MalariaSED: a deep learning framework to decipher the regulatory contributions of noncoding variants in malaria parasites

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Abstract

Malaria remains one of the deadliest infectious diseases. Transcriptional regulation effects of noncoding variants in this unusual genome of malaria parasites remain elusive. We developed a sequence-based, *ab initio* deep learning framework, MalariaSED, for predicting chromatin profiles in malaria parasites. The MalariaSED performance was validated by published ChIP-qPCR and TF motifs results. Applying MalariaSED to ~1.3 million variants showed that geographically differentiated noncoding variants are associated with parasite invasion and drug resistance. Further analysis revealed chromatin accessibility changes at *P. falciparum* rings are partly associated with artemisinin resistance. MalariaSED illuminates the potential functional roles of noncoding variants in malaria parasites.

Introduction

Malaria remains one of the largest global public health challenges, with an estimated ~241 million cases worldwide in 2020 despite remarkable achievements in reducing this deadly disease over the past decade. It has been reported that some noncoding regions in *P. falciparum* are associated with artemisinin resistance (ART-R)[1,2] or regulate a group of genes related to crucial biological processes, such as antigen variation[3,4], merozoite invasion[5-7] and gametocytogenesis[6]. Therefore, understanding the effects of genomic variants in malaria parasites is critical for monitoring the spread of drug resistance and the escape of the host immune response. Unlike genomic variants in protein-coding regions whose impacts can often be deduced from resultant codon changes, functional effects associated with noncoding variants cannot be so easily assessed. Despite the large number of noncoding genomic variants recorded in the public variant-tracking resource MalariaGEN[8] (~1.31 million noncoding vs. ~1.35 million coding), detecting the contribution of mutations located at noncoding regions is still challenging, and establishing the role of the vast noncoding space in the genetic basis of crucial parasite phenotypes remains difficult.

Genome-wide association studies (GWASs) have been widely used to identify variants in the *P. falciparum* genome significantly associated with drug resistance[1,9-18]. However, GWAS is an a posteriori approach requiring many historical samples that take time to acquire. Although functional screens have been implemented previously[15,19], these had limited throughput for testing the whole repertoire of noncoding variants, and these procedures are also time-consuming and expensive[20].

Sequence-based prediction models provide a new perspective on the effects of genomic variants on gene regulation. However, all currently available models were developed in model

organisms[21-24], which are not suitable for malaria parasites due to several unusual features of their genome architecture[25]. The most striking trait of the *P. falciparum* genome is its high AT content (~80% on average, rising to ~92% in intergenic regions). Additional features complicating the applicability of existing predictive models include the apparent absence of some subunits of the preinitiation complex[26], as well as the outsized contributions of a relatively small 27-member sequence-specific transcription factor (TF) family known as ApiAP2s to transcriptional regulation[27]. In comparison, the similarly sized genome of the yeast *Saccharomyces cerevisiae* contains ~170 sequence-specific TFs[28]. A sequence-based model accounting for these peculiarities is required for accurate prediction of gene expression profiles associated with noncoding variants specifically for malaria parasites.

In this work, we have leveraged recent advancements in deep learning (DL) to develop a sequence-based computational framework, MalariaSED, for noncoding variant effect prediction in malaria parasites. To our knowledge, this is the first time in malaria parasites using DL to learn regulatory sequence patterns, including TF binding, chromatin accessibility and histone modification profiles. This framework is complementary to experimental approaches at higher throughput and with lower cost. MalariaSED predicts epigenetic effects based upon sequence alterations at single-nucleotide resolution, enabling us to trace the associated biological process naturally-selected on regulatory sequences and to improve the prioritization of functional variants associated with important phenotypes.

Results

MalariaSED accurately predicts chromatin profiles from genomic sequences in malaria parasites

The unusual genome property of malaria parasites requires the development of a novel computational framework to understand the regulatory sequence code from parasite noncoding sequences. To this end, we developed the first sequence-based deep learning framework, MalariaSED (Supplement table 1), for malaria parasites, which learns the sequence code of transcriptional regulatory elements by training in large-scale epigenetic experimental data, including open chromatin accessibility (ATAC-Seq)[29], TF binding, and histone-mark profile (ChIP-Seq)[5-7,30,31]. MalariaSED computes a chromatin score $f(s)$ for a sequence s using three stages:

$$f(s) = net(LSTM(Conv(s)))$$

Here, *s* is an input sequence represented as a $4 \times l$ matrix, where each row represents a nucleotide, and each column indicates a position in the length l sequence. '1' represents a

specific nucleotide present at a specific position of the sequence, and '0' does not (Fig. 1A). Our results indicate that the 1 kb sequence context substantially improves the performance of MalariaSED (Supplement figure 1). The convolution layer (Conv) is a motif scanner analogizing the position weight matrix. The bidirectional long short-term memory (LSTM) uses forward and reverse complement features as input to capture the relationship between a set of sequence information outputted from the convolution layer. The LSTM is crucial since TF binding sites may have a group of sequence patterns separated from each other by a long distance. We used the flatten layer and sigmoid function ('net' in the equation) to generate chromatin prediction scores. The high predictive performance for eight chromatin profiles at multiple parasite stages in both *P. falciparum* and *P. berghei* demonstrates that the MalariaSED architecture can capture important patterns in DNA sequences (average Area Under the Receiver Operating Characteristic curve (auROC) > 0.96, Fig. 1B, Supplement table 2). Fig. 2 illustrates an agreement between the predicted PfAP2-G binding profile and ChIP-seq/Input ratio[6] across a genomic region of chromosome 10 throughout commitment and early gametocytogenesis. We also tested the previously DL model trained in the mammalian genome[21], which can only obtain an average auROC of 0.81 (Supplement figure 2). We reasoned that the outperformance of MalariaSED is due to the structural discrepancy between the malaria parasite genome and that of model organisms, such as the number of TFs and highly skewed AT content.

CRISPR/Cas9 mutations in ApiAP2 binding sites demonstrate the capacity of MalariaSED to identify damaging genetic mutations

Unidirectional flow from sequence information to consequent epigenetic status in MalariaSED framework provides a novel technique for estimating the noncoding variant effects in *malaria* genomes. We tested this capability in predicting ChIP profile changes at multiple TF binding motif mutations generated by the CRISPR–Cas9 system. These ChIP-qPCR profiles are currently available for three TFs, PfAP2-G5[32], PfAP2-G[6] and PfAP2-I[5,] in *P. falciparum*. Four PfAP2-G5 motif mutations, S1-mutation (mut), S2-mut and S3-mut and S3-deletion (del), were generated at the *ap2-g* gene upstream regulatory region[32]. Their surrounding [-100, +100] bp regions are illustrated in Fig. 3A as S1, S2 and S3. The PfAP2-G5 ChIP-Seq results indicate a strong binding affinity of PfAP2-G5 at S1 and S2, whereas S3 presents a moderate level. The mutations, S1-mut and S2-mut from the original ChIP-qPCR profiles showed minor binding effects, but S3-mut and S3-del strongly weakened PfAP2-G5 binding sites. Further comparison between S3-del and S3-mut indicated higher binding effects at S3-mut. We validated the predictions from MalariaSED by these ChIP profiles. The estimated PfAP2-G5 binding

probability from MalariaSED, represented as S1-WT, S2-WT and S3-WT in Fig 3B, is correlated with the ChIP-Seq profiles. In contrast, the binding effects shown as S1-mut, S2-mut, S3-mut and S3-del in Fig.3B are in concordance with the original ChIP-qPCR results. We also used MalariaSED to predict the PfAP2-G binding change at a region containing eight GTAC motifs (S4 in Fig. 3C). A previous study showed that mutation of the three motifs by CRISPR/Cas9 significantly reduced binding of PfAP2-G[6]. This result supports the predictions from MalariaSED, which shows a strong decrease in PfAP2-G binding probability in Fig. 3D. An exceptional case for MalariaSED prediction occurred at the promoter region of the *pfmsp5* gene, where ChIP-qPCR showed PfAP2-I binding but was not identified by the original ChIP-Seq signal[5]. Since MalariaSED was trained by the ChIP-Seq results, it only predicted a PfAP2-I binding probability lower than 0.1 for both the WT and ATGCA motif mutations.

Single-nucleotide substitutions at the TF motif weaken TF binding

The successful validations of TF binding changes in CRISPR/Cas9-generated motif mutations imply potential DNA motif contributions to MalriaSED prediction. To validate this globally, we used MalariaSED to interrogate the average TF binding effects of all possible single nucleotide substitutions (SNSs) at the reported motifs (Supplement Table 3, see Methods for definition). Basically, for each site of a reported motif, the reference nucleotide was in turn changed by all other three nucleotides as motif mutation sets (e.g., a DNA motif comprising 5 nucleotides generates 5 x 4 = 20 motif mutations). Each generated sequence in the mutation sets was individually selected to replace the TF motifs that appeared in all the predicted TF binding regions. The average binding effect of all these SNSs was used to evaluate the motif contribution to TF binding. All other k-mer sequences with the same length as the investigated motif were used as the control sets (shown as ‘ALL’ in Fig. 4). We used this strategy to check the reported motifs of the parasite-specific TF PfAP2-G, a key determinant of sexual commitment in *P. falciparum*. We observed a significantly reduced binding affinity of PfAP2-G after incorporating SNSs at its binding motif GTRC (committed schizonts) and GTAC (sexual rings and stage I gametocytes) (Fig. 4A, Wilcoxon test $p < 2.2e-16$). Similarly, for PfAP2-I, a key regulator of red blood cell invasion, SNSs at the reported motif (GTGCA) significantly weakened its binding affinity at the schizont stage (Fig. 4B, Wilcoxon test $p < 2.2e-16$). Previous results indicate that two motifs (GGTCG and CTTGC) at other DNA-binding domains of PfAP2-I are not required for PfAP2-I binding during the RBC stage[5]. MalariaSED prediction also showed that SNSs in these two motifs did not significantly reduce PfAP2-I binding (Fig. 4B). When another invasion regulator, PfBDP1, was examined, SNSs introduced into the PfAP2-I GTGCA motif led

