**Supplementary file 1a. Sulfur-dependent pathways present in *P. falciparum*.**

\*Although thiamine biosynthesis pathway is present in *P. falciparum*, the parasite depends on exogenous supplementation from the host (1), # From genome wide saturation piggyBac transposon mutagenesis study (2).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Pathways that require sulfur  | Presence in asexual blood stage | Essentiality in asexual blood stage | Localization | Reference |
| FeS assembly | Present | Essential | Apicoplast and mitochondria | (3, 4) |
| Lipoic acid biosynthesis | Present | Non-essential | Apicoplast | (5, 6) |
| Biotin biosynthesis | Absent |  |  | (7, 8)  |
| Molybdopterin biosynthesis | Absent |  |  | (7, 9) |
| Selenocysteine biosynthesis | Present | Non-essential# | Cytosol | (10, 11) |
| Thiamine biosynthesis\* | Present | Essential | Cytosol | (1, 12, 13) |
| Thio-modification of tRNA | Present | Essential# | Apicoplast | (14) |

**Supplementary file 1b. Summary of attempted transfections in *P. falciparum* to knockout genes of interest**

|  |  |  |  |
| --- | --- | --- | --- |
| Parental line | Gene of interest | No. of attempted independent transfection | No. of transgenic parasite lines with successful deletion |
| PfMev | *sufC*  | 8 | 5 |
| *sufD*  | 8 | 5 |
| *sufE*  | 3 | 3 |
| *sufS*  | 10 | 8 |
| *mnmA*  | 3 | 3 |
| ∆*sufD* | *sufC* | 4 | 4 |
| *bsmnmA*+ | *mnmA*  | 4 | 1 |
| *bsmnmA*-*yrvO*+ | *mnmA*  | 4 | 4 |
| *sufS*  | 2 | 2 |

**Supplementary file 1c. Plasmids used for generation of gene deletion lines**

|  |  |  |  |
| --- | --- | --- | --- |
| Gene deletion lines | Target gene | Cas9 plasmid | Repair plasmid |
| ∆*sufC* | *sufC* | pCasG- *sufC* | pRSng-*sufC* |
| ∆*sufD* | *sufD* | pCasG-*sufD* | pRSng-*sufD* |
| ∆*sufC/sufD* | *sufC* *sufD* | pCasG-*sufC*pCasG-*sufD* | pRSng-(BSD)-*sufC*pRSng-*sufD* |
| ∆*sufE* | *sufE* | pCasG-*sufE* | pRSng-*sufE* |
| ∆*sufS* | *sufS* | pUF1-Cas9 | pRS-*sufS* |
| ∆*mnmA* | *mnmA* | pCasG-*mnmA* | pRSng-*mnmA* |
| *bsmnmA+* Δ*mnmA* | *mnmA* | pCasG-*mnmA* | pRSng-*mnmA* |
| *bsmnmA-yrvO+* Δ*mnmA* | *mnmA* | pCasG-*mnmA* | pRSng-*mnmA* |
| *bsmnmA-yrvO+* Δ*mnmA* | *sufS* | pUF1-Cas9 | pRS-*sufS* |

**Supplementary file 1d. Primers used in this study.** Restriction enzyme sites are single underlined, linker regions are double underlined.

