A Plasmodium falciparum C-mannosyltransferase is dispensable for parasite asexual blood stage development

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Abstract

C-mannosylation was recently identified in the thrombospondin-related anonymous protein (TRAP) from Plasmodium falciparum salivary gland sporozoites. A candidate P. falciparum C-mannosyltransferase (PfDPY-19) was demonstrated to modify thrombospondin type 1 repeat (TSR) domains in vitro, exhibiting a different acceptor specificity than their mammalian counterparts. According to the described minimal acceptor of PfDPY19, several TSR domain-containing proteins of P. falciparum could be C-mannosylated in vivo. However, the relevance of this protein modification for the parasite viability remains unknown. In the present study, we used CRISPR/Cas9 technology to generate a PfDPY19 null mutant, demonstrating that this glycosyltransferase is not essential for the asexual blood development of the parasite. PfDPY19 gene disruption was not associated with a growth phenotype, not even under endoplasmic reticulum-stressinducing conditions that could impair protein folding. The data presented in this work strongly suggest that PfDPY19 is unlikely to play a critical role in the asexual blood stages of the parasite, at least under in vitro conditions.

Introduction

Malaria remains one of the most serious global infectious diseases. Only in 2017, it was responsible for 219 million cases and 435,000 deaths worldwide (World Health Organization, 2018). It is generally accepted that malaria elimination from the remaining endemic countries will not be possible with currently available strategies (Alonso et al., 2011). In this regard, a better understanding of the numerous gaps of knowledge in the complex biology of the parasite would facilitate the rationale design of new interventions.

The thrombospondin type 1 repeat (TSR) superfamily is a diverse group of proteins often involved in interactions with the extracellular matrix (Tucker, 2004; Morahan et al., 2009). Particularly, the functional characterization of P. falciparum TSR domain-containing proteins revealed the essential roles that these effectors play in processes involved in cell invasion, egress and transmission of the parasite through the mosquito stages. Thus, circumsporozoite- and TRAP-related protein (CTRP) is essential for ookinete invasion of the mosquito midgut epithelium and oocyst development (Dessens et al., 1999), whereas thrombospondin-related protein 1 is required for the subsequent sporozoite egress from oocytes (Klug and Frischknecht, 2017). TRAP, circumsporozoite protein (CSP) and TRAP-related protein are all involved in the sporozoite invasion of the mosquito salivary glands; in the vertebrate host, CSP and TRAP also bind to the hepatocyte surface during sporozoite invasion (Ménard et al., 1997; Sultan et al., 1997; Combe et al., 2009). Finally, TRAP-like protein and thrombospondin-related sporozoite protein have been described to play an important role in hepatocyte cell traversal (Labaied et al., 2007; Moreira et al., 2008). Other TSR domain-containing proteins are expressed in the blood stages of the parasite. Previously described to be essential for the merozoite invasion of erythrocytes (Baum et al., 2006), more recent studies revealed that the merozoite TRAP family protein (MTRAP) is completely dispensable for the viability of asexual parasites. Instead, MTRAP was demonstrated to be essential for gametocyte egress and, thus, for transmission to the mosquito (Bargieri et al., 2016). On the other hand, the thrombospondin-related apical merozoite protein (TRAMP) seems to be involved in the invasion of erythrocytes, since antibodies raised against this protein block merozoite invasion (Siddiqui et al., 2013). Interestingly, a secreted protein with altered thrombospondin repeat domain (SPATR) is expressed at multiple stages of the parasite such as sporozoites, asexual blood stages and gametocytes. Antibodies raised against SPATR block hepatocyte invasion by sporozoites; however, the biological relevance of SPATR during other stages of the parasite has not been addressed (Chattopadhyay et al., 2003).

