Supplemental Information

A License to Switch: Hemizygosity Enables a Mutational Transition Governing Fungal Virulence and Commensalism

Shen-Huan Liang, Matthew Z. Anderson, Matthew P. Hirakawa, Joshua M. Wang, Corey Frazer, Leenah M. Alaalm, Gregory J. Thomson, Iuliana V. Ene, and Richard J. Bennett

Table S1. RNA-sequencing data for *C. albicans* cells in different cell states and genetic backgrounds. Related to Figure 3. TPM values, differentially expressed genes and GO term analysis are listed in separate sheets. * indicates cells derived from *efg1Δ/EFG1* white state.

Table S2. Oligos used in this study. Related to STAR Methods.

Oligo	Sequence
11	CTCAACCATAGCAATCATGG
12	GCGAAAAAGTGGGCACTAAG
25	GAATATGAACCCATGCCAATGAC
26	CCATATGTTGGATGAAGATACGG
397	GGGACATTACAATTGTCTCTC
398	GAATCACAGAGTCTAACCAC
690	ATTAGATACGTTGGTGGTTC
691	AACACAACTGCACAATCTGG
692	AGAATTCCCAACTTTGTCTG
693	AAACTTTGAACCCGGCTGCG
819	CATTGATCCGATTTTCACAACG
828	CCTACAACAGATATCAGTATCC
1473	AACCTTCTGGCATGGCAGAC
1838	CGGCCGGGTACCATTGCCCTACCCATCTACTCGC
1839	GGCCGCGGGCCCTGTCAATGGATTTGGGAGAAG
1840	GGGGAAGCAAAACTAAGAAAAGTAG
2251	ACATCCAGCCAACCCACTTA
2252	CACGGCGCGCCTAGCAGCGGGCAGTTGTTTGCTGGGGCATA
2253	GTCAGCGGCCGCATCCCTGCTGACTCAAGGTTCAGTTCACC
2254	CCTTCCGCATTAGACGCTTA
2284	TTGCCCTACCCATCTACTCG
2285	CATCACCTGTAACTCGTGTCG
2286	TTGCTGCTGTTTGTTGTTTG
2291	AACAGACTGGACAGACAGCAGGAC
2292	TAGTAGTAGCAGCAGCAACGGGTG
2381	GCCGGCGTCGACCAACTCAGAGCACGCTAGAC
2609	ACCCCAAAATCCACCACAGG
2610	ACATGACGGTCGAAGATGGT
2909	GCAGGTGGGCCCATGTCAACGTATTCTATACCC
2910	GGTGGTGGATCCCGTTCATGTCAATGGATTTG
2933	CTCGTGGTCTGATTCCTGGT
3055	CCCAGATGCGAAGTTAAGTGCGCAG
3056	AAAAGGCCTGATAAGGAGAGATCCATTAAGAGCA
3265	GACGTCTCTGTAGATGGGTT
3266	AAGTCGGTGCCCAAGTAATG
3269	GGAACTTCCTGCGGTCATTA
3270	CGGTGCATCGGTAAACGATT
3429	TTGTACTCTTCTGGTAGAAC
3430	GTTTGGTCAATACCAGCAGC
3497	ACCAGGAATCAGACCACG
3499	GGCAACAGTGCTAGCTGAT
3685	ATTTGTCCCAAATAGTATAAATTCGTTCATGTCAATGGATTTGGGAGAAGATTATGATCTATACTATTT CAGGGAACAAAAGCTGGAGCT
3687	ACAAATGGTCAAGGCGTCATCATATCATTCATTTATCTATTTGTTGCGGCAATATTACAATTCCCTTCAT GAGGGAACAAAAGCTGGAGCT
3819	TACTAATGGAACATTGGTTGCTGCTGGAACAGATGACGCAGTTGGAAATTCTTCTGGGTCGTATTACA CCGGTACTCGGATCCCCGGGTTAATTAACGGT
3821	TCAAGGTTCAGTTCACCCTTCACCCCAACAACATCAAGCTAATCAATCAGCTAGCACTGTTGCCAAAGA AGAAAAGCGGATCCCCGGGTTAATTAACGGT

4104 GGAGCGGGGCCCACATCCAGCCAACCCACTTA
4105 GGAGCGCTCGAGGCAGTTGTTTGCTGGGGCATA
4106 GGAGCGCCGCGGTGACTCAAGGTTCAGTTCACC
4107 GGAGCGGAGCTCCCTTCCGCATTAGACGCTTA
4114 TGTGGTAGGGGAGGCC
4115 AGGTGTGTACATCAAGGTGG
4290 GGCCCAAATGACACGTGATAGAAGAGATGGG
4291 CCCATCTCTTCTATCACGTGTCATTTGGGCC
4476 ACCAACCAACCCTTAACCCA
4480 TGCCCAAATGTCTTCCGAT
4481 GAGGTAAGGGTTCAAGTCCA
4483 TTCGATATCAGTGAGCCAAC
4484 CCAAATCCAAAGCCTGTTGA
4532 GGACCGGGGCCCCTTTTCTTCTTTGGCAACAGTGC
4577 TCTCGTTTGCATGCGAGATT
4578 CCTATCTCCAAGCGTCAGAT
4680 GATAGACTCTGTGCATTGTC
4681 ACCAACCAAGTCTATTTGAT
4716 GGCGCCCCGCGGGTGTGTAAGTCATTGTCTTTTCC
4864 ACCAAAAAGAATCAACTTATACATTACTAATCTATTCATCTTATTCTCATCACACACGCATATACAAGCA CTACACATAACCAGTGTGATGGATATCTGC
4865 CACGGATAGATCTGTTAGAGATGTGGTGGATTGGTATTTCTTTCTGTGATTCACCGAAAGTATTATCAA CTAAGAAGGGAGCTCGGATCCACTAGTAACG
4928 TCCTAGTTCACCACATAATACTCT
4929 GTCTCACAAGGGTCAAGGT
4930 CACCAACAACAACCAGAGTGA
4945 ACAGAATTAGTTTGGAACTCAAGCA
4946 TGGTTGTTCACGTAATTGTCCT
4992 ACCGAGATATGATACAGGTTGC
4993 TCCGATTCCAACTGTGGAG

Table S3. *C. albicans* strains used in this study. Related to STAR Methods.

