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Supplementary Information

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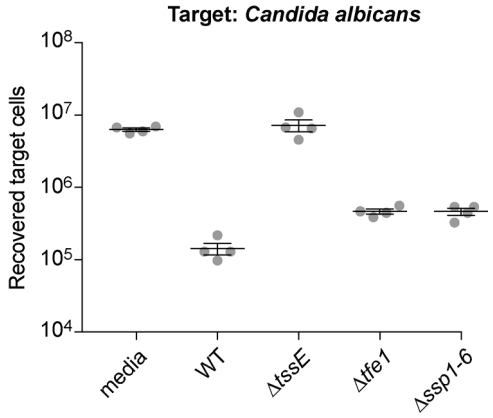
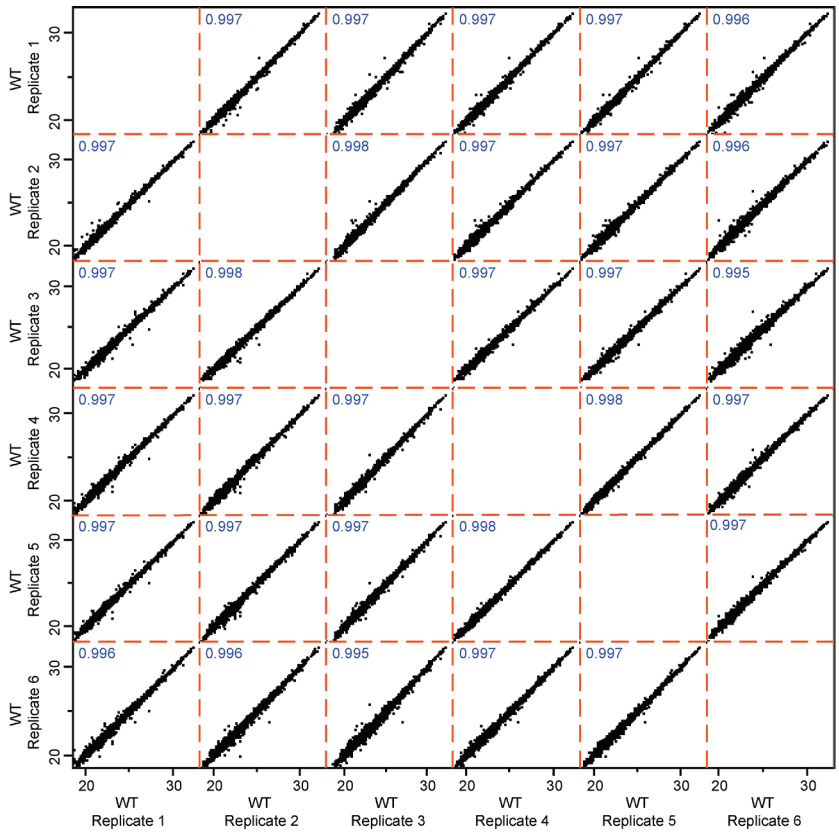


Supplementary Figure 1. The effectors Ssp1, Ssp2, Ssp4, Ssp5 and Ssp6 do not contribute to the anti-fungal activity of the *Serratia marcescens* Db10 Type VI secretion system. Shown is the number of recovered cells of *Candida albicans* SC5314 following co-culture with wild type (WT) or mutant (Δ*tssE*, Δ*tfe1* and Δ*ssp1-ssp6*) strains of *S. marcescens* Db10. Cells of *C. albicans* were also cultured in the presence of sterile media alone (media). Individual data points are overlaid with the mean +/- SEM (n=4 biological replicates).

