SUPPLEMENTAL FIGURES

FIGURE S1: **Phylogenetic analysis of the MCRA protein family.** Phylogenetic tree of 148 representatives of the MCRA protein family. Clades with bootstrap support less than 0.6 are not marked, *clades with 0.8 – 1 bootstrap support, **clades with 0.6 – 0.8 bootstrap support. Names of the taxonomic phyla are indicated in the parentheses for every sequence analyzed.

FIGURE S2: **Phylogenetic tree and multiple sequence alignment of FAD binding motif of MCRA from Streptococcus, Lactobacillus and Staphylococcus species.** On the left panel value of non-synonymous/synonymous substitutions rate is indicated for the branches if excides 1. On the right panel alignment of FAD binding motifs from corresponding proteins are shown. Clusters supported by phylogenetic analysis and including majority of the sequences form Streptococcus, Lactobacillus or Staphylococcus species are marked respectively as "Streptococcus", "Lactobacillus" and "Staphylococcus" on the right side of aligned sequences. Residues that are either identical or similar in >85% sequences in each cluster are shown on black and gray backgrounds, respectively. Gly and Glu residues of the central FAD binding motif are marked with *.

FIGURE S3: **SPH purification**. *A.* Non-tagged SPH, the crude cells extract (1), Source Q eluate (2) and Superdex S200 eluate (3). *B.* N-His-tagged SPH, HisTrap eluate. The 12% SDS-PAGE gels were stained by Coomassie blue for 20 min and distained by subsequent washing in distillated water.

FIGURE S4: **UV-Vis spectra of SPH and its supernatant after heat precipitation (SPHsup), showing flavin-like absorbance.** SPH spectra were recorded at the enzyme concentration of 10 mg/mL. For the reference, spectra of FAD and FMN are shown.

FIGURE S5: **UV-Vis spectra of holo- and apo-SPH.** Both holo- and apo-SPH were at concentration of 1 mg/mL. For the reference, a spectrum of FAD is shown.

FIGURE S6: **Stop-flow kinetic analysis of holo-SPH reduction by linoleic acid.** *A & B.* Stop-flow kinetic plots for 36-fold and 360-fold excess of linoleic acid. The reactions were performed at 37 ºC by mixing equal volumes of the enzyme and the substrate solutions in buffer containing 50 mM MES/NaOH (pH 6.1), 50 mM NaCl, 2 % EtOH and 10 % glycerol with final concentration of the enzyme 5 μM and linoleic acid 180 μM and 1.8 mM respectively. Representative data for one out of five repetitions for the respective condition is presented.

FIGURE S7: **Confirmation of *sph* deletion knockout in *S. pyogenes* M49 by PCR on genomic and mRNA levels.** *A.* PCR of genomic DNA from the wild type (1) and the mutant (2), fragment of 1782 bp was formed from the wild type DNA but not from the mutant DNA upon amplification of *sph* ORF, quality of the DNA (DNAq) was checked with the genome specific primers homologous to the upstream region of *sph* gene, negative control (-). *B.* RT-PCR of total mRNA from the wild type (Wt) and the mutant (*Δ sph*) confirmed *sph* expression in the wild type and no expression in the mutant during the exponential phase of growth, gyrA was used as the internal control, presence of genomic DNA was checked by genome specific primers as above, negative control (-), positive control (+).

FIGURE S8: **Growth phenotypes of wild type and Δ *sph* strains of *S. pyogenes* M49 without or with additives.** *A.* Growth of the wild type and mutant strains in THY media. *B & C.* Growth of the wild type (*B*) and the mutant (*C*) in THY media with 10 % human blood serum (Serum), 10 μg/mL linoleic acid (18:2) and oleic acid (18:1). Representative data for one out of two repetitions for the respective condition is presented.

FIGURE S9: **Effect of fatty acids in different formulations on minimal inhibitory concentration of cerulenin for wild type and Δ *sph* strains of *S. pyogenes* M49.** *A.* Growth in presence of 10 μg/mL oleic (18:1) and linoleic acid (18:2). *B.* Addition of 1 % human albumin (Alb) and 10 μg/mL palmitic acid (16:0) with equal concentrations of oleic and linoleic acid. *C.* Effect on growth of 10 % human blood serum (Serum). Optical density was measured after overnight growth in presence of different concentrations of the respective compounds. Data represent means of two independent experiments.

