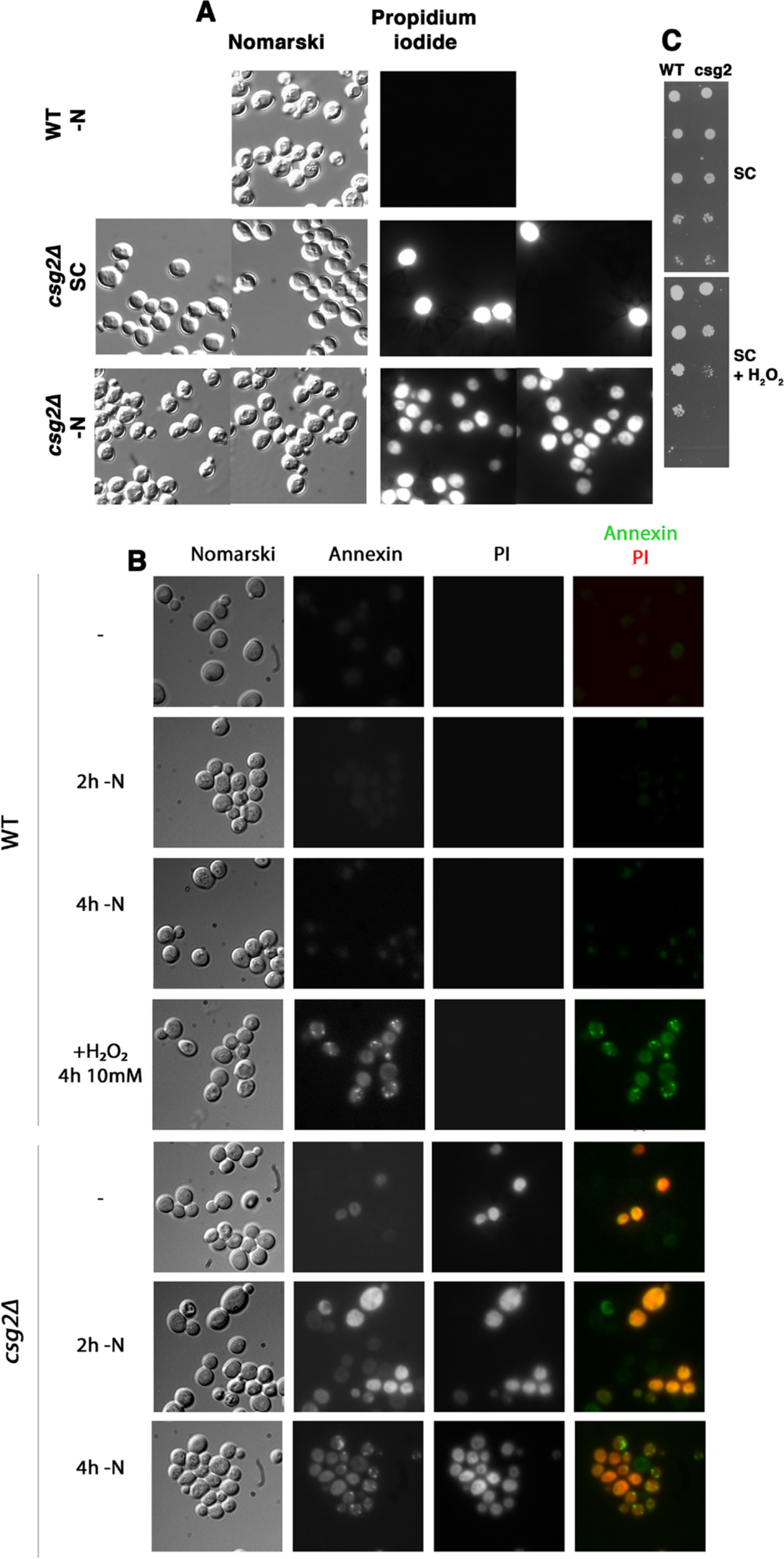
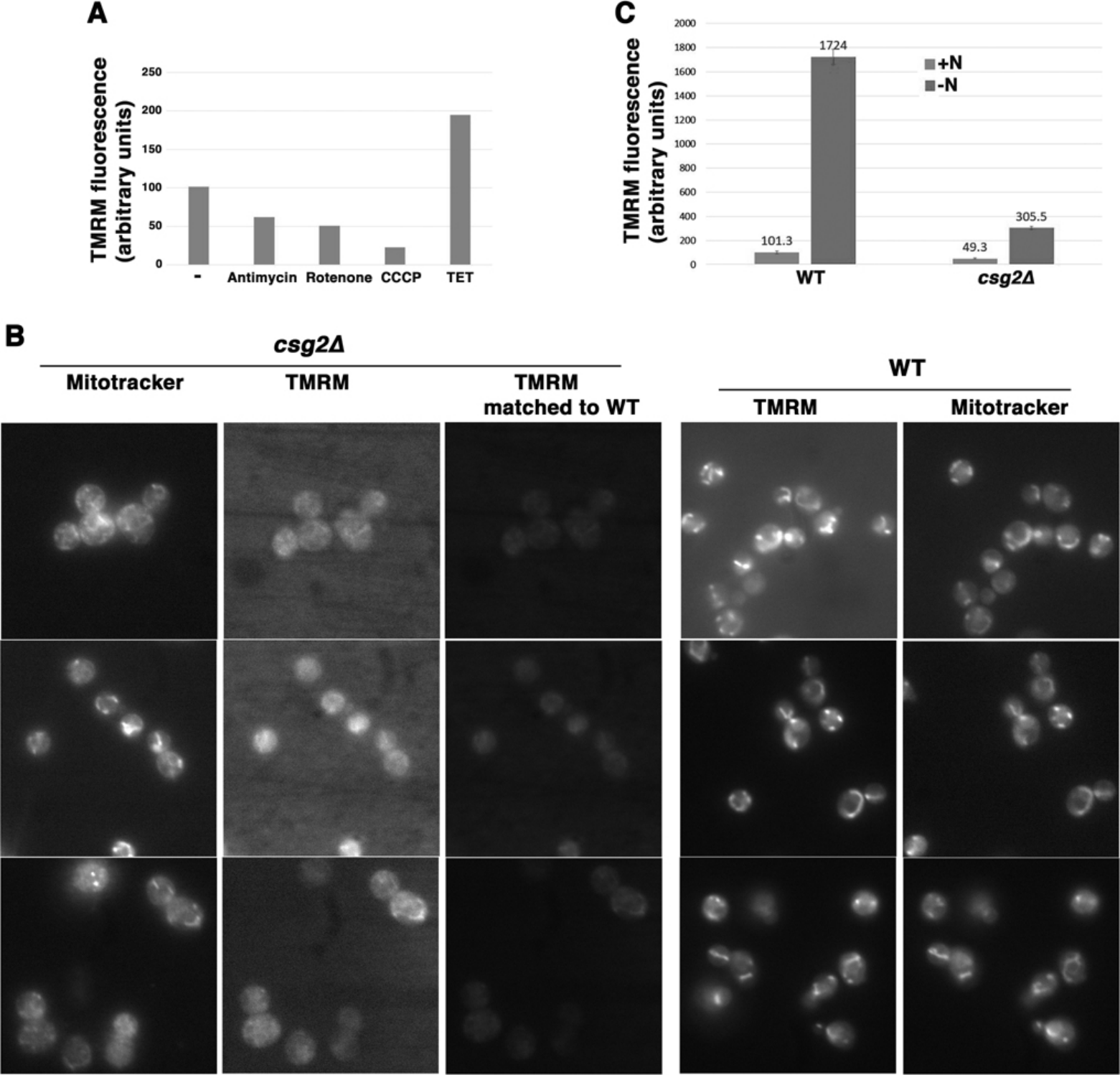
Supplemental Table 1-Mean colony numbers +/- standard deviation for cell viability assays.. Cell counts were in duplicate, n=3.

