38 do not significantly affect the ability of the peptide to activate the PAC1 receptor although the binding affinity of the peptide is reduced. PACAP27, PACAP(1-23), and PACAP(1-21) all act as full PAC1 receptor agonists despite lacking the C-terminal 11, 15, and 17 amino acids, respectively (Inooka et al., Nature Structural Biology, Vol. 8: 161-165, 2001; Bourgault *at al.*, J. Mol. Neurosci., Vol. 36: 260-269, 2008). Consistent with the apparent lack of importance of the C-terminal amino acids in receptor activation, an antibody that bound to a peptide consisting of amino acids 31-38 of PACAP38 had no inhibitory effect on PACAP38-induced cAMP production in rat PC12 cells (i.e. the antibody had no neutralization activity). *See* U.S. Patent No. 5,486,472. Given these reports in the field, it is surprising and unexpected that the three monoclonal antibodies that bind to PACAP38 within amino acids 28-38 (mAbs 1, 2, and 3) were more potent in inhibiting PACAP38-induced PAC1 receptor activation than the antibodies that bind to the N-terminus of PACAP38.

**Example 3. Epitope Mapping of Anti-PACAP Antibodies**

**[0170]** The binding epitopes of anti-PACAP antibodies were evaluated by using the Biacore 3000 biosensor-based instrument (GE Healthcare USA). The Biacore 3000 instrument utilizes surface plasmon resonance (SPR) technology to measure mass increase on the sensor-chip surface. The binding interactions are measured in real-time and recorded as Response Units (RU). Each binding profile (cycle) is stored in a "sensorgram" in real-time. Generally, Biacore technology entails immobilizing a ligand of interest on to a flow-cell of the sensor-chip via a coupling chemistry. The analyte (e.g. putative binding partner to the ligand of interest) is injected across the immobilized ligand allowing it to bind to the ligand-immobilized surface.

The magnitude of the response is directly proportional to the mass increase on the immobilized surface due to the binding of the analyte.

**[0171]** In this experiment, various peptide fragments of PACAP38 were incubated with each of the six antibodies (mAb1, mAb2, mAb3, mAb4, mAb5, and mAb6) described in Example 2 and evaluated for their ability to inhibit binding of the antibodies to full-length PACAP38 immobilized on the sensor-chip. The binding epitopes for each of the antibodies were deduced from the set of peptides that were able to inhibit the binding of each antibody to full-length PACAP38.

**[0172]** Full-length PACAP38 peptide (SEQ ID NO: 1) was diluted to 2 μM in acetate buffer and immobilized on to flow cell 2 or 4 of a CM5 sensor chip by amine coupling chemistry. The targeted immobilization density was estimated to be around 1000 RU. A blank, reference surface on flow cell 1 or 3 was also prepared using an amine coupling chemistry to calculate the net binding using the following equation:

Net binding = Active surface RU – Reference surface RU

**[0173]** Each of the six anti-PACAP antibodies was incubated at a 1:200 molar ratio (Ab: peptide fragment) with each of the peptide fragments in Table 9 at an ambient temperature for 2 hours prior to analysis. Full-length PACAP38 peptide (SEQ ID NO: 1) was also mixed in with each antibody separately as a control to demonstrate inhibition of antibody binding, thereby confirming the specificity of the antibody for PACAP38. A second control containing 6.5 nM antibody was prepared in sample diluent to be used as the untreated control sample for each antibody. Full-length PACAP38, buffer, and each peptide fragment were also tested to evaluate non-specific binding to the immobilized PACAP38 surface.

**Table 9. PACAP38 Peptide Fragments used for Epitope Mapping**

| Peptide No. | Amino Acid Positions within PACAP38 | Sequence | SEQ ID NO. |
|---|---|---|---|
| 1 | 1-27 | HSDGIFTDSYSRYRKQMAVKKYLAAVL | 2 |
| 2 | 26-33 | VLGKRYKQ | 112 |
| 3 | 6-37 | FTDSYSRYRKQMAVKKYLAAVLGKRYKQRVKN | 113 |
| 4 | 6-35 | FTDSYSRYRKQMAVKKYLAAVLGKRYKQRV | 114 |

| Peptide No. | Amino Acid Positions within PACAP38 | Sequence | SEQ ID NO. |
|---|---|---|---|
| 5 | 31-38 | YKQRVKNK | 115 |
| 6 | 27-38 | LGKRYKQRVKNK | 116 |
| 7 | 14-38 | RKQMAVKKYLAAVLGKRYKQRVKNK | 117 |
| 8 | 11-27 | SRYRKQMAVKKYLAAVL | 118 |
| 9 | 4-27 | GIFTDSYSRYRKQMAVKKYLAAVL | 119 |
| 10 | 1-13 | HSDGIFTDSYSRY | 5 |
| 11 | 6-38 | FTDSYSRYRKQMAVKKYLAAVLGKRYKQRVKNK | 120 |
| 12 | 6-36 | FTDSYSRYRKQMAVKKYLAAVLGKRYKQRVK | 121 |
| 13 | 6-20 | FTDSYSRYRKQMAVK | 122 |
| 14 | 9-18 | SYSRYRKQMA | 123 |
| 15 | 16-23 | QMAVKKYL | 124 |
| 16 | 19-25 | VKKYLAA | 125 |
| 17 | 34-38 | RVKNK | 126 |
| 18 | 28-38 | GKRYKQRVKNK | 4 |
| 19 | 30-37 | RYKQRVKN | 127 |

**[0174]** Each sample was injected at 5 µl/min for 60 seconds and it travelled either through flow cell 1 (blank surface) into flow cell 2 (PACAP38 surface) or through flow cell 3 (blank surface) into flow cell 4 (PACAP38 surface). A baseline report was taken at 15 seconds prior to each sample injection. The sample binding report was taken at 45 seconds after completion of each injection. The chip surface was regenerated after each sample using a 100 mM HCl injection.

**[0175]** Untreated antibody samples for each of the six antibodies were tested before and after the testing of samples comprising the antibody/peptide mixtures (i.e. treated antibody samples) to assess the immobilized PACAP38 surface integrity for its binding capability over time. An average of the untreated antibody binding response was used to calculate percent inhibition by each peptide fragment using the following equation:

Percent inhibition = {(Avg. untreated Ab RU - Treated Ab RU)/Avg. untreated Ab RU} x 100

**[0176]** The anti-PACAP antibody was considered to be reactive to the peptide fragment if the percent inhibition was greater than 50%.

**[0177]** The results of the analysis for each of the six antibodies are shown in **Figures 3A-3F**. Consistent with the results from the ELISA-based screening assay described in Example 1, mAb1, mAb2, and mAb3 bound to PACAP38 between amino acids 28 to 38. As shown in **Figure 3A**, the binding of mAb1 to full-length PACAP38 was nearly completely inhibited by peptide fragments comprising amino acids 28-38 as well as a peptide consisting of amino acids 6-37, suggesting that the binding epitope for mAb1 is within amino acids 28 to 37 of PACAP38. The inhibition of mAb1 binding diminished with peptides containing C-terminal truncations (compare % inhibition for peptide fragments 6-35, 6-36, 6-37, and 6-38) and was completely eliminated by deletion of amino acids 34-38 (see % inhibition for peptide fragment consisting of amino acids 26-33), suggesting that the final four C-terminal amino acid residues are important for mAb1 binding to PACAP38. Similar results were obtained for mAb2, except that a peptide fragment consisting of amino acids 34 to 38 of PACAP38 was also able to nearly completely block binding of mAb2 to PACAP38, indicating that the binding epitope for this antibody can be narrowed down to amino acids 34 to 38 of PACAP38 (**Figure 3B**). Binding of mAb3 to PACAP38 was substantially inhibited by peptide fragments comprising amino acids 28-38 as well as a peptide fragment comprising amino acids 6-37 (**Figure 3C**). These results suggest that the binding epitope for mAb3 is located within amino acid residues 28 to 37 of PACAP38.

