Supplementary Information

Reconstruction and flux-balance analysis of the *Plasmodium falciparum* metabolic network

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SUPPLEMENTARY FIGURES

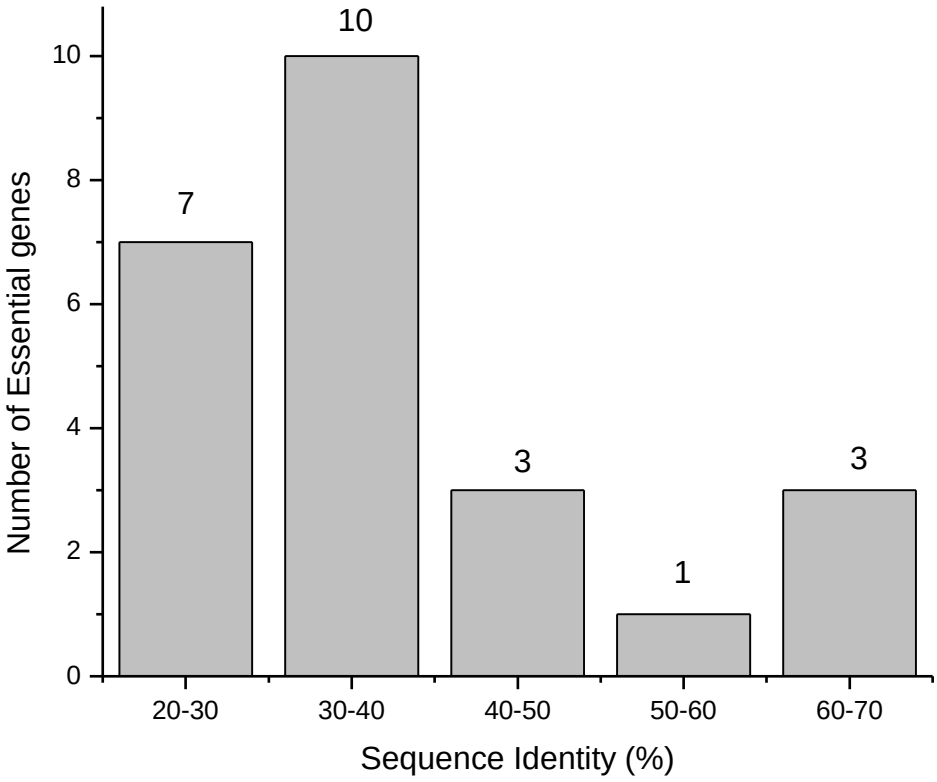

Figure S1| Distribution of sequence identities to human transcripts for *Plasmodium* proteins predicted as essential for growth.