|  |  |
| --- | --- |
| Primer name | Sequence (5’🡪3’) |
| Primers to amplify homology arms for genetic knockouts |
| SufC.HA1F | GCCACGAGCGGCCTATGCATCCTTTTGAAACCCTTCAACT |
| SufC.HA1R | AAGCGCAGCGGCCTTGTTATTTTCCATATGCACGACATTC |
| SufC.HA2F | CGACAGACGCCGGAGGAGAAAAAAAAAGAAATGAAATATT |
| SufC. HA2R | GGCCACCAGCCGGCTATTCCTTAAGAAATTGAGAGTACCCC |
| SufD.HA1F | GCCACGAGCGGCCTTTGATTATGTTGAGAGTAAACATTGG |
| SufD.HA1R | AAGCGCAGCGGCCAAATGGGTAGCATTATTAATCTTTAGA |
| SufD.HA2F | CGACAGACGCCGGATGGATTCTCAAGAATTTGTTTAGAAG |
| SufD.HA2R | GGCCACCAGCCGGGTGATTTGCACATTCTATATCACTTGG |
| SufE.HA1F | GCCACGAGCGGCCTTGTTTTCGGGCATACCATTTAATATGC |
| SufE.HA1R | AAGCGCAGCGGCCTGGAGATTACTGAAAAATAAATTGGAGTCA |
| SufE.HA2F | CGACAGACGCCGGCAGGGGTGTCAATCGGTAGTATATATTTAC |
| SufE.HA2R | GGCCACCAGCCGGTGTCCATATTCTTCAATATATTGGTGCAGA |
| SufS.HA1F | GCCACGAGCGGCGCTTAATAAATATTTTCTATTATGTCAT |
| SufS.HA1R | AAGCGCAGCGGCATTATATTATTTCTTCATTTTATTACC |
| SufS.HA2F | CGACAGACGCCGGTAGTAATATAATAACTAATGTTTCTAA |
| SufS.HA2R | GGCCACCAGCCGGTGTGTTATAAAAATATATGGATATCC  |
| MnmA.HA1F | GCCACGAGCGGCCATCATTTTGTAAATCTATTAAGGTCACACA |
| MnmA.HA1R | AAGCGCAGCGGCCTATAGCCATAAATAAAATTTCATCACACC |
| MnmA.HA2F | CGACAGACGCCGGAGGGGAAATTACCTGCATGTATAAAAAAG |
| MnmA.HA2R | GGCCACCAGCCGGCAAGAAATTGATTACTTATTGTTGCTGAAC |
| Primers for gRNA to generate knockout lines |
| SufC.gRNA.F | TAAGTATATAATATTGTTAATTATGGATTTAGTGGGTTTTAGAGCTAGAA |
| SufC.gRNA.R | TTCTAGCTCTAAAACCCACTAAATCCATAATTAACAATATTATATACTTA |
| SufD.gRNA.F | TAAGTATATAATATTAGTAATAGTGGACTAGTTAAGTTTTAGAGCTAGAA |
| SufD.gRNA.R | TTCTAGCTCTAAAACTTAACTAGTCCACTATTACTAATATTATATACTTA |
| SufE.gRNA.F | TAAGTATATAATATTATATAATCTACACATAACAGGTTTTAGAGCTAGAA |
| SufE.gRNA.R | TTCTAGCTCTAAAACCTGTTATGTGTAGATTATATAATATTATATACTTA |
| SufS.gRNA.F | TAAGTATATAATATTAAATTTAAACCATTGTTATAGTTTTAGAGCTAGAA |
| SufS.gRNA.R | TTCTAGCTCTAAAACTATAACAATGGTTTAAATTTAATATTATATACTTA |
| MnmA.gRNA.F | TAAGTATATAATATTACAATATAAAATGGATAGAGGTTTTAGAGCTAGAA |
| MnmA.gRNA.R | TTCTAGCTCTAAAACCTCTATCCATTTTATATTGTAATATTATATACTTA |
| Primers for gene knockout confirmation |
| SufC.5F | GGGATTTGTCATACATATAAATATGTATAAAAAGGTCC |
| SufC.3R | TAAGTACAAAATAATATCATATGTACATTATAAAATCCAC |
| SufC.5WTR | CTTCTTCTAAATTTTTTAATAAAGATAATCTATCCATATC |
| SufC.3WTF | GAGATAAGTGTAAGTGAATTTAATTTAATGATGATAGAAG |
| SufD.5F | GTGTATATAATTTTTTGAAAAAAATGATTTAGCTAACACA |
| SufD.3R | CAGATATTCCTCTTGTCATTAATGAGAAGATTGG |
| SufD.5WTR | GTGTTTTCTCTTCATTTTGATGTCTATAGTTTTTTATAC |
| SufD.3WTF | CCAATAACAAATCCTAGATTAGTTGTATATGTAAAAGG |
| SufE.5F | CATTTCATTCATTTTGGTAGTTAAAAAGAAAAGG |
| SufE.3R | TCATTTTATTAAAAACATATTTCATTTAATTTGTAAGA |
| SufE.5WTR | CATGAGCATATTATATATAAAACAAAGAAATGTGC |
| SufE.3WTF | CCTGATAAACATAAAATTAGACAAAATCAAGTTTTGG |
| SufS.5F | TTTTCAGAAAATGAGTGAGTTTTATAAAAAGGAAAACTCGA |
| SufS.3R | CAATTTGCTGTGCCAAATATTTGATTTC |
| SufS.5WTR | CAATTTGCTGTGCCAAATATTTGATTTCCTG |
| SufS.3WTF | CCAGATATATTAATAACATCTGGTCATAAATTTTG |
| MnmA.5F | CTACTTTGATGTTTTTTTATTTTTGCACATTTTATG |
| MnmA.3R | GGTATTTTTTATTTGCTCATGTTTAAATCATTTATCTG |
| MnmA.5WTR | CTTTATACTATTGTTTCTTTGTTCGTCTTCATCAATTTG |
