Supplemental Methods

**Creation of *P. falciparum* pdh e1α**

Targeting sequences 5’ and 3’ to *P. falciparum* PDH E1α (PF3D7_1124500) were amplified from *P. falciparum* NF54 genomic DNA and cloned into plasmid pCC1 to facilitate positive–negative selection (1). Restriction sites in the multiple cloning sites used were Sac II/Spe I for the 5’ flank and Neo I/Avr II for the 3’ flank (Fig. S1A). Sequencing was performed to confirm inserts and primers used are detailed in Table S1. Plasmid DNA was extracted using a Maxiprep Kit (Qiagen). For transfection, the NF54 line of the *P. falciparum* parasite was synchronized at ring stage with sorbitol two days prior. Transfection of *P. falciparum* ring stages with 100 μg of plasmid DNA was performed by electroporation at 0.31 kV and 950 μF with a Bio-Rad Gene Pulser (Bio-Rad, La Jolla, CA). Cultures were placed on the positive selection drug WR99210 (Jacobus Pharmaceuticals, Princeton, NJ) 6 hours post-transfection and maintained as described to select for a population of parasites resistant to WR99210 and thus harboring the plasmid (2). This was followed by negative selection against the cytosine deaminase/uracil phosphoribosyl transferase gene product with 5-fluorocytosine in order to obtain a parental line with double crossover homologous recombination (3), which thus results in the specific gene deletion of PDH E1α and the creation of *Pf* pdh e1α-. To test for gene disruption, genomic DNA was extracted and analysis was performed by comparing the parental transgenic parasite and wild type genomic DNA by Southern Blot. Genomic DNA was digested for with Bgl II and Eco RI and separated on a 1 % TAE agarose gel. Following DNA depurination, denaturation and neutralization, the digested DNA was transferred to Hybond membrane overnight. Digoxigenin-labelled 5’ and 3’ probes were prepared from the same targeting sequences and used to probe the transferred DNA to confirm the presence of a population of parasites with double cross over homologous recombination (Fig. S1).

Clones of *pf*pdh e1α- were isolated by limiting dilution. Gene disruption was confirmed by PCR using oligonucleotide primers that specifically amplified DNA across both the 5’ and 3’ recombination sites as well as primers that amplified the *PDH E1α* open reading frame, a PCR product that would be absent from the knockout parasite but present in the wild type parasite (Fig. S1B). Genomic DNA was isolated from both the knockout parasites and wild type parasites for PCR.

In vitro growth assay: Tightly synchronous ring-stage NF54 and pdh e1α- G2/B9 clones were adjusted to 1 % parasitemia and 2 % hematocrit. Media was changed daily and cultures were diluted with fresh uninfected erythrocytes when necessary. Thin-blood smears were taken and an aliquot of each culture was collected and stored at -20 °C until analysis. After 15 days, all aliquots were thawed and the relative DNA content was assessed via the syb-green assay (4). Growth curves were constructed by blank subtraction and dilution-factor correction.

**mRNA time course and microarray:**

For each time point 0.5 mL of packed infected RBC culture (10 % parasitemia) were harvested from 25 ml of culture suspension at each time-point (an additional 25ml was harvested at time 0 and 6 h to ensure sufficient amounts of RNA could be extracted) by centrifugation at 1,500rpm for 5min. Total RNA was extracted and purified using TriZol reagent (Invitrogen, Grand Island, NY, USA) at a volume of 5 ml reagent to 1 ml of packed parasitized erythrocytes as previously described (5). Quality and quantity of total RNA extracted was assessed using a ND-1000 (NanoDrop Technologies, Thermo
cDNA was generated, dye coupled and hybridized to *P. falciparum* 8x15K Agilent Arrays as previously described (6,7). Hybridized arrays were then scanned using an Agilent G2505B Microarray Scanner (Agilent Technologies, Santa Clara, CA, USA) with 5 μm resolution at wavelengths of 532 nm (Cy3) and 633 nm (Cy5) employing an extended dynamic range (10-100 %) setting. Normalized intensities were extracted using Agilent Feature Extractor Software Version 9.5 was utilized to extract normalized intensities by the standard GE2-v5.95_Feb07_no_spikein extraction protocol and uploaded to the Princeton University Microarray Database (PUMA.princeton.edu) for analysis.

