Supporting information

for Cabrera and González-Montelongo et al. 2013

TABLE S1 Strains used in this work.

Strain	Genotype	Source
WT*	*NCYC495 leu2::p18B1(LEU2)::pBSURA3(URA3)*	Laboratory collection
ynr1Δ	*ynr1Δ::URA3*	Laboratory collection
yni1Δ	*yni1Δ::URA3*	Laboratory collection
GPβ1	*NCYC495 leu2:: pGPβ1 (P_{YNR1}-lacZ HpLEU2)*	Laboratory collection
EC0010	*ssu1Δ::URA3*	This work
EC0012	*ssu2Δ::ble*	This work
EC0016	*ssu2Δ::ble leu2::pSSU2-LEU2*	This work
EC0014	*ssu1Δ::URA3 ssu2Δ::ble*	This work
EC0034	*NCYC495:: leu2::pSSU2-lacZ (P_{SSU2}-lacZ HpLEU2)*	This work
EC0036	*ynr1Δ::URA3 leu2::pSSU2-lacZ (P_{SSU2}-lacZ HpLEU2)*	This work
EC0042	*ssu2Δ::ble leu2::pScSSU1 (P_{SSU2}-ScSSU1 HpLEU2)*	This work
EC0044.1	*NCYC495::leu2::pScSSU1(P_{SSU2}-ScSSU1 HpLEU2)*	This work
EC0044.4	*NCYC495::leu2::pScSSU1(P_{SSU2}-ScSSU1 HpLEU2)*	This work
RG0058	*nar1Δ:: URA3 leu2:: pNAR1-LEU2*	This work
EC0022	*ynr1Δ::URA3 ssu2Δ::ble*	This work
EC0024	*ynr1Δ::URA3 leu2::pSSU2-LEU2 (nSSU2)*	This work
RG0070	*ynr1Δ::LEU2 nar1Δ::URA3*	This work
RG0079	*ynr1Δ::*URA3 *nar1Δ::LEU2 ssu2Δ::ble*	This work

EC0046.10	*ynr1*Δ::*neo ssu2*Δ::*ble leu2:: pScSSU1 (P_{SSU2}-ScSSU1 HpLEU2)*	This work
EC0028	*yni1*Δ*::URA3 ssu2*Δ*::bleo*	This work
EC0052	*ssu2*Δ::*ble leu2:: pSSU2-LEU2(nSSU2)*	This work
EC0054	NCYC495::*ura3::pSSU2-URA3::ssu2*Δ*::ble leu2::pSSU2-LEU2(nSSU2)*	This work
EC0032	*ssu2*Δ*::bleo ynr1*Δ*::URA3 leu2::pGPβ1 (P_{YNR1}-lacZ HpLEU2)*	This work
RG0059	*nar1*Δ*::URA3 (P_{YNR1}-lacZ), LEU2*	This work
RG0062	*NAR1-GFP (ble)*	This work
EC0040	*SSU2-GFP (ble)*	This work

All strains are derivatives of NCYC495 *leu2 ura3*. WT* was obtained by trasnforming this strain with integrative vectors p18B1(*LEU2*) or pBSURA3(*URA3*) bearing *HpLEU2* and *URA3*, linearized either at *LEU2* or *URA3* to target the integration to *leu2* or *ura3* loci.

TABLE S2 Primers used in this work.

