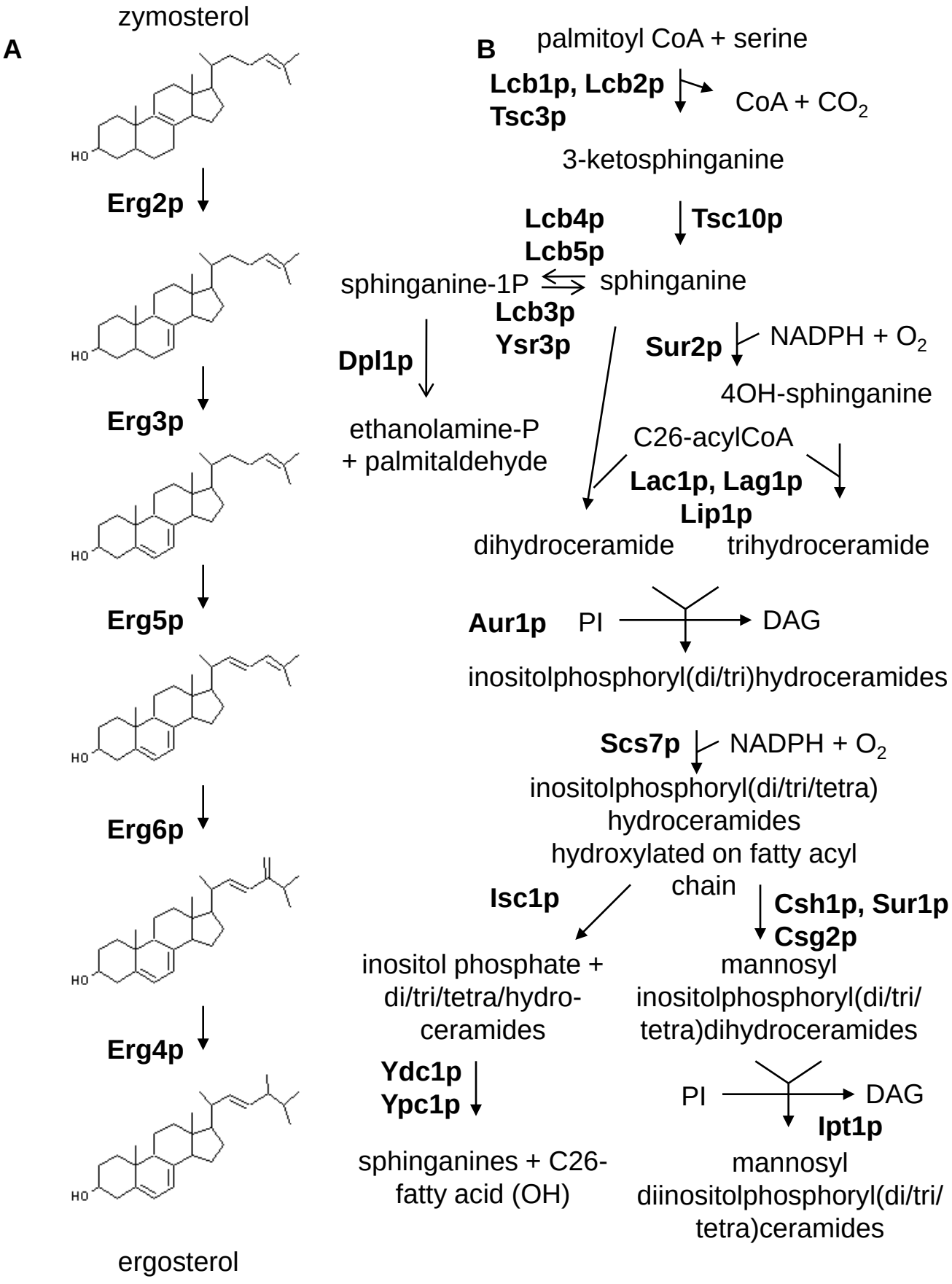
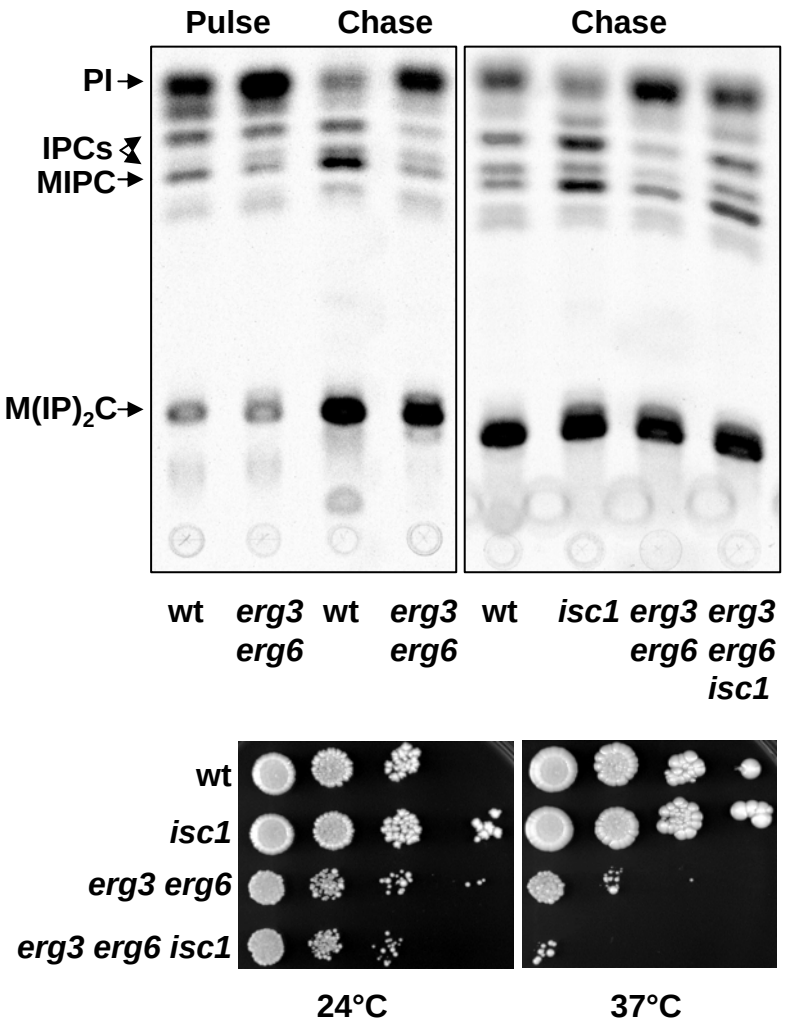


Supp Fig 1, Guan et al.