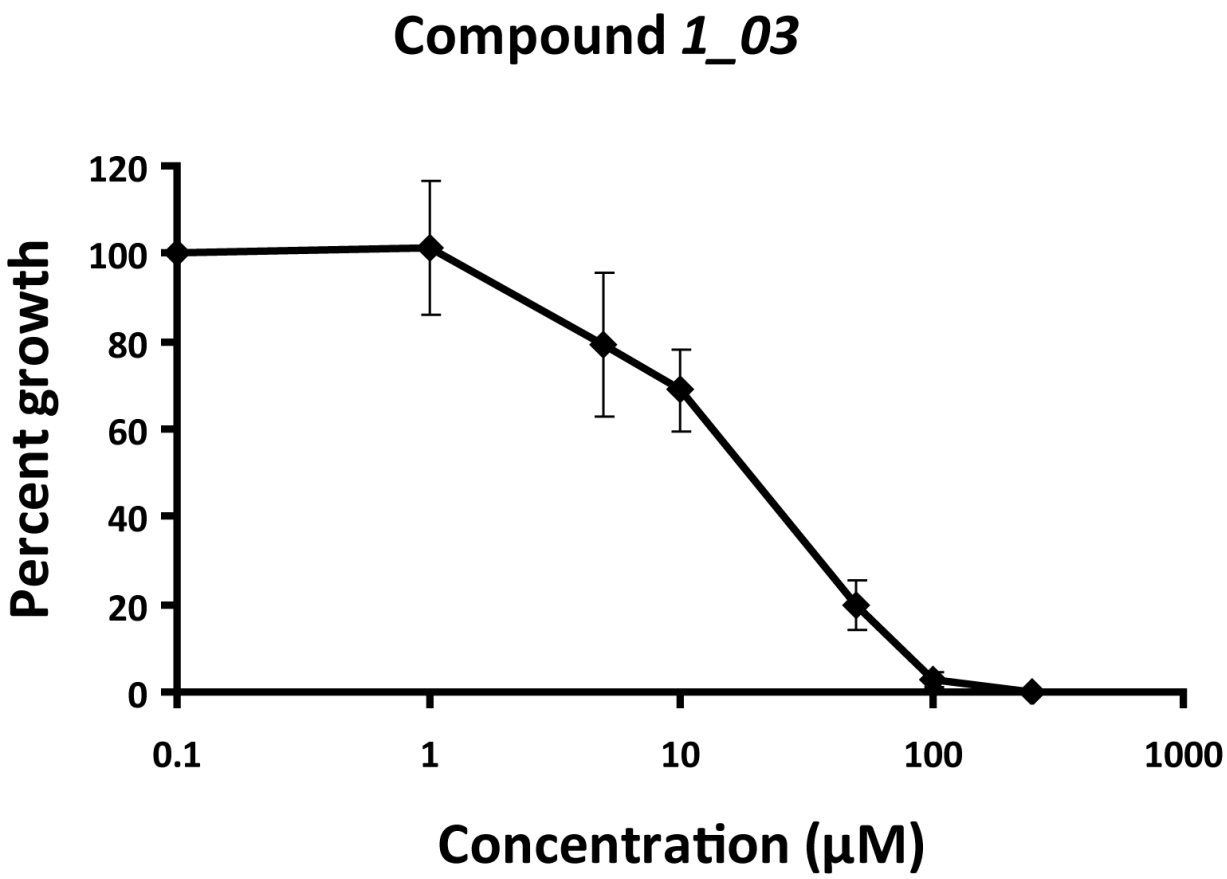

Figure S2| Growth inhibition profile of compound ***1_03*** in *in vitro* cultures of *P. falciparum*. See Methods for experimental details. Error bars represent the standard deviation of the mean based on triplicate experiments.

