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(54) **Titre : ANTIGENES DE SPOROZOITES DE PLASMODIUM FALCIPARUM EN PHASE HEPATIQUE**
(54) **Title: PLASMODIUM FALCIPARUM SPOROZOITE AND LIVER STAGE ANTIGENS**

(57) **Abrégé/Abstract:**

The invention provides novel malaria polypeptides expressed at the preerythrocytic stage of the malaria life-cycle. The antigens can be utilized to induce an immune response against malaria in a mammal by administering the antigens in vaccine formulations or expressing the antigens in DNA or other nucleic acid expression systems delivered as a vaccine formulation.

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(54) Title: PLASMODIUM FALCIPARUM SPOROZOITE AND LIVER STAGE ANTIGENS

(57) Abstract: The invention provides novel malaria polypeptides expressed at the preerythrocytic stage of the malaria life-cycle. The antigens can be utilized to induce an immune response against malaria in a mammal by administering the antigens in vaccine formulations or expressing the antigens in DNA or other nucleic acid expression systems delivered as a vaccine formulation.



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PLASMODIUM FALCIPARUM SPOROZOITE AND LIVER STAGE ANTIGENS

BACKGROUND OF INVENTION

1. Field of Invention

[0002] The inventive subject matter relates to DNA sequences and polypeptides from *Plasmodium falciparum* for use as an anti-malaria vaccine component and methods of inducing an immune response to these antigens.

2. Background Art

[0003] Malaria is caused by the vector borne organism *Plasmodium spp.* The parasite has a complex lifecycle requiring stage specific expression of proteins. These proteins can be expressed at different stages or be specific to stages. Malaria is an extremely important disease, with over 3 billion people living in malaria endemic areas. Over 1 million deaths are attributable to malaria per year. The emergence of drug resistant strains has compounded the problem of treating the disease. Unfortunately, no FDA-approved vaccine exists.

[0004] The entire genomic sequence of *P. falciparum* has been sequenced (Bowman et al., Nature, 400: 532-538 (1999), Gardner, et al., Nature, 419: 498-511 (2002)). The rodent malaria parasite, *P. yoelii* has also been sequenced (Carlton et al., Nature, 419: 512-519 (2002)). Despite this, however, the development of

efficacious anti-malaria vaccines has been severely hampered by the paucity of promising antigens. Sequencing of the *Plasmodium falciparum* and *Plasmodium yoelii* genomes yielding identification over 5,200 genes in the genome. However, despite the large number of potential gene targets, use of the data set alone will not likely result in new vaccine constructs. Consequently, only 0.2% of the *P. falciparum* proteome is undergoing clinical testing. Moreover, these vaccine candidate antigens have failed to induce high grade protection in volunteers. Nevertheless, immunization of mice and humans with radiation-attenuated sporozoites results in a high-grade immunity (>90%), suggesting that development of effective anti-malaria vaccines is possible. This protective immunity appears to target multiple sporozoite and liver stage antigens.

SUMMARY OF THE INVENTION

[0005] The invention relates DNA sequences encoding recombinant *Plasmodium falciparum* proteins. The proteins were identified from a large panel of *P. falciparum* proteins. These were evaluated based on a number of criteria, judged to be relevant to protection against malaria. The sequences can be utilized to express the encoded proteins for use as subunit immunogenic antigens or can be incorporated into vectors suitable for *in vivo* expression in a host in order to induce an immunogenic response. The proteins can be utilized in combination or singly in immunogenic formulations.

[0006] In one embodiment, the compounds can be used as immunogenic proteins. In this embodiment, the proteins can be produced by first inserting the DNA encoding the proteins in suitable expression systems. The expressed and purified proteins can

then be administered in one or multiple doses to a mammal, such as humans. In this embodiment, the purified proteins can be expressed individually or DNA encoding specific proteins can be recombinantly associated to form a single immunogenic composition. These immunogenic compositions can then be administered in one or multiple doses to induce an immunogenic response.

[0007] In an alternative embodiment, DNA encoding the proteins can be inserted into suitable vector expression systems. These include, for example, adenoviral based systems, such as in Bruder, et al (patent application publication number US 20080248060, published Oct 9, 2008) or a DNA plasmid system.

[0007a] In accordance with an aspect of the present invention, there is provided an immunogenic composition for inducing an immune response to *Plasmodium falciparum* comprising an isolated *Plasmodium falciparum*-derived polypeptide and a pharmaceutically acceptable carrier, wherein the isolated polypeptide comprises the amino acid sequence defined by SEQ ID NO: 10.

[0007b] In accordance with another aspect of the present invention, there is provided a use of the composition described herein for inducing an immune response against malaria in a mammal, wherein said composition further comprises one or more isolated nucleic acid molecules, inserted into a suitable expression vector, encoding isolated malaria polypeptides, said isolated nucleic acid molecules comprising the nucleic acid sequence according to SEQ ID NO: 9.

BRIEF DESCRIPTION OF THE DRAWINGS

[0008] FIG. 1. Protection of mice immunized (primed) with a with 100 µg of DNA *P. yoelii* vector containing the indicated insert. Mice receiving more than one insert are indicated as receiving multiple doses (i.e., dose = 1X to 3X). The mice were boosted, on day 40, with vaccinia-*P. yoelii* vector containing the same insert as priming dosage. On day 50, the mice were challenged with *P. yoelii* sporozoites and at day 61-68, parasitemia was evaluated. In these experiments, N=14.

[0009] FIG. 2. Protection of mice immunized as in FIG 1. In these studies, N=14. Mice receiving more than one insert are indicated as receiving multiple doses (i.e., dose = 1X or 3X).

[0010] FIG 3. ELISpot screening of PF106 antigen using HLA-specific peptide pools as stimulants (IFN- γ spot-forming cells (SFC)/million peripheral blood mononuclear cells (PBMC)). A star indicates the highest IFN- γ induction.

[0011] FIG 4. ELISpot screening of PF 61 antigen using HLA-specific peptide pools as stimulants (IFN- γ spot-forming cells (SFC)/million PBMC). A star indicates the highest IFN- γ induction.

[0012] FIG 5. ELISpot screening of PF 56 antigen using HLA-specific peptide pools as stimulants (IFN- γ spot-forming cells (SFC)/million PBMC). A star indicates the highest IFN- γ induction.

[0013] FIG 6. ELISpot screening of PF 121 antigen using HLA-specific peptide pools as stimulants (IFN- γ spot-forming cells (SFC)/million PBMC). A star indicates the highest IFN- γ induction.

[0014] FIG 7. ELISpot screening of PF 144 antigen using HLA-specific peptide pools as stimulants (IFN- γ spot-forming cells (SFC)/million PBMC). A star indicates the highest IFN- γ induction.

[0015] FIG 8. ELISpot screening of PF 144 antigen using HLA-restricted (A2, A3 and B7) peptide pools as stimulants (IFN- γ spot-forming cells(SFC)/million PBMC). PBMC's were obtained from a subject pre- or post-immunization with irradiated sporozoite vaccine (ISV).

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

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[0016] One embodiment of the invention relates to isolated proteins expressed at the pre-erythrocytic stage of the malaria (i.e., *Plasmodium falciparum*) life-cycle.

The isolated proteins can be incorporated in immunogenic formulations in order to induce an immune response. In this embodiment, the proteins can be incorporated singly or in combination. The immunogenic compositions of the invention also include adjuvants to improve or enhance the immune response elicited by the polypeptides. A further aspect of the invention is the ability of the proteins to induce an humoral and/or T-cell immune response.

[0017] As used herein, the term “polypeptide” refers to a polymer of amino acids and does not refer to a specific length of the product. Proteins are included within the definition of polypeptides. The term “mer”, in conjunction with a number, such as 15-mer, refers to the length of a polypeptide in numbers of amino acids.

[0018] Methods of predicting immunogenic regions, including predicting T-cell epitopes or HLA binding regions in a polypeptide are well-known in the art. The term “motif” refers to a polypeptide with a specific amino acid sequence that has been predicted to associate or bind to an HLA molecule.

[0019] As used herein, the proteins may be prepared for inclusion of an effective amount of one or more polypeptides described herein into an immunogenic composition by first expressing the appropriate gene fragments by molecular methods, expression from plasmids or other expression systems such as viral systems and then isolated.

[0020] An embodiment of the invention is the incorporation of DNA encoding the polypeptides in vector expression systems, wherein the system permits expression of

one or more polypeptides in mammalian host cells, such as in humans to induce an immune response. The expression systems can be DNA plasmids or viral systems. Methods for preparing and administering a DNA vaccine expressing Plasmodium proteins are well known in the art and have been previously described (e.g., Doolan and Hoffman (2001), Int J. Parasitol. 31: 753-62; U.S. Patent Publication 2008/0248060 (Oct 9, 2008)).

[0021] In another embodiment, derivatives of the proteins can be used in immunogenic compositions. In a variant of this embodiment, the immunogenic derivatives of the *P. falciparum* proteins include at least 10 contiguous amino acids of an amino acid sequence of a full length polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 4, 6, 8, 10, 12 and 14. Immunogenic derivatives of the polypeptides may be prepared by expression of the appropriate gene fragments or by other methods such as by peptide synthesis. Additionally, derivatives may be a fusion polypeptide containing additional sequence encoding one or more epitopes of the *P. falciparum* polypeptides of selected from the group consisting of SEQ ID NOs: 2, 4, 6, 8, 10, 12 and 14. In these embodiments, the proteins can be directly incorporated in immunogenic formulations or expressed from DNA plasmids or viral expression systems.

[0022] In some embodiments, the *P. falciparum* polypeptides include immunogenic derivatives with more than 80% amino acid sequence identity to the sequences of selected from the group consisting of SEQ ID NOs: 2, 4, 6, 8, 10, 12 and 14. In this context, the term "identity" refers to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues

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that are the same, when aligned for maximum correspondence. Where sequences differ in conservative substitutions, i.e., substitution of residues with identical properties, the percent sequence identity may be adjusted upwards to correct for the conservative nature of the substitution.

[0023] Immunization of mice with irradiation-attenuated sporozoites elicits a strong protective immunity (Clyde et al., *Am.J.Med.Sci*, 266: 398-401 (1973a); Clyde et al., *AM.J.Med.Sci*, 266: 169-177 (1973b); Nussenzweig et al., *Nature*, 216: 160-162 (1967)). Use of irradiation-attenuated sporozoites, as a vaccine approach, appears to target multiple antigens, many of which are pre-erythrocytic, as evidenced by the identification of genetically restricted responses to pre-erythrocytic stage antigens other than CSP in volunteers immunized with the irradiated sporozoite vaccine (ISV). (Kryzch et al., *J.Immunol*, 155: 4072-4077 (1995); Wize et al., *J.Immunol.*, 155: 766-775 (1995a); Wize et al., *J.Exp.Med.*, 182: 1435 -1445 (1995b)). Targeting of pre-erythrocytic stage antigens with the irradiation-attenuated vaccine leads to immunity against sporozoites or infected hepatocytes and, therefore, prevents the onset of blood infection by blocking the release of primary merozoites into the blood stream.