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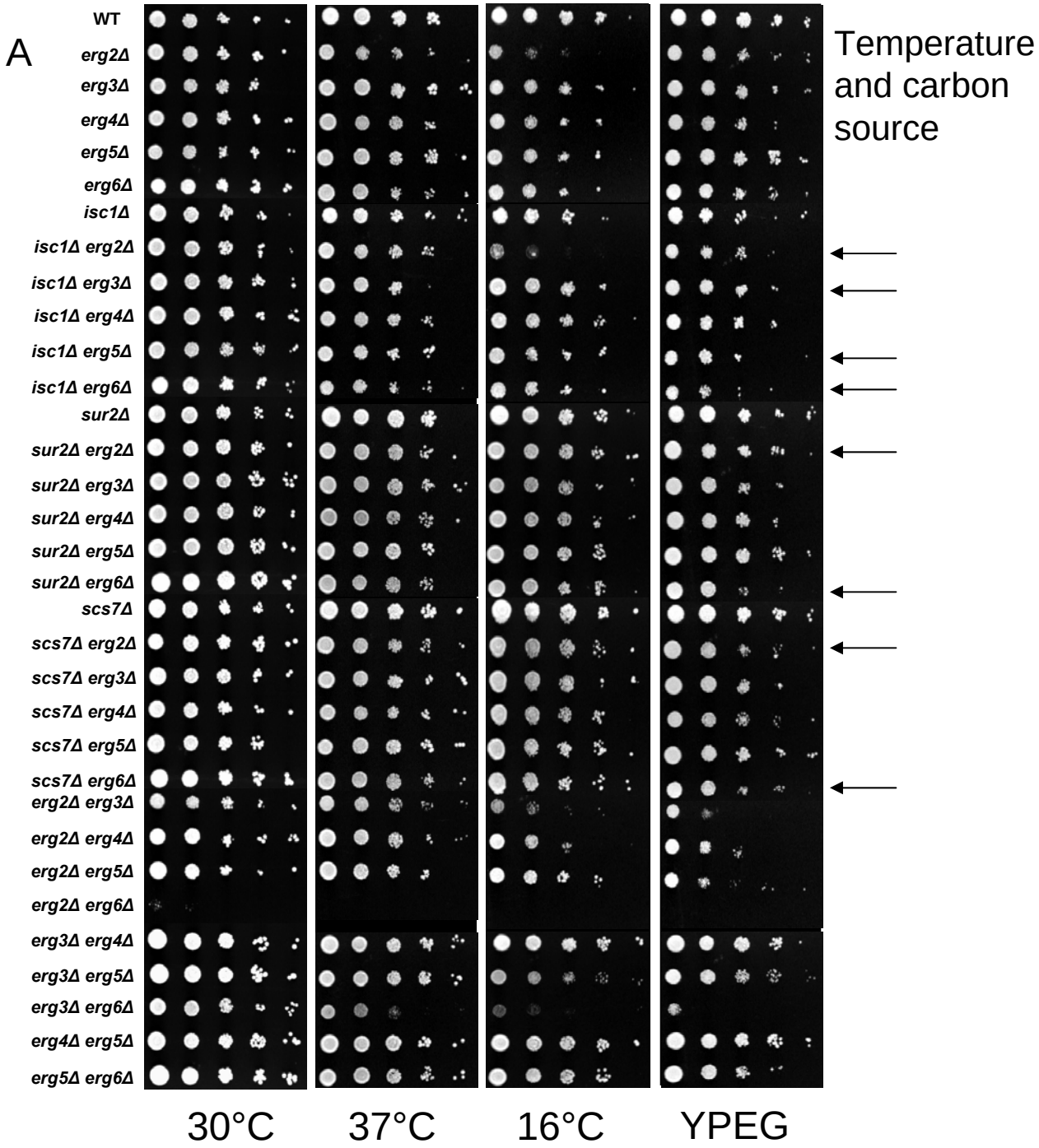
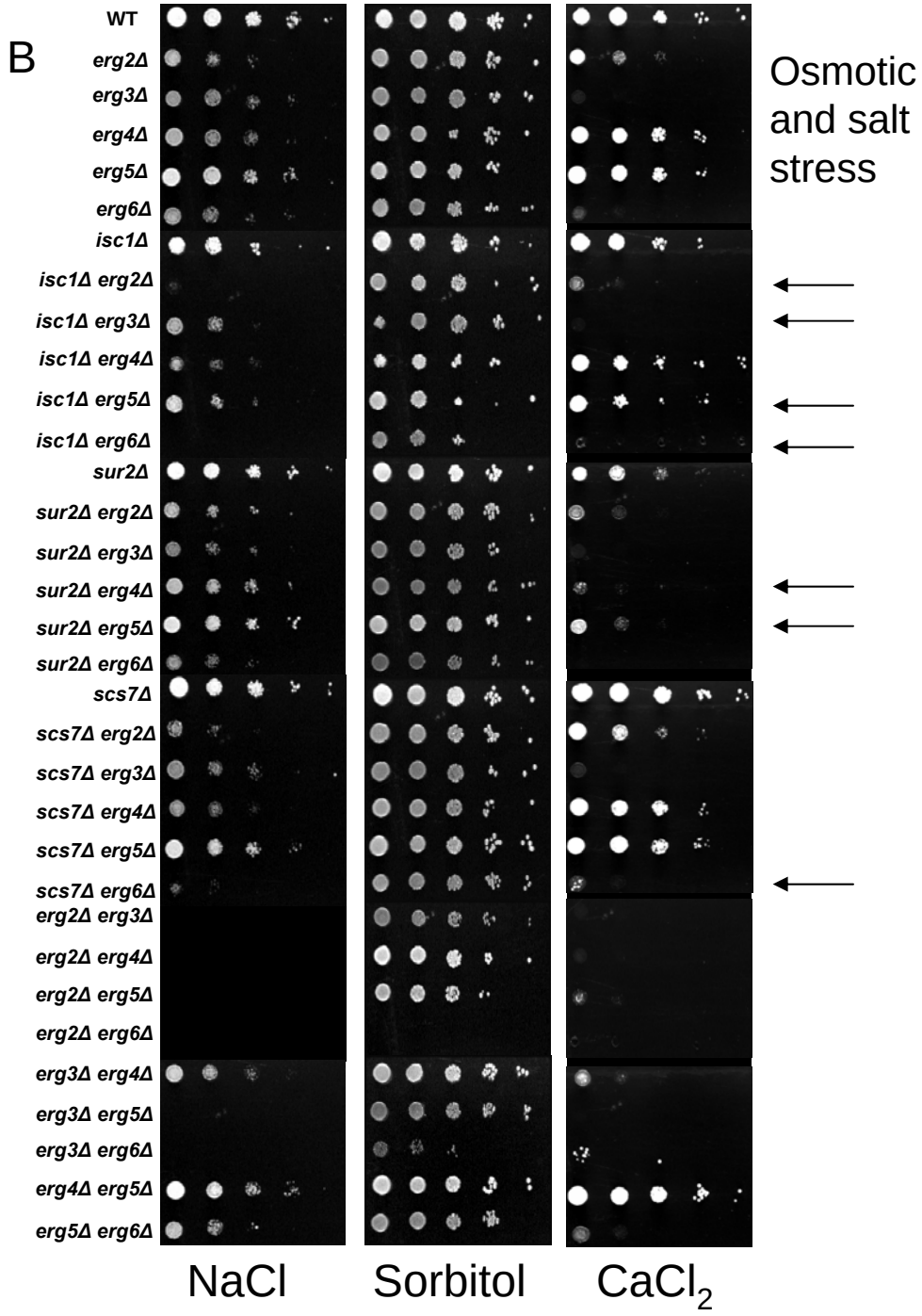
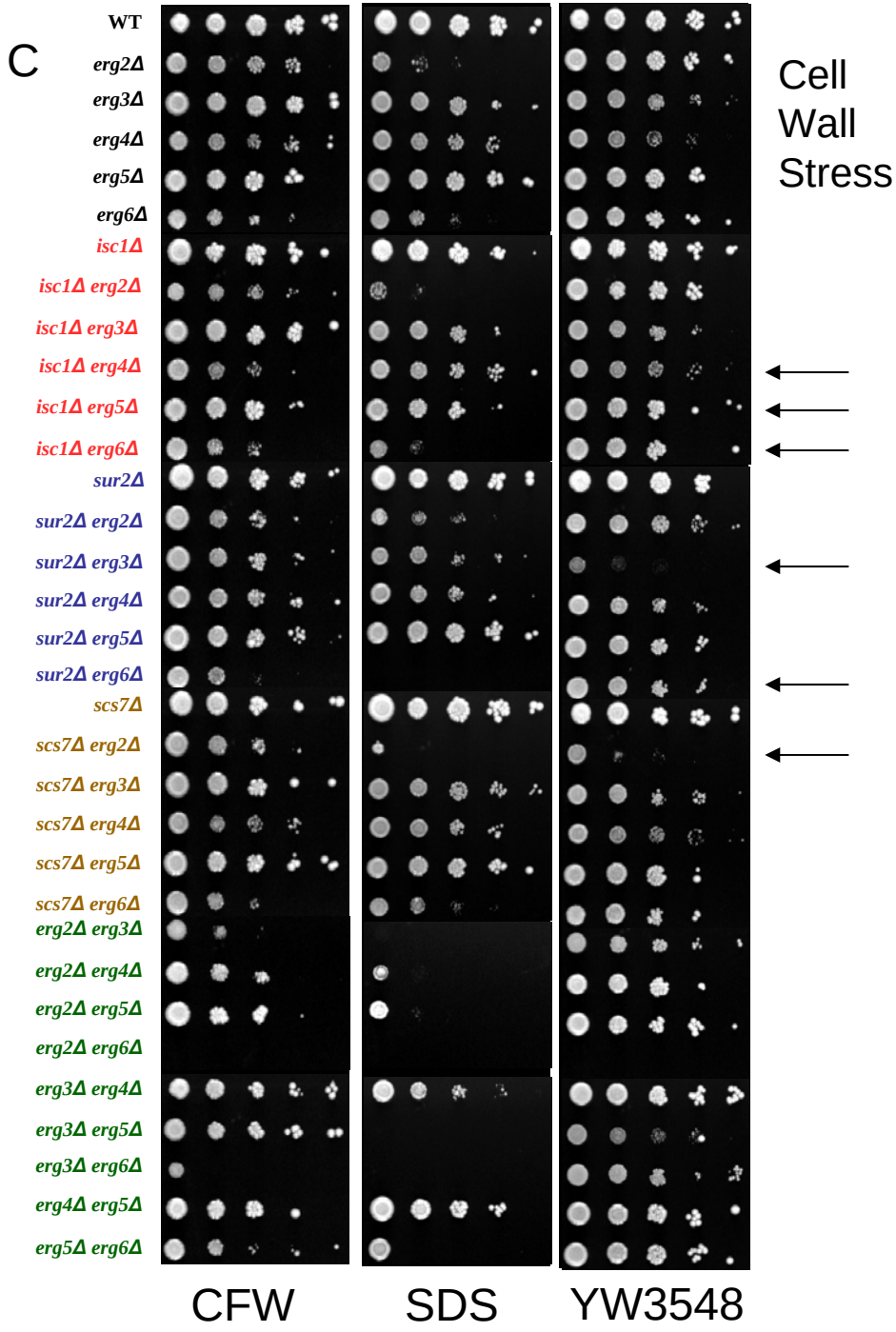


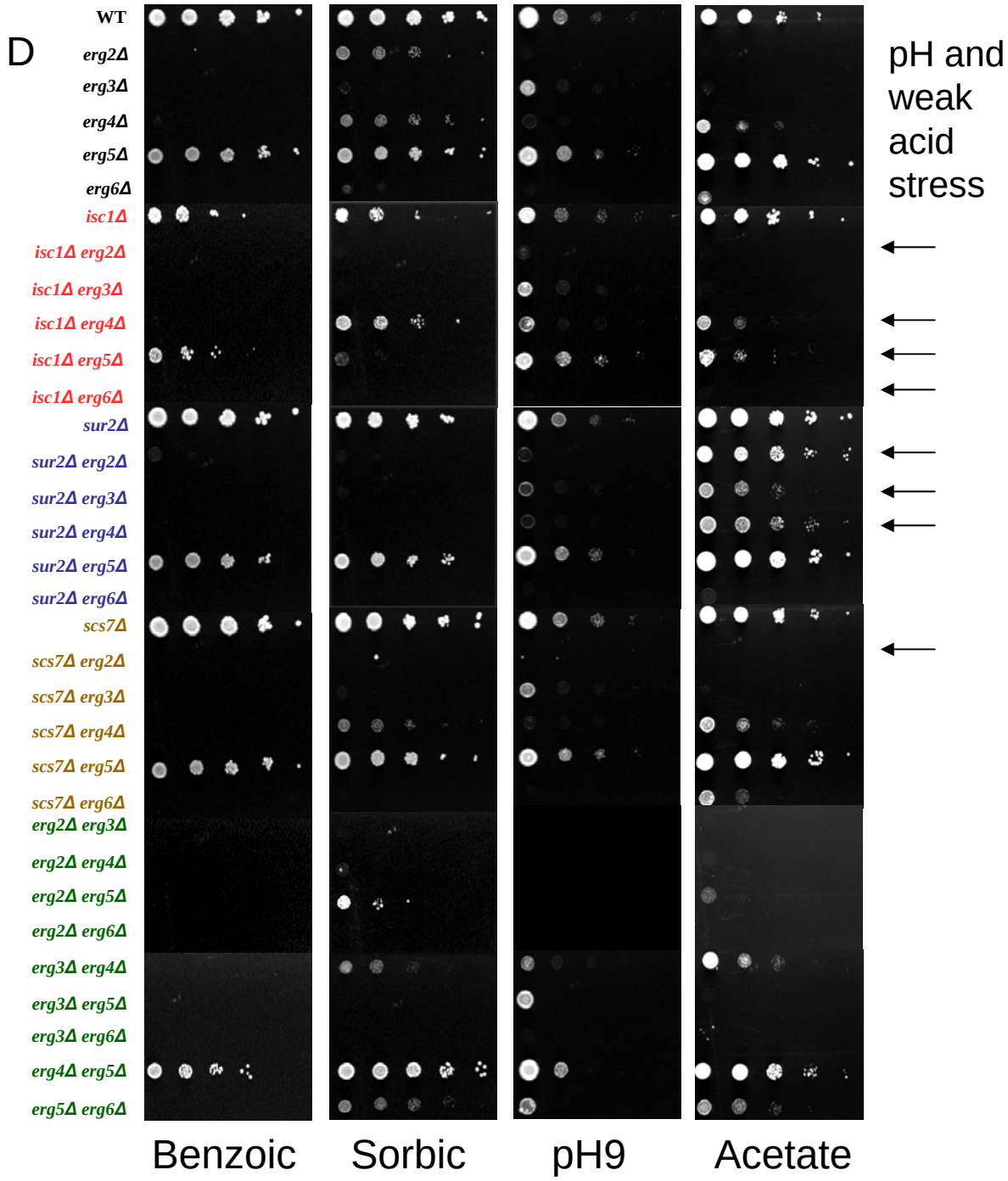
Supp Fig 3, Guan et al.



Supp Fig 3, Guan et al.



Supp Fig 3, Guan et al.



