Supplemental Materials

Molecular Biology of the Cell

Swinnen et al.

SUPPLEMENTARY DATA

SUPPLEMENTARY TABLES

Table S1: Strains used in this study.

Name	Genotype and markers	Reference
W303-1A (WT)	MATa *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1*	*(Thomas and Rothstein, 1989)*
JW 00 145	MATα *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1*	(Thomas and Rothstein, 1989)
BS_188	W303-1A *TRP1+*	This study
JW 00 035	W303-1A with *sch9::TRP1*	(Roosen *et al.*, 2005)
JW 01 418	W303-1A with *sch9::NATMX4*	This study
BY4741 (WT)	MATa *his3Δ1 leu2Δ0 ura3Δ0 met15Δ0*	Y.K.O. Collection
BY4742	MATα *his3Δ1 leu2Δ0 ura3Δ0 lys2Δ0*	Y.K.O. Collection
JW 01 306	BY4741 with *sch9::HIS3*	(Swinnen *et al.*, 2005)
JW 03 038	BY4741 with *sch9::NATMX4*	This study
isc1Δ	BY4741 with *isc1::KANMX4*	Y.K.O. Collection
sur2Δ	BY4742 with *sur2::KANMX4*	Y.K.O. Collection
lcb4Δ	BY4742 with *lcb4::KANMX4*	Y.K.O. Collection
lcb5Δ	BY4742 with *lcb5::KANMX4*	Y.K.O. Collection
lcb3Δ	BY4742 with *lcb3::KANMX4*	Y.K.O. Collection
ysr3Δ	BY4742 with *ysr3::KANMX4*	Y.K.O. Collection
lag1Δ	BY4742 with *lag1::KANMX4*	Y.K.O. Collection
lac1Δ	BY4742 with *lac1::KANMX4*	Y.K.O. Collection
sur4Δ	BY4742 with *sur4::KANMX4*	Y.K.O. Collection
ydc1Δ	BY4742 with *ydc1::KANMX4*	Y.K.O. Collection
ypc1Δ	BY4742 with *ypc1::KANMX4*	Y.K.O. Collection
csg2Δ	BY4742 with *csg2::KANMX4*	Y.K.O. Collection
sur1Δ	BY4742 with *sur1::KANMX4*	Y.K.O. Collection
ipt1Δ	BY4742 with *ipt1::KANMX4*	Y.K.O. Collection
isc1Δ	BY4742 with *isc1::KANMX4*	Y.K.O. Collection
svf1Δ	BY4742 with *svf1::KANMX4*	Y.K.O. Collection
sch9Δ sur2Δ	BY with *sch9::HIS3 sur2::KANMX4*	This study(1)
sch9Δ lcb4Δ	BY with *sch9::HIS3 lcb4::KANMX4*	This study(1)
sch9Δ lcb5Δ	BY with *sch9::HIS3 lcb5::KANMX4*	This study(1)
sch9Δ lcb4Δ lcb5Δ	BY with *sch9::HIS3 lcb4::KANMX4 lcb5::KANMX4*	This study(1)
sch9Δ lcb3Δ	BY with *sch9::HIS3 lcb3::KANMX4*	This study(1)
sch9Δ ysr3Δ	BY with *sch9::HIS3 ysr3::KANMX4*	This study(1)
sch9Δ lcb3Δ ysr3Δ	BY with *sch9::HIS3 lcb3::KANMX4 ysr3::KANMX4*	This study(1)
sch9Δ lac1Δ	BY with *sch9::HIS3 lac1::KANMX4*	This study(1)
sch9Δ lag1Δ	BY with *sch9::HIS3 lag1::KANMX4*	This study(1)
sch9Δ sur4Δ	BY with *sch9::HIS3 sur4::KANMX4*	This study(1)
sch9Δ ydc1Δ	BY with *sch9::HIS3 ydc1::KANMX4*	This study(1)
sch9Δ ypc1Δ	BY with *sch9::HIS3 ypc1::KANMX4*	This study(1)
sch9Δ csg2Δ	BY with *sch9::HIS3 csg2::KANMX4*	This study(1)
sch9Δ sur1Δ	BY with *sch9::HIS3 sur1::KANMX4*	This study(1)
sch9Δ ipt1Δ	BY with *sch9::HIS3 ipt1::KANMX4*	This study(1)
sch9Δ isc1Δ	BY with *sch9::HIS3 isc1::KANMX4*	This study(1)
sch9Δ svf1Δ	BY with *sch9::HIS3 svf1::KANMX4*	This study(1)
JW 04 601	BY4741 with C-terminal 9MYC tagged *LAG1*	This study (2)
JW 04 602	JW 03 038 with C-terminal 9MYC tagged *LAG1*	This study (2)
JW 04 603	BY4741 with C-terminal 9MYC tagged *LAC1*	This study (2)

