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(71) Applicants *(for all designated States except US)*: **THE UNITED STATES OF AMERICA, AS REPRESENTED BY THE SECRETARY, DEPARTMENT OF HEALTH AND HUMAN SERVICES** [US/US]; Office of Technology Transfer, National Institutes of Health, 6701 Rockledge Drive, Suite 700, MSC 7788, Bethesda, Maryland 20892-7788 (US). **THE UNITED STATES OF AMERICA, AS REPRESENTED BY THE SECRETARY OF AGRICULTURE** [US/US]; 1400 Independence Ave., S.W., Washington, District of Columbia 20250 (US). **BIOLOGICKE CENTRUM AV CR, V. V. I.** [CZ/CZ]; Branisovska 31, 370 05 Ceske Budejovice (CZ).

(72) Inventors; and

(71) Applicants *(for US only)*: **GRIGG, Michael E.** [CA/US]; NIAID, NIH, Molecular Parasitology Section, 4 Center Drive, Room B1-06, Bethesda, Maryland 20892 (US). **PSZENNY, Viviana** [US/US]; NIAID, NIH, Molecular Parasitology Section, 4 Center Drive, Room B1-06, Bethesda, Maryland 20892 (US). **SARDINHA DA SILVA, Aline** [BR/US]; NIAID, NIH, Molecular Parasitology Section, 4 Center Drive, Room B1-06, Bethesda, Maryland 20892 (US). **DUBEY, Jitender P.** [US/US]; 10300 Baltimore Ave, Building 1040, USDA, Animal Parasitic Diseases Laboratory, Beltsville, Maryland 20705-2350 (US). **LUKES, Julius** [CZ/CZ]; University of Bohemia, Institute of Parasitology, Branisovska 31, 370 05 Ceske Budejovice (CZ).

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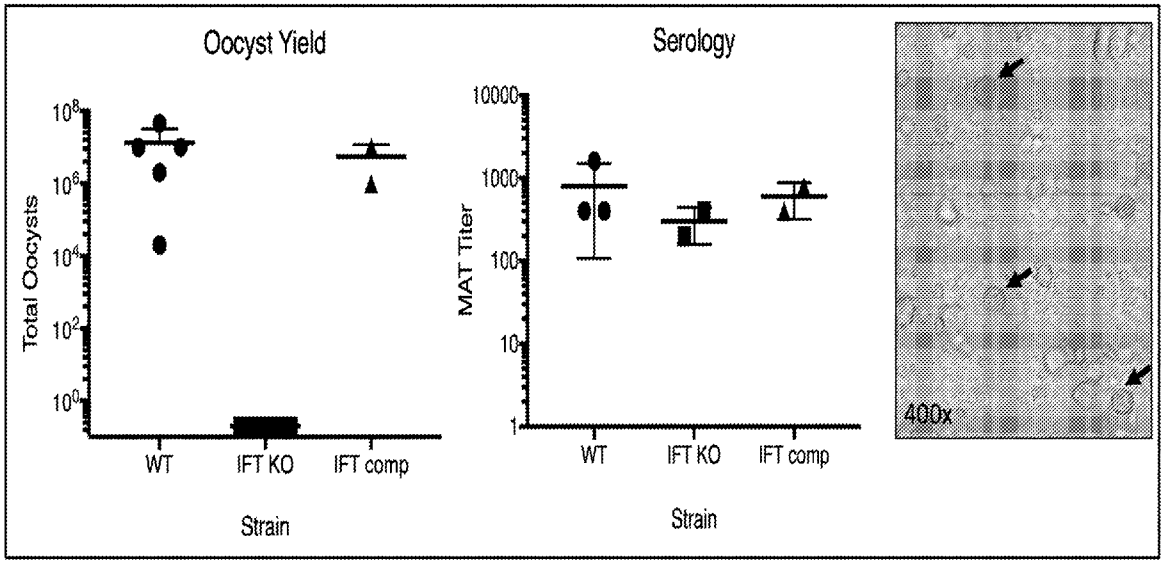
(54) Title: VACCINE FOR CATS TO BLOCK TOXOPLASMA OOCYST SHEDDING AND TRANSMISSION

Figure 1



(57) Abstract: The invention provides a recombinant parasite, which is a member of the phylum *Apicomplexa or Euglenozoa*, and which comprises a genome which lacks a native gene encoding *Toxoplasma gondii* IFT88, SRS15A, SRS15B, and/or SRS15C, SRS26B, a native homolog or ortholog thereof, or a combination of two or more of these. The invention also provides a method for producing the recombinant parasite, which involves knocking out one or more of these genes from the genome of the parasite. Reagents for accomplishing this are provided, as are vaccine compositions comprising the inventive recombinant parasites and vaccine compositions comprising a *Toxoplasma gondii* IFT88, SRS15A, SRS15B, and/or SRS15C, SRS26B protein or homolog or ortholog thereof, or a combination of two or more thereof. Methods of vaccinating animals using such vaccine compositions also are provided.

[Continued on next page]

(74) **Agent: HEFNER, M. Daniel** et al.; Leydig, Voit & Mayer, Ltd., Two Prudential Plaza, Suite 4900, 180 North Stetson Avenue, Chicago, Illinois 60601 (US).

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VACCINE FOR CATS TO BLOCK TOXOPLASMA OOCYST SHEDDING AND TRANSMISSION

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This patent application claims the benefit of U.S. Provisional Patent Application No. 63/470,773 filed June 2, 2023, which is incorporated by reference.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0002] This invention was made with Government support under project number AI001018 by the National Institutes of Health, National Institute of Allergy and Infectious Diseases. The Government has certain rights in the invention.

INCORPORATION-BY-REFERENCE OF MATERIAL SUBMITTED ELECTRONICALLY

[0003] Incorporated by reference in its entirety herein is a computer-readable nucleotide/amino acid sequence listing submitted concurrently herewith and identified as follows: One 49,203 Byte XML file named "771108.xml," dated June 3, 2024.

BACKGROUND OF THE INVENTION

[0004] Toxoplasmosis is a major public health concern and also economically disruptive to agriculture involving livestock. *Toxoplasma gondii* is the zoonotic causative agent of toxoplasmosis. A member of the phylum *Apicomplexa*, *Toxoplasma* can infect almost any cell type found in mammals and birds. It has been estimated that more than 30% of the world population over the age of six has been infected with *T. gondii*, with some regions having over 60% of the population test seropositive. There are multiple transmission pathways, including consumption of undercooked meat from infected animals, consumption of unwashed plants, contaminated water supplies, blood transfers, and congenital transfer. Additionally, direct or indirect transmission can occur via contact with the stool of infected felids.

[0005] Transmission of *Toxoplasma* in nature is highly dependent on the parasite's sexual cycle, which occurs exclusively in felids. However, the molecular pathways promoting sexual stage development in *T. gondii* are largely unknown. Transmission of *T. gondii* to cats is almost entirely through the consumption of infected prey. The parasite can undergo sexual reproduction in the GI tracts of cats (in other animals, reproduction occurs asexually). *T. gondii* generates oocysts which are expelled in the stool. These infectious oocysts containing sporozoites are extremely hardy (they are kept in weak sulfuric acid for long term storage in laboratories) and persist in the environment for many years. In moist environments, oocysts undergo sporulation. These sporulated oocysts can then be consumed by animals through consumption of unwashed grass or other plants grown in the infected soil, or by consumption of contaminated water. In infected animals, including humans, the parasite will find its way to tissues and form a cyst. Thus, infected cats can shed high numbers of persistent and infectious *T. gondii* oocysts, which are capable of causing toxoplasmosis outbreaks in both animals and people.

[0006] In humans, the parasite is capable of infecting anyone (healthy and immunocompromised individuals). The cysts persist in the dormant phase and are present in the muscles and in the brain of infected individuals. However, individuals with weakened immune systems can develop several symptoms of Toxoplasmosis disease. Toxoplasmosis can present with fever, swollen lymph nodes, headaches, muscle aches, and skin rash. In some cases, *Toxoplasma* can infect the eye. In individuals with weakened immune systems, *Toxoplasma* can affect the lungs or the brain. Toxoplasmosis also is a significant concern for pregnant persons. *Toxoplasma* can induce a miscarriage or cause medical problems which will only be apparent at birth. These problems can include hydrocephalus, eye infections, brain tissue anomalies, or an enlarged liver or spleen. Further, the infant may present with developmental issues, blindness, hearing loss, seizures, heart disorders, jaundice, or a rash. In some cases, an infant with *Toxoplasma* may appear fine but develop conditions later in life.

[0007] In livestock, Toxoplasmosis is typically subclinical due to control by the host immune system, and treatment often is unnecessary. In young animals whose immune systems are not robust, however, Toxoplasmosis can cause a host of issues across a range of tissues, in some cases resulting in death. *T. gondii* is an important cause of abortion and stillbirth in sheep, goats,

cervids, and sometimes pigs. However, infection prevention in livestock is key to not produce meat for consumption that is infected with cysts, and prevention of shedding of oocysts from felines is important to protect environmental risk.

[0008] While a few drugs are available to treat *Toxoplasma* infection, once *Toxoplasma* enters the tissue and forms cysts, such drugs become less effective. Moreover, no vaccine is currently available to prevent Toxoplasmosis in humans; however, a live attenuated vaccine is approved for sheep, TOXOVAX® (MSD Animal Health), to prevent abortions caused by *Toxoplasma* infections. TOXOVAX® uses the *T. gondii* S48 strain, which cannot generate tissue cysts in animal hosts. It is administered prior to mating and does not require a booster shot. Currently, TOXOVAX® is only available for use in the United Kingdom, New Zealand, France, and Ireland.

[0009] From the foregoing, it is apparent that current approaches to controlling Toxoplasmosis are suboptimal. As noted, drugs available for veterinary use are few, only moderately effective, and at an early stage of infection. A single vaccine is available in but a few countries, and it relies on a strain that cannot generate cysts. The present invention addresses these concerns.

BRIEF SUMMARY OF THE INVENTION

[0010] The invention provides a recombinant parasite, which is a member of the phyla *Apicomplexa* or *Euglenozoa*, and which comprises a genome which lacks a native gene homologous to (including identical to if within *Toxoplasma gondii* or homologs in species other than *T. gondii*) *T. gondii* IFT88, one or more genes from the *T. gondii* tandem SRS15 array (e.g., SRS15A, SRS15B, and/or SRS15C), *T. gondii* SRS26B, or a combination of two or more thereof. The invention also provides a method for producing the recombinant parasite, which involves knocking out *T. gondii* IFT88, one or more genes from the *T. gondii* SRS15 array (e.g., SRS15A, SRS15B, and/or SRS15C), *T. gondii* SRS26B, or homologs thereof (such as in species other than *T. gondii*), or a combination of two or more thereof from the genome of the parasite. Reagents for accomplishing this are provided, as are vaccine compositions comprising the inventive recombinant parasites and vaccine compositions comprising *T. gondii* IFT88, one or

more genes from the *T. gondii* SRS15 array (e.g., SRS15A, SRS15B, and/or SRS15C), *T. gondii* SRS26B, *T. gondii*, including homologs thereof (such as in species other than *T. gondii*), or a combination of two or more thereof. Methods of vaccinating animals using such vaccine compositions also are provided.