Strains	*MTL*	Genotype[a]	Background	Phenotype[b]	Source
SN87	**a**/α	*his1Δ/his1Δ leu2Δ/leu2Δ**	SC5314	WH	(Noble and Johnson, 2005)
TF156	**a**/α	*arg4Δ/arg4Δ his1Δ/his1Δ leu2Δ/leu2Δ efg1Δ::C.d. HIS1/efg1Δ::*C.m *LEU2*	SC5314	GR	(Homann et al., 2009)
RBY717	**a/a**	Wildtype*	SC5314	WH/OP	(Bennett et al., 2003)
RBY1132	**a/a**	*arg4Δ/arg4Δ his1Δ/his1Δ leu2Δ/leu2Δ**	SC5314	WH/OP	(Sherwood and Bennett, 2008)
RBY1177	**a/a**	*his1Δ/his1Δ::HIS1 leu2Δ/leu2Δ**	SC5314	WH/OP	(Bennett and Johnson, 2006)
CAY701	**a**/α	Wildtype P37037	P37037	WH	(Wu et al., 2007)
CAY709	α/α	Wildtype GC75	GC75	WH	(Wu et al., 2007)
CAY710	**a/a**	Wildtype P94015	P94015	GR/OP	(Wu et al., 2007)
CAY711	**a**/α	Wildtype P75063	P75063	WH	(Wu et al., 2007)
CAY712	**a**/α	Wildtype P76055	P76055	WH	(Wu et al., 2007)
CAY714	α/α	Wildtype P57072	P57072	WH	(Wu et al., 2007)
CAY716	**a/a**	Wildtype P37005	P37005	WH	(Wu et al., 2007)
CAY6911	**a/a**	*arg4Δ/arg4Δ his1Δ/his1Δ leu2Δ/leu2Δ efg1Δ::C.d. HIS1/efg1Δ::*C.m *LEU2::EFG1-SAT1-FLIP**	SC5314	WH	This study
CAY6970	**a/a**	*arg4Δ/arg4Δ his1Δ/his1Δ leu2Δ/leu2Δ efg1Δ::C.d. HIS1/EFG1**	SC5314	WH	This study
CAY6973	**a/a**	*arg4Δ/arg4Δ his1Δ/his1Δ leu2Δ/leu2Δ efg1Δ::C.d. HIS1/EFG1**	SC5314	WH	This study
CAY6976	**a/a**	*arg4Δ/arg4Δ his1Δ/his1Δ leu2Δ/leu2Δ efg1Δ::C.d. HIS1/EFG1**	SC5314	WH	This study
CAY6977	**a/a**	*arg4Δ/arg4Δ his1Δ/his1Δ leu2Δ/leu2Δ efg1Δ::C.d. HIS1/efg1Δ::*C.m *LEU2**	SC5314	GR/OP	This study
CAY7022	**a/a**	*arg4Δ/arg4Δ his1Δ/his1Δ leu2Δ/leu2Δ efg1Δ::C.d. HIS1/efg1$^{\#}$**	SC5314	GR	This study
CAY7023	**a/a**	*arg4Δ/arg4Δ his1Δ/his1Δ leu2Δ/leu2Δ efg1Δ::C.d. HIS1/efg1$^{\#}$**	SC5314	OP	This study
CAY7064	**a**/α	*his1Δ/his1Δ leu2Δ/leu2Δ efg1Δ::C.d. HIS1/EFG1**	SC5314	WH	This study
CAY7065	**a**/α	*his1Δ/his1Δ leu2Δ/leu2Δ efg1Δ::*C.d. *HIS1/efg1$^{\#}$**	SC5314	GR	This study
CAY7286	**a/a**	*arg4Δ/arg4Δ his1Δ/his1Δ WOR1/WOR1-GFP-SAT1-FLIP**	SC5314	WH/OP	This study
CAY7292	**a/a**	*arg4Δ/arg4Δ his1Δ/his1Δ EFG1/EFG1-GFP-SAT1-FLIP**	SC5314	WH/OP	This study
CAY7364	**a**/α	Wildtype HJ039	HJ039	GR	(Tao et al., 2014)
CAY7365	**a**/α	Wildtype HJ071	HJ071	GR	(Tao et al., 2014)
CAY7369	**a**/α	Wildtype BJ1097	BJ1097	WH	(Tao et al., 2014)
CAY7370	**a**/α	*EFG1^{Q61*}/EFG1$^{Q61*\#}$*	BJ1097	GR	(Tao et al., 2014)
CAY7637	**a**/α	Wildtype 945	945	GR	(Perea et al., 2001)
CAY7638	**a**/α	Wildtype 1619	1619	WH	(Perea et al., 2001)
CAY7639	**a**/α	Wildtype 2823	2823	WH	(Perea et al., 2001)
CAY7640	**a**/α	Wildtype 4018	4018	WH	(Perea et al., 2001)
CAY7683	**a**/α	*EFG1/EFG1::EFG1$_{SC5314}$*	P75063	WH	This study
CAY7768	**a**/α	*his1Δ/his1Δ leu2Δ/leu2Δ efg1Δ::C.d. HIS1/EFG1 neut5lΔ::NAT1/NEUT5L**	SC5314	WH	This study
CAY7769	**a**/α	*his1Δ/his1Δ leu2Δ/leu2Δ efg1Δ::C.d. HIS1/efg1 neut5lΔ::NAT1/NEUT5L$^{\#}$**	SC5314	GR	This study
CAY7770	**a**/α	*his1Δ/his1Δ leu2Δ/leu2Δ neut5lΔ::NAT1/NEUT5L**	SC5314	WH	This study
CAY7868	**a/a**	*arg4Δ/arg4Δ his1Δ/his1Δ leu2Δ/leu2Δ efg1Δ::C.d. HIS1/efg1 neut5lΔ::NAT1/NEUT5L$^{\#}$**	SC5314	OP	This study