to a significantly reduced binding level in schizonts (Fig. 4C, Wilcoxon test $p < 2.2e-16$), supporting the conclusion that PfBDP1 is likely to form a protein complex with PfAP2-I to regulate invasion genes[5,7,33]. For the parasite trophozoite stage, we also checked the DNA binding change of PfBDP1 by introducing SNSs at the PfAP2-I motif GTGCA (Fig. 4C) and did not observe significant changes compared with other 5-mer sequences. This result supports that PfBDP1 binds many gene promoters not bound by PfAP2-I in trophozoites and are in line with the conclusion that PfBDP1 and PfAP2-I are involved in a protein complex in trophozoites but not in schizonts[7]. Furthermore, the predicted results from MalaraSED do not show significant binding effects of the motifs TAACT and ACAAC on PfBDP1 binding sites (Supplement Figure 3). It has been shown that TAACT, the second motif of PfBDP1 binding, co-occurs with the micronemal DNA motif ACAAC in the promoters of several micronemal and rhoptry neck genes[5]. The limited contributions of these two motifs suggest that some other unidentified TFs may recruit PfBDP1 to regulate micronemal gene expression. We also tested MalariaSED on two AP2 family TFs, PbAP2-G2 and PbAP2-O, in *P. berghei*[30,31]. PbAP2-G2 represses asexual genes to support conversion to the sexual stage, while AP2-O plays a critical role in regulating mosquito midgut invasion. MalariaSED identifies SNSs in two binding motifs, GTTG for PbAP2-G2 and GCTA for PbAP2-O, which are more likely to abolish their binding (Fig. 4D, Wilcoxon test $p < 2.2e-16$). Taken together, these results suggest that MalariaSED can capture crucial DNA sequences responsible for TF binding. Critically, MalariaSED predicted disrupted TF binding in mutants with mutations at TF binding motifs from sequence data alone, demonstrating the utility of sequence-based models for interpreting functional variants in malaria parasites.

Geographically differentiated variants at noncoding regions are more likely to alter their surrounding chromatin environments

Approximately six million curated genomic variants (including SNPs and short indels) from 7,000 *P. falciparum* samples recently made available on MalariaGEN show the enrichment of geographically differentiated SNPs in nonsynonymous variants[8]. Further analysis indicates that genes harboring the top geographically variable nonsynonymous SNPs tend to be involved in the process of parasite transmission by the mosquito vector and are associated with drug resistance. Despite noncoding variants showing the second-highest geographic differentiation after nonsynonymous variants, the limited functional annotation of malaria parasite noncoding regions restricts our understanding of noncoding-variant contributions to this process. Our initial analysis of ~1.3 million noncoding variants[8] (trimming off ~1.4 noncoding loci with low

Low_VQSLOD provided by MalariaGEN) indicates that noncoding variants with high levels of geographic differentiation (global $F_{ST} > 0.1$) are located closer to their nearest gene (Fig. 5A, Wilcoxon test $p < 2.2e-16$ compared with genomic variants showing global $F_{ST} < 0.1$), suggesting that the differentiation process at the noncoding region may partly be due to the selection of regulatory activity. We used MalariaSED to predict the impact of chromatin profiles of noncoding variants (Supplement Figure 4, Supplement Table 4). We observed significant alteration of chromatin profiles in the group of genomic variants presenting strong geographic differentiation (Fig 5B, Wilcoxon test $p < 2.2e-16$ compared with noncoding variants showing limited geographic differentiation). The higher chromatin effects observed at these geographically differentiated variants indicate that *P. falciparum* may use epigenetic mechanisms to locally regulate the expression of genes of certain biological pathways to benefit its survival.

Drug resistance and red blood cell invasion-related pathways are associated with geographic differentiation in *P. falciparum* noncoding regions

We next investigated the biological pathways associated with strong geographic differentiation at noncoding regions by assigning variants to their nearest genes ($F_{ST} > 0.1$, distance to the nearest gene < 3 kb). Only the noncoding variants leading to significantly predicted chromatin profile changes (the top 1% of chromatin effects) were selected. Pathway enrichment analysis indicated that oxidative stress defense (S-nitrosylation and S-glutathionylation), proteasome degradation (ubiquitin-protein conjugates) and food vacuoles were associated with noncoding variants predicted to cause significant chromatin accessibility changes in the ring and early trophozoite stages (Fig. 6A, B, Supplement table 5 for the complete list of enriched pathways). Other enriched pathways included protein transport (PTEX export), epigenetic modifications and the palmitome. Notably, most of these pathways have been previously demonstrated to be associated with altered transcription profiles in ART-R parasites[34,35]. Our results suggest that geographically diverse noncoding variants may enable the parasite to modulate the transcription of key pathways underlying the artemisinin response, ultimately allowing survival under drug pressure. Interrogating enriched genes around each geographically differentiated variant leading to binding affinity changes for the TFs PfBDP1, PfAP2-G and PfAP2-I (Fig. 6C-E), we identified a group of genes related to merozoite invasion, including *EBA181, AARP, RhopH2, RhopH3* and *SRA, RON2, MSP9, RAP1*. PfBDP1 is a chromatin-associated protein responsible for regulating the expression of genes involved in red blood cell (RBC) invasion[7]. TF PfAP2-I has been shown to associate with PfBDP1 and positively regulate the transcription of invasion-

related genes[5]. As a known pivotal activator of early gametocyte genes, PfAP2-G was also recently reported to have a potential role in regulating genes important for RBC invasion[6]. *P. falciparum* strains present broad diversity in invasion phenotypes and gene expression across populations [36]. The TF binding affinity changes surrounding noncoding variants upstream of invasion-related genes may explain how parasites maintain this diversity of gene expression.

Significant chromatin accessibility changes surround artemisinin resistance-related eQTLs

The large-scale Expression quantitative trait loci (eQTL) and Transcriptome-wide association studies (TWAS) study conducted by Tracking Resistance to Artemisinin Collaboration I (TRACI) identified more than 13,000 SNS-expression linkages in 773 parasite isolates from the Greater Mekong subregion (GMS) [1]. The result provides a new perspective to understand ART-R associated with SNSs through the unidirectional flow of information from sequence changes to consequent gene expression changes and, ultimately, phenotype alteration. However, the extensive gaps in our understanding of genomic changes and the resulting transcriptional alterations in malaria parasites impede the use of TWAS. Considering the correlations between chromatin accessibility and neighboring gene activity[29], we reasoned that the surrounding chromatin profile changes resulting from eQTLs would lead to gene transcription changes. To test the associations between the eQTL and its surrounding chromatin profiles, we investigated the capacity of different chromatin profile effects to distinguish local intergenic eQTLs from SNS reported in MalariaGEN[8]. Our results show that chromatin accessibility in rings outperforms other epigenetic markers in different parasite stages as an indicator/predictor of eQTLs (auROC = 0.7, Fig. 7A, Supplement table 6), consistent with skewness toward the ring-stage parasites collected in the TRACI study[1]. We further tested this on a group of local eQTLs linked with ART-R by regulating ART-R-related gene expression (see Methods). Ring-stage chromatin accessibility also presented the highest predictive performance in identifying ART-R-associated eQTLs (auROC = 0.73, Fig. 7B), indicating that transcriptional regulatory activity changes may lead to neighboring gene transcriptional alterations associated with ART-R phenotypes. Taken together, these results demonstrate that MalariaSED is informative for predicting the transcriptional impact of common noncoding variants in *P. falciparum*, such as eQTLs.

Multiple chromatin profile alterations at the schizont stage show signatures of intergenic selection

Previous analysis of single-cell sequencing data revealed strong evidence that *de novo* mutations are under selection in *P. falciparum* populations[37]. This evidence includes the skewness toward protein-coding regions, high nonsynonymous vs. synonymous substitution ratio, and elevated expression levels of *de novo* mutations-targeted genes. Because of limited functional annotation of *P. falciparum* intergenic regions, this analysis was focused on coding regions. We used MalariaSED to investigate the chromatin effects of the 35 noncoding *de novo* mutations reported in the SCS study. Compared with the genome-wide background distribution generated from all SNSs reported in MalariaGEN[8], significant changes were observed in schizonts, including chromatin accessibility, H3K9ac and two TFs, PfAP2-I and PfAP2-G binding affinities (Fig 8, Wilcoxon test $p < 0.05$). Our results show signatures of selection for intergenic variants in schizonts, which could result in neighboring gene expression changes.

Discussion

This work presents the first sequence-based computational framework, MalariaSED, that learns regulatory sequence codes in malaria parasites. In malaria parasite genomes, MalariaSED outperforms two DL models previously developed for mammalian genomes, Enrofmer[21] and Basenji2[23]. The DL-based framework enables us to predict the chromatin effects of sequence alterations with single-nucleotide sensitivity, thereby providing a means to assess the functional relevance of noncoding variants. We demonstrated the accuracy of this approach using previously published ChIP–qPCR data from CRISPR/Cas9-generated mutations in AP2 TF-family binding motifs[6,32]. One failure case occurred at the *pfmsp5* promoter, which was not identified in PfAP2-I ChIP-seq but detected in the ChIP–qPCR experiment[5]. MalariaSED could not predict PfAP2-I binding in either the wild type or parasites carrying mutations at the PfAP2-I binding motif at the *pfmsp5* promoter. We reason that some DNA sequence patterns may be related to discrepancies between the ChIP–qPCR and ChIP-Seq results. This DNA sequence pattern is reflected at the *pfmsp5* promoter and cannot be captured by MalariaSED trained in ChIP-seq data. We also demonstrated the significant contributions of previously identified DNA motifs to known TF binding, such as the members in the PfAP2 family, PfBDP1, PbAP2-O and PbAP2-G2[5-7,30,31]. We are not clear the reasons for MalariaSED not capturing previously reported PfAP2-G5 binding motifs, although it achieves prediction performance auROC > 0.95 and validates previous CRISPR/Cas9 results. One explanation is that MalariaSED may use DNA patterns where nucleotides are intermittently arranged for AP2-G5 binding predictions.

