Supplementary Data (Calvo *et al.*, 2012)

Supplementary Table S1. Strains used in this study

Strain	Genotype	Origin
972	*h⁻*	(39)
NG25	*h⁺ caf4/trr1::ura4⁺ ura4-D18*	(40)
EHH14	*h⁻ his2 ura4 pap1::ura4⁻ leu1–32 nmt::GFP-pap1::leu1⁺*	(41)
EHH14.C523D	*h⁻ his2 ura4 pap1::ura4-D18 leu1–32 nmt::GFP pap1C523D::leu1⁺*	(41)
AV19	*h⁺ trr1::ura4⁺ ura4-D18 ade6-704 leu1-32 nmt::GFP-pap1::leu1*	This work
caf1⁺::ura4⁺	*h⁺ caf1/hba1::ura4⁺ ura4-D18 ade6-704 leu1-32*	(42)
EA33	*h⁺ caf1::ura4⁺ ura4-D18 ade6-704 leu1–32 nmt::GFP-pap1::leu1⁺*	(41)
IC1	*h⁻ pap1::ura4⁺ ura4-D18 leu1-32*	This work
IC2	*h⁻ pap1 ura4-D18 leu1-32*	This work
IC2.C523D	*h⁻ pap1.C523D ura4-D18 leu1-32*	This work
MS46	*h⁺ atf1::kanMX6 ura4-D18 leu1-32*	This work
MC16	*h⁻ prr1::kanMX6 ura4-D18 leu1-32*	This work
MC18	*h⁻ pap1::ura4⁺ ura4-D18 prr1::kanMX6 leu1-32*	This work
MC40	*h⁻ prr1-HA::kanMX6 ura4-D18 leu1-32*	This work
IC64	*h⁻ prr1-HA::kanMX6 pap1::natMX6 ura4-D18 leu1-32*	This work
MC41	*h⁻ prr1-GFP::kanMX6 ura4-D18 leu1-32*	This work
MC42	*h⁻ pap1-GFP::kanMX6 ura4-D18 leu1-32*	This work
IC81	*h⁻ prr1::kanMX6 leu1-32 nmt::GFP-pap1::leu1⁺ ura4-D18*	This work
IC83	*h⁺ pap1::ura4⁺ prr1-GFP::kanMX6 his2 ura4-D18 leu1-32*	This work
PG3	*h⁺ prr1-HA::kanMX6 pap1.C523D::natMX6 ura4-D18 leu1-32*	This work
PG7	*h⁻ caf4::ura4⁺ prr1-HA::kanMX6 leu1-32*	This work
IC70	*h⁻ pap1-HA::kanMX6 ura4-D18 leu1-32*	This work
IC102	*h⁻ trr1::ura4⁺ prr1-GFP::natMX6 leu1-32*	This work
PG20	*h⁻ hba1::ura4⁺ prr1-GFP::natMX6 ade6-704 leu1-32*	This work
IC97	*h⁻ pap1.C523D::ura4⁺ prr1-GFP::natMX6 leu1-32*	This work
PG21	*h⁻ prr1::kanMX6 pap1::natMX6 ura4-29 leu1-32*	This work
PG15	*h⁻ pap1::ura4⁺ atf1::natMX6 leu1-32*	This work
EP137	*h⁻ mpr1::natMX6 ura4-D18 leu1-32*	This work
IC86	*h⁻ mpr1::natMX6 prr1-HA::kanMX6 ura4-D18 leu1-32*	This work

