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(54) **Title:** HUMAN MONOCLONAL AUTOANTIBODIES TO ADAMTS13 AND USES THEREOF

(57) **Abstract:** The present invention relates to compositions and methods of use of anti-ADAMTS13 autoantibodies and fragments thereof. In one aspect, the invention includes a composition comprising an isolated anti-ADAMTS13 autoantibody or fragment thereof. In other aspects, methods are described for generating an in vivo model of thrombotic thrombocytopenic purpura (TTP) comprising introducing at least one anti-ADAMTS13 autoantibody or fragment thereof into a model organism and identifying an anti-autoimmune reagent for treating TTP.



TITLE OF THE INVENTION

Human Monoclonal Autoantibodies to ADAMTS13 and Uses Thereof

CROSS-REFERENCE TO RELATED APPLICATION

5 The present application is entitled to priority under 35 U.S.C. § 119(e) to U.S. Provisional Patent Application No. 62/144,186, filed April 7, 2015, which is hereby incorporated by reference in its entirety herein.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR
DEVELOPMENT

This invention was made with government support under Grant No. HL81012, HL007775, HL110860 and HL115187 awarded by the National Institutes of Health. The government has certain rights in the invention.

BACKGROUND OF THE INVENTION

15 Current therapeutic approaches to the treatment of antibody-mediated autoimmune disease are generally limited to the use of systemic immunosuppression with its attendant side effects rather than therapy that solely targets just the pathogenic autoantibodies.

The acquired form of thrombotic thrombocytopenic purpura (TTP) is an example of an autoimmune disorder that the majority of patients have reduced activity levels of the VWF-cleaving protease ADAMTS13 due to the development of autoantibodies that inhibit its function. Decreased ADAMTS13 activity results in the accumulation of ultralarge VWF (UL-VWF) multimers that foster systemic platelet aggregation in the microcirculation when coincident with additional factors, such as endothelial injury, and can lead to severe thrombocytopenia, microangiopathic hemolytic anemia, varying degrees of organ dysfunction, and death.

First-line therapy for TTP is non-specific comprising daily therapeutic plasma exchange (TPE), which reduces mortality from ~90% to ~20%, presumably by repeated depletion of a fraction of circulating autoantibodies as well as replenishment of ADAMTS13 levels until the disease resolves on its own. Depending on a patient's response to TPE, systemic immunosuppressive agents including corticosteroids and the B-cell depletion agent rituximab, and, less commonly, cyclophosphamide, vincristine, or cyclosporine, may be used in conjunction with TPE. Notwithstanding improvements in the recognition, early initiation

of therapy, and the use of combination therapies, the mortality for patients diagnosed with TTP has remained relatively constant since the initial introduction of TPE over 25 years ago.

Recently, a number of alternative therapeutic approaches for TTP have been proposed including the infusion of excess quantities of recombinant ADAMTS13 to override
5 autoantibody inhibition, and the development of agents that would target UL-VWF multimers either by reducing their size or by blocking their interactions with platelets. However, it is not clear that these approaches would necessarily obviate the need for TPE and/or agents that induce generalized immunosuppression since these modalities affect pathogenesis downstream of the effects of the autoantibody-mediated ADAMTS13 inhibition, rather than
10 by affecting the pathogenic ADAMTS13 inhibitors themselves. Moreover, there is the potential downside of inhibiting normal hemostatic processes that are mediated through some of the same pathways.

There is a need in the art for the development of more effective and targeted therapies to treat thrombotic thrombocytopenic purpura (TTP). The present invention
15 addresses this need.

SUMMARY OF THE INVENTION

As disclosed herein, the present invention includes compositions and methods of use of anti-ADAMTS13 autoantibodies and fragments thereof.

In one aspect, the invention includes an isolated anti-ADAMTS13
20 autoantibody or fragment thereof.

In one aspect, the invention includes isolated nucleic acid sequence encoding an anti-ADAMTS13 autoantibody or fragment thereof.

In another aspect, the invention includes a method for generating an *in vivo* model of thrombotic thrombocytopenic purpura (TTP) comprising introducing at least one
25 anti-ADAMTS13 autoantibody or fragment thereof into a model organism.

In another aspect, the invention includes an anti-autoimmune reagent, wherein the anti-autoimmune reagent specifically binds to an anti-ADAMTS13 autoantibody or fragment thereof.

In yet another aspect, the invention includes a method for identifying an anti-
30 autoimmune reagent for treating thrombotic thrombocytopenic purpura (TTP). The method of the invention comprises contacting a panel of agents with at least one anti-ADAMTS13 autoantibody or fragment thereof and identifying the agents that bind to the anti-ADAMTS13 autoantibody or fragment thereof.

In still another aspect, the invention includes a method of inhibiting the binding of an anti-ADAMTS13 autoantibody or fragment thereof to ADAMTS13. The method comprises contacting the anti-ADAMTS13 autoantibody or fragment thereof with a composition comprising an anti-autoimmune reagent that specifically binds to the anti-ADAMTS13 autoantibody or fragment thereof.

In another aspect, the invention includes a method of identifying an ADAMTS13 variant that does not bind an anti-ADAMTS13 autoantibody or fragment thereof. The method comprises contacting an ADAMTS13 protein with an anti-ADAMTS13 autoantibody or fragment thereof, wherein when the ADAMTS13 protein does not bind the anti-ADAMTS13 autoantibody or fragment thereof, then the ADAMTS13 protein is a variant that does not bind an anti-ADAMTS13 autoantibody or fragment thereof.

In yet another aspect, the invention includes a method for treating thrombotic thrombocytopenic purpura (TTP) in a subject in need thereof, the method comprising administering to the subject a composition comprising an effective amount of an ADAMTS13 variant, wherein the ADAMTS13 variant is resistant to inhibition by an anti-ADAMTS13 autoantibody or fragment thereof.

In various embodiments of the above aspects or any other aspect of the invention delineated herein, the anti-ADAMTS13 autoantibody or fragment thereof comprises a heavy chain selected from the group consisting of SEQ ID NOs: 46-90. In another embodiment, the anti-ADAMTS13 autoantibody or fragment thereof comprises a light chain selected from the group consisting of SEQ ID NOs: 142-192.

In another embodiment, the anti-ADAMTS13 autoantibody or fragment thereof comprises a single chain variable fragment (scFv). In another embodiment, the anti-ADAMTS13 autoantibody or fragment thereof is capable of decreasing ADAMTS13 activity. In yet another embodiment, the ADAMTS13 activity is selected from the group consisting of proteolytic activity, disulfide reducing activity, interacting or attaching to an endothelial cell surface, and any combination thereof.

In a further embodiment, the anti-ADAMTS13 autoantibody or fragment thereof binds at least one of the ADAMTS13 region selected from the group consisting of amino-terminal (MDT1) domain, carboxy-terminal (T5-8/CUB) domain and cysteine-rich/spacer region.

In one embodiment, the isolated nucleic acid sequence encoding an anti-ADAMTS13 autoantibody or fragment thereof comprises a heavy chain nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1-45. In another embodiment,

the isolated nucleic acid sequence encoding the anti-ADAMTS13 autoantibody or fragment thereof comprises a light chain nucleic acid sequence selected from the group consisting of SEQ ID NOs: 91-141.

5 In another embodiment, the isolated nucleic acid sequence encodes a single chain variable fragment (scFv).

In yet another embodiment, the isolated nucleic acid sequence encoding an anti-ADAMTS13 autoantibody or fragment thereof has an identity of at least 80% to at least one heavy chain nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1-45. In yet another embodiment, the isolated nucleic acid sequence encoding an anti-
10 ADAMTS13 autoantibody or fragment thereof has an identity of at least 80% to at least one light chain nucleic acid sequence selected from the group consisting of SEQ ID NOs: 91-141.

In one embodiment, the *in vivo* model organism of thrombotic thrombocytopenic purpura (TTP) of the invention is selected from the group consisting of a non-mammalian organism and a non-human mammalian organism. In another embodiment,
15 the mammalian organism is selected from the group consisting of a non-human primate, an ovine, a bovine, a porcine, a canine, a feline and a murine organism.

In another embodiment, the method of the invention for generating an *in vivo* model of TTP comprises introducing the anti-ADAMTS13 autoantibody or fragment thereof by formulating the anti-ADAMTS13 autoantibodies or fragments thereof in a composition for
20 administration to the model organism. In yet another embodiment, the introduction of the anti-ADAMTS13 autoantibody or fragment thereof further comprises injecting the anti-ADAMTS13 autoantibodies or fragments thereof into the model organism. In yet another embodiment, the introduction of the anti-ADAMTS13 autoantibody or fragment thereof comprises inducing *in vivo* expression in the model organism. In a further embodiment, the *in*
25 *vivo* expression comprises delivering nucleic acids to the model organism. In yet a further embodiment, the delivery of the nucleic acids is through a method selected from the group consisting of injection through hydrodynamic delivery, electroporation, transfection, transduction and other methods of viral delivery, and any combination thereof.

In one embodiment, the anti-autoimmune reagent of the invention blocks
30 binding of the anti-ADAMTS13 autoantibody or fragment thereof to ADAMTS13. In another embodiment, the anti-autoimmune reagent specifically binds to at least one amino acid sequence selected from the group consisting of SEQ ID NOs: 46-90; 142-192.

In one embodiment, the identification of agents that bind to the anti-ADAMTS13 autoantibody or fragment thereof of the invention comprises identifying agents

that block binding of the anti-ADAMTS13 autoantibody or fragment thereof to ADAMTS13. In another embodiment, the binding of the anti-ADAMTS13 autoantibody or fragment thereof to ADAMTS13 is blocked to at least one of the ADAMTS13 regions selected from the group consisting of amino-terminal (MDT1) domain, carboxy-terminal (T5-8/CUB) domain and cysteine-rich/spacer region.

In one embodiment, the ADAMTS13 variant that does not bind an anti-ADAMTS13 autoantibody or fragment thereof comprises a preserved or enhanced proteolytic activity as compared to a native ADAMTS13. In another embodiment, the ADAMTS13 variant is useful for treating thrombotic thrombocytopenic purpura (TTP). In yet another embodiment, the ADAMTS13 variant is resistant to inhibition by an anti-ADAMTS13 autoantibody or fragment thereof comprising at least one amino acid sequence selected from the group consisting of SEQ ID NOs: 46-90; 142-192.

BRIEF DESCRIPTION OF THE DRAWINGS

The following detailed description of preferred embodiments of the invention will be better understood when read in conjunction with the appended drawings. For the purpose of illustrating the invention, there are shown in the drawings embodiments that are presently preferred. It should be understood, however, that the invention is not limited to the precise arrangements and instrumentalities of the embodiments shown in the drawings.

FIGS. 1A-1B are series of histograms demonstrating the inhibitory activity of human anti-ADAMTS13 monoclonal antibodies. ADAMTS13 activities of normal human plasma were measured using FRET-S-VWF73 in the presence of (FIG. 1A) recombinant anti-ADAMTS13 scFv clones or PBS control (defined as 100% activity) or (FIG. 1B) >5-fold more of an irrelevant keratinocyte-binding human V_H1-69-encoded scFv PX4-3. Values for each scFv are the averages of 3 independent measurements (+/- SD). For reference, germline V_H genes from Table 2 are indicated above each bar.

FIG. 2 is a graph illustrating epitope specificities of anti-ADAMTS13 scFv. Using overlapping fragments of ADAMTS13 and immunoprecipitation with selected scFv, binding regions for antibodies were derived and indicated in a cartoon map of ADAMTS13. For reference, heavy chain germline V_H genes from Table 2 and ADAMTS13 inhibitory activities (percent residual activity from Fig. 1) are indicated in parentheses next to the name of each clone. Raw data for this experiment are in Table 5 and FIGS. 10A-10B. Domain abbreviations: M, metalloprotease; D, disintegrin; 1 through 8, thrombospondin type 1 motifs 1 through 8; C, cysteine-rich domain; S, spacer domain; CUB, pair of CUB domains (complement C1r/C1s, Uegf, bone morphogenic protein 1).

FIGS. 3A-3C are series of histograms demonstrating that ADAMTS13 inhibitory antibodies are blocked by rabbit anti-idiotypic IgG. FIG. 3A: scFv 1-416 inhibited ADAMTS13 in the absence of rabbit IgG and in the presence of pre-immune rabbit IgG, but not in the presence of post-immune anti-scFv 1-416 rabbit IgG (right-hand set of bars; *, P=0.006; **, P=0.008; N.S., not significant). No scFv or irrelevant human anti-Rh(D) scFv E1M2 had any effect on ADAMTS13 activity under any rabbit IgG conditions (left-hand and middle sets of bars). Residual ADAMTS13 activity with scFv 1-416 and no rabbit IgG or pre-immune IgG (white and gray bars in right-hand set of bars) is higher than in FIG. 1A because the amount of scFv was 2.5-fold lower here to increase the sensitivity of rabbit post-immune IgG blocking. FIG. 3B: In the presence of scFv 1-428, ADAMTS13 activity was inhibited when mixed with no rabbit or pre-immune rabbit IgG, but was “rescued” in presence of post-immune rabbit IgG (4th set of bars) as for scFv 1-416 in FIG. 3A (*, P=0.002; **, P=0.0006). Results were unchanged if performed in the presence of an 8-fold excess of human V_H3-33-encoded anti-Rh(D) scFv E1M2 (5th set of bars) or human anti-keratinocyte V_H1-69-encoded PX4-3 (6th set of bars). First 3 sets of bars are controls showing show that scFv’s E1M2 and PX4-3 had no effect on ADAMTS13 activity themselves. In this experiment, the amount of rabbit IgG was titrated down to the point of just being able to block 1-428 in order to increase its sensitivity to any effects of E1M2 or PX4-3. FIG. 3C: Gray bars show reduction of ADAMTS13 activity in normal plasma when mixed with heat-treated (56°C for 30 min to destroy any residual patient plasma ADAMTS13) TTP4-TTP7 plasmas and pre-immune rabbit IgG. Black bars show various degrees of “rescue” of ADAMTS13 activity if TTP4-TTP7 plasmas were pre-incubated with post-immune rabbit anti-idiotypic IgG raised against one of 4 inhibitory scFv’s as indicated. All differences significant (P range, 0.005 – 0.041) except for those marked “N.S.” (P range, 0.059 – 0.098). ADAMTS13 activities are averages of 2 measurements except for TTP7/1-431 which were measured once (*).

FIGS. 4A-4B are series of graphs illustrating the inhibition of murine ADAMTS13 by human anti-ADAMTS13. FIG. 4A: Dose response curve showing residual murine ADAMTS13 activity when normal mouse plasma was mixed *in vitro* with increasing amounts of human ADAMTS13 inhibitory scFv’s 1-416, 1-420, 1-428, 1-431 or irrelevant anti-Rh(D) scFv negative control E1M2. FIG. 4B: Time course of murine ADAMTS13 inhibition *in vivo* following intraperitoneal injection of scFv 1-420 (inset: over 24 hour period).

FIGS. 5A-5C are series of graphs and histograms illustrating the inhibition of murine ADAMTS13 by *in vivo*-expressed human anti-ADAMTS13 scFv. FIG. 5A: Time

course of murine ADAMTS13 inhibition *in vivo* following hydrodynamic tail vein delivery into 2 pairs of mice with pLIVE plasmid containing scFv 1-420 cDNA (triangles and circles) or negative control human platelet factor 4 scFv X24-3 cDNA (diamonds and squares). FIG. 5B: Western blot of plasma immunoprecipitated *in vivo*-expressed scFv drawn 7 days post scFv cDNA injection. Lanes 1-3 show negative control scFv X24-3 for 3 mice; lanes 4-6 show scFv 1-420 for 3 mice; lane 7 represents the amount of scFv 1-420 immunoprecipitated from an equivalent volume of mouse plasma spiked with 20 ng purified scFv 1-420 protein. FIG. 5C: ADAMTS13 “inhibitor assay” performed on a 1:1 mix of normal human plasma and heat-inactivated murine plasma (56°C for 30 min to destroy endogenous mouse ADAMTS13) derived from 2 untransfected mice (left-hand set of bars), 2 mice transfected with control scFv plasmid (middle set of bars), and 5 mice transfected with plasmid containing scFv 1-420 cDNA (right-hand set of bars).

FIGS. 6A-6B are series of graphs exemplifying the platelet thrombus formation after cremaster arteriole injury in mice transfected with human anti-ADAMTS13 cDNA. FIG. 6A: Intravital microscopy and time-lapse video illustrating formation of platelet thrombus over a 180-second period post laser injury. Platelets were labeled with Alexa⁴⁸⁸-conjugated F(ab')₂ fragments of anti-mouse CD41 in control scFv-transfected animal (normal ADAMTS13 activity, left panel) and scFv 1-420-transfected animal (<10% normal ADAMTS13 activity, right panel). Blood flow in direction of arrows. Bar represents 30 μm in all video frames. FIG. 6B: Length/width aspect ratios for platelet thrombi were averaged for 28 injuries in 5 control mice and 33 injuries in 7 scFv 1-420-transfected mice and plotted ±SEM as a function of time. Arterioles of 20–40 μm diameter were selected. To the right of each tracing, VWF multimer analyses of plasma from representative pair of control and ADAMTS13-inhibited mice shows accumulation of UL-VWF in scFv 1-420-transfected mouse.

FIGS. 7A-7C are series of graphs illustrating thrombocytopenia, thrombus formation, and death after injection of Shigatoxin-2 in mice transfected with human anti-ADAMTS13 cDNA. FIG. 7A: Platelet counts in mice expressing control scFv (upper panel) and 1-420 scFv (lower panel) antibodies following Shigatoxin-2 (Stx-2) injection on Day 0. ADAMTS13 activities prior to Stx-2 injections are listed on the right-hand side of each panel. FIG. 7B: Kaplan-Meier survival curves for mice expressing control scFv and scFv 1-420. P value determined using GraphPad Prism software (La Jolla, CA) and the log-rank Mantel-Cox test. FIG. 7C: Peripheral blood smears (panels 1, 2) and organ histology (panels 3 through 8) in representative control scFv (left-hand panel set) and scFv 1-420 (right-hand

panel set) expressing mice. Arrows in panel 2 point to schistocytes. Original magnifications, $\times 100$; insets, $\times 500$.

FIG. 8 is a table comparing CDR2 amino acid residues for VH1-69 encoded antibody heavy chains. Antibodies are grouped by identical CDR2 amino acid residues in positions 56-62. Replacement mutations are indicated with letters, identities with “.”, and gaps with “--”. CDR2 numbering designations per Brochet et al., (Nucl. Acids Res., 2008, 36:W503-W508)..

FIGS. 9A-9B are series of tables showing the alignment of anti-ADAMTS13 (FIG. 9A, five panels from the left toward the right) heavy and (FIG. 9B, five panels from the left toward the right) light chains to their most likely germline immunoglobulin genes. Antibodies are grouped by B cell clonotype based on CDR3 region as described in text. Clonotypes are grouped by V_H or V_L genes then by J_H or J_L genes (italicized letters and shaded lines). Replacement mutations are indicated with letters, silent mutations with “*”, identities with “.”, and gaps with “--”. Numbering and framework/CDR regions designations per Brochet et al., Nucl. Acids Res., 2008, 36:W503-W508. Framework and CDR1/CDR2 replacement and silent mutations and their respective replacement-to-silent mutation ratios (“R:S”) are indicated to the right of each sequence. In general, mutations in CDR3 regions are difficult to assess due to junctional additions and deletions of nucleotides so mutations were not scored in these regions.

FIGS. 10A-10B are series of graphs demonstrating the identification of anti-ADAMTS13 scFv binding domains. FIG. 10A: Cartoon depicting the ADAMTS13 constructs used for epitope determination (based on Zheng et al., Haemat., 2010, 95:1555-1562). Domain abbreviations as defined in the legend to FIG. 2. FIG. 10B: Representative examples of Western blots of full-length and truncated ADAMTS13 constructs immunoprecipitated by the scFv indicated at the bottom left or bottom right of each panel. Included are examples of scFv specific for the six types of binding specificities shown in FIG. 2: CS domain (1-416, 1-304, 1-420, 1-428), MDT domain (1-437), CUB domain (1-441), T5-8 + CUB domain (z1-303), CS/CUB domains (1-404), CS/ T5-8 + CUB domains (1-410). ScFv E1M2 is anti-red blood cell Rh(D) antigen negative control.

FIG. 11 is a schematic diagram of pLIVE plasmid vector modified for *in vivo* scFv expression. Shown are positions of Ig-kappa leader sequence, Sfi I restriction sites for inserting pComb3X-derived scFv construct, and V5-tag sequence.

DETAILED DESCRIPTION

Definitions

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to whom the invention pertains. Although any methods and materials similar or equivalent to those described herein can be used in the practice for testing of the present invention, the preferred materials and methods are described herein. In describing and claiming the present invention, the following terminology will be used.

It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting.

The articles “a” and “an” are used herein to refer to one or to more than one (*i.e.*, to at least one) of the grammatical object of the article. By way of example, “an element” means one element or more than one element.

“About” as used herein when referring to a measurable value such as an amount, a temporal duration, and the like, is meant to encompass variations of $\pm 20\%$ or $\pm 10\%$, more preferably $\pm 5\%$, even more preferably $\pm 1\%$, and still more preferably $\pm 0.1\%$ from the specified value, as such variations are appropriate to perform the disclosed methods.

The term “ADAMTS13” refers to a disintegrin and/or metalloproteinase with a thrombospondin type 1 motif, member 13, also known as von Willebrand Factor-cleaving protease (VWFCP). ADAMTS13 is a zinc-containing metalloprotease enzyme that cleaves von Willebrand factor (vWF). It is generally secreted into the blood and degrades vWF into smaller subunits to decrease its activity. ADAMTS13 shares many properties with the other family members in the ADAMTS family, all of which are characterized by a protease domain, an adjacent disintegrin domain, and one or more thrombospondin domains.

ADAMTS13 has 8 thrombospondin domains and lacks a hydrophobic transmembrane domain, thus it is not anchored in the cell membrane.

As used herein, to “alleviate” a disease, disorder or condition means reducing the severity of one or more symptoms of the disease, disorder or condition.

The term “antibody,” as used herein, refers to an immunoglobulin molecule that specifically binds with an antigen. Antibodies can be intact immunoglobulins derived from natural sources or from recombinant sources and can be immunoreactive portions of intact immunoglobulins. Antibodies are typically tetramers of immunoglobulin molecules. Tetramers may be naturally occurring or reconstructed from single chain antibodies or

antibody fragments. Antibodies also include dimers that may be naturally occurring or constructed from single chain antibodies or antibody fragments. The antibodies in the present invention may exist in a variety of forms including, for example, polyclonal antibodies, monoclonal antibodies, Fv, Fab and F(ab')₂, as well as single chain antibodies (scFv),
5 humanized antibodies, and human antibodies (Harlow et al., 1999, In: Using Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, NY; Harlow et al., 1989, In: Antibodies: A Laboratory Manual, Cold Spring Harbor, New York; Houston et al., 1988, Proc. Natl. Acad. Sci. USA 85:5879-5883; Bird et al., 1988, Science 242:423-426).

The term “antibody fragment” refers to a portion of an intact antibody and
10 refers to the antigenic determining variable regions of an intact antibody. Examples of antibody fragments include, but are not limited to, Fab, Fab', F(ab')₂, and Fv fragments, linear antibodies, scFv antibodies, single-domain antibodies, such as camelid antibodies (Riechmann, 1999, Journal of Immunological Methods 231:25-38), composed of either a VL or a VH domain that exhibit sufficient affinity for the target, and multispecific antibodies
15 formed from antibody fragments. The antibody fragment also includes a human antibody or a humanized antibody or a portion of a human antibody or a humanized antibody.

An “antibody heavy chain,” as used herein, refers to the larger of the two types of polypeptide chains present in all antibody molecules in their naturally occurring conformations.

20 An “antibody light chain,” as used herein, refers to the smaller of the two types of polypeptide chains present in all antibody molecules in their naturally occurring conformations. κ and λ light chains refer to the two major antibody light chain isotypes.

By the term “synthetic antibody” as used herein, is meant an antibody that is generated using recombinant DNA technology, such as, for example, an antibody expressed
25 by a bacteriophage as described herein. The term should also be construed to mean an antibody that has been generated by the synthesis of a DNA molecule encoding the antibody and the DNA molecule expresses an antibody protein, or an amino acid sequence specifying the antibody, wherein the DNA or amino acid sequence has been obtained using synthetic DNA or amino acid sequence technology that is available and well known in the art.

30 As used herein, an “autoantibody” or an “autoimmune antibody” is an antibody produced by the immune system that is directed against one or more of the host's own proteins. Autoantibodies may be produced by a host's immune system when it fails to distinguish between “self” and “non-self” proteins. Usually the immune system is able to

discriminate by recognizing foreign substances (“non-self”) and ignoring the host's own cells (“self”). When the immune system ceases to recognize one or more of the host's normal constituents as “self,” it may produce to autoantibodies that attack its own cells, tissues, and/or organs.

5 As used herein, an “anti-autoimmune reagent” refers to an agent that is capable of binding to an autoimmune antibody and/or inhibiting function of an autoimmune antibody, such as disrupting the interaction of the autoantibody with its antigen. Examples of anti-autoimmune reagents include but are not limited to an antibody against an anti-ADAMTS13 antibody, idiotypic antibody, peptide, polypeptide, small molecule or other
10 agent that binds to the anti-ADAMTS13 antibody and/or prevents binding between the anti-ADAMTS13 antibody and ADAMTS13. The anti-autoimmune reagent can be any agent that binds to an anti-ADAMTS13 antibody or inhibits its function. In some instances, the anti-autoimmune reagent is an agent that disrupts binding between the anti-ADAMTS13 antibody and ADAMTS13. In another aspect, the anti-autoimmune reagent is an idiotypic antibody,
15 peptide, polypeptide, small molecule or other agent that can bind to an anti-ADAMTS13 antibody.

By the term “Fab/phage” as used herein, is meant a phage particle that expresses the Fab portion of an antibody.

20 By the term “scFv/phage” as used herein, is meant a phage particle that expresses the Fv portion of an antibody as a single chain.

“Phage,” or “phage particle,” as these terms are used herein, include bacteriophage that contain phage nucleic acid encoding, inter alia, an antibody. This is because, as would be appreciated by the skilled artisan, unlike peptide phage display (where the peptide DNA insert is small and it is actually cloned into the phage DNA), the larger scFv
25 or Fab DNA inserts are actually cloned into, among other things, a plasmid. Thus, the nucleic acid encoding the antibody, e.g., a plasmid such as, but not limited to, pComb3X, not only comprises a plasmid origin of replication, but also a phage (e.g., M13) origin of replication sequence and an M13 packaging sequence, so that when the nucleic acid is produced, a helper phage can be used to provide the required phage (e.g., M13) proteins in trans to make
30 “phage-like” particles. That is, these particles resemble phage on the outside, but on the inside they contain plasmid (also referred to as a “phagemid”) DNA. In other words, the phagemid DNA need not encode any M13 phage proteins, except a piece of M13 gene III fused to the DNA for antibody or peptide. Thus, it should be understood that the terms

“phage,” “phage particle,” “phage-like particle” and “phagemid” are used interchangeably herein.

The term “antigen” or “Ag” as used herein is defined as a molecule that provokes an immune response. This immune response may involve either antibody
5 production, or the activation of specific immunologically-competent cells, or both. The skilled artisan will understand that any macromolecule, including virtually all proteins or peptides, can serve as an antigen. Furthermore, antigens can be derived from recombinant or genomic DNA. A skilled artisan will understand that any DNA, which comprises a nucleotide sequences or a partial nucleotide sequence encoding a protein that elicits an
10 immune response therefore encodes an “antigen” as that term is used herein. Furthermore, one skilled in the art will understand that an antigen need not be encoded solely by a full length nucleotide sequence of a gene. It is readily apparent that the present invention includes, but is not limited to, the use of partial nucleotide sequences of more than one gene and that these nucleotide sequences are arranged in various combinations to elicit the desired
15 immune response. Moreover, a skilled artisan will understand that an antigen need not be encoded by a “gene” at all. It is readily apparent that an antigen can be generated synthesized or can be derived from a biological sample. Such a biological sample can include, but is not limited to a tissue sample, a tumor sample, a cell or a biological fluid.

“Non-self” antigens are those antigens on substances entering or present in the
20 body that are detectably different or foreign from the animal's own constituents, whereas “self” antigens are those that, in the healthy animal, are not detectably different or foreign from its own constituents. However, under certain conditions, including in certain disease states, an individual's immune system will identify its own constituents as “non-self,” and initiate an immune response against “self” material, at times causing more damage or
25 discomfort as from an invading microbe or foreign material, and often producing serious illness in an individual.

As used herein, the term “autologous” is meant to refer to any material derived from the same individual to whom it is later to be re-introduced into the individual.

“Derivative” in the context of proteins and peptides includes any purposefully
30 generated amino acid sequence that in its entirety, or in part, comprises a substantially similar amino acid sequence to a desired protein. The term derivative can also be applied to the antibodies described herein such that “derivative” includes any purposefully generated peptide, which in its entirety, or in part, comprises a substantially similar amino acid sequence to an anti-ADAMTS13 antibody or an anti-idiotypic antibody that is capable of

specifically binding to an anti-ADAMTS13 antibody. Derivatives of the antibodies may be characterized by single or multiple amino acid substitutions, deletions, additions, or replacements. Derivatives may include: (a) derivatives that one or more amino acid residues are substituted with conservative or non-conservative amino acids; (b) derivatives that one or more amino acids are added; (c) derivatives that one or more of the amino acids of the amino acid sequence includes a substituent group; (d) derivatives that amino acid sequences or a portion thereof is fused to another peptide (e.g., serum albumin or protein transduction domain); (e) derivatives that one or more nonstandard amino acid residues (e.g., those other than the 20 standard L-amino acids found in naturally occurring proteins) are incorporated or substituted into the amino acid sequences; (f) derivatives that one or more non-amino acid linking groups are incorporated into or replace a portion of the amino acids; and (g) derivatives that one or more amino acid is modified by glycosylation, acetylation, myristoylation, and the like.

The terms “disease,” “disorder,” and “condition” refer to thrombotic thrombocytopenic purpura (TTP) is an example of an autoimmune disorder that the majority of patients have reduced activity levels of the VWF-cleaving protease ADAMTS13 due to the development of autoantibodies that inhibit its function. Decreased ADAMTS13 activity results in the accumulation of ultralarge VWF (UL-VWF) multimers that foster systemic platelet aggregation in the microcirculation when coincident with additional factors, such as endothelial injury, and can lead to severe thrombocytopenia, microangiopathic hemolytic anemia, varying degrees of organ dysfunction, and death.

“Encoding” refers to the inherent property of specific sequences of nucleotides in a polynucleotide, such as a gene, a cDNA, or an mRNA, to serve as templates for synthesis of other polymers and macromolecules in biological processes having either a defined sequence of nucleotides (*i.e.*, rRNA, tRNA and mRNA) or a defined sequence of amino acids and the biological properties resulting therefrom. Thus, a gene encodes a protein if transcription and translation of mRNA corresponding to that gene produces the protein in a cell or other biological system. Both the coding strand, the nucleotide sequence that is identical to the mRNA sequence and is usually provided in sequence listings, and the non-coding strand, used as the template for transcription of a gene or cDNA, can be referred to as encoding the protein or other product of that gene or cDNA.

Unless otherwise specified, a “nucleotide sequence encoding an amino acid sequence” includes all nucleotide sequences that are degenerate versions of each other and

that encode the same amino acid sequence. Nucleotide sequences that encode proteins and RNA may include introns.

“Effective amount” or “therapeutically effective amount” are used interchangeably herein, and refer to an amount of a compound, formulation, material, or composition, as described herein effective to achieve a particular biological result. Such results may include, but are not limited to, the inhibition of virus infection as determined by any means suitable in the art.

As used herein “endogenous” refers to any material from or produced inside an organism, cell, tissue or system.

10 As used herein, the term “exogenous” refers to any material introduced from or produced outside an organism, cell, tissue or system.

The term “expression” as used herein is defined as the transcription and/or translation of a particular nucleotide sequence driven by its promoter.

“Expression vector” refers to a vector comprising a recombinant polynucleotide comprising expression control sequences operatively linked to a nucleotide sequence to be expressed. An expression vector comprises sufficient cis-acting elements for expression; other elements for expression can be supplied by the host cell or in an in vitro expression system. Expression vectors include all those known in the art, such as cosmids, plasmids (*e.g.*, naked or contained in liposomes) and viruses (*e.g.*, lentiviruses, retroviruses, adenoviruses, and adeno-associated viruses) that incorporate the recombinant polynucleotide.

20 As used herein, the term “fragment,” as applied to a nucleic acid, refers to a subsequence of a larger nucleic acid. A “fragment” of a nucleic acid can be at least about 15 nucleotides in length; for example, at least about 50 nucleotides to about 100 nucleotides; at least about 100 to about 500 nucleotides, at least about 500 to about 1000 nucleotides; at least about 1000 nucleotides to about 1500 nucleotides; about 1500 nucleotides to about 2500 nucleotides; or about 2500 nucleotides (and any integer value in between).

25 As used herein, the term “fragment,” as applied to a protein or peptide, refers to a subsequence of a larger protein or peptide. A “fragment” of a protein or peptide can be at least about 20 amino acids in length; for example, at least about 50 amino acids in length; at least about 100 amino acids in length; at least about 200 amino acids in length; at least about 300 amino acids in length; or at least about 400 amino acids in length (and any integer value in between).

As used herein, the terms “gene” and “recombinant gene” refer to nucleic acid molecules comprising an open reading frame encoding a polypeptide. Such natural allelic

variations can typically result in 1-5% variance in the nucleotide sequence of a given gene. Alternative alleles can be identified by sequencing the gene of interest in a number of different individuals. This can be readily carried out by using hybridization probes to identify the same genetic locus in a variety of individuals. Any and all such nucleotide variations and resulting amino acid polymorphisms or variations that are the result of natural allelic variation and that do not alter the functional activity are intended to be within the scope of the invention.

Moreover, nucleic acid molecules encoding proteins from other species (homologs), which have a nucleotide sequence that differs from that of the human proteins described herein are within the scope of the invention. Nucleic acid molecules corresponding to natural allelic variants and homologs of a cDNA of the invention can be isolated based on their identity to human nucleic acid molecules using the human cDNAs, or a portion thereof, as a hybridization probe according to standard hybridization techniques under stringent hybridization conditions.

“Homologous” as used herein, refers to the subunit sequence identity between two polymeric molecules, *e.g.*, between two nucleic acid molecules, such as, two DNA molecules or two RNA molecules, or between two polypeptide molecules. When a subunit position in both of the two molecules is occupied by the same monomeric subunit; *e.g.*, if a position in each of two DNA molecules is occupied by adenine, then they are homologous at that position. The homology between two sequences is a direct function of the number of matching or homologous positions; *e.g.*, if half (*e.g.*, five positions in a polymer ten subunits in length) of the positions in two sequences are homologous, the two sequences are 50% homologous; if 90% of the positions (*e.g.*, 9 of 10), are matched or homologous, the two sequences are 90% homologous.

As applied to a protein sequence, “homology” as used herein refers to a protein sequence that has about 50% sequence similarity. More preferably, the sequence has about 75% sequence similarity, even more preferably, has at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence similarity.

“Humanized” forms of non-human (*e.g.*, murine) antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', F(ab')₂ or other antigen-binding subsequences of antibodies) that contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in that residues from a complementary-determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species

(donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity, and capacity. In some instances, Fv framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies can comprise residues that are found neither in the recipient antibody nor in the imported CDR or framework sequences. These modifications are made to further refine and optimize antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, that all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see Jones et al., Nature, 321: 522-525, 1986; Reichmann et al., Nature, 332: 323-329, 1988; Presta, Curr. Op. Struct. Biol., 2: 593-596, 1992.

“Fully human” refers to an immunoglobulin, such as an antibody, where the whole molecule is of human origin or consists of an amino acid sequence identical to a human form of the antibody.

The term “hydrodynamic delivery” refers to the delivery of nucleic acids while controlling the hydrodynamic pressure in capillaries to enhance endothelial and parenchymal cell permeability to the nucleic acids. Hydrodynamic delivery uses a hydrodynamic force generated by a pressurized injection of a large volume of a nucleic acid solution into the blood vessel so as to permeabilize the capillary endothelium and generate pores in the plasma membrane of the surrounding parenchyma cells so that the nucleic acids or other macromolecules of interest may reach the cell interior. See also Zhang, et al., Gene Ther., 2000, 7:1344-1349 and Miao, et al., Mol. Ther., 2001, 3:947-957.

“Identity” as used herein refers to the subunit sequence identity between two polymeric molecules particularly between two amino acid molecules, such as, between two polypeptide molecules. When two amino acid sequences have the same residues at the same positions; *e.g.*, if a position in each of two polypeptide molecules is occupied by an Arginine, then they are identical at that position. The identity or extent that two amino acid sequences have the same residues at the same positions in an alignment is often expressed as a percentage. The identity between two amino acid sequences is a direct function of the number of matching or identical positions; *e.g.*, if half (*e.g.*, five positions in a polymer ten amino acids in length) of the positions in two sequences are identical, the two sequences are 50% identical; if 90% of the positions (*e.g.*, 9 of 10), are matched or identical, the two amino acids

sequences are 90% identical. As applied to nucleic acid sequences, “identity” as used herein refers to a sequence that has about 50% sequence identity. More preferably, the homologous sequence has about 75% sequence identity, even more preferably, has at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence identity.

5 “Immunization” is the process of administering an immunogenic composition and stimulating an immune response to an antigen in a host (i.e., rodents and rabbits). Preferred hosts are mammals, such as primates (e.g., humans) as well as veterinary animals and agricultural animals.

10 An “immunogen” is an immunogenic composition used to immunized the host. “Immunogen” also refers to a substance that is able to stimulate or induce a humoral antibody and/or cell-mediated immune response in a mammal. In some instances, the immunogen comprises an anti-ADAMTS13 pathogenic antibody or any fragment thereof.

15 An “immune response” refers to the activities of the immune system, including activation and proliferation of specific cytotoxic T-cells and B-cells resulting in antigen-specific antibody production, after contact with an antigen.

As used herein, an “instructional material” includes a publication, a recording, a diagram, or any other medium of expression that can be used to communicate the usefulness of the compositions and methods of the invention. The instructional material of the kit of the invention may, for example, be affixed to a container that contains the nucleic acid, peptide, and/or composition of the invention or be shipped together with a container that contains the nucleic acid, peptide, and/or composition. Alternatively, the instructional material may be shipped separately from the container with the intention that the instructional material and the compound be used cooperatively by the recipient.

25 “Isolated” means altered or removed from the natural state. For example, a nucleic acid or a peptide naturally present in a living animal is not “isolated,” but the same nucleic acid or peptide partially or completely separated from the coexisting materials of its natural state is “isolated.” An isolated nucleic acid or protein can exist in substantially purified form, or can exist in a non-native environment such as, for example, a host cell.

30 An “isolated nucleic acid” refers to a nucleic acid segment or fragment that has been separated from sequences that flank it in a naturally occurring state, e.g., a DNA fragment that has been removed from the sequences that are normally adjacent to the fragment, e.g., the sequences adjacent to the fragment in a genome that it naturally occurs. The term also applies to nucleic acids that have been substantially purified from other components that naturally accompany the nucleic acid, e.g., RNA or DNA or proteins, that

naturally accompany it in the cell. The term therefore includes, for example, a recombinant DNA that is incorporated into a vector, into an autonomously replicating plasmid or virus, or into the genomic DNA of a prokaryote or eukaryote, or that exists as a separate molecule (e.g., as a cDNA or a genomic or cDNA fragment produced by PCR or restriction enzyme digestion) independent of other sequences. It also includes a recombinant DNA that is part of a hybrid gene encoding additional polypeptide sequence.

The term “model organism” refers to a non-human species that is easy to maintain and breed in a laboratory setting and has particular experimental advantages. Model organisms as used herein provide an *in vivo* model to research the effects of a human disease and/or biological activities associated with a disease, such as thrombotic thrombocytopenic purpura (TTP).

As used herein, the term “modulate” is meant to refer to any change in biological state, i.e. increasing, decreasing, and the like. For example, the term “modulate” refers to the ability to regulate positively or negatively the expression or activity of, for example, an anti-ADAMTS13 pathogenic antibody, including but not limited to transcription of the desired anti-ADAMTS13 pathogenic antibody mRNA, stability of the desired anti-ADAMTS13 pathogenic antibody mRNA, translation of the desired anti-ADAMTS13 pathogenic antibody mRNA, stability of the desired anti-ADAMTS13 pathogenic antibody polypeptide, post-translational modifications of the desired anti-ADAMTS13 pathogenic antibody, or any combinations thereof. Further, the term modulate can be used to refer to an increase, decrease, masking, altering, overriding or restoring of activity of an anti-ADAMTS13 pathogenic antibody.

In the context of the present invention, the following abbreviations for the commonly occurring nucleic acid bases are used. “A” refers to adenosine, “C” refers to cytosine, “G” refers to guanosine, “T” refers to thymidine, and “U” refers to uridine.

Unless otherwise specified, a “nucleotide sequence encoding an amino acid sequence” includes all nucleotide sequences that are degenerate versions of each other and that encode the same amino acid sequence. The phrase nucleotide sequence that encodes a protein or an RNA may also include introns to the extent that the nucleotide sequence encoding the protein may in some version contain an intron(s).

The term “oligonucleotide” typically refers to short polynucleotides, generally, no greater than about 50 nucleotides. It will be understood that when a nucleotide sequence is represented by a DNA sequence (i.e., A, T, G, C), this also includes an RNA sequence (i.e., A, U, G, C) that “U” replaces “T.”

The term “operably linked” refers to functional linkage between a regulatory sequence and a heterologous nucleic acid sequence resulting in expression of the latter. For example, a first nucleic acid sequence is operably linked with a second nucleic acid sequence when the first nucleic acid sequence is placed in a functional relationship with the second nucleic acid sequence. For instance, a promoter is operably linked to a coding sequence if the promoter affects the transcription or expression of the coding sequence. Generally, operably linked DNA sequences are contiguous and, where necessary to join two protein coding regions, in the same reading frame.

“Parenteral” administration of an immunogenic composition includes, *e.g.*, subcutaneous (s.c.), intravenous (i.v.), intramuscular (i.m.), or intrasternal injection, or infusion techniques.