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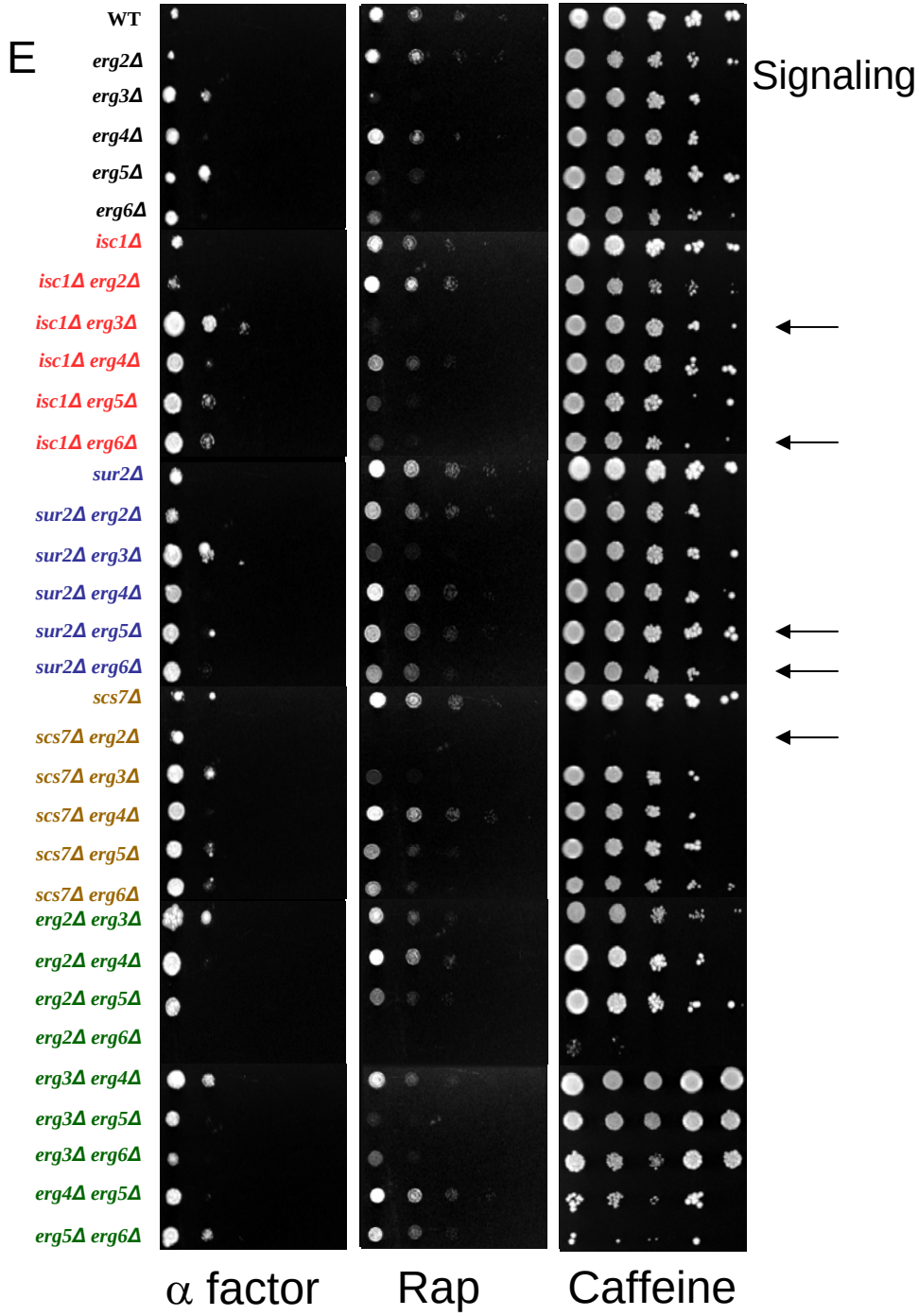
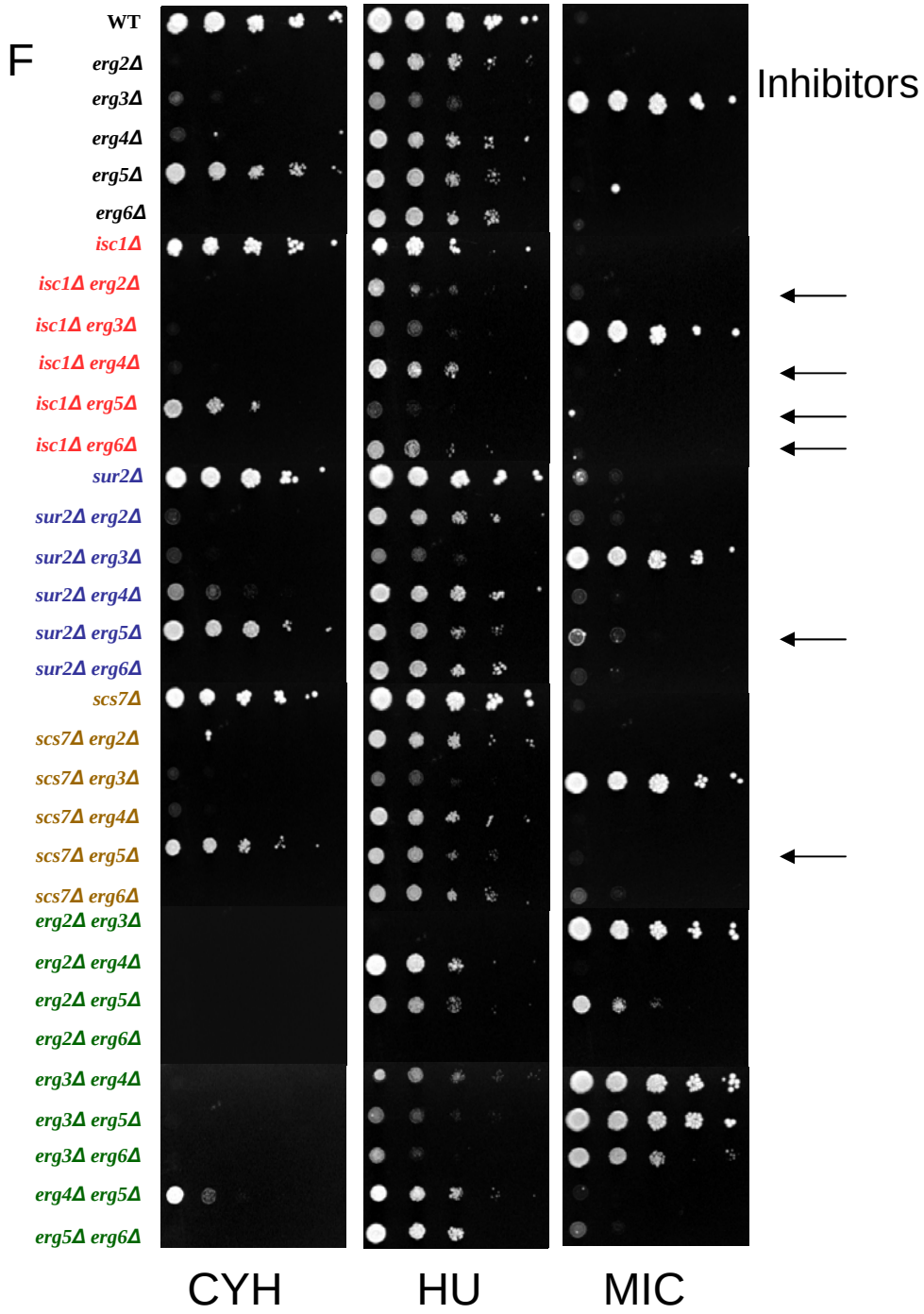
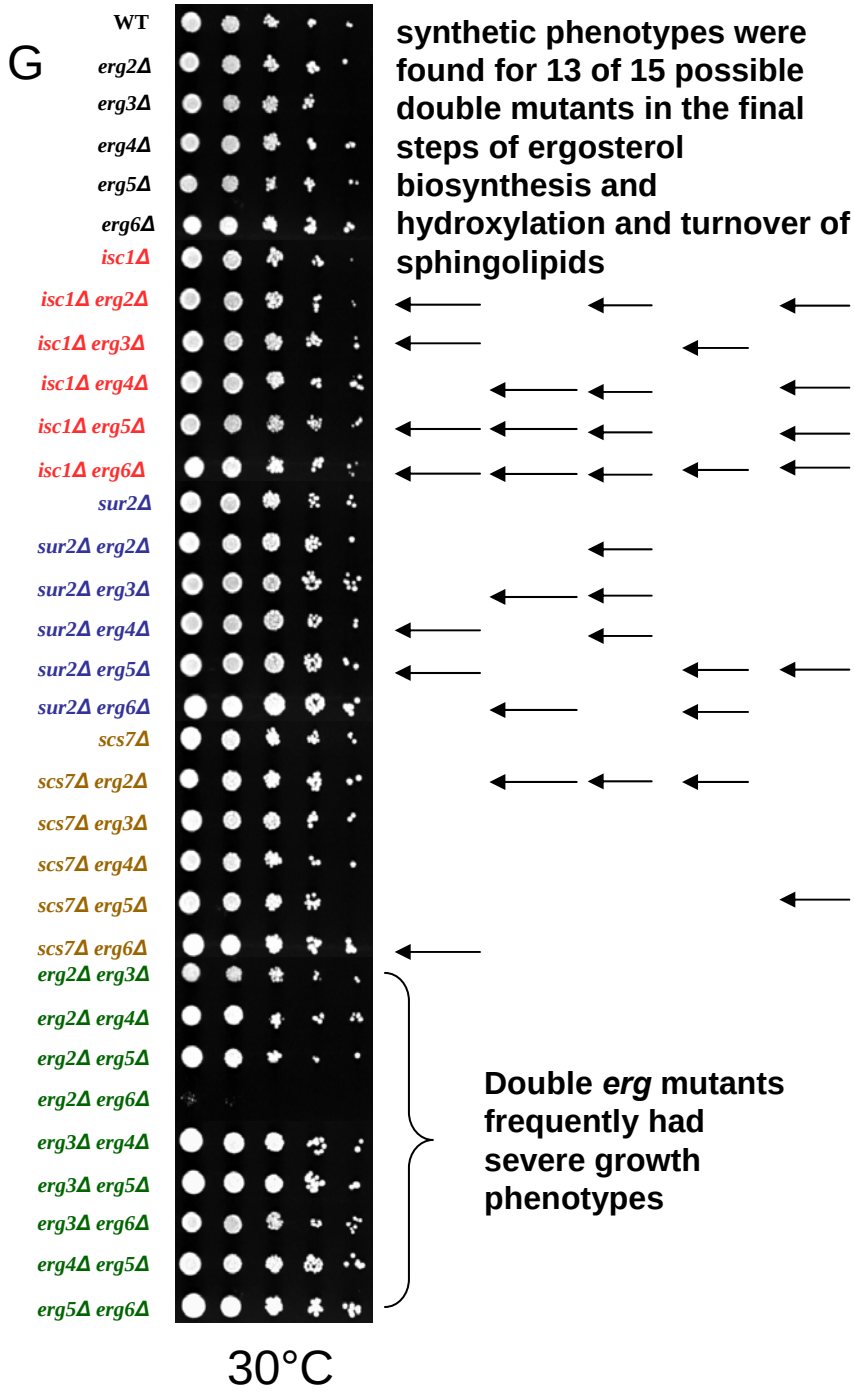


Supp Fig 3, Guan et al.

