

Transcriptional and post translational roles of calcineurin in cationic stress and glycerol biosynthesis in *Cryptococcus neoformans*

Authors

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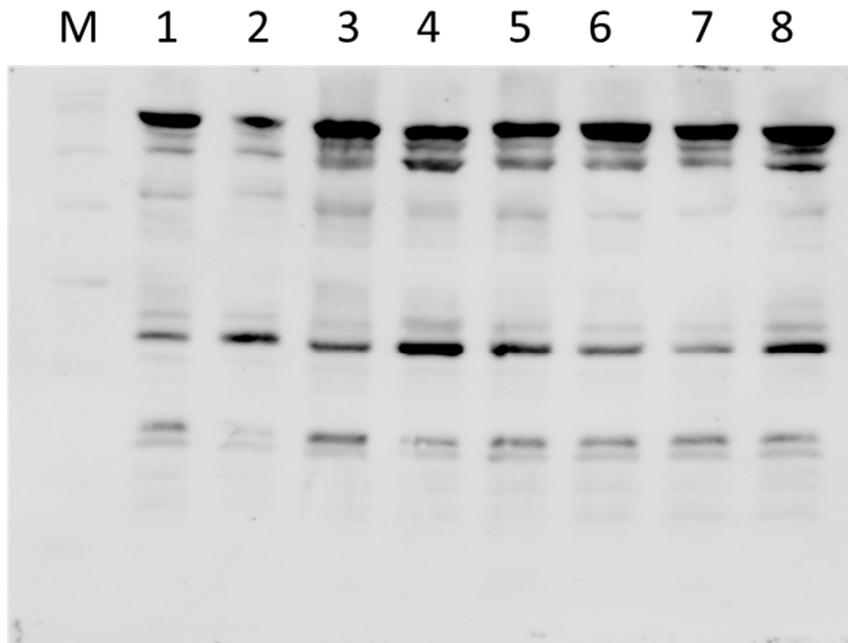
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Keywords: *Cryptococcus neoformans*; calcineurin; glycerol phosphate phosphatase;  
osmotic stress; cationic stress, *GPP2*

Supplementary Figure S1: Original membrane image presented in the western blot, after 2 hours of induction at NaCL (Figure 2A). The first 3 lanes were cropped.



