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(71) **Demandeur/Applicant:**
PETMEDIX LTD, GB

(72) **Inventeurs/Inventors:**
LI, MENG AMY, GB;
BARDELLI, MARCO, GB

(74) **Agent:** SMART & BIGGAR LP

(54) **Titre : PROTEINES COMPRENANT LE DOMAINE EXTRACELLULAIRE DE P75NTR**
(54) **Title: PROTEINS COMPRISING THE EXTRACELLULAR DOMAIN OF P75NTR**

Figure 1a.

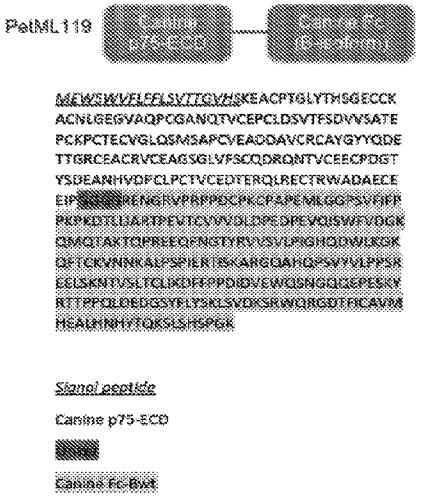
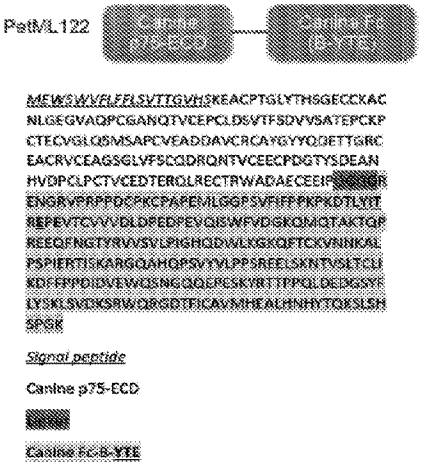


Figure 1b.



(57) **Abrégé/Abstract:**
The present invention relates to effective pain therapies in companion animals. In particular, an isolated companion animal p75NTR protein or a fusion protein containing the same or portions thereof are contemplated. Nucleic acids encoding the proteins are also encompassed in the invention as well as methods of using the same.

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(71) Applicant: **PETMEDIX LTD** [GB/GB]; The Glenn Berge Building, Building 940, Babraham Research Campus, Babraham, Cambridge Cambridgeshire CB22 3FH (GB).

(72) Inventors: **LI, Meng Amy**; The Glenn Berge Building, Building 940, Babraham Research Campus, Babraham, Cambridge Cambridgeshire CB22 3FH (GB). **BARDELLI, Marco**; The Glenn Berge Building, Building 940, Babraham Research Campus, Babraham, Cambridge Cambridgeshire CB22 3FH (GB).

(74) Agent: **APPLEYARD LEES IP LLP**; 15 Clare Road, Halifax Yorkshire HX1 2HY (GB).

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(54) Title: PROTEINS COMPRISING THE EXTRACELLULAR DOMAIN OF P75NTR

Figure 1a.

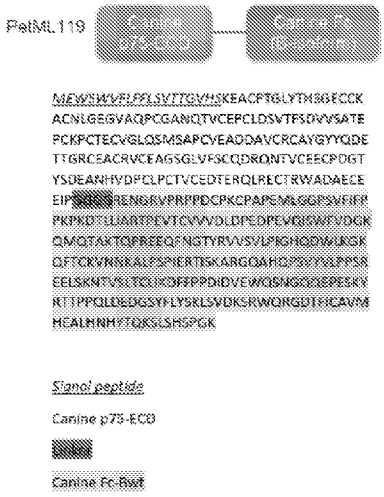
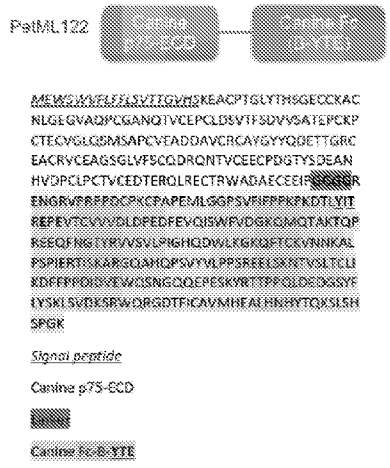


Figure 1b.



(57) Abstract: The present invention relates to effective pain therapies in companion animals. In particular, an isolated companion animal p75NTR protein or a fusion protein containing the same or portions thereof are contemplated. Nucleic acids encoding the proteins are also encompassed in the invention as well as methods of using the same.

PROTEINS COMPRISING THE EXTRACELLULAR DOMAIN OF P75NTR

Related Applications and Incorporation by Reference

All documents cited or referenced herein ("herein cited documents"), and all documents cited or referenced in herein cited documents, together with any manufacturer's instructions, descriptions, product specifications, and product sheets for any products mentioned herein or in any document incorporated by reference herein, are hereby incorporated herein by reference, and may be employed in the practice of the invention. More specifically, all referenced documents are incorporated by reference to the same extent as if each individual document was specifically and individually indicated to be incorporated by reference.

Field of the Invention

The present invention relates to effective pain therapies in companion animals.

Introduction

There is a huge need for therapies in veterinary medicine, in particular for pain relief.

Pain relief treatments for dogs currently include nonsteroidal anti-inflammatory drugs, commonly called NSAIDs, and several nonsteroidal anti-inflammatory drugs which help to control pain and inflammation associated with osteoarthritis. Several NSAIDs have been approved by the FDA. However, there is a need for further effective pain treatments in companion animals with minimal side effects.

Nerve growth factor (NGF) was firstly discovered in 1950s. The NGF protein was then cloned and identified as part of brain-derived neurotrophic factor (BDNF), neurotrophin-3 and 4/5 (NT-3, NT-4-5). All of these are secreted proteins that promote survival and growth of the peripheral nervous system.

NGF causes peripheral sensitization both in vitro and in vivo, as illustrated by the increased response of DRG neurons to temperature or capsaicin in its presence. NGF also leads to transcriptional regulation after retrograde axonal transport, as illustrated by immunostaining showing upregulation of BDNF after intrathecal NGF treatment. Furthermore, NGF can cause sprouting of peripheral afferents into diseased joints and cancerous tissue (Denk et al, Annual Review of Neuroscience, Vol. 40:307-325, 2017).

NGF is expressed at low levels in adulthood, but injury, inflammation or release of NGF cause activation of inflammatory cells. These cells in turn produce and secrete NGF as well and this leads to short-term

and long-term effects. NGF has a well-known and multifunctional role in nociceptive processing, although the precise signaling pathways downstream of NGF receptor activation that mediate nociception are complex and not completely understood. The role of NGF in nociception and the generation and/or maintenance of chronic pain has led to it becoming an attractive target of pain therapeutics for the treatment of chronic pain conditions (Barker et al, Journal of Pain Research, 2020:13 1223–1241).

Very low doses of monoclonal antibodies (mAbs) directed against NGF can reduce chronic pain. However, during clinical trials in humans, a small subset of patients treated with mAbs directed against NGF developed rapidly progressive joint degeneration due to interaction with NSAIDs treatment. Complete NGF removal is shown to cause impaired bone and cartilage repairing (Denk et al, Annual Review of Neuroscience, Vol. 40:307-325, 2017). Treatments for use in dogs and cats based on species-specific mAbs that target NGF are now being developed for the management of osteoarthritis (OA)-associated pain (Enomoto et al, Vet Rec. 2019 Jan 5;184(1):23 and WO2019177690). However, given the side effects that occurred in clinical trials in humans, there is a need to develop alternative treatments that target NGF.

Citation or identification of any document in this application is not an admission that such document is available as prior art to the present invention.

Summary of the invention

The invention is aimed at addressing a need for effective pain therapies in companion animals.

The invention provides a pain therapy for companion animals with reduced side effects compared to anti-NGF antibody therapies. The proteins of the invention bind NGF which is at elevated levels in pain conditions, thus binding to excess NGF to restore normal NGF levels without completely blocking NGF signalling. Without wishing to be bound by theory, the inventors believe that this ensures a level of NGF signalling which is required for healthy functions. Furthermore, it is believed that the fusion proteins of the invention can operate at a very low dose, but are highly efficacious.

The inventors have used an analgesic strategy to reduce, but not completely deplete, NGF in circulation. To this end, the Extracellular Domain (ECD) of p75 neurotrophin receptor (p75NTR) was used, fused to Fc to increase its half-life. p75NTR binds NGF and other brain-derived neurotrophic factors (NT3, NT4) and mediates different cellular activities.

The use of human p75 has been described for treatment of pain in humans (WO2013136078).

Thus, in a first aspect, the invention relates to an isolated companion animal p75NTR protein or a portion thereof. The invention also relates to an isolated nucleic acid encoding the protein or portion thereof.
The invention also relates to a vector comprising a nucleic acid as described above.
The invention further relates to a host cell comprising a nucleic acid as described above.
In another aspect, the invention relates to a fusion protein comprising an isolated companion animal p75NTR extracellular domain or portion thereof and a half-life extending moiety.
In another aspect, the invention relates to a nucleic acid encoding a fusion protein as described above.
In another aspect, the invention relates to a vector comprising a nucleic acid as described above.
In another aspect, the invention relates to a host cell comprising a nucleic acid as described above or a vector as described above.
In another aspect, the invention relates to a pharmaceutical composition comprising an isolated companion animal p75NTR protein as described above and herein, or a fusion protein as described above and herein.
In another aspect, the invention relates to a method for treating an NGF-related disorder in a companion animal comprising administering an isolated companion animal p75NTR protein as described above, a fusion protein as described above and herein or a pharmaceutical composition as described above and herein.
In another aspect, the invention relates to the use of an isolated companion animal p75NTR protein as described above, a fusion protein as described above or a pharmaceutical composition as described above and herein in the treatment of an NGF-related disorder in a companion animal.
In another aspect, the invention relates to a method of inhibiting NGF activity in a companion animal comprising administering an isolated companion animal p75NTR protein as described above, a fusion protein as described above and herein or a pharmaceutical composition as described above and herein.
In another aspect, the invention relates to a kit comprising an isolated companion animal p75NTR protein as described above, a fusion protein as described above or a pharmaceutical composition as described above and optionally instructions for use.

Accordingly, it is an object of the invention not to encompass within the invention any previously known product, process of making the product, or method of using the product such that Applicants reserve the right and hereby disclose a disclaimer of any previously known product, process, or method. It is further noted that the invention does not intend to encompass within the scope of the invention any product, process, or making of the product or method of using the product, which does not meet the written description and enablement requirements of the USPTO (35 U.S.C. §112, first paragraph) or the EPO (Article 83 of the EPC), such that Applicants reserve the right and hereby disclose a disclaimer of any previously described product, process of making the product, or method of using the product. It may be advantageous in the practice of the invention to be in compliance with Art. 53(c) EPC and Rule 28(b) and (c) EPC. All rights to explicitly disclaim any embodiments that are the subject of any granted patent(s) of applicant in the lineage of this application or in any other lineage or in any prior filed application of any third party is explicitly reserved. Nothing herein is to be construed as a promise.

It is noted that in this disclosure and particularly in the claims and/or paragraphs, terms such as "comprises", "comprised", "comprising" and the like can have the meaning attributed to it in U.S. Patent law; e.g., they can mean "includes", "included", "including", and the like; and that terms such as "consisting essentially of" and "consists essentially of" have the meaning ascribed to them in U.S. Patent law, e.g., they allow for elements not explicitly recited, but exclude elements that are found in the prior art or that affect a basic or novel characteristic of the invention.

These and other embodiments are disclosed or are obvious from and encompassed by, the following Detailed Description.

Description of Figures

The invention is described in the following non-limiting figures.

Figure 1. Exemplary fusion proteins (a) PetML119 and (b) PetML122.

Figure 2. a) Sequence alignment of part of the ECD of p75NTR as used in the fusion proteins of the invention. There is a very high similarity between species:

Canine vs Feline: 1/164 (more than 99% identical);

Canine vs Equine: 4/164 (97.6% identical);

Feline vs Equine: 5/164 (97% identical);

Canine vs Bovine: 2/164 (98.8% identical)

b) Sequence alignment of the Fc region (including CH3, CH2 and CH2-CH1 hinge) from different species/different isotypes.

Figure 3. Purity of isolated fusion proteins. a) H-SEC b) H-SCX. PetML119 appears to be highly pure by analytical size exclusion chromatography. b) Cation exchange chromatography showed presence of one main species (~80%) and two minor charge variants.

Figure 4. PetML119 Stability.

a) Tonset analysis.

b) Thermal and chemical stress.

c) Freeze and thaw stress.

d) Thermal stability.

e) Aggregation analysis.

Figure 5. Protein A binding.

a) and b) PetML119 Protein A binding.

c) and d) PetML122 Protein A binding.

e) Bedinvetmab Protein A binding. Bedinvetmab is an anti-NGF IgG control.

f) Table showing kinetics.

Figure 6. PetML119/122 canine and murine FcRN binding.

a) Canine FcRN binding PetML119.

b) Murine FcRN binding PetML119.

c) Affinity table of canine and murine binding FcRN.

d) Canine FcRN binding PetML122.

e) Murine FcRN binding PetML122.

f) Affinity table of canine and murine FcRN binding.

Figure 7. NGF binding.

a) PetML119 binding to human NGF.

b) PetML119 to rat NGF.

c) Kinetics affinity table.

d) Bedinvetmab binding to human NGF.

e) Kinetics affinity table.

Figure 8. TF-1 cell proliferation assay. TF-1 labelled with CFSE-cell trace at 2.5uM 30' RT. TF-1 seeded at 104 cells/well in 96 well plate. Positive ctr: TF-1 cells in RPMI media + 10% FBS + 10ng/mL hNGF 3 timepoints (t 2hrs, t 4d and t 7d).

a) Activity of NGF: 2ng/mL EC50.

b) TF-1 cell proliferation assay read out.

c) TF-1 cell proliferation assay read out using PetML119, PetML122 and Bedinvetmab.

Figure 9. *In vivo* model.

Figure 10. Analgesic effects *in vivo*.

a) 2 hours post IV treatment.

b) 3 days post IV treatment.

c) 11 days post IV treatment.

d) 18 days post IV treatment.

Figure 11. Joint Diametre and body weight.

a) Joint Diametre analysis.

b) body weight analysis.

Figure 12. pk analysis- half life.

a) Standard curve for PetML119 in rat serum.

b) Standard curve for PetML122 in rat serum.

c-f) Rat study pK results. Calculated half-life for PetML119 at different concentration is between 56-70hrs.

Figure 13. Safety analysis of PetML119/ PetML122 in dogs.

a) Body weight. Top row in top table shows the individual animals in Group 1, bottom row in top table shows the individual animals in Group 2. Animals in Group 1 were treated with PetML119, animals in Group 2 were treated with PetML122 (see Table 3). Bottom table shows mean values.

b) Anti-Drug Antibodies (ADA) analysis. Top panel PetML119, bottom panel PetML120.

Figure 14. a-d Efficacy analysis of PetML119/ PetML122 in dogs.

Force plate analysis and visual lameness analysis of PetML119/ PetML122.

Figure 15. pk analysis- half life for dog.
Top (a) shows endpoint Absorbance at 650nm and 450nm using sera. Bottom (b) shows one phase decay fitting to estimate half-life for PetML119/ PetML122.

Detailed description

The present invention will now be further described. In the following passages, different aspects of the invention are defined in more detail. Each aspect so defined may be combined with any other aspect or aspects unless clearly indicated to the contrary. In particular, any feature indicated as being preferred or advantageous may be combined with any other feature or features indicated as being preferred or advantageous.

Generally, nomenclatures used in connection with, and techniques of, cell and tissue culture, pathology, oncology, molecular biology, immunology, microbiology, genetics and protein and nucleic acid chemistry and hybridization described herein are those well-known and commonly used in the art. The methods and techniques of the present disclosure are generally performed according to conventional methods well-known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification unless otherwise indicated. See, e.g., Green and Sambrook et al., Molecular Cloning: A Laboratory Manual, 4th ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (2012); Therapeutic Monoclonal Antibodies: From Bench to Clinic, Zhiqiang An (Editor), Wiley, (2009); and Antibody Engineering, 2nd Ed., Vols. 1 and 2, Ontermann and Duebel, eds., Springer-Verlag, Heidelberg (2010).

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

The invention provides biological therapeutics for veterinary use, in particular fusion proteins for use in the treatment of companion animals such as dogs, cats, bovines or horses.

In a first aspect, the invention relates to an isolated companion animal p75NTR protein or a portion thereof.

As used herein, the term p75NTR protein refers to a p75NTR protein that binds NGF and/or other neurotrophins (BDNF, NT-3 and/or NT-4/5). As used herein, this means that the protein is capable of binding to NGF and inhibiting NGF biological activity and/or downstream pathway(s) mediated by NGF

signalling. An NGF binding protein reduces NGF biological activity, including downstream pathways mediated by NGF signalling and/or reduces the amount of NGF that is in circulation and which can bind to its receptors trkA and NGFR (p75NTR).

The term companion animal as used herein refers to a dog, cat or horse. In one embodiment, the companion animal is a dog. In another embodiment, the animal to be treated may be a cow or pig.

The term "isolated" protein or polypeptide refers to a protein or polypeptide that is substantially free of other proteins or polypeptides, having different antigenic specificities. Moreover, protein or polypeptide may be substantially free of other cellular material and/or chemicals. Thus, the protein, nucleic acids and polypeptides described herein are preferably isolated. Thus, as used herein, an "isolated" protein, or polypeptide means protein or polypeptide that has been identified and separated and/or recovered from a component of its natural cell culture environment. Contaminant components of its natural environment are materials that would interfere with diagnostic or therapeutic uses for the protein or polypeptide, and may include enzymes, hormones, and other proteinaceous or non-proteinaceous solutes.

The terms "polypeptide" and "protein" are used interchangeably to refer to a polymer of amino acid residues and are not limited to a minimum length. Peptides, oligopeptides, dimers, multimers, and the like, are also composed of linearly arranged amino acids linked by peptide bonds, and whether produced biologically, recombinantly, or synthetically and whether composed of naturally occurring or non-naturally occurring amino acids, are included within this definition. Both full-length proteins and fragments thereof are encompassed by the definition. The terms also include co-translational and post-translational modifications of the polypeptide, such as, for example, disulfide-bond formation, glycosylation, acetylation, phosphorylation, proteolytic cleavage (e.g., cleavage by furins or metalloproteases and prohormone convertases (PCs)), and the like. Furthermore, for purposes of the present invention, a "polypeptide" encompasses a protein that includes modifications, such as deletions, additions, and substitutions (generally conservative in nature as would be known to a person in the art), to the native sequence, as long as the protein maintains the desired activity. These modifications can be deliberate, as through site-directed mutagenesis, or can be accidental, such as through mutations of hosts that produce the proteins, or errors due to PCR amplification or other recombinant DNA methods. Polypeptides or proteins are composed of linearly arranged amino acids linked by peptide bonds, but in contrast to peptides, have a well-defined conformation.

Proteins, as opposed to peptides, generally consist of chains of 50 or more amino acids. For the purposes of the present invention, the term "peptide" as used herein typically refers to a sequence of amino acids of made up of a single chain of D- or L-amino acids or a mixture of D- and L-amino acids joined by peptide bonds. Generally, peptides contain at least two amino acid residues and are less than about 50 amino acids in length.

By "amino acid" herein is meant one of the 20 naturally occurring amino acids or any non- natural analogues that may be present at a specific, defined position. Amino acid encompasses both naturally occurring and synthetic amino acids. Although in most cases, when the protein is to be produced recombinantly, only naturally occurring amino acids are used.

In one embodiment, the isolated companion animal p75NTR protein comprises or consists of a canine, feline or bovine p75NTR protein or a portion or a variant thereof. Therefore, in one embodiment, the isolated companion animal p75NTR protein comprises or consists of SEQ ID No. 1, 3, 5 or 36, a portion or a variant thereof or a variant of a portion.

The p75 neurotrophin receptor p75NTR in its native form exists as a transmembrane glycoprotein. Family members are characterised by multiple cysteine-rich domains for ligand binding, a single transmembrane sequence extracellular domain (ECD), and a non-catalytic cytoplasmic domain.

Endogenous soluble ECD of p75NTR is produced by regulated proteolysis by α- secretase and γ-secretase that cleaves the protein near the membrane junction of the ECD. This is cleavage results in the release of the cytoplasmic domain which is free to bind NGF as a natural antagonist to NGF signalling.

In one embodiment, the isolated companion animal p75NTR protein or a portion thereof comprises or consists of the extracellular domain (ECD) or part thereof or a variant thereof. In one embodiment, the isolated companion animal p75NTR protein or a portion thereof comprises or consists of the extracellular domain (ECD). In one embodiment, the isolated companion animal p75NTR protein or a portion thereof comprises the extracellular domain (ECD) and additional C-terminal amino acids of the companion animal p75NTR protein adjacent to the ECD. For example, the portion of the companion animal p75NTR protein may comprise the ECD and at least 1- 5 or 5-10 amino acids, e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 C-terminal amino acids of the companion animal p75NTR protein adjacent to the ECD; or the ECD and at least 10-20 amino acids, or the ECD and at least 20-30 amino acids, or the ECD and at least 30-40 amino acids, or the ECD and at least 40-50 amino acids, or the ECD and at least 50-60 amino acids, or the ECD and at least 60-70 amino acids, or the ECD and at least 70-80 amino acids, or the ECD and at least 80-90 amino acids, or the ECD and at least 90-100 amino acids, or the ECD and at least 100-110 amino acids, or the ECD and at least 110-120 amino acids, or the ECD and at least 120-130 amino acids, or the ECD and at least 130-140 amino acids, or the ECD and at least 140-150 amino acids, or the ECD and at least 150-160 amino acids, or the ECD and at least 160-170 amino acids or the ECD and at least 170-180 amino acids C-terminal amino acids of the companion animal p75NTR protein adjacent to the ECD.

In one embodiment, α- secretase and γ-secretase cleavage sites within the ECD are removed. In one embodiment, the stalk region is removed. In one embodiment, the stalk region and α- secretase and γ-secretase cleavage sites within the ECD are removed.

In one embodiment, the isolated companion animal p75NTR extracellular domain is canine and comprises or consists of SEQ ID No. 7 or a variant thereof or a portion thereof, for example SEQ ID No. 34.
In another embodiment, the isolated companion animal p75NTR extracellular domain is feline and comprises or consists of SEQ ID No. 38 or a variant thereof or a portion thereof.

The ECD of p75NTR has a stalk region (e.g. SEQ ID No. 9, canine stalk region) that is prone to O-glycosylation. Glycosylation in proteins can cause manufacturing difficulties. Thus, in one embodiment, the isolated companion animal ECD may comprise deletions in the stalk region to reduce the number of O-glycosylation sites within the stalk region e.g. to form a truncated stalk region. A truncated stalk region may comprise any number of the amino acids of the stalk region. For example, the stalk region may comprise 1-10, 1-20, 1-30 amino acids. The stalk region may be removed in embodiments of the fusion protein described herein. Thus, a portion of the ECD as used herein may be the ECD without the stalk region and 3' sequences α- secretase and γ-secretase cleavage sites (e.g. canine sequence SEQ ID No. 34).

Thus, in one embodiment, the isolated companion animal p75NTR is a truncated protein which has the O-glycosylation stalk region removed.

In one embodiment, the isolated companion animal p75NTR protein may be a variant of the wild type protein which comprises one or more amino acid modification compared to the wild type protein. The modification may be a substitution, deletion or addition of an amino acid.

By "variant" or "mutant" herein is meant a polypeptide sequence that differs from that of a wild-type sequence by virtue of at least one amino acid modification. As used herein, a "substitution of an amino acid residue" with another amino acid residue in an amino acid sequence of a protein or polypeptide as described herein, is equivalent to "replacing an amino acid residue" with another amino acid residue and denotes that a particular amino acid residue at a specific position in the original (e.g. wild type / germline) amino acid sequence has been replaced by (or substituted for) by a different amino acid residue. This can be done using standard techniques available to the skilled person, e.g. using recombinant DNA technology. The amino acids are changed relative to the native (wild type / germline) sequence as found in nature in the wild type (wt), but may be made in IgG molecules that contain other changes relative to the native sequence.

Variants of the p75NTR protein or portions thereof as used herein retain the biological function of the wild type protein, that is binding to NGFs.

Amino acid modifications in general refer to and include substitutions, insertions and deletions, with the former being preferred in many cases. The variants of the invention include amino acid substitutions, and they can include any number of further modifications, as long as the function of the protein is still present, as described herein. In one embodiment, from 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 modifications are generally utilized as often the goal is to alter function with a minimal number of modifications. A variant polypeptide sequence will preferably possess at least about 80%, 85%, 90%, 95% or up to 98% or 99% identity to the wild-type sequences or the parent sequences. It should be noted that depending on the size of the sequence, the percent identity will depend on the number of amino acids. Variants do not include human sequences.

By "protein variant" or "variant protein" herein is meant a protein that differs from a wild-type protein by virtue of at least one amino acid modification. The parent polypeptide may be a naturally occurring or wild-type (WT) polypeptide, or may be a modified version of a WT polypeptide. Variant polypeptide may refer to the polypeptide itself, a composition comprising the polypeptide, or the amino sequence that encodes it. Preferably, the variant polypeptide has at least one amino acid modification compared to the parent polypeptide, e.g. from about one to about ten amino acid modifications, and preferably from about one to about five amino acid modifications compared to the parent. The variant polypeptide sequence herein will preferably possess at least about 80% identity with a parent polypeptide sequence, and most preferably at least about 90% identity, more preferably at least about 95% identity. Variants do not include human sequences.

By "parent polypeptide", "parent protein" as used herein is meant an unmodified polypeptide that is subsequently modified to generate a variant. Said parent polypeptide may be a naturally occurring polypeptide, or a variant or engineered version of a naturally occurring polypeptide. Parent polypeptide may refer to the polypeptide itself, compositions that comprise the parent polypeptide, or the amino acid sequence that encodes it.

By "wild type" or "WT", “wt” or "native" herein is meant an amino acid sequence or a nucleotide sequence that is found in nature, including allelic variations. A WT protein, polypeptide, Fc domain, immunoglobulin etc. has an amino acid sequence or a nucleotide sequence that has not been intentionally modified.

In another aspect, the invention relates to an isolated nucleic acid encoding a companion animal p75NTR protein or a portion thereof, e.g. the ECD or a portion thereof or a variant. As used herein, a portion of p75NTR or a portion of the ECD of p75NTR includes at least one neurotrophin binding domain. In one embodiment, the companion animal is a dog. For example, the isolated nucleic acid comprises or consists of SEQ ID No. 2, 4, 6, 8 or 37 or a variant thereof or a portion thereof.

"Isolated nucleic acid molecule" means a DNA or RNA of genomic, mRNA, cDNA, or synthetic origin or some combination thereof which is not associated with all or a portion of a polynucleotide in which the isolated polynucleotide is found in nature or is linked to a polynucleotide to which it is not linked in nature.

In another aspect, the invention relates to a vector, plasmid, transcription, expression cassette or nucleic acid construct comprising a nucleic acid encoding a companion animal p75NTR protein or a portion thereof, e.g. the ECD or portion thereof as described above.

The construct may include a suitable leader sequence. The term leader sequence is used interchangeably with signal sequence. Thus, in some embodiments, the nucleic acid sequence / nucleic acid construct encoding the fusion protein may also comprise a leader sequence. The leader sequence is made as part of the protein and then cleaved off when the protein is secreted. Any suitable leader sequence may be used, including a native immunoglobulin germline leader sequence, such as the endogenous p75 leader of the relevant species (e.g. canine, equine, feline, bovine), the endogenous p75 leader of a different species e.g. human, canine, equine, feline, bovine or a mouse IgG leader or another leader sequences known in the art, e.g. the Campath leader sequence (see US 8,362,208 B2) or an artificial sequence. Such leader sequences can aid in enhancing protein expression.

In another aspect, the invention relates to a host cell comprising a nucleic acid encoding a companion animal p75NTR protein or a portion thereof, e.g. the ECD, or a vector, plasmid, vector, transcription, expression cassette or construct as described above.

Expression vectors of use in the invention may be constructed from a starting vector such as a commercially available vector. After the vector has been constructed and the nucleic acid molecule has been inserted into the proper site of the vector, the completed vector may be inserted into a suitable host cell for amplification and/or polypeptide expression.

The term “vector” means a construct, which is capable of delivering, and in some aspects expressing one or more gene(s) or sequence(s) of interest in a host cell. Examples of vectors include, but are not limited to, viral vectors, naked DNA or RNA expression vectors, plasmid, cosmid or phage vectors, DNA or RNA expression vectors associated with cationic condensing agents, DNA or RNA expression vectors encapsulated in liposomes, and certain eukaryotic cells, such as producer cells.

The invention also relates to an isolated recombinant host cell comprising one or more nucleic acid molecule plasmid, vector, transcription or expression cassette as described above. The transformation of an expression vector 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.

The host cell may be eukaryotic or prokaryotic, for example a bacterial, viral, plant, fungal, mammalian or other suitable host cell. In one embodiment, the cell is an E. coli cell. In another embodiment, the cell is a yeast cell. In another embodiment, the cell is a Chinese Hamster Ovary (CHO) cell, HeLa cell or other cell that would be apparent to the skilled person. 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) and any cell lines used in an expression system known in the art can be used to make the recombinant polypeptides of the invention.

In general, host cells are transformed with a recombinant expression vector that comprises DNA encoding a protein. Among the host cells that may be employed are prokaryotes, yeast or higher eukaryotic cells. Prokaryotes include gram negative or gram-positive organisms, for example E. coli or bacilli. Higher eukaryotic cells include insect cells and established cell lines of mammalian origin. Examples of suitable mammalian host cell lines include the COS-7 cells, L cells, CI27 cells, 3T3 cells, Chinese hamster ovary (CHO) cells, or their derivatives and related cell lines which grow in serum free media, HeLa cells, BHK cell lines, the CVIIEBNA cell line, human embryonic kidney cells such as 293, 293 EBNA or MSR 293, human epidermal A431 cells, human Colo205 cells, other transformed primate cell lines, normal diploid cells, cell strains derived from in vitro culture of primary tissue, primary explants, HL-60, U937, HaK or Jurkat cells. Optionally, mammalian cell lines such as HepG2/3B, KB, NIH 3T3 or S49, for example, can be used for expression of the polypeptide when it is desirable to use the polypeptide in various signal transduction or reporter assays.

Other suitable host cells include insect cells, using expression systems such as baculovirus in insect cells, plant cells, transgenic plants and transgenic animals, and by viral and nucleic acid vectors.

Alternatively, it is possible to produce the polypeptide in lower eukaryotes such as fungal cell lines and yeast or in prokaryotes such as bacteria. Suitable yeasts include *S. cerevisiae*, *S. pombe*, *Kluyveromyces* strains, *Pichia pastoris*, *Candida*, or any yeast strain capable of expressing heterologous polypeptides. Suitable bacterial strains include *E. coli*, *B. subtilis*, *S. typhimurium*, or any bacterial strain capable of expressing heterologous polypeptides. If the protein is made in yeast or bacteria, it may be desirable to modify the product produced therein, for example by phosphorylation or glycosylation of the appropriate sites, in order to obtain a functional product. Such covalent attachments can be accomplished using known chemical or enzymatic methods.