JW 04 604	JW 03 038 with C-terminal 9MYC tagged *LAC1*	This study [(2)]
JW 04 606	BY4741 with C-terminal 9MYC tagged *ISC1*	This study [(2)]
JW 04 607	JW 03 038 with C-terminal 9MYC tagged ISC*1*	This study [(2)]
JW 04 610	BY4741 with C-terminal 9MYC tagged *YDC1*	This study [(2)]
JW 04 612	JW 03 038 with C-terminal 9MYC tagged *YDC1*	This study [(2)]
JW 04 613	BY4741 with C-terminal 9MYC tagged *YPC1*	This study [(2)]
JW 04 615	JW 03 038 with C-terminal 9MYC tagged *YPC1*	This study [(2)]

[(1)] Strains were created by crossing JW 01 306 (BY4741 with *sch9::HIS3*) with BY4742 strains containing the desired *KANMX4* deletions, followed by sporulation and tetrad dissection. Triple deletion strains were made using appropriate single and double deletion strains for crossing and sporulation.
[(2)] For C-terminal 9Myc tagging, the Euroscarf plasmid pYM20 (9Myc tag, hphNT1 marker) was used as a template for PCR using primers containing 60bp overlap with the region directly before and after the stop codon of the target gene, as described in (Janke *et al.*, 2004). Transformants were selected on hygromycin B containing medium and checked with colony PCR for correct integration of the 9Myc tag.

Table S2: Plasmids used in this study.

Name	backbone	insert	marker	Reference
FBp87	Yeplac195	p$_{SCH9pr}$-*SCH9*	*URA3*	(Reinders *et al.*, 1998)
p$_{CUP}$-*LAG1*	pYEX 4T-1	p$_{CUP1pr}$-GST-*LAG1*	*URA3*	(Martzen *et al.*, 1999)
p$_{CUP}$-*LAC1*	pYEX 4T-1	p$_{CUP1pr}$-GST-*LAG1*	*URA3*	(Martzen *et al.*, 1999)
FBp431	pYES2	*ISC1-GFPuv*	*URA3*	(Vaena de Avalos *et al.*, 2004)
FBp459	pHS12	p$_{ADHpr}$-*preCox4-mCherry*	*LEU2*	Addgene 25444 (Benjamin Glick)
FBp701	YEp357	*LAG1* promoter (-780/+30)	*URA3*	this study
FBp702	YEp357	*LAC1* promoter (-750/+18)	*URA3*	this study
FBp703	YEp357	*YDC1* promoter (-750/+30)	*URA3*	this study
FBp704	YEp357	*YPC1* promoter (-750/+30)	*URA3*	this study
FBp705	pRS415	p$_{SCH9pr}$-*SCH9*	*LEU2*	this study
FBp706	pRS415	p$_{SCH9pr}$-*SCH9*^{5A}	*LEU2*	this study
FBp707	pRS415	p$_{SCH9pr}$-*SCH9*^{2D3E}	*LEU2*	this study
FBp708	pRS426	p$_{ISC1pr}$-*ISC1-GFPuv*	*URA3*	this study
FBp709	pYX242	*KAR2*$_{(1\rightarrow 135)}$-mCherry-HDEL	*LEU2*	this study

Table S3: Primers used in this study.

