Passage through the mammalian gut triggers a phenotypic switch that promotes *Candida albicans* commensalism

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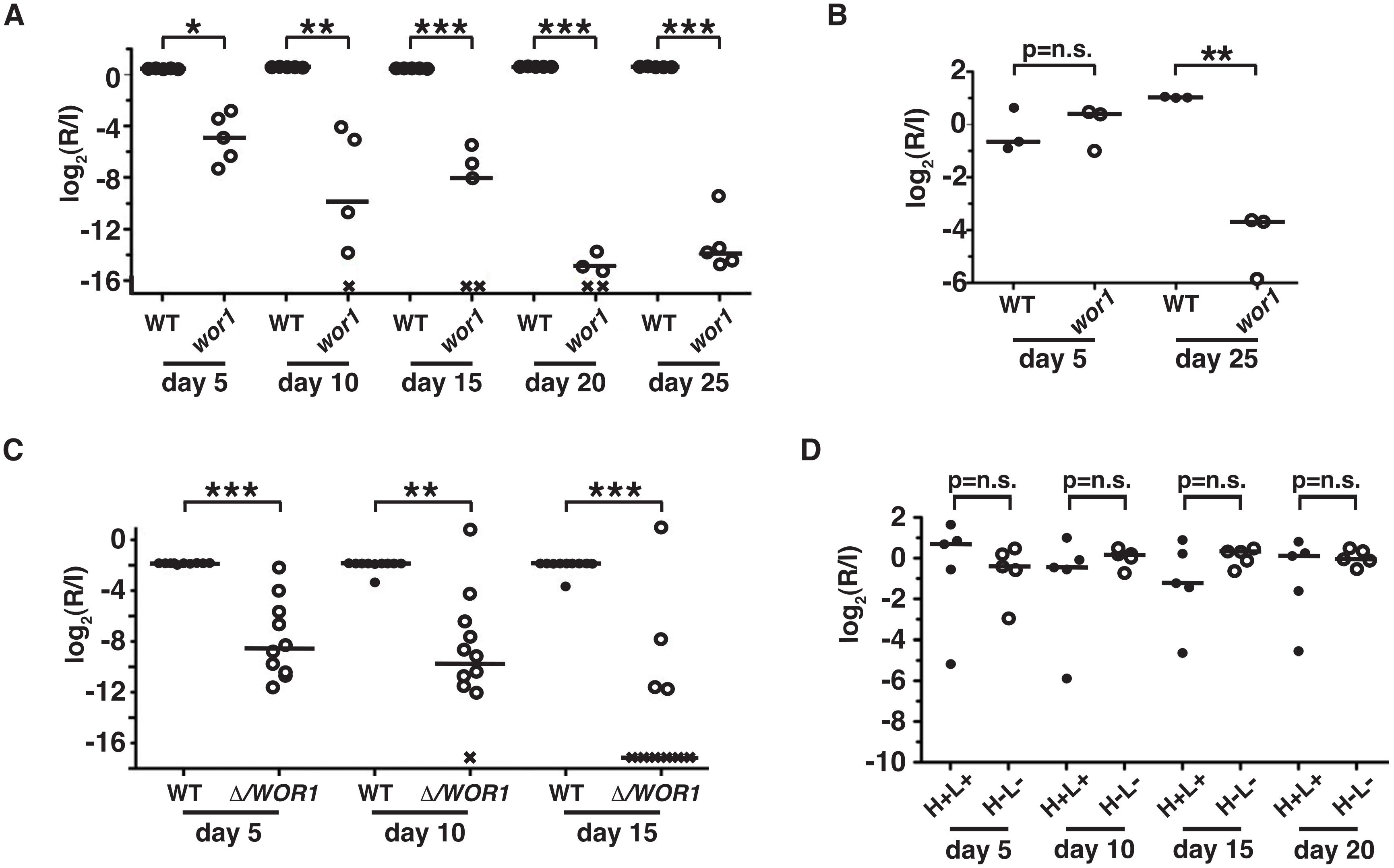
Supplementary Information

Supplementary References

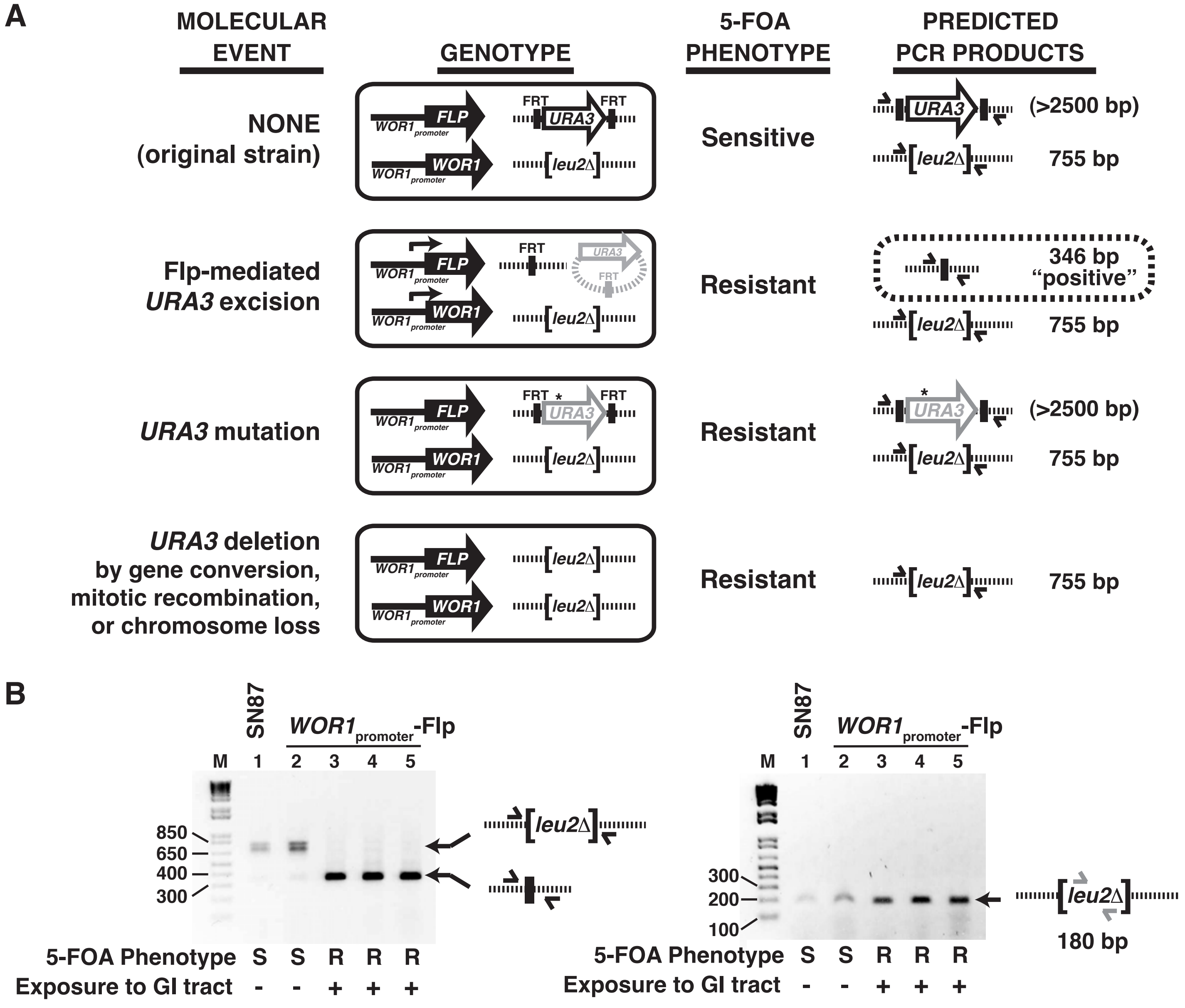
42. Noble, S.M., French, S., Kohn, L.A., Chen, V. & Johnson, A.D. Systematic screens of a Candida albicans homozygous deletion library decouple morphogenetic switching and pathogenicity. *Nat Genet* **42**, 590-598 (2010).
43. Tsong, A.E., Miller, M.G., Raisner, R.M. & Johnson, A.D. Evolution of a combinatorial transcriptional circuit: a case study in yeasts. *Cell* **115**, 389-399 (2003).
44. Tuch, B.B., *et al.* The transcriptomes of two heritable cell types illuminate the circuit governing their differentiation. *PLoS Genet* **6**, e1001070 (2011).

