



HAL
open science

Comparative proteomics uncovers low asparagine content in Plasmodium tRip-KO proteins

Martina Pitolli, Marta Cela, Delphine Kapps, Johana Chicher, Laurence Despons, Magali Frugier

► **To cite this version:**

Martina Pitolli, Marta Cela, Delphine Kapps, Johana Chicher, Laurence Despons, et al.. Comparative proteomics uncovers low asparagine content in Plasmodium tRip-KO proteins. IUBMB Life, In press, 10.1002/iub.2891 . hal-04645678

HAL Id: hal-04645678

<https://hal.science/hal-04645678v1>

Submitted on 11 Jul 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 This is the pre-peer reviewed version of the following article: Pitolli, M. Cela, M.,
2 Kapps, D., Chicher, J. Despons, L. and Frugier M. (2024), Comparative
3 proteomics uncovers low asparagine content in *Plasmodium* tRip-KO proteins.
4 *IUBMB Life*, which has been published in final form at
5 <https://doi.org/10.1002/iub.2891>. This article may be used for non-commercial
6 purposes in accordance with Wiley Terms and Conditions for Use of Self-
7 Archived Versions.

8
9 **Comparative proteomics uncovers low asparagine content in**
10 ***Plasmodium* tRip-KO proteins.**

11 Martina Pitolli¹, Marta Cela^{1#a}, Delphine Kapps¹, Johana Chicher², Laurence Despons¹ and Magali
12 Frugier^{1*}

13 ¹Université de Strasbourg, CNRS, Architecture et Réactivité de l'ARN, UPR 9002, F-67084 Strasbourg,
14 France

15 ²Strasbourg-Esplanade Proteomics facility, Université de Strasbourg, F-67084 Strasbourg, France.

16 #Current address: Molecular Microbiology and Structural Biochemistry, CNRS UMR 5086, Université
17 Claude Bernard Lyon 1, Lyon, France

18 *Corresponding author: m.frugier@ibmc-cnrs.unistra.fr

19
20 **Abstract**

21 tRNAs are not only essential for decoding the genetic code, but their abundance also has a strong
22 impact on the rate of protein production, folding, and on the stability of the translated messenger RNAs.
23 *Plasmodium* expresses a unique surface protein called tRip, involved in the import of exogenous tRNAs
24 into the parasite. Comparative proteomic analysis of the blood stage of wild-type and tRip-KO variant of
25 *P. berghei* parasites revealed that down-regulated proteins in the mutant parasite are distinguished by
26 a bias in their asparagine content. Furthermore, the demonstration of the possibility of charging host
27 tRNAs with *Plasmodium* aminoacyl-tRNA synthetases, led us to propose that, imported host tRNAs
28 participate in parasite protein synthesis. These results also suggest a novel mechanism of translational
29 control in which import of host tRNAs emerge as regulators of gene expression in the *Plasmodium*
30 developmental cycle and pathogenesis, by enabling the synthesis of asparagine-rich regulatory proteins
31 that efficiently and selectively control the parasite infectivity.

32 Introduction

33 We have discovered the only example to date of an exogenous tRNA import pathway,
34 summarized in Fig. 1 [1–4]. In *Plasmodium*, the malaria parasite, *P. berghei* sporozoites (the
35 extracellular infective form of the parasite, Supplementary Fig. S1a) isolated from the salivary glands of
36 infected mosquitoes import exogenous tRNAs *via* a surface protein, named tRip (tRNA import protein).
37 However, the absence of homologous mechanisms in other organisms raises the question of the role of
38 this unique tRNA import and its mode of action. It has been established that (i) tRip is a homodimeric
39 protein made of an N-terminal GST-like domain and a C-terminal EMAPII-like tRNA binding domain; (ii)
40 tRip binds human tRNAs with high affinity *in vitro* and with a stoichiometry of one tRNA per tRip dimer;
41 (iii) tRip is anchored to the parasite's plasma membrane with its tRNA binding domain exposed to the
42 host system; (iv) immunolocalization experiments found tRip expressed both in the liver and blood
43 stages in the vertebrate host, as well as in the intestine and salivary glands of the mosquito; (v) *in vitro*,
44 exogenous tRNAs enter living sporozoites; (vi) the knockout parasite, tRip-KO, does not import tRNAs,
45 its protein biosynthesis is significantly reduced, and its growth is decreased in vertebrate blood
46 compared to the wild-type parasite.

47 Recently, the crystal structure of the dimeric N-terminal GST-like domain of *P. vivax* tRip was
48 solved and revealed a unique homodimerization interface [5]. We confirmed by SAXS that this unusual
49 interface exists in solution and that it allows Multi-Synthetase Complex (MSC) formation [3,4]. Indeed,
50 tRip is an aminoacyl-tRNA synthetase interacting multifunctional protein (AIMP) since it specifically co-
51 immunoprecipitate with three aminoacyl-tRNA synthetases (aaRS), namely glutamyl- (ERS), glutaminyl-
52 (QRS) and methionyl- (MRS) tRNA synthetases; These aaRSs all contain an N-terminal GST-like
53 domain involved in MSC assembly. Unexpectedly, these proteins form two exclusive heterotrimeric
54 MSCs with a 2:2:2 stoichiometry: a Q-complex (tRip:ERS:QRS) and an M-complex (tRip:ERS:MRS)
55 characterized by different biophysical properties and interaction networks (Fig. 1). We could also identify
56 a set of host tRNAs preferentially bound by tRip (Fig. 1) and potentially best imported into the parasite.
57 Interestingly, tRip does not bind to tRNAs in a sequence-dependent manner, but would rather recognize
58 post-transcriptional modifications that modulate this interaction [2]. In contrast to what has been shown
59 for its cytosolic homologue Arc1p in *Saccharomyces cerevisiae* [6], the tRNAs that best bind tRip do not
60 match the aaRSs that compose MSCs. This suggests that, although tRip is an AIMP, it is not dedicated

61 to the aminoacylation of specific tRNAs (2), thereby leading us to search for a novel function for this
62 unique membrane protein and the tRNA import with which it is associated.

63 The AIMP tRip is not the only protein that has been characterized as cytosolic in other organisms
64 and is localized on the surface of *Plasmodium*. This is also the case for other RNA-binding proteins such
65 as the poly-A binding protein-1 (PABP-1) [7] and the glyceraldehyde-3 phosphate dehydrogenase
66 (GAPDH) [8], which are both found on the surface of the sporozoites. The presence of different RNA-
67 binding proteins at the parasite-host interface is another indication that RNA exchange might control
68 unsuspected host-parasite interactions that take place during the parasite life cycle. In the present study,
69 we compared the proteomes of the wild-type (WT) and the tRip-KO blood stage parasites with the
70 objective of understanding the fate of imported tRNAs in *Plasmodium* protein synthesis and its infectious
71 process.

72

73 **Results**

74 **Host tRNAs are aminoacylated by the cognate parasite aaRSs *in vitro***

75 Cross-aminoacylation reactions were tested using the aspartyl-, tyrosyl-, and asparaginyl-tRNA
76 synthetases from *H. sapiens* and *P. falciparum* (Supplementary Fig. S2) *in vitro* to determine their ability
77 to aminoacylate human tRNA^{Asp}, tRNA^{Tyr}, and tRNA^{Asn}, respectively using crude human tRNA (Fig. 2).
78 These three aminoacylation systems are representative of the tRNAs recognized weakly (aspartate),
79 moderately (tyrosine) and preferentially (asparagine) by tRip [2]. Aminoacylation plateaus show that all
80 three *Plasmodium* aaRSs can aminoacylate nearly the same level of tRNA as their human counterparts,
81 although asparaginylation by the parasite enzyme is effective on about 80% of human tRNA^{Asn}
82 isodecoders. It indicates that host tRNAs, when imported into parasites by tRip can be aminoacylated
83 by parasite aaRSs to be used in protein translation.

84 **Comparative proteomics of tRip-KO versus wild-type (WT) schizonts.** We investigated the
85 relative protein abundance of tRip-KO *versus* WT in the schizont stage of synchronized parasites
86 (Supplementary Fig. S1b) using liquid chromatography coupled to tandem mass spectrometry (LC-
87 MS/MS). To highlight the most relevant effects associated with the deletion of tRip, we chose to compare
88 equal amounts of proteins, even though tRip-KO parasites grow slower than wild-type parasites and
89 their translation efficiency is significantly reduced [1]. Three biological replicates for tRip-KO and WT
90 were defined for the schizonts samples (Supplementary Dataset 1). The amounts of proteins (KO and

91 WT) were comparable as most proteins were stable in the proteomic data. This is notably the case for
92 four constitutively highly expressed proteins: EF1- α (PBANKA_1133300), Hsp70 (PBANKA_0914400),
93 enolase (PBANKA_1214300) and histone H4 (PBANKA_941900) (Supplementary Dataset 1). Protein
94 abundance was calculated using the intensity of *P. berghei* peptides, which identified between 347 and
95 469 proteins. Metric multidimensional scaling (MDS) (included in Supplementary Dataset 1) indicated
96 clustering of the tRip-KO and WT parasites, suggesting there are distinct groups of differentially
97 abundant proteins between the two genotypes. Among these proteins, only 8 were significantly up-
98 regulated and 49 were down-regulated in the tRip-KO parasites compared to the WT (*p-value* < 0.05)
99 with a at least a 2-fold change in abundance; they are highlighted in the volcano plots in orange and
100 green, respectively (Fig. 3a). Most of down-regulated proteins are involved in translation (31% ribosomal
101 proteins, initiation, and elongation factors); the two other abundant categories were surface proteins
102 involved in transport and invasion (16%) and proteins with unknown functions (14%) (Supplementary
103 Dataset 1). Interestingly, the deregulated proteins include several aminoacyl-tRNA synthetases
104 (aaRSs). The glutamyl-tRNA synthetase (ERS), which is tightly associated to tRip in the two plasmodium
105 MSCs is reduced in the tRip-KO sample. **However, the other two aaRSs associated with *Plasmodium***
106 **MSCs, i.e. glutamyl- and methionyl-tRNA synthetases, are detected but their expression is not**
107 **significantly affected. Moreover,** both the lysyl- (KRS) and the asparaginyl (NRS) -tRNA synthetases are
108 increased in the mutant compared to the WT parasite (Supplementary Dataset 1), NRS and ERS being
109 just below the threshold with *p-values* of 0.0723 and 0.0627, respectively.

110 **Amino acid compositions of tRip-KO down-regulated proteins in schizonts.** Amino acid
111 usage (percentage of amino acids) in the 100 most expressed stable proteins in the tRip-KO parasite
112 was compared with that of the 49 significantly down-regulated proteins (Fig. 3b). There is no major
113 increase in glutamate, methionine or glutamine usage in down-regulated proteins compared to the
114 proteins efficiently expressed in the tRip-KO parasite, indicating that the dissociation of the two MSCs
115 caused by tRip deletion does not directly affect protein synthesis in the mutant parasite. On the other
116 hand, we clearly observe a 70% increase in asparagine in the proteins down-regulated in the tRip-KO
117 parasite (Supplementary Dataset 2, 11.6% in downregulated proteins *versus* 6.8% stable proteins), thus,
118 suggesting that the tRip-KO parasite has difficulty synthesizing asparagine-rich proteins. **However, our**
119 **analysis is global and our results do not mean that all down-regulated proteins in the tRip-KO parasite**

120 are asparagine-rich. Only some of them are, but they are so rich in asparagine that they bias the content
121 of the whole group.

122 **Quantitative proteomics of tRip-KO versus WT in “blood stages” samples.** Three biological
123 replicates for tRip-KO and WT containing all blood stages (rings, trophozoites, and schizonts) were used
124 for comparative proteomics (Supplementary Fig. S1b and Dataset 3). Triplicate proteomic data sets (KO
125 and WT) were compared and identified between 617 and 1017 proteins each time. Sixty-nine proteins
126 were significantly deregulated (at least a 2-fold change in abundance), 8 were up-regulated and 61 were
127 down-regulated in the tRip-KO parasites compared to the WT (p -value < 0.05) (Fig. 3c). The few up-
128 regulated proteins are especially rhoptry associated proteins (a specialized organelle in *Plasmodia*) and
129 proteins involved in mobility and invasion (Supplementary Dataset 3), while down-regulated proteins
130 were distributed across different functional families: Apart from the 20% of proteins with unknown
131 function, most are involved in DNA replication (41%). Yet, none were common to the schizonts samples
132 (compare Supplementary Data sets. S1 and S3). Amino acid usage in the tRip-KO down-regulated
133 proteins shows also that these proteins contain more asparagine residues (10.5% at tRip-KO versus
134 7.8% at WT, i.e. 34% more) than the 150 most expressed stable proteins (Fig. 3d, and Supplementary
135 Dataset 2), leading to the same conclusion as above: in the absence of tRip, asparagine-rich proteins
136 are less efficiently synthesized.

137 The mRNAs encoding 3 down-regulated proteins (MCM7, ChAF1-C and one protein with
138 unknown function) were quantified by qRT-PCR. They all showed a strong decrease in the tRip-KO
139 parasite compared to the WT parasite (Supplementary Fig. S3b), suggesting that down-regulated
140 proteins are controlled at the level of mRNA turnover (either transcription or degradation).

141 **Identification of conserved asparagine-rich proteins in several *Plasmodia* species.** Since
142 both "schizonts" and "blood stages" samples strongly suggest that the tRip-KO parasite is hindered in
143 inserting asparagine into proteins, the complete proteomes of six *Plasmodium* lineages (*P. falciparum*-
144 5476 proteins, *P. berghei*-5076 proteins, *P. yoelii*-6097 proteins, *P. chabaudi*-5222 proteins, *P.*
145 *knowlesi*-5340 proteins and *P. vivax*-6708 proteins) as well as *Toxoplasma gondii* (control, 8322
146 proteins) were retrieved from ApiDB [9] and analyzed for their asparagine content (Supplementary Fig.
147 S4a). *Plasmodium* proteins contain more asparagine residues than *Toxoplasma*. Yet, the *P. knowlesi*
148 and *P. vivax* proteomes contain fewer asparagine residues (about 8 %) than the 4 other *Plasmodia*
149 strains (about 12%) (Supplementary Dataset 2 and Fig. S4a). Proteins were then ranked from highest

150 to lowest asparagine contents to identify those that might be impacted by decreased asparagine
151 decoding efficiency. For all proteomes, only the top 0.5% proteins were considered to search for
152 conserved proteins with the highest asparagine content. Depending on the strain, 25 to 30 proteins were
153 selected (Fig. 4). Besides many proteins with unknown functions and different AP2 domain transcription
154 factors, only 2 proteins are strictly conserved in all strains. They correspond to the CCR4-associated
155 factor-1 (CAF-1) and the poly-A binding protein-3 (PABP-3). Both share the same ligand (poly-A
156 sequence) and a peculiar modular organization. They feature a high-complexity N-terminal domain and
157 a long low-complexity C-terminal domain containing a very large amount of asparagine residues
158 (Supplementary Figs. S4b and S4c). The proportion of asparagine varies locally between 35 and 60%
159 in *P. falciparum*, *P. yoelii*, *P. chabaudi* and *P. berghei* and between 20 and 25% in *P. knowlesi* and *P.*
160 *vivax* (i.e. at least 3 times higher than their respective average usage of asparagine). Yet these two
161 proteins are not detected or by only a few spectral counts in our proteomic data (Supplementary Data
162 sets S1 and S3 in both WT and KO parasites).

163

164 Discussion

165 To achieve efficient and specific protein synthesis, codon usage of mRNA must be balanced with
166 the availability of corresponding aminoacylated tRNAs in the cell (i.e.[10–13]). Any discrepancy can
167 affect the rate of protein elongation in ribosomes and result in pauses in translation that lead to mRNA
168 degradation [14]. It is interesting to note that blood contains reticulocytes, precursors of red blood cells
169 (RBCs), which are very active in translation and therefore rich in tRNAs [15]. Both reticulocytes (about
170 2%) and mature RBC can be infected by *Plasmodia*. While reticulocytes are characterized by a dynamic
171 protein synthesis, RBC are hyper-specialized anucleate cells that retain about 10 % of the protein
172 synthesis observed in reticulocytes [16]. Despite their scarcity, *P. berghei* [17], *P.chabaudi* [18], *P. yoelii*
173 [19] and *P. vivax* [20] have been shown to preferentially invade these tRNA-rich reticulocytes compared
174 to mature RBCs.

175 Moreover, by comparing the proteomes of wild-type and tRip-KO *P. berghei*, we observed in two
176 independent experiments that down-regulated proteins in the tRip-KO parasite are asparagine-rich
177 proteins. Asparagine is the most used amino acid in the *P. berghei* proteome and is often found in long
178 homorepeats [21,22]. This asparagine abundance is also found in *P. falciparum*, *P. chabaudi*, *P. yoelii*
179 and to a lesser extent in *P.knowlesi* and *P. vivax* (Supplementary Fig. S4a), however proteins in the

180 latter two parasites do not contain long asparagine homorepeats. Based on these results, we propose
181 that import of host tRNA^{Asn} by tRip would ensure correct translation of asparagine-rich protein domains.
182 In other words, the absence of tRNA import in the tRip-KO parasite would prevent the accumulation of
183 host tRNA^{Asn} and would thus explain why asparagine-rich proteins are poorly translated. **This doesn't**
184 **mean that two proteins with the same percentage of asparagine will necessarily be synthesized in the**
185 **same way; the synthesis of a protein containing asparagine clusters will be reduced in the tRip-KO**
186 **parasite, whereas the synthesis of a protein whose asparagines are evenly distributed in the sequence**
187 **will be unaffected.** This hypothesis is strongly supported by several observations: (i) asparaginyl- and
188 lysyl-tRNA synthetases are overexpressed in the proteomes of tRip-KO schizonts (Fig. 3a,
189 Supplementary Dataset 1). Both aaRSs aminoacylate tRNAs that are most used to synthesize *P.*
190 *berghei* proteins. In general, increased expression of aminoacyl-tRNA synthetase correlates with a
191 decrease in cognate aminoacyl-tRNA and/or amino acid [23,24]. This observation suggests, that
192 tRNA^{Asn} and tRNA^{Lys} levels are low in tRip-KO schizonts. (ii) Furthermore, mammalian (human) tRNA^{Asn}
193 is among the tRNAs with the highest affinity for *P. falciparum* tRip, along with tRNA^{Ser}_{AGA} and tRNA^{Leu}_{hGA}
194 (Fig. 1) [2]; It is reasonable to assume that the tRNAs with the highest affinity for tRip are also those that
195 are most efficiently imported into the parasite. (iii) Finally, compensation for low tRNA^{Asn} by tRip-
196 mediated tRNA import is only possible if the host tRNA^{Asn} is a substrate for the parasite AsnRS. This is
197 indeed the case (Fig. 2) indicating that once in the parasite, at least some host tRNA^{Asn} isoacceptors
198 can be efficiently aminoacylated and used in parasite mRNA translation. We have previously shown that
199 the *P. falciparum* AsnRS aminoacylates the human tRNA^{Asn} transcript 8-fold less efficiently than its
200 homologous *P. falciparum* transcript³⁸. In contrast, in the present study, human tRNA^{Asn} is efficiently
201 charged by the *P. falciparum* enzyme, indicating that post-transcriptional modifications of human
202 tRNA^{Asn} are positively recognized by the parasite synthetase.

