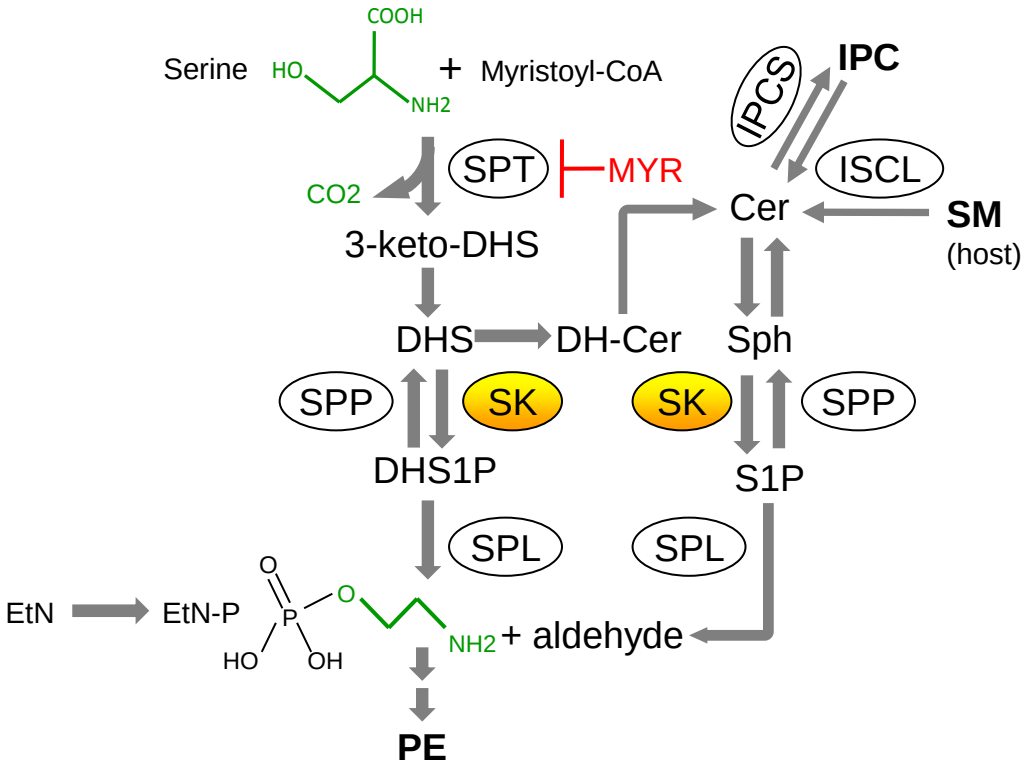
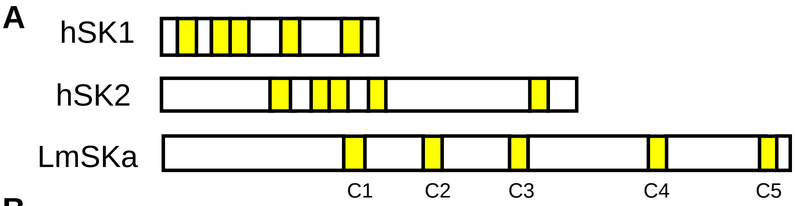


Figure S1. SL metabolism in *Leishmania*. Serine, CO_2, and part of ethanolamine phosphate (EtN-P) are shown in green to illustrate the conversion. Abbreviations for enzymes (shown in circles): SPT, serine palmitoyltransferase; SK, sphingosine kinase; SPP, sphingosine-1-phosphate phosphatase; SPL, sphingosine-1-phosphate lyase; IPCS, IPC synthase; ISCL, inositol phosphosphingolipid phospholipase C-like protein. Abbreviations for metabolites: DHS, dihydrosphingosine; Sph, sphingosine; DHS1P, dihydrosphingosine-1-phosphate; S1P, sphingosine-1-phosphate; DH-cer, dihydroceramide; Cer, ceramide; IPC, inositol phosphorylceramide; SM, sphingomyelin; MYR, myriocin (an inhibitor of SPT); EtN-P, ethanolamine-phosphate; PE: phosphatidylethanolamine.

