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(54) **NOVEL NICOTINE-BINDING ANTIBODIES**

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(57) **ABSTRACT**

Described are novel nicotine-binding antibodies and meth-
ods of using them for treating nicotine addiction and/or
facilitating smoking cessation, or for treating nicotine over-
dose or nicotine poisoning.

Specification includes a Sequence Listing.

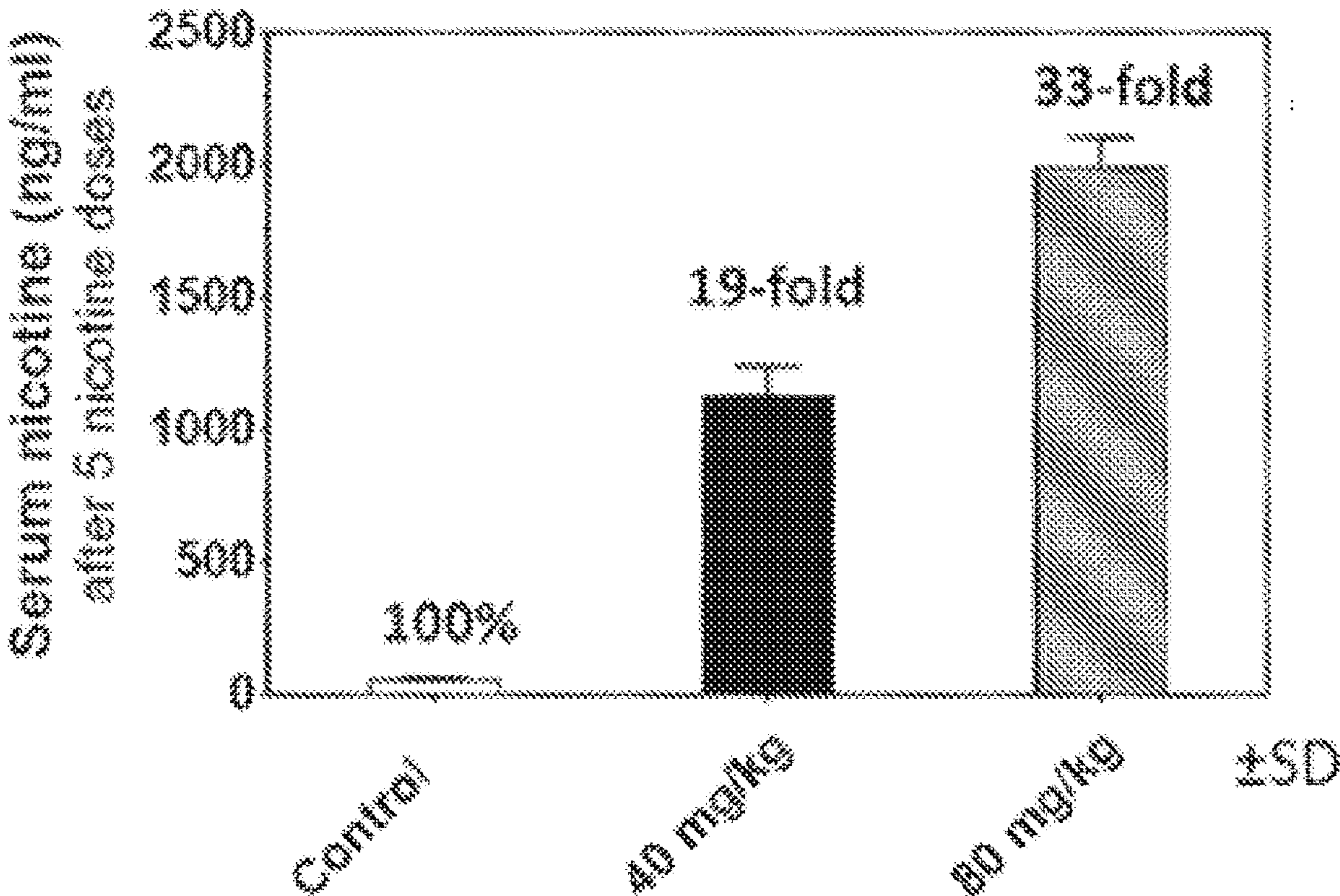


Fig 1A

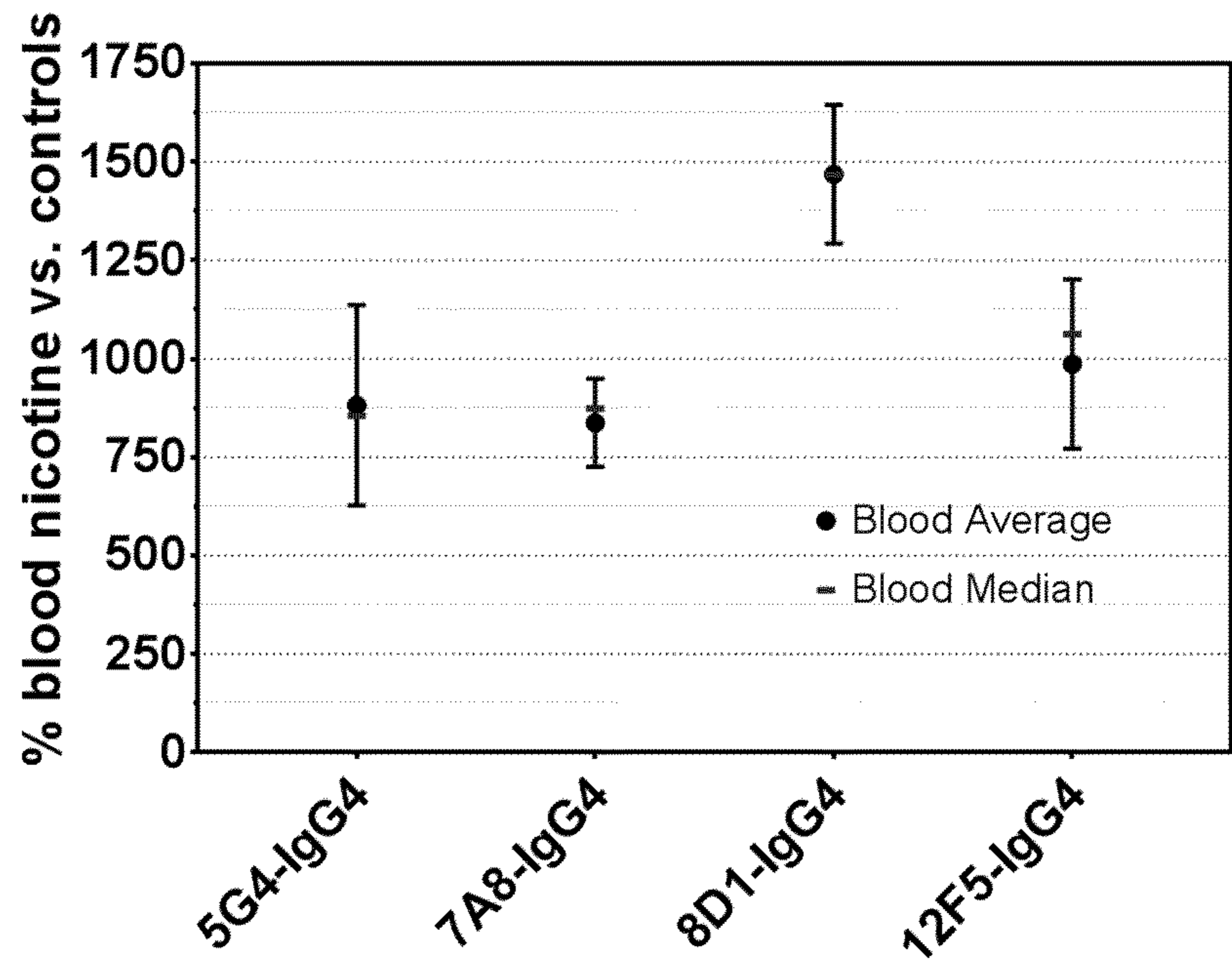


Fig 1B

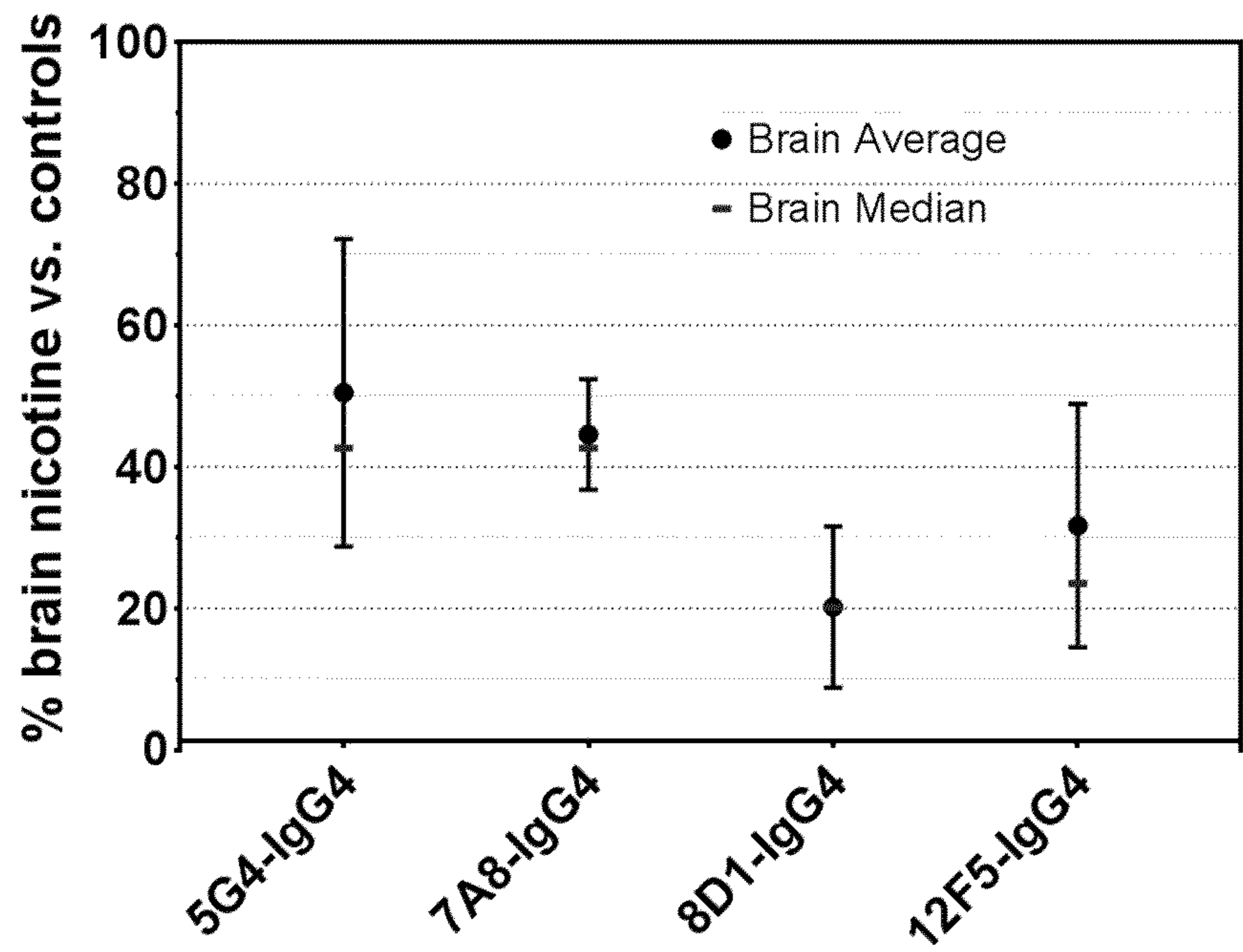


Fig 2A

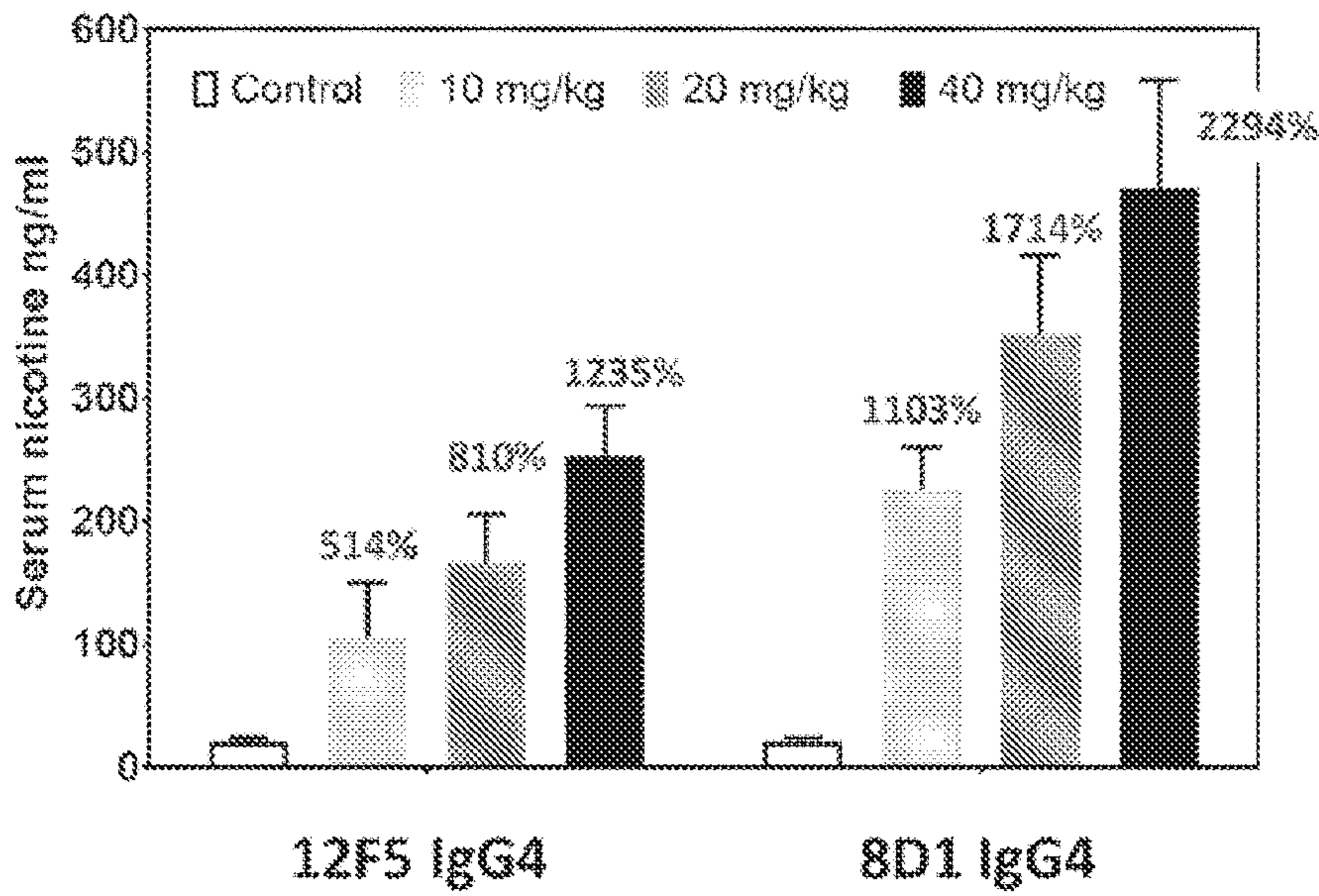


Fig 2B

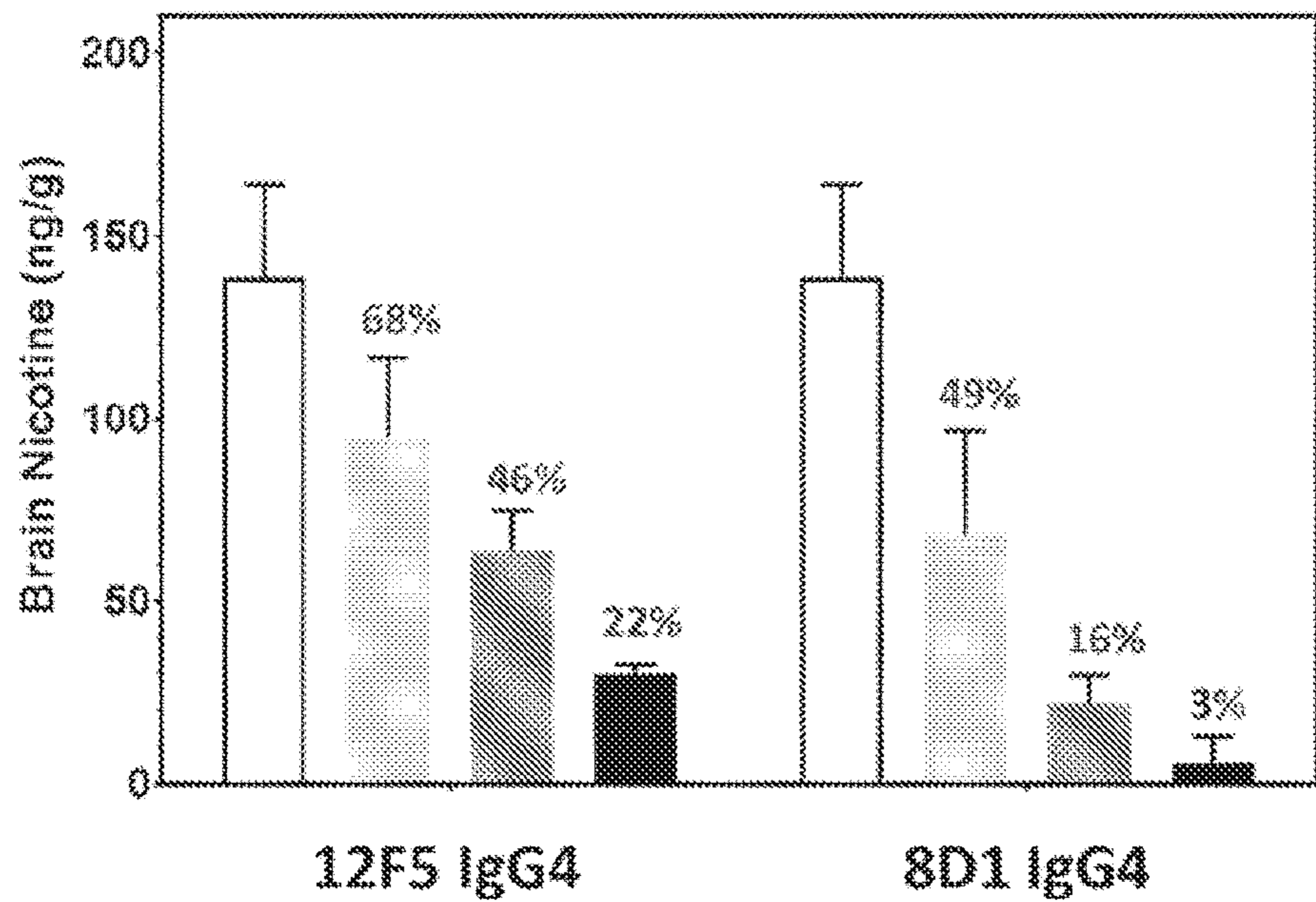


Fig 3A

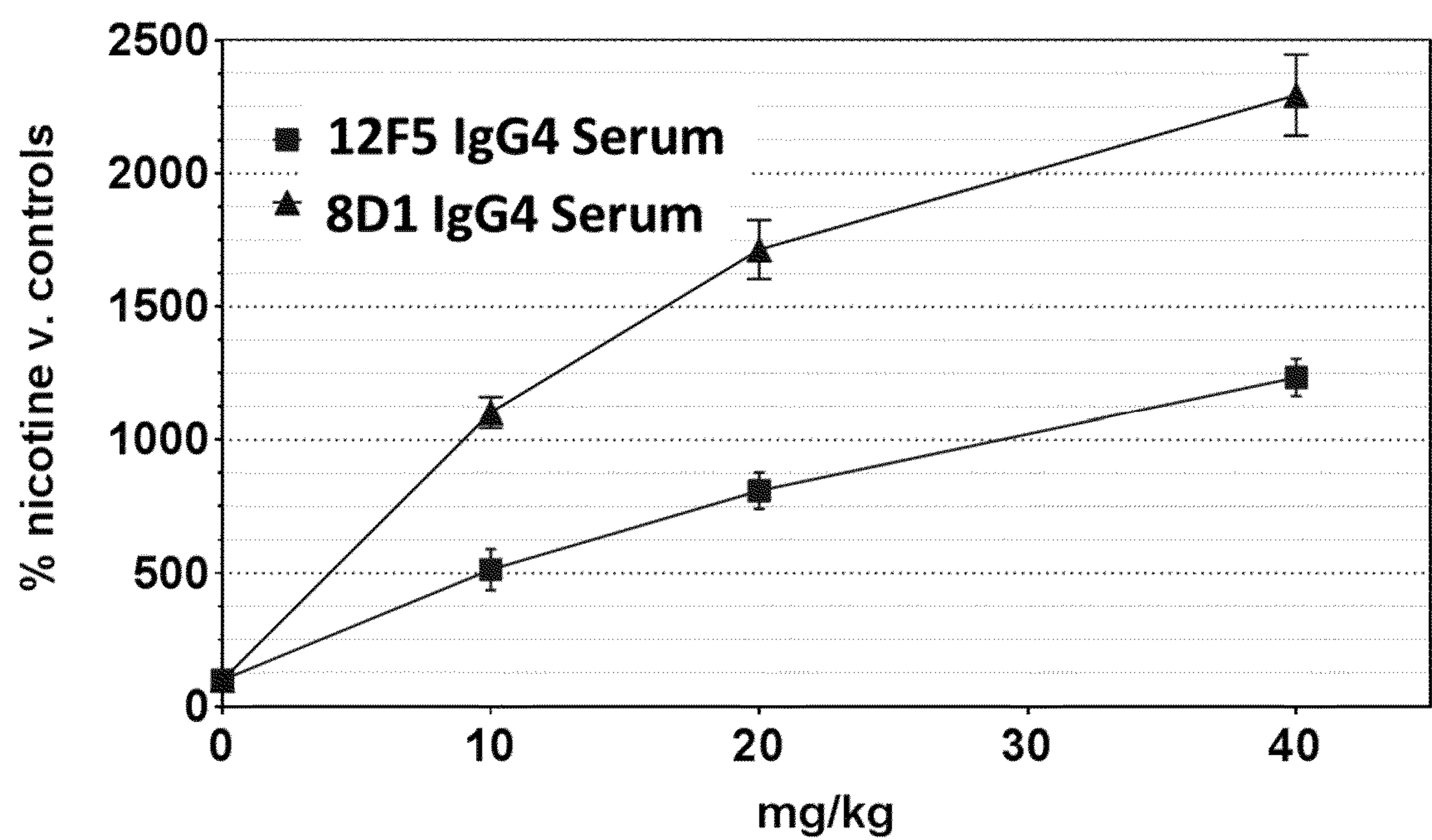


Fig 3B

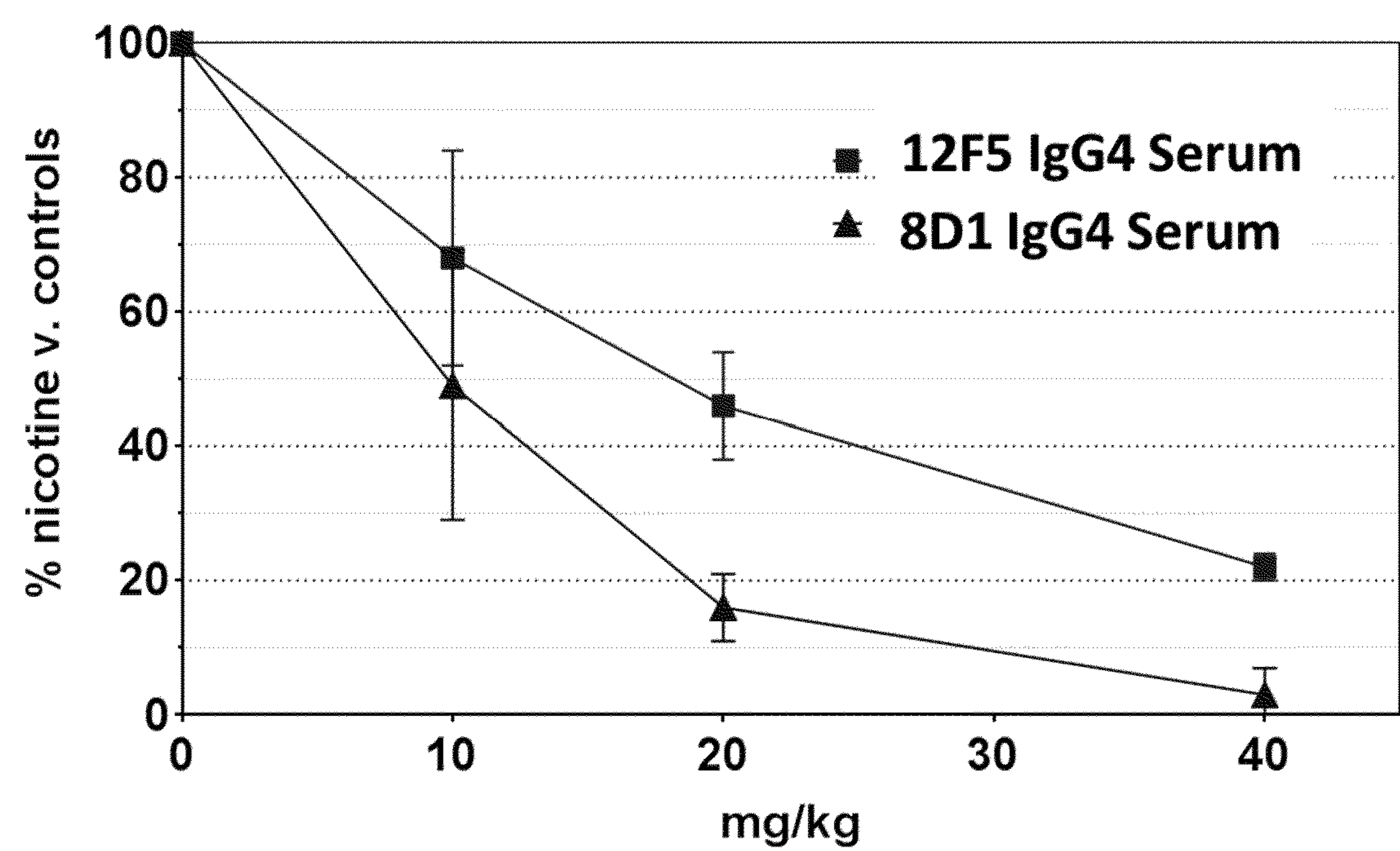


Fig 4A

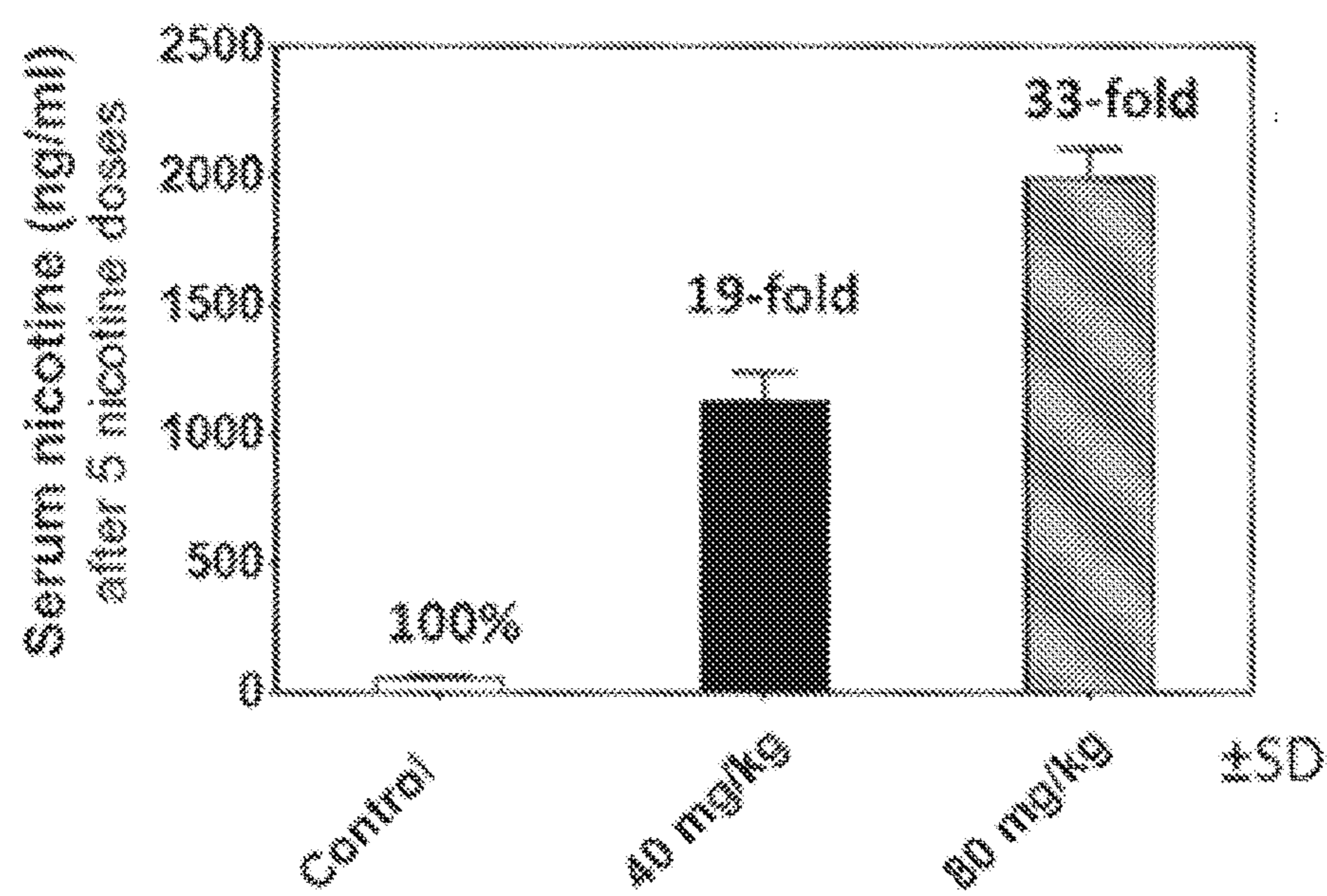


Fig 4B

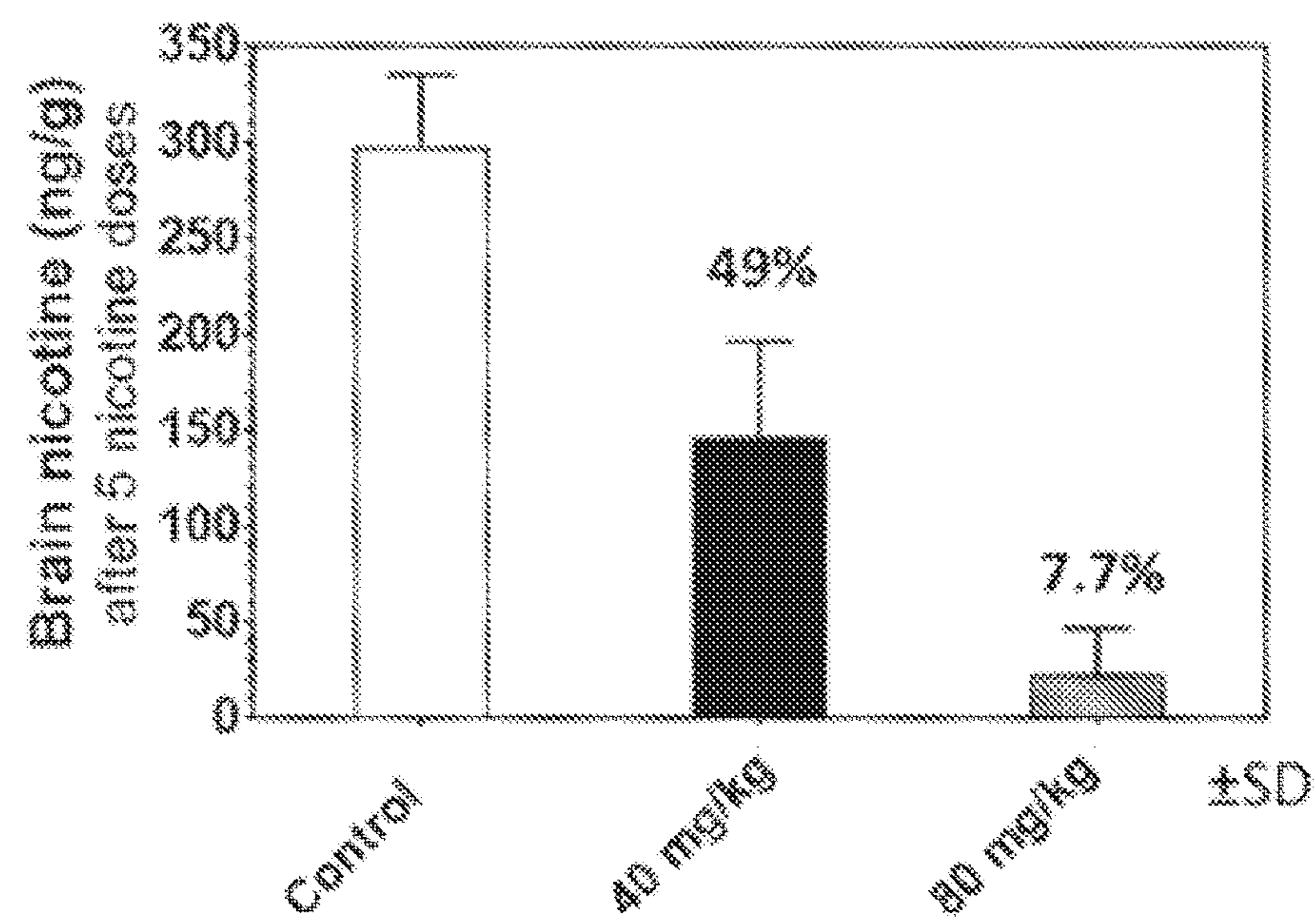


Fig 5

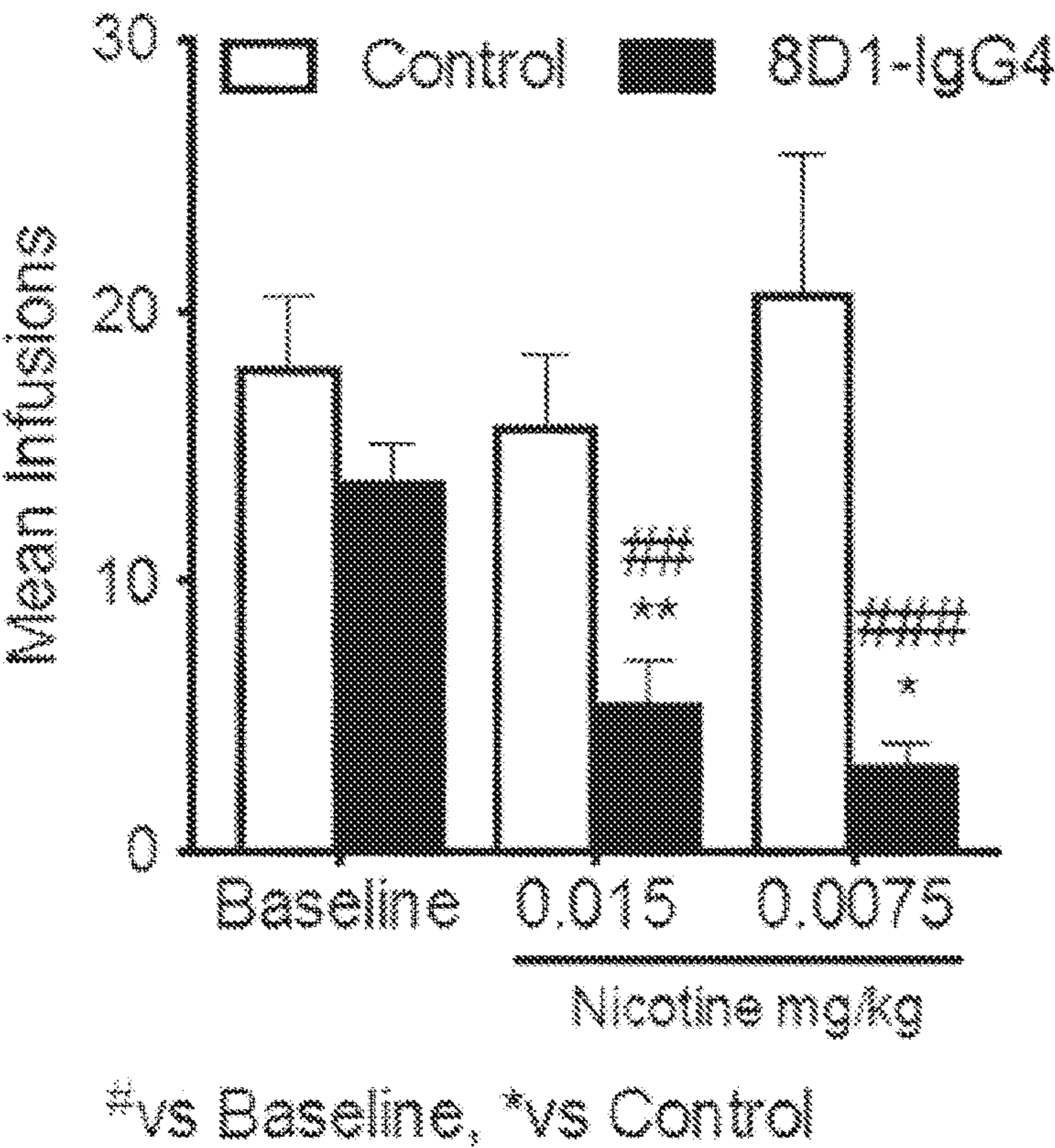


Fig 6

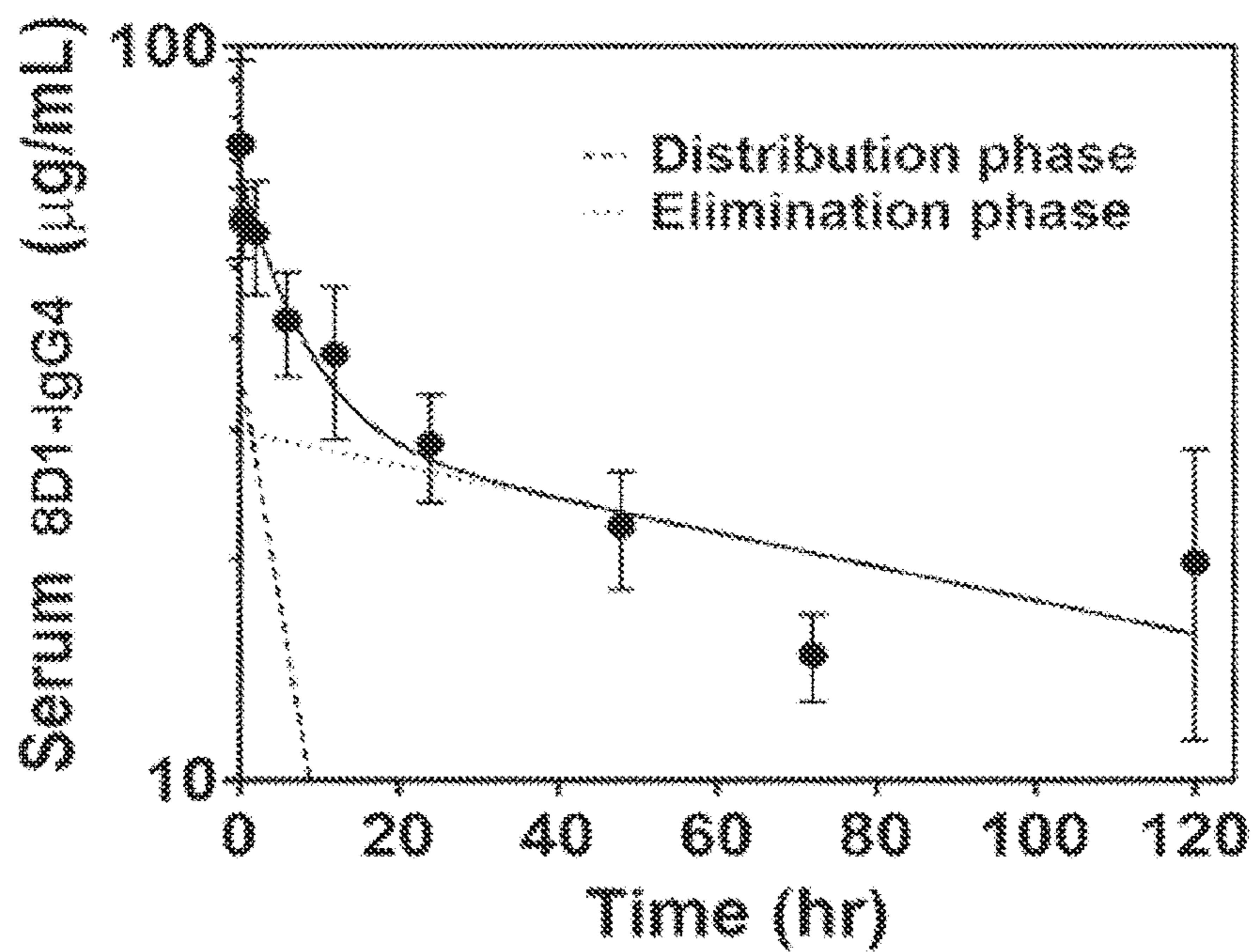
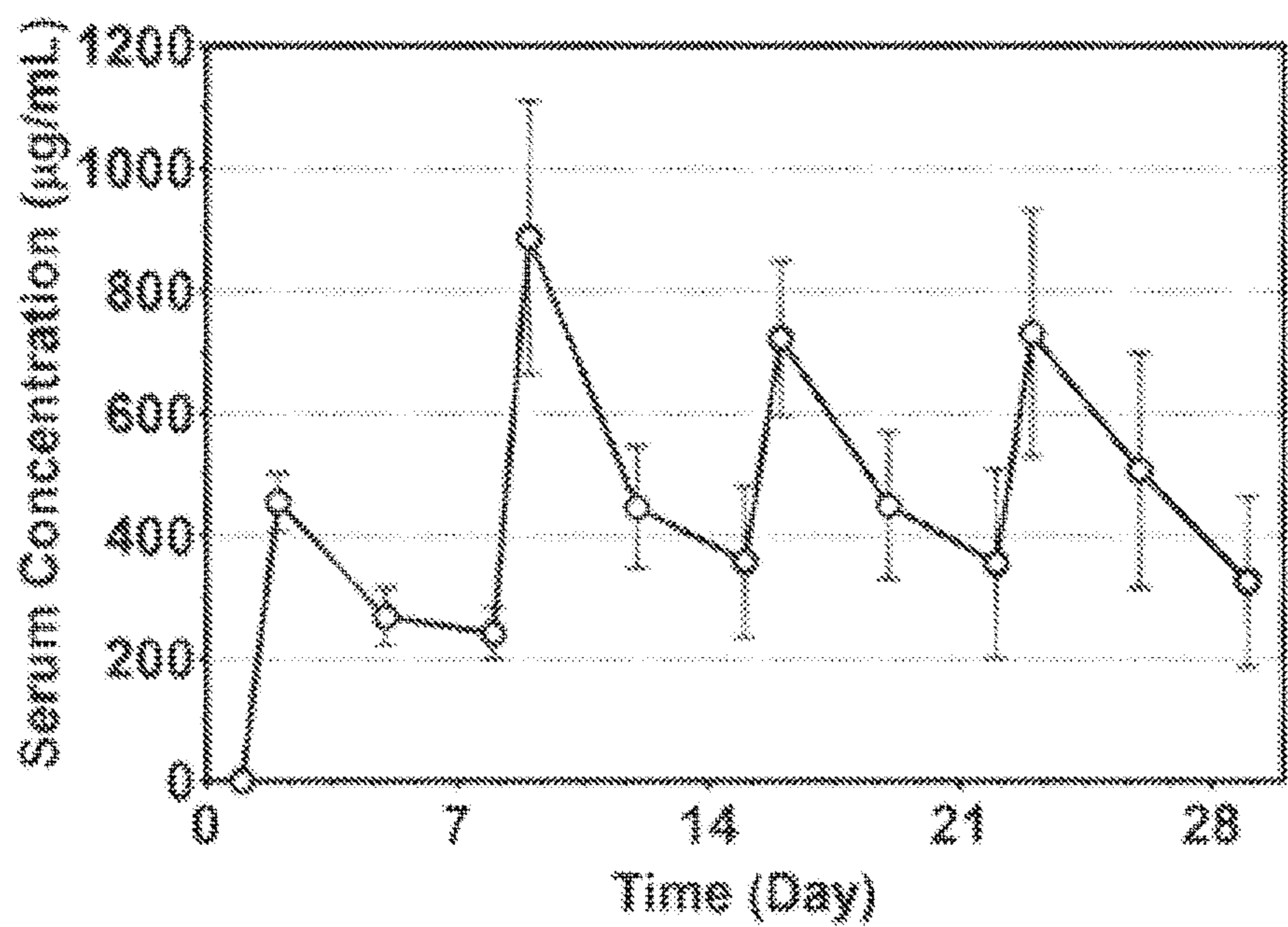


Fig 7



NOVEL NICOTINE-BINDING ANTIBODIES**CROSS-REFERENCE TO RELATED APPLICATIONS**

[0001] This application is a continuation of U.S. application Ser. No. 16/639,050, filed Feb. 13, 2020, which is the U.S. National Stage of International Application PCT/US2018/046621, which claims priority under 35 U.S.C. § 119(e) to U.S. Provisional Application 62/545,696 filed Aug. 15, 2017, the entire contents of which are incorporated herein by reference.

GOVERNMENT LICENSE RIGHTS

[0002] This invention was made with Government support under National Institutes of Health grant RO1 DA038877 awarded by the PHS. The Government has certain rights in the invention.

SEQUENCE LISTING

[0003] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety.

FIELD

[0004] The present disclosure relates generally to the field of antibody therapeutics, specifically antibodies that bind to nicotine. The disclosed nicotine-binding antibodies can be used in methods of aiding smoking cessation and methods of treating nicotine toxicity, including nicotine poisoning and nicotine overdose.

BACKGROUND

[0005] The following discussion is merely provided to aid the reader in understanding the disclosure and is not admitted to describe or constitute prior art thereto.