203 Two asparagine-rich proteins stand out and are conserved in the six *Plasmodium* species
204 analyzed (Fig. 4): the CCr4-associated factor 1 (CAF-1), involved in the poly-(A) decay of mRNAs [25],
205 and to the poly-(A) binding protein-3 (PABP3). Not only are both proteins involved in poly-(A) recognition,
206 but they also adopt a common global structure with long asparagine-rich C-terminal domains found only
207 in *Plasmodia* (Supplementary Figs. S4b and S4c). While PABP3 has not yet been characterized, CAF-
208 1 has been studied in detail in *P. falciparum*. CAF-1 is an essential nuclease that degrades mRNAs
209 enriched with non-optimally decoded codons. It belongs to the eukaryotic CCr4-Not complex, which

210 binds to the empty E-site of the ribosome and properly positions CAF-1 to initiate the decay of the 3'-
211 poly(A)-tails of stalled mRNAs [26]. However, the asparagine-rich C-terminal domain of *P. falciparum*
212 CAF-1 is not essential and it has been shown that its deletion leads to the premature release of non-
213 infectious merozoites *in vitro* [27] and to the inappropriate development of gametocytes *in vivo* [28].
214 Interestingly, disruption of tRip and of the C-terminal domain of CAF-1 not only lead to the same
215 phenotype: parasites have reduced infectivity and multiplication in the blood stage [1,27], but also results
216 in overexpression of the same gene products involved in parasite egress and invasion: the rhoptry
217 proteins, RAP1, RAP2/3 and the major surface proteins AMA-1 and MSP1 (Supplementary Dataset 3
218 and Fig. S4d). This suggests that tRip and CAF-1 may be linked in their cellular functions, i.e. in the
219 tRip-KO parasite the absence of host tRNA^{Asn} import would hinder the synthesis of the asparagine-rich
220 C-terminus of CAF-1, and lead to the specific and premature expression of mRNAs encoding the
221 proteins responsible for merozoite release and infectivity. Interestingly, this hypothesis is supported not
222 only by the low level of tRip expression observed in this study at the schizont stage (compare tRip in
223 Figs. 3a and 3c), but also by several transcriptomic datasets obtained on the individual blood stages of
224 *P. berghei* and *P. falciparum* (<https://plasmodb.org>). Transcriptomes clearly show a significant reduction
225 in tRip mRNA content in schizonts compared with ring and trophozoite stages. Thus, in the WT parasite,
226 low tRip expression at the schizont stage could mimic what is proposed in this study for the tRip-KO
227 parasite, i.e. lead to partial CAF-1 synthesis and timely release of mature merozoites from schizonts.

228 The complex life cycle of *Plasmodium* is highly regulated, involving tight transcriptional and
229 especially post-transcriptional controls each time the parasite moves from one stage to another [29,30].
230 Translational regulation of specific proteins which depends on the availability of host tRNAs would
231 enable efficient and rapid control of the transition from one stage to another during development. Failure
232 to import host tRNAs could indicate to the parasite that the cell has run out of resources and that it's
233 time to leave and infect another cell. In this respect, tissue-specific variations in tRNA levels and in their
234 post-transcriptional modifications [31,32] represent diverse sources of tRNAs for import. Depending on
235 the available tRNAs isodecoders, their concentration and their post-transcriptional modifications in the
236 host cell, tRNA import could differentially control parasite translation and enable it to develop optimally
237 not only in blood, but also in liver or mosquito. In this model, host tRNA supply could play a major role
238 in parasite development by modulating the translation efficiency of certain mRNAs, while complying with
239 the "just-in-time" translation model [29].

240

241 **Methods**

242 **Ethic Statement:** Experiments were carried out in conformity with the 2010/63/EU directive of
243 the European regulation on the protection of animals used for scientific purposes. Our animal care facility
244 received agreement #I-67-482-2 from the veterinary services of the département du Bas Rhin (Direction
245 Départementale de la Protection des Populations). All experimental protocols including the animals was
246 approved by the French ministry of higher education, research, and innovation under the agreements
247 number APAFIS#11124-2018010312571506 v2. All experiments were performed in accordance with
248 the ARRIVE guidelines.

249 **Parasite production:** Four- to six-week-old female mice (C57BL/6), weighing approximately 20
250 g, were injected intraperitoneally with 200 μ L of frozen infected red blood cells (10-15% parasitemia
251 diluted in phosphate-buffered saline (PBS)) either with wild-type (WT, *Pb* gfpGOMO14) or with tRip-KO
252 (*Pb* tRip-KO mCherry) malaria parasites derived from the *P. berghei* (ANKA strain). Parasitemia was
253 monitored daily by cytometry (BD Accuri C6). Mice with parasitemia between 5 and 10% were selected.
254 Therefore, in this study, only mice without or low symptoms were used. These parasitemia levels were
255 reached 3-6 days after parasite injection. Mice were put to sleep and blood was collected by intracardiac
256 puncture (about 1 to 1.5 mL) and parasites were directly purified (all blood stages) or cultured for 24
257 hours in RPMI at 37°C under 5% CO₂ (only schizonts) (Supplementary Fig. S1b) as described in [33].

258 infected blood was filtered through a Plasmodipur filter (Europroxima) to remove mouse
259 leukocytes, centrifuged for 10 min at 450 g and recovered in 4 mL of RPMI. A 7.2 mL cushion of 60%
260 isotonic Percoll was gently pipetted under the red blood cells and the tube was centrifuged for 20 min
261 at 1450 g (swinging buckets), to separate infected red blood cells (iRBC) at the Percoll/RPMI interface
262 from non-infected RBC at the bottom of the tube. Parasitized RBCs were recovered in 2 tubes, washed
263 3 times in 1 mL of PBS and combined with 200 μ L each of PBS. Infected RBCs were then lysed with
264 0.02% saponin for 5 min in ice (in 400 μ L). Free parasites were recovered by centrifugation for 5 min at
265 2000 g and washed in 500 μ L of PBS. The two pellets were resuspended either in 50 μ L of protein
266 loading buffer and stored at -80°C until mass spectrometry analysis, or as is and placed at -80°C for
267 RNA preparation.

268 **Mass spectrometry (MS) and data analyses.** Approximately 10 μ g of protein was obtained from
269 the half of the blood (~0.75 mL of a 5-10% infected mouse). Protein concentrations were determined by

270 Bradford assay using bovine serum albumin as the standard. Proteins were precipitated, reduced, and
271 alkylated as described in [3]. After digestion overnight with sequencing grade porcine trypsin (300 ng,
272 w/w, Promega, Fitchburg, MA, USA), the generated peptides were analyzed either using Easy-nanoLC-
273 1000 system coupled to a Q-Exactive Plus mass spectrometer (Thermo Fisher Scientific, Germany) with
274 160-minutes gradients (blood stages) or a NanoLC-2DPlus system (nanoFlexChiP module; Eksigent,
275 ABSciex, Concord, Ontario, Canada) coupled to a TripleTOF 5600 mass spectrometer (ABSciex)
276 operating in positive mode with 120-minutes gradients (schizonts). Data were searched using the
277 Mascot algorithm (version 2.6.2, Matrix Science) against the Uniprot database with *P. Berghei* taxonomy
278 (release 2021_03, 4 927 sequences) with a decoy strategy. The resulting .dat Mascot files were then
279 imported into Proline version 2.0 software [34] to align the identified proteins. Proteins were then
280 validated with Mascot pretty rank equal to 1, and 1% false discovery rate (FDR) on both peptide
281 spectrum matches (PSM) and protein sets (based on Mascot score). As expected for blood cells from
282 infected mice, the number of contaminating mouse proteins represents between 40 and 50% of the
283 proteins identified by mass spectrometry (Sup datasets 1 and 3).

284 For statistical analyses, raw Spectral Count values were imported into R (v. 3.5.0) where the
285 number of spectra were first normalized using the DESeq2 median of ratio normalization method. A
286 negative-binomial test using an edgeR GLM regression generated for each identified protein a p-value
287 and a protein fold-change (FC). The R script used to process the dataset is published on Github [35].
288 Proteins were statistically enriched or decreased with a p-value < 0.05 and a minimum fold change (FC)
289 of 2 or 0.5, respectively. Mass spectrometry proteomic data has been deposited within the
290 ProteomeXchange Consortium via the PRIDE partner repository [36] with the dataset ID PXD043916
291 (Username: reviewer_pxd043916@ebi.ac.uk, Password: xu4rkrPE).

292 **RNA purification and QRT-PCR.** Total RNA was extracted from parasites using the RNeasy
293 Mini Kit (Qiagen) according to the manufacturer's protocol and was subjected to DNase treatment with
294 the Rapid OUT DNA removal kit (Thermo Scientific). Total RNA was analyzed and quantified on
295 Bioanalyser (puce PICO). Each sample was reverse transcribed in a 20 μ L reaction volume containing
296 10 μ L (0.16 to 0.5 μ g) of RNA, using the SuperScript II reverse transcriptase (Invitrogen) according to
297 the manufacturer's protocol. The mRNAs levels were measured by RT-PCR (25 μ L containing 4 μ L of
298 cDNA) on a CFX94 (Bio-Rad) using the Syber Green kit (Thermo Scientific). Oligonucleotides used for
299 qRT-PCR are listed in the Supplementary Fig. S3a. qRT-PCR reactions were designed according MIQE

300 guidelines [37], the specificity of the oligonucleotides was validated, and the amplification efficiencies of
301 the primer sets are all between 90 and 110% and r^2 values greater than 0.96. The mRNAs levels were
302 calculated according to the ΔCq method and normalized by the mRNA level of both the EF1- α and
303 Hsp70 in each sample. Raw data are indicated as mean of three measurements, and results were
304 expressed as the average of 3 biological samples \pm standard error of the mean (SEM).

305 **Purification of aminoacyl-tRNA synthetases and aminoacylation assays.** Recombinant *P.*
306 *falciparum* and *H. sapiens* NRS and DRS were cloned, expressed and purified as described in
307 references [38,39] respectively. The gene encoding the *P. falciparum* TyrRS was amplified by PCR from
308 *P. falciparum* cDNA and cloned into pQE30 (Qiagen) and the plasmid encoding the *H. sapiens* YRS
309 was a gift from P. Schimmel. Both *P. falciparum* and *H. sapiens* YRS were expressed and purified as
310 described for NRS [38]. Purified enzymes are shown in Supplementary Fig. S2. *H. sapiens* crude tRNAs
311 was prepared as described in [2].

312 Aminoacylation assays were performed under the same conditions: at 37 °C for 2, 4 and 6 min in
313 50 mM HEPES-KOH (pH 7.5), 20 mM KCl, 10 mM MgCl₂, 2 mM ATP, 6 μ M total tRNA from HeLa cells,
314 in the presence of 20 μ M of the corresponding L-[¹⁴C]-amino acid (Perkin Elmer). AaRSs were diluted
315 in 100 mM HEPES-KOH pH 7.5, 1 mM DTT, 5 mg/mL BSA, and 10% glycerol and used at a final
316 concentration of 200 nM in the aminoacylation assay. The aminoacylation plateaus are the mean of 3
317 independent experiments \pm standard deviation (SD).

318 **Bioinformatics.** Protein sequences as well as proteomes from all *Plasmodium* strains were
319 retrieved from PlasmoDB. A home-made Python 2.7 script was used to calculate the asparagine content
320 for each protein sequence. *Toxoplasma gondii* was the outgroup species.

321

322 **References**

- 323 [1] T. Bour, N. Mahmoudi, D. Kapps, S. Thiberge, D. Bargieri, R. Ménard, M. Frugier, *Apicomplexa* -
324 specific tRip facilitates import of exogenous tRNAs into malaria parasites, *Proc Natl Acad Sci USA*
325 113 (2016) 4717–4722. <https://doi.org/10.1073/pnas.1600476113>.
- 326 [2] M. Cela, A. Théobald-Dietrich, J. Rudinger-Thirion, P. Wolff, R. Geslain, M. Frugier, Identification
327 of host tRNAs preferentially recognized by the *Plasmodium* surface protein tRip, *Nucleic Acids*
328 *Research* (2021) gkab769. <https://doi.org/10.1093/nar/gkab769>.

- 329 [3] J.R. Jaramillo Ponce, D. Kapps, C. Paulus, J. Chicher, M. Frugier, Discovery of two distinct
330 aminoacyl-tRNA synthetase complexes anchored to the Plasmodium surface tRNA import protein,
331 Journal of Biological Chemistry 298 (2022) 101987. <https://doi.org/10.1016/j.jbc.2022.101987>.
- 332 [4] J.R. Jaramillo Ponce, A. Théobald-Dietrich, P. Bénas, C. Paulus, C. Sauter, M. Frugier, Solution
333 X-ray scattering highlights discrepancies in *Plasmodium* MULTI-AMINOACYL-TRNA synthetase
334 complexes, Protein Science 32 (2023). <https://doi.org/10.1002/pro.4564>.
- 335 [5] S. Gupta, J. Chhibber-Goel, M. Sharma, S. Parvez, K. Harlos, A. Sharma, M. Yogavel, Crystal
336 structures of the two domains that constitute the Plasmodium vivax p43 protein, Acta Crystallogr
337 D Struct Biol 76 (2020) 135–146. <https://doi.org/10.1107/S2059798319016413>.
- 338 [6] K. Deinert, F. Fasiolo, E.C. Hurt, G. Simos, Arc1p Organizes the Yeast Aminoacyl-tRNA
339 Synthetase Complex and Stabilizes Its Interaction with the Cognate tRNAs, Journal of Biological
340 Chemistry 276 (2001) 6000–6008. <https://doi.org/10.1074/jbc.M008682200>.
- 341 [7] A.M. Minns, K.J. Hart, S. Subramanian, S. Hafenstein, S.E. Lindner, Nuclear, Cytosolic, and
342 Surface-Localized Poly(A)-Binding Proteins of Plasmodium yoelii, mSphere 3 (2018).
343 <https://doi.org/10.1128/mSphere.00435-17>.
- 344 [8] S.-J. Cha, M.-S. Kim, A. Pandey, M. Jacobs-Lorena, Identification of GAPDH on the surface of
345 Plasmodium sporozoites as a new candidate for targeting malaria liver invasion, Journal of
346 Experimental Medicine 213 (2016) 2099–2112. <https://doi.org/10.1084/jem.20160059>.
- 347 [9] C. Aurrecochea, M. Heiges, H. Wang, Z. Wang, S. Fischer, P. Rhodes, J. Miller, E. Kraemer, C.J.
348 Stoeckert, D.S. Roos, J.C. Kissinger, ApiDB: integrated resources for the apicomplexan
349 bioinformatics resource center, Nucleic Acids Research 35 (2007) D427–D430.
350 <https://doi.org/10.1093/nar/gkl880>.
- 351 [10] E.M. Novoa, L. Ribas de Pouplana, Speeding with control: codon usage, tRNAs, and ribosomes,
352 Trends in Genetics 28 (2012) 574–581. <https://doi.org/10.1016/j.tig.2012.07.006>.
- 353 [11] J.D. Richter, J. Collier, Pausing on Polyribosomes: Make Way for Elongation in Translational
354 Control, Cell 163 (2015) 292–300. <https://doi.org/10.1016/j.cell.2015.09.041>.
- 355 [12] M. Torrent, G. Chalancon, N.S. de Groot, A. Wuster, M. Madan Babu, Cells alter their tRNA
356 abundance to selectively regulate protein synthesis during stress conditions, Sci. Signal. 11 (2018)
357 eaat6409. <https://doi.org/10.1126/scisignal.aat6409>.

- 358 [13] P.C. Dedon, T.J. Begley, Dysfunctional tRNA reprogramming and codon-biased translation in
359 cancer, *Trends in Molecular Medicine* 28 (2022) 964–978.
360 <https://doi.org/10.1016/j.molmed.2022.09.007>.
- 361 [14] Q. Wu, S.G. Medina, G. Kushawah, M.L. DeVore, L.A. Castellano, J.M. Hand, M. Wright, A.A.
362 Bazzini, Translation affects mRNA stability in a codon-dependent manner in human cells, *eLife* 8
363 (2019) e45396. <https://doi.org/10.7554/eLife.45396>.
- 364 [15] D.W.E. Smith, R.F. Randazzo, A.L. McNamara, The tRNA content of non-hemoglobinized red cell
365 precursors: Evidence that tRNA content is controlled by tRNA utilization, *Biochemical and*
366 *Biophysical Research Communications* 95 (1980) 468–473. [https://doi.org/10.1016/0006-](https://doi.org/10.1016/0006-291X(80)90761-5)
367 [291X\(80\)90761-5](https://doi.org/10.1016/0006-291X(80)90761-5).
- 368 [16] S.D. Kumar, D. Kar, M.N. Akhtar, B. Willard, D. Roy, T. Hussain, P.I. Rajyaguru, S.M. Eswarappa,
369 Evidence for low-level translation in human erythrocytes, *MBoC* 33 (2022) br21.
370 <https://doi.org/10.1091/mbc.E21-09-0437>.
- 371 [17] D. Cromer, K.J. Evans, L. Schofield, M.P. Davenport, Preferential invasion of reticulocytes during
372 late-stage *Plasmodium berghei* infection accounts for reduced circulating reticulocyte levels,
373 *International Journal for Parasitology* 36 (2006) 1389–1397.
374 <https://doi.org/10.1016/j.ijpara.2006.07.009>.
- 375 [18] R. Antia, A. Yates, J.C. De Roode, The dynamics of acute malaria infections. I. Effect of the
376 parasite's red blood cell preference, *Proc. R. Soc. B.* 275 (2008) 1449–1458.
377 <https://doi.org/10.1098/rspb.2008.0198>.
- 378 [19] B. Mons, Preferential invasion of malarial merozoites into young red blood cells, *Blood Cells* 16
379 (1990) 299–312.
- 380 [20] N. Thawani, M. Tam, M.-J. Bellemare, D.S. Bohle, M. Olivier, J.B. De Souza, M.M. Stevenson,
381 *Plasmodium* Products Contribute to Severe Malarial Anemia by Inhibiting Erythropoietin-Induced
382 Proliferation of Erythroid Precursors, *The Journal of Infectious Diseases* 209 (2014) 140–149.
383 <https://doi.org/10.1093/infdis/jit417>.
- 384 [21] S.R. Chaudhry, N. Lwin, D. Phelan, A.A. Escalante, F.U. Battistuzzi, Comparative analysis of low
385 complexity regions in *Plasmodia*, *Sci Rep* 8 (2018) 335. [https://doi.org/10.1038/s41598-017-](https://doi.org/10.1038/s41598-017-18695-y)
386 [18695-y](https://doi.org/10.1038/s41598-017-18695-y).