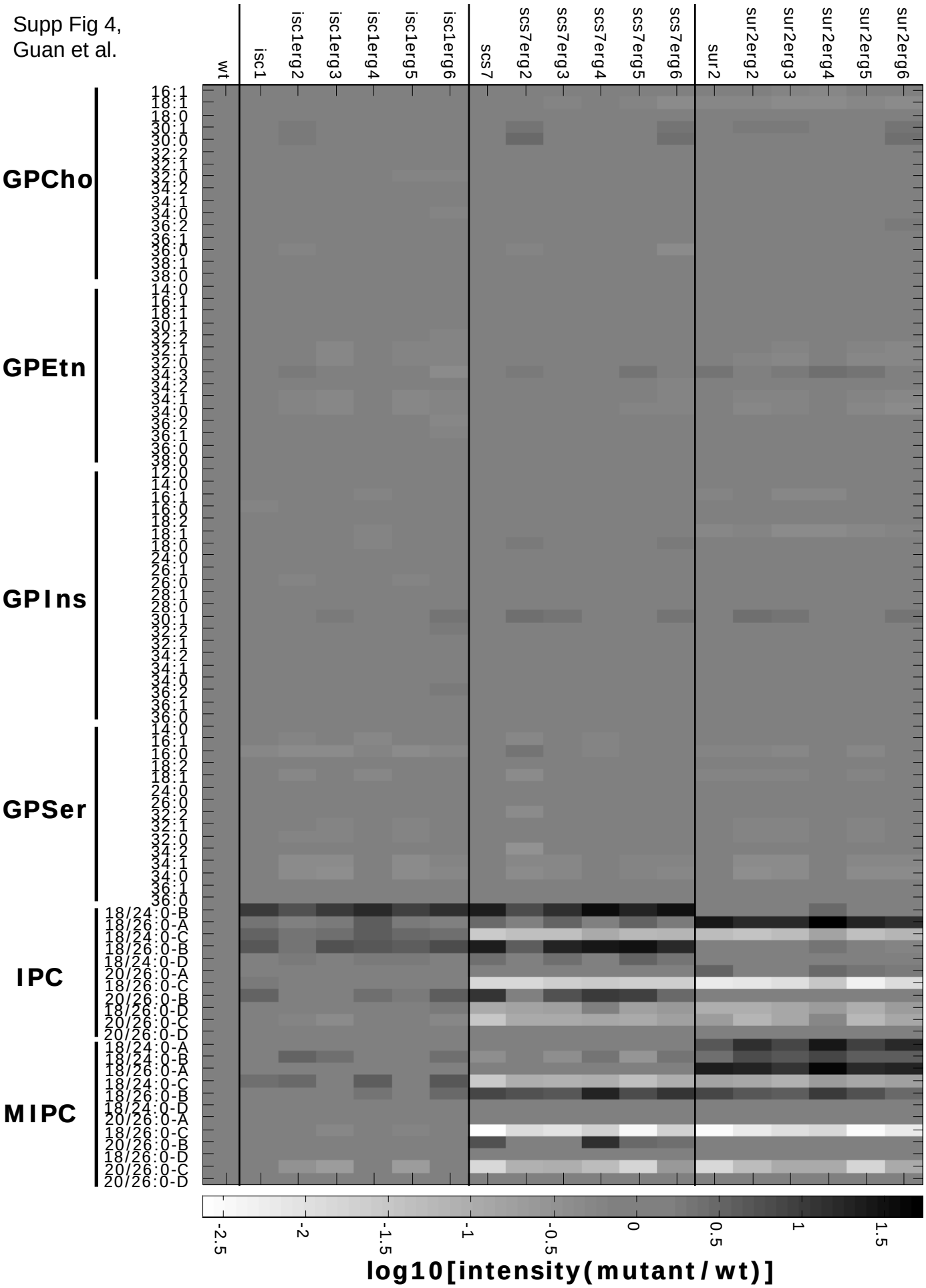


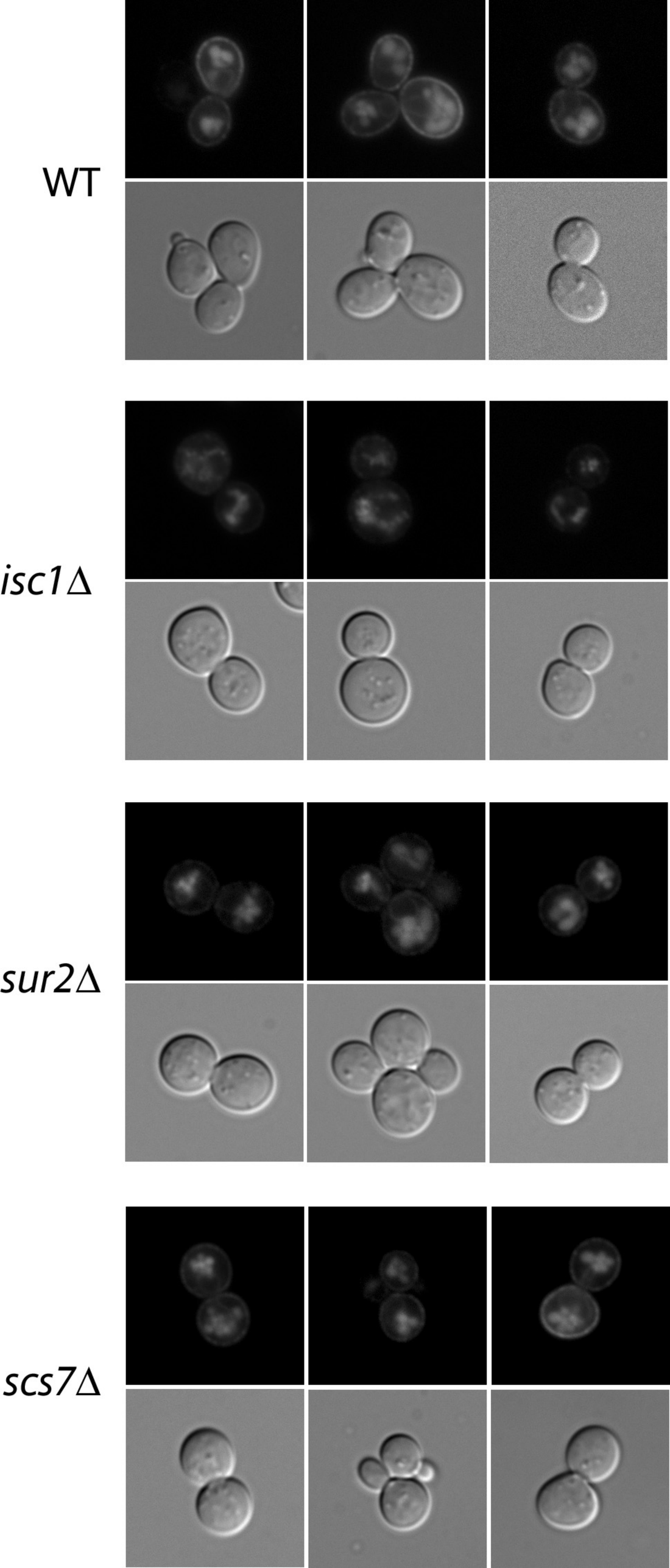
Supp Fig 3, Guan et al.

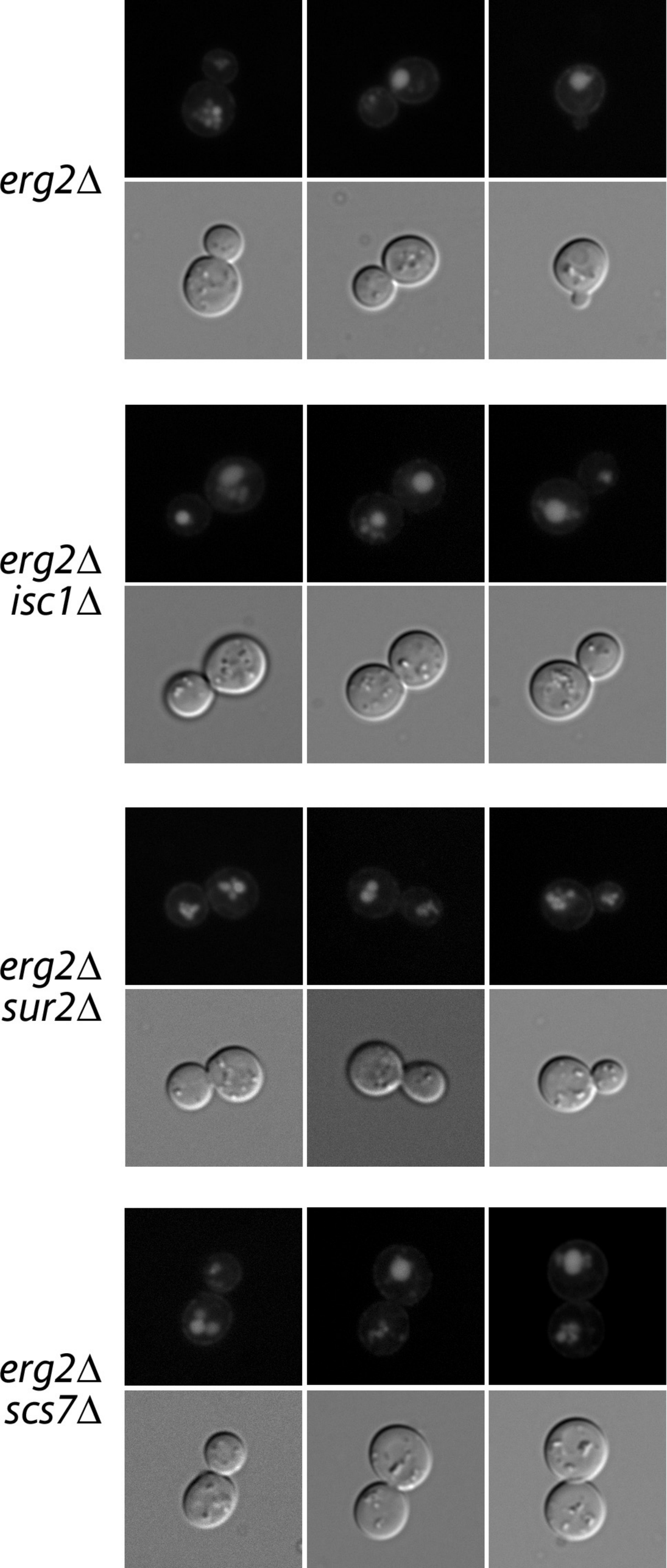


Supp Fig 3, Guan et al.

Supp Fig 4,
Guan et al.




WT
isc1Δ
sur2Δ
scs7Δ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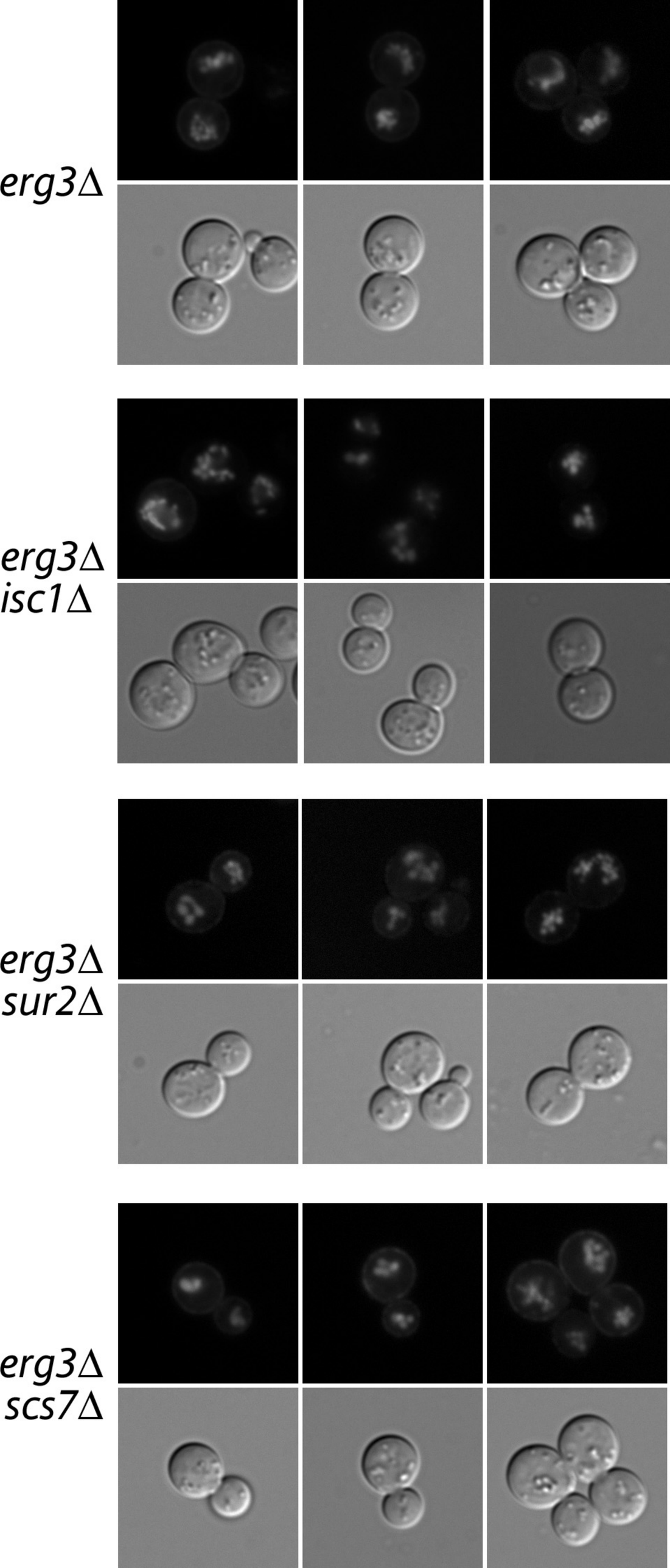
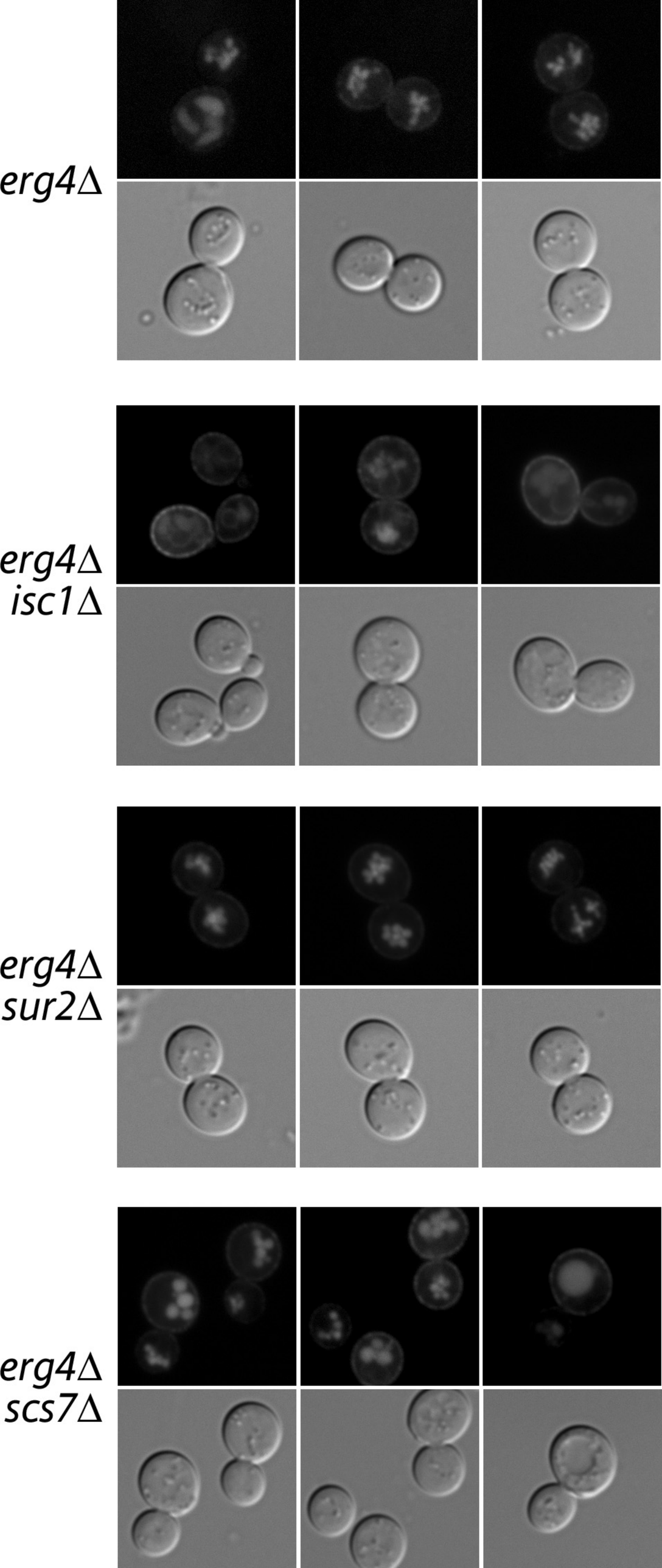
erg2Δ
isc1Δ
erg2Δ
sur2Δ
erg2Δ
scs7Δ


