

Anti-netrin-1 monoclonal antibody for treating endometriosis and associated pains

The present invention relates to medicaments and methods for the treatment of endometriosis, allowing a reduction or a disappearance of endometriosis lesions, and, more specifically, combining a favorable therapeutic effect on endometriosis and the lesions that mark it, and on associated pain(s) including vaginal hyperalgesia and on other comorbidities.

Background

Endometriosis is a chronic inflammatory gynaecological disorder affecting approximately 10 % of females of reproductive age, which imparts an economic load of almost \$80 billion/year in the USA alone. Chronic pelvic pain (CPP) is the most debilitating symptom experienced by women with endometriosis. In addition, endometriosis patients are commonly co-diagnosed with visceral comorbidities, including irritable bowel syndrome (IBS) and overactive bladder syndrome (OAB). Current research into mechanisms underlying pain in endometriosis primarily focus on endometriotic lesions and their inflammatory environment as the source of CPP. However, surgical removal of endometriotic lesions does not reliably provide pain relief, and adequate treatments remain lacking.

According to World Health Organization, endometriosis is a disease characterized by the presence of tissue resembling endometrium (the lining of the uterus) outside the uterus. It causes a chronic inflammatory reaction that may result in the formation of scar tissue (adhesions, fibrosis) within the pelvis and other parts of the body. Several lesion types have been described, such as superficial endometriosis found mainly on the pelvic peritoneum, cystic ovarian endometriosis (endometrioma) found in the ovaries, deep endometriosis found in the recto-vaginal septum, bladder, and bowel, and, in rare cases, endometriosis has also been found outside the pelvis. Symptoms associated with endometriosis vary, and include a combination of painful periods, chronic pelvic pain, pain during and/or after sexual intercourse, painful bowel movements, painful urination, fatigue, depression or anxiety, abdominal bloating and nausea.

At present, there is no known cure for endometriosis. Treatment is usually aimed at controlling symptoms and this is clearly insufficient.

There is thus a need for new therapeutic treatments capable of reducing, limiting or regressing the lesions due to endometriosis, and preferably, at the same time, capable of reducing endometriosis-related hyperalgesia or pain, and, possibly, other comorbidities or comorbidity symptoms as well.

Summary of the invention

NP137 (a monoclonal antibody (mAb) against Netrin-1 developed by Patrick Mehlen [23], has demonstrated anti-cancer effects in pre-clinical mouse models [15], and it is currently in Phase II clinical trial for advanced-stage cancer (<https://clinicaltrials.gov/ct2/show/NCT02977195>). It is shown here for the first time that NP137 has *in vivo* the unusual and surprising capacity to reduce both the development of endometriosis lesions, and endometriosis-associated chronic pelvic pain by providing vaginal hyperalgesia relief. Convincing data were obtained in two well characterised mouse models of endometriosis that exhibit similar symptoms to those observed in women with endometriosis (i.e the development of endometrial lesions and widespread chronic pelvic pain).

In an aspect, the invention relates to an anti-netrin-1 antibody or an antigen-binding fragment thereof, or a pharmaceutical composition containing an anti-netrin-1 antibody or an antigen-binding fragment thereof and a pharmaceutically acceptable vehicle, for use in treating endometriosis, said treating comprising a reduction of the endometriosis lesions, especially uterine and/or uterine horn endometriosis lesions.

In an embodiment, said anti-netrin-1 antibody and antigen-binding fragment thereof specifically bind to a netrin-1 epitope or binding region having the amino acid sequence SEQ ID NO: 3 or 33.

In another embodiment, said anti-netrin-1 antibody and antigen-binding fragment thereof is as defined hereinafter with the disclosed set of 6 CDRs, or the disclosed specific VH and VL regions.

In preferred embodiments, said anti-netrin-1 antibody and antigen-binding fragment comprise the pairs of VH and VL of any one of the humanized monoclonal antibodies HUM01-10.

In a preferred embodiment, said anti-netrin-1 antibody is NP137.

In a further aspect, said treating comprises a reduction of both, the endometriosis lesions developed within the peritoneal cavity (i.e around the uterus and/or uterine horns), and chronic pelvic pain relief in endometriosis.

In particular, said treating comprises reduction of the endometriosis lesions and/or the hemorrhagic lesions. By imaging, one can observe, measure and take into account the reduction in the total volume of lesions and/or the reduction in the volume of hemorrhagic lesions. We consider that a reduction is significant when the total volume of the lesions is reduced by more than 20%. In an embodiment, the antibody of the invention allows a lesion reduction of more than 20, 30, 40 or 50%. Imaging may be performed by the imaging methods in use for endometriosis imaging, such as transvaginal ultrasound, magnetic resonance imaging (MRI, for more details see [30]) and computed tomography.

Moreover, treatment with NP137 results in improvement of the vaginal hyperalgesia developed in endometriosis, as assessed in our model by electromyography (EMG) signal analysis of the visceromotor responses (VMR) evoked by vaginal distension (VD), as described in the section 4. In women, we use the VAS (visual analogue score). A treatment is usually considered to have a certain degree of effectiveness in painful endometriosis when the pain scales show a reduction of at least 2 VAS points (out of 10). In an embodiment, the antibody of the invention allows a reduction of at least 2, 3 or 4 VAS. For more details on VAS see [10].

In further aspects, said treating may further comprise one or several of the additional following technical effects or therapeutic functions:

- a reduction of the colorectal hyperalgesia developed in endometriosis,
- an improvement 75% recover of the bladder dysfunction developed in endometriosis,
- a reduction of the enhanced cutaneous sensitivity to thermal stimuli developed in endometriosis,
- a reduction of enhanced cutaneous sensitivity to mechanical stimuli developed in endometriosis,
- improvement of the overall wellbeing which was altered by endometriosis.

In an aspect, the invention relates to a method of treatment of endometriosis in a women in need thereof, comprising administering to said women an efficient amount of an anti-netrin-1 antibody or an antigen-binding fragment thereof, or a pharmaceutical composition containing an anti-netrin-1 antibody or an antigen-binding fragment thereof and a pharmaceutically acceptable vehicle.

The method comprises reducing endometriosis lesions, especially uterine and/or uterine horn endometriosis lesions.

In a further aspect, said method comprises reducing endometriosis lesions, especially uterine and/or uterine horn endometriosis lesions, and reducing a chronic pelvic pain relief, or a vaginal hyperalgesia relief.

In further aspects, said method further comprises one or several of the additional following effects:

- a reduction of colorectal hyperalgesia developed in endometriosis.
- an improvement of bladder dysfunction developed in endometriosis.
- a reduction of enhanced cutaneous sensitivity to thermal stimuli developed in endometriosis.
- a reduction of enhanced cutaneous sensitivity to mechanical stimuli developed in endometriosis.

- a improvement of the overall wellbeing altered by the endometriosis.

The above disclosed % of reduction or improvement of these effects apply here as well.

In another aspect, the invention relates to the use of an anti-netrin-1 antibody or an antigen-binding fragment thereof, as disclosed herein, for the manufacture of a medicament for treating endometriosis, and preferably endometriosis and pain, as disclosed herein.

Detailed description

The antibody may be a polyclonal or monoclonal antibody specifically binding to netrin-1 (NTN1) (anti-netrin-1 antibody or antibody binding to netrin-1), especially human netrin-1.

An anti-netrin-1 polyclonal antibody may, inter alia, be obtained by immunizing an animal such as a rabbit, a mouse and the like with the aid of the selected amino acid sequence, collecting and then depleting the antiserum obtained on, for example, an immunoabsorbent containing the receptor according to methods known per se to a person skilled in the art.

The netrin-1 amino acid sequence is as depicted on SEQ ID NO: 1 and netrin-1 may be used in whole or in part to generate polyclonal or monoclonal antibodies.

Generally, monoclonal antibodies may be obtained according to the conventional method of lymphocyte fusion and hybridoma culture described by Köhler and Milstein, (Nature, 1975, 256(5517): 495-7). Other methods for preparing monoclonal antibodies are also known (Harlow et al., ed., 1988 "Antibodies: a laboratory manual"). The monoclonal antibodies may be prepared by immunizing a mammal (for example a mouse, a rat, a rabbit or even a human being, and the like) and using the lymphocyte fusion technique leading to hybridoma (Köhler and Milstein, 1975). Alternative techniques to this customary technique exist. It is possible, for example, to produce monoclonal antibodies by expressing a nucleic acid cloned from a hybridoma. It is also possible to produce antibodies by the phage display technique by introducing cDNAs for antibodies into vectors, which are typically filamentous phages which exhibit gene libraries V at the surface of the phage (for example fUSE5 for E. coli, Scott J.K., Smith G.P. Science 1990; 249:386-390). Protocols for constructing these antibody libraries are described in J.D. Marks et al., J. Mol. Biol., 222 (1991), p. 581). The cDNA corresponding to full length netrin-1 with signal sequence (SEQ ID NO: 2) or to a suitable fragment thereof may be used to produce monoclonal antibodies according to these methods.

The anti-netrin-1 monoclonal antibody (mAb) may be a murine, a chimeric, a humanized or a full-human monoclonal antibody. The fragment may be any type of mAb fragment that keeps substantially the ability of the whole antibody to bind to Netrin-1, it can

be for example a Fab or a F(ab')₂. In particular, a monoclonal antibody is one disclosed in WO2015/104360 or US10,494,427, which documents are incorporated herein by reference, and disclose useful murine, chimeric and humanized monoclonal antibodies, and methods for preparing the various mAb presented herein (e.g. HUM01-10 and the like). These are antibodies or fragments thereof, which specifically bind to a netrin-1 epitope or to a polypeptide having the amino acid sequence SEQ ID NO: 3 or 33, or a variant thereof.

The antibodies useful in the invention may be defined by their CDRs. In particular these CDRs are derived from the murine antibody 4C11 disclosed in WO2015/104360 or US10,494,427, which antibody specifically binds to the polypeptide having the amino acid sequence SEQ ID NO: 3 or 33. Preferably, the antibody is a monoclonal antibody or an antigen-binding fragment thereof, comprising

a variable domain VH comprising:

- a H-CDR1 having a sequence set forth as SEQ ID NO: 5;
- a H-CDR2 having a sequence set forth as SEQ ID NO: 6;
- a H-CDR3 having a sequence set forth as SEQ ID NO: 7;

a variable domain VL comprising:

- a L-CDR1 having a sequence set forth as SEQ ID NO: 8;
- a L-CDR2 having a sequence YAS;
- a L-CDR3 having a sequence set forth as SEQ ID NO: 9;

or

a variable domain VH comprising:

- a H-CDR1 having a sequence set forth as SEQ ID NO: 28;
- a H-CDR2 having a sequence set forth as SEQ ID NO: 29;
- a H-CDR3 having a sequence set forth as SEQ ID NO: 30;

a variable domain VL comprising:

- a L-CDR1 having a sequence set forth as SEQ ID NO: 31;
- a L-CDR2 having a sequence set forth as SEQ ID NO: 32;
- a L-CDR3 having a sequence set forth as SEQ ID NO: 9.

In a first series of embodiments, the antibody of the invention comprises an amino acid sequence SEQ ID NO: 10, 11, 12 or 13. Typically, it comprises a VH of sequence SEQ ID NO: 10 and a VL of sequence SEQ ID NO: 11, or a heavy chain of sequence SEQ ID NO: 12 and a light chain of sequence SEQ ID NO: 13.

In a second series of embodiments, the antibody is chimeric. Preferably, it comprises a VH of sequence SEQ ID NO: 27 and a VL of sequence SEQ ID NO: 19.

In a third series of embodiments, the antibody is humanized. Preferably, it comprises an amino acid sequence selected from the group of SEQ ID NO: 14 to 18 (VL) and/or from

the group of SEQ ID NO: 20 to 26 (VH). Typically, the antibody is humanized and comprises a VH having an amino acid sequence selected from the group of SEQ ID NO: 14 to 18 and a VL having an amino acid sequence selected from the group of SEQ ID NO: 20 to 26.

5 The antibody preferably comprises a monoclonal antibody (mAb) or an antigen-binding fragment thereof, wherein the mAb or its fragment specifically binds to Netrin-1. The mAb may be a murine, a chimeric, a humanized or a full-human monoclonal antibody. The fragment may be any type of mAb fragment that keeps substantially the ability of the whole antibody to bind to Netrin-1, it can be for example a Fab or a F(ab')₂.

