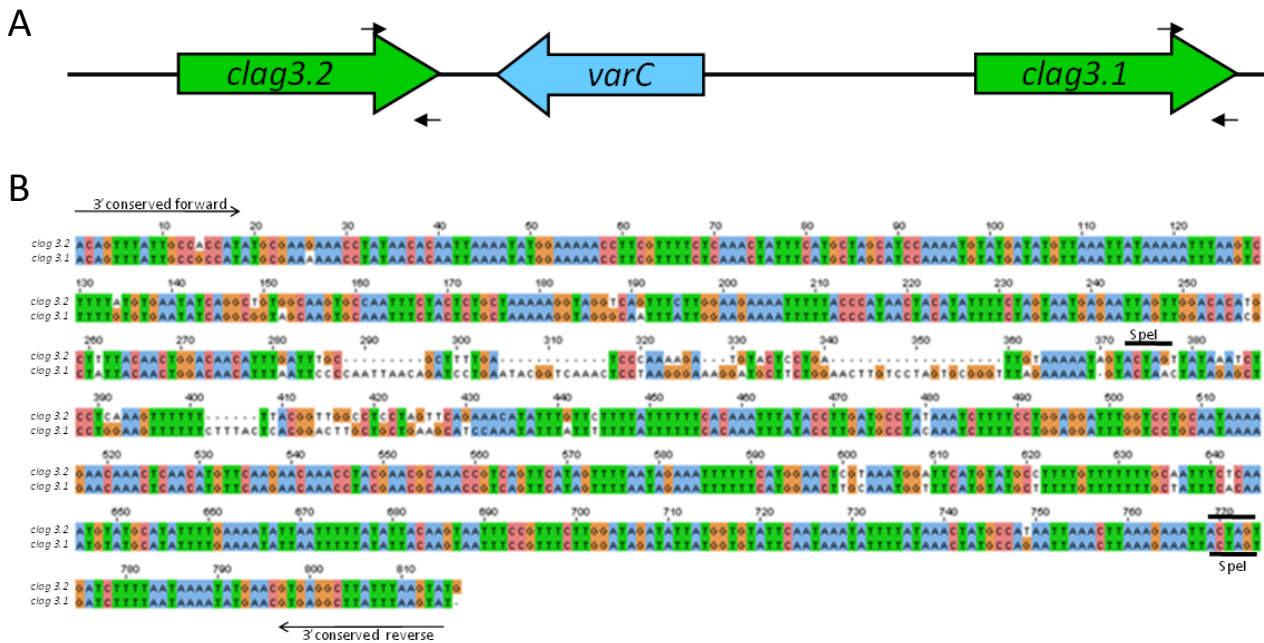


SUPPLEMENTAL FIGURE 1

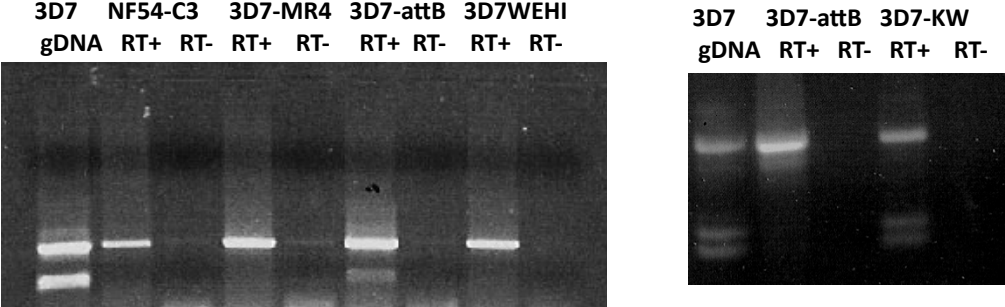


Supplemental figure 1: Schematic of *clag 3* PCR-RFLP assay.

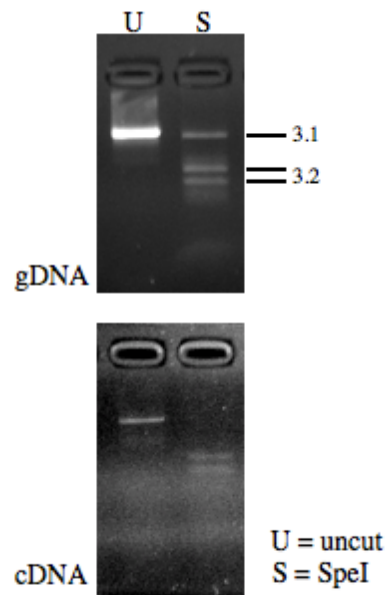
A. Diagram of the chromosome 3 region containing both *clag 3* paralogs with the 3' region amplified from each *clag 3* gene with conserved primers marked with thin black arrows.