Figure	Strain	SC+gluc +/-SD	-N +/-SD	-leu +/-SD	-N-gluc +/-SD	-N+glyc +/-SD	Ras2^{Val19} +/-SD
Fig. 1B	WT	121.5+/-7.1	92.4+/-9.5	95.6+/-8.8			
	csg2	102.3+/-9.9	10.8+/-5.5	8.6+/-3.6			
	csg2 kei1	114.7+/-11.6	63.6+/-15.4				
	kei1	141.1+/-5.9	123.6+/-11.6				
	ipt1 csg2	115.7+/-7.3	15.2+/-5.5				
	ipt1	134.8+/-12.3	106.1+/-20.1				
Fig. 1C	WT *rho0*	89.5+/-17.7	78.8+/-9.4				
	WT *[CTA1]*	131.2+/-6.2	112+/-7.6				
	WT +CCCP	145.7+/-9.2	132.2+/-5.0				
	csg2 [CTA1]	99.7+/-3.6	71.5+/-5.9				
	csg2 rho0	88.1+/-8.1	75+/-3.5				
	csg2 +CCCP	122.1+/-4.8	94.6+/-10.2				
Fig. 2D	WT	101.4+/-2.1			82.6+/-10.2	93.5+/-6.8	
	csg2	88.4+/-7.4			66.7+/-5.4	61.4+/-11.6	
Fig. 4D	WT *[Ras2^{Val19}]*	103.4+/-9.2	90.2+/-9		85.4+/-5.4		
	csg2 [Ras2^{Val19}]	79.5+/-10.6	2.5+/-4.3		4.5+/-3.6		
	ras2 csg2	134.7+/-3.8	59.8+/-13.3				
	WT	94.1+/-9.4					89.8+/-11.6
	csg2	85.4+/-8.0					41.9+/-20.8
Fig. 5B	*snf1*	151.3+/-11.8	27.5+/-9.0				
	reg1 csg2	99.1+/-11.3	61.9+/-7.4				
	csg2 [SNF1-G53R]	115.9+/-14.0	74.8+/-11.1				
	reg1	121+/-4.3	94.3+/-7.1				