The elucidation of the crystal structure of TSR domains revealed an unusual three-stranded fold known as the tryptophan ladder, also found in type 1 cytokine receptors and characterized by a motif of stacked aromatic and basic amino acids stabilized by disulfide bonds (Tan et al., 2002; Olsen and Kragelund, 2014). In other organisms, TSR domains can be modified by two types of glycosylation, namely O-fucosylation and C-mannosylation. The protein O-fucosyltransferase 2 (PoFUT2) catalyses the O-fucose modification of TSR domains, which in turn may be elongated by a β3-glucosyltransferase (Kozma et al., 2006; Luo et al.,...
2006). On the other hand, DPY19 enzymes have been identified as the glycosyltransferases responsible for TSR C-mannosylation (Buettner et al., 2013; Shcherbakova et al., 2017). Recent works revealed that TRAP and CSP are both modified in vivo by a deox-yhexose and a hexose (presumably forming a glucose-β1,3-fucose O-glycan) in *P. falciparum* and *P. vivax* salivary gland sporozoites. Additionally, TRAP was also shown to be modified by an additional hexose (presumably C-linked mannone) in *P. falciparum* (Swearingen et al., 2016) but, interestingly, not in *P. vivax* (Swearingen et al., 2017). These, and other works (Sanz et al., 2013; Cova et al., 2015; Bandini et al., 2019), are renewing the interest of the *Plasmodium* research community on the extent and relevance of the parasite glycosylation.

*Pf*PoFUT2 was demonstrated to be dispensable for the asexual and sexual blood stage development of the parasite, in agreement with the absence of predicted acceptors with the TSR O-fucosylation consensus sequence (Lopatiicki et al., 2017; Sanz et al., 2019). On the contrary, the relevance of *Pf*PoFUT2 for the establishment of the infection in the mosquito host remains a controversial issue. While Lopaticki et al. reported that *Pf*PoFUT2 gene disruption resulted in a deficient ookinete invasion and a reduced sporozoite gliding motility and cell traversal activity, Sanz et al. did not observe any evident phenotype secondary to *Pf*PoFUT2 ablation, neither in *P. falciparum* nor in the murine model *P. berghei*. Interestingly, the phenotype described by Lopaticki et al. was attributed to a deficient secretion of certain *Pf*PoFUT2 acceptors, in accordance with the role played by this modification in the stabilization of the tryptophan ladder (Buettner et al., 2013; Shcherbakova et al., 2017). Taking into consideration the proposed roles for some of the putative acceptors of *Pf*DPY19 in the asexual blood stages, we aimed to describe the relevance of the gene for the viability of *P. falciparum* by disrupting it and analysing the growth phenotype of Δ*Pf*DPY19 mutant parasites.

**Material and methods**

**Construction of plasmids for CRISPR/Cas9-mediated *Pf*DPY19 knock out**

A single guide RNA (sgRNA) targeting the *Pf*DPY19 genomic locus (PF3D7_0806200) was designed with the Eukaryotic Pathogen gRNA Design Tool (Peng and Tarleton, 2015). To generate the plasmid expressing the Streptococcus pyogenes Cas9 and the sgRNA, the primers 5′-ATGGTATATATTATTTTTGCTCTCAGGATCGTTCGTCGACTCTAGAGGATCCACGTCTC-3′ and 5′-TTCTTTCATCTAACCACCTGGGAAGCGCTTTAAATTTAAA-TTAGGGGTTCCCACTATGTTTTAGACGCTAA-3′ and 5′-TTCTTTCATCTAACCACCTGGGAAGCGCTTTAAATTTAAA-TTAGGGGTTCCCACTATGTTTTAGACGCTAA-3′ were annealed and ligated into a BbsI-digested pDC2-Cas9-hDHFRyFCU plasmid (a generous gift from Ellen Kneuper) (Kneuper et al., 2017). To generate the rescue plasmid, two homology regions for the *Pf*DPY19 gene were amplified from a *Pf*530 bp were amplified from *Pf*BD7 1.2B genomic DNA with the primer pairs

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**Table 1. Conservation of the apicomplexan DPY19 C-mannosyltransferase consensus sequence among TSR domain-containing proteins expressed in *P. falciparum***