CAY7869	**a/a**	*arg4Δ/arg4Δ his1Δ/his1Δ leu2Δ/leu2Δ efg1Δ::C.d. HIS1/efg1 neut5lΔ::NAT1/NEUT5L*[#]*	SC5314	GR	This study
CAY8128	**a/a**	*arg4Δ/arg4Δ his1Δ/his1Δ leu2Δ/leu2Δ efg1Δ::C.d. HIS1/EFG1-GFP-SAT1-FLIP**	SC5314	WH	This study
CAY8131	**a/a**	*arg4Δ/arg4Δ his1Δ/his1Δ leu2Δ/leu2Δ efg1Δ::C.d. HIS1/EFG1 WOR1/WOR1-GFP-SAT1-FLIP**	SC5314	WH	This study
CAY8162	**a/a**	*leu2Δ/leu2Δ EFG1/EFG1-myc-SAT1-FLIP**	SC5314	WH/OP	This study
CAY8282	**a**/α	*his1Δ/his1Δ::HIS1 leu2Δ/leu2Δ**	SC5314	WH	This study
CAY8646	**a/a**	*arg4Δ/arg4Δ his1Δ/his1Δ leu2Δ/leu2Δ efg1Δ::C.d. HIS1/efg1Δ::C.m. LEU2::EFG1*^{G252D}*-SAT1-FLIP**	SC5314	GR	This study
CAY8673	**a**/α	*efg1Δ::SAT1-FLIP/EFG1*	P76055	WH	This study
CAY8718	α/α	*efg1Δ::SAT1-FLIP/EFG1*	GC75	WH	This study
CAY8721	α/α	*efg1Δ::SAT1-FLIP/EFG1*	P57072	WH	This study
CAY8724	**a/a**	*efg1Δ::SAT1-FLIP/EFG1*	P37005	WH	This study
CAY8940	**a/a**	*leu2Δ/leu2Δ efg1Δ::SAT1-FLIP/EFG1-myc::FRT**	SC5314	WH	This study
CAY9155	**a**/α	*EFG1*^{G252D}*/EFG1-GFP-SAT1*	P37037	WH	This study
CAY9159	**a**/α	*EFG1*^{insert22}*/EFG1-GFP-SAT1*	P75063	WH	This study
CAY9165	**a**/α	*EFG1*^{Q37*}*/EFG1-GFP-SAT1*	1619	WH	This study
CAY9195	**a**/α	*EFG1*^{Q61*}*/EFG1-GFP-SAT1*	BJ1097	WH	This study
CAY9628	**a/**α	*leu2Δ/leu2Δ his1Δ/his1Δ ade2Δ::C.d. HIS1/ADE2**	SC5314	WH	This study
CAY9962	**a/a**	*his1Δ/his1Δ::HIS1 leu2Δ/leu2Δ arg4Δ/arg4Δ**	SC5314	WH/OP	This study

Only one representative strain was listed for each genetic and epigenetic background.
* indicates that strains contain the genotype *ura3Δ::imm*^{434}*/URA3 iro1Δ::imm*^{434}*/IRO1*.
[#] indicates strains were derived from *EFG1*$^{+/-}$ white cells through *de novo* mutations
[a] *C.d., C. dubliniensis*; *C.m.*, *C. maltosa*.
[b] WH, white; GR, gray; OP, opaque.

Table S4. Set of SNP-RFLP markers used in this study. Related to STAR Methods.

Chr	Position (bp)	SNP	Size of the marker (bp)	Oligos	RE	Size of digestion products (bp)	Genetic background	Ref
RL	679112	T/C	956	4480/4481	AseI	93/863	P75063, SC5314	(Forche et al., 2009) and this study
RL	1602285	A/G	139	3265/3266	DdeI	62/77	SC5314	(Forche et al., 2009)
RL	1686418	C/T	181	4483/4484	SmlI	138/43	SC5314	(Forche et al., 2009)
RL	1712897	T/C	600	4992/4993	BclI	228/372	P75063, P37037	This study
RL	1720595	T/C	430	4945/4946	XhoI	283/147	P37037	This study
RL	1721256	A/G	436	4928/4929	BstBI	241/195	P75063	This study
RL	1721280	T/C	436	4928/4929	AluI	265/171	P75063	This study
RL	1722874	A/G	906	2284/2252	PsiI	114/792	P75063, SC5314, P37037	This study
RL	1723718	G/A	816	2909/819	NheI	132/684	1619	This study
RL	1723738	T/A	816	2909/819	SphI	149/667	1619	This study
RL	1724302	T/C	816	2909/819	BclI	712/104	P75063, 1619	This study
RL	1724343	G/A	816	2909/819	XcmI	748/68	P37037	This study
RL	1724772	T/C	1650	2909/4532	KpnI	1182/468	1619, P37037	This study
RL	1725663	A/G	524	4716/2285	BstBI	105/419	P75063	This study
RL	1725946	A/G	524	4716/2285	BsrI	384/140	P37037	This study
RL	1726064	T/G	675	4716/4930	SalI	504/171	P75063	This study
RL	1727072	T/C	765	4577/4578	Bpu10I	460/305	SC5134	This study
RR	1781488	A/G	187	3269/3270	DdeI	71/116	SC5314	(Forche et al., 2009)

RE, restriction enzyme

RL, left of chromosome R centromere; RR, right of chromosome R centromere.