a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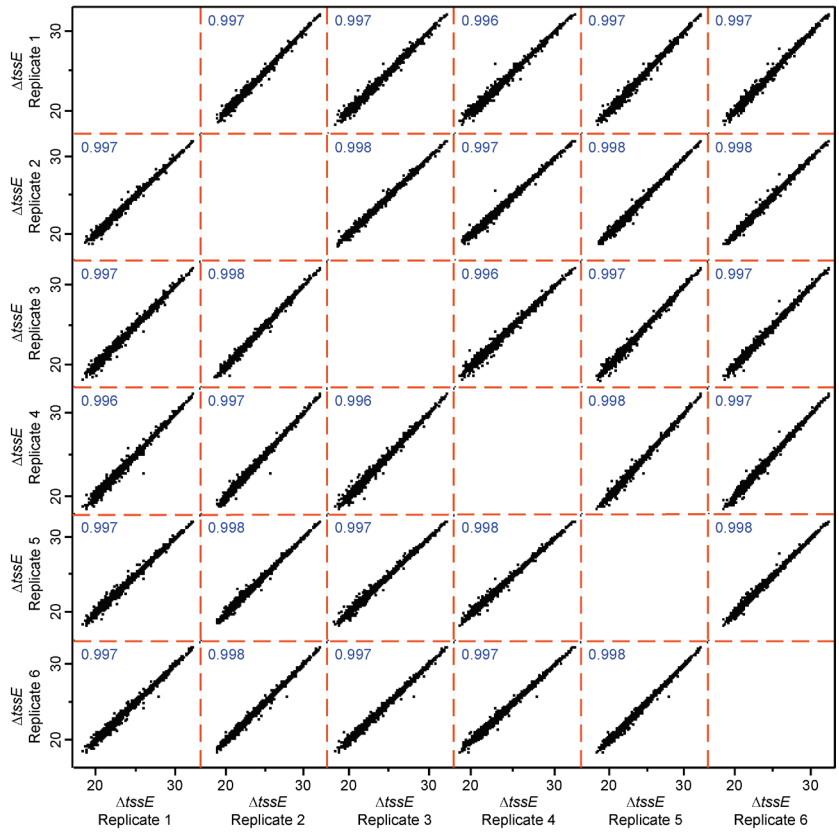
b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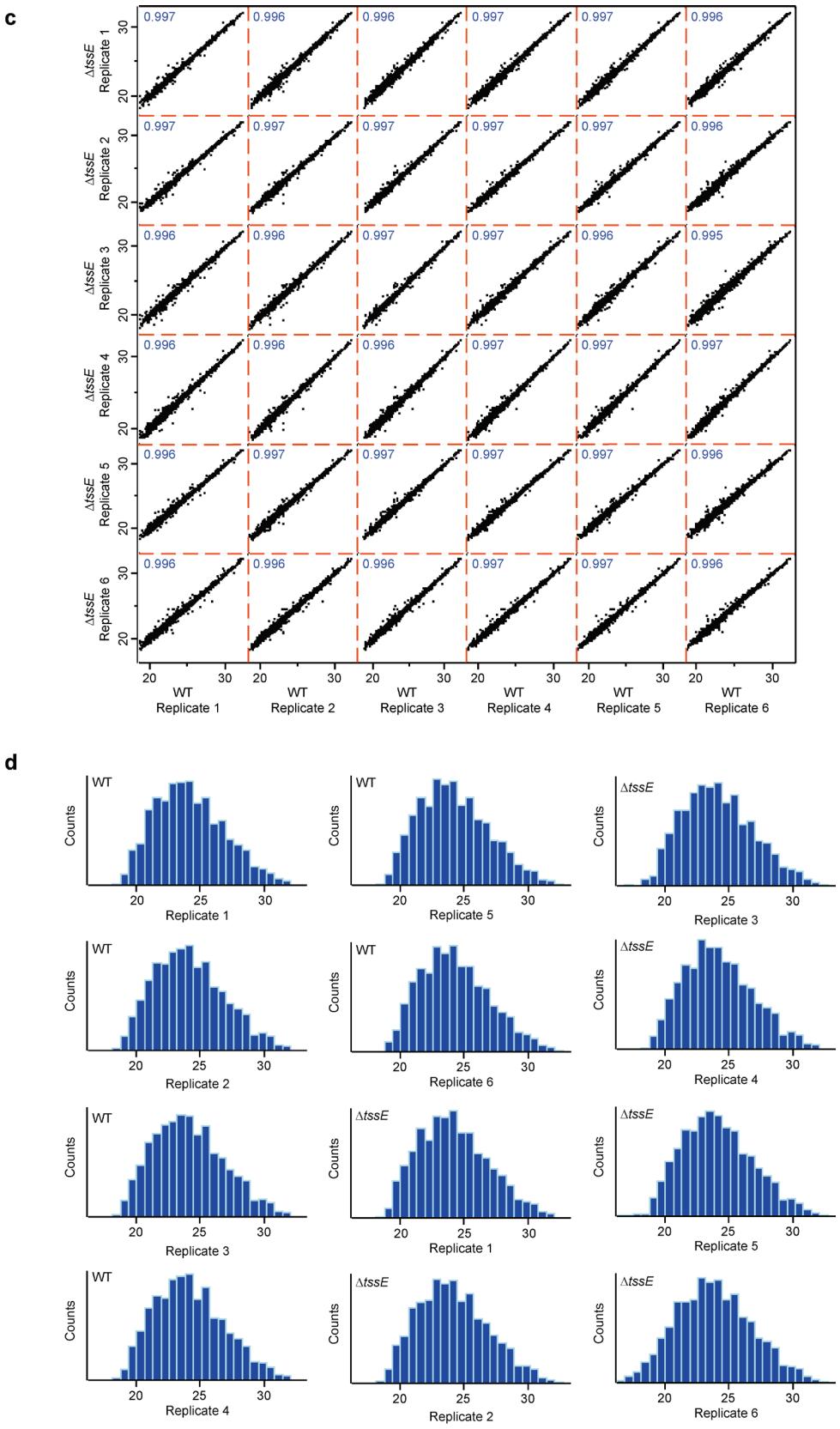
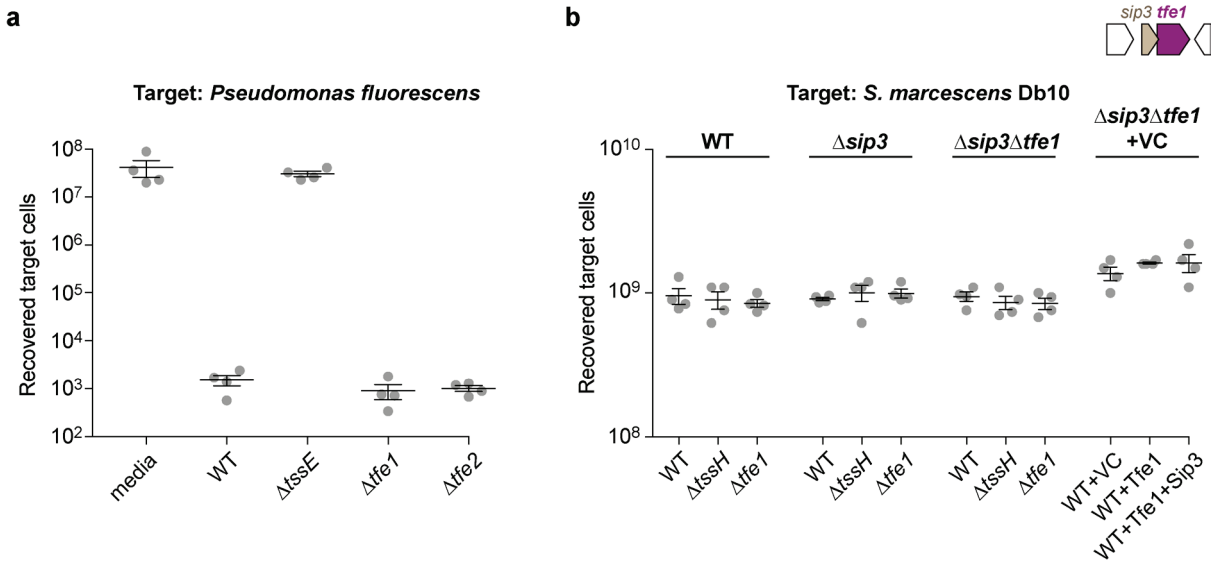
20 30

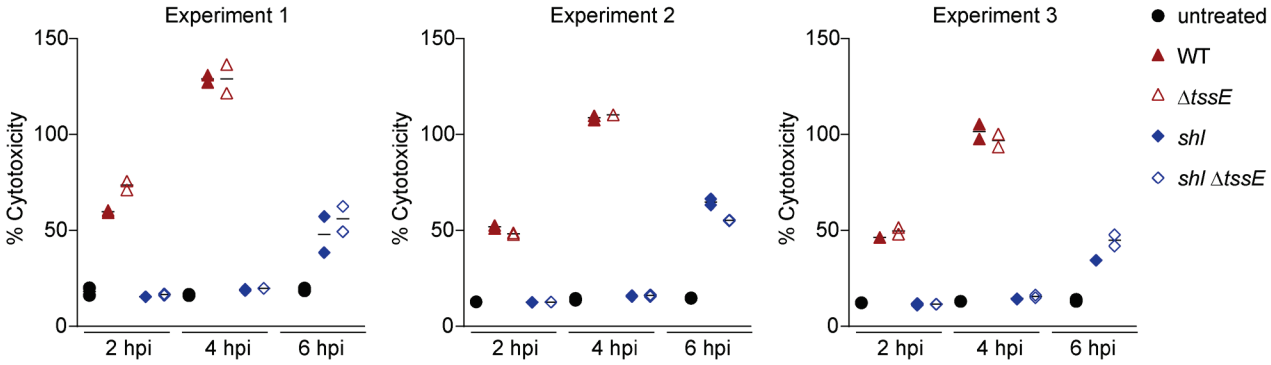


Supplementary Figure 2. Multi-scatter plots and histograms showing the reproducibility of the LFQ proteomics experiments. (a-c) Multi-scatter plots show high reproducibility between six individual biological replicate samples of *S. marcescens* Db10 (WT) (a), between six replicates of Δ*tssE* (b), and between Db10 and Δ*tssE* samples (c). The calculated Pearson correlation is displayed in blue. (d) Intensity distribution histograms of each replicate show log-normal distributions and equal loading of samples.



