SUPPLEMENTAL FIGURE TITLES AND TEXT

Recording of DNA binding events during gut commensalism reveals the action of a repurposed *Candida albicans* regulatory network

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Figure S1. *C. albicans* sexual regulators bind to each other's promoters in white and opaque a cells (related to Figure 1). Cartoons of DNA binding activity in white **a** cells (left) and opaque **a** cells (right) are based on the results of ChIP experiments reported by other groups (Hernday et al., 2013; Hernday et al., 2016; Lohse and Johnson, 2016). Each dot indicates a direct binding interaction, and only the boxed regulators are expressed in each cell type. Cells used for ChIP experiments were propagated *in vitro.*

Figure S2. Validation of commensal phenotypes with independent isolates of *wor2, wor3, wor4, czf1,* and *ahr1* (related to Figures 1 and 2). A-E. Independently generated isolates of each mutant were competed against WT in the murine gut colonization model. A. WT (ySN250) v. *wor2* (ySN1143). B. WT (ySN250) v. *wor3* (ySN1433). C. WT (ySN250) v. *wor4* (ySN1494). D. WT (ySN250) v. *czf1* (ySN1383). E. WT (ySN250) v. *ahr1* (ySN1188). Paired student's t-test ns=not significant, *p<0.05, ** p<0.01, *** p<0.001. F. *ssn6* exhibits colony growth and wrinkling (a marker of filamentation) defects on multiple types of laboratory media. Single colonies of WT and *ssn6* were propagated on YEPD, Lee's glucose, and Spider plates incubated at 30°C and 37°C. G. Growth under anaerobic conditions does not correlate with commensal phenotypes. Serial dilutions of WT and transcription factor mutants were spotted onto YEPD medium and

incubated at 30°C for 2 days in an aerobic environment or for 3 days in an anaerobic environment. H-I. Repeats of commensalism experiments shown in Figure 2C and 2D. H. *efg1* vs. *efg1wor1* using the same strains as in Figure 2C. I. WT vs. *efg1czf1,* using an independent isolate of *efg1czf1.* Paired student's t-test, ns=not significant, *p<0.05, ** p<0.01.

Figure S3. Features of the Calling Card-Seq method (related to Figures 3 and 4). A. Schematic of PB transposons at the donor site. *C.d.HIS1*- and, in strains containing two transposons, *C.m.LEU2*-marked PB are flanked by a split *ARG4* gene (from *Candida dubliniensis)*. The split *ARG4*-transposon constructs are integrated into the chromosomal *leu2*Δ locus (both endogenous alleles of the *LEU2* ORF are deleted in the SN152 parent strain). B. Use of auxotrophic markers to monitor transposon excision and reintegration events. Prior to transposase activity, strains containing one copy of PB have a His+Arg- phenotype. Strains that have undergone transposon excision and loss of PB have a His-Arg+ phenotype. Strains that have undergone transposon excision and reintegration into the genome have a His+Arg+ phenotype. C. Schematic of method to create PBase^{OE} strains. The insert from PmeI-digested pSN422 undergoes recombination at the *TDH3* locus, resulting in replacement of the *TDH3* ORF. As a result, untethered PBase is expressed at high levels via the strong, constitutively active *TDH3* promoter. D. Growth of strains containing only the PB-*HIS1* transposon (top) vs. PB-*HIS1* plus untethered PBase (bottom) on various media. Both strains grow well on YEPD/agar (left, nonselective) and on SD-his/agar (middle, selective for presence of the transposon), but only the strain expressing PBase grows on SD-arg (right; selective for the presence of the transposon and for excision of the transposon from the donor site).

Figure S4. Additional Cht2 experiments (related to Figure 4). A. Spot plate growth assays of *cht2* under cell wall stress conditions. B. Spot plates growth assays of *CHT2* mutants under

aerobic, microaerophilic and anaerobic conditions. Strains were spotted onto YPD and incubated in air or in microaerophilic or anaerobic chambers for 5 days at 30°C.