A



B

```
hSK1    ------------------------------------------------------------
hSK2    ------------------------------------------------------------
SKa     MHSLSSNNTPSPHPATHRHTNHSSTHTSSLLPPTASPVLQASCMSAKEDHIGADAQGIPS 60

hSK1    ------------------------------------------------------------
hSK2    ----------------MAPPPPPLAASTPLLHGEFGSYPARGPRFALTLTS--------- 35
SKa     SALFTPKPRIFAVPASTESPPSPGATCHHAPTGGINVSAATGVSAAHHSDSGSNTAAPTA 120

hSK1    ----------------------------------MDPAGGPRG----------------- 9
hSK2    -------------------------QALHIQRLRPKPEARPRGGLVPLAEVSGCCTLRSR 70
SKa     SSSSPALQKQQPEHCVPAAYRGKLDDAPHAVDDNMEAVILSKGRICTLAYFPSRGSFRIT 180
                                           ..    .:*

hSK1    ------------------------------------------------------------
hSK2    SPSDSAAYFCIYTYPRGRRGARRRATRTFRADGAATYEENRAEAQRWATALTCLLRGLPL 130
SKa     HVSSNGKTRVVFNIPVRMIINIETAAERDARQRARQANDDTIKLVFAESGRNTRSLLCSF 240

hSK1    -------------------------VLPRPC----------------------------- 15
hSK2    PG---------------DGEITPDLLPRPP------------------------------ 145
SKa     PSGAGEDNEGVVLAHSVNTASLTLTSLLGGPPPTPTASHFGAHTAACAAATAAPSIRYYVH 300
                                 :*  *

hSK1    -------------------------------------------RVLVLLNPRGGKGKALQ 32
hSK2    -------------------------------------------RLLLLVNPFGGRGLAWQ 162
SKa     YVQQRNKENPSIRTLEFQSSGPAETVQHVVSTVVQHIYQKGSKHIIAFISAKSGKGKGEH 360
                                                   :::  ::.. .*:*  . :

hSK1    LFRSHVQPLLAEAEISFTLMLTERRNHARELVRSEE--LGRWDALVVMSGDGLMHEVVNG 90
hSK2    WCKNHVLPMISEAGLSFNLIQTERQNHARELVQGLS--LSEWDGIVTVSGDGLLHEVLNG 220
SKa     IFEKHVRPLLHFSRHTYQAHVTRRAHDCEDYVANLENPMDSNTVIAAVGGDGMIHETVNG 420
          ..** *::  :  ::    *.* :...: *  .  .  :.    :..:.***::**.:**

hSK1    LMER------------------------------------------------PDWETA 100
hSK2    LLDR------------------------------------------------PDWEEA 230
SKa     VHRRKLALVRWLRSVTANVSTGNGSVVSPDLSVHLNEERCAAVLLKSGSANKVVHHGEAS 480
        :  *                                                . * :

hSK1    IQKP-----------------------------------------------LCSLP 109
hSK2    VKMP-----------------------------------------------VGILP 239
SKa     VDSPSSPFIELGCEENKRDGYGGNGVTPSAEATAREAYRLARCLVQGGWDALMPLVATVA 540
        :. *                                                :  :.

hSK1    AGSGNALAASLNHYAGYEQVTNEDLLTNCTLLLCRRLLSPMN---------LLSLHTASG 160
hSK2    CGSGNALAGAVNQHGGFEPALGLDLLLNCSLLLCRGGGHPLD---------LLSVTLASG 290
SKa     TGSACGLAKSLDVLSVTEAALSLVHLSTVHMDLLLLNFTPNEDMVEFHRCRMSSRRLDAA 600
         **. .** :::  .  *  . .   *  .  : *     * :      : *   :.

hSK1    LRLFS------------------------------------------------------- 165
hSK2    SRCFS------------------------------------------------------- 295
SKa     QREFSRYKEDKAAELQERSRLGEAPQLPPRTLTAADCMTPFLKDGSNVYRDAVSCAMRMP 660
         * **

hSK1    -------VLSLAWGFIADVDLESEKYRRLGEMRFTLG-TFLRLAALRTYRGRLAYLP--- 214
hSK2    -------FLSVAWGFVSDVDIQSERFRALGSARFTLG-TVLGLATLHTYRGRLSYLPATV 347
SKa     ELHSRVAFMSLSFGSANDIDHGSESLRWMGNARFQVYGGYMILRGLKRYKGMLRYLPWGS 720
               .:*:::*   *:*  **  * .* *  **  .    . *  *. *.* * ***

hSK1    -------------------------------------------------VGRVG------- 219
hSK2    EPASPTPAHSLPRAKSELTLTPDPAPPMAHSPLHRSVSDLPLPLPQPALASPGSPEPLPI 407
SKa     KAG---KTVEKLHTRCKMPSTDDFPLCTMRESCPHCRQYVFVHCGAPSLSSIQGDDTHPG 777

hSK1    ----------------------------SKTPASPVVVQQ-------------------- 231
hSK2    LSLNGGGPELAGDWGGAGDAPLSPDPLLSSPPGSPKAALHSPVSEGAPVIPPSSGLPLPT 467
SKa     ------------------------PTPNTSRSTAQPISAAEVLAP--------------- 798
                                    *  ....*   . .

hSK1    ----------GPVDAHLVPLEEPVPSHWTVVPDEDFVLVLALLHSHLGSEMFAAPMGRCA 281
hSK2    PDARVGASTCGPPDHLLPPLGTPLPPDWVTLEG-DFVLMLAISPSHLGADLVAAPHARFD 526
SKa     ------YTDQQLLDEDVVDFKDERLP-WVTVRG-DFCIALLCNVRDVAQDMLMAPLAHMS 850
                          *  :  :     . *..: . ** : *     .:. ::. ** .:

hSK1    AGVMHLFYVRAG-----VSRAMLLRLFLAMEKGRHMEYECPYLVYVPVVAFRLEPKDGKG 336
hSK2    DGLVHLCWVRSG-----ISRAALLRLFLAMERGSHFSLGCPQLGYAAARAFRLEPLTPRG 581
SKa     DGAIDIVYCRVDPITGRGGRMEMLKFVIGLESGSHVNLDFVNYVKARALEIKVD----AG 906
         *  :.: : * .      .*  :*::.:.:* * *..      . .   ::::      *

hSK1    VFAVDGELMVSEAVQGQVHPNYFWMVSGCVEPPPSWKPQQMPPPEEPL 384
hSK2    VLTVDGEQVEYGPLQAQMHPGIGTLLTG----PPGC------PGREP- 618
SKa     ISMSDGELMPLSSVRVTKMRGSVQFVRSG------------------- 935
        :   *** :   .::     .    :: .
```

C1 C2 C3 C4 C5

Figure S2. Comparison of SKs from human and *L. major*. (**A**) Schematic representation of human SK1 (NM_001142601), human SK2 (AF245447), and *L. major* SKa (Tritrypdb LmjF26.0710). Yellow boxes indicate the five conserved regions (C1-C5) found in all SKs. (**B**) ClustalW alignment of *L. major* SKa, human SK1 and human SK2. The underlined sequence (amino acid 343-421 of SKa) represents the predicted diacylglycerol kinase catalytic domain. Asterisks (*): fully conserved residues; colons (:): highly similar residues; periods (.): weakly similar residues.