| Protein     | Gene ID         | W   | X   | X   | W   | X   | X   | W   | X   | C   |
|-------------|-----------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| **Sporozoite** |                 |     |     |     |     |     |     |     |     |     |
| TRAP        | PF3D7_1335900   | W   | D   | E   | W   | S   | P   | C   | Y   |
| TLP         | PF3D7_0616500   | W   | S   | E   | W   | S   | P   | C   | Y   |
| TRSP        | PF3D7_0104000   | W   | S   | E   | W   | S   | A   | C   | Y   |
| TRP1        | PF3D7_0822700   | W   | S   | E   | W   | S   | T   | C   | Y   |
| CSP         | PF3D7_0304600   | S   | T   | E   | W   | S   | P   | C   | N   |
| TREP        | PF3D7_1442600   | W   | S   | E   | W   | S   | G   | P   | N   |
| **Ookinete** |                 |     |     |     |     |     |     |     |     |     |
| CTRP        | PF3D7_0315200   | W   | E   | E   | W   | S   | P   | C   | Y   |
|             |                 | F   | G   | E   | W   | S   | E   | C   | N   |
|             |                 | W   | S   | D   | W   | S   | S   | C   | Y   |
|             |                 | W   | S   | D   | W   | S   | E   | C   | Y   |
|             |                 | W   | E   | T   | W   | V   | E   | C   | Y   |
|             |                 | W   | E   | E   | W   | G   | D   | C   | Y   |
| **Blood**   |                 |     |     |     |     |     |     |     |     |     |
| MTRAP       | PF3D7_1028700   | W   | S   | E   | W   | S   | A   | C   | Y   |
| SPATR       | PF3D7_0212600   | W   | G   | E   | W   | S   | E   | C   | Y   |
| PTRAMP      | PF3D7_1218000   | W   | G   | E   | W   | S   | N   | C   | Y   |

CSP, circumsporozoite protein; CTRP, circumsporozoite and TRAP-related protein; MTRAP, merozoite TRAP-like protein; PTRAMP, Plasmodium thrombospondin-related apical merozoite protein; SPATR, secreted protein with altered thrombospondin repeat; TLP, TRAP-like protein; TRAP, thrombospondin-related anonymous protein; TREP, TRAP-related protein; TREP1, thrombospondin-related protein 1; TRSP, thrombospondin-related sporozoite protein. Residues of the apicomplexan C-mannosylation consensus sequence are marked in bold, when conserved. *TSR domains with or without a conserved C-mannosylation consensus sequence are marked as Y and N, respectively.**
Parasite culture and transfection

*Plasmodium falciparum* 3D7 1.2B line (kindly provided by Cortés) (Cortés, 2005) was used for transfection and parasite maintenance. *Pf*3D7 1.2B was cultured with B+ human erythrocytes in Roswell Park Memorial Institute (RPMI) medium supplemented with Albumax-II at 37 °C under an atmosphere of 92% N2, 3% O2 and 5% CO2, following standard methods (Trager and Jensen, 1976). For *Pf*DPY19 gene disruption, 60 µg of pDC2-Cas9-sgRNA-hDHFRyFCU plasmids and 15 µg of Scal-linearized pUC19-PfDPY19 plasmids (Fig. 1A) were transfected into Percoll-purified segmented schizonts by electroporation with the Amaxa 4D system, as previously described (Moon et al., 2013). Drug selection (i.e. 10 nM WR99210 from Jacobus Pharmaceuticals) was first applied ∼20 h post-transfection and maintained for 4 days with daily media changes. The emergence of resistant parasites was monitored by visualizing Giemsa-stained blood smears by light microscopy. Viable parasites were screened by PCR for *Pf*DPY19 gene disruption and treated for 1 week with 1 µM 5-fluorocytosine for negative selection of parasites containing the pDC2-Cas9-sgRNA-hDHFRyFCU plasmid, prior to subcloning by limiting dilution.

In vitro growth assay of parasite asexual development

To assess the biological relevance of *Pf*DPY19 for the asexual blood stage development of the parasite, the growth of two independent clones of *Pf*DPY19 null mutants was compared with that of the wild-type parental line in three biological replicates. Cultures were synchronized at ring stages by sorbitol synchronization and parasitaemias were determined by flow cytometry (FACSCalibur, BD Biosciences, Franklin Lakes, NJ, USA) using the Syto 11 dye to discriminate between infected and uninfected erythrocytes, as described elsewhere (Urbán et al., 2011). Cultures were adjusted to ∼1.5% parasitaemia and 1% haematocrit. After incubation for one complete intraerythrocytic cycle
To assess the ability of Oxidative stress induction and parasite survival assay between the final and the initial parasitaemia. Multiplication rates for each strain were calculated as the ratio between the final and the initial parasitaemia.