Supplementary Figure 1. ***WOR1*** **is required for wild-type commensal fitness**



Legend: A) and B) Two independent isolates of *wor1*ΔΔ are attenuated in the murine commensal model relative to isogenic wild-type strains. These mutants were generated independently from the *wor1*ΔΔ isolate tested in Figure 1C. C) A *wor1*Δ/*WOR1* heterozygous deletion mutant is attenuated for commensalism. Note that the haploinsufficiency phenotype can be complemented by increasing *WOR1* expression from the remaining allele by means of the *TDH3* promoter (*WOR1*^{OE} strain, Figure 3A). D) A His-Leu- double auxotroph exhibits wild-type commensalism. Strain designations are: (A) *wor1*ΔΔ (SN1064), WT (SN425); B) *wor1*ΔΔ (RZY244), WT (QMY23); C) *wor1* Δ/*WOR1* (SN999), WT (SN235); D) His-Leu- auxotroph (SN87), prototroph (SN425). Significance was determined using the t-test: A) *$p<0.005$, **$p<0.001$, ***$p<0.0001$; B) n.s. not significant, **$p<0.002$; C) **$p=0.0002$, ***$p<0.0001$; D) n.s. not significant.

Supplementary Figure 2. Estimation of *WOR1* gene expression in *MTLa/*α cells

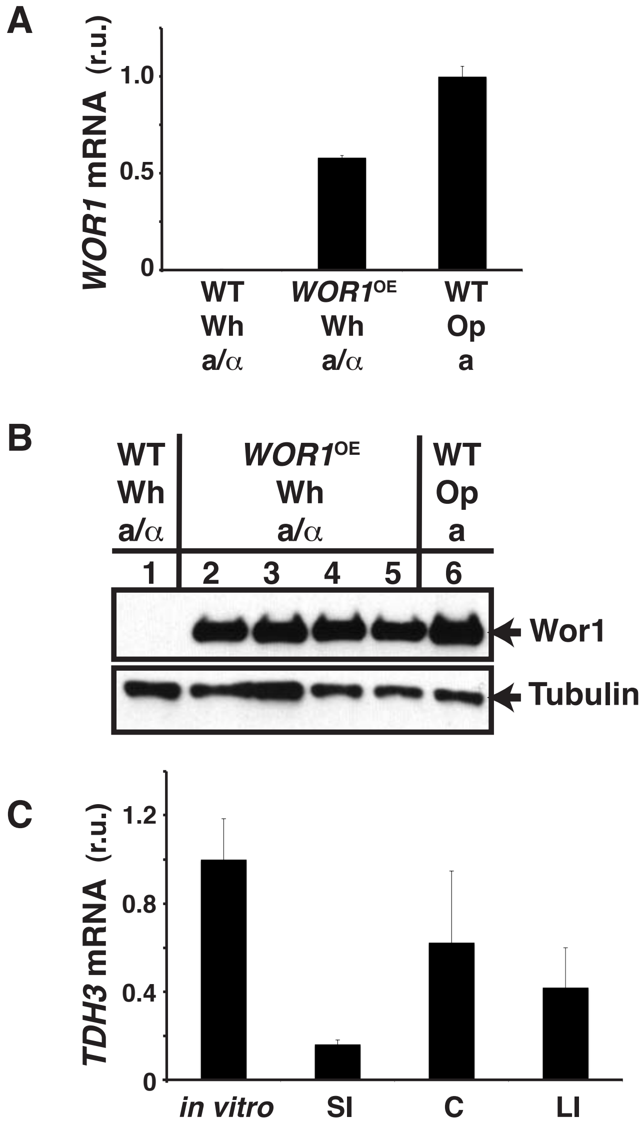


C

	# Cells	# 5-FOA-resistant	# 5-FOA-resistant, PCR-positive	*WOR1* expression frequency	Standard deviation
in vivo	1.60 x 10e6	3.17 x 10e4	3.14 x 10e4	1.94%	0.55%
in vitro	1.79 x 10e6	47	4	0.0002%	0.0001%

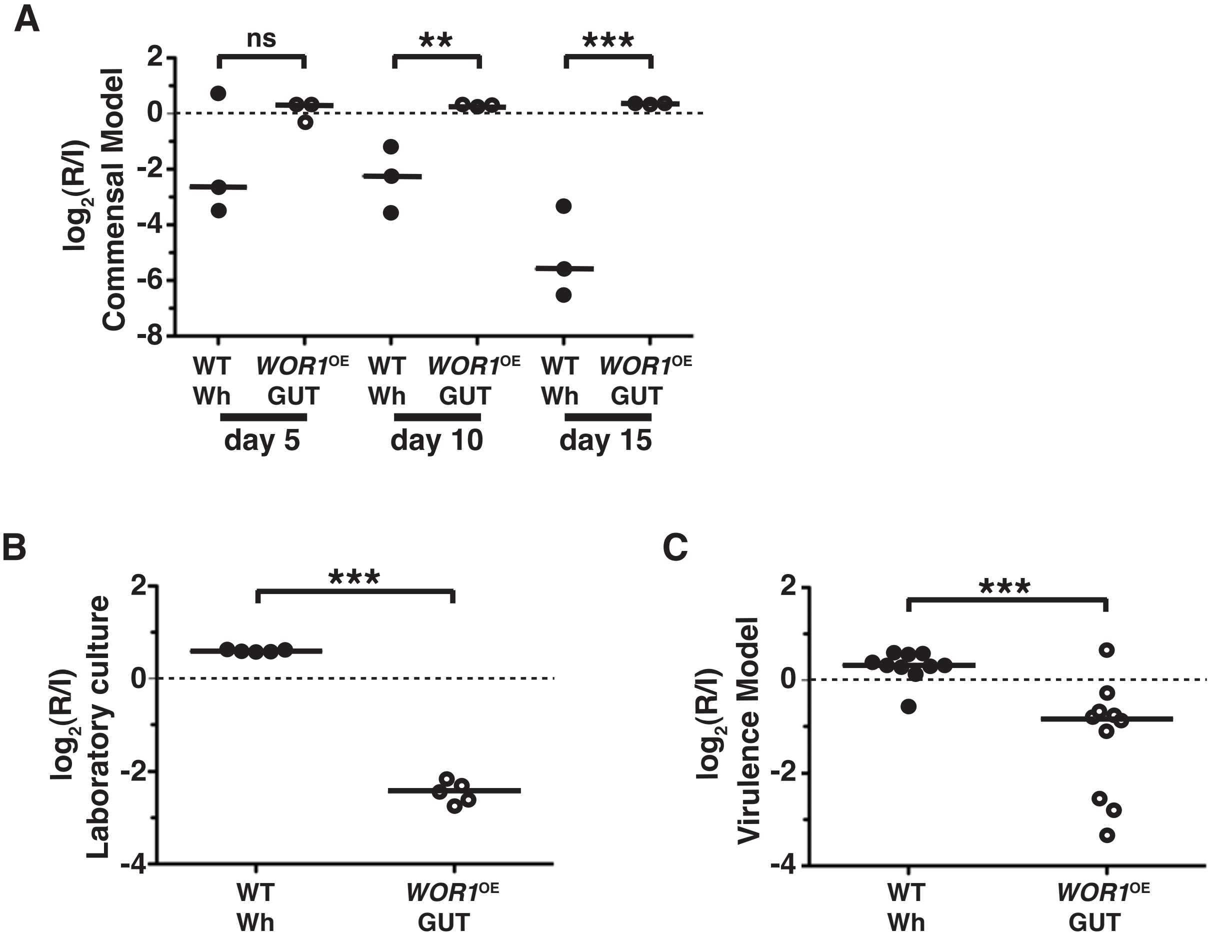
Supplementary Figure 2 Legend: Estimation of *WOR1* gene expression in *MTL*a/α cells. A) Strategy for capturing *WOR1* expression events. The *WOR1*$_{promoter}$-*FLP* strain (SN1020) has the following genotype: *wor1*Δ::*WOR1*$_{promoter}$-*FLP*/*WOR1, leu*2Δ::FRT-*URA3*-FRT/leu2Δ. Activation of the *WOR1* promoter in this strain results in transcription of *FLP*, followed by Flp-mediated excision of *URA3*, and acquisition of resistance to 5-FOA. Note that 5-FOA-resistance may alternatively arise from mutation of the *URA3* gene or loss of *URA3* through mitotic recombination, gene conversion (via homologous sequences on the other copy of Chromosome 7), or chromosome loss. Colony PCR is performed to screen for products of Flp-mediated recombination, which generate a unique 346 bp product ("positive" test). B) Representative PCR results. On the left, PCR for Flp-mediated events is shown for SN87 (lane 1), the reference strain used to construct *WOR1*$_{promoter}$-*FLP*; the *WOR1*$_{promoter}$-*FLP* starting strain (lane 2); and three 5-FOA-resistant isolates of *WOR1*$_{promoter}$-*FLP* (lanes 3-5) that were recovered from animals. Note that the trans-FRT reaction in Flp-recombinant strains outcompetes the trans- *leu*2Δ reaction, such that only the 346 bp product is apparent in lanes 3-5. On the right, PCR using primers internal to the *leu*2Δ locus confirms that all five strains retain this locus. C) Frequency of Flp-mediated *URA3* excision *in vivo* and *in vitro*. The *WOR1*$_{promoter}$-*FLP* strain was propagated for three days in the commensal model (n=6 mice) or for 8 generations *in vitro* at 37°C (n=4 cultures). The frequency of cells expressing *WOR1* propagated under a given condition was estimated from the ratio of (5-FOA^{R}, PCR-positive colonies) / (total number of colonies). Diagnostic PCR was performed on 288 of the 31,700 5-FOA-resistant isolates recovered from mice and on all 47 5-FOA-resistant isolates obtained *in vitro*. 277/278 *in vivo* strains and 4/47 *in vitro* strains tested positive for the 346 bp marker of Flp-mediated recombination. Average values with standard deviations were plotted.