A host cell, when cultured under appropriate conditions, can be used to express a 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.

In another aspect, the invention also relates to the use of an isolated companion animal p75NTR protein or a portion thereof as described above in a fusion protein with another moiety, e.g. with a half-life extending moiety as described in more detail below. Therefore, the p75NTR protein or a portion thereof can be provided covalently linked or couple to a half-life extending moiety. Alternatively, it may be provided incorporated in a liposome. The invention further relates to an isolated companion animal p75NTR protein or a portion thereof for use in therapy. Further, there is provided an isolated companion animal p75NTR protein or a portion thereof for use in the treatment of a pain related disease. Such diseases are described in more detail below.

In some embodiments, to improve its pharmacokinetic (PK) properties, the half-life of the p75NTR protein is extended.

Fusion proteins

Thus, in another aspect, the invention relates to a fusion protein comprising an isolated companion animal p75NTR protein or portion thereof, e.g. the extracellular domain, as described above and another moiety.

For example, the other moiety may be a half-life extending moiety. Thus, the p75NTR protein or portion thereof is coupled to a half-life extending moiety. As described above, the p75NTR protein or portion thereof may be canine, feline or equine. The p75NTR protein or portion thereof used in the fusion protein may thus comprise or consist of a sequence selected from SEQ ID NO. 1, 3, 5, 7, 34, 36 or 38 or a portion or a variant thereof. In one embodiment of the canine p75NTR protein or portion, the stalk region (e.g. SEQ ID NO. 9) is removed.

Half-life extending moieties have been described. For example, the half-life extending moiety may be selected from the following non-limiting list: a companion animal immunoglobulin Fc domain, polyethylene glycol (PEG), PEG derivatives, simple lipids, lipid dicarboxylic acids, lipids with additional moieties, companion animal serum albumin binders, e.g. small-molecule binders or antibodies/antibody fragments that bind companion animal serum albumin, companion animal serum albumin, or streptococcal protein G's albumin-binding domain (ABD). Examples of lipids include glucagon-like peptide 1 (GLP-1), the analogs GLP-1 liraglutide and semaglutide or cholesterol. Advantageously, using an immunoglobulin Fc domain facilitates purification of the protein. In particular, Fc binding to Protein A can be used in purification procedures. The presence of an immunoglobulin Fc domain can also stabilise the overall folding of the fusion protein as well as extending its half-life.

In one embodiment, where the half-life extending moiety is a companion animal Fc domain, companion animal serum albumin binder or companion animal serum albumin, the p75NTR protein or portion and half-life extending moiety are from / specific to the same companion animal. For example, in one embodiment, the half-life extending moiety is a companion animal Fc domain of the corresponding companion animal. For example, if the p75NTR protein or portion thereof, e.g. the extracellular domain is canine, the Fc domain is canine. If the p75NTR protein or portion thereof, e.g. the extracellular domain is feline, the Fc domain is feline. If the p75NTR protein or portion thereof, e.g. the extracellular domain is equine, the Fc domain is equine. If the p75NTR protein or portion thereof, e.g. the extracellular domain is bovine, the Fc domain is bovine.

However, given the high sequence similarity between companion animal p75 protein, in another embodiment, where the half-life extending moiety is a companion animal Fc domain, companion animal serum albumin binder or companion animal serum albumin, the p75NTR protein or portion and half-life extending moiety are not from / specific to the same companion animal. For example, in one embodiment, the half-life extending moiety is the companion animal Fc domain of the corresponding companion animal, but the p75 protein or portion thereof is that of a different companion animal. For example, for treatment of dogs, if the Fc domain is canine, the p75NTR protein or portion thereof, e.g. the extracellular domain is may be from a different animal, e.g. cat, horse or cow. For example, for treatment of cats, if the Fc domain is feline, the p75NTR protein or portion thereof, e.g. the extracellular domain is may be from a different animal, e.g. dog, cow or horse. For example, for treatment of cats, if the Fc domain is equine, the p75NTR protein or portion thereof, e.g. the extracellular domain is may be from a different animal, e.g. cat, cow dog. In yet another embodiment, human p75 or a portion thereof fused to companion animal Fc can be used.

The companion animal serum albumin binder, e.g. antibody or fragment thereof, may be canine or caninized, feline of felinized, equine or equinized. The companion animal serum albumin binder may bind to canine, feline or equine serum albumin.

In one embodiment, the half-life extending moiety is a wild type or variant Fc domain. The term variant is as defined above. For example, an Fc domain variant may have modified half-life compared to the wild type Fc domain. In one embodiment, the Fc domain is a canine Fc domain, that is a wild type domain or a variant thereof. Variant Fc domains are described, for example in WO2020/142625.

By "Fc" or "Fc region" or "Fc domain" as used herein is meant the polypeptide comprising the constant region of an antibody excluding the first constant region immunoglobulin domain (CH1) and, in some cases, part of the hinge. In one embodiment, the Fc domain includes constant region immunoglobulin domains CH2, CH3 and the hinge region between CH1 and CH2 or part of the hinge region.

Proteolytic digestion of antibodies releases different fragments termed Fv (Fragment variable), Fab (Fragment antigen binding) and Fc (Fragment crystallisation). The Fc fragment comprises the carboxy-terminal portions of both H chains held together by disulfides. The constant domains of the Fc fragment are responsible for mediating the effector functions of an antibody.

In canine, there are four IgG heavy chains referred to as A, B, C, and D. These heavy chains represent four different subclasses of dog IgG, which are referred to as IgG-A, IgG-B, IgG-C and IgG-D. The DNA and amino acid sequences of these four heavy chains were first identified by Tang et al. (Vet. Immunol. Immunopathol. 80: 259-270 (2001)). Exemplary amino acid and DNA sequences for these heavy chains are also available from the GenBank data bases (IgGA: accession number AAL35301.1, IgGB: accession number AAL35302.1, IgGC: accession number AAL35303.1, IgGD: accession number AAL35304.1). Amino acid sequences for IgG-A, IgG-B, IgG-C and IgG-D as used by the inventors and according to the aspects and embodiments of the invention are provided as SEQ ID Nos. 15, 16, 17, 18).

In human, Fc refers to the last two constant region immunoglobulin domains of IgA, IgD, and IgG, the last three constant region immunoglobulin domains of IgE and IgM, and the flexible hinge N-terminal to these domains. For IgA and IgM, Fc may include the J chain. For IgG, the Fc domain comprises immunoglobulin domains CH2 and CH3 and the lower hinge region between CH1 and CH2. Although the boundaries of the Fc region may vary, the human IgG heavy chain Fc region is usually defined to include residues C226 or P230 to its carboxyl- terminus, wherein the numbering is according to the EU index as in Kabat.

Fc as used herein may refer to the Fc region in isolation, or this region in the context of an Fc fusion ("fusion composition" or "fusion construct"), as described herein. Fc domains include all or part of an Fc region; that is, N- or C- terminal sequences may be removed from wild-type or variant Fc domains, as long as this does not affect function.

Briefly, IgG functions are generally achieved via interaction between the Fc region of the Ig and an Fcγ receptor (FcγR) or another binding molecule, sometimes on an effector cell. This can trigger the effector cells to kill target cells to which the antibodies are bound through their variable (V) regions. Also, antibodies directed against soluble antigens might form immune complexes which are targeted to FcγRs which result in the uptake (opsonisation) of the immune complexes or in the triggering of the effector cells and the release of cytokines.

By "Fc gamma receptor", " FcγR " or "FcgammaR" as used herein is meant any member of the family of proteins that bind the IgG antibody Fc region and is encoded by an FcγR gene.

In humans, three classes of FcγR have been characterised, although the situation is further complicated by the occurrence of multiple receptor forms. The three classes are:
(i) FcγRI (CD64) including isoforms FcγRIa, FcγRIb, and FcγRIc binds monomeric IgG with high affinity and is expressed on macrophages, monocytes, and sometimes neutrophils and eosinophils;
(ii) FcγRII (CD32) binds complexed IgG with medium to low affinity and is widely expressed. These receptors can be divided into two important types, FcγRIIa and FcγRIIb. The 'a' form of the receptor is found on many cells involved in killing (e. g. macrophages, monocytes, neutrophils) and seems able to activate the killing process and occurs as two alternative alleles. The 'b' form seems to play a role in inhibitory processes and is found on B-cells, macrophages and on mast cells and eosinophils. On B-cells it seems to function to suppress further immunoglobulin production and isotype switching to for example, the IgE class. On macrophages, the b form acts to inhibit phagocytosis as mediated through FcγRIIa. On eosinophils and mast cells the b form may help to suppress activation of these cells through IgE binding to its separate receptor and
(iii) FcγRIII (CD16) binds IgG with medium to low affinity and exists as two types. FcγRIIIa is found on NK cells, macrophages, eosinophils and some monocytes and T cells and mediates ADCC. FcγRIIIb is highly expressed on neutrophils. Both types have different allotypic forms.

Canine Fc receptors are described in Bergeron et al L.M. Bergeron et al.; Veterinary Immunology and Immunopathology 157 (2014) 31– 41. Canine has RI, RIIb, RIII, but not Riia.

As well as binding to FcγRs, IgG antibodies can activate complement and this can also result in cell lysis, opsonisation or cytokine release and inflammation. The Fc region also mediates such properties as the transportation of IgGs to the neonate (via the so-called "FcRn"), increased half-life (also believed to be effected via an FcRn-type receptor) and self-aggregation. The Fc-region is also responsible for the interaction with protein A and protein G (which interaction appears to be analogous to the binding of FcRn).

By "effector function" as used herein is meant a biochemical event that results from the interaction of an antibody Fc region with an Fc receptor or ligand. Effector functions include but are not limited to antibody-dependent cell-mediated cytotoxicity (ADCC), antibody-dependent cell-mediated phagocytosis (ADCP) and complement-dependent cytotoxicity (CDC).

In one embodiment, the companion animal p75NTR protein or portion thereof, e.g. the extracellular domain, and the other moiety are linked with a linker moiety or otherwise conjugated, attached or covalently or non-covalently linked. Suitable linkers are known to the skilled person. For example, the linker is a peptide linker, such as a glycine and/or alanine and/or threonine and/or serine-rich linker e.g. a glycine-serine linker, such as $(G_4S)_n$ wherein n is 1 to 4.

In another embodiment, the linker can be cleavable.

In one embodiment, the companion animal p75NTR protein or portion thereof comprises or consists of a canine p75NTR ECD or portion thereof. In one embodiment, the ECD comprises of consists of SEQ No. 7 or a variant thereof.

Thus, in one embodiment, the invention relates to a fusion protein comprising a canine p75NTR ECD linked to a canine Fc domain. In one embodiment, the ECD comprises of consists of SEQ No. 7 or a variant thereof.

In one embodiment, the fusion protein of the present invention preferably binds to any one or more of NGF, BDNF, NT3 or NT4/5 with a binding affinity (Kd) of between about lpM to about 100 nM. In some preferred embodiments, the binding affinity (Kd) is between about 5pM and any of about 10 pM, 20pM, 40pM, 50pM l00pM, 0.2nM, 0.5nM, lnM 1.5nM 2 nM, 2.5 nM, 3 nM, 3.5 nM, 4 nM, 4.5 nM, 5 nM, 5.5 nM, 6 nM, 6.5 nM, 7 nM, 7.5 nM, 8 nM, 8.5 nM, 9 nM, 9.5 nM, 10 nM, 15 nM, 20 nM, 25 nM, 30 nM, 35 nM, 40 nM, 45 nM, 50 nM or 100 nM as measured in an in vitro binding assay for NGF, BDNF, NT3 or NT4/5 such as described herein. Subnanomolar range is preferred.

In one embodiment, the fusion protein comprises or consists of SEQ ID NO. 11 or 13 or a variant thereof. These fusion proteins include the p75 ECD operably linked to a canine Fc domain as shown in Figure 1. The Fc domain in the construct of SEQ ID NO. 11 is a wild type canine Fc domain. The Fc domain in the construct of SEQ ID NO. 13 is a variant canine Fc domain which has been modified to increase half-life. In this domain, the mutation YTE has been introduced at residues Y252 – T254 –of the wt Fc domain using EU numbering.

In another embodiment, the fusion protein comprises or consists of SEQ ID NO. 39. Such a fusion proteins includes the p75 ECD operably linked to a feline Fc domain. The Fc domain in the construct of SEQ ID NO. 39 is a wild type feline Fc domain.

Therefore, modified companion animal Fc domains that include this mutation, e.g. canine, feline or equine Fc domains, can be used in the fusion proteins of the invention. A skilled person would know that any other known mutations that increase half-life could also be introduced in the Fc domain.

According to the present invention, the fusion proteins demonstrate advantageous biological properties including improved solubility, stability and/or improved serum half-life. Improved solubility and stability is, for example, demonstrated in Examples 4 and 9. These examples show that the described molecules are very stable in both temperature and chemical stress, showing unfolding only when incubated at temperature higher than 70°C with no aggregation up to 95°C with Tm1 around 67°C. Improved half-life is, for example, demonstrated in Examples 11 and 12. Improved half-life allows for less frequent dosing (a single administration in comparison to existing treatments where daily administration is required).

This effect is demonstrated whilst showing strong analgesic effects. In one embodiment, the fusion protein of the invention has a half-life in-vivo of about or more than any one of 2, 4, 6, 8,10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 1 10, 1 12, 1 14, 1 16, 1 18, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152,154, 156, 158, 160, 62, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202, 204, 206, 208 or 210 hours +/- 1 hour, further preferably the p75NTR(NBP)-Fc fusion protein of the invention has a half-life in-vivo of about or more than 24 hours.

In another embodiment, the fusion protein of the invention has a half-life in-vitro of about or more than any one of 2, 4, 6, 8,10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152,154, 156, 158, 160, 62, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202, 204, 206, 208 or 210 days +/- 1 day, further the fusion protein has a half-life in-vitro of about or more than 6 days or more than 1 month. In one embodiment, the half life is 14 days.

According to the foregoing preferred embodiments, the in-vivo half-life can be the half-life in rat or in the corresponding companion animal, e.g. in a dog.

Fusion proteins of the invention can operate at a very low dose, but are highly efficacious. This is, for example, demonstrated in Examples 11 and 12 where administration of PetML119 and PetML122 in rats and dogs outcompete existing treatments.

According to the present invention, the fusion proteins display a good safety profile. This is, for example, demonstrated in Example 12, where animals maintain normal body weight and haematological parameters and do not generate anti-Drug Antibodies after administration of fusion proteins. The maintenance of normal body weight after administration is, for example, also demonstrated in Example 11. This is in contrast to the administration of dexamethasone which decreased body weight over time.

In another aspect, the invention relates to an isolated nucleic acid encoding a fusion protein as described above, for example a fusion protein encoding SEQ ID NO. 11,13 or 39. In one embodiment, the nucleic acid is selected from SEQ ID NO. 12, 14 or 40.

In another aspect, the invention relates to a vector, plasmid, vector, transcription, expression cassette or construct comprising a nucleic acid described above.

In another aspect, the invention relates to a host cell comprising a nucleic acid vector, plasmid, vector, transcription, expression cassette or construct as described above. Suitable host cells are described elsewhere herein.

In another embodiment, the P75NTR protein, portion thereof or fusion protein is labelled with a detectable or functional label. A label can be any molecule that produces or can be induced to produce a signal, including but not limited to fluorophores, fluorescers, radiolabels, enzymes, chemiluminescers, a nuclear magnetic resonance active label or photosensitizers. Thus, the binding may be detected and/or measured by detecting fluorescence or luminescence, radioactivity, enzyme activity or light absorbance.

In another aspect, there is provided a pharmaceutical composition comprising a p75NTR protein or portion thereof or a fusion protein of the invention. The fusion protein or pharmaceutical composition described herein can be administered by any convenient route, including but not limited to oral, topical, parenteral, sublingual, rectal, vaginal, ocular, intranasal, pulmonary, intradermal, intravitrial, intratumoural, intramuscular, intraperitoneal, intravenous, subcutaneous, intracerebral, transdermal, transmucosal, by inhalation, or topical, particularly to the ears, nose, eyes, or skin or by inhalation. In another embodiment, delivery is of the nucleic acid encoding the drug, e.g. a nucleic acid encoding the molecule of the invention is delivered.

Parenteral administration includes, for example, intravenous, intramuscular, intraarterial, intraperitoneal, intranasal, rectal, intravesical, intradermal, topical, intra-articular or subcutaneous administration. Preferably, the compositions are administered parenterally.

The pharmaceutically acceptable carrier or vehicle can be particulate, so that the compositions are, for example, in tablet or powder form. The term "carrier" refers to a diluent, adjuvant or excipient, with which a drug antibody conjugate of the present invention is administered. Such pharmaceutical carriers can be liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. The carriers can be saline, gum acacia, gelatin, starch paste, talc, keratin, colloidal silica, urea, and the like. In addition, auxiliary, stabilizing, thickening, lubricating and coloring agents can be used. In one embodiment, when administered to an animal, the polypeptide of the present invention or compositions and pharmaceutically acceptable carriers are sterile. Water is a preferred carrier when the drug antibody conjugates of the present invention are administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical carriers also include excipients such as starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The present

compositions, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents.

The pharmaceutical composition can be in the form of a liquid, *e.g.*, a solution, syrup, solution, emulsion or suspension. The liquid can be useful for oral administration or for delivery by injection, infusion (*e.g.*, IV infusion) or sub-cutaneous.

When intended for oral administration, the composition can be in solid or liquid form, where semi-solid, semi-liquid, suspension and gel forms are included within the forms considered herein as either solid or liquid.

As a solid composition for oral administration, the composition can be formulated into a powder, granule, compressed tablet, pill, capsule, chewing gum, wafer or the like form. Such a solid composition typically contains one or more inert diluents. In addition, one or more of the following can be present: binders such as carboxymethylcellulose, ethyl cellulose, microcrystalline cellulose, or gelatin; excipients such as starch, lactose or dextrins, disintegrating agents such as alginic acid, sodium alginate, corn starch and the like; lubricants such as magnesium stearate; glidants such as colloidal silicon dioxide; sweetening agents such as sucrose or saccharin; a flavoring agent such as peppermint, methyl salicylate or orange flavoring; and a coloring agent. When the composition is in the form of a capsule (e. g. a gelatin capsule), it can contain, in addition to materials of the above type, a liquid carrier such as polyethylene glycol, cyclodextrin or a fatty oil.

When intended for oral administration, a composition can comprise one or more of a sweetening agent, preservatives, dye/colorant and flavor enhancer. In a composition for administration by injection, one or more of a surfactant, preservative, wetting agent, dispersing agent, suspending agent, buffer, stabilizer and isotonic agent can also be included.

Compositions can take the form of one or more dosage units.

In specific embodiments, it can be desirable to administer the composition locally to the area in need of treatment, or by intravenous injection or infusion.

The amount of the polypeptide, Fc domain or pharmaceutical composition described herein that is effective/active in the treatment of a particular disease or condition will depend on the nature of the disease or condition and can be determined by standard clinical techniques. In addition, *in vitro* or *in vivo* assays can optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the compositions will also depend on the route of administration, and the seriousness of the disease or disease, and should be decided according to the judgment of the practitioner and each patient's circumstances. Factors like age, body weight, sex, diet, time of administration, rate of excretion,

condition of the host, drug combinations, reaction sensitivities and severity of the disease shall be taken into account.

Typically, the amount is at least about 0.01% of a polypeptide of the present invention by weight of the composition. When intended for oral administration, this amount can be varied to range from about 0.1 % to about 80% by weight of the composition. Preferred oral compositions can comprise from about 4% to about 50% of the polypeptide of the present invention by weight of the composition.

Compositions can be prepared so that a parenteral dosage unit contains from about 0.01 % to about 2% by weight of the polypeptide of the present invention.

For administration by injection, the composition can comprise from about typically about 0.1 mg/kg to about 250 mg/kg of the animal's body weight, preferably, between about 0.1 mg/kg and about 20 mg/kg of the animal's body weight, and more preferably about 1 mg/kg to about 10 mg/kg of the animal's body weight. In one embodiment, the composition is administered at a dose of about 1 to 30 mg/kg, e.g., about 5 to 25 mg/kg, about 10 to 20 mg/kg, about 1 to 5 mg/kg, or about 3 mg/kg. The dosing schedule can vary from e.g., once a week to once every 2, 3, or 4 weeks or more.

Treatment can for example be once a month or bi-monthly. This is advantageous over daily administration as this improves compliance and minimises stress to the animal.

As used herein, "treat", "treating" or "treatment" means inhibiting or relieving a disease or disease. For example, treatment can include a postponement of development of the symptoms associated with a disease or disease, and/or a reduction in the severity of such symptoms that will, or are expected, to develop with said disease. The terms include ameliorating existing symptoms, preventing additional symptoms, and ameliorating or preventing the underlying causes of such symptoms. Thus, the terms denote that a beneficial result is being conferred on at least some of the mammals, e.g., canine patients, being treated. Many medical treatments are effective for some, but not all, patients that undergo the treatment.

The term "subject" or "patient" refers to an animal which is the object of treatment, observation, or experiment, suitably a companion animal, specifically a canine or a feline.

In another aspect, the invention relates to the use of a p75NTR protein or portion thereof, fusion protein or pharmaceutical composition described herein in the treatment or prevention of a disease. In another aspect, the disclosure relates to the use of a polypeptide, Fc domain or pharmaceutical composition described herein in the manufacture of a medicament for the treatment or prevention of a disease as listed herein. The invention further relates to a method of treating a disease in a subject comprising an

effective amount of the polypeptide, Fc domain or pharmaceutical composition as described herein to said subject.

For example, the disease is a NGF related disorder.

In one embodiment, the NGF related disorder is selected from the group consisting of: cardiovascular diseases, atherosclerosis, obesity, type 2 diabetes, metabolic syndrome, pain and inflammation. In one embodiment, the NGF related disorder comprises pain. In one embodiment, the pharmaceutical composition is used in the treatment of pain. In one embodiment, the pharmaceutical composition is used for the treatment of a pain and the type of pain is selected from osteoarthritis pain, rheumatoid arthritis pain, surgical and postsurgical pain, incisional pain, general inflammatory pain, cancer pain, pain from trauma, neuropathic pain, neuralgia, diabetic neuropathy pain, pain associated with rheumatic diseases, pain associated with musculoskeletal diseases, visceral pain, and gastrointestinal pain. In one embodiment, the pain comprises osteoarthritis pain. In one embodiment, the pain comprises surgical and post-surgical pain. In one embodiment, the pain comprises cancer pain.

In one or more embodiments, the p75NTR protein or portion thereof, fusion protein or pharmaceutical composition of the invention is for use in a canine. In one or more embodiments, the p75NTR protein or portion thereof, fusion protein or pharmaceutical composition of the invention is for use in felines. In one or more embodiments, the p75NTR protein or portion thereof, fusion protein or pharmaceutical composition of the invention is for use in equine.

In one embodiment, the p75NTR protein or portion thereof, fusion protein or pharmaceutical composition of the invention is administered together with one or more therapeutic agent, for example a therapeutic agent to treat pain.

The polypeptide, the p75NTR protein or portion thereof, fusion protein or pharmaceutical composition may be administered at the same time or at a different time as the other therapy or therapeutic compound or therapy, e.g., simultaneously, separately or sequentially.

The invention also provides an in vitro or in vivo method for inhibiting NGF activity in a companion animal comprising administering the p75NTR protein or portion thereof, fusion protein or pharmaceutical composition of the invention.

In one or more aspects, the present invention provides a method of producing the fusion protein of the invention by culturing the host cell of the invention under conditions that result in production of the fusion protein and subsequently isolating the fusion protein from the host cell or culture medium of the host cell.

In another aspect, the invention provides a kit for the treatment or prevention of a disease, diagnosis, prognosis or monitoring disease comprising a the p75NTR protein or portion thereof, fusion protein or pharmaceutical composition of the invention of the invention. Such a kit may contain other components, packaging and/or instructions.

The invention in another aspect provides a the p75NTR protein or portion thereof, fusion protein or pharmaceutical composition of the invention packaged in lyophilized form or packaged in an aqueous medium.

In another aspect, a p75NTR protein or portion thereof, fusion protein or pharmaceutical composition of the invention as described herein is used for non-therapeutic purposes, such as diagnostic tests and assays. Thus, the present invention also provides the above p75NTR proteins and fusion proteins for use in diagnostic methods for detecting NGF in species, particularly canines and felines, known to be or suspected of having an NGF related disorder. Methods for detecting NGF in species, particularly canines and felines, known to be or suspected of having an NGF related disorder may include exposing a sample from the animal to a labelled protein of the invention and detecting said labelled protein. may be used to quantitatively or qualitatively detect the NGF in a sample or to detect presence of cells that express the NGF.

Further aspects and embodiments of the invention will be apparent to those skilled in the art given the present disclosure including the following experimental exemplification.

Unless otherwise defined herein, scientific and technical terms used in connection with the present disclosure shall have the meanings that are commonly understood by those of ordinary skill in the art. While the foregoing disclosure provides a general description of the subject matter encompassed within the scope of the present invention, including methods, as well as the best mode thereof, of making and using this invention, the following examples are provided to further enable those skilled in the art to practice this invention and to provide a complete written description thereof. However, those skilled in the art will appreciate that the specifics of these examples should not be read as limiting on the invention, the scope of which should be apprehended from the claims and equivalents thereof appended to this disclosure. Various further aspects and embodiments of the present invention will be apparent to those skilled in the art in view of the present disclosure.

All documents mentioned in this specification are incorporated herein by reference in their entirety, including any references to gene accession numbers and references to patent publications.

"and/or" where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. For example, "A and/or B" is to be taken as specific disclosure of each of (i) A, (ii) B and (iii) A and B, just as if each is set out individually herein. Unless context dictates

otherwise, the descriptions and definitions of the features set out above are not limited to any particular aspect or embodiment of the invention and apply equally to all aspects and embodiments which are described.

The invention is further described in the non-limiting examples.

Examples

Methods:

1. Protein constructs and CHO-s transfection/expression (Figure 1)

For protein production, DNA constructs were generated to encode chimeric Fc fusion protein comprising selected canine IgG constant regions (between hinge and C-terminus) fused to the extracellular domain of canine p75 lacking the predicted O-glycosylation rich stalk region and the α- and γ-secretase sites. The amino acid sequences for PetML119 (SEQ ID NO: 11) and PetML122 (SEQ ID NO: 13) are given in Figure 1. An alignment of canine, feline, bovine and equine p75 sequence portions is provided in Figure 2.

Both the canine IgG-B Fc domain and the p75 extracellular domain (res 31-194 from UniProtKB - J9PAM0) were codon optimised and synthesised by GeneArt (Thermo Fisher). Both genes were PCR amplified using Q5 high fidelity DNA polymerase (using specific primers including overlapping regions to allow assembly) and assembled into mammalian expression vector PetML119 (Fc-Bwt) and PetML122 (Fc-B-YTE with the mutated residues Y252 – T254 – E256 using EU numbering) using NEBuilder HIFI DNA Assembly (New England Biolabs). In the expression vector, the fusion protein chain and the antibiotic resistant gene expression units are flanked by DNA transposon *piggyBac* terminal inverted repeats to mediate stable integration into host cells in the presence of piggyBac transposase. Both expression vectors also contain puromycin resistant cassette which is located within the piggyBac terminal repeats to facilitate selection for stable integration. To generate stable expression cell lines, PetML119 or PetMl122 was transfected into a suitable mammalian cell line such as CHO cells together with PiggyBac transposase followed by puromycin selection at 10-30 µg/ml for at least 8-10 days. For fusion protein production, 1 × 10^6/mL selected CHO cells are seeded in 800mL culture media (F17 + 4mM l-Gln + 0.3%P188 + 1:500 ACA) and incubated at 32°C, 8% CO_2 with shaking at 130 rpm. 2 % HyClone Cell Boost 7a supplement + 0.2 % HyClone Cell Boost 7b supplement 2mM glucose is added to the media daily from the 4th day of overproduction. Culture supernatants are collected on day 10 and the protein concentration is determined using surface plasmon resonance using protein A chip (Biacore 8K, Cytiva Life Sciences). Typically, PetML119/122 showed peak of expression at 10day in production reaching around 70mg/L.

2. PetML119-122 Purification

Cell suspensions from PetML119 and PetML122 stable transfected clones, cultured as described for at least 7days, were filtered using 0.22um filters after being incubated for 10 minutes with Sartoclear Dynamics® Lab V (SDLV-0500-20C—E). Cleared supernatants have been loaded into Mabselect sure LX prepacked 20mL column (17547402), pre-equilibrated with PBS. Column was washed with 40mLs of PBS (2CV) and then fusion proteins were eluted using gradient (0-100% in 2CV) of 0.1M Glycine pH2.7. Fractionations containing fusion proteins have been pooled together and neutralised with 100mM TRIS pH8 (final concentration).
Neutralised fusion protein pooled fractions were concentrated till 5mL and loaded into PBS pre-equilibrated HiLoad 16/600 Superdex 200 pg (28989335) as second step purification. Monomeric fractions (based on previously analysed protein standards' retention times) were pooled and protein concentration was assessed using NanoDrop™ One (Thermo Scientific™).
Around 30mg/L of purified product was obtained following the above mentioned protocol.

PetML119 showed 34mg/L final recovery after two step purification (with acceptable Endotoxin and HCP using standard protocols and buffers).

PetML122 showed 28mg/L final recovery after two step purification (with acceptable Endotoxin and HCP using standard protocols and buffers)

3. HPLC Analytical chromatography (Figure 3)

Purified material purity was assessed using both Size Exclusion Chromatography (SEC), for oligomerisation analyses, and cation exchange chromatography (SCX) for charge variants analyses.
HPLC-SEC chromatography (column: BioResolve SEC mAb 200A, 2.5um column WATERS) was performed using ACQUITY H-class Bio from WATERS using PBS as mobile phase with isocratic 0.575mL/min flow rate.
HPLC-SCX chromatography (column: BioResolve SCX mAb Column, 3 µm, 4.6 mm x 100 mm) was performed using ACQUITY H-class Bio from WATERS using MES pH5 as mobile phase with salt gradient used to separate charge variants at 0.9mL/min flow rate.
10uL of each sample were injected into both H-SEC / H-SCX using the above-mentioned protocol. Percentage of monomeric species and Area (indicative of protein concentration) were determined for each molecule.
Both PetML119/122 showed very high purity (more than 99%) by HSEC and few charge variants (potentially corresponding to different glycoforms) were observed by HSCX.

4. Stress tests (Figure 4)

Freeze and thaw test:
50uL aliquots of PetML119 at 5mg/mL in PBS were prepared and stored at either 4°C (1 aliquot per molecule) or -80°C (3 aliquots per molecules) in sealed PCR tubes. -80°C aliquots were thawed at room temperature in 5 minutes and stored back at -80°C leaving one aliquot out (1 freeze and thaw). This have been repeated two more times (2 and 3 freeze and thaw cycles) and then 10uL of each sample (including 4°C controls) were injected into H-SEC using same method as above. Percentage of monomeric species and Area (indicative of protein concentration) were determined for each molecule/freeze&thaw cycle and compared to a reference run (samples stored at 4°C).
PetML119 showed good resistance to Freeze and Thaw, with only minor protein loss upon 3 cycles.