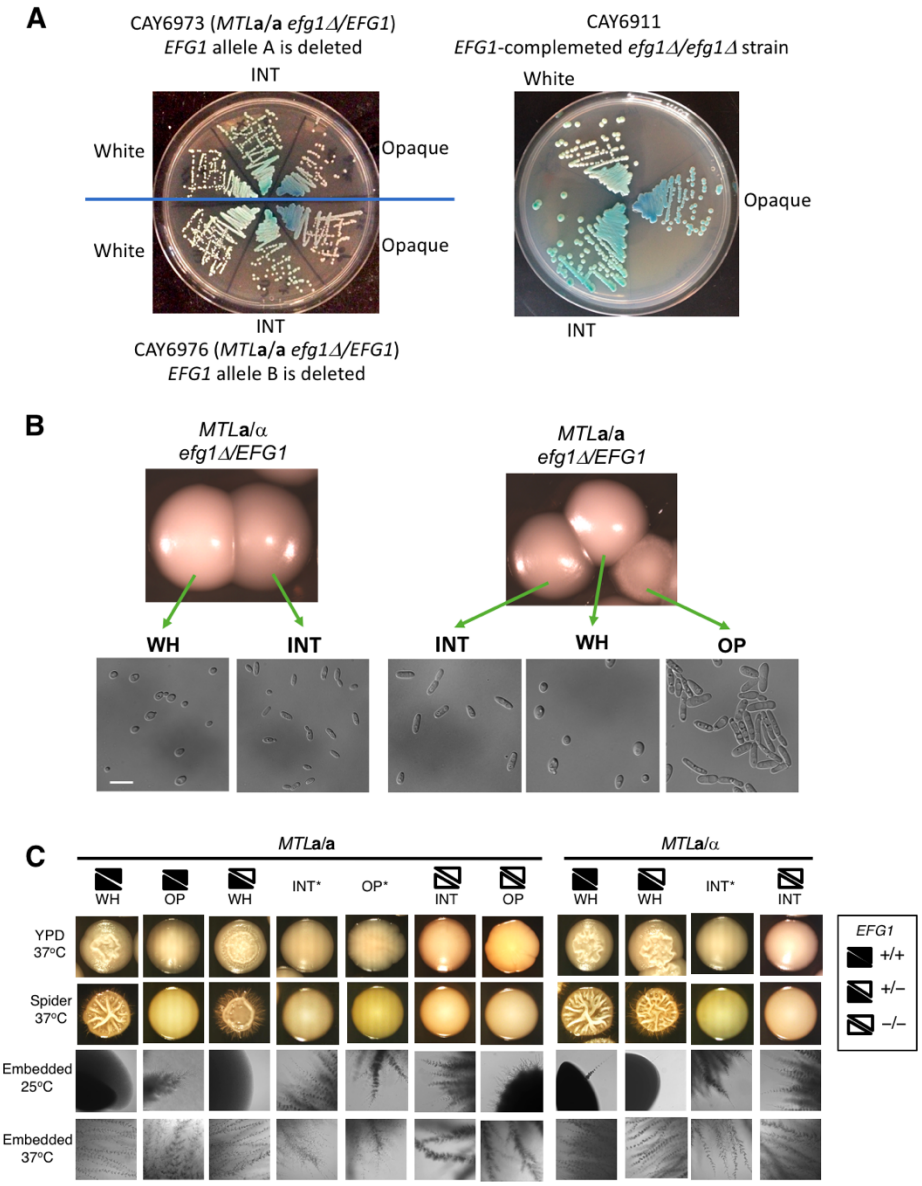

Figure S1. Morphology of SC5314 white, INT and opaque cells. Related to Figure 1.

(A) *C. albicans* SC5314 cells can access the 'INT' cell state in *EFG1* hemizygous strains. Left panel, SC5314 *MTL***a**/**a** *efg1Δ/EFG1* cells exhibit white-INT-opaque transitions regardless of whether allele A (CAY6973) or allele B (CAY6976) is deleted. Right panel, SC5314 *MTL***a**/**a** *efg1Δ/Δ* cells can form INT and opaque states, but re-integration of one *EFG1* allele allows cells to also form the white state. * indicates cells originated from *efg1Δ/EFG1* white state.

(B) Morphology of white, INT and opaque cells on YPD medium. Colonies were grown on YPD for 2 days. White cells form white colonies with a smooth surface. INT colonies have a smooth surface but a darker color than white cells. In contrast, opaque cells form rougher colonies. INT and opaque cells are elongated on YPD medium, whereas white cells are oval. Opaque cells are larger than INT or white cells. Scale bar, 10 μm. *MTL***a**/α *efg1Δ/EFG1*; CAY7064. *MTL***a**/**a** *efg1Δ/EFG1*; CAY6970. * indicates cells derived from *efg1Δ/EFG1* white state.

(C) Filamentation of white, INT and opaque cells. SC5314-derived cells were grown on YPD or Spider media at 37°C or embedded in YPS medium at 37°C or 22°C for 5 days. *MTL***a**/α *EFG1/EFG1*; SC5314. *MTL***a**/α *efg1Δ/EFG1*; CAY7064. *MTL***a**/α *efg1Δ/efg1Δ*; TF156. *MTL***a**/**a** *EFG1/EFG1*; RBY717. *MTL***a**/**a** *efg1Δ/EFG1*; CAY6970. *MTL***a**/**a** *efg1Δ/efg1Δ*; CAY6977. * indicates cells generated from *efg1Δ/EFG1* white state.