Oxidative stress induction and parasite survival assay

To assess the ability of PfDPY19 null mutants to cope with the endoplasmic reticulum (ER)-stressing conditions, parasite cultures were subjected to different concentrations of dithiothreitol (DTT) as an oxidative stress proxy. Cultures were treated with DTT at a final concentration of 0.05 and 0.15 M or, alternatively, with phosphate buffered saline (PBS) as a negative control. After ~48 h of incubation, parasitaemia was determined by flow cytometry as described previously. The survival rate for each strain was expressed as the ratio between the parasitaemia of the cultures treated with DTT and the negative controls incubated with PBS.

Results

PfDPY19 is not essential for the asexual blood development of *P. falciparum*

To investigate the relevance of the PfDPY19 C-mannosyltransferase, we disrupted the gene coding for this glycosyltransferase (Fig. 1). As demonstrated by PCR, PfDPY19 gene was truncated in edited parasites. Remarkably, the deleted gene fragment (see striped box in Fig. 1B) codes for eight out of the nine predicted transmembrane domains of the native protein. Moreover, the deleted fragment also codes for the conserved amino acids that have been predicted to bind to the dolichol-phosphate mannose precursor (Buettner et al., 2013). Given the magnitude of the gene deletion, it is highly unlikely that the generated PfDPY19 null mutants could yield a functional C-mannosyltransferase activity. Thus, the viability of these mutants strongly suggests that PfDPY19 is not essential for the asexual blood stage development of the parasite.

PfDPY19 gene disruption does not affect parasite growth

*Plasmodium falciparum* expresses three TSR domain-containing proteins during the blood stage development of the parasite, all of which contain at least one TSR domain that conserves the apicomplexan C-mannosylation consensus sequence W-X-W-X-X-C (see Table 1). Specifically, antibodies raised against TRAMP block the erythrocyte invasion of *P. falciparum* merozoites (Siddiqui et al., 2013). To assess if PfDPY19 gene disruption could have an impact on the secretion of TRAMP (or other acceptors) and, consequently, on parasite growth, we compared the multiplication rate of PfDPY19 null mutants with that of the parental wild-type line (Fig. 2A). However, we did not observe any difference across three biological replicates, suggesting that PfDPY19 disruption does not affect the parasite’s asexual blood stage in vitro growth rate.

During the intraerythrocytic development, the parasite is exposed to oxidative stress due to the host immune responses and the products generated during haemoglobin degradation. Thus, the parasite is especially vulnerable to redox imbalances that affect protein folding in the ER (Chaubey et al., 2014). Given the alleged role of C-mannosylation in the stabilization of the tryptophan ladder of TSR domains (Buettner et al., 2013; Shcherbakova et al., 2017), we compared the survival rate of PfDPY19 null mutants with that of the parental wild-type line when subjected to different concentrations of DTT (Fig. 2B) to assess the impact of the absence of a PfDPY19 C-mannosyltransferase activity when coping with such conditions. However, no significant differences were observed at any of the DTT concentrations tested.

Discussion

The recent functional characterization of *P. falciparum* PoFUT2 suggests a critical role of O-fucosylation during the infection of the mosquito host (Lopaticki et al., 2017), although these results could not be replicated in an independent study (Sanz et al., 2019). According to the work conducted by Lopaticki et al., PoFUT2 disruption results in a deficient secretion of TRAP, which probably affects the parasite ability to invade hepatocytes. Moreover, it is likely that other acceptors are also affected by the absence of O-fucosyltransferase activity, as evidenced by the deficient invasion of the mosquito midgut epithelium. Although TRAP has also been demonstrated to be C-mannosylated in salivary gland sporozoites (Swearingen et al., 2016), the relevance of this modification remains unknown. The essential role of C-mannosylation for protein secretion observed in other organisms (Buettner et al., 2013; Shcherbakova et al., 2017) prompted us to investigate the contribution of PfDPY19 to parasite survival and infectivity.