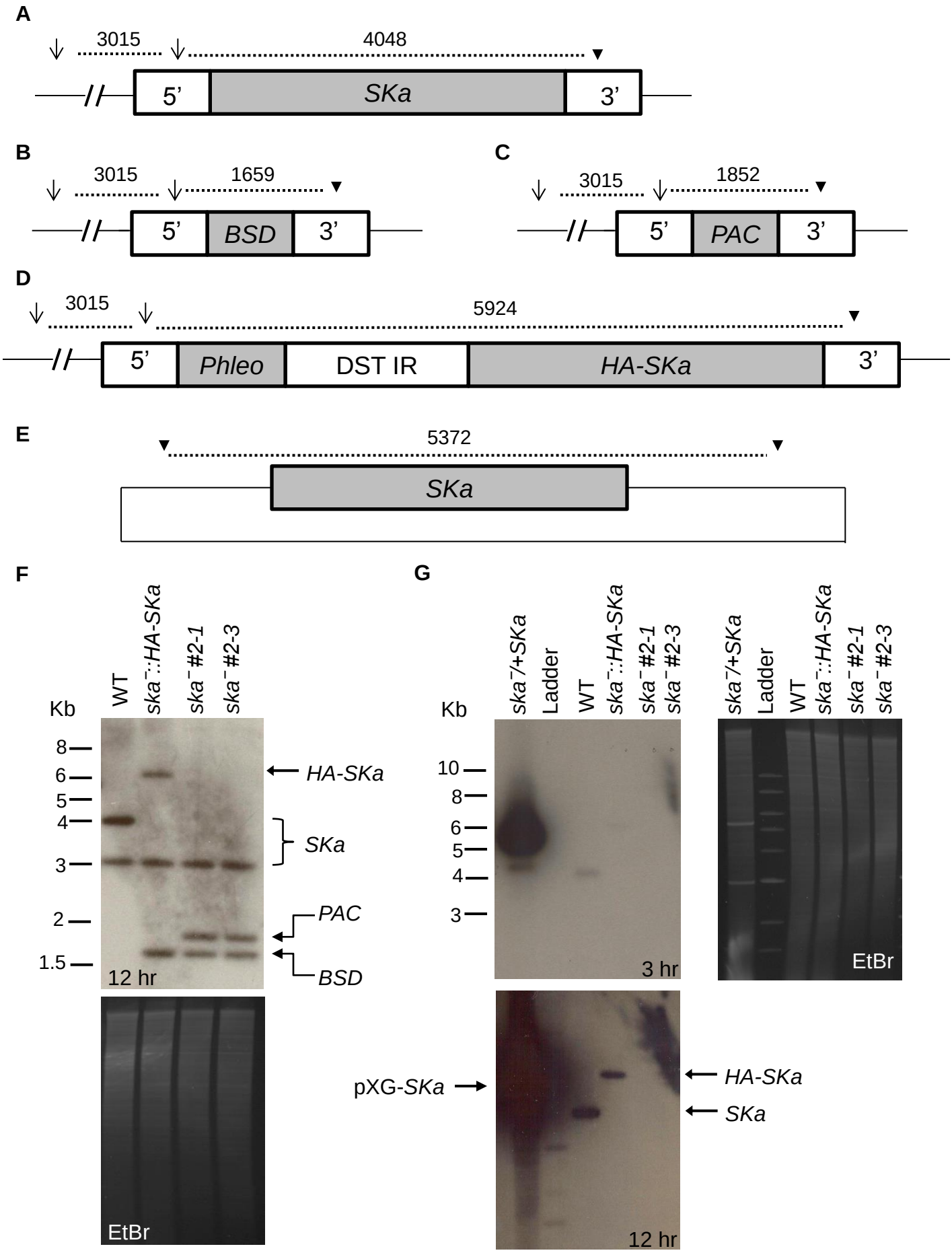

A
3015
4048
5'
SKa
3'
B
3015
1659
5'
BSD
3'
C
3015
1852
5'
PAC
3'
D
3015
5924
5'
Phleo
DST IR
HA-SKa
3'
E
5372
SKa
F
Kb
WT
ska⁻::HA-SKa
ska⁻#2-1
ska⁻#2-3
8
6
5
4
3
2
1.5
HA-SKa
SKa
PAC
BSD
12 hr
EtBr
G
Kb
ska⁻/+SKa
Ladder
WT
ska⁻::HA-SKa
ska⁻#2-1
ska⁻#2-3
10
8
6
5
4
3
3 hr
EtBr
pXG-SKa
HA-SKa
SKa
12 hr

Figure S3. Targeted deletion of *SKa* in *L. major*. (**A-E**) Schematic representations of the WT *SKa* allele (**A**), the replacement of *SKa* with *BSD* (**B**) or *PAC* (**C**), the chromosomal knockin of *HA-SKa* (**D**), and the episomal expression of *SKa* from pXG-*SKa* (**E**). 5': Upstream flanking region; 3': downstrean flanking region; DST IR: downstream intergenic region. (**F-G**) Southern blot analyses of genomic DNA digested with *EcoRI* (↓ in **A-E**) and *HindIII* (▼in **A-E**). Blots were hybridized with the 5' probe (**F**) or the *SKa* ORF probe (**G**). Dotted lines in **A-E** indicate the expected fragment sizes in bp (not drawn to scale). Exposure times and DNA loading controls (EtBr staining) are included in **F** and **G**.