10 Specific embodiments disclosed in this prior document and that can be used herein are the following antibodies listed in Table 1. The first listed antibody is a chimeric 4C11 antibody, comprising the murine VH and VL of the murine 4C11 antibody. HUM00 listed in Table 1 corresponds to the grafting of the murine 4C11 CDRs into a human IgG1. The ten humanized mAb HUM01 to HUM10 correspond to humanized mAbs derived from HUM00 with the same CDRs, but specific modifications in the FR regions of the human IgG. HUM03
15 is also publicly known as NP137 and is currently under clinical trials. Sequences of the human IgG1 CH come from Genbank AEL33691.1 modified R97K. Sequences of the human IgG1 CL (Kappa) come from Genbank CAC20459.1. The other allotypes may be used as well. Specific binding of all these mAbs, murine, chimeric and humanized HUM01-HUM10, Fab fragments and F(ab')₂ fragments, to Netrin-1 and their ability to inhibit binding
20 of netrin-1 to its receptor UNC5B, are demonstrated in US 10,494,427 (Example 3).

In particular these antibodies specifically bind to the polypeptide having the amino acid sequence SEQ ID NO: 33.

Table 1	VH SEQ ID NO:	Constant heavy chain	VL SEQ ID NO:	Constant light chain
4C11	10	Human IgG1	11	Human IgG1
HUM00	27	Human IgG1	19	Human IgG1
HUM01	20	Human IgG1	14	Human IgG1
HUM02	21	Human IgG1	15	Human IgG1
HUM03	22	Human IgG1	16	Human IgG1
HUM04	23	Human IgG1	17	Human IgG1
HUM05	24	Human IgG1	17	Human IgG1
HUM06	25	Human IgG1	16	Human IgG1
HUM07	26	Human IgG1	17	Human IgG1

HUM08	22	Human IgG1	17	Human IgG1
HUM09	25	Human IgG1	18	Human IgG1
HUM10	21	Human IgG1	16	Human IgG1

Preferably, the antibody is a monoclonal antibody or an antigen-binding fragment thereof, comprising a pair of VH and VL sequences selected from the following pairs: SEQ ID NO: 27 and 19, SEQ ID NO: 20 and 14, SEQ ID NO: 21 and 15, SEQ ID NO: 22 and 16, SEQ ID NO: 23 and 17, SEQ ID NO: 24 and 17, SEQ ID NO: 25 and 16, SEQ ID NO: 26 and 17, SEQ ID NO: 22 and 17, SEQ ID NO: 25 and 18, SEQ ID NO: 21 and 16. More preferably, the antibody is a monoclonal antibody or an antigen-binding fragment thereof, comprising a pair of VH and VL sequences SEQ ID NO: 22 and 16.

The anti-netrin-1 antibody may further comprise a Human IgG1 Constant heavy chain (CH) and/or a Human IgG1 Constant light chain (CL), in particular a human kappa constant domain.

In an embodiment, sequences of the human IgG1 CH come from Genbank AEL33691.1 modified R97K. Sequences of the human IgG1 CL (Kappa) come from Genbank CAC20459.1. In an embodiment, the mAb is NP137 (AB_2811180 in The Antibody Registry) and comprises SEQ ID NO: 22 and 16 as VH, respectively VL sequences, and those specific IgG1 CH and CL.

The term “antigen-binding fragment” of an antibody (or “antibody-binding portion”), as used herein, refers to one or more fragments of an antibody that retain the ability to specifically bind to netrin-1. An antibody fragment may include, for example, a Fab fragment, a F(ab')₂ fragment, a Fv fragment, a dAb fragment, a fragment containing a CDR, or an isolated CDR. In an aspect, the fragment comprises the VH and VL sequences of an antibody selected from HUM00 to HUM10.

As anti-netrin-1 antibodies that may be used, one may cite other antibodies, especially monoclonal antibodies, or their antigen-binding fragments, developed against human netrin-1 or against animal netrin-1, netrin-1 being very homologous among species. May be cited: Abcam antibodies ab126729, ab122903, ab201324, ab39370; AF1109, AF6419, AF128.

In one embodiment, an antibody or antigen-binding fragment thereof having applications in a use or method of the present invention is administered as a single dose, or an initial dose followed by administration of a second or a plurality of subsequent doses of the antibody or antigen-binding fragment thereof, wherein the subsequent doses are separated by at least one day; at least one week, at least 2 weeks; at least 3 weeks; at least 4 weeks; at least 5 weeks; at least 6 weeks; at least 7 weeks; at least 8 weeks; at least 9 weeks; at least 10 weeks; at least 12 weeks; or at least 14 weeks. The dose may vary

depending upon the age and the weight of a subject to be administered, target disease, conditions, route of administration, and the like. When the antibody of the present invention is used for treating pain in an adult patient, it is advantageous to administer the antibody of the present invention either intravenously, subcutaneously, or intraperitoneally. One single
5 dose or the subsequent doses may comprise an amount of antibody or fragment thereof of at least 0.1 or 1 mg/kg body weight, e.g. about 1 mg/kg body weight to about 100 mg/kg body weight, in particular about 10 mg/kg body weight to about 60 mg/kg body weight of the subject. Depending on the severity of the endometriosis, such as the number and extent of lesions, and the degree of pain and/or comorbidities, the frequency and the duration of
10 the treatment can be adjusted.

Definitions and further embodiments, variants and alternatives of the invention:

Throughout the instant application, the term "comprising" is to be interpreted as encompassing all specifically mentioned features as well optional, additional, unspecified ones. As used herein, the use of the term "comprising" also discloses the embodiment
15 wherein no features other than the specifically mentioned features are present (i.e. "consisting of", "contributing to").

In the context of the invention, the term "treating" or "treatment", as used herein, means obtaining a reduction or a disappearance of the lesions and, preferably, a reduction or alleviating of endometriosis-related pain and/or a comorbidity associated with
20 endometriosis, as disclosed herein.

By a "therapeutically effective amount" of the antibody of the invention is meant a sufficient amount thereof to treat said pain, at a reasonable benefit/risk ratio applicable to any medical treatment. It will be understood, however, that the total daily usage of the antibody on will be decided by the attending physician within the scope of sound medical
25 judgment. The specific therapeutically effective dose level for any particular patient will depend upon a variety of factors including the severity of the endometriosis, such as the number and extent of lesions, and the degree of pain and/or comorbidities; activity of the specific antibody employed; the specific composition employed, the age, body weight, general health, and diet of the women; the time of administration, route of administration,
30 and rate of excretion of the specific antibody employed; the duration of the treatment; drugs used in combination or coincidental with the specific antibody employed; and like factors well known in the medical arts.

"Pharmaceutically" or "pharmaceutically acceptable" refers to molecular entities and compositions that do not produce an adverse, allergic or other untoward reaction when
35 administered to a mammal, especially a human, as appropriate. A pharmaceutically

acceptable vehicle (or carrier or excipient) refers to a non-toxic solid, semi-solid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type.

The form of the pharmaceutical compositions including the antibody of the invention and the route of administration naturally depend upon the condition to be treated, the severity of the illness, the age, weight, and gender of the patient, etc.

The antibody of the invention can be formulated for a topical, oral, parenteral, intranasal, intravenous, intramuscular, subcutaneous or intraocular administration and the like. In a particular embodiment, the antibody of the invention is administered intravenously.

In particular, the pharmaceutical compositions including the antibody of the invention may contain vehicles, which are pharmaceutically acceptable for a formulation capable of being injected. These may be in particular isotonic, sterile, saline solutions (monosodium or disodium phosphate, sodium, potassium, calcium or magnesium chloride and the like or mixtures of such salts), or dry, especially freeze-dried compositions which upon addition, depending on the case, of sterilized water or physiological saline, permit the constitution of injectable solutions.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases, the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi.

The vehicle or carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like) and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants, stabilizing agents, cryoprotectants or antioxidants. The prevention of the action of microorganisms can be brought about by antibacterial and antifungal agents. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride.

Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with several of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum-drying and freeze-drying techniques which

yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Upon formulation, solutions will be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically effective. The formulations are easily administered in a variety of dosage forms, such as the type of injectable solutions described above, but drug release capsules and the like can also be employed.

For parenteral administration in an aqueous solution, for example, the solution should be suitably buffered if necessary and the liquid diluent first rendered isotonic with sufficient saline or glucose. These particular aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous and intraperitoneal administration. In this connection, sterile aqueous media which can be employed will be known to those of skill in the art in light of the present disclosure. For example, one dosage could be dissolved in 1 mL of isotonic NaCl solution and either added to 1000 mL of hypodermoclysis fluid or injected at the proposed site of infusion, (see for example, "Remington's Pharmaceutical Sciences" 15th Edition, pages 1035-1038 and 1570-1580). Some variation in dosage will necessarily occur depending on the condition of the subject being treated. The person responsible for administration will, in any event, determine the appropriate dose for the individual subject.

Table 2: Description of the sequences:

SEQ ID NO:	Description	Sequence
1	Netrin-1 amino acid (aa) sequence (seq.) with signal peptide in bold and linear epitope mapping in bold and underlined	MMRAVWEALAALA AVACLVGAVRGG PGLSMFAGQAAQ PDPCSDENGHPRCIPDFVNAAF GKDVRSSTCGRPPA RYCVVSEERGEERLRSCHLCNASDPKKAHPPAFLTDLNNP HN LTCWQSENYLQFPHNVTLTLSLGKKFEVTYVSLQFCS PRPESMAIYKSMDYGRTWVPFQFYSTQCRKMYNRPHRA PITKQNEQEAVCTDSHTDMRPLSGGLIAFSTLDGRPSAHD FDNSPVLQDWVTATDIRVAFSRLHTFGDENEDDSELARD SYFYAVSDLQVGGRCKCNGHAARCVRDRDDSLVCDCRH NTAGPECDRCKPFHYDRPWQRATAREANEC <u>VACNCNL</u> <u>HARRCRFNMELYKL</u> <u>SGRKSGGVCLNCRHNTAGRHCY</u> CKEGYYRDMGKPITHRKACKACDCHPVGAAGKTCNQTT GQCPCKDGVTGITCNRCAGYQQSRSPIAPCIKIPVAPPT TAASSVEEPEDCDSYCKASKGKLKINMKKYCKKDYAVQIH ILKADKAGDWWKFTVNIISVYKQGTSRIIRRGDQSLWIRSR

		DIACKCPKIKPLKKYLLLGNAEDSPDQSGIVADKSSLVIQW RDTWARRLRKFQQREKKGKCKKA
2	Netrin-1 nucleic acid seq.	ATGATGCGCGCAGTGTGGGAGGCGCTGGCGGCGCTG GCGGCGGTGGCGTGCCTGGTGGGCGCGGTGCGCGG CGGGCCCCGGGCTCAGCATGTTGCGGGGCCAGGCGGC GCAGCCCATCCCTGCTCGGACGAGAACGGCCACCC GCGCCGCTGCATCCCGGACTTTGTCAATGCGGCCTTC GGCAAGGACGTGCGCGTGTCCAGCACCTGCGGCCGG CCCCCGGCGCGCTACTGCGTGGTGAGCGAGCGCGGC GAGGAGCGGCTGCGCTCGTGCCACCTCTGCAACGCGT CCGACCCCAAGAAGGCGCACCCGCCCGCCTTCCTCAC CGACCTCAACAACCCGCACAACCTGACGTGCTGGCAG TCCGAGAACTACCTGCAGTTCCCGCACACGTACAGCT CACACTGTCCCTCGGCAAGAAGTTCGAAGTGACCTAC GTGAGCCTGCAGTTCTGCTCGCCGCGGCCCGAGTCCA TGGCCATCTACAAGTCCATGGACTACGGGCGCACGTG GGTGCCCTTCCAGTTCTACTCCACGCAGTGCCGCAAG ATGTACAACCGGCCGCACCGCGCGCCCATCACCAAGC AGAACGAGCAGGAGGCCGTGTGCACCGACTCGCACAC CGACATGCGCCCGCTCTCGGGCGGCCTCATCGCCTTC AGCACGCTGGACGGGCGGCCCTCGGCGCACGACTTC GACAACTCGCCCGTGCTGCAGGACTGGGTACGGCCA CAGACATCCGCGTGGCCTTCAGCCGCTGCACACGTT CGGCGACGAGAACGAGGACGACTCGGAGCTGGCGCG CGACTCGTACTTCTACGCGGTGTCCGACCTGCAGGTG GGCGGCCGGTGCAAGTGCAACGGCCACGCGGCCCGC TGCGTGCGCGACCGCGACGACAGCCTGGTGTGCGACT GCAGGCACAACACGGCCGGCCCGGAGTGCGACCGCT GCAAGCCCTTCCACTACGACCGGCCCTGGCAGCGCGC CACAGCCCGCGAAGCCAACGAGTGCGTGGCCTGTAAC TGCAACCTGCATGCCCGGCGCTGCCGCTTCAACATGG AGCTCTACAAGCTTTCGGGGCGCAAGAGCGGAGGTGT CTGCCTCAACTGTCGCCACAACACCGCCGGCCGCCAC TGCCATTACTGCAAGGAGGGCTACTACCGCGACATGG GCAAGCCCATCACCCACCGGAAGGCCTGCAAAGCCTG TGATTGCCACCCTGTGGGTGCTGCTGGCAAAACCTGC