[0011] The invention facilitates the generation of live, attenuated oral vaccines and/or nanoparticle oral or mucosal vaccines that can be employed by caregivers of cats worldwide to reduce the burden of Toxoplasmosis globally. Moreover, in endemic areas, as a public health effort, vaccination of stray cats in regions were *T. gondii* transmission by oocysts is high, through the use of the inventive attenuated oral vaccines and/or nanoparticle oral or mucosal vaccines, can further reduce the transmission of *T. gondii* in food and water sources destined for human consumption or consumption by livestock (such as sheep, goats, cervids, pigs, and others potentially infected with parasites of the phylum *Apicomplexa*). These advantages, and additional inventive features, will be apparent upon reviewing the following detailed description and the accompanying drawings.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWING(S)

[0012] **Figure 1** presents data concerning cumulative oocyst yield (left panel) and serology titer (middle panel) among experimental cats challenged with bradyzoites either from wildtype ("WT") CZ1, a knock-out ("KO") strain deficient for IFT88 ("IFT88-KO"), or IFT88-KO complemented with a heterologous allele of IFT88. The micrograph (right panel) shows *Toxoplasma* oocysts obtained from floating fecal pellets from cats infected with WT CZ1.

[0013] **Figure 2** presents data concerning cumulative oocyst yield (left panel) and serology titer (middle panel) among cats first challenged with bradyzoites from WT CZ1 or SRS15B-KO. Cats infected with WT CZ1 shed oocysts, but SRS15B-KO infected cats did not. Both cats seroconverted, indicating that they generated robust titers of anti-*Toxoplasma*-specific IgG. Immunized cats were then re-infected with WT CZ1 (representing a secondary, homologous challenge) at 6 months post first infection to assess protection from oocyst shedding. The SRS15B-KO vaccinated cats were immune to secondary challenge with WT CZ1 bradyzoites and did not shed oocysts. As a control, the WT CZ1 infected cats were re-infected with WT CZ1

bradyzoites, and did not shed oocysts, as expected. The micrograph (right panel) shows *Toxoplasma* oocysts obtained from floating fecal pellets from cats infected with WT CZ1, which, as noted above, is one method used to quantify oocyst numbers defecated from cats during their patency period of secretion.

DETAILED DESCRIPTION OF THE INVENTION

[0014] In a first aspect, the invention provides a recombinant parasite (i.e., a live organism), which is a member of the phyla *Apicomplexa* or *Euglenozoa*, which comprises a genome which lacks native genes corresponding to or homologous to *T. gondii* IFT88, one or more genes from the *T. gondii* SRS15 array (e.g., SRS15A, SRS15B, and/or SRS15C), *T. gondii* SRS26B, or a combination of two or more thereof, or a combination of two or more thereof. The inventive recombinant parasite can be an organism from any genus within these phyla, such as being a member of the genera *Toxoplasma*, *Cryptosporidium*, *Neospora*, or *Sarcocystis*. While the experiments reported herein involve a cosmopolitan Type II strain (CZ1) from the species *T. gondii*, the invention can be applied to other strains of *T. gondii* as well.

[0015] As noted, the inventive recombinant parasite comprises a genome, which is recombinant through being engineered to lack a native genetic sequence encoding a homolog of *T. gondii* IFT88, one or more genes from the *T. gondii* SRS15 array (e.g., SRS15A, SRS15B, and/or SRS15C), SRS26B, or a combination of two or more thereof. By "native" in this context is meant that the recombinant parasite lacks a gene that naturally occurs in a wildtype organism from which the inventive parasite is derived. This is in contradistinction to organisms which may not natively possess a homolog (ortholog) of *T. gondii* IFT88, one or more genes from the *T. gondii* SRS15 array (e.g., SRS15A, SRS15B, and/or SRS15C), SRS26B, or a combination of two or more thereof.

[0016] Within *T. gondii*, the IFT88 gene encodes a protein involved in intraflagellar transport. Without wishing to be bound by theory, it is believed that due to the absence of the IFT88 gene product, male gametes of the inventive recombinant parasite cannot effectively swim, which thus prevents them from contacting and fertilizing female gametes and the subsequent production of oocysts. Within *T. gondii*, SRS15B is a 6-CYS fold surface antigen

found at the interface between the male and female gametes. Without wishing to be bound by theory, it is believed that due to the absence of the SRS15B gene product, the male and female gametes are attenuated in fusing to form a zygote, or highly attenuated in doing so. This also effectively prevents fertilization and the subsequent production of oocysts. SRS15B is part of a tandem array of three genes that have the 6-CYS fold established in plasmodium to promote male-female gamete recognition and fertilization (see, e.g., He *et al.* 2002. *Nat Struct Biol* 9:606-611. PMID: 12091874 and Tonkin *et al.* 2013. *J Biol Chem.* 288(18):12805-17 PMID: 23511632, both of which are incorporated herein in their entireties by reference). Thus, while experiments herein conclusively establish SRS15B to be one gene product, which, when knocked out, blocks oocyst formation, it is to be understood that the inventive method, reagents, and resulting organisms, can apply equally to SRS15A and SRS15C, which, together with SRS15B, form the tandem *T. gondii* SRS15 array alluded to here. Also, SRS26B appears to be required for successful sexual reproduction of these organisms.

[0017] Thus, the inventive recombinant parasite is engineered to be unable to reproduce sexually, i.e., to produce oocysts by the definitive host (felines). However, the inventive recombinant parasite is nonetheless immunogenic within host animals. Thus, the inventive recombinant parasite can serve as a vaccine for cats. For example, when the inventive recombinant parasite is *T. gondii*, such can be orally or mucosally administered to a felid (e.g., a domestic or wild cat), which, as noted above, is the host animal necessary for sexual reproduction of *T. gondii*. As demonstrated in the Examples below, felids inoculated with recombinant *T. gondii* deficient for either IFT88 or SRS15B developed persistent immunity to the *T. gondii* but were not observed to shed oocysts.

[0018] In other members of the phyla *Apicomplexa* or *Euglenozoa*, the inventive approach can be similarly employed. For example, IFT88 in particular is highly conserved throughout *Apicomplexa* as well as other parasitic protozoa in other phyla, such as the *Euglenozoa (i.e. Leishmania spp)*. For example, IFT88 ortholog sequences have been reported for organisms such as *Hammondia hammondi* (HHA_207410), *Eimeria* spp. (ETH2_0205400), *Sarcocystis neurona* (SN3_00600560 and SN3_00600565), and *Neospora caninum* (XP_003879797.1), among others. Thus, a similar approach for knocking out the gene homologous or orthologous to

T. gondii IFT88 in other parasite members of the phyla *Apicomplexa* and *Euglenozoa* (such as those noted above) can, in certain embodiments, similarly impede the reproduction of recombinant parasites. Similarly, where the genomes of species from these phyla also have native homologs or orthologs of one or more genes from the *T. gondii* SRS15 array (e.g., SRS15A, SRS15B, and/or SRS15C) and/or *T. gondii* SRS26B, these likewise can be targeted in such other species. Thus, inventive recombinant parasites drawn from genera, such as *Cryptosporidium*, *Eimeria*, *Hammondia*, *Neospora*, and *Sarcocystis*, in addition to *Toxoplasma*, similarly can serve as vaccines for use in their host animals required for sexual reproduction.

[0019] For producing the inventive recombinant parasite comprising a genome lacking a native homolog of *T. gondii* IFT88, SRS15 array (e.g., SRS15A, SRS15B, and/or SRS15C), SRS26B, or a combination of two or more thereof, techniques such as CRISPR, the use of Transcription Activator-like Effector Nucleases (TALENs) or Zinc Finger proteins can be employed. CRISPR-Cas systems employ a tracrRNA which plays a role in the maturation of crRNA. The tracrRNA is partially complementary to and base pairs with a pre-crRNA forming an RNA duplex. This is cleaved by RNase III to form a crRNA/tracrRNA hybrid, which acts as a guide for the endonuclease Cas9, which cleaves the invading nucleic acid. Typically, for CRISPR applications, the Cas9 nickase enzyme is co-transfected with a guide RNA ("gRNA") to effectuate gene editing. However, enzymes other than Cas9 (such as, for example Cas12a) can be suitably employed in some embodiments.

[0020] CRISPR technology is well known to persons of ordinary skill in the art, and any suitable protocol can be employed in the context of the present invention. As information concerning the genetic sequences for *T. gondii* IFT88, SRS15 array, and SRS26B homologs is known (see, e.g., SEQ ID Nos: 6-9 and 18-20 herein), suitable gRNAs for use in targeting CRISPR-Cas9 (or other suitable CRISPR system) to knock out all or a portion of the native *T. gondii* genes encoding IFT88, a gene within the SRS15 array, SRS26B, or a combination of two or more thereof (including their homologs and orthologs) can readily be designed by persons of ordinary skill in the art. Non-limiting examples of sequences for constructing CRISPR-Cas9 gRNAs for knocking out genes encoding IFT88, a gene within the SRS15 array, and SRS26B homologs in *T. gondii* are provided herein as SEQ ID Nos:10-12 and 21-14.

[0021] As noted, other methods for gene editing can be employed as alternatives to CRISPR to generate embodiments of the inventive recombinant parasite comprising a genome lacking a native homolog of a gene encoding either *T. gondii* IFT88, SRS15 array (e.g., SRS15A, SRS15B, and/or SRS15C), SRS26B, or a combination of two or more thereof (applicable as well for embodiments in which the inventive cell lacks functional expression of IFT88, SRS15 array (e.g., SRS15A, SRS15B, and/or SRS15C), and/or SRS26B homologs). Such methods include those employing transcription activator like effector nucleases (TALENs) and the use of Zinc finger proteins, for example. For each application, standard methodology known to those of ordinary skill can be employed to generate recombinant parasite of the present invention. TALENs are customized artificial restriction nucleases that can be readily constructed to target a known genetic sequence, using methods known to persons of ordinary skill in the art. Similarly, Zinc finger domains can be engineered using methods known to persons of ordinary skill in the art to target specific desired DNA sequences, which thus enables Zinc finger nucleases to target unique sequences within complex genomes to alter the chromosomal DNA of cells. Thus, knowledge of the sequences for genes encoding *T. gondii* IFT88, the *T. gondii* SRS15 array (e.g., SRS15A, SRS15B, and/or SRS15C), and *T. gondii* SRS26B, and their native homologs in other species, as set forth herein and otherwise known in the art, can facilitate the design and construction of TALENs and Zinc finger nucleases, such as targeting the same genome loci that gRNAs bind (see SEQ ID Nos:6-12, 18-19, and 23-24), suitable for generating the inventive recombinant parasite in which native genes encoding *T. gondii* IFT88, one or more of the SRS15 array (e.g., SRS15A, SRS15B, and/or SRS15C), SRS26B, or a combination of two or more thereof, or their homologs in other species, are "knocked out."