Primer	5'-3' sequence	Utility
SSU1-F	TTTGACCAGCTTCTTGAGGA	*SSU1* disruption
SSU1-R	AAAGCCAAGTACGATGCTGA	*SSU1* disruption
SSU1int- F	CCACATGAGCGAAACACAACT	*SSU1* disruption confirmation
Ura3 ext II-F	TGGATATTGGATTGCAAGCAG	*SSU1* and *NAR1* disruption confirmation
SSU2-F	TCCCTTCCTGAGTGTATGGCA	*SSU2* disruption
SSU2-R	TGCGGATGGTATACACGAAA	*SSU2* disruption
SSU2int-R	ACAATACGAGCGCAAACTAGA	*SSU2* disruption confirmation
ZeoC-R	TATCGACAAAGGAAAAGGGG	*SSU2* disruption confirmation
334int-F	CACATAAACGAGTACCATCCG	*NAR1* disruption
334int-R	AACAGAATAAAGCGGCCAAG	*NAR1* disruption
ext334	TGGATATGGGAGTGCAGAAGA	*NAR1* disruption confirmation
ScSSU1-F	AGATCTATGGTTGCCAATTGGGTA	Sc*SSU1* amplification
ScSSU1ter-R	GGATCCTGCTAAACGCGTAAAATCTA	Sc*SSU1* amplification
kanMX-F	AGGCCTACTTGAACGGATCCACTAGCT	*kanMX* amplification
kanMX-R	AGGCCTTTCTTTCCTGCGTTATCCCCT	*kanMX* amplification
SSU2Prom-F	GGATCCTCTCCCTTCCTGAGTGTATGG	*SSU2*-lacZ fusion
SSU2Prom-R	GCATGCACGGAGGCACCGTCGTCTCGG	*SSU2*-lacZ fusion
SSU2orf-F	AGATCTATGGCATCTTCTCTCTCATC	*SSU2*-6HA and *eGFP* fusion
SSU2orf-R	AGATCTGACGTCATGCTTTCGAATAG	*SSU2*-6HA and *eGFP* fusion
3-F	AAGCTTATGGCAGATGACACATACTAT	*NAR1*-6HA and *eGFP* fusion
3-R	AGATCTATTTGCGTCTCTCTTCTCGT	*NAR1*-6HA and *eGFP* fusion
tag-R	AGAGGTCGACGTGAATGATCGTTCCACTTTT	HA fusion confirmation
G2	ATGAACTTCAGGGTCAGCTTG	*eGFP* fusion confirmation
proNAR1-F	GGATCCCACAAAGAAGAGAAGAGACTG	*NAR1*-lacZ fusion
proNAR1-R	GCATGCAGTATGTGTCATCTGCCAT	*NAR1-lacZ* fusion
QSSU2-F	GCGTATTCCTTGGAGCAGAG	qRT-PCR
QSSU2-R	CGTGGAAGAAGCAACTGTCA	qRT-PCR
Q-ACT1-F	GAGGTTACACGTTCTCCACCA	qRT-PCR
Q-ACT1-R	ACCTGTCAATCAGGCAACTC	qRT-PCR

TABLE S3 Plasmid used in this work.

Plasmid	Characteristic	Origin
pGEM-T Easy	Used to clone PCR products. Amp^r, *lacZ*	Promega, EEUU
pBS	pBluescript KS(+).Amp^r.	Stratagene, EEUU
pBSURA3	pBluescript derivative containing the 2 kbp *H. polymorpha URA3* genomic fragment, Amp^r.	Laboratory collection
p18B1	pTZ18R derivative containing the 2.5 kbp *H. polymorpha LEU2* genomic fragment. Amp^r.	(1)
pANL31	pBS derivative containing eGFP without the start codon. Amp^r, Zeo^r.	(2)
pGEM-ble	pGEM-T Easy derivative containing the 1312 bp fragmente of *ble* gene marker. Zeo^r.	Laboratory collection
pGEM-LEU2	pGEM-T Easy derivative containing the 1.5 kbp *H. polymorpha LEU2* genomic fragment. Amp^r.	Laboratory collection
pHA1	pANL31 derivative replacing eGFP gene by a PCR fragment encoding for six copies of the HA epitope. Amp^r, Zeo^r.	Laboratory collection
pHPI 359	Yep356 derivative replacing *S. cerevisiae URA3* gene by a 2.5 kbp fragment containing the *H. polymorpha LEU2* gene obtained from p18B1. Amp^r, *LEU2.*	(3)
pP_{SSU2}-lacZ	pHPI359 derivative expressing *lacZ* gene under control of 5' non-coding region of *H.polymorpha SSU2* gene. Amp^r, *LEU2*.	This work
pNAR1-lacZ	pHPI359 derivative expressing *lacZ* gene under control of 5' non-coding region of *H.polymorpha NAR1* gene. Amp^r, *LEU2*.	This work
pGEMT-P_{SSU2I}	pGEM-T Easy derivative. It carries a 1883 bp fragment containing the *S. cerevisiae SSU1* ORF plus 503 bp corresponding to the 3' non-coding region. Amp^r.	This work
pP_{SSU2I}-ScSSU1	pGEMT-P_{SSU2I} derivative expressing *S. cerevisiae SSU1* gene under control of 5' non-coding region of *H. polymorpha SSU2* gene. Amp^r, *LEU2*.	This work
pP_{SSU2I}-ScSSU1LEU2	pP_{SSU2I}-ScSSU1 derivative containing *H. polymorpha LEU2* gene. Amp^r.	This work
pSSU2-GFP	pANL31 derivative. pANL31 with 1182 kbp fragment containing the *SSU2* gene lacking stop codon fused in frame to the *eGFP* gene. Amp^r, Zeo^r.	This work
pNAR1-GFP	pANL31 derivative. pANL31 with 1440 kbp fragment containing the *NAR1* gene lacking stop codon fused in frame to the *eGFP* gene. Amp^r, Zeo^r.	This work
pEYFP-N1	Containing enhanced yellow fluorescent protein (*EYFP*) gene. Kan^r,Zeo^r.	Takara, EEUU
pSSU2-YFP	pEYFP-N1 derivative. Used to SSU2-*EYFP* fusion. Kan^r, Zeo^r.	This work
pGEMHE	Used in the synthesis of mRNA in *Xenopus*. Contains 1098 bp which are not transcribed at the 5' region	(4)

