Supplemental Materials

Molecular Biology of the Cell

Laborenz *et al*.

Supplemental Figures

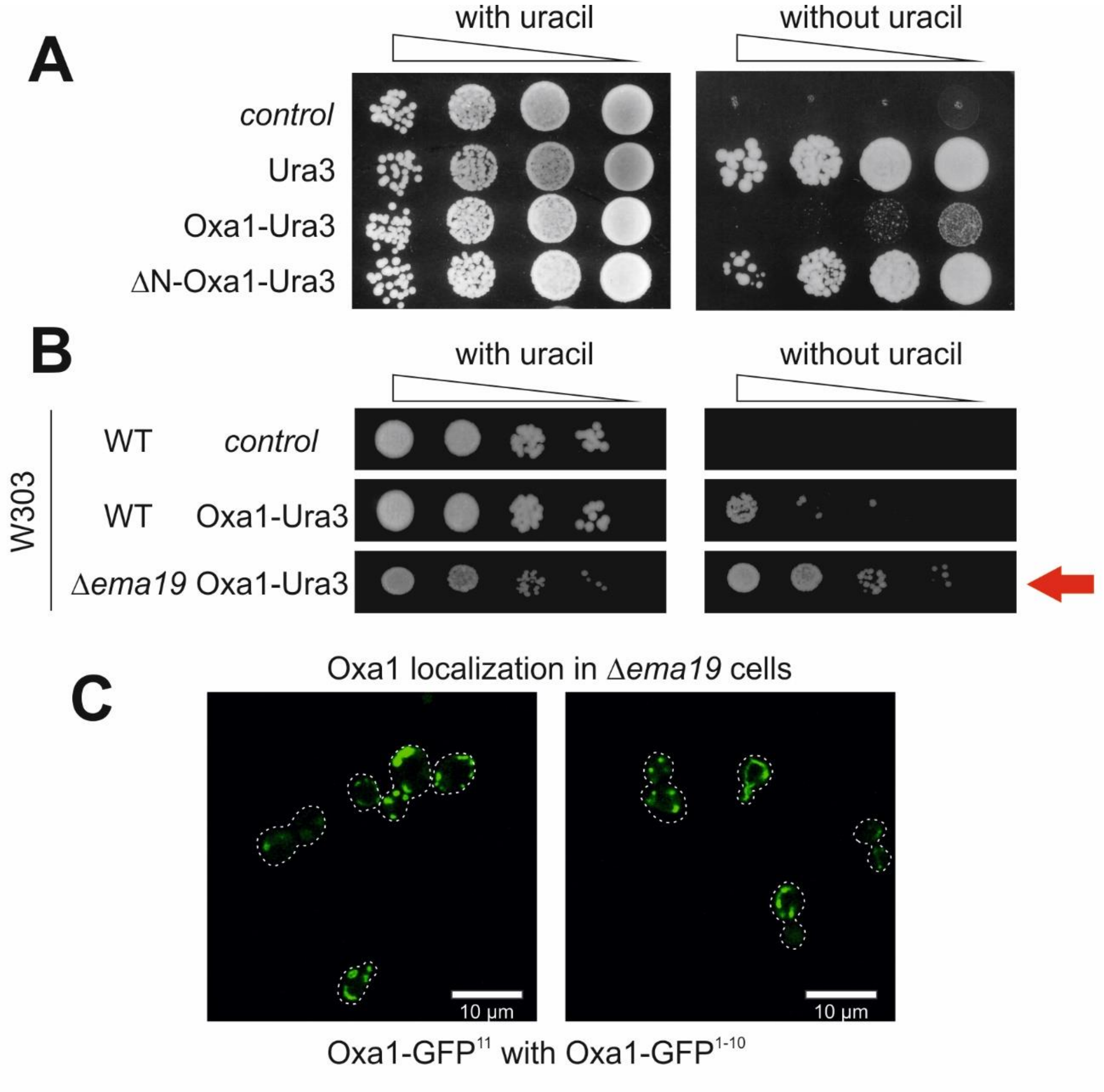


Figure S1. The uracil-independent growth of Oxa1-Ura3-expressing *Δema19* cells is influenced by the strain background. A, B. The indicated strains were generated in the wild type background YPH (A), W303 or BY4742 and analyzed as described for Fig. 1. **C.** Cells expressing Oxa1-GFP^{11} and Sec63-GFP$^{1-10}$ were analyzed by fluorescence microscopy.