**[0178]** The binding of mAb4 to PACAP38 was inhibited by PACAP27 and peptide fragments consisting of amino acids 4-27, amino acids 6-20, amino acids 6-35, amino acids 6-36, amino acids 6-37, and amino acids 6-38 (**Figure 3D**). Mab4 binding was not inhibited by a peptide fragment consisting of amino acids 27-38. Based on these results, mAb4 most likely binds to PACAP38 within amino acid residues 6 to 20. The binding of mAb5 to PACAP38 was almost completely inhibited by PACAP27 and peptide fragments consisting of amino acids 4-27, amino acids 11-27, amino acids 6-35, and amino acids 14-38, but was not inhibited by peptide fragments consisting of amino acids 26-33 or amino acids 27-38 (**Figure 3E**). Therefore, the binding epitope for mAb5 can be conservatively estimated to be between 14-27 amino acids of PACAP38. Similar results were obtained with mAb6 also suggesting that the binding epitope for mAb6 is between 14-27 amino acids of PACAP38 (**Figure 3F**). In contrast to the results with

the ELISA-based screening assay described in Example 1, mAb4, mAb5, and mAb6 did not bind to the N-terminal peptide consisting of amino acids 1-13 of PACAP38. The reason for this result is unclear. However, mAb4, mAb5, and mAb6 did not bind to the C-terminal peptide consisting of amino acids 28-38 and remained selective towards the N-terminal region of PACAP38.

**Example 4. Inhibition of Vasodilation by Anti-PACAP Antibodies**

**[0179]** PACAP38 is a vasodilatory peptide that when administered intradermally can cause an increase in local dermal blood flow in rodents, cynomolgus monkeys (cynos) and humans. This increase in local dermal blood flow (DBF) can be measured by laser Doppler imaging and the prevention of this effect by an anti-PACAP antibody can serve as a translational pharmacodynamic (PD) model of antagonism of PACAP biological activity.

**[0180]** To evaluate the efficacy of the anti-PACAP antibodies of the invention in inhibiting receptor activation *in vivo*, the ability of the antibodies to inhibit DBF induced by intradermal administration of PACAP38 in rats, cynos, and/or humans is tested. Specifically, animals are administered an anti-PACAP antibody (e.g. mAbs 1, 2, 3, 4, 5, or 6) or placebo subcutaneously or intravenously at one or more concentrations. Subsequently, PACAP38 peptide is injected intradermally into the abdomen (rats) or forearm or thigh (cynos or humans) and local DBF in the area of the injection site is measured by laser Doppler imaging prior to and periodically (e.g. every five or ten minutes) after injection. The detected intensity fluctuations are processed to give parameters of flux (proportional to tissue blood flow) and concentration (proportional to the concentration of moving blood cells). DBF is measured as Flux (relative units) or % change from baseline [100 x (individual post-PACAP flux-individual baseline flux)/individual baseline flux]. An IC50 value for the anti-PACAP antibody can be calculated from a dose-response curve of antibody concentration vs. Flux. Ability of the anti-PACAP antibody to inhibit PACAP38-induced DBF can be used to predict the *in vivo* efficacy of the antibody to inhibit PACAP38-induced receptor activation and possible efficacy in treating PACAP/PAC1 receptor-mediated disorders, such as cluster headache and migraine.

**[0181]** All publications, patents, and patent applications discussed and cited herein are hereby incorporated by reference in their entireties. It is understood that the disclosed invention is not limited to the particular methodology, protocols and materials described as these can vary. It is

also understood that the terminology used herein is for the purposes of describing particular embodiments only and is not intended to limit the scope of the appended claims.

**[0182]** Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

# CLAIMS

What is claimed:

1. A method for inhibiting activation of human PAC1 receptor in a patient having a headache condition comprising administering to the patient an effective amount of a monoclonal antibody or binding fragment thereof, wherein the monoclonal antibody or binding fragment specifically binds to human pituitary adenylate cyclase-activating polypeptide (PACAP) at an epitope within residues 28 to 38 of human PACAP38 (SEQ ID NO: 1), and wherein the monoclonal antibody or binding fragment inhibits PACAP38-induced activation of human PAC1 receptor with an IC50 less than 5 nM as measured by a cell-based cAMP assay.

2. A method for treating or preventing a headache condition in a patient in need thereof comprising administering to the patient an effective amount of a monoclonal antibody or binding fragment thereof, wherein the monoclonal antibody or binding fragment specifically binds to human PACAP at an epitope within residues 28 to 38 of human PACAP38 (SEQ ID NO: 1), and wherein the monoclonal antibody or binding fragment inhibits PACAP38-induced activation of human PAC1 receptor with an IC50 less than 5 nM as measured by a cell-based cAMP assay.

3. The method of claim 1 or 2, wherein the headache condition is migraine.

4. The method of claim 3, wherein the migraine is episodic migraine.

5. The method of claim 3, wherein the migraine is chronic migraine.

6. The method of claim 1 or 2, wherein the headache condition is cluster headache.

7. The method of claim 1 or 2, wherein the monoclonal antibody or binding fragment binds an epitope within residues 28 to 37 of human PACAP38 (SEQ ID NO: 1).

8. The method of claim 1 or 2, wherein the monoclonal antibody or binding fragment binds an epitope within residues 34 to 38 of human PACAP38 (SEQ ID NO: 1).

9. The method of claim 1 or 2, wherein the monoclonal antibody or binding fragment thereof binds to a polypeptide consisting of the sequence of SEQ ID NO: 4, but does not significantly bind to a polypeptide consisting of the sequence of SEQ ID NO: 5.

10. The method of claim 1 or 2, wherein the monoclonal antibody or binding fragment thereof binds to a polypeptide consisting of the sequence of SEQ ID NO: 4 with a binding affinity at least 2-fold greater than that for a polypeptide consisting of the sequence of SEQ ID NO: 115.

11. The method of claim 1 or 2, wherein the monoclonal antibody or binding fragment thereof binds to a polypeptide consisting of the sequence of SEQ ID NO: 4.

12. The method of claim 1 or 2, wherein the monoclonal antibody or binding fragment thereof binds to a polypeptide consisting of the sequence of SEQ ID NO: 126.

13. The method of any one of claims 1 to 12, wherein the monoclonal antibody or binding fragment does not significantly inhibit the binding of PACAP27 to human PAC1 receptor.

14. The method of any one of claims 1 to 13, wherein the monoclonal antibody or binding fragment inhibits PACAP38-induced activation of human PAC1 receptor with an IC50 less than 1 nM as measured by a cell-based cAMP assay.

15. The method of any one of claims 1 to 14, wherein the monoclonal antibody or binding fragment does not significantly inhibit PACAP27-induced activation of human PAC1 receptor.

16. The method of any one of claims 1 to 15, wherein the monoclonal antibody or binding fragment competes with a reference antibody for binding to human PACAP38, wherein the reference antibody comprises:

(a) a light chain variable region comprising the sequence of SEQ ID NO: 18 and a heavy chain variable region comprising the sequence of SEQ ID NO: 19;

(b) a light chain variable region comprising the sequence of SEQ ID NO: 20 and a heavy chain variable region comprising the sequence of SEQ ID NO: 21; or

(c) a light chain variable region comprising the sequence of SEQ ID NO: 22 and a heavy chain variable region comprising the sequence of SEQ ID NO: 23.

17. The method of claim 16, wherein the reference antibody comprises:

(a) a light chain comprising the sequence of SEQ ID NO: 6 and a heavy chain comprising the sequence of SEQ ID NO: 7;

(b) a light chain comprising the sequence of SEQ ID NO: 8 and a heavy chain comprising the sequence of SEQ ID NO: 9; or

(c) a light chain comprising the sequence of SEQ ID NO: 10 and a heavy chain comprising the sequence of SEQ ID NO: 11.

