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(54) **Title:** PROTEIN M ANALOGS AND FUSION PROTEINS AND THEIR USE FOR INHIBITING ANTIBODY FUNCTION

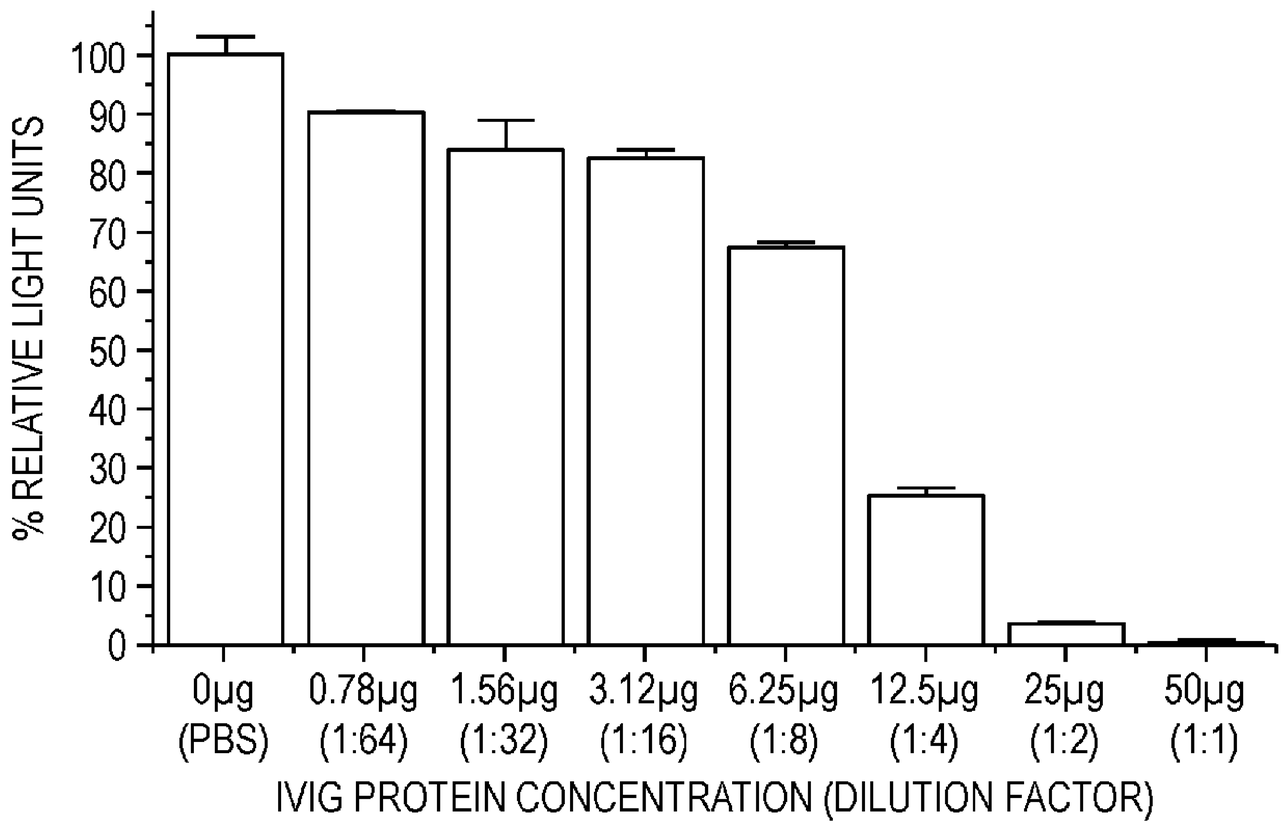


FIG. 1

(57) **Abstract:** This invention relates to methods and compositions for binding antibodies. The methods may be used to isolate antibodies, treat disorders related to excess antibodies, acutely block antibodies to stop an autoimmune or inflammatory response, and inhibit neutralizing antibodies. In embodiments, the invention relates to methods of inhibiting neutralizing antibodies against a heterologous agent when the heterologous agent is administered to a subject, comprising administering to the subject an effective amount of mycoplasma protein M or a functional fragment or derivative thereof, thereby inhibiting neutralization of the heterologous agent. The invention further relates to modified mycoplasma protein M or functional fragments thereof having increased thermostability relative to wild-type protein M and their use in the methods of the invention.

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PROTEIN M ANALOGS AND FUSION PROTEINS AND THEIR USE FOR INHIBITING ANTIBODY FUNCTION

STATEMENT OF PRIORITY

[0001] This application claims the benefit of U.S. Provisional Application Serial No. 63/145,188, filed February 3, 2021, the entire contents of which are incorporated by reference herein.

FIELD OF THE INVENTION

[0002] This invention relates to methods and compositions for binding antibodies. The methods may be used to isolate antibodies, treat disorders related to excess antibodies, acutely block antibodies to stop an autoimmune or inflammatory response, and inhibit neutralizing antibodies. In embodiments, the invention relates to methods of inhibiting neutralizing antibodies against a heterologous agent when the heterologous agent is administered to a subject, comprising administering to the subject an effective amount of mycoplasma protein M or a functional fragment or derivative thereof, thereby inhibiting neutralization of the heterologous agent. The invention further relates to modified mycoplasma protein M or functional fragments thereof having increased thermostability relative to wild-type protein M and their use in the methods of the invention.

BACKGROUND OF THE INVENTION

[0003] Approximately 1 in 10 people in the US suffers from a rare genetic disease, which can seriously impact life-span, quality of life, independence, and economic potential. Gene therapy is the most promising form of treatment for the correction of heritable diseases. Among gene therapy delivery vehicles, adeno-associated virus (AAV) vectors have showed therapeutic effect in numerous clinical trials. The first FDA approved gene therapy has been used in patients with blindness. The number of clinical trials for AAV gene therapy has grown substantially due to its safety and success in targeting many different types of organs for long-term therapeutic gene expression. Despite clinical success, the major barrier to AAV mediated gene delivery is the high prevalence of neutralizing antibodies (NAbs), which block vector transduction of target tissues. Greater than 90% of the general population has been exposed to AAV through natural infection, and over 50% of people are serum positive for NAbs against AAV. Identification of pre-existing NAbs above a specific threshold during clinical trial

screening disqualifies patients from enrollment, since NAbs severely attenuate therapeutic effectiveness and cause outcome variability.

[0004] To overcome AAV NAbs, several approaches have been employed: serial plasmapheresis, immune suppressive drugs, and alteration of the vector capsid to eliminate immune epitopes. Plasmapheresis is inefficient, requiring multiple rounds that remove 2-3 fold of the remaining NAbs each session and is only capable of addressing low titer of NAbs. Plasmapheresis is also time consuming and puts patients at risk for communication of nosocomial infections through simultaneous depletion of antibodies and repeated intravenous needle exposure. Steroids or pharmacological immune suppression to kill B-cells carries significant health risks and requires prolonged regimens to reduce NAbs by even 10-fold. AAV capsid engineering is an innovative method but ultimately inadequate against polyclonal anti-AAV sera, where a modest 10-fold increase in antibody escape is observed *in vivo*. Additionally, modification of the capsid structure to alter NAb recognition epitopes typically results in less potent vectors and defective vector production because those altered surface regions are multifunctional. None of the current approaches successfully overcome pre-existing anti-AAV NAbs above the typical thresholds set for clinical trial exclusion, or allow repeat administration of the same AAV vectors.

[0005] Mycoplasma protein M has been identified as capable of non-specifically binding antibodies and blocking their ability to bind antigen (US Patent No. 9,593,150; US Publication No, 2017/0320921).

[0006] The present invention overcomes shortcomings in the art by providing a vector independent protein based method to universally block Nabs and other methods and compositions based on antibody binding.

SUMMARY OF THE INVENTION

[0007] The present invention is based, in part, on the development of a vector independent protein based method to universally block NAbs and demonstration that this method is effective against a broad range of pre-existing NAb concentrations. The invention pioneers the use of a unique mycoplasma-derived protein and its analogues, termed protein M, to enable successful gene delivery by preventing neutralization of heterologous agents (*e.g.*, AAV vectors) by NAbs upon administration of the heterologous agent to a subject. Protein M was shown to block mammalian IgG, IgM, and IgA antibody classes in a species and antigen independent manner by universally binding to conserved regions on the antibody light and heavy chains, causing structural interference with the antigen recognizing CDR regions. Protein M binds to

antibodies with nanomolar affinity, and prevents antigen-antibody union for a variety of different tested immunoglobulin/antigen pairs. The inventors discovered that protein M blocks antibody recognition of AAV and prevents antibody-mediated neutralization of AAV. It has been demonstrated that the level of AAV vector escape from NAbs is proportional to the NAb titer and volume, amount of protein M, and amount of AAV. Protein M mediated NAb escape has been validated *in vitro* and *in vivo* using human serum (IVIG) and serum from AAV immunized mice. It was demonstrated that protein M can be administered alone prior to AAV administration, or formulated with AAV for NAb evasion. The effectiveness of this approach depends on the interaction of protein M with immunoglobulin before AAV is neutralized. This approach can be used to overcome NAbs to multiple heterologous agents (*e.g.*, AAV vector serotypes) while maintaining the unique or beneficial properties of each agent for specific gene therapy applications.

[0008] The invention further relates to the use of protein M in other methods in which binding of antibodies is beneficial, including treatment of autoimmune disorders, treatment of disorders associated with excess antibodies, methods of isolating antibodies, and methods of performing immunoassays.

[0009] The invention further relates to modified protein M proteins having increased thermostability and/or other advantageous characteristics (e.g., increased or maintained pH stability relative to wild-type mycoplasma protein M or a functional fragment thereof) for carrying out the methods of the invention *in vivo* or at elevated temperatures.

[0010] Thus, one aspect of the invention relates to a modified mycoplasma protein M or a functional fragment thereof, having one or more amino acid mutations that increase or maintain thermostability and/or pH stability of the mycoplasma protein M or a functional fragment thereof relative to wild-type mycoplasma protein M or a functional fragment thereof. In some embodiments, the thermostability is maintained up to 75 °C, 70 °C, 65 °C, 60 °C, 55 °C, 50 °C, 45 °C, 40 °C, or 38 °C and/or the pH stability is maintained from a pH of 2 to 8, from a pH of 2.5 to 7.5, or from a pH of 4.5 to 7.5.

[0011] Another aspect of the invention relates to a fusion protein comprising a modified mycoplasma protein M or a functional fragment thereof, a linker, and a peptide.

[0012] An additional aspect of the invention relates to a polynucleotide encoding the modified mycoplasma protein M or a functional fragment thereof of the invention and a vector or transformed cell comprising the same.

[0013] Another aspect of the invention relates to a method of inhibiting neutralization of a heterologous agent by neutralizing antibodies upon administration of the heterologous agent to

a subject, comprising administering to the subject an effective amount of mycoplasma protein M or a functional fragment or derivative thereof, thereby inhibiting neutralization of the heterologous agent.

[0014] A further aspect of the invention relates to a method of expressing a polypeptide or functional nucleic acid in a subject, comprising administering to the subject (a) a nucleic acid delivery vector encoding the polypeptide or functional nucleic acid, and (b) an effective amount of mycoplasma protein M or a functional fragment or derivative thereof, thereby expressing the polypeptide or functional nucleic acid in the subject.

[0015] An additional aspect of the invention relates to a method of treating a disorder in a subject in need thereof, wherein the disorder is treatable by expressing a polypeptide or functional nucleic acid in the subject, comprising administering to the subject (a) a therapeutically effective amount of a nucleic acid delivery vector encoding the polypeptide or functional nucleic acid, and (b) an effective amount of mycoplasma protein M or a functional fragment or derivative thereof, thereby treating the disorder in the subject.

[0016] Another aspect of the invention relates to a method of editing a gene in a subject, comprising administering to the subject (a) a gene editing complex, and (b) an effective amount of mycoplasma protein M or a functional fragment or derivative thereof, thereby expressing the polypeptide or functional nucleic acid in the subject.

[0017] An additional aspect of the invention relates to a method of treating an autoimmune disease in a subject in need thereof, comprising administering to the subject an effective amount of mycoplasma protein M or a functional fragment or derivative thereof, thereby treating the autoimmune disease.

[0018] A further aspect of the invention relates to a method of treating a disorder associated with excess antibodies in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of mycoplasma protein M or a functional fragment or derivative thereof, thereby treating the disorder associated with excess antibodies.

Another aspect of the invention relates to a method of treating and/or preventing acute transplant rejection of solid organs associated with recognition of the transplant by recipient antibodies in a subject in need thereof, comprising administering to the subject prior to and/or after a transplant, a therapeutically effective amount of the mycoplasma protein M or a functional fragment or derivative thereof.

[0019] Another aspect of the invention relates to a method of isolating a compound comprising an antibody light chain variable region and/or heavy chain variable region from a sample, the method comprising contacting the compound with the modified mycoplasma

protein M or a functional fragment thereof of the invention attached to a solid support, then eluting the compound from the modified mycoplasma protein M or a functional fragment thereof.

[0020] An additional aspect of the invention relates to a method of performing an immunoassay, the method comprising using the modified mycoplasma protein M or a functional fragment thereof of the invention to bind a compound comprising an antibody light chain variable region and/or heavy chain variable region.

[0021] A further aspect of the invention relates to a kit comprising the modified mycoplasma protein M or a functional fragment thereof of the invention.

[0022] These and other aspects of the invention are set forth in more detail in the description of the invention below.

BRIEF DESCRIPTION OF THE DRAWINGS

[0023] Figure 1 shows human IVIG contains AAV neutralizing antibodies that dose-dependently inhibit AAV transduction. A 2-fold dilution series of human IVIG was used to generate an AAV2 neutralization plot. Beginning with 50 µg, IVIG was diluted stepwise to less than 1 µg, and each dilution was incubated for 1 h at 4 °C with $2x10^8$ viral genomes of AAV2-Luciferase before seeding the wells. The luminescent reporter signal generated by the AAV2 transgene expression is a functional read-out of AAV transduction and is proportional to the amount of AAV that was available to transduce cultured HEK-293 cells. In this experiment, each well of a 48-well plate was seeded with $1x10^5$ cells and transduced at an MOI of 2,000 in serum free media (n=3 technical replicates per condition). Measurement of luciferase activity was performed at 48 h post-transduction on a plate reader after cell lysis and addition of luciferin. The relationship between the percent of AAV neutralized for a given IVIG quantity was determined at 12.5 µg of IVIG to be 72% (+/- 1.3%) neutralization compared to the no-IVIG control that contained an equivalent volume of phosphate buffered saline (PBS). Furthermore, 25 µg of IVIG neutralized 96% (+/- 0.2%) while 50 µg of IVIG neutralized 99% (+/- 0.17%) of AAV2.

[0024] Figure 2 shows protein M protects AAV from neutralizing antibodies present in human IVIG. Using 12.5 µg IVIG which was established in the previous figure to neutralize ~70% of AAV2 at a dose of $2x10^8$ vg, escape from antibody mediated neutralization was investigated using a 2-fold dilution series of protein M. In this case, the experiment began with a molar ratio of 8 protein M molecules to 1 IgG molecule, which is a 4-fold excess of protein M given that 1 IgG molecule has 2 protein M binding sites that must be occupied for complete

antibody inactivation. In each well, IVIG was first incubated with individual protein M dilutions for 1 h at 4 °C, followed by addition of AAV2-Luciferase ($2x10^8$ vg) and incubation for an additional 1 h at 4 °C. HEK-293 cells ($1x10^5$) were then added to the wells and incubated for 48 h before measuring the luciferase reporter signal. Wells that contained IVIG but no protein M demonstrated a ~68% (+/- 3.3%) reduction in AAV signal as compared to no-IVIG control wells containing phosphate buffered saline (PBS). However, protein M dose dependently blocked neutralization of AAV at molar ratios greater than 2:1, which completely prevented neutralization (108%-193% of no-IVIG control) while ratios of 1:1 or 0.5:1 only partially blocked neutralization (52% and 40% of no-IVIG control respectively). Additionally, at ratios of 8:1 and 4:1 a dose dependent increase of AAV luciferase signal over the no-IVIG control was observed. All wells were done in triplicate in serum free media (n=3 technical replicates per condition) in a 48-well plate and luciferase signal was measured 48 h post-transduction.

[0025] Figure 3 shows excess protein M provides little additional protection from NAbs at molar ratios greater than 8:1 and protein M enhances AAV transduction. In an effort to establish the upper limit of protein M molar ratios that protect or enhance transduction of AAV, with or without neutralizing antibody conditions, the NAb escape assay was repeated with molar ratios of 8:1 or 20:1. Addition of 12.5 µg of IVIG neutralized 80% (+/- 2.6%) of the AAV2-Luciferase signal compared with the no-IVIG PBS control wells. Similar to the previous experiment, protein M pre-incubated with 12.5 µg IVIG (8:1 molar ratio, 33 µg protein M) for 1 h at 4 °C followed by 1 h at 4 °C incubation with AAV prevented AAV neutralization and enhanced luciferase signal to 194% (+/- 24%) of the no-IVIG control with PBS. At 10-times the amount of protein M necessary for NAb escape (20:1 molar ratio, 75 µg protein M), a modest increase in luciferase activity was observed to 256% (+/- 44%) over the no-IVIG control containing PBS. The luciferase signal of the 20:1 ratio was not statistically different from the 8:1 ratio in the presence of IVIG. Furthermore, without IVIG when either 75 µg or 33 µg of protein M were incubated alone with AAV2-Luciferase for 1 h at 4 °C, there was no significant difference in the amount of luciferase signal (208% vs 216% respectively), confirming that the increase in luciferase signal relative to the PBS control is due to protein M based enhancement. Each well was seeded with $1x10^5$ HEK-293 cells in serum free media and transduced at an MOI of 2,000 (n=3 technical replicates per well condition) in a 48-well plate format.

[0026] Figure 4 shows protein M enhancement of AAV transduction is dose dependent. A 2-fold dilution series of protein M, beginning with 33 µg, demonstrates that incubation of

AAV2-Luciferase with protein M results in an increase in luciferase signal relative to the PBS+AAV control. Enhancement of the luciferase signal is abolished at concentrations of protein M below 2 μg for $2x10^8$ viral genomes, or a molar ratio of 40,000 protein M molecules to 1 genome containing AAV2-Luciferase particle. Enhancement begins to saturate at about 33 μg of protein M for $2x10^8$ vector particles, or roughly 700,000 molecules of protein M to 1 genome containing AAV2-Luciferase particle. Protein M dilutions were incubated with AAV for 1 h at 4 °C prior to transduction. Each well was seeded with $1x10^5$ HEK-293 cells in serum free media and transduced at an MOI of 2,000 (n=3 technical replicates per well condition) in a 48-well plate format.

[0027] Figure 5 shows protein M based enhancement of AAV transduction is dependent on protein M direct interaction with the AAV capsid. Three different conditions were tested with AAV2-Luceiferase: protein M pre-incubation with AAV for 1 h prior to transduction (-1 h), addition of protein M to AAV at the peri-transduction time point (0 h), or addition of protein M to the wells 18 h post-transduction by AAV (18 h). Two negative control conditions without protein M were used: AAV was incubated alone for 1 h at 4 °C in cell culture media prior to cell seeding and transduction (representative of conditions in the pre-incubation and peri-transduction groups), or AAV was diluted in PBS and added to cell culture at the time of cell seeding and transduction (representative of the conditions in the post-transduction group). At 18 h post-transduction, an additional volume of PBS was added to the PBS control group to mimic conditions in which an equivalent volume of protein M was added to the post-transduction group. No enhancement of AAV luciferase signal was seen in the wells where protein M was added peri-transduction or post-transduction, while dose dependent enhancement was observed in the pre-incubation group, indicating protein M interaction with AAV was necessary for the enhancement mechanism. All wells were seeded with $1x10^5$ Huh7 cells and each well was transduced by $2x10^8$ vg of AAV, n=3 technical replicates per well condition in a 48-well plate format.

[0028] Figure 6 shows protein M does not prevent AAV neutralization after NAbs have bound the capsid. AAV2-Luciferase was first incubated with different dilutions of IVIG at 4 °C for 1 h, then protein M was added and incubated for an additional 1 h at 4 °C. A double negative control group received AAV only, while a positive control received 33 μg of protein M incubated with AAV for 1 h at 4 °C before transduction. Three different dilutions of IVIG were used that contained either 200 μg, 50 μg, or 12.5 μg. Compared to the protein M negative control groups that contained only AAV incubated with dilutions of IVIG, 33 μg of protein M does not enable post-neutralization enhancement or escape from neutralizing antibodies when

99% of AAV has already been neutralized by either 50 μg or 200 μg of IVIG. However, when 12.5 μg of IVIG is incubated with AAV about 30% of the vector remains un-neutralized (see **FIGS. 1, 2, and 3**), and 33 μg of protein M was able to enhance transduction of this fraction post-neutralization compared to the protein M negative control wells containing AAV and 12.5 μg IVIG. Unlike previous figures, luciferase measurements were taken at only 24 h post transduction, which could explain why luciferase signal from the group with 12.5 μg IVIG plus AAV is less than 30% of the double negative control. Each well was seeded with $1x10^5$ HEK-293 cells in serum free media and transduced at an MOI of 2,000 (n=3 technical replicates per well condition) in a 48-well plate format.

[0029] Figure 7 shows pre-incubation of protein M with AAV protects the vector from later neutralization by IVIG. For this neutralization assay the quantity of protein M was kept the same (8.25 μg) while various amounts of IVIG were used to achieve different molar ratios (from 50 μg to 3.12 μg IVIG). Protein M was first incubated with AAV2-Luciferase for 1 h at 4 °C, followed by addition of IVIG for 1 h at 4 °C before transducing the cell cultures. Positive control groups contained AAV plus either 33 μg, 8.25 μg, or 1 μg protein M, while the negative control group contained only AAV incubated with PBS. Protein M mediated enhancement of the non-neutralized fraction of AAV by 2-3 fold could be observed. Each well was seeded with $1x10^5$ HEK-293 cells in serum free media and transduced at an MOI of 2,000 (n=3 technical replicates per well condition) in a 48-well plate format. Luciferase measurements were taken 24 h post transduction.

[0030] Figure 8 shows pre-incubating protein M with AAV protects the vector from neutralization by IVIG in an excess of background serum immunoglobulins. For this experiment 25 μg of IVIG was used, which was previously shown to neutralize ~95% of AAV2-Luciferase at a dose of $2x10^8$ viral genomes. The 25 μg of Human IVIG was added to cell culture wells containing 10% Fetal Bovine Serum (FBS), with an estimated Bovine IgG concentration of 350 μg as determined by ELISA performed by the manufacturer. Another set of cell culture wells containing the same quantity of 10% FBS served as a no-IVIG control. Protein M at molar ratios of 4:1, 2:1, and 1:1 (based on Bovine IgG content) was incubated with AAV for 1 h at 4 °C before being added the no-IVIG control wells or 25 μg IVIG containing wells each with 10% FBS. AAV was incubated with PBS instead of protein M in the negative control group, and added to wells containing either 10% FBS or 10% FBS with 25 μg of IVIG. The results demonstrate that protein M pre-incubation protected AAV2-Luciferase from neutralization by IVIG even at a ratio of 1 protein M molecule to 1 Bovine IgG molecule, although this amount of protein M (108 μg) was still in a 12-fold greater ratio

than Human IVIG (25 μg). Each well was seeded with $1x10^5$ HEK-293 cells and transduced at an MOI of 2,000 (n=3 technical replicates per well condition) in a 48-well plate format. Luciferase measurements were taken 24 h post transduction.

[0031] Figure 9 shows protein M allows *in vitro* escape of AAV8 from neutralizing antibodies found in pooled polyclonal serum collected from AAV8 immunized mice. For this experiment 10 μl of pooled polyclonal serum from AAV8 immunized mice was serially diluted in PBS, first 10-fold to generate 1 μl and 0.1 μl serum containing wells. The 0.1 μl serum well was then further diluted in a 2-fold dilution series. AAV8-Luciferase vector ($2x10^8$ viral genomes per well) was incubated with the dilutions of neutralizing serum for 1 h at 4 °C before transduction. Protein M was added to a 10-fold dilution series of the same AAV8 neutralizing mouse serum in a ratio of 2 protein M molecules to an estimated 1 immunoglobulin molecule. Beginning with 6.5 μg of protein M to an estimated 10 μg of immunoglobulin in 1 μl of neutralizing mouse serum, each of the protein M and serum samples were reciprocally diluted separately before being combined for a 1 h incubation at 4 °C. All samples were then incubated for 1 h at 4 °C with AAV8-Luciferase before being added to cells. Data from the resulting neutralization experiment were normalized to no serum control wells containing only AAV8, and the luciferase signals were then fit with double exponential decay functions to estimate the curve between data points. Using the modeled curve it was found that 50% neutralization of AAV8 by the polyclonal serum occurred at a volume of 0.0039 μl for an effective titer of 1:2,564. However, 50% neutralization of the same serum incubated with a 2:1 molar ratio of protein M occurred at a serum volume of 0.2744 μl resulting in an estimated effective titer of 1:36. This result demonstrates that protein M is capable of protecting AAV over nearly a 100-fold difference in serum concentration *in vitro*. All wells were seeded with $5x10^4$ HEK-293 cells in a 96-well plate format (MOI of 4,000), n=3 technical replicates per well condition, and luciferase was measured 48 h post-transduction.

[0032] Figure 10 shows *in vivo* administration of protein M to mice with passive immunity to AAV8 results in neutralizing antibody escape. In this experiment mice were given various volumes of polyclonal AAV8 serum (titer 1:2,564 from the previous figure) through passive transfer via IV injection, followed within 15-20 minutes by IV administration of $2x10^{10}$ viral genomes of AAV8-Luciferase. This established a neutralization curve based on serum volume delivered to each mouse. It was found that over 50% neutralization of the AAV8-Luciferase signal occurred from 1 μl to 0.001 μl of transferred serum as compared to a group of naïve mice without passive transfer of serum. However, when an estimated 2:1 ratio of protein M (6.3 mg) was delivered to mice after passive transfer but before AAV administration, then

complete escape from neutralizing antibodies was achieved at transferred serum volumes of 0.3 µl and 1 µl indicated protein M mediated escape from neutralizing antibodies of a 1,000-fold change in NAb concentration. Furthermore, escape from neutralization was dose dependent for the 0.3 µl passively transferred serum group, demonstrating NAb escape is decreased for 1:1 (3.15 mg) and 0.5:1 (1.58 mg) estimated molar ratios compared to the no-serum control group. For all groups n=5 mice per group condition and luciferase signal from the liver was measured at 24 h post AAV administration.

[0033] Figure 11 shows the averaged raw luminescence quantified from the results in Figure 10.

[0034] Figure 12 shows the protein M/antibody complex is stable after formation *in vitro*. In this experiment 1 µl of polyclonal serum (titer 1:2,564) containing an estimated 10 µg of immunoglobulin was used to incubate with protein M (2:1 molar ratio, 6.6 µg) for various durations before addition of AAV8-Luciferase. The negative control group contained neutralizing serum plus media, while the positive control groups contained either media plus PBS or protein M plus media. Incubation intervals of 72 h, 48 h, 24 h, 16 h, 4 h, 2 h, and 1 h were applied to all groups. All incubation durations showed protein M mediated protection of AAV8 from neutralization compared to the media plus PBS negative control. AAV8-Luciferase was added to the wells at the time of seeding (0 h) with $5x10^4$ HEK-293 cells and transduced at an MOI of 4,000 (n=3 technical replicates per well condition) in a 96-well plate format. Luciferase measurements were taken 48 h post transduction.

[0035] Figure 13 shows the potency of neutralizing antibody escape by protein M *in vivo* is unstable. The same experiment from **FIGS. 10 and 11** was performed but this time AAV8 was added at either 5 minutes post protein M administration, or 3 h post protein M administration in order to test the durability of NAb escape. AAV8-Luciferase signal was about 1/3 of the no-serum control group after waiting 3 h to administer AAV8 while the luciferase signal was roughly equivalent to the no-serum control group when waiting only 5 minutes to administer AAV8 after protein M. An estimated 2:1 ratio of protein M (6.3 mg) was delivered to mice after passive transfer but before AAV administration, and n=5 mice per group condition except for the 3 h interval group which contained n=3 mice. Luciferase signal from the liver was measured at 24 h post AAV administration.

[0036] Figure 14 shows truncated protein M from *M. genitalium* (MG WT) is unstable at body temperature. Melting temperature (Tm) was determined with nano differential scanning fluoroscopy (NanoDSF). Inflection points of the first derivative indicate Tm. Proteins were recombinantly expressed in *E. coli* and purified with nickel column chromatography before

measurement. A circular dichroism assay demonstrated that MG WT is stable for at least 2 h at 20°C, along with no visible precipitate forming. However, at 37°C MG WT began to unfold after 15 minutes, along with production of visible precipitate. This indicates the protein is unstable near standard human body temperature. A temperature ramp from 0-100°C demonstrated instantaneous unfolding of MG WT and a melting temperature (Tm) of about 41.2°C. Unfolding was accompanied by aggregation of a visible precipitate.

[0037] Figures 15A-15B show melting temperatures of truncated protein M from *M. genitalium* (MG WT) and *M. pneumoniae* (MP WT), as well as analogs of protein M that improved melting temperature over MG WT by at least 1 degree (A) or maintain the melting temperature of MG WT (B). Melting temperatures were determined by differential scanning fluoroscopy (NanoDSF) for WT and mutant protein analogs generated by small scale protein production from *E.coli*. The number of mutations and their corresponding amino-acid substitutions are listed to the right. The analogs demonstrate a range of thermostability, with multiple mutations resulting in an additive effect to increase melting temperature.

[0038] Figure 16 shows example data using differential scanning fluoroscopy (DSF) to measure the melting temperature of MG WT and MG 29. Melting temperatures were determined for all analogs, with the results of MG WT (Tm=41.9°C) and MG 29 (Tm=55.2°C) pictured here. Inflection points of the first derivative indicate melting temperatures (Tm). Proteins were recombinantly expressed in *E. coli* and purified with nickel column chromatography before measurement.

[0039] Figures 17A-17C show soluble fraction assessment of protein M analogs determined by SDS-PAGE. Seven aliquots of each protein (0.4 mg/mL) were incubated at 37°C for varying amounts of time. Precipitated protein was pelleted by centrifuging the samples at 15,000 x G for 10 minutes before running the soluble fraction on an SDS-PAGE gel. Proteins were heated for either 0, 1, 4, 24, 48, 72, or 96 h prior to assessment. Results demonstrate MG WT (A) begins to precipitate out of solution between 1-4 h post heating. MG 27 (B) and MG 29 (A) remain soluble for over 72 h, while MG 31 (B) and MG 40 (C) remain soluble for over 24 h.

[0040] Figures 18A-18C show different protein M analogs block pooled human Intravenous Immunoglobulin Gamma (IVIG) to prevent AAV2-Luciferase vector neutralization during an *in vitro* neutralization assay: comparison of 4°C vs. 37°C thermal challenge for 1 h (A and B) or 24 h (C) incubation. Ratio of 4:1 protein M to IVIG. Relative light units generated by luciferase activity were measured from cell cultures 24 h after

transduction by an AAV2-Luciferase reporter vector (2E8 vg) performed in triplicate in a 96-well plate format. Results were normalized to wells containing only AAV2 and phosphate buffered saline (PBS), white bar. AAV2 incubated with IVIG (12 μg) for 1 h demonstrated near complete neutralization of the AAV. Protein M analogs (16 μg) incubated at 4°C prevented neutralization of AAV by IVIG when incubated together for 1 h prior to AAV. This was compared to protein M analogs incubated at 4°C but without IVIG incubation, (not done for MG 8 and MG 24). MG WT and some analogs with lower melting temperatures did not protect AAV from neutralization when the analogs were thermally challenged at 37°C prior to incubation with IVIG, while other analogs with higher Tm retained the ability to protect AAV from neutralization. Most mutant MG analogs retained neutralizing antibody blocking ability after 37°C challenge for 1 h. However, MG 27, MG 29, and MG 46 prevented AAV neutralization after 37°C challenge for 24 h.

[0041] Figure 19 shows MG WT blocks NAbs for AAV re-dosing 1-month after primary AAV administration. Wild-type C57BL6 female mice were immunized via intraperitoneal administration of AAV8-GFP (1E9 vg), and serum was collected one month later to assess AAV neutralizing antibody titer *in vitro*. Mice were then administered AAV8-Luciferase reporter vectors intramuscularly (1E9 vg per leg), where AAV was formulated as a simple admixture with MG WT (33 μg per leg) to the mouse's right leg, or AAV was formulated with phosphate buffered saline as a vehicle control to the mouse's left leg. Luminescent imaging of the leg muscles was performed 2 weeks after AAV8-Luciferase administration. Three mice with an AAV neutralizing antibody titer of 1:10 demonstrated neutralization of the AAV luciferase reporter vector in the saline formulated leg, while the AAV luciferase reporter vector was protected from neutralizing antibodies in the MG WT formulated leg of the same mouse. Neutralization of AAV in the saline leg produced an average luciferase signal of 80% less than that of the MG WT formulated leg. This result demonstrates the ability to successfully re-dose AAV using protein M after generation of neutralizing antibodies elicited by a previous AAV dose.

[0042] Figure 20 shows MG 29 blocks NAbs for AAV re-dosing 1-month after primary AAV administration. Wild-type C57BL6 female mice were immunized via intraperitoneal administration of AAV8-GFP (5E8 vg), and serum was collected one month later to assess AAV neutralizing antibody titer *in vitro*. Mice were then administered AAV8-Luciferase reporter vectors intramuscularly (2E9 vg per leg), where AAV was formulated as a simple admixture with the engineered analog MG 29 (500 μg per leg) to the mouse's right leg, or AAV

was formulated with phosphate buffered saline as a vehicle control to the mouse's left leg. Luminescent imaging of the leg muscles was performed 2 weeks after AAV8-Luciferase administration. Three mice each with an AAV neutralizing antibody titer less than 1:100 (individually 1:8, 1:32, and 1:64) demonstrated neutralization of the AAV luciferase reporter vector in the saline formulated leg, while the AAV luciferase reporter vector was protected from neutralizing antibodies in the MG 29 formulated leg of the same mouse. Neutralization of AAV in the saline leg produced an average luciferase signal at least 98% less than that of the MG WT formulated leg. This result demonstrates the ability to successfully re-dose AAV using an engineered protein M analog after generation of neutralizing antibodies elicited by a previous AAV dose.

[0043] Figure 21 shows engineered protein M analogs demonstrate different affinities for IgG. Affinity of specific protein M analogs were measured using biolayer inferometry (BLI). Binding constants (KD) are calculated using association and dissociation rates (kinetic analysis) over a protein M concentration range of 15.6 nM - 250 nM. Results demonstrate enhanced or decreased affinity to IgG affixed to the BLI probe compared to MG WT.

[0044] Figure 22 shows an example of BLI affinity data generated by kinetic analysis. The curve is used to predict the kinetic K_D value. The curve fit is overlaid on collected data.

[0045] Figure 23 shows mutant stabilization success rate. The frequency that mutations predicted by Rosetta for MG WT increased (Tm +1 °C), decreased (Tm -1 °C), or had no effect on stability are shown. 'Combined mutations' represent constructs built by merging mutant constructs that stabilized MG WT individually.

[0046] Figure 24 shows MP WT sequence conservation. The homology model of MP WT is depicted in two orientations, colored by conservation to the MG WT sequence according to the BLOSUM62 matrix. Residues are colored according to degree of conservation, ranging from white (identical) to black (significantly different). The homology model was built with PDB ID: 4NZR as a template.

[0047] Figure 25 shows codon optimization of protein M DNA sequences enhances manufacturing yield. DNA plasmids encoding for MG WT protein were generated using either bacterial codons native to MG281 (Original PM), or codons optimized to both bacteria and human codon usage (Optimized PM). Three pooled bacterial colonies transformed by equivalent concentrations of either the Original PM plasmid or Optimized PM plasmid were cultured and propagated in equivalent growth media volumes (10 mL) for an equivalent overnight time period. Bacteria were pelleted and crude lysates were generated by freeze thaw and lysis in equivalent volumes of lysis buffer. Crude lysate was centrifuged and the

supernatant collected for protein separation on an SDS-PAGE gel. Equal volumes from each lysate were run on the SDS-PAGE gel, transferred to a western blot, and then probed with a mouse antibody against the 6X Histidine tag present on the amino terminus of MG WT, followed by a goat anti-mouse secondary antibody conjugated to horseradish peroxidase (HRP). Band intensity of MG WT protein was measured using a luminol chemiluminescence assay. The resulting MG WT yield was calculated based on western blot band intensity normalized to the weight of the bacterial pellet. The Optimized PM plasmid generated roughly a 4 fold increase in MG WT protein yield when compared to the Original PM plasmid.

[0048] Figure 26 shows improved pH stability of mutants. The melting temperature (Tm) was determined with NanoDSF at varying pH conditions. The protein was purified in PBS via SEC and buffer-exchanged into glycine (pH 2.5 & 3.5), acetate (pH 4.5 & 5.5), and phosphate (pH 6.5 and 7.5) buffers.

[0049] Figures 27A-27D show a sequence alignment of wild-type *M. genitalium* protein M amino acids 74-479 (SEQ ID NO:3) and modified protein M MG1 (SEQ ID NO:4), MG8 (SEQ ID NO:5), MG13 (SEQ ID NO:6), MG15 (SEQ ID NO:7), MG21 (SEQ ID NO:8), MG22 (SEQ ID NO:9), MG23 (SEQ ID NO:10), MG24 (SEQ ID NO:11), MG27 (SEQ ID NO:12), MG28 (SEQ ID NO:13), MG29 (SEQ ID NO:14), MG31 (SEQ ID NO:15), MG33 (SEQ ID NO:16), MG38 (SEQ ID NO:17), MG40 (SEQ ID NO:18), MG43 (SEQ ID NO:19), MG44 (SEQ ID NO:20), MG45 (SEQ ID NO:21), and MG46 (SEQ ID NO:22).

[0050] Figure 28 shows a sequence alignment of wild-type *M. genitalium* protein M amino acids 74-479 (SEQ ID NO:3) and the equivalent fragment of *M. pneumoniae* protein M (SEQ ID NO:23).

[0051] Figure 29 is a bar graph of luminescence after administration. Figure 29 shows *in vivo* antibody neutralization 7 days after administration. Serum was collected and pooled from mice serially immunized with AAV8. In vitro neutralization assay determined that the anti-AAV8 neutralizing titer of the pooled serum to be roughly 1:10,000. Different quantities of the anti-AAV8 serum (determined by serum volume) were administered via IV injection to groups of AAV naive mice in a passive transfer experiment, followed 20-25 minutes later by IV injection of 2e10 viral genomes (vg) of AAV8-Luciferase reporter vector. Some groups of mice were given protein M via IV injection 5 minutes before the AAV administration. Non-invasive in vivo imaging of luciferase activity was performed 7 days later to quantify luminescence as a proxy of gene expression from the AAV8 vector, and any decrease in luminescence signal is indicative of AAV8-Luciferase neutralization by the serum. Mice given 0.0003ul of serum failed to neutralize the AAV8 and resulted in nearly 100% luminescence signal as compared

to naive mice without serum injected with 2e10 vg AAV8-Luciferase alone. Groups of mice given increasing quantities of neutralizing serum neutralized progressively more and more AAV8-Luciferase, where 0.001ul to 0.003ul serum neutralized approximately half of the AAV and 0.3ul serum or greater neutralized over 99% of the AAV. Groups of mice administered 3ul of serum, followed by MG88 or MG118 and then 2e10 vg AAV8-Luciferase, resulted in gene expression indicative of escape from neutralization due to suppression of neutralizing antibodies by the protein M fusion proteins MG29 (6.3 mg), MG29* (6.3 mg), and MGWT* (6.3 mg), MG118 (9 mg), and MG88 (6.3 mg).

[0052] Figure 30 shows an *in vitro* neutralization assay where high titer anti-AAV8 serum from mice was titrated in 2-fold dilutions, and added to the well as indicated on the X-axis. Then the different Protein M analogs (MG29, MG66, MG71, and MG64) were added to the well in the molecular ratio indicated in the figure legend and incubated for 1 hour. Saline was added to the serum only well (black circles). Then AAV8-Luciferase ($2x10^{10}$ viral genomes) was added to each well and incubated 1 hr, before adding $5x10^4$ Huh7 cells in serum free media. The cells were cultured 48 hrs and then the luciferase reporter signal, which is a proxy for neutralization, was read and quantified. These data demonstrate these analogs have neutralizing antibody blocking functionality, thereby shifting the neutralization curve, and that the MG64 Fc-Protein M fusion dimer (Fc-PM) shifts the curve greater than the Protein M monomer analogs.

DETAILED DESCRIPTION OF THE INVENTION

[0053] The present invention is explained in greater detail below. This description is not intended to be a detailed catalog of all the different ways in which the invention may be implemented, or all the features that may be added to the instant invention. For example, features illustrated with respect to one embodiment may be incorporated into other embodiments, and features illustrated with respect to a particular embodiment may be deleted from that embodiment. In addition, numerous variations and additions to the various embodiments suggested herein will be apparent to those skilled in the art in light of the instant disclosure which do not depart from the instant invention. Hence, the following specification is intended to illustrate some particular embodiments of the invention, and not to exhaustively specify all permutations, combinations and variations thereof.

[0054] Unless the context indicates otherwise, it is specifically intended that the various features of the invention described herein can be used in any combination. Moreover, the present invention also contemplates that in some embodiments of the invention, any feature or

combination of features set forth herein can be excluded or omitted. To illustrate, if the specification states that a complex comprises components A, B and C, it is specifically intended that any of A, B or C, or a combination thereof, can be omitted and disclaimed singularly or in any combination.

[0055] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. The terminology used in the description of the invention herein is for the purpose of describing particular embodiments only and is not intended to be limiting of the invention.

[0056] Nucleotide sequences are presented herein by single strand only, in the 5' to 3' direction, from left to right, unless specifically indicated otherwise. Nucleotides and amino acids are represented herein in the manner recommended by the IUPAC-IUB Biochemical Nomenclature Commission, or (for amino acids) by either the one-letter code, or the three letter code, both in accordance with 37 C.F.R. §1.822 and established usage.

[0057] Except as otherwise indicated, standard methods known to those skilled in the art may be used for production of recombinant and synthetic polypeptides, antibodies or antigen-binding fragments thereof, manipulation of nucleic acid sequences, production of transformed cells, the construction of rAAV constructs, modified capsid proteins, packaging vectors expressing the AAV rep and/or cap sequences, and transiently and stably transfected packaging cells. Such techniques are known to those skilled in the art. *See, e.g.,* SAMBROOK *et al.*, MOLECULAR CLONING: A LABORATORY MANUAL 2nd Ed. (Cold Spring Harbor, NY, 1989); F. M. AUSUBEL *et al.* CURRENT PROTOCOLS IN MOLECULAR BIOLOGY (Green Publishing Associates, Inc. and John Wiley & Sons, Inc., New York).

[0058] All publications, patent applications, patents, nucleotide sequences, amino acid sequences and other references mentioned herein are incorporated by reference in their entirety.

Definitions

[0059] As used in the description of the invention and the appended claims, the singular forms "a," "an" and "the" are intended to include the plural forms as well, unless the context clearly indicates otherwise.

[0060] As used herein, "and/or" refers to and encompasses any and all possible combinations of one or more of the associated listed items, as well as the lack of combinations when interpreted in the alternative ("or").

[0061] Moreover, the present invention also contemplates that in some embodiments of the invention, any feature or combination of features set forth herein can be excluded or omitted.

[0062] Furthermore, the term "about," as used herein when referring to a measurable value such as an amount of a compound or agent of this invention, dose, time, temperature, and the like, is meant to encompass variations of ± 10%, ± 5%, ± 1%, ± 0.5%, or even ± 0.1% of the specified amount.

[0063] As used herein, the transitional phrase "consisting essentially of" is to be interpreted as encompassing the recited materials or steps and those that do not materially affect the basic and novel characteristic(s) of the claimed invention. Thus, the term "consisting essentially of" as used herein should not be interpreted as equivalent to "comprising."

[0064] The term "consists essentially of" (and grammatical variants), as applied to a polynucleotide or polypeptide sequence of this invention, means a polynucleotide or polypeptide that consists of both the recited sequence (*e.g.,* SEQ ID NO) and a total of ten or less (*e.g.,* 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) additional nucleotides or amino acids on the 5' and/or 3' or N-terminal and/or C-terminal ends of the recited sequence or between the two ends (*e.g.,* between domains) such that the function of the polynucleotide or polypeptide is not materially altered. The total of ten or less additional nucleotides or amino acids includes the total number of additional nucleotides or amino acids added together. The term "materially altered," as applied to polynucleotides of the invention, refers to an increase or decrease in ability to express the encoded polypeptide of at least about 50% or more as compared to the expression level of a polynucleotide consisting of the recited sequence. The term "materially altered," as applied to polypeptides of the invention, refers to an increase or decrease in biological activity of at least about 50% or more as compared to the activity of a polypeptide consisting of the recited sequence.

[0065] The term "parvovirus" as used herein encompasses the family *Parvoviridae*, including autonomously-replicating parvoviruses and dependoviruses. The autonomous parvoviruses include members of the genera *Parvovirus*, *Erythrovirus*, *Densovirus*, *Iteravirus*, and *Contravirus*. Exemplary autonomous parvoviruses include, but are not limited to, minute virus of mouse, bovine parvovirus, canine parvovirus, chicken parvovirus, feline panleukopenia virus, feline parvovirus, goose parvovirus, H1 parvovirus, muscovy duck parvovirus, snake parvovirus, and B19 virus. Other autonomous parvoviruses are known to those skilled in the art. *See, e.g.,* FIELDS *et al.*, VIROLOGY, volume 2, chapter 69 (4th ed., Lippincott-Raven Publishers).

[0066] The genus *Dependovirus* contains the adeno-associated viruses (AAV), including but not limited to, AAV type 1, AAV type 2, AAV type 3 (including types 3A and 3B), AAV type 4, AAV type 5, AAV type 6, AAV type 7, AAV type 8, AAV type 9, AAV type 10, AAV type 11, AAV type 12, AAV type 13, avian AAV, bovine AAV, canine AAV, goat AAV, snake AAV, equine AAV, and ovine AAV. *See, e.g.,* FIELDS *et al.*, VIROLOGY, volume 2, chapter 69 (4th ed., Lippincott-Raven Publishers); and **Table 1**.

[0067] The term "adeno-associated virus" (AAV) in the context of the present invention includes without limitation AAV type 1, AAV type 2, AAV type 3 (including types 3A and 3B), AAV type 4, AAV type 5, AAV type 6, AAV type 7, AAV type 8, AAV type 9, AAV type 10, AAV type 11, avian AAV, bovine AAV, canine AAV, equine AAV, and ovine AAV and any other AAV now known or later discovered. *See, e.g.,* BERNARD N. FIELDS *et al.*, VIROLOGY, volume 2, chapter 69 (4th ed., Lippincott-Raven Publishers). A number of additional AAV serotypes and clades have been identified (*see, e.g.,* Gao *et al.*, (2004) *J. Virol.* 78:6381-6388 and **Table 1**), which are also encompassed by the term "AAV."

[0068] The parvovirus particles and genomes of the present invention can be from, but are not limited to, AAV. The genomic sequences of various serotypes of AAV and the autonomous parvoviruses, as well as the sequences of the native ITRs, Rep proteins, and capsid subunits are known in the art. Such sequences may be found in the literature or in public databases such as GenBank. *See*, *e.g.,* GenBank Accession Numbers NC_002077, NC_001401, NC_001729, NC_001863, NC_001829, NC_001862, NC_000883, NC_001701, NC_001510, NC_006152, NC_006261, AF063497, U89790, AF043303, AF028705, AF028704, J02275, J01901, J02275, X01457, AF288061, AH009962, AY028226, AY028223, AY631966, AX753250, EU285562, NC_001358, NC_001540, AF513851, AF513852 and AY530579; the disclosures of which are incorporated by reference herein for teaching parvovirus and AAV nucleic acid and amino acid sequences. *See also, e.g.,* Bantel-Schaal *et al.*, (1999) *J. Virol.* 73: 939; Chiorini *et al.*, (1997) *J. Virol.* 71:6823; Chiorini *et al.*, (1999) *J. Virol.* 73:1309; Gao *et al.*, (2002) *Proc. Nat. Acad. Sci. USA* 99:11854; Moris *et al.*, (2004) *Virol.* 33-:375-383; Mori *et al.*, (2004) *Virol.* 330:375; Muramatsu *et al.*, (1996) *Virol.* 221:208; Ruffing *et al.*, (1994) *J. Gen. Virol.* 75:3385; Rutledge *et al.*, (1998) *J. Virol.* 72:309; Schmidt *et al.*, (2008) *J. Virol.* 82:8911; Shade *et al.*, (1986) *J. Virol.* 58:921; Srivastava *et al.*, (1983) *J. Virol.* 45:555; Xiao *et al.*, (1999) *J. Virol.* 73:3994; international patent publications WO 00/28061, WO 99/61601, WO 98/11244; and U.S. Patent No. 6,156,303; the disclosures of which are incorporated by reference herein for teaching parvovirus and AAV nucleic acid and amino acid sequences. *See also* **Table 1**. An early description of the AAV1, AAV2 and AAV3 ITR sequences is provided

by Xiao, X., (1996), "Characterization of Adeno-associated virus (AAV) DNA replication and integration," Ph.D. Dissertation, University of Pittsburgh, Pittsburgh, PA (incorporated herein it its entirety).

[0069] A "chimeric" AAV nucleic acid capsid coding sequence or AAV capsid protein is one that combines portions of two or more capsid sequences. A "chimeric" AAV virion or particle comprises a chimeric AAV capsid protein.

[0070] The term "tropism" as used herein refers to preferential but not necessarily exclusive entry of the vector (*e.g.,* virus vector) into certain cell or tissue type(s) and/or preferential but not necessarily exclusive interaction with the cell surface that facilitates entry into certain cell or tissue types, optionally and preferably followed by expression (*e.g.,* transcription and, optionally, translation) of sequences carried by the vector contents (*e.g.,* viral genome) in the cell, *e.g.,* for a recombinant virus, expression of the heterologous nucleotide sequence(s). Those skilled in the art will appreciate that transcription of a heterologous nucleic acid sequence from the viral genome may not be initiated in the absence of trans-acting factors, *e.g.,* for an inducible promoter or otherwise regulated nucleic acid sequence. In the case of a rAAV genome, gene expression from the viral genome may be from a stably integrated provirus and/or from a non-integrated episome, as well as any other form which the virus nucleic acid may take within the cell.

[0071] The term "tropism profile" refers to the pattern of transduction of one or more target cells, tissues and/or organs. Representative examples of chimeric AAV capsids have a tropism profile characterized by efficient transduction of cells of the central nervous system (CNS) with only low transduction of peripheral organs (*see e.g.* US Patent No. 9,636,370 McCown *et al.*, and US patent publication 2017/0360960 Gray *et al.*). Vectors (*e.g.,* virus vectors, *e.g.,* AAV capsids) expressing specific tropism profiles may be referred to as "tropic" for their tropism profile, *e.g.,* neuro-tropic, liver-tropic, *etc.*

[0072] As used herein, "transduction" of a cell by a virus vector (*e.g.,* an AAV vector) means entry of the vector into the cell and transfer of genetic material into the cell by the incorporation of nucleic acid into the virus vector and subsequent transfer into the cell via the virus vector.

Table 1

AAV Serotypes/Isolates	GenBank Accession Number		AAV Serotypes/Isolates	GenBank Accession Number		AAV Serotypes/Isolates	GenBank Accession Number
Clonal Isolates			Hu52	AY530614		Cy3	AY243019
Avian AAV ATCC VR-865	AY186198, AY629583, NC_004828		Hu T41	AY695378		Cy5	AY243017
Avian AAV strain DA-1	NC_006263, AY629583		Hu S17	AY695376		Rh13	AY243013
Bovine AAV	NC_005889, AY388617		Hu T88	AY695375			
AAV4	NC_001829		Hu T71	AY695374		**Clade E**	
AAV5	AY18065, AF085716		Hu T70	AY695373		Rh38	AY530558
Rh34	AY243001		Hu T40	AY695372		Hu66	AY530626
Rh33	AY243002		Hu T32	AY695371		Hu42	AY530605
Rh32	AY243003		Hu T17	AY695370		Hu67	AY530627
AAV10	AY631965		Hu LG15	AY695377		Hu40	AY530603
AAV11	AY631966					Hu41	AY530604
AAV12	DQ813647		**Clade C**			Hu37	AY530600
AAV13	EU285562		**AAV 3**	NC_001729		Rh40	AY530559
			AAV 3B	NC_001863		Rh2	AY243007
Clade A			Hu9	AY530629		Bb1	AY243023
AAV1	NC_002077, AF063497		Hu10	AY530576		Bb2	AY243022
AAV6	NC_001862		Hu11	AY530577		**Rh10**	AY243015
Hu.48	AY530611		Hu53	AY530615		Hu17	AY530582
Hu 43	AY530606		Hu55	AY530617		Hu6	AY530621
Hu 44	AY530607		Hu54	AY530616		Rh25	AY530557
Hu 46	AY530609		Hu7	AY530628		Pi2	AY530554
			Hu18	AY530583		Pi1	AY530553
Clade B			Hu15	AY530580		Pi3	AY530555
Hu19	AY530584		Hu16	AY530581		Rh57	AY530569
Hu20	AY530586		Hu25	AY530591		Rh50	AY530563
Hu23	AY530589		Hu60	AY530622		Rh49	AY530562
Hu22	AY530588		Ch5	AY243021		Hu39	AY530601
Hu24	AY530590		Hu3	AY530595		Rh58	AY530570
Hu21	AY530587		Hu1	AY530575		Rh61	AY530572
Hu27	AY530592		Hu4	AY530602		Rh52	AY530565
Hu28	AY530593		Hu2	AY530585		Rh53	AY530566
Hu29	AY530594		Hu61	AY530623		Rh51	AY530564
Hu63	AY530624					Rh64	AY530574
Hu64	AY530625		**Clade D**			Rh43	AY530560
Hu13	AY530578		Rh62	AY530573		**AAV8**	AF513852
Hu56	AY530618		Rh48	AY530561		Rh8	AY242997
Hu57	AY530619		Rh54	AY530567		Rh1	AY530556
Hu49	AY530612		Rh55	AY530568			
Hu58	AY530620		Cy2	AY243020		**Clade F**	
Hu34	AY530598		**AAV7**	AF513851		**AAV9** (Hu14)	AY530579
Hu35	AY530599		Rh35	AY243000		Hu31	AY530596
AAV2	NC_001401		Rh37	AY242998		Hu32	AY530597
Hu45	AY530608		Rh36	AY242999			
Hu47	AY530610		Cy6	AY243016			
Hu51	AY530613		Cy4	AY243018			

[0073] Unless indicated otherwise, "efficient transduction" or "efficient tropism," or similar terms, can be determined by reference to a suitable positive or negative control (*e.g.*, at least about 50%, 60%, 70%, 80%, 85%, 90%, 95% or more of the transduction or tropism, respectively, of a positive control or at least about 110%, 120%, 150%, 200%, 300%, 500%, 1000% or more of the transduction or tropism, respectively, of a negative control).

[0074] Similarly, it can be determined if a virus "does not efficiently transduce" or "does not have efficient tropism" for a target tissue, or similar terms, by reference to a suitable control. In particular embodiments, the virus vector does not efficiently transduce (*i.e.*, does not have efficient tropism for) tissues outside the CNS, *e.g.*, liver, kidney, gonads and/or germ cells. In particular embodiments, undesirable transduction of tissue(s) (*e.g.*, liver) is 20% or less, 10% or less, 5% or less, 1% or less, 0.1% or less of the level of transduction of the desired target tissue(s) (*e.g.*, CNS cells).

[0075] The terms "5' portion" and "3' portion" are relative terms to define a spatial relationship between two or more elements. Thus, for example, a "3' portion" of a polynucleotide indicates a segment of the polynucleotide that is downstream of another segment. The term "3' portion" is not intended to indicate that the segment is necessarily at the 3' end of the polynucleotide, or even that it is necessarily in the 3' half of the polynucleotide, although it may be. Likewise, a "5' portion" of a polynucleotide indicates a segment of the polynucleotide that is upstream of another segment. The term "5' portion" is not intended to indicate that the segment is necessarily at the 5' end of the polynucleotide, or even that it is necessarily in the 5' half of the polynucleotide, although it may be.

[0076] As used herein, the term "polypeptide" encompasses both peptides and proteins, unless indicated otherwise.

[0077] A "polynucleotide," "nucleic acid," or "nucleotide sequence" may be of RNA, DNA or DNA-RNA hybrid sequences (including both naturally occurring and non-naturally occurring nucleotides), but is preferably either a single or double stranded DNA sequence.

[0078] The term "regulatory element" refers to a genetic element which controls some aspect of the expression of nucleic acid sequences. For example, a promoter is a regulatory element which facilitates the initiation of transcription of an operably linked coding region. Other regulatory elements are splicing signals, polyadenylation signals, termination signals, *etc*. The region in a nucleic acid sequence or polynucleotide in which one or more regulatory elements are found may be referred to as a "regulatory region."

[0079] The term "fragment," as applied to a polypeptide, will be understood to mean an amino acid sequence of reduced length relative to a reference polypeptide or amino acid

sequence and comprising, consisting essentially of, and/or consisting of an amino acid sequence of contiguous amino acids identical to the reference polypeptide or amino acid sequence. In some embodiments, such fragments can comprise, consist essentially of, and/or consist of peptides having a length of at least about 4, 6, 8, 10, 12, 15, 20, 25, 30, 35, 40, 45, 50, 75, 100, 150, 200, or more consecutive amino acids of a polypeptide or amino acid sequence according to the invention. In some embodiments, such fragments can comprise, consist essentially of, and/or consist of peptides having a length of less than about 4, 6, 8, 10, 12, 15, 20, 25, 30, 35, 40, 45, 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, or 500 consecutive amino acids of a polypeptide or amino acid sequence according to the invention.

[0080] As used herein, a "functional fragment" is one that substantially retains at least one biological activity normally associated with that polypeptide (*e.g.*, antibody binding). In "functional fragment" substantially retains all of the activities possessed by the unmodified polypeptide. By "substantially retains" biological activity, it is meant that the polypeptide retains at least about 20%, 30%, 40%, 50%, 60%, 75%, 85%, 90%, 95%, 97%, 98%, 99%, or more, of the biological activity of the native polypeptide (and can even have a higher level of activity than the native polypeptide). A "non-functional" polypeptide is one that exhibits little or essentially no detectable biological activity normally associated with the polypeptide (*e.g.*, at most, only an insignificant amount, *e.g.*, less than about 10% or even 5%). Biological activities such as antibody binding can be measured using assays that are well known in the art and as described herein.

