SUPPLEMENTARY METHODS

Southern blot

Southern blotting was conducted as described previously (7). Briefly, 5 µg of genomic DNA were digested by overnight incubation with appropriate restriction enzyme (see Supplementary Figures 1 to 4 for details), separated on a 1% agarose gel electrophoresis, then transferred by vacuum blotting to a GeneScreen Plus Hybridization Transfer nylon membrane (Perkin Elmer) and UV-cross-linked (GS Gene Linker UV chamber, BioRad). Probes were obtained by α^{32}P-dATP labelling of either PCR products or cloned fragments using the Megaprime DNA labelling system kit (GE Healthcare) following manufacturer's recommendations. Radioactive signals were obtained by exposure of a Storage Phosphor Screen (Amersham Biosciences) and revealed with a Typhoon Trio imager (Ge Healthcare).

^{3}H-fluconazole accumulation

Accumulation of ^{3}H-fluconazole was quantified as previously described (7). Briefly, 2.5 x 10^{8} cells from 20 ml cultures in SC medium were incubated at 30°C with 2.165 nmol of ^{3}H-fluconazole with a specific activity of 5 x 10^{6} dpm.nmol^{-1} (Amersham Biosciences), and 100 µl of the cell suspension were sampled at 0, 15, and 30 min. Accumulation was stopped by dilution of each sample in SC medium containing 20 µM cold fluconazole, and unincorporated ^{3}H-fluconazole was removed by 3 washes in the same medium. Intracellular ^{3}H-fluconazole was quantified on a Wallac 1409 scintillation counter (Perkin Elmer; Waltham, MA) after alkaline cell lysis and resuspension of the supernatant in scintillation liquid (UltimaGold; Packard, Groningen, The Netherlands). All experiments were performed in triplicate and statistically analyzed by repeated mesure ANOVA at $p = 0.05$ with Dunnett multiple test comparison using Prism software (version 5.04, GraphPad).

Transmission electron microscopy

Ultrastructure of *C. albicans* cells was observed by transmission electron microscopy following standard protocol with osmium tetroxyde fixation and Epon resin inclusion (8).

SUPPLEMENTARY TABLES

Table S2. Plasmids used in this study.

Plasmid	Origin	Description	Reference
CIp10	see reference	integration of the *URA3* gene at the *RPS1* locus	(6)
CIp30	see reference	integration of the *URA3*, *ARG4*, and *HIS1* genes at the *RPS1* locus	(3)
pMTL21	see reference	cloning vector	(1)
pBluescript KS+	purchased	cloning vector	Invitrogen
pMB-7	see ref	URA-blaster deletion strategy	(4)
pPV1	pKS+	orf19.6102 with UTRs cloned at XhoI/BamHI sites	This study
pPV7	pKS+	orf19.909 with UTRs cloned at XhoI/BamHI sites	This study
pPV13	pKS+	orf19.7371 with UTRs cloned at XhoI/BamHI sites	This study
pPV25	pKS+	orf19.6781 with UTRs cloned at XhoI/BamHI sites	This study
pPV29	pKS+	orf19.5617 with UTRs cloned at XhoI/BamHI sites	This study
pPV46	pMB-7	URA-blaster flanking deletion cassette for orf19.6102 (inverse PCR from pPV1 cloned at PstI/BglII sites)	This study
pPV47	pMB-7	URA-blaster internal deletion cassette for orf19.6102 (inverse PCR from pPV1 cloned at PstI/BglII sites)	This study
pPV48	pMB-7	URA-blaster flanking deletion cassette for orf19.7371 (inverse PCR from pPV13 cloned at PstI/BglII sites)	This study
pPV52	pMB-7	URA-blaster flanking deletion cassette for orf19.6781 (inverse PCR from pPV25 cloned at PstI/BglII sites)	This study
pPV53	pMB-7	URA-blaster internal deletion cassette for orf19.6781 (inverse PCR from pPV25 cloned at PstI/BglII sites)	This study
pPV54	pMB-7	URA-blaster flanking deletion cassette for orf19.5617 (inverse PCR from pPV29 cloned at PstI/BglII sites)	This study
pPV55	pMB-7	URA-blaster internal deletion cassette for orf19.5617 (inverse PCR from pPV29 cloned at PstI/BglII sites)	This study
pPV59	pKS+	orf19.2747 with UTRs cloned at XhoI/BamHI sites	This study
pPV60	pMB-7	URA-blaster flanking deletion cassette for orf19.909 (inverse PCR from pPV7 cloned at PstI/BglII sites)	This study
pPV64	pMB-7	URA-blaster internal deletion cassette for orf19.2747 (inverse PCR from pPV59 cloned at PstI/BglII sites)	This study
pPV66	pMB-7	URA-blaster flanking deletion cassette for orf19.2747 (inverse PCR from pPV59 cloned at PstI/BglII sites)	This study
pPV68	pKS+	orf19.7381 with UTRs cloned at XhoI/BamHI sites	This study
pPV69	pMB-7	URA-blaster flanking deletion cassette for orf19.7381 (inverse PCR from pPV68 cloned at PstI/BglII sites)	This study
pPV70	pMB-7	URA-blaster internal deletion cassette for orf19.7381 (inverse PCR from pPV68 cloned at PstI/BglII sites)	This study
pPV76	CIp10	orf19.2545 reversion at the *RPS1* locus (entire ORF with UTRs cloned at XhoI/MluI sites)	This study
pPV77	CIp10	orf19.6102 reversion at the *RPS1* locus (entire ORF with UTRs cloned at XhoI/MluI sites)	This study
pPV78	CIp10	orf19.6713 reversion at the *RPS1* locus (entire ORF with UTRs cloned at XhoI/MluI sites)	This study
pPV79	CIp10	orf19.7381 reversion at the *RPS1* locus (entire ORF with UTRs cloned at XhoI/MluI sites)	This study
pPV80	CIp10	orf19.5617 reversion at the *RPS1* locus (entire ORF with UTRs cloned at XhoI/MluI sites)	This study
pPV81	CIp10	orf19.6781 reversion at the *RPS1* locus (entire ORF with UTRs cloned at XhoI/MluI sites)	This study
pPV83	pMB-7	URA-blaster internal deletion cassette for orf19.1168 (inverse PCR from pDS1670 cloned at PstI/BglII sites)	This study

Table S2 (continued).