| MnmA.3WTF | CTTACTAAAAATTATGACCAAGATTTATTTACACATATACG |
| pRS.R | TACAAAATGCTTAAGCGCAGCGGCC |
| pRS.F | CATATTTATTAAATCTAGAATTCGACAGACGCCG |
| Primers to amplify representative genes from nuclear and organellar genome |
| LDH.F | GGAGATGTAGTTTTGTTCGATATTG |
| LDH.R | CTTGTAAAGGGATACCACCTACAG |
| SufB.F | CATGTAGCTATAGTAGAAATAATAGTAAAAGATTATGG  |
| SufB.R | GACTCTGAAATACTTAAACCACGTTGC |
| Cox1.F | CTTCATCTTTAAGAATAATTGCACAAGAAAATGTAAATC |
| Cox1.R | GTACATATGATGTACCCATACTAAGCTTCC |
| Primers for generation of pCLD-*bsmnmA*-mCherry-10xapt and pCLD-*bsmnmA*-*yrvO*-mCherry-10xapt plasmids |
| MnmA.BspEI.InF.F | GTTAGAAGGTTCCGGAATGGAAAAACGGCCGGAGG |
| MnmA.BsiWI.InF.R | gcccttgctCgtacgTACGTACCACAATTTTGTTCCGTCTTTG |
| MnmA.Link.R | CCGTTCCATaccagatccactacCTACGTACCACAATTTTGTTCCGTCTTTG |
| YrvO.Link.F | GGTACGTAGgtagtggatctggtATGGAACGGATTTATTTAGATCATGCCG |
| YrvO.BsiWI.InF.R | gcccttgctCgtacgTGTCAGCCGTTTGACAACGTCG |
| MY Apt PspOMI F | tatgcaTAAgggcccactgtCtggcaag |
| MY Apt XmaI R | gacCTTAAGcccgggtcagtcagagcag |
| Primers for generation of pKD-*mnmA*-2xFLAG-10xapt |
| FLAG.F | GATGTGTCAAGACGTCGATTATAAAGATCATGATGGTGATTATAAGGATCATGATATAGA |
| FLAG.R | CCAGACAGTGGGCCCTTATTTATCATCATCATCCTTATAATCTATATCATGATCCTTATA |
| MnmAKD.HA1F  | GATATCgtccacctgGATATCagtcaaaaatattccccatgatga |
| MnmAKD.HA1R | CTTTATAATCgacgtctttgctcatgtttaaatcatttatctg |
| MnmAKD.HA2F | Ccctttccgggcgcgccatatggttgtaatacattttgtatcct |
| MnmAKD.HA2R | GATATCcaggtggacGATATCtatcgtgaataagatatttctggaaatt |
| Primers for gRNA to generate *mnmA*-flag  |
| MnmAKD.gRNA.F | TAAGTATATAATATTtatttgtatgtgtacttatatttGTTTTAGAGCTAGAA |
| MnmAKD.gRNA.R | TTCTAGCTCTAAAACaaatataagtacacatacaaataAATATTATATACTTA |
| Primers for confirmation of *mnmA*-flag genotype |
| MnmAKD.5F | TTCATGTTTATAATAATATTCAAGAGAGTGG |
| MnmAKD.3R | TGATATGAACATATATAAAGGAGTTTGTAC |
| NewApt.5R | ctcgcTATCAAGGAATCgagtcc |
| HSP86KD.5F | GGAATACTAAATATATATCCAATGGCC |
| Primers for confirmation of gene knock-in |
| attB.Int.F | GCAGTGTGGAATTCCCTGCA |
| attB.Int.R | TTAAGTGTAGTTAATTCATCAAATAGCATGC |
| P230p.out.HA.F | Ggttgtgatttttcaggtgattcc |
| P230p.out.HA.R | gaaaattgtaggggcagctaaatccgac​ |
| Primers for plasmid sequencing |
| pRS.R | TACAAAATGCTTAAGCGCAGCGGCC |
| pRS.F | CATATTTATTAAATCTAGAATTCGACAGACGCCG |
| MnmA.seqF | GCTGCGCGGCATTGATG |
| MnmA.seqR | CGCCGATCGGGAACATGAC |
| Primers for *P. falciparum* SufS PCR amplification for sequencing |
| SufS.SeqF1 | ctATGTTAAGAGGCCCTAGATG |
| SufS.SeqR3 | CGAGTTTTCCTTTTTATAAAACTCACTCA |
| SufS.SeqF3 | TGAGTGAGTTTTATAAAAAGGAAAACTCG |
| SufS.SeqR4 | CCAGGAATATTTTGTGTTCCTGTTTC |
| SufS.SeqF2 | TCATTCAAATATTATTCCCTGGCAAG |
| SufS.SeqR2 | tTCATTTTTCATTTTTCATTTCATTTAACAT |
| Primers for *P. falciparum* SufS amplicon sequencing |
| SufS.SeqF1 | ctATGTTAAGAGGCCCTAGATG |
| SufS.SeqR1 | CCAATAACATTAGATGCATGACATATAG |
| SufS.SeqF2 | TCATTCAAATATTATTCCCTGGCAAG |
| SufS.SeqR2 | tTCATTTTTCATTTTTCATTTCATTTAACAT |
| SufS.SeqF3 | TGAGTGAGTTTTATAAAAAGGAAAACTCG |
| SufS.SeqR3 | CGAGTTTTCCTTTTTATAAAACTCACTCA |
| SufS.SeqF4 | GAAACAGGAACACAAAATATTCCTGG |
| SufS.SeqR4 | CCAGGAATATTTTGTGTTCCTGTTTC |

