SUPPORTING INFORMATION

A Novel Small Molecule Methyltransferase is Important for Virulence in *Candida albicans*

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Supplementary Figure Legends:

Supplementary Figure 1

A. Diagram of cloning and subcloning steps of the synthesized *CaCRG1* onto BG1805 vector.
B. Analysis of the levels of wt and mutated CaCrg1 proteins by immunoblotting. Cells were grown overnight in SD-ura, diluted to OD_{600} of 0.2 and grown to mid-exponential stage (OD_{600} of 0.8). The expression was induced in YP and galactose (2%) for 3 hours. The cell lysates were analyzed by anti-HA antibody.

Supplementary Figure 2

Possible effectors of CaCrg1 enzyme activity show little effect on methylation of cantharidin. Addition of 1 mM DTT, 2 mM EGTA, 0.5 mM imidazole, or 0.2 mM sodium chloride to reactions with 0.0225 µg of CaCrg1 enzyme do not significantly alter enzyme activity as measured by acid-labile methylation assays. Error bars represent standard deviation from duplicate samples in one single experiment.

Supplementary Figure 3

CaCRG1 is required for adhesion of cells to plastic surface at log phase at 37ºC. Wt and *cacrg1Δ/Δ* mutants were grown overnight in liquid YPD, diluted to OD_{600} of 0.2 and grown to mid-exponential stage (OD_{600} of 0.8) in SC media. Cells were diluted to 0.5 OD_{600} in SC media and incubated at 30ºC or 37ºC for 2hrs. * p-value <0.01.

Supplementary Figure 4

A. Simplified diagram of endocytic pathway. CaCrg1 interacts with phosphoinositides PI(3)*P* and PI(3,5)*P2* known to be associated with the membranes of early and later endosomes.
B. Fluorescence imaging of co-localization of GFP-tagged ScCrg1 and vacuolar membrane stain FM4-64 in the baker's yeast after overnight treatment with cantharidin (50 µM). .

Supplementary Figure 5

A. C16-ceramide - CaCrg1 overlay assay. Biologically active C16-ceramide was spotted onto nitrocellulose membrane. Affinity purified CaCrg1 (19 pmol; 1 ug/ml) was incubated with C16-ceramide overnight at 4ºC and its binding was assessed with anti-HA antibody. Quantification of relative binding of CaCrg1 to ceramide was performed with ImageJ software.
B. Sphingolipid-protein overlay assay of CaCrg1. 6xHis tagged CaCrg1 was expressed in baker's yeast followed by affinity purification. CaCrg1 (38 pmol; 2ug/ml) was incubated with lipid-spotted membrane overnight at 4ºC. After rigorous washing, the binding of CaCrg1 to sphingolipids (100 pmol per spot) was detected using anti-HA antibody.

Supplementary Figure 6

A. *CaCRG1* interacts with GlcCer-related genes in a condition-dependent manner. Overnight cultures were 10-fold diluted, spotted onto SC defined medium with or without cantharidin and incubated at various temperatures (37 ºC and 43 ºC). The unexpected phenotypes for double deletion mutants (relative to wt and *crg1*Δ/Δ mutant) are highlighted: "+" denotes positive genetic interactions, "-" denotes negative genetic interaction.
B. Morphology of *C. albicans* wt and mutant cells. Cells were streaked onto solid YPD media and incubated at 37 °C for 5 days. Bar, 2mm.

Supplementary Table 1. Differentially expressed genes in *C. albicans* wt cells treated with cantharidin (2 mM, 30 min).

Supplementary Table 2. Bioactive lipids present on the array.

Supplementary Table 3. Yeast strains used in this study.

Supplementary Table 4. Plasmids used in this study.

Supplementary Table 5. Primers used in this study.

Supplementary Table 1. Differentially expressed genes in *C. albicans* wt cells treated with cantharidin (2 mM, 30 min).

ORF	***GENE***	**Mean DMSO**	**Mean drug**	**Log2 (drug/DMSO)**	**T-test, *p*-val (2,2)**
orf19.6475		53.03	9379.51	7.5	0.00010
orf19.1048	*IFD6*	18.93	3283.63	7.4	0.00571
orf6.2005		2.54	316.44	7.0	0.00875
orf19.4173	*ScDPH2*	55.97	6731.72	6.9	0.00434
orf19.2713	*ScMSH5*	8.39	868.15	6.7	0.00355
orf19.5604	*MDR1*	16.94	1338.45	6.3	0.00347
orf19.629	*IFD7*	6.67	425.83	6.0	0.00150
orf19.3848		53.61	1814.50	5.1	0.00922
orf19.4309	*GRP2*	44.31	993.20	4.5	0.00012
orf19.7296		29.42	618.37	4.4	0.00319
orf6.4916		12.31	210.82	4.1	0.00016
orf19.2451	*PGA45*	32.88	533.30	4.0	0.00431
orf19.3735		18.27	228.68	3.6	0.00100
orf19.4477	*CSH1*	33.28	369.28	3.5	0.00157
orf19.2023	*HGT7*	133.77	1039.73	3.0	0.02302
orf19.7085		78.12	525.47	2.7	0.01160
orf19.633	*CaCRG1*	200.37	986.84	2.3	0.00090
orf19.251	*ScHSP31*	119.47	544.13	2.2	0.00274
orf19.5806	*ALD5*	609.95	2671.22	2.1	0.00336
orf19.4631	*ERG251*	327.36	1410.31	2.1	0.00686
orf19.1426		534.51	137.64	-2.0	1.9E-04
orf19.7492	*SWC4*	233.12	59.97	-2.0	2.6E-05
orf19.5599	*MDL2*	243.86	62.69	-2.0	9.1E-04
orf19.1607	*ALR1*	374.28	96.22	-2.0	4.8E-03
orf6.1737		773.62	198.45	-2.0	6.7E-03
orf19.1490	*MSB2*	310.23	79.38	-2.0	3.3E-03
orf19.6114		1517.96	387.56	-2.0	8.5E-03
orf19.1623	*CAP1*	247.04	62.60	-2.0	2.4E-03
orf19.866	*RAD32*	312.07	78.96	-2.0	5.5E-04
orf19.3752	*RAD51*	360.51	91.11	-2.0	1.4E-02
orf19.5676		210.81	53.23	-2.0	1.5E-03
orf19.5148	*CYR1*	284.23	71.69	-2.0	6.6E-03
orf19.6476	*ScAVL9*	245.29	61.80	-2.0	6.8E-04
orf19.789	*PYC2*	241.54	60.77	-2.0	2.5E-02
orf19.5908	*TEC1*	486.13	122.17	-2.0	4.5E-03