[0006] Nicotine is a bitter-tasting, parasympathomimetic alkaloid compound that naturally occurs in large amounts in the leaves of tobacco plants. Nicotine is a nicotinic acetylcholine receptor (nAChR) agonist and functions physiologically as a stimulant. Nicotine is both addictive and toxic, and its ingestion and inhalation have been associated with cardiovascular disease, potential birth defects, and poisoning.

[0007] Smoking is a global healthcare problem, largely due to the addictiveness of nicotine. The World Health Organization estimates that there are 1.3 billion smokers worldwide today and nearly five million tobacco-related deaths each year. If current smoking patterns continue, smoking will cause some 10 million deaths each year by 2020. According to the U.S. Center for Disease Control (CDC), tobacco use is the single leading preventable cause of death in the U.S., responsible for approximately 438,000 deaths each year. In addition, it is estimated that smoking results in an annual health-related economic cost of approximately \$157 billion. The CDC estimates that, among the 45 million adult smokers in the U.S., 70% want to quit, but less than five percent of those who try to quit remain smoke-free after 12 months.

[0008] Addiction to the nicotine in cigarettes and other tobacco products makes it difficult for individuals to quit smoking or using tobacco products. Nicotine is a small molecule that upon inhalation or ingestion into the body quickly passes into the bloodstream and subsequently

reaches the brain by crossing the blood-brain barrier. Once in the brain, the nicotine binds to nicotinic receptors, which results in the release of stimulants, such as dopamine, activating the reward system and providing the smoker with a positive and pleasurable re-enforcing experience, which leads to addiction.

[0009] Nicotine poisoning, which results from ingestion or inhalation of too much nicotine, is another nicotine-related health problem. The LD₅₀ of nicotine is 50 mg/kg for rats and 3 mg/kg for mice. A dose as low as 30-60 mg (0.5-1.0 mg/kg) may be lethal for adult humans, while children may become ill following ingestion of one cigarette, and ingestion of more than this may cause a child to become severely ill. On the other hand, some evidence suggests that a lethal dose may be as high as 500 mg or more (1.0-7.1 mg/kg) for a human adult. In either case, acute nicotine poisoning usually occurs in children who accidentally chew on nicotine gum or patches or ingest the “e-liquid” of electronic cigarettes. In rare instances, children have also been known to become ill after ingesting cigarettes. There are several hundred cases of acute nicotine poisoning reported every month in the United States alone.

[0010] Symptoms of nicotine poisoning can include abdominal cramping, agitation, restlessness, or excitement, a burning sensation in the mouth, headache, vomiting, muscle twitching, fainting, rapid breathing and heartrate, and weakness, as well as more serious complications like convulsions and seizures, coma, and potentially death. The ultimate outlook for a person depends on the amount of nicotine at issue and how quickly treatment is received. The faster a person gets medical help, the better the chance for recovery.

[0011] Typically, initial treatment of nicotine poisoning may include the administration of activated charcoal to try to reduce gastrointestinal absorption, while additional treatment may address the symptoms that result from nicotine poisoning.

[0012] Thus, there remains a need for effective agents, compositions and methods for aiding smoking cessation and treating nicotine poisoning.

SUMMARY

[0013] Described herein are antibodies that bind nicotine, compositions comprising the antibodies, and methods using them for aiding smoking cessation and treating nicotine toxicity, including nicotine poisoning and nicotine overdose.

[0014] In one aspect, the present disclosure provides nicotine-binding antibodies or nicotine-binding fragments thereof, comprising the complementarity determining regions (CDRs), the variable regions, or the full heavy chain and light chain of the sequences selected from: the heavy chain sequence of SEQ ID NO: 1 and the light chain sequence of SEQ ID NO: 2; the heavy chain sequence of SEQ ID NO: 3 and the light chain sequence of SEQ ID NO: 4; the heavy chain sequence of SEQ ID NO: 5 and the light chain sequence of SEQ ID NO: 6; the heavy chain sequence of SEQ ID NO: 7 and the light chain sequence of SEQ ID NO: 8; the heavy chain sequence of SEQ ID NO: 9 and the light chain sequence of SEQ ID NO: 10; the heavy chain sequence of SEQ ID NO: 11 and the light chain sequence of SEQ ID NO: 12; the heavy chain sequence of SEQ ID NO: 13 and the light chain sequence of SEQ ID NO: 14; the heavy chain sequence of SEQ ID NO: 15 and the light chain sequence of SEQ ID NO: 16; the heavy chain sequence of

SEQ ID NO: 17 and the light chain sequence of SEQ ID NO: 18; the heavy chain sequence of SEQ ID NO: 19 and the light chain sequence of SEQ ID NO: 20; the heavy chain sequence of SEQ ID NO: 21 and the light chain sequence of SEQ ID NO: 22; the heavy chain sequence of SEQ ID NO: 23 and the light chain sequence of SEQ ID NO: 24; the heavy chain sequence of SEQ ID NO: 25 and the light chain sequence of SEQ ID NO: 26; the heavy chain sequence of SEQ ID NO: 27 and the light chain sequence of SEQ ID NO: 28; the heavy chain sequence of SEQ ID NO: 29 and the light chain sequence of SEQ ID NO: 30; the heavy chain sequence of SEQ ID NO: 31 and the light chain sequence of SEQ ID NO: 32; the heavy chain sequence of SEQ ID NO: 33 and the light chain sequence of SEQ ID NO: 34; the heavy chain sequence of SEQ ID NO: 35 and the light chain sequence of SEQ ID NO: 36; the heavy chain sequence of SEQ ID NO: 37 and the light chain sequence of SEQ ID NO: 38; and the heavy chain sequence of SEQ ID NO: 39 and the light chain sequence of SEQ ID NO: 40.

[0015] In some embodiments the antibody or fragment may be an IgG4 or derived from an IgG4, and in some embodiments the antibody or fragment may comprise a S228P substitution in its Fc domain.

[0016] In some embodiments, the antibody or fragment may be a long-acting variant, such as an antibody or fragment that is conjugated to polyethylene glycol ("PEG"; i.e., the antibody or fragment is PEGylated).

[0017] In some embodiments, the antibody or fragment has a K_D for S-(−)-nicotine of less than about 100 nM. For example, in some embodiments, the K_D for S-(−)-nicotine may be less than about 60 nM, less than about 30 nM, less than about 10 nM, or less than about 5 nM.

[0018] In some embodiments, the antibody or fragment is substantially not cross-reactive with cotinine or other non-nicotine molecules. For example, in some embodiments, the antibody or fragment is substantially not cross-reactive with one or more nicotine-related compounds selected from cotinine, nicotinamide, B-nicotinamide adenine dinucleotide and nornicotine. In some embodiments, the antibody or fragment is substantially not cross-reactive with one or more smoking-cessation drugs selected from bupropion, varenicline, and cytosine. In some embodiments, the antibody or fragment is substantially not cross-reactive with one or more neurotransmitters selected from acetylcholine chloride, 3-hydroxytyramine (dopamine), serotonin, and norepinephrine.

[0019] In another aspect, the present disclosure provides pharmaceutical compositions comprising a nicotine-binding antibody or nicotine-binding fragment thereof according to of any one of the embodiments above or disclosed herein and a pharmaceutically acceptable carrier. In some embodiments, the pharmaceutical composition may be formulated for injection or infusion.

[0020] In another aspect, the present disclosure provides methods of treating nicotine addiction or facilitating smoking cessation, comprising administering to a mammalian subject in need thereof a therapeutically effective amount of a nicotine-binding antibody or nicotine-binding fragment thereof according to of any one of the embodiments above or disclosed herein, or a pharmaceutical composition comprising the same. In some embodiments, the therapeutically effective amount is effective to reduce plasma levels of nicotine and/or to reduce levels of nicotine localized in the brain. In some embodiments, the subject is a human. In some

embodiments, the nicotine addiction is associated with the consumption of a nicotine product selected from tobacco products and electronic cigarettes. In some embodiments, at least one symptom of nicotine withdrawal is reduced, ameliorated, or eliminated.

[0021] In some embodiments, the nicotine-binding antibody or nicotine-binding fragment is administered a route of administration selected from the group consisting of intravenously, subcutaneously, intramuscularly, intraperitoneally, orally, nasally, pulmonarily, ocularly, vaginally, or rectally.

[0022] In another aspect, the present disclosure provides uses of a nicotine-binding antibody or nicotine-binding fragment thereof according to any one of the embodiments above or disclosed herein in the manufacture of a medicament for the treatment of nicotine addiction or facilitating smoking cessation.

[0023] In another aspect, the present disclosure provides nicotine-binding antibodies or nicotine-binding fragments thereof according to any one of the embodiments above or disclosed herein, for use in the treatment of nicotine addiction or facilitating smoking cessation.

[0024] In another aspect, the present disclosure provides methods of treating nicotine overdose or nicotine poisoning, comprising administering to a mammalian subject in need thereof a therapeutically effective amount of a nicotine-binding antibody or nicotine-binding fragment thereof according to of any one of the embodiments above or disclosed herein, or a pharmaceutical composition comprising the same. In some embodiments, the therapeutically effective amount is effective to reduce plasma levels of nicotine and/or to reduce levels of nicotine localized in the brain. In some embodiments, the subject is a mammal selected from the group consisting of canines, felines, equines, bovines, and humans. For examples, in some embodiments, the subject is a human child.

[0025] In some embodiments, the antibody or nicotine-binding fragment is administered a route of administration selected from the group consisting of intravenously, subcutaneously, intramuscularly, intraperitoneally, orally, nasally, pulmonarily, ocularly, vaginally, or rectally.

[0026] In some embodiments, the methods of treating nicotine poisoning or toxicity may further comprise administration of a second compound for treating nicotine overdose or nicotine poisoning, such as activated charcoal.

[0027] In another aspect, the present disclosure provides uses of a nicotine-binding antibody or nicotine-binding fragment thereof according to any one of the embodiments above or disclosed herein in the manufacture of a medicament for the treatment of nicotine overdose or nicotine poisoning.

[0028] In another aspect, the present disclosure provides nicotine-binding antibodies or nicotine-binding fragments thereof according to any one of the embodiments above or disclosed herein, for use in the treatment of nicotine overdose or nicotine poisoning.

[0029] The foregoing general description and following detailed description are exemplary and explanatory and are intended to provide further explanation of the invention.

BRIEF DESCRIPTION OF DRAWINGS

[0030] FIGS. 1A and 1B show the results of a nicotine pharmacokinetic study in rats. FIG. 1A shows the serum concentration of nicotine in rats that were pre-treated with the disclosed antibodies as a percent of serum levels in

control rats that were not treated with antibody. FIG. 1B shows the concentration of nicotine in the brains of rats that were pre-treated with the disclosed antibodies as a percent of the brain levels of control rats that were not treated with antibody.

[0031] FIGS. 2A and 2B show the results of a dose response study of exemplary nicotine-binding antibodies in rats. FIG. 2A shows the serum concentration of nicotine (ng/ml) of rats that were pre-treated with the disclosed antibodies at a dose of 10, 20, or 40 mg/kg. FIG. 2B shows the concentration of nicotine in the brains (ng/g) of rats that were pre-treated with the disclosed antibodies at the same doses.

[0032] FIGS. 3A and 3B show the results from a dose response study of the exemplary nicotine-binding antibodies in rats. FIG. 3A shows the serum concentration of nicotine as a percent of serum levels in control rats that were not treated with antibody. FIG. 3B shows the concentration of nicotine in the brain as a percent of brain levels in control rats that were not treated with antibody.

[0033] FIGS. 4A and 4B shows the impact of multiple doses of nicotine—simulating a heavy smoker—after pre-treatment with disclosed nicotine-binding antibodies. When pretreated with 8D1-IgG4, rats showed an increase in serum nicotine levels after 5 nicotine doses (FIG. 4A) and a decrease in brain nicotine levels (FIG. 4B).

[0034] FIG. 5 shows that treatment with disclosed nicotine-binding antibodies reduces nicotine self-administration in rats. When rats were treated with 8D1-IgG4 the mean (\pm SEM) number of self-administered infusions during the last three sessions before (Baseline) was significantly higher than the number of infusions during antibody treatment at each unit nicotine dose.

[0035] FIG. 6 shows the single dose pharmacokinetics of 8D1-IgG4 in rats when administered at a dose of 20 mg/kg.

[0036] FIG. 7 shows the pharmacokinetics of repeated dosing of 8D1-IgG4 in rats over an extended period of time. Rats were administered a 40 mg/kg dose once per week for 4 weeks.

DETAILED DESCRIPTION

[0037] Described herein are nicotine-binding antibodies, compositions comprising the antibodies, and methods using them, including for treating nicotine addiction and facilitating nicotine cessation (e.g., smoking cessation) and treating nicotine toxicity, including nicotine poisoning and nicotine overdose.

I. Definitions

[0038] As used in the description of the invention and the appended claims, the singular forms “a”, “an” and “the” are used interchangeably and intended to include the plural forms as well and fall within each meaning, unless the context clearly indicates otherwise. Also, as used herein, “and/or” refers to and encompasses any and all possible combinations of one or more of the listed items, as well as the lack of combinations when interpreted in the alternative (“or”).

[0039] As used herein, the term “about” will be understood by persons of ordinary skill in the art and will vary to some extent depending upon the context in which it is used. If there are uses of the term which are not clear to persons

of ordinary skill in the art given the context in which it is used, “about” will mean up to plus or minus 10% of the particular term.

[0040] As used herein, the phrases “therapeutically effective amount” and “therapeutic level” mean that drug dosage or plasma concentration in a subject that provides the specific pharmacological effect for which the drug is administered in a subject in need of such treatment, i.e. to reduce, ameliorate, or eliminate the symptoms or effects of nicotine poisoning or nicotine overdose, and/or treat nicotine addiction and/or facilitate smoking cessation. It is emphasized that a therapeutically effective amount or therapeutic level of a drug will not always be effective in treating the conditions described herein, even though such dosage is deemed to be a therapeutically effective amount by those of skill in the art. For convenience only, exemplary dosages, drug delivery amounts, therapeutically effective amounts, and therapeutic levels are provided below. Those skilled in the art can adjust such amounts in accordance with standard practices as needed to treat a specific subject and/or condition. The therapeutically effective amount may vary based on the route of administration and dosage form, the age and weight of the subject, and/or the subject’s condition, including the amount of nicotine ingested and/or the subject’s plasma levels of nicotine at the time of treatment and/or the amount of nicotine localized in the brain at the time of treatment.

[0041] The terms “treatment” or “treating” as used herein with reference to nicotine toxicity, nicotine poisoning, and nicotine overdose refer to reducing, ameliorating or eliminating one or more symptoms or effects of nicotine and/or reducing the subject’s plasma levels of nicotine and/or reducing the amount of nicotine localized in specific tissues of the subject (e.g., brain/central nervous system, heart and vasculature, etc.).

[0042] Alternatively, the terms “treatment” or “treating” as used herein with reference to nicotine addiction or smoking cessation refers to one or more of: reducing, ameliorating or eliminating one or more symptoms or effects of nicotine withdrawal; reducing the daily number of cigarettes or the daily amount of nicotine consumed by a subject; and/or reducing the subject’s plasma levels of nicotine and/or reducing the amount of nicotine localized in specific tissues of the subject (e.g., brain/central nervous system, heart and vasculature, etc.).

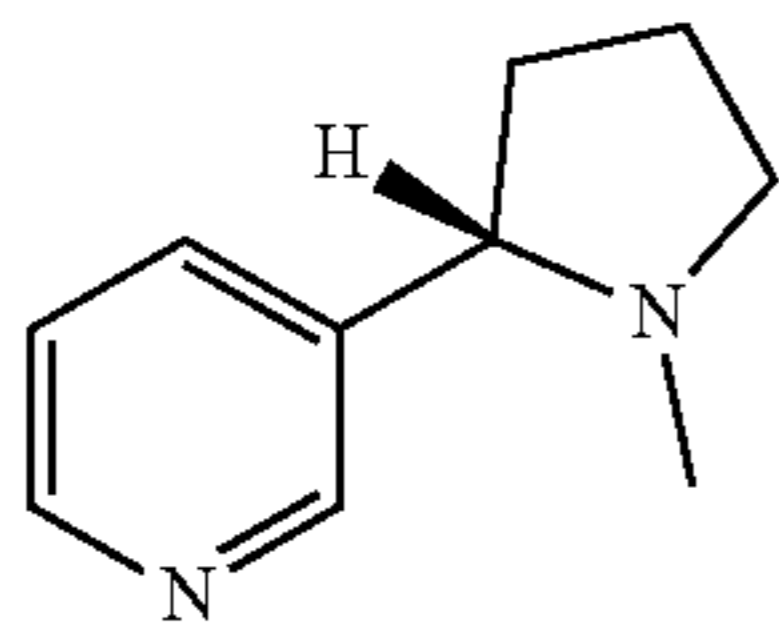
[0043] The terms “individual,” “subject,” and “patient” are used interchangeably herein, and refer to any individual mammal subject, e.g., bovine, canine, feline, equine, or human.

[0044] As used herein, “child” refers to a human subject from 0 through about 18 years of age. A child can be a subject that begins a course of treatment prior to turning about 18 years of age, even if the subject continues treatment beyond 18 years of age.

II. Nicotine, Addiction, and Toxicity

[0045] Nicotine is a nitrogen-containing chemical made by several types of plants including tobacco and other members of the nightshade family. When humans, mammals and most other types of animals are exposed to nicotine, it increases their heart rate, heart muscle oxygen consumption rate, and heart stroke volume. The consumption of nicotine is also linked to raised alertness, euphoria, and a sensation of being relaxed. However, nicotine is highly addictive.

[0046] By binding to nicotinic acetylcholine receptors in the brain, nicotine elicits its psychoactive effects and increases the levels of several neurotransmitters in various brain structures. Nicotine has a higher affinity for nicotinic receptors in the brain than those in skeletal muscle, though at toxic doses it can induce contractions and respiratory paralysis. Nicotine's selectivity is thought to be due to a particular amino acid difference on these receptor subtypes. The structure of nicotine is shown in Formula I below.



Formula I

[0047] People who regularly consume nicotine and then suddenly stop experience withdrawal symptoms, which may include cravings, a sense of emptiness, anxiety, depression, moodiness, irritability, and inattentiveness. The American Heart Association says that nicotine (from smoking tobacco) is one of the hardest substances to quit, at least as hard as heroin.

[0048] The methods described herein useful in treating nicotine addiction and/or facilitating smoking cessation (or the cessation of use of other tobacco or nicotine products) in a mammalian subject in need thereof, use nicotine-binding antibodies, which bind nicotine and prevent it from interacting with nicotinic acetylcholine receptors.

[0049] Nicotine poisoning or nicotine overdose can occur when an individual consumes loose tobacco, cigarettes, nicotine gum, patches, or the “e-liquid” of electronic cigarettes (e.g., the nicotine-containing liquid that is used in electronic cigarettes and other vaporizing devices). Indeed, a recent study showed that the incidence of nicotine poisoning from exposure to e-cigarettes increased 1492.9% between January 2012 and April 2015 (Kamboj et al. *PEDIATRICS* 137(6): e20160041 (2016)). Although exposure can occur through inhalation of tobacco smoke (either primary or second hand), nicotine poisoning or nicotine overdose more commonly results when a subject (typically a child) ingests nicotine, for example by chewing or ingesting nicotine gum, ingesting cigarettes or other tobacco leaf products, ingesting nicotine patches, or ingesting e-liquid. Additionally, nicotine can be dermally absorbed, and therefore nicotine poisoning can result from toxic levels of nicotine coming into direct contact with the skin.

[0050] Nicotine poisoning can produce neurological symptoms (convulsions, coma, depression, confusion, fainting, headache), cardiovascular symptoms (rapid heartbeat, high blood pressure), respiratory symptoms (difficulty breathing, rapid breathing), gastrointestinal symptoms (increased salivation, abdominal cramps, vomiting), and musculoskeletal symptoms (Muscular twitching, weakness), as well as death.

[0051] The methods described herein for treating nicotine toxicity, including nicotine poisoning and nicotine overdose, use an antibody that binds nicotine, thereby sequestering it and preventing the nicotine from binding a cognate receptor or crossing the blood-brain barrier. In some embodiments, a pharmaceutical composition comprising such an antibody is

administered in a therapeutically effective amount, such as an amount effective to reduce plasma levels of nicotine and/or to reduce levels of nicotine localized in the brain.

III. Nicotine-Binding Antibodies

[0052] In some embodiments, the disclosed methods comprise administering to a mammalian subject in need thereof a therapeutically effective amount of a nicotine-binding antibody, a nicotine-binding fragment thereof, a related construct capable of binding nicotine, or a pharmaceutical composition comprising the same. For convenience, these agents are referred to collectively herein as “nicotine-binding antibodies.”

[0053] Anti-nicotine antibodies have been previously developed, primarily for the purpose of facilitating smoking cessation. See, e.g., WO 2002/058635; WO 2000/032239; WO 2003/082329; U.S. Patent Application Publication 2006/111271; U.S. Pat. Nos. 8,344,111; 8,232,072; 6,232,082; 7,547,712; 7,446,205; and Carrera et al., “Investigations using immunization to attenuate the psychoactive effects of nicotine,” *Bioorg Med Chem* 12(3):563-70 (2004). These patents, applications, and non-patent literature are incorporated by reference herein to the extent that they relate to anti-nicotine antibodies and related constructs including nicotine-binding antibody fragments. However, the antibodies disclosed herein are novel, and may be used not only for facilitating smoking cessation, but also for treating nicotine toxicity.

[0054] Nicotine is a small, haptenic molecule and typically is coupled to an immunogenic carrier, such as an immunogenic protein, to elicit an immune response and induce the production of nicotine-binding antibodies. General techniques for making antibodies can be employed. See, e.g., Kohler and Milstein, *Eur. J. Immunol.*, 5: 511-519 (1976); Harlow and Lane (eds.), *ANTIBODIES: A LABORATORY MANUAL*, CSH Press (1988); C. A. Janeway et al. (eds.), *IMMUNOBIOLOGY*, 5th Ed., Garland Publishing, New York, N.Y. (2001).

[0055] Anti-nicotine antibodies useful in the methods described herein can be obtained by any means, including via in vitro sources (e.g., a hybridoma or a cell line producing an antibody recombinantly) and in vivo sources (e.g., rodents, rabbits, humans, etc.). Human, partially humanized, fully humanized, and chimeric antibodies can be made by methods known in the art, such as using a transgenic animal (e.g., a mouse) wherein one or more endogenous immunoglobulin genes are replaced with one or more human immunoglobulin genes. Examples of transgenic mice wherein endogenous antibody genes are effectively replaced with human antibody genes include, but are not limited to, the HUMAB-MOUSE™, the Kirin TC MOUSE™, and the KM-MOUSE™ (see, e.g., Lonberg, *Nat. Biotechnol.*, 23(9): 1117-25 (2005), and Lonberg, *Handb. Exp. Pharmacol.*, 181: 69-97 (2008)).

[0056] Nicotine-binding antibodies used in the methods disclosed herein generally will be monoclonal and/or recombinant. Monoclonal antibodies (mAbs) may be obtained by methods known in the art, for example, by fusing antibody-producing cells with immortalized cells to obtain a hybridoma, and/or by generating mAbs from mRNA extracted from bone marrow, B cells, and/or spleen cells of immunized animals using combinatorial antibody library technology and/or by isolating monoclonal antibodies from serum from subjects immunized with a nicotine antigen.

