**Supplementary Information for Zhang et al.**

*Cloning of L. major SPT2 and molecular constructs.*

The putative *SPT2* gene was PCR amplified from *L. major* genomic DNA using the 5’ primer SMB1480 (5’-gctagcggatccacc-ATGATTCACGATGCTGTCC, capitalized letters are from *SPT2*) and 3’ primer SMB1481 (5’-gagctaggatccTTACCGCAGCGGGTTCGTGCTG). The resulting fragment was digested with *BamHI* and cloned in the *BamHI* site of an expression vector pXG (Ha et al., 1996) to make pXG-*SPT2* (B4511), and the correct *SPT2* sequence was confirmed.

To introduce the K372A mutation in *SPT2*, first a 515 bp DNA fragment of the 3’-end of *SPT2* was PCR amplified using primer SMB1481 and SMB1876 (TGCTCATGGGTACCTTCACGgcgAGCTTCGGCTCCATCGG, the lower case letters represent the K373A mutation). The resulting DNA fragment was cut with *KpnI* and *BamHI*, combined with a 1108 bp *BamHI*-*KpnI* fragment of the 5’-end of *SPT2* (cleaved out of pXG-*SPT2*, B4511), and ligated into the *BamHI* site of pXG to form pXG-*SPT2* (B4923). The *SPT2* sequence was confirmed.

Two *SPT2* deletion constructs were created: pUC-*SPT2-KO-HYG* (B4539) and pUC-*SPT2-KO-PAC* (B4575). Briefly, two 1 Kb DNA fragments immediately upstream and downstream of the predicted *SPT2* ORF were PCR amplified from *L. major* genomic DNA and cloned into vector pUC18 in a “head-to-tail” manner to make pUC-*SPT2-KO-5’-3’* (B4533, in which the end of the upstream fragment was linked to the start of the downstream fragment). Then DNA fragments corresponding to the *HYG* (confers resistance to hygromycin) and *PAC* (confers resistance to puromycin) markers were inserted in between the upstream and downstream regions. The resulting constructs

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(pUC-SPT2-KO-HYG and pUC-SPT2-KO-PAC) were digested with SacI and HindIII to obtain linear SPT2 deletion fragments (2.9 Kb for HYG-cassette and 2.5 Kb for PAC-cassette).

A GFP::SPT2 fusion protein expression vector (pXG-GFP-SPT2, B4512) was constructed by inserting the 1.6 Kb BamHI SPT2 ORF fragment of pXG-SPT2 into the BamHI site of pXG-GFP+2/ vector (B2952; Zufferey et al., 2003).

A His-tagged SPT2 expression construct (pET-SPT2, B4924) was created by inserting the 1.6 Kb SPT2 ORF fragment into pET-15b. Recombinant N-terminally His-tagged SPT2 protein was prepared using a pET-15ba expression system, and rabbit anti-SPT2 antisera were made by the Proteintech Group Inc (Chicago, IL). Affinity purified antiserum was made by absorbing 0.5 ml of the starting antisera to ~0.2 mg His-tagged SPT2 separated by SDS-PAGE and bound to nitrocellulose (Boumba and Seferiadis, 2002), and used at a dilution of 1:100 (Fig. 1A).

**Deletion of SPT2 from Leishmania major.**

The first allele of SPT2 was disrupted by transfecting WT cells with 5 μg of the 2.9-kb SacI-HindIII SPT2::HYG fragment from pUC-SPT2-KO-HYG and plating on semisolid media containing hygromycin B(50 μg/ml). Heterozygote SPT2 replacements (SPT2/Δspt2::HYG) were subjected to the second round of gene disruption with 5 μg of the 2.5-kb SacI-HindIII SPT2::PAC fragment (from pUC-SPT2-KO-PAC). Eight clones (spt2-1-8) that grew in the presence of hygromycin B (50 μg/ml) and puromycin (10 μg/ml) were selected as spt2- null mutants (Δspt2::HYG/Δspt2::PAC). Successful replacements of SPT2 alleles in these clones were confirmed by Southern blot analysis.
To restore gene expression, spt2^- mutants were transfected with pXG-SPT2 and selected for clonal lines that were resistant to G418 (10 µg/ml) as spt2^-/+ SPT2. Two independent SPT2 null clones, spt2^- #3 and #7, as well as their respective add-backs were used in all the studies; these behaved similarly and only results from spt2^- #3 and its add-back are shown.