[0022] Also, while approaches for removing functional copies of native genes encoding *T. gondii* IFT88, SRS15A, B, or C, SRS26B, or a combination of two or more thereof, or their native homologs in other species, other methods for attenuating the expression of these genes can alternatively be employed in some embodiments. As an example, the genetic regulatory elements controlling expression of native genes encoding *T. gondii* IFT88, SRS15 array (e.g., SRS15A, SRS15B, and/or SRS15C), SRS26B, or a combination of two or more thereof, or their homologs

in other species, can be altered or removed from the parasite genomes to attenuate transcription, if one or both of their coding sequences is not "knocked out."

[0023] Alternatively, in performing the inventive method, the genetic manipulation can involve using RNA interference to block or reduce translation of native genes encoding *T. gondii* IFT88, the SRS15 array (e.g., SRS15A, SRS15B, and/or SRS15C), SRS26B, homologs or orthologs, or a combination of two or more thereof in certain species, particularly those other than from the genus *Toxoplasma*, as RNAi is generally technically problematic in *Toxoplasma*. Persons of ordinary skill in the art will be able to design suitable interfering RNA sequences for attenuating ("knocking down") the expression of genes encoding homologs or orthologs of IFT88, SRS15B and/or SRS26B in such species.

[0024] It will be observed that the capacity of the inventive recombinant parasite to both effectively immunize the definitive host animal required for its sexual reproduction and highly attenuate or effectively eliminate the production of oocysts provides the use of the inventive recombinant parasite as a vaccine for cats. Accordingly, an aspect of the invention provides a vaccine composition comprising the inventive recombinant parasite and a carrier. In some embodiments, the recombinant parasite can exist in the form of an active cell, while in other embodiments, the parasite can exist in the form of a bradyzoite cyst.

[0025] In the context of the present invention, the "carrier" within the inventive vaccine composition is any suitable vehicle to deliver the inventive recombinant parasite to the host animal needed for sexual reproduction of the respective species of inventive recombinant parasite. Thus, for example, where the parasite of interest is *T. gondii*, the host animal will be a cat species, and the route of administration can suitably be oral or mucosal. For such an embodiment, the inventive vaccine composition can be orally or mucosally admissible, such as being a capsule, tablet, or food substance or food additive, which the cat can ingest. In other embodiments, it may be desirable to formulate the inventive vaccine composition for delivery through other means, such as via injection, transdermally, transmucosally, intranasally, etc.

[0026] For such applications, the formulation can be manufactured in accordance with standard methodology in pharmaceutics, and the carrier(s) can be selected from those ordinarily used to formulate pharmaceutical compositions (e.g., saline for injection, petrolatum).

Moreover, such compositions can include other excipients and agents as desired, such as stabilizing agents, preservatives, humectants, etc. Indeed, in certain embodiments, the formulation can include nanoparticles (such as virus-like particles).

[0027] In another embodiment, the invention comprises a composition comprising a homolog of *T. gondii* IFT88, SRS15A, B and/or C, SRS26B protein (from *T. gondii* or another species), or a combination of two or more thereof, for use as a vaccine. To make such a composition, the homolog of *T. gondii* IFT88, SRS15A, B and/or C, SRS26B, or a combination of two or more thereof can be recombinantly synthesized as a secreted protein and then formulated with a suitable pharmaceutically acceptable carrier, as described herein. Specific to the *T. gondii* IFT88, SRS15A, B and/or C, and SRS26B species-specific homologs, cDNA and/or their encoded transcripts are set forth as SEQ ID NOs: 1-4 and 17 below. Production of such proteins can be achieved by employing standard methodology. In preferred embodiments, the homolog of *T. gondii* IFT88, SRS15A, B and/or C, SRS26B, or a combination of two or more thereof can be immobilized or conjugated to supports within the composition, such as nanoparticles or virus-like particles (VLPSs) pseudotyped with the homolog of IFT88, SRS15A, B and/or C, SRS26B, or a combination of two or more thereof.

[0028] Using such formulations, in another aspect, the invention provides a method of vaccinating an animal. In accordance with the method, the animal to be vaccinated is one which is a competent host for sexual reproduction of the species of recombinant parasite from which the homolog of *T. gondii* IFT88, SRS15A, B and/or C, SRS26B, or a combination of two or more thereof is drawn. Thus, for example, where the inventive recombinant parasite is *T. gondii*, the animal to be vaccinated suitably can be a felid.

[0029] Furthermore, the inventive method of vaccinating the animal involves administering the inventive composition to the animal in an amount and via a route suitable for infecting the animal. Again, with reference to *T. gondii*, the preferred route to administer the inventive composition to a cat is orally or mucosally. For other types of parasites and host animals, the composition may be administered via other routes (e.g., via injection, transdermally, transmucosally, intranasally, etc., as noted above).

[0030] The amount of the inventive composition and inventive recombinant parasite delivered to the animal in accordance with the method of vaccinating the animal can vary, and it will depend on the animal and route of administration. For oral administration to cats, as was observed in the Examples below, no specific measurement of the quantity of recombinant parasites was made, where live parasites constituted the vaccine composition. In general, as *T. gondii* is highly transmissible, one or a very few (e.g., 40 or less individual) bradyzoite cysts may be sufficient to infect a cat. In this respect, feeding cats the macerated brains of mice infected with the recombinant parasite, which had harbored the parasites for 42 days, provided a sufficient load of recombinant parasite to both immunize the cats and to prevent the formation of oocysts. In the performance of embodiments involving a more precise pharmaceutical preparation, the dose (number or weight of parasites/ml or bradyzoite cysts/ml or per weight of the animal to be inoculated, nanoparticles or VLPs/ml or per weight of the animal to be inoculated, etc.) can be more closely titrated. In experimental work, the lowest doses tested were 40 and 80 cysts (bradyzoites) per cat, but a dosage range of from about 40 to about 400 individuals per cat may be typically employed. However, as mentioned, as few as a single cyst (bradyzoite) can lead to infection in cats and can serve as an effective dose. Similarly, in embodiments in which the composition comprises the homolog of *T. gondii* IFT88, SRS15A, B and/or C, SRS26B, or a combination of two or more thereof as a protein, such as conjugated to a nanoparticle or VLP, an attending veterinarian can optimize the dosage.

SEQUENCES

[0031] The following biological sequences are presented:

SEQ ID NO:1 - SRS15B cDNA sequence:

ATGGCGCGGTCAGGAGGGATGCTGCAACGTGGTCGCGGTTTCAAGTCACGAGCCCGAAAGCTGATGGCAT
TGTGCATGGGTGGAGTTTTGCTGTTTTCCGGTGGACAAGCTGCTGCAGAGCCTTGGCCTGAAGGTATGAA
ACATCGGAATTTGGAAGCAACGTTTACGGCTGCTGAGCCAGAATACACGGATGCGGTCGCCACCTGTGAT
CTAACAAGCGGAGCTGCGGCAGCTGCCGCGCCAGCTGTTGAATCCTTGACGCTTTCTCAAAAAAGTCTCA
CCGCTACTTTGGTATGTACTGGAGGAGCCGACGCCGAAATAAATAGTGTTCCGCCTACCTTGGAGAACGT
ATGTGACCCAACGAAAACAAGTGATAACACCAAATGTACTTTTGCCGGGAGCAGTGCTGAAGGCGGAGAG
GTGAAGCTGAATGAATTGCTTGGAACGAAACGCAGTGTTACGTGGACGAAGACGAACACGAATACGGAAA
GGACAAAGAAAGAAACATGGACACTGCAGCTGGAGGATGGTGATATTCCTTTGACCGACAAGACCTTCCT

TGTCGGGTGTCAAAACACAAAAAATGCTTCATCAGGTACTAAGAAGGCATGCAAGGTCACGGTGAACGTG
CAAGCGAGAGCTTCCTCTGTTGCAGATAATAACGTTGTCACATGCGCGTACGGCCAAAACAGCAACGACG
CGCCTTTGAGACTTGAGATGACAACAGAGAAGAACAGGCTCACTCTGATATGTGGTTCCGCGGGTTCTCT
CAAACCTACAACCTACAAAACCCAGTACTGCGATCCTCAGGAAGATGTGAAGAAGTGCACAGAGAAGAAG
TTCGAAGATATTCTTCCGACCATCGCGGAGAGCTGGTGGACGGCGGATACGACGACGAATTCCGCGACGC
TGACAATCCCGGCGACGGAATTTCCAGAAGCGGAACAGCAGTTTCGTTTGCAGTGCGTCCGCAATAAGCC
AAATTCGCCGGCATCAGGTGCACACGGAAAAACGGAAAAAACTCCTGGCGCCGAAGGGTCTGATACGTCG
GTTTGCAATGTGATTGTGACGGTCAAGTCAGGAAGCTCTGCATCGTCAGCCGGTCAAATGGTAGCTACGG
TCTCTGGTGTGGCTGCTTTGGCAGGATTCCTCGTTGGGTCCTTGTAA

SEQ ID NO:2 - SRS15B protein sequence:

MARSGGMLQRGRGFKSRARKLMALCMGGVLLFSGGQAAAEPWPEGMKHRNLEATFTAAEPEYTDAVATCD
LTSGAAAAAAPAVESLTLSQKSLTATLVCTGGADAEINSVPPTLENVCDPTKTSDNTKCTFAGSSAEGGE
VKLNELLGTKRSVTWTKTNTNTERTKKETWTLQLEDGDIPLTDKTFLVGCQNTKNASSGTKKACKVTVNV
QARASSVADNNVVTCAYGQNSNDAPLRLEMTTEKNRLTLICGSAGSLKPTTYKTQYCDPQEDVKKCTEKK
FEDILPTIAESWWTADTTTNSATLTIPATEFPEAEQQFRLQCVRNKPNSPASGAHGKTEKTPGAEGSDTS
VCNVIVTVKSGSSASSAGQMVATVSGVAALAGFLVGSL

SEQ ID NO:3 - IFT88 cDNA sequence:

ATGCACGAGGGAATTGTTCTTTTCTGTCGACTGCGTGCTGTCCGTAGCGTCCGGGTTAACATTGGCAACA
TTTACCTCGCCCAAGGCAGGCACGGCGAGGCTCTGAAGATGTACCACATCGCCTTGGATTCGACTCCGGC
AAACAGCAATTTTCTCCGTTGCAAGTTACTGAAGACAATTGGGGACGCATACTTCCTCGCAGGAAAGTTT
ACGGAGGCAACAGCAACATACGAACAGCTTCTCAAAGTCCGAGCGCAACTGGGCTGGAGCGTCTCAGCCT
TCTTGCCACGTGGCCTTCGAGGTCCCAGCCGCTGTGTCTCTGTCGAGAGCGAGAGTCTAAACCTGCTTCT
GTGTTACTATGCCGCCGGGAACAAAGCGAAGATGCAAGAGCACTTCTTGCACATGCTTGTTATGCAGGAC
GACATGTCGGGCACAGATGAACTGGTTGAGGATGATGAGGATGACGACGAGGATCAGCTCTCGCCTTCGT
CGATGTCTCCGCTTATCGTCGCAGCGCAGAGCGCTTCTTTCTGCGGTGCTTCCTCGTGTTGCCCGTTCCC
TGACAACTCACCGAATCATGGTGCTGCTTGTTCTCCTCCATCGTCACAAAACTCATCATCCAGTGGAGCG
GACTTATCCCCTGCATCCGGGGAGCCAGACGGTTTCACTCAATTGATCTGCCAGCGACGTAAACAGACCT
ACCGGAGACTGGTTTTGGCAGGTGGCCTCATTGCGCAAGCCCTGGCCGGGAATGTCACAGGATCTCTTGC
TGCGAATTTGCAGTCCGCATTCGACTGGGTAATCGAATGTTTCAGAAGCCATGGGTACAGAGAGCTGGCA
TACGAGATCGAGATGCACAAGGCCAACGCACTTTTGCGCCTGAAATGCGGGGTGGATACGGCGGTGAAGA
TCTACAGAAGCTTTGAACACGTTGAACGGCACCATGCAGTTCTGACGCCGCGAGCCTCGACGAATCTATC
CTTCATTTACATTCTCGAAAACGACCTCCCGCAGGCCACGAAATACGCTGATATCGCTCTCTCTGCGGAC
CGGTACAACGCACATGCTCTCGTTAACAAGGGGTGCTGCCTACTCCTCGCCGGCAAGCGACAGGAAGCGC
GACACCACTTCCTGGAGGCACTTGGGCTCGATGCTGAGTGTGTTGAGGCCCTCTACAATTTTGGCCTTGC
CTGCAAAATCGATGACCAACTTGACGAGGCTCTAGAGGCATTCTCAAGGTTCAACCAAATCCTTCCGAGT
CAGCCCGAGGTTCTGTACCACCTCGGGGATATCAGCGAGTCTATGGGTCAGTACGAGAAAACGATGGAGT
GGTTTTCGCTGCTCACATCTCCTGGAGTTCGGCCGACGGATGCCCGCATCCTGGGACGCATGGGTGCAGC
GGCGGCCGCCCATTCTCAAGACGACGAGGATAAACAAGCGCTCCACTATTTCCTCAGCTCATACAGCTAC
TACCCGTACTCGGTGGATGTCATCACGTGGCTCGGCGTGTACTTCGTGAAGCAGCAGCTGTTTGACAAGG
CCGCAGAGTTTTTCCACCGCGCGGCAGCTCTGCAACCTGCAGAACCTAAGTGGCTGCTCATGGTCGCGTC
CTGCTACCGCCGAAGCGAGAATTTCCCTCTCGCTCTCGCCCTATACAAAAAGGTGCACCGCGAGCACCCG
GCTGACGTCGAGTGCCTCCGGTGTCTAGTCACCGTCTGCAAAGAAATGCGGATCCCGGACGACCAGTATG
CGGCGCTCCTGCGAAAAGCAGAAAATGCGCAGCAGATAATTTACGGGCTTCAGCCCCGCGGTGCGTCTTC

TGCACGTCTGGAGGAACGACAACCAATAGTCCAGAGTGGGAGGACGACGCAGATCCGCTCCCTGGGAGCG
CGACAGAAAACGCTGGCGCTAACTGACGGCGCGGCACCAGAAGAGGATATGGCGATCATAGAGAACTTGG
TTTTCTGA

SEQ ID NO:4 - IFT88 protein sequence:

MHEGIVLFCRLRAVRSVRVNIGNIYLAQGRHGEALKMYHIALDSTPANSNFLRCKLLKTIGDAYFLAGKF
TEATATYEQLLKVRAQLGWSVSAFLPRGLRGPSRCVSVESESLNLLLCYYAAGNKAKMQEHFLHMLVMQD
DMSGTDELVEDDEDDDEDQLSPSSMSPLIVAAQSASFCGASSCCPFPDNSPNHGAACSPPSSQNSSSSGA
DLSPASGEPDGFTQLICQRRKQTYRRLVLAGGLIAQALAGNVTGSLAANLQSAFDWVIECFRSHGYRELA
YEIEMHKANALLRLKCGVDTAVKIYRSFEHVERHHAVLTPRASTNLSFIYILENDLPQATKYADIALSAD
RYNAHALVNKGCCLLLAGKRQEARHHFLEALGLDAECVEALYNFGLACKIDDQLDEALEAFSRFNQILPS
QPEVLYHLGDISESMGQYEKTMEWFSLLTSPGVRPTDARILGRMGAAAAAHSQDDEDKQALHYFLSSYSY
YPYSVDVITWLGVYFVKQQLFDKAAEFFHRAAALQPAEPKWLLMVASCYRRSENFPLALALYKKVHREHP
ADVECLRCLVTVCKEMRIPDDQYAALLRKAENAQQIIYGLQPRGASSARLEERQPIVQSGRTTQIRSLGA
RQKTLALTDGAAPEEDMAIIENLVF

SEQ ID NO:5 - HPT drug selectable marker sequence:

tttgtacaaaaaagcaggcttcATAACTTCGTATAATGTATGCTATACGAAGTTATcaGcacgaaaccttt
gcattcaaacccgcccgcggaagatccgatcttgctgctgttcgcagtcccagtagcgtcctgtcggccg
cgccgtctctgttggtgggcagccgctacacctgttatctgactgccgtgcgcgaaaatgacgccatttt
tgggaaaatcggggaacttcattctttaaaagtatgcggaggtttccttttttcttctgttcgtttctttt
tctcgggtttgataaccgtgttcgatgtaagcactttccgtctctcctccgtgctttgttcgacatcgag
accaggtgtgcagatccttcgcttgtcgatccggagacgcgtgtctcgtagaacctttttcattttaccac
acggcagtgcggagcactgctctgagtgcagcagggacgggtgaagtttcgctttagtagtgcgtttctg
ctctacggggcgttgtcagatccagcaaaATGGCGTCCAAACCCATTGAAGACTACGGCAAGGGCAAGGG
CCGTATTGAGCCCATGTATATCCCCGACAACACCTTCTACAACGCTGATGACTTTCTTGTGCCCCCCCAC
TGCAAGCCCTACATTGACAAAATCCTCCTCCCTGGTGGATTGGTCAAGGACAGAGTTGAGAAGTTGGCGT
ATGACATCCACAGAACTTACTTCGGCGAGGAGTTGCACATCATTTGCATCCTGAAAGGCTCTCGCGGCTT
CTTCAACCTTCTGATCGACTACCTTGCCACCATACAGAAGTACAGTGGTCGTGAGTCCAGCGTGCCCCCC
TTCTTCGAGCACTATGTCCGCCTGAAGTCCTACCAGAACGACAACAGCACAGGCCAGCTCACCGTCTTGA
GCGACGACTTGTCAATCTTTCGCGACAAGCACGTTCTGATTGTTGAGGACATCGTCGACACCGGTTTCAC
CCTCACCGAGTTCGGTGAGCGCCTGAAAGCCGTCGGTCCCAAGTCGATGAGAATCGCCACCCTCGTCGAG
AAGCGCACAGATCGCTCCAACAGCTTGAAGGGCGACTTCGTCGGCTTCAGCATTGAAGACGTCTGGATCG
TTGGTTGCTGCTACGACTTCAACGAGATGTTCCGCGACTTCGACCACGTCGCCGTCCTGAGCGACGCCGC
TCGCAAAAAGTTCGAGAAGTAAaccctgcatagcccacagaagctgcccgtctctcgttttcctctcttt
tcggagggatcagggagagtgcctcgggtcggagagagctgacgagggggtgccagagacccctgtgtcc
tttatcgaagaaaagggatgactcttcatgtggcatttcacacagtctcacctcgccttgttttcttttt
gtcaatcagaacgaaagcgagttgcgggtgacgcagatgtgcgtgtatccactcgtgaatgcgttatcgt
tctgtatgccgctagagtgctggactgttgctgtctgcccacgacagcagacaactttccttctatgcac
ttATAACTTCGTATAATGTATGCTATACGAAGTTATgacccagctttcttgtacaaa

SEQ ID NO:6 - HPT drug selectable marker sequence flanked by [30 base pairs homology to SRS15B sequence]:

[CAAGCTGCTGCAGAGCCTTGGCCTGAAGGT]tttgtacaaaaaagcaggcttcATAACTTCGTATAATG
TATGCTATACGAAGTTATcaGcacgaaaccttgcattcaaacccgcccgcggaagatccgatcttgctgc
tgttcgcagtcccagtagcgtcctgtcggccgcgccgtctctgttggtgggcagccgctacacctgttat
ctgactgccgtgcgcgaaaatgacgccatttttgggaaaatcggggaacttcattctttaaaagtatgcg
gaggtttccttttttcttctgttcgtttctttttctcgggtttgataaccgtgttcgatgtaagcactttc
cgtctctcctccgtgctttgttcgacatcgagaccaggtgtgcagatccttcgcttgtcgatccggagac
gcgtgtctcgtagaacctttttcatttttaccacacggcagtgcggagcactgctctgagtgcagcagggac
gggtgaagtttcgctttagtagtgcgtttctgctctacggggcgttgtcagatccagcaaaATGGCGTCC
AAACCCATTGAAGACTACGGCAAGGGCAAGGGCCGTATTGAGCCCATGTATATCCCCGACAACACCTTCT
ACAACGCTGATGACTTTCTTGTGCCCCCCCACTGCAAGCCCTACATTGACAAAATCCTCCTCCCTGGTGG
ATTGGTCAAGGACAGAGTTGAGAAGTTGGCGTATGACATCCACAGAACTTACTTCGGCGAGGAGTTGCAC
ATCATTTGCATCCTGAAAGGCTCTCGCGGCTTCTTCAACCTTCTGATCGACTACCTTGCCACCATACAGA
AGTACAGTGGTCGTGAGTCCAGCGTGCCCCCCTTCTTCGAGCACTATGTCCGCCTGAAGTCCTACCAGAA
CGACAACAGCACAGGCCAGCTCACCGTCTTGAGCGACGACTTGTCAATCTTTCGCGACAAGCACGTTCTG
ATTGTTGAGGACATCGTCGACACCGGTTTCACCCTCACCGAGTTCGGTGAGCGCCTGAAAGCCGTCGGTC
CCAAGTCGATGAGAATCGCCACCCTCGTCGAGAAGCGCACAGATCGCTCCAACAGCTTGAAGGGCGACTT
CGTCGGCTTCAGCATTGAAGACGTCTGGATCGTTGGTTGCTGCTACGACTTCAACGAGATGTTCCGCGAC
TTCGACCACGTCGCCGTCCTGAGCGACGCCGCTCGCAAAAAGTTCGAGAAGTAAaccctgcatagcccac
agaagctgcccgtctctcgttttcctctctttcggagggatcagggagagtgcctcgggtcggagagag
ctgacgaggggtgccagagacccctgtgtcctttatcgaagaaaagggatgactcttcatgtggcattt
cacacagtctcacctcgccttgttttctttttgtcaatcagaacgaaagcgagttgcgggtgacgcagat
gtgcgtgtatccactcgtgaatgcgttatcgttctgtatgccgctagagtgctggactgttgctgtctgc
ccacgacagcagacaactttccttctatgcacttATAACTTCGTATAATGTATGCTATACGAAGTTATga
cccagctttcttgtacaaa[CGGAATTTGGAAGCAACGTTTACGGCTGCT]