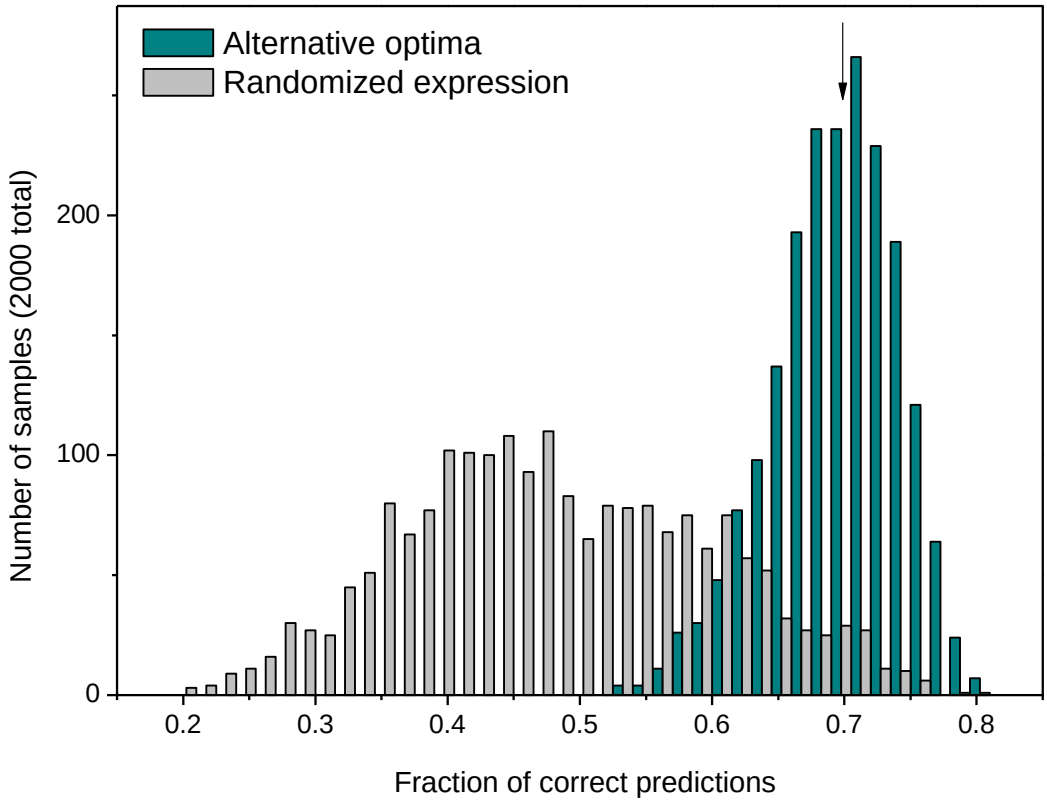

Figure S3| The accuracy of the metabolite exchange predictions made using 2000 trials with randomized expression data (the distribution in grey), and prediction based on sampling of 2000 alternative FBA optimal solutions (the distribution in cyan). The single prediction discussed in the paper (70% accuracy) is shown using a vertical black arrow. In the randomized trials expression values were randomly shuffled between parasite metabolic genes. Only in about 2% of the randomized trials the prediction accuracy was higher than the average accuracy obtained using the true (non-shuffled) expression values. Alternative optima were obtained using the artificial centering hit-and-run algorithm (Kaufman & Smith, 1998), implemented in the COBRA toolbox (Becker et al, 2007), with biomass fluxes fixed to their maximum value. The difference in the distributions is highly statistically significant (Mann-Whitney U *P*-value $<10^{-10}$).

SUPPLEMENTARY TABLES

Table S1 | Biomass components and cofactors which can be synthesized or imported by the reconstructed metabolic network of *P. falciparum*.

Amino acids	dGTP
Alanine	dTTP
Arginine	**Cofactors**
Asparagine	10-Formyltetrahydrofolate
Aspartate	2-Octaprenyl-6-hydroxyphenol
Cysteine	Coenzyme A
Glutamine	Flavin adenine dinucleotide
Glutamate	5,10-Methylenetetrahydrofolate
Glycine	Nicotinamide adenine dinucleotide
Histidine	Nicotinamide adenine dinucleotide phosphate
Isoleucine	Protoheme
Leucine	Pyridoxal 5-phosphate
Lysine	Riboflavin
Methionine	5,6,7,8-Tetrahydrofolate
Phenylalanine	Thiamine diphosphate
Proline	**Polyamines**
Serine	Putrescine
Threonine	Spermidine
Tryptophan	**Lipids**
Tyrosine	Phosphatidylethanolamine
Valine	Phosphatidylcholine
Ribonucleotides	Cholesterol
ATP	**Others**
CTP	S-Adenosyl-L-methionine
GTP	Ammonium
UTP	Fe^{2+}
Deoxyribonucleotides	Water
dATP	
dCTP	

Table S2 | Single deletions displaying a phenotype in the unconstrained metabolic network of *P. falciparum.* L: Lethal, GR: Growth Reducing, SGR: Slight Growth Reducing

