Yeast Genome-wide Screens to Ascertain the Genetic Landscape for Barth Syndrome

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Barth Syndrome Foundation

Using Systematic Genetics to Identify Modifies Genes that Affect Fitness of *taz1*Δ yeast

Much easier to identify modifier genes in inbred/isogenic organisms

Negative modifier genes – those that decrease fitness of cells containing an inactivated *TAZ1* gene (focus of this talk)
Positive modifier genes – those that increase the fitness of cells containing an inactivated *TAZ1* gene

*are these 'genetic interactions' conserved in other models of Barth syndrome (*Drosophila*, zebrafish, mouse, human)
**are there polymorphisms in these genes in Barth patients that could these be prognostic for either disease progression or treatment(s) (once available)

Negative modifier genes: inactivation of what other gene(s) in combination with inactivation of the *TAZ1* gene result in a sick cell?

A systematic genome-wide analysis

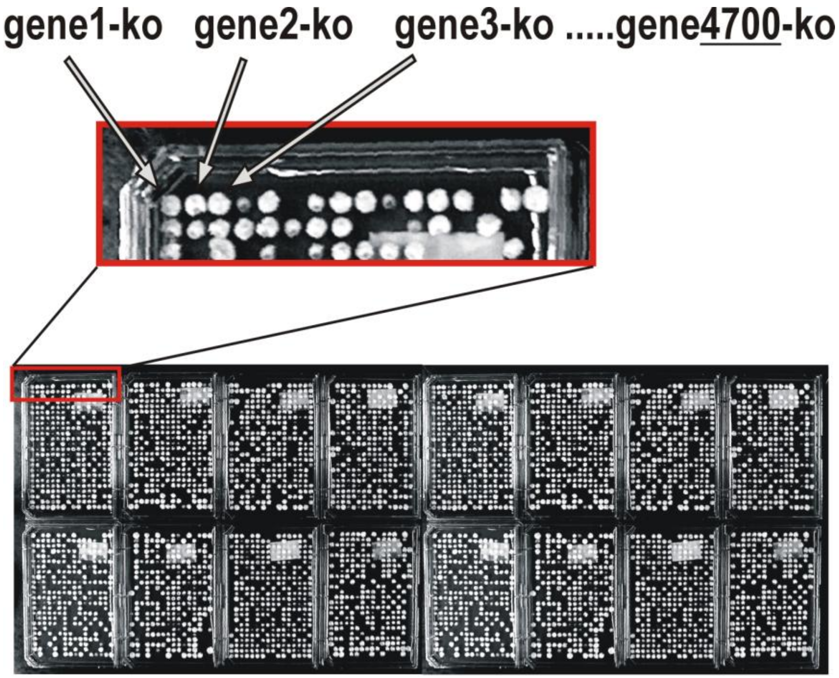
Does loss of any one of the ~4700 non-essential genes in yeast result in a sicker cell when the *TAZ1* gene is also inactivated?

Systematic construction of:

- *taz1 xxx1*
- *taz1 xxx1*
- *taz1 xxx3*
- *taz1 xxx4*

…in combination with every non-essential yeast gene…………………
taz1 xxx4700 (in triplicate)

In plain English: who dies?



taz1 xxx1

taz1 xxx2

taz1 xxx3

Major Functions that when Deficient Compromise Fitness of Yeast Cells with an Inactivated *TAZ1* Gene

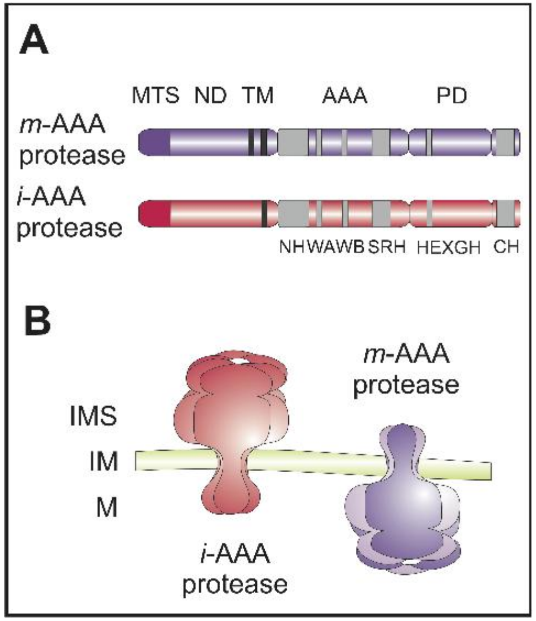
- Mitochondrial Protein Import
- Mitochondrial Protein Stability
- Argininosuccinate shunt
- Mitochondrial Translation
- Endosome Trafficking

Mitochondrial Protein Stability/Quality Control – *YME1*

- *YME1* - Catalytic subunit of the mitochondrial inner membrane *i*-AAA protease complex

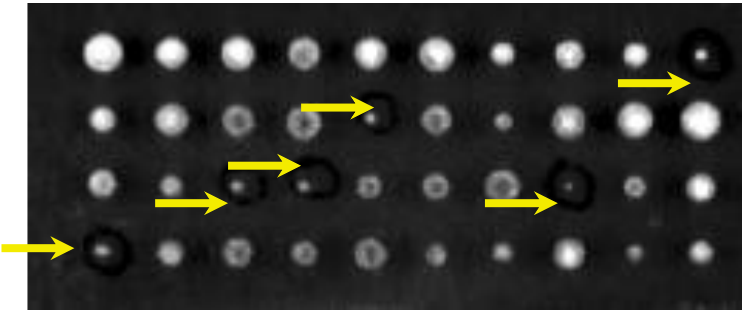
  - responsible for degradation of unfolded or misfolded mitochondrial proteins in the IM space including components of complex IV of the electron transport chain

  - also implicated in induction of mitophagy



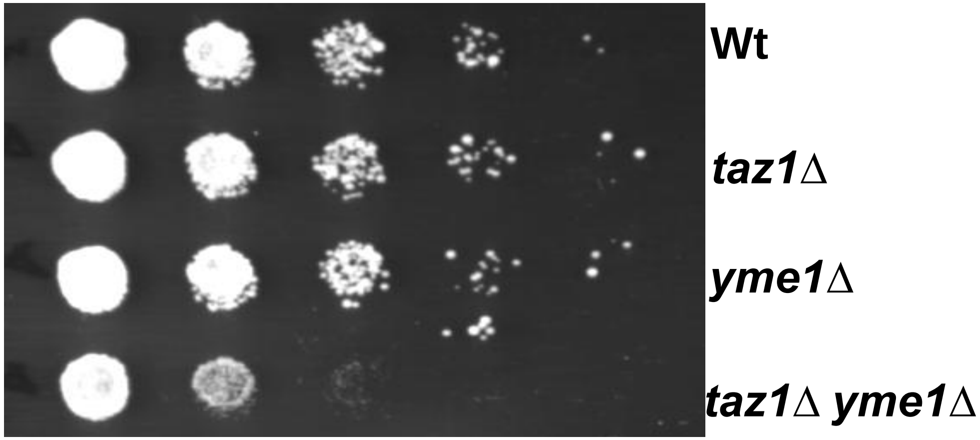
Confirmation that inactivation of the *YME1* gene is synthetic sick with inactivation of the *TAZ1* gene

*yme1*Δ X *taz1*Δ



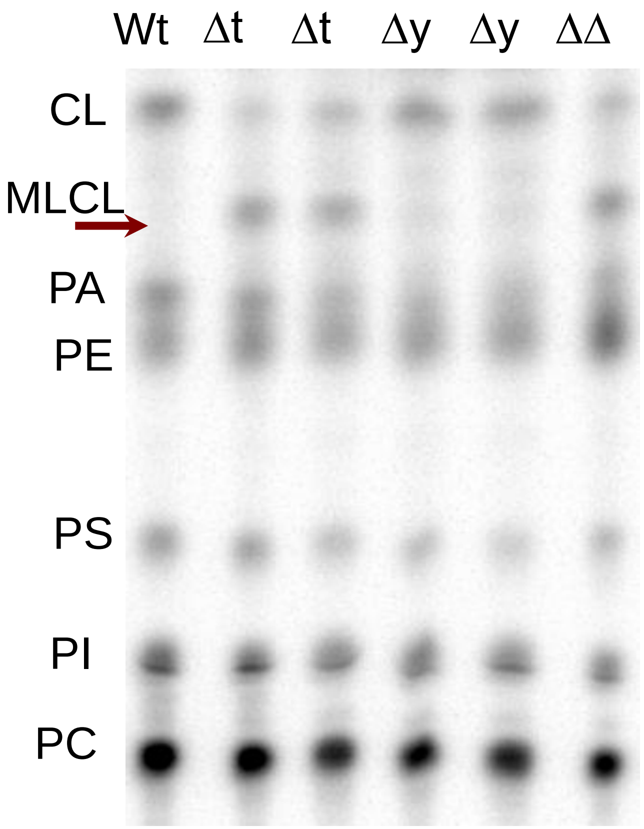
Tetrad dissection

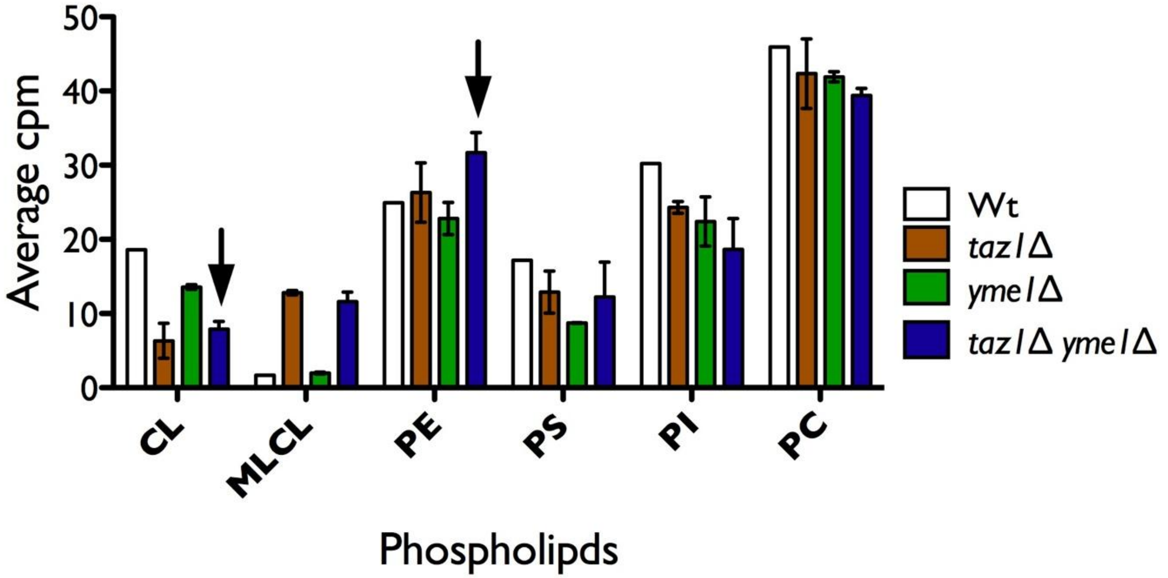
Confirmation that inactivation of the *YME1* gene is synthetic sick with inactivation of the *TAZ1* gene