Name	sequence
LAG1-lacZFw	CCGGGATCCGATCTGTTGCCTTCAACAGTGCATTCC (bp -780 → -754, BamHI)
LAG1-lacZRv	CCGGTCGACATCGATAGATTTGTCCGTAGCTGATGTC (bp 30 → 3, SalI)
LAC1-lacZFw	CCGGGATCCTTTCTGTCGCTCTTATGATCCGTTGG (bp -750 → -725, BamHI)
LAC1-lacZRv	CCGGTCGACTGGCTTTATTGTGGACATAGCTCTTGTTTATTGATACTGTG (bp 18 → -21, (bp6 mutated (C→G) to remove endogenous SalI site), SalI)
YDC1-lacZFw	CCGGGATCCCACGGACAGGATTAAGAGTGATCC (bp -750 → -727, BamHI)

Primer	Sequence
YDC1-lacZRv	CCGGTCGACGGCTTCTGGATAAGGCCAGCTGAACAGC (bp 30 → 3, SalI)
YPC1-lacZFw	CCGGGATCCTGCGTAGTGTGAGTGCCACTAG (bp -750 → -729, BamHI)
YPC1-lacZRv	CCGGTCGACCTCTGGATAGTTCCAACGAAATATTCCC (bp 30 → 3, SalI)
ISC1-fus1	ccgggccccccctcgaggtcgacggtatcgataagcttgatatcgGCCGCGAGGTCGCCCTGGACGG (ISC1: bp -795 → - 774)
ISC1-RVC	GAGTCTTTCTTTACGGTGTTTGG (ISC1: bp 180 → 158)
ISC1-fus5	ATGTACAACAGAAAAGACAGAGATG (ISC1: bp 1 → 25)
ISC1-fus4	gcggtggcggccgctctagaactagtggatcccccgggctgcaggGCAAATTAAAGCCTTCGAGCG (CYC1ter: bp 248 → 229)
KAR2-F1	gaattcaccatggatcctagggcccacaagcttacgcgtcgacccgggATGTTTTTCAACAGACTAAGCGC (KAR2: bp 1 → 23)
KAR2-F2	gaactccttgatgatggccatgttatcctcctcgcccttgctcacATCATCGGCACCTCTAACTAAAAC (KAR2: bp 135 → 112)
mCherry-F1	aattctttccactcctccaatgttttagttagaggtgccgatgatGTGAGCAAGGGCGAGGAGG (mCherry 4 → 22)
mCherry-F2	ggaaaaacgttcattgttccttattcagttagctagctgagctcgagatatctcagagctcatcgtg CTTGTACAGCTCGTCCATGC (mCherry: bp 705 → 686)