erg3Δ
erg3Δ
isc1Δ
erg3Δ
sur2Δ
erg3Δ
scs7Δ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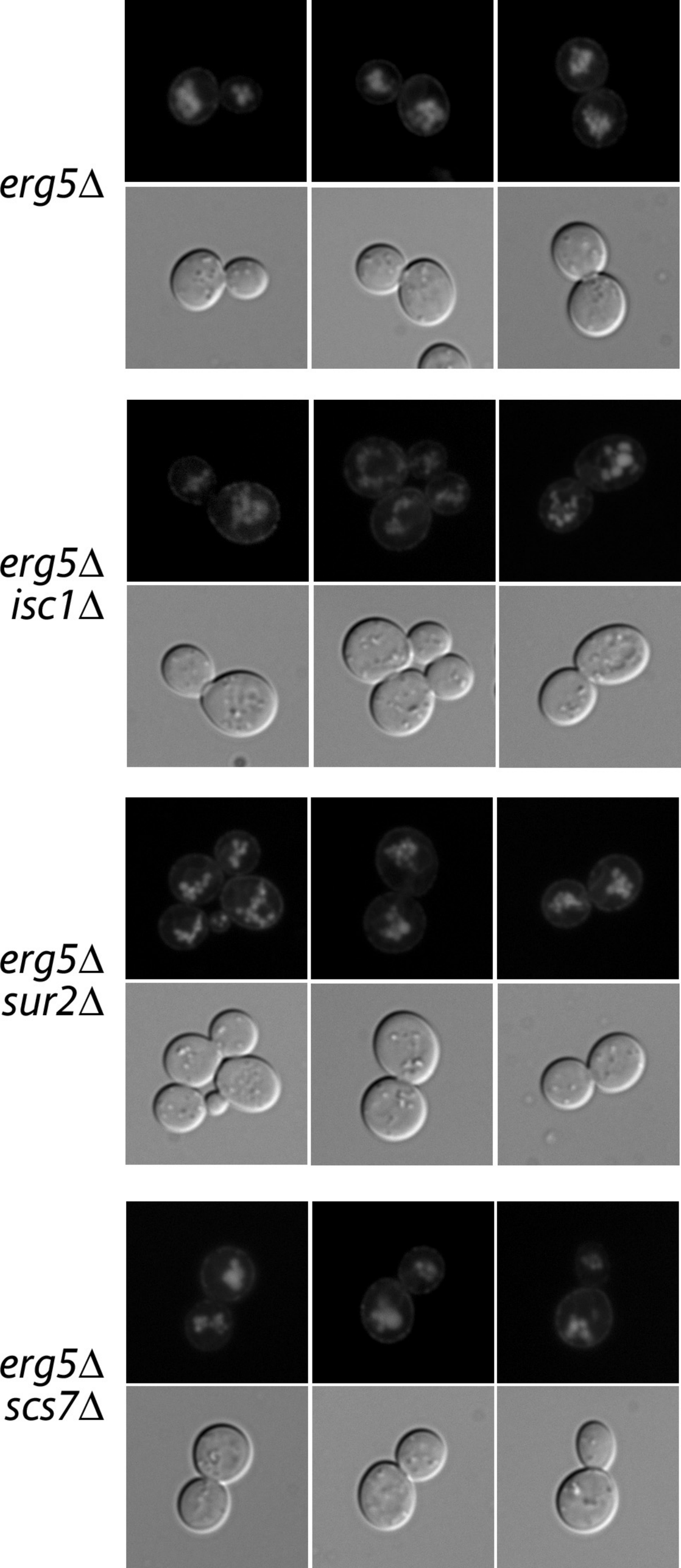
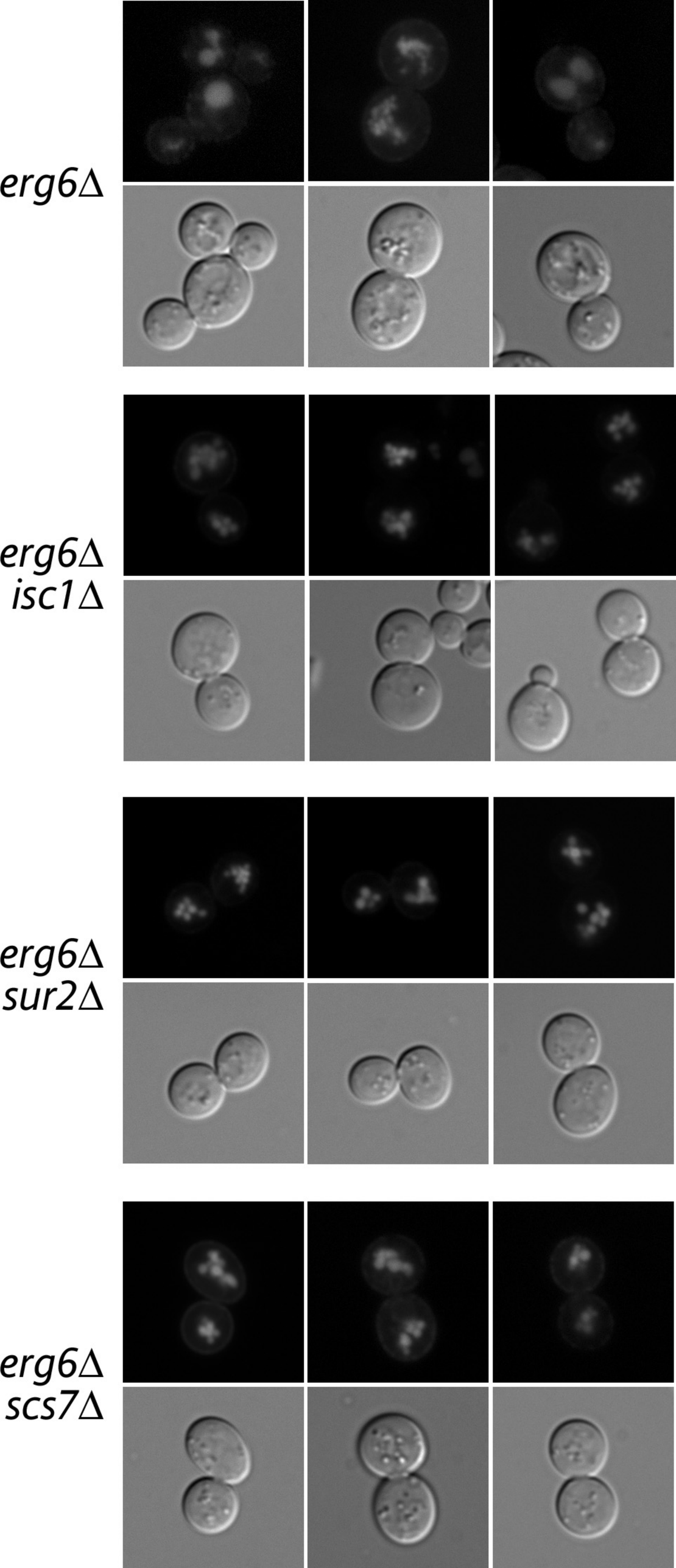
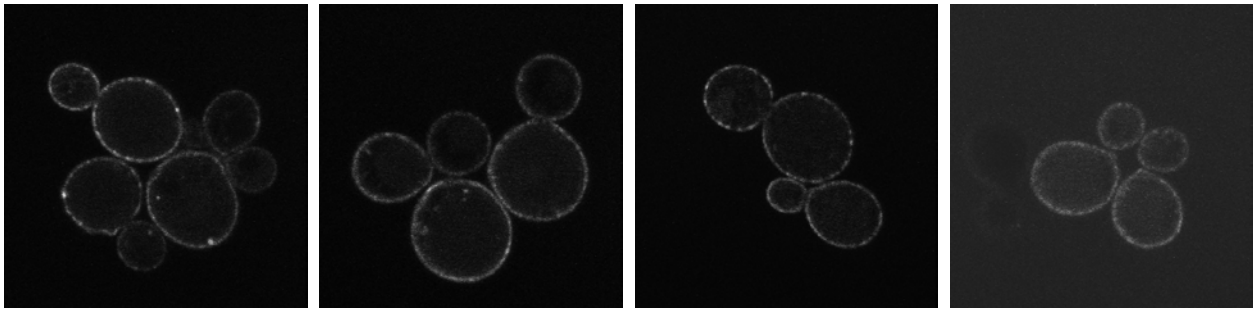
erg4Δ
isc1Δ
erg4Δ
sur2Δ
erg4Δ
scs7Δ