C and E. Competitive fitness assays in the mouse model of gut colonization. C. WT (SN250) vs. *cht2* (SN2157) (independent *cht2* isolate compared to Figure 4C) D. Alignment of *C. albicans* Cht2 and *S. cerevisiae* Cts1. Boxes indicate conserved residues of the DxE motif and chitin binding pocket that were mutated to alanine for assessment of Cht2 chitinase activity. E. *cht2*+*CHT2* (SN2225) vs. *cht2*+*CHT2*^{D151A,E153A} (SN2230). Paired student's t-test, ns = not significant, *p<0.05, **p<0.01.

SUPPLEMENTAL TABLE TITLES

Table S1. Efg1-PBase in vitro

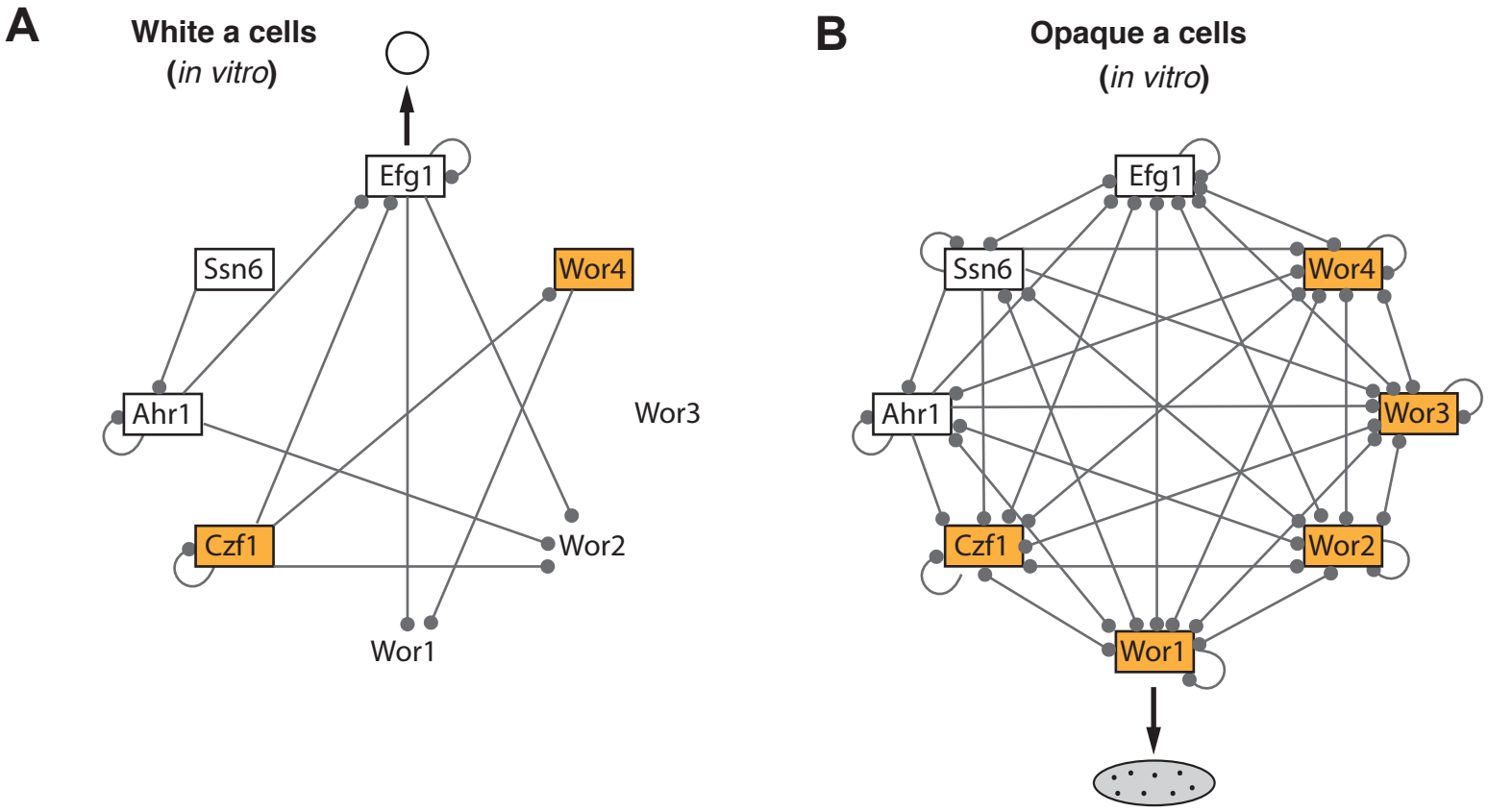
Table S2. CCS in host

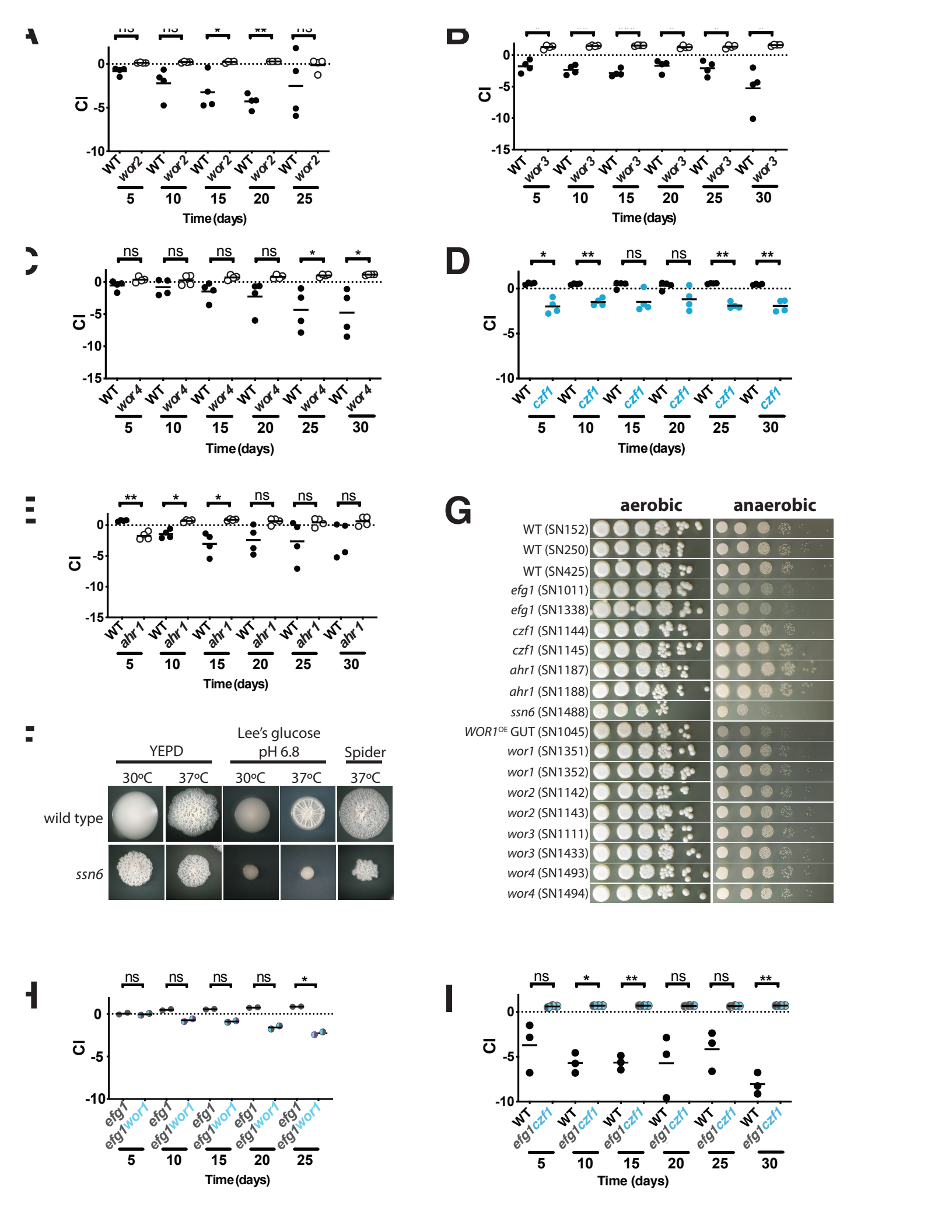
Table S3. RNA-Seq

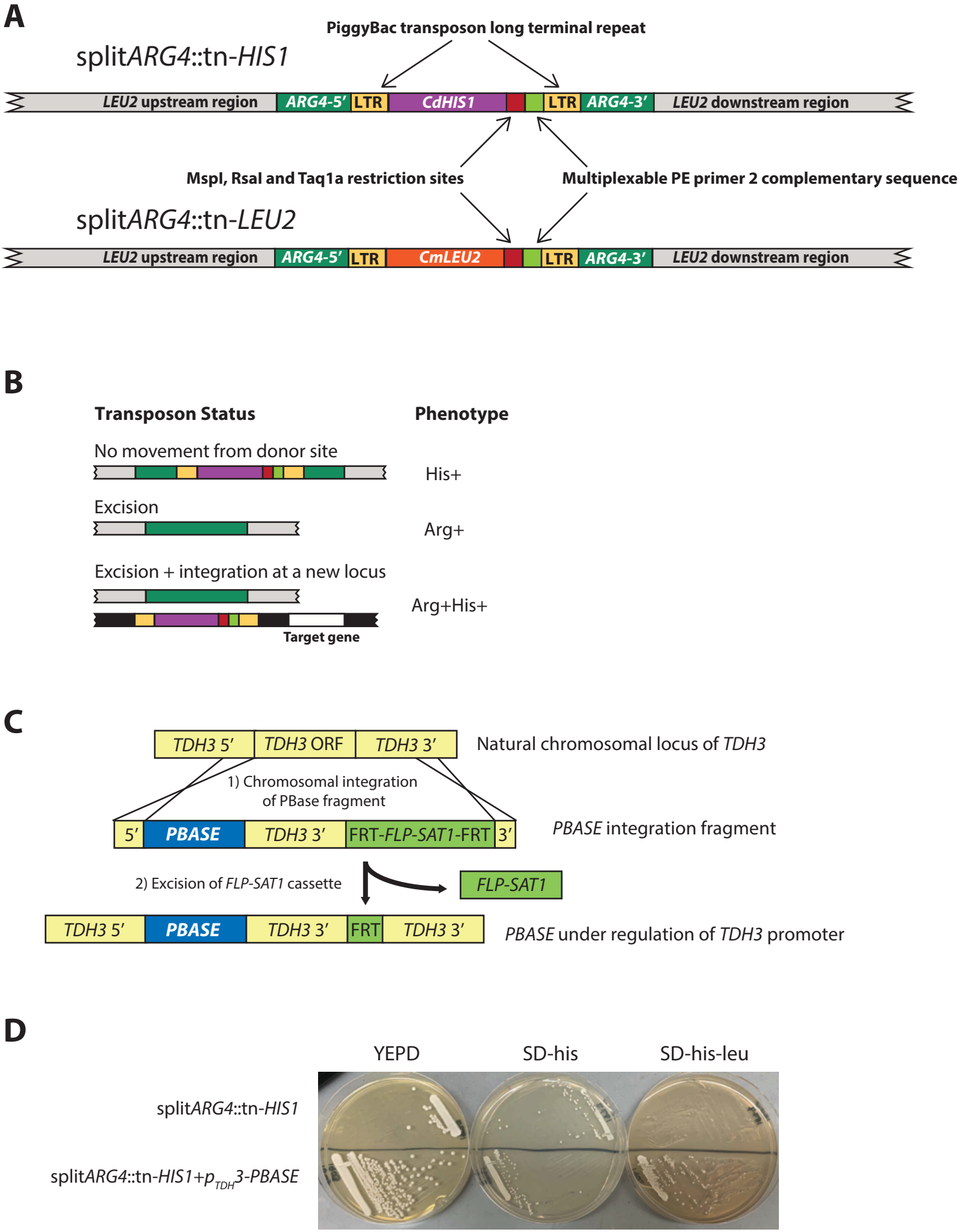
Table S4. Strains

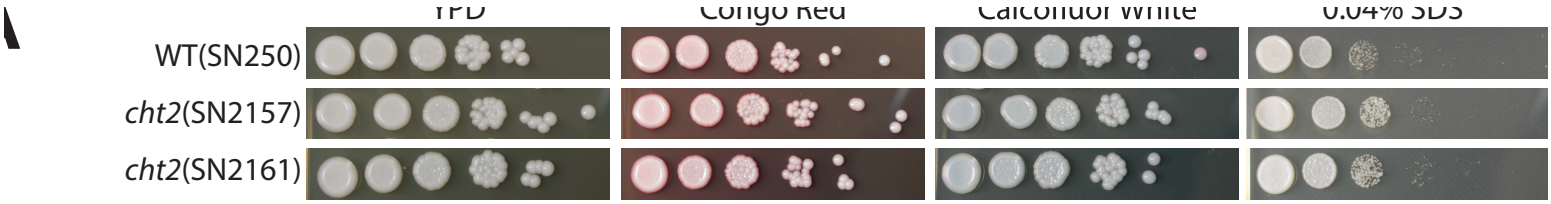