orf19.3858		224.49	56.14	-2.0	3.8E-06
orf19.2990	*XOG1*	231.64	57.89	-2.0	7.6E-03
orf6.2673		2955.41	735.23	-2.0	8.7E-05
orf19.4772	*SSU81*	402.77	100.17	-2.0	3.7E-04
orf19.7123		481.84	119.78	-2.0	5.3E-03
orf19.6745	*TPI1*	3343.17	830.10	-2.0	1.5E-03
orf19.3926		266.56	66.17	-2.0	2.4E-03
orf19.1806		214.78	53.30	-2.0	2.6E-03
orf19.1658		549.76	136.27	-2.0	2.2E-03
orf19.1135	*CAS1*	452.47	111.72	-2.0	8.5E-04
orf19.3630	*RRP8*	863.37	212.94	-2.0	6.6E-05
orf19.2051	*ScRPN4*	336.69	83.02	-2.0	5.9E-03
orf19.4281		310.53	76.38	-2.0	2.3E-04
orf19.3644		978.79	240.18	-2.0	8.1E-05
orf19.6121	*MNL1*	214.67	52.14	-2.0	4.5E-03
orf19.6493		559.48	135.80	-2.0	9.7E-04
orf19.4820		224.86	54.10	-2.1	4.9E-04
orf19.6573	*BEM2*	883.57	212.34	-2.1	1.7E-03
orf19.723	*BCR1*	358.56	85.78	-2.1	3.0E-03
orf19.5071	*NRP1*	450.09	106.93	-2.1	4.9E-05
orf19.6202	*RBT4*	394.16	93.41	-2.1	3.8E-03
orf19.4346		933.18	220.99	-2.1	4.8E-03
orf19.1685		253.81	59.98	-2.1	1.3E-02
orf19.7278		1644.19	387.85	-2.1	3.9E-02
orf19.5041		300.70	70.82	-2.1	2.7E-04
orf19.4728	*ScHOS4*	294.68	69.33	-2.1	9.5E-04
orf19.7079		208.27	48.97	-2.1	1.1E-03
orf6.326		498.90	117.17	-2.1	1.5E-04
orf19.5678	*ScDPH2*	296.46	69.58	-2.1	7.9E-04
orf19.1842		352.63	82.70	-2.1	1.2E-04
orf19.4775	*CTA8*	639.66	149.36	-2.1	9.8E-06
orf19.7150	*NRG1*	829.72	193.69	-2.1	3.1E-03
orf19.2460		231.70	53.87	-2.1	1.3E-02
orf19.6186		252.94	58.57	-2.1	3.7E-03
orf19.6713		424.30	98.23	-2.1	6.9E-05
orf19.1281		208.12	48.00	-2.1	1.3E-04
orf19.3252		219.57	50.21	-2.1	1.8E-05
orf19.4377	*KRE1*	702.67	160.37	-2.1	7.7E-04
orf19.182		1709.35	389.72	-2.1	5.8E-03
orf19.2331	*ADA2*	205.35	46.78	-2.1	3.3E-03
orf19.2877	*PDC11*	7456.90	1691.80	-2.1	9.4E-03
orf19.5903	*RAX1*	273.83	61.83	-2.1	2.9E-03
orf6.895		373.42	83.86	-2.2	6.1E-05
orf19.2812		1543.60	346.39	-2.2	7.2E-03
orf19.932		510.83	114.09	-2.2	3.9E-04
orf19.3678		281.27	62.73	-2.2	2.9E-03
orf19.1741		4474.98	996.82	-2.2	2.0E-03
orf19.5469	*HPR5*	447.01	99.42	-2.2	2.8E-02
orf19.3728	*ScGIP4*	246.09	54.60	-2.2	2.5E-03
trna6.2289.1.5prime		201.30	44.44	-2.2	7.9E-05
orf19.5483		224.08	49.33	-2.2	7.5E-05
orf6.579		684.20	150.59	-2.2	3.9E-04
orf19.4225	*LEU3*	263.29	57.84	-2.2	1.8E-04
orf19.2501	*FLC1*	350.16	76.46	-2.2	3.7E-02
orf19.1871	*ScSWR1*	432.63	94.24	-2.2	1.6E-06
orf6.3074		938.23	204.29	-2.2	2.8E-03