FIGURE S1


XP 389374.1 Gibberella zeae PH-1 (ascomycetes)
XP 001216855.1 Aspergillus terreus NIH2624 (ascomycetes)
XP 682566.1 Aspergillus nidulans FGSC A4 (ascomycetes)
XP 748677.1 Aspergillus fumigatus Af293 (ascomycetes)
XP 001268275.1 Aspergillus clavatus NRRL 1 (ascomycetes)
XP 001821606.1 Aspergillus oryzae RIB40 (ascomycetes)
XP 002379842.1 Aspergillus flavus NRRL3357 (ascomycetes)
XP 001216512.1 Aspergillus terreus NIH2624 (ascomycetes)
XP 001398622.1 Aspergillus niger (ascomycetes)
CAP97292.1 Penicillium chrysogenum Wisconsin 54-1255 (ascomycetes)
CAP93148.1 Penicillium chrysogenum Wisconsin 54-1255 (ascomycetes)
XP 661386.1 Aspergillus nidulans FGSC A4 (ascomycetes)
CAP93045.1 Penicillium chrysogenum Wisconsin 54-1255 (ascomycetes)
XP 001270501.1 Aspergillus clavatus NRRL 1 (ascomycetes)
XP 001213001.1 Aspergillus terreus NIH2624 (ascomycetes)
XP 002152225.1 Penicillium marneffei ATCC 18224 (ascomycetes)
CAP96477.1 Penicillium chrysogenum Wisconsin 54-1255 (ascomycetes)
XP 001216186.1 Aspergillus terreus NIH2624 (ascomycetes)
XP 001401212.1 Aspergillus niger (ascomycetes)
XP 002384726.1 Aspergillus flavus NRRL3357 (ascomycetes)
XP 001274306.1 Aspergillus clavatus NRRL 1 (ascomycetes)
XP 001259694.1 Neosartorya fischeri NRRL 181 (ascomycetes)
XP 753643.2 Aspergillus fumigatus Af293 (ascomycetes)
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ZP 02885383.1 Burkholderia graminis C4D1M (b-proteobacteria)
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ZP 03756041.1 Clostridium asparagiforme DSM 15981 (firmicutes)
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YP 002420162.1 Methylobacterium chloromethanicum CM4 (a-proteobacteria)
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YP 155984.1 Idiomarina loihiensis L2TR (g-proteobacteria)
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ACT54545.1 Elizabethkingia meningoseptica (CFB)
