Supplemental information for "Methylene tetrahydrofolate dehydrogenase/cyclohydrolase and the synthesis of 10-CHO-THF are essential in *Leishmania major", by* Silvane M. F. Murta, Tim J. Vickers, David A. Scott, and Stephen M. Beverley.

Supplemental methods

Expression and purification of recombinant LmjDHCH1

The pET15b-*LmjDHCH1* vector was transformed into *E. coli* strain Rosetta2(DE3)/pLysS. Cells were grown at 37°C in 800 ml LB media containing 100 μg ml^{-1} carbenicillin and 12.5 μg ml^{-1} chloramphenicol to an OD of 0.6. Expression was induced for 4 h with 1 mM isopropyl-β-D-galactopyranoside and the cells then harvested and frozen.

Frozen cells were thawed on ice and resuspended in 30 ml of lysis buffer (75 mM Na-phosphate, pH 7.5, 500 mM NaCl, 1 mM benzamidine, 3 μg ml^{-1} leupeptin, 250 μM 4-(2-amino-ethyl)benzenesulphonyl fluoride, 10 U ml^{-1} DNAase I (Sigma), 10,000 U ml^{-1} lysozyme (Sigma), 1 mM 2-mercaptoethanol, 10% (v/v) glycerol). The cells were then lysed by sonication, (4 x 30 s bursts), with cooling to <4°C between pulses. An identical extraction procedure was used to produce extracts of *L. major* for enzyme assay. After centrifugation (30,000 x g, 1 h, 4°C), clarified extract was applied at 2 ml min^{-1} at 4°C to a nickel-charged 20 ml (1.2 x 11 cm) Ni-NTA chelating agarose column (Qiagen) equilibrated with binding buffer (50 mM Na-phosphate, pH 7.5, 200 mM NaCl, 10% (v/v) glycerol). Unbound protein was removed by washing with 50 ml of binding buffer. Retained proteins were eluted with a linear gradient at 1 ml min^{-1} of 0-100% of elution buffer containing (50 mM Na-phosphate, pH 7.5, 200 mM NaCl, 10% (v/v) glycerol, 500 mM imidazole). Fractions containing DHCH1 were pooled.

Western blot analysis

Whole cell lysates from *L. major* lines were prepared from stationary phase parasites in SDS sample buffer; 5 X 10^6 cell equivalents/lane. The samples were separated by electrophoresis on a 10% SDS polyacrylamide gel and electrotransferred onto nitrocellulose membranes (Hybond-ECL; Amersham Biosciences). Antiserum raised against *Lmj*DHCH1 and *Lmj*FTL were used at a 1:3,000 and 1:1,000 dilution, respectively. Secondary antibodies conjugated to peroxidase were from Amersham Biosciences, and ECL reactions were conducted using PerkinElmer Life Sciences chemiluminescence kit. In western blot analysis both the anti-FTL and DHCH1 antisera identify a major protein band of the expected molecular weights. The assignment of these bands to FTL and DHCH1 was confirmed by western blots against null or overexpressor mutants.

Table S1. Oligonucleotide primers used in this work.

Primer number	Sequence
SMB 3528	CATATGCCGTCTGCTCAGATCATTGA
SMB 3529	GGATCCCTATGATACGCCCAACGCAGCCT
SMB 2557	ggtaacggtgcgggctgacgCCACCATGACCGAGTACAAGCCC
SMB 2558	cgagatcccacgtaaggtgcTCAGGCACCGGGCTTGCG
SMB 2561	ggtaacggtgcgggctgacgCCACCATGAAAAGCCTGAACTC
SMB 2562	cgagatcccacgtaaggtgcCTATTCCTTTGCCCTCG
SMB 2656	cgtcagcccgcaccgttaccATGATCTGAGCAGACGGCATC
SMB 2657	gcaccttacgtgggatctcgGGCACGTGCGCTCCACTTC
SMB 2731	CGTGTGACACTTTCAAGGTGCGA
SMB 2771	ATTCGACGTTGCTTGCGGT
SMB 2772	GCATGCACGCAAACGGATTGGATGTGT
SMB 2773	GGATCCCACGTGTCTTTGGAACAGAGTG
SMB 3123	AGATCT**CCACC**ATGGCCACCCGGAAGCTGCA
SMB 3124	AGATCTTTACGAAAGACCCACAATCC
SMB 3125	AGATCT**CCACC**ATGCCGTCTGCTCAGATCAT
SMB 3126	AGATCTCTATGATACGCCCAACGCAG

Restriction sites are underlined small type is linker sequence.