Contrary to O-fucosylation (Lopaticki et al., 2017; Sanz et al., 2019), C-mannosylation is also expected to occur during the blood stages of the parasite, as supported by the conservation of the apicomplexan consensus sequence (Hoppe et al., 2018) in the TSR domain-containing proteins PTRAMP, MTRAP and...
SPATR (Table 1). However, the successful generation of a parasite line with a severely truncated PfDPY19 gene strongly suggests that this modification is not essential for the asexual development of the parasite. Furthermore, parasite growth was not affected in ΔPfDPY19 mutants, suggesting that the gene is not relevant during the asexual multiplication of the parasite, at least under the tested conditions. This finding is in partial agreement with a previous high-throughput screening study that described the gene coding for PfDPY19 as dispensable, but conferring significant fitness to P. falciparum in the asexual blood stages (Zhang et al., 2018).

The discrepancy might arise from slight deviations associated with the comparison of sequencing reads as a proxy for competitive growth fitness in this particular gene or genomic region. The contribution of PfDPY19 to the secretion of the putative acceptors expressed in the blood stages has not been directly assessed. Nevertheless, if there is an effect on protein secretion secondary to PfDPY19 gene disruption, it does not seem to affect neither parasite growth nor the parasite’s ability to cope with DTT-induced oxidative stress. Contrary to this, a recent work reports that Toxoplasma gondii DPY19 may be important for the growth fitness of T. gondii tachyzoites, a related apicomplexan (Gas-Pascual et al., 2019).

The O-fucosylation dependency for protein secretion seems to be variable among the different acceptors that are expressed in the mosquito stages of P. falciparum (Lopaticki et al., 2017). These findings are in agreement with similar observations made with human TLR domain-containing proteins after the loss of the enzyme responsible for O-fucose elongation with glucose (Vasudevan et al., 2015). Similarly, C-mannosylation seems to be required for the secretion of only a subset of its acceptors in C. elegans (Buettner et al., 2013; Shterbakova et al., 2017). Thus, this modification may be more relevant in other stages of the parasite, where other putative effectors have also been described to play essential roles for parasite viability and infectivity. For instance, when given MTRAP essentiality for gametocyte egress (Bargieri et al., 2016), a reduced secretion of this protein may result in a deficient transmission to the mosquito. Likewise, a reduced secretion of CTRP or TRAP as it has been reported after PfPoUT2 gene disruption (Lopaticki et al., 2017) may cause a deficient mosquito midgut colonization and hepatocyte invasion, respectively. Furthermore, specific in vivo environmental conditions, such as temperature variations or other ER stress inducers, might affect the secretion of different acceptors along the parasite life cycle, including the asexual blood stages. Hence, further studies are required to define the function of PfDPY19 C-mannosyltransferase in Plasmodium parasites.

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References

Alonso PL, Brown G, Arevalo-Herrera M, Binka F, Chitnis C, Collins F, Doumbo OK, Greenwood B, Hall BF, Levine MM, Mendis K, Newman RD, Plowe CV, Rodriguez MH, Singen R, Slutske I and Tanner M (2011) A research agenda to underpin malaria eradication. PLoS Medicine 8, e1000406.

Bandini G, Albuquerque-Wendt A, Hegermann J, Samuelson J and Rountier FH (2019) Protein-O- and C-glycosylation pathways in Toxoplasma gondii and Plasmodium falciparum. Parasitology, 1–12. doi: 10.1017/S0031182019000010.

Bargieri DY, Thibierge S, Tay CL, Carey AF, Rantz A, Hischen F, Lorthiois A, Straschil U, Singh P, Singh S, Trigila T, Tsuboi T, Cowman A, Chitnis C, Alano P, Baum J, Pradel G, Lavazec C and Ménard R (2016) Plasmodium merozoite TRAP family protein is essential for vacuole membrane disruption and gamete egress from erythrocytes. Cell Host and Microbe 20, 618–630.

Baum J, Richard D, Healer J, Rug M, Krams Z, Gilberger T-W, Green JL, Holder AA and Cowman AF (2006) A conserved molecular motor drives cell invasion and gliding motility across malaria life cycle stages and other apicomplexan parasites. The Journal of Biological Chemistry 281, 5197–5208.

Buettner FFR, Ashikov A, Tiemann B, Leile I and Bakker H (2013) C. elegans DPY-19 Is a C-mannosyltransferase glycosylating thrombospondin repeats. Molecular Cell 50, 295–302.