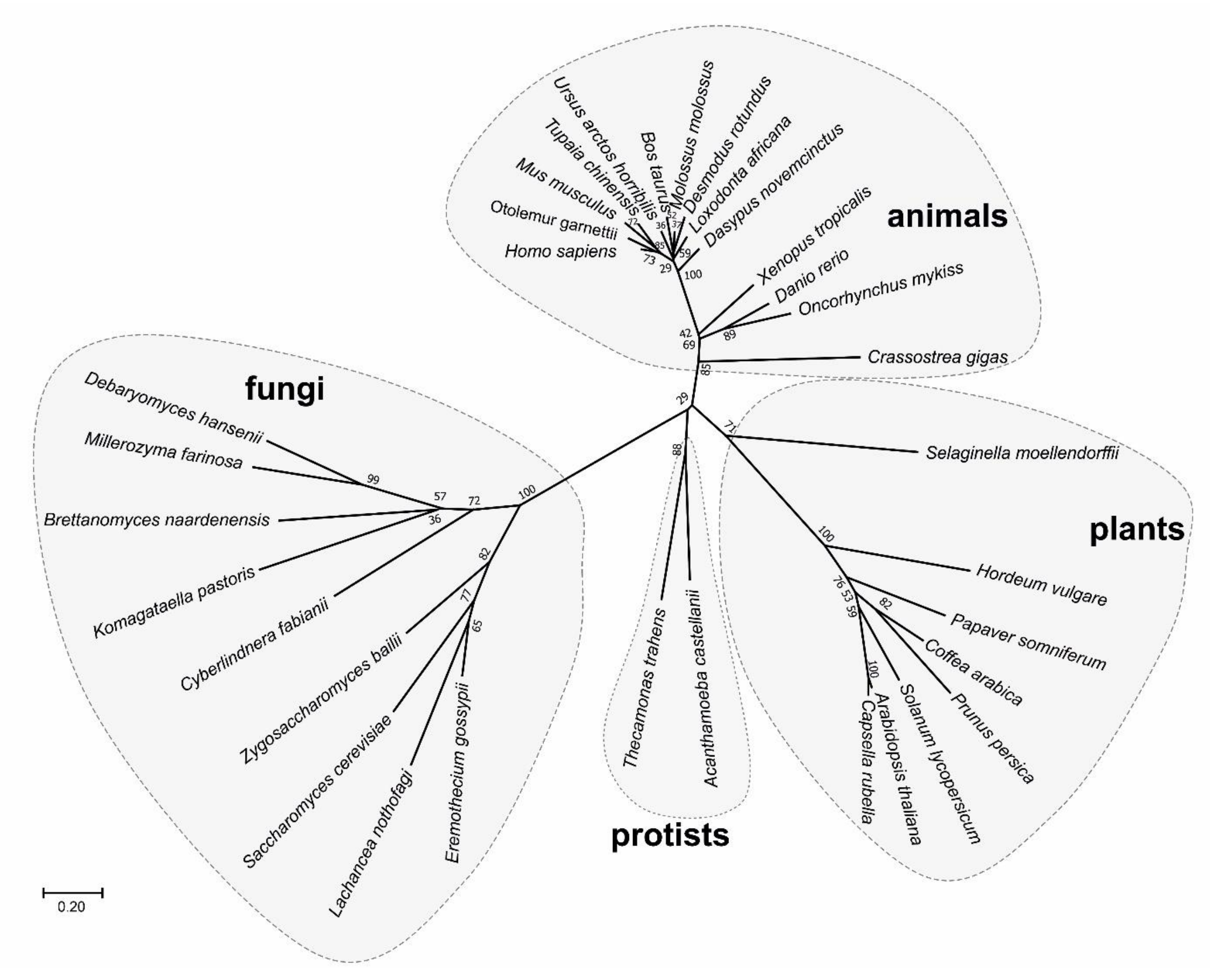
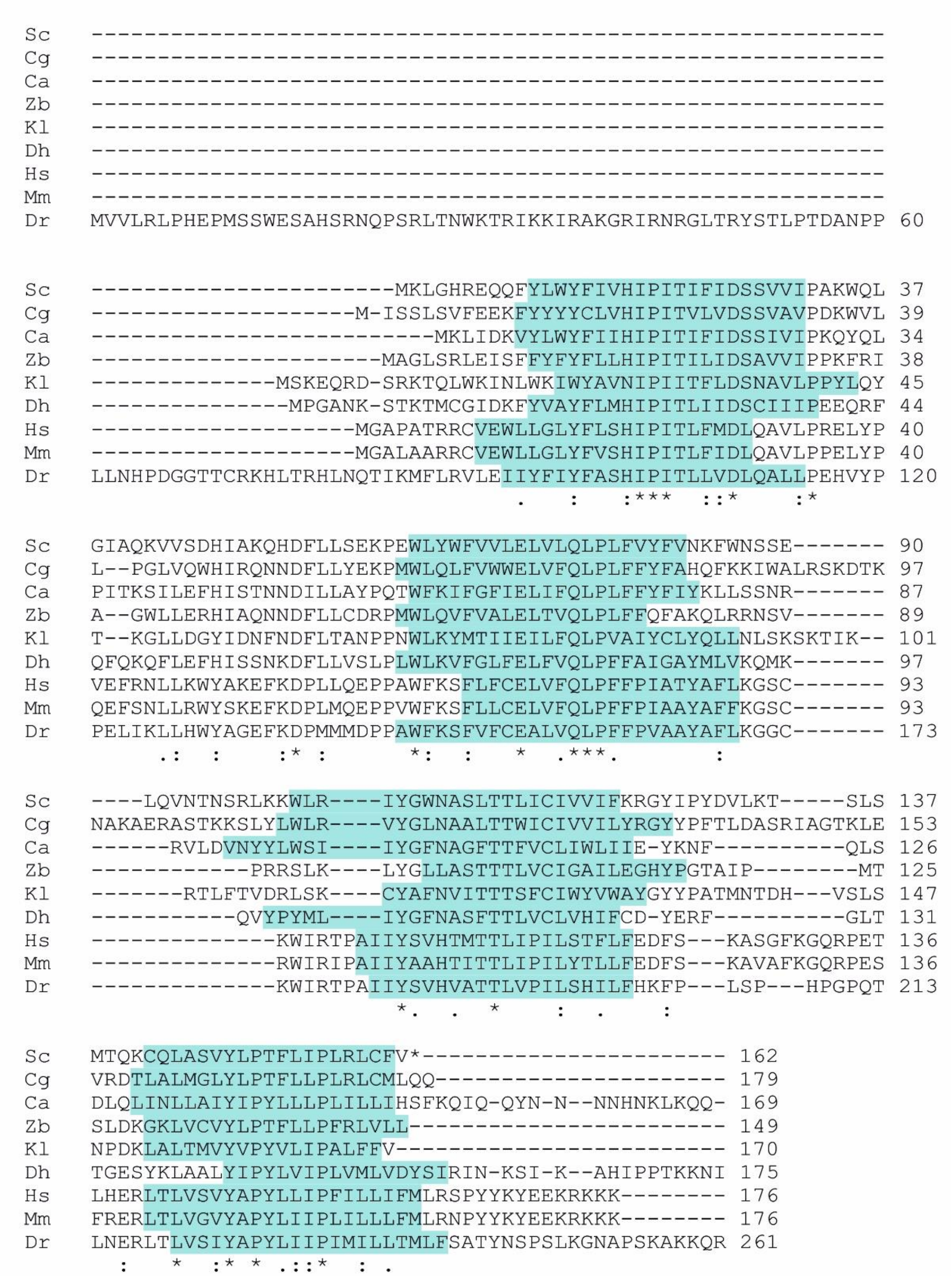