Supplementary Figure 3. Characterization of the *WOR1*$^{\mathrm{OE}}$ strain



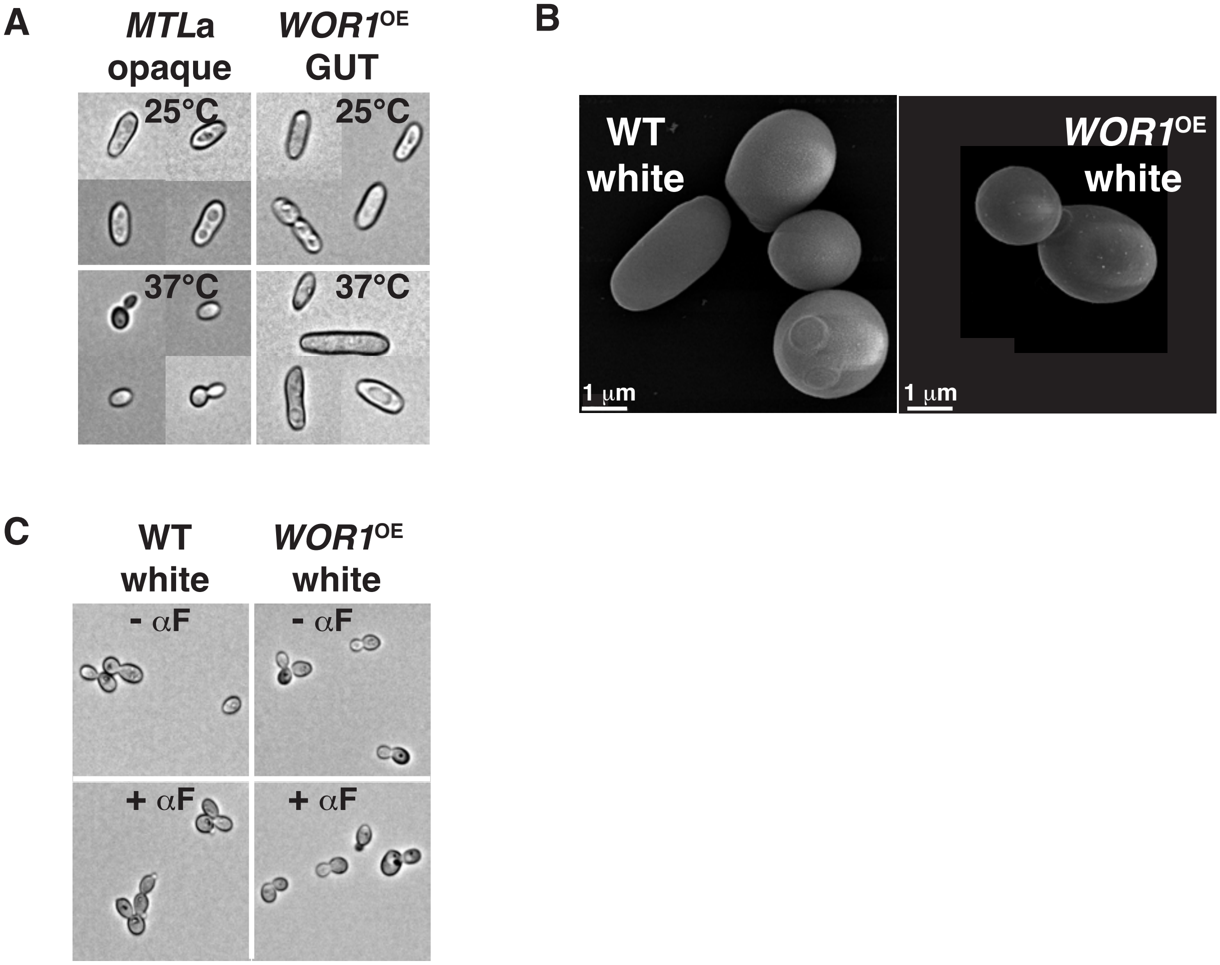
Legend: A) *WOR1* mRNA is highly expressed in the *WOR1*$^{\mathrm{OE}}$ (*TDH3*$_{\mathrm{promoter}}$-*WOR1*) strain. RT-qPCR was performed on three biological replicates each of wild-type white (*MTL***a**/α, SN425), *WOR1*$^{\mathrm{OE}}$ white (*MTL***a**/α, SN928), and opaque (*MTL***a**, SN967) strains. Median values are shown, along with standard deviations. Relative to its expression is WT cells, *WOR1* is induced 102-fold in the *WOR1*$^{\mathrm{OE}}$ strain and 175-fold in opaque cells. B) Wor1 protein is highly expressed in the *WOR1*-overexpression strain. Immunoblot of Wor1 protein prepared from wild-type white (*MTL***a**/α, SN425), *WOR1*$^{\mathrm{OE}}$ white (*MTL***a**/α, SN828 and three additional isolates), and opaque (*MTL***a**, SN967) strains. C) In the mammalian GI tract, the *TDH3* promoter is active but not induced relative to *in vitro*. RT-qPCR of mRNA from wild-type white cells (*MTL***a**/α, SN425) after 8 doublings in liquid YEPD medium at 37°C (*in vitro*) or directly recovered from small intestines (SI), ceca, or large intestines (LI) after 10 days in the murine commensal model. Medians with standard deviations are shown for 5 (laboratory culture) or 3 (commensalism model) biological replicates.

Supplementary Figure 4. *MTLa*/α GUT cells display an early fitness advantage in the mammalian GI tract, but are less fit in laboratory culture and in blood-stream infection



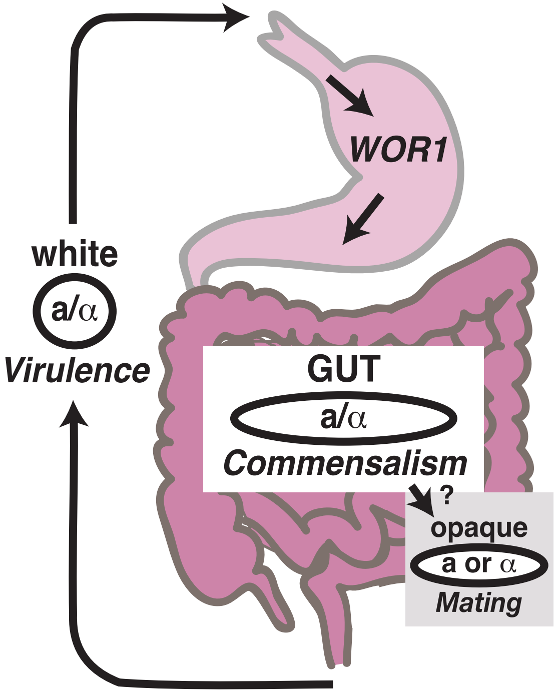
Legend: A) Commensal competition experiment between WT *MTL***a**/α white cells (SN425) and *WOR1*^{OE} *MTL***a**/α GUT cells (SN1045). Note that, in contrast to the case with white phase *WOR1*^{OE} (Figure 2A), GUT phase cells are highly competitive from the beginning of the time course. ns nonsignificant, ** $p<0.02$, *** $p<0.005$. B) *in vitro* competition between the same two strains. Strains were co-cultured for 8 generations in liquid YEPD medium at 37°C. Relative abundances of strains at the beginning (I) and end (R) of the time course were determined by qPCR. *** $p<0.0001$. C) Competition between the same two strains in a murine model of disseminated disease. Strains were co-infected into BALB/c mice via lateral tail vein, *C. albicans* was recovered from kidneys of moribund animals after a median of 5 days post infection (range 4 to 6 days), and relative abundances in the inoculum (I) and after recovery from kidneys (R) was determined by qPCR.