[0024] Pre-erythrocytic proteins are likely critical in conferring protective immunity against malaria. Despite the relatively large number of malaria genes that have been identified, following sequencing of the malaria parasite genome, identification of anti-malaria drug vaccine candidates has been hampered, to a great extent, by the relatively complex life-cycle of malaria parasite. Furthermore, many genes of the malaria parasite are poorly defined, antigenically, as well as functionally.

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Therefore, high-throughput characterization of antigens encoded by numerous genes was undertaken in order to ascertain potential protective responses. Based on these responses specific genes were selected as potential vaccine formulations.

Example 1: Identification and Expression of P. falciparum proteins

[0025] Characterization of identified sequences requires the availability of effective protein expression platforms. This limitation has been problematic in the development of anti-malaria vaccines. In order to circumvent this restriction, a modified wheat germ cell-free system was employed for parallel expression and screening of recombinant proteins. A panel of 150 putative *P. falciparum* pre-erythrocytic stage genes was established, based on bioinformatic analysis and transcriptome and proteome expression databases for sporozoite and liver stage parasites. Most of the genes in this list encode hypothetical proteins with unknown function. It was hypothesized that some of the genes in this panel could serve as vaccine antigens, as evidenced by immune reactivity to sera from irradiated sporozoite vaccine (ISV) - immunized volunteers.

Use of other expression systems proved difficult for further, necessary down-stream analysis and characterization. In order to improve expression, a modified wheat germ cell-free expression system was utilized. This system permitted expression of approximately 90% of clones evaluated. Genes selected, for further expression and characterization, were identified, based on proteome and/or transcriptome datasets, by their expression at the pre-erythrocytic stage. A total of 155 genes of *P. falciparum*

were used to generate protein expression DNA constructs as GST-and 6xHis-fusions using the pE-E01-GST-TEV-GW and pEU-E01-His-TEV-GW plasmids, respectively (Tsuboi, et al.; 2008).

[0026] Expression of recombinant proteins.

The expression of recombinant proteins was conducted utilizing a wheat germ cell-free system. Gene-specific RNAs to be used as template in the *in vitro* translation reactions were synthesized in two scales:

- a. Small-scale. The small-scale batch-reaction was performed with all clones by first generating RNase-free PCR DNAs and using them as templates in transcription reactions. Individual genes were PCR-amplified from plasmid mini-prep DNA using pEU-E01-specific primers and adapters. Primers used were SP6: 5' AGAGCGCGCAAGACGCGCAGGACCG 3'; E01: 5' AGAGAGAGAGAGA ACAACAACACAAACA 3'. All PCR-amplified gene sizes were confirmed in an agarose gel. Transcription reactions were set up with 2 μ l of PCR DNA template, 25 mM dNTPs, 1 U/ μ l RNase inhibitor, 1.2 U/ μ l SP6 Polymerase and transcription buffer for 6 hours at 37°C. The sizes of the gene-specific transcripts were verified by agarose gel electrophoresis. The RNAs were ethanol-precipitated to remove unwanted transcription reagents, dissolved in RNase-free water and used for protein expression reactions.
- b. Large-scale. The synthesis of RNAs for large-scale protein expression was performed using RNase-free plasmid DNA as template, purified by a standard

DNA isolation kit, and added directly into the transcription reactions as follows:
25 µg of plasmid DNA were mixed with 2.5 mM of NTPs, 1 U/µl of RNAsin, 1 U/µl of SP6 polymerase in transcription buffer described above. The total reaction volume was 250 µl and incubated at 37°C for 6 hours. The qualities of all expressed RNAs were analyzed by agarose gel electrophoresis prior to setting up the translation reactions.

[0027] As with RNA synthesis, recombinant proteins were expressed in two scales throughout this study, as follows:

a. Small Scale. Batch reactions were set up in 96-well U-bottom plates using a protocol described earlier (Sawasaki et al., FEBS Lett, 514: 102-105 (2002b)). Briefly, the reaction was assembled by overlaying 40 µl of substrate mix (0.45 mg/ml Creatine kinase, 20U of RNasin, 24 mM Hepes/KOH pH 7.8, 100 mM KOAc, 2.7 mM Mg(OAc), 0.4 mM Spermedine, 2.5 mM DTT, 1.2 mM ATP, 0.25 mM GTP, 16 mM Creatine-Phosphate, 0.005% NaN₃ and 0.3 mM of each of the amino acids including [¹⁴C]leucine (2 µCi/ml)) over 10 µl of translation mix containing 2 µl of each RNA and 8 µl of wheat germ extract OD240 (OD60 final concentration). The extract was purchased from CFSciences, Yokohama, Japan. However, other comparable sources can be used. The plate was covered with parafilm and incubated at 26 °C for 16 hours.

b. Large-Scale. Large amounts of recombinant proteins were synthesized following general guidelines recommended by the manufacturer of the wheat germ extract (i.e., CFSciences, Yokohama, Japan). Depending on the specific

quantity needs for each protein, variable numbers of reactions were set up using flat bottom 6-well plates. Each translation reaction contains a bilayer of 2 mixtures; the lower portion includes a total of 500 μ l of the transcribed RNA and the wheat germ extract and the upper mixture consists of 5.5 ml of dialysis buffer. In brief, the lower reaction mixture was prepared by mixing 250 μ l of the RNA reaction, 40 mg/ml creatine kinase and 120 OD/ml of wheat germ extract (WGE OD240, CFSciences, Yokohama, Japan). This lower mixture was transferred to a 6-well and carefully overlaid with 5.5 ml of sub-admix buffer supplied by the manufacturer (CFSciences, Yokohama, Japan) in order to form a bilayer reaction. Plates were sealed with parafilm and translation occurred at room temperature overnight.

[0028] Purification of proteins.

Recombinant proteins tagged with GST were affinity-purified using a procedure that has been previously described (Tsuboi et al., Infect. Immun., 76: 1702-1708 (2008)). After translation reaction was complete, the protein-containing mix was absorbed into Glutathione Sepharose 4B resin (GE healthcare, Piscataway, NJ) followed by protein elution with 20 U of tobacco etch virus protease (AcTEVTM, Invitrogen, Carlsbad, CA) and 1 mM of DTT. Eluted proteins were confirmed by SDS-PAGE stained by Coomassie blue or silver stains. Purified protein concentrations were determined by both Bradford protein assay kit and spectrophotometer and reported as μ g/ml.

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[0029] Expressed proteins were further characterized by *in vitro* and *in vivo* analysis. *In vitro* analysis included antibody based and T-cell mediated assays. Analysis of the selected genes were analyzed using the following methodologies:

Example 2: Assay methods in analysis of pre-erythrocytic gene products

[0030] Indirect Fluorescence Antibody Assay (IFA).

Sera from both mice and rabbits immunized with all proteins were tested for immune-reactivity to sporozoite, 4- and 6-day old exo-erythrocytic, asexual and sexual erythrocytic stages of *P. falciparum*. IFA protocols for testing sera against sporozoite and erythrocytic stages were as described in Aguiar et al., Vaccine, 20: 275-280 (2001).

[0031] Liver stage parasites were generated and tested by two different methods. The first used *in vitro* generated liver stages by infecting 5×10^4 HC04 hepatocytes (Sattabongkot et al., Am J Trop Med Hyg., 74: 708-715 (2006)) with 5×10^5 *P. falciparum* sporozoites. After a brief incubation period of 4-6 hours, cells were washed and parasites were left to develop for a period of 2-, 4- and 6-days. Before infected hepatocytes were tested in IFA, cells were stained with lysotrackerTM (Invitrogen, Carlsbad, CA) following manufacturer's protocol and fixed with acetone. The second method used *P. falciparum*-infected liver stages parasite generated in chimeric mice as reported in a study describing this novel method (Sacci et al., Int J Parasitol., 36: 353-360 (2006)).

[0032] In summary, mice were infected with 1.5×10^6 *P. falciparum* sporozoites and their livers harvested between 3 and 8 days post-infection for cryosectioning.

Selected lobes were embedded in tissue-Tek O.C.T.TM compound (Miles Scientific, Naperville, IL) and frozen in an isopentane/liquid nitrogen bath. Tissue sections (7 μ m) were cut on a Leica CM1900TM (Leica Microsystems, Deerfield, IL.), fixed in absolute methanol and stored at -80° C until used. IFA on both types of infected hepatocytes were done with mouse and rabbit polyclonal sera at single 1:100 dilution. As a positive control, anti-heat shock protein-70 antibody was utilized. The IFA was developed by incubating cells with FITC-conjugated anti-mouse or anti-rabbit IgG and staining the nuclei with Hoechst 33258 pentahydrate. Slides were mounted in anti-fading VectashieldTM (Vector Laboratories, Burlingame, CA) and visualized by confocal microscopy.

[0033] Immuno Electron Microscopy.

The ultrastructural localization of *Plasmodium* antigen expression was examined by immunoelectron microscopy. Briefly, *P. falciparum* infected red blood cells and salivary glands *P. falciparum* infected *Anopheles stephensi* were fixed in PBS containing 1% paraformaldehyde, and 0.1% glutaraldehyde, for 24 h at room temperature. Ultra thin sections, embedded in LR-WhiteTM resin (Polyscience, Inc., Warrington, PA) were cut and placed on nickel grids. The sections on the grids were etched by incubation with freshly prepared, saturated sodium-m-periodate for 5 minutes, followed by rinsing 3 times in deionized water. The grids were quenched with 0.1 M glycine in phosphate buffer for 20 minutes to prevent any free aldehyde groups from binding to the primary antibody. The grids were blocked by incubation in PBS, 1% BSA, 5% Fish gelatin for 30 minutes.

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[0034] Grids were incubated with the primary antibody (diluted 1:50) in a humidified environment for 2 hours, followed by washing 5 times in PBS-0.1% Tween-20 (polyoxyethylene (20) sorbitan monolaurate). The grids were then incubated for 30 minutes with a goat anti-mouse or rabbit antibody conjugated to 10 nm gold particles. The grids were washed as described above, then post-stained with 2% Uranyl acetate and rinsed with water.

[0035] The sections were examined with a transmission electron microscope. Negative controls included uninfected cells and the use of nonspecific, irrelevant antibodies as the primary antibody.

