Supplementary information

Methods

1. ^{14}C-label distribution in free parasite culture

For ^{14}C-label distribution between spent medium and parasite lysate, saponin released erythrocyte free parasites were incubated with 1 μCi (0.5 mM) of ^{14}C-2,3-fumarate for 8 h.

After incubation, the spent medium was treated with 10 % trichloroacetic acid (TCA) for 15 min. on ice followed by boiling for 10 min. The protein precipitate was removed by centrifugation for 30 min. at 4 ºC. 10 μl of the supernatant was spotted on a silica-TLC plate to separate ^{14}C-fumarate from ^{14}C malate (TLC conditions are as described in Materials and methods of the main paper for the separation of aspartate and fumarate) and counts under malate spot were obtained. It should be noted here that the spot corresponding to the mobility of malate might as well include other polar metabolites such as aspartate, pyruvate and lactate. The parasite cell pellet obtained after centrifugation was washed twice with 1X PBS and was then lysed by sonication in sterile distilled water. Following centrifugation to remove the cell debris, the supernatant was counted for radioactivity. In each case, the radioactive counts were normalized to the total volume of the solution and expressed as fraction of the input radioactive counts of 1μCi of ^{14}C-2,3-fumarate.

2. Cloning, expression and purification of *P. falciparum* malate dehydrogenase and *P. falciparum* aspartate aminotransferase

P. falciparum malate dehydrogenase gene (single exon) was amplified using parasite genomic DNA as template, a forward primer, Pf $MDH_{fwd.}$ with *Nco* I and reverse primer, $PfMDH_{rev.}$ with *Xho* I restriction sites.

$PfMDH_{fwd.}$: $^{5'}$GCT**CCATGG**CTAAAATTGCCTTAATAGGTAGTGGTCAAATCGG $^{3'}$
$PfMDH_{rev.}$: $^{5'}$ATC**CTCGAG**TTTAATTAAGTCGAAAGCTTTTTGTGTGTTGC $^{3'}$

Similarly, *P. falciparum* aspartate amintransferase (AAT) gene was amplified using the forward primer $PfAAT_{fwd.}$ with *Nco* I and reverse primer $PfAAT_{rev.}$ with *Xho* I restriction sites.

$PfAAT_{fwd.}$: $^{5'}$CATG**CCATGG**ATAAGTTATTAAGCAGCTTAGAAAATATCG $^{3'}$
$PfAAT_{rev.}$: $^{5'}$ CCG**CTCGAG**TATTTGACTTAGCGAAAGACAAATTTTGTCGGC $^{3'}$

Amplification yielded DNA fragments corresponding to 0.9 Kb for PfMDH and 1.2 Kb for PfAAT that were digested with *Nco* I and *Xho* I and cloned in pET 28b expression vector. The identity of the clone was confirmed by sequencing. PfMDH protein was expressed with a hexahistidine tag at the C-terminus in *E. coli* BL21-CodonPlus™ (DE3)-RIL cells (Stratagene, USA). Transformed cultures were grown in terrific broth containing kanamycin (50 μg ml^{-1}) and chloramphenicol (40 μg ml^{-1}) and induced with IPTG at a final concentration of 0.75 mM. Induction was carried out at 16 °C for 12 hours. PfAAT was expressed in C41 DE3 strain of *E. coli* (Miroux and Walker, 1996). Induction was carried out for 12 hours at 27 °C. For protein purification, the cell pellets were resuspended in lysis buffer containing 50 mM potassium phosphate, pH 7.4, 100 mM NaCl, 2 mM dithiothreitol (DTT) and 10 % glycerol, lysed using

French press and centrifuged. The supernatant was incubated with nickel-nitrilotriacetic acid (Ni-NTA) agarose beads after which the beads were washed extensively with wash buffer (50 mM potassium phosphate, pH 7.4, 100 mM NaCl, 30 mM imidazole, 2 mM DTT, 10 % glycerol). Bound proteins were eluted with elution buffer, pH 7.4 (wash buffer containing 200 mM imidazole). The eluted protein was concentrated and further purified on Sephacryl 200 gel filtration column pre-equilibrated with 50 mM potassium phosphate, pH 7.4, 100 mM NaCl, 2 mM DTT, 1 mM EDTA and 10 % glycerol. The eluted protein was again concentrated and used for further experiments. Protein concentrations were determined by the method of Bradford (Bradford, 1976) with bovine serum albumin (BSA) as standard.

PfAAT was assayed as described by Winter and Dekker (17). The consumption of oxaloacetate was monitored as a time dependent decrease in absorbance at 270 nm with a molar extinction coefficient of 782 M^{-1} cm^{-1}.

3. Sequences of primers used in RT-PCR.

Pf Fumarate hydratase
Forward: 5' CAGGTAAACTTGTTGTTGCTAGAGATAG 3'
Reverse: 5' CAACGACGTCAATTAAATGAACGGCTTCC 3'

Pf Malate Quinone oxidoreductase
Forward: 5' GACTTAACATATTGTGTTGGTTATGGAGG 3'
Reverse: 5' TTAACGGGATATTCGCCTTCATATAAATA 3'

Pf Aspartate aminotransferase
Forward: 5' ATACATACGAATCGTATTCTATGCCAACT 3'
Reverse: 5' TGCTCAGCAATTTTGGCTAGTAGAGGAACG 3'

Pf Malate oxoglutarate translocator
Forward: 5' TGAAAGCAGATCCTGTTACTAAGAAAATG 3'
Reverse: 5' AAGATTATTTAGATAATCTACAGTTATGAGTG 3'