B. Sequence alignment of the resulting amplicon of *clag 3.1* and *clag 3.2*, with primer binding regions and sites of restriction enzymes used in assay denoted. A *SpeI* site that is present in both sequences is marked by a black line both above and below the sequence, whereas the unique *SpeI* site found only in *clag 3.2* is denoted by a single black line.

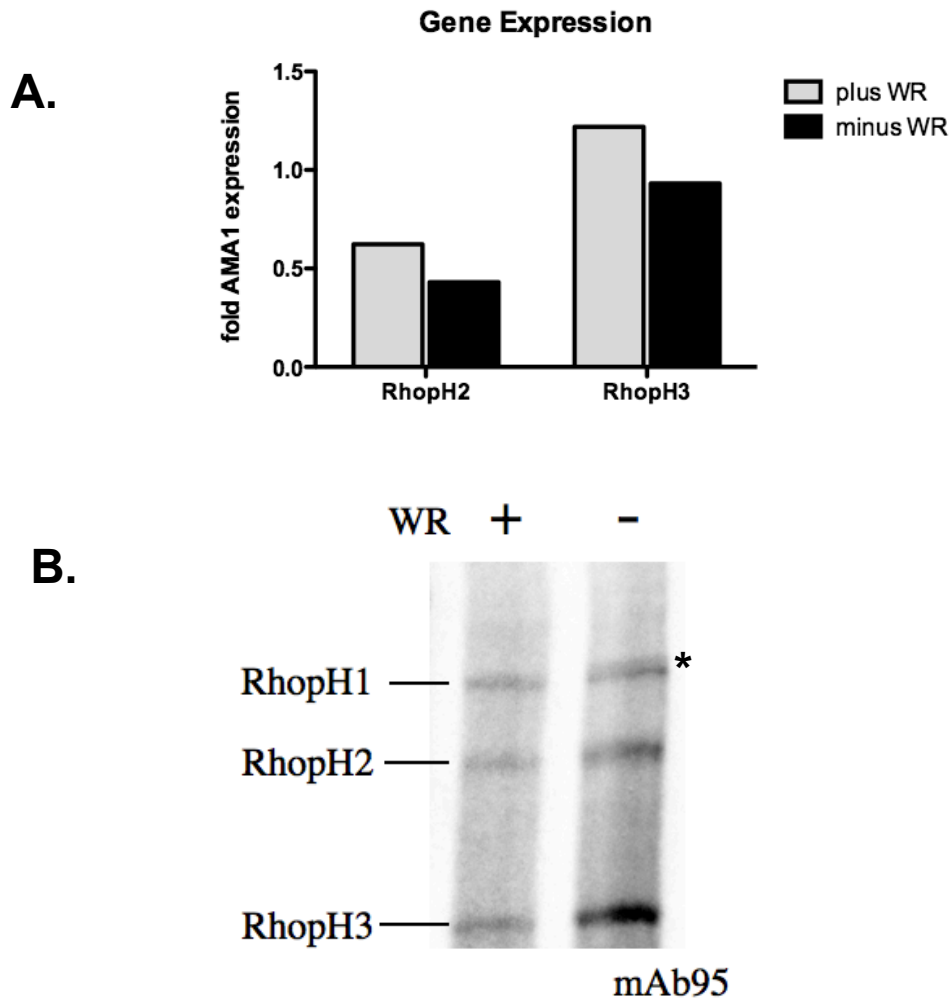
SUPPLEMENTAL FIGURE 2



Supplemental figure 2: Screen of 4 3D7 isogenic lines (3D7 sources and NF54) for clag 3.2 expression. Only 3D7-attB appears to express a low level of 3.2. Further experimentation with 3D7attB after additional time in culture (6 weeks between these gels) revealed only minimal 3.2 expression; B. An additional source of 3D7 (3D7-KW) expressed clag 3.1 and clag 3.2 in an approximately 1:1 ratio, making recovering clag 3.2 expressing clones more feasible.