Supplementary references

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41. Castillo, E.A., Ayte, J., Chiva, C., Moldon, A., Carrascal, M., Abian, J., Jones, N. and Hidalgo, E. (2002) Diethylmaleate activates the transcription factor Pap1 by covalent modification of critical cysteine residues. *Mol.Microbiol.*, **45**, 243-254.
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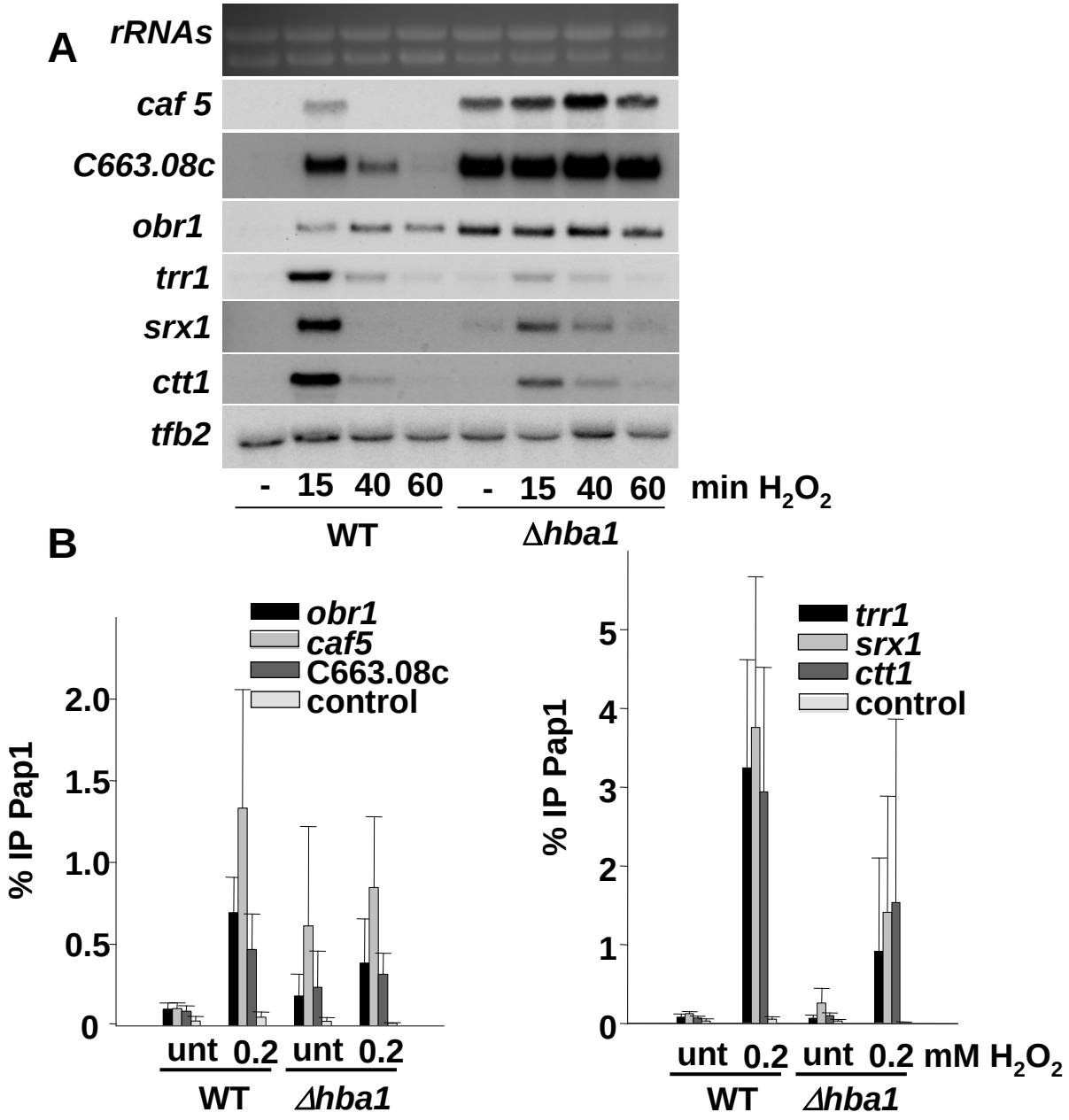
SUPPLEMENTARY FIGURE LEGENDS – Calvo *et al.* 2012

Supplementary Figure S1. Constitutively reduced/nuclear Pap1 can only bind and activate a subset of all Pap1-dependent genes. (**A**) Stress-dependent transcriptional analysis of wild-type and mutant strains. Total RNA from strains IC2 (WT) and Δ*hba1* (*caf1*::*ura4*$^{+}$) was obtained from cultures treated or not with 0.2 mM H_2O_2 for the indicated times and analyzed by Northern blot as described in Figure 2A. (**B**) Binding of reduced/nuclear Pap1 to DNA *in vivo.* Cultures of strains such as those used in A were treated with 0.2 mM H_2O_2 for 5 min. ChIP analysis was performed as described in Figure 2B.