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ZP 03752189.1 Roseburia inulinivorans DSM 16841 (firmicutes)
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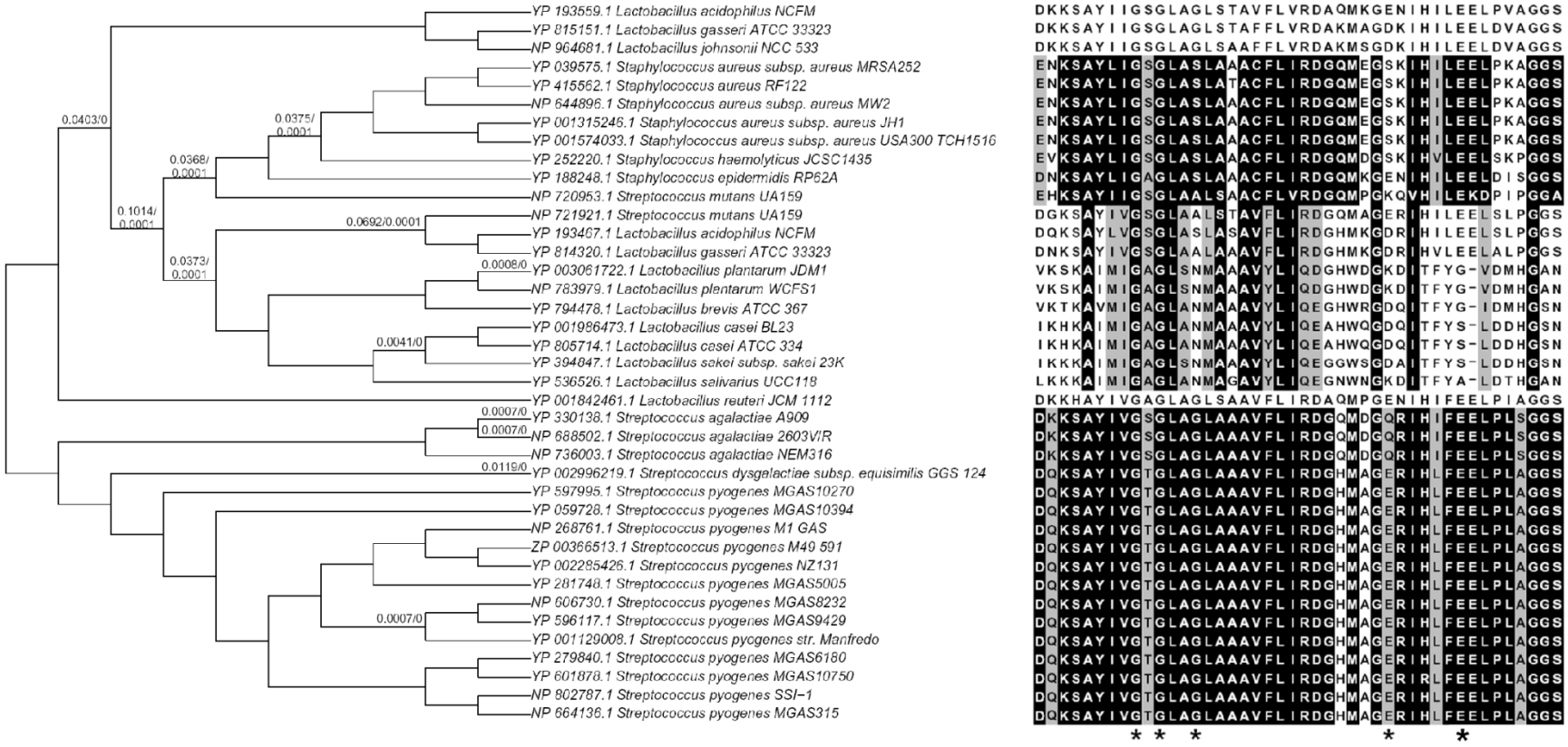
FIGURE S2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YP 415562.1 Staphylococcus aureus RF122
NP 644896.1 Staphylococcus aureus subsp. aureus MW2
YP 001315246.1 Staphylococcus aureus subsp. aureus JH1
YP 001574033.1 Staphylococcus aureus subsp. aureus USA300 TCH1516
YP 252220.1 Staphylococcus haemolyticus JCSC1435
YP 188248.1 Staphylococcus epidermidis RP62A
NP 720953.1 Streptococcus mutans UA159
NP 721921.1 Streptococcus mutans UA159
YP 193407.1 Lactobacillus acidophilus NCFM
YP 814320.1 Lactobacillus gasseri ATCC 33323
YP 003061722.1 Lactobacillus plantarum JDM1
NP 783979.1 Lactobacillus plantarum WCFS1
YP 794478.1 Lactobacillus brevis ATCC 367
YP 001986473.1 Lactobacillus casei BL23
YP 805714.1 Lactobacillus casei ATCC 334
YP 394847.1 Lactobacillus sakei subsp. sakei 23K
YP 536526.1 Lactobacillus salivarius UCC118
YP 001842461.1 Lactobacillus reuteri JCM 1112
YP 330138.1 Streptococcus agalactiae A909
NP 688502.1 Streptococcus agalactiae 2603V/R
NP 736003.1 Streptococcus agalactiae NEM316
YP 002996219.1 Streptococcus dysgalactiae subsp. equisimilis GGS 124
YP 597995.1 Streptococcus pyogenes MGAS10270
YP 059728.1 Streptococcus pyogenes MGAS10394
NP 268761.1 Streptococcus pyogenes M1 GAS
ZP 00366513.1 Streptococcus pyogenes M49 591
YP 002295426.1 Streptococcus pyogenes NZ131
YP 281748.1 Streptococcus pyogenes MGAS5005
NP 606730.1 Streptococcus pyogenes MGAS8232
YP 596117.1 Streptococcus pyogenes MGAS9429
YP 001129008.1 Streptococcus pyogenes str. Manfredo
YP 279840.1 Streptococcus pyogenes MGAS6180
YP 601878.1 Streptococcus pyogenes MGAS10750
NP 802787.1 Streptococcus pyogenes SSI-1
NP 664136.1 Streptococcus pyogenes MGAS315
0.0403/0
0.0375/
0.0001
0.0368/
0.0001
0.1014/
0.0001
0.0692/0.0001
0.0373/
0.0001
0.0008/0
0.0041/0
0.0007/0
0.0007/0
0.0119/0
0.0007/0