[0036] Western Blotting. Recombinant proteins were analyzed by Western blot using two protocol, summarized as follows:

- a. For the high throughput expression, [¹⁴C]Leucine-labeled proteins were separated by SDS-PAGE as three fractions; 2 µl of the total translation reaction (T), after the 96-well plate was spun 2 µl of supernatant (S) fraction and lastly the pellet (P) was re-suspended in sample buffer and separated as pellet fraction. Recombinant proteins were identified by autoradiography using an imaging analyzer.
- b. Purified recombinant proteins were screened using the regular Western blot protocol. Ten micrograms of each protein were separated on a pre-cast 4–20% gradient SDS polyacrylamide gel, and subsequently electrotransferred onto a PVDF membrane. Western blot with a 1:500 dilution of rabbit antisera immunized with the proteins or human immune sera. Peroxidase-conjugated goat anti-rabbit or human IgG antibody was used as the secondary antibody at a

dilution of 1:10,000. The reaction was developed using ECL-Plus™ (General Electric Healthcare, Piscataway, NJ) western blotting detection system according to the manufacturer's instructions.

[0037] Enzyme-Linked ImmunoSorbent Assay (ELISA).

Human sera, from volunteers immunized with ISV, were tested at 1:100 dilutions for antigen-specific reactivity measured by ELISA using recombinant proteins expressed by both small and large scale methodologies, as described in Example 1. Negative (GST and wheat germ extract) and positive (CSP and SSP2/Trap) control proteins were also expressed in the same scale. Immunolon II™ ELISA plates (Dynatech Laboratory Inc., Chantilly, VA) were coated with 2-5 µl of recombinant protein and wheat germ extract in PBS overnight at room temperature. Wells were washed three times with PBS containing 0.05% Tween 20 (polyoxethylene (20) sorbitan monolaurate) (washing buffer) and blocked with 100 µl of 5% non-fat dry milk in PBS (blocking buffer) for 2 hours at 4°C. After washing three times, wells were incubated for 2 hours with 50 µl of 1:100 dilution of test human sera. The wells were again washed three times and incubated for 1 hour with peroxidase-labeled goat anti-human IgG (KPL). After a three washes the wells were incubated for 20 minutes with 100 µl of a solution containing ABTS substrate [2,2'-azino-di-(3 ethylbenzthiazoline sulfonate)] and H₂O₂. Color reaction was measured in a micro-ELISA automated reader at OD 410 nm. ELISA data were presented as the average of OD readings for tested sera dilutions. The sporozoite-specific antibodies in these human sera were assessed by the indirect fluorescent antibody test (IFAT) as

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previously described. Results were reported as the endpoint dilution, representing the last serum dilution at which fluorescence was scored as positive.

[0038] Interferon γ ELISpot assay.

Multiscreen MAIPS4510TM plates (Millipore, Billerica, MA) were coated with 100 μ l/well of sterile carbonate/bicarbonate buffer containing 15 μ g/ml of anti-human IFN- γ mAb 1-D1K (purchased from Mabtech, Cincinnati, OH) overnight at 4°C. Plates were washed five times with 100 μ l/well RPMI medium containing 25 mM HEPES buffer and L-glutamine.

[0039] The plates were blocked with 200 μ l/well HR-10 media containing 1% of the following: 200 mM L-glutamine, Pen/Strep, 10 mM MEM, 10% Human AB Serum and 87% RPMI medium. The plates were incubated with blocking solution at 4°C for at least 24 hours. PBMCs (20×10^6) were thawed at 37°C and resuspended in 10 mls R-10 media containing 10% FBS, 1% 200 mM L-glutamine, 1% Pen/Strep and 88% RPMI. The cells were washed twice at 1200 rpm, 25°C, 10 minutes and again with 10 mls R-10 and pelleted at 1000rpm, 25°C, 10 minutes. The cells were re-suspended in an appropriate volume of HR-10 for counting. The volume of the cell suspension was increased with HR-10 and rested overnight at 37°C/5% CO₂ incubator. Cells were counted again to calculate recovery and viability. The plates were washed from their block solution six times with RPMI medium and left at room temperature until the stimulants and cell suspension were prepared. The stimulants were diluted with HR-10 media to its optimal concentration. The cell suspension was diluted with HR-10 to its optimal concentration. The stimulants were added first to the α -IFN- γ coated wells in quadruplicate at 100 μ l/well followed by the addition of

100 µl/well cell suspension at a final concentrations of 100,000, 200,000 and 400,000 cells/well. Non-stimulant containing wells contained HR-10 media and the cell suspension were used as background controls. Plates were incubated for 36 hours at 37°C/5%CO₂ then washed six times with 1xPBS+0.05% Tween-20 (polyoxethylene (20) sorbitan monolaurate) solution. ELIspot was developed by adding 100 µl of 1 µg/ml biotinylated α-IFN-γ mAb 7-B6-1 (purchased from Mabtech, Cincinnati, Ohio) and incubated at room temperature for three hours. Plates were washed six times with 1x PBS+0.05% Tween-20 (polyoxethylene (20) sorbitan monolaurate) solution. Development was performed by adding 100 µl of a 1:1000 streptavidin-alkaline phosphatase solution and incubated at room temperature for one hour. Plates were washed six times with 1x PBS+0.05% Tween-20TM (polyoxethylene (20) sorbitan monolaurate) followed by three washes with 1x PBS solution. Alkaline Phosphatase Conjugate at 1:25 dilution was added and incubated at room temperature for 15 minutes. The chromogenic reaction was stopped by extensively washing the plates with water. Once dry, the spots were counted using the AIDTM ELIspot plate reader (Strassberg, Germany).

Example 3: Immunogenicity and life cycle expression of pre-erythrocytic P. falciparum genes

[0040] A summary of the expression and reactivity of 22 genes is shown in Table 1. An important aspect of the proteins in Table 1 is that they were recognized by human antibody and/or T-cells from volunteers immunized with IVS. Therefore,

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since they are recognized within the natural immune response against malaria sporozoites these proteins may be valuable in conferring immunity against malaria.

[0041] Expressed proteins from all genes in Table 1 were recognized by ELISA and/or by Western blot analysis using ISV-immunized human sera. Additionally, some antigens were also recognized by immune T cells. This illustrates that the novel antigens exist within the natural structure of malaria sporozoites used for ISV immunization. Furthermore, the antigens are recognized as foreign to the human body and are therefore capable of eliciting a protective immune response. Additionally, reactivity of the sera also indicates that these antigens are associated with complete protection against malaria infection in these volunteers, which further indicates that these antigens are potential vaccine candidate antigens. The genes shown in Table 1 have been verified as being expressed at the pre-erythrocytic (sporozoite and liver) parasite stages by IFA. The antigen's subcellular localization was also determined by electron microscopy (EM).

[0042] *P. falciparum* proteins in Table 1 expressed from the cloned sequences were recognized by human antibodies and/or T cells from volunteers immunized with ISV. As such, the novel proteins are recognized within the natural immune response against malaria sporozoites. Therefore, these proteins may be important in immunity against malaria.

Table 1

	Antigen ID #	<i>P. falciparum</i> Gene Locus	Screen Immune T cell	Screen Immune antibody	IFA Sporozoite Stage	IFA Liver Stage	IFA Blood Stage	<i>P. yoelii</i> Ortholog Locus	Individual Py Protection	Combined Py Protection
1	PF26	PF11425w	Negative	Positive	Negative	Positive	Negative	PY00232	ND	ND
2	PF56	PF08_0008	Positive	Positive	Positive	Positive	Positive	PY07130	ND	ND
3	PF61	PF10_0138	Positive	Positive	Positive	Positive	Positive	PY04748	ND	ND
4	PF106	PFI0580c	Positive	Positive	Positive	Positive	Positive	PY03424	7%	43%
5	PF116	PFI0460w	Negative	Positive	Positive	Positive	Negative	PY03130	ND	ND
6	PF121	PF10_0319	Positive	Positive	Positive	Positive	Positive	PY02600	ND	ND
7	PF01	PF10_0098	ND	Positive	ND	ND	ND	PY04010	ND	ND
8	PF08	PFC0555c	ND	Positive	Positive	Pending	ND	PY03661	0%	43%
9	PF09	MAL7P1.32	ND	Positive	ND	ND	ND	PY04689	ND	ND
10	PF13	PFC0700c	ND	Positive	ND	ND	ND	PY03459	ND	ND
11	PF24	PFC1055w	ND	Positive	ND	ND	ND	PY00359	ND	ND
12	PF47	PF11_0156	ND	Positive	ND	ND	ND	PY02926	ND	ND
13	PF51	PFE0565w	ND	Positive	ND	ND	ND	PY00913	ND	ND
14	PF59	PF14_0495	ND	Positive	ND	ND	ND	PY06813	ND	ND
15	PF68	PF14_0722	ND	Positive	ND	ND	ND	PY00150	ND	ND
16	PF72	MAL13P1.25	ND	Positive	NDd	ND	ND	PY05161	ND	ND
17	PF93	PF13_0012	ND	Positive	ND	ND	ND	PY03011	7%	43%
18	PF119	PFD0235c	ND	Positive	Positive	Positive	Positive	PY01067	ND	ND
19	PF78	MAL8P1.78	Positive	Positive	Positive	Positive	Negative	PY00566	ND	ND
20	PF02	PFE0785c	ND	Positive	Positive	Positive	Positive	PY00232	30%	ND
21	PF144	PF14_0467	Positive	Positive	Positive	Positive	Positive	PY05966	ND	ND
22	PF131	MAL13P1.107	ND	Positive	ND	ND	ND	PY131	ND	ND

Example 4: Immunogenic protection by P. yoelii orthologous genes

[0043] *P. yoelii* genes, listed in Table 1, that are orthologous to *P. falciparum* genes were evaluated for their ability to protect mice from challenge. For protection studies, CD1 mice were injected intramuscularly in the tibialis anterior muscle with 100 μ l of vaccine (50 μ l in each leg). The DNA vaccine vectors were prepared in 1X Phosphate Buffered Saline (PBS) and diluted to the appropriate concentration for vaccination in 1X PBS. The vaccinia vaccine vectors were prepared in 1 mM Tris (9.0) and diluted to the appropriate concentration for vaccination in 1X PBS. Mice

were challenged intravenously in the tail vein with 300 *P. yoelii* (17XNL) sporozoites. Sporozoites were hand dissected from infected mosquito salivary glands and diluted for challenge in M199 medium containing 5% normal mouse serum.

[0044] In the first study, 14 mice per group were primed on day 0 with 100 µg of DNA-*P.yoelii* vaccine vector and boosted on day 40 with 5×10^7 plaque forming units (pfu) of vaccinia-*P.yoelii* vaccine vector containing the same *P. yoelii* insert. Mice immunized with a combination of vectors expressing PY03011, PY03424 and PY03661 were primed with a total of 300 µg of DNA-*P.yoelii* vectors and boosted with a total of 1.5×10^8 pfu of the vaccinia-*P.yoelii* vectors. On day 50, the mice were bled and sera prepared. On day 54, the mice were challenged with 300 *P. yoelii* sporozoites. On days 61-68, parasitemia was evaluated by visualization of Giemsa-stained blood smears. Mice were considered positive if parasites were observed in any sample. To gauge the severity of the challenge, 4 groups of naïve CD1 mice were challenged with 100, 33.3, 11.1 or 3.7 *P.yoelii* sporozoites. The results from these infectivity control mice indicated that the mice injected with 300 *P.yoelii* sporozoites were challenged with a dose equivalent to 7 times the ID₅₀ dose.