Supplementary Figure 5. Additional phenotypes of GUT and white cells



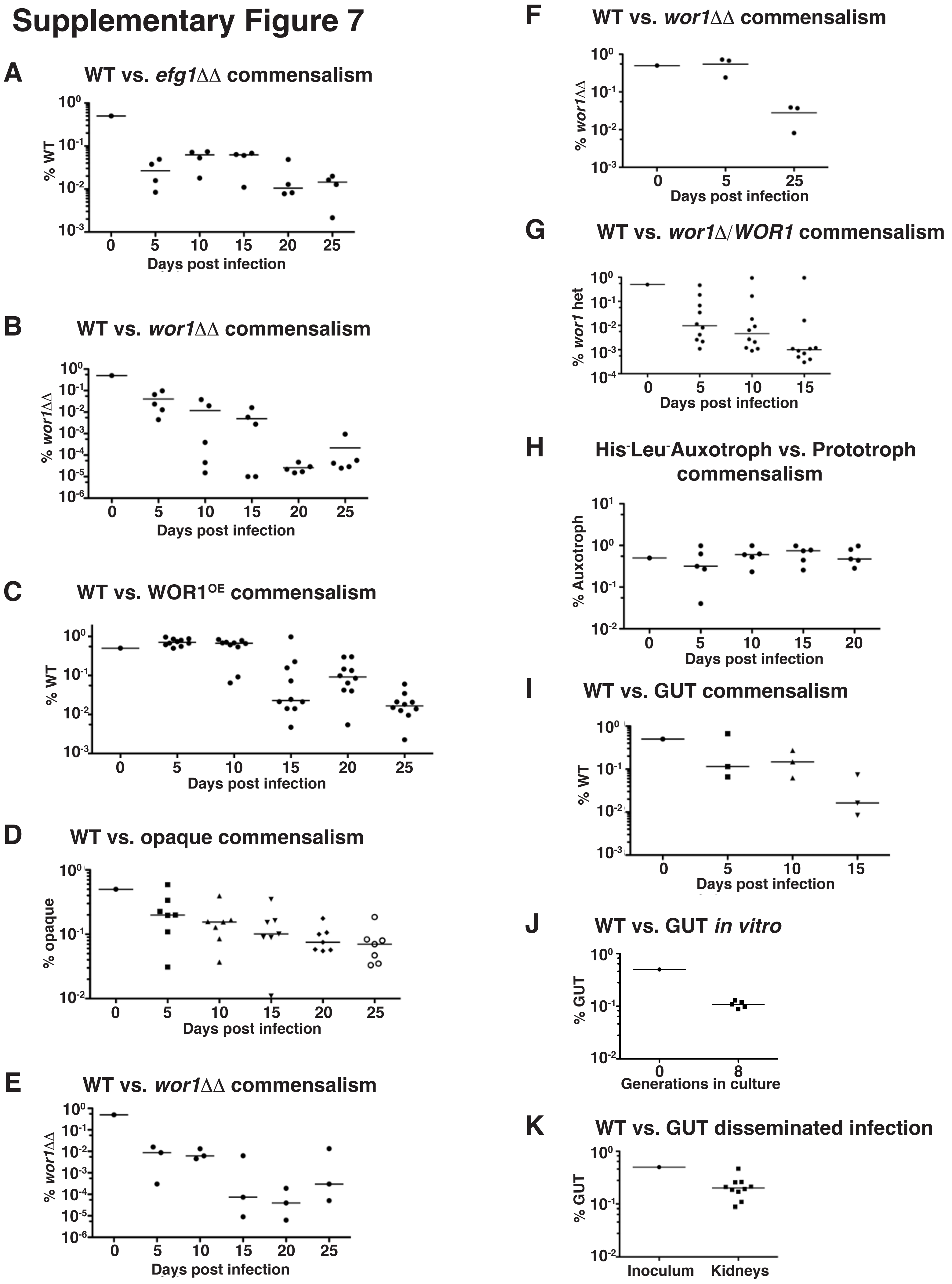
Legend: A) The GUT phenotype is stable at 37°C. After 3 days of incubation on YEPD medium at room temperature (~25°C) or 37°C, *MTL***a**/α GUT (SN1045) and *MTL***a** opaque (SN967) cells were visualized by light microscopy. B) SEM images of white phase WT (SN425) and *WOR1*^{OE} (SN928) cells, which lack pimple structures. C) White phase *MTL***a** (SN966) and *WOR1*^{OE} (SN1044) strains fail to produce germ tubes in response to mating pheromone.

Supplementary Figure 6. Model of phenotypic switching in the host



Legend: According to this model, *MTL***a**/α white cells exposed to the mammalian GI tract encounter signals triggering *WOR1* expression and a shift to the GUT state, which is specialized for commensalism. GUT cells exiting this space rapidly convert back to the white state, which is virulent. Additional signals may alternatively trigger GUT cells to undergo loss of one allele of *MTL* (e.g. through loss of Chromosome 5) and conversion to the sexually-competent opaque state.

Supplementary Figure 7



Supplementary Figure 7 Legend: Competition experiments in "% abundance of the less fit strain" format. A) WT (SN250) is outcompeted by *efg1*ΔΔ **(**SN1011) in the murine commensal model. Plot is derived from the same data as depicted in **Figure 1A**. B) *wor1*ΔΔ (SN881) is outcompeted by WT (SN250) in the murine commensal model. Plot is derived from the same data as depicted in **Figure 1C**. C) WT (SN425) is outcompeted by *WOR1*OE (SN928) in the murine commensal model. Plot is derived from the same data as depicted in **Figure 2A**. D) Opaque cells (SN967) are outcompeted by WT white cells (SN425) in the murine commensal model. Plot is derived from the same data as depicted in **Figure 3C**. E) *wor1*ΔΔ (SN1064) is outcompeted by WT (SN425) in the murine commensal model. Plot is derived from the same data as depicted in **Supplementary Figure 1A**. F) *wor1*ΔΔ (RZY244) is outcompeted by WT (QMY23) in the murine commensal model. Plot is derived from the same data as depicted in **Supplementary Figure 1B**. G) *wor1*Δ/*WOR1* heterozygous knockout (SN999) is outcompeted by WT (SN235) in the murine commensal model. Plot is derived from the same data as depicted in **Supplementary Figure 1C**. H) A His-Leu- double auxotroph (SN87) is as fit as a prototroph (SN425) in the murine commensal model. Plot is derived from the same data as depicted in **Supplementary Figure 1D**. I) White phase WT (SN425) is outcompeted by GUT cells (SN1045) in the murine commensal model. Plot is derived from the same data as depicted in **Supplementary Figure 4A**. J) GUT cells (SN1045) are outcompeted by white phase WT (SN425) in liquid culture medium. Plot is derived from the same data as depicted in **Supplementary Figure 4B**. K) GUT cells (SN1045) are outcompeted by white phase WT (SN425) in a murine model of disseminated candidiasis. Plot is derived from the same data as depicted in **Supplementary Figure 4C**.

Supplementary Table 1. *MTL***a** and *MTL*α Genotypes of White and GUT Cells After Recovery from the Murine Commensal Model

Experiment	Phenotype	*MTL***a** present	*MTL*α present	Colonies analyzed	% *MTL***a**/α
1	white	44	44	44	100
1	GUT	32	40	40	80
2	white	53	52	53	98
2	GUT	7	8	8	88
3	white	24	24	24	100
3	GUT	24	24	24	100
4	white	24	24	24	100
4	GUT	24	24	24	100
Total	white	145	144	145	99
Total	GUT	87	96	96	91

Supplementary Table 2. Mating Efficiencies of Various Strains

Cross	Strain and Phase		Mating Efficiency (His$^+$Arg$^+$/His$^+$Arg$^-$)		
	Strain 1 (His$^+$Arg$^-$)	Strain 2 (His$^-$ Arg$^+$)	3 days	5 days	13 Days
1	*MTL*α opaque	*MTL***a** opaque	3.3 x 10-1	8.0 x 10-1	1.5 x 10-1
2	*MTL***a**/α white	*MTL***a** opaque	<6 x 10-7	<2 x 10-7	<1 x 10-7
3	*WOR1*^{OE} *MTL***a**/α white	*MTL***a** opaque	4.6 x 10-6	4.2 x 10-6	5.7 x10-6
4	*WOR1*^{OE} *MTL***a**/α GUT	*MTL***a** opaque	<2 x 10-7	<3 x 10-7	<2 x 10-7

