Supporting Information for

"Redox-dependent lipoylation of mitochondrial proteins in *Plasmodium falciparum*"

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Figure S1

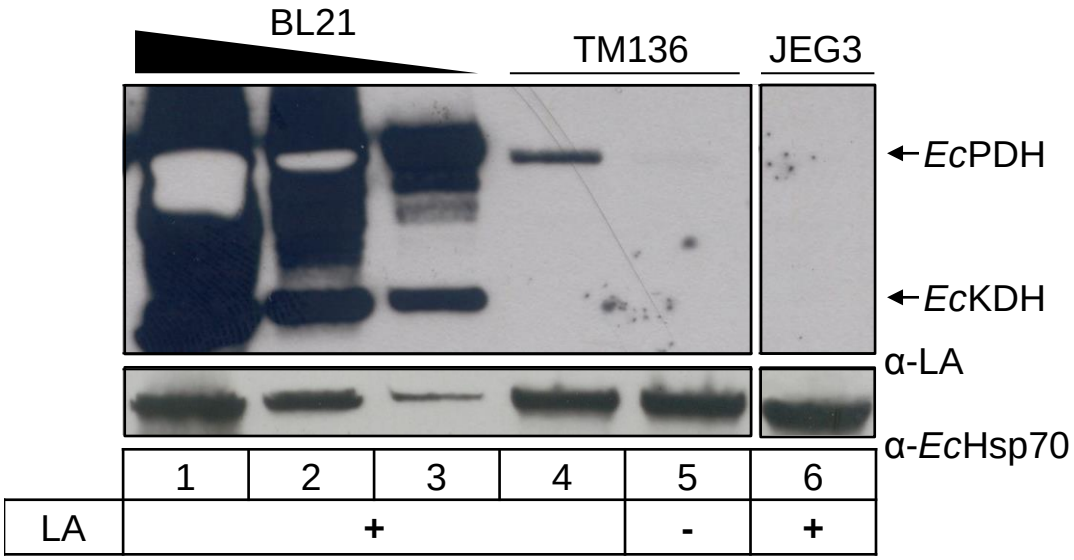

Fig. S1. Analysis of *lplA*-/*lipB*- knockout *E. coli* cell line JEG3. Cultures of three different *E. coli* cell lines (BL21, TM136 and JEG3) were normalized by OD_{600} before analysis by western blot using antibodies specific for lipoylated proteins (α-LA) and antiserum specific for *E. coli* Hsp70 (α-Hsp70) as a loading control. Lipoate (LA) was added to the cultures grown in minimal medium (containing succinate and acetate) to assess lipoate ligase activity which is still present in the TM136 *lplA*-/*lipB*- disruption cell line, but not the JEG3 *lplA*-/*lipB*- knockout cell line.

Figure S2

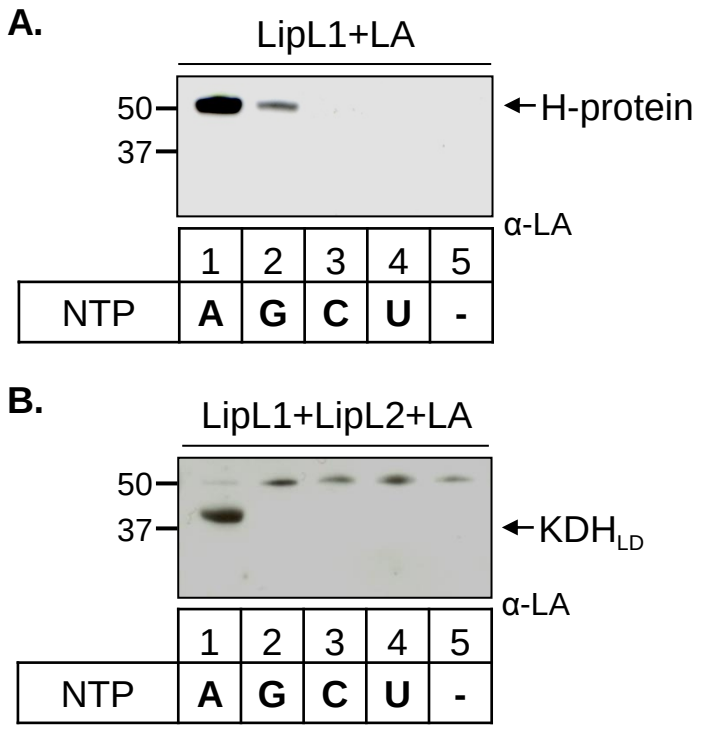

Fig. S2. Ligation reactions proceed through an ATP-dependent reaction.