orf19.1277		486.81	105.82	-2.2	3.7E-03
orf6.2111		203.57	43.93	-2.2	2.3E-03
orf19.4555	*ALS4;*	133.41	28.70	-2.2	2.4E-03
orf19.3083		337.63	72.50	-2.2	6.7E-03
orf19.1103		324.62	69.61	-2.2	9.4E-04
orf19.1393		216.91	46.48	-2.2	2.3E-03
orf19.2296		503.18	107.47	-2.2	6.8E-03
orf19.7506		210.82	45.02	-2.2	2.9E-03
orf19.469	*HST7*	369.95	78.94	-2.2	9.9E-04
orf19.1259		224.63	47.75	-2.2	6.4E-05
orf19.4099	*ECM17*	358.01	75.87	-2.2	7.1E-06
orf19.6760	*MDS3*	279.29	59.16	-2.2	6.5E-04
orf19.4192	*CDC14*	232.73	48.75	-2.3	2.8E-02
orf19.3967	*PFK1*	1129.72	236.63	-2.3	3.2E-03
orf19.655	*PHO84*	814.54	170.44	-2.3	1.9E-02
orf19.2724		211.55	43.68	-2.3	7.5E-03
orf19.1617		203.45	41.89	-2.3	5.2E-03
orf19.5877		665.62	136.78	-2.3	3.4E-04
orf19.7450		210.61	43.21	-2.3	1.1E-04
orf19.7254		212.34	43.52	-2.3	1.9E-04
orf19.1133	*MSB1*	232.83	47.44	-2.3	4.8E-05
orf19.6414		234.29	47.71	-2.3	1.5E-05
orf19.5372		1994.33	406.04	-2.3	2.8E-02
orf19.4076	*MET10*	373.72	75.64	-2.3	3.9E-05
orf19.3728		344.00	68.76	-2.3	2.3E-04
orf19.4712	*FGR6-3*	243.90	48.06	-2.3	4.5E-03
orf19.3211	*RCF3*	440.08	86.65	-2.3	8.5E-03
orf19.5406		776.40	152.72	-2.3	8.9E-03
orf19.3487		206.57	40.33	-2.4	3.8E-02
orf19.4890	*CLA4*	609.07	118.73	-2.4	2.8E-05
orf19.3789	*RPL24A*	994.33	192.29	-2.4	2.3E-03
orf19.132	*ScSIF2*	282.25	53.96	-2.4	2.6E-04
orf19.6960		229.99	43.77	-2.4	2.6E-02
orf19.2374		1166.05	220.97	-2.4	3.0E-03
orf19.3447		646.09	122.31	-2.4	1.2E-02
orf19.7017	*YOX1*	206.09	38.90	-2.4	4.9E-04
orf19.3207	*CCN1*	248.32	46.72	-2.4	3.1E-04
orf19.3764	*GSG1*	240.36	45.11	-2.4	1.1E-02
orf19.3555	*BUD14*	211.38	39.59	-2.4	3.2E-03
orf19.580		1248.70	233.73	-2.4	1.5E-02
orf19.7272		418.26	78.22	-2.4	9.7E-04
orf19.914		272.21	50.89	-2.4	6.7E-04
orf19.2467	*PRN1*	260.22	48.48	-2.4	5.0E-04
orf19.173		446.49	82.60	-2.4	2.9E-02
orf19.7506		235.88	43.56	-2.4	1.6E-04
orf19.985		474.61	87.59	-2.4	1.6E-04
orf19.6276		436.39	80.40	-2.4	2.7E-03
orf19.1409	*VAC7*	233.22	42.58	-2.5	1.3E-04
orf19.3624	*ScDSS1*	210.32	38.25	-2.5	1.4E-03
orf6.2737		262.92	47.05	-2.5	1.3E-03
orf19.5292	*AXL2*	498.94	88.86	-2.5	9.9E-05
orf19.2641	*ARP1*	441.08	78.14	-2.5	5.2E-04
orf19.2608	*ADH5*	38.02	6.67	-2.5	5.0E-02
orf19.2680	*ScDNF3*	546.73	95.12	-2.5	4.4E-04
orf19.3622	*ANP1*	290.44	50.36	-2.5	6.0E-03
orf19.6686	*ENP2*	1002.10	173.09	-2.5	6.4E-06

orf19.3820		218.27	37.27	-2.6	6.1E-03
orf19.3089		484.42	82.28	-2.6	1.3E-03
orf19.5518		572.37	96.50	-2.6	8.2E-05
orf19.2372		928.93	156.31	-2.6	7.1E-03
orf19.3469		371.38	62.47	-2.6	6.1E-03
orf19.1119	*MTR10*	298.82	50.06	-2.6	4.9E-04
orf19.4365		830.24	139.05	-2.6	8.1E-05
orf6.279		1568.85	261.70	-2.6	2.1E-03
orf19.3144		270.44	44.67	-2.6	6.7E-04
orf19.5531	*CDC37*	406.64	66.93	-2.6	3.8E-03
orf19.4552		550.39	90.48	-2.6	5.0E-04
orf19.4284	*BUR2*	238.06	39.08	-2.6	1.6E-05
orf19.2827		310.53	50.90	-2.6	6.1E-03
orf19.2236		306.91	50.24	-2.6	2.9E-03
orf19.946	*MET14*	394.41	64.24	-2.6	4.7E-03
orf19.1351		205.74	33.43	-2.6	2.6E-04
orf19.7359	*CRZ1*	309.84	49.73	-2.6	3.1E-05
orf19.564	*KAR3*	269.41	43.03	-2.6	2.8E-03
orf19.4276		266.44	42.42	-2.7	3.3E-05
orf6.1533	*CDC39*	1021.42	162.41	-2.7	1.1E-03
orf19.3345	*SIZ1*	289.83	44.90	-2.7	6.2E-04
orf19.4365		663.84	102.14	-2.7	1.1E-03
orf19.1666		253.45	38.96	-2.7	8.5E-04
orf19.5280	*MUP1*	660.51	98.83	-2.7	7.2E-03
25S.6.3p	*N/A*	2066.83	309.08	-2.7	4.6E-02
orf19.68		423.42	63.31	-2.7	2.9E-03
orf19.6514	*CUP9*	323.39	48.20	-2.7	2.5E-03
orf19.4722		309.70	44.97	-2.8	3.4E-03
orf19.5755		291.21	42.26	-2.8	4.4E-04
orf19.6537		231.79	32.73	-2.8	6.0E-03
orf19.4643		263.80	36.29	-2.9	5.6E-03
orf19.1383		201.85	27.60	-2.9	2.5E-03
orf19.2028	*MXR1*	1632.44	222.54	-2.9	2.9E-03
orf19.4952		647.02	87.72	-2.9	2.4E-03
orf19.6978		244.63	33.09	-2.9	3.9E-03
orf6.4835		439.29	58.84	-2.9	3.5E-03
orf19.414		274.37	36.51	-2.9	1.7E-04
orf19.660		278.97	36.90	-2.9	1.8E-04
orf19.3111	*PRA1*	449.24	58.86	-2.9	4.2E-05
orf19.422	*SPT20*	224.95	29.41	-2.9	1.9E-03
orf19.3148		602.75	77.88	-3.0	5.5E-04
orf19.5953		569.52	73.11	-3.0	7.2E-05
orf19.4553		335.99	43.10	-3.0	1.0E-04
orf19.1479		432.98	55.50	-3.0	2.0E-04
orf19.4912		234.89	30.08	-3.0	4.1E-03
orf19.522		1516.38	193.77	-3.0	1.8E-04
orf19.5528	*MOB1*	1124.01	143.39	-3.0	8.1E-04
orf19.6209		1201.19	153.15	-3.0	5.8E-03
orf19.3202		268.62	34.18	-3.0	4.5E-05
orf19.3840		442.03	55.67	-3.0	6.5E-06
orf6.1268		1505.45	188.98	-3.0	1.4E-03
orf19.4666		887.20	108.32	-3.0	1.0E-03
orf19.3142		287.08	34.62	-3.1	2.8E-03
orf19.267		943.48	113.24	-3.1	3.0E-03
orf19.2550		1797.45	214.88	-3.1	4.2E-03
orf19.5056		85.29	9.97	-3.1	2.3E-02