FIGURE S3

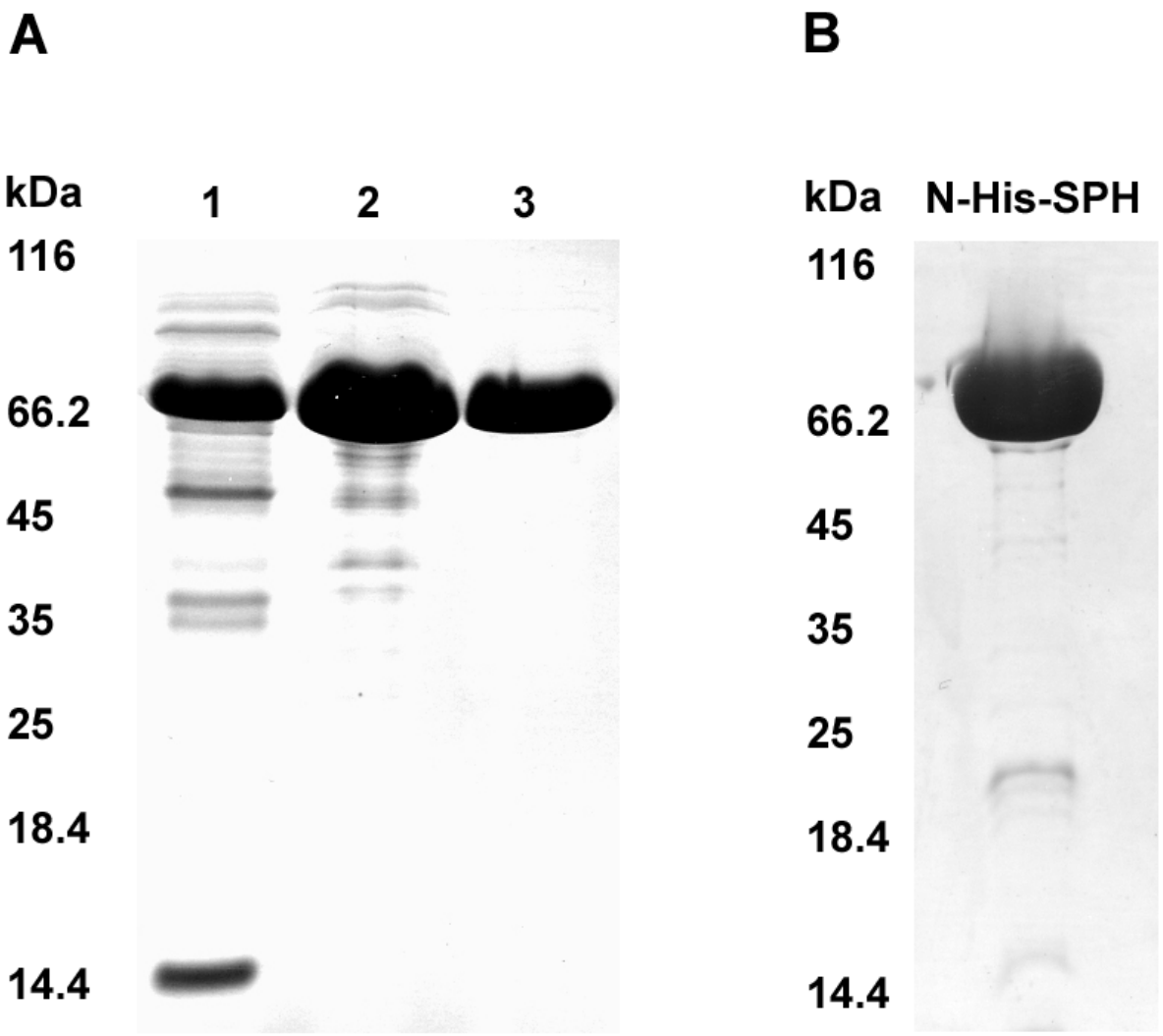

A
kDa
1
2
3
116
66.2
45
35
25
18.4
14.4
B
kDa
N-His-SPH
116
66.2
45
35
25
18.4
14.4

FIGURE S4

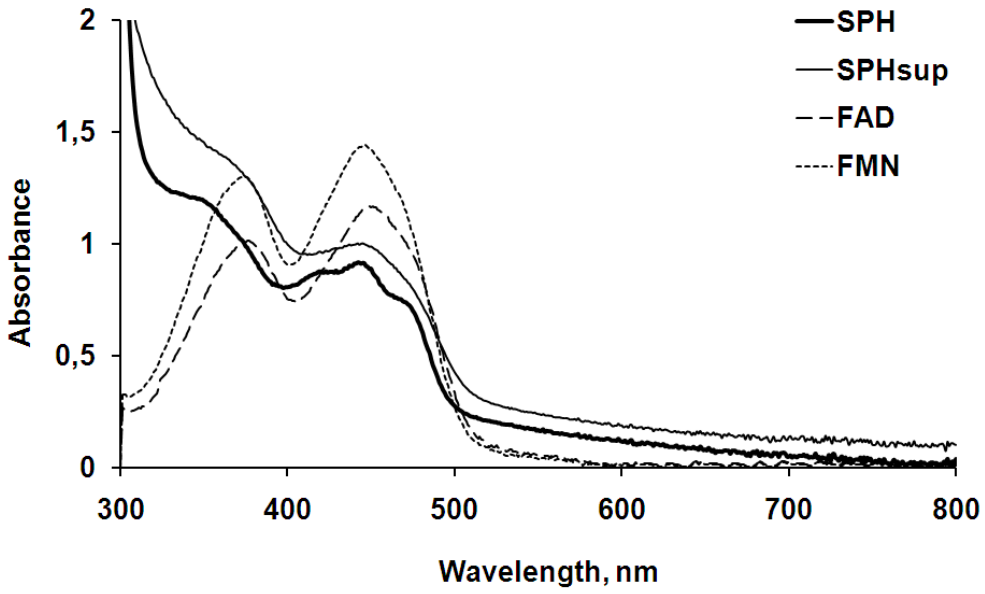

SPH
SPHsup
FAD
FMN
Absorbance
2
1,5
1
0,5
0
300
400
500
600
700
800
Wavelength, nm