(A) Western blot analysis of ligation reactions catalyzed by LipL1 showing that the H-protein is preferentially lipoylated using ATP (A) as a nucleotide source with minor activity observed for GTP (G), but not for CTP (C) or UTP (U).

(B) Western blot analysis of ligation reactions catalyzed by LipL1 and LipL2 showing that the KDH_{LD} is preferentially lipoylated using ATP (A). Note a minor population of *E. coli* E2-KDH found in purified LipL2.

Figure S3

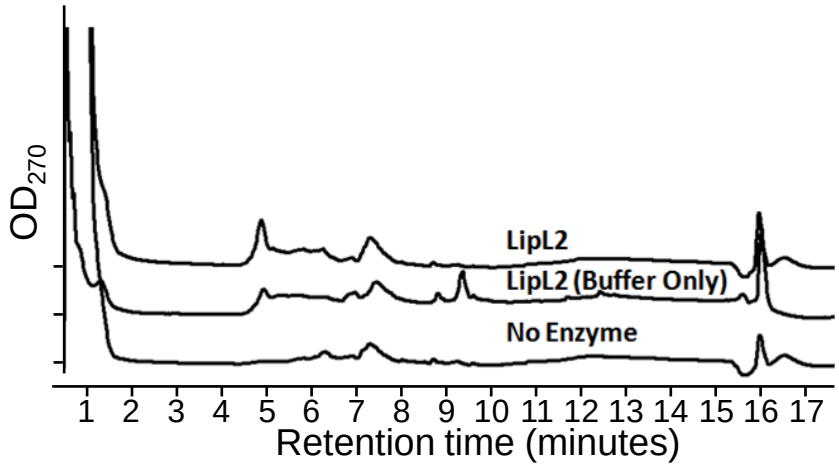

Fig. S3. HPLC traces for LipL2 reaction and controls. LipL2 was incubated in the presence of LA and ATP as described in the Methods (LipL2 trace). As a control we ran a sample of LipL2 with no reactants (Buffer only) and a sample with all reactants but no enzyme (No Enzyme).

Figure S4

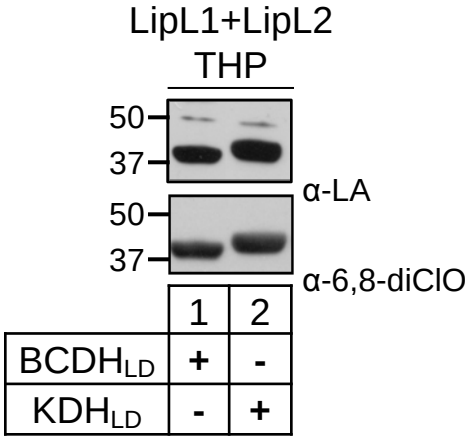

Fig. S4. Modification of $BCDH_{LD}$ and KDH_{LD} in the presence of THP.

LipL1 and LipL2 can modify both substrates with LA (top panel) or 6,8-diClO (lower panel) in a coupled reaction in the presence of the strong reducing agent THP, similar to the results with TCEP (see Fig. 4B).

Figure S5

Harmonized LipL2 nucleotide sequence.