Chattopadhyay R, Rathore D, Fujioka H, Kumar S, de la Vega P, Haynes D, Moch K, Fryauf D, Wang R, Carucci DJ and Hoffman SL (2003) Plasmatr, a Plasmodium falciparum protein containing an altered thrombospondin type I repeat domain is expressed at several stages of the parasite life cycle and is the target of inhibitory antibodies. The Journal of Biological Chemistry 278, 25977–25981.

Chabey S, Grover M and Tatu U (2014) Endoplasmic reticulum stress triggers gametocytogenesis in the malaria parasite. The Journal of Biological Chemistry 289, 16662–16674.

Combe A, Moreira C, Ackerman S, Thibierge S, Templeton TJ and Menard R (2009) TREP, a novel protein necessary for gliding motility of the malaria sporozoite. International Journal for Parasitology 39, 489–496.

Cortés A (2005) A chimeric Plasmodium falciparum Pfnbp2b/Pfnpb2a gene originated during asexual growth. International Journal for Parasitology 35, 125–130.

Cova M, Rodrigues JA, Smith TK and Izquierdo L (2015) Sugar activation and glycosylation in Plasmodium. Malaria Journal 14, 427.

Dessens JT, Beetsma AL, Dimopoulos G, Wengelnik K, Crisanti A, Kafatos FC and Sinden RE (1999) CTRP is essential for mosquito infection by malaria ookinetes. The EMBO Journal 18, 6221–6227.

Gas-Pascual E, Ichikawa HT, Sheikh MO, Serji MI, Deng B, Mandalasi M, Bandini G, Samuelson J, Wells L and West CM (2019) CRISPR/Cas9 and glycomics tools for Toxoplasma glycobiology. The Journal of Biological Chemistry 294, 1104–1125.

Hoppe CM, Albuquerque-Wendt A, Bandini G, Leon DR, Shterbakova A, Buettner FFR, Izquierdo L, Costello CE, Bakker H and Rountier FH (2018) Apicomplexan C-mannosyltransferases modify thrombospondin type I-containing adhesins of the TRAP family. Glycobiology 28, 333–343.

Klug D and Frischknecht F (2017) Motility precedes egress of malaria parasites from oocysts. eLife 6, 1–32.

Kneuper E, Napikorska M, van Ooj C and Holder AA (2017) Generating conditional gene knockouts in Plasmodium—A protocol to produce stable DiCre recombinase-expressing parasite lines using CRISPR/Cas9. Scientific Reports 7, 3881.

Kozma K, Keusch JJ, Hegemann B, Luther KB, Klein D, Hess D, Haltiwanger RS and Hofsteenge J (2006) Identification and characterisation of abeta1,3-glucosyltransferase that synthesize the Glc-beta1,3-Fuc disaccharide on thrombospondin type 1 repeats. The Journal of Biological Chemistry 281, 36742–36751.

Krogh A, Larsson B, von Heijne G and Sonnhammer EL (2001) Predicting transmembrane protein topology with a hidden Markov model: application to complete genomes. Journal of Molecular Biology 305, 567–580.

Labatied M, Camargo N and Kappe SH (2007) Depletion of the Plasmodium berghei thrombospondin-related sporozoite protein reveals a role in host cell entry by sporozoites. Molecular and Biochemical Parasitology 153, 158–166.

Lopaticki S, Yang ASP, John A, Scott NE, Lindford JP, O’Neill MT, Erickson SM, McKenzie NG, Jennison C, Whitehead LW, Douglas DN, Kneteman NM, Goddard-Borger ED and Boddey JA (2017) Protein O-fucosylation in Plasmodium falciparum ensures efficient infection of mosquito and vertebrate hosts. Nature Communications 8, 561. doi: 10.1038/s41467-017-00571-y.
Luo Y, Koles K, Vorndam W, Haltiwanger RS and Panin VM (2006) Protein O-fucosyltransferase 2 adds O-fucose to thrombospondin type 1 repeats. Journal of Biological Chemistry 281, 9393–9399.