Figure S1. Purified *Lm*DHCH1.

A 5 μg sample of purified recombinant DHCH1 was separated on a 12% SDS-PAGE gel and stained with Coomassie Blue.

Figure S2. Alignment of human cytoplasmic and mitochondrial DHCH domains with *Leishmania major* DHCH1.

The sequences are L. major DHCH1 and the DHCH domains of the human cytoplasmic C1-synthase (Swiss-Prot P11586) and mitochondrial DHCH (P13995). Residues identical in all three proteins are shown boxed and shaded. Structural features have been depicted based upon the structure of the human C1-synthase DHCH domain (Schmidt et al., 2000). The active site lysine (K56, ▼) and proximal residues (*) as well as Arg173 implicated in NADP+ binding are marked (*). Residues implicating in $NADP^+$ binding are shown boxed with a blue outline, while residues implicated in DHCH inhibitor binding are shown boxed with a gray outline.

Figure S3. PCR confirming heterozygous *DHCH1* replacements.

PCR tests of planned *HYG* (**A**) or *PAC* (**B**) replacements at *DHCH1*. Homologous replacement was visualized by PCR amplification using a primer located on the *DHCH1* chromosome outside of the targeting fragment on the 5' site (SMB2731) and a primer directed against sequences located with the HYG (SMB2562) or PAC ORF (SMB2558) within the targeting fragment. The sizes of the predicted PCR products are shown (**C**). #1-4, four independent heterozygous lines with HYG replacement +/ *HYG* and #1-12, twelve independent heterozygous lines with PAC replacement +/ *PAC*.

Figure S4. Overexpression of FTL in pXNG4-*FTL* transfectants

Western blot analysis of FTL protein detected with polyclonal rabbit antisera against *Lmj*FTL (1:1,000) (Vickers et al., 2008) in extracts of the *L. major* WT, WT+pXNG4-*FTL* and heterozygous +/*PAC* or +/*HYG* lines transfected with pXNG4-*FTL*. Protein loading was assessed with a rabbit anti-*L. major* histone H2A polyclonal antibody (Iris Wong and Stephen M. Beverley, manuscript in preparation) at 1: 100,000 dilution.

Fig. S1 Purification of recombinant *L. major* DHCH1 protein

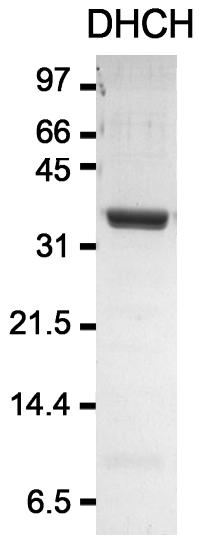

Fig. S2. Alignment of human DHCH domains and *Leishmania major* DHCH1

showing homology (shading) and features based on human cDHCH domain structure (Schmidt *et al* 2000)