Recombinant antibodies may be obtained by methods known in the art, for example, using phage display technologies, yeast surface display technologies (Chao et al., Nat. Protoc., 1(2): 755-68 (2006)), mammalian cell surface display technologies (Beerli et al., PNAS, 105(38): 14336-41 (2008), and/or expressing or co-expressing antibody polypeptides. Other techniques for making antibodies are known in the art, and can be used to obtain antibodies used in the methods described herein.

[0057] Typically, an antibody consists of four polypeptides: two identical copies of a heavy (H) chain polypeptide and two copies of a light (L) chain polypeptide. Typically, each heavy chain contains one N-terminal variable (V_H) region and three C-terminal constant (C_{H1} , C_{H2} and C_{H3}) regions, and each light chain contains one N-terminal variable (V_L) region and one C-terminal constant (C_L) region. The variable regions of each pair of light and heavy chains form the antigen binding site of an antibody.

[0058] The terms “antibody fragment” and “nicotine-binding fragment,” as used herein, refer to one or more portions of a nicotine-binding antibody that exhibits the ability to bind nicotine. Examples of binding fragments include (i) Fab fragments (monovalent fragments consisting of the V_L , V_H , C_L and C_{H1} domains); (ii) F(ab')₂ fragments (bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region); (iii) Fd fragments (comprising the V_H and C_{H1} domains); (iv) Fv fragments (comprising the V_L and V_H domains of a single arm of an antibody), (v) dAb fragments (comprising a V_H domain);

and (vi) isolated complementarity determining regions (CDR), e.g., V_H CDR3. Other examples include single chain Fv (scFv) constructs. See e.g., Bird et al., Science, 242:423-26 (1988); Huston et al., Proc. Natl. Acad. Sci. USA, 85:5879-83 (1988). Other examples include nicotine-binding domain immunoglobulin fusion proteins comprising (i) a nicotine-binding domain polypeptide (such as a heavy chain variable region, a light chain variable region, or a heavy chain variable region fused to a light chain variable region via a linker peptide) fused to an immunoglobulin hinge region polypeptide, (ii) an immunoglobulin heavy chain C_{H2} constant region fused to the hinge region, and (iii) an immunoglobulin heavy chain C_{H3} constant region fused to the C_{H2} constant region, where the hinge region may be modified by replacing one or more cysteine residues with, for example, serine residues, to prevent dimerization. See, e.g., U.S. Patent Application 2003/0118592; U.S. Patent Application U.S. 2003/0133939.

[0059] In some embodiments, a nicotine-binding antibody as disclosed herein is a human IgG1 antibody or a human IgG4 antibody. In some embodiments, the nicotine-binding antibody is mammalian, human, humanized, or chimeric.

[0060] In some embodiments, nicotine-binding antibodies as disclosed herein comprise one or more mutations that make the antibody more suitable in a therapeutic context.

[0061] Heavy and light chain sequences of exemplary novel IgG1 nicotine-binding antibodies are disclosed in Table 1 below. Heavy and light chain sequences of exemplary novel IgG4 nicotine-binding antibodies are disclosed in Table 2 below.

TABLE 1

Heavy and Light Chain Sequences of IgG1 Nicotine-Binding Antibodies		
Antibody Chain	Amino Acid Sequence	SEQ ID NO:
8D1 Heavy	QVRLQESGPGLVKPSGTLSTCAVSGGSIYSSNWWTWVRQPPGKGLE WVGEIHIRGTTYNPSLNSRVTISLDKSNQVSLRLTSVTAADSAVY YCVSQEVGGPDLWGQGLTVTVSSASTKGPSVFPLAPSSKSTSGGTAAL GCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSWTVPSSSLG TQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFP PKPKDTEMISRTPEVTCWVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPR EPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTT PVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSP GK	1
8D1 Light	NFMLTQPHSVSESPGKTVTISCTRSGGSIATYYVQWYQORPGSAPT NVIYKYDQRPSPGVPDRFSGSIDSSNSASLTISGLKTEDEADYYCQSYDN NIQVFGGGYKLYVLGQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPG AVTVAWKADGSPVKAGVETTKPSKQSNKYYAAASYLSLTPEQWKSHRYS CQVTHEGSTVEKTVAPTECS	2
12F5 Heavy	QLQLQESGPGLVKPSSETLSLICTVSGGSIIRKNNNEWAWIRQAPGKGL EWIGSLSYTGRTVYNPSLKSRTISTDTSETQFSLKVNSTVTAADTAVY YCARLSPFVGAAWFDPWGQGLTVTVSSASTKGPSVFPLAPSSKSTSG GGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSWT VPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGG PSVFLFPPKPKDTEMISRTPEVTCWVDVSHEDPEVKFNWYVDGVEVHNA KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISK AKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPE NNYKTTFPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYT QKSLSLSPGK	3
12F5 Light	EVVLTQSPGTLSSLSPGERATLSCRASQSVSSRYLAWYQQKPGQAPRL LIYGASSRAIGTPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYAYSP PAITYGGGYKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREA KVQWKVDNALQSGNSQESVTEQDSKSTYLSLSSTLTLSKADYEKHKVYAC EVTHQGLSSPVTKSFNRGEC	4

TABLE 1-continued

Heavy and Light Chain Sequences of IgG1 Nicotine-Binding Antibodies		
Antibody Chain	Amino Acid Sequence	SEQ ID NO:
7A8 Heavy	QLQLQESGPGLLKPSETLSLTCTVSGGSVTTS PD WWAWLRQSPGKGL EWIGSV SYTGRT VYNPSLKSRVTISLDTSKNHLSLRMTSATAADTAVF YC ARLTPIDRFSADYYVLDI WGQGATVTVSS ASTKGPSVFPLAPSSKST <u>SGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSW</u> <u>TVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLG</u> <u>GPSVFLFPPKPKDTEMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN</u> <u>AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIS</u> <u>KAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQP</u> <u>ENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT</u> <u>QKSLSLSPGK</u>	5
7A8 Light	EIVMTQSPATLSVSPGERATLSCRAS QSISSN LAWFQHKPGQAPRLLI F RSSTRAT GTTPRFSGSGSGTEFTLTITSS LQSE DAVYFC QHYSYWPLI TYGGGY YAPEAK RTVAAPSVFIFPPSDEQLKSGTASV VCLLNNFY P REAKVQ <u>WKVDNALQSGNSQESVTEQDSKDYSLSSITLTLSKADYEKHKVYACEVTH</u> <u>QGLSSPVTKSFNRGEC</u>	6
5D1 Heavy	QLQLRESGPGLVKPSETLSLTCSVSGGS IS SSSYWG WIR QPPGKGLE WIGS IYYTGRT YYNPSLESRVTI SVDTSKNQFSLKLSSVTAADTAVYY CAGLHYSWSALGGYFYGM DVWGQGT TVTVSSASTKGPSVFPLAPS <u>SKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL</u> <u>SSWTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPE</u> <u>LLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE</u> <u>VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE</u> <u>KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESN</u> <u>GQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH</u> <u>NHYTQKSLSLSPGK</u>	7
5D1 Light	EIVLTQSPGTL SLSP GERATLSCRAS QSVSSRDL VWYQ QKPGQAPRLLI YGAS TRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC QKYGSSPP RITYG PGYKNDIK RTVAAPSVFIFPPSDEQLKSGTASV VCLLNNFY P REAK <u>VQWKVDNALQSGNSQESVTEQDSKDYSLSSITLTLSKADYEKHKVYACEV</u> <u>THQGLSSPVTKSFNRGEC</u>	8
5G4 Heavy	QLQLQESGPGLVKPSETLSLTCSVSGGS IS SSSYWGWSRQSPGKGLE WIAS IYYSGST YYNPSLKSRVTIFIDTSKNQFSLKLSSVTAADTAIYYC ARVGTSAMSRAFD MWGQGT MVTVSSASTKGPSVFPLAPSSKSTSGGT <u>AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSWTVPSS</u> <u>SLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVF</u> <u>LFPPKPKDTEMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP</u> <u>REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ</u> <u>PREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT</u> <u>TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL</u> <u>SPGK</u>	9
5G4 Light	DIVMTQSP LSLP VT PGE PASISCRSS QSL LQ SN GYNYLDWYLQKPGQS PQLLIY LGS NRASGVPDRFSGSGSGTDFTLKISKVEAEDGVGYFC CMQ ALQIPWTF GQGYKVEIK RTVAAPSVFIFPPSDEQLKSGTASV VCLLNNFY <u>PREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSSITLTLSKADYEKHK</u> <u>VYACEVTHQGLSSPVTKSFNRGEC</u>	10
5H1 Heavy	QVQLQESGPGLVKPSETLSLTCTVSGGS ISRRNDY WAWIRQSPGKDL EWIGT ISFSGST FYNPSLKSRVTISADTFNNHFSRLRLDAVAAADTAVY YC ARLSPPFVGAAWFD PWGPGTLVTVSS ASTKGPSVFPLAPSSKSTSG <u>GTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSWTV</u> <u>PSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPS</u> <u>VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT</u> <u>KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK</u> <u>GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQENN</u> <u>YKTTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKS</u> <u>LSLSPGK</u>	11
5H1 Light	EIVLTQSPGTL SLSP GERATLSCRAS QSLSSNYL GWYQ QKPGQAPRLLI YGAS NRATGIPDRFSGSGSGTDFTLTISRLEPEDFGVYYC QRYGRSPP AIT FGGYKVEIK RTVAAPSVFIFPPSDEQLKSGTASV VCLLNNFY P REAK <u>VQWKVDNALQSGNSQESVTEQDSKDYSLSSITLTLSKADYEKHKVYACEV</u> <u>THQGLSSPVTKSFNRGEC</u>	12
15A4 Heavy	QLQLQESGPGLVKPSETLSLTCTAS GSITNNIDY WVWIRQPPGRGLE WIGT IYYSGST FYNPSLKSRVTISVDTSNNQFSLNLSMSAADTAVYY CARLRYYYDSNGYLPYWID SWGQGT LTVTVSSASTKGPSVFPLAPSSKS <u>TSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV</u>	13

TABLE 1-continued

Heavy and Light Chain Sequences of IgG1 Nicotine-Binding Antibodies		
Antibody Chain	Amino Acid Sequence	SEQ ID NO:
	<u>VTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLG</u> <u>GPSVFLFPPKPKDTEMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN</u> <u>AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIS</u> <u>KAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQP</u> <u>ENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVMEALHNHYT</u> <u>QKSLSLSPGK</u>	
15A4 Light	EIVLTQSPGTLSSLSPGERATLSCRAS <u>QSISSSY</u> LGWYQQKPGQAPRLLI Y <u>GASS</u> RATGIPDRFSGSGSGTDFTLTIS <u>SLEPEDFAVYFCQLYRRSPPR</u> LTFGGGTKVEIKRTVAAPSVFIFPPSDE <u>QLKSGTASVVCLLNNFYPREAKV</u> <u>QWKVDNALQSGNSQESVTEQDSKDYSLSSSTLTLSKADYEKHKVYACEVT</u> <u>HQGLSSPVTKSFNRGEC</u>	14
2E11 Heavy	QLQLQESGPGLVKPS <u>ESLSLTCTVSGGSIISNDYY</u> WAWIRQSPGKGLE WIGS <u>INYRGST</u> FYSPSLNSRVTTSDTSKNQFFLKLTSTVTAADTAMYF <u>CTRLHGRYRGVGRLAFDY</u> WGQGLTVTVSS <u>ASTKGPSVFPLAPSSKST</u> <u>SGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV</u> <u>TVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLG</u> <u>GPSVFLFPPKPKDTEMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN</u> <u>AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIS</u> <u>KAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQP</u> <u>ENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVMEALHNHYT</u> <u>QKSLSLSPGK</u>	15
2E11 Light	DIQMTQSPSTLSASVGDIVTITCRAS <u>QSIGDW</u> LAWYQQKPGKAPKLLI Y <u>KASN</u> LESGVPSRFSGSGSGTEFTLTIS <u>SLQSDDFATYYCQQYDSYSV</u> <u>TFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQ</u> <u>WKVDNALQSGNSQESVTEQDSKDYSLSSSTLTLSKADYEKHKVYACEVTH</u> <u>QGLSSPVTKSFNRGEC</u>	16
13F7 Heavy	QVQLQEAGPGLVKPS <u>ETLSLTCTVSGGSINTRNYY</u> WGWVRQPPGKG LEWIAS <u>VYYTGST</u> FYDPSLRSRVTISIDTPRNQFSLRVSSVDAGDMGV YY <u>CVRLDGGYNNGY</u> YY <u>GMDV</u> WGQGSTVTVSS <u>ASTKGPSVFPLAPS</u> <u>SKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL</u> <u>SSWTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPE</u> <u>LLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE</u> <u>VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE</u> <u>KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESN</u> <u>GQ</u> <u>PENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVMEALH</u> <u>NHYTQKSLSLSPGK</u>	17
13F7 Light	GVQMTQSPSTLSASVGERVTVTTCRAS <u>RPIS</u> NWLSWYQQKPGRAPKLL IY <u>GTST</u> LESGVPSRFSGSGSGTEFTLTITNLQPD <u>DFATYYCQEHNL</u> YTI <u>TFGPGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQ</u> <u>WKVDNALQSGNSQESVTEQDSKDYSLSSSTLTLSKADYEKHKVYACEVTH</u> <u>QGLSSPVTKSFNRGEC</u>	18
8H5 Heavy	QLQLQESGPGLVKPS <u>ETLSLSCAVSGASIRSNTYY</u> WGWIRQPPGRGLE WIGS <u>ISHRGDA</u> HYSPSLKSPVTISVDTSKNEFS <u>LKATS</u> VTAADTAVYY <u>CVSLAYSFSWNTYYFYGMDV</u> WGHGITVTVSS <u>ASTKGPSVFPLAPSSKS</u> <u>TSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV</u> <u>VTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLG</u> <u>GPSVFLFPPKPKDTEMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN</u> <u>AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIS</u> <u>KAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQP</u> <u>ENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVMEALHNHYT</u> <u>QKSLSLSPGK</u>	19
8H5 Light	DIVLTQSPGTLSSLSPGEGATLSCRAS <u>QSVNSGY</u> LAWYQQKPGQPRLLI VF <u>AASS</u> RATGIADRFSGSGSGTDFTLTITRLEPEDFAVYY <u>CQLYGHSP</u> <u>ARIT</u> FGQGRLETKRTVAAPSVFIFPPSDE <u>QLKSGTASVVCLLNNFYPREA</u> <u>KVQWKVDNALQSGNSQESVTEQDSKDYSLSSSTLTLSKADYEKHKVYAC</u> <u>EVTHQGLSSPVTKSFNRGEC</u>	20

Heavy and light chain complementarity determining regions (CDRs) are shown in bold, underlined text. CDR annotation was made according to IMGT numbering. Constant regions are denoted in italicized, underlined text.

TABLE 2

Heavy and Light Chain Sequences of IgG4 Nicotine-Binding Antibodies		
Antibody Chain	Amino Acid Sequence	SEQ ID NO:
5G4-IgG4 Heavy	QLQLQESGPGLVKPSETLSLTCSVSGGSISSSSYWGWSRQSPGKGLE WIASIYYSGSTYYNP SLKSRVTIFIDTSKNQFSLKLSSVTAADTAIYYC ARVGTSA MSRAFD MWGQGTMTVSSASTKGPSVFPLAPCSRSTSESTA ALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSS LGTKTYTCNV DHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPP KPKDTEMISR TPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREE QFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTI SKAKGQPRE PQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTT P PVLDS DGSFFLYSRLTVDKSRWQEGNVFSCSV MHEALHNHYTQKSLSL SL GK	21
5G4-IgG4 Light	DIVMTQSPLSLPVTPGEPASISCRSSQSL LQSN GYNYLDWYLQKPGQS PQLLIYLG SNRASGV PDRFSGSGSGTDFTLKI SKVEAEDGVGYFCMQ ALQIPYWTFGQGYKVEIKRTVAAPSVFI FPPSDEQLKSGTASVVC LLNNFY PREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKADYEKHK VYACEVTHQGLSSPVTKSFNRGEC	22
7A8-IgG4 Heavy	QLQLQESGPGLLKPSETLSLTCTVSGGSVTTSPDWAWLRQSPGKGL EWIGSVSYTGRTVYNP SLKSRVTISLDTSKNHLSLRMTSATAADTAVF YCARLTPIDRFSADYYVLDI WGQGATVTVSSASTKGPSVFPLAPCSRS TSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV TVPSSSLGTKTYTCNV DHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSV FLFPPKPKDTEMISR TPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKT KPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTI SKAK GQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTT PVLDS DGSFFLYSRLTVDKSRWQEGNVFSCSV MHEALHNHYTQKS LSLSLGK	23
7A8-IgG4 Light	EIVMTQSPATLSVSPGERATLSCRASQSI SSNLAWFQHKPGQAPRLLI F RSSTRATGT PPRFSGSGSGTEFTLT ISSLQSEDFAVYFCQHYSYWPLLI TFGQGTRLEIKRTVAAPSVFI FPPSDEQLKSGTASVVC LLNNFYPREAKVQ WKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKADYEKHKVYACEVTH QGLSSPVTKSFNRGEC	24
12F5-IgG4 Heavy	QLQLQESGPGLVKPSETLSLICTVSGGSIRKNNEWAWIRQAPGKGL EWIGSLSYTGRTVYNP SLKSRVTISTDTSETQFSLKVNSVTAADTAVY YCARLSFFVGA AWWFDPWGQGT LVTVSSASTKGPSVFPLAPCSRSTS ESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTV PSSSLGTKTYTCNV DHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFL FPPKPKDTEMISR TPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKP REEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTI SKAKGQ PREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK TTPPVLDS DGSFFLYSRLTVDKSRWQEGNVFSCSV MHEALHNHYTQKSLS LSLGK	25
12F5-IgG4 Light	EVVLTQSPGTL SLSLSPGERATLSCRASQSVSSRYLAWYQQKPGQAPRL LIYGASSRAIGTPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYAYSP PAITFGGGYKVEIKRTVAAPSVFI FPPSDEQLKSGTASVVC LLNNFYPREA KVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKADYEKHKVYAC EVTHQGLSSPVTKSFNRGEC	26
8D1-IgG4 Heavy	QVRLQESGPGLVKP SGTLSLTCAVSGGSIIYSSNWWTWVRQPPGKGLE WVGEIHIRGTTYYNP SLNSRVTISLDKSNNQVSLRLTSVTAADSAVY YCVSQEVGGPDLWGQGT LVTVSSASTKGPSVFPLAPCSRSTSESTAALG CLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGT KTYTCNV DHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPK DTLMISR TPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFN STYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTI SKAKGQPREPQV YTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTT PVL DSDGSFFLYSRLTVDKSRWQEGNVFSCSV MHEALHNHYTQKSLSLSL GK	27
8D1-IgG4 Light	NFMLTQPHSVSESPGKTVTISCTRSGGSIIATYYVQWYQQRPGSAPT N VIYKYDQRPSGVPDRFSGSIDSSNSASLTISGLKTEDEADYYCQSYDN NIQVFGGGTKLTVLGQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPG AVTVAWKADGSPVKAGVETTKPSKQSNKNKYAASSYLSTLPEQWKSHRSYSC QVTHEGSTVEKTVAPTECS	28
5D1-IgG4 Heavy	QLQLRESGPGLVKPSETLSLTCSVSGGSISSSSYWGWI RQPPGKGLE WIGSIYYTGRTYYNP SLESRVTISVDTSKNQFSLKLSSVTAADTAVYY CAGLHYSWALGGYIFYGMDVWGQGT TTVTVSSASTKGPSVFPLAPC SRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL SVVTVPSSSLGTKTYTCNV DHKPSNTKVDKRVESKYGPPCPPCPAPEFLGG	29

TABLE 2-continued

Heavy and Light Chain Sequences of IgG4 Nicotine-Binding Antibodies		
Antibody Chain	Amino Acid Sequence	SEQ ID NO:
	<u>PSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNA</u> <u>KTTPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTI</u> <u>AKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPE</u> <u>NNYKTTTPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQ</u> <u>KSLSLSLGK</u>	
5D1-IgG4 Light	EIVLTQSPGTL SL SPGERATLSCRAS QSVSSRD LWVYQQKPGQAPRLLI YGAS TRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYY CQKYGS SPP RIT FGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFY PREAK <u>VQWKVDNALQSGNSQESVTEQDSKDYSLSSLTLSKADYEKHKVYACEV</u> <u>THQGLSSPVTKSFNRGEC</u>	30
5H1-IgG4 Heavy	QVQLQESGPGLVKPSETLSLTCTV SGGSI SRNDY WAWIRQSPGKDL EWIGT ISFSGST FYNPSLKSRTISADTFNNHFSRLDAVAADTAVY YCARLS PFVGA AWFDP WGPGTLVTVSSASTKGPSVFPLAPCSR STSE <u>STAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTV</u> <u>SSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLF</u> <u>PPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPR</u> <u>EEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTI</u> <u>SKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQ</u> <u>PENNYKTTTPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQ</u> <u>KSLSLSLGK</u>	31
5H1-IgG4 Light	EIVLTQSPGTL SL SPGERATLSCRAS QSLSSNY LGWYQQKPGQAPRLLI YGAS NRATGIPDRFSGSGSGTDFTLTISRLEPEDFGVYY CQRYGR SPP AIT YGGGKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFY PREAK <u>VQWKVDNALQSGNSQESVTEQDSKDYSLSSLTLSKADYEKHKVYACEV</u> <u>THQGLSSPVTKSFNRGEC</u>	32
15A4-IgG4 Heavy	QLQLQESGPGLVKPSETLSLTCTAS GGSI TNNIDY WVWIRQPPGRGLE WIGT IYYSGST FYNPSLKSRTISVDTSNNQFSLNLSMSAADTAVYY CARLRY YDSNGYL PYWID SWGQGLVTVSSASTKGPSVFPLAPCSR STSE STAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV <u>TVTPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPS</u> <u>VFLFPPKPKDTEMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAK</u> <u>TPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTI</u> <u>SKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQ</u> <u>PENNYKTTTPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQ</u> <u>KSLSLSLGK</u>	33
15A4-IgG4 Light	EIVLTQSPGTL SL SPGERATLSCRAS QSISSSY LGWYQQKPGQAPRLLI YGAS SRATGIPDRFSGSGSGTDFTLTISLLEPEDFAVY FCQLYRR SPPR LTF GGGGKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFY PREAKV <u>QWKVDNALQSGNSQESVTEQDSKDYSLSSLTLSKADYEKHKVYACEV</u> <u>THQGLSSPVTKSFNRGEC</u>	34
2E11-IgG4 Heavy	QLQLQESGPGLVKPSESLSLTCTV SGGSI ISNDYY WAWIRQSPGKGLE WIGS INRYRGST FYSPSLNSRVTTSDVTSKNQFFLKLTSVTAADTAMYF CTRLHGRYRGVGR LAFDY WGQGLVTVSSASTKGPSVFPLAPCSR ST <u>SESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV</u> <u>TVTPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSV</u> <u>FLFPPKPKDTEMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAK</u> <u>TPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTI</u> <u>SKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQ</u> <u>PENNYKTTTPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQ</u> <u>KSLSLSLGK</u>	35
2E11-IgG4 Light	DIQMTQSPSTLSASVGDIVTITCRAS QSIGD WLAWYQQKPGKAPKLLI YKAS NLESGVPSRFSGSGSGTEFTLTISSLQSDDFATYY CQQYDSYSV TFG QGTKVEIKGTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFY PREAKVQ <u>WKVDNALQSGNSQESVTEQDSKDYSLSSLTLSKADYEKHKVYACEV</u> <u>THQGLSSPVTKSFNRGEC</u>	36
13F7-IgG4 Heavy	QVQLQEAGPGLVKPSETLSLTCTV SGGSI INTRNYY WGWVRQPPGKG LEWIAS VYYTGST FYDPSLRSRVTISIDTPRNQFSLRVSSVDAGDMGV YY CVRLDGGYNN GYYYYGMDV WGQGTSTVTVSSASTKGPSVFPLAP CSRST SESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL <u>SSWTVTPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLG</u> <u>GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVH</u> <u>NAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTI</u> <u>SKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQ</u> <u>PENNYKTTTPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYT</u>	37

TABLE 2-continued

Heavy and Light Chain Sequences of IgG4 Nicotine-Binding Antibodies		
Antibody Chain	Amino Acid Sequence	SEQ ID NO:
	<u>QKSLSLSLGK</u>	
13F7-IgG4 Light	GVQMTQSPSTLSASVGERVTVTTCRAS <u>RPISNWLSWYQQKPGRAPKLL</u> IY <u>GT</u> STLESQVPSRFSGSGSGTEFTLTITNLQPDDEFATYYC <u>QEHNLYTI</u> <u>TFGPGTKVEIKRTVAAPSVEIFPPSDEQLKSGTASVVCLLNNFYPREAKVQ</u> <u>WKVDNALQSGNSQESVTEQDSKDYSLSSLTLSKADYEKHKVYACEVTH</u> <u>QGLSSPVTKSFNRGEC</u>	38
8H5-IgG4 Heavy	QLQLQESGPGLVKPSETLSLSCAVSG <u>ASIRSNTYY</u> WGWIROPGRGLE WIGS <u>ISHRGDA</u> HYSPSLKSPVTISVDTSKNEFSLKATSVTAADTAVYY <u>CVSLAYSFSWNTYYFYGM</u> DVWGHGIVTVSSASTKGPSVFPLAPCSR <u>STSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV</u> <u>VTVPSSSLGTQTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPS</u> <u>VFLFPPKPKDTEMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKT</u> <u>KPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTI</u> <u>SKAK</u> <u>GQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN</u> <u>YKTTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHREALHNYTQKS</u> <u>LSLSLGK</u>	39
8H5-IgG4 Light	DIVLTQSPGTLSSLSPGEGATLSCRAS <u>QSVNSGY</u> LAWYQQKPGQPRL VF <u>AASS</u> RATGIADRFRGSGSGTDFTLTITRLEPEDFAVYYC <u>QLYGHSP</u> <u>ARIT</u> FGQTRLET <u>KRTVAAPSVEIFPPSDEQLKSGTASVVCLLNNFYPREA</u> <u>KVQWKVDNALQSGNSQESVTEQDSKDYSLSSLTLSKADYEKHKVYAC</u> <u>EVTHQGLSSPVTKSFNRGEC</u>	40

Heavy and light chain complementarity determining regions (CDRs) are shown in bold, underlined text. CDR annotation was made according to IMGT numbering. Constant regions are denoted in italicized, underlined text.