Table S5. Plasmids

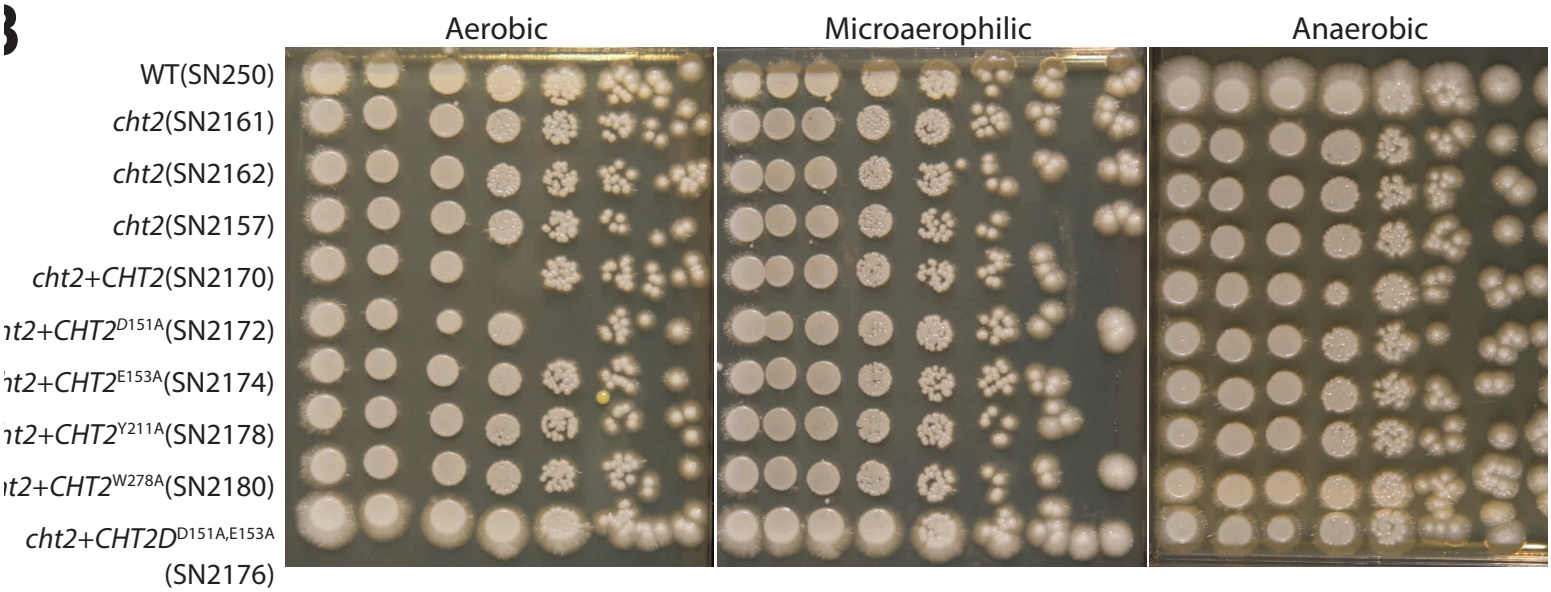
Table S6. Primers

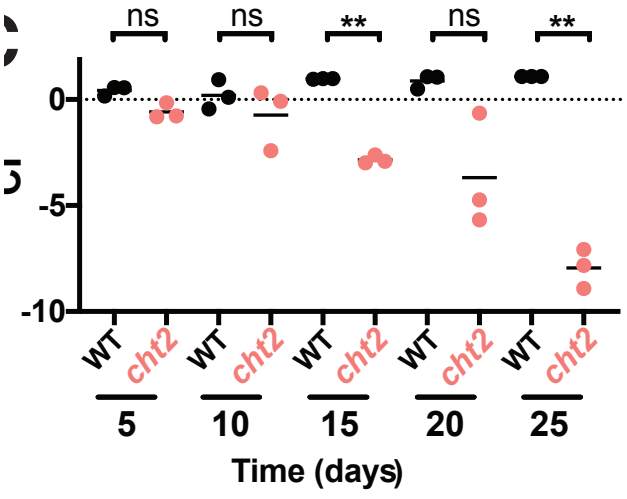

A
White a cells
(in vitro)
Efg1
Ssn6
Wor4
Ahr1
Wor3
Czf1
Wor2
Wor1
B
Opaque a cells
(in vitro)
Efg1
Ssn6
Wor4
Ahr1
Wor3
Czf1
Wor2
Wor1

