Supplementary information:

Methods:

Table S1:

<table>
<thead>
<tr>
<th>Primer Name</th>
<th>Nucleotide sequence (5’-3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DBL3-F</td>
<td>tcc ccg cgg agt gaa aca tca tgt gac tg</td>
</tr>
<tr>
<td>DBL3-R</td>
<td>gac tag ttt ctt tca ata aat cac tgc c</td>
</tr>
<tr>
<td>DBL5-F</td>
<td>cgc cct agg tgc att tcc gat gtt tg</td>
</tr>
<tr>
<td>DBL5-R</td>
<td>cgc cct agg tgc ttc att tcc gat gtt tg</td>
</tr>
<tr>
<td>CHC-F</td>
<td>tcc atg aat ttt cat cac atg</td>
</tr>
<tr>
<td>CHC-R</td>
<td>tga aat tat ttt gtg gag gc</td>
</tr>
<tr>
<td>CS2-1F</td>
<td>atg gtg atg caa ggt cct cgt gtt gg</td>
</tr>
<tr>
<td>CS2-1R</td>
<td>acc tcc tgg tgt taa atc acg tac gc</td>
</tr>
<tr>
<td>CS2-2F</td>
<td>aac agt cat agt gga gca tgt atg cc</td>
</tr>
<tr>
<td>CS2-2R</td>
<td>gca gtt tgg gta tgg tca cta gtt gg</td>
</tr>
<tr>
<td>CS2-3F</td>
<td>caa att gct gct gca act gat aaa gg</td>
</tr>
<tr>
<td>CS2-3R</td>
<td>tta tat atc cca cac atc tgt gat cag</td>
</tr>
<tr>
<td>var-1F</td>
<td>tgg cac gct atg tta tgt gg</td>
</tr>
<tr>
<td>var1-R</td>
<td>tac gca aca agt cca cag acg</td>
</tr>
<tr>
<td>PfCX</td>
<td>ggc gcg t g/a a acc ct g/a aac cct g/a aa ccc</td>
</tr>
</tbody>
</table>

PCR

PCR analysis was performed to show the presence and the integrity of the \textit{var1csa} and \textit{var-}
CS2 genes in the genome of the FCR3\textDelta var2csa clones. \textit{Var-CS2} was amplified using the following primer combinations: CS2-1F / CS2-1R or CS2-2F / CS2-2R or CS2-3F / CS2-3R. PCR was carried out using the Expand High Fidelity PCR System (Roche) according to the manufacturer’s instructions. The primers var1-F / var2-R were used to amplify the complete intron sequence and parts of \textit{var1csa} exon II. The primer pair var1-F / PfCX was used to amplify the truncated 3D7\textit{var1csa}.
Flow cytometry

IE were incubated for 30 min at 37°C with ethidium bromide (10 µg/ml), washed twice and incubated with sera of malaria-exposed multigravidae or men from Senegal or non-immune French donors (diluted 1/20) at 4°C for 30 min. IE were washed twice, and incubated with a goat anti-human IgG as a secondary antibody (1/120; Sigma) and with a chicken anti-goat Alexa Fluor 488 as a tertiary antibody (1/200; Molecular Probes) for 30 min at 4°C. Immunofluorescence staining was analyzed using a Coulter EPICS XL flow cytometer (Coultronics, France).
Figure S1.

A

B

C

D

PCR1

PCR2

PCR3

PCR4

PCR5

PCR4

PCR5

PCR1

PCR2

PCR3

ran1csa

ran2csa

ran3csa

ran4csa
Figure S1 legend.

Var1csa and var-CS2 genes are present in FCR3Δvar2csa mutants.

A. Schematic representation of the var-CS2 gene and the genomic loci for FCR3var1csa and 3D7var1csa. The different Duffy binding-like domains (DBL), the cysteine-rich interdomain regions (CIDR) and the C-terminal cytoplasmic domain (exon II) of the genes are shown. Telomeric repeats at the chromosome end and the position of the primer pairs are indicated.

B. PCR analysis of genomic DNA from FCR3 wt and the two mutant clones 1F1 and 2A5 using three different primer pairs, designed to amplify the var-CS2 gene. Bands of the expected sizes are observed for all parasite clones.

C. PCR analysis of genomic DNA from 3D7 wt, NF54 wt, FCR3 wt and the two mutant clones 1F1 and 2A5. The upper panel shows a band in 3D7 wt and NF54 wt corresponding to the truncated var1csa region. The lower panel shows a band in FCR3 wt, 1F1 and 2A5 corresponding to parts of var1DBL7-e, the 230 bp intron and parts of var1exon II. Var1csa is not truncated in FCR3 wt and in the FCR3Δvar2csa clones.

D. The var1csa gene is transcribed in FCR3Δvar2csa mutants. Northern Blot analysis of total RNA isolated from ring (R) and trophozoite stage parasites (T) FCR3-CSA, FCR3-CD36 and one representative FCR3Δvar2csa clone. The membrane was hybridized with a probe specific for var1csa DBL3-γ.

Figure S1 results and discussion.

Var1csa is transcribed and var-CS2 is present in the genome of the FCR3Δvar2csa mutants

Two other var genes of the FCR3 genetic background, var1csa and var-CS2 were described earlier, to be involved in cytoadhesion of P. falciparum IE to CSA (Buffet et al, 1999; Reeder et al, 1999). We performed PCR analysis to confirm that the var-CS2 gene is present in the genome. Three primer pairs were designed to amplify overlapping fragments of var-CS2 (Fig.
S1A). PCR fragments of the expected size were obtained for wildtype FCR3 as well as for the FCR3∆var2csa mutant clones, showing that full-length var-CS2 is present in the genome of these parasites (Fig. S1B).

As var1csa is truncated in 3D7, we PCR amplified either the truncated 3’ region of the open reading frame of var1csa up to the telomeric repeats for the truncated gene or a fragment starting in the sequence for DBL7-ε up to exon II for full-length var1csa (Fig. S1A). The PCR results showed that NF54 has a truncated var1csa gene as is already known for the NF54 derived clone 3D7 (Fig. S1C). In contrast, full-length var1csa was detected in FCR3 wild type and in the two FCR3∆var2csa clones 1F1 and 2A5.

To confirm that var1csa is transcribed in the wild type parasites with CSA- and CD36-binding phenotypes as well as in the mutants, a Northern analysis was performed on total RNA isolated from ring and trophozoite stage parasites. A high molecular transcript for var1csa was detected in FCR3-CSA, FCR3-CD36 and in FCR3∆var2csa as described earlier (Kyes et al, 2003).

These results indicate that var1csa and var-CS2 are not involved in CSA specific cytoadhesion of erythrocytes infected with late stages of FCR3∆var2csa.
Figure S2.

MULTIGRAVIDAE
SERUM N°9S Senegal

MULTIGRAVIDAE
SERUM N°11S Senegal

Male SERUM
N°90S Senegal

FCR3-CSA

FCR3-CD36

1F1-KO

2A5-KO
**Figure S2 legend.**

Sera of malaria-exposed multigravidae do not recognize the surface of FCR3Δvar2csa IE. The surface of intact erythrocytes infected with FCR3-CSA, FCR3-CD36, 1F1 and 2A5 were immunostained with sera of malaria-exposed multigravidae (sera N°9S and N°11S) or male (serum N°90S) from Senegal (line) and with serum of non-immune French donors (solid grey) and analyzed by flow cytometry.