

Supplemental Materials

Molecular Biology of the Cell

Stauffer and Powers et al.

Figure S1

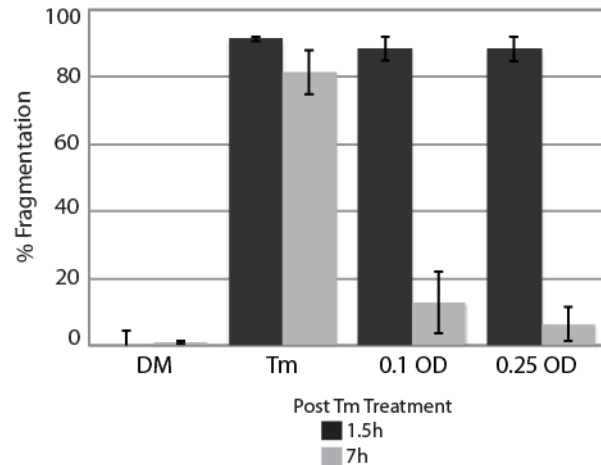


FIGURE S1: Reversibility of fragmentation following Tm treatment. (A) WT (W303 α) cells were grown overnight at 30°C to early log phase in YPD+1 μ M FM4-64. Cells were then either treated with DM or 1 μ g/mL Tm for 2h. A portion of Tm-treated cells were centrifuged and re-suspended in fresh YPD at the OD₆₀₀ indicated. Cells were centrifuged and immediately visualized at the indicated times using the Nikon microscope as described in (Materials and Methods). Quantification of vacuolar morphology was done as described in Figure 1. Depicted is the percentage of cells containing greater than 5 vacuoles/cell. The averages of three independent experiments are presented \pm SEM. In a separate experiment not shown, we observed fragmentation levels at 4h after treatment are comparable to fragmentation at 1.5h.

Figure S2

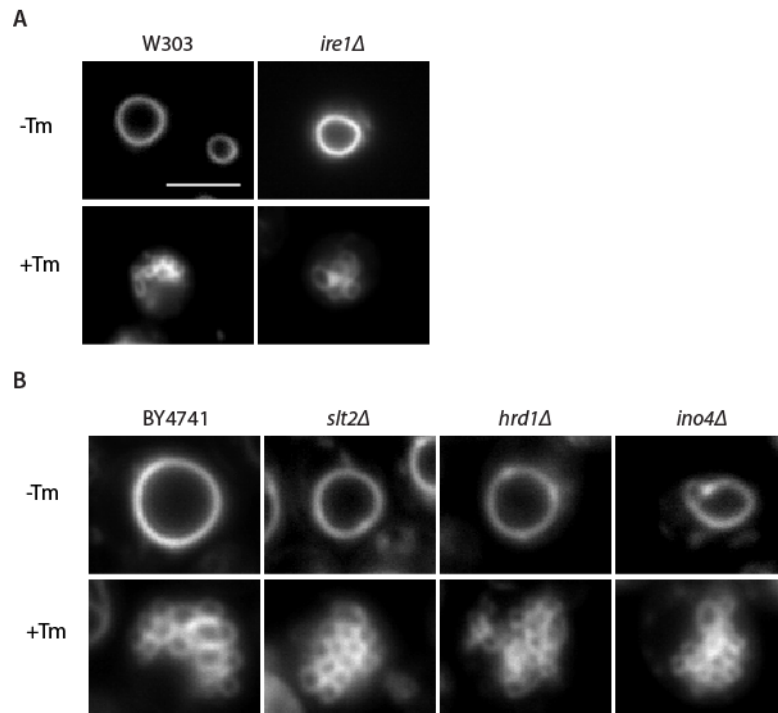


FIGURE S2: Images of cells used in Figure 2. (A) Cells were grown, treated, and quantified as described in Figure 2. Images were obtained with the Nikon microscope described in (Materials and Methods) using a 60x objective lens. (B) Images were obtained as described in (A), using a 100x objective lens. Scale bars 5 μ m.