	(5'UTR) and 279 bp in the region 3'(3'UTR). Amp^r	
pGEMT-SSU1	pGEM-T Easy derivative containing 2.8 kpb corresponding to *SSU1*. Amp^r	This work
pssu1ΔURA3	pGEMT-SSU1 derivative containing a deletion of 1642 pb and the *URA3* marker insertion. Amp^r. *URA3*.	This work
pGEMT-SSU2	pGEM-T Easy derivative containing 2.8 kpb corresponding to *SSU2*. Amp^r	This work
pssu2Δble	pGEMT-SSU2 derivative containing a deletion of 1599 pb and the *ble* marker insertion. Amp^r. *ble.*	This work
pNAR1	pGEM-T Easy derivative. It carries a 2876 bp fragment used to *NAR1* disruption with *URA3* or *LEU2* gene marker. Amp^r.	This work
pnar1Δ	pNAR1 derivative containing the 1970 bp fragment used to disruption with *URA3* gene marker. Amp^r, *URA3.*	This work
pSSU2-URA3	pBSURA3 derivative containing 2.8 kpb of *SSU2* gene (ORF plus 1008 pb and 682 pb from *SSU2* 5´and 3´ non-coding región). *Amp*R, *URA3*	This work
pSSU2-LEU2	pGEMT-SSU2 derivative containing containing *H. polymorpha LEU2* gene. Amp^r. *LEU2*.	This work
pNAR1-LEU2	pGEM-LEU2 derivative containing a 2492 bp *H. polymorpha NAR1* fragment obtained from pGEM-LEU2. Amp^r.	This work

1. **Agaphonov MO, Poznyakovski AI, Bogdanova AI, Ter-Avanesyan MD.** 1994. Isolation and characterization of the *LEU2* gene of *Hansenula polymorpha.* Yeast. **10:**509-513.

2. **Leão-Helder AN, Krikken AM, van der Klei IJ, Kiel JKAW, Veenhuis M.** 2003. Transcriptional Down-regulation of Peroxisome Numbers Affects Selective Peroxisome Degradation in *Hansenula polymorpha.* J. Biol. Chem. **278:**40749-40756.

3. **Brito N, Pérez MD, Perdomo G, González C, García-Lugo P, Siverio JM.** 1999. A set of *Hansenula polymorpha* integrative vectors to construct *lacZ* fusions. Appl. Microbiol. Biotechnol. **53:**23-29.

4. **Liman ER, Tytgat J, Hess P.** 1992. Subunit stoichiometry of a mammalian K^+ channel determined by construction of multimeric cDNAs. Neuron. **9:**861-871.