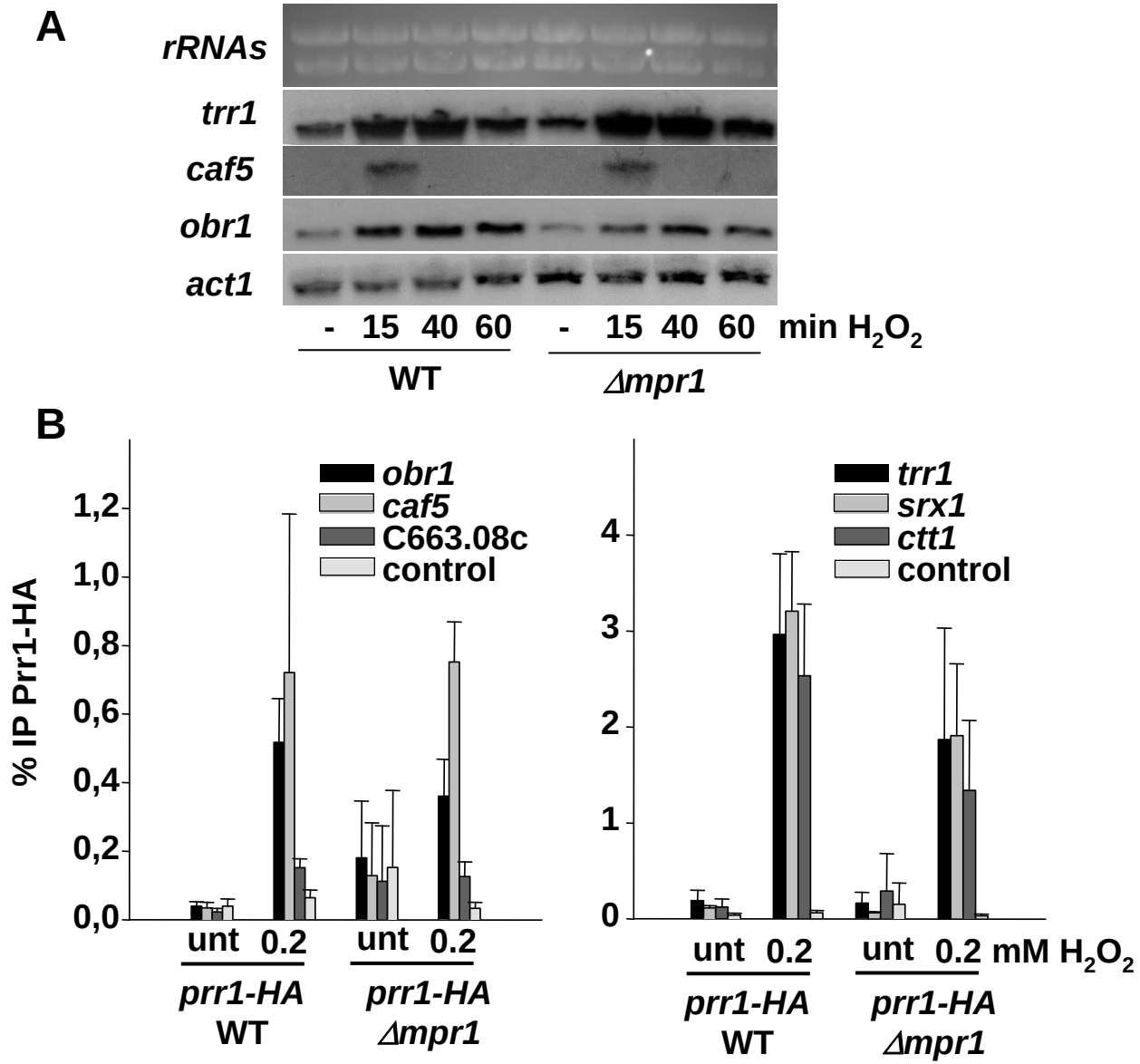
Supplementary Figure S2. Regulation of Prr1 by Mpr1 is not necessary for induction of Pap1-dependent genes. (**A**) Total RNA from strains IC2 (WT) and EP137 (Δ*mpr1*) was obtained from cultures treated with 0.2 mM H_2O_2 for the indicated times, and analyzed by Northern blot as described in Figure 2A. **(B)** Binding of Prr1-HA to DNA *in vivo* in Δ*mpr1* strain. Cultures of strains MC40 (*prr1-HA*) and IC86 (Δ*mpr1 prr1-HA*) were treated or not with 0.2 mM H_2O_2 for 5 min. ChIP of Prr1-HA using anti-HA antibody was performed as described in Figure 2B.