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ATGCGCATCATTAAGTGTCTAGATCAGATTTTCCGGCCGGTACTGCCGAATGTGAACATTAACA
ACATTCAGAAGAACAAAAAAATTAATATCCTCTATTTCATCGATGTATCAAAATTTCATGTGTT
TGAACAACTTCTGCTGGAAGAGAGCCTGTTTCGGATTAGCAACAACACCACCGAAGGCCTGAAT
AACATTGGTTTTGTAATCGTGAACAACACCTGCGAAGAAATGAACGAGTCGAAAGGCAATGAAT
GTATCTTTAACAACAAGAAATGCGTCATTCTGGGTATCAGCAACAAAATTAAAGATCATATTAA
AGATACGAACTATATCAAAGAAAACAAAATTTCACTGATCAAACGCTTTACCGGCGGTGGCACG
ATTTACATTAACAAAAACAGCCTGCTGGTTTCTCTGATTCTCCCACATAAATTTGAAAAAAACA
AAAAAATCTATCCGAGCAACATCACAGAATGGTCCTATAACTATTTCTACAACACCTCAAAACA
GATTTATGATAAAACCCAGATTAATAACGAAAAAAACTCCCTGAATAAAAACCATATCTTATTT
AACCAGTATTTTAACTATTATGAAAACGATTATGTGTATAAAGATTATGATGAACATAACAAAA
ACATAATCCTGAAAAAAGTCGGCGGTAACGCACAGAGCTTCGCCCGTAACTATTTCGTGCATCA
CACGAGCTATATTTGGACCTGCGATTACAAGGAAATGAACAACATTCTGCTGAACCCGTCAAAG
CAGCCGATTTACCGTAACAAACGCAAACATCAGCACTTTCTTCAAAGCATAAAACTGTGCCTTC
ATGATGATATTCACACCCCAAACATTTTTATCGAAAAACTGATCAAACATATTAAACACATAAT
TAATTATAAAAACATCACGGATCAGCATGATTACTGGTTCTTTAATAAAATCAACCTGAAAAAC
ATCAACGATCATATTCTGCGTAACTCAGAACACTTTGATGATATTTATGTAGCGGATATGAACT
TGCTGCAGTGTATCTTTAATTATTATAACAACAGCAGCTTGTTCAACAACATGCGCAGTACGTA
TTTCCTGGATTTAGAGGGAAAAAAAGTTAGTGATCGGTATTACGATATTCCGACCTATTTTCTC
TAATAA
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Harmonized LipL2 amino acid sequence.

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MRIIKCLDQIFRPVLPNVNINNIQKNKKINILYFIDVSKFHVFEQLLLEESLFRISNNTTEGLN
NIGFVIVNNTCEEMNESKGNECIFNNKKCVILGISNKIKDHIKDTNYIKENKISLIKRFTGGGT
IYINKNSLLVSLILPHKFEKNKKIYPSNITEWSYNYFYNTSKQIYDKTQINNEKNSLNKNHILF
NQYFNYYENDYVYKDYDEHNKNIILKKVGGNAQSFARNYFVHHTSYIWTCDYKEMNNILLNPSK
QPIYRNKRKHQHFLQSIKLCLHDDIHTPNIFIEKLIKHIKHIINYKNITDQHDYWFFNKINLKN
INDHILRNSEHFDDIYVADMNLLQCIFNYYNNSSLFNNMRSTYFLDLEGKKVSDRYYDIPTYFL
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Fig. S5. Nucleotide and amino acid sequence for synthetic harmonized LipL2 based on gene PF3D7_0923600. Cysteine 93 and cysteine 276 are conserved in the sequences of LipL2 proteins from malaria parasites and are highlighted in yellow.

Table S1. Primers and plasmids used in this study.