The term “polynucleotide” as used herein is defined as a chain of nucleotides. Furthermore, nucleic acids are polymers of nucleotides. Thus, nucleic acids and polynucleotides as used herein are interchangeable. One skilled in the art has the general knowledge that nucleic acids are polynucleotides, which can be hydrolyzed into the monomeric “nucleotides.” The monomeric nucleotides can be hydrolyzed into nucleosides. As used herein polynucleotides include, but are not limited to, all nucleic acid sequences that are obtained by any means available in the art, including, without limitation, recombinant means, *i.e.*, the cloning of nucleic acid sequences from a recombinant library or a cell genome, using ordinary cloning technology and PCR™, and the like, and by synthetic means.

As used herein, the terms “peptide,” “polypeptide,” and “protein” are used interchangeably, and refer to a compound comprised of amino acid residues covalently linked by peptide bonds. A protein or peptide must contain at least two amino acids, and no limitation is placed on the maximum number of amino acids that can comprise a protein’s or peptide’s sequence. Polypeptides include any peptide or protein comprising two or more amino acids joined to each other by peptide bonds. As used herein, the term refers to both short chains, which also commonly are referred to in the art as peptides, oligopeptides and oligomers, for example, and to longer chains, which generally are referred to in the art as proteins, that there are many types. “Polypeptides” include, for example, biologically active fragments, substantially homologous polypeptides, oligopeptides, homodimers, heterodimers, variants of polypeptides, modified polypeptides, derivatives, analogs, fusion proteins, among others. The polypeptides include natural peptides, recombinant peptides, synthetic peptides, or a combination thereof.

A “recombinant polypeptide” is one that is produced upon expression of a recombinant nucleic acid.

The term “promoter” as used herein is defined as a DNA sequence recognized by the synthetic machinery of the cell, or introduced synthetic machinery, required to initiate
5 the specific transcription of a polynucleotide sequence.

As used herein, the term “promoter/regulatory sequence” means a nucleic acid sequence that is required for expression of a gene product operably linked to the promoter/regulatory sequence. In some instances, this sequence may be the core promoter sequence and in other instances, this sequence may also include an enhancer sequence and
10 other regulatory elements that are required for expression of the gene product. The promoter/regulatory sequence may, for example, be one that expresses the gene product in a tissue specific manner.

A “constitutive” promoter is a nucleotide sequence that, when operably linked with a polynucleotide that encodes or specifies a gene product, causes the gene product to be
15 produced in a cell under most or all physiological conditions of the cell.

An “inducible” promoter is a nucleotide sequence that, when operably linked with a polynucleotide that encodes or specifies a gene product, causes the gene product to be produced in a cell substantially only when an inducer that corresponds to the promoter is present in the cell.

20 A “tissue-specific” promoter is a nucleotide sequence that, when operably linked with a polynucleotide encodes or specified by a gene, causes the gene product to be produced in a cell substantially only if the cell is a cell of the tissue type corresponding to the promoter.

A “signal transduction pathway” refers to the biochemical relationship
25 between a variety of signal transduction molecules that play a role in the transmission of a signal from one portion of a cell to another portion of a cell. The phrase “cell surface receptor” includes molecules and complexes of molecules capable of receiving a signal and transmitting signal across the plasma membrane of a cell. An example of a “cell surface receptor” is human GFR α 4.

30 “Single chain antibodies” refer to antibodies formed by recombinant DNA techniques that immunoglobulin heavy and light chain fragments are linked to each other using an engineered span of amino acids to recapitulate the Fv region of an antibody as a single polypeptide. Various methods of generating single chain antibodies are known, including those described in U.S. Pat. No. 4,694,778; Bird (1988) Science 242:423-442;

Huston et al. (1988) Proc. Natl. Acad. Sci. USA 85:5879-5883; Ward et al. (1989) Nature 334:54454; Skerra et al. (1988) Science 242:1038-1041.

By the term “specifically binds,” as used herein, is meant a compound, e.g., a protein, a nucleic acid, an antibody, and the like, which recognizes and binds a specific molecule, but does not substantially recognize or bind other molecules in a sample.

The term “subject” is intended to include living organisms that an immune response can be elicited (e.g., mammals). A “subject” or “patient,” as used therein, may be a human or non-human mammal. Non-human mammals include, for example, livestock and pets, such as ovine, bovine, porcine, canine, feline and murine mammals. Preferably, the subject is human.

By “transgenic” is meant any animal that includes a nucleic acid sequence that is inserted by artifice into a cell and becomes a part of the genome of the animal that develops from that cell. Such a transgene may be partly or entirely heterologous to the transgenic animal. Although transgenic mice represent another embodiment of the invention, other transgenic mammals including, without limitation, transgenic rodents (for example, hamsters, guinea pigs, rabbits, and rats), and transgenic pigs, cattle, sheep, and goats are included in the definition.

As used herein, a “substantially purified” cell is a cell that is essentially free of other cell types. A substantially purified cell also refers to a cell that has been separated from other cell types that it is normally associated in its naturally occurring state. In some instances, a population of substantially purified cells refers to a homogenous population of cells. In other instances, this term refers simply to cell that have been separated from the cells that they are naturally associated in their natural state. In some embodiments, the cells are cultured *in vitro*. In other embodiments, the cells are not cultured *in vitro*.

The term “therapeutic” as used herein means a treatment and/or prophylaxis. A therapeutic effect is obtained by suppression, remission, or eradication of a disease state.

As used herein, a “therapeutic agent” is a molecule or atom, which is conjugated to an anti-autoimmune reagent to produce a conjugate that is useful for therapy. Examples of therapeutic agents include drugs, toxins, enzymes, hormones, cytokines, immunomodulators, anti-tumor agents, chemotherapeutic agents, anti-cell proliferation agents, boron compounds, and therapeutic radioisotopes.

The term “transfected” or “transformed” or “transduced” as used herein refers to a process that exogenous nucleic acid is transferred or introduced into the host cell. A “transfected” or “transformed” or “transduced” cell is one that has been transfected,

transformed or transduced with exogenous nucleic acid. The cell includes the primary subject cell and its progeny.

As used herein, to “treat” means reducing the frequency that symptoms of a disease, disorder, or adverse condition, and the like, are experienced by a patient. Such non-limiting conditions include bona fide illness as well as cosmetic or other conditions.

The phrase “under transcriptional control” or “operatively linked” as used herein means that the promoter is in the correct location and orientation in relation to a polynucleotide to control the initiation of transcription by RNA polymerase and expression of the polynucleotide.

A “vector” is a composition of matter that comprises an isolated nucleic acid and that can be used to deliver the isolated nucleic acid to the interior of a cell. Numerous vectors are known in the art including, but not limited to, linear polynucleotides, polynucleotides associated with ionic or amphiphilic compounds, plasmids, and viruses. Thus, the term “vector” includes an autonomously replicating plasmid or a virus. The term should also be construed to include non-plasmid and non-viral compounds that facilitate transfer of nucleic acid into cells, such as, for example, polylysine compounds, liposomes, and the like. Examples of viral vectors include, but are not limited to, adenoviral vectors, adeno-associated virus vectors, retroviral vectors, lentiviral vectors, and the like.

Ranges: throughout this disclosure, various aspects of the invention can be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 2.7, 3, 4, 5, 5.3, and 6. This applies regardless of the breadth of the range.

Detailed Description

Anti-ADAMTS13 Autoantibodies

The present invention relates to compositions and methods of use of anti-ADAMTS13 autoantibodies and fragments thereof. The invention described herein includes

autoantibodies or fragments thereof and methods that utilize such autoantibodies and fragments thereof. A better understanding of the repertoire of autoantibody expression within an individual patient and across multiple patients on a molecular level may be achieved by examining autoantibody clonality, epitope specificity, idiotypic relatedness, and functional significance *in vivo*. This knowledge is important for designing innovative therapies that specifically target pathogenic autoantibodies or the B cells that produce them, and providing animal models to test such approaches.

In one aspect, the invention includes a composition comprising at least one isolated anti-ADAMTS13 autoantibody or fragment. The anti-ADAMTS13 autoantibody or fragment is further identified as comprising a heavy chain selected from the group consisting of SEQ ID NOs: 46-90 or comprising a light chain selected from the group consisting of SEQ ID NOs: 142-192. In one embodiment, the anti-ADAMTS13 autoantibody or fragment comprises a single chain variable fragment (scFv). In another embodiment, the anti-ADAMTS13 autoantibody or fragment thereof binds at least one of the ADAMTS13 region selected from the group consisting of amino-terminal (MDT1) domain, carboxy-terminal (T5-8/CUB) domain and cysteine-rich/spacer region. In yet another embodiment, the anti-ADAMTS13 autoantibody or fragment thereof is capable of decreasing ADAMTS13 activity, such as proteolytic activity, disulfide reducing activity (Yeh, et al., J. Thromb. Haemost., 2010, 8:2778-2788), interaction or attachment to an endothelial cell surface, other functions of ADAMTS13, and any combination thereof.

In another embodiment, the anti-ADAMTS13 autoantibody or fragment thereof has a homology of at least 80% to at least one heavy chain selected from the group consisting of SEQ ID NOs: 46-90. In yet another embodiment, the anti-ADAMTS13 autoantibody or fragment thereof has a homology of at least 80% to at least one light chain selected from the group consisting of SEQ ID NOs: 142-192. The isolated anti-ADAMTS13 autoantibody or fragment thereof may share at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more homology with at least one antibody selected from the group consisting of SEQ ID NOs: 52-102.

In another aspect, the invention includes a composition comprising at least one isolated nucleic acid sequence encoding an anti-ADAMTS13 autoantibody or fragment thereof. The isolated nucleic acid sequence comprises a heavy chain nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1-45 and a light chain nucleic acid sequence selected from the group consisting of SEQ ID NOs: 91-141. In one embodiment, the isolated nucleic acid sequence encodes a single chain variable fragment (scFv).

In another embodiment, the isolated nucleic acid sequence has an identity of at least 80% to at least one heavy chain nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1-45. In yet another embodiment, the isolated nucleic acid sequence has an identity of at least 80% to at least one light chain nucleic acid sequence selected from the group consisting of SEQ ID NOs: 91-141. The isolated nucleic acid sequence may share at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identity with either a heavy chain or a light chain nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1-45 or 91-141, respectively.

Much of what is known about ADAMTS13-specific autoantibodies has been obtained from the analysis of polyclonal mixtures of immunoglobulin derived from TTP patients. Such studies have shown that anti-ADAMTS13 autoantibodies are predominantly of the IgG isotype subclass IgG1 and IgG4 many that recognize epitopes in the spacer domain of the protease. Binding of autoantibodies to the spacer domain of ADAMTS13 is thought to inhibit cleavage of VWF by blocking amino acid motifs involved in complex formation of ADAMTS13 with the unfolded VWF A2 domain. However, nearly all patient plasmas have circulating autoantibodies that recognize at least one additional domain of ADAMTS13 other than the spacer domain. The potential functional significance of autoantibodies directed to non-spacer domains has been largely unexplored due to the inherent difficulty in studying complex autoantibody repertoires using heterogeneous mixtures of IgG contained in patient plasma.

A limited number of inhibitory anti-ADAMTS13 monoclonal autoantibodies directed to the spacer domain of ADAMTS13 have been cloned from the peripheral blood lymphocytes or spleen of four TTP patients. It is not clear whether human anti-ADAMTS13 autoantibodies specific for other domains also inhibit ADAMTS13 proteolytic activity or interfere with other functions of the protease. A few of these human spacer domain-specific inhibitory antibodies cloned from different patients were found to be encoded by the human germline heavy chain variable region gene V_H1-69 suggesting a potential property of ADAMTS13 inhibitory antibodies one might exploit as a therapeutic target. However, the structural features conferred by this single immunoglobulin gene that confer ADAMTS13 inhibition are not clear nor is the extent that V_H1-69-encoded antibodies are representative of circulating autoantibodies expressed in TTP patient plasma. Furthermore, the clinical relevance of the *in vivo* properties of these cloned human autoantibodies in an animal model has not been tested. Indeed, to date, there are no published reports describing animal models

of human autoantibody-mediated TTP. Models have been developed that ADAMTS13 deficiency in mice is mediated by xenoantibodies to ADAMTS13, e.g. by rabbit polyclonal anti-human ADAMTS13 antibodies, or by mouse monoclonal antibodies to the ADAMTS13 metalloprotease domain in baboons. These antibodies and models may not be suitable to test agents that block the idiotopes of human anti-ADAMTS13 autoantibodies or to test novel engineered preparations of ADAMTS13 that have been designed to be uninhibitable by human antibodies directed to human autoepitopes.

In one embodiment, the invention includes anti-autoimmune antibodies or reagents directed against anti-ADAMTS13 antibodies. The anti-autoimmune antibodies of the invention can be monoclonal antibodies (mAb) in some aspects, or polyclonal antibodies in other aspects. The anti-autoimmune antibodies of the invention that are useful for the compositions, methods and kits of the invention can be from any source, and in addition may be chimeric. In one embodiment, sources of anti-autoimmune antibodies can be from a mouse, or a rat, a plant, or a human in other embodiments. Anti-autoimmune antibodies of the invention that are useful for the compositions, and methods of the invention have reduced antigenicity in humans (to reduce or eliminate the risk of formation of anti-human antibodies), and in another embodiment, are not antigenic in humans. Chimeric anti-autoimmune antibodies for use the invention contain in one embodiment, human amino acid sequences and include humanized antibodies that are non-human antibodies substituted with sequences of human origin to reduce or eliminate immunogenicity, but retains the antigen binding characteristics of the non-human antibody. In one embodiment, the anti-autoimmune antibodies of the invention directed against anti-ADAMTS13 antibodies are used therapeutically to treat TTP. In some embodiments, TTP is prevented or reduced.

In another aspect, the invention includes a method of identifying an ADAMTS13 variant that does not bind an anti-ADAMTS13 autoantibody or fragment thereof. The method comprises contacting an ADAMTS13 protein with an anti-ADAMTS13 autoantibody or fragment thereof, wherein when the ADAMTS13 protein does not bind the anti-ADAMTS13 autoantibody or fragment thereof, then the ADAMTS13 protein is a variant that does not bind an anti-ADAMTS13 autoantibody or fragment thereof.

In yet another aspect, the invention includes a method for treating thrombotic thrombocytopenic purpura (TTP) in a subject in need thereof, the method comprising administering to the subject a composition comprising an effective amount of an ADAMTS13 variant, wherein the ADAMTS13 variant is resistant to inhibition by an anti-ADAMTS13 autoantibody or fragment thereof.

In one embodiment, the ADAMTS13 variant is resistant to inhibition by an anti-ADAMTS13 autoantibody or fragment thereof comprising at least one amino acid sequence selected from the group consisting of SEQ ID NOs: 46-90; 142-192. In another embodiment, the ADAMTS13 variant that does not bind an anti-ADAMTS13 autoantibody or fragment thereof comprises a preserved or enhanced proteolytic activity as compared to a native ADAMTS13. In yet another embodiment, the ADAMTS13 variant is useful for treating thrombotic thrombocytopenic purpura (TTP).

Peptidomimetic compounds can also be made where individual amino acids are replaced by analogous structures, for example gem-diaminoalkyl groups or alkylmalonyl groups, with or without modified termini or alkyl, acyl or amine substitutions to modify their charge. The use of such alternative structures can provide significantly longer half-life in the body, since they are more resistant to breakdown under physiological conditions.

Methods for combinatorial synthesis of peptide analogs and for screening of peptides and peptide analogs are well known in the art (see, for example, Gallop et al., 1994 J. Med. Chem. 37: 1233). It is particularly contemplated that the compounds of the invention are useful as templates for design and synthesis of compounds of improved activity, stability and bioavailability. Preferably where amino acid substitution is used, the substitution is conservative, i.e. an amino acid is replaced by one of similar size and with similar charge properties. As used herein, the term "conservative substitution" denotes the replacement of an amino acid residue by another, biologically similar residue. Examples of conservative substitutions include the substitution of one hydrophobic residue such as isoleucine, valine, leucine, alanine, cysteine, glycine, phenylalanine, proline, tryptophan, tyrosine, norleucine or methionine for another, or the substitution of one polar residue for another, such as the substitution of arginine for lysine, glutamic acid for aspartic acid, or glutamine for asparagine, and the like. Neutral hydrophilic amino acids that can be substituted for one another include asparagine, glutamine, serine and threonine. The term "conservative substitution" also includes the use of a substituted amino acid in place of an unsubstituted parent amino acid.

The antibodies of the present invention include those cloned from a phage antibody library, as described in detail elsewhere herein. For example, a cDNA library is generated from mRNA obtained from a population of antibody-producing cells. The mRNA encodes rearranged immunoglobulin genes and thus, the cDNA encodes the same. Amplified cDNA is cloned into expression vectors creating a library of phage that express human Fab or scFv fragments on their surface. Phage that display the antibody of interest are selected by

antigen binding and are propagated in bacteria to produce soluble human Fab or scFv immunoglobulin. Thus, in contrast to conventional monoclonal antibody synthesis, this procedure immortalizes DNA encoding human immunoglobulin rather than cells that express human immunoglobulin.

5 Using the information provided herein, the antibodies of the present invention can be produced recombinantly using standard techniques well known to those of skill in the art. For example, the sequences provided herein can be used to express one or more antibodies. The nucleic acid sequence may be optimized to reflect particular codon "preferences" for various expression systems according to standard methods well known to
10 those of skill in the art.

 Using the sequence information provided herein, the nucleic acids may be synthesized according to a number of standard methods known to those of skill in the art. Oligonucleotide synthesis, is preferably carried out on commercially available solid phase oligonucleotide synthesis machines or manually synthesized using the solid phase
15 phosphoramidite triester method described by Beaucage et. al., 1981, Tetrahedron Letts. 22:1859-1862.

 Once a nucleic acid encoding an antibody is synthesized, it may be amplified and/or cloned according to standard methods in order to produce recombinant antibodies of the invention. Molecular cloning techniques to achieve these ends are known in the art. A
20 wide variety of cloning and in vitro amplification methods suitable for the construction of recombinant nucleic acids are known to those skilled in the art. Examples of these techniques and instructions sufficient to direct the skilled artisan are found in Berger and Kimmel, Guide to Molecular Cloning Techniques, Methods in Enzymology volume 152 Academic Press, Inc., San Diego, Calif. (Berger); Sambrook et al., 2002 Molecular Cloning. A Laboratory
25 Manual Vol. 1-3, Cold Spring Harbor Laboratory, Cold Spring Harbor Press, NY, (Sambrook). Methods of producing recombinant immunoglobulins are also known in the art. See, Cabilly, U.S. Pat. No. 4,816,567; and Queen et al., 1989 Proc. Nat'l Acad. Sci. USA.

 Examples of techniques sufficient to direct persons of skill through in vitro amplification methods, including the polymerase chain reaction (PCR), the ligase chain
30 reaction (LCR), and other DNA or RNA polymerase-mediated techniques are found in Berger, Sambrook, and Ausubel, as well as U.S. Pat. No. 4,683,202 and U.S. Pat. No. 5,426,039.

 Once the nucleic acid encoding a desired antibody is isolated and cloned, a skilled artisan may express the recombinant gene(s) in a variety of engineered cells known to

those of skill in the art. Examples of such cells include bacteria, yeast, filamentous fungi, insect (especially employing baculoviral vectors), and mammalian cells. It is expected that those of skill in the art are knowledgeable in the numerous expression systems available for expression of the desired antibodies.

5 For some uses of the anti-autoimmune antibodies or reagents directed against anti-ADAMTS13 antibodies, including *in vivo* in humans and *in vitro* detection assays, it may be preferable to use chimeric, hybrid, primatized, humanized, or human antibodies. Methods for producing chimeric and hybrid antibodies are known in the art. See e.g., Morrison, 1985 Science 229: 1202-1207; U.S. Pat. Nos. 6,965,024, 5,807,715; 4,816,567;
10 and 4,816,397. Humanized antibodies are antibody molecules from non-human species that bind the desired antigen having one or more complementarity determining regions (CDRs) from the non-human species and framework regions and constant domains from a human immunoglobulin molecule. Often, framework residues in the human framework regions are substituted with the corresponding residue from the CDR donor antibody to alter and in some
15 instances improve, antigen binding. These framework substitutions are identified by methods well known in the art, e.g., by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence comparison to identify unusual framework residues at particular positions. See, e.g., Queen et al., U.S. Pat. No. 5,585,089. Antibodies can be humanized using a variety of techniques
20 known in the art including, for example, CDR-grafting and chain shuffling. Humanized antibodies may be generated using any of the methods disclosed in U.S. Pat. No. 5,693,762, U.S. Pat. No. 5,693,761, U.S. Pat. No. 5,585,089, U.S. Pat. No. 6,180,370.

Models of Thrombotic Thrombocytopenic Purpura (TTP)

25 The present invention also includes the generation of an *in vivo* model of thrombotic thrombocytopenic purpura (TTP). In one aspect, the invention includes a method for generating an *in vivo* model of thrombotic thrombocytopenic purpura (TTP) comprising introducing at least one anti-ADAMTS13 autoantibody or fragment thereof into a model organism. The model organism may include a non-mammalian organism or a non-human
30 mammalian organism, such as a non-human primate, an ovine, a bovine, a porcine, a canine, a feline and a murine organism.

For *in vivo* models of TTP of the present invention, the generation of anti-ADAMTS13 autoantibodies or fragments thereof may be expressed or introduced into a model organism, preferably a model organism that does not spontaneously develop

autoimmune diseases. In one embodiment, introducing the anti-ADAMTS13 autoantibody or fragment thereof comprises formulating the anti-ADAMTS13 autoantibodies or fragments thereof in a composition for administration to the model organism. Such an embodiment may further comprise injecting the anti-ADAMTS13 autoantibodies or fragments thereof into the model organism. In another embodiment, introducing the anti-ADAMTS13 autoantibody or fragment thereof comprises inducing *in vivo* expression. One example of inducing *in vivo* expression is through injection of nucleic acids, such as hydrodynamic delivery, to the model organism. Other examples of methods of inducing *in vivo* expression may include electroporation, transfection, transduction and other methods of viral delivery, and any combination thereof. Introducing the anti-ADAMTS13 autoantibodies or fragments thereof may be exemplified by methods known in the art and is not limited to the methods described herein.

In vivo models generated by the methods described herein may be useful for further characterizing TTP, identifying anti-autoimmune reagents or therapeutic agents, characterizing an anti-autoimmune reagent or therapeutic agent, or any other purpose described herein or known in the art.

Screening for Anti-Autoimmune Reagents

The present invention is partly based on the identification of an anti-autoimmune reagent or therapeutic agent, such as peptides or small molecules, that bind a desired autoantibody or fragment thereof. In some instances, the autoantibody or fragment thereof is a disease associated-pathogenic antibody, for example a pathogenic anti-ADAMTS13 autoantibody. Accordingly, a peptide that binds to a disease associated-pathogenic antibody is an example of an anti-autoimmune reagent. However, the invention also includes anti-autoimmune reagents that bind to non-pathogenic antibodies. In one aspect, the invention includes a method for identifying an anti-autoimmune reagent for treating thrombotic thrombocytopenic purpura (TTP) comprising contacting a panel of agents with at least one anti-ADAMTS13 autoantibody or fragment thereof and identifying the agents that bind to the anti-ADAMTS13 autoantibody or fragment thereof. In one embodiment, identifying the agents comprises identifying agents that blocks binding of the anti-ADAMTS13 autoantibody or fragment thereof to ADAMTS13.

There are several examples of methods that use peptides or nucleotides to develop libraries of potential receptor, enzyme, or antibody interacting peptides. These libraries have been incorporated into systems that allow the expression of random peptides on

the surface of different phage or bacteria. The use of phage display technology to produce and screen libraries of polypeptides for binding to a selected target has been widely used. A basic concept of phage display methods is the establishment of a physical association between DNA encoding a polypeptide to be screened and the target polypeptide. This physical association is provided by the phage particle, which displays a polypeptide as part of a capsid enclosing the phage genome that encodes the polypeptide. The establishment of a physical association between polypeptides and their genetic material allows simultaneous mass screening of very large numbers of phage bearing different polypeptides. Phage displaying a polypeptide with affinity to a target bind to the target and these phage are enriched by affinity screening to the target. The identity of polypeptides displayed from these phage can be determined from their respective genomes. Using these methods a polypeptide identified as having a binding affinity for a desired target can then be synthesized in bulk by conventional means.

A phage display library may be screened to identify peptides that bind to an anti-ADAMTS13 antibody (e.g., a pathogenic anti-ADAMTS13 antibody). Accordingly, the invention includes peptides that specifically bind pathogenic anti-ADAMTS13 antibodies. However, the invention should not be limited to only these peptides. Rather, the invention encompasses using any disease-associated pathogenic antibody to screen libraries of peptides or small molecules to identify therapeutic reagents. However, the invention also contemplates peptides and small molecules that bind to non-pathogenic antibodies. This is because a non-pathogenic can be used in the phage display library screening procedure to identify the corresponding binding molecule, and in some cases non-pathogenic antibodies when used in combination or under certain conditions may prove to cause pathology.

Anti-Autoimmune Reagent

The invention provides a composition comprising an anti-autoimmune reagent. The anti-autoimmune reagent includes any agent that is capable of binding to an autoimmune antibody. In one aspect, an anti-autoimmune reagent specifically binds to an anti-ADAMTS13 antibody. In such an aspect, the anti-autoimmune reagent blocks binding of the anti-ADAMTS13 autoantibody or fragment thereof to ADAMTS13. In one embodiment, the anti-autoimmune reagent is an antibody that binds to an autoantibody or fragment thereof. In another embodiment, the anti-autoimmune reagent is a peptide or small molecule that binds to an autoantibody or fragment thereof. For example, the anti-autoimmune reagent binds to a pathogenic autoantibody or the anti-ADAMTS13 autoantibody or fragment thereof.

In one embodiment, the anti-autoimmune reagent blocks the binding of the anti-ADAMTS13 autoantibody or fragment thereof to at least one of the ADAMTS13 regions selected from the group consisting of amino-terminal (MDT1) domain, carboxy-terminal (T5-8/CUB) domain and cysteine-rich/spacer region. In another embodiment, the anti-autoimmune reagent for
5 treating TTP specifically binds to at least one amino acid sequence selected from the group consisting of SEQ ID NOs: 46-90; 142-192.

Also described herein are compositions and methods for treatment of diseases or conditions, such as thrombotic thrombocytopenic purpura (TTP). In one aspect, the invention includes a method of inhibiting the binding of an anti-ADAMTS13 autoantibody or
10 fragment thereof to ADAMTS13 comprising contacting the anti-ADAMTS13 autoantibody or fragment thereof with a composition comprising an anti-autoimmune reagent that specifically binds to the anti-ADAMTS13 autoantibody or fragment thereof. The composition may include a pharmaceutical composition and further include a pharmaceutically acceptable carrier. A therapeutically effective amount of the
15 pharmaceutical composition comprising the anti-autoimmune reagent directed against the anti-ADAMTS13 autoantibody or fragment thereof may be administered.

In one aspect, the invention includes identifying an anti-autoimmune reagent for treating thrombotic thrombocytopenic purpura (TTP) comprising contacting a panel of agents with at least one anti-ADAMTS13 autoantibody or fragment thereof and identifying
20 the agents that bind to the anti-ADAMTS13 autoantibody or fragment thereof. In this embodiment, identifying the agents comprises identifying agents that blocks binding of the anti-ADAMTS13 autoantibody or fragment thereof to ADAMTS13. In another aspect, the invention includes a method of inhibiting the binding of an anti-ADAMTS13 autoantibody or fragment thereof to ADAMTS13 comprising contacting the anti-ADAMTS13 autoantibody
25 or fragment thereof with a composition comprising an anti-autoimmune reagent that specifically binds to the anti-ADAMTS13 autoantibody or fragment thereof.

The compositions comprising a therapeutic agent described herein can be administered to an animal, preferably a mammal, even more preferably a human, to suppress an immune reaction, such as those common to autoimmune diseases such as TTP.

30 Compositions of the invention can be administered in dosages and routes and at times to be determined in appropriate pre-clinical and clinical experimentation and trials. The compositions of the invention may be administered multiple times at dosages within these ranges. Administration of these compositions may be combined with other methods useful to treat the desired disease or condition as determined by those of skill in the art.

The administration of the compositions of the invention may be carried out in any convenient manner known to those of skill in the art. The compositions of the present invention may be administered to a subject by aerosol inhalation, injection, ingestion, transfusion, implantation or transplantation. The compositions described herein may be administered to a patient transarterially, subcutaneously, intradermally, intratumorally, intranodally, intramedullary, intramuscularly, by intravenous (*i.v.*) injection, or intraperitoneally. In other instances, the cells of the invention are injected directly into a site of inflammation in the subject, a local disease site in the subject, a lymph node, an organ, a tumor, and the like.

Pharmaceutical compositions

Pharmaceutical compositions of the present invention may comprise a therapeutic agent as described herein, in combination with one or more pharmaceutically or physiologically acceptable carriers, diluents or excipients. Such compositions may comprise buffers such as neutral buffered saline, phosphate buffered saline and the like; carbohydrates such as glucose, mannose, sucrose or dextrans, mannitol; proteins; polypeptides or amino acids such as glycine; antioxidants; chelating agents such as EDTA or glutathione; adjuvants (*e.g.*, aluminum hydroxide); and preservatives. Compositions of the present invention are preferably formulated for intravenous administration.

Pharmaceutical compositions of the present invention may be administered in a manner appropriate to the disease to be treated (or prevented). The quantity and frequency of administration will be determined by such factors as the condition of the patient, and the type and severity of the patient's disease, although appropriate dosages may be determined by clinical trials.

When "an immunologically effective amount", "an anti-immune response effective amount", "an immune response-inhibiting effective amount", or "therapeutic amount" is indicated, the precise amount of the compositions of the present invention to be administered can be determined by a physician with consideration of individual differences in age, weight, immune response, and condition of the patient (subject). It can generally be stated that a pharmaceutical composition comprising the therapeutic agents described herein may be administered at a dosage of 10^4 to 10^9 cells/kg body weight, preferably 10^5 to 10^6 cells/kg body weight, including all integer values within those ranges. Therapeutic compositions may also be administered multiple times at these dosages. The therapeutic agents can be administered by using infusion techniques that are commonly known in

immunotherapy (see, *e.g.*, Rosenberg et al., New Eng. J. of Med. 319:1676, 1988). The optimal dosage and treatment regime for a particular patient can readily be determined by one skilled in the art of medicine by monitoring the patient for signs of disease and adjusting the treatment accordingly.

5 In certain embodiments of the present invention, the methods described herein, or other methods known in the art where therapeutic agents are directed to autoantibodies and administered to a patient alone or in conjunction with (*e.g.*, before, simultaneously or following) any number of relevant treatment modalities, including but not limited to treatment with agents such as antiviral therapy, cidofovir and interleukin-2, Cytarabine (also
10 known as ARA-C) or natalizumab treatment for MS patients or efalizumab treatment for psoriasis patients or other treatments for PML patients. In further embodiments, the therapeutic agents of the invention may be used in combination with chemotherapy, radiation, immunosuppressive agents, such as cyclosporin, azathioprine, methotrexate, mycophenolate, and FK506, antibodies, or other immunoablative agents such as CAM PATH, anti-CD3
15 antibodies or other antibody therapies, cytoxin, fludaribine, cyclosporin, FK506, rapamycin, mycophenolic acid, steroids, FR901228, cytokines, and irradiation. These drugs inhibit either the calcium dependent phosphatase calcineurin (cyclosporine and FK506) or inhibit the p70S6 kinase that is important for growth factor induced signaling (rapamycin). (Liu et al., Cell 66:807-815, 1991; Henderson et al., Immun. 73:316-321, 1991; Bierer et al., Curr. Opin.
20 Immun. 5:763-773, 1993). In a further embodiment, the therapeutic agents of the present invention are administered to a patient in conjunction with (*e.g.*, before, simultaneously or following) bone marrow transplantation, T cell ablative therapy using either chemotherapy agents such as, fludarabine, external-beam radiation therapy (XRT), cyclophosphamide, or antibodies such as OKT3 or CAMPATH. In another embodiment, the cell compositions of
25 the present invention are administered following B-cell ablative therapy such as agents that react with CD20, *e.g.*, Rituxan. For example, in one embodiment, subjects may undergo standard treatment with high dose chemotherapy followed by peripheral blood stem cell transplantation. In certain embodiments, following the transplant, subjects receive an infusion of the expanded immune cells of the present invention. In an additional
30 embodiment, expanded cells are administered before or following surgery.

 The dosage of the above treatments to be administered to a patient will vary with the precise nature of the condition being treated and the recipient of the treatment. The scaling of dosages for human administration can be performed according to art-accepted practices. The dose for CAMPATH, for example, will generally be in the range 1 to about

100 mg for an adult patient, usually administered daily for a period between 1 and 30 days. The preferred daily dose is 1 to 10 mg per day although in some instances larger doses of up to 40 mg per day may be used (described in U.S. Patent No. 6,120,766).

It should be understood that the method and compositions that would be useful in the present invention are not limited to the particular formulations set forth in the examples. The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the cells, expansion and culture methods, and therapeutic methods of the invention, and are not intended to limit the scope of what the inventors regard as their invention.

The practice of the present invention employs, unless otherwise indicated, conventional techniques of molecular biology (including recombinant techniques), microbiology, cell biology, biochemistry and immunology, which are well within the purview of the skilled artisan. Such techniques are explained fully in the literature, such as, “Molecular Cloning: A Laboratory Manual”, fourth edition (Sambrook, 2012); “Oligonucleotide Synthesis” (Gait, 1984); “Culture of Animal Cells” (Freshney, 2010); “Methods in Enzymology” “Handbook of Experimental Immunology” (Weir, 1997); “Gene Transfer Vectors for Mammalian Cells” (Miller and Calos, 1987); “Short Protocols in Molecular Biology” (Ausubel, 2002); “Polymerase Chain Reaction: Principles, Applications and Troubleshooting”, (Babar, 2011); “Current Protocols in Immunology” (Coligan, 2002). These techniques are applicable to the production of the polynucleotides and polypeptides of the invention, and, as such, may be considered in making and practicing the invention. Particularly useful techniques for particular embodiments will be discussed in the sections that follow.

EXPERIMENTAL EXAMPLES

The invention is further described in detail by reference to the following experimental examples. These examples are provided for purposes of illustration only, and are not intended to be limiting unless otherwise specified. Thus, the invention should in no way be construed as being limited to the following examples, but rather, should be construed to encompass any and all variations that become evident as a result of the teaching provided herein.

Without further description, it is believed that one of ordinary skill in the art can, using the preceding description and the following illustrative examples, make and utilize the compounds of the present invention and practice the claimed methods. The following

working examples therefore, specifically point out the preferred embodiments of the present invention, and are not to be construed as limiting in any way the remainder of the disclosure.

The materials and methods employed in these experiments are now described.

Isolation of human anti-ADAMTS13 monoclonal autoantibodies

5 Antibody phage display libraries expressing IgG₁₋₄ κ/λ -derived single chain variable region fragments (scFv) were created from the splenic B-cells or peripheral blood B cells of 4 unrelated patients with autoantibody-mediated TTP (“TTP1”-“TTP4”) (Table 1) using methods previously described (Siegel, Cold Spring Harbor, NY: Cold Spring Harbor Press, 2001:23.1-32; Roark et al., Blood 2002;100:1388-98; Payne et al., J Clin Invest 10 2005;115:888-99). Briefly, cDNAs encoding the rearranged immunoglobulin heavy chain and light chain variable regions were amplified by PCR and cloned into the pComb3X phagemid vector (Scripps Research Institute, La Jolla, CA). After electroporation into XL1-Blue *E. coli* (Stratagene, La Jolla) and co-infection with VCSM13 helper phage (Stratagene), DNA encoding each scFv sequence was packaged into a filamentous phage particle 15 expressing the encoded protein on its surface.

Relevant demographic and clinical data are shown in Table 1. Peripheral blood (in 0.38% citrate) for serological and ADAMTS13 activity assays and for isolation of mononuclear cells for library construction (in 16 U/ml sodium heparin) were collected from TTP2, TTP3, and TTP4 prior to their first plasma exchange. Citrated blood was centrifuged 20 at 1500g for 15 min at 25°C and plasma was collected and stored at -80°C. Mononuclear cells were isolated by density sedimentation with Ficoll-Paque (GE Healthcare Life Sciences, Pittsburgh, PA) following manufacturer’s instructions and stored at -80°C. The TTP1 antibody library was generated from 15 g of splenic tissue following splenectomy after a 42-week relapsing/remitting course.

25 Antibody libraries were selected against full-length human recombinant ADAMTS13 coated in wells of enzyme immunoassay plates (Technoclone GmbH, Vienna, Austria) using previously-described methods for solid phase selection (Payne, et al., J. Clin. Invest., 2005, 115:888-899). For TTP1, the library was also selected against the TSP1 5-8/CUB fragment of ADAMTS13 prepared as described (Ai, et al., J. Biol. Chem., 2005, 30 280:29428-29434). After 4 rounds of selection for each library, randomly picked antibody clones were assessed for binding to ADAMTS13 by phage ELISA using ADAMTS13-coated plates, HRP-conjugated anti-M13 antibody (GE Healthcare Life Sciences) as described (Payne, et al., J. Clin. Invest., 2005, 115:888-899). Positive binding clones (>10-fold

absorbance above background with irrelevant phage-displayed scFv) were identified from each patient's library. Nucleotide sequences of the heavy and light chains of each scFv were determined using pComb3X-specific sequencing primers to identify unique antibodies.

5 Nucleotide sequences were analyzed for homology to known human V_H, D, J_H, V_κ, J_κ, V_λ, and J_λ immunoglobulin heavy or light chain germline gene segments using IMGT/V-QUEST (www.imgt.org/).

Production of soluble scFv antibody fragments in bacteria

Soluble scFv preparations (i.e. antibodies unlinked to phage) of each positive
10 clone identified through phage ELISA were used to confirm ADAMTS13 binding and to perform ADAMTS13 inhibition assays, epitope mapping, and generation of rabbit anti-idiotypic antibodies as detailed below. The TOP10F' non-suppressor strain of *E. coli* (Invitrogen, Life Technologies, Grand Island, NY) was infected with individual phage clones and scFv molecules with carboxy terminus 6xHis sequence (for purification) and a
15 hemagglutinin (HA) peptide tag (for detection) were then purified from the bacterial periplasmic space using sucrose shock and nickel-chelation affinity chromatography. ScFv's were dialyzed against PBS, concentrated by Amicon ultrafiltration (Millipore, Billerica, MA), and quantified by SDS-PAGE using molecular weight standards of known mass (Novex NuPAGE, Life Technologies). Binding of ADAMTS13 by soluble scFv (150 ng scFv per
20 well) was assessed by ELISA with recombinant ADAMTS13-coated enzyme immunoassay plate wells and HRP-conjugated anti-HA secondary antibody (Roche Diagnostics, Indianapolis, IN). Negative controls included identically-prepared scFv specific for irrelevant antigens including E1M2, a red cell Rh(D)-specific scFv, PX4-3, a keratinocyte desmoglein-specific scFv, and X24-3, a human platelet factor 4-specific scFv.

25

Production of soluble scFv antibody fragments in insect cell culture

Soluble scFv of selected ADAMTS13-positive clones were expressed in
Drosophila S2 cells to prepare antibodies for injection into mice and for experiments requiring antibodies expressing a V5 vs. HA tag. The multiple cloning site of the plasmid
30 pMT/BiP/V5-His A (Invitrogen) was modified to introduce a pair of Sfi I sites so that scFv constructs could be shuttled easily from their Sfi I cloning sites in the pComb3X phagemid vector. To express a phage display-derived scFv in S2 cells, the desired scFv construct was removed from the pComb3X phagemid by Sfi I digestion, gel purified, and ligated into Sfi I-

digested and gel-purified PMT vector from above. PMT plasmids and pCoBlast (Invitrogen) were co-transfected into S2 cells at a ratio of 19:1 using FuGene6 (Promega, Madison, WI), stable transfectants were established using Blasticidin (Invitrogen), and induction of scFv expression with CuSO₄ was performed as per manufacturer's instructions as modified as previously described (Zaitsev, et al, J. Pharmacol. Exp. Ther., 2010, 332:1022-1031). V5-tagged scFv's were separated from cell media components by dialysis into 300mM NaCl/50 mM NaPO₄, pH 8.00 buffer and a first-round purification using Ni-NTA agarose (Qiagen, Germantown, MD) following manufacturer's instructions. After Centricon ultrafiltration (10 kDa MW, Millipore) and further purification by size exclusion gel chromatography on Sephadex G-75 (GE Healthcare Life Sciences), scFv preparations were once again concentrated by ultrafiltration and stored at -80°C until use.

Epitope mapping

Epitope mapping of selected scFv was performed by immunoprecipitation (IP) of mammalian cell culture-produced full-length and truncated forms of ADAMTS13 modified for IP with HA-tagged scFv instead of polyclonal TTP patient serum IgG. Incubation mixtures comprising 500 µL of PBS containing 25-100 ng of an ADAMTS13 construct, 600 ng of *E. coli*-produced scFv, 0.1% protease inhibitor cocktail (#P8849, Sigma, St. Louis, MO), 0.05% Tween 20 (Pierce Biotechnologies, Rockford, IL), and 1% bovine serum albumin were rotated overnight at 4°C followed by addition of 30 µL anti-HA agarose beads (Roche). Beads were incubated for 2 hours at room temperature, washed 5 times with 1 mL of 0.05% Tween 20/PBS, and the final bead pellet was resuspended in 50 µL NuPAGE LDS gel electrophoresis sample buffer containing DTT (Novex) and heated at 85°C for 5 min. Electrophoresis and Western blotting were performed per manufacturer's instructions using NuPAGE 4-12% Bis-Tris gels loaded with 15 µL sample per well. Immunoprecipitated ADAMTS13 constructs were visualized on X-ray film with a chemiluminescent substrate (ECL, Amersham, GE Healthcare Life Sciences) on PVDF membranes developed with HRP-conjugated mouse anti-V5 antibody (Invitrogen) at 1:5000 dilution in blocking buffer (2.5% non-fat dry milk, 0.5% Tween, TBS).

