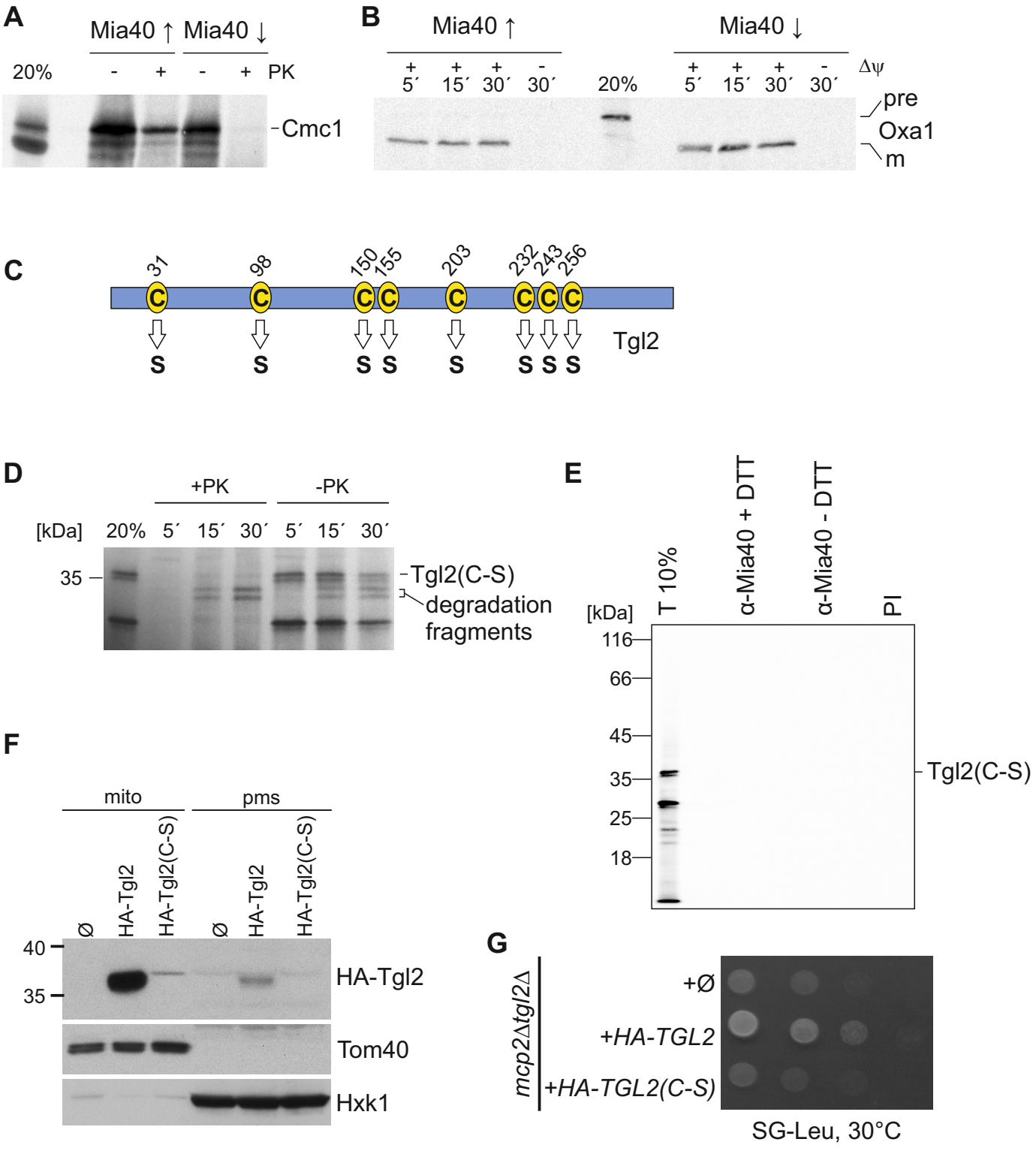
Supplemental Materials