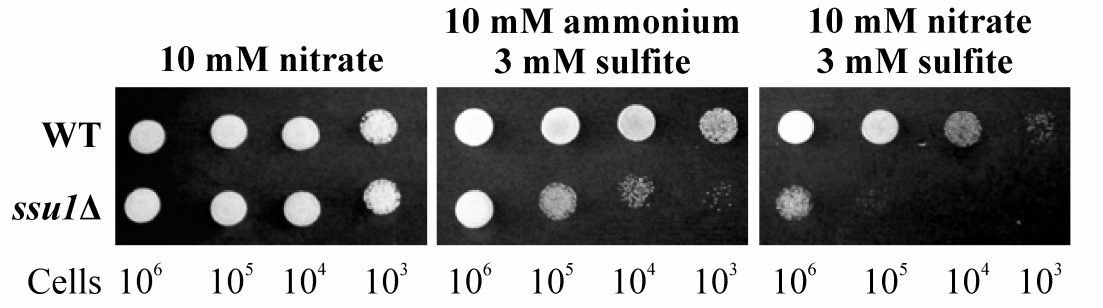

FIG S1 Sensitivity to sulfite is increased when nitrate is added. Serial 10-fold dilutions of the indicated strains were spotted on synthetic medium buffered at pH 3.5 plus 10 mM nitrate, 5 mM ammonium plus 3 mM sulfite and 10 mM nitrate plus 3 mM sulfite and 1 mM proline. Cells were incubated at 37ºC for 2 days.

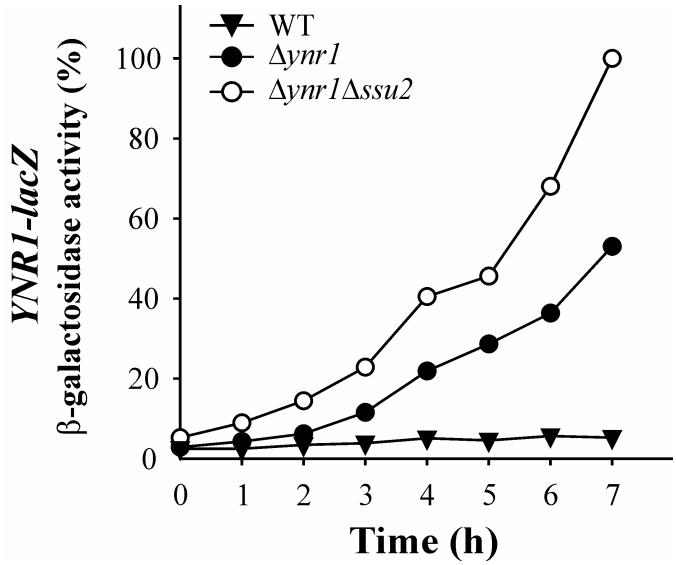

FIG S2 *SSU2* deletion affects the expression levels of genes induced by nitrate. Ammonium-grown cells were resuspended and incubated in synthetic medium plus 1 μM nitrate for 7 h. The experiments were repeated three times without significant differences; data from only one experiment are shown.

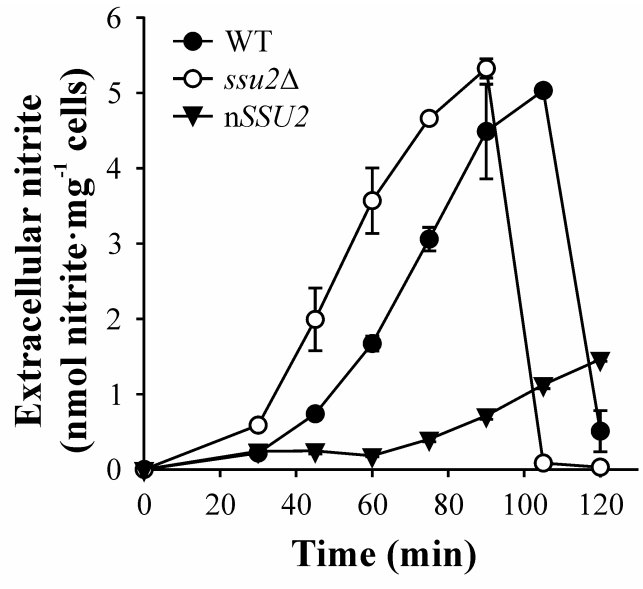

FIG S3 Kinetic nitrite excretion in WT, *ssu2*Δ and n*SSU2*. Ammonium-grown cells were incubated in nitrogen free medium buffered at pH 5.5 for 120 min before to added 1 mM sodium nitrate. Data are expressed as mean values ± S.E. from three independent experiments.