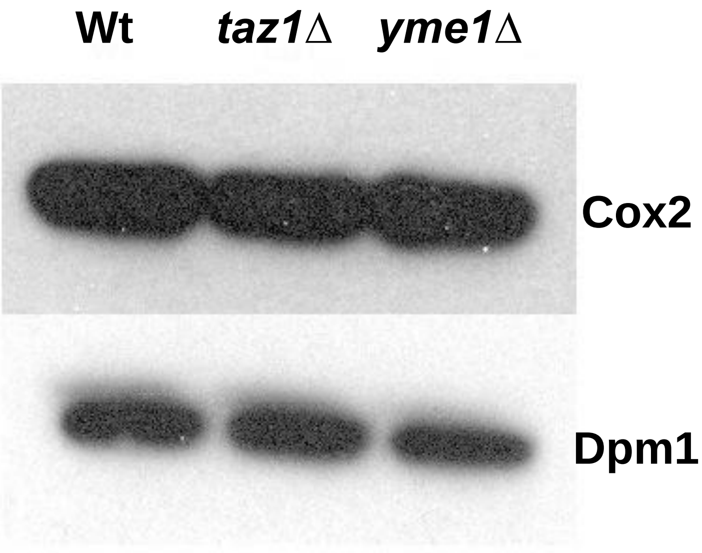
Serial dilutions

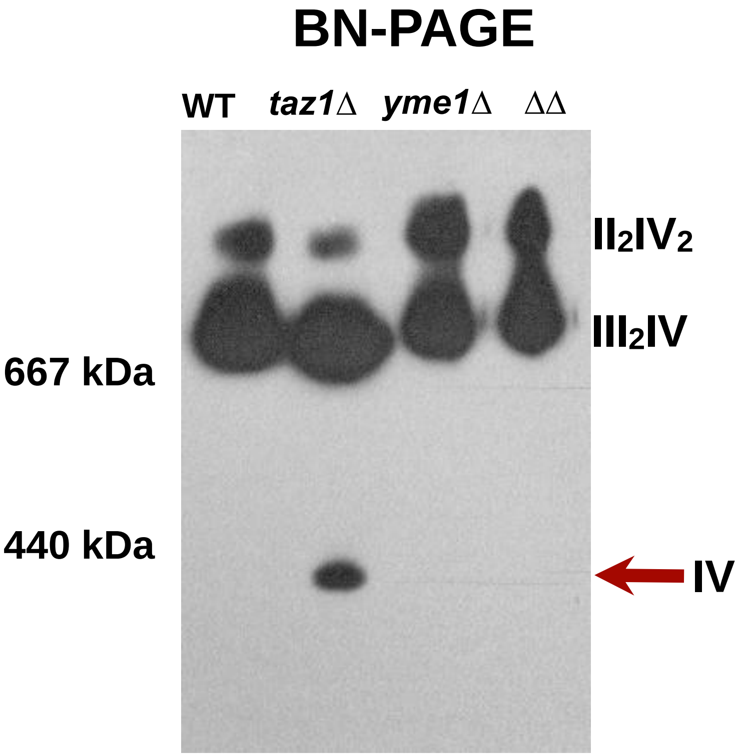
Phospholipid levels are not altered in *taz1*Δ *yme1*Δ cells



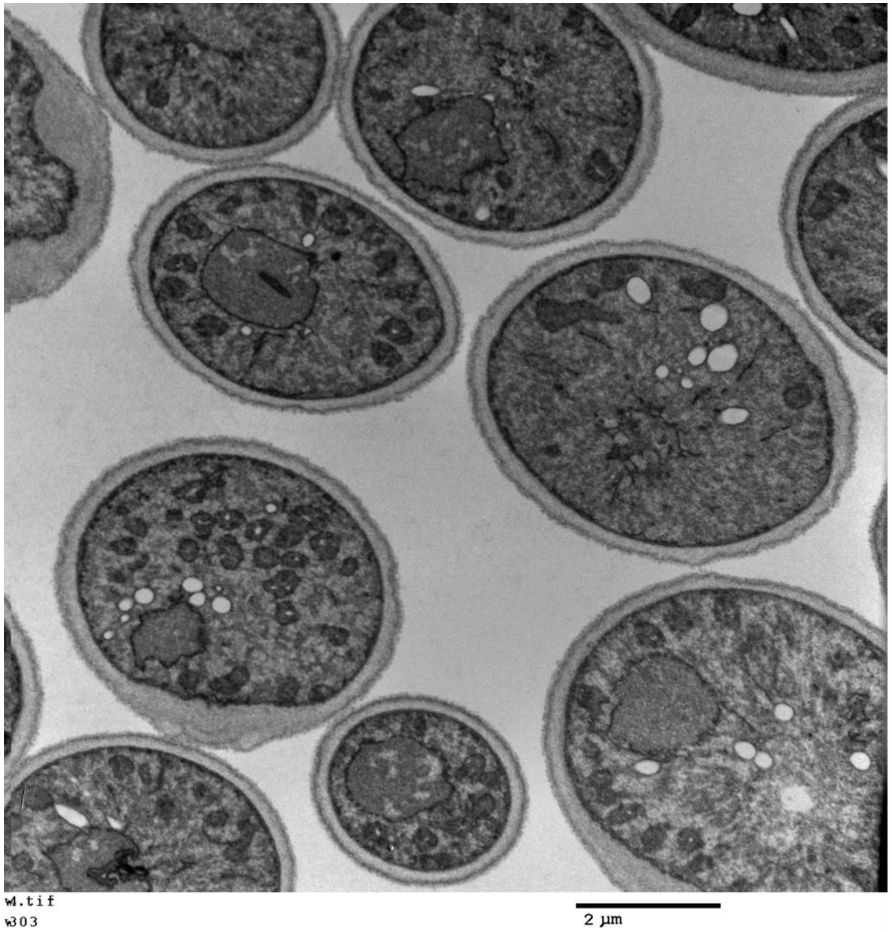


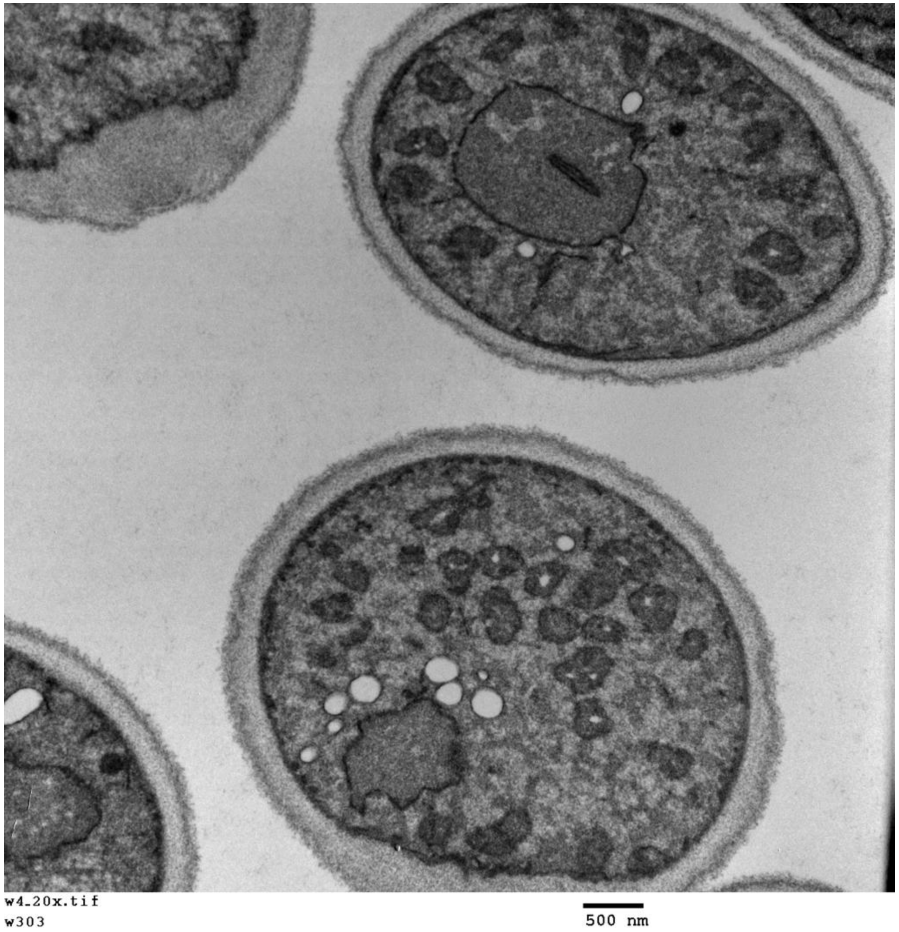
Electron chain supercomplex formation is not affected in *taz1Δ yme1Δ* cells