We used MalariaSED to explore the epigenetic effects of large amounts of noncoding variants released from MalariaGEN partner studies[38]. The results indicate that noncoding

variants that acquire high levels of geographic differentiation significantly likely impact the surrounding epigenetic profiles. This suggests that some noncoding regions have been at least in part subjected to natural selection, which is consistent with the previous analysis results on coding regions. It is unclear why all epigenetic markers present significant changes surrounding geographic differentiation variants. Further study is needed to understand the dependence of each chromatin profile. We speculate that other histone modifications may recruit some TFs and that the binding affinity alterations may result from these marker changes. Our reanalysis of ~1.3 million noncoding variants also provides new insight into parasite local geographical adaptation driven by noncoding variations. The associated potential transcriptional change in protein trafficking, epigenetic and translational machinery, and the alternations of many PfAP2-G binding affinities provide new evidence that parasites trade off reproduction (transmission to new hosts) and growth (within-host replication) to adapt to different local regions where parasites present diverse transmission intensity[39]. We need to point out that the PfAP2-G binding change regions tend to be located upstream of genes important for RBC invasion. This is further demonstrated by the overlapping gene list with PfAP2-I and PfBDP1, as well as the limited functional enrichment in sexual rings and stage I gametocytes for PfAP2-G binding site changes. We also observed that a group of geographic differentiation variants located at noncoding regions are likely to regulate gene functions in food vacuoles, oxidative stress defense, and proteasome degradation. This reflects the evolution of genetically distinct *P. falciparum* subpopulations that are thought to occur due to high drug pressure and oxidative stress[1,40].

To test the epigenetic contributions resulting from intergenic variants to transcriptional change, we used chromatin effects to discriminate reported eQTLs[1] against the nontrait-associated SNSs collected in MalariaGEN partner studies[38]. We observed high concordance between the chromatin accessibility change at the ring stage and the local eQTL discovered in *P. falciparum* clinical isolates. This result is also shown up in the subset of eQTLs linked with ART-R via transcriptional profiles. The agreement between the clinical isolate stage (high skewness toward rings) and the ring stage of the chromatin profile supports a previous study showing that the chromatin accessibility pattern correlates with neighboring gene expression values[29]. Furthermore, MalariaSED bridges the gap in our understanding between DNA sequence changes and the resulting epigenetic profile alterations in malaria parasites. Building on the known association between gene transcription and parasite phenotype, the directional flow of information from sequence variation to consequent chromatin profile change to gene transcription alternation and further to ART-R effect will allow us to extract the causality of

variant(s). Based on currently available chromatin profiles, we are not able to identify any chromatin profiles associated with distal eQTLs (auROC < 0.65 for all 13 chromatin profiles here). This may be due to limited epigenetic data available in *P. falciparum* and the fact that only one chromatin profile in rings can be implemented in MalariaSED. It will be interesting to study these distal eQTL hotspots when new epigenetic data are available for MalariaSED training.

MalariaSED facilitates the functional explanations of *de novo* mutations discovered in SCS studies. The significant *de novo* mutations enrichment in coding regions and the disproportionate genes targeted by *de novo* mutations suggested strong selection in the *P. falciparum* population collected in Chikhwawa, Malawi[37,41]. This includes the ApiAP2 gene family, implying selection in the epigenetic regulation mechanism. We used *de novo* mutations to investigate the chromatin effects of intergenic *de novo* mutations and identified significant changes in schizonts, including H3K9ac, PfAP2-G and PfAP2-I binding affinities, as well as chromatin accessibility. However, due to the limited number of intergenic *de novo* mutations in the single cell sequencing study, we are hesitant to make conclusions of potential functions associated with these 35 noncoding *de novo* mutations. Further study is required when more single cell sequencing datasets are generated.

In the long run, we expected that sequence-based regulation analysis would become a crucial part of malaria parasite research, especially to help unveil the regulatory activity of noncoding genomic regions, which are currently poorly understood in malaria parasites. Such analyses could, in the future, be implemented to prioritize noncoding variants potentially perturbing gene expression contributing to parasite immune escape and drug resistance. With the increasing data availability of functional variants, the approach can be readily trained, adapted, and further improved.

Methods

Datasets for MalariaSED development

The previously published ATAC-Seq datasets[29], nine ChIP-seq datasets, including PfAP2-I[5], PfAP2-G[6], PfAP2-G5[32] and PfBDP1[7], from *P. falciparum* RBCs to the gametocyte stage and two ChIP-Seq datasets for PbAP2-O and PbAP2-G2[30,31] in *P. berghei* were used to develop the MalariaSED framework. The whole genome is divided into bins of 200 bp, yielding more than 50,000 bins. A bin is labeled "positive" if more than 60% of it overlaps with ChIP or ATAC peaks, while the remaining bins are used as negative datasets. We used the ratio 7/1.5/1.5 to divide all

extracted bins into training, test, and validation sets. The number of bins used for MalariaSED training for different epigenetic markers is listed in supplement table 2.

Deep neural network models with long short-term memory (LSTM) layers

For each bin 200 bp in length, we extended it by 400 bp in both directions, resulting in a 1 kb input sequence. We used one-hot encoding to convert each 1 kb sequence into a 1000 x 4 bit matrix, where '1' represents a specific nucleotide present and '0' does not. The architecture of MalariaSED framework is shown in Fig. 1A. Two 1D convolutional layers followed up with max pooling and drop-out layers are combined with bidirectional LSTM to capture dependencies of DNA sequence patterns contributing to specific epigenetic markers. Regularization and constraint are applied to all layers to minimize overfitting. After the flatten layer, the final predictions are computed through sigmoid activation in dense layers. The Adam optimizer was used to search for model parameters based on weighted binary cross-entropy. We use early stopping to seize the parameter optimization process if there is no improvement in validation auPRC for ten consecutive epochs. Bayesian optimization[42] was used to optimize the hyperparameters.

Sequence pattern contribution to MalariaSED

To estimate the sequence contributions to MalariaSED predictions, we predict chromatin profile changes by generating all possible SNSs along with each site of the k-mer sequence. Each 200 bp region predicted as positive from MalariaSED was extended by 400 bp in both directions, resulting in a 1 kb sequence window. SNS generation was then performed for every positive 1 kb bp region covering all 4-mer or 5-mer sequences. For each 4/5-mer sequence, we took the average chromatin effects resulting from all SNSs as the contribution to MalariaSED. The effect of an SNS on a specific chromatin marker was measured by the absolute value of log2-fold change of odds predicted from MalariaSED. This measurement was also successfully utilized to evaluate the chromatin effects of genomic variants in the human genome[24,43].

Functional enrichment for geographic differential variants at intergenic regions

The intergenic variants presenting high geographic differential (Fst > 0.1) from MalariaGEN partner studies[38] were selected for this analysis. We further picked up the geographic differential variants predicted to change the particular chromatin profile at the top 1% level to explore the potential biological function change. A gene is used for functional enrichment analysis if its distance to at least one selected variant uses the abovementioned criteria shorter than 3 kb. To

identify GO terms enriched in the gene list of interest, Fisher's exact test was used to test whether more genes with this GO term were observed compared with the background comprising the whole genome.

Chromatin profile prediction surrounding eQTLs

We downloaded 13,257 eQTLs from a previous TRACI study[1]. Only local intergenic eQTLs (15 kb between an eQTL and its regulated target(s)) were selected for the positive dataset in DL predictions. We strengthened the ART-R-associated eQTLs by the cutoff of an absolute value of 0.1 for the adjusted Spearman correlation between the eQTL-regulated transcriptional profile and parasite clearance time. All SNSs gathered in MalariaGene[8] are used as the negative dataset. Each reported eQTL or SNS is extended to include the surrounding 1 kb DNA sequence to fit the input requirement of MalariaSED. The chromatin effects of each variant were measured by the log2-fold change difference of odds, which was previously used in human genetic studies[43].

Data and code availability

The data supporting the findings of the study are available within the paper and its supplementary information files. The MalariaSED web portal for 15 epigenetic profile predictions for malaria parasites is at malariased.charleywanglab.org. The source code for running and training MalariaSED models is available at https://github.com/CharleyWang/MalariaSED.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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FIGURE LEGENDS

Figure 1. MalaiaSED accurately predicts chromatin profiles in malaria parasites. A. The general framework of the deep learning model. The input DNA sequence is encoded into the four rows matrix by one hot encoder, where each element represents the appearance of a nucleotide at a specific location. The following two convolutional layers capture cumulative effects of short sequence patterns by analogy to motif scan. The bidirectional LSTM layer follows up to summarize long-term dependencies between captured DNA patterns. Outputs are fed into the flatten and full connection layers. We calculate the final score by dense layer with sigmoid activation. **B.** Performances of the deep learning framework are measured by AUC in multiple experimental epigenetic profiles in *P. falciparum* and *P. berghei*.

Figure 2. MalariaSED can predict the binding dynamic of PfAP2-G throughout commitment and early gametocytogenesis. High agreement between log2-transformed PfAP2-G ChIP/input ratio and predicted genomic tracks from MalariaSED over a region of chromosome 10. The PfAP2-G ChIP intensity covers parasite stage committed schizonts, sexual ring and stage I gametocyte.

Figure 3. PfAP2-G5 and PfAP2-G binding affinity predicted from MalariaSED are consistent with previous published ChIP-qPCR results. A. PfAP2-G5 ChIP-Seq intensity along the promoter region of *ap2-g* during Trophozoits stage. ChIP-seq results come from a recent study[32]. The flanking region [-100,+100] bp of each mutation on PfAP2-G5-binding sites is highlighted as S1 (dark red), S2 (Black), and S3 (Brown). **B**. The binding affinity predicted

from MalariaSED is consistent with the recently published ChIP-qPCR results[32].The height of each bar indicates the predicted binding affinity of PfAP2-G5 in three mutants highlighted in A. **C**. PfAP2-G ChIP-Seq intensity[6] along the promoter region of *ap2-g* in early sexual rings. The S4 (Dark yellow) indicates flanking region [-100,+100]bp of the mutation of the PfAP2-G motif. **D**. Similar to B, but showing the predicted binding affinity of the mutant S4 in C. The results are in concordance with the published ChIP-qPCR result[6] that PfAP2-G is not able to bind in the mutant.