Generation of rabbit anti-ADAMTS13 anti-idiotypic antibodies

A pair of New Zealand White rabbits were each immunized with 1 of 4 purified HA-tagged *E. coli*-produced scFv preparations (scFv 1-416, 1-420, 1-428, or 1-431)

over a period of 90 days by Pocono Rabbit Farm & Laboratory (Canadensis, PA), a laboratory animal care facility accredited, registered, and assured by AAALAC International, the USDA, and OLAW, respectively, following their standard IACUC-approved protocol for rabbit protein immunization. Rabbit IgG was purified using recombinant Protein G agarose (Invitrogen) following manufacturer's instructions from sera. Rabbit IgG was quantified by OD₂₈₀ using an extinction coefficient of 1.4. Immunity to recombinant scFv was verified by comparing respective rabbit pre-immune sera with post-immune sera by ELISA using plates pre-coated with 10 µg/mL mouse anti-V5 tag antibody (Invitrogen) to capture S2 cell-produced V5-tagged scFv's and developed with a 1:5000 dilution of HRP-conjugated donkey anti-rabbit IgG (Amersham). V5-tagged scFv were used here and in all subsequent assays of scFv with rabbit IgG to avoid any anti-HA tag antibodies that may have been produced from HA-tagged scFv immunogens. Reactivity of rabbit IgG to V5-tagged scFv's pre-/post-immunization increased nearly 100-fold.

Intraperitoneal injection of anti-ADAMTS13 antibodies in mice

Two- to three-month-old mice (C57BL/6 or CAST/Ei, Jackson Laboratory, Bar Harbor, ME) were anesthetized with ketamine/xylazine and blood samples (50 µL) were obtained from their jugular veins before and after intraperitoneal injection of 100 µL PBS containing 30 µg *Drosophila* S2 cell-produced scFv. Blood samples were taken at various time points and anticoagulated with 5 µL PBS containing 5 U/mL heparin. Plasma was separated from cells at 1500g for 15 min and frozen at -80°C for ADAMTS13 activity assays and/or VWF multimer analyses.

In vivo production of human scFv antibodies in mice by hydrodynamic delivery

ScFv cDNA was cloned into the pLIVE *in vivo* expression vector (Mirus, Madison, WI) and injected via tail vein into 2- to 3-month-old mice (C57BL/6 or CAST/Ei) per manufacturer's instructions. Briefly, mice were warmed by heat lamp for several minutes and 30 µg of pLIVE/scFv DNA diluted in 2 mL *TransIT-QR* hydrodynamic delivery solution were injected in the tail over 4 to 7 seconds using a syringe equipped with a 30-gauge needle. To facilitate the cloning of scFv constructs from the pComb3X phagemid vector into pLIVE with the subsequent secretion of antibody from murine liver, the multiple cloning site of the pLIVE vector was first modified with an immunoglobulin kappa-chain leader sequence followed by Sfi I restriction sites and a V5-tag sequence for subsequent detection in mouse

plasma (FIG. 11 for details). Plasmid DNA for injection was produced in XL1-Blue and purified using an endotoxin-free plasmid purification kit (Qiagen). Just prior to and after DNA delivery, blood samples (~100 μ L) were collected from the retro-orbital sinus under light anesthesia with isoflurane into heparinized capillary tubes at various time points and
 5 transferred to 10 μ L of 1.9% sodium citrate. Plasma was separated by centrifugation and stored at -80°C.

Cremaster arteriole laser injury model in scFv-expressing mice

Platelet thrombus formation in C57BL/6 mice pre-injected 7 days earlier with
 10 pLIVE expression vector containing scFv 1-420 anti-ADAMTS13 antibody or control scFv cDNA was examined by intravital video-microscopy following cremaster arteriole laser injury. Thrombi were imaged using an Olympus BX61WI microscope (Olympus, Center Valley, PA) with a 60 $\times$ /0.9 numeric aperture water immersion objective and captured using a Cooke SensiCam CCD camera (Cooke, Auburn Hills, MI) coupled to a Lambda DG4
 15 widefield excitation system (Sutter, Novato, CA). The microscope, camera, and DG4 were all controlled using Slidebook 5.0 software (Intelligent Imaging Innovations). F(ab')₂ fragments of rat anti-mouse CD41 IgG (BD Pharmingen, San Diego, CA) were F(ab')₂ conjugated to Alexa⁴⁸⁸ according to the manufacturer's instructions (Life Technologies). F(ab')₂ fragments were infused via jugular vein (0.1 mg/kg) immediately prior to first injury. Arterioles of 20–
 20 40 μ m were selected. Vascular injury was induced with a pulsed nitrogen dye laser (SRS NL100, Photonic Instruments, St. Charles, IL) focused on the vessel wall through the microscope objective. Analysis of time-lapse videos (750 frames per injury) was performed using Slidebook 5.0 (Intelligent Imaging Innovations, Denver, CO). After background fluorescence was subtracted from all images in one injury video, the resulting thrombus
 25 fluorescence was analyzed in the software to calculate an X/Y aspect ratio. A median filter was applied to each injury before taking the average of all injuries.

Shigatoxin challenge in scFv-expressing mice

CAST/Ei mice were injected with pLIVE expression vector containing scFv 1-
 30 420 anti-ADAMTS13 or control scFv cDNA and monitored for 10 days for ADAMTS13 activity, VWF multimer size, and scFv expression prior to Shigatoxin-2 challenge (Stx-2, Toxin Technology, Sarasota, FL). At day 10, Stx-2 (50 pg/g body weight) was injected via tail vein. Complete blood counts were performed just prior to and for up to 10 days following Stx-2 using a Hemavet M2950HV analyzer (Drew Scientific, Waterbury, CT). Blood smears

were stained with Wright stain and tissues were processed and stained with hematoxylin-eosin.

ADAMTS13 activity assays and VWF multimer analysis

Human and murine ADAMTS13 activities were measured in the presence or absence of various factors (recombinant scFv, TTP patient plasma IgG, rabbit anti-idiotypic IgG) using a commercial FRETs-VWF73 peptide (Peptide International, Louisville, KY). Components to be measured were mixed in a volume of 8 μ L as described in FIG. legends, added to 42 μ L of substrate buffer and 50 μ L of diluted FRETs-VWF73 reagent.

Fluorescence emission from cleavage of FRETs-VWF73 was measured using a Synergy 2 Multi-Mode Reader (BioTek, Winooski, VT) equipped with 340 nm excitation and 440 nm emission filters. vWF multimers were visualized as previously described (Laje, et al., Blood, 2009, 113:2172-2180; Niiya, et al., Mol. Ther., 2009, 17:34-41).

Adapting pMT/BiP/V5-His A plasmid vector for expression of pComb3X phagemid-derived scFv antibody clones

The multiple cloning site of the plasmid vector pMT/BiP/V5-His A was modified to incorporate Sfi I restriction sites to facilitate easy shuttling of scFv sequences from the pComb3X phage display vector. Modification of the pMT vector also required removal of an endogenous Sfi I site in the BiP secretion signal sequence. This was accomplished by digesting pMT/BiP/V5-His A with Sfi I and BstB I and ligating a double-stranded oligonucleotide formed by annealing single-stranded oligonucleotides “PMT FOR” (5’-TT GCC TTT GTT GGC CTC TCG CTC GGG AGA TCT GCG **GCC CAG GCG** **GCC** CCA TGG CCC GGG GTA CCT ACT AGT **GGC CAG GCC GGC** CAG TT-3’ SEQ ID NO: 193) and “PMT REV” (5’-C GAA CTG **GCC GGC CTG GCC** ACT AGT AGG TAC CCC GGG CCA TGG **GGC CGC CTG GGC CGC** AGA TCT CCC GAG CGA GAG GCC AAC AAA GGC AAC GA-3’ SEQ ID NO: 194), where the single-underlined bases on the 5’ forward strand alter the BiP Sfi I site, the double-underlined bases at the 3’ forward end complete a BstB I site, and the 2 sets of bases in bold provide the new Sfi I sites, 5’-GGCCNNNNNGGCC-3’ SEQ ID NO: 195, where N’s match those on either side of scFv sequences in the pComb3X vector. To shuttle a scFv, pComb3X DNA is digested with Sfi I restriction enzyme and ligated into Sfi I-cut modified pMT/BiP/V5-His A.

Adapting pLIVE in vivo expression vector for secretion of pComb3X phagemid-derived scFv antibody clones

The multiple cloning site of pLIVE plasmid vector was modified to contain an Ig-kappa leader sequence upstream from Sfi I restriction sites and a V5-tag sequence to facilitate secretion of V5-tagged scFv antibody fragments from murine liver following hydrodynamic delivery. pLIVE vector was first digested with Nhe I and Xho I restriction enzymes and a double-stranded oligonucleotide formed from the annealing of “V5 FOR” (5’-

CTAGCACTAGTGGCCAGGCCGGCCAGTTCTGAAGGTAAGCCTATCCCTAACCCCTC
TCCTCGGTCTCGATTCTACGCGTACCGGT*TAGC*-3’ SEQ ID NO: 196) and “V5 REV”

(5’-
TCGAGCTAACCGGTACGCGTAGAATCGAGACCGAGGAGAGGGTTAGGGATAGGC
TTACCTTCGAACTGGCCGGCCTGGCCACTAGTG-3’ SEQ ID NO: 197) was ligated in

to the cut and gel-purified vector to introduce the 3’ Sfi I restriction site (bold), the V5 tag sequence (underlined), and a stop codon (italics). The resultant plasmid was digested with

Nhe I and Sfi I and a second double-stranded oligonucleotide formed from the annealing of “LEAD FOR” (5’-

CTAGCATGGAGACAGACACACTCCTGCTATGGGTACTGCTGCTCTGGGTTCCAGG
TTCCACTGGTGACGGAGCTGCGGCCCAAGCGGCCCCCATGGCCCGGGGTACCTA
CTAGTGGCCAGGC-3’ SEQ ID NO: 198) and “LEAD REV” (5’-

TGGCCACTAGTAGGTACCCCGGGCCATGGGGCCGCCTGGGCCGCAGCTCCGTC
ACCAGTGGAACCTGGAACCCAGAGCAGCAGTACCCATAGCAGGAGTGTGTCTGT
CTCCATG-3’ SEQ ID NO: 199) was ligated in to the cut and gel-purified vector to introduce

the Ig kappa-chain leader sequence (underline) and 5’ Sfi I restriction site (bold). ScFv constructs were cloned into the modified pLIVE vector using Sfi I sites as above for the

modified pMT vector.

Tables

Table 1 lists patient demographics, clinical data, and number of anti-ADAMTS13 antibodies isolated.

5 Table 2 lists the genetic features and clonality of anti-ADAMTS13 heavy chains.

Table 3 lists the genetic features and clonality of anti-ADAMTS13 light chains.

Table 4 lists the percentage of ADAMTS13 activity in presence of scFv inhibitors: rabbit anti-idiotypic IgG.

Table 5 Lists ADAMTS13 domains bound by human recombinant scFv.

10

The results of the experiments are now described.

Cloning human anti-ADAMTS13 autoantibodies from TTP patients

Antibody phage display libraries expressing IgG₁₋₄ κ/λ isotypes of single chain
 15 variable region fragments (scFv) (i.e. heavy and light chain variable regions tethered together by a short peptide linker) were created from the splenic B-cells ("TTP1") or peripheral blood B cells ("TTP2", "TTP3" and "TTP4") of 4 unrelated patients with autoantibody-mediated TTP. Patients were diagnosed with acquired TTP on the basis of thrombocytopenia, microangiopathic hemolytic anemia, and <10% plasma ADAMTS13 activity in the setting of inhibitory
 20 immunoglobulin. Table 1 shows relevant demographic and clinical data. Peripheral blood for library construction was collected from TTP2-TTP4 just prior to their first plasma exchange and comprised $\sim 1 \times 10^6$ IgG-positive B-cells. Splenic tissue from TTP1 was obtained following splenectomy after a 42-week relapsing/remitting course and comprised $\sim 6 \times 10^9$ IgG-positive B-cells. Antibody libraries contained 4.6×10^8 , 3.6×10^8 , 7.4×10^8 , and 6.6×10^7 independent
 25 transformants, respectively, which represent complexities within (or higher) than the range considered ideal for libraries constructed from immune vs. non-immune sources (Rader et al., Phage Display: A Laboratory Manual. Cold Spring Harbor, NY: Cold Spring Harbor Press, 2001:10.1-20). Antibody libraries for each patient were each selected ("panned") against full-length human recombinant ADAMTS13. Library TTP1 was also panned against recombinant
 30 TSP1 5-8/CUB fragment of ADAMTS13 (Zhang et al., Blood 2007;110:1887-94). After four rounds of panning for each library, antigen-positive clones were identified by ADAMTS13

ELISA. Nucleotide sequences for the heavy and light chains of each were determined to identify the number of unique antibodies obtained from each patient. The last column of Table 1 tabulates the number of antibody clones sampled from each patient library, the number of positives, and, of these, the number of unique antibodies. In sum, a cohort of 51 unique human monoclonal anti-ADAMTS13 antibodies was assembled for further study. Nomenclature for antibody clones are in the form “X-Y” where “X” is TTP patient number and “Y” is an arbitrary number.

TABLE 1. Patient demographics, clinical data, and number of anti-ADAMTS13 antibodies isolated

Patient	Age/Sex/Race	Hgb (g/dL)	Plt ($\times 10^3$ /L)	LDH (U/L)	Cr (mg/dL)	No. TPE	Source of B cells	No. scFv sampled/ positive/unique
TTP1*	10y/M/W	8.7	9	8845	0.9	109	spleen [†]	75/67/31 [‡]
TTP2§	28y/F/W	9.6	7	2763	0.5	64	PB	32/22/14
TTP3§	47y/F/B	11.5	35	761	1.1	48	PB	16/12/4
TTP4#	59y/M/W	9	14	3427	1.5	13	PB	17/10/2

Patients were diagnosed with acquired TTP on the basis of thrombocytopenia, microangiopathic hemolytic anemia, and <10% plasma ADAMTS13 activity in the setting of inhibitory IgG.

M indicates male; F, female; W, White; B, Black; Hgb, hemoglobin concentration; Plt, platelet count; LDH, lactate dehydrogenase; Cr, creatinine; TPE, therapeutic plasma exchange procedure; PB, peripheral blood

* Patient's course of TTP relapsed/remitted over period of 42 weeks until splenectomy. No recurrence in 12 years of follow-up.

[†] Antibody library constructed from 15 g spleen comprising $\sim 6 \times 10^8$ IgG-positive B cells.

[‡] Of 31 unique positive clones, 28 were obtained from selection against full-length ADAMTS13 and 3 were obtained from selection against the TSP1 5-8/CUB fragment of ADAMTS13.

[§] Patient had history of well-controlled lupus since 18 years of age. Experienced miscarriage a few months before diagnosis of TTP. Clinical course complicated by multiple subarachnoid hemorrhages and grand mal seizures. Diagnosed with lymphoma 12 years post-TTP.

^{||} Antibody library constructed from $\sim 1 \times 10^6$ IgG-positive B cells isolated from 50 ml peripheral blood collected prior to first plasma exchange.

[¶] This episode of TTP was a relapse from initial diagnosis of TTP made 2 years earlier. Had splenectomy after this episode and no recurrence in over 10 years.

[#] Patient responded rapidly to TPE and has not had relapse in the past 8 years.

10 Structural analysis of anti-ADAMTS13 autoantibodies shows evidence of clonal expansion and somatic mutation

The nucleotide sequences of the 51 antibodies showed use of the human heavy chain variable region gene V_H1-69 for 75% of the anti-ADAMTS13 antibodies (Table 2), a bias reported previously (Pos et al., J Thromb Haemost 2009;7:421-8). However, 13 of the 51 antibodies were derived from diverse V_H3- and V_H6-family genes as well. That splenic tissue derived from TTP1 vs. peripheral blood lymphocytes from TTP2-TTP4 yielded the largest

number of unique antibodies with the greatest genetic diversity was not surprising given that the TTP1 library was constructed from >1000-fold more B-cells. Furthermore, the spleen may be a reservoir for long-lived memory B-cells producing ADAMTS13 autoantibodies given that splenectomy is associated with long-term remission in TTP patients (Kappers-Klunne et al., Br J Haematol 2005;130:768-76).

To rule out the possibility that the relatively high gene usage of V_H1-69 for anti-ADAMTS13 antibodies across all 4 patients was due to preselection factors (i.e. cloning artifacts during library construction), the diversity of each unpanned scFv patient library was assessed. By sequencing dozens of randomly-picked clones, it was determined that there was significant heterogeneity in V_H gene representation before selection similar to that typically found for IgG-secreting lymphocytes in the repertoire of adults.

Table 2 also shows the D and J_H gene segments that had rearranged with V_H genes to form the entire heavy chain variable regions (i.e., V_HDJ_H) and their complementarity determining region-3 (HC-CDR3), the region of greatest diversity in an antibody's heavy chain.

By exploiting the fact that there is only a remote probability that two B-cells will not only randomly select an identical combination of V_H, D, and J_H, but will also splice the genes together to create identical HC-CDR3 regions (theoretical probably <1 in 10¹¹), one can use an HC-CDR3 to identify a B-cell clonotype. This is indicated in Table 2 by each separate line. For example, the heavy chains of antibodies 1-416, 1-428 and 1-304 would be predicted to have each been derived from the same original parental B-cell within patient TTP1 because they each share the identical HC-CDR3 ("AMDSVYGNFDF"; SEQ ID NO: 200) though those 3 heavy chains are otherwise unique due to somatic mutation elsewhere in the heavy chain (FIG. 9A). Counting the number of different HC-CDR3 regions suggests that the 51 scFv heavy chains were derived from clonal expansion of 30 individual B-cells. Antibody light chain analysis showed use of both kappa and lambda, but light chains lack D segments which make it difficult to confidently assign discrete B-cell origins to every light chain that share similar CDR3's (Table 3). FIGS. 9A-9B provide sequence alignments of all 51 antibody heavy and light chains and indicates positions of replacement and silent mutations with respect to their most likely immunoglobulin germline genes.

Together, these data (Tables 2 and 3 and FIGS. 9A-9B) demonstrate that within each of the four TTP patients, the autoimmune response to ADAMTS13 was oligoclonal with

multiple B-cells expanding to produce groups of related antibodies that underwent further somatic mutation. The relatively high ratios of replacement-to-silent mutations in HC-CDR1 and HC-CDR2 (>4.7 , FIG. 9A) are characteristic of antigen-driven clonal expansion (Dorner et al., J Immunol 1998;160:2831-41) as it was previously found in the autoimmune repertoires in
5 idiopathic thrombocytopenic purpura (Roark et al., Blood 2002;100:1388-98) and pemphigus (Payne et al., J Clin Invest 2005;115:888-99), and alloimmune Rh(D) repertoire (Chang et al., Blood 1998;91:3066-78).

TABLE 2. Genetic features and clonality of anti-ADAMTS13 heavy chains

Patient	Antibodies grouped by heavy chain clonotype	V _H family	V _H gene	D gene	J _H gene	HC-CDR3
TTP1	1-416, 1-428, 1-304	1	1-69*09	D5-12*01	J4*02	AMDSVYGNFDF (SEQ ID NO: 200)
	1-431, 1-417, 1-303	1	1-69*09	D221*02	J4*02	ARDLGDFGDS (SEQ ID NO: 201)
	1-408	1	1-69*09	D1-20*01	J4*02	ARDSVIGTSD (SEQ ID NO: 202)
	1-406	1	1-69*09	D4-23*01	J4*02	ARDVGDFGDS (SEQ ID NO: 203)
	1-458†, 1-401†, 1-420	1	1-69*09	D1-26*01	J4*02	AREFSGGNYFDF (SEQ ID NO: 204)
	1-438, 1-434	1	1-69*09	D2-8*01	J6*02	ARFLWGLDV (SEQ ID NO: 206)
	1-432	1	1-69*09	D6-13*01	J3*01	ARGVAAGWNAFDV (SEQ ID NO: 206)
	1-405	1	1-69*09	D3-22*01	J4*02	ARSSYYSTFDY (SEQ ID NO: 207)
	1-450	1	1-69*09	D1-26*01	J6*02	ASGDYYDMAY (SEQ ID NO: 208)
	1-423	1	1-69*09	D3-16*01	J4*02	SIGRYTYGHFDT (SEQ ID NO: 209)
	1-418, 1-413	1	1-69*09	D6-19*01	J4*02	(TV)SNGWSNFDF (SEQ ID NO: 210)
	1-437	3	3-21*01	D3-3*01	J6*02	AAAYDFWSGYYF (SEQ ID NO: 211)
	1-404, 1-441	3	3-30*04	D2-21*01	J4*02	ARDLRGGEDY (SEQ ID NO: 212)
	1-403†, 1-415†	3	3-30*04	D3-3*01	J4*02	ARDTFSYYDFWRAFDY (SEQ ID NO: 213)
	z1-402	3	3-30*04	D2-2*01	J4*02	AASSYFPFDF (SEQ ID NO: 214)
	1-410, 1-407	3	3-43*01	D3-9*01	J4*02	AKDNGYDILTQYLD(S/Y) (SEQ ID NO: 215)
	1-440, 1-451, z1-201	3	3-9*01	D3-22*01	J4*02	AKDPNSLYRSGSFDY (SEQ ID NO: 216)
	z1-303	6	6-1*01	D6-19*01	J6*02	AREGQWLPNYFDP (SEQ ID NO: 217)
TTP2	2-204, 2-102	1	1-69*09	D2-8*01	J4*02	ARDKGYANNYGAY (SEQ ID NO: 218)
	2-207†, 2-301†, 2-304	1	1-69*09	D2-15*01	J4*02	ARDQGYANDYGAY (SEQ ID NO: 219)
	2-103, 2-106, 2-305†, 2-406†	1	1-69*09	D2-8*01	J4*02	ARDQGYANNYGAY (SEQ ID NO: 220)
	2-302	1	1-69*09	D6-6*01	J4*02	ARDQVFGAY (SEQ ID NO: 221)
	2-203	1	1-69*09	D3-16*01	J4*02	ARDRGYANTYGAY (SEQ ID NO: 222)
	2-206	1	1-69*09	D3-16*01	J4*02	ARDRGYDNKYGAY (SEQ ID NO: 223)
	2-408	1	1-69*09	D2-8*01	J4*02	ARDRGYSNNYGAY (SEQ ID NO: 224)
	2-108	3	3-7*01	D1-14*01	J4*02	ARSPGYFFDY (SEQ ID NO: 225)
TTP3	3-305†, 3-405†, 3-302†	1	1-69*01	D1-26*01	J3*01	AREARDSFDF (SEQ ID NO: 226)
	3-301	1	1-69*10	D2-8*02	J4*02	ARDDTGRDDYFEY (SEQ ID NO: 227)
TTP4	4-307	1	1-69*01	D5-12*01	J4*02	ARSGYSDAFDI (SEQ ID NO: 228)
	4-303	1	1-69*09	D1-26*01	J4*02	ARGGGSYDFFDY (SEQ ID NO: 229)

All antibodies were obtained by selecting phage display antibody libraries against full-length ADAMTS13 except those with names beginning with “z” which were isolated by selecting the TTP1 library against the TSP1 5-8/CUB fragment of ADAMTS13.

5

HC-CDR3 indicates heavy chain complementarity determining region 3.

† Members of a clonotype identical at the heavy chain amino acid level but comprise unique antibodies due to mutations in the associated light chains (see Table 5 and FIG. 9B).

Relationship of ADAMTS13 inhibitory autoantibodies to genetic background and epitope specificity

Inhibitory activities of anti-ADAMTS13 antibodies varied from 0% to ~100% residual ADAMTS13 activity (FIG. 1A). For reference, the germline V_H gene from which the particular recombinant scFv was derived is shown above each bar. With only two exceptions (1-437 and 1-404), antibodies that significantly inhibited ADAMTS13 were encoded by V_H1-69 ($P = 1.4 \times 10^{-6}$). In contrast, anti-keratinocyte PX4-3 scFv is also encoded by V_H1-69 (FIG. 1B) and had no effect on ADAMTS13 activity even when incubated with ADAMTS13 at a 5-fold greater concentration, indicating inhibitory activity is not conferred simply by the use of this heavy chain gene.

Epitope mapping was performed with a subset of 23 scFv's with different genetic backgrounds and inhibitory activities in order to investigate whether the ability of an anti-ADAMTS13 antibody to inhibit ADAMTS13 proteolytic activity *in vitro* is related to where it binds the enzyme. FIG. 2 summarizes the results (raw data in FIGS. 10A-10B and Table 5 below) and illustrates a diversity in epitope specificities similar to that found in patient plasma (Soejima et al., Blood 2003;102:3232-7; Pos et al., Haematologica 2011;96:1670-7).

With only one clear exception (1-437), antibodies that inhibit ADAMTS13 proteolytic activity *in vitro* require the cysteine-rich/spacer region for binding. This finding is consistent with previous studies suggesting that antibodies that bind to the cysteine-rich/spacer region interfere with engagement of ADAMTS13 with VWF substrate (Soejima et al., Blood 2003;102:3232-7; Akiyama et al., Proc Natl Acad Sci U S A 2009;106:19274-9). The fact that all 13 V_H1-69-encoded scFv's in this group require this region for binding is consistent with other reports (Luken et al., J Thromb Haemost 2006;4:2355-64; Pos et al., J Thromb Haemost 2009;7:421-8; Schaller et al., Blood 2014;124:3469-79) and suggests that there is a feature expressed by V_H1-69 (independent of HC-CDR3 that is encoded primarily by the D gene, not V_H) that is either permissive or required (but not sufficient vis-a-vis anti-keratinocyte PX4-3 above) for an antibody to recognize features presented by immunodominant residues in cysteine-rich/spacer region-containing domains of ADAMTS13. For scFv's 1-420, 1-416, and 3-301, these results are also consistent with those of a separate study using hydrogen-deuterium

exchange mass spectrometry in which their specificity for the ADAMTS13 spacer region was shown at near amino acid resolution (Casina et al., Proc Natl Acad Sci U S A 2015;112:9620-5).

Of the non-V_H1-69 inhibitory antibodies, 1-437 maps to a fragment containing the metalloprotease domain (potentially explaining its inhibitory activity), and the idiotope of 1-404
5 appears to make contact independently with both cysteine-rich/spacer-containing and CUB domains, perhaps stabilizing ADAMTS13 in a "closed" inactive conformation (South et al., Proc Natl Acad Sci U S A 2014;111:18578-83). ScFv 1-410 also binds to both cysteine-rich/spacer-containing domains and TSP 5-8/CUB domains but is not inhibitory. The 7 remaining scFv's target the C-terminal domains and do not inhibit enzymatic activity *in vitro*. It
10 should be recognized, however, that ADAMTS13 activity assayed by measuring the cleavage of VWF peptides vs. VWF multimers may miss pathogenic effects of certain antibodies including those that target the C-terminal domains of ADAMTS13.

Table 5. ADAMTS13 domains bound by human recombinant scFv

scFv	FL-A13	delCUB	MDTCS	MDT	T2-8	T5-8 + CUB	CUB
E1M2	-	-	-	-	-	-	-
1-437	+	+	+	+	-	-	-
1-303	+	+	+	-	-	-	-
1-304	+	+	+	-	-	-	-
1-416	+	+	+	-	-	-	-
1-428	+	+	+	-	-	-	-
1-431	+	+	+	-	-	-	-
1-420	+	+	+	-	-	-	-
1-434	+	+	+	-	-	-	-
3-301	+	+	+	-	-	-	-
2-102	+	+	+	-	-	-	-
2-103	+	+	+	-	-	-	-
1-438	+	+	+	-	-	-	-
3-302	+	+	+	-	-	-	-
3-405	+	+	+	-	-	-	-
1-441	+	-	-	-	-	+	+
1-403	+	-	-	-	-	+	+
z1-402	+	-	-	-	-	+	+
1-440	+	-	-	-	-	+	+
z1-201	+	-	-	-	-	+	+
1-407	+	-	-	-	-	+	+
z1-303	+	-	-	-	-	+	-
1-404	+	+	+	-	-	+	W
1-410	+	W	W	-	-	+	-

Plus signs denote positive reactivity toward the respective ADAMTS13 construct; negative signs, no signal detected; w, weak signal detected.

Abbreviations for ADAMTS13 constructs illustrated and defined herein (FIGS. 10A-10B).

5 E1M2 is negative control scFv specific for human red cell Rh(D) antigen.

ADAMTS13 autoantibodies share cross-reactive idiotypes

10 The observation that 13 of the 15 inhibitory scFv's in the present subset of antibodies were bound to identical ADAMTS13 regions and were encoded by the same V_H gene suggested that their idiotypes (the areas of their variable regions that make contact with ADAMTS13) shared common structural features. If inhibitory anti-ADAMTS13 antibodies did share idiotypes within and across patients, there would be rationale for developing therapies that recognize these common features to block antibody binding or attenuate their production.

However, in general, the most important contributing factors to the structure of an antibody's idio-
type are its heavy and light chain CDR3 loops which, for these antibodies, appeared to be
quite varied in length and amino acid sequence (Table 2 above and Table 3 below, FIGS. 9A-
9B). This would suggest that their idiotypes were quite different.

5 To explore idiotypic diversity within a set of ADAMTS13 autoantibodies, rabbit
antisera were raised to V_H1-69-encoded 1-416, 1-420, 1-428, and 1-431. Antibodies 1-416 and 1-
428 shared the same heavy chain CDR3 while the heavy chain CDR3's of antibodies 1-420 and
1-431 were each distinct (Table 2) as were the light chain CDR3's in each of the 4 antibodies
(Table 3).

10

Table 3. Genetic features and clonality of anti-ADAMTS13 light chains

Patient	Antibodies grouped by similar LC-CDR3	Isotype	V _L family	V _L gene	J _L gene	LC-CDR3
TTP1	1-407, 1-410	kappa	κ1	KV1-33*01	KJ4*01	QQY(A/D)NLPLT (SEQ ID NO: 230)
	1-403	kappa	κ1	KV1-39*01	KJ4*01	QQSHNVPLT (SEQ ID NO: 231)
	1-440, 1-451, z1-201	kappa	κ1	KV1-39*01	KJ3*01	QQSYSTP(F/Y)T (SEQ ID NO: 232)
	z1-402	kappa	κ2	KV2-28*01	KJ2*01	MQALQTPQT (SEQ ID NO: 233)
	1-404, 1-441	lambda	λ1	LV1-51*01	LJ2*01	GTWDSLSAVV (SEQ ID NO: 234)
	1-415	lambda	λ2	LV2-14*01	LJ2*01	SSYTSSTTVV (SEQ ID NO: 235)
	1-437	lambda	λ2	LV2-14*01	LJ1*01	SSYTSSTPYV (SEQ ID NO: 236)
	1-458	lambda	λ3	LV3-21*01	LJ2*01	QVWDRSSDHVV (SEQ ID NO: 237)
	1-432	lambda	λ3	LV3-21*02	LJ3*02	QVWDSRSDHQV (SEQ ID NO: 238)
	1-438, 1-434	lambda	λ3	LV3-21*02	LJ2*01	QVWDSSTDH(K/V)V (SEQ ID NO: 239)
	z1-303	lambda	λ3	LV3-21*02	LJ2*01	QVWDSRSDHVV (SEQ ID NO: 240)
	1-423	lambda	λ3	LV3-21*03	LJ3*02	QVWDSNSDHQV (SEQ ID NO: 241)
	1-401	lambda	λ3	LV3-21*03	LJ2*01	QVWDSNDHSV (SEQ ID NO: 242)
	1-417	lambda	λ3	LV3-21*03	LJ7*01	QVWDSNDQVV (SEQ ID NO: 243)
	1-303	lambda	λ3	LV3-21*03	LJ1*01	QVWDSSDHYV (SEQ ID NO: 244)
	1-420	lambda	λ3	LV3-21*03	LJ2*01	QVWDSSTDYVV (SEQ ID NO: 245)
	1-406	lambda	λ3	LV3-25*01	LJ2*01	QSADSNQWVL (SEQ ID NO: 246)
	1-405	lambda	λ3	LV3-25*01	LJ2*01	QSEDSSGIL (SEQ ID NO: 247)
	1-416	lambda	λ3	LV3-25*02	LJ1*01	QSTDSSDTWV (SEQ ID NO: 248)
	1-431	lambda	λ3	LV3-25*03	LJ3*02	QSADSSGTYV (SEQ ID NO: 249)
	1-450	lambda	λ3	LV3-25*03	LJ2*01	QSAYSSGTVV (SEQ ID NO: 250)
	1-304	lambda	λ3	LV3-25*03	LJ3*02	QSSDINGTSWI (SEQ ID NO: 251)
	1-418, 1-413	lambda	λ3	LV3-25*03	LJ3*02	QSVDSNDTSWI (SEQ ID NO: 252)
	1-408	lambda	λ3	LV3-25*03	LJ3*02	QSADSSGTSWV (SEQ ID NO: 253)
	1-428	lambda	λ3	LV3-25*03	LJ2*01	QSADSSGTYEV (SEQ ID NO: 254)
TTP2	2-108	lambda	λ1	LV1-47*01	LJ1*01	AAWDDSLRVYV (SEQ ID NO: 255)
	2-102	lambda	λ3	LV3-21*02	LJ3*02	QVWDSRSDYVV (SEQ ID NO: 256)
	2-103	lambda	λ3	LV3-21*02	LJ3*02	QVWDSSTDHWV (SEQ ID NO: 257)
	2-106	lambda	λ3	LV3-21*02	LJ3*02	QVWDSSTDHWV (SEQ ID NO: 257)
	2-406	lambda	λ3	LV3-21*02	LJ3*02	QVWESSDHWV (SEQ ID NO: 258)
	2-204	lambda	λ3	LV3-21*02	LJ1*01	QVWESTTDHYV (SEQ ID NO: 259)
	2-306	lambda	λ3	LV3-21*02	LJ3*02	QVWDSNSDHWV (SEQ ID NO: 260)
	2-301	lambda	λ3	LV3-21*02	LJ3*02	QVWHSSSDHWV (SEQ ID NO: 261)
	2-207	lambda	λ3	LV3-21*02	LJ3*02	QVWDSSTDHWV (SEQ ID NO: 257)
	2-304	lambda	λ3	LV3-21*02	LJ3*02	QVWDSSTDHWV (SEQ ID NO: 257)
	2-203	lambda	λ3	LV3-21*02	LJ3*02	QVWDSSTDHWV (SEQ ID NO: 257)
	2-206	lambda	λ3	LV3-21*02	LJ3*02	QVWDSSTDHWV (SEQ ID NO: 257)
	2-302	lambda	λ3	LV3-21*02	LJ3*02	QVWESSDHWV (SEQ ID NO: 258)
	2-408	lambda	λ3	LV3-21*02	LJ2*01	QVWDSSTDHV (SEQ ID NO: 262)
TTP3	3-302	lambda	λ3	LV3-1*01	LJ2*01	QVWESSDHLV (SEQ ID NO: 263)
	3-405	lambda	λ3	LV3-21*01	LJ2*01	QVWDSSTDQRV (SEQ ID NO: 264)
	3-305	lambda	λ3	LV3-21*03	LJ3*02	QVWDSSTDHWV (SEQ ID NO: 257)
	3-301	lambda	λ3	LV3-25*03	LJ3*02	QSADSSNGTYKV (SEQ ID NO: 265)
TTP4	4-303	lambda	λ3	LV3-21*01	LJ2*01	EVWDSLTDRVV (SEQ ID NO: 266)
	4-307	lambda	λ3	LV3-21*01	LJ2*01	QVWDSSTDQGV (SEQ ID NO: 267)

Legend for Table 3:

Antibody nomenclature same as in Table 2.

LC-CDR3 indicates light chain complementarity determining region 3

Each line indicates one or more light chains with the identical LC-CDR3 and VL and JL

5 consistent with those light chains being clonally related. Antibodies listed on separate lines that have identical LC-CDR3 have patterns of somatic mutation in their VL and/or JL that suggests different clonal origins (See FIG. 9B).

Binding of post-immune rabbit IgG to its scFv immunogen was ~100-fold greater
 10 than pre-immune IgG by ELISA. Post-immune, but not pre-immune, IgG blocked its respective scFv's ability to inhibit ADAMTS13 as illustrated for 1-416 (FIG. 3A). Similar patterns of reactions were found for rabbit IgG raised against 1-420, 1-428, and 1-431. The ability to block scFv-mediated inhibition of ADAMTS13 was not simply due to rabbit IgG molecules directed to human isotype (e.g., Fc γ) because scFv have no isotype (they comprise only V_H and V_L chains).
 15 The ability to block scFv-mediated inhibition could not be due to rabbit IgG being directed to conserved human V_H/V_L framework regions or even to conserved V_H1-69-specific structural elements because the ability of rabbit IgG raised against 1-428 to block 1-428's inhibition of ADAMTS13 was unaffected by the presence of 8-fold excess human V_H3-33-encoded E1M2 (an anti-Rh(D) scFv) or human V_H1-69-encoded PX4-3 (an anti-keratinocyte scFv, Fig. 3B). In the
 20 present investigation, the amount of rabbit IgG was titrated down to the point of just being able to block 1-428 in order to increase its sensitivity to any effects of E1M2 or PX4-3.

To explore whether the idiotypes among the four scFv's share common features, anti-idiotypic IgG raised against a given scFv was tested for its ability to block the inhibition of ADAMTS13 by the other 3 scFv's. To increase the sensitivity of these assays, the amounts of
 25 scFv used were 2.5-fold less than the amounts used in FIGS. 1A-1B. As shown in Table 4, there was evidence of broad cross reactivity among 3 of the 4 scFv. Marked inhibition of ADAMTS13 by 1-420 could not be blocked by anti-idiotypic IgG to any of the other 3 scFv's nor could anti-idiotypic IgG raised against 1-420 block the inhibition of ADAMTS13 by the other 3 scFv's. This finding served as a convenient internal control showing that the rabbit IgG were not acting
 30 by binding to common human or V_H1-69 structures. This finding also suggested that for

inhibitory antibodies directed to cysteine-rich/spacer region-containing domains, V_H1-69 features alone did not define the antibody's idiotype.

These data suggest that with one exception, a small set of ADAMTS13 inhibitory monoclonal antibodies derived from a single TTP patient share idiotypic determinants that can be targeted to prevent inhibition of ADAMTS13. The larger question is how representative these

TABLE 4. Blocking of scFv-induced ADAMTS13 inhibition by rabbit anti-idiotypic IgG

Rabbit IgG added	% ADAMTS13 activity in presence of scFv inhibitor			
	scFv 1-416	scFv 1-420	scFv 1-428	scFv 1-431
none	20*	0	7*	19*
anti-scFv 1-416	93	0	84	13
anti-scFv 1-420	0	99	0	0
anti-scFv 1-428	75	8	100	72
anti-scFv 1-431	70	3	94	92

* Residual ADAMTS13 activities with scFv's alone are higher than in FIGS. 1A-1B because a 2.5-fold lower amount of scFv was used in order to increase sensitivity of blocking scFv by rabbit IgG

To address this question, inhibition of ADAMTS13 by plasma from TTP4 and three additional TTP patients (TTP5-TTP7) was measured in the presence of anti-idiotypic IgG. As shown in FIG. 3C, inhibition of ADAMTS13 by polyclonal patient plasma-derived immunoglobulin was blocked to varying extents by anti-idiotypic IgG generated to a single monoclonal scFv from a completely unrelated TTP patient. The ability to block ADAMTS13 inhibition was striking in some cases (e.g., TTP7) with each of the anti-idiotypic IgG. These results could not be attributed to rabbit antibodies to patient IgG Fc domains because the rabbits were immunized with scFv. That rabbit antisera might have pre-existing reactivity to human IgG was ruled out through the use of preimmune sera. Therefore, these data provide support for the clinical relevance of these cloned scFv's and suggest their use as targets for the design of small molecules that could block enough ADAMTS13 inhibitory IgG to raise ADAMTS13 activity above a clinical threshold.

IP injections of human anti-ADAMTS13 scFv cross react and inhibit murine ADAMTS13 *in vivo*

Data presented herein describes a set of human monoclonal anti-ADAMTS13 single chain antibody fragments (scFv's) cloned from four unrelated TTP patients that displayed characteristics *in vitro* one would expect from disease-related pathogenic antibodies, e.g., ability to inhibit ADAMTS13 enzymatic activity, epitope specificities shared with patient plasma IgG, etc. To test the clinical relevance of these recombinant antibodies and to provide an *in vivo* system for studying the pathophysiology of TTP, these *in vitro* findings were extended to the development of a murine model of acquired TTP.

First, human scFv antibody fragments were screened for their ability to inhibit the activity of murine plasma ADAMTS13 *in vitro*, and the majority of scFv's that inhibited human ADAMTS13 activity were found to also inhibit murine ADAMTS13. Of the human scFv's that cross reacted with murine ADAMTS13, antibody clones 1-416, 1-420, 1-428, and 1-431 were chosen to pursue because characterization of these antibodies *in vitro* showed potent inhibition of ADAMTS13 activity, binding to human ADAMTS13 epitopes most commonly targeted by patient plasma IgG, and idiotopes shared by patient plasma IgG.

Dose response inhibition curves with mouse plasma revealed 1-420 to be most potent (FIG. 4A) so it was chosen for evaluation *in vivo*. Mice were given intraperitoneal injections of 30 µg 1-420 and mouse plasma showed rapid inhibition of ADAMTS13 resulting in <10% pre-injection activity within ~2 hours (FIG. 4B) which persisted for ~24 hours (FIG. 4B, inset).

Transfection of mouse liver with scFv cDNA leads to prolonged ADAMTS13 deficiency

Next, a sustained antibody-mediated ADAMTS13 deficiency was created in order to simulate TTP disease and observe the effect of such deficiency on thrombus formation in the settings of focal and systemic vascular injury. To accomplish this, the cDNA for scFv 1-420 was cloned into the pLIVE plasmid vector, a vector designed for the hydrodynamic gene transfer of naked DNA. The vector was modified to facilitate the insertion of phage display-derived scFv antibody fragment cDNA's upstream of a liver-specific promoter composed of the minimal mouse albumin promoter and the mouse α -fetoprotein enhancer (FIG. 11). Tail vein injection of a 2-mL solution of pLIVE plasmid over 4 - 7 seconds delivers the scFv cDNA to mouse liver by

hydrostatic force. Both control scFv cDNA and 1-420-containing plasmids initially led to a drop in ADAMTS13 activity *in vivo* for several days that was then followed by recovery of ADAMTS13 in the control group after the expected physiological effects of rapid hydrodynamic injection of a one blood volume-equivalent through the portal circulation to the liver resolved.

- 5 Inhibition of ADAMTS13 activity by *in vivo*-expressed 1-420 persisted for over 2 weeks (FIG. 5A) and for as long as 3 months.