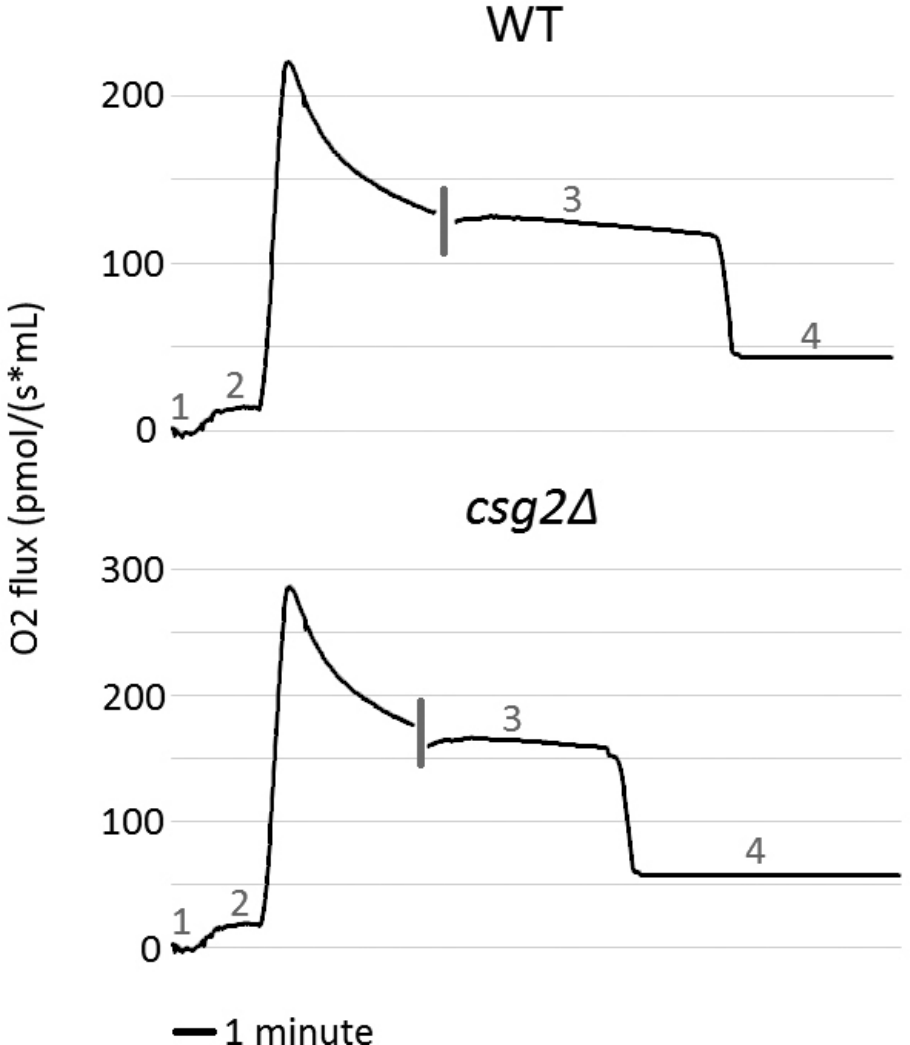
Supplemental Fig. 1-(A) Cell staining with propidium iodide confirms rapid and massive cell death in *csg2Δ* cells deprived of nitrogen. (B) Cells were shifted to nitrogen deprivation medium for various times and stained with propidium iodide and annexin V-FITC (Clontech Laboratories) according to manufacturer's instructions. As a positive control for apoptotic death, wild-type cells were exposed to 10 mM H_2O_2 for 4h before annexin V-FITC staining. (C) Growth of *csg2Δ* cells on SC plates with H_2O_2.