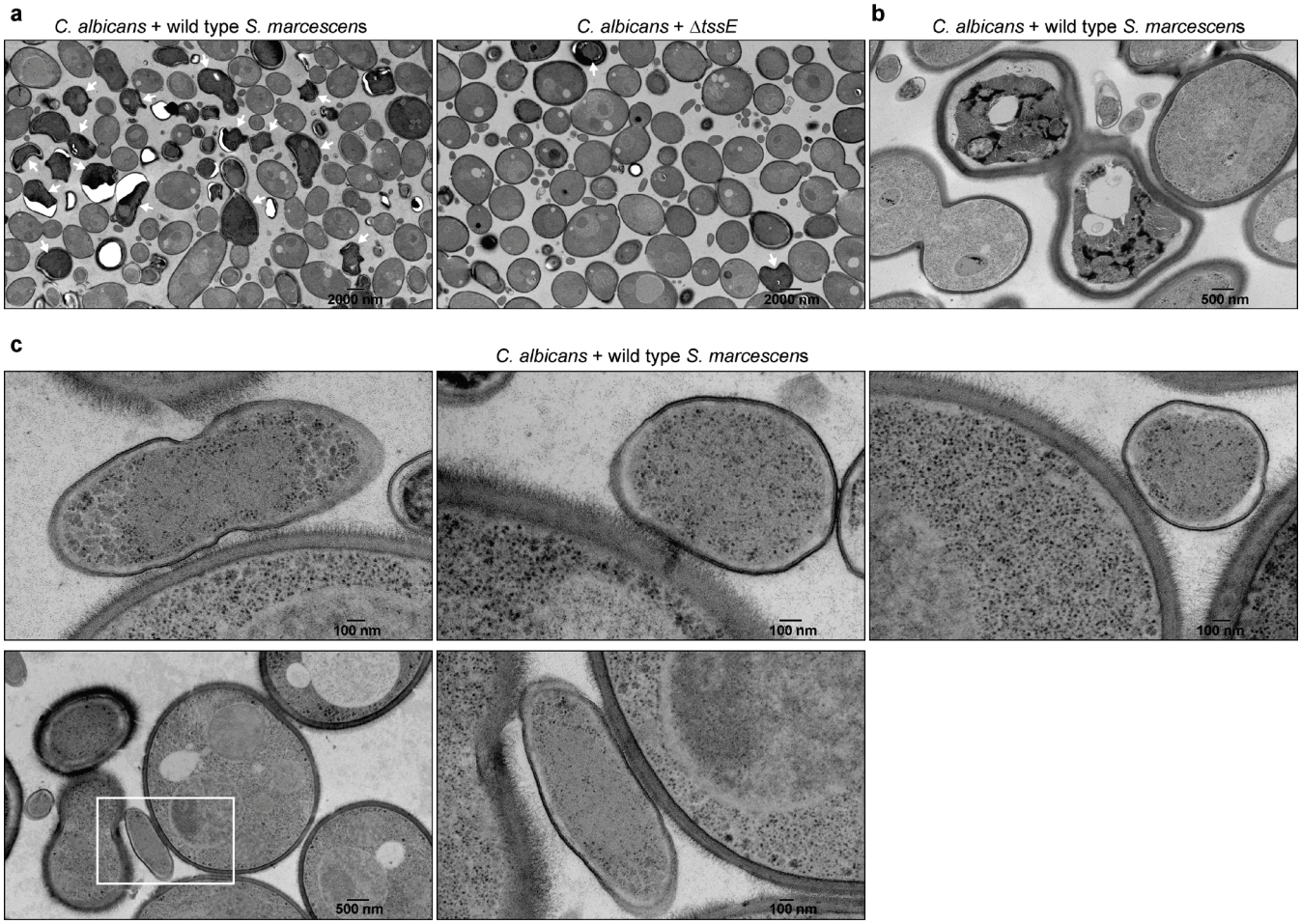
Supplementary Figure 3. The anti-fungal effectors Tfe1 and Tfe2 do not contribute to function or anti-bacterial activity of the Type VI secretion system. (a) Loss of Tfe1 or Tfe2 does not affect T6SS-dependent anti-bacterial activity against *Pseudomonas fluorescens*. Recovery of *P. fluorescens* following co-culture with wild type (WT) or mutant (Δ*tssE*, Δ*tfe1* and Δ*tfe2*) strains of *Serratia marcescens* Db10 as attacker, or following culture with sterile media alone (media). (b) A mutant lacking the cognate 'immunity protein' for Tfe1, Sip3, is not susceptible to wild type *S. marcescens*. Recovery of different target strains of *S. marcescens*, wild type (WT, Db11), Δ*sip3* (KT69), Δ*sip3*Δ*tfe1* (KT98), or Δ*sip3*Δ*tfe1* carrying the vector control plasmid (+VC, pBAD18Kn), following co-culture with attacking strains of *S. marcescens* Db10. These attackers were wild type Db10 (WT), the T6SS-inactive mutant Δ*tssH* and the effector mutant Δ*tfe1*, or the wild type carrying either the vector control plasmid (+VC) or a plasmid directing the expression of Tfe1 (+Tfe1, pSC1231) or of the immunity-effector pair Sip3-Tfe1 (+Tfe1+Sip3, pSC1232). In each case, the target strain additionally carries streptomycin resistance (Supplementary Table 2). Inset, arrangement of *sip3* and *tfe1* (formerly known as *ssp3*) genes. (a,b) data points are overlaid with the mean +/- SEM (n=4 biological replicates).

Supplementary Figure 4

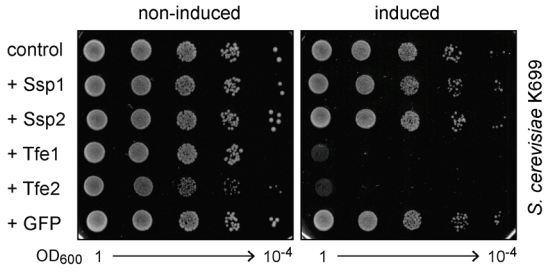


Supplementary Figure 4. The *S. marcescens* Type VI secretion system has no impact on cytotoxicity towards RAW264.7 cells. RAW264.7 macrophage-like cells were infected with strains of *S. marcescens* at a multiplicity of infection of 10:1 and lactate dehydrogenase (LDH) release measured after 2, 4 or 6 hours. Cytotoxicity is expressed as a percentage of the value of extracellular LDH obtained from total lysis of the RAW cells using 0.1% Triton X-100. Strains of *S. marcescens* used were were wild type Db10 (WT), a T6SS mutant (Δ*tssE*), a mutant unable to produce secreted haemolysin (*shl*), and the T6SS mutant in the haemolysin-null background (*shl* Δ*tssE*); as negative control no bacteria were added (untreated). The Shl haemolysin is the primary virulence factor of *Serratia marcescens* and thus could potentially have masked any T6SS-dependent effect. Three independent experiments are shown, with individual values from duplicate culture wells given.