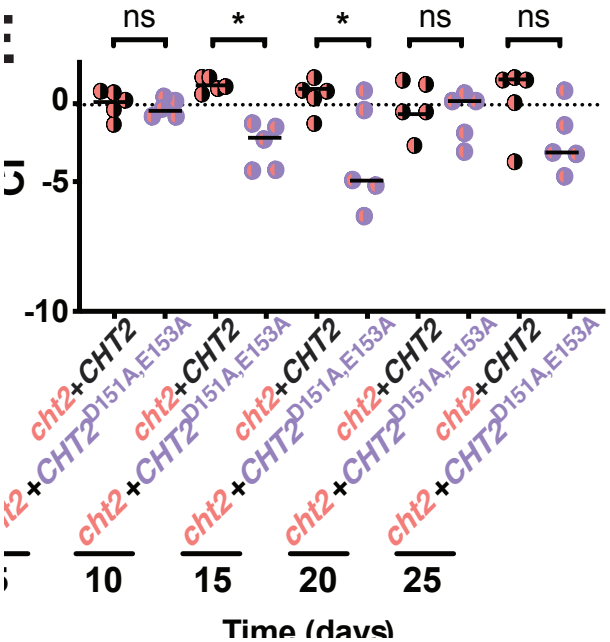

A
B
C
D
E
F
G
H
I
CI
Time (days)
WT
wor2
wor3
wor4
czf1
ahr1
ns
*
**
YEPD
Lee's glucose pH 6.8
Spider
30°C
37°C
wild type
ssn6
aerobic
anaerobic
WT (SN152)
WT (SN250)
WT (SN425)
efg1 (SN1011)
efg1 (SN1338)
czf1 (SN1144)
czf1 (SN1145)
ahr1 (SN1187)
ahr1 (SN1188)
ssn6 (SN1488)
WOR1OE GUT (SN1045)
wor1 (SN1351)
wor1 (SN1352)
wor2 (SN1142)
wor2 (SN1143)
wor3 (SN1111)
wor3 (SN1433)
wor4 (SN1493)
wor4 (SN1494)
efg1
efg1wor1
efg1czf1


A
PiggyBac transposon long terminal repeat
splitARG4::tn-HIS1
LEU2 upstream region
ARG4-5′
LTR
CdHIS1
LTR
ARG4-3′
LEU2 downstream region
MspI, RsaI and Taq1a restriction sites
Multiplexable PE primer 2 complementary sequence
splitARG4::tn-LEU2
LEU2 upstream region
ARG4-5′
LTR
CmLEU2
LTR
ARG4-3′
LEU2 downstream region
B
Transposon Status
Phenotype
No movement from donor site
His+
Excision
Arg+
Excision + integration at a new locus
Arg+His+
Target gene
C
TDH3 5′
TDH3 ORF
TDH3 3′
Natural chromosomal locus of TDH3
1) Chromosomal integration of PBase fragment
5′
PBASE
TDH3 3′
FRT-FLP-SAT1-FRT
3′
PBASE integration fragment
2) Excision of FLP-SAT1 cassette
FLP-SAT1
TDH3 5′
PBASE
TDH3 3′
FRT
TDH3 3′
PBASE under regulation of TDH3 promoter
D
YEPD
SD-his
SD-his-leu
splitARG4::tn-HIS1
splitARG4::tn-HIS1+p_TDH3-PBASE









D

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Cht2      1 ------MLSFKSLLAAAVVASSALASASNQVALYWGQNGAGGQERLAQYC
                  :|.|...|.....|..  .||:..:|:|||||.|||.||.||
ScCts1    1 MSLLYIILLFTQFLLLPTDAFD--RSANTNIAVYWGQNSAGTQESLATYC