[0045] *P. yoelii* genes were cloned in a DNA and orthopox viruses, as above. The *P. yoelii* orthologous genes were cloned into plasmids for generating DNA vaccines. Poxvirus and adenovirus vectors expressing the *P. yoelii* orthologs were also generated. The DNA, poxvirus and adenovirus-based vaccine constructs were tested in mice using a prime-boost regimen and the mice challenged with *P. yoelii* sporozoites to assess protection.

[0046] In the second study, 14 mice per group were primed on day 0 with 100 μ g of DNA-*P. yoelii* vaccine vector and 30 μ g of DNA vector expressing murine granulocyte-macrophage colony-stimulating factor (mGM-CSF) and boosted on day 42 with 3.3×10^7 pfu of vaccinia-*P. yoelii* vaccine vector. Mice immunized with 2 or 3 DNA-*P. yoelii* vectors were primed with a total of 200 μ g or 300 μ g of the DNA-*P. yoelii* vectors and 30 μ g of the DNA-mGM-CSF vector and boosted with a total of 6.6×10^7 pfu or 1×10^8 pfu of the vaccinia-*P. yoelii* vectors, respectively. Three separate groups for the negative control mice were immunized with 3 different doses of the DNA and vaccinia vectors that do not express a *P. yoelii* antigen. One group was primed with 100 μ g of an "empty" DNA vector and 30 μ g of a DNA-mGM-CSF vector and boosted with 3.3×10^7 pfu of an "empty" vaccinia vector. A second group was primed with 200 μ g of an "empty" DNA vector and 30 μ g of a DNA-mGM-CSF vector and boosted with 6.6×10^7 pfu of an "empty" vaccinia vector. A third group was primed with 300 μ g of an "empty" DNA vector and 30 μ g of a DNA-mGM-CSF vector and boosted with 1×10^8 pfu of an "empty" vaccinia vector. On day 52, the mice were bled and sera prepared. On day 57, the mice were challenged with 300 *P. yoelii* sporozoites. On days 64-71, parasitemia was evaluated by visualization of Giemsa-stained blood smears. Mice were considered positive if parasites were observed in any sample. To gauge the severity of this challenge, 4 groups of naïve mice were challenged with 100, 33.3, 11.1 or 3.7 *P. yoelii* sporozoites. The results from these infectivity control mice indicated that the mice injected with 300 *P. yoelii* sporozoites were challenged with a dose equivalent to 13.6 times the ID₅₀ dose.

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[0047] It should be noted that the regimens for the two protection studies were slightly different. For example, the dose of the individual vaccinia-*P. yoelii* vectors was slightly higher in protection study one (5×10^7 pfu) than protection study 2 (3.3×10^7 pfu). Consequently, the total dose of the trivalent vaccine was 1.5×10^8 pfu in protection study one and 1×10^8 pfu in protection study two. Additionally, in protection study 2, the DNA vectors were mixed with a DNA-mGM-CSF plasmid. Although previous studies had indicated that co-administration of a DNA-PyCSP vector with the DNA-mGM-CSF plasmid could enhance the immunogenicity and efficacy of a DNA-vaccinia prime-boost regimen, the DNA-mGM-CSF plasmid did not appear to enhance the efficacy of the PyCSP or trivalent *P. yoelii* vaccines in protection study two, relative to one.

[0048] As illustrated in Table 1 and FIG 1 (panel A), none of the 14 mice immunized with vectors that express PY03011 or PY03661 were sterilely protected (i.e., 0% protection). Additionally, 2 out of 14 mice immunized with PY03424 were protected (i.e., 14%). However, when all three antigens were used (i.e., PY03011; PY03424 and PY03661), 57% protection was observed. This was greater than that seen with PyCSP.

[0049] The results of a second study, FIG 1 (panel B) and Table 1, further confirmed that additive protective effect of the *P. yoelii* polypeptides. As illustrated in Table 1 and in FIG 1 (panel B), none of the mice immunized with vectors that express PY03661 were protected and only 1 of 14 mice immunized with vectors that express PY03011 or PY03424 were protected (7% protection). However, 6 of 14 mice immunized with PY03011 and PY03424 were protected (i.e., 43%), 3 of 14

mice immunized with PY03424 and PY03661 were protected (i.e., 21% protection) and 6 of 14 mice immunized with all 3 *P. yoelii* antigens were protected (i.e., 43% protection). The protection elicited by PY03011 and PY03424 in this study is statistically significant (PY03011/PY03424 (dose=2X vs Neg control (dose=2X), $p=0.0159$). Similar to the previous study, the protection elicited by the combination of PY03011 and PY03424 or all 3 antigens was greater than the protection elicited by PyCSP (i.e., 43% vs. 14%).

[0050] In another study, FIG. 2, 14 CD1 mice were again immunized using a DNA/adenovirus prime boost regime. In this study, a greater response was observed using PY3011 or PY3424, singly. In fact, the response to PY3424 was equivalent to that observed for PYCSP. A third antigen, PY4748 resulted in 1 out of 14 mice were protected. Interestingly, when all three antigens (i.e., PY3011, PY3424 and PY4748) were used to immunize CD1 mice, no protection was observed. The causation of this result is not clear.

[0051] The results of this study illustrate the potential value of *P. yoelii* proteins in conferring immunity against *P. yoelii*. Of interest is that the additive effect of the proteins resulted in protection often beyond that which would have been predicted from mice immunized with only a single protein. For example, in FIG. 1, panel A, PY03011 or PY03661 did not result in protection. Similarly, immunization with PY03424 resulted in only 14% protection. However, immunization of the genes in combination, *albeit* at a higher dose due to all three being administered simultaneously, resulted in protection higher than PyCSP.

[0052] From these analyses, the orthologous *P. falciparum* genes would be important candidates to be included in compositions for the induction of immunity against malaria. The *P. falciparum* orthologs are listed in Table 1. For all of these *P. yoelii* gene products tested in these murine protection studies, the *P. falciparum* orthologs reacted positively to either immune human serum and/or T-cell. A summary of the DNA and protein sequences are summarized in Table 2.

Table 2

SEQ ID No.	Clone	Gene Name	Protein or DNA
SEQ 1	PF56	PF08_0008	DNA
SEQ 2	PF56	PF08_0008	protein
SEQ 3	PF61	PF10_0138	DNA
SEQ 4	PF61	PF10_0138	protein
SEQ 5	PF106	PF10580c	DNA
SEQ 6	PF106	PF10580c	protein
SEQ 7	PF121	PF10_0319	DNA
SEQ 8	PF121	PF10_0319	protein
SEQ 9	PF08	PFC0555c	DNA
SEQ 10	PF08	PFC0555c	protein
SEQ 11	PF144	PF14_0467	DNA
SEQ 12	PF144	PF14_0467	Protein
SEQ 13	PF93	PF13_0012	DNA
SEQ 14	PF93	PF13_0012	Protein

Example 5: Immune recognition of P. falciparum genes orthologous to P. yoelii

[0053] From the *P. yoelii* protection data, orthologous *P. falciparum* genes were identified and further evaluated for their ability to confer cellular immunity. .

Illustrated in FIG 3 are the results of utilizing the *P. falciparum* ortholog to PY3424, PF 106, as an antigen in inducing a T-cell response, as evidenced by its ability to

induce IFN- γ in PBMCs in ELISpot assays. The results are presented in spot-forming cells (SPC)/million.

[0054] In FIG 3, the “pool” represents 15-mer peptides, overlapping by 10-mers, encompassing the entire length of the PF 106 protein. Additionally, HLA class I-specific 15-mer peptide pools were developed and also used as antigen. The HLA class I-specific pools contained 12 to 15 peptides, each containing an HLA class I binding motifs for specific HLA class I alleles. The HLA genotypes of the PBMCs are summarized in Table 3. All volunteers had been immunized with ISV, with some exhibiting a protective immune response. The results of these studies are illustrated in FIG 3.

Table 3

Volunteer	Sex	Age	HLA-A	HLA-B	HLA DR	Cohort
DF0043	Male	37	A26, A'68	B15, B38/39	DRB1*13, DRB3*02/03	Not protected
MF0052	Male	39	A3002, A'3402	B'53, B0812	DRB1*03/06/11, DRB3*02/03	Not protected
MR0053	Male	45	A1, A'6802	B7, B18	DRB1*1503, DRB1*07MT, DRB4*01, DRB5BFB	Not protected
JS0065	Male	26	A1, A'24	B8, B44	DRB1*13, DRB1*08DKZ, DRB3*01	Not protected
DC0020	Male	41	A2, A'32	B15, B'44	DRB1*04BMS, DRB1*14APF, DRB3*02CAY	Not protected
GB0021	Male	29	A1, A11	B35, B57	DRB1*01AD, DRB1*07AC	Protected
DF0030	Male	47	A2, A3	B7, B40DGE	DRB1*01AD, DRB1.2 03YH, DRB3.1 01MN	Protected
WW0058	Male	32	A2, A'3	B7, B44	DRB1*02, DRB5*01/02	Protected
ND0064	Male	21	A2, A'3	B14, B'51	DRB1*02, DRB1*03/06, DRB3*02/03, DRB5*01BFB	Protected
HG0066	Male	38	A2, A'2	B18, B39	DRB1*08/14/(03), DRB3*02/03	Protected

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[0055] As illustrated in FIG 3, the peptide pool generally induced a significant T-cell response with a greater response in PBMC's from immunized volunteers, compared to pre-immunized PBMC. Post-immune PBMC's from several individuals, e.g., GB21; DF0030, showed relatively high induction of IFN- γ . In fact, the volunteer GB21 exhibited a protective immune response against malaria.

Additionally, peptide antigens containing HLA class I binding motifs generally resulted in significant IFN- γ induction. Interestingly, PBMCs from GB21, which gave the overall greatest IFN- γ induction, yielded a level of IFN- γ SPC, using A11 HLA pools as antigen, equivalent to that observed with the pool of 15-mers. This suggests that PF106 not only contains anti-*P. falciparum* epitopes but that some epitopes may be important in immunity against malaria. Furthermore, regions of PF106 operate in an HLA-restricted fashion to induce class I T-cell immunity.

[0056] In addition to PF106, other *P. falciparum* proteins were utilized as antigens in IFN- γ ELISpot assays resulted in HLA class I specific induction of IFN- γ . These include PF61, PF56, PF121 and PF144. The results of these studies are illustrated in FIG's 3-7, respectively. Additionally, the results are summarized in Table 1. The DNA and protein sequence identification numbers are summarized in Table 2.