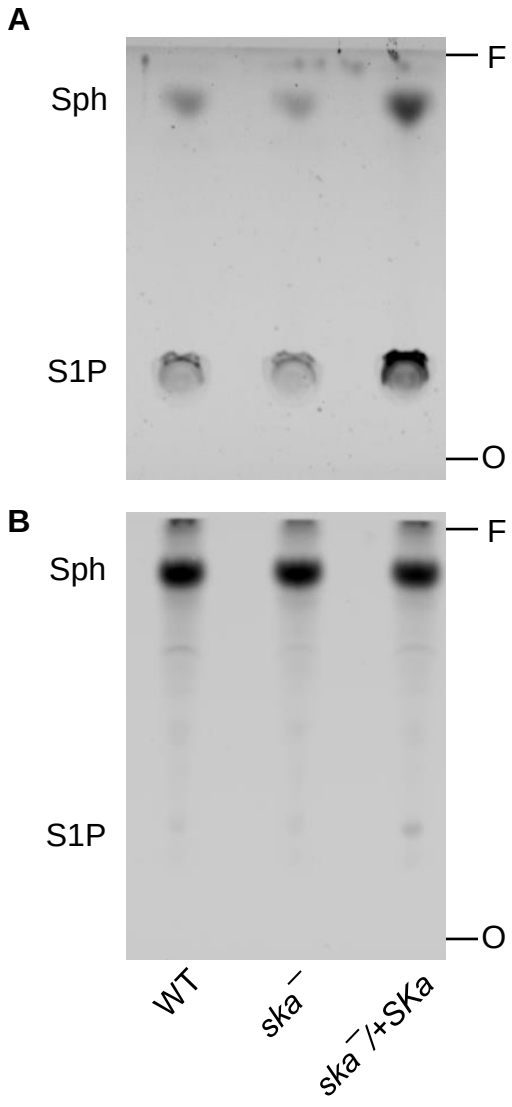


Figure S4. SK activity in *L. major* promastigotes. (**A**-**B**) Lysates from WT, *ska*$^{-}$, or *ska*$^{-}$*/+SKa* promastigotes were incubated with NBD-Sph as described in Experimental Procedures. After lipid extraction, each sample was partitioned into approximately equal volumes of aqueous phase and organic phase. 5 μl of the aqueous phase (**A**) and 5 μl of organic phase (**B**) from each sample were resolved by TLC. O: origin of migration; F: solvent front.

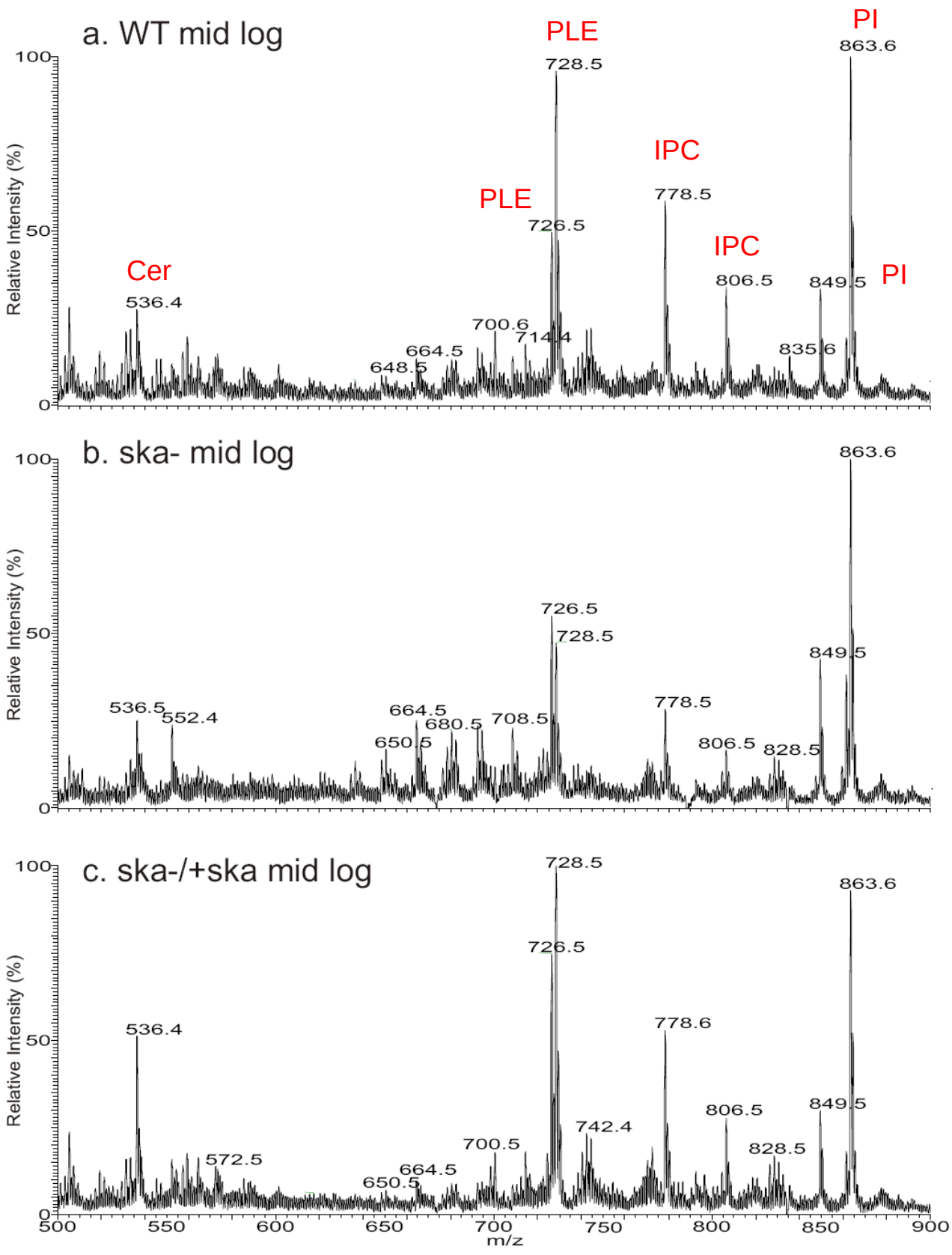


Figure S5. Negative-ion ESI/MS spectra of total lipids from log phase promastigotes of WT (a), *ska*$^{-}$ (b), and *ska*$^{-}$*/+SKa* (c). $[M\text{-}H]^{-}$ ions representing ceramide, PLE, IPC, and PI are labeled in red.