orf6.3626		1478.49	172.21	-3.1	1.2E-03
orf19.4347		280.17	32.17	-3.1	3.0E-04
orf19.1555	*SAC3*	1566.52	179.34	-3.1	3.2E-03
orf19.3603		984.41	111.29	-3.1	6.6E-04
orf19.4741		1243.92	139.75	-3.2	1.8E-03
orf19.3956		2761.71	304.61	-3.2	4.0E-04
orf19.5645	*MET15*	994.34	109.00	-3.2	4.9E-03
orf19.750		1506.08	159.66	-3.2	4.6E-03
orf19.1353		1922.26	203.26	-3.2	1.5E-03
orf19.6309		880.34	93.02	-3.2	4.0E-03
orf19.1960	*CLN3*	303.77	30.57	-3.3	6.1E-03
orf19.4750		309.87	29.88	-3.4	9.0E-05
orf19.156	*FGR51*	259.83	23.70	-3.5	7.7E-04
orf19.176	*OPT4*	1212.63	110.14	-3.5	4.6E-03
orf19.4699		1255.18	113.67	-3.5	1.2E-03
orf19.6981		237.14	21.04	-3.5	5.8E-05
orf19.5842		596.72	52.35	-3.5	2.8E-03
orf19.1960	*CLN3*	914.76	77.71	-3.6	6.4E-03
orf19.3563		245.46	20.69	-3.6	1.1E-03
orf19.4322	*DAP2*	278.76	22.18	-3.7	2.0E-03
orf19.2555	*URA5*	1160.78	90.13	-3.7	1.0E-03
orf19.1868	*RNR22*	123.73	9.60	-3.7	5.2E-03
orf19.177		267.06	20.33	-3.7	2.5E-05
orf19.843		1387.07	102.46	-3.8	1.4E-04
orf19.3695		858.38	60.32	-3.8	4.9E-03
orf6.2920		1559.11	104.55	-3.9	2.6E-03
orf19.5742	*ALS9*	1606.72	105.02	-3.9	2.0E-03
orf6.4638		219.69	11.73	-4.2	1.7E-06
orf19.1995	*ScMNN2*	266.12	13.48	-4.3	6.1E-05
orf6.2374		338.80	17.03	-4.3	7.0E-04
orf19.3869		1808.51	87.68	-4.4	3.0E-05
orf19.334		468.07	10.90	-5.4	1.5E-03

Supplementary Table 2. Bioactive lipids present on the array.

Name	Conc.	MW
5(S)-HETE	0.1mM	320.2
(±)5-HETE	0.1mM	320.2
(±)5-HETE LACTONE	0.1mM	302.2
8(S)-HETE	0.1mM	320.2
9(S)-HETE	0.1mM	320.2
EMPTY		
12(S)-HETE	0.1mM	320.2
12(R)-HETE	0.1mM	320.2
15(S)-HETE	0.1mM	320.2
15(S)-HEDE	0.1mM	324.3
(±)5-HETrE	0.1mM	322.3
TETRANOR-12(R)-HETE	0.1mM	266.2
15(S)-HETrE	0.1mM	322.3
(±)5-HEPE	0.1mM	318.2
15(S)-HEPE	0.1mM	318.2
5(S)-HPETE	0.1mM	336.2
12(S)-HPETE	0.1mM	336.2
15(S)-HPETE	0.1mM	336.2