Supplementary Table 4. Regulated Genes Sets in GUT and opaque cells

Gene Name Standard Name	**Description of Gene Product**	**Cellular Process**	**Reported Specificity** (Wh vs. Op)			**RNA Expression Level**					**Relative Expression**		
			Lan et al.	**Tsong et al.**	**Tuch et al.**	**WT Wh**	***WOR1* Wh**	***WOR1* GUT**	***MTLa* Wh**	***MTLa* Op**	**GUT/ Wh**	**Op/ Wh**	**GUT/ Op**
UPREGULATED IN GUT AND OPAQUE (VS. WH CONTROLS)													
PXP2 *orf19.1655*	Putative acyl-CoA oxidase	Lipid Utilization	**Op**	**Op**	**Op**	1.7	7.6	30.0	1.0	47.7	**17.8***	**47.7***	0.6
ANT1 *orf19.6254*	Putative adenine nucleotide transmembrane transporter	Lipid Utilization	**Op**	**Op**	**Op**	1.1	2.7	6.8	1.0	5.7	**6.4***	**5.7***	1.2
FOX2 *orf19.1288*	3-hydroxyacyl-CoA epimerase	Lipid Utilization	**Op**			1.2	3.3	5.0	1.0	6.1	**4.1***	**6.1***	0.8
POX1 *orf19.5723*	Putative acyl-CoA oxidase	Lipid Utilization	**Op**		**Op**	1.1	2.2	4.1	1.0	5.0	**3.9***	**5.0***	0.8
ECI1 *orf19.6445*	Ortholog of S. cerevisiae Eci1p, which is involved in fatty acid oxidation	Lipid Utilization			**Op**	1.1	2.2	3.1	1.0	5.2	**2.9***	**5.2***	**0.6***
POX1-3 *orf19.1652*	Putative acyl-CoA oxidase	Lipid Utilization				1.0	1.8	3.9	1.0	3.1	**3.8***	**3.1***	1.3
CAT2 *orf19.4591*	Major carnitine acetyl transferase	Lipid Utilization		**Op**		1.1	1.8	2.5	1.0	2.6	**2.2***	**2.6***	0.9
POT1 *orf19.7520*	Putative peroxisomal 3-oxoacyl CoA thiolase	Lipid Utilization				1.0	1.9	2.8	1.0	2.7	**2.7***	**2.7***	1.1
SPS20 *orf19.4157*	Peroxisomal 2,4-dienoyl-CoA reductase	Lipid Utilization	**Op**	**Op**	**Op**	1.0	1.7	3.0	1.0	5.2	**2.9***	**5.2***	0.6
NAG1 *orf19.2156*	Glucosamine-6-phosphate deaminase	NAG Utilization	**Op**		**Op**	1.0	4.2	17.7	1.0	10.6	**17.4***	**10.6***	**1.7***
DAC1 *orf19.2157*	N-acetylglucosamine-6-phosphate (GlcNAcP) deacetylase	NAG Utilization		**Op**	**Op**	1.2	2.5	10.0	1.0	5.5	**8.3***	**5.5***	**1.8***
HXK1 *orf19.2154*	N-acetylglucosamine (GlcNAc) kinase	NAG Utilization			**Op**	1.0	1.5	2.1	1.0	2.2	**2.1***	**2.2***	0.9

UPREGULATED IN OPAQUE ONLY (VS. WH CONTROLS)													
SAP2 *orf19.3708*	Secreted aspartyl proteinase	Protein Utilization		**Op**	**Wh**	1.2	1.2	1.3	1.0	4.4	1.1	**4.4***	**0.3***
SAP3 *orf19.6001*	Secreted aspartyl proteinase	Protein Utilization	**Op**	**Op**		1.0	1.2	1.1	1.0	2.5	1.1	**2.4***	**0.5***
SAP4 *orf19.5716*	Secreted aspartyl proteinase	Protein Utilization		**Op**		1.3	1.7	1.7	1.0	11.9	1.3	**11.9***	**0.1***
SAP6 *orf19.5542*	Secreted aspartyl proteinase	Protein Utilization			**Wh**	1.5	1.7	1.6	1.0	12.0	1.1	**12.0***	**0.1***
SAP7 *orf19.756*	Secreted aspartyl proteinase	Protein Utilization				1.9	4.5	1.0	1.9	5.5	0.5	**2.9***	**0.2***
SAP99 *orf19.853*	Putative secreted aspartyl protease	Protein Utilization	**Op**		**Op**	1.1	1.3	1.4	1.0	7.3	1.3	**7.3***	**0.2***
DOWNREGULATED IN GUT AND OPAQUE (VS. WH CONTROLS)													
ALS2 *orf19.1097*	ALS family protein with roles in adhesion, biofilm formation, germ tube induction	Adhesion	**Wh**		**Wh**	5.5	1.4	1.0	4.6	1.0	**0.2***	**0.2***	1.0
ADH1 *orf19.3997*	Alcohol dehydrogenase	Adhesion			**Wh**	20.9	4.7	1.0	21.7	2.6	**0.0***	**0.1***	**0.4**
orf19.4216	*Putative heat shock protein*	Adhesion			**Wh**	37.1	28.2	3.1	38.9	1.0	**0.1***	**0.0***	**3.1**
CSH1 *orf19.4477*	Aldo-keto reductase family member with role in fibronectin adhesion	Adhesion			**Wh**	2.2	2.0	1.0	2.4	1.0	**0.5***	**0.4***	1.0
ALS4 *orf19.4555*	ALS family protein with roles in adhesion and germ tube induction	Adhesion	**Wh**	**Wh**	**Wh**	8.4	2.0	1.0	7.6	1.2	**0.1***	**0.2***	0.8
EFG1 *orf19.610*	Transcriptional repressor required for white-phase cell type, hyphal growth, metabolism with roles in adhesion and virulence	Adhesion	**Wh**	**Wh**	**Wh**	5.3	2.0	1.1	4.8	1.0	**0.2***	**0.2***	1.1

DOWNREGULATED IN GUT ONLY (VS. WH CONTROLS)													
FRE10 *orf19.1415*	Major cell-surface ferric reductase	Iron uptake			**Wh**	11.0	5.1	1.0	9.6	2.9	**0.1***	**0.3***	**0.3***
CFL4 *orf19.1932*	Putative ferric reductase	Iron uptake			**Wh**	7.0	2.0	1.0	9.1	2.0	**0.1***	**0.2**	**0.5**
SIT1 *orf19.2179*	Transporter of ferrichrome siderophores	Iron uptake		**Wh**		2.1	1.9	1.0	1.9	1.6	**0.5***	0.8	0.6
FET34 *orf19.4215*	Putative multicopper ferroxidase	Iron uptake	**Wh**		**Wh**	29.0	2.1	1.0	23.7	1.3	**0.0***	**0.1**	0.8
FTH1 *orf19.4802*	Protein similar to S. cerevisiae Fth1p, a high affinity iron transporter	Iron uptake			**Wh**	2.1	1.2	1.0	2.0	1.4	**0.5***	0.7	0.7
FTR1 *orf19.7219*	High-affinity iron permease	Iron uptake			**Wh**	7.1	1.9	1.0	6.2	1.8	**0.1***	**0.3***	**0.5***
FTR2 orf19.7231	High-affinity iron permease	Iron uptake			**Op**	2.8	1.3	1.0	2.4	1.5	**0.4***	0.6	0.7*
PDC11 *orf19.2877*	Putative pyruvate decarboxylase	Glucose Utilization				10.1	3.9	1.0	7.0	1.9	**0.1***	**0.3**	**0.5**
PGK1 *orf19.3651*	Phosphoglycerate kinase	Glucose Utilization		**Wh**		2.1	1.6	1.0	1.8	1.6	**0.5***	0.9	**0.6***
PFK1 *orf19.3967*	Phosphofructokinase alpha subinit	Glucose Utilization		**Wh**		2.1	1.5	1.0	2.0	1.5	**0.5***	0.8	**0.7***
ADH1 *orf19.3997*	Alcohol dehydrogenase	Glucose Utilization			**Wh**	20.9	4.7	1.0	21.7	2.6	**0.0***	**0.1***	**0.4**
FBA1 *orf19.4618*	Putative fructose-bisphosphate aldolase	Glucose Utilization		**Wh**		2.2	1.4	1.0	1.7	1.4	**0.5***	0.9	0.7
ADH2 *orf19.5113*	Alcohol dehydrogenase	Glucose Utilization	**Op**	**Wh**	**Op**	11.8	3.1	1.0	9.6	2.6	**0.1***	**0.3***	0.4
HXK2 *orf19.542*	Hexokinase II	Glucose Utilization		**Wh**		2.1	1.5	1.0	1.9	1.6	**0.5***	0.8	**0.6***

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HIGHER IN OPAQUE THAN GUT (DIRECT COMPARISON, IN ADDITION TO SAP GENES)													
STE4 *orf19.799*	Beta subunit of heterotrimeric G protein required for mating	Mating			**Op**	1.2	1.5	1.0	3.7	15.9	0.9	**4.3***	**0.1***
CAG1 *orf19.4015*	Alpha subunit of heterotrimeric G protein required for mating	Mating				1.0	1.3	1.2	14.2	20.7	1.2	1.5	**0.1***
STE18 *orf19.6551.1*	Gamma subunit of heterotrimeric G protein involved in mating	Mating				1.0	1.1	1.7	12.1	15.2	1.7	1.3	**0.1***
STE2 *orf19.696*	Receptor for alpha factor mating pheromone	Mating				1.1	1.2	1.0	3.7	4.0	0.9	1.1	**0.2***
HIGHER IN GUT THAN OPAQUE													
GIT2 *orf19.1978*	glycerophosphoinositol permease	Phosphate transport			**Wh**	3.6	2.9	2.2	4.3	1.0	**0.6***	**0.2***	**2.2***
GIT3 *orf19.1979*	*Putative glycerophosphoinositol permease*	Phosphate transport	**Wh**	**Wh**	**Wh**	28.1	8.0	2.8	26.7	1.0	**0.1***	**0.0***	**2.8***
PHO84 *orf19.655*	High-affinity phosphate transporter	Phosphate transport	**Op**		**Op**	1.3	4.7	14.4	1.0	4.2	**10.7***	**4.2***	**3.5***
orf19.6888	Putative transcription factor with Gal4p-like DNA-binding domain	Transcription Factor			**Wh**	2.0	1.9	2.9	1.9	1.0	1.5	**0.5***	**2.9***
orf19.4914	Putative transcription factor	Transcription Factor			**Wh**	2.1	1.8	2.8	2.2	1.0	1.3	**0.5***	**2.8***
LEU3 *orf19.4225*	Putative transcription factor with zinc-finger domain	Transcription Factor				3.8	3.4	3.2	1.1	1.0	0.8	0.9	**3.2***
orf19.2726	Putative plasma membrane protein	Cell Wall or Secreted	**Wh**	**Wh**	**Wh**	10.2	6.6	4.3	9.2	1.0	**0.4***	**0.1***	**4.3***
orf19.5070	Putative cell-wall mannoprotein	Cell Wall or Secreted	**Wh**			1.8	2.0	3.0	1.7	1.0	**1.7***	0.6	**3.0***
orf19.1258	Putative adhesin-like protein	Cell Wall or Secreted	**Wh**	**Wh**	**Wh**	6.3	6.9	2.9	6.5	1.0	**0.5***	**0.2***	**2.9***
RBT4 *orf19.6202*	Putative secreted protein that is required for virulence	Cell Wall or Secreted			**Wh**	7.1	3.8	2.7	6.7	1.0	**0.4***	**0.1***	**2.7***