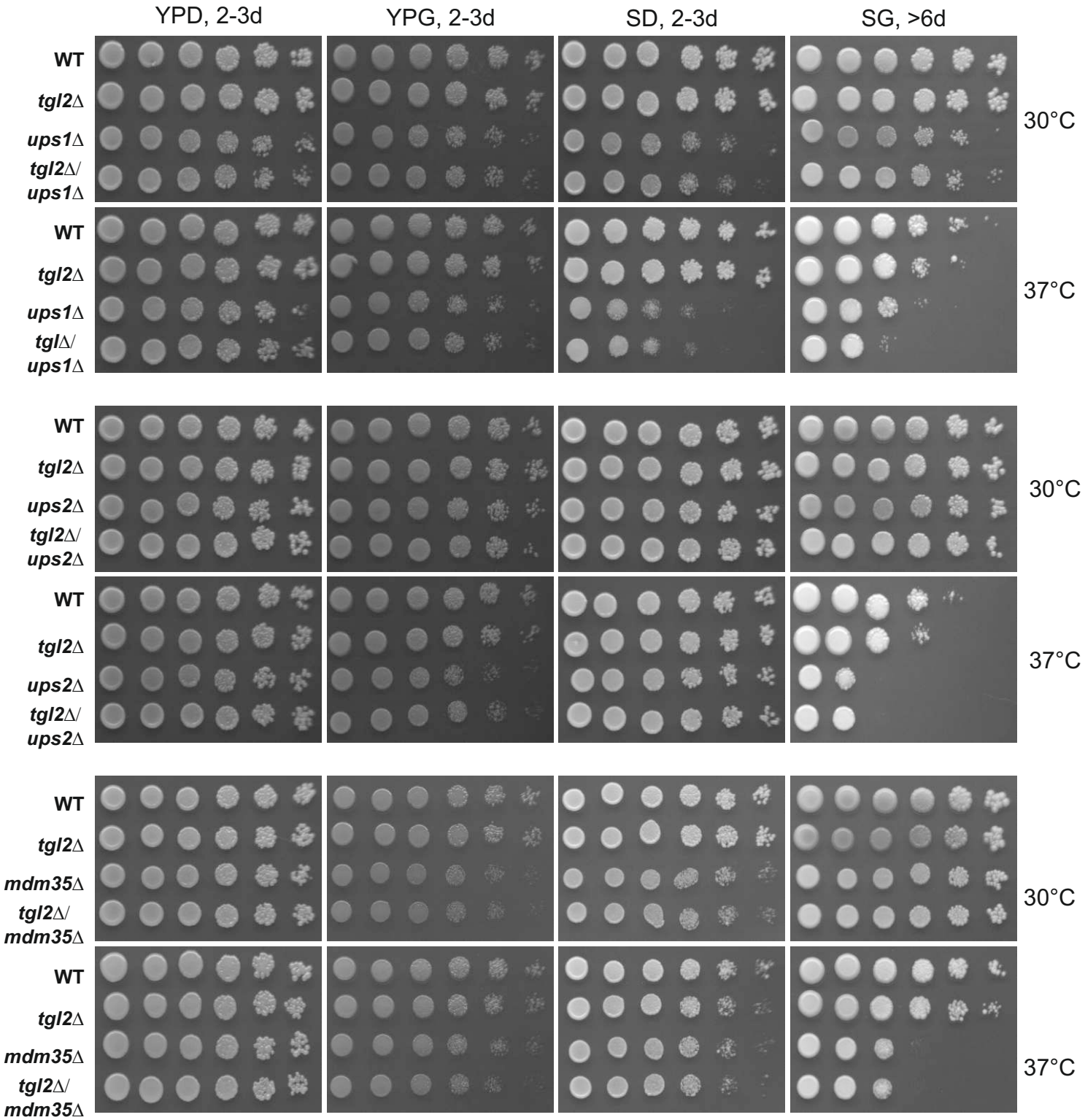
Molecular Biology of the Cell

Odendall et al.