That the recombinant human antibodies were expressed in mouse plasma was confirmed by immunoprecipitation of scFv 7 days after scFv DNA vector injection (FIG. 5B). To estimate plasma levels of scFv *in vivo*, an aliquot of normal mouse plasma was spiked with 20 ng
10 of recombinant 1-420, and immunoprecipitation was performed in parallel. Quantification using the spiked sample as a reference led to estimates of 0.4 - 0.8 µg/ml mouse plasma for scFv's from 3 control mice and 0.4 - 10 µg/ml mouse plasma for 1-420 from 3 experimental mice. If the concentration of mouse plasma ADAMTS13 was similar to that in humans (~1 µg/ml), the stoichiometric ratio of scFv to ADAMTS13 was ~3 to ~5 except for one of the 1-420 mice in
15 which it was ~10-fold higher. Excess unbound plasma scFv was affirmed by mixing plasma derived from cDNA-transfected mice 1:1 with normal human plasma, analogous to the "inhibitor assay" used clinically to diagnose patients with antibody-mediated ADAMTS13 deficiency (FIG. 5C).

20 **Laser-induced vascular injury in scFv-transfected mice leads to thrombus elongation**

The data described herein demonstrate that DNA transfection of human anti-ADAMTS13 antibodies using hydrodynamic delivery leads to rapid and stable ADAMTS13 inhibition *in vivo*. Next the effects of ADAMTS13 inhibition on the temporal and spatial aspects of platelet thrombus formation was examined in these mice using the cremaster arteriole laser
25 injury model (FIG. 6A). Thrombi in control mice enlarged spherically around the ~1 µm injury site to a diameter of ~30 µm and then remained relatively constant in size during the 3-minute observation period due to frequent embolization. In clear contrast, thrombi in ADAMTS13-inhibited mice grew in an asymmetrically elongating fashion to a length >80 µm beyond the injury site ($P < 0.0001$) consistent with the presence of strings of unprocessed UL-VWF
30 multimers, although a contribution from adhesion of plasma VWF multimers to the damaged vasculature could not be excluded. Length/width aspect ratio measurements of thrombi

quantified these differences (FIG. 6B). Agarose gel electrophoresis of plasma confirmed the presence of UL-VWF multimers in mice expressing 1-420 (FIG. 6B, inset) relating the findings to underlying mechanism.

5

Shigatoxin challenge induces the TTP phenotype in scFv-transfected mice

Though pathogenesis of TTP has been linked to ADAMTS13 deficiency, the natural history of the disease has suggested that additional genetic or environmental factors were required for the onset of disease. In murine models of congenital TTP that a state of ADAMTS13
10 deficiency was induced genetically, the administration of the bacterial agent Shigatoxin-2 (Stx-2) was found to precipitate disease phenotype presumably due to endothelial injury. To further test the clinical relevance of the cloned human scFv, it was determined whether “TTP” could be induced in mice expressing human anti-ADAMTS13 inhibitory antibodies following challenge with Stx-2. CAST/Ei strain mice, which have 5 times more circulating VWF and are sensitive to
15 the development of TTP in ADAMTS13 knock-out mice, were injected hydrodynamically with scFv 1-420 or scFv X24-3 control antibody plasmid, challenged with a sublethal dose of Stx-2 10 days later after full recovery from the side effects of hydrodynamic challenge, and followed for an additional 10 days. As shown in FIG. 7A, 6 of 10 control mice survived and maintained platelet counts in the normal range throughout the 10-day post-Stx-2 period. However, all mice
20 (10/10) rendered ADAMTS13 deficient by scFv 1-420 died by day 4 post-Stx-2 (half within 24-48 hours of injection, FIG. 7B) and each suffered a fall in platelet count to less than one-third its starting value. Schistocytes were seen on peripheral blood smear and thrombi were readily detected in brain, heart and kidney (FIG. 7C, right panel), but not in control animals (FIG. 7C, left panel) that had died from Stx-2 challenge alone. These experiments demonstrated that the *in vivo* expression of the variable region of a single human anti-ADAMTS13 inhibitory antibody
25 fragment in mice sufficed to mediate the most salient features of TTP in an animal model.

Acquired TTP is a potentially fatal disease with a mortality that has remained relatively constant over the past 25 years despite improvements in diagnosis, early initiation of
30 therapy, and the use of adjunctive immunosuppressive agents when plasma exchange alone is not entirely effective. An understanding of the repertoire of ADAMTS13 autoantibodies on a

molecular level is a prerequisite to the development of innovative, targeted therapies and to the design of animal models to evaluate such approaches.

By analogy to acquired TTP, pemphigus vulgaris is a potentially fatal blistering skin disease caused by autoantibodies to the keratinocyte adhesion protein desmoglein. Current therapies are non-specific and limited to systemic immunosuppression. Previous studies conducted in the laboratory using an antibody phage display cloning approach similar to that used in the study described herein were successful in defining the genetic origins of pathogenic and non-pathogenic desmoglein autoantibodies and their autoepitopes. This information has recently facilitated the engineering of T lymphocytes expressing novel chimeric autoantibody/T cell receptors that specifically kill anti-desmoglein antibody producing cells and lead to prolonged survival in a mouse model of pemphigus vulgaris. Critical to the success of these studies was the ability to clone repertoires of desmoglein autoantibodies that were representative of the diversity of epitope specificities contained within patient plasma, and the ability to assess the clinical relevance of the cloned antibodies in an animal model of the disease. To apply this approach for destruction of anti-ADAMTS13 producing B cells, or for the development of other targeted therapeutic approaches for acquired TTP, such as autoantibody blocking with idiotope-directed agents or the use of ADAMTS13 preparations engineered to lack such idiotopes, an animal model utilizing human autoantibodies recognizing human autoepitopes would be required. To date, animal models of the disease have been limited to the use of xenoantibodies to human ADAMTS13 made in rabbits or mice to simulate disease pathophysiology in mice or baboons, respectively.

Though the plasma of patients with acquired TTP contains IgG to multiple domains of ADAMTS13, it is only those directed at the spacer domain that human monoclonal antibodies have been described so far (Luken, et al., J. Thromb. Haemost., 2006, 4:2355-2364, Pos, et al., J. Thromb. Haemost., 2009, 7:421-428, and Schaller, et al., Blood 2014, 124:3469-3479). It is unknown whether anti-ADAMTS13 autoantibodies directed to epitopes other than those expressed within the spacer domain are inhibitory or may in some other way contribute to disease pathogenesis. Furthermore, no animal models for assessing the clinical relevance of cloned human ADAMTS13 autoantibodies have been described.

In the current study, 51 unique human ADAMTS13 autoantibodies from 4 unrelated TTP patients with respect to their genetic origins, clonality, and ADAMTS13

inhibitory activity were cloned and characterized. Although 75% of the antibodies used the V_H1-69 heavy chain and bound to epitopes in the Cys-rich/spacer domain, antibodies encoded by 6 other V_H genes were also represented in the group and included specificities for each of the domains targeted by IgG in a large cohort of patient plasmas (Table 2, FIG. 3). With respect to antibody light chains, the cohort of antibodies described herein was encoded by a large diversity of light chain germline genes with the use of lambda light chain gene segments predominating (Table 2). The relatively high prevalence of lambda light chain usage was also seen in both antibody phage display technology and single B cell cloning in 2 TTP patients (Schaller, et al., Blood, 2014, 28:3469-3479). Kappa light chains dominated anti-ADAMTS13 antibodies in a set of 9 antibodies described previously using phage display (Luken, et al., J. Thromb. Haemost., 2006, 4:2355-2364; Pos, et al., J. Thromb. Haemost., 2009, 7:421-428), but the light chains in those antibodies were derived from a normal healthy donor so the relationship of the associated light chains to those present in the TTP patients in these studies was not clear.

Analysis of the heavy chain CDR3 regions within the cohort of antibodies described herein indicates that they were derived from the clonal expansion of 30 individual B cells across the 4 TTP patients (Table 2, FIGS. 9A and 9B). This relatively large number of clonally-unrelated anti-ADAMTS13 antibodies may explain the diversity in epitope specificities that was found (FIG. 3) when compared to antibodies characterized in previous studies derived from 6 (Pos, et al., J. Thromb. Haemost., 2009, 7:421-428) and 12 (Schaller, et al., Blood 2014, 124:3469-3479) B cell clonotypes where all but one antibody were identified as being specific to the spacer domain. Analysis of the pattern of somatic mutation in nearly half of the 30 B cell clonotypes showed replacement-to-silent mutation ratios greater than 4.7 in heavy chain CDR1 and CDR2 regions (FIG. 9A) that is characteristic of antigen-driven clonal expansion in the setting of autoimmune disease. These observations along with predominant use of a particular heavy chain germline gene (V_H1-69) were analogous to those found previously for human alloantibodies to the red cell Rh(D) antigen (V_H3-33 gene), for human autoantibodies against platelets in ITP (V_H3-30 gene), and to human autoantibodies against desmoglein that cause pemphigus vulgaris (V_H1-46 gene).

Initial observations identifying the use of the V_H1-69 heavy chain gene for spacer domain-specific inhibitory antibodies cloned from TTP patients led investigators to hypothesize a “shape complementarity” between V_H1-69-encoded variable domain residues and exposed

exosites in the spacer domain. It was noted that the heavy chain CDR2 of the V_H1-69 germline gene contained a unique hydrophobic “Ile-Ile-Pro-Ile-Phe” motif that might facilitate interaction with hydrophobic residues present on the antigenic surface of the spacer domain, including Tyr661 and Tyr665 (Akiyama, et al., PNAS, 2009, 106:19274-19279). More recently, four
5 additional V_H1-69-encoded spacer domain-directed patient antibodies were reported that also have an “Ile-Ile-Pro-Ile-Phe” in their CDR2 (Schaller et al., Blood 2014;124:3469-79).

Alignment of these seven previously-reported V_H1-69-encoded antibodies bearing this CDR2 motif with the 38 V_H1-69-encoded antibodies reported herein revealed some variability in amino acid residues occupying these CDR2 positions, though much of the variability was conservative
10 (FIG. 8). The variability was, in part, because only certain alleles of the V_H1-69 gene (e.g., *I-69*01*) encoded the “Ile-Ile-Pro-Ile-Phe” motif that was described initially. Of note, 37 of the 38 V_H1-69-encoded antibodies were derived from *I-69*09* or *I-69*10* alleles that have a leucine at position 62. Overall, all antibodies retained a proline at position 58, and two of the previously-reported antibodies, II-1 and 3b have substituted a tyrosine for the phenylalanine at position 62.

Also shown in FIG. 8, is the CDR2 region of PX4-3, a V_H1-69-encoded anti-keratinocyte autoantibody that did not bind to or inhibit ADAMTS13 (FIGS. 1A-1B) or prevent anti-idiotypic IgG from neutralizing ADAMTS13 inhibitory scFv (FIG. 3B). Unless amino acid residues isoleucine, valine, or methionine at position 59 could not be replaced by threonine as in PX4-3, the present results suggest that the hydrophobic CDR2 motifs of V_H1-69-encoded heavy
20 chains could not be solely responsible for ADAMTS13 binding. The present findings that anti-idiotypic IgG cross react and block ADAMTS13 inhibition mediated by 1-416, 1-428, and 1-431, but not 1-420 (Table 4), when considered in the context of their CDR2 residues (FIG. 8), also suggest that the idiotopes of spacer domain-directed V_H1-69-encoded inhibitory antibodies comprise more than just their V_H CDR2 regions.

25 The collection of anti-ADAMTS13 autoantibody clones described herein closely mimics the diversity of ADAMTS13 binding domains found in the plasma of patients with acquired TTP. The idiotypic relatedness of the present set of inhibitory antibodies to patient IgG supported their clinical relevance and might serve as useful targets for the design of therapeutic agents that block IgG binding.

30 Given the observation that viral infections often precede initial or recurrent episodes of TTP, it has also been suggested that preferential use of V_H1-69 to encode

ADAMTS13 autoantibodies may have resulted from the presence of pre-existing, cross-reactive antibodies to the hemagglutinin (HA) ectodomain of influenza A virus, which also preferentially use the V_H1-69 germline gene. Twelve of the 38 V_H1-69-encoded antibodies (1-416, 1-420, 1-428, 1-431, 1-303, 1-438, 1-434, 2-102, 2-103, 3-301, 3-302 and 3-405) did not bind to the hemagglutinin ectodomain of four strains of influenza virus (H1/PR/8/34, H3/Perth/16/2009, H3/Perth/1609, and H5/Vietnam/1203/2004) by either immunoassay or flow cytometry. These results, however, do not rule out the possibility that infection with influenza activated a pool of naïve B-cells that underwent somatic mutation and divergence into distinct populations of HA-binding and ADAMTS13-binding clones. It may be possible to test this hypothesis by panning a TTP patient antibody libraries for HA binders and comparing HC-CDR3 domains with ADAMTS13 antibodies for identical V_H-D-J_H rearrangements within the same patient.

Described in this invention are the first examples of human antibodies specific for ADAMTS13 amino-terminal (MDT1) and carboxy-terminal (T5-8/CUB) domains, and their apparent diversity in V_H gene usage (Table 2) contrasts significantly to the marked V_H1-69 restriction of antibodies targeting ADAMTS13 domains containing the cysteine-rich/spacer region. Antibodies directed toward these amino- and carboxy-terminal domains are known to be present in TTP patient plasma and correlate with platelet count at disease onset (Zheng et al., *Haematologica* 2010;95:1555-62), but their ability to inhibit ADAMTS13 proteolytic activity has not been demonstrated. In the present invention, MDT1-binding 1-437 was found to be a potent inhibitor as any cysteine-rich/spacer region-directed antibody, perhaps by interfering with catalysis mediated by the metalloprotease domain. The present antibody cohort includes 6 CUB-specific antibodies and one TSP 2-8/CUB-specific antibody, none of which inhibit ADAMTS13 activity as assessed by cleavage of VWF peptide. However, 1-404 is an inhibitory antibody and independently binds to cysteine-rich/spacer-containing fragments and CUB regions, suggesting that its epitope comprises amino acid residues located in both regions. In light of recent reports proposing that ADAMTS13 normally circulates in a “closed” inactive form comprising an intramolecular CUB-to-spacer binding interaction subject to allosteric activation by VWF (Muia et al., *Proc Natl Acad Sci U S A* 2014;111:18584-9; South et al., *Proc Natl Acad Sci U S A* 2014;111:18578-83), the antibody 1-404 may be exemplary of a class of autoantibodies that exert their pathogenic effect by stabilizing the enzyme’s closed conformation. Though CUB-binding antibodies could reduce ADAMTS13 activity by enhanced clearance or by inhibiting

other functions of the protease, they might function synergistically to stabilize the enzyme in an open conformation allowing spacer domain-specific antibodies to bind and block VWF binding to ADAMTS13.

The present invention discloses human anti-ADAMTS13 autoantibodies that
5 function in an animal model. Whether by injection of scFv protein (FIGS. 4B-4C) or by *in vivo* expression of antibody mediated by hydrodynamic gene transfer of scFv DNA-containing plasmids (FIGS. 5A-5C and FIGS. 6A-6B), murine ADAMTS13 proteolytic activity was inhibited, resulting in the accumulation of UL-VWF multimers. After triggering endothelial injury with Shigatoxin-2, key pathologic features of TTP were observed including
10 thrombocytopenia, microangiopathic hemolytic anemia, formation of platelet thrombi in vital organs, and death (FIGS. 7A-7C). As in patients with antibody-mediated ADAMTS13 deficiency, these mice exhibited sustained inhibition of ADAMTS13 (<5%) and “positive inhibitor assays”, i.e. their plasmas inhibited ADAMTS13 activity when mixed with normal human plasma (FIG. 5C). The altered clot morphology revealed by focal arteriole injury via laser
15 and intravital video-microscopy in the setting of prolonged autoantibody-mediated ADAMTS13 inhibition (FIGS. 6A-6B) may illustrate the process by which clots extend linearly and cause the blockage of microvessels in patients with acquired TTP. The present observation that inhibition of ADAMTS13 function and the subsequent pathology that develops can be mediated by a monomeric antibody fragment lacking a constant region domain suggests that antibody-mediated
20 clearance of ADAMTS13 or other effector functions conferred by IgG Fc domains may not be necessary for the expression of disease in patients.

To date, animal models of acquired TTP have been limited to the use of rabbit or mouse antibodies to human ADAMTS13 that produce transient enzyme inhibition (Chauhan et al., J Exp Med 2006;203:767-76; Chauhan et al., J Thromb Haemost 2007;5:583-9; Feys et al.,
25 Blood 2010;116:2005-10). Such xenoantibodies would not be expected to necessarily primarily target human autoepitopes and, if so, would therefore not be helpful for testing novel therapies such as altered forms of recombinant ADAMTS13 that are engineered to be unrecognizable by human pathogenic autoantibodies (Jian et al., Blood 2012;119:3836-43; Zheng et al., Annu Rev Med 2015;66:211-25). In point of fact, antibodies to human ADAMTS13 generated by mice are
30 not expected to bind to the same epitopes as the present clones 1-416, 1-420, 1-428, and 1-431 because these four human antibodies also cross react with murine ADAMTS13 (FIGS. 4A-4C).

Tolerance mechanisms in healthy murine immune systems would not permit such antibodies to be made because they would be autoreactive.

Of the 51 anti-ADAMTS13 scFv antibodies described herein, 1-416, 1-420, 1-428, and 1-431 were initially selected for further evaluation not only because of their ability to inhibit ADAMTS13 *in vitro* but because rabbit anti-idiotypic antisera raised to each of these scFv demonstrated the presence of cross reactive idiotypes in a number of unrelated patient plasma samples. Of the four antibodies, scFv 1-420 was then chosen to pursue in an animal model because it appeared the most potent (FIGS. 4A-4C).

The use of rapid, large volume intravenous injection of plasmid DNA for transfer of exogenous genes into mice is a much simpler approach than those employing viral vectors for transfection and avoids the laborious steps necessary for virus preparation and purification as well as safety concerns associated with systemic administration of recombinant virus to animals. There are numerous examples in the literature of hydrodynamic-based transfections of plasmid DNA in animals used to study the effects of *in vivo*-expressed transgenes such as those encoding recombinant enzymes, hormones, cytokines and other proteins but not antibody fragments. The approach described herein could be useful for exploring the pathophysiological effects of autoantibodies in other disorders where the antibodies, as in acquired TTP, may not require the expression of full-length IgG for bivalency or Fc domains for effector function. In addition to its utility for the study of acquired TTP, the sustained inhibition of ADAMTS13 mediated by anti-ADAMTS13 DNA transfection may prove useful in murine models of other disease states such as ischemic stroke, myocardial infarction, atherosclerosis, malignant (cerebral) malaria, and pre-eclampsia where perturbations in ADAMTS13 is believed to play a role in disease pathogenesis.

The disclosures of each and every patent, patent application, and publication cited herein are hereby incorporated herein by reference in their entirety. While this invention has been disclosed with reference to specific embodiments, it is apparent that other embodiments and variations of this invention may be devised by others skilled in the art without departing from the true spirit and scope of the invention. The appended claims are intended to be construed to include all such embodiments and equivalent variations.

Antibody Heavy Chains – nucleic acid sequences

1-303, SEQ ID NO: 1

CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGTTTGGGTCCTCAGTGAA
GGTCTCCTGCAAGGCTTCTGGAGGCACCTTCAGCAGTTATACTATGAGCTGGGTGCG
ACAGGCCCCCTGGGCAAGGGCTTGAGTGGATGGGAAGTTTCATCCCTATTCTTGAGAG
AGCAAACCTACGCACAGAAAGTTCCAGGGCAGAGTCACTTTAACCGCGGACAAAAGTA
5 CGAGCACAGCCTACATGGAGCTGGGCAGCCTGAGATCTGAGGACACGGCCGTGTAT
TATTGTGCGAGAGACCTTGGGGACTTCGGTGACTCCTGGGGCCAGGGAACCCTGGTC
ACCGTCTCCTCAG

1-304, SEQ ID NO: 2

10 CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGTCCTCGGTGAG
GGTCTCCTGCAAGGCTTCTGGAAGCAATTCAGCAGCCACACCATCAATTGGGTACG
ACAGGCCCCCTGGACACGGGCTTGAGTGGATGGGCAAGATCATCCCTGTCCTTGATAT
ATCTAAACACGCACAGACATTCTGGGCAGAGTCATAATTACCGCGGACAAATCCA
CGAGCACAGCCTACATGGAGCTGAGCAGCCTGAGATCTGAGGACACGGCCATTTAT
15 TACTGTGCGATGGATAGTGTCTACGGCAACTTTGACTTTTGGGGCCAGGGAACCCCG
GTCACCGTCTCCTCAG

1-401 (same heavy chain as antibody 1-458), SEQ ID NO: 3

20 CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGTTGAAGAAGCCTGGGTCCTCGGTGAA
GGTCTCCTGCAAGGCTTCTGGAGGCGCCTTCACCATGTACACTATCAACTGGGTGCG
ACAGGCCCCCTGGACGAGGGCTTGAGTGGATGGGAAGGATCATACCTATTCTAGGTA
TAACAGACTACGCACAGAAATTCCAGGGCAGAGGCACGATTACCGCGGACAAATCC
ACAAGCACAGCCTACCTGGAGCTGAGCGGCCTGACTTCTGAGGACACGGCCGTGTA
TTACTGTGCGAGAGAGTTTAGTGGGGGCAACTATTTGACTTCTGGGGCCAGGGAAC
25 CCTGGTCACCGTCTCCTCAG

1-403 (same heavy chain as antibody 1-415), SEQ ID NO: 4

30 CAGGTGCAGCTGCAGGAGTCGGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAG
ACTCTCCTGTGCAGCCTCTGGATTACCTTCAGGAAGTATGCTATGCACTGGGTCCG
CCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGCAGTTATATCATATGATGGAGGTA
AGGAATACTACGCAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCC
AAGAACACGCTGTATTTGCAAATGAACAGCCTGAGAGCTGAGGACACGGCTGTGTA
TTACTGTGCGAGAGATACTTTTTCGTATTACGATTTTGGAGGGCTTTTGACTACTGG
GGCCAGGGAACCCTGGTCACCGTCTCCTCAG

35

1-404, SEQ ID NO: 5

CAGGTGCAGCTGCAGGAGTCGGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAG
ACTCTCCTGTACAGACTCTGGATTACCTTCAGTCGCTATGTTATGCACTGGGTCCGC
CAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGCAGGTATATCATATGATGGAAGTTA
40 TGAATACTACGCAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCA
AGAACACGCTATATGTGCAAATGAACAGCCTGAGAGGTGAGGACACGGCTGTGTAT
TACTGTGCGAGAGATTTACGTGGTGGGGAAGACTACTGGGGCCAGGGAACCCTGGT
CACCGTCTCCTCAG

45 1-405, SEQ ID NO: 6

GAGGTGCAGCTGGTGGAGTCTGGGGCTGAGGTGAAGAAGCCTGGGTCCTCGGTGAA
GGTCTCCTGCAAGGCTTCTGGAGGCATCTTCGGCACCTATACTATCAGCTGGGTGCG
ACAGGCCCCCTGGACAAGGGCTTGAGTGGATGGGAGGGATCATCCCTGTCCTTGATGT
CACTCACTACGCGCAGGATTTCCAGGACAGAGTCACCATTACCGCGGACAAGTCCA
5 CGAGCACTGCCTCCATGGAGCTGAGCAGCCTGAAATCTGACGACACGGCCATATAT
TACTGTGCGAGAAGTTCCTATTATAGCACCTTTGACTACTGGGGCCAGGGAACCCTT
GTCACCGTCTCCTCAG

1-406, SEQ ID NO: 7

10 CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGTTTGGGTCCTCAGTGAA
GGTCTCCTGCAAGGCTTCTGGAGGCATGTTTCAGCAGTTATACTATCAGCTGGGTGCG
ACAGGGCCCCTGGACAAGGGCTTGAGTGGATGGGAAGTTTCATCCCTATTCTTGAGAG
AGCAAACCTACGCACAGAAGTTCCAGGGCAGAGTCTCTTTTACCGCGGACAAAAGCA
CGAGCACAGCCTACATGGAGCTGGGCAGCCTGACATCTGAGGACACGGCCGTGTAT
15 TTTTGTGCGAGAGACGTTGGGGACTTCGGTGACTCCTGGGGCCAGGGAACCCTGGTC
ACCGTCTCCTCAG

1-407, SEQ ID NO: 8

20 GAGGTGCAGCTGGTGGAGTCTGGGGGAGTCGTGGTACAGCCTGGGGGGTCCCTGAG
ACTCTCCTGTGCAGCCTCTGGATTACCTTTGATGATTATAACCATGCACTGGGTCCGT
CAAGCTCCGGGGGAAGGGTCTGGAGTGGGTCTCTCTTATTAGTTGGGATGGTGGTAGC
ACATACTATGCAGACTCTGTGAAGGGCCGATTACCATCTCCAGAGACAACAGCAA
AAACTCCCTGTATCTGCAAATGAACAGTCTGAGAACTGAGGACACCGCCTTGTATTA
CTGTGCAAAAGATAACGGTTACGATATTTTGACTGATTATCTTGACTACTGGGGCCA
25 GGAACCCTGGTCACCGTCTCCTCAG

1-408, SEQ ID NO: 9

30 CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGTCCTCGGTGAA
GGTCTCCTGCAAGGCTTCTGGAGGCTCCTTCAGCAATTATACTATCACCTGGGTGCG
CCAGGCCCCCTGGACAAGGACTTGAGTGGATGGGAAGGATCATCCCTGTCCTTGGTCT
GACAGACTCCGCACAGAAGTTCAAGGGCAGAGTCACGATTACCGCGGACAAGTCCA
CGAGCACAGCCTACATGGAGCTGAGCAGCCTGACATCTGAAGACACGGCCGTGTAT
TACTGTGCGAGAGATTTCGGTAATTGGAACGTCCGACTGGGGCCAGGGAACCCTGGT
CACCGTCTCCTCAG

1-410, SEQ ID NO: 10

40 CAGGTGCAGCTGCAGGAGTCGGGGGGGGGCGTGGTACAGCCTGGGGGGTCCCTGAG
ACTCTCCTGTGCAGCCTCTGGATTACCTTTGATGATTATAACCATGCACTGGGTCCGT
CAAGCTCCGGGGGAAGGGTCTGGAGTGGGTCTCTCTTATTAGTTGGGATGGTGGTAGC
ACATACTATGCAGACTCTGTGAAGGGCCGATTACCATCTCCAGAGACAACAGCAA
AAACTCCCTGTATCTGCAAATGAACAGTCTGAGAACTGAGGACACCGCCTTGTATTA
CTGTGCAAAAGATAACGGTTACGATATTTTGACTGATTATCTTGACTCCTGGGGCCA
GGGAACCCTGGTCACCGTCTCCTCAG

45 1-413, SEQ ID NO: 11

CAGGTGCAGCTGGTGCAGTCTGGGTCTGAGGTGAAGAAGCCTGGGTCCTCGGTGAA
AGTCTCCTGCAAGGCTTCTGGAGGCACCTTCAGCAGTCATACTCTCAGCTGGGTACG
ACAGGCCCCTGGACAAGGGCTTGAGTGGATGGGAGAGATCATCCCTATCCTTGATA
GAGTGAAGTATTCACAGAACTTCCAGGGCAGAGTCACGATTACCGCGGACAAATCC
5 ACAAACACAACCTACATGGAGCTGAGCAGCGTGAGATCTGAGGACACGGCCGTATA
CTATTGTGTTAGCAATGGCTGGTCCAACCTTGACTTCTGGGGCCAGGGAACCCTGGT
CACCGTCTCCCCAG

1-416, SEQ ID NO: 12

10 CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGTCCTCGGTGAG
GGTCTCCTGCACGGCTTCTGGAAGCAATTCAGCAGCCACACCATCAACTGGGTACG
ACAGGCCCCTGGACAAGGACTTGAGTGGATGGGCAAGGTCATCCCTGTCCTTGATAT
ATCAAAACACGCACAGACATTCCTGGGCAGAGTCATTATTACCGCGGACAAATCCA
CGAGCACAGCCTACTTGAGCTGAGCAGCCTGAGATCTGAGGACACGGCCATTTATT
15 ACTGTGCGATGGATAGTGTCTACGGCAACTTTGACTTTTGGGGTCAGGGAACCCTGG
TCACCGTCTCCTCAG

1-417, SEQ ID NO: 13

20 CAGGTGCAGCTGGTGCAGTCTGGGACTGAGGTGAAGAAGTTTGGGTCCTCGGTGAA
GGTCTCCTGCAAGGCTTCTGGAGGCAGCTTCAGTAGTTATACTATCAGCTGGGTGCG
ACAGGCCCCTGGACAAGGGCTTGAGTGGATGGGAAGTTTCATCCCTATCCTTGAGAG
AGCAAACCTACGCACAGAAGTTCCAGGGCAGAGTCACCTTTTACCGCGGACAAAACCA
CGAGCACAGCCTACATGGAGCTGGGCGGCCTGAGATCTCAGGACACGGCCGTCTAT
TATTGTGCGAGAGACCTTGGGGACTTCGGTGACTCCTGGGGCCAGGGAACCCTGGTC
25 ACCGTCTCCTCAG

1-418, SEQ ID NO: 14

30 CAGGTGCAGCTGGTGCAGTCTGGGTCTGAGGTGAAGAAGCCTGGGTCCTCGGTGAA
GGTCTCCTGCAAGGCTTCTGGAGGCACCTTCAGCAGTCACACTGTCAGCTGGGTACG
ACAGGCCCCTGGACAAGGGCTTGAATGGATGGGAGAGATCATCCCTATCCTTGATA
GAGTGAAGTATGCAGAGAACTTCCAGGGCAGAGTCACGATTACCGCGGACAAAGTCC
ACGAATACAACCTATATGGACCTGAGCAGCCTGAGATCTGAGGACACGGCCGTATA
TTATTGTACTAGCAATGGCTGGTCCAACCTTGACTTCTGGGGCCAGGGAACCCTGGT
35 CACCGTCTCCTCAG

1-420, SEQ ID NO: 15

40 CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGTCCTCGGTGAA
GGTCTCCTGCAAGGCTTCTGGAGGCGCCTTCACCATGTACACTATCAACTGGGTGCG
ACAGGCCCCTGGACGAGGGCTTGAGTGGATGGGAAGGATCATACCTATTCTAGGTA
TAACAGACTACGCACAGAAATTCAGGGCAGAGGCACGATTACCGCGGACAAATCC
ACAAGCACAGCCTACCTGGAGCTGAGCGGCCTGACTTCTGAGGACACGGCCGTGTA
T TACTGTGCGAGAGAGTTT AGTGGGGGCAACTATTT CGACTTCTGGGGCCAGGGAAC
CCTGGTCACCGTCTCCTCAG

45 1-423, SEQ ID NO: 16

CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAGGCCTGGGTCCTCGGTGAA
GGTCTCCTGCAAGGCCTCTGGAGGCACCTTCAGCAGCTACACTATCAGCTGGGTGCG
ACAGGCCCCAGGACAAGGGCTTGAATGGATGGGAAGTATCATCCCTGTCCTTGATGT
CACATCCTACGCACAGCAATTCCAGGGCAGAGTCACTATTACCGCGGACAAATCCA
5 CGAAGACAGCCTACATGGACCTGAGCAGCCTAACATTTGAGGACACGGCCCTGTAT
TTCTGTTCGATTGGTAGATATACTTATGGACACTTTGACACCTGGGGCCAGGGAACC
CAGGTCACCGTCTCCTCAG

1-428, SEQ ID NO: 17

10 GAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGTCCTCGGTGAA
GGTCTCCTGCAAGGCCTCTGGAAGCAATTCAGCAGCCACACCATCAATTGGGTCCG
ACAGGCCCCCTGGACACGGGCTTGAGTGGATGGGAAAGATCATCCCTGTCCTTGATAT
ATCAAAAGACGCAGAGACATTCCTGGGCAGAGTCGTAATTACCGCGGACAAGTCCA
CGAGCACAGCCTACATGGAGCTGAGCAGCCTGAGATCTGAGGACACGGCCATTTAT
15 TACTGTGCGATGGATAGTGTCTACGGCAACTTTGACTTTTGGGGCCAGGGAACCCTG
GTCACCGTCTCCTCAG

1-431, SEQ ID NO: 18

20 CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGTTTGGGTCCTCAGTGAA
GGTCTCCTGCAAGGCTTCTGGAGGCACCTTCAGCAGTTATACTATGAGCTGGGTGCG
ACAGGCCCCCTGGACAAGGGCTTGAGTGGATGGGAAGTTTCGTCCCTATTCTTGAGAG
AGCAAACCTACGCACAGGGATTCCAGGGCAGAGTCACTTTTACCGCGGACAAAAGCA
CGAGCACAGCCTACATGGAGCTGGGCAGCCTGAGATCTGAGGACACGGCCGTGTAT
TATTGTGCGAGAGACCTTGGGGACTTCGGTGACTCCTGGGGCCAGGGAACCCTGGTC
25 ACCGTCTCCTCAG

1-432, SEQ ID NO: 19

30 CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTCTGTCCTCAGTGAA
GGTCTCCTGCAAGGCCTCTGGAGGCACCTTCAACATGTATGATATCAACTGGGTGCG
ACAGGCCCCCTGGACAAGGGCTTGAGTGGATGGGAGGGATCGTCCCTATTCTTGGTGT
GACAAACCTACGCACAGAACTTCCAGGGCAGACTAACAATTACCGCGGACAAATCAA
CGAGCACAGCCTACATGGAGCTGAGCAGCCTGAGATCTGAGGACACGGCCGTGTAT
TACTGTGCGAGAGGCGTGGCAGCAGGATGGAATGCTTTTGATGTCTGGGGCCAAGG
GACAATGGTCACCGTCTCTGCAG
35

1-434, SEQ ID NO: 20

40 CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGTCCTCGGTGAA
GGTCTCCTGCAAGGCTTCTGGAGGCAGCTTCAGTGATTATACTATCATTGTTGCG
ACAGGCCCCGTGGACACGGGCTTGAGTGGATGGGAAAAATCGTCCCTATACTTGGTG
TCACAACCTACGCACAGGAGTTCCAGGGCAGAATCACGATCACCGCGGACAGGTCC
ACGAGCACAGCCTACATGGAGCTGAGCAGCCTGAGATCTGAGGACACGGCCGTCTA
TTACTGTGCGAGGTTCTTGTGGGGTTTGGACGTCTGGGGCCAAGGGACCACGGTCAC
CGTCTCCTCA

45 1-437, SEQ ID NO: 21

CAGGTGCAGCTGGTGCAGTCTGGGGGAGGCCTGGTCAAGCCTGGGGGGTCCCTGAG
ACTCTCCTGTGCAGCCTCTGGATTTCATCTTCAGTAACTATATCATGAACTGGGTCCGC
CAGGCTCCAGGGAAGGGGCTGGAGTGGGTCTCGTCCATTAGTAGTAGTGGTAGTTA
CATATACTACGCAGACTCAGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCA
5 AGAACTCAATGTATCTGCAAATGAACAGTCTGAGAGGCGAGGACACGGCTGTGTAT
TACTGTGCGGCCGCTTACGATTTTTGGAGTGGTTATTATTTCTGGGGCCAAGGGACC
ACGGTCACCGTCTCCTCA

1-438, SEQ ID NO: 22

10 CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGTCCCTCGGTGAA
GGTCTCCTGCAAGGCTTCTGGAGGCACCTTCAGCAACTATGATATCACCTGGGTGCG
ACAGGCCCCAGGACAAGGGCTTGAGTGGGTGGGAAAAGTCATCCCTATCCTTGATG
TAACAAACTACGCACAGAAGTTCCAGGGCAGAGTCACTATTACCGCGGACAAATCC
ACGAGCACAGCCTACATGGAGCTGAGCAACCTGACATCTGAGGACACGGCCGTTTA
15 TTTCTGTGCGAGGTTCTTATGGGGTTTGGACGTCTGGGGCCAAGGGACCATGGTCAC
CGTCTCCTCA

1-440, SEQ ID NO: 23

20 GAGGTGCAGCTGTTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGCAGGTCCCTGAG
ACTCTCCTGTGCAGCCTCTGGATTTCATCTTTGATGATTATGCCATGCACTGGGTCCGG
CAAGCTCCAGGGAAGGGCCTGGAGTGGGTCTCAGGTATTAGTTGGAATAGTGGTAG
CATAGGCTATGCGGACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCA
AGAACTCCCTGTATCTGCAAATGAACAGTCTGAGAGCTGAGGACACGGCCTTGTATT
ACTGTGCAAAAGACCCTAATTCGCTGTATAGAAGTGGTTCCTTTGACTACTGGGGCC
25 AGGGAACGCTGGTTCACCGTCTCCTCAG

1-441, SEQ ID NO: 24

30 CAGGTGCAGCTGCAGGAGTCGGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAG
ACTCTCCTGTACAGACTCTGGATTTCACCTTCAGTCGCTATGCTATGCACTGGGTCCGC
CAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGCAGGTATATCATATGATGGAAGTTA
TGAATACTACGCAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCA
AGAACACGCTATATGTGCAAATGAACAGCCTGAGAGGTGAGGACACGGCTGTGTAT
TACTGTGCGAGAGATCTACGTGGTGGGGAAGACTACTGGGGCCAGGGAACCCTGGT
CACCGTCTCCTCAG
35

1-450, SEQ ID NO: 25

40 CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGTCCCTCGATGAA
TGTCTCCTGCAAGGCTTCTGGAGGCACCTTCAGCAGGCATACCATCAACTGGGTGCG
ACAGGCCCCCTGGACAAGGGCTTGAGTGGATGGGAAGCATCATCCCTATTCTTGGTAT
AACAAACTACGCACAGAACTTCCAGGGCAGACTCACGTTTAGCGCGGACAAATCCA
CGAACACAGCCTATGTGGAGTTGAGTGGCCTGAGATCTGAGGACACGGCCGTCTATT
ACTGTGCGAGTGGGGACTACTACTATGACATGGCCGTTTGGGGCCAAGGGACCACG
GTCGCCGTCACCTCA

45 1-451, SEQ ID NO: 26

CAGGTGCAGCTGGTGCAGTCTGGGGGAGGCTTGGTACAGCCTGGCAGGTCCCTGAG
ACTCTCCTGTGCAGCCTCTGGATTTCATCTTTGATGATTATGCCATGCACTGGGTCCGG
CAAGCTCCAGGGAAGGGCCTGGAGTGGGTCTCAGGTATTAGTTGGAATAGTGGTAG
CATAGGCTATGCGGACTCTGTGAAGGGCCGATTACCATCTCCAGAGACAACGCCA
5 AGAACTCCCTGTATCTGCAAATGAACAGTCTGAGAGCTGAGGACACGGCCTTGTATT
ACTGTGCAAAAGACCCTAATTCGCTGTATAGAAGTGGTTCCTTTGACTACTGGGGCC
AGGGAACGCTGGTACCGTCTCCTCAG

2-102, SEQ ID NO: 27

10 CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGTCCCTCGGTGAA
GGTCTCCTGCAGGGCTTCTGGAGGCACCTTCAGCAGCTATACTATCAACTGGGTGCG
ACAGGCCCCTGGACAAGGGCTTGAGTGGATGGGAAGGGTCGTCCCTATCCTTGGTGT
AACAACTACGCACAGAAGTTCCAGGGCAGAGTCACCATTACCGCGGACAAATCCA
CGAGCACAGCCTACATGGAGCTGAGCAGCCTGAGATCTGAGGACACGGCCGTGTAT
15 TACTGTGCGAGAGATAAGGGCTATGATAATAATTACGGGGCCTACTGGGGCCAGGG
AACCTGGTACCGTCTCCTCAG

2-103, SEQ ID NO: 28

20 CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGTCCCTCGGTGAA
GGTCTCCTGCAAGGCTTCTGGAGGCACCTTCAGCAGCTATGATATCAACTGGGTGCG
ACAGGCCCCTGGACAAGGGCTTGAGTGGATGGGAAGCATCATCCCTATCCTTGGTAT
ATCAAACTACGCACAGAAGTTCCAGGGCAGAGTCACGATTACCGCGGACAAATCCA
CGAGCACAGCCTACATGGAGCTGAGCAGCCTGAGATCTGAGGACACGGCCGTGTAT
TACTGTGCGAGAGATCAGGGCTATGCCAATAATTACGGGGCCTACTGGGGCCAGGG
25 AACCTGGTACCGTCTCCTCAG

2-106, SEQ ID NO: 29

30 CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGTCCCTCGGTGAA
GGTCTCCTGCAAGGCTTCTGGAGGCACCTTCAGCAGCTATGATATCAATTGGGTGCG
ACAGGCCCCTGGACAAGGGCTTGAGTGGATGGGAAGCATCATCCCTATCCTTGGTAT
ACGAAATTACGCACAGAAGTTCCAGGGCAGAGTCACGATTACCGCGGACAAATCCA
CGAGCACAGCCTACATGGAGCTGAGCAGCCTGAGATCTGAGGACACGGCCGTGTAT
TACTGTGCGAGAGATCAGGGCTATGCTAATAATTACGGGGCCTACTGGGGCCAGGG
35 AACCTGGTACCGTCTCCTCAG

2-108, SEQ ID NO: 30

40 GAGGTGCAGCTGGTGCAGTCTGGGGGAGGCTTGGTCCAGCCTGGGGGGTCCCTGAG
ACTCTCCTGTGCAGCCTCTGGATTACGTTTAGTAGCTATTGGATGAGCTGGGTCCG
CCAGGCTCCAGGGAAGGGGCTGGAGTGGGTGGCCAACATAAAGCAAGATGGAAGT
GAGAAACACTATGTGGACTCTATGAAGGGCCGATTACCATCTCCAGAGACAACGG
CAAGAACTCACTGTATCTGCAAATGAACAGCCTGAGAGCCGAGGACACGGCTGTGT
ATTACTGTGCGAGGTCCCCGGGATACTACTTTGACTACTGGGGCCAGGGAACCCTGG
TCACCGTCTCCTCAG

45 2-203, SEQ ID NO: 31

CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGTCCTCGGTGAA
GGTCTCCTGCAAGGCTTCTGGAGGCACCTTCAGCAGCTATGCTATCGACTGGGTGCG
ACAGGCCCCTGGACAAGGGCTTGAGTGGATGGGAAGGATCGTCCCTATCCTTGGTAT
AGCAAACCTACGCACAGAAGTTCCAGGGCAGAGTCACGATTACCGCGGACAAATCCA
5 CGAGCACGGCCTACATGGAAGTGCAGCAGCCTGAGATCTGACGACACGGCCGTATAT
TACTGTGCGAGAGATCGGGGCTATGCTAATACTTACGGGGCCTACTGGGGCCAGGG
AACCTGGTCAACGTCTCCTCAG