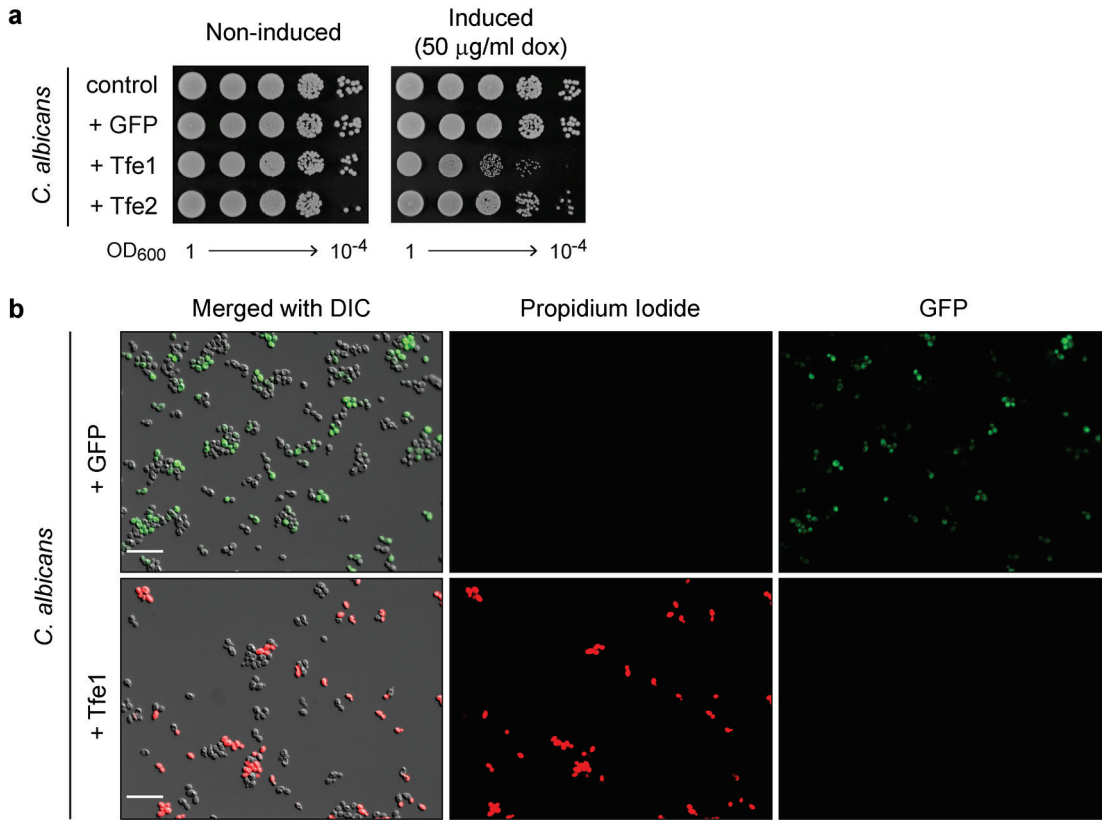
Supplementary Figure 5



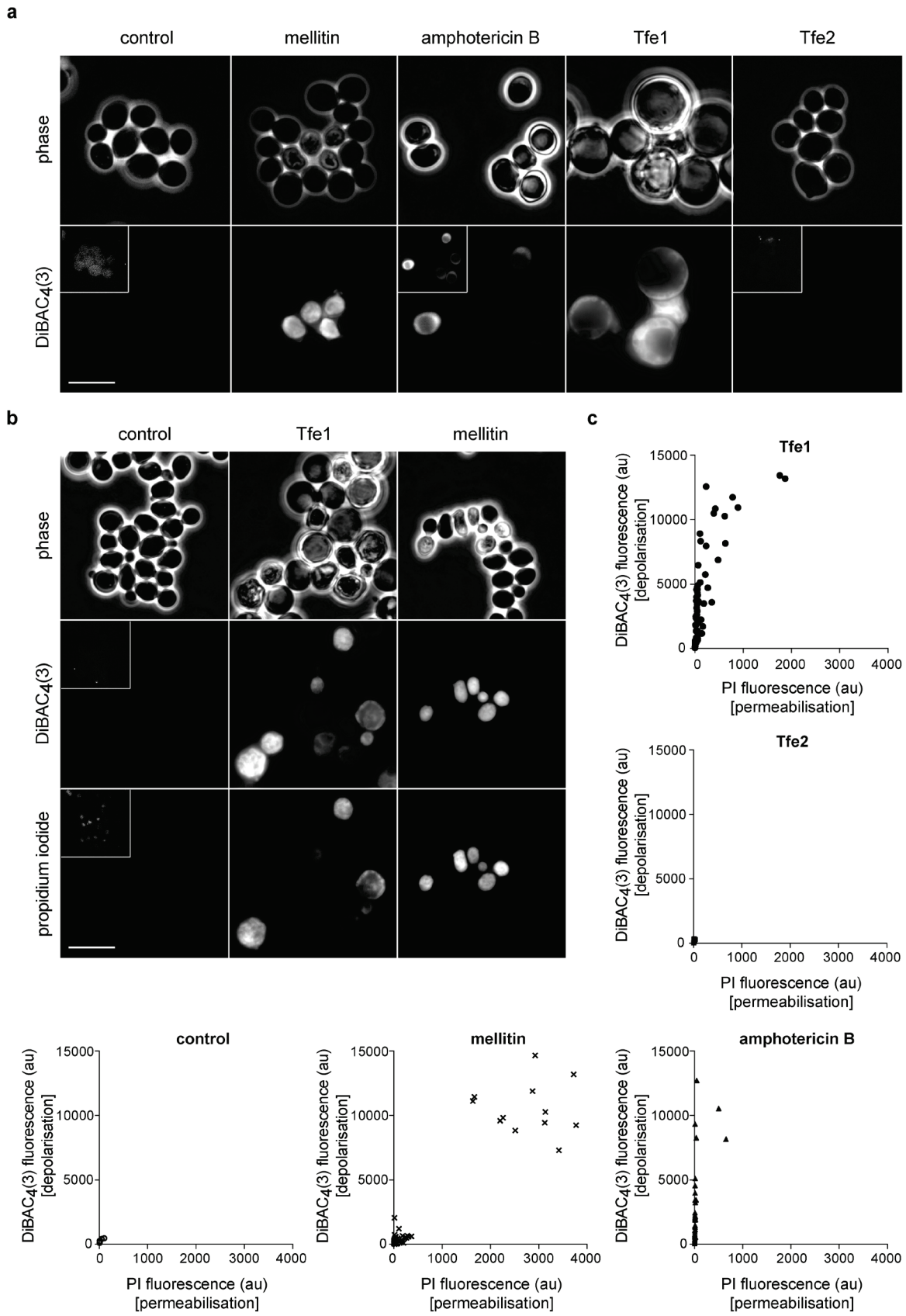
Supplementary Figure 5. Cryo-TEM reveals Type VI secretion system-dependent necrosis and intimate association between cells of *Candida albicans* and *Serratia marcescens*. (a) Cryo-TEM images of multiple cells from co-cultures of *C. albicans* SC5314 with either wild type (left) or Δ*tssE* (right) *S. marcescens* Db10. Examples of necrotic *C. albicans* cells are indicated with white arrows. (b) Higher magnification image of two necrotic *C. albicans* cells. (c) Cryo-TEM images of individual cells from co-cultures of *C. albicans* with *S. marcescens*, showing a very tight interface between the bacterial and fungal cells. The white box shows an area enlarged in the adjacent panel. Images were acquired on one occasion and are representative of three sections and at least 60 *C. albicans* cells observed at high magnification.