[0081] As used herein with respect to nucleic acids, the term "operably linked" refers to a functional linkage between two or more nucleic acids. For example, a promoter sequence may be described as being "operably linked" to a heterologous nucleic acid sequence because the promoter sequences initiates and/or mediates transcription of the heterologous nucleic acid sequence. In some embodiments, the operably linked nucleic acid sequences are contiguous and/or are in the same reading frame.

[0082] The term "open reading frame (ORF)," as used herein, refers to the portion of a polynucleotide (*e.g.,* a gene) that encodes a polypeptide, and is inclusive of the initiation start site (*i.e.,* Kozak sequence) that initiates transcription of the polypeptide. The term "coding region" may be used interchangeably with open reading frame.

[0083] The term "codon-optimized," as used herein, refers to a gene coding sequence that has been optimized to increase expression by substituting one or more codons normally present in a coding sequence (for example, in a wildtype sequence, including, *e.g.,* a coding sequence for protein M) with a codon for the same (synonymous) amino acid. In this manner, the protein

encoded by the gene is identical, but the underlying nucleobase sequence of the gene or corresponding mRNA is different. In some embodiments, the optimization substitutes one or more rare codons (that is, codons for tRNA that occur relatively infrequently in cells from a particular species) with synonymous codons that occur more frequently to improve the efficiency of translation. For example, in human codon-optimization one or more codons in a coding sequence are replaced by codons that occur more frequently in human cells for the same amino acid. Codon optimization can also increase gene expression through other mechanisms that can improve efficiency of transcription and/or translation. Strategies include, without limitation, increasing total GC content (that is, the percent of guanines and cytosines in the entire coding sequence), decreasing CpG content (that is, the number of CG or GC dinucleotides in the coding sequence), removing cryptic splice donor or acceptor sites, and/or adding or removing ribosomal entry and/or initiation sites, such as Kozak sequences. Desirably, a codon-optimized gene exhibits improved protein expression, for example, the protein encoded thereby is expressed at a detectably greater level in a cell compared with the level of expression of the protein provided by the wildtype gene in an otherwise similar cell. Codon-optimization also provides the ability to distinguish a codon-optimized gene and/or corresponding mRNA from an endogenous gene and/or corresponding mRNA *in vitro* or *in vivo*.

[0084] The term "sequence identity," as used herein, has the standard meaning in the art. As is known in the art, a number of different programs can be used to identify whether a polynucleotide or polypeptide has sequence identity or similarity to a known sequence. Sequence identity or similarity may be determined using standard techniques known in the art, including, but not limited to, the local sequence identity algorithm of Smith & Waterman, *Adv. Appl. Math. 2*:482 (1981), by the sequence identity alignment algorithm of Needleman & Wunsch, *J. Mol. Biol. 48*:443 (1970), by the search for similarity method of Pearson & Lipman, *Proc. Natl. Acad. Sci. USA 85*:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Drive, Madison, WI), the Best Fit sequence program described by Devereux *et al.*, *Nucl. Acid Res. 12*:387 (1984), preferably using the default settings, or by inspection.

[0085] An example of a useful algorithm is PILEUP. PILEUP creates a multiple sequence alignment from a group of related sequences using progressive, pairwise alignments. It can also plot a tree showing the clustering relationships used to create the alignment. PILEUP uses a

simplification of the progressive alignment method of Feng & Doolittle, *J. Mol. Evol. 35*:351 (1987); the method is similar to that described by Higgins & Sharp, *CABIOS 5*:151 (1989).

[0086] Another example of a useful algorithm is the BLAST algorithm, described in Altschul *et al.*, *J. Mol. Biol. 215*:403 (1990) and Karlin *et al., Proc. Natl. Acad. Sci. USA 90*:5873 (1993). A particularly useful BLAST program is the WU-BLAST-2 program which was obtained from Altschul *et al.*, *Meth. Enzymol.*, *266*:460 (1996); blast.wustl/edu/blast/README.html. WU-BLAST-2 uses several search parameters, which are preferably set to the default values. The parameters are dynamic values and are established by the program itself depending upon the composition of the particular sequence and composition of the particular database against which the sequence of interest is being searched; however, the values may be adjusted to increase sensitivity.

[0087] An additional useful algorithm is gapped BLAST as reported by Altschul *et al., Nucleic Acids Res. 25*:3389 (1997).

[0088] A percentage amino acid sequence identity value is determined by the number of matching identical residues divided by the total number of residues of the "longer" sequence in the aligned region. The "longer" sequence is the one having the most actual residues in the aligned region (gaps introduced by WU-Blast-2 to maximize the alignment score are ignored).

[0089] In a similar manner, percent nucleic acid sequence identity is defined as the percentage of nucleotide residues in the candidate sequence that are identical with the nucleotides in the polynucleotide specifically disclosed herein.

[0090] The alignment may include the introduction of gaps in the sequences to be aligned. In addition, for sequences which contain either more or fewer nucleotides than the polynucleotides specifically disclosed herein, it is understood that in one embodiment, the percentage of sequence identity will be determined based on the number of identical nucleotides in relation to the total number of nucleotides. Thus, for example, sequence identity of sequences shorter than a sequence specifically disclosed herein, will be determined using the number of nucleotides in the shorter sequence, in one embodiment. In percent identity calculations relative weight is not assigned to various manifestations of sequence variation, such as insertions, deletions, substitutions, *etc.*

[0091] In one embodiment, only identities are scored positively (+1) and all forms of sequence variation including gaps are assigned a value of "0," which obviates the need for a weighted scale or parameters as described below for sequence similarity calculations. Percent sequence identity can be calculated, for example, by dividing the number of matching identical residues by the total number of residues of the "shorter" sequence in the aligned region and

multiplying by 100. The "longer" sequence is the one having the most actual residues in the aligned region.

[0092] As used herein, an "isolated" nucleic acid or nucleotide sequence (*e.g.*, an "isolated DNA" or an "isolated RNA") means a nucleic acid or nucleotide sequence separated or substantially free from at least some of the other components of the naturally occurring organism or virus, for example, the cell or viral structural components or other polypeptides or nucleic acids commonly found associated with the nucleic acid or nucleotide sequence.

[0093] Likewise, an "isolated" polypeptide means a polypeptide that is separated or substantially free from at least some of the other components of the naturally occurring organism or virus, for example, the cell or viral structural components or other polypeptides or nucleic acids commonly found associated with the polypeptide.

[0094] As used herein, the term "modified," as applied to a polynucleotide or polypeptide sequence, refers to a sequence that differs from a wild-type sequence due to one or more deletions, additions, substitutions, or any combination thereof.

[0095] As used herein, by "isolate" (or grammatical equivalents) a virus vector, it is meant that the virus vector is at least partially separated from at least some of the other components in the starting material.

[0096] By the term "treat," "treating," or "treatment of" (or grammatically equivalent terms) is meant to reduce or to at least partially improve or ameliorate the severity of the subject's condition and/or to alleviate, mitigate or decrease in at least one clinical symptom and/or to delay the progression of the condition.

[0097] As used herein, the term "prevent," "prevents," or "prevention" (and grammatical equivalents thereof) means to delay or inhibit the onset of a disease. The terms are not meant to require complete abolition of disease, and encompass any type of prophylactic treatment to reduce the incidence of the condition or delays the onset of the condition.

[0098] A "treatment effective" amount as used herein is an amount that is sufficient to provide some improvement or benefit to the subject. Alternatively stated, a "treatment effective" amount is an amount that will provide some alleviation, mitigation, decrease or stabilization in at least one clinical symptom in the subject. Those skilled in the art will appreciate that the therapeutic effects need not be complete or curative, as long as some benefit is provided to the subject.

[0099] A "prevention effective" amount as used herein is an amount that is sufficient to prevent and/or delay the onset of a disease, disorder and/or clinical symptoms in a subject and/or to reduce and/or delay the severity of the onset of a disease, disorder and/or clinical

symptoms in a subject relative to what would occur in the absence of the methods of the invention. Those skilled in the art will appreciate that the level of prevention need not be complete, as long as some benefit is provided to the subject.

[0100] A "heterologous nucleotide sequence" or "heterologous nucleic acid," with respect to a virus, is a sequence or nucleic acid, respectively, that is not naturally occurring in the virus. Generally, the heterologous nucleic acid or nucleotide sequence comprises an open reading frame that encodes a polypeptide and/or a nontranslated RNA.

[0101] A "vector" refers to a compound used as a vehicle to carry foreign genetic material into another cell, where it can be replicated and/or expressed. A cloning vector containing foreign nucleic acid is termed a recombinant vector. Examples of nucleic acid vectors are plasmids, viral vectors, cosmids, expression cassettes, and artificial chromosomes. Recombinant vectors typically contain an origin of replication, a multicloning site, and a selectable marker. The nucleic acid sequence typically consists of an insert (recombinant nucleic acid or transgene) and a larger sequence that serves as the "backbone" of the vector. The purpose of a vector which transfers genetic information to another cell is typically to isolate, multiply, or express the insert in the target cell. Expression vectors (expression constructs or expression cassettes) are for the expression of the exogenous gene in the target cell, and generally have a promoter sequence that drives expression of the exogenous gene/ORF. Insertion of a vector into the target cell is referred to transformation or transfection for bacterial and eukaryotic cells, although insertion of a viral vector is often called transduction. The term "vector" may also be used in general to describe items to that serve to carry foreign genetic material into another cell, such as, but not limited to, a transformed cell or a nanoparticle.

[0102] As used herein, the term "vector," "virus vector," "delivery vector" (and similar terms) in a specific embodiment generally refers to a virus particle that functions as a nucleic acid delivery vehicle, and which comprises the viral nucleic acid (*i.e.,* the vector genome) packaged within the virion. Virus vectors according to the present invention comprise a chimeric AAV capsid according to the invention and can package an AAV or rAAV genome or any other nucleic acid including viral nucleic acids. Alternatively, in some contexts, the term "vector," "virus vector," "delivery vector" (and similar terms) may be used to refer to the vector genome (*e.g.,* vDNA) in the absence of the virion and/or to a viral capsid that acts as a transporter to deliver molecules tethered to the capsid or packaged within the capsid.

[0103] The virus vectors of the invention can further be duplexed parvovirus particles as described in international patent publication WO 01/92551 (the disclosure of which is

incorporated herein by reference in its entirety). Thus, in some embodiments, double stranded (duplex) genomes can be packaged.

[0104] A "recombinant AAV vector genome" or "rAAV genome" is an AAV genome (*i.e.,* vDNA) that comprises at least one inverted terminal repeat (*e.g.,* one, two or three inverted terminal repeats) and one or more heterologous nucleotide sequences. rAAV vectors generally retain the 145 base terminal repeat(s) (TR(s)) in *cis* to generate virus; however, modified AAV TRs and non-AAV TRs including partially or completely synthetic sequences can also serve this purpose. All other viral sequences are dispensable and may be supplied in *trans* (Muzyczka, (1992) *Curr. Topics Microbiol. Immunol.* 158:97). The rAAV vector optionally comprises two TRs (*e.g.,* AAV TRs), which generally will be at the 5' and 3' ends of the heterologous nucleotide sequence(s), but need not be contiguous thereto. The TRs can be the same or different from each other. The vector genome can also contain a single ITR at its 3' or 5' end.

[0105] The term "terminal repeat" or "TR" includes any viral terminal repeat or synthetic sequence that forms a hairpin structure and functions as an inverted terminal repeat (ITR) (*i.e.,* mediates the desired functions such as replication, virus packaging, integration and/or provirus rescue, and the like). The TR can be an AAV ITR or a non-AAV TR. For example, a non-AAV TR sequence such as those of other parvoviruses (*e.g.,* canine parvovirus (CPV), mouse parvovirus (MVM), human parvovirus B-19) or the SV40 hairpin that serves as the origin of SV40 replication can be used as a TR, which can further be modified by truncation, substitution, deletion, insertion and/or addition. Further, the TR can be partially or completely synthetic, such as the "double-D sequence" as described in United States Patent No. 5,478,745 to Samulski *et al.*

[0106] Parvovirus genomes have palindromic sequences at both their 5' and 3' ends. The palindromic nature of the sequences leads to the formation of a hairpin structure that is stabilized by the formation of hydrogen bonds between the complementary base pairs. This hairpin structure is believed to adopt a "Y" or a "T" shape. *See*, *e.g.,* FIELDS *et al.*, VIROLOGY, volume 2, chapters 69 & 70 (4th ed., Lippincott-Raven Publishers).

[0107] An "AAV inverted terminal repeat" or "AAV ITR" may be from any AAV, including but not limited to serotypes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or 11 or any other AAV now known or later discovered (*see, e.g.,* **Table 1**). An AAV ITR need not have the native ITR sequence (*e.g.,* a native AAV ITR sequence may be altered by insertion, deletion, truncation and/or missense mutations), as long as the ITR mediates the desired functions, *e.g.,* replication, virus packaging, integration, and/or provirus rescue, and the like.

[0108] The terms "rAAV particle" and "rAAV virion" are used interchangeably here. A "rAAV particle" or "rAAV virion" comprises a rAAV vector genome packaged within an AAV capsid.

[0109] The virus vectors of the invention can further be "targeted" virus vectors (*e.g.*, having a directed tropism) and/or a "hybrid" parvovirus (*i.e.*, in which the viral ITRs and viral capsid are from different parvoviruses) as described in international patent publication WO 00/28004 and Chao *et al.*, (2000) *Mol. Therapy* 2:619.

[0110] Further, the viral capsid or genomic elements can contain other modifications, including insertions, deletions and/or substitutions.

[0111] As used herein, the term "amino acid" encompasses any naturally occurring amino acids, modified forms thereof, and synthetic amino acids, including non-naturally occurring amino acids.

[0112] Naturally occurring, levorotatory (L-) amino acids are shown in **Table 2**.

Table 2

Amino Acid Residue	Abbreviation	
	Three-Letter Code	One-Letter Code
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid (Aspartate)	Asp	D
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic acid (Glutamate)	Glu	E
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V

[0113] Alternatively, the amino acid can be a modified amino acid residue (nonlimiting examples are shown in **Table 3**) or can be an amino acid that is modified by post-translation modification (*e.g.*, acetylation, amidation, formylation, hydroxylation, methylation, phosphorylation or sulfatation).

Table 3: Amino Acid Residue Derivatives

Modified Amino Acid Residue	Abbreviation
2-Aminoadipic acid	Aad
3-Aminoadipic acid	bAad
beta-Alanine, beta-Aminoproprionic acid	bAla
2-Aminobutyric acid	Abu
4-Aminobutyric acid, Piperidinic acid	4Abu
6-Aminocaproic acid	Acp
2-Aminoheptanoic acid	Ahe
2-Aminoisobutyric acid	Aib
3-Aminoisobutyric acid	bAib
2-Aminopimelic acid	Apm
t-butylalanine	t-BuA
Citrulline	Cit
Cyclohexylalanine	Cha
2,4-Diaminobutyric acid	Dbu
Desmosine	Des
2,2'-Diaminopimelic acid	Dpm
2,3-Diaminoproprionic acid	Dpr
N-Ethylglycine	EtGly
N-Ethylasparagine	EtAsn
Homoarginine	hArg
Homocysteine	hCys
Homoserine	hSer
Hydroxylysine	Hyl
Allo-Hydroxylysine	aHyl
3-Hydroxyproline	3Hyp
4-Hydroxyproline	4Hyp
Isodesmosine	Ide
allo-Isoleucine	aIle
Methionine sulfoxide	MSO
N-Methylglycine, sarcosine	MeGly
N-Methylisoleucine	MeIle
6-N-Methyllysine	MeLys
N-Methylvaline	MeVal
2-Naphthylalanine	2-Nal
Norvaline	Nva
Norleucine	Nle
Ornithine	Orn
4-Chlorophenylalanine	Phe(4-Cl)
2-Fluorophenylalanine	Phe(2-F)
3-Fluorophenylalanine	Phe(3-F)
4-Fluorophenylalanine	Phe(4-F)
Phenylglycine	Phg
Beta-2-thienylalanine	Thi

[0114] Further, the non-naturally occurring amino acid can be an "unnatural" amino acid as described by Wang *et al.*, (2006) *Annu. Rev. Biophys. Biomol. Struct.* 35:225-49. These unnatural amino acids can advantageously be used to chemically link molecules of interest to the AAV capsid protein.

[0115] Conservative amino acid substitutions are known in the art. In particular embodiments, a conservative amino acid substitution includes substitutions within one or more of the following groups: glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid; asparagine, glutamine; serine, threonine; lysine, arginine; and/or phenylalanine, tyrosine.

[0116] The term "template" or "substrate" is used herein to refer to a polynucleotide sequence that may be replicated to produce the parvovirus viral DNA. For the purpose of vector production, the template will typically be embedded within a larger nucleotide sequence or construct, including but not limited to a plasmid, naked DNA vector, bacterial artificial chromosome (BAC), yeast artificial chromosome (YAC) or a viral vector (*e.g.,* adenovirus, herpesvirus, Epstein-Barr Virus, AAV, baculoviral, retroviral vectors, and the like). Alternatively, the template may be stably incorporated into the chromosome of a packaging cell.

[0117] As used herein, parvovirus or AAV "Rep coding sequences" indicate the nucleic acid sequences that encode the parvoviral or AAV non-structural proteins that mediate viral replication and the production of new virus particles. The parvovirus and AAV replication genes and proteins have been described in, *e.g.,* FIELDS *et al.*, VIROLOGY, volume 2, chapters 69 & 70 (4th ed., Lippincott-Raven Publishers).

[0118] The "Rep coding sequences" need not encode all of the parvoviral or AAV Rep proteins. For example, with respect to AAV, the Rep coding sequences do not need to encode all four AAV Rep proteins (Rep78, Rep 68, Rep52 and Rep40), in fact, it is believed that AAV5 only expresses the spliced Rep68 and Rep40 proteins. In representative embodiments, the Rep coding sequences encode at least those replication proteins that are necessary for viral genome replication and packaging into new virions. The Rep coding sequences will generally encode at least one large Rep protein (*i.e.,* Rep78/68) and one small Rep protein (*i.e.,* Rep52/40). In particular embodiments, the Rep coding sequences encode the AAV Rep78 protein and the AAV Rep52 and/or Rep40 proteins. In other embodiments, the Rep coding sequences encode the Rep68 and the Rep52 and/or Rep40 proteins. In a still further embodiment, the Rep coding sequences encode the Rep68 and Rep52 proteins, Rep68 and Rep40 proteins, Rep78 and Rep52 proteins, or Rep78 and Rep40 proteins.

[0119] As used herein, the term "large Rep protein" refers to Rep68 and/or Rep78. Large Rep proteins of the claimed invention may be either wildtype or synthetic. A wildtype large Rep protein may be from any parvovirus or AAV, including but not limited to serotypes 1, 2, 3a, 3b, 4, 5, 6, 7, 8, 9, 10, 11, or 13, or any other AAV now known or later discovered (*see,*

e.g., **Table 1**). A synthetic large Rep protein may be altered by insertion, deletion, truncation and/or missense mutations.

[0120] Those skilled in the art will further appreciate that it is not necessary that the replication proteins be encoded by the same polynucleotide. For example, for MVM, the NS-1 and NS-2 proteins (which are splice variants) may be expressed independently of one another. Likewise, for AAV, the p19 promoter may be inactivated and the large Rep protein(s) expressed from one polynucleotide and the small Rep protein(s) expressed from a different polynucleotide. Typically, however, it will be more convenient to express the replication proteins from a single construct. In some systems, the viral promoters (*e.g.*, AAV p19 promoter) may not be recognized by the cell, and it is therefore necessary to express the large and small Rep proteins from separate expression cassettes. In other instances, it may be desirable to express the large Rep and small Rep proteins separately, *i.e.*, under the control of separate transcriptional and/or translational control elements. For example, it may be desirable to control expression of the large Rep proteins, so as to decrease the ratio of large to small Rep proteins. In the case of insect cells, it may be advantageous to down-regulate expression of the large Rep proteins (*e.g.*, Rep78/68) to avoid toxicity to the cells (*see, e.g.*, Urabe *et al.*, (2002) *Human Gene Therapy* 13:1935).

[0121] As used herein, the parvovirus or AAV "cap coding sequences" encode the structural proteins that form a functional parvovirus or AAV capsid (*i.e.*, can package DNA and infect target cells). Typically, the cap coding sequences will encode all of the parvovirus or AAV capsid subunits, but less than all of the capsid subunits may be encoded as long as a functional capsid is produced. Typically, but not necessarily, the cap coding sequences will be present on a single nucleic acid molecule.

[0122] The capsid structure of autonomous parvoviruses and AAV are described in more detail in BERNARD N. FIELDS *et al.*, VIROLOGY, volume 2, chapters 69 & 70 (4th ed., Lippincott-Raven Publishers).

[0123] By "substantially retain" a property, it is meant that at least about 75%, 85%, 90%, 95%, 97%, 98%, 99% or 100% of the property (*e.g.*, activity or other measurable characteristic) is retained.

Methods of Using Protein M and Derivatives Thereof to Bind Antibodies

[0124] One aspect of the present invention relates to a method of inhibiting neutralization of a heterologous agent by neutralizing antibodies upon administration of the heterologous agent to a subject, comprising administering to the subject an effective amount of mycoplasma

protein M or a functional fragment or derivative thereof or a fusion protein comprising an effective amount of mycoplasma protein M or a functional fragment or derivative thereof, thereby inhibiting neutralization of the heterologous agent.

[0125] Another aspect of the invention relates to a method of expressing a polypeptide or functional nucleic acid in a subject, comprising administering to the subject (a) a nucleic acid delivery vector encoding the polypeptide or functional nucleic acid, and (b) an effective amount of mycoplasma protein M or a functional fragment or derivative thereof or a fusion protein comprising mycoplasma protein M or a functional fragment or derivative thereof, thereby expressing the polypeptide or functional nucleic acid in the subject.

[0126] A further aspect of the invention relates to a method of editing a gene in a subject, comprising administering to the subject (a) a gene editing complex, and (b) an effective amount of mycoplasma protein M or a functional fragment or derivative thereof or a fusion protein comprising an effective amount of mycoplasma protein M or a functional fragment or derivative thereof, thereby expressing the polypeptide or functional nucleic acid in the subject.

[0127] As used herein, the term "heterologous agent" refers to an agent that is not naturally found in the subject to which the agent is to be administered. The term also includes recombinant or synthetic versions of agents that are naturally found in the subject. The heterologous agent may be one for which neutralizing antibodies are present in the subject prior to administration of the heterologous agent or one that is likely to raise neutralizing antibodies upon administration to the subject. The heterologous agent may be one that has never been administered to the subject. The heterologous agent may be one that has previously been administered to the subject.

[0128] As used herein, the term "neutralizing antibodies" refers to antibodies that specifically bind to a heterologous agent and inhibit one or more biological activities of the heterologous agent after it has been administered to a subject.

[0129] In some embodiments, the heterologous agent may be a nucleic acid delivery vector, *e.g.*, a viral vector or a non-viral vector. In some embodiments, the viral vector is an oncolytic vector. In some embodiments, the viral vector is an adeno-associated virus, retrovirus, lentivirus, poxvirus, alphavirus, baculovirus, vaccinia virus, herpes virus, Epstein-Barr virus, polio virus, or adenovirus vector. In some embodiments, the non-viral vector is a plasmid, liposome, electrically charged lipid, nucleic acid-protein complex, or biopolymer.

[0130] In some embodiments, the heterologous agent is a gene editing complex, *e.g.*, a CRISPR complex.

[0131] In some embodiments, the heterologous agent is a protein or nucleic acid. In some embodiments, the protein is an enzyme, a regulatory protein, or a structural protein, *e.g.*, one that can substitute for a missing or defective protein in a subject. In some embodiments, the nucleic acid is a functional nucleic acid, *e.g.*, an antisense nucleic acid or an inhibitory RNA.

[0132] The effective amount of protein M or a functional fragment or derivative thereof or a fusion protein comprising an effective amount of mycoplasma protein M or a functional fragment or derivative thereof is an amount that at least partially blocks the inhibition of the heterologous agent by neutralizing antibodies and/or outcompetes self-neutralizing antibodies ability to bind to the heterologous agent. In some embodiments, the effective amount of protein M is an amount sufficient to inhibit neutralization by at least about 50%, 60%, 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98, 99%, 99.5%, or 99.9%. In some embodiments, the effective amount of protein M or a functional fragment or derivative thereof or a fusion protein comprising an effective amount of mycoplasma protein M or a functional fragment or derivative thereof is an amount sufficient to produce a ratio of protein M to total immunoglobulin in the subject of about 0.5:1 to about 8:1 on a molar basis or any range therein, *e.g.*, about 0.5:1, 1:1, 1.5:1, 2:1, 2.5:1, 3:1, 3.5:1, 4:1, 4.5:1, 5.5:1, 6:1, 6.5:1, 7:1, 7.5:1, or 8:1 or any range therein. In other embodiments, the ratio is from about 8:1 to about 50:1. In some embodiments, the ratio is about 0.5:1 to about 6:1, about 0.5:1 to about 4:1, about 0.5:1 to about 2.5:1, about 0.5:1 to about 2:1, about 1:1 to about 8:1, about 1.5:1 to about 8:1, or about 2:1 to about 8:1. In one embodiment, the ratio is about 1:1 to about 3:1, *e.g.*, about 2:1.

[0133] In some embodiments, the administration comprises from about 1e9 vg/kg to about 1e14 vg/kg, from about 1e10 vg/kg to about 1e13 vg/kg, or from about 1e11 vg/kg to about 1e12 vg/kg of AAV, from about 1 μl to about 6 L of serum, and from about 1 mg/kg to about 1000 mg/kg, from about 10 mg/kg to about 900 mg/kg, from about 25 mg/kg to about 800 mg/kg, from about 50 mg/kg to about 600 mg/kg, from about 100 mg/kg to about 500 mg/kg, or from about 200 mg/kg to about 400 mg/kg of Protein M.

[0134] Total immunoglobulin may be total serum immunoglobulin (*e.g.*, for systemic administration of protein M). Total immunoglobulin may be the total level in a localized fluid or tissue (*e.g.*, for specific delivery to the eye, ear, lung, brain, muscle, joint, *etc.*). Total immunoglobulin may be measured by any technique known in the art, such as by performing an ELISA on serum using either an antibody that binds the Fc region of immunoglobulins or by using Protein A or G to bind immunoglobulins. Additionally, for mice it is known that their serum contains between 5 mg/ml to 10 mg/ml immunoglobulin. The normal range for serum immunoglobulin in humans is 8-10 mg/ml. For *in vivo* estimates, the high end of 10 mg/ml

can be used to calculate the ratio. Local immunoglobulin content can be estimated based on tissue weight (40 mL of serum per 1 kg weight), or concentration of Ig in specific body fluids and the body fluid volume in that organ if it is less than blood serum (*e.g.*, eye, cerebrospinal fluid).

[0135] The protein M may be administered to the subject by any schedule found to be effective to block inhibition of the heterologous agent by neutralizing antibodies. In some embodiments, the protein M or a functional fragment or derivative thereof or a fusion protein comprising an effective amount of mycoplasma protein M or a functional fragment or derivative thereof is administered to the subject prior to administration of the heterologous agent, *e.g.*, at least about 1, 5, 10, 15, 20, 30, 40, or 50 minutes or at least about 1, 2, 3, 4, 5, 6, 12, 18, 24. 48, or 72 hours prior to administration of the heterologous agent. In some embodiments, the protein M or a functional fragment or derivative thereof or a fusion protein comprising an effective amount of mycoplasma protein M or a functional fragment or derivative thereof is administered to the subject concurrently with administration of the heterologous agent. As used herein, the term "concurrently" means sufficiently close in time to produce a combined effect (that is, concurrently can be simultaneously, or it can be two or more events occurring within a short time period before or after each other).

[0136] In some embodiments, the heterologous agent is combined with the protein M or a functional fragment or derivative thereof or a fusion protein comprising an effective amount of mycoplasma protein M or a functional fragment or derivative thereof prior to administration to the subject, *e.g.*, the two components are mixed together prior to administration in a single composition. The heterologous agent may be combined with the protein M or a functional fragment or derivative thereof or a fusion protein comprising an effective amount of mycoplasma protein M or a functional fragment or derivative thereof at least about 1, 5, 10, 15, 20, 30, 40, or 50 minutes or at least about 1, 2, 3, 4, 5, 6, 12, 18, or 24 hours prior to administration to the subject. In other embodiments, the protein M or a functional fragment or derivative thereof or a fusion protein comprising an effective amount of mycoplasma protein M or a functional fragment or derivative thereof and the heterologous agent are administered in separate compositions.

[0137] In some embodiments, it may be necessary to administer the heterologous agent and/or the protein M or a functional fragment or derivative thereof or a fusion protein comprising an effective amount of mycoplasma protein M or a functional fragment or derivative thereof to the subject more than once to provide a therapeutic or otherwise beneficial effect. The protein M or a functional fragment or derivative thereof or a fusion protein

comprising an effective amount of mycoplasma protein M or a functional fragment or derivative thereof may be administered, *e.g.*, 1, 2, 3, 4 or more times. The administrations may be days, weeks, months, or years apart (*e.g.*, from about two days to about 10 years or more). In some embodiments, the administration decreases the amount of antibody cycling and the onset of the decrease is from about 1 hour to about 3 hours. In some embodiments, the administration decreases the amount of antibody cycling and the onset of the decrease is from about 5 minutes to about 8 weeks, from about 5 minutes to about 1 hour, from about 1 hour to about 2 days, or from about 2 days to about 8 weeks. In some embodiments, the protein M or a functional fragment or derivative thereof or a fusion protein comprising an effective amount of mycoplasma protein M or a functional fragment or derivative thereof is administered to the subject each time the heterologous agent is administered to the subject, *e.g.*, in the same manner as described above, *e.g.*, before or currently with the heterologous agent. The use of protein M with each administration of the heterologous agent may inhabit the effect of NAb against the heterologous agent which is often an issue upon readministration. In some embodiments, the same protein M or a functional fragment or derivative thereof or a fusion protein comprising an effective amount of mycoplasma protein M or a functional fragment or derivative thereof is administered each time. In other embodiments, a different protein M or a functional fragment or derivative thereof or a fusion protein comprising an effective amount of mycoplasma protein M or a functional fragment or derivative thereof is administered each time, *e.g.*, a different modified protein M as describe further below. Without being bound by theory, it is thought that the use of a different protein M derivative with each administration may limit the effect of inhibitory antibodies against protein M that may occur with readministration of the same protein. It is also thought that administration of saturating doses of protein M or a functional fragment or derivative thereof or a fusion protein comprising an effective amount of mycoplasma protein M or a functional fragment or derivative thereof will outcompete any inhibitory antibodies to protein M and prevent antigen recognition. In some embodiments, administrations outcompete self-neutralizing antibodies.

[0138] The ability of protein M or a functional fragment or derivative thereof or a fusion protein comprising an effective amount of mycoplasma protein M or a functional fragment or derivative thereof to non-specifically bind antibodies advantageously may be used in other methods where it is beneficial to inhibit antibody binding to antigen, *e.g.*, where immunosuppression is desirable or where excess antibodies are present.

[0139] An additional aspect of the invention relates to a method of treating an autoimmune disease in a subject in need thereof, comprising administering to the subject a therapeutically

effective amount of mycoplasma protein M or a functional fragment or derivative thereof or a fusion protein comprising an effective amount of mycoplasma protein M or a functional fragment or derivative thereof, thereby treating the autoimmune disease.

[0140] The term "autoimmune disease," as used herein, refers to any disorder associated with an autoimmune reaction. Examples include, without limitation, multiple sclerosis, Crohn's disease, ulcerative colitis, systemic lupus erythematosus, rheumatoid arthritis, inflammatory bowel syndrome, irritable bowel syndrome, uveitis, insulin-dependent diabetes mellitus, hemolytic anemias (e.g., warm autoimmune hemolytic anemia), rheumatic fever, Goodpasture's syndrome, Guillain-Barre syndrome, psoriasis, thyroiditis, Graves' disease, myasthenia gravis, glomerulonephritis, autoimmune hepatitis, immune thrombocytopenic purpura, acute inflammatory demyelinating polyneuropathy, antibody-mediated rapidly progressive glomerulonephritis, chronic inflammatory demyelinating polyradiculoneuropathy, hyper-viscosity syndrome, recurrent focal segmental glomerulosclerosis, neuromyelitis optica spectrum disorder, cryoglobulinemia, autoimmune encephalitis, autoimmune neuropathy, ANCA vasculitis, autoimmune neutropenia, anti-GBM disease, pemphigus vulgaris, Sjogren's syndrome, lupus nephritis, and immune cytopenias.

[0141] Another aspect of the invention relates to a method of treating a disorder associated with excess antibodies in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of mycoplasma protein M or a functional fragment or derivative thereof or a fusion protein comprising an effective amount of mycoplasma protein M or a functional fragment or derivative thereof, thereby treating the disorder associated with excess antibodies. The term "disorder associated with excess antibodies," as used herein, refers to any disorder wherein the cause or at least one symptom of the disorder is due to greater than average levels of antibodies in the blood or elsewhere in the body. Examples include, without limitation, multiple myeloma, monoclonal gammopathy of undetermined significance (MGUS), Waldenstrom macroglobulinemia, cytokine release syndrome, or acute autoimmune attacks such as severe autoimmune vasculitis with sudden onset. The method may also be useful for an acute blockade of all antibodies to rapidly stop an autoimmune event such as cytokine release syndrome or acute autoimmune attacks such as severe autoimmune vasculitis with sudden onset, or preventing damage to transplant tissue and/or transplant organ caused by antibody mediated immune complex formation.

[0142] For any of the methods of the invention, the protein M or a functional fragment or derivative thereof or a fusion protein comprising an effective amount of mycoplasma protein

M or a functional fragment or derivative thereof may be administered to the subject by any route of administration found to be effective (*e.g.*, local or systemic).

[0143] The most suitable route will depend on the subject being treated and the disorder or condition being treated. In some embodiments, the protein M or a functional fragment or derivative thereof or a fusion protein comprising an effective amount of mycoplasma protein M or a functional fragment or derivative thereof is administered to the subject by a route selected from oral, rectal, transmucosal, intranasal, inhalation (*e.g.*, via an aerosol), buccal (*e.g.*, sublingual), vaginal, intrathecal, intraocular, intravitreal, intracochlear, transdermal, intraendothelial, *in utero* (or *in ovo*), parenteral (*e.g.*, intravenous, subcutaneous, intradermal, intracranial, intramuscular [including administration to skeletal, diaphragm and/or cardiac muscle], intrapleural, intracerebral, and intraarticular), topical (*e.g.*, to both skin and mucosal surfaces, including airway surfaces, and transdermal administration), intralymphatic, and the like, as well as direct tissue or organ injection (*e.g.*, to liver, eye, skeletal muscle, cardiac muscle, diaphragm muscle or brain).

[0144] The protein M or a functional fragment or derivative thereof or a fusion protein comprising an effective amount of mycoplasma protein M or a functional fragment or derivative thereof may be delivered or targeted to any tissue or organ in the subject. In some embodiments, the protein M or a functional fragment or derivative thereof or a fusion protein comprising an effective amount of mycoplasma protein M or a functional fragment or derivative thereof is administered to, *e.g.*, a skeletal muscle, a smooth muscle, the heart, the diaphragm, the airway epithelium, the liver, the kidney, the spleen, the pancreas, the skin, the lung, the ear, and the eye. In some embodiments, the protein M or a functional fragment or derivative thereof or a fusion protein comprising an effective amount of mycoplasma protein M or a functional fragment or derivative thereof is administered to a diseased tissue or organ, *e.g.*, a tumor.

[0145] In some embodiments, the heterologous agent and the protein M or a functional fragment or derivative thereof or a fusion protein comprising an effective amount of mycoplasma protein M or a functional fragment or derivative thereof are administered by the same route. In other embodiments, the heterologous agent and the protein M or a functional fragment or derivative thereof or a fusion protein comprising an effective amount of mycoplasma protein M or a functional fragment or derivative thereof are administered by different routes, *e.g.*, the protein M or a functional fragment or derivative thereof or a fusion protein comprising an effective amount of mycoplasma protein M or a functional fragment or

derivative thereof is administered intravenously and the heterologous agent is administered locally to a target tissue or organ (*e.g.*, diseased tissue or organ).

[0146] Any of the above methods may further comprise administering to the subject an additional treatment for reducing antibody concentration or inhibiting antibody function in the subject. The additional treatment may be any method known in the art and incudes, without limitation, plasmapheresis, administration of an antibody digesting enzyme such as IdeS or IdeZ, administration of an antibody that binds the FcRn receptor, administration of an Fc fragment that binds the FcRn receptor, administration of an immunosuppressant drug (*e.g.*, corticosteroids (*e.g.*, prednisone, budesonide, prednisolone), Janus kinase inhibitors (*e.g.*, tofacitinib), calcineurin inhibitors (*e.g.*, cyclosporine, tacrolimus), mTOR inhibitors (*e.g.*, sirolimus, everolimus), IMDH inhibitors (*e.g.*, azathioprine, leflunomide, mycophenolate), or biologics (*e.g.*, abatacept, adalimumab, anakinra, certolizumab, etanercept, golimumab, infliximab, ixekizumab, natalizumab, rituximab, secukinumab, tocilizumab, ustekinumab, vedolizumab, basiliximab, daclizumab), administration of an antibody that binds CD19 or an antibody that binds CD20 on B cells and depletes them or other treatments designed to inhibit or destroy B cells (*e.g.*, splenectomy, chemotherapy, immunotherapy, radiation therapy). The additional treatment may be administered before, during, and/or after administration of the protein M or a functional fragment or derivative thereof or a fusion protein comprising an effective amount of mycoplasma protein M or a functional fragment or derivative thereof.

[0147] The ability of protein M or a functional fragment or derivative thereof or a fusion protein comprising an effective amount of mycoplasma protein M or a functional fragment or derivative thereof to non-specifically bind antibodies advantageously may be used in purification methods. While purification of antibodies often relies on agents that bind to the Fc region of the antibody (such as protein A and protein G), protein M non-specifically binds to the variable region of the antibody. Thus, protein M can be used to isolate antibody fragments and antibody derivatives that do not contain an Fc region (such as single chain variable fragments) and other molecules that incorporate an antibody variable region.

[0148] Thus, one aspect of the invention relates to a method of isolating a compound comprising an antibody light chain variable region and/or heavy chain variable region from a sample, the method comprising contacting the compound with the modified mycoplasma protein M or a functional fragment thereof or a fusion protein comprising an effective amount of mycoplasma protein M or a functional fragment or derivative thereof of the invention attached to a solid support, then eluting the compound from the modified mycoplasma protein M or a functional fragment thereof or a fusion protein comprising an effective amount of

mycoplasma protein M or a functional fragment or derivative thereof. In some embodiments, the compound comprising an antibody light chain variable region and/or heavy chain variable region is an antibody or an antigen-binding fragment thereof. In some embodiments, the compound comprising an antibody light chain variable region and/or heavy chain variable region is an antibody derivative, an immunoglobulin scaffold, or the like. The modified protein M or a functional fragment thereof or a fusion protein comprising an effective amount of mycoplasma protein M or a functional fragment or derivative thereof of the invention is advantageous over wild-type protein M due to increased thermostability. This allows the protein M to be reusable for multiple purifications and permits the use of elution conditions that would destabilize wild-type protein M.

[0149] The method may be carried using techniques well known in the art of affinity purification. The solid support may be any material that is suitable for affinity chromatography or batch purification. Suitable materials include, without limitation, agarose, polyacrylamide, dextran, cellulose, polysaccharide, nitrocellulose, silica, alumina, aluminum oxide, titania, titanium oxide, zirconia, styrene, polyvinyldifluoride nylon, copolymer of styrene and divinylbenzene, polymethacrylate ester, derivatized azlactone polymer or copolymer, glass, or cellulose. In some embodiments, the solid support is a resin. In some embodiments, the solid support is a bead or particle. In some embodiments, the solid support is a surface, *e.g.*, of a plate, vial, or column.

[0150] The contacting step may be carried out by any suitable method, *e.g.*, by passing a sample comprising the compound over the modified mycoplasma protein M or a functional fragment thereof or a fusion protein comprising an effective amount of mycoplasma protein M or a functional fragment or derivative thereof in a column or incubating the composition comprising the compound with the modified mycoplasma protein M or a functional fragment thereof or a fusion protein comprising an effective amount of mycoplasma protein M or a functional fragment or derivative thereof in a vessel or the well of a plate. The contacting step may be carried out for a sufficient amount of time to allow the compound to bind to the modified mycoplasma protein M or a functional fragment thereof or a fusion protein comprising an effective amount of mycoplasma protein M or a functional fragment or derivative thereof. After washing, centrifugation, or other forms of separation of the compound bound to the modified mycoplasma protein M or a functional fragment thereof or a fusion protein comprising an effective amount of mycoplasma protein M or a functional fragment or derivative thereof from other components in the sample, the compound is eluted from the modified mycoplasma protein M or a functional fragment thereof or a fusion protein

comprising an effective amount of mycoplasma protein M or a functional fragment or derivative thereof. The elution may be carried out by any method known in the art, *e.g.*, a change in ion concentration, temperature, *etc*. In one embodiment, the elution is carried out by a change in pH. The modified mycoplasma protein M or a functional fragment thereof of the present invention advantageously is stable over a wider range of pH than wild-type protein M. This allows the modified protein M to remain stable at lower pHs that permit elution of the compound.

[0151] In some embodiments, the contacting step is carried out in a binding buffer (*e.g.*, at neutral pH) and the elution is carried out with a low pH buffer (*e.g.*, 0.1 M glycine pH 2-3.5 or 0.1 M acetate pH 3.5-4.5) into a neutralization buffer (*e.g.*, a high-ionic strength alkaline buffer such as 1 M phosphate or 1 M Tris at pH 8-9).

[0152] A further aspect of the invention relates to the modified mycoplasma protein M or a functional fragment thereof or a fusion protein comprising an effective amount of mycoplasma protein M or a functional fragment or derivative thereof attached to a solid support as described above. The modified mycoplasma protein M or a functional fragment thereof or a fusion protein comprising an effective amount of mycoplasma protein M or a functional fragment or derivative thereof may be attached to the solid support by any means known in the art, *e.g.*, covalently linked, *e.g.*, using a linker molecule.

[0153] The ability of protein M or a functional fragment or derivative thereof or a fusion protein comprising an effective amount of mycoplasma protein M or a functional fragment or derivative thereof to non-specifically bind antibodies advantageously may be used in any immunoassay that involves a step of binding an antibody or fragment or derivative thereof or a fusion protein comprising an effective amount of mycoplasma protein M or a functional fragment or derivative thereof.

[0154] Thus, one aspect of the invention relates to a method of performing an immunoassay, the method comprising using the modified mycoplasma protein M or a functional fragment thereof or a fusion protein comprising an effective amount of mycoplasma protein M or a functional fragment or derivative thereof of the invention to bind a compound comprising an antibody light chain variable region and/or heavy chain variable region.

[0155] The modified mycoplasma protein M or a functional fragment thereof or a fusion protein comprising an effective amount of mycoplasma protein M or a functional fragment or derivative thereof can substitute for any generic or specific antibody binding molecule, *e.g.*, protein A, protein G, or a secondary antibody. The modified mycoplasma protein M or a functional fragment thereof or a fusion protein comprising an effective amount of mycoplasma

protein M or a functional fragment or derivative thereof may be labeled as is well known in the art, *e.g.*, for radioactive, chemiluminescent, or enzymatic detection.

[0156] Examples of immunoassays include, without limitation, radio-immunoassays (RIA), enzyme-linked immunosorbent assays (ELISA) assays, enzyme immunoassays (EIA), sandwich assays, gel diffusion precipitation reactions, immunodiffusion assays, agglutination assays, immunofluorescence assays, fluorescence activated cell sorting (FACS) assays, immunohistochemical assays, protein A immunoassays, protein G immunoassays, protein L immunoassays, biotin/avidin assays, biotin/streptavidin assays, immunoelectrophoresis assays, precipitation/flocculation reactions, immunoblots (Western blot; dot/slot blot); immunodiffusion assays; liposome immunoassay, chemiluminescence assays, library screens, expression arrays, immunoprecipitation, competitive binding assays, and immunohistochemical staining.

Protein M and Derivatives Thereof

[0157] The protein M or a functional fragment or derivative thereof used in the methods of the invention may be from any mycobacterial species that produces a protein M that binds to antibodies. In some embodiments, the protein M or a functional fragment or derivative thereof is from *Mycoplasma genitalium, Mycoplasma pneumoniae,* or *Mycoplasma penetrans*.

[0158] In some embodiments, the protein M or a functional fragment or derivative thereof may be any of the protein M sequences described in PCT Publication No. WO 2014/014897 and US Publication No. 2017/0320921, incorporated by reference herein in their entirety. In some embodiments, the protein M or a functional fragment or derivative thereof is a *M. genitalium* protein M (MG281, SEQ ID NO:1) or a functional fragment or derivative thereof (*e.g.*, the fragment shown in SEQ ID NO:3 or a derivative thereof). In some embodiments, the protein M or a functional fragment or derivative thereof is a *M. pneumoniae* protein M (MPN400, SEQ ID NO:23) or a functional fragment or derivative thereof (*e.g.*, the fragment shown in SEQ ID NO:24 or a derivative thereof). In some embodiments, the protein M or a functional fragment or derivative thereof is a *Mycoplasma penetrans* protein M or a functional fragment or derivative thereof. In some embodiments, the protein M or a functional fragment or derivative thereof is a protein M fragment or a derivative of the fragment, *e.g.*, a fragment that does not contain the transmembrane domain and/or does not contain the C-terminus, *e.g.*, a functional fragment comprising, consisting essentially of, or consisting of about amino acid residues 17-537, 37-556, 37-482, 37-468, 37-442, 74-468, 74-479, 74-482, 74-468, 74-442, or 74-556 of *M. genitalium* protein M (SEQ ID NO:1) or the equivalent residues from another

protein M. The term "about" as applied to the termini of each of the listed fragments means that one or both of the terminal residues may be varied to a small extent, *e.g.*, by about 5, 4, 3, or 2 amino acids on either side of the recited residue. The equivalent residues from another protein M can readily be determined by the skilled artisan by performing a sequence alignment between *M. genitalium* protein M and the other protein M. For example, **FIG. 27** showing a sequence alignment of wild-type *M. genitalium* protein M amino acids 74-479 (SEQ ID NO:3) and the equivalent fragment of *M. pneumoniae* protein M (SEQ ID NO:24). In some embodiments, the protein M or a functional fragment or derivative thereof comprises, consists essentially of, or consists of the amino acid sequence of SEQ ID NO:2, which is a soluble form of protein M (amino acid residues 37-556 of SEQ ID NO:1) with an N-terminal 6-His tag followed by a thrombin cleavage site.

[0159] As used herein, the term "derivative" is used to refer to a polypeptide which differs from a naturally occurring Protein M or Protein M functional fragment by minor modifications to the naturally occurring polypeptide, but which significantly retains a biological activity of Protein M. Minor modifications include, without limitation, changes in one or a few amino acid side chains, changes to one or a few amino acids (including deletions, insertions, and/or substitutions), changes in stereochemistry of one or a few atoms (*e.g.*, D-amino acids), and minor derivatizations, including, without limitation, methylation, glycosylation, phosphorylation, acetylation, myristoylation, prenylation, palmitation, amidation, and addition of glycosylphosphatidyl inositol. The term "substantially retains," as used herein, refers to a fragment, derivative, or other variant of a polypeptide that retains at least about 20% of the activity of the naturally occurring polypeptide (*e.g.*, antibody binding), *e.g.*, about 30%, 40%, 50% or more. In some embodiments, the derivative of Protein M or Protein M functional fragment contains mutations (deletions, insertions, and/or substitutions in any combination) of 20 or fewer amino acid residues, *e.g.*, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, or 2 or fewer mutations. In some embodiments, the protein M derivative comprises an amino acid sequence that is at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 99.9% identical to the amino acid sequence of SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3 of *M. pneumoniae* protein M or the wild-type sequence of another mycoplasma Protein M or a functional fragment thereof.

[0160] In some embodiments, the protein M or a functional fragment or derivative thereof can be modified for *in vivo* use by the addition, at the amino- and/or carboxyl-terminal ends, of a blocking agent to facilitate survival of the relevant polypeptide *in vivo*. This can be useful in those situations in which the peptide termini tend to be degraded by proteases. Such blocking

agents can include, without limitation, additional related or unrelated peptide sequences that can be attached to the amino and/or carboxyl terminal residues of the protein to be administered. This can be done either chemically during the synthesis of the protein or by recombinant DNA technology by methods familiar to artisans of average skill. Alternatively, blocking agents such as pyroglutamic acid or other molecules known in the art can be attached to the amino and/or carboxyl terminal residues, or the amino group at the amino terminus or carboxyl group at the carboxyl terminus can be replaced with a different moiety. Likewise, the proteins can be covalently or noncovalently coupled to pharmaceutically acceptable "carrier" proteins prior to administration.

[0161] In one aspect of the invention, the protein M derivative is a modified mycoplasma protein M or a functional fragment thereof comprising mutations that increase or at least maintain the thermostability of protein M. These modified protein M derivatives have increased suitability for use in *in vivo* methods and other methods that require elevated temperatures (*e.g.*, about 37 °C) where wild-type protein M may denature.

[0162] In some embodiments, the protein M derivative is a modified mycoplasma protein M or a functional fragment thereof, having one or more amino acid mutations that increase or maintain thermostability of the mycoplasma protein M or a functional fragment thereof relative to wild-type mycoplasma protein M or a functional fragment thereof. In some embodiments, the modified protein M or a functional fragment thereof has an increased melting temperature (Tm) that is at least 0.5 °C than the Tm of wild-type protein M or a functional fragment thereof, *e.g.*, 0.5 °C, 1.0 °C, 1.5 °C, 2.0 °C, 2.5 °C, 3.0 °C, 3.5 °C, 4.0 °C, 4.5 °C, 5.0 °C, 5.5 °C, 6.0 °C, 6.5 °C, 7.0 °C, 7.5 °C, 8.0 °C, 8.5 °C, 9.0 °C, 9.5 °C, 10.0 °C, 11.0 °C, 12.0 °C, 13.0 °C, 14.0 °C, 15.0 °C, 16.0 °C, 17.0 °C, 18.0 °C, 19.0 °C, 20.0 °C, 25.0 °C, 30.0 °C or more higher. In some embodiments, the modified protein M or a functional fragment thereof has a Tm that is maintained (*i.e.*, is within 0.5 °C) relative to the Tm of wild-type protein M or a functional fragment thereof. The Tm may be measured by differential scanning fluoroscopy or any other suitable technique. The Tm of wild type *Mycoplasma genitalium* protein M is about 41.9 °C and the Tm of wild type *Mycoplasma pneumoniae* protein M is about 44.1 °C.

[0163] In some embodiments, the modified mycoplasma protein M or a functional fragment thereof may have 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 or more mutations. In some embodiments, the modified mycoplasma protein M or a functional fragment thereof may have 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or fewer mutations.

[0164] In some embodiments, the modified mycoplasma protein M or a functional fragment thereof is derived from protein M of *Mycoplasma genitalium, Mycoplasma pneumoniae,* or *Mycoplasma penetrans*.

[0165] In some embodiments, the modified mycoplasma protein M or a functional fragment thereof is a fragment from about residue 74 (*e.g.*, residue 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79) to about residue 479 (*e.g.*, residue 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484) of *M. genitalium* protein M (SEQ ID NO:3) or the equivalent residues of *M. pneumoniae* protein M (SEQ ID NO:24).

[0166] In some embodiments, the one or more mutations are located in portions of protein M that are known to effect thermostability. In some embodiments, the one or more mutations are not located in proteins of protein M that are known to have other roles in the biological activity of protein M. In one embodiment, the one or more mutations are not at a residue within 5 Å of the antibody-binding site of protein M (*i.e.*, residues 95, 99, 102, 103, 105, 106, 107, 109, 110, 114, 116, 117, 118, 119, 120, 144, 158, 160, 161, 162, 163, 177, 178, 179, 180, 181, 186, 187, 188, 191, 321, 338, 340, 341, 345, 381, 384, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 426, 427, 429, 436, 438, 439, 440, 441, 442, 444, 445, 446, 447, 448, 449, 452, 453, 455, 456, 457, 462, 466) of *M. genitalium* protein M (SEQ ID NO:1) or the equivalent residues of *M. pneumoniae* protein M (SEQ ID NO:23). In one embodiment, the one or more mutations are not at a residue within 5 Å of the antibody-binding site of protein M (*i.e.*, residues 100, 104, 107, 108, 110, 111, 112, 114, 115, 119, 121, 122, 123, 124, 125, 149, 163, 165, 166, 167, 168, 182, 183, 184, 185, 186, 192, 193, 196, 337, 338, 354, 356, 357, 399, 402, 404, 405, 406, 407, 408, 409, 410, 411, 412, 442, 443, 445, 454, 455, 456, 457, 458, 460, 461, 462, 463, 464, 465, 468, 469, 472, 473, 478) of *M. pneumoniae* protein M (SEQ ID NO:23). In one embodiment, the one or more mutations is not at any of residues 469-479 of *M. genitalium* protein M (SEQ ID NO:1) or the equivalent residues of *M. pneumoniae* protein M (SEQ ID NO:23).

[0167] The present inventors have used computational analysis to identify residues in protein M that are predicted to increase or maintain the Tm of the protein when mutated. Thus, in some embodiments, the one or mutations are at residue 78, 81, 83, 84, 85, 89, 90, 91, 92, 93, 94, 96, 97, 100, 101, 108, 111, 112, 113, 122, 123, 125, 126, 127, 128, 130, 131, 133, 134, 136, 137, 139, 141, 142, 146, 147, 148, 149, 150, 153, 154, 155, 156, 164, 167, 170, 175, 176, 184, 185, 189, 192, 193, 196, 198, 201, 202, 204, 205, 206, 207, 209, 211, 215, 218, 220, 224, 225, 226, 227, 231, 232, 234, 235, 236, 237, 239, 241, 243, 244, 245, 246, 247, 249, 250, 252, 253, 254, 255, 256, 257, 258, 259, 264, 269, 270, 272, 274, 275, 276, 279, 282, 284, 286, 287, 288,

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[0168] In some embodiments, one or mutations are at residue 83, 90, 92, 94, 137, 142, 147, 150, 156, 184, 196, 198, 205, 211, 215, 225, 231, 232, 234, 235, 236, 237, 239, 243, 245, 250, 255, 256, 259, 264, 272, 274, 275, 276, 279, 282, 297, 300, 302, 310, 320, 326, 331, 332, 335, 342, 343, 347, 348, 355, 357, 361, 371, 374, 378, 385, 401, 402, 409, 413, 424, 460, 463, 464, 468, or any combination thereof of *M. genitalium* protein M (SEQ ID NO:1) or the equivalent residues of *M. pneumoniae* protein M (SEQ ID NO:23). In some embodiments, the one or mutations is a mutation listed in **Table 5** or any combination thereof.

[0169] The present inventors have prepared and tested numerous mutations from the predicted residue lists alone or in combination with a high success rate of increased thermostability (stabilizing mutations) or at least maintained thermostability (neutral mutations). See **FIG. 23**, which shows that 79% of point mutations tested were stabilizing or neutral. The data further show that combining point mutations that increase Tm tends to produce modified protein M having even higher Tms (see **FIG. 15A**).

[0170] Thus, in some embodiments, the one or more mutations are at a residue that has been demonstrated to increase Tm either alone or in combination with other mutations, *e.g.*, wherein the one or mutations are at residue 150, 196, 198, 201, 205, 224, 232, 237, 274, 282, 342, 355, 373, 400, 402, 407, 409, 413, 135, or any combination thereof of *M. genitalium* protein M (SEQ ID NO:1) or the equivalent residues of *M. pneumoniae* protein M (SEQ ID NO:23).

[0171] In some embodiments, the one or more mutations are at residues selected from the following residues or combinations of residues:

a) 237 (MG1);

b) 232 (MG8);

c) 282 (MG13);

d) 150, 196, 198, 400, 402, 407, 409 (MG15);

e) 413, 435 (MG21);

f) 373, 400 (MG22);

g) 402, 407, 409, 413 (MG23);

h) 342 (MG24);

i) 150, 196, 198, 232, 237, 282, 342, 373, 400, 402, 407, 409, 413, 435 (MG27);

j) 274 (MG28);

k) 150, 196, 198, 232, 237, 342, 400, 402, 407, 409 (MG29);

l) 373, 413, 435 (MG31)

m) 205 (MG33);

n) 355 (MG38, MG40);

o) 150, 196, 198, 342, 373, 400, 402, 407, 409 (MG43);

p) 150, 196, 198, 232, 237, 342, 373, 400, 402, 407, 409 (MG44);

q) 201, 224 (MG45);

r) 150, 196, 198, 201, 224, 232, 237, 342, 400, 402, 407, 409 (MG46);

s) 150, 196, 198, 232, 237, 342, 390, 400, 402, 407, 409, 444 (MG47);

t) 150, 196, 198, 201, 205, 224, 232, 237, 274, 342, 355, 400, 402, 407, 409 (MG48); or

u) 150, 196, 198, 232, 237, 342, 391, 400, 402, 407, 409 (MG49)

of *M. genitalium* protein M (SEQ ID NO:1) or the equivalent residues of *M. pneumoniae* protein M (SEQ ID NO:23).

[0172] In some embodiments, the one or mutations are selected from:

a) F237T (MG1);

b) S232Q (MG8);

c) Q282D (MG13);

d) S150E, S196R, S198P, V400I, N402I, K407P, S409V (MG15);

e) L413I, T435I (MG21);

f) V373I, V400I (MG22);

g) N402L, K407P, S409V, L413I (MG23);

h) A342V (MG24);

i) S150E, S196R, S198P, S232Q, F237T, Q282D, A342V, V373I, V400I, N402I, K407P, S409V, L413I, T435I (MG27);

j) N274D (MG28);

k) S150E, S196R, S198P, S232Q, F237T, A342V, V400I, N402I, K407P, S409V (MG29);

l) V373I, L413I, T435I (MG31)

m) A205P (MG33);

n) T355D (MG38);

o) T355P (MG40);

p) S150E, S196R, S198P, A342V, V373I, V400I, N402I, K407P, S409V (MG43);

q) 150, 196, 198, 232, 237, 342, 373, 400, 402, 407, 409 (MG44);

r) S201C, A224C (MG45);

s) S150E, S196R, S198P, S201C, A224C, S232Q, F237T, A342V, V400I, N402I, K407P, S409V (MG46)

t) S150E, S196R, S198P, S232Q, F237T, A342V, F390E, V400I, N402I, K407P, S409V Y444K (MG47);

u) S150E, S196R, S198P, S201C, A205P, A224C, S232Q, F237T, N274D, A342V, T355P, V400I, N402I, K407P, S409V (MG48); or

v) S150E, S196R, S198P, S232Q, F237T, A342V, A391P, V400I, N402I, K407P, S409V (MG49)

of *M. genitalium* protein M (SEQ ID NO:1) or the equivalent residues of *M. pneumoniae* protein M (SEQ ID NO:23).