A
Nomarski
Propidium iodide
WT -N
csg2Δ SC
csg2Δ -N
C
WT csg2
SC
SC + H_2O_2
B
Nomarski
Annexin
PI
Annexin PI
WT
-
2h -N
4h -N
+H_2O_2 4h 10mM
csg2Δ
-
2h -N
4h -N

Supplemental Fig. 2-Relative mitochondrial membrane potential, assayed by TMRM fluorescence. Fluorescence images were quantitated by Image J; 50 cells/experiment; n = 3. (A) ETC inhibitors (antimycin A, 100 μM; Na azide, 10 μM; CCCP, 10 μM; TET, 100 μM) were added for 60 min before staining with TMRM, as described in Methods. (B) Representative images of TMRM fluorescence with Mitotracker green to localize mitochondria in mid-log cells. Matched exposure indicates *csg2Δ* cells photographed at the same exposure time with images adjusted at the same Photoshop settings as those for wild-type cells. (C) Quantitation of TMRM fluorescence in mid-log cells and after shifting to nitrogen deprivation medium for 1h.


A
TMRM fluorescence (arbitrary units)
250
200
150
100
50
0
-
Antimycin
Rotenone
CCCP
TET
C
TMRM fluorescence (arbitrary units)
2000
1800
1600
1400
1200
1000
800
600
400
200
0
1724
101.3
49.3
305.5
+N
-N
WT
csg2Δ
B
csg2Δ
Mitotracker
TMRM
TMRM matched to WT
WT
TMRM
Mitotracker