Supplementary Figure 6. The anti-bacterial effectors Ssp1 and Ssp2 do not cause toxicity upon artificial expression in yeast. Growth of *S. cerevisiae* K699 carrying the empty vector (pRB1438, control) or plasmids directing expression of Ssp1, Ssp2, Tfe1, Tfe2 or GFP on non-inducing (2% glucose) and inducing (2% raffinose) media. Representative of two independent experiments.



Supplementary Figure 7. Tfe1 displays toxicity when artificially expressed in cells of *C. albicans*. (a) Growth of *C. albicans* SC5314 (control) and derivatives expressing GFP (+GFP, KT158), Tfe1 (+ Tfe1, KT159) or Tfe2 (+ Tfe2, KT160) on non-inducing and inducing media (dox, doxycycline). Representative of three independent experiments. (b) Fluorescence microscopy of *C. albicans* SC5314 expressing Tfe1 or control protein GFP, and subjected to propidium iodide staining following a 3.5 h induction period. Shown are the single and merged GFP and propidium iodide channels, and their overlays on the corresponding DIC images. Representative of two independent experiments. Scale bar, 25 μm.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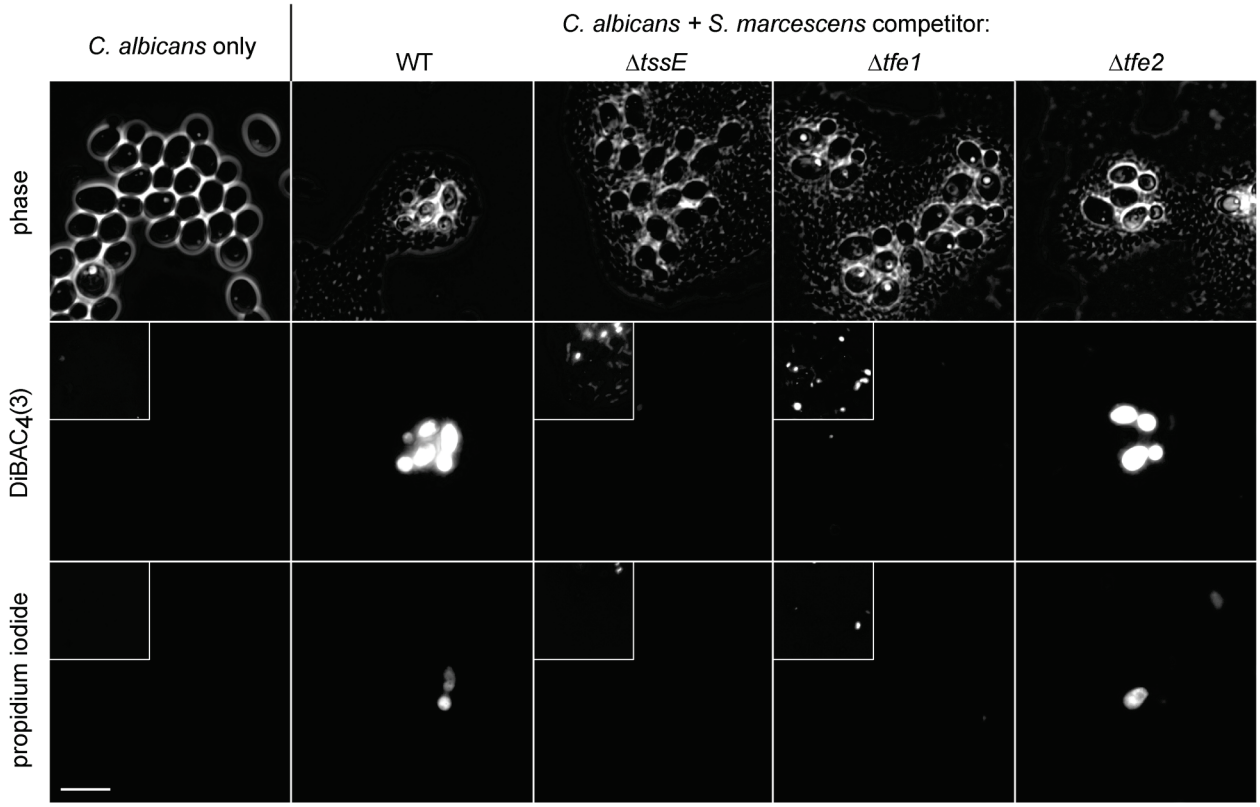
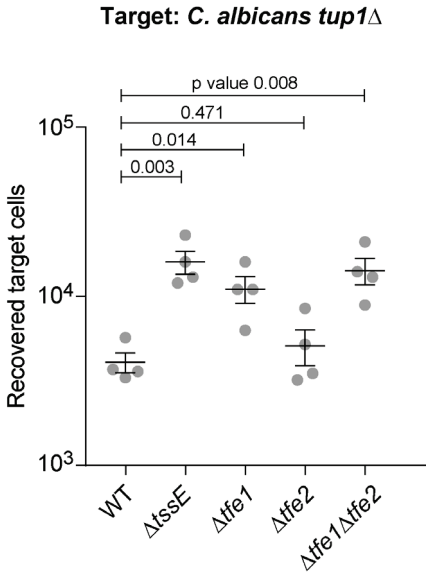
control
mellitin
amphotericin B
Tfe1
Tfe2
phase
DiBAC4(3)
b
control
Tfe1
mellitin
phase
DiBAC4(3)
propidium iodide
c
Tfe1
Tfe2
control
mellitin
amphotericin B
DiBAC4(3) fluorescence (au) [depolarisation]
PI fluorescence (au) [permeabilisation]
0
5000
10000
15000
1000
2000
3000
4000

