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**Supplementary Figure 1.** Sequence alignment of DPY19 from *P. falciparum* (PF3D7\_0806200) and *C. elegans* (CCD62139.1), showing identical (dark grey), similar (light grey) and essential residues for enzyme activity (red).

**Diagram

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**Supplementary Figure 2.** **(a)** Strategy for inserting a C-terminal triple hemagglutinin (HA) epitope tag into the *P. falciparum* *DPY19* locus. **(b)** Southern blot analysis of parental NF54 and DPY19-HA parasites after digestion of genomic DNA with *Eco*RI/*Cla*I/*Eco*RV. The probe used (orange bar) for genotyping and the expected fragment sizes after digestion of the loci is shown.

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**Supplementary Figure 3.** Southern blot analysis of parental NF54, D*DPY19* and D*POFUT2*/D*DPY19* clones after digestion of genomic DNA with *Eco*RI/*Cla*I. The NF54 and mutant band sizes are as expected indicating all mutant clones have the D*DPY19* locus.

Diagram

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**Supplementary Figure 4.** Occupancy and localization oftryptophan C-mannosylation on rSPATR. **(a)** Tandem mass spectra of the most abundant C-mannosylated peptide from rSPATR enabled localization of the modification to first tryptophan of the WxxW motif. **(b)** The relative abundance of the unglycosylated, mono-glycosylated, and di-glycosylated peptides from rSPATR, as determined by LC-MS.Graphical user interface, diagram

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**Supplementary Figure 5.** Localization oftryptophan C-mannosylation on rMTRAP. Tandem mass spectra of C-mannosylated peptides from rMTRAP for the **(a)** di-glycosylated peptide DTCDEWSEWSACTHGISTR and **(b** and **c)** mono-glycosylated peptides DTCDEWSEWSACTHGISTR and CDKWGEWSECKDGR.

**a**

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**bChart, box and whisker chart

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**c**

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**d**

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**Supplementary Figure 6.** Occupancy oftryptophan C-mannosylation on rMTRAP. The relative abundance of glycoforms for the DTCDEWSEWSACTHGISTR peptide in rMTRAP was determined by LC-MS for samples expressed **(a)** with and **(b)** without *Ce*DPY19. The relative abundance of glycoforms for the CDKWGEWSECKDGR peptide in rMTRAP was determined by LC-MS for samples expressed **(c)** with and **(d)** without *Ce*DPY19.

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**Supplementary Figure 7. (a)** No difference in stage V gametocytemia was observed between NF54, Δ*DPY19* and Δ*DPY19* parasites. Data shown are n=3 experiments. n.s., not significant. **(b)** SPATR and PTRAMP are not expressed in stage V gametocytes. **(c)** Exflagellation by NF54 and Δ*DPY19* microgametes. Scale bar, 5 mm. **(d)** Quantification of exflagellation by NF54 and Δ*DPY19* microgametes using light microscopy, represented as a percentage of total (infected and uninfected) erythrocytes. n= 3 experiments. **(e)** Quantification of NF54 and Δ*DPY19* ookinetes in the bloodmeal bolus dissected from groups of 20 *Anopheles stephensi* mosquitoes per parasite strain 23 hours post bloodmeal. n=3 experiments. Data are mean ± s.e.m. and were analyzed by one-way ANOVA (Kruskal-Wallis test). n.s., not significant.

Timeline

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**Supplementary Figure 8.** Complementation of Δ*DPY19* parasites with the *DPY19* gene to restore expression. **(a)** Schematic of the strategy to complement the Δ*DPY19* c1 and c2 clones. **(b)** Southern blot analysis of parental NF54, two clonal Δ*DPY19* parasite lines (c1 and c2) and the complemented Δ*DPY19* Comp clones after digestion of genomic DNA with *EcoR*I/*Cla*I/*Kpn*I. The probe used (orange bar) for genotyping and the expected fragment sizes after digestion of the loci are shown.

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**Supplementary Figure 9.** Human HC-04 hepatocyte infection assays. (a) Flow cytometric quantification of cell traversal by *P.* *falciparum* sporozoites after incubation for 4 hours using FITC-dextran uptake to label the wounded cells. n=4 experiments. (b) Flow cytometric quantification of intracellular *P. falciparum* liver stages after incubation with sporozoites for 24 hours using CSP-positive antibody staining of fixed, permeabilized cells. n=2 experiments. Variance between assays occurred and was likely due to very low D*DPY19* sporozoite yields from the low oocyst intensities, which restricted the multiplicity of infection to 0.02-0.35, as indicated. Data are mean ± s.e.m.