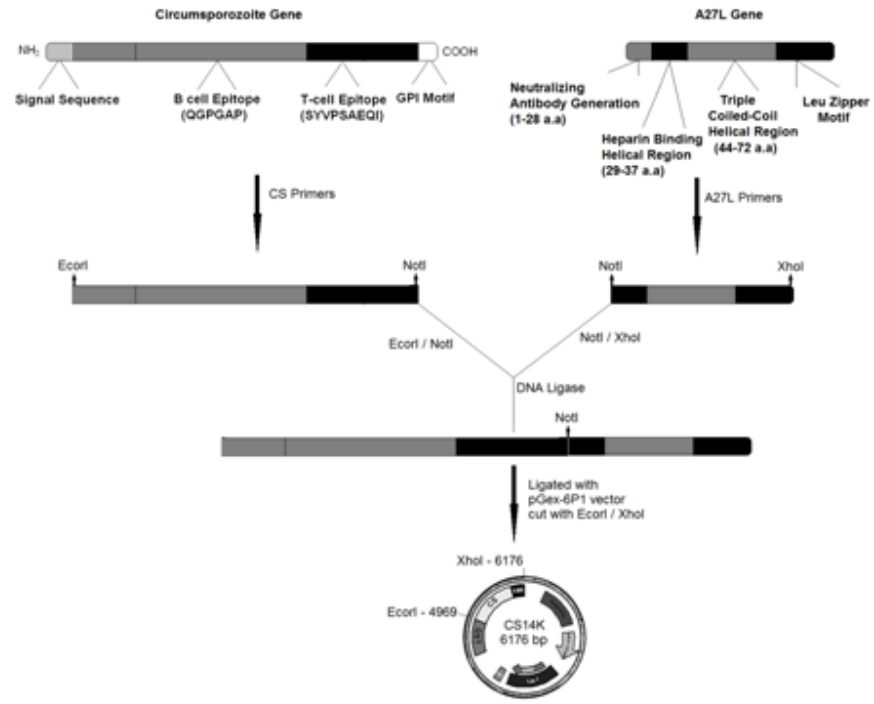
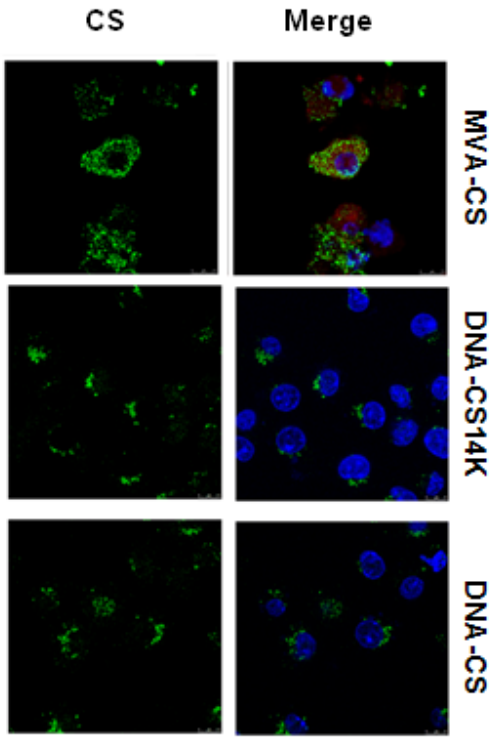
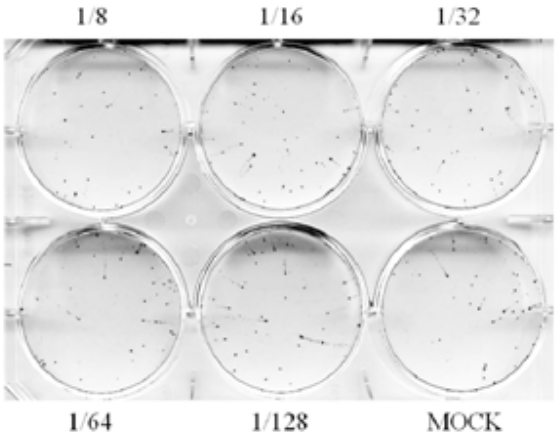
A.