Figure 4. TFs binding effects of genomic variants predicted by the DL framework demonstrate the contribution of previously identified sequence motif. A. Potential single-nucleotide substitutions (SNSs) at reported pfAP2-G motif, GTRC (committed schizonts), GTAC (sexual rings and stage I gametocytes) significantly reduce the binding affinity of PfAP2-G. Comparisons are between reported PfAP2-G motifs and all 4-mers (ALL) appeared on predicted ApAP2-G binding sites. **B.** The PfAP2-I binding effects of base substitutions at the reported motif GTGCA is significant compared with all 5-mers (ALL) in *P. falciparum* genome during schizonts. The other two motifs (GGTCG and CTTGC) from PfAP2-I binding domains 1 and 2 are not able to show significantly reduced PfAP2-I affinity, which is consistent with experimental results[5]. **C.** The significantly reduced binding affinity of SNSs at reported PfBDP1 binding motif GTGCA in schizonts support the previous conclusion that PfAP2-I and PfBDP1 may form a protein complex to bind DNA. However, SNSs at GTGCA motif are not likely to disrupt PfBDP1 binding at trophozoite stage. **D,E.** Single-nucleotide substitutions at two reported binding motifs in *P. Berghei* significantly weaken PbAP2-G2 and PbAP2-O binding sites. '***' indicates Wilcoxon test compared with whole-genome background $p < 2.2e-16$.

Figure 5. Noncoding variants with high levels of geographic differentiation tend to alter their surrounding chromatin profiles. A. Non-coding variants strongly differentiated at the global level are closer to their neighboring genes. We used global Fst > 0.1 to identify variants with high levels of geographic differentiation. '***' indicates Wilcoxon test compared with remaining non-coding variants, $p < 2.2e-16$. **B.** Non-coding variants acquired high geographic differentiation (Fst > 0.1) are predicted to bring in significant epigenetic changes at their +/-100bp surrounding regions.

Figure 6. The enriched functions or pathways upon noncoding variants with strong geographic differentiation. The height of yellow bar indicates the number of genes assigned

to noncoding variants that are strongly geographically differentiated and significantly change the chromatin profile. To estimate the number of genes expected to observe for each function or pathway term, the gene fraction in *P. falciparum* genome presenting the specific functional term is multiplied by the number of assigned genes to geographic differential variants exhibiting the high chromatin effects. (Hypergeometric test, FDR-corrected P-value < 0.1, check supplement table X for full list).

Figure 7. Chromatin accessibility alternation is surrounding previously identified eQTLs in the TRACI study. A. The chromatin effects of reported eQTLs in the TRACI study are used to discriminate them from all SNPs gathered in Pf3K. **B.** The eQTLs linked with ART-R associated transcriptions were also investigated. The eQTL prediction performance of different chromatin profiles is measured by AUC.

Figure 8. The results from MalariaSED indicates de novo mutations at non-coding regions discovered from the single cell study have higher chance to alter their surrounding chromatin profiles in schizonts. '*' indicates Wilcoxon test compared with non-coding SNPs collected from MalariaGEN, p < 0.05.

SUPPLEMENTARY FIGURE LEGENDS

Supplement figure S1. The same DL architecture as MalariaSED was trained on 200, 700, 1000 and 1400 bp DNA sequences, respectively. We present the auROC of each model as a point and use a box plot to show the distribution of the models inputting the same DNA sequence length. The results indicate 1000bp input outperforms other length input ('***' indicates Wilcoxon test p < 0.01 compared between the 1kb input sequence and any other length listed here).

Supplement figure S2. The prediction performance between MalariaSED and Enformer. We present the auROC of each model as a point and use a box plot to show the distribution of the models. We used 1280bp DNA sequence as the input to match the requirement of Enformer. ('***' indicates Wilcoxon test p < 0.01).

Supplement figure S3. TFs binding effects of single nucleotide substitutions at 5-mers sequences predicted by MalariaSED in *P. falciparum.* '***' indicates Wilcoxon test compared with whole-genome background p < 2.2e-16.

Supplement figure S4. MalariaSED prediction for epigenetic markers of ~1.3 millions variants gathered by Pf3K. The horizontal and vertical direction represent, respectively, the predicted probabilities that the sequences carrying the reference allele and alternative allele.

Supplement figure S5. The MalariaSED prediction results for de novo mutations at non-coding regions discovered from the single cell study. '*' indicates Wilcoxon test compared with non-coding SNPs collected from MalariaGEN, $p < 0.05$.

SUPPLEMENTARY TABLES LEGENDS

Supplement table S1. MalariaSED model configuration.

Supplement table S2. The prediction performance of MalariaSED in the independent test sets.

Supplement table S3. The average SNS effects at each DNA motif through all predicted TF binding sites.

Supplement table S4. The chromatin effects prediction from MalariaSED for all reported genomic variants collected by malariaGEN.

Supplement table S5. The GO and MPMP enrichment for geographically differentiated variants presenting high chromatin effects.

Supplement table S6. The chromatin effects prediction from MalairaSED for local eQTLs reported in TRACKI study.

References

1 Zhu, L. *et al.* The origins of malaria artemisinin resistance defined by a genetic and transcriptomic background. *Nat Commun* **9**, 5158, doi:10.1038/s41467-018-07588-x (2018).

2 Gibbons, J. *et al.* Altered expression of K13 disrupts DNA replication and repair in Plasmodium falciparum. *BMC Genomics* **19**, 849, doi:10.1186/s12864-018-5207-7 (2018).

3 Wang, C., Adapa, S. R., Gibbons, J., Sutton, S. & Jiang, R. H. Punctuated chromatin states regulate Plasmodium falciparum antigenic variation at the intron and 2 kb upstream regions. *BMC Genomics* **17**, 652, doi:10.1186/s12864-016-3005-7 (2016).

4 Swamy, L., Amulic, B. & Deitsch, K. W. Plasmodium falciparum var gene silencing is determined by cis DNA elements that form stable and heritable interactions. *Eukaryot Cell* **10**, 530-539, doi:10.1128/EC.00329-10 (2011).

5 Santos, J. M. *et al.* Red Blood Cell Invasion by the Malaria Parasite Is Coordinated by the PfAP2-I Transcription Factor. *Cell Host Microbe* **21**, 731-741 e710, doi:10.1016/j.chom.2017.05.006 (2017).

562 6 Josling, G. A. *et al.* Dissecting the role of PfAP2-G in malaria gametocytogenesis. *Nat*
563 *Commun* **11**, 1503, doi:10.1038/s41467-020-15026-0 (2020).
564 7 Josling, G. A. *et al.* A Plasmodium Falciparum Bromodomain Protein Regulates Invasion
565 Gene Expression. *Cell Host Microbe* **17**, 741-751, doi:10.1016/j.chom.2015.05.009
566 (2015).
567 8 MalariaGen *et al.* An open dataset of Plasmodium falciparum genome variation in 7,000
568 worldwide samples. *Wellcome Open Res* **6**, 42, doi:10.12688/wellcomeopenres.16168.2
569 (2021).
570 9 Cerqueira, G. C. *et al.* Longitudinal genomic surveillance of Plasmodium falciparum
571 malaria parasites reveals complex genomic architecture of emerging artemisinin
572 resistance. *Genome Biol* **18**, 78, doi:10.1186/s13059-017-1204-4 (2017).
573 10 Wang, Z. *et al.* Genome-wide association analysis identifies genetic loci associated with
574 resistance to multiple antimalarials in Plasmodium falciparum from China-Myanmar
575 border. *Sci Rep* **6**, 33891, doi:10.1038/srep33891 (2016).
576 11 Takala-Harrison, S. *et al.* Independent emergence of artemisinin resistance mutations
577 among Plasmodium falciparum in Southeast Asia. *The Journal of infectious diseases* **211**,
578 670-679, doi:10.1093/infdis/jiu491 (2015).
579 12 Wendler, J. P. *et al.* A genome wide association study of Plasmodium falciparum
580 susceptibility to 22 antimalarial drugs in Kenya. *PLoS One* **9**, e96486,
581 doi:10.1371/journal.pone.0096486 (2014).
582 13 Borrmann, S. *et al.* Genome-wide screen identifies new candidate genes associated with
583 artemisinin susceptibility in Plasmodium falciparum in Kenya. *Sci Rep* **3**, 3318,
584 doi:10.1038/srep03318 (2013).
585 14 Miotto, O. *et al.* Multiple populations of artemisinin-resistant Plasmodium falciparum in
586 Cambodia. *Nat Genet* **45**, 648-655, doi:10.1038/ng.2624 (2013).
587 15 Van Tyne, D. *et al.* Identification and functional validation of the novel antimalarial
588 resistance locus PF10_0355 in Plasmodium falciparum. *PLoS Genet* **7**, e1001383,
589 doi:10.1371/journal.pgen.1001383 (2011).
590 16 Mu, J. *et al.* Plasmodium falciparum genome-wide scans for positive selection,
591 recombination hot spots and resistance to antimalarial drugs. *Nat Genet* **42**, 268-271,
592 doi:10.1038/ng.528 (2010).
593 17 Miotto, O. *et al.* Genetic architecture of artemisinin-resistant Plasmodium falciparum.
594 *Nat Genet* **47**, 226-234, doi:10.1038/ng.3189 (2015).
595 18 Mok, S. *et al.* Drug resistance. Population transcriptomics of human malaria parasites
596 reveals the mechanism of artemisinin resistance. *Science* **347**, 431-435,
597 doi:10.1126/science.1260403 (2015).
598 19 Pradhan, A. *et al.* Chemogenomic profiling of Plasmodium falciparum as a tool to aid
599 antimalarial drug discovery. *Sci Rep* **5**, 15930, doi:10.1038/srep15930 (2015).
600 20 Visscher, P. M. *et al.* 10 Years of GWAS Discovery: Biology, Function, and Translation.
601 *Am J Hum Genet* **101**, 5-22, doi:10.1016/j.ajhg.2017.06.005 (2017).
602 21 Avsec, Z. *et al.* Effective gene expression prediction from sequence by integrating long-
603 range interactions. *Nat Methods* **18**, 1196-1203, doi:10.1038/s41592-021-01252-x
604 (2021).