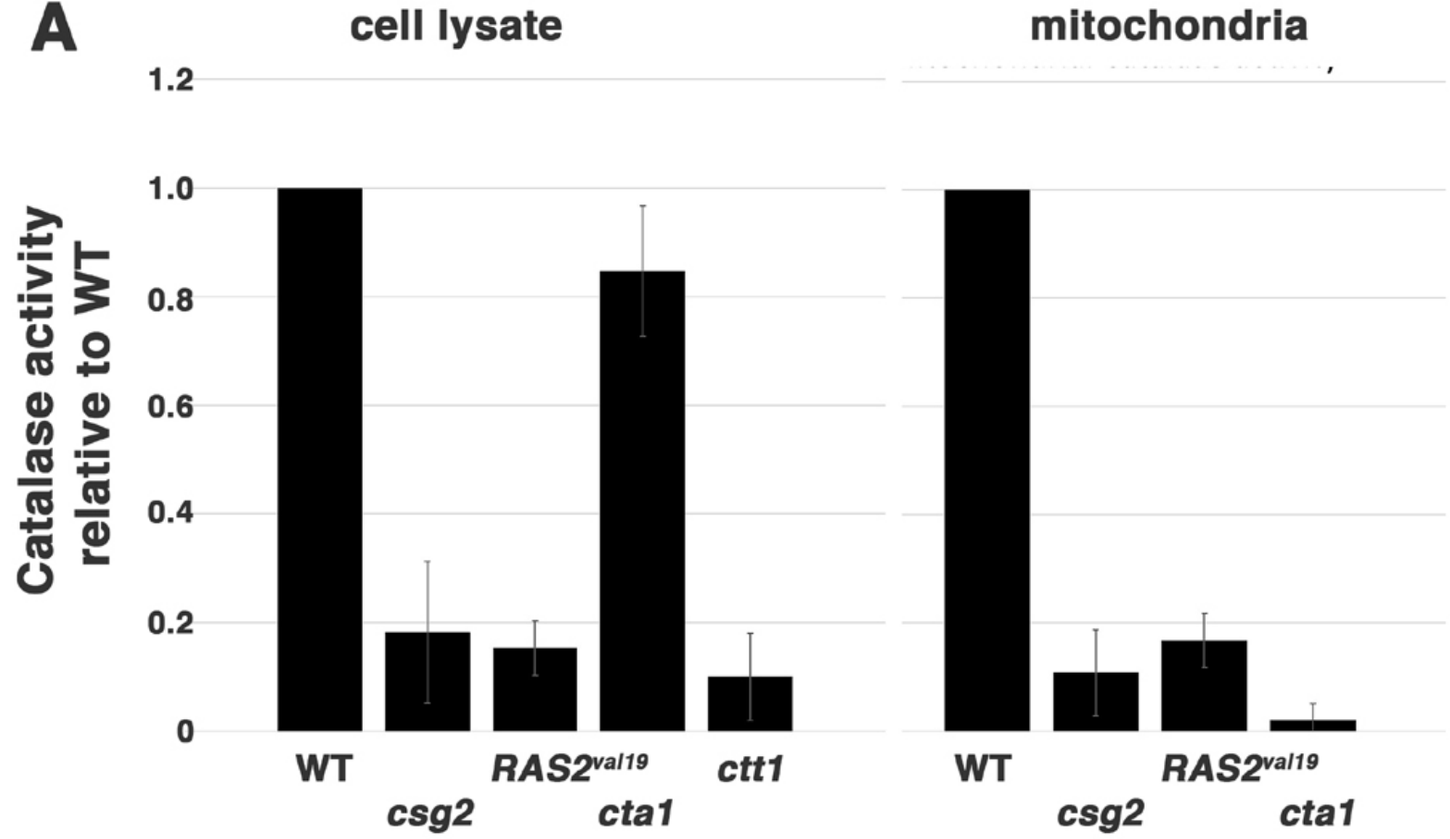
Supplemental Fig. 3- Oxygraph measurement of O_2 consumption by mitochondria isolated from WT and *csg2Δ* cells growing in SC medium with 3% glycerol. Mitochondria were normalized to protein. State (1) indicates basal mitochondrial O_2 consumption. State (2) indicates O_2 consumption after addition of 10 μM α ketoglutarate. State (3) is after addition 500 μM ADP, reflecting Vmax respiration. State (4) occurs after consumption of ADP.