**Confirmation of *P. falciparum* PDH presence during the asexual blood stage**

Synchronous parasite cultures were pelleted and extracted into solublization buffer (8M guanidine HCl, 100 mM Tris pH 8, 50 mM DTT) then subjected to dissolution, thiol reduction and alkylation, and overnight trypsin digestion according to the FASP methodology (8). Digest solution was subjected to 10 KDa cutoff membrane ultrafiltration (Microcon YM-10, Millipore) and the filter was washed with 50 % acetonitrile (ACN), 0.1 % formic acid (FA). The final peptide liquor was concentrated in a speedvac until near dryness. Peptides were desalted using StageTip micro-scale reversed-phase chromatography (9) and then subjected to reversed-phase nano-UPLC-MS and MS/MS performed on an Easy-nLC Ultra 1000 nanoflow capillary UPLC system (ThermoFisher Scientific, San Jose, CA) coupled to a VelosPro-Orbitrap Elite hybrid mass spectrometer (ThermoFisher Scientific) using a Flex ion source (Proxeon, Odense, Denmark). Separation and data acquisition were achieved via methods previously described (10). The resulting LC-MS/MS data were processed into peak-list files (mgf) using ProteomeDiscoverer (v. 1.4, ThermoFisher), and then searched against a concatenated database consisting of the proteomes of *Plasmodium falciparum* (PlasmoDB v. 9.2) and Human erythrocytes (derived from (11)) using the Mascot search engine (v. 2.2.7, Matrix Science), allowing for an initial mass error of 6 ppm for precursor and 1.2 Da for fragment ion species, ≤ 3 missed trypsin cleavages, carbamidomethylation of cysteines as a fixed modification, with methionine oxidation and N-terminal protein acetylation as variable modifications. Aggregate search results were collated, consolidated, and subject to spectral counting as previously described (10) and the protein false discovery rate was estimated to be ≤ 0.1 %. Fragmentation spectral assignments were subject to manual inspection and validation using the original tandem mass spectra acquired in profile mode using Xcalibur software (ThermoFisher).

**Supplemental Figure 1.** Rapid \[^{13}\text{C}-6\]glucose labeling of glycolysis. RPMI containing 11 mM \[^{13}\text{C}-6\]glucose was added to enriched *P. falciparum*-infected and uninfected erythrocyte suspensions at a 1:1 ratio. Rapid quenching and metabolite extraction was performed and metabolic flux assessed via LC/MS. Percent labeling was adjusted for the maximal theoretical enrichment for equal \(^ {12}\text{C}:^{13}\text{C}\) glucose mixing and data are presented as intracellular concentration (top inset) and % of total metabolite pool (bottom inset) as mean ± SEM from n =4 experiments.

**Supplemental Figure 2.** LC/MS-MS detection of PDH subunits from blood-stage *P. falciparum*. MS-MS spectra of unique peptides for PDH E1α (A-B), PDH E1β (C-D), PDH E2 (E-F), and PDH E3 (G-H). The peptide sequence is listed above with the B (red) and Y (blue) fragments detected denoted as corresponding colored dashes between amino acid residues.