		AACCAAACCACCGGCCAGTGTCCCTGCAAGGACGGCG TGACGGGTATCACCTGCAACCGCTGCGCCAAAGGCTA CCAGCAGAGCCGCTCTCCCATCGCCCCCTGCATAAAG ATCCCTGTAGCGCCGCCGACGACTGCAGCCAGCAGCG TGGAGGAGCCTGAAGACTGCGATTCTACTGCAAGGC CTCCAAGGGGAAGCTGAAGATTAACATGAAAAAGTACT GCAAGAAGGACTATGCCGTCCAGATCCACATCCTGAA GGCGGACAAGGCGGGGGGACTGGTGGAAAGTTCACGGT GAACATCATCTCCGTGTATAAGCAGGGCACGAGCCGC ATCCGCCCGCGGTGACCAGAGCCTGTGGATCCGCTCGC GGGACATCGCCTGCAAGTGTCCCAAATCAAGCCCT CAAGAAGTACCTGCTGCTGGGCAACGCGGAGGACTCT CCGGACCAGAGCGGCATCGTGGCCGATAAAAGCAGCC TGGTGATCCAGTGGCGGGACACGTGGGCGCGGCGGC TGCGCAAGTTCCAGCAGCGTGAGAAGAAGGGCAAGTG CAAGAAGGCCTAGCG
3	Netrin-1 aa epitopic seq.	VACNCNLHARRCRFMELYKLSGRKSGGVCLNCRHNTA GRHCH
4	Netrin-1 epitopic cDNA seq.	GTGGCCTGTAAGTCAACCTGCATGCCCGGCGCTGCC GCTTCAACATGGAGCTCTACAAGCTTTCGGGGCGCAA GAGCGGAGGTGTCTGCCTCAACTGTCGCCACAACACC GCCGGCCGCCACTGCCAT
5	aa seq. of CDR1-H (IMGT)	GYTFTSYN
6	aa seq. of CDR2-H (IMGT)	IYPGNGDT
7	aa seq. of CDR3-H (IMGT)	ARGGTGFAY
8	aa seq. of CDR1-L (IMGT)	QSVSND
-	aa seq. of CDR2-L (IMGT)	YAS
9	aa seq. Of CDR3-L (IMGT et Kabat)	QQDYSSPWT

10	aa sequence of mouse 4C11 VH	QAYLQQSGAELVRPGASVKMSCKAS GYTFTSYNMHWVK QTPRQGLEWIGAI YPGNGDTSYNQKFKGKATLTVDKSSS TAYMQLSSLTSEDSAVYFC ARGGTGFAYWGQGT LVTVS A
11	aa sequence of mouse 4C11 VL	SIVMTQTPKFLLVSAGDRVITITCKAS QSVSNDVAWYQQK PGQSPKLLIY YASNRYTGVPDRFTGSGYGT DFTFTISTVQ AEDLAVYFC QQDYSSPWT FGGGTKLEIK
12	Full aa sequence of 4C11 (VH + mouse IgG1 CH)	QAYLQQSGAELVRPGASVKMSCKAS GYTFTSYNMHWVK QTPRQGLEWIGAI YPGNGDTSYNQKFKGKATLTVDKSSS TAYMQLSSLTSEDSAVYFC ARGGTGFAYWGQGT LVTVS AAKTPPSVYPLAPGSAAQTNSMVTLGCLVKGYFPEPVT VTWNSGSLSSGVHTFPAVLES DL YTLSSSVTPSSPRPS ETVTCNVAHPASSTKVDKKIVPRDCGCKPCICTVPEVSSV FIFPPKPKDVLITITLTPKVT CV VDDISKDDPEVQFSWFVDD VEVHTAQTQPREEQFNSTFRSVSELPIMHQDWLNGKEFK CRVNSAAFPAPIEKTISKTKGRPKAPQVYTIPPPKEQMAK DKVSLTCMITDFFPEDITVEWQWNGQPAENYKNTQPIMN TNGSYFVYSKLN VQ SNWEAGNTFTCSVLHEGLHNHHT EKSLSHSPGK
13	Full aa sequence of 4C11 (VL + mouse Kappa CL)	SIVMTQTPKFLLVSAGDRVITITCKAS QSVSNDVAWYQQK PGQSPKLLIY YASNRYTGVPDRFTGSGYGT DFTFTISTVQ AEDLAVYFC QQDYSSPWT FGGGTKLEIKRADAAPTVSIFP PSSEQLTSGGASVVCFLNNFYPKDINV KW KIDGSERQNG VLNSWTDQDSKDYMSSTLT LTK DEYERHNSYTCEAT HKTSTSPIVKSFNRNEC
14	VL aa sequence of humanized variant of 4C11	EIVMTQSPATLSVSPGERATLSCKAS QSVSNDVAWYQQK PGKAPKLLIY YASNRYTGIPPRFSGSGYGT DFTLTINNIES EDAAYYFC QQDYSSPWT FGQG
15	VL aa sequence of humanized variant of 4C11	DIQMTQSPSSLSASVGDRVITITCKAS QSVSNDVAWFQQR PGQSPRRLIY YASNRYTGVP SRFSGSGSGTDFTFTISSLE AEDAATYYC QQDYSSPWT FGQG
16	VL aa sequence of humanized variant of 4C11	DIQMTQSPSSLSASVGDRVITITCKAS QSVSNDVAWYQQK PGQAPRLLIY YASNRYTGIPPRFSGSGYGT DFTLTINNIES EDAAYYFC QQDYSSPWT FGQG

17	VL aa sequence of humanized variant of 4C11	DIQMTQSPSSLSASVGDRVITITCKAS QSVSND VAWYLQK PGQSPQLLIY YASN RYTGVPSPRFSGSGSGTDFTFTISSLE AEDAATYYC QQDYSSPWTF GQG
18	VL aa sequence of humanized variant of 4C11	DIVMTQTPLSLPVTPGEPASISCKAS QSVSND VAWYQQK PGQAPRLLIY YASN RYTGIPPRFSGSGYGTDFTLTINNIES EDAAYYFC QQDYSSPWTF GQG
19	VL aa sequence of humanized variant of 4C11	EIVMTQSPATLSVSPGERATLSCRAS QSVSND VAWYQQK PGQAPRLLIY YASN RYTGIPARFSGSGSGTEFTLTISLQS EDFAVYYC QQDYSSPWTF GQG
20	VH aa sequence of humanized variant of 4C11	QVQLVQSGAEVKKPGASVKVSC KASGYTFTSYNM HWVR QATGQGLEWMGAI YPGN GDTSYNQKFKGRVTITADKSTS TAYMELSSLRSEDTAVYYC CARGGTGFAYW GQG
21	VH aa sequence of humanized variant of 4C11	QVQLQQSGPGLVKPSQTLSTCAIS GYTFTSYNM HWIRQ PPGKGLEWIGAI YPGN GDTSYNQKFKGRVTITADKSTSTA YMELSSLRSEDTAVYYC CARGGTGFAYW GQG
22	VH aa sequence of humanized variant of 4C11	QVQLQQSGPGLVKPSQTLSTCAIS GYTFTSYNM HWVRQ ATGQGLEWMGAI YPGN GDTSYNQKFKGRLTISKDTSKNQ VVLTMNMDPVDATYYC CARGGTGFAYW GQG
23	VH aa sequence of humanized variant of 4C11	EVQLVQSGAEVKKPGESLRISCKGS GYTFTSYNM HWVR QATGQGLEWMGAI YPGN GDTSYNQKFKGRFTISRDDSK NTAYLQMNSLKTEDTAVYYC CARGGTGFAYW GQG
24	VH aa sequence of humanized variant of 4C11	QVQLQESGPGLVKPSQTLSTCTV SGYTFTSYNM HWVR QAPGQGLEWMGAI YPGN GDTSYNQKFKGRVTISVDTSK NQFSLKLSSVTAADTAVYYC CARGGTGFAYW GQG
25	VH aa sequence of humanized variant of 4C11	QVQLVQSGAEVKKPGASVKVSC KASGYTFTSYNM HWVR QATGQGLEWMGAI YPGN GDTSYNQKFKGRVTITADKSTS TAYMELSSLRSEDTAVYYC CARGGTGFAYW GQG
26	VH aa sequence of humanized variant of 4C11	QVQLQQSGPGLVKPSQTLSTCAIS GYTFTSYNM HWVRQ ATGQGLEWMGAI YPGN GDTSYNQKFKGRVTITADKSTST AYMELSSLRSEDTAVYYC CARGGTGFAYW GQG
27	VH aa sequence of humanized variant of 4C11	QVQLVQSGAEVKKPGASVKVSC KASGYTFTSYNM HWVR QAPGQGLEWMGAI YPGN GDTSYAQKFKGRVTMTRDTST STVYMELSSLRSEDTAVYYC CARGGTGFAYW GQ
28	aa seq. of CDR1-H (Kabat)	SYNMH

29	aa seq. of CDR2-H (Kabat)	AIYPGNGDTSYNQKFKG
30	aa seq. of CDR3-H (Kabat)	GGTGFAY
31	aa seq. of CDR1-L (Kabat)	KASQSVSNDVA
32	aa seq. of CDR2-L (Kabat)	YASNRYT
33	Netrin-1 aa epitopic seq.	ARRCRFMELYKLSGRKSGGVC
34	Netrin-1 epitopic cDNA seq.	GCCCGGCGCTGCCGCTTCAACATGGAGCTCTACAAGC TTTCGGGGCGCAAGAGCGGAGGTGTCTGC

CDRs under IMGT are highlighted in bold in Table 1 where appropriate.

Definitions and further embodiments, variants and alternatives of the invention:

As used herein, a sequence “at least 85% identical to a reference sequence” is a sequence having, on its entire length, 85%, or more, in particular 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9% or 100% sequence identity with the entire length of the reference sequence.

A percentage of “sequence identity” may be determined by comparing the two sequences, optimally aligned over a comparison window, wherein the portion of the polypeptide sequence in the comparison window may comprise additions or deletions (i.e. gaps) as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison and multiplying the result by 100 to yield the percentage of sequence identity. Optimal alignment of sequences for comparison is conducted by global pairwise alignment, e.g. using the algorithm of Needleman and Wunsch (1970) J. Mol. Biol. 48: 443. The percentage of sequence identity can be readily determined for instance using the program Needle, with the BLOSUM62 matrix, and the following parameters gap-open=10, gap-extend=0.5.

In the context of the invention, a “conservative amino acid substitution” is one in which an amino acid residue is substituted by another amino acid residue having a side chain group with similar chemical properties (e.g., charge or hydrophobicity). In general, a conservative amino acid substitution will not substantially change the functional properties of a protein. Examples of groups of amino acids that have side chains with similar chemical

properties include 1) aliphatic side chains: glycine, alanine, valine, leucine, and isoleucine; 2) aliphatic-hydroxyl side chains: serine and threonine; 3) amide-containing side chains: asparagine and glutamine; 4) aromatic side chains: phenylalanine, tyrosine, and tryptophan; 5) basic side chains: lysine, arginine, and histidine; 6) acidic side chains: aspartic acid and glutamic acid; and 7) sulfur-containing side chains: cysteine and methionine. Conservative amino acids substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine-tryptophan, lysine-arginine, alanine-valine, glutamate-aspartate, and asparagine-glutamine.

An "antibody" may be a natural or conventional antibody in which two heavy chains are linked to each other by disulfide bonds and each heavy chain is linked to a light chain by a disulfide bond. There are two types of light chain, lambda and kappa. There are five main heavy chain classes (or isotypes) which determine the functional activity of an antibody molecule: IgM, IgD, IgG, IgA and IgE. Each chain contains distinct sequence domains. The light chain includes two domains or regions, a variable domain (VL) and a constant domain (CL). The heavy chain includes four domains, a variable domain (VH) and three constant domains (CH1, CH2 and CH3, collectively referred to as CH). The variable regions of both light (VL) and heavy (VH) chains determine binding recognition and specificity to the antigen. The constant region domains of the light (CL) and heavy (CH) chains confer important biological properties such as antibody chain association, secretion, trans-placental mobility, complement binding, and binding to Fc receptors (FcR). The Fv fragment is the N-terminal part of the Fab fragment of an immunoglobulin and consists of the variable portions of one light chain and one heavy chain. The specificity of the antibody resides in the structural complementarity between the antibody combining site and the antigenic determinant. Antibody combining sites are made up of residues that are primarily from the hypervariable or complementarity determining regions (CDRs). Occasionally, residues from non-hypervariable or framework regions (FR) influence the overall domain structure and hence the combining site.