Thermal & Chemical stress:
50uL aliquots of PetML119 at 5mg/mL in PBS or PBS+100mM DTT were prepared and incubated for 30' at different temperatures (25°C, 37°C, 60°C, 70°C and 95°C).
40uL of each condition were diluted with 10uL of NuPAGE™ LDS Sample Buffer (NP0007). 10uL were loaded into NuPAGE™ 10%, Bis-Tris, 1.0 mm, Mini Protein Gel, 12-well (NP0302BOX) and run at 120V for 45'. Gel was stained with InstantBlue® Coomassie Protein Stain (ISB1L) (ab119211) for 10' and destained in ddH2O for an 1hr. SDS-PAGE was then acquired using standard transilluminator equipment.
Results showed that PetML119 is very stable in both temperature and chemical stress, showing unfolding only when incubated at temperature higher than 70°C.

5. Protein A binding affinity validation (Figure 5)

Purified fusion proteins (PetML119 and PetML122) in PBS were concentrated using centrifugal concentrators (Sartorious - VS02H22) to 5mg/mL Protein concentration was assessed using UV absorbance at 280nm with NanoDrop™ One (Thermo Scientific™).

Binding affinity of fusion proteins to Protein A was assessed using Biacore 8K (Cytiva).

Briefly, Sensor Chip Protein A (Cytiva) was docked into Biacore 8K, equilibrated for 30' at RT and then Running Buffer (10mM HEPES pH7.4 150mM NaCl 3mM EDTA and 0.005% Tween20) was applied to the SPR chip surface.

Fusion protein dilutions were prepared diluting PetML119/122 from 1uM to 4nM (6 concentrations with 1:3 dilutions) in Running Buffer and kinetics was assessed using single cycle kinetics method (Biacore Assay Handbook, Cytiva). Kinetics and/or Affinity quantification have been performed using Biacore Insight following standard analyses methods.

The results show that protein A binding for PetML122 is slightly affected by YTE mutation introduced, a faster dissociation is observed compared to PetML119. This results in lower yield post protein A purification although the quality of protein is comparable.

6. FcRn binding affinity validation (Figure 6)

Purified fusion proteins (PetML119 and PetML122) in PBS were concentrated using centrifugal concentrators (Sartorious - VS02H22) to 5mg/mL Protein concentration was assessed using UV absorbance at 280nm with NanoDrop™ One (Thermo Scientific™).
Binding affinity of fusion proteins to Fc Neonatal Receptor was assessed using Biacore 8K (Cytiva).

Briefly, CM5 Sensor Chip (Cytiva) was docked into Biacore 8K, equilibrated for 30' at RT and then Running Buffer (10mM HEPES pH6 150mM NaCl 3mM EDTA and 0.005% Tween20) was applied to the SPR chip surface.
Canine and murine FcRn-B2M recombinant protein (Immunitrack, ITF12 - ITF08) was diluted into 10mM acetate buffer pH4.5 at 4nM (1:2000 dilution from stock) and immobilised using standard amine coupling reaction

Fusion protein dilutions were prepared from 3uM to 37nM (5 concentrations with 1:3 dilutions) in Running Buffer and kinetics was assessed using multi-cycle kinetics method (120sec association – 300sec dissociation). Kinetics and/or Affinity quantification have been performed using Biacore Insight following standard analyses methods.

A 10-fold increase in binding affinity to FcRn has been seen for PetML122, confirming the triple mutant YTE had a positive effect on binding to both canine and murine neonatal receptor. In particular, an extended dissociation rate was observed.

7. Human and rat Nerve Growth Factor (h-rNGF) binding affinity determination (Figure 7)

Purified fusion protein (PetML119) in PBS was concentrated using centrifugal concentrators (Sartorious - VS02H22) to 5mg/mL Protein concentration was assessed using UV absorbance at 280nm with NanoDrop™ One (Thermo Scientific™).

Binding affinity of fusion proteins to human and rat NGF was assessed using Biacore 8K (Cytiva).

Briefly, Protein A Sensor Chip (Cytiva) was docked into Biacore 8K, equilibrated for 30' at RT and then Running Buffer (10mM HEPES pH7.4 150mM NaCl 3mM EDTA and 0.005% Tween20) was applied to the SPR chip surface.

PetML119 was diluted into running buffer at 6nM concentration. These have been immobilised using 90sec association at 10uL/min as capturing step, followed by injection of running buffer to remove any unbound product.

Human and rat NGF (from Bio-Techne Ltd - 556-NG/CF / 256-GF-100/CF) was diluted in Running Buffer at 100nM with 1:2 further dilutions down to 4.68nM. Kinetics were assessed using multi-cycle kinetics with capture step method (30sec association – 120sec dissociation) followed by regeneration step (0.1M Glycine pH2.2 contact time 60sec FR 30uL/min). Kinetics quantification have been performed using Biacore Insight following standard analyses methods.

Results showed subnanomolar KD for both human and rat NGF with PetML119.

8. Intact Mass analyses

Purified fusion proteins (PetML119 and PetML122) in PBS were concentrated using centrifugal concentrators (Sartorious - VS02H22) to 1mg/mL Protein concentration was assessed using UV absorbance at 280nm with NanoDrop™ One (Thermo Scientific™).

LC-MS analyses have been performed on BioAccord using BioResolve RP mAb Polyphenyl Column, 450 Å, 2.7 µm, 2.1 x 50 mm and ACQUITY UPLC® I-Class Plus from WATERS.
Briefly, 3pmol (diluted in LC-MS grade H2O) of each fusion protein were injected in RP column and a eluate peaks were analysed by MS. Intact mass analyses showed presence of multiple species corresponding to different degree of glycosylation, consistently on what observed by H-SCX.

The intact molecule appears to be a mixture of species. The observed mass is 96158-97836 Da. The mass of the base species (96158Da) is consistent with a dimer of Fc fusion aa 20-241 + 4 glycans which is consistent with the total number of glycosylation sites expected in the molecule. Additional species are consistent with additional decoration of the glycans.

9. Unfolding and oligomerisation determination (Figure 4)

Purified fusion proteins (PetML119) in PBS were concentrated using centrifugal concentrators (Sartorious - VS02H22) to 3mg/mL Protein concentration was assessed using UV absorbance at 280nm with NanoDrop™ One (Thermo Scientific™).
Tm and Tagg analyses have been performed on UnCle from Unchained labs using standard protocol.
Briefly, 10uL of fusion protein have been used to determine unfolding and aggregation events during a temperature ramp (from 25°C till 95°C). As anticipated from previous results, PetML119 showed no aggregation up to 95°C with Tm1 around 67°C.

10. In vitro NGF inhibition assay (Figure 8)

To assess biological activity of our p75 fusion protein, we used an NGF-dependent (cell line TF-1 cell line). The cells are completely dependent on interleukin 3 (IL-3) or granulocyte-macrophage colony-stimulating factor (GM-CSF) for long term growth. The cells do not respond to interleukin 5 (IL-5). TF-1 cells respond to a variety of other lymphokines and cytokines such as interleukin 1 (IL-1), interleukin 4 (IL-4), interleukin 6 (IL-6), interleukin 9 (IL-9), Interleukin 11 (IL-11), interleukin 13 (IL-13), stem cell factor (SCF), leukemia inhibitory factor (LIF) and nerve growth factor (NGF).

Proficient sequestration of NGF by our fusion protein will result in slower proliferation in comparison to control.

TF-1 cell line was bought from ATCC (CRL-2003) and kept in culture using standard aseptic methods using complete RPMI (10% FBS + 2mM l-Gln + 10ng/mL hNGF).
2 million TF-1 cells were labelled with 2.5uM CFSE cell trace (Invitrogen - C34554) in 1mL of RPMI only for 30' at RT in the dark. Cells have been then washed 2x in complete RPMI media, counted again and seeded at 10000cells/mL (1mL total volume per well) in 24-well plate.
Dilution of PetML119/122 or controls (Bedinvetmab – anti-NGF IgG control, unrelated fusion protein and RPMI without NGF as negative control WHO Drug Information, Vol. 32, No. 4, 2018 -pg568) were diluted in 100uL of RPMI media from 3uM concentration till 91.25nM. 100uL of protein dilution was added to each well. Briefly, published Bedinvetmab aa sequence for both heavy chain and light chain have been used to generate codon optimised (for CHO expression) cDNA. Both HC and LC cDNA have been cloned in frame with standard peggy-bac plasmid. CHO have been then stably transfected as above.
Plates were analysed after 3 days. Briefly, 1mL cell suspension were centrifuged 5' at 300g RT, washed 2times with FACS buffer (PBS + 3% FBS + 3mM EDTA) and finally resuspended in 100uL of FACS buffer. Cells were acquired using CytoFLEX Flow Cytometer using following parameters (FSC:20 ; SSC:50 ; FITC:1 ; threshold: 1313131). Cells were gated based on FITC fluorescence (more fluorescence less proliferation) and % of proliferation inhibition was calculated considering 100% inhibition TF-1 cells cultured in RPMI without NGF and 0% inhibition cells cultured with complete RPMI media. Graph pad was used to calculate IC50 values.

Both PetML119/122 and Bedinvetmab were able to inhibit TF-1 proliferation in a dose dependent manner.
Table 1

	PetML119	PetML122	BedinvetmAb
IC50 (nM)	15.25	5.91	1.645

11. MIA-induced OA in rats (efficacy and pK) – Figures 9 to 12)

Efficacy and half-life for PetML119/122 were analysed using a rat model of OA. The detailed protocol is shown below. The model is shown in Figure 9.

Induction of Arthritis

Osteoarthritis was induced chemically by an intra-articular (I.A.) injection of 3 mg of monosodium-iodoacetate (MIA) (in 25 µL saline) into the right hind limb knee joint of the rat given under isoflurane anesthesia. While under anesthesia, ophthalmic ointment was applied to both eyes. The day of I.A. injection of MIA was counted as Day 0.

Allocation to Treatment Groups

Baseline dynamic weight bearing (DWB) were measured for all rats. Body weight (BW) was also measured at the same time. Rats were anesthetized and MIA injected into the right Knee joint through the middle of the patellar tendon approximately perpendicular to the tibia (Intra-articular (I.A.)). Dose level for I.A. injection of MIA was selected based on previous literature report in rodents (Bove et al.: Weight bearing as a measure of disease progression and efficacy of anti-inflammatory compounds in a model of monosodium iodoacetate-induced osteoarthritis. Osteoarthritis Cartilage. 2003 Nov;11(11):821-830). Animals showing a significant weight bearing difference between the MIA injected limb (right) and the healthy limb (left) were assigned to the study. Randomization was done based on *both* baseline DWB and BW (two variables randomization).

Dynamic Weight Bearing (DWB) Evaluation

Dynamic Weight Bearing was evaluated using the BioSeb® automated DWB system according to the manufacturer's manual. A two-minute recording was done for each rat. Analysis of dynamic weight bearing data was done off-line using the BioSeb® software. The system automatically calculated the weight borne by each limb and the tail. Body weight was measured for each rat immediately before the DWB for each time of testing. DWB measurement was done at different time points as per schedule in Study Design. Total distance travelled was also noted during DWB data analysis.

Dosing with test items

Group 1-2 rats received intravenous (IV) injections of vehicle and Group 3-7 rats received IV injections with the test items at designated doses once on Day 3 as depicted in the table below. Group 8 rats received oral gavage dosing with dexamethasone once daily from Day 3-21 as depicted in the table below.

Monosodium-Iodoacetate **(MIA)-INDUCED OA RAT STUDY WITH PETML119/122**

Study design

Table 2

*Gr.	Group Treatment	TI Dose level	TI Route, Volume	TI Dosing schedule	DWB testing	N
1	Naive/ Vehicle PBS	0	IV, 5mL/kg	Day 3, QD	Day 1 (baseline), Days 3, 6, 14, 21	10
2	3mg MIA/ Vehicle PBS	0	IV, 5mL/kg	Day 3, QD	Day 1 (baseline), Days 3, 6, 14, 21	10
3	3mg MIA/ Sample 1 (PetML119), low dose	0.5 mg/kg	IV, 5mL/kg	Day 3, QD	Day 1 (baseline), Days 3, 6, 14, 21	10
4	3mg MIA/ Sample 1 (PetML119), mid dose	1.5 mg/kg	IV, 5mL/kg	Day 3, QD	Day 1 (baseline), Days 3, 6, 14, 21	10
5	3mg MIA/ Sample 1 (PetML119), high dose	5 mg/kg	IV, 5mL/kg	Day 3, QD	Day 1 (baseline), Days 3, 6, 14, 21	10
6	3mg MIA/ Sample 2 (PetML122)	5 mg/kg	IV, 5mL/kg	Day 3, QD	Day 1 (baseline), Days 3, 6, 14, 21	10
7	3mg MIA/ Sample 3 (Bedinvetmab)	5 mg/kg	IV, 5mL/kg	Day 3, QD	Day 1 (baseline), Days 3, 6, 14, 21	10
8	3mg MIA/ Dexamethasone	1 mg/kg from Day 1-7 and 0.3mg/kg from Day 8-21	PO, 10ml/kg	Days 3-21 QD	Day 1 (baseline), Days 3, 6, 14, 21	10

*Animals from Groups 2 to 8 plus spares receive a single intra-articular (IA) injection with MIA (3mg/25µL saline) and the **day of MIA injection is considered as Day 0** (in the right knee joint).

IV: Intravenous (tail vein); PO: oral gavage; QD-once daily

Treated animals were observed for any clinical signs during the study. DWB was analysed on Days 3, 6, 14 and 21. Joint diameter was measured using a caliper on the right knee joint (medio-laterally) on days 3, 6, 14 and 21.

Briefly, both PetML119 (at different doses) and 122 (single dose) showed good analgesic effect outcompeting anti-NGF benchmark (Bedinvetmab) (Figure 10). They also showed a similar activity to daily administration of dexamethasone while not showing side effects of NSAIDs (body weight loss – Figure 11).

PK bleeds from 5 rats per group were taken using standard procedures on alternative days.

Serum pk analysis (Figure 12)

Sandwich ELISA to quantify serum levels of our fusion protein were set up as follows:

- 30uL of 2ug/mL of Capturing antibody (Mouse anti-canine p75 Ab --> MAB367-SP (Novus Bio)) diluted in PBS + 0.1M sodium bicarbonate were immobilised on half-area ELISA plates (MICROPLATE, 96 WELL, PS, HALF AREA, CLEAR, Item No.: 675061) overnight at 4°C.
- Plates were washed 2x with 200uL blocking solution (PBS + 5%DNFM + 0.2% Tween20) and blocking have been performed with 150uL of blocking solution across all wells for 3hrs at RT.
- Sera from different timepoints/groups of rat study were diluted 100x in blocking solution (2uL serum + 198uL blocking solution) and 30uL were added to relevant wells. Standards from PetML119/122 were prepared diluting fusion protein into rat serum from 100ug/mL till 1ng/mL with 1:5 dilutions. Standards were then diluted 100x in blocking solution and 30uL have been added to relevant wells.
- Sera were incubated for 1hr at RT with 450rpm shaking.
- Plates were washed 2x with 200uL blocking solution; detection antibody (SA5-10309 (ThermoFisher)) have been diluted 1:40000 in blocking solution and 30uL were added to each well and left 30' at RT with 450rpm shaking.
- Plates were washed 2x with 200uL blocking solution; developing HRP-conjugated antibody (A16035 (ThermoFisher)) were diluted 1:10000 in blocking solution and 30uL were added to each well and left 30' at RT with 450rpm shaking.
- Plates were washed 2x with 200uL blocking solution then 2x with 200uL of PBS + 0.2%Tween20 and finally 50uL of TMB (TMB Chromogen Solution (for ELISA) --> 002023) were added to each well. After 10', when standard curve showed saturation in first two points, the reaction was stopped adding 50uL of 1M Sulphuric acid.
- All wells were read with CLARIOstar Plus (BMG LABTECH) using endpoint Absorbance at 650nm and 450nm. Values were imported in Graph Pad and one-phase decay fitting have been applied to estimate half-life of these.

PetML119, from different doses, showed similar half-life (around 70 hrs) while PetML122 showed an extended half-life (around 10.5 days), see figure 12.

12. Pharmacokinetic and pharmacodynamic study of PetML119 and PetML122 after OA induction in dogs

The safety profile, pharmacokinetic and pharmacodynamic parameters of PetML119 and PetML122 were evaluated after a single intravenous administration at 1.5 mg/kg in dogs after induction of OA (urate crystals model). The study was carried out similar to the protocol in Toutian et al, J. vet. Pharmacol. Therap. 24, 43-55, 2001.

This study was performed according to a 2-groups, 4-period, 4-treatments, and 3 sequences. Ten dogs were used.

Four treatments were tested:

A. Positive Control (Meloxicam) Per Os.

B. Negative Control (Vehicle) Intravenous infusion.

C. PetML119 (1.5 mg/kg) Intravenous infusion.

D. PetML122 (1.5 mg/kg) Intravenous infusion

Table 3

Group No.	Period 1	Period 2	Period 3	Period 4
1	A	B	C	/
2	B	A	D	/

A reversible urate crystal synovitis model was used to induce an experimental acute synovitis. A sodium urate crystal suspension was prepared at a concentration of 10 mg/mL. General anaethesia was induced via an intra-articular injection of propofol. Alternate stifle joints were used in each period.

Vehicle and test item (PetML119/PetML122) treatment administrations (by intravenous delivery) were performed three days before acute synovitis induction (at Day-3). Meloxicam treatment administrations were performed right before acute synovitis induction (at T-0.5h). Treatment administrations were designed to obtain maximum treatment effect at the time of maximal induced lameness (i.e. 2 h-3 h after acute synovitis induction).

Table 4

Period	**Study day**	**Event**
Period 1	Day -4 to day -1 13 to 16 JAN 2022	- Efficacy assessments. Pre-induction (visual lameness score and measure of the peak vertical force) **- Vehicle administration (3 days before synovitis induction) group 2** - Overnight fasting (12h at least)
	Day 0 17 JAN 2022	Animal weighing **- Meloxicam administration (30mins before synovitis induction) group 1** - Synovitis induction (T0) - Efficacy assessments: T1.5h, T2.75h post urate crystals induction - Food distribution (after T2.75 h assessments) - Efficacy assessments: T4h, T5.25h, T6.5h post urate crystals induction
Period 2	Day -4 to day -1 27 to 30 JAN 2022	- Efficacy assessments. Pre-induction (visual lameness score and measure of the peak vertical force) **- Vehicle administration (3 days before synovitis induction) group 1**

		- Overnight fasting (12h at least)
	Day 0 31 JAN 2022	- Animal weighing **- Meloxicam administration (30mins before synovitis induction) group.** - Synovitis induction (T0) - Efficacy assessments: T1.5h, T2.75h post urate crystals induction - Food distribution (after T2.75 h assessments) - Efficacy assessments: T4h, T5.25h, T6.5h post urate crystals induction
Period 3	Day -4 10 FEB 2022	- Blood sampling for hematology biochemistry and plasma biomarkers T-24h before test item administration - Efficacy assessments Pre-induction (visual lameness score and measure of the peak vertical force)
	Day -3 11 FEB 2022	- Predose blood sampling **- Test item administration (3 days before synovitis induction) all groups** - Overnight fasting (12h at least)
	Day-2 12 FEB 2022	- Blood sampling T24h after test item administration.
	Day-1 13 FEB 2022	- Blood sampling T48h after test item administration.
	Day 0 14 FEB 2022	- Blood sampling T72h after test item administration. - Animal weighing - Synovitis induction (T0) - Efficacy assessments: T1.5h, T2.75h post urate crystals induction - Food distribution (after T2.75 h assessments) - Efficacy assessments: T4h, T5.25h, T6.5h post urate crystals induction
	Day4 18 FEB 2022	- Blood sampling T168h after test item administration.
	Day11 25 FEB 2022	- Blood sampling T336h after test item administration. - Blood sampling for hematology biochemistry T336h after test item administration
	Day18 04 MAR 2022	Blood sampling T504h after test item administration.
Period 4	Day -1 10 MAR 2022	- Efficacy assessments Pre-induction (visual lameness score and measure of the peak vertical force) - Overnight fasting (12h at least)
	Day 0 11 MAR 2022	Blood sampling T672h after test item administration. Animal weighing Synovitis induction (T0) Efficacy assessments: T1.5h, T2.75h post urate crystals induction Food distribution (after T2.75 h assessments) Efficacy assessments: T4h T5.25h, T6.5h post urate crystals induction
Animal Follow-up	28 MAR 2022	Blood sampling T1080h (Day 45) after test item administration
	12 APR 2022	Blood sampling T1440h (Day 60) after test item administration

Safety

Each animal was observed at least once daily and no abnormal findings were reported. Haematological parameters were collected and animals weighed at the time points indicated in Table 4. Blood samples were drawn from the jugular or cephalic vein at alternate sites.

Sandwich ELISA to identify potential anti-Drug Antibodies (ADA) in the serum generated after treatment with our fusion protein *in-vivo* were set up as follows:

- 30uL of 2ug/mL of either PetML119 or PetML122 (two plates) diluted in PBS + 0.1M sodium bicarbonate were immobilised on half-area ELISA plates (MICROPLATE, 96 WELL, PS, HALF AREA, CLEAR, Item No.: 675061) overnight at 4°C.
- Plates were washed 2x with 200uL blocking solution (PBS + 5%DNFM + 0.2% Tween20) and blocking have been performed with 150uL of blocking solution across all wells for 3hrs at RT.
- Sera from different timepoints/groups of rat/dog study were diluted 100x in blocking solution (2uL serum + 198uL blocking solution) and 30uL were added to relevant wells. Standards Standard anti PetML119/122 Ab (MAB367 from 1ng/mL) have been prepared in 20uL of rat/dog serum + Ab and then diluted with a 1 in 2 dilution. Standards were then diluted 100x in blocking solution and 30uL have been added to relevant wells.
- Sera were incubated for 1hr at RT with 450rpm shaking.
- Plates were washed 2x with 200uL blocking solution; detection antibody (SA5-10309 (ThermoFisher)) have been diluted 1:20000 in blocking solution and 30uL were added to each well and left 30' at RT with 450rpm shaking.
- Plates were washed 2x with 200uL blocking solution; developing HRP-conjugated antibody (A16035 (ThermoFisher)) were diluted 1:10000 in blocking solution and 30uL were added to each well and left 30' at RT with 450rpm shaking.
- Plates were washed 2x with 200uL blocking solution then 2x with 200uL of PBS + 0.2%Tween20 and finally 50uL of TMB (TMB Chromogen Solution (for ELISA) --> 002023) were added to each well. After 10', when standard curve showed saturation in first two points, the reaction was stopped adding 50uL of 1M Sulphuric acid.
- All wells were read with CLARIOstar Plus (BMG LABTECH) using endpoint Absorbance at 650nm and 450nm. Values were imported in Graph Pad and one-phase decay fitting have been applied to estimate half-life of these.

Results

Bodyweight (Figure 13a) and haematological parameters were unaltered after PetML119/ PetML122 administration.

For ADA analysis (Figure 13b), both sera from animals treated with PetML119/PetML122 showed basal signal comparable with the pre-dose control, indicating no anti-drug antibodies had been generated during the experimental time frame.

Efficacy

To assess the anti-inflammatory and analgesic efficacy of the test item, two parameters were investigated: visual lameness score (VLS, determined using a scoring system) and the vertical force (VF, expressed in Newtons) applied to the ground for the hind limb measured with a force plate. The force plate was connected to a computer equipped with a digital analogical acquisition card and a signal processing software. The force plate (made with 8 scales) was inserted in a path 50 cm wide and about 5 m long along which the dogs were trained to walk on a lead at a constant and similar speed. Based on the maximum treatment effect and maximal induced lameness, the efficacy assessment was performed pre-induction and 1.5 h, 2.75 h, 4 h, 5.25 h and 6.5 h (± 10 min per timepoint) post-induction.

Lameness ratio

The VF values were obtained as the limb is placed on the force plate. The dogs passed at least 5 times in the path of the force plate in order to obtain 3 interpretable values for the hind limb, to calculate the mean force of the limb. The ratio between the force applied after treatment and the force applied on Predose (reference force) of the same hind limb was referred to as the 'lameness ratio.' This parameter describes the force applied by the induced limb in relation to the force applied in the absence of inflammation. If severe lameness with no weight bearing is observed during walking phase, the vertical force was not measured and the lameness ratio was considered equal to zero.

Visual Lameness score

The dogs were scored for lameness whilst standing and whilst walking (for approximately one minute before scoring), according to the semi-quantitative scales detailed as follows:

Table 5

Observation whilst standing	**Score**
Full weight bearing (touching of all digital pads on the ground)	0
Partial weight bearing (touching of just a part of digital pads on the ground)	1
No weight bearing to toe touching	2

Table 6

Observation whilst walking	**Score**
Full weight bearing, no lameness	0
Slight lameness with partial weight bearing: lameness barely perceptible	1
Moderate lameness with partial weight bearing: the animal rests the limb on the ground slightly	2

Severe lameness with no weight bearing: the animal uses its limb but does not put its weight on the limb and/or avoid putting the limb on the ground	3

The combined lameness visual score was considered as the sum of the standing and walking phase scores.

Results

After treatment, both PetML119 and PetML122 exhibit improved efficacy to the gold standard for acute pain, Meloxicam (Figure 14). Furthermore, PetML119 and PetML122 have the additional benefit of only requiring a single dose for extended pain relief. In contrast, Meloxicam or other existing treatments require daily administration which reduces compliance and increases stress to the animal.

Serum pk analysis

Blood samples were collected before and during period 3 at the following target time points:
After intravenous test item administration at T24h, T48h, T72h, (pre-induction Period 3) and T168h, T336h, T504h (pre-induction Period 4) and T672h post dosing. Additional blood samples were collected at 45 and 60 days post dosing.

Sandwich ELISA to measure pK values are as described in Example 11 above.

Results

PetML122 showed an extended half-life (around 19 days) in comparison to the half-life of PetML119 (around 4 days), see figure 15.

Sequences

SEQ ID NO. 1 canine p75NTR protein

KEACPTGLYTHSGECCKACNLGEGVAQPCGANQTVCEPCLDSVTFSDVVSATEPCKPCTE
CVGLQSMSAPCVEADDAVCRCAYGYYQDETTGRCEACRVCEAGSGLVFSCQDRQNTVCEE
CPDGTYSDEANHVDPCLPCTVCEDTERQLRECTRWADAECEEIPGRWITRSTPSEDSDST
APSTEEPELPPDQEIIASTMADVVTTVMGSSQPVVTRGTADNLIPVYCSILAAVVVGLVA
YIAFKRWNSCKQNKQGANSRPVNQTPPPEGEKLHSDSGISVDSQSLHDQQPHTQTAAGQA
LKGDGGLYSSLPPAKREEVEKLLNGSAGDTWRHLAGELGYQPEHIDSFTHEACPARALLA
SWAAQDSATLDALLAALRRIQRADIVESLCSESTATSPV

ECD is underlined

SEQ ID NO. 2 canine p75NTR nucleic acid sequence

ATGGACGGGCCGCGCCTGCTGCTGCTGCTGCTGCTCCTGGGGGTGTCCCTTGGAGGTGCC
AAGGAGGCATGTCCCACTGGCCTGTACACCCACAGCGGCGAGTGCTGCAAAGCCTGCAATCTGG
GTGAGGGGGTGGCCCAGCCTTGCGGAGCCAACCAGACCGTGTGTGAGCCCTGCCTGGACAGCG
TGACCTTCTCGGACGTGGTGAGCGCCACCGAGCCGTGCAAGCCGTGCACCGAGTGCGTGGGGC
TGCAGAGCATGTCGGCGCCGTGCGTGGAGGCGGACGACGCCGTGTGCCGCTGCGCCTACGGC
TACTACCAGGACGAGACGACGGGCCGCTGCGAGGCGTGCCGCGTGTGCGAGGCGGGCTCGGG
GCTCGTGTTCTCGTGCCAGGACAGGCAGAACACCGTGTGCGAGGAGTGTCCCGACGGCACGTA
CTCCGACGAGGCCAACCACGTGGACCCGTGCCTGCCCTGCACCGTGTGCGAGGACACCGAGCG
CCAGCTGCGCGAGTGCACGCGCTGGGCCGACGCCGAGTGCGAGGAGATCCCTGGCCGTTGGA
TTACCCGGTCCACACCCTCAGAGGACTCGGACAGCACCGCCCCCAGCACAGAGGAGCCAGAGC
TACCTCCAGATCAAGAAATCATAGCCAGCACCATGGCAGATGTGGTGACCACAGTGATGGGCAG
CTCTCAGCCTGTAGTGACCCGAGGAACCGCTGACAACCTCATCCCTGTCTACTGCTCCATCCTGG
CCGCCGTGGTTGTGGGCTTAGTGGCCTACATTGCCTTCAAGAGGTGGAACAGCTGCAAGCAGAA
CAAGCAAGGAGCCAACAGCCGGCCCGTGAACCAGACGCCTCCGCCGGAGGGAGAAAAGCTCCA
CAGTGACAGTGGCATCTCTGTGGACAGCCAGAGCCTGCATGACCAGCAGCCCCACACACAGACG
GCCGCAGGCCAGGCCCTCAAGGGGGATGGAGGTCTCTACAGCAGCCTGCCACCAGCCAAGCGG
GAGGAGGTGGAGAAGCTGCTCAATGGCTCTGCGGGGGACACCTGGCGGCACCTGGCAGGTGA
GCTGGGCTACCAGCCTGAGCACATAGACTCCTTCACCCACGAGGCCTGCCCAGCCCGAGCCCT
GCTTGCCAGCTGGGCCGCCCAGGACAGCGCGACGCTCGACGCCCTCCTGGCTGCTCTGCGCCG
CATCCAGCGAGCCGACATCGTGGAGAGCCTGTGTAGCGAGTCCACGGCCACGTCTCCAGTGTG
A

Leader sequence is underlined

SEQ ID NO. 3 feline p75NTR protein

KEACPTGLFTHSGECCKACNLGEGVAQPCGANQTVCEPCLDSVTFSDVVSATEPCKPCTE
CVGLQSMSAPCVEADDAVCRCAYGYYQDETTGRCEACRVCEAGSGLVFSCQDRQNTVCEE
CPDGTYSDEANHVDPCLPCTVCEDTERQLRECTRWADAECEEIPGRWITRSTPSEGSDST
APSTEEPEVPPEQDLIASTVADVVTTVMGSSQPVVTRGTADNLIPVYCSILAAVVVGLVA
YIAFKRWNSCKQDKQGANSRPVNQTPPPEGEKLHSDSGISVDSQSLHDQQSHTQTAAGQA
LKGDGGLYSSLPSAKREEVEKLLNGSAGDTWRHLAGELGYQPEHIDSFTREACPARALLA
SWAAQDSATLDALLAALRRIQRADIVESLCSESTATSPV