pPV84	CIp10	orf19.909 reversion at the *RPS1* locus (entire ORF with UTRs cloned at XhoI/MluI sites)	This study
pPV85	CIp10	orf19.2747 reversion at the *RPS1* locus (entire ORF with UTRs cloned at XhoI/MluI sites)	This study
pPV86	CIp10	orf19.3865 reversion at the *RPS1* locus (entire ORF with UTRs cloned at XhoI/MluI sites)	This study
pPV87	CIp10	orf19.5676 reversion at the *RPS1* locus (entire ORF with UTRs cloned at XhoI/MluI sites)	This study
pPV88	CIp10	orf19.7371 reversion at the *RPS1* locus (entire ORF with UTRs cloned at XhoI/MluI sites)	This study
pPV89	pMB-7	URA-blaster internal deletion cassette for orf19.6227 (inverse PCR from pDS1671 cloned at PstI/BglII sites)	This study
pPV111	CIp10	orf19.1168 reversion at the *RPS1* locus (entire ORF with UTRs cloned at XhoI/MluI sites)	This study
pPV112	CIp10	orf19.6227 reversion at the *RPS1* locus (entire ORF with UTRs cloned at XhoI/MluI sites)	This study
pDS1669	pKS+	orf19.2545 with UTRs cloned at XhoI/BamHI sites	This study
pDS1670	pKS+	orf19.1168 with UTRs cloned at XhoI/BamHI sites	This study
pDS1671	pKS+	orf19.6227 with UTRs cloned at XhoI/BamHI sites	This study
pDS1672	pKS+	orf19.3865 with UTRs cloned at XhoI/BamHI sites	This study
pDS1674	pKS+	orf19.5676 with UTRs cloned at XhoI/BamHI sites	This study
pDS1675	pMB-7	URA-blaster flanking deletion cassette for orf19.2545	This study
pDS1676	pMB-7	URA-blaster flanking deletion cassette for orf19.6227 (inverse PCR from pDS1671 cloned at PstI/BglII sites)	This study
pDS1678	pMB-7	URA-blaster flanking deletion cassette for orf19.3865 (inverse PCR from pDS1672 cloned at PstI/BglII sites)	This study
pDS1680	pKS+	orf19.6713with UTRs cloned at XhoI/BamHI sites	This study
pDS1681	pMB-7	URA-blaster flanking deletion cassette for orf19.5676 (inverse PCR from pDS1674 cloned at PstI/BglII sites)	This study
pDS1682	pMB-7	URA-blaster flanking deletion cassette for orf19.6713 (inverse PCR from pDS1680 cloned at PstI/BglII sites)	This study
pDS1685	pMB-7	URA-blaster flanking deletion cassette for orf19.1168 (inverse PCR from pDS1670 cloned at PstI/BglII sites)	This study

Table S3. Strains used in this study.

Strain	Parent	Phenotype	Genotype[a]	Reference
CAF4-2	CAF2-1	ura-	*ura3*Δ::λ*imm434/ura3*Δ::λ*imm434*	(4)
BWP17	SC5314	arg-, his-, ura-	*ura3*Δ::λ*imm434/ura3*Δ::λ*imm434 his1*::*hisG/his1*::*hisG arg4*::*hisG/arg4*::*hisG*	(9)
DAY286	BWP17	arg+, his-, ura+	*ura3*Δ::λ*imm434/ura3*Δ::λ*imm434 his1*::*hisG/his1*::*hisG arg4*::*hisG/pARG4::URA3::arg4*:.*hisG*	(2)
SFY87	BWP17	arg+, his+, ura+	*RPS1*/*rps1*::[CIp30]	This study
PVY121	CAF4-2	ura+	*RPS1*/*rps1*::[CIp10]	This study
PVY44	CAF4-2	ura+	orf19.2545/*orf19.2545*::*hisG-URA3-hisG*	This study
PVY56	PVY44	ura-	orf19.2545/*orf19.2545*::*hisG*	This study
PVY62	PVY56	ura+	*orf19.2545*::*hisG-URA3-hisG /orf19.2545*::*hisG*	This study
PVY106	PVY62	ura-	*orf19.2545*::*hisG /orf19.2545*::*hisG*	This study
PVY119	PVY106	ura+, *orf19.2545*Δ	*orf19.2545*::*hisG /orf19.2545*::*hisG, rps1/rps1*::[CIp10]	This study
PVY128	PVY106	ura+, orf19.2545rev	*orf19.2545*::*hisG /orf19.2545*::*hisG, rps1/rps1*::[pPV76]	This study
PVY45	CAF4-2	ura+	orf19.6227/*orf19.6227*::*hisG-URA3-hisG*	This study
PVY57	PVY45	ura-	orf19.6227/*orf19.6227*::*hisG*	This study
PVY68	PVY57	ura+	*orf19.6227*::*hisG-URA3-hisG /orf19.6227*::*hisG*	This study
PVY189	PVY68	ura-	*orf19.6227*::*hisG /orf19.6227*::*hisG*	This study
PVY224	PVY189	ura+, *orf19.6227*Δ	*orf19.6227*::*hisG /orf19.6227*::*hisG, rps1/rps1*::[CIp10]	This study
PVY222	PVY189	ura+, orf19.6227rev	*orf19.6227*::*hisG /orf19.6227*::*hisG, rps1/rps1*::[pPV112]	This study
PVY46	CAF4-2	ura+	orf19.3865/*orf19.3865*::*hisG-URA3-hisG*	This study
PVY58	PVY46	ura-	orf19.3865/*orf19.3865*::*hisG*	This study
PVY63	PVY58	ura+	*orf19.3865*::*hisG-URA3-hisG /orf19.3865*::*hisG*	This study
PVY107	PVY63	ura-	*orf19.3865*::*hisG /orf19.3865*::*hisG*	This study
PVY120	PVY107	ura+, *orf19.3865*Δ	*orf19.3865*::*hisG /orf19.3865*::*hisG, rps1/rps1*::[CIp10]	This study
PVY137	PVY107	ura+, orf19.3865rev	*orf19.3865*::*hisG /orf19.3865*::*hisG, rps1/rps1*::[pPV86]	This study
PVY47	CAF4-2	ura+	orf19.5676/*orf19.5676*::*hisG-URA3-hisG*	This study
PVY59	PVY47	ura-	orf19.5676/*orf19.5676*::*hisG*	This study
PVY64	PVY59	ura+	*orf19.5676*::*hisG-URA3-hisG /orf19.5676*::*hisG*	This study
PVY98	PVY64	ura-	*orf19.5676*::*hisG /orf19.5676*::*hisG*	This study
PVY111	PVY98	ura+, *orf19.5676*Δ	*orf19.5676*::*hisG /orf19.5676*::*hisG, rps1/rps1*::[CIp10]	This study
PVY133	PVY98	ura+, orf19.5676rev	*orf19.5676*::*hisG /orf19.5676*::*hisG, rps1/rps1*::[pPV87]	This study
PVY48	CAF4-2	ura+	orf19.6713/*orf19.6713*::*hisG-URA3-hisG*	This study
PVY55	PVY48	ura-	orf19.6713/*orf19.6713*::*hisG*	This study
PVY60	PVY55	ura+	*orf19.6713*::*hisG-URA3-hisG /orf19.6713*::*hisG*	This study
PVY97	PVY60	ura-	*orf19.6713*::*hisG /orf19.6713*::*hisG*	This study
PVY110	PVY97	ura+, *orf19.6713*Δ	*orf19.6713*::*hisG /orf19.6713*::*hisG, rps1/rps1*::[CIp10]	This study
PVY132	PVY97	ura+, orf19.6713rev	*orf19.6713*::*hisG /orf19.6713*::*hisG, rps1/rps1*::[pPV78]	This study
PVY65	CAF4-2	ura+	orf19.6102/*orf19.6102*::*hisG-URA3-hisG*	This study
PVY70	PVY65	ura-	orf19.6102/*orf19.6102*::*hisG*	This study
PVY80	PVY70	ura+	*orf19.6102*::*hisG-URA3-hisG /orf19.6102*::*hisG*	This study
PVY104	PVY80	ura-	*orf19.6102*::*hisG /orf19.6102*::*hisG*	This study
PVY117	PVY104	ura+, *orf19.6102*Δ	*orf19.6102*::*hisG /orf19.6102*::*hisG, rps1/rps1*::[CIp10]	This study
PVY127	PVY104	ura+, orf19.6102rev	*orf19.6102*::*hisG /orf19.6102*::*hisG, rps1/rps1*::[pPV77]	This study
PVY67	CAF4-2	ura+	orf19.5617/*orf19.5617*::*hisG-URA3-hisG*	This study
PVY87	PVY67	ura-	orf19.5617/*orf19.5617*::*hisG*	This study
PVY89	PVY87	ura+	*orf19.5617*::*hisG-URA3-hisG /orf19.5617*::*hisG*	This study
PVY100	PVY89	ura-	*orf19.5617*::*hisG /orf19.5617*::*hisG*	This study
PVY113	PVY100	ura+, *orf19.5617*Δ	*orf19.5617*::*hisG /orf19.5617*::*hisG, rps1/rps1*::[CIp10]	This study
PVY129	PVY100	ura+, orf19.5617rev	*orf19.5617*::*hisG /orf19.5617*::*hisG, rps1/rps1*::[pPV80]	This study
PVY71	CAF4-2	ura+	orf19.7371/*orf19.7371*::*hisG-URA3-hisG*	This study
PVY76	PVY71	ura-	orf19.7371/*orf19.7371*::*hisG*	This study
PVY84	PVY76	ura+	*orf19.7371*::*hisG-URA3-hisG /orf19.7371*::*hisG*	This study
PVY105	PVY84	ura-	*orf19.7371*::*hisG /orf19.7371*::*hisG*	This study
PVY118	PVY105	ura+, *orf19.7371*Δ	*orf19.7371*::*hisG /orf19.7371*::*hisG, rps1/rps1*::[CIp10]	This study
PVY136	PVY105	ura+, orf19.7371rev	*orf19.7371*::*hisG /orf19.7371*::*hisG, rps1/rps1*::[pPV88]	This study