Figure S2. Phylogenetic analysis of the members of Ema19/TMEM97/sigma 2 receptor family. For the phylogenetic tree construction, pairwise sequence alignments were generated using ClustalW with default settings and subjected to the calculation of maximum likelihood distances implementing a JTT matrix (Jones *et al.*, 1992). This matrix was then used to calculate a neighbor-joining tree. Given bootstrap values are based on 1000 random reconstructed trees and indicate the confidence of the respective branches. All analyses were conducted in MegaX (Kumar *et al.*, 2018). Sequences and corresponding accession numbers are listed in the Supplementary Tables file.

Jones, D.T., Taylor, W.R., and Thornton, J.M. (1992). The rapid generation of mutation data matrices from protein sequences. CABIOS *8*, 275-282.

Kumar, S., Stecher, G., Li, M., Knyaz, C., and Tamura, K. (2018). MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. Mol Biol Evol *35*, 1547-1549.



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Sc    ------------------------------------------------------------
Cg    ------------------------------------------------------------
Ca    ------------------------------------------------------------
Zb    ------------------------------------------------------------
Kl    ------------------------------------------------------------
Dh    ------------------------------------------------------------
Hs    ------------------------------------------------------------
Mm    ------------------------------------------------------------
Dr    MVVLRLPHEPMSSWESAHSRNQPSRLTNWKTRIKKIRAKGRIRNRGLTRYSTLPTDANPP 60

Sc    -----------------------MKLGHREQQFYLWYFIVHIPITIFIDSSVVIPAKWQL 37
Cg    --------------------M-ISSLSVFEEKFYYYYCLVHIPITVLVDSSVAVPDKWVL 39
Ca    --------------------------MKLIDKVYLWYFIIHIPITIFIDSSIVIPKQYQL 34
Zb    ----------------------MAGLSRLEISFFYFYFLLHIPITILIDSAVVIPPKFRI 38
Kl    --------------MSKEQRD-SRKTQLWKINLWKIWYAVNIPIITFLDSNAVLPPYLQY 45
Dh    ---------------MPGANK-STKTMCGIDKFYVAYFLMHIPITLIIDSCIIIPEEQRF 44
Hs    --------------------MGAPATRRCVEWLLGLYFLSHIPITLFMDLQAVLPRELYP 40
Mm    --------------------MGALAARRCVEWLLGLYFVSHIPITLFIDLQAVLPPELYP 40
Dr    LLNHPDGGTTCRKHLTRHLNQTIKMFLRVLEIIYFIYFASHIPITLLVDLQALLPEHVYP 120
                                      .   :   :***  ::*    :*

Sc    GIAQKVVSDHIAKQHDFLLSEKPEWLYWFVVLELVLQLPLFVYFVNKFWNSSE------- 90
Cg    L--PGLVQWHIRQNNDFLLYEKPMWLQLFVWWELVFQLPLFFYFAHQFKKIWALRSKDTK 97
Ca    PITKSILEFHISTNNDILLAYPQTWFKIFGFIELIFQLPLFFYFIYKLLSSNR------- 87
Zb    A--GWLLERHIAQNNDFLLCDRPMWLQVFVALELTVQLPLFFQFAKQLRRNSV------- 89
Kl    T--KGLLDGYIDNFNDFLTANPPNWLKYMTIIEILFQLPVAIYCLYQLLNLSKSKTIK-- 101
Dh    QFQKQFLEFHISSNKDFLLVSLPLWLKVFGLFELFVQLPFFAIGAYMLVKQMK------- 97
Hs    VEFRNLLKWYAKEFKDPLLQEPPAWFKSFLFCELVFQLPFFPIATYAFLKGSC------- 93
Mm    QEFSNLLRWYSKEFKDPLMQEPPVWFKSFLLCELVFQLPFFPIAAYAFFKGSC------- 93
Dr    PELIKLLHWYAGEFKDPMMMDPPAWFKSFVFCEALVQLPFFPVAAYAFLKGGC------- 173
           .:  :    :* :      *:  :   *  .***.       :

Sc    ----LQVNTNSRLKKWLR----IYGWNASLTTLICIVVIFKRGYIPYDVLKT-----SLS 137
Cg    NAKAERASTKKSLYLWLR----VYGLNAALTTWICIVVILYRGYYPFTLDASRIAGTKLE 153
Ca    ------RVLDVNYYLWSI----IYGFNAGFTTFVCLIWLIIE-YKNF----------QLS 126
Zb    ------------PRRSLK----LYGLLASTTTLVCIGAILEGHYPGTAIP--------MT 125
Kl    -------RTLFTVDRLSK----CYAFNVITTTSFCIWYVWAYGYYPATMNTDH---VSLS 147
Dh    -----------QVYPYML----IYGFNASFTTLVCLVHIFCD-YERF----------GLT 131
Hs    --------------KWIRTPAIIYSVHTMTTLIPILSTFLFEDFS---KASGFKGQRPET 136
Mm    --------------RWIRIPAIIYAAHTITTLIPILYTLLFEDFS---KAVAFKGQRPES 136
Dr    --------------KWIRTPAIIYSVHVATTLVPILSHILFHKFP---LSP---HPGPQT 213
                             *.  .  *    :  .    :