[0057] The T-cell response elicited by PF144 was particularly noteworthy. As shown in FIG 7, PBMC's from four individuals, exposed to PF144, resulted in a high level of IFN- γ secreting cells. Of note is that three of the four individuals were previously shown to have elicited a protective immune responses against malaria, suggesting the importance of this protein in protection. Furthermore, peptides

containing HLA binding motifs to class I alleles induced an IFN- γ response, in some cases, such as in GB21, WW58 and DF30, equivalent or even greater than that observed when the pool of 15-mer peptides were used.

[0058] In furtherance to the notion that PF144 contains T-cell epitopes important in conferring immunity against malaria, polypeptide containing motifs associated with B7 were analyzed in greater detail for their ability to induce IFN- γ . As illustrated in FIG 8, the IFN- γ responses to peptide pools were analyzed in PBMCs from WW58. As seen in FIG 8, the results using A2 and A3 pools were consistent to that illustrated in FIG 7. However, in using B7 pools as antigen, one peptide in particular, KLRHFFSILLKSLVI, containing a B7 binding motif, induced a high IFN- γ response in the immune PBMCs. This suggests the likely importance of B7 allele in class I T-cell immunity against PF144.

[0059] The data in FIG 1-8 strongly suggest that the protein antigens PF106, 61, 56, 121 and 144 contain epitopes important in conferring protection against *P. falciparum* malaria. Furthermore, the proteins are able to induce class I specific responses, as evidenced by their ability to induce a T-cell response to antigen containing specific HLA class I peptide binding motifs.

Example 6: Incorporation into expression systems

[0060] This example illustrates prophetic uses of the recombinant genes encoding the novel antigens by incorporating one or more of the antigens of Table 2, or nucleic acid encoding the antigens into vaccine formulations. Alternatively, immunogenic fragments of the antigens or derivatives of the antigens of Table 2 can be utilized. It

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is contemplated that the antigens can be expressed either as a component of a DNA vaccine or other platform system. An example of a contemplated expression system includes, but is not limited to, viral systems, including replicating and nonreplicating vectors. Examples of contemplated viral vectors include adenovirus, alphavirus, posvirus, cytopomegalovirus, canine distemper virus and yellow fever virus. The antigen could be incorporated as an insert of a DNA or other vaccine expression system, either as a single antigen or multiple antigen expression systems from a single or multiple promoters.

[0061] The contemplated invention includes a method for inducing an immune response in mammals, including humans. In this example, antigens, either as polypeptide or incorporated into a nucleic acid expression system, such as a DNA or viral system, are administered in one or more doses. The method also contemplates inducing an immune response utilizing a prime-boost immunization regimen. In this embodiment, one or more priming immunization doses would be administered followed by one or more boosting immunizations.

[0062] The priming and boosting immunization comprises a composition containing one or more malaria polypeptides, wherein the polypeptides contains the amino acid sequence of SEQ ID Nos. 2, 4, 6, 8, 10, 12 or 14, or immunogenic derivatives, thereof. Alternatively, the immunogenic composition can be comprised of an expression system capable of expressing the polypeptides. In this embodiment, nucleic acid molecules, encoding these polypeptides, with the nucleic acid sequences of SEQ ID Nos. 1, 3, 5, 7, 9, 11 or 13, can be inserted into a DNA plasmid or a viral expression vector. Examples of viral expression vector systems include: alphavirus

(and alphavirus replicons), adenovirus, poxvirus, adeno-associated virus, cytomegalovirus, canine distemper virus, yellow fever virus and retrovirus.

[0063] The contemplated methods include immunization regimens wherein the priming immunization comprises malarial peptides expressed from a DNA plasmid expression vector or an adenovirus, while the boosting immunization includes malaria peptides expressed from either: adenovirus, adenovirus that is heterologous to the priming adenovirus, poxvirus or one or more malaria polypeptides. The malaria polypeptides include polypeptides with the amino acid sequences of SEQ ID NO. 2, 4, 6, 8, 10, 12 or 14, or immunogenic derivatives, thereof.

[0064] Having described the invention, one of skill in the art will appreciate in the appended claims that many modifications and variations of the present invention are possible in light of the above teachings. It is therefore, to be understood that, within the scope of the appended claims, the invention may be practiced otherwise than as specifically described.

What is claimed is:

1. An immunogenic composition for inducing an immune response to *Plasmodium falciparum* comprising an isolated *Plasmodium falciparum*-derived polypeptide and a pharmaceutically acceptable carrier, wherein the isolated polypeptide comprises the amino acid sequence defined by SEQ ID NO: 10.
2. The immunogenic composition of claim 1, wherein said isolated polypeptide is encoded by the nucleic acid sequence according to SEQ ID NO: 9.
3. The immunogenic composition of claim 1, wherein said polypeptide contains, within the amino acid sequence defined by SEQ ID NO: 10, an HLA class I epitope.
4. The immunogenic composition of claim 2, wherein said nucleic acid sequence encodes an HLA class I epitope.
5. The immunogenic composition of claim 2, wherein said nucleic acid sequence is inserted into, and the polypeptide is expressed from a DNA plasmid or viral expression system, wherein said viral expression system is replicating or nonreplicating.
6. The immunogenic composition of claim 5, wherein said viral expression system is selected from the group consisting of alphavirus replicon, adenovirus, poxvirus, adeno-associated virus, cytomegalovirus, canine distemper virus, yellow fever virus and retrovirus.

7. Use of the composition of any one of claims 1 to 6 for inducing an immune response against malaria in a mammal, wherein said composition further comprises one or more isolated nucleic acid molecules, inserted into a suitable expression vector, encoding isolated malaria polypeptides, said isolated nucleic acid molecules comprising the nucleic acid sequence according to SEQ ID NO: 9.
8. The use of claim 7, where the suitable expression system is a DNA plasmid or replicating or nonreplicating viral vector.
9. The use of claim 7, wherein said isolated nucleic acid molecules encode HLA class I epitopes.
10. The use of claim 7, further comprising at least one of a priming immunogenic composition and a boosting immunogenic composition, wherein said priming or boosting immunogenic composition comprises one or more *Plasmodium falciparum*-derived polypeptides comprising the amino acid sequence defined by SEQ ID NO: 10 or comprises a composition comprising one or more isolated nucleic acid molecules defined by the nucleic acid sequence according to SEQ ID NO: 9 inserted into a suitable expression vector.
11. The use of claim 7, wherein said suitable expression vector is selected from the group consisting of DNA plasmid, alphavirus replicon, adenovirus, poxvirus, adenoassociated virus, cytomegalovirus, canine distemper virus, yellow fever virus and retrovirus.

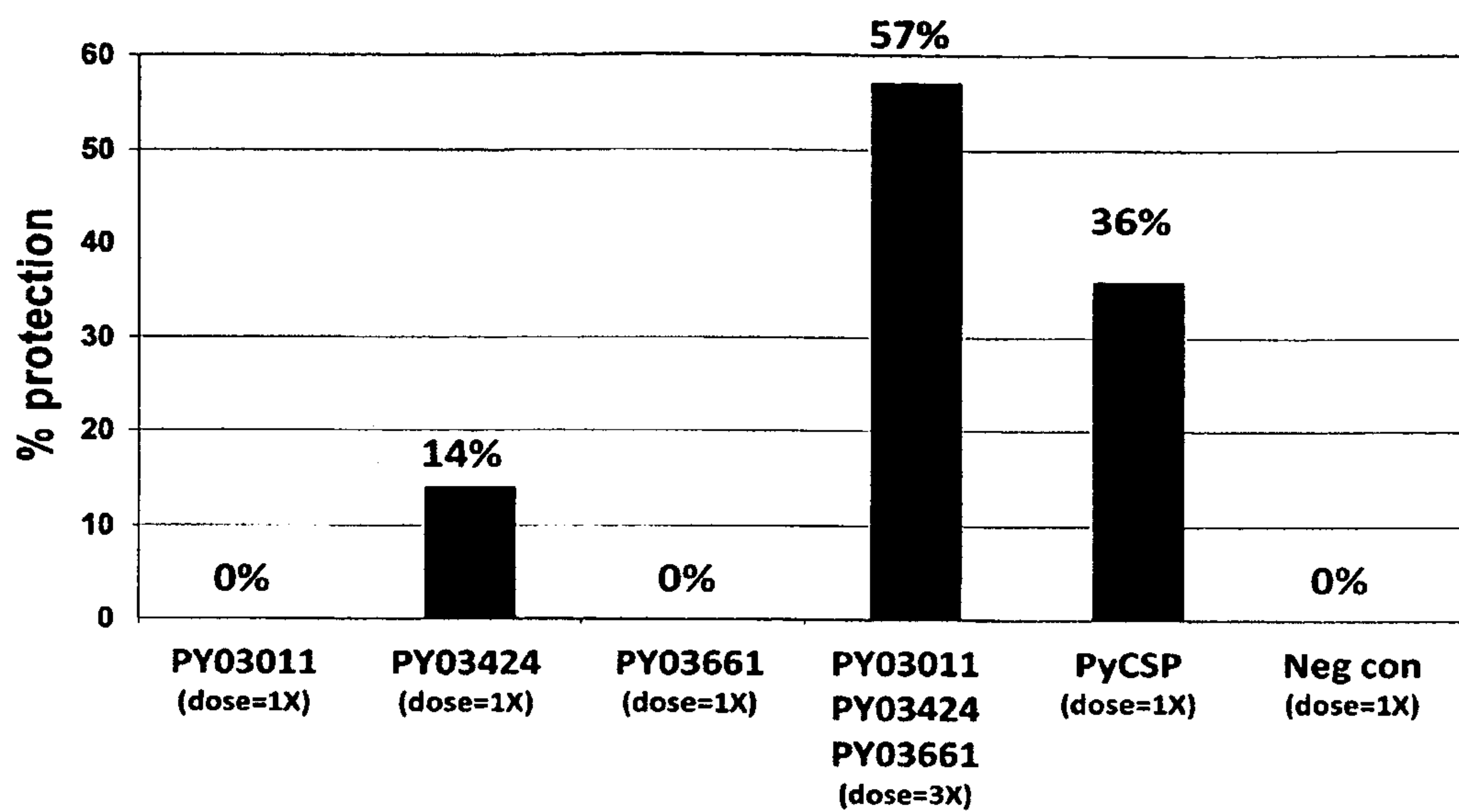
12. The use of claim 10, wherein said suitable expression vector is selected from the group consisting of DNA plasmid, alphavirus replicon, adenovirus, poxvirus, adenoassociated virus, cytomegalovirus, canine distemper virus, yellow fever virus and retrovirus.
13. The use of claim 10, wherein said priming immunogenic composition comprises a vector, which is an alphavirus vector and said boosting immunogenic composition comprises a non-alphavirus vector.
14. The use of claim 10, wherein said priming immunogenic composition comprises an expression vector that is a DNA plasmid or an adenovirus and the boosting immunogenic composition comprises an expression vector selected from the group consisting of adenovirus, adenovirus heterologous to the priming adenovirus, poxvirus and polypeptide, wherein said polypeptide comprises the amino acid sequence according to SEQ ID NO: 10.
15. The use of claim 12, wherein the alphavirus replicon is selected from the group consisting of RNA replicons, DNA replicons and alphavirus replicon particles.
16. The use of claim 12, wherein the alphavirus is selected from the group consisting of Venezuelan Equine Encephalitis Virus, Semliki Forest Virus and Sindbis Virus.