Figure S3

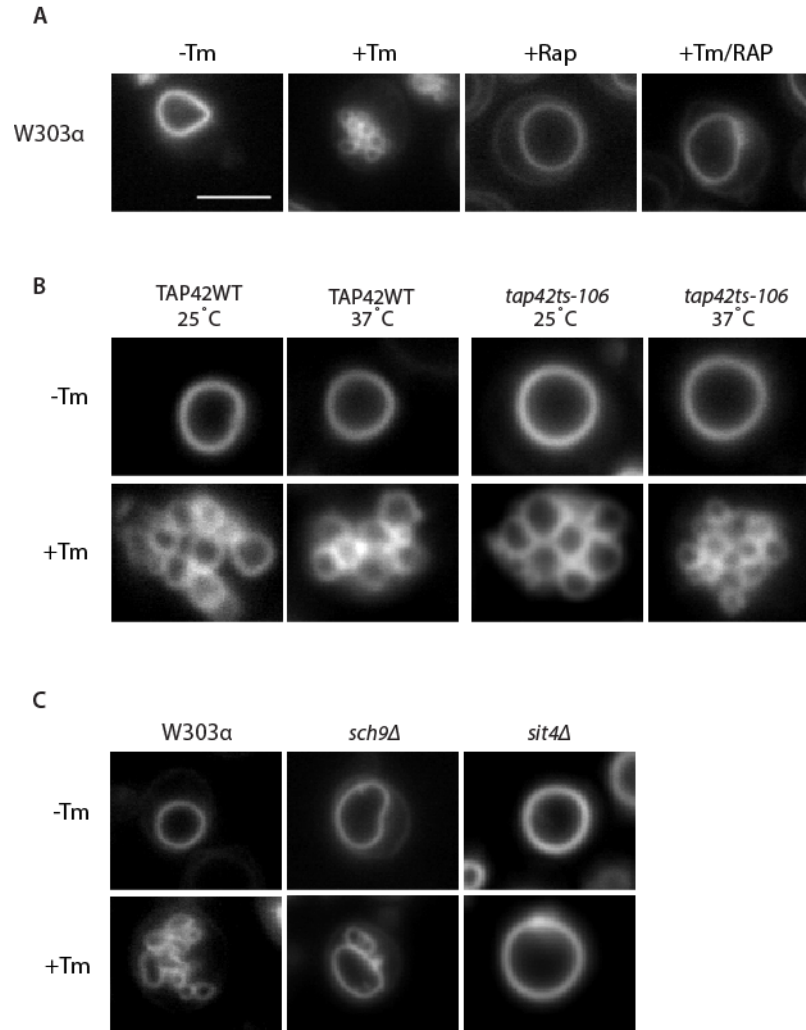


FIGURE S3: Images of cells used in Figures 3&4. Cells were grown, treated and quantified as described in figures 3&4. Images were obtained with the Nikon microscope described in (Materials and Methods) using either a 60x objective (A,C) or 100x objective (B). Scale bars 5μm.