Table S3 (continued).

PVY73	CAF4-2	ura+	orf19.6781/*orf19.6781*::*hisG-URA3-hisG*	This study
PVY78	PVY73	ura-	orf19.6781/*orf19.6781*::*hisG*	This study
PVY82	PVY78	ura+	*orf19.6781*::*hisG-URA3-hisG* /*orf19.6781*::*hisG*	This study
PVY99	PVY82	ura-	*orf19.6781*::*hisG* /*orf19.6781*::*hisG*	This study
PVY112	PVY99	ura+, *orf19.6781*Δ	*orf19.6781*::*hisG* /*orf19.6781*::*hisG, rps1/rps1*::[CIp10]	This study
PVY130	PVY99	ura+, orf19.6781rev	*orf19.6781*::*hisG* /*orf19.6781*::*hisG, rps1/rps1*::[pPV81]	This study
PVY74	CAF4-2	ura+	orf19.909/*orf19.909*::*hisG-URA3-hisG*	This study
PVY91	PVY74	ura-	orf19.909/*orf19.909*::*hisG*	This study
PVY93	PVY91	ura+	*orf19.909*::*hisG-URA3-hisG* /*orf19.909*::*hisG*	This study
PVY102	PVY93	ura-	*orf19.909*::*hisG* /*orf19.909*::*hisG*	This study
PVY115	PVY102	ura+, *orf19.909*Δ	*orf19.909*::*hisG* /*orf19.909*::*hisG, rps1/rps1*::[CIp10]	This study
PVY135	PVY102	ura+, orf19.909rev	*orf19.909*::*hisG* /*orf19.909*::*hisG, rps1/rps1*::[pPV84]	This study
PVY75	CAF4-2	ura+	orf19.2747/*orf19.2747*::*hisG-URA3-hisG*	This study
PVY88	PVY75	ura-	orf19.2747/*orf19.2747*::*hisG*	This study
PVY90	PVY88	ura+	*orf19.2747*::*hisG-URA3-hisG* /*orf19.2747*::*hisG*	This study
PVY101	PVY90	ura-	*orf19.2747*::*hisG* /*orf19.2747*::*hisG*	This study
PVY114	PVY101	ura+, *orf19.2747*Δ	*orf19.2747*::*hisG* /*orf19.2747*::*hisG, rps1/rps1*::[CIp10]	This study
PVY134	PVY101	ura+, orf19.2747rev	*orf19.2747*::*hisG* /*orf19.2747*::*hisG, rps1/rps1*::[pPV85]	This study
PVY81	CAF4-2	ura+	orf19.7381/*orf19.7381*::*hisG-URA3-hisG*	This study
PVY92	PVY81	ura-	orf19.7381/*orf19.7381*::*hisG*	This study
PVY94	PVY92	ura+	*orf19.7381*::*hisG-URA3-hisG* /*orf19.7381*::*hisG*	This study
PVY103	PVY94	ura-	*orf19.7381*::*hisG* /*orf19.7381*::*hisG*	This study
PVY116	PVY103	ura+, *orf19.7381*Δ	*orf19.7381*::*hisG* /*orf19.7381*::*hisG, rps1/rps1*::[CIp10]	This study
PVY126	PVY103	ura+, orf19.7381rev	*orf19.7381*::*hisG* /*orf19.7381*::*hisG, rps1/rps1*::[pPV79]	This study
PVY131	CAF4-2	ura+	orf19.1168/*orf19.1168*::*hisG-URA3-hisG*	This study
PVY138	PVY131	ura-	orf19.1168/*orf19.1168*::*hisG*	This study
PVY139	PVY138	ura+	*orf19.1168*::*hisG-URA3-hisG* /*orf19.1168*::*hisG*	This study
PVY140	PVY139	ura-	*orf19.1168*::*hisG* /*orf19.1168*::*hisG*	This study
PVY221	PVY140	ura+, orf19.1168rev	*orf19.1168*::*hisG* /*orf19.1168*::*hisG, rps1/rps1*::[pPV111]	This study
PVY223	PVY140	ura+, *orf19.1168*Δ	*orf19.1168*::*hisG* /*orf19.1168*::*hisG, rps1/rps1*::[CIp10]	This study
HLC67	CAF4-2	ura+	*efg1*::*hisG/efg1*::*hisG-URA3-hisG*	(5)
PVY252	HLC67	ura-	*efg1*::*hisG/efg1*::*hisG*	This study
PVY253[b]	PVY252	ura+	orf19.6102/*orf19.6102*::*hisG-URA3-hisG*	This study
PVY254[b]	PVY252	ura+, *efg1*Δ	*rps1/rps1*::[CIp10]	This study
PVY255[b]	PVY253	ura-	orf19.6102/*orf19.6102*:: *hisG*	This study
PVY256[b]	PVY255	ura+	*orf19.6102*::*hisG-URA3-hisG* /*orf19.6102*::*hisG*	This study
PVY257[b]	PVY256	ura-	*orf19.6102*:: *hisG* /*orf19.6102*::*hisG*	This study
PVY258[b]	PVY257	ura+, *efg1*Δ *rca1*Δ	*orf19.6102*:: *hisG* /*orf19.6102*::*hisG, rps1/rps1*::[CIp10]	This study

[a]All PVY strains are *ura3*Δ::λ*imm434/ura3*Δ::λ*imm434*.

[b]All HLC67-derived strains are *efg1*::*hisG/efg1*::*hisG*.

Table S4. Primers used in this study.