2-204, SEQ ID NO: 32

10 CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGTCCTCGGTGAA
GGTCTCCTGCAAGGCTTCTGGAGGCACCTTCAGCAGTTATGCTATTAGCTGGGTGCG
ACAGGCCCCTGGACAGGGGCTTGAGTGGATGGGAAGCATCGTCCCTATCCTTGGTGT
AGTAAACTACGCACAGAAGTTCCAGGGCAGAGTCACGATTACCGCGGACAAATCCA
CGAGCACAGCCTACATGGAGCTGAGCAGCCTGAGATCTGAGGACACGGCCGTGTAT
15 TACTGTGCGAGAGATAAGGGGTATGCTAATAATTACGGGGCCTACTGGGGCCAGGG
AACCTGGTCAACGTCTCCTCAG

2-206, SEQ ID NO: 33

20 CAGGTGCAGCTGGTGCAGTCTGGGGCTGACGTGAAGAAGCCTGGGTCCTCGGTGAA
GGTCTCCTGCAAGGCTTCTGGAGGCACCTTCAGCAGCTATACTATCAACTGGGTGCG
ACAGGCCCCTGGACAAGGGCTTGAGTGGATGGGAAGGATCGTCCCTATCCTTGATAT
TGCAAACCTACGCACAGAAGTTCCAGGGCAGAGTCACGATTACCGCGGACAAATCCA
CGAGCACAGCCTACATGGAGCTGAGCAGCCTGACATCTGAGGACACGGCCGTGTAT
TATTGTGCGAGAGATCGGGGCTATGATAATAAATACGGGGCCTACTGGGGCCAGGG
25 AACCTGGTCAACGTCTCCTCAG

2-207 (same heavy chain as antibody 2-301), SEQ ID NO: 34

30 CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGTCCTCGGTGAA
GGTCTCCTGCAAGGCTTCTGGAGGCACCTTCAGCAGCTATGCTATCAGCTGGGTGCG
ACAGGCCCCTGGACAAGGGCTTGAGTGGATGGGAGGGATCACCCCTATCCTTGGTG
TAACAACTACTCACAGAAGTTCCAGGGCAGAGTCACCGTTACCGCGGACATATCC
ACGACCACAGCCTACATGGAGCTGAGCAGCCTGACATCTGAGGACACGGCCGTGTA
TACTGTGCGAGAGATCAGGGGCTATGCTAATGATTACGGGGCCTACTGGGGCCAGG
35 GAACCCTGGTCAACGTCTCCTCAG

2-302, SEQ ID NO: 35

40 GAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGTCCTCGGTGAA
GGTCTCCTGCAAGGCTTCTGGAGGCACCTTCAGCAGCTATACTATCAACTGGGTGCG
ACAGGCCCCTGGACAAGGACTTGAGTGGATGGGAAGGATCATCCCTATCCTTGGTAT
AGAAAACCTACGCACAGAAGTTCCAGGGCAGAGTCACGATTTCCGCGGACAAATCCA
CGAGCACAGCCTACATGGAGCTGAGCAGCCTGAGATCTGACGACACGGCCGTGTAT
TACTGTGCGAGAGATCAGGTCTTCGGGGCCTACTGGGGCCCGGGAACCCTGGTCAC
CGTCTCCTCAG

45 2-304, SEQ ID NO: 36

CAGGTGCATCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGTCCTCGGTGAA
GGTCTCCTGCAAGGCTTCTGGAGGCACCTTCAGCAGCTATGCTATCAGCTGGGTGCG
ACAGGCCCCTGGACAAGGGCTTGAGTGGATGGGAGGGATCACCCCTATCCTTGGTG
TAACAACTACTCACAGAAGTTCCAGGGCAGAGTCACCGTTACCGCGGACATATCC
5 ACGACCACAGCCTACATGGAGCTGAGCAGCCTGACATCTGAGGACACGGCCGTGTA
T TACTGTGCGAGAGATCAGGGCTATGCTAATGATTACGGGGCCTACTGGGGCCAGG
GAACCCTGGTCACCGTCTCCTCAG

2-305 (same heavy chain as antibody 2-406), SEQ ID NO: 37

10 CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGTCCTCGGTGAA
GGTCTCCTGCAAGGCTTCTGGAGGCACCTTCAGCAGCTATGAAATCAGTTGGGTGCG
ACAGGCCCCTGGACAAGGGCTTGAGTGGATGGGGAGGATCGTCCCTATCCTGGGTTT
GGCAAACCTACGCACAGAACTTCCAGGGCAGAGTCACCATTACCGCGGACAAATCCA
CGAGCACAGCCTACATGGAGCTGAGCAGCCTGAGATTCGAGGATACGGCCGTGTAT
15 TACTGTGCGAGAGATCAGGGCTATGCTAATAATTACGGGGCCTACTGGGGCCAGGG
AACCCCTGGTCAGCGTCTCCTCAG

2-408, SEQ ID NO: 38

20 CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGTCCTCGGTGAA
GGTCTCCTGCGAGGCTTCTGGAGGCACCTTCAGCAGCTATGCTATCACCTGGGTGCG
ACAGGCCCCTGGACAAGGGCTTGAGTGGATGGGAAGCATCCTCCCTATCCTTGATAT
AACAACTACGCACAGAACTTCCAGGGCAGAGTCACGCTTACCGCGGACAAATCCA
CGAGCACAGCCTACATGGAGCTGAGCAGCCTGAGATCTGAGGACACGGCCGTGTAT
TTCTGTGCGAGAGATCGGGGCTATAGTAATAATTATGGGGCCTACTGGGGCCAGGG
25 AACCCCTGGTCACCGTCTCCTCAG

3-301, SEQ ID NO: 39

30 GAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGTCCTCAGTGAA
GGTCTCCTGCAAGGCTTCTGGAGGCACCTTCAGCAGCTATGCTATCAGCTGGGTGCG
ACAGGCCCCTGGACAAGGGCTTGAGTGGATGGGAGGGATCATCCCTATCCTTGGTAT
AACAACTACGCACAGAACTTCCAGGGCAGAGTCACGATTACCGCGGACGAATCCA
CGGGCGCAGCCTACATGGAGCTGAGCAGCCTGGCATCTGAGGACACGGCCGTATAT
TACTGTGCGAGAGATGATACTGGCCGGGACGACTACTTTGAGTACTGGGGTCAGGG
AACCCCTGGTCACCGTCTCCTCAG
35

3-302 (same heavy chain as antibodies 3-305 and 3-405), SEQ ID NO: 40

40 CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAGGCCTGGGTCCTCAGTGAA
GGTCTCCTGCAAGGCTTCTGGAAACACCTTCAGTAACTATCATATCAACTGGGTGCG
ACAGGCCCCTGGACAAGGGCTTCAGTGGATGGGAGGAATCATCCCTATTCTTGGGA
GAACAACTACGCACAGAACTTCCAGGGCAGAGTCACGATTACCACGGACGAATCA
ACGAACACAGCCTACATGGAGCTGACTAGCCTGAGATCCGAGGACACGGCCGTTTA
TTATTGTGCGAGGGAGGCCCGGGATAGTTTTGATTTCTGGGGCCAAGGGACAATGGT
CACCGTCTCTTCAG

45 4-303, SEQ ID NO: 41

CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGTCTCAGTGAA
GGTCTCCTGCAAGGCTTCTGGAGGCACCTTCAGCAACTATGGTATCAGCTGGGTGCG
ACAGGCCCCTGGACAAGGGCTTGAGTGGATGGGAAGATCATCCCTATCCTTGGTAT
AACAACTACGCACAGAAGTTCCAGGGCAGGGTCACGATTACCGCGGACAAATCCA
5 CGAGCACAGCCTACATGGAGCTGAGCAGCCTGAGATCTGAGGACACGGCCGTGTAT
TACTGTGCGAGAGGGGGTGGGAGCTACGACTTCTTTGACTACTGGGGCCAGGGAAC
GCTGGTCACCGTCTCCTCAG

4-307, SEQ ID NO: 42

10 CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGTCTCAGTGAA
GGTCTCCTGCAAGGCTTCTGGTGGCACCTTCAGCACCTATACTATCAACTGGATGCG
ACAGGCCCCTGGACAAGGGCTTGAGTGGATGGGAAGGATCATCCCTATGTTTCGGTA
CAGCAAACTACGCACAGAAGTTCCGGGGCAGAGTCACGATTACCGCGGACGAATCC
ACGAGCACAGCCTACATGGAGCTGAGCAGCCTGATATCTGAGGACACGGCCATCTA
15 TTA CTGTGCGAGAAGTGGCTACAGTGATGCTTTTGATATCTGGGGCCAAGGGACAAT
GGTCACCGTCTCTTCAG

z1-201, SEQ ID NO: 43

20 CAGGTGCAGCTGGTGCAGTTTGGGGGAGGCTTGGTACAGCCTGGCAGGTCCCTGAG
ACTCTCCTGTGCAGCCTCTGGATTTCATCTTTGATGATTATGCCATGCACTGGGTCCGG
CAAGCTCCAGGGAAGGGCCTGGAGTGGGTCTCAGGTATTAGTTGGAATAGTGGTAG
CATAGGCTATGCGGACTCTGTGAAGGGCCGATTACCATCTCCAGAGACAACGCCA
AGA ACTCCCTGTATCTGCAAATGAACAGTCTGAGAGCTGAGGACACGGCCTTGTATT
ACTGTGCAAAAGACCCTAATTCGCTATATAGAAGTGGTTCCTTTGACTACTGGGGCC
25 AGGGAACGCTGGTCACCGTCTCCTCAG

z1-303, SEQ ID NO: 44

30 CAGGTGCAGCTGCAGGAGTCGGGTCCAGGACTGGTGAGGCCCTCGCAGACCCTCTC
ACTCACCTGTGCCATCTCCGGGGACAGTGTCTCAAACAACAATGCTGCTTGGA ACTG
GATTAGGCAGTCCCCATCGAGAGGCCTTGAGTGGCTGGGAAGGACATTCTACAGGT
CCAGGTGGTATAATGATTATGCAGTTTCTGTGAAAAGTCGAATAATCATCAACCCAG
ACACATCCAAGAACCAATTCTCCCTGGACCTGACCTCTGTGACTCCCGAAGACACGG
CTGTGTATTTCTGTGCAAGAGAAGGACAGTGGCTGCCCACTACTTCGACCCCTGGG
GCCAGGGGACCCTGGTCACCGTCTCCTCAG

z1-402, SEQ ID NO: 45

40 CAGGTGCAGCTGGTGCAGTCTGGGGGAGGCGTCGTCCAGCCTGGGACGTCCCTAAG
ACTCTCCTGTGCAGCCTCTGGATTACCTTCCGCAACCATGCTATGCACTGGGTCCG
CCAGGCTCCAGGGAGGGGGCTGGAGTGGGTGGCAGATATACTGTACGATTCAAGTA
ACAAATACTACGCAGACTCTGTGAAGGGCCGATTACCATCTCCAGAGACAATTCC
AAGAACACGCTGTATCTGCAAATGAGCAGCCTGAGAGCTGAGGACACGGCCGTCTA
TTTCTGTGCGGCCAGTTCATATTTCCATTTGACTTCTGGGGCCAGGGAACCCTGGTC
ACCGTCTCCTCAG

45

Antibody Heavy Chains – amino acid sequences

1-303, SEQ ID NO: 46

QVQLVQSGAEVKKFGSSVKVSCKASGGTFSSYTMSWVRQAPGQGLEWMGSFIPILERA
NYAQKFQGRVTLTADKSTSTAYMELGSLRSEDNAVYYCARDLGDFGDSWGQGTLLTVV
SS

1-304, SEQ ID NO: 47

QVQLVQSGAEVKKPGSSVRVSCKASGSNFSSTINWVRQAPGHGLEWMGKIIPVLDISK
HAQTFLGRVIITADKSTSTAYMELSSLRSEDNAIYYCAMDSVYGNFDFWGQGTPTVTVSS

1-401 (same heavy chain as antibody 1-458), SEQ ID NO: 48

QVQLVQSGAELKKPGSSVKVSCKASGGAFTMYTINWVRQAPGRGLEWMGRIIPILGITD
YAKKFQGRGTITADKSTSTAYLELSGLTSEDNAVYYCAREFSGGNYFDFWGQGTLLTVVS
S

1-403 (same heavy chain as antibody 1-415), SEQ ID NO: 49

QVQLQESGGGVVQPGRSLRLSCAASGFTFRNYAMHWVRQAPGKGLEWVAVISYDGGK
EYYADSVKGRFTISRDN SKNTLYLQMNSLR AEDNAVYYCARDTFSSYDFWRAFDYWG
QGTLLTVTVSS

1-404, SEQ ID NO: 50

QVQLQESGGGVVQPGRSLRLSCTDSGFTFSRYVMHWVRQAPGKGLEWVAGISYDGSY
EYYADSVKGRFTISRDN SKNTLYVQMNSLRGEDNAVYYCARDLRGGEDYWGQGTLLTV
TVSS

1-405, SEQ ID NO: 51

EVQLVESGAEVKKPGSSVKVSCKASGGIFGTYTISWVRQAPGQGLEWMGGIIPVLDVTH
Y AQDFQDRVTITADKSTSTASME LSS LKSDDTAIYYCARSSYSTFDYWGQGTLLTVTVSS

1-406, SEQ ID NO: 52

QVQLVQSGAEVKKFGSSVKVSCKASGGMFSSYTISWVRQGPQGLEWMGSFIPILERAN
YAKKFQGRVSFTADKSTSTAYMELGSLTSEDNAVYFCARDVGDFGDSWGQGTLLTVTVSS

1-407, SEQ ID NO: 53

EVQLVESGGVVVQPGGSLRLSCAASGFTFDDYTMHWVRQAPGKGLEWVSLISWDGGS
TYYADSVKGRFTISRDN SKNSLYLQMNSLR TEDTA LY YCAKDNGYDILTDYLDYWGQG
TLVTVTVSS

1-408, SEQ ID NO: 54

QVQLVQSGAEVKKPGSSVKVSCKASGGSFSSNYTITWVRQAPGQGLEWMGRIIPVLGLT
DSAQKFQGRVTITADKSTSTAYMELSSLTSEDNAVYYCARDSVIGTSDWGQGTLLTVTVSS

1-410, SEQ ID NO: 55

QVQLQESGGGVVQPGGSLRLSCAASGFTFDDYTMHWVRQAPGKGLEWVSLISWDGGS
TYYADSVKGRFTISRDN SKNSLYLQMNSLR TEDTA LY YCAKDNGYDILTDYLDYWGQG
TLVTVTVSS

1-413, SEQ ID NO: 56

QVQLVQSGSEVKKPGSSVKVSCKASGGTFSSHTLSWVRQAPGQGLEWMGEIIPILDRVK
YSQNFQGRVTITADKSTNTTYMELSSVRSEDTAVYYCVSNGWSNFDWFVGQGTLLTVSP

5

1-416, SEQ ID NO: 57

QVQLVQSGAEVKKPGSSVRVSCTASGSNFSSHTINWVRQAPGQGLEWMGKVIPVLDISK
HAQTFGLGRVIITADKSTSTAYLELSSLRSEDTAIYYCAMDSVYGNFDWFVGQGTLLTVSS

10 1-417, SEQ ID NO: 58

QVQLVQSGTEVKKFGSSVKVSCKASGGSFSSYTISWVRQAPGQGLEWMGSFIPILERAN
YAQKFQGRVTFTADKTTSTAYMELGGLRSQDTAVYYCARDLGDFGDSWGQGTLLTVS
S

15 1-418, SEQ ID NO: 59

QVQLVQSGSEVKKPGSSVKVSCKASGGTFSSHTVSWVRQAPGQGLEWMGEIIPILDRVN
YAENFQGRVTITADKSTNTTYMDLSSLRSEDTAVYYCTSNNGWSNFDWFVGQGTLLTVSS

1-420, SEQ ID NO: 60

20 QVQLVQSGAEVKKPGSSVKVSCKASGGAFTMYTINWVRQAPGRGLEWMGRIIPILGITD
YAQKFQGRGTITADKSTSTAYLELSGLTSEDTAVYYCAREFSGGNYFDWFVGQGTLLTVS
S

1-423, SEQ ID NO: 61

25 QVQLVQSGAEVKRPGSSVKVSCKASGGTFSSYTISWVRQAPGQGLEWMGSIIPVLDVTS
YAQQFQGRVTITADKSTKTAYMDLSSLTFEDTALYFCSIGRYTYGHFDTWGQGTQVTVS
S

1-428, SEQ ID NO: 62

30 EVQLVQSGAEVKKPGSSVKVSCKASGSNFSSHTINWVRQAPGHGLEWMGKIIPVLDISK
DAETFLGRVVITADKSTSTAYMELSSLRSEDTAIYYCAMDSVYGNFDWFVGQGTLLTVSS

1-431, SEQ ID NO: 63

35 QVQLVQSGAEVKKFGSSVKVSCKASGGTFSSYTMSWVRQAPGQGLEWMGSFVPILERA
NYAQQFQGRVTFTADKSTSTAYMELGSLRSEDTAVYYCARDLGDFGDSWGQGTLLTV
SS

1-432, SEQ ID NO: 64

40 QVQLVQSGAEVKKPLSSVKVSCKASGGTFNMYDINWVRQAPGQGLEWMGGIVPILGV
TNYAQNFQGRLTITADKSTSTAYMELSSLRSEDTAVYYCARGVAAGWNAFDVWGQGT
MVTVSA

1-434, SEQ ID NO: 65

45 QVQLVQSGAEVKKPGSSVKVSCKASGGSFSDYTIWLRQARGHGLEWMGKIVPILGVTT
YAQEFQGRITITADRSTSTAYMELSSLRSEDTAVYYCARFLWGLDVWGQGTTVTVSS

1-437, SEQ ID NO: 66

QVQLVQSGGGLVKPGGSLRLSCAASGFIFSNYIMNWVRQAPGKGLEWVSSISSSGSYIY
YADSVKGRFTISRDNANKNSMYLQMNSLRGEDTAVYYCAAAYDFWSGYFFWGQGTTV
TVSS

5

1-438, SEQ ID NO: 67

QVQLVQSGAEVKKPGSSVKVSCKASGGTFSNYDITWVRQAPGQGLEWVGKVIPLDVT
NYAQKFQGRVTITADKSTSTAYMELSNLTSEDVAVYFCARFLWGLDVWGQGTMTVTS
S

10

1-440, SEQ ID NO: 68

EVQLLES GGGLVQPGRSLRLSCAASGFIFDDYAMHWVRQAPGKGLEWVSGISWNSGSI
GYADSVKGRFTISRDNANKNSLYLQMNSLRAEDTALYYCAKDPNSLYRSGSFDYWGQGT
LVTVSS

15

1-441, SEQ ID NO: 69

QVQLQESGGGVVQPGRSLRLSCTDSGFTFSRYAMHWVRQAPGKGLEWVAGISYDGSY
EYYADSVKGRFTISRDNANKNTLYVQMNSLRGEDTAVYYCARDLRGGEDYWGQGTLV
VSS

20

1-450, SEQ ID NO: 70

QVQLVQSGAEVKKPGSSMNVSCKASGGTFSRHTINWVRQAPGQGLEWMGSIIPILGITN
Y AQNFQGRLTFSADKSTNTAYVELSGLRSEDVAVYYCASGDYYDMAVWGQGTTVAV
TS

25

1-451, SEQ ID NO: 71

QVQLVQSGGGLVQPGRSLRLSCAASGFIFDDYAMHWVRQAPGKGLEWVSGISWNSGSI
GYADSVKGRFTISRDNANKNSLYLQMNSLRAEDTALYYCAKDPNSLYRSGSFDYWGQGT
LVTVSS

30

2-102, SEQ ID NO: 72

QVQLVQSGAEVKKPGSSVKVSCRASGGTFSSYTINWVRQAPGQGLEWMGRVVPILGVT
NYAQKFQGRVTITADKSTSTAYMELSSLRSEDVAVYYCARDKGYDNNGAYWGQGT
LVTVSS

35

2-103, SEQ ID NO: 73

QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYDINWVRQAPGQGLEWMGSIIPILGISN
Y AQKFQGRVTITADKSTSTAYMELSSLRSEDVAVYYCARDQGYANNYGAYWGQGT
LVTVSS

40

2-106, SEQ ID NO: 74

QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYDINWVRQAPGQGLEWMGSIIPILGIRN
Y AQKFQGRVTITADKSTSTAYMELSSLRSEDVAVYYCARDQGYANNYGAYWGQGT
LVTVSS

45

2-108, SEQ ID NO: 75

EVQLVQSGGGLVQPGGSLRLSCAASGFTFSSYWMSWVRQAPGKGLEWVANIKQDGSE
KHYVDSMKGRFTISRDNKGKNSLYLQMNSLRAEDTAVYYCARSPGYFDYWGQGTLVT
VSS

5 2-203, SEQ ID NO: 76

QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAIDWVRQAPGQGLEWMGRIVPILGIA
NYAQKFQGRVTITADKSTSTAYMELSSLRSDDTAVYYCARDRGYANTYGAYWGQGTL
VTVSS

10 2-204, SEQ ID NO: 77

QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGSIVPILGVV
NYAQNFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCARDKGYANNYGAYWGQGTL
VTVSS

15 2-206, SEQ ID NO: 78

QVQLVQSGADVKKPGSSVKVSCKASGGTFSSYTINWVRQAPGQGLEWMGRIVPILDIA
NYAQKFQGRVTITADKSTSTAYMELSSLTSEDTAVYYCARDRGYDNKYGAYWGQGTL
VTVSS

20 2-207 (same heavy chain as antibody 2-301), SEQ ID NO: 79

QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGITPILGVT
NYSQKFQGRVTITADISTTTAYMELSSLTSEDTAVYYCARDQGYANDYGAYWGQGTL
VTVSS

25 2-302, SEQ ID NO: 80

EVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYTINWVRQAPGQGLEWMGRIIPILGIEN
YAKKFQGRVTISADKSTSTAYMELSSLRSDDTAVYYCARDQVFGAYWGPGLTVTVSS

2-304, SEQ ID NO: 81

30 QVHLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGITPILGVT
NYSQKFQGRVTITADISTTTAYMELSSLTSEDTAVYYCARDQGYANDYGAYWGQGTL
VTVSS

2-305 (same heavy chain as antibody 2-406), SEQ ID NO: 82

35 QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYEISWVRQAPGQGLEWMGRIVPILGLA
NYAQNFQGRVTITADKSTSTAYMELSSLRFEDTAVYYCARDQGYANNYGAYWGQGTL
VSVSS

2-408, SEQ ID NO: 83

40 QVQLVQSGAEVKKPGSSVKVSCEASGGTFSSYAITWVRQAPGQGLEWMGSILPILDITN
YAKKFQGRVTLTADKSTSTAYMELSSLRSEDTAVYFCARDRGYSNNYGAYWGQGLV
TVSS

3-301, SEQ ID NO: 84

EVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIIPILGITN
YAQKFQGRVTITADESTGAAYMELSSLASEDTAVYYCARDDTGRDDYFEYWGQGT
LVTVSS

5 3-302 (same heavy chain as antibodies 3-305 and 3-405), SEQ ID NO: 85
QVQLVQSGAEVKRPGSSVKVSCKASGNTFSNYHINWVRQAPGQGLQWMGGIIPILGRT
NYAQNFQGRVTITTDESTNTAYMELTSLRSEDVAVYYCAREARDSFDFWGQGTMTVVS
S

10 4-303, SEQ ID NO: 86
QVQLVQSGAEVKKPGSSVKVSCKASGGTFSNYGISWVRQAPGQGLEWMGKIIPILGITN
YAQKFQGRVTITADKSTSTAYMELSSLRSEDVAVYYCARGGGSYDFFDYWGQGT
LVTVSS

15 4-307, SEQ ID NO: 87
QVQLVQSGAEVKKPGSSVKVSCKASGGTFSTYTINWMRQAPGQGLEWMGRIIPMFGTA
NYAQKFRGRVTITADESTSTAYMELSSLISEDVAVYYCARSGYSDAFDIWGQGT
MTVTVSS

20 z1-201, SEQ ID NO: 88
QVQLVQFGGGLVQPGRSLRLSCAASGFIFDDYAMHWVRQAPGKGLEWVSGISWNSGSI
GYADSVKGRFTISRDNANKNSLYLQMNSLRAEDTALYYCAKDPNSLYRSGSFDYWGQGT
LVTVSS

25 z1-303, SEQ ID NO: 89
QVQLQESGPGLVPSQTLSTCAISGDSVSNNNAAWNWIRQSPSRGLEWLGRTFYRSRW
YNDYAVSVKSRIINPDTSKNQFSLDLTSVTPEDTAVYFCAREGQWLPNYFDPWGQGT
LVTVSS

30 z1-402, SEQ ID NO: 90
QVQLVQSGGGVVQPGTSLRLSCAASGFTFRNHAMHWVRQAPGRGLEWVADILYDSSN
KYYADSVKGRFTISRDNANKNTLYLQMSSLRAEDTAVYFCAASSYFPDFWGQGT
LVTVSS

35 Antibody Light Chains – nucleic acid sequences

1-303, SEQ ID NO: 91
GAGCTCGAGCTGACTCAGCCACCCTCAGTGTCAGTGGCCCCAGGAAAGACGGCCAG
GATTACCTGTGGGGGAAACAACATTGGAAGTAAAAGTGTGCACTGGTACCAGCAGA
40 AGCCAGGCCAGGCCCTGTGCTGGTCGTCTATGATGATAGCGACCGGCCCTCAGGG
ATCCCTGAGCGATTCTCTGGCTCCAACCTCTGGGAACACGGCCACCCTGACCATCAGC
AGGGTCGAAGCCGGGGATGAGGCCGACTATTACTGTCAGGTGTGGGATAGTAGTAG
TGATCATTATGTCTTCGGAACCTGGGACCAAGGTGACCGTCCTAG

45 1-304, SEQ ID NO: 92

GAGCTCGTGCTGACTCAGCCACCCTCGGTGTCAGTGTCCCCAGGACAGACGGCCAG
GATCACCTGCTCTGGAGATGCATTGCCAAAGCATTATGCTTATTGGTACCAGCAGAA
GCCAGGCCAGGCCCTGTGGTGGTTATATATAAAGACACTGAGAGGCCCTCAGGGA
TCCCTGAGCGATTCTCTGGCGCCACCTCAGGGACAACAACACGTTGACCATCAGTG
5 GAGTCCAGGCAGAAGACGAGGCTGACTATTTTTGTCAATCATCAGACATCAATGGTA
CATCTTGATATTCGGCGGCGGCACCAAGCTGACCGTCCTAG

1-401, SEQ ID NO: 93

10 GAGCTCGTGCTGACTCAGCCACCCTCAGTGTGAGTGGCCCCAGGAAAGACGGCCAG
GATGACCTGTGGGGGAAACAACATTGGAGGTTATAGTGTGCACTGGTACCAGCAGA
AGCCAGGCCAGGCCCTGTGCTGGTCTATCATGATAGCGACCGGCCCTCAGGG
ATCCCTGAGCGATTCACTGGCTCCAATTCTGGGAACATGGCCACCCTGACCATCAGC
AGGGTCGAGGCCGGGGATGAGGCCGACTATTACTGTGAGGTGTGGGATAGTAGTAA
TGATCATTCGGTATTCGGCGGAGGCACCGAGCTGACCGTCCTCG

1-403, SEQ ID NO: 94

15 GAGCTCGTGATGACCCAGTCTCCATCGTCTCTGTCTGCATCTGTAGGAGACACCGTC
ACCATCACTTGCCGGGCAAGTCAGAGCCTTAGGGGATATTTAAATTGGTATCAACAG
AAGCCAGGGGAAGCCCCTAACTCCTCATCTACGCTGCGTCCACTTTGCGGGGCTGGG
20 GTCCCACCAAGGTTCAGTGGCGCCGGGTATGAGACAGATTTTCAGTCTCACCATCAGC
AATCTGCAACTTGAAGATTTTGCAACTTACTATTGTCAACAGTCTCACAATGTCCCCC
TCACCTTCGGCGGAGGGACCAAGGTGGAAATCAAAG

1-404, SEQ ID NO: 95

25 GAGCTCGTGGTGACGCAGCCGCCCTCAGTGTCTGCGGCCCCAGGACAGAAGGTCAC
CATCTCCAGCTCTGGAAGCACCTCCAACATTGGGAACAATTATGTATCCTGGTACCA
GCAGTTCCACGAACAGCCCCCAAACCTCATTATGACAATAATAAGCGACCCTC
AGGGATTCTGACCGATTCTCTGGCTCCAAGTCTGGCACGTCAGCCACCCTGGGCAT
CACCGGGCTCCAGACTGGGGACGAGGCCGATTATTACTGCGGAACATGGGATAGCA
30 GCCTGAGTGCTGTGGTATTCGGCGGAGGCACCAAGGTGACCGTCCTAG

1-405, SEQ ID NO: 96

35 GAGCTCGAGCTGATTCAGCCACCCTCAGTGTGAGTGTCCCCAGGACAGACGGCCAG
GATCACCTGCTCTGGAGATGCATTGCCAAAGCAATACGCTTATTGGTACCAGCAGAA
GCCAGGCCAGGCCCTGCGATGGTGATATATAAAGACACTGAGAGGCCCCCAGGGA
TCTCTGAGCGAATCTCTGGCTTCATCTCAGGGACAACAGCCACGTTGACCATCAGTG
GAGTCCAGGCAGAGGACGAGGCTGACTATTACTGTCAATCAGAAGACAGCAGTGGT
ATCCTTTTTGGCGGAGGGACCAAGCTGACCGTCCTAG

1-406, SEQ ID NO: 97

40 GAGCTCGAGCTGACTCAGCCACCCTCAGTGTGAGTTTTCCCAGGACAGACGGCCAG
GATCACCTGTTCTGGAGATACATTGCCAAAGCAATACGGTAATTGGTACCAGCAGA
AGCCAGGCCAGGCCCTGTGGTCTGATATATAAAGACACTGAGAGGCCCTCAGGG
ATCCCTGAGCGATTCTCTGGCTCCAGTTCAGGGACAACAGCCACGTTGACCATCAGT
45 GGGGTCCAGGCAGAAGACGAGGCTGATTATTACTGTCAATCAGCAGACAGCAATGA
TTGGGTGCTATTCGGCGGAGGGACCAAGCTGACCGTCCTAG

1-407, SEQ ID NO: 98

GAGCTCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTC
ACCATCACTTGCCAGGCGAGTCAGGACATTAGCAACTATTTAAATTGGTATCAGCAG
5 AAACCAGGGAAAGCCCCCTAAGCTCCTGATCTACGATGCATCCAATTTGGAAACAGG
GGTCCCATCAAGGTTTCAGTGGAGGTGGGTCTGGGACAGACTTTACCTTCACCATCAG
CAGCCTGCAGGCTGAAGATTTTGCAACATATTATTGTCAACAGTATGCTAATCTCCC
GCTCACTTTCGGCGGAGGGACCAAGCTGGAGATCAAAG

1-408, SEQ ID NO: 99

GAGCTCGTGCTGACTCAGCCACCCTCGGTGTCAGTGTCCCCAGGACAGACGGCCAG
GATCACCTGCTCTGGAGATGCATTGCCAAAGCAATATGCTTATTGGTACCAGCAGAA
GCCAGGCCAGGCCCTGTGCTGGTGATATATAAAGACAGTGAGAGGCCCTCAGGGA
TCCCTGAGCGATTCTCTGGCTCCAGCTCAGGGACAACAGTCACGTTGACCATCAGTG
15 GAGTCCAGGCAGAAGACGAGGCTGACTATTACTGTCAATCAGCAGACAGCAGTGGT
ACTTCCTGGGTGTTTCGGCGGAGGCACCAAGCTGACCGTCCTAG

1-410, SEQ ID NO: 100

GAGCTCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTC
20 ACCATCACTTGCCAGGCGAGTCAGGACATTAGCAACTATTTAAATTGGTATCAGCAG
AAACCAGGGAAAGCCCCCTAAGCTCCTGATCTACGATGCATCCAATTTGGAAACAGG
GGTCCCATCAAGGTTTCAGTGGAAAGTGGATCTGGGACAGATTTTACTTTCACCATCAG
CAGCCTGCAGCCTGAAGATATTGCAACATATTACTGTCAACAGTATGATAATCTCCC
CCTCACCTTCGGCCAAGGGACACGACTGGAGATTAAAG

1-413, SEQ ID NO: 101

GAGCTCGAGCTGACTCAGCCACCCTCAGTGTCTGAGTGTCCCCAGGACAGACGGCCAG
GATCACCTGCTCTGGAGGTGCATTGCCAAAGCATTATGTTTATTGGTACCAGCAGAA
GCCAGGCCAGGCCCTGCGGTGGTAATATATAAAGACACTGAGAGGCCCTCAGGGA
30 TCCCTGAGCGATTCTCTGGCTCCACCTCAGGGGCAACAGTCACGTTGACCATCAGTG
GAGTCCAGGCAGATGACGACGCTGTCTATTTCTGTCAATCAGTAGACAGCAATGATA
CTTCTTGATATTCGGCGGAGGCACCAAGCTGACCGTCCTAG

1-415, SEQ ID NO: 102

GAGCTCGAGCTGACTCAGCCACCCTCAGTGTCTGGGTCTCCTGGACAGTCGATCACC
35 ATCTCCTGCACTGGAACCAGCAGTGACGTTGGTGGTTATAACTATGTCTCCTGGTAC
CAACAGCACCCAGGCAAAGCCCCCAAACATCATGATTTATGAGGTCAGTAATCGGCC
CTCAGGGGTTTCTAATCGCTTCTCTGGCTCCAAGTCTGGCAACACGGCCTCCCTGAC
CATCTCTGGGCTCCAGGCTGAGGACGAGGCTGATTATTACTGCAGCTCATATACAAG
40 CAGCAGCACTGTGGTATTCGGCGGAGGCACCAAGCTGACCGTCCTAG

1-416, SEQ ID NO: 103

GAGCTCGTGCTGACTCAGCCACCCTCGGTGTCAGTGTCCCCAGGACAGACGGCCAG
GATCACCTGCTCTGGAGATGCATTTCTAAGCACTATGCTTATTGGTACCAGCAGAA
45 GCCAGGCCAGGCCCTGTATTGGTGATCTATAAAGACACTGAGAGGCCCTCAGGGA
TACCTGAGCGATTCTCTGGCTCCAGCTCAGGGACAACAGCCACGTTGACCATCAGTG

GAGTCCAGGCAGAAGATGAAGCTGACTATTACTGTCAATCAACAGACTCCAGTGAT
ACCTGGGTCTTCGGAGCTGGCACCAAGGTGACCGTCCTAG

1-417, SEQ ID NO: 104

5 GAGCTCGTGCTGACTCAGCCACCCTCAGTGTCAGTGGCCCCAGGAAAGACGGCCAG
AATTACCTGTGGGGGAAACAACATCGGAAGTAAAACCTGTGCACTGGTACCAGCAGA
AGCCAGGCCAGGCCCTGTGGTGGTCGTCTCTGATGATAGCGAACGGCCCTCAGGG
ATCCCTGAGCGATTCTCTGGCTCCAACCTCTGGGAACACGGCCACCCTTACCATCAGC
AGGGTCGAAGGCGGGGATGAGGCCGACTATTACTGTGAGGTGTGGGATAGCAGTAA
10 TGATCAGGTAGTGTTTCGGCGGAGGCACCGAGCTGACCGTCCTCG

1-418, SEQ ID NO: 105

GAGCTCGTGCTGACTCAGCCACCCTCGGTGTCAGTGTCCCCAGGACAGACGGCCAG
GATCACCTGCTCTGGAGGTGCATTGCCAAAGCATTATGCCTATTGGTACCAGCAGAA
15 GCCAGGCCAGGCCCTGCGGTGGTAATATATAAAGACACTGAGAGGCCCTCCGGGA
TCCCTGAGCGATTCTCTGGCTCCACCTCAGGGACAACAGTCACGTTGACCATCAGTG
GAGTCCAGGCAGATGACGACGCTGTCTATTTCTGTCAATCAGTAGACAGCAATGATA
CTTCTTGATATTCGGCGGAGGCACTAAGCTGACCGTCCTAG

20 1-420, SEQ ID NO: 106

GAGCTCGAGCTGACTCAGCCACCCTCAGTGTC AATTTCCCCGGGAAAGACGGCCAA
GATTTTCTGTGGGGGAAACAGCATTGGACGTAAAAGTGTGCACTGGTACCAGCAGA
AGCCAGGCCAGGCCCTGTGCTGGTCGTCTATGATGATAGCGACCGGCCCTCAGGG
ATCCCTGAGCGATTCTCTGGCTCCAACCTCTGGGAACACGGCCACCCTGACCATCAGC
25 AGGGTCGAAGCCGGGGATGAGGCCGACTATTACTGTGAGGTGTGGGATAGTAGTAG
TGATTACGTGGTATTCGGCGGAGGCACCAAGCTGACCGTCCTAG

1-423, SEQ ID NO: 107

GAGCTCGTGCTGACTCAGCCACCCTCAGTGTCAGTGGCCCCAGGAAAGACGGCCAG
30 GGTTACCTGTGGGGGAGGCAACATTGGAGATAAAGCTGTACACTGGTACCAGCAGA
GGCCAGGCCAGGCCCTGTGCTGGTCGTCTTTGGTGATAGCGCCCGGCCCTCAGGGA
TCCCTGAGCGATTCTCTGGCTCCAACCTCTGGGAACACGGCCACCCTGACCATCAGCA
GGGTCTGAAGTCGGGGATGAGGCCGACTATTACTGTGAGGTGTGGGATAGTAATAGT
GATCATCAGGTGTTCGGCGGAGGCACCAAGCTGACCGTCCTAG

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1-428, SEQ ID NO: 108

GAGCTCGAGCTGACTCAGCCGCCCTCGGTGTCAGTGTCCCCAGGACAGACGGCCAG
GATCACCTGCTCTGGAGATGCATTGCCAAAGCAATATGCTTATTGGTACCAGCAGAA
GCCAGGCCAGGCCCTGTGCTGGTGATATATAAAGACAGTGAGAGGCCCTCAGGGA
40 TCCCTGAGCGATTCTCTGGCTCCAGCTCAGGGACAACAGTCACGTTGACCATCAGTG
GAGTCCAGGCAGAAGACGAGGCTGACTATTACTGTCAATCAGCAGACAGCAGTGGT
ACTTATGAGGTGTTCGGCGGAGGGACCAAGCTGACCGTCCTAG

1-431, SEQ ID NO: 109

45 GAGCTCGAGCTGACTCAGCCACCCTCAGTGTCAGTGTCCCCAGGACAGACGGCCAG
GATCACCTGCTCTGGAGATGCATTGCCAAAGCAATATGCTTATTGGTACCAGCAGAA

GCCAGGCCAGGCCCTGTGCTGGTGATATATAAAGACAGTGAGAGGCCCTCAGGGA
TCCCTGAGCGATTCTCTGGCTCCAGCTCAGGGACAACAGTCACGTTGACCATCAGTG
GAGTCCAGGCAGAAGACGAGGCTGACTATTACTGTCAATCAGCAGACAGCAGTGGT
ACTTACGTGTTTCGGCGGAGGCACCAAGCTGACCGTCCTAG

5

1-432, SEQ ID NO: 110

GAGCTCGTGCTGACTCAGCCACCTTCGATGTCAGTGGTCCCAGGACAGACGGCCAG
GATTACCTGTGGGGGAGACAACATTGGAAGTAAAAGTGTTTCATTGGTACCAGCAAA
AGCCAGGCCAGGCCCTGTTCTGGTTCGTCATGATGATACCGAGCGGCCCTCAGGA
ATCCCCGACCGATTCTCTGGCTCCAACCTCTGGGAACACGGCCACCCTGGTCATCAGC
AGGGTCGGGGCCGGGGATGAGGCCGACTATTTCTGTCAGGTGTGGGATAGCAGGAG
TGATCATCAGGTGTTTCGGCGGAGGCACCAAGCTGACCGTCCTAG

10

1-434, SEQ ID NO: 111

GAGCTCGTGTTGACGCAGCCGCCCTCGGTGTCAGTGGCCCCAGGACAGACGGCCAG
GATTACCTGTGGGGGAAACAACATTGGAAGTAAAAGTGTCAGTGGTACCAGCAGA
AGCCAGGCCAGGCCCTGTGCTGGTTCGTCATGATGATAGCGACCGGCCCTCAGGG
ATCCCTGAGCGATTCTCTGGCTCCAATTCTGGGAACACGGCCACCCTGACCATCAGC
AGGGTCGAAGCCGGGGATGAGGCCGACTATTACTGTCAGGTGTGGGATAGTAGTAG
TGATCATGTGGTATTCGGCGGAGGGACCGAGCTGACCGTCCTCG

20

1-437, SEQ ID NO: 112

GAGCTCGCCCTGACTCAGCCTCCCTCCGTGTCTGGGTCTCCTGGACAGTCGATCACC
ATCTCCTGCACTGGAACCAGCAGTGACGTTGGTGGTTATAACTATGTCTCCTGGTAC
CAACAGCACCCAGGCAAAGCCCCCAAATCATGATTTATGAGGTCAGTAATCGGCC
CTCAGGGGTTTCTAATCGCTTCTCTGGCTCCAAGTCTGGCAACACGGCCTCCCTGAC
CATCTCTGGGCTCCAGGCTGAGGACGAGGCTGATTATTACTGCAGCTCATATACAAG
CAGCACCCCTTATGTCTTCGGAAGTGGGACCAAGGTGACCGTCCTAG

25

1-438, SEQ ID NO: 113

GAGCTCGTGCTGACTCAGCCACCTTCGGTGTGTCAGTGGCCCCAGGACAGACGGCCAG
GATTACCTGTGGGGGAAACAACATTGGAAGTAAAAGTGTCAGTGGTACCAGCAGA
AGCCAGGCCAGGCCCTGTGCTGGTTCGTCATGATGATAGCGACCGGCCCTCAGGG
ATCCCTGAGCGATTCTCTGGCTCCAACCTCTGGGAACACGGCCACCCTGACCATCAGC
AGGGTCGAAGCCGGGGATGAGGCCGACTATTACTGTCAGGTGTGGGATAGTAGTAG
TGATCATAAGGTATTCGGCGGAGGGACCGAGCTGACCGTCCTCG