SEQ ID NO:7 - HPT drug selectable marker sequence flanked by [40 base pairs homology to IFT88 sequence]:

[AGCGTCCGGGTTAACATTGGCAACATTTACCTCGCCCAAG]tttgtacaaaaaagcaggcttcATAACT
TCGTATAATGTATGCTATACGAAGTTATcaGcacgaaaccttgcattcaaacccgcccgcggaagatccg
atcttgctgctgttcgcagtcccagtagcgtcctgtcggccgcgccgtctctgttggtgggcagccgcta
cacctgttatctgactgccgtgcgcgaaaatgacgccatttttgggaaaatcggggaacttcattcttta
aaagtatgcggaggtttccttttttcttctgttcgtttctttttctcgggtttgataaccgtgttcgatgt
aagcactttccgtctctcctccgtgctttgttcgacatcgagaccaggtgtgcagatccttcgcttgtcg
atccggagacgcgtgtctcgtagaacctttttcatttttaccacacggcagtgcggagcactgctctgagtg
cagcagggacgggtgaagtttcgctttagtagtgcgtttctgctctacggggcgttgtcagatccagcaa
aATGGCGTCCAAACCCATTGAAGACTACGGCAAGGGCAAGGGCCGTATTGAGCCCATGTATATCCCCGAC
AACACCTTCTACAACGCTGATGACTTTCTTGTGCCCCCCCACTGCAAGCCCTACATTGACAAAATCCTCC
TCCCTGGTGGATTGGTCAAGGACAGAGTTGAGAAGTTGGCGTATGACATCCACAGAACTTACTTCGGCGA
GGAGTTGCACATCATTTGCATCCTGAAAGGCTCTCGCGGCTTCTTCAACCTTCTGATCGACTACCTTGCC
ACCATACAGAAGTACAGTGGTCGTGAGTCCAGCGTGCCCCCCTTCTTCGAGCACTATGTCCGCCTGAAGT
CCTACCAGAACGACAACAGCACAGGCCAGCTCACCGTCTTGAGCGACGACTTGTCAATCTTTCGCGACAA
GCACGTTCTGATTGTTGAGGACATCGTCGACACCGGTTTCACCCTCACCGAGTTCGGTGAGCGCCTGAAA

GCCGTCGGTCCCAAGTCGATGAGAATCGCCACCCTCGTCGAGAAGCGCACAGATCGCTCCAACAGCTTGA
AGGGCGACTTCGTCGGCTTCAGCATTGAAGACGTCTGGATCGTTGGTTGCTGCTACGACTTCAACGAGAT
GTTCCGCGACTTCGACCACGTCGCCGTCCTGAGCGACGCCGCTCGCAAAAAGTTCGAGAAGTAAaccctg
catagcccacagaagctgcccgtctctcgttttcctctctttcggagggatcagggagagtgcctcggg
tcggagagagctgacgaggggtgccagagacccctgtgtcctttatcgaagaaaagggatgactcttca
tgtggcatttcacacagtctcacctcgccttgttttctttttgtcaatcagaacgaaagcgagttgcggg
tgacgcagatgtgcgtgtatccactcgtgaatgcgttatcgttctgtatgccgctagagtgctggactgt
tgctgtctgcccacgacagcagacaactttccttctatgcacttATAACTTCGTATAATGTATGCTATAC
GAAGTTATgacccagctttcttgtacaaa[AGAAGAGGATATGGCGATCATAGAGAACTTGGTTTTCTGA
]

SEQ ID NO:8 - SRS15B genomic locus after insertion of HPT sequence to generate the knockout:

ATGGCGCGGTCAGGAGGGATGCTGCAACGTGGTCGCGGTTTCAAGTCACGAGCCCGAAAGCTGATGGCAT
TGTGCATGGGTGGAGTTTTGCTGTTTTCCGGTGGACAAGCTGCTGCAGAGCCTTGGCCTGAAGGTtttgt
acaaaaaagcaggcttcATAACTTCGTATAATGTATGCTATACGAAGTTATcaGcacgaaaccttgcatt
caaacccgcccgcggaagatccgatcttgctgctgttcgcagtcccagtagcgtcctgtcggccgcgccg
tctctgttggtgggcagccgctacacctgttatctgactgccgtgcgcgaaaatgacgccatttttggga
aaatcggggaacttcattctttaaaagtatgcggaggtttccttttttcttctgttcgtttcttttttctcg
ggtttgataaccgtgttcgatgtaagcactttccgtctctcctccgtgctttgttcgacatcgagaccag
gtgtgcagatccttcgcttgtcgatccggagacgcgtgtctcgtagaacctttttcattttaccacacggc
agtgcggagcactgctctgagtgcagcagggacgggtgaagtttcgctttagtagtgcgtttctgctcta
cggggcgttgtcagatccagcaaaATGGCGTCCAAACCCATTGAAGACTACGGCAAGGGCAAGGGCCGTA
TTGAGCCCATGTATATCCCCGACAACACCTTCTACAACGCTGATGACTTTCTTGTGCCCCCCCACTGCAA
GCCCTACATTGACAAAATCCTCCTCCCTGGTGGATTGGTCAAGGACAGAGTTGAGAAGTTGGCGTATGAC
ATCCACAGAACTTACTTCGGCGAGGAGTTGCACATCATTTGCATCCTGAAAGGCTCTCGCGGCTTCTTCA
ACCTTCTGATCGACTACCTTGCCACCATACAGAAGTACAGTGGTCGTGAGTCCAGCGTGCCCCCCTTCTT
CGAGCACTATGTCCGCCTGAAGTCCTACCAGAACGACAACAGCACAGGCCAGCTCACCGTCTTGAGCGAC
GACTTGTCAATCTTTCGCGACAAGCACGTTCTGATTGTTGAGGACATCGTCGACACCGGTTTCACCCTCA
CCGAGTTCGGTGAGCGCCTGAAAGCCGTCGGTCCCAAGTCGATGAGAATCGCCACCCTCGTCGAGAAGCG
CACAGATCGCTCCAACAGCTTGAAGGGCGACTTCGTCGGCTTCAGCATTGAAGACGTCTGGATCGTTGGT
TGCTGCTACGACTTCAACGAGATGTTCCGCGACTTCGACCACGTCGCCGTCCTGAGCGACGCCGCTCGCA
AAAAGTTCGAGAAGTAAaccctgcatagcccacagaagctgcccgtctctcgttttcctctctttcggga
gggatcagggagagtgcctcgggtcggagagagctgacgaggggtgccagagaccctgtgtcctttat
cgaagaaaagggatgactcttcatgtggcatttcacacagtctcacctcgccttgttttctttttgtcaa
tcagaacgaaagcgagttgcgggtgacgcagatgtgcgtgtatccactcgtgaatgcgttatcgttctgt
atgccgctagagtgctggactgttgctgtctgcccacgacagcagacaactttccttctatgcacttATA
ACTTCGTATAATGTATGCTATACGAAGTTATgacccagctttcttgtacaaaCGGAATTTGGAAGCAACG
TTTACGGCTGCTGAGCCAGAATACACGGATGCGGTCGCCACCTGTGATCTAACAAGCGGAGCTGCGGCAG
CTGCCGCGCCAGCTGTTGAATCCTTGACGCTTTCTCAAAAAAGTCTCACCGCTACTTTGGTATGTACTGG
AGGAGCCGACGCCGAAATAAATAGTGTTCCGCCTACCTTGGAGAACGTATGTGACCCAACGAAAACAAGT
GATAACACCAAATGTACTTTTGCCGGGAGCAGTGCTGAAGGCGGAGAGGTGAAGCTGAATGAATTGCTTG
GAACGAAACGCAGTGTTACGTGGACGAAGACGAACACGAATACGGAAAGGACAAAGAAAGAAACATGGAC
ACTGCAGCTGGAGGATGGTGATATTCCTTTGACCGACAAGACCTTCCTTGTCGGGTGTCAAAACACAAAA
AATGCTTCATCAGGTACTAAGAAGGCATGCAAGGTCACGGTGAACGTGCAAGCGAGAGCTTCCTCTGTTG
CAGATAATAACGTTGTCACATGCGCGTACGGCCAAAACAGCAACGACGCGCCTTTGAGACTTGAGATGAC
AACAGAGAAGAACAGGCTCACTCTGATATGTGGTTCCGCGGGTTCTCTCAAACCTACAACCTACAAAACC

CAGTACTGCGATCCTCAGGAAGATGTGAAGAAGTGCACAGAGAAGAAGTTCGAAGATATTCTTCCGACCA
TCGCGGAGAGCTGGTGGACGGCGGATACGACGACGAATTCCGCGACGCTGACAATCCCGGCGACGGAATT
TCCAGAAGCGGAACAGCAGTTTCGTTTGCAGTGCGTCCGCAATAAGCCAAATTCGCCGGCATCAGGTGCA
CACGGAAAAACGGAAAAAACTCCTGGCGCCGAAGGGTCTGATACGTCGGTTTGCAATGTGATTGTGACGG
TCAAGTCAGGAAGCTCTGCATCGTCAGCCGGTCAAATGGTAGCTACGGTCTCTGGTGTGGCTGCTTTGGC
AGGATTCCTCGTTGGGTCCTTGTAA