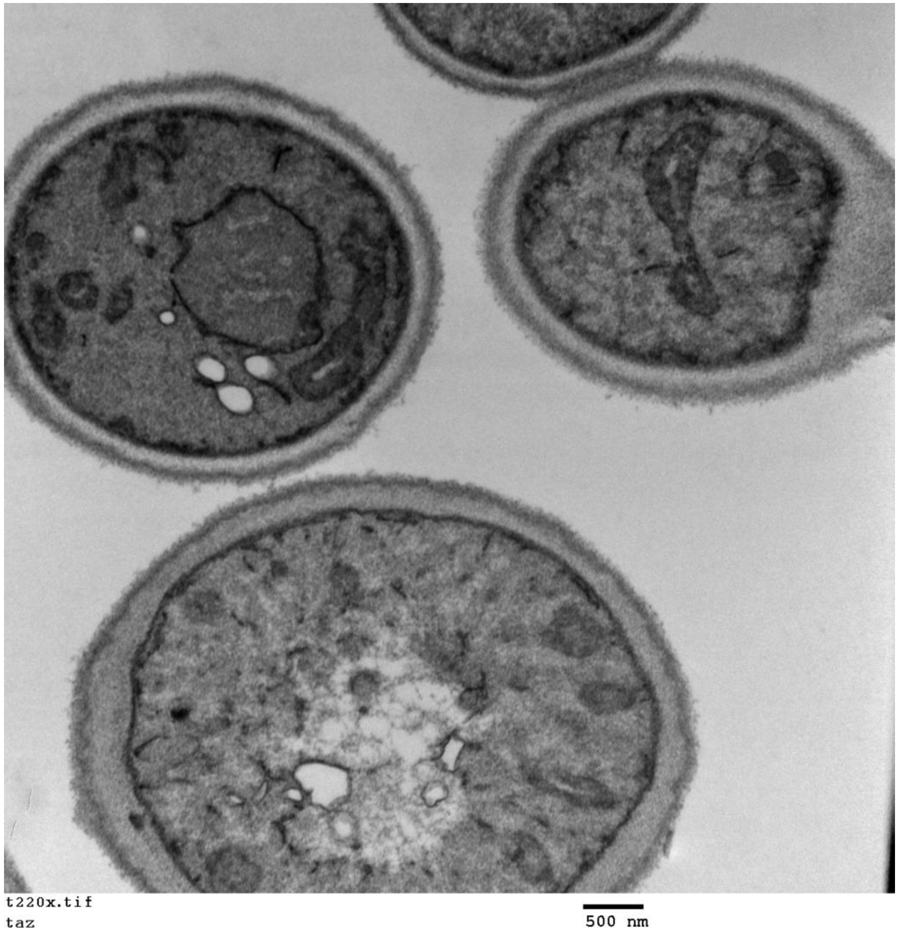


Electron microscopy to examine mitochondrial morphology – wild type cells

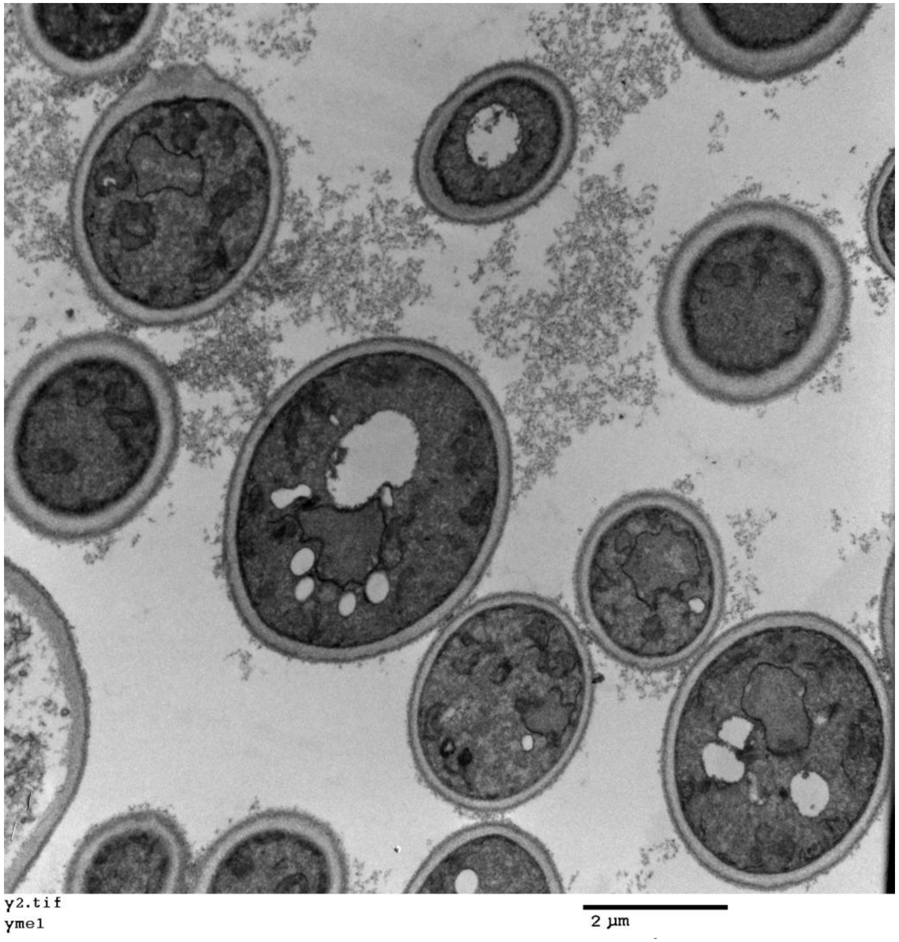


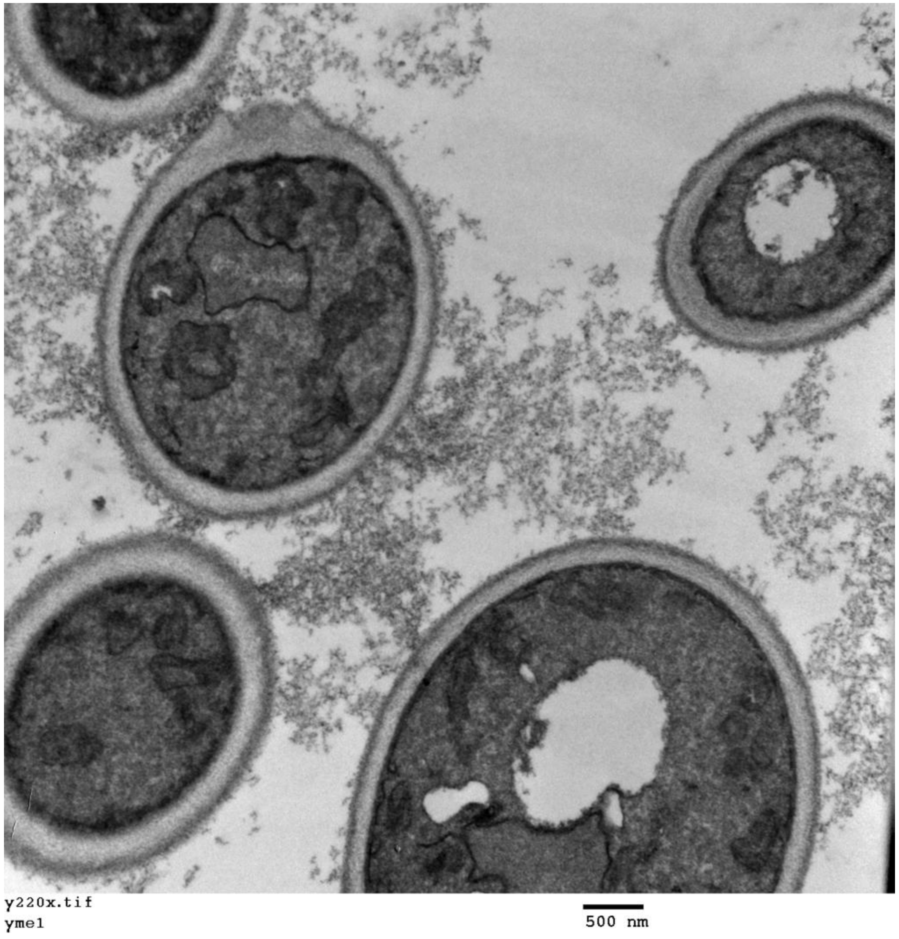


Electron microscopy to examine mitochondrial morphology – *taz1*Δ

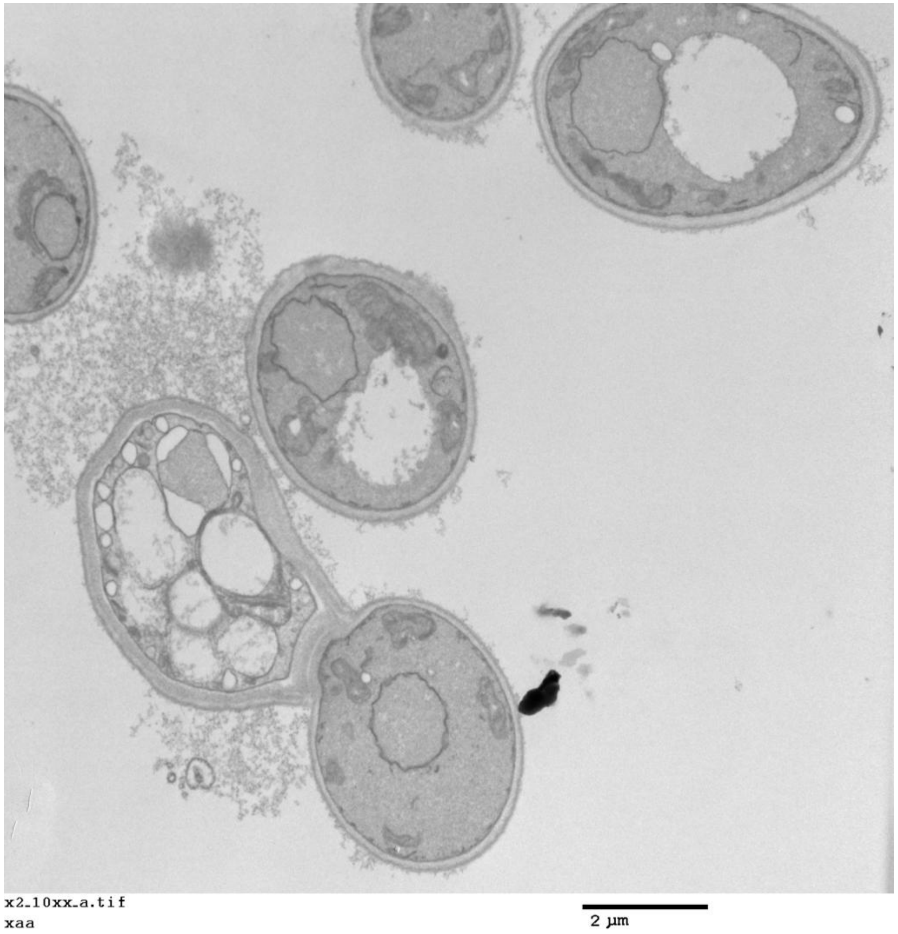


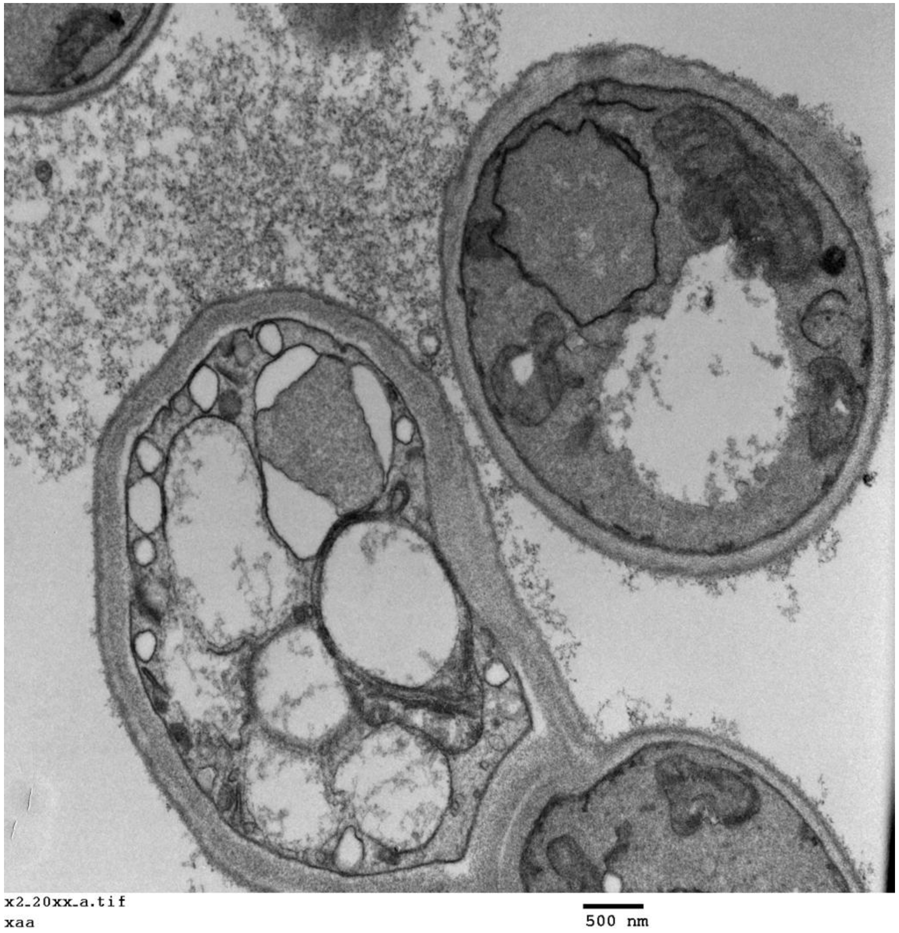
Electron microscopy to examine mitochondrial morphology – *yme1*Δ