Ménard R, Sultan AA, Cortes C, Altszuler R, van Dijk MR, Janse CJ, Waters AP, Nussenzeew RS and Nussenzeew V (1997) Circumsporozoite guide RNAs for eukaryotic pathogens. Nature 385, 336–340.

Moon RW, Hall J, Rangkuti F, Ho YS, Almond N, Mitchell GH, Pain A, Holder AA and Blackman MJ (2013) Adaptation of the genetically tractable malaria pathogen Plasmodium knowlesi to continuous culture in human erythrocytes. Proceedings of the National Academy of Sciences 110, 531–536.

Morahan BJ, Wang L and Coppel RL (2009) No TRAP, no invasion. Trends in Parasitology 25, 77–84.

Moreira CK, Templeton TJ, Lavazec C, Hayward RE, Hobbs CV, Kroeze H, Janse CJ, Waters AP, Sinnis P and Coppi A (2008) The Plasmodium TRAP/MIC2 family member, TRAP-Like Protein (TLP), is involved in tissue traversal by sporozoites. Cellular Microbiology 10, 1505–1516.

Olsen JG and Kragelund BB (2014) Who climbs the tryptophan ladder? On the structure and function of the WSXWS motif in cytokine receptors and thrombospondin repeats. Cytokine & Growth Factor Reviews 25, 337–341.

Peng D and Tarleton R (2015) EuPaGDT: a web tool tailored to design CRISPR guide RNAs for eukaryotic pathogens. Microbial Genomics 1, e000033.

Sanz S, Bandini G, Ospina D, Bernabeu M, Marino K, Fernandez-Becerra C and Ménard R, Sultan AA, Cortes C, Altszuler R, van Dijk MR, Janse CJ, Waters AP, Sinnis P and Coppi A (2008) The Plasmodium TRAP/MIC2 family member, TRAP-Like Protein (TLP), is involved in tissue traversal by sporozoites. Cellular Microbiology 10, 1505–1516.

Sanz S, Aquilini E, Tweedell RE, Verma G, Hamerly T, Hritzo B, Ménard R, Sultan AA, Cortes C, Altszuler R, van Dijk MR, Janse CJ, Waters AP, Sinnis P and Coppi A (2008) The Plasmodium TRAP/MIC2 family member, TRAP-Like Protein (TLP), is involved in tissue traversal by sporozoites. Cellular Microbiology 10, 1505–1516.

Sanz S, Bandini G, Ospina D, Bernabeu M, Marino K, Fernandez-Becerra C and Ménard R, Sultan AA, Cortes C, Altszuler R, van Dijk MR, Janse CJ, Waters AP, Sinnis P and Coppi A (2008) The Plasmodium TRAP/MIC2 family member, TRAP-Like Protein (TLP), is involved in tissue traversal by sporozoites. Cellular Microbiology 10, 1505–1516.

Tucker RP (2004) The thrombospondin type 1 repeat superfamily. The International Journal of Biochemistry & Cell Biology 36, 969–974.

Urbaní P, Estelrich J, Cortés A and Fernández-Busquets X (2011) A novel vector with complete discrimination for targeted delivery to Plasmodium falciparum-infected versus non-infected red blood cells in vitro. Journal of Controlled Release 151, 202–211.

Vasudevan D and Haltiwanger RS (2014) Novel roles for O-linked glycans in protein folding. Glycoconjugate Journal 31, 417–426.

Vasudevan D, Takeuchi H, Johar SS, Majerus E and Haltiwanger RS (2015) Peters plus syndrome mutations disrupt a noncanonical ER quality-control mechanism. Current Biology 25, 286–295.

World Health Organization (2018) World Malaria Report 2018. Geneva.

Zhang M, Wang C, Otto TD, Oberstaller J, Liao X, Adapa SR, Udenze K, Bronner IF, Casandra D, Mayho M, Brown J, Li S, Swearingen KE, Lindner SE, Flannery EL, Vaughan AM, Morrison RD, Patrapuvich R, KoepflI C, Muller I, Jex A, Moritz RL, Kappe SHI, Sattabongkot J and Mikolajczak SA (2017) Proteogenomic analysis of the total and surface-exposed proteomes of Plasmodium vivax salivary gland sporozoites. PLoS Neglected Tropical Diseases 11, e0005791.