SEQ ID NO:9 - IFT88 genomic locus after insertion of HPT sequence to generate the knockout:

ATGCACGAGGGAATTGTTCTTTTCTGTCGACTGCGTGCTGTCCGTAGCGTCCGGGTTAACATTGGCAACA
TTTACCTCGCCCAAGtttgtacaaaaaagcaggcttcATAACTTCGTATAATGTATGCTATACGAAGTTA
TcaGcacgaaaccttgcattcaaacccgcccgcggaagatccgatcttgctgctgttcgcagtcccagta
gcgtcctgtcggccgcgccgtctctgttggtgggcagccgctacacctgttatctgactgccgtgcgcga
aaatgacgccatttttgggaaaatcggggaacttcattctttaaaagtatgcggaggtttccttttctt
ctgttcgtttctttttctcgggtttgataaccgtgttcgatgtaagcactttccgtctctcctccgtgct
ttgttcgacatcgagaccaggtgtgcagatccttcgcttgtcgatccggagacgcgtgtctcgtagaacc
ttttcattttaccacacggcagtgcggagcactgctctgagtgcagcagggacgggtgaagtttcgcttt
agtagtgcgtttctgctctacggggcgttgtcagatccagcaaaATGGCGTCCAAACCCATTGAAGACTA
CGGCAAGGGCAAGGGCCGTATTGAGCCCATGTATATCCCCGACAACACCTTCTACAACGCTGATGACTTT
CTTGTGCCCCCCCACTGCAAGCCCTACATTGACAAAATCCTCCTCCCTGGTGGATTGGTCAAGGACAGAG
TTGAGAAGTTGGCGTATGACATCCACAGAACTTACTTCGGCGAGGAGTTGCACATCATTTGCATCCTGAA
AGGCTCTCGCGGCTTCTTCAACCTTCTGATCGACTACCTTGCCACCATACAGAAGTACAGTGGTCGTGAG
TCCAGCGTGCCCCCCTTCTTCGAGCACTATGTCCGCCTGAAGTCCTACCAGAACGACAACAGCACAGGCC
AGCTCACCGTCTTGAGCGACGACTTGTCAATCTTTCGCGACAAGCACGTTCTGATTGTTGAGGACATCGT
CGACACCGGTTTCACCCTCACCGAGTTCGGTGAGCGCCTGAAAGCCGTCGGTCCCAAGTCGATGAGAATC
GCCACCCTCGTCGAGAAGCGCACAGATCGCTCCAACAGCTTGAAGGGCGACTTCGTCGGCTTCAGCATTG
AAGACGTCTGGATCGTTGGTTGCTGCTACGACTTCAACGAGATGTTCCGCGACTTCGACCACGTCGCCGT
CCTGAGCGACGCCGCTCGCAAAAAGTTCGAGAAGTAAaccctgcatagcccacagaagctgcccgtctct
cgttttcctctcttttcggagggatcagggagagtgcctcgggtcggagagagctgacgaggggtgcca
gagacccctgtgtcctttatcgaagaaaagggatgactcttcatgtggcatttcacacagtctcacctcg
ccttgttttctttttgtcaatcagaacgaaagcgagttgcgggtgacgcagatgtgcgtgtatccactcg
tgaatgcgttatcgttctgtatgccgctagagtgctggactgttgctgtctgcccacgacagcagacaac
tttccttctatgcacttATAACTTCGTATAATGTATGCTATACGAAGTTATgacccagctttcttgtaca
aaAGAAGAGGATATGGCGATCATAGAGAACTTGGTTTTCTGA

SEQ ID NO:10 - SRS15B guide sequence: GGCCTGAAGGTATGAAACAT (DNA) or GGCCUGAAGGUAUGAAACAU (RNA)

SEQ ID NO:11 - IFT88 guide sequence: AAGGCAGGCACGGCGAGGCT (DNA) or AAGGCAGGCACGGCGAGGCU (RNA)

SEQ ID NO:12 – SRS26B guide sequence: GCATTGCACCAGATATCGGT (DNA) or GCAUUGCACCAGAUAUCGGU (RNA)

SEQ ID NO:13 - SRS15B forward PCR primer: GTGGTCGCGGTTTCAAGTC

SEQ ID NO:14 - IFT88 forward PCR primer: ATGCACGAGGGAATTGTTCT

SEQ ID NO:15 – SRS26B forward PCR primer: GGGGTGCAACATGCATCTG

SEQ ID NO:16 - HPT reverse PCR primer: GTCTTCAATGGGTTTGGACG

SEQ ID NO:17 – SRS26B protein sequence:

MGCNMHLCLLCVFAVACFVTGSASALNSIAPDIGRHKSTAVALSPPGDNNTCTNEKQKIKITIAATQTDA
TFKCGGTVTTLHPADCTSSSSCPPEALSSEDRPVVPRICETENCDKPRILTDVFPGATRVDDSDTQVYQL
TIPKGNRPKKDVWYHCKSEGGDNICKVQISVAAALADPPDDHQCSDTKPTITVDVGVEEEEATFKCGDTL
TTLDPQDCSGDSCQEEVAHDNDEPVSMIYEDESCSTAKHLDQVFPGATRHDDATNHVYKLTIPKEGRTTK
AAWYQCKGSERNSNTLCKIKINVTAALPTPPTPEAKNKCTAAVEELNLSASPQLPLTFVCPHDLPLKPSE
TRVYDNRDGQCTNEVDLSSLVDATLSGTTQVDTLAPGDTTYTLTVRRLPPERALLCYRCSGRSAFSESFR
KARGPVAKE

SEQ ID NO:18 – HPT drug selectable marker sequence flanked by 30 base pairs homology to SRS26B sequence:

TCTGCCTCTGCATTAAACAGCATTGCACCAtttgtacaaaaaagcaggcttcATAACTTCGTATAATGTA
TGCTATACGAAGTTATcaGcacgaaaccttgcattcaaacccgcccgcggaagatccgatcttgctgctg
ttcgcagtcccagtagcgtcctgtcggccgcgccgtctctgttggtgggcagccgctacacctgttatct
gactgccgtgcgcgaaaatgacgccatttttgggaaaatcggggaacttcattctttaaaagtatgcgga
ggtttccttttttcttctgttcgtttctttttctcgggtttgataaccgtgttcgatgtaagcactttccg
tctctcctccgtgctttgttcgacatcgagaccaggtgtgcagatccttcgcttgtcgatccggagacgc
gtgtctcgtagaacctttcattttaccacacggcagtgcggagcactgctctgagtgcagcagggacgg
gtgaagtttcgctttagtagtgcgtttctgctctacggggcgttgtcagatccagcaaaATGGCGTCCAA
ACCCATTGAAGACTACGGCAAGGGCAAGGGCCGTATTGAGCCCATGTATATCCCCGACAACACCTTCTAC
AACGCTGATGACTTTCTTGTGCCCCCCCACTGCAAGCCCTACATTGACAAAATCCTCCTCCCTGGTGGAT
TGGTCAAGGACAGAGTTGAGAAGTTGGCGTATGACATCCACAGAACTTACTTCGGCGAGGAGTTGCACAT
CATTTGCATCCTGAAAGGCTCTCGCGGCTTCTTCAACCTTCTGATCGACTACCTTGCCACCATACAGAAG
TACAGTGGTCGTGAGTCCAGCGTGCCCCCCTTCTTCGAGCACTATGTCCGCCTGAAGTCCTACCAGAACG
ACAACAGCACAGGCCAGCTCACCGTCTTGAGCGACGACTTGTCAATCTTTCGCGACAAGCACGTTCTGAT
TGTTGAGGACATCGTCGACACCGGTTTCACCCTCACCGAGTTCGGTGAGCGCCTGAAAGCCGTCGGTCCC

AAGTCGATGAGAATCGCCACCCTCGTCGAGAAGCGCACAGATCGCTCCAACAGCTTGAAGGGCGACTTCG
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SEQ ID NO:19 – SRS26B genomic locus after insertion of HPT sequence to generate the knockout:

ATGGGGTGCAACATGCATCTGTGTTTGCTGTGTGTATTTGCTGTCGCCTGCTTTGTTACCGGCTCTGCCT
CTGCATTAAACAGCATTGCACCAtttgtacaaaaaagcaggcttcATAACTTCGTATAATGTATGCTATA
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AA

SEQ ID NO:20 – Genomic sequence of IFT88 plus the flanking sequences from genomic DNA of GT1 *T. gondii* strain:

GAAGGTGAACCGAAGTCAAGACGAGTTATGTCGTCTGCCTTATCGTTCAGCTTTGCTATATCTCAACGAA
ACGCGTGTATCTGTTTCATTACCTCTAACACCTTTCGTAGAGACTGGTCCCACTGTCTCTGtggtGCTCG
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Sequence Length: 5924 bp; Sequence + flanking: 8464 bp

SEQ ID NO2:21-22 - Primers to amplify the construct of SEQ ID NO:20:

SEQ ID NO:21 - GA-5'IFT88flkFW

5'GAAGGTGAACCGAAGTCAAGACGAG 3'

SEQ ID NO:22 - GA-3'IFT88flkRev

5' CACATCGACTTCTGGTCAAGGCTC 3'

SEQ ID NOs:23-24- sgRNA used to remove HPT gene from SEQ ID NO:20:

SEQ ID NO:23 - 5'sgRNA compIFT88

CAAGTTGATGCGTCAGTACAGGAACAGTTTTA
GTTCAACTACGCAGTCATGTCCTTGTCAAAAT

SEQ ID NO:24 - 3'sgRNA compIFT88

CAAGTTGCCATCTTGTACCTTCCTATGTTTTA
GTTCAACGGTAGAACATGGAAGGATACAAAAT

EXAMPLE 1

[0032] This experimental Example further illustrate the invention but, of course, should not be construed as in any way limiting its scope.

[0033] The experiments in this Example demonstrate the construction of two different mutant *Toxoplasma gondii* strains, respectively deficient (knocked out (KO)) in either SRS15B (TGME49_320240) or IFT88 (TGME49_207410) genes. Each of these is demonstrated to be reproductively deficient in cats, does not shed oocysts, and immunizes cats against rechallenge. Specifically, cats fed bradyzoites from tissue cysts collected from laboratory animals infected with each of these attenuated strains developed immunity against subsequent re-infection with homologous or heterologous strains of *T. gondii*. Immunized cats developed high levels of *Toxoplasma*-specific IgG in their blood and did not shed infectious oocysts. The vaccine is an attenuated strain (CZ1) of the cosmopolitan Type II *T. gondii* parasite that commonly infects people and livestock.