17. The use of claim 13, wherein the non-alphavirus viral expression system is selected from the group consisting of poxvirus, adenovirus, adeno-associated virus and retrovirus.
18. The use of claim 17, wherein the poxvirus is selected from the group consisting of cowpox, canarypox, vaccinia, modified vaccinia Ankara, and fowlpox.
19. The immunogenic composition of claim 1, wherein said composition also comprises one or more polypeptides selected from the group consisting of SEQ ID NOs. 2, 4, 6, 8, 12 and 14 encoded by one or more nucleic acids sequences selected from the group consisting of SEQ ID NOs. 1, 3, 5, 7, 11 and 13.
20. The immunogenic composition of claim 19, wherein said nucleic acid sequence contains a region encoding an HLA class I epitope.
21. The immunogenic composition of claim 19, wherein said nucleic acid sequence is inserted into, and the polypeptide is expressed from a DNA plasmid or viral expression system, wherein said viral expression system is replicating or nonreplicating.
22. The immunogenic composition of claim 19, for inducing an immune response against malaria in a mammal, wherein said composition further comprises one or more isolated nucleic acid molecules, inserted into a suitable expression

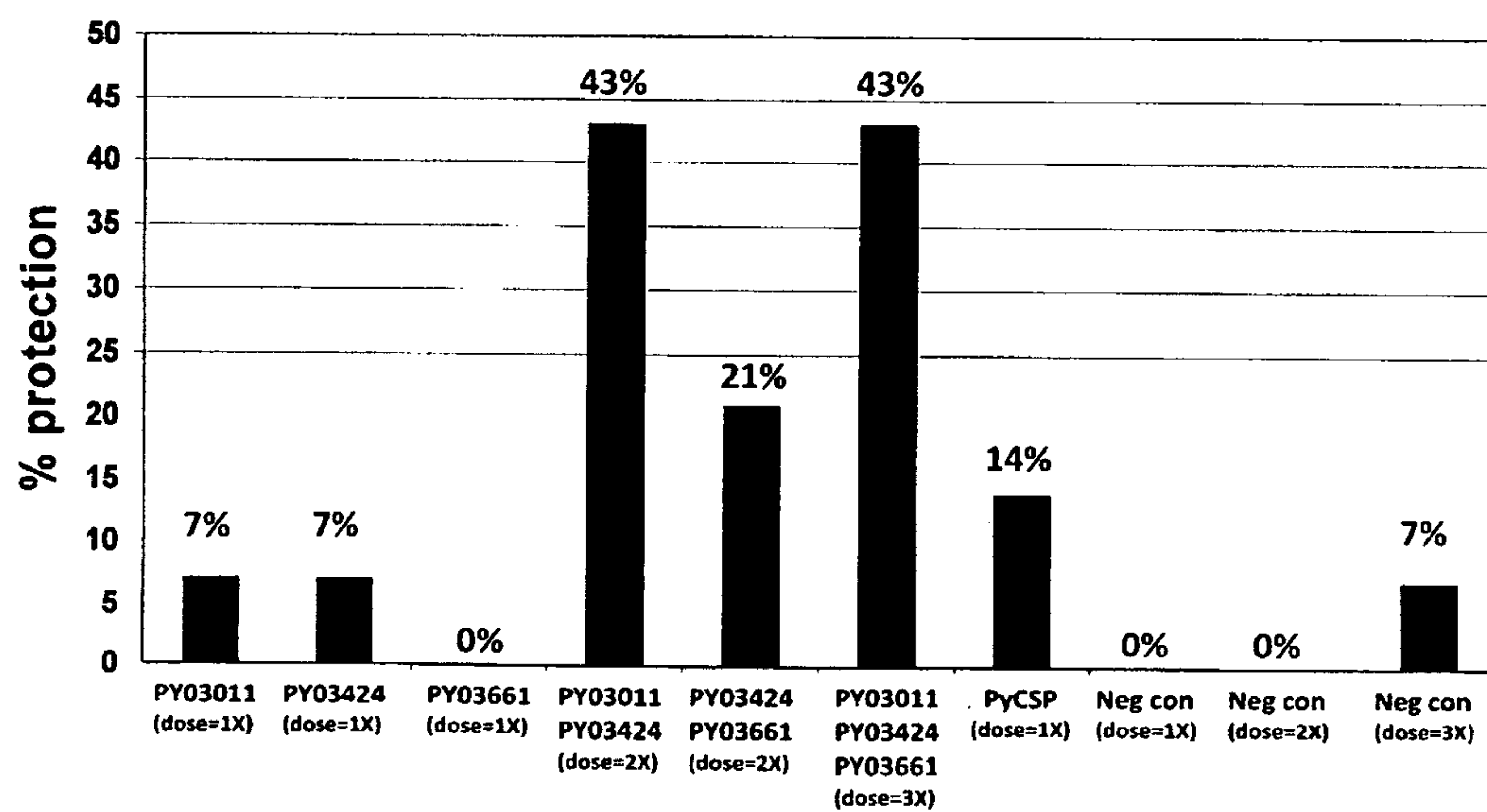
vector, encoding isolated malaria polypeptides, said isolated nucleic acid molecules comprising a nucleic acid sequence according to SEQ ID NO: 9.

