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Supplemental Information

A Specific PfEMP1 Is Expressed in *P. falciparum* Sporozoites and Plays a Role in Hepatocyte Infection

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SUPPLEMENTARY FIGURE & LEGENDS :

Figure S1:

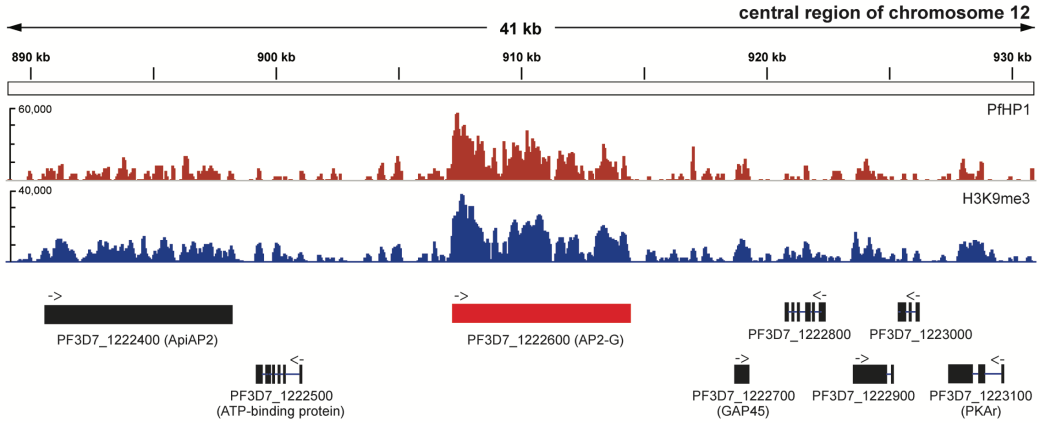


Figure S1. Related to Figure 2. H3K9me3 and PfHP1 are enriched at the AP2-G genomic locus on chromosome 12 in sporozoites. ChIP-seq analysis shows the enrichment of PfHP1 (red) and H3K9me3 (blue) at the AP2-G locus (highlighted in red), with genomic position indicated at the top in kilobases. Coverage plots are represented as average reads per million reads mapped (RPM) over bins of 1 nucleotide with the maximum value of y-axis indicated. Data are representative of three or more independent experiments.

Figure S2:

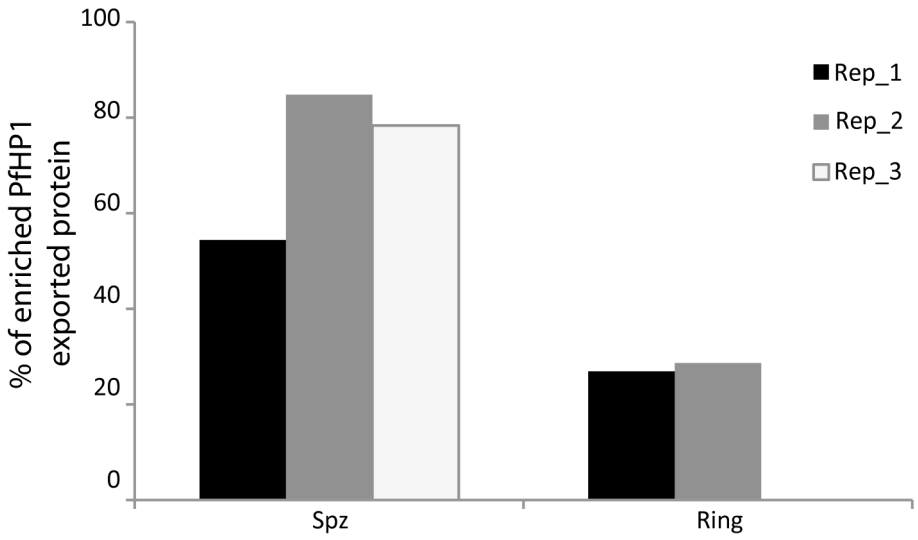
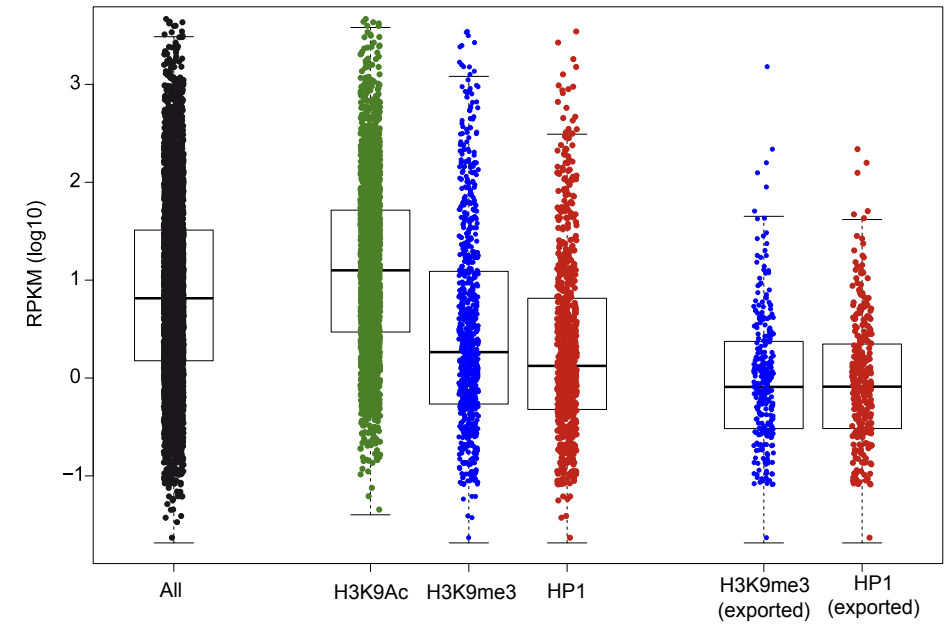