**Fluorescence microscopy.**

Parasites were attached to poly-lysine coated cover slips and fixed in 3.5% formaldehyde (1 min at room temperature) followed by ethanol permeabilization (15 min on ice). A rabbit anti-*T. brucei* BIP polyclonal antibody (provided by Dr. E. Handman, Australia) was used to reveal the ER (1:1000, 20 min at room temperature), followed by Texas Red labeled goat anti-rabbit IgG secondary antibody (1:1000, 20 min at room temperature). Localizations of LPG and gp63 were determined using monoclonal antibody WIC79.3 (de Ibarra et al., 1982) and anti-gp63 (Cedarlane, Ontario, CANADA), respectively, followed by FITC labeled goat anti-rabbit IgG secondary antibody as described above.

**Electron microscopy.**

Parasites were fixed in freshly prepared 2% paraformaldehyde/2.5% glutaraldehyde (Polysciences Inc., Warrington, PA) in 100 mM phosphate buffer, pH 7.2 for 1 hr at room temperature. Following three washes in phosphate buffer, parasites were post-fixed in 1% osmium tetroxide (Polysciences Inc.) for 1 hr. Samples were then rinsed extensively in dH$_2$O to remove excess phosphate prior to en bloc staining with 1% aqueous uranyl acetate (Ted Pella Inc., Redding, CA) for 1 hr. Following several rinses in
dH₂O, samples were dehydrated in a graded series of ethanol and embedded in Eponate 12 resin (Ted Pella Inc.). Sections of 70-80 nm were cut, stained with uranyl acetate and lead citrate, and viewed on a JEOL 1200 EX transmission electron microscope (JEOL USA, Inc., Peabody, MA).

**Mass spectrometry.**

Mass spectrometry was performed on a Finnigan (San Jose, CA) TSQ-7000 triple stage quadrupole mass spectrometer equipped with an electrospray ion source and controlled by Finnigan ICIS software operated on a DEC alpha station. Samples were diluted in methanol (1:5) and infused (1 µl/min) into the ESI source with a Harvard (Holliston, MA) syringe pump. The analysis was carried out in the negative-ion mode. The skimmer was at ground potential and the electrospray needle was set at 4.5 KV. The heated capillary temperature was 260 °C. The mass spectrometer was tuned to unit-mass resolution. Structural assignments of lipid molecular species were based on analyses of tandem mass spectra from collisionally activated dissociation of precursor ions (Hsu and Turk, 2000a; Hsu and Turk, 2000b; Hsu and Turk, 2002). Such ESI/MS/MS spectra were produced by selection of precursor ions (e.g., [M-H]⁻ ions) in the first quadrupole (Q1), acceleration (35-40 eV collision energy) into an rf-only second quadrupole (Q2) collision cell filled with argon gas (2.3 mtorr), and mass analysis of the resultant product ions in the third quadrupole (Q3). Typically, ESI/MS or ESI/MS/MS spectra were generated by a 1 min or 1-20 min period respectively of signal averaging of repeated scans.


Stationary spt2- parasites do not go exhibit features characteristic of apoptosis/programmed cell death. (A) Transmission EM graphs showing nuclear morphology of log and stationary (day 3) phase promastigotes of WT and spt2- parasites. Arrows pointed to nuclei; the bar represents 500 nm. (B) and (C) Stationary (day 3) phase spt2- parasites were labeled with PI (Y axis) and annexin (B, X axis) or YOPRO-1 (C, X axis) and analyzed by flow cytometry.