Primer name	Description	Sequence (5'-3')*
orf19.3865-XhoI-F	Cloning into pBluescript KS+	TTTGCTCGAGCTTACAAACTAGAATTTGTG
orf19.3865-XbaI-R	Cloning into pBluescript KS+	AAATTATCTAGACATCTATTTTCAACCAAT
orf19.3865-PstI-F	Cloning into pMB-7 (flanking cassette)	TGTGACTGCAGAGGTACATTTTGTTGATAA
orf19.3865-BglII-R	Cloning into pMB-7 (flanking cassette)	AAAAAGATCTATTCAGAATAATAATAATAA
orf19.3865-MluI-rev	Cloning into CIp10	ATTCAATTACGCGTGTTTTAAGA
orf19.5676-XhoI-F	Cloning into pBluescript KS+	GCTCTCGAGTGGTATCATTTATTGGCGGAA
orf19.5676-XbaI-R	Cloning into pBluescript KS+	GATTCTAGACCAAAGTCACATGAGATATGA
orf19.5676-PstI-F	Cloning into pMB-7 (flanking cassette)	TGTGTCTGCAGTTATGAAATGTAAAGTGTG
orf19.5676-BglII-R	Cloning into pMB-7 (flanking cassette)	TCAAGATCTCATTAGGTATTTCATTTTCTC
orf19.5676-XhoI3	Cloning into CIp10	TCTCGCTCGAGGAAAAATGT
orf19.5676-MluI-rev	Cloning into CIp10	TTCCAATTACGCGTACACCAA
orf19.2545-XhoI-F	Cloning into pBluescript KS+	AAATCCTCGAGGATCATCTTGAAAACTTGA
orf19.2545-XbaI-R	Cloning into pBluescript KS+	TATAATCTAGAATTGGTGAATCGATGGGAA
orf19.2545-HindIII-F	Cloning into pMB-7 (flanking cassette)	TGAAAGCTTAAATGAAACCAAGTTTAATTC
orf19.2545-BglII-R	Cloning into pMB-7 (flanking cassette)	TATGAGATCTCCTTGGTGGTGGTGGTAGTG
orf19.2545-MluI-rev	Cloning into CIp10	CAAAACGCGTACTAGATGAAG
orf19.1168-XhoI-F	Cloning into pBluescript KS+	TGTGACTCGAGCCAGTCTTAAAACTATTGT
orf19.1168-XbaI-R	Cloning into pBluescript KS+	TGTGTCTTCTAGAGTACTATACGGCAAAGAA
orf19.1168-PstI-F	Cloning into pMB-7 (flanking cassette)	AGTAACTGCAGTACATAATGCAAAAATAAA
orf19.1168-BglII-R	Cloning into pMB-7 (flanking cassette)	CAACTAGATCTTGGATGATACTATGATTGA
orf19.1168-PstI-F2	Cloning into pMB-7 (internal cassette)	AGTAACTGCAGTACATAATGCAAAAATAAA
orf19.1168-MluI-rev	Cloning into CIp10	AGAAAGACGCGTCTGATTACTTGTTAGATT
orf19.6713-XhoI-F	Cloning into pBluescript KS+	GTTCTCGAGACTATCTTTGAAGTAAAGTGT
orf19.6713-XbaI-R	Cloning into pBluescript KS+	ACATCTAGATCTATGGTTTTAAACTTATGG
orf19.6713-PstI-F	Cloning into pMB-7 (flanking cassette)	TTCCTGCAGCCCATTACTAATGCTACGACTG
orf19.6713-BglII-R	Cloning into pMB-7 (flanking cassette)	ATTGAGATCTTGAAGCAAAATTGGCTTGTT
orf19.6713-MluI-rev	Cloning into CIp10	ATGGACGCGTTTAGCTTGAGT
orf19.6227-XhoI-F	Cloning into pBluescript KS+	TGCAGCTCGAGAAAATGGAGAAATATTGCTGG
orf19.6227-XbaI-R	Cloning into pBluescript KS+	TATAACTCTAGAATCCCAAATATACAGTAG
orf19.6227-PstI-F	Cloning into pMB-7 (flanking cassette)	ATCTGCTGCAGTAGTTATATGTTCCTTATT
orf19.6227-BglII-R	Cloning into pMB-7 (flanking cassette)	AATAGATCTCAGAAAAGGAGGGGGTTC
orf19.6227-Rint	Verification of homozygous deletion	TACGTGCATGATACGCCAGAT
orf19.6227-MluI-rev	Cloning into CIp10	AAGGACGCGTAATATTTCAAT
orf19.6102-XhoI-F	Cloning into pBluescript KS+	CGCGAAACTCGAGTCAGCGACGACAAGAAAGAT
orf19.6102-BamHI-R	Cloning into pBluescript KS+	CGCGAAAGGATCCAGGTTGTAGTGTGAGTAGGCG
orf19.6102-BglII-F	Cloning into pMB-7 (flanking cassette)	CGCGAAAAGATCTTTAATGATTCACTAGTTTCT

Table S4 (continued).

orf19.6102-PstI-R	Cloning into pMB-7 (flanking cassette)	CGCGAAACTGCAGCCACCCTTACCAGTTCGACT
orf19.6102-PstI-F	Cloning into pMB-7 (internal cassette)	CGCGAAACTGCAGGGATTGCTGCGTCAAAGTGT
orf19.6102-BglII-R	Cloning into pMB-7 (internal cassette)	CGCGAAAAGATCTTCAAAGAGTGGTTGTTCAAA
orf19.6102-MluI-rev	Cloning into CIp10	GAGTACGCGTAGTAGTCGATG
orf19.7371-F	Cloning into pBluescript KS+	TCGCTAAACAGAAGCGCAAA
orf19.7371-XhoI-R	Cloning into pBluescript KS+	CGCGAAACTCGAGCTGATACTGATTTATCCGTCA
orf19.7371-BglII-F	Cloning into pMB-7 (flanking cassette)	CGCGAAAAGATCTGTTGCTGTTCGTGGTTTATT
orf19.7371-PstI-R	Cloning into pMB-7 (flanking cassette)	CGCGAAACTGCAGTTAATATAGAGAAATAGATA
orf19.7371-PstI-F	Cloning into pMB-7 (internal cassette)	CGCGAAACTGCAGAATACAATATTATCATTTTT
orf19.7371-BglII-R	Cloning into pMB-7 (internal cassette)	CGCGAAAAGATCTAACAGAATATCCCACCTCAT
orf19.7371-Rint	Verification of homozygous deletion	GCCATCACCTTCTTCGGGTGCC
orf19.7371-MluI-rev	Cloning into CIp10	ATCGAAACGCGTTGTCATAGTA
orf19.909-XhoI-F	Cloning into pBluescript KS+	CGCGAAACTCGAGGTGGAACGACAATCATTTTGA
orf19.909-BamHI-R	Cloning into pBluescript KS+	CGCGAAAGGATCCTGCCTTGTTGTTGCTGTCTT
orf19.909-BglII-F	Cloning into pMB-7 (flanking cassette)	CGCGAAAAGATCTATGAAGAGAGTTAAATTAAG
orf19.909-PstI-R	Cloning into pMB-7 (flanking cassette)	CGCGAAACTGCAGGTTTTAATTGATATTATAGT
orf19.909-PstI-F	Cloning into pMB-7 (internal cassette)	CGCGAAACTGCAGGATGTGATACATTCAAAAAT
orf19.909-BglII-R	Cloning into pMB-7 (internal cassette)	CGCGAAAAGATCTAAGGAATGTATTGATGGTAA
orf19.909-Rint	Verification of homozygous deletion	GAAACCAAACAATCGTCCTCA
orf19.909-MluI-rev	Cloning into CIp10	CGCGAAAACGCGTTGCCTTGTTGTTGCTGTCTT
orf19.6781-XhoI-F	Cloning into pBluescript KS+	CGCGAAACTCGAGTCTTCTCCAACTATTAAGCCA
orf19.6781-BamHI-R	Cloning into pBluescript KS+	CGCGAAAGGATCCCACCAATAGATCAACAACGA
orf19.6781-BglII-F	Cloning into pMB-7 (flanking cassette)	CGCGAAAAGATCTATAGGTGTATACCTATATGT
orf19.6781-PstI-R	Cloning into pMB-7 (flanking cassette)	CGCGAAACTGCAGTGCGAAAATGAAAATGATAA
orf19.6781-PstI-F	Cloning into pMB-7 (internal cassette)	CGCGAAACTGCAGATTATGGAACATTAAATGGT
orf19.6781-BglII-R	Cloning into pMB-7 (internal cassette)	CGCGAAAAGATCTACCGGCAGAGAATATTCTGT
orf19.6781-MluI-rev	Cloning into CIp10	TTTAAACGCGTAATACTACAC
orf19.5617-XhoI-F	Cloning into pBluescript KS+	CGCGAAACTCGAGACAAGTTACACCAAGAGCTAG
orf19.5617-BamHI-R	Cloning into pBluescript KS+	CGCGAAAGGATCCCCACAACCACAATCACATCA
orf19.5617-BglII-F	Cloning into pMB-7 (flanking cassette)	CGCGAAAAGATCTTTATATTGCTGTTGCTGAAT
orf19.5617-PstI-R	Cloning into pMB-7 (flanking cassette)	CGCGAAACTGCAGAGAAAAGTTTGTTTGCTATT
orf19.5617-PstI-F	Cloning into pMB-7 (internal cassette)	CGCGAAACTGCAGCAAGGATGTGGAATTCTGGT
orf19.5617-BglII-R	Cloning into pMB-7 (internal cassette)	CGCGAAAAGATCTAAACAATAATAATAATAACA
orf19.5617-MluI-rev	Cloning into CIp10	CAATATACGCGTCGATTGAAT