[0062] Also encompassed by the present disclosure are nicotine-binding antibodies and nicotine-binding fragments thereof comprising the same CDR sequences and/or the same framework region sequences and/or the same variable region sequences as one or more of the novel antibodies disclosed in Tables 1 and 2. In this regard, although the novel nicotine-binding antibodies disclosed in Tables 1 and 2 are IgG1 and IgG4 antibodies, respectively, other nicotine-binding antibodies within the scope of this disclosure may be IgG2, IgG3, IgA1, IgA2, IgE, IgH, or IgM, for example.

[0063] Human immunoglobulin IgG4 antibodies are good candidates for antibody-based therapy when, as here, reduced effector functions are desirable. However, IgG4 antibodies are dynamic molecules able to undergo a process known as Fab arm exchange (FAE). See, e.g., Labrijn et al., *Therapeutic IgG4 antibodies engage in Fab-arm exchange with endogenous human IgG4 in vivo*, NATURE BIOTECH 27(8): 767-71 (2009). This results in functionally monovalent, bispecific antibodies (bsAbs) with unknown specificity and hence, potentially, reduced therapeutic efficacy. FAE can be prevented by introducing a S228P mutation into the hinge region of the antibody. Thus, in some embodiments, a nicotine-binding antibody as disclosed herein comprises a S228P substitution. The novel antibodies disclosed in Table 2 comprise such a S228P substitution. In other embodiments, a nicotine-binding antibody as disclosed herein does not comprise a S228P substitution.

[0064] In some embodiments, a nicotine-binding antibody as disclosed herein comprises one or more additional or alternative substitutions, insertions, or deletions beyond the aforementioned S228P substitution. For example, in some embodiments, a nicotine-binding antibody of the present disclosure comprises heavy and light chains with at least about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%,

about 95%, about 96%, about 97%, about 98%, about 99%, or about 100% identity to one or more of the heavy and light chain sequences disclosed in Tables 1 and 2, respectively. In some embodiments, a nicotine-binding antibody of the present disclosure comprises heavy and light chains with at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity to one or more of the heavy and light chain sequences disclosed in Tables 1 and 2, respectively.

[0065] In some embodiments, the antibodies disclosed herein bind nicotine with a high affinity. As shown in Table 3 below, the novel antibodies of Tables 1 and 2 can bind to free S-nicotine with a K_D in the nanomolar range. The K_D values reported below were determined by Surface Plasmon Resonance Biosensor. Other methodology for determining binding affinity also can be used, such as equilibrium dialysis.

TABLE 3

Nicotine Binding Affinity	
Antibody	K_D (nM) (S-Nicotine; 25° C.)
8D1	5
12F5	29
7A8	30
5D1	30
5G4	31
5H1	37
15A4	40
2E11	61
13F7	62

TABLE 3-continued

Nicotine Binding Affinity	
Antibody	K_D (nM) (S-Nicotine; 25° C.)
8H5	67
5G4 IgG4	31
7A8 IgG4	30
12F5 IgG4	20
8D1 IgG4	5

[0066] Thus, in some embodiments, the nicotine-binding antibodies or fragments thereof disclosed herein have a K_D of less than 100 nM. For example, in some embodiment, the nicotine-binding antibodies or fragments thereof have a K_D for nicotine of less than about 1.5×10^{-7} , less than about 1.0×10^{-7} , less than about 0.5×10^{-7} , less than about 9.5×10^{-8} , less than about 9.0×10^{-8} , less than about 8.5×10^{-8} , less than about 8.0×10^{-8} , less than about 7.5×10^{-8} , less than about 7.0×10^{-8} , less than about 6.5×10^{-8} , less than about 6.0×10^{-8} , less than about 5.5×10^{-8} , less than about 5.0×10^{-8} , less than about 4.5×10^{-8} , less than about 4.0×10^{-8} , less than about 3.5×10^{-8} , less than about 3.0×10^{-8} , less than about 2.5×10^{-8} , less than about 2.0×10^{-8} , less than about 1.5×10^{-8} , less than about 1.0×10^{-8} , less than about 0.5×10^{-8} , less than about 9.5×10^{-9} , less than about 9.0×10^{-9} , less than about 8.5×10^{-9} , less than about 8.0×10^{-9} , less than about 7.5×10^{-9} , less than about 7.0×10^{-9} , less than about 6.5×10^{-9} , less than about 6.0×10^{-9} , less than about 5.5×10^{-9} , less than about 5.0×10^{-9} , less than about 4.5×10^{-9} , less than about 4.0×10^{-9} , less than about 3.5×10^{-9} , less than about 3.0×10^{-9} , less than about 2.5×10^{-9} , less than about 2.0×10^{-9} , less than about 1.5×10^{-9} , less than about 1.0×10^{-9} , less than about 0.5×10^{-9} , less than about 9.5×10^{-10} , less than about 9.0×10^{-10} , less than about 8.5×10^{-10} , or less than about 8.0×10^{-10} M. In some embodiment, the nicotine-binding antibodies or fragments thereof have a K_D for nicotine of less than 1.5×10^{-7} , less than 1.0×10^{-7} , less than 0.5×10^{-7} , less than 9.5×10^{-8} , less than 9.0×10^{-8} , less than 8.5×10^{-8} , less than 8.0×10^{-8} , less than 7.5×10^{-8} , less than 7.0×10^{-8} , less than 6.5×10^{-8} , less than 6.0×10^{-8} , less than 5.5×10^{-8} , less than 5.0×10^{-8} , less than 4.5×10^{-8} , less than 4.0×10^{-8} , less than 3.5×10^{-8} , less than 3.0×10^{-8} , less than 2.5×10^{-8} , less than 2.0×10^{-8} , less than 1.5×10^{-8} , less than 1.0×10^{-8} , less than 0.5×10^{-8} , less than 9.5×10^{-9} , less than 9.0×10^{-9} , less than 8.5×10^{-9} , less than 8.0×10^{-9} , less than 7.5×10^{-9} , less than 7.0×10^{-9} , less than 6.5×10^{-9} , less than 6.0×10^{-9} , less than 5.5×10^{-9} , less than 5.0×10^{-9} , less than 4.5×10^{-9} , less than 4.0×10^{-9} , less than 3.5×10^{-9} , less than 3.0×10^{-9} , less than 2.5×10^{-9} , less than 2.0×10^{-9} , less than 1.5×10^{-9} , less than 1.0×10^{-9} , less than 0.5×10^{-9} , less than 9.5×10^{-10} , less than 9.0×10^{-10} , less than 8.5×10^{-10} , or less than 8.0×10^{-10} M.

[0067] In some embodiments, the disclosed nicotine-binding antibodies or fragments thereof have a K_D for nicotine between 100 nM and 0.01 nM, between 90 nM and 0.05 nM, between 80 nM and 0.1 nM, between 70 nM and 0.5 nM, between 70 nM and 1.0 nM, between 60 nM and 30 nM, or any value in between. For example, in some embodiments, the disclosed nicotine-binding antibodies or fragments

thereof have a K_D for nicotine of less than 100 nM, less than 60 nM, less than 30 nM, less than 10 nM, less than 5 nM, or less than 1 nM.

[0068] Nicotine has two enantiomers: S-(–)-nicotine and R-(+)-nicotine, with the S-enantiomer known to be the most physiologically active. In some embodiments, the disclosed nicotine-binding antibodies exhibit selectivity for one enantiomer over the other. For instance, in some embodiments, a nicotine-binding antibody selectively binds to S-(–)-nicotine with a higher affinity than it binds to R-(+)-nicotine, while in some embodiments a nicotine-binding antibody may bind S-(–)-nicotine and substantially not bind to R-(+)-nicotine. For example, 8D1-IgG4 and 12F5-IgG4 preferentially bind to S-(–)-nicotine. In this regard, 8D1-IgG4 has a K_D for R-(+)-nicotine of 92 nM and 12F5-IgG4 has a K_D for R-(+)-nicotine of 1.2 μ M. These disclosed antibodies exhibit greater binding affinity and selectivity for S-(–)-nicotine than has previously been reported for previously described nicotine-binding antibodies, such as the Nic12 mAb, which is disclosed in U.S. Pat. No. 8,344,111 and Tars et al., J. Mol. Bio., 415: 118-127 (2012).

[0069] Alternatively, in some embodiments, a nicotine-binding antibody may selectively bind to R-(+)-nicotine with a higher affinity than it binds to S-(–)-nicotine, while in some embodiments a nicotine-binding antibody may bind to R-(+)-nicotine and substantially not bind to S-(–)-nicotine.

[0070] In some embodiments, a nicotine-binding antibody may bind to both enantiomers of nicotine with comparable affinity.

[0071] In some embodiments, the disclosed nicotine-binding antibodies have a strong binding affinity for nicotine (one or both enantiomers) and a comparatively weak binding affinity for other molecules that may be present in a subject being treated, including molecules that are chemically- and/or structurally-related to nicotine, metabolites or byproducts of nicotine (e.g., cotinine), molecules that are ligands of or that bind to nicotinic receptors, drugs (e.g., small molecule drugs) used to aid smoking cessation (e.g., bupropion, varenicline, and cytisine) and/or treat nicotine addiction and/or nicotine toxicity, and/or other endogenous or exogenous molecules that may be present in a subject's blood, including neurotransmitters and other molecules that may be administered to diagnose or treat a condition in the subject or to maintain or support normal physiology. In other words, in some embodiments, the disclosed nicotine-binding antibodies do not cross-react with molecules that are not nicotine, i.e., “off-target compounds”.

[0072] ION The percent of cross reactivity (% cross reactivity to mAb ($IC_{50, Nicotine}/IC_{50, compound} \times 100\%$)) of the disclosed antibodies against several exemplary molecules is shown in Table 4 below. Of these, cotinine, nicotinamide, B-nicotinamide adenine dinucleotide, and nornicotine are nicotine-related molecules; bupropion, varenicline and cytisine are smoking-cessation drugs, and acetylcholine chloride, 3-hydroxytyramine (dopamine), serotonin, and norepinephrine are neurotransmitters. A cross-reactivity of less than 0.1%, less than 0.05%, less than 0.01%, or less than 0.005%, or less than 0.001%, or less than 0.0005%, or less than 0.0001% is considered to be substantially not cross-reactive.

TABLE 4

Cross Reactivity of Exemplary Antibodies				
Compound	8D1	12F5	7A8	5G4
S-Nicotine affinity K_D (nM)	5	29	30	31
S-Nicotine	100	100	100	100
Cotinine	NCR*	NCR	0.0938	0.0352
Acetylcholine Chloride	NCR	NCR	NCR	NCR
Nicotinamide	NCR	NCR	NCR	0.0004
3-HydroxytyramineHCl (Dopamine HCl)	0.0003	NCR	NCR	NCR
Serotonin Hydrochloride	NCR	NCR	NCR	NCR
(+/-)-Norepinephrine (+)- Bitartrate Salt	0.0005	0.0296	0.0020	0.0015
Normicotine	NCR	NCR	0.0558	0.1971
Bupropion	NCR	NCR	NCR	0.0106
Cytisine	0.0001	NCR	NCR	NCR
Varenicline tartrate	0.0002	NCR	NCR	0.0018
B-Nicotinamide Adenine Dinucleotide	0.0002	NCR	0.0036	0.0037

*NCR = no cross reactivity detected

Values are shown as percent of cross reactivity, which was calculated using the equation: $(IC_{50}, \text{Nicotine} / IC_{50}, \text{Compound} \times 100\%)$

[0073] Binding affinity for nicotine over cotinine is particularly advantageous because cotinine is the major human metabolite of nicotine and has a longer half-life than nicotine, so it often accumulates at high concentrations relative to nicotine in smokers and other individuals who consume nicotine-based products. Indeed, this is a reason that cotinine is used for testing to determine if someone is a smoker. Given the high levels of circulating cotinine found in individuals that consume nicotine-based products (e.g., cigarettes, e-cigarettes, smokeless tobacco, etc.), a nicotine-binding antibody that also exhibits substantial binding affinity for cotinine would be less effective for treating nicotine poisoning or facilitating smoking cessation, since the antibody would bind to cotinine as well as nicotine, limiting its efficacy at binding (and sequestering) nicotine. Thus, the binding selectivity of the specific antibodies disclosed herein is a significant advantageous property that supports their efficacy in clinical applications.

[0074] Binding affinity for nicotine over bupropion, varenicline and/or cytisine also is advantageous because those drugs are commonly used for smoking cessation. The binding selectivity of the specific antibodies disclosed herein and their lack of binding affinity for bupropion, varenicline and cytisine indicates that they could be used in combination with bupropion, varenicline and/or cysteine, since the antibodies would not bind those drugs. Thus, in some embodiments, the methods disclosed herein include administering an antibody as disclosed herein that does not exhibit binding affinity to bupropion, varenicline and/or cytisine (such as any of the antibodies set forth in the Table 4) in a combination therapy with a smoking cessation drug (such as bupropion, varenicline and/or cytisine), wherein the antibodies and drugs may be administered substantially simultaneously or sequentially in any order. Such embodiments may be particularly advantageous in methods for facilitating smoking cessation, quitting smoking (or quitting using other nicotine products), maintaining abstinence from smoking (or use of other nicotine products), or decreasing consumption of nicotine products.

[0075] The data shown in Table 4 also indicate that the disclosed antibodies do not bind to neurotransmitters. This type of binding selectivity is advantageous because it indi-

cates that the disclosed antibodies are not likely to interfere with normal brain physiology/pharmacology.

[0076] In some embodiments, the nicotine-binding antibody or fragment is a long-acting variant that has been modified in order to extend its half-life in vivo (after administration). Various techniques are known in the art for extending the circulating half-life of peptides, such as antibodies. For example, in some embodiments the antibody carries mutations in the Fc region with enhanced FcRn-mediated recycling such as “YTE” (M252Y/S254T/T256E), see e.g., Dall’Acqua et al., J Biol Chem., 281:23514-24 (2006), or “Xtend” Fc domain mutations from Xencor (US 2014/0056879 A1). In other embodiments, the antibody or fragment thereof is conjugated to polyethylene glycol (PEG; i.e., the antibody is PEGylated) or a similar polymer that prolongs half-life. In some embodiments, the antibody is fused to an albumin-binding peptide, an albumin-binding protein domain, human serum albumin, or an inert polypeptide. Exemplary inert polypeptides that have been used to increase the circulating half-life of peptides include, but are not limited to, XTEN® (also known as recombinant PEG or “rPEG”), a homo-amino acid polymer (HAP; HAPylation), a proline-alanine serine polymer (PAS; PASylation), or an elastin-like peptide (ELP; ELPylation). As used herein, “fused to” includes genetic fusion, directly or through a linker, resulting in a single polypeptide containing multiple domains, unless otherwise specified.

[0077] The nicotine-binding antibody or a nicotine-binding fragment thereof can be formulated in a pharmaceutical composition suitable for administration to the target subject by the intended route of administration, as discussed in more detail below.

IV. Pharmaceutical Compositions

[0078] Pharmaceutical compositions suitable for use in the methods described herein can include the disclosed nicotine-binding antibodies or fragments thereof and a pharmaceutically acceptable carrier or diluent.

[0079] The composition may be formulated for intravenous, subcutaneous, intraperitoneal, intramuscular, oral, nasal, pulmonary, ocular, vaginal, or rectal administration. In some embodiments, nicotine-binding antibodies are for-

mulated for intravenous, subcutaneous, intraperitoneal, or intramuscular administration, such as in a solution, suspension, emulsion, liposome formulation, etc. The pharmaceutical composition can be formulated to be an immediate-release composition, sustained-release composition, delayed-release composition, etc., using techniques known in the art.

[0080] Pharmacologically acceptable carriers for various dosage forms are known in the art. For example, excipients, lubricants, binders, and disintegrants for solid preparations are known; solvents, solubilizing agents, suspending agents, isotonicity agents, buffers, and soothing agents for liquid preparations are known. In some embodiments, the pharmaceutical compositions include one or more additional components, such as one or more preservatives, antioxidants, colorants, sweetening/flavoring agents, adsorbing agents, wetting agents and the like.

[0081] In some embodiments, the disclosed nicotine-binding antibodies or fragments thereof may be formulated for administration by injection or infusion. In some embodiments, the nicotine-binding antibody or fragment thereof is formulated for administration by a non-oral route since nicotine poisoning may induce vomiting, thus limiting the effectiveness of oral administration for that particular indication.

V. Methods Of Treating Nicotine Poisoning

[0082] As noted above, in some aspects the methods of treating nicotine overdose or nicotine poisoning described herein comprise administering to a mammalian subject in need thereof a nicotine-binding antibody or nicotine-binding fragment thereof as disclosed herein, or a pharmaceutical composition comprising the same. In some embodiments, the methods comprise administering a nicotine-binding antibody or nicotine-binding fragment thereof to a subject that has ingested or consumed a toxic amount of nicotine. In some embodiments, the methods may comprise administering both a nicotine-binding antibody or nicotine-binding fragment thereof and another compound that is useful for treating nicotine poisoning, such as activated charcoal. In such embodiments, the antibody or fragment and the second compound (e.g., activated charcoal) can be administered sequentially or simultaneously, from the same or different compositions. Thus, the treatment may include administering activated charcoal and/or other supportive treatments to address the symptoms and/or effects of nicotine poisoning.

[0083] In some embodiments, the therapeutically effective amount of the nicotine-binding antibody or fragment thereof is effective to reduce plasma levels of nicotine, and/or to reduce levels of nicotine localized in the brain, and/or to reduce, ameliorate, or eliminate one or more symptoms or effects of nicotine poisoning or overdose. The specific amount administered may depend on one or more of the age and/or weight of the subject, the amount of nicotine believed to have been ingested, and/or the subject's plasma level of nicotine at the time of treatment, and/or the subject's brain level of nicotine at the time of treatment.

[0084] In some embodiments, the nicotine-binding antibody is administered at a dose of from about 50 to about 1000 mg/kg, about 150 mg/kg to about 850 mg/kg, about 250 mg/kg to about 750 mg/kg, about 350 mg/kg to about 650 mg/kg, or about 450 mg/kg to about 550 mg/kg. In some embodiments, the nicotine-binding antibody is administered at a dose of from 50 to 1000 mg/kg, 150 mg/kg to 850

mg/kg, 250 mg/kg to 750 mg/kg, 350 mg/kg to 650 mg/kg, or 450 mg/kg to 550 mg/kg. In some embodiments, the nicotine-binding antibody is administered at a dose of about 50 mg/kg, about 100 mg/kg, about 150 mg/kg, about 200 mg/kg, about 250 mg/kg, about 300 mg/kg, about 350 mg/kg, about 400 mg/kg, about 450 mg/kg, about 500 mg/kg, about 550 mg/kg, about 600, about 650 mg/kg, about 700 mg/kg, about 750 mg/kg, about 800 mg/kg, about 850 mg/kg, about 900 mg/kg, about 950 mg/kg, or about 1000 mg/kg. In some embodiments, the nicotine-binding antibody is administered at a dose of 50 mg/kg, 100 mg/kg, 150 mg/kg, 200 mg/kg, 250 mg/kg, 300 mg/kg, 350 mg/kg, 400 mg/kg, 450 mg/kg, 500 mg/kg, 550 mg/kg, 600, 650 mg/kg, 700 mg/kg, 750 mg/kg, 800 mg/kg, 850 mg/kg, 900 mg/kg, 950 mg/kg, or 1000 mg/kg. In some embodiments, the nicotine-binding antibody is administered at a dose of about 3000 mg, about 3500 mg, about 4000 mg, about 4500 mg, about 5000 mg, about 5500 mg, about 6000, about 6500 mg, about 7000 mg, about 7500 mg, about 8000 mg, about 8500 mg, about 9000 mg, about 9500 mg, about 10000 mg, about 10500 mg, about 11000 mg, about 11500 mg, or about 12000 mg. In some embodiments, the nicotine-binding antibody is administered at a dose of 3000 mg, 3500 mg, 4000 mg, 4500 mg, 5000 mg, 5500 mg, 6000, 6500 mg, 7000 mg, 7500 mg, 8000 mg, 8500 mg, 9000 mg, 9500 mg, 10000 mg, 10500 mg, 11000 mg, 11500 mg, or 12000 mg. In some embodiments, the nicotine-binding antibody is administered at a dose of up to about 10 g. When other antibody-related constructs are used, such as antibody fragments, they can be used at comparable doses adjusted for their different molecular weights and/or binding affinities. For example, the dose of a fragment can be chosen to achieve comparable C_{max} and/or AUC parameters as the corresponding full-length antibody, or to achieve binding of a comparable amount of nicotine.