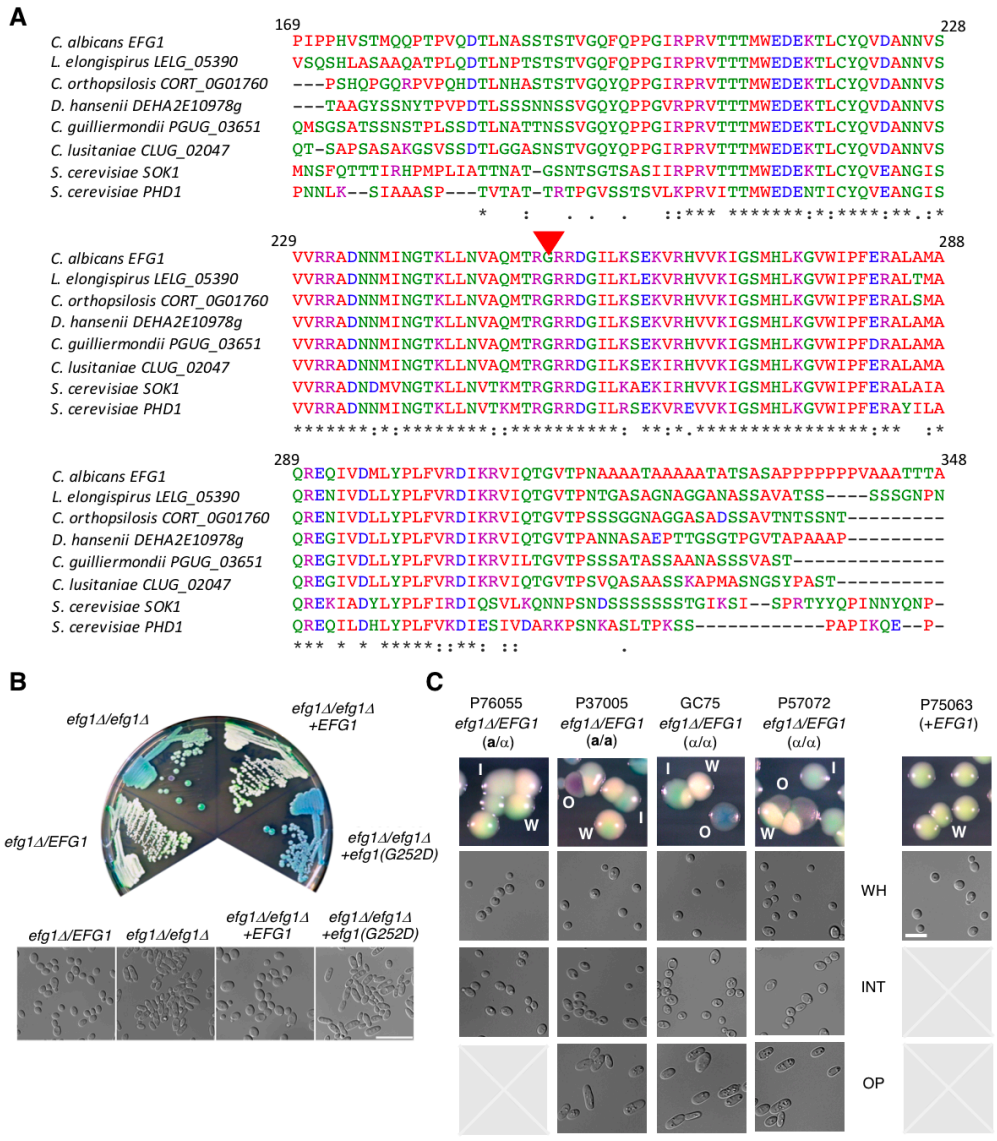

Figure S2. Contribution of *EFG1* loci to phenotypic states in clinical strains of *C. albicans*. Related to Figure 2.

(A) Alignment of Efg1 protein sequences including *C. albicans* (allele B) and orthologs from the *Candida* CTG clade and *S. cerevisiae* showing conservation of amino acids within the APSES DNA-binding domain (amino acids 207-317 in *C. albicans* Efg1). Alignment performed with Clustal Omega (Sievers et al., 2011). An inverted red triangle shows the position of G252 that is naturally mutated in one *EFG1* allele in clinical isolate P37037.

(B) CHROMagar plate and cell images show that reconstruction of the G252D mutation in SC5314 *EFG1* led to an allele that was nonfunctional when re-introduced into SC5314 *efg1Δ/Δ* cells as it did not promote cell state switching. Scale bar, 20 μm. Strains *efg1Δ/EFG1* (CAY6970) and *efg1Δ/Δ+EFG1* (CAY6911) are shown in the white state while *efg1Δ/Δ* (CAY6977) and *efg1Δ/Δ+EFG1(G252D)* (CAY8646) strains are in the INT state.

(C) An *MTL***a**/α *EFG1*$^{+/+}$ strain (P76055) was locked in the white state but exhibited white-to-INT transitions upon deletion of one *EFG1* allele. *MTL* homozygous strains (P37005, GC75, and P57072) were also naturally *EFG1*$^{+/+}$ and formed white, INT and opaque states when one allele of *EFG1* was deleted. These experiments phenocopy our observations with *EFG1* and *MTL* mutants of SC5314 (see Figure 1 and Figure S1). P75063 is an *MTL***a**/α strain that is naturally hemizygous for *EFG1* and exhibits white-to-INT transitions (see Fig. 4B). Integration of a functional SC5314 *EFG1* allele into P75063 (resulting in an *EFG1*$^{+/+}$ strain) blocked formation of the INT state.

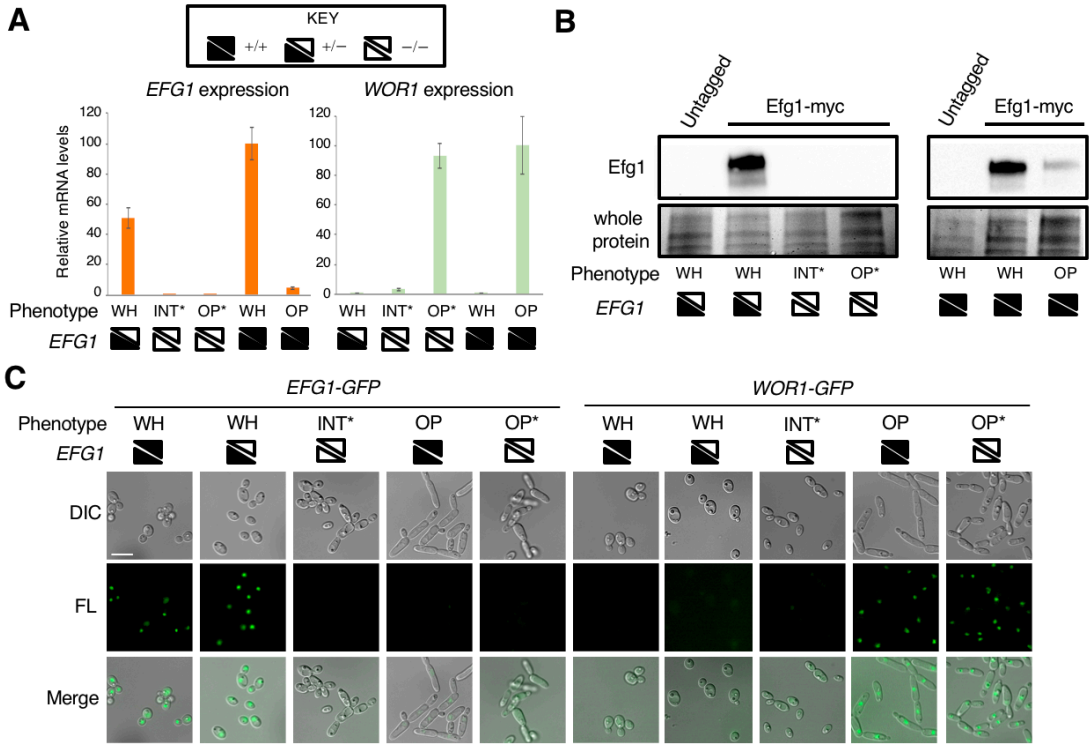

Figure S3. Expression of *EFG1* and *WOR1* in different cell states. Related to Figure 1.

(A) RT-PCR of *EFG1* and *WOR1* expression in different cell types (*MTL***a**/**a** strain). Expression levels normalized to actin controls. *EFG1* levels displayed relative to those in *EFG1*$^{+/+}$ white cells and *WOR1* levels relative those in *EFG1*$^{+/+}$ opaque cells. *MTL***a**/**a** *EFG1*$^{+/+}$; RBY717. *MTL***a**/**a** *efg1Δ/EFG1*; CAY6970. * indicates cells derived from *efg1Δ/EFG1* white state.

(B) Top panels, western blot of Efg1-myc expression. Bottom panels, total protein. *EFG1/EFG1-myc*; CAY8162. *efg1Δ/EFG1-myc*; CAY8940. * indicates cells derived from *efg1Δ/EFG1* white cells.