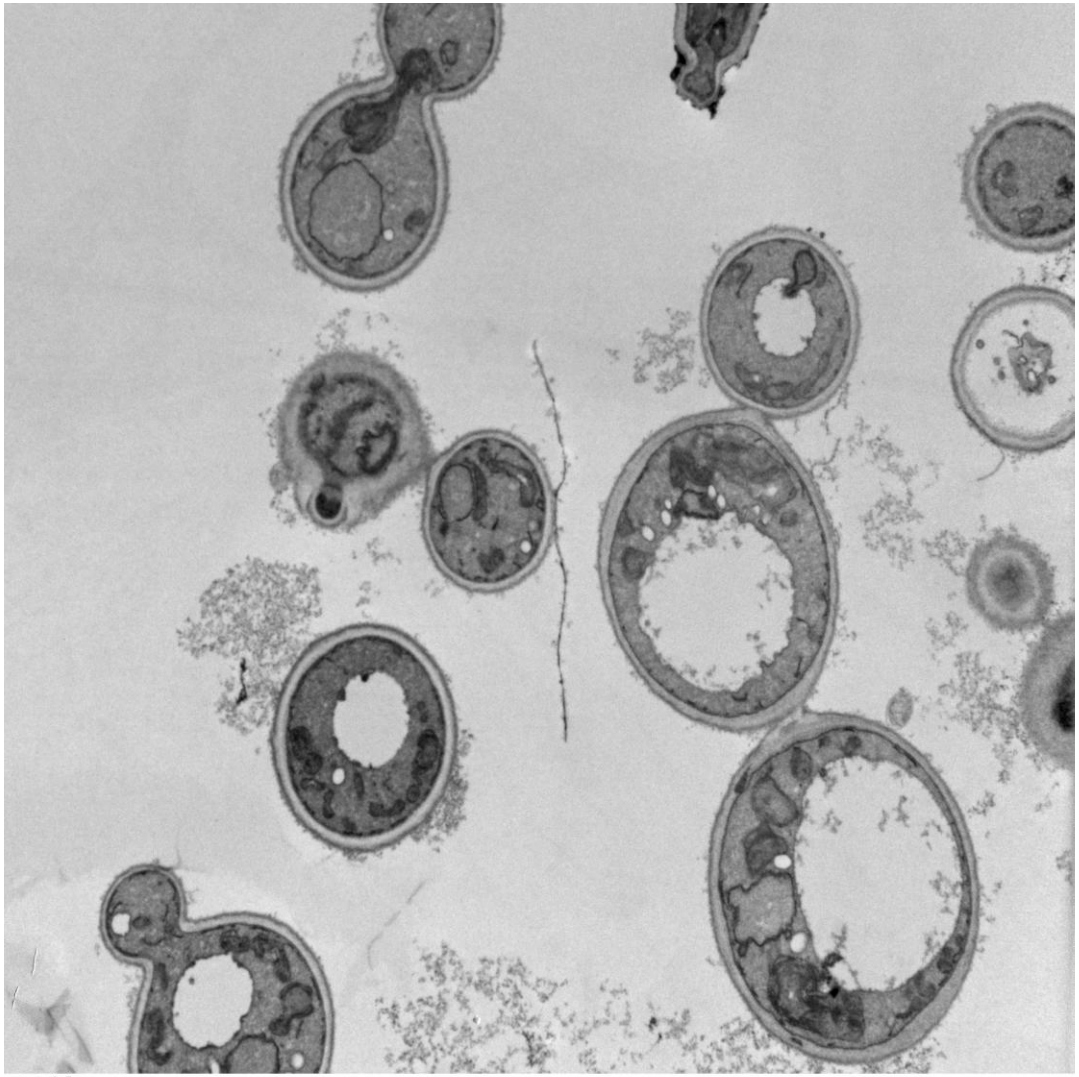


Electron microscopy to examine mitochondrial morphology – *taz1Δ yme1Δ*

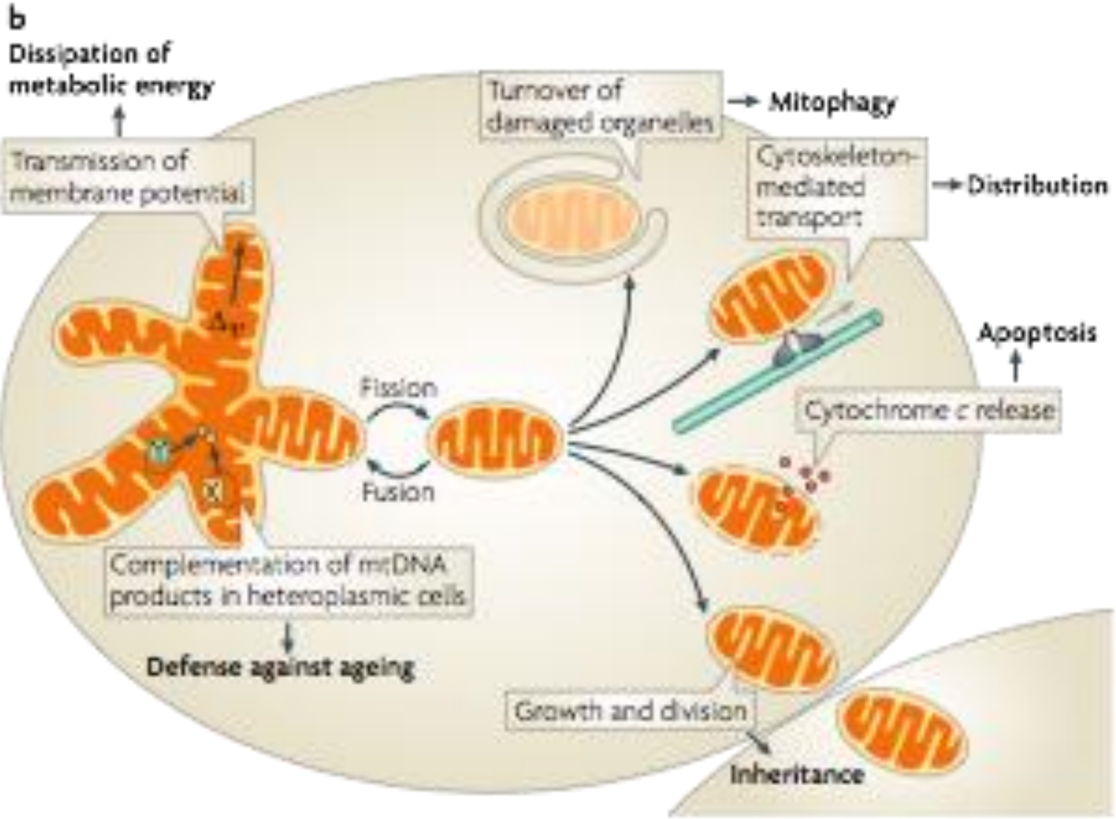




Electron microscopy to examine mitochondrial morphology – *taz1Δ yme1Δ*

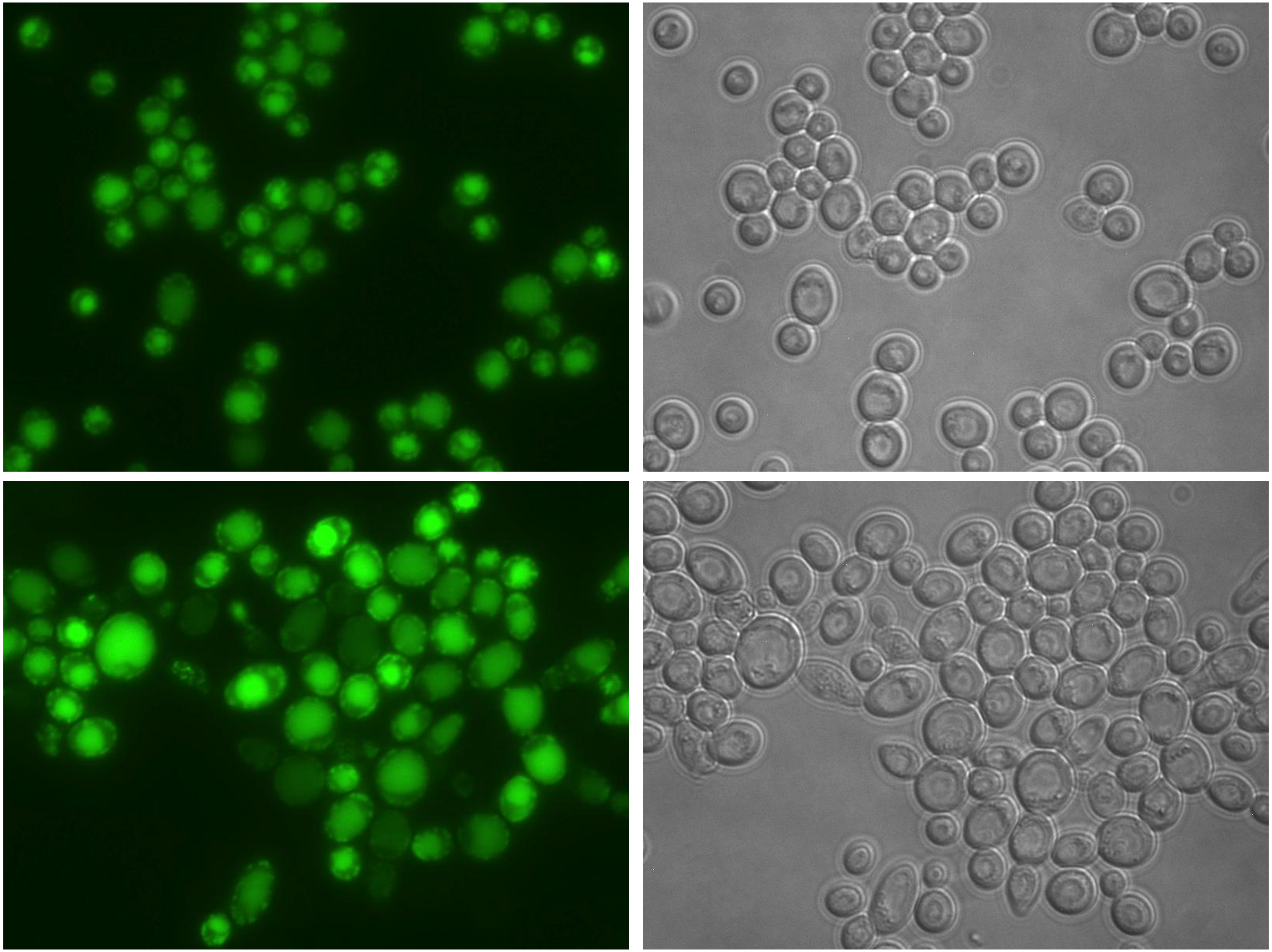


Roles for mitochondrial fission of fusion



NATURE REVIEWS 872 | DECEMBER 2010 | VOLUME 11

Mitophagy assay (OM45-GFP) in wild type and *taz1*Δ cells

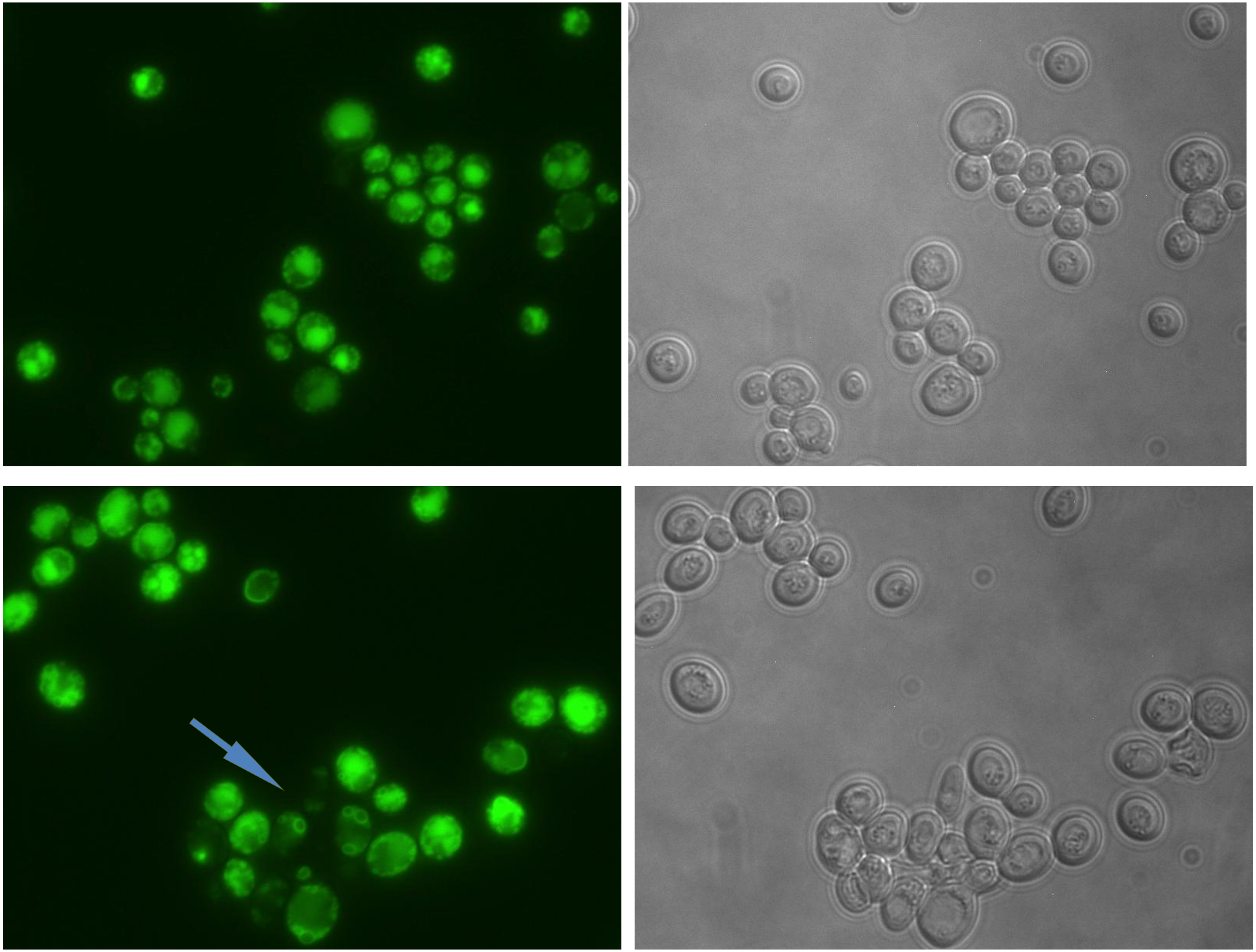


Wt

2%lactate minus a.a 24hrs

***taz1*Δ**

Mitophagy assay (OM45-GFP) in *yme1Δ* and *taz1Δ yme1Δ* cells

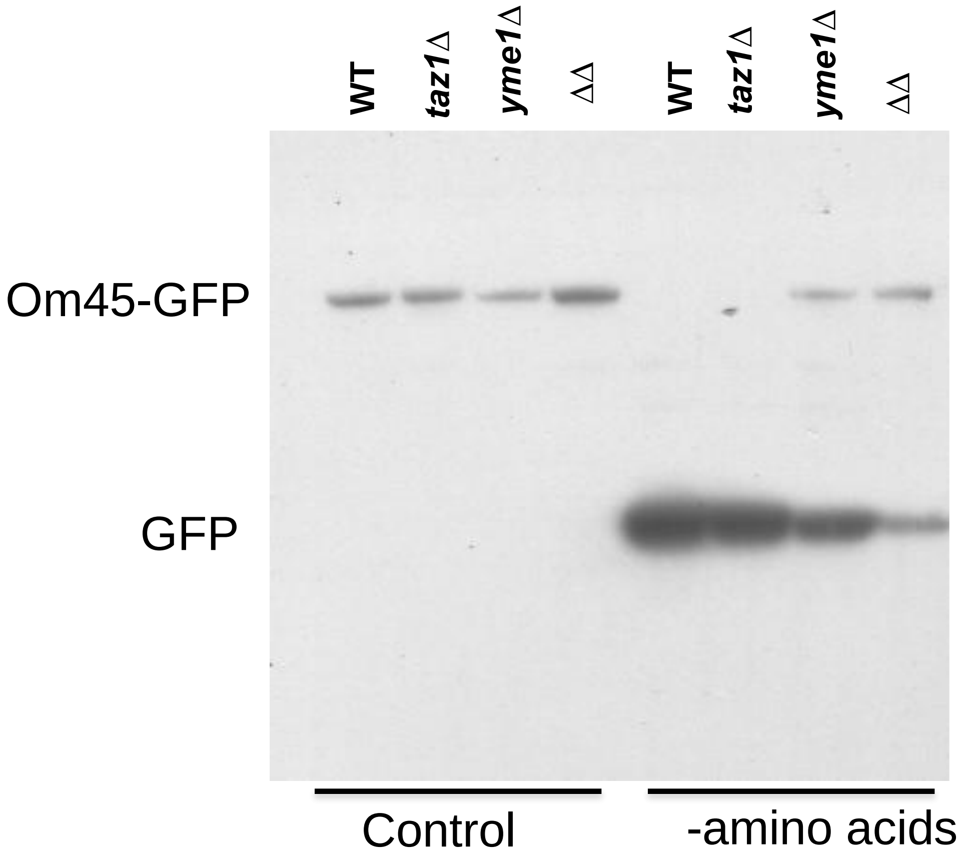


yme1Δ

2% lactate
minus a.a
24hrs

taz1Δ yme1Δ

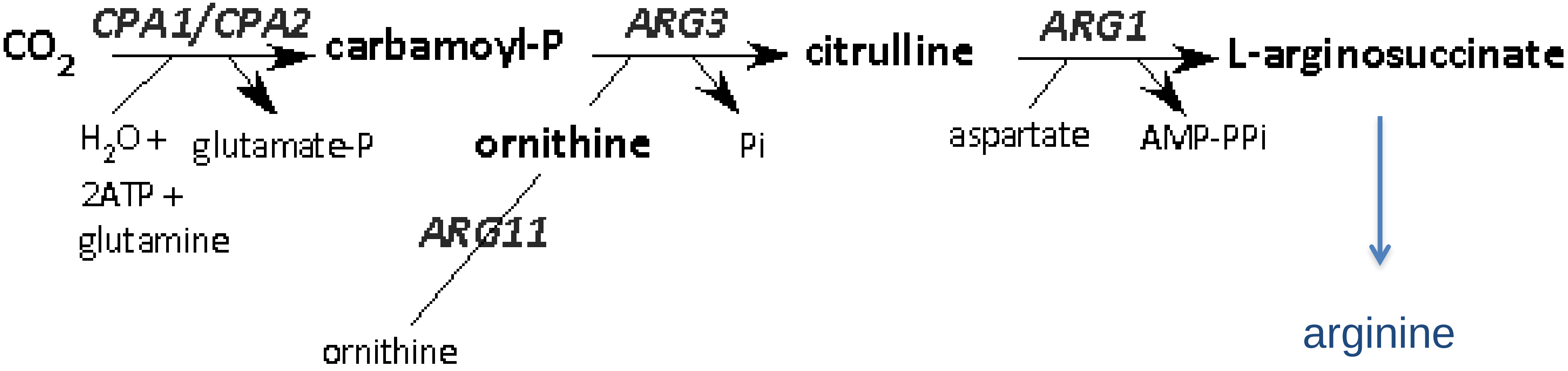
Mitophagy Assay – Om45-GFP quantified by western blot



The main take home message