30

35

1-440, SEQ ID NO: 114

GAGCTCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTC
ACCATCACTTGCCGGGCAAGTCAGAGCATTAGCAGCTATTTAAATTGGTATCAGCAG
AAACCAGGGAAAGCCCCCTAAGCTCCTGATCTATGCTGCATCCAGTTTGGAAAGTGG
GGTCCCATCAAGGTTTCAGTGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAG
CAGTCTGCAACCTGAAGATTTTGCAAGTTACTACTGTCAACAGAGTTACAGTACCCC
ATTCATTTTCGGCCCTGGGACCAAGTGGATATCAAAG

45

1-441, SEQ ID NO: 115

GAGCTCGTGGTGACGCAGCCGCCCTCAGTGTCTGCGGCCCCAGGACAGAAGGTCAC
CATCTCCTGCTCTGGAAGCACCTCCAACATTGGGAACAATTATGTATCCTGGTACCA
GCAGTTCCCAGGAACAGCCCCCAAACCTCCTCATTATGACAATAATAAGCGACCCTC
AGGGATTCTGACCGATTCTCTGGCTCCAAGTCTGGCACGTCAGCCACCCTGGGCAT
5 CACCGGGCTCCAGACTGGGGACGAGGCCGATTATTACTGCGGAACATGGGATAGCA
GCCTGAGTGCTGTGGTATTCGGCGGAGGCACCAAGGTGACCGTCCTAG

1-450, SEQ ID NO: 116

10 GAGCTCGTGTGCTGACTCAGCCACCCTCGGTGTCAGTGTCCCCAGGACAGACGGCCAG
GATCACCTGCTCTGGAGATGCATTGCCAAAGCAATATGCTTATTGGTACCAGCAGAA
GCCAGGCCAGGCCCTGTGCTGGTGATATATAAAGACAGTGAGAGGCCCTCAGGGA
TCCCTGAGCGATTCTCTGGCTCCAGCTCAGGGACAACAGCCACGTTGACCATTAGTG
GAGTCCAGGCAGAAGACGAGGCTGACTATTACTGTCAATCAGCATAACAGCAGTGGT
ACTGTGGTATTCGGCGGAGGGACCGAGCTGACCGTCCTCG

15 1-451, SEQ ID NO: 117

20 GAGCTCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTC
ACCATCACTTGCCGGGCAAGTCAGAGCATTAGCAGCTATTTAAATTGGTATCAGCAG
AAACCAGGGAAAGCCCCCTAAGCTCCTGATCTATGCTGCATCCAGTTTGCAAAGTGGG
GTCCCATCAAGGTTTCAGTGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGC
AGTCTGCAACCTGAAGATTTTGCAACTTACTACTGTCAACAGAGTTACAGTACCCCG
TACACTTTTGGCCAGGGGACCAAGCTGGAGATCAAAG

1-458, SEQ ID NO: 118

25 GAGCTCGTGTGCTGACTCAGCCACCCTCAGTGTGAGTGGCCCCAGGAAAGACGGCCAG
GATTCCCTGTGGGGGAAACAACATTGGAGGTAAAAGTGTGCACTGGTACCAGCAGA
GGCCAGGCCAGGCCCTGTGCTGGTCGTCTATTCTGATAGCGTCCGGGCCCTCGGGGA
TCCCTGAGCGATTCTCTGGCTCCAACCTCTGGGAACACGGCCACCCTGACCATCAGCA
GGGTCGAAGCCGGGGATGAGGCCGACTATTACTGTCAGGTGTGGGATAGAAGTAGT
30 GATCATGTGGTATTCGGCGGAGGCACCCAGCTGACCGTCCTCG

2-102, SEQ ID NO: 119

35 GAGCTCGTGGTGACGCAGCCGCCCTCGGTGTCAGTGGCCCCAGGACAGACGGCCAG
GATTACCTGTGGGGGAAACAACATTGGAAGTATAAATGTGCACTGGTACCAGCAGA
AGCCAGGCCAGGCCCTGTACTGGTCGTCTATGATGATAGCGCCCGGCCCTCAGGG
ATCCCTGAGCGATTTTCTGGCTCCAACCTCTGGGAACACGGCCACCCTGACCATCAGC
AGGGTCGAAGCCGGGGATGAGGCCGACTATTACTGTCAGGTGTGGGATAGTAGAAG
TGATTATTGGGTGTTTCGGCGGAGGCACCAAGGTGACCGTCCTAG

2-103, SEQ ID NO: 120

40 GAGCTCGTGTGCTGACTCAGCCACCCTCGGTGTCAGTGGCCCCAGGACAGACGGCCAG
GATTACCTGTGGGGGAAACAACATTGGAAGTAAAAGTGTGCACTGGTACCAGCAGA
AGCCAGGCCAGGCCCTGTAGTGGTCGTCTATGATGATAGCGACCGGCCCTCAGGG
ATCCCTGAGCGATTCTCTGGCTCCAACCTCTGGGAACACGGCCACCCTGACCATCAGC
45 AGGGTCGAAGCCGGGGATGAGGCCGACTATTACTGTCAGGTGTGGGATAGTAGTAG
TGATCATTGGGTGTTTCGGCGGAGGCACCGAGCTGACCGTCCTCG

2-106, SEQ ID NO: 121

GAGCTCGTGCTGACTCAGCCACCCTCGGTGTCAGTGGCCCCAGGACAGACGGCCAG
GATTACCTGTGGGGGAAACAACATTGGAAGTAAAAGTGTGCACTGGTACCAGCAGA
5 AGCCAGGCCAGGCCCTGTGCTGGGCGTCTATGATGATAGCAACCGGCCCTCAGGG
ATCCCTGAGCGATTCTCTGGCTCCAACCTCTGGGAACACGGCCACCCTGACCATCAGC
AGGGTCGAAGCCGGGGATGAGGCCGACTATTACTGTCAGGTGTGGGATAGTAGTAG
TGATCATTGGGTGTTTCGGCGGAGGCACCCAGCTGACCGTCCTCG

2-108, SEQ ID NO: 122

GAGCTCGTGGTGACGCAGCCGCCCTCAGCGTCTGGGACCCCCGGGCAGAGGGTCAC
CATCTCTTGTCTGGAAGCAGCTCCAACATCGGAAGTAATTATGTATACTGGTACGA
GCAGCTCCCAGGAACGGCCCCCAAACCTCCTCATCTATAGGAATAATCAGCGGCCCTC
AGGGGTCCCTGACCGATTCTCTGGCTCCAAGTCTGGCACCTCAGCCTCCCTGGCCAT
15 CAGTGGGCTCCGGTCCGAGGATGAGGCTGATTATTACTGTGCAGCATGGGATGACA
GCCTGCGTGTTTATGTCTTCGGAAGTGGCACCAAGCTGACCGTCCTAG

2-203, SEQ ID NO: 123

GAGCTCGTGCTGACTCAGCCACCTTCGGTGTGTCAGTGGCCCCAGGACAGACGGCCAG
20 GATTACCTGTGGGGGAAACAACATTGGAAGTAAAGGTGTGCACTGGTACCAGCAGA
AGCCAGGCCAGGCCCTGTACTGGTCGTCTATGATGATAGCGACCGGCCCTCAGGG
ATCCCTGAGCGATTATCTGGCTCCAACCTCTGGGAACACGGCCACCCTGACCATCAGC
AGGGTCGAAGCCGGGGATGAGGCCGACTATTACTGTCAGGTGTGGGATAGTAGTAG
TGATCATTGGGTGTTTCGGCGGAGGCACCGAGCTGACCGTCCTCG

2-204, SEQ ID NO: 124

GAGCTCGTGCTGACTCAGCCACCCTCGGTGTCAGTGGCCCCAGGACAGACGGCCAC
GCTTACGTGTGGGGGGAACAACATTGGAAGTAGAAGTGTGCACTGGTACCAGCAGA
AGCCAGGCCAGGGCCCTGTGCTGGTCGTCTATGATGATAGCGACCGGCCCTCAGGG
30 ATCCCTGAGCGATTCTCTGGCTCCAACCTCTGGGAACACGGCCACCCTGACTATCAGC
AGGGTCGAAGCCGGGGATGACGCCGACTATTACTGTCAGGTGTGGGAGAGTACTAC
TGATCATTATGTCTTCGGAAGTGGGACCAAGCTGACCGTCCTAG

2-206, SEQ ID NO: 125

GAGCTCGTGTTGACGCAGCCGCCCTCGGTGTCAGTGGCCCCAGGACAGACGGCCAG
35 GATTACCTGTGGGGGAAACAACATTGGAAGTAAAATGTGCACTGGTACCAGCAGA
AGCCAGGCCAGGCCCTGTGCTGGTCGTCTATGATGATAGCGACCGACCCTCAGGG
ATCCCTGAGCGATTCTCTGGCTCCAACCTCTGGGAACACGGCCACCCTGACCATCAGC
AGGGTCGAAGCCGGGGATGAGGCCGACTATTACTGTCAGGTGTGGGATAGTAGTAG
40 TGATCATTGGGTGTTTCGGCGGAGGCACCAAGGTGACCGTCCTAG

2-207, SEQ ID NO: 126

GAGCTCGTGCTGACTCAGCCACCTTCGGTGTGTCAGTGGCCCCAGGACAGACGGCCAG
GATTACCTGTGGGGGAAACAACATTGGAAGTAAAAGTGTGCACTGGTACCAGCAGA
45 AGCCAGGCCAGGCCCTGTGCTGGTCGTCCATGATGATAGCGACCGGCCCTCAGGG
ATCCCTGAGCGATTCTCTGGCTCCAACCTCTGGGAACACGGCCACCCTGACCATCGGC

AGGGTCGGAGCCGGGGATGAGGCCGACTATTACTGTCAGGTGTGGGATAGTAGTAG
TGATCATTGGGTGTTTCGGCGGAGGCACCCAGCTGACCGTCCTCG

2-301, SEQ ID NO: 127

5 GAGCTCGTGCTGACTCAGCCACCCTCGGTGTCAGTGGCCCCAGGACAGACGGCCAG
GATTATCTGTGGGGGAAACAACATTGGAAGTAAAAGTGTGCACTGGTACCAGCAGA
AGCCAGGCCAGGCCCTGTGTTGGTCGTCTATGATGATAGCGACCGGCCCTCAGGG
ATCCCTGAGCGATTCTCTGGCTCCAACCTCTGGGAACACGGCCGCCCTGACCATCAGC
10 AGGGTCGAAGCCGGGGATGAGGCCGACTATTATTGTCAGGTGTGGCATAGTAGTAG
TGATCATTGGGTGTTTCGGCGGAGGCACCAAGGTGACCGTCCTAG

2-302, SEQ ID NO: 128

GAGCTCGTGCTGACTCAGCCACCCTCGGTGTCAGTGGCCCCAGGACAGACGGCCAT
AATTACCTGTGGGGGAAAGCAACATTGGAAGTAAAAGTGTGCACTGGTATCAGCAGA
15 AGTCAGGCCAGGCCCTGTGCTGGTCGTCCATGATGATGCCACCGGCCCTCAGGGA
TCCCTGAGCGATTCTCTGGCTCCAACCTCTGGGAACACGGCCACCCTGACCATCAGCA
GGGTCGAAGCCGGGGATGAGGCCGACTATTACTGTCAGGTGTGGGAAAGTAGTAGT
GATCATTGGGTGTTTCGGCGGAGGCACCAAGGTGACCGTCCTAG

2-304, SEQ ID NO: 129

20 GAGCTCGTGCTGACTCAGCCACCCTCGGTGTCAGTGGCACCAGGACAGACGGCCAG
GATTACCTGTGGGGGAAACAACATTGGAAGTAAAAGTGTGCACTGGTACCAGCAGA
AGCCAGGCCAGGCCCTGTGCTGGTCGTCTATGATGATAGCGGCCGGCCCTCAGGG
ATCCCTGAGCGATTCTCTGGCTCCAACCTCTGGGAACACGGCCACCCTGACCATCAGC
25 AGGGTCGAAGCCGGGGATGAGGCCGACTATTACTGTCAGGTGTGGGATAGTAGTAG
TGATCATTGGGTGTTTCGGCGGAGGCACCAAGGTGACCGTCCTAG

2-305, SEQ ID NO: 130

30 GAGCTCGTGCTGACTCAGCCACCCTCGGTGTCAGTGGCACCAGGACAGACGGCCAG
GATTACCTGTGGGGGAAACAACATTGGAAGTAAAAGTGTGCACTGGTACCAGCAGA
AGCCAGGCCAGGCCCTGTGCTGGTCGTCTATGATGATAGCGACCGGCCCTCAGGG
ATCCCTGAGCGATTCTCTGGCTCCAACCTCTGGGAACACGGCCACCCTGACCATCAGC
AGGGTCGAAGCCGGGGATGAGGCCGACTATTACTGTCAGGTGTGGGATAGTAATAG
TGATCATTGGGTGTTTCGGCGGAGGCACCGAGCTGACCGTCTTCG

35

2-406, SEQ ID NO: 131

GAGCTCGTGCTGACTCAGCCACCCTCGGTGTCAGTGGCCCCAGGACAGACGGCCAG
GATTACCTGTGGGGGAAACAACATTGGAAGTAAAAGTGTGCACTGGTACCAGCAGA
AGCCAGGCCGGGCCCTGTGCTGGTCGTCTATGATGATAGCGAGCGGCCCTCAGGG
40 ATCCCTGAGCGATTCTCTGGCTCCAACCTCTGGGAACACGGCCACCCTGACCATCAGC
AGGGTCGAAGCCGGGGATGAGGCCGACTATTACTGTCAGGTGTGGGAGAGTAGTAG
TGATCATTGGGTGTTTCGGCGGAGGGACCAAGGTGACCGTCCTAG

2-408, SEQ ID NO: 132

45 GAGCTCGTGCTGACTCAGCCACCCTCGGTGTCAGTGGCCCCAGGACAGACGGCCAG
GATTACCTGTGGGGGAAACAACATTGGAAGTAAAAGTGTGCACTGGTACCAGCAGA

AGCCAGGCCAGGCCCTCTACTGGTCGTCTATGATGATAGCGACCGGCCCTCAGGG
ATCCCTGAGCGATTCTCTGGCTCCAACCTCTGGGAACATGGCCACCCTGACCATCAGC
AGGGTCGAAGCCGGGGATGAGGCCAACTATTACTGTCAGGTGTGGGATAGTAGTAG
TGATCATGTGGTATTCGGCGGAGGGACCAAGCTGACCGTCCTAG

5

3-301, SEQ ID NO: 133

GAGCTCGTGCTGACTCAGCCACCCTCGGTGTCAGTGTCCCCAGGACAGACGGCCAG
GATCACCTGCTCTGGAGATGCATTGCCAAAGCAATATGCTTATTGGTACCAGCAGAA
GCCAGGCCAGGCCCTGTGTTGGTGATATATAAAGACAGTGAGAGGCCCTCAGGGA
10 TCCCTGAGCGATTCTCTGGCTCCAGCTCAGGGACAACAGTCACGTTGACCATCAGTG
GAGTCCAGGCAGAAGACGAGGCTGACTATTACTGTCAATCAGCAGACAGCAATGGT
ACTTATAAGGTGTTTCGGCGGAGGCACCGAGCTGACCGTCCTCG

3-302, SEQ ID NO: 134

15 GAGCTCGTGCTGACTCAGCCACCCTCAGTGTCCGTGTCCCCGGGACAGACAGCCAGC
ATCACCTGCTCTGGAGATAAATTGGGACATACATACACTTCCTGGTATCAACAGAAG
CCAGGCCAGTCCCCTGTCTAGTCATCTATCAAGATAACAGGCGGCCCTCAGGGCTC
CCTGAGCGATTCTCTGGCTCCAACCTCTGGGAACACGGCCACCCTGACCATCAGCAGG
GTCGAAGCCGGGGATGAGGCCGACTATTACTGTCAGGTGTGGGAAAGTAGTAGTA
20 TCATCTTGTATTTCGGCGGAGGCACCGAGCTGACCGTCCTCG

3-305, SEQ ID NO: 135

GAGCTCGTGCTGACTCAGCCACCCTCAGTGTGTCAGTGGCCCCAGGAAAGACGGCCAG
GATTACCTGTGGGGGAAACAACATTGGAAGTAAAAGTGTGCACTGGTACCAGCAGA
25 AGCCAGGCCAGGCCCTGTGCTGGTCGTCTATGATGATAGCGACCGGCCCTCAGGG
ATCCCTGAGCGATTCTCTGGCTCCAACCTCTGGGAACACGGCCACCCTGACCATCAGC
AGGGTCGAAGCCGGGGATGAGGCCGACTATTACTGTCAGGTGTGGGATAGTAGTAG
TGATCATTGGGTGTTTCGGCGGAGGGACCCAGCTGACCGTCCTCG

3-405, SEQ ID NO: 136

GAGCTCGTGCTGACTCAGCCACCCTCAGTGTGTCAGTGGCCCCAGGAAAGACGGCCAG
GATTACCTGTGGGGGAAACAACATTGGAAGTAAAAGTGTGCACTGGTACCAGCAGA
AGCCAGGCCAGGCCCTGTCTGGTCATCTATTATGATACCGACCGGCCCTCAGGGA
TCCCTGCGCGCTTCTCTGGCTCCAACCTCTGGGAACACGGCCACCCTGACCATCAGCA
35 GGGTCGAAGCCGGGGATGAGGCCGACTATTACTGTCAGGTGTGGGATAGTAGTAGT
GACCAGAGGGTATTCGGCGGAGGCACCGAGCTGACCGTCCTCG

4-303, SEQ ID NO: 137

GAGCTCGAGCTGACTCAGCCACCCTCAGTGTGTCAGTGGCCCCAGGAAAGGCGGCCAG
40 ACTTTCCTGCGGGGGAGAGGACATTGGAATTAAAAGTGTCCACTGGTACCAACAGA
AGACAGGCCGGGCCCTGTGTTGGTCATCTATAATGATGACGACCGGCCCTCAGGG
ATCCCTGAGCGGTTTCGCTGGCTCCAATTCTGGGAACACGGCCACCCTGACCATCAGC
AGGGTCGAGGCCGGGGATGAGGCCGACTACTATTGTGAGGTGTGGGACAGTCTTAC
TGATCGTGTCTGTTCGGCGGAGGCACCAAGCTGACCGTCCTAG

45

4-307, SEQ ID NO: 138

GAGCTCGTGCTGACTCAGCCACCCTCAGTGTGAGTGGCCCCAGGAACGACGGCCAG
 GATTACCTGTGGGGGAAACAACATTGGAAGTAAAAGTGTGCACTGGTACCAGCAGA
 AGCCAGGCCAGGCCCTGTGCTGGTCATTTATTATGATAGCGACCGGCCCTCAGGGA
 TCCCTGAGCGATTCTCTGGCTCCAACCTCTGGGAACACGGCCACCCTGACCATCAGCA
 5 GGGTCGAAGCCGGGGATGAGGCCGACTATTACTGTCAGGTGTGGGATAGTAGTAGT
 GATCAGGGGGTATTCGGCGGAGGGACCCAGCTGACCGTCCTCG

z1-201, SEQ ID NO: 139

10 GAGCTCGTGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTC
 ACCATCACTTGCCGGGCAAGTCAGAGCATTAGCAGCTATTTAAATTGGTATCAGCAG
 AAACCAGGGAAAGCCCCCTAAGCTCCTGATCTATGCTGCATCCAGTTTGCAAAGTGGG
 GTCCCATCAAGGTTCAAGTGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGC
 AGTCTGCAACCTGAAGATTTTGCAACTTACTACTGTCAACAGAGTTACAGTACCCCG
 TACACTTTTGGCCAGGGGACCAAGCTGGAGATCAAAG

15 z1-303, SEQ ID NO: 140

GAGCTCGTGCTGACTCAGCCACCCTCGGTGTGAGTGGCCCCAGGACAGACGGCCAG
 GATTACCTGTGGGGGAAACAACATTGGAAGTAAAAGTGTGCACTGGTACCAGCAGA
 AGCCAGGCCAGGCCCTGTGTTGGTCTATGATGATAGCGACCGGCCCTCAGGG
 20 ATCCCTGAGCGATTCTCTGGCTCCAACCTCTGGGAACACGGCCACCCTGACCATCAGC
 GGGGTGCAAGTCGGGGATGAGGCCGACTATTACTGTCAGGTGTGGGATAGTAGTCG
 TGATCATGTGGTATTCGGCGGAGGGACCGAGCTGACCGTCCTCG

z1-402, SEQ ID NO: 141

25 GAGCTCGTGATGACCCAGTCTCCACTCTCCCTGCCCCGTCACCCCTGGAGAGCCGGCC
 TCCATCTCCTGCAGGTCTAGTCAGAGCCTCCTGCATAGTAATGGATACAACATTTG
 GATTGGTACCTGCAGAAGCCAGGGCAGTCTCCACAGCTCCTGATCTATTTGGGTTCT
 AATCGGGCCTCCGGGGTCCCTGACAGGTTCAAGTGGCAGTGGATCAGGCACAGATTTT
 AACTGAAAATCAGCAGAGTGGAGGCTGAGGATGTTGGGGTTTATTACTGTATGCA
 30 AGCTCTACAACTCCTCAGACTTTTGGCCAGGGGACCAAGCTGGAGATCAAAG

Antibody Light Chains – amino acid sequences

35 1-303, SEQ ID NO: 142

ELELTQPPSVSVAPGKTARITCGGNNIGSKSVHWYQQKPGQAPVLVVYDDSDRPSGIPE
 RFSGSNSGNTATLTISRVEAGDEADYYCQVWDSSSDHYVFGTGTKVTVL

1-304, SEQ ID NO: 143

40 ELVLTQPPSVSVSPGQTARITCSGDALPKHYAYWYQQKPGQAPVVVIYKDTERPSGIPER
 FSGATSGTTTTLTISGVQAEDEADYFCQSSDINGTSWIFGGGTKLTVL

1-401, SEQ ID NO: 144

45 ELVLTQPPSVSVAPGKTARMTCTCGGNNIGGYSVHWYQQKPGQAPVLVVYHDSRPSGIP
 ERFTGSNSGNMATLTISRVEAGDEADYYCQVWDSSNDHSVFGGGTELTVL

1-403, SEQ ID NO: 145

ELVMTQSPSSLSASVGDVTITCRASQSLRGYLNWYQQKPGEAPKLLIYAAS TL RAGVP
PRFSGAGYETDFSLTISNLQLEDFATYYCQQSHNVPLTFGGG TKVEIK

5 1-404, SEQ ID NO: 146

ELVVTQPPSVSAAPGQKV TISSSGSTSNIGNNYVSWYQQFPRTAPKLLIYDNNKRPSGIPD
RFSGSKSGTSATLGITGLQTGDEADYYCGTWDSSL SAVVF GG GTKVTVL

1-405, SEQ ID NO: 147

10 ELELIQPPSVSVSPGQTARITCSGDALPKQYAYWYQQKPGQAPAMVIYKDTERPPGISER
ISGFISGTTATLTISGVQAEDEADYYCQSEDSSGILF GG GTKLTVL

1-406, SEQ ID NO: 148

15 ELELTQPPSVSVFPGQTARITCSGDTLPKQYGNWYQQKPGQAPVVVIYKDTERPSGIPER
FSGSSSGTTATLTISGVQAEDEADYYCQSADSNDWVLF GG GTKLTVL

1-407, SEQ ID NO: 149

20 ELQMTQSPSSLSASVGDRVTITCQASQDISNYLNWYQQKPGKAPKLLIYDASNLETGVPS
RFSGGGSGTDFTF TISSLQAEDFATYYCQQYANLPLTF GG GTKLEIK

1-408, SEQ ID NO: 150

ELVLTQPPSVSVSPGQTARITCSGDALPKQYAYWYQQKPGQAPVLVIYKDSERPSGIPER
FSGSSSGTTVTLTISGVQAEDEADYYCQSADSSGTSWVF GG GTKLTVL

25 1-410, SEQ ID NO: 151

ELQMTQSPSSLSASVGDRVTITCQASQDISNYLNWYQQKPGKAPKLLIYDASNLETGVPS
RFSGSGSGTDFTF TISSLQPEDIA TYYCQQYDNLPLTFGQGT RLEIK

1-413, SEQ ID NO: 152

30 ELELTQPPSVSVSPGQTARITCSGGALPKHYVYWYQQKPGQAPAVVIYKDTERPSGIPER
FSGSTSGATVTLTISGVQADDDAVYFCQSVDSNDTSWIF GG GTKLTVL

1-415, SEQ ID NO: 153

35 ELELTQPPSVSGSPGQSITISCTGTSSDVGGYNYVSWYQQHPGKAPKLMIEVSNRPSGV
SNRFSGSKSGNTASLTISGLQAEDEADYYC SSYTSSSTVVF GG GTKLTVL

1-416, SEQ ID NO: 154

40 ELVLTQPPSVSVSPGQTARITCSGDAFPKHYAYWYQQKPGQAPVLVIYKDTERPSGIPER
FSGSSSGTTATLTISGVQAEDEADYYCQSTDSSDTWVF GAGTKVTVL

1-417, SEQ ID NO: 155

ELVLTQPPSVSVAPGKTARITCGGNNIGSKTVHWYQQKPGQAPVVVVSDDSERPSGIPE
RFSGSNSGNTATLTISRVEGGDEADYYCQVWDSSNDQVVF GG GTELTVL

45 1-418, SEQ ID NO: 156

ELVLTQPPSVSVSPGQTARITCSGGALPKHYAYWYQQKPGQAPAVVIYKDTERPSGIPER
FSGSTSGTTVTLTISGVQADDDAVYFCQSVDSNDTSWIFGGGTKLTVL

1-420, SEQ ID NO: 157

5 ELELTQPPSVSISPGKTAKIFCGGNSIGRKS VHWYQQKPGQAPVLV VYDDSDRPSGIPERF
SGSNSGNTATLTISRVEAGDEADYYCQVWDSSSDYVVFGGGTKLTVL

1-423, SEQ ID NO: 158

10 ELVLTQPPSVSVAPGKTARVTCGGGNIGDKAVHWYQQRPGQAPVLV VFGDSARPSGIPE
RFGSGNSGNTATLTISRVEVGDEADYYCQVWDSDHSDHGVFGGGTKLTVL

1-428, SEQ ID NO: 159

15 ELELTQPPSVSVSPGQTARITCSGDALPKQYAYWYQQKPGQAPVLVIYKDSERPSGIPER
FSGSSSGTTVTLTISGVQAEDEADYYCQSDSSGTVEVFGGGTKLTVL

1-431, SEQ ID NO: 160

ELELTQPPSVSVSPGQTARITCSGDALPKQYAYWYQQKPGQAPVLVIYKDSERPSGIPER
FSGSSSGTTVTLTISGVQAEDEADYYCQSDSSGTVEVFGGGTKLTVL

1-432, SEQ ID NO: 161

20 ELVLTQPPSM SVVPGQTARITCGGDNIGSKSVHWYQQKPGQAPVLV VND DTERPSGIPD
RFGSGNSGNTATLVISR VGAGDEADYFCQVWDSDHSDHGVFGGGTKLTVL

1-434, SEQ ID NO: 162

25 ELVLTQPPSVSVAPGQTARITCGGNNIGSKSVHWYQQKPGQAPVLV VYDDSDRPSGIPE
RFGSGNSGNTATLTISRVEAGDEADYYCQVWDSSSDHVVFGGGTELTVL

1-437, SEQ ID NO: 163

30 ELALTQPPSVSGSPGQSITISCTGTSSDVGGYNYVSWYQQHPGKAPKLM IYEVSNRPSGV
SNRFGSGSKSGNTASLTISGLQAEDEADYYCSSYTSSTPYVFGTGTKVTVL

1-438, SEQ ID NO: 164

35 ELVLTQPPSVSVAPGQTARITCGGNNIGSKSVHWYQQKPGQAPVLV VYDDSDRPSGIPE
RFGSGNSGNTATLTISRVEAGDEADYYCQVWDSSSDHKVFGGGTELTVL

1-440, SEQ ID NO: 165

ELQMTQSPSSL SASVGDRTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLESGVPS
RFGSGSGTDFTLTISSLQPEDFASYCQSYSTPFTFGPGTKVDIK

1-441, SEQ ID NO: 166

40 ELVVTQPPSVSAAPGQKVTISCSGTSNIGNNYVSWYQQFPGTAPKLLIYDNNKRPSGIP
DRFGSGSKSGTSATLGITGLQTGDEADYYCGTWDSSLSAVVFGGGTKVTVL

1-450, SEQ ID NO: 167

45 ELVLTQPPSVSVSPGQTARITCSGDALPKQYAYWYQQKPGQAPVLVIYKDSERPSGIPER
FSGSSSGTTATLTISGVQAEDEADYYCQSA YSSGT VVFGGGTELTVL

1-451, SEQ ID NO: 168

ELQMTQSPSSLASVGDRTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPS
RFSGSGSGTDFTLTISLQPEDFATYYCQQSYSTPYTFGQGTKLEIK

5

1-458, SEQ ID NO: 169

ELVLTQPPSVSVAPGKTARIPCGGNNIGGKSVHWYQQRPGQAPVLVVYSDSVRPSGIPE
RFSGSNSGNTATLTISRVEAGDEADYYCQVWDRSSDHVVFGGGTQLTVL

10 2-102, SEQ ID NO: 170

ELVVTQPPSVSVAPGQTARITCGGNNIGSINVHWYQQKPGQAPVLVVYDDSDRPSGIPE
RFSGSNSGNTATLTISRVEAGDEADYYCQVWDSRSDYWVFGGGTGKTVL

2-103, SEQ ID NO: 171

15 ELVLTQPPSVSVAPGQTARITCGGNNIGSKSVHWYQQKPGQAPVVVVYDDSDRPSGIPE
RFSGSNSGNTATLTISRVEAGDEADYYCQVWDSSSDHWVFGGGTQLTVL

2-106, SEQ ID NO: 172

20 ELVLTQPPSVSVAPGQTARITCGGNNIGSKSVHWYQQKPGQAPVLGVYDDSDNRPSGIPE
RFSGSNSGNTATLTISRVEAGDEADYYCQVWDSSSDHWVFGGGTQLTVL

2-108, SEQ ID NO: 173

25 ELVVTQPPSASGTPGQRTVITSCGSSSNIGSNYVYWYEQLPGTAPKLLIYRNNQRPSGVP
DRFSGSKSGTSASLAISGLRSEDEADYYCAAWDDSLRVYVFGTGTKLTVL

2-203, SEQ ID NO: 174

ELVLTQPPSVSVAPGQTARITCGGNNIGSKGVHWYQQKPGQAPVLVVYDDSDRPSGIPE
RLSGNSGNTATLTISRVEAGDEADYYCQVWDSSSDHWVFGGGTQLTVL

30 2-204, SEQ ID NO: 175

ELVLTQPPSVSVAPGQTATLTCTGGNNIGSRSVHWYQQKPGQGPVLVVYDDSDRPSGIPE
RFSGSNSGNTATLTISRVEAGDDADYYCQVWESTTDHYVFGTGTKLTVL

2-206, SEQ ID NO: 176

35 ELVLTQPPSVSVAPGQTARITCGGNNIGSKNVHWYQQKPGQAPVLVVYDDSDRPSGIPE
RFSGSNSGNTATLTISRVEAGDEADYYCQVWDSSSDHWVFGGGTGKTVL

2-207, SEQ ID NO: 177

40 ELVLTQPPSVSVAPGQTARITCGGNNIGSKSVHWYQQKPGQAPVLVVHDDSDRPSGIPE
RFSGSNSGNTATLTIGRVGAGDEADYYCQVWDSSSDHWVFGGGTQLTVL

2-301, SEQ ID NO: 178

45 ELVLTQPPSVSVAPGQTARIICGGNNIGSKTVHWYQQKPGQAPVLVVYDDSDRPSGIPER
FSGNSGNTAALTISRVEAGDEADYYCQVWHSSSDHWVFGGGTGKTVL

2-302, SEQ ID NO: 179

ELVLTQPPSVSVAPGQTAITCGGSNIGTKSVHWYQQKSGQAPVLVVHDDAHRPSGIPER
FSGSNSGNTATLTISRVEAGDEADYYCQVWESSSDHWVFGGGTKVTVL

2-304, SEQ ID NO: 180

5 ELVLTQPPSVSVAPGQTARITCGGNNIESKNVHWYQQKPGQAPVLVVYDDSGRPSGIPE
RFGSNSGNTATLTISRVEAGDEADYYCQVWDSSSDHWVFGGGTKLTVL

2-305, SEQ ID NO: 181

10 ELVLTQPPSVSVAPGQTAITCGGNNIGSKSVHWYQQKPGQAPVLVVYDDSDRPSGIPE
RFGSNSGNTATLTISRVEAGDEADYYCQVWDSSSDHWVFGGGTCLTVF

2-406, SEQ ID NO: 182

15 ELVLTQPPSVSVAPGQTAITCGGNNIGSKSVHWYQQKPGRAPVLVVYDDSERPSGIPER
FSGSNSGNTATLTISRVEAGDEADYYCQVWESSSDHWVFGGGTKVTVL

2-408, SEQ ID NO: 183

ELVLTQPPSVSVAPGQTAITCGGNNIGSKNVHWYQQKPGQAPLLVVYDDSDRPSGIPE
RFGSNSGNTATLTISRVEAGDEADYYCQVWDSSSDHVFGGGTKLTVL

3-301, SEQ ID NO: 184

20 ELVLTQPPSVSVSPGQTAITCSGDALPKQYAYWYQQKPGQAPVLVIYKDSERPSGIPER
FSGSSSGTTVTLTISGVQAEDEADYYCQSADSNGTYKVFGGGTCLTVL

3-302, SEQ ID NO: 185

25 ELVLTQPPSVSVSPGQTASITCSGDKLGHTYTSWYQQKPGQSPVLVIYQDNRRPSGLPER
FSGSNSGNTATLTISRVEAGDEADYYCQVWESSSDHLVFGGGTCLTVL

3-305, SEQ ID NO: 186

30 ELVLTQPPSVSVAPGKTARITCGGNNIGSKSVHWYQQKPGQAPVLVVYDDSDRPSGIPE
RFGSNSGNTATLTISRVEAGDEADYYCQVWDSSSDHWVFGGGTCLTVL

3-405, SEQ ID NO: 187

35 ELVLTQPPSVSVAPGKTARITCGENNIGSKSVHWYQQKPGQAPVLVIYYDTDRPSGIPAR
FSGSNSGNTATLTISRVEAGDEADYYCQVWDSSSDQRVFGGGTCLTVL

4-303, SEQ ID NO: 188

ELELTQPPSVSVAPGKAARLSCGGEDIGIKSVHWYQQKTGRAPVLVIYNDDDRPSGIPER
FAGSNSGNTATLTISRVEAGDEADYYCEVWDSLTDREVFGGGTKLTVL

4-307, SEQ ID NO: 189

40 ELVLTQPPSVSVAPGTTARITCGGNNIGSKSVHWYQQKPGQAPVLVIYYDSDRPSGIPER
FSGSNSGNTATLTISRVEAGDEADYYCQVWDSSSDQGVFGGGTCLTVL

z1-201, SEQ ID NO: 190

45 ELVMTQSPSSLASVGDRTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPS
RFGSGSGTDFTLTISSLQPEDFATYYCQSYSTPYTFGGGTCLTKLEIK

z1-303, SEQ ID NO: 191

ELVLTQPPSVSVAPGQTARITCGGNNIGSKSVHWYQQKPGQAPVLVVYDDSDRPSGIPE
RFGSNSGNTATLTISGVEVGDEADYYCQVWDSSRDHVVFGGGTELTVL

5

z1-402, SEQ ID NO: 192

ELVMTQSPLSLPVTGPASISCRSSQSLHNSNGYNYLDWYLQKPGQSPQLLIYLGSNRA
SGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQALQTPQTFGQGTKLEIK

10

Table 6: Sequence listings of heavy and light chains.

SEQ ID NO	Nucleic acid Ab	Chain	SEQ ID NO	Amino Acid Ab	Chain
1	1-303	Heavy chain	46	1-303	Heavy chain
2	1-304	Heavy chain	47	1-304	Heavy chain
3	1-401	Heavy chain	48	1-401	Heavy chain
4	1-403	Heavy chain	49	1-403	Heavy chain
5	1-404	Heavy chain	50	1-404	Heavy chain
6	1-405	Heavy chain	51	1-405	Heavy chain
7	1-406	Heavy chain	52	1-406	Heavy chain
8	1-407	Heavy chain	53	1-407	Heavy chain
9	1-408	Heavy chain	54	1-408	Heavy chain
10	1-410	Heavy chain	55	1-410	Heavy chain
11	1-413	Heavy chain	56	1-413	Heavy chain
12	1-416	Heavy chain	57	1-416	Heavy chain
13	1-417	Heavy chain	58	1-417	Heavy chain
14	1-418	Heavy chain	59	1-418	Heavy chain
15	1-420	Heavy chain	60	1-420	Heavy chain
16	1-423	Heavy chain	61	1-423	Heavy chain
17	1-428	Heavy chain	62	1-428	Heavy chain
18	1-431	Heavy chain	63	1-431	Heavy chain
19	1-432	Heavy chain	64	1-432	Heavy chain
20	1-434	Heavy chain	65	1-434	Heavy chain
21	1-437	Heavy chain	66	1-437	Heavy chain
22	1-438	Heavy chain	67	1-438	Heavy chain
23	1-440	Heavy chain	68	1-440	Heavy chain
24	1-441	Heavy chain	69	1-441	Heavy chain
25	1-450	Heavy chain	70	1-450	Heavy chain
26	1-451	Heavy chain	71	1-451	Heavy chain
27	2-102	Heavy chain	72	2-102	Heavy chain
28	2-103	Heavy chain	73	2-103	Heavy chain
29	2-106	Heavy chain	74	2-106	Heavy chain
30	2-108	Heavy chain	75	2-108	Heavy chain

31	2-203	Heavy chain	76	2-203	Heavy chain
32	2-204	Heavy chain	77	2-204	Heavy chain
33	2-206	Heavy chain	78	2-206	Heavy chain
34	2-207	Heavy chain	79	2-207	Heavy chain
35	2-302	Heavy chain	80	2-302	Heavy chain
36	2-304	Heavy chain	81	2-304	Heavy chain
37	2-305	Heavy chain	82	2-305	Heavy chain
38	2-408	Heavy chain	83	2-408	Heavy chain
39	3-301	Heavy chain	84	3-301	Heavy chain
40	3-302	Heavy chain	85	3-302	Heavy chain
41	4-303	Heavy chain	86	4-303	Heavy chain
42	4-307	Heavy chain	87	4-307	Heavy chain
43	z1-201	Heavy chain	88	z1-201	Heavy chain
44	z1-303	Heavy chain	89	z1-303	Heavy chain
45	z1-402	Heavy chain	90	z1-402	Heavy chain
91	1-303	Light chain	142	1-303	Light chain
92	1-304	Light chain	143	1-304	Light chain
93	1-401	Light chain	144	1-401	Light chain
94	1-403	Light chain	145	1-403	Light chain
95	1-404	Light chain	146	1-404	Light chain
96	1-405	Light chain	147	1-405	Light chain
97	1-406	Light chain	148	1-406	Light chain
98	1-407	Light chain	149	1-407	Light chain
99	1-408	Light chain	150	1-408	Light chain
100	1-410	Light chain	151	1-410	Light chain
101	1-413	Light chain	152	1-413	Light chain
102	1-415	Light chain	153	1-415	Light chain
103	1-416	Light chain	154	1-416	Light chain
104	1-417	Light chain	155	1-417	Light chain
105	1-418	Light chain	156	1-418	Light chain
106	1-420	Light chain	157	1-420	Light chain
107	1-423	Light chain	158	1-423	Light chain
108	1-428	Light chain	159	1-428	Light chain
109	1-431	Light chain	160	1-431	Light chain
110	1-432	Light chain	161	1-432	Light chain
111	1-434	Light chain	162	1-434	Light chain
112	1-437	Light chain	163	1-437	Light chain
113	1-438	Light chain	164	1-438	Light chain
114	1-440	Light chain	165	1-440	Light chain
115	1-441	Light chain	166	1-441	Light chain
116	1-450	Light chain	17	1-450	Light chain

117	1-451	Light chain	168	1-451	Light chain
118	1-458	Light chain	169	1-458	Light chain
119	2-102	Light chain	170	2-102	Light chain
120	2-103	Light chain	171	2-103	Light chain
121	2-106	Light chain	172	2-106	Light chain
122	2-108	Light chain	173	2-108	Light chain
123	2-203	Light chain	174	2-203	Light chain
124	2-204	Light chain	175	2-204	Light chain
125	2-206	Light chain	176	2-206	Light chain
126	2-207	Light chain	177	2-207	Light chain
127	2-301	Light chain	178	2-301	Light chain
128	2-302	Light chain	179	2-302	Light chain
129	2-304	Light chain	180	2-304	Light chain
130	2-305	Light chain	181	2-305	Light chain
131	2-306	Light chain	182	2-306	Light chain
132	2-408	Light chain	183	2-408	Light chain
133	3-301	Light chain	184	3-301	Light chain
134	3-302	Light chain	185	3-302	Light chain
135	3-305	Light chain	186	3-305	Light chain
136	3-405	Light chain	187	3-405	Light chain
137	4-303	Light chain	188	4-303	Light chain
138	4-307	Light chain	189	4-307	Light chain
139	z1-201	Light chain	190	z1-201	Light chain
140	z1-303	Light chain	191	z1-303	Light chain
141	z1-402	Light chain	192	z1-402	Light chain

CLAIMS

What is claimed is:

1. A composition comprising an isolated anti-ADAMTS13 autoantibody or fragment thereof.
2. The composition of claim 1, wherein the anti-ADAMTS13 autoantibody or fragment thereof comprises a heavy chain selected from the group consisting of SEQ ID NOs: 46-90.
3. The composition of claim 1, wherein the anti-ADAMTS13 autoantibody or fragment thereof comprises a light chain selected from the group consisting of SEQ ID NOs: 142-192.
4. The composition of claim 1, wherein the anti-ADAMTS13 autoantibody or fragment thereof comprises a single chain variable fragment (scFv).
5. The composition of claim 1, wherein the anti-ADAMTS13 autoantibody or fragment thereof is capable of decreasing ADAMTS13 activity.
6. The composition of claim 5, wherein the ADAMTS13 activity is selected from the group consisting of proteolytic activity, disulfide reducing activity, interacting or attaching to an endothelial cell surface, and any combination thereof.
7. The composition of claim 1, wherein the anti-ADAMTS13 autoantibody or fragment thereof binds at least one of the ADAMTS13 region selected from the group consisting of amino-terminal (MDT1) domain, carboxy-terminal (T5-8/CUB) domain and cysteine-rich/spacer region.
8. A composition comprising an isolated nucleic acid sequence encoding an anti-ADAMTS13 autoantibody or fragment thereof.
9. The composition of claim 8, wherein the isolated nucleic acid sequence comprises a heavy chain nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1-45.