15(S)-HPEDE	0.1mM	340.3
15(S)-HPEPE	0.1mM	334.2
(±)4-HYDROXYNON-2-ENAL	1mM	156.1
HEPOXILIN A3	0.1mM	336.2
HEPOXILIN B3	0.1mM	336.2
12(S),20-DIHETE	0.1mM	336.2
5(S),15(S)-DIHETE	0.1mM	336.2
8(S),15(S)-DIHETE	0.1mM	336.2
5(S),6(R)-DIHETE	0.1mM	336.2
5(S),12(R)-DIHETE all trans	0.1mM	336.2
8(R),15(S)-DIHETE all trans	0.1mM	336.2
5(S),12(S)-DIHETE all trans	0.1mM	336.2
8(S),15(S)-DIHETE all trans	0.1mM	336.2
5,6-EPOXYEICOSATRIENOIC ACID	0.1mM	320.2
8,9-EPOXYEICOSATRIENOIC ACID	0.1mM	320.2
11,12-EPOXYEICOSATRIENOIC ACID	0.1mM	320.2
14,15-EPOXYEICOSATRIENOIC ACID	0.1mM	320.2
5-KETOEICOSATETRAENOIC ACID	0.1mM	318.2
15-KETOEICOSATETRAENOIC ACID	0.1mM	318.2
13-KETOOCTADECADIENOIC ACID	0.1mM	294.2
LEUKOTRIENE B3	0.1mM	338.2
LEUKOTRIENE B4	0.1mM	336.2
20-HYDROXY-LEUKOTRIENE B4	0.1mM	352.2
20-CARBOXY-LEUKOTRIENE B4	0.1mM	366.2
LEUKOTRIENE C4	0.1mM	625.3
LEUKOTRIENE D4	0.1mM	496.3
LEUKOTRIENE E4	0.1mM	439.2
N-ACETYL-LEUKOTRIENE E4	0.1mM	481.2
LIPOXIN A4	0.1mM	352.2
EPOXY-OLEIC ACID	0.1mM	298.3
PROSTAGLANDIN A1	1mM	336.2
PROSTAGLANDIN A2	1mM	334.2
PROSTAGLANDIN B1	1mM	336.2
PROSTAGLANDIN B2	1mM	334.2
PROSTAGLANDIN D2	1mM	352.2
PROSTAGLANDIN E1	1mM	354.2
PROSTAGLANDIN E2	1mM	352.2
PROSTAGLANDIN F2a	1mM	354.2
PROSTAGLANDIN F1a	1mM	356.3
PROSTAGLANDIN I2 Na	1mM	352.2
15-KETO-PROSTAGLANDIN E2	1mM	350.2
15-KETO-PROSTAGLANDIN F2a	1mM	352.2
13,14-DIHYDRO-15-KETO-PGF2a	1mM	354.2
6-KETO-PROSTAGLANDIN F1a	1mM	370.2
16,16-DIMETHYL-PROSTAGLANDIN E2	1mM	380.3
U-46619	1mM	350.2
9b,11a PROSTAGLANDIN F2	1mM	354.2
9a,11b PROSTAGLANDIN F2	1mM	354.2
PROSTAGLANDIN J2	1mM	334.2
2,3-DINOR-6-KETO-PGF1a	0.1mM	342.2
CARBACYCLIN	1mM	350.2
(±)13-AZAPROSTANOIC ACID	1mM	311.3
19(R)-HYDROXY-PROSTAGLANDIN E2	1mM	368.2
19(R)-HYDROXY-PROSTAGLANDIN F2a	0.1mM	370.2

17-PHENYL-TRINOR-PGE2	1mM	386.2
D12-PROSTAGLANDIN J2	1mM	334.2
13,14-DIHYDRO-PGE1	1mM	356.3
8-EPI-PROSTAGLANDIN F2a	1mM	354.2
15d-PGJ2	1mM	316.2
MISOPROSTOL, FREE ACID	1mM	368.3
THROMBOXANE B2	1mM	370.2
11-DEHYDRO-THROMBOXANE B2	1mM	368.2
ANANDAMIDE (20:4, n-6)	1mM	347.3
PALMITYLETHANOLAMIDE	1mM	299.3
ANANDAMIDE (18:2,n-6)	1mM	323.3
ANANDAMIDE (20:3,n-6)	1mM	349.3
ANANDAMIDE (22:4,n-6)	1mM	375.3
MEAD ETHANOLAMIDE	1mM	349.3
(R)-METHANANDAMIDE	1mM	361.3
BML-190	1mM	426.1
N-Arachidonylglycine	1mM	361.3
EMPTY		
WIN 55,212-2	1mM	426.2
ARACHIDONAMIDE	1mM	303.3
LINOLEAMIDE	1mM	279.3
9,10-OCTADECENOAMIDE	1mM	281.3
ACETYL-FARNESYL-CYSTEINE	1mM	367.2
S-FARNESYL-L-CYSTEINE ME	1mM	339.2
AGGC	1mM	435.3
AGC	1mM	299.2
FARNESYLTHIOACETIC ACID	1mM	296.2
9(S)-HODE	0.1mM	296.2
(±)9-HODE	0.1mM	296.2
13(S)-HODE	0.1mM	296.2
(±)13-HODE	0.1mM	296.2
13(S)-HOTE	0.1mM	294.2
9(S)-HPODE	0.1mM	312.2
13(S)-HPODE	0.1mM	312.2
LEUKOTOXIN A (9,10-EODE)	0.1mM	296.2
LEUKOTOXIN B (12,13-EODE)	0.1mM	296.2
12(S)-HHT	0.1mM	280.2
25-HYDROXYVITAMIN D3	1mM	400.3
1,25-DIHYDROXYVITAMIN D3	1mM	416.3
24,25-DIHYDROXYVITAMIN D3	1mM	416.3
RETINOIC ACID, ALL TRANS	1mM	300.2
9-CIS RETINOIC ACID	1mM	300.2
13-CIS RETINOIC ACID	1mM	300.2
4-HYDROXYPHENYLRETINAMIDE	1mM	391.3
AM-580	1mM	351.2
TTNPB	1mM	348.2
METHOPRENE ACID	1mM	268.2
WY-14643	1mM	323.0
CIGLITAZONE	1mM	333.1
CLOFIBRATE	1mM	242.1
5,8,11-EICOSATRIYNOIC ACID	1mM	300.2
5,8,11,14-EICOSATETRAYNOIC ACID	1mM	296.2
1,2-DIDECANOYL-GLYCEROL (10:0)	1mM	400.3
1,2-DIOCTANOYL-SN-GLYCEROL	1mM	344.3