Cht2     45 QEADVDIILLSFLNLFPDPLNVNFANQCGNTFESGLLHCSQIGADIKTCQ
             :.:|.||.|||||.|| .|.:||||.|.:||..||||:||..||:|||
ScCts1   49 ESSDADIFLLSFLNQFP-TLGLNFANACSDTFSDGLLHCTQIAEDIETCQ

Cht2     95 SLGKTVLLSLGGGVGDYGFSDVASATKFADTLWNKFGAGED-PERPFDDA   1
            |||||.|||||||..|.|.|||.:.|..||.|||:.||.|.. .||||||.|
ScCts1   98 SLGKKVLLSLGGASGSYLFSDDSQAETFAQTLWDTFGEGTGASERPFDSA   1
                 DxE
Cht2    144 VVDGFDFDIEHGGATGYPELATALRGKFAKDTSKNYFLSAAPQCPYPDAS   1
            |||||||||||:....||..|||.||..||:.| |.|:|||||||||||||
ScCts1  148 VVDGFDFDIENNNEVGYSALATKLRTLFAEGT-KQYYLSAAPQCPYPDAS   1
                          Y
Cht2    194 LGDLLSKVPLDFAFIQFYNNYCSINGQFNYDTWSKFADS-APNKNIKLFV   2
            :||||....:||||||||||||::||||:|||..:|.: :|||||||:|
ScCts1  197 VGDLLENADIDFAFIQFYNNYCSVSGQFNWDTWLTYAQTVSPNKNIKLFL   2
                                                  W
Cht2    243 GVPATSNIA--GYV-DTSKLSSAIEEIKCDSHFAGVSIWDASGAWLNTDE   2
            |:|.::::.|   ||: |||.|.|.|.:|...|.|.|::|||||||.|:.| :.
ScCts1  247 GLPGSASAAGSGYISDTSLLESTIADIASSSSFGGIAIWDASQAFSN-EL   2

Cht2    290 KGENFVVQVKNVLNQNACVAPSSSATTQSTTTTSSAVTQSTTTTSAAITQ   3
            .||.:|..:||:|..     |..::.||.:|:.||:|.|.|.:|:||:.:|
ScCts1  296 NGEPYVEILKNLLTS----ASQTATTTVATSKTSAASTSSASTSSASTSQ   3

Cht2    340 SATTTSAAVATKSNQIVTSSSSSSSSIFYGNSTTESSTGIATGTVLPTGS   3
            ..||.| ..:|:|...||.|.::||:|  ..|.|:::.:.:.|  .|.|
ScCts1  342 KKTTQS-TTSTQSKSKVTLSPTASSAI--KTSITQTTKTLTSST--KTKS   3

Cht2    390 NENAATTGSGSNTKLAISTVTDVQKTVIT--ITSCSEHKCVATP--VTTG   4
            :....||.|..|: :||:::     ||.::  |||.:    :.||  .||.
ScCts1  387 SLGTTTTESTLNS-VAITSM----KTTLSSQITSAA----LVTPQTTTTS   4

Cht2    436 VVVVTDIDTVYT-TYCPLTNSQVYVSVKTVVCTEETCVPSPTSTSQKPKA   4
            :|....|.|..| |..|.|.|...||::|  .|..|..|:.||||....:
ScCts1  428 IVSSAPIQTAITSTLSPATKSSSVVSLQT--ATTSTLSPTTTSTSSGSTS   4

Cht2    485 S------TTIKGVEKGQTTSYPVVGTTEGVKKIVTTSAQTVGSSTKYVTI   5
            |       :|.:.:.|.....| ..|...| |...|.......:..|:...
ScCts1  476 SGSTSSDSTARTLAKELNAQY-AAGKLNG-KSTCTEGEIACSADGKFAVC   5

Cht2    529 ELTSTITPVTYPTSVASNGTNTTVPVFTFEGGAAVANSLN-----SVWFT   5
            :.:::.:      ....::||.    .:.:::.|.:|....|     |.:|.
ScCts1  524 DHSAWV------YMECASGTT----CYAYDSGDSVYTQCNFSYLESNYF*   5

Cht2    574 VPFLLAAFAF*    584
ScCts1  564 ----------    563
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