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Panel A



Panel B

FIG 1

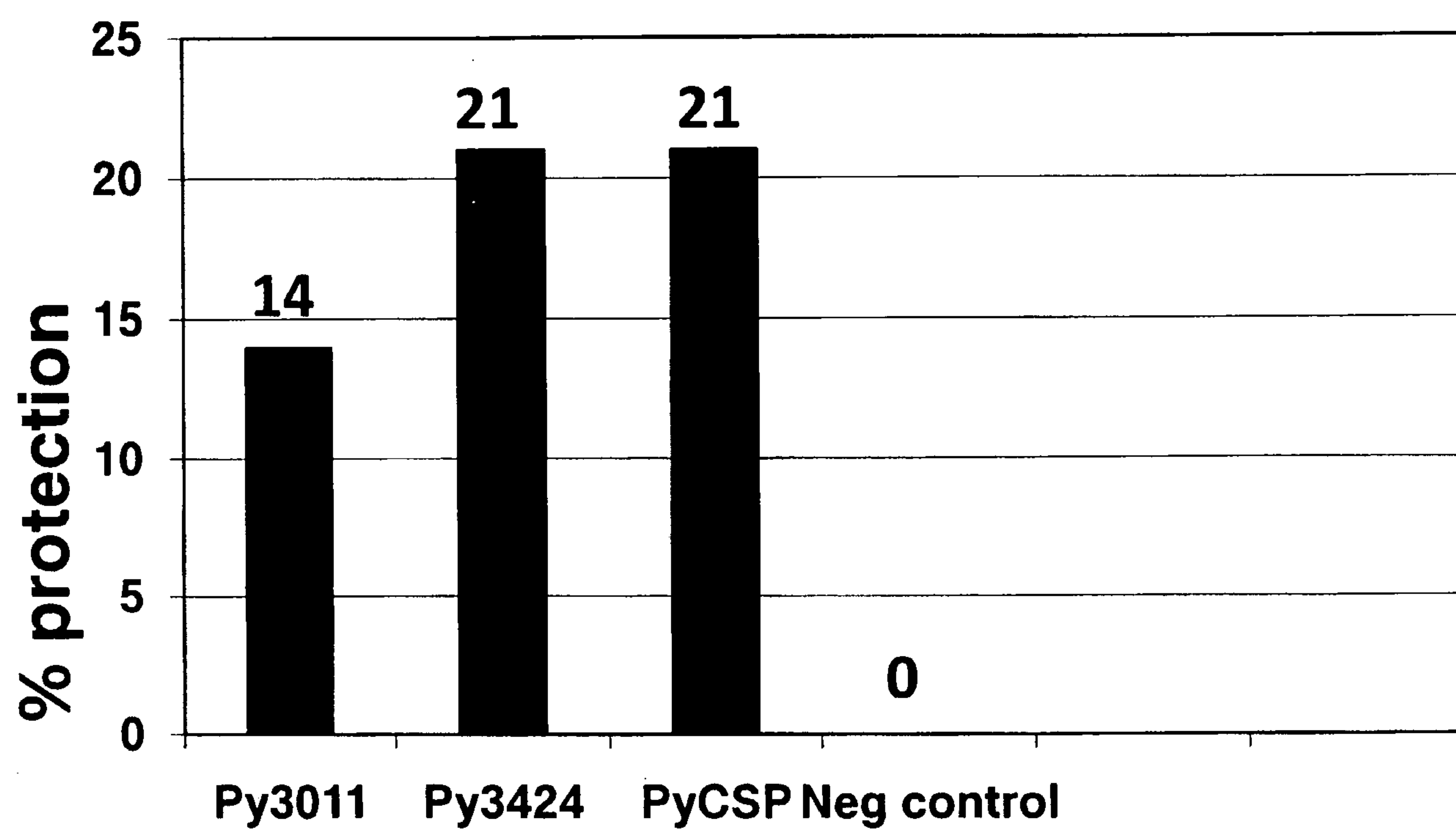


FIG. 2

PF 106

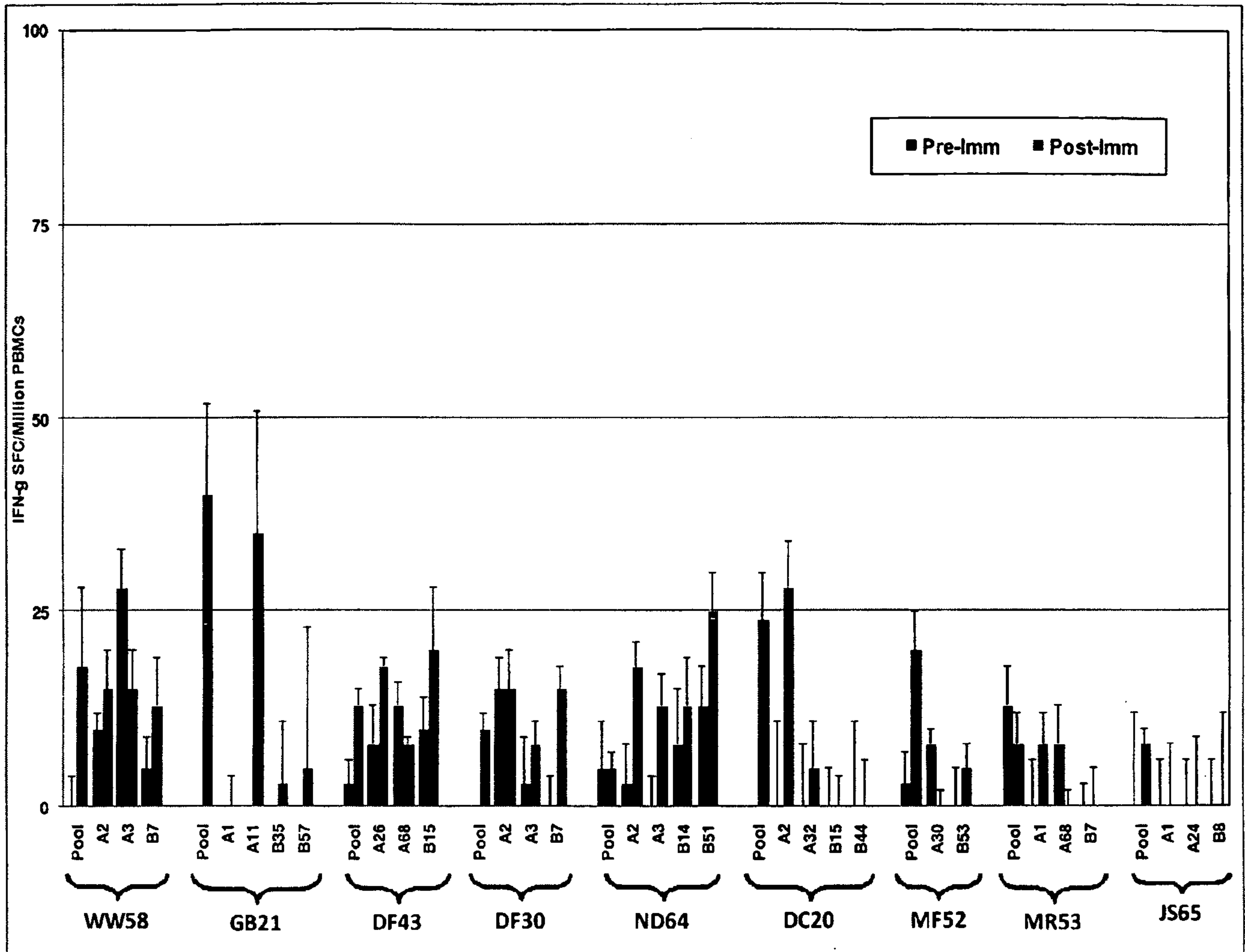


FIG. 3

PF 61

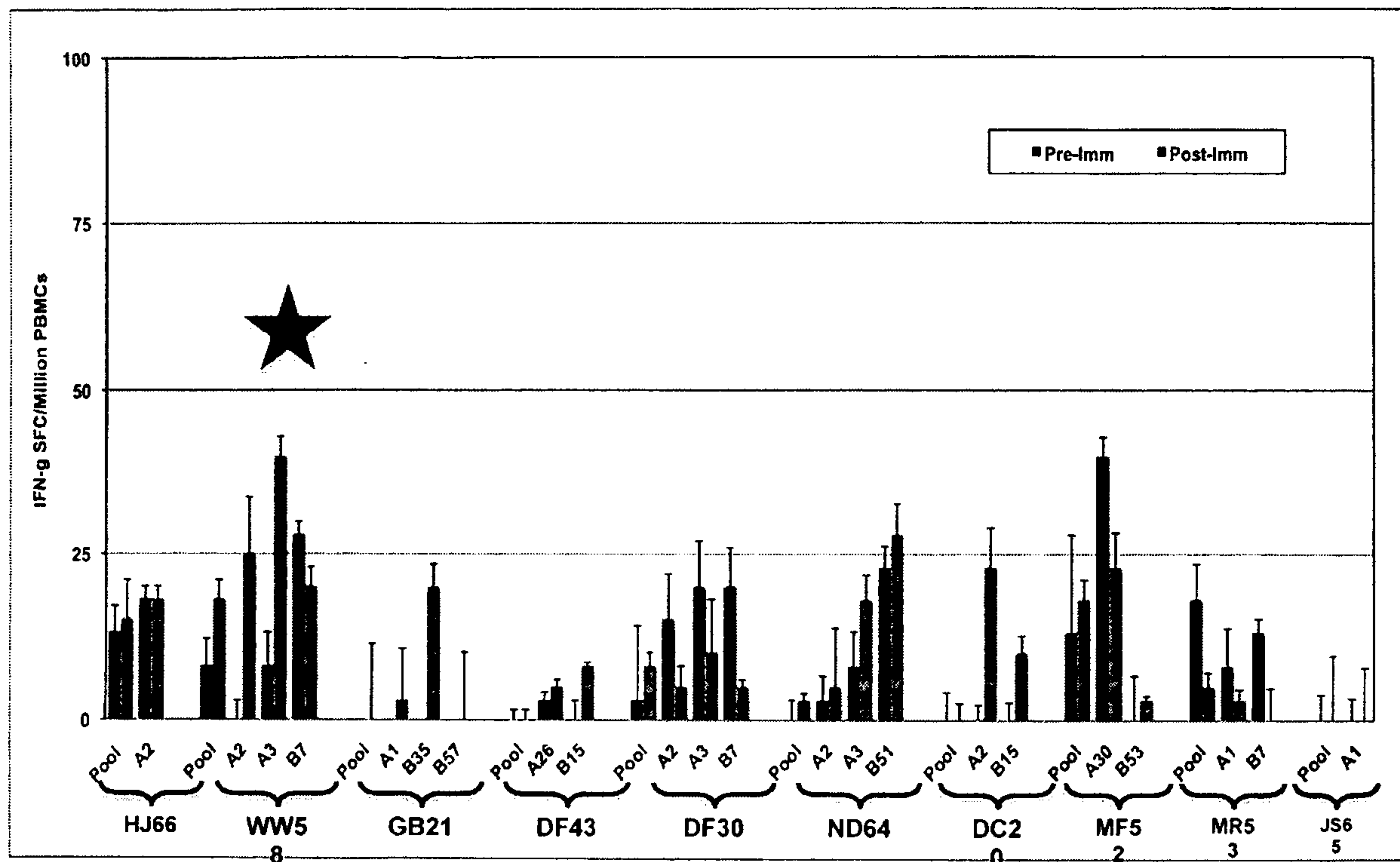


FIG. 4

PF 56