(C) Fluorescence microscopy of *MTL***a**/**a** cells expressing Efg1-GFP or Wor1-GFP in different cell states. Exposure time and settings are constant between panels. DIC – Differential Interference Contrast. FL=GFP channel. Scale bar, 10 µm. *EFG1/EFG1-GFP*; CAY7292. *efg1Δ/EFG1-GFP*; CAY8128. *EFG1/EFG1 WOR1-GFP*; CAY7286. *efg1Δ/EFG1 WOR1-GFP*; CAY8131. * indicates cells derived from *efg1Δ/EFG1* white state.

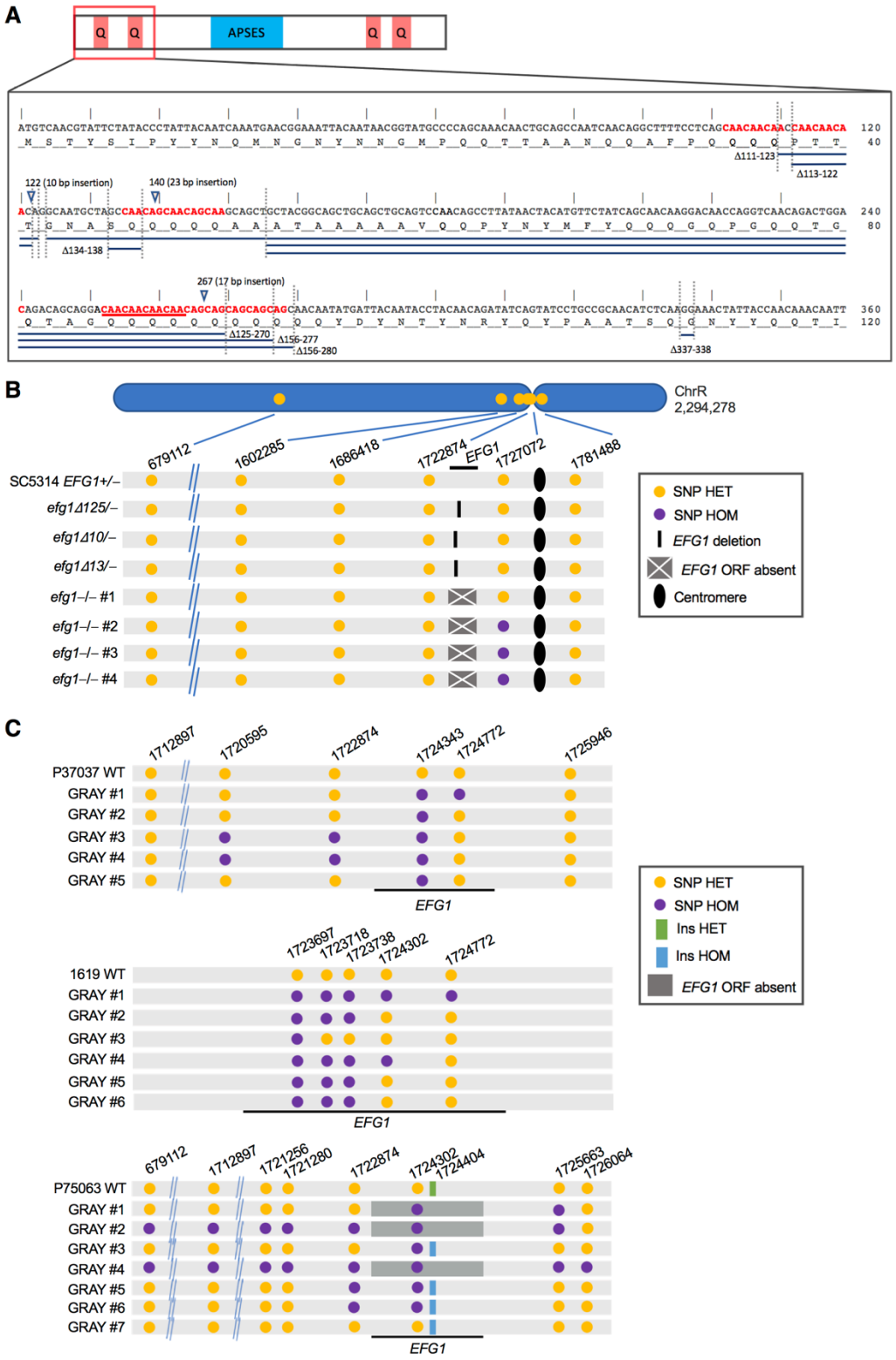

Figure S4. Summary of *de novo* mutations and gene conversion tracts in gray cells. Related to Figure 3.

(A) *De novo* mutations arising in *EFG1* in Figure 2A are summarized here. Several repetitive sequences that are repeatedly mutated are highlighted in red.

(B) Summary of heterozygous positions along Chromosome R examined by SNP-RFLP analysis in SC5314. The parental *EFG1* hemizygous strain (*EFG1*$^{+/-}$) is heterozygous for 6 SNPs (nucleotide positions indicated) and 7 independently formed gray lineages were evaluated for heterozygosity at each of these positions. *EFG1* and *CENR* are located at 1,723,589-1,725,166 and 1,743,190-1,747,664, respectively.

(C) Heterozygous positions on Chromosome R examined by SNP-RFLP analysis in three clinical isolates. The intact *EFG1* allele in the parental isolates was tagged with GFP. *EFG1* ORF absent (gray box) indicates that no PCR fragment was amplified by primers that include the GFP tag. The P37037 strain was analyzed at 6 heterozygous SNP positions. Position 1724343 is the G252D mutation, which was defined by both Sanger sequencing and SNP-RFLP in the gray cells. Strain 1619 was analyzed at only 4 SNP positions (as much of Chr R was homozygous). Position 1723697 is the Q37* mutation, which was defined by Sanger sequencing. P75063 was analyzed at 8 SNPs along Chr R. The insertion (ins) was defined by Sanger sequencing.