1,2-DIOLEOYL-GLYCEROL (18:1)	1mM	620.5
1-OLEOYL-2-ACETYL-GLYCEROL	1mM	398.3
1-STEAROYL-2-ARACHIDONOYL-GLYCEROL	1mM	644.5
RICINOLEIC ACID	1mM	298.3
1-HEXADECYL-2-ARACHIDONOYL-GLYCEROL	1mM	602.5
1-HEXADECYL-2-O-METHYL-GLYCEROL	1mM	330.3
1-HEXADECYL-2-O-ACETYL-GLYCEROL	1mM	358.3
2,3-DINOR-THROMBOXANE B2	0.1mM	342.2
14,15-DEHYDRO-LEUKOTRIENE B4	0.1mM	334.2
REV-5901	1mM	335.2
LY-171883	1mM	318.2
U-75302	0.1mM	361.3
SQ-29548	1mM	387.2
FLUPROSTENOL	1mM	458.2
CLOPROSTENOL Na	1mM	424.2
EICOSAPENTAENOIC ACID (20:5 n-3)	1mM	302.2
DOCOSAHEXAENOIC ACID (22:6 n-3)	1mM	328.2
ARACHIDONIC ACID (20:4 n-6)	1mM	304.2
MEAD ACID (20:3 n-9)	1mM	306.3
LINOLENIC ACID (18:3 n-3)	1mM	278.2
GAMMA-LINOLENIC ACID (18:3 n-6)	1mM	278.2
EICOSA-5,8-DIENOIC ACID (20:2 n-12)	1mM	308.3
EICOSADIENOIC ACID (20:2 n-6)	1mM	308.3
7,7-DIMETHYLEICOSADIENOIC ACID	1mM	336.3
EICOSATRIENOIC ACID (20:3 n-3)	1mM	306.3
DIHOMO-GAMMA-LINOLENIC ACID	1mM	306.3
DOCOSATRIENOIC ACID (22:3 n-3)	1mM	334.3
ADRENIC ACID (22:4 n-6)	1mM	332.3
DOCOSAPENTAENOIC ACID	1mM	330.3
LINOLEIC ACID	1mM	280.2
17-OCTADECYNOIC ACID	1mM	280.2
2-HYDROXYMYRISTIC ACID	1mM	244.2
2-FLUOROPALMITIC ACID	1mM	274.2
4-OXATETRADECANOIC ACID	1mM	230.2
12-METHOXYDODECANOIC ACID	1mM	230.2
SPHINGOSINE	1mM	299.3
C2 CERAMIDE	1mM	341.3
C2 DIHYDROCERAMIDE	1mM	343.3
N,N-DIMETHYLSPHINGOSINE	1mM	327.3
C8 CERAMIDE	1mM	425.4
C8 DIHYDROCERAMIDE	1mM	427.4
C16 CERAMIDE	1mM	537.5
DIHYDROSPHINGOSINE	1mM	301.3
SPHINGOMYELIN *	100nmol	730.6
SPHINGOSINE-1-PHOSPHATE *	100nmol	379.2
SPHINGOSYLPHOSPHORYL CHOLINE *	100nmol	464.3
DIHYDROSPHINGOSINE-1-PHOSPHATE *	100nmol	381.3
C8 CERAMINE	1mM	411.4
DL-DIHYDROSPHINGOSINE	1mM	301.3
DL-PDMP	1mM	390.3
DL-PPMP	1mM	474.4
MAPP, D-erythro	1mM	361.3

MAPP, L-erythro	1mM	361.3
PAF C16	1mM	523.4
LYSO-PAF C16	1mM	481.4
PAF C18	1mM	551.4
LYSO-PAF C18 *	100nmol	509.4
PAF C18:1	1mM	549.4
ENANTIO-PAF C16	1mM	523.4
ARACHIDONOYL-PAF	1mM	767.6
2-EPA-PAF	1mM	751.6
2-DHLA-PAF	1mM	769.6
DCHA-PAF	1mM	791.6
1-HEXADECYL-2-METHYLGLYCERO-3 PC	1mM	495.4
1-OCTADECYL-2-METHYLGLYCERO-3 PC	1mM	523.4
C-PAF	1mM	538.4
1-ACYL-PAF	1mM	537.3
LYSOPHOSPHATIDIC ACID	1mM	436.3
L-NASPA	1mM	423.2
PHOSPHATIDIC ACID, DIPALMITOYL	1mM	648.5
AM251	1mM	554.0
2-ARACHIDONOYLGLYCEROL	1mM	378.3
6-FORMYLINDOLO [3,2-B] CARBAZOLE	1mM	284.1
DIINDOLYLMETHANE	1mM	246.1
N-LINOLEOYLGLYCINE	1mM	337.3
PALMITOYL DOPAMINE	1mM	391.3
OLEOYL DOPAMINE	1mM	417.3
ARACHIDONOYL DOPAMINE	1mM	439.3

Supplementary Table 3. Yeast strains used in this study

Name	Genotype	Source
CaLC238	SN87, derivative of SC5314 leu2Δ/leu2Δ his1Δ/his1Δ URA3/ura3::imm434 IRO1/iro1::imm434	Noble
CaEL1 (wild type)	Isogenic to SN87, except his1::CdHIS1/his1Δ leu2::CmLEU2/leu2Δ	This study
CaLC941 (*orf19.633*Δ/Δ)	Isogenic to SN87, except orf19.633::CdHIS1/orf19.633::CmLEU2	Gift from Leah Cowen
*sld1*Δ/Δ	Isogenic to CaEL1, except sld1Δ/sld1::CaNAT	This study
*mts1*Δ/Δ	Isogenic to CaEL1, except mts1Δ/mts1::CaNAT	This study
*orf19.752*Δ/Δ	Isogenic to CaEL1, except orf19.752Δ/orf19.752::CaNAT	This study
*hsx11*Δ/Δ	Isogenic to CaEL1, except hsx11Δ/hsx11::CaNAT	This study
*sld1*Δ/Δ *orf19.633*Δ/Δ	Isogenic to CaLC941, except sld1Δ/sld1::CaNAT	This study
*mts1*Δ/Δ	Isogenic to CaLC941, except	This study

*orf19.633*Δ/Δ	mts1Δ/mts1::CaNAT	
*orf19.752*Δ/Δ	Isogenic to CaLC941, except	This study
*orf19.633*Δ/Δ	orf19.752Δ/orf19.752::CaNAT	
*hsx11*Δ/Δ	Isogenic to CaLC941, except	This study
*orf19.633*Δ/Δ	hsx11Δ/hsx11::CaNAT	
BY4743	MATa/α his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 LYS2/lys2Δ0	Brachmann
(wild-type)	met15Δ0/MET15 ura3Δ0/ura3Δ0	
*crg1*Δ/Δ	Isogenic to BY4743, except for crg1::KanMX	Giaever
	crg1::KanMX	

Brachmann CB, Davies A, Cost GJ, Caputo E, Li J, *et al.* (1998) Designer deletion strains derived from *Saccharomyces cerevisiae* S288C: a useful set of strains and plasmids for PCR-mediated gene disruption and other applications. Yeast 14: 115-132.
Giaever G, Chu AM, Ni L, Connelly C, Riles L, *et al.* (2002) Functional profiling of the *Saccharomyces cerevisiae* genome. Nature 418: 387-391.
Noble SM, Johnson AD (2005) Strains and strategies for large-scale gene deletion studies of the diploid human fungal pathogen Candida albicans. Eukaryot Cell 4: 298-309.