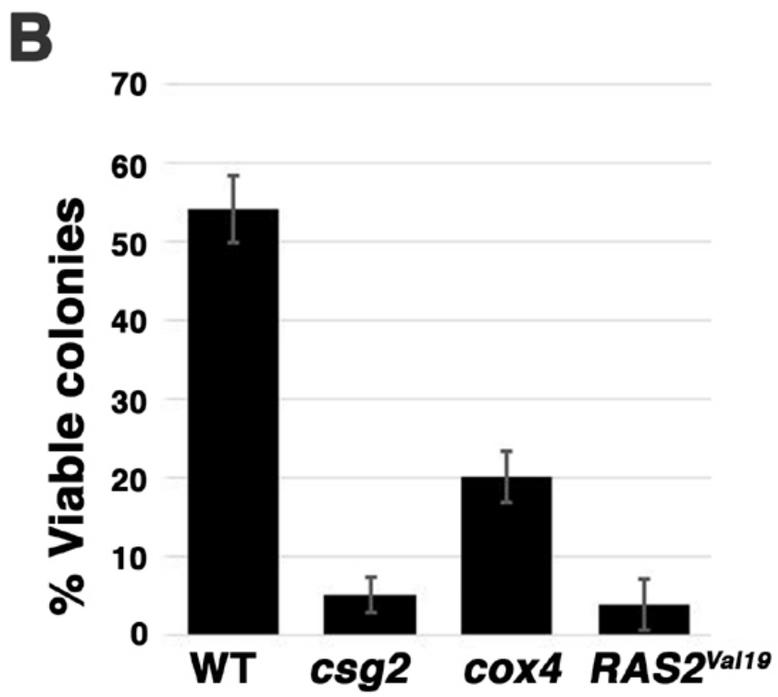

WT
200
100
0
1
2
3
4
O2 flux (pmol/(s*mL)
csg2Δ
300
200
100
0
1
2
3
4
1 minute

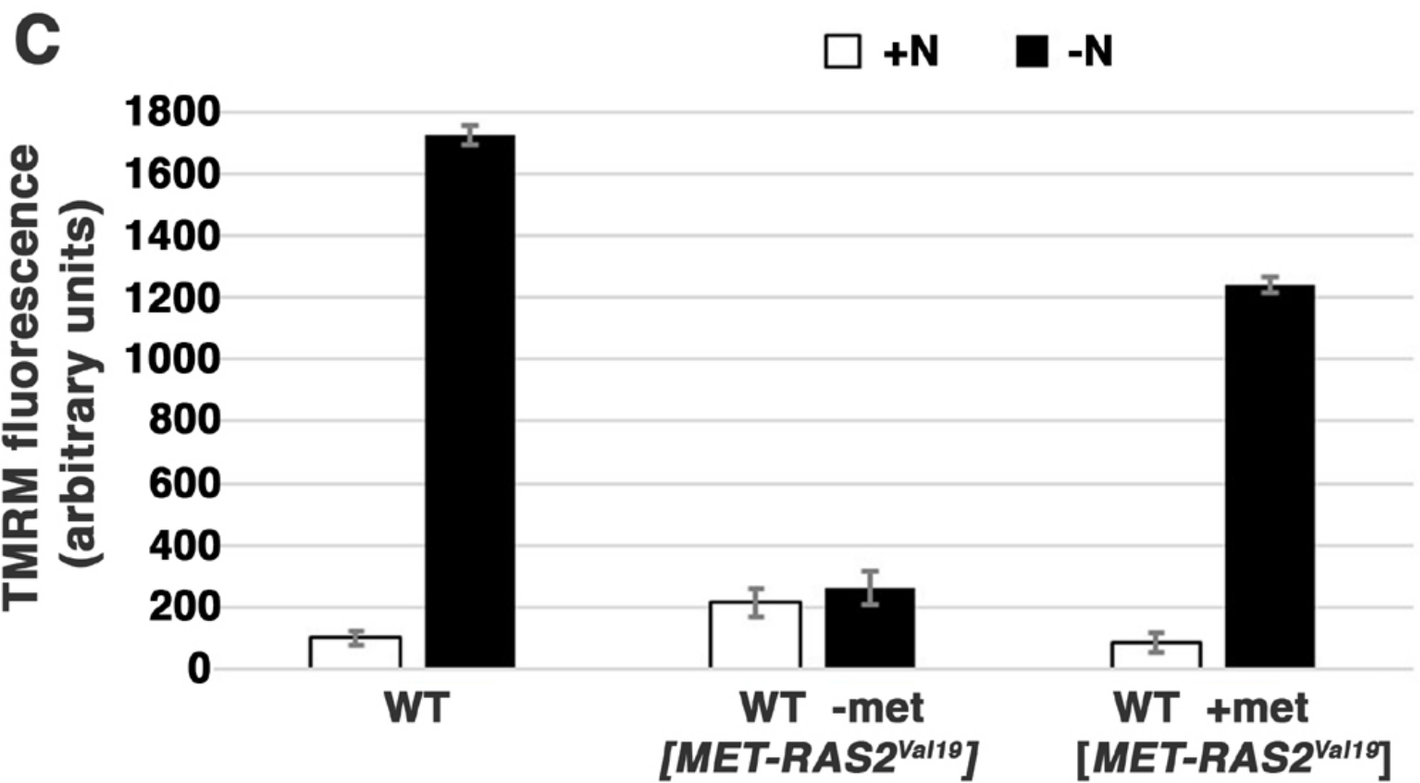
Supplemental Fig. 4- (A) Cytoplasmic catalase activity, measured in cell lysate, and mitochondrial catalase activity, measured in isolated mitochondria, are decreased in cells expressing *MET-RAS2*^{Val19}, and in *csg2Δ* cells. Hyperactive Ras was induced by washing exponentially growing cells with water and resuspending in methionine-free medium for 1h. *CTT1* and *CTA1* encode cytoplasmic and mitochondrial catalase activity, respectively.

(B) Heat shock sensitivity was measured by shifting cells to 52°C for 10 min and then plating for viability. Colony numbers were expressed as a percent of those formed from cells that were not temperature-shifted but plated directly.