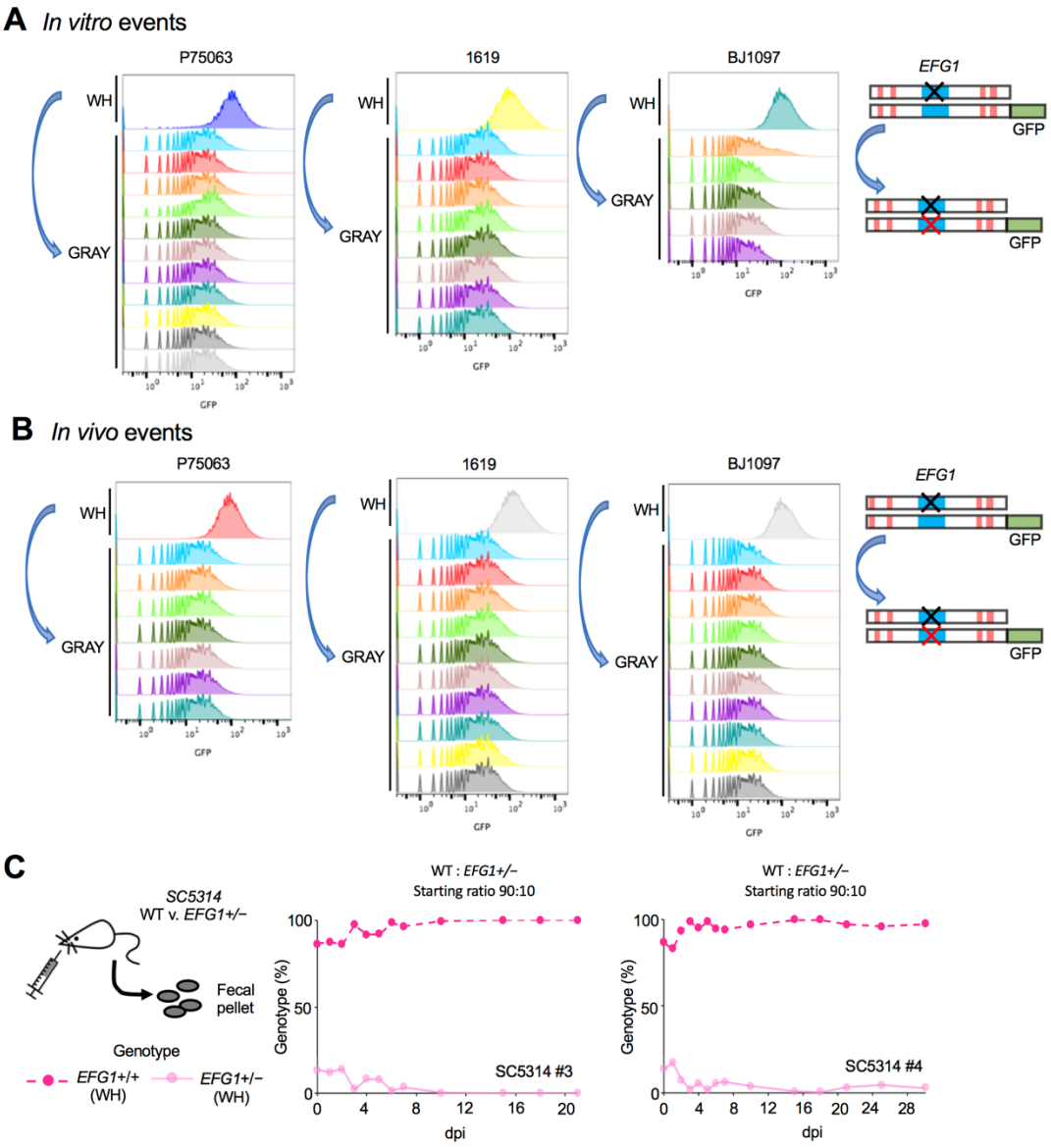

Figure S5. Loss of *EFG1* expression upon white-to-gray switching and competition between wildtype and *EFG1*$^{+/-}$ strains in the GI tract. Related to Figures 3 and 4.

(A) *EFG1* expression was monitored by flow cytometry prior to and after white-to-gray switching. The intact *EFG1* allele in *EFG1*$^{+/-}$ strains was expressed as a GFP fusion protein in isolates P75063, 1619 and BJ1097. Following independent white-to-gray transition events cells were analyzed by flow cytometry to examine expression of the GFP marker. GFP-labeled strains used in flow cytometry analysis: P75063, CAY9159; 1619, CAY9165; BJ1097, CAY9195.

(B) Experiments were performed as in (A) for cells from gray colonies recovered from mouse fecal samples.

(C) Competition between SC5314 *EFG1*$^{+/+}$ and *EFG1*$^{+/-}$ cells (strains CAY8282 and 7768) in the mouse GI tract. Isolates were inoculated at a 90:10 ratio. Genotype was determined by the NAT^R:NAT^S ratio in cells recovered from fecal samples. Gray cells emerged in two of four mice infected with this strain combination (see Figure 4D), while the other two mice lost the *EFG1*$^{+/-}$ cells or maintained a low population of *EFG1*$^{+/-}$ cells. Mice were housed separately

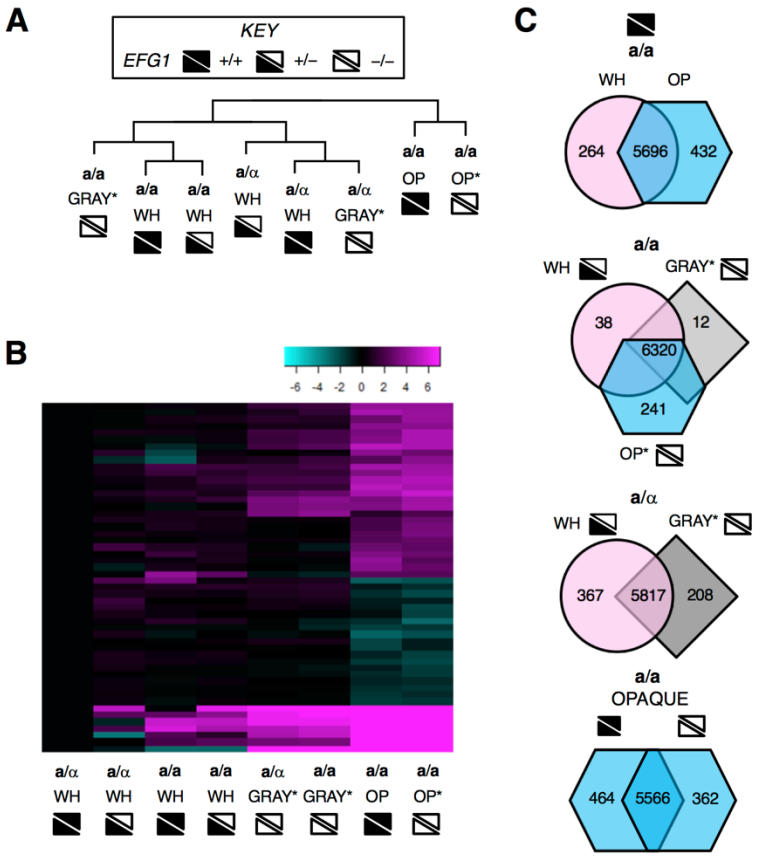

Figure S6. Transcriptional profiles of cells in different states and genetic backgrounds. Related to Figure 1.