[0085] In some embodiments, the nicotine-binding antibody is administered as a dose based on the molar ratio of antibody to nicotine. For instance, in some embodiments, the ratio of antibody:nicotine is 10:1, 9:1, 8:1, 7:1, 6:1, 5:1, 4:1, 3:1, 2:1, 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9, or 1:10. The disclosed nicotine-binding antibodies possess two nicotine binding sites per antibody, while a Fab of the disclosed nicotine-binding antibodies may only have one nicotine binding site. Accordingly, the dose may be adjusted based on the number of nicotine binding sites per molecule. For example, if one assumes that the MW for a full length antibody is 150 KD and 50 KD for a Fab, then an "equimolar dose amount" adjusted for the number of nicotine binding sites would be equivalent to a 50% higher dose amount (in mg/kg) for the full length antibody versus the Fab. These amounts are based on the assumption that the pharmacokinetic profile is substantially the same between the full-length antibody and the Fab; if that is not the case, those of ordinary skill in the art can adjust the amounts as needed in the event that the pharmacokinetic profiles are different.

[0086] In some embodiments, the method comprises administering a single dose of a pharmaceutical composition comprising a nicotine-binding antibody or nicotine-binding fragment thereof, or a single dose of a pharmaceutical composition comprising a nicotine-binding antibody or nicotine-binding fragment thereof and another compound. In other embodiments, the method comprises administering repeated doses of the pharmaceutical composition(s) until the symptoms or effects of nicotine poisoning or nicotine

overdose are reduced, ameliorated, or eliminated. For instance, a subject with nicotine poisoning or overdose may be evaluated for the presence and/or severity of signs and symptoms associated with nicotine poisoning, including, but not limited to, seizures, coma, shortness of breath, and increased heart rate, and treated with one or more pharmaceutical composition(s) as described herein until one or more of the signs/symptoms is reduced, ameliorated, or eliminated after treatment. In some embodiments, samples are taken to monitor nicotine levels in the subject's plasma or brain. In some embodiments, treatment is repeated with additional doses of the pharmaceutical composition(s) if signs/symptoms/effects persist and/or if nicotine plasma or brain levels remain elevated, and can be continued (repeated) until one or more symptoms or effects of nicotine poisoning or nicotine overdose are reduced, ameliorated, or eliminated, and/or until plasma levels and/or brain levels are reduced.

[0087] In some embodiments, treating a subject with nicotine poisoning or overdose may comprise extracorporeal detoxification of the subject's blood. For instance, the disclosed nicotine-binding antibodies or nicotine-binding fragments thereof can be attached to an affinity column through which the subject's blood can be circulated. This process can remove circulating nicotine from the subject's blood.

VI. Methods of Aiding in Smoking Cessation

[0088] As noted above, the antibodies described herein are useful in methods of treating nicotine addiction and/or facilitating smoking cessation (or the cessation of use of other nicotine products) in a mammalian subject in need thereof. In some embodiments, the subject is a human subject addicted to nicotine or desiring to quit smoking (or quit using other nicotine products) or maintain abstinence from smoking or consumption of other nicotine products.

[0089] As disclosed in the Examples section below, in some embodiments, the disclosed nicotine-binding antibodies or nicotine-binding fragments thereof attenuate nicotine's effects and do not induce withdrawal symptoms at predicted therapeutic doses, and have been demonstrated to aid in smoking cessation and the maintenance of abstinence in pre-clinical studies. The results have been noteworthy, as the negative affective consequences of early nicotine withdrawal are recognized as significant contributors to relapse to tobacco smoking during quit attempts, and the maintenance of compulsive nicotine use. In addition, the enhancement by nicotine of the reward value of other environmental rewarding stimuli is considered critical in the maintenance of nicotine dependence. Thus, blockade of nicotine-induced reward enhancement without inducing strong withdrawal effects are desirable properties of nicotine-binding antibodies and nicotine-binding fragments thereof as a putative anti-smoking medications that may play an important role in preventing relapse within the quit process and in the maintenance of abstinence.

[0090] Furthermore, the ligand-binding approach of the disclosed nicotine-binding antibodies and nicotine-binding fragments thereof is complementary to the pharmacodynamic mechanisms of non-nicotine pharmacotherapies, such as varenicline and bupropion. Without being bound by theory, the mechanism of the disclosed antibodies and fragments may be that when a smoker quits and then slips or relapses, the attenuation of nicotine's reinforcing effects helps to prevent resumption of regular smoking. Further, in

clinical trials, a greater number of quit attempts per subject were made in the high antibody group, as compared to placebo, consistent with this postulated relapse-prevention mechanism.

[0091] The methods generally involve administering a therapeutically effective amount of a nicotine-binding antibody or nicotine-binding fragment thereof as described herein (or a pharmaceutical composition comprising the same) to the subject. However, in some embodiments, the methods comprise administering a nucleic acid encoding the nicotine-binding antibody in a construct that expresses the antibody in vivo. For example, in such embodiments, the nucleic acid can be provided in a suitable vector, such as an adeno-associated virus (AAV) gene transfer vector. Other exemplary vectors that are suitable for use in such methods are known in the art. See, e.g., Lukashev and Zamyatnin, *Biochem.*, 81(7): 700-8 (2016)). Exemplary vectors may include one or more enhancers (e.g., a cytomegalovirus (CMV) enhancer), promoters (e.g., chicken β -actin promoter), and/or other elements enhancing the properties of the expression cassette. Methods of making suitable vectors and general methods of using expression vectors in vivo are known in the art. See, e.g., (see Hicks et al., *Sci. Transl. Med.*, 4(140): 140ra87 (2012)).

[0092] In some embodiments, a subject in need of treatment for nicotine addiction or facilitation of smoking cessation is a human subject who consumes nicotine products, such as smoking tobacco, chewing tobacco, electronic cigarettes, and/or other nicotine delivery devices. Such a subject may or may not be physically addicted to nicotine and/or psychologically addicted to consuming nicotine products. Typical subjects in need of smoking cessation treatment smoke or use tobacco or other nicotine products daily, such as smoking at least 1 or more cigarettes a day, such as at least about 5, at least about 10, at least about 15, at least about 20 or more, cigarettes per day, including fewer than 10, 10-20, 20-30, 30-40, or 40 or more (or the equivalent use of other tobacco or nicotine products).

[0093] In some embodiments, a therapeutically effective amount of a nicotine-binding antibody is an amount effective to reduce plasma levels of nicotine, to reduce levels of nicotine localized in the brain, or both.

[0094] Nicotine exerts many of its significant effects after it crosses the blood brain barrier. In some embodiments, the methods and uses described herein reduce or prevent nicotine from crossing the blood-brain-barrier. Thus, in some embodiments, administration of a nicotine-binding antibody as described herein binds up or sequesters nicotine circulating in the bloodstream of the subject, thereby reducing or preventing the nicotine from crossing the blood-brain-barrier. Thus, in some embodiments, the methods described herein reduce or prevent the physiological and psychological effects of nicotine that originate in the brain. Because the subject will experience a lessening or cessation of these effects, he/she will lose the desire to consume nicotine products. Additionally or alternatively, the disclosed nicotine-binding antibody may exert an effect by affecting the ability of nicotine to stimulate the peripheral nervous system.

[0095] The specific amount of a nicotine-binding antibody or nicotine-binding fragment thereof that is administered may depend on one or more of the age and/or weight of the subject, the amount of nicotine routinely consumed (e.g., smoked, chewed, or inhaled), and/or the level of nicotine in

the subject's brain or plasma at the time of treatment. For instance, in some embodiments, the nicotine-binding antibody is administered at a dose of from about 50 to about 1000 mg/kg, about 150 mg/kg to about 850 mg/kg, about 250 mg/kg to about 750 mg/kg, about 350 mg/kg to about 650 mg/kg, or about 450 mg/kg to about 550 mg/kg. In some embodiments, the nicotine-binding antibody is administered at a dose of from 50 to 1000 mg/kg, 150 mg/kg to 850 mg/kg, 250 mg/kg to 750 mg/kg, 350 mg/kg to 650 mg/kg, or 450 mg/kg to 550 mg/kg. In some embodiments, the nicotine-binding antibody is administered at a dose of about 50 mg/kg, about 100 mg/kg, about 150 mg/kg, about 200 mg/kg, about 250 mg/kg, about 300 mg/kg, about 350 mg/kg, about 400 mg/kg, about 450 mg/kg, about 500 mg/kg, about 550 mg/kg, about 600, about 650 mg/kg, about 700 mg/kg, about 750 mg/kg, about 800 mg/kg, about 850 mg/kg, about 900 mg/kg, about 950 mg/kg, or about 1000 mg/kg. In some embodiments, the nicotine-binding antibody is administered at a dose of 50 mg/kg, 100 mg/kg, 150 mg/kg, 200 mg/kg, 250 mg/kg, 300 mg/kg, 350 mg/kg, 400 mg/kg, 450 mg/kg, 500 mg/kg, 550 mg/kg, 600, 650 mg/kg, 700 mg/kg, 750 mg/kg, 800 mg/kg, 850 mg/kg, 900 mg/kg, 950 mg/kg, or 1000 mg/kg. In some embodiments, the nicotine-binding antibody is administered at a dose of about 3000 mg, about 3500 mg, about 4000 mg, about 4500 mg, about 5000 mg, about 5500 mg, about 6000, about 6500 mg, about 7000 mg, about 7500 mg, about 8000 mg, about 8500 mg, about 9000 mg, about 9500 mg, about 10000 mg, about 10500 mg, about 11000 mg, about 11500 mg, or about 12000 mg. In some embodiments, the nicotine-binding antibody is administered at a dose of 3000 mg, 3500 mg, 4000 mg, 4500 mg, 5000 mg, 5500 mg, 6000, 6500 mg, 7000 mg, 7500 mg, 8000 mg, 8500 mg, 9000 mg, 9500 mg, 10000 mg, 10500 mg, 11000 mg, 11500 mg, or 12000 mg. In some embodiments, the nicotine-binding antibody is administered at a dose of up to about 10 g. When other antibody-related constructs are used, such as antibody fragments, they can be used at comparable doses adjusted for their different molecular weights and/or binding affinities. For example, the dose of a fragment can be chosen to achieve comparable C_{max} and/or AUC parameters as the corresponding full-length antibody, or to achieve binding of a comparable amount of nicotine.

[0096] In some embodiments, the methods comprise administering a single dose of a nicotine-binding antibody(s) or nicotine-binding fragment(s) thereof (or composition comprising the same). In some embodiments, the method comprises administering repeated doses, such as for a pre-determined period of time or until the symptoms or effects of nicotine addiction are reduced, ameliorated, or eliminated or until the subject has ceased smoking or otherwise consuming nicotine. In some embodiments, treatment is repeated with additional doses of the variant(s) if signs/symptoms/effects persist or if the subject continues to have nicotine cravings or experiences them anew.

[0097] In some embodiments, the methods comprise administering a nicotine-binding antibody(s) or nicotine-binding fragment(s) thereof (or composition comprising the same) three or more times a day, twice a day, or once a day. In some embodiments, the methods comprise administering a nicotine-binding antibody(s) or nicotine-binding fragment(s) thereof (or composition comprising the same) once every other day, three times a week, twice a week, once a week, once every other week, once every three weeks, once a

month, or less frequently. In such embodiments, the nicotine-degrading enzyme variant may be a long-acting nicotine-binding antibody as described above.

[0098] In some embodiments, treatment may continue for 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or 21 or more days; 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, or 18 or weeks months; or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 or more months; or 1, 2, or 3 or more years or until the subject no longer experiences nicotine cravings or other nicotine withdrawal symptoms, or has ceased smoking or using other tobacco products.

[0099] As noted above, in some embodiments, the methods disclosed herein include administering an antibody as disclosed herein that does not exhibit binding affinity to smoking cessation drug (such as bupropion, varenicline and/or cytidine) in a combination therapy with a smoking cessation drug (such as bupropion, varenicline and/or cytidine, respectively), wherein the antibodies and drugs may be administered substantially simultaneously or sequentially in any order. Such embodiments may be particularly advantageous in methods for facilitating smoking cessation, quitting smoking (or quitting using other nicotine products), maintaining abstinence from smoking (or use of other nicotine products), or decreasing consumption of nicotine products. One skilled in the art will readily appreciate that the present disclosure is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those inherent therein. Modifications therein and other uses will occur to those skilled in the art. These modifications are encompassed within the spirit of the disclosure.

[0100] The following examples illustrate the invention. It should be understood, however, that the invention is not to be limited to the specific conditions or details described in these examples. All printed publications referenced herein are specifically incorporated by reference.

EXAMPLES

Example 1—Treatment of a Pediatric Patient with an Anti-Nicotine Antibody

[0101] This example illustrates methods using anti-nicotine antibodies in the treatment of nicotine poisoning or nicotine overdose.

[0102] A child known to have or suspected of having ingested nicotine is administered a therapeutically effective amount of a pharmaceutical composition comprising a nicotine-binding antibody, by intravenous, intramuscular, or subcutaneous injection. The child is evaluated for the presence and/or severity of signs and symptoms associated with nicotine poisoning, including, but not limited to, seizures, coma, shortness of breath, and increased heart rate, and the child is treated until one or more signs/symptoms is reduced, ameliorated, or eliminated. Optionally, another dose of the pharmaceutical composition is administered if signs/symptoms persist and/or if nicotine plasma levels remain elevated.

Example 2—Treating Nicotine Addiction And/Or Facilitating Smoking Cessation

[0103] This example illustrates methods of using a variant as described herein to treat nicotine addiction and/or facilitate smoking cessation in a human adult.

[0104] An adult human subject who regularly smokes cigarettes but wishes to quit is administered a therapeutically effective amount of a pharmaceutical compositions comprising a nicotine-binding antibody (e.g., the antibodies disclosed in Tables 1 and 2, or a long-acting version thereof) by intravenous, intramuscular, or subcutaneous injection. The subject is evaluated for levels of nicotine circulating in plasma, as well as for the presence and/or severity of signs and symptoms associated with nicotine withdrawal, such as headache, irritability, anxiety, and sleeplessness, as well as the number of cigarettes smoked in a given day. The subject is treated with repeated administrations of the antibody until levels of nicotine circulating in plasma reach a target (reduced) level, and/or until one or more signs/symptoms of nicotine withdrawal are reduced, ameliorated, or eliminated, and/or until the subject has reduced the level of consumption of nicotine products (e.g., is smoking fewer cigarettes per day), and/or until the subject has ceased consumption of nicotine products (e.g., has quit smoking).

Example 3—In Vivo Kinetic Studies

[0105] A single dose nicotine pharmacokinetic study was carried out in rats (N=8). Rats were pre-treated with 20 mg/kg of 5G4 IgG4, 7A8 IgG4, 12F5 IgG4, or 8D1 IgG4, and then 0.03 mg/kg of nicotine was administered intravenously. The nicotine dose was administered in less than 10 seconds (it takes roughly 10 minutes to smoke a cigarette. Three minutes later, animals were sacrificed and the amount of nicotine in their blood and brains was quantified.

[0106] FIGS. 1A and 1B show the blood and brain concentrations, respectively, as a percent of levels in control rats not pre-treated with antibody. Each antibody reduced the levels of nicotine in the brain compared to control animals that were not pre-treated with antibody. For example, the 8D1 IgG4 antibody produced an 80% decrease in the level of nicotine localized in the brain.

levels of nicotine in the brain, a 40 mg/kg dose of the 8D1 IgG4 antibody decreased the amount of nicotine localized in the brain by more than 95%. FIGS. 3A and 3B show the same data, but as a percentage of levels in control rats not pre-treated with antibody.

[0109] The 0.03 mg/kg dose of nicotine is equivalent to 2 cigarettes (mg/kg basis) and was administered as a rapid bolus (10s) in contrast to 5-10 minutes to smoke one cigarette. Serum levels of antibody were measured using ELISA and rats that had less than 5 µg/mL serum antibody level (due to incomplete administration) were excluded from the analyses. The excluded animals had an average serum antibody level of 0.73 µg/mL, while the rats included in the analysis had an average serum antibody level of 302 µg/mL. Compared to a control serum level of 21 ng/mL nicotine, single doses of 10, 20, and 40 mg/kg 8D1-IgG4 produced serum nicotine levels of 226, 351, and 470 ng/mL, corresponding respectively to 11-, 17-, and 22-fold of the control level (p=0.0057 by one-way ANOVA with Bonferroni correction for multiple comparisons). Compared to a control brain level of 139 ng/g nicotine, single doses of 10, 20, and 40 mg/kg 8D1-IgG4 produced brain nicotine levels of 68, 22, and 4 ng/g, corresponding respectively to 49%, 16%, and 3% of the control level (p=0.0045).

Example 5—Accelerated Stability Study

[0110] To determine the relative stability of exemplary nicotine-binding antibodies, antibodies 8D1-IgG4 and 12F5 IgG4 were formulated in phosphate buffer saline (PBS) at a concentration of approximately 10 mg/ml and incubated at 40° C. or 5° C. Samples were taken after 2 weeks and 4 weeks for analysis by Size Exclusion Chromatography and functional assay (direct binding to nicotine conjugate). The results of the stability studies are shown in Table 5 below.

TABLE 5

Stability of Exemplary Nicotine-Binding Antibodies					
Sample ID	Stability time point	Storage condition	Main Peak Area	% Aggregates	% Fragments
12F5-IgG4	2 week	5° C.	461,532	0	0
		40° C.	523,855	1.2	0
	4 week	5° C.	524,356	0	0
		40° C.	611,147	2.7	0
8D1-IgG4	2 week	5° C.	500,715	0.98	0
		40° C.	523,434	2.9	0
	4 week	5° C.	476,309	0	0
		40° C.	586,613	4.0	0

Example 4—In Vivo Dose-Response Studies

[0107] A single dose nicotine dose-response study was carried out in rats (N=8). Rats were used since their nicotine metabolism is generally similar to humans in rate and range of metabolites. Rats were pre-treated with 10, 20, or 40 mg/kg of 12F5 IgG4 or 8D1 IgG4. Subsequently, 0.03 mg/kg of nicotine was administered intravenously in less than 10 seconds. Three minutes later, the animals were sacrificed and the amount of nicotine in their serum and brains was quantified.

[0108] FIGS. 2A and 2B show the serum and brain concentrations, respectively. While both antibodies reduced the

[0111] Overall, stability of the nicotine-binding antibodies was acceptable, with a similar amount of monomer loss at week 4 for both antibodies that were tested. The functional (ELISA) assays showed identical functional binding to a nicotine-conjugate after 2 weeks storage.

Example 6—In Vivo Study of Acute Heavy Smoking

[0112] To test the effects of 8D1-IgG4 in a simulated scenario of acute heavy smoking, rats (N=10; 5 male and 5 female SD rats) pre-treated with 8D1-IgG4 or control IgG, received a series of 5 repeated intravenous nicotine doses

spaced 10 minutes apart (FIG. 4). Total serum nicotine increased as a function of accumulated nicotine dosing, and in an 8D1-IgG4 dose-dependent manner (FIG. 4A). After the fifth nicotine dose, brain nicotine levels were reduced by more than 90% at the 80 mg/kg 8D1-IgG4 dose level, and by a more moderate 51% at the 40 mg/kg dose level, compared to control IgG (FIG. 4B). Compared to an average control serum level of 60 ng/mL nicotine following the 5th nicotine dose, single doses of 40 and 80 mg/kg 8D1-IgG4 produced total serum nicotine levels of 1130 and 1987 ng/mL (<2% free nicotine, see below), corresponding respectively to 19 and 33-fold of the control level ($p < 0.0001$ by one-way ANOVA with Bonferroni's correction). Compared to an average control brain level of 298 ng/g nicotine following the 5th nicotine dose, single doses of 40 and 80 mg/kg 8D1-IgG4 produced brain levels of 146 and 23 ng/g, corresponding respectively to 49% and 8% of the control level ($p = 0.0006$ by one-way ANOVA with Bonferroni's correction). These data indicate 8D1-IgG4 is well maintained at nicotine dosing rates simulating very heavy smoking (10 cigarettes over 40 minutes).

Example 7—In Vivo Study on Nicotine Self-Administration

[0113] To assess whether 8D1-IgG4 could reduce self-administration, rats were initially trained for nicotine self-administration (NSA) using a unit nicotine dose of 0.03 mg/kg under a fixed-ratio (FR) 3 schedule during 2 hour sessions. After stable NSA was established, the unit dose was reduced to 0.015 mg/kg, which results in serum nicotine concentrations more similar to smoking in humans. After NSA stabilized at this unit dose, rats received twice-weekly i.v. infusions of 160 mg/kg 8D1-IgG4 (N=7) or 160 mg/kg Gammagard (control mAb, N=7) 30 minutes prior to the session while rats continued NSA at the 0.015 mg/kg dose for 10 consecutive sessions. Then, the unit nicotine dose was reduced to 0.0075 mg/kg for another 10 consecutive sessions while mAb treatment continued. FIG. 5 shows the mean (\pm SEM) number of infusions during the last three sessions before (Baseline) and during mAb treatment at each unit nicotine dose. Rats given 8D1-IgG4 exhibited a significant decrease in NSA at both unit doses compared to their respective baseline and to control rats. These findings demonstrate that 8D1-IgG4 reduces the reinforcing effects of nicotine. Although the dose of 8D1-IgG4 was high, the effective dose for smoking cessation in humans will likely be much lower because people will be motivated to quit. As a point of reference, the potency of varenicline was considerably higher in clinical trials for smoking cessation than it was in preclinical nicotine self-administration studies in rats. Rollema, H. et al., *Neuropharmacology*, 52: 985-994 (2007).

Example 8—In Vivo Pharmacokinetic Studies

[0114] The pharmacokinetics of 8D1-IgG4 were tested in rats following a single dose (20 mg/kg; FIG. 6) and repeated doses (40 mg/kg; FIG. 7) of 8D1-IgG4 dosed weekly for 4 weeks in rats (N=6). Residual mAb concentrations were measured at various time points after i.v. dosing. The ELISA detection assay employed relies on binding to the nicotine conjugate 3'Am-S(-)Nic-polyglutamic acid, and thus reflects functional mAb levels binding S(-)-nicotine in

serum. Parameters estimated by non-compartmental analysis of 8D1-IgG4 concentrations include an elimination phase half-life of 131 h, clearance of 0.10 mL/min/kg, and a steady-state $VD = 79.2$ mL/kg, respectively. Rodent PK assays of mAb's are not always predictive of PK in humans but are often used as a measure of "in vivo fitness" in the lead selection process. While not seen for 8D1-IgG4, an abnormally fast antibody clearance can be a sign of unwanted nonspecific interactions, so these assays are used to identify antibodies with high nonspecific disposition PK. At the end of this study, rats were dosed with 0.03 mg/kg i.v. nicotine and sacrificed 3 minutes later and samples were analyzed to assess the amount of unbound nicotine by before and after ultrafiltration. All samples had <2% unbound nicotine (data not shown).