Figure S4

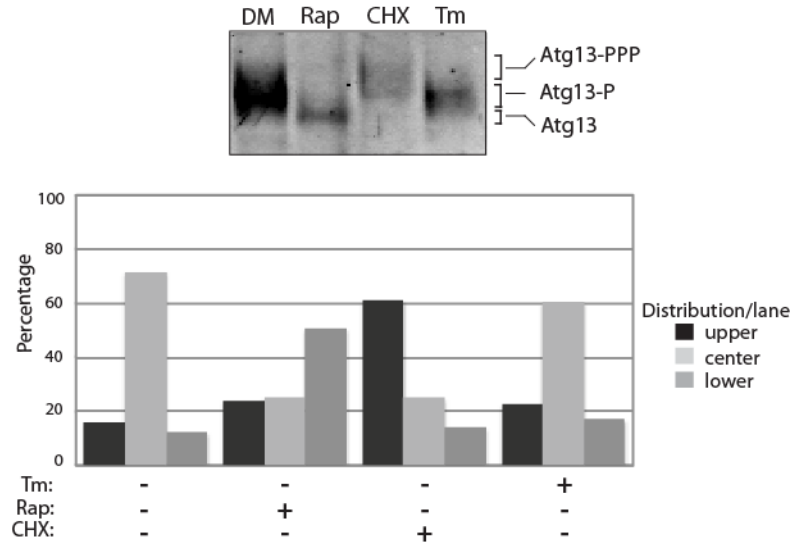


FIGURE S4: ER stress does not affect TORC1 activity towards Atg13. WT (W303 α) cells were grown to in YPD to early log phase, then treated with DM, Tm (1 μ g/mL), Rap (200ng), or CHX (25 μ g/mL) for 2h. Atg13 phosphorylation was monitored by whole cell extraction and western blot analysis using the α -Atg13 antibody. Phosphorylation was quantified as described in Figure 6.

Figure S5

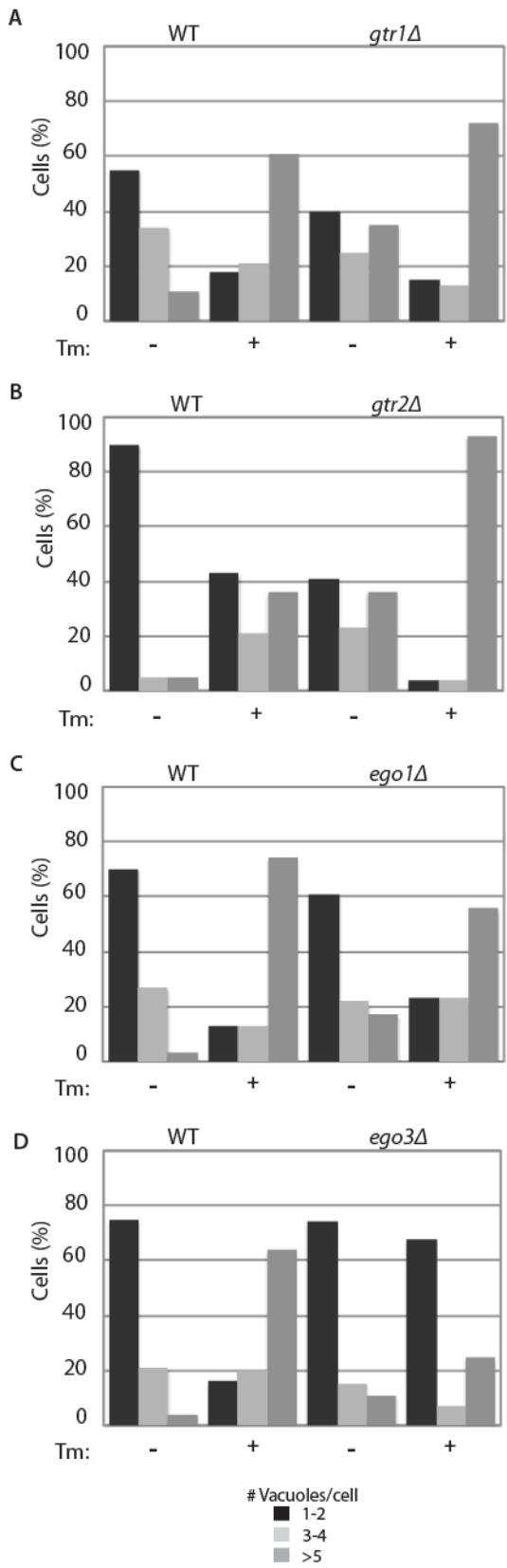


FIGURE S5: Ego3 is required for ER stress-induced vacuolar fragmentation. (A-D) WT (BY4741), *gtr1Δ*, *gtr2Δ*, *ego1Δ*, and *ego3Δ* cells were grown at 30°C overnight in YPD+1μM FM4-64 to OD₆₀₀ = 0.25, then treated with DM or 1μg/mL Tm for 2h. Cells were centrifuged and immediately visualized using fluorescence microscopy. Vacuolar morphology was quantified as described in Figure 1.