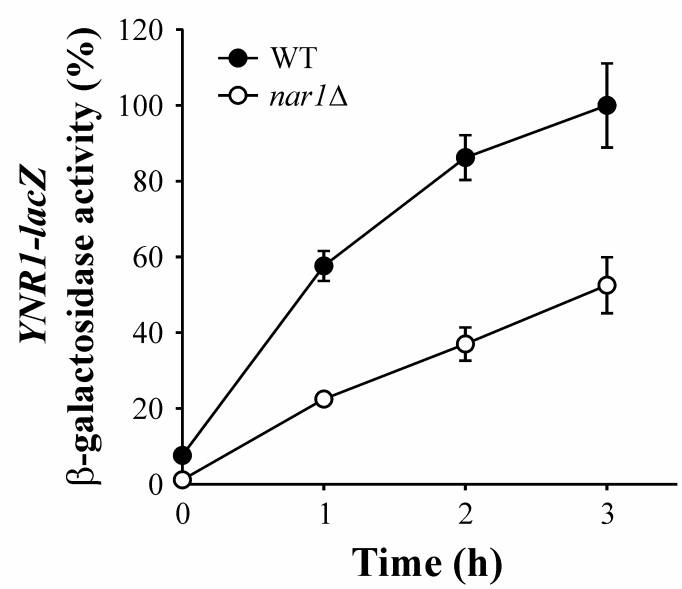

FIG S4 Effect of *NAR1* deletion on *YNR1* expression. *YNR1* expression was followed by assaying β-galactosidase activity in WT and *nar1*Δ bearing *YNR1-lacZ*. Cells were grown in synthetic medium plus 5 mM ammonium at OD_{660} of 2-3 and were resuspended in synthetic medium plus 5 mM nitrate for 3 hours. Values are expressed as percentage of β-galactosidase activity ± S.E from three independent experiments.