[0173] In some embodiments, the one or more mutations are at a residue that has been demonstrated to maintain Tm either alone or in combination with other mutations, *e.g.*, wherein the one or mutations are at residue 147, 150, 156, 225, 232, 245, 272, 276, 277, 279, 300, 310, 355, 378, 468, or any combination thereof of *M. genitalium* protein M (SEQ ID NO:1) or the equivalent residues of *M. pneumoniae* protein M (SEQ ID NO:23).

[0174] In some embodiments, the one or more mutations are at residues selected from the following residues or combinations of residues:

a) 468 (MG2);

b) 150 (MG4);

c) 147 (MG5);

d) 272 (MG10);

e) 355 (MG12);

f) 276,277,279 (MG17);

g) 300 (MG18);

h) 378 (MG20);

i) 156 (MG32);

j) 232 (MG34);

k) 245 (MG35);

l) 276 (MG36)

m) 225 (MG41); or

n) 310 (MG42)

of *M. genitalium* protein M (SEQ ID NO:1) or the equivalent residues of *M. pneumoniae* protein M (SEQ ID NO:23).

[0175] In some embodiments, the one or mutations are selected from:

a) R468Q (MG2);

b) S150E (MG4);

c) H147F (MG5);

d) S272G (MG10);

e) T355G (MG12);

f) S276E,Q277L,N279R (MG17);

g) N300Q (MG18);

h) N378Y (MG20);

i) S156K (MG32);

j) S232L (MG34);

k) A245Q (MG35);

l) S276D (MG36)

m) K225P (MG41); or

n) V310E (MG42)

of *M. genitalium* protein M (SEQ ID NO:1) or the equivalent residues of *M. pneumoniae* protein M (SEQ ID NO:23).

[0176] In some embodiments, the one or mutations are selected from:

a) A77E;

b) K125R;

c) V165Q;

d) H170T; or

e) A280V

of *M. pneumoniae* protein M (SEQ ID NO:23).

[0177] In some embodiments, the one or mutations are selected from:

a) 159;

b) 182;

c) 102;

d) 161;

e) 86;

f) 101;

g) 147;

h) 236;

i) 348;

j) 424;

k) 147;

l) 321;

m) 389;

n) 442;

o) 119;

p) 179;

q) 186; or

r) 103

of *M. genitalium* protein M (SEQ ID NO:1) or the equivalent residues of *M. pneumoniae* protein M (SEQ ID NO:23) or any combination thereof.

[0178] In some embodiments, the one or mutations are selected from:

a) Q159C and A182C;

b) A102C and T161C; or

c) I86C and F101C

of *M. genitalium* protein M (SEQ ID NO:1) or the equivalent residues of *M. pneumoniae* protein M (SEQ ID NO:23) or any combination thereof, wherein the modified residues in a), b), or c) are capable of forming a di-sulfide bond between the modified residues.

[0179] In some embodiments, the one or mutations are selected from:

a) H147Y;

b) H236Y;

c) H348Y;

d) H424Y; or

e) H147Q

of *M. genitalium* protein M (SEQ ID NO:1) or the equivalent residues of *M. pneumoniae* protein M (SEQ ID NO:23) or any combination thereof, wherein the mutation can prevent histidine protonation at that site at low pH and thereby prevent protein destabilization.

[0180] In some embodiments, the one or mutations are selected from:

a) N321H;

b) Y389H;

c) N442H;

d) P119H;

e) T179H;

f) G186H; or

g) N103H

of *M. genitalium* protein M (SEQ ID NO:1) or the equivalent residues of *M. pneumoniae* protein M (SEQ ID NO:23) or any combination thereof, wherein the mutation can increase pH sensitivity thereby improving elution conditions for antibody purification.

[0181] In some embodiments, the one or mutations are at residues 155, 203, 243, 248, and 358 of *M. pneumoniae* protein M (SEQ ID NO:23).

[0182] In some embodiments, the one or mutations are A155E, K203R, H243T, V248Q, and A358V of *M. pneumoniae* protein M (SEQ ID NO:23).

[0183] In some embodiments, the mutation is at residue 338 of *M. genitalium* protein M (SEQ ID NO:1) (*e.g.*, Y388N).

[0184] The modified mycoplasma protein M or a functional fragment thereof may contain additional modifications beyond mutations in the amino acid sequence. In some embodiments, one or more glycosylation sites in the protein M sequence are removed, *e.g.*, 1, 2, or 3 glycosylation sites. Three N-glycosylation sites are predicted in *M. genitalium* protein M based on both sequence and structural analysis with the NGlycPred server. These include N177, N213, and N274. Two O-glycosylation sites are predicted in *M. genitalium* protein M based on structural analysis with the NetOGlyc 4.0 server. These include T110 and T206. Suitable mutations include, without limitation, N177D, T215Y, N274D, S112I, and T206Y in any combination. In some embodiments, one or more glycosylation sites are added to the modified mycoplasma protein M or a functional fragment thereof. Changes in glycosylation patterns may add to the thermostability of the protein and/or alter the immunogenicity of the protein by blocking antibody recognition.

[0185] For expression and purification purposes, the modified mycoplasma protein M or a functional fragment may comprise a secretion peptide, *e.g.*, at the N-terminus, so that the expressed protein may be secreted from the cell in which it is expressed and collected from the culture medium. Suitable secretion peptides include, without limitation, those from human serum albumin, interleukin-2, CD5, immunoglobulin Kappa light chain, trypsinogen, or prolactin for mammalian cells and Sec or Tat for bacterial cells. The secretion peptide may or may not be removed from the protein M before it is used in the methods of the invention.

[0186] In some embodiments, the modified mycoplasma protein M or a functional fragment may comprise one or more additional mutations that alter one or more biological functions or physical characteristics of the protein. In some embodiments, the modified mycoplasma protein M or a functional fragment may comprise one or more additional mutations that alter the affinity of the protein for antibodies. The present inventors have used computational analysis to identify residues in protein M that are predicted to increase the affinity of the protein for antibodies when mutated. Thus, in some embodiments, the one or mutations are at residue 95, 102, 103, 106, 107, 114, 116, 160, 161, 162, 163, 181, 186, 321, 381, 384, 389, 390, 391, 396, 397, 426, 429, 436, 438, 439, 441, 442, 447, 448, 449, 452, 453, 455, 456, 462, or 466, or any combination thereof of *M. genitalium* protein M (SEQ ID NO:1) or the equivalent residues of *M. pneumoniae* protein M (SEQ ID NO:23). In some embodiments, the one or mutations is a mutation listed in **Table 6** or any combination thereof. In some embodiments, the one or mutations are at residue 100, 104, 107, 108, 110, 111, 112, 114, 115, 119, 121, 122, 123, 124, 125, 149, 163, 165, 166, 167, 168, 182, 183, 184, 185, 186, 192, 193, 196, 337, 338, 354, 356, 357, 399, 402, 404, 405, 406, 407, 408, 409, 410, 411, 412,

442, 443, 445, 454, 455, 456, 457, 458, 460, 461, 462, 463, 464, 465, 468, 469, 472, 473, 478, or any combination thereof of *M. pneumoniae* protein M (SEQ ID NO:23).

[0187] In some embodiments, the modified mycoplasma protein M or a functional fragment may comprise one or more additional mutations that alter the affinity of the protein for antibodies by modifying the pH sensitivity of the protein. The present inventors have used computational analysis to identify residues in protein M that are predicted to increase the affinity for antibodies by modifying pH sensitivity when mutated. These mutants may be particularly useful for antibody isolation due to the ability to use change in pH for elution. Thus, in some embodiments, the one or mutations are at residue 95, 103, 116, 186, 321, 389, 429, 442, or 466, or any combination thereof of *M. genitalium* protein M (SEQ ID NO:1) or the equivalent residues of *M. pneumoniae* protein M (SEQ ID NO:23). In some embodiments, the one or mutations is a mutation listed in **Table 7** or any combination thereof.

[0188] In some embodiments, the modified mycoplasma protein M or a functional fragment may comprise one or more additional mutations that reduce or eliminate affinity for antibodies. Examples include, without limitation, mutations at residues 390 and 444, *e.g.*, 390E and Y444K of *M. genitalium* protein M (SEQ ID NO:1) or the equivalent residues of *M. pneumoniae* protein M (SEQ ID NO:23).

[0189] In some embodiments, the modified mycoplasma protein M or a functional fragment may comprise a deletion from about residue 185 to about residue 342 of *M. genitalium* protein M (SEQ ID NO:1) or the equivalent residues of another mycoplasma protein M. In some embodiments, the deleted amino acids are replaced by a short linker. In some embodiments, the deletion creates newly exposed hydrophobic residues and these newly exposed residues have one or more mutations.

[0190] In one aspect of the invention, a fusion protein comprises a modified mycoplasma protein M or a functional fragment thereof, a linker, and a peptide. In some embodiments, the fusion protein has a modified mycoplasma protein M or a functional fragment thereof with one or more mutations that enhance the affinity or avidity of the modified mycoplasma protein M or a functional fragment thereof to antibodies. In some embodiments, the peptide is a dimerizing peptide (e.g., IL-GCN4, C-IL-GCN4). In other embodiments, the peptide is a Fab binding polypeptide or an Fc binding polypeptide. In some embodiments, the Fab binding polypeptide is Protein L, protein A, or protein G. In some embodiments, the Fab binding polypeptide binds to the Fc portion of immunoglobulins and/or decreases Fc receptor recycling of the immunoglobulins and/or increases binding of the fusion protein to immunoglobulins. In other embodiments, the peptide comprises a dimerizing peptide and Protein L. In one

embodiment, the fusion protein, starting from the N-terminus, comprises in order a dimerizing peptide, a first linker, Protein L, a second linker, and a modified mycoplasma protein M or a functional fragment thereof.

[0191] In other embodiments, the peptide is a Fc binding polypeptide, which can be a Fc receptor polypeptide. In some embodiments, the peptide comprises a Fc binding polypeptide and Protein L. In one embodiment, the fusion protein, starting from the N-terminus, comprises in order a Fc binding polypeptide, a first linker, Protein L, a second linker, and the modified mycoplasma protein M or a functional fragment thereof. In some embodiments, the peptide is a Fc domain, which may include complement binding residues for binding to a Fc receptor polypeptide and/or one or more mutations of complement binding residues for binding to a Fc receptor polypeptide. In some embodiments, the peptide is a Fc receptor protein and the fusion protein decreases the cellular uptake of antibodies, decreases antibody recycling and causes immunoglobulin depletion by decreasing antibody recycling, and/or increases affinity of the fusion protein for immunoglobulins. In other embodiments, the fusion protein comprises two or more modified mycoplasma protein M or a functional fragment thereof. In other embodiments, the fusion protein comprises half-life extension moieties, such as human albumin or addition of polyethylene glycol (PEG), protein modifications such as acylation, methylation, phosphorylation, sulfation, farnesylation, ubiquitination, site-selective conjugation, glycosylation, introduction of PTMs, PEGylation, ligation, polymerization of protein-based initiators, or any modifications that may alter binding, affinity, binding targets, or any combination thereof.

[0192] The protein M proteins according to the invention are produced and characterized by methods well known in the art and as described herein, such as recombinant expression.

[0193] An additional aspect of the invention provides an isolated polynucleotide encoding the protein M or a functional fragment or derivative thereof of this invention and an expression cassette for producing the protein M or a functional fragment or derivative thereof.

[0194] The polynucleotide may be operably linked to regulatory elements to aid in expression of the protein. In some embodiments, the polynucleotide is operably linked to a promoter. The promoter may be a bacterial promoter (*e.g.*, operable in *E. coli*) or a mammalian promoter (*e.g.*, a human promoter).

[0195] In some embodiments, the polynucleotide may be codon optimized to enhance expression of the protein in a host cell. In one embodiment, the polynucleotide is codon optimized for expression in bacteria, such as *E. coli*. In another embodiment, the polynucleotide is codon optimized for expression in a mammalian cell, such as a human cell.

One example is the sequence of SEQ ID NO:26, which is a codon optimized for expression in human cells, or a sequence at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.5% identical thereto. In a further embodiment, the polynucleotide is codon optimized for expression in both bacteria, such as *E. coli*, and a mammalian cell, such as a human cell. One example is the sequence of SEQ ID NO:25, which is a codon optimized for expression in both *E. coli* and human cells, or a sequence at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.5% identical thereto.

[0196] Another aspect of the invention is a vector, *e.g.*, an expression vector, comprising the polynucleotide of the invention. The vector may be any type of vector known in the art, including, without limitation, plasmid vectors and viral vectors. The vector may be, for example, a bacterial vector (*e.g.*, an *E. coli* vector) or a mammalian cell vector (*e.g.*, a human cell vector).

[0197] A further aspect of the invention relates to a cell comprising the polynucleotide and/or vector of the invention (*e.g.*, an isolated cell, a transformed cell, a recombinant cell, *etc.*). Thus, various embodiments of the invention are directed to recombinant host cells containing the vector (*e.g.*, expression cassette). Such a cell can be an isolated cell. In some embodiments, the polynucleotide is stably incorporated into the genome of the cell. In some embodiments, the cell may be a bacterial cell, such as *E. coli*, or a mammalian cell, such as a human cell.

[0198] A further aspect of the invention relates to a kit comprising the modified mycoplasma protein M or a functional fragment thereof, polynucleotide, vector, and/or transformed cell of the invention. The kit may include additional reagents for carrying out one of the methods described herein. The reagents may be included in suitable packages or containers. Additional reagents include, without limitation, buffers, labels, enzymes, detection reagents, *etc.*

[0199] When a kit is supplied, the different components may be packaged in separate containers and admixed immediately before use. Such packaging of the components separately may permit long-term storage without losing the active components' functions. Kits may also be supplied with instructional materials. Instructions may be printed on paper or other substrate, and/or may be supplied as an electronic-readable medium.

Heterologous Agents

[0200] As described above, the heterologous agent may be one for which neutralizing antibodies are present in the subject prior to administration of the heterologous agent or one

that is likely to raise neutralizing antibodies upon administration to the subject. In some embodiments, the heterologous agent may be a nucleic acid delivery vector (*e.g.*, a viral vector or a non-viral vector), a gene editing complex (*e.g.*, a CRISPR complex), a protein, or a nucleic acid.

[0201] Any nucleic acid sequence(s) of interest may be delivered in the nucleic acid delivery vectors of the present invention. Nucleic acids of interest include nucleic acids encoding polypeptides, including therapeutic (*e.g.*, for medical or veterinary uses), immunogenic (*e.g.*, for vaccines), or diagnostic polypeptides.

[0202] Therapeutic polypeptides include, but are not limited to, cystic fibrosis transmembrane regulator protein (CFTR), dystrophin (including mini- and micro-dystrophins (*see, e.g.*, Vincent *et al.*, (1993) *Nature Genetics* 5:130; U.S. Patent Publication No. 2003/017131; International publication WO/2008/088895, Wang *et al.*, *Proc. Natl. Acad. Sci. USA* 97:13714-13719 (2000); and Gregorevic *et al.*, *Mol. Ther.* 16:657-64 (2008)), myostatin propeptide, follistatin, activin type II soluble receptor, IGF-1, anti-inflammatory polypeptides such as the Ikappa B dominant mutant, sarcospan, utrophin (Tinsley *et al.*, (1996) *Nature* 384:349), mini-utrophin, clotting factors (*e.g.*, Factor VIII, Factor IX, Factor X, *etc.*), erythropoietin, angiostatin, endostatin, catalase, tyrosine hydroxylase, superoxide dismutase, leptin, the LDL receptor, lipoprotein lipase, ornithine transcarbamylase, β-globin, α-globin, spectrin, α_1-antitrypsin, adenosine deaminase, hypoxanthine guanine phosphoribosyl transferase, β-glucocerebrosidase, sphingomyelinase, lysosomal hexosaminidase A, branched-chain keto acid dehydrogenase, RP65 protein, cytokines (*e.g.*, α-interferon, β-interferon, interferon-γ, interleukin-2, interleukin-4, granulocyte-macrophage colony stimulating factor, lymphotoxin, and the like), peptide growth factors, neurotrophic factors and hormones (*e.g.*, somatotropin, insulin, insulin-like growth factors 1 and 2, platelet derived growth factor, epidermal growth factor, fibroblast growth factor, nerve growth factor, neurotrophic factor –3 and –4, brain-derived neurotrophic factor, bone morphogenic proteins [including RANKL and VEGF], glial derived growth factor, transforming growth factor –α and –β, and the like), lysosomal acid α-glucosidase, α-galactosidase A, receptors (*e.g.*, the tumor necrosis growth factorα soluble receptor), S100A1, parvalbumin, adenylyl cyclase type 6, a molecule that effects G-protein coupled receptor kinase type 2 knockdown such as a truncated constitutively active bARKct, anti-inflammatory factors such as IRAP, anti-myostatin proteins, aspartoacylase, and monoclonal antibodies (including single chain monoclonal antibodies; an exemplary Mab is the Herceptin® Mab). Other illustrative heterologous nucleic acid sequences

encode suicide gene products (*e.g.*, thymidine kinase, cytosine deaminase, diphtheria toxin, and tumor necrosis factor), proteins conferring resistance to a drug used in cancer therapy, tumor suppressor gene products (*e.g.*, p53, Rb, Wt-1), TRAIL, FAS-ligand, and any other polypeptide that has a therapeutic effect in a subject in need thereof. Parvovirus vectors can also be used to deliver monoclonal antibodies and antibody fragments, for example, an antibody or antibody fragment directed against myostatin (*see, e.g.*, Fang *et al.*, *Nature Biotechnol.* 23:584-590 (2005)).

[0203] Nucleic acid sequences encoding polypeptides include those encoding reporter polypeptides (*e.g.*, an enzyme). Reporter polypeptides are known in the art and include, but are not limited to, Green Fluorescent Protein, β-galactosidase, alkaline phosphatase, luciferase, and chloramphenicol acetyltransferase gene.

[0204] Alternatively, in particular embodiments of this invention, the nucleic acid may encode a functional nucleic acid, *i.e.*, nucleic acid that functions without getting translated into a protein, *e.g.*, an antisense nucleic acid, a ribozyme (*e.g.*, as described in U.S. Patent No. 5,877,022), RNAs that effect spliceosome-mediated *trans*-splicing (*see*, Puttaraju *et al.*, (1999) *Nature Biotech.* 17:246; U.S. Patent No. 6,013,487; U.S. Patent No. 6,083,702), interfering RNAs (RNAi) including siRNA, shRNA or miRNA that mediate gene silencing (*see*, Sharp *et al.*, (2000) *Science* 287:2431), and other non-translated RNAs, such as "guide" RNAs (Gorman *et al.*, (1998) *Proc. Nat. Acad. Sci. USA* 95:4929; U.S. Patent No. 5,869,248 to Yuan *et al.*), and the like. Exemplary untranslated RNAs include RNAi against a multiple drug resistance (MDR) gene product (*e.g.*, to treat and/or prevent tumors and/or for administration to the heart to prevent damage by chemotherapy), RNAi against myostatin (*e.g.*, for Duchenne muscular dystrophy), RNAi against VEGF (*e.g.*, to treat and/or prevent tumors), RNAi against phospholamban (*e.g.*, to treat cardiovascular disease, *see, e.g.*, Andino *et al.*, *J. Gene Med.* 10:132-142 (2008) and Li *et al.*, *Acta Pharmacol Sin.* 26:51-55 (2005)); phospholamban inhibitory or dominant-negative molecules such as phospholamban S16E (*e.g.*, to treat cardiovascular disease, *see*, *e.g.*, Hoshijima *et al. Nat. Med.* 8:864-871 (2002)), RNAi to adenosine kinase (*e.g.*, for epilepsy), RNAi to a sarcoglycan [*e.g.*, α, β, γ], RNAi against myostatin, myostatin propeptide, follistatin, or activin type II soluble receptor, RNAi against anti-inflammatory polypeptides such as the Ikappa B dominant mutant, and RNAi directed against pathogenic organisms and viruses (*e.g.*, hepatitis B virus, human immunodeficiency virus, CMV, herpes simplex virus, human papilloma virus, *etc.*).

[0205] Alternatively, in particular embodiments of this invention, the nucleic acid may encode protein phosphatase inhibitor I (I-1), serca2a, zinc finger proteins that regulate the phospholamban gene, Barkct, β2-adrenergic receptor, β2-adrenergic receptor kinase (BARK), phosphoinositide-3 kinase (PI3 kinase), a molecule that effects G-protein coupled receptor kinase type 2 knockdown such as a truncated constitutively active bARKct; calsarcin, RNAi against phospholamban; phospholamban inhibitory or dominant-negative molecules such as phospholamban S16E, enos, inos, or bone morphogenic proteins (including BNP 2, 7, *etc.*, RANKL and/or VEGF).

[0206] The nucleic acid delivery vectors may also comprise a nucleic acid that shares homology with and recombines with a locus on a host chromosome. This approach can be utilized, for example, to correct a genetic defect in the host cell.

[0207] The present invention also provides nucleic acid delivery vectors that express an immunogenic polypeptide, *e.g.*, for vaccination. The nucleic acid may encode any immunogen of interest known in the art including, but not limited to, immunogens from human immunodeficiency virus (HIV), simian immunodeficiency virus (SIV), influenza virus, HIV or SIV gag proteins, tumor antigens, cancer antigens, bacterial antigens, viral antigens, and the like.

[0208] The use of parvoviruses as vaccine vectors is known in the art (*see*, *e.g.*, Miyamura *et al.*, (1994) *Proc. Nat. Acad. Sci USA* 91:8507; U.S. Patent No. 5,916,563 to Young *et al.*, U.S. Patent No. 5,905,040 to Mazzara *et al.*, U.S. Patent No. 5,882,652, U.S. Patent No. 5,863,541 to Samulski *et al.*). The antigen may be presented in the parvovirus capsid. Alternatively, the antigen may be expressed from a nucleic acid introduced into a recombinant vector genome. Any immunogen of interest as described herein and/or as is known in the art can be provided by the nucleic acid delivery vectors.

[0209] An immunogenic polypeptide can be any polypeptide suitable for eliciting an immune response and/or protecting the subject against an infection and/or disease, including, but not limited to, microbial, bacterial, protozoal, parasitic, fungal and/or viral infections and diseases. For example, the immunogenic polypeptide can be an orthomyxovirus immunogen (*e.g.*, an influenza virus immunogen, such as the influenza virus hemagglutinin (HA) surface protein or the influenza virus nucleoprotein, or an equine influenza virus immunogen) or a lentivirus immunogen (*e.g.*, an equine infectious anemia virus immunogen, a Simian Immunodeficiency Virus (SIV) immunogen, or a Human Immunodeficiency Virus (HIV) immunogen, such as the HIV or SIV envelope GP160 protein, the HIV or SIV matrix/capsid proteins, and the HIV or SIV *gag*, *pol* and *env* genes products). The immunogenic polypeptide

can also be an arenavirus immunogen (*e.g.,* Lassa fever virus immunogen, such as the Lassa fever virus nucleocapsid protein and the Lassa fever envelope glycoprotein), a poxvirus immunogen (*e.g.*, a vaccinia virus immunogen, such as the vaccinia L1 or L8 gene products), a flavivirus immunogen (*e.g.,* a yellow fever virus immunogen or a Japanese encephalitis virus immunogen), a filovirus immunogen (*e.g.*, an Ebola virus immunogen, or a Marburg virus immunogen, such as NP and GP gene products), a bunyavirus immunogen (*e.g.*, RVFV, CCHF, and/or SFS virus immunogens), or a coronavirus immunogen (*e.g.*, an infectious human coronavirus immunogen, such as the human coronavirus envelope glycoprotein, or a porcine transmissible gastroenteritis virus immunogen, or an avian infectious bronchitis virus immunogen). The immunogenic polypeptide can further be a polio immunogen, a herpes immunogen (*e.g.*, CMV, EBV, HSV immunogens) a mumps immunogen, a measles immunogen, a rubella immunogen, a diphtheria toxin or other diphtheria immunogen, a pertussis antigen, a hepatitis (*e.g.*, hepatitis A, hepatitis B, hepatitis C, etc.) immunogen, and/or any other vaccine immunogen now known in the art or later identified as an immunogen.

[0210] Alternatively, the immunogenic polypeptide can be any tumor or cancer cell antigen. Optionally, the tumor or cancer antigen is expressed on the surface of the cancer cell. Exemplary cancer and tumor cell antigens are described in S.A. Rosenberg (*Immunity* 10:281 (1991)). Other illustrative cancer and tumor antigens include, but are not limited to: BRCA1 gene product, BRCA2 gene product, gp100, tyrosinase, GAGE-1/2, BAGE, RAGE, LAGE, NY-ESO-1, CDK-4, β-catenin, MUM-1, Caspase-8, KIAA0205, HPVE, SART-1, PRAME, p15, melanoma tumor antigens (Kawakami *et al.*, (1994) *Proc. Natl. Acad. Sci. USA* 91:3515; Kawakami *et al.*, (1994) *J. Exp. Med.*, 180:347; Kawakami *et al.*, (1994) *Cancer Res.* 54:3124), MART-1, gp100 MAGE-1, MAGE-2, MAGE-3, CEA, TRP-1, TRP-2, P-15, tyrosinase (Brichard *et al.,* (1993) *J. Exp. Med.* 178:489); HER-2/neu gene product (U.S. Patent No. 4,968,603), CA 125, LK26, FB5 (endosialin), TAG 72, AFP, CA19-9, NSE, DU-PAN-2, CA50, SPan-1, CA72-4, HCG, STN (sialyl Tn antigen), c-erbB-2 proteins, PSA, L-CanAg, estrogen receptor, milk fat globulin, p53 tumor suppressor protein (Levine, (1993) *Ann. Rev. Biochem.* 62:623); mucin antigens (International Patent Publication No. WO 90/05142); telomerases; nuclear matrix proteins; prostatic acid phosphatase; papilloma virus antigens; and/or antigens now known or later discovered to be associated with the following cancers: melanoma, adenocarcinoma, thymoma, lymphoma (*e.g.*, non-Hodgkin's lymphoma, Hodgkin's lymphoma), sarcoma, lung cancer, liver cancer, colon cancer, leukemia, uterine cancer, breast cancer, prostate cancer, ovarian cancer, cervical cancer, bladder cancer, kidney

cancer, pancreatic cancer, brain cancer and any other cancer or malignant condition now known or later identified (*see, e.g.*, Rosenberg, (1996) *Ann. Rev. Med.* 47:481-91).

[0211] It will be understood by those skilled in the art that the nucleic acid(s) of interest can be operably associated with appropriate control sequences. For example, the heterologous nucleic acid can be operably associated with expression control elements, such as transcription/translation control signals, origins of replication, polyadenylation signals, internal ribosome entry sites (IRES), promoters, and/or enhancers, and the like.

[0212] Those skilled in the art will appreciate that a variety of promoter/enhancer elements can be used depending on the level and tissue-specific expression desired. The promoter/enhancer can be constitutive or inducible, depending on the pattern of expression desired. The promoter/enhancer can be native or foreign and can be a natural or a synthetic sequence. By foreign, it is intended that the transcriptional initiation region is not found in the wild-type host into which the transcriptional initiation region is introduced.

[0213] In particular embodiments, the promoter/enhancer elements can be native to the target cell or subject to be treated. In representative embodiments, the promoters/enhancer element can be native to the nucleic acid sequence. The promoter/enhancer element is generally chosen so that it functions in the target cell(s) of interest. Further, in particular embodiments the promoter/enhancer element is a mammalian promoter/enhancer element. The promoter/enhancer element may be constitutive or inducible.

[0214] Inducible expression control elements are typically advantageous in those applications in which it is desirable to provide regulation over expression of the nucleic acid sequence(s). Inducible promoters/enhancer elements for gene delivery can be tissue-specific or –preferred promoter/enhancer elements, and include muscle specific or preferred (including cardiac, skeletal and/or smooth muscle specific or preferred), neural tissue specific or preferred (including brain-specific or preferred), eye specific or preferred (including retina-specific and cornea-specific), liver specific or preferred, bone marrow specific or preferred, pancreatic specific or preferred, spleen specific or preferred, and lung specific or preferred promoter/enhancer elements. Other inducible promoter/enhancer elements include hormone-inducible and metal-inducible elements. Exemplary inducible promoters/enhancer elements include, but are not limited to, a Tet on/off element, a RU486-inducible promoter, an ecdysone-inducible promoter, a rapamycin-inducible promoter, and a metallothionein promoter.

[0215] In embodiments wherein the nucleic acid sequence(s) is transcribed and then translated in the target cells, specific initiation signals are generally included for efficient translation of inserted protein coding sequences. These exogenous translational control

sequences, which may include the ATG initiation codon and adjacent sequences, can be of a variety of origins, both natural and synthetic.

[0216] The nucleic acid delivery vectors provide a means for delivering nucleic acids into a broad range of cells, including dividing and non-dividing cells. The nucleic acid delivery vectors can be employed to deliver a nucleic acid of interest to a cell *in vitro*, *e.g.*, for *ex vivo* gene therapy. The nucleic acid delivery vectors are additionally useful in a method of delivering a nucleic acid to a subject in need thereof, *e.g.*, to express an immunogenic or therapeutic polypeptide or a functional RNA. In this manner, the polypeptide or functional RNA can be produced *in vivo* in the subject. The subject can be in need of the polypeptide because the subject has a deficiency of the polypeptide. Further, the method can be practiced because the production of the polypeptide or functional RNA in the subject may impart some beneficial effect.

[0217] The nucleic acid delivery vectors can also be used to produce a polypeptide of interest or functional RNA in a subject (*e.g.*, using the subject as a bioreactor to produce the polypeptide or to observe the effects of the functional nucleic acid on the subject, for example, in connection with screening methods).

[0218] In general, the nucleic acid delivery vectors of the present invention can be employed to deliver a nucleic acid encoding a polypeptide or functional nucleic acid to treat and/or prevent any disease state for which it is beneficial to deliver a therapeutic polypeptide or functional nucleic acid. Illustrative disease states include, but are not limited to: cystic fibrosis (cystic fibrosis transmembrane regulator protein) and other diseases of the lung, hemophilia A (Factor VIII), hemophilia B (Factor IX), thalassemia (ß-globin), anemia (erythropoietin) and other blood disorders, Alzheimer's disease (GDF; neprilysin), multiple sclerosis (ß-interferon), Parkinson's disease (glial-cell line derived neurotrophic factor [GDNF]), Huntington's disease (RNAi to remove repeats), amyotrophic lateral sclerosis, epilepsy (galanin, neurotrophic factors), and other neurological disorders, cancer (endostatin, angiostatin, TRAIL, FAS-ligand, cytokines including interferons; RNAi including RNAi against VEGF or the multiple drug resistance gene product), diabetes mellitus (insulin), muscular dystrophies including Duchenne (dystrophin, mini-dystrophin, insulin-like growth factor I, a sarcoglycan [*e.g.*, α, β, γ], RNAi against myostatin, myostatin propeptide, follistatin, activin type II soluble receptor, anti-inflammatory polypeptides such as the Ikappa B dominant mutant, sarcospan, utrophin, mini-utrophin, RNAi against splice junctions in the dystrophin gene to induce exon skipping [*see, e.g.*, WO/2003/095647], antisense against U7 snRNAs to induce exon skipping [*see, e.g.*, WO/2006/021724], and antibodies or antibody fragments

against myostatin or myostatin propeptide) and Becker, Gaucher disease (glucocerebrosidase), Hurler's disease (α-L-iduronidase), adenosine deaminase deficiency (adenosine deaminase), glycogen storage diseases (*e.g.*, Fabry disease [α-galactosidase] and Pompe disease [lysosomal acid α-glucosidase]) and other metabolic defects, congenital emphysema (α1-antitrypsin), Lesch-Nyhan Syndrome (hypoxanthine guanine phosphoribosyl transferase), Niemann-Pick disease (sphingomyelinase), Tay Sachs disease (lysosomal hexosaminidase A), Maple Syrup Urine Disease (branched-chain keto acid dehydrogenase), retinal degenerative diseases (and other diseases of the eye and retina; *e.g.*, PDGF for macular degeneration), diseases of solid organs such as brain (including Parkinson's Disease [GDNF], astrocytomas [endostatin, angiostatin and/or RNAi against VEGF], glioblastomas [endostatin, angiostatin and/or RNAi against VEGF]), liver, kidney, heart including congestive heart failure or peripheral artery disease (PAD) (*e.g.*, by delivering protein phosphatase inhibitor I (I-1), serca2a, zinc finger proteins that regulate the phospholamban gene, Barkct, β2-adrenergic receptor, β2-adrenergic receptor kinase (BARK), phosphoinositide-3 kinase (PI3 kinase), S100A1, parvalbumin, adenylyl cyclase type 6, a molecule that effects G-protein coupled receptor kinase type 2 knockdown such as a truncated constitutively active bARKct; calsarcin, RNAi against phospholamban; phospholamban inhibitory or dominant-negative molecules such as phospholamban S16E, *etc.*), arthritis (insulin-like growth factors), joint disorders (insulin-like growth factor 1 and/or 2), intimal hyperplasia (*e.g.*, by delivering enos, inos), improve survival of heart transplants (superoxide dismutase), AIDS (soluble CD4), muscle wasting (insulin-like growth factor I), kidney deficiency (erythropoietin), anemia (erythropoietin), arthritis (anti-inflammatory factors such as IRAP and TNFα soluble receptor), hepatitis (α-interferon), LDL receptor deficiency (LDL receptor), hyperammonemia (ornithine transcarbamylase), Krabbe's disease (galactocerebrosidase), Batten's disease, spinal cerebral ataxias including SCA1, SCA2 and SCA3, phenylketonuria (phenylalanine hydroxylase), autoimmune diseases, and the like. The invention can further be used prior to and/or following organ transplantation to increase the success of the transplant and/or to reduce the negative side effects of organ transplantation or adjunct therapies (*e.g.*, by administering immunosuppressant agents or inhibitory nucleic acids to block cytokine production). As another example, bone morphogenic proteins (including BNP 2, 7, *etc.*, RANKL and/or VEGF) can be administered with a bone allograft, for example, following a break or surgical removal in a cancer patient.

[0219] Gene transfer has substantial potential use for understanding and providing therapy for disease states. There are a number of inherited diseases in which defective genes are known

and have been cloned. In general, the above disease states fall into two classes: deficiency states, usually of enzymes, which are generally inherited in a recessive manner, and unbalanced states, which may involve regulatory or structural proteins, and which are typically inherited in a dominant manner. For deficiency state diseases, gene transfer can be used to bring a normal gene into affected tissues for replacement therapy, as well as to create animal models for the disease using antisense mutations. For unbalanced disease states, gene transfer can be used to create a disease state in a model system, which can then be used in efforts to counteract the disease state. Thus, nucleic acid delivery vectors permit the treatment and/or prevention of genetic diseases.

[0220] The nucleic acid delivery vectors may also be employed to provide a functional nucleic acid to a cell *in vitro* or *in vivo*. Expression of the functional nucleic acid in the cell, for example, can diminish expression of a particular target protein by the cell. Accordingly, functional nucleic acid can be administered to decrease expression of a particular protein in a subject in need thereof.

[0221] Nucleic acid delivery vectors find use in diagnostic and screening methods, whereby a nucleic acid of interest is transiently or stably expressed in a transgenic animal model.

[0222] The nucleic acid delivery vectors can also be used for various non-therapeutic purposes, including but not limited to use in protocols to assess gene targeting, clearance, transcription, translation, *etc.*, as would be apparent to one skilled in the art. The nucleic acid delivery vectors can also be used for the purpose of evaluating safety (spread, toxicity, immunogenicity, *etc.*). Such data, for example, are considered by the United States Food and Drug Administration as part of the regulatory approval process prior to evaluation of clinical efficacy.

[0223] As a further aspect, the nucleic acid delivery vectors of the present invention may be used to produce an immune response in a subject. According to this embodiment, a nucleic acid delivery vectors comprising a nucleic acid sequence encoding an immunogenic polypeptide can be administered to a subject, and an active immune response is mounted by the subject against the immunogenic polypeptide. Immunogenic polypeptides are as described hereinabove. In some embodiments, a protective immune response is elicited.

[0224] Alternatively, the nucleic acid delivery vectors may be administered to a cell *ex vivo* and the altered cell is administered to the subject. The nucleic acid delivery vectors comprising the nucleic acid is introduced into the cell, and the cell is administered to the subject, where the nucleic acid encoding the immunogen can be expressed and induce an immune response in

the subject against the immunogen. In particular embodiments, the cell is an antigen-presenting cell (*e.g.*, a dendritic cell).

[0225] An "active immune response" or "active immunity" is characterized by "participation of host tissues and cells after an encounter with the immunogen. It involves differentiation and proliferation of immunocompetent cells in lymphoreticular tissues, which lead to synthesis of antibody or the development of cell-mediated reactivity, or both." Herbert B. Herscowitz, *Immunophysiology: Cell Function and Cellular Interactions in Antibody Formation, in* IMMUNOLOGY: BASIC PROCESSES 117 (Joseph A. Bellanti ed., 1985). Alternatively stated, an active immune response is mounted by the host after exposure to an immunogen by infection or by vaccination. Active immunity can be contrasted with passive immunity, which is acquired through the "transfer of preformed substances (antibody, transfer factor, thymic graft, interleukin-2) from an actively immunized host to a non-immune host." *Id.*

[0226] A "protective" immune response or "protective" immunity as used herein indicates that the immune response confers some benefit to the subject in that it prevents or reduces the incidence of disease. Alternatively, a protective immune response or protective immunity may be useful in the treatment and/or prevention of disease, in particular cancer or tumors (*e.g.*, by preventing cancer or tumor formation, by causing regression of a cancer or tumor and/or by preventing metastasis and/or by preventing growth of metastatic nodules). The protective effects may be complete or partial, as long as the benefits of the treatment outweigh any disadvantages thereof.

[0227] In particular embodiments, the nucleic acid delivery vector or cell comprising the nucleic acid can be administered in an immunogenically effective amount, as described below.

[0228] The nucleic acid delivery vectors can also be administered for cancer immunotherapy by administration of a nucleic acid delivery vector expressing one or more cancer cell antigens (or an immunologically similar molecule) or any other immunogen that produces an immune response against a cancer cell. To illustrate, an immune response can be produced against a cancer cell antigen in a subject by administering a nucleic acid delivery vectors comprising a nucleic acid encoding the cancer cell antigen, for example to treat a patient with cancer and/or to prevent cancer from developing in the subject. The nucleic acid delivery vectors may be administered to a subject *in vivo* or by using *ex vivo* methods, as described herein. Alternatively, the cancer antigen can be expressed as part of the nucleic acid delivery vectors.

[0229] As another alternative, any other therapeutic nucleic acid (*e.g.*, RNAi) or polypeptide (*e.g.*, cytokine) known in the art can be administered to treat and/or prevent cancer.

[0230] As used herein, the term "cancer" encompasses tumor-forming cancers. Likewise, the term "cancerous tissue" encompasses tumors. A "cancer cell antigen" encompasses tumor antigens.

[0231] The term "cancer" has its understood meaning in the art, for example, an uncontrolled growth of tissue that has the potential to spread to distant sites of the body (*i.e.*, metastasize). Exemplary cancers include, but are not limited to melanoma, adenocarcinoma, thymoma, lymphoma (*e.g.*, non-Hodgkin's lymphoma, Hodgkin's lymphoma), sarcoma, lung cancer, liver cancer, colon cancer, leukemia, uterine cancer, breast cancer, prostate cancer, ovarian cancer, cervical cancer, bladder cancer, kidney cancer, pancreatic cancer, brain cancer and any other cancer or malignant condition now known or later identified. In representative embodiments, the invention provides a method of treating and/or preventing tumor-forming cancers.

[0232] The term "tumor" is also understood in the art, for example, as an abnormal mass of undifferentiated cells within a multicellular organism. Tumors can be malignant or benign. In representative embodiments, the methods disclosed herein are used to prevent and treat malignant tumors.

[0233] By the terms "treating cancer," "treatment of cancer" and equivalent terms it is intended that the severity of the cancer is reduced or at least partially eliminated and/or the progression of the disease is slowed and/or controlled and/or the disease is stabilized. In particular embodiments, these terms indicate that metastasis of the cancer is prevented or reduced or at least partially eliminated and/or that growth of metastatic nodules is prevented or reduced or at least partially eliminated.

[0234] By the terms "prevention of cancer" or "preventing cancer" and equivalent terms it is intended that the methods at least partially eliminate or reduce and/or delay the incidence and/or severity of the onset of cancer. Alternatively stated, the onset of cancer in the subject may be reduced in likelihood or probability and/or delayed.

[0235] In particular embodiments, cells may be removed from a subject with cancer and contacted with a nucleic acid delivery vectors. The modified cell is then administered to the subject, whereby an immune response against the cancer cell antigen is elicited. This method can be advantageously employed with immunocompromised subjects that cannot mount a sufficient immune response *in vivo* (*i.e.*, cannot produce enhancing antibodies in sufficient quantities).

[0236] It is known in the art that immune responses may be enhanced by immunomodulatory cytokines (*e.g.*, α-interferon, β-interferon, γ-interferon, ω-interferon, τ-interferon, interleukin-1α, interleukin-1β, interleukin-2, interleukin-3, interleukin-4, interleukin 5, interleukin-6, interleukin-7, interleukin-8, interleukin-9, interleukin-10, interleukin-11, interleukin 12, interleukin-13, interleukin-14, interleukin-18, B cell Growth factor, CD40 Ligand, tumor necrosis factor-α, tumor necrosis factor-β, monocyte chemoattractant protein-1, granulocyte-macrophage colony stimulating factor, and lymphotoxin). Accordingly, immunomodulatory cytokines (preferably, CTL inductive cytokines) may be administered to a subject in conjunction with the virus vector.

[0237] Cytokines may be administered by any method known in the art. Exogenous cytokines may be administered to the subject, or alternatively, a nucleic acid encoding a cytokine may be delivered to the subject using a suitable vector, and the cytokine produced *in vivo*.

Subjects, Pharmaceutical Formulations, and Modes of Administration

[0238] The methods of the present invention find use in both veterinary and medical applications. Suitable subjects include avians, reptiles, amphibians, fish, and mammals. The term "mammal" as used herein includes, but is not limited to, humans, primates, non-human primates (*e.g.,* monkeys and baboons), cattle, sheep, goats, pigs, horses, cats, dogs, rabbits, rodents (*e.g.,* rats, mice, hamsters, and the like), *etc.* Human subjects include neonates, infants, juveniles, and adults. Optionally, the subject is "in need of" the methods of the present invention, *e.g.,* because the subject has or is believed at risk for a disorder including those described herein or that would benefit from the delivery of a polynucleotide including those described herein. As a further option, the subject can be a laboratory animal and/or an animal model of disease. Preferably, the subject is a human.

[0239] In certain embodiments, the heterologous agent and protein M or a functional fragment or derivative thereof is administered to a subject in need thereof as early as possible in the life of the subject, *e.g.,* as soon as the subject is diagnosed with a disease or disorder. In some embodiments, the method are carried out on a newborn subject, *e.g.,* after newborn screening has identified a disease or disorder. In some embodiments, methods are carried out on a subject prior to the age of 10 years, *e.g.,* prior to 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 years of age. In some embodiments, the methods are carried out on juvenile or adult subjects after the age of 10 years. In some embodiments, the methods are carried out on a fetus *in utero*, *e.g.,* after

prenatal screening has identified a disease or disorder. In some embodiments, the methods are carried out on a subject as soon as the subject develops symptoms associated with a disease or disorder. In some embodiments, the methods are carried out on a subject before the subject develops symptoms associated with a disease or disorder, *e.g.,* a subject that is suspected or diagnosed as having a disease or disorder but has not started to exhibit symptoms.

[0240] In particular embodiments, the present invention provides one or more pharmaceutical compositions comprising protein M or a functional fragment or derivative thereof, alone or together with a heterologous agent, in a pharmaceutically acceptable carrier and, optionally, other medicinal agents, pharmaceutical agents, stabilizing agents, buffers, carriers, adjuvants, diluents, *etc.* For injection, the carrier will typically be a liquid. For other methods of administration, the carrier may be either solid or liquid. For inhalation administration, the carrier will be respirable, and optionally can be in solid or liquid particulate form.

[0241] By "pharmaceutically acceptable" it is meant a material that is not toxic or otherwise undesirable, *i.e.*, the material may be administered to a subject without causing any undesirable biological effects.

[0242] One aspect of the present invention is a method of transferring a nucleic acid to a cell *in vitro*, *e.g.*, as part of an *ex vivo* method. The heterologous agent (*e.g.*, nucleic acid delivery vector, *e.g.*, viral vector) may be introduced into the cells at the appropriate amount, *e.g.*, multiplicity of infection according to standard transduction methods suitable for the particular target cells. Titers of virus vector to administer can vary, depending upon the target cell type and number, and the particular virus vector, and can be determined by those of skill in the art without undue experimentation. In representative embodiments, at least about 10^3 infectious units, more preferably at least about 10^5 infectious units are introduced to the cell.

[0243] The cell(s) into which the nucleic acid delivery vector is introduced can be of any type, including but not limited to neural cells (including cells of the peripheral and central nervous systems, in particular, brain cells such as neurons and oligodendrocytes), lung cells, cells of the eye (including retinal cells, retinal pigment epithelium, and corneal cells), blood vessel cells (*e.g.*, endothelial cells, intimal cells), epithelial cells (*e.g.*, gut and respiratory epithelial cells), muscle cells (*e.g.*, skeletal muscle cells, cardiac muscle cells, smooth muscle cells and/or diaphragm muscle cells), dendritic cells, pancreatic cells (including islet cells), hepatic cells, kidney cells, myocardial cells, bone cells (*e.g.*, bone marrow stem cells), hematopoietic stem cells, spleen cells, keratinocytes, fibroblasts, endothelial cells, prostate cells, germ cells, and the like. In representative embodiments, the cell can be any progenitor

cell. As a further possibility, the cell can be a stem cell (*e.g.*, neural stem cell, liver stem cell). As still a further alternative, the cell can be a cancer or tumor cell. Moreover, the cell can be from any species of origin, as indicated above.

[0244] The nucleic acid delivery vectors can be introduced into cells *in vitro* for the purpose of administering the modified cell to a subject. In particular embodiments, the cells have been removed from a subject, the nucleic acid delivery vector is introduced therein, and the cells are then administered back into the subject. Methods of removing cells from subject for manipulation *ex vivo*, followed by introduction back into the subject are known in the art (*see, e.g.*, U.S. Patent No. 5,399,346). Alternatively, the nucleic acid delivery vectors can be introduced into cells from a donor subject, into cultured cells, or into cells from any other suitable source, and the cells are administered to a subject in need thereof (*i.e.*, a "recipient" subject).

[0245] Suitable cells for *ex vivo* gene delivery are as described above. Dosages of the cells to administer to a subject will vary upon the age, condition and species of the subject, the type of cell, the nucleic acid being expressed by the cell, the mode of administration, and the like. Typically, at least about 10^2 to about 10^8 cells or at least about 10^3 to about 10^6 cells will be administered per dose in a pharmaceutically acceptable carrier. In particular embodiments, the cells transduced with the nucleic acid delivery vector are administered to the subject in a treatment effective or prevention effective amount in combination with a pharmaceutical carrier.

[0246] In some embodiments, the nucleic acid delivery vector is introduced into a cell and the cell can be administered to a subject to elicit an immunogenic response against the delivered polypeptide (*e.g.*, expressed as a transgene or in the capsid). Typically, a quantity of cells expressing an immunogenically effective amount of the polypeptide in combination with a pharmaceutically acceptable carrier is administered. An "immunogenically effective amount" is an amount of the expressed polypeptide that is sufficient to evoke an active immune response against the polypeptide in the subject to which the pharmaceutical formulation is administered. In particular embodiments, the dosage is sufficient to produce a protective immune response (as defined above). The degree of protection conferred need not be complete or permanent, as long as the benefits of administering the immunogenic polypeptide outweigh any disadvantages thereof.

[0247] A further aspect of the invention is a method of administering the heterologous agent (*e.g.*, nucleic acid delivery vector) to subjects. Administration of the nucleic acid delivery vectors to a human subject or an animal in need thereof can be by any means known

in the art. Optionally, the nucleic acid delivery vector is delivered in a treatment effective or prevention effective dose in a pharmaceutically acceptable carrier.

[0248] The nucleic acid delivery vectors can further be administered to elicit an immunogenic response (*e.g.*, as a vaccine). Typically, immunogenic compositions of the present invention comprise an immunogenically effective amount of nucleic acid delivery vector in combination with a pharmaceutically acceptable carrier. Optionally, the dosage is sufficient to produce a protective immune response (as defined above). The degree of protection conferred need not be complete or permanent, as long as the benefits of administering the immunogenic polypeptide outweigh any disadvantages thereof. Subjects and immunogens are as described above.

[0249] Dosages of the nucleic acid delivery vector (*e.g.*, viral vector) to be administered to a subject depend upon the mode of administration, the disease or condition to be treated and/or prevented, the individual subject's condition, the particular nucleic acid delivery vector, and the nucleic acid to be delivered, and the like, and can be determined in a routine manner. Exemplary doses for achieving therapeutic effects are titers of at least about 10^5, 10^6, 10^7, 10^8, 10^9, 10^{10}, 10^{11}, 10^{12}, 10^{13}, 10^{14}, 10^{15} transducing units, optionally about 10^8 – 10^{13} transducing units.

[0250] In particular embodiments, more than one administration (*e.g.*, two, three, four or more administrations) may be employed to achieve the desired level of gene expression over a period of various intervals, *e.g.*, daily, weekly, monthly, yearly, *etc.*

[0251] Exemplary modes of administration include oral, rectal, transmucosal, intranasal, inhalation (*e.g.*, via an aerosol), buccal (*e.g.*, sublingual), vaginal, intrathecal, intraocular, transdermal, intraendothelial, *in utero* (or *in ovo*), parenteral (*e.g.*, intravenous, subcutaneous, intradermal, intracranial, intramuscular [including administration to skeletal, diaphragm and/or cardiac muscle], intrapleural, intracerebral, and intraarticular), topical (*e.g.*, to both skin and mucosal surfaces, including airway surfaces, and transdermal administration), intralymphatic, and the like, as well as direct tissue or organ injection (*e.g.*, to liver, eye, skeletal muscle, cardiac muscle, diaphragm muscle or brain).

[0252] Administration can be to any site in a subject, including, without limitation, a site selected from the group consisting of the brain, a skeletal muscle, a smooth muscle, the heart, the diaphragm, the airway epithelium, the liver, the kidney, the spleen, the pancreas, the skin, and the eye.

[0253] Administration can also be to a tumor (*e.g.*, in or near a tumor or a lymph node). The most suitable route in any given case will depend on the nature and severity of the

condition being treated and/or prevented and on the nature of the particular vector that is being used.

[0254] Administration to skeletal muscle according to the present invention includes but is not limited to administration to skeletal muscle in the limbs (*e.g.*, upper arm, lower arm, upper leg, and/or lower leg), back, neck, head (*e.g.*, tongue), thorax, abdomen, pelvis/perineum, and/or digits. Suitable skeletal muscles include but are not limited to abductor digiti minimi (in the hand), abductor digiti minimi (in the foot), abductor hallucis, abductor ossis metatarsi quinti, abductor pollicis brevis, abductor pollicis longus, adductor brevis, adductor hallucis, adductor longus, adductor magnus, adductor pollicis, anconeus, anterior scalene, articularis genus, biceps brachii, biceps femoris, brachialis, brachioradialis, buccinator, coracobrachialis, corrugator supercilii, deltoid, depressor anguli oris, depressor labii inferioris, digastric, dorsal interossei (in the hand), dorsal interossei (in the foot), extensor carpi radialis brevis, extensor carpi radialis longus, extensor carpi ulnaris, extensor digiti minimi, extensor digitorum, extensor digitorum brevis, extensor digitorum longus, extensor hallucis brevis, extensor hallucis longus, extensor indicis, extensor pollicis brevis, extensor pollicis longus, flexor carpi radialis, flexor carpi ulnaris, flexor digiti minimi brevis (in the hand), flexor digiti minimi brevis (in the foot), flexor digitorum brevis, flexor digitorum longus, flexor digitorum profundus, flexor digitorum superficialis, flexor hallucis brevis, flexor hallucis longus, flexor pollicis brevis, flexor pollicis longus, frontalis, gastrocnemius, geniohyoid, gluteus maximus, gluteus medius, gluteus minimus, gracilis, iliocostalis cervicis, iliocostalis lumborum, iliocostalis thoracis, illiacus, inferior gemellus, inferior oblique, inferior rectus, infraspinatus, interspinalis, intertransversi, lateral pterygoid, lateral rectus, latissimus dorsi, levator anguli oris, levator labii superioris, levator labii superioris alaeque nasi, levator palpebrae superioris, levator scapulae, long rotators, longissimus capitis, longissimus cervicis, longissimus thoracis, longus capitis, longus colli, lumbricals (in the hand), lumbricals (in the foot), masseter, medial pterygoid, medial rectus, middle scalene, multifidus, mylohyoid, obliquus capitis inferior, obliquus capitis superior, obturator externus, obturator internus, occipitalis, omohyoid, opponens digiti minimi, opponens pollicis, orbicularis oculi, orbicularis oris, palmar interossei, palmaris brevis, palmaris longus, pectineus, pectoralis major, pectoralis minor, peroneus brevis, peroneus longus, peroneus tertius, piriformis, plantar interossei, plantaris, platysma, popliteus, posterior scalene, pronator quadratus, pronator teres, psoas major, quadratus femoris, quadratus plantae, rectus capitis anterior, rectus capitis lateralis, rectus capitis posterior major, rectus capitis posterior minor, rectus femoris, rhomboid major, rhomboid minor, risorius, sartorius, scalenus minimus, semimembranosus, semispinalis capitis, semispinalis cervicis,

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[0255] The heterologous agent can be delivered to skeletal muscle by intravenous administration, intra-arterial administration, intraperitoneal administration, limb perfusion, (optionally, isolated limb perfusion of a leg and/or arm; *see, e.g.* Arruda *et al.*, (2005) *Blood* 105: 3458-3464), and/or direct intramuscular injection. In particular embodiments, the heterologous agent is administered to a limb (arm and/or leg) of a subject (*e.g.*, a subject with muscular dystrophy such as DMD) by limb perfusion, optionally isolated limb perfusion (*e.g.*, by intravenous or intra-articular administration. In embodiments of the invention, the heterologous agent can advantageously be administered without employing "hydrodynamic" techniques. Tissue delivery (*e.g.*, to muscle) of prior art vectors is often enhanced by hydrodynamic techniques (*e.g.*, intravenous/intravenous administration in a large volume), which increase pressure in the vasculature and facilitate the ability of the agent to cross the endothelial cell barrier. In particular embodiments, the heterologous agent can be administered in the absence of hydrodynamic techniques such as high volume infusions and/or elevated intravascular pressure (*e.g.*, greater than normal systolic pressure, for example, less than or equal to a 5%, 10%, 15%, 20%, 25% increase in intravascular pressure over normal systolic pressure). Such methods may reduce or avoid the side effects associated with hydrodynamic techniques such as edema, nerve damage and/or compartment syndrome.

[0256] Administration to cardiac muscle includes administration to the left atrium, right atrium, left ventricle, right ventricle and/or septum. The heterologous agent can be delivered to cardiac muscle by intravenous administration, intra-arterial administration such as intra-aortic administration, direct cardiac injection (*e.g.*, into left atrium, right atrium, left ventricle, right ventricle), and/or coronary artery perfusion.

[0257] Administration to diaphragm muscle can be by any suitable method including intravenous administration, intra-arterial administration, and/or intra-peritoneal administration.

[0258] Administration to smooth muscle can be by any suitable method including intravenous administration, intra-arterial administration, and/or intra-peritoneal administration.

In one embodiment, administration can be to endothelial cells present in, near, and/or on smooth muscle.

[0259] Delivery to a target tissue can also be achieved by delivering a depot comprising the heterologous agent. In representative embodiments, a depot comprising the heterologous agent is implanted into skeletal, smooth, cardiac and/or diaphragm muscle tissue or the tissue can be contacted with a film or other matrix comprising the heterologous agent. Such implantable matrices or substrates are described in U.S. Patent No. 7,201,898.

[0260] In particular embodiments, a heterologous agent is administered to skeletal muscle, diaphragm muscle and/or cardiac muscle (*e.g.*, to treat and/or prevent muscular dystrophy or heart disease [for example, PAD or congestive heart failure]).

[0261] In representative embodiments, the invention is used to treat and/or prevent disorders of skeletal, cardiac and/or diaphragm muscle.

[0262] In a representative embodiment, the invention provides a method of treating and/or preventing muscular dystrophy in a subject in need thereof, the method comprising: administering a treatment or prevention effective amount of a heterologous agent to a mammalian subject, wherein the heterologous agent comprises a nucleic acid encoding dystrophin, a mini-dystrophin, a micro-dystrophin, myostatin propeptide, follistatin, activin type II soluble receptor, IGF-1, anti-inflammatory polypeptides such as the Ikappa B dominant mutant, sarcospan, utrophin, a micro-dystrophin, laminin-α2, α-sarcoglycan, β-sarcoglycan, γ-sarcoglycan, δ-sarcoglycan, IGF-1, an antibody or antibody fragment against myostatin or myostatin propeptide, and/or RNAi against myostatin. In particular embodiments, the heterologous agent can be administered to skeletal, diaphragm and/or cardiac muscle as described elsewhere herein.

[0263] Alternatively, the invention can be practiced to deliver a nucleic acid to skeletal, cardiac or diaphragm muscle, which is used as a platform for production of a polypeptide (*e.g.*, an enzyme) or functional nuclei acid (*e.g.*, functional RNA, *e.g.*, RNAi, microRNA, antisense RNA) that normally circulates in the blood or for systemic delivery to other tissues to treat and/or prevent a disorder (*e.g.*, a metabolic disorder, such as diabetes (*e.g.*, insulin), hemophilia (*e.g.*, Factor IX or Factor VIII), a mucopolysaccharide disorder (*e.g.*, Sly syndrome, Hurler Syndrome, Scheie Syndrome, Hurler-Scheie Syndrome, Hunter's Syndrome, Sanfilippo Syndrome A, B, C, D, Morquio Syndrome, Maroteaux-Lamy Syndrome, *etc.*) or a lysosomal storage disorder (such as Gaucher's disease [glucocerebrosidase], Pompe disease [lysosomal acid α-glucosidase] or Fabry disease [α-galactosidase A]) or a glycogen storage disorder (such

as Pompe disease [lysosomal acid α glucosidase]). Other suitable proteins for treating and/or preventing metabolic disorders are described above. The use of muscle as a platform to express a nucleic acid of interest is described in U.S. Patent Publication No. 2002/0192189.