RBE1 *orf19.7218*	Cell wall protein	Cell Wall or Secreted				1.3	1.6	2.4	1.1	1.0	**1.9***	0.9	**2.4***
orf19.5267	Putative cell wall adhesin-like protein	Cell Wall or Secreted			**Op**	1.2	1.3	5.6	1.0	2.2	**4.9***	**2.2***	**2.6***
RHD1 *orf19.54*	Putative beta-mannosyltransferase	Cell Wall Structure	**Wh**	**Wh**	**Wh**	7.1	4.0	4.8	6.5	1.0	0.7	**0.2***	**4.8***
CIS2 *orf19.6053*	Putative role in regulation of biogenesis of the cell wall	Cell Wall Structure			**Wh**	2.3	1.9	2.4	2.4	1.0	1.1	**0.4***	**2.4***
SKN2 *orf19.348*	Protein with a potential role in beta-1,6 glucan biosynthesis	Cell Wall Structure			**Wh**	2.5	1.6	2.4	2.9	1.0	1.0	**0.3***	**2.4***

Descriptions of published white (w) and opaque (o) gene sets refer to: [28] Lan et al.(2002) PNAS 23: 14902-12; [43] Tsong et al. (2003) Cell 115: 389-99; [44] Tuch et al. (2010) PLoS Genetics 6: e1001070. *Denotes a significant difference using BAGEL analysis (Supp. Table 3).

Supplementary Table 5. Strains Used in this Study

Strain	Nickname	*MTL* genotype	Phase	Full Genotype	Parent	Reference
SN78	Wild type	*MTL***a**/α	white	*leu2Δ/leu2Δ, ura3Δ/ura3Δ, his1Δ/his1Δ iro1Δ/iro1Δ, MTL***a***/MTLα*	RM1000 #2	14
SN87	Wild type	*MTL***a**/α	white	*leu2Δ/leu2Δ, ura3Δ/URA3, his1Δ/his1Δ iro1Δ/IRO1, MTL***a***/MTLα*	RM1000 #2	14
SN152	Wild type	*MTL***a**/α	white	*leu2Δ/leu2Δ, ura3Δ/URA3, his1Δ/his1Δ, arg4Δ/arg4Δ, iro1Δ/IRO1, MTL***a***/MTLα*	RM1000 #2	14
SN235	Wild type	*MTL***a**/α	white	*leu2Δ::C.d.HIS1/leu2Δ, ura3Δ/URA3, his1Δ/his1Δ, arg4Δ/arg4Δ, iro1Δ/IRO1, MTL***a***/MTLα*	SN152	This study
SN250	Wild type	*MTL***a**/α	white	*leu2Δ::C.m.LEU2/leu2Δ::C.d.HIS1, ura3Δ/URA3, his1Δ/his1Δ,* arg4Δ/*arg4Δ, iro1Δ/IRO1, MTL***a***/MTLα*	SN152	42
SN425	Wild type	*MTL***a**/α	white	*leu2Δ::C.d.HIS1/leu2Δ::C.m.LEU2, ura3Δ/URA3, his1Δ/his1Δ, arg4Δ/arg4Δ::C.d.ARG4, iro1Δ/IRO1, MTL***a***/MTLα*	SN152	42
QMY23	Wild type	*MTL***a**/α	white	*leu2Δ::C.m.LEU2/leu2Δ::C.d.HIS1, ura3Δ/URA3, his1Δ/his1Δ, iro1Δ/IRO1, MTL***a***/MTLα*	SN87	39
SN1011	*efg1Δ/Δ*	*MTL***a**/α	white	*efg1Δ::C.m.LEU2/efg1Δ::C.d.HIS1, leu2Δ/leu2Δ, ura3Δ/URA3, his1Δ/his1Δ, arg4Δ/arg4Δ, iro1Δ/IRO1, MTL***a***/MTLα*	SN152	14
SN999	*wor1Δ/ WOR1*	*MTL***a**/α	white	*WOR1/wor1Δ::C.d.HIS1, leu2Δ/leu2Δ, ura3Δ/URA3, his1Δ/his1Δ, arg4Δ/arg4Δ, iro1Δ/IRO1, MTL***a***/MTLα*	SN152	This study
SN881	*wor1Δ/Δ*	*MTL***a**/α	white	*wor1Δ::C.m.LEU2/wor1Δ::C.d.HIS1, leu2Δ/leu2Δ, ura3Δ/URA3, his1Δ/his1Δ, arg4Δ/arg4Δ, iro1Δ/IRO1, MTL***a***/MTLα*	SN152	This study
RZY244	*wor1Δ/Δ*	*MTL***a**/α	white	*wor1Δ::C.m.LEU2/wor1Δ::C.d.HIS1, leu2Δ/leu2Δ, ura3Δ/URA3, his1Δ/his1Δ, iro1Δ/IRO1, MTL***a***/MTLα*	SN87	Zordan & Johnson, unpublished
SN1064	*wor1Δ/Δ*	*MTL***a**/α	white	*wor1Δ::C.m.LEU2/wor1Δ::C.d.HIS1, leu2Δ/leu2Δ, ura3Δ/URA3, his1Δ/his1Δ, arg4Δ/ARG4, iro1Δ/IRO1, MTL***a***/MTLα*	SN152	This study
SN1014	*WOR1*$_{prom}$-*FLP* intermediate	*MTL***a**/α	white	*wor1Δ::FLP-SAT1/WOR1, leu2Δ/leu2Δ, ura3Δ/ura3Δ, his1Δ/his1Δ, iro1Δ/iro1Δ, MTL***a***/MTLα*	SN78	This study
SN1020	*WOR1*$_{prom}$-*FLP*	*MTL***a**/α	white	*wor1Δ::FLP-SAT1/WOR1, leu2Δ::FRT-URA3-FRT/leu2Δ, ura3Δ /ura3Δ, his1Δ/his1Δ, iro1Δ/iro1Δ, MTL***a***/MTLα*	SN1014	This study
SN967*	*MTL***a** opaque	*MTL***a**/**a**	opaque	*leu2Δ::C.m.LEU2/leu2Δ::C.d.HIS1, ura3Δ/URA3, his1Δ/his1Δ, iro1Δ/IRO1, MTL***a***/MTL***a**	QMY23	35
SN966	*MTL***a**	*MTL***a**/**a**	white	*leu2Δ::C.m.LEU2/leu2Δ::C.d.HIS1, ura3Δ/URA3, his1Δ/his1Δ, iro1Δ/IRO1, MTL***a***/MTL***a**	SN967	This study
SN1008	*MTLα* opaque	*MTLΔ/α*	opaque	*leu2Δ/leu2::SAT1, ura3Δ/URA3, his1Δ/his1Δ, arg4Δ/arg4Δ, iro1Δ/IRO1,*	SN152	This study