Mitophagy defects are excaberbated in the absence of the *TAZ1* gene when the i-AAA protease Yme1 involved in mitochondrial inner membrane space quality control is defective

What next: Barth syndrome and the argininosuccinate shunt



Inactivation of *TAZ1* is synthetic sick with all of the above…

Decrease in arginine level in Barth patients…

Thank You

Barth Syndrome Foundation

DALHOUSIE
UNIVERSITY
Inspiring Minds

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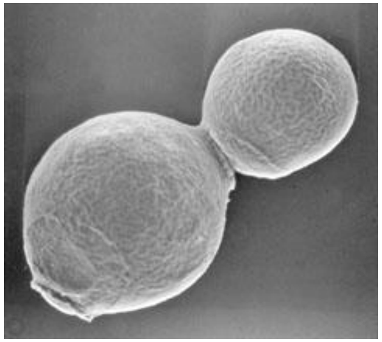
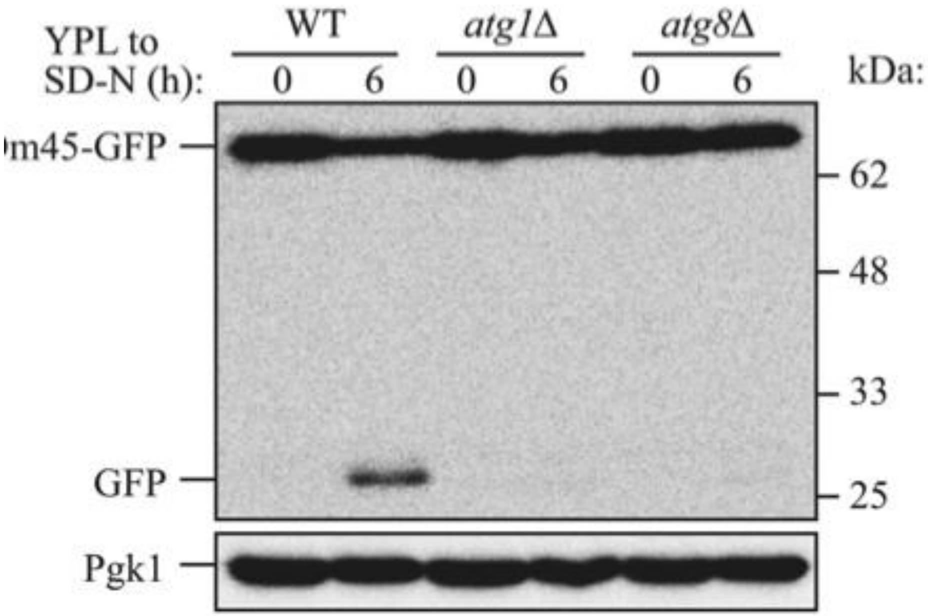
A Yeast Model of Barth Syndrome



- Yeast contain a homologue of the gene defective in Barth syndrome patients

- Inactivation of the yeast Barth syndrome gene results in the same problems in the cell as is found in humans