M- Molecular weight marker

1- CNU151 0M

5- CNU151 0M BR2

2- CNU151 1.5M

6- CNU151 1.5M BR2

3- CNU151 0M BR1

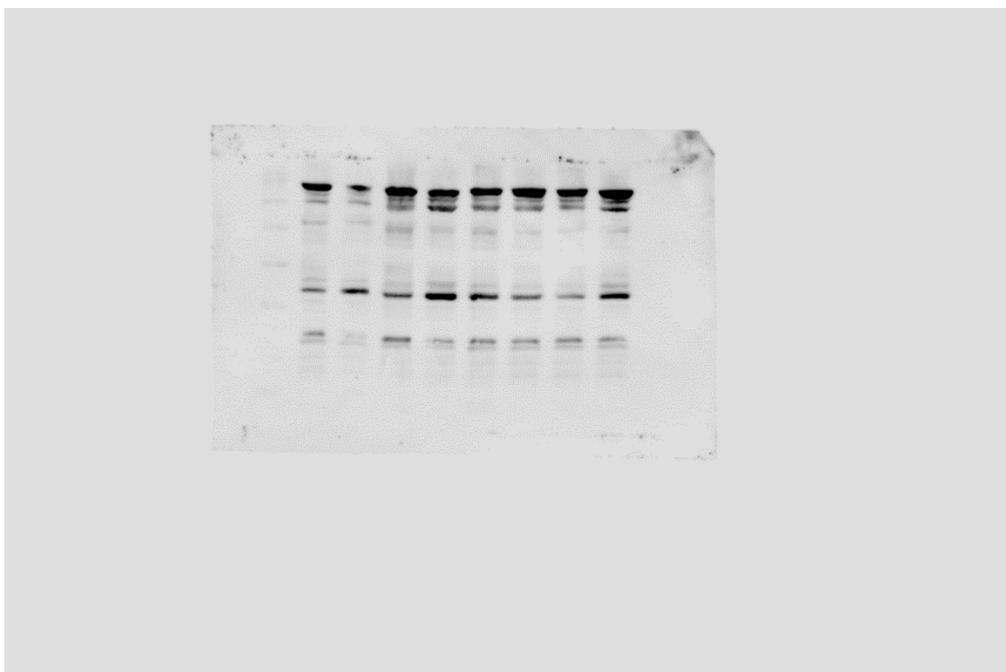
7- CNU151 0M BR3

4- CNU151 1.5M BR1

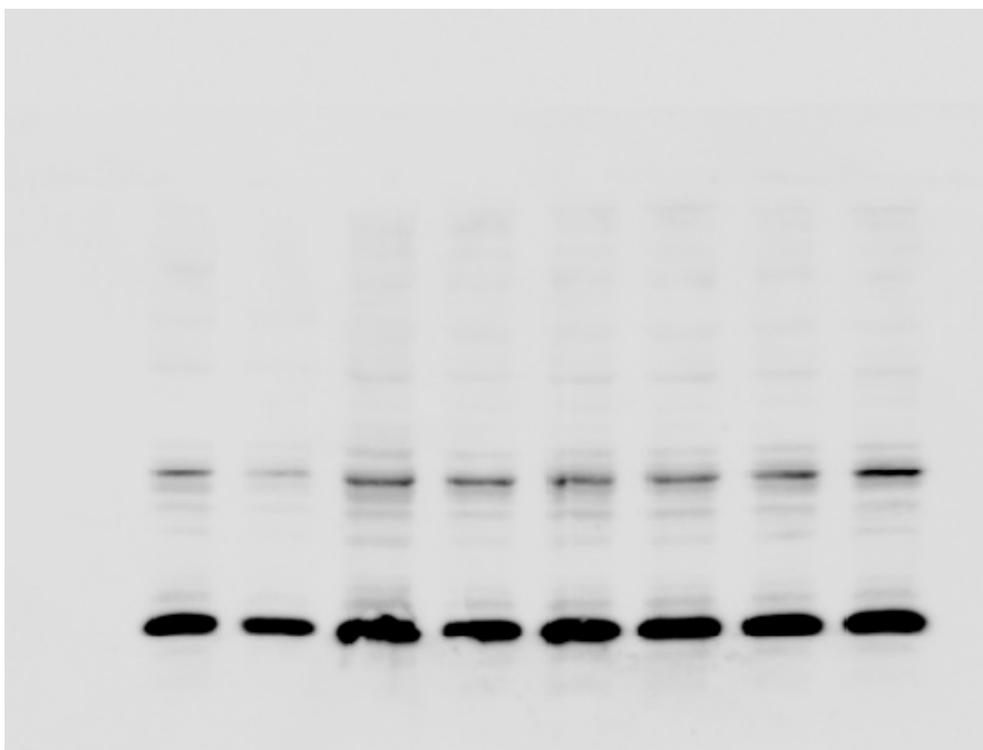
8- CNU151 1.5M BR3

\*BR- Biological Replica

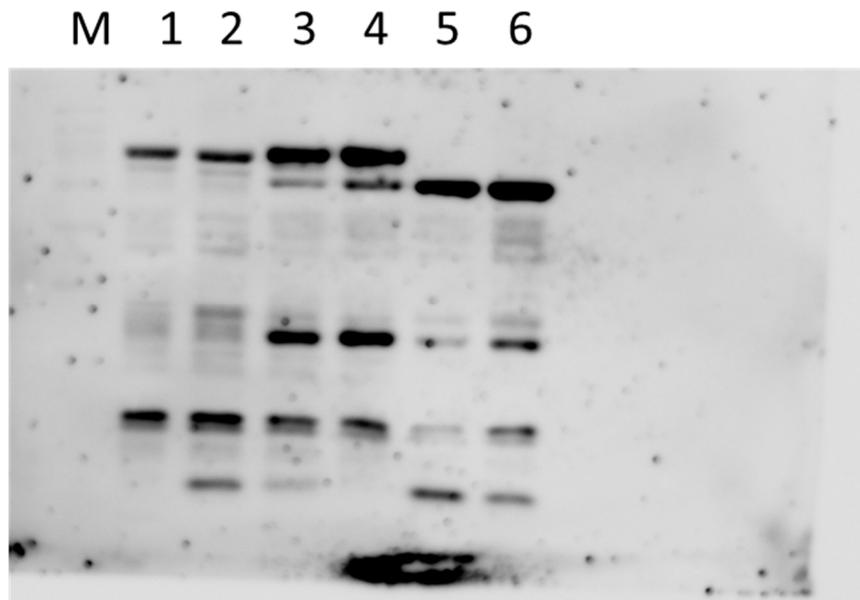
Supplementary Figure S2: Original western blot membrane that originated Figure 2A, detecting Gfp-Gpp2. Anti-Gfp antibody.



Supplementary Figure S3: Original western blot membrane that originated Figure 2A - normalizer Histone H3. Anti-Histone antibody.



Supplementary Figure S4: Original membrane image presented in the western blot, after 2 hours of induction at NaCl (Figure 2B). The first 3 lanes were cropped.