- 387 [22] F.U. Battistuzzi, K.A. Schneider, M.K. Spencer, D. Fisher, S. Chaudhry, A.A. Escalante, Profiles
388 of low complexity regions in Apicomplexa, *BMC Evol Biol* 16 (2016) 47.
389 <https://doi.org/10.1186/s12862-016-0625-0>.
- 390 [23] Harald Putzer, Laalami Soumaya, Regulation of the Expression of Aminoacyl-tRNA Synthetases
391 and Translation Factors, in: *Madame Curie Bioscience Database*, Landes Bioscience, 2000.
- 392 [24] O. Levi, S. Garin, Y. Arava, RNA mimicry in post-transcriptional regulation by aminoacyl tRNA
393 synthetases, *WIREs RNA* 11 (2020). <https://doi.org/10.1002/wrna.1564>.
- 394 [25] M.A. Collart, The Ccr4-Not complex is a key regulator of eukaryotic gene expression, *WIREs RNA*
395 7 (2016) 438–454. <https://doi.org/10.1002/wrna.1332>.
- 396 [26] R. Buschauer, Y. Matsuo, T. Sugiyama, Y.-H. Chen, N. Alhusaini, T. Sweet, K. Ikeuchi, J. Cheng,
397 Y. Matsuki, R. Nobuta, A. Gilmozzi, O. Berninghausen, P. Tesina, T. Becker, J. Coller, T. Inada,
398 R. Beckmann, The Ccr4-Not complex monitors the translating ribosome for codon optimality,
399 *Science* 368 (2020) eaay6912. <https://doi.org/10.1126/science.aay6912>.
- 400 [27] B. Balu, S.P. Maher, A. Pance, C. Chauhan, A.V. Naumov, R.M. Andrews, P.D. Ellis, S.M. Khan,
401 J. Lin, C.J. Janse, J.C. Rayner, J.H. Adams, CCR4-Associated Factor 1 Coordinates the
402 Expression of *Plasmodium falciparum* Egress and Invasion Proteins, *Eukaryot Cell* 10 (2011)
403 1257–1263. <https://doi.org/10.1128/EC.05099-11>.
- 404 [28] K.J. Hart, J. Oberstaller, M.P. Walker, A.M. Minns, M.F. Kennedy, I. Padykula, J.H. Adams, S.E.
405 Lindner, *Plasmodium* male gametocyte development and transmission are critically regulated by
406 the two putative deadenylases of the CAF1/CCR4/NOT complex, *PLoS Pathog* 15 (2019)
407 e1007164. <https://doi.org/10.1371/journal.ppat.1007164>.
- 408 [29] S.S. Vembar, D. Droll, A. Scherf, Translational regulation in blood stages of the malaria parasite
409 *Plasmodium spp.* : systems-wide studies pave the way: Translational regulation in blood stages of
410 the malaria parasite, *WIREs RNA* 7 (2016) 772–792. <https://doi.org/10.1002/wrna.1365>.
- 411 [30] S. Bennink, G. Pradel, The molecular machinery of translational control in malaria parasites, *Mol*
412 *Microbiol* 112 (2019) 1658–1673. <https://doi.org/10.1111/mmi.14388>.
- 413 [31] K.A. Dittmar, J.M. Goodenbour, T. Pan, Tissue-Specific Differences in Human Transfer RNA
414 Expression, *PLoS Genet* 2 (2006) e221. <https://doi.org/10.1371/journal.pgen.0020221>.

- 415 [32] O. Pinkard, S. McFarland, T. Sweet, J. Collier, Quantitative tRNA-sequencing uncovers metazoan
416 tissue-specific tRNA regulation, *Nat Commun* 11 (2020) 4104. [https://doi.org/10.1038/s41467-](https://doi.org/10.1038/s41467-020-17879-x)
417 [020-17879-x](https://doi.org/10.1038/s41467-020-17879-x).
- 418 [33] C. Ramakrishnan, M.J. Delves, K. Lal, A.M. Blagborough, G. Butcher, K.W. Baker, R.E. Sinden,
419 Laboratory Maintenance of Rodent Malaria Parasites, in: R. Ménard (Ed.), *Malaria*, Humana
420 Press, Totowa, NJ, 2012: pp. 51–72. https://doi.org/10.1007/978-1-62703-026-7_5.
- 421 [34] D. Bouyssié, A.-M. Hesse, E. Mouton-Barbosa, M. Rompais, C. Macron, C. Carapito, A. Gonzalez
422 de Peredo, Y. Couté, V. Dupierris, A. Burel, J.-P. Menetrey, A. Kalaitzakis, J. Poisat, A. Romdhani,
423 O. Burlet-Schiltz, S. Cianférani, J. Garin, C. Bruley, Proline: an efficient and user-friendly software
424 suite for large-scale proteomics, *Bioinformatics* 36 (2020) 3148–3155.
425 <https://doi.org/10.1093/bioinformatics/btaa118>.
- 426 [35] L. Kuhn, T. Vincent, P. Hammann, H. Zuber, Exploring Protein Interactome Data with IPInquiry:
427 Statistical Analysis and Data Visualization by Spectral Counts, in: T. Burger (Ed.), *Statistical*
428 *Analysis of Proteomic Data*, Springer US, New York, NY, 2023: pp. 243–265.
429 https://doi.org/10.1007/978-1-0716-1967-4_11.
- 430 [36] Y. Perez-Riverol, J. Bai, C. Bandla, D. García-Seisdedos, S. Hewapathirana, S. Kamatchinathan,
431 D.J. Kundu, A. Prakash, A. Frericks-Zipper, M. Eisenacher, M. Walzer, S. Wang, A. Brazma, J.A.
432 Vizcaíno, The PRIDE database resources in 2022: a hub for mass spectrometry-based proteomics
433 evidences, *Nucleic Acids Research* 50 (2022) D543–D552. <https://doi.org/10.1093/nar/gkab1038>.
- 434 [37] S.A. Bustin, V. Benes, J.A. Garson, J. Hellems, J. Huggett, M. Kubista, R. Mueller, T. Nolan,
435 M.W. Pfaffl, G.L. Shipley, J. Vandesompele, C.T. Wittwer, The MIQE Guidelines: Minimum
436 Information for Publication of Quantitative Real-Time PCR Experiments, *Clinical Chemistry* 55
437 (2009) 611–622. <https://doi.org/10.1373/clinchem.2008.112797>.
- 438 [38] D. Filisetti, A. Théobald-Dietrich, N. Mahmoudi, J. Rudinger-Thirion, E. Candolfi, M. Frugier,
439 Aminoacylation of *Plasmodium falciparum* tRNAAsn and Insights in the Synthesis of Asparagine
440 Repeats, *Journal of Biological Chemistry* 288 (2013) 36361–36371.
441 <https://doi.org/10.1074/jbc.M113.522896>.
- 442 [39] T. Bour, A. Akaddar, B. Lorber, S. Blais, C. Balg, E. Candolfi, M. Frugier, Plasmodial aspartyl-
443 tRNA synthetases and peculiarities in *Plasmodium falciparum*, *The Journal of Biological Chemistry*
444 284 (2009) 18893–903.

445 [40] P.P. Chan, T.M. Lowe, GtRNAdb 2.0: an expanded database of transfer RNA genes identified in
446 complete and draft genomes, *Nucleic Acids Research* 44 (2016) D184–D189.
447 <https://doi.org/10.1093/nar/gkv1309>.
448
449

450 **Acknowledgments**

451 We are grateful to Philippe Hammann, Lauriane Kuhn, and Béatrice Chane Woon Ming for LC-
452 MS/MS analysis, Fabrice Auge and Eric Marois for animal experiments, and to Dr Alain Lescure and
453 Prof Tamara Hendrickson for providing comments on this manuscript.

454 This work was performed under the framework of the Interdisciplinary Thematic Institute IMCBio,
455 as part of the ITI 2021-2028 program of the University of Strasbourg, CNRS and Inserm. It was
456 supported by IdEx Unistra (ANR-10-IDEX-0002), by SFRI-STRAT'US project (ANR 20-SFRI-0012), and
457 EUR IMCBio (IMCBio ANR-17-EURE-0023) under the framework of the French Investments for the
458 Future Program », by previous Labex NetRNA (ANR-10-LABX-0036), by the CNRS and the Université
459 de Strasbourg, IdEx "Équipement mi-lourd" (2015) and Equipement d'Excellence (EquipEx) I2MC (ANR-
460 11-EQPX-0022), and by the Fondation pour la Recherche Médicale (FRM) (grant number
461 FDT201704337050) to Marta Cela.

462

463 **Authors Contributions**

464 MP, DK and MC performed experiments, data acquisition, and analysis, JC performed mass
465 spectrometry analysis and LD provided computer programming. MF managed the conception, design,
466 interpretation of data and funding acquisition and wrote, reviewed, and edited the manuscript.

467

468 **Data availability statement**

469 All relevant data are within the manuscript and its Supporting Information files.

470

471 **Additional information**

472 **Competing Interest Statement:** The authors declare no conflict of interest.

473 1 pdf with 4 Supplementary Figures and 1 excel document containing 3 supplementary Data sets.

474

475

476 **Figure legends**

477 **Figure 1. *P. berghei* membrane-bound multi-synthetase complexes.** *Plasmodium* is
478 characterized by the presence of two multisynthetase complexes (MSC) named Q-complex and M-
479 complex. The Q-complex is composed of glutamyl- (ERS) and glutaminy- (QRS) tRNA synthetases
480 linked to a dimer of tRip whereas the M-complex is composed of tRip and Methionyl-tRNA synthetase
481 (MRS) organized around a dimer of ERS. tRip is therefore an AIMP (Aminoacyl-tRNA synthetase
482 Interacting Multifunctional Protein). tRip, ERS, QRS, and MRS are schematized and colored in grey,
483 black, cyan, and orange, respectively. the GST-like domains are shown as a drop and the C-terminal
484 domains of tRip, QRS and MRS involved in tRNA binding are either EMAPII-like domains (tRip and
485 MRS, grey diamonds) or a positively charged α -helix (QRS, shown as a grey helix). The characteristic
486 feature of *Plasmodium* MSCs is that tRip is a membrane protein (the transmembrane helix is shown in
487 red) with the GST-like domain required for MSC formation localized inside the parasite and the tRNA
488 binding domain exposed outside the parasite to host tRNAs. This unique organization justifies that only
489 the EMAPII-like domain of tRip (tRip₂₀₀₋₄₀₂) is fused at the C-terminal domain of a GST domain and used
490 as a target for the selection of aptamers capable of inhibiting tRip tRNA binding. Interfaces 1, 1' or 2,
491 involved in protein-protein interactions, are indicated in the corresponding GST-like domains. For the
492 sake of simplicity, this illustration does not show the parasitophorous membrane that surrounds the
493 parasite in the infected red blood cell.

494 **Figure 2. Cross aminoacylation of host tRNAs by the parasite aminoacyl-tRNA**
495 **synthetases.** Six aaRSs, aspartyl-, asparaginyl- and tyrosyl-tRNA synthetases from *H. sapiens* (in blue)
496 or *P. falciparum* (in orange) were tested with crude human tRNAs under the same experimental
497 conditions (6 μ M crude tRNA and 0.2 μ M aaRSs). Aminoacylation plateaus were measured at 6 min of
498 incubation. Human crude tRNAs are potentially transcribed from 420 genes, amongst which 19 genes
499 encode tRNA^{Asp}, 34 genes encoding tRNA^{Asn} and 15 genes encoding tRNA^{Tyr} [40]. Error bars represent
500 the standard deviation (SEM) of three independent experiments.

501 **Figure 3. Comparative proteomic analysis and amino acid usage of proteins identified in**
502 **WT and tRip-KO samples containing schizonts (a and b) or all blood stages (c and d).** (a) Volcano
503 plot of all quantified proteins from WT and tRip-KO parasites displaying the relationship between
504 statistical significance ($-\log_{10}(p\text{-value})$, y-axis) and log fold change (FC) of each protein ($\log_2(\text{FC})$, x-
505 axis). Statistics are based on three independent experiments (Supplementary Dataset 1). Deregulated

506 proteins in tRip-KO parasite compared to the WT parasite are shown in orange (up-regulated, $FC \geq 2$ and
507 $p\text{-value} \leq 0.05$) and green (down-regulated, $FC \leq -1$ and $p\text{-value} \leq 0.05$). tRip is shown in red and black
508 dots represent proteins with no significant change. ERS, KRS and NRS correspond to glutamyl-, lysyl-
509 and asparaginyI-tRNA synthetases; ERS and NRS are shown as black circles surrounded by green and
510 orange, respectively because their $p\text{-values}$ are slightly greater than 0.05. **(b)** Comparison of amino acid
511 usage (%) of proteins whose expression is stable in the tRip-KO parasite (the 100 most expressed
512 proteins, black) and all proteins that are down-regulated in the tRip-KO parasite (green). Amino acids
513 are designated by their one letter symbol and the total number of amino acids used in the analyses is
514 specified. **(c)** Volcano plot of all quantified proteins from WT and tRip-KO parasites. Statistics are based
515 on three independent experiments (Supplementary Dataset 3). **(d)** Comparison of amino acid usage (%)
516 between proteins whose expression is stable in the tRip-KO parasite (the 150 most expressed proteins,
517 black) and proteins down-regulated in the tRip-KO parasite (green).

518 **Figure 4. Top 0.5% of the most asparagine-rich proteins in 6 *Plasmodium* strains.** Six
519 *Plasmodium* species were selected: *P. falciparum*, *P. yoelii*, *P. berghei*, *P. chabaudi*, all featuring high
520 asparagine usage (around 12%, Supplementary Fig. S4a) often localized in homorepetitions and *P.*
521 *knowlesi* and *P. vivax* which show lower asparagine usage (around 8%, Supplementary Fig. S4a) and
522 no homorepeats. Proteins highlighted in yellow are proteins of unknown function, light green indicate
523 AP2 family regulatory proteins and dark green show the two proteins conserved in all 6 species,
524 regardless of their asparagine usage.

27 **Introduction**

28 We have discovered the only example to date of an exogenous tRNA import pathway,
29 summarized in Fig. 1 [1–4]. In *Plasmodium*, the malaria parasite, *P. berghei* sporozoites (the
30 extracellular infective form of the parasite, Supplementary Fig. S1a) isolated from the salivary glands of
31 infected mosquitoes import exogenous tRNAs *via* a surface protein, named tRip (tRNA import protein).
32 However, the absence of homologous mechanisms in other organisms raises the question of the role of
33 this unique tRNA import and its mode of action. It has been established that (i) tRip is a homodimeric
34 protein made of an N-terminal GST-like domain and a C-terminal EMAPII-like tRNA binding domain; (ii)
35 tRip binds human tRNAs with high affinity *in vitro* and with a stoichiometry of one tRNA per tRip dimer;
36 (iii) tRip is anchored to the parasite's plasma membrane with its tRNA binding domain exposed to the
37 host system; (iv) immunolocalization experiments found tRip expressed both in the liver and blood
38 stages in the vertebrate host, as well as in the intestine and salivary glands of the mosquito; (v) *in vitro*,
39 exogenous tRNAs enter living sporozoites; (vi) the knockout parasite, tRip-KO, does not import tRNAs,
40 its protein biosynthesis is significantly reduced, and its growth is decreased in vertebrate blood
41 compared to the wild-type parasite.

42 Recently, the crystal structure of the dimeric N-terminal GST-like domain of *P. vivax* tRip was
43 solved and revealed a unique homodimerization interface [5]. We confirmed by SAXS that this unusual
44 interface exists in solution and that it allows Multi-Synthetase Complex (MSC) formation [3,4]. Indeed,
45 tRip is an aminoacyl-tRNA synthetase interacting multifunctional protein (AIMP) since it specifically co-
46 immunoprecipitate with three aminoacyl-tRNA synthetases (aaRS), namely glutamyl- (ERS), glutaminyl-
47 (QRS) and methionyl- (MRS) tRNA synthetases; These aaRSs all contain an N-terminal GST-like
48 domain involved in MSC assembly. Unexpectedly, these proteins form two exclusive heterotrimeric
49 MSCs with a 2:2:2 stoichiometry: a Q-complex (tRip:ERS:QRS) and an M-complex (tRip:ERS:MRS)
50 characterized by different biophysical properties and interaction networks (Fig. 1). We could also identify
51 a set of host tRNAs preferentially bound by tRip (Fig. 1) and potentially best imported into the parasite.
52 Interestingly, tRip does not bind to tRNAs in a sequence-dependent manner, but would rather recognize
53 post-transcriptional modifications that modulate this interaction [2]. In contrast to what has been shown
54 for its cytosolic homologue Arc1p in *Saccharomyces cerevisiae* [6], the tRNAs that best bind tRip do not
55 match the aaRSs that compose MSCs. It suggests that, although tRip is an AIMP, it is not dedicated to

56 the aminoacylation of specific tRNAs (2), thereby leading us to search for a novel function for this unique
57 membrane protein and the tRNA import with which it is associated.

58 The AIMP tRip is not the only protein that has been characterized as cytosolic in other organisms
59 and is localized on the surface of *Plasmodium*. This is also the case for other RNA-binding proteins such
60 as the poly-A binding protein-1 (PABP-1) [7] and the glyceraldehyde-3 phosphate dehydrogenase
61 (GAPDH) [8], which are both found on the surface of the sporozoites. The presence of different RNA-
62 binding proteins at the parasite-host interface is another indication that RNA exchange might control
63 unsuspected host-parasite interactions that take place during the parasite life cycle. In the present study,
64 we compared the proteomes of the wild-type (WT) and the tRip-KO blood stage parasites with the
65 objective of understanding the fate of imported tRNAs in *Plasmodium* protein synthesis and its infectious
66 process.

67

68 **Results**

69 **Host tRNAs are aminoacylated by the cognate parasite aaRSs *in vitro***

70 Cross-aminoacylation reactions were tested using the aspartyl-, tyrosyl-, and asparaginyl-tRNA
71 synthetases from *H. sapiens* and *P. falciparum* (Supplementary Fig. S2) *in vitro* to determine their ability
72 to aminoacylate human tRNA^{Asp}, tRNA^{Tyr}, and tRNA^{Asn}, respectively using crude human tRNA (Fig. 2).
73 These three aminoacylation systems are representative of the tRNAs recognized weakly (aspartate),
74 moderately (tyrosine) and preferentially (asparagine) by tRip [2]. Aminoacylation plateaus show that all
75 three *Plasmodium* aaRSs can aminoacylate nearly the same level of tRNA as their human counterparts,
76 although asparaginylation by the parasite enzyme is effective on about 80% of human tRNA^{Asn}
77 isodecoders. It indicates that host tRNAs, when imported into parasites by tRip can be aminoacylated
78 by parasite aaRSs to be used in protein translation.

79 **Comparative proteomics of tRip-KO versus wild-type (WT) schizonts.** We investigated the
80 relative protein abundance of tRip-KO *versus* WT in the schizont stage of synchronized parasites
81 (Supplementary Fig. S1b) using liquid chromatography coupled to tandem mass spectrometry (LC-
82 MS/MS). To highlight the most relevant effects associated with the deletion of tRip, we chose to compare
83 equal amounts of proteins, even though tRip-KO parasites grow slower than wild-type parasites and
84 their translation efficiency is significantly reduced [1]. Three biological replicates for tRip-KO and WT
85 were defined for the schizonts samples (Supplementary Dataset 1). The amounts of proteins (KO and

86 WT) were comparable as most proteins were stable in the proteomic data. This is notably the case for
87 four constitutively highly expressed proteins: EF1- α (PBANKA_1133300), Hsp70 (PBANKA_0914400),
88 enolase (PBANKA_1214300) and histone H4 (PBANKA_941900) (Supplementary Dataset 1). Protein
89 abundance was calculated using the intensity of *P. berghei* peptides, which identified between 347 and
90 469 proteins. Metric multidimensional scaling (MDS) (included in Supplementary Dataset 1) indicated
91 clustering of the tRip-KO and WT parasites, suggesting there are distinct groups of differentially
92 abundant proteins between the two genotypes. Among these proteins, only 8 were significantly up-
93 regulated and 49 were down-regulated in the tRip-KO parasites compared to the WT (*p-value* < 0.05)
94 with a at least a 2-fold change in abundance; they are highlighted in the volcano plots in orange and
95 green, respectively (Fig. 3a). Most of down-regulated proteins are involved in translation (31% ribosomal
96 proteins, initiation, and elongation factors); the two other abundant categories were surface proteins
97 involved in transport and invasion (16%) and proteins with unknown functions (14%) (Supplementary
98 Dataset 1). Interestingly, the deregulated proteins include several aminoacyl-tRNA synthetases. The
99 glutamyl-tRNA synthetase (ERS), which is tightly associated to tRip in the two plasmodium MSCs is
100 reduced in the tRip-KO sample and both the lysyl- (KRS) and the asparaginyl (NRS) -tRNA synthetases
101 are increased in the mutant compared to the WT parasite (Supplementary Dataset 1), NRS and ERS
102 being just below the threshold with *p-values* of 0.0723 and 0.0627, respectively.