Gene	Enzyme name	EC Number	Pred.
MAL13P1_186	1-deoxy-D-xylulose-5-phosphate synthase	2.2.1.7	L
MAL13P1_221	1-deoxy-D-xylulose-5-phosphate synthase	2.1.3.2	L
MAL13P1_292	FAD synthetase, riboflavin kinase	2.7.7.2, 2.7.1.26	L
MAL13P1_326	Ferrochelatase	4.99.1.1	L
MAL8P1_81	Phosphopantothenoylcysteine decarboxylase	4.1.1.36	L
PF07_0018	Pantetheine-phosphate adenylyltransferase	2.7.7.3	L
PF08_0095	2-amino-4-hydroxy-6-hydroxymethyldihydropteridine diphosphokinase, dihydropteroate synthase	2.7.6.3, 2.5.1.15	L
PF10_0121	Hypoxanthine phosphoribosyltransferase	2.4.2.8	L
PF10_0155	Phosphopyruvate hydratase	4.2.1.11	L
PF10_0221	(E)-4-hydroxy-3-methylbut-2-enyl-diphosphate synthase	1.17.7.1	L
PF10_0225	Orotidine-5'-phosphate decarboxylase	4.1.1.23	L
PF10_0275	Protoporphyrinogen oxidase	1.3.3.4	L
PF10_0363	Pyruvate kinase	2.7.1.40	L
PF11_0059	Pantothenate transporter	--	L
PF11_0169	Pyridoxine/pyridoxal 5-phosphate biosynthesis enzyme	--	L
PF11_0295	Geranyltranstransferase, dimethylallyltranstransferase	2.5.1.10, 2.5.1.1	L
PF11_0410	Carbonate dehydratase	4.2.1.1	L
PF11_0436	Coproporphyrinogen oxidase	1.3.3.3	L
PF13_0044	Carbamoyl-phosphate synthase (ammonia), carbamoyl-phosphate synthase (glutamine-hydrolysing)	6.3.4.16, 6.3.5.5	L
PF13_0140	Dihydrofolate synthase, tetrahydrofolate synthase	6.3.2.12, 6.3.2.17	L
PF13_0159	Nicotinate-nucleotide adenylyltransferase	2.7.7.18	L
PF13_0287	Adenylosuccinate synthase	6.3.4.4	L
PF14_0415	Dephospho-CoA kinase	2.7.1.24	L
PF14_0598	Glyceraldehyde-3-phosphate dehydrogenase (phosphorylating)	1.2.1.12	L
PF14_0641	1-deoxy-D-xylulose-5-phosphate reductoisomerase	1.1.1.267	L
PF14_0697	Dihydroorotase	3.5.2.3	L
PFA0225w	4-hydroxy-3-methylbut-2-enyl diphosphate reductase	1.17.1.2	L
PFA0340w	2-C-methyl-D-erythritol 4-phosphate cytidylyltransferase	2.7.7.60	L
PFB0200c	Aspartate transaminase, tyrosine transaminase, phenylalanine(histidine) transaminase	2.6.1.1, 2.6.1.5, 2.6.1.58	L
PFB0280w	Shikimate kinase, 3-phosphoshikimate 1-carboxyvinyltransferase	2.7.1.71, 2.5.1.19	L
PFB0295w	Adenylosuccinate lyase	4.3.2.2	L
PFB0420w	Adenylate cyclase, 2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase	4.6.1.1, 4.6.1.12	L
PFC0831w	Triose-phosphate isomerase	5.3.1.1	L
PFD0830w	Thymidylate synthase, dihydrofolate reductase	2.1.1.45, 1.5.1.3	L
PFE0150c	4-(cytidine 5'-diphospho)-2-C-methyl-D-erythritol kinase	2.7.1.148	L