Supplementary Figure S3. Over-expression of reduced/nuclear Pap1 triggers constitutive transcription of not only *caf5* but also *trr1*. (**A**) The redox state and relative protein levels of Pap1 of extracts from strains EHH14 (Δ*pap1 nmt::pap1;* the expression of GFP-Pap1 is under the control of the *nmt41* promoter), EHH14.C523D (Δ*pap1 nmt::pap1.C523D*), IC2 (WT) and IC2.C523D (*pap1.C523D*), before (-) or after 5 min of 0.2 mM H_2O_2 stress, was analyzed by Western blot using polyclonal anti-Pap1 antibodies. (**B**) Northern blot analysis of antioxidant Pap1-dependent gene (*trr1)* and drug tolerance gene (*caf5*) expression. Total RNA from strains as in Figure S3A was obtained from cultures treated or not (-) with 0.2 mM H_2O_2 for 15 min and analyzed by Northern blot as described in Figure 2A. **(C)** Prr1 over-expression does not trigger induction of Pap1-dependent genes. Total RNA from strains IC2 (WT), IC1 (Δ*pap1*), MC16 (Δ*prr1),* PG21 (Δ*pap1* Δ*prr1),* PG15 (Δ*pap1* Δ*atf1) and* PG21 with p397.41 (Δ*pap1* Δ*prr1 with pnmt41::prr1*), treated or not (-) with 0.2 mM H_2O_2 for 15 min, was obtained and analyzed by Northern blot as described in Figure 2A.

