SUPPLEMENTARY INFORMATION FOR:

The Golgi-localized Sphingosine-1-Phosphate Phosphatase is Indispensable for *Leishmania major*

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Supplementary Table S1. Oligonucleotide primers used in this study

Primer number	Primer Sequence	Purpose
915	CGTACGGATCCATGTCAACAGCCACACACCCAATG	Forward primer to clone the full length *SPP* ORF (BamHI)
472	CCTCGCGGATCCTCACTCTGGCGACGGTG	Reverse primer to clone the full length *SPP* ORF (BamHI)
916	CCTCGCCCGGGATGTCAACAGCCACACACCCAATG	Forward primer to clone the full length *SPP* ORF (XmaI)
505	CCTCCGGATATCCTCTGGCGACGGTGGTGGTG	Reverse Primer to clone *SPP* without the stop codon (ECORV)
564	GCGAGGggtaccACATCCCTTCACATTCGTCG	Forward primer to amplify a 2 Kb upstream region of *SPP* (KpnI)
565	GCGAGGactagtTGGGTGTGTGGCTGTTGAC	Reverse primer to amplify a 2 Kb upstream region of *SPP* (SpeI)
566	GCGAGGactagtGGCTAGagatctACTACGAAGGAGAAGGAGAGAAG	Forward primer to amplify a 2 Kb downstream region of *SPP* (SpeI+BglII)
567	CCTCGCggatccATACTCAGTTAACGCTCGTTTTCG	Reverse primer to amplify a 2 Kb downstream region of *SPP* (BamHI)

SUPPLEMENTARY FIGURE LEGENDS

Figure S1. Alignment of SPPs from yeast, *L. major*, human and mouse using the CLUSTAL Omega Alignment. Genbank accession numbers for *Saccharomyces cerevisiae* SPP, *L. major* SPP, *Homo sapiens* SPP2, and *Mus musculus* SPP2 are P47013, Q4Q546, Q8IWX5 and Q810K3, respectively. Asterisks (*) represent fully conserved residues; colons (:) represent strongly similar residues; and periods (.) represent weakly similar residues.

Figure S2. Schematic illustration of the knockout strategy for *L. major SPP*. One of the two chromosomal *SPP* alleles in wild type (WT) *L. major* was replaced by *BSD* to generate the heterozygous *SPP* knockout (*SPP*+/-). A pXNG4-*SPP* plasmid was introduced into *SPP*+/- to generate *SPP*+/- +pXNG4-*SPP*. The second chromosomal *SPP* allele was to be replaced by *HYG* (not feasible in this study).

Figure S3. Southern blot analysis of attempted deletion of chromosomal *SPP* in the absence of episomal *SPP*. (**a**-**b**) Genomic DNA samples from WT (lane 1) and three *SPP*+/- clones (lane 2-4; lane 3 was under-loaded) were digested with SpeI and subjected to Southern blot analysis using a *SPP* flanking region probe (**a**: autoradiograph, **b**: ethidium bromide staining). (**c**-**d**) Genomic DNA samples from WT (lane 1) and five putative *spp*¯ clones (lane 2-6) were digested with XhoI and subjected to Southern blot using a *SPP* ORF probe (**c**: autoradiograph, **d**: ethidium bromide staining). Expected positions for the chromosomal *SPP* and *BSD* genes are indicated.

Figure S4. Full-length images for the Southern blot analyses in Figure 5. (**a**) flanking region probe; (**b**) a *SPP* ORF probe; top: Southern blot results; and bottom: ethidium bromide staining results.

Figure S5. Attempts to delete *L. major SPP* or *FPPS* in the presence of episomal complementing genes. Genetic manipulations of *SPP* or *FPPS* were performed as illustrated in Fig. S2 and results were examined by PCR. In **a**-**c**, genomic DNA samples from WT, *SPP*+/-, *SPP*+/- +pXNG4-SPP, and a putative *spp*$^{-}$ +pXNG4-SPP clone #3 were used as templates. In **d**-**f**, genomic DNA samples from WT, *FPPS*+/-, and two *fpps*$^{-}$ +pXNG4-FPPS clones were used as templates. Primers were designed to amplify the chromosomal *SPP* and *FPPS* alleles (**a** and **d**, respectively), the *HYG* resistance gene used to replace the second chromosomal allele of *SPP* and *FPPS* (**b** and **e**, respectively), or control regions (**c**: a 500-bp fragment of the dihydroceramide synthase ORF; and **f**: a 6000-bp region including the SPP ORF plus ~2 Kb of upstream and downstream flanking sequences). Red arrows indicate the expected sizes of amplicons.

Figure S6. Full-length images for the Western blot analyses in Figure 7. (**a**) anti-LPG. (**b**) anti-α-tubulin. Promastigote lysates from log phase (day 1 and day 2) and stationary phase (day 2) were analyzed.

Figure S7. *SPP* overexpression does not affect sensitivity to sodium fluoride. Promastigotes of WT, *SPP*+/-, and *SPP*+/- +pXNG4-SPP were cultivated in various concentrations of sodium fluoride; culture densities were determined after 48 hours; and effects on growth were measured in comparison to cells grown in the absence of sodium fluoride. Error bars represent standard deviations from four experiments.