M- Molecular weight marker

1- CNU151 0M

5- CNU193 0M

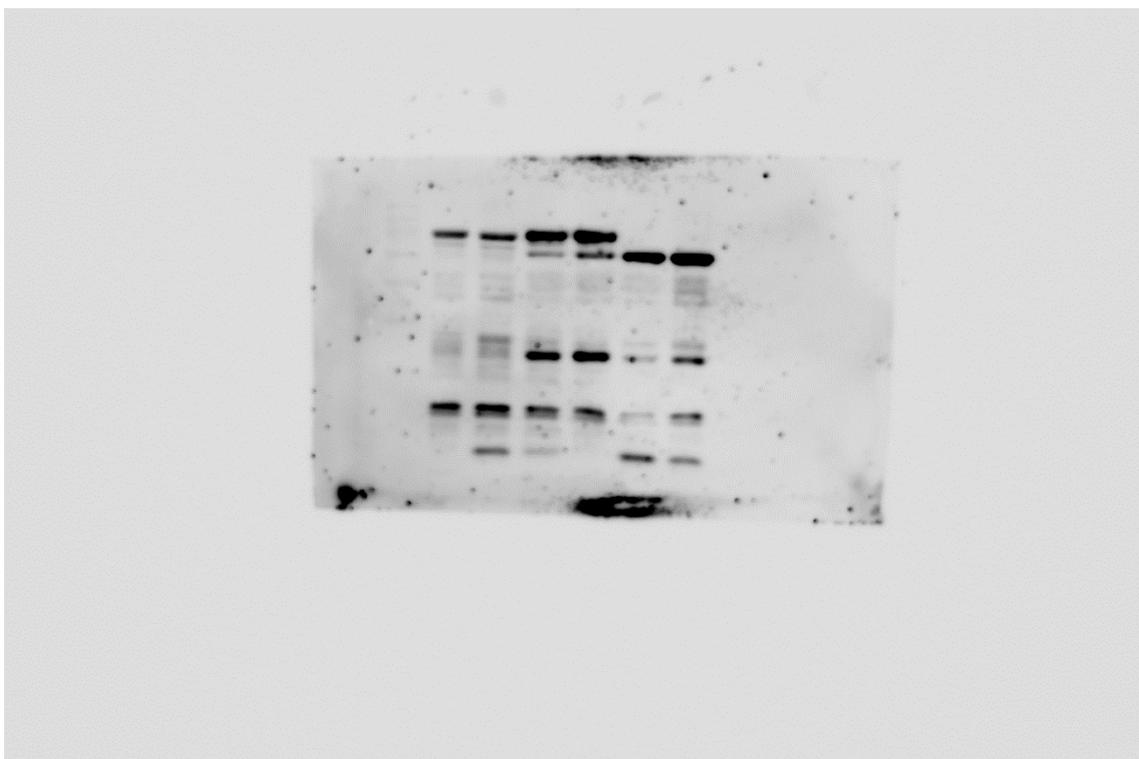
2- CNU151 1.5M

6- CNU193 1.5M

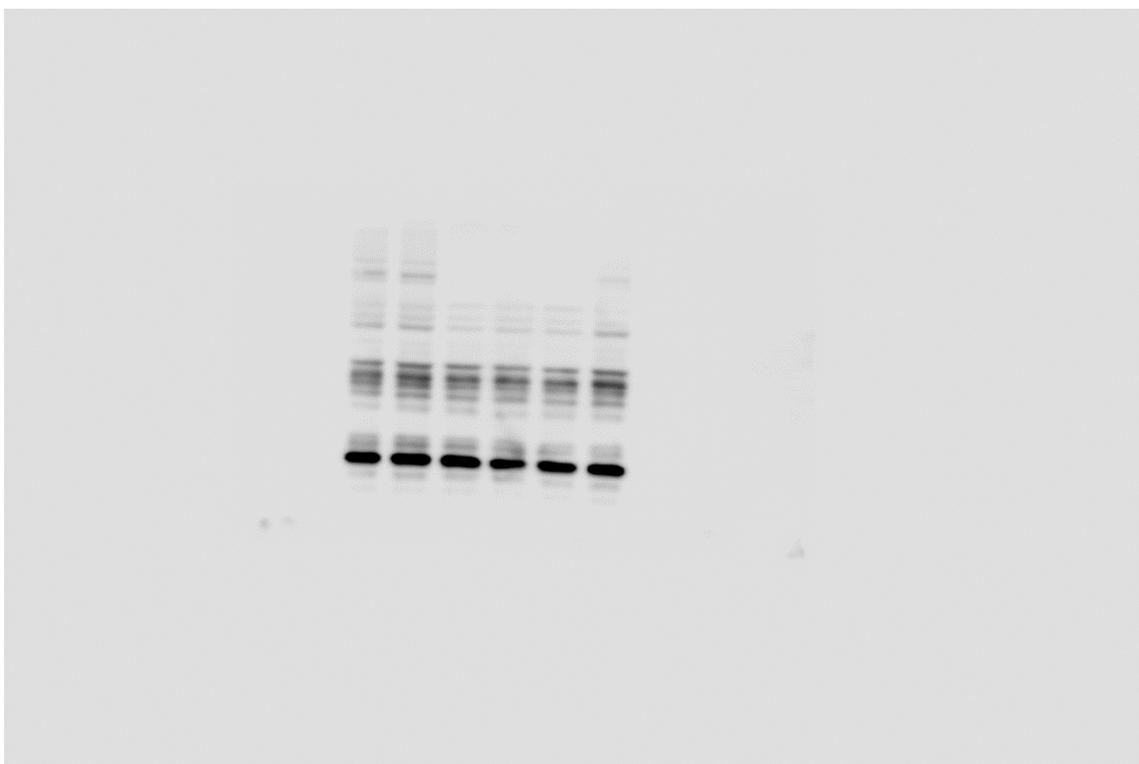
3- CNU 189 0M

4- CNU189 1.5M

Supplementary Figure S5: Original western blot membrane that originated Figure 2B, detecting Gfp-Gpp2. Anti-Gfp antibody.

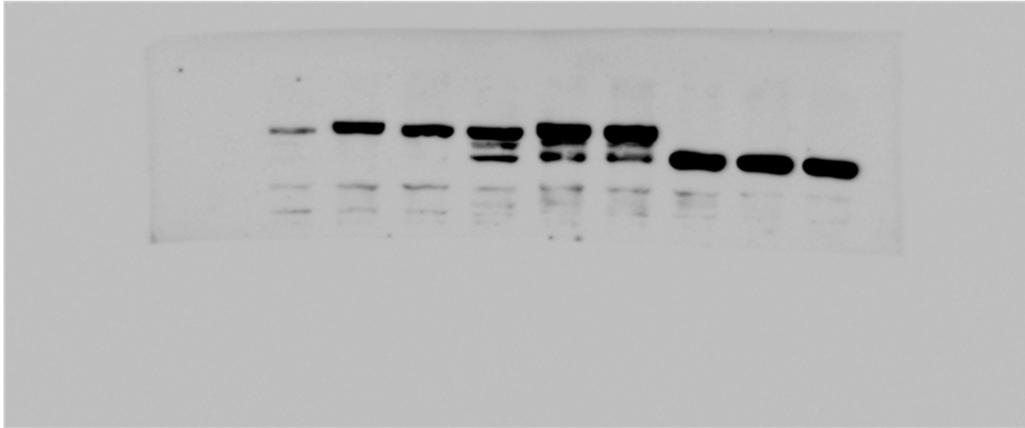


Supplementary Figure S6: Original western blot membrane that originated Figure 2B - normalizer Histone H3. Anti-Histone antibody.



Supplementary Figure S7: Original membrane image presented in the western blot, after 0, 2 and 4 hours of exposure on cyclosporine A CsA (Figure 2C).

M 1 2 3 4 5 6 7 8 9



M- Molecular weight marker

1- CNU151 0h CsA

2- CNU151 2h CsA

3- CNU 151 4h CsA

4- CNU189 0h CsA

5- CNU189 2h CsA

6- CNU189 4h CsA

7- CNU193 0h CsA

8- CNU193 2h CsA

9- CNU193 4h CsA

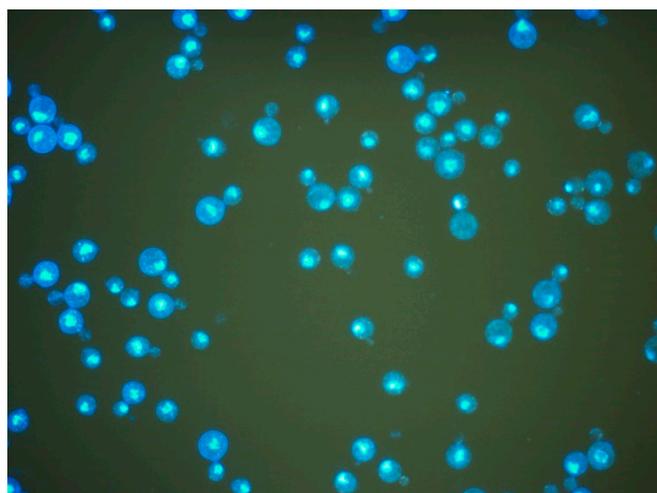
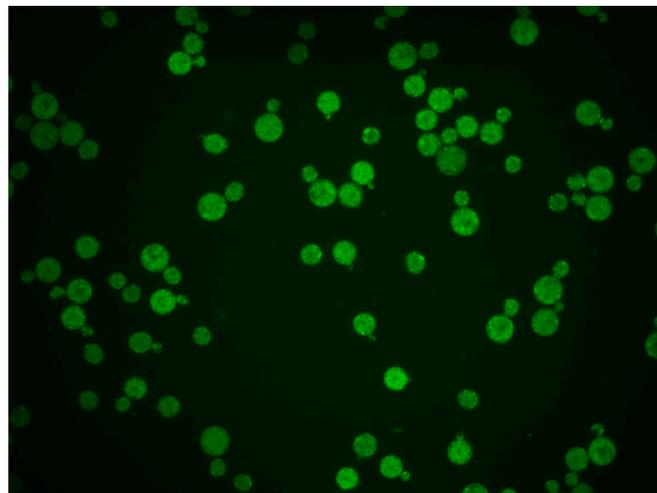
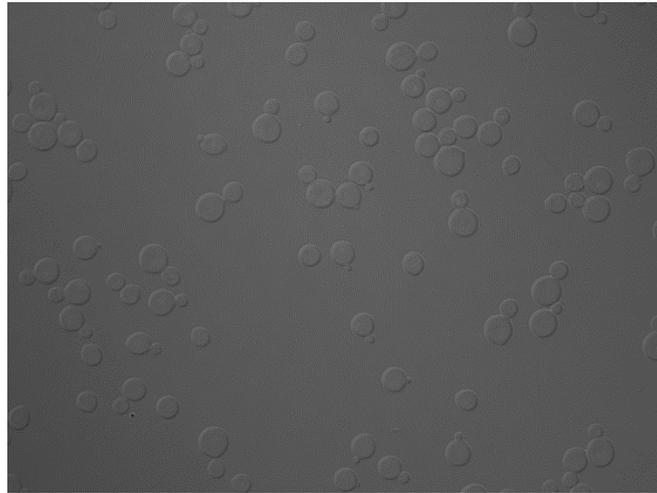
Supplementary Figure S8: Original western blot membrane that originated Figure 2C, detecting Gfp-Gpp2. Anti-Gfp antibody.