"Complementarity Determining Regions" or "CDRs" refer to amino acid sequences which together define the binding affinity and specificity of the natural Fv region of a native immunoglobulin binding site. The light and heavy chains of an immunoglobulin each have three CDRs, designated CDR1-L, CDR2-L, CDR3-L and CDR1-H, CDR2-H, CDR3-H, respectively. A conventional antibody antigen-binding site, therefore, includes six CDRs, comprising the CDR set from each of a heavy and a light chain V region.

"Framework Regions" (FRs) refer to amino acid sequences interposed between CDRs, i.e. to those portions of immunoglobulin light and heavy chain variable regions that are relatively conserved among different immunoglobulins in a single species. The light and

heavy chains of an immunoglobulin each have four FRs, designated FR1-L, FR2-L, FR3-L, FR4-L, and FR1-H, FR2-H, FR3-H, FR4-H, respectively.

As used herein, a "human framework region" is a framework region that is substantially identical (about 85%, or more, in particular 90%, 95%, 97%, 99% or 100%) to the framework region of a naturally occurring human antibody.

In the context of the invention, CDR/FR definition in an immunoglobulin light or heavy chain is to be determined based on Kabat or IMGT definitions.

The residues in antibody variable domains are conventionally numbered according to a system devised by Kabat et al. This system is set forth in Kabat et al., 1987, in Sequences of Proteins of Immunological Interest, US Department of Health and Human Services, NIH, USA (hereafter "Kabat et al."). This numbering system is used in the present specification. The Kabat residue designations do not always correspond directly with the linear numbering of the amino acid residues in SEQ ID sequences. The actual linear amino acid sequence may contain fewer or additional amino acids than in the strict Kabat numbering corresponding to a shortening of, or insertion into, a structural component, whether framework or complementarity determining region (CDR), of the basic variable domain structure. The correct Kabat numbering of residues may be determined for a given antibody by alignment of residues of homology in the sequence of the antibody with a "standard" Kabat numbered sequence. The CDRs of the heavy chain variable domain are located at residues 31-35B (H-CDR1), residues 50-65 (H-CDR2) and residues 95-102 (H-CDR3) according to the Kabat numbering system. The CDRs of the light chain variable domain are located at residues 24-34 (L-CDR1), residues 50-56 (L-CDR2) and residues 89-97 (L-CDR3) according to the Kabat numbering system. (<http://www.bioinf.org.uk/abs/#cdrdef>)

In the context of the invention, the amino acid residues of the antibody of the invention may be numbered according to the IMGT numbering system. The IMGT unique numbering has been defined to compare the variable domains whatever the antigen receptor, the chain type, or the species (Lefranc M.-P., "Unique database numbering system for immunogenetic analysis" Immunology Today, 18, 509 (1997) ; Lefranc M.-P., "The IMGT unique numbering for Immunoglobulins, T cell receptors and Ig-like domains" The Immunologist, 7, 132-136 (1999).; Lefranc, M.-P., Pommié, C., Ruiz, M., Giudicelli, V., Foulquier, E., Truong, L., Thouvenin-Contet, V. and Lefranc, G., "IMGT unique numbering for immunoglobulin and T cell receptor variable domains and Ig superfamily V-like domains" Dev. Comp. Immunol., 27, 55-77 (2003).). In the IMGT unique numbering, the conserved amino acids always have the same position, for instance cysteine 23, tryptophan 41, hydrophobic amino acid 89, cysteine 104, phenylalanine or tryptophan 118. The IMGT unique numbering provides a standardized delimitation of the framework regions (FR1-

IMGT: positions 1 to 26, FR2-IMGT: 39 to 55, FR3-IMGT: 66 to 104 and FR4-IMGT: 118 to 128) and of the complementarity determining regions: CDR1-IMGT: 27 to 38, CDR2-IMGT: 56 to 65 and CDR3-IMGT: 105 to 117. If the CDR3-IMGT length is less than 13 amino acids, gaps are created from the top of the loop, in the following order 111, 112, 110, 113, 109, 114, etc. If the CDR3-IMGT length is more than 13 amino acids, additional positions are created between positions 111 and 112 at the top of the CDR3-IMGT loop in the following order 112.1, 111.1, 112.2, 111.2, 112.3, 111.3, etc. (<http://www.imgt.org/IMGTScientificChart/Nomenclature/IMGT-FRCDRdefinition.html>).

As used herein, the term “antibody” denotes conventional antibodies and fragments thereof, as well as single domain antibodies and fragments thereof, in particular variable heavy chain of single domain antibodies, and chimeric, humanized, bispecific or multispecific antibodies.

As used herein, antibody or immunoglobulin also includes “single domain antibodies” which have been more recently described and which are antibodies whose complementary determining regions are part of a single domain polypeptide. Examples of single domain antibodies include heavy chain antibodies, antibodies naturally devoid of light chains, single domain antibodies derived from conventional four-chain antibodies, engineered single domain antibodies. Single domain antibodies may be derived from any species including, but not limited to mouse, human, camel, llama, goat, rabbit and bovine. Single domain antibodies may be naturally occurring single domain antibodies known as heavy chain antibody devoid of light chains. In particular, Camelidae species, for example camel, dromedary, llama, alpaca and guanaco, produce heavy chain antibodies naturally devoid of light chain. Camelid heavy chain antibodies also lack the CH1 domain.

The variable heavy chain of these single domain antibodies devoid of light chains are known in the art as “VHH” or “nanobody”. Similar to conventional VH domains, VHHs contain four FRs and three CDRs. Nanobodies have advantages over conventional antibodies: they are about ten times smaller than IgG molecules, and as a consequence properly folded functional nanobodies can be produced by in vitro expression while achieving high yield. Furthermore, nanobodies are very stable, and resistant to the action of proteases. The properties and production of nanobodies have been reviewed by Harmsen and De Haard (2007) *Appl. Microbiol. Biotechnol.* 77:13-22.

The term “monoclonal antibody” or “mAb” as used herein refers to an antibody molecule of a single amino acid composition that is directed against a specific antigen, and is not to be construed as requiring production of the antibody by any particular method. A monoclonal antibody may be produced by a single clone of B cells or hybridoma, but may also be recombinant, i.e. produced by protein engineering.

"Fragments" of (conventional) antibodies comprise a portion of an intact antibody, in particular the antigen binding region or variable region of the intact antibody. Examples of antibody fragments include Fv, Fab, F(ab')₂, Fab', dsFv, (dsFv)₂, scFv, sc(Fv)₂, diabodies, bispecific and multispecific antibodies formed from antibody fragments. A fragment of a conventional antibody may also be a single domain antibody, such as a heavy chain antibody or VHH.

The term "Fab" denotes an antibody fragment having a molecular weight of about 50,000 Da and antigen binding activity, in which about a half of the N-terminal side of H chain and the entire L chain, among fragments obtained by treating IgG with a protease, papain, are bound together through a disulfide bond.

The term "F(ab')₂" refers to an antibody fragment having a molecular weight of about 100,000 Da and antigen binding activity, which is slightly larger than the Fab bound via a disulfide bond of the hinge region, among fragments obtained by treating IgG with a protease, pepsin.

A single chain Fv ("scFv") polypeptide is a covalently linked VH::VL heterodimer which is usually expressed from a gene fusion including VH and VL encoding genes linked by a peptide-encoding linker. The human scFv fragment of the invention includes CDRs that are held in appropriate conformation, in particular by using gene recombination techniques. Divalent and multivalent antibody fragments can form either spontaneously by association of monovalent scFvs, or can be generated by coupling monovalent scFvs by a peptide linker, such as divalent sc(Fv)₂.

"dsFv" is a VH::VL heterodimer stabilized by a disulphide bond.

"(dsFv)₂" denotes two dsFv coupled by a peptide linker.

The term "bispecific antibody" or "BsAb" denotes an antibody which combines the antigen-binding sites of two antibodies within a single molecule. Thus, BsAbs are able to bind two different antigens simultaneously. Genetic engineering has been used with increasing frequency to design, modify, and produce antibodies or antibody derivatives with a desired set of binding properties and effector functions as described for instance in EP 2 050 764 A1.

The term "multispecific antibody" denotes an antibody which combines the antigen-binding sites of two or more antibodies within a single molecule.

The term "diabodies" refers to small antibody fragments with two antigen-binding sites, which fragments comprise a heavy-chain variable domain (VH) connected to a light-chain variable domain (VL) in the same polypeptide chain (VH-VL). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced

to pair with the complementary domains of another chain and create two antigen-binding sites.

In a particular embodiment, the epitope-binding fragment is selected from the group consisting of Fv, Fab, F(ab')₂, Fab', dsFv, (dsFv)₂, scFv, sc(Fv)₂, diabodies and VHH.

5 A "chimeric antibody", as used herein, is an antibody in which the constant region, or a portion thereof, is altered, replaced, or exchanged, so that the variable region is linked to a constant region of a different species, or belonging to another antibody class or subclass. "Chimeric antibody" also refers to an antibody in which the variable region, or a portion thereof, is altered, replaced, or exchanged, so that the constant region is linked to a variable
10 region of a different species, or belonging to another antibody class or subclass.

The term "humanized antibody" refers to an antibody which is initially wholly or partially of non-human origin and which has been modified to replace certain amino acids, in particular in the framework regions of the heavy and light chains, in order to avoid or minimize an immune response in humans. The constant domains of a humanized antibody
15 are most of the time human CH and CL domains. In an embodiment, a humanized antibody has constant domains of human origin. As used herein, the term "humanized antibody" refers to a chimeric antibody which contain minimal sequence derived from non-human immunoglobulin, e.g. the CDRs.

The term "antibody" is used to encompass all these kinds of antibodies, fragments or
20 combination thereof.

The goal of humanization is a reduction in the immunogenicity of a xenogenic antibody, such as a murine antibody, for introduction into a human, while maintaining the full antigen binding affinity and specificity of the antibody. Humanized antibodies, or antibodies adapted for non-rejection by other mammals, may be produced using several
25 technologies such as resurfacing and CDR grafting. As used herein, the resurfacing technology uses a combination of molecular modeling, statistical analysis and mutagenesis to alter the non-CDR surfaces of antibody variable regions to resemble the surfaces of known antibodies of the target host.

Antibodies can be humanized using a variety of other techniques including CDR-grafting (EP0239400; WO91/09967; U.S. Patent Nos. 5,530,101 and 5,585,089), veneering
30 or resurfacing (EP0592106; EP0519596; Padlan (1991) Molecular Immunology 28(4/5):489-498; Studnicka et al. (1994) Protein Engineering 7(6):805-814; Roguska et al. (1994) Proc. Natl. Acad. Sci U.S.A. 91:969-973), and chain shuffling (U.S. Patent No. 5,565,332). Human antibodies can be made by a variety of methods known in the art
35 including phage display methods. See also U.S. Patent Nos. 4,444,887, 4,716,111,

5,545,806, and 5,814,318; and International patent application WO98/46645, WO98/50433, WO98/24893, WO98/16654, WO96/34096, WO96/33735, and WO91/10741.

As used herein, the term “specificity” refers to the ability of an antibody to detectably bind an epitope presented on an antigen, such as netrin-1, while having relatively little detectable reactivity with non-netrin-1 proteins or structures (such as other proteins presented on cancer cells, or on other cell types). Specificity can be relatively determined by binding or competitive binding assays, using, e.g., Biacore instruments, as described elsewhere herein. Specificity can be exhibited by, e.g., an about 10:1, about 20:1, about 50:1, about 100:1, 10,000:1 or greater ratio of affinity/avidity in binding to the specific antigen versus nonspecific binding to other irrelevant molecules (in this case the specific antigen is netrin-1).

The term “affinity”, as used herein, means the strength of the binding of an antibody to an epitope. The affinity of an antibody is given by the dissociation constant K_d , defined as $[1] \times [1] / [Ab-Ag]$, where $[Ab-Ag]$ is the molar concentration of the antibody-antigen complex, $[1]$ is the molar concentration of the unbound antibody and $[Ag]$ is the molar concentration of the unbound antigen. The affinity constant K_a is defined by $1/K_d$. Preferred methods for determining the affinity of mAbs can be found in Harlow, et al., *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1988), Coligan et al., eds., *Current Protocols in Immunology*, Greene Publishing Assoc. and Wiley Interscience, N.Y., (1992, 1993), and Muller, *Meth. Enzymol.* 92:589-601 (1983), which references are entirely incorporated herein by reference. One preferred and standard method well known in the art for determining the affinity of mAbs is the use of Biacore instruments.