103 **Amino acid compositions of tRip-KO down-regulated proteins in schizonts.** Amino acid
104 usage (percentage of amino acids) in the 100 most expressed stable proteins in the tRip-KO parasite
105 was compared with that of the 49 significantly down-regulated proteins (Fig. 3b). There is no major
106 increase in glutamate, methionine or glutamine usage in down-regulated proteins compared to the
107 proteins efficiently expressed in the tRip-KO parasite, indicating that the dissociation of the two MSCs
108 caused by tRip deletion does not directly affect protein synthesis in the mutant parasite. On the other
109 hand, we clearly observe a 70% increase in asparagine in the proteins down-regulated in the tRip-KO
110 parasite (Supplementary Dataset 2, 11.6% in downregulated proteins *versus* 6.8% stable proteins), thus,
111 suggesting that the tRip-KO parasite has difficulty synthesizing asparagine-rich proteins.

112 **Quantitative proteomics of tRip-KO versus WT in “blood stages” samples.** Three biological
113 replicates for tRip-KO and WT containing all blood stages (rings, trophozoites, and schizonts) were used
114 for comparative proteomics (Supplementary Fig. S1b and Dataset 3). Triplicate proteomic data sets (KO
115 and WT) were compared and identified between 617 and 1017 proteins each time. Sixty-nine proteins

116 were significantly deregulated (at least a 2-fold change in abundance), 8 were up-regulated and 61 were
117 down-regulated in the tRip-KO parasites compared to the WT (p -value < 0.05) (Fig. 3c). The few up-
118 regulated proteins are especially rhoptry associated proteins (a specialized organelle in *Plasmodia*) and
119 proteins involved in mobility and invasion (Supplementary Dataset 3), while down-regulated proteins
120 were distributed across different functional families: Apart from the 20% of proteins with unknown
121 function, most are involved in DNA replication (41%). Yet, none were common to the schizonts samples
122 (compare Supplementary Data sets. S1 and S3). Amino acid usage in the tRip-KO down-regulated
123 proteins shows also that these proteins contain more asparagine residues (10.5% at tRip-KO versus
124 7.8% at WT, i.e. 34% more) than the 150 most expressed stable proteins (Fig. 3d, and Supplementary
125 Dataset 2), leading to the same conclusion as above: in the absence of tRip, asparagine-rich proteins
126 are less efficiently synthesized.

127 The mRNAs encoding 3 down-regulated proteins (MCM7, ChAF1-C and one protein with
128 unknown function) were quantified by qRT-PCR. They all showed a strong decrease in the tRip-KO
129 parasite compared to the WT parasite (Supplementary Fig. S3b), suggesting that down-regulated
130 proteins are controlled at the level of mRNA turnover.

131 **Identification of conserved asparagine-rich proteins in several *Plasmodia* strains.** Since
132 both "schizonts" and "blood stages" samples strongly suggest that the tRip-KO parasite is hindered in
133 inserting asparagine into proteins, the complete proteomes of six *Plasmodium* lineages (*P. falciparum*-
134 5476 proteins, *P. berghei*-5076 proteins, *P. yoelii*-6097 proteins, *P. chabaudi*-5222 proteins, *P.*
135 *knowlesi*-5340 proteins and *P. vivax*-6708 proteins) as well as *Toxoplasma gondii* (control, 8322
136 proteins) were retrieved from ApiDB [9] and analyzed for their asparagine content (Supplementary Fig.
137 S4a). *Plasmodium* proteins contain more asparagine residues than *Toxoplasma*. Yet, the *P. knowlesi*
138 and *P. vivax* proteomes contain fewer asparagine residues (about 8 %) than the 4 other *Plasmodia*
139 strains (about 12%) (Supplementary Dataset 2 and Fig. S4a). Proteins were then ranked from highest
140 to lowest asparagine contents to identify those that might be impacted by decreased asparagine
141 decoding efficiency. For all proteomes, only the top 0.5% proteins were considered to search for
142 conserved proteins with the highest asparagine content. Depending on the strain, 25 to 30 proteins were
143 selected (Fig. 4). Besides many proteins with unknown functions and different AP2 domain transcription
144 factors, only 2 proteins are strictly conserved in all strains. They correspond to the CCR4-associated
145 factor-1 (CAF-1) and the poly-A binding protein-3 (PABP-3). Both share the same ligand (poly-A

146 sequence) and a peculiar modular organization. They feature a high-complexity N-terminal domain and
147 a long low-complexity C-terminal domain containing a very large amount of asparagine residues
148 (Supplementary Figs. S4b and S4c). The proportion of asparagine varies locally between 35 and 60%
149 in *P. falciparum*, *P. yoelii*, *P. chabaudi* and *P. berghei* and between 20 and 25% in *P. knowlesi* and *P.*
150 *vivax* (i.e. at least 3 times higher than their respective average usage of asparagine). Yet these two
151 proteins are not detected or by only a few spectral counts in our proteomic data (Supplementary Data
152 sets S1 and S3).

153

154 Discussion

155 To achieve efficient and specific protein synthesis, codon usage of mRNA must be balanced with
156 the availability of corresponding aminoacylated tRNAs in the cell (i.e.[10–13]). Any discrepancy can
157 affect the rate of protein elongation in ribosomes and result in pauses in translation that lead to mRNA
158 degradation [14]. It is interesting to note that blood contains reticulocytes, precursors of RBCs, which
159 are very active in translation and therefore rich in tRNAs [15]. Both reticulocytes (about 2%) and mature
160 red blood cells (RBC) can be infected by *Plasmodia*. While reticulocytes are characterized by a dynamic
161 protein synthesis, RBC are hyper-specialized anucleate cells that retain about 10 % of the protein
162 synthesis observed in reticulocytes [16]. Despite their scarcity, *P. berghei* [17], *P.chabaudi* [18], *P. yoelii*
163 [19] and *P. vivax* [20] have been shown to preferentially invade these tRNA-rich reticulocytes compared
164 to mature RBCs.

165 Moreover, by comparing the proteomes of wild-type and tRip-KO *P. berghei*, we observed in two
166 independent experiments that down-regulated proteins in the tRip-KO parasite are asparagine-rich
167 proteins. Asparagine is the most used amino acid in the *P. berghei* proteome and is often found in long
168 homorepeats [21,22]. This asparagine abundance is also found in *P. falciparum*, *P. chabaudi*, *P. yoelii*
169 and to a lesser extent in *P.knowlesi* and *P. vivax* (Supplementary Fig. S4a), however proteins in the
170 latter two parasites do not contain long asparagine repeats. Based on these results, we propose that
171 import of host tRNA^{Asn} by tRip would ensure correct translation of asparagine-rich protein domains. In
172 other words, the absence of tRNA import in the tRip-KO parasite would prevent the accumulation of host
173 tRNA^{Asn} and would thus explain why asparagine-rich proteins are poorly translated. This hypothesis is
174 strongly supported by several observations: (i) asparaginyl- and lysyl-tRNA synthetases are
175 overexpressed in the proteomes of tRip-KO schizonts (Fig. 3a, Supplementary Dataset 1). Both aaRSs

176 aminoacylate tRNAs that are most used to synthesize *P. berghei* proteins. In general, increased
177 expression of aminoacyl-tRNA synthetase correlates with a decrease in cognate aminoacyl-tRNA and/or
178 amino acid [23,24]. This observation suggests, that tRNA^{Asn} and tRNA^{Lys} levels are low in tRip-KO
179 schizonts. (ii) Furthermore, mammalian (human) tRNA^{Asn} is among the tRNAs with the highest affinity
180 for *P. falciparum* tRip, along with tRNA^{Ser_{AGA}} and tRNA^{Leu_{hGA}} (Fig. 1) [2]; It is reasonable to assume that
181 the tRNAs with the highest affinity for tRip are also those that are most efficiently imported into the
182 parasite. (iii) Finally, compensation for low tRNA^{Asn} by tRip-mediated tRNA import is only possible if the
183 host tRNA^{Asn} is a substrate for the parasite AsnRS. This is indeed the case (Fig. 2) indicating that once
184 in the parasite, at least some host tRNA^{Asn} isoacceptors can be efficiently aminoacylated and used in
185 parasite mRNA translation. We have previously shown that the *P. falciparum* AsnRS aminoacylates the
186 human tRNA^{Asn} transcript 8-fold less efficiently than its homologous *P. falciparum* transcript³⁸. In
187 contrast, in the present study, human tRNA^{Asn} is efficiently charged by the *P. falciparum* enzyme,
188 indicating that post-transcriptional modifications of human tRNA^{Asn} are positively recognized by the
189 parasite synthetase.

190 Two asparagine-rich proteins stand out and are conserved in the six *Plasmodium* species
191 analyzed (Fig. 4): the CCr4-associated factor 1 (CAF-1), involved in the poly-(A) decay of mRNAs [25],
192 and to the poly-(A) binding protein-3 (PABP3). Not only are both proteins involved in poly-(A) recognition,
193 but they also adopt a common global structure with long asparagine-rich C-terminal domains found only
194 in *Plasmodia* (Supplementary Figs. S4b and S4c). While PABP3 has not yet been characterized, CAF-
195 1 has been studied in detail in *P. falciparum*. CAF-1 is an essential nuclease that degrades mRNAs
196 enriched with non-optimally decoded codons. It belongs to the eukaryotic CCr4-Not complex, which
197 binds to the empty E-site of the ribosome and properly positions CAF-1 to initiate the decay of the 3'-
198 poly(A)-tails of stalled mRNAs [26]. However, the asparagine-rich C-terminal domain of *P. falciparum*
199 CAF-1 is not essential and it has been shown that its deletion leads to the premature release of non-
200 infectious merozoites *in vitro* [27] and to the inappropriate development of gametocytes *in vivo* [28].
201 Interestingly, disruption of tRip and of the C-terminal domain of CAF-1 not only lead to the same
202 phenotype: parasites have reduced infectivity and multiplication in the blood stage [1,27], but also results
203 in overexpression of the same gene products involved in parasite egress and invasion: the rhoptry
204 proteins, RAP1, RAP2/3 and the major surface proteins AMA-1 and MSP1 (Supplementary Dataset 3
205 and Fig. S4d). It suggests that tRip and CAF-1 may be linked in their cellular functions, i.e. in the tRip-

206 KO parasite the absence of host tRNA^{Asn} import would hinder the synthesis of the asparagine-rich C-
207 terminus of CAF-1, and lead to the specific and premature expression of mRNAs encoding the proteins
208 responsible for merozoite release and infectivity.

209 The complex life cycle of *Plasmodium* is highly regulated, involving tight transcriptional and
210 especially post-transcriptional controls each time the parasite moves from one stage to another [29,30].
211 Translational regulation of specific proteins which depends on the availability of host tRNAs would
212 enable efficient and rapid control of the transition from one stage to another during development. Failure
213 to import host tRNAs could indicate to the parasite that the cell has run out of resources and that it's
214 time to leave and infect another cell. In this respect, tissue-specific variations in tRNA levels and in their
215 post-transcriptional modifications [31,32] represent diverse sources of tRNAs for import. Depending on
216 the available tRNAs isodecoders, their concentration and their post-transcriptional modifications in the
217 host cell, tRNA import could differentially control parasite translation and enable it to develop optimally
218 not only in blood, but also in liver or mosquito. In this model, host tRNA supply could play a major role
219 in parasite development by modulating the translation efficiency of certain mRNAs, while complying with
220 the "just-in-time" translation model [29].

221

222 **Methods**

223 **Ethic Statement:** Experiments were carried out in conformity with the 2010/63/EU directive of
224 the European regulation on the protection of animals used for scientific purposes. Our animal care facility
225 received agreement #I-67-482-2 from the veterinary services of the département du Bas Rhin (Direction
226 Départementale de la Protection des Populations). All experimental protocols including the animals was
227 approved by the French ministry of higher education, research, and innovation under the agreements
228 number APAFIS#11124-2018010312571506 v2. All experiments were performed in accordance with
229 the ARRIVE guidelines.

230 **Parasite production:** Four- to six-week-old female mice (C57BL/6), weighing approximately 20
231 g, were injected intraperitoneally with 200 µL of frozen infected red blood cells (10-15% parasitemia
232 diluted in phosphate-buffered saline (PBS)) either with wild-type (WT, *Pb* gfpGOMO14) or with tRip-KO
233 (*Pb* tRip-KO mCherry) malaria parasites derived from the *P. berghei* (ANKA strain). Parasitemia was
234 monitored daily by cytometry (BD Accuri C6). Mice with parasitemia between 5 and 10% were selected.
235 Therefore, in this study, only mice without or low symptoms were used. These parasitemia levels were

236 reached 3-6 days after parasite injection. Mice were put to sleep and blood was collected by intracardiac
237 puncture (about 1 to 1.5 mL) and parasites were directly purified (all blood stages) or cultured for 24
238 hours in RPMI at 37°C under 5% CO₂ (only schizonts) (Supplementary Fig. S1b) as described in [33].

239 infected blood was filtered through a Plasmodipur filter (Europroxima) to remove mouse
240 leukocytes, centrifuged for 10 min at 450 g and recovered in 4 mL of RPMI. A 7.2 mL cushion of 60%
241 isotonic Percoll was gently pipetted under the red blood cells and the tube was centrifuged for 20 min
242 at 1450 g (swinging buckets), to separate infected red blood cells (iRBC) at the Percoll/RPMI interface
243 from non-infected RBC at the bottom of the tube. Parasitized RBCs were recovered in 2 tubes, washed
244 3 times in 1 mL of PBS and combined with 200 µL each of PBS. Infected RBCs were then lysed with
245 0.02% saponin for 5 min in ice (in 400 µL). Free parasites were recovered by centrifugation for 5 min at
246 2000 g and washed in 500 µL of PBS. The two pellets were resuspended either in 50 µL of protein
247 loading buffer and stored at -80°C until mass spectrometry analysis, or as is and placed at -80°C for
248 RNA preparation.

249 **Mass spectrometry (MS) and data analyses.** Approximately 10 µg of protein was obtained from
250 the half of the blood (~0.75 mL of a 5-10% infected mouse). Protein concentrations were determined by
251 Bradford assay using bovine serum albumin as the standard. Proteins were precipitated, reduced, and
252 alkylated as described in [3]. After digestion overnight with sequencing grade porcine trypsin (300 ng,
253 w/w, Promega, Fitchburg, MA, USA), the generated peptides were analyzed either using Easy-nanoLC-
254 1000 system coupled to a Q-Exactive Plus mass spectrometer (Thermo Fisher Scientific, Germany) with
255 160-minutes gradients (blood stages) or a NanoLC-2DPlus system (nanoFlexChiP module; Eksigent,
256 ABSciex, Concord, Ontario, Canada) coupled to a TripleTOF 5600 mass spectrometer (ABSciex)
257 operating in positive mode with 120-minutes gradients (schizonts). Data were searched using the
258 Mascot algorithm (version 2.6.2, Matrix Science) against the Uniprot database with *P. Berghei* taxonomy
259 (release 2021_03, 4 927 sequences) with a decoy strategy. The resulting .dat Mascot files were then
260 imported into Proline version 2.0 software [34] to align the identified proteins. Proteins were then
261 validated with Mascot pretty rank equal to 1, and 1% false discovery rate (FDR) on both peptide
262 spectrum matches (PSM) and protein sets (based on Mascot score). As expected for blood cells from
263 infected mice, the number of contaminating mouse proteins represents between 40 and 50% of the
264 proteins identified by mass spectrometry (Sup datasets 1 and 3).

265 For statistical analyses, raw Spectral Count values were imported into R (v. 3.5.0) where the
266 number of spectra were first normalized using the DESeq2 median of ratio normalization method. A
267 negative-binomial test using an edgeR GLM regression generated for each identified protein a p-value
268 and a protein fold-change (FC). The R script used to process the dataset is published on Github [35].
269 Proteins were statistically enriched or decreased with a p-value < 0.05 and a minimum fold change (FC)
270 of 2 or 0.5, respectively. Mass spectrometry proteomic data has been deposited within the
271 ProteomeXchange Consortium via the PRIDE partner repository [36] with the dataset ID PXD043916
272 (Username: reviewer_pxd043916@ebi.ac.uk, Password: xu4rkrPE).

273 **RNA purification and QRT-PCR.** Total RNA was extracted from parasites using the RNeasy
274 Mini Kit (Qiagen) according to the manufacturer's protocol and was subjected to DNase treatment with
275 the Rapid OUT DNA removal kit (Thermo Scientific). Total RNA was analyzed and quantified on
276 Bioanalyser (puce PICO). Each sample was reverse transcribed in a 20 μ L reaction volume containing
277 10 μ L (0.16 to 0.5 μ g) of RNA, using the SuperScript II reverse transcriptase (Invitrogen) according to
278 the manufacturer's protocol. The mRNAs levels were measured by RT-PCR (25 μ L containing 4 μ L of
279 cDNA) on a CFX94 (Bio-Rad) using the Syber Green kit (Thermo Scientific). Oligonucleotides used for
280 qRT-PCR are listed in the Supplementary Fig. S3a. qRT-PCR reactions were designed according MIQE
281 guidelines [37], the specificity of the oligonucleotides was validated, and the amplification efficiencies of
282 the primer sets are all between 90 and 110% and r^2 values greater than 0.96. The mRNAs levels were
283 calculated according to the Δ Cq method and normalized by the mRNA level of both the EF1- α and
284 Hsp70 in each sample. Raw data are indicated as mean of three measurements, and results were
285 expressed as the average of 3 biological samples \pm standard error of the mean (SEM).

286 **Purification of aminoacyl-tRNA synthetases and aminoacylation assays.** Recombinant *P.*
287 *falciparum* and *H. sapiens* NRS and DRS were cloned, expressed and purified as described in
288 references [38,39] respectively. The gene encoding the *P. falciparum* TyrRS was amplified by PCR from
289 *P. falciparum* cDNA and cloned into pQE30 (Qiagen) and the plasmid encoding the *H. sapiens* YRS
290 was a gift from P. Schimmel. Both *P. falciparum* and *H. sapiens* YRS were expressed and purified as
291 described for NRS [38]. Purified enzymes are shown in Supplementary Fig. S2. *H. sapiens* crude tRNAs
292 was prepared as described in [2].

293 Aminoacylation assays were performed under the same conditions: at 37 °C for 2, 4 and 6 min in
294 50 mM HEPES-KOH (pH 7.5), 20 mM KCl, 10 mM MgCl₂, 2 mM ATP, 6 μ M total tRNA from HeLa cells,

295 in the presence of 20 μ M of the corresponding L-[¹⁴C]-amino acid (Perkin Elmer). AaRSs were diluted
296 in 100 mM HEPES-KOH pH 7.5, 1 mM DTT, 5 mg/mL BSA, and 10% glycerol and used at a final
297 concentration of 200 nM in the aminoacylation assay. The aminoacylation plateaus are the mean of 3
298 independent experiments \pm standard deviation (SD).

299 **Bioinformatics.** Protein sequences as well as proteomes from all *Plasmodium* strains were
300 retrieved from PlasmoDB. A home-made Python 2.7 script was used to calculate the asparagine content
301 for each protein sequence. *Toxoplasma gondii* was the outgroup species.