605 22 Zrimec, J. *et al.* Deep learning suggests that gene expression is encoded in all parts of a
606 co-evolving interacting gene regulatory structure. *Nat Commun* **11**, 6141,
607 doi:10.1038/s41467-020-19921-4 (2020).
608 23 Kelley, D. R. *et al.* Sequential regulatory activity prediction across chromosomes with
609 convolutional neural networks. *Genome Res* **28**, 739-750, doi:10.1101/gr.227819.117
610 (2018).
611 24 Zhou, J. *et al.* Deep learning sequence-based ab initio prediction of variant effects on
612 expression and disease risk. *Nat Genet* **50**, 1171-1179, doi:10.1038/s41588-018-0160-6
613 (2018).
614 25 Hollin, T. & Le Roch, K. G. From Genes to Transcripts, a Tightly Regulated Journey in
615 Plasmodium. *Front Cell Infect Microbiol* **10**, 618454, doi:10.3389/fcimb.2020.618454
616 (2020).
617 26 Callebaut, I., Prat, K., Meurice, E., Mornon, J. P. & Tomavo, S. Prediction of the general
618 transcription factors associated with RNA polymerase II in Plasmodium falciparum:
619 conserved features and differences relative to other eukaryotes. *BMC Genomics* **6**, 100,
620 doi:10.1186/1471-2164-6-100 (2005).
621 27 Campbell, T. L., De Silva, E. K., Olszewski, K. L., Elemento, O. & Llinas, M. Identification
622 and genome-wide prediction of DNA binding specificities for the ApiAP2 family of
623 regulators from the malaria parasite. *PLoS Pathog* **6**, e1001165,
624 doi:10.1371/journal.ppat.1001165 (2010).
625 28 Templeton, T. J. *et al.* Comparative analysis of apicomplexa and genomic diversity in
626 eukaryotes. *Genome Res* **14**, 1686-1695, doi:10.1101/gr.2615304 (2004).
627 29 Toenhake, C. G. *et al.* Chromatin Accessibility-Based Characterization of the Gene
628 Regulatory Network Underlying Plasmodium falciparum Blood-Stage Development. *Cell*
629 *Host Microbe* **23**, 557-569 e559, doi:10.1016/j.chom.2018.03.007 (2018).
630 30 Kaneko, I., Iwanaga, S., Kato, T., Kobayashi, I. & Yuda, M. Genome-Wide Identification of
631 the Target Genes of AP2-O, a Plasmodium AP2-Family Transcription Factor. *PLoS Pathog*
632 **11**, e1004905, doi:10.1371/journal.ppat.1004905 (2015).
633 31 Yuda, M., Iwanaga, S., Kaneko, I. & Kato, T. Global transcriptional repression: An initial
634 and essential step for Plasmodium sexual development. *Proc Natl Acad Sci U S A* **112**,
635 12824-12829, doi:10.1073/pnas.1504389112 (2015).
636 32 Shang, X. *et al.* A cascade of transcriptional repression determines sexual commitment
637 and development in Plasmodium falciparum. *Nucleic Acids Res* **49**, 9264-9279,
638 doi:10.1093/nar/gkab683 (2021).
639 33 Oehring, S. C. *et al.* Organellar proteomics reveals hundreds of novel nuclear proteins in
640 the malaria parasite Plasmodium falciparum. *Genome Biol* **13**, R108, doi:10.1186/gb-
641 2012-13-11-r108 (2012).
642 34 Rocamora, F. *et al.* Oxidative stress and protein damage responses mediate artemisinin
643 resistance in malaria parasites. *PLoS Pathog* **14**, e1006930,
644 doi:10.1371/journal.ppat.1006930 (2018).
645 35 Mok, S. *et al.* Artemisinin-resistant K13 mutations rewire Plasmodium falciparum's intra-
646 erythrocytic metabolic program to enhance survival. *Nat Commun* **12**, 530,
647 doi:10.1038/s41467-020-20805-w (2021).

36 Bowyer, P. W. *et al.* Variation in Plasmodium falciparum erythrocyte invasion phenotypes and merozoite ligand gene expression across different populations in areas of malaria endemicity. *Infect Immun* **83**, 2575-2582, doi:10.1128/IAI.03009-14 (2015).

37 Dia, A. *et al.* Single-genome sequencing reveals within-host evolution of human malaria parasites. *Cell Host Microbe* **29**, 1496-1506 e1493, doi:10.1016/j.chom.2021.08.009 (2021).

38 MalariaGen *et al.* An open dataset of Plasmodium falciparum genome variation in 7,000 worldwide samples. *Wellcome Open Res* **6**, 42, doi:10.12688/wellcomeopenres.16168.1 (2021).

39 Rono, M. K. *et al.* Adaptation of Plasmodium falciparum to its transmission environment. *Nat Ecol Evol* **2**, 377-387, doi:10.1038/s41559-017-0419-9 (2018).

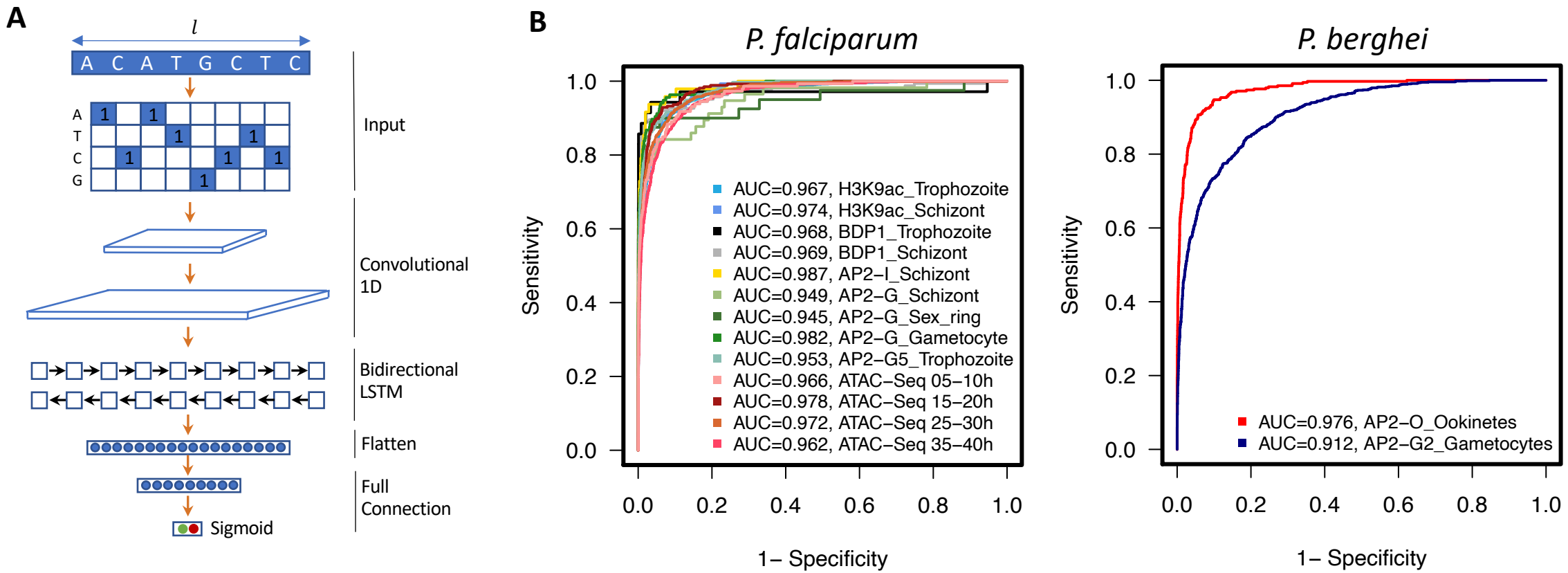
40 Amaratunga, C. *et al.* Artemisinin-resistant Plasmodium falciparum in Pursat province, western Cambodia: a parasite clearance rate study. *Lancet Infect Dis* **12**, 851-858, doi:10.1016/S1473-3099(12)70181-0 (2012).

41 Nkhoma, S. C. *et al.* Co-transmission of Related Malaria Parasite Lineages Shapes Within-Host Parasite Diversity. *Cell Host Microbe* **27**, 93-103 e104, doi:10.1016/j.chom.2019.12.001 (2020).

42 Snoek, J., Larochelle, H. & Adams, R. Practical bayesian optimization of machine learning algorithms. *arXiv.*, 1206.2944 (2012).

43 Zhou, J. & Troyanskaya, O. G. Predicting effects of noncoding variants with deep learning-based sequence model. *Nat Methods* **12**, 931-934, doi:10.1038/nmeth.3547 (2015).