[0264] Thus, as one aspect, the invention further encompasses a method of treating and/or preventing a metabolic disorder in a subject in need thereof, the method comprising: administering a treatment or prevention effective amount of a heterologous agent to a subject (*e.g.*, to skeletal muscle of a subject), wherein the heterologous agent comprises a nucleic acid encoding a polypeptide, wherein the metabolic disorder is a result of a deficiency and/or defect in the polypeptide. Illustrative metabolic disorders and nucleic acids encoding polypeptides are described herein. Optionally, the polypeptide is secreted (*e.g.*, a polypeptide that is a secreted polypeptide in its native state or that has been engineered to be secreted, for example, by operable association with a secretory signal sequence as is known in the art). Without being limited by any particular theory of the invention, according to this embodiment, administration to the skeletal muscle can result in secretion of the polypeptide into the systemic circulation and delivery to target tissue(s). Methods of delivering heterologous agent to skeletal muscle are described in more detail herein.

[0265] The invention can also be practiced to produce antisense RNA, RNAi or other functional RNA (*e.g.*, a ribozyme) for systemic delivery.

[0266] The invention also provides a method of treating and/or preventing congenital heart failure or PAD in a subject in need thereof, the method comprising administering a treatment or prevention effective amount of a heterologous agent of the invention to a mammalian subject, wherein the heterologous agent comprises a nucleic acid encoding, for example, a sarcoplasmic endoreticulum Ca^{2+}-ATPase (SERCA2a), an angiogenic factor, phosphatase inhibitor I (I-1), RNAi against phospholamban; a phospholamban inhibitory or dominant-negative molecule such as phospholamban S16E, a zinc finger protein that regulates the phospholamban gene, β2-adrenergic receptor, β2-adrenergic receptor kinase (BARK), PI3 kinase, calsarcan, a β-adrenergic receptor kinase inhibitor (βARKct), inhibitor 1 of protein phosphatase 1, S100A1, parvalbumin, adenylyl cyclase type 6, a molecule that effects G-protein coupled receptor kinase type 2 knockdown such as a truncated constitutively active bARKct, Pim-1, PGC-1α, SOD-1, SOD-2, EC-SOD, kallikrein, HIF, thymosin-β4, mir-1, mir-133, mir-206 and/or mir-208.

[0267] Injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution or suspension in liquid prior to injection, or as

emulsions. Alternatively, one may administer the heterologous agent in a local rather than systemic manner, for example, in a depot or sustained-release formulation. Further, the heterologous agent can be delivered adhered to a surgically implantable matrix (*e.g.*, as described in U.S. Patent Publication No. 2004-0013645).

[0268] The heterologous agent disclosed herein can be administered to the lungs of a subject by any suitable means, optionally by administering an aerosol suspension of respirable particles comprised of the heterologous agent, which the subject inhales. The respirable particles can be liquid or solid. Aerosols of liquid particles comprising the heterologous agent may be produced by any suitable means, such as with a pressure-driven aerosol nebulizer or an ultrasonic nebulizer, as is known to those of skill in the art. *See, e.g.*, U.S. Patent No. 4,501,729. Aerosols of solid particles comprising the heterologous agent may likewise be produced with any solid particulate medicament aerosol generator, by techniques known in the pharmaceutical art.

[0269] The heterologous agent can be administered to tissues of the CNS (*e.g.*, brain, eye) and may advantageously result in broader distribution of the heterologous agent than would be observed in the absence of the present invention.

[0270] In particular embodiments, the heterologous agent may be administered to treat diseases of the CNS, including genetic disorders, neurodegenerative disorders, psychiatric disorders and tumors. Illustrative diseases of the CNS include, but are not limited to Alzheimer's disease, Parkinson's disease, Huntington's disease, Canavan disease, Leigh's disease, Refsum disease, Tourette syndrome, primary lateral sclerosis, amyotrophic lateral sclerosis, progressive muscular atrophy, Pick's disease, muscular dystrophy, multiple sclerosis, myasthenia gravis, Binswanger's disease, trauma due to spinal cord or head injury, Tay Sachs disease, Lesch-Nyan disease, epilepsy, cerebral infarcts, psychiatric disorders including mood disorders (*e.g.*, depression, bipolar affective disorder, persistent affective disorder, secondary mood disorder), schizophrenia, drug dependency (*e.g.*, alcoholism and other substance dependencies), neuroses (*e.g.*, anxiety, obsessional disorder, somatoform disorder, dissociative disorder, grief, post-partum depression), psychosis (*e.g.*, hallucinations and delusions), dementia, paranoia, attention deficit disorder, psychosexual disorders, sleeping disorders, pain disorders, eating or weight disorders (*e.g.*, obesity, cachexia, anorexia nervosa, and bulemia) and cancers and tumors (*e.g.*, pituitary tumors) of the CNS.

[0271] Disorders of the CNS include ophthalmic disorders involving the retina, posterior tract, and optic nerve (*e.g.*, retinitis pigmentosa, diabetic retinopathy and other retinal degenerative diseases, uveitis, age-related macular degeneration, glaucoma).

[0272] Most, if not all, ophthalmic diseases and disorders are associated with one or more of three types of indications: (1) angiogenesis, (2) inflammation, and (3) degeneration. The heterologous agent of the present invention can be employed to deliver anti-angiogenic factors; anti-inflammatory factors; factors that retard cell degeneration, promote cell sparing, or promote cell growth and combinations of the foregoing.

[0273] Diabetic retinopathy, for example, is characterized by angiogenesis. Diabetic retinopathy can be treated by delivering one or more anti-angiogenic factors either intraocularly (*e.g.*, in the vitreous) or periocularly(*e.g.*, in the sub-Tenon's region). One or more neurotrophic factors may also be co-delivered, either intraocularly (*e.g.*, intravitreally) or periocularly.

[0274] Uveitis involves inflammation. One or more anti-inflammatory factors can be administered by intraocular (*e.g.*, vitreous or anterior chamber) administration of a delivery vector of the invention.

[0275] Retinitis pigmentosa, by comparison, is characterized by retinal degeneration. In representative embodiments, retinitis pigmentosa can be treated by intraocular (*e.g.*, vitreal administration) of a heterologous agent encoding one or more neurotrophic factors.

[0276] Age-related macular degeneration involves both angiogenesis and retinal degeneration. This disorder can be treated by administering a heterologous agent encoding one or more neurotrophic factors intraocularly (*e.g.*, vitreous) and/or one or more anti-angiogenic factors intraocularly or periocularly (*e.g.*, in the sub-Tenon's region).

[0277] Glaucoma is characterized by increased ocular pressure and loss of retinal ganglion cells. Treatments for glaucoma include administration of one or more neuroprotective agents that protect cells from excitotoxic damage using the heterologous agent. Such agents include N-methyl-D-aspartate (NMDA) antagonists, cytokines, and neurotrophic factors, delivered intraocularly, optionally intravitreally.

[0278] In other embodiments, the present invention may be used to treat seizures, *e.g.*, to reduce the onset, incidence or severity of seizures. The efficacy of a therapeutic treatment for seizures can be assessed by behavioral (*e.g.*, shaking, ticks of the eye or mouth) and/or electrographic means (most seizures have signature electrographic abnormalities). Thus, the invention can also be used to treat epilepsy, which is marked by multiple seizures over time.

[0279] In one representative embodiment, somatostatin (or an active fragment thereof) is administered to the brain using a heterologous agent of the invention to treat a pituitary tumor. According to this embodiment, the heterologous agent encoding somatostatin (or an active fragment thereof) is administered by microinfusion into the pituitary. Likewise, such treatment

can be used to treat acromegaly (abnormal growth hormone secretion from the pituitary). The nucleic acid (*e.g.*, GenBank Accession No. J00306) and amino acid (*e.g.*, GenBank Accession No. P01166; contains processed active peptides somatostatin-28 and somatostatin-14) sequences of somatostatins as are known in the art.

[0280] In particular embodiments, the heterologous agent can comprise a secretory signal as described in U.S. Patent No. 7,071,172.

[0281] In representative embodiments of the invention, the heterologous agent is administered to the CNS (*e.g.*, to the brain or to the eye). The heterologous agent may be introduced into the spinal cord, brainstem (medulla oblongata, pons), midbrain (hypothalamus, thalamus, epithalamus, pituitary gland, substantia nigra, pineal gland), cerebellum, telencephalon (corpus striatum, cerebrum including the occipital, temporal, parietal and frontal lobes. cortex, basal ganglia, hippocampus and portaamygdala), limbic system, neocortex, corpus striatum, cerebrum, and inferior colliculus. The heterologous agent may also be administered to different regions of the eye such as the retina, cornea and/or optic nerve.

[0282] The heterologous agent may be delivered into the cerebrospinal fluid (*e.g.*, by lumbar puncture) for more disperse administration of the heterologous agent. The heterologous agent may further be administered intravascularly to the CNS in situations in which the blood-brain barrier has been perturbed (*e.g.*, brain tumor or cerebral infarct).

[0283] The heterologous agent can be administered to the desired region(s) of the CNS by any route known in the art, including but not limited to, intrathecal, intra-ocular, intracerebral, intraventricular, intravenous (*e.g.*, in the presence of a sugar such as mannitol), intranasal, intra-aural, intra-ocular (*e.g.*, intra-vitreous, sub-retinal, anterior chamber) and peri-ocular (*e.g.*, sub-Tenon's region) delivery as well as intramuscular delivery with retrograde delivery to motor neurons.

[0284] In particular embodiments, the heterologous agent is administered in a liquid formulation by direct injection (*e.g.*, stereotactic injection) to the desired region or compartment in the CNS. In other embodiments, the heterologous agent may be provided by topical application to the desired region or by intra-nasal administration of an aerosol formulation. Administration to the eye, may be by topical application of liquid droplets. As a further alternative, the heterologous agent may be administered as a solid, slow-release formulation (*see, e.g.,* U.S. Patent No. 7,201,898).

[0285] In yet additional embodiments, the heterologous agent can used for retrograde transport to treat and/or prevent diseases and disorders involving motor neurons (*e.g.*,

amyotrophic lateral sclerosis (ALS); spinal muscular atrophy (SMA), etc.). For example, the heterologous agent can be delivered to muscle tissue from which it can migrate into neurons.

[0286] The protein M or a functional fragment or derivative thereof may be administered by any of the routes or schedules described above for the heterologous agent The protein M or a functional fragment or derivative thereof may be administered by a different route or schedule than the heterologous agent.

[0287] Having described the present invention, the same will be explained in greater detail in the following examples, which are included herein for illustration purposes only, and which are not intended to be limiting to the invention.

EXAMPLES

Example 1: Methods

[0288] **AAV virus production**: AAV vectors were produced using a standard approach with three-plasmid transfection in HEK293 cells. Briefly, the AAV transgene plasmid pTR-CBA-Luc was co-transfected with an AAV Rep/Cap helper plasmid (pXR2 or pXR8) and adenovirus helper plasmid pXX6-80. 72 hours later, cell cultures were harvested and lysed by freeze thaw and ultra sonication. Clarified cell lysate was DNAse treated and ultra-centrifuged in a 15%/25%/40%/60% iodixanol step gradient and purified by anion exchange Q-column. Purified AAV vector was tittered by qPCR with primers directed to amplify a segment of the packaged AAV transgene.

[0289] **Protein Production**: The plasmid pET-28b(+) which encodes the protein M, a truncated *M. genitalium* Protein MG281 lacking the transmembrane domain (amino acids 74 to 479) and carries a N-terminal His-Tag and a thrombin cleavage site, was generously provided by Rajesh. Plasmids were propagated in electro-competent DH10B cells and purified using a PureLink Maxi-Prep kit from Invitrogen. The pET-28b(+) plasmid was transiently transfected into BL21/DE3 cells using an overnight starter culture. Auto-induction media (Magic media from Invitrogen) was seeded with the starter culture and grown at 18 °C for 3 days in 1 L to 4 L culture volumes. The culture was then pelleted by centrifugation and frozen down at -80 °C.

[0290] **Protein Purification**: The frozen bacterial cell culture pellet was thawed, lysed by sonication, DNAse treated, and clarified by centrifugation. Clarified bacterial lysate was dialyzed into nickel-binding buffer (20 mM imidazole, 50 mM sodium phosphate pH 7.4, 500 mM NaCl, 0.02% sodium azide) and passed through a nickel His-trap FF column using FPLC. Protein M bound by the nickel column was then eluted by the same buffer base but containing

500 mM imidazole. Protein M was then run through an S-100 size exclusion column, dialyzed into phosphate buffered saline with 2% glycerol, and quantified using spectrophotometry. Protein M identity was confirmed using SDS-PAGE protein gel electrophoresis for protein separation followed by a Coomassie blue protein stain to correctly identify the 48 kDa Protein M band.

[0291] **Western Blot**: After protein separation on an 8% SDS-PAGE gel, the proteins were transferred onto a PVDF membrane. Immunoblotting was performed in 5% non-fat milk using anti-His primary antibody 1:1000 dilution (10 μg/ml). The secondary goat anti-human IgG antibody was conjugated to horseradish peroxidase (1:10,000 dilution).

[0292] **Cell Culture**: HEK-293 cells and Huh7 cells were used for all *in vitro* AAV neutralization experiments and for enhancement of transduction, respectively. Cells were maintained at 37 °C in 5% CO_2 with Dulbecco's modified Eagle's medium supplemented with 10% bovine calf serum and penicillin–streptomycin.

[0293] **Human IVIG and immunized mouse serum**: 10% Human IVIG (Gamunex) was purchased from Grifols Therapeutics Inc. (Research Triangle Park, NC, USA). Serum was collected and pooled from 12 different mice (50% male, 50% female) after IP administration of $3x10^{10}$ viral genomes of AAV8-FVIII followed by a boost administration of the same vector 2 weeks later, and a second boost 6 weeks after the first administration. Human IVIG and mouse serum were aliquoted and stored at −80 °C for future use.

[0294] ***In vitro* AAV Neutralization Assay**: NAb analyses were performed as described previously with slight modification. Cells were pelleted by centrifugation and resuspended in serum free X-Vivo 10 medium, then plated in either a 48-well or 96-well plate format. Human IVIG or serum was serially diluted either two-fold or ten-fold. AAV-Luc was incubated with either human IVIG or mouse serum for 1 h at 4 °C before adding AAV and incubating an additional 1 h at 4 °C. AAV+serum incubations were then mixed with cells suspended in serum free media at the time of plating. In the neutralization experiments using protein M, 3 different incubations scenarios were tested with each step for 1 h at 4 °C: neutralizing serum incubated first with protein M followed by AAV, AAV incubated first with neutralizing serum followed by protein M, and AAV incubated first with protein M followed by neutralizing serum. Cells were seeded in a 48-well plate in 200 μl media, or in a 96-well plate in 100 μl media. After transduction, cells were cultured for 24-48 h at 37 °C to allow for AAV-Luciferase transgene expression. To measure Luc activity, cells were lysed with passive lysis buffer (Promega, Madison, WI, USA) and luciferase signal was measured with a Wallac1420 Victor 2 automated

plate reader. NAb titers were defined as the highest dilution for which luciferase activity was 50% lower than serum-free controls.

[0295] ***In Vivo*** **AAV Neutralization and Passive Transfer of NAb Serum**: Different amounts of serum containing AAV NAbs were injected into the retro-orbital vein of C57BL/6 mice and 20-minutes later AAV was injected. For mice receiving protein M treatment before AAV administration, protein M (either 6.3 mg for 2:1 ratio, 3.15 mg for 1:1 ratio, or 1.58 mg for 0.5:1 ratio) was delivered by retro-orbital vein 5-15 minutes after serum injection. AAV was administered 5 minutes later by systemic injection of 2×10^{12} particles per kg of AAV-Luc vector. Imaging was performed 1 day after AAV administration, as well as at 1 week and 9 day time points post-injection.

Example 2: Protein M ablates the inhibition activity of IVIG on AAV transduction

[0296] In order to study the antibody blocking function of protein M, *in vitro* neutralization assays of AAV by human intravenous immunoglobulin gamma (IVIG) were set up. Serial dilutions of IVIG were used to perform an AAV neutralization assay to determine the quantity of IVIG that will neutralize a given amount of AAV2. Luciferase activity generated by cellular transduction with AAV-Luciferase vectors served as a functional read out of gene expression. The neutralization was determined as the percent of luciferase activity normalized to no IVIG controls. It was found that 12.5 μg of IVIG neutralizes 75% (+/-5%) of AAV2 (**FIG. 1**) and this quantity of IVIG was chosen to move forward with experiments design to test protein M blocking of IVIG for AAV neutralization. Next, a dose dilution series was conducted of protein M (SEQ ID NO:2) which was collected by removing His-tag by thrombin cleavage and it's ability to block 12.5 μg of IVIG (**FIG. 2**), where protein M was incubated with IVIG for 1 h followed by AAV2 for 1 h at 4 °C before cell culture transduction. It was found that a molar ratio of 2 protein M molecules to 1 IgG molecule was sufficient to prevent neutralization to an equivalent level as the no IVIG control. Furthermore, it was found that higher molar ratios of protein M to IVIG enhanced the luciferase signal to levels greater than the no IVIG control (**FIG. 2**). 8 protein M molecules to 1 IgG increased luciferase expression by 2-fold. In order to see if enhancement increases further with greater molar ratios, an 8:1 and a 20:1 ratio of protein M to IVIG was tested in the same experimental conditions. It was found that increasing the protein M dose by 2.5-fold (from 8:1 to a 20:1 ratio) provided litter further enhancement since the corresponding signal increased by only 0.25-fold over the 8:1 ratio (**FIG. 3**). Additionally, it was found that protein M blocking activity of immunoglobulin does not depend

on cleavage of the N-terminal His tag, therefore Protein M without cleavage of His tag was used for the next experiments.

Example 3: Interaction of protein M with AAV vector virions enhances AAV transduction

[0297] Previous studies from the inventors have demonstrated that interaction of serum proteins with AAV virions is able to enhance AAV transduction. In order to further characterize the enhancement function of protein M on AAV transduction, a dose-response assay was performed without IVIG where protein M at serial 2-fold dilution was incubated with AAV for 1 h prior to cell culture transduction. In **FIG. 4** it was found that the 8:1 ratio (33 μg protein M) of protein M from the previous experiments was able to dose dependently enhance AAV transduction in the absence of IVIG, and that enhancement was lost at dilutions below 2 μg of protein M for $2x10^8$ viral particles. The equivalent molar ratio of protein M molecules to AAV particles at which enhancement was lost was below 40,000:1. Next, it was demonstrated that protein M enhancement of transduction is dependent on incubation of protein M with AAV. In **FIG. 5** a cell culture transduction assay was performed where protein M was added to AAV either 1 h before adding to the cells (pre-incubation, -1 h time point), added at the time of transduction without pre-incubation (peri-transduction, 0 h time point), or 18 h after adding AAV to the cells (post-transduction, 18 h time point). It was discovered that dose-dependent enhancement was seen in the pre-incubation group similar to that in **FIG. 4**, but not in the other 2 groups where protein M and AAV2 were not incubated together prior to the assay. Furthermore, this result also argues that the mechanism of protein M enhancement involves interaction with the vector capsid, and not a biological effect of protein M on the cells since adding protein M to the cells at the time of transduction had no impact on luciferase signal. Finally, it was demonstrated that protein M is not able to block neutralization when IVIG is first pre-incubated with AAV2 for 1 h followed by a 1 h incubation with protein M, and that protein M enhancement of AAV2 luciferase signal is only present when AAV2 is not fully neutralized by IVIG. For this assay 12.5 μg of IVIG (which neutralizes 75-80% of AAV2), 50 μg of IVIG (which neutralizes 99% of AAV2, from **FIG. 1**), or 200 μg of IVIG (which neutralizes 100% of AAV2) was used. In **FIG. 6** it was observed that when 99-100% of AAV2 was neutralized by IVIG (50 μg and 200 μg), then protein M is incapable of blocking neutralization and cannot provide transduction enhancement. In combination with the data in **FIGS. 2 and 3**, this result implicates that the excess amount of protein M after binding to immunoglobulin is able to interact with AAV virions and results in enhanced transduction.

Example 4: Pre-incubation of protein M with AAV vector virions protects AAV neutralization of IVIG

[0298] It was next decided to investigate the effect of pre-incubating protein M first with AAV and then IVIG instead of allowing protein M to first interact with IVIG. The ability of protein M to block neutralization was examined when protein M was incubated with AAV2 for 1 h followed by addition of IVIG and incubation for an additional 1 h at 4 °C before cell transduction. In **FIG. 7** it was found that when protein M concentrations are keep static (8.25 µg), and the dose of IVIG is serially diluted from 50 µg to 3.12 µg (1:2 to 8:1 molar ratio of protein M to IVIG) then protection of the vector from neutralization is achieved at a 2:1 ratio or greater, which is similar to the previous results when protein M was incubated with IVIG prior to AAV addition. In an effort to simulate an environment closer to the *in vivo* situation, where other types of proteins are present in the serum besides IgG, a similar *in vitro* neutralization test was performed in the presence of 10% Fetal Bovine Serum (FBS). As per the manufacturer's IgG quantifications for the bovine serum, it was estimated the media volume contained ~350 µg of bovine IgG per well. 25 µg IVIG or serum free media was then spiked in half the wells to neutralize AAV or serve as a control without neutralizing activity. In **FIG. 8,** when protein M was incubated with AAV for 1 h prior to transduction, and total IgG was added (human and bovine) at different molar ratios of 4:1, 2:1, and 1:1 (protein M to IgG), then protection from neutralization was observed even at the 1:1 ratio. These results indicate that protein M is able to bind and block immunoglobulins even in the presence of other serum proteins.

Example 5: Efficient blocking function of protein M on neutralizing activity of murine serum to AAV *in vitro*

[0299] To translate the finding from IVIG to a real situation, *in vitro* neutralization experiments were first performed with AAV8-Luciferase vectors using serum from mice that were immunized with an AAV8 capsid vector. When both the serum from immunized mice and protein M were serially diluted to maintain a molar ratio of 2:1 the results compared to serum only controls, it was found that protein M allows escape from neutralization over an estimated ~100-fold dilution of neutralizing serum at a titer of 1:2,564 (50% neutralization at 0.0039 µl of serum vs 0.2744 µl of serum in the presence of protein M, **FIG. 9**).

Example 6: Efficient blocking function of protein M on neutralizing activity of murine serum to AAV *in vivo*

[0300] Next, *in vivo* experiments were carried out where different volumes of neutralizing serum were passively transferred into naïve mice via retro-orbital injection. After letting the serum perfuse through the mice for 5-15 minutes, protein M was administered systemically to the mice also via retro-orbital injection followed 5 minutes later by AAV8-Luc ($2x10^{10}$ viral genomes). **FIG. 10** shows that protein M, at an estimated molar ratio of 2:1 (6.3 mg) to total immunoglobulin in the mouse, is able to prevent neutralization of AAV8 by 1 µl of AAV8-NAb serum (titer 1:2,564). This is compared to an *in vivo* neutralization assay of serially diluted anti-AAV8 serum where greater than 50% of AAV8-Luc is neutralized at serum volumes between 1 µl to 0.001 µl, representing AAV NAb escape over a 1,000-fold difference in concentration (**FIG. 11**).

Example 7: The stability of protein M/immunoglobulin complex

[0301] To study how stable the complex of protein M with immunoglobulins is, the assay was first carried out *in vitro*. Protein M was pre-incubated with mouse sera at molecular ratio of 2:1 at 37 °C for different duration from 1 h to 72 h, and then AAV8 vectors added for one more hour at 4 °C for neutralizing analysis. As shown in **FIG. 12**, the pre-formed complex between anti-AAV8 immunoglobulins in mouse serum and protein M was stable for greater than 72 h when incubated at 37 °C before being added to cell culture, as compared to controls containing neutralizing serum without protein M, or containing only PBS or media. This result suggests the formed complex between protein M and immunoglobulin is highly stable *in vitro*.

[0302] Next, *in vivo* stability of protein M was examined after passive transfer of 0.3 µl of AAV8 neutralizing serum. It was found that if protein M was administered and waited 3 h instead of 5 minutes, then the antibody blocking function of protein M is reduced by ~70% from $3.5x10^5$ to $1x10^5$ photons/second/cm^2/spontaneous rate (**FIG. 13**). This result indicates the NAb blocking effect of protein M is short lived *in vivo*.

[0303] Gene therapy with AAV vectors has been successful in clinical trials. However, the high prevalence of AAV neutralizing antibodies in human population prevents more patients from benefitting from this effective gene delivery. In this study, it was found that the protein M derived from mycoplasma is able to interact with NAbs and enhance AAV transduction. The minimum dose of protein M needed to block NAb activity was 2 fold more molecules than immunoglobulin. Direct interaction of protein M with AAV virions also increased AAV transduction. Neutralizing antibody assay showed that approximate 100 fold of NAb activity

was decreased when AAV immunized mouse sera were incubated with protein M *in vitro*, and over 1000 fold protection was observed in mice with adoptive transfer of NAb positive sera when protein M was applied. Although the complex of protein M with immunoglobulin was stable *in vitro* over time, the protein M gradually loses its protect function from the neutralization of immunoglobulins *in vivo*.

[0304] To overcome AAV NAbs, a number of strategies have been exploited in the laboratory. One approach is to mask the AAV surface for blocking Nab recognition using a polymer or exosome for coating. While promising, this approach may change the AAV transduction profile. A second approach is to use error-prone PCR or DNA shuffling to generate a library of AAV capsid variants and select NAb escape mutants in the presence of NAbs *in vitro* and *in vivo*. This approach has yielded novel capsids; however, it bears the potential limitation of generating capsids with unknown transduction efficiency in human since these mutants are isolated from and tested in animal tissues and the data from animal studies do not always translate into human as well as no authentic systems are available to predict AAV transduction in human tissues. A third approach has been to use alternative serotypes of AAV that show low or absent NAb cross-reactivity. While this popular strategy is logical and successful in animal models, concerns remain about the existence of cross-reactivity in most humans that may not be predicted in the animals. A final laboratory approach is to rationally engineer the NAb binding domains on the AAV capsid surface to eliminate the NAb binding sites. This strategy requires information about monoclonal antibody epitopes and the structure of AAV virion, and is inherently limited due to the fact that the NAbs from human sera are polyclonal and it is impossible to obtain mAbs from humans that represent all generated NAbs. Several clinical related approaches have also been studied: one example is to perform plasmapheresis prior to vector delivery. However, due to the relative inefficiency of each round of apheresis and the fact that even low titers of NAbs (<1:5) can abrogate AAV transduction, this strategy is only suitable for patients with lower starting titers of AAV NAbs and requires multiple sessions of apheresis. Similarly, the use of anti-CD20 antibody (Rituximab) can achieve B cell depletion, but it takes a long time (about 6-9 months) and is only effective in reducing AAV NAb in a minority of subjects. A final clinical approach is to use excessive empty AAV capsids as decoys for NAbs. The concern remains that the addition of empty particles increases the AAV capsid load which potentially increases capsid specific cytotoxic immune response mediated the elimination of AAV transduced cells and perhaps competes with full AAV particles for effective transduction. Additionally, empty capsids may induce a greater liver inflammation than full AAV vectors. Compared to the low efficiency of NAb

protection or complications in the above approaches, in this study, a strategy with greater potential to block neutralizing antibody activity was demonstrated using immunoglobulin binding protein M. The NAb evasion ability was achieved for 100 fold *in vitro* and over 1000 fold *in vivo* when protein M was used.

[0305] Protein M functions as a universal antibody binding protein which blocks mammalian IgG, IgM, and IgA antibody classes by universally binding to conserved regions on the antibody light and heavy chains, causing structural interference with the antigen recognizing or CDR regions. Protein M binds antibodies and prevents antigen-antibody union but does not disrupt previously formed antigen-antibody complexes. The interaction of protein M with antibodies has been validated by western blot, ELISA, x-ray crystallography, electron microscopy, and biolayer interferometry. Since protein M can be used independently of the vector, it could be incorporated into a treatment regimen that includes previously FDA approved gene therapies. This is an advantage over capsid based immune evasion, since each individual capsid must go through its own clinical trial for each disease target. Based on the properties of protein M, this protein can be not only applied for any viral vector mediated gene delivery, but also for transient protein therapy such as CRISPR/cas9 in case to avoid humoral immune response mediated clearance. Also, protein M has potential to treat autoimmune disorders resulted from autoantibodies.

[0306] In the present study, it was demonstrated that at least 2 or more molecules of protein M are required to block one molecule of immunoglobulin, which may need high amount of protein M to interact with all immunoglobulin in blood for NAb evasion after systemic administration. Although high dose of recombinant proteins such as human serum albumin and immunoglobulin has been used in clinical trials, the concern from potential complications of high dose of protein M needs to be addressed in big animals before clinical trials. It may not be a major issue if protein M is locally used for AAV administration.

[0307] The *in vitro* study showed that the complex of protein M/immunoglobulin was stable for a relatively long time, while the *in vivo* experiment manifested a gradual loss of protein M function when AAV vectors were administered much later after protein M application. It is important to figure out the dynamics and kinetics of protein M/immunoglobulin complexes in blood; this information will provide valuable information about the window for AAV injection after administration of protein M.

[0308] The other concern is immunogenicity of protein M for repeat administration. Since protein M is a foreign protein, it will elicit a humoral immune response to produce antibodies. Protein M executes its protection function by binding all immunoglobulins and the amount of

specific Ig to protein M only accounts for a very small portion of all Igs, therefore, when a high dose of protein M is used for the purpose of blocking AAV NAbs, the amount of protein M specific Igs should only neutralize a very small amount of protein M and then could not influence the function of administered protein M to protect AAV from AAV NAbs. Protein M could bind to B cell surface and has the potential to stimulate B cell proliferation. Previous studies have demonstrated that intact protein M is able to induce B cell proliferation, however, truncated protein M loses this function. Long-term follow up should be performed *in vivo* after protein M administration.

[0309] In conclusion, the present results demonstrate that protein M prevents immunoglobulins from neutralizing AAV when protein M is present at a molecular ratio equal to or greater than 2 molecules to 1 IgG molecule. Protection of the AAV vector is dependent on protein M interacting with immunoglobulin prior to immunoglobulin neutralization of AAV. Protein M can protect AAV from neutralization spanning a 1,000-fold range of NAb titer *in vivo*. This study has provided the important insight to use protein M for protection of AAV vector virions from NAbs activity in future AAV clinical trials in patients with NAbs or for re-administration.

Example 8: Engineered protein M Variants with Enhanced Properties

[0310] The above examples describe the use of mycoplasma protein M as an antibody blocking protein in order to escape neutralizing antibodies against gene therapy vectors. While naturally occurring protein M homologs from different mycoplasma species bind most mammalian antibody classes with nanomolar affinity (Grover *et al.*, Science 343:656 (2014)) many features of the naturally occurring protein are unsuitable for use as a therapeutic. Native protein M is not soluble, but is membrane bound by an N-terminal transmembrane domain that anchors it in the bacterial plasma membrane. Additionally, the native protein has a disordered C-terminus that may behave unpredictably as a drug-like molecule. Investigations were made using a truncated version of the native protein M (protein M TD from Grover *et al.*, Science 343:656 (2014)) lacking the N-terminal transmembrane domain and the disordered C-terminal segment as a therapeutic antibody-blocking molecule, but a key discovery was made that this protein lacks structural stability when incubated at body temperature (37 °C).

[0311] Exposing the truncated protein M (SEQ ID NO:3) to 37 °C in a simple *in vitro* buffer suspension caused visible precipitation and aggregation to occur within a short period of time (15 min to 1 h). Upon analysis using circular dichroism, it was discovered that heating

the protein to 37 °C was associated with a distinct protein unfolding event within 1 h and instantaneous melting of the protein occurred at 41.2 °C, while no unfolding event occurred at 20 °C incubation lasting over 2 h (**FIG. 14**). Unfolding of the protein also correlated with a measured decrease of the soluble fraction in solution assessed by western blot. To overcome therapeutic limitations of using an unstable protein, a rational design approach was used to generate mutant analogs of truncated protein M, including mutant homologs from *M. genitalium* (MG281) and *M. pneumoniae* (MPN400). This approach used crystal structures and amino acid sequences of protein M (PDB ID: 4NZR and 4NZT) as input into the protein modeling and engineering software called Rosetta. Free energy calculations and 3D modeling were used to predict which amino acid sequence changes would result in stabilization of the tertiary structure, outputting a list of over 850+ amino acid substitutions. Through DNA synthesis of individual mutants and combined multiple mutants, a rationally designed library of variants were used to produce mutant proteins. **Table 4** lists 885 computationally identified point mutations that scored better than the wild-type protein for thermostability. **Table 5** lists 165 computationally identified point mutations that scored substantially better (greater than -3.0 delta score) than the wild-type protein for thermostability.

[0312] Different protein M mutants were validated using differential scanning fluoroscopy to calculate protein melting temperature (**FIGS. 15, 16**). Mutants were then subjected to temperature challenge at 37 °C for varying periods of time to measure solubility (**FIGS. 17A-17C**) and the ability to prevent AAV neutralization by pooled human intravenous IgG (IVIG) in an *in vitro* neutralization assay (**18A-18C**). In addition to testing analogs of protein M derived from *M. genitalium*, analogs derived from *M. pneumonia* were also produced. Some mutants were then tested by Bio-Layer Interferometry (BLI) to determine affinity of the mutant protein to mouse IgG, and demonstrate the ability of mutants to alter affinity of the mutants for immunoglobulin substrates (**FIGS. 21, 22**). Mutants also showed increased stability as evidenced by stability over a broader pH range than wild-type protein M (**FIG. 26**). MG 29, one of the lead analogs with increased stability at 37°C and maintenance of nano-molar affinity for IgG, was used to test blockade of AAV neutralizing antibodies *in vivo* after direct immunization of the mouse against the AAV capsid. Results from intramuscular injection in one leg with AAV formulated with phosphate buffered saline, and the other leg with AAV formulated with MG 29 demonstrated that MG 29 blocks AAV neutralizing antibodies in AAV immunized mice to enable effective gene delivery and redosing of AAV (**FIGS. 19, 20**).

[0313] A second round of rational engineering strategies using Rosetta software included alteration of mutant protein affinity using an averaged model of many human immunoglobulin structures to predict modifications of the binding site of mutant protein M analogs to enhance or diminish affinity and binding. Furthermore, a third round of rational design strategies incorporated using both Rosetta modeling and the diversity of naturally occurring protein M sequences from different mycoplasma species to create antigenically distinct analogs that do not exist in nature. These analogs can be chimeric, mosaic, or de-novo rationally altered amino acid mutants of the whole protein or individual epitopes. The same or different analogs can then be used in conjunction with AAV for multiple rounds of redosing, even in the presence of inhibitory antibodies generated against protein M analogs. This is because protein M analogs directly compete for antibody binding and blocking of antigen recognition, even recognition of itself by inhibitory antibodies. Saturating doses of protein M analogs will be in excess of the small portion of immunoglobulins raised against protein M analogs after initial immune exposure. Additionally, generation of protein M analogs with increased epitope diversity can provide an additional layer of immune evasion from protein M inhibitory antibodies. It is believed that each of the listed engineering strategies can be combined or overlayed on top of each other to create protein M analogs with multiple different properties.

[0314] Finally, DNA codon optimized versions of protein M were produced that enhanced protein product yield (**FIG. 25**). Two codon optimized versions of the parental truncated protein M sequence have been generated: one sequence optimized for both *E. coli* and H. *sapiens* codon usage, and the other optimized for *H. sapiens* codon usage only. The dual *E. coli* and *H. sapiens* codon construct was used to produce protein in *E. coli* and demonstrates a ~ 4-fold increase in protein yield compared to the parental unoptimized plasmid. The *H. sapiens* only optimized construct contains an IL-2 secretion peptide or albumin secretion peptide sequence to enable secretion of protein from mammalian cell lines for protein M analog harvest from the culture media. Protein M mutant analogs are cloned or synthesized into both types of optimized DNA sequences, and used for enhanced protein production. Protein M analogs that are stable at 37°C can be grown in human cells without susceptibility to protein unfolding after secretion. Additionally, glycosylation sites of protein M analogs are substituted to eliminate glycosylation at the binding site or add glycosylation to the external surface, such as analog MG 28 (N274D). Addition of glycosylation sites to the external surface is another mechanism to enhance immune evasion of protein M analogs and reduce protein M inhibitory antibody recognition for multiple administration of protein M analogs. Finally, DNA sequences of protein M analogs will be stably integrated into mammalian cell lines in order to produce

secreted protein that is stable at 37°C and has glycosylation sites either removed or new ones added.

Methods

[0315] **AAV virus production**: AAV vectors were produced using a standard approach with three-plasmid transfection in HEK293 cells. Briefly, the AAV transgene plasmid pTR-CBA-Luc was co-transfected with an AAV Rep/Cap helper plasmid (pXR2 or pXR8) and adenovirus helper plasmid pXX6-80. 72 h later, cell cultures were harvested and lysed by freeze thaw and ultra sonication. Clarified cell lysate was DNAse treated and ultra-centrifuged in a 15%/25%/40%/60% iodixanol step gradient and purified by anion exchange Q-column. Purified AAV vector was titered by qPCR with primers directed to amplify a segment of the packaged AAV transgene.

[0316] **Protein Production**: The plasmid pET-28b(+) which encodes the protein M analogs, truncated proteins derived from *M. genitalium* or *M. pneumonia* lacking the transmembrane domain, and carries a N-terminal His-Tag and a thrombin cleavage site. Plasmids were propagated in electro-competent DH10B cells and purified using a PureLink Maxi-Prep kit from Invitrogen. The pET-28b(+) plasmid was transiently transfected into BL21/DE3 cells using an overnight starter culture. Auto-induction media (Magic media from Invitrogen) was seeded with the starter culture and grown at 18°C for 3 days. The culture was then pelleted by centrifugation and frozen down at -80°C.

[0317] **Protein Purification**: The frozen bacterial cell culture pellet was thawed, lysed by sonication, DNAse treated, and clarified by centrifugation. Clarified bacterial lysate was dialyzed into nickel-binding buffer (20 mM imidazole, 50 mM sodium phosphate pH 7.4, 500 mM NaCl, 0.02% sodium azide) and passed through a nickel His-trap FF column using FPLC. Protein M bound by the nickel column was then eluted by the same buffer base but containing 500 mM imidazole. Protein M was then run through an S-100 size exclusion column, dialyzed into phosphate buffered saline with 2% glycerol, and quantified using spectrophotometry. Protein M identity was confirmed using SDS-PAGE protein gel electrophoresis for protein separation followed by a coomassie blue protein stain to correctly identify the 45kDa - 48kDa protein M band.

[0318] For some protein production, either of two strategies were implemented to purify the protein M variants. The first protocol (Ni-Purity) was for small-scale production for NanoDSF. Lysis was performed by mixing cell pellets with a buffer consisting of 2.5 mg/mL Lysozyme, 25% B-PER, 75% Phosphate Buffered Saline (PBS) pH 7.4, and protease inhibitors

(PMSF, Bestatin, and Pepstatin). After 30 min of mixing at room temperature, the crude lysates were centrifuged at 15,000 rcf for 20 min and the supernatant was incubated with Ni resin for 1 h at 4°C. The resin was then washed (PBS + 20 mM imidazole) and the protein was eluted with (PBS + 500 mM imidazole). The eluted protein was exchanged into PBS pH 7.4 with Zeba desalting columns before conducting the stability assay. The second protocol (SEC-Purity) was for large-scale production and removed additional contaminants/aggregates from the protein samples. The cells were lysed via sonication, purified with nickel resin (same buffers as the first protocol), and finished with size-exclusion chromatography into PBS + 2% glycerol before freezing the samples.

[0319] **Western Blot**: After protein separation on an 8% SDS-PAGE gel, the proteins were transferred onto a PVDF membrane. Immunoblotting was performed in 5% non-fat milk using anti-His primary antibody 1:1000 dilution (10 μg/ml). The secondary goat anti-human IgG antibody conjugated to horseradish peroxidase (1:10,000 dilution).

[0320] **Determination of Protein Melting Temperature and Unfolding**: Melting temperatures (Tm) were determined with nano differential scanning fluorimetry (NanoDSF), and protein unfolding was determined at different temperatures using a circular dichroism assay. Inflection points of the first derivative indicate Tm or unfolding. Proteins were purified according to protocol 1 (Ni-Purity).

[0321] **Solubility Assay**: A thermostability time-course was performed by incubating seven aliquots of each SEC-purified protein (0.4 mg/mL) at 37°C for varying amounts of time. Precipitated protein was pelleted by centrifuging the samples at 15,000 x G for 10 minutes before loading a gel for SDS-PAGE. Proteins were purified according to protocol 2 (SEC-Purity).

[0322] **Affinity Assessment by Bio-Layer Interferometry**: Bio-Layer Interferometry was performed at 37°C to assess the affinity of the Protein M constructs. Proteins were purified according to protocol 2 (SEC-Purity). A two-fold dilution series of each M construct ranging from 1000 nM to 15.6 nM was performed with ForteBio Kinetics buffer (PBS + detergent). Binding was assessed to Anti-Mouse IgG Fc Capture biosensors. Each protocol included a baseline, association, and dissociation step for 10, 5, and 5 minutes, respectively. Data was subtracted from a reference sensor run in parallel with an association step in buffer. Affinity data were calculated based on a 1:1 curve fit within the ForteBio Data Analysis v9.0 software. Reported steady-state binding constants were calculated based on the maximum response at each concentration (n=7). To minimize X2, the kinetic data only included data for the 250-15.6 nM dilution range (n=5).

[0323] Structural Modeling and Rational Protein Engineering: Optimization of analogs of protein M (PM) thermal stability was achieved using an in silico rational protein engineering approach based on existing crystal structures (4NZT and 4NZR) of PM bound to immunoglobulins. The Rosetta software package was employed to identify mutations that stabilize the PM peptide. The first protocol performed site saturation mutagenesis in silico, allowing the amino acid neighbors to repack around the mutated residue. A second protocol generated combinatorial mutations in regions that are under-packed within the crystal structure. The mutants were ranked according to the difference in score between the wild type amino acid and the substitutions. Additional visual inspection was performed to identify preferred mutants. The mutations were cloned into the pET-28b+ vector by Twist Biosciences and synthesized. Additional design strategies for alteration of binding site affinity and generation of PM analogs with distinct antigenicity were employed.

[0324] A homology model of MP WT was constructed with the Swiss-Model webserver (**FIG. 24**). Conservation scores between the MG and MP WT isoforms were calculated according to the BLOSUM90 matrix. The image was rendered in PyMol.

[0325] Cell Culture: HEK-293 cells or Huh7 cells were used for all *in vitro* AAV neutralization experiments. Cells were grown on 15 cm tissue culture plates and maintained at 37 °C in 5% CO_2 with Dulbecco's modified Eagle's medium supplemented with 10% bovine calf serum and penicillin–streptomycin.

[0326] Human IVIG for *in vitro* neutralization experiments: A stock of 10% Human IVIG (Gamunex) was purchased from Grifols Therapeutics Inc. (Research Triangle Park, NC, USA). Individual aliquots were diluted to 1 mg/mL in phosphate buffered saline and stored at −80 °C for future use. Serum was collected and pooled from 12 different mice (50% male, 50% female) after IP administration of $3x10^{10}$ viral genomes of AAV8-FVIII followed by a boost administration of the same vector 2 weeks later, and a second boost 6 weeks after the first administration. Mouse serum were aliquoted and stored at −80 °C for future use.

[0327] *In vitro* AAV Neutralization Assay: Nab analyses were performed as described previously with slight modification (Wang *et al.*, Gene Ther. 22:984 (2015)). Cells were pelleted by centrifugation and resuspended in serum free X-Vivo 10 medium, then plated in either a 48-well or 96-well plate format. Human IVIG or serum was serially diluted either two-fold or ten-fold. AAV-Luc was incubated with either human IVIG or mouse serum for 1 h at 4°C before adding AAV and incubating an additional 1 h at 4 °C. AAV+serum incubations were then mixed with cells suspended in serum free media at the time of plating. In the neutralization experiments using protein M, 3 different incubations scenarios were tested with

each step for 1 h at 4°C: neutralizing serum incubated first with protein M followed by AAV, AAV incubated first with neutralizing serum followed by protein M, and AAV incubated first with protein M followed by neutralizing serum. Cells were seeded in a 48-well plate in 200 µl media, or in a 96-well plate in 100 µl media. After transduction, cells were cultured for 24-48 h at 37°C to allow for AAV-Luciferase transgene expression. To measure Luc activity, cells were lysed with passive lysis buffer (Promega, Madison, WI, USA) and luciferase signal was measured with a Wallac1420 Victor 2 automated plate reader. Nab titers were defined as the highest dilution for which luciferase activity was 50% lower than serum-free controls.

[0328] ***In Vivo*** **AAV Redosing in AAV Immunized Mice**: C57BL/6 mice were immunized by I.P. injection of either 8E5vg or 1E9vg AAV8-GFP. Approximately 1-month later serum was collected from the mice for titering by AAV8 *in vitro* neutralization assay. A day later I.M. injection was performed in each leg containing equal doses of either 1E9vg or 2E9vg AAV8-Luciferase, where one leg was administered AAV formulated with phosphate buffered saline, and the other leg was administered AAV formulated with a protein M analog in a simple admixture just prior to injection. A total volume of 200 µl was injected per leg. Luminescent imaging was performed 2-weeks after AAV-Luc administration after I.P. administration of D-Luciferin substrate.

Example 9: Kinetics of Modified Protein M and Fusion Proteins with Protein M

[0329] Biolayer interferometry was performed on a serial dilution of protein M concentrations. **Fig. 22** shows exemplary data for MG29. Data from MG29 and other Protein M constructs are summarized in **Tables 8, 9** and **Fig. 21**.

TABLE 8

Sample	KD (nM)	kon(1/Ms)	kdis(1/s)	Full X^2	Full R^2	Max Conc. (nM)	# Points in Fit
WT*	0.80	8.38E+04	6.73E-05	1.55	1.00	50	6
21*	0.74	5.23E+04	3.85E-05	0.48	1.00	50	6
29*	0.92	8.00E+04	7.35E-05	0.80	1.00	50	6
27*	1.05	5.65E+04	5.95E-05	0.61	1.00	50	6
66*	0.69	8.01E+04	5.55E-05	0.84	1.00	50	6

TABLE 9

Sample ID	KD (nM)	kon(1/Ms)	kdis(1/s)	Full X^2	Full R^2	Max Conc. (nM)	Num Dilutions
MG64	< 0.001	2.21E+04	<1.0E-07	0.09	1.00	500	5
MG29	7.9	4.3E+04	3.4E-04	1.7	1.00	250	5

[0330] Anti-human IgG Fc capture (AHC) biosensors and anti-mouse IgG Fc capture biosensors (AMC) were purchased from ForteBio. The experiments were performed at 37 °C with 10 min association and dissociation. The results were fit with a 1:1 binding curve. An asterisk* denotes amino acid Y338. Analogs without an * are N338.

Example 10: *in vivo* Antibody Neutralization

[0331] Serum was collected and pooled from mice serially immunized with AAV8. In vitro neutralization assay determined that the anti-AAV8 neutralizing titer of the pooled serum to be roughly 1:10,000. Different quantities of the anti-AAV8 serum (determined by serum volume) were administered via IV injection to groups of AAV naive mice in a passive transfer experiment, followed 20-25 minutes later by IV injection of 2e10 viral genomes (vg) of AAV8-Luciferase reporter vector. Some groups of mice were given protein M via IV injection 5 minutes before the AAV administration. Non-invasive in vivo imaging of luciferase activity was performed 7 days later to quantify luminescence as a proxy of gene expression from the AAV8 vector, and any decrease in luminescence signal is indicative of AAV8-Luciferase neutralization by the serum. Results are shown in **Fig. 29**. Mice given 0.0003ul of serum failed to neutralize the AAV8 and resulted in nearly 100% luminescence signal as compared to naive mice without serum injected with 2e10 vg AAV8-Luciferase alone. Groups of mice given increasing quantities of neutralizing serum neutralized progressively more and more AAV8-Luciferase, where 0.001ul to 0.003ul serum neutralized approximately half of the AAV and 0.3ul serum or greater neutralized over 99% of the AAV. Groups of mice administered 3ul of serum, followed by MG88 or MG118 and then 2e10 vg AAV8-Luciferase, resulted in gene expression indicative of escape from neutralization due to suppression of neutralizing antibodies by the protein M fusion proteins. The specific doses of MG88, MG29, MG29*, and MGWT* were 6.3 mg and the dose of MG118 was 9 mg per mouse, with an average mouse weight of 21 grams.

Example 11: Thermostability of disulfide mutations of Protein M

[0332] Mutations were added to a stabilized version of protein M (Start) that included the following mutations: S150E, S196R, S198P, F237T, S232Q, A342V, N274D, A205P, and T355P (**Table 10**). Melting temperatures (Tm) were determined with NanoDSF. Two capillaries were run on the same sample (Tm1 & Tm2). ΔTm represents the difference between the average of Tm1 & Tm2 compared to the Start construct.

Table 10

Internal Number	Name	Tm1	Tm2	ΔTm	Mutations*
66	Start*	61.2°C	61.2°C	0	
48	Disulf1	64.9°C	64.9°C	3.7	S201C & A224C
87	Disulf2	64.6°C	64.5°C	3.4	Q159C & A182C
91	Disulf3	60.8°C	60.7°C	-0.5	A102C & T161C
92	Disulf4	61.0°C	61.1°C	-0.1	I86C & F101C
112	Disulf1&2	68.6°C	68.5°C	7.3	

Example 12: *In vitro* Antibody Neutralization

[0333] **Fig. 30** shows an in vitro neutralization assay where high titer anti-AAV8 serum from mice was titrated in 2-fold dilutions, and added to the well as indicated on the X-axis. Then the different Protein M analogs (MG29, MG66, MG71, and MG64) were added to the well in the molecular ratio indicated in the figure legend and incubated for 1 hour. Saline was added to the serum only well (black circles). Then AAV8-Luciferase ($2x10^{10}$ viral genomes) was added to each well and incubated 1 hr, before adding $5x10^4$ Huh7 cells in serum free media. The cells were cultured 48 hrs and then the luciferase reporter signal, which is a proxy for neutralization, was read and quantified.

[0334] This data demonstrates these analogs have neutralizing antibody blocking functionality, thereby shifting the neutralization curve, and that the MG64 Fc-Protein M fusion dimer (Fc-PM) shifts the curve greater than the Protein M monomer analogs. Based on biolayer inferometry, MG64 has sub-nanomolar affinity for immunoglobulin.

[0335] The foregoing examples are illustrative of the present invention, and are not to be construed as limiting thereof. Although the invention has been described in detail with reference to preferred embodiments, variations and modifications exist within the scope and spirit of the invention as described and defined in the following claims.

Table 4: 885 point mutations predicted to improve stability of MG WT. Delta Score refers to the score of the mutant – score of the WT residue. This list considered residues 78-468 EXCEPT for residues within 5 A of the antibody interface within PDB ID: 4NZR.