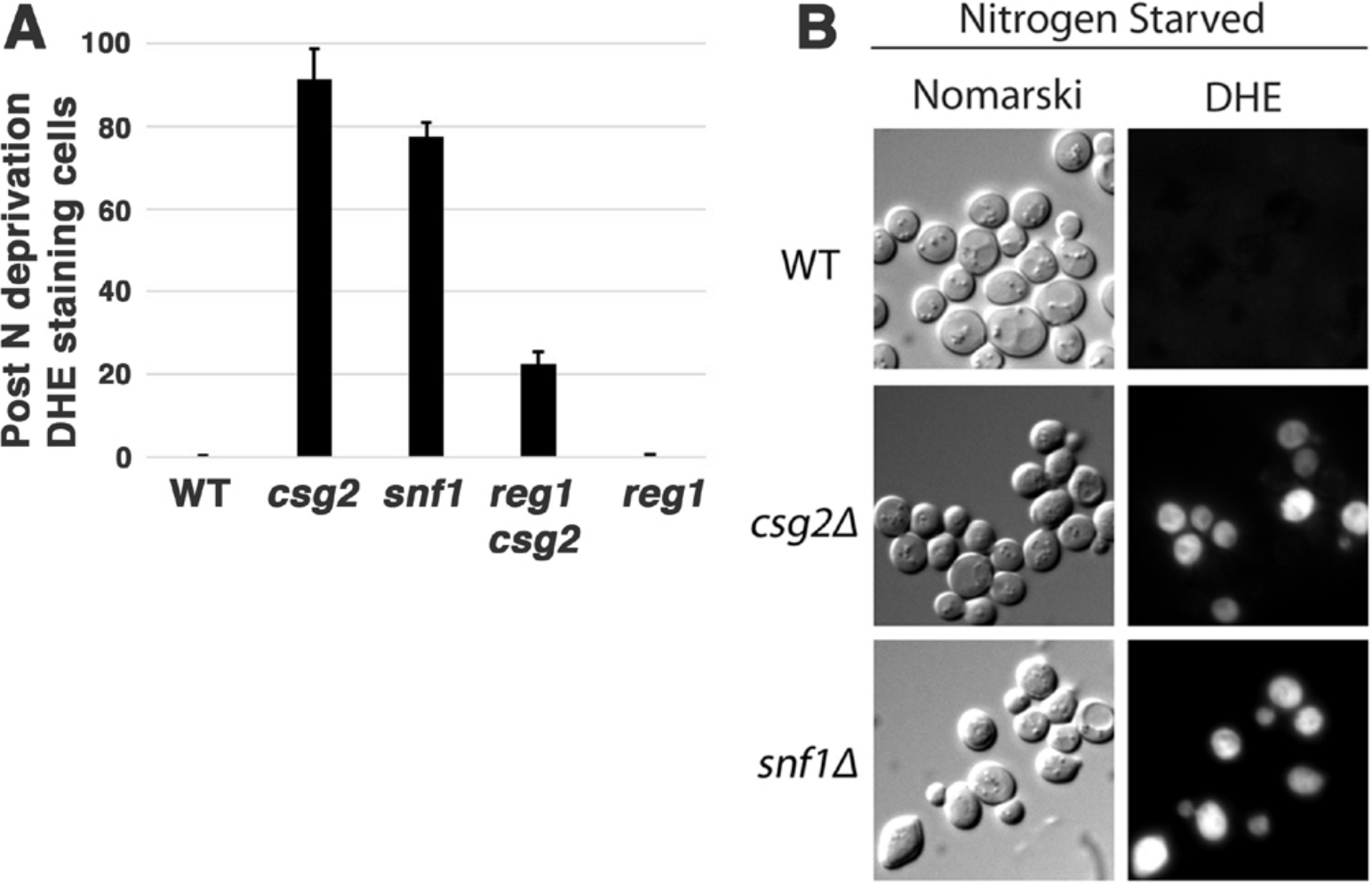
(C) Hyperactive Ras2^{Val19} prevents increased MMP during nitrogen deprivation. Exponentially growing wild-type cells bearing *pMET-RAS2*^{Val19} were washed with water and incubated in medium with or without methionine for 3h; cells were then washed with water and resuspended in nitrogen deprivation medium for 1h. Relative MMP was measured by TMRM fluorescence; fluorescence in 50 cells were quantitated by Image J/experiment; n = 3.


A
cell lysate
mitochondria
Catalase activity relative to WT
1.2
1.0
0.8
0.6
0.4
0.2
0
WT
csg2
RAS2val19
cta1
ctt1
WT
csg2
RAS2val19
cta1


B
% Viable colonies
70
60
50
40
30
20
10
0
WT
csg2
cox4
RAS2Val19


C
+N
-N
TMRM fluorescence (arbitrary units)
1800
1600
1400
1200
1000
800
600
400
200
0
WT
WT -met [MET-RAS2Val19]
WT +met [MET-RAS2Val19]

Supplemental Fig. 5- ROS accumulation in *csg2Δ* cells is abrogated by *reg1Δ*, constitutively activating Snf1; ROS accumulates in *snf1Δ* cells during nitrogen deprivation. (A) Quantitation of DHE stained cells post-nitrogen deprivation; fluorescence in 150 total cells quantitated by Image J; n = 3. (B) DHE staining of cells after nitrogen deprivation for 4h.


A
Post N deprivation
DHE staining cells
100
80
60
40
20
0
WT
csg2
snf1
reg1
csg2
reg1
B
Nitrogen Starved
Nomarski
DHE
WT
csg2Δ
snf1Δ