Example 9—In Vivo Toxicity Study

[0115] To assess the toxicity of high doses of 8D1-IgG4, a non-GLP 4-week repeated, high-dose toxicology study of 8D1-IgG4 with and without concurrent administration of nicotine was conducted in rats to evaluate if any significant toxicity signals were observed. Four groups of 16 rats per group (8 male and 8 female) were tested: vehicle control, 8D1-IgG4 only, nicotine only, and 8D1-IgG4 plus nicotine—the latter to assess the safety of the nicotine:antibody complex. 8D1-IgG4 was dosed i.v. once weekly at 200 mg/kg. Nicotine was dosed continuously via infusion pump into the subcutaneous space (1 mg/kg/day for 28d).

[0116] Assessment of toxicity was based on mortality, clinical observations, and body weight during the course of the 28-day study, and at the end of study organ weights, gross anatomic pathology, hematology, serum clinical chemistry, and coagulation was performed. Histopathology of selected tissues (heart, liver, lung, kidney, spleen, skeletal muscle, brain, colon, stomach, ovary, and testis) is pending. Tissues were fixed immediately in formalin, and processed for embedding in paraffin, staining with H&E, and review by a veterinary pathologist.

[0117] 8D1-IgG4 was well-tolerated with no obvious pathology in the treatment groups. All animals received the full dose and no mortality was induced in any animals. Daily clinical observations found no observable behavioral changes or modifications in feeding or grooming in any groups. Body weight was monitored twice weekly for the duration of the study and no significant differences between treatment groups was found. At the end of the study animals were necropsied and major organs (liver, lung, spleen, heart, kidneys, testis or ovaries) were isolated and weighed. No gross pathological findings were noted and no statistically significant changes in organ weights were found. Blood was collected, and complete blood count performed to determine any changes in hematological parameters. While occasional animals had values outside the normal range (e.g. slightly decreased lymphocytes or hemoglobin) no significant changes or trends were found in any group. There was a trend to have slight polychromasia in some of the animals that received nicotine. Serum clinical chemistry of 23 different analytes and plasma coagulation did not find any notable changes between treatment groups.

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QYNSTYRVVS		VLTVLHQDWL		NGKEYKCKVS	NKALPAPIEK	TISKAKGQPR	EPQVYTLPPS	360	
RDELTKNQVS		LTCLVKGFYP		SDIAVEWESN	GQPENNYKTT	PPVLDSGGSF	FLYSKLTVDK	420	
SRWQQGNVFS		CSVMHEALHN		HYTQKSLSLS	PGK			453	
SEQ ID NO: 4		moltype = AA length = 217							
FEATURE		Location/Qualifiers							
source		1..217							
		mol_type = protein							
		organism = synthetic construct							
		note = Description of Artificial Sequence: Synthetic polypeptide							
SEQUENCE: 4									
EVVLTQSPGT		LSLSPGERAT		LSCRASQSVS	SRYLAWYQQK	PGQAPRLLIY	GASSRAIGTP	60	
DRFSGSGSGT		DFTLTISRLE		PEDFAVYYCQ	QYAYSPPAIT	FGGGTKVEIK	RTVAAPSVFI	120	
FPPSDEQLKS		GTASVVCLLN		NFYFPREAKVQ	WKVDNALQSG	NSQESVTEQD	SKDSTYSLSS	180	
TLTLSKADYE		HKHVVACEVT		HQGLSSPVTK	SFNRGEC			217	
SEQ ID NO: 5		moltype = AA length = 456							
FEATURE		Location/Qualifiers							
source		1..456							
		mol_type = protein							
		organism = synthetic construct							
		note = Description of Artificial Sequence: Synthetic polypeptide							
SEQUENCE: 5									
QLQLQESGPG		LLKPSETLSL		TCTVSGGSVT	TSPDWWAWLR	QSPGKGLEWI	GSVSYTGRTV	60	
YNPSLKSRVT		ISLDTSKNHL		SLRMTSATAA	DTAVFYCARL	TPIDRFSADY	YVLDIWGQGA	120	
TVTVSSASTK		GPSVFPLAPS		SKSTSGGTAA	LGCLVKDYFP	EPVTVSWNSG	ALTSGVHTFP	180	
AVLQSSGLYS		LSSVVTVPSS		SLGTQTYICN	VNHKPSNTKV	DKKVEPKSCD	KTHTCPPCPA	240	
PELLGGPSVF		LFPPKPKDTL		MISRTPEVTC	VVVDVSHEDP	EVKFNWYVDG	VEVHNAKTKP	300	
REEQYNSTYR		VVSVLTVLHQ		DWLNGKEYKC	KVSNKALPAP	IEKTISKAKG	QPREPQVYTL	360	

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PPSRDELTKN	QVSLTCLVKG	FYPSDIAVEW	ESNGQPENNY	KTPPVLDSD	GSFFLYSKLT	420
VDKSRWQQGN	VFSCSVMHEA	LHNHYTQKSL	SLSPGK			456
SEQ ID NO: 6	moltype = AA length = 216					
FEATURE	Location/Qualifiers					
source	1..216					
	mol_type = protein					
	organism = synthetic construct					
	note = Description of Artificial Sequence: Synthetic polypeptide					
SEQUENCE: 6						
EIVMTQSPAT	LSVSPGERAT	LSCRASQSIG	SNLAWFQHKP	GQAPRLLIFR	SSTRATGTPP	60
RFSGSGSGTE	FTLTISLQS	EDFAVYFCQH	YSWPPPLITF	GQGTRLEIKR	TVAAPSVFIF	120
PPSDEQLKSG	TASVVCLLN	FYPREAKVQW	KVDNALQSGN	SQESVTEQDS	KDSTYLSST	180
LTLSKADYEK	HKVYACEVTH	QGLSSPVTKS	FNRGEC			216
SEQ ID NO: 7	moltype = AA length = 458					
FEATURE	Location/Qualifiers					
source	1..458					
	mol_type = protein					
	organism = synthetic construct					
	note = Description of Artificial Sequence: Synthetic polypeptide					
SEQUENCE: 7						
QLQLRESGPG	LVKPSETLSL	TCSVSGGSIS	SSSYWGWIR	QPPGKGLEWI	GSIIYTGRTY	60
YNPSLESRV	ISVDTSKNQF	SLKLSSVTAA	DTAVYYCAGL	HYSWSALGGY	YFYGMDVWGQ	120
GTTVTVSSAS	TKGPSVFPLA	PSSKSTSGGT	AALGCLVKDY	FPEPVTVSWN	SGALTSGVHT	180
FPAVLQSSGL	YSLSSVVTVP	SSSLGTQTYI	CNVNHKPSNT	KVDKKVEPKS	CDKTHTCPPC	240
PAPELLGGPS	VFLFPPKPKD	TLMISRTPEV	TCVVDVSHE	DPEVKFNWYV	DGVEVHNAKT	300
KPREEQYNST	YRVVSVLTVL	HQDWLNGKEY	KCKVSNKALP	APIEKTISKA	KGQPREPQVY	360
TLPPSRDEL	KNQVSLTCLV	KGFYPSDIAV	EWESNGQPEN	NYKTPPVLD	SDGSFFLYSK	420
LTVDKSRWQQ	GNVFSCSVMH	EALHNHYTQK	SLSLSPGK			458
SEQ ID NO: 8	moltype = AA length = 217					
FEATURE	Location/Qualifiers					
source	1..217					
	mol_type = protein					
	organism = synthetic construct					
	note = Description of Artificial Sequence: Synthetic polypeptide					
SEQUENCE: 8						
EIVLTQSPGT	LSLSPGERAT	LSCRASQSVS	SRDLVWYQQK	PGQAPRLLIY	GASTRATGIP	60
DRFSGSGSGT	DFTLTISRLE	PEDFAVYQC	KYGSSPPRIT	FGPGTKVDIK	RTVAAPSVFI	120
FPPSDEQLKS	GTASVVCLLN	NFYPREAKVQ	WKVDNALQSG	NSQESVTEQD	SKDSTYLSLS	180
TLTLKADYE	KHKVYACEVT	HQGLSSPVTK	SFNRGEC			217
SEQ ID NO: 9	moltype = AA length = 452					
FEATURE	Location/Qualifiers					
source	1..452					
	mol_type = protein					
	organism = synthetic construct					
	note = Description of Artificial Sequence: Synthetic polypeptide					
SEQUENCE: 9						
QLQLQESGPG	LVKPSETLSL	TCSVSGGSIS	SSSYWGWSR	QSPGKGLEWI	ASIIYSGSTY	60
YNPSLKSRVT	IFIDTSKNQF	SLKLSSVTAA	DTAIYYCARV	GTSAMSRAFD	MWGQGTMTV	120
SSASTKGPSV	FPLAPSSKST	SGGTAALGCL	VKDYFPEPVT	VSWNSGALTS	GVHTFPAVLQ	180
SSGLYSLSSV	VTVPSSSLGT	QTYICNVNHK	PSNTKVDKKV	EPKSCDKTHT	CPPCPAPELL	240
GGPSVFLFPP	KPKDTLMISR	TPEVTCVVVD	VSHEDPEVKF	NWYVDGVEVH	NAKTKPREEQ	300
YNSTYRVVSV	LTVLHQDWLN	GKEYKCKVSN	KALPAPIEKT	ISKAKGQPRE	PQVYTLPPSR	360
DELTKNQVSL	TCLVKGFYPS	DIAVEWESNG	QPENNYKTPP	PVLDSGGSFF	LYSKLTVDKS	420
RWQQGNVFSC	SVMHEALHNH	YTQKSLSLSP	GK			452
SEQ ID NO: 10	moltype = AA length = 219					
FEATURE	Location/Qualifiers					
source	1..219					
	mol_type = protein					
	organism = synthetic construct					
	note = Description of Artificial Sequence: Synthetic polypeptide					
SEQUENCE: 10						
DIVMTQSPLS	LPVTPGEPAS	ISCRSSQSLL	QSNGYNYLDW	YLQKPGQSPQ	LLIYLGSNRA	60
SGVPDRFSGS	GSGETDFTLKI	SKVEAEDVGV	YFCMQALQIP	WTFGQGTKVE	IKRTVAAPSV	120
FIFPPSDEQL	KSGTASVVCL	LNNFYPREAK	VQWKVDNALQ	SGNSQESVTE	QDSKDSTYSL	180
SSTLTLSKAD	YEKHKVYACE	VTHQGLSPV	TKSFNRGEC			219

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SEQ ID NO: 11	moltype = AA	length = 453
FEATURE	Location/Qualifiers	
source	1..453	
	mol_type = protein	
	organism = synthetic construct	
	note = Description of Artificial Sequence: Synthetic polypeptide	
SEQUENCE: 11		
QVQLQESGPG	LVKPSETLSL	TCTVSGGSIS RRNDYWAWIR QSPGKDLEWI GTISFSGSTF 60
YNPSLKSRVT	ISADTFNNHF	SLRLDAVAAA DTAVYYCARL SPFVGAAWWF DPWGPGLVT 120
VSSASTKGPS	VFPLAPSSKS	TSGGTAALGC LVKDYFPEPV TVSWNSGALT SGVHTFPAVL 180
QSSGLYSLSS	VVTVPSSSLG	TQTYICNVNH KPSNTKVDKK VEPKSCDKTH TCPPCPAPEL 240
LGGPSVFLFP	PKPKDTLMIS	RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV HNAKTKPREE 300
QYNSTYRVVS	VLTVLHQDWL	NGKEYKCKVS NKALPAPIEK TISKAKGQPR EPQVYTLPPS 360
RDELTKNQVS	LTCLVKGFYP	SDIAVEWESN GQPENNYKTT PPVLDSGGSF FLYSKLTVDK 420
SRWQQGNVFS	CSVMHEALHN	HYTQKSLSLS PGK 453
SEQ ID NO: 12	moltype = AA	length = 217
FEATURE	Location/Qualifiers	
source	1..217	
	mol_type = protein	
	organism = synthetic construct	
	note = Description of Artificial Sequence: Synthetic polypeptide	
SEQUENCE: 12		
EIVLTQSPGT	LSLSPGERAT	LSCRASQSLS SNYLGWYQQK PGQAPRLLIY GASNRATGIP 60
DRFSGSGSGT	DFTLTISRLE	PEDFGVYYCQ RYGRSPPAIT FGGGTKVEIK RTVAAPSVFI 120
FPPSDEQLKS	GTASVCLLN	NFYPREAKVQ WKVDNALQSG NSQESVTEQD SKDSTYSLSS 180
TLTLKADYE	KHKVYACEVT	HQGLSSPVTK SFNRGEC 217
SEQ ID NO: 13	moltype = AA	length = 457
FEATURE	Location/Qualifiers	
source	1..457	
	mol_type = protein	
	organism = synthetic construct	
	note = Description of Artificial Sequence: Synthetic polypeptide	
SEQUENCE: 13		
QLQLQESGPG	LVKPSETLSL	TCTASGGSIT NNIDYWVWIR QPPGRGLEWI GTIYYSGSTF 60
YNPSLKSRVT	ISVDTSNNQF	SLNLNSMSAA DTAVYYCARL RYYYDSNGYL PYWIDSWGQG 120
TLVTVSSAST	KGPSVFPLAP	SSKSTSGGTA ALGCLVKDYF PEPVTVSWNS GALTSGVHTF 180
PAVLQSSGLY	SLSSVTVPS	SSLGTQTYIC NVNHKPSNTK VDKKVEPKSC DKTHTCPPCP 240
APELLGGPSV	FLFPPKPKDT	LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNAKTK 300
PREEQYNSTY	RVVSVLTVLH	QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT 360
LPSPRDELTK	NQVSLTCLVK	GFYPSDIAVE WESNGQPENN YKTPPVLDSDGSFFLYSKL 420
TVDKSRWQQG	NVFSCSVMHE	ALHNHYTQKS LSLSPGK 457
SEQ ID NO: 14	moltype = AA	length = 217
FEATURE	Location/Qualifiers	
source	1..217	
	mol_type = protein	
	organism = synthetic construct	
	note = Description of Artificial Sequence: Synthetic polypeptide	
SEQUENCE: 14		
EIVLTQSPGT	LSLSPGERAT	LSCRASQSIS SSYLGWYQQK PGQAPRLLIY GASSRATGIP 60
DRFSGSGSGT	DFTLTISSLE	PEDFAVYFCQ LYRRSPPRLT FGGGTKVEIK RTVAAPSVFI 120
FPPSDEQLKS	GTASVCLLN	NFYPREAKVQ WKVDNALQSG NSQESVTEQD SKDSTYSLSS 180
TLTLKADYE	KHKVYACEVT	HQGLSSPVTK SFNRGEC 217
SEQ ID NO: 15	moltype = AA	length = 455
FEATURE	Location/Qualifiers	
source	1..455	
	mol_type = protein	
	organism = synthetic construct	
	note = Description of Artificial Sequence: Synthetic polypeptide	
SEQUENCE: 15		
QLQLQESGPG	LVKPSESLSL	TCTVSGGSII SNDYYWAWIR QSPGKGLEWI GSINYRGSTF 60
YPSLNSRVVT	TSVDTSKNQF	FLKLTSVTAA DTAMYFCTRL HGRYRGVGRL AFDYWGGGTL 120
VTVSSASTKG	PSVFPLAPSS	KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA 180
VLQSSGLYSL	SSVVTVPSSS	LGTQTYICNV NHKPSNTKVD KKVEPKSCDK THTCPPCPAP 240
ELLGGPSVFL	FPPKPKDTLM	ISRTPEVTCV VVDVSHEDPE VKFNWYVDGV EVHNAKTKPR 300
EEQYNSTYRV	VSVLTVLHQD	WLNGKEYKCK VSNKALPAPI EKTISKAKGQ PREPQVYTL 360
PSRDELTKNQ	VSLTCLVKGF	YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSKLTV 420
DKSRWQQGNV	FSCSVMHEAL	HNHYTQKSLS LSPGK 455

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SEQ ID NO: 16	moltype = AA	length = 214
FEATURE	Location/Qualifiers	
source	1..214	
	mol_type = protein	
	organism = synthetic construct	
	note = Description of Artificial Sequence: Synthetic	
	polypeptide	
SEQUENCE: 16		
DIQMTQSPST LSASVGDIVT	ITCRASQSIG DWLAWYQQKP	GKAPKLLIYK ASNLESGVPS 60
RFGSGSGSGTE FTLTISSLQS	DDFATYYCQQ YDSYSVTFGQ	GTKVEIKGTV AAPSVFIFPP 120
SDEQLKSGTA SVVCLLNNFY	PREAKVQWKV DNALQSGNSQ	ESVTEQDSKD STYLSSTLT 180
LSKADYEKHK VYACEVTHQG	LSSPVTKSFN RGE	C 214
SEQ ID NO: 17	moltype = AA	length = 456
FEATURE	Location/Qualifiers	
source	1..456	
	mol_type = protein	
	organism = synthetic construct	
	note = Description of Artificial Sequence: Synthetic	
	polypeptide	
SEQUENCE: 17		
QVQLQEAGPG LVKPSETLSL	TCTVSGGSIN TRNYYGWVR	QPPGKGLEWI ASVYYTGSTF 60
YDPSLRSRVT ISIDTPRNQF	SLRVSSVDAG DMGVYVCVRL	DGGYNNGYYY YGMDVWGQGT 120
SVTVSSASTK GPSVFPLAPS	SKSTSGGTAA LGCLVKDYFP	EPVTVSWNSG ALTSGVHTFP 180
AVLQSSGLYS LSSVTVPS	SLGTQTYICN VNHKPSNTKV	DKKVEPKSCD KTHTCPPCPA 240
PELLGGPSVF LFPPKPKDTL	MISRTPEVTC VVVDVSHEDP	EVKFNWYVDG VEVHNAKTKP 300
REEQYNSTYR VVSVLTVLHQ	DWLNGKEYKC KVSNAKALPAP	IEKTISKAKG QPREPQVYTL 360
PPSRDELTKN QVSLTCLVKG	FYPQSDIAVEW ESNGQPENNY	KTTTPVLDS GSFFLYSKLT 420
VDKSRWQQGN VFSCVMHEA	LHNHYTQKSL SLSPGK	456
SEQ ID NO: 18	moltype = AA	length = 214
FEATURE	Location/Qualifiers	
source	1..214	
	mol_type = protein	
	organism = synthetic construct	
	note = Description of Artificial Sequence: Synthetic	
	polypeptide	
SEQUENCE: 18		
GVQMTQSPST LSASVGERVT	VTCRASRPIS NWLSWYQQKP	GRAPKLLIYG TSTLESGVPS 60
RFGSGSGSGTE FTLTITNLQP	DDFATYYCQE HNLYTITFGP	GTKVEIKRTV AAPSVFIFPP 120
SDEQLKSGTA SVVCLLNNFY	PREAKVQWKV DNALQSGNSQ	ESVTEQDSKD STYLSSTLT 180
LSKADYEKHK VYACEVTHQG	LSSPVTKSFN RGE	C 214
SEQ ID NO: 19	moltype = AA	length = 457
FEATURE	Location/Qualifiers	
source	1..457	
	mol_type = protein	
	organism = synthetic construct	
	note = Description of Artificial Sequence: Synthetic	
	polypeptide	
SEQUENCE: 19		
QLQLQESGPG LVKPSETLSL	SCAVSGASIR SNTYYGWIR	QPPGRGLEWI GSISHRGDAH 60
YSPSLKSPVT ISVDTSKNEF	SLKATSVTAA DTAVYVCVSL	AYSFSWNTYY FYGMDVWGHH 120
ITVTVSSAST KGPSVFPLAP	SSKSTSGGTA ALGCLVKDYF	PEPVTVSWNS GALTSGVHTF 180
PAVLQSSGLY SLSSVTVPS	SSLGTQTYIC NVNHKPSNTK	VDKKVEPKSC DKTHTCPPCP 240
APELLGGPSV FLFPPKPKDT	LMISRTPEVT CVVVDVSHED	PEVKFNWYVD GVEVHNAKTK 300
PREEQYNSTY RVVSVLTVLH	QDWLNGKEYK CKVSNKALPA	PIEKTISKAK GQPREPQVYT 360
LPSPSRDELTK NQVSLTCLVK	GFYPQSDIAVE WESNGQPENN	YKTTTPVLDS DGSFFLYSKL 420
TVDKSRWQQG NVFSCVMHE	ALHNHYTQKS LSLSPGK	457
SEQ ID NO: 20	moltype = AA	length = 217
FEATURE	Location/Qualifiers	
source	1..217	
	mol_type = protein	
	organism = synthetic construct	
	note = Description of Artificial Sequence: Synthetic	
	polypeptide	
SEQUENCE: 20		
DIVLTQSPGT LSLSPGEGAT	LSCRASQSVN SGYLAWYQQK	PGQPPRLLVF AASSRATGIA 60
DRFRGSGSGT DFTLTITRLE	PEDFAVYYCQ LYGHSPARIT	FGQGRLETK RTVAAPSVFI 120
FPPSDEQLKS GTASVCLLN	NFYPPREAKVQ WKVDNALQSG	NSQESVTEQD SKDSTYSLSS 180
TLTSLKADYE KHKVYACEVT	HQGLSSPVTK SFNRGEC	217
SEQ ID NO: 21	moltype = AA	length = 449
FEATURE	Location/Qualifiers	