[0034] The experimental procedures and reagents employed in these experiments were as follows:

Selection and Preparation of *Toxoplasma gondii* Strain

[0035] A clonal isolate of the cosmopolitan Type II CZ1 strain of *Toxoplasma gondii* was generated to be susceptible to drug selection using Mycophenolic Acid and Xanthine by a deletion in the Hypoxanthine-Xanthine-Guanine Phosphoribosyl Transferase (HXGPRT) gene. This strain was selected because it commonly infects both humans and livestock.

[0036] The strain, once engineered to be susceptible to drug selection using Mycophenolic Acid and Xanthine, was next rendered deficient in Non-Homologous End-Joining (NHEJ) repair by deletion of the KU80 gene to facilitate highly specific and efficient CRISPR/Cas9 mediated gene editing by double crossover homologous recombination. The mutant isolate ("CZ1ΔhptΔku80") was then tested to confirm cat-competency; it produced in excess of $10e^6$ infectious oocysts upon challenge in seronegative cats.

Generation of SRS15B and IFT88 knockout *Toxoplasma gondii*

[0037] The cat-competent CZ1ΔhptΔku80 mutant isolate was used to generate SRS15B and IFT88 knockout strains. Firstly, the SRS15B and IFT88 genes were replaced by the drug selectable marker HPT (HXGPRT – hypoxanthine-xanthine-guanine phosphoribosyl transferase) cassette (SEQ ID NO:5) that was flanked by 30 or 40 base pairs of homology (as indicated with brackets "[]" in the sequences set forth above) to SRS15B (SEQ ID NO:6) or to IFT88 (SEQ ID NO:7) to promote targeted deletion of the respective genes by CRISPR/Cas9 gene editing using guide RNAs specific to the homology flanks for each gene (SRS15B guide sequence: GGCCTGAAGGTATGAAACAT (SEQ ID NO:10) and IFT88 guide sequence: AAGGCAGGCACGGCGAGGCT (SEQ ID NO:11). Parasites were transfected by electroporation using the 4D NUCLEOFECTOR system (LONZA BIOSCIENCE) using the FI111 program.

[0038] The parasites were then transferred to T25 flasks containing a confluent monolayer of HFF cells (*Human Foreskin Fibroblast cells*) for parasite growth and selection for SRS15B or IFT88 deletion using 10% FBS-DMEM with mycophenolic acid (MPA) and xanthine at 50μg/ml (HPT positive selection) (together referred to as "MPA/X selection").

[0039] After four to six passages under MPA/X selection, the drug-selected parasite population was tested by PCR to confirm HPT insertion into, respectively, the SRS15B (SEQ ID NO:8) or IFT88 (SEQ ID NO:9) genomic locus, and then cloned. Briefly, SRS15B-KO and IFT88-KO parasites were identified by PCR of the integrated HPT into the open reading frame ("ORF") using the following primers: SRS15B forward: GTGGTCGCGGTTTCAAGTC (SEQ ID NO:13); IFT88 forward: ATGCACGAGGGAATTGTTCT (SEQ ID NO:14), and HPT reverse: GTCTTCAATGGGTTTGGACG (SEQ ID NO:16). This strategy generated SRS15B and IFT88 KO parasites; no wildtype (WT) parasites were selected out of both knockout populations.

Immunization of test animals (*Felis catus*)

[0040] To produce infectious cysts to feed to experimental cat subjects, first mice were infected by intraperitoneal injection of 5,000 to 10,000 tachyzoites of either the SRS15B-KO or IFT88-KO parasites, which had been harvested from the infected-HFF culture flasks referred to above. Forty-two days post-infection, mouse brains containing cysts (bradyzoites) were harvested and used to feed the cats. After feeding the cats (infection), stool samples were collected up to 21 days post-infection and oocyst presence/absence was assessed by microscopy and/or PCR.

[0041] The results of these experiments are presented in Table 1 and Figures 1 and 2. In particular, Table 1 presents data representing cat infections with CZ1 and various engineered parasites deficient in IFT88 and SRS15B. Seroconversion after primary challenge was assessed by either a Modified Agglutination Test (MAT) or reactivity to recombinant SAG1 (a Toxoplasma-specific IgG ELISA).

Table 1

Toxoplasma Strain	Bradyzoite Cysts	Patency Period	# of Oocysts	Seropositivity (MAT or ELISA)
WT CZ1 (ΔhptΔku80)	100	8-12	> 1 x 10^7	++
WT CZ1 (ΔhptΔku80)	500	6-12	2 x 10^6	400
WT CZ1 (ΔhptΔku80)	80	7-9	Not assessed	+
WT CZ1 (ΔhptΔku80)	10	5-8	2 x 10^4	+
IFT88 (Δift88Δku80)	100	NEG	NEG	++
IFT88 (Δift88Δku80)	300	NEG	NEG	200
IFT88 (Δift88Δku80)	250	NEG	NEG	400
Complement IFT88 (ΔhptΔku80)	400	6-9	3 x 10^6	++
Complement IFT88 (ΔhptΔku80)	600	5-10	> 1 x 10^6	600
WT CZ1 (ΔhptΔku80)	600	7-9	2.4 x 10^4	+
SRS15B (Δsrs15bΔku80)	600	NEG	NEG	++

[0042] Figure 1 presents data concerning cumulative oocyst yield (left panel) and serology titer (middle panel) among experimental cats challenged with bradyzoites either from wildtype WT CZ1, IFT88-KO, or IFT88-KO further engineered to be complemented with a heterologous allele of IFT88 from a different strain (GT1 (some sequence info presented in SEQ ID NO:20)) to restore expression of IFT88 and, therefore, wildtype phenotype. The use of this heterologous allele to rescue the phenotype permits a determination that the endogenous locus was correctly targeted.

[0043] The micrograph (right panel of Figure 1) shows *Toxoplasma* oocysts obtained from floating fecal pellets from cats infected with WT CZ1, which is one method commonly used to quantify oocyst numbers defecated from cats during their patency period of secretion.

[0044] Figure 2 presents data concerning cumulative oocyst yield (left panel) and serology titer (middle panel) among cats first challenged with bradyzoites from WT CZ1 or SRS15B-KO. Cats infected with WT CZ1 shed oocysts, but SRS15B-KO-infected cats did not. Both cat cohorts seroconverted, indicating that they generated robust titers of anti-*Toxoplasma*-specific IgG. Immunized cats were then re-infected with WT CZ1 (representing a secondary, homologous challenge) at 6 months post first infection to assess protection from oocyst shedding. The SRS15B KO vaccinated cats were immune to secondary challenge with WT CZ1 bradyzoites and did not shed oocysts. As a control, the WT CZ1 infected cats were re-infected with WT CZ1

bradyzoites, and did not shed oocysts, as expected. The micrograph (right panel) shows *Toxoplasma* oocysts obtained from floating fecal pellets from cats infected with WT CZ1, which, as noted above, is one method used to quantify oocyst numbers defecated from cats during their patency period of secretion.

[0045] Taken together, the results of these experiments demonstrated that SRS15B-KO- and IFT88-KO-infected cats did not shed oocysts in their feces during all time points analyzed, whereas WT-infected cats shed between 150 to 200 oocysts per gram of feces. When WT-, SRS15B-KO- or IFT-88-KO-infected cats were challenged with WT cysts 6 months post-infection, all cats were naturally vaccinated and did not produce oocysts. These results demonstrate that the inventive approach effectively prevents oocyst formation in *Toxoplasma*, thus preventing the spread of the parasite, yet still effectively immunizes animals from challenge from infectious parasites for at least six months.

EXAMPLE 2

[0046] Transmission of *Toxoplasma gondii* in nature is highly dependent on the parasite's sexual cycle, which occurs exclusively in felids. Infected cats shed high numbers of infectious oocysts, which are capable of causing toxoplasmosis outbreaks in both animals and people. The molecular pathways promoting sexual stage development in *T. gondii* are largely unknown. Identifying essential genes that regulate oocyst formation is central to blocking the parasite's transmission. Previous RNA-Seq work identified stage-specific merozoite transcripts, that are conserved across the Apicomplexa, and are thought to facilitate sexual competency. These include surface antigen genes related to 6-CYS proteins in *Plasmodium*, genes required to form flagella, a family of transcription factors (ApiAP2s), and a large family of secreted proteins expressed exclusively in merozoites (Families A-D).

[0047] To identify genes that impact oocyst formation, a forward genetic signature-tag mutagenesis screen was undertaken, using CRISPR/Cas9 to generate a library of 192 *T. gondii* strains that each possesses a unique barcode and are each deficient in a single gene predicted to be highly expressed in the parasite's sexual stages. These knock-out strains were pooled, used to

infect mice, then used to challenge cats with brain cysts from the infected mice to perform an input/output screen that can identify sexual stage-specific genes critical for oocyst formation.

[0048] Using Mi-Seq analysis, three genes were thusly identified: IFT88, SRS15, and SRS26B, which failed to produce oocysts in cats. When assayed individually through cats, as noted in Example 1, IFT88- and SRS15-KO strains were significantly attenuated in oocyst production. Work is underway in generating IFT88- and SRS15-specific antibodies and in engineering a complemented strain to demonstrate that IFT88 is a pivotal antigen regulating sexual competence in *Toxoplasma* development.

[0049] All references, including publications, patent applications, and patents, cited herein are hereby incorporated by reference to the same extent as if each reference were individually and specifically indicated to be incorporated by reference and were set forth in its entirety herein.

[0050] The use of the terms "a" and "an" and "the" and "at least one" and similar referents in the context of describing the invention (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. The use of the term "at least one" followed by a list of one or more items (for example, "at least one of A and B") is to be construed to mean one item selected from the listed items (A or B) or any combination of two or more of the listed items (A and B), unless otherwise indicated herein or clearly contradicted by context. The terms "comprising," "having," "including," and "containing" are to be construed as open-ended terms (i.e., meaning "including, but not limited to,") unless otherwise noted. Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., "such as") provided herein, is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention unless otherwise claimed. No

language in the specification should be construed as indicating any non-claimed element as essential to the practice of the invention.

[0051] Preferred embodiments of this invention are described herein, including the best mode known to the inventors for carrying out the invention. Variations of those preferred embodiments may become apparent to those of ordinary skill in the art upon reading the foregoing description. The inventors expect skilled artisans to employ such variations as appropriate, and the inventors intend for the invention to be practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contradicted by context.

CLAIM(S):

1. A recombinant parasite, which is a member of the phyla *Apicomplexa or Euglenozoa*, which comprises a genome which lacks a native homolog of the *Toxoplasma gondii* IFT88 gene, the *Toxoplasma gondii* SRS15A, SRS15B, and/or SRS15C gene, the *Toxoplasma gondii* SRS26B gene, or a combination of two or more thereof.