Delta Score	WT Residue	Residue Number	Mutant Residue
-1.941	N	78	A
-1.209	N	78	D
-2.37	N	78	K
-2.042	N	78	P
-0.503	N	78	R
-2.53	S	81	D
-1.123	S	81	Q
-1.913	Q	83	F
-1.083	Q	83	H
-0.893	Q	83	K
-2.313	Q	83	W
-3.922	Q	83	Y
-0.688	S	84	I
-0.532	S	84	N
-1.437	S	84	T
-0.66	E	85	G
-0.797	E	85	M
-0.601	S	89	R
-0.891	G	90	A
-1.407	G	90	D
-2.616	G	90	E
-2.329	G	90	F
-2.57	G	90	H
-2.587	G	90	K
-1.955	G	90	L
-0.961	G	90	M
-2.926	G	90	Q
-1.886	G	90	R
-1.851	G	90	S
-0.542	G	90	T
-0.794	G	90	W
-3.813	G	90	Y
-1.071	G	91	D
-0.548	G	91	S
-1.752	A	92	D
-1.759	A	92	E
-1.217	A	92	F
-3.819	A	92	G
-2.717	A	92	H
-1.365	A	92	K
-2.814	A	92	L
-1.631	A	92	M
-0.523	A	92	N
-2.818	A	92	Q
-2.113	A	92	R
-1.364	A	92	S
-1.66	A	92	T
-1.995	A	92	W
-3.614	A	92	Y
-0.571	N	93	D
-0.867	N	93	E
-3.022	F	94	T
-0.67	F	94	V
-1.789	E	96	D
-0.618	K	97	Y
-0.729	N	100	E
-0.506	N	100	G
-0.817	N	100	K
-0.705	N	100	S
-0.569	N	100	T
-2.662	F	101	W
-0.531	A	108	H
-1.192	A	108	V
-1.094	A	108	W
-0.723	N	111	H
-1.94	N	111	K
-1.372	N	111	R
-0.545	N	111	Y
-1.2	S	112	A
-3	S	112	I
-0.791	S	112	N
-1.232	S	112	Q
-2.525	S	112	T
-1.568	S	112	V
-2.517	S	112	Y
-0.871	P	113	E
-0.828	K	122	Q
-1.059	K	122	R
-0.876	T	123	F
-0.857	T	123	R
-2.035	T	123	V
-0.542	T	123	Y

-0.636	I	125	V
-1.039	S	126	A
-1.739	S	126	T
-2.338	S	126	V
-0.914	S	126	Y
-1.447	G	127	V
-0.932	L	128	I
-0.743	L	128	R
-0.66	L	128	V
-0.978	K	130	L
-0.557	K	130	R
-1.688	K	130	Y
-2.35	T	131	L
-0.832	D	133	W
-1.585	N	134	I
-1.077	N	134	K
-1.851	N	134	L
-1.405	N	134	M
-0.653	N	134	R
-0.558	N	134	W
-0.966	N	134	Y
-1.303	I	136	Y
-1.332	T	137	C
-4.319	T	137	I
-0.647	T	137	M
-2.796	T	137	V
-2.106	S	139	D
-0.656	S	139	N
-1.302	S	139	P
-0.713	K	141	I
-0.59	K	141	L
-0.687	K	141	V
-1.754	K	141	W
-3.179	A	142	I
-1.581	A	142	T
-3.178	A	142	V
-0.904	D	146	F
-1.939	D	146	I
-2.182	D	146	L
-0.705	D	146	V
-1.628	D	146	W
-1.335	D	146	Y
-5.122	H	147	F
-2.485	H	147	L
-2.909	H	147	M
-0.596	H	147	W
-3.907	H	147	Y
-2.12	V	148	F
-1.976	V	148	I
-1.402	V	148	L
-1.182	V	148	Q
-0.697	V	148	W
-1.809	V	148	Y
-1.083	A	149	L
-2.315	A	149	T
-1.928	A	149	V
-2.153	S	150	A
-3.033	S	150	D
-7.399	S	150	E
-2.785	S	150	H
-2.321	S	150	K
-1.154	S	150	L
-1.469	S	150	N
-2.975	S	150	Q
-1.326	S	150	R
-0.648	S	150	Y
-1.674	S	153	R
-1.26	L	154	I
-0.729	L	155	R
-1.276	L	155	Y
-0.943	S	156	C
-2.615	S	156	F
-4.064	S	156	I
-4.341	S	156	K
-3.463	S	156	L
-2.776	S	156	M
-0.936	S	156	N
-1.309	S	156	P
-0.846	S	156	R
-4.236	S	156	T
-3.759	S	156	V
-4.005	S	156	W
-2.623	S	156	Y
-1.007	F	164	L
-0.618	F	164	Y
-0.571	R	167	F
-2.262	R	167	W
-1.335	M	170	F
-2.097	T	175	P
-0.937	T	176	N
-1.299	N	184	E
-1.904	N	184	F

-2.794	N	184	I
-1.554	N	184	T
-3.268	N	184	V
-1.558	N	184	Y
-0.501	R	185	F
-1.262	R	185	I
-2.285	R	185	L
-2.347	R	185	M
-0.999	D	189	Q
-0.831	R	192	D
-2.382	R	192	G
-1.519	R	192	T
-1.168	R	192	V
-0.527	Q	193	K
-1.143	Q	193	R
-0.963	S	196	E
-1.34	S	196	F
-1.583	S	196	I
-1.214	S	196	K
-1.886	S	196	L
-0.669	S	196	M
-0.517	S	196	N
-3.217	S	196	Q
-3.52	S	196	R
-0.976	S	196	V
-3.279	S	196	Y
-1.692	S	198	A
-2.806	S	198	F
-1.083	S	198	H
-2.867	S	198	I
-3.325	S	198	K
-3.234	S	198	L
-2.335	S	198	M
-1.628	S	198	N
-3.686	S	198	P
-2.556	S	198	Q
-3.722	S	198	R
-0.646	S	198	T
-1.334	S	198	V
-6.127	S	198	Y
-1.375	S	201	D
-1.334	S	201	E
-1.914	S	201	F
-1.701	S	201	G
-0.869	S	201	H
-1.426	S	201	K

-1.078	S	201	N
-1.07	S	201	Q
-2.311	S	201	T
-1.826	S	201	V
-2.775	S	201	Y
-0.788	N	202	A
-2.384	N	202	F
-2.528	N	202	I
-0.825	N	202	S
-2.151	N	202	T
-1.783	N	202	V
-2.941	N	202	W
-2.296	N	202	Y
-2.388	K	204	P
-1.227	K	204	Q
-0.519	A	205	C
-2.228	A	205	I
-1.657	A	205	L
-3.12	A	205	P
-1.408	A	205	Q
-0.669	A	205	T
-0.817	A	205	V
-1.201	T	206	D
-0.502	T	206	E
-0.83	T	206	F
-1.418	T	206	I
-0.654	T	206	L
-0.73	T	206	Q
-1.067	T	206	V
-1.448	T	206	Y
-0.588	T	207	R
-0.717	S	209	E
-1.577	S	209	F
-1.686	S	209	I
-0.958	S	209	K
-1.783	S	209	L
-1.567	S	209	M
-0.791	S	209	Q
-1.454	S	209	R
-1.638	S	209	T
-1.984	S	209	V
-1.384	S	209	W
-2.308	S	209	Y
-1.891	S	211	D
-3.197	S	211	K
-0.9	S	211	N

-0.542	S	211	P
-2.202	S	211	Q
-1.875	T	215	F
-1.234	T	215	H
-1.064	T	215	Q
-1.375	T	215	W
-3.349	T	215	Y
-0.856	K	218	I
-0.888	K	218	R
-1.308	K	218	V
-0.656	T	220	F
-1.52	T	220	I
-1.372	T	220	K
-0.646	T	220	L
-0.945	T	220	R
-1.702	T	220	V
-0.666	T	220	W
-0.891	T	220	Y
-1.113	F	222	R
-2.105	A	224	E
-1.543	A	224	Q
-0.698	K	225	E
-3.025	K	225	P
-1.562	G	226	D
-0.743	S	227	T
-2.441	D	231	C
-3.049	D	231	I
-2.346	D	231	K
-0.937	D	231	L
-0.738	D	231	R
-3.258	D	231	T
-0.971	S	232	A
-1.24	S	232	C
-5.712	S	232	D
-6.694	S	232	E
-3.533	S	232	F
-3.132	S	232	H
-6.649	S	232	I
-4.075	S	232	K
-6.718	S	232	L
-4.104	S	232	M
-3.012	S	232	N
-6.497	S	232	Q
-4.977	S	232	R
-3.937	S	232	V
-1.104	S	232	W

-1.517	S	232	Y
-1.935	P	234	A
-3.303	P	234	D
-1.38	P	234	E
-1.214	P	234	F
-3.816	P	234	K
-2.003	P	234	L
-4.159	P	234	Q
-4.154	P	234	R
-2.314	P	234	S
-2.296	P	234	T
-0.924	P	234	W
-1.709	P	234	Y
-3.955	N	235	W
-0.751	H	236	A
-4.755	H	236	F
-3.111	H	236	I
-1.635	H	236	K
-3.932	H	236	M
-2.882	H	236	T
-5.392	H	236	V
-2.542	H	236	W
-3.223	H	236	Y
-1.625	F	237	C
-3.832	F	237	D
-3.074	F	237	E
-3.752	F	237	I
-4.054	F	237	K
-2.101	F	237	L
-1.774	F	237	N
-1.465	F	237	P
-1.24	F	237	Q
-4.499	F	237	R
-3.617	F	237	T
-3.417	F	237	V
-2.078	E	239	D
-4.044	E	239	F
-1.146	E	239	I
-0.577	E	239	K
-0.717	E	239	L
-1.1	E	239	R
-0.829	E	239	S
-0.933	E	239	T
-0.887	E	239	V
-1.193	E	239	W
-3.362	E	239	Y

-2.128	V	241	I
-0.684	V	241	M
-2.872	T	243	I
-3.525	T	243	V
-1.174	L	244	M
-2.811	A	245	D
-1.161	A	245	E
-1.134	A	245	F
-3.259	A	245	I
-3.917	A	245	L
-2.947	A	245	M
-5.98	A	245	Q
-2.926	A	245	R
-0.579	A	245	S
-4.056	A	245	V
-0.829	I	246	Y
-1.007	D	247	E
-1.005	D	247	F
-1.068	D	247	G
-2.402	D	247	H
-1.724	D	247	I
-0.822	D	247	L
-0.793	D	247	M
-1.467	D	247	N
-1.068	D	247	V
-1.243	D	247	W
-1.512	D	247	Y
-0.559	K	249	N
-1.533	K	249	R
-2.761	D	250	E
-0.872	D	250	F
-1.268	D	250	H
-1.039	D	250	I
-0.698	D	250	M
-1.318	D	250	N
-3.546	D	250	Q
-0.744	D	250	R
-3.286	D	250	W
-1.838	D	250	Y
-1.447	S	252	G
-2.581	A	253	K
-0.599	A	253	N
-1.189	A	253	Q
-1.419	A	253	R
-0.52	L	254	H
-4.297	K	255	C
-2.226	K	255	F
-0.65	K	255	G
-4.582	K	255	I
-6.318	K	255	L
-4.961	K	255	M
-1.004	K	255	N
-1.368	K	255	Q
-1.305	K	255	R
-1.12	K	255	T
-2.993	K	255	V
-0.79	K	255	W
-2.48	K	255	Y
-1.663	T	256	A
-4.605	T	256	D
-3.077	T	256	E
-2.088	T	256	F
-1.105	T	256	H
-1.273	T	256	I
-2.344	T	256	K
-1.261	T	256	L
-2.018	T	256	N
-1.721	T	256	Q
-1.135	T	256	R
-1.085	T	256	S
-3.685	T	256	W
-3.064	T	256	Y
-1.052	T	257	E
-0.905	T	257	F
-1.179	T	257	H
-1.169	T	257	L
-2.225	T	257	M
-1.898	T	257	V
-0.719	T	257	W
-2.493	T	257	Y
-1.846	I	258	F
-0.602	D	259	I
-2.52	D	259	L
-2.002	D	259	M
-2.419	D	259	N
-4.395	D	259	R
-0.504	D	259	T
-1.555	D	259	Y
-3.113	T	264	D
-0.619	T	264	G
-0.606	T	264	H
-1.036	T	264	I

-2.271	T	264	K
-2.231	T	264	N
-1.027	T	264	Q
-1.297	T	264	R
-1.789	T	264	V
-1.162	R	269	F
-2.384	R	269	W
-0.916	R	269	Y
-1.668	G	270	K
-1.018	G	270	N
-7.501	S	272	G
-6.59	N	274	D
-1.29	N	274	H
-1.438	N	274	K
-1.345	N	274	Q
-1.128	N	274	R
-1.046	N	274	S
-6.425	G	275	A
-6.719	G	275	C
-3.004	G	275	E
-3.439	G	275	F
-2.989	G	275	H
-5.834	G	275	L
-5.771	G	275	M
-4.083	G	275	N
-2.832	G	275	Q
-5.141	G	275	S
-6.48	S	276	D
-4.126	S	276	E
-4.296	S	276	F
-3.057	S	276	G
-1.099	S	276	H
-1.014	S	276	L
-1.983	S	276	M
-4.191	S	276	Q
-1.244	S	276	R
-4.129	S	276	T
-2.589	S	276	W
-3.747	S	276	Y
-1.295	N	279	D
-1.316	N	279	E
-1.933	N	279	F
-0.519	N	279	M
-2.816	N	279	R
-1.667	N	279	S
-2.151	N	279	W

-5.197	N	279	Y
-5.082	Q	282	D
-1.912	Q	282	E
-3.221	Q	282	G
-2.608	Q	282	H
-3.413	Q	282	N
-1.175	P	284	A
-0.563	P	284	C
-1.726	P	284	D
-0.948	P	284	R
-0.824	S	286	D
-0.7	S	286	T
-0.836	V	287	I
-1.126	V	287	L
-0.679	K	288	R
-1.935	S	291	T
-4.25	T	297	D
-5.165	T	297	G
-2.506	T	297	H
-0.602	T	297	K
-0.653	T	297	L
-3.168	T	297	N
-3.251	T	297	Q
-0.569	T	297	R
-0.607	T	297	Y
-0.622	V	299	I
-3.283	N	300	A
-0.67	N	300	D
-2.389	N	300	E
-4.801	N	300	F
-0.955	N	300	G
-2.175	N	300	H
-2.688	N	300	K
-1.342	N	300	L
-1.566	N	300	M
-4.67	N	300	Q
-4.369	N	300	R
-2.253	N	300	T
-3.152	N	300	W
-4.116	N	300	Y
-0.711	A	302	D
-1.057	A	302	K
-2.367	A	302	N
-3.445	A	302	P
-1.437	A	302	Q
-1.562	A	302	R

-1.241	A	302	S
-1.665	A	302	T
-0.918	A	302	V
-1.631	K	303	M
-1.841	Q	304	P
-1.3	I	305	L
-0.931	A	307	G
-1.57	N	308	I
-1.084	N	308	R
-1.003	N	308	Y
-0.919	V	309	I
-1.968	V	309	L
-3.222	V	310	D
-4.277	V	310	E
-1.284	V	310	G
-2.278	V	310	I
-1.206	V	310	K
-2.64	V	310	L
-1.981	V	310	M
-1.643	V	310	Q
-0.603	V	310	R
-1.476	V	310	Y
-0.969	E	311	D
-0.741	E	313	T
-0.584	T	317	L
-1.56	T	317	Q
-0.974	S	318	T
-1.689	K	319	G
-1.245	K	319	P
-0.645	K	319	Q
-0.662	K	319	S
-1.445	A	320	N
-2.732	A	320	S
-3.457	A	320	T
-1.693	S	322	D
-1.149	S	322	E
-2.31	S	322	F
-0.547	S	322	L
-1.208	S	322	Q
-1.229	S	322	R
-1.363	S	322	W
-2.665	S	322	Y
-1.806	N	326	C
-1.348	N	326	E
-6.807	N	326	I
-3.008	N	326	L
-4.117	N	326	M
-0.912	N	326	Q
-3.518	N	326	T
-6.143	N	326	V
-1.18	N	326	W
-1.944	P	327	I
-0.929	P	327	L
-1.154	V	329	I
-1.555	G	331	C
-7.293	G	331	D
-1.759	G	331	E
-2.826	G	331	H
-0.631	G	331	I
-1.148	G	331	K
-2.247	G	331	M
-5.615	G	331	N
-3.757	G	331	Q
-1.709	G	331	R
-5.193	G	331	S
-6.058	G	331	T
-2.025	G	331	V
-1.709	S	332	D
-4.232	S	332	G
-1.172	K	333	D
-0.711	K	333	F
-1.439	K	333	H
-1.286	K	333	I
-0.779	K	333	M
-0.688	K	333	N
-0.984	K	333	Q
-0.517	K	333	R
-0.911	K	333	V
-2.311	N	335	F
-0.994	N	335	H
-1.861	N	335	I
-0.569	N	335	K
-1.288	N	335	Q
-1.41	N	335	R
-1.375	N	335	V
-2.167	N	335	W
-4.264	N	335	Y
-1.842	I	337	L
-2.65	A	342	F
-2.784	A	342	I
-3.644	A	342	L
-4.974	A	342	V

-2.055	S	343	F
-1.081	S	343	H
-2.498	S	343	I
-0.647	S	343	L
-1.148	S	343	M
-2.983	S	343	T
-5.103	S	343	V
-3.4	T	347	L
-0.929	T	347	Y
-2.024	H	348	F
-1.537	H	348	I
-3.436	H	348	L
-0.527	H	348	T
-0.875	H	348	W
-2.087	H	348	Y
-0.64	L	351	W
-2.674	L	351	Y
-0.9	V	354	I
-0.894	V	354	W
-2.123	T	355	A
-6.547	T	355	D
-2.908	T	355	E
-0.505	T	355	F
-6.168	T	355	G
-1.467	T	355	H
-1.937	T	355	I
-2.431	T	355	K
-1.713	T	355	L
-1.509	T	355	M
-2.926	T	355	N
-4.599	T	355	P
-2.548	T	355	Q
-1.312	T	355	R
-1.839	T	355	S
-1.912	T	355	V
-0.851	T	355	W
-3.208	T	355	Y
-1.345	Q	357	A
-2.15	Q	357	D
-1.018	Q	357	E
-2.172	Q	357	F
-0.616	Q	357	K
-0.946	Q	357	L
-0.974	Q	357	M
-2.632	Q	357	N
-3.698	Q	357	P
-1.047	Q	357	R
-3.433	Q	357	S
-1.903	Q	357	T
-2.693	Q	357	W
-3.419	Q	357	Y
-1.398	N	358	A
-0.821	N	358	T
-0.616	N	358	V
-1.062	N	358	Y
-1.438	S	359	D
-1.059	S	359	E
-1.378	S	359	K
-2.104	S	359	R
-1.28	D	360	N
-2.902	N	361	D
-3.808	N	361	G
-0.729	N	361	Q
-3.46	N	361	S
-0.856	S	362	T
-0.513	A	363	F
-0.78	A	363	N
-0.774	A	363	R
-0.558	A	363	T
-0.668	A	363	Y
-1.701	N	367	D
-2.021	N	367	E
-2.692	N	367	R
-1.089	N	367	T
-1.429	L	369	F
-1.334	L	369	Y
-0.878	K	370	Q
-1.132	Q	371	E
-4.072	Q	371	G
-1.78	Q	371	K
-0.776	Q	371	N
-1.402	Q	371	S
-1.278	Q	371	T
-1.584	A	372	F
-1.167	A	372	M
-0.734	A	372	Q
-1.863	A	372	Y
-1.946	V	373	F
-1.549	V	373	I
-0.698	V	373	L
-2.254	V	373	W
-2.226	V	373	Y

-2.604	G	374	A
-0.584	G	374	C
-1.9	G	374	D
-2.244	G	374	E
-1.248	G	374	F
-2.467	G	374	H
-2.244	G	374	I
-2.238	G	374	K
-2.56	G	374	L
-2.589	G	374	M
-1.682	G	374	N
-2.086	G	374	Q
-1.912	G	374	R
-2.461	G	374	S
-1.343	G	374	T
-1.743	G	374	V
-1.968	G	374	W
-3.292	G	374	Y
-2.883	D	375	E
-2.01	D	375	F
-2.704	D	375	Y
-1.033	N	378	D
-2.421	N	378	F
-0.527	N	378	H
-4.306	N	378	I
-2.095	N	378	L
-2.91	N	378	V
-2.298	N	378	W
-5.452	N	378	Y
-1.64	Q	385	I
-1.14	Q	385	L
-6.284	Q	385	Y
-1.657	L	399	Y
-2.025	V	400	I
-1.497	V	400	L
-0.776	V	400	M
-4.382	K	401	G
-0.99	N	402	D
-0.666	N	402	H
-3.648	N	402	I
-0.629	N	402	K
-3.676	N	402	L
-1.291	N	402	M
-4.123	N	402	Q
-1.9	N	402	R
-0.632	N	402	T
-2.535	N	402	V
-2.104	N	402	Y
-1.381	T	405	D
-1.16	T	405	E
-1.456	T	405	G
-0.661	T	405	N
-1.568	T	405	P
-0.659	T	405	S
-1.621	N	406	D
-1.036	K	407	Q
-0.624	D	408	E
-1.367	S	409	A
-1.017	S	409	D
-1.012	S	409	E
-2.884	S	409	F
-2.887	S	409	G
-3.223	S	409	H
-2.537	S	409	I
-2.811	S	409	K
-1.925	S	409	L
-2.672	S	409	N
-0.58	S	409	Q
-2.919	S	409	R
-2.456	S	409	V
-1.9	S	409	W
-3.769	S	409	Y
-0.703	D	411	I
-0.601	D	411	N
-1.019	D	411	W
-2.19	D	411	Y
-3.008	L	413	I
-0.851	L	413	R
-0.557	L	413	T
-2.019	V	414	F
-1.445	V	414	W
-2.099	V	414	Y
-1.502	S	417	A
-1.726	S	417	L
-1.482	S	417	M
-1.798	S	417	T
-2.722	S	417	V
-2.372	S	417	W
-1.977	S	417	Y
-1.184	L	418	F
-0.84	L	418	Y
-0.759	K	419	E

-1.798	K	419	I
-2.514	K	419	L
-1.942	K	419	M
-1.308	K	419	Q
-3.11	H	424	F
-1.633	H	424	L
-1.004	H	424	M
-3.125	H	424	Y
-0.972	A	428	C
-1.093	A	428	I
-1.48	A	428	L
-2.906	A	428	T
-2.715	A	428	V
-2.242	N	434	A
-1.994	N	434	F
-1.556	N	434	G
-1.092	N	434	I
-0.835	N	434	L
-1.969	N	434	Q
-2.092	N	434	R
-1.518	N	434	T
-0.503	N	434	V
-0.931	T	435	I
-1.41	T	435	V
-1.764	Y	443	E
-2.209	Y	450	W
-0.751	S	459	K
-1.234	S	459	L
-1.791	S	459	M
-2.107	S	459	P
-0.934	E	460	D
-3.904	E	460	G
-1.489	E	460	K
-1.046	E	460	Q
-1.919	E	460	R
-0.581	N	463	A
-2.529	N	463	D
-3.986	N	463	E
-4.058	N	463	K
-0.527	N	463	L
-2.682	N	463	Q
-2.561	N	463	R
-0.51	N	463	T
-1.797	N	463	V
-3.545	E	464	A
-3.37	E	464	D
-2.285	E	464	H
-2.454	E	464	I
-5.122	E	464	K
-2.639	E	464	L
-3.909	E	464	M
-2.144	E	464	N
-2.078	E	464	Q
-3.742	E	464	R
-2.292	E	464	S
-2.385	E	464	T
-1.377	E	464	W
-0.612	E	464	Y
-2.49	I	465	L
-1.611	I	465	Y
-1.788	R	468	G
-0.909	R	468	K
-1.169	R	468	L
-4.089	R	468	Q
-0.664	R	468	S
-1.506	R	468	T
-1.095	R	468	W

Table 5: 165 point mutations predicted to improve stability of MG WT. Delta Score refers to the score of the mutant – score of the WT residue. This list considered residues 78-468 EXCEPT for residues within 5 A of the antibody interface within PDB ID: 4NZR.

Delta Score	WT Residue	Residue Number	Mutant Residue
-3.9	Q	83	Y
-3.8	G	90	Y
-3.8	A	92	G
-3.6	A	92	Y
-3.0	F	94	T
-4.3	T	137	I
-3.2	A	142	I
-3.2	A	142	V
-5.1	H	147	F
-3.9	H	147	Y
-3.0	S	150	D
-7.4	S	150	E
-4.1	S	156	I
-4.3	S	156	K
-3.5	S	156	L
-4.2	S	156	T
-3.8	S	156	V
-4.0	S	156	W
-3.3	N	184	V
-3.2	S	196	Q
-3.5	S	196	R
-3.3	S	196	Y
-3.3	S	198	K
-3.2	S	198	L
-3.7	S	198	P
-3.7	S	198	R
-6.1	S	198	Y
-3.1	A	205	P
-3.2	S	211	K
-3.3	T	215	Y
-3.0	K	225	P
-3.0	D	231	I
-3.3	D	231	T
-5.7	S	232	D
-6.7	S	232	E
-3.5	S	232	F
-3.1	S	232	H
-6.6	S	232	I
-4.1	S	232	K
-6.7	S	232	L
-4.1	S	232	M
-3.0	S	232	N
-6.5	S	232	Q
-5.0	S	232	R
-3.9	S	232	V
-3.3	P	234	D
-3.8	P	234	K
-4.2	P	234	Q
-4.2	P	234	R
-4.0	N	235	W
-4.8	H	236	F
-3.1	H	236	I
-3.9	H	236	M
-5.4	H	236	V
-3.2	H	236	Y
-3.8	F	237	D
-3.1	F	237	E
-3.8	F	237	I
-4.1	F	237	K
-4.5	F	237	R
-3.6	F	237	T
-3.4	F	237	V
-4.0	E	239	F
-3.4	E	239	Y
-3.5	T	243	V
-3.3	A	245	I
-3.9	A	245	L
-6.0	A	245	Q
-4.1	A	245	V
-3.5	D	250	Q
-3.3	D	250	W
-4.3	K	255	C
-4.6	K	255	I
-6.3	K	255	L
-5.0	K	255	M
-4.6	T	256	D
-3.1	T	256	E
-3.7	T	256	W
-3.1	T	256	Y
-4.4	D	259	R
-3.1	T	264	D
-7.5	S	272	G
-6.6	N	274	D

-6.4	G	275	A
-6.7	G	275	C
-3.0	G	275	E
-3.4	G	275	F
-5.8	G	275	L
-5.8	G	275	M
-4.1	G	275	N
-5.1	G	275	S
-6.5	S	276	D
-4.1	S	276	E
-4.3	S	276	F
-3.1	S	276	G
-4.2	S	276	Q
-4.1	S	276	T
-3.7	S	276	Y
-5.2	N	279	Y
-5.1	Q	282	D
-3.2	Q	282	G
-3.4	Q	282	N
-4.3	T	297	D
-5.2	T	297	G
-3.2	T	297	N
-3.3	T	297	Q
-3.3	N	300	A
-4.8	N	300	F
-4.7	N	300	Q
-4.4	N	300	R
-3.2	N	300	W
-4.1	N	300	Y
-3.4	A	302	P
-3.2	V	310	D
-4.3	V	310	E
-3.5	A	320	T
-6.8	N	326	I
-3.0	N	326	L
-4.1	N	326	M
-3.5	N	326	T
-6.1	N	326	V
-7.3	G	331	D
-5.6	G	331	N
-3.8	G	331	Q
-5.2	G	331	S
-6.1	G	331	T
-4.2	S	332	G
-4.3	N	335	Y
-3.6	A	342	L
-5.0	A	342	V
-5.1	S	343	V
-3.4	T	347	L
-3.4	H	348	L
-6.5	T	355	D
-6.2	T	355	G
-4.6	T	355	P
-3.2	T	355	Y
-3.7	Q	357	P
-3.4	Q	357	S
-3.4	Q	357	Y
-3.8	N	361	G
-3.5	N	361	S
-4.1	Q	371	G
-3.3	G	374	Y
-4.3	N	378	I
-5.5	N	378	Y
-6.3	Q	385	Y
-4.4	K	401	G
-3.6	N	402	I
-3.7	N	402	L
-4.1	N	402	Q
-3.2	S	409	H
-3.8	S	409	Y
-3.0	L	413	I
-3.1	H	424	F
-3.1	H	424	Y
-3.9	E	460	G
-4.0	N	463	E
-4.1	N	463	K
-3.5	E	464	A
-3.4	E	464	D
-5.1	E	464	K
-3.9	E	464	M
-3.7	E	464	R
-4.1	R	468	Q

Table 6: 128 point mutations that are predicted to improve affinity of MG WT to antibodies. A Delta Score refers to the score of the mutant – score of the WT residue. This list considered 70 residues that were within 5 Å of the antibody interface within PDB ID: 4NZR.

Delta Score	WT Residue	Residue Number	Mutant Residue
-1.6	R	95	E
-0.6	A	102	I
-0.7	A	102	L
-1.3	N	103	A
-0.9	N	103	C
-1.5	N	103	D
-2.5	N	103	E
-0.8	N	103	F
-0.7	N	103	H
-3.5	N	103	I
-2.6	N	103	K
-0.8	N	103	L
-1.5	N	103	M
-1.8	N	103	Q
-2.5	N	103	R
-1.0	N	103	S
-2.3	N	103	T
-2.1	N	103	V
-1.3	N	103	Y
-0.8	S	106	A
-0.9	E	107	K
-0.7	E	107	L
-0.7	K	114	F
-0.7	K	114	L
-1.1	K	114	M
-2.1	K	114	T
-1.6	L	116	D
-1.4	L	116	E
-2.4	L	116	K
-3.9	L	116	R
-1.0	L	116	W
-1.3	S	160	A
-1.5	S	160	P
-0.9	T	161	I
-0.9	T	161	L
-2.3	T	161	P
-0.6	E	162	L
-0.5	Y	163	F
-1.0	M	181	F
-0.8	M	181	I
-1.8	M	181	W
-0.6	G	186	A
-0.5	G	186	C
-0.5	G	186	D
-1.6	G	186	F
-2.3	G	186	H
-0.8	G	186	K
-0.5	G	186	L
-0.6	G	186	Q
-2.4	G	186	R
-3.0	G	186	S
-3.0	G	186	T
-1.8	N	321	H
-0.8	N	321	K
-1.9	R	381	F
-1.6	R	381	W
-2.0	R	381	Y
-0.7	R	384	L
-0.5	Y	389	A
-2.3	Y	389	D
-2.5	Y	389	E
-1.7	Y	389	K
-1.1	Y	389	L
-2.4	Y	389	P
-1.2	Y	389	Q
-0.8	Y	389	S
-0.8	F	390	Y
-2.9	A	391	I
-0.8	A	391	T
-2.8	A	391	V
-1.7	D	396	A
-0.9	K	397	I
-0.8	K	397	V
-1.6	E	426	I
-1.0	E	426	L
-0.9	Y	429	E
-0.9	Y	436	L
-1.5	R	438	F
-1.6	R	438	I
-0.6	R	438	Q
-0.7	R	438	V
-3.0	V	439	F

-1.2	V	439	L
-1.4	V	439	Y
-1.1	E	441	Q
-1.0	N	442	D
-3.5	N	442	E
-4.0	N	442	F
-0.6	N	442	H
-1.7	N	442	I
-1.2	N	442	L
-1.2	N	442	M
-1.9	N	442	V
-1.6	N	442	W
-4.4	N	442	Y
-1.0	A	447	L
-0.5	A	447	N
-0.8	S	448	Y
-0.7	I	449	K
-2.5	I	449	R
-0.7	N	452	A
-3.5	N	452	F
-1.1	N	452	K
-0.6	N	452	Q
-1.1	E	453	K
-1.3	E	453	L
-2.0	E	453	Q
-1.7	E	453	R
-0.6	A	455	M
-2.0	S	456	A
-3.7	S	456	F
-4.7	S	456	K
-0.6	S	456	L
-1.7	S	456	M
-1.0	S	456	N
-3.2	S	456	R
-1.3	S	456	Y
-0.8	Q	462	A
-0.6	Q	462	F
-1.4	Q	462	K
-1.8	Q	462	L
-2.7	Q	462	R
-1.1	Q	462	Y
-0.5	L	466	D
-1.2	L	466	E
-1.5	L	466	K
-1.3	L	466	R
-0.7	L	466	Y

Table 7: 17 point mutations that are predicted to improve affinity of MG WT to antibodies. A delta score refers to the score of the mutant – score of the wt residue. This list considered 70 residues that were within 5 Å of the antibody interface within PDB ID: 4NZR.

Delta Score	WT Residue	Residue Number	Mutant Residue
-0.7	N	103	H
-2.3	G	186	H
-1.8	N	321	H
-0.6	N	442	H
-1.5	N	103	D
-1.6	L	116	D
-0.5	G	186	D
-2.3	Y	389	D
-1.0	N	442	D
-0.5	L	466	D
-1.6	R	95	E
-2.5	N	103	E
-1.4	L	116	E
-2.5	Y	389	E
-0.9	Y	429	E
-3.5	N	442	E
-1.2	L	466	E

SEQUENCES

SEQ ID NO:1: Wild-type *M. genitalium* protein M

```
Met Gln Phe Lys Lys His Lys Asn Ser Val Lys Phe Lys Arg Lys Leu
1               5                  10                  15

Phe Trp Thr Ile Gly Val Leu Gly Ala Gly Ala Leu Thr Thr Phe Ser
            20                  25                  30

Ala Val Met Ile Thr Asn Leu Val Asn Gln Ser Gly Tyr Ala Leu Val
        35                  40                  45

Ala Ser Gly Arg Ser Gly Asn Leu Gly Phe Lys Leu Phe Ser Thr Gln
    50                  55                  60

Ser Pro Ser Ala Glu Val Lys Leu Lys Ser Leu Ser Leu Asn Asp Gly
65                  70                  75                  80

Ser Tyr Gln Ser Glu Ile Asp Leu Ser Gly Gly Ala Asn Phe Arg Glu
                85                  90                  95

Lys Phe Arg Asn Phe Ala Asn Glu Leu Ser Glu Ala Ile Thr Asn Ser
            100                 105                 110

Pro Lys Gly Leu Asp Arg Pro Val Pro Lys Thr Glu Ile Ser Gly Leu
        115                 120                 125

Ile Lys Thr Gly Asp Asn Phe Ile Thr Pro Ser Phe Lys Ala Gly Tyr
    130                 135                 140

Tyr Asp His Val Ala Ser Asp Gly Ser Leu Leu Ser Tyr Tyr Gln Ser
145                 150                 155                 160

Thr Glu Tyr Phe Asn Asn Arg Val Leu Met Pro Ile Leu Gln Thr Thr
                165                 170                 175

Asn Gly Thr Leu Met Ala Asn Asn Arg Gly Tyr Asp Asp Val Phe Arg
            180                 185                 190

Gln Val Pro Ser Phe Ser Gly Trp Ser Asn Thr Lys Ala Thr Thr Val
        195                 200                 205

Ser Thr Ser Asn Asn Leu Thr Tyr Asp Lys Trp Thr Tyr Phe Ala Ala
    210                 215                 220

Lys Gly Ser Pro Leu Tyr Asp Ser Tyr Pro Asn His Phe Phe Glu Asp
225                 230                 235                 240

Val Lys Thr Leu Ala Ile Asp Ala Lys Asp Ile Ser Ala Leu Lys Thr
                245                 250                 255

Thr Ile Asp Ser Glu Lys Pro Thr Tyr Leu Ile Ile Arg Gly Leu Ser
            260                 265                 270

Gly Asn Gly Ser Gln Leu Asn Glu Leu Gln Leu Pro Glu Ser Val Lys
        275                 280                 285

Lys Val Ser Leu Tyr Gly Asp Tyr Thr Gly Val Asn Val Ala Lys Gln
    290                 295                 300
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Ile Phe Ala Asn Val Val Glu Leu Glu Phe Tyr Ser Thr Ser Lys Ala
305                 310                 315                 320

Asn Ser Phe Gly Phe Asn Pro Leu Val Leu Gly Ser Lys Thr Asn Val
                325                 330                 335

Ile Tyr Asp Leu Phe Ala Ser Lys Pro Phe Thr His Ile Asp Leu Thr
            340                 345                 350

Gln Val Thr Leu Gln Asn Ser Asp Asn Ser Ala Ile Asp Ala Asn Lys
        355                 360                 365

Leu Lys Gln Ala Val Gly Asp Ile Tyr Asn Tyr Arg Arg Phe Glu Arg
    370                 375                 380

Gln Phe Gln Gly Tyr Phe Ala Gly Gly Tyr Ile Asp Lys Tyr Leu Val
385                 390                 395                 400

Lys Asn Val Asn Thr Asn Lys Asp Ser Asp Asp Asp Leu Val Tyr Arg
                405                 410                 415

Ser Leu Lys Glu Leu Asn Leu His Leu Glu Glu Ala Tyr Arg Glu Gly
            420                 425                 430

Asp Asn Thr Tyr Tyr Arg Val Asn Glu Asn Tyr Tyr Pro Gly Ala Ser
        435                 440                 445

Ile Tyr Glu Asn Glu Arg Ala Ser Arg Asp Ser Glu Phe Gln Asn Glu
    450                 455                 460

Ile Leu Lys Arg Ala Glu Gln Asn Gly Val Thr Phe Asp Glu Asn Ile
465                 470                 475                 480

Lys Arg Ile Thr Ala Ser Gly Lys Tyr Ser Val Gln Phe Gln Lys Leu
                485                 490                 495

Glu Asn Asp Thr Asp Ser Ser Leu Glu Arg Met Thr Lys Ala Val Glu
            500                 505                 510

Gly Leu Val Thr Val Ile Gly Glu Glu Lys Phe Glu Thr Val Asp Ile
        515                 520                 525

Thr Gly Val Ser Ser Asp Thr Asn Glu Val Lys Ser Leu Ala Lys Glu
    530                 535                 540

Leu Lys Thr Asn Ala Leu Gly Val Lys Leu Lys Leu
545                 550                 555
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SEQ ID NO:2: Soluble form of *M. genitalium* protein M (amino acid residues 37-556 of SEQ ID NO:1) with an N-terminal 6-His tag followed by a thrombin cleavage site

```
His His His His His His Ser Ser Gly Leu Val Pro Arg Gly Ser His
1               5                   10                  15

Met Thr Asn Leu Val Asn Gln Ser Gly Tyr Ala Leu Val Ala Ser Gly
            20                  25                  30

Arg Ser Gly Asn Leu Gly Phe Lys Leu Phe Ser Thr Gln Ser Pro Ser
        35                  40                  45
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Ala Glu Val Lys Leu Lys Ser Leu Ser Leu Asn Asp Gly Ser Tyr Gln
    50                  55                  60

Ser Glu Ile Asp Leu Ser Gly Gly Ala Asn Phe Arg Glu Lys Phe Arg
65                  70                  75                  80

Asn Phe Ala Asn Glu Leu Ser Glu Ala Ile Thr Asn Ser Pro Lys Gly
                85                  90                  95

Leu Asp Arg Pro Val Pro Lys Thr Glu Ile Ser Gly Leu Ile Lys Thr
            100                 105                 110

Gly Asp Asn Phe Ile Thr Pro Ser Phe Lys Ala Gly Tyr Tyr Asp His
        115                 120                 125

Val Ala Ser Asp Gly Ser Leu Leu Ser Tyr Tyr Gln Ser Thr Glu Tyr
    130                 135                 140

Phe Asn Asn Arg Val Leu Met Pro Ile Leu Gln Thr Thr Asn Gly Thr
145                 150                 155                 160

Leu Met Ala Asn Asn Arg Gly Tyr Asp Asp Val Phe Arg Gln Val Pro
                165                 170                 175

Ser Phe Ser Gly Trp Ser Asn Thr Lys Ala Thr Thr Val Ser Thr Ser
            180                 185                 190

Asn Asn Leu Thr Tyr Asp Lys Trp Thr Tyr Phe Ala Ala Lys Gly Ser
        195                 200                 205

Pro Leu Tyr Asp Ser Tyr Pro Asn His Phe Phe Glu Asp Val Lys Thr
    210                 215                 220

Leu Ala Ile Asp Ala Lys Asp Ile Ser Ala Leu Lys Thr Thr Ile Asp
225                 230                 235                 240

Ser Glu Lys Pro Thr Tyr Leu Ile Ile Arg Gly Leu Ser Gly Asn Gly
                245                 250                 255

Ser Gln Leu Asn Glu Leu Gln Leu Pro Glu Ser Val Lys Lys Val Ser
            260                 265                 270

Leu Tyr Gly Asp Tyr Thr Gly Val Asn Val Ala Lys Gln Ile Phe Ala
        275                 280                 285

Asn Val Val Glu Leu Glu Phe Tyr Ser Thr Ser Lys Ala Asn Ser Phe
    290                 295                 300

Gly Phe Asn Pro Leu Val Leu Gly Ser Lys Thr Asn Val Ile Tyr Asp
305                 310                 315                 320

Leu Phe Ala Ser Lys Pro Phe Thr His Ile Asp Leu Thr Gln Val Thr
                325                 330                 335

Leu Gln Asn Ser Asp Asn Ser Ala Ile Asp Ala Asn Lys Leu Lys Gln
            340                 345                 350

Ala Val Gly Asp Ile Tyr Asn Tyr Arg Arg Phe Glu Arg Gln Phe Gln
        355                 360                 365
```

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Gly Tyr Phe Ala Gly Gly Tyr Ile Asp Lys Tyr Leu Val Lys Asn Val
    370                 375                 380

Asn Thr Asn Lys Asp Ser Asp Asp Asp Leu Val Tyr Arg Ser Leu Lys
385                 390                 395                 400

Glu Leu Asn Leu His Leu Glu Glu Ala Tyr Arg Glu Gly Asp Asn Thr
                405                 410                 415

Tyr Tyr Arg Val Asn Glu Asn Tyr Tyr Pro Gly Ala Ser Ile Tyr Glu
            420                 425                 430

Asn Glu Arg Ala Ser Arg Asp Ser Glu Phe Gln Asn Glu Ile Leu Lys
        435                 440                 445

Arg Ala Glu Gln Asn Gly Val Thr Phe Asp Glu Asn Ile Lys Arg Ile
    450                 455                 460

Thr Ala Ser Gly Lys Tyr Ser Val Gln Phe Gln Lys Leu Glu Asn Asp
465                 470                 475                 480

Thr Asp Ser Ser Leu Glu Arg Met Thr Lys Ala Val Glu Gly Leu Val
                485                 490                 495

Thr Val Ile Gly Glu Glu Lys Phe Glu Thr Val Asp Ile Thr Gly Val
            500                 505                 510

Ser Ser Asp Thr Asn Glu Val Lys Ser Leu Ala Lys Glu Leu Lys Thr
        515                 520                 525

Asn Ala Leu Gly Val Lys Leu Lys Leu
    530                 535
```

SEQ ID NO:3: Wild-type *M. genitalium* protein M fragment 74-479

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SLSLNDGSYQSEIDLSGGANFREKFRNFANELSEAITNSPKGLDRPVPKTEISGLIKTGDNF
ITPSFKAGYYDHVASDGSLLSYYQSTEYFNNRVLMPILQTTNGTLMANNRGYDDVFRQVPSF
SGWSNTKATTVSTSNNLTYDKWTYFAAKGSPLYDSYPNHFFEDVKTLAIDAKDISALKTTID
SEKPTYLIIRGLSGNGSQLNELQLPESVKKVSLYGDYTGVNVAKQIFANVVELEFYSTSKAN
SFGFNPLVLGSKTNVINDLFASKPFTHIDLTQVTLQNSDNSAIDANKLKQAVGDIYNYRRFE
RQFQGYFAGGYIDKYLVKNVNTNKDSDDDLVYRSLKELNLHLEEAYREGDNTYYRVNENYYP
GASIYENERASRDSEFQNEILKRAEQNGVTFDEN
```

SEQ ID NO:4: Modified *M. genitalium* protein M MG1