302

303 **References**

- 304 [1] T. Bour, N. Mahmoudi, D. Kapps, S. Thiberge, D. Bargieri, R. Ménard, M. Frugier, *Apicomplexa* -
305 specific tRip facilitates import of exogenous tRNAs into malaria parasites, *Proc Natl Acad Sci USA*
306 113 (2016) 4717–4722. <https://doi.org/10.1073/pnas.1600476113>.
- 307 [2] M. Cela, A. Théobald-Dietrich, J. Rudinger-Thirion, P. Wolff, R. Geslain, M. Frugier, Identification
308 of host tRNAs preferentially recognized by the *Plasmodium* surface protein tRip, *Nucleic Acids*
309 *Research* (2021) gkab769. <https://doi.org/10.1093/nar/gkab769>.
- 310 [3] J.R. Jaramillo Ponce, D. Kapps, C. Paulus, J. Chicher, M. Frugier, Discovery of two distinct
311 aminoacyl-tRNA synthetase complexes anchored to the Plasmodium surface tRNA import protein,
312 *Journal of Biological Chemistry* 298 (2022) 101987. <https://doi.org/10.1016/j.jbc.2022.101987>.
- 313 [4] J.R. Jaramillo Ponce, A. Théobald-Dietrich, P. Bénas, C. Paulus, C. Sauter, M. Frugier, Solution
314 X-ray scattering highlights discrepancies in *Plasmodium* MULTI-AMINOACYL-TRNA synthetase
315 complexes, *Protein Science* 32 (2023). <https://doi.org/10.1002/pro.4564>.
- 316 [5] S. Gupta, J. Chhibber-Goel, M. Sharma, S. Parvez, K. Harlos, A. Sharma, M. Yogavel, Crystal
317 structures of the two domains that constitute the Plasmodium vivax p43 protein, *Acta Crystallogr*
318 *D Struct Biol* 76 (2020) 135–146. <https://doi.org/10.1107/S2059798319016413>.
- 319 [6] K. Deinert, F. Fasiolo, E.C. Hurt, G. Simos, Arc1p Organizes the Yeast Aminoacyl-tRNA
320 Synthetase Complex and Stabilizes Its Interaction with the Cognate tRNAs, *Journal of Biological*
321 *Chemistry* 276 (2001) 6000–6008. <https://doi.org/10.1074/jbc.M008682200>.
- 322 [7] A.M. Minns, K.J. Hart, S. Subramanian, S. Hafenstein, S.E. Lindner, Nuclear, Cytosolic, and
323 Surface-Localized Poly(A)-Binding Proteins of Plasmodium yoelii, *mSphere* 3 (2018).
324 <https://doi.org/10.1128/mSphere.00435-17>.

- 325 [8] S.-J. Cha, M.-S. Kim, A. Pandey, M. Jacobs-Lorena, Identification of GAPDH on the surface of
326 Plasmodium sporozoites as a new candidate for targeting malaria liver invasion, *Journal of*
327 *Experimental Medicine* 213 (2016) 2099–2112. <https://doi.org/10.1084/jem.20160059>.
- 328 [9] C. Aurrecochea, M. Heiges, H. Wang, Z. Wang, S. Fischer, P. Rhodes, J. Miller, E. Kraemer, C.J.
329 Stoeckert, D.S. Roos, J.C. Kissinger, ApiDB: integrated resources for the apicomplexan
330 bioinformatics resource center, *Nucleic Acids Research* 35 (2007) D427–D430.
331 <https://doi.org/10.1093/nar/gkl880>.
- 332 [10] E.M. Novoa, L. Ribas de Pouplana, Speeding with control: codon usage, tRNAs, and ribosomes,
333 *Trends in Genetics* 28 (2012) 574–581. <https://doi.org/10.1016/j.tig.2012.07.006>.
- 334 [11] J.D. Richter, J. Collier, Pausing on Polyribosomes: Make Way for Elongation in Translational
335 Control, *Cell* 163 (2015) 292–300. <https://doi.org/10.1016/j.cell.2015.09.041>.
- 336 [12] M. Torrent, G. Chalancon, N.S. de Groot, A. Wuster, M. Madan Babu, Cells alter their tRNA
337 abundance to selectively regulate protein synthesis during stress conditions, *Sci. Signal.* 11 (2018)
338 eaat6409. <https://doi.org/10.1126/scisignal.aat6409>.
- 339 [13] P.C. Dedon, T.J. Begley, Dysfunctional tRNA reprogramming and codon-biased translation in
340 cancer, *Trends in Molecular Medicine* 28 (2022) 964–978.
341 <https://doi.org/10.1016/j.molmed.2022.09.007>.
- 342 [14] Q. Wu, S.G. Medina, G. Kushawah, M.L. DeVore, L.A. Castellano, J.M. Hand, M. Wright, A.A.
343 Bazzini, Translation affects mRNA stability in a codon-dependent manner in human cells, *eLife* 8
344 (2019) e45396. <https://doi.org/10.7554/eLife.45396>.
- 345 [15] D.W.E. Smith, R.F. Randazzo, A.L. McNamara, The tRNA content of non-hemoglobinized red cell
346 precursors: Evidence that tRNA content is controlled by tRNA utilization, *Biochemical and*
347 *Biophysical Research Communications* 95 (1980) 468–473. [https://doi.org/10.1016/0006-](https://doi.org/10.1016/0006-291X(80)90761-5)
348 [291X\(80\)90761-5](https://doi.org/10.1016/0006-291X(80)90761-5).
- 349 [16] S.D. Kumar, D. Kar, M.N. Akhtar, B. Willard, D. Roy, T. Hussain, P.I. Rajyaguru, S.M. Eswarappa,
350 Evidence for low-level translation in human erythrocytes, *MBoC* 33 (2022) br21.
351 <https://doi.org/10.1091/mbc.E21-09-0437>.
- 352 [17] D. Cromer, K.J. Evans, L. Schofield, M.P. Davenport, Preferential invasion of reticulocytes during
353 late-stage *Plasmodium berghei* infection accounts for reduced circulating reticulocyte levels,

354 International Journal for Parasitology 36 (2006) 1389–1397.
355 <https://doi.org/10.1016/j.ijpara.2006.07.009>.

356 [18] R. Antia, A. Yates, J.C. De Roode, The dynamics of acute malaria infections. I. Effect of the
357 parasite's red blood cell preference, *Proc. R. Soc. B.* 275 (2008) 1449–1458.
358 <https://doi.org/10.1098/rspb.2008.0198>.

359 [19] B. Mons, Preferential invasion of malarial merozoites into young red blood cells, *Blood Cells* 16
360 (1990) 299–312.

361 [20] N. Thawani, M. Tam, M.-J. Bellemare, D.S. Bohle, M. Olivier, J.B. De Souza, M.M. Stevenson,
362 Plasmodium Products Contribute to Severe Malarial Anemia by Inhibiting Erythropoietin-Induced
363 Proliferation of Erythroid Precursors, *The Journal of Infectious Diseases* 209 (2014) 140–149.
364 <https://doi.org/10.1093/infdis/jit417>.

365 [21] S.R. Chaudhry, N. Lwin, D. Phelan, A.A. Escalante, F.U. Battistuzzi, Comparative analysis of low
366 complexity regions in Plasmodia, *Sci Rep* 8 (2018) 335. [https://doi.org/10.1038/s41598-017-](https://doi.org/10.1038/s41598-017-18695-y)
367 [18695-y](https://doi.org/10.1038/s41598-017-18695-y).

368 [22] F.U. Battistuzzi, K.A. Schneider, M.K. Spencer, D. Fisher, S. Chaudhry, A.A. Escalante, Profiles
369 of low complexity regions in Apicomplexa, *BMC Evol Biol* 16 (2016) 47.
370 <https://doi.org/10.1186/s12862-016-0625-0>.

371 [23] Harald Putzer, Laalami Soumaya, Regulation of the Expression of Aminoacyl-tRNA Synthetases
372 and Translation Factors, in: *Madame Curie Bioscience Database*, Landes Bioscience, 2000.

373 [24] O. Levi, S. Garin, Y. Arava, RNA mimicry in post-transcriptional regulation by aminoacyl tRNA
374 synthetases, *WIREs RNA* 11 (2020). <https://doi.org/10.1002/wrna.1564>.

375 [25] M.A. Collart, The Ccr4-Not complex is a key regulator of eukaryotic gene expression, *WIREs RNA*
376 7 (2016) 438–454. <https://doi.org/10.1002/wrna.1332>.

377 [26] R. Buschauer, Y. Matsuo, T. Sugiyama, Y.-H. Chen, N. Alhusaini, T. Sweet, K. Ikeuchi, J. Cheng,
378 Y. Matsuki, R. Nobuta, A. Gilmozzi, O. Berninghausen, P. Tesina, T. Becker, J. Coller, T. Inada,
379 R. Beckmann, The Ccr4-Not complex monitors the translating ribosome for codon optimality,
380 *Science* 368 (2020) eaay6912. <https://doi.org/10.1126/science.aay6912>.

381 [27] B. Balu, S.P. Maher, A. Pance, C. Chauhan, A.V. Naumov, R.M. Andrews, P.D. Ellis, S.M. Khan,
382 J. Lin, C.J. Janse, J.C. Rayner, J.H. Adams, CCR4-Associated Factor 1 Coordinates the

- 383 Expression of Plasmodium falciparum Egress and Invasion Proteins, *Eukaryot Cell* 10 (2011)
384 1257–1263. <https://doi.org/10.1128/EC.05099-11>.
- 385 [28] K.J. Hart, J. Oberstaller, M.P. Walker, A.M. Minns, M.F. Kennedy, I. Padykula, J.H. Adams, S.E.
386 Lindner, Plasmodium male gametocyte development and transmission are critically regulated by
387 the two putative deadenylases of the CAF1/CCR4/NOT complex, *PLoS Pathog* 15 (2019)
388 e1007164. <https://doi.org/10.1371/journal.ppat.1007164>.
- 389 [29] S.S. Vembar, D. Droll, A. Scherf, Translational regulation in blood stages of the malaria parasite
390 *Plasmodium spp.* : systems-wide studies pave the way: Translational regulation in blood stages of
391 the malaria parasite, *WIREs RNA* 7 (2016) 772–792. <https://doi.org/10.1002/wrna.1365>.
- 392 [30] S. Bennink, G. Pradel, The molecular machinery of translational control in malaria parasites, *Mol*
393 *Microbiol* 112 (2019) 1658–1673. <https://doi.org/10.1111/mmi.14388>.
- 394 [31] K.A. Dittmar, J.M. Goodenbour, T. Pan, Tissue-Specific Differences in Human Transfer RNA
395 Expression, *PLoS Genet* 2 (2006) e221. <https://doi.org/10.1371/journal.pgen.0020221>.
- 396 [32] O. Pinkard, S. McFarland, T. Sweet, J. Collier, Quantitative tRNA-sequencing uncovers metazoan
397 tissue-specific tRNA regulation, *Nat Commun* 11 (2020) 4104. [https://doi.org/10.1038/s41467-](https://doi.org/10.1038/s41467-020-17879-x)
398 [020-17879-x](https://doi.org/10.1038/s41467-020-17879-x).
- 399 [33] C. Ramakrishnan, M.J. Delves, K. Lal, A.M. Blagborough, G. Butcher, K.W. Baker, R.E. Sinden,
400 Laboratory Maintenance of Rodent Malaria Parasites, in: R. Ménard (Ed.), *Malaria*, Humana
401 Press, Totowa, NJ, 2012: pp. 51–72. https://doi.org/10.1007/978-1-62703-026-7_5.
- 402 [34] D. Bouyssié, A.-M. Hesse, E. Mouton-Barbosa, M. Rompais, C. Macron, C. Carapito, A. Gonzalez
403 de Peredo, Y. Couté, V. Dupierris, A. Burel, J.-P. Menetrey, A. Kalaitzakis, J. Poisat, A. Romdhani,
404 O. Bulet-Schiltz, S. Cianférani, J. Garin, C. Bruley, Proline: an efficient and user-friendly software
405 suite for large-scale proteomics, *Bioinformatics* 36 (2020) 3148–3155.
406 <https://doi.org/10.1093/bioinformatics/btaa118>.
- 407 [35] L. Kuhn, T. Vincent, P. Hammann, H. Zuber, Exploring Protein Interactome Data with IPInquiry:
408 Statistical Analysis and Data Visualization by Spectral Counts, in: T. Burger (Ed.), *Statistical*
409 *Analysis of Proteomic Data*, Springer US, New York, NY, 2023: pp. 243–265.
410 https://doi.org/10.1007/978-1-0716-1967-4_11.
- 411 [36] Y. Perez-Riverol, J. Bai, C. Bandla, D. García-Seisdedos, S. Hewapathirana, S. Kamatchinathan,
412 D.J. Kundu, A. Prakash, A. Frericks-Zipper, M. Eisenacher, M. Walzer, S. Wang, A. Brazma, J.A.

413 Vizcaíno, The PRIDE database resources in 2022: a hub for mass spectrometry-based proteomics
414 evidences, *Nucleic Acids Research* 50 (2022) D543–D552. <https://doi.org/10.1093/nar/gkab1038>.

415 [37] S.A. Bustin, V. Benes, J.A. Garson, J. Hellems, J. Huggett, M. Kubista, R. Mueller, T. Nolan,
416 M.W. Pfaffl, G.L. Shipley, J. Vandesompele, C.T. Wittwer, The MIQE Guidelines: Minimum
417 Information for Publication of Quantitative Real-Time PCR Experiments, *Clinical Chemistry* 55
418 (2009) 611–622. <https://doi.org/10.1373/clinchem.2008.112797>.

419 [38] D. Filisetti, A. Théobald-Dietrich, N. Mahmoudi, J. Rudinger-Thirion, E. Candolfi, M. Frugier,
420 Aminoacylation of *Plasmodium falciparum* tRNA^{Asn} and Insights in the Synthesis of Asparagine
421 Repeats, *Journal of Biological Chemistry* 288 (2013) 36361–36371.
422 <https://doi.org/10.1074/jbc.M113.522896>.

423 [39] T. Bour, A. Akaddar, B. Lorber, S. Blais, C. Balg, E. Candolfi, M. Frugier, Plasmodial aspartyl-
424 tRNA synthetases and peculiarities in *Plasmodium falciparum*, *The Journal of Biological Chemistry*
425 284 (2009) 18893–903.

426 [40] P.P. Chan, T.M. Lowe, GtRNADB 2.0: an expanded database of transfer RNA genes identified in
427 complete and draft genomes, *Nucleic Acids Research* 44 (2016) D184–D189.
428 <https://doi.org/10.1093/nar/gkv1309>.

429

430

431 **Aknowledgments**

432 We are grateful to Philippe Hammann, Lauriane Kuhn, and Béatrice Chane Woon Ming for LC-
433 MS/MS analysis, Fabrice Auge and Eric Marois for animal experiments, and to Dr Alain Lescure and
434 Prof Tamara Hendrickson for providing comments on this manuscript.

435 This work was performed under the framework of the Interdisciplinary Thematic Institute IMCBio,
436 as part of the ITI 2021-2028 program of the University of Strasbourg, CNRS and Inserm. It was
437 supported by IdEx Unistra (ANR-10-IDEX-0002), by SFRI-STRAT'US project (ANR 20-SFRI-0012), and
438 EUR IMCBio (IMCBio ANR-17-EURE-0023) under the framework of the French Investments for the
439 Future Program », by previous Labex NetRNA (ANR-10-LABX-0036), by the CNRS and the Université
440 de Strasbourg, IdEx "Equipement mi-lourd" (2015) and Equipement d'Excellence (EquipEx) I2MC (ANR-
441 11-EQPX-0022), and by the Fondation pour la Recherche Médicale (FRM) (grant number
442 FDT201704337050) to Marta Cela.

443

444 **Authors Contributions**

445 MP, DK and MC performed experiments, data acquisition, and analysis, JC performed mass
446 spectrometry analysis and LD provided computer programming. MF managed the conception, design,
447 interpretation of data and funding acquisition and wrote, reviewed, and edited the manuscript.

448

449 **Data availability statement**

450 All relevant data are within the manuscript and its Supporting Information files.

451

452 **Additional information**

453 **Competing Interest Statement:** The authors declare no conflict of interest.

454 1 pdf with 4 Supplementary Figures and 1 excel document containing 3 supplementary Data sets.

455

456

457 **Figure legends**

458 **Figure 1. *P. berghei* membrane-bound multi-synthetase complexes.** *Plasmodium* is
459 characterized by the presence of two multisynthetase complexes (MSC) named Q-complex and M-
460 complex. The Q-complex is composed of glutamyl- (ERS) and glutaminy- (QRS) tRNA synthetases
461 linked to a dimer of tRip whereas the M-complex is composed of tRip and Methionyl-tRNA synthetase
462 (MRS) organized around a dimer of ERS. tRip is therefore an AIMP (Aminoacyl-tRNA synthetase
463 Interacting Multifunctional Protein). tRip, ERS, QRS, and MRS are schematized and colored in grey,
464 black, cyan, and orange, respectively. the GST-like domains are shown as a drop and the C-terminal
465 domains of tRip, QRS and MRS involved in tRNA binding are either EMAPII-like domains (tRip and
466 MRS, grey diamonds) or a positively charged α -helix (QRS, shown as a grey helix). The characteristic
467 feature of *Plasmodium* MSCs is that tRip is a membrane protein (the transmembrane helix is shown in
468 red) with the GST-like domain required for MSC formation localized inside the parasite and the tRNA
469 binding domain exposed outside the parasite to host tRNAs. This unique organization justifies that only
470 the EMAPII-like domain of tRip (tRip₂₀₀₋₄₀₂) is fused at the C-terminal domain of a GST domain and used
471 as a target for the selection of aptamers capable of inhibiting tRip tRNA binding. Interfaces 1, 1' or 2,
472 involved in protein-protein interactions, are indicated in the corresponding GST-like domains. For the
473 sake of simplicity, this illustration does not show the parasitophorous membrane that surrounds the
474 parasite in the infected red blood cell.

475 **Figure 2. Cross aminoacylation of host tRNAs by the parasite aminoacyl-tRNA**
476 **synthetases.** Six aaRSs, aspartyl-, asparaginyl- and tyrosyl-tRNA synthetases from *H. sapiens* (in blue)
477 or *P. falciparum* (in orange) were tested with crude human tRNAs under the same experimental
478 conditions (6 μ M crude tRNA and 0.2 μ M aaRSs). Aminoacylation plateaus were measured at 6 min of
479 incubation. Human crude tRNAs are potentially transcribed from 420 genes, amongst which 19 genes
480 encode tRNA^{Asp}, 34 genes encoding tRNA^{Asn} and 15 genes encoding tRNA^{Tyr} [40]. Error bars represent
481 the standard deviation (SEM) of three independent experiments.