Sc    MTQKCQLASVYLPTFLIPLRLCFV*----------------------- 162
Cg    VRDTLALMGLYLPTFLLPLRLCMLQQ---------------------- 179
Ca    DLQLINLLAIYIPYLLLPLILLIHSFKQIQ-QYN-N--NNHNKLKQQ- 169
Zb    SLDKGKLVCVYLPTFLLPFRLVLL------------------------ 149
Kl    NPDKLALTMVYVPYVLIPALFFV------------------------- 170
Dh    TGESYKLAALYIPYLVIPLVMLVDYSIRIN-KSI-K--AHIPPTKKNI 175
Hs    LHERLTLVSVYAPYLLIPFILLIFMLRSPYYKYEEKRKKK-------- 176
Mm    FRERLTLVGVYAPYLIIPLILLLFMLRNPYYKYEEKRKKK-------- 176
Dr    LNERLTLVSIYAPYLIIPIMILLTMLFSATYNSPSLKGNAPSKAKKQR 261
        :   *  :* * .::*  : .
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Figure S3. Alignment of sequences of members of the Ema19/TMEM97 protein family. Sc, *Saccharomyces cerevisiae*; Cg, *Candida glabrata* KTA97029; Ca, *Candida albicans* EEQ46884; Zb, *Zygosaccharomyces bailii* AQZ15181; Kl, *Kluyvermyces lactis* CAH00503; Dh, *Debaromyces hansenii* CAG89731; Hs, *Homo sapiens* TMEM97; Mm, *Mus musculus* TMEM97; Dr, *Danio rerio* tmem97. Transmembrane domains are highlighted in blue.

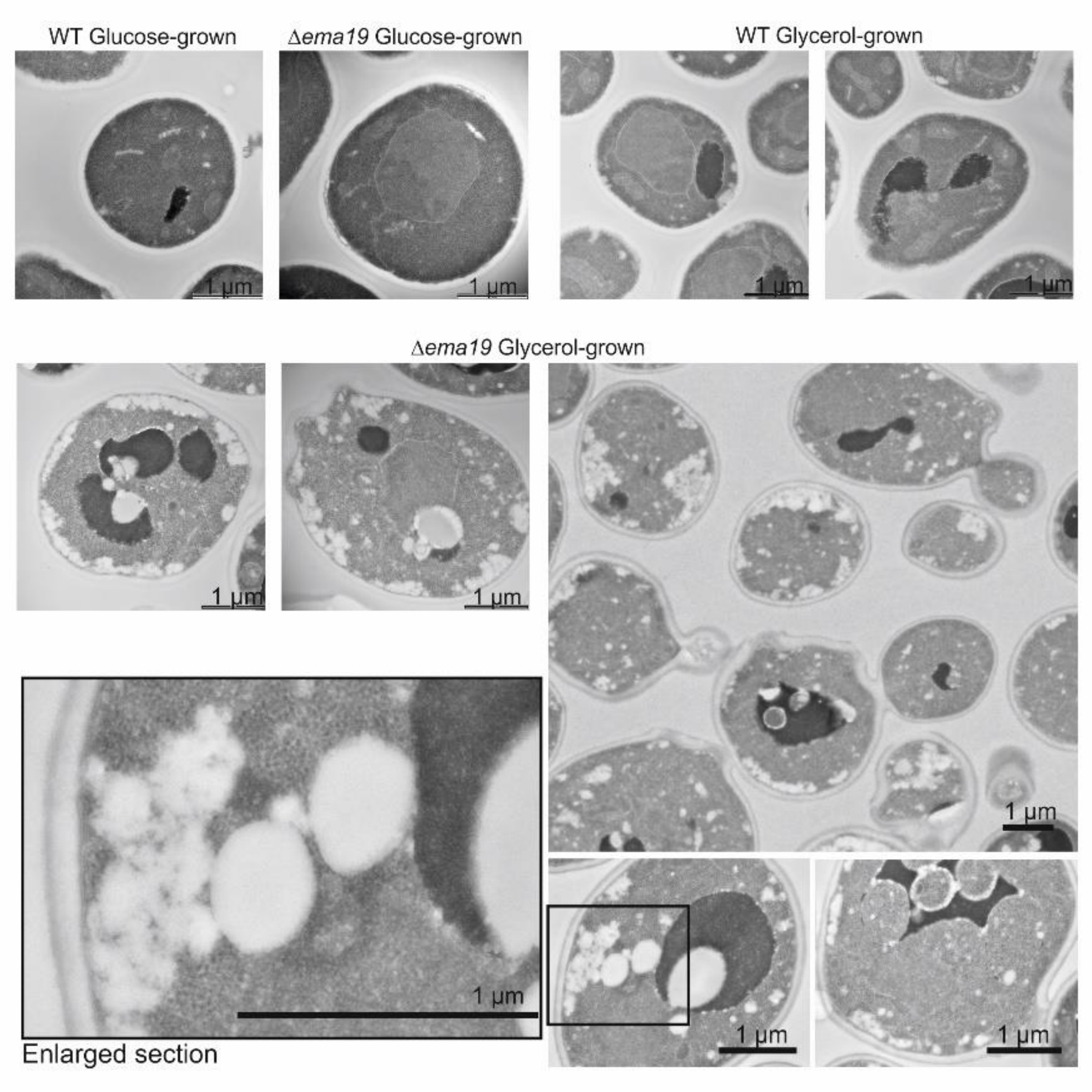

Figure S4. *Δema19* cells show morphological changes upon growth on glycerol but staining with BODIPY does not show obvious changes. Yeast cells were visualized by electron microscopy as described for Fig. 3A.