```
SLSLNDGSYQSEIDLSGGANFREKFRNFANELSEAITNSPKGLDRPVPKTEISGLIKTGDNF
ITPSFKAGYYDHVASDGSLLSYYQSTEYFNNRVLMPILQTTNGTLMANNRGYDDVFRQVPSF
SGWSNTKATTVSTSNNLTYDKWTYFAAKGSPLYDSYPNHTFEDVKTLAIDAKDISALKTTID
SEKPTYLIIRGLSGNGSQLNELQLPESVKKVSLYGDYTGVNVAKQIFANVVELEFYSTSKAN
SFGFNPLVLGSKTNVINDLFASKPFTHIDLTQVTLQNSDNSAIDANKLKQAVGDIYNYRRFE
RQFQGYFAGGYIDKYLVKNVNTNKDSDDDLVYRSLKELNLHLEEAYREGDNTYYRVNENYYP
GASIYENERASRDSEFQNEILKRAEQNGVTFDEN
```

SEQ ID NO:5: Modified *M. genitalium* protein M MG8

SLSLNDGSYQSEIDLSGGANFREKFRNFANELSEAITNSPKGLDRPVPKTEISGLIKTGDNF
ITPSFKAGYYDHVASDGSLLSYYQSTEYFNNRVLMPILQTTNGTLMANNRGYDDVFRQVPSF
SGWSNTKATTVSTSNNLTYDKWTYFAAKGSPLYDQYPNHFFEDVKTLAIDAKDISALKTTID
SEKPTYLIIRGLSGNGSQLNELQLPESVKKVSLYGDYTGVNVAKQIFANVVELEFYSTSKAN
SFGFNPLVLGSKTNVINDLFASKPFTHIDLTQVTLQNSDNSAIDANKLKQAVGDIYNYRRFE
RQFQGYFAGGYIDKYLVKNVNTNKDSDDDLVYRSLKELNLHLEEAYREGDNTYYRVNENYYP
GASIYENERASRDSEFQNEILKRAEQNGVTFDEN

SEQ ID NO:6: Modified *M. genitalium* protein M MG13

SLSLNDGSYQSEIDLSGGANFREKFRNFANELSEAITNSPKGLDRPVPKTEISGLIKTGDNF
ITPSFKAGYYDHVASDGSLLSYYQSTEYFNNRVLMPILQTTNGTLMANNRGYDDVFRQVPSF
SGWSNTKATTVSTSNNLTYDKWTYFAAKGSPLYDSYPNHFFEDVKTLAIDAKDISALKTTID
SEKPTYLIIRGLSGNGSQLNELDLPESVKKVSLYGDYTGVNVAKQIFANVVELEFYSTSKAN
SFGFNPLVLGSKTNVINDLFASKPFTHIDLTQVTLQNSDNSAIDANKLKQAVGDIYNYRRFE
RQFQGYFAGGYIDKYLVKNVNTNKDSDDDLVYRSLKELNLHLEEAYREGDNTYYRVNENYYP
GASIYENERASRDSEFQNEILKRAEQNGVTFDEN

SEQ ID NO:7: Modified *M. genitalium* protein M MG15

SLSLNDGSYQSEIDLSGGANFREKFRNFANELSEAITNSPKGLDRPVPKTEISGLIKTGDNF
ITPSFKAGYYDHVAEDGSLLSYYQSTEYFNNRVLMPILQTTNGTLMANNRGYDDVFRQVPRF
PGWSNTKATTVSTSNNLTYDKWTYFAAKGSPLYDSYPNHFFEDVKTLAIDAKDISALKTTID
SEKPTYLIIRGLSGNGSQLNELQLPESVKKVSLYGDYTGVNVAKQIFANVVELEFYSTSKAN
SFGFNPLVLGSKTNVINDLFASKPFTHIDLTQVTLQNSDNSAIDANKLKQAVGDIYNYRRFE
RQFQGYFAGGYIDKYLVKNVNTNKDSDDDLVYRSLKELNLHLEEAYREGDNTYYRVNENYYP
GASIYENERASRDSEFQNEILKRAEQNGVTFDEN

SEQ ID NO:8: Modified *M. genitalium* protein M MG21

SLSLNDGSYQSEIDLSGGANFREKFRNFANELSEAITNSPKGLDRPVPKTEISGLIKTGDNF
ITPSFKAGYYDHVASDGSLLSYYQSTEYFNNRVLMPILQTTNGTLMANNRGYDDVFRQVPSF
SGWSNTKATTVSTSNNLTYDKWTYFAAKGSPLYDSYPNHFFEDVKTLAIDAKDISALKTTID
SEKPTYLIIRGLSGNGSQLNELQLPESVKKVSLYGDYTGVNVAKQIFANVVELEFYSTSKAN
SFGFNPLVLGSKTNVINDLFASKPFTHIDLTQVTLQNSDNSAIDANKLKQAVGDIYNYRRFE
RQFQGYFAGGYIDKYLIKIVNTNPDVDDDIVYRSLKELNLHLEEAYREGDNIYYRVNENYYP
GASIYENERASRDSEFQNEILKRAEQNGVTFDEN

SEQ ID NO:9: Modified *M. genitalium* protein M MG22

SLSLNDGSYQSEIDLSGGANFREKFRNFANELSEAITNSPKGLDRPVPKTEISGLIKTGDNF
ITPSFKAGYYDHVASDGSLLSYYQSTEYFNNRVLMPILQTTNGTLMANNRGYDDVFRQVPSF
SGWSNTKATTVSTSNNLTYDKWTYFAAKGSPLYDSYPNHFFEDVKTLAIDAKDISALKTTID
SEKPTYLIIRGLSGNGSQLNELQLPESVKKVSLYGDYTGVNVAKQIFANVVELEFYSTSKAN
SFGFNPLVLGSKTNVINDLFASKPFTHIDLTQVTLQNSDNSAIDANKLKQAIGDIYNYRRFE
RQFQGYFAGGYIDKYLIKNVNTNKDSDDDLVYRSLKELNLHLEEAYREGDNTYYRVNENYYP
GASIYENERASRDSEFQNEILKRAEQNGVTFDEN

SEQ ID NO:10: Modified *M. genitalium* protein M MG23

SLSLNDGSYQSEIDLSGGANFREKFRNFANELSEAITNSPKGLDRPVPKTEISGLIKTGDNF
ITPSFKAGYYDHVASDGSLLSYYQSTEYFNNRVLMPILQTTNGTLMANNRGYDDVFRQVPSF
SGWSNTKATTVSTSNNLTYDKWTYFAAKGSPLYDSYPNHFFEDVKTLAIDAKDISALKTTID
SEKPTYLIIRGLSGNGSQLNELQLPESVKKVSLYGDYTGVNVAKQIFANVVELEFYSTSKAN
SFGFNPLVLGSKTNVINDLFASKPFTHIDLTQVTLQNSDNSAIDANKLKQAVGDIYNYRRFE
RQFQGYFAGGYIDKYLVKLVNTNPDVDDDIVYRSLKELNLHLEEAYREGDNTYYRVNENYYP
GASIYENERASRDSEFQNEILKRAEQNGVTFDEN

SEQ ID NO:11: Modified *M. genitalium* protein M MG24

SLSLNDGSYQSEIDLSGGANFREKFRNFANELSEAITNSPKGLDRPVPKTEISGLIKTGDNF
ITPSFKAGYYDHVASDGSLLSYYQSTEYFNNRVLMPILQTTNGTLMANNRGYDDVFRQVPSF
SGWSNTKATTVSTSNNLTYDKWTYFAAKGSPLYDSYPNHFFEDVKTLAIDAKDISALKTTID
SEKPTYLIIRGLSGNGSQLNELQLPESVKKVSLYGDYTGVNVAKQIFANVVELEFYSTSKAN
SFGFNPLVLGSKTNVINDLFVSKPFTHIDLTQVTLQNSDNSAIDANKLKQAVGDIYNYRRFE
RQFQGYFAGGYIDKYLVKNVNTNKDSDDDLVYRSLKELNLHLEEAYREGDNTYYRVNENYYP
GASIYENERASRDSEFQNEILKRAEQNGVTFDEN

SEQ ID NO:12: Modified *M. genitalium* protein M MG27

SLSLNDGSYQSEIDLSGGANFREKFRNFANELSEAITNSPKGLDRPVPKTEISGLIKTGDNF
ITPSFKAGYYDHVAEDGSLLSYYQSTEYFNNRVLMPILQTTNGTLMANNRGYDDVFRQVPRF
PGWSNTKATTVSTSNNLTYDKWTYFAAKGSPLYDQYPNHTFEDVKTLAIDAKDISALKTTID
SEKPTYLIIRGLSGNGSQLNELDLPESVKKVSLYGDYTGVNVAKQIFANVVELEFYSTSKAN
SFGFNPLVLGSKTNVINDLFVSKPFTHIDLTQVTLQNSDNSAIDANKLKQAIGDIYNYRRFE
RQFQGYFAGGYIDKYLIKIVNTNPDVDDDIVYRSLKELNLHLEEAYREGDNIYYRVNENYYP
GASIYENERASRDSEFQNEILKRAEQNGVTFDEN

SEQ ID NO:13: Modified *M. genitalium* protein M MG28

SLSLNDGSYQSEIDLSGGANFREKFRNFANELSEAITNSPKGLDRPVPKTEISGLIKTGDNF
ITPSFKAGYYDHVASDGSLLSYYQSTEYFNNRVLMPILQTTNGTLMANNRGYDDVFRQVPSF
SGWSNTKATTVSTSNNLTYDKWTYFAAKGSPLYDSYPNHFFEDVKTLAIDAKDISALKTTID
SEKPTYLIIRGLSGDGSQLNELQLPESVKKVSLYGDYTGVNVAKQIFANVVELEFYSTSKAN
SFGFNPLVLGSKTNVINDLFASKPFTHIDLTQVTLQNSDNSAIDANKLKQAVGDIYNYRRFE
RQFQGYFAGGYIDKYLVKNVNTNKDSDDDLVYRSLKELNLHLEEAYREGDNTYYRVNENYYP
GASIYENERASRDSEFQNEILKRAEQNGVTFDEN

SEQ ID NO:14: Modified *M. genitalium* protein M MG29

SLSLNDGSYQSEIDLSGGANFREKFRNFANELSEAITNSPKGLDRPVPKTEISGLIKTGDNF
ITPSFKAGYYDHVAEDGSLLSYYQSTEYFNNRVLMPILQTTNGTLMANNRGYDDVFRQVPRF
PGWSNTKATTVSTSNNLTYDKWTYFAAKGSPLYDQYPNHTFEDVKTLAIDAKDISALKTTID
SEKPTYLIIRGLSGNGSQLNELQLPESVKKVSLYGDYTGVNVAKQIFANVVELEFYSTSKAN
SFGFNPLVLGSKTNVINDLFVSKPFTHIDLTQVTLQNSDNSAIDANKLKQAVGDIYNYRRFE
RQFQGYFAGGYIDKYLVKNVNTNKDSDDDLVYRSLKELNLHLEEAYREGDNTYYRVNENYYP
GASIYENERASRDSEFQNEILKRAEQNGVTFDEN

SEQ ID NO:15: Modified *M. genitalium* protein M MG31

SLSLNDGSYQSEIDLSGGANFREKFRNFANELSEAITNSPKGLDRPVPKTEISGLIKTGDNF
ITPSFKAGYYDHVASDGSLLSYYQSTEYFNNRVLMPILQTTNGTLMANNRGYDDVFRQVPSF
SGWSNTKATTVSTSNNLTYDKWTYFAAKGSPLYDSYPNHFFEDVKTLAIDAKDISALKTTID
SEKPTYLIIRGLSGNGSQLNELQLPESVKKVSLYGDYTGVNVAKQIFANVVELEFYSTSKAN
SFGFNPLVLGSKTNVINDLFASKPFTHIDLTQVTLQNSDNSAIDANKLKQAIGDIYNYRRFE
RQFQGYFAGGYIDKYLIKIVNTNPDVDDDIVYRSLKELNLHLEEAYREGDNIYYRVNENYYP
GASIYENERASRDSEFQNEILKRAEQNGVTFDEN

SEQ ID NO:16: Modified *M. genitalium* protein M MG33

SLSLNDGSYQSEIDLSGGANFREKFRNFANELSEAITNSPKGLDRPVPKTEISGLIKTGDNF
ITPSFKAGYYDHVASDGSLLSYYQSTEYFNNRVLMPILQTTNGTLMANNRGYDDVFRQVPSF
SGWSNTKPTTVSTSNNLTYDKWTYFAAKGSPLYDSYPNHFFEDVKTLAIDAKDISALKTTID
SEKPTYLIIRGLSGNGSQLNELQLPESVKKVSLYGDYTGVNVAKQIFANVVELEFYSTSKAN
SFGFNPLVLGSKTNVINDLFASKPFTHIDLTQVTLQNSDNSAIDANKLKQAVGDIYNYRRFE
RQFQGYFAGGYIDKYLVKNVNTNKDSDDDLVYRSLKELNLHLEEAYREGDNTYYRVNENYYP
GASIYENERASRDSEFQNEILKRAEQNGVTFDEN

SEQ ID NO:17: Modified *M. genitalium* protein M MG38

SLSLNDGSYQSEIDLSGGANFREKFRNFANELSEAITNSPKGLDRPVPKTEISGLIKTGDNF
ITPSFKAGYYDHVASDGSLLSYYQSTEYFNNRVLMPILQTTNGTLMANNRGYDDVFRQVPSF
SGWSNTKATTVSTSNNLTYDKWTYFAAKGSPLYDSYPNHFFEDVKTLAIDAKDISALKTTID
SEKPTYLIIRGLSGNGSQLNELQLPESVKKVSLYGDYTGVNVAKQIFANVVELEFYSTSKAN
SFGFNPLVLGSKTNVINDLFASKPFTHIDLTQVDLQNSDNSAIDANKLKQAVGDIYNYRRFE
RQFQGYFAGGYIDKYLVKNVNTNKDSDDDLVYRSLKELNLHLEEAYREGDNTYYRVNENYYP
GASIYENERASRDSEFQNEILKRAEQNGVTFDEN

SEQ ID NO:18: Modified *M. genitalium* protein M MG40

SLSLNDGSYQSEIDLSGGANFREKFRNFANELSEAITNSPKGLDRPVPKTEISGLIKTGDNF
ITPSFKAGYYDHVASDGSLLSYYQSTEYFNNRVLMPILQTTNGTLMANNRGYDDVFRQVPSF
SGWSNTKATTVSTSNNLTYDKWTYFAAKGSPLYDSYPNHFFEDVKTLAIDAKDISALKTTID
SEKPTYLIIRGLSGNGSQLNELQLPESVKKVSLYGDYTGVNVAKQIFANVVELEFYSTSKAN
SFGFNPLVLGSKTNVINDLFASKPFTHIDLTQVPLQNSDNSAIDANKLKQAVGDIYNYRRFE
RQFQGYFAGGYIDKYLVKNVNTNKDSDDDLVYRSLKELNLHLEEAYREGDNTYYRVNENYYP
GASIYENERASRDSEFQNEILKRAEQNGVTFDEN

SEQ ID NO:19: Modified *M. genitalium* protein M MG43

SLSLNDGSYQSEIDLSGGANFREKFRNFANELSEAITNSPKGLDRPVPKTEISGLIKTGDNF
ITPSFKAGYYDHVAEDGSLLSYYQSTEYFNNRVLMPILQTTNGTLMANNRGYDDVFRQVPRF
PGWSNTKATTVSTSNNLTYDKWTYFAAKGSPLYDSYPNHFFEDVKTLAIDAKDISALKTTID
SEKPTYLIIRGLSGNGSQLNELQLPESVKKVSLYGDYTGVNVAKQIFANVVELEFYSTSKAN
SFGFNPLVLGSKTNVINDLFVSKPFTHIDLTQVTLQNSDNSAIDANKLKQAIGDIYNYRRFE
RQFQGYFAGGYIDKYLIKNVNTNKDSDDDLVYRSLKELNLHLEEAYREGDNTYYRVNENYYP
GASIYENERASRDSEFQNEILKRAEQNGVTFDEN

SEQ ID NO:20: Modified *M. genitalium* protein M MG44

SLSLNDGSYQSEIDLSGGANFREKFRNFANELSEAITNSPKGLDRPVPKTEISGLIKTGDNF
ITPSFKAGYYDHVAEDGSLLSYYQSTEYFNNRVLMPILQTTNGTLMANNRGYDDVFRQVPRF
PGWSNTKATTVSTSNNLTYDKWTYFAAKGSPLYDQYPNHTFEDVKTLAIDAKDISALKTTID
SEKPTYLIIRGLSGNGSQLNELQLPESVKKVSLYGDYTGVNVAKQIFANVVELEFYSTSKAN
SFGFNPLVLGSKTNVINDLFVSKPFTHIDLTQVTLQNSDNSAIDANKLKQAIGDIYNYRRFE
RQFQGYFAGGYIDKYLIKNVNTNKDSDDDLVYRSLKELNLHLEEAYREGDNTYYRVNENYYP
GASIYENERASRDSEFQNEILKRAEQNGVTFDEN

SEQ ID NO:21: Modified *M. genitalium* protein M MG45

SLSLNDGSYQSEIDLSGGANFREKFRNFANELSEAITNSPKGLDRPVPKTEISGLIKTGDNF
ITPSFKAGYYDHVASDGSLLSYYQSTEYFNNRVLMPILQTTNGTLMANNRGYDDVFRQVPSF
SGWCNTKATTVSTSNNLTYDKWTYFACKGSPLYDSYPNHFFEDVKTLAIDAKDISALKTTID
SEKPTYLIIRGLSGNGSQLNELQLPESVKKVSLYGDYTGVNVAKQIFANVVELEFYSTSKAN
SFGFNPLVLGSKTNVINDLFASKPFTHIDLTQVTLQNSDNSAIDANKLKQAVGDIYNYRRFE
RQFQGYFAGGYIDKYLVKNVNTNKDSDDDLVYRSLKELNLHLEEAYREGDNTYYRVNENYYP
GASIYENERASRDSEFQNEILKRAEQNGVTFDEN

SEQ ID NO:22: Modified *M. genitalium* protein M MG46

SLSLNDGSYQSEIDLSGGANFREKFRNFANELSEAITNSPKGLDRPVPKTEISGLIKTGDNF
ITPSFKAGYYDHVAEDGSLLSYYQSTEYFNNRVLMPILQTTNGTLMANNRGYDDVFRQVPRF
PGWCNTKATTVSTSNNLTYDKWTYFACKGSPLYDQYPNHTFEDVKTLAIDAKDISALKTTID
SEKPTYLIIRGLSGNGSQLNELQLPESVKKVSLYGDYTGVNVAKQIFANVVELEFYSTSKAN
SFGFNPLVLGSKTNVINDLFVSKPFTHIDLTQVTLQNSDNSAIDANKLKQAVGDIYNYRRFE
RQFQGYFAGGYIDKYLVKNVNTNKDSDDDLVYRSLKELNLHLEEAYREGDNTYYRVNENYYP
GASIYENERASRDSEFQNEILKRAEQNGVTFDEN

SEQ ID NO:23: Wild-type *M. pneumoniae* protein M

MKLNFKIKDKKTLKRLKKGGFWALGLFGAAINAFSAVLIVNEVLRLQSGETLIASGRSGNLS
FQLYSKVNQNAKSKLNSISLTDGGYRSEIDLGDGSNFREDFRNFANNLSEAITDAPKDLLRP
VPKVEVSGLIKTSSTFITPNFKAGYYDQVAADGKTLKYYQSTEYFNNRVVMPILQTTNGTLT
ANNRAYDDIFVDQGVPKFPGWFHDVDKAYYAGSNGQSEYLFKEWNYYVANGSPLYNVYPNHH
FKQIKTIAFDAPRIKQGNTDGINLNLKQRNPDYVIINGLTGDGSTLKDLELPESVKKVSIYG
DYHSINVAKQIFKNVLELEFYSTNQDNNFGFNPLVLGDHTNIIYDLFASKPFNYIDLTSLEL
KDNQDNIDASKLKRAVSDIYIRRRFERQMQGYWAGGYIDRYLVKNTNEKNVNKDNDTVYAAL
KDINLHLEETYTHGGNTMYRVNENYYPGASAYEAERATRDSEFQKEIVQRAELIGVVFEYGV
KNLRPGLKYTVKFESPQEQVALKSTDKFQPVIGSVTDMSKSVTDLIGVLRDNAEILNITNVS
KDETVVAELKEKLDRENVFQEIRT

SEQ ID NO:24: Wild-type *M. pneumoniae* protein M fragment

SISLTDGGYRSEIDLGDGSNFREDFRNFANNLSEAITDAPKDLLRPVPKVEVSGLIKTSSTF
ITPNFKAGYYDQVAADGKTLKYYQSTEYFNNRVVMPILQTTNGTLTANNRAYDDIFVDQGVP
KFPGWFHDVDKAYYAGSNGQSEYLFKEWNYYVANGSPLYNVYPNHHFKQIKTIAFDAPRIKQ
GNTDGINLNLKQRNPDYVIINGLTGDGSTLKDLELPESVKKVSIYGDYHSINVAKQIFKNVL
ELEFYSTNQDNNFGFNPLVLGDHTNIIYDLFASKPFNYIDLTSLELKDNQDNIDASKLKRAV
SDIYIRRRFERQMQGYWAGGYIDRYLVKNTNEKNVNKDNDTVYAALKDINLHLEETYTHGGN
TMYRVNENYYPGASAYEAERATRDSEFQKEIVQRAELIGVVFE

SEQ ID NO:25: Polynucleotide encoding *M. genitalium* protein M codon-optimized for both bacterial and human expression

ATGGGCAGCAGCCATCACCATCATCATCACAGCAGCGGTCTGGTGCCGCGTGGT
AGCCACATGAGCCTGAGCCTGAACGATGGTAGCTACCAGAGCGAGATCGACCTG
AGCGGCGGTGCCAACTTCCGTGAAAAATTCCGCAACTTTGCTAACGAGCTGAGC
GAAGCCATTACCAATAGCCCAAAGGGCCTGGATCGTCCAGTGCCGAAAACCGAG
ATCAGCGGCCTGATTAAGACCGGTGACAACTTTATCACCCCGAGCTTCAAGGCGG
GCTACTATGATCACGTGGCTGAGGACGGTAGCCTGCTGAGCTACTATCAGAGCAC
CGAGTACTTTAACAACCGTGTGCTGATGCCGATTCTGCAGACCACCAACGGCACC
CTGATGGCCAACAACCGTGGTTATGACGACGTGTTCCGTCAGGTGCCGCGTTTCC
CGGGCTGGAGCAACACCAAAGCGACCACCGTGAGCACCAGCAACAACCTGACCT
ACGACAAGTGGACCTATTTCGCCGCGAAAGGTAGCCCGCTGTACGATCAGTATCC
GAACCACACCTTTGAGGACGTGAAAACCCTGGCTATCGATGCCAAGGACATTAG
CGCGCTGAAAACCACCATCGATAGCGAAAAGCCGACCTACCTGATCATTCGCGG
TCTGAGCGGCAACGGTAGCCAGCTGAACGAGCTGCAGCTGCCGGAAAGCGTGAA
GAAAGTGAGCCTGTACGGCGACTATACCGGTGTGAACGTGGCCAAACAGATTTT
TGCGAACGTGGTGGAGCTGGAATTCTATAGCACCAGCAAGGCGAACAGCTTCGG
CTTTAACCCGCTGGTGCTGGGTAGCAAAACCAACGTGATCAACGACCTGTTCGTG
AGCAAGCCGTTCACCCACATTGACCTGACCCAGGTGACCCTGCAGAACAGCGAT
AACAGCGCGATCGACGCTAACAAGCTGAAACAGGCTGTGGGCGATATCTACAAC
TATCGTCGCTTCGAGCGTCAGTTTCAGGGTTACTTCGCCGGCGGTTACATCGATA
AGTATCTGGTGAAAAACGTGAACACCAACAAAGACAGCGACGATGACCTGGTGT
ACCGCAGCCTGAAGGAACTGAACCTGCACCTGGAGGAAGCTTATCGTGAGGGCG
ACAACACCTACTATCGCGTGAACGAAAACTACTATCCGGGTGCCAGCATCTACG
AGAACGAACGTGCGAGCCGCGATAGCGAGTTTCAGAACGAAATTCTGAAGCGTG
CGGAGCAGAACGGCGTGACCTTCGACGAAAACTAATAA

SEQ ID NO:26: Polynucleotide encoding *M. genitalium* protein M codon-optimized for human expression

ATGAGCCTGAGCCTGAACGATGGCAGCTACCAGAGCGAGATCGACCTGTCTGGC
GGAGCCAACTTCAGAGAGAAGTTCAGAAACTTCGCCAACGAGCTGAGCGAGGCC
ATCACAAACAGCCCCAAAGGCCTGGACAGACCCGTGCCTAAGACAGAGATCAGC
GGCCTGATCAAGACCGGCGACAACTTCATCACCCCTAGCTTCAAGGCCGGCTACT
ACGATCACGTGGCCTCTGATGGCAGCCTGCTGAGCTACTACCAGTCCACCGAGTA
CTTCAACAACCGGGTGCTGATGCCCATCCTCCAGACCACCAATGGCACCCTGATG
GCCAACAACAGAGGCTACGACGACGTGTTCAGACAGGTGCCCAGCTTTAGCGGC
TGGTCCAATACCAAGGCCACCACCGTGTCCACCAGCAACAACCTGACCTACGAC
AAGTGGACCTACTTCGCCGCCAAGGGCAGCCCTCTGTACGACAGCTACCCCAAC
CACTTCTTCGAGGACGTGAAAACCCTGGCCATCGACGCCAAGGATATCAGCGCC
CTGAAAACCACCATCGACAGCGAGAAGCCCACCTACCTGATCATCAGAGGACTG
AGCGGCAACGGCAGCCAGCTGAATGAACTCCAGCTGCCTGAGAGCGTGAAGAAG
GTGTCCCTGTACGGCGATTACACCGGCGTGAACGTGGCCAAGCAGATCTTCGCCA
ATGTGGTGGAACTGGAATTCTACAGCACCAGCAAGGCCAACAGCTTCGGCTTCA
ACCCTCTGGTGCTGGGCAGCAAGACCAACGTGATCAACGACCTGTTCGCCAGCA
AGCCCTTCACACACATCGATCTGACCCAAGTGACCCTCCAGAACAGCGACAACA
GCGCCATTGATGCCAACAAGCTGAAACAGGCCGTGGGCGACATCTACAACTACA
GAAGATTCGAGCGGCAGTTCCAGGGCTACTTCGCTGGCGGCTACATCGACAAGT
ACCTGGTCAAGAACGTGAACACCAACAAGGACAGCGACGACGACCTGGTGTACA

GAAGCCTGAAAGAGCTGAACCTGCACCTGGAAGAGGCCTACAGAGAGGGCGAC
AACACCTACTACAGAGTGAACGAGAACTACTACCCAGGCGCCAGCATCTACGAG
AACGAGAGAGCCAGCAGAGACAGCGAGTTCCAGAACGAGATCCTGAAGCGGGC
CGAGCAGAATGGCGTGACCTTCGACGAGAACTGATGA

SEQ ID NO:27: Modified *M. genitalium* protein M MG47

SLSLNDGSYQSEIDLSGGANFREKFRNFANELSEAITNSPKGLDRPVPKTEISGLIKTG
DNFITPSFKAGYYDHVAEDGSLLSYYQSTEYFNNRVLMPILQTTNGTLMANNRGYD
DVFRQVPRFPGWSNTKATTVSTSNNLTYDKWTYFAAKGSPLYDQYPNHTFEDVKTL
AIDAKDISALKTTIDSEKPTYLIIRGLSGNGSQLNELQLPESVKKVSLYGDYTGVNVA
KQIFANVVELEFYSTSKANSFGFNPLVLGSKTNVINDLFVSKPFTHIDLTQVTLQNSD
NSAIDANKLKQAVGDIYNYRRFERQFQGYEAGGYIDKYLVKNVNTNKDSDDDLVY
RSLKELNLHLEEAYREGDNTYYRVNENYKPGASIYENERASRDSEFQNEILKRAEQN
GVTFDEN

SEQ ID NO:28: Modified *M. genitalium* protein M MG48

SLSLNDGSYQSEIDLSGGANFREKFRNFANELSEAITNSPKGLDRPVPKTEISGLIKTG
DNFITPSFKAGYYDHVAEDGSLLSYYQSTEYFNNRVLMPILQTTNGTLMANNRGYD
DVFRQVPRFPGWCNTKPTTVSTSNNLTYDKWTYFACKGSPLYDQYPNHTFEDVKTL
AIDAKDISALKTTIDSEKPTYLIIRGLSGDGSQLNELQLPESVKKVSLYGDYTGVNVA
KQIFANVVELEFYSTSKANSFGFNPLVLGSKTNVINDLFVSKPFTHIDLTQVPLQNSD
NSAIDANKLKQAVGDIYNYRRFERQFQGYFAGGYIDKYLVKNVNTNKDSDDDLVY
RSLKELNLHLEEAYREGDNTYYRVNENYYPGASIYENERASRDSEFQNEILKRAEQN
GVTFDEN

SEQ ID NO:29: Modified *M. genitalium* protein M MG49

SLSLNDGSYQSEIDLSGGANFREKFRNFANELSEAITNSPKGLDRPVPKTEISGLIKTG
DNFITPSFKAGYYDHVAEDGSLLSYYQSTEYFNNRVLMPILQTTNGTLMANNRGYD
DVFRQVPRFPGWSNTKATTVSTSNNLTYDKWTYFAAKGSPLYDQYPNHTFEDVKTL
AIDAKDISALKTTIDSEKPTYLIIRGLSGNGSQLNELQLPESVKKVSLYGDYTGVNVA
KQIFANVVELEFYSTSKANSFGFNPLVLGSKTNVINDLFVSKPFTHIDLTQVTLQNSD
NSAIDANKLKQAVGDIYNYRRFERQFQGYFPGGYIDKYLVKNVNTNKDSDDDLVYR
SLKELNLHLEEAYREGDNTYYRVNENYYPGASIYENERASRDSEFQNEILKRAEQNG
VTFDEN

SEQ ID NO:30: Modified *M. pneumoniae* protein M MP29

SISLTDGGYRSEIDLGDGSNFREDFRNFANNLSEAITDAPKDLLRPVPKVEVSGLIKTS
STFITPNFKAGYYDQVAEDGKTLKYYQSTEYFNNRVVMPILQTTNGTLTANNRAYD
DIFVDQGVPRFPGWFHDVDKAYYAGSNGQSEYLFKEWNYYVANGSPLYNQYPNHT
FKQIKTIAFDAPRIKQGNTDGINLNLKQRNPDYVIINGLTGDGSTLKDLELPESVKKVS
IYGDYHSINVAKQIFKNVLELEFYSTNQDNNFGFNPLVLGDHTNIIYDLFVSKPFNYID
LTSLELKDNQDNIDASKLKRAVSDIYIRRRFERQMQGYWAGGYIDRYLVKNTNEKN
VNKDNDTVYAALKDINLHLEETYTHGGNTMYRVNENYYPGASAYEAERATRDSEF
QKEIVQR

SEQ ID NO:31: Modified *M. genitalium* protein M MG64 (Secretion Peptide + Fc from IgG1 + 15xGS Linker + MG29dGly1):

MYRMQLLSCIALSLALVTNSAPLEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL
YITREPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLT
VLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVS
LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMHEALKFHYTQKSLSLSPGAGGGSGGGSGGGSGGGMSLSLNDGSYQSEI
DLSGGANFREKFRNFANELSEAITNSPKGLDRPVPKTEISGLIKTGDNFITPSFKAGYY
DHVAEDGSLLSYYQSTEYFNNRVLMPILQTTDGTLMANNRGYDDVFRQVPRFPGWS
NTKATTVSTSNNLYYDKWTYFAAKGSPLYDQYPNHTFEDVKTLAIDAKDISALKTTI
DSEKPTYLIIRGLSGDGSQLNELQLPESVKKVSLYGDYTGVNVAKQIFANVVELEFYS
TSKANSFGFNPLVLGSKTNVINDLFVSKPFTHIDLTQVTLQNSDNSAIDANKLKQAVG
DIYNYRRFERQFQGYFAGGYIDKYLVKNVNTNKDSDDDLVYRSLKELNLHLEEAYR
EGDNTYYRVNENYYPGASIYENERASRDSEFQNEILKRAEQNGVTFDEN**

SEQ ID NO:32: Modified *M. genitalium* protein M MG88 (Secretion Peptide + Fc from IgG2 + 15xGS Linker + MG29dGly1):

MYRMQLLSCIALSLALVTNSAPLERKSSVECPPCPAPPAAASSVFLFPPKPKDTLMISR
TPEVTCVVVDVSAEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVLHQ
DWLNGKEYKCKVSNKGLPSSIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCL
VKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFS
CSVMHEALHNHYTQKSLSLSPGAGGGSGGGSGGGSGGGMSLSLNDGSYQSEIDLSG
GANFREKFRNFANELSEAITNSPKGLDRPVPKTEISGLIKTGDNFITPSFKAGYYDHVA
EDGSLLSYYQSTEYFNNRVLMPILQTTDGTLMANNRGYDDVFRQVPRFPGWSNTKA
TTVSTSNNLYYDKWTYFAAKGSPLYDQYPNHTFEDVKTLAIDAKDISALKTTIDSEK
PTYLIIRGLSGDGSQLNELQLPESVKKVSLYGDYTGVNVAKQIFANVVELEFYSTSKA
NSFGFNPLVLGSKTNVINDLFVSKPFTHIDLTQVTLQNSDNSAIDANKLKQAVGDIYN
YRRFERQFQGYFAGGYIDKYLVKNVNTNKDSDDDLVYRSLKELNLHLEEAYREGDN
TYYRVNENYYPGASIYENERASRDSEFQNEILKRAEQNGVTFDEN**

SEQ ID NO:33: Modified *M. genitalium* protein M MG118 (Protein A (Z-domain) + 10x GS Linker + MG29):

MKFNKEQQNAFYEILHLPNLNEEQRNAFIQSLKDDPSQSANLLAEAKKLNDAQAPG
GSGGSGGSGSLSLNDGSYQSEIDLSGGANFREKFRNFANELSEAITNSPKGLDRPVPK
TEISGLIKTGDNFITPSFKAGYYDHVAEDGSLLSYYQSTEYFNNRVLMPILQTTNGTL
MANNRGYDDVFRQVPRFPGWSNTKATTVSTSNNLTYDKWTYFAAKGSPLYDQYPN
HTFEDVKTLAIDAKDISALKTTIDSEKPTYLIIRGLSGNGSQLNELQLPESVKKVSLYG
DYTGVNVAKQIFANVVELEFYSTSKANSFGFNPLVLGSKTNVINDLFVSKPFTHIDLT
QVTLQNSDNSAIDANKLKQAVGDIYNYRRFERQFQGYFAGGYIDKYLVKNVNTNKD
SDDDLVYRSLKELNLHLEEAYREGDNTYYRVNENYYPGASIYENERASRDSEFQNEI
LKRAEQNGVTFDEN*

SEQ ID NO:34: Modified *M. genitalium* protein M MG66 (having the following mutations: S150E, S196R, S198P, F237T, S232Q, A342V, N274D, A205P, and T355P):

SLSLNDGSYQSEIDLSGGANFREKFRNFANELSEAITNSPKGLDRPVPKTEISGLIKTG
DNFITPSFKAGYYDHVAEDGSLLSYYQSTEYFNNRVLMPILQTTNGTLMANNRGYD

DVFRQVPRFPGWSNTKPTTVSTSNNLTYDKWTYFAAKGSPLYDQYPNHTFEDVKTL
AIDAKDISALKTTIDSEKPTYLIIRGLSGDGSQLNELQLPESVKKVSLYGDYTGVNVA
KQIFANVVELEFYSTSKANSFGFNPLVLGSKTNVINDLFVSKPFTHIDLTQVPLQNSD
NSAIDANKLKQAVGDIYNYRRFERQFQGYFAGGYIDKYLVKNVNTNKDSDDDLVY
RSLKELNLHLEEAYREGDNTYYRVNENYYPGASIYENERASRDSEFQNEILKRAEQN
GVTFDEN*

SEQ ID NO:35: Modified *M. genitalium* protein M MG71 (Protein L B1 Domain + 5xGS Linker + MG29 with an N-terminal deletion of SLSLND):

MEEVTIKANLILPGCCTQTAEFKGTFEKATSEAYAYADTLKKDNGEWTVDVADKGY
TLNIKFAGGSGGSGSYQSEIDLSGGANFREKFRNFANELSEAITNSPKGLDRPVPKTEI
SGLIKTGDNFITPSFKAGYYDHVAEDGSLLSYYQSTEYFNNRVLMPILQTTNGTLMA
NNRGYDDVFRQVPRFPGWSNTKATTVSTSNNLTYDKWTYFAAKGSPLYDQYPNHT
FEDVKTLAIDAKDISALKTTIDSEKPTYLIIRGLSGNGSQLNELQLPESVKKVSLYGDY
TGVNVAKQIFANVVELEFYSTSKANSFGFNPLVLGSKTNVINDLFVSKPFTHIDLTQV
TLQNSDNSAIDANKLKQAVGDIYNYRRFERQFQGYFAGGYIDKYLVKNVNTNKDSD
DDLVYRSLKELNLHLEEAYREGDNTYYRVNENYYPGASIYENERASRDSEFQNEILK
RAEQNGVTFDEN*

SEQ ID NO:36: Modified *M. genitalium* protein M MG86 (a dimer containing GCN4 helix + 15x GS + MG66)

GGCGGMKQLEDKIEELLSKIYHLENEIARLKKLIGEGGGSGGGSGGGSGGGMSLSLN
DGSYQSEIDLSGGANFREKFRNFANELSEAITNSPKGLDRPVPKTEISGLIKTGDNFITP
SFKAGYYDHVAEDGSLLSYYQSTEYFNNRVLMPILQTTNGTLMANNRGYDDVFRQ
VPRFPGWSNTKPTTVSTSNNLTYDKWTYFAAKGSPLYDQYPNHTFEDVKTLAIDAK
DISALKTTIDSEKPTYLIIRGLSGDGSQLNELQLPESVKKVSLYGDYTGVNVAKQIFAN
VVELEFYSTSKANSFGFNPLVLGSKTNVINDLFVSKPFTHIDLTQVPLQNSDNSAIDA
NKLKQAVGDIYNYRRFERQFQGYFAGGYIDKYLVKNVNTNKDSDDDLVYRSLKEL
NLHLEEAYREGDNTYYRVNENYYPGASIYENERASRDSEFQNEILKRAEQNGVTFDE
N*

What is claimed is:

1. A modified mycoplasma protein M or a functional fragment thereof, having one or more amino acid mutations that increase or maintain pH stability or thermostability of the mycoplasma protein M or a functional fragment thereof relative to wild-type mycoplasma protein M or a functional fragment thereof, wherein thermostability is maintained up to 75 degrees, 70 degrees, 65 degrees, 60 degrees, 55 degrees, 50 degrees, 45 degrees, 40 degrees, or 38 degrees and/or wherein pH stability is maintained from a pH of 2 to 8, from a pH of 2.5 to 7.5, or from a pH of 4.5 to 7.5.

2. The modified mycoplasma protein M or a functional fragment thereof of claim 1, having one or more amino acid mutations that increase pH stability or thermostability of the mycoplasma protein M or a functional fragment relative to wild-type mycoplasma protein M or a functional fragment thereof.

3. The modified mycoplasma protein M or a functional fragment thereof of claim 1 or 2, wherein the protein M or a functional fragment or derivative thereof is a protein M fragment.

4. The modified mycoplasma protein M or a functional fragment thereof of any one of claims 1-3, derived from protein M of *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, or *Mycoplasma penetrans*.

5. The modified mycoplasma protein M or a functional fragment thereof of any one of claims 1-4, wherein the protein M functional fragment comprises amino acid residues 17-537, 37-556, 37-482, 37-468, 37-442, 74-468, 74-479, 74-482, 74-468, or 74-442 of *M. genitalium* protein M (SEQ ID NO:1) or the equivalent residues from another protein M.

6. The modified mycoplasma protein M or a functional fragment thereof of any one of claims 1-5, being a fragment from about residue 74 to about residue 479 of *M. genitalium* protein M (SEQ ID NO:3) or the equivalent residues of *M. pneumoniae* protein M (SEQ ID NO:23).

7. The modified mycoplasma protein M or a functional fragment thereof of any one of claims 1-6, wherein the one or more mutations are at residues 77, 125, 165, 170, and 280 of *M. pneumoniae* protein M (SEQ ID NO:23) or any combination thereof.

8. The modified mycoplasma protein M or a functional fragment thereof of claim 7, wherein the one or more mutations are A77E, K125R, V165Q, H170T, and A280V of *M. pneumoniae* protein M (SEQ ID NO:23) or any combination thereof.

9. The modified mycoplasma protein M or a functional fragment thereof of any one of claims 1-6, wherein the one or more mutations are at residues selected from:

a) 159;

b) 182;

c) 102;

d) 161;

e) 86;

f) 101;

g) 147;

h) 236;

i) 348;

j) 424;

k) 147;

l) 321;

m) 389;

n) 442;

o) 119;

p) 179;

q) 186; or

r) 103

of *M. genitalium* protein M (SEQ ID NO:1) or the equivalent residues of *M. pneumoniae* protein M (SEQ ID NO:23) or any combination thereof.

10. The modified mycoplasma protein M or a functional fragment thereof of claim 9, wherein the one or more mutations are selected from:

a) Q159C and A182C;

b) A102C and T161C; or

c) I86C and F101C

of *M. genitalium* protein M (SEQ ID NO:1) or the equivalent residues of *M. pneumoniae* protein M (SEQ ID NO:23) or any combination thereof, wherein the modified residues in a), b), or c) are capable of forming a di-sulfide bond between the modified residues (*e.g.*, SEQ ID NO:34).

11. The modified mycoplasma protein M or a functional fragment thereof of claim 9, wherein the one or more mutations are selected from:

a) H147Y;

b) H236Y;

c) H348Y;

d) H424Y; or

e) H147Q

of *M. genitalium* protein M (SEQ ID NO:1) or the equivalent residues of *M. pneumoniae* protein M (SEQ ID NO:23) or any combination thereof, wherein the mutation can prevent histidine protonation at that site at low pH and thereby prevent protein destabilization.

12. The modified mycoplasma protein M or a functional fragment thereof of claim 9, wherein the one or more mutations are selected from:

a) N321H;

b) Y389H;

c) N442H;

d) P119H;

e) T179H;

f) G186H; or

g) N103H

of *M. genitalium* protein M (SEQ ID NO:1) or the equivalent residues of *M. pneumoniae* protein M (SEQ ID NO:23) or any combination thereof, wherein the mutation can increase pH sensitivity thereby improving elution conditions for antibody purification.

13. The modified mycoplasma protein M or a functional fragment thereof of any one of claims 1-6, wherein the mutation is at residue 338 of *M. genitalium* protein M (SEQ ID

NO:1) (*e.g.*, Y388N) or the equivalent residues of *M. pneumoniae* protein M (SEQ ID NO:23).

14. The modified mycoplasma protein M or a functional fragment thereof of any one of claims 1-13, wherein the modified mycoplasma protein M or a functional fragment thereof is further modified to remove one or more glycosylation sites.

15. A modified mycoplasma protein M or a functional fragment thereof, having a deletion from about residue 185 to about residue 342 of *M. genitalium* protein M (SEQ ID NO:1) or the equivalent residues of another mycoplasma protein M.

16. The modified mycoplasma protein M or a functional fragment thereof of claim 15, wherein the deletion is amino acids 185-342.

17. The modified mycoplasma protein M or a functional fragment thereof of claim 15 or 16, wherein the deleted amino acids are replaced by a short linker.

18. The modified mycoplasma protein M or a functional fragment thereof of any one of claims 15-17, wherein newly exposed hydrophobic residues have one or more mutations.

19. A fusion protein comprising:

the modified mycoplasma protein M or a functional fragment thereof of any one of claims 1-18;

a linker; and

a peptide.

20. The fusion protein of claim 19, wherein the modified mycoplasma protein M or a functional fragment thereof has one or more mutations that enhance the affinity or avidity of the modified mycoplasma protein M or a functional fragment thereof to antibodies.

21. The fusion protein of claim 19 or 20, wherein the peptide is a dimerizing peptide (e.g., IL-GCN4, C-IL-GCN4, SEQ ID NO:36).

22. The fusion protein of claim 19 or 20, wherein the peptide is a Fab binding polypeptide or an Fc binding polypeptide.

23. The fusion protein of claim 22, wherein the Fab binding polypeptide is Protein L.

24. The fusion protein of claim 22, wherein the Fab or Fc binding polypeptide is protein A or a functional fragment thereof.

25. The fusion protein of claim 24, wherein the fusion protein binds to the Fc portion of immunoglobulins and/or decreases Fc receptor recycling of the immunoglobulins and/or increases binding of the fusion protein to immunoglobulins.

26. The fusion protein of claim 22, wherein the Fab or Fc binding polypeptide is protein G or a functional fragment thereof.

27. The fusion protein of claim 26, wherein and the fusion protein binds to the Fc portion of immunoglobulins and/or decreases Fc receptor recycling of the immunoglobulins and/or increases binding of the fusion protein to immunoglobulins.

28. The fusion protein of claim 19 or 20, wherein the peptide comprises a dimerizing peptide and Protein L.

29. The fusion protein of claim 28, wherein the fusion protein, starting from the N-terminus, comprises in order a dimerizing peptide, a first linker, Protein L, a second linker, and the modified mycoplasma protein M or a functional fragment thereof of any one of claims 1-18.

30. The fusion protein of claim 19 or 20, wherein the peptide is a Fc binding polypeptide.

31. The fusion protein of claim 19 or 20, wherein the Fc binding polypeptide is a Fc receptor.

32. The fusion protein of claim 19 or 20, wherein the peptide comprises a Fab binding polypeptide and Protein L.

33. The fusion protein of claim 32, wherein the fusion protein, starting from the N-terminus, comprises in order a Fc binding polypeptide, a first linker, Protein L, a second linker, and the modified mycoplasma protein M or a functional fragment thereof of any one of claims 1-18.

34. The fusion protein of claim 19 or 20, wherein the peptide is a Fc domain.

35. The fusion protein of claim 34, wherein the Fc domain comprises complement binding residues for binding to a Fc receptor.

36. The fusion protein of claim 35, wherein the Fc domain has one or more mutations of complement binding residues for binding to a Fc receptor.

37. The fusion protein of claim 19 or 20, wherein the peptide is a Fc receptor protein and wherein the fusion protein decreases the cellular uptake of antibodies, decreases antibody recycling and causes immunoglobulin depletion by decreasing antibody recycling, or increases affinity of the fusion protein for immunoglobulins.

38. The fusion protein of any one of claims 19-37, further comprising a second modified mycoplasma protein M or a functional fragment thereof of any one of claims 1-18.

39. The modified mycoplasma protein M or a functional fragment thereof of claims 1-18 or the fusion protein of any one of claims 19-38, further comprising half-life extension moieties, such as human albumin or addition of polyethylene glycol (PEG), protein modifications such as acylation, methylation, phosphorylation, sulfation, farnesylation, ubiquitination, site-selective conjugation, glycosylation, introduction of PTMs, PEGylation, ligation, polymerization of protein-based initiators, or any modifications that may alter binding, affinity, binding targets, or any combination thereof.

40. A polynucleotide encoding the modified mycoplasma protein M or a functional fragment thereof of any one of claims 1-18 or the fusion protein of any one of claims 19-38.

41. The polynucleotide of claim 40, operably linked to a promoter.

42. The polynucleotide of claim 41, wherein the promoter is a bacterial promoter.

43. The polynucleotide of claim 41, wherein the promoter is a mammalian promoter.

44. The polynucleotide of any one of claims 40-43, wherein the polynucleotide is codon optimized for expression in bacteria, such as *E. coli*.

45. The polynucleotide of any one of claims 40-43, wherein the polynucleotide is codon optimized for expression in a mammalian cell, such as a human cell.

46. The polynucleotide of any one of claims 40-43, wherein the polynucleotide is codon optimized for expression in both bacteria, such as *E. coli*, and a mammalian cell, such as a human cell.

47. A vector comprising the polynucleotide of any one of claims 40-46.

48. The vector of claim 47, wherein the vector is a bacterial vector.

49. The vector of claim 47, wherein the vector is a mammalian vector.

50. A transformed cell comprising the polynucleotide of any one of claims 40-46 and/or the vector of any one of claims 47-49.

51. The transformed cell of claim 50, which is a bacterial cell, such as *E. coli*.

52. The transformed cell of claim 50, which is a mammalian cell, such as a human cell.

53. The transformed cell of any one of claims 50-52, wherein the polynucleotide is stably incorporated into the genome of the cell.

54. A method of inhibiting neutralization of a heterologous agent by neutralizing antibodies upon administration of the heterologous agent to a subject, comprising administering to the subject an effective amount of the mycoplasma protein M or a functional

fragment or derivative thereof of any one of claims 1-18 or the fusion protein of any one of claims 19-38, thereby inhibiting neutralization of the heterologous agent.

55. The method of claim 54, wherein the heterologous agent is a nucleic acid delivery vector.

56. The method of claim 55, wherein the nucleic acid delivery vector is a viral vector.

57. The method of claim 56, wherein the viral vector is an oncolytic vector.

58. The method of claim 56, wherein the viral vector is an adeno-associated virus, retrovirus, lentivirus, poxvirus, alphavirus, baculovirus, vaccinia virus, herpes virus, Epstein-Barr virus, polio virus, or adenovirus vector.

59. The method of claim 55, wherein the nucleic acid delivery vector is a non-viral vector.

60. The method of claim 59, wherein the non-viral vector is a plasmid, liposome, electrically charged lipid, nucleic acid-protein complex, or biopolymer (*e.g.*, PEG).

61. The method of claim 54, wherein the heterologous agent is a gene editing complex.

62. The method of claim 61, wherein the gene editing complex is a CRISPR complex.

63. The method of claim 54, wherein the heterologous agent is a protein or nucleic acid.

64. The method of claim 63, wherein the protein is an enzyme.

65. The method of claim 63, wherein the nucleic acid is an antisense nucleic acid or an inhibitory RNA.

66. The method of any one of claims 54-65, wherein the effective amount of protein M is an amount sufficient to inhibit neutralization by at least about 50%, 60%, 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98, 99%, 99.5%, or 99.9%.

67. The method of any one of claims 54-66, wherein the effective amount of protein M is an amount sufficient to produce a ratio of protein M to total immunoglobulin in the subject of about 0.5:1 to about 5:1, e.g., about 2:1 on a molar basis.

68. The method of any one of claims 54-67, wherein the protein M or a functional fragment or derivative thereof or the fusion protein is administered to the subject prior to administration of the heterologous agent (*e.g.*, from about 1 minute to about 3 days prior, from about 5 minutes to about 48 hours prior, from about 10 minutes to about 24 hours prior, from about 20 minutes to about 12 hours prior, from about 30 minutes to about 6 hours prior, or from about 1 hour to about 3 hours prior).

69. The method of any one of claims 54-67, wherein the protein M or a functional fragment or derivative thereof or the fusion protein is administered to the subject concurrently with administration of the heterologous agent.

70. The method of claim 69, wherein the heterologous agent is combined with the protein M or a fragment or derivative thereof or the fusion protein prior to administration to the subject.

71. The method of any one of claims 54-70, wherein the protein M or a functional fragment or derivative thereof is administered to the subject more than once.

72. The method of claim 71, wherein a second dose is administered from about two days to about 10 years or more after a first dose.

73. The method of claim 71, wherein the protein M or a functional fragment or derivative thereof is administered to the subject each time the heterologous agent is administered to the subject.

74. The method of any one of claims 71-73, wherein the same protein M or a functional fragment or derivative thereof is administered each time.

75. The method of any one of claims 71-73, wherein a different protein M or a functional fragment or derivative thereof is administered each time.

76. The method of any one of claims 54-75, further comprising administering to the subject an additional treatment for reducing antibody concentration in the subject.

77. The method of claim 76, wherein the additional treatment is plasmaphereses, administration of an antibody digesting enzyme such as IdeS or IdeZ, administration of an antibody that binds the FcRn receptor, administration of an Fc fragment that binds the FcRn receptor, administration of an antibody that binds CD19 or an antibody that binds CD20 on B cells and depletes them, or other B cell depletion methods such as splenectomy, chemotherapy, immunotherapy, or radiation therapy.

78. The method of claim 76 or 77, wherein the additional treatment is administered before, during, and/or after administration of the protein M or a functional fragment or derivative thereof.

79. The method of any one of claims 54-78, wherein the administration is local.

80. The method of any one of claims 54-78, wherein the administration is systemic.

81. The method of any one of claims 54-80, wherein the administration decreases the amount of antibody cycling and the onset of the decrease is from about 5 minutes to about 8 weeks, from about 5 minutes to about 1 hour, from about 1 hour to about 2 days, or from about 2 days to about 8 weeks.

82. The method of any one of claims 54-80, wherein the administration decreases the amount of antibody cycling and the onset of the decrease is from about 1 hour to about 3 hours.

83. The method of any one of claims 54-82, wherein the administration comprises from about 1e9 vg/kg to about 1e14 vg/kg, from about 1e10 vg/kg to about 1e13 vg/kg, or from about 1e11 vg/kg to about 1e12 vg/kg of AAV, from about 1 μl to about 6 L of serum, and from about 1 mg/kg to about 1000 mg/kg, from about 10 mg/kg to about 900 mg/kg, from about 25 mg/kg

to about 800 mg/kg, from about 50 mg/kg to about 600 mg/kg, from about 100 mg/kg to about 500 mg/kg, or from about 200 mg/kg to about 400 mg/kg of Protein M.

84. The method of any one of claims 54-82, wherein the administration comprises sufficient Protein M to achieve about 0.5:1 to about 50:1 molar ratio of Protein M to serum immunoglobulins.

85. The method of any one of claims 54-84, wherein administration outcompetes self-neutralizing antibodies.

86. A method of expressing a polypeptide or functional nucleic acid in a subject, comprising administering to the subject (a) a nucleic acid delivery vector encoding the polypeptide or functional nucleic acid, and (b) an effective amount of the mycoplasma protein M or a functional fragment or derivative thereof of any one of claims 1-18 or the fusion protein of any one of claims 19-38, thereby expressing the polypeptide or functional nucleic acid in the subject.

87. A method of treating a disorder in a subject in need thereof, wherein the disorder is treatable by expressing a polypeptide or functional nucleic acid in the subject, comprising administering to the subject (a) a therapeutically effective amount of a nucleic acid delivery vector encoding the polypeptide or functional nucleic acid, and (b) an effective amount of the mycoplasma protein M or a functional fragment or derivative thereof of any one of claims 1-18 or the fusion protein of any one of claims 19-38, thereby treating the disorder in the subject.

88. A method of editing a gene in a subject, comprising administering to the subject (a) an effective amount of a gene editing complex, and (b) an effective amount of the mycoplasma protein M or a functional fragment or derivative thereof of any one of claims 1-18 or the fusion protein of any one of claims 19-38, thereby expressing the polypeptide or functional nucleic acid in the subject.

89. A method of treating an autoimmune disease in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of the mycoplasma protein M

or a functional fragment or derivative thereof of any one of claims 1-18 or the fusion protein of any one of claims 19-38, thereby treating the autoimmune disease.

90. A method of treating a disorder associated with excess antibodies in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of the mycoplasma protein M or a functional fragment or derivative thereof of any one of claims 1-18 or the fusion protein of any one of claims 19-38, thereby treating the disorder associated with excess antibodies.

91. The method of claim 90, wherein the disorder associated with excess antibodies is multiple myeloma, monoclonal gammopathy of undetermined significance (MGUS), Waldenstrom macroglobulinemia, cytokine release syndrome, or acute autoimmune attacks such as severe autoimmune vasculitis with sudden onset.

92. A method of preventing and/or treating acute transplant rejection of solid organs associated with recognition of a transplant by recipient antibodies in a subject in need thereof, comprising administering to the subject prior to and/or after the transplant a therapeutically effective amount of the mycoplasma protein M or a functional fragment or derivative thereof of any one of claims 1-18 or the fusion protein of any one of claims 19-38, thereby treating the acute transplant rejection.

93. The method of any one of claims 54-92, wherein the mycoplasma protein M or a functional fragment or derivative thereof is administered to the subject by a route selected from oral, rectal, transmucosal, intranasal, inhalation, buccal (*e.g.*, sublingual), vaginal, intrathecal, intraocular, intravitreal, intracochlear, transdermal, intraendothelial, *in utero* (or *in ovo*), parenteral (*e.g.*, intravenous, subcutaneous, intradermal, intracranial, intramuscular [including administration to skeletal, diaphragm and/or cardiac muscle], intrapleural, intracerebral, and intraarticular), topical (*e.g.*, to both skin and mucosal surfaces, including airway surfaces, and transdermal administration), intralymphatic, and the like, as well as direct tissue or organ injection (*e.g.*, to liver, eye, skeletal muscle, cardiac muscle, diaphragm muscle or brain).

94. The method of any one of claims 54-92, wherein the mycoplasma protein M or a functional fragment or derivative thereof is administered to a skeletal muscle, a smooth

muscle, the heart, the diaphragm, the airway epithelium, the liver, the kidney, the spleen, the pancreas, the skin, the lung, the ear, or the eye.

95. The method of any one of claims 54-94, wherein the mycoplasma protein M or a functional fragment or derivative thereof is administered to a diseased tissue or organ.

96. The method of any one of claims 54-95, wherein the subject is a human.

97. A method of isolating a compound comprising an antibody light chain variable region and/or heavy chain variable region from a sample, the method comprising contacting the compound with the modified mycoplasma protein M or a functional fragment thereof of any one of claims 1-18 or the fusion protein of any one of claims 19-38 attached to a solid support, then eluting the compound from the modified mycoplasma protein M or a functional fragment thereof or the fusion protein.

98. The method of claim 97, wherein the compound comprising an antibody light chain variable region and/or heavy chain variable region is an antibody or an antigen-binding fragment thereof.

99. The method of claim 97 or 98, wherein the solid support is a bead or particle.

100. The method of any one of claims 97-99, wherein the solid support comprises agarose, polyacrylamide, dextran, cellulose, polysaccharide, nitrocellulose, silica, alumina, aluminum oxide, titania, titanium oxide, zirconia, styrene, polyvinyldifluoride nylon, copolymer of styrene and divinylbenzene, polymethacrylate ester, derivatized azlactone polymer or copolymer, glass, or cellulose.

101. The method of any one of claims 97-100, wherein the compound is eluted by a change in pH.

102. The modified mycoplasma protein M or a functional fragment thereof of any one of claims 1-18 or the fusion protein of any one of claims 19-38, attached to a solid support.

103. A method of performing an immunoassay, the method comprising using the modified mycoplasma protein M or a functional fragment thereof of any one of claims 1-18 or the fusion protein of any one of claims 19-38 to bind a compound comprising an antibody light chain variable region and/or heavy chain variable region.

104. The method of claim 103, wherein the immunoassay is selected from radio-immunoassays (RIA), enzyme-linked immunosorbent assays (ELISA) assays, enzyme immunoassays (EIA), sandwich assays, gel diffusion precipitation reactions, immunodiffusion assays, agglutination assays, immunofluorescence assays, fluorescence activated cell sorting (FACS) assays, immunohistochemical assays, protein A immunoassays, protein G immunoassays, protein L immunoassays, biotin/avidin assays, biotin/streptavidin assays, immunoelectrophoresis assays, precipitation/flocculation reactions, immunoblots (Western blot; dot/slot blot); immunodiffusion assays; liposome immunoassay, chemiluminescence assays, library screens, expression arrays, immunoprecipitation, competitive binding assays, and immunohistochemical staining.

105. A kit comprising the modified mycoplasma protein M or a functional fragment thereof of any one of claims 1-18 or the fusion protein of any one of claims 19-38 or the solid support of claim 102.

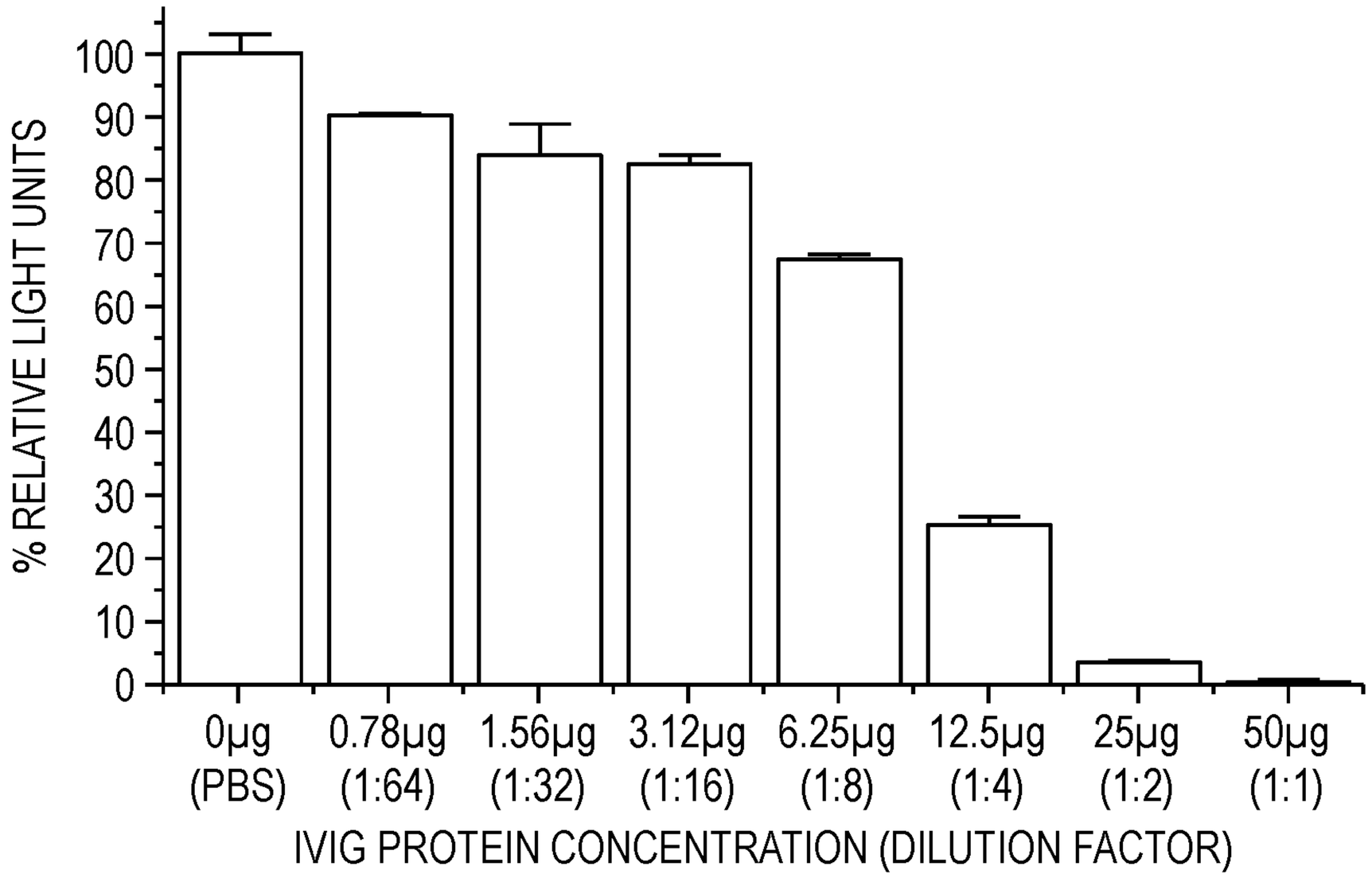

% RELATIVE LIGHT UNITS
100
90
80
70
60
50
40
30
20
10
0
0µg (PBS)
0.78µg (1:64)
1.56µg (1:32)
3.12µg (1:16)
6.25µg (1:8)
12.5µg (1:4)
25µg (1:2)
50µg (1:1)
IVIG PROTEIN CONCENTRATION (DILUTION FACTOR)

FIG. 1

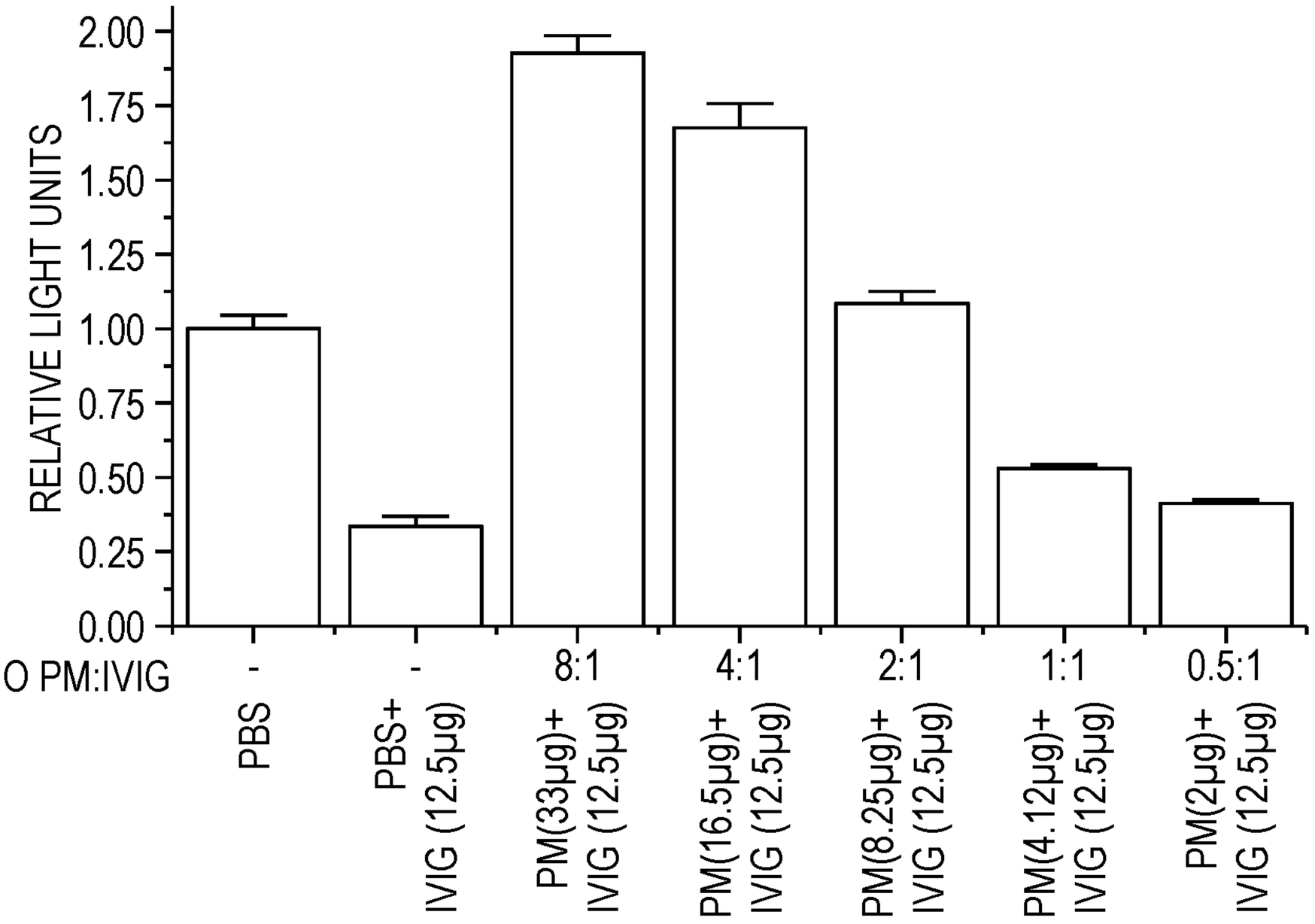

RELATIVE LIGHT UNITS
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
RATIO PM:IVIG
-
-
8:1
4:1
2:1
1:1
0.5:1
PBS
PBS+ IVIG (12.5µg)
PM(33µg)+ IVIG (12.5µg)
PM(16.5µg)+ IVIG (12.5µg)
PM(8.25µg)+ IVIG (12.5µg)
PM(4.12µg)+ IVIG (12.5µg)
PM(2µg)+ IVIG (12.5µg)

FIG. 2

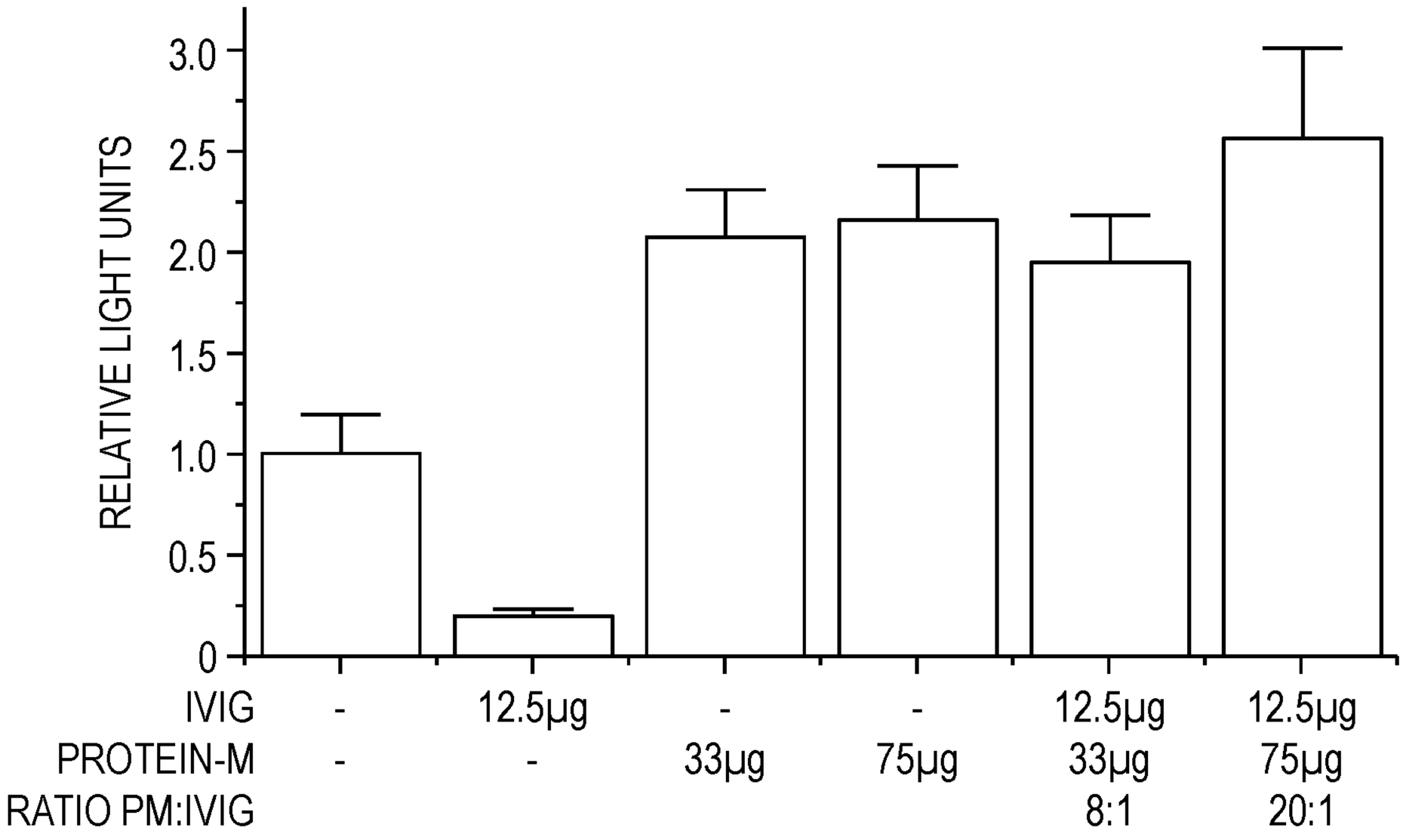

RELATIVE LIGHT UNITS
3.0
2.5
2.0
1.5
1.0
0.5
0
IVIG - 12.5µg - - 12.5µg 12.5µg
PROTEIN-M - - 33µg 75µg 33µg 75µg
RATIO PM:IVIG 8:1 20:1

FIG. 3

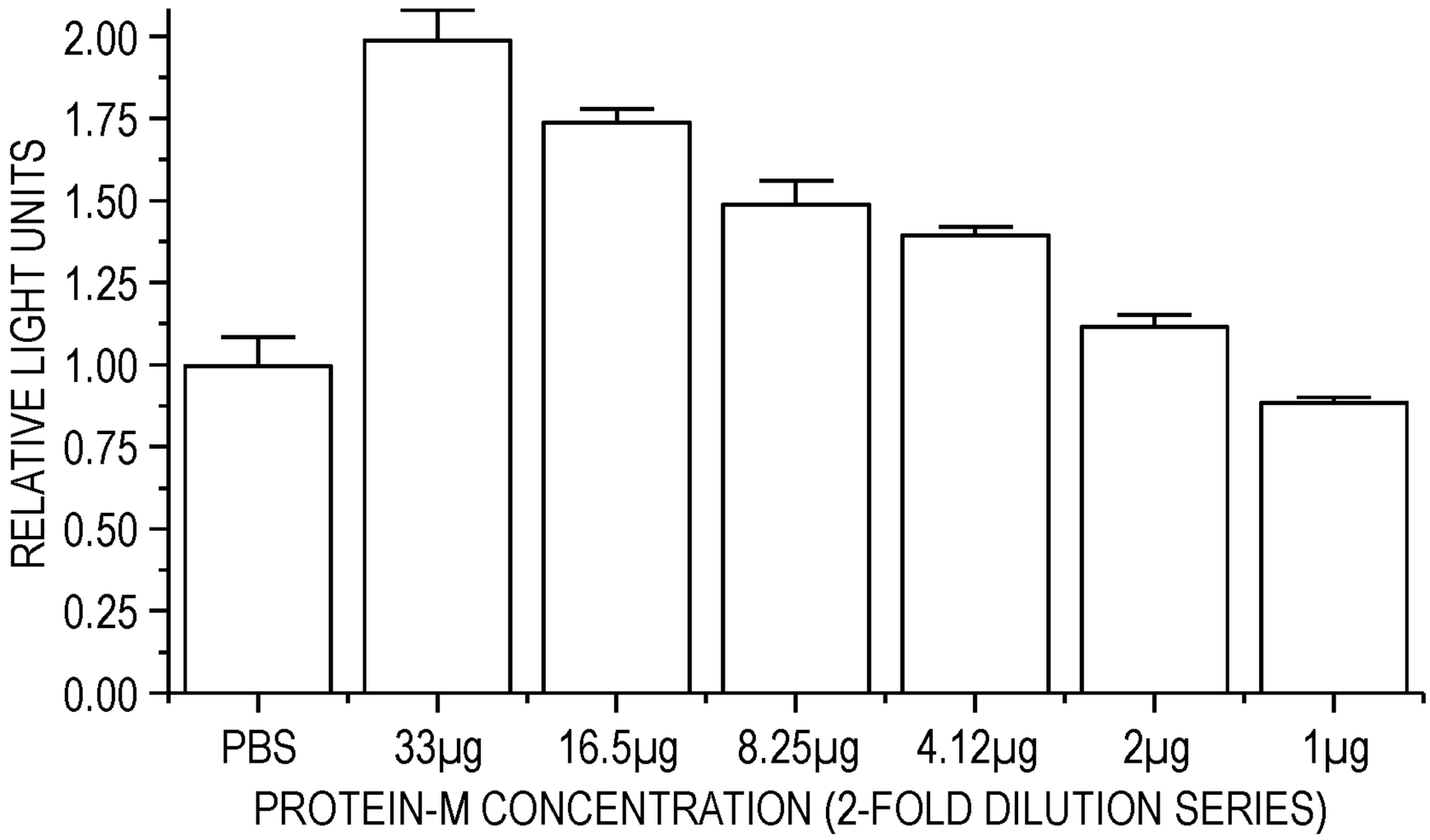

RELATIVE LIGHT UNITS
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
PBS 33µg 16.5µg 8.25µg 4.12µg 2µg 1µg
PROTEIN-M CONCENTRATION (2-FOLD DILUTION SERIES)

FIG. 4

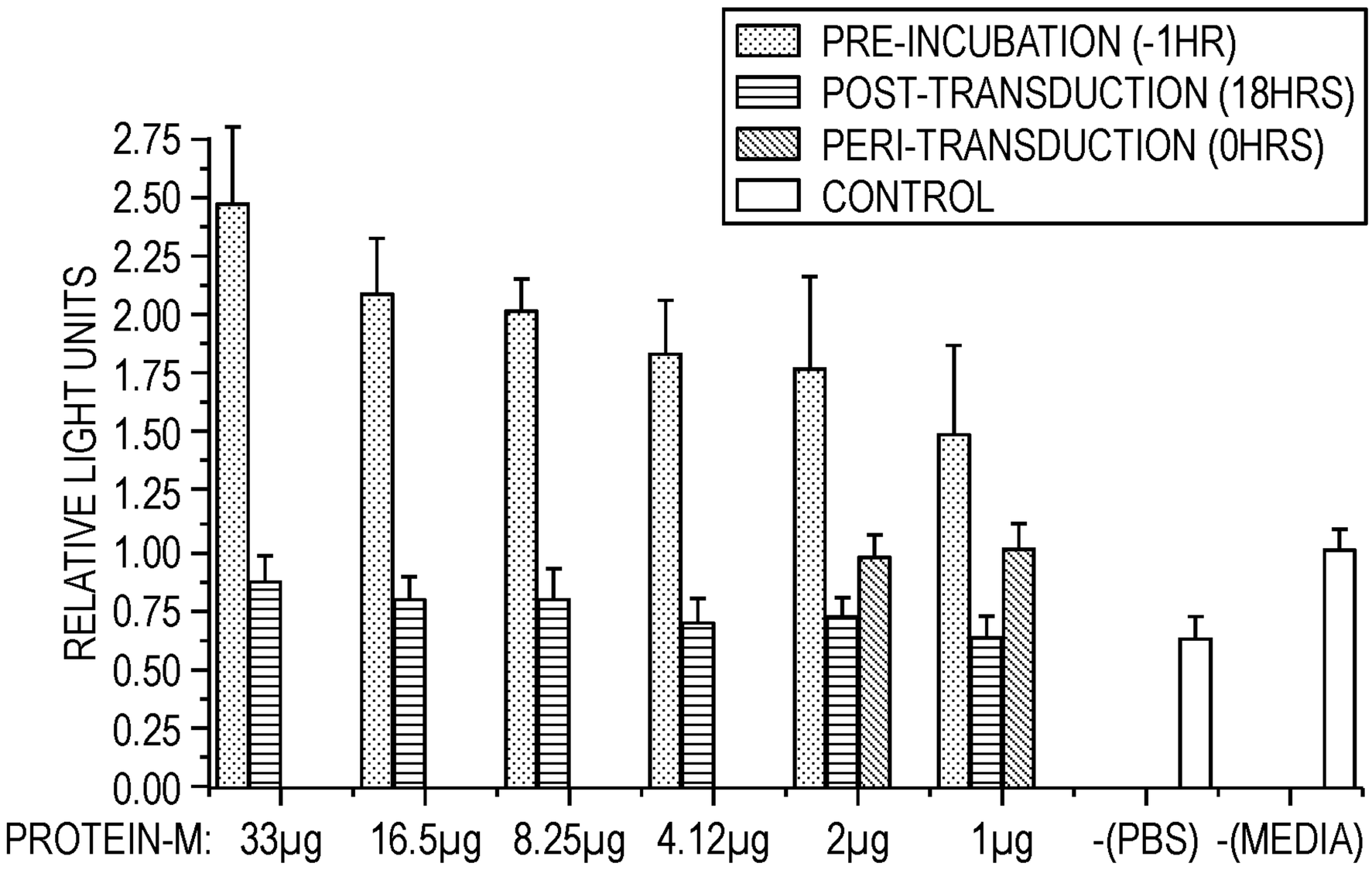

PRE-INCUBATION (-1HR)
POST-TRANSDUCTION (18HRS)
PERI-TRANSDUCTION (0HRS)
CONTROL
RELATIVE LIGHT UNITS
2.75
2.50
2.25
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
PROTEIN-M: 33µg 16.5µg 8.25µg 4.12µg 2µg 1µg -(PBS) -(MEDIA)

FIG. 5

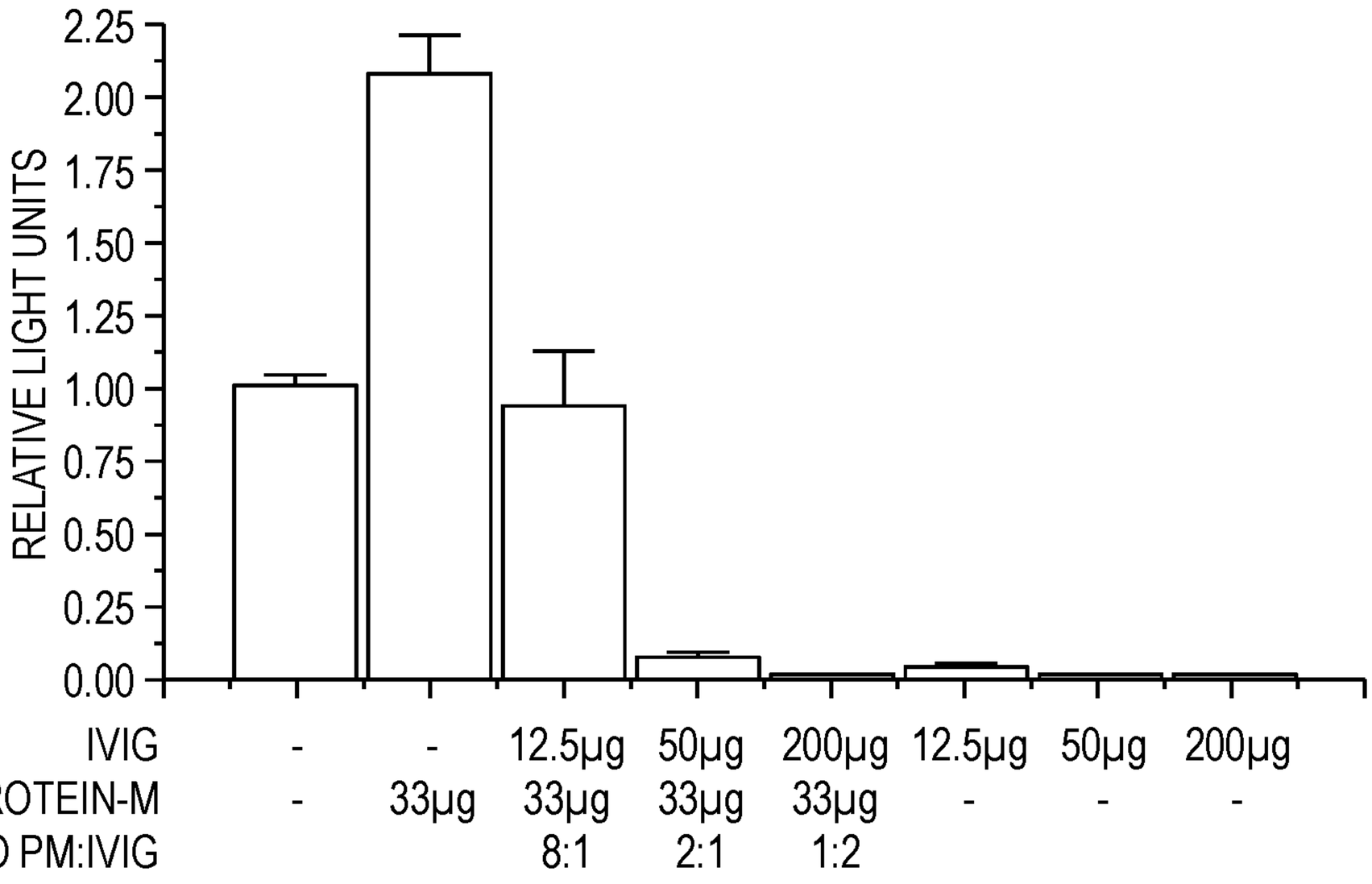

RELATIVE LIGHT UNITS
2.25
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
IVIG - - 12.5µg 50µg 200µg 12.5µg 50µg 200µg
PROTEIN-M - 33µg 33µg 33µg 33µg - - -
RATIO PM:IVIG 8:1 2:1 1:2

FIG. 6

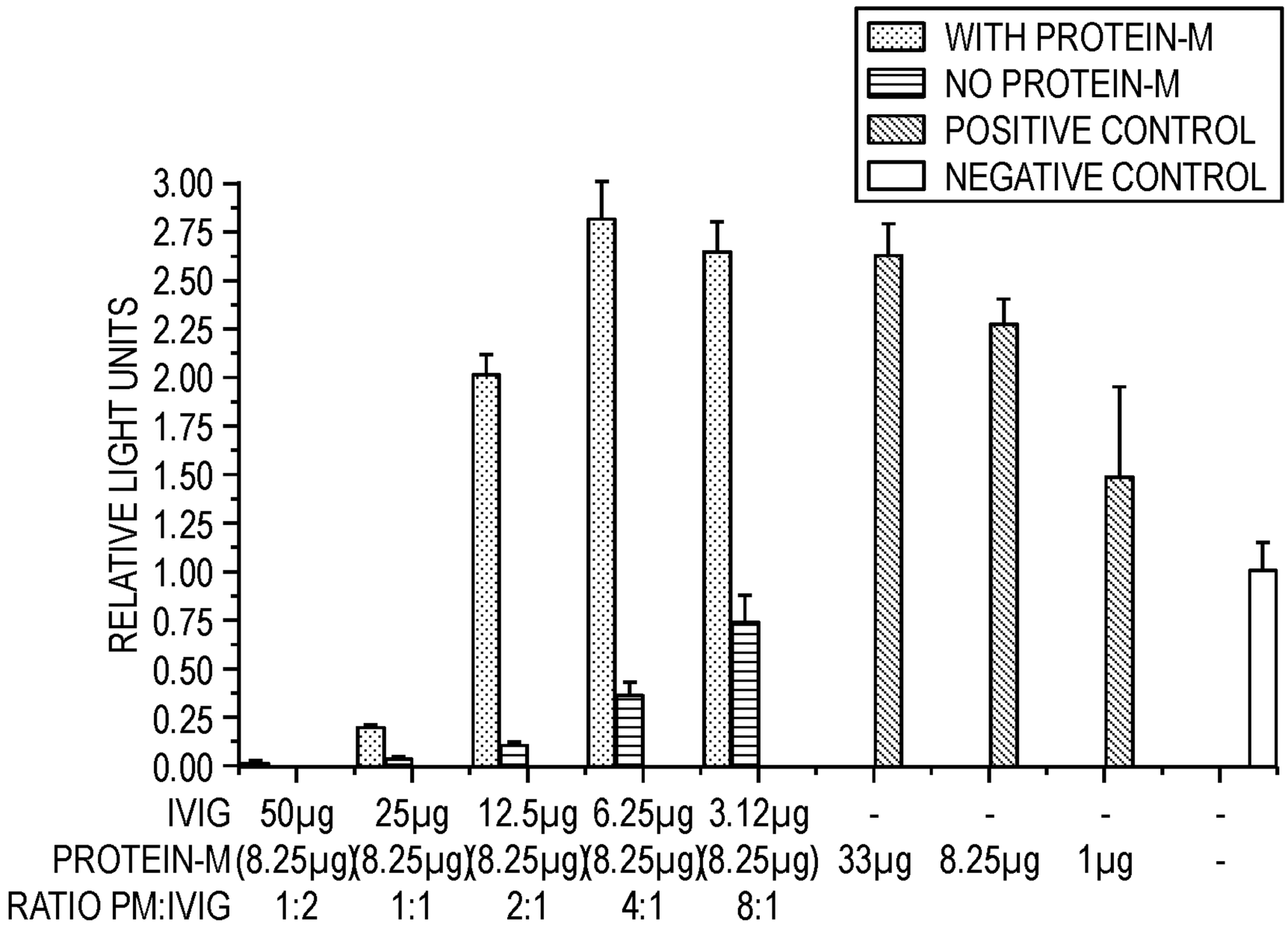
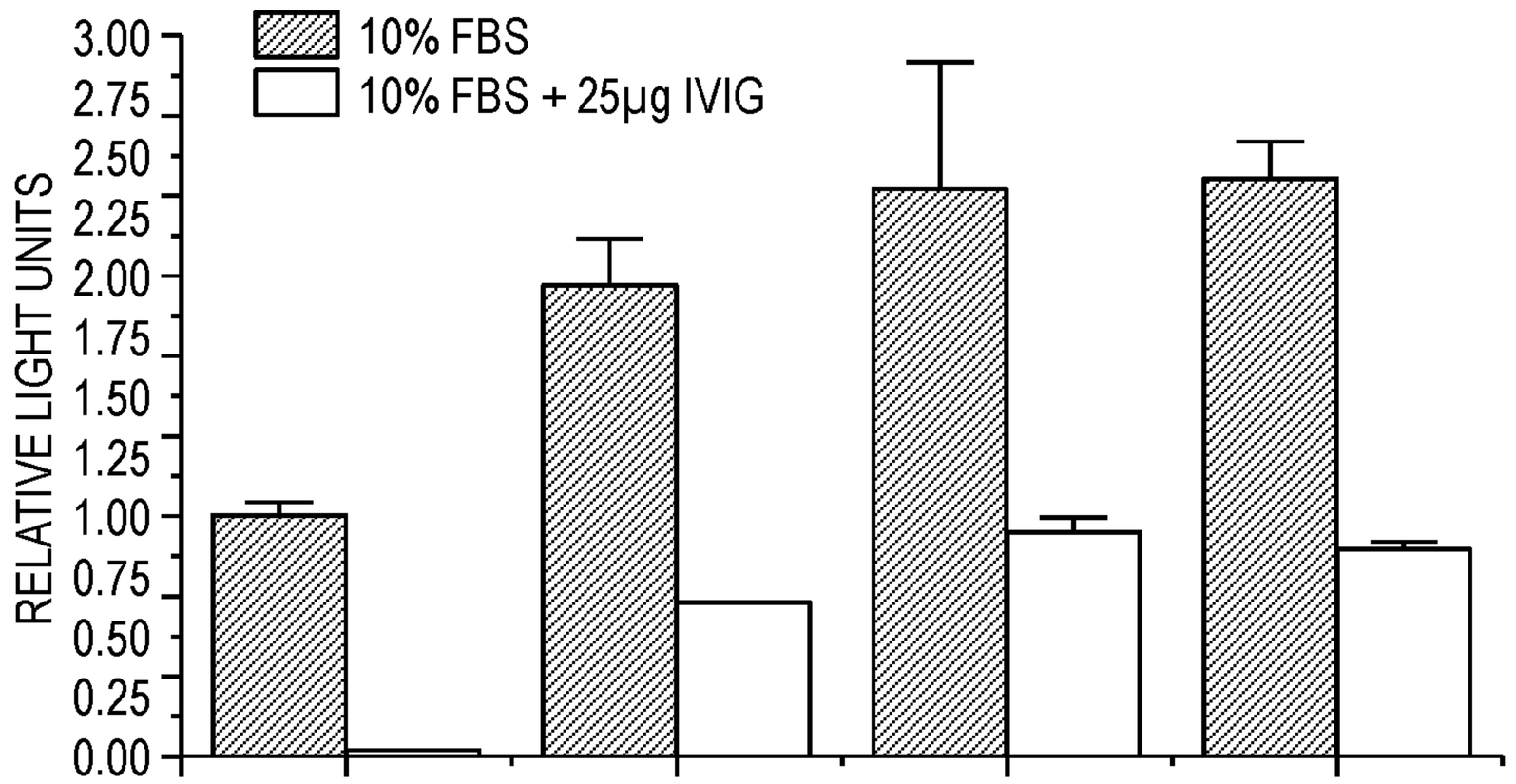


FIG. 7



PROTEIN-M	-	433µg	217µg	108µg
HUMAN IVIG	-/(25µg)	-/(25µg)	-/(25µg)	-/(25µg)
BOVINE SERUM IgG	350µg	350µg	350µg	350µg
RATIO PM:TOTAL IVIG	-	~4:1	~2:1	~1:1

FIG. 8

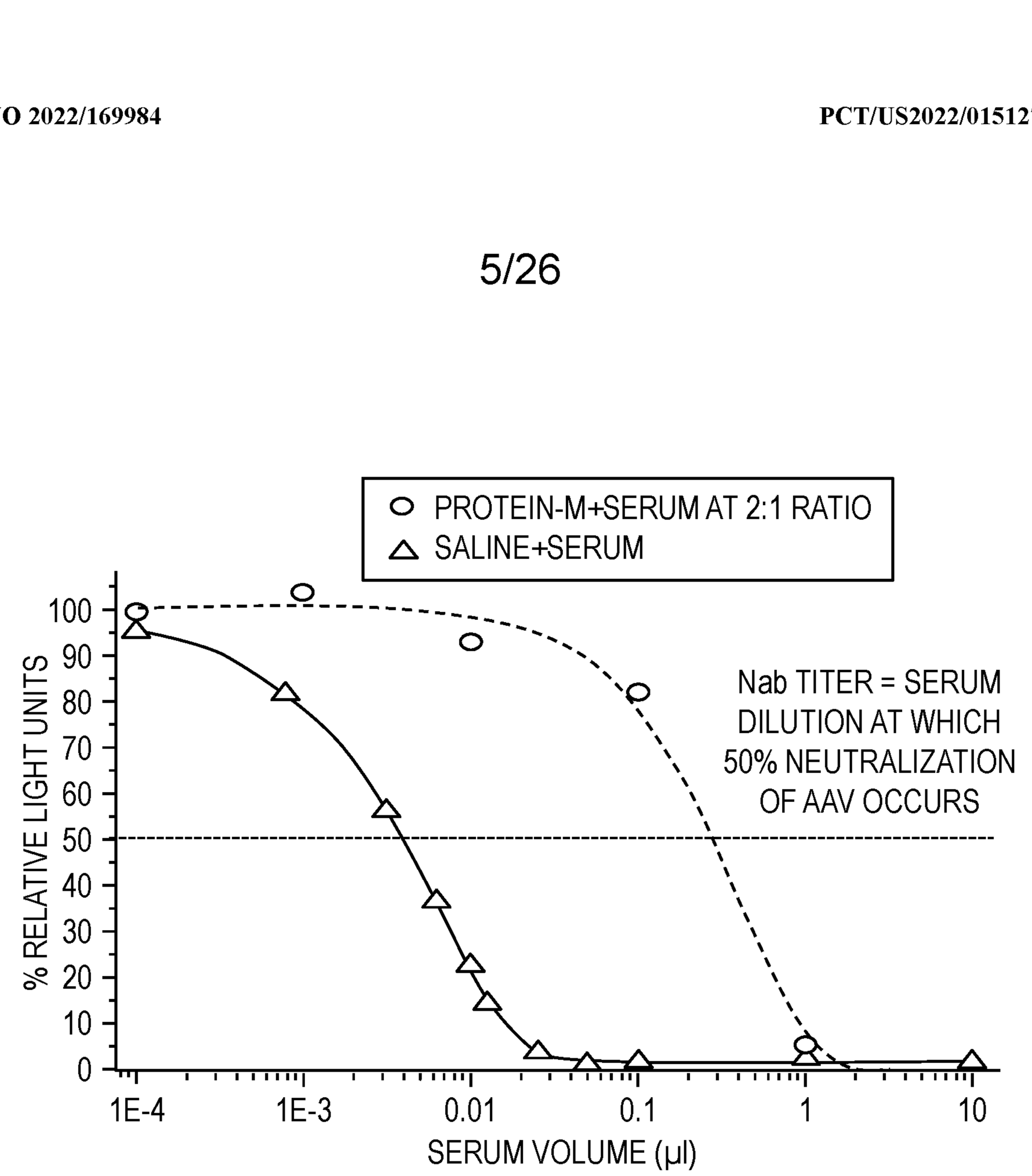

PROTEIN-M+SERUM AT 2:1 RATIO
SALINE+SERUM
% RELATIVE LIGHT UNITS
100
90
80
70
60
50
40
30
20
10
0
Nab TITER = SERUM DILUTION AT WHICH 50% NEUTRALIZATION OF AAV OCCURS
1E-4
1E-3
0.01
0.1
1
10
SERUM VOLUME (µl)

FIG. 9

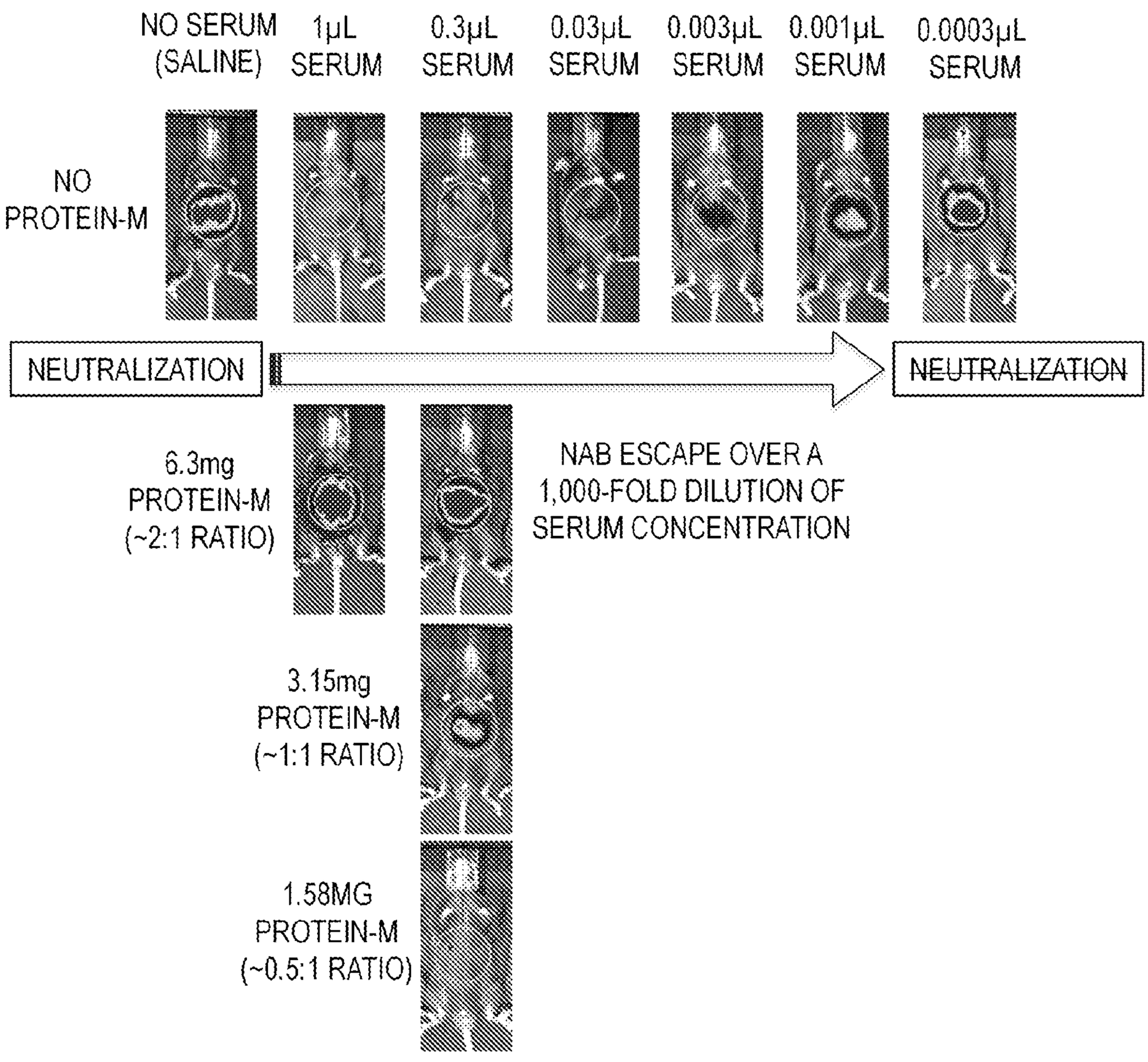

NO SERUM (SALINE)
1µL SERUM
0.3µL SERUM
0.03µL SERUM
0.003µL SERUM
0.001µL SERUM
0.0003µL SERUM
NO PROTEIN-M
NEUTRALIZATION
NEUTRALIZATION
6.3mg PROTEIN-M (~2:1 RATIO)
NAB ESCAPE OVER A 1,000-FOLD DILUTION OF SERUM CONCENTRATION
3.15mg PROTEIN-M (~1:1 RATIO)
1.58MG PROTEIN-M (~0.5:1 RATIO)

FIG. 10

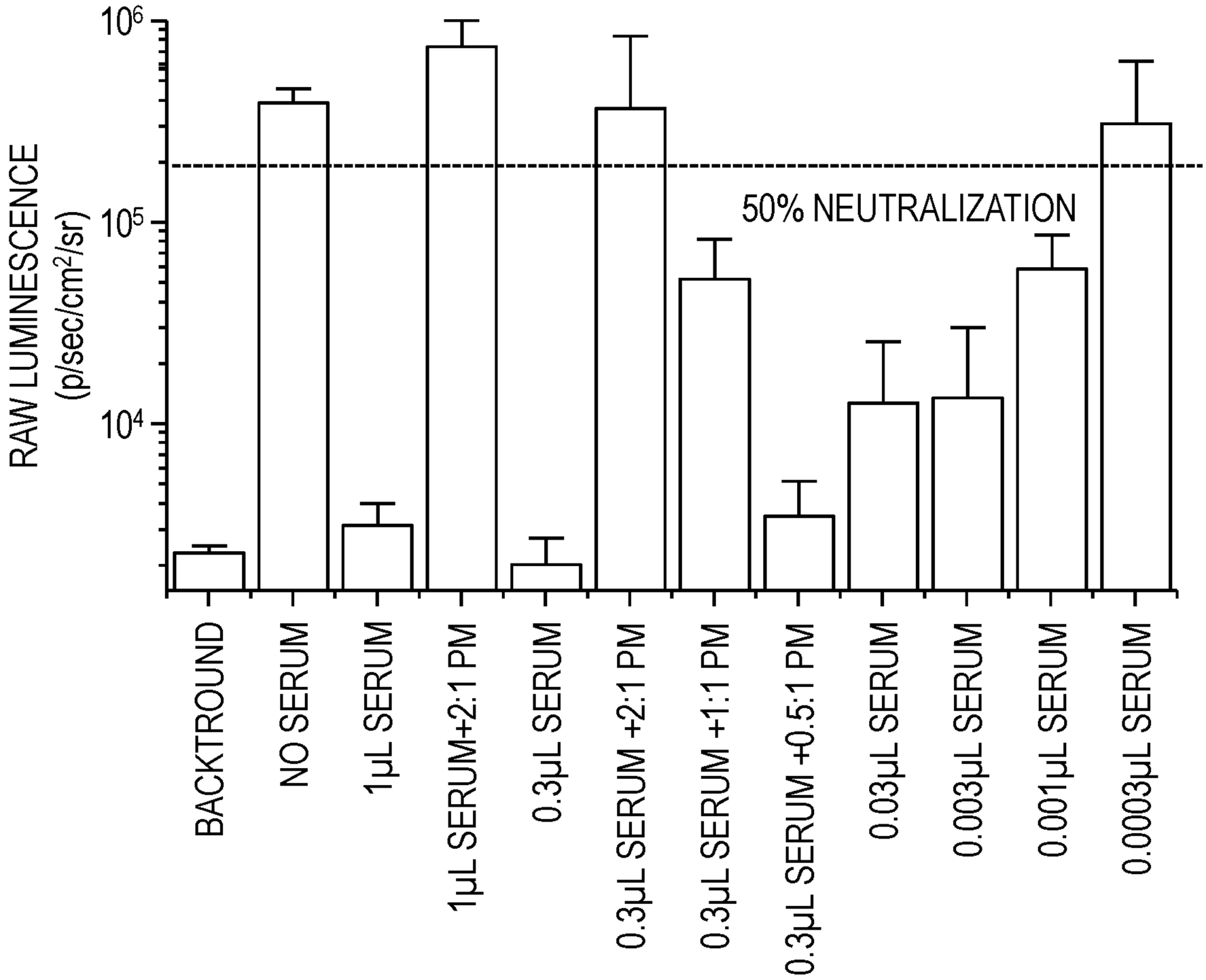

RAW LUMINESCENCE (p/sec/cm²/sr)
10⁶
10⁵
10⁴
50% NEUTRALIZATION
BACKTROUND
NO SERUM
1µL SERUM
1µL SERUM+2:1 PM
0.3µL SERUM
0.3µL SERUM +2:1 PM
0.3µL SERUM +1:1 PM
µL SERUM +0.5:1 PM
0.03µL SERUM
0.003µL SERUM
0.001µL SERUM
0.0003µL SERUM

FIG. 11

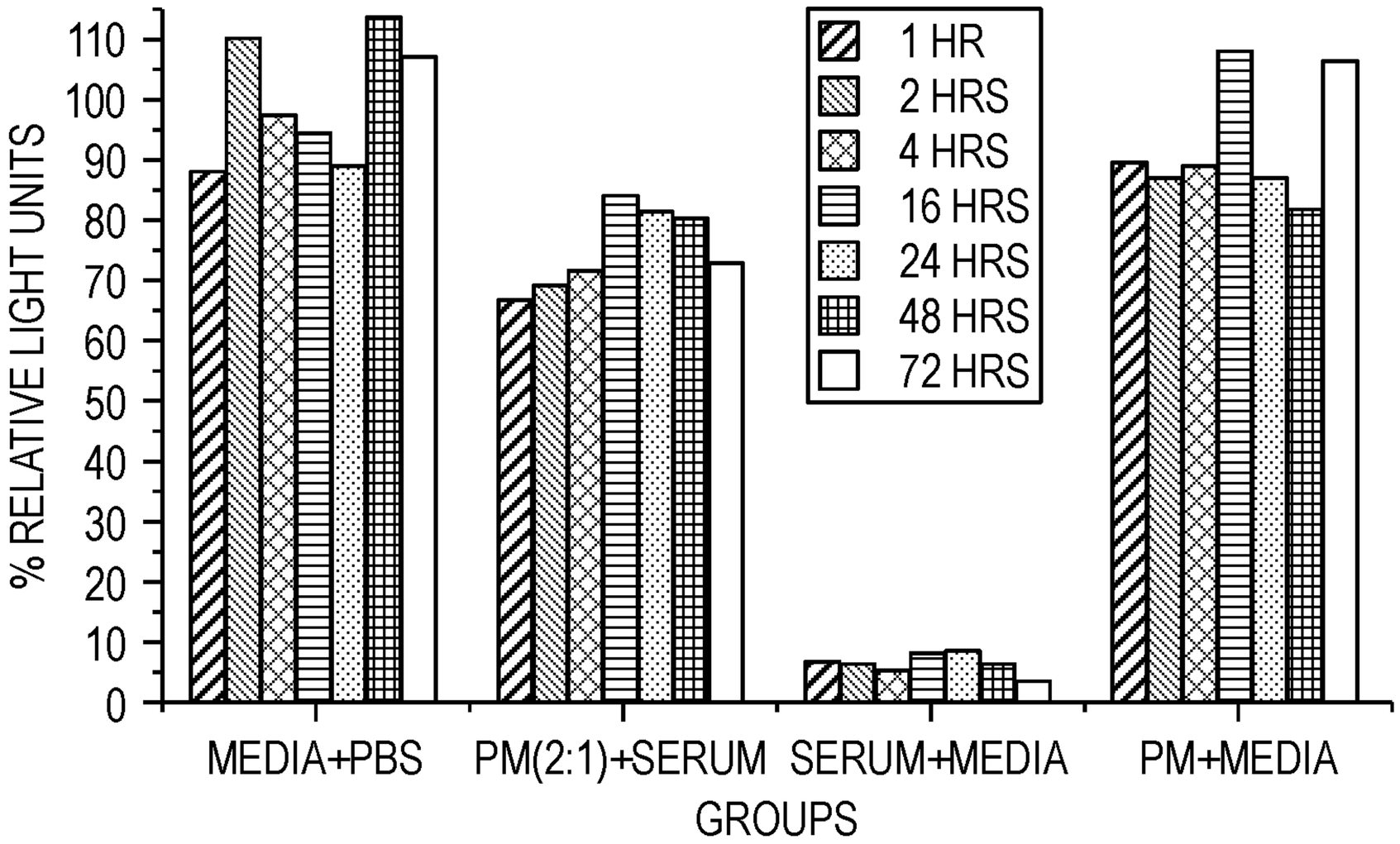

% RELATIVE LIGHT UNITS
110
100
90
80
70
60
50
40
30
20
10
0
1 HR
2 HRS
4 HRS
16 HRS
24 HRS
48 HRS
72 HRS
MEDIA+PBS
PM(2:1)+SERUM
SERUM+MEDIA
PM+MEDIA
GROUPS

FIG. 12

RAW LUMINESCENCE
4.0X10^5
3.5X10^5
3.0X10^5
2.5X10^5
2.0X10^5
1.5X10^5
1.0X10^5
0.5X10^4
0.0
BACKGROUND
NO SERUM+AAV8
0.3µl SERUM+AAV8
0.3µl SERUM+PM 2:1+ (5 MIN)+AAV8
0.3µl SERUM+PM 2:1+ (3HR)+AAV8

FIG. 13

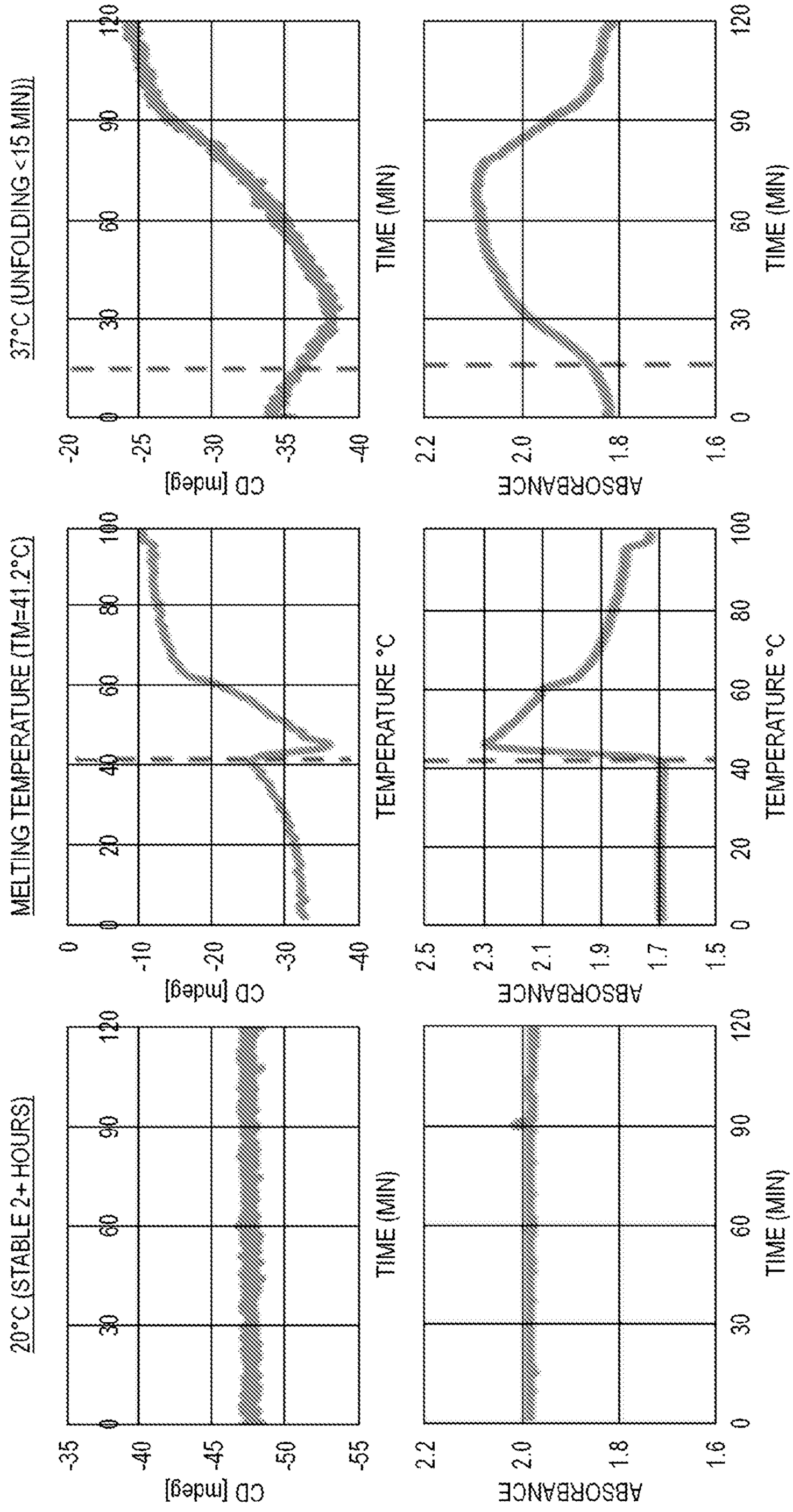

37°C (UNFOLDING <15 MIN))
MELTING TEMPERATURE (TM=41.2°C)
20°C (STABLE 2+ HOURS)
CD [mdeg]
ABSORBANCE
TIME (MIN)
TEMPERATURE °C

FIG. 14

NAME	Tm (°C)	NUMBER OF MUTATIONS	MUTATIONS
MG WT	41.9	--	---
MG 1	43.6	1	F237T
MG 8	44.9	1	S232Q
MG 13	43	1	Q282D
MG 15	49.3	3	S150E, S196R, S198P
MG 21	44.7	6	V400I, N402I, K407P, S409V, L413I, T435I
MG 22	44.1	2	V373I, V400I
MG 23	43.2	4	N402L, K407P, S409V, L413I
MG 24	44.7	1	A342V
MG 27	58.3	14	MG 29+MG 31+MG 13
MG 28	43.9	1	N274D
MG 29	55.2	6	MG 15+MG 8+MG 1+MG 24
MG 31	45.2	7	MG 21+V373I (FROM 22)
MG 33	44.2	1	A205P
MG 38	43.4	1	T355D
MG 40	43	1	T355P
MG 43	51.4	6	MG 15+MG 22+MG 24
MG 44	54.7	8	MG 22+MG 29
MG 45	43.1	2	S201C & A224C
MG 46	58.5	8	MG 29+MG 45
MG 47	56.9	8	MG 29+F390E+Y444K
MG 48	64.9	11	MG 46+MG 28+MG 33+MG 40
MG 49	55.8	7	MG 29+A391P
MP WT	44.1	--	---
MP 29	45.6	5	A77E, K125R, H170T, V165Q, A280V

FIG. 15A

NAME	Tm (°C)	NUMBER OF MUTATIONS	MUTATIONS
MG 2	42.1	1	R468Q
MG 4	42.7	1	S150E
MG 5	41.0	1	H147F
MG 10	41.5	1	S272G
MG 12	42.1	1	T355G
MG 17	41.6	3	S276E, Q277L, N279R
MG 18	42.3	1	N300Q
MG 20	41.0	1	N378Y
MG 32	42.3	1	S156K
MG 34	42.5	1	S232L
MG 35	41.0	1	A245Q
MG 36	41.7	1	S267D
MG 41	42.6	1	K225P
MG 42	41.2	1	V310E

FIG. 15B

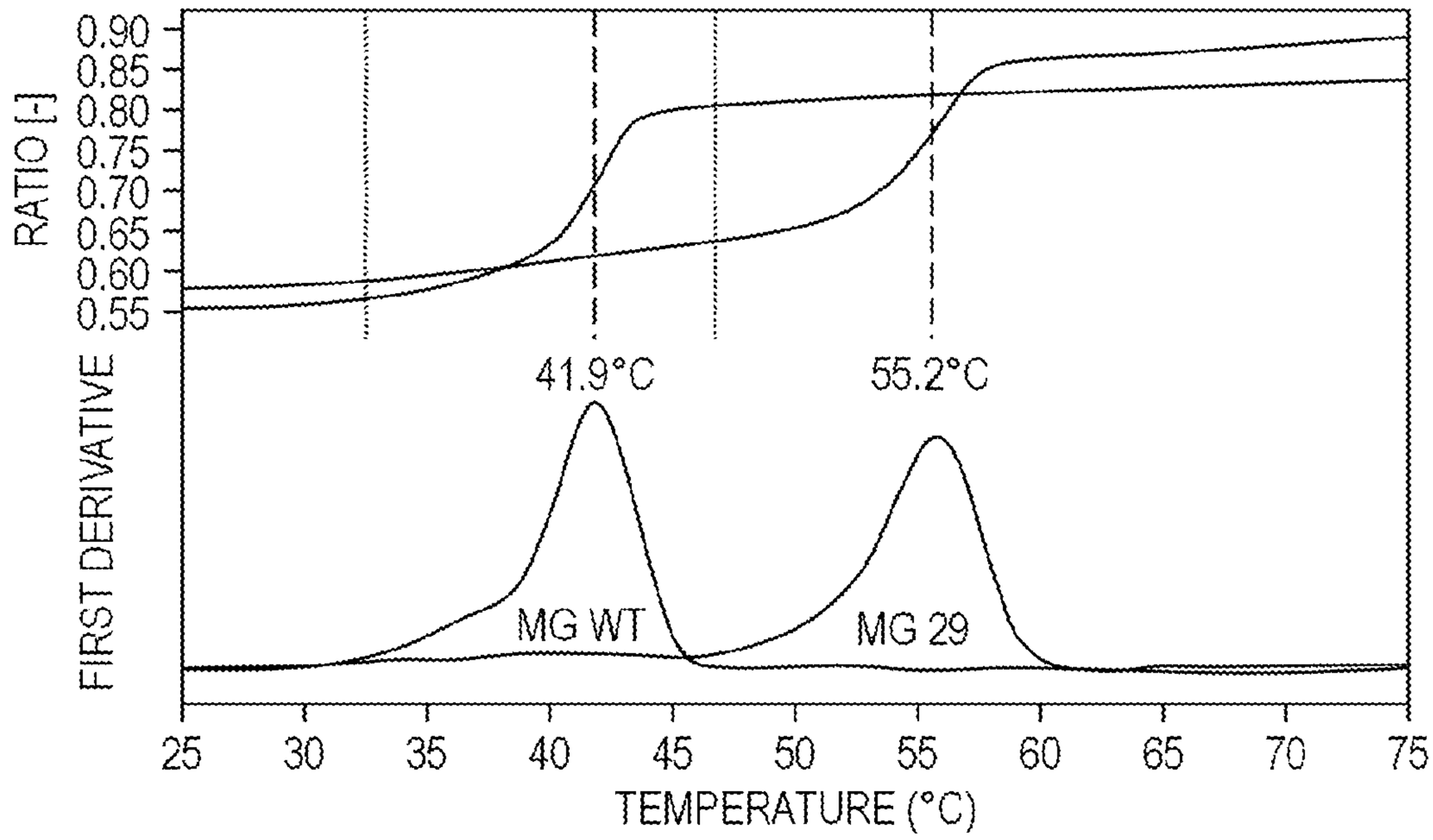


FIG. 16

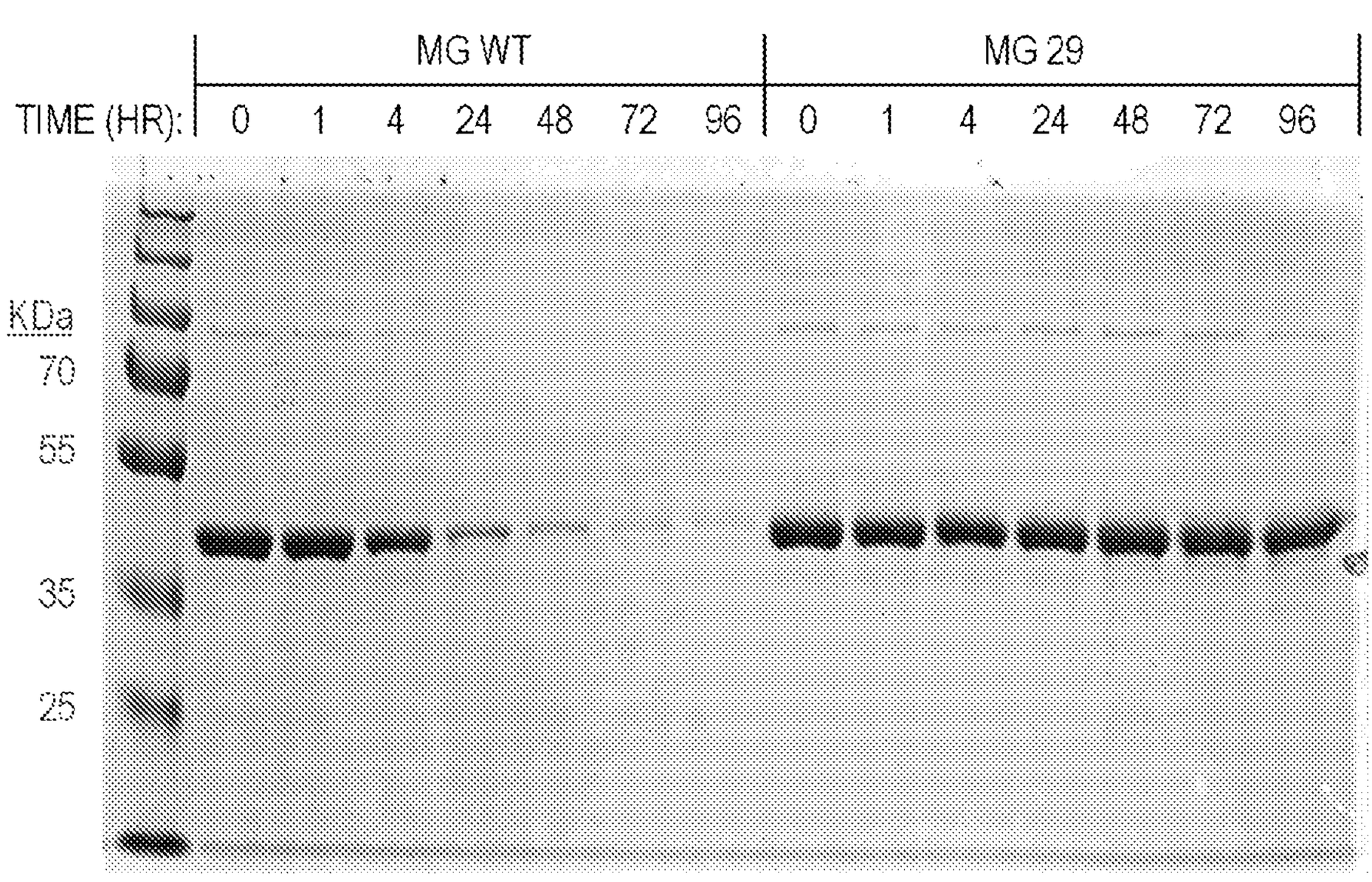


FIG. 17A

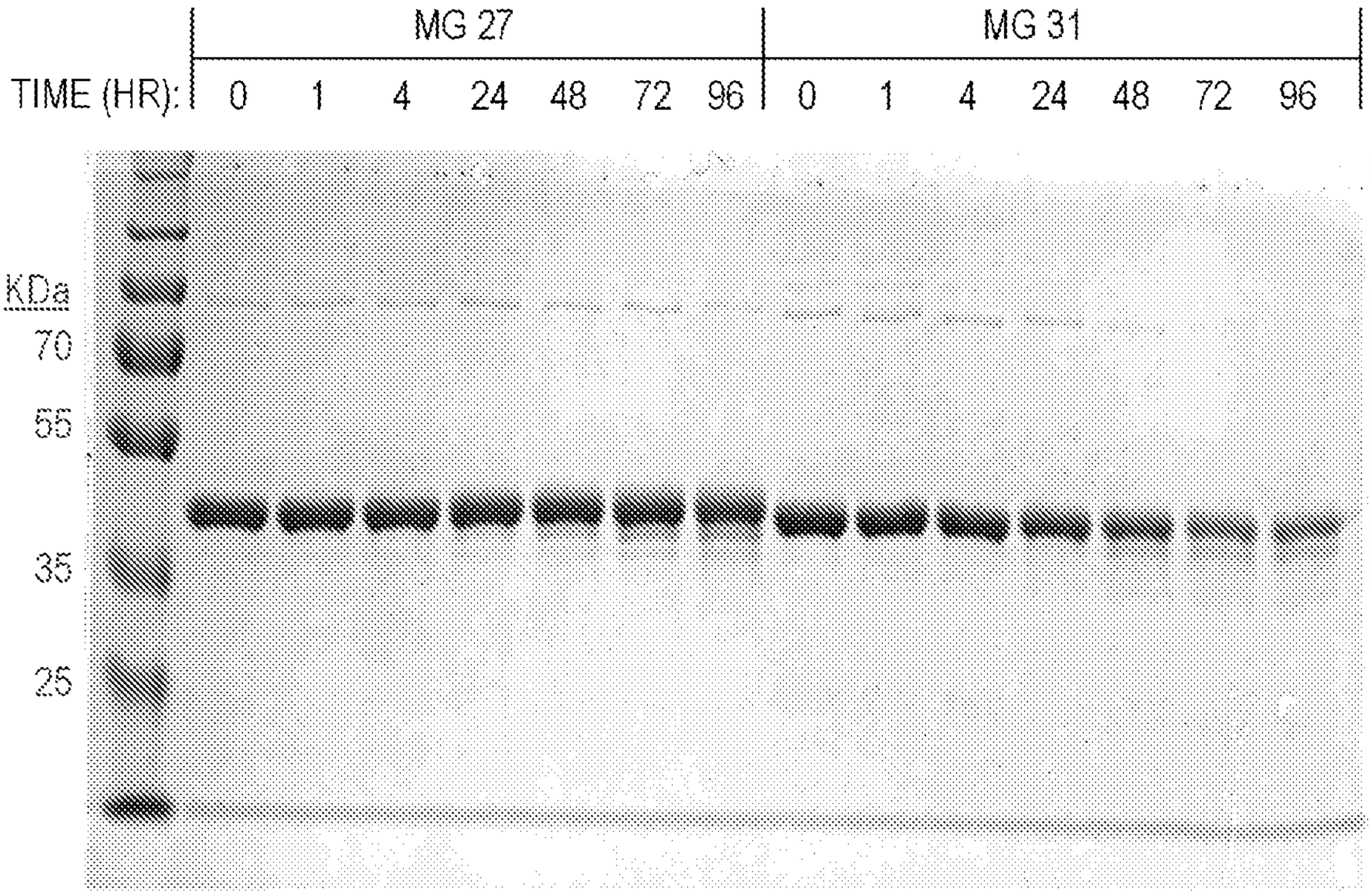


FIG. 17B

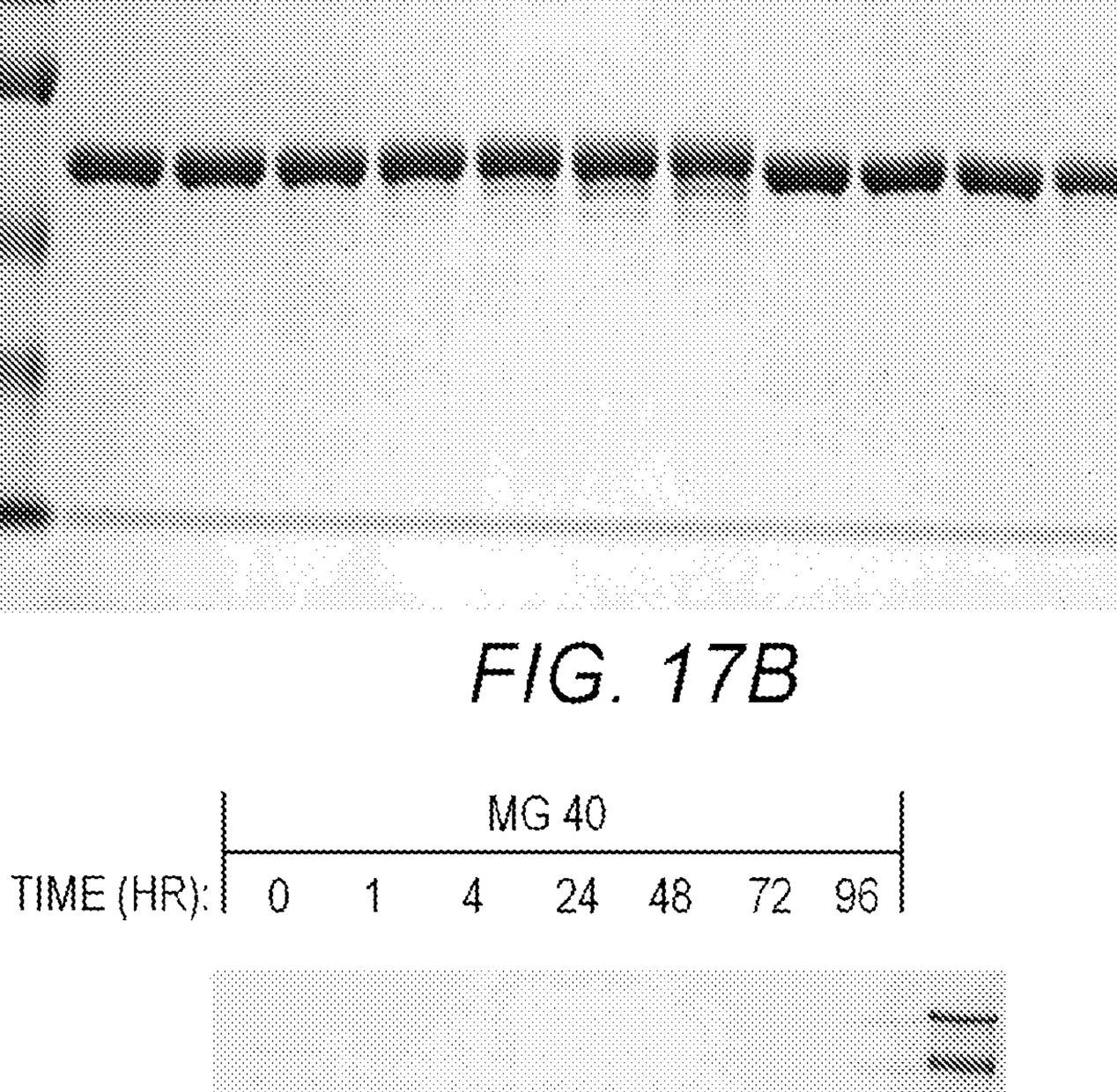


FIG. 17C

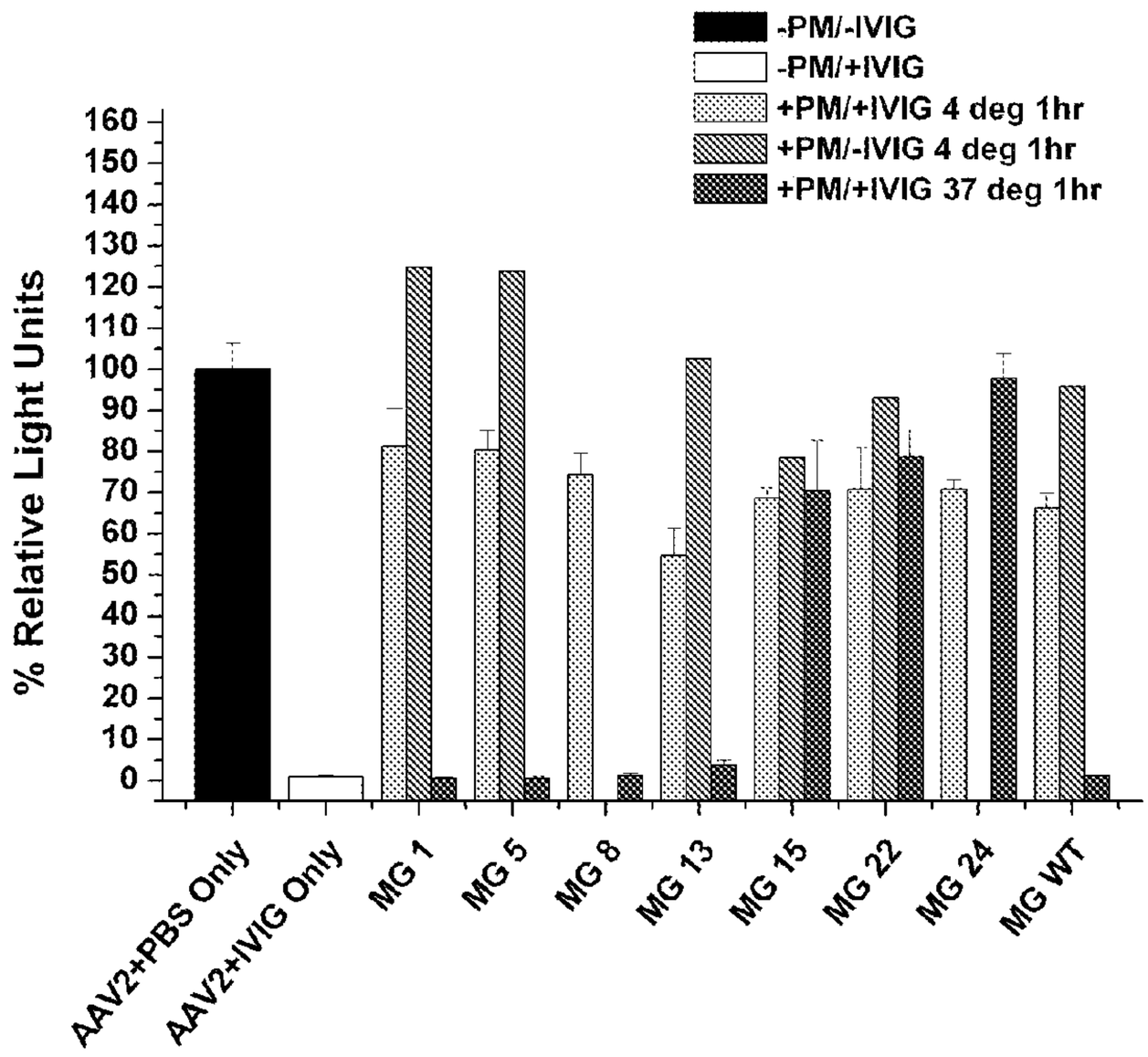


FIG. 18A

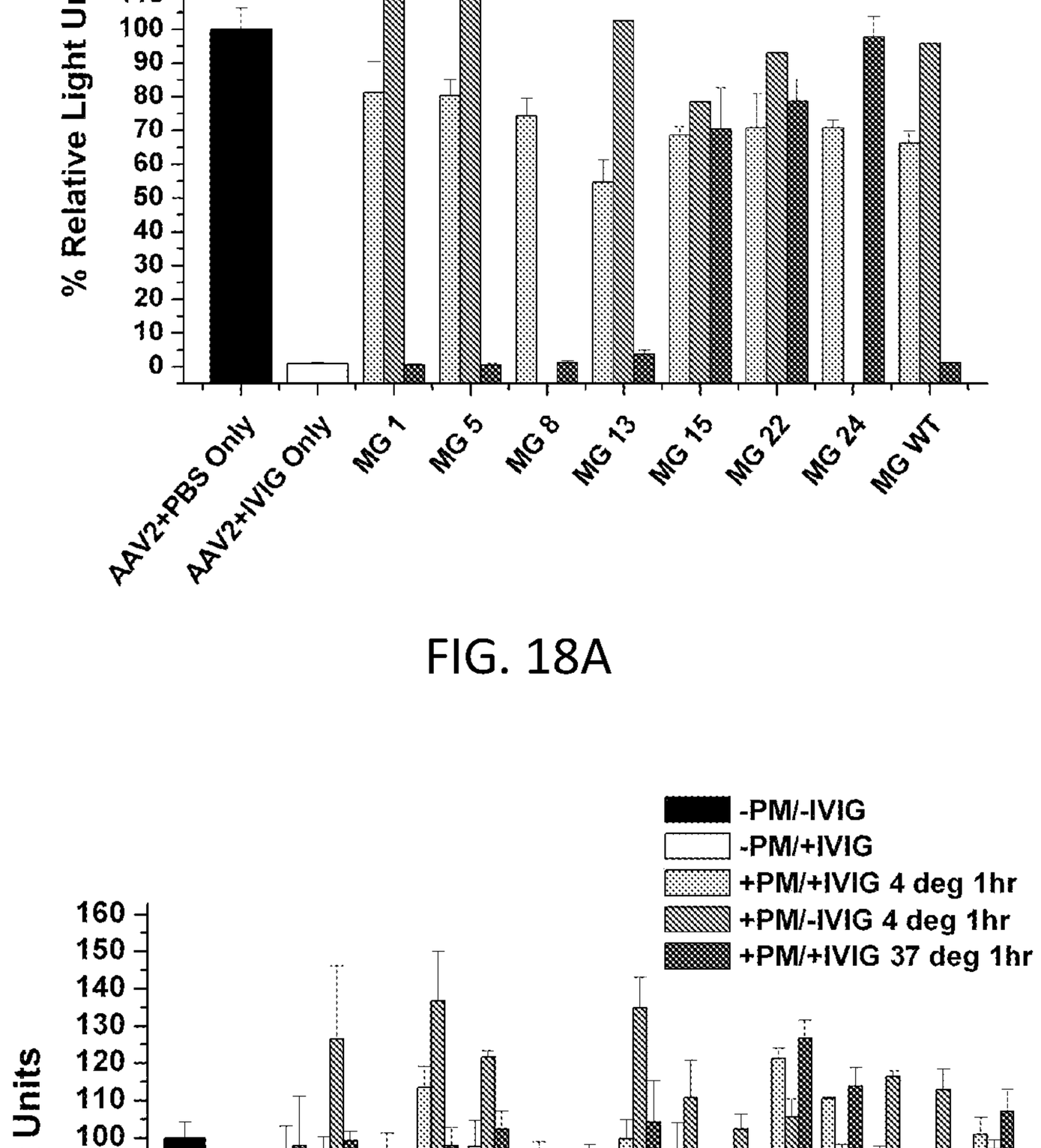


FIG. 18B

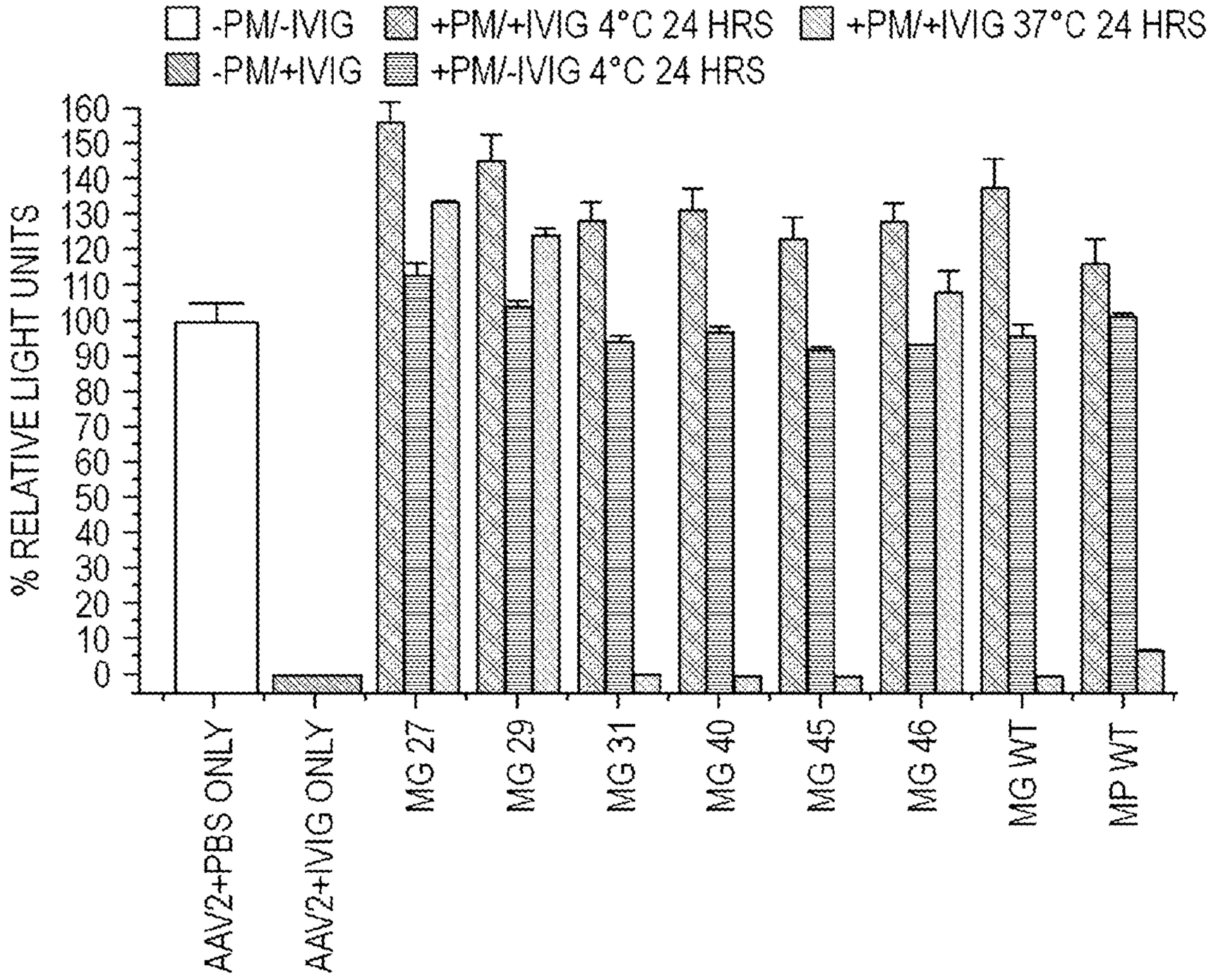
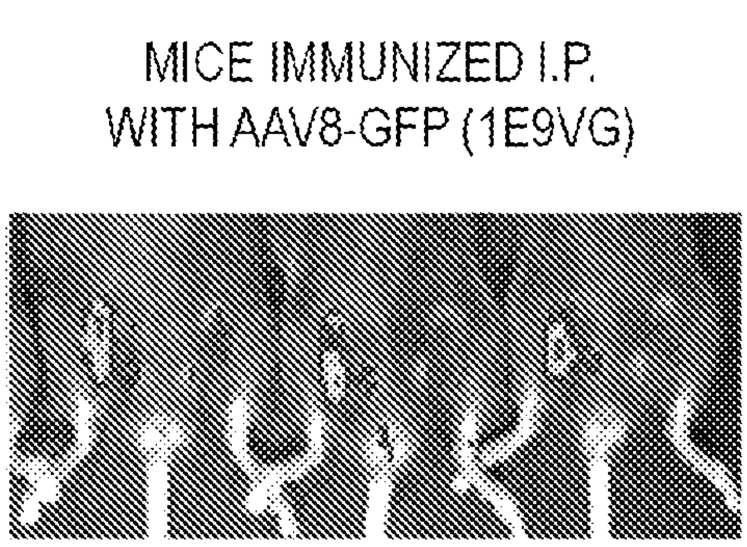
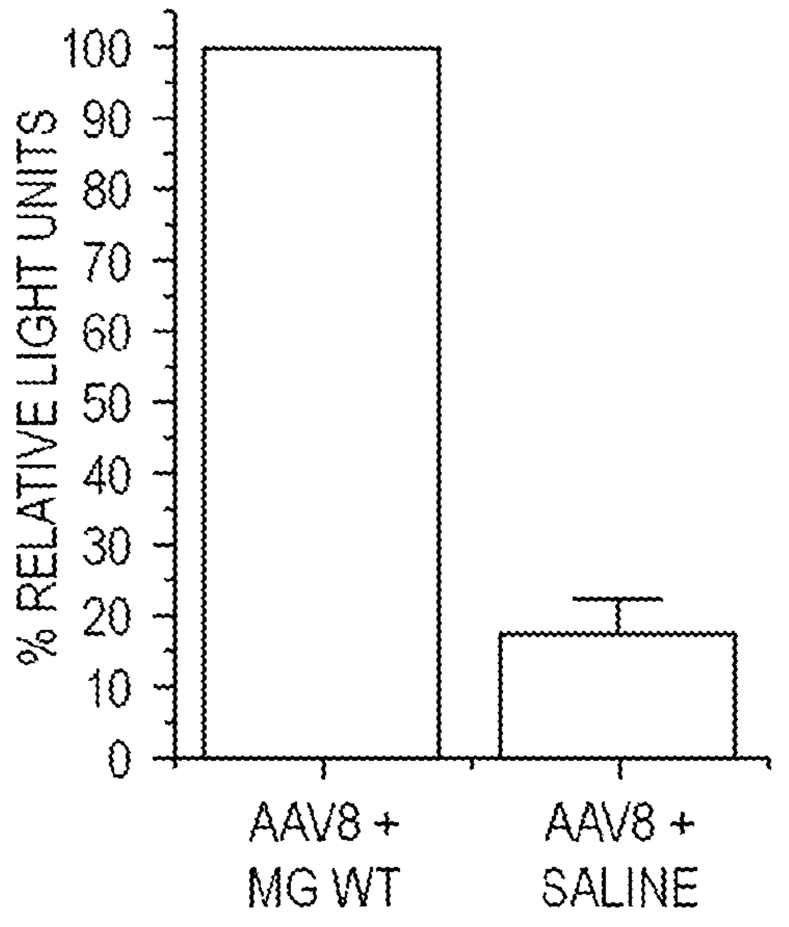


FIG. 18C

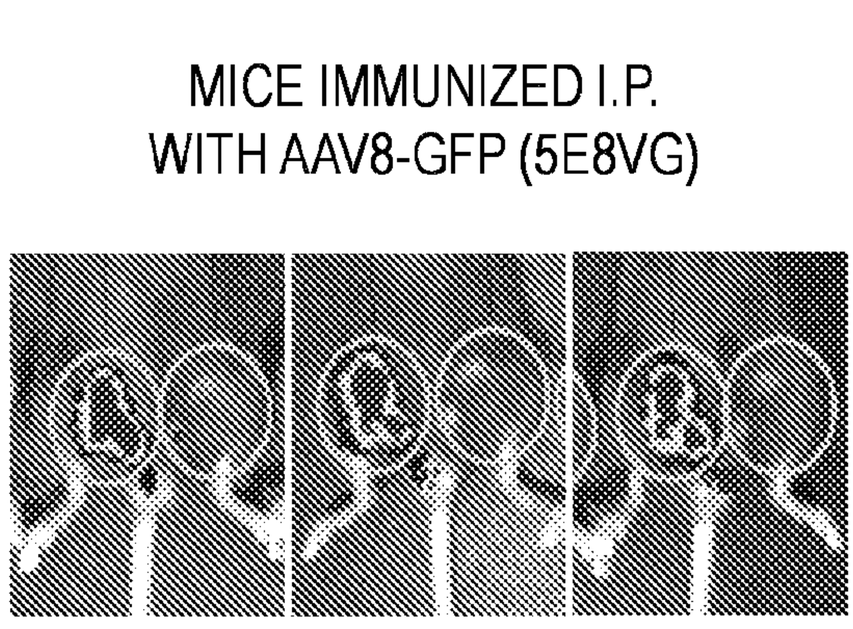


I.M. INJECTION OF AAV8-LUCIFERASE (1E9VG) EACH LEG
RIGHT LEG: AAV FORMULATED WITH MG WT (33µg)
LEFT LEG: AAV FORMULATED WITH SALINE



AVERAGE SIGNAL FROM MICE WITH AAV8 SERUM TITER 1:10

FIG. 19



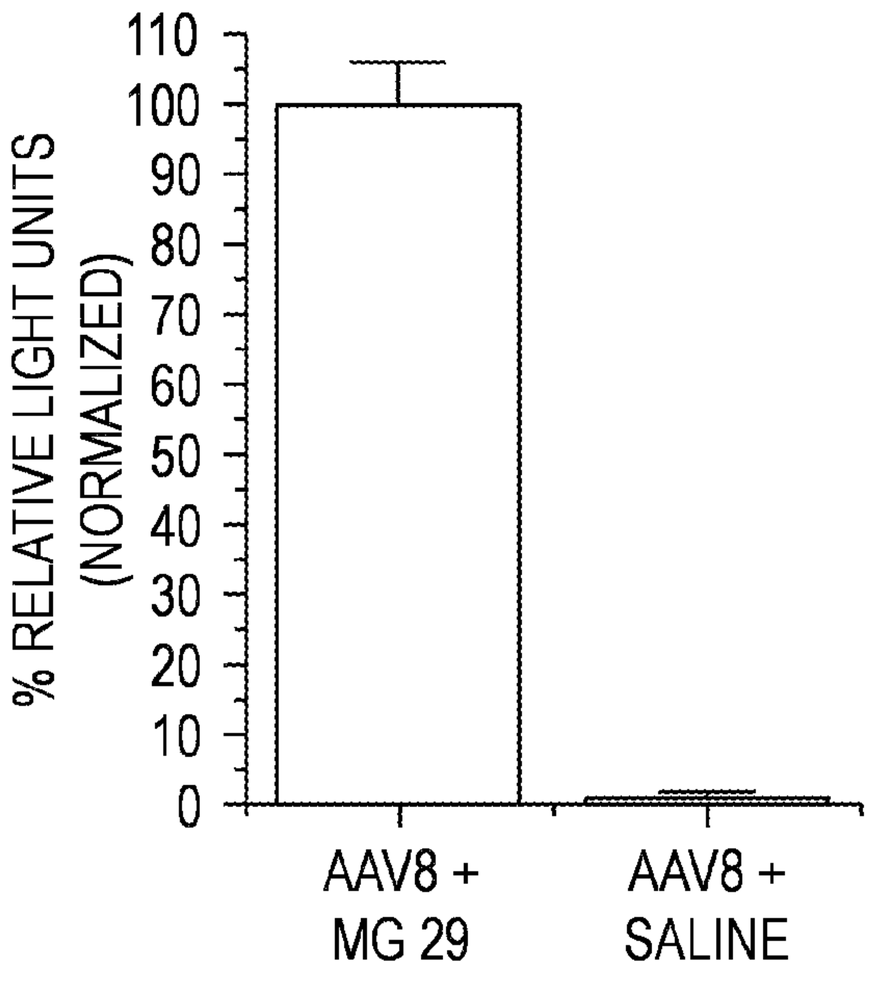


FIG. 20

NAME	K_D (nM)	K_D ERROR (nM)	K_{ON} (1/Ms)	K_{OFF} (1/s)	X^2	R^2
MG WT	11.9	0.2	2.8E+0.4	3.3E-0.4	2.5	1.00
MG 27	16.3	0.5	2.6E+0.4	4.3E-0.4	1.8	0.99
MG 29	7.9	0.1	4.3E+0.4	3.4E-0.4	1.7	1.00
MG 31	15.5	0.5	3.0E+0.4	4.7E-0.4	2.4	0.99
MG 40	10.1	0.2	3.1E+0.4	3.1E-0.4	1.9	1.00

FIG. 21

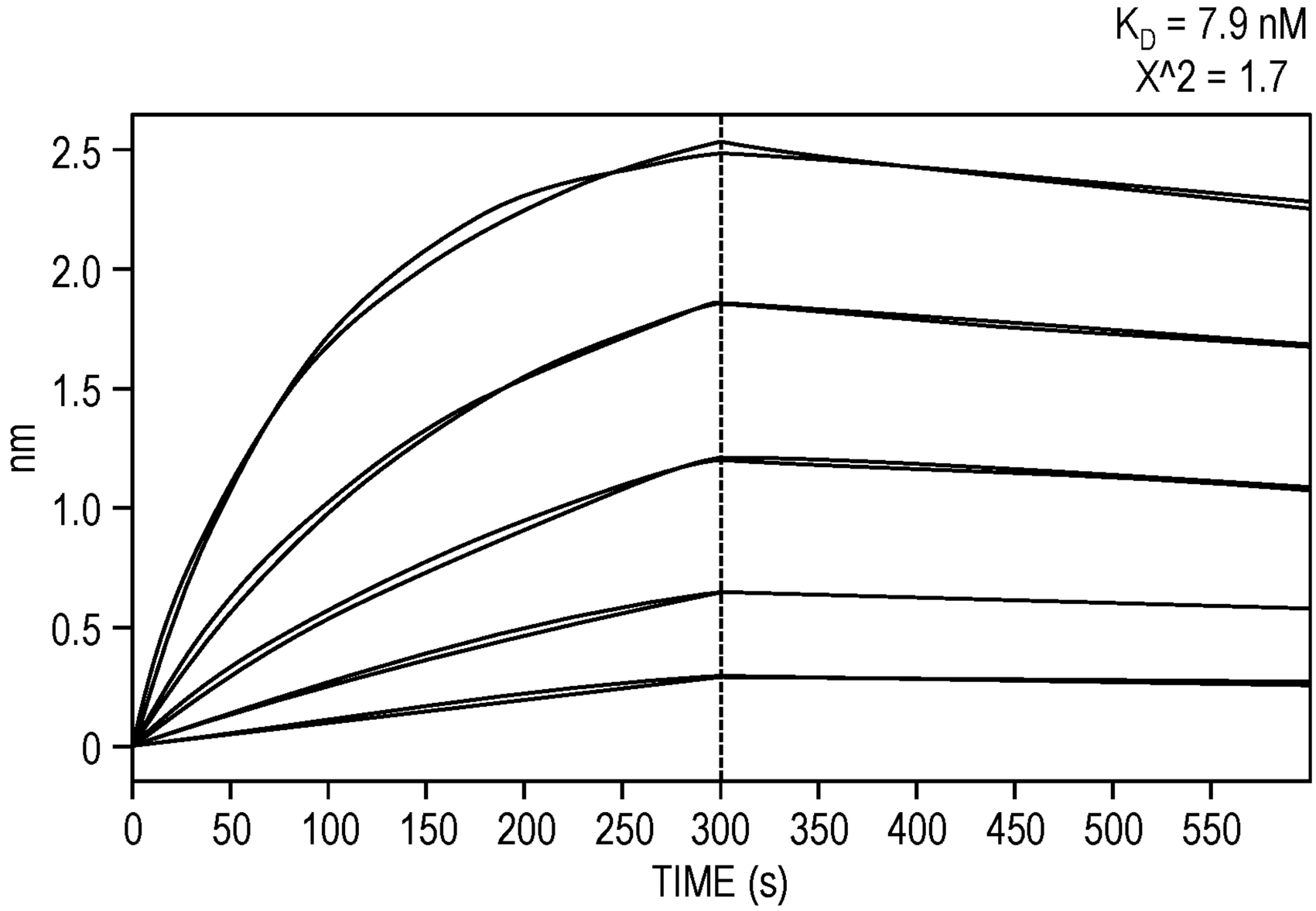


FIG. 22

	STABILIZING	NEUTRAL	DE-STABILIZING
POINT MUTANTS (N=28)	29%	50%	21%
MULTIPLE MUTANTS (N=8)	63%	25%	13%
COMBINED MUTATIONS (N=6)	100%	0%	0%
TOTAL (N=42)	45%	38%	17%

FIG. 23

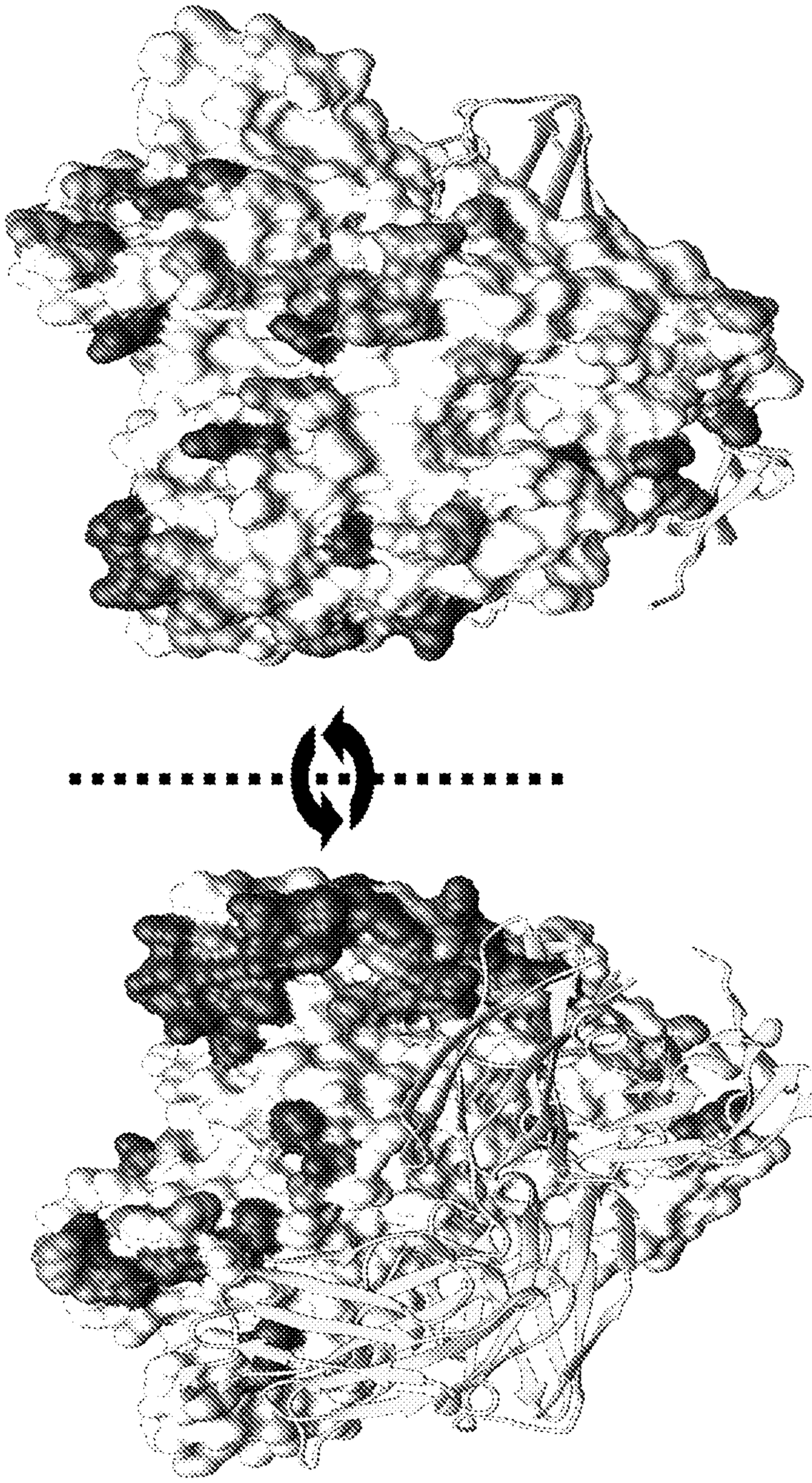


FIG. 24

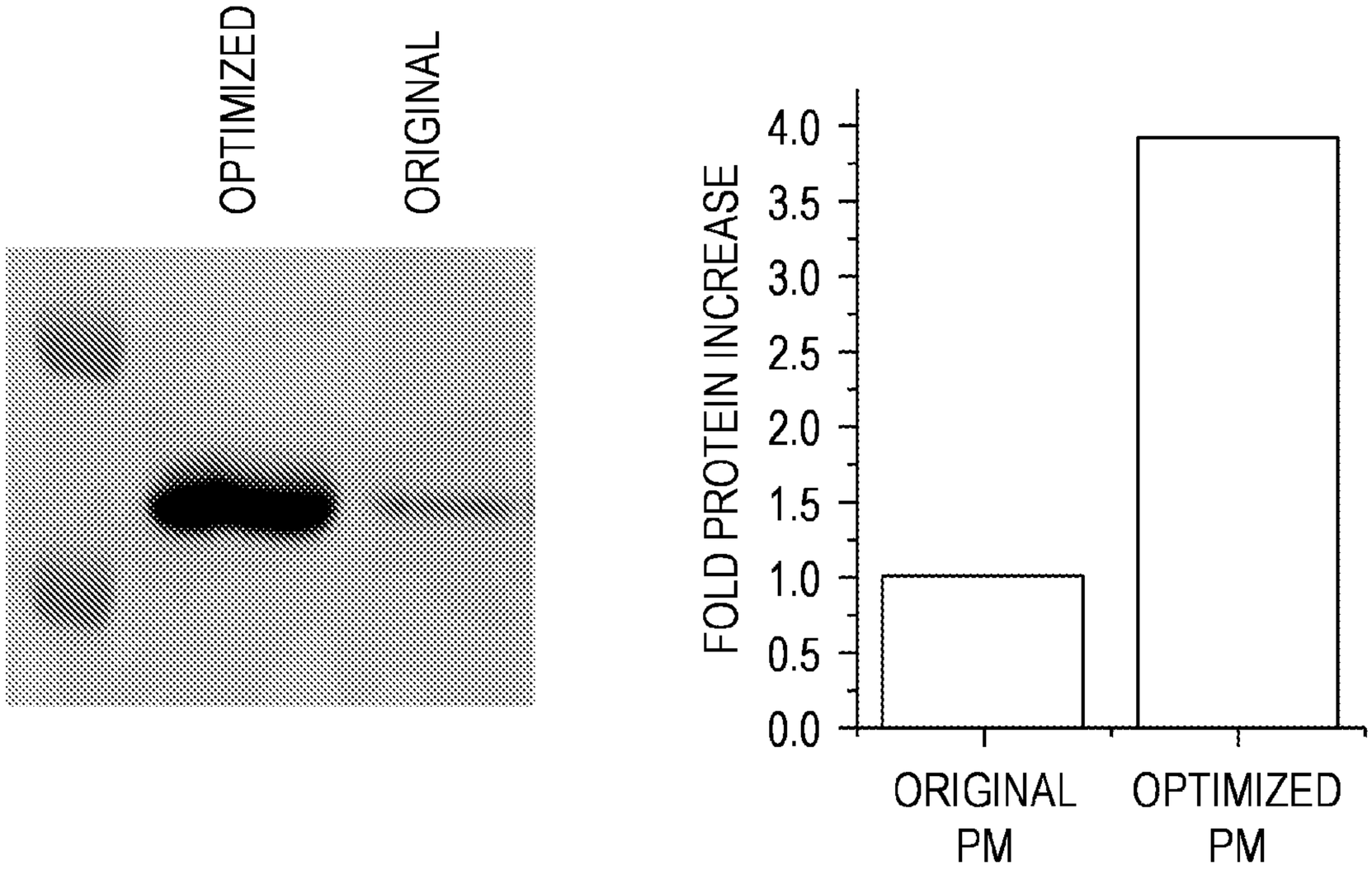

OPTIMIZED
ORIGINAL
FOLD PROTEIN INCREASE
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
ORIGINAL PM
OPTIMIZED PM

FIG. 25

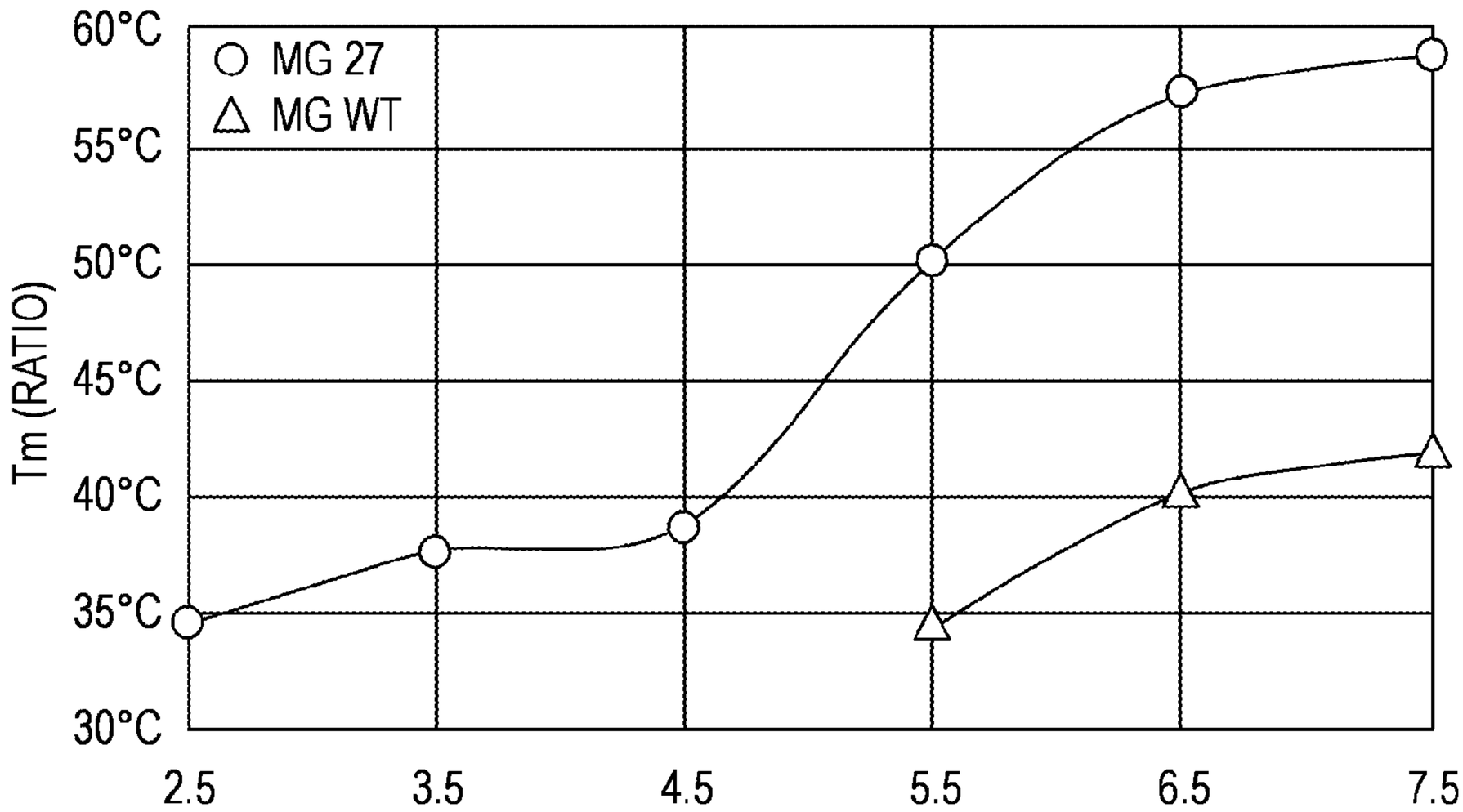

60°C
55°C
50°C
45°C
40°C
35°C
30°C
MG 27
MG WT
Tm (RATIO)
2.5
3.5
4.5
5.5
6.5
7.5
pH

FIG. 26