FIGURE S5

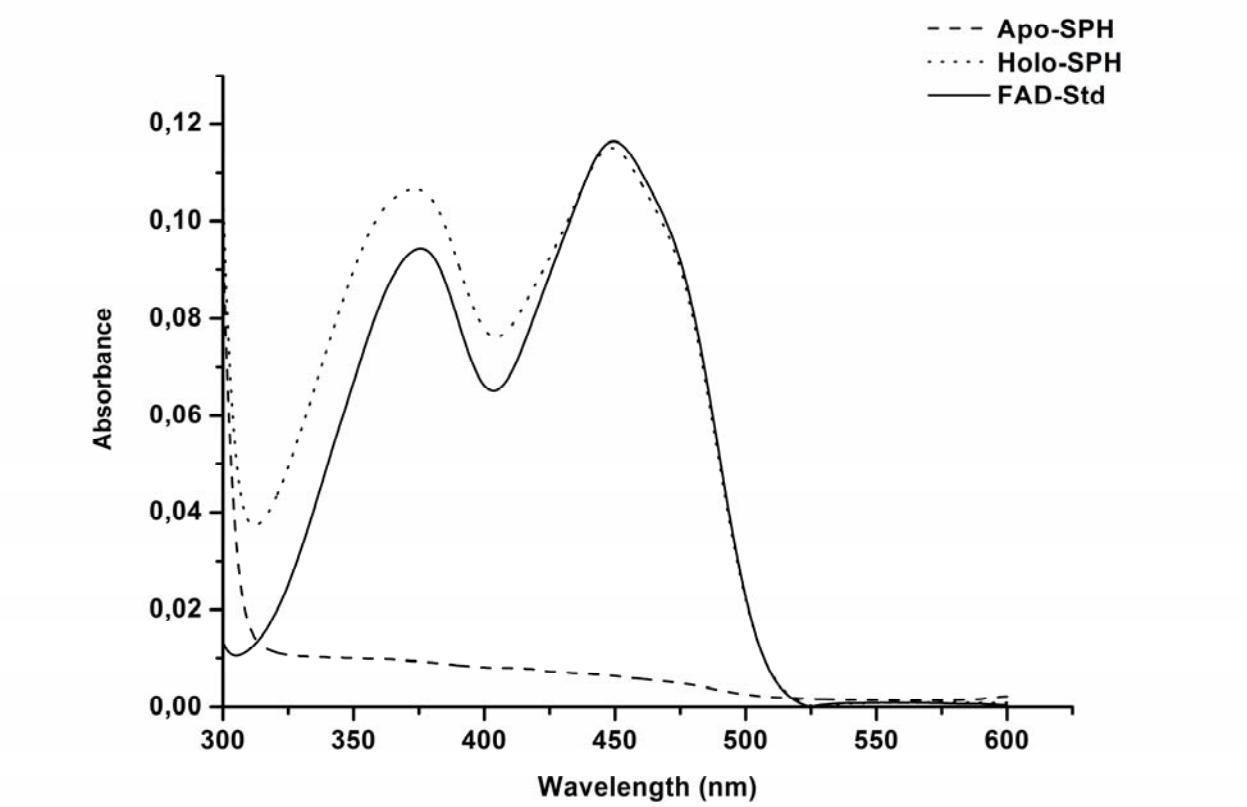

Apo-SPH
Holo-SPH
FAD-Std
Absorbance
0,12
0,10
0,08
0,06
0,04
0,02
0,00
300
350
400
450
500
550
600
Wavelength (nm)