4. Indirect immunofluorescence

Parasitized erythrocytes were washed thrice in 1 X PBS and then fixed with 4 % paraformaldehyde and 0.0075 % glutaraldehyde in PBS for 30 min. Following washing with 1 X PBS, the cells were permeabilized with 0.05 % saponin solution (prepared in 1 X PBS) for 2 min. Following another PBS wash, cells were blocked with 3 % BSA in 1 X PBS for one hour. Anti-PfMDH (diluted 1:200) or anti-PfAAT antiserum (diluted 1:200) was added to the blocking solution and cells were incubated for further one hour. Following washing, the cells were incubated with anti-rabbit IgG (for MDH) or anti-mouse IgG (for AAT) conjugated with AlexaFluor dyes (Molecular Probes®, USA) for one hour. After washes with wash buffer the nuclei were stained with Hoechst stain (Molecular Probes®, USA) (1:10,000 dilution) for 20 min. After washing, the cell suspension was placed on poly-lysine coated coverslips, mounted on a glass microscope slide in 50 % glycerol (in 1X PBS) and sealed. The glass slides were visualized by using a Carl-Zeiss laser scanning confocal microscope (Carl Zeiss, Inc., USA).

Reference:
Miroux, B., and Walker, J.E. (1996) *J. Mol. Biol.* **260**:289-98.

Figure legends and Figures

Fig. S1: U-[^{14}C]-aspartate incorporation in nucleic acids and proteins by parasitized erythrocytes and free parasites.

(A) Radio-TLC of U-[^{14}C]-aspartate (200 mCi mmol^{-1}). (B) U-[^{14}C]-aspartate incorporation in the nucleic acids by parasitized erythrocytes after 24 and 48 h. 1 μCi of the radioactive tracer was added to parasitized erythrocytes and erythrocytes and were harvested onto glass fiber filters using a Combi-12 automated cell harvester (Molecular Devices, Sunnyvale, CA), washed extensively, dried and the incorporated radioactivity measured as counts per minute (cpm) using a Wallac 1409 (Wallac Oy, Turku, Finland) liquid scintillation counter. (C) Parasitized erythrocytes were incubated with 1 μCi of U-[^{14}C]-aspartate for 24 h and were then treated with 0.15 % saponin solution to release the parasites from the erythrocytes. The parasite pellet was resuspended and subjected to SDS-PAGE and the gel was dried and developed using Fuji FLA 5000 phosphorimager. (D) U-[^{14}C]-aspartate incorporation in saponin released erythrocyte free parasites.

Fig. S2: Dilution of ^{14}C fumarate incorporation by unlabelled fumarate, malate and aspartate

(A) ^{14}C-2,3-fumarate incorporation by parasitized erythrocytes in the presence or absence of 0.5 mM fumarate, malate and aspartate. (B) [^{3}H]-hypoxanthine incorporation by parasitized erythrocytes in the presence or absence of 0.5 mM fumarate, malate and aspartate.

Figure S1

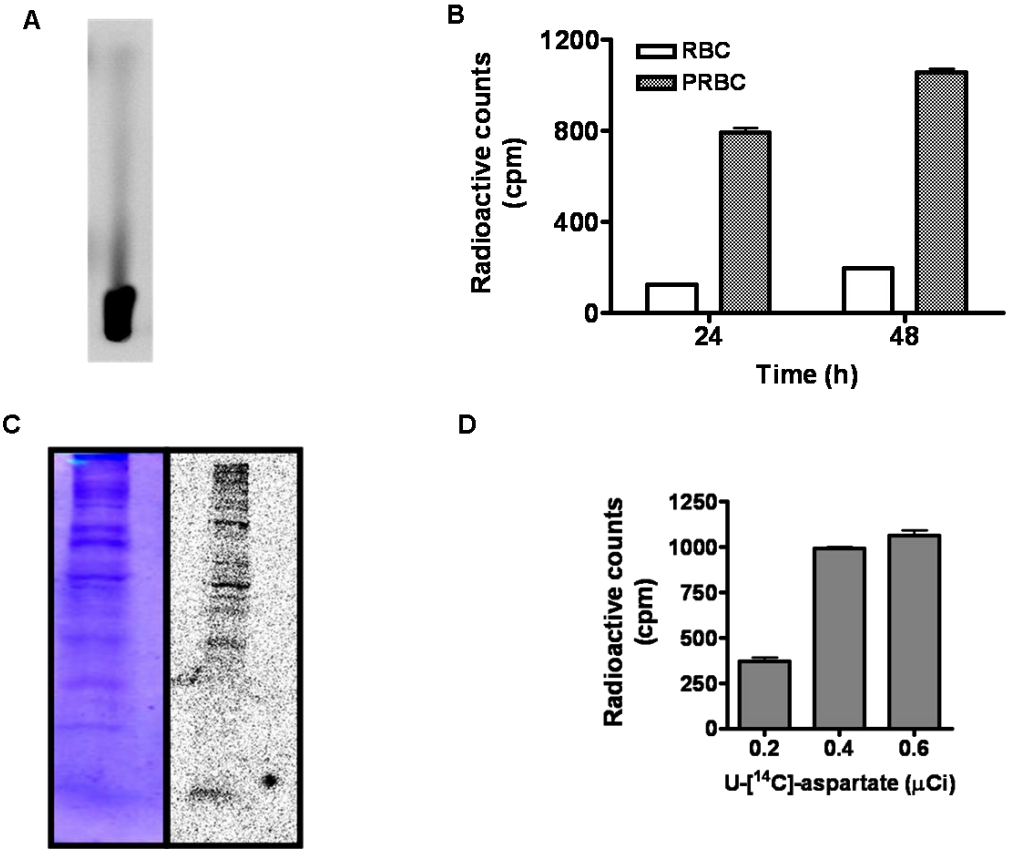

Figure S2