482 **Figure 3. Comparative proteomic analysis and amino acid usage of proteins identified in**
483 **WT and tRip-KO samples containing schizonts (a and b) or all blood stages (c and d).** (a) Volcano
484 plot of all quantified proteins from WT and tRip-KO parasites displaying the relationship between
485 statistical significance ($-\log_{10}(p\text{-value})$, y-axis) and log fold change (FC) of each protein ($\log_2(\text{FC})$, x-
486 axis). Statistics are based on three independent experiments (Supplementary Dataset 1). Deregulated

487 proteins in tRip-KO parasite compared to the WT parasite are shown in orange (up-regulated, $FC \geq 2$ and
488 $p\text{-value} \leq 0.05$) and green (down-regulated, $FC \leq -1$ and $p\text{-value} \leq 0.05$). tRip is shown in red and black
489 dots represent proteins with no significant change. ERS, KRS and NRS correspond to glutamyl-, lysyl-
490 and asparaginyI-tRNA synthetases; ERS and NRS are shown as black circles surrounded by green and
491 orange, respectively because their $p\text{-values}$ are slightly greater than 0.05. **(b)** Comparison of amino acid
492 usage (%) of proteins whose expression is stable in the tRip-KO parasite (the 100 most expressed
493 proteins, black) and all proteins that are down-regulated in the tRip-KO parasite (green). Amino acids
494 are designated by their one letter symbol and the total number of amino acids used in the analyses is
495 specified. **(c)** Volcano plot of all quantified proteins from WT and tRip-KO parasites. Statistics are based
496 on three independent experiments (Supplementary Dataset 3). **(d)** Comparison of amino acid usage (%)
497 between proteins whose expression is stable in the tRip-KO parasite (the 150 most expressed proteins,
498 black) and proteins down-regulated in the tRip-KO parasite (green).

499 **Figure 4. Top 0.5% of the most asparagine-rich proteins in 6 *Plasmodium* strains.** Six
500 *Plasmodium* species were selected: *P. falciparum*, *P. yoelii*, *P. berghei*, *P. chabaudi*, all featuring high
501 asparagine usage (around 12%, Supplementary Fig. S4a) often localized in homorepetitions and *P.*
502 *knowlesi* and *P. vivax* which show lower asparagine usage (around 8%, Supplementary Fig. S4a) and
503 no homorepeats. Proteins highlighted in yellow are proteins of unknown function, light green indicate
504 AP2 family regulatory proteins and dark green show the two proteins conserved in all 6 species,
505 regardless of their asparagine usage.

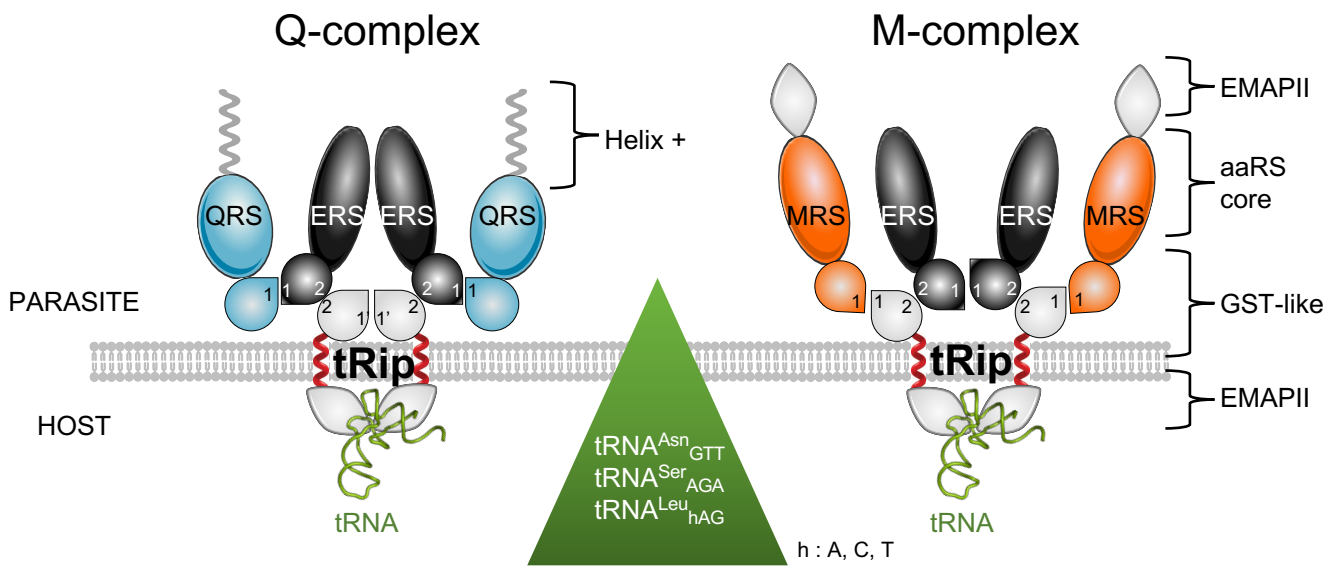


Fig. 1

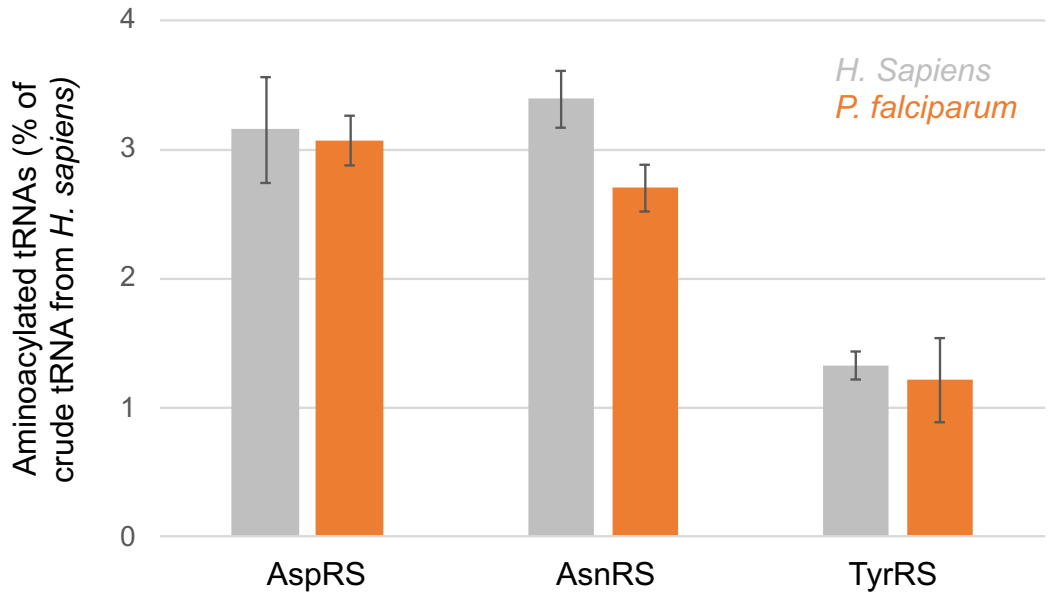


Fig. 2

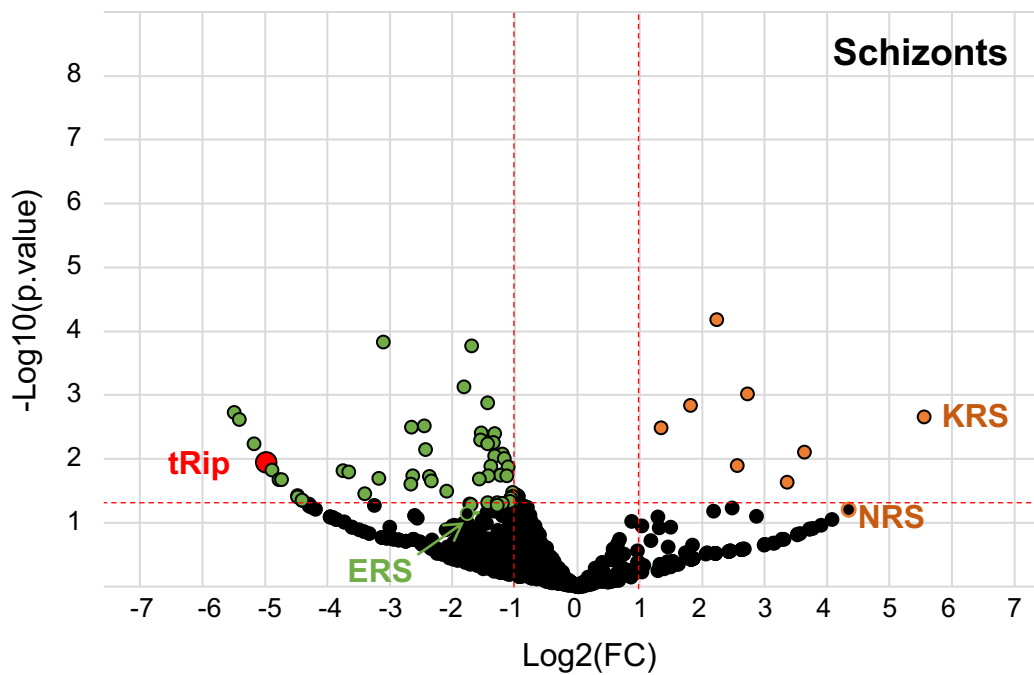
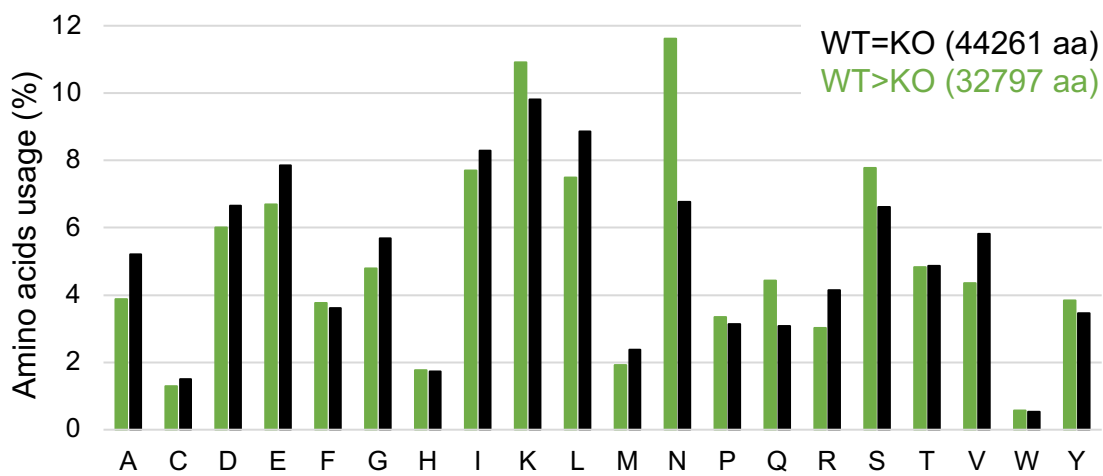
a**b**

Fig. 3

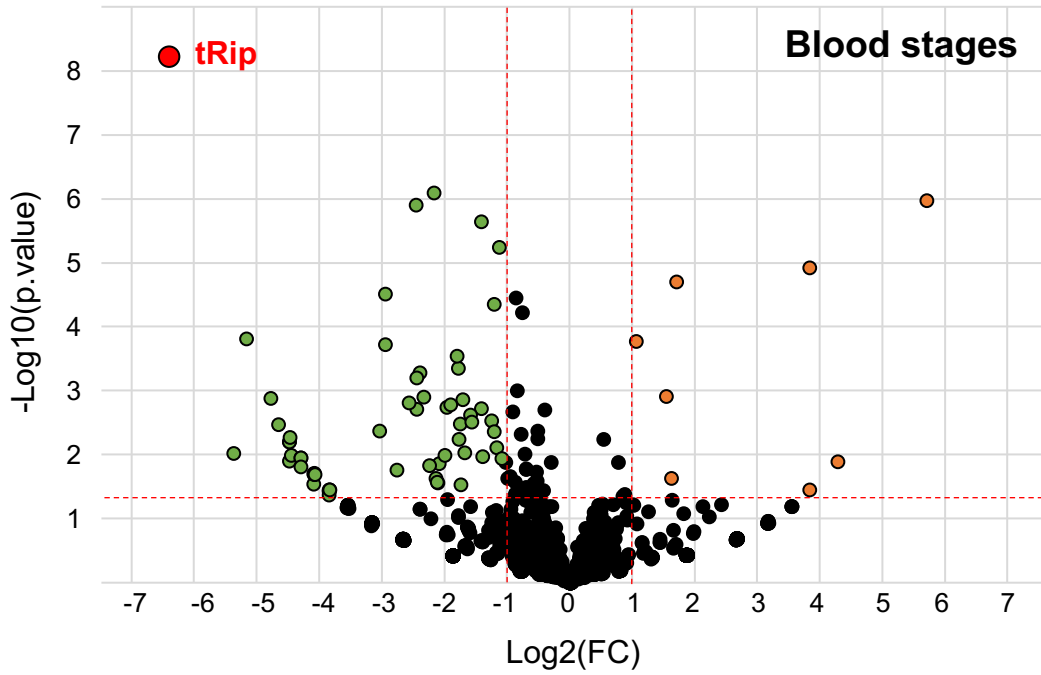
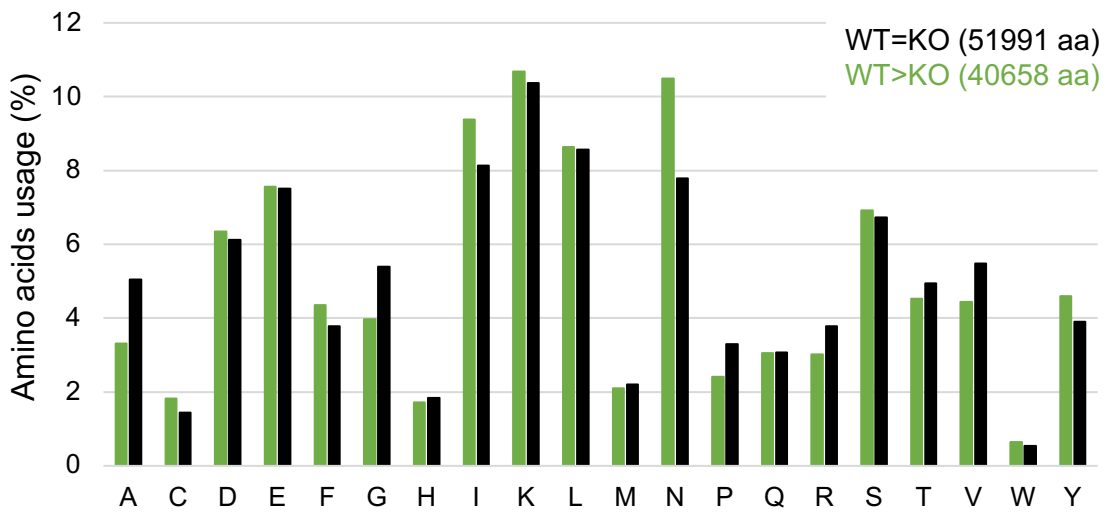
c**d**

Fig. 3

<i>P. falciparum</i>			
Protein id	Prot length	Asn (%)	function
PF3D7_0923500	357	42.017	cyclin-dependent kinases regulatory subunit
PF3D7_1366900	685	34.891	conserved protein, unknown function
PF3D7_0218200	807	33.333	SUZ domain-containing protein, putative
PF3D7_1439500	1074	32.961	oocyst rupture protein 2, putative
PF3D7_0706500	1706	32.298	conserved Plasmodium protein, unknown function
PF3D7_0811300	1774	32.187	CCR4-associated factor 1
PF3D7_0611800	3248	31.989	conserved Plasmodium protein, unknown function
PF3D7_1249700	908	31.608	conserved Plasmodium protein, unknown function
PF3D7_0817300	2235	31.588	conserved Plasmodium protein, unknown function
PF3D7_1236100	445	31.236	clustered-asparagine-rich protein
PF3D7_0525300	481	31.185	conserved protein, unknown function
PF3D7_0323800	977	31.116	conserved Plasmodium protein, unknown function
PF3D7_0212100	1844	31.074	conserved Plasmodium protein, unknown function
PF3D7_1329600	301	30.897	conserved Plasmodium protein, unknown function
PF3D7_1473500	675	30.667	conserved Plasmodium protein, unknown function
PF3D7_0926100	3178	30.585	protein kinase, putative
PF3D7_0411000	1570	30.382	AP2 domain transcription factor AP2-Z
PF3D7_1146800	3188	30.332	conserved Plasmodium protein, unknown function
PF3D7_1337500	3334	30.324	conserved Plasmodium protein, unknown function
PF3D7_1317200	2672	30.202	AP2 domain transcription factor AP2-FG
PF3D7_0602000	1437	29.923	zinc finger protein, putative
PF3D7_1222600	2432	29.852	AP2 domain transcription factor AP2-G
PF3D7_0906600	843	29.775	zinc finger protein, putative
PF3D7_1205500	1785	29.636	zinc finger protein, putative
PF3D7_0629400	780	29.487	polyadenylate-binding protein 3, putative
PF3D7_0304600	397	29.471	circumsporozoite (CS) protein

<i>P. yoelii</i>			
Protein id	Prot length	Asn (%)	function
PY17X_1428300	1807	30.825	CCR4-associated factor 1, putative
PY17X_1125700	535	30.654	polyadenylate-binding protein 3
PY17X_0111700	2872	28.482	conserved Plasmodium protein, unknown function
PY17X_1225100	169	28.402	conserved Plasmodium protein, unknown function
PY17X_1213800	261	27.969	rhopty associated adhesin, putative
PY17X_1341400	610	27.869	conserved Plasmodium protein, unknown function
PY17X_1334500	967	27.611	AP2 domain transcription factor AP2-SP, putative
PY17X_0929400	122	27.049	heat shock factor-binding protein 1
PY17X_1453200	411	26.764	clustered-asparagine-rich protein, putative
PY17X_1356400	1912	26.569	conserved Plasmodium protein, unknown function
PY17X_0309500	987	26.444	conserved Plasmodium protein, unknown function
PY17X_1307200	960	26.354	oocyst rupture protein 2, putative
PY17X_1110800	1966	26.144	KH domain-containing protein, putative
PY17X_0614700	1230	25.528	apicomplexan kinetochore protein 3, putative
PY17X_1313200	552	25.362	RNA-binding protein, putative
PY17X_1334300	2085	25.324	conserved Plasmodium protein, unknown function
PY17X_0103500	483	25.259	RNA-binding protein, putative
PY17X_1440000	2341	25.16	AP2 domain transcription factor AP2-G
PY17X_0806800	1567	25.144	conserved Plasmodium protein, unknown function
PY17X_0315400	830	25.06	SUZ domain-containing protein, putative
PY17X_1417400	2502	25.02	AP2 domain transcription factor AP2-SP, putative
PY17X_1403500	312	25	LiSH domain-containing protein, putative
PY17X_0930400	329	24.924	conserved Plasmodium protein, unknown function
PY17X_1426800	201	24.876	U1 small nuclear ribonucleoprotein C, putative
PY17X_1454600	1693	24.867	conserved Plasmodium protein, unknown function
PY17X_1205700	1022	24.853	E3 ubiquitin-protein ligase, putative
PY17X_0903500	3240	24.846	sporozoite and liver stage asparagine-rich protein
PY17X_1457300	1863	24.745	RNA-binding protein, putative
PY17X_1465200	746	24.665	conserved Plasmodium protein, unknown function
PY17X_0943000	142	24.648	conserved Plasmodium protein, unknown function