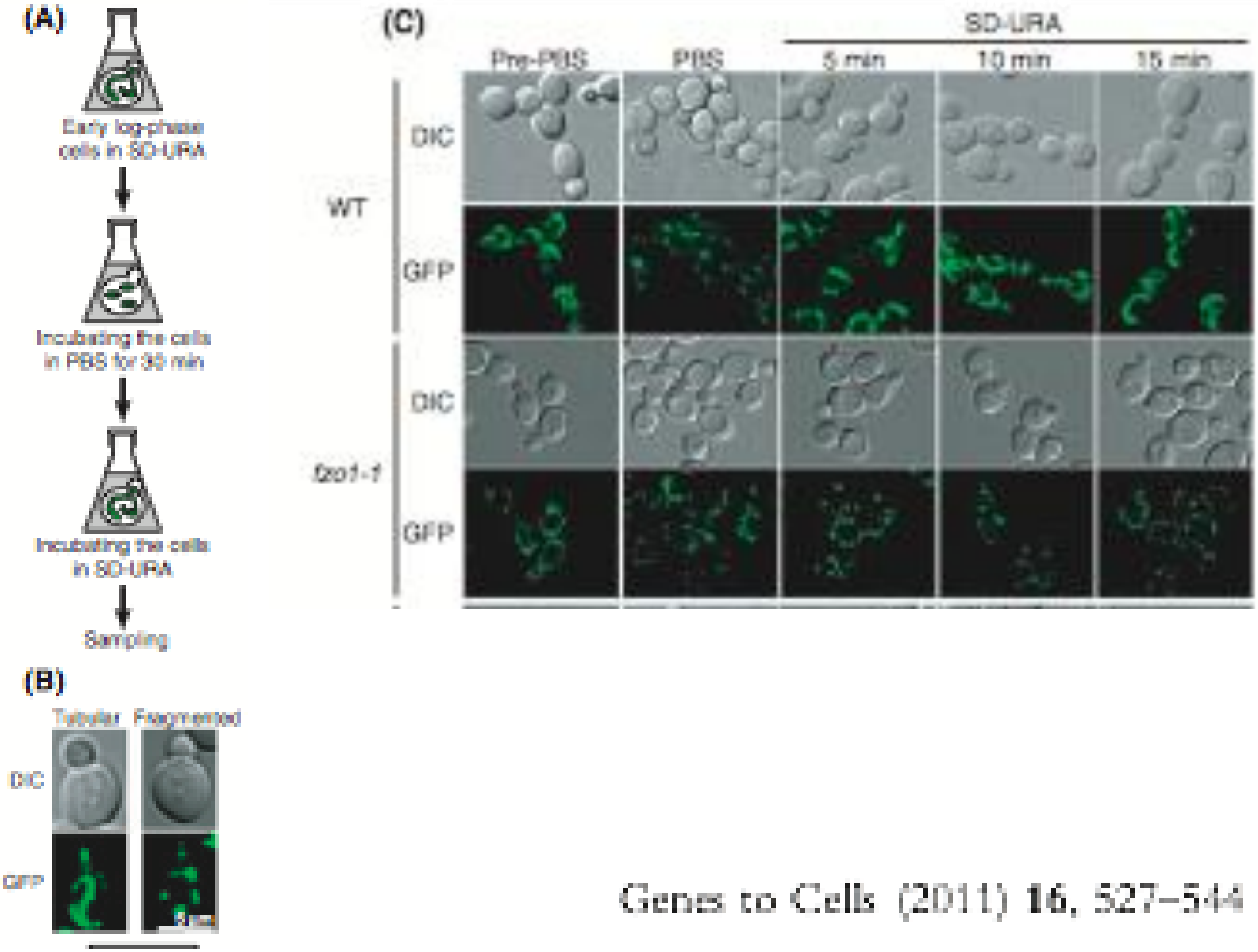
- Expression of human *TAZ* in *taz1*Δ yeast restores known defective phenotypes


YPL to SD-N (h):
WT
atg1Δ
atg8Δ
0
6
0
6
0
6
kDa:
m45-GFP
GFP
Pgk1
62
48
33
25

Future Work

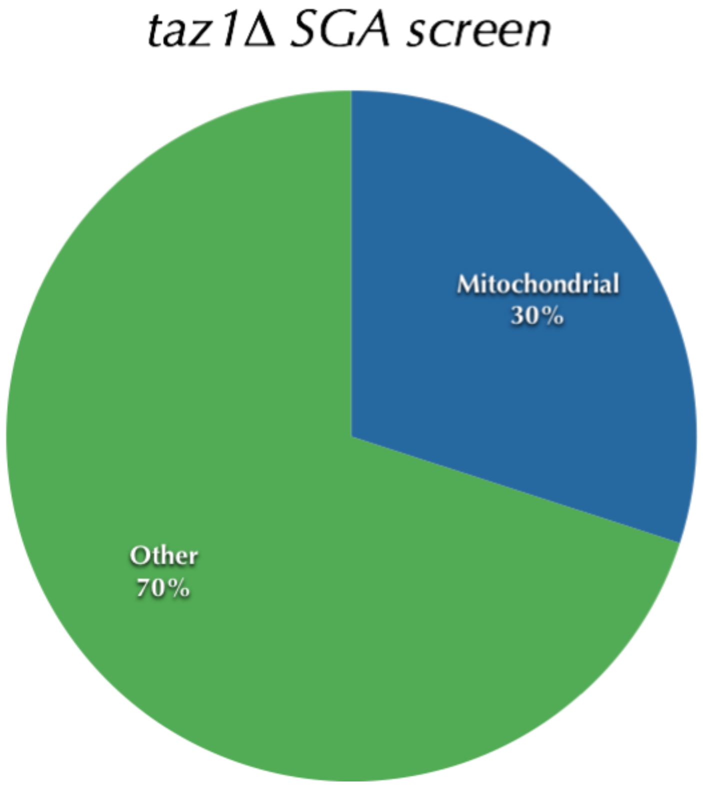
- Assess mitophagy.
- - Assess cleavage of GFP from Om-45 by western blotting.
- - Assess mitophagy in SD-N media.

- Assess ROS production in ΔΔ cells.
- - Peroxide production : Amplex red assay.
- - Oxygen consumption : Oximeter.



Genes to Cells (2011) **16**, 527–544

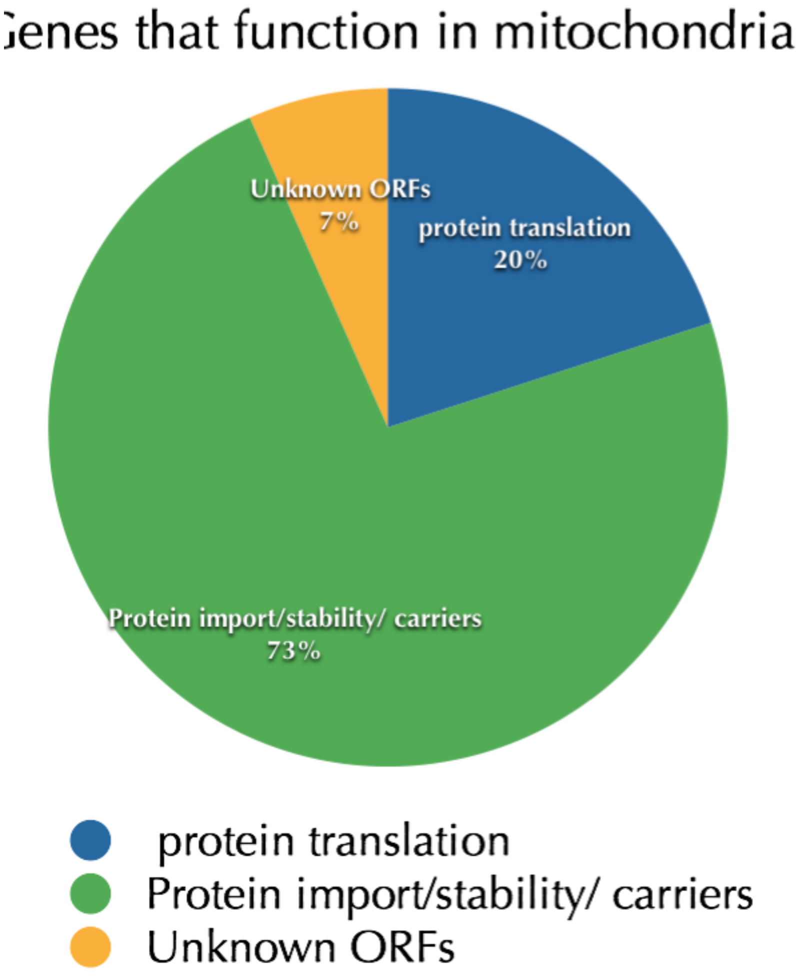
Genes deletions that reduce fitness of *taz1*Δ strain.



Arginine metabolism

Endosome trafficking

Vacuolar biogenesis



Mitochondrial Protein Import – *TOM5*

Current Biology *19*, 2133–2139, December 29, 2009 ©2009 Elsevier Ltd All rights reserved DOI 10.1016/j.cub.2009.10.074

Report

Mitochondrial Cardiolipin Involved in Outer-Membrane Protein Biogenesis: Implications for Barth Syndrome

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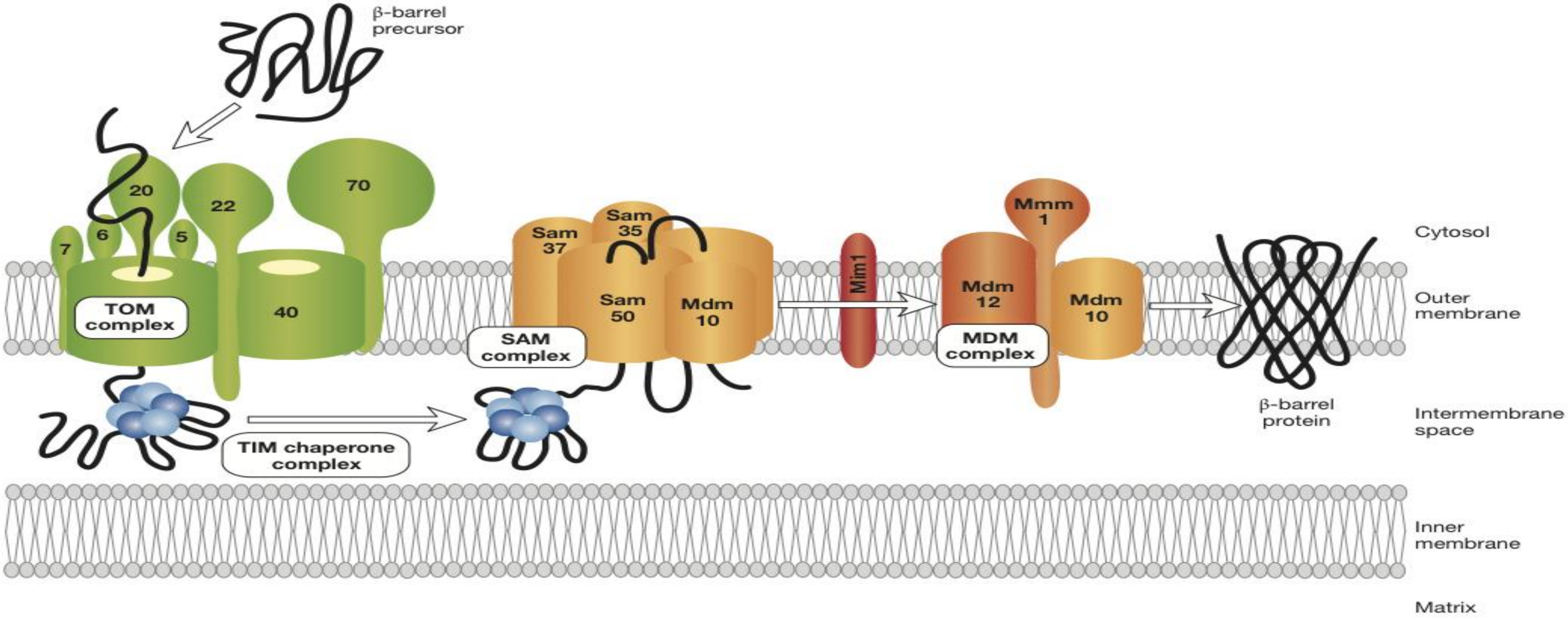
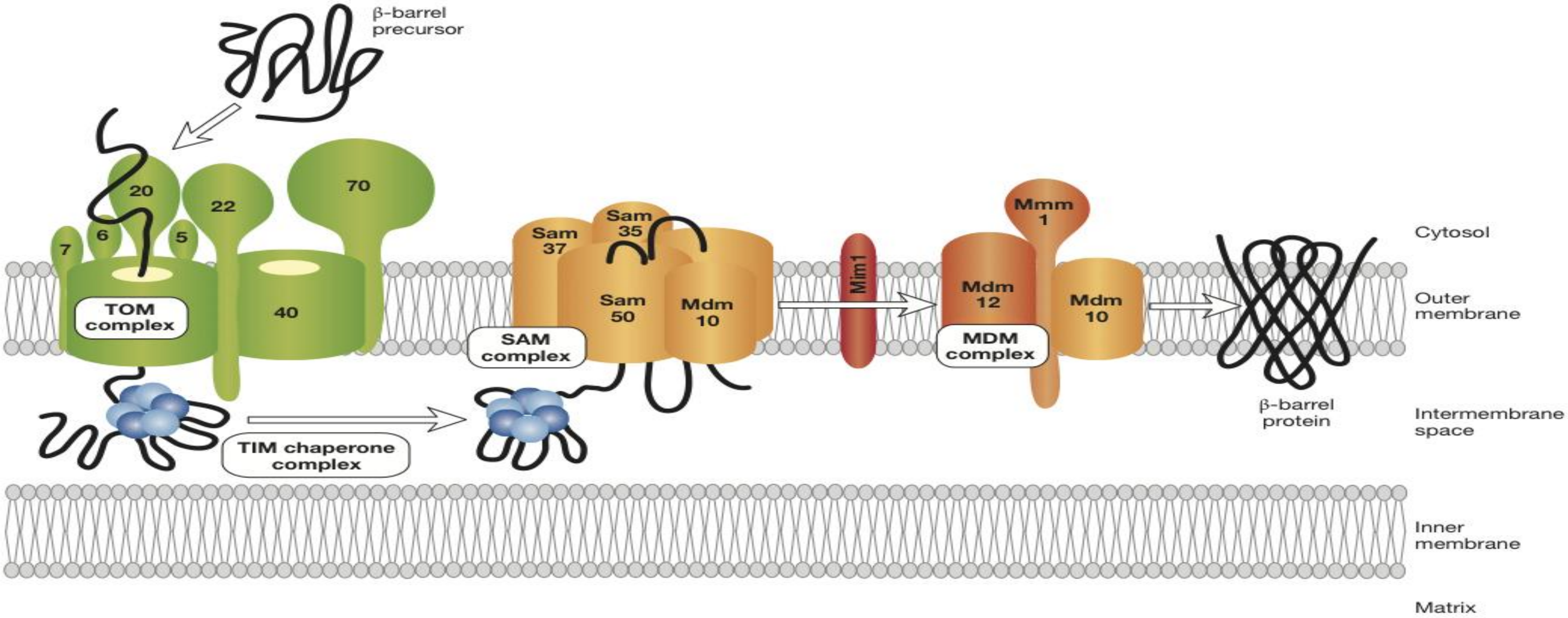
*taz1*Δ *tom5*Δ cells are synthetic lethal (do not grow)