Table S4 (continued).

orf19.7381-SacI-F	Cloning into pBluescript pMTL21	CGCGAAAGAGCTCTAACAAACCAACCCCCTACCT
orf19.7381-SphI-R	Cloning into pBluescript pMTL21	CGCGAAAGCATGCCTATACATATTTCGGCAAAAC
orf19.7381-KpnI-F	Cloning into pMB-7 (flanking cassette)	CGCGAAAGGTACCGACTATTGGATATGGATCTT
orf19.7381-PstI-R	Cloning into pMB-7 (flanking cassette)	CGCGAAACTGCAGTAAATTCACATCTATATACT
orf19.7381-PstI-F	Cloning into pMB-7 (internal cassette)	CGCGAAACTGCAGACTGACAATGAAGTAGTCAA
orf19.7381-KpnI-R	Cloning into pMB-7 (internal cassette)	CGCGAAAGGTACCCGAATTTATATTCCAACCCGG
orf19.7381-MluI-rev	Cloning into CIp10	TATCACGCGTCTAATCTTCTA
orf19.2747-XhoI-F	Cloning into pBluescript KS+	CGCGAAACTCGAGAGTTATTTGCTGGTGGGAGGA
orf19.2747-BamHI-R	Cloning into pBluescript KS+	CGCGAAAGGATCCTGTCAGATTAATTTATGGTCA
orf19.2747-BglII-F	Cloning into pMB-7 (flanking cassette)	CGCGAAAAGATCTAAGAGTAGGAAAAAAAAAAA
orf19.2747-SphI-R	Cloning into pMB-7 (flanking cassette)	CGCGAAAGCATGCAAAAAGAAAAGTTAGCATAC
orf19.2747-SphI-F	Cloning into pMB-7 (internal cassette)	CGCGAAAGCATGCTTAGCTTTGGTTGATGCTTG
orf19.2747-BglII-R	Cloning into pMB-7 (internal cassette)	CGCGAAAAGATCTACAACAATAACAATACTTCC
orf19.2747-Rint	Verification of homozygous deletion	TGAAACTAATCCCCGCGTTA
orf19.2747-MluI-rev	Cloning into CIp10	CGCGAAAACGCGTTGTCAGATTAATTTATGGTCA
OCH1-RT-F	Quantitative PCR	GGCATACCATCATCTTTCCA
OCH1-RT-R	Quantitative PCR	ATGTCAATCAAATGGGTGCT
CHT2-RT-F	Quantitative PCR	CGGTGCATACAACAGTTTGA
CHT2-RT-F	Quantitative PCR	AATGTGTTGCCACTCCAGTT
PGA13-RT-F	Quantitative PCR	CTAGTGCCAGTGCCGGTGCC
PGA13-RT-R	Quantitative PCR	TGTGGTTGGGTGACATTCGTTGT
HWP1-RT-F	Quantitative PCR	ACAACCACAAGAACCTTGCGACA
HWP1-RT-R	Quantitative PCR	CAGGCTGATCAGGTTGAGGAGGA
ECE1-RT-F	Quantitative PCR	AAGGCCAACATCTGGAACGCCA
ECE1-RT-R	Quantitative PCR	TGCCGTCGTCAGATTGCCAGA
CDR4-RT-F	Quantitative PCR	CGCCACTTGGCTGCAGTGGT
CDR4-RT-R	Quantitative PCR	ACGCTCACCACCAGAAACCCC
HGC1-RT-F	Quantitative PCR	TCGGTTTTGAATGCTTCGGA
HGC1-RT-R	Quantitative PCR	ACCACTATTACCAAACCCGCCA
ACT1-RT-F	Quantitative PCR	GTTCCCAGGTATTGCTGAAC
ACT1-RT-R	Quantitative PCR	CAATGGATGGACCAGATTCG
5' adapter	Strand specific library	rGUUrCrArGrArGUUrCUrArCrArGurCrCrGrArCrGrAUrC
3' adapter	Strand specific library	rAATCTCGTATGCCGTCTTCTGCTTGddC
SRA-RT	Strand specific library	CAAGCAGAAGACGGCATACGA
GX1	Strand specific library	CAAGCAGAAGACGGCATACGA
GX2	Strand specific library	AATGATACGGCGACCACCGACAGGTTCAGAGTTCTACAGTCCGA

*Artificially added resctrictions sites are underlined whenever appropriate.