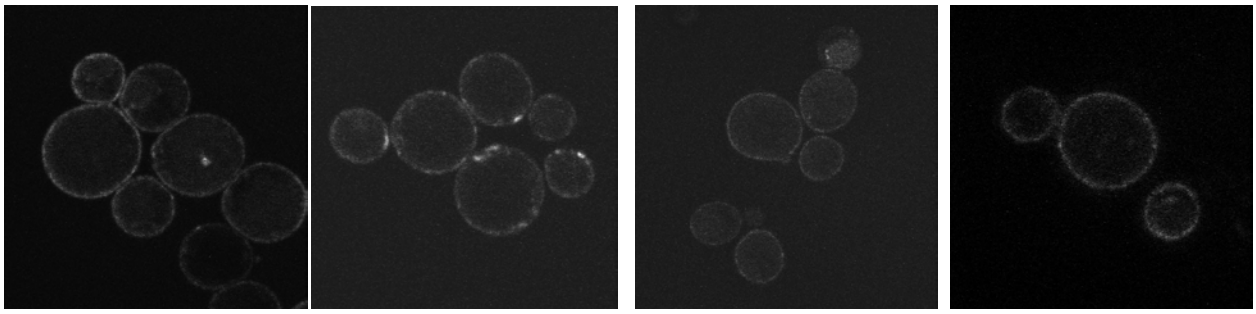

erg5Δ
erg5Δ isc1Δ
erg5Δ sur2Δ
erg5Δ scs7Δ


erg6Δ
erg6Δ isc1Δ
erg6Δ sur2Δ
erg6Δ scs7Δ

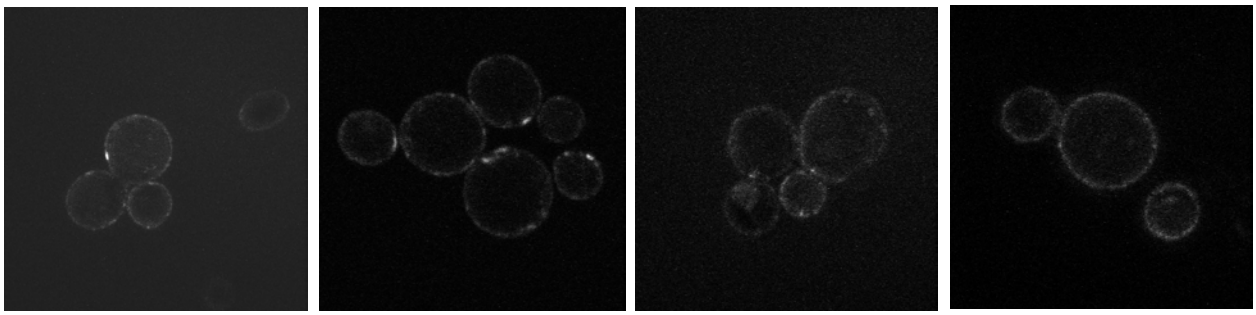
Can1-GFP localization



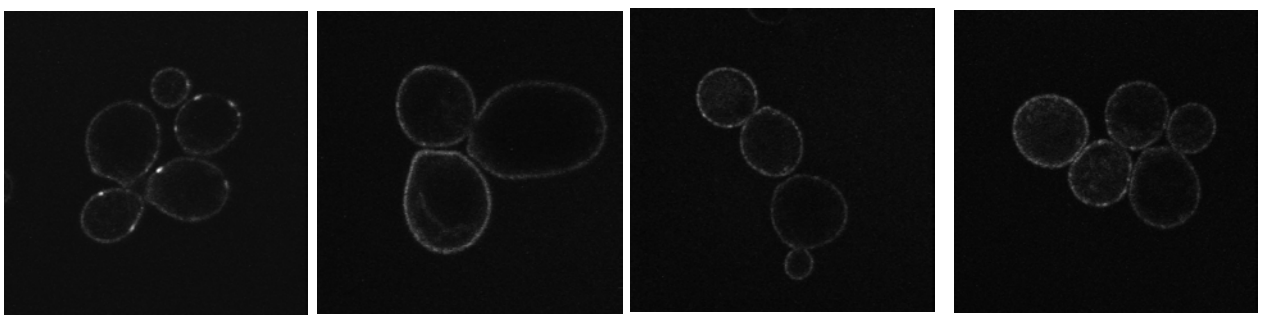
Wild type | *isc1* | *sur2* | *scs7*



erg2 | *erg2 isc1* | *erg2 sur2* | *erg2 scs7*



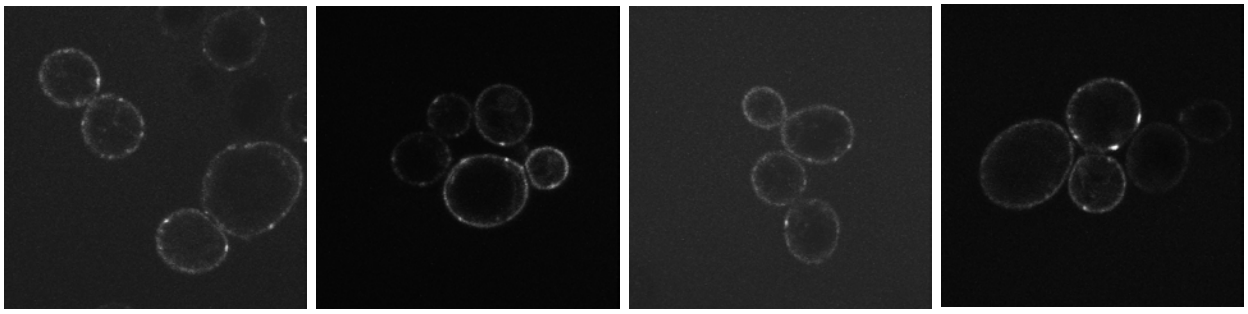
erg3 | *erg3 isc1* | *erg3 sur2* | *erg3 scs7*



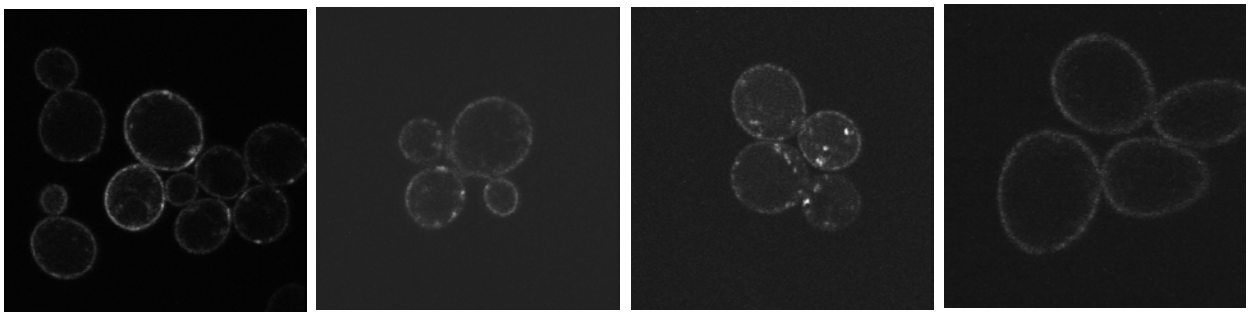
erg4 | *erg4 isc1* | *erg4 sur2* | *erg4 scs7*

Guan *et al* Supp Fig 5B

Can1-GFP localization



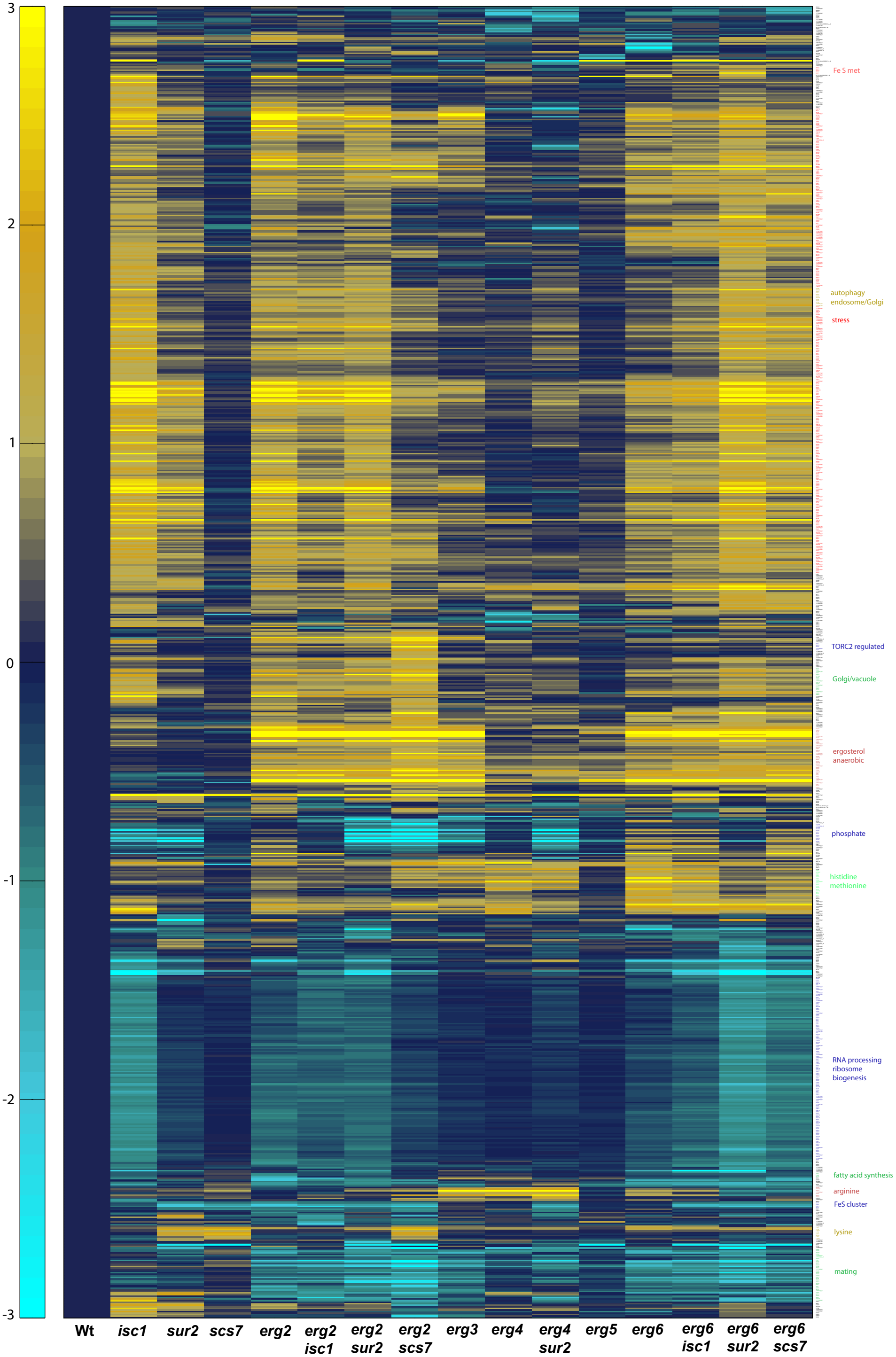
erg5 *erg5 isc1* *erg5 sur2* *erg5 scs7*



erg6 *erg6 isc1* *erg6 sur2* *erg6 scs7*

Guan *et al* Supp Fig 5B

Supp Fig 6, Guan et al.



Supplementary Figure Legends

Supplementary Figure 1. Ergosterol and sphingolipid synthesis and turnover[1]. A) Synthesis of ergosterol from zymosterol. The Erg proteins used at each enzymatic step are shown. Synthesis of ergosterol from zymosterol is not a linear sequence. The only reaction that strictly depends upon a previous reaction is that of Erg4p, which depends upon prior action of Erg6p. Erg2p is an Δ8,Δ7 isomerase, Erg3p a Δ5 desaturase, Erg4p a Δ24(28) desaturase, Erg5p a Δ22 desaturase, Erg6p a Δ24 methyltransferase. Erg2p functions inefficiently in the erg6 mutant[2] (Supp Table II). B) Synthesis and turnover of sphingolipids. Sphinganine (commonly called dihydrosphingosine) is synthesized starting from palmitoyl-CoA and serine. If can be hydroxylated by Sur2p to form 4OH-sphinganine (commonly called phytosphingosine). The di- and tri-hydroceramides are made by condensing a C26 fatty acid onto either sphingoid base. The hydroxyceramides are converted to inositol(di/tri)hydroceramides in the Golgi and can be further hydroxylated on the C26 fatty acyl chain by Scs7p. These sphingolipids can be converted to mannosyl and mannosyldiinositolhydroceramides. An additional hydroxylation can occur on the complex sphingolipids (not shown). The head group of the inositolhydroceramides and mannosylated versions can be recycled by action of Isc1p, the first step in sphingolipid degradation which yields inositol phosphate and (di/tri/tetra)hydroceramides. Ceramides are deacylated by the Ydc1p and Ypc1p. Phosphorylated sphingoid bases are removed from the pathway by the sphingoid base phosphate lyase, Dpl1p. Structures were drawn with ACD/ChemSketch freeware.