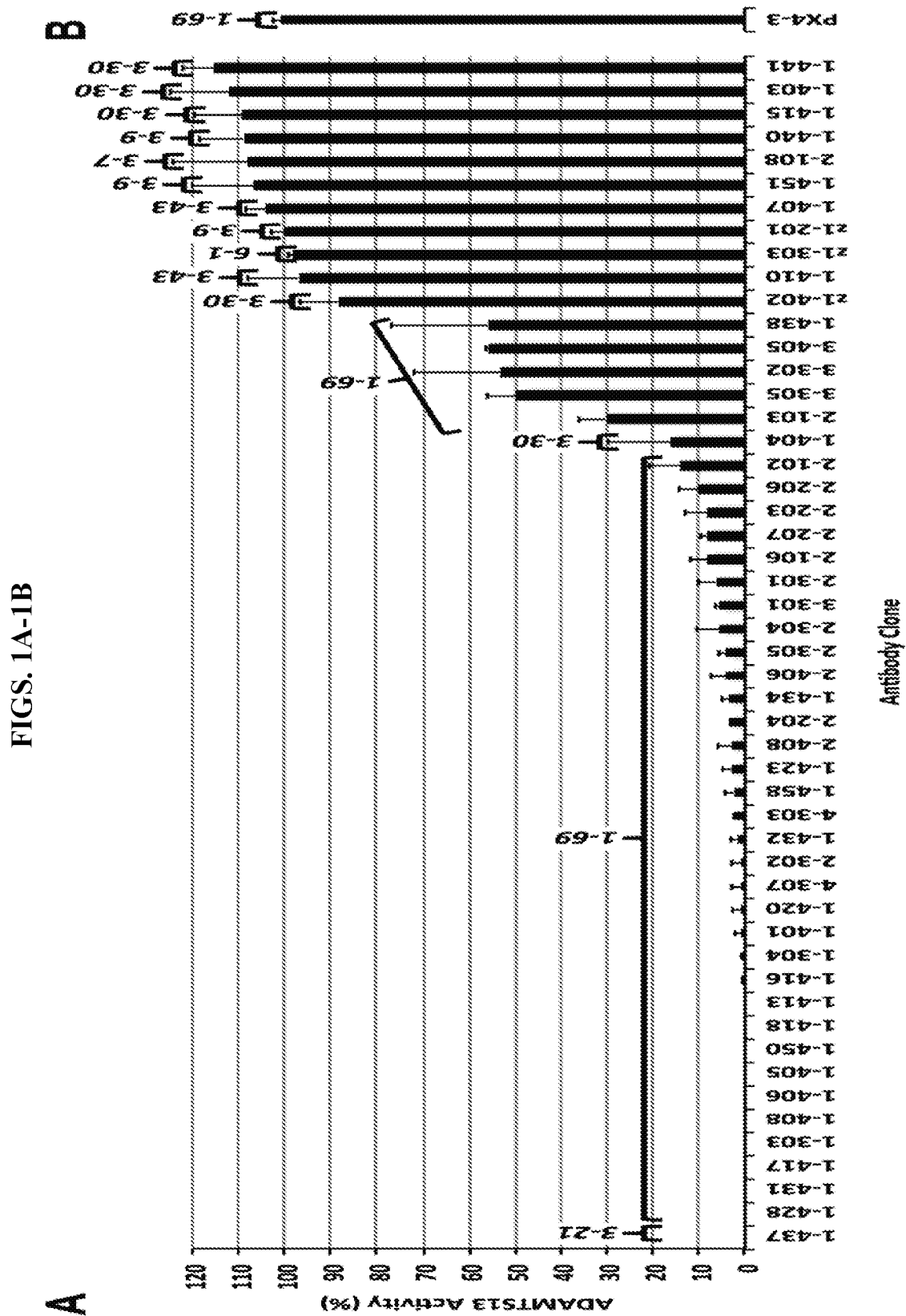
10. The composition of claim 8, wherein the isolated nucleic acid sequence comprises a light chain nucleic acid sequence selected from the group consisting of SEQ ID NOs: 91-141.
11. The composition of claim 8, wherein the isolated nucleic acid sequence encodes a single chain variable fragment (scFv).
12. The composition of claim 8, wherein the isolated nucleic acid sequence has an identity of at least 80% to at least one heavy chain nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1-45.
13. The composition of claim 8, wherein the isolated nucleic acid sequence has an identity of at least 80% to at least one light chain nucleic acid sequence selected from the group consisting of SEQ ID NOs: 91-141.
14. A method for generating an *in vivo* model of thrombotic thrombocytopenic purpura (TTP) comprising introducing at least one anti-ADAMTS13 autoantibody or fragment thereof into a model organism.
15. The method of claim 14, wherein the model organism is selected from the group consisting of a non-mammalian organism and a non-human mammalian organism.
16. The method of claim 15, wherein the mammalian organism is selected from the group consisting of a non-human primate, an ovine, a bovine, a porcine, a canine, a feline and a murine organism.
17. The method of claim 14, wherein introducing the anti-ADAMTS13 autoantibody or fragment thereof comprises formulating the anti-ADAMTS13 autoantibodies or fragments thereof in a composition for administration to the model organism.
18. The method of claim 17, wherein introducing the anti-ADAMTS13 autoantibody or fragment thereof further comprises injecting the anti-ADAMTS13 autoantibodies or fragments thereof into the model organism.
19. The method of claim 14, wherein introducing the anti-ADAMTS13 autoantibody or fragment thereof comprises inducing *in vivo* expression in the model organism.

20. The method of claim 19, wherein inducing *in vivo* expression comprises delivering nucleic acids to the model organism.
21. The method of claim 20, wherein delivering the nucleic acids through a method selected from the group consisting of injection through hydrodynamic delivery, electroporation, transfection, transduction and other methods of viral delivery, and any combination thereof.
22. The method of claim 14, wherein the anti-ADAMTS13 autoantibody or fragment thereof comprises a single chain variable fragment (scFv).
23. The method of claim 14 wherein the anti-ADAMTS13 autoantibody or fragment thereof comprises a heavy chain selected from the group consisting of SEQ ID NOs: 46-90.
24. The method of claim 14, wherein the anti-ADAMTS13 autoantibody or fragment thereof comprises a light chain selected from the group consisting of SEQ ID NOs: 142-192.
25. The method of claim 14, wherein the anti-ADAMTS13 autoantibody or fragment thereof comprises a heavy chain encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1-45.
26. The method of claim 14, wherein the anti-ADAMTS13 autoantibody or fragment thereof comprises a light chain encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 91-141.
27. The method of claim 14, wherein the anti-ADAMTS13 autoantibody or fragment thereof binds at least one of the ADAMTS13 region selected from the group consisting of amino-terminal (MDT1) domain, carboxy-terminal (T5-8/CUB) domain and cysteine-rich/spacer region.
28. An anti-autoimmune reagent, wherein the anti-autoimmune reagent specifically binds to an anti-ADAMTS13 autoantibody or fragment thereof.
29. The reagent of claim 28, wherein the anti-autoimmune reagent blocks binding of the anti-ADAMTS13 autoantibody or fragment thereof to ADAMTS13.

30. The reagent of claim 28, wherein the anti-autoimmune reagent specifically binds to at least one amino acid sequence selected from the group consisting of SEQ ID NOs: 46-90; 142-192.
31. A method for identifying an anti-autoimmune reagent for treating thrombotic thrombocytopenic purpura (TTP) comprising contacting a panel of agents with at least one anti-ADAMTS13 autoantibody or fragment thereof and identifying the agents that bind to the anti-ADAMTS13 autoantibody or fragment thereof.
32. The method of claim 31, wherein identifying the agents comprises identifying agents that block binding of the anti-ADAMTS13 autoantibody or fragment thereof to ADAMTS13.
33. The method of claim 32, wherein the binding of the anti-ADAMTS13 autoantibody or fragment thereof to ADAMTS13 is blocked to at least one of the ADAMTS13 regions selected from the group consisting of amino-terminal (MDT1) domain, carboxy-terminal (T5-8/CUB) domain and cysteine-rich/spacer region.
34. A method of inhibiting the binding of an anti-ADAMTS13 autoantibody or fragment thereof to ADAMTS13 comprising contacting the anti-ADAMTS13 autoantibody or fragment thereof with a composition comprising an anti-autoimmune reagent that specifically binds to the anti-ADAMTS13 autoantibody or fragment thereof.
35. The method of claim 34, wherein the anti-autoimmune reagent specifically binds to at least one amino acid sequence selected from the group consisting of SEQ ID NOs: 46-90; 142-192.
36. The method of claim 34, wherein the anti-autoimmune reagent blocks the binding of the anti-ADAMTS13 autoantibody or fragment thereof to at least one of the ADAMTS13 regions selected from the group consisting of amino-terminal (MDT1) domain, carboxy-terminal (T5-8/CUB) domain and cysteine-rich/spacer region.
37. A method of identifying an ADAMTS13 variant that does not bind an anti-ADAMTS13 autoantibody or fragment thereof, the method comprising contacting an ADAMTS13 protein with an anti-ADAMTS13 autoantibody or fragment thereof, wherein when the

ADAMTS13 protein does not bind the anti-ADAMTS13 autoantibody or fragment thereof, then the ADAMTS13 protein is a variant that does not bind an anti-ADAMTS13 autoantibody or fragment thereof.

38. The method of claim 37, wherein the anti-ADAMTS13 autoantibody or fragment thereof comprises at least one amino acid sequence selected from the group consisting of SEQ ID NOs: 46-90; 142-192.
39. The method of claim 37, wherein the ADAMTS13 variant comprises a preserved or enhanced proteolytic activity as compared to a native ADAMTS13.
40. The method of claim 37, wherein the ADAMTS13 variant is useful for treating thrombotic thrombocytopenic purpura (TTP).
41. A method for treating thrombotic thrombocytopenic purpura (TTP) in a subject in need thereof, the method comprising administering to the subject a composition comprising an effective amount of an ADAMTS13 variant, wherein the ADAMTS13 variant is resistant to inhibition by an anti-ADAMTS13 autoantibody or fragment thereof.
42. The method of claim 41, wherein the anti-ADAMTS13 autoantibody or fragment thereof comprises at least one amino acid sequence selected from the group consisting of SEQ ID NOs: 46-90; 142-192.
43. The method of claim 41, wherein the ADAMTS13 variant comprises a preserved or enhanced proteolytic activity as compared to a native ADAMTS13.



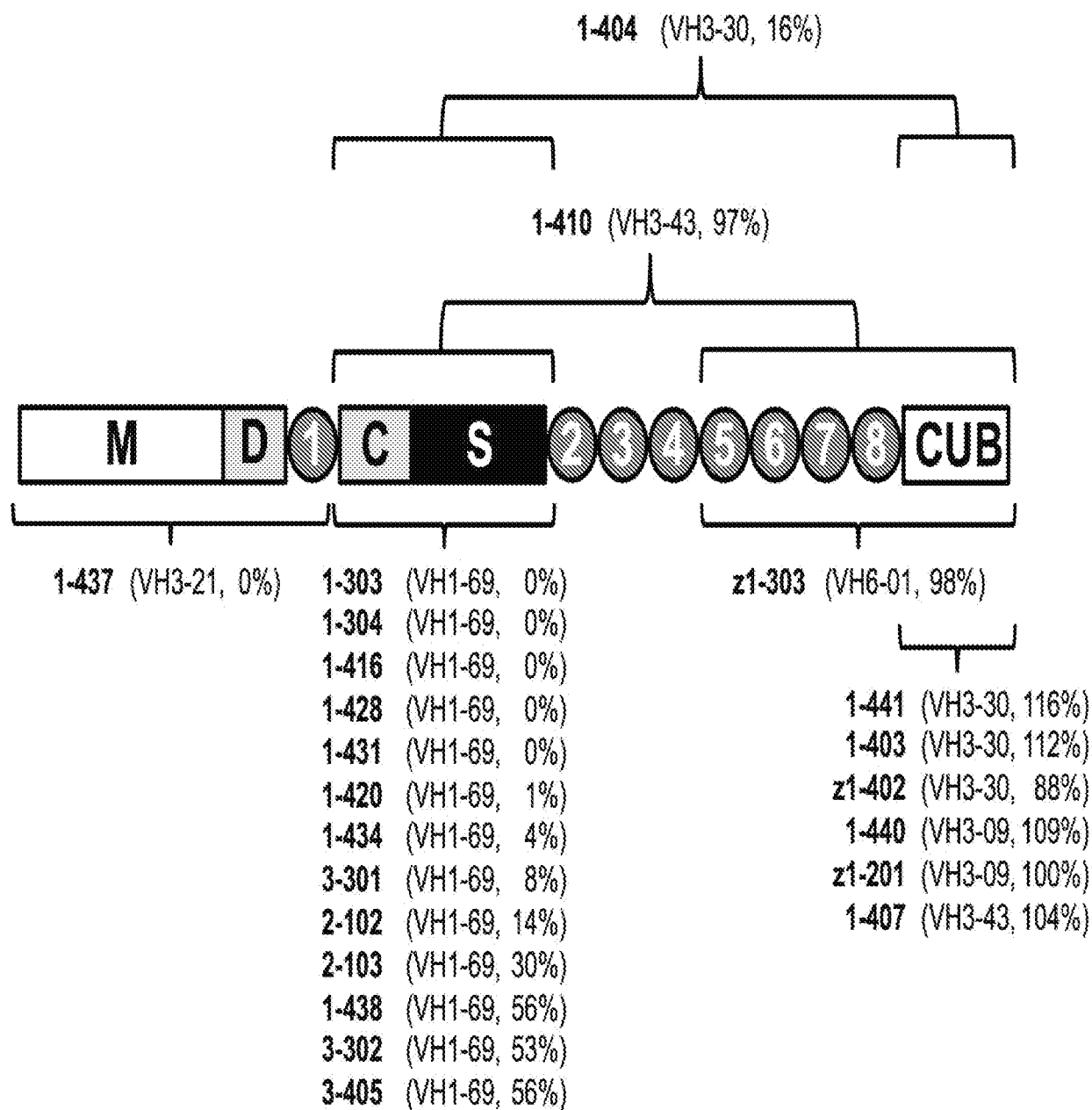
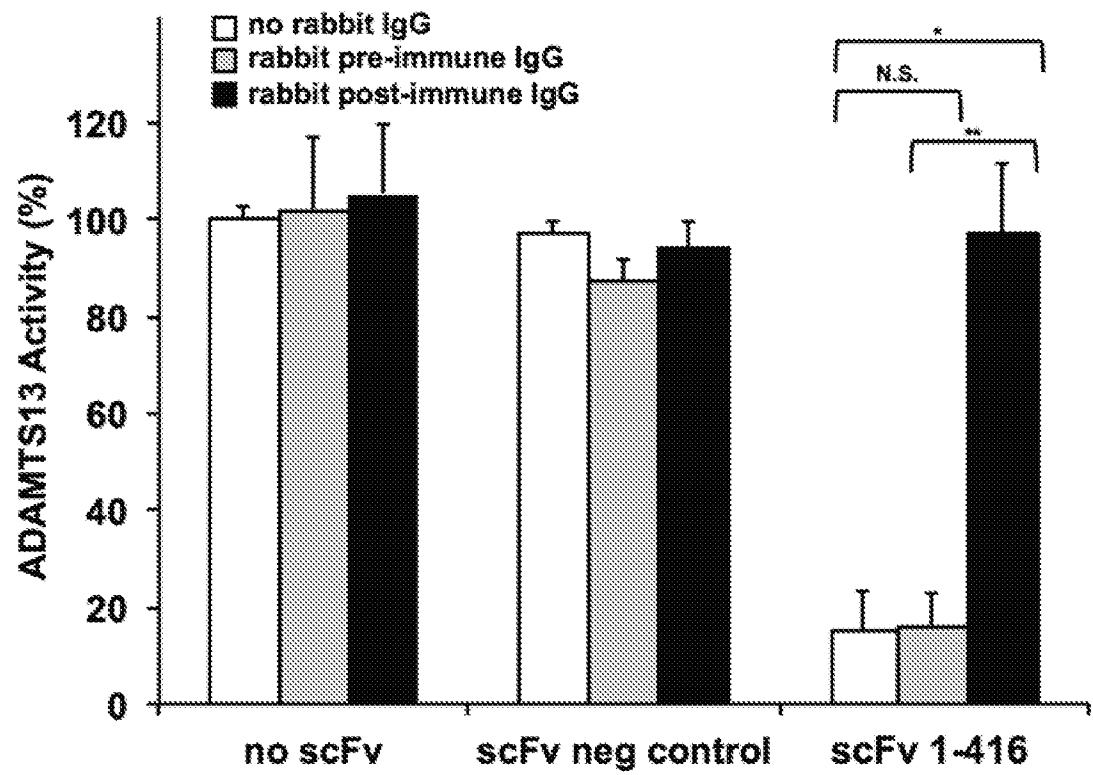
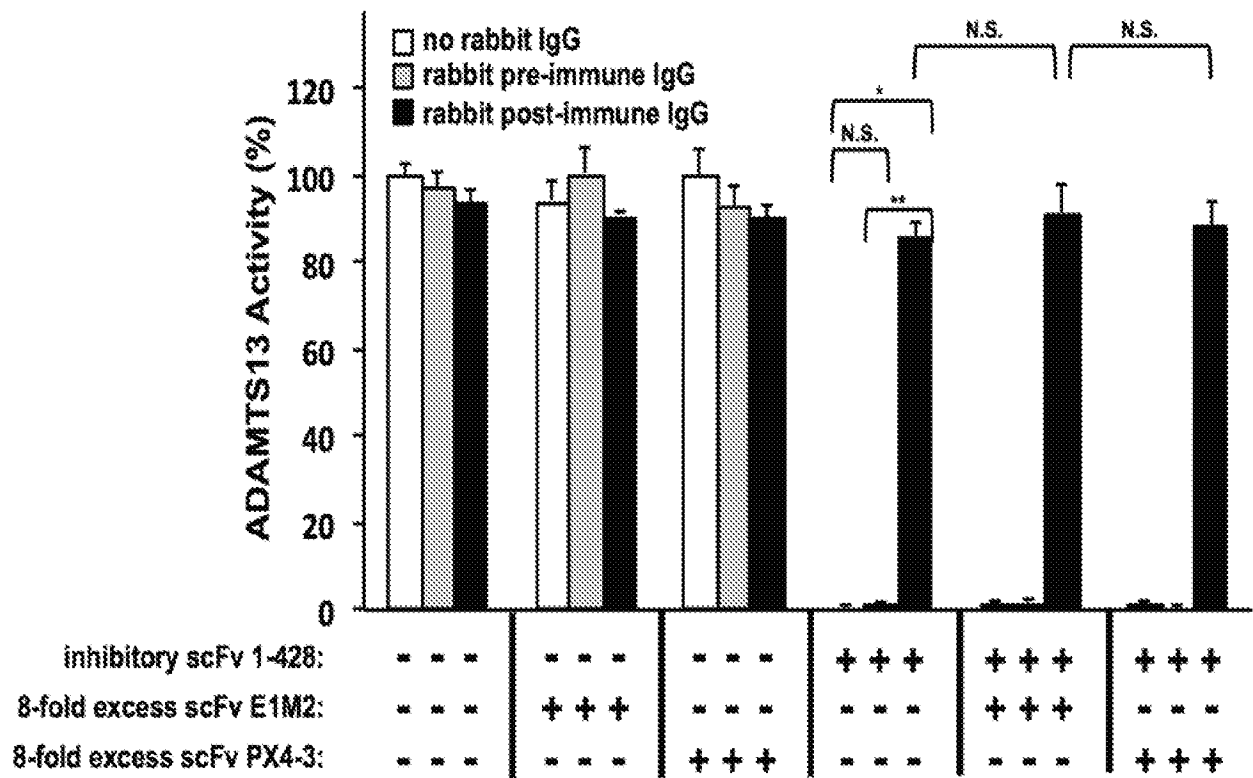


FIG. 2

A

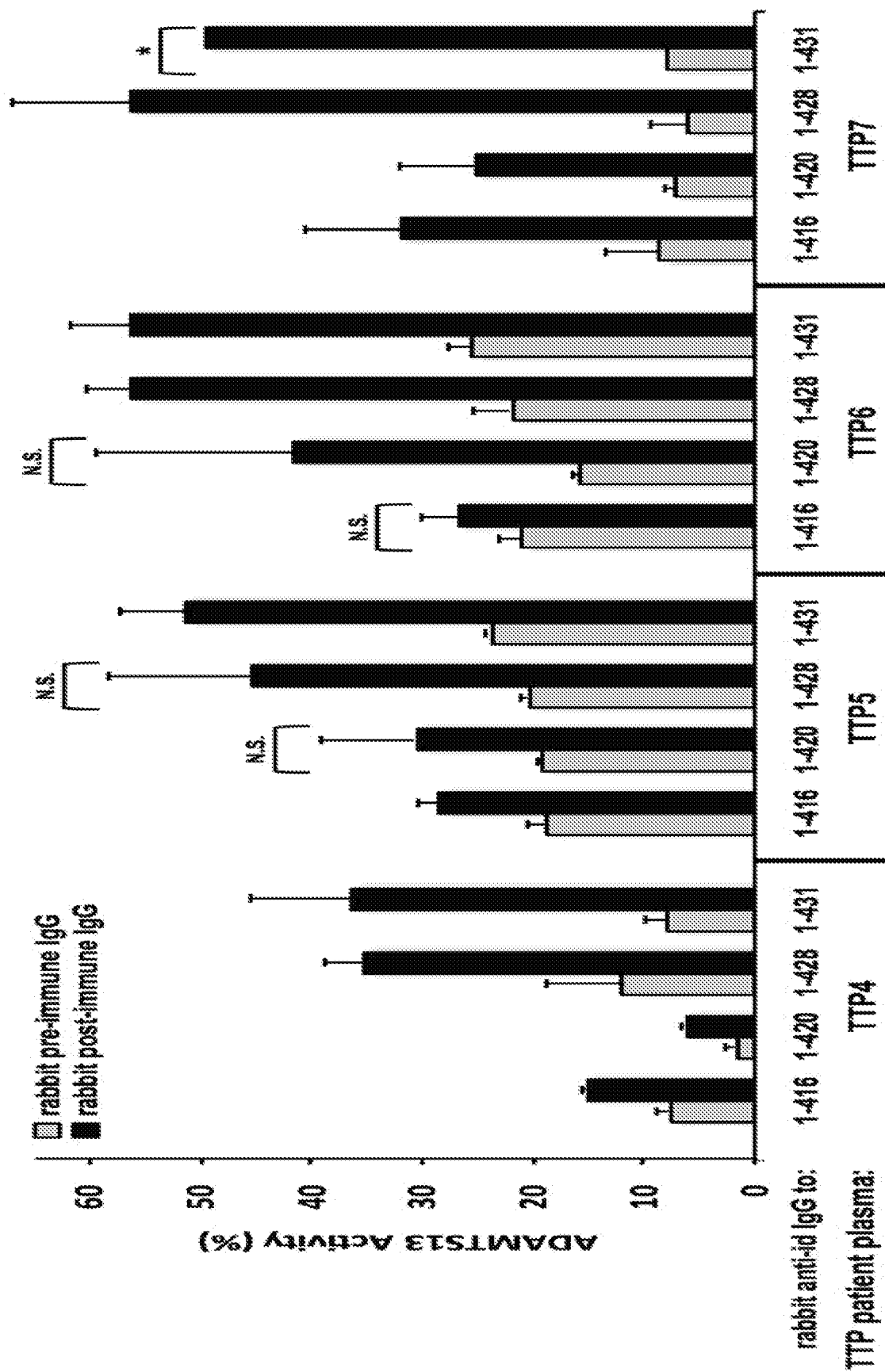


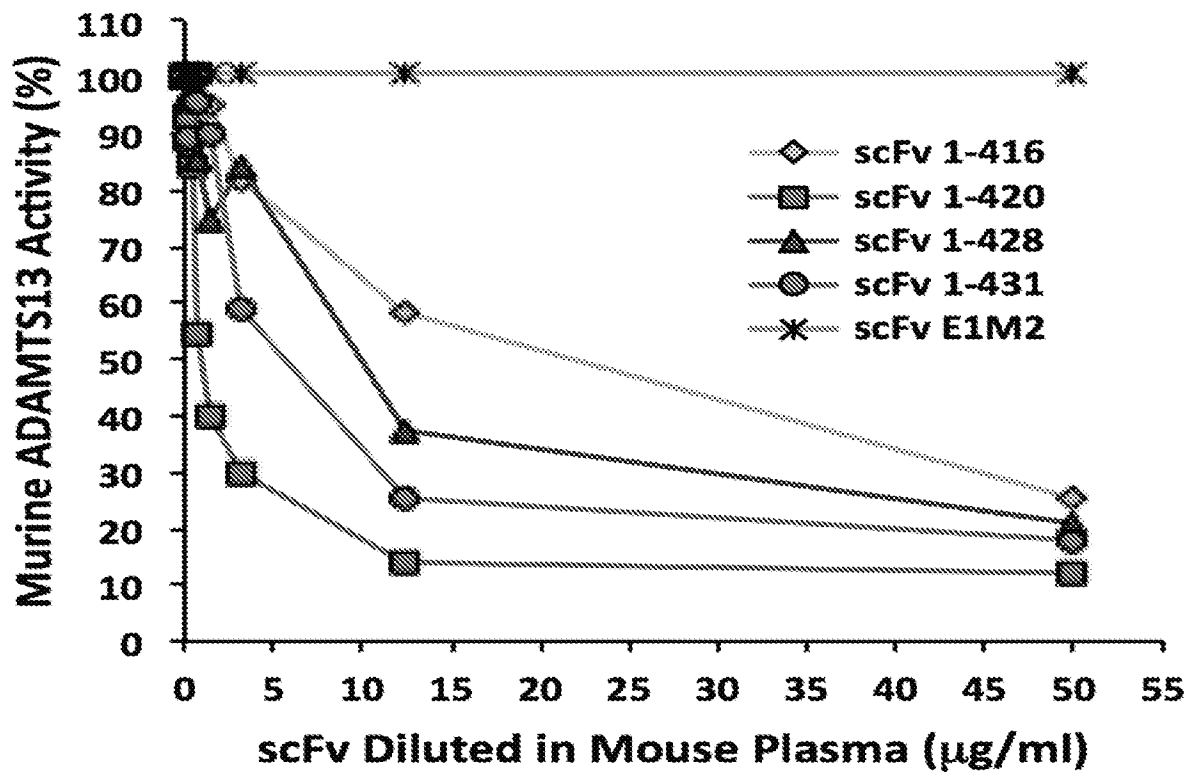
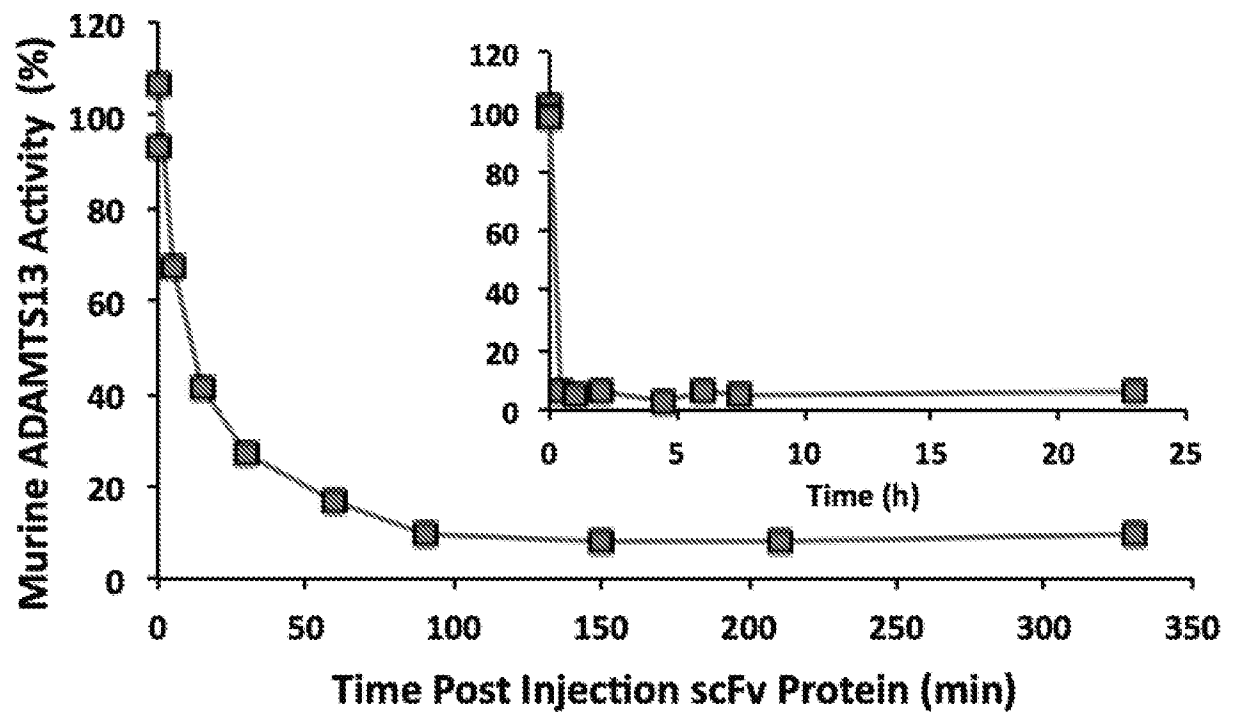
B



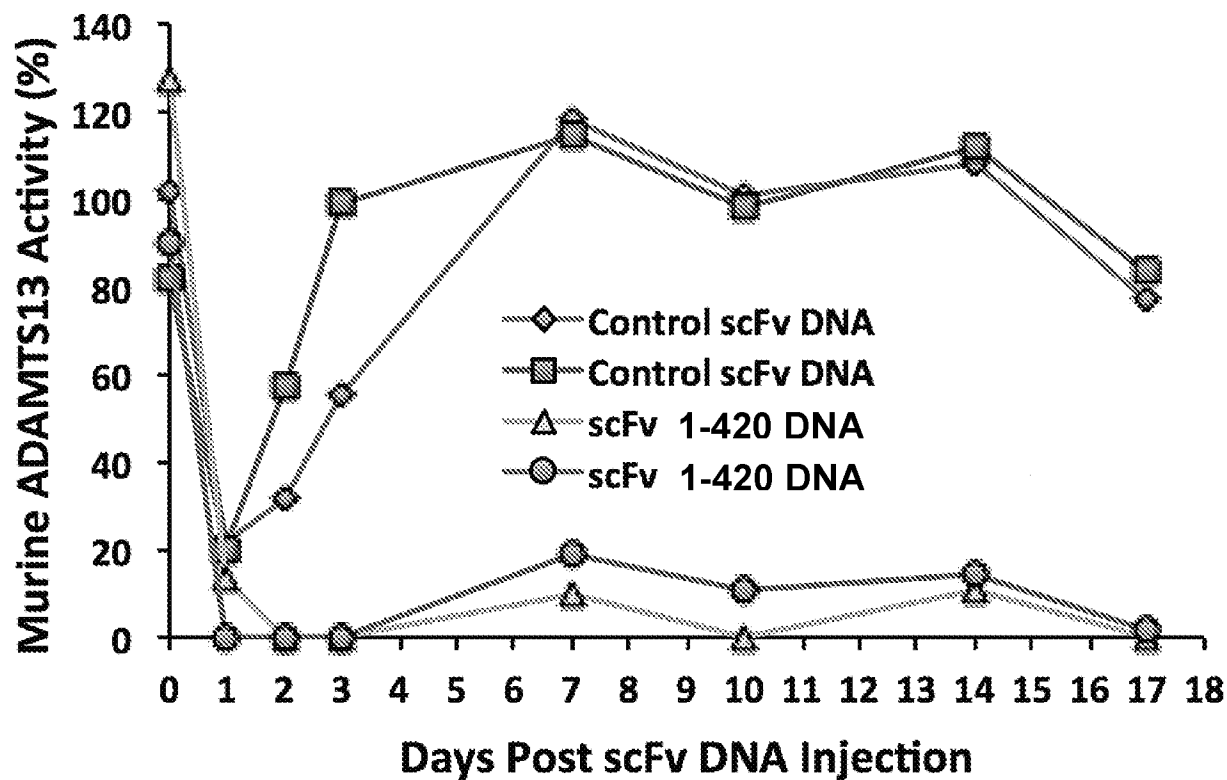
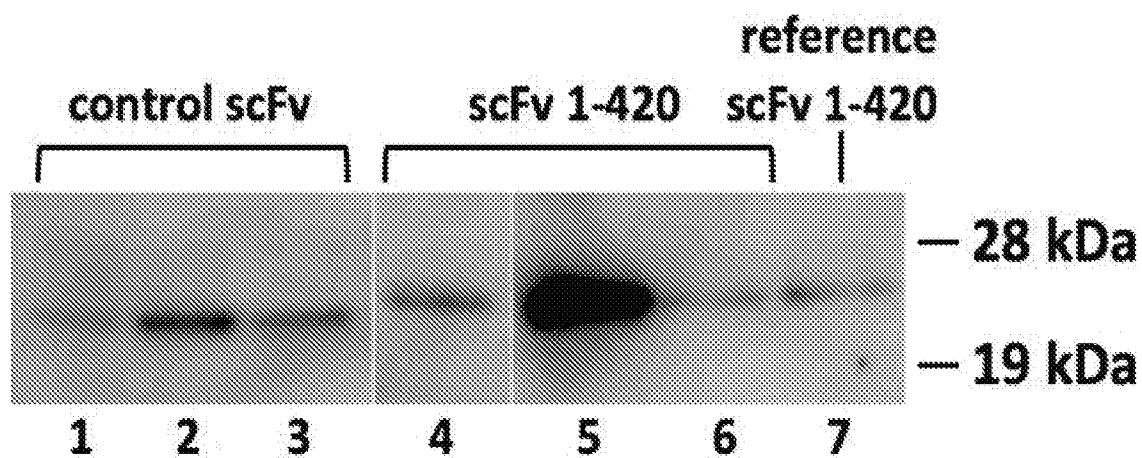
FIGS. 3A-3B

FIG. 3C



A**B**

FIGS. 4A-4B

A**B**

FIGS. 5A-5B

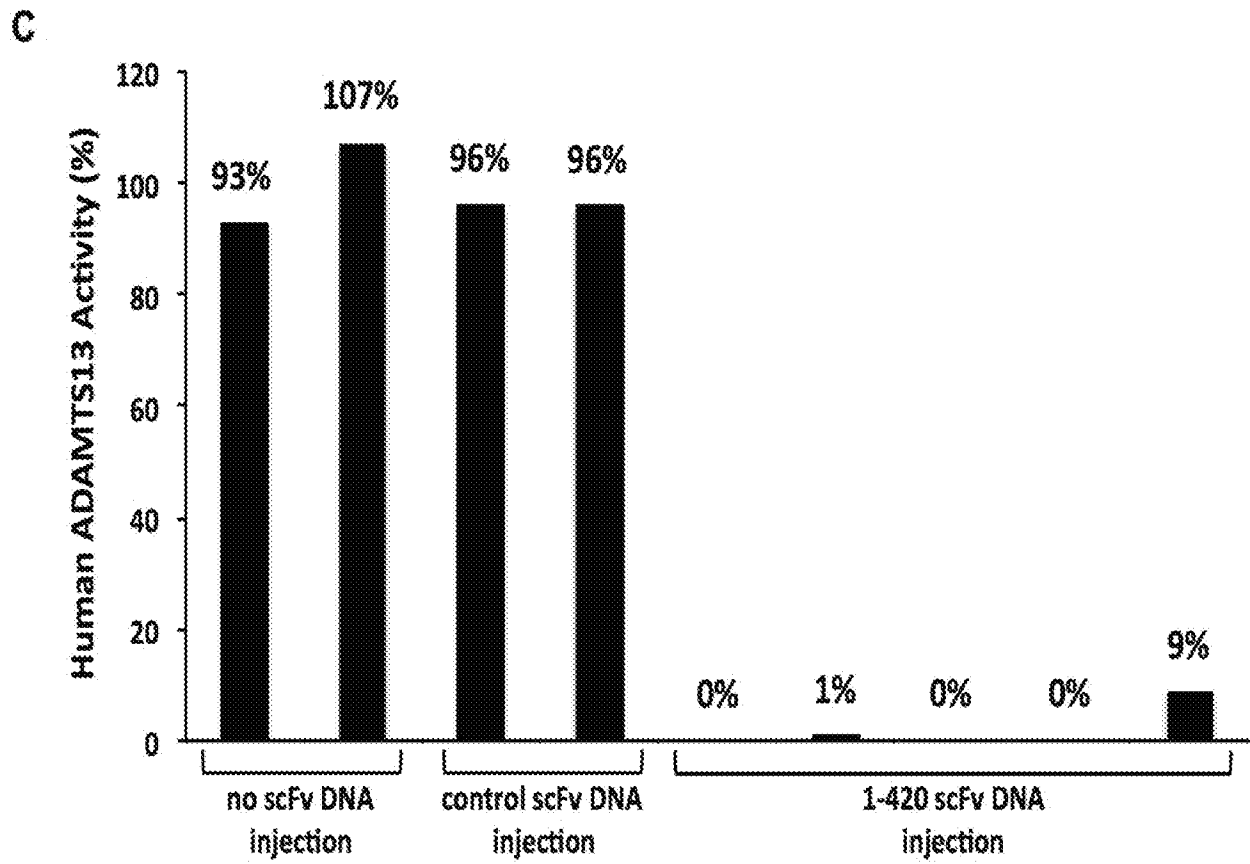
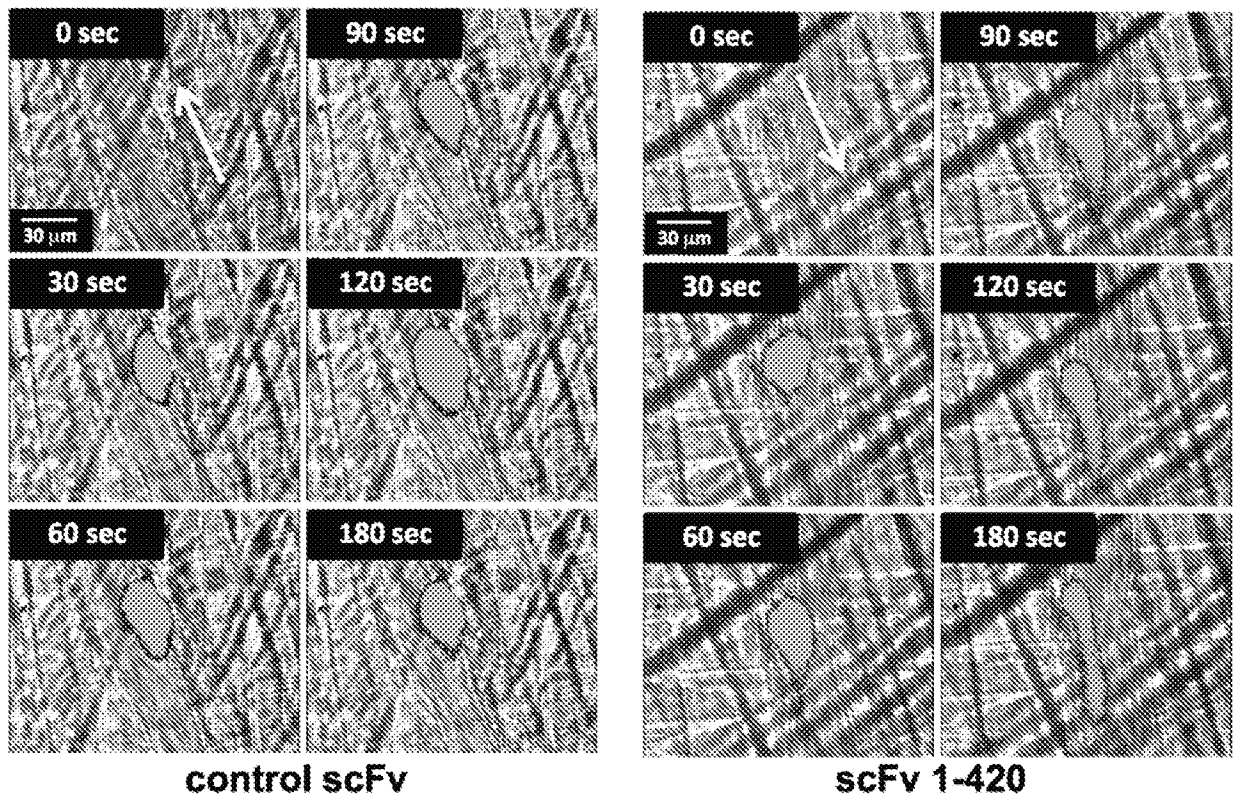
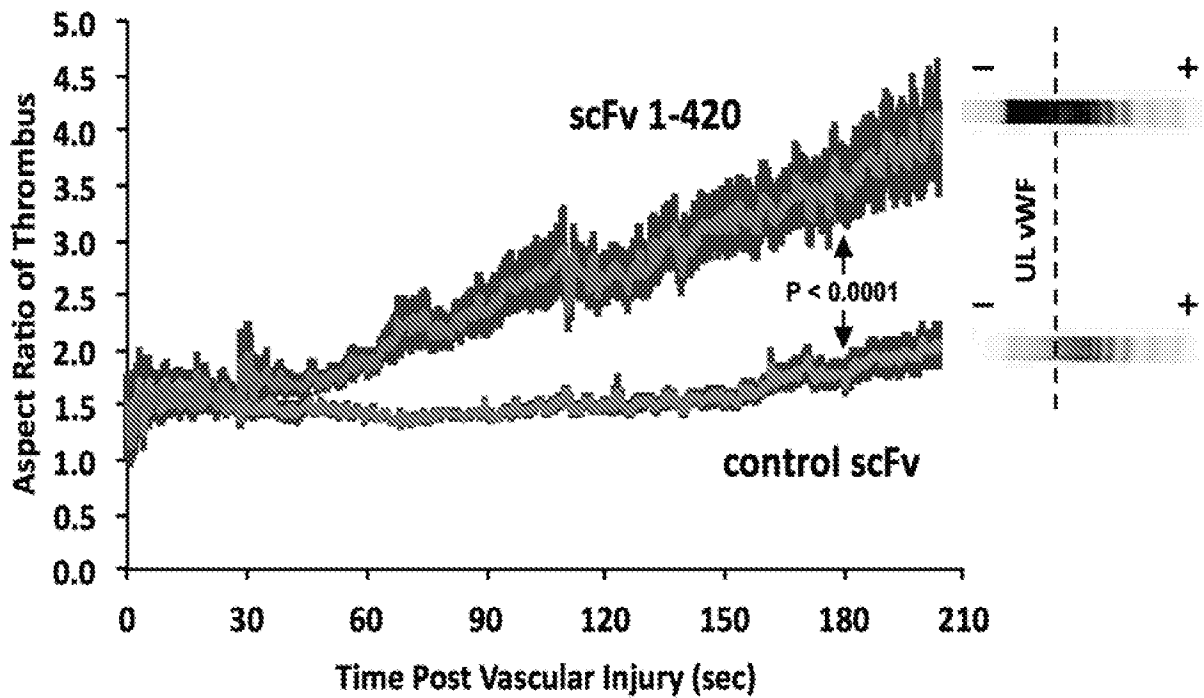