Fig. 1


A
l
A C A T G C T C
A
T
C
G
Input
Convolutional 1D
Bidirectional LSTM
Flatten
Full Connection
Sigmoid
B
P. falciparum
Sensitivity
1– Specificity
AUC=0.967, H3K9ac_Trophozoite
AUC=0.974, H3K9ac_Schizont
AUC=0.968, BDP1_Trophozoite
AUC=0.969, BDP1_Schizont
AUC=0.987, AP2–I_Schizont
AUC=0.949, AP2–G_Schizont
AUC=0.945, AP2–G_Sex_ring
AUC=0.982, AP2–G_Gametocyte
AUC=0.953, AP2–G5_Trophozoite
AUC=0.966, ATAC–Seq 05–10h
AUC=0.978, ATAC–Seq 15–20h
AUC=0.972, ATAC–Seq 25–30h
AUC=0.962, ATAC–Seq 35–40h
P. berghei
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1– Specificity
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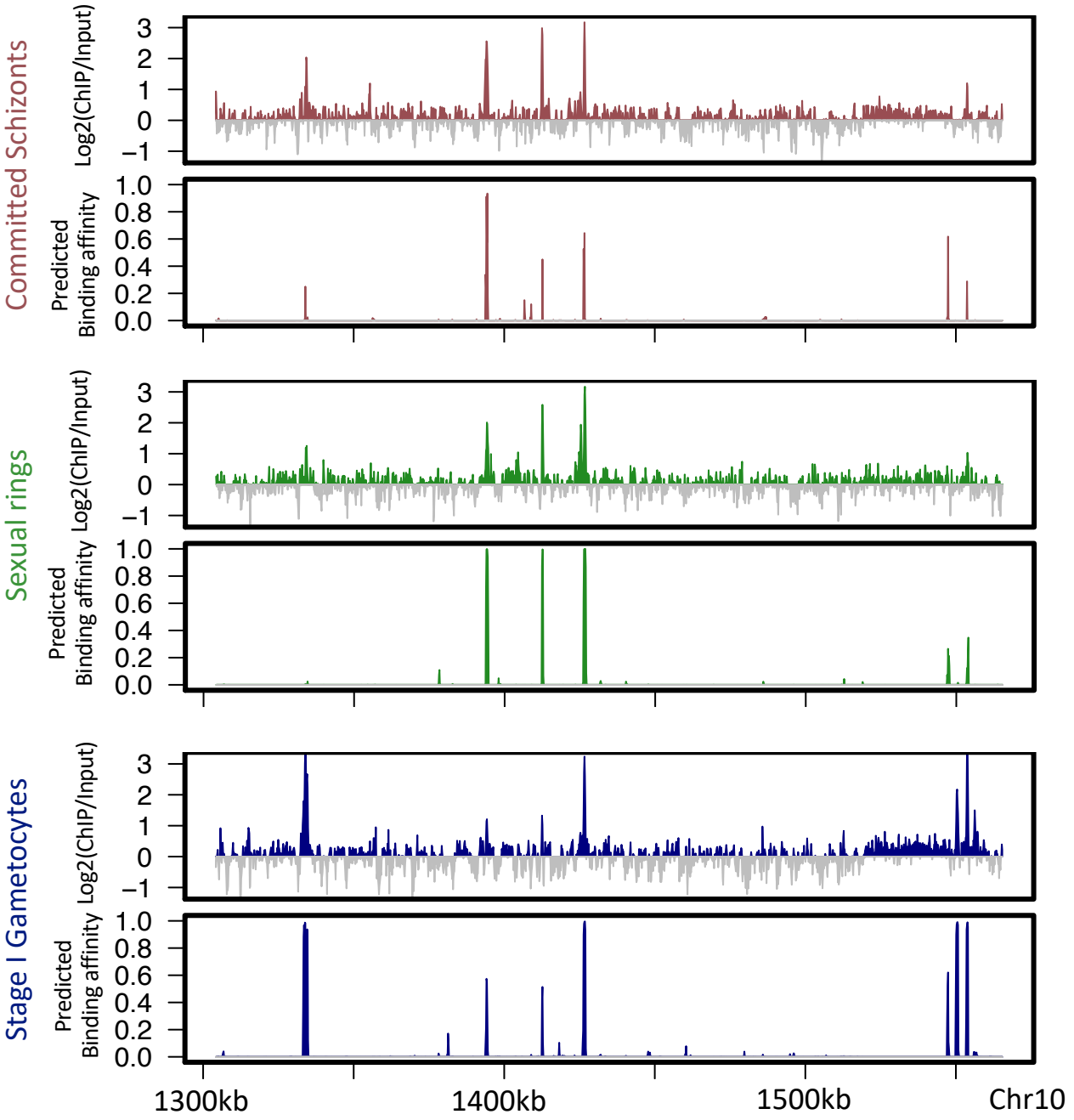
Fig. 2


Committed Schizonts
Sexual rings
Stage I Gametocytes
Log2(ChIP/Input)
Predicted Binding affinity
3
2
1
0
−1
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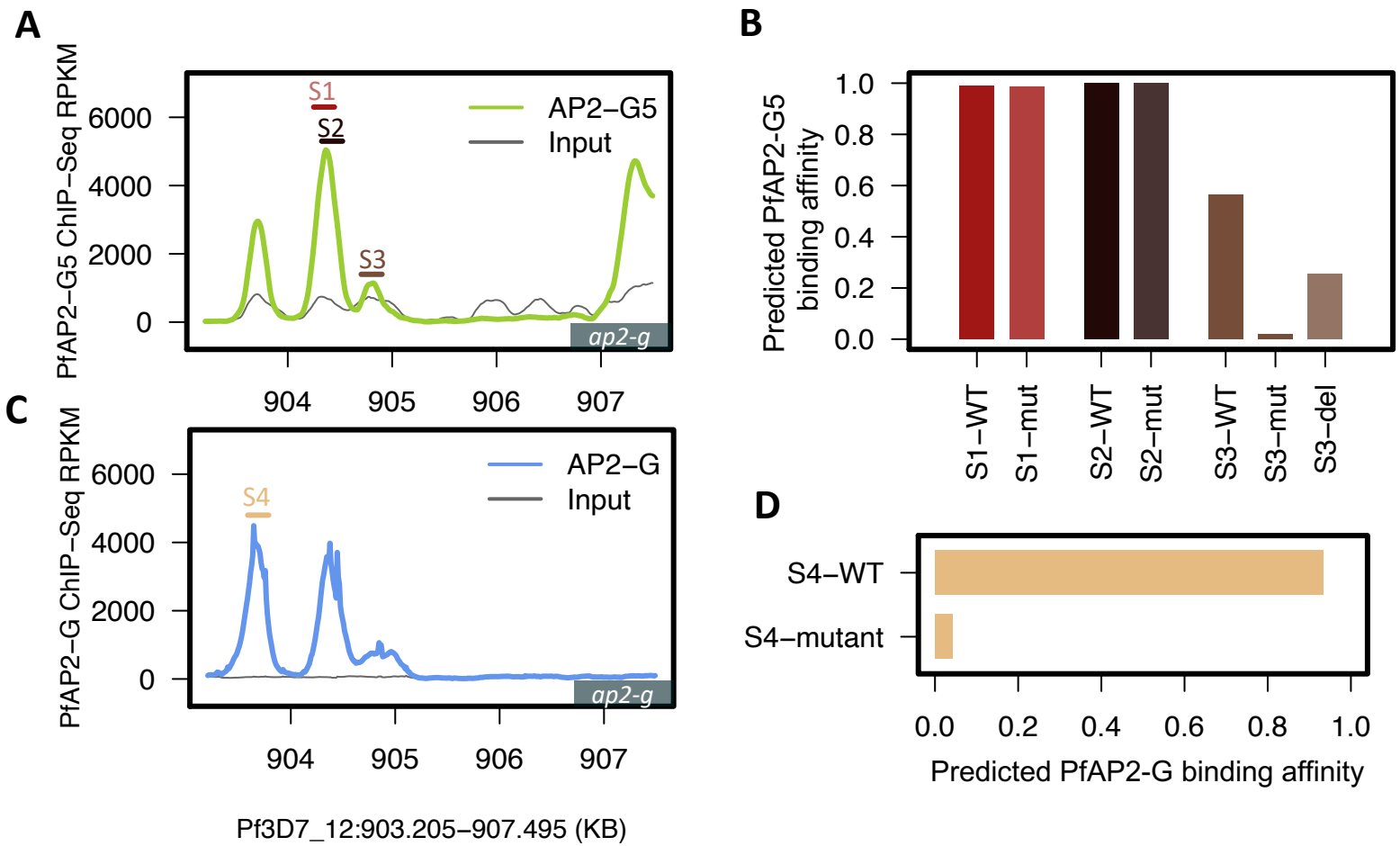
0.8
0.6
0.4
0.2
0.0
1300kb
1400kb
1500kb
Chr10

Fig. 3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4000
2000
0
S1
S2
S3
AP2–G5
Input
ap2-g
904
905
906
907
B
Predicted PfAP2-G5 binding affinity
1.0
0.8
0.6
0.4
0.2
0.0
S1–WT
S1–mut
S2–WT
S2–mut
S3–WT
S3–mut
S3–del
C
PfAP2–G ChIP–Seq RPKM
6000
4000
2000
0
S4
AP2–G
Input
ap2-g
904
905
906
907
Pf3D7_12:903.205–907.495 (KB)
D
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S4–mutant
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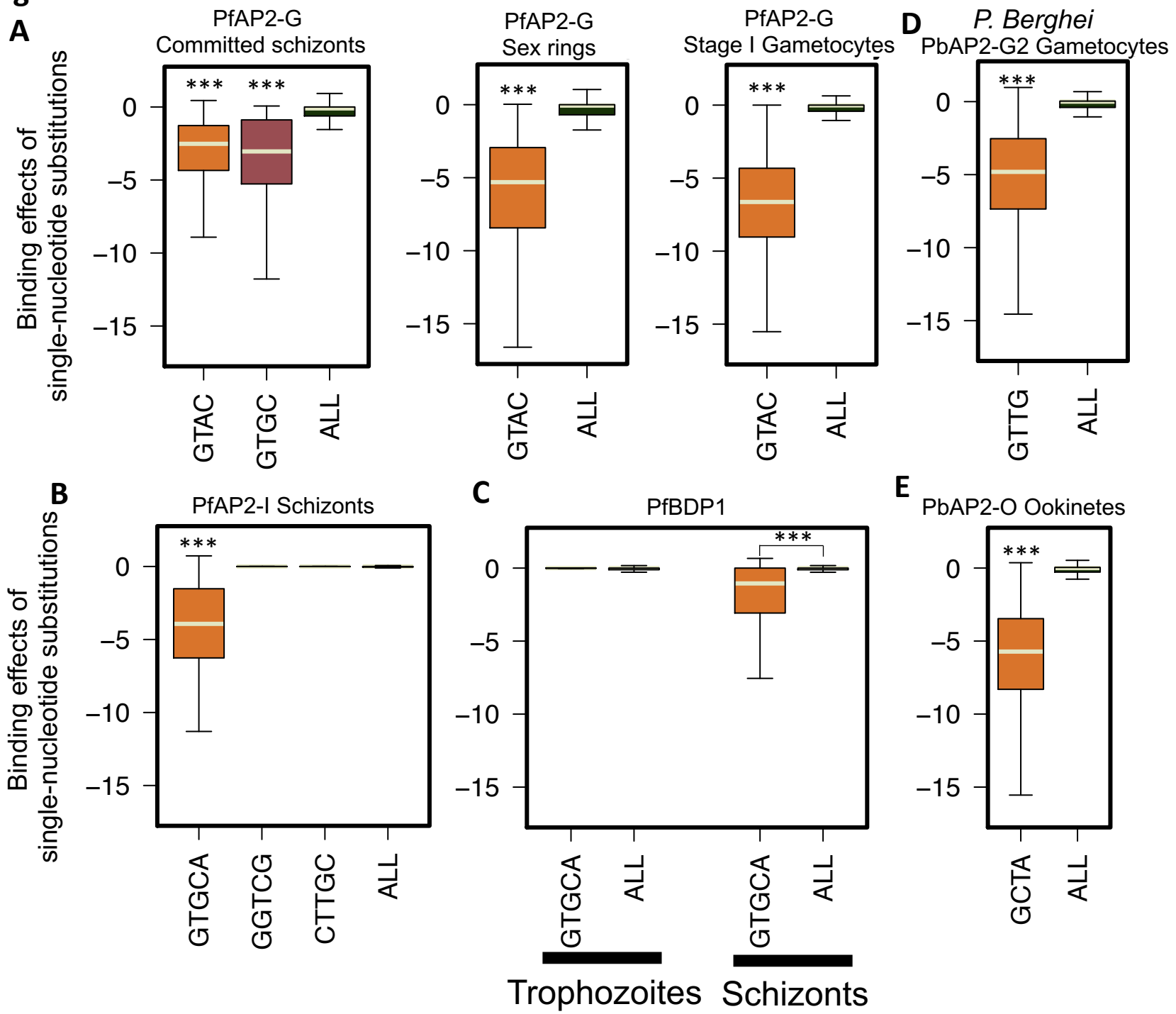
0.2
0.4
0.6
0.8
1.0
Predicted PfAP2-G binding affinity