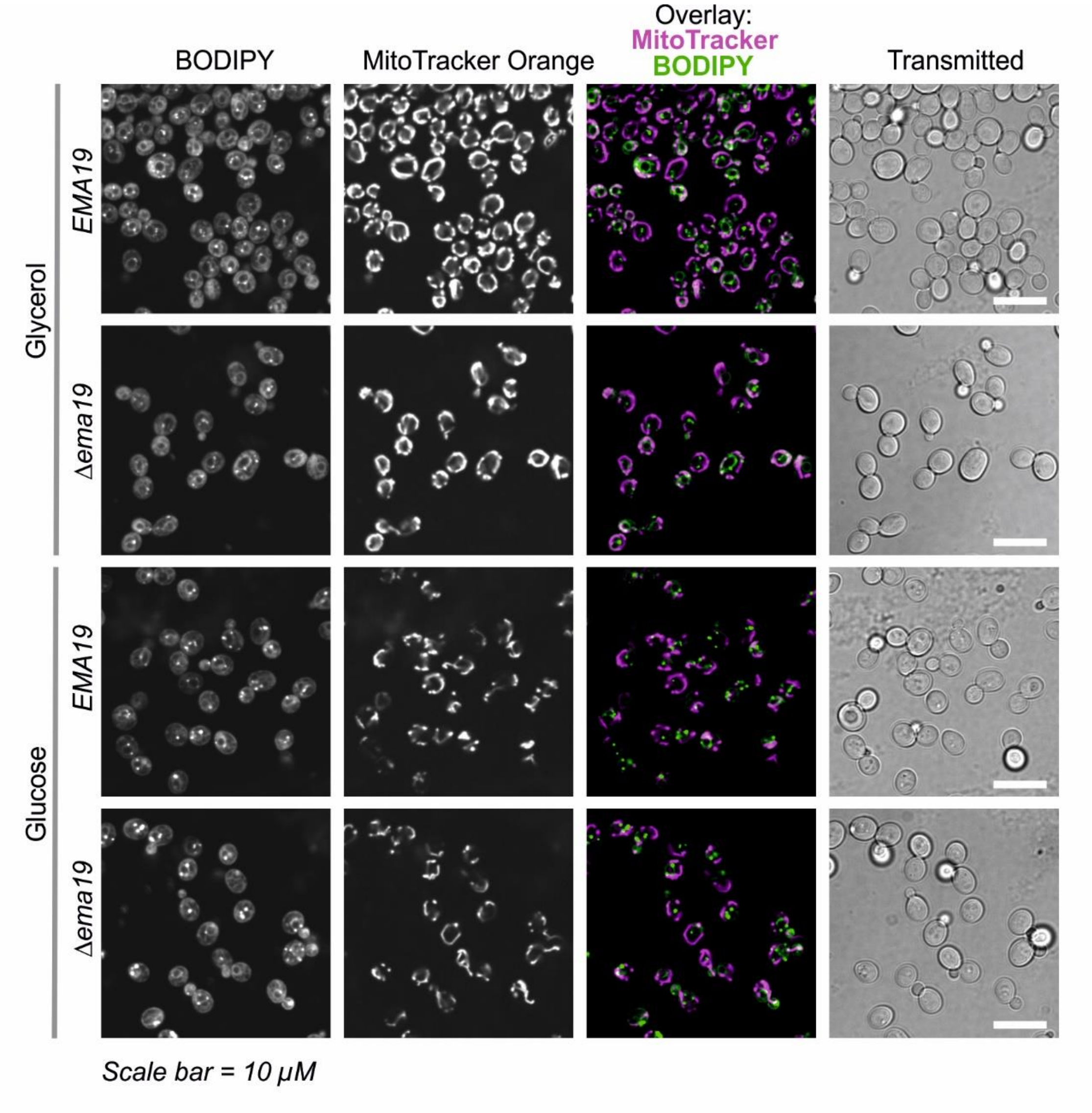

Figure S5. BODIPY staining does not indicate the accumulation of lipid droplets in *Δema19* cells. Cells were grown in glycerol medium before mitochondria and lipid bodies were visualized by fluorescence microscopy using MitoTracker Orange and BODIPY 493/503, respectively.