SUPPLEMENTARY FIGURES

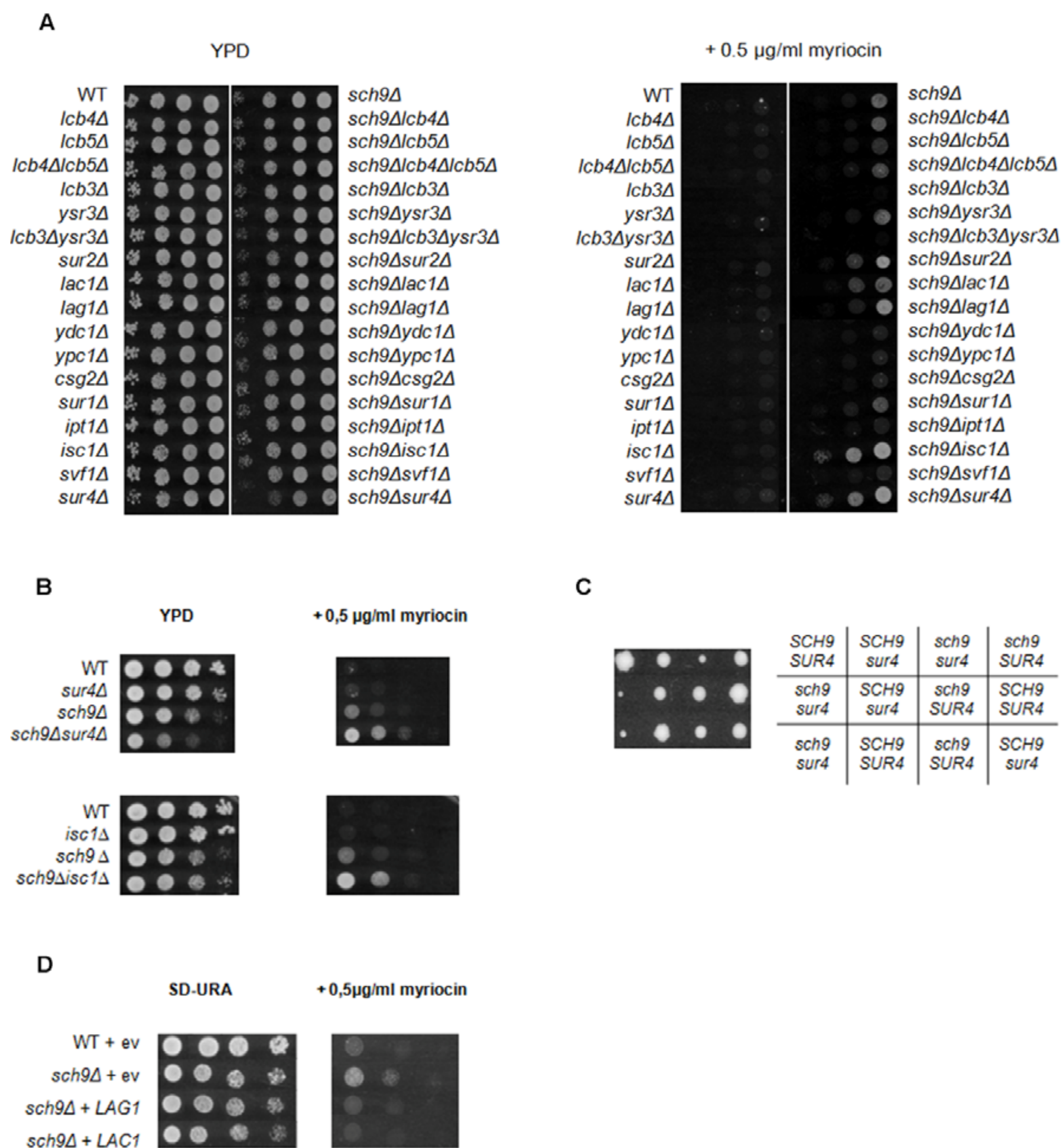

Figure S1: Genetic interaction of *SCH9* with enzymes involved in sphingolipid metabolism.
(A) Serial dilutions of exponential growing wild type, single, double and triple deletion strains on YPD medium with or without the addition of 0.5 µg/ml myriocin. The wild type and the single and double deletion mutants with a BY background are segregants from a diploid strain, created by crossing the *sch9Δ* (JW 01 306; background BY4741) strain with the respective single deletion strains with background BY4742. Triple deletions are segregants from a diploid strain, created by crossing a double deletion mutant with the single deletion mutant carrying the third deletion. **(B)** Additional deletion of *SUR4* or *ISC1* in the *sch9Δ* strain increases myriocin resistance. Double deletion strains were spotted directly below the *sch9Δ* strain, to better observe the increase in myriocin resistance. **(C)** Synthetic sick phenotype of a *sch9Δ sur4Δ* strain. Diploid cells, heterozygous for *SCH9* and *SUR4* were sporulated, and tetrads were dissected on YPD (in horizontal rows). Genotypes are indicated. **(D)** Overexpression of the ceramide synthases *LAG1* or *LAC1* suppresses the myriocin

resistance of the *sch9Δ* strain. Spottest on SD-URA with or without addition of myriocin of the WT (BY4741) and the *sch9Δ* strain (JW 03 038) transformed with plasmids with Cu^{2+} inducible over-expression of *LAC1* and *LAG1* (Martzen *et al.*, 1999) or an empty vector (ev).