18. The method of any one of claims 1 to 17, wherein the monoclonal antibody or binding fragment thereof is a chimeric antibody or binding fragment thereof.

19. The method of any one of claims 1 to 17, wherein the monoclonal antibody or binding fragment thereof is a humanized antibody or binding fragment thereof.

20. The method of claims 18 or 19, wherein the chimeric or humanized antibody comprises a human IgG1, IgG2, IgG3, or IgG4 Fc region.

21. The method of any one of claims 1 to 20, wherein the monoclonal antibody or binding fragment thereof is administered to the patient parenterally.

22. The method of claim 21, wherein the parenteral administration is subcutaneous or intravenous administration.

23. The method of any one of claims 2 to 22, wherein the monoclonal antibody or binding fragment thereof is administered to the patient as a prophylactic treatment.

24. An isolated monoclonal antibody or binding fragment thereof that specifically binds to human pituitary adenylate cyclase-activating polypeptide (PACAP), wherein the antibody or binding fragment binds an epitope within residues 28 to 38 of human PACAP38 (SEQ ID NO: 1), and wherein the monoclonal antibody or binding fragment inhibits PACAP38-induced activation of human PAC1 receptor with an IC50 less than 5 nM as measured by a cell-based cAMP assay.

25. The isolated monoclonal antibody or binding fragment thereof of claim 24, wherein the monoclonal antibody or binding fragment binds an epitope within residues 28 to 37 of human PACAP38 (SEQ ID NO: 1).

26. The isolated monoclonal antibody or binding fragment thereof of claim 24, wherein the monoclonal antibody or binding fragment binds an epitope within residues 34 to 38 of human PACAP38 (SEQ ID NO: 1).

27. The isolated monoclonal antibody or binding fragment thereof of claim 24, wherein the antibody or binding fragment does not significantly bind to a polypeptide consisting of the sequence of SEQ ID NO: 2 or a polypeptide consisting of the sequence of SEQ ID NO: 3.

28. The isolated monoclonal antibody or binding fragment thereof of any one of claims 24 to 27, wherein the monoclonal antibody or binding fragment thereof binds to a polypeptide consisting of the sequence of SEQ ID NO: 4 with a binding affinity at least 2-fold greater than that for a polypeptide consisting of the sequence of SEQ ID NO: 115.

29. The isolated monoclonal antibody or binding fragment thereof of any one of claims 24 to 27, wherein the monoclonal antibody or binding fragment thereof binds to a polypeptide consisting of the sequence of SEQ ID NO: 4.

30. The isolated monoclonal antibody or binding fragment thereof of any one of claims 24 to 27, wherein the monoclonal antibody or binding fragment thereof binds to a polypeptide consisting of the sequence of SEQ ID NO: 126.

31. The isolated monoclonal antibody or binding fragment thereof of any one of claims 24 to 30, wherein the antibody or binding fragment does not significantly inhibit the binding of PACAP27 to the human PAC1 receptor.

32. The isolated monoclonal antibody or binding fragment thereof of any one of claims 24 to 31, wherein the antibody or binding fragment inhibits PACAP38-induced activation of human PAC1 receptor with an IC50 less than 1 nM as measured by a cell-based cAMP assay.

33. The isolated monoclonal antibody or binding fragment thereof of any one of claims 24 to 32, wherein the antibody or binding fragment does not significantly inhibit PACAP27-induced activation of human PAC1 receptor.

34. The isolated monoclonal antibody or binding fragment thereof of claim 24, wherein the antibody or binding fragment competes with a reference antibody for binding to human PACAP38, wherein the reference antibody comprises:

(a) a light chain variable region comprising the sequence of SEQ ID NO: 18 and a heavy chain variable region comprising the sequence of SEQ ID NO: 19;

(b) a light chain variable region comprising the sequence of SEQ ID NO: 20 and a heavy chain variable region comprising the sequence of SEQ ID NO: 21; or

(c) a light chain variable region comprising the sequence of SEQ ID NO: 22 and a heavy chain variable region comprising the sequence of SEQ ID NO: 23.

35. The isolated monoclonal antibody or binding fragment thereof of claim 34, wherein the reference antibody comprises:

(a) a light chain comprising the sequence of SEQ ID NO: 6 and a heavy chain comprising the sequence of SEQ ID NO: 7;

(b) a light chain comprising the sequence of SEQ ID NO: 8 and a heavy chain comprising the sequence of SEQ ID NO: 9; or

(c) a light chain comprising the sequence of SEQ ID NO: 10 and a heavy chain comprising the sequence of SEQ ID NO: 11.

36. The isolated monoclonal antibody or binding fragment thereof of any one of claims 24 to 35, wherein the antibody or binding fragment comprises a light chain variable region comprising complementarity determining regions CDRL1, CDRL2, and CDRL3, wherein CDRL1 has the sequence of SEQ ID NO: 30 or 31, CDRL2 has the sequence of SEQ ID NO: 35 or 36, and CDRL3 has the sequence of SEQ ID NO: 40 or 41.

37. The isolated monoclonal antibody or binding fragment thereof of any one of claims 24 to 36, wherein the antibody or binding fragment comprises a heavy chain variable region comprising complementarity determining regions CDRH1, CDRH2, and CDRH3, wherein CDRH1 has a sequence selected from SEQ ID NOs: 45-47, CDRH2 has the sequence of SEQ ID NO: 51 or 52, and CDRH3 has a sequence selected from SEQ ID NOs: 56-58.

38. The isolated monoclonal antibody or binding fragment thereof of any one of claims 24 to 37, wherein the antibody or binding fragment comprises a light chain variable region comprising a sequence that is at least 90% identical to a sequence selected from SEQ ID NOs: 18, 20, or 22.

39. The isolated monoclonal antibody or binding fragment thereof of any one of claims 24 to 38, wherein the antibody or binding fragment comprises a heavy chain variable region comprising a sequence that is at least 90% identical to a sequence selected from SEQ ID NOs: 19, 21, or 23.

40. The isolated monoclonal antibody or binding fragment thereof of any one of claims 24 to 39, wherein the antibody or binding fragment thereof is a chimeric antibody or binding fragment thereof.

41. The isolated monoclonal antibody or binding fragment thereof of any one of claims 24 to 39, wherein the antibody or binding fragment thereof is a humanized antibody or binding fragment thereof.

42. The isolated monoclonal antibody or binding fragment thereof of claims 40 or 41, wherein the chimeric or humanized antibody comprises a human IgG1, IgG2, IgG3, or IgG4 Fc region.

43. An isolated monoclonal antibody or binding fragment thereof comprising (i) a light chain variable region comprising complementarity determining regions CDRL1, CDRL2, and CDRL3, and (ii) a heavy chain variable region comprising complementarity determining regions CDRH1, CDRH2, and CDRH3, wherein CDRL1 has a sequence selected from SEQ ID NOs: 30-34, CDRL2 has a sequence selected from SEQ ID NOs: 35-39, CDRL3 has a sequence selected from SEQ ID NOs: 40-44, CDRH1 has a sequence selected from SEQ ID NOs: 45-50, CDRH2 has a sequence selected from SEQ ID NOs: 51-55, and CDRH3 has a sequence selected from SEQ ID NOs: 56-61.