Figure S6

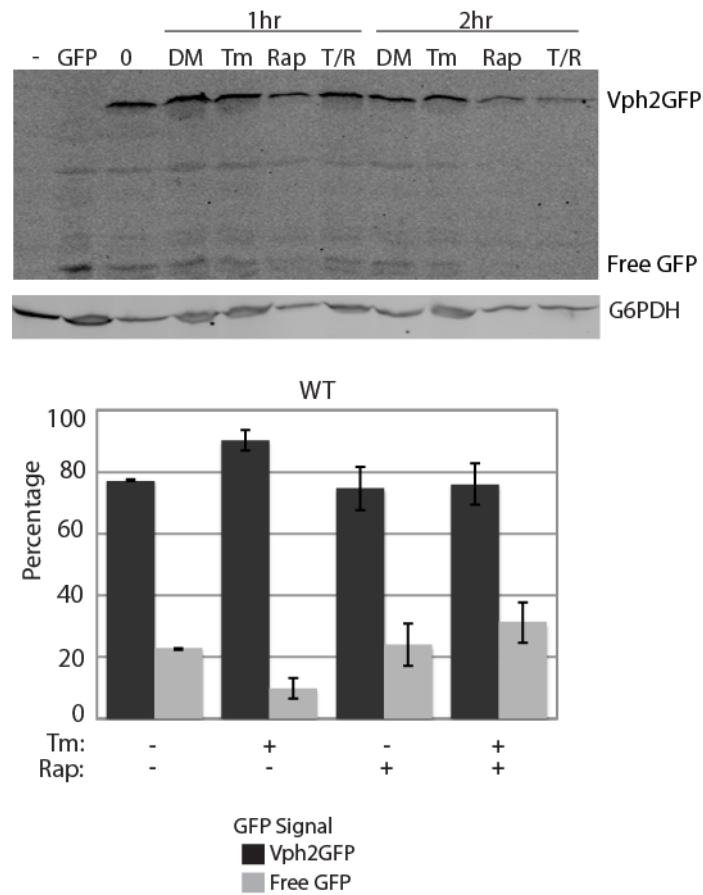


FIGURE S6: Vph2GFP is not cleaved upon ER stress. PLY1641 cells were grown at 30°C overnight to OD₆₀₀ = 0.25, then treated with DM, 1µg/mL Tm, Rap (200ng), or CHX (25µg/mL). Cells were analyzed at indicated time points by whole cell extraction and western blot analysis using α-GFP and α-G6PDH antibodies. Quantification of the amount of GFP signal per lane was performed by measuring the level of GFP signal in each lane, and dividing either the Vph2GFP or free GFP portion by the total amount of signal in each lane. Averages of three independent experiments are presented ± SEM.