Fig. 4


A
PfAP2-G
Committed schizonts
PfAP2-G
Sex rings
PfAP2-G
Stage I Gametocytes
D
P. Berghei
PbAP2-G2 Gametocytes
Binding effects of
single-nucleotide substitutions
***
0
−5
−10
−15
GTAC
GTGC
ALL
GTAC
ALL
GTAC
ALL
GTTG
ALL
B
PfAP2-I Schizonts
C
PfBDP1
E
PbAP2-O Ookinetes
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CTTGC
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GTGCA
ALL
GTGCA
ALL
GCTA
ALL
Trophozoites
Schizonts

Fig. 5

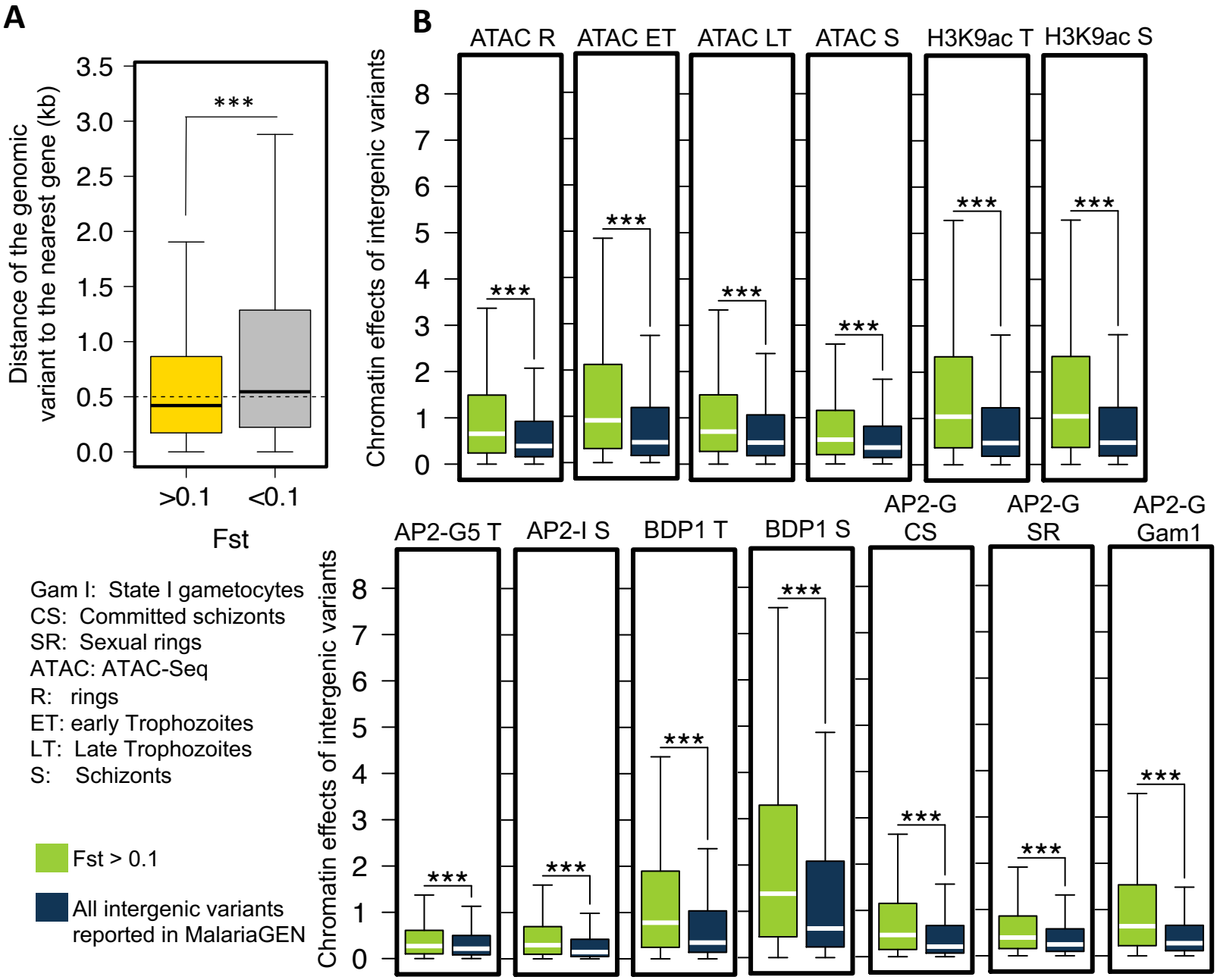
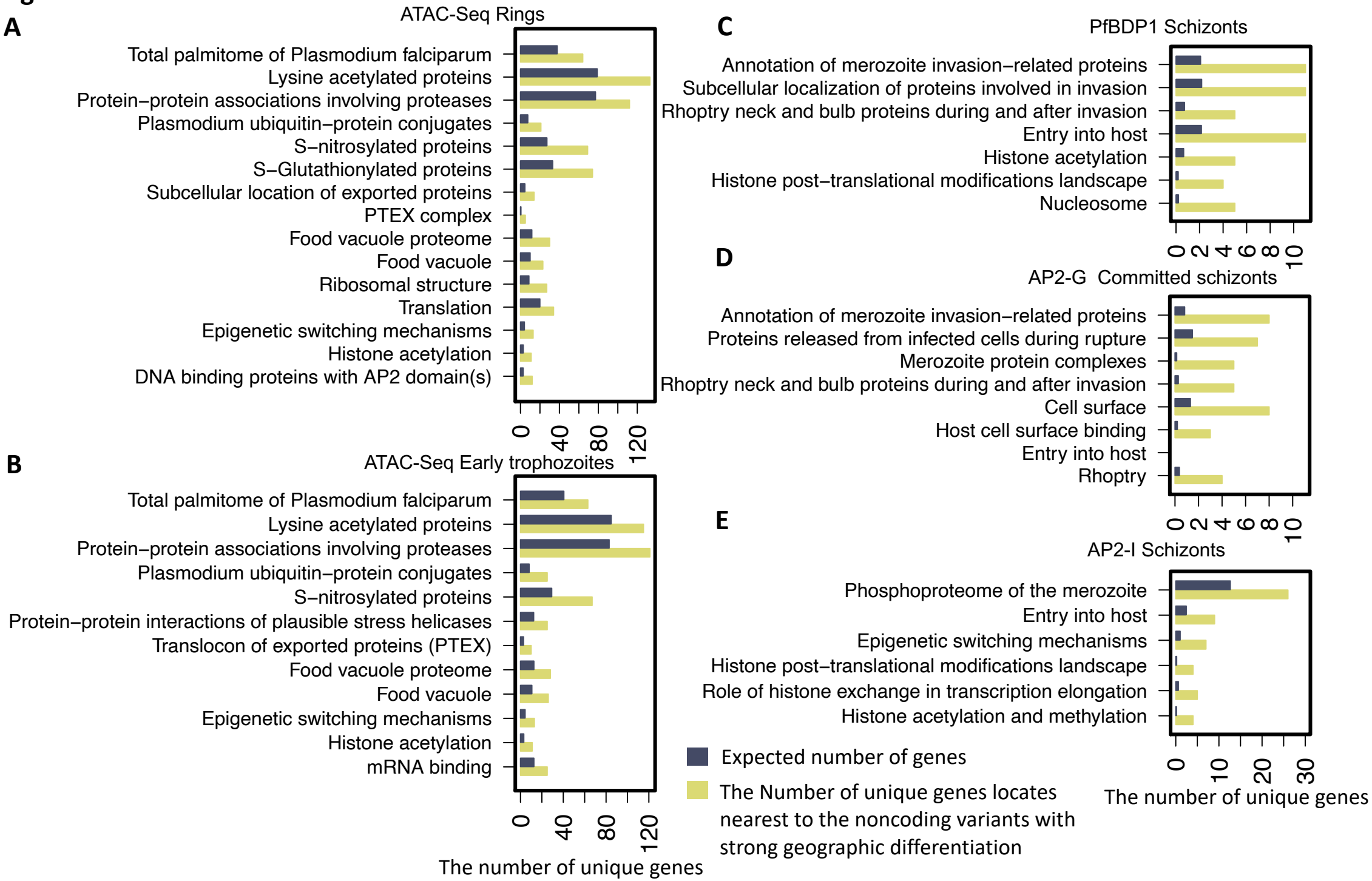

A
Distance of the genomic variant to the nearest gene (kb)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
***
>0.1
<0.1
Fst
B
Chromatin effects of intergenic variants
ATAC R
ATAC ET
ATAC LT
ATAC S
H3K9ac T
H3K9ac S
AP2-G5 T
AP2-I S
BDP1 T
BDP1 S
AP2-G CS
AP2-G SR
AP2-G Gam1
Gam I: State I gametocytes
CS: Committed schizonts
SR: Sexual rings
ATAC: ATAC-Seq
R: rings
ET: early Trophozoites
LT: Late Trophozoites
S: Schizonts
Fst > 0.1
All intergenic variants reported in MalariaGEN