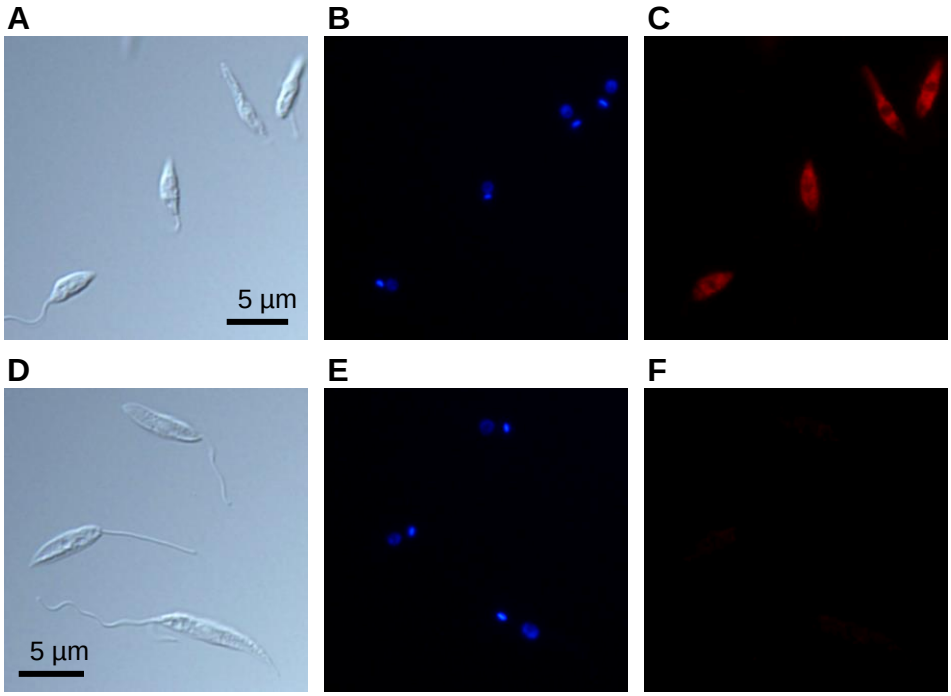


Figure S6. Cytoplasmic localization of HA-SKa expressed from the endogenous locus of *SKa*. Log phase promastigotes of *ska*$^{-}$*::HA-SKa* (**A-C**) or *ska*$^{-}$ (**D-F**) were permeabilized and subjected to immunofluorescence microscopy. (**A**, **D**) Differential interference contrast images; (**B**, **E**) DNA staining with Hoechst 33242; (**C**, **F)** rabbit anti-HA polyclonal antibody labeling followed by incubation with a Texas Red-conjugated goat anti-rabbit IgG antibody.

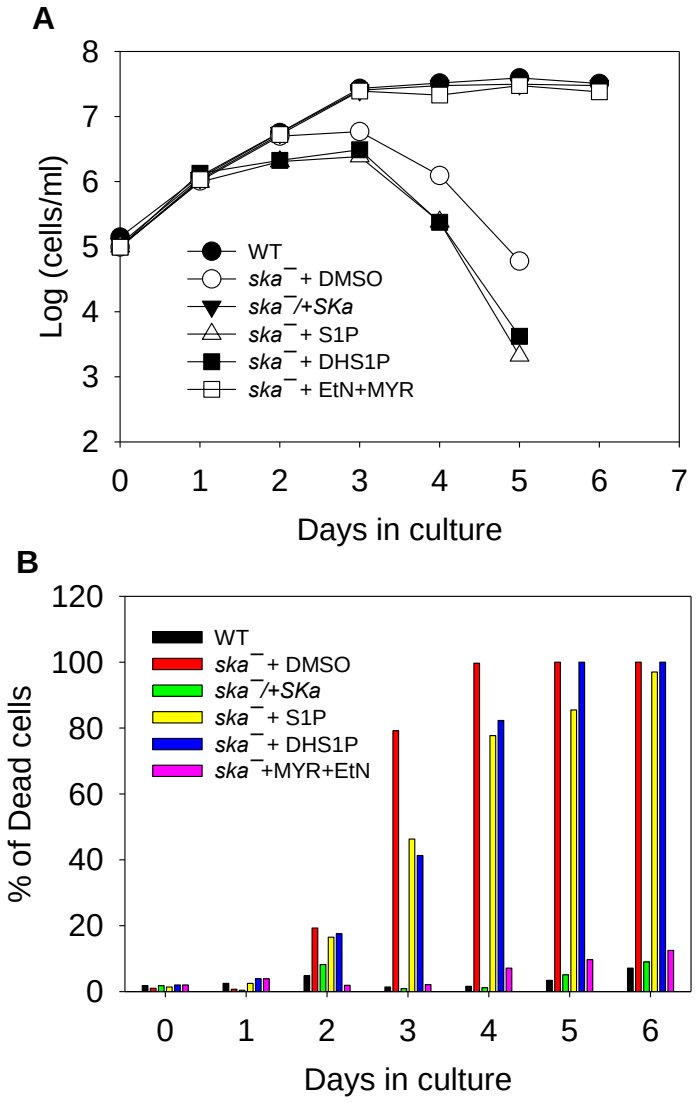


Figure S7. S1P or DHS1P fail to reverse the growth defect of *ska*¯ mutants in culture. Promastigotes were inoculated at 1.0 x 10^5 cells/ml and incubated at 27 °C. Culture densities (**A**) and percentages of dead cells (**B**) were monitored daily. *Ska*¯ mutants were supplemented with S1P (2 µM), DHS1P (2 µM), EtN + MYR (200 µM and 1 µM, respectively), or 0.1% of DMSO (solvent control). Experiments were repeated three times and results from one representative set were shown here.