Mitochondrial Protein Import

- *TOM5* - Component of the TOM (translocase of outer membrane) complex responsible for recognition and initial import of mitochondrial directed proteins



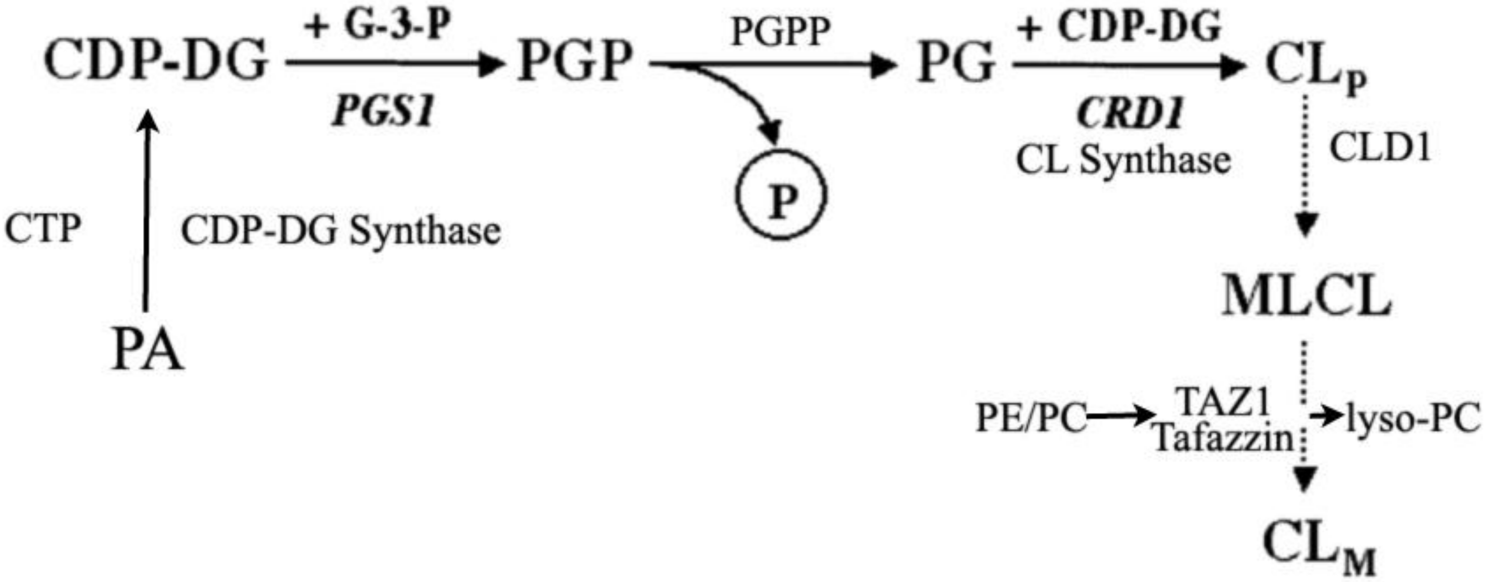


- *tom5*Δ *taz1*Δ double mutant is ‘synthetic lethal’
- assembly pathway of an outer membrane β-barrel protein is impaired in *taz1*Δ yeast cells and assembly kinetics of mouse β-barrel outer membrane protein VDAC1 is delayed in lymphoblasts from BTHS patients
- why just *TOM5* and not other components of the TOM complex - imply some specificity?

My wish list ☺

- CL and MLCL analysis by mass spec
- Mitochondrial respiration measurements
- Mitochondrial membrane potential measurements
- Urea cycle perhaps?
- Knockdowns of the genes we found in flies, fish, mice, et al and analysis of the above in these as well as yeast…
- Worth looking at Barth patients for polymorphisms in any of these genes to predict disease severity – too early perhaps…

CL synthesis and remodeling



In Barth Syndrome CL remodeling is affected

1. CL levels are reduced
2. MLCL levels are increased
3. CL has more saturated fatty acyl species

Molecular Microbiology 51(1), 149-158 (2004)

Arginine Synthesis/Urea Cycle

- *ARG11* - Ornithine transporter of the mitochondrial inner membrane, exports ornithine from mitochondria as part of arginine biosynthesis
- *ARG1* - Arginosuccinate synthetase, catalyzes the formation of L-argininosuccinate from citrulline and L-aspartate in the arginine biosynthesis pathway
- *ARG3* - Ornithine carbamoyltransferase catalyzes the sixth step in the biosynthesis of the arginine precursor ornithine
- *CPA1* - Small subunit of carbamoyl phosphate synthetase, which catalyzes a step in the synthesis of citrulline, an arginine precursor
- *CPA2* - Large subunit of carbamoyl phosphate synthetase, which catalyzes a step in the synthesis of citrulline, an arginine precursor
- *CPA1* uORF - Arginine attenuator peptide, regulates translation of the *CPA1* mRNA
- YHR091c - Mitochondrial arginyl-tRNA synthetase
- YML014w - tRNA methyltransferase, catalyzes esterification of modified uridine nucleotides in tRNA(Arg3) and tRNA(Glu)

Mitochondrial Translation

- *YMR31*-Mitochondrial ribosomal protein of the small subunit
- RSM24 -Mitochondrial ribosomal protein of the small subunit
- YKL155c- Mitochondrial ribosomal protein of the small subunit
- MRPL32-Mitochondrial ribosomal protein of the large subunit
- YEL050c - Mitochondrial ribosomal protein of the large subunit
- YMR286w - Mitochondrial ribosomal protein of the large subunit
- YLR069c - Mitochondrial translation elongation factor
- YOR187w - Mitochondrial translation elongation factor

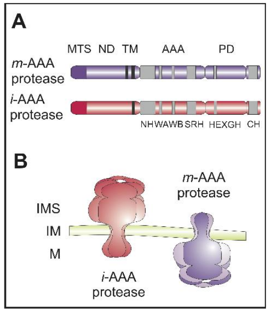
Endosome trafficking (autophagy?)

- *VPS34* - Phosphatidylinositol 3-kinase responsible for the synthesis of PI-3P; activated by the GTP-bound form of Gpa1
- *VPS15* - functions as a membrane-associated complex with Vps34; interacts with the GDP-bound form of Gpa1
- *SST2* - GTPase-activating protein for Gpa1
- *PEP7* - Multivalent adaptor protein that facilitates vesicle-mediated vacuolar protein sorting; essential for targeting of vesicles to the endosome
- *SNF7* - One of four subunits of the endosomal sorting complex required for transport III (ESCRT-III); involved in the sorting of transmembrane proteins into the endosome
- *SNF8* - Component of the ESCRT-II complex, which is involved in ubiquitin dependent sorting of proteins into the endosome
- *VPS36* - Component of the ESCRT-II complex; involved in interactions with ESCRT-I and ubiquitin-dependent sorting of proteins into the endosome

Chaperones

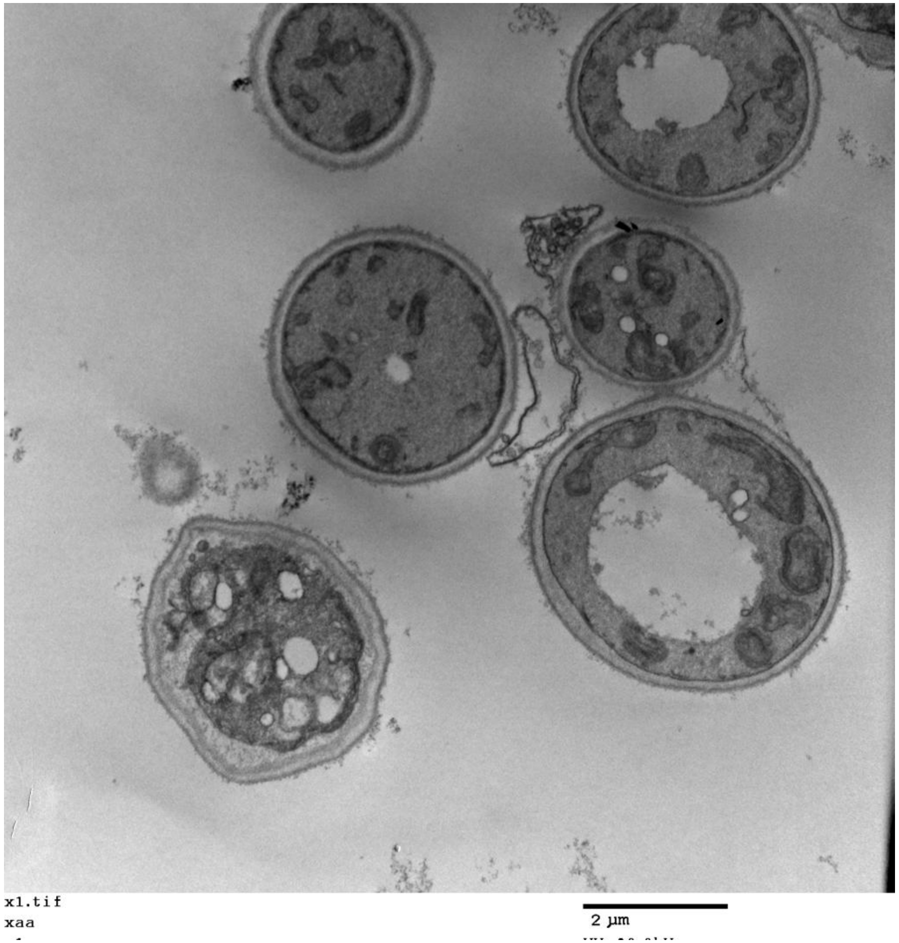
- *ZUO1* - cytosolic ribosome-associated chaperone (a DnaJ homolog)
- *SSZ1* - Hsp70 protein that interacts with Zuo1 to form a ribosome-associated complex that binds the ribosome via the Zuo1 subunit