Supplementary Figure 2. Sphingolipid turnover and consequences in the *erg3 erg6* mutant. The indicated yeast strains were grown to early log phase and 1 ml of cells (10^8 cells/ml) were labeled with ^{3}H-inositol (25 μCi) for 1 hour, then for an additional 18 hours in presence of

unlabeled inositol (180 μg/0.5 ml). Lipids were extracted and equal amounts of radioactive lipids were analyzed by thin layer chromatography and imaged using a Cyclone phosphorimager[3] (Upper panels). One can see that there is little difference in the labeling patterns of the wild type (wt) and *erg3 erg6* mutant cells after a pulse, indicating that synthesis of the major inositol-containing lipids is normal. However, after a chase period it can be seen that there are less IPCs and MIPC in the double erg mutant, but more PI (upper left panel). The *ISC1* gene product is required to generate this difference because addition of the *isc1* mutation to *erg3 erg6* restores its ^{3}H-inositol chase-labeling pattern to wild type (upper right panel). In the lower panel the indicated strains were grown to stationary phase and serial dilutions were prepared and plated onto YPUADT plates (1% yeast extract, 2% peptone, 2% glucose, 40 mg/l uracil, adenine and tryptophan, 2% agar) and grown for 5 days at 24°C or 37°C, then photographed. The addition of the *isc1* mutation to the *erg3 erg6* strain caused a synthetic growth defect as the triple mutant grew worse than either of the parents.

Supplementary Figure 3. Growth phenotypes of ergosterol and sphingolipid biosynthesis mutants. The indicated strains were grown until stationary phase and diluted to 1.4 OD_{600}/ml with water and serial 10 fold dilutions were prepared in microtiter dishes. The dilutions were pinned onto YPD (1% Yeast extract, 2% Peptone, 2% glucose, 40 mM MES, pH 5.5, 2% agar) or YPEG (1% Yeast Extract, 2% Peptone, 3% ethanol, 3% glycerol, 40 mM MES, pH 5.5, 2% agar) plates and then grown at 30°C except when indicated. Plates were photographed after 2 to 6 days depending upon the growth rate on the different plates. An arrow is placed next to the double mutants (ergosterol and sphingolipid) where introduction of the sphingolipid mutation changed growth of the erg mutant (synthetic growth phenotype or improved growth). The following plate compositions were used. A) YPD (30°C, 37°C, 16°C) and YPEG, B) YPD plus 1M NaCl, 1.7M sorbitol, 200 mM $CaCl_2$, C) 10 mg/l calcofluor

white, 0.01% SDS, 1 mg/l YW3548[4], D) 2 mM benzoic acid, pH 4.5, 1 mM sorbic acid, pH 4.5, YPD adjusted to pH 9, 200 mM sodium acetate, E) 0.1 mg/l alpha factor, 0.02 mg/l rapamycin, 2 g/l caffeine, F) 0.1 mg/l cycloheximide, 100 mM hydroxyurea, 1 mg/l miconazole. G contains a summary of the suppression and synthetic growth phenotypes, indicated by arrows, seen in the double (ergosterol and sphingolipid) mutants. Some combinations have synthetic phenotypes under several growth conditions.

Supplementary Figure 4. Lipidome of the double mutants. Isogenic wild type, ergosterol, sphingolipid, and double mutant strains were grown in duplicate overnight in rich medium, harvested, washed three times and frozen. Lipid standards (5μg dimyristoyl GPCho, 20μg dimyristoyl GPEtn, 4μg dioctyl GPIns and 15μg didocosahexaenoyl GPSer) were added to 50 OD-equivalent of cells and lipids were extracted as described and measured using negative ion electrospray ionization mass spectrometry (ESI-MS)[5]. The quantities of lipids are expressed as ion intensities relative to wild type levels, converted to a log10 scale. Glycerophospholipids: GPCho, glycerophosphocholine; GPEtn, glycerolphosphoethanolamine; GPIns, glycerophosphoinositol; GPSer, glycerophosphoserine; Sphingolipids: IPC, inositolphosphoceramide; MIPC, mannosyl inositolphosphoceramide. The suffixes -B, -C, and -D on IPC and MIPC denote hydroxylation states, having two, three, or four hydroxyl groups respectively.

Supplementary Figure 5. Fluorescence microscopy of Tat2-mRFP and Can1-GFP. Fluorescent proteins were visualized on log phase cells as described in Experimental Procedures. Representative images are shown for each protein in the wild type and 15 double mutants.

Supplementary Figure 6. Transcriptome analysis of single and double mutants. The transcript data was obtained and treated as described in Experimental Procedures. Gene names or identifiers are shown on the right. The scale is log2.

1. Souza CM, Pichler H (2006) Lipid requirements for endocytosis in yeast. Biochim Biophys Acta.
2. Heese-Peck A, Pichler H, Zanolari B, Watanabe R, Daum G, et al. (2002) Multiple functions of sterols in yeast endocytosis. Mol Biol Cell 13: 2664-2680.
3. Funato K, Riezman H (2001) Vesicular and nonvesicular transport of ceramide from ER to the Golgi apparatus in yeast. J Cell Biol 155: 949-959.
4. Sutterlin C, Horvath A, Gerold P, Schwarz RT, Wang Y, et al. (1997) Identification of a species-specific inhibitor of glycosylphosphatidylinositol synthesis. Embo J 16: 6374-6383.
5. Guan XL, Wenk MR (2006) Mass spectrometry-based profiling of phospholipids and sphingolipids in extracts from Saccharomyces cerevisiae. Yeast 23: 465-477.

Supplementary Table I. Strains used in this study.

Name	Genotype
RH448	MATa *his4 ura3 lys2 leu2 can1 bar1*
RH5812	MATa *erg2Δ::LEU2 his4 ura3 lys2 leu2 can1 bar1*
RH4213	MATa *erg3Δ::LEU2 his4 ura3 lys2 leu2 can1 bar1*
RH4217	MATa *erg4Δ::URA3 his4 ura3 lys2 leu2 can1 bar1*
RH6969	MATa *erg4Δ::ura3 his4 ura3 lys2 leu2 can1 bar1*
RH6774	MATa *erg5Δ::KanMx his4 ura3 lys2 leu2 can1 bar1*
RH5684	MATa *erg6Δ::KanMx his4 ura3 lys2 leu2 can1 bar1*
RH5912	MATa *isc1Δ::KanMx his4 ura3 lys2 leu2 can1 bar1*
RH4348	MATa *sur2Δ::LEU2 his4 ura3 leu2 can1 bar1*
RH4524	MATa *scs7Δ::LEU2 his4 ura3 leu2 can1 bar1*
RH5913	MATa *erg2Δ::LEU2 isc1Δ ::KanMx his4 ura3 lys2 leu2 can1 bar1*
RH5935	MATa *erg3Δ::LEU2 isc1Δ ::KanMx his4 ura3 lys2 leu2 can1 bar1*
RH5916	MATa *erg4Δ::LEU2 isc1Δ ::KanMx his4 ura3 lys2 leu2 can1 bar1*
RH5917	MATa *erg5Δ::LEU2 isc1Δ ::KanMx his4 ura3 lys2 leu2 can1 bar1*
RH6787	MATa *erg6Δ::LEU2 isc1Δ ::KanMx his4 ura3 lys2 leu2 can1 bar1*
RH5818	MATa *erg3Δ::LEU2 erg6Δ::LEU2 isc1Δ::KanMx his4 ura3 lys2 leu2 can1 bar1*
RH6711	MATa *erg2Δ::LEU2 sur2Δ::LEU2 his4 ura3 lys2 leu2 can1 bar1*
RH6749	MATa *erg3Δ::LEU2 sur2Δ::LEU2 his4 ura3 lys2 leu2 can1 bar1*
RH6718	MATa *erg4Δ::URA3 sur2Δ::LEU2 his4 ura3 lys2 leu2 can1 bar1*
RH6915	MATa *erg4Δ::ura3 sur2Δ::LEU2 his4 ura3 lys2 leu2 can1 bar1*
RH6732	MATa *erg5Δ ::KanMx sur2Δ::LEU2 his4 ura3 lys2 leu2 can1 bar1*
RH6744	MATa *erg6Δ::KanMx sur2Δ::LEU2 his4 ura3 lys2 leu2 can1 bar1*
RH6709	MATa *erg2Δ ::LEU2 scs7Δ ::LEU2 his4 ura3 leu2 can1 bar1*
RH6741	MATa *erg3Δ::LEU2 scs7Δ::LEU2 his4 ura3 lys2 leu2 can1 bar1*
RH6714	MATa *erg4Δ::URA3 scs7Δ::LEU2 his4 ura3 lys2 leu2 can1 bar1*
RH6916	MATa *erg4Δ::ura3 scs7Δ::LEU2 his4 ura3 lys2 leu2 can1 bar1*
RH6734	MATa *erg5Δ::KanMx scs7Δ::LEU2 his4 ura3 lys2 leu2 can1 bar1*
RH6752	MATa *erg6Δ::KanMx scs7Δ::LEU2 his4 ura3 lys2 leu2 can1 bar1*
RH5928	MATa *erg2Δ::LEU2 erg3Δ::LEU2 his4 ura3 lys2 leu2 can1 bar1*
RH5864	MATa *erg2Δ::LEU2 erg4Δ::LEU2 his4 ura3 lys2 leu2 can1 bar1*
RH5866	MATa *erg2Δ::LEU2 erg5Δ::LEU2 his4 ura3 lys2 leu2 can1 bar1*
RH3616	MATa *erg2Δ::URA3 erg6Δ ura3 leu2 can1 bar1*
RH5868	MATa *erg3Δ::LEU2 erg4Δ::LEU2 his4 ura3 lys2 leu2 can1 bar1*
RH5871	MATa *erg3Δ::LEU2 erg5Δ::LEU2 his4 ura3 lys2 leu2 can1 bar1*
RH5930	MATa *erg3Δ::LEU2 erg6Δ::LEU2 his4 ura3 lys2 leu2 can1 bar1*
RH5873	MATa *erg4Δ::LEU2 erg5Δ::LEU2 his4 ura3 lys2 leu2 can1 bar1*
RH5874	MATa *erg5Δ::LEU2 erg6Δ::LEU2 his4 ura3 lys2 leu2 can1 bar1*
RH6971	Matα *PDR12::CFP::HygB ura3 leu2 his4 lys2 can1 bar1*
RH6926	Matα *sur2Δ::LEU2 PDR12::CFP::HygB ura3 leu2 his4 lys2 can1 bar1*
RH6919	Matα *erg4Δ::URA3 PDR12::CFP::HygB ura3 leu2 his4 lys2 can1 bar1*
RH6930	Matα *erg4Δ::URA3 isc1Δ::KanMx PDR12::CFP::HygB ura3 leu2 his4 lys2 can1 bar1*

RH6925	Matα *erg4Δ::URA3 sur2Δ::LEU2 PDR12::CFP::HygB ura3 leu2 his4 lys2 can1 bar1*
RH6922	Matα *erg4Δ::URA3 scs7Δ::LEU2 PDR12::CFP::HygB ura3 leu2 his4 lys2 can1 bar1*

Yeast strains were constructed using standard gene replacement and tagging methods and double mutants were generated by standard genetic techniques of crossing and tetrad dissection. All strains were generated in the Riezman laboratory.

ura3 derivatives of *URA3* strains were selected on plates containing 5-fluoroorotic acid(Rothstein, 1991).

Reference

Rothstein, R. (1991). Targeting, disruption, replacement, and allele rescue: integrative DNA transformation in yeast. Methods Enzymol *194*, 281-301.