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source	1..449				
	mol_type = protein				
	organism = synthetic construct				
	note = Description of Artificial Sequence: Synthetic polypeptide				
SEQUENCE: 21					
QLQLQESGPG	LVKPSETLSL	TCSVSGGSIS	SSSYWGWSR	QSPGKGLEWI	ASIYYSGSTY 60
YNPSLKSRVT	IFIDTSKNQF	SLKLSSVTAA	DTAIYYCARV	GTSAMSRAFD	MWGQGTMTVT 120
SSASTKGPSV	FPLAPCSRST	SESTAALGCL	VKDYFPEPVT	VSWNSGALTS	GVHTFPAVLQ 180
SSGLYSLSSV	VTVPSSSLGT	KTYTCNVDHK	PSNTKVDKRV	ESKYGPPCPP	CPAPEFLGGP 240
SVFLFPPKPK	DTLMISRTP	VTCVVVDVSQ	EDPEVQFNWY	VDGVEVHNAK	TKPREEQFNS 300
TYRVVSVLTV	LHQDWLNGKE	YKCKVSNKGL	PSSIEKTISK	AKGQPREPQV	YTLPPSQEEM 360
TKNQVSLTCL	VKGFYPSDIA	VEWESNGQPE	NNYKTTPPVL	DSDGSFFLYS	RLTVDKSRWQ 420
EGNVFSCSVM	HEALHNHYTQ	KSLSLSLGK			449
SEQ ID NO: 22	moltype = AA	length = 219			
FEATURE	Location/Qualifiers				
source	1..219				
	mol_type = protein				
	organism = synthetic construct				
	note = Description of Artificial Sequence: Synthetic polypeptide				
SEQUENCE: 22					
DIVMTQSPLS	LPVTPGEPAS	ISCRSSQSLL	QSNGYNYLDW	YLQKPGQSPQ	LLIYLGSNRA 60
SGVPDRFSGS	GSGTDFTLKI	SKVEAEDVGV	YFCMQALQIP	WTFGQGTKVE	IKRTVAAPSV 120
FIFPPSDEQL	KSGTASVVCL	LNNFYPREAK	VQWKVDNALQ	SGNSQESVTE	QDSKDYSTYSL 180
SSTLTLSKAD	YEKHKVYACE	VTHQGLSSPV	TKSFNRGEC		219
SEQ ID NO: 23	moltype = AA	length = 453			
FEATURE	Location/Qualifiers				
source	1..453				
	mol_type = protein				
	organism = synthetic construct				
	note = Description of Artificial Sequence: Synthetic polypeptide				
SEQUENCE: 23					
QLQLQESGPG	LLKPSETLSL	TCTVSGGSVT	TSPDWWAWLR	QSPGKGLEWI	GSVSYTGRTV 60
YNPSLKSRVT	ISLDTSKNHL	SLRMTSATAA	DTAVFYCARL	TPIDRFSADY	YVLDIWGQGA 120
TVTVSSASTK	GPSVFPLAPC	SRSTSESTAA	LGCLVKDYFP	EPVTVSWNSG	ALTSGVHTFP 180
AVLQSSGLYS	LSSVTVVPSS	SLGTKTYTCN	VDHKPSNTKV	DKRVESKYGP	PCPPCPAPEF 240
LGGPSVFLFP	PKPKDTLMIS	RTPEVTCVVV	DVSQEDPEVQ	FNWYVDGVEV	HNAKTKPREE 300
QFNSTYRVVS	VLTVLHQDWL	NGKEYKCKVS	NKGLPSSIEK	TISKAKGQPR	EPQVYTLPPS 360
QEEMTKNQVS	LTCLVKGFYP	SDIAVEWESN	GQPENNYKTT	PPVLDSGGSF	FLYSRLTVDK 420
SRWQEGNVFS	CSVMHEALHN	HYTQKSLSL	LGK		453
SEQ ID NO: 24	moltype = AA	length = 216			
FEATURE	Location/Qualifiers				
source	1..216				
	mol_type = protein				
	organism = synthetic construct				
	note = Description of Artificial Sequence: Synthetic polypeptide				
SEQUENCE: 24					
EIVMTQSPAT	LSVSPGERAT	LSCRASQSIG	SNLAWFQHKP	GQAPRLLIFF	SSTRATGTPP 60
RFGSGSGSTE	FTLTISSLQS	EDFAVYFCQH	YSYWPPLITF	GQGRLEIKR	TVAAPSVFIF 120
PPSDEQLKSG	TASVVCLLNN	FYPREAKVQW	KVDNALQSGN	SQESVTEQDS	KDSTYLSLST 180
LTLISKADYEK	HKVYACEVTH	QGLSSPVTKS	FNRGEC		216
SEQ ID NO: 25	moltype = AA	length = 450			
FEATURE	Location/Qualifiers				
source	1..450				
	mol_type = protein				
	organism = synthetic construct				
	note = Description of Artificial Sequence: Synthetic polypeptide				
SEQUENCE: 25					
QLQLQESGPG	LVKPSETLSL	ICTVSGGSIR	KNNEWWAWIR	QAPGKGLEWI	GSLSYTGRTV 60
YNPSLKSRVT	ISTDTSETQF	SLKVNSVTAA	DTAVYYCARL	SPFVGAAWWF	DPWGQGTTLVT 120
VSSASTKGPS	VFPLAPCSRS	TSESTAALGC	LVKDYFPEPV	TVSWNSGALT	SGVHTFPAVL 180
QSSGLYSLSS	VVTVPSSSLG	TKTYTCNVDH	KPSNTKVDKR	VESKYGPPCP	PCPAPEFLGG 240
PSVFLFPPKP	KDTLMISRTP	EVTCVVVDVS	QEDPEVQFNW	YVDGVEVHNA	KTKPREEQFN 300
STYRVVSVLT	VLHQDWLNGK	EYKCKVSNKG	LPSSIEKTIS	KAKGQPREPQ	VYTLPPSQEE 360
MTKNQVSLTC	LVKGFYPSDI	AVEWESNGQP	ENNYKTTPPV	LDSDGSFFLY	SRLTVDKSRW 420
QEGNVFSCSV	MHEALHNHYT	QKSLSLSLGK			450
SEQ ID NO: 26	moltype = AA	length = 217			

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FEATURE	Location/Qualifiers
source	1..217 mol_type = protein organism = synthetic construct note = Description of Artificial Sequence: Synthetic polypeptide
SEQUENCE: 26	
EVVLTQSPGT LSLSPGERAT	LSCRASQSVS SRYLAWYQQK PGQAPRLLIY GASSRAIGTP 60
DRFSGSGSGT DFTLTISRLE	PEDFAVYYCQ QYAYSPPAIT FGGGTKVEIK RTVAAPSVFI 120
FPPSDEQLKS GTASVVCLLN	NFYPPREAKVQ WKVDNALQSG NSQESVTEQD SKDSTYSLSS 180
TLTLSKADYE KHKVYACEVT	HQGLSSPVTK SFNRGEC 217
SEQ ID NO: 27	moltype = AA length = 444
FEATURE	Location/Qualifiers
source	1..444 mol_type = protein organism = synthetic construct note = Description of Artificial Sequence: Synthetic polypeptide
SEQUENCE: 27	
QVRLQESGPG LVKPSGTLSL	TCAVSGGSIY SSNWWTWVRQ PPGKGLEWVG EHIRGTTY 60
NPSLNSRVTI SLDKSNNQVS	LRLTSVTAAD SAVYYCVSQE VGGPDLWGQG TLVTVSSAST 120
KGPSVFPLAP CSRSTSESTA	ALGCLVKDYF PEPVTVSWNS GALTSGVHTF PAVLQSSGLY 180
SLSSVVTVPS SSLGKTYTC	NVDHKPSNTK VDKRVESKYG PPCPPCPAPE FLGGPSVFLF 240
PPKPKDTLMI SRTPEVTCVV	VDVSQEDPEV QFNWYVDGVE VHNAKTKPRE EQFNSTYRVV 300
SVLTVLHQDW LNGKEYKCKV	SNKGLPSSIE KTISKAKGQP REPQVYTLPP SQEEMTKNQV 360
SLTCLVKGFY PSDIAVEWES	NGQPENNYKT TPPVLDSGGS FFLYSRLTVD KSRWQEGNVF 420
SCSVMHEALH NHYTQKSLSL	SLGK 444
SEQ ID NO: 28	moltype = AA length = 216
FEATURE	Location/Qualifiers
source	1..216 mol_type = protein organism = synthetic construct note = Description of Artificial Sequence: Synthetic polypeptide
SEQUENCE: 28	
NFMLTQPHSV SESPGKTVTI	SCTRSGGSIA TYYVQWYQQR PGSAPTNVIY KYDQRPSGVP 60
DRFSGSIDSS SNSASLTISG	LKTEDEADYY CQSYDNNIQV FGGGTKLTVL GQPKAAPSVT 120
LFPPSSEELQ ANKATLVCLI	SDFYPGAVTV AWKADGSPVK AGVETTKPSK QSNNKYAASS 180
YLSLTPEQWK SHRSYSCQVT	HEGSTVEKTV APTECS 216
SEQ ID NO: 29	moltype = AA length = 455
FEATURE	Location/Qualifiers
source	1..455 mol_type = protein organism = synthetic construct note = Description of Artificial Sequence: Synthetic polypeptide
SEQUENCE: 29	
QLQLRESGPG LVKPSETLSL	TCSVSGGSIS SSSYYWGWIR QPPGKGLEWI GSIYYTGRTY 60
YNPSLESRVIT ISVDTSKNQF	SLKLSSVTAA DTAVYYCAGL HYSWSALGGY YFYGMVDVWGQ 120
GTTVTVSSAS TKGPSVFPLA	PCSRSTSEST AALGCLVKDY FPEPVTVSWN SGALTSGVHT 180
FPAVLQSSGL YSLSSVTVP	SSSLGKTYT CNVDHKPSNT KVDKRVESKY GPPCPPCPAP 240
EFLGGPSVFL FPPKPKDTLM	ISRTPEVTCV VVDVSQEDPE VQFNWYVDGV EVHNAKTKPR 300
EEQFNSTYRV VSVLTVLHQD	WLNGKEYKCK VSNKGLPSSI EKTISKAKGQ PREPQVYTL 360
PSQEEMTKNQ VSLTCLVKGF	YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSRLTV 420
DKSRWQEGNV FSCSVMHEAL	HNHYTQKSLS LSLGK 455
SEQ ID NO: 30	moltype = AA length = 217
FEATURE	Location/Qualifiers
source	1..217 mol_type = protein organism = synthetic construct note = Description of Artificial Sequence: Synthetic polypeptide
SEQUENCE: 30	
EIVLTQSPGT LSLSPGERAT	LSCRASQSVS SRDLVWYQQK PGQAPRLLIY GASTRATGIP 60
DRFSGSGSGT DFTLTISRLE	PEDFAVYYCQ KYGSSPPRIT FGPGTKVDIK RTVAAPSVFI 120
FPPSDEQLKS GTASVVCLLN	NFYPPREAKVQ WKVDNALQSG NSQESVTEQD SKDSTYSLSS 180
TLTLSKADYE KHKVYACEVT	HQGLSSPVTK SFNRGEC 217
SEQ ID NO: 31	moltype = AA length = 450
FEATURE	Location/Qualifiers
source	1..450 mol_type = protein

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	organism = synthetic construct				
	note = Description of Artificial Sequence: Synthetic polypeptide				
SEQUENCE: 31					
QVQLQESGPG	LVKPSETLSL	TCTVSGGSIS	RRNDYWAWIR	QSPGKDLEWI	GTISFSGSTF 60
YNPSLKSRVT	ISADTFNNHF	SLRLDAVAAA	DTAVYYCARL	SPFVGAAWWF	DPWGPGLVT 120
VSSASTKGPS	VFPLAPCSRS	TSESTAALGC	LVKDYFPEPV	TVSWNSGALT	SGVHTFPAVL 180
QSSGLYSLSS	VVTVPSSSLG	TKTYTCNVDH	KPSNTKVDKR	VESKYGPPCP	PCPAPEFLGG 240
PSVFLFPPKP	KDTLMISRTP	EVTCTVVVDVS	QEDPEVQFNW	YVDGVEVHNA	KTKPREEQFN 300
STYRVVSVLT	VLHQDWLNGK	EYKCKVSNKG	LPSSIEKTIS	KAKGQPREPQ	VYTLPPSQEE 360
MTKNQVSLTC	LVKGFYPSDI	AVEWESNGQP	ENNYKTTPPV	LDSDGSFFLY	SRLTVDKSRW 420
QEGNVFSCSV	MHEALHNHYT	QKSLSLSLGK			450
SEQ ID NO: 32	moltype = AA length = 217				
FEATURE	Location/Qualifiers				
source	1..217				
	mol_type = protein				
	organism = synthetic construct				
	note = Description of Artificial Sequence: Synthetic polypeptide				
SEQUENCE: 32					
EIVLTQSPGT	LSLSPGERAT	LSCRASQSLS	SNYLGWYQQK	PGQAPRLLIY	GASNRATGIP 60
DRFSGSGSGT	DFTLTISRLE	PEDFGVYYCQ	RYGRSPPAIT	FGGGTKVEIK	RTVAAPSVFI 120
FPPSDEQLKS	GTASVVCLLN	NFYFPREAKVQ	WKVDNALQSG	NSQESVTEQD	SKDSTYSLSS 180
TLTLKADYE	KHKVYACEVT	HQGLSSPVTK	SFNRGEC		217
SEQ ID NO: 33	moltype = AA length = 454				
FEATURE	Location/Qualifiers				
source	1..454				
	mol_type = protein				
	organism = synthetic construct				
	note = Description of Artificial Sequence: Synthetic polypeptide				
SEQUENCE: 33					
QLQLQESGPG	LVKPSETLSL	TCTASGGSIT	NNIDYWVWIR	QPPGRGLEWI	GTIYYSGSTF 60
YNPSLKSRVT	ISVDTSNNQF	SLNLNSMSAA	DTAVYYCARL	RYYYDSNGYL	PYWIDSWGQG 120
TLVTVSSAST	KGPSVFPLAP	CSRSTSESTA	ALGCLVKDYF	PEPVTVSWNS	GALTSGVHTF 180
PAVLQSSGLY	SLSSVTVPS	SSLGKTYTC	NVDHKPSNTK	VDKRVESKYG	PPCPPCPAPE 240
FFLGGPSVFLF	PPKPKDTLMI	SRTPEVTCVV	VDVSQEDPEV	QFNWYVDGVE	VHNAKTKPRE 300
EQFNSTYRVV	SVLTVLHQDW	LNGKEYKCKV	SNKGLPSSIE	KTISKAKGQP	REPQVYTLPP 360
SQEEMTKNQV	SLTCLVKGFY	PSDIAVEWES	NGQPENNYKT	TPPVLDSDGS	FFLYSRLTVD 420
KSRWQEGNVF	SCSVMHEALH	NHYTQKSLSL	SLGK		454
SEQ ID NO: 34	moltype = AA length = 217				
FEATURE	Location/Qualifiers				
source	1..217				
	mol_type = protein				
	organism = synthetic construct				
	note = Description of Artificial Sequence: Synthetic polypeptide				
SEQUENCE: 34					
EIVLTQSPGT	LSLSPGERAT	LSCRASQSIS	SSYLGWYQQK	PGQAPRLLIY	GASSRATGIP 60
DRFSGSGSGT	DFTLTISSLE	PEDFAVYFCQ	LYRRSPPRLT	FGGGTKVEIK	RTVAAPSVFI 120
FPPSDEQLKS	GTASVVCLLN	NFYFPREAKVQ	WKVDNALQSG	NSQESVTEQD	SKDSTYSLSS 180
TLTLKADYE	KHKVYACEVT	HQGLSSPVTK	SFNRGEC		217
SEQ ID NO: 35	moltype = AA length = 452				
FEATURE	Location/Qualifiers				
source	1..452				
	mol_type = protein				
	organism = synthetic construct				
	note = Description of Artificial Sequence: Synthetic polypeptide				
SEQUENCE: 35					
QLQLQESGPG	LVKPSESLSL	TCTVSGGSII	SNDYYWAWIR	QSPGKGLEWI	GSINYRGSTF 60
YSPSLNSRVVT	TSVDTSKNQF	FLKLTSVTAA	DTAMYFCTRL	HGRYRGVGR	AFDYWGQGT 120
VTVSSASTKG	PSVFPLAPCS	RSTSESTAAL	GCLVKDYFPE	PVTVSWNSGA	LTSGVHTFPA 180
VLQSSGLYSL	SSVTVPS	LGTKTYTCNV	DHKPSNTKVD	KRVESKYGPP	CPPCPAPEFL 240
GGPSVFLFPP	KPKDTLMISR	TPEVTCVVVD	VSQEDPEVQF	NWYVDGVEVH	NAKTKPREEQ 300
FNSTYRVVSV	LTVLHQDWLN	GKEYKCKVSN	KGLPSSIEKT	ISKAKGQPRE	PQVYTLPPSQ 360
EEEMTKNQVSL	TCLVKGFYPS	DIAVEWESNG	QPENNYKTP	PVLDSGGSFF	LYSRLTVDKS 420
RWQEGNVFSC	SVMHEALHNH	YTQKSLSLSL	GK		452
SEQ ID NO: 36	moltype = AA length = 214				
FEATURE	Location/Qualifiers				
source	1..214				

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	mol_type = protein				
	organism = synthetic construct				
	note = Description of Artificial Sequence: Synthetic polypeptide				
SEQUENCE: 36					
DIQMTQSPST	LSASVGDIVT	ITCRASQSIG	DWLAWYQQKP	GKAPKLLIYK	ASNLESGVPS 60
RFSGSGSGTE	FTLTISLQ	DDFATYYCQQ	YDSYSVTFGQ	GTKVEIKGTV	AAPSVFIFPP 120
SDEQLKSGTA	SVVCLLNNFY	PREAKVQWKV	DNALQSGNSQ	ESVTEQDSKD	STYLSSTLT 180
LSKADYEKHK	VYACEVTHQG	LSSPVTKSFN	RGEC		214
SEQ ID NO: 37					
moltype = AA length = 453					
Location/Qualifiers					
source 1..453					
mol_type = protein					
organism = synthetic construct					
note = Description of Artificial Sequence: Synthetic polypeptide					
SEQUENCE: 37					
QVQLQEAGPG	LVKPSETLSL	TCTVSGGSIN	TRNYWGWVR	QPPGKGLEWI	ASVYYTGSTF 60
YDPSLRSRVT	ISIDTPRNQF	SLRVSSVDAG	DMGVYCVRL	DGGYNNGYYY	YGMDVWGQGT 120
SVTVSSASTK	GPSVFPLAPC	SRSTSESTAA	LGCLVKDYFP	EPVTVSWNSG	ALTSGVHTFP 180
AVLQSSGLYS	LSSVVTVPSS	SLGTKYTCN	VDHKPSNTKV	DKRVESKYGP	PCPPCPAPEF 240
LGGPSVFLFP	PKPKDTLMIS	RTPEVTCVVV	DVSQEDPEVQ	FNWYVDGVEV	HNAKTKPREE 300
QFNSTYRVVS	VLTVLHQDWL	NGKEYKCKVS	NKGLPSSIEK	TISKAKGQPR	EPQVYTLPPS 360
QEEMTKNQVS	LTCLVKGFYP	SDIAVEWESN	GQPENNYKTT	PPVLDSDGSF	FLYSRLTVDK 420
SRWQEGNVFS	CSVMHEALHN	HYTQKSLSLS	L GK		453
SEQ ID NO: 38					
moltype = AA length = 214					
Location/Qualifiers					
source 1..214					
mol_type = protein					
organism = synthetic construct					
note = Description of Artificial Sequence: Synthetic polypeptide					
SEQUENCE: 38					
GVQMTQSPST	LSASVGERVT	VTCRASRPIS	NWLSWYQQKP	GRAPKLLIYG	TSTLESGVPS 60
RFSGSGSGTE	FTLTITNLQP	DDFATYYCQE	HNLYTITFGP	GTKVEIKRTV	AAPSVFIFPP 120
SDEQLKSGTA	SVVCLLNNFY	PREAKVQWKV	DNALQSGNSQ	ESVTEQDSKD	STYLSSTLT 180
LSKADYEKHK	VYACEVTHQG	LSSPVTKSFN	RGEC		214
SEQ ID NO: 39					
moltype = AA length = 454					
Location/Qualifiers					
source 1..454					
mol_type = protein					
organism = synthetic construct					
note = Description of Artificial Sequence: Synthetic polypeptide					
SEQUENCE: 39					
QLQLQESGPG	LVKPSETLSL	SCAVSGASIR	SNTYYWGWIR	QPPGRGLEWI	GSISHRGDAH 60
YSPSLKSPVT	ISVDTSKNEF	SLKATSVTAA	DTAVYYCVSL	AYSFSWNTYY	FYGMDVWGHG 120
ITVTVSSAST	KGPSVFPLAP	CSRSTSESTA	ALGCLVKDYF	PEPVTVSWNS	GALTSGVHTF 180
PAVLQSSGLY	SLSSVVTVP	SSLGTKYTC	NVDHKPSNTK	VDKRVESKYG	PPCPPCPAPE 240
FLGGPSVFLF	PPKPKDTLMI	SRTPEVTCVV	VDVSQEDPEV	QFNWYVDGVE	VHNAKTKPRE 300
EQFNSTYRVV	SVLTVLHQDW	LNGKEYKCKV	SNKGLPSSIE	KTISKAKGQP	REPQVYTLPP 360
SQEEMTKNQV	SLTCLVKGFY	PSDIAVEWES	NGQPENNYKT	TPPVLDSDGS	FFLYSRLTVD 420
KSRWQEGNVF	SCSVMHEALH	NHYTQKSLSL	SLGK		454
SEQ ID NO: 40					
moltype = AA length = 217					
Location/Qualifiers					
source 1..217					
mol_type = protein					
organism = synthetic construct					
note = Description of Artificial Sequence: Synthetic polypeptide					
SEQUENCE: 40					
DIVLTQSPGT	LSLSPGEGAT	LSCRASQSVN	SGYLAWYQQK	PGQPPRLLVF	AASSRATGIA 60
DRFRGSGSGT	DFTLTITRLE	PEDFAVYYCQ	LYGHSPARIT	FGQGTRLETK	RTVAAPSVFI 120
FPPSDEQLKS	GTASVVCLLN	NFYPREAKVQ	WKVDNALQSG	NSQESVTEQD	SKDSTYLSLS 180
TLTLKADYE	KHKVYACEVT	HQGLSSPVTK	SFNRGEC		217

wherein nicotine-binding antibody or nicotine-binding fragment thereof has a K_D for S-(–)-nicotine of less than about 100 nM.

3. The nicotine-binding antibody or nicotine-binding fragment thereof according to claim **2**, wherein the nicotine-binding antibody or nicotine-binding fragment thereof has a K_D for S-(–)-nicotine between 90 nM and 0.05 nM.

4. The nicotine-binding antibody or nicotine-binding fragment thereof according to claim **3**, wherein the antibody or fragment is an IgG4.

5. The nicotine-binding antibody or nicotine-binding fragment thereof according to claim **4**, wherein the antibody or fragment comprises the substitution S228P.

6. The nicotine-binding antibody or nicotine-binding fragment thereof according to claim **3**, wherein the antibody or fragment is a long-acting variant.

7. The nicotine-binding antibody or nicotine-binding fragment thereof according to claim **6**, wherein the antibody or fragment is PEGylated.

8. The nicotine-binding antibody or nicotine-binding fragment thereof according to claim **1**, wherein the antibody or fragment has a K_D for S-(–)-nicotine of less than about 100 nM.

9. The nicotine-binding antibody or nicotine-binding fragment thereof according to claim **1**, wherein the antibody or fragment has a K_D for S-(–)-nicotine of less than about 60 nM.

10. The nicotine-binding antibody or nicotine-binding fragment thereof according to claim **1**, wherein the antibody or fragment has a K_D for S-(–)-nicotine of less than about 30 nM.

11. The nicotine-binding antibody or nicotine-binding fragment thereof according to claim **1**, wherein the antibody or fragment has a K_D for S-(–)-nicotine of less than about 10 nM.

12. The nicotine-binding antibody or nicotine-binding fragment thereof according to claim **1**, wherein the antibody or fragment has a K_D for S-(–)-nicotine of less than about 5 nM.

13. The nicotine-binding antibody or nicotine-binding fragment thereof according to claim **1**, wherein the antibody or fragment is substantially not cross-reactive with cotinine.

14. The nicotine-binding antibody or nicotine-binding fragment thereof according to claim **3**, wherein the antibody or fragment is substantially not cross-reactive with one or more nicotine-related compounds selected from cotinine, nicotinamide, B-nicotinamide adenine dinucleotide and nor-nicotine.

15. A pharmaceutical composition comprising a nicotine-binding antibody or nicotine-binding fragment thereof according to claim **3** and a pharmaceutically acceptable carrier.

16. The pharmaceutical composition according to claim **15**, wherein the composition is formulated for injection or infusion.

17. A method of treating nicotine addiction or facilitating smoking cessation, comprising administering to a human subject in need thereof a therapeutically effective amount of a nicotine-binding antibody or nicotine-binding fragment thereof according to claim **3**.

18. A method of treating nicotine overdose or nicotine poisoning, comprising administering to a mammalian subject in need thereof a therapeutically effective amount of a nicotine-binding antibody or nicotine-binding fragment thereof according to claim **3**.

19. The method of claim **18**, wherein the subject is a human child.

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