2. The recombinant parasite of claim 1, which is a member of the genus *Cryptosporidium*, *Eimeria*, *Hammondia, Neospora*, *Sarcocystis*, or *Toxoplasma*.

3. The recombinant parasite of claim 1 or 2, which is a member of the species *Toxoplasma gondii*.

4. The recombinant parasite of claim 3, which is a member of a cosmopolitan Type II strain of *Toxoplasma gondii.*

5. The recombinant parasite of claim 3, which is a member of the Castells strain of *Toxoplasma gondii.*

6. A method for producing the recombinant parasite of any one of claims 1-5, comprising knocking out the homolog of the *Toxoplasma gondii* IFT88 gene, the *Toxoplasma gondii* SRS15A, SRS15B, and/or SRS15C gene, the *Toxoplasma gondii* SRS26B gene, or a combination of two or more thereof from the genome of the parasite to generate the recombinant parasite lacking the homolog.

7. The method of claim 6, wherein knocking out the homolog is accomplished using CRISPR/Cas9 methodology.

8. The method of claim 7, wherein the CRISPR/Cas9 methodology employs a guide RNA selected from SEQ ID NOs: 10, 11, 12, 23 or 24.

9. A vaccine composition comprising the recombinant parasite of any one of claims 1-5 and a carrier.

10. A vaccine composition comprising a *Toxoplasma gondii* IFT88, SRS15A, SRS15B, and/or SRS15C, SRS26B protein, a homolog or ortholog thereof, or a combination thereof, and a carrier.

11. The composition of claim 10, wherein the carrier comprises a nanoparticle.

12. The composition of claim 11, wherein the nanoparticle comprises a virus-like particle ("VLP"), which is pseudotyped with the *Toxoplasma gondii* IFT88, SRS15A, SRS15B, and/or SRS15C, SRS26B protein, a homolog or ortholog thereof, or a combination thereof.

13. The composition of any one of claims 9-12, which is orally or mucosally admissible.

14. The composition of any one of claims 9-12, which is formulated as a food additive.

15. The composition of any one of claims 9-12, which is formulated for injection.

16. A method of vaccinating an animal comprising administering the composition of any one of claims 9-15 to an animal, wherein the animal is a competent host for sexual reproduction of the species of parasite from which the homolog or ortholog of *Toxoplasma gondii* IFT88, SRS15A, SRS15B, SRS15C, and/or SRS26B is drawn.

17. The method of claim 16, wherein the animal is a felid and the parasite is a member of the species *Toxoplasma gondii*.

18. The method of claim 16 or 17, wherein the composition is administered to the animal orally or mucosally.

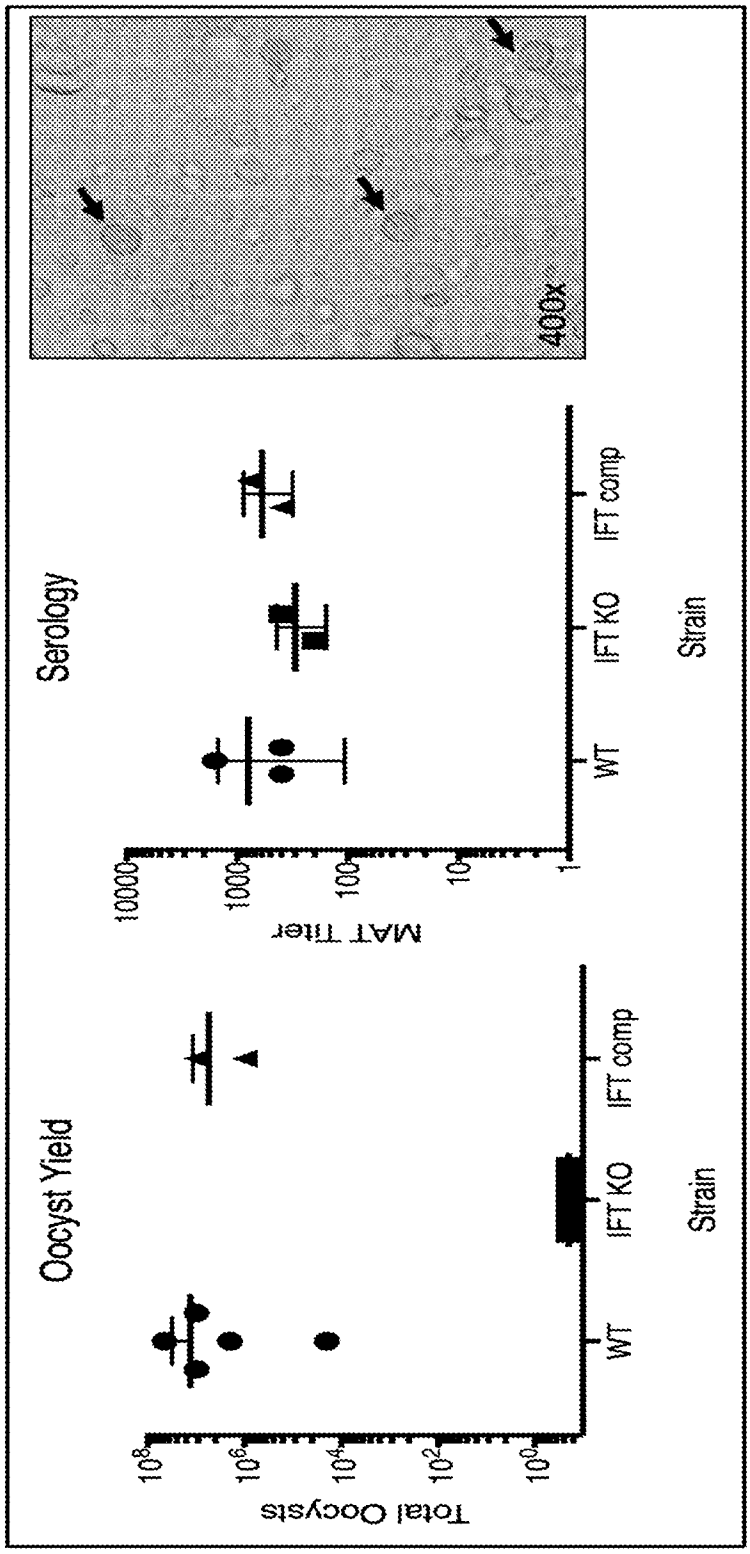
Oocyst Yield
Total Oocysts
10^8
10^6
10^4
10^2
10^0
WT
IFT KO
IFT comp
Strain
Serology
MAT Titer
10000
1000
100
10
1
WT
IFT KO
IFT comp
Strain
400x

Figure 1

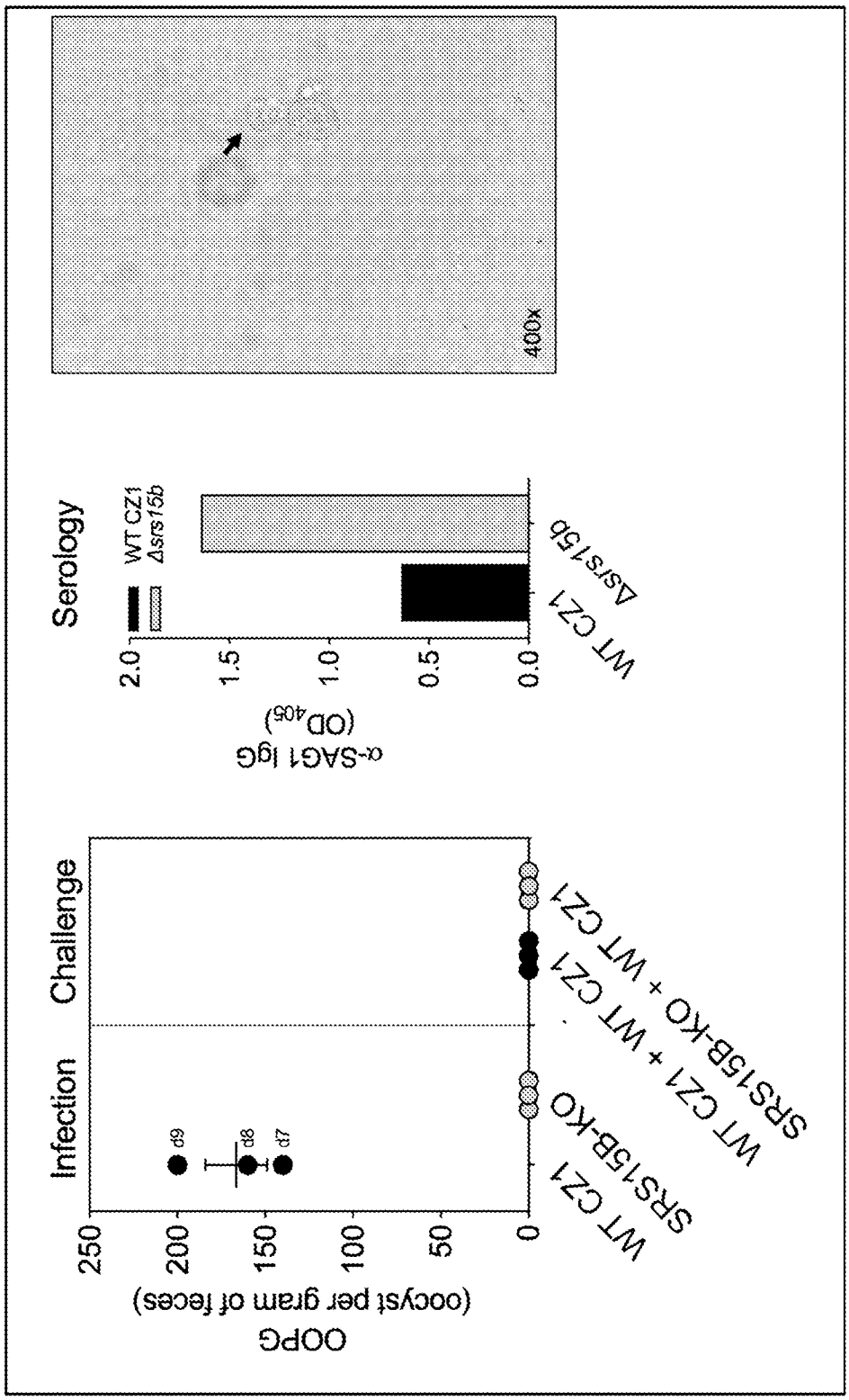
OOPG
(oocyst per gram of feces)
250
200
150
100
50
0
Infection
Challenge
d9
d8
d7
WT CZ1
SRS15B-KO
WT CZ1 + WT CZ1
SRS15B-KO + WT CZ1
α-SAG1 IgG
(OD405)
2.0
1.5
1.0
0.5
0.0
Serology
WT CZ1
Δsrs15b
WT CZ1
Δsrs15b
400x

Figure 2