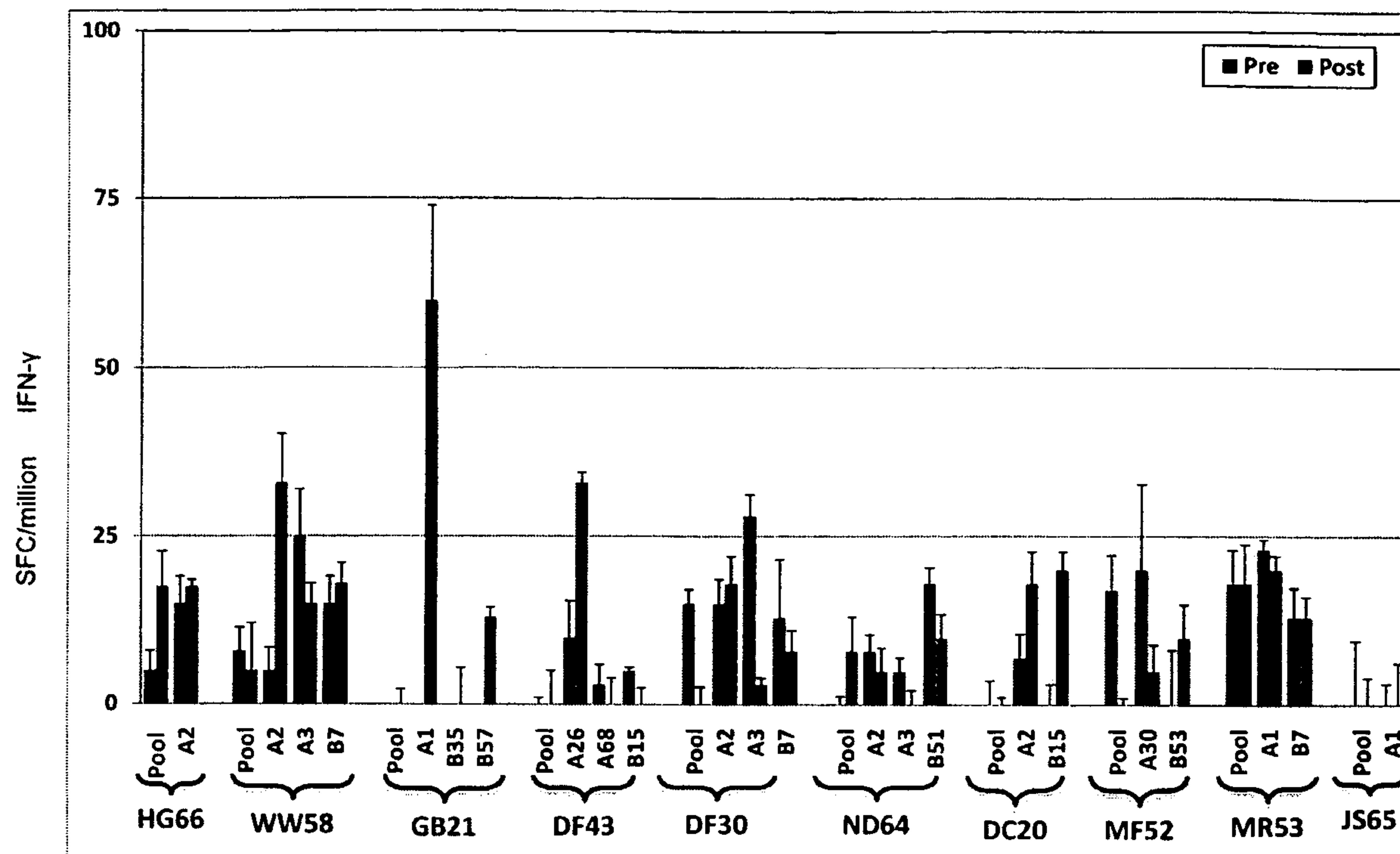


FIG. 5

PF 121

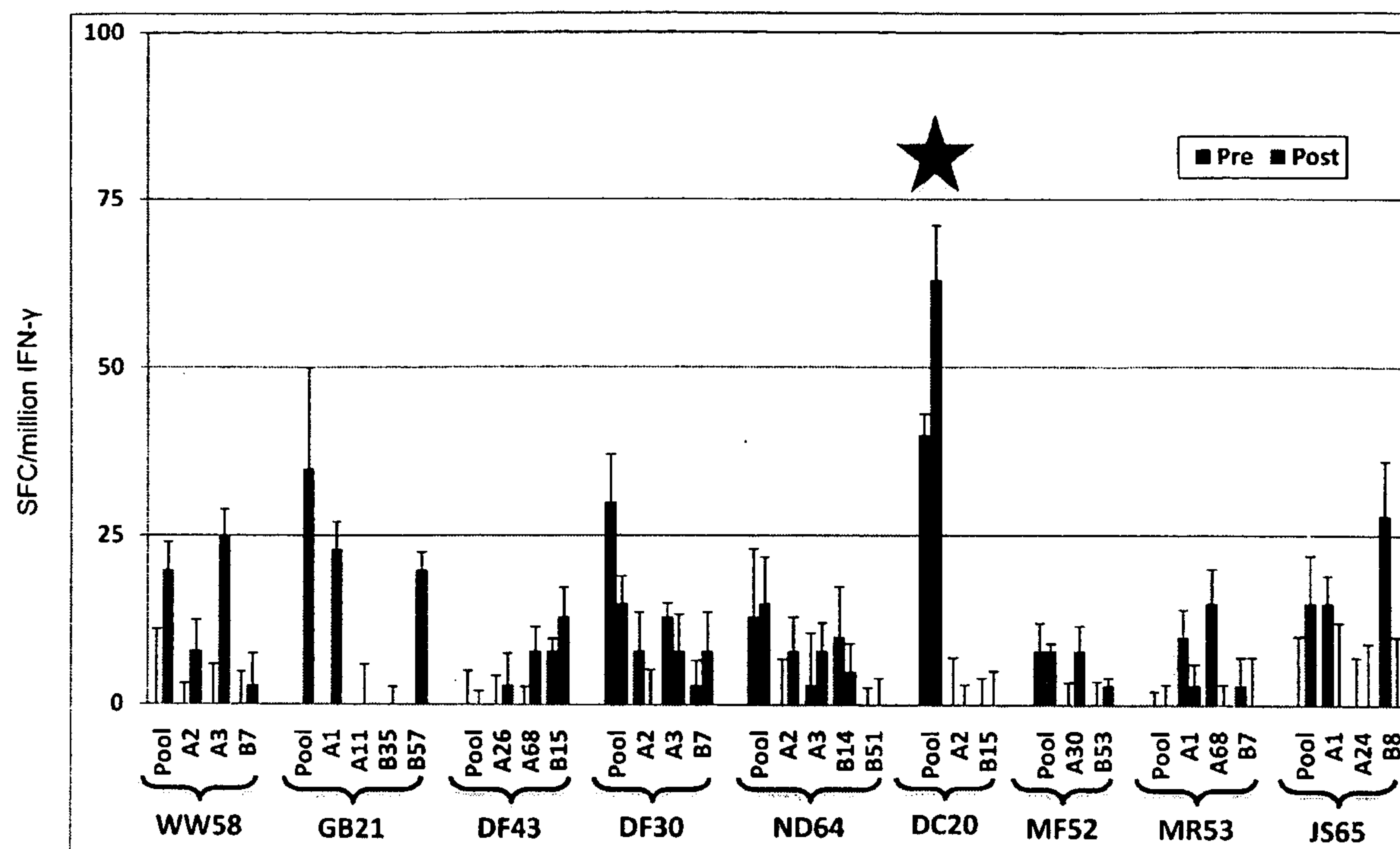


FIG. 6

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PF 144

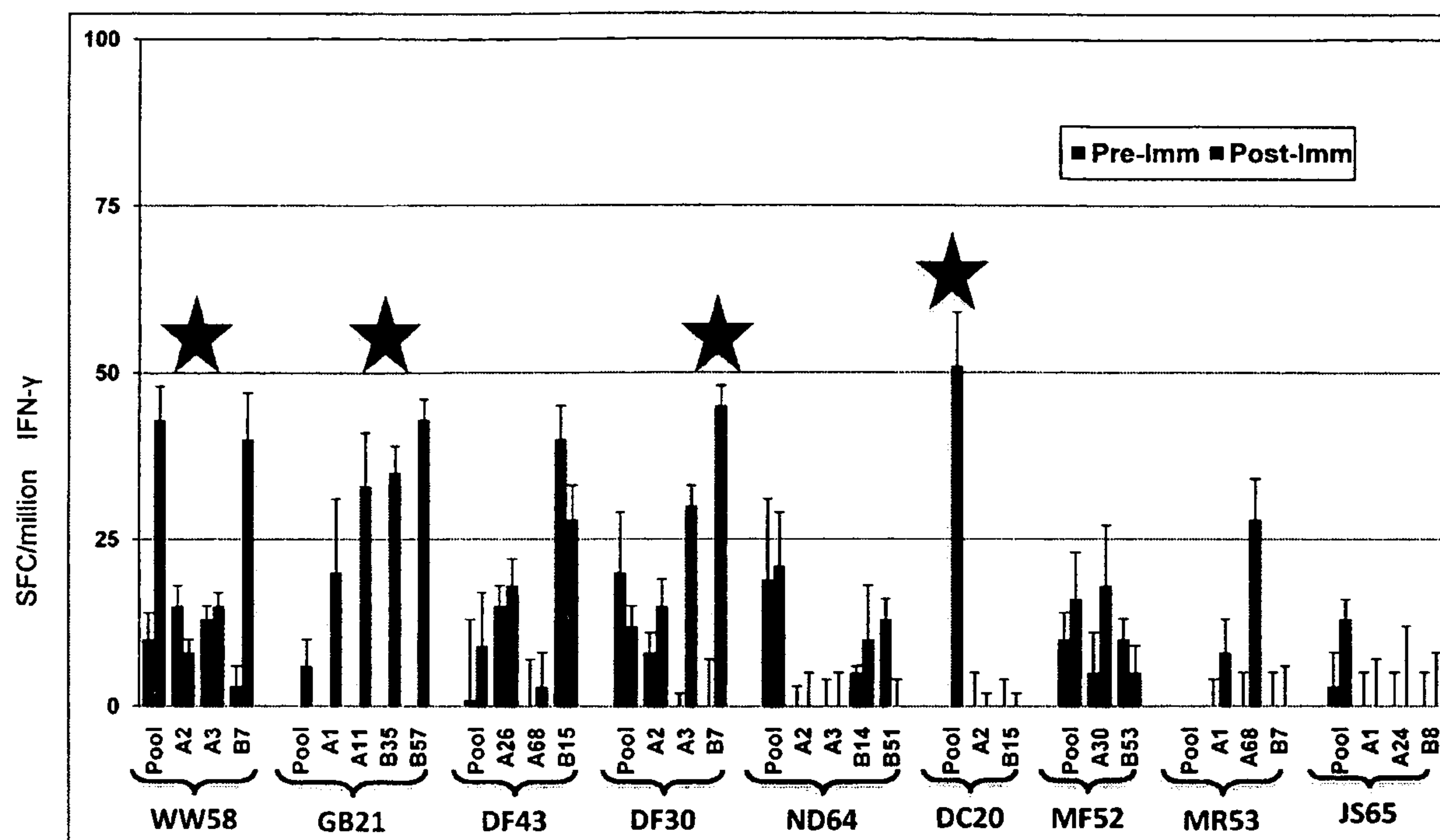


FIG. 7

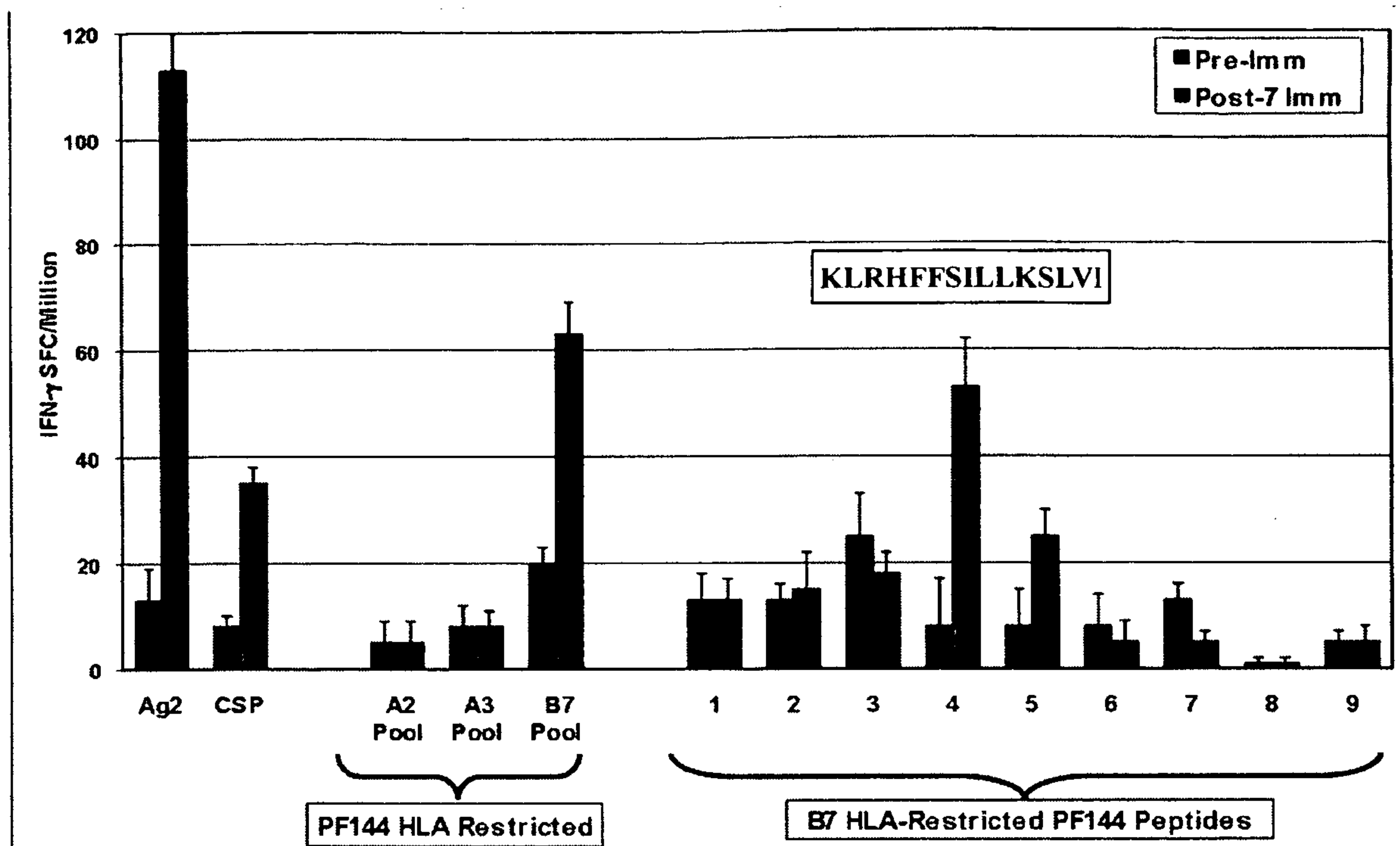


FIG. 8