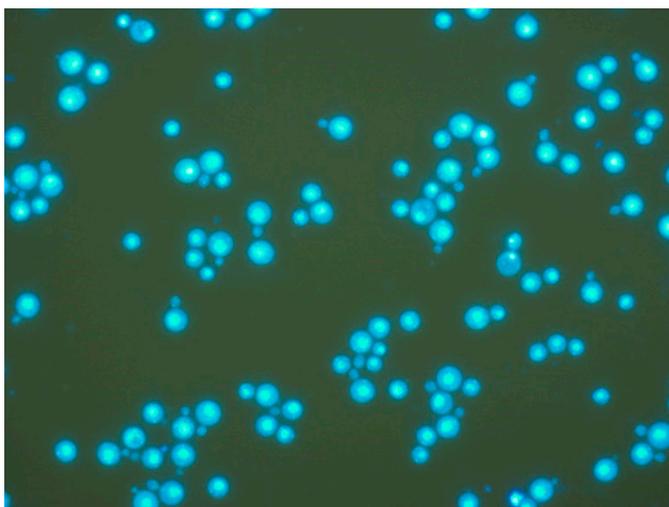
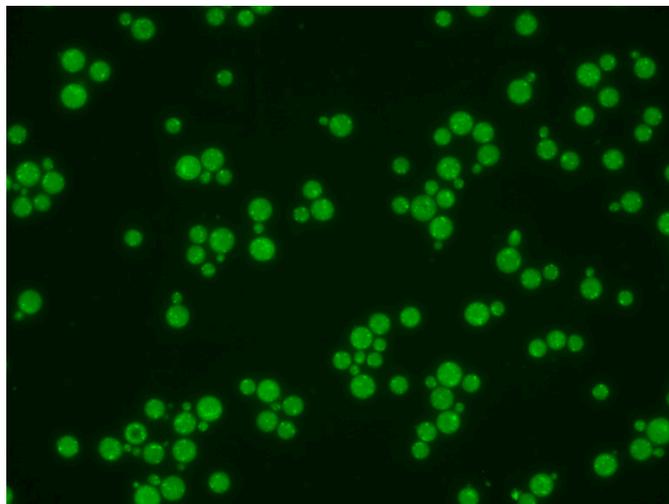
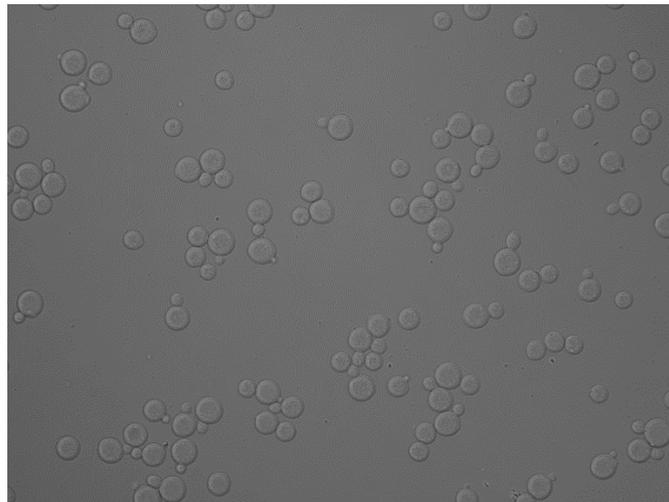
Supplementary Figure S9: Original western blot membrane that originated Figure 2C - normalizer Histone H3. Anti-Histone antibody.



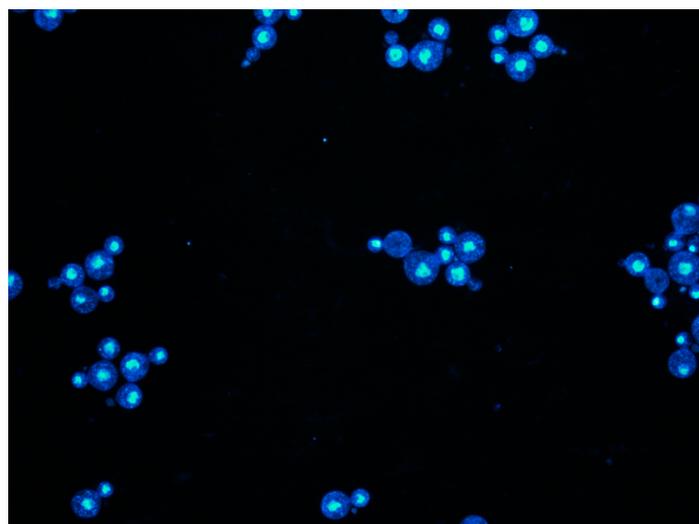
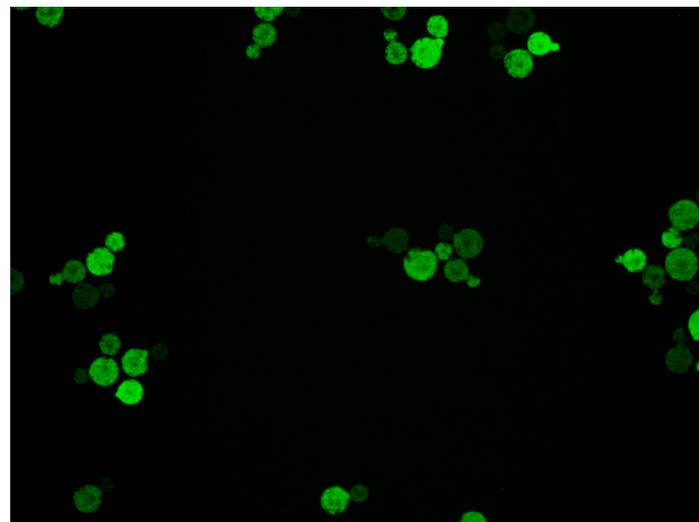
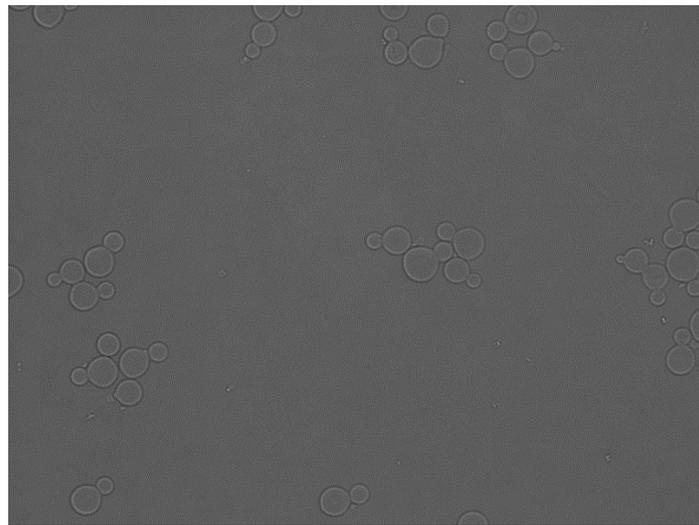
Supplementary Figure S10: Original microscopy image of Gfp-Gpp2 protein of the strain CNU151 (wild type) presented in the (figure 3A) after induction in 0M NaCl for 2 hours.