Supplementary Table II. Sterol compositions in the yeast strains used in this study (single determinations)

A. Wild type and sphingolipid mutant cells.

strain		wt	*isc1*	*sur2*	*scs7*
µg sterols / 10^8 cells		26	33	30	31
sterol	mass				
Cholesta-5,8,24(25)-trienol	382	1.0 %	0.6 %	0.9 %	0.6 %
Cholesta-8,24(25)-dienol	384	9.6 %	7.9 %	8.6 %	9.3 %
Ergosta-5,8,14,22-tetraenol *	394	4.8 %	3.7 %	4.9 %	4.6 %
Ergosta-5,7,22,24(28)-tetraenol	394	2.8 %	2.9 %	3.3 %	3.0 %
Ergosta-5,7,22-trienol	396	58.5 %	59.1 %	61.4 %	64.7 %
Ergosta-5,8,14-trienol *	396	1.4 %	1.7 %	1.2 %	1.1 %
Ergosta-7,22,24(28)-trienol *	396	2.0 %	1.0 %	1.2 %	0.9 %
Ergosta-8,24(28)-dienol	398	1.0 %	1.5 %	2.6 %	2.1 %
Ergosta-5,7-dienol	398	16.5 %	17.2 %	12.2 %	11.7 %
Ergosta-7,24(28)-dienol	398	1.4 %	1.5 %	1.5 %	1.0 %
4,4,14-Trimethyl cholesta-8,24(25)-dienol	426	1.0 %	0.9 %	1.2 %	0.4 %

B. *erg2* mutant and *erg2*-derived strains.

strain		***erg2***	***isc1 erg2***	***sur2 erg2***	***scs7 erg2***
µg sterols / 10^8 cells		**62**	**52**	**51**	**59**
sterol	**mass**				
Cholesta-5,8,14,24(25)-tetraenol *	**380**	**9.3 %**	**8.5 %**	**8.9 %**	**6.2 %**
Cholesta-8,24(25)-dienol	**384**	**1.5 %**	**1.3 %**	**1.2 %**	**2.4 %**
Ergosta-5,8,14,22-tetraenol *	**394**	**2.9 %**	**2.5 %**	**2.3 %**	**1.6 %**
Ergosta-5,8,22-trienol	**396**	**23.1 %**	**22.6 %**	**27.1 %**	**20.7 %**
Ergosta-5,8,24(28)-trienol *	**396**	**2.7 %**	**1.8 %**	**1.5 %**	**1.9 %**
??	**396**	**2.0 %**	**1.2 %**	**1.1 %**	**1.5 %**
Ergosta-8,22-dienol	**398**	**1.7 %**	**2.0 %**	**1.6 %**	**1.2 %**
Ergosta-5,8-dienol	**398**	**3.8 %**	**5.4 %**	**5.0 %**	**3.0 %**
Ergosta-8,24(28)-dienol	**398**	**24.5 %**	**20.0 %**	**19.4 %**	**32.9 %**
??	**398**	**3.6 %**	**3.9 %**	**3.6 %**	**3.2 %**
Ergosta-8-enol	**400**	**23.8 %**	**29.9 %**	**27.6 %**	**24.5 %**
4,4,14-Trimethyl cholesta-8,24(25)-dienol	**426**	**0.4 %**	**0.3 %**	**0.2 %**	**0.5 %**

B. *erg3* mutant and *erg3*-derived strains.

strain		***erg3***	***isc1 erg3***	***sur2 erg3***	***scs7 erg3***
µg sterols / 10^8 cells		**63**	**52**	**51**	**62**
sterols	**mass**				
Cholesta-7,22,24(25)-trienol	**382**	**0.2 %**	**0.2 %**	**0.2 %**	**0.3 %**
Cholesta-8,24(25)-dienol	**384**	**2.5 %**	**2.4 %**	**2.8 %**	**4.2 %**
Ergosta-8,22,24(28)-trienol	**396**	**0.5 %**	**0.7 %**	**0.5 %**	**0.4 %**
Ergosta-8,14,24(28)-trienol *	**396**	**1.0 %**	**1.0 %**	**0.8 %**	**0.7 %**
Ergosta-7,22,24(28)-trienol	**396**	**1.2 %**	**1.3 %**	**1.0 %**	**0.9 %**
Ergosta-8,22-dienol	**398**	**1.6 %**	**1.7 %**	**1.8 %**	**1.6 %**
Ergosta-7,22-dienol	**398**	**44.3 %**	**41.1 %**	**41.2 %**	**40.4 %**
Ergosta-8,24(28)-dienol	**398**	**6.4 %**	**6.5 %**	**7.5 %**	**7.5 %**
Ergosta-7,24(28)-dienol	**398**	**15.5 %**	**15.0 %**	**15.5 %**	**18.7 %**
Ergosta-8-enol	**400**	**4.2 %**	**4.5 %**	**4.7 %**	**4.3 %**
Ergosta-7-enol	**400**	**20.9 %**	**23.1 %**	**22.8 %**	**19.0 %**
4,4,14-Trimethyl cholesta-8,24(25)-dienol	**426**	**0.1 %**	**0.3 %**	**0.2 %**	**0.2 %**

C. *erg4* mutant and *erg4*-derived strains**.

strain		*erg4*	*isc1 erg4*	*sur2 erg4*
µg sterols / 10^8 cells		43	57	43
sterols	mass			
Cholesta-8,24(25)-dienol	384	3.7 %	2.3 %	3.4%
Ergosta-5,8,14,22,24(28)-pentaenol *	392	3.3 %	2.8 %	2.7%
Ergosta-5,7,14,22,24(28)-pentaenol *	392	1.9 %	3.1 %	2.9 %
??	392	~ 6 %	~ 7 %	~6 %
Ergosta-5,8,22,24(28)-tetraenol	394	1.3 %	1.0 %	1.1 %
Ergosta-5,7,22,24(28)-tetraenol	394	79.2 %	79.1 %	80.5%
Ergosta-5,8,24(28)-trienol	396	~ 2 %	~ 2 %	~2 %
4-Methyl cholesta-8,24(25)-dienol	398	0.8 %	0.7 %	0.6 %
Ergosta-7,24(28)-dienol	398	0.7 %	0.9 %	0.7 %
4,4,14-Trimethyl cholesta-8,24(25)-dienol	426	0.4 %	0.5 %	0.4 %

strain		*erg4*	*erg4 scs7*
µg sterols / 10^8 cells		39	38
sterols	mass		
Cholesta-8,24(25)-dienol	384	2.1 %	3.0 %
Ergosta-5,8,14,22,24(28)-pentaenol *	392	0.8 %	0.8 %
Ergosta-5,7,22,24(28)-tetraenol	394	86.8 %	85.5 %
Ergosta-5,8,22,24(28)-tetraenol *	394	1.0 %	1.0 %
Ergosta-5,8,24(28)-trienol	396	~6 %	~5 %
4-Methyl cholesta-8,24(25)-dienol	398	1.4 %	1.2 %
4,4-Dimethyl cholesta-8,24(25)-dienol	412	0.6 %	0.8 %
4,4,14-Trimethyl cholesta-8,24(25)-dienol	426	0.9 %	1.7 %

D. *erg5* mutant and *erg5*-derived strains.

strain		***erg5***	***isc1 erg5***	***sur2 erg5***	***scs7 erg5***
µg sterols / 10^8 cells		**54**	**32**	**43**	**35**
sterols	**mass**				
Cholesta-8,24(25)-dienol	**384**	**5.6 %**	**4.2 %**	**5.0%**	**6.1 %**
Ergosta-5,8,14-trienol *	**396**	**4.5 %**	**5.3 %**	**5.6 %**	**5.8 %**
Ergosta-5,7,14-trienol *	**396**	**5.3 %**	**5.6 %**	**5.6 %**	**5.3 %**
Ergosta-5,7,24(28)-trienol	**396**	**2.3 %**	**2.3 %**	**3.2 %**	**2.2 %**
Ergosta-5,8-dienol	**398**	**1.5 %**	**1.6 %**	**1.5 %**	**1.3 %**
Ergosta-5,7-dienol	**398**	**77.2 %**	**78.1 %**	**76.0 %**	**77.0 %**
Ergosta-8,24(28)-dienol	**398**	**0.8 %**	**0.6%**	**1.0 %**	**0.8 %**
Ergosta-8-enol	**400**	**0.1 %**	**-**	**0.2 %**	**-**
4,4,14-Trimethyl cholesta-8,24(25)-dienol	**426**	**1.4%**	**1.3 %**	**1.4 %**	**0.9 %**

E. erg6 mutant and erg6-derived strains**.

strain		*erg6*	*sur2 erg6*	*scs7 erg6*
µg sterols / 10^8 cells		43	29	42
sterols	mass			
Cholesta-5,8,14,24(25)-tetraenol *	380	2.9 %	3.2 %	2.5 %
??	380	6.6 %	3.9 %	5.0 %
Cholesta-8,22,24(25)-trienol *	382	0.7 %	0.7 %	0.5 %
Cholesta-5,8,24(25)-trienol	382	5.8 %	7.8 %	6.3 %
Cholesta-7,22,24(25)-trienol *	382	2.1 %	2.5 %	~ 3 %
Cholesta-5,7,24(25)-trienol	382	34.5 %	27.4 %	30.1 %
Cholesta-8,24(25)-dienol	384	41.1 %	44.7 %	~ 46 %
Cholesta-7,24(25)-dienol	384	4.1 %	4.8 %	3.3 %
4-Methyl cholesta-8,24(25)-dienol	398	0.6 %	0.8 %	0.5 %
4,4-Dimethyl cholesta-8,24(25)-dienol	412	0.9 %	1.0 %	0.8 %
4,4,14-Trimethyl cholesta-8,24(25)-dienol	426	0.5 %	1.1 %	0.4 %

strain		*erg6*	*erg6 isc1*
µg sterols / 10^8 cells		48	39
sterols	mass		
Cholesta-5,8,14,24(25)-tetraenol *	380	0.9 %	0.7 %
??	380	7.8 %	8.3 %
Cholesta-5,8,24(25)-trienol	382	7.1 %	6.9 %
Cholesta-5,7,24(25)-trienol	382	46.6 % %	50.2 %
Cholesta-8,24(25)-dienol	384	25.1 %	23.7 %
Cholesta-7,24(25)-dienol	384	5.8 %	5.9 %
4-Methyl cholesta-8,24(25)-dienol	398	1.5 %	1.1 %

4,4-Dimethyl cholesta-8,24(25)-dienol	**412**	**1.9 %**	**1.1 %**
4,4,14-Trimethyl cholesta-8,24(25)-dienol	**426**	**2.0 %**	**1.2 %**

* denotes sterols whose identity is not certain.

**Sterol determinations for some of the *erg4* and *erg6* strains were determined in two separate experiments. The data from each experiment is presented in a separate table.

Data on some minor sterols (less than 2% of total) whose identity was not certain is not shown.

Isogenic wild type and ergosterol mutant strains were grown overnight in 2% peptone, 1% yeast extract, 2% glucose, 20 mM MES, 40 mg/l each adenine, uracil, tryptophan at 30°C, harvested at 1-2 OD600/ml and washed three times with water. 4 μg of cholesterol was added as an internal standard to 5 x 10^8 cells and total sterols were extracted, derivatized and analyzed as described previously[1]. One can see that there are some differences in sterols between experiments, however these differences sometimes exceed those found between *erg* and *erg*-derived strains in a single experiment. Therefore, we cannot find any significant differences in sterol composition in *erg* strains that are caused by introduction of the sphingolipid mutations. In particular, in the wild type sterol background no substantial differences in sterol amounts or composition were detected (A). With the possible exception of the *sur2 erg6* strain all *erg* mutant strains show an increase in total sterols over wild type cells, although the sterol overproduction varies greatly between *erg* mutants. We have not determined whether the increased sterol amount is due to an increase in free and/or esterified sterols, but find it more likely that the increases are mainly reflected in esterified sterols.

1. Heese-Peck, A. et al. Multiple functions of sterols in yeast endocytosis. *Mol Biol Cell* **13**, 2664-80 (2002).