Plasmid	Primer Name	Primer Sequence
pMALcHT-LipL1	n/a	pMA006 as described in (Allary *et al.*, 2007).
pMALcHT-LipL2	n/a	none - excised using *Eco*RI and *Hind*III from synthesized gene
pMALcHT-LipL1$_{K160A}$	LipL1$_{K160A}$.F	GAAACGATATTACAGTAAATGATCAAgcaTGTTCAGGTTCTGCTTTTAAAAAAA
	LipL1$_{K160A}$.R	TTTTTTTAAAAGCAGAACCTGAACATGCTTGATCATTTACTGTAATATCGTTTC
pMALcHT-LipL2$_{C93A}$	LipL2$_{C93A}$.F	GTATCTTTAACAACAAGAAAgctGTCATTCTGGGTATCAG
	LipL2$_{C93A}$.R	CTGATACCCAGAATGACAGCTTTCTTGTTGTTAAAGATAC
pMALcHT-LipL2$_{C276A}$	LipL2$_{C276A}$.F	CTTCAAAGCATAAAACTGgctCTTCATGATGATATTCAC
	LipL2$_{C276A}$.R	GTGAATATCATCATGAAGAGCCAGTTTTATGCTTTGAAG
pMALcHT -LplA	LplA.BamHI.F	GGTGGT**GGATCC***ATG*TCCACATTACGCCTGCTCATCTC
	LplA.SalI.R	GGTGGT**GTCGAC***CTA*CCTTACAGCCCCCGCCATC
pGEXT-BCDH$_{LD}$	BCDH.BamHI.F100	GGCGGC**GGATCC**GTGAAATGCAAATTATTTGATATAGG
	BCDH.EcoRI.R417	GGCGGC**GAATTC***TTA*TAAACTTAAATCACTTTCACCATC
pGEXT-KDH$_{LD}$	KDH.BamHI.F103	GGCGGC**GGATCC**ATTGAAGGATCACTTAAGAGATATTTTTC
	KDH.EcoRI.R456	GGCGGC**GAATTC***TTA*ATTTAATTGATTAAATGTATAATTATTTTC
pGEXTK	Kan.BspHI.F	GGTGGT**TCATGA**GGATGGCTTTCTTGCCGCCAAGG
	Kan.BspHI.R	GGTGGT**TCATGA**CGCCCGGCGGCAACCGAG
pGEXTK-BCDH$_{LD}$	n/a	none - excised using *Bam*HI and *Sal*I from pGEXT-BCDH$_{LD}$
pGEXTK-KDH$_{LD}$	n/a	none - excised using *Bam*HI and *Sal*I from pGEXT-KDH$_{LD}$
pGEXTK-Hprot	Hprot.BamHI.F	GGTGGT**GGATCC**GAATATATAAAAATTGAGGATGGAAATTTGAAC
	Hprot.SalI.R	GGTGGT**GTCGAC***TTA*TTTCCCCCCTTGCCCTTTATTTTC
pRL2-BCDH$_{30}$GFP	BCDH$_{30}$.AvrII.F	GGTGGTGCTAGC**CCTAGG***ATG*TTTGTGAAGAATGTACTAAACGTGC
	BCDH$_{30}$.BsiWI.R	GGTGGT**CGTACG**ATGTATTGCACTCGTGTTCAGGTAATG
pRL2-KDH$_{30}$GFP	KDH$_{30}$.AvrII.F	GGTGGT**CCTAGG***ATG*ACAAAGAATCTTGTATTTCGATTAAATAAATC
	KDH$_{30}$.BsiWI.R	GGTGGT**CGTACG**ATTATACTTATGGTTTAAAAATGTTCTATTAAAACTTTT
	LplA.H.F	AAAGCGAGAAAAAAGAGTGACCCATTACTACAAGAAAGGAAATCGTT*ATG*
	LplA.H.R	AAGAGAAAGTTGCCCGCATGGGCGGGTAA*CTA*CCTTACAGCCCCCGCCAT
	K1.int.F	AGGCTATTCGGCTATGACTG
	K2.int.R	GCAGTTCATTCAGGGCACCG
	LplA.U	CTGGCGAAAGCGTCGAAGTG
	LplA.D	GCGTGATGCTGCCATTGAGG
	LipB.U	AGTGTAAATTGGGCCATTGATGTATGG
	LipB.D	CCCACTTTTACTCATTCTCCACGGAGATGCCG

The annealing portions of these sequences are underlined, endonuclease sites are marked by boldface type, italics marks relevant start and stop codons, and lower case text marks mutation sites.

Allary, M., J. Z. Lu, L. Zhu & S. T. Prigge, (2007) Scavenging of the cofactor lipoate is essential for the survival of the malaria parasite Plasmodium falciparum. *Mol Microbiol* **63**: 1331-1344.