44. The isolated monoclonal antibody or binding fragment thereof of claim 43, wherein the monoclonal antibody or binding fragment comprises:

(a) CDRL1, CDRL2, and CDRL3 sequences of SEQ ID NOs: 30, 35, and 40, respectively, and CDRH1, CDRH2, and CDRH3 sequences of SEQ ID NOs: 45, 51, and 56, respectively;

(b) CDRL1, CDRL2, and CDRL3 sequences of SEQ ID NOs: 31, 36, and 41, respectively, and CDRH1, CDRH2, and CDRH3 sequences of SEQ ID NOs: 46, 52, and 57, respectively;

(c) CDRL1, CDRL2, and CDRL3 sequences of SEQ ID NOs: 30, 35, and 40, respectively, and CDRH1, CDRH2, and CDRH3 sequences of SEQ ID NOs: 47, 51, and 58, respectively;

(d) CDRL1, CDRL2, and CDRL3 sequences of SEQ ID NOs: 32, 37, and 42, respectively, and CDRH1, CDRH2, and CDRH3 sequences of SEQ ID NOs: 48, 53, and 59, respectively;

(e) CDRL1, CDRL2, and CDRL3 sequences of SEQ ID NOs: 33, 38, and 43, respectively, and CDRH1, CDRH2, and CDRH3 sequences of SEQ ID NOs: 49, 54, and 60, respectively; or

(f) CDRL1, CDRL2, and CDRL3 sequences of SEQ ID NOs: 34, 39, and 44, respectively, and CDRH1, CDRH2, and CDRH3 sequences of SEQ ID NOs: 50, 55, and 61, respectively.

45. The isolated monoclonal antibody or binding fragment thereof of claim 43 or 44, wherein the monoclonal antibody or binding fragment comprises a light chain variable region comprising a sequence that is at least 90% identical to a sequence selected from SEQ ID NOs: 18, 20, 22, 24, 26, or 28; and a heavy chain variable region comprising a sequence that is at least 90% identical to a sequence selected from SEQ ID NOs: 19, 21, 23, 25, 27, or 29.

46. The isolated monoclonal antibody or binding fragment thereof of any one of claims 43 to 45, wherein the monoclonal antibody or binding fragment comprises:

(a) a light chain variable region comprising the sequence of SEQ ID NO: 18 and a heavy chain variable region comprising the sequence of SEQ ID NO: 19;

(b) a light chain variable region comprising the sequence of SEQ ID NO: 20 and a heavy chain variable region comprising the sequence of SEQ ID NO: 21;

(c) a light chain variable region comprising the sequence of SEQ ID NO: 22 and a heavy chain variable region comprising the sequence of SEQ ID NO: 23;

(d) a light chain variable region comprising the sequence of SEQ ID NO: 24 and a heavy chain variable region comprising the sequence of SEQ ID NO: 25;

(e) a light chain variable region comprising the sequence of SEQ ID NO: 26 and a heavy chain variable region comprising the sequence of SEQ ID NO: 27; or

(f) a light chain variable region comprising the sequence of SEQ ID NO: 28 and a heavy chain variable region comprising the sequence of SEQ ID NO: 29.

47. The isolated monoclonal antibody or binding fragment thereof of any one of claims 43 to 46, wherein the monoclonal antibody comprises a light chain comprising a sequence selected from SEQ ID NOs: 6, 8, 10, 12, 14, or 16, and a heavy chain comprising a sequence selected from SEQ ID NOs: 7, 9, 11, 13, 15, or 17.

48. The isolated monoclonal antibody of claim 47, wherein the monoclonal antibody comprises:

(a) a light chain comprising the sequence of SEQ ID NO: 6 and a heavy chain comprising the sequence of SEQ ID NO: 7;

(b) a light chain comprising the sequence of SEQ ID NO: 8 and a heavy chain comprising the sequence of SEQ ID NO: 9;

(c) a light chain comprising the sequence of SEQ ID NO: 10 and a heavy chain comprising the sequence of SEQ ID NO: 11;

(d) a light chain comprising the sequence of SEQ ID NO: 12 and a heavy chain comprising the sequence of SEQ ID NO: 13;

(e) a light chain comprising the sequence of SEQ ID NO: 14 and a heavy chain comprising the sequence of SEQ ID NO: 15; or

(f) a light chain comprising the sequence of SEQ ID NO: 16 and a heavy chain comprising the sequence of SEQ ID NO: 17.

49. An isolated polynucleotide encoding the monoclonal antibody or binding fragment thereof of any one of claims 24 to 48.

50. The isolated polynucleotide of claim 49, wherein the polynucleotide comprises a sequence that is at least 80% identical to SEQ ID NOs: 72-77.

51. The isolated polynucleotide of claim 49, wherein the polynucleotide comprises a sequence that is at least 80% identical to SEQ ID NOs: 78-83.

52. An expression vector comprising the polynucleotide of any one of claims 49 to 51.

53. A host cell comprising the expression vector of claim 52.

54. A method of producing an anti-PACAP monoclonal antibody or binding fragment thereof comprising culturing the host cell of claim 53 under conditions that allow expression of the

antibody or binding fragment; and recovering the antibody or binding fragment from the culture medium or host cell.

55. A composition comprising the monoclonal antibody or binding fragment thereof of any one of claims 24 to 48 and a pharmaceutically acceptable diluent, excipient, or carrier.

56. A method for inhibiting activation of human PAC1 receptor in a patient having a headache condition comprising administering to the patient an effective amount of a monoclonal antibody or binding fragment thereof of any one of claims 43 to 48.

57. A method for treating or preventing a headache condition in a patient in need thereof comprising administering to the patient an effective amount of a monoclonal antibody or binding fragment thereof of any one of claims 43 to 48.

58. The method of claim 56 or 57, wherein the headache condition is migraine.

59. The method of claim 58, wherein the migraine is episodic migraine.

60. The method of claim 58, wherein the migraine is chronic migraine.

61. The method of claim 56 or 57, wherein the headache condition is cluster headache.

62. The method of any one of claims 56 to 61, wherein the monoclonal antibody or binding fragment thereof is administered to the patient parenterally.

63. The method of claim 62, wherein the parenteral administration is subcutaneous or intravenous administration.

64. The method of any one of claims 57 to 63, wherein the monoclonal antibody or binding fragment thereof is administered to the patient as a prophylactic treatment.