ECD is underlined

SEQ ID NO. 4 feline p75NTR nucleic acid sequence

ATGGACGGGCCGCGCCCGCTGCTGCTGCTGTTGCCGCTGCTCCTGGGGGTGTCCCTTGGAGGT
GCCAAGGAGGCATGTCCCACGGGCCTGTTCACCCACAGCGGCGAGTGCTGTAAAGCCTGCAAC
CTGGGAGAGGGCGTAGCCCAGCCTTGCGGAGCCAACCAGACCGTGTGTGAGCCCTGCCTGGAC
AGCGTGACCTTCTCGGACGTGGTGAGCGCCACGGAGCCGTGCAAGCCGTGCACCGAGTGCGTG
GGCCTGCAGAGCATGTCGGCGCCGTGCGTGGAGGCCGACGACGCCGTGTGTCGCTGCGCCTA
CGGCTACTACCAGGACGAGACGACGGGCCGCTGCGAGGCGTGCCGCGTGTGCGAGGCGGGCT
CCGGCCTGGTGTTCTCGTGCCAGGACCGGCAGAATACCGTGTGCGAGGAGTGTCCCGACGGCA
CGTACTCGGACGAGGCCAACCACGTGGACCCGTGCCTGCCCTGCACCGTGTGCGAGGACACCG
AGCGCCAGCTGCGCGAGTGCACGCGCTGGGCCGACGCCGAGTGCGAGGAGATCCCTGGCCGT
TGGATTACTCGGTCTACACCTTCGGAGGGCTCGGACAGCACCGCCCCCAGCACGGAGGAGCCA
GAGGTACCTCCAGAGCAAGACCTCATAGCCAGCACGGTGGCAGATGTGGTGACCACAGTGATGG
GCAGCTCTCAGCCCGTAGTGACCCGAGGCACCGCCGACAACCTCATCCCTGTCTATTGTTCCAT
CCTGGCCGCTGTGGTTGTGGGCCTGGTGGCCTACATTGCCTTCAAGAGGTGGAACAGCTGCAAA
CAGGACAAGCAAGGCGCCAACAGCCGGCCCGTGAACCAGACGCCCCCGCCCGAGGGAGAAAA
GCTCCACAGTGACAGTGGCATCTCTGTGGACAGCCAGAGCCTGCATGACCAGCAGTCCCACACG
CAGACGGCCGCCGGCCAGGCCCTCAAGGGGGACGGAGGTCTCTACAGCAGCCTGCCGTCAGC
CAAGCGGGAGGAGGTAGAGAAACTGCTCAACGGCTCTGCGGGGGACACGTGGCGGCACCTGG
CGGGCGAGCTGGGCTACCAGCCTGAGCACATAGACTCCTTCACCCGCGAGGCCTGCCCAGCCC
GGGCCCTGCTCGCCAGCTGGGCCGCCCAGGACAGCGCGACGCTCGACGCCCTCCTGGCCGCC
CTGCGCCGCATCCAGCGGGCCGACATCGTCGAGAGCCTGTGCAGCGAGTCCACGGCCACGTCC
CCGGTGTGA

Leader sequence is underlined

SEQ ID NO. 5 equine p75NTR protein

KEVCPTDLYTHSGECCKACNLGEGVAQPCGANQTVCEPCLDSVTFSDVVSATEPCKPCTE
CVGLQSMSAPCVEADDAVCRCAYGYYQDETTGRCEACQVCEAGSGLVFSCQDKQNTVCEE
CPDGTYSDEANHVDPCLPCTVCEDTERQLRECTRWADAECEEIPSRWITRATPPEGSDST
APSTQEPEGPPEKDLVASTVADVVTTVMGSSQPVVTRGTTDNLIPVYCSILAAVVVGLVA
YIAFKRWNSCKQNKQGANSRPVNQTPPPEGEKLHSDSGISVDSQSLHDQQPHTQTAAGQA
LKGDGGLYSSLPLAKREEVEKLLNGSAGDTWRHLAGLVGQGLLRLELVSVFQGPAHGGML
PPATPSLQAPVWLGPEGCSEKWDQRGNAARRAGLRVWPMEGLSQV

ECD is underlined

SEQ ID NO. 6 equine p75NTR nucleic acid sequence

ATGAGGGCAGGTGCCGCCGACTGCGCCATGGACGGACCGCGCCTTCTGCTGCTGCTTCTGCTC
TTGGGGGTGTGCCTGCTGGGAGGTGCCAAGGAGGTGTGCCCCACAGACCTGTACACCCACAGC

GGCGAGTGCTGCAAAGCCTGCAACCTGGGCGAGGGTGTGGCCCAGCCTTGCGGAGCCAACCAG
ACTGTGTGTGAACCCTGCCTGGACAGCGTGACGTTCTCGGACGTGGTGAGCGCCACAGAGCCAT
GTAAGCCGTGCACCGAGTGCGTGGGCCTGCAGAGCATGTCGGCGCCATGCGTGGAGGCCGAC
GACGCGGTGTGCCGCTGCGCCTATGGCTACTACCAGGACGAGACGACGGGCCGCTGCGAGGC
GTGCCAGGTGTGCGAGGCGGGCTCGGGCCTCGTGTTCTCGTGCCAGGACAAGCAGAACACCGT
GTGCGAGGAATGCCCCGACGGCACGTACTCCGACGAGGCCAACCACGTGGACCCGTGCCTGCC
CTGCACCGTGTGCGAGGACACCGAGCGCCAGCTGCGAGAGTGCACGCGCTGGGCCGACGCCG
AGTGCGAGGAGATCCCCAGCCGTTGGATTACACGGGCCACGCCGCCGGAGGGCTCAGACAGCA
CTGCCCCCAGCACCCAGGAGCCCGAGGGACCTCCAGAGAAAGACCTTGTAGCCAGCACGGTGG
CGGATGTGGTGACCACAGTGATGGGCAGCTCTCAGCCCGTGGTGACCCGAGGCACCACGGACA
ACCTCATCCCCGTCTATTGCTCCATCCTGGCCGCTGTGGTTGTGGGCCTTGTGGCCTACATCGC
CTTCAAGAGGTGGAACAGCTGCAAGCAGAACAAGCAAGGAGCCAACAGCCGACCCGTGAACCA
GACACCACCACCCGAGGGAGAAAAACTCCACAGCGACAGCGGCATCTCTGTGGACAGCCAGAG
CCTGCATGACCAGCAGCCTCACACACAGACAGCCGCAGGCCAGGCCCTCAAGGGAGATGGAGG
CCTCTACAGCAGCCTGCCACTGGCCAAGAGGGAAGAGGTGGAGAAGCTACTCAATGGCTCCGCA
GGGGACACCTGGCGGCACCTGGCGGGTGAGCTGGGCTACCAGCCCGAGCACATAGACTCCTTC
ACCCACGAGGCCTGCCCCGTCCGCGCCCTGCTTGCCAGCTGGGCCGCCCAGGACAGTGCGACA
TTCGATGCCCTCCTGACCGCCCTGCGCCGCATCCAGCGAGCCGACATTGTCGAGAGCCTGTGCA
GCGAGTCCACCGCCACATCCCCGGTGTGA

Leader sequence is underlined

SEQ ID NO. 7 canine p75NTR protein ECD

KEACPTGLYTHSGECCKACNLGEGVAQPCGANQTVCEPCLDSVTFSDVVSATEPCKPCTECVGLQS
MSAPCVEADDAVCRCAYGYYQDETTGRCEACRVCEAGSGLVFSCQDRQNTVCEECPDGTYSDEAN
HVDPCLPCTVCEDTERQLRECTRWADAECEEIPGRWITRSTPSEDSDSTAPSTEEPELPPDQEIIAST
MADVVTTVM**GSSQPVVTRGTADN**

The wt ECD region includes the stalk region (underlined) and alpha and gamma secretase cleavage 3' of the stalk region (in bold)

SEQ ID NO. 8 canine p75NTR ECD nucleic acid sequence

AAGGAGGCATGTCCCACTGGCCTGTACACCCACAGCGGCGAGTGCTGCAAAGCCTGCAATCTGG
GTGAGGGGGTGGCCCAGCCTTGCGGAGCCAACCAGACCGTGTGTGAGCCCTGCCTGGACAGCG
TGACCTTCTCGGACGTGGTGAGCGCCACCGAGCCGTGCAAGCCGTGCACCGAGTGCGTGGGGC
TGCAGAGCATGTCGGCGCCGTGCGTGGAGGCGGACGACGCCGTGTGCCGCTGCGCCTACGGC
TACTACCAGGACGAGACGACGGGCCGCTGCGAGGCGTGCCGCGTGTGCGAGGCGGGCTCGGG

GCTCGTGTTCTCGTGCCAGGACAGGCAGAACACCGTGTGCGAGGAGTGTCCCGACGGCACGTA
CTCCGACGAGGCCAACCACGTGGACCCGTGCCTGCCCTGCACCGTGTGCGAGGACACCGAGCG
CCAGCTGCGCGAGTGCACGCGCTGGGCCGACGCCGAGTGCGAGGAGATCCCTGGCCGTTGGA
TTACCCGGTCCACACCCTCAGAGGACTCGGACAGCACCGCCCCCAGCACAGAGGAGCCAGAGC
TACCTCCAGATCAAGAAATCATAGCCAGCACCATGGCAGATGTGGTGACCACAGTGATGGGCAG
CTCTCAGCCTGTAGTGACCCGAGGAACCGCTGACAAC

SEQ ID NO. 9 canine ECD of p75NTR stalk region protein

WITRSTPSEDSDSTAPSTEEPELPPDQEIIASTMADVVTTVM

SEQ ID NO. 10 canine ECD of p75NTR stalk region nucleic acid sequence

TGGATTACCCGGTCCACACCCTCAGAGGACTCGGACAGCACCGCCCCCAGCACAGAGGAGCCA
GAGCTACCTCCAGATCAAGAAATCATAGCCAGCACCATGGCAGATGTGGTGACCACAGTGATG

SEQ ID NO. 11 canine p75NTR ECD – canine IgGB wt Fc protein fusion

MEWSWVFLFFLSVTTGVHSKEACPTGLYTHSGECCKACNLGEGVAQPCGANQTVCEPCLDSVTFSD
VVSATEPCKPCTECVGLQSMSAPCVEADDAVCRCAYGYYQDETTGRCEACRVCEAGSGLVFSCQD
*RQNTVCEECPDGTYSDEANHVDPCLPCTVCEDTERQLRECTRWADAECEEIP***GGGG**RENGRVPRP
PDCPKCPAPEMLGGPSVFIFPPKPKDTLLIARTPEVTCVVVDLDPEDPEVQISWFVDGKQMQTAKTQP
REEQFNGTYRVVSVLPIGHQDWLKGKQFTCKVNNKALPSPIERTISKARGQAHQPSVYVLPPSREELS
KNTVSLTCLIKDFFPPDIDVEWQSNGQQEPESKYRTTPPQLDEDGSYFLYSKLSVDKSRWQRGDTFIC
AVMHEALHNHYTQKSLSHSPGK

Signal peptide

Canine p75-ECD

Linker GGGG

Canine Fc-B wt

SEQ ID NO. 12 canine p75NTR ECD– canine IgGB wt Fc nucleic acid sequence
ATGGAATGGTCCTGGGTGTTCCTGTTCTTCCTGTCCGTGACCACCGGCGTGCACTCCAAAGAGG
CTTGTCCTACCGGCCTGTACACCCACTCTGGCGAGTGTTGCAAGGCCTGTAATCTCGGCGAAGG
CGTGGCACAACCTTGTGGCGCTAATCAGACAGTGTGCGAGCCTTGCCTGGACTCCGTGACCTTC
TCTGATGTGGTGTCTGCCACCGAGCCATGCAAGCCTTGTACCGAGTGTGTGGGCCTGCAGTCCA
TGTCTGCCCCTTGTGTGGAAGCCGACGACGCCGTGTGTAGATGTGCCTACGGCTACTACCAGGA
CGAGACAACCGGAAGATGCGAGGCCTGCAGAGTGTGTGAAGCTGGCTCTGGACTGGTGTTCTCC

TGCCAAGACAGACAGAACACCGTGTGCGAGGAATGCCCTGACGGCACCTACTCTGATGAGGCCA
ATCACGTGGACCCCTGCCTGCCTTGTACTGTGTGCGAAGATACCGAGCGGCAGCTGCGCGAGTG
TACCAGATGGGCTGATGCCGAGTGCGAAGAGATCCCTGGAGGTGGCGGACGCGAGAATGGCAG
AGTGCCTAGACCTCCTGACTGCCCTAAGTGCCCTGCTCCTGAAATGCTCGGCGGACCCTCCGTG
TTCATCTTCCCACCTAAGCCTAAGGACACCCTGCTGATCGCTCGGACCCCTGAAGTGACATGCGT
GGTGGTGGATCTGGACCCCGAGGATCCTGAGGTGCAGATCAGTTGGTTCGTGGACGGCAAGCA
GATGCAGACCGCTAAGACCCAGCCTAGAGAGGAACAGTTCAACGGCACCTACAGAGTGGTGTCT
GTGCTGCCTATCGGCCACCAGGATTGGCTGAAGGGCAAGCAGTTTACCTGCAAAGTGAACAACA
AGGCCCTGCCTTCTCCAATCGAGCGGACCATCTCTAAGGCCAGAGGCCAGGCTCATCAGCCTTC
CGTGTATGTCCTGCCACCTAGCCGCGAGGAACTGTCCAAGAACACCGTGTCTCTGACCTGCCTG
ATCAAGGACTTCTTCCCTCCTGACATCGACGTGGAATGGCAGTCCAACGGCCAGCAAGAGCCCG
AGTCTAAGTACCGGACAACCCCTCCACAGCTGGACGAGGACGGCTCCTACTTCCTGTACTCCAA
GCTGTCCGTGGACAAGTCTCGGTGGCAGAGAGGCGACACCTTCATCTGTGCTGTGATGCACGAG
GCCCTGCACAACCACTACACCCAGAAGTCCCTGTCTCACTCCCCTGGCAAGTGA

Leader sequence is underlined

SEQ ID NO. 13 canine p75NTR ECD – canine Fc YTE protein fusion

MEWSWVFLFFLSVTTGVHSKEACPTGLYTHSGECCKACNLGEGVAQPCGANQTVCEPCL
DSVTFSDVVSATEPCKPCTECVGLQSMSAPCVEADDAVCRCAYGYYQDETTGRCEACRVC
EAGSGLVFSCQDRQNTVCEECPDGTYSDEANHVDPCLPCTVCEDTERQLRECTRWADAE
*CEEIP***GGGG**RENGRVPRPPDCPKCPAPEMLGGPSVFIFPPKPKDTL**Y**I**T**R**E**PEVTCVVVDL
DPEDPEVQISWFVDGKQMQTAKTQPREEQFNGTYRVVSVLPIGHQDWLKGKQFTCKVNNK
ALPSPIERTISKARGQAHQPSVYVLPPSREELSKNTVSLTCLIKDFFPPDIDVEWQSNGQQE
PESKYRTTPPQLDEDGSYFLYSKLSVDKSRWQRGDTFICAVMHEALHNHYTQKSLSHSPGK

Signal peptide

Canine p75-ECD

Linker GGGG

Canine Fc-B-**YTE**

SEQ ID NO. 14 canine p75NTR ECD - Fc YTE nucleic acid sequence

ATGGAATGGTCCTGGGTGTTCCTGTTCTTCCTGTCCGTGACCACCGGCGTGCACTCCAAAGAGG
CTTGTCCTACCGGCCTGTACACCCACTCTGGCGAGTGTTGCAAGGCCTGTAATCTCGGCGAAGG
CGTGGCACAACCTTGTGGCGCTAATCAGACAGTGTGCGAGCCTTGCCTGGACTCCGTGACCTTC
TCTGATGTGGTGTCTGCCACCGAGCCATGCAAGCCTTGTACCGAGTGTGTGGGCCTGCAGTCCA

TGTCTGCCCCTTGTGTGGAAGCCGACGACGCCGTGTGTAGATGTGCCTACGGCTACTACCAGGA
CGAGACAACCGGAAGATGCGAGGCCTGCAGAGTGTGTGAAGCTGGCTCTGGACTGGTGTTCTCC
TGCCAAGACAGACAGAACACCGTGTGCGAGGAATGCCCTGACGGCACCTACTCTGATGAGGCCA
ATCACGTGGACCCCTGCCTGCCTTGTACTGTGTGCGAAGATACCGAGCGGCAGCTGCGCGAGTG
TACCAGATGGGCTGATGCCGAGTGCGAAGAGATCCCTGGAGGTGGCGGACGCGAGAATGGCAG
AGTGCCTAGACCTCCTGACTGCCCTAAGTGCCCTGCTCCTGAAATGCTCGGCGGACCCTCCGTG
TTCATCTTCCCACCTAAGCCTAAGGACACCCTGTATATCACTCGGGAACCTGAAGTGACATGCGT
GGTGGTGGATCTGGACCCCGAGGATCCTGAGGTGCAGATCAGTTGGTTCGTGGACGGCAAGCA
GATGCAGACCGCTAAGACCCAGCCTAGAGAGGAACAGTTCAACGGCACCTACAGAGTGGTGTCT
GTGCTGCCTATCGGCCACCAGGATTGGCTGAAGGGCAAGCAGTTTACCTGCAAAGTGAACAACA
AGGCCCTGCCTTCTCCAATCGAGCGGACCATCTCTAAGGCCAGAGGCCAGGCTCATCAGCCTTC
CGTGTATGTCCTGCCACCTAGCCGCGAGGAACTGTCCAAGAACACCGTGTCTCTGACCTGCCTG
ATCAAGGACTTCTTCCCTCCTGACATCGACGTGGAATGGCAGTCCAACGGCCAGCAAGAGCCCG
AGTCTAAGTACCGGACAACCCCTCCACAGCTGGACGAGGACGGCTCCTACTTCCTGTACTCCAA
GCTGTCCGTGGACAAGTCTCGGTGGCAGAGAGGCGACACCTTCATCTGTGCTGTGATGCACGAG
GCCCTGCACAACCACTACACCCAGAAGTCCCTGTCTCACTCCCCTGGCAAGTGA

Leader sequence is underlined

SEQ ID NO. 15 IgG-A

MEFVLGWVFLVAILQGVQGEVQLVESGGDLVKPAGSLRLSCVASGFTFSNNAMNWVRQAPGKGLQ
WVAGINSGGSTASADAVKGRFTISRDNAKNTVYLQMNSLTAEDTAVYYCAKVIGNWIATSDLDYWGQ
GTLVIVSSASTTAPSVFPLAPSCGSTSGSTVALACLVSGYFPEPVTVSWNSGSLTSGVHTFPSVLQSS
GLYSLSSMVTVPSSRWPSETFTCNVVHPASNTKVDKPVFNECRCTDTPPCPVPEPLGGPSVLIFPPK
PKDILRITRTPEVTCVVLDLGREDPEVQISWFVDGKEVHTAKTQSREQQFNGTYRVVSVLPIEHQDWL
TGKEFKCRVNHIDLPSPIERTISKARGRAHKPSVYVLPPSPKELSSSDTVSITCLIKDFYPPDIDVEWQS
NGQQEPERKHRMTPPQLDEDGSYFLYSKLSVDKSRWQQGDPFTCAVMHETLQNHYTDLSLSHSPG
K

SEQ ID NO. 16 IgG-B

MEFVLGWVFLVAILQGVQGEVQLVESGGDLVKPAGSLRLSCVASGFTFSNNAMNWVRQAPGKGLQ
WVAGINSGGSTASADAVKGRFTISRDNAKNTVYLQMNSLTAEDTAVYYCAKVIGNWIATSDLDYWGQ
GTLVIVSSASTTAPSVFPLAPSCGSTSGSTVALACLVSGYFPEPVTVSWNSGSLTSGVHTFPSVLQSS
GLYSLSSMVTVPSSRWPSETFTCNVAHPASKTKVDKPVPKRENGRVPRPPDCPKCPAPEMLGGPSV
FIFPPKPKDTLLIARTPEVTCVVVDLDPEDPEVQISWFVDGKQMQTAKTQPREEQFNGTYRVVSVLPIG
HQDWLKGKQFTCKVNNKALPSPIERTISKARGQAHQPSVYVLPPSREELSKNTVSLTCLIKDFFPPDID

VEWQSNGQQEPESKYRTTPPQLDEDGSYFLYSKLSVDKSRWQRGDTFICAVMHEALHNHYTQKSLS
HSPGK

SEQ ID NO. 17 IgG-C

MEFVLGWVFLVAILQGVQGEVQLVESGGDLVKPAGSLRLSCVASGFTFSNNAMNWVRQAPGKGLQ
WVAGINSGGSTASADAVKGRFTISRDNAKNTVYLQMNSLTAEDTAVYYCAKVIGNWIATSDLDYWGQ
GTLVIVSSASTTAPSVFPLAPSCGSQSGSTVALACLVSGYIPEPVTVSWNSGSLTSGVHTFPSILQSSG
LYSLSSMVTVPSSRWPSETFTCNVAHPATNTKVDKPVVKECECKCNCNNCPCPGCGLLGGPSVFIFP
PKPKDILVTARTPTVTCVVVDLDPENPEVQISWFVDSKQVQTANTQPREEQSNGTYRVVSVLPIGHQD
WLSGKQFKCKVNNKALPSPIEEIISKTPGQAHQPNVYVLPPSRDEMSKNTVTLTCLVKDFFPPEIDVE
WQSNGQQEPESKYRMTPPQLDEDGSYFLYSKLSVDKSRWQRGDTFICAVMHEALHNHYTQKSLSH
SPGK

SEQ ID NO. 18 IgG-D

MEFVLGWVFLVAILQGVQGEVQLVESGGDLVKPAGSLRLSCVASGFTFSNNAMNWVRQAPGKGLQ
WVAGINSGGSTASADAVKGRFTISRDNAKNTVYLQMNSLTAEDTAVYYCAKVIGNWIATSDLDYWGQ
GTLVIVSSASSTAPSVFPLAPSCGSTSGSTVALACLVSGYFPEPVTVSWNSGSLTSGVHTFPSVLKSS
GLYSLSSMVTVPSSRLPSETFTCNVVHPATNTKVDKPVPKESTCKCISPCPVPESLGGPSVFIFPPKPK
DILRITRTPEVTCVVLDLGREDPEVQISWFVDGKEVHTAKTQPREQQFNSTYRVVSVLPIEHQDWLTG
KEFKCRVNHIGLPSPIERTISKARGQAHQPGVYVLPPSPKELSSSDTVTLTCLIKDFFPPEIDVEWQSN
GQPEPESKYHTTAPQLDEDGSYFLYSKLSVDKSRWQQGDPFTCAVMHEALQNHYTDLSLSHSPGK

SEQ ID NO. 19
DOGA constant region

FNECRCTDTPPCPVPEPLGGPSVLIFPPKPKDILRITRTPEVTCVVLDLGREDPEVQISWFVDGKEVHT
AKTQSREQQFNGTYRVVSVLPIEHQDWLTGKEFKCRVNHIDLPSPIERTISKARGRAHKPSVYVLPPS
PKELSSSDTVSITCLIKDFYPPDIDVEWQSNGQQEPERKHRMTPPQLDEDGSYFLYSKLSVDKSRWQ
QGDPFTCAVMHETLQNHYTDLSLSHSPGK

SEQ ID NO. 20
DOGB constant region
RENGRVPRPPDCPKCPAPEMLGGPSVFIFPPKPKDTLLIARTPEVTCVVVDLDPEDPEVQISWFVDGK
QMQTAKTQPREEQFNGTYRVVSVLPIGHQDWLKGKQFTCKVNNKALPSPIERTISKARGQAHQPSVY
VLPPSREELSKNTVSLTCLIKDFFPPDIDVEWQSNGQQEPESKYRTTPPQLDEDGSYFLYSKLSVDKS
RWQRGDTFICAVMHEALHNHYTQKSLSHSPGK

SEQ ID NO. 21

DOGB-YTE constant region

RENGRVPRPPDCPKCPAPEMLGGPSVFIFPPKPKDTLYITREPEVTCVVVDLDPEDPEVQISWFVDGK
QMQTAKTQPREEQFNGTYRVVSVLPIGHQDWLKGKQFTCKVNNKALPSPIERTISKARGQAHQPSVY
VLPPSREELSKNTVSLTCLIKDFFPPDIDVEWQSNGQQEPESKYRTTPPQLDEDGSYFLYSKLSVDKS
RWQRGDTFICAVMHEALHNHYTQKSLSHSPGK

SEQ ID NO. 22

DOGC constant region

ECECKCNCNNCPCPGCGLLGGPSVFIFPPKPKDILVTARTPTVTCVVVDLDPENPEVQISWFVDSKQV
QTANTQPREEQSNGTYRVVSVLPIGHQDWLSGKQFKCKVNNKALPSPIEEIISKTPGQAHQPNVYVLP
PSRDEMSKNTVTLTCLVKDFFPPEIDVEWQSNGQQEPESKYRMTPPQLDEDGSYFLYSKLSVDKSR
WQRGDTFICAVMHEALHNHYTQKSLSHSPGK

SEQ ID NO. 23

DOGD constant region

ESTCKCISPCPVPESLGGPSVFIFPPKPKDILRITRTPEVTCVVLDLGREDPEVQISWFVDGKEVHTAKT
QPREQQFNSTYRVVSVLPIEHQDWLTGKEFKCRVNHIGLPSPIERTISKARGQAHQPGVYVLPPSPKE
LSSSDTVTLTCLIKDFFPPEIDVEWQSNGQPEPESKYHTTAPQLDEDGSYFLYSKLSVDKSRWQQGD
PFTCAVMHEALQNHYTDLSLSHSPGK

SEQ ID NO. 24

CAT_IGG1V1 constant region

TDHPPGPKPCDCPKCPPPEMLGGPSIFIFPPKPKDTLSISRTPEVTCLVVDLGPDDSDVQITWFVDNT
QVYTAKTSPREEQFNSTYRVVSVLPILHQDWLKGKEFKCKVNSKSLPSPIERTISKAKGQPHEPQVYV
LPPAQEELSRNKVSVTCLIKSFHPPDIAVEWEITGQPEPENNYRTTPPQLDSDGTYFVYSKLSVDRSH
WQRGNTYTCSVSHEALHSHHTQKSLTQSPGK

SEQ ID NO. 25

CAT_IGG1V2 constant region

TDHPPGPKPCDCPKCPPPEMLGGPSIFIFPPKPKDTLSISRTPEVTCLVVDLGPDDSDVQITWFVDNT
QVYTAKTSPREEQFNSTYRVVSVLPILHQDWLKGKEFKCKVNSKSLPSPIERTISKAKGQPHEPQVYV
LPPAQEELSRNKVSVTCLIKSFHPPDIAVEWEITGQPEPENNYRTTPPQLDSDGTYFVYSKLSVDRSH
WQRGNTYTCSVSHEALHSHHTQKSLTQSPGK

SEQ ID NO. 26

CAT_IGG2 constant region

KTASTIESKTGEGPKCPVPEIPGAPSVFIFPPKPKDTLSISRTPEVTCLVVDLGPDDSNVQITWFVDNTE
MHTAKTRPREEQFNSTYRVVSVLPILHQDWLKGKEFKCKVNSKSLPSAMERTISKAKGQPHEPQVYV

LPPTQEELSENKVSVTCLIKGFHPPDIAVEWEITGQPEPENNYQTTPPQLDSDGTYFLYSRLSVDRSH
WQRGNTYTCSVSHEALHSHHTQKSLTQSPGK

SEQ ID NO. 27

HORSE_IGHG1 constant region

VIKECNGGCPAECLQVGPSVFIFPPKPKDVLMISRTPTVTCVVVDVGHDFPDVQFNWYVDGVETHTA
TTEPKQEQFNSTYRVVSVLPIQHKDWLSGKEFKCKVNNKALPAPVERTISKPTGQPREPQVYVLAPH
RDELSKNKVSVTCLVKDFYPTDIDIEWKSNGQPEPETKYSTTPAQLDSDGSYFLYSKLTVETNRWQQ
GTTFTCAVMHEALHNHYTEKSVSKSPGK

SEQ ID NO. 28

HORSE_IGHG2 constant region

CVLSAEGVIPIPSVPKPQCPPYTHSKFLGGPSVFIFPPNPKDALMISRTPVVTCVVVNLSDQYPDVQFS
WYVDNTEVHSAITKQREAQFNSTYRVVSVLPIQHQDWLSGKEFKCSVTNVGVPQPISRAISRGKGPS
RVPQVYVLPPHPDELAKSKVSVTCLVKDFYPPDISVEWQSNRWPELEGKYSTTPAQLDGDGSYFLYS
KLSLETSRWQQVESFTCAVMHEALHNHFTKTDISESLGK

SEQ ID NO. 29

HORSE_IGHG3

TTPPCPCECPKCPAPELLGGPSVFIFPPKPKDVLMITRTPEVTCLVVDVSHDSSDVLFTWYVDGTEVK
TAKTMPNEEQNNSTYRVVSVLRIQHQDWLNGKKFKCKVNNQALPAPVERTISKATGQTRVPQVYVLA
PHPDELSKNKVSVTCLVKDFLPTDITVEWQSNEHPEPEGKYRTTEAQKDSDGSYFLYSKLTVETDRW
QQGTTFTCVVMHEALHNHVMQKNVSHSPGK

SEQ ID NO. 30

HORSE_IGHG4 constant region

VIKECNGGCPAECLQVGPSVFIFPPKPKDVLMISRTPTVTCVVVDVGHDFPDVQFNWYVDGVETHTA
TTEPKQEQFNSTYRVVSVLPIQHKDWLSGKEFKCKVNNKALPAPVERTISKPTGQPREPQVYVLAPH
RDELSKNKVSVTCLVKDFYPTDIDIEWKSNGQPEPETKYSTTPAQLDSDGSYFLYSKLTVETNRWQQ
GTTFTCAVMHEALHNHYTEKSVSKSPGK

SEQ ID NO. 31

HORSE_IGHG5 constant region

VVKGSPCPKCPAPELPGGPSVFIFPPKPKDVLKISRKPEVTCVVVDLGHDDPDVQFTWFVDGVETHT
ATTEPKEEQFNSTYRVVSVLPIQHQDWLSGKEFKCSVTNKALPAPVERTTSKAKGQLRVPQVYVLAP
HPDELAKNTVSVTCLVKDFYPPEIDVEWQSNEHPEPEGKYSTTPAQLNSDGSYFLYSKLSVETSRWK
QGESFTCGVMHEAVENHYTQKNVSHSPGK

SEQ ID NO. 32

>HORSE_IGHG6 constant region
KEPCCCPKCPGRPSVFIFPPNPKDTLMISRTPEVTCVVVDVSQENPDVKFNWYVDGVEAHTATTKAK
EKQDNSTYRVVSVLPIQHQDWRRGKEFKCKVNNRALPAPVERTITKAKGELQDPKVYILAPHREEVTK
NTVSVTCLVKDFYPPDINVEWQSNEEPEPEVKYSTTPAQLDGDGSYFLYSKLTVETDRWEQGESFTC
VVMHEAIRHTYRQKSITNFPGK