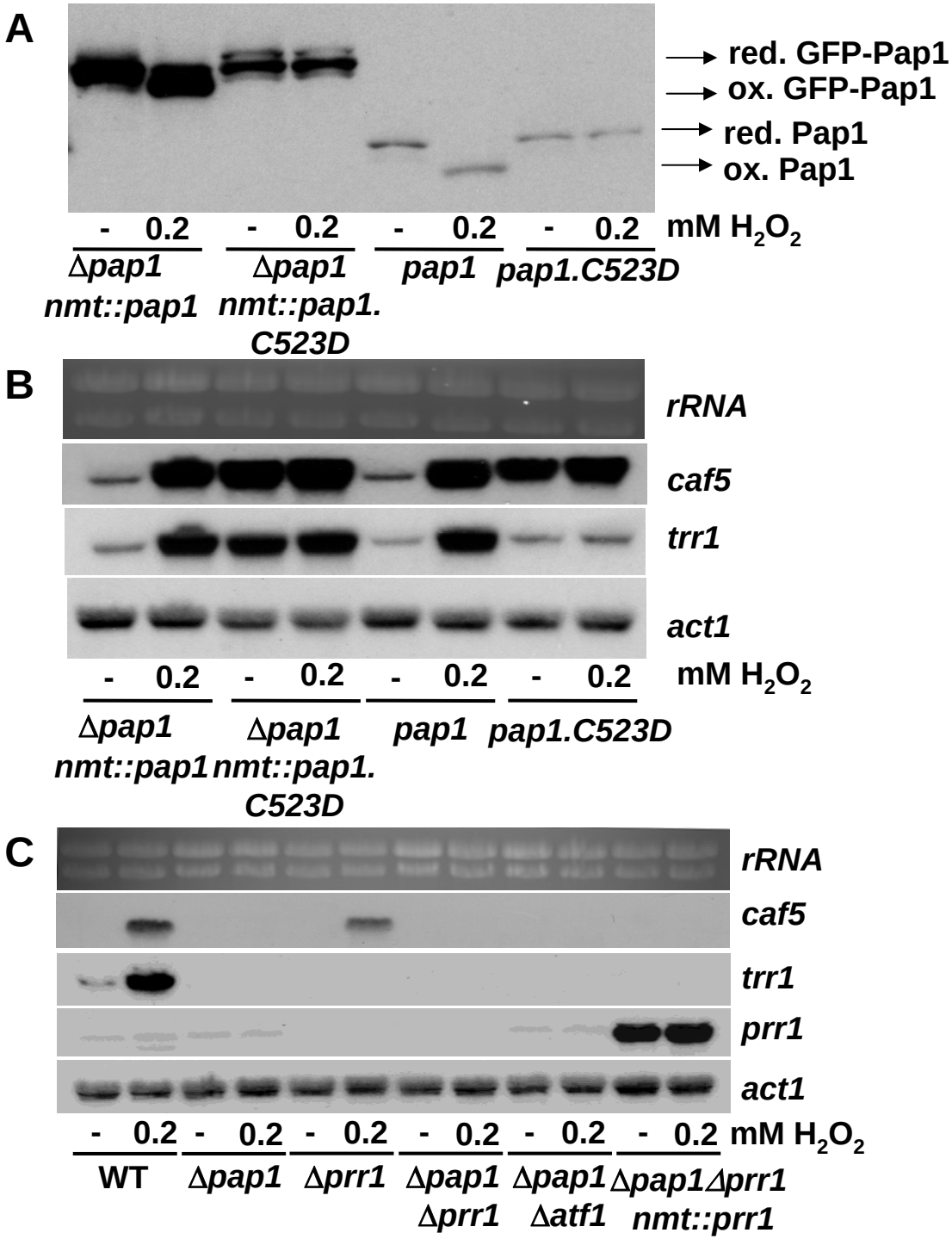
Supplementary Figure S4. Diethylmaleate and Leptomycin B do not induce the antioxidant response. **(A)** Total RNA from strains IC2 (WT) and IC1 (Δ*pap1*) was obtained from cultures treated with 0.2 mM H_2O_2 for 15 min (H) and 4 mM diethylmaleate for 30 min (D) and analyzed by Northern blot as described in Figure 2A. **(B)** RNA from wild-type strain IC2 was obtained from cultures treated with 0.2 mM H_2O_2 for 15 min (H) or 100 uM of Leptomycin B (LMB) for 60 and 90 min and analyzed by Northern blot as described in Figure 2A.



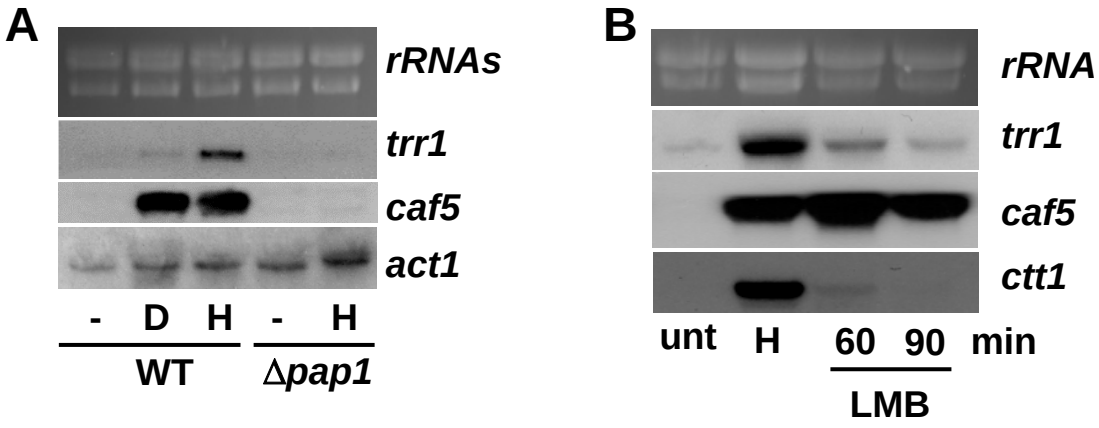
Calvo et al. Supplementary Figure S1



Calvo et al. Supplementary Figure S2



Calvo et al. Supplementary Figure S3



Calvo et al. Supplementary Figure S4