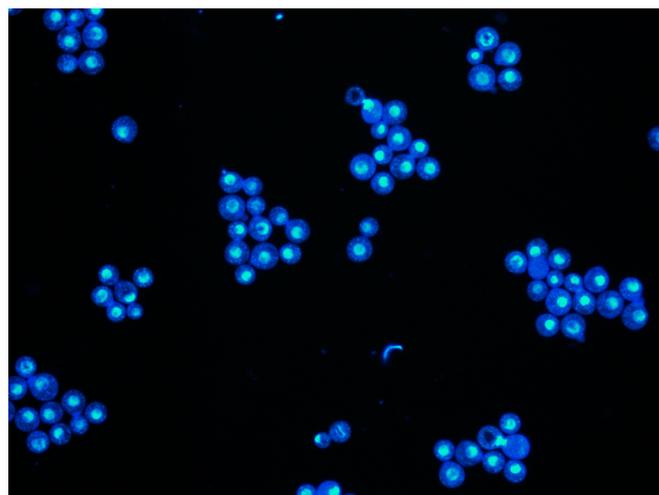
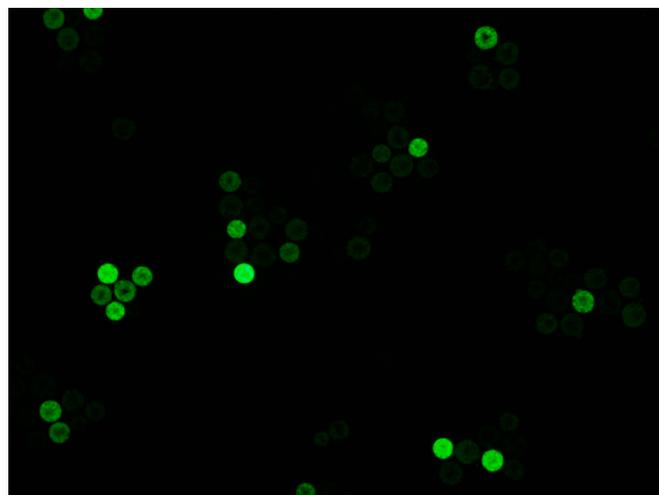
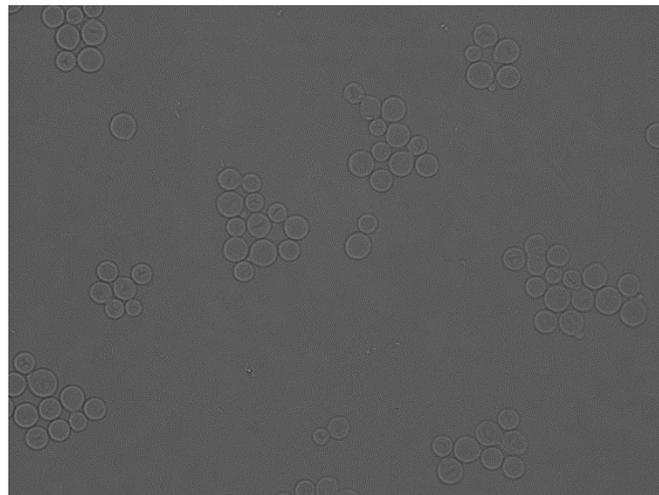
Supplementary Figure S11: Original microscopy image of Gfp-Gpp2 protein of the strain CNU151 (wild type) presented in the (figure 3A) after induction in 1,5M NaCl for 2 hours.



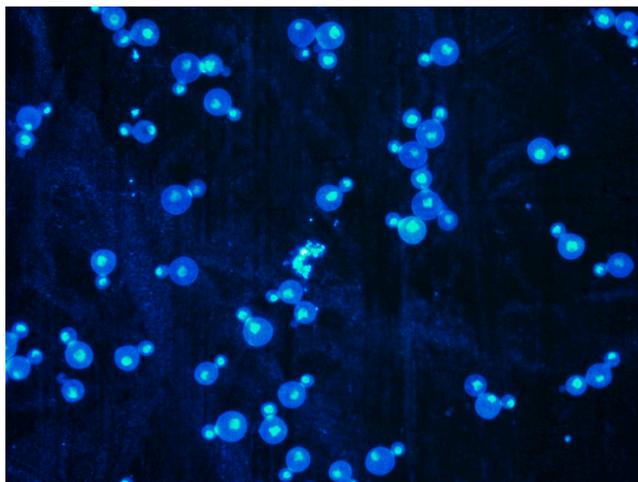
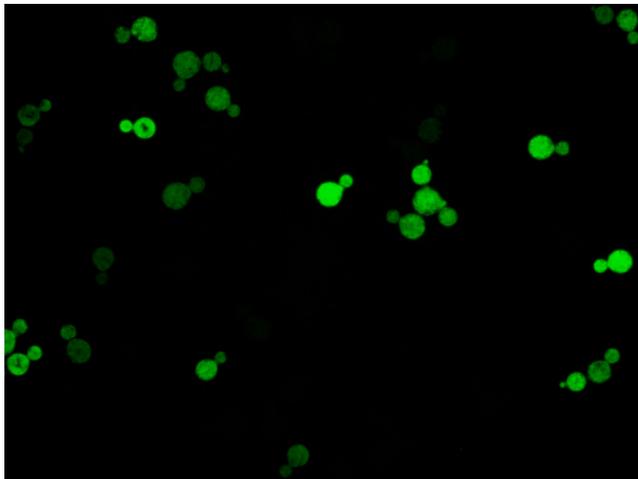
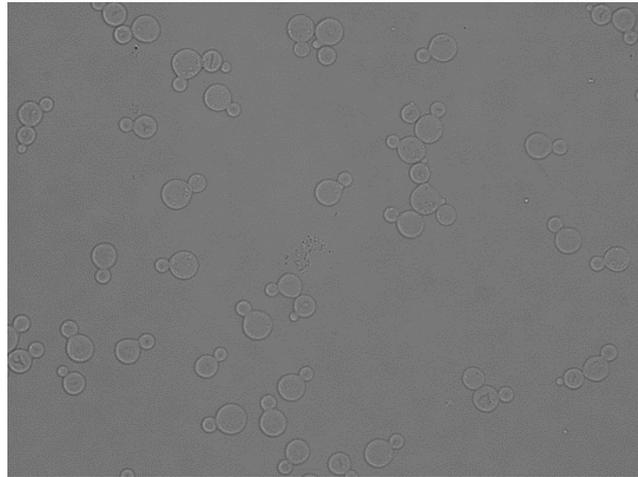
Supplementary Figure S12: Original microscopy image of Gfp-Gpp2 protein of the mutant strain *cnb1* $\Delta$  presented in the (figure 3C) after induction in 0M NaCl for 2 hours.