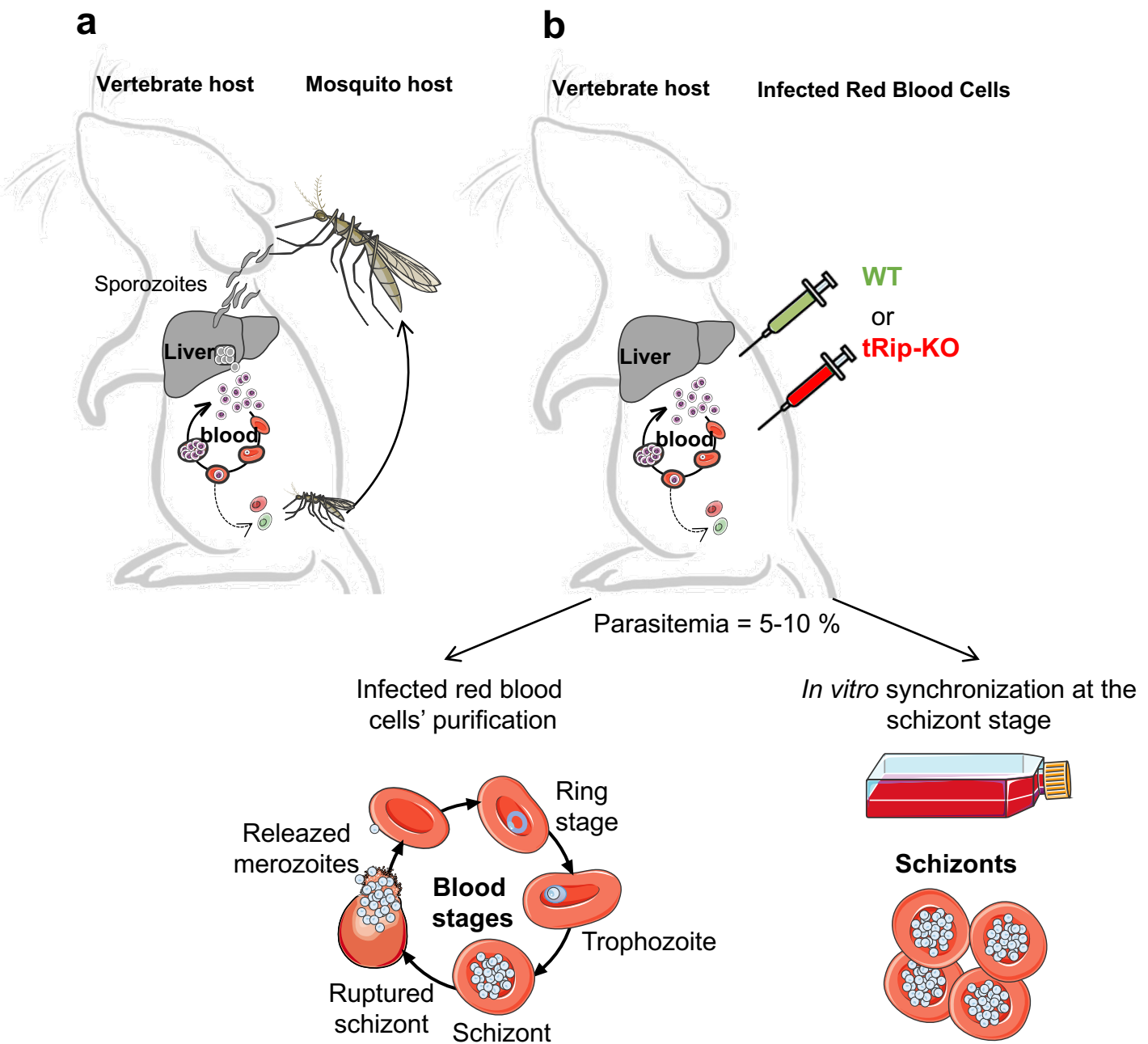
<i>P. berghei</i>			
Protein id	Prot length	Asn (%)	function
PBANKA_1426200	1801	30.372	CCR4-associated factor 1
PBANKA_0927400	122	28.689	heat shock factor-binding protein 1
PBANKA_0928400	358	28.492	conserved Plasmodium protein, unknown function
PBANKA_1112300	511	27.984	polyadenylate-binding protein 3
PBANKA_1210600	256	27.734	rhopty associated adhesin, putative
PBANKA_1303400	875	27.429	oocyst rupture protein 2
PBANKA_0612200	1251	27.338	apicomplexan kinetochore protein 3
PBANKA_0939600	3296	27.306	polyadenylate-binding protein-interacting protein 1
PBANKA_1336700	607	27.183	conserved Plasmodium protein, unknown function
PBANKA_1329600	2115	27.139	conserved Plasmodium protein, unknown function
PBANKA_1107200	677	27.031	protein CAF40, putative
PBANKA_1245900	412	26.942	Plasmodium exported protein, unknown function
PBANKA_0110100	2611	26.848	conserved Plasmodium protein, unknown function
PBANKA_1351300	1864	26.019	conserved Plasmodium protein, unknown function
PBANKA_0101900	478	25.941	RNA-binding protein, putative
PBANKA_1100600	54	25.926	PIR protein, pseudogene
PBANKA_1329800	943	25.875	AP2 domain transcription factor AP2-SP
PBANKA_0804100	1572	25.509	conserved Plasmodium protein, unknown function
PBANKA_0103300	2033	25.43	GYF domain-containing protein, putative
PBANKA_0308900	918	25.272	conserved Plasmodium protein, unknown function
PBANKA_1450700	397	25.189	clustered-asparagine-rich protein, putative
PBANKA_0604500	2264	25.044	high mobility group protein B3, putative
PBANKA_1415700	2487	24.568	AP2 domain transcription factor AP2-FG
PBANKA_1109700	1930	24.508	KH domain-containing protein, putative
PBANKA_0514300	947	24.498	conserved Plasmodium protein, unknown function

<i>P. chabaudi</i>			
Protein id	Prot length	Asn (%)	function
PCHAS_1428000	1775	29.972	CCR4-associated factor 1
PCHAS_1341300	641	29.173	conserved Plasmodium protein, unknown function
PCHAS_0915900	329	28.875	conserved Plasmodium protein, unknown function
PCHAS_0916900	122	27.869	heat shock factor-binding protein 1, putative
PCHAS_1127700	510	27.647	polyadenylate-binding protein 3
PCHAS_1211300	246	26.423	rhopty associated adhesin, putative
PCHAS_1306600	957	26.332	oocyst rupture protein 2, putative
PCHAS_0804400	1528	26.113	conserved Plasmodium protein, unknown function
PCHAS_0110700	2594	25.867	conserved Plasmodium protein
PCHAS_1426400	533	25.328	protein KIC7, putative
PCHAS_1453000	396	25.253	clustered-asparagine-rich protein, putative
PCHAS_0613900	1198	25.209	apicomplexan kinetochore protein 3, putative
PCHAS_1334400	984	24.695	AP2 domain transcription factor AP2-SP
PCHAS_1142300	556	24.46	conserved protein, unknown function
PCHAS_0311100	953	24.449	conserved Plasmodium protein, unknown function
PCHAS_0625600	336	24.405	fam-c protein
PCHAS_0514400	948	24.262	conserved Plasmodium protein, unknown function
PCHAS_1417500	2390	24.226	AP2 domain transcription factor AP2-FG
PCHAS_0703300	3131	24.082	sporozoite and liver stage asparagine-rich protein
PCHAS_1454400	1526	24.05	conserved Plasmodium protein, unknown function
PCHAS_0102600	488	23.975	RNA-binding protein, putative
PCHAS_1109400	1837	23.925	KH domain-containing protein
PCHAS_1464900	726	23.416	conserved Plasmodium protein, unknown function
PCHAS_0520100	1543	23.396	conserved Plasmodium protein
PCHAS_0413300	893	23.292	RING finger protein RNF1, putative
PCHAS_1457100	1728	23.09	RNA-binding protein, putative

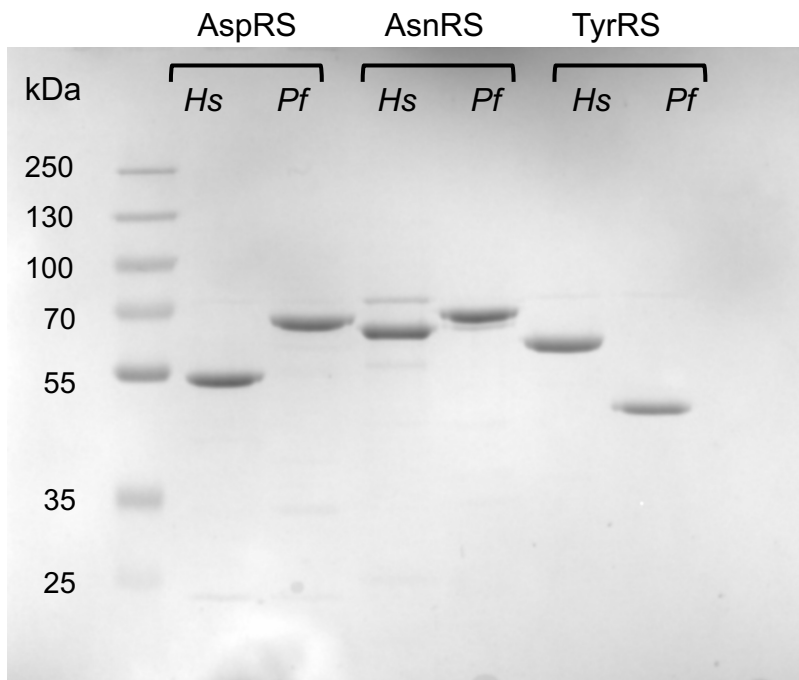
<i>P. knowlesi</i>			
Protein id	Prot length	Asn (%)	function
PKNH_0515300	239	23.013	apical asparagine-rich protein AARP
PKNH_API05400	97	21.649	hypothetical protein
PKNH_0934400	741	20.783	conserved Plasmodium protein, unknown function
PKNH_1427000	689	20.174	conserved protein, unknown function
PKNH_1245200	334	20.06	trailer hitch homolog, putative
PKNH_1236600	775	19.613	RNA-binding protein, putative
PKNH_1429400	1971	19.533	CCR4-associated factor 1, putative
PKNH_API04400	125	19.2	apicoplast ribosomal protein L11, putative
PKNH_0826500	1208	19.123	zinc finger protein, putative
PKNH_1427400	615	19.024	protein KIC7, putative
PKNH_1007500	471	18.684	conserved protein, unknown function
PKNH_0301400	75	18.667	RNA-binding protein, putative
PKNH_0812000	281	18.505	rhopty associated adhesin, putative
PKNH_1430100	908	18.392	ATP-dependent RNA helicase DBP1, putative
PKNH_1215100	1094	18.282	AP2 domain transcription factor AP2-EXP, putative
PKNH_1007800	880	18.182	zinc finger protein, putative
PKNH_0101500	143	18.182	conserved Plasmodium protein, unknown function
PKNH_1455800	474	18.143	clustered-asparagine-rich protein, putative
PKNH_API02500	89	17.978	apicoplast ribosomal protein S19, putative
PKNH_1348500	497	17.706	CUGBP Elav-like family member 2, putative
PKNH_1264600	147	17.687	parasitophorous vacuolar protein 3, putative
PKNH_0811000	119	17.647	BET1-like protein, putative
PKNH_0917400	721	17.614	conserved Plasmodium protein, unknown function
PKNH_1120100	644	17.547	polyadenylate-binding protein 3, putative
PKNH_API03300	80	17.5	hypothetical protein
PKNH_API04900	103	17.476	hypothetical chloroplast reading frame 93, putative
PKNH_0827400	1201	17.402	RING finger protein RNF1, putative
PKNH_API05300	1021	17.042	DNA-directed RNA polymerase subunit beta, putative
PKNH_0621400	1861	17.034	conserved Plasmodium protein, unknown function

<i>P. vivax</i>			
Protein id	Prot length	Asn (%)	function
PVP01_0937100	779	24.904	conserved Plasmodium protein, unknown function
PVP01_1001950	86	22.093	PIR protein, pseudogene
PVP01_API04400	97	21.649	hypothetical protein
PVP01_0531900	297	20.539	apical asparagine-rich protein AARP
PVP01_1426900	699	19.886	conserved protein, unknown function
PVP01_API01500	89	19.101	apicoplast ribosomal protein S19, putative
PVP01_1269800	341	19.062	trailer hitch homolog, putative
PVP01_API02300	80	18.75	hypothetical protein
PVP01_0421700	91	18.681	conserved protein, unknown function
PVP01_0811300	118	18.644	BET1-like protein, putative
PVP01_API03400	125	18.4	apicoplast ribosomal protein L11, putative
PVP01_API01200	202	18.317	S05 ribosomal protein L4, putative
PVP01_1401600	205	18.049	Plasmodium exported protein, unknown function
PVP01_1218000	139	17.986	parasitophorous vacuolar protein 3, putative
PVP01_1429000	2030	17.98	CCR4-associated factor 1, putative
PVP01_0705100	842	17.815	zinc finger protein, putative
PVP01_API04100	975	17.744	DNA-directed RNA polymerase subunit beta ¹ , putative
PVP01_1429700	923	17.66	ATP-dependent RNA helicase DBP1, putative
PVP01_API03900	121	17.355	hypothetical chloroplast reading frame 93, putative
PVP01_API04300	1021	17.336	DNA-directed RNA polymerase subunit beta, putative
PVP01_1404300	1063	17.31	LiSH domain-containing protein, putative
PVP01_1339100	503	17.296	CUGBP Elav-like family member 2, putative
PVP01_1120100	670	17.164	polyadenylate-binding protein 3, putative
PVP01_0010790	112	16.964	PIR protein
PVP01_1427300	661	16.944	protein KIC7, putative
PVP01_0103500	143	16.783	conserved Plasmodium protein, unknown function
PVP01_API02500	132	16.667	apicoplast ribosomal protein S11, putative
PVP01_1001850	42	16.667	PIR protein, pseudogene
PVP01_1008800	1006	16.501	zinc finger protein, putative
PVP01_1420400	75	16	conserved protein, unknown function

Fig. 4



Supplementary Fig. S1. Mice infections and sample preparation. (a) *Plasmodium* life cycle. The cycle of vertebrate infection starts when a mosquito injects sporozoites into the host. Sporozoites invade hepatocytes (liver stage), multiply, and produce tens of thousands of merozoites per infected hepatocyte¹. Merozoites exit the liver and develop in red blood cells (blood stages) to produce 10 to 30 new merozoites per intra-erythrocytic cycle². Some of the erythrocytic merozoites differentiate into gametocytes (sexual forms, dashed lines)³. Fertilization takes place in mosquitoes where gametocytes are ingested during a blood meal. In 8-15 days, sporozoites invade the mosquito salivary glands, completing the cycle. (b) Alternatively, mice are infected with frozen stocks of red blood cells infected with wild-type (GFP) or tRip-KO (mCherry) *P. berghei* parasites. Three to six days later (5-10% parasitemia), infected blood is collected and used as is for analysis (all blood stages) or synchronized *in vitro* to the schizont stage⁴. "All blood stages" samples contain rings, trophozoites and merozoites.



Supplementary Fig. S2. Gel analysis of aminoacyl-tRNA synthetases. One μg of each recombinant enzyme aspartyl- (DRS), asparaginyl- (NRS) and tyrosyl-(YRS) tRNA synthetases from *H. sapiens* (*Hs*) or *P. falciparum* (*Pf*) was run on a 10 % SDS PAGE.

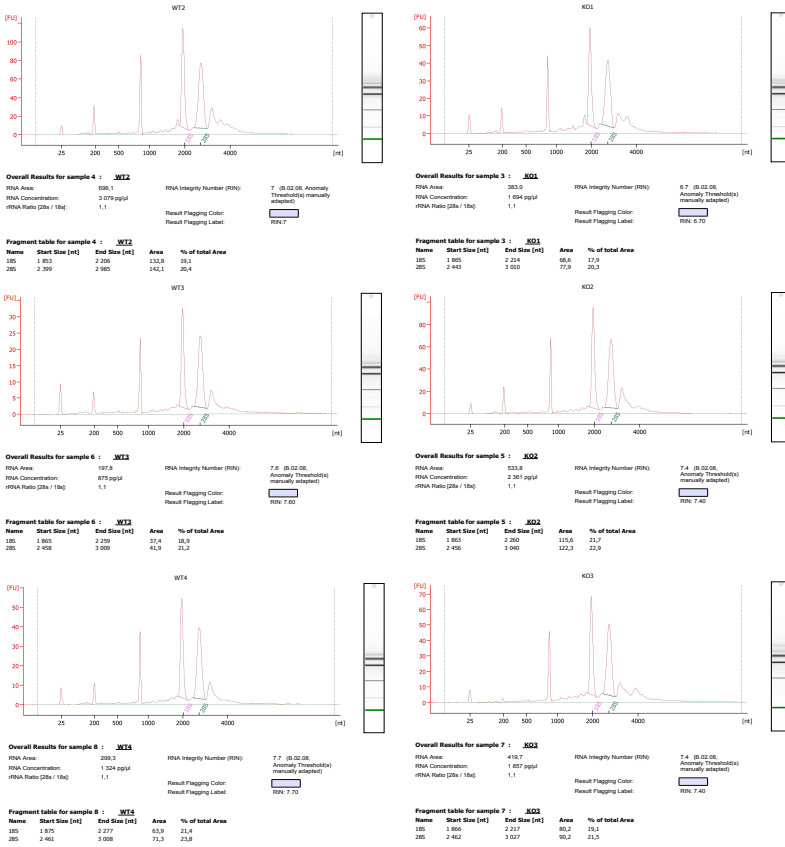
a

Genotype	Sample	Average Ct (triplicate)			ΔCt			Efficiency			Mean	RO	Mean RO	SD	SEM
		MCM7	Hsp70	EF1-α	MCM7	Hsp70	EF1-α	MCM7	Hsp70	EF1-α					
WT	2	19.82	19.77	17.86	-1.10	-0.59	0.88	2.14	1.50	0.54	0.90	2.37			
WT	3	20.73	21.54	18.21	-0.19	1.18	-0.77	1.14	0.44	1.71	0.87	1.32	1.77	0.49	0.28
WT	4	20.79	20.86	17.50	-0.13	0.60	0.52	1.09	0.66	0.70	0.68	1.62			
KO	1	21.87	19.99	16.73	0.95	-0.37	-0.25	0.52	1.29	1.19	1.24	0.42	0.62	0.25	0.14
KO	2	21.27	19.75	16.30	0.35	-0.61	-0.68	0.78	1.52	1.60	1.56	0.50			
KO	4	21.04	20.14	17.27	0.12	-0.22	0.29	0.92	1.16	0.82	0.97	0.94			
Mean		20.92	20.36	16.98											<i>p</i> -value = 0.03

Genotype	Sample	Average Ct (triplicate)			ΔCt			Efficiency			Mean	RO	Mean RO	SD	SEM
		ChAF1-C	Hsp70	EF1-α	ChAF1-C	Hsp70	EF1-α	ChAF1-C	Hsp70	EF1-α					
WT	2	17.89	19.37	15.75	-3.29	-2.68	-2.32	9.75	6.42	4.98	5.65	1.72	2.70	0.88	0.51
WT	3	24.23	26.49	23.51	3.06	4.44	5.45	0.12	0.05	0.02	0.03	3.70			
WT	4	21.34	23.53	19.75	0.16	1.48	1.69	0.89	0.36	0.31	0.33	2.67			
KO	1	23.80	23.57	16.11	2.63	1.52	-1.96	0.16	0.35	3.88	1.16	0.14	0.48	0.27	0.15
KO	2	19.23	18.95	15.72	-1.95	-3.10	-2.35	3.85	8.59	5.08	6.61	0.58			
KO	4	20.56	20.41	17.55	-0.62	-1.64	-0.52	1.53	3.12	1.43	2.11	0.72			
Mean		21.18	22.05	16.07											<i>p</i> -value = 0.02