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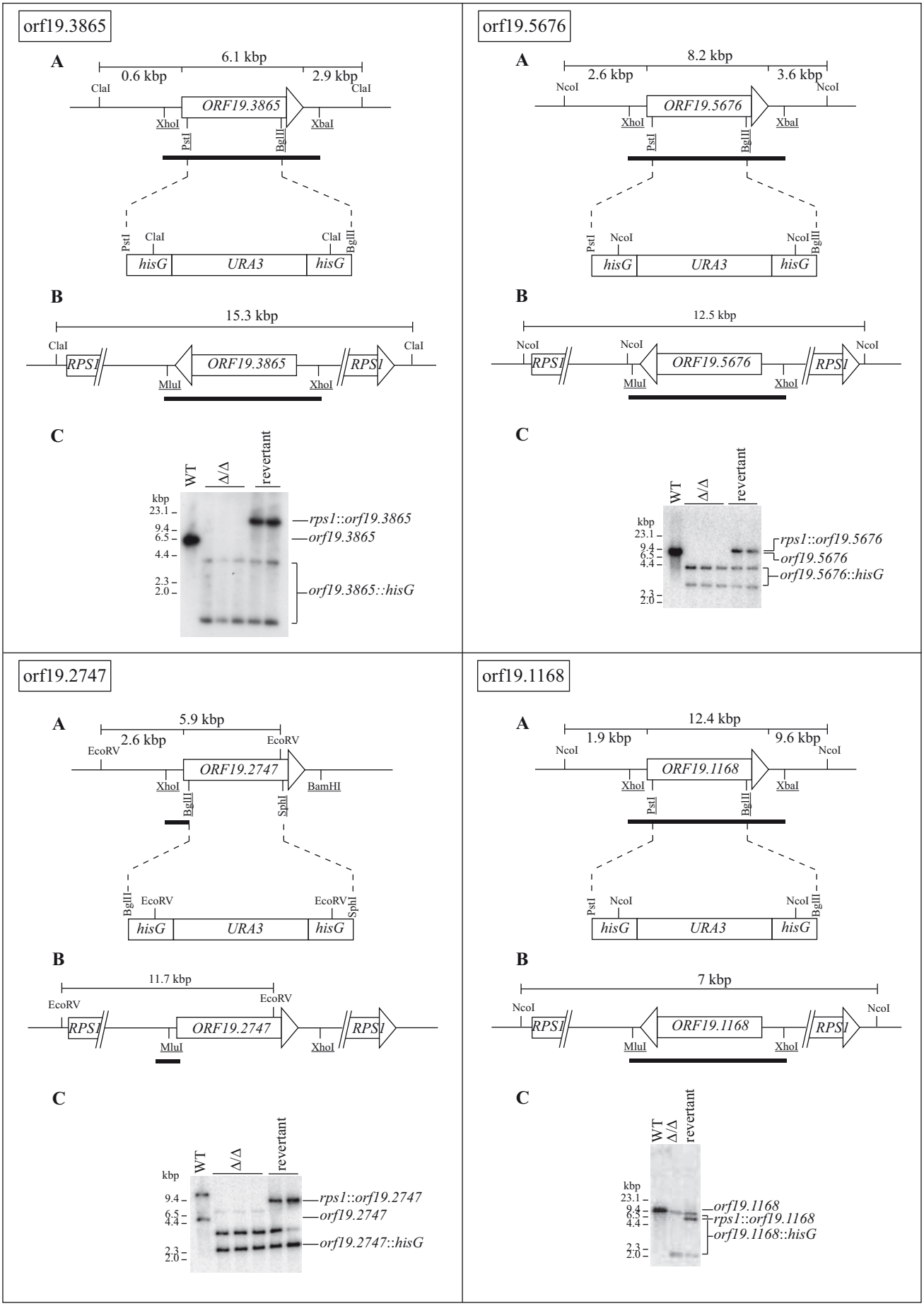

Figure S1: Genotype analysis of reconstructed mutant and revertant strains for orf19.3865, orf19.5676, orf19.2747, and orf19.1168. (**A**) Restriction maps of selected TFs loci and (**B**) of TF ORFs integrated at the *RPS1* locus. Underlined restriction sites were created by PCR for construction of recombination cassette (lines not scaled). Probe position is indicated by thick black lines under each loci. (**C**) Southern blot analysis of WT, homozygous deletion mutant and revertant strains for selected TFs. Each expected band is identified on the right and molecular sizes are indicated on the left (see Materials and Methods for details).

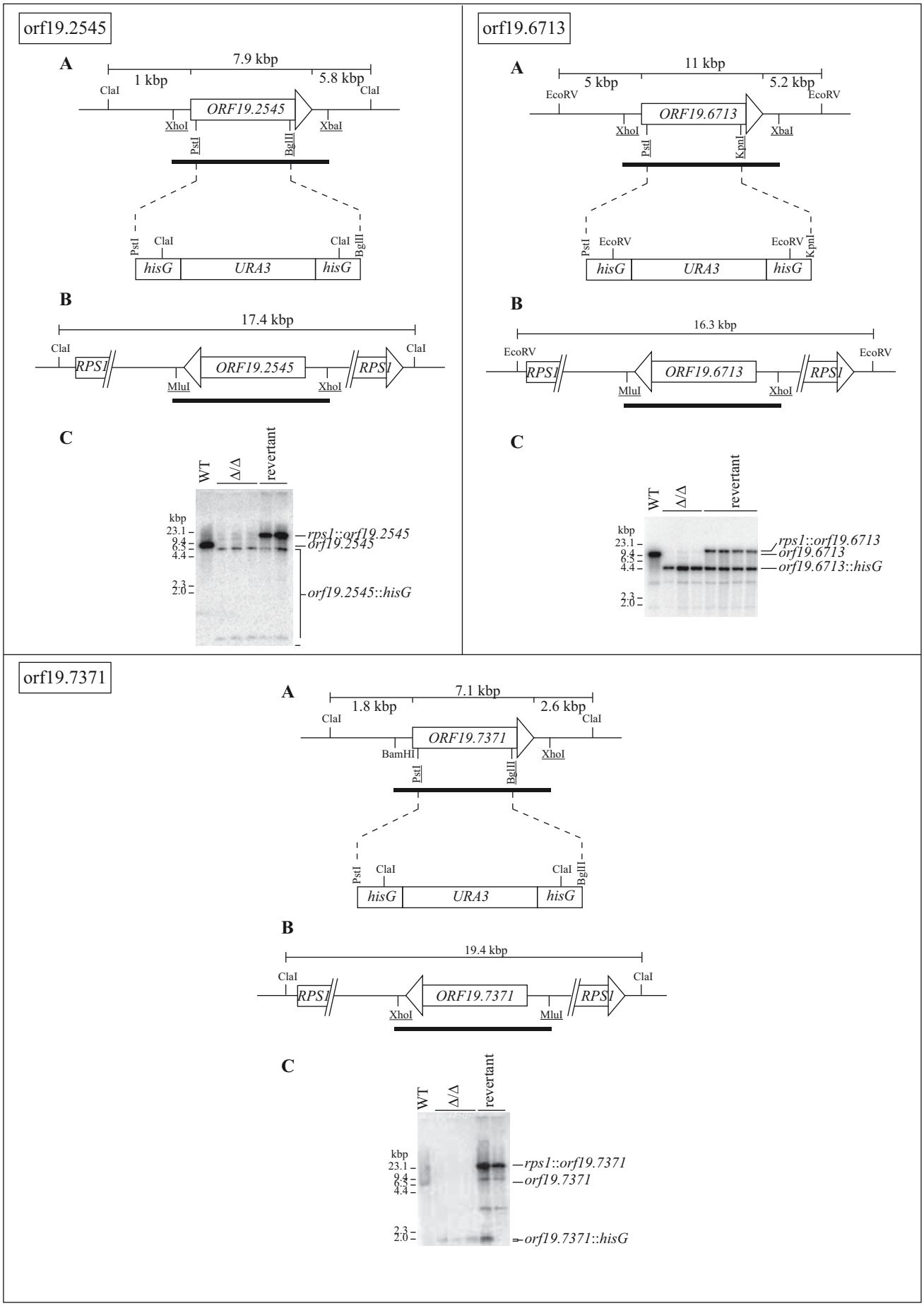

Figure S2: Genotype analysis of reconstructed mutant and revertant strains for orf19.2545, orf19.6713, and orf19.7371. (**A**) Restriction maps of selected TFs loci and (**B**) of TF ORFs integrated at the *RPS1* locus. Underlined restriction sites were created by PCR for construction of recombination cassette (lines not scaled). Probe position is indicated by thick black lines under each loci. (**C**) Southern blot analysis of WT, homozygous deletion mutant and revertant strains for selected TFs. Each expected band is identified on the right and molecular sizes are indicated on the left (see Materials and Methods for details).