PFE0410w	Dihydroxyacetone phosphate transporter (apicoplast)	--	L
PFE0630c	Orotate phosphoribosyltransferase	2.4.2.10	L
PFE1510c	Phosphoenolpyruvate transporter (apicoplast)	--	L
PFF0160c	Dihydroorotate oxidase	1.3.3.1	L
PFF0360w	Uroporphyrinogen decarboxylase	4.1.1.37	L
PFF0370w	3-octaprenyl-4-hydroxybenzoate carboxy-lyase	4.1.1.-	L
PFF0450c	Iron transporter	--	L
PFF0530w	Transketolase	2.2.1.1	L
PFF1105c	Chorismate synthase	4.2.3.5	L
PFF1410c	Nicotinate phosphoribosyltransferase	2.4.2.11	L
PFF1490w	Methylenetetrahydrofolate dehydrogenase (NADP+), methenyltetrahydrofolate cyclohydrolase	1.5.1.5, 3.5.4.9	L
PFI1090w	Methionine adenosyltransferase	2.5.1.6	L
PFI1100w	Aminodeoxychorismate synthase, aminodeoxychorismate lyase	2.6.1.85, 4.1.3.38	L
PFI1105w	Phosphoglycerate kinase	2.7.2.3	L
PFI1170c	Thioredoxin-disulfide reductase	1.8.1.9	L
PFI1195c	Thiamine diphosphokinase	2.7.6.2	L
PFI1310w	NAD+ synthase (glutamine-hydrolysing)	6.3.5.1	L
PFI1420w	Guanylate kinase	2.7.4.8	L
PFL2210w	5-aminolevulinate synthase	2.3.1.37	L
PFL2465c	dTMP kinase	2.7.4.9	L
PF14_0378	Triose-phosphate isomerase	5.3.1.1	GR
PF14_0425	Fructose-bisphosphate aldolase	4.1.2.13	GR
PFL0960w	Ribulose-phosphate 3-epimerase	5.1.3.1	GR
MAL13P1_206	Phosphate transporter (citoplasm)	--	SGR
MAL13P1_82	CDP-diacylglycerol-inositol 3-phosphatidyltransferase	2.7.8.11	SGR
MAL13P1_86	Choline-phosphate cytidylyltransferase	2.7.7.15	SGR
PF10_0122	Phosphoglucomutase	5.4.2.2	SGR
PF13_0259	dCTP deaminase	3.5.4.13	SGR
PF14_0097	phosphatidate cytidylyltransferase	2.7.7.41	SGR
PF14_0341	Glucose-6-phosphate isomerase	5.3.1.9	SGR
PF14_0511	6-phosphogluconolactonase, glucose-6-phosphate dehydrogenase	3.1.1.31, 1.1.1.49	SGR
PF14_0520	phosphogluconate dehydrogenase (decarboxylating)	1.1.1.44	SGR
PFE0660c	Purine-nucleoside phosphorylase	2.4.2.1	SGR
PFF1215w	Sphingomyelin synthase	2.7.8.27	SGR
PFF1375c	Ethanolaminephosphotransferase, diacylglycerol cholinephosphotransferase	2.7.8.1, 2.7.8.2	SGR
PFL1870c	Sphingomyelin phosphodiesterase	3.1.4.12	SGR

Table S3| Single deletions of orphan metabolic activities predicted to be lethal in the unconstrained metabolic network of *P. falciparum*

Reaction Name	Enzyme	EC Number	Biological Process
R_NADK	NAD+ kinase	2.7.1.23	Nicotinate and nicotinamide metabolism
R_UPP3S_ap	Uroporphyrinogen-III synthase	4.2.1.75	Porphyrin metabolism
R_DDPA	3-deoxy-7-phosphoheptulonate synthase	2.5.1.54	Shikimate biosynthesis
R_DHQS	3-dehydroquinate synthase	4.2.3.4	Shikimate biosynthesis
R_DHQTi	3-dehydroquinate dehydratase	4.2.1.10	Shikimate biosynthesis
R_SHK3Dr	Shikimate dehydrogenase	1.1.1.25	Shikimate biosynthesis
R_CHRPL	Chorismate lyase	4.1.3.40	Ubiquinone metabolism
R_OPHBDC_mt	3-octaprenyl-4-hydroxybenzoate carboxy-lyase	4.1.1.-	Ubiquinone metabolism
R_OPHHX_mt	2-octaprenylphenol hydroxylase	1.14.13.-	Ubiquinone metabolism

Table S4| Double deletions predicted to be lethal in the unconstrained metabolic network of *P. falciparum.* *Isoenzyme pairs