**Supplementary references**

1. X. W. Chan *et al.*, Chemical and genetic validation of thiamine utilization as an antimalarial drug target. *Nat Commun* **4**, 2060 (2013).

2. M. Zhang *et al.*, Uncovering the essential genes of the human malaria parasite *Plasmodium falciparum* by saturation mutagenesis. *Science* **360**, eaap7847 (2018).

3. T. A. Dellibovi-Ragheb, J. E. Gisselberg, S. T. Prigge, Parasites FeS up: iron-sulfur cluster biogenesis in eukaryotic pathogens. *PLoS Pathog* **9**, e1003227 (2013).

4. J. E. Gisselberg, T. A. Dellibovi-Ragheb, K. A. Matthews, G. Bosch, S. T. Prigge, The suf iron-sulfur cluster synthesis pathway is required for apicoplast maintenance in malaria parasites. *PLoS Pathog* **9**, e1003655 (2013).

5. J. Storm, S. Müller, Lipoic acid metabolism of *Plasmodium*-a suitable drug target. *Curr Pharm Des* **18**, 3480-3489 (2012).

6. S. Günther *et al.*, Apicoplast lipoic acid protein ligase B is not essential for *Plasmodium falciparum*. *PLoS Pathog* **3**, e189 (2007).

7. A. Krishnan, J. Kloehn, M. Lunghi, D. Soldati-Favre, Vitamin and cofactor acquisition in apicomplexans: synthesis versus salvage. *J Biol Chem* **295**, 701-714 (2020).

8. T. A. Dellibovi-Ragheb *et al.*, Host biotin is required for liver stage development in malaria parasites. *Proc Natl Acad Sci* **115**, E2604-E2613 (2018).

9. E. Kemen *et al.*, Gene gain and loss during evolution of obligate parasitism in the white rust pathogen of *Arabidopsis thaliana*. *PLoS Biol* **9**, e1001094 (2011).

10. A. V. Lobanov *et al.*, The *Plasmodium* selenoproteome. *Nucleic Acids Res* **34**, 496-505 (2006).

11. A. Röseler *et al.*, Insight into the selenoproteome of the malaria parasite *Plasmodium falciparum*. *Antioxid Redox Signal* **17**, 534-543 (2012).

12. M. L. Eschbach, I. B. Muller, T. W. Gilberger, R. D. Walter, C. Wrenger, The human malaria parasite *Plasmodium falciparum* expresses an atypical N-terminally extended pyrophosphokinase with specificity for thiamine. *Biol Chem* **387**, 1583-1591 (2006).

13. C. Wrenger *et al.*, Vitamin B1 de novo synthesis in the human malaria parasite *Plasmodium falciparum* depends on external provision of 4-amino-5-hydroxymethyl-2-methylpyrimidine. *Biol Chem* **387**, 41-51 (2006).

14. S. A. Ralph *et al.*, Metabolic maps and functions of the *Plasmodium falciparum* apicoplast. *Nat Rev Microbiol* **2**, 203-216 (2004).