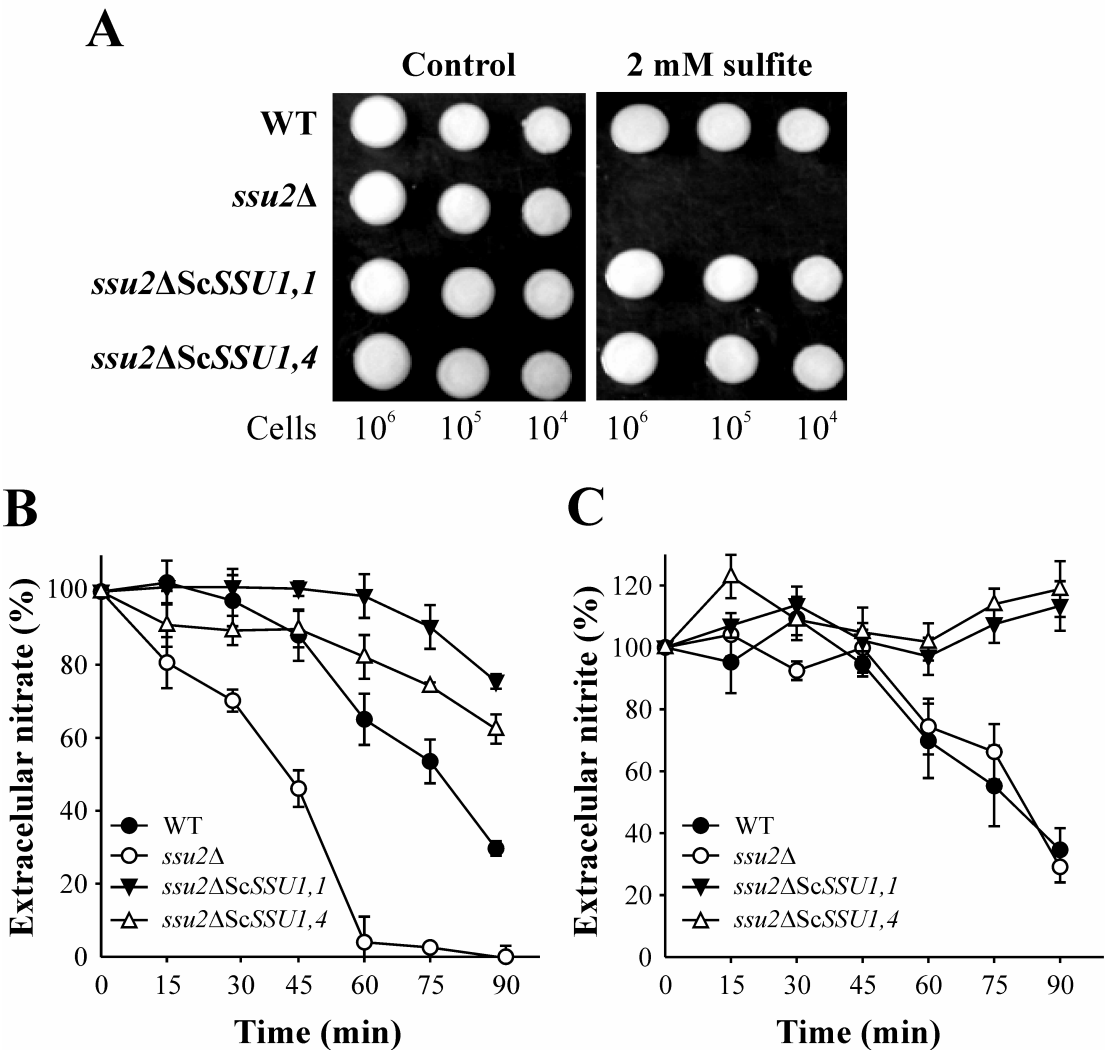

FIG S5 Sc*SSU1* complements sulfite sensibility and nitrate and nitrite efflux in Hps*su2*Δ expressing Sc*SSU1*. (A) To determinate sulfite sensiblility, cells were grown in YPD. Serial 10-fold dilutions were spotted on synthetic medium buffered at pH 3.5 containing 5 mM of ammonium and the sulfite concentration indicated. (B) Nitrate uptake. Cells were grown in 5 mM of ammonium, were nitrogen starved for 90 min in nitrogen free medium buffered to pH 5.5. Nitrate uptake rate assays were triggered with 0.5 mM nitrate. Values are expressed as percentage of extracellular nitrate ± S.E. from three independent experiments. (C) Nitrite

uptake. Ammonium-grown cells were nitrogen starved for 90 min on pH 5.5 buffered synthetic medium. Nitrite uptake rate assays were triggered with 0.5 mM nitrite. Values are expressed as percentage of extracellular nitrite ± S.E. from three independent experiments.

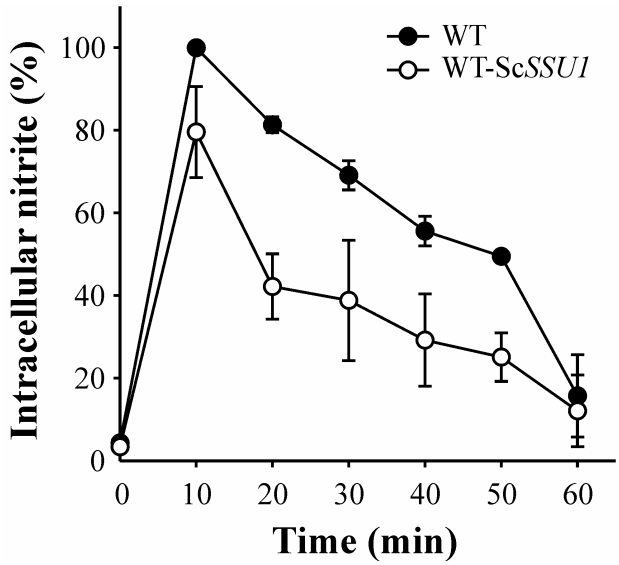

FIG S6 WT expressing Sc*SSU1* efflux nitrite faster than WT. Nitrite accumulations was determinate in ammonium-grown cells starved for 120 min in nitrogen free medium. Nitrite accumulation assays were triggered with 1 mM nitrite. Values are expressed as a percentage of intracellular nitrite ± S.E. from three independent experiments.