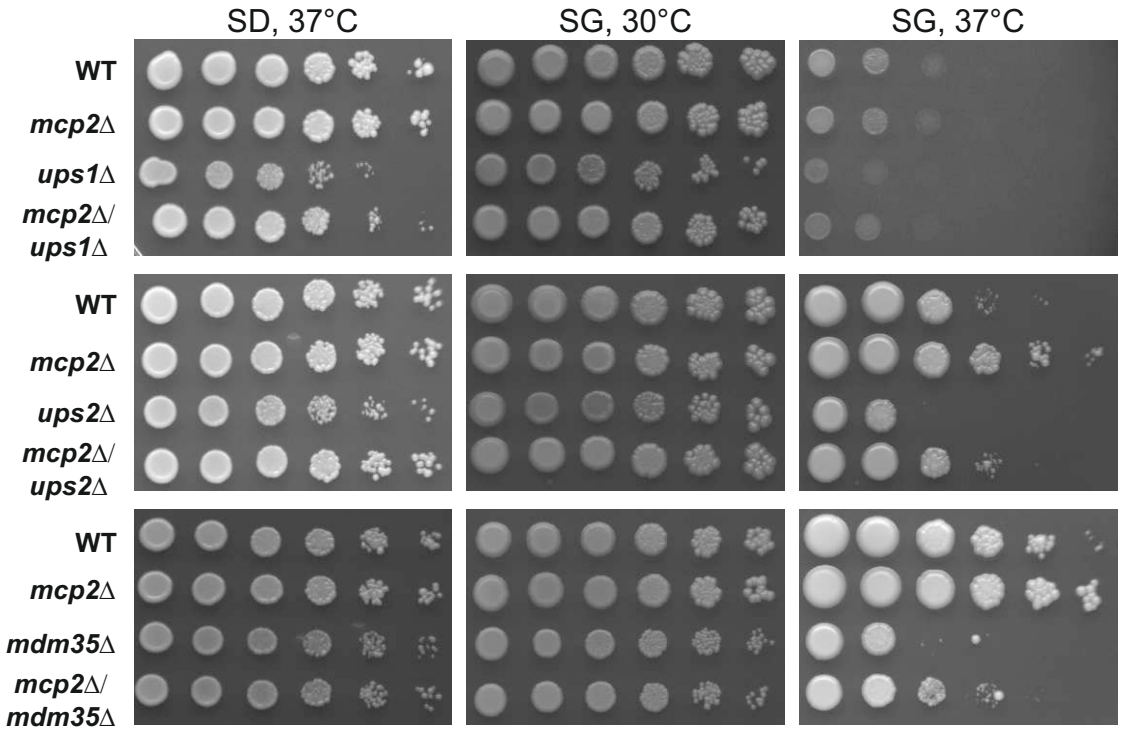


Odendall *et al.*, Supplementary Figure S1

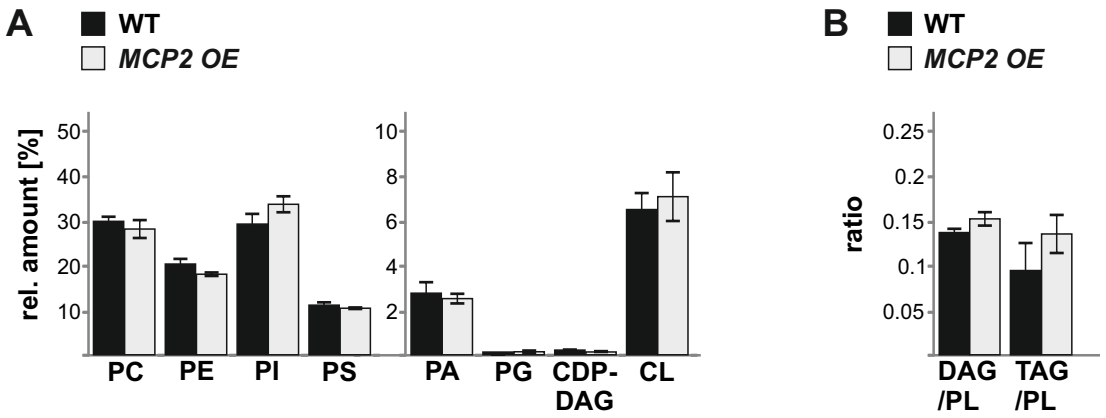
Supplementary Figure S1. The cysteine residues of Tgl2 are critical for its stability in the IMS. **(A)** Radiolabeled Cmc1 was incubated for 15 min at 25°C with mitochondria containing (Mia40 ↑) or depleted of (Mia40 ↓) Mia40. Mitochondria were treated without or with proteinase K (PK). Proteins were resolved by SDS-PAGE and visualized by autoradiography. 20% of the Cmc1 protein used for one import sample was loaded for control. Note, that upon depletion of Mia40, Cmc1 is not imported.**(B)** Radiolabeled Oxa1 precursor (pre) was incubated for the times indicated with mitochondria containing (Mia40 ↑) or depleted (Mia40 ↓) of Mia40. For the samples labeled with – $\Delta\psi$, the mitochondrial membrane potential was dissipated by addition of a cocktail of valinomycin, antimycin and oligomycin. Mitochondria were treated without or with PK. Proteins were resolved by SDS-PAGE and visualized by autoradiography. 20% of the Oxa1 precursor protein used for one import sample was loaded for control; m, mature form of Oxa1. Note, that upon depletion of Mia40, Oxa1 is still efficiently imported. **(C)** Schematic representation of Tgl2 with indicated cysteine residues. For the experiments shown in panels D-G, a Tgl2(C-S) variant was used in which all 8 cysteines were replaced by serine residues. **(D)** Radiolabeled Tgl2(C-S) was incubated for different time points at 25°C with wild-type mitochondria. After import mitochondria were treated without or with PK. Proteins were resolved by SDS-PAGE and visualized by autoradiography. 20% of the Tgl2(C-S) protein used for one import sample was loaded for control. Note that degradation fragments accumulate during the import reaction that are protease protected. These were not formed by the added PK but presumably by a protease present in the mitochondrial IMS. **(E)** Radiolabeled Tgl2(C-S) was incubated with isolated mitochondria before Mia40 was isolated by immunoprecipitation as described for Figure 3C. Note, that this variant does not form SDS-stable interactions with Mia40. **(F)** Expression levels of HA-tagged Tgl2 and Tgl2(C-S). Cells were grown to mid-logarithmic phase and, crude mitochondrial fractions and supernatant after precipitation of mitochondria (pms) were analyzed by SDS-PAGE and immunodecoration with antibodies against the HA-tag, or Tom40, a MOM protein, and Hxk1 a cytosolic protein as loading control. Ø, empty plasmid control. **(G)** Expression of Tgl2(C-S) does not rescue the growth defect of *mcp2*Δ*tgl2*Δ cells. Cells lacking endogenous Mcp2 and Tgl2 were transformed with the empty plasmid pYX142 (∅), or with pYX142 encoding *HA-TGL2 or HA-TGL2(C-S)* under control of the TPI promoter. Cells were grown to logarithmic phase and spotted on SG-Leu plates in a 1:5 dilution series. Plates were incubated for growth at 30°C.