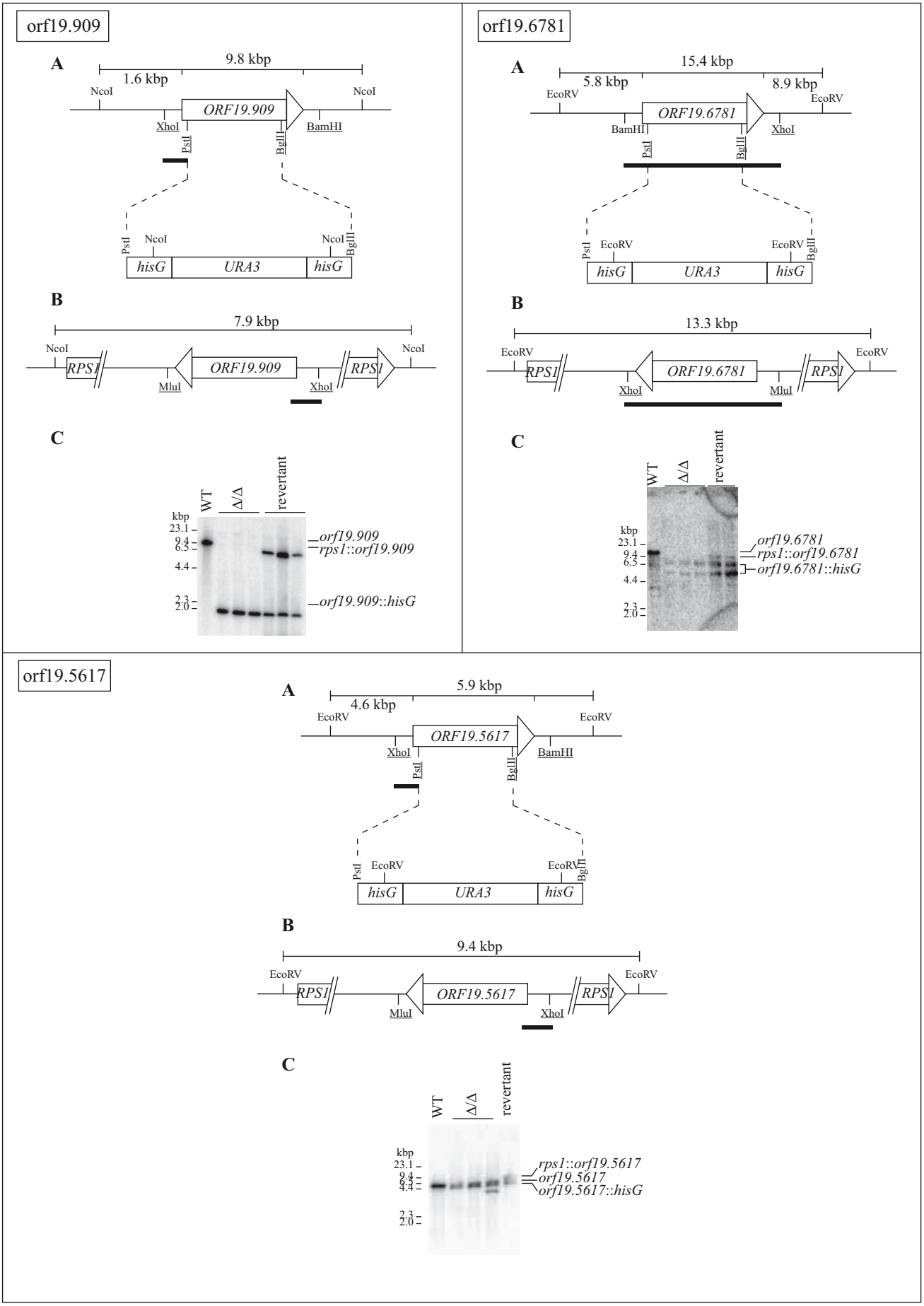

Figure S3: Genotype analysis of reconstructed mutant and revertant strains for orf19.909, orf19.6781, and orf19.5617. (**A**) Restriction maps of selected TFs loci and (**B**) of TF ORFs integrated at the *RPS1* locus. Underlined restriction sites were created by PCR for construction of recombination cassette (lines not scaled). Probe position is indicated by thick black lines under each loci. (**C**) Southern blot analysis of WT, homozygous deletion mutant and revertant strains for selected TFs. Each expected band is identified on the right and molecular sizes are indicated on the left (see Materials and Methods for details).