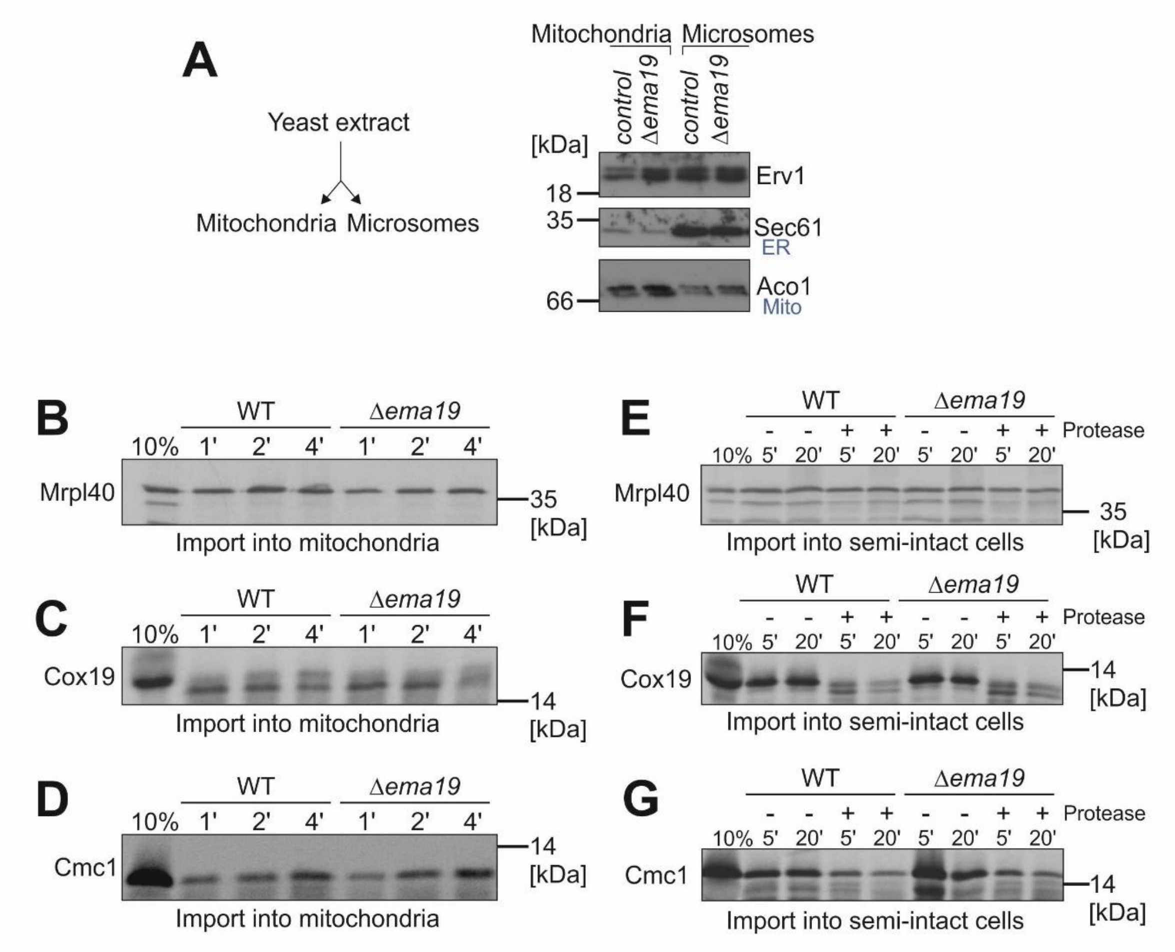

Figure S6. Ema19 is not critical for the import of mitochondrial proteins. A. Microsomes and mitochondrial fractions were isolated from cells expressing or lacking Ema19. The levels of the indicated proteins were analyzed by Western blotting. **B.-G.** *In vitro* import experiments with isolated mitochondria or semi-intact cells as described in the legend to Fig. 5.