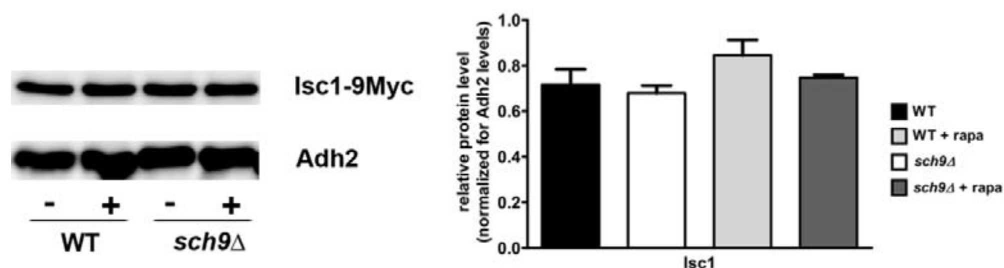

Figure S2: Deletion of *SCH9* or rapamycin treatment of cells does not affect Isc1 protein levels.
The gene encoding the inositol phosphosphingolipid phospholipase C (*ISC1*) was genomically tagged with a 9Myc tag in both WT BY4741 and *sch9Δ* (JW 03 038) strains. Overnight precultures were inoculated at an initial OD600 of 0.3 and grown to OD600 2.0, after which a sample was withdrawn for protein extraction (noted as -). The remainder of the culture was treated with 200nM rapamycin and grown for an additional hour, after which a second sample for protein extraction was taken (noted as +). After TCA protein extraction, equal protein amounts were run on a SDS-PAGE. The tagged enzyme was detected using an anti-Myc antibody, the yeast Adh2 serves as an internal control. A representative result for the western blot is shown in **(A)**. Signals for Myc detection were quantified and normalized for Adh2 levels. Average results of two independent cultures are shown in **(B),** with standard deviations noted as error bars.

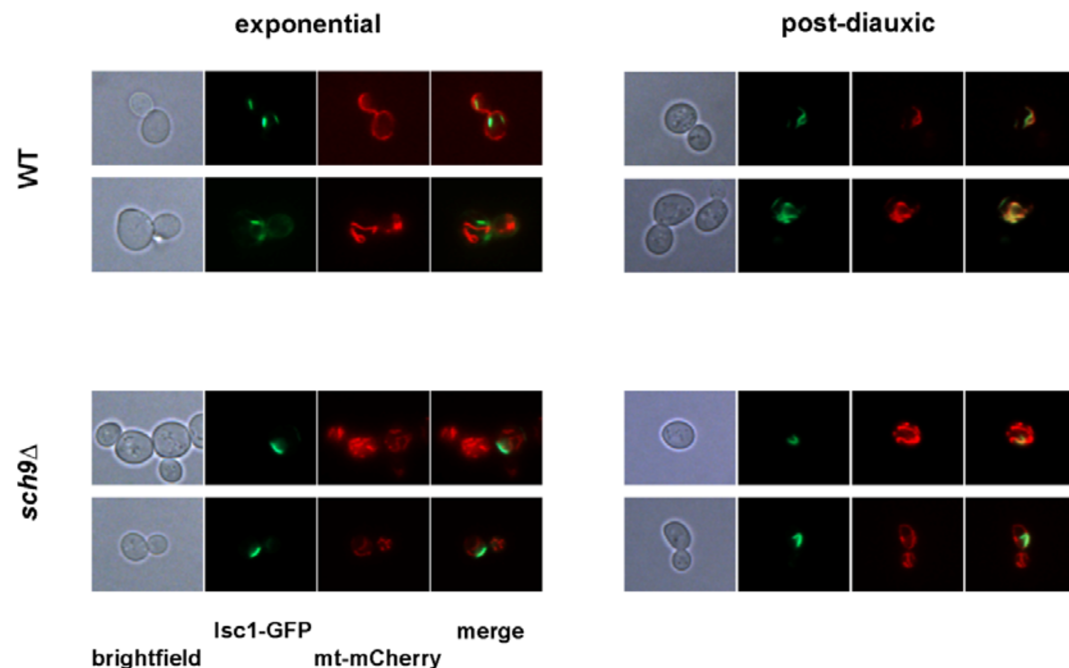

Figure S3: Mitochondrial translocation of Isc1 on galactose medium is impaired in the absence of Sch9.
Wild type (BY4741) and *sch9*Δ (JW 01 306) expressing Isc1-GFP (from a galactose-inducible promoter, plasmid FBp431) and a red fluorescent mitochondrial marker protein (plasmid FBp459, containing fluorescent mCherry targeted to the mitochondria by the Cox4 presequence) were grown in YPGAL medium and localization was analyzed at the early exponential and post-diauxic growth phase using fluorescence microscopy. Bright field, single channel and the corresponding merge are shown for both growth phases. Representative pictures are shown.