SEQ ID NO. 33
HORSE_IGHG7 constant region
VIKECGGCPTCPECLSVGPSVFIFPPKPKDVLMISRTPTVTCVVVDVGHDFPDVQFNWYVDGVETHTA
TTEPKQEQNNSTYRVVSILAIQHKDWLSGKEFKCKVNNQALPAPVQKTISKPTGQPREPQVYVLAPHR
DELSKNKVSVTCLVKDFYPTDIDIEWKSNGQPEPETKYSTTPAQLDSDGSYFLYSKLTVETNRWQQG
TTFTCAVMHEALHNHYTEKSVSKSPGK

SEQ ID NO. 34 portion of canine ECD as used in the fusion constructs (without stalk and without alpha and gamma secretase cleavage 3' of the stalk region)

KEACPTGLYTHSGECCKACNLGEGVAQPCGANQTVCEPCLDSVTFSDVVSATEPCKPCTE
CVGLQSMSAPCVEADDAVCRCAYGYYQDETTGRCEACRVCEAGSGLVFSCQDRQNTVCEE
CPDGTYSDEANHVDPCLPCTVCEDTERQLRECTRWADAECEEIPKEACPTGLYTHSGECCKACNLG
EGVAQPCGANQTVCEPCLDSVTFSDVVSATEPCKPCTECVGLQSMSAPCVEADDAVCRCAYGYYQ
DETTGRCEACRVCEAGSGLVFSCQDRQNTVCEECPDGTYSDEANHVDPCLPCTVCEDTERQLRECT
RWADAECEEIP

SEQ ID NO. 35 canine p75NTR ECD nucleic acid sequence as used in the fusion constructs (without stalk and without alpha and gamma secretase cleavage 3' of the stalk region)

AAGGAGGCATGTCCCACTGGCCTGTACACCCACAGCGGCGAGTGCTGCAAAGCCTGCAATCTGG
GTGAGGGGGTGGCCCAGCCTTGCGGAGCCAACCAGACCGTGTGTGAGCCCTGCCTGGACAGCG
TGACCTTCTCGGACGTGGTGAGCGCCACCGAGCCGTGCAAGCCGTGCACCGAGTGCGTGGGGC
TGCAGAGCATGTCGGCGCCGTGCGTGGAGGCGGACGACGCCGTGTGCCGCTGCGCCTACGGC
TACTACCAGGACGAGACGACGGGCCGCTGCGAGGCGTGCCGCGTGTGCGAGGCGGGCTCGGG
GCTCGTGTTCTCGTGCCAGGACAGGCAGAACACCGTGTGCGAGGAGTGTCCCGACGGCACGTA
CTCCGACGAGGCCAACCACGTGGACCCGTGCCTGCCCTGCACCGTGTGCGAGGACACCGAGCG
CCAGCTGCGCGAGTGCACGCGCTGGGCCGACGCCGAGTGCGAGGAGATCCCTGGCCGT

SEQ ID NO. 36 bovine p75 NTR protein
KEACLTGLYTHSGECCKACNLGEGVAQPCGANQTVCEPCLDSVTFSDVVSATEPCKPCTECVGLQS
MSAPCVEADDAVCRCAYGYYQDETTGRCEACRVCEAGSGLVFSCQDKQNTVCEECPDGTYSDEAN
HVDPCLPCTVCEDTERQLRECTRWADAECEEIPGRWITRATPPEGSDSTDPSTQEPEVPPEQDLVTS

TVSDVVTTVMGSSQPVVTRGTADNLIPVYCSILAAVVVGLVAYIAFKRWNSCKQNKQGANSRPVNQT
PPPEGEKLHSDSGISVDSQSLHDQQPHTQTAAGQALKGDGGLYSSLPLAKREEVEKLLNGSAGDTW
RHLAGELGYQPEHIDSFTHEACPARALLASWAAQDSATLDTLLAALRRIQRADLVESLCSESTATSPV

ECD is underlined

SEQ ID NO. 37 bovine p75 NTR nucleic acid

ATGGGGTCAGGTGCCGCCGGCCGCGCCATGGACGGGCCGCGCCTGCTGCTGCTGCTGCTGCT
GCTCCTGGGGGGTGTCCCTTGGAGGTGCCAAGGAAGCATGCCTCACGGGCCTGTACACCCACAG
CGGAGAGTGCTGCAAAGCCTGCAACCTGGGCGAGGGTGTGGCCCAGCCTTGTGGAGCCAACCA
GACCGTGTGTGAACCCTGCCTGGACAGCGTGACCTTCTCGGACGTGGTGAGCGCCACGGAGCC
GTGTAAGCCGTGCACGGAGTGCGTGGGACTGCAGAGCATGTCGGCGCCCTGCGTGGAGGCCGA
CGACGCCGTGTGCCGCTGCGCCTACGGCTATTACCAGGACGAGACGACCGGCCGCTGCGAGGC
GTGCCGCGTGTGCGAGGCGGGCTCGGGGCTCGTGTTCTCGTGCCAGGACAAGCAGAACACCGT
CTGCGAGGAGTGCCCCGACGGCACGTACTCCGACGAGGCCAACCACGTGGACCCCTGCCTGCC
CTGCACGGTGTGCGAGGACACGGAGCGCCAGCTGCGCGAGTGCACGCGCTGGGCCGACGCCG
AGTGCGAGGAGATCCCTGGACGTTGGATTACACGGGCCACGCCCCCTGAGGGCTCCGACAGCA
CAGACCCCAGCACCCAGGAGCCCGAGGTACCTCCAGAGCAAGATCTGGTAACCAGCACTGTGTC
AGATGTGGTGACCACGGTGATGGGCAGCTCCCAGCCTGTGGTGACCCGAGGTACCGCCGACAA
CCTCATCCCTGTCTATTGCTCCATCCTGGCTGCTGTGGTTGTGGGCCTTGTGGCCTACATCGCCT
TCAAGAGGTGGAACAGCTGCAAGCAGAACAAGCAAGGAGCCAACAGCCGACCTGTGAACCAGAC
ACCCCCACCAGAGGGGGAAAAGCTACACAGCGATAGCGGCATCTCTGTGGACAGCCAGAGCCT
GCATGACCAGCAGCCCCACACGCAGACTGCCGCAGGCCAGGCCCTCAAGGGTGATGGAGGCCT
CTACAGCAGCCTGCCGCTGGCCAAGCGGGAGGAGGTGGAGAAGCTGCTCAACGGCTCTGCGGG
GGACACCTGGCGGCATCTGGCAGGCGAGTTGGGTTACCAGCCTGAGCACATAGACTCCTTCACC
CACGAGGCCTGCCCAGCCCGCGCCCTGCTGGCCAGCTGGGCTGCCCAGGACAGCGCCACGCT
CGACACCCTCCTTGCGGCCCTGCGCCGCATCCAGCGCGCCGACATCGTGGAGAGCCTGTGCAG
CGAGTCCACGGCCACGTCCCCCGTGTGA

SEQ ID NO. 38 feline p75NTR protein ECD

KEACPTGLFTHSGECCKACNLGEGVAQPCGANQTVCEPCLDSVTFSDVVSATEPCKPCTE
CVGLQSMSAPCVEADDAVCRCAYGYYQDETTGRCEACRVCEAGSGLVFSCQDRQNTVCEE
CPDGTYSDEANHVDPCLPCTVCEDTERQLRECTRWADAECEEIPGRWITRSTPSEGSDST
APSTEEPEVPPEQDLIASTVADVVTTVMGSSQPVVTRGTADN

SEQ ID NO. 39 feline p75NTR ECD – feline IgG1 wt Fc protein fusion

MDGPRPLLLLLPLLLGVSLGGAKEACPTGLFTHSGECCKACNLGEGVAQPCGANQTVCEPCLDSVTF
SDVVSATEPCKPCTECVGLQSMSAPCVEADDAVCRCAYGYYQDETTGRCEACRVCEAGSGLVFSC
*QDRQNTVCEECPDGTYSDEANHVDPCLPCTVCEDTERQLRECTRWADAECEEIPGR***GGGG**VPKTA

STIESKTCDCPKCPVPEIPGAPSVFIFPPKPKDTLSISRTPEVTCLVVDLGPDDSNVQITWFVDNTEMHT
AKTRPREEQFNSTYRVVSVLPILHQDWLKGKEFKCKVNSKSLPSAMERTISKAKGQPHEPQVYVLPPT
QEELSENKVSVTCLIKGFHPPDIAVEWEITGQPEPENNYQTTPPQLDSDGTYFLYSRLSVDRSHWQR
GNTYTCSVSHEALHSHHTQKSLTQSPGK

Signal peptide

Feline p75-ECD

Linker GGGG

Feline Fc-IgG1

SEQ ID NO. 40 feline p75NTR ECD– feline IgG1 wt Fc nucleic acid sequence

ATGGATGGACCTAGACCTCTGCTGCTGCTCCTGCCTCTGCTGTTGGGAGTTTCTCTCGGCGGAG
CCAAAGAGGCTTGTCCTACCGGCCTGTTTACCCACTCTGGCGAGTGTTGCAAGGCCTGTAATCTC
GGCGAAGGCGTGGCACAACCTTGTGGCGCTAATCAGACAGTGTGCGAGCCTTGCCTGGACTCC
GTGACCTTCTCTGATGTGGTGTCTGCCACCGAGCCATGCAAGCCTTGTACCGAGTGTGTGGGCC
TGCAGTCCATGTCTGCCCCTTGTGTGGAAGCCGACGACGCCGTGTGTAGATGTGCCTACGGCTA
CTACCAGGACGAGACAACCGGAAGATGCGAGGCCTGCAGAGTGTGTGAAGCTGGCTCTGGACT
GGTGTTCTCCTGCCAAGACAGACAGAACACCGTGTGCGAGGAATGCCCTGACGGCACCTACTCT
GATGAGGCCAATCACGTGGACCCCTGCCTGCCTTGTACTGTGTGCGAAGATACCGAGCGGCAGC
TGCGCGAGTGTACCAGATGGGCTGATGCCGAGTGCGAAGAGATCCCTGGAAGAGGCGGAGGCG
GAGTGCCTAAGACCGCTTCTACCATCGAGTCCAAGACCTGCGACTGCCCTAAGTGCCCTGTGCC
TGAAATTCCTGGCGCTCCCTCCGTGTTCATCTTCCCACCTAAGCCTAAGGACACCCTGTCCATCT
CTCGGACCCCTGAAGTGACCTGCCTGGTGGTTGATCTGGGCCCTGACGACTCCAACGTGCAGAT
CACTTGGTTTGTGGACAACACCGAGATGCACACCGCCAAGACCAGACCTAGAGAGGAACAGTTC
AACTCCACCTACAGAGTGGTGTCCGTGCTGCCCATCCTGCACCAGGATTGGCTGAAGGGCAAAG
AATTCAAGTGCAAAGTGAACTCCAAGAGCCTGCCTTCCGCCATGGAACGGACCATCTCTAAGGCT
AAGGGCCAGCCTCATGAGCCCCAGGTGTACGTTCTGCCTCCTACACAAGAGGAACTGTCCGAGA
ACAAAGTGTCCGTGACATGCCTGATCAAGGGCTTTCACCCTCCTGATATCGCCGTGGAATGGGA
GATCACCGGACAGCCTGAGCCTGAGAACAACTACCAGACCACACCTCCTCAGCTGGACAGCGAC
GGAACCTACTTCCTGTACTCCCGGCTGTCCGTGGACAGATCCCATTGGCAGAGAGGCAACACCT
ACACCTGTTCCGTGTCTCACGAGGCCCTGCACTCTCATCACACCCAGAAGTCCCTGACACAGTCC
CCTGGCAAG

SEQ ID NO: 41 feline IgG3

LPPCKCPKCPVPEIPGGPSVFIFPPKPKDTLSISRTPEVTCLVVDLGPDDSNVQITWFVDNTEMHTAKT
RPREEQFNSTYRVVSVLPIVHQDWLTGKEFKCKVNSKALPSAIERTISKAKGQPHEPQVYVLPPAQEE
LSENKVCVTCLIKGFYPPDIAVEWEITGQPEPENNYRTTPPQLDSDGTYFVYSRLSMDRSRWQSGNT
YTCSVSHEALHSHHTQKSLTQSPGK

* * *

Having thus described in detail preferred embodiments of the present invention, it is to be understood that the invention defined by the above paragraphs is not to be limited to particular details set forth in the above description as many apparent variations thereof are possible without departing from the spirit or scope of the present invention.

CLAIMS:

1. An isolated companion animal p75NTR protein or a portion thereof.
2. The isolated companion animal p75NTR protein or a portion thereof according to claim 1 wherein the companion animal is a cat, dog, pig, cow or horse.
3. The isolated companion animal p75NTR protein or a portion thereof according to claim 2 wherein the companion animal is a dog; optionally wherein the isolated companion animal p75NTR protein or a portion thereof comprises SEQ ID NO. 1.
4. The isolated companion animal p75NTR protein or a portion thereof according to claim 2 wherein the companion animal is a cat; optionally wherein the isolated companion animal p75NTR protein or a portion thereof comprises SEQ ID NO. 3.
5. The isolated companion animal p75NTR protein or a portion thereof according to any one of claims 1 to 4 wherein the p75NTR protein or a portion thereof comprises the extracellular domain or part thereof; optionally wherein the companion animal is a dog and the p75NTR extracellular domain comprises SEQ ID NO. 7; or optionally wherein the companion animal is a cat and the p75NTR extracellular domain comprises SEQ ID NO. 38.
6. An isolated nucleic acid encoding a companion animal p75NTR protein or a portion thereof according to any one of claims 1 to 5; optionally wherein the companion animal is a dog and the nucleic acid comprises SEQ ID NO. 2; or optionally wherein the companion animal is a cat and the nucleic acid comprises SEQ ID NO. 4.
7. A vector comprising a nucleic acid according to claim 6.
8. A host cell comprising a nucleic acid according to claim 6 or a vector according to claim 7.
9. A fusion protein comprising an isolated companion animal p75NTR extracellular domain or portion thereof and a half-life extending moiety.
10. The fusion protein according to claim 9 wherein the half-life extending moiety is selected from an Fc domain, a serum albumin binder or PEG; optionally wherein the half-life extending moiety is a wild type or mutant Fc domain.
11. The fusion protein according to claim 9 or 10 wherein the half-life extending moiety is an Fc domain and the p75NTR extracellular domain or portion thereof and the Fc domain are linked with a linker; optionally wherein the linker is a peptide linker, further optionally wherein the peptide linker is $(G_4S)_n$ wherein n is 1 to 4.
12. The fusion protein according to any one of claims 9 to 11 wherein the companion animal is a cat, dog or horse.
13. The fusion protein according to claim 12 wherein the companion animal is a dog; optionally wherein the p75NTR extracellular domain comprises SEQ ID NO. 7 or a portion thereof such as SEQ ID NO. 34.

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14. The fusion protein according to any one of claims 9 to 13 wherein the Fc domain is a canine Fc domain; optionally wherein the fusion protein comprises SEQ ID NOs. 11 or 13.
15. The fusion protein according to claim 12 wherein the companion animal is a cat.
16. The fusion protein according to claim 26 wherein the p75NTR extracellular domain comprises SEQ ID No. 38; optionally wherein the fusion protein comprises SEQ ID NO. 39.
17. A nucleic acid encoding a fusion protein according to any one of claims 9 to 16.
18. A vector comprising a nucleic acid according to claim 17.
19. A host cell comprising a nucleic acid according to claim 17 or a vector according to claim 18.
20. A pharmaceutical composition comprising an isolated companion animal p75NTR protein according to any one of claims 1 to 5, or a fusion protein according to any one of claims 9 to 16.
21. Use of the isolated companion animal p75NTR protein according to any one of claims 1 to 5, the fusion protein according to any one of claims 9 to 16 or the pharmaceutical composition of claim 20 for treating an NGF-related disorder in a companion animal; optionally wherein the NGF-related disorder is cardiovascular diseases, atherosclerosis, obesity, type 2 diabetes, metabolic syndrome, pain and inflammation.
22. The use of claim 21 wherein the NGF-related disorder is a pain related disorder; optionally wherein pain is selected from osteoarthritis pain, rheumatoid arthritis pain, surgical and postsurgical pain, incisional pain, general inflammatory pain, cancer pain, pain from trauma, neuropathic pain, neuralgia, diabetic neuropathy pain, pain associated with rheumatic diseases, pain associated with musculoskeletal diseases, visceral pain, and gastrointestinal pain; and/or optionally further comprising use of a second compound that treats pain.
23. Use of the isolated companion animal p75NTR protein according to any one of claims 1 to 5, the fusion protein according to any one of claims 9 to 16 or the pharmaceutical composition of claim 20 to inhibiting NGF activity in a companion animal; optionally further comprising use of a second compound that treats pain.
24. A kit comprising the isolated companion animal p75NTR protein according to any one of claims 1 to 5, a fusion protein according to any one of claims 9 to 16 or a pharmaceutical composition of claim 20 and instructions for use.

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Figure 1a.

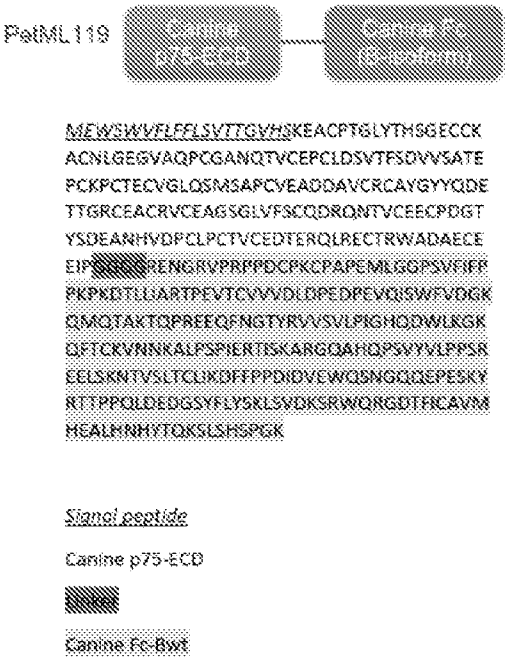


Figure 1b.

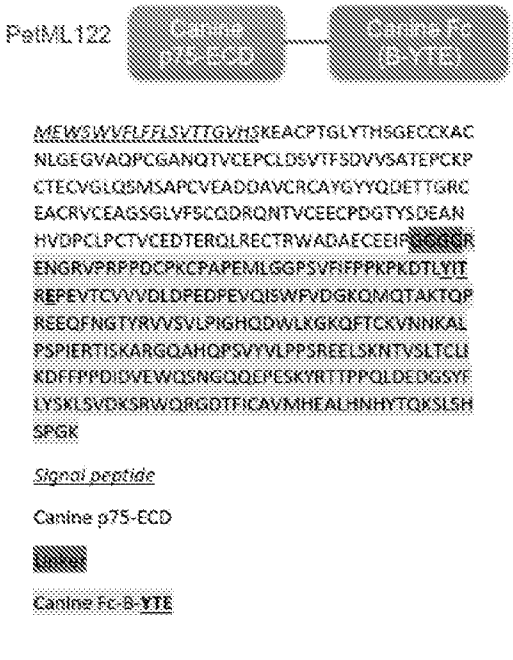


Figure 2

a.

```
Horse       KEVCPTDLYTHSGECCKACNLGEGVAQPCGANQTVCEPCLDSVTFSDVVSATEPCKPCTE    60
Bovine      KEACLTGLYTHSGECCKACNLGEGVAQPCGANQTVCEPCLDSVTFSDVVSATEPCKPCTE    60
Dog         KEACPTGLYTHSGECCKACNLGEGVAQPCGANQTVCEPCLDSVTFSDVVSATEPCKPCTE    60
Cat         KEACPTGLFTHSGECCKACNLGEGVAQPCGANQTVCEPCLDSVTFSDVVSATEPCKPCTE    60
            **.* *.*:************************************************

Horse       CVGLQSMSAPCVEADDAVCRCAYGYYQDETTGRCEACQVCEAGSGLVFSCQDKQNTVCEE    120
Bovine      CVGLQSMSAPCVEADDAVCRCAYGYYQDETTGRCEACRVCEAGSGLVFSCQDKQNTVCEE    120
Dog         CVGLQSMSAPCVEADDAVCRCAYGYYQDETTGRCEACRVCEAGSGLVFSCQDRQNTVCEE    120
Cat         CVGLQSMSAPCVEADDAVCRCAYGYYQDETTGRCEACRVCEAGSGLVFSCQDRQNTVCEE    120
            ************************************:**************:*******

Horse       CPDGTYSDEANHVDPCLPCTVCEDTERQLRECTRWADAECEEIP    164
Bovine      CPDGTYSDEANHVDPCLPCTVCEDTERQLRECTRWADAECEEIP    164
Dog         CPDGTYSDEANHVDPCLPCTVCEDTERQLRECTRWADAECEEIP    164
Cat         CPDGTYSDEANHVDPCLPCTVCEDTERQLRECTRWADAECEEIP    164
            *******************************************
```

b.

```
CAT_IGG1V1      ------TDHPPGPKPCDC--PKCPPPEMLGGPSIFIFPPKPKDTLSISRTPEVTCLVVDLG    53
CAT_IGG1V2      ------TDHPPGPKPCDC--PKCPPPEMLGGPSIFIFPPKPKDTLSISRTPEVTCLVVDLG    53
CAT_IGG2        -----KTASTIESKTGEG--PKCPVPEIPGAPSVFIFPPKPKDTLSISRTPEVTCLVVDLG    54
DOGA            --------FN--ECRCTD-TPPCPVPEPLGGPSVLIFPPKPKDILRITRTPEVTCVVLDLG    50
DOGD            ----------ESTCKC-ISPCPVPESLGGPSVFIFPPKPKDILRITRTPEVTCVVLDLG    48
DOGC            ---------ECECKCNCNNCPCPGCGLLGGPSVFIFPPKPKDILVTARTPTVTCVVVDLD    51
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DOGB            ------RENGRVPRPPDCPK--CPAPEMLGGPSVFIFPPKPKDTLLIARTPEVTCVVVDLD   53
DOGB-YTE        ------RENGRVPRPPDCPK--CPAPEMLGGPSVFIFPPKPKDTLYITREPEVTCVVVDLD   53
HORSE_IGHG2     CVLSAEGVIPIPSVPKPQCPPYTHSKFLGGPSVFIFPPNPKDALMISRTPVVTCVVVNLS   60
HORSE_IGHG6     ----------K---EPCCCPKCP-----GRPSVFIFPPNPKDTLMISRTPEVTCVVVDVS   42
HORSE_IGHG3     -------TTPP---CPCECPKCPAPELLGGPSVFIFPPKPKDVLMITRTPEVTCLVVDVS   50
HORSE_IGHG5     ---------VV---KGSPCPKCPAPELPGGPSVFIFPPKPKDVLKISRKPEVTCVVVDLG   48
HORSE_IGHG1     --------VIKE---CNGGCP--AE--CLQVGPSVFIFPPKPKDVLMISRTPTVTCVVVDVG   47
HORSE_IGHG4     --------VIKE---CNGGCP--AE--CLQVGPSVFIFPPKPKDVLMISRTPTVTCVVVDVG   47
HORSE_IGHG7     --------VIKE---CG--GCPTCPE--CLSVGPSVFIFPPKPKDVLMISRTPTVTCVVVDVG   48
                                            **::****:*** *  :* * ***:*:::.

CAT_IGG1V1      PDDSDVQITWFVDNTQVYTAKTSPREEQFNSTYRVVSVLPILHQDWLKGKEFKCKVNSKS   113
CAT_IGG1V2      PDDSDVQITWFVDNTQVYTAKTSPREEQFNSTYRVVSVLPILHQDWLKGKEFKCKVNSKS   113
CAT_IGG2        PDDSNVQITWFVDNTEMHTAKTRPREEQFNSTYRVVSVLPILHQDWLKGKEFKCKVNSKS   114
DOGA            REDPEVQISWFVDGKEVHTAKTQSREQQFNGTYRVVSVLPIEHQDWLTGKEFKCRVNHID   110
DOGD            REDPEVQISWFVDGKEVHTAKTQPREQQFNSTYRVVSVLPIEHQDWLTGKEFKCRVNHIG   108
DOGC            PENPEVQISWFVDSKQVQTANTQPREEQSNGTYRVVSVLPIGHQDWLSGKQFKCKVNNKA   111
DOGB            PEDPEVQISWFVDGKQMQTAKTQPREEQFNGTYRVVSVLPIGHQDWLKGKQFTCKVNNKA   113
DOGB-YTE        PEDPEVQISWFVDGKQMQTAKTQPREEQFNGTYRVVSVLPIGHQDWLKGKQFTCKVNNKA   113
HORSE_IGHG2     DQYPDVQFSWYVDNTEVHSAITKQREAQFNSTYRVVSVLPIQHQDWLSGKEFKCSVTNVG   120
HORSE_IGHG6     QENPDVKFNWYVDGVEAHTATTKAKEKQDNSTYRVVSVLPIQHQDWRRGKEFKCKVNNRA   102
HORSE_IGHG3     HDSSDVLFTWYVDGTEVKTAKTMPNEEQNNSTYRVVSVLRIQHQDWLNGKKFKCKVNNQA   110
HORSE_IGHG5     HDDPDVQFTWFVDGVETHTATTEPKEEQFNSTYRVVSVLPIQHQDWLSGKEFKCSVTNKA   108
HORSE_IGHG1     HDFPDVQFNWYVDGVETHTATTEPKQEQFNSTYRVVSVLPIQHKDWLSGKEFKCKVNNKA   107
HORSE_IGHG4     HDFPDVQFNWYVDGVETHTATTEPKQEQFNSTYRVVSVLPIQHKDWLSGKEFKCKVNNKA   107
HORSE_IGHG7     HDFPDVQFNWYVDGVETHTATTEPKQEQNNSTYRVVSILAIQHKDWLSGKEFKCKVNNQA   108
                 :  :* :.*:**. :  :* *  .: * *.******:* * *:**  **:*.* *.

CAT_IGG1V1      LPSPIERTISKAKGQPHEPQVYVLPPAQEELS-RNKVSVTCLIKSFHPPDIAVEWEITGQ   172
CAT_IGG1V2      LPSPIERTISKAKGQPHEPQVYVLPPAQEELS-RNKVSVTCLIKSFHPPDIAVEWEITGQ   172
CAT_IGG2        LPSAMERTISKAKGQPHEPQVYVLPPTQEELS-ENKVSVTCLIKGFHPPDIAVEWEITGQ   173
DOGA            LPSPIERTISKARGRAHKPSVYVLPPSPKELSSSDTVSITCLIKDFYPPDIDVEWQSNGQ   170
DOGD            LPSPIERTISKARGQAHQPGVYVLPPSPKELSSSDTVTLTCLIKDFFPPEIDVEWQSNGQ   168
DOGC            LPSPIEEIISKTPGQAHQPNVYVLPPSRDEMS-KNTVTLTCLVKDFFPPEIDVEWQSNGQ   170
DOGB            LPSPIERTISKARGQAHQPSVYVLPPSREELS-KNTVSLTCLIKDFFPPDIDVEWQSNGQ   172
DOGB-YTE        LPSPIERTISKARGQAHQPSVYVLPPSREELS-KNTVSLTCLIKDFFPPDIDVEWQSNGQ   172
HORSE_IGHG2     VPQPISRAISRGKGPSRVPQVYVLPPHPDELA-KSKVSVTCLVKDFYPPDISVEWQSNRW   179
HORSE_IGHG6     LPAPVERTITKAKGELQDPKVYILAPHREEVT-KNTVSVTCLVKDFYPPDINVEWQSNEE   161
HORSE_IGHG3     LPAPVERTISKATGQTRVPQVYVLAPHPDELS-KNKVSVTCLVKDFLPTDITVEWQSNEH   169
HORSE_IGHG5     LPAPVERTTSKAKGQLRVPQVYVLAPHPDELA-KNTVSVTCLVKDFYPPEIDVEWQSNEH   167
HORSE_IGHG1     LPAPVERTISKPTGQPREPQVYVLAPHRDELS-KNKVSVTCLVKDFYPTDIDIEWKSNGQ   166
HORSE_IGHG4     LPAPVERTISKPTGQPREPQVYVLAPHRDELS-KNKVSVTCLVKDFYPTDIDIEWKSNGQ   166
HORSE_IGHG7     LPAPVQKTISKPTGQPREPQVYVLAPHRDELS-KNKVSVTCLVKDFYPTDIDIEWKSNGQ   167
                :*  :..  ::  *  : * **:* *  .*::  ..*::***:*.* * :* :**: .

CAT_IGG1V1      PEPENNYRTTPPQLDSDGTYFVYSKLSVDRSHWQRGNTYTCSVSHEALHSHHTQKSLTQS   232
CAT_IGG1V2      PEPENNYRTTPPQLDSDGTYFVYSKLSVDRSHWQRGNTYTCSVSHEALHSHHTQKSLTQS   232
CAT_IGG2        PEPENNYQTTPPQLDSDGTYFLYSRLSVDRSHWQRGNTYTCSVSHEALHSHHTQKSLTQS   233
DOGA            QEPERKHRMTPPQLDEDGSYFLYSKLSVDKSRWQQGDPFTCAVMHETLQNHYTDLSLSHS   230
DOGD            PEPESKYHTTAPQLDEDGSYFLYSKLSVDKSRWQQGDPFTCAVMHEALQNHYTDLSLSHS   228
DOGC            QEPESKYRMTPPQLDEDGSYFLYSKLSVDKSRWQRGDTFICAVMHEALHNHYTQKSLSHS   230
DOGB            QEPESKYRTTPPQLDEDGSYFLYSKLSVDKSRWQRGDTFICAVMHEALHNHYTQKSLSHS   232
DOGB-YTE        QEPESKYRTTPPQLDEDGSYFLYSKLSVDKSRWQRGDTFICAVMHEALHNHYTQKSLSHS   232
HORSE_IGHG2     PELEGKYSTTPAQLDGDGSYFLYSKLSLETSRWQQVESFTCAVMHEALHNHFTKTDISES   239
HORSE_IGHG6     PEPEVKYSTTPAQLDGDGSYFLYSKLTVETDRWEQGESFTCVVMHEAIRHTYRQKSITNF   221
HORSE_IGHG3     PEPEGKYRTTEAQKDSDGSYFLYSKLTVETDRWQQGTTFTCVVMHEALHNHVMQKNVSHS   229
HORSE_IGHG5     PEPEGKYSTTPAQLNSDGSYFLYSKLSVETSRWKQGESFTCGVMHEAVENHYTQKNVSHS   227
HORSE_IGHG1     PEPETKYSTTPAQLDSDGSYFLYSKLTVETNRWQQGTTFTCAVMHEALHNHYTEKSVSKS   226
HORSE_IGHG4     PEPETKYSTTPAQLDSDGSYFLYSKLTVETNRWQQGTTFTCAVMHEALHNHYTEKSVSKS   226
HORSE_IGHG7     PEPETKYSTTPAQLDSDGSYFLYSKLTVETNRWQQGTTFTCAVMHEALHNHYTEKSVSKS   227
                 * * ::  *  * : **:**:**:*::: .:*::   : * * **::.    . .::.

CAT_IGG1V1      PGK         235
CAT_IGG1V2      PGK         235
CAT_IGG2        PGK         236
DOGA            PGK         233
DOGD            PGK         231
DOGC            PGK         233
DOGB            PGK         235
DOGB-YTE        PGK         235
HORSE_IGHG2     LGK         242
HORSE_IGHG6     PGK         224
HORSE_IGHG3     PGK         232
HORSE_IGHG5     PGK         230
```

```
HORSE_IGHG1       PGK         229
HORSE_IGHG4       PGK         229
HORSE_IGHG7       PGK         230
                   **
```

Figure 3a.