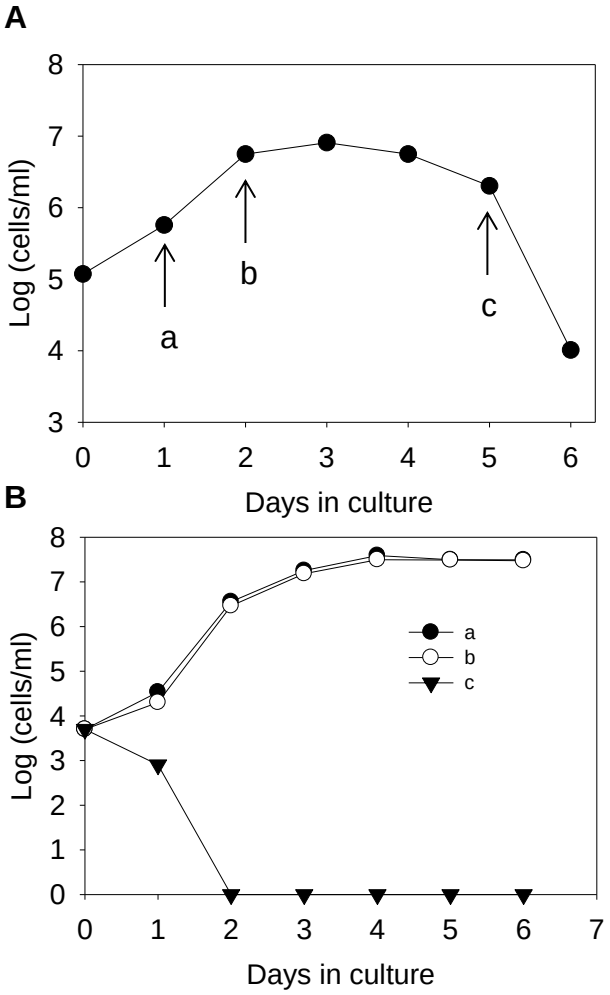


Figure S8. *Ska*$^{-}$-conditioned medium is toxic. (**A**) *Ska*$^{-}$ promastigotes were inoculated in M199 medium at 1.0 x 10^5 cells/ml and incubated at 27 °C (without MYR or EtN supplements). Culture densities were monitored daily. At day 1, 2, and 5, conditioned medium was collected by centrifugation (to remove cells) and designated as a, b, and c, respectively. (**B**) WT promastigotes were inoculated in *ska*$^{-}$-conditioned medium (a, b, or c as indicated in **A**) at 6 × 10^3 cell/ml. Culture densities were monitored daily. Experiments were repeated twice and results from one representative set were shown here.

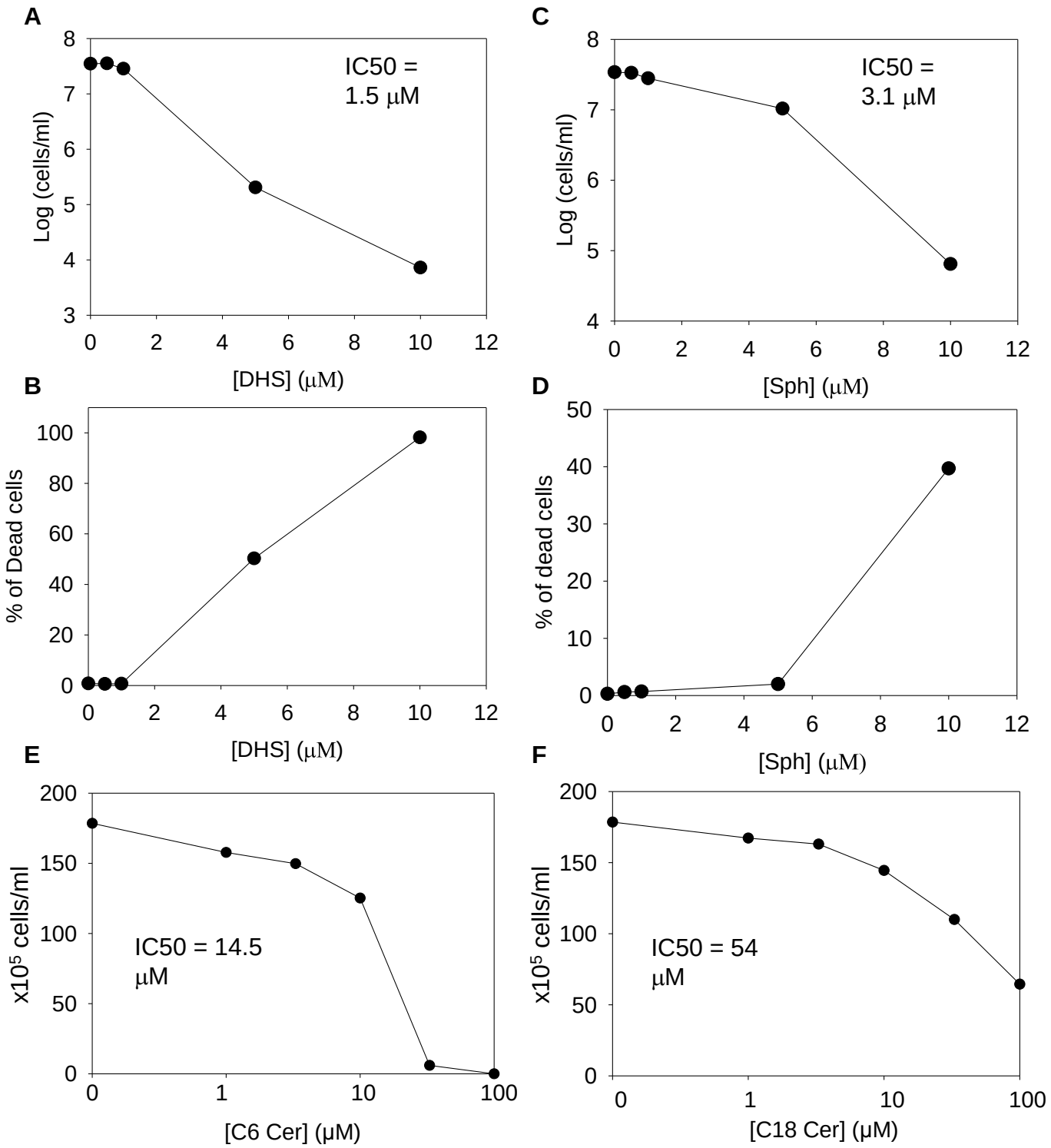


Figure S9. Effects of exogenous sphingoid bases or ceramides on *L. major* promastigotes. WT promastigotes were inoculated in 0-10 μM of DHS (**A-B**), 0-10 μM of Sph (**C-D**), 0-100 μM of C6-ceramide (**E**), or 0-100 μM of C18-ceramide (**F**) at 2.0 × 10^5 cell/ml. Culture densities (**A**, **C, E,** and **F**) and percentages of dead cells (**B**, **D**) were determined after 48 hours. Experiments were repeated three times and results from one representative set were shown here. IC50 values represent the concentrations required to inhibit growth by half.