Supplementary Figure 8. Determination of the impact of Tfe1 and Tfe2 expression on membrane potential and permeability in *Sacchromyces cerevisiae*. (a) Phase contrast (top) and fluorescence microscopy of *S. cerevisiae* K699 cells stained with the potential-sensitive dye $DiBAC_4(3)$, which stains cells in a depolarisation-dependent manner (bottom). The strains used were K699 chromosomal integration strains harbouring the empty promoter construct (control) or derivatives expressing Tfe1 or Tfe2. The control cells were additionally treated with 10 µM mellitin (membrane depolarization through pore formation) or 3 µg/ml amphotericin B (membrane depolarization without pore formation) as positive controls. The large fluorescence images depict the cells with identical contrast settings. In the smaller inserts, the contrast of the fluorescence image is increased to reveal weakly stained cells. Scale bar, 8 µm. (b) As part a, but including co-staining with propidium iodide, which can cross permeabilised membranes, for control, Tfe1-expressing and mellitin-treated cells (bottom panel). Scale bar, 8 µm. (c) Cellular $DiBAC_4(3)$ and propidium iodide fluorescence values were quantified for control cells, those expressing Tfe1 or Tfe2, and those treated with mellitin or amphotericin B. Scatter plots depict fluorescence intensity values of individual cells for both dyes. Data shown are representative of two independent experiments.

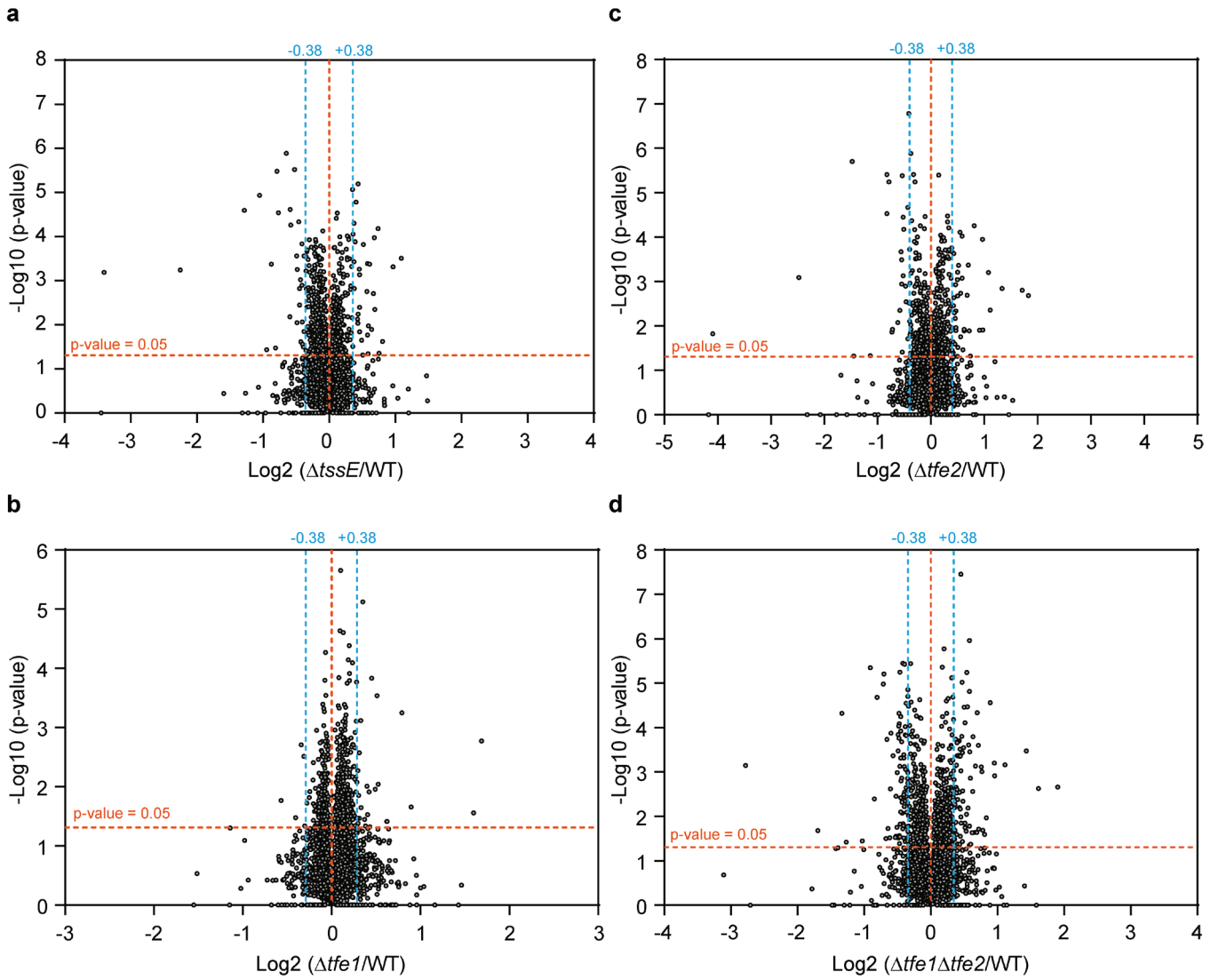
Supplementary Figure 9



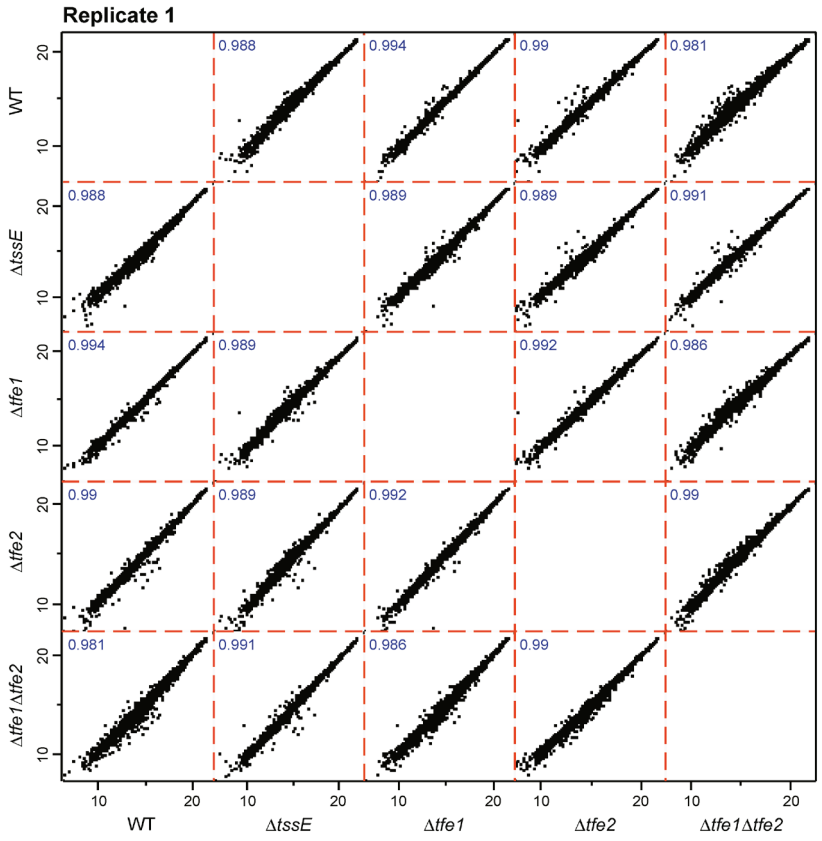
Supplementary Figure 9. Determination of the impact of Tfe1 and Tfe2 delivery on membrane potential and permeability of *C. albicans* when co-cultured with strains of *S. marcescens*. Phase contrast (top panels), and fluorescence microscopy of *C. albicans* SC5314 cells, following co-culture with the indicated strains of *S. marcescens* and co-staining with the potential-sensitive dye $DiBAC_4(3)$ which stains cells in a depolarisation-dependent manner (middle panels), and propidium iodide which can cross permeabilised membranes (bottom panels). The large fluorescence images depict the cells with identical contrast settings. In the smaller inserts, the contrast of the fluorescence image is increased to reveal weakly stained cells. Scale bar, 8 μm. Data shown are representative of two independent experiments.