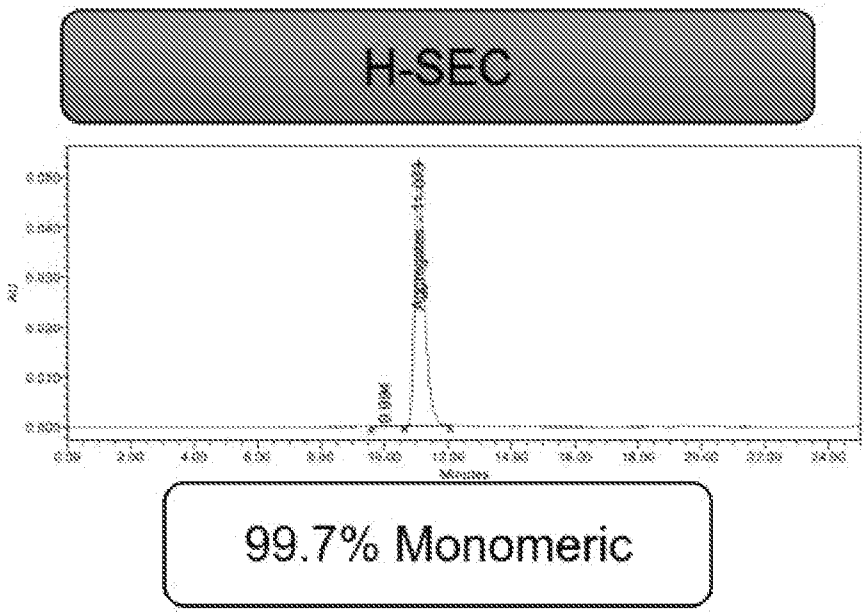


Figure 3b.

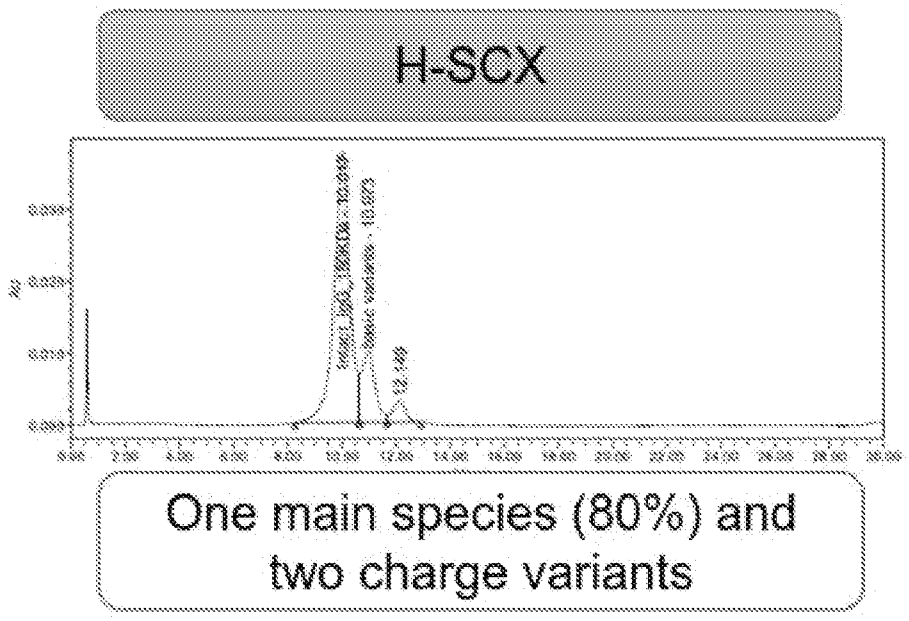


Figure 4a.

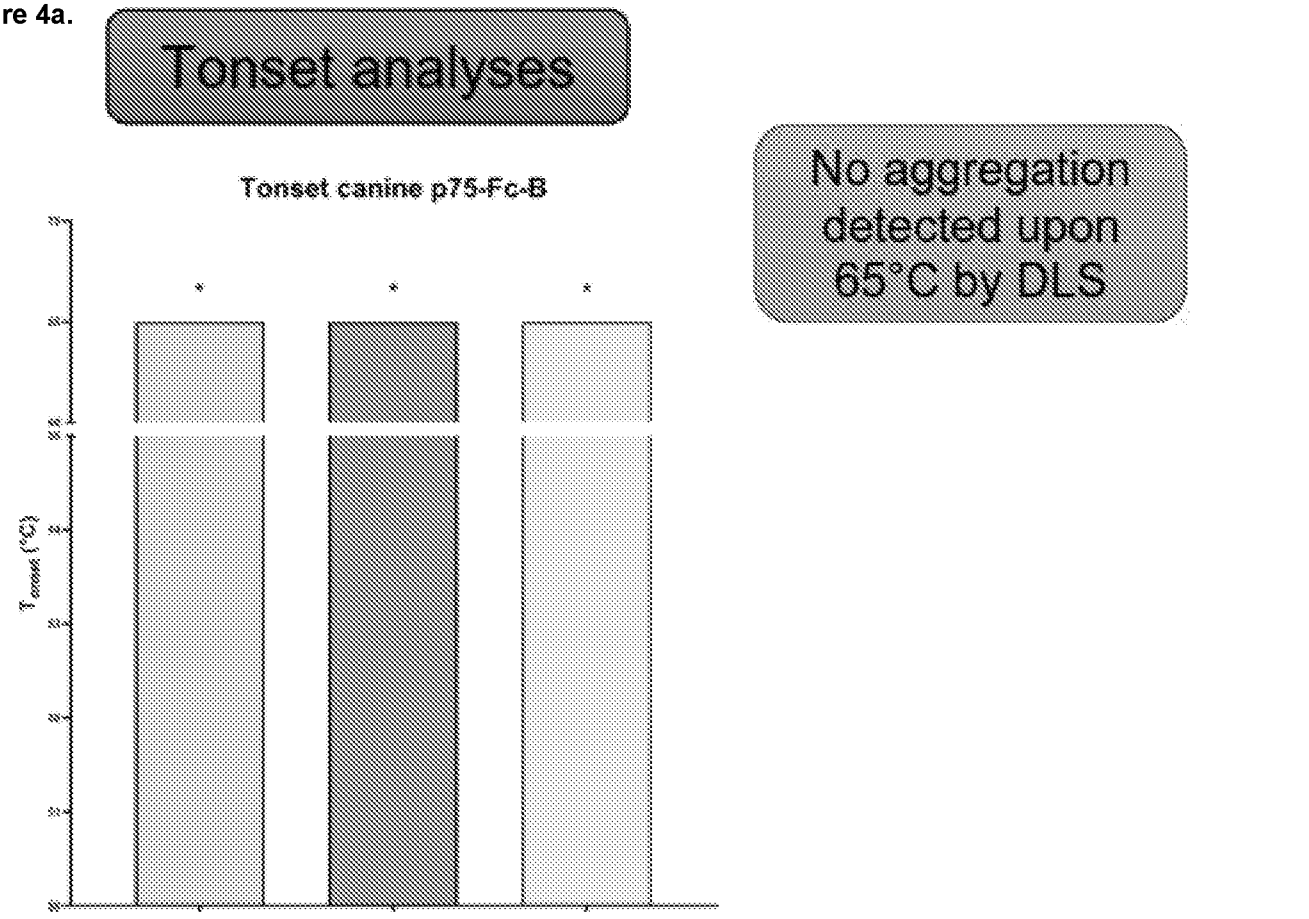


Figure 4b.

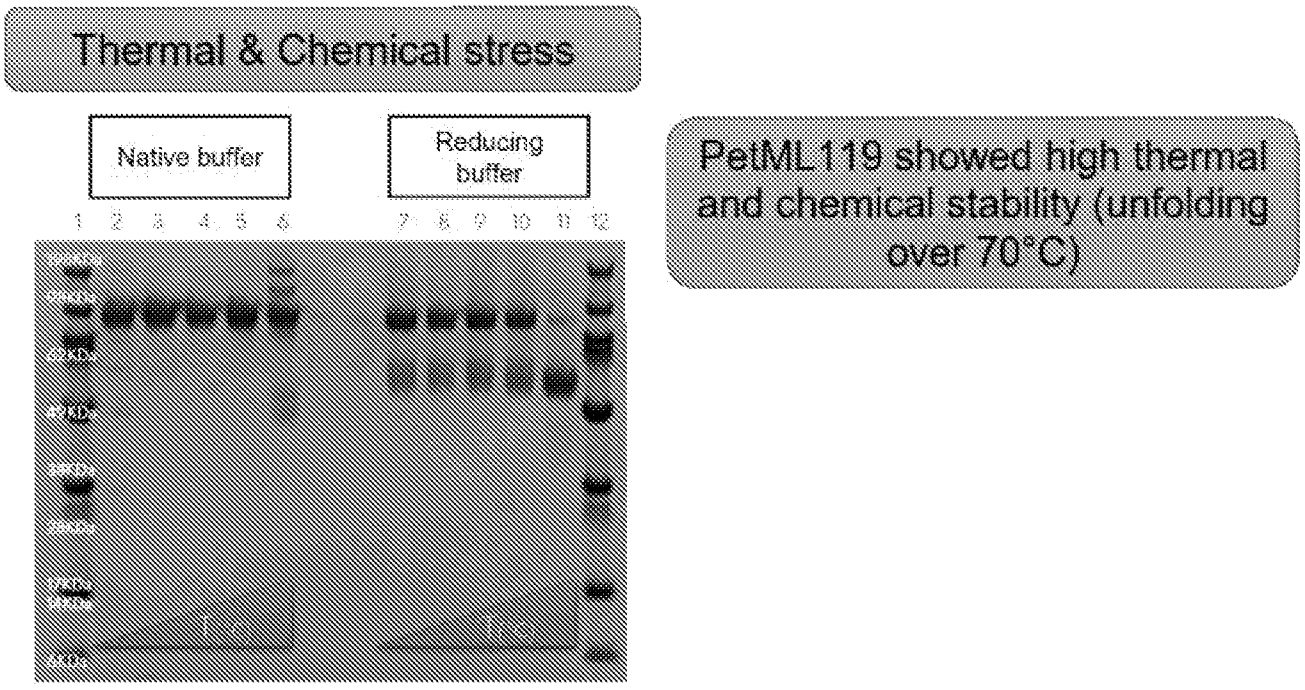


Figure 4c.

Freeze & Thaw stress

Sample	% Purity	Area	Δconc after FT
PetML119	99.39	333981	0
PetML119 1FT	99.39	322675	-3.38522
PetML119 2FT	99.42	311886	-6.61565

PetML119 showed good stability after 2 FT, showing no changes in oligomerisation with only minor protein loss (~5%)

Figure 4d.

#	Sample name	Sample MW	Sample conc	Buffer type	pH	T_{m1} (°C)	%CV	T_{m2} (°C)	%CV
1	Protein_1*	92 kDa	3 mg/ml	PBS	7.4	**67.4**	0.24	**77.4**	0.25
2	Protein_1*	92 kDa	1.5 mg/ml	PBS	7.4	**67.1**	0.31	**77**	0.71
3	Protein_1*	92 kDa	0.75 mg/ml	PBS	7.4	**66.9**	0.06	**76.6**	0.22

Figure 4e.

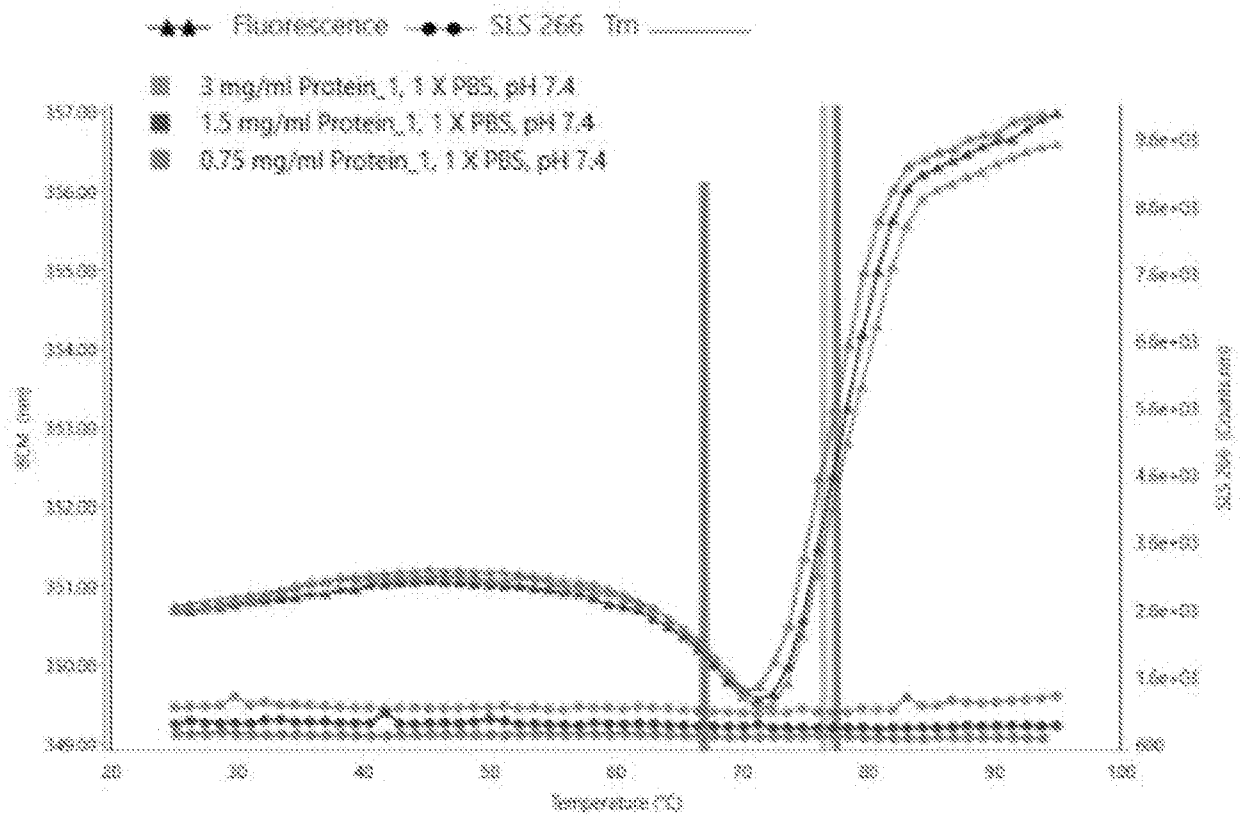

Fluorescence
SLS 266
Tm
3 mg/ml Protein_1, 1 X PBS, pH 7.4
1.5 mg/ml Protein_1, 1 X PBS, pH 7.4
0.75 mg/ml Protein_1, 1 X PBS, pH 7.4
Temperature (°C)

Figure 5a.

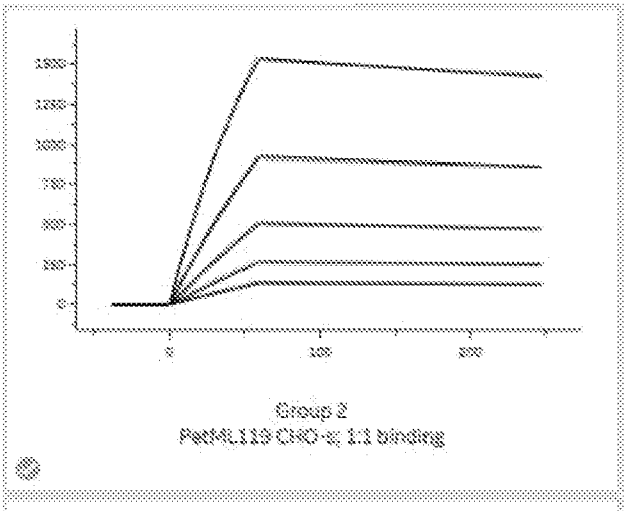


Figure 5b.

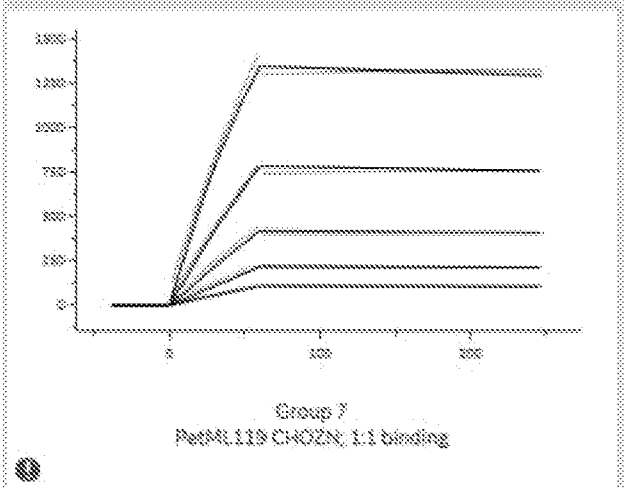


Figure 5c.

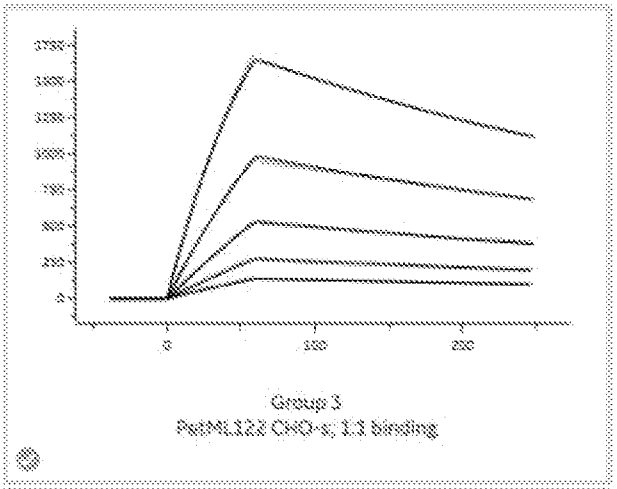


Figure 5d.

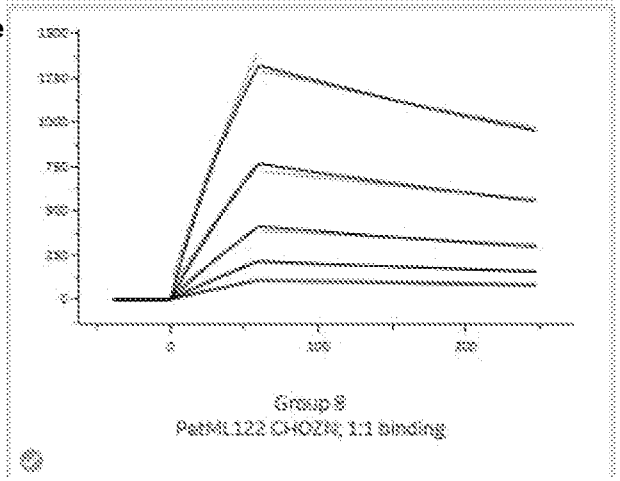


Figure 5e.

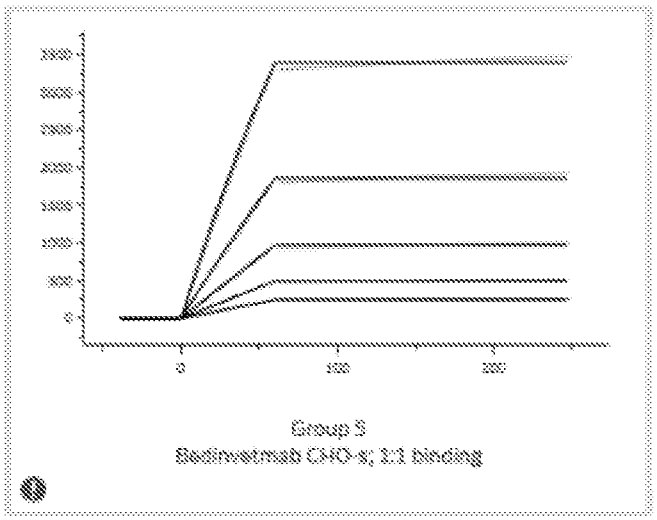


Figure 5f.

Sample	Kinetics affinity KD (nM)
Bedinvetmab : ProtA	0.01
PetML119 (CHO-s) : ProtA	2.8
PetML119 (CHOZN) : ProtA	1.3
PetML122 (CHO-s) : ProtA	10.6
PetML122 (CHOZN) : ProtA	15

Figure 6a.

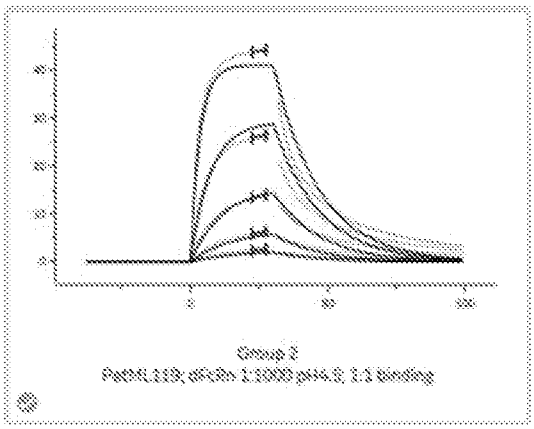


Figure 6b.

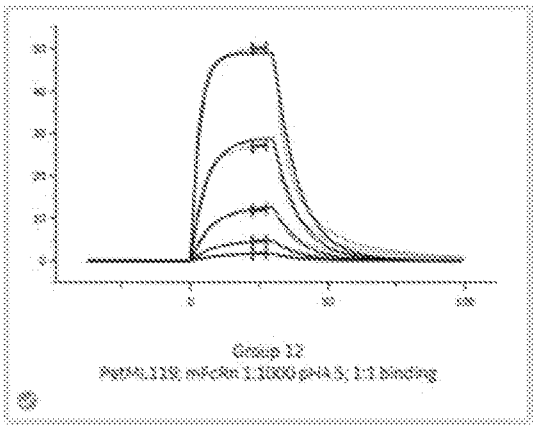


Figure 6c.

Sample	Steady state affinity KD (µM)
PetML119 : dog FcRn	6
PetML119 : mouse FcRn	2

Figure 6d.

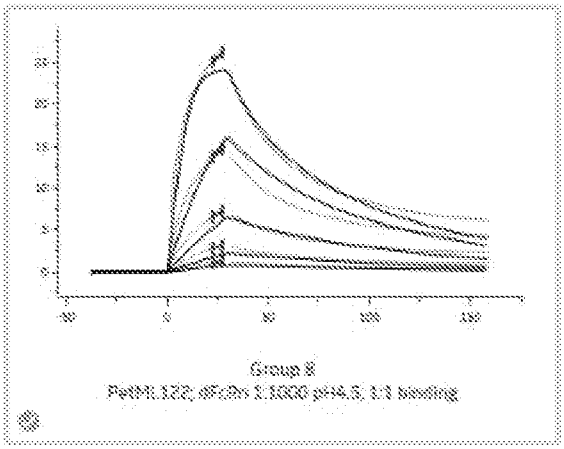


Figure 6e.

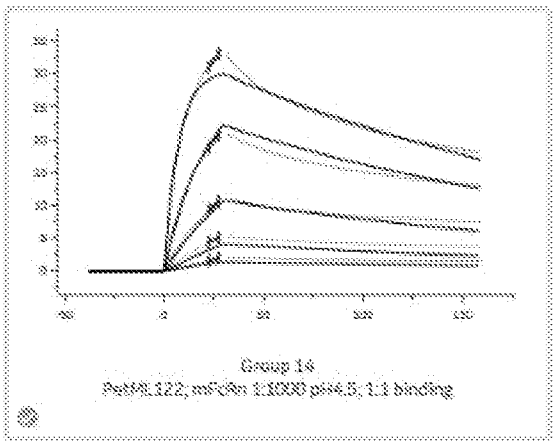


Figure 6f.

Sample	Steady state affinity KD (µM)
PetML122 : dog FcRn	0.4
PetML122 : mouse FcRn	0.09

Figure 7a.

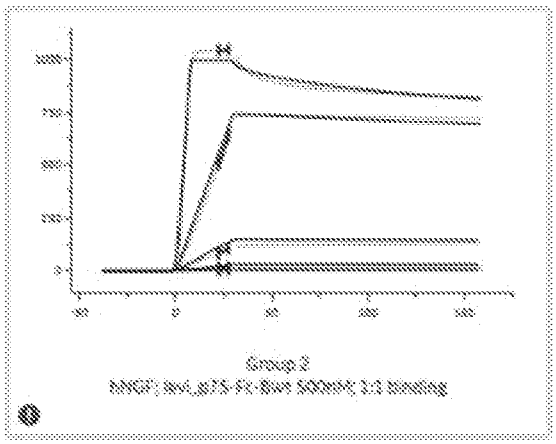


Figure 7b.

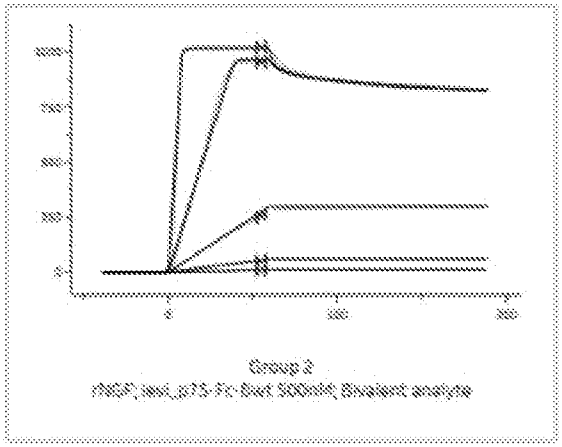


Figure 7c.

Sample	Kinetics affinity KD (nM)
PetML119 : human NGF	0.12
PetML119 : rat NGF	0.12

Figure 7d.

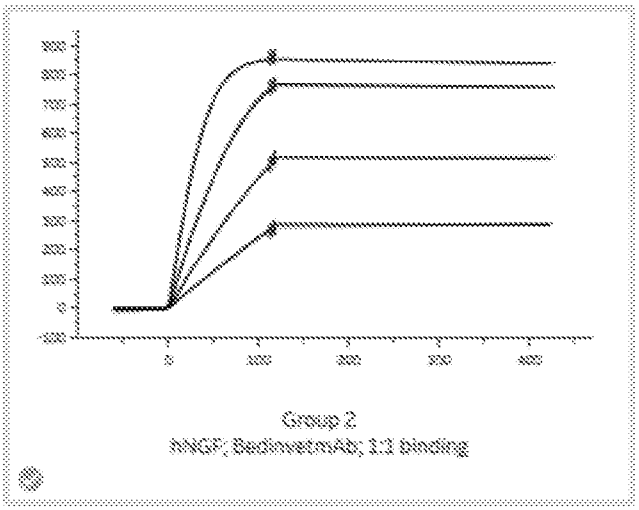


Figure 7e.

Sample	Kinetics affinity KD (nM)
Bedinvetmab : human NGF	0.073

Figure 8a.

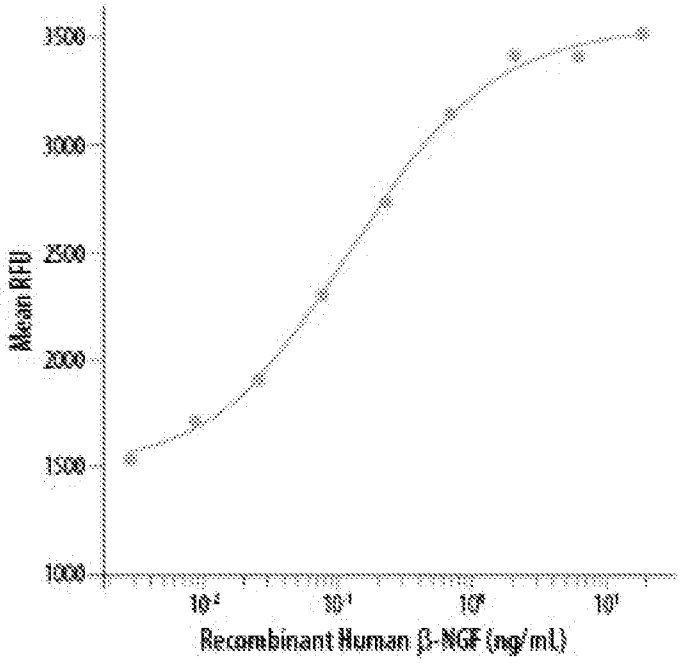


Figure 8b.

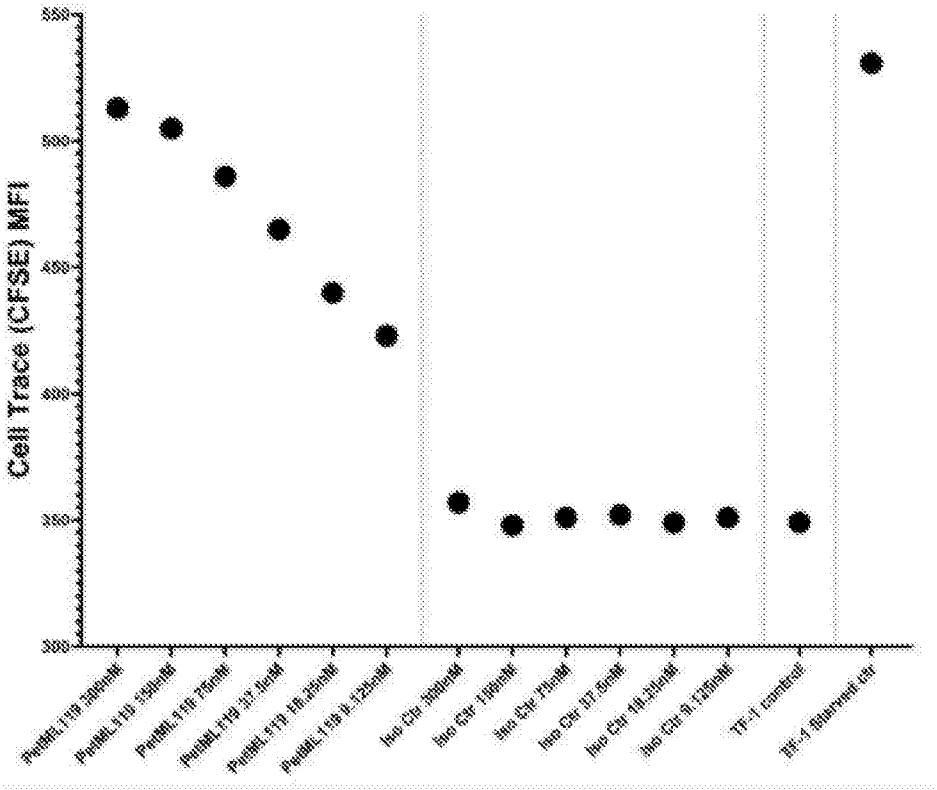


Figure 8c.