Figure S2. Related to Figure 3. Comparison of PfHP1 enrichment in genes encoding exported proteins in sporozoites and asexual ring stage parasites. The percentage of genes encoding erythrocyte stage exported proteins (containing a PEXEL motif) with PfHP1 enrichment in three biological replicates of sporozoites is increased when compared to asexual blood stage parasites (two biological replicates). Rep 1=Pilot, Rep 2=L1 and Rep 3=L3 for the sporozoite samples and Rep 1=A and Rep2 =B for the asexual blood stage samples (Refer to Supplementary Table S6).

Figure S3:

A



B

	H3K9Ac	H3K9me3	HP1
Total peaks	4620	1572	1606
peaks in genes (# unique genes)	3773 (2612)	1322 (945)	1210 (904)
Transcription Level			
High	846	146	88
Medium	741	111	103
Moderate	580	164	171
Low	377	403	429

Figure S3 Related to Figure 3 and Figure 4. Correlation of PfHP1 and H3K9me3 occupancy within gene bodies to transcriptional levels in sporozoite stages. A) Distribution of steady state mRNA levels (y-axis; calculated as $\log_{10}$RPKM (reads per kilobase of exon model per one million mapped reads); Refer to Supplementary Table S3) of all *P. falciparum* genes (in black) is compared to genes enriched with either H3K9Ac (green), H3K9me3 (blue) or PfHP1 (red) in sporozoites. The presence of H3K9Ac leads to overall higher transcription, whereas the presence of H3K9me3 and PfHP1 leads to transcriptional repression. Genes encoding proteins that are predicted to be exported to the RBC surface

during the blood stages (and containing a PEXEL motif) show low to zero transcription in sporozoites. **B**) Top: A summary of H3K9Ac, H3K9me3 and PfHP1 peaks within genes, as determined by MACS2 peak calling analysis (Refer to Supplementary Table S2). Bottom: Steady state mRNA levels of all genes were sorted into four equally sized groups in decreasing order of magnitude of expression (*i.e.*, RPKM) and analyzed for the occupancy of the different epigenetic marks. In general, genes associated with H3K9Ac have higher steady state mRNA levels as compared to genes associated with either PfHP1 or H3K9me3.

Figure S4:

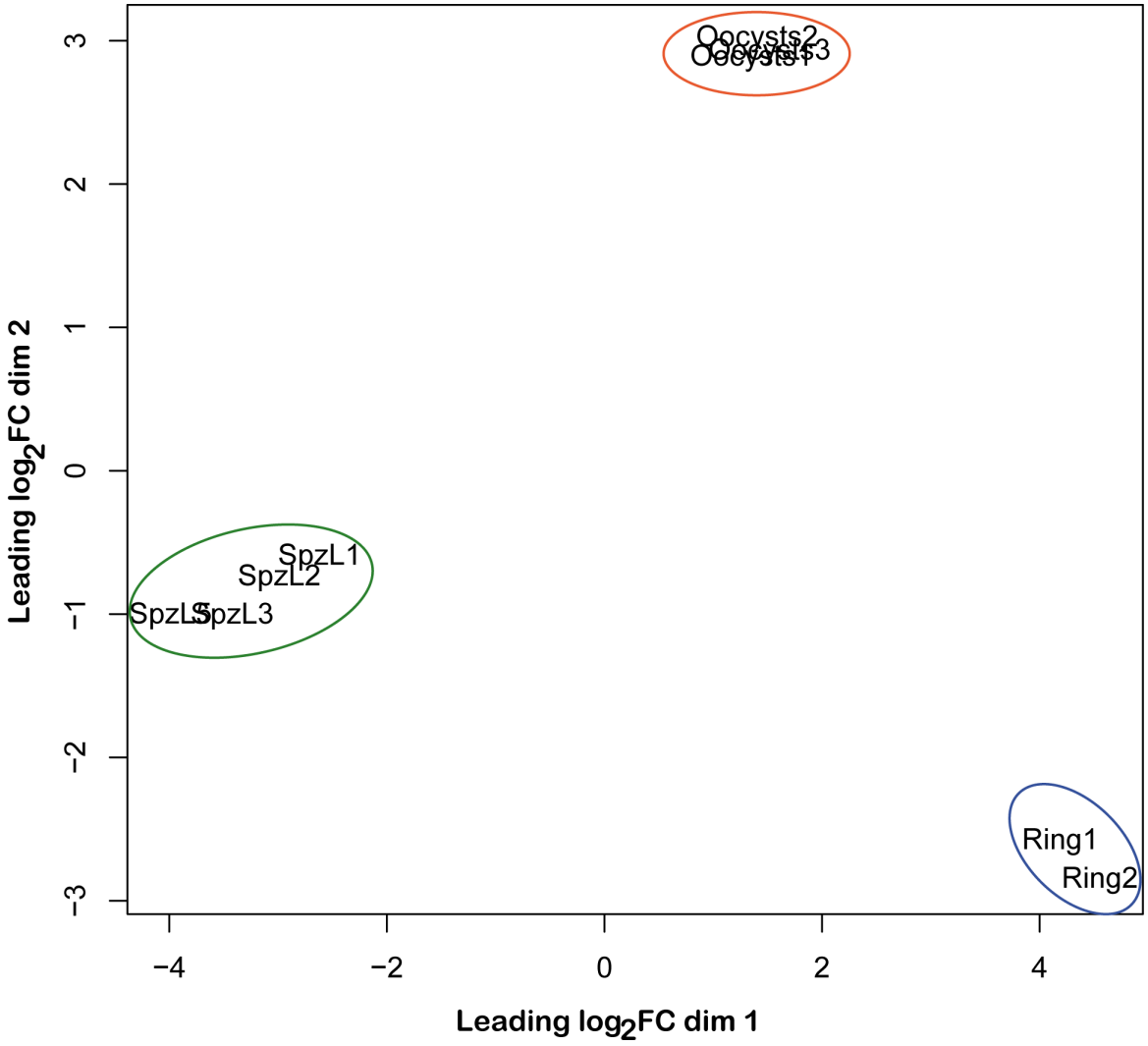


Figure S4. Related to Figure 4. Similarity of the transcriptomes of the different life cycle stages of *P. falciparum* analyzed in this study. A multidimensional scaling plot representing each RNA-seq dataset in two dimensions, such that the distances between each dataset reflect the typical $\log_2$(fold change) ($\log_2$FC) between them. The clustering of similar datasets is highlighted to emphasize the separation of samples into natural groups, based on life cycle stage. See Supplementary Table S3 for more details.

Figure S5:

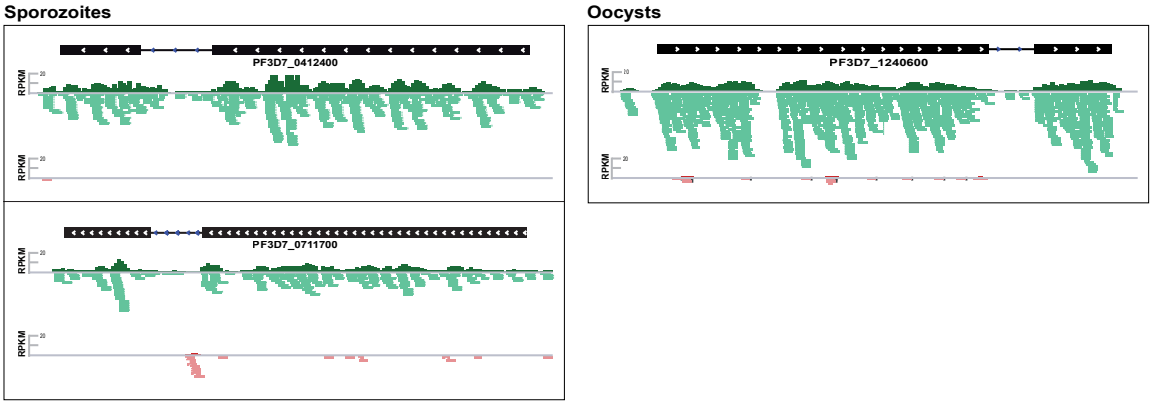


Figure S5. Related to figure 4. Coverage plot of *var* gene expression analyzed by RNA-seq. Top: Transcriptional levels are indicated as RPKM, *i.e.*, reads per kilobase of exon per one million mapped reads, Bottom: mapped reeds. For sense RNA (light green) and antisense RNA (red). See Supplementary Table S4 for more details

Figure S6:

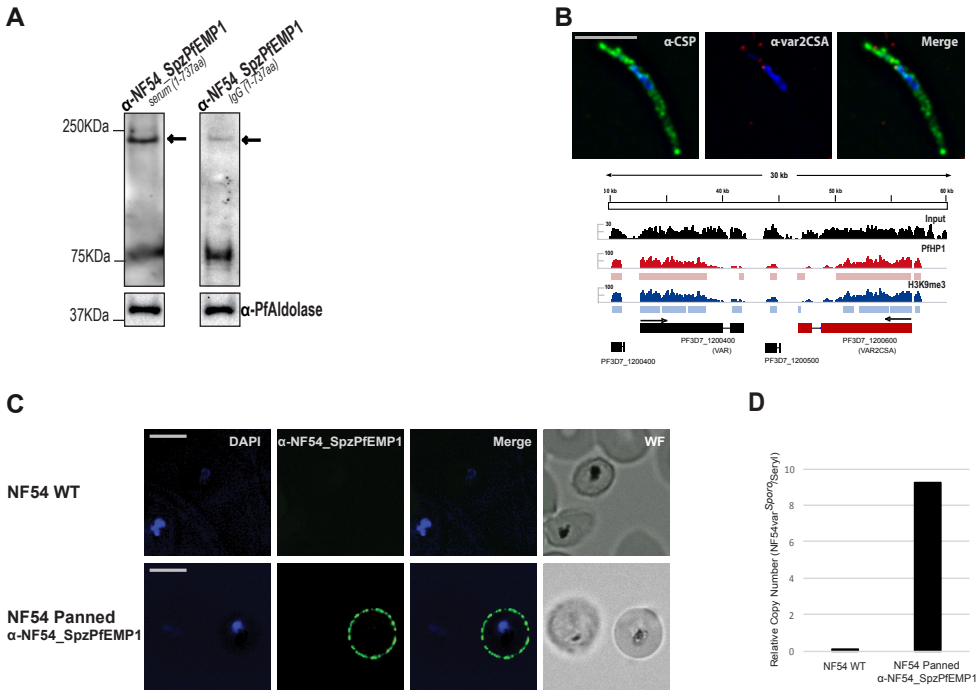


Figure S6. Related to Figure 5. Immunofluorescence analysis of PfEMP1 protein expression in sporozoites and infected red blood cells (iRBCs). (**A**) Western blot analysis of sporozoite extracts using anti-NF54_SpzPfEMP1 antibodies. Anti-PfAldolase antibodies served as a positive control for protein extraction. Molecular weights are shown at the left of the blot and PfEMP1 is indicated with an arrow. (**B**) Top: Immunofluorescence analysis of fixed and permeabilized sporozoites using anti-var2CSA (red) and anti-CSP (green) antibodies. Bottom: Coverage plot (average RPM over bins of 1 nucleotide) representing the enrichment levels of PfHP1 and H3K9me3 at the *var2CSA* locus. The genomic position is indicated at the top in kb. For all panels, DNA was stained with DAPI (blue), indicating the nucleus, and the scale bar is 5 µm. (**C**) Surface immunofluorescence of living iRBCs at 30 hours post infection using anti-NF54_SpzPfEMP1 1/1000 dilution (green). Top panel shows WT NF54 trophozoites and lower panel panned NF54 parasites. DNA was stained with DAPI (blue) and the wide-field (WF) image is shown at the right. Scale bar = 5 µm. (**D**) RT-qPCR analysis of the WT NF54 and antibody NF54_SpzPfEMP1-panned NF54 parasites. RNA transcripts from highly synchronized ring stage parasites 12 hours post-infection. *var* gene cDNA levels (NF54*var*^{sporo}) are normalized to those of seryl tRNA synthetase.