C.



B.



D.

MCYNEENDNKLYHVLNSKNGKIYNRNIVNRLLGDALNGKPEE
KKDDPPKDGNKDDLPKEEKKDDLPKEEKKDDPPKDPKKDDPPK
EAQNKLNQPVVADENVDQGPGAPQGPGAPQGPGAPQGPG
APQGPGAPQGPGAPQGPGAPQGPGAPQGPGAPQGPGAP
QGPGAPQGPGAPQGPGAPQGPGAPQGPGAPQEPPQQPPQ
QPPQQPPQQPPQQPPQQPPQQPRPQPDGNNNNNNNNNGNNN
EDSYVPSAEQILEFVKQISSQLTEEWSQCSVTCGSGVRVRKR
KNVNKQPENLTLEDIDTEICKMDKCSSIFNSGREAKREAIVKAD
EDDNEETLKQRLTNLEKKITNVTTKFEQIEKCCKRNDEVLFRLE
NHAETLRAAMISLAKKIDVQTGRRPYE*

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Fig S1. Related to Figure 1. (A) Design and construction of plasmid encoding CS14K. A schematic representation of gene encoding the CS protein of *P.yoelii* and that of 14K of vaccinia virus is shown. For generating the CS fragment of chimeric protein, the signal sequence represented by 37 amino acids of amino terminal and GPI motif involving the 19 amino acids at the carboxy terminal of CS gene was removed by PCR amplification. The C terminal of amplified CS fragment was then fused in frame to the N terminal of gene, A27L of vaccinia, lacking first 28 amino acids. All PCR amplifications were carried out using appropriate primers carrying restriction enzyme sites. This fusion construct was then fused in frame with the GST tag present within the pGEX-6P-1 vector. **(B) CS14K fusion protein does not induce neutralizing antibodies against MVA.** Serum from rabbits injected with CS14K protein was not able to neutralize the infectivity of MVA virus *in-vitro*. 200 PFU of MVA virus were incubated with two fold dilutions of serum from rabbit injected with CS-14K protein. Following infection of DF-1 cells with the serum treated virus, immunostaining was carried out using anti-vaccinia antibody used at a dilution of 1:1000. Virus incubated with rabbit serum was used as mock control. The numbers of plaques formed by the viruses were counted to determine the extent of infection. **(C) Localization of proteins in macrophages:** Confocal microscopy analysis of CS and CS14K localization in macrophages using NYS1 antibody at a dilution of 1:500. Pattern of CS expression by MVA-CS. Confluent J774 cells were infected with MVA-CS at an MOI of 5 PFU/cell for 18 hours. Protein localisation in transfected macrophages. CS and CS14K encoding plasmids, pCINeo-CS (10 μg) and pCDNA-CS14K (10 μg), both lacking the signal and GPI motifs were transfected into confluent mono layers of J774 cells for 24 hours. **(D) Amino acid sequence of CS14K:** Amino acid composition of CS14K protein is represented with the 14K sequence under lined.

