1   **Supporting Information**

2   **Figure S1.** *LACK/LACK* **and** *LACK/- L. major* **cultures show comparable percentages of live**

3   **cells at 35°C**. The indicated parasite lines were incubated for four days at 35°C then diluted 1 in

4   10 and enumerated by hemocytometer under phase-contrast illumination. After normalizing for

5   cell density, equal numbers of parasites were stained with FDA then FDA-positive cells were

6   counted by fluorescence microscopy, as described in Experimental Procedures.

7

8   **Figure S2. Identified peptides matching the sequence of LmCOX4, as predicted by** *L. major*

9   **orf LmjF.12.0670.** Mass spectrometric identification of matching LmCOX4 peptides (single

10   letter amino acid designation) isolated from the 2D-DIGE analysis protein spot highlighted in

11   Fig. 1. High-confidence peptides are indicated in blue, underlined. Modest-confidence peptides

12   are indicated in red, underlined (see Experimental Procedures for definitions).

13

14   **Figure S3. Sequence alignment of LmCOX4 with its trypanosomatid orthologs.** A. Amino

15   acid sequence alignments for LmCOX4, *L. major* COX4; LdCOX4, *L. donovani* COX4;

16   LbCOX4, *L. brasiliensis* COX4; TbCOX4, Trypanosoma *brucei* COX4 (COIV); TcCOX4,

17   *Trypanosoma cruzi* COX4. Asterisks, colons and periods denote identical, strongly similar and

18   weakly similar residues, respectively. B. Table showing overall percentage identities between

19   trypanosomatid COX4 orthologs. C. Phylogenetic tree indicating relative relatedness of the

20   trypanosomatid COX4 orthologs. These analyses were performed using the Clustal Omega

21   online sequence analysis program (http://www.ebi.ac.uk/Tools/msa/clustalo/) with default

22   parameters.

23

24 **Table S1. Total number of 2D-DIGE protein spots detected and analysed.** Data from each of

25   the four gels, representing the experimental replicates, are shown. Spots were excluded from the

26   analysis if the spot was outside the area of interest, the spot was not a protein (i.e.dust specks), or

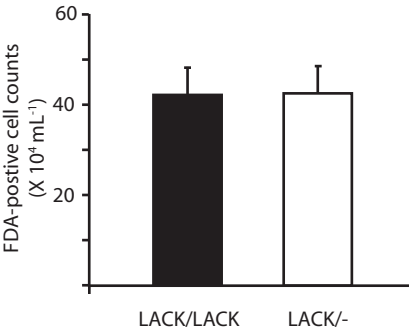27   the spot was not consistently resolved.

28

Figure S1

Y-axis: FDA-postive cell counts ($\times 10^4$ mL^{-1})

X-axis categories: LACK/LACK, LACK/–

Figure S2

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mltrravssavgaamvtsssvsmqrrydhdrwyghaleldthnykfngeppswmktrakTEEETSFAKsvl 71
phidfassyecllfdadrLNTNLNRkEFGNEIKyrlekqantvarAQQLLRdkkagtgpdaekVENTLIAR 142
ifdeehvqaemkyvkcirANELAEDNRLDILPGGSPNSLRektrwnlntelhpadrAEIGARLTAWLPEKy 213
hivyfddfqtvaandatarKEMLEIVESVQKeytaeakEGGYESDLKEAVAELMDDVDPTRTITMEAIKsc 284
kdlqqledwsrqvheyngddrIIAIYARaaeitknvehqalvrqmrewrkLATKNESKl           343
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Figure S3

A

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LmCOX4    MLTRRAVSSAVGAA---------------MV-TSSSVSMQRRYDHDRWYGHALELDTHN
LdCOX4    MFTRRSVSAAVGAA---------------MA-TSSSLSMQRRYDHDRWYGHALELDTHN
LbCOX4    MFTRRAVSSALGAA---------------AVAMSSSLSVQRRYDHDRWYGHALELDTHN
TbCOX4    MFARRSLIATVAAATATKPTSSAAQSNANGTGATQSTLLQQRRYDHDRWYGHALELDSHN
TcCOX4    MLSRRSLTAA----------------FAAMTKQPLMQQRRHDHDRWYGHALELDSHN
          *::**:: ::                     .. :  ***:*************:**

LmCOX4    YKFNGEPPSWMKTRAKTEEETSFAKSVLPHIDFASSYECLLFDADRLNTNLNRKEFGNEI
LdCOX4    YKFNGEPPSWMKTHAKTEEETSFAKSVLPHIDFASSYECLLFDADRLNTNLNRKEFGNEI
LbCOX4    YKFNGEPPSWMKARAKTEEETNFAKSVLPHIDFASSYECLLFDADRLNTHLNRKEFGNEI
TbCOX4    YKFTGEPPSWMRVRERTEEETNFAKSVLPHVDFASSYECLLFDADRLNGTLNRKEFGNEV
TcCOX4    YKFTGEPPSWMRQRERTEEETAFAKSVLPHVDFASSYECLLFDADRLNGHLNRKEFGNEV
          ***.*******: : :***** ********:****************  *********:

LmCOX4    KYRLEKQANTVARAQQLLRDKKAGTGPDAEKVENTLIARIFDEEHVQAEMKYVKCIRANE
LdCOX4    KYRLEKQANTVARAQQLLRDKKAGTGPDAEKVENTLIARIFDEEHVQAEMKYVKCIRANE
LbCOX4    KYRLEKQANTVARAQQLLKDRKAGTGPDAEKSENALIARVFDEEHVQAEMKYVKCIRANE
TbCOX4    RFRLEKQSNTVARAQQLLKDGRAGG---DERVENAMIARIFDEEHVQAEMRYVKCIRANE
TcCOX4    TFRLEKQSNTVARAQQMLKEKKSSS---DERLENTMIARIFDEEHVQAEMKYVKCIRANE
           :*****:*********:*:: ::.     *: **::***:***********:*********

LmCOX4    LAEDNRLDILPGGSPNSLREKTRWNLNTELHPADRAEIGARLTAWLPEKYHIVYFDDFQT
LdCOX4    LAEDNRLDILPGGSPNSLREKTRWNLNTELHPADRAEIGARLTAWLPEKYHIVYFDDFQT
LbCOX4    LAEDNRLDILPGGSPNSLREKTRWNLNTELHPADRAEIGARLTAWLPEKYHIVYFDDFQT
TbCOX4    LAEDNRLDILPGGSPNSLREKTRWNVNTELHPADRAEIASRLTAWLPEKYHIVYVDDFQT
TcCOX4    LAEDNRLDILPGGSPNSLREKTRWNVNTELHPADRAEIGARLTAWLPEKYHIVYFDDFQT
          ***********************:*********** .:*************** .*****

LmCOX4    VAANDATARKEMLEIVESVQKEYTAEAKEGGYESDLKEAVAELMDDVDPTRTITMEAIKS
LdCOX4    VAANDATARKEMLEIVESVQKEYTAEAKECGYENDLKETVAELMDDVDPTRTITMEAIKS
LbCOX4    VAANDTTARREMLEIVESVRKEYTAEAKEGGYESDLRETVAELLDDVDPTRAITMEAIKG
TbCOX4    VAANDSCARDAMLRIVEGVSKEYEAEARSSGYERDLREVVQELLDDVDPSRHITSEAIKA
TcCOX4    VAANDPSARREMLNIVQNVEREYADEAKSSGYEKDLKEVVNELLDDVDPSRHITSEAIKA
          *****  **  **.**: * :** **:. *** **:*.* **:*****:* ** ****.

LmCOX4    CKDLQQLEDWSRQVHEYNGDDRIIAIYARAAEITKNVEHQALVRQMREWRKLATKNESKL
LdCOX4    CKDLQQLEDWSRQVHEYNGDDRIIEIYARAAEITKNAEHQALVRQMREWRKLATKNESRL
LbCOX4    CSNLQQLEDWSHQVHEYNGDHRIIEIYARAAEITKNAEHQELVKQMREWRKLATRN----
TbCOX4    ATDLTQLEEWSRVVHEYNGDERILDIYSRAAELTRNEEHKKLVEDMRQWKKLSNKI----
TcCOX4    CTDLNQLEEWSRVVHEYNGDDRILDIYARAAELTKNADHQALVKNMKEWRKLANKI----
          ..:* ***:**: *******.**: **:****:*:* :*: **.:*::*:**::
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B

	LmCOX4	LdCOX4	LbCOX4	TbCOX4	TcCOX4
LmCOX4	100.00	96.50	90.86	75.30	78.01
LdCOX4	96.50	100.00	90.56	76.19	78.92
LbCOX4	90.86	90.56	100.00	75.37	76.88
TbCOX4	75.30	76.19	75.37	100.00	82.04
TcCOX4	78.01	78.92	76.88	82.04	100.00

C

LmCOX4	0.019
LdCOX4	0.01598
LbCOX4	0.05136
TbCOX4	0.1014
TcCOX4	0.07824
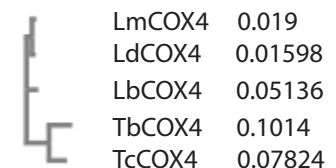
Table S1

2D-DIGE Gel ID	Total Spots Detected	Spots included in Analysis
Gel 1	2324	448
Gel 2	2215	607
Gel 3	2168	350
Gel 4	2431	667