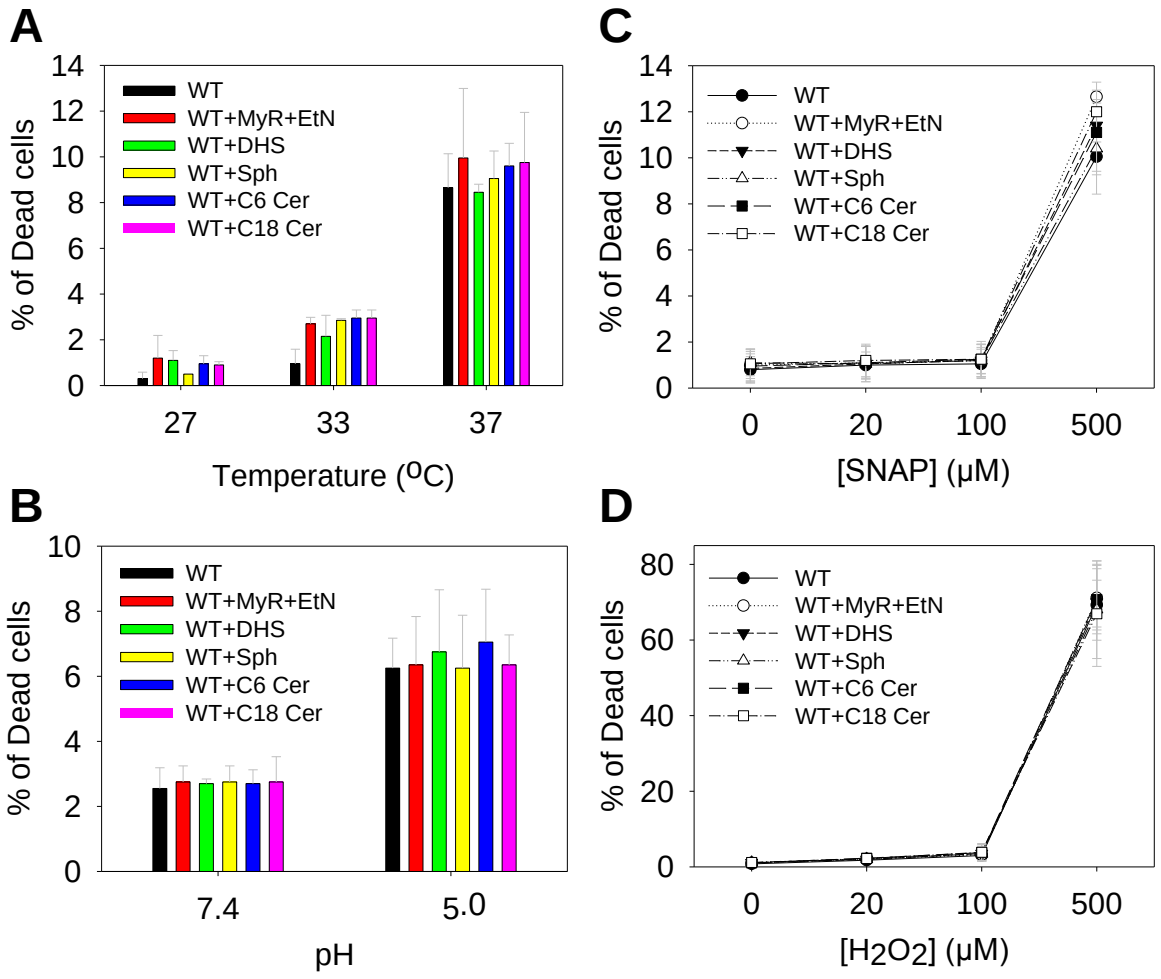


Figure S10. WT promastigotes treated with sphingolipid metabolites are not hypersensitive to stress conditions. (**A-B**) WT promastigotes were cultured under regular condition (27 °C, pH 7.4) to stationary phase before stress treatments. In **A**, parasites were transferred to 37 °C, 33 °C, or remained at 27 °C. In **B**, half of the parasites were transferred to an acidic medium (pH 5.0) while the other half remained in regular medium (pH 7.4) for 72 hours at 27 °C. (**C-D**) Stationary phase promastigotes were challenged with 0-500 μM of SNAP (**C**) or hydrogen peroxide (**D**). In these experiments, WT promastigotes were supplemented with MYR+EtN (1 μM and 200 μM, respectively), or 0.5 μM of DHS/Sph/C6 ceramide/C18 ceramide daily. Percentages of cell death were determined at 24 (**A**), 48 (**C-D**), or 72 (**B**) hours post treatments; and error bars indicate standard deviations from two independent repeats.