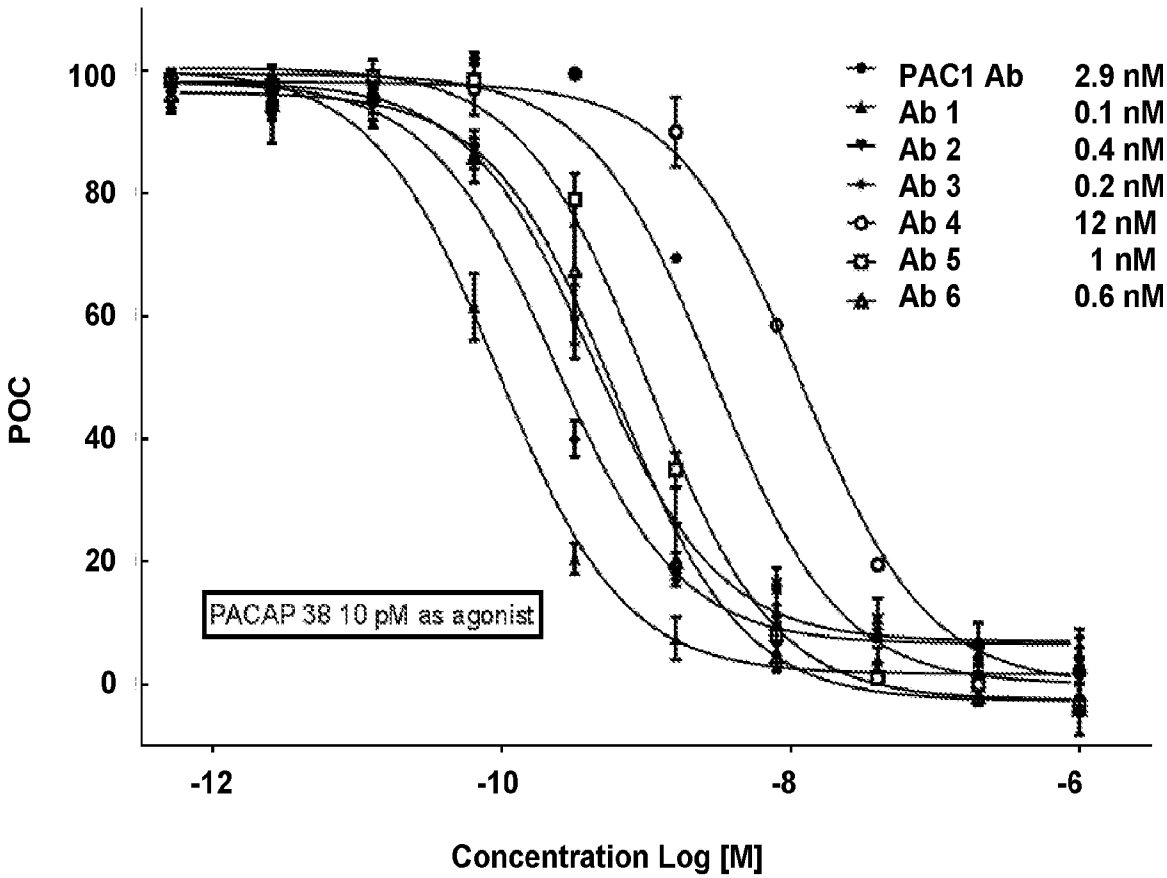

100
80
60
40
20
0
POC
-12
-10
-8
-6
Concentration Log [M]
PACAP 38 10 pM as agonist
PAC1 Ab 2.9 nM
Ab 1 0.1 nM
Ab 2 0.4 nM
Ab 3 0.2 nM
Ab 4 12 nM
Ab 5 1 nM
Ab 6 0.6 nM

**FIG. 1**

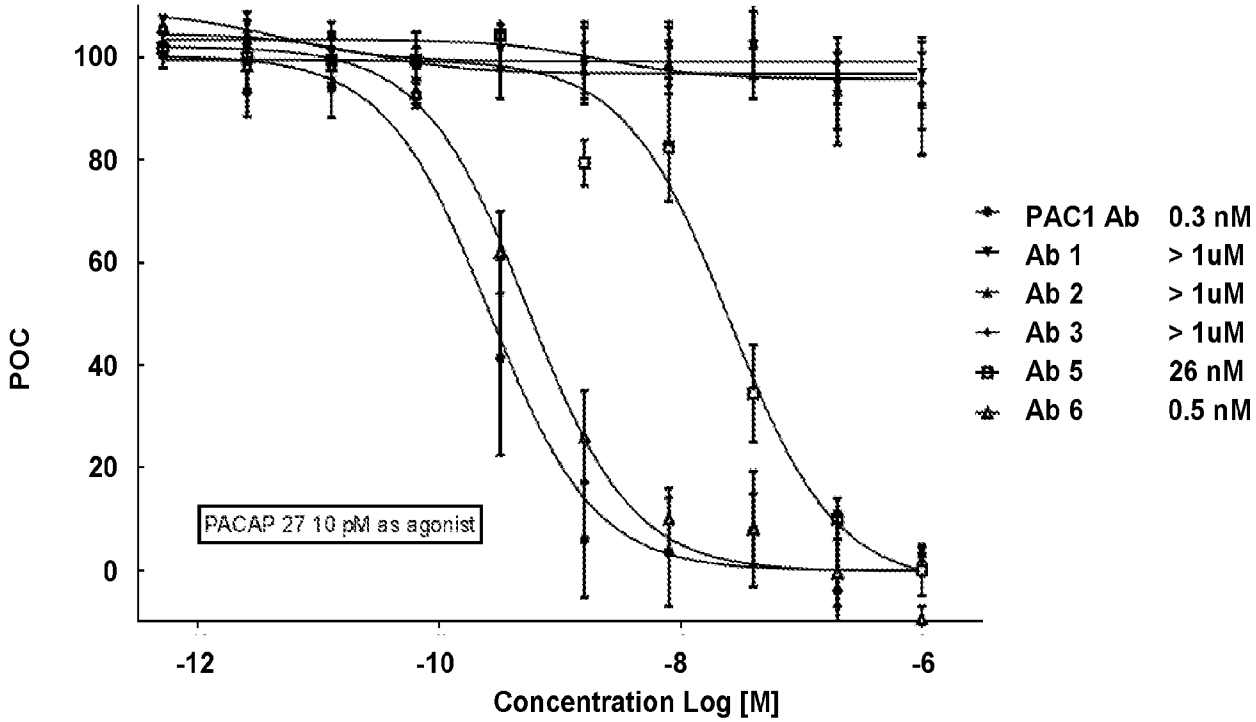
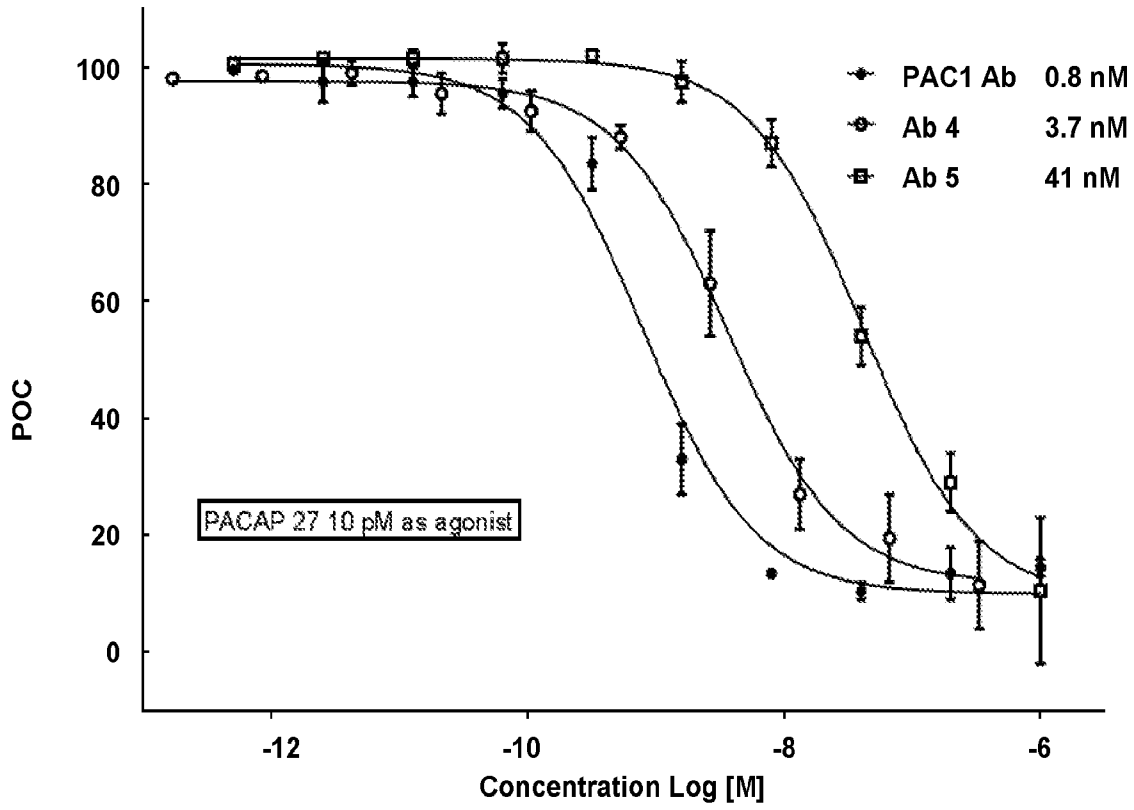

100
80
60
40
20
0
POC
PACAP 27 10 pM as agonist
-12
-10
-8
-6
Concentration Log [M]
PAC1 Ab 0.3 nM
Ab 1 > 1uM
Ab 2 > 1uM
Ab 3 > 1uM
Ab 5 26 nM
Ab 6 0.5 nM