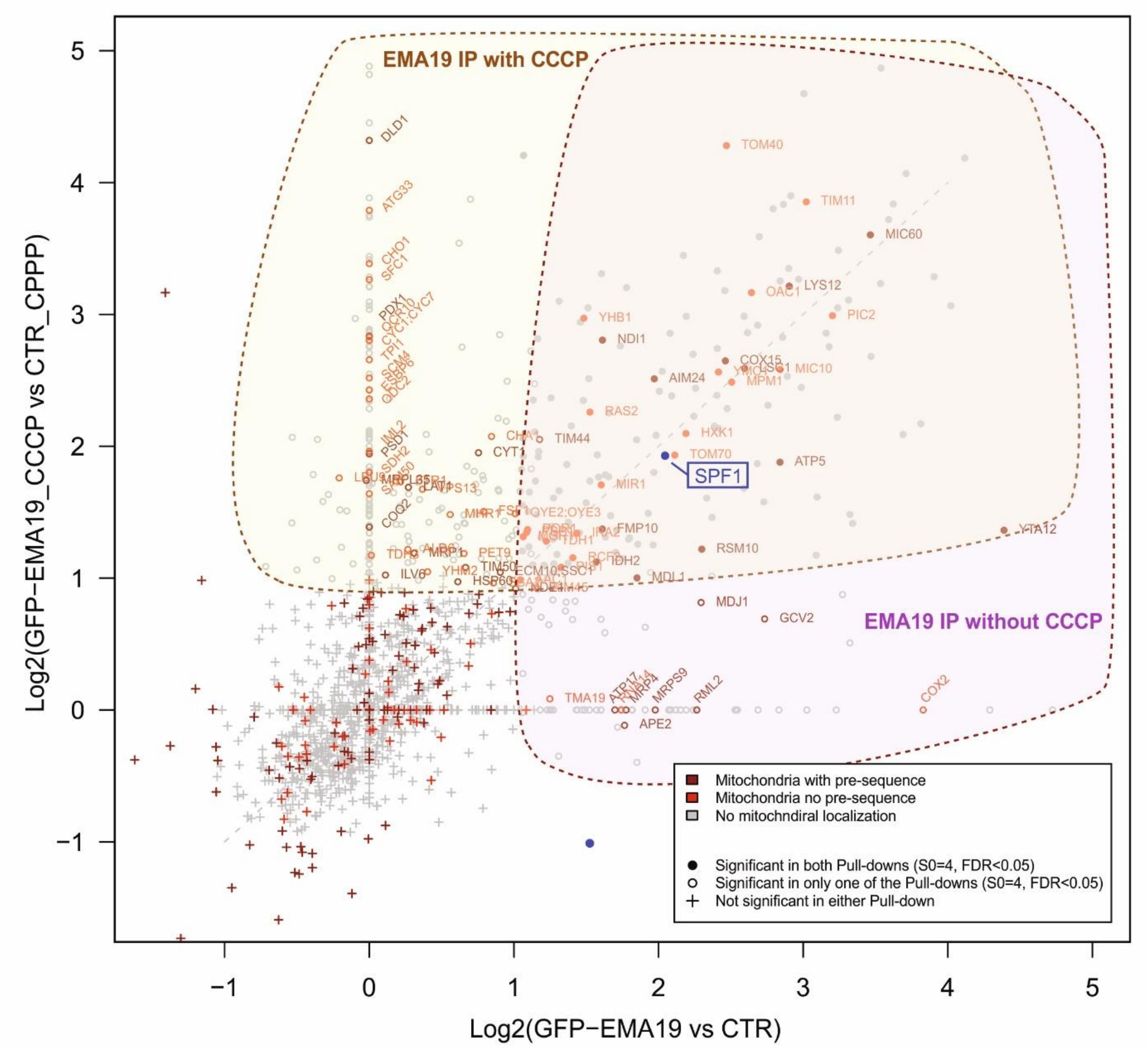

Figure S7. Pull-down experiments indicate that the mitochondrial membrane potential influences the interaction partners of Ema19. The log2-fold change of proteins in Ema19-GFP pulldowns in the presence or absence of 200 µM CCCP was plotted against each other (see Table S5 for data). To visualize proteins that were identified only in one of the two pull-down experiments, missing values were replaced by 0 (indicated by rotated labels). Proteins with a predicted mitochondrial localization are shown in red, proteins with a predicted matrix targeting sequence are shown in dark red. Proteins with a significant enrichment in both pull-down experiments are shown as filled circles. Proteins with significant enrichment in only one of the two pull-down experiments are shown in non-filled circles (four replicates, FDR<0.05). Note that Ema19 (fold change = 9.14 / 9.22) has been omitted for clarity.

Supplemental Table S1 listing the strains used in this study

Strain	Genotype	Reference
BY4742 WT	MATα *his3 leu2 lys2 ura3*	
YPH499 WT	MATa *ura3 lys2 ade2 trp1 his3 leu2*	
W303 WT	MATa *ura3-1 ade2-1 his3-11 leu2-3,112 trp1Δ2*	
Δ*ema19*	MATa *ura3 lys2 ade2 trp1 his3 leu2 EMA19::NatNT2*	This study
Δ*ema19*	MATa *ura3-1 ade2-1 his3-11 leu2-3,112 trp1Δ2 EMA19::NatNT2*	This study
Δ*ema19*	MATα *his3 leu2 lys2 ura3 EMA19::NatNT2*	This study
Δ*djp1*	MATa *ura3 lys2 ade2 trp1 his3 leu2 DJP1::KanMX4*	This study
Δ*ema19*Δ*djp1*	MATa *ura3 lys2 ade2 trp1 his3 leu2 EMA19::NatNT2 DJP1::KanMX4*	This study
Δ*oxa1*	MATa *ura3 lys2 ade2 trp1 his3 leu2 OXA1::KanMX4*	This study
Δ*ema19*Δ*oxa1*	MATa *ura3 lys2 ade2 trp1 his3 leu2 EMA19::NatNT2 OXA1::KanMX4*	This study
Δ*cox23*	MATa *ura3 lys2 ade2 trp1 his3 leu2 COX23::KanMX4*	This study
Δ*ema19*Δ*cox23*	MATa *ura3 lys2 ade2 trp1 his3 leu2 EMA19::NatNT2 COX23::KanMX4*	This study
Δ*qcr2*	MATa *ura3 lys2 ade2 trp1 his3 leu2 QCR2::KanMX4*	This study
Δ*ema19*Δ*qcr2*	MATa *ura3 lys2 ade2 trp1 his3 leu2 EMA19::NatNT2 QCR2::KanMX4*	This study
Δ*coa4*	MATa *ura3 lys2 ade2 trp1 his3 leu2 COA4::KanMX4*	This study
Δ*ema19*Δ*coa4*	MATa *ura3 lys2 ade2 trp1 his3 leu2 EMA19::NatNT2 COA4::KanMX4*	This study
Δ*ema19*-ΔN-Oxa1-HA	MATa *ura3 lys2 ade2 trp1 his3 leu2 EMA19::NatNT2* pYX223-ΔN-Oxa1-HA	This study
ΔN-Oxa1-HA	MATa *ura3 lys2 ade2 trp1 his3 leu2* pYX223- ΔN-Oxa1-HA	This study
Δ*ema19*-Erv1-HA	MATa *ura3 lys2 ade2 trp1 his3 leu2 EMA19::NatNT2* pYX232-Erv1-HA	This study
Erv1-HA	MATa *ura3 lys2 ade2 trp1 his3 leu2* pYX232-Erv1-HA	This study
Δ*ema19*-Oxa1-Ura3	MATa *ura3 lys2 ade2 trp1 his3 leu2 EMA19::NatNT2* pRS313 Oxa1-Ura3	This study
YPH499-Oxa1-Ura3	MATa *ura3 lys2 ade2 trp1 his3 leu2* pRS313 Oxa1-Ura3	This study
Δ*ema19*-Oxa1-Ura3	MATα *his3 leu2 lys2 ura3 EMA19::NatNT2* pRS313 Oxa1-Ura3	This study
BY4742-Oxa1-Ura3	MATα *his3 leu2 lys2 ura3* pRS313 Oxa1-Ura3	This study
Δ*ema19*-Oxa1-Ura3	MATa *ura3-1 ade2-1 his3-11 leu2-3,112 trp1Δ2 EMA19::NatNT2* pRS313 Oxa1-Ura3	This study
W303-Oxa1-Ura3	MATa *ura3-1 ade2-1 his3-11 leu2-3,112 trp1Δ2* pRS313 Oxa1-Ura3	This study
Δ*ema19*-Oxa1-GFP^{11} + Oxa1-GFP$^{1-10}$	MATa *ura3 lys2 ade2 trp1 his3 leu2 EMA19::NatNT2* pYX142-Oxa1-GFP^{11} + pYX122-Oxa1-GFP$^{1-10}$	This study