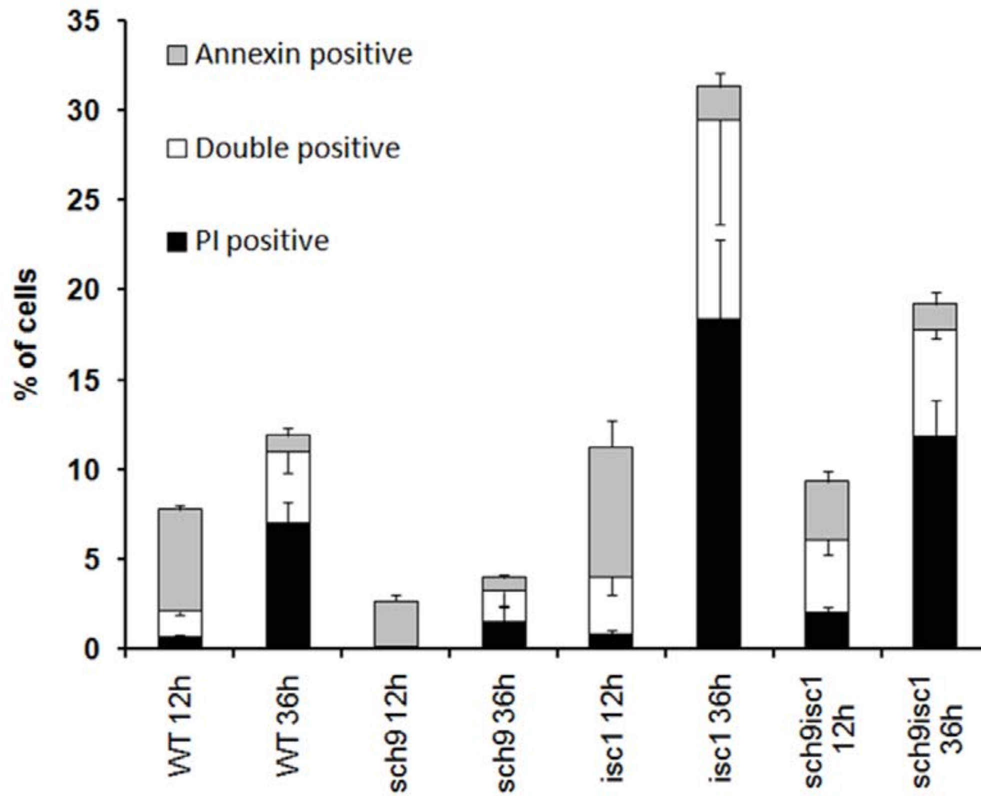

Figure S4: Sch9 and Isc1 modulate apoptotic cell death.
Tests for cell death markers of apoptosis and necrosis (Annexin/PI co-staining) were performed as described by (Buttner *et al.*, 2008). The wild type (BY4741), *sch9Δ* (JW 01 306), *isc1Δ* and *sch9Δisc1Δ* strains were inoculated in fresh synthetic complete medium at an OD600 0,1. After 12h (exponential phase) and 36h (post-diauxic phase) cells were stained using Annexin-V-FITC and propidium iodide (PI), to detect phosphatidyl serine externalization and loss of membrane integrity, respectively, as described by (Buttner *et al.*, 2008). Quantitative flow cytometric analysis, using a BD influx flow cytometer, on the apoptotic/necrotic markers was performed and analysis was carried out using FlowJo software. Results depicted are mean values ± SD of three independent cultures.

SUPPLEMENTARY REFERENCES

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