FIG. 2A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POC
PACAP 27 10 pM as agonist
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-8
-6
Concentration Log [M]
PAC1 Ab 0.8 nM
Ab 4 3.7 nM
Ab 5 41 nM

FIG. 2B

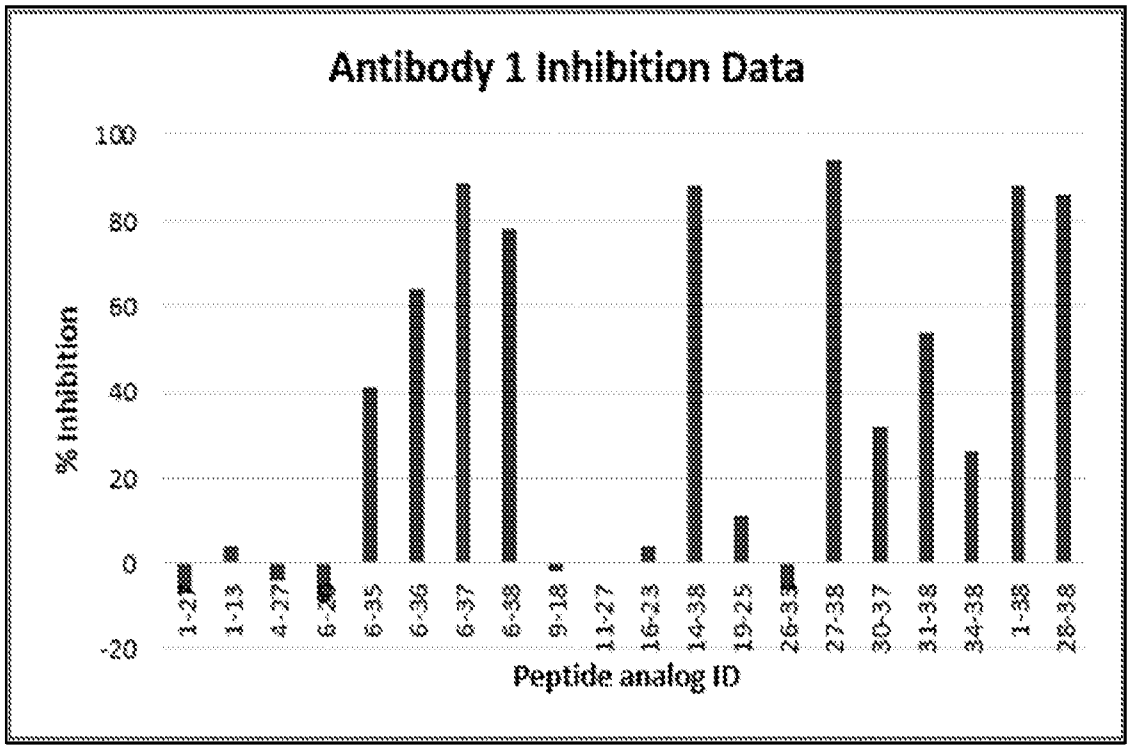
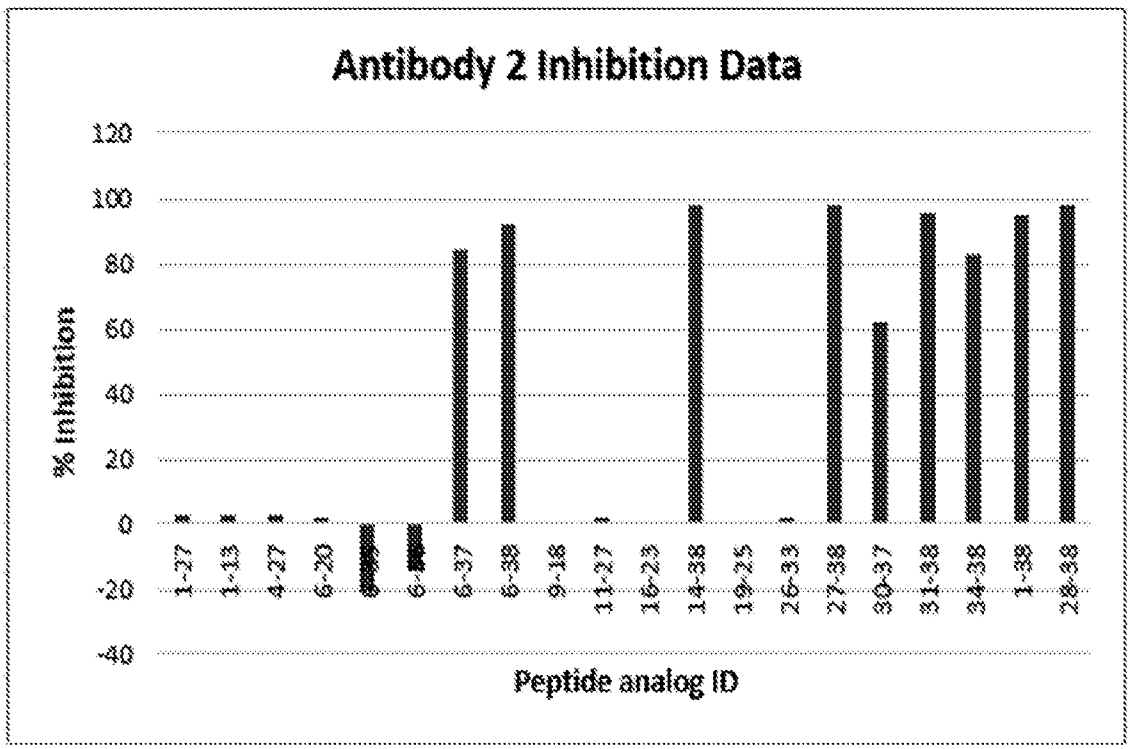

Antibody 1 Inhibition Data
% Inhibition
100
80
60
40
20
0
-20
1-27
1-13
4-27
6-20
6-35
6-36
6-37
6-38
9-18
11-27
16-23
14-38
19-25
26-33
27-38
30-37
31-38
34-38
1-38
28-38
Peptide analog ID

FIG. 3A


Antibody 2 Inhibition Data
% Inhibition
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80
60
40
20
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-20
-40
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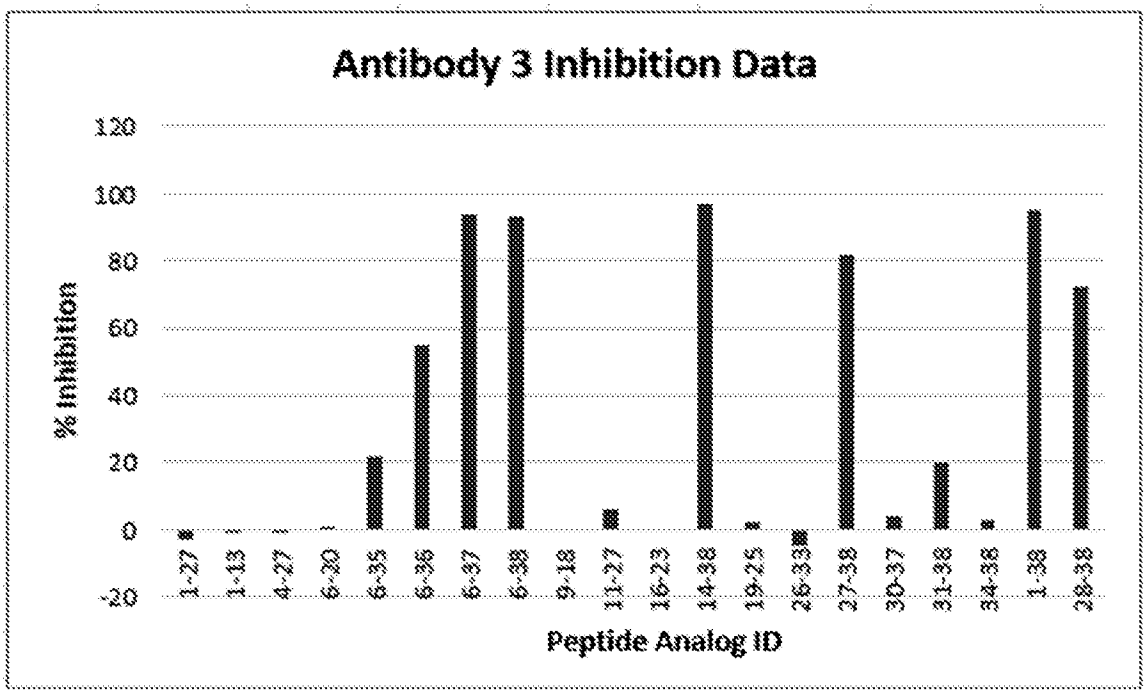
1-13
4-27
6-20
6-37
6-38
9-18
11-27
16-23
14-38
19-25
26-33
27-38
30-37
31-38
34-38
1-38
28-38
Peptide analog ID