Supplementary Figure 3. RFLP analysis of Clag3.1 and Clag3.2 from the parasite strain T9/94. Top panel. RFLP (digestion with SpeI) of Clag 3 amplified from genomic DNA. Restriction digestion reveals one copy of Clag 3.1 and one copy of Clag 3.2 Lower panel. RFLP (digestion with SpeI) of Clag 3 amplified from schizont cDNA of T9/94. Only clag 3.2 is expressed.



Supplementary Figure 4.

A. RhopH2 and RhopH3 expression is unchanged in Clag3.2ins cl5 WR + and WR- lines

B. Immunoprecipitation of RhopH complex members with antibodies specific to RhopH2 (mAb95). This was carried out by the method described in Baldi et al. *Infection & Immunity*, 70, 5236-45, 2002.

*:Clag 3.1 band that is only present in WR- and not in WR+. Quantity of members of RhopH1 complex does not change in Clag3.2 cl5 WR + and WR- lines. Clag3.1 is lost in the Clag3.2 cl5 WR +, IPed with mAb95 (described in Doury et al. *Parasitology*, 108, 269-280,1994, a kind gift from Dr. Dubremetz INSERM, France).

SUPPLEMENTAL TABLE 1: qRT-PCR primers for measuring RhopH expression levels

Primer name	Gene	Sequence (5'→3')
clag2ccF/R	PFB0935w	gaagtgtttctaaatgacag catcggattatctgattgag
clag3.1ccF/R	PFC0120w (5'UTR)	tggacaaatagattttgtac gtacagaattgaatagtac
clag3.2ccF/R	PFC0110w (5'UTR)	ggatcatcatcggattgtac ccgtattcaaaaaggagtacac
clag8ccF/R	MAL7P1.229	ctgtcgacatatgtag gccttactatttgacg
clag9ccF/R	PFI1730w	ggctatgtcaagagct ggagtaatgtcagtacc
RhopH2ccF/R	PFI1445w	cacatatcttacagaacc ttggatcctcagatac
RhopH3ccF/R		
AMA1F/R	PF11_0344	tcccatcccatcgaagttgaa tgatgcaattataatttcat

Supplemental table: qPCR primers for *clag 3* ChIP assays

Primer name	Relative position to region's genes	Sequence (5'→3')
c3chip1F/R	Far upstream <i>clag3.2</i>	acgatgatgattacgaggag gatcagcatcatgttcttgtc
c3chip2F/R	Far upstream <i>clag3.2</i>	atagagatgaccagaatg ctgtatatatttacttgtag
c3chip3F/R	Close upstream <i>clag3.2</i>	ggatcatcatcggattgtac ccgtattcaaaaaggagtacac
c3chip4F/R	Within 3' coding region of <i>clag 3.2</i>	atcccaaaagatgtactcctg gtttctgaactaggaggccaa
c3chip5F/R	Within 3' coding region of <i>varC</i> pseudogene	cgattagtactaatcgttattg gttgagatctcaatcg
c3chip6F/R	Middle coding region of <i>varC</i> pseudogene	cgtgccattccatcaacca cgaactaccaaaaacatcacc
c3chip7F/R	Upstream <i>varC</i> /far upstream <i>clag 3.1</i>	ctataataaatccattattgc gaatggttattaactattatg
c3chip8F/R	Upstream <i>varC</i> /far upstream <i>clag 3.1</i>	tctgcaggttttatatattcc tcattcagatggagaaaatg
c3chip9F/R	Upstream <i>varC</i> /far upstream <i>clag 3.1</i>	gagacagctgtatcaccca cacaaaacattttaggtgtcatg
c3chip10F/R	Upstream <i>clag 3.1</i> / far upstream <i>varC</i>	agaatccttgtgcaatac gcacttaattcttgttgac
c3chip11F/R	Upstream <i>clag 3.1</i> / far upstream <i>varC</i>	caataacgtattaggaatgttac gtagttacgtaaacgtttc
c3chip12F/R	Upstream <i>clag 3.1</i> / far upstream <i>varC</i>	taaacataagagggtga cattcgagaatatatg
c3chip13F/R	Upstream <i>clag 3.1</i> / far upstream <i>varC</i>	tggacaaatagattttgtac gtacagaattgaatagtac
c3chip14F/R	Within 3' coding region of <i>clag 3.1</i>	gtcccaagttctactgctaag cttcagcagcaagtccgtgagtgaa g