				*mtl**a**Δ::C.d.HIS1/MTLα*		
SN1038	*MTL**a*** opaque	*MTL**a**/Δ*	opaque	*leu2Δ/leu2Δ, ura3Δ/URA3, his1Δ/his1Δ, arg4Δ/arg4Δ, iro1Δ/IRO1, MTL**a**/mtlαΔ::C.d.ARG4*	SN152	This study
SN927	*WOR1*^{OE}*/WOR1*	*MTL**a**/α*	white	*SAT1-TDH3*$_{prom}$*-WOR1/WOR1, leu2Δ::C.m.LEU2/leu2Δ::C.d.HIS1, ura3Δ/URA3, his1Δ/his1Δ, arg4Δ/arg4, Δiro1Δ/IRO1, MTL**a**/MTLα*	SN250	This study
SN928	*WOR1*^{OE}	*MTL**a**/α*	white	*SAT1-TDH3*$_{prom}$*-WOR1/wor1Δ::C.d.ARG4, leu2Δ::C.m.LEU2/leu2Δ::C.d.HIS1, ura3Δ/URA3, his1Δ/his1Δ, arg4Δ/arg4Δ, iro1Δ/IRO1, MTL**a**/MTLα*	SN927	This study
SN1001	*WOR1*^{OE}	*MTL**a**/α*	white	*SAT1-TDH3* $_{prom}$*-WOR1/wor1Δ::C.d.HIS1, leu2Δ/leu2Δ, ura3Δ/URA3, his1Δ/his1Δ, iro1Δ/IRO1, MTL**a**/MTLα*	SN999	This study
SN1044	*WOR1*^{OE} (post-mouse)	*MTL**a**/α*	white	*SAT1-TDH3*$_{prom}$*-WOR1/wor1Δ::C.d.ARG4, leu2Δ::C.m.LEU2/leu2Δ::C.d.HIS1, ura3Δ/URA3, his1Δ/his1Δ, arg4Δ/arg4Δ, iro1Δ/IRO1, MTL**a**/MTLα*	SN928	This study
SN1045	*WOR1*^{OE} GUT (post-mouse)	*MTL**a**/α*	GUT	*SAT1-TDH3*$_{prom}$*-WOR1/wor1Δ::C.d.ARG4, leu2Δ::C.m.LEU2/leu2Δ::C.d.HIS1, ura3Δ/URA3, his1Δ/his1Δ, arg4Δ/arg4Δ, iro1Δ/IRO1, MTL**a**/MTLα*	SN928	This study
SN1046	*WOR1*^{OE} GUT (post-mouse)	*MTL**a**/α*	GUT	*SAT1-TDH3* $_{prom}$*-WOR1/wor1Δ::C.d.HIS1, leu2Δ/leu2Δ, ura3Δ/URA3, his1Δ/his1Δ, iro1Δ/IRO1, MTL**a**/MTLα*	SN1001	This study

C.d. ARG4 denotes *Candida dubliniensis ARG4, C.d. HIS1* denotes *Candida dubliniensis HIS1,* and *C.m. LEU2* denotes *Candida maltosa LEU2.*

* SN967 was constructed by Mitrovich et al. [35] but was not assigned a unique name to distinguish it from its *MTL**a**/α* parent (QMY23).

Supplementary Table 6. Plasmids Used in this Study

Plasmid	Insert	Use	Selectable Markers	Vector
pSN209	*PmeI*-sequence ending 120 bp upstream of *WOR1* ORF-*SAT1* gene-*TDH3* promoter-1st 300 bp of *WOR1* ORF-*PmeI*	Replacement of *WOR1* promoter with (*SAT1* gene and) *TDH3* promoter	AmpR, *ScURA3*, NatR	pRS316
pSN288	*PmeI*-final 500 bp of *WOR1* promoter-*FLP* ORF-*SAT1* gene-*WOR1* downstream sequence-*PmeI*	Replacement of *WOR1* ORF with *FLP* ORF	AmpR, *ScURA3,* NatR	pRS316
pSN290	*PmeI*-*LEU2* upstream sequence-FRT-*C. albicans URA3*-FRT-*LEU2* downstream sequence- *PmeI*	Introduction of *FRT-URA3-FRT* at the *LEU2* locus	AmpR, *ScURA*	pRS316

PmeI denotes the recognition sequence for this restriction enzyme; *ScURA3* denotes the *URA3* gene from *Saccharomyces cerevisiae,* AmpR denotes resistance to ampicillin, NatR denotes resistance to nourseothricin.

Supplementary Table 7. Primers Used in this Study

Primer Name	Purpose	Sequence (5’ to 3’)
SNO1122	*wor1*-ko gene disruption primer 5' flank, forward (SN881 and SN1064)	TCCTGTATTGGTATTGGTGAG
SNO1123	*wor1*-ko gene disruption primer 5' flank, reverse (SN881 and SN1064)	CACGGCGCGCCTAGCAGCGGTAAGACAGATATTGAAACTTCAC
SNO1124	*wor1*-ko gene disruption primer 3' flank, forward (SN881 and SN1064)	GTCAGCGGCCGCATCCCTGCATCTTATATATGTGGGTCTGTG
SNO1125	*wor1*-ko gene disruption primer 3' flank, reverse (SN881 and SN1064)	TTCAGCTCTCTAAGTAGTATTG
SNO1271	Common primer for amplifying His, Leu and Arg casettes, forward	CCGCTGCTAGGCGCGCCGTGACCAGTGTGATGGATATCTGC
SNO1272	Common primer for amplifying His, Leu and Arg casettes, reverse	GCAGGGATGCGGCCGCTGACAGCTCGGATCCACTAGTAACG
SNO1126	*Wor1* ORF check primer, forward	GACGACGAGTACGACCACAA
SNO1127	*Wor1* ORF check primer, reverse	CAGCTTTCCCTTCCATGTGT
SNO1128	*Wor1* integration verification check left, forward	TTTTCTTTCCAAAACCCTGCC
SNO1129	*Wor1* integration verification check right, reverse	TCCCTTCATGAATAGTTTCCC
SNO1130	*C. dubliniensis HIS1* integration verification check left, reverse	ATTAGATACGTTGGTGGTTC
SNO1131	*C. dubliniensis HIS1* integration verification check right, forward	AACACAACTGCACAATCTGG
SNO1132	*C. maltosa LEU2* integration verification check left, reverse	AGAATTCCCAACTTTGTCTG
SNO1133	*C. maltosa LEU2* integration verification check right, forward	AAACTTTGAACCCGGCTGCG
SNO143	*C. dubliniensis ARG4* integration on *C. albicans* ARG4 locus, 5' flank, forward	GGACAGAAAGTTATTGTACAG
SNO144	*C. dubliniensis ARG4* integration on *C. albicans* ARG4 locus, 5' flank, reverse	TCACGGCGCGCCTAGCAGCGGGATTATTCTTGATAGCTGTTATG
SNO1385	*C. dubliniensis ARG4* integration on *C. albicans* ARG4 locus, ARG4 insert, forward	CCGCTGCTAGGCGCGCCGTGAAACGAATCAGACTCTGATACCCAGTGTGATGGATATCTGC
SNO241	*C. dubliniensis ARG4* integration on *C. albicans* ARG4 locus, ARG4 insert, reverse	GCAGGGATGCGGCCGCTGACAGCTCGGATCCACTAGTAACG
SNO145	*C. dubliniensis ARG4* integration on *C. albicans* ARG4 locus, 3' flank, forward	GTCAGCGGCCGCATCCCTGCGTCATATAATAATCACAGTATTG
SNO146	*C. dubliniensis ARG4* integration on *C. albicans* ARG4 locus, 3' flank, reverse	TCAGACGATCTTTACAATGG
SNO187	*C. dubliniensis ARG4* integration verification check, left, forward	CAAGAGTAGTCTCAAATAAACC
SNO263	*C. dubliniensis ARG4* integration verification check, left, reverse, qPCR for *wor1ko* (SN1064), reverse	TTCAACCTTTCAAACGATGC
SNO264	*C. dubliniensis ARG4* integration verification check right, forward	TCGATACATTTGCGGTACAG
SNO188	*C. dubliniensis ARG4* integration verification check, right, reverse	CGTTTGGAAGCTGTATATCG
SNO183	qPCR for *SN425 WT, SN250 WT* and *QMY23 WT*, forward	GATGCCTTAGCTCATTCTTC
SNO322	Common qPCR primer for *SN425 WT, SN250 WT, SN235 WT, efg1ko and wor1ko* (SN881), reverse	TGAGCACCATAAGGACGTTC