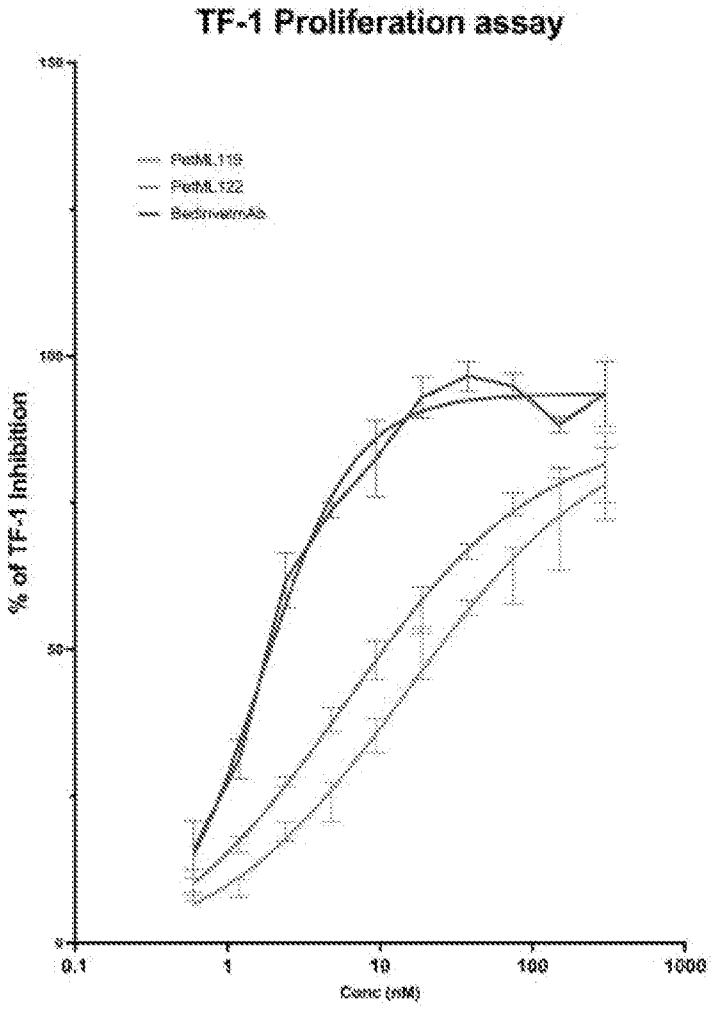

TF-1 Proliferation assay
PetM.119
PetM.122
Bedinvetmab
% of TF-1 Inhibition
0
50
100
0.1
1
10
100
1000
Conc (nM)

Figure 9.

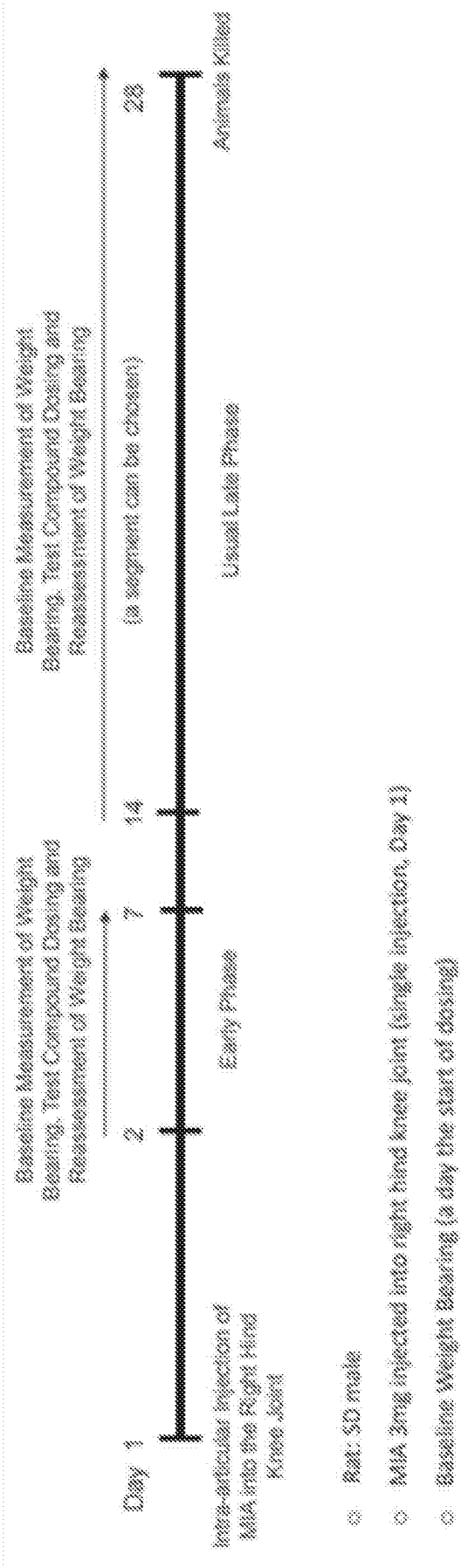

Day 1
2
7
14
28
Intra-articular Injection of MIA into the Right Hind Knee Joint
Baseline Measurement of Weight, Bearing, Test Compound Dosing and Reinforcement of Weight Bearing
Baseline Measurement of Weight, Bearing, Test Compound Dosing and Reinforcement of Weight Bearing (a segment can be chosen)
Early Phase
Usual Late Phase
Animals Killed
Rat: SD male
MIA 2mg injected into right hind knee joint (single injection, Day 1)
Baseline Weight Bearing (a day the start of dosing)

Figure 10a.

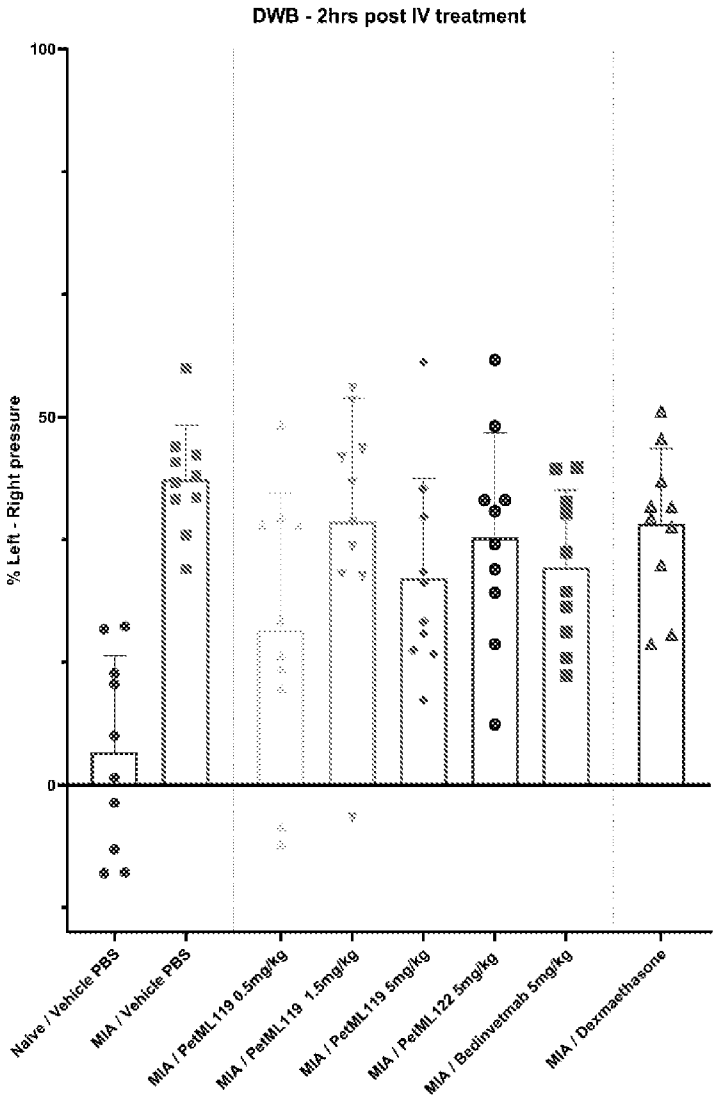


Figure 10b.

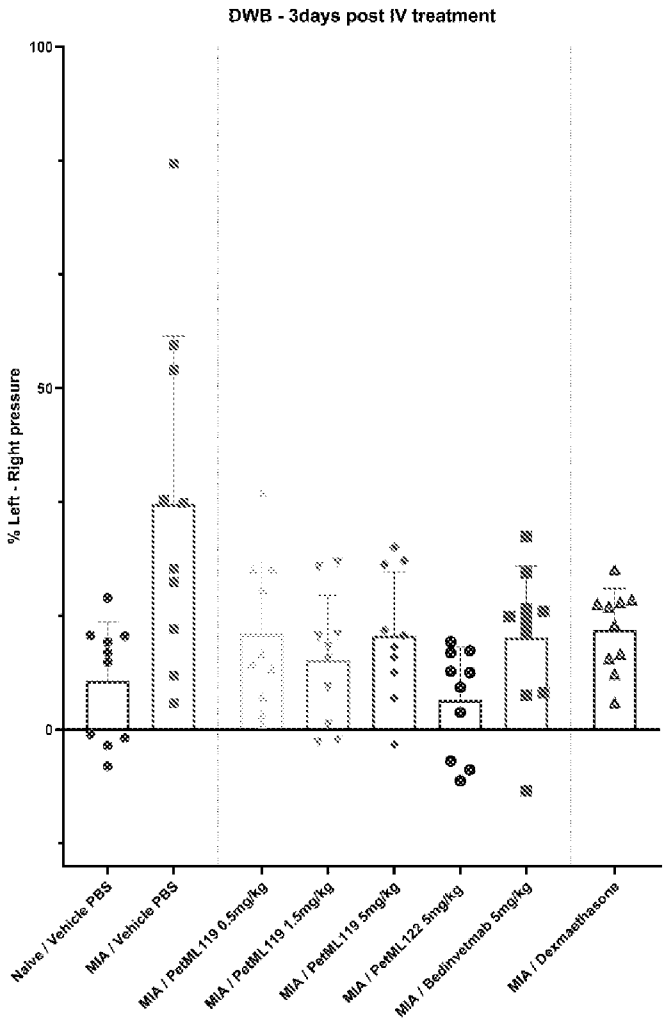


Figure 10c.

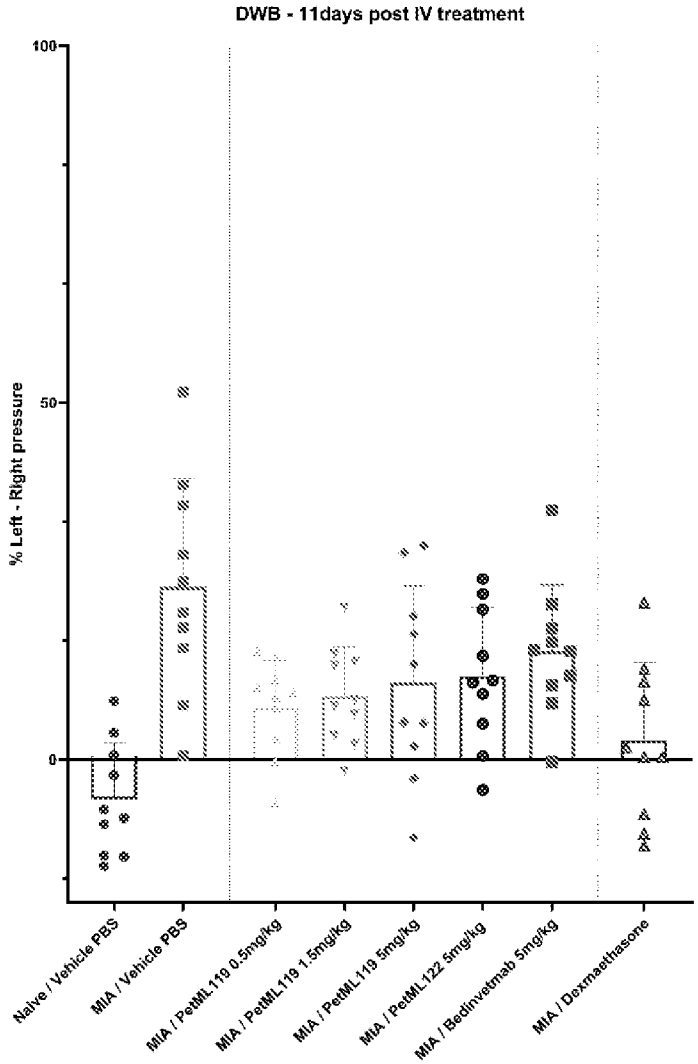


Figure 10d.

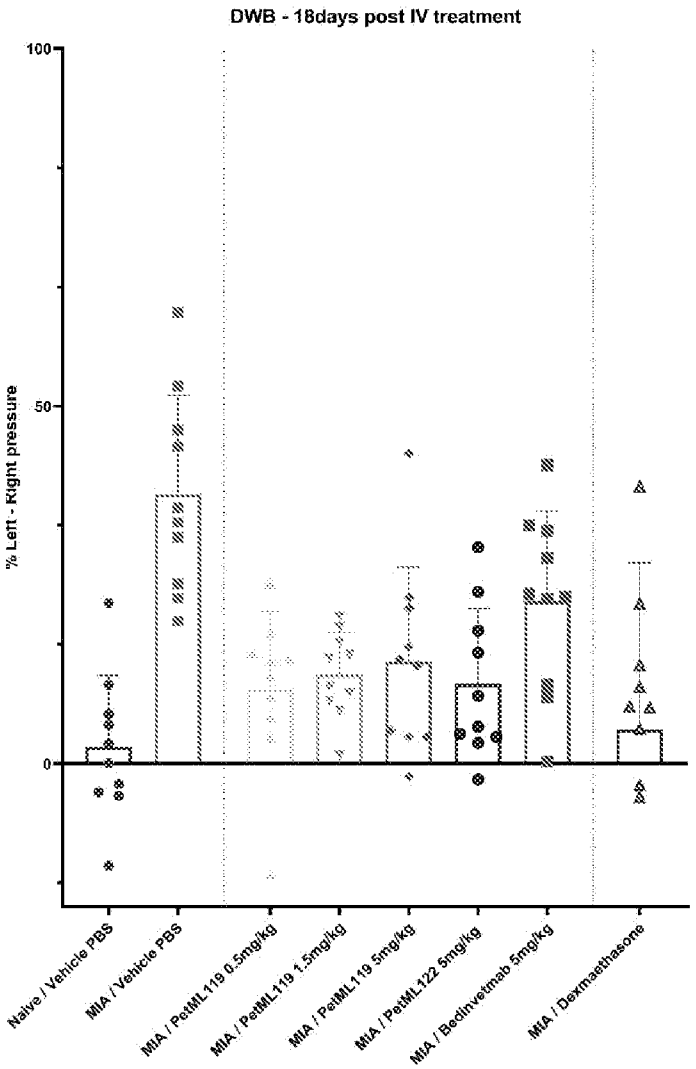


Figure 11a.

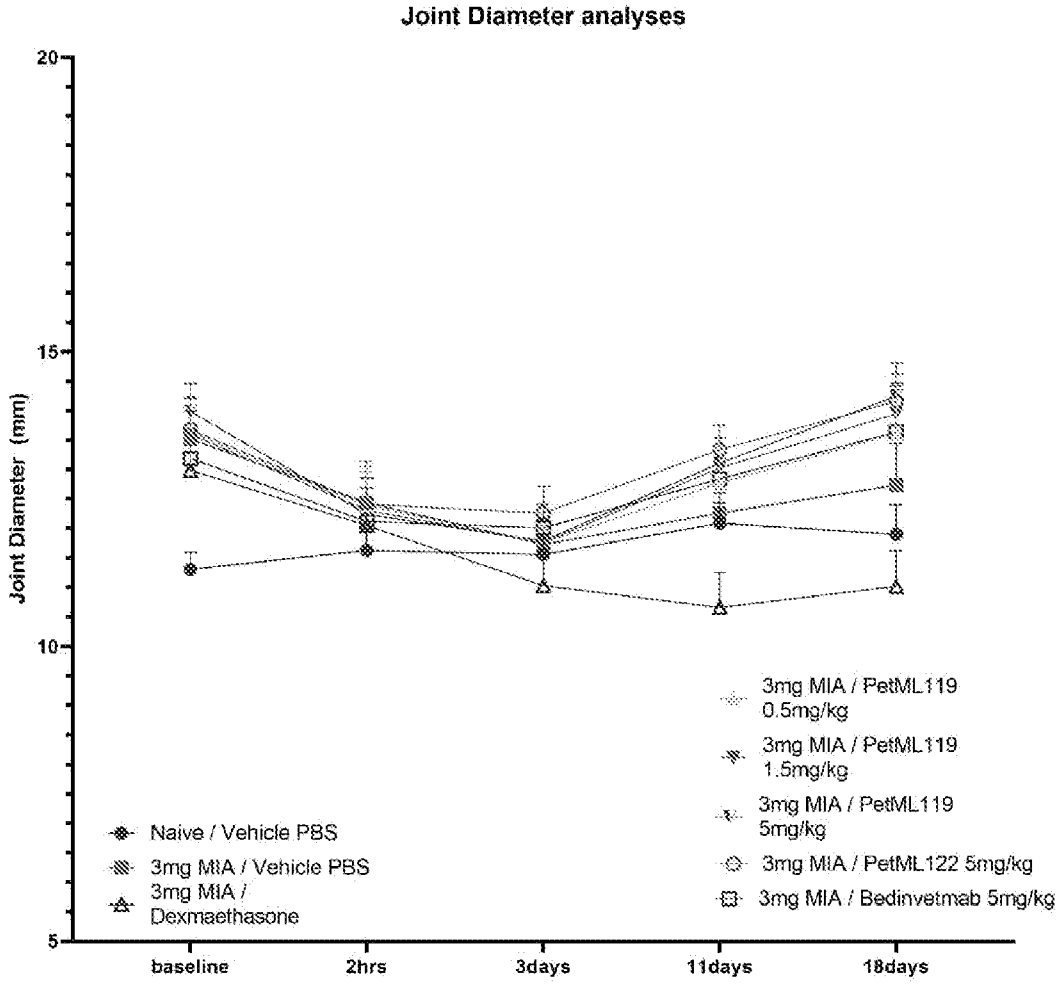

Joint Diameter analyses
Joint Diameter (mm)
20
15
10
5
baseline
2hrs
3days
11days
18days
Naive / Vehicle PBS
3mg MIA / Vehicle PBS
3mg MIA / Dexmaethasone
3mg MIA / PetML119 0.5mg/kg
3mg MIA / PetML119 1.5mg/kg
3mg MIA / PetML119 5mg/kg
3mg MIA / PetML122 5mg/kg
3mg MIA / Bedinvetmab 5mg/kg

Figure 11b.

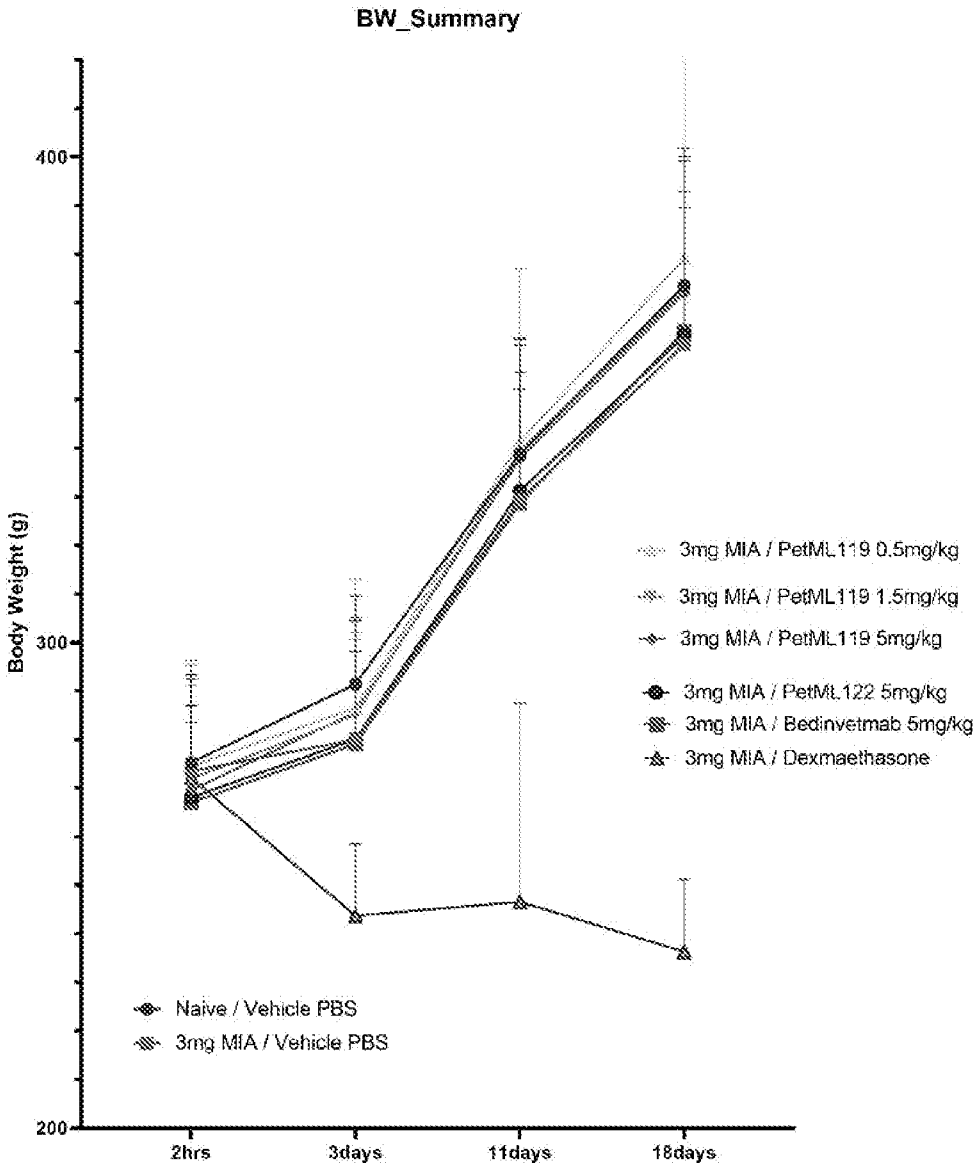

BW_Summary
Body Weight (g)
400
300
200
2hrs
3days
11days
18days
3mg MIA / PetML119 0.5mg/kg
3mg MIA / PetML119 1.5mg/kg
3mg MIA / PetML119 5mg/kg
3mg MIA / PetML122 5mg/kg
3mg MIA / Bedinvetmab 5mg/kg
3mg MIA / Dexmaethasone
Naive / Vehicle PBS
3mg MIA / Vehicle PBS

Figure 12a.

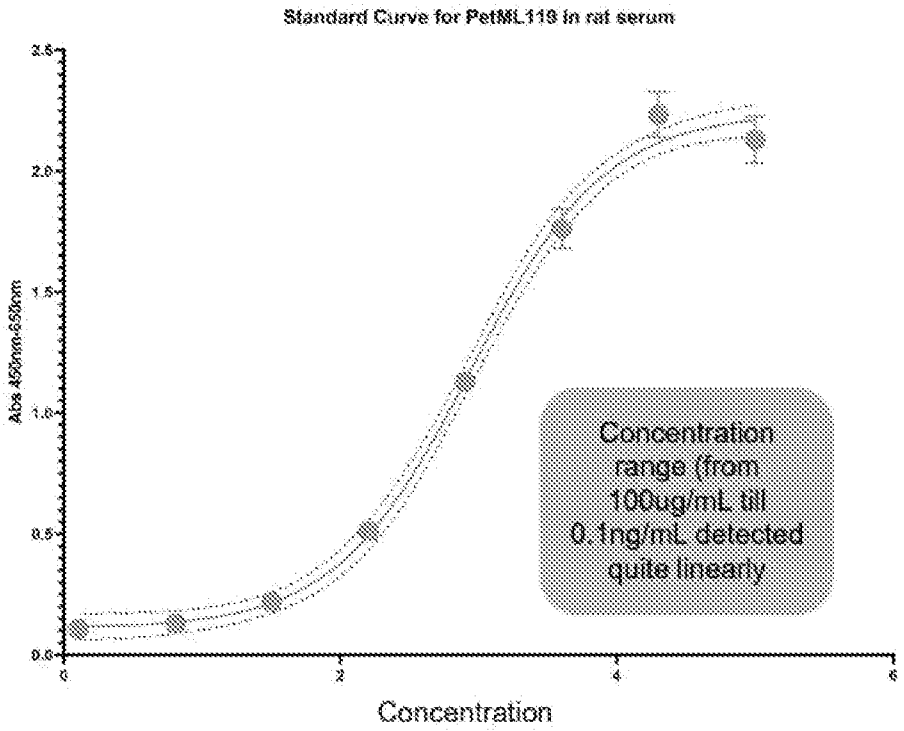


Figure 12b.

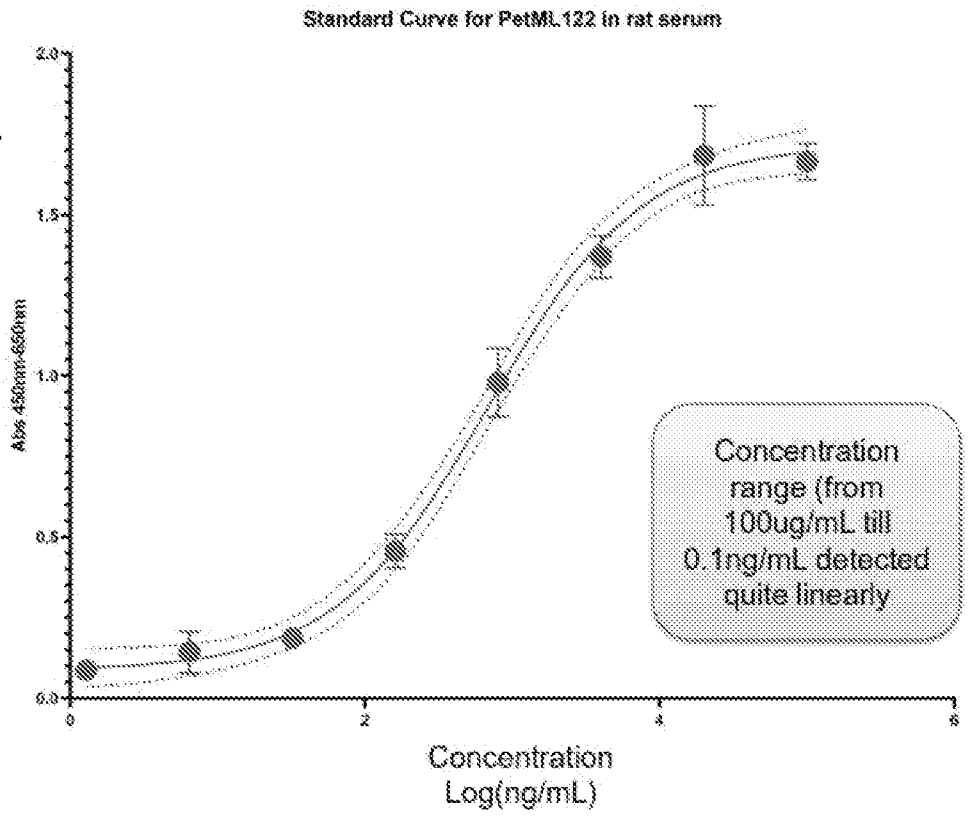


Figure 12c.

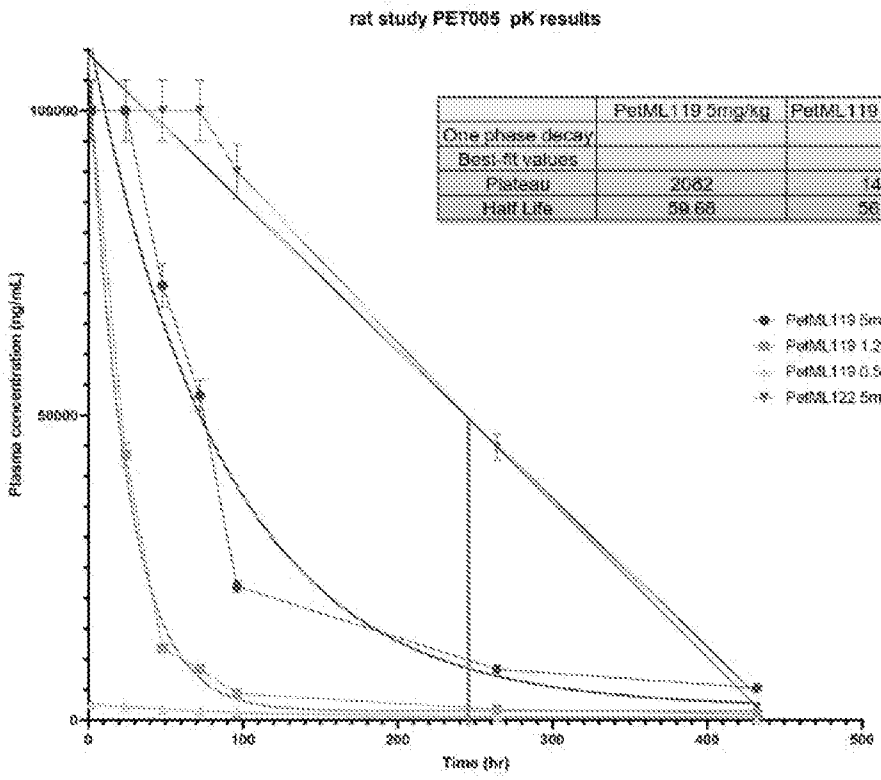
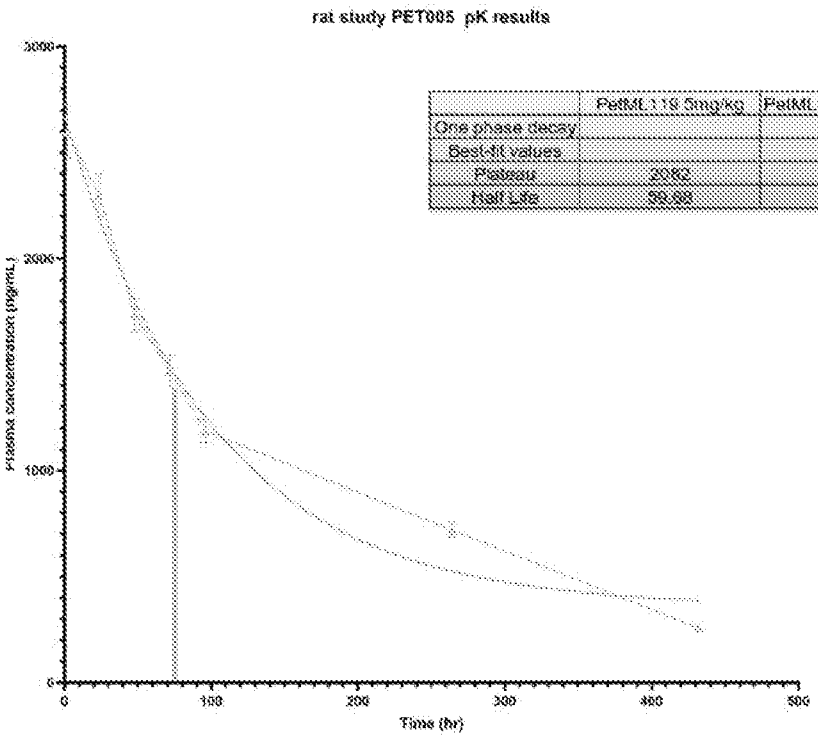


Figure 12d.