YME1: i-AAA Protease

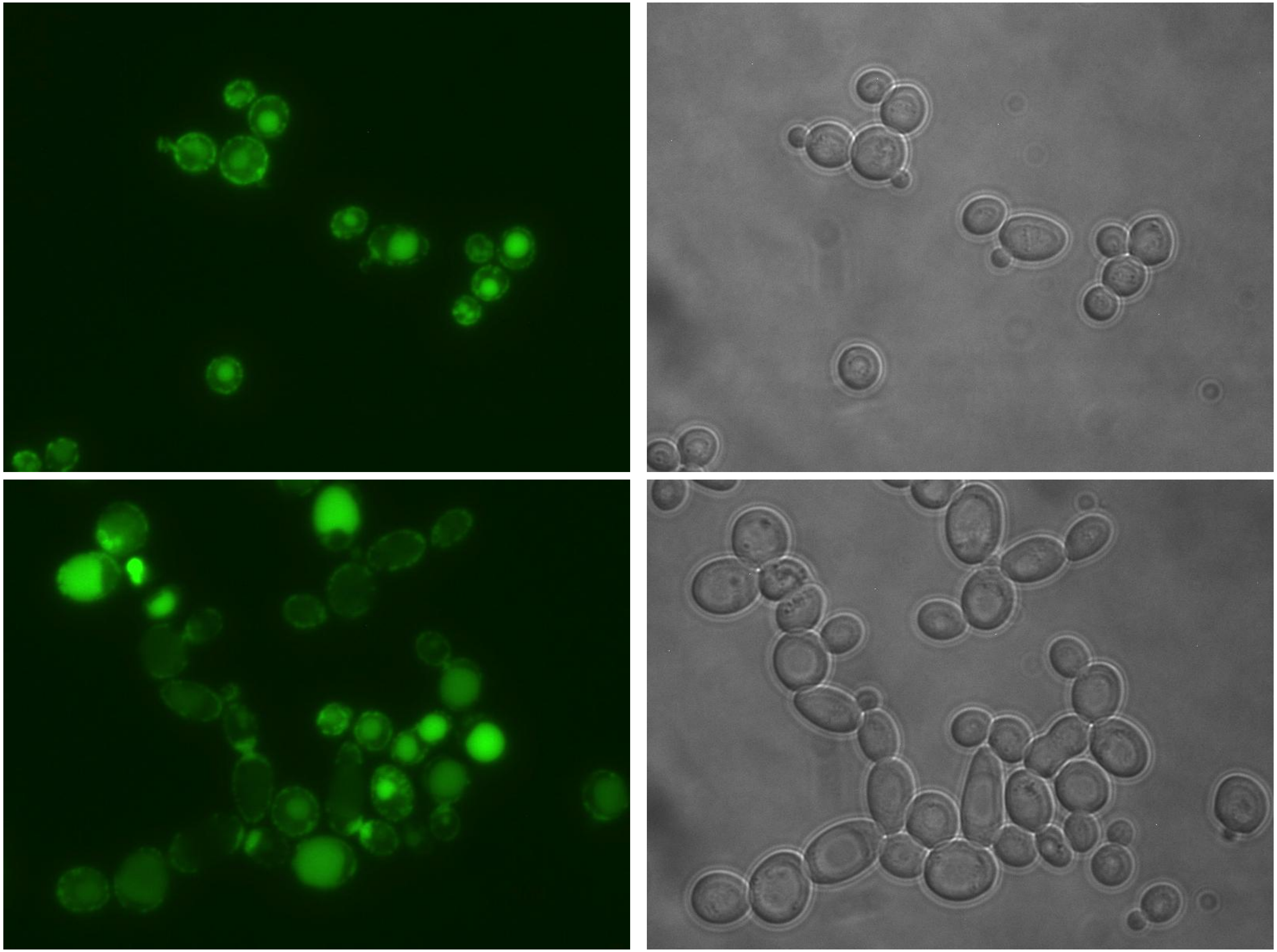


- Yme1 degrades improperly folded cytochrome c oxidase - cytochrome c association with CL is lost when CL is oxidized – is this a mimic of less unsaturated CL as is found in Barth syndrome patients/yeast? – is this occurring in cells with an inactive *TAZ1* gene resulting in Yme1 mediated proteolysis and decreasing cytochrome c oxidase function
- grow poorly and are respiratory deficient at high temperatures in non-fermentative media (similar to cells with an inactive *TAZ1* gene)
- have mitochondria that are swollen and lack reticulated network (similar to cells with an inactive *TAZ1* gene)
- role in induction of mitophagy – mitophagy is also observed in cells with an inactive *TAZ* gene

Electron microscopy to examine mitochondrial morphology – *taz1Δ yme1Δ*



Mitophagy assay (OM45-GFP) in wild type and *taz1*Δ cells



Wt

2% lactate

***taz1*Δ**

Double mutants showed defects in mitophagy

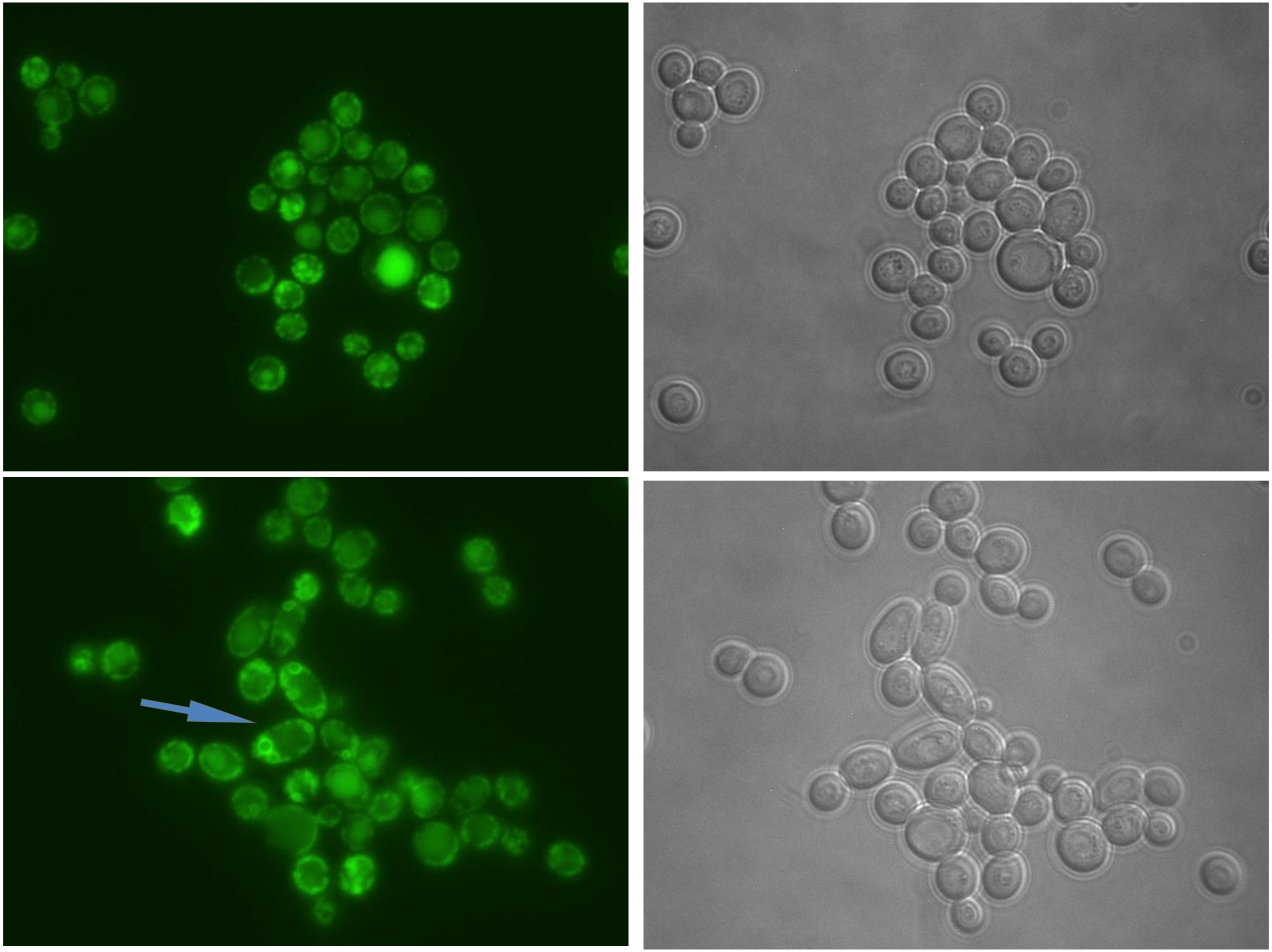
yme1Δ

OM45-GFP::TRP

YP 2% lactate

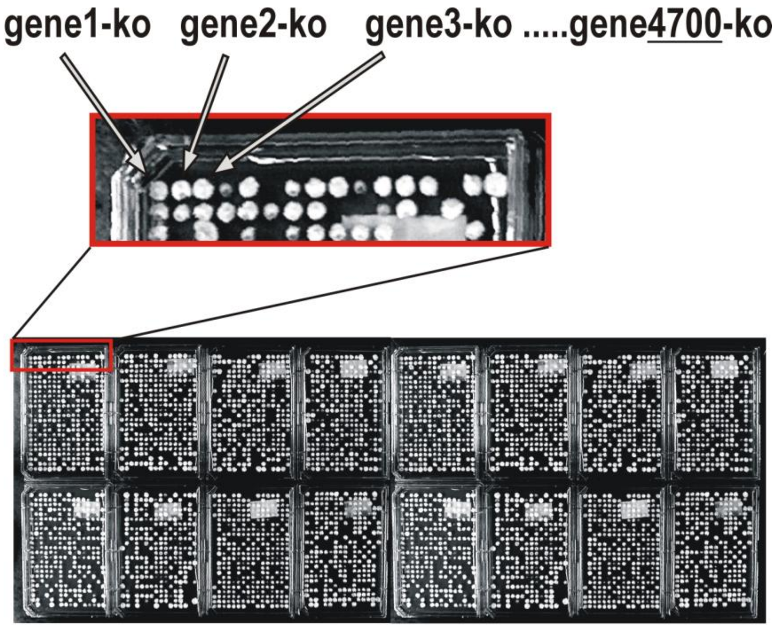
taz1Δ yme1Δ

OM45-GFP::TRP

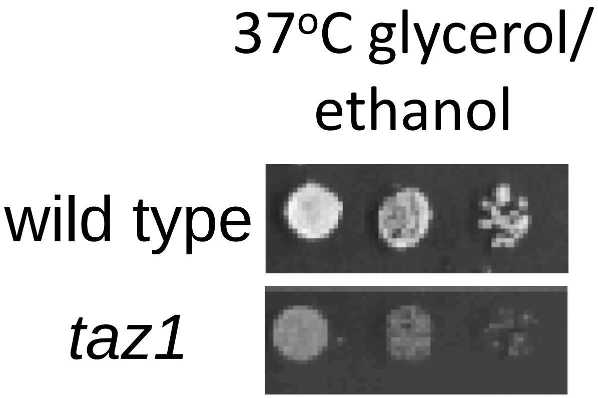


Positive modifier genes

Inactivation of what other gene(s) in combination with *TAZ1* result in a happier cell (drug targets)?



*technical difficulties



Positive modifier drugs?

Addition of what drug to a yeast cell with an inactivated *TAZ1* gene results in a happier cell?