The present invention will now be described in more detail using the following non-limiting experiments referring to the Figures.

Description of the Figures:

Figure 1: Schematic diagram showing timeline for different interventions performed in this study. (A) Mice were ovariectomised one-week (-1 Wk) prior to surgical induction of uterine horns fragments (Endo) or suture only (Sham) at day 0 (d0). All mice were injected weekly with intraperitoneal (i.p.) estradiol benzoate (vertical red ticks) to maintain steady levels of circulating estrogen throughout the study. Treatment started at 4 weeks post-surgical induction, with groupings consisting of Endo mice treated with NP137 (Endo + NP137 (N =15)) or control antibody NP001 (Endo + Control Ab (N =16)) and Sham with control Ab NP001 (Sham + control Ab (N =16)). Bi-weekly i.p. treatment was maintained throughout the duration of the study. *In vivo* pain assessment studies, comparing Sham (control Ab) and Endo (control Ab and NP137), were performed between weeks 8-10 post-

surgical induction (shaded box). Following the final behavioural assessment, tissue was collected from all mice (diamonds). The total number of mice used for this study was N = 16 Sham (control Ab), N = 16 Endo (control Ab) and N=15 Endo (NP137). **(B)** Mice were ovariectomised one-week (-1 Wk) prior to inoculation of uterine horns fragments (Endo) or saline only (Sham) at day 0 (d0). All mice were injected weekly with intraperitoneal (i.p.) estradiol benzoate (vertical ticks) to maintain steady levels of circulating estrogen throughout the study. Treatment started at 4 weeks post-surgical induction, with groupings consisting of Endo mice treated with NP137 (Endo + NP137) or control antibody NP001 (Endo + Control Ab (N =8)) and Sham with control Ab NP001 (Sham + control Ab (N =4)). Bi-weekly i.p. treatment was maintained throughout the duration of the study. *In vivo* pain assessment studies, comparing Sham (control Ab) and End o (control Ab and NP137), were performed between weeks 8-10 post-surgical induction (shaded box). Following behavioural assessment suing hot plate test, tissue was collected from all mice (diamonds). The total number of mice used was N = 4 Sham (control Ab), N = 8 Endo (control Ab) and N=7 Endo (NP137).

Figure 2: NP137 treatment reduces endometriosis lesion size.

(A1) Representative images of the reproductive tract at 10 weeks post-induction surgery for a Sham mouse treated with control Ab (left box, sutures only on the uterine horns and alongside the uterus (arrows, and arrows heads indicate placement)), Endo mouse (endometrial fragments attached on the uterine horns and alongside the uterus (arrows indicate placement)) treated with either control Ab (middle box) or NP137 (right box).

(A2) Representative images of dissected and cleaned endometriosis lesions collected from the uterus and uterine horns of both Endo mice treated with control Ab (4 mice, left box) and NP137 (3 mice, right box). Measurement bars represent 5 mm.

(B) Grouped data shows a significant reduction in average lesion weight at 10 weeks post-Endo induction following chronic treatment with NP137 compared to control Ab. Grouped data is displayed as **(B1)** the average lesion weight of all lesions per animal (g), **(B2)** the average lesions weight of lesions located on the uterus (g) and **(B3)** the average lesion weight of lesions located on the uterine horns (g).

(C) Grouped data shows a significant reduction in average maximum lesion diameter at 10 weeks post-Endo induction following chronic treatment with NP137 compared to control Ab. Grouped data is displayed as **(C1)** the average maximum lesion diameter of all lesions per animal (mm), **(C2)** the average maximum lesion diameter of lesions located on the uterus (mm) and **(C3)** the average maximum lesion diameter of lesions located on the uterine horns (mm). Data represent mean $\pm$ SEM. NS, $P > 0.05$, $**P < 0.01$, $***P < 0.001$,

Mann-Whitney non-parametric test for 2 groups with unequal variances from N = 9 to 11 mice and n = 29 to 35 lesions per treatment group.

Figure 3: NP137 treatment reduces vaginal hyperalgesia developed in endometriosis mice.

5 (A) Grouped data showing an elevated visceromotor responses (VMR) to vaginal distension (VD) in Endo mice treated with control Ab compared to Sham mice when treated with control Ab from 20 mm Hg – 70 mm Hg. The elevated response in Endo mice was significantly reduced at non-noxious distension pressures in mice treated with NP137. ^ Represents Sham control Ab vs. Endo Control Ab and * represents Endo control Ab vs. Endo NP137. Data represent mean $\pm$ SEM with * or ^P < 0.05, ** or ^^P < 0.01, **** or ^^^P < 0.0001, generalised estimating equations followed by LSD post hoc test.

(B-D) Grouped data expressed as the total area under the curve of the VMR to (B) all distension pressures (20-80 mm Hg), as well as separated into (C) low (20-40 mm Hg) distension pressures and (D) high (60-80 mm Hg) distension pressures of VD shows that the elevated responses seen in Endo mice treated with control Ab compared to Sham mice treated with control Ab is normalised by treatment with NP137. Data represent mean $\pm$ SEM with NS = not significant, *P < 0.05 and **P < 0.01, an ordinary one-way ANOVA with Sidak's multiple comparison post-hoc test for groups of equal variances, from N = 12 – 14 mice per treatment group. Each dot represents the AUC from an individual animal.

20 **Figure 4: NP137 treatment reduces colonic hyperalgesia developed in endometriosis mice.**

(A) Grouped data showing increased visceromotor responses (VMR) to colorectal distension (CRD) in Endo mice (yellow symbols) compared to Sham mice when treated with control Ab (blue symbols). The elevated response to CRD in Endo mice was significantly reduced in mice treated with NP137 (green symbols), from 20 mm Hg – 60 mm Hg. ^ Represents Sham control Ab vs Endo Control Ab and * represents Endo control Ab vs Endo NP137. Data represents mean $\pm$ SEM with * or ^P < 0.05, *** or ^^P < 0.001, **** or ^^^P < 0.0001, generalised estimating equations followed by LSD post hoc test. (B-D) Grouped data expressed as the total area under the curve of the VMR to (B) all distension pressures (20-80 mm Hg), as well as separated into (C) low (20-40 mm Hg) distension pressures and (D) high (50-80 mm Hg) distension pressures of CRD shows that the elevated responses seen in Endo mice treated with control Ab compared to Sham mice treated with control Ab is normalised by treatment with NP137. Data represent mean $\pm$ SEM with NS = not significant, **P < 0.01, ***P < 0.001 and ****P < 0.0001, Kruskal-Wallis test with Dunn's multiple comparisons for groups with unequal variances, from N = 12 – 14 mice per treatment group. Each dot represents the AUC from an individual animal.

Figure 5: NP137 treatment reverts bladder dysfunction developed in endometriosis mice.

(A) Grouped data of total urine spot count in Sham mice treated with control Ab, Endo mice treated with control Ab and Endo mice treated with NP137 showing the significant increase in total spot count produced by Endo mice was normalised with treatment of NP137.

(B) Grouped data showing the voiding patterns (total urine spots) separated into small (100-1,000 dpi) medium (1,001-100,000 dpi) or large (>100,001 dpi) urine spots in Sham mice treated with control Ab (blue symbols), Endo mice treated with control Ab (yellow symbols) and Endo mice treated with NP137 (green symbols). Data shows that the significant increase in small spots produced by Endo mice is normalised with treatment of NP137. Data represent mean $\pm$ SEM with NS = not significant, *P < 0.05 and **P < 0.01, ordinary one-way ANOVA with Turkey's multiple comparison post-hoc test for groups of equal variances; or Kruskal-Wallis test with Dunn's multiple comparisons for groups with unequal variances, from N = 13 – 16 mice per treatment group. Each dot represents an individual animal.

Figure 6: NP137 treatment reverts mechanical and thermal cutaneous hypersensitivity developed in endometriosis mice.

(A) Grouped data displaying the mechanical paw withdrawal threshold induced by Electronic von Frey (EvF) filament stimulation of the hind-paw (g) in Sham mice treated with control Ab, Endo mice treated with control Ab and Endo mice treated with NP137. Data shows the enhanced sensitivity to mechanical stimuli displayed by Endo mice compared to Sham mice treated with control Ab is normalised with NP137 treatment.

(B) Grouped data displaying the latency of reaction to the heat plate (sec) in Sham mice treated with control Ab, Endo mice treated with control Ab and Endo mice treated with NP137. Data shows the enhanced sensitivity to noxious thermal stimuli displayed by Endo mice compared to Sham mice treated with control Ab is absent in NP137 treated Endo mice. Data represent mean $\pm$ SEM with NS = not significant, *P < 0.05, **P < 0.01 and ****P < 0.0001, ordinary one-way ANOVA with Turkey's multiple comparison post-hoc test for groups of equal variances; or Kruskal-Wallis test with Dunn's multiple comparisons for groups with unequal variances, from N = 14 – 16 mice per treatment group. Each dot represents an individual animal.

Figure 7: NP137 treatment improves signs of reduced overall wellbeing displayed in the nest building of endometriosis mice. Grouped data displaying the nest building score (scale 1-5) in Sham mice treated with control Ab, Endo mice treated with control Ab and Endo mice treated with NP137 shows that reduced nesting behaviours seen

in Endo mice treated with control Ab are normalised with NP137 treatment. Data represent mean $\pm$ SEM with NS = not significant and *P < 0.05, Kruskal-Wallis test with Dunn's multiple comparisons for groups with unequal variances, from N = 14 – 16 mice per treatment group. Each dot represents an individual animal.

5 **Figure 8: NP137 treatment tends to revert thermal cutaneous hypersensitivity developed in the syngeneic mouse model of endometriosis.**

Grouped data displaying the latency of reaction to the heat plate (sec) in Sham mice treated with control Ab, Endo mice treated with control Ab and Endo mice treated with NP137. Data shows the tendency to an enhanced in sensitivity to noxious thermal stimuli displayed by Endo mice, compared to Sham mice treated with control Ab, which is partially
10 reverted in NP137 treated Endo mice. Data represent mean $\pm$ SEM with NS = not significant, ordinary one-way ANOVA with Turkey's multiple comparison post-hoc test for groups of equal variances; or Kruskal-Wallis test with Dunn's multiple comparisons for groups with unequal variances, from N = 4 – 8 mice per treatment group. Each dot
15 represents an individual animal.

Figure 9: NP137-induced improvement on overall wellbeing of mice with Endo is confirmed in a new cohort of animals. Grouped data displaying the nest building score (scale 1-5) in Sham mice treated with control Ab, Endo mice treated with control Ab and Endo mice treated with NP137 shows that reduced nesting behaviours seen in Endo mice
20 treated with control Ab are normalised with NP137 treatment. Data represent mean $\pm$ SEM with NS = not significant and *P < 0.05, Kruskal-Wallis test with Dunn's multiple comparisons for groups with unequal variances, from N = 20 – 21 mice per treatment group. Each dot represents an individual animal.

25 **Figure 10: NP137 treatment tends to reduce endometriosis lesion size and the number of lesions developed in the syngeneic mouse model of endometriosis.**

(A) Grouped data shows a slight reduction in average maximum lesion diameter at 10 weeks post-Endo induction following chronic treatment with NP137 compared to control Ab. (B) Grouped data shows a slight reduction in average lesion size per animal at 10 weeks post-Endo induction following chronic treatment with NP137 compared to control Ab. (C)
30 Grouped data shows a slight reduction in the number of lesions developed per animal at 10 weeks post-Endo induction following chronic treatment with NP137 compared to control Ab. Data represent mean $\pm$ SEM. NS, P>0.05, , Mann-Whitney non-parametric test for 2 groups with unequal variances and unpaired TTest for 2 groups with equal variances from N = 5 mice and n = 24 to 33 lesions per treatment group.

35 **Figure 11: NP137-induced reduction of endometriosis lesion size is confirmed in a new cohort of animals.**

(A1) Representative images of the reproductive tract at 10 weeks post-induction surgery for a Sham mouse treated with control Ab (upper panel, sutures only on the uterine horns and alongside the uterus (arrows heads indicate placement)), Endo mouse (endometrial fragments attached on the uterine horns and alongside the uterus (arrows head indicate placement)) treated with either control Ab (middle panel) or NP137 (bottom panel).

(A2) Representative images of dissected and cleaned endometriosis lesions collected from the uterus and uterine horns of both Endo mice treated with control Ab (4 mice, upper panel) and NP137 (4 mice, bottom panel).