Supplementary Table 4. Plasmids used in this study

Plasmid name	Description	Source
pJK863 (pLC49)	FLP-CaNAT, ampR	Shen
pUC57	With optimized sequence orf19.633, AmpR	This study
BG1805	2 µm, URA3, GAL1prom, triple affinity tag (His6-HAepitope-3Cprotease site-ZZproteinA) at C-terminal	Lissina
orf19.633	As BG1805, with orf19.633	This study
D48A	As BG1805, orf19.633–D48A	This study
E153A/R156G	As BG1805 orf19.633–E153A/R156G	This study
Motif III Δ	As BG1805 orf19.633–Motif III Δ	This study

Supplementary Table 5. Primers used in this study

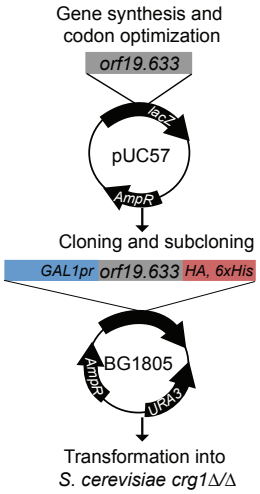
Primer name	Purpose	Oligonucleotide sequence (5'-3')
orf19.633 (mid)_F	qRT-PCR	ATCACCGGTGGAGAAATACG
orf19.633 (mid)_R	qRT-PCR	TGATTCATATCCTGCTTCTAATGC
ACT1 (3')_F	qRT-PCR	AGGTTTGGAAGCTGCTGGTA
ACT1 (3')_R	qRT-PCR	AGCAATACCTGGGAACATGG

D48A_F	Orf19.633 mutagenesis	TTCAAGATTTTAGCTGTTGGGTGCGGTCCTGG
D48A_R	Orf19.633 mutagenesis	GTTTGGTTTTATCAGTGGAATGACGTACTTAGC
E153A/R156G_F	Orf19.633 mutagenesis	GAAGCCTTGAAGGCATTGAAAGGAGTTACGAAACCAG
E153A/R156G_R	Orf19.633 mutagenesis	GATTGGATTCTGTAAATGAATGATCACCTGG
Motif III_F	Orf19.633 mutagenesis	ATCTGTATTAGAGATGCAGATTTGGAATCTAGTATAG
Motif III_R	Orf19.633 mutagenesis	CAGTTCATAGATAGAACCAATTTGAAACGAAATATTAGTC
MTS1_F	Double mutant construction	GTTTTCGTCTTTTGTCGAGTTTAACATTTCAATTGAATATCAATTTTTGTAACAATGCGTATGTTGTGTGGAATTGTGAG
MTS1_R	Double mutant construction	TATTCAGATCAGAATAAATAAAAATCTATACAAATACACCATAAAAGCTCAACCAGTTTAGGCGATTAAGTTGGGTAACG
SLD1_F	Double mutant construction	TTATTTATTTATTGTTATTTTTTTTTTTCACCTTCGTTAACAAACCTTTATGAGATAAACCATGCGTATGTTGTGTGGAATTGTGAG
SLD1_R	Double mutant construction	GTCTCTATATATATATCTATACCCTCATATAAATTTGCATAAAAGTTAAGAAAACAGGATACTGTCTAGGCGATTAAGTTGGGTAACG
HSX11_F	Double mutant construction	TACATTTGATCTTGTTTCTTTTATCAACACTGTGAAATATCGTAATCTTTTGTTCTTCTTTCTAATGCGTATGTTGTGTGGAATTGTGAG
HSX11_R	Double mutant construction	TTACATTTAACTTTTACTTATCTATATATACATGTCCTGTCTATCTATCTATAACAATTGGCGTATCAGGCGATTAAGTTGGGTAACG
orf19.752_F	Double mutant construction	TTCTTTGTATTATTGGTTAGATTTCCATTCCATATACACACAAGATGCGTATGTTGTGTGGAATTGTGAG
orf19.752_R	Double mutant construction	TTGAAGTGAGTTGATGTAGTAAATTATGTATGAATGTATATAAACCCTCAGGCGATTAAGTTGGGTAACG
confirm_Flip_NAT_F	Confirmation	CAACCACAAATGACCAGCAC
confirm_Flip_NAT_R	Confirmation	GTGATTTGGCTGGTTTCGTT
confirm_MTS1_F	Confirmation	CACATTTGCCCATCACTCTG
confirm_MTS1_R	Confirmation	CGAAGTCATGTTCGTGCATC
confirm orf19.752_F	Confirmation	CAAGCCGAGTCGGAAAACTA
confirm orf19.752_R	Confirmation	TGTGTTCGACACATCCTGGT
confirm_HSX11_F	Confirmation	TCGAACTGTCATCCTGTCCA
confirm_HSX11_R	Confirmation	TACAACCAAGCTGCGAAAAA

confirm_SLD1_F	Confirmation	CTTTTGGCAGGATTCTTGGA
confirm_SLD1_R	Confirmation	CCAAAAGAACCAGAGCTTGC
confirm_SLD1_1100bp_F	Confirmation	TGGACGTTGATTGTCCTGAA
confirm_HSX11_430bp_F	Confirmation	AATCGATTGGAGGGGAAGAC
confirm_MTS1_990bp_F	Confirmation	AGAGACGCTTTGGAAGACGA
confirm_orf19.752_995bp_F	Confirmation	CGACCTAATTGTTGCCAAGG
GAL1_orf19.633_F	*19.633* cloning	TTAACGTCAAGGAGAAGGAATTATCAAGTTTGTACAATGAGCGGTGCTAACAACAACCATCAAGTG
Cterm_orf19.633_R	*19.633* cloning	ATGGTGATGATGATGTCTAGACACATCAACCACTTTTGTACACACTTGACGTGTAGTTGTTGGTTTTTGGTA