**Supplemental Figure 3.** Creation of a *PDH E1α* gene knockout in *P. falciparum* NF54 (*Pf pdh e1α*). (A) *Pf pdh e1α*- was created by double crossover homologous recombination by transfecting ring stage parasites with the plasmid pCC1 (1) containing the 5’ and 3’ flanks of *PDH E1α*. The mutant human dihydrofolate reductase cassette (HsDHFR) allowed for positive selection of plasmid integration with WR999210. The cytosine deaminase/uracil phosphoribosyl transferase cassette (FCU) allowed for negative selection with 5-fluorocytosine to ensure for double crossover (3). Oligonucleotide primers were
used to test for the presence of the PDH E1α open reading frame (OF/OR), presence of 5’ integration (T1F/T1R) and the presence of 3’ integration (T2F/T2R) in the parental population after selection and clones isolated by limiting dilution. Sizes of the expected amplicons are noted in base pairs. (B) Following transfection and both positive and negative selection genomic DNA was extracted from the parental parasite line (e1α-) and the wild type (WT), digested with Bgl II and Eco RI and separated by agarose electrophoresis. Southern blot hybridization was performed with digoxygenin labeled 5’ and 3’ probes (the targeting sequences used for knockout). Band sizes in kilobases (kb) are shown and were of the expected size for both WT and e1α- based on the Bgl II and Eco RI digestion. (C) Agarose gel electrophoresis of PCR products from the parental population and the two Pf pdh e1α- clones used in this study (B9 and G2). Note the faint band for the open reading frame product in the parental population (white arrow) is absent from the clones and the PCR products are of the expected size.

Supplemental Figure 4. In vitro growth of P. falciparum pdh e1α- parasites. To assess blood stage growth, synchronous parasite cultures of both NF54 and the pdh e1α- clone B8 were adjusted to 1 % parasitemia 2 % hematocrit. Cultures were maintained under standard culturing conditions and media changed daily. Aliquots of culture were collected daily for 15 days and growth was assessed via syb-green fluorescence measurement. The Y-axis is set to the initial parasitemia of 1 (%) but is expressed as arbitrary growth units as log10. The inset represents growth over day 10 to 14 expressed as arbitrary growth units. The data is presented as mean ± SEM from n =3.

Supplemental Figure 5. Oxythiamine inhibition of P. falciparum metabolism. (A) [13C-2]Citrate (B) [13C-2]N-acetyl-glutamate (C) [13C-5]AMP (D) total succinate pool presented as total ion count/10⁸ cells. Data are presented as mean ± SEM from n = 3 independent experiments.

Supplemental Figure 6. ¹H-¹³C HSQC NMR spectra of infected erythrocytes labeled with [¹³C-6]glucose for 2 hours. Pure standards were used for assignment and quantification. Values represent the mean ± SEM from n = 3 experiments and represented as the ¹³C-labeled intracellular concentration assuming the water volume for an infected erythrocyte is 75 fL. Dotted lines indicate that a pure standard was not run for that compound and assignment was made using the Madison Metabolomics Consortium Database (MMCD) (12). Peak intensity is presented using the following colors; pink = negative, blue – white = increasingly positive. Metabolites presented are as follows; acetate (Ace), alanine (Ala), AMP, ATP, Glucose (Glc), glutamine (Gln), glutamate (Glu), glycerol (Glyc), lactate (Lac), NAD, pyruvate (Pyr), UMP, and an unknown peak (Ukn).

Supplemental Table 1. Kinetic flux analysis of glycolysis in P. falciparum-infected and uninfected erythrocytes. Flux rates for each detectable intermediate of glycolysis were determined from the data presented in Figure 1 and S1, fitting a straight line from 0 to 30 seconds and converting the rate to nmol/10⁸ cells/min. In some instances the estimated flux through a particular reaction is likely to be an underestimate, considering non-linear ¹³C-labeling of several intermediates had already occurred at the first time point measured.

Supplemental Table 2. Peptide confirmation of PDH subunits.

Supplemental Table 3. Genes with significantly different transcript abundance between NF54 and pdh e1α-. RNA was extracted at six hour intervals and analyzed via microarray. Normalized ratios of each time point RNA to total RNA was extracted and significance was determined using a false-discovery rate of 5 %.