(A) Hierarchical clustering of RNA-Seq profiles for white, gray and opaque cells with different *MTL* and *EFG1* configurations. Asterisks indicate gray/opaque cells that arose from *EFG1* hemizygous parental cells and are *efg1* null.

(B) Heatmap of RNA-Seq data shows significant differential gene expression relative to *MTL***a**/α *EFG1*$^{+/+}$ cells (>2-fold expression change, q<0.05). Scale bar represents log base 2 values.

(C) Venn diagram comparing gene expression between cells in different phenotypic states. Numbers indicate differentially expressed genes (>2-fold expression change, q<0.05).

Strains used are SC5314 derivatives: *MTL***a**/**a** *EFG1*$^{+/+}$; RBY717, *MTL***a**/**a** *EFG1*$^{+/-}$; CAY6970, *MTL***a**/α *EFG1*$^{+/+}$; SN87, *MTL***a**/α *EFG1*$^{+/-}$; CAY7064.

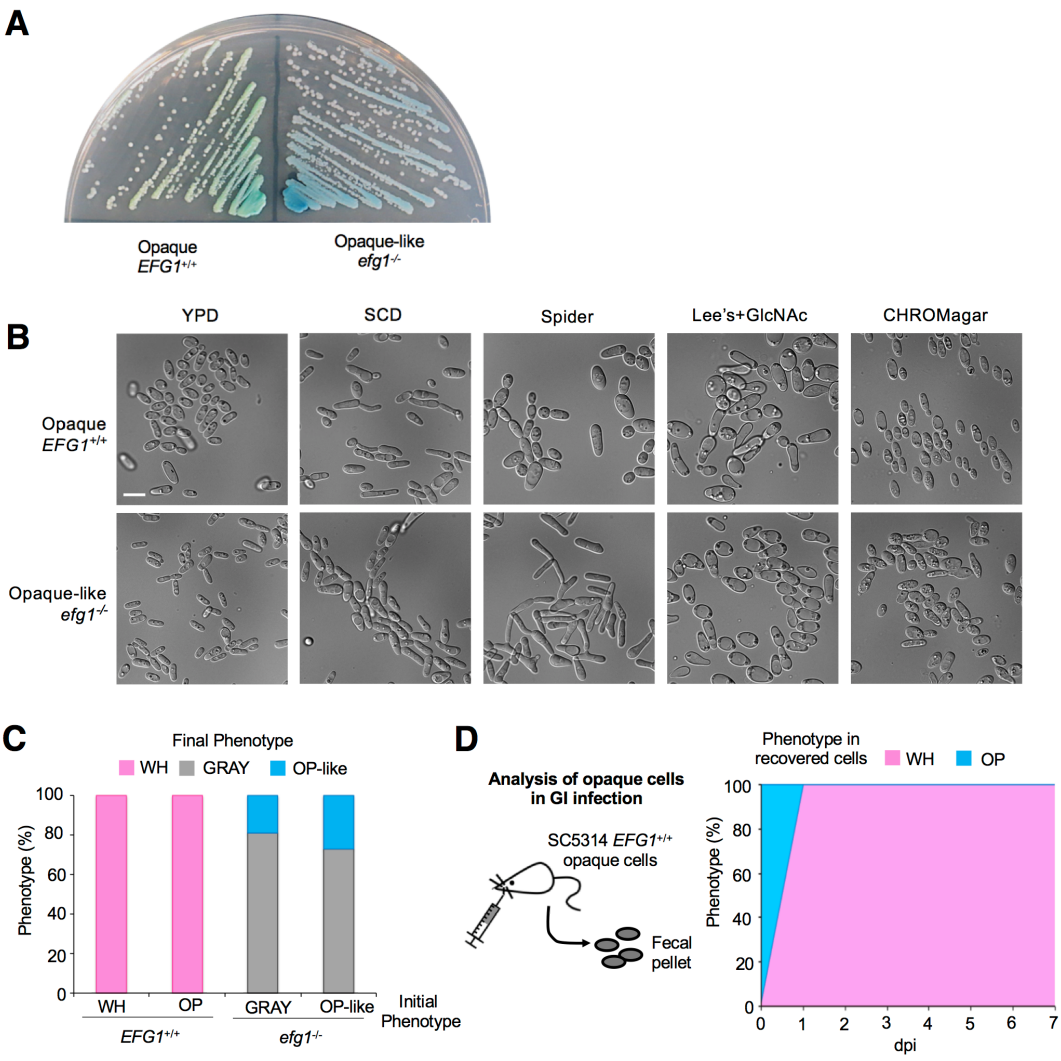

Figure S7. Comparison of opaque cell phenotypes in *MTL* homozygous *EFG1*$^{+/+}$ and *efg1*$^{-/-}$ strains. Related to Figure 1 and 6.

(A) *EFG1*$^{+/+}$ opaque and *efg1* null opaque colonies show different colors on CHROMagar. Cells grown on CHROMagar at 22°C for 3 days.

(B) Morphologies of *EFG1*$^{+/+}$ (RBY717) and *efg1*$^{-/-}$ (derived from CAY6970) opaque cells grown on different media. Cells were grown on YPD, SCD, Spider, Lee's+GlcNAc and CHROMagar at 22°C for 5 days. Scale bar, 10 μm.

(C) Cells in the indicated states were grown on YPD medium at 37°C for 7 days and subsequently analyzed for phenotypes by plating on CHROMagar. Strains were *EFG1*$^{+/+}$

(RBY1177) and *efg1*$^{-/-}$ (derived from CAY6970 via an LOH at *EFG1*). The experiment was performed twice independently. Mean values are plotted.

(D) Analysis of *EFG1*$^{+/+}$ opaque cells in the mouse GI tract. Opaque cells (from CAY9962) were inoculated into the GI tract and cells recovered daily from fecal pellets to examine their phenotype. *n*=2 mice housed separately.