```
          74                                                      123
MG WT:  SLSLNDGSYQSEIDLSGGANFREKFRNFANELSEAITNSPKGLDRPVPKT
MG1:    SLSLNDGSYQSEIDLSGGANFREKFRNFANELSEAITNSPKGLDRPVPKT
MG8:    SLSLNDGSYQSEIDLSGGANFREKFRNFANELSEAITNSPKGLDRPVPKT
MG13:   SLSLNDGSYQSEIDLSGGANFREKFRNFANELSEAITNSPKGLDRPVPKT
MG15:   SLSLNDGSYQSEIDLSGGANFREKFRNFANELSEAITNSPKGLDRPVPKT
MG21:   SLSLNDGSYQSEIDLSGGANFREKFRNFANELSEAITNSPKGLDRPVPKT
MG22:   SLSLNDGSYQSEIDLSGGANFREKFRNFANELSEAITNSPKGLDRPVPKT
MG23:   SLSLNDGSYQSEIDLSGGANFREKFRNFANELSEAITNSPKGLDRPVPKT
MG24:   SLSLNDGSYQSEIDLSGGANFREKFRNFANELSEAITNSPKGLDRPVPKT
MG27:   SLSLNDGSYQSEIDLSGGANFREKFRNFANELSEAITNSPKGLDRPVPKT
MG28:   SLSLNDGSYQSEIDLSGGANFREKFRNFANELSEAITNSPKGLDRPVPKT
MG29:   SLSLNDGSYQSEIDLSGGANFREKFRNFANELSEAITNSPKGLDRPVPKT
MG31:   SLSLNDGSYQSEIDLSGGANFREKFRNFANELSEAITNSPKGLDRPVPKT
MG33:   SLSLNDGSYQSEIDLSGGANFREKFRNFANELSEAITNSPKGLDRPVPKT
MG38:   SLSLNDGSYQSEIDLSGGANFREKFRNFANELSEAITNSPKGLDRPVPKT
MG40:   SLSLNDGSYQSEIDLSGGANFREKFRNFANELSEAITNSPKGLDRPVPKT
MG43:   SLSLNDGSYQSEIDLSGGANFREKFRNFANELSEAITNSPKGLDRPVPKT
MG44:   SLSLNDGSYQSEIDLSGGANFREKFRNFANELSEAITNSPKGLDRPVPKT
MG45:   SLSLNDGSYQSEIDLSGGANFREKFRNFANELSEAITNSPKGLDRPVPKT
MG46:   SLSLNDGSYQSEIDLSGGANFREKFRNFANELSEAITNSPKGLDRPVPKT