(B) Grouped data shows a significant reduction in average lesion weight at 10 weeks post-Endo induction following chronic treatment with NP137 compared to control Ab. Grouped data is displayed as (B1) the average lesion weight of all lesions per animal (g), (B2) the average lesions weight of lesions located on the uterus (g) and (B3) the average lesion weight of lesions located on the uterine horns (g).

(C) Grouped data shows a significant reduction in average maximum lesion diameter at 10 weeks post-Endo induction following chronic treatment with NP137 compared to control Ab. Grouped data is displayed as (C1) the average maximum lesion diameter of all lesions per animal (mm), (C2) the average maximum lesion diameter of lesions located on the uterus (mm) and (C3) the average maximum lesion diameter of lesions located on the uterine horns (mm). Data represent mean $\pm$ SEM. NS, $P > 0.05$, * $P < 0.05$, ** $P < 0.01$, **** $P < 0.0001$, Mann-Whitney non-parametric test for 2 groups with unequal variances from $N = 12$ to 16 mice and $n = 44$ to 53 lesions per treatment group.

Figure 12: NP137-induced reduction of thermal cutaneous hypersensitivity is confirmed in a new cohort of animals.

(A) Grouped data displaying the mechanical paw withdrawal threshold induced by Electronic von Frey (EvF) filament stimulation of the hind-paw (g) in Sham mice treated with control Ab, Endo mice treated with control Ab and Endo mice treated with NP137. Data shows the enhanced sensitivity to mechanical stimuli displayed by Endo mice compared to Sham mice treated with control Ab is normalised with NP137 treatment.

(B) Grouped data displaying the latency of reaction to the heat plate (sec) in Sham mice treated with control Ab, Endo mice treated with control Ab and Endo mice treated with NP137. Data shows the enhanced sensitivity to noxious thermal stimuli displayed by Endo mice compared to Sham mice treated with control Ab is absent in NP137 treated Endo mice. Data represent mean $\pm$ SEM with NS = not significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ and **** $P < 0.0001$, ordinary one-way ANOVA with Turkey's multiple comparison post-hoc

test for groups of equal variances; or Kruskal-Wallis test with Dunn's multiple comparisons for groups with unequal variances, from N = 20 – 22 mice per treatment group. Each dot

1. Animals:

The Animal Ethics Committee of the South Australian Health and Medical Research
5 Institute (SAHMRI) approved all experiments involving animals (ethics number SAM342).
All animal experiments conformed to the relevant regulatory standards and the ARRIVE
guidelines. Female C57BL/6J mice at 6 weeks of age were used and acquired from an in-
house C57BL/6J breeding programme (Jax strain #000664; originally purchased from The
10 Jackson Laboratory (breeding barn MP14; Bar Harbor, ME; USA) within SAHMRI's specific
and opportunistic pathogen-free animal care facility. All female mice used in this study were
virgin (never been mated) and housed in the absence of males from weaning. Mice were
individually housed following surgery within individually ventilated cages (IVC) filled with
coarse chip dust-free aspen bedding (PURA®; Cat# – ASPJMAEB-CA, Niederglatt,
15 Switzerland). Animal cages were stored on IVC racks in housing rooms within a humidity
and temperature-controlled environment, maintaining 22±1 °C and a 12 h light/12 h dark
cycle. Mice had free access to LabDiet® JL Rat and Mouse/Auto6F chow (Cat# 5K52, St.
Louis, MO; USA) and were provided with autoclaved reverse osmosis purified water.

2. Mouse model of surgically induced endometriosis:

For this study, we used an adapted version of our previously established autologous
20 mouse model of surgically induced endometriosis [9] (the whole content of this article is
incorporated herein by reference). For this study, a total of 4 donor uterine horn fragments
were surgically sutured alongside the female reproductive tract, with 2 alongside the uterus
(1 on either side) and 2 attached to the uterine horns (1 on each horn). See Figure 1 for
chematic diagram showing timeline for different interventions performed in this study.

2.1. Ovariectomy surgery:

Seven days prior to surgical induction of endometriosis, female mice were
ovariectomised to deplete endogenous steroid production. Briefly, mice were anesthetized
under isoflurane (2.5% / 0.5 L O₂) and given a low dose (0.05 mg/kg) of analgesic
buprenorphine prior to the commencement of surgery. Following aseptic conditions, a small
30 longitudinal paralumbar incision of the skin and dorsal abdominal muscle was performed to
expose the ovaries on either side. Ovaries were exteriorised and oviduct/ovarian artery and
vein/ovarian ligaments were severed using cauterisation. The uterine horns were then
returned to the abdominal cavity and the superficial skin layer was closed using 9 mm clips
(AutoClip® System, FST). Ovariectomised mice were allowed to recover for a minimum of
35 5 days before removal of surgical clips. As endometriosis is an estrogen dependant disease,
it is important to maintain steady levels of circulating estrogen and minimize any difference

related to the stage of the estrous cycle. With these factors considered, all mice were given an intraperitoneal injection of 100 µg/kg estradiol benzoate (Progynon-B) following ovariectomy, which continued weekly throughout the duration of the study.

2.2. Endometriosis (Endo) and Sham surgeries:

5 Seven days after ovariectomy, experimental mice were surgically induced with endometriosis using uterine horn tissue collected from donor mice. To collect donor tissue, uterine horns were excised and pinned out in a dissecting dish with ice-cold sterile phosphate buffered saline (PBS) containing penicillin (100 U/ml) and streptomycin (100 µg/ml) (Sigma-Aldrich, St. Louis, MO). Uterine horns were longitudinally opened, with 4 x 2
10 mm sections of uterine horn tissue collected using a 2 mm biopsy punch (Kai medical, KAI00010). Recipient experimental mice were then anesthetized under isoflurane and given a low dose (0.05 mg/kg) of analgesic buprenorphine prior to the commencement of surgery. Following aseptic conditions, a small midline abdominal incision was made to expose the abdominal cavity and the 2 mm circular fragments of donor uterine horn tissue were sutured
15 alongside the uterus (2 fragments) and uterine horns (1 fragment on each horn) using 6.0 Prolene sutures. For Sham surgeries, a similar length of the 6.0 Prolene sutures were attached to the same surgical sites as those in Endo surgery, but in the absence of any tissue. Following this, the abdominal muscle was sutured closed, and the superficial skin layer closed using 9 mm clips (AutoClip® System, FST). Throughout the surgery and during
20 the recovery period, animals were positioned on a heating mat to maintain body temperature and monitored daily for complications. No signs of distress or unusual pain behaviours were observed during recovery.

2.3. Mouse model of syngeneic endometriosis:

In this study we used a second model of endometriosis called the syngeneic mouse
25 model of endometriosis. This clinically relevant mouse model is generated by inoculating uterine horn fragments into the peritoneal cavity as described in publication which is incorporated herein by reference [21]. This a minimally invasive model which resembles the naturally occurrence of endometriosis lesions in humans due to the fact that the inoculated fragments randomly distributed throughout the peritoneal cavity, where they implant and
30 developed into lesions [21]. This model developed both lesions and widespread chronic pelvic pain, which are both hallmarks of endometriosis in humans.

2.4. Endometriosis (Endo) and Sham surgeries for the syngeneic model:

Seven days after ovariectomy, experimental mice were induced with endometriosis by
inoculating fragments of uterine horn tissue collected from donor mice into a recipient
35 mouse. To collect donor tissue, uterine horns were excised and pinned out in a dissecting dish with 0.5 ml ice-cold sterile phosphate buffered saline (PBS) containing penicillin (100

U/ml) and streptomycin (100 µg/ml) (Sigma-Aldrich, St. Louis, MO). Uterine horns were longitudinally opened and cut in small fragments. Recipient experimental mice were then anesthetized under isoflurane and given a low dose (0.05 mg/kg) of analgesic buprenorphine prior to the commencement of induction. Following aseptic conditions, a small midline abdominal incision was made to expose the abdominal cavity and half of the collected fragments of donor uterine horn tissue were inoculated, using a 1ml pipette, into the peritoneal cavity of one recipient mouse (total volume of ~0.2ml solution containing fragments). For Sham surgeries, a similar procedure was undertaken, but 0.2ml sterile phosphate buffered saline (PBS) containing penicillin (100 U/ml) and streptomycin (100 µg/ml) (Sigma-Aldrich, St. Louis, MO), were inoculated instead. Following this, the abdominal muscle was sutured closed, and the superficial skin layer closed using 9 mm clips (AutoClip® System, FST). Throughout the surgery and during the recovery period, animals were positioned on a heating mat to maintain body temperature and monitored daily for complications. No signs of distress or unusual pain behaviours were observed during recovery.

3. Characterization of endometriosis lesions growth and development:

Endometriosis lesions growing from the surgically transplanted uterine horn fragments were collected and measured at the completion of all behavioural assays to track endometriosis lesions development following treatment with either control Ab or NP137. All tissue was collected at least 24 hours following the last control Ab or NP137 treatment to minimise any acute effects of either treatment, and at least 3 days post-estradiol administration to minimise estrogen variation between time points.

3.1 Collection and measurement of endometriotic lesions:

Lesions were located, dissected, measured, and weighed post-mortem from Endo (control Ab) and Endo (NP137) mice. A total of 4 endometriosis lesions were collected from each mouse (2 x uterine and 2 x uterine horn lesions). Lesions that were damaged during dissection, or could not be cleanly separated from surrounding tissue, were not included in lesion growth analysis. Measurements at the largest point of the lesion were taken using callipers (Castroviejo callipers 8.5 cm / 3-1/4").

3.2. Statistical analysis of lesion growth:

Data are presented as mean ± SEM, N represents the number of animals and n represents the number of individual lesions per group. Data were graphed using Prism 9 software (GraphPad Software, San Diego, CA, USA). Data was statistically analysed using the Mann-Whitney non-parametric test for 2 groups with unequal variances. Differences were considered statistically significant at ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

4. In vivo assessment of chronic pelvic pain by quantification of visceromotor responses (VMR).

The visceromotor response (VMR) is a nociceptive brainstem reflex consisting of the contraction of the abdominal muscles in response to noxious distension of hollow organs such as the vagina and the colorectum [4, 6, 7, 19, 25, 27, 16]. We recorded the VMR to vaginal distension (VD) or colorectal distension (CRD) as an objective measurement of vaginal and colonic sensitivity to pain in fully conscious animals [14, 3, 22, 24, 5, 2, 8]. Using this technique, we have previously shown that mice with endometriosis develop both colonic and vaginal hypersensitivity [9, 21]. To determine whether chronic treatment with NP137 was able to reduce the vaginal and colonic hypersensitivity developed in a mouse model of endometriosis we used VMR to determine any differences between Sham (control Ab) and Endo mice (control Ab or NP137). All mice were studied at least 24 hours following the last control Ab or NP137 treatment to minimise any acute effects of either treatment, and at least 3 days post-estradiol administration to minimise estrogen variation between time points

4.1. Surgical implantation of electromyography (EMG) electrodes:

The VMR is objectively assessed by recording the electrical activity (electromyography (EMG)) produced by abdominal muscle contractions in response to non-noxious and noxious vaginal or colorectal distensions. To enable this, three days prior to VMR and under isoflurane anaesthesia, the bare endings of two teflon-coated stainless-steel wires (Advent Research Materials Ltd, Oxford, UK) were sutured into the right external oblique abdominal muscle of Sham or Endo mice and tunnelled subcutaneously to be exteriorized at the base of the neck for future access. At the end of the surgery, mice received prophylactic antibiotic (Baytril®; 5 mg/kg s.c.) and analgesic (buprenorphine; 0.5 mg/10 kg s.c.) and returned to their individual housing and allowed to recover for a minimum three days, as previously described [21, 9, 8].

4.2. Assessing visceromotor responses (VMR) to vaginal distension (VD) or colorectal distension (CRD):

On the day of VMR assessment, mice were temporarily anaesthetized using inhaled isoflurane and a saline enema administered via catheter to either the colorectum (100 µL) or vaginal cavity (50 µL) and a balloon inserted into either the colorectum or vaginal cavity for VMR assessment, as previously described [21, 9, 6, 7]. Briefly, a lubricated balloon was gently introduced through the anus and inserted into the colorectum up to 0.25 cm past the anal verge (2.5 cm length latex balloon) for CRD or gently passed through the vagina and inserted up to 1 mm proximal to the vaginal verge (3 mm length latex balloon) for VD. The balloon catheter was secured to the base of the tail with surgical tape and connected to a

barostat (Isobar 3, G&J Electronics, Willowdale, Canada) for graded and pressure-controlled balloon distension. Mice were gently restrained in a mouse restrainer with dorsal access and allowed to recover from anaesthesia for 10 minutes prior to initiation of the distension sequence. Distension sequences were pre-set and applied via the barostat to 20-40-50-60-70-80 mm Hg (20 seconds duration) at 4 minute-intervals for CRD or 20-30-40-60-70-80 mm Hg (30 seconds duration) applied at 3 minute-intervals for VD. The EMG electrodes were relayed to a data acquisition system and the signal was recorded (NL100AK headstage), amplified (NL104), filtered (NL 125/126, Neurolog, Digitimer Ltd, bandpass 50–5000 Hz) and digitized (CED 1401, Cambridge Electronic Design, Cambridge, UK) to a PC for off-line analysis using Spike2 (Cambridge Electronic Design), as previously described [6]. Mice were allowed to recover from their first distension protocol (randomised to either VD or CRD) for a minimum of 3 hours before the next distension protocol. Following this final distension, mice were returned to their IVC cages and returned to the rack for final i.p. treatment and tissue collection.