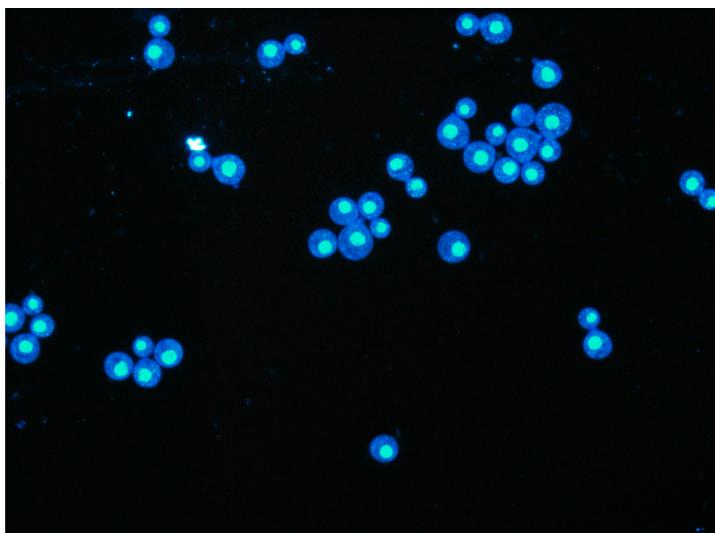
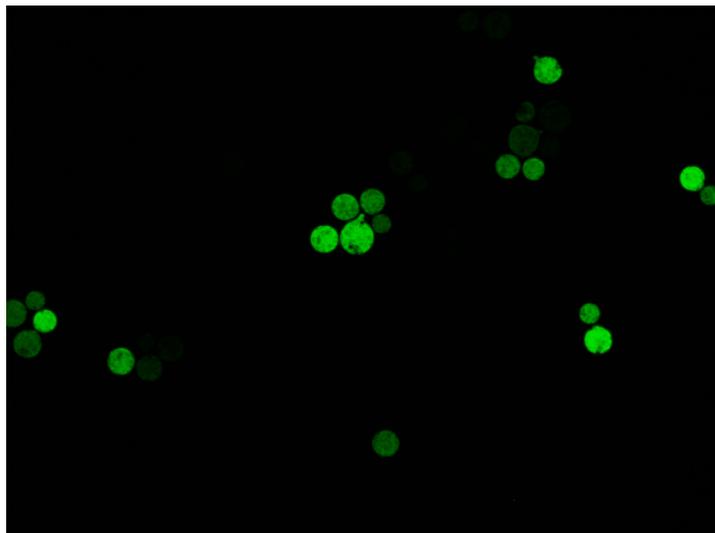
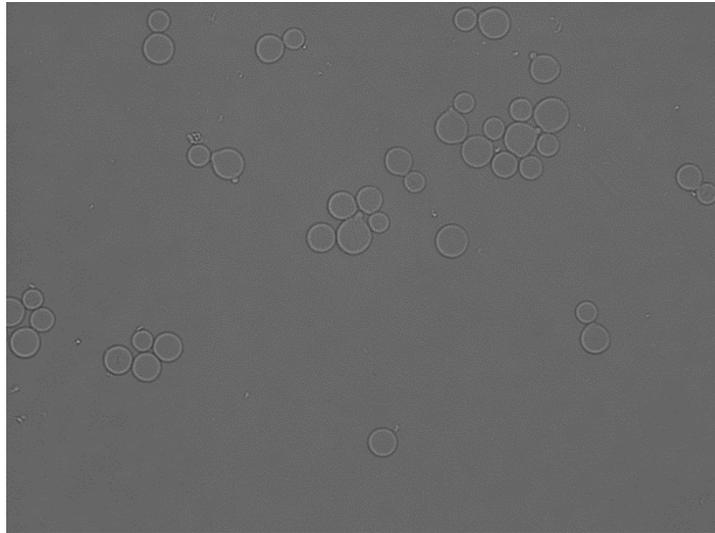
Supplementary Figure S13: Original microscopy image of Gfp-Gpp2 protein of the mutant strain *cnb1* $\Delta$  presented in the (figure 3C) after induction in 1,5M NaCl for 2 hours.



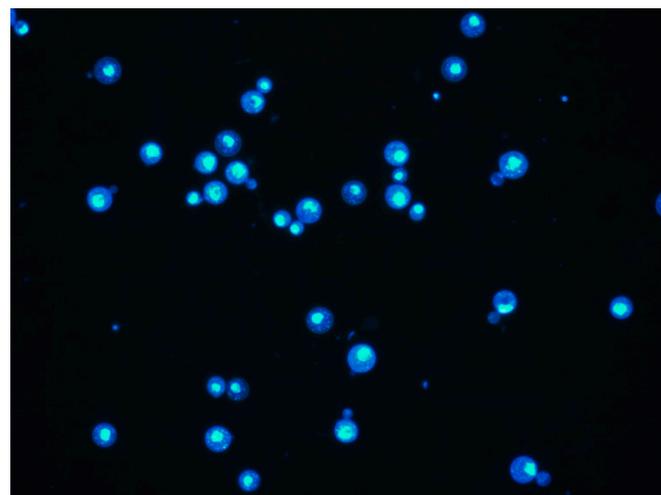
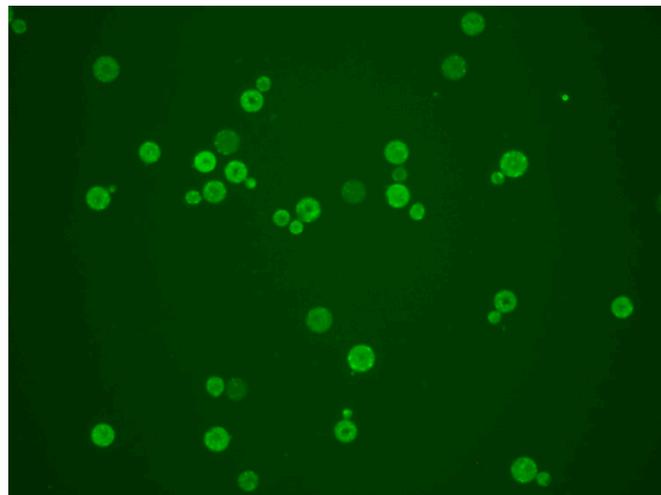
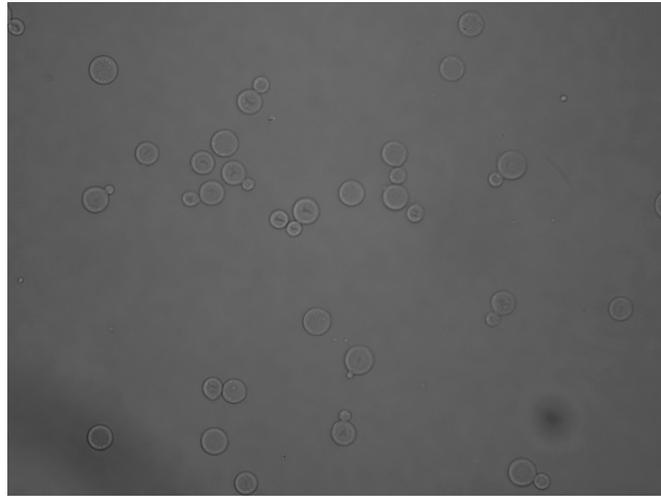
Supplementary Figure S14: Original microscopy image of Gfp-Gpp2 protein of the mutant strain *cna1* $\Delta$  presented in the (figure 3C) after induction in 0M NaCl for 2 hours.