Supplemental Table 1: Genome-wide screen hits

Vacuolar Proteins

PHO91

VTC4

VCX1

CLA4

AVT7

V-ATPase

VMA8

VMA7

VMA22

VMA6

VMA5

VPH2

VMA21

VMA16

YOR331C

VMA3

VMA2

VMA10

VMA13

VMA11

Vacuolar Protein Sorting & Trafficking

VPS15

PEP7

VPS45

VPS70

AP-3 complex

APL6

APS3

APM3

APL5

CORVET tethering Complex

VPS3

VPS8

PI(3,5)P2 Synthesis

FAB1

VAC14

FIG4

ER/Golgi Proteins

N linked glycosylation

ALG8

ALG9

CAX4
KTR2
YUR1
ER
EPS1
SGT2
CPR5
ATG8
HER1
YDL121C
DIE2
Golgi
GYP5
SVP26
GVP36
SYS1
VPS74
COG7
AGE2
KRE2

Mitochondrial Proteins

CAF4
FIS1
TOM7
TOM70
TOM71
MGR2
PAM17
GTF1
MRPL28
MRP2
PDR18
MMT2
MRS3
FSF1
TUM1
CTP1
COA6
ATP4
COR1
SUE1
MIG2
DLD1

RRG9

YLR346C

YIL077C

YPR098C

YLR281C

YPL168W

YPR099C

Peroxisome Function

PEX7

PEX28

FOX2

PEX3

Cell Signaling

TOR Signaling

VPS9

MDS3

EGO3

NPR1

VHS1

other

HAL5

GPB2

GRE2

WHI2

TRK1

PPZ1

SYG1

BDH2

MTL1

PPQ1

DLD3

FUS3

Vesicular Transport/endocytosis

SNC2

PEP12

SEE1

DNF3

BRE4

RIM8

ART1

CAN1

SNX4

YGR122W

YAP1801

TVP38

Cytoskeleton

VRP1

RVS161

RVS167

TPM2

HOF1

YDL162C

Metabolism

ADO1

AAH1

CYS4

MET5

YPR127W

FAA4

BIO2

DUG3

PRM15

RHR2

ARA1

ZWF1

PDC1

RIB4

DAL2

BUD17

LYP1

OSM1

CAR1

NMA2

GLG1

PIG2

GLC3

LSB6

PDR17

PDR16

INP54

DDP1

FAA3

ELO1

EEB1

ECM22

DNA functions

DNA Repair

HIM1

HNT3

APN2

POL II

SRB8

SSN8

CSE2

SIN4

TAF14

Telomere

HAT2

RSC2

TEL1

mismatch repair

PMS1

MLH1

MLH3

Other

NNF2

RAD5

ALK1

PSY2

RAD16

SDS3

ZIP2

SSP1

MND2

SPT7

HIR1

HIR3

SWD3

CNN1

GAL3

TUP1

YOX1

AFT1

HAA1

SLI15

NHP6B

HST3

YNG1

HTA1

IRC4
CTF18
DPH1
DPH6
AMA1
IML3

Translation

CLU1
DMA2
HPM1
RPL16
CCR4
POP2
ECM23
ARC1
CTL1
RPL22A
DMA2
REF2
RPL31A
RQC1
SQS1
RPL20A
RPL19-A
SNT309
ITT1
BUD23
RNP1
TAE2
SCD6
EGD2
PUF2
RPS7A
BRR1
DUS4
ELP6

Other

MUB1
UBP7
DUF1
FUB1
YBR071W
AQR1

SPO22
RMD6
GRX4
KEL2
MKT1
STB6
PDR10
TIR4
APQ12
NUP188
PHM6
ECM7
FPR3
AHP1
EPO1
HSL7
ZDS2
SFA1
YGR250C
RAM1
GRX2
STB2
GAS1
HXT14
GUP2
Unknown
YLR358C
YIL092W
YNL095C
YJR129C
YML053C
YGL235W
YML083C
YBL081W
YKL202W
YJL215C
YDR442W
YHR130C
BUD26
YNL303W
YFR056C
YEL028W
YNL324W

YAR030C
YML057C-A
YJR154W
YPR015C
RBD2
MTC4
YPL225W
YGR018C
YMR244W
HOR7
YDL218W
YHR182W
YPR174C
YHL017W
YKR075C
YDR336W
YOR097C
YBL096C
YJL163C
YOL014W
YFR018C
YFR020W
YPR114W
YOR131C
YFR006W
YNR025C
VPS69
YBR174C
YER188W
YPL073C
YOR263C
YOR364W
YKL115C
YKR033C
YGL217C
YIL028W
YPR053C
YLR402W
YOR225W
YCR102W-A
YOL153C
YDR426C