4.3. Statistical analysis of VMR to VD and CRD

The analogue EMG signal was rectified and integrated and used for analysis of VMR. To quantify the magnitude of the VMR at each distension pressure, the area under the curve (AUC) during the distension was corrected for baseline activity (AUC pre-distension). The AUC was quantified by calculating the AUC of the EMG signal at each distension pressure. In addition, the sum of all the AUC for all distension pressures combined (total AUC) is also analysed as an overall parameter of VMR responses for each individual mouse. VMR data are presented as mean $\pm$ SEM, and N represents the number of animals. Analysis and figures were prepared in GraphPad Prism software (Version 9, San Diego, CA, USA). VMR data for the AUC at each distension pressure were statistically analysed by generalised estimating equations (GEE) followed by least significant difference (LSD) post hoc test when appropriate using SPSS 23.0. Data for total AUC were statistically analysed using Prism 9 software (GraphPad Software, San Diego, CA, USA) and were analysed where appropriate, using an ordinary one-way ANOVA with Sidak's multiple comparison post-hoc test for groups of equal variances; or Kruskal-Wallis test with Dunn's multiple comparisons for groups with unequal variances. Differences were considered statistically significant at $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $****P < 0.0001$.

5. In vivo assessment of evoked cutaneous sensitivity to thermal and mechanical stimuli.

To determine whether chronic treatment with NP137 was able to reduce the elevated evoked pain like behaviours developed in a mouse model of endometriosis, we used both hot plate (thermal sensitivity) and electronic von Frey (EvF) testing (mechanical sensitivity)

to assess cutaneous sensitivity. To ensure consistency and reduce bias, the same investigator carried out all measurements in a blinded fashion. All mice were studied at least 24 hours following the last control Ab or NP137 treatment to minimise any acute effects of either treatment, and at least 3 days post-estradiol administration to minimise estrogen variation between time points.

5.1. Assessing cutaneous sensitivity to mechanical stimuli with electronic von Frey (EvF) reflex:

The Electronic von Frey (EvF) test is used to assess mechanical allodynia to mechanical stimuli by measuring the withdrawal to a stimulus that is not normally painful [12]. We have previously used this technique to demonstrate the development of mechanical sensitivity in a mouse model of endometriosis [9]. Before EvF testing, mice were habituated to the enclosure made of clear Plexiglas (observation chamber approx. 230x240x146 mm, BSBIO PVF, Panlab, Spain) and placed on an elevated wire mesh stand, for 30 minutes each day, over 2 days. On the day of testing, mice were moved to the testing room in their IVC and allowed to acclimatize for a minimum of 15 minutes. Animals were individually placed into the testing arenas, with an aerated lid placed on top, and left undisturbed for 15 minutes. The EvF unit (hand-held force transducer, BSBIOEVF4s, Panlab, Spain) was fitted with a semi-flexible tip and zeroed. Once the animal was still and quiet, the force transducer was applied perpendicularly to the animal's hind paw or lower abdomen, from below. Force was gradually increased until a clear withdrawal response was observed. The maximum force applied (in grams) that elicited the paw or abdominal withdrawal was noted as the withdrawal threshold. Each area was stimulated 5 times, with the average recorded as the experimental withdrawal threshold for each mouse. At the end of testing, mice were returned to their home cage and returned to their IVC rack.

Statistical analysis:

The average force in grams required to elicit a withdrawal response using EvF was recorded for analysis. Data are presented as mean $\pm$ SEM, and N represents the number of animals. Data were statistically analysed using Prism 9 software (GraphPad Software, San Diego, CA, USA) and were analysed where appropriate, using an ordinary one-way ANOVA with Turkey's multiple comparison post-hoc test for groups of equal variances; or Kruskal-Wallis test with Dunn's multiple comparisons for groups with unequal variances. Differences were considered statistically significant at $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $****P < 0.0001$.

5.2. Assessing cutaneous sensitivity to noxious thermal stimuli with hot plate latency:

The hot plate test is a popular test used to measure acute thermal nociception by monitoring reflexive behaviours to thermal stimuli. Using this technique, we have previously

demonstrated the development of thermal hypersensitivity in a mouse model of endometriosis [21, 9]. Prior to testing, mice were moved to the testing room in their IVC and allowed to acclimatize for a minimum of 15 minutes. Mice are then individually placed on a preheated hot plate (LE7406, Harvard Apparatus, Panlab, Spain), set at $54 \pm 1^\circ\text{C}$, which is shown to be a noxious stimuli [12], and surrounded by a clear methacrylate protection casing. The time between being placed on the hot plate and their first sign of pain response, including hind paw licking, hind paw flicking, or jumping (whichever comes first), was recorded as their hot plate latency. Mice were allowed to recover in their cage for 24 hours, and the test was repeated. The average response latency of both tests was recorded as their final hot plate latency. Mice were then returned to their home cage and IVC rack.

Statistical analysis:

The average response, in seconds, was taken from both exposures to the heat plate was recorded for analysis. Data are presented as mean $\pm$ SEM, and N represents the number of animals. Data were statistically analysed using Prism 9 software (GraphPad Software, San Diego, CA, USA) and were analysed using Kruskal-Wallis test with Dunn's multiple comparisons for groups with unequal variances. Differences were considered statistically significant at $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $****P < 0.0001$.

6. In vivo assessment of spontaneous behaviour:

To determine whether chronic treatment with NP137 was able to reduce the enhanced spontaneous pain like behaviours developed in a mouse model of endometriosis, we used voiding pattern analysis together with overnight nest building analysis to determine any differences between Sham (control Ab) and Endo mice (control Ab or NP137). All mice were assessed at least 24 hours following the last control Ab or NP137 treatment to minimise any acute effects of either treatment, and at least 3 days post-estradiol administration to minimise estrogen variation between time points. To ensure consistency and reduce bias, the same investigator carried out all measurements in a blinded fashion. At the end of each testing protocol, mice were returned to their home cage and returned to their IVC rack for subsequent assays.

6.1 In vivo assessment of bladder dysfunction by voiding pattern analysis:

Voiding pattern analysis is a micturition assessment tool that provides information about changes in spontaneous behavioural patterns related to urinary tract pathologies, including overactive bladder (OAB) and interstitial cystitis/painful bladder syndrome (IC/PBS) [16, 17]. Voiding pattern analysis was performed as previously described for assessing changes in bladder function following the development of endometriosis in mice [9, 21].

Voiding pattern analysis was performed from all mice, at 8-weeks post endometriosis or Sham induction surgery and 4 weeks of either control Ab or NP137 treatment. For this, all bedding was removed from each individual Endo and Sham mice home cage and filter paper was positioned on the bottom of each cage. Mice remained in their lined cages for 3 hours, between 9 AM and 12 PM, with free access to food and water. Throughout this time, mice produce spontaneous voiding patterns on the filter paper. Filter paper was collected and stored for imaging, bedding was returned to IVCs and mice returned to the IVC rack.

Urine collected by the filter paper was imaged using an ultraviolet trans-illuminator (Bio-Rad, California, USA) and digitised into binary images using ImageJ software (NIH, ImageJ, 2.0.0). The number and size of urine spots was determined using pre-set thresholds within ImageJ software, and for the purposes of this study, the number of small (100-1,000 pixels), medium (1,001-100,000 pixels) and large (>100,000 pixels) spots were quantified.

Statistical analysis:

Data are presented as mean $\pm$ SEM, and N represents the number of animals. Data were graphed using Prism 9 software (GraphPad Software, San Diego, CA, USA) and, where appropriate, were analysed using an ordinary one-way ANOVA with Turkey's multiple comparison post-hoc test for groups of equal variances; or Kruskal-Wallis test with Dunn's multiple comparisons for groups with unequal variances. Differences were considered statistically significant at * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

6.2. Assessment of overall animal wellbeing by spontaneous overnight nesting building analysis:

Spontaneous nesting analysis is a sensitive assessment tool that provides information about changes in spontaneous behavioural patterns related to overall wellbeing in mice [13]. In this study, we performed overnight nesting analysis using a predefined 5-point scale to assess the effect of chronic NP137 dosing on overall animal wellbeing, as previously described [11].

Nesting pattern analysis was performed on all mice, at 8-weeks post endometriosis or sham induction surgery and 4 weeks of either control Ab or NP137 treatment. For this, all environmental enrichment items (including the original nestlet material, cardboard tunnels and tissue) were removed from each individual Endo and Sham mice home cage, leaving only the original bedding material at the base of each cage. A pre-weighed Nestlet (a 5-cm squares of cotton wafer - approx. 3g) was placed into each cage and mice were returned to the IVC rack and left undisturbed to nest overnight (4 PM to 9 AM). The following morning, individual nests were assessed in a blinded fashion and rated on a definitive predetermined 5-point nest-rating scale ranging from 1-5, with a score of 1 reflecting an untouched nestlet

and a score of 5 representing a fully torn nestlet with high and fluffy walls, as previously described [11]. Environmental enrichment was returned to the home cage and mice were returned to their IVC racks.

Statistical analysis:

Data are presented as mean $\pm$ SEM, and N represents the number of animals. Data were graphed using Prism 9 software (GraphPad Software, San Diego, CA, USA) and were analysed using Kruskal-Wallis test with Dunn' multiple comparisons. Differences were considered statistically significant at $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $****P < 0.0001$.

7. Results and conclusions:

7.1. Mice with endometriosis developed cyst-like endometriotic lesions:

The mouse model of endometriosis used in this study is an adaption of the previously established model of endometriosis induced by surgical transplantation [9]. In this model we transplanted uterine horn fragments on each side of the uterus dome, and at the end of the uterine horns, as detailed in the method section (also **Figure 2A1**). Control mice (Sham mice) were generated following the same surgical procedure, but in the absence of uterine horn tissue (**Figure 2A1**). As previously described [9], transplanted fragments developed into endometriotic lesions 10 weeks after transplantation (also **Figure 2A1**).

7.2. NP137 treatment reduced endometriotic lesions development

To investigate whether NP137 treatment could limit lesion development and provide analgesia to mice with endometriosis we examined the growth of endometrial lesions. The efficacy of NP137 treatment was quantified by comparing the degree of endometriosis development in Endo mice treated with NP137 to that observed in Endo mice treated with the control antibody NP001 (control Ab) (**Figure 2A1**). As previously described, Sham mice do not develop endometrial lesions at the site of surgical sutures (**Figure 2A1**).

Examination of the female reproductive tract of mice with endometriosis shows that chronic NP137 treatment reduced the size of the lesions developed (**Figure 2A1-2**). Reduction in lesions' size is visually evident on **Figure 2A2**, which shows endometrial lesions collected from 4 Endo mice treated with NP001 (control Ab) (**Figure 2A2**, left panel) and endometrial lesions collected from 3 Endo mice treated with NP137 (**Figure 2A2**, right panel). Quantitative analysis of the collected lesions shows NP137 treatment significantly reduced both the weight and size of these endometriotic lesions (**Figure 2B-C**). Interestingly, NP137-induced reduction in lesion size was specific to lesions developed around the uterus, and not to those growing at the end of the uterine horns (**Figure 2A2 and 2B-C**). Remarkably, a separate set of treatments (an additional 4 Endo mice treated with NP001 and 5 Endo mice treated with NP137) confirmed the initial findings showing NP137's ability to significantly reduced lesion size in mice with Endo (**Figure 11A1-2**).

Moreover, inclusion of the data generated from these additional mice, into the overall data (**Figure 11 B-C**), eliminates the regional differences observed in the first set of treatments (**Figure 2A2 and 2B-C**). Therefore, NP137 is efficient on reducing endometriosis lesions development regardless the locations they developed on (**Figure 11B2-3 and 11C2-3**).