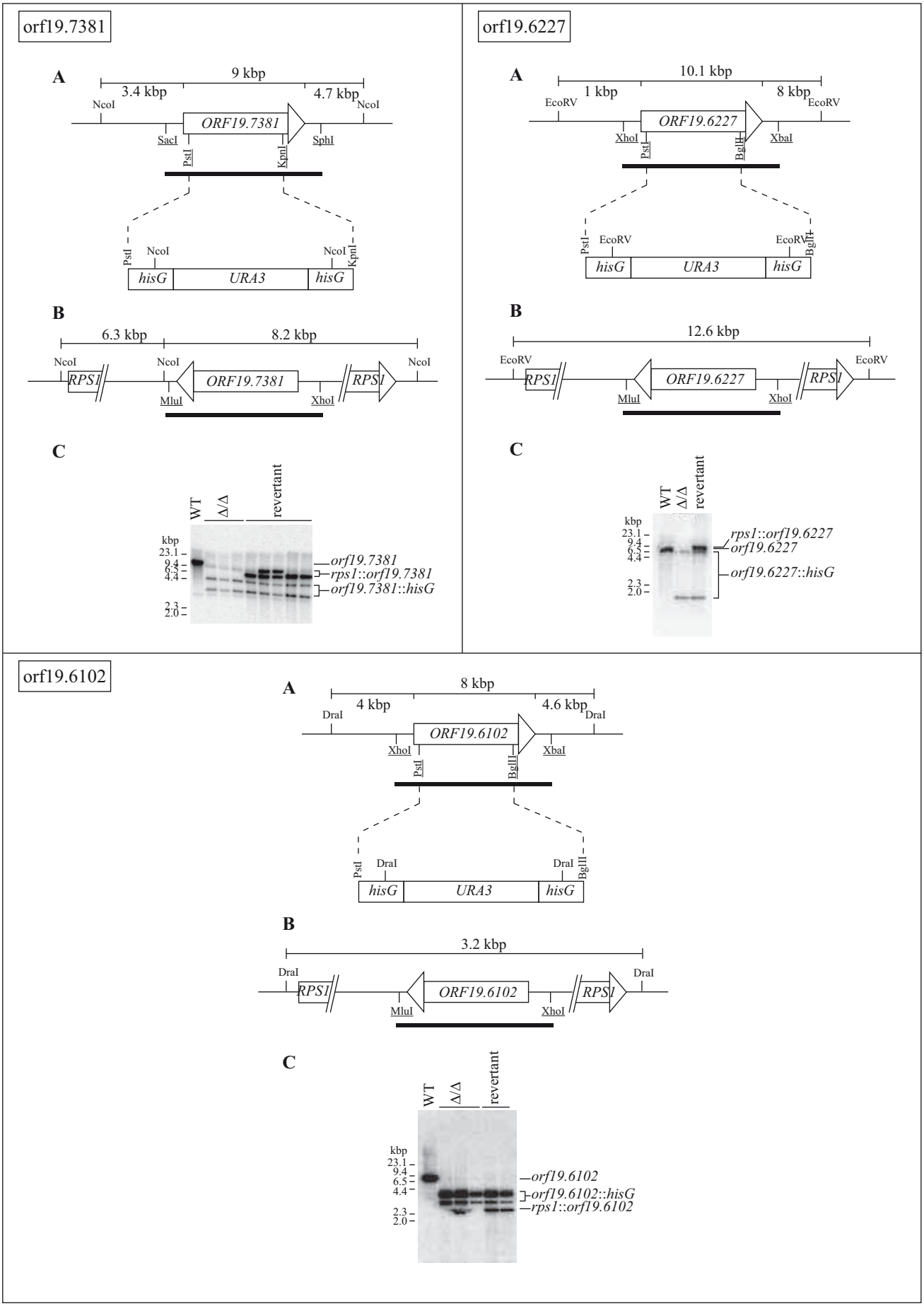

Figure S4: Genotype analysis of reconstructed mutant and revertant strains for orf19.7381, orf19.6227, and orf19.6102. (**A**) Restriction maps of selected TFs loci and (**B**) of TF ORFs integrated at the *RPS1* locus. Underlined restriction sites were created by PCR for construction of recombination cassette (lines not scaled). Probe position is indicated by thick black lines under each loci. (**C**) Southern blot analysis of WT, homozygous deletion mutant and revertant strains for selected TFs. Each expected band is identified on the right and molecular sizes are indicated on the left (see Materials and Methods for details).

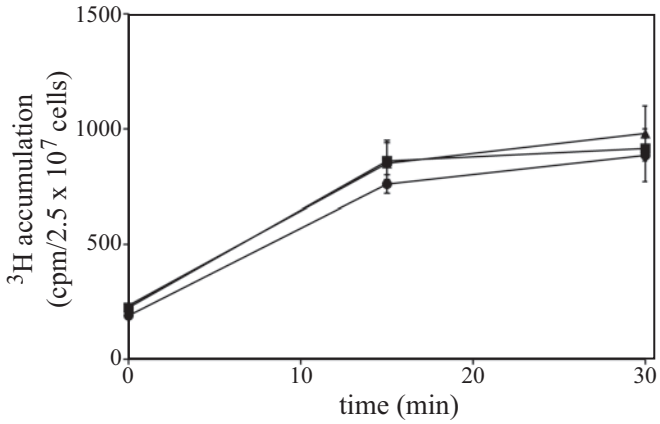

Figure S5: ^{3}H-fluconazole accumulation. No difference in ^{3}H-fluconazole accumulation was observed for *RCA1* mutant cells (squares), as compared to the wild type (circles) or revertant strain (triangles).

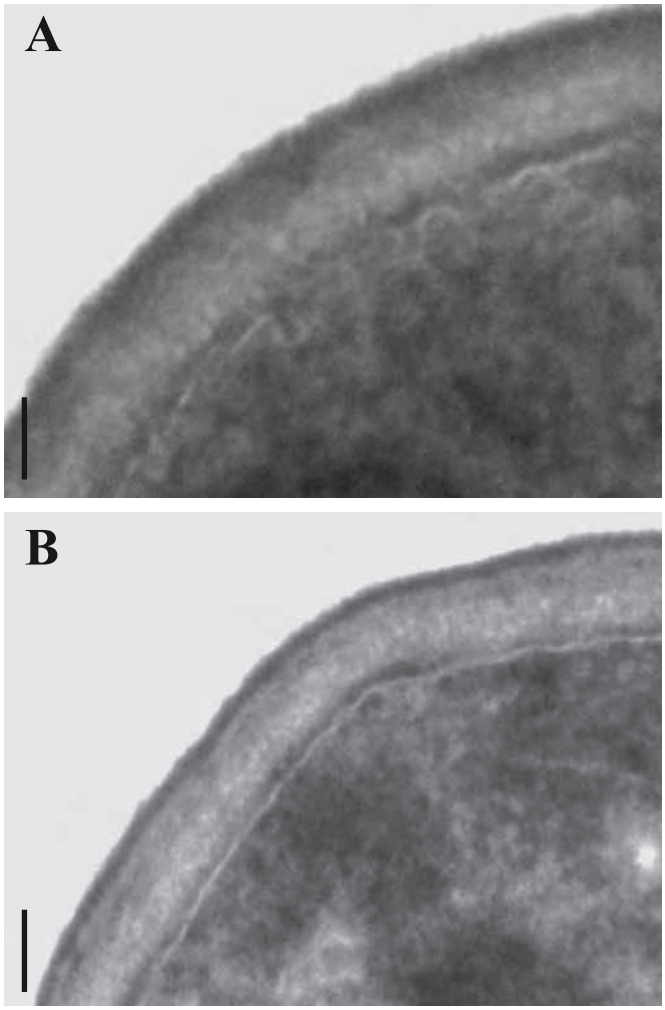

Figure S6: Cell wall ultrastructure. Transmission electron micrographs of *C. albicans RCA1* revertant (**A**) and mutant (**B**) strains revealed no differences. Bars: 100 nm.

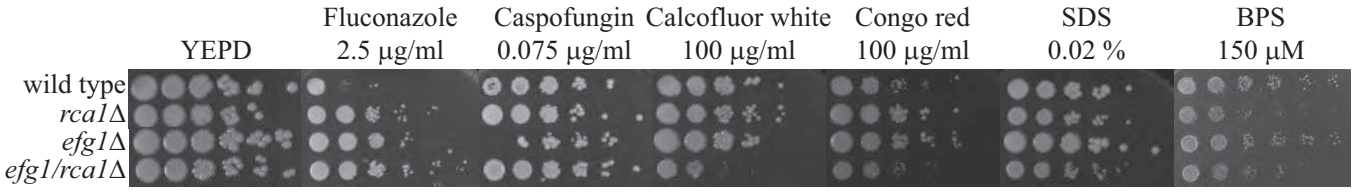

Figure S7: Susceptibility of *RCA1* and *EFG1* mutants, and of *EFG1*/*RCA1* double mutant to antifungals, cell wall perturbing agents, and iron chelator. Susceptibility to FLC, CAS, calcofluor white, Congo red, SDS and bathophenantroline di-sulfonic acid (BPS) was determined by serial dilution assay. The *EFG1* mutant and the *EFG1*/*RCA1* double mutant, as the *RCA1* mutant, were resistant to FLC. The *EFG1*/*RCA1* double mutant had an increased susceptibility to all cell wall perturbing agents tested but was resistant to CAS. On the opposite, the *EFG1* mutant had an increased susceptibility to CAS but was resistant to SDS, calcofluor white, and Congo red. Only *RCA1* mutant and *RCA1*/*EFG1* double mutant were more susceptible to iron chelation as compared to the wild type. The *EFG1* deletion alone had no effect on BPS susceptibility, suggesting that this phenotype is mediated by the putative interaction between Rca1 and a transcriptional repressor.