FIG. 3B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% Inhibition
120
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80
60
40
20
0
-20
1-27
1-13
4-27
6-20
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6-37
6-38
9-18
11-27
16-23
14-38
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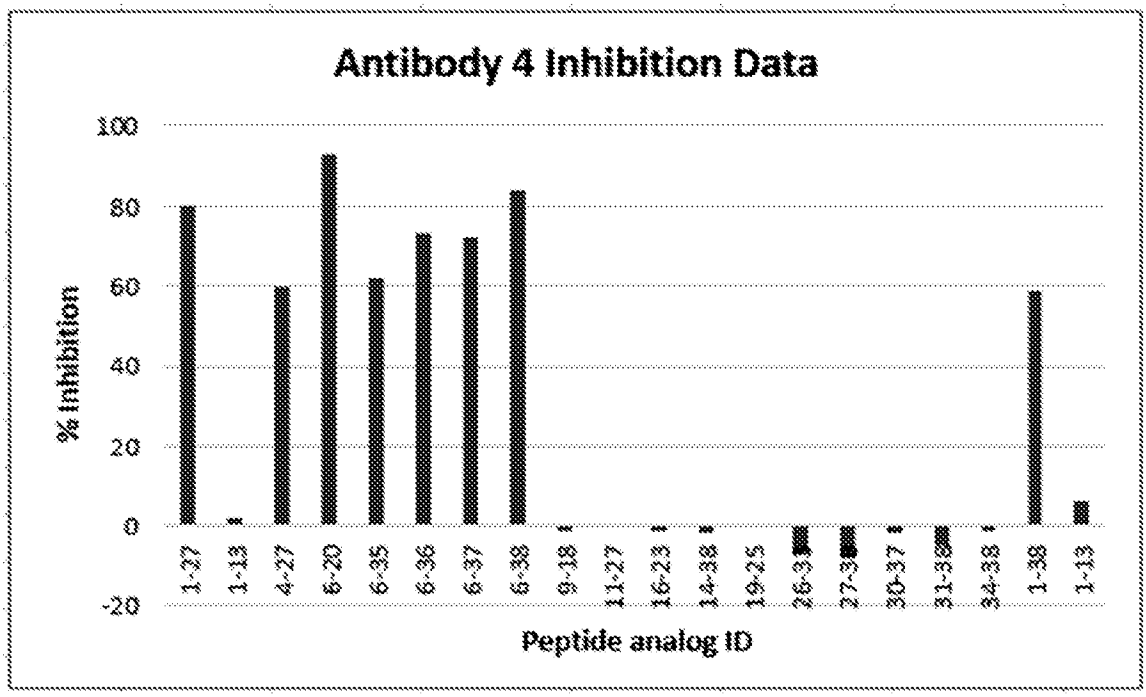
30-37
31-38
34-38
1-38
28-38
Peptide Analog ID

FIG. 3C


Antibody 4 Inhibition Data
% Inhibition
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80
60
40
20
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-20
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4-27
6-20
6-35
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6-37
6-38
9-18
11-27
16-23
14-38
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26-3
27-3
30-37
31-38
34-38
1-38
1-13
Peptide analog ID

FIG. 3D

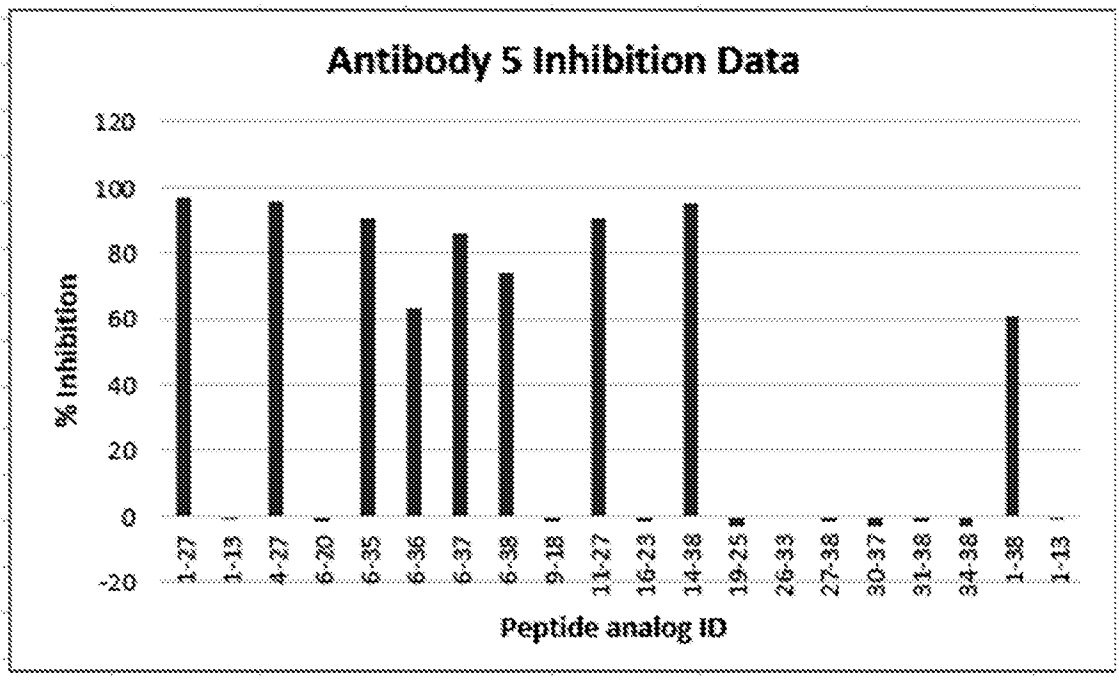
Antibody 5 Inhibition Data
% Inhibition
120
100
80
60
40
20
0
-20
1-27
1-13
4-27
6-20
6-35
6-36
6-37
6-38
9-18
11-27
16-23
14-38
19-25
26-33
27-38
30-37
31-38
34-38
1-38
1-13
Peptide analog ID