	PetML119 5mg/kg	PetML119 1.25mg/kg	PetML119 0.5mg/kg	PetML122 5mg/kg
One phase decay				
Best-fit values				
Plateau	2062	1471	359.7	Unstable
Half Life	59.68	56.66	70.31	418874

Figure 12e.



	PetML119 5mg/kg	PetML119 1.25mg/kg	PetML119 0.5mg/kg	PetML122 5mg/kg
One phase decay				
Best-fit values				
Plateau	2082	1471	356.7	Unstable
Half Life	59.69	56.66	70.01	418974

Figure 12f.

Figure 13a

Animal ID working number	order number	Bodyweight (kg) 14 jan 2022	17 jan 2022	28 jan 2022	31 jan 2022	11 feb 2022	14 feb 2022	11 mar 2022
469045	1	-	10.657	11.066	10.731	10.918	10.824	10.909
008438	2	-	11.044	11.423	10.950	11.261	11.069	10.647
008373	3	-	10.498	10.748	10.379	10.485	10.471	10.605
008423	4	-	10.660	11.015	10.597	10.692	10.832	10.834
430040	5	-	10.111	10.659	9.985	10.029	10.118	9.882
430031	6	11.178	10.851	-	10.760	10.740	10.781	10.685
430022	7	10.361	10.099	-	10.080	10.337	10.180	10.163
839431	8	11.035	10.914	-	10.708	10.584	10.558	10.703
840259	9	10.745	10.767	-	10.797	10.634	10.732	10.746
839425	10	10.324	9.947	-	10.121	10.136	10.219	10.169
Total	**Mean**	NA	10.555	NA	10.511	10.582	10.578	10.534
	SD	NA	0.380	NA	0.344	0.363	0.323	0.340
Group 1	**Mean**	NA	10.594	10.982	10.528	10.677	10.663	10.575
	SD	NA	0.337	0.301	0.368	0.463	0.372	0.408
Group 2	**Mean**	10.729	10.516	NA	10.493	10.486	10.494	10.493
	SD	0.386	0.456	NA	0.360	0.345	0.282	0.300

Figure 13b

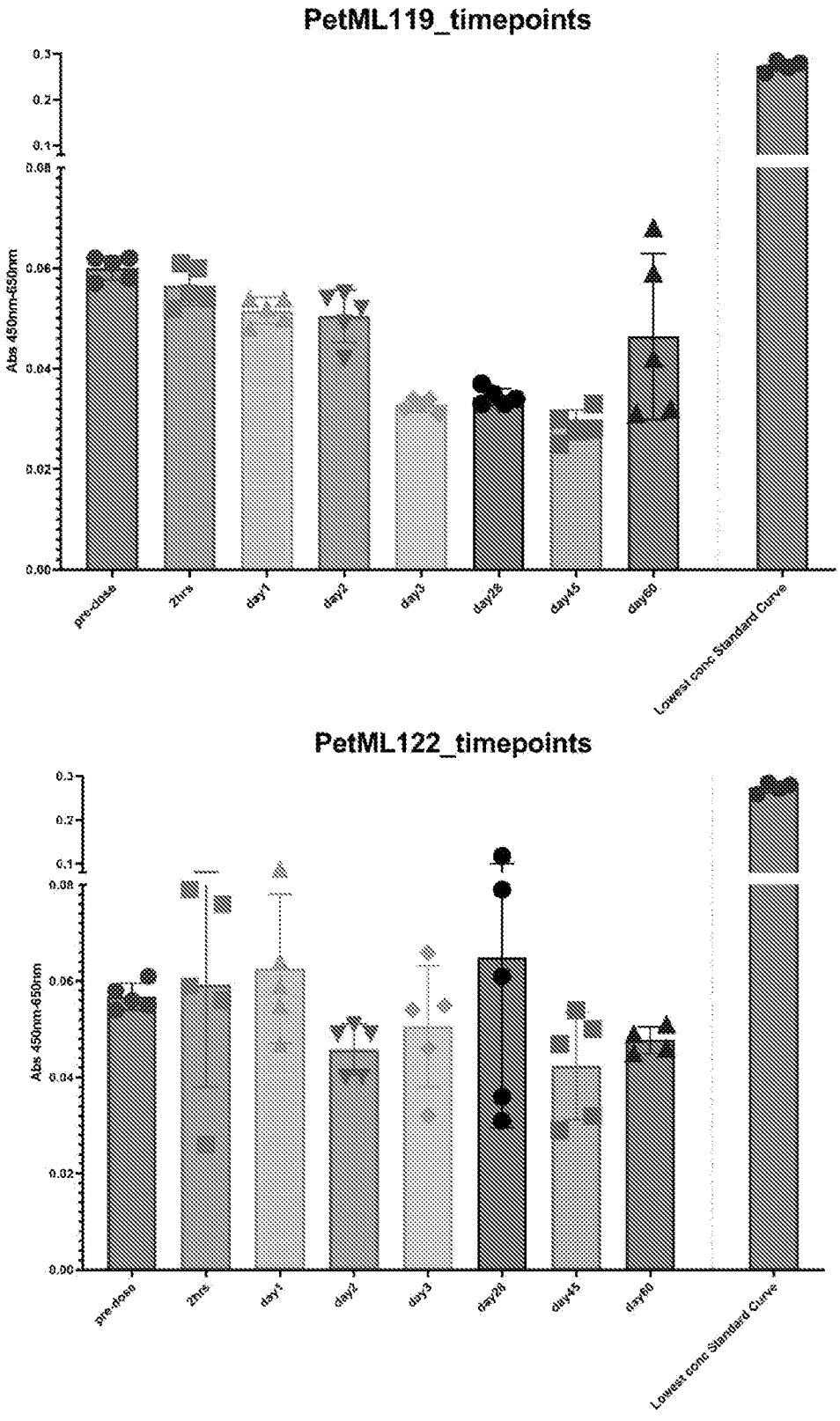
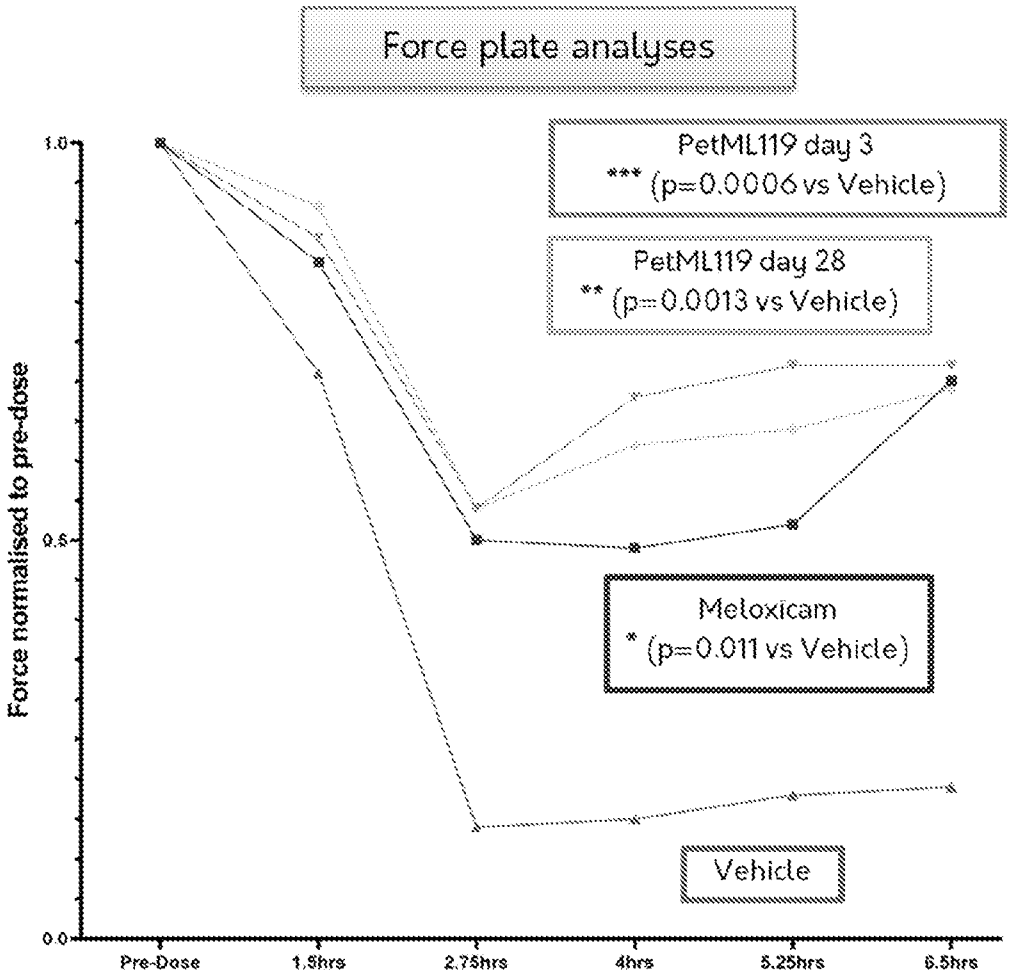
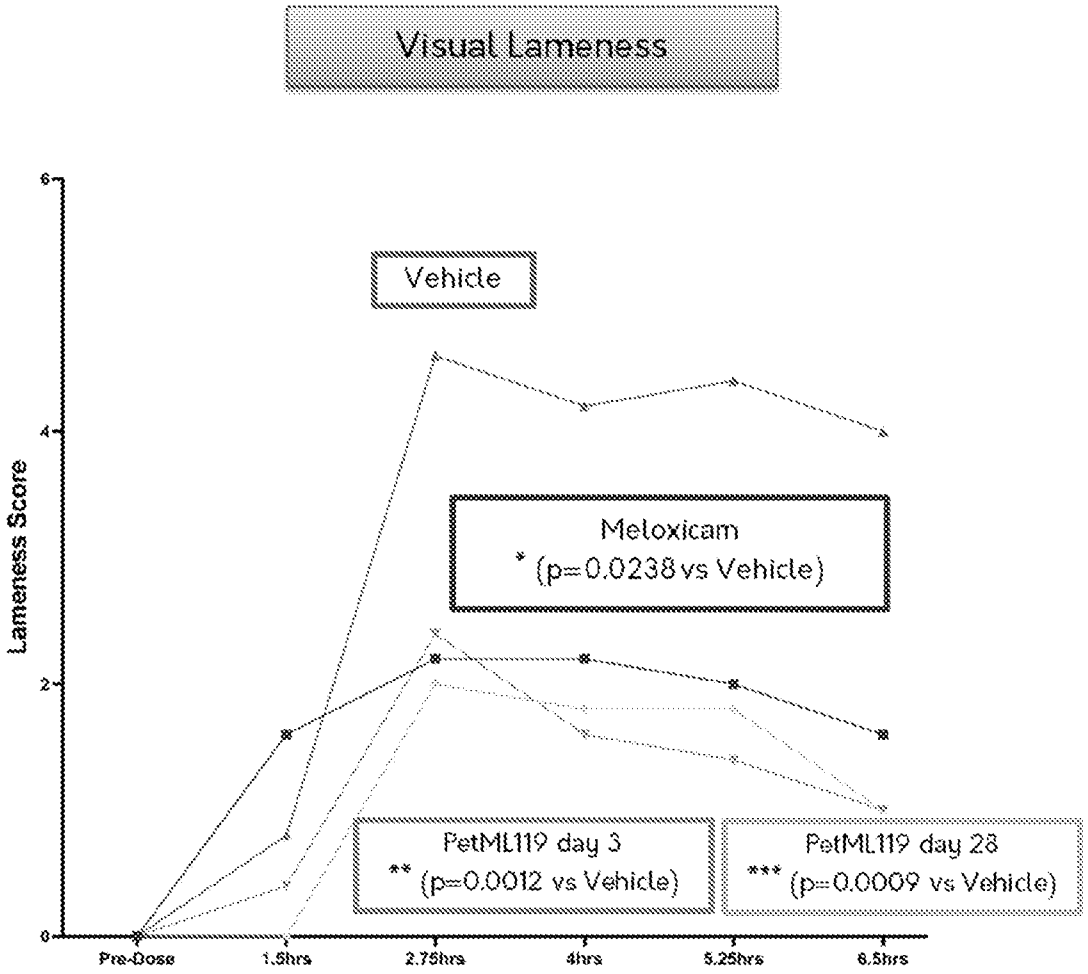

PetML119_timepoints
Abs 450nm-650nm
0.3
0.2
0.1
0.08
0.06
0.04
0.02
0.00
pre-dose
2hrs
day1
day2
day3
day28
day45
day60
Lowest conc Standard Curve
PetML122_timepoints
Abs 450nm-650nm
0.3
0.2
0.1
0.08
0.06
0.04
0.02
0.00
pre-dose
2hrs
day1
day2
day3
day28
day45
day60
Lowest conc Standard Curve

Figure 14

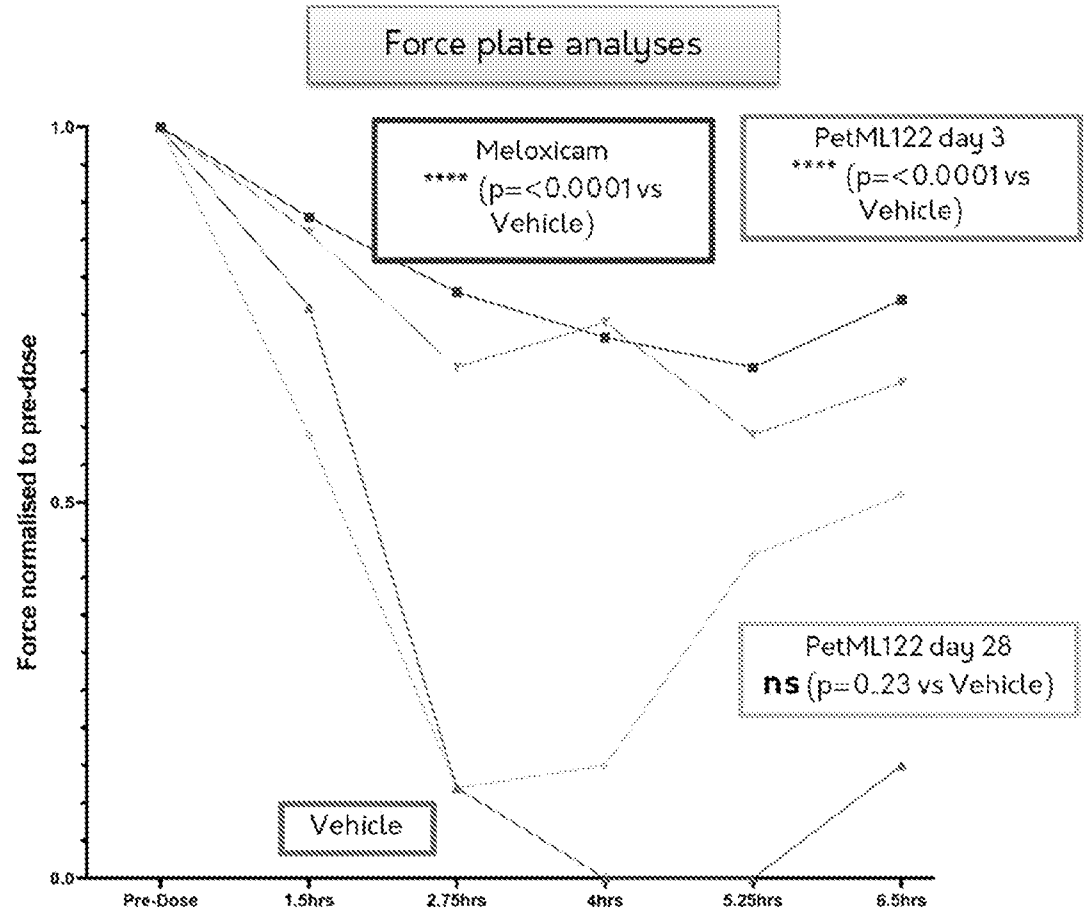
a


Force plate analyses
PetML119 day 3
*** (p=0.0006 vs Vehicle)
PetML119 day 28
** (p=0.0013 vs Vehicle)
Meloxicam
* (p=0.011 vs Vehicle)
Vehicle
Force normalised to pre-dose
1.0
0.5
0.0
Pre-Dose
1.5hrs
2.75hrs
4hrs
5.25hrs
6.5hrs

b


Visual Lameness
Vehicle
Meloxicam
* (p=0.0238 vs Vehicle)
PetML119 day 3
** (p=0.0012 vs Vehicle)
PetML119 day 28
*** (p=0.0009 vs Vehicle)
Lameness Score
0
2
4
6
Pre-Dose
1.5hrs
2.75hrs
4hrs
5.25hrs
6.5hrs

C


Force plate analyses
Meloxicam
**** (p=<0.0001 vs Vehicle)
PetML122 day 3
**** (p=<0.0001 vs Vehicle)
PetML122 day 28
ns (p=0.23 vs Vehicle)
Vehicle
Force normalised to pre-dose
1.0
0.5
0.0
Pre-Dose
1.5hrs
2.75hrs
4hrs
5.25hrs
6.5hrs

d

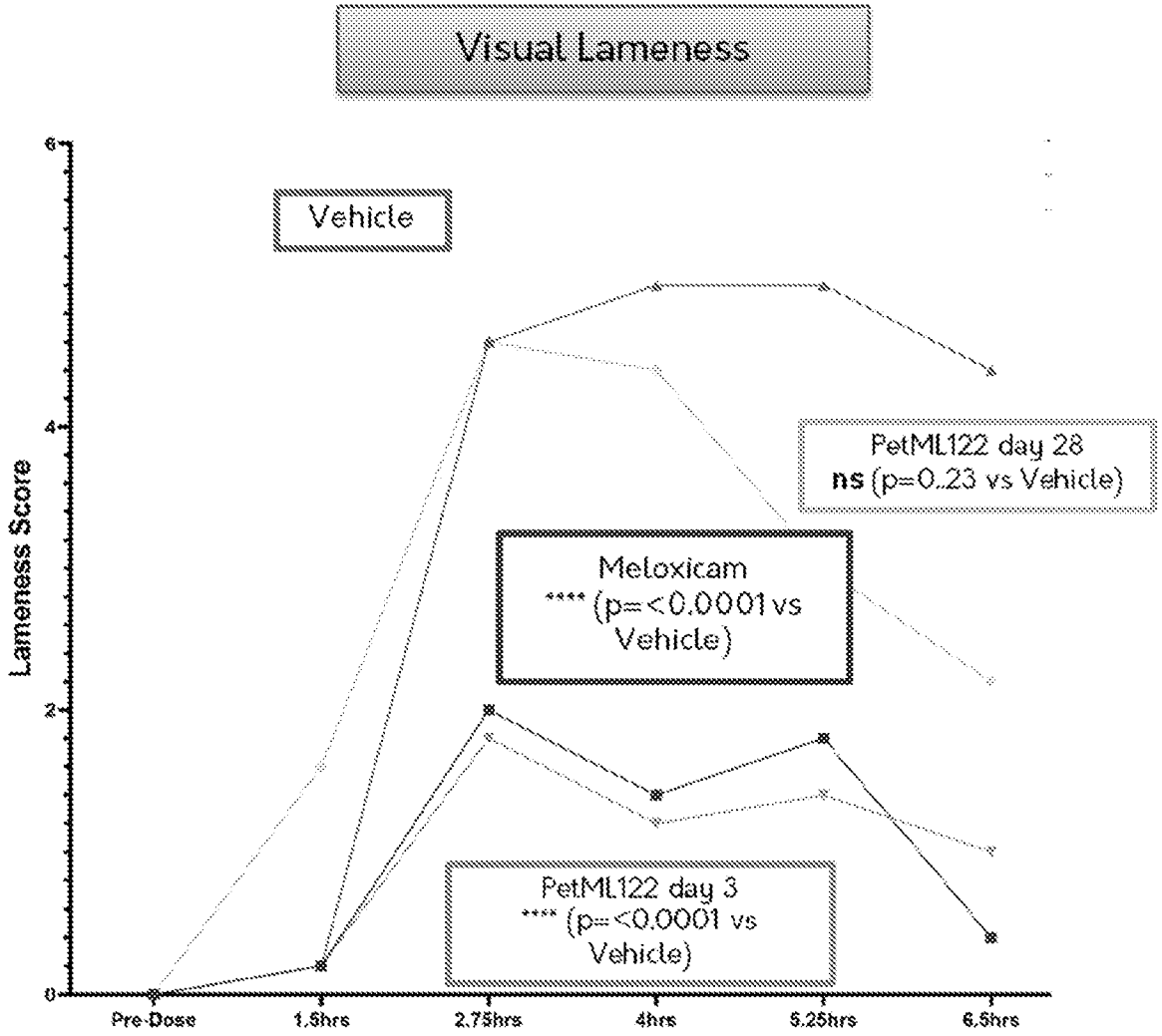
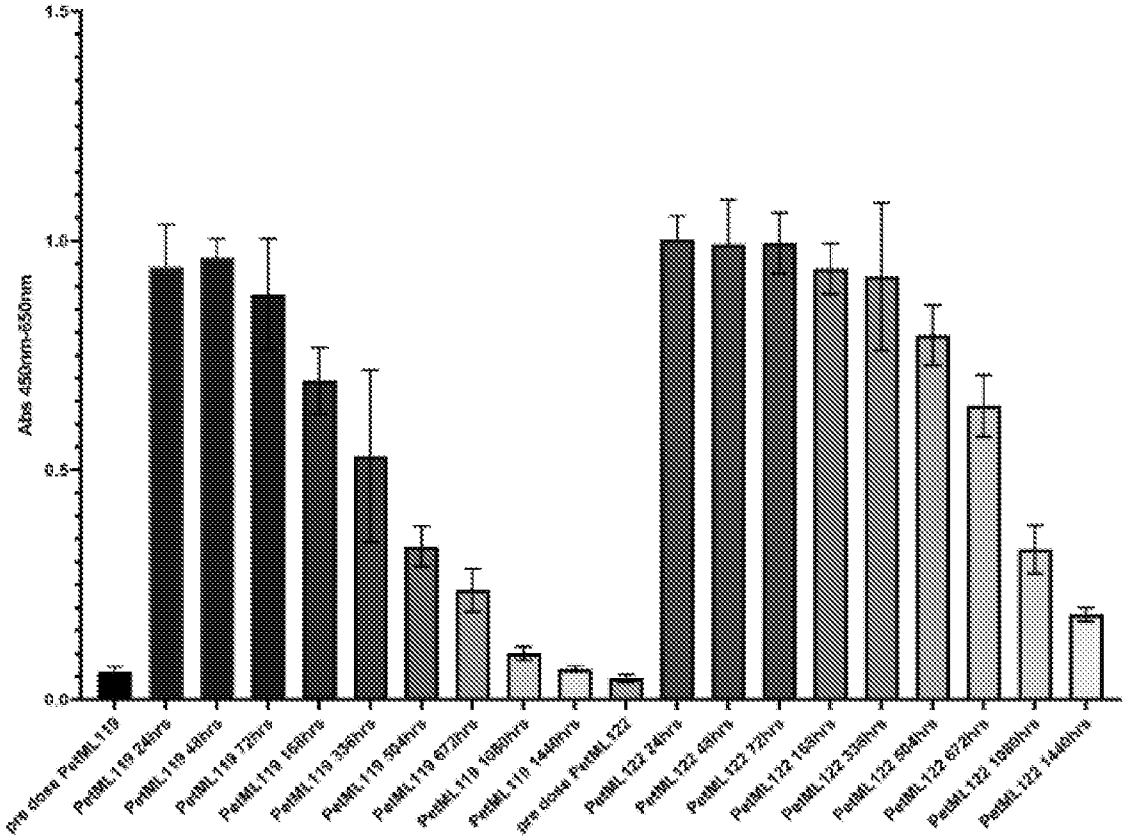
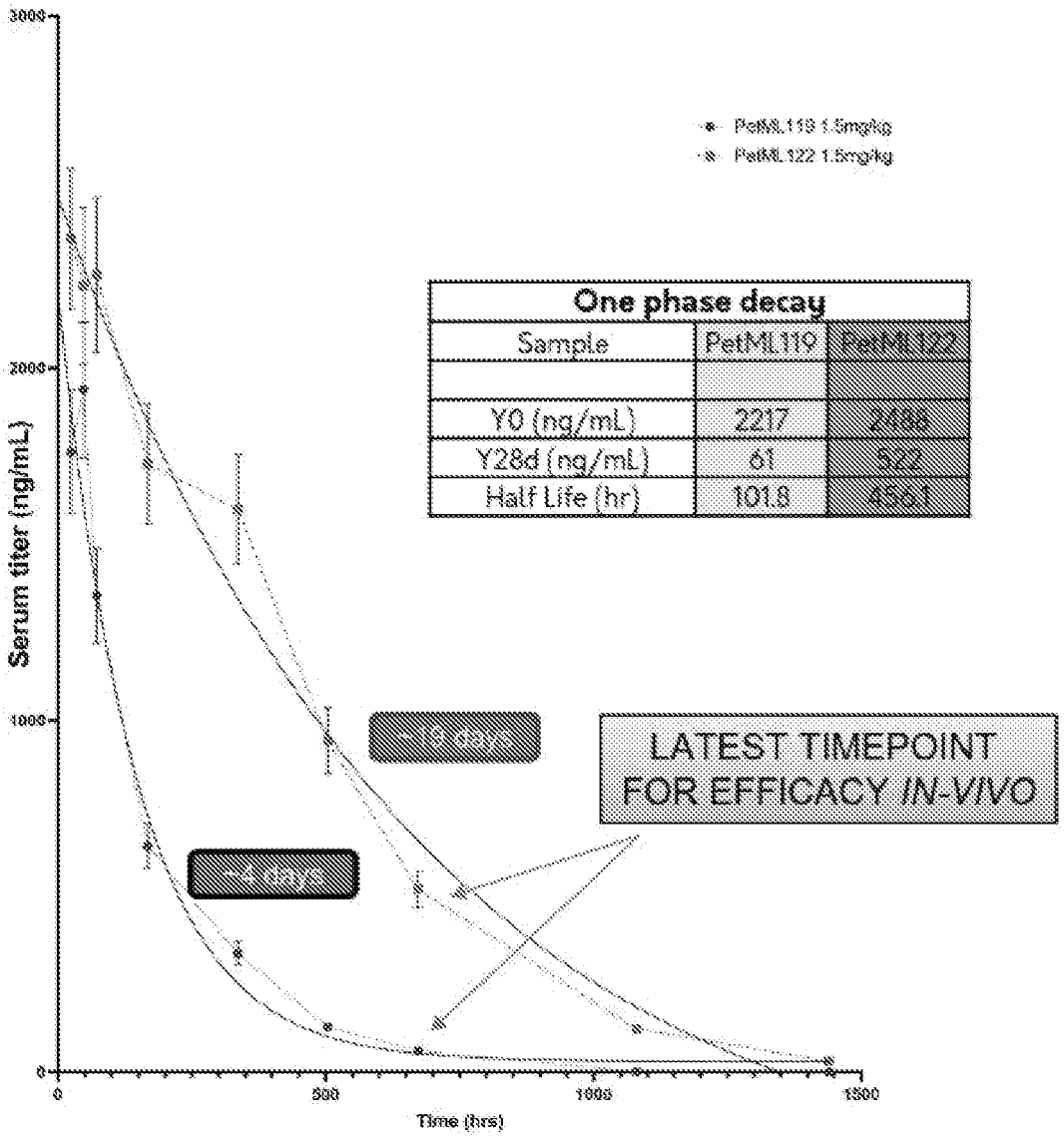

Visual Lameness
Vehicle
PetML122 day 28
ns (p=0.23 vs Vehicle)
Meloxicam
**** (p=<0.0001 vs Vehicle)
PetML122 day 3
**** (p=<0.0001 vs Vehicle)
Lameness Score
0
2
4
6
Pre-Dose
1.5hrs
2.75hrs
4hrs
5.25hrs
6.5hrs

Figure 15 a


Abs 450nm-650nm
1.5
1.0
0.5
0.0
pre dose PetML119
PetML119 24hrs
PetML119 48hrs
PetML119 72hrs
PetML119 168hrs
PetML119 336hrs
PetML119 504hrs
PetML119 672hrs
PetML119 1008hrs
PetML119 1440hrs
pre dose PetML122
PetML122 24hrs
PetML122 48hrs
PetML122 72hrs
PetML122 168hrs
PetML122 336hrs
PetML122 504hrs
PetML122 672hrs
PetML122 1008hrs
PetML122 1440hrs

b



One phase decay		
Sample	PetML119	PetML122
Y0 (ng/mL)	2217	2186
Y28d (ng/mL)	61	522
Half Life (hr)	101.8	456.1

Figure 1a.



*MEWSWVFLFFLSVTTGVHS*KEACPTGLYTHSGECCK
ACNLGEGVAQPCGANQTVCEPCLDSVTFSDVVSATE
PCKPCTECVGLQSMSAPCVEADDAVCRCAYGYYQDE
TTGRCEACRVCEAGSGLVFSCQDRQNTVCEECPDGT
YSDEANHVDPCLPCTVCEDTERQLRECTRWADAECE
EIPGRWRENGNRVPRPPDCPKCPAPEMLGGPSVFIFP
PKPKDTLLIARTPEVTCVVVDLDPEDPEVQISWFVDGK
QMQTAKTQPREEQFNGTYRVVSVLPIGHQDWLKGK
QFTCKVNNKALPSPIERTISKARGQAHQPSVYVLPPSR
EELSKNTVSLTCLIKDFFPPDIDVEWQSNGQQEPESKY
RTTPPQLDEDGSYFLYSKLSVDKSRWQRGDTFICAVM
HEALHNHYTQKSLSHSPGK

Signal peptide

Canine p75-ECD

GGGG

Canine Fc-Bwt

Figure 1b.



*MEWSWVFLFFLSVTTGVHS*KEACPTGLYTHSGECCKAC
NLGEGVAQPCGANQTVCEPCLDSVTFSDVVSATEPCKP
CTECVGLQSMSAPCVEADDAVCRCAYGYYQDETTGRC
EACRVCEAGSGLVFSCQDRQNTVCEECPDGTYSDEAN
HVDPCLPCTVCEDTERQLRECTRWADAECEEIPGRWR
ENGNRVPRPPDCPKCPAPEMLGGPSVFIFPPKPKDTLYIT
REPEVTCVVVDLDPEDPEVQISWFVDGKQMQTAKTQP
REEQFNGTYRVVSVLPIGHQDWLKGKQFTCKVNNKAL
PSPIERTISKARGQAHQPSVYVLPPSREELSKNTVSLTCLI
KDFFPPDIDVEWQSNGQQEPESKYRTTPPQLDEDGSYF
LYSKLSVDKSRWQRGDTFICAVMHEALHNHYTQKSLSH
SPGK

Signal peptide

Canine p75-ECD

GGGG

Canine Fc-**YTE**