Δ*ema19*- ΔN-Oxa1-GFP^{11} + Sec63-GFP$^{1-10}$	MATa *ura3 lys2 ade2 trp1 his3 leu2 EMA19::NatNT2* pYX142-Oxa1-GFP^{11} + pYX122-Sec63-GFP$^{1-10}$	This study
YPH499-ΔN-Oxa1-GFP^{11} + Sec63-GFP$^{1-10}$	MATa *ura3 lys2 ade2 trp1 his3 leu2* pYX142-Oxa1-GFP^{11} + pYX122-Sec63-GFP$^{1-10}$	This study
Ema19-GFP Sec63-mCherry	MATa *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 EMA19-GFP::HIS SEC63-mCherry::KanMX*	This study
GFP-Ema19 Sec63-mCherry	MATa *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 hphΔn::URA3::SpNOP1pr-sfGFP-EMA19 SEC63-mCherry::KanMX*	This study
GFP-Ema19	MATa *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 hphΔn::URA3::SpNOP1pr-sfGFP-EMA19*	Hansen et al., 2018

Supplemental Table S2 listing the plasmids used in this study

Plasmid	**Description**	**Reference**
pYX223-ΔN-Oxa1-HA	2µ, GAL-ΔN-Oxa1-HA	Hansen et al., 2018
pYX232-Erv1-HA	2µ, TPI-Erv1-HA	Bien et al., 2010
pRS313	CEN, empty vector	Hansen et al., 2018
pRS313-Oxa1-Ura3	CEN, Oxa1-Ura3	Hansen et al., 2018
pGem4-Mrpl40		Herrmann lab collection
pGem4-Cox19		Herrmann lab collection
pGem4-Cmc1		Herrmann lab collection
pGem4-Oxa1		Herrmann lab collection
pYX142-Oxa1-GFP^{11}	CEN, TPI-Oxa1- GFP^{11}	This study
pYX122-Oxa1-GFP$^{1-10}$	CEN, TPI-Oxa1-GFP$^{1-10}$	This study
pYX142-ΔN-Oxa1-GFP^{11}	CEN, TPI-ΔN-Oxa1-GFP^{11}	This study
pYX122-Sec63-GFP$^{1-10}$	CEN, TPI-Sec63-GFP$^{1-10}$	This study

Supplemental Table S3 listing the antibodies used in this study

Antibodies	Source	Identifier
anti-Ilv5	Herrmann lab collection	Peleh et al., 2017
anti-Oxa1	Herrmann lab collection	
anti-Atp23	Herrmann lab collection	
Anti-Cmc1	Herrmann lab collection	
anti-Erv1	Herrmann lab collection	
anti-Rpl6B	Herrmann lab collection	
anti-HA-Peroxidase	Roche	12 013 819 001
anti-rabbit secondary antibody	BioRad	172-1019

Supplemental Table S4 listing organisms, accession numbers and protein names of the sequences used for the unrooted neighbor-joining tree shown in Fig. S2

	Organism	Protein	Accession Number
fungi	*Brettanomyces naardenensis*	DEKNAAC105048	VEU23783.1
	Cyberlindnera fabianii	TMEM97	ONH65138.1
	Debaryomyces hansenii	DEHA2F22616p	XP_461326.1
	Eremothecium gossypii	AFR508Wp	NP_986055.1
	Komagataella pastoris	BA75_00687T0	ANZ74746.1
	Lachancea nothofagi	LANO_0B02652g1_1	SCU81319.1
	Millerozyma farinosa	Piso0_004871	CCE86385.1
	Saccharomyces cerevisiae	Ema19p	NP_013151.1
	Zygosaccharomyces bailii	ZYBA0S09-00276g1_1	CDF90900.1
protists	*Acanthamoeba castellanii*	TMEM97	XP_004367690.1
	Thecamonas trahens	TMEM97	XP_013756112.1
plants	*Arabidopsis thaliana*	TMEM97	NP_565742.1
	Capsella rubella	sigma intracellular receptor 2	XP_006295112.1
	Coffea arabica	sigma intracellular receptor 2	XP_027063739.1
	Hordeum vulgare	TMEM97	KAE8810566.1
	Papaver somniferum	sigma intracellular receptor 2	XP_026392546.1
	Prunus persica	TMEM97	XP_007216330.1
	Selaginella moellendorffii	sigma intracellular receptor 2	XP_002969527.1
	Solanum lycopersicum	sigma intracellular receptor 2	XP_004243464.1
animals	*Bos taurus*	sigma intracellular receptor 2	NP_001029575.1
	Crassostrea gigas	sigma intracellular receptor 2	XP_011421127.1
	Danio rerio	sigma intracellular receptor 2	NP_001313327.1
	Dasypus novemcinctus	sigma intracellular receptor 2	XP_004450017.2
	Desmodus rotundus	sigma intracellular receptor 2	XP_024420904.1
	Homo sapiens	TMEM97	AAH91504.1
	Loxodonta africana	sigma intracellular receptor 2	XP_003416883.2
	Molossus molossus	TMEM97	KAF6417492.1
	Mus musculus	sigma intracellular receptor 2	NP_598467.1
	Oncorhynchus mykiss	sigma intracellular receptor 2	XP_021444210.2
	Otolemur garnettii	sigma intracellular receptor 2	XP_012665224.1

Tupaia chinensis	sigma intracellular receptor 2	XP_006165494.1
Ursus arctos horribilis	sigma intracellular receptor 2	XP_026373477.1
Xenopus tropicalis	sigma intracellular receptor 2	sp|Q6DFQ5.1