FIG. 5C

A**B**

FIGS. 6A-6B

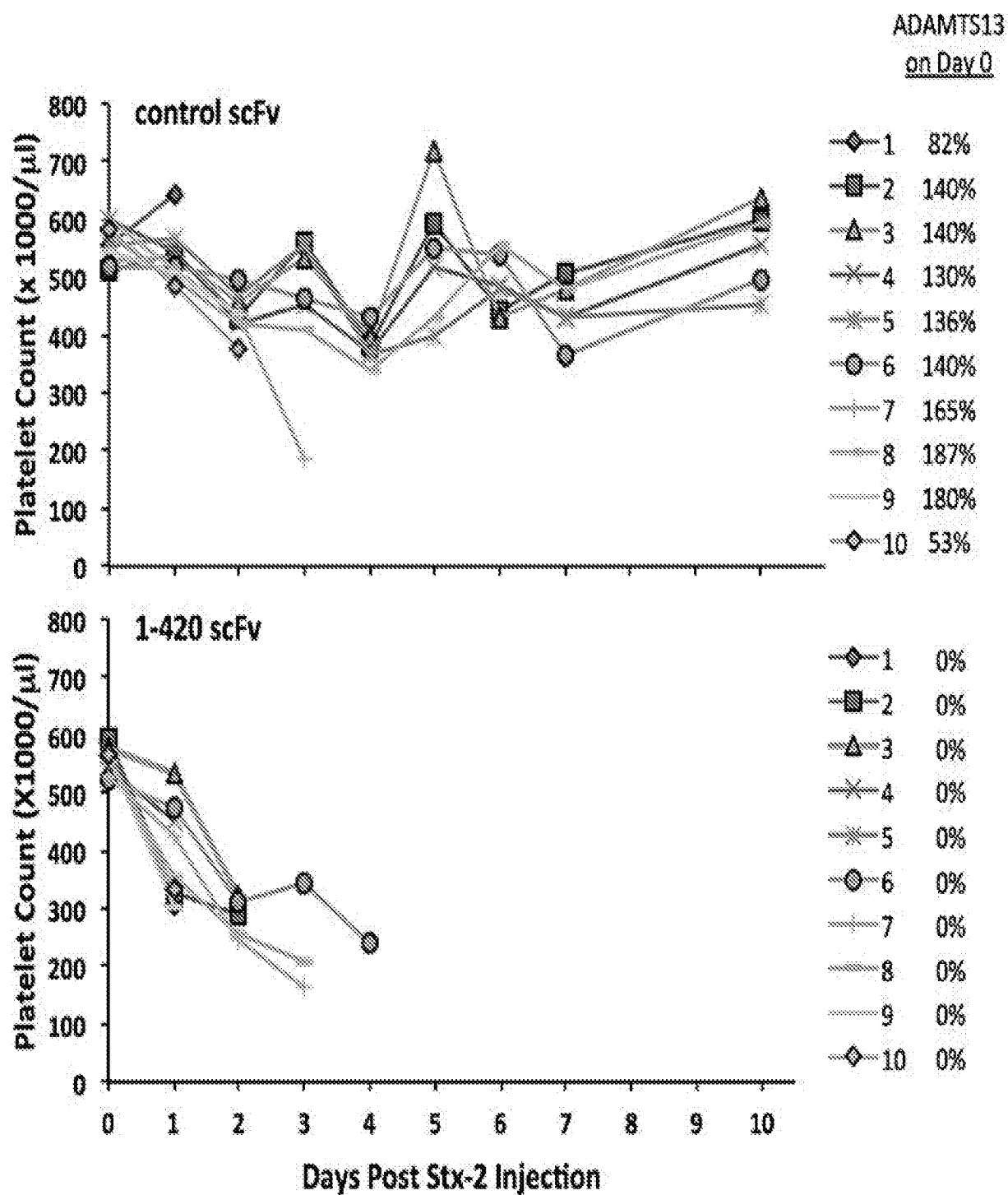


FIG. 7A

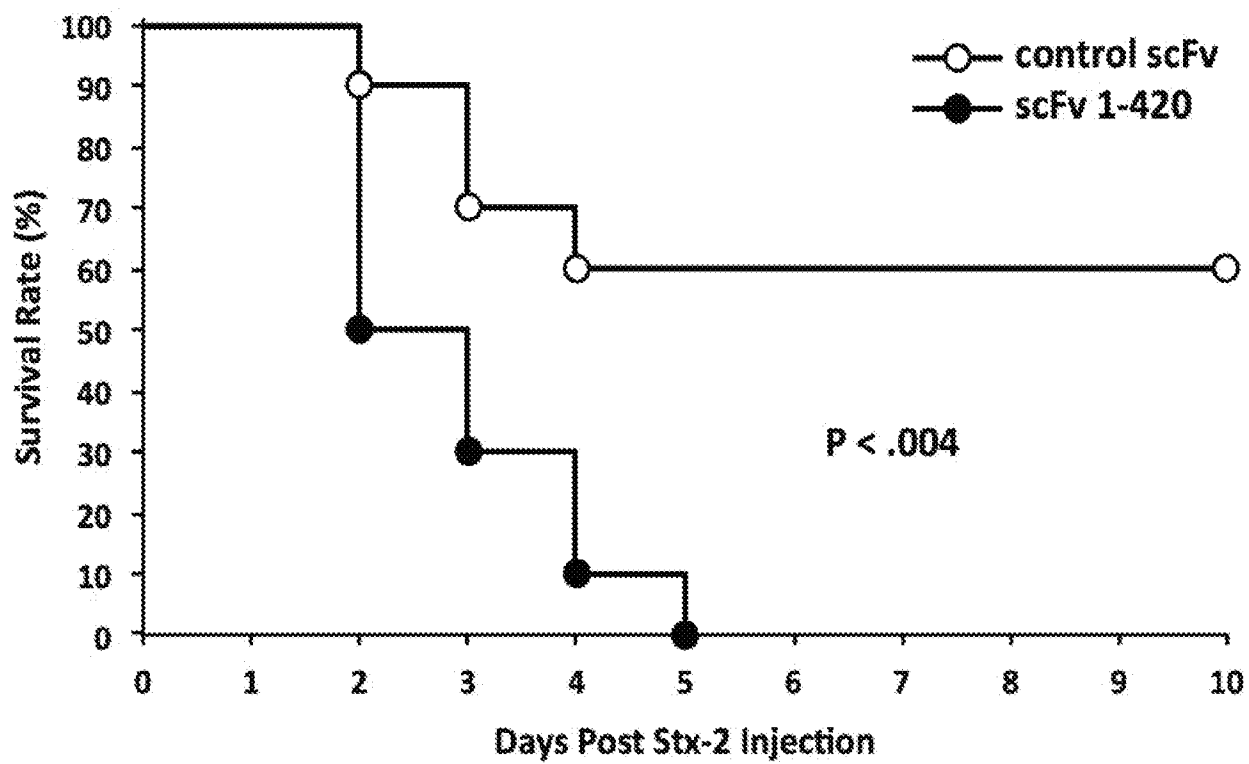


FIG. 7B

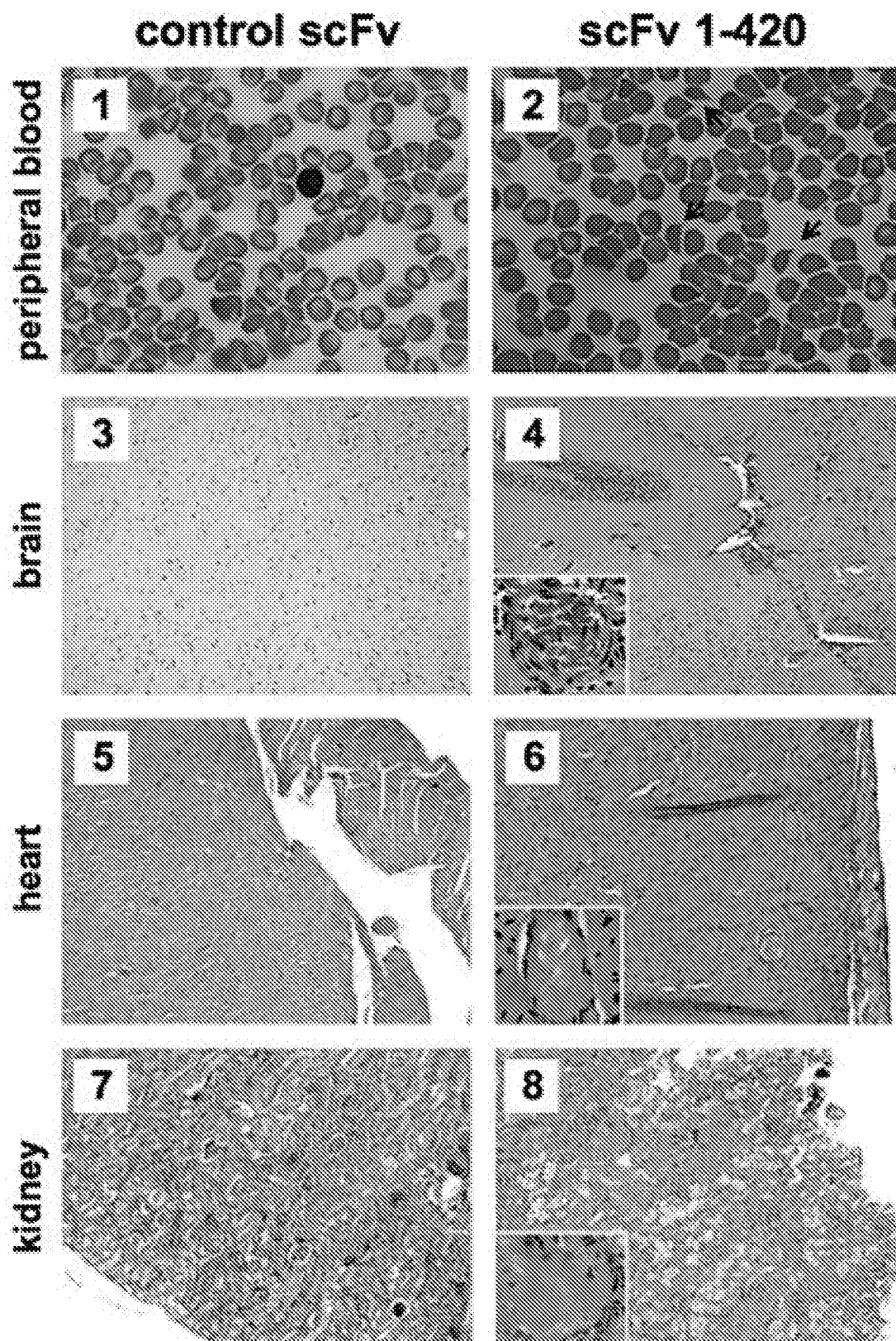


FIG. 7C

FIG. 8
Comparison of CDR2 amino acid residues for V_H1-69 encoded antibody heavy chains

Antibody	Heavy chain amino acid							Reference
	56	57	58	59	60	61	62	
1-69*01 germline gene	I	I	P	I	--	--	F	IMGT ⁽¹⁾
1-69*09 & *10 germline genes	.	.	.	.	--	--	L	IMGT ⁽¹⁾
I-9, I-10, I-27	.	.	.	.	--	--	.	Luken ⁽²⁾ , Pos ⁽³⁾
B-2, B-6, B-5, 3j	.	.	.	.	--	--	.	Schaller ⁽⁴⁾
II-1	.	.	.	.	--	--	Y	Pos ⁽³⁾
3b	.	.	.	.	--	--	Y	Schaller ⁽⁴⁾
1-416	V	.	.	V	--	--	L	
1-420, 1-401, 1-458, 1-450, 1-418, 1-413, 2-103, 2-106, 2-302, 4-303, 3-302, 3-305, 3-405, 3-301	.	.	.	.	--	--	L	
1-428, 1-304, 1-408, 1-405, 1-423	.	.	.	V	--	--	L	
1-431	F	V	.	.	--	--	L	
1-417, 1-303, 1-406	F	.	.	.	--	--	L	Present
1-438	V	.	.	.	--	--	L	Invention
1-434, 1-432, 2-204, 2-305, 2-406, 2-203, 2-206, 2-408	.	V	.	.	--	--	L	
2-102	V	V	.	.	--	--	L	
2-207, 2-301, 2-304	.	T	.	.	--	--	L	
4-307	.	.	.	M	--	--	.	
PX4-3	.	.	.	T	--	--	L	Payne ⁽⁵⁾

Antibodies are grouped by identical CDR2 amino acid residues in positions 56-62. Replacement mutations are indicated with letters, identities with ".", and gaps with "--". CDR2 numbering designations per Brochet et al.⁽¹⁾

- (1) Brochet et al., Nucleic Acids Research 2008;36:W503-W8
- (2) Luken et al., J Thromb Haemost 2006;4:2355-64
- (3) Pos et al., J Thromb Haemost 2009;7:421-8
- (4) Schaller et al., Blood 2014;124:3469-79
- (5) Payne et al., J Clin Invest 2005;115:888-99

Antibody	VH GENE	D GENE	JH GENE	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
	1-69*09		J4*02	FR1																									
				Q	V	Q	L	V	Q	S	G	A	--	S	V	K	P	G	S	S	V	K	V	S	C	K	A	S	
1-416	.	D6-12*01	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
1-428	.	D6-12*01	.	E	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
1-304	.	D6-12*01	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
1-431	.	D2-21*02	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
1-417	.	D2-21*02	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
1-303	.	D2-21*02	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
1-408	.	D1-26*01	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
1-406	.	D4-23*01	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
1-401, 1-458	.	D1-26*01	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
1-420	.	D1-26*01	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
			J6*02																										
1-438	.	D2-8*01	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
1-434	.	D2-8*01	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
			J3*01																										
1-432	.	D6-13*01	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
			J4*02																										
1-405	.	D3-22*01	.	E	.	.	.	E	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
			J6*02																										
1-450	.	D1-26*01	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
			J4*02																										
1-423	.	D3-16*01	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
1-418	.	D6-19*01	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
1-413	.	D6-19*01	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
2-204	.	D2-8*01	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
2-102	.	D2-8*01	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
2-207, 2-301	.	D2-15*01	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
2-304	.	D2-15*01	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
2-305, 2-406	.	D2-8*01	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
2-103	.	D2-8*01	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
2-106	.	D2-8*01	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
2-302	.	D6-6*01	.	E	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
2-203	.	D3-16*01	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
2-206	.	D3-16*01	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
2-408	.	D2-8*01	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
4-303	.	D1-26*01	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	1-59*01		J3*01	Q	V	Q	L	V	Q	S	G	A	--	E	V	K	K	P	G	S	S	V	K	V	S	C	K	A	S
3-302, 3-305, 3-405	.	D1-26*01	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
			J4*02																										
4-307	.	D6-12*01	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	1-59*10		J4*02	Q	V	Q	L	V	Q	S	G	A	--	E	V	K	K	P	G	S	S	V	K	V	S	C	K	A	S
3-301	.	D2-8*02	.	E	*	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	3-21*01		J6*02	E	V	Q	L	V	E	S	G	G	--	G	L	V	K	P	G	G	S	L	R	L	S	C	A	A	S
1-437	.	D3-3*01	.	Q	.	.	.	Q	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	3-30*04		J4*02	Q	V	Q	L	V	E	S	G	G	--	G	V	V	Q	P	G	R	S	L	R	L	S	C	A	A	S
1-404	.	D2-21*01	.	.	.	.	Q	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
1-441	.	D2-21*01	.	.	.	.	Q	*	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
1-403, 1-415	.	D3-3*01	.	.	.	.	Q	*	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
z1-402	.	D2-2*01	.	.	.	.	Q	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	3-43*01		J4*02	E	V	Q	L	V	E	S	G	G	--	V	V	V	Q	P	G	G	S	L	R	L	S	C	A	A	S
1-410	.	D3-9*01	.	Q	.	.	Q	*	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
1-407	.	D3-9*01	.	*	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	3-7*01		J4*02	E	V	Q	L	V	E	S	G	G	--	G	L	V	Q	P	G	G	S	L	R	L	S	C	A	A	S
2-108	.	D1-14*01	.	.	.	.	Q	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	3-9*01		J4*02	E	V	Q	L	V	E	S	G	G	--	G	L	V	Q	P	G	R	S	L	R	L	S	C	A	A	S
1-440	.	D3-22*01	.	*	.	.	L	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
1-451	.	D3-22*01	.	Q	.	.	Q	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
z1-201	.	D3-22*01	.	Q	.	.	Q	*	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	6-1*01		J6*02	Q	V	Q	L	Q	Q	S	G	P	--	G	L	V	K	P	S	Q	T	L	S	L	T	C	A	I	S
z1-303	.	D6-19*01	.	.	*	.	.	E	*	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

FIG. 9A (Part 1/5 – From left to right)

FIG. 9A (Part 2/5 – From left to right)

[illegible]

<u>Antibody</u>	<u>Total Framework Replacement Mutations</u>	<u>Total Framework Silent Mutations</u>	<u>Total CDR1 & CDR2 Replacement Mutations</u>
1-416	11	4	8
1-428	11	3	7
1-304	11	2	7
1-431	8	4	5
1-417	8	3	5
1-303	5	5	4
1-408	5	4	6
1-406	8	2	5
1-401, 1-456	8	2	5
1-420	7	2	5
1-438	7	3	6
1-434	9	2	6
1-432	6	3	6
1-405	10	4	7
1-450	13	5	4
1-423	11	5	5
1-418	8	5	5
1-413	10	7	5
2-204	2	2	3
2-102	2	1	5
2-207, 2-301	6	1	3
2-304	7	1	3
2-305, 2-406	3	4	3
2-103	2	0	2
2-106	2	1	2
2-302	5	1	2
2-203	2	3	1
2-206	3	1	3
2-408	5	0	3
4-303	1	3	3
3-302, 3-305, 3-405	7	6	6
4-307	7	3	4
3-301	5	3	1
1-437	4	2	4
1-404	6	2	4
1-441	6	2	3
1-403, 1-415	1	2	5
21-402	6	5	5
1-410	3	2	0
1-407	0	1	0
2-108	4	0	0
1-440	1	2	1
1-451	2	1	1
21-201	3	1	1
21-303	6	9	4

FIG. 9A (Part 4/5 – From left to right)

Total CDR1 & CDR2 Silent Mutations	Total Replacement Mutations	Total Silent Mutations	R:S ratio	
			FR	CDR1 & CDR2
0	19	4	2.8	>6.0
0	18	3	3.7	>7.0
0	18	2	5.6	>7.0
1	11	5	1.5	5.0
2	13	5	2.7	2.5
2	9	7	1.0	2.0
0	11	4	1.3	>6.0
1	13	3	4.0	5.0
3	13	5	4.0	1.7
3	12	5	3.5	1.7
0	13	3	2.3	>6.0
2	15	4	4.8	3.0
1	12	4	2.0	6.0
0	17	4	2.5	>7.0
1	17	6	2.6	4.0
1	16	6	2.2	5.0
1	13	5	1.6	5.0
1	15	5	1.4	5.0
1	5	3	1.0	3.0
0	7	1	2.0	>5.0
0	9	1	6.0	>3.0
0	10	1	7.0	>3.0
1	6	5	0.8	3.0
0	4	0	>2.0	>2.0
0	4	1	2.0	>2.0
0	7	1	5.0	>2.0
0	3	3	0.7	>1.0
1	6	3	3.0	3.0
0	8	0	>5.0	>3.0
0	4	3	0.3	>3.0
2	13	8	1.2	3.0
1	13	4	2.3	4.0
0	6	3	1.7	>1.0
0	8	2	2.0	>4.0
0	10	2	3.0	>4.0
0	9	2	3.0	>3.0
0	6	2	0.5	>5.0
1	11	5	1.2	5.0
0	3	2	1.5	-
0	0	1	0.0	-
1	4	1	>4.0	0.0
0	2	2	0.5	>1.0
0	3	1	2.0	>1.0
0	4	1	3.0	>1.0
1	10	8	0.9	4.0

FIG. 9A (Part 5/5 – From left to right)

Antibody	VL GENE	JL GENE	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	
	KV1-33*01	KJ4*01	FR1																										
1-107	-	-	D	I	D	N	T	Q	S	P	E	S	E	S	A	S	V	S	D	S	V	S	F	T	C	G	A	S	
1-410	-	-	E	L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	KV1-39*01	KJ4*01	D	I	D	N	T	Q	S	P	E	S	E	S	A	S	V	S	D	S	V	S	F	T	C	G	A	S	
1-403	-	-	E	L	V	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	KJ3*01	-	E	L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
1-440	-	-	E	L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
1-481	-	-	E	L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
21-201	-	-	E	L	V	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	KV2-28*01	KJ2*01	D	I	V	N	T	Q	S	P	L	E	S	P	V	T	P	G	E	F	A	S	E	S	C	R	S	S	
21-402	-	-	E	L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	LV1-47*01	LJ1*01	Q	S	V	E	T	Q	F	P	S	-	-	A	S	D	T	P	S	Q	S	V	T	I	S	C	S	G	S
2-108	-	-	E	L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	LV1-51*01	LJ2*01	Q	S	V	E	T	Q	F	P	S	-	-	V	G	A	A	P	Q	Q	S	V	T	I	S	C	S	G	S
1-406	-	-	E	L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
1-441	-	-	E	L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	LV2-14*01	LJ2*01	Q	S	A	E	T	Q	P	A	S	-	-	V	S	G	S	P	G	Q	S	E	T	I	S	C	T	G	T
1-415	-	-	E	L	K	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
1-437	-	-	E	L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	LV3-1*01	LJ2*01	S	T	E	E	T	Q	P	P	S	-	-	V	S	V	S	P	Q	Q	T	A	R	I	T	C	R	G	S
3-302	-	-	E	L	V	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	LV3-21*01	LJ2*01	S	T	V	E	T	Q	P	P	S	-	-	V	S	V	A	P	G	K	T	A	R	I	T	C	G	G	N
1-158	-	-	E	L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
3-405	-	-	E	L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
4-303	-	-	E	L	K	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
4-307	-	-	E	L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	LV3-21*02	LJ2*01	S	T	V	E	T	Q	P	P	S	-	-	V	S	V	A	P	Q	Q	T	A	R	I	T	C	G	G	N
1-438	-	-	E	L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
1-438	-	-	E	L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
21-303	-	-	E	L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
2-408	-	-	E	L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	LJ3*02	-	E	L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
1-432	-	-	E	L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
2-102	-	-	E	L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
2-103	-	-	E	L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
2-108	-	-	E	L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
2-406	-	-	E	L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
2-305	-	-	E	L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
2-301	-	-	E	L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
2-207	-	-	E	L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
2-304	-	-	E	L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
2-203	-	-	E	L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
2-208	-	-	E	L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
2-302	-	-	E	L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	LJ1*01	-	E	L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
2-204	-	-	E	L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	LV3-21*03	LJ3*02	S	T	V	E	T	Q	F	P	S	-	-	V	S	V	A	P	G	K	T	A	R	I	T	C	G	G	N
1-423	-	-	E	L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
3-305	-	-	E	L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	LJ2*01	-	E	L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
1-401	-	-	E	L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
1-420	-	-	E	L	E	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	LJ*01	-	E	L	E	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
1-417	-	-	E	L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
1-303	-	-	E	L	K	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	LV3-25*01	LJ2*01	S	T	E	E	T	Q	P	P	S	-	-	V	S	V	S	P	Q	Q	T	A	R	I	T	C	R	G	S
1-405	-	-	E	L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	LV3-25*02	LJ1*01	S	T	E	E	T	Q	F	P	S	-	-	V	S	V	S	P	Q	Q	T	A	R	I	T	C	R	G	S
1-416	-	-	E	L	V	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	LV2-28*02	LJ3*02	S	T	E	E	T	Q	P	P	S	-	-	V	S	V	S	P	Q	Q	T	A	R	I	T	C	R	G	S
1-431	-	-	E	L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
1-306	-	-	E	L	V	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
1-418	-	-	E	L	V	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
1-413	-	-	E	L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
1-400	-	-	E	L	V	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
3-301	-	-	E	L	V	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	LJ2*01	-	E	L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
1-428	-	-	E	L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
1-450	-	-	E	L	V	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
1-408	-	-	E	L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	

FIG. 9B (Part 1/5 – From left to right)

[illegible]

105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124	125	126	127	Antibody	Total Framework Replacement Mutations	Total Framework Silent Mutations		
CDR3													FH4														
G	C	F	D	N									F	C	C	C	F	K	V	E	I	N	1-437	8	4		
*	*	*	R										*		Q	*	*	R	L	*	*	*	1-418	5	2		
G	C	S	F	S									F	C	C	C	T	K	V	E	I	N	1-433	15	9		
*	*	*	H	S									*						*	*	*	*					
																							1-440	4	0		
													*		Q	*	*	L	N	*	*	*	1-451	5	2		
													*		Q	*	*	L	E	*	*	*	21-201	6	2		
N	D	A	D	D									F	C	C	C	T	K	Z	S	E	N	21-402	2	2		
L	C	F	D	N									L	C	F	D	N										
A	A	N	D	D									F	C	C	C	T	K	V	T	V	E	2-108	5	3		
*	*	*											*		*	*		L	*	*	*	*					
C	F	D	S	S									F	C	C	C	T	K	L	C	V	E	1-434	7	2		
*	*	*	A	-									*		*	*		U	*	*	*	*	1-441	5	2		
													*		*	*		V	*	*	*	*					
N	S	Y	T	S									F	C	C	C	T	K	Z	Y	V	E	1-415	4	3		
*	*	*											*														
													V	V	F	C	C	C	S	N	V	T	V	L	1-437	5	1
													*		*	*											
G	A	N	D	S									F	C	C	C	T	K	L	C	V	E	3-302	13	9		
V	E	*											*		*	*		E	*	*	*	*					
O	V	N	D	S									F	C	C	C	T	K	L	C	V	E	1-456	8	3		
*	*	*	R										*		*	*		Q	*	*	*	*					
													*		*	*		S	*	*	*	*	2-435	5	4		
R	*	*	L										*		*	*							4-303	13	10		
*	*	*	F										*		*	*		Q	*	*	*	*	4-307	4	3		
O	V	N	D	S									F	C	C	C	T	K	L	C	V	E	1-438	4	3		
*	*	*											*		*	*		E	*	*	*	*					
													*		*	*		E	*	*	*	*	1-434	3	6		
													*		*	*		E	*	*	*	*	21-303	5	2		
													*		*	*							2-408	5	0		
													*		*	*							1-432	13	6		
*	*	*	A	A									*		*	*		V	*	*	*	*	2-102	5	5		
													*		*	*		E	*	*	*	*	2-103	4	3		
													*		*	*		Q	*	*	*	*	2-108	5	2		
													*		*	*							2-406	5	0		
													*		*	*		E	*	*	*	*	2-306	4	3		
													*		*	*		V	*	*	*	*	2-301	5	4		
													*		*	*		Q	*	*	*	*	2-207	6	3		
													*		*	*							2-304	3	2		
													*		*	*		E	*	*	*	*	2-303	4	4		
													*		*	*		L	V	*	*	*	2-208	3	6		
													*		*	*		V	*	*	*	*	2-302	8	3		
													*		*	*		L	*	*	*	*	2-204	6	3		
O	V	N	D	S									F	C	C	C	T	K	L	C	V	E	1-423	8	3		
*	*	*	S										*		*	*		Q	*	*	*	*	3-305	3	2		
													F	C	C	C	T	K	L	C	V	E	1-401	8	5		
													*		*	*		E	*	*	*	*	1-420	7	3		
													A	V	F	C	C	C	F	Q	L	S	V	L	1-417	7	4
													*		*	*		E	*	*	*	*	1-303	3	1		
													*		*	*											
G	R	A	D	S									F	C	C	C	T	K	L	Z	S	E	1-405	13	4		
*	*	*	F										*		*	*											
G	S	A	D	S									F	C	C	C	T	K	V	Y	V	E	1-416	8	3		
*	*	*	T	*									*		*	*		*	*	*	*	*					
G	S	A	D	S									F	C	C	C	T	K	L	Z	S	E	1-431	2	3		
*	*	*											*		*	*		*	*	*	*	*	1-304	8	4		
													*		*	*		*	*	*	*	*	1-418	13	6		
													*		*	*		*	*	*	*	*	1-413	12	4		
													*		*	*							1-408	3	2		
													*		*	*		E	*	*	*	*	3-301	4	4		
													F	C	C	C	T	K	Z	Y	V	E	1-428	3	2		
													*		*	*							1-450	5	2		
													*		*	*		E	*	*	*	*	1-406	7	6		

FIG. 9B (Part 4/5 – From left to right)

Total CDR1 & CDR2 Replacement Mutations	Total CDR1 & CDR2 Silent Mutations	Total Replacement Mutations	Total Silent Mutations	R:S ratio	
				FR	CDR1 & CDR2
0	0	0	10	1.0	-
0	0	0	7	2.0	-
3	1	18	27	1.7	3.0
0	0	4	4	>4.0	-
0	0	0	7	2.5	-
0	0	0	8	3.0	-
0	0	2	4	1.0	-
0	0	0	8	1.7	-
3	1	8	10	3.8	1.0
3	1	0	8	2.5	1.0
0	0	4	7	1.3	-
0	0	3	4	3.0	-
4	1	18	24	1.3	4.0
3	0	0	12	2.7	>1.0
3	0	0	10	1.3	>1.0
3	1	14	24	1.3	3.0
0	0	4	0	2.0	-
0	0	4	0	2.0	-
0	0	3	3	0.0	-
0	0	0	7	2.0	-
3	0	0	8	>5.0	>1.0
3	0	12	18	1.0	>1.0
0	0	7	18	1.0	>3.0
0	0	4	7	1.3	-
0	0	0	7	2.0	-
0	0	0	5	>5.0	-
0	0	4	0	2.0	-
3	0	0	0	1.7	>1.0
0	0	0	0	2.0	-
3	0	0	7	1.5	>2.0
3	0	0	0	1.0	>1.0
3	0	4	0	0.0	>1.0
3	0	10	13	2.7	>2.0
3	0	7	10	2.0	>1.0
3	0	11	13	4.0	>3.0
0	0	3	0	1.0	-
3	0	0	14	1.3	-
0	0	0	12	2.3	-
3	1	8	12	1.8	1.0
0	0	3	4	3.0	-
3	1	12	18	2.8	1.0
3	0	10		2.7	0.3
0	0	2	5	0.7	-
3	0	10	14	2.0	>2.0
0	0	13	19	1.8	>2.0
0	0	14	18	2.0	>2.0
0	0	3	5	1.5	-
0	0	4	3	1.0	-
0	0	3	4	1.0	-
0	0	0	7	2.0	-
3	0	0	15	1.2	>2.0

FIG. 9B (Part 5/5 – From left to right)

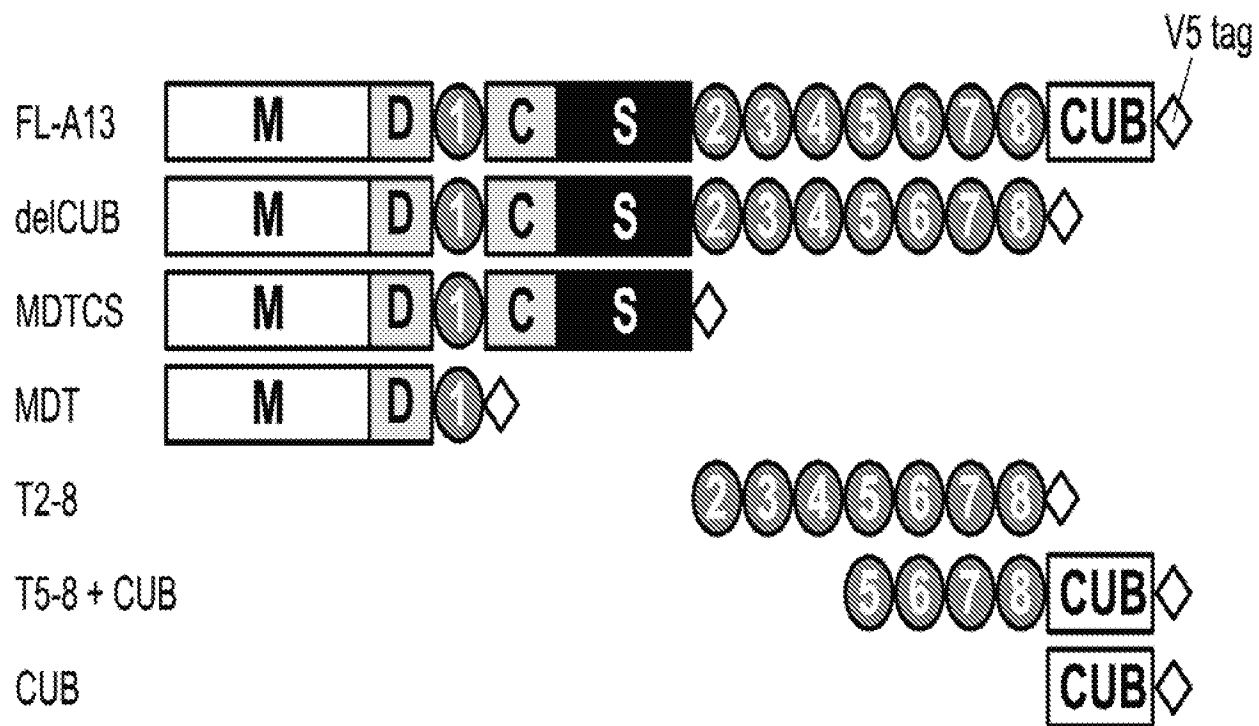


FIG. 10A

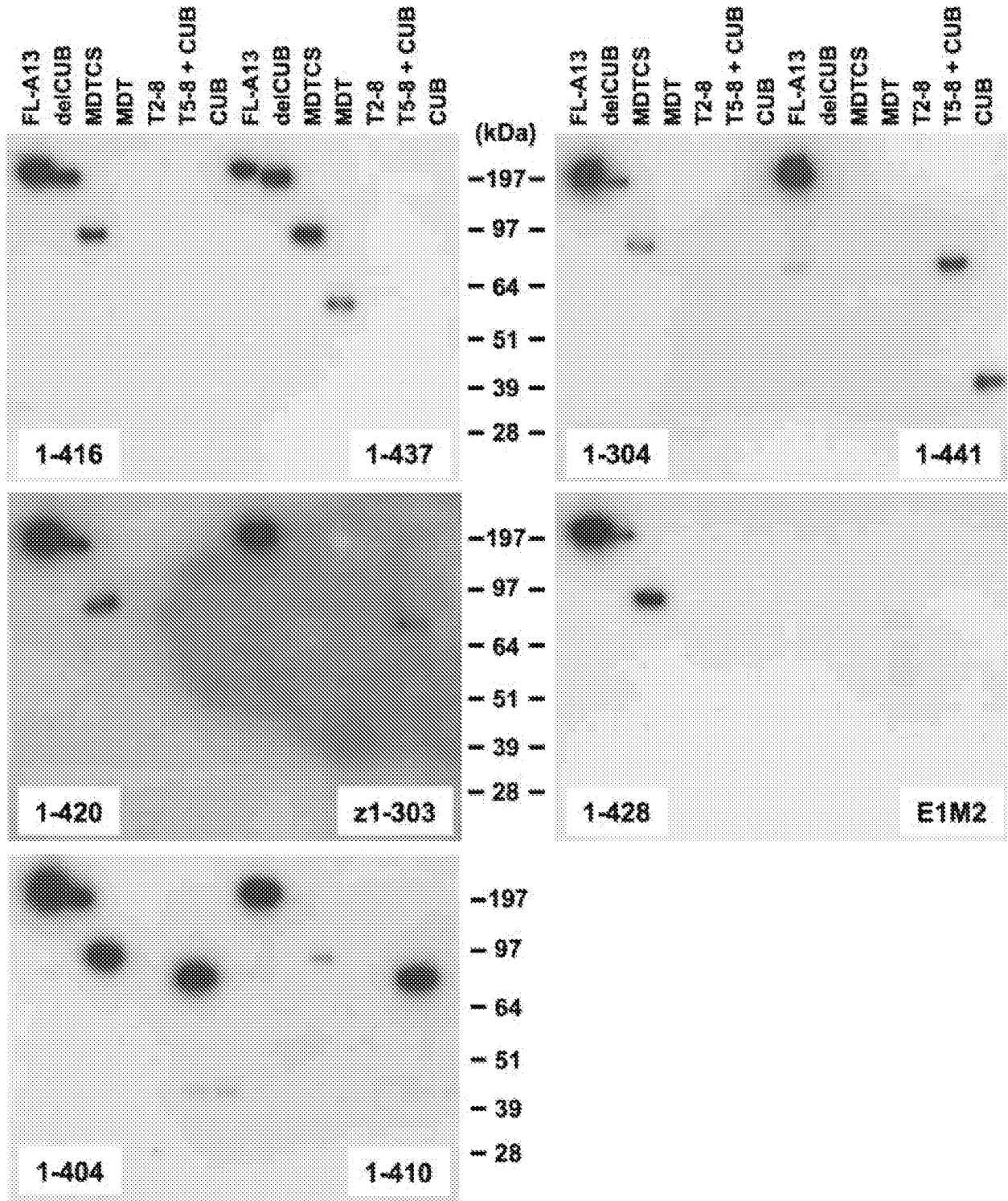


FIG. 10B

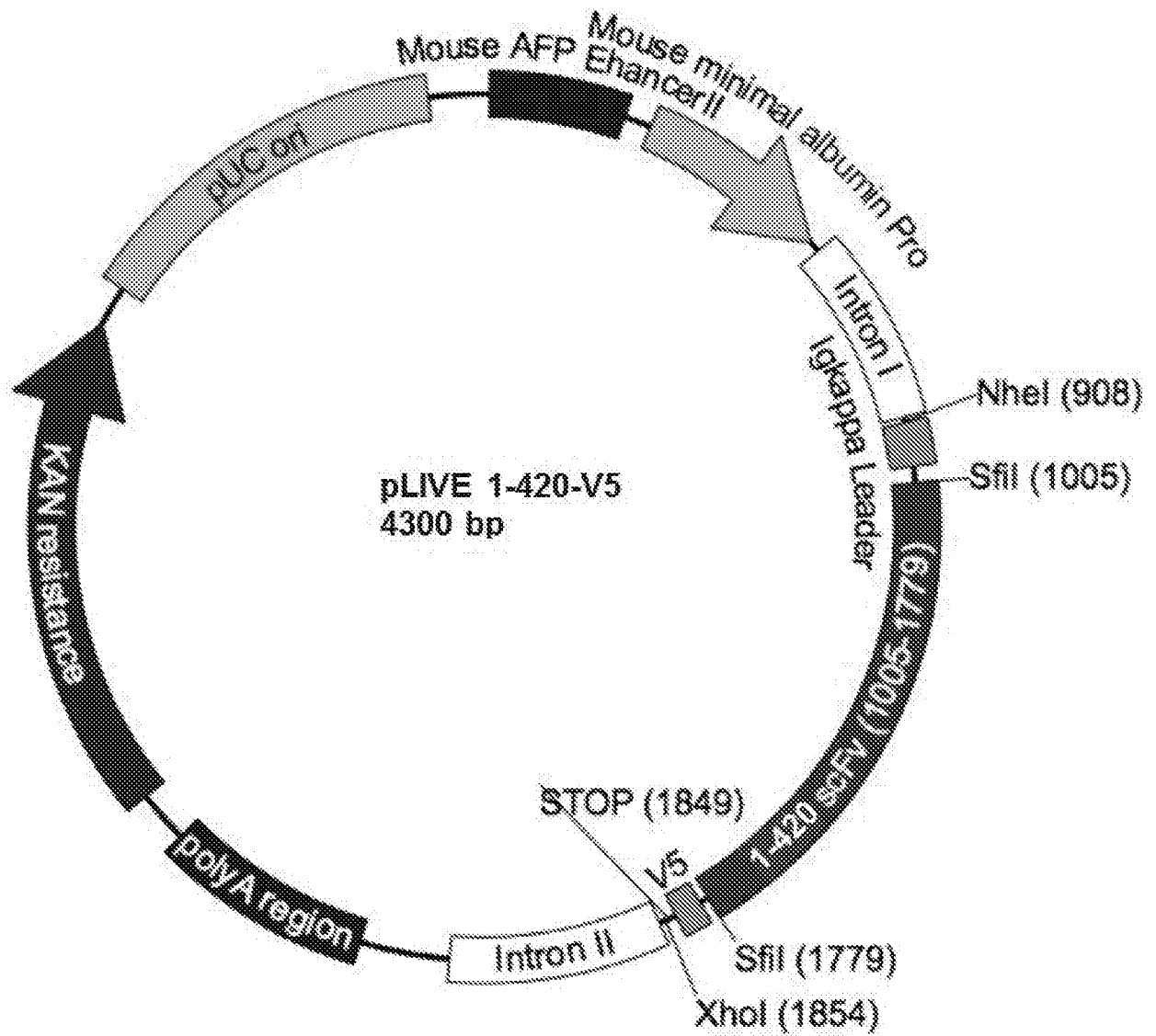


FIG. 11