Supplementary Figure S2. Growth analysis of double deletion mutants lacking *TGL2* and either *UPS1*, *UPS2* or *MDM35*. Cells of the indicated genotype in the W303a wild-type background were grown to logarithmic phase on full medium containing fermentable carbon source glucose (YPD) to logarithmic phase and spotted on full (YP) or synthetic (S) media containing either glucose (D) or the non-fermentable carbon source glycerol (G) in a 1:5 dilution series. Plates were analyzed for growth at either 30°C or 37°C.



Supplementary Figure S3. Growth analysis of double deletion mutants lacking *MCP2* and either *UPS1*, *UPS2* or *MDM35*. Cells of the indicated genotype in the W303 wild-type background were grown to logarithmic phase on full medium containing fermentable carbon source glucose (YPD) to logarithmic phase and spotted on synthetic (S) media containing either glucose (D) or the non-fermentable carbon source glycerol (G) in a 1:5 dilution series. Plates were analyzed for growth at either 30°C or 37°C.



Supplementary Figure S4. Strong overexpression of Mcp2 does not result in significant alterations in phospholipid composition or neutral lipid ratios. Lipids were extracted from yeast cells grown on SGal-Ura medium to logarithmic phase (OD_{600} = 1,5), lipids were extracted and then analyzed by mass spectrometric analysis. **(A)** Phospholipid composition. The level of each phospholipid species in [%] of total PL is shown as mean with standard deviation bars (n=3). **(B)** TAG/PL ratio. The average ratio with standard deviation bars is shown (n=3).

Supplementary Table 1: Lipidomic analyses of yeast cells of the indicated genotype grown on YPD at 30°C to logarithmic phase.

	WT	***mcp2Δ/tgl2Δ***	
Phospholipid	*amount [%]*	*amount [%]*	*significance (p-value, n=6)*
PC	35.557 ± 0.414	38.165 ± 1.168	0.01411
PE	21.329 ± 1.270	17.812 ± 0.701	0.04297
PI	28.149 ± 1.599	30.149 ± 1.379	0.06362
PS	10.012 ± 0.123	9.519 ± 1.144	0.13932
PA	1.815 ± 0.276	1.647 ± 0.246	0.94345
PG	0.299 ± 0.050	0.327 ± 0.045	0.77718
CL	2.813 ± 0.147	2.349 ± 0.201	0.03152
CDP-DAG	0.026 ± 0.012	0.032 ± 0.009	0.24863
neutral lipid / phospholipid ratio	*ratio*	*ratio*	*significance (p-value, n=6)*
DAG/PL	0.188 ± 0.026	0.192 ± 0.023	0.46576
TAG/PL	0.105 ± 0.002	0.12 ± 0.011	0.03306

Supplementary Table 2: Lipidomic analysis of yeast cells with strong overexpression of Mcp2. Cells were grown on SGal-Ura medium at 30°C to logarithmic phase.

	WT	***mcp2Δ/tgl2Δ***
Phospholipid	*amount [%]*	*amount [%]*
PC	29.829 ± 1.025	28.105 ± 2.002
PE	20.285 ± 1.150	18.008 ± 0.391
PI	29.213 ± 2.278	33.639 ± 1.786
PS	11.094 ± 0.752	10.406 ± 0.171
PA	2.754 ± 0.491	2.511 ± 0.215
PG	0.122 ±0.008	0.136 ± 0.053
CDP-DAG	0.210 ± 0.098	0.127 ± 0.023
CL	6.493 ± 0.737	7.069 ± 0.722
neutral lipid / PL ratio	*ratio*	*ratio*
DAG/PL	0.137 ± 0.004	0.152 ± 0.007
TAG/PL	0.094 ± 0.033	0.135 ± 0.021