FIG. 3E

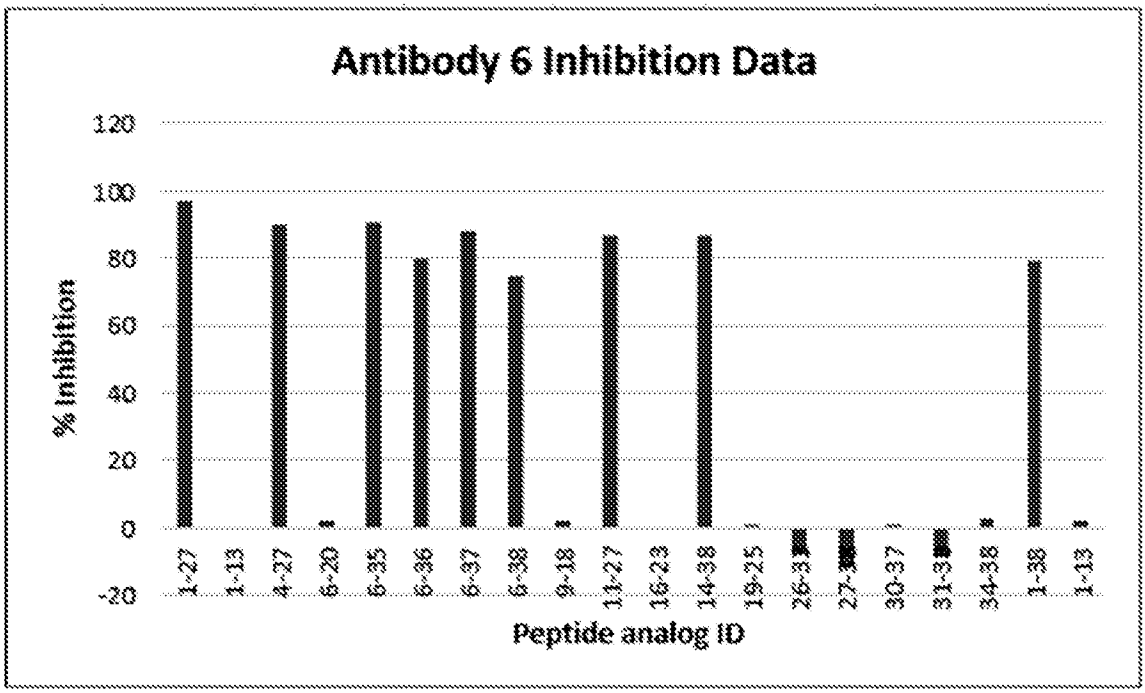
Antibody 6 Inhibition Data
% Inhibition
120
100
80
60
40
20
0
-20
1-27
1-13
4-27
6-20
6-35
6-36
6-37
6-38
9-18
11-27
16-23
14-38
19-25
26-33
27-38
30-37
31-38
34-38
1-38
1-13
Peptide analog ID

FIG. 3F

# INTERNATIONAL SEARCH REPORT

International application No
PCT/US2016/067054

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/26
ADD. A61K39/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | RAINER SCHWARZHOFF ET AL: "Specific monoclonal antibodies neutralize the action of PACAP 1-27 or PACAP 1-38 on intestinal muscle strips in vitro", REGULATORY PEPTIDES., vol. 55, no. 1, 1 January 1995 (1995-01-01), pages 57-66, XP055351445, NL ISSN: 0167-0115, DOI: 10.1016/0167-0115(94)00092-C abstract; figure 1 page 63, left-hand column, paragraph 1 page 65, left-hand column, paragraph 1 page 61, right-hand column, paragraph 1 table 1 ----- -/-- | 24-55 |

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 3 March 2017 | 17/05/2017 |

| Name and mailing address of the ISA/ | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040,<br>Fax: (+31-70) 340-3016 | Malamoussi, A |

# INTERNATIONAL SEARCH REPORT

International application No
PCT/US2016/067054

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | NOBUHIRO SUZUKI ET AL: "Production of Immunoreactive Pituitary Adenylate Cyclase Activating Polypeptide (PACAP) by Human Neuroblastoma Cells, IMR-32: Detection and Characterization with Monoclonal and Polyconal Antibodies against Different Epitopes of PACAP", JOURNAL OF BIOCHEMISTRY, vol. 113, no. 5, 1 May 1993 (1993-05-01), pages 549-556, XP055351080, GB ISSN: 0021-924X, DOI: 10.1093/oxfordjournals.jbchem.a124081 page 552, left-hand column, paragraph 1 page 553, left-hand column, paragraph 1 ----- | 24-55 |
| X | WO 2004/062684 A2 (COLLEN RES FOUNDATION VZW ONDE [BE]; FRESON KATHLEEN [BE]; VAN GEET CH) 29 July 2004 (2004-07-29) page 30, line 5 - line 19 page 29, line 6 - line 14 ----- | 24-55 |
| X | ALESSANDRO S. ZAGAMI ET AL: "Pituitary adenylate cyclase activating polypeptide and migraine", ANNALS OF CLINICAL AND TRANSLATIONAL NEUROLOGY, vol. 1, no. 12, 12 November 2014 (2014-11-12), pages 1036-1040, XP055351305, GB ISSN: 2328-9503, DOI: 10.1002/acn3.113 abstract page 1038, left-hand column, paragraph 4 page 1039, left-hand column, paragraph 2 ----- | 1-23, 56-64 |
| X,P | WO 2016/168757 A1 (ALDER BIOPHARMACEUTICALS INC [US]; THE UNIV OF IOWA RES FOUND [US]; AL) 20 October 2016 (2016-10-20) examples 1-12 ----- | 24-55 |
| A | ARSALAN U SYED ET AL: "Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP) Potently Dilates Middle Meningeal Arteries: Implications for Migraine", JOURNAL OF MOLECULAR NEUROSCIENCE, HUMANA PRESS INC, NEW YORK, vol. 48, no. 3, 6 July 2012 (2012-07-06), pages 574-583, XP035115144, ISSN: 1559-1166, DOI: 10.1007/S12031-012-9851-0 abstract ----- -/-- | 1-64 |

# INTERNATIONAL SEARCH REPORT

International application No
PCT/US2016/067054

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | BERNADETT TUKA ET AL: "Peripheral and central alterations of pituitary adenylate cyclase activating polypeptide-like immunoreactivity in the rat in response to activation of the trigeminovascular system", PEPTIDES, ELSEVIER, AMSTERDAM, NL, vol. 33, no. 2, 29 December 2011 (2011-12-29), pages 307-316, XP028462579, ISSN: 0196-9781, DOI: 10.1016/J.PEPTIDES.2011.12.019 [retrieved on 2012-01-08] abstract<br>---<br>H. W. SCHYTZ ET AL: "PACAP38 induces migraine-like attacks in patients with migraine without aura", BRAIN., vol. 132, no. 1, 11 November 2008 (2008-11-11), pages 16-25, XP055128665, GB ISSN: 0006-8950, DOI: 10.1093/brain/awn307 abstract<br>--- | 1-64<br><br>1-64 |

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2016/067054

**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-64(partially)

**Remark on Protest**

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

**FURTHER INFORMATION CONTINUED FROM PCT/ISA/** 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-64(partially)

   relating to the antibody clone Ab1 with HCDR1-3 and LCDR3 with SEQ ID NO 30, 35, 40, 45, 51 and 56, respectively, and its use in therapy

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2. claims: 1-64(partially)

   relating to the antibody clone Ab2 with HCDR1-3 and LCDR3 with SEQ ID NO 31, 36, 41, 46, 52 and 57, respectively, and its use in therapy.

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3. claims: 1-64(partially)

   relating to the antibody clone Ab3 with HCDR1-3 and LCDR3 with SEQ ID NO 30, 35, 40, 47, 51 and 58, respectively, and its use in therapy.

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4. claims: 43-64(partially)

   relating to the antibody clone Ab4 with HCDR1-3 and LCDR3 with SEQ ID NO 32, 37, 42, 48, 53 and 59, respectively, and its use in therapy.

   ---

5. claims: 43-64(partially)

   relating to the antibody clone Ab5 with HCDR1-3 and LCDR3 with SEQ ID NO 33, 38, 43, 49, 54 and 60, respectively, and its use in therapy.

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6. claims: 43-64(partially)

   relating to the antibody clone Ab6 with HCDR1-3 and LCDR3 with SEQ ID NO 34, 39, 44, 50, 55 and 61, respectively, and its use in therapy.

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# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2016/067054

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