          124                                                     173
MG WT:  EISGLIKTGDNFITPSFKAGYYDHVASDGSLLSYYQSTEYFNNRVLMPIL
MG1:    EISGLIKTGDNFITPSFKAGYYDHVASDGSLLSYYQSTEYFNNRVLMPIL
MG8:    EISGLIKTGDNFITPSFKAGYYDHVASDGSLLSYYQSTEYFNNRVLMPIL
MG13:   EISGLIKTGDNFITPSFKAGYYDHVASDGSLLSYYQSTEYFNNRVLMPIL
MG15:   EISGLIKTGDNFITPSFKAGYYDHVAEDGSLLSYYQSTEYFNNRVLMPIL
MG21:   EISGLIKTGDNFITPSFKAGYYDHVASDGSLLSYYQSTEYFNNRVLMPIL
MG22:   EISGLIKTGDNFITPSFKAGYYDHVASDGSLLSYYQSTEYFNNRVLMPIL
MG23:   EISGLIKTGDNFITPSFKAGYYDHVASDGSLLSYYQSTEYFNNRVLMPIL
MG24:   EISGLIKTGDNFITPSFKAGYYDHVASDGSLLSYYQSTEYFNNRVLMPIL
MG27:   EISGLIKTGDNFITPSFKAGYYDHVAEDGSLLSYYQSTEYFNNRVLMPIL
MG28:   EISGLIKTGDNFITPSFKAGYYDHVASDGSLLSYYQSTEYFNNRVLMPIL
MG29:   EISGLIKTGDNFITPSFKAGYYDHVAEDGSLLSYYQSTEYFNNRVLMPIL
MG31:   EISGLIKTGDNFITPSFKAGYYDHVASDGSLLSYYQSTEYFNNRVLMPIL
MG33:   EISGLIKTGDNFITPSFKAGYYDHVASDGSLLSYYQSTEYFNNRVLMPIL
MG38:   EISGLIKTGDNFITPSFKAGYYDHVASDGSLLSYYQSTEYFNNRVLMPIL
MG40:   EISGLIKTGDNFITPSFKAGYYDHVASDGSLLSYYQSTEYFNNRVLMPIL
MG43:   EISGLIKTGDNFITPSFKAGYYDHVAEDGSLLSYYQSTEYFNNRVLMPIL
MG44:   EISGLIKTGDNFITPSFKAGYYDHVAEDGSLLSYYQSTEYFNNRVLMPIL
MG45:   EISGLIKTGDNFITPSFKAGYYDHVASDGSLLSYYQSTEYFNNRVLMPIL
MG46:   EISGLIKTGDNFITPSFKAGYYDHVAEDGSLLSYYQSTEYFNNRVLMPIL
```

FIG. 27A

```
        174                                                                 223
MG WT:  QTTNGTLMANNRGYDDVFRQVPSFSGWSNTKATTVSTSNNLTYDKWTYFA
MG1:    QTTNGTLMANNRGYDDVFRQVPSFSGWSNTKATTVSTSNNLTYDKWTYFA
MG8:    QTTNGTLMANNRGYDDVFRQVPSFSGWSNTKATTVSTSNNLTYDKWTYFA
MG13:   QTTNGTLMANNRGYDDVFRQVPSFSGWSNTKATTVSTSNNLTYDKWTYFA
MG15:   QTTNGTLMANNRGYDDVFRQVPRFPGWSNTKATTVSTSNNLTYDKWTYFA
MG21:   QTTNGTLMANNRGYDDVFRQVPSFSGWSNTKATTVSTSNNLTYDKWTYFA
MG22:   QTTNGTLMANNRGYDDVFRQVPSFSGWSNTKATTVSTSNNLTYDKWTYFA
MG23:   QTTNGTLMANNRGYDDVFRQVPSFSGWSNTKATTVSTSNNLTYDKWTYFA
MG24:   QTTNGTLMANNRGYDDVFRQVPSFSGWSNTKATTVSTSNNLTYDKWTYFA
MG27:   QTTNGTLMANNRGYDDVFRQVPRFPGWSNTKATTVSTSNNLTYDKWTYFA
MG28:   QTTNGTLMANNRGYDDVFRQVPSFSGWSNTKATTVSTSNNLTYDKWTYFA
MG29:   QTTNGTLMANNRGYDDVFRQVPRFPGWSNTKATTVSTSNNLTYDKWTYFA
MG31:   QTTNGTLMANNRGYDDVFRQVPSFSGWSNTKATTVSTSNNLTYDKWTYFA
MG33:   QTTNGTLMANNRGYDDVFRQVPSFSGWSNTKPTTVSTSNNLTYDKWTYFA
MG38:   QTTNGTLMANNRGYDDVFRQVPSFSGWSNTKATTVSTSNNLTYDKWTYFA
MG40:   QTTNGTLMANNRGYDDVFRQVPSFSGWSNTKATTVSTSNNLTYDKWTYFA
MG43:   QTTNGTLMANNRGYDDVFRQVPRFPGWSNTKATTVSTSNNLTYDKWTYFA
MG44:   QTTNGTLMANNRGYDDVFRQVPRFPGWSNTKATTVSTSNNLTYDKWTYFA
MG45:   QTTNGTLMANNRGYDDVFRQVPSFSGWCNTKATTVSTSNNLTYDKWTYFA
MG46:   QTTNGTLMANNRGYDDVFRQVPRFPGWCNTKATTVSTSNNLTYDKWTYFA

        224                                                                 273
MG WT:  AKGSPLYDSYPNHFFEDVKTLAIDAKDISALKTTIDSEKPTYLIIRGLSG
MG1:    AKGSPLYDSYPNHTFEDVKTLAIDAKDISALKTTIDSEKPTYLIIRGLSG
MG8:    AKGSPLYDQYPNHFFEDVKTLAIDAKDISALKTTIDSEKPTYLIIRGLSG
MG13:   AKGSPLYDSYPNHFFEDVKTLAIDAKDISALKTTIDSEKPTYLIIRGLSG
MG15:   AKGSPLYDSYPNHFFEDVKTLAIDAKDISALKTTIDSEKPTYLIIRGLSG
MG21:   AKGSPLYDSYPNHFFEDVKTLAIDAKDISALKTTIDSEKPTYLIIRGLSG
MG22:   AKGSPLYDSYPNHFFEDVKTLAIDAKDISALKTTIDSEKPTYLIIRGLSG
MG23:   AKGSPLYDSYPNHFFEDVKTLAIDAKDISALKTTIDSEKPTYLIIRGLSG
MG24:   AKGSPLYDSYPNHFFEDVKTLAIDAKDISALKTTIDSEKPTYLIIRGLSG
MG27:   AKGSPLYDQYPNHTFEDVKTLAIDAKDISALKTTIDSEKPTYLIIRGLSG
MG28:   AKGSPLYDSYPNHFFEDVKTLAIDAKDISALKTTIDSEKPTYLIIRGLSG
MG29:   AKGSPLYDQYPNHTFEDVKTLAIDAKDISALKTTIDSEKPTYLIIRGLSG
MG31:   AKGSPLYDSYPNHFFEDVKTLAIDAKDISALKTTIDSEKPTYLIIRGLSG
MG33:   AKGSPLYDSYPNHFFEDVKTLAIDAKDISALKTTIDSEKPTYLIIRGLSG
MG38:   AKGSPLYDSYPNHFFEDVKTLAIDAKDISALKTTIDSEKPTYLIIRGLSG
MG40:   AKGSPLYDSYPNHFFEDVKTLAIDAKDISALKTTIDSEKPTYLIIRGLSG
MG43:   AKGSPLYDSYPNHFFEDVKTLAIDAKDISALKTTIDSEKPTYLIIRGLSG
MG44:   AKGSPLYDQYPNHTFEDVKTLAIDAKDISALKTTIDSEKPTYLIIRGLSG
MG45:   CKGSPLYDSYPNHFFEDVKTLAIDAKDISALKTTIDSEKPTYLIIRGLSG
MG46:   CKGSPLYDQYPNHTFEDVKTLAIDAKDISALKTTIDSEKPTYLIIRGLSG
```

FIG. 27B

```
        274                                                                 323
MG WT:  NGSQLNELQLPESVKKVSLYGDYTGVNVAKQIFANVVELEFYSTSKANSF
MG1:    NGSQLNELQLPESVKKVSLYGDYTGVNVAKQIFANVVELEFYSTSKANSF
MG8:    NGSQLNELQLPESVKKVSLYGDYTGVNVAKQIFANVVELEFYSTSKANSF
MG13:   NGSQLNELDLPESVKKVSLYGDYTGVNVAKQIFANVVELEFYSTSKANSF
MG15:   NGSQLNELQLPESVKKVSLYGDYTGVNVAKQIFANVVELEFYSTSKANSF
MG21:   NGSQLNELQLPESVKKVSLYGDYTGVNVAKQIFANVVELEFYSTSKANSF
MG22:   NGSQLNELQLPESVKKVSLYGDYTGVNVAKQIFANVVELEFYSTSKANSF
MG23:   NGSQLNELQLPESVKKVSLYGDYTGVNVAKQIFANVVELEFYSTSKANSF
MG24:   NGSQLNELQLPESVKKVSLYGDYTGVNVAKQIFANVVELEFYSTSKANSF
MG27:   NGSQLNELDLPESVKKVSLYGDYTGVNVAKQIFANVVELEFYSTSKANSF
MG28:   DGSQLNELQLPESVKKVSLYGDYTGVNVAKQIFANVVELEFYSTSKANSF
MG29:   NGSQLNELQLPESVKKVSLYGDYTGVNVAKQIFANVVELEFYSTSKANSF
MG31:   NGSQLNELQLPESVKKVSLYGDYTGVNVAKQIFANVVELEFYSTSKANSF
MG33:   NGSQLNELQLPESVKKVSLYGDYTGVNVAKQIFANVVELEFYSTSKANSF
MG38:   NGSQLNELQLPESVKKVSLYGDYTGVNVAKQIFANVVELEFYSTSKANSF
MG40:   NGSQLNELQLPESVKKVSLYGDYTGVNVAKQIFANVVELEFYSTSKANSF
MG43:   NGSQLNELQLPESVKKVSLYGDYTGVNVAKQIFANVVELEFYSTSKANSF
MG44:   NGSQLNELQLPESVKKVSLYGDYTGVNVAKQIFANVVELEFYSTSKANSF
MG45:   NGSQLNELQLPESVKKVSLYGDYTGVNVAKQIFANVVELEFYSTSKANSF
MG46:   NGSQLNELQLPESVKKVSLYGDYTGVNVAKQIFANVVELEFYSTSKANSF

        324                                                                 373
MG WT:  GFNPLVLGSKTNVINDLFASKPFTHIDLTQVTLQNSDNSAIDANKLKQAV
MG1:    GFNPLVLGSKTNVINDLFASKPFTHIDLTQVTLQNSDNSAIDANKLKQAV
MG8:    GFNPLVLGSKTNVINDLFASKPFTHIDLTQVTLQNSDNSAIDANKLKQAV
MG13:   GFNPLVLGSKTNVINDLFASKPFTHIDLTQVTLQNSDNSAIDANKLKQAV
MG15:   GFNPLVLGSKTNVINDLFASKPFTHIDLTQVTLQNSDNSAIDANKLKQAV
MG21:   GFNPLVLGSKTNVINDLFASKPFTHIDLTQVTLQNSDNSAIDANKLKQAV
MG22:   GFNPLVLGSKTNVINDLFASKPFTHIDLTQVTLQNSDNSAIDANKLKQAI
MG23:   GFNPLVLGSKTNVINDLFASKPFTHIDLTQVTLQNSDNSAIDANKLKQAV
MG24:   GFNPLVLGSKTNVINDLFVSKPFTHIDLTQVTLQNSDNSAIDANKLKQAV
MG27:   GFNPLVLGSKTNVINDLFVSKPFTHIDLTQVTLQNSDNSAIDANKLKQAI
MG28:   GFNPLVLGSKTNVINDLFASKPFTHIDLTQVTLQNSDNSAIDANKLKQAV
MG29:   GFNPLVLGSKTNVINDLFVSKPFTHIDLTQVTLQNSDNSAIDANKLKQAV
MG31:   GFNPLVLGSKTNVINDLFASKPFTHIDLTQVTLQNSDNSAIDANKLKQAI
MG33:   GFNPLVLGSKTNVINDLFASKPFTHIDLTQVTLQNSDNSAIDANKLKQAV
MG38:   GFNPLVLGSKTNVINDLFASKPFTHIDLTQVDLQNSDNSAIDANKLKQAV
MG40:   GFNPLVLGSKTNVINDLFASKPFTHIDLTQVPLQNSDNSAIDANKLKQAV
MG43:   GFNPLVLGSKTNVINDLFVSKPFTHIDLTQVTLQNSDNSAIDANKLKQAI
MG44:   GFNPLVLGSKTNVINDLFVSKPFTHIDLTQVTLQNSDNSAIDANKLKQAI
MG45:   GFNPLVLGSKTNVINDLFASKPFTHIDLTQVTLQNSDNSAIDANKLKQAV
MG46:   GFNPLVLGSKTNVINDLFVSKPFTHIDLTQVTLQNSDNSAIDANKLKQAV
```

FIG. 27C

```
        374                                                     423
MG WT:  GDIYNYRRFERQFQGYFAGGYIDKYLVKNVNTNKDSDDDLVYRSLKELNL
MG1:    GDIYNYRRFERQFQGYFAGGYIDKYLVKNVNTNKDSDDDLVYRSLKELNL
MG8:    GDIYNYRRFERQFQGYFAGGYIDKYLVKNVNTNKDSDDDLVYRSLKELNL
MG13:   GDIYNYRRFERQFQGYFAGGYIDKYLVKNVNTNKDSDDDLVYRSLKELNL
MG15:   GDIYNYRRFERQFQGYFAGGYIDKYLVKNVNTNKDSDDDLVYRSLKELNL
MG21:   GDIYNYRRFERQFQGYFAGGYIDKYLIKIVNTNPDVDDDIVYRSLKELNL
MG22:   GDIYNYRRFERQFQGYFAGGYIDKYLIKNVNTNKDSDDDLVYRSLKELNL
MG23:   GDIYNYRRFERQFQGYFAGGYIDKYLVKIVNTNPDVDDDIVYRSLKELNL
MG24:   GDIYNYRRFERQFQGYFAGGYIDKYLVKNVNTNKDSDDDLVYRSLKELNL
MG27:   GDIYNYRRFERQFQGYFAGGYIDKYLIKIVNTNPDVDDDIVYRSLKELNL
MG28:   GDIYNYRRFERQFQGYFAGGYIDKYLVKNVNTNKDSDDDLVYRSLKELNL
MG29:   GDIYNYRRFERQFQGYFAGGYIDKYLVKNVNTNKDSDDDLVYRSLKELNL
MG31:   GDIYNYRRFERQFQGYFAGGYIDKYLIKIVNTNPDVDDDIVYRSLKELNL
MG33:   GDIYNYRRFERQFQGYFAGGYIDKYLVKNVNTNKDSDDDLVYRSLKELNL
MG38:   GDIYNYRRFERQFQGYFAGGYIDKYLVKNVNTNKDSDDDLVYRSLKELNL
MG40:   GDIYNYRRFERQFQGYFAGGYIDKYLVKNVNTNKDSDDDLVYRSLKELNL
MG43:   GDIYNYRRFERQFQGYFAGGYIDKYLIKNVNTNKDSDDDLVYRSLKELNL
MG44:   GDIYNYRRFERQFQGYFAGGYIDKYLIKNVNTNKDSDDDLVYRSLKELNL
MG45:   GDIYNYRRFERQFQGYFAGGYIDKYLVKNVNTNKDSDDDLVYRSLKELNL
MG46:   GDIYNYRRFERQFQGYFAGGYIDKYLVKNVNTNKDSDDDLVYRSLKELNL

        424                                                     473
MG WT:  HLEEAYREGDNTYYRVNENYYPGASIYENERASRDSEFQNEILKRAEQNG
MG1:    HLEEAYREGDNTYYRVNENYYPGASIYENERASRDSEFQNEILKRAEQNG
MG8:    HLEEAYREGDNTYYRVNENYYPGASIYENERASRDSEFQNEILKRAEQNG
MG13:   HLEEAYREGDNTYYRVNENYYPGASIYENERASRDSEFQNEILKRAEQNG
MG15:   HLEEAYREGDNTYYRVNENYYPGASIYENERASRDSEFQNEILKRAEQNG
MG21:   HLEEAYREGDNIYYRVNENYYPGASIYENERASRDSEFQNEILKRAEQNG
MG22:   HLEEAYREGDNTYYRVNENYYPGASIYENERASRDSEFQNEILKRAEQNG
MG23:   HLEEAYREGDNTYYRVNENYYPGASIYENERASRDSEFQNEILKRAEQNG
MG24:   HLEEAYREGDNTYYRVNENYYPGASIYENERASRDSEFQNEILKRAEQNG
MG27:   HLEEAYREGDNIYYRVNENYYPGASIYENERASRDSEFQNEILKRAEQNG
MG28:   HLEEAYREGDNTYYRVNENYYPGASIYENERASRDSEFQNEILKRAEQNG
MG29:   HLEEAYREGDNTYYRVNENYYPGASIYENERASRDSEFQNEILKRAEQNG
MG31:   HLEEAYREGDNIYYRVNENYYPGASIYENERASRDSEFQNEILKRAEQNG
MG33:   HLEEAYREGDNTYYRVNENYYPGASIYENERASRDSEFQNEILKRAEQNG
MG38:   HLEEAYREGDNTYYRVNENYYPGASIYENERASRDSEFQNEILKRAEQNG
MG40:   HLEEAYREGDNTYYRVNENYYPGASIYENERASRDSEFQNEILKRAEQNG
MG43:   HLEEAYREGDNTYYRVNENYYPGASIYENERASRDSEFQNEILKRAEQNG
MG44:   HLEEAYREGDNTYYRVNENYYPGASIYENERASRDSEFQNEILKRAEQNG
MG45:   HLEEAYREGDNTYYRVNENYYPGASIYENERASRDSEFQNEILKRAEQNG
MG46:   HLEEAYREGDNTYYRVNENYYPGASIYENERASRDSEFQNEILKRAEQNG
```

FIG. 27D

```
          474  480
MG WT:    VTFDEN
MG1:      VTFDEN
MG8:      VTFDEN
MG13:     VTFDEN
MG15:     VTFDEN
MG21:     VTFDEN
MG22:     VTFDEN
MG23:     VTFDEN
MG24:     VTFDEN
MG27:     VTFDEN
MG28:     VTFDEN
MG29:     VTFDEN
MG31:     VTFDEN
MG33:     VTFDEN
MG38:     VTFDEN
MG40:     VTFDEN
MG43:     VTFDEN
MG44:     VTFDEN
MG45:     VTFDEN
MG46:     VTFDEN
```

FIG. 27E

MG WT-1 SLSLNDGSYQSEIDLSGGANFREKFRNFANELSEAITNSP
MP WT-1 SISLTDGGYRSEIDLGDGSNFREDFRNFANNLSEAITDAP

MG WT-1 KGLDRPVPKTEISGLIKTGDNFITPSFKAGYYDHVASDGS
MP WT-1 KDLLRPVPKVEVSGLIKTSSTFITPNFKAGYYDQVAADGK

MG WT-1 LLSYYQSTEYFNNRVLMPILQTTNGTLMANNRGYDDVFR-
MP WT-1 TLKYYQSTEYFNNRVVMPILQTTNGTLTANNRAYDDIFVD

MG WT-1 -QVPSFSGWSNTKATTV----STSNNLTYDKWTYFAAKGS
MP WT-1 QGVPKFPGWFHDVDKAYYAGSNGQSEYLFKEWNYYVANGS

MG WT-1 PLYDSYPNHFFEDVKTLAIDAKDIS-----ALKTTIDSEK
MP WT-1 PLYNVYPNHHFKQIKTIAFDAPRIKQGNTDGINLNLKQRN

MG WT-1 PTYLIIRGLSGNGSQLNELQLPESVKKVSLYGDYTGVNVA
MP WT-1 PDYVIINGLTGDGSTLKDLELPESVKKVSIYGDYHSINVA

MG WT-1 KQIFANVVELEFYSTSKANSFGFNPLVLGSKTNVINDLFA
MP WT-1 KQIFKNVLELEFYSTNQDNNFGFNPLVLGDHTNIIYDLFA

MG WT-1 SKPFTHIDLTQVTLQNSDNSAIDANKLKQAVGDIYNYRRF
MP WT-1 SKPFNYIDLTSLELKDN-QDNIDASKLKRAVSDIYIRRRF

MG WT-1 ERQFQGYFAGGYIDKYLVKNVNTN-KDSDDDLVYRSLKEL
MP WT-1 ERQMQGYWAGGYIDRYLVKNTNEKNVNKDNDTVYAALKDI

MG WT-1 NLHLEEAYREGDNTYYRVNENYYPGASIYENERASRDSEF
MP WT-1 NLHLEETYTHGGNTMYRVNENYYPGASAYEAERATRDSEF

MG WT-1 QNEILKRAEQNGVTFDEN

MP WT-1 QKEIVQR----------

FIG. 28

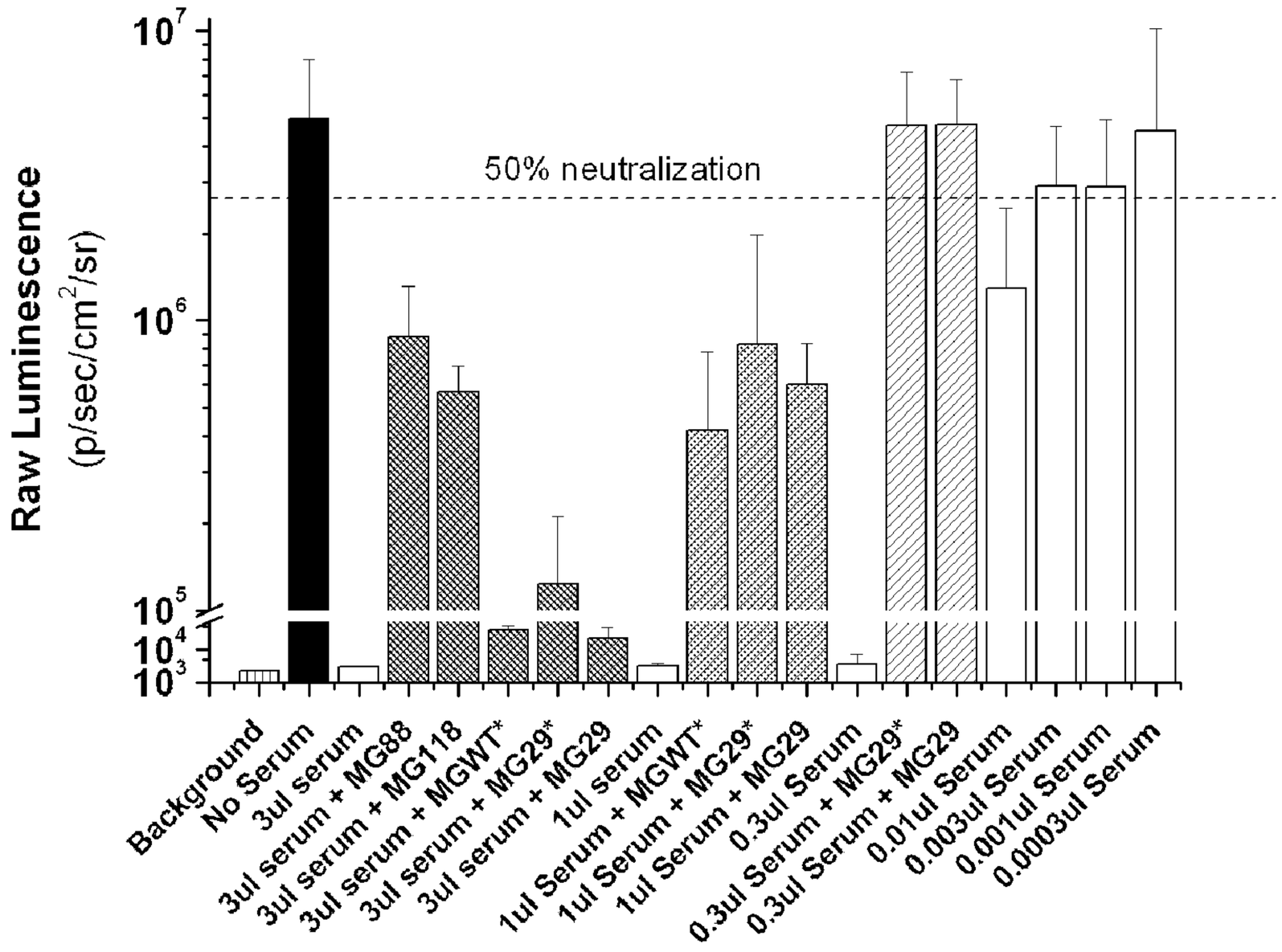

Raw Luminescence
(p/sec/cm²/sr)
10⁷
10⁶
10⁵
10⁴
10³
50% neutralization
Background
No Serum
3ul serum
3ul serum + MG88
3ul serum + MG118
3ul serum + MGWT*
3ul serum + MG29*
3ul serum + MG29
1ul serum
1ul Serum + MGWT*
1ul Serum + MG29*
1ul Serum + MG29
0.3ul Serum
0.3ul Serum + MG29*
0.3ul Serum + MG29
0.01ul Serum
0.003ul Serum
0.001ul Serum
0.0003ul Serum

FIG. 29

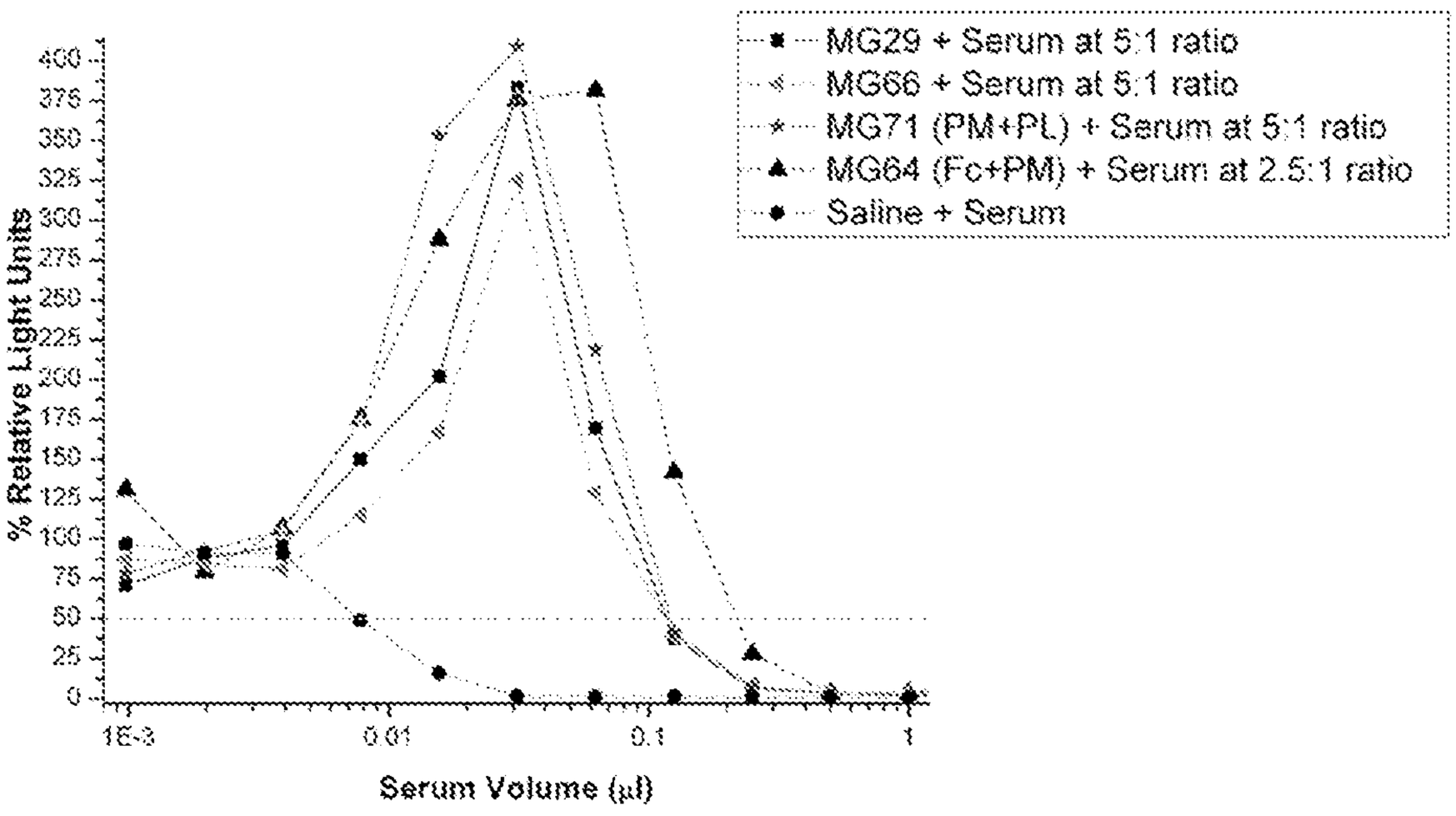

MG29 + Serum at 5:1 ratio
MG66 + Serum at 5:1 ratio
MG71 (PM+PL) + Serum at 5:1 ratio
MG64 (Fc+PM) + Serum at 2.5:1 ratio
Saline + Serum
% Relative Light Units
400
375
350
325
300
275
250
225
200
175
150
125
100
75
50
25
0
1E-3
0.01
0.1
1
Serum Volume (µl)

FIG. 30