Table S2 : Top 77 Hits

ORF	# vacuole/cell (TM)		
	1 to2	3 to 4	>5
WT	11.7%	8.0%	80.3%
apl6	95.0%	5.0%	0.0%
apl5	92.0%	3.0%	5.0%
vps15	91.3%	3.3%	5.4%
vma7	90.5%	9.5%	0.0%
vma6	90.5%	7.2%	2.3%
apm3	90.1%	6.8%	3.2%
fab1	79.6%	4.1%	16.3%
aps3	76.5%	11.0%	12.5%
atg8	76.0%	12.0%	12.0%
vma3	75.7%	13.7%	10.5%
vps3	74.0%	15.0%	11.0%
vma21	73.8%	16.6%	9.6%
pep12	70.3%	15.8%	13.9%
vma16	69.9%	15.4%	14.7%
srb8	69.8%	11.3%	18.9%
pep7	67.0%	15.6%	17.5%
sit4	60.8%	15.3%	23.9%
cys4	56.3%	21.4%	22.4%
ypr099c	55.7%	25.7%	18.6%
vma8	50.7%	26.8%	22.6%
vps9	49.2%	23.4%	27.4%
vma5	46.5%	29.0%	24.5%
vph2	36.9%	1.6%	11.5%
him1	36.5%	13.5%	50.0%
grx4	36.2%	15.3%	48.5%
yor331c	32.0%	15.0%	52.9%
vma2	31.2%	33.4%	35.4%
eps1	30.2%	14.4%	55.4%
hpm1	29.9%	20.4%	49.7%
aqr1	29.5%	13.2%	57.4%
ylr358c	28.9%	20.3%	50.8%
gvp36	28.6%	12.1%	59.3%
ynl095c	28.2%	13.3%	58.5%
vma13	27.7%	18.6%	53.7%
ypl168w	26.8%	13.4%	59.9%
aah1	25.9%	10.8%	63.4%
ykl202w	25.8%	9.4%	64.8%
hal5	25.0%	11.5%	63.5%
fig4	24.8%	25.8%	49.5%

met5	22.2%	6.9%	70.9%
rpl16	21.5%	12.5%	66.0%
vma10	21.2%	17.2%	61.6%
ado1	20.5%	15.0%	64.5%
pms1	20.0%	14.6%	65.4%
spo22	19.9%	12.2%	67.9%
alg9	19.7%	10.3%	70.0%
kel2	18.0%	8.9%	73.1%
vac14	17.1%	4.1%	78.8%
yil092w	16.8%	12.8%	70.4%
gtf1	16.8%	2.9%	80.4%
whi2	16.5%	9.0%	74.5%
ccr4	16.2%	4.2%	79.6%
ypr127w	15.4%	5.5%	79.2%
vma22	15.3%	1.6%	33.1%
tum1	15.1%	9.3%	75.6%
mlh1	14.4%	12.6%	73.0%
nnf2	13.9%	19.5%	66.6%
caf4	13.8%	11.8%	74.4%
vps38	13.8%	7.1%	79.1%
yjr128c	13.7%	19.3%	66.9%
rad5	12.4%	9.8%	77.9%
gre2	11.6%	4.5%	83.9%
mtl1	11.5%	5.0%	83.5%
dld3	11.5%	6.7%	81.8%
vps30	11.0%	8.9%	80.1%
ppq1	10.4%	8.5%	81.1%
fub1	9.7%	2.2%	88.2%
trk1	8.5%	18.8%	72.7%
mds3	7.1%	2.4%	90.5%
fus3	6.5%	3.5%	90.0%
ego3	6.4%	0.5%	93.1%
tco89	6.1%	2.0%	91.9%
lsb6	6.0%	8.5%	85.5%
npr1	5.3%	0.0%	94.7%
bdh2	4.5%	8.0%	87.5%
gpb2	3.6%	2.9%	93.5%
syg1	3.2%	0.5%	96.3%
ppz1	3.1%	5.7%	91.2%
vhs1	2.5%	3.5%	94.0%

Highlighted cells represent overlap of ER stress and NaCl- induced fragmentation screens from this study and Michailat L, Mayer A. PloS one 2013