Pair	Gene Name	Enzyme Name	EC Number	Biological Process
1*	PF11_0036	Phosphopantothenate-cysteine ligase	6.3.2.5	CoA biosynthesis
	PFD0610w	Phosphopantothenate-cysteine ligase	6.3.2.5	CoA biosynthesis
2*	PF11_0208	Phosphoglycerate mutase	5.4.2.1	Glycolysis
	PFD0660w	Phosphoglycerate mutase	5.4.2.1	Glycolysis
3	PF14_0378	Triose-phosphate isomerase	5.3.1.1	Glycolysis, isoprenoid metabolism
	PFL0960w	Ribulose-phosphate 3-epimerase	5.1.3.1	Pentose phosphate cycle
4	PF10_0122	Phosphoglucomutase	5.4.2.2	Glycolysis, pentose phosphate cycle
	PFE0730c	Ribose-5-phosphate isomerase	5.3.1.6	Pentose phosphate cycle
5*	PF13_0143	Ribose-phosphate diphosphokinase	2.7.6.1	Pentose phosphate cycle
	PF11_0157	Ribose-phosphate diphosphokinase	2.7.6.1	Pentose phosphate cycle
6	PFE0660c	Purine-nucleoside phosphorylase	2.4.2.1	Purine, methionine and polyamine metabolism
	PFE0730c	Ribose-5-phosphate isomerase	5.3.1.6	Pentose phosphate cycle
7*	PF10_0086	Adenylate kinase	2.7.4.3	Purine metabolism
	PFD0755c	Adenylate kinase	2.7.4.3	Purine metabolism
8*	PF14_0541	Inorganic diphosphatase	3.6.1.1	Purine, terpenoid metabolism
	PFC0710w	Inorganic diphosphatase	3.6.1.1	Purine, terpenoid metabolism

9	MAL13P1_206	Phosphate transporter	--	Transport
	PFL0305c	5'-nucleotidase	3.1.3.5	Purine metabolism
10*	PF13_0349	Nucleoside-diphosphate kinase	2.7.4.6	Pyrimidine, purine, dolichol metabolism
	PFF0275c	Nucleoside-diphosphate kinase	2.7.4.6	Pyrimidine, purine, dolichol metabolism
11	MAL13P1_82	CDP-diacylglycerol-inositol 3-phosphatidyltransferase	2.7.8.11	Inositol phosphate metabolism
	PF14_0100	CTP synthase	6.3.4.2	Pyrimidine metabolism
12	MAL13P1_82	CDP-diacylglycerol-inositol 3-phosphatidyltransferase	2.7.8.11	Inositol phosphate metabolism
	MAL13P1_206	Phosphate transporter	--	Transport
13	PF14_0097	Phosphatidate cytidylyltransferase	2.7.7.41	Phosphatidylethanolamine, phosphatidylserine metabolism
	PF14_0100	CTP synthase	6.3.4.2	Pyrimidine metabolism
14	MAL13P1_206	Phosphate transporter	--	Transport (Pi)
	PF14_0097	Phosphatidate cytidylyltransferase	2.7.7.41	Phosphatidylethanolamine, phosphatidylserine Metabolism
15	PFC0725c	NO_2 transporter	--	Transport
	PFI0735c	NADH dehydrogenase (ubiquinone)	1.6.5.3	Mitochondrial electron flow
16	PF10_0334	Succinate dehydrogenase (ubiquinone)	1.3.5.1	Mitochondrial electron flow
	PFC0725c	NO_2 transporter	--	Transport

Table S5| Essentiality in *S. cerevisiae* of the orthologs for essential and non-essential *Plasmodium* metabolic genes. While all of the orthologs for essential metabolic genes in yeast are also essential in *Plasmodium*, only about half of the orthologs for *Plasmodium* essential metabolic genes are essential in yeast. Fisher's exact test *P*-value =0.04.

	Yeast essential	Yeast non-essential	**Total**
Plasmodium essential	6	4	10
Plasmodium non-essential	0	5	5
Total	6	9	15

References

Becker SA, Feist AM, Mo ML, Hannum G, Palsson BO, Herrgard MJ (2007) Quantitative prediction of cellular metabolism with constraint-based models: the COBRA Toolbox. *Nat Protoc* **2:** 727-738

Kaufman DE, Smith RL (1998) Direction choice for accelerated convergence in hit-and-run sampling. *Operations Research* **46:** 84-95