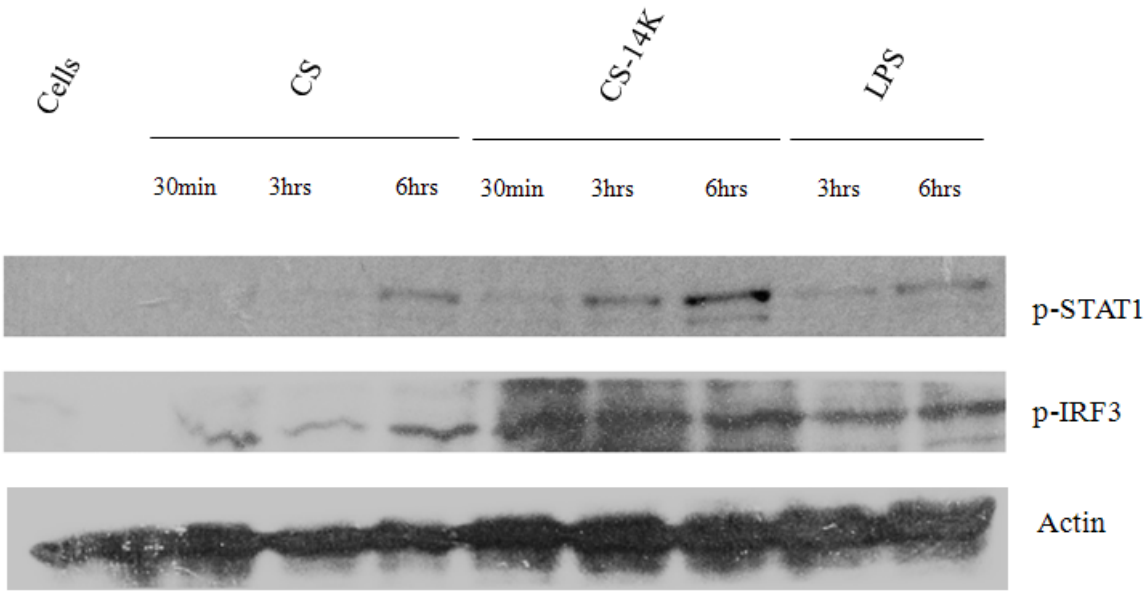

Fig S2. Related to Figure 2. IRF-3 and Stat-1 activation by CS14K is independent of ds-RNA: Proteins (5 *µg/ml*) and LPS (1 *µg/ml*) were treated with 2 µl of Shortcut®RNase III for 30 minutes at 37ºC. Following incubation the proteins were mixed with RPMI medium containing 10% serum to inhibit RNase III and incubated with 2×10^6 cells of THP-1 for defined time intervals. The levels of phospho IRF-3 and Stat-1 were detected as mentioned in *Materials and Methods.*

Priming Agent.	^{62}CS (DNA)	^{49}CS^{GPI-} (DNA)	^{15}CS+GM-CSF (DNA)	CS14K (Protein)	^{63}CSgp64 [a] (Protein)	^{64}Sb824/pST-TB [b] (Protein) (3)	^{65}ADPyCS [d] + 7DW8-5
Boosting Agent (Number of Boost).	MVA-CS (1)	CS^{GPI-} (3)	NYVAC-K1L-CS (1)	MVA-CS (1)	CSgp64 (3)	ACT-CS [c] (1)	-
Antibody Levels.	ND	+++	++	++++	+++	ND	++
Antibody Avidity.	ND	ND	ND	+++	ND	ND	ND
Total CD8$^+$ T-Cells Secreting Cytokines.	ND	ND	+++	+++	ND	+++	ND
ELISPOT (IFN-γ).	+++	ND	+++	+++	+	ND	++++
Nitric Oxide.	ND	ND	ND	Yes	ND	ND	ND
Protection	Yes	Yes	Yes	Yes	Yes	Yes	Yes

[a] CSgp64 : Recombinant Baculovirus expressing CS fused with baculovirus envelope protein gp64.

[b] Sb824/pST-TB : *Salmonella* expressing fusion protein between YoPE and CS peptide.

[c] ACT-CS : *Bordetella pertussis* adenylate cyclase toxoid fused with CS peptide.

[d] ADPyCS + 7DW8-5 : Adenovirus expressing CS mixed with a synthetic analogue of Alpha-Galactosylceramide.

+ - Low Levels	(Elispot: Less than 400 spots.)	(CD8 T-cells: Less than 1%.)	(Antibody Levels: < 1:50,000)
++ - Moderate Levels	(Elispot: 450-850 spots.)	(CD8 T-cells: 1% to 3%.)	(Antibody Levels: 1:60,000 to 100,000)
+++ - High Levels	(Elispot: 900-1200 spots.)	(CD8 T-cells: 4% to 6%.)	(Antibody Levels: 1:200,000 to 1:500,000)
++++ - Very High Levels	(Elispot: 1500-2000 spots.)	(CD8 T-cells: 10% and above.)	(Antibody Levels: > 1:800,000)

Fig S3. Comparative analysis of immunogenicity of various CS based vaccine regime. A detailed comparison of various murine plasmodial CS based vaccine regime against CS14K protein prime and MVA-CS boost. Experiments were appropriate studies were not carried out are represented as ND.