REFERENCES


Figure S1

1. [IC](μM) %[^{13}C-6]G-6-P
2. [IC](μM) %[^{13}C-6]FBP
3. [IC](μM) %[^{13}C-3]Gly-P
4. [IC](μM) %[^{13}C-3]Gly-P
5. Ion Count (x10^6) / 10^6 cells %[^{13}C-3]1,3-BPG
6. [IC](μM) %[^{13}C-3]3-PG
7. [IC](μM) %[^{13}C-3]PEP
8. [IC](μM) %[^{13}C-3]Pyr
9. [IC](μM) %[^{13}C-3]Malate
10. [IC](μM) %[^{13}C-2]Ac-CoA
11. [IC](μM) %[^{13}C-3]Lactate

Graphs 1-9 show the percentage of labeled metabolites over time for different conditions.

Graph 5 shows ion count over time for different conditions.

Graphs 10 and 11 show the percentage of labeled metabolites for different conditions.

Legend:
- uRBC
- NF54
- e1α

Metabolite Pathway:
1. Glucose-6-P
2. Fructose-1,6-P
3. Glycerone-P
4. Sn-glycerol-3-P
5. Glycerate-1,3-P
6. Glycerate-3-P
7. PEP
8. Pyruvate
9. Malate
10. Acetyl-CoA
11. Lactate

* Cannot differentiate between 1,3-BPG and 2,3-BPG
A. Pyruvate dehydrogenase E1 alpha subunit (PF11_0256)

Peptide spectral match of the MS/MS spectrum for the [M+2H]^{2+} precursor at m/z 717.3996

B. Pyruvate dehydrogenase E1 alpha subunit (PF11_0256)

Peptide spectral match of the MS/MS spectrum for the [M+2H]^{2+} precursor at m/z 807.9428

C. Pyruvate dehydrogenase E1 beta subunit (PF14_0441)

Peptide spectral match of the MS/MS spectrum for the [M+2H]^{2+} precursor at m/z 393.2575

D. Pyruvate dehydrogenase E1 beta subunit (PF14_0441)

Peptide spectral match of the MS/MS spectrum for the [M+2H]^{2+} precursor at m/z 422.2478
E. dihydrolipoamide acyltransferase component E2 (DLAT, PF3D7_1020800)

\[\text{263DNEKEKIEEPFKNK}_{276}\]

Peptide spectral match of the MS/MS spectrum for the [M+2H]^2+ precursor at m/z 874.4401

G. Lipoamide dehydrogenase (aLipDH, PF3D7_0815900)

\[\text{434NPNTQLNLGEKLK}_{446}\]

Peptide spectral match of the MS/MS spectrum for the [M+2H]^2+ precursor at m/z 734.9054

H. Lipoamide dehydrogenase (aLipDH, PF3D7_0815900)

\[\text{63NILNLK}_{68}\]

Peptide spectral match of the MS/MS spectrum for the [M+2H]^2+ precursor at m/z 357.7268
Figure S3

A

- Wild type locus
- Plasmid
- Recombinant locus

B

- Probe: 5' flank
  - DNA: WT eIα-
  - 4.8 kb >
  - < 1.8 kb

- 3' flank
  - WT eIα-
  - 4.8 kb >
  - < 2.1 kb

C

- Parental
- Clone B9
- Clone G2
Figure S4

Arbitrary Growth Units

Day

NF54

e1α−
Figure S5

A

% $[^{13}\text{C}-2]\text{Citrate}$

B

% $[^{13}\text{C}-2]\text{Ac-Glut}$

C

% $[^{13}\text{C}-2]\text{AMP}$

D

Ion Count (x10$^5$) / 10$^5$ Cells

13C-U-Glu - + + +
Thiamine + + - -
Oxy-T - - - +
Figure S6

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**[Intracellular]** (μM)

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Supplemental Table 1

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