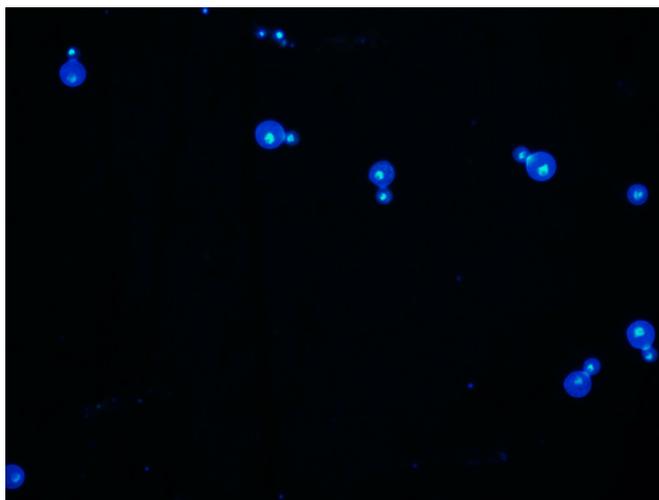
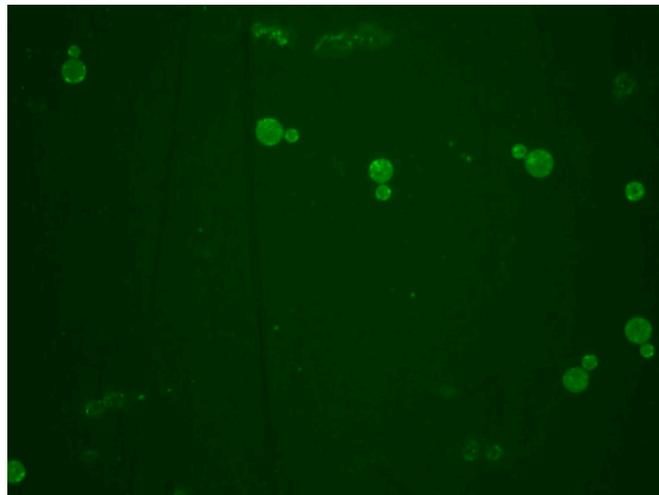
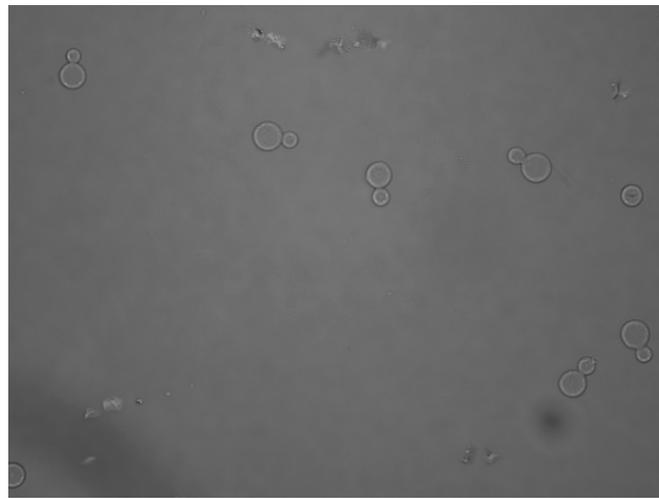
Supplementary Figure S15: Original microscopy image of Gfp-Gpp2 protein of the mutant strain *cna1* $\Delta$  presented in the (figure 3C) after induction in 1,5M NaCl for 2 hours.



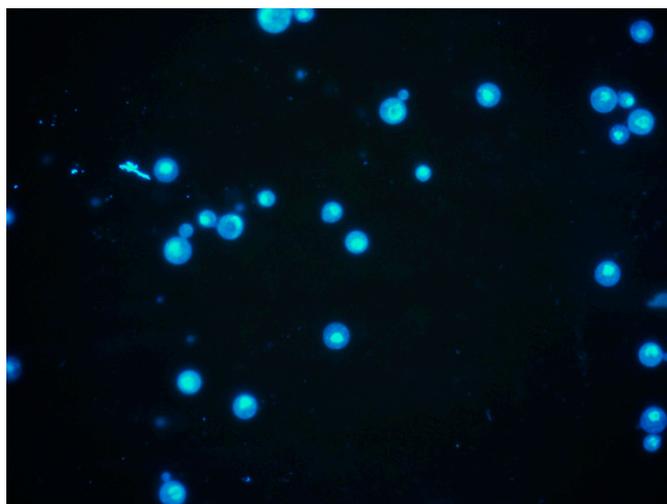
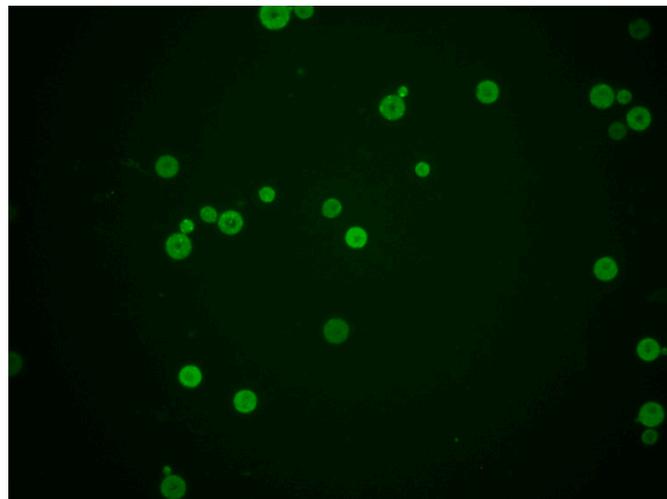
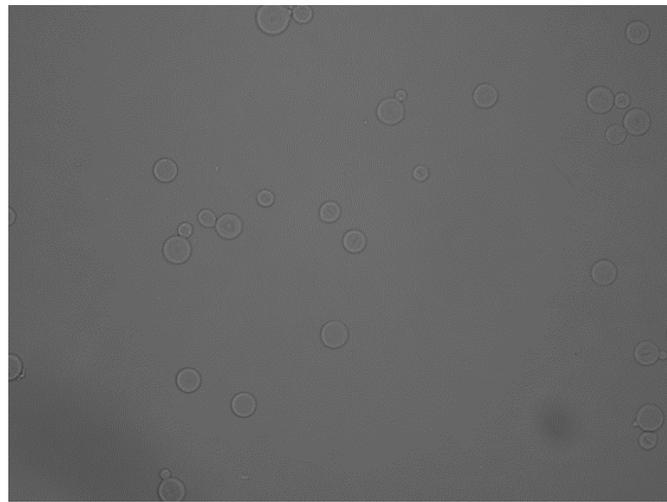
Supplementary Figure S16: Original microscopy image of Gfp-Gpp2 protein of the strain CNU151 (wild type) presented in the (figure 3D) after exposure with 0  $\mu\text{g/mL}$  CsA for 2 hours.