SNO1003	Common qPCR primer for *QMY23 WT* and *wor1ko* (RZY244), reverse	CCGGTTTACTTGGATCTTCG
SNO1007	qPCR for *wor1ko* (RZY244), forward	GTACACTGACATCTCAAACATCAA
ST49	qPCR for *wor1ko* (SN1064), forward	AACGAATCAGACTCTGATAC
STO8	qPCR for *efg1ko*, forward	TTTTGTGGAGCCTTTCATGA
STO2	qPCR for *wor1ko* (SN881) and *wor1het* (SN999), forward	AAGATTGGTTTCTGTCGACA
SNO1697	qPCR for *wor1het* (SN999), reverse	CGTAGCCATGAGCACCATAA
SNO1134	*WOR1*-OE and *WOR1p-FLP* strain construction; amplifies *WOR1* upstream region, forward	GGCGAATTGGAGCTCCACCGCGGTGGCGGCCGCTCTAGAACTAGTGGATCGTTTAAACCTTTCCAAAACCCTGCCTTT
SNO1135	*WOR1*-OE strain construction; amplifies *WOR1* upstream region, reverse	GGGACGAGGCAAGCTTGATTCTTTGATGTTTGAGATGTCAGTG
SNO1136	*WOR1*-OE strain construction; amplifies *SAT1* casette, forward	CTGACATCTCAAACATCAAAGAATCAAGCTTGCCTCGTCCCC
SNO1137	*WOR1*-OE strain construction; amplifies *SAT1* casette, reverse	GACTATACTTGAATTAGACATATTTGAATTCAATTGTGATG
SNO1138	*WOR1*-OE strain construction; amplifies *WOR1* ORF fragment, forward	CATCACAATTGAATTCAAATATGTCTAATTCAAGTATAGTCC
SNO1139	*WOR1*-OE strain construction; amplifies *WOR1* ORF fragment, reverse	GTCGACGGTATCGATAAGCTTGATATCGAATTCCTGCAGCCCGGGGGATCGTTTAAACTTGTGGTCGTACTCGTCGTCGGGACC
SNO1187	*WOR1*-OE 5' integration verification check, forward	CCTATTGTTATTGCAGCAACAG
SNO1188	*WOR1*-OE 5' integration verification check, reverse	GTATTCTGGGCCTCCATGTC
SNO1185	*WOR1*-OE 3' integration verification check, forward	AACCCTTGAAATTCCCTTCA
SNO1186	*WOR1*-OE 3' integration verification check, reverse	GTGGTTACCATACCCACCAG
SNO1342	qPCR for *SN425 WT*, reverse	TCAAACGAAGGTCACACTGACT
SNO1343	qPCR for *SN425 WT*, forward	AACAGCTATCAAGAATAATCCCGC
STO50	alternate qPCR for *SN425 WT*, forward	TATGCAGAGAGATATACATC
SNO1355	qPCR for *WOR1-OE*, forward	CCAATTTGAAGACCATTTACGC
SNO1361	qPCR for *WOR1-OE*, reverse	GCACGTCAAGACTGTCAAGGAGG
SNO1521	qPCR for *SN967 WT*, forward	TCTAGTGGTGAATTTGGGTTC
SNO1522	qPCR for *SN967 WT*, reverse	TAAGTAGATGGAATAGATTTGATC
SNO1008	PCR check for the presence of *OBPa*, forward	GCATATTGCACCAAAGGCAG
SNO1009	PCR check for the presence of *OBPa*, reverse	GATTTCCATGACCTCGTTCC
SNO1010	PCR check for the presence of *OBPalpha*, forward	GAAGATGACTCAGGTCATGC
SNO1011	PCR check for the presence of *OBPalpha*, reverse	CTTCAATTGCATCGTAAGTACC
SNO1531	*WOR1p-FLP* strain construction; amplifies *WOR1* upstream region, reverse	CATAATATATCAAATTGTGGCATTGCTTAATATTGAATTGAATTATAC
SNO1532	*WOR1p-FLP* strain construction; amplifies *FLP-SAT1* casette, forward	GTATAATTCAATTCAATATTAAGCAATGCCACAATTTGATATATTATG
SNO1533	*WOR1p-FLP* strain construction; amplifies *FLP-SAT1* casette, reverse	CCTGAATGAGCCCCAAAATAATAACAGGACCACCTTTGATTGTAAA
SNO1534	*WOR1p-FLP* strain construction; amplifies ~450bp of *WOR1* 3'UTR, forward	TTTACAATCAAAGGTGGTCCTGTTATTATTTTGGGGCTCATTCAGG
SNO1352	*WOR1p-FLP* strain construction; amplifies ~450bp of *WOR1* 3'UTR, reverse	GTCGACGGTATCGATAAGCTTGATATCGAATTCCTGCAGCCCGGGGGATCGTTTAAACGTCAAGGCGTCATCATATCATTC
SNO1535	*WOR1p-FLP* strain verification check, left,	AGAAAGAAAGAGAGAGAGGGAACG

	forward	
SNO1536	*WOR1p-FLP* strain verification check, left, reverse	CTGTTCCGTTATGTGTAATCATCC
SNO1537	*WOR1p-FLP* strain verification check, right forward	CGCCTAACATATGTGAAGTGTGA
SNO1354	*WOR1p-FLP* strain verification check, right, reverse	CGTTCAGATATTCATACATCCACCT
SNO464	*Leu2-FRT-URA-FRT* strain construction; amplifies *Leu2* 5' flank, forward	GGCGAATTGGAGCTCCACCGCGGTGGCGGCCGCTCTAGAACTAGTGGATCGTTTAAACTTGGTAGATTTACAACTGAAGCCG
SNO1538	*Leu2-FRT-URA-FRT* strain construction; amplifies *Leu2* 5' flank, reverse	CAGTAGCTCGAGTTAAATCCGAAGTTCCTATTCTCTAGAAAGTATAGGAACTTCCTCGAGGAAAAGGGGAGTATTTCTGGAGTGAA
SNO1539	*Leu2-FRT-URA-FRT* strain construction; amplifies *C.a. URA3* casette, forward	AGAAATACTCCCCTTTTCCTCGAGGAAGTTCCTATACTTTCTAGAGAATAGGAACTTCGGATTTAACTCGAGCTACTGATATCAATGC
SNO1540	*Leu2-FRT-URA-FRT* strain construction; amplifies *C.a. URA3* casette, reverse	GCTAACTACTGTATATACTGGGATCTGAAGTTCCTATTCTCTAGAAAGTATAGGAACTTCCTGCTTAAATCGATATAACTTGGTTTGA
SNO1541	*Leu2-FRT-URA-FRT* strain construction; amplifies *Leu2* 3' flank, forward	CAAGTTATATCGATTTAAGCAGGAAGTTCCTATACTTTCTAGAGAATAGGAACTTCAGATCCCAGTATATACAGTAGTTAGCATTTA
SNO467	*Leu2-FRT-URA-FRT* strain construction; amplifies *Leu2* 3' flank, reverse	GTCGACGGTATCGATAAGCTTGATATCGAATTCCTGCAGCCCGGGGGATCGTTTAAACTCGAAAACGATGTTTGCACCACCG
SNO1443	*Leu2-FRT-URA-FRT* strain verification check, left. forward	GCTTTGAGTTCTGGGTCAGC
SNO1547	*Leu2-FRT-URA-FRT* strain verification check, left. reverse	GTTGACCGTATCAGTAGCATCATC
SNO1447	*Leu2-FRT-URA-FRT* strain verification check, right. reverse	CGCTACCCAACAAAGAGACC
SNO1542	*Leu2-FRT-URA-FRT* strain verification check, right. forward	GCAATCGAAGTAGCTGGGATA
SNO509	URA excision verification primer, and qPCR for *SN235 WT*, forward	GTTGTGATTTTGCTATTCCGGCGCT
SNO840	URA excision verification primer, reverse	TCTCTCCGAATGAAGAGCC
SNO1650	*Leu2* knockout verification primer for strains *SN78* and *SN1020*, forward	GAAATGCTGGTTGGAATGCT
SNO1652	*Leu2* knockout verification primer for strains *SN78* and *SN1020*, reverse	GCGGTCTAGAAGGACCACCT
SNO819	qRTPCR for *ACT1* ORF, forward	GTGGTACTACCATGTTCCCAGG
SNO820	qRTPCR for *ACT1* ORF, reverse	GATAGAACCACCAATCCAGACAGAG
SNO1154	qRTPCR for *WOR1* ORF, forward	TGCTGGTGGATCTAGTAGTGTAGC
SNO1155	qRTPCR for *WOR1* ORF, reverse	AGTACCGGTGTAATACGACCCAGA
SNO1603	qRTPCR for *TDH3* ORF, forward	GCTCCAGACTATGCTGCTTACAT
SNO1604	qRTPCR for *TDH3* ORF, reverse	GGAATGTTAGCTGGGTCTCTTTC
SNO1332	*MTLa*-ko gene disruption primer 3' flank, forward	GGAATTATTGTCTAACCTGCCCGTGG
SNO1333	*MTLa*-ko gene disruption primer 3' flank, reverse	CGTTACTAGTGGATCCGAGCTGCGGCCGCATATACACTGGAAATAAGTGGTGGTAGT
SNO1336	*MTLa*-ko gene disruption primer 5' flank, forward	TCAATCAGGTTGCGGTGTGGCGCGCCTATATGTACATACAAAACTGGTTATTGTAGCAGG
SNO1335	*MTLa*-ko gene disruption primer 5' flank, reverse	CCCTTGGGTAGTAAAGGTAAAGCCAATGCCG
SNO1315	*MTLa*-ko gene disruption, primer to amplify *C. dubliniensis HIS1* casette, forward	TATATGCGGCCGCAGCTCGGATCCACTAGTAACG

SNO1318	*MTLa*-ko gene disruption, primer to amplify *C. dubliniensis HIS1* casette, reverse	ATATAGGCGCGCCACACCGCAACCTGATTGATACCAGTGTGATGGATATCTGC
SNO1337	*MTLa*-ko 3' integration verification check, reverse	GTTGGTGAACCTAAAGTACTTTATTGG
SNO1339	*MTLa*-ko 3' integration verification check, forward	AGTGGATCCGAGCTGCGGCCGCATATA
SNO1340	*MTLa*-ko 5' integration verification check, forward	TTGAGTGTTGCTGGTCTTGC
SNO1341	*MTLa*-ko 5' integration verification check, reverse	TGCAGATATCCATCACACTGG