Supplementary Figure 10. Activity of the *S. marcescens* T6SS against hyphae of *C. albicans* measured using viable cell counting. Recovery of viable cells (colony forming units) of the constitutively filamentous mutant of *C. albicans*, *tup1Δ*, following co-culture with wild type or mutant (Δ*tssE*, Δ*tfe1*, Δ*tfe2* and Δ*tfe1*Δ*tfe2*) strains of *S. marcescens* Db10. Note that, as observed in Figure 4, only a fraction of individual cells within the hyphal filament are intoxicated, explaining why the T6SS-dependent reduction in viable cell counts is smaller than that observed using yeast form cells (Figure 1). Individual data points are overlaid with the mean +/- SEM (n=4 biological replicates) and p values are shown from unpaired, two-sided t-test.



Supplementary Figure 11. Volcano plot of *Candida albicans* proteins from 'in competition' proteomics experiments. Volcano plots summarising the changes in *C. albicans* proteins following co-culture of *C. albicans* with wild type *S. marcescens* Db10 (WT) compared to co-culture with Δ*tssE* (a), Δ*tfe1* (b), Δ*tfe2* (c) or Δ*tfe1*Δ*tfe2* (d) mutants, using TMT-based quantification (n=6 biological replicates). The log2 of the ratios of protein intensities between the wild type and the Δ*tssE,* Δ*tfe1,* Δ*tfe2* or Δ*tfe1*Δ*tfe2* mutants are plotted against the -log10 of t-test p-values. Cut-off values of log2 ratios and -log10 of t-test p-value are represented in blue (± 1.3-fold) and orange (p-value =0.05), respectively.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0.99
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0.992
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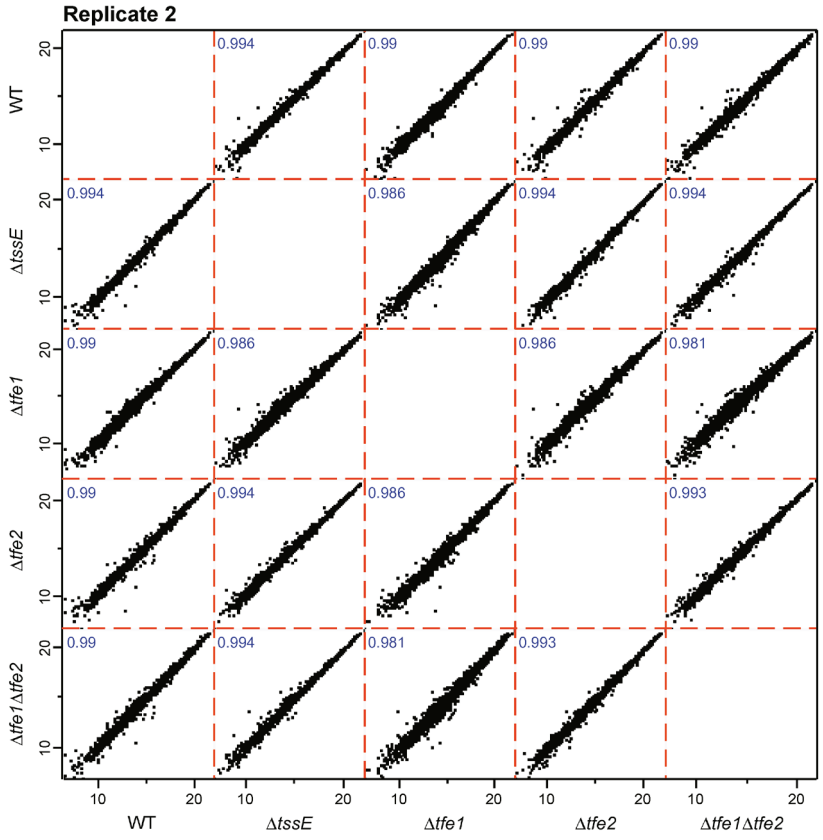
Δtfe1
Δtfe2
Δtfe1Δtfe2
10
20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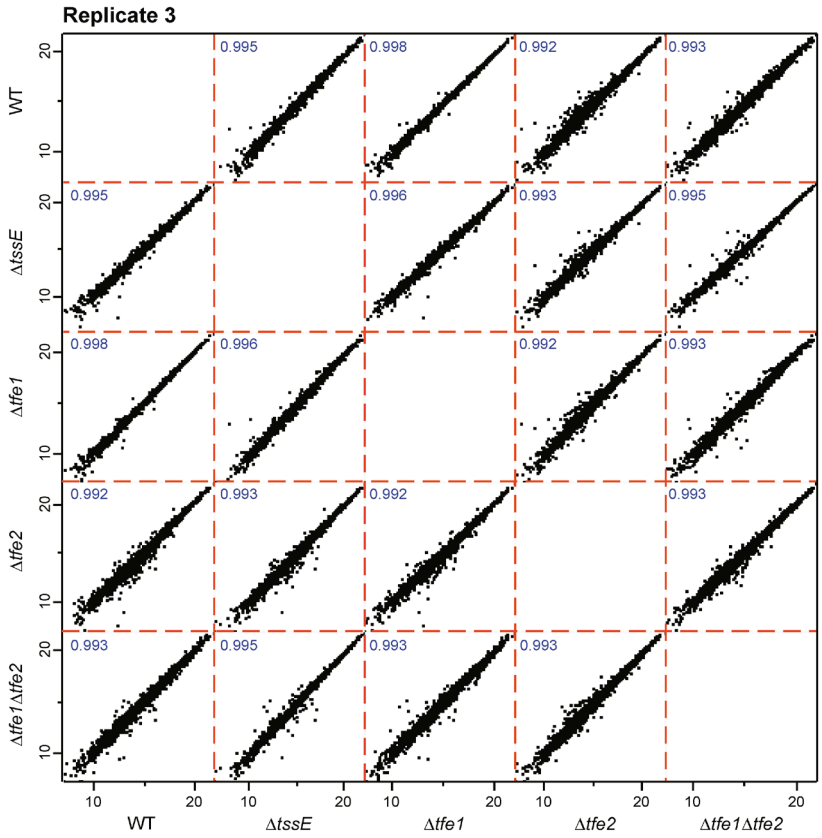
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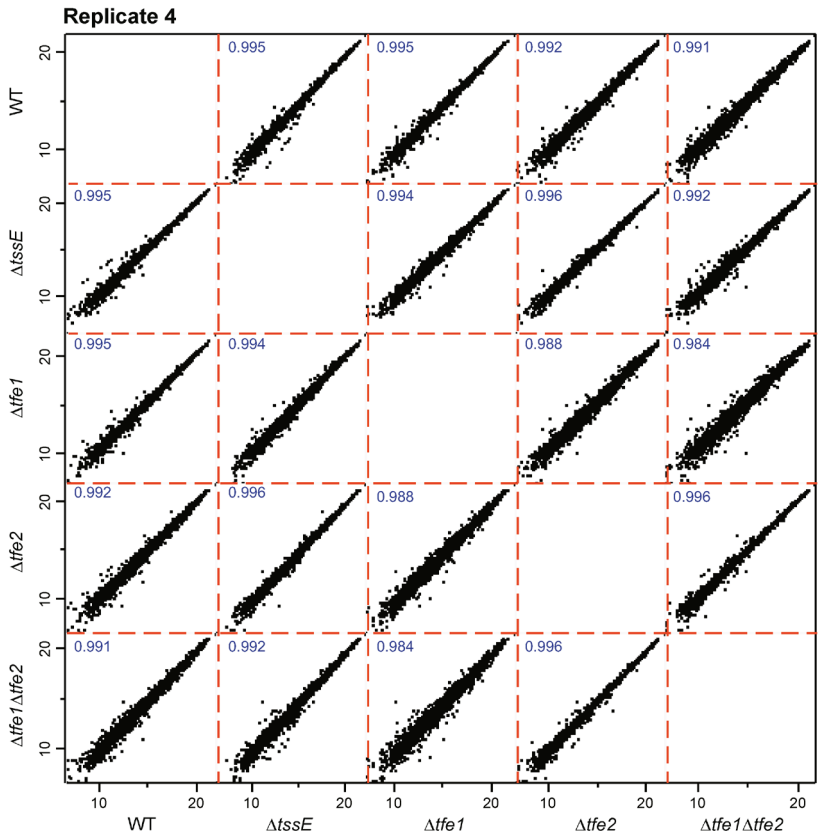
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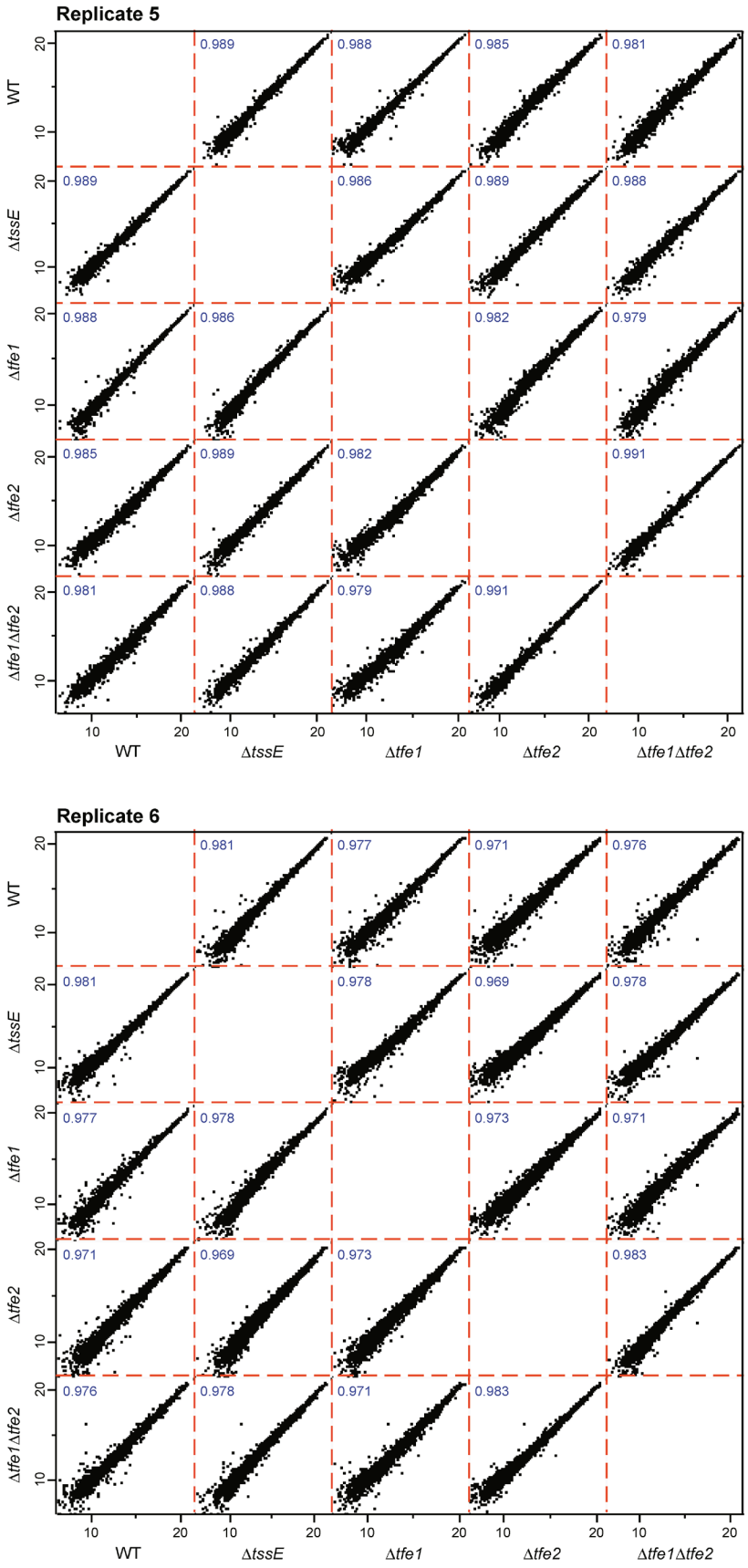