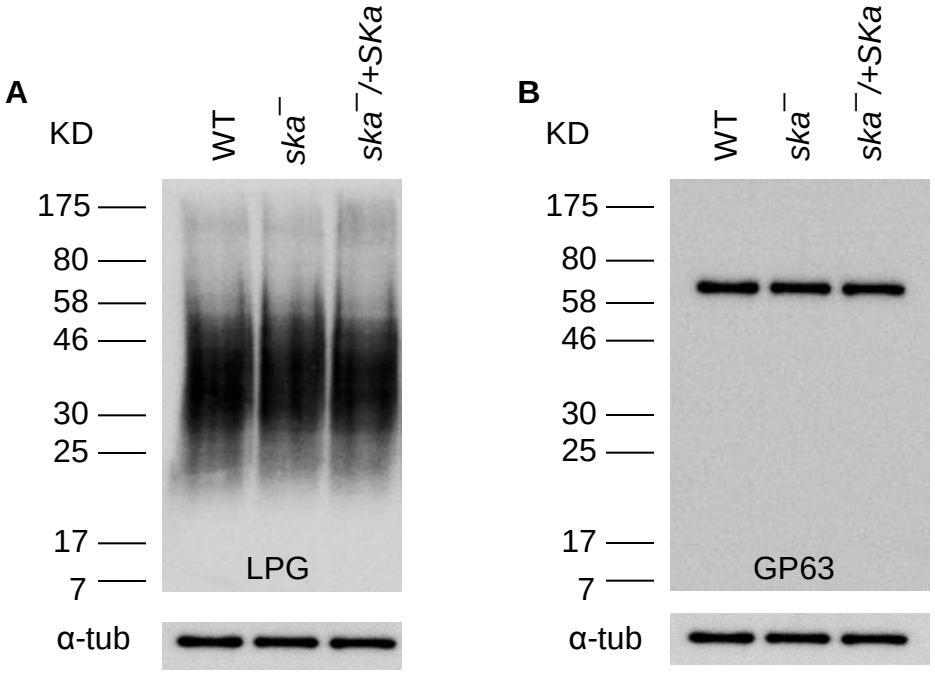


Figure S11. Expression of LPG and GP63 is not altered in *ska*$^{-}$ mutants. Whole cell lysates from stationary phase promastigotes were resolved by SDS-PAGE and transferred to PVDF membranes. Monoclonal antibodies WIC79.3 and 96-126 (Cedarlane Labs) were used to detect LPG (**A**) and GP63 (**B**), respectively. A monoclonal anti-α-tubulin antibody was used to control loading (bottom).

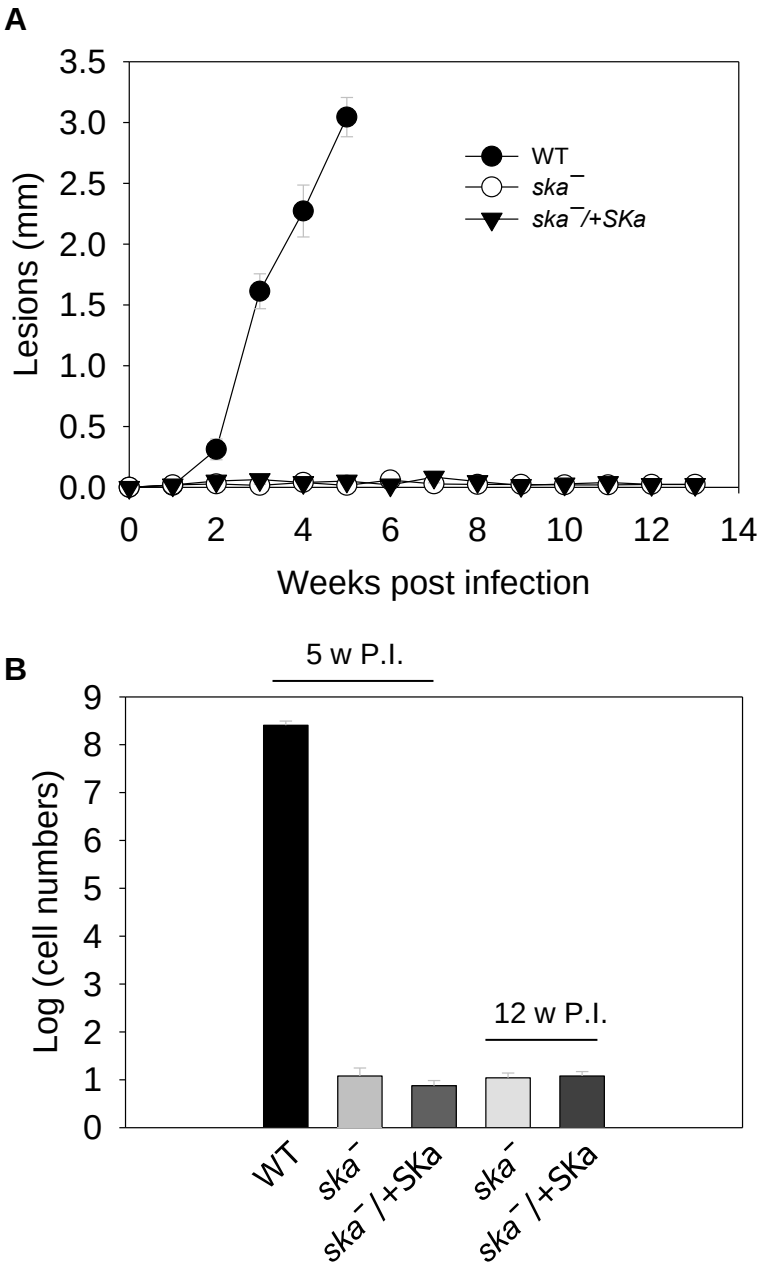


Figure S12. Metacyclics of *ska*$^{-}$ and *ska*$^{-}$*/+SKa* fail to induce pathology in BALB/c mice. BALB/c mice were infected in the footpads with metacyclics (2.0 x 10^5 cells/mouse, 5 mice/group). (**A**) Lesion sizes of infected footpads were measured weekly with a caliper. (**B**) Parasite numbers in the infected footpads were determined at 5 or 12 weeks post infection (w P.I.) by limiting dilution assay. Error bars represent standard deviations.