FIGURE S6

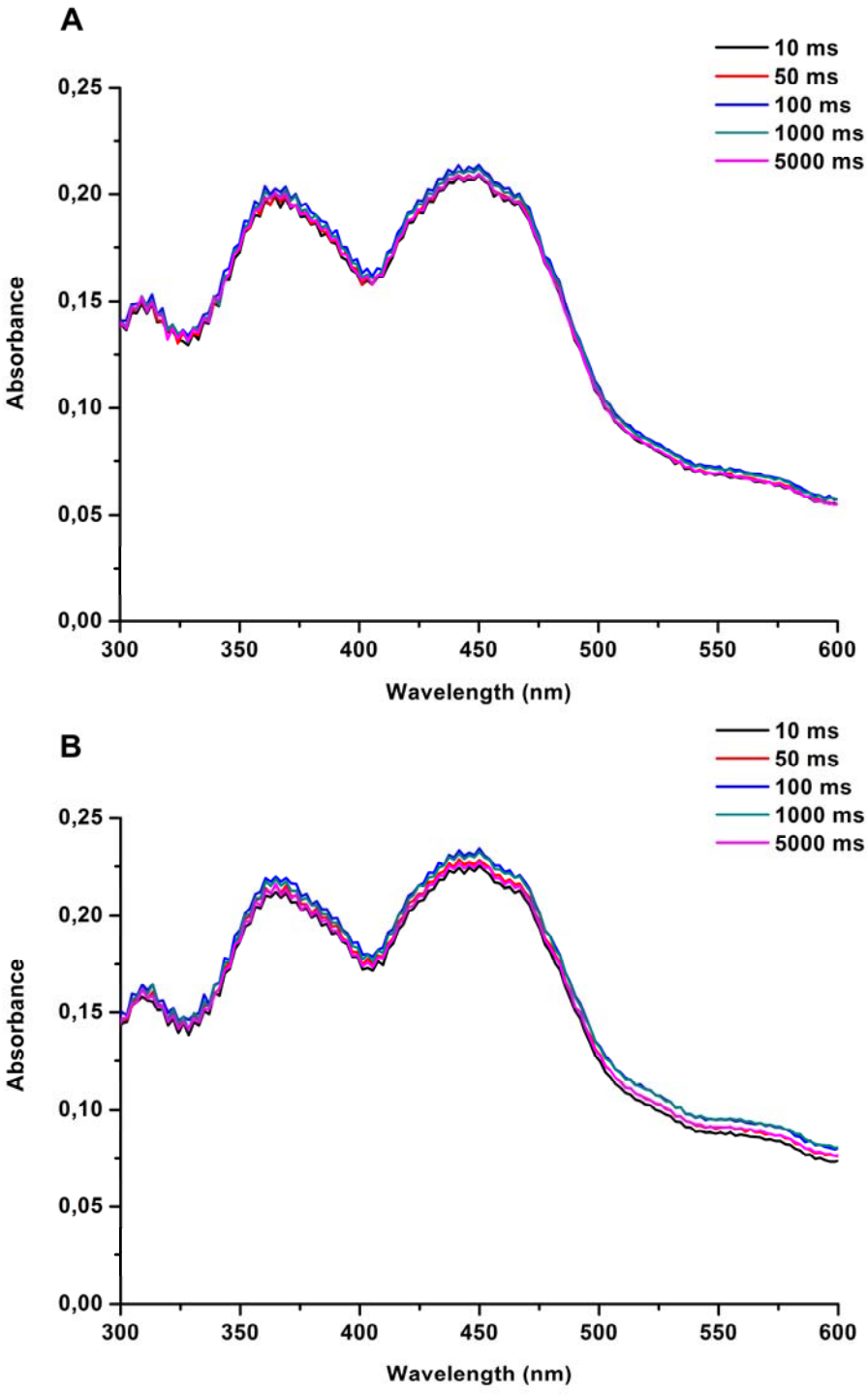

A
10 ms
50 ms
100 ms
1000 ms
5000 ms
Absorbance
0,25
0,20
0,15
0,10
0,05
0,00
300
350
400
450
500
550
600
Wavelength (nm)
B
10 ms
50 ms
100 ms
1000 ms
5000 ms
Absorbance
0,25
0,20
0,15
0,10
0,05
0,00
300
350
400
450
500
550
600
Wavelength (nm)