5 Interestingly, we also tested the effect of NP137 on reducing lesions developed in a different mouse model of endometriosis known as the syngeneic mouse model of endometriosis (**Figure 10**). This a minimally invasive model which resembles the naturally occurrence of endometriosis lesions in humans due to the fact that the inoculated fragments randomly distributed throughout the peritoneal cavity, where they implant and developed
10 into lesions. Remarkably, NP137 treatment tends to reduce the size and the number of the lesions developed within the peritoneal cavity of the syngeneic mouse model of endometriosis (**Figure 10A-C**). This suggested that NP137 is not only efficient on reducing the growth of the lesions once they are formed but potentially impede the formation of the lesions itself.

15 **7.3. NP137 treatment reduced vaginal hyperalgesia developed in endometriosis.**

Visceral pain was measured *in vivo* by recording the visceromotor pain response (VMR) evoked by distension of the vagina (VD). VMR is the gold standard to measure pain arising from hollow organs such as the vagina [3, 26] and the colon (as per our previous studies [6, 16]). VMR responses were quantified by recording electromyography (EMG)
20 activity from electrodes surgically implanted into the abdominal muscles of the mouse, as previously described [8].

In Sham mice, distension of the vagina increases VMR responses, with the degree of VMR related to the pressure applied (**Figure 3A**). We found that mice with fully developed endometriosis displayed a significant increase in VMR responses to VD at 20 and 40-70 mmHg (**Figure 3A**), and to all distensions combined (**Figure 3B**); indicating the development of vaginal hyperalgesia in endometriosis mice. Remarkably, compared to Endo mice treated with control Ab, treatment with NP137 significantly reduced the VMR response in Endo mice, specifically at lower (<40mmHg) distension pressures (**Figure 3C**). Moreover, the overall effect of NP137 was to normalise the VMR responses to those
30 displayed by Sham control mice (**Figure 3D**).

Overall, these results demonstrates that treatment with the monoclonal antibody NP137 is an effective approach to relieve vaginal hyperalgesia associated with endometriosis.

35 **7.4. NP137 treatment reduced colorectal hyperalgesia developed in endometriosis.**

Women with endometriosis are commonly co-diagnosed with other chronic visceral comorbidities such as irritable bowel syndrome (IBS) [29]. We next aimed to determine whether mice with fully developed endometriosis also have enhanced pain sensitivity evoked by colorectal distension (CRD), and whether NP137 treatment was able to provide analgesia.

We found that, compared to Sham mice treated with control Ab, Endo mice treated with control Ab displayed significant hypersensitivity to CRD at all the distensions tested 20-80 mmHg (**Figure 4A**), and to all distensions combined (**Figure 4B**); indicating the development of colorectal hyperalgesia in endometriosis mice. Similar to that seen with VD when compared to Endo mice treated with control Ab, treatment with NP137 significantly reduced the VMR response in Endo mice, specifically at lower (<40mmHg) distension pressures (**Figure 4C**). Moreover, the overall effect of NP137 was to normalise the VMR responses to that displayed by Sham control mice (**Figure 4D**).

Importantly, these findings are clinically relevant, as women with endometriosis often experience severe CPP involving both the colon and the vagina [20, 29]. Overall, these results show that treatment with the monoclonal anti-netrin antibody, NP137, is an effective approach to relieve colorectal hyperalgesia associated with endometriosis.

7.5. NP137 treatment normalised the altered bladder function developed in endometriosis.

Women with endometriosis often suffer from visceral comorbidities including overactive bladder (OAB) and interstitial cystitis/painful bladder syndrome (IC-PBS). In this study we aimed to determine whether our mouse model of endometriosis developed altered bladder function, and importantly, whether NP137 treatment could normalise function. To investigate this, we examined and compared the spontaneous bladder-voiding patterns of Sham and Endo mice treated with control Ab with Endo mice treated with NP137, as an indication of altered bladder function.

We found that, compared to Sham mice, mice with fully developed endometriosis displayed alterations in bladder voiding patterns, evidenced by an increase in the total number of urine spots (**Figure 5A**). This disrupted, scattered voiding pattern, characterised by an increase in the number of small sized urine spots (**Figure 5B**) is indicative of the OAB/IC-PBS symptoms of urgency and frequency [17].

Interestingly, treatment with NP137 normalised the bladder dysfunction developed in mice with endometriosis, as evidenced by a reduction in the total number of urine spots (**Figure 5A**) observed in Endo mice treated with NP137, specifically the small sized urine spots (**Figure 5B**). Moreover, the overall effect of NP137 was to normalise the voiding patterns to that displayed by Sham control mice (**Figure 5A-B**).

7.6. NP137 treatment normalise the enhanced cutaneous sensitivity to thermal and mechanical stimuli developed in endometriosis

In addition to suffering from comorbidities associated with visceral organs, women with endometriosis can also experience alterations in cutaneous sensation [18, 28]. Here, we determine whether our mouse model of endometriosis displayed altered cutaneous sensitivity, and whether NP137 treatment could correct it. To determine whether the development of endometriosis influences cutaneous sensitivity evoked by thermal and mechanical stimuli, mice underwent hot plate and Electronic von Frey (EvF) testing.

We found that mice with fully developed endometriosis treated with control Ab exhibited an enhanced sensitivity to mechanical stimulation of their hind paw, indicated by a significantly reduced force required to elicit a response compared with their Sham counterparts (**Figure 6A**). The force required to elicit a response is termed the mechanical threshold, with a reduction in this threshold indicating enhanced sensitivity to mechanical stimuli. We also found that Endo control Ab treated mice displayed an enhanced sensitivity to thermal stimuli, indicated by a significantly reduced latency of response to the hot plate compared to their Sham counterparts (**Figure 6B**). These results indicate that our mouse model of endometriosis indeed experiences enhanced sensitivity to cutaneous mechanical and thermal stimuli compared to Sham mice.

Interestingly, we found that treatment with NP137, efficiently reversed the increased sensitivity to both mechanical and thermal stimuli observed in Endo mice treated with control Ab (**Figure 6A-B**). Importantly, the reduction seen was to levels similar to those observed in Sham mice treated with control Ab (**Figure 6A-B**). Remarkably, a separate set of treatments (an additional 6 Endo mice treated with NP001 and 6 Endo mice treated with NP137) confirmed the initial findings showing NP137's ability to significantly reduced both cutaneous mechanical and thermal hypersensitivity in mice with Endo (**Figure 12A-B**).

Moreover, we also found that mice with Endo induced by inoculation of uterine horn fragments into the peritoneal cavity (the syngeneic mouse model), displayed a discrete enhanced sensitivity to thermal stimuli, indicated by a discrete reduction in the latency of response to the hot plate, compared to their Sham counterparts (**Figure 8**). Interestingly, we found that treatment with NP137, tends to reverse this effect observed in Endo mice treated with control Ab (**Figure 8**).

7.7. NP137 improves signs of reduced overall wellbeing displayed in endometriosis

Finally, we tested whether the development of endometriosis alters spontaneous behaviour in a way which is indicative of the deterioration of an animal's overall wellbeing, and whether NP137 treatment could help maintain an animal's overall wellbeing.

Spontaneous nest building analysis is a sensitive assessment tool that provides information about changes in spontaneous behavioural patterns related to overall wellbeing in mice [13].

In this study we analysed the overnight nesting behaviour of Sham and Endo mice treated with control Ab and found that mice with fully developed endometriosis had a reduced capacity to build their nest during this period (**Figure 7**). This reduction in nest building indicates a lack of nocturnal activity, which suggest the animal's wellbeing is reduced by the development of endometriosis. We found that treatment with NP137 partially recovers the nesting behaviours in mice with endometriosis (**Figure 7**), with Endo mice treated with NP137 having similar nesting behaviour to that displayed by Sham mice (**Figure 7**). Remarkably, a separate set of treatments (an additional 6 Endo mice treated with NP001 and 5 Endo mice treated with NP137) confirmed the initial findings showing NP137's ability to significantly improve the overall wellbeing of mice with Endo (**Figure 9**). Inclusion of the data generated from these additional mice, into the overall data shown that NP137 treatment completely reverted the deficiency on overnight nesting generated on mice with Endo. This is evident by the fact mice with Endo treated with NP137 exhibited similar nesting behaviours patterns to those exhibited by Sham mice treated with NP001 (**Figure 9**), indicating its beneficial effect on reducing anxiety like behaviours associated with endometriosis.

7.8. Conclusion

Overall, the results from this pre-clinical study is the demonstration that chronic treatment with NP137, a clinically approved mAb anti-netrin-1, is effective towards reducing the size of endometriotic lesions developed in a clinically relevant mouse model of endometriosis. In addition, NP137 treatment was able to revert the altered sensitivity to pain across multiple peripheral organs, reducing the chronic pelvic pain experienced by mice with fully developed endometriosis. These results strongly suggest NP137 is a feasible therapeutical strategy to provide endometriosis lesions reduction or disappearance and chronic pelvic pain relief in women with endometriosis.

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CLAIMS

1. An anti-netrin-1 antibody or an antigen-binding fragment thereof, for use in treating endometriosis, said treating comprising a reduction of the endometriosis lesions, especially the uterine and/or uterine horn endometriosis lesions.
2. The antibody or antigen-binding fragment thereof for the use of claim 1, wherein said treating further comprises a chronic pelvic pain relief.
3. The antibody or antigen-binding fragment thereof for the use of claim 1 or 2, wherein said treating further comprises a vaginal hyperalgesia relief.
4. The antibody or antigen-binding fragment thereof for the use of any one of claims 1 to 3, wherein said treating further comprises:
- a reduction of colorectal hyperalgesia developed in endometriosis;
 - an improvement of bladder dysfunction developed in endometriosis;
 - a reduction of enhanced cutaneous sensitivity to thermal and/or mechanical stimuli developed in endometriosis;
 - an improvement of the overall wellbeing altered by the endometriosis.
5. The anti-netrin-1 antibody or an antigen-binding fragment thereof for the use of any one of claims 1 to 4, wherein the antibody or antigen-binding fragment specifically binds to a polypeptide of sequence SEQ ID NO: 33.
6. The anti-netrin-1 antibody or an antigen-binding fragment thereof for the use of any one of claims 1 to 5, wherein the antibody is a monoclonal antibody or an antigen-binding fragment thereof, wherein the antibody or fragment comprises
- a variable domain VH comprising:
 - a H-CDR1 having a sequence set forth as SEQ ID NO: 5;
 - a H-CDR2 having a sequence set forth as SEQ ID NO: 6;
 - a H-CDR3 having a sequence set forth as SEQ ID NO: 7;
 - a variable domain VL comprising:
 - a L-CDR1 having a sequence set forth as SEQ ID NO: 8;
 - a L-CDR2 having a sequence YAS;
 - a L-CDR3 having a sequence set forth as SEQ ID NO: 9;
- or
- a variable domain VH comprising:
 - a H-CDR1 having a sequence set forth as SEQ ID NO: 28;
 - a H-CDR2 having a sequence set forth as SEQ ID NO: 29;
 - a H-CDR3 having a sequence set forth as SEQ ID NO: 30;
 - a variable domain VL comprising:

- a L-CDR1 having a sequence set forth as SEQ ID NO: 31;
- a L-CDR2 having a sequence set forth as SEQ ID NO: 32;
- a L-CDR3 having a sequence set forth as SEQ ID NO: 9.

5 7. The anti-netrin-1 antibody or an antigen-binding fragment thereof for the use
of any one of claims 1 to 6, wherein the antibody is a monoclonal antibody or an antigen-
binding fragment thereof, and the antibody or fragment thereof comprises a pair of VH and
VL sequences selected from the following pairs: SEQ ID NO: 27 and 19, SEQ ID NO: 20
and 14, SEQ ID NO: 21 and 15, SEQ ID NO: 22 and 16, SEQ ID NO: 23 and 17, SEQ ID
NO: 24 and 17, SEQ ID NO: 25 and 16, SEQ ID NO: 26 and 17, SEQ ID NO: 22 and 17,
10 SEQ ID NO: 25 and 18, SEQ ID NO: 21 and 16, and preferably the antibody or fragment
thereof comprises a pair of VH and VL sequences SEQ ID NO: 22 and 16.

8. The anti-netrin-1 antibody for the use of any one of claims 1 to 7, comprising
a Human IgG1 Constant heavy chain (CH) and/or a Human IgG1 Constant light chain (CL),
in particular a human kappa constant domain.

15 9. The antibody for the use of any one of claims 1 to 8, comprising a VH of
sequence of SEQ ID NO: 22 and a VL of sequence SEQ ID NO: 16.

10. The antibody for the use of to claim 9, further comprising a human IgG1 CH
Genbank AEL33691.1 modified R97K, and a human IgG1 CL Kappa Genbank
CAC20459.1.