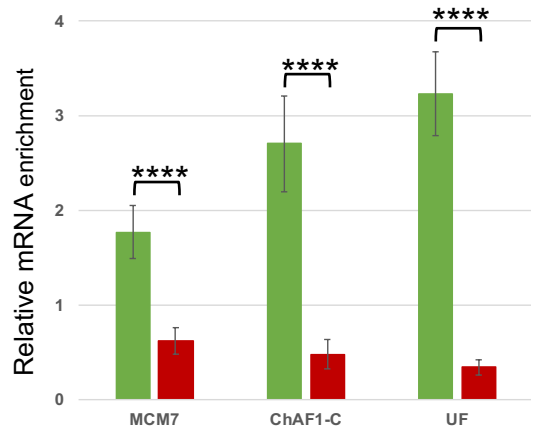
Genotype	Sample	Average Ct (triplicate)			ΔCt			Efficiency			Mean	RO	Mean RO	SD	SEM
		UF	Hsp70	EF1-α	UF	Hsp70	EF1-α	UF	Hsp70	EF1-α					
WT	2	19.91	19.78	16.19	-2.09	-0.81	-0.72	4.26	1.53	1.54	1.58	2.68			
WT	3	21.13	21.74	17.98	-0.87	1.35	1.07	1.83	0.39	0.48	0.43	4.23	3.23	0.77	0.44
WT	4	20.98	20.92	17.30	-1.02	0.53	0.39	2.03	0.69	0.76	0.73	2.79			
KO	1	24.04	19.92	16.70	2.04	-0.47	-0.21	0.24	1.39	1.15	1.27	0.19	0.34	0.14	0.08
KO	2	22.68	19.47	16.91	0.68	-0.93	-0.90	0.62	1.90	1.86	1.88	0.53			
KO	4	23.26	20.54	17.26	1.26	0.15	0.35	0.42	0.90	0.78	0.84	0.50			
Mean		22.00	20.40	16.91											<i>p</i> -value = 0.0046

RNA analyses



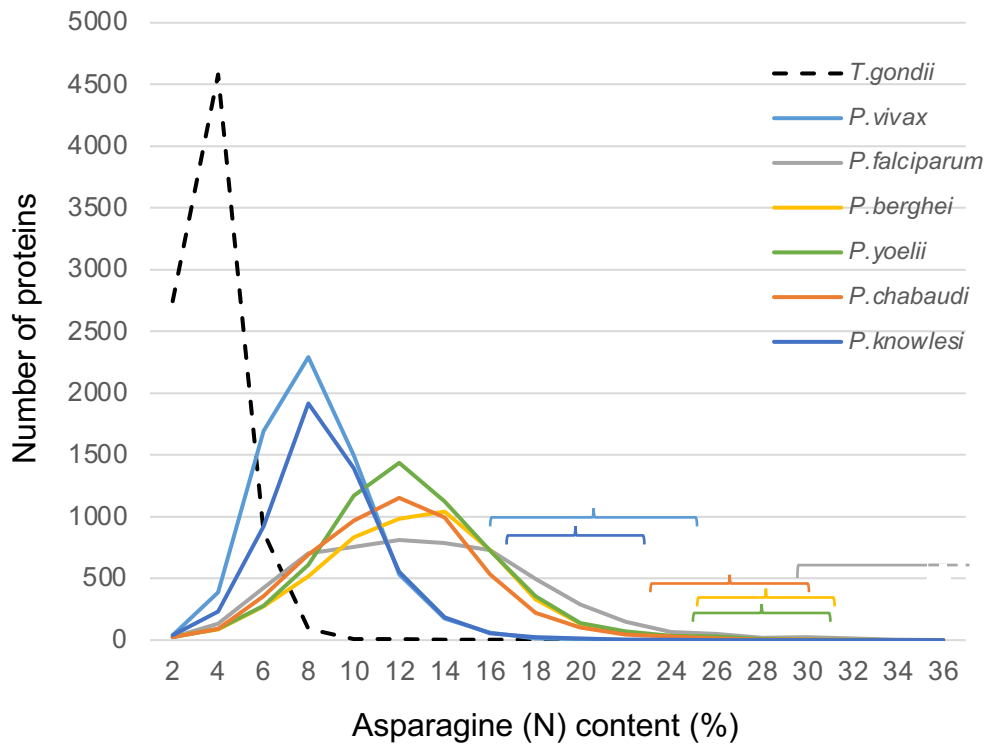
Gene ID and name (expression status in tRip-KO)	Primers	Primer efficiency	r ²
PBANKA_0803100 DNA replication licensing factor MCM7 (down regulated)	Forward CAACCATTTCCCTGAAC Reverse CACTATCTCTACCCCTGCC	105.4 %	0.965
	this study		
PBANKA_0203000 chromatin assembly 1 (down regulated)	Forward GGACATTTTGCATCGAAG Reverse GCATCTAAGCTTCACCGAT	99.8 %	0.98
	this study		
PBANKA_1029400 unknown function (down regulated)	Forward TAGCTACTTGTTCACGCCAA Reverse TGAGGCCCTTTAATACCTCTGT	96.0 %	0.990
	this study		
Reference genes			
PBANKA_1133300 EF1-α	Forward TGGACCCACCCAAAGACCA Reverse ACAACAGCAGATGGAGCGAA	100.7 %	1
	Tokunaga et al., 2019		
PBANKA_0914400 Hsp70	Forward AGAGACGACGTGAACACGC Reverse TCCCTTTAATAATCATGGC	106.9 %	0.999
	Sanyal et al., 2013		

b

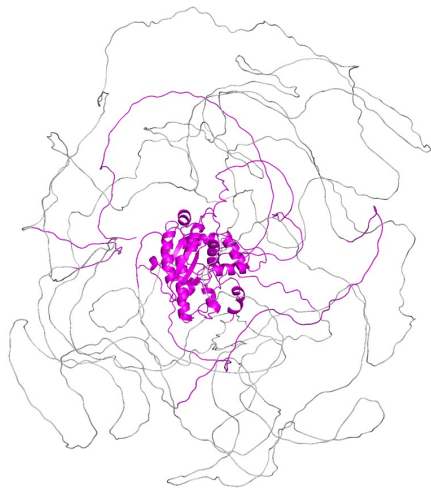


Supplementary Fig. S3. qRT-PCR analysis of the mRNAs coding for deregulated proteins in the tRip-KO parasite.

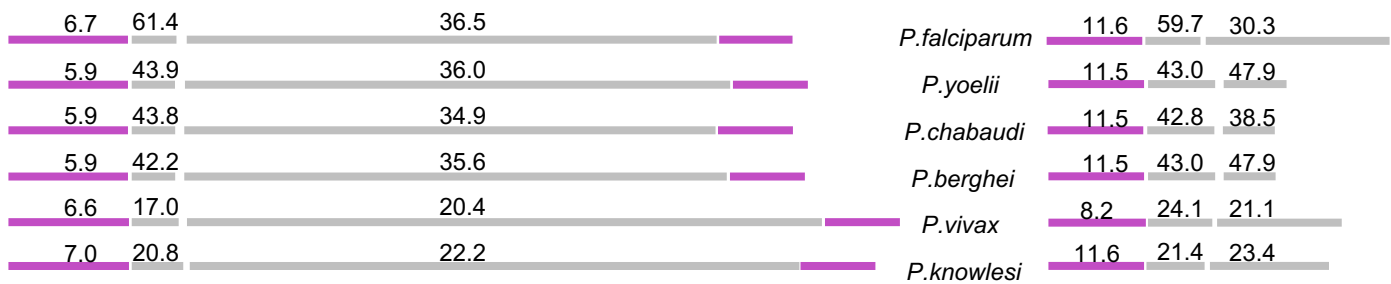
The relative enrichment of mRNA was measured by qRT-PCR and determined by the $\Delta\Delta C_t$ method using both EF1- α (PBANKA_1133300) and Hsp70 (PBANKA_0914400) as normalizers. mRNAs are coding for down-regulated proteins: the DNA replication licensing factor MCM7 (MCM7, FC = 5.7, *p*-value = 1.3×10^{-6}), chromatin assembly factor 1-subunit C (ChAF1-C, FC = 2.2, *p*-value = 5.7×10^{-6}) and a protein with unknown function (UF, FC = 3.6, *p*-value = 2.9×10^{-4}). (a) QRT-PCR raw data. For each “all blood stages” samples, qRT-PCR data the primers as well as the quality control of the RNA samples are shown. (b) Error bars represent the standard errors of the means (SEM) of three independent experiments performed on “All blood stages” samples, each corresponding to triplicate qRT-PCR measurements (S4 Table). Asterisks indicate statistically significant differences with the WT control mRNA: **** *p*-value < 0.0001 based on Student’s *t* test.

a**b**

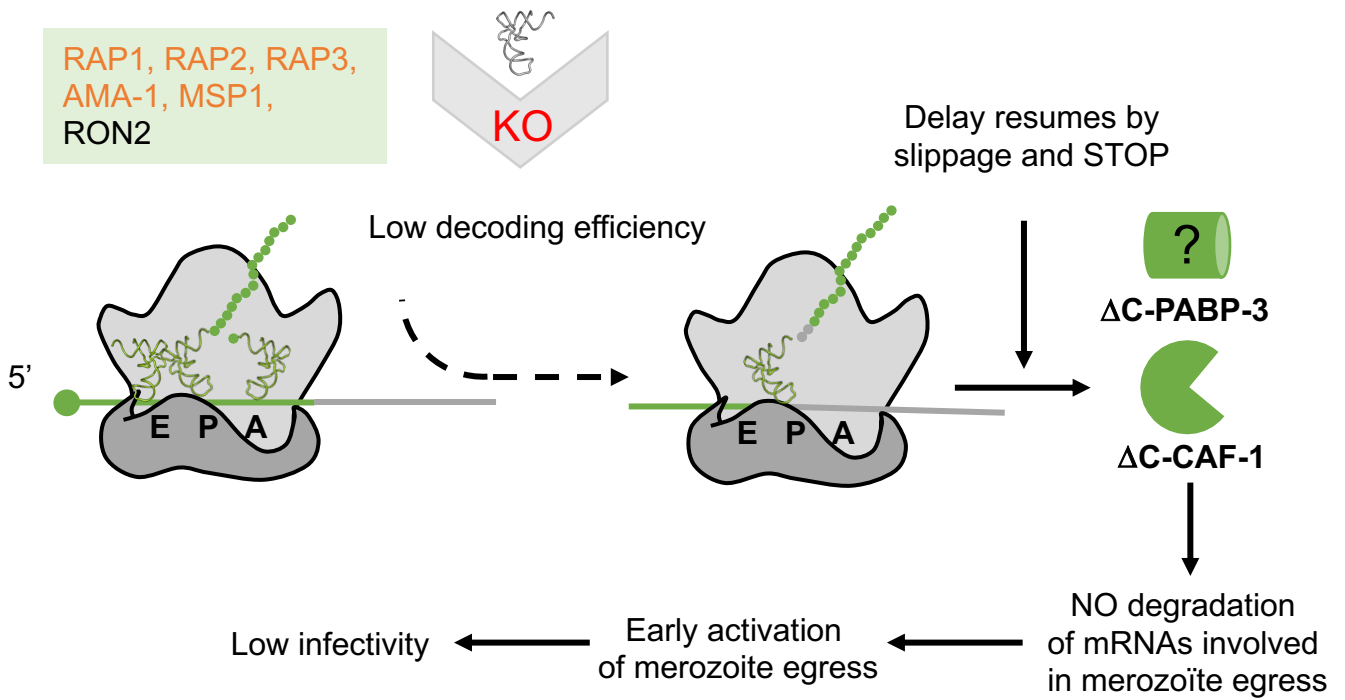
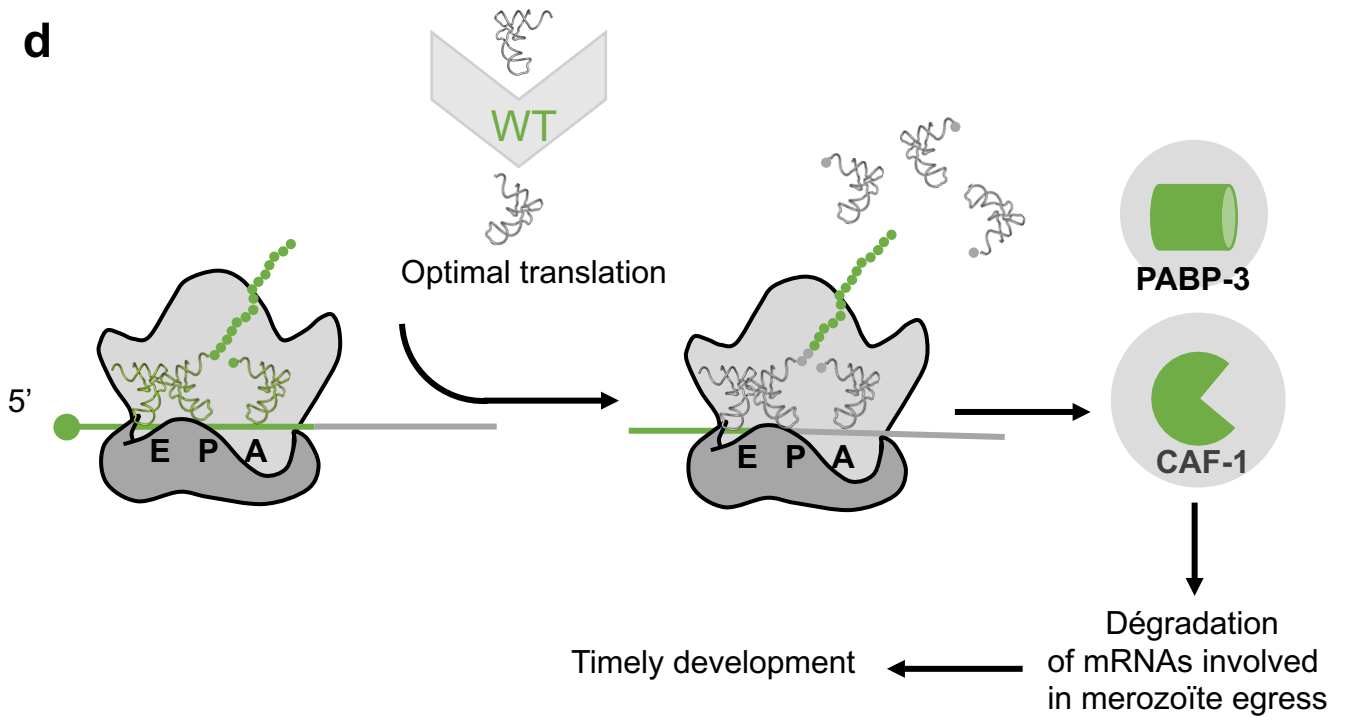
CAF-1 (*P. falciparum*)
AF-C0H4T9



PABP-3 (*P. falciparum*)
AF-C6KTC9

**c**

d



RAP1, RAP2, RAP3, AMA-1, MSP1, RhopH2, RhopH3, GAP50, MSP2, EBA140, EBA175, EBA181, P12, P38, GAP45, MTIP, MyoA, SUB1, CDPK5

Supplementary Fig. S4. Asparagine frequency in *Plasmodium* proteins and functional link between tRip and CAF-1. (a) Proteomes of *P. falciparum*, *P. yoelii*, *P. berghei*, *P. chabaudi*, *P. knowlesi* and *P. vivax* were compared and the proteome of *Toxoplasma gondii* was used as a negative control⁵. For each species, the number of proteins is indicated for a given asparagine content. Brackets show the top 0.5 % proteins containing the highest percentage of asparagine residues. (b) AlphaFold models of *P. falciparum* CAF-1 and PABP-3 (10). Both models show conserved domains (pink) and an unfolded asparagine-rich *Plasmodium* specific C-terminal extensions (grey). (c) Domain alignments of CAF-1 and PABP-3. The corresponding domains are shown with the same color code and the corresponding percentage of asparagine. (d) In WT parasites, the asparagine-rich C-terminal domains of *Plasmodium* CAF-1 and PABP-3 are efficiently translated thanks to tRNA^{Asn} provided by tRip-mediated tRNA import from the host. Full-length CAF-1 can thus down-regulate specific mRNAs including those involved in egress and invasion that adequately control parasite development. On the contrary, in the tRip-KO parasite, translation of the C-terminal domains of CAF-1 and PABP-3 is hindered by the absence of host tRNA^{Asn}. The delay in translation can lead to frameshifting and the occurrence of stop codons. The resulting DC-CAF-1 protein can no longer regulate the translation of genes involved in parasite egress/invasion leading to early release of infectivity-deficient immature merozoites. Genes up-regulated in both mutants are highlighted in orange, they correspond to roptry-associated proteins 1, 2 and 3 (RAP1, PF3D7_1410400/PBANKA_1032100; RAP2/3, PF3D7_0501600/0501500, PBANKA_1101400), apical membrane antigen (AMA1, PF3D7_1133400/PBANKA_0915000) and merozoite surface protein 1 (MSP1, PF3D7_0930300/PBANKA_0831000). Up-regulated genes involved in merozoite's egress either in *P. falciparum* Δ C-CAF1 mutant or *P. berghei* tRip-KO parasite are listed inside green boxes: genes not in common between both strains are indicated in black: merozoite surface protein 2 (MSP2, PF3D7_0206800), glideosome associated protein 50 (GAP50, PF3D7_0918000/PBANKA_0819000), high molecular weight roptry proteins 2 and 3 (RhopH2, PF3D7_0929400/PBANKA_0830200 and RhopH3, PF3D7_0905400/PBANKA0416000) and Rhoptry neck protein 2 (RON2, PBANKA_1315700); Ten genes were up-regulated in the Δ C-CAF-1 mutant but were not detectable in blood stages proteomes. They correspond to: erythrocyte binding antigens 140, 175 and 181 (EBA140, PF3D7_1301600; EBA175, PF3D7_0731500; EBA181, PF3D7_0102500), 6-cysteine proteins P12 and P38 (PF3D7_0612700 and PF3D7_0508000), glideosome-associated protein 45 (GAP45, PF3D7_1222700), myosin A-tail interaction protein (MTIP, PF3D7_1246400/PBANKA_1459500), myosin A (MyoA, PF3D7_1342600), subtilisin-like protease 1 (SUB1, PF3D7_0507500) and calcium-dependent protein kinase 5 (CDPK5, PF3D7_1337800).

References for supplementary figures

1. Rankin, K. E., Graewe, S., Heussler, V. T. & Stanway, R. R. Imaging liver-stage malaria parasites. *Cellular Microbiology* **12**, 569–579 (2010).
2. Cowman, A. F., Tonkin, C. J., Tham, W.-H. & Duraisingh, M. T. The Molecular Basis of Erythrocyte Invasion by Malaria Parasites. *Cell Host & Microbe* **22**, 232–245 (2017).
3. Talman, A. M., Clain, J., Duval, R., Ménard, R. & Ariey, F. Artemisinin Bioactivity and Resistance in Malaria Parasites. *Trends in Parasitology* **35**, 953–963 (2019).
4. Ramakrishnan, C. *et al.* Laboratory Maintenance of Rodent Malaria Parasites. in *Malaria* (ed. Ménard, R.) vol. 923 51–72 (Humana Press, 2012).
5. Amos, B. *et al.* VEuPathDB: the eukaryotic pathogen, vector and host bioinformatics resource center. *Nucleic Acids Research* **50**, D898–D911 (2022).