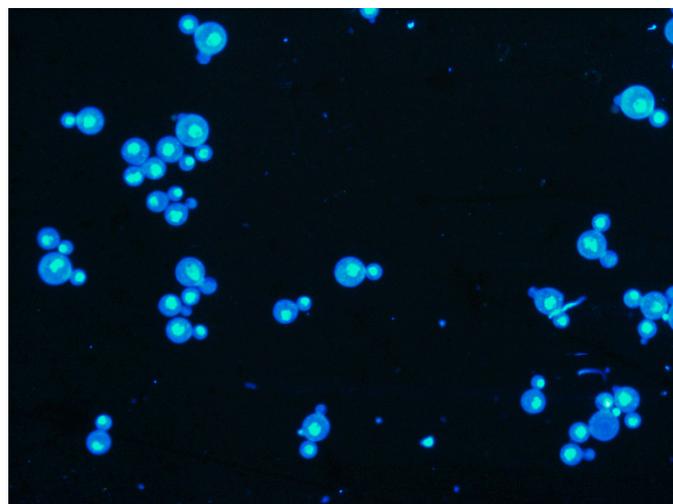
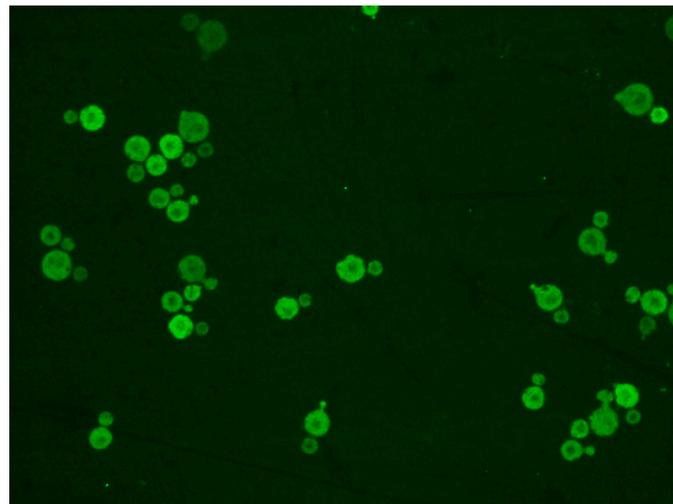
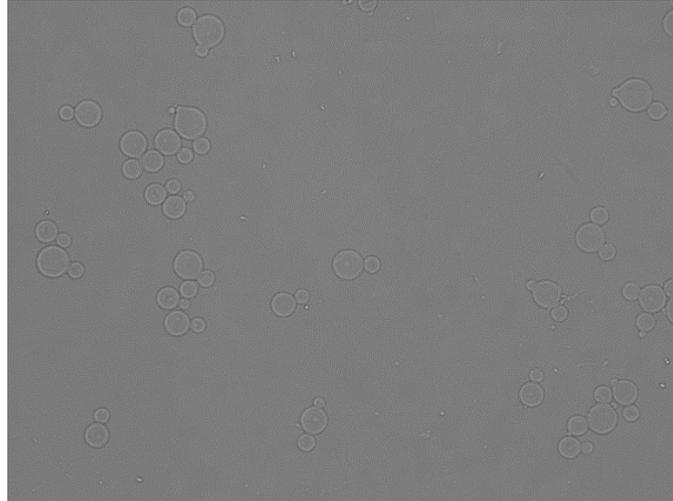
Supplementary Figure S17: Original microscopy image of Gfp-Gpp2 protein of the strain CNU151 (wild type) presented in the (figure 3D) after exposure with 100  $\mu\text{g}/\text{mL}$  CsA for 2 hours.



Supplementary Figure S18: Original microscopy image of Gfp-Gpp2 protein of the strain CNU151 (wild type) presented in the (figure 3D) after exposure with 0  $\mu\text{g/mL}$  CsA for 4 hours.



Supplementary Figure S19: Original microscopy image of Gfp-Gpp2 protein of the strain CNU151 (wild type) presented in the (figure 3D) after exposure with 100  $\mu\text{g}/\text{mL}$  CsA for 4 hours.



Supplementary Table S1: Plasmids used in this work.

Plasmid code	Vector	Gene	E. coli antibiotic resistance	Fungal Gene marker	Source
pRCP115	pCN19	<i>GFP:GPP2</i>	Amp/Kan	NAT	This work

Supplementary Table S2: Strains used in this work.

Strain Code	Background strain	Gene marker	Origen
<i>C. neoformans</i>			
CNU151	H99	<i>GFP:GPP2:Neo<sup>R</sup></i>	This work
CNU080	H99	<i>GFP::CSY3 Neo<sup>R</sup></i>	De Melo et al., 2019.
CNU160	H99	<i>cnb1Δ::Neo<sup>R</sup></i>	This work
CNU166	H99	<i>cnalΔ::Hph<sup>R</sup></i>	Da Silva et al., 2023
CNU189	CNU160	<i>GFP:GPP2::cnb1Δ</i>	This work
CNU193	CNU166	<i>GFP:GPP2::cnalΔ</i>	This work
CNU153	CNU080	<i>met3Δ::NAT<sup>R</sup></i>	Da Silva et al., 2023
CNU 125	CNU080	<i>gpp2Δ::Nat<sup>R</sup></i>	Martho et al., 2019

Supplementary Table S3: Primers used in this work.

Primer	Sequence	F or R	Use
PRCP407	GTTCATGTACAGCTACACTTTGC	F	CNB1 deletion
PRCP408	CTCCAGCTCACATCCTCGCATGCAATAAGGCGGTATTGATGATG	R	CNB1 deletion
PRCP409	CATCATCAATACCGCCTTATTGCATGCGAGGATGTGAGCTGGAG	F	CNB1 deletion
PRCP410	GTATATGGGGTAGGAATGAGAAAGAAGAGATGTAGAAACTAGCTTCC	R	CNB1 deletion
PRCP411	GGAAGCTAGTTTCTACATCTCTTCTTCTCATTCCACCCATATAC	F	CNB1 deletion
PRCP412	CGCAGGAAGTCGTTGGAATC	R	CNB1 confirmation
PRCP472	GTACGAGCTCGGATCCATGTCCGTCTTACCAAGTCTGCC	F	GFP-GPP2
PRCP473	GGCGGCCGTTACTAGTTGGCAGTCTTTATTCTTACCCAGC	R	GFP-GPP2