Supplementary Figure 1

A



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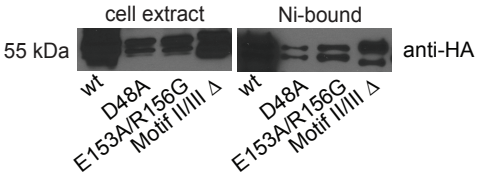
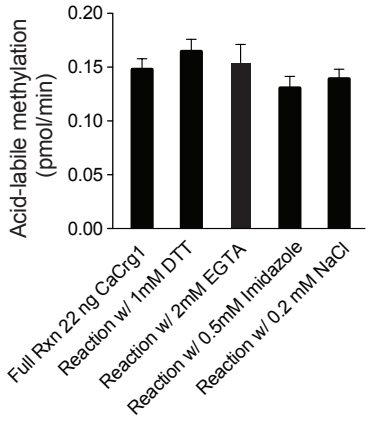
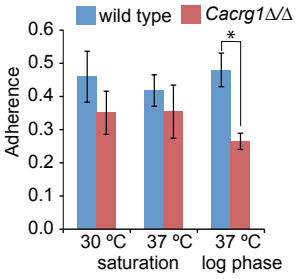


Supplementary Figure 2

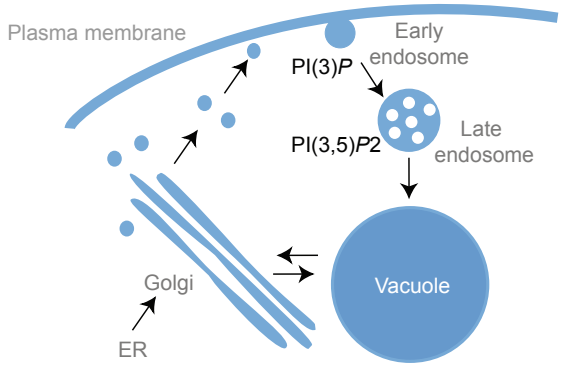


Supplementary Figure 3



Supplementary Figure 4

A



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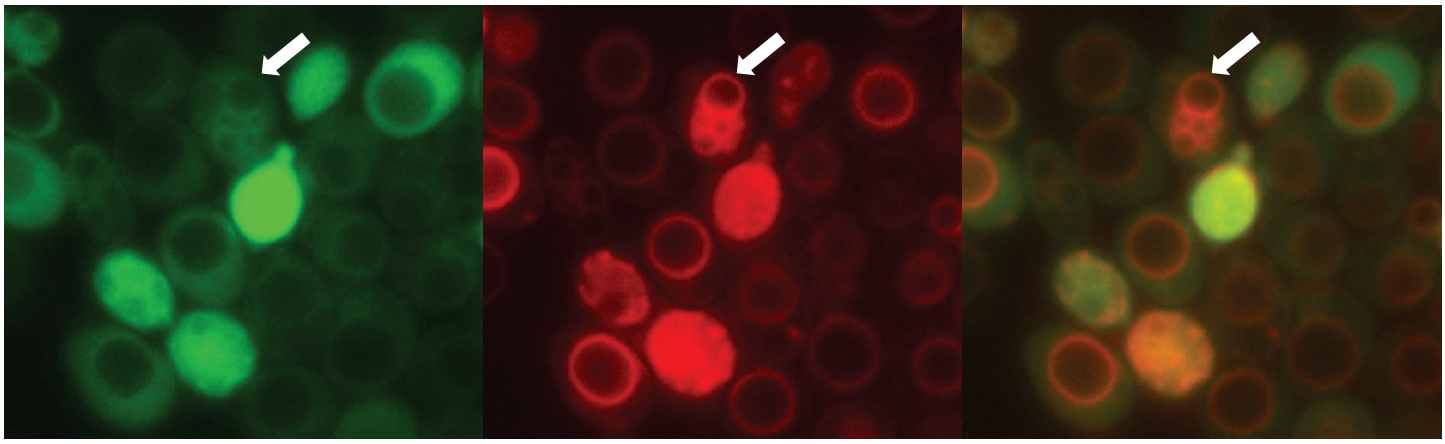

Supplementary Figure 5

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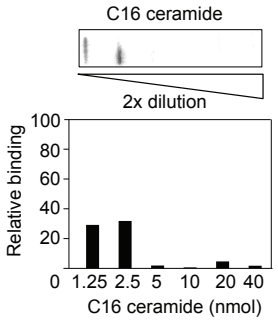

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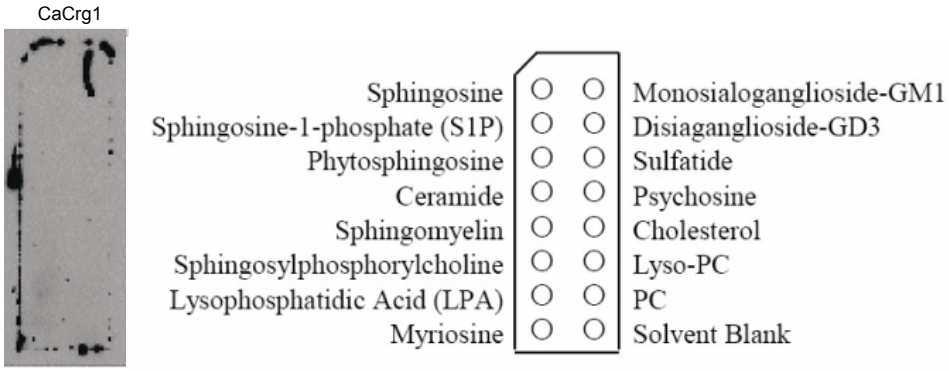

Supplementary Figure 6