FIGURE S7

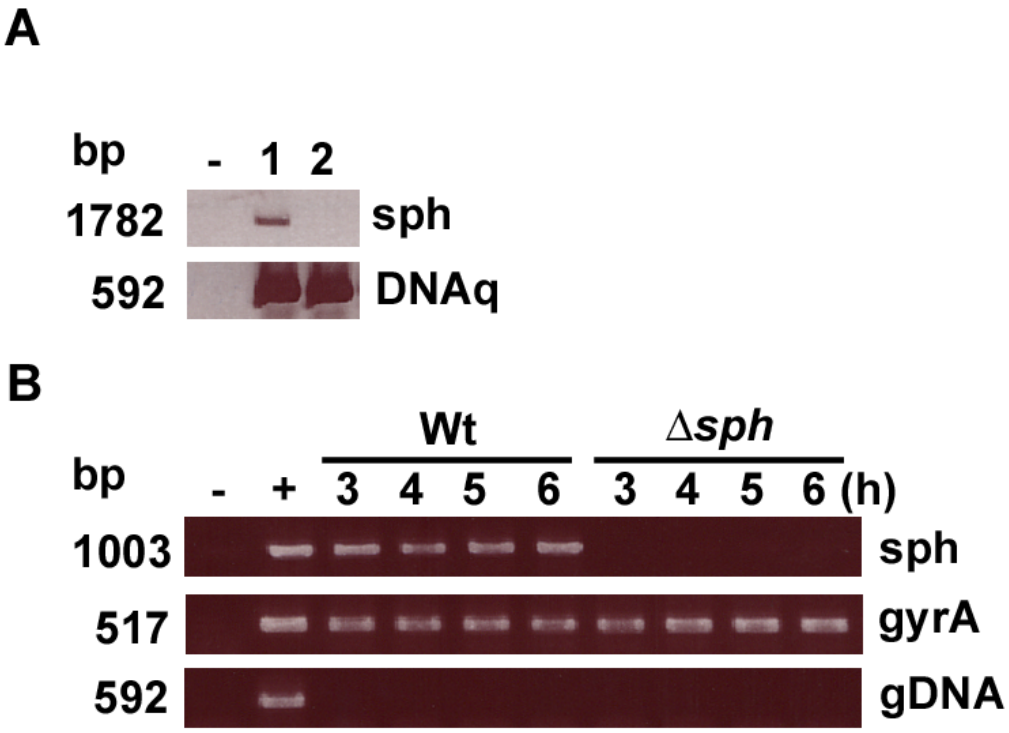

A
bp
- 1 2
1782
sph
592
DNAq
B
Wt
Δsph
bp
- + 3 4 5 6 3 4 5 6 (h)
1003
sph
517
gyrA
592
gDNA

FIGURE S8

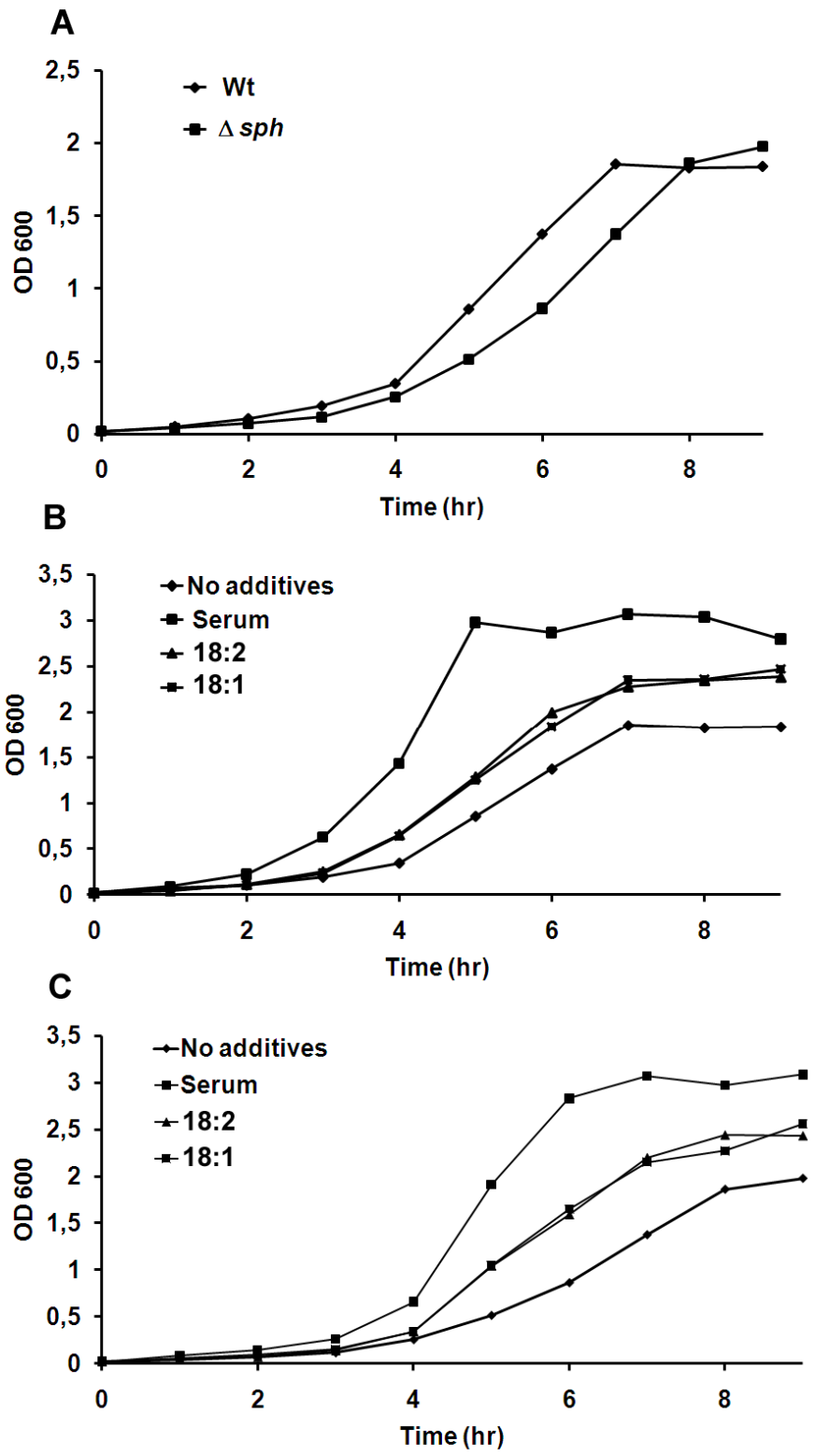
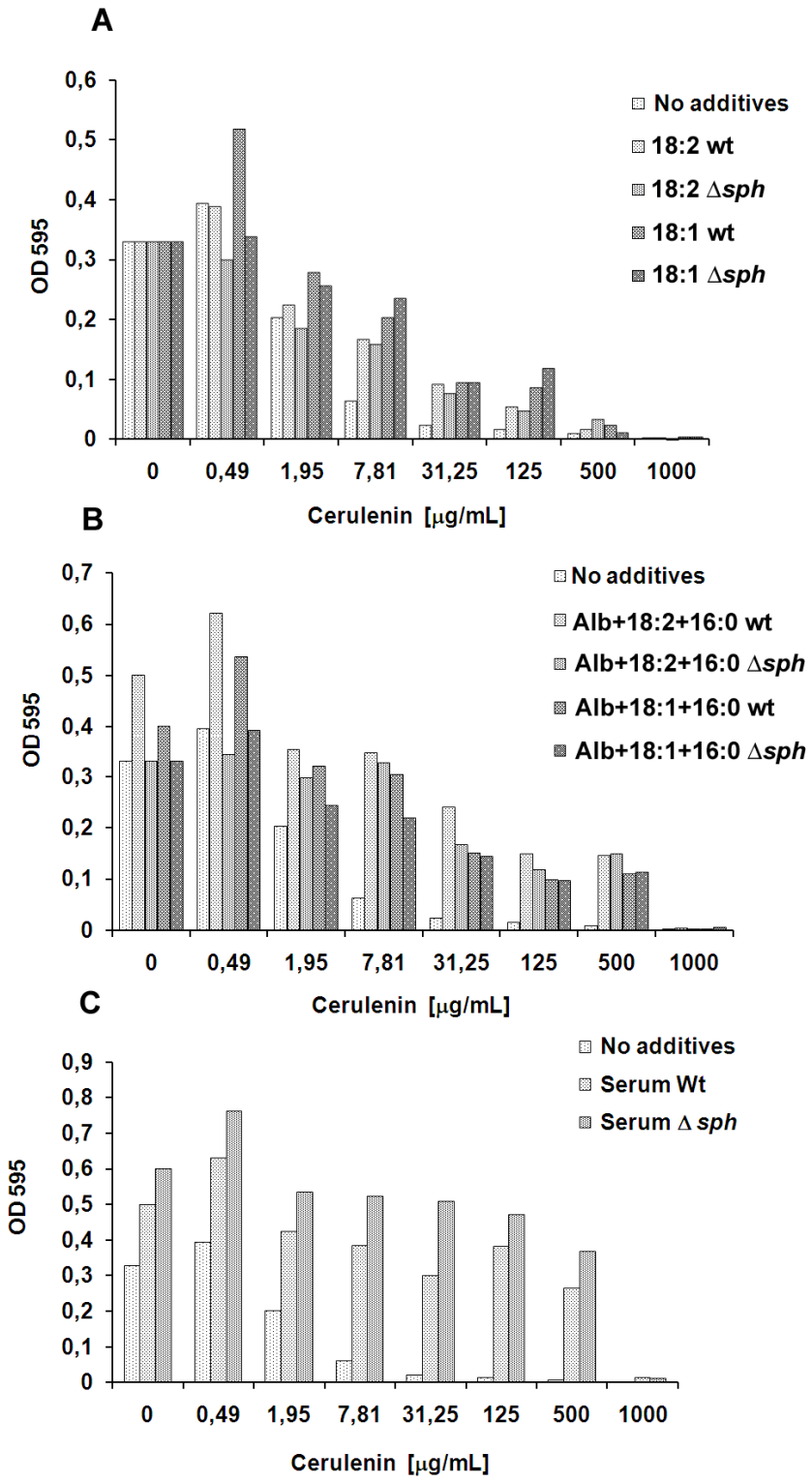

A
2,5
2
1,5
1
0,5
0
OD 600
Wt
Δ sph
0
2
4
6
8
Time (hr)
B
3,5
3
2,5
2
1,5
1
0,5
0
OD 600
No additives
Serum
18:2
18:1
0
2
4
6
8
Time (hr)
C
3,5
3
2,5
2
1,5
1
0,5
0
OD 600
No additives
Serum
18:2
18:1
0
2
4
6
8
Time (hr)

FIGURE S9


A
OD 595
0,6
0,5
0,4
0,3
0,2
0,1
0
0
0,49
1,95
7,81
31,25
125
500
1000
Cerulenin [μg/mL]
No additives
18:2 wt
18:2 Δsph
18:1 wt
18:1 Δsph
B
OD 595
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0
0
0,49
1,95
7,81
31,25
125
500
1000
Cerulenin [μg/mL]
No additives
Alb+18:2+16:0 wt
Alb+18:2+16:0 Δsph
Alb+18:1+16:0 wt
Alb+18:1+16:0 Δsph
C
OD 595
0,9
0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0
0
0,49
1,95
7,81
31,25
125
500
1000
Cerulenin [μg/mL]
No additives
Serum Wt
Serum Δ sph