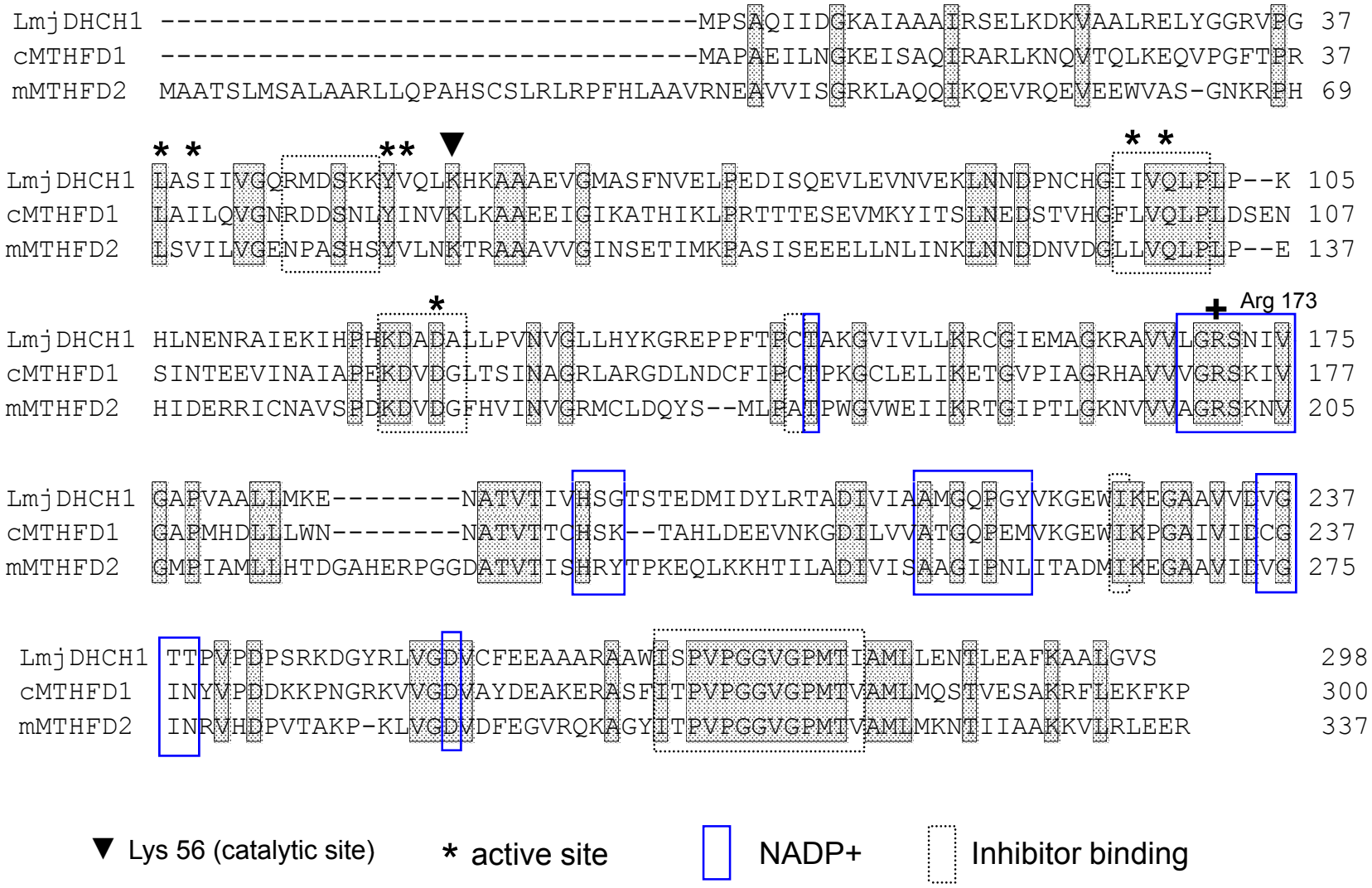

Fig. S3 PCR confirming *DHCH1* heterozygotes

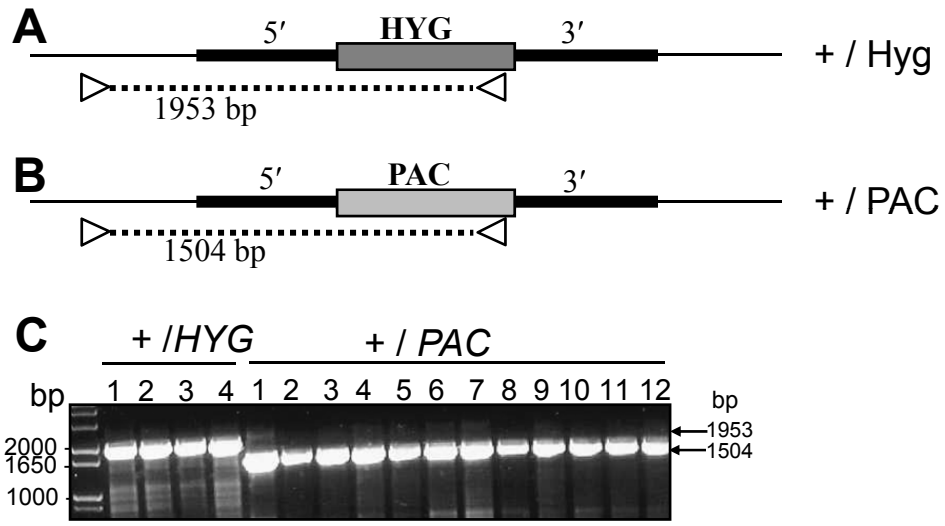

Fig. S4 Overexpression of FTL in pXNG4-FTL transfectants