Fig. 6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ATAC-Seq Rings
Total palmitome of Plasmodium falciparum
Lysine acetylated proteins
Protein–protein associations involving proteases
Plasmodium ubiquitin–protein conjugates
S–nitrosylated proteins
S–Glutathionylated proteins
Subcellular location of exported proteins
PTEX complex
Food vacuole proteome
Food vacuole
Ribosomal structure
Translation
Epigenetic switching mechanisms
Histone acetylation
DNA binding proteins with AP2 domain(s)
0
40
80
120
B
ATAC-Seq Early trophozoites
Total palmitome of Plasmodium falciparum
Lysine acetylated proteins
Protein–protein associations involving proteases
Plasmodium ubiquitin–protein conjugates
S–nitrosylated proteins
Protein–protein interactions of plausible stress helicases
Translocon of exported proteins (PTEX)
Food vacuole proteome
Food vacuole
Epigenetic switching mechanisms
Histone acetylation
mRNA binding
0
40
80
120
The number of unique genes
C
PfBDP1 Schizonts
Annotation of merozoite invasion–related proteins
Subcellular localization of proteins involved in invasion
Rhoptry neck and bulb proteins during and after invasion
Entry into host
Histone acetylation
Histone post–translational modifications landscape
Nucleosome
0
2
4
6
8
10
D
AP2-G Committed schizonts
Annotation of merozoite invasion–related proteins
Proteins released from infected cells during rupture
Merozoite protein complexes
Rhoptry neck and bulb proteins during and after invasion
Cell surface
Host cell surface binding
Entry into host
Rhoptry
0
2
4
6
8
10
E
AP2-I Schizonts
Phosphoproteome of the merozoite
Entry into host
Epigenetic switching mechanisms
Histone post–translational modifications landscape
Role of histone exchange in transcription elongation
Histone acetylation and methylation
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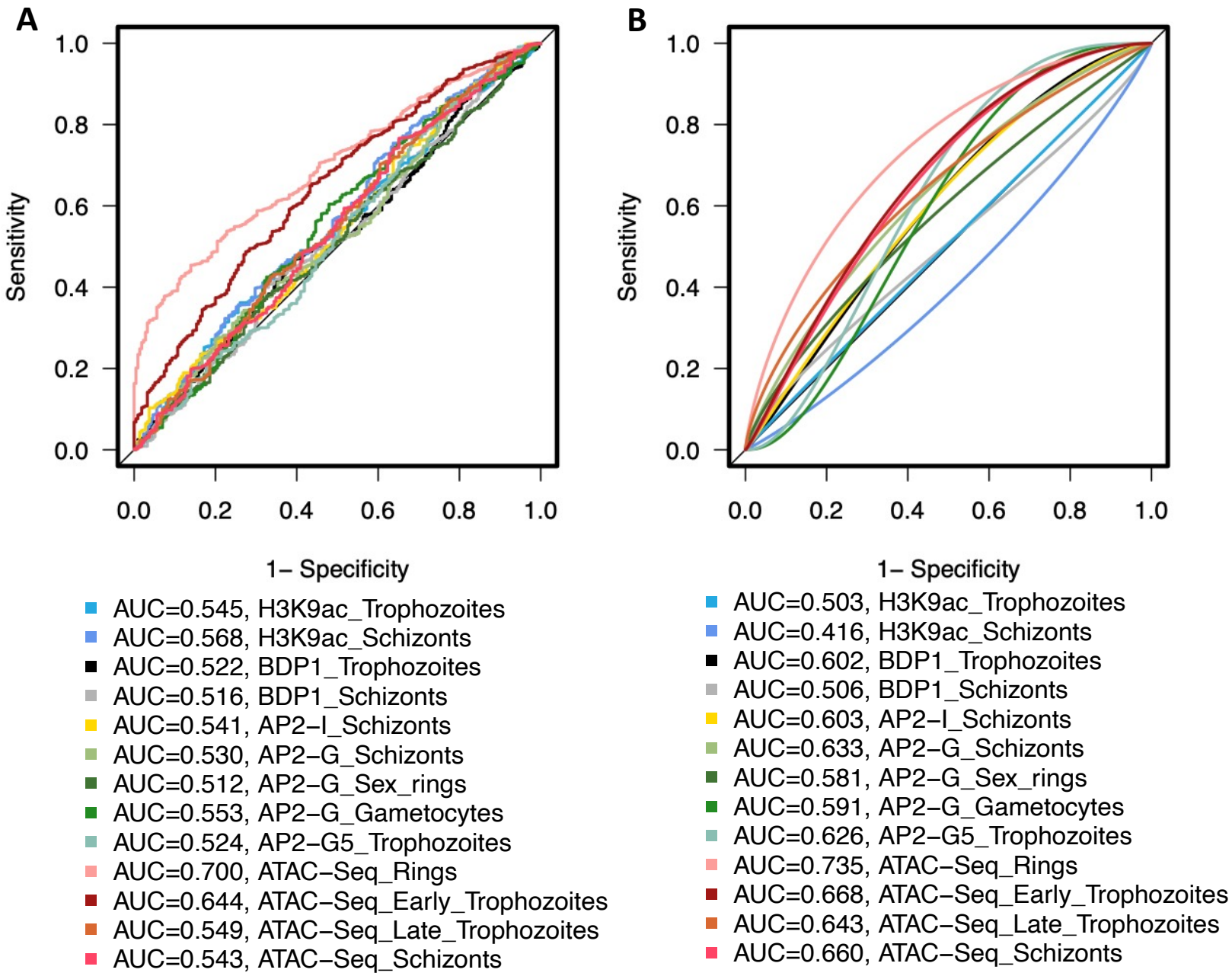
10
20
30
The number of unique genes
Expected number of genes
The Number of unique genes locates nearest to the noncoding variants with strong geographic differentiation

Fig. 7


A
B
Sensitivity
1– Specificity
0.0
0.2
0.4
0.6
0.8
1.0
AUC=0.545, H3K9ac_Trophozoites
AUC=0.568, H3K9ac_Schizonts
AUC=0.522, BDP1_Trophozoites
AUC=0.516, BDP1_Schizonts
AUC=0.541, AP2–I_Schizonts
AUC=0.530, AP2–G_Schizonts
AUC=0.512, AP2–G_Sex_rings
AUC=0.553, AP2–G_Gametocytes
AUC=0.524, AP2–G5_Trophozoites
AUC=0.700, ATAC–Seq_Rings
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AUC=0.549, ATAC–Seq_Late_Trophozoites
AUC=0.543, ATAC–Seq_Schizonts
AUC=0.503, H3K9ac_Trophozoites
AUC=0.416, H3K9ac_Schizonts
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AUC=0.506, BDP1_Schizonts
AUC=0.603, AP2–I_Schizonts
AUC=0.633, AP2–G_Schizonts
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AUC=0.591, AP2–G_Gametocytes
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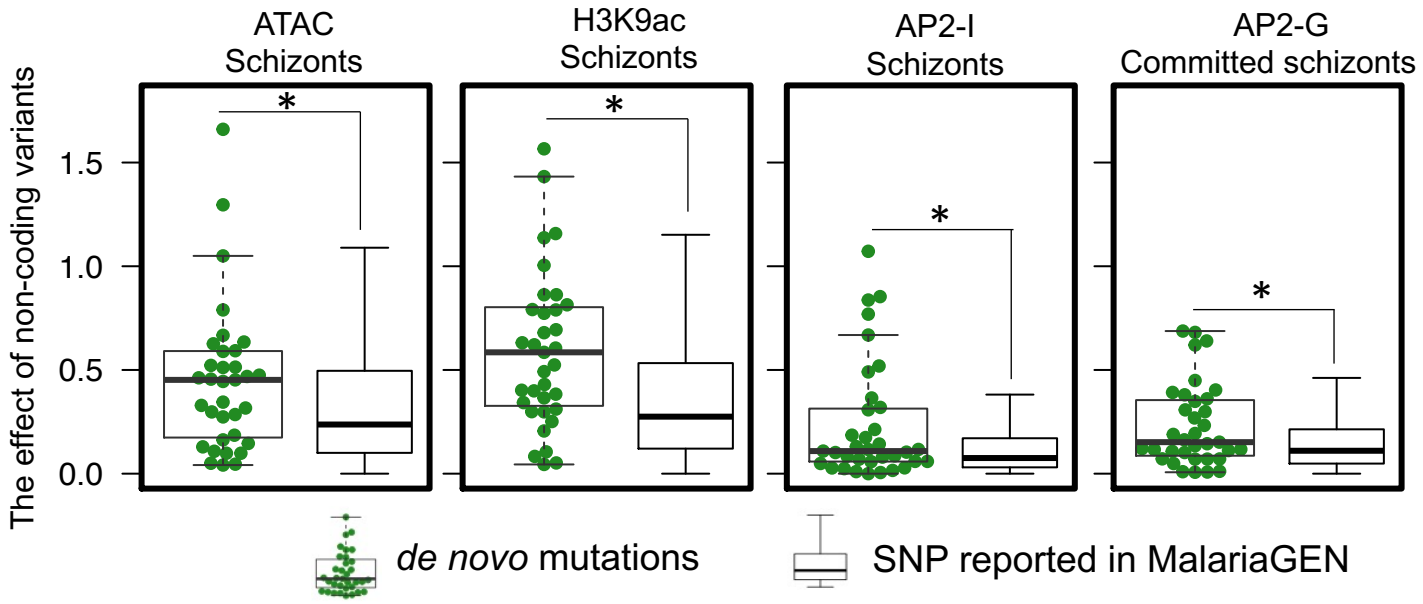
AUC=0.660, ATAC–Seq_Schizonts

Fig. 8


ATAC
Schizonts
H3K9ac
Schizonts
AP2-I
Schizonts
AP2-G
Committed schizonts
The effect of non-coding variants
1.5
1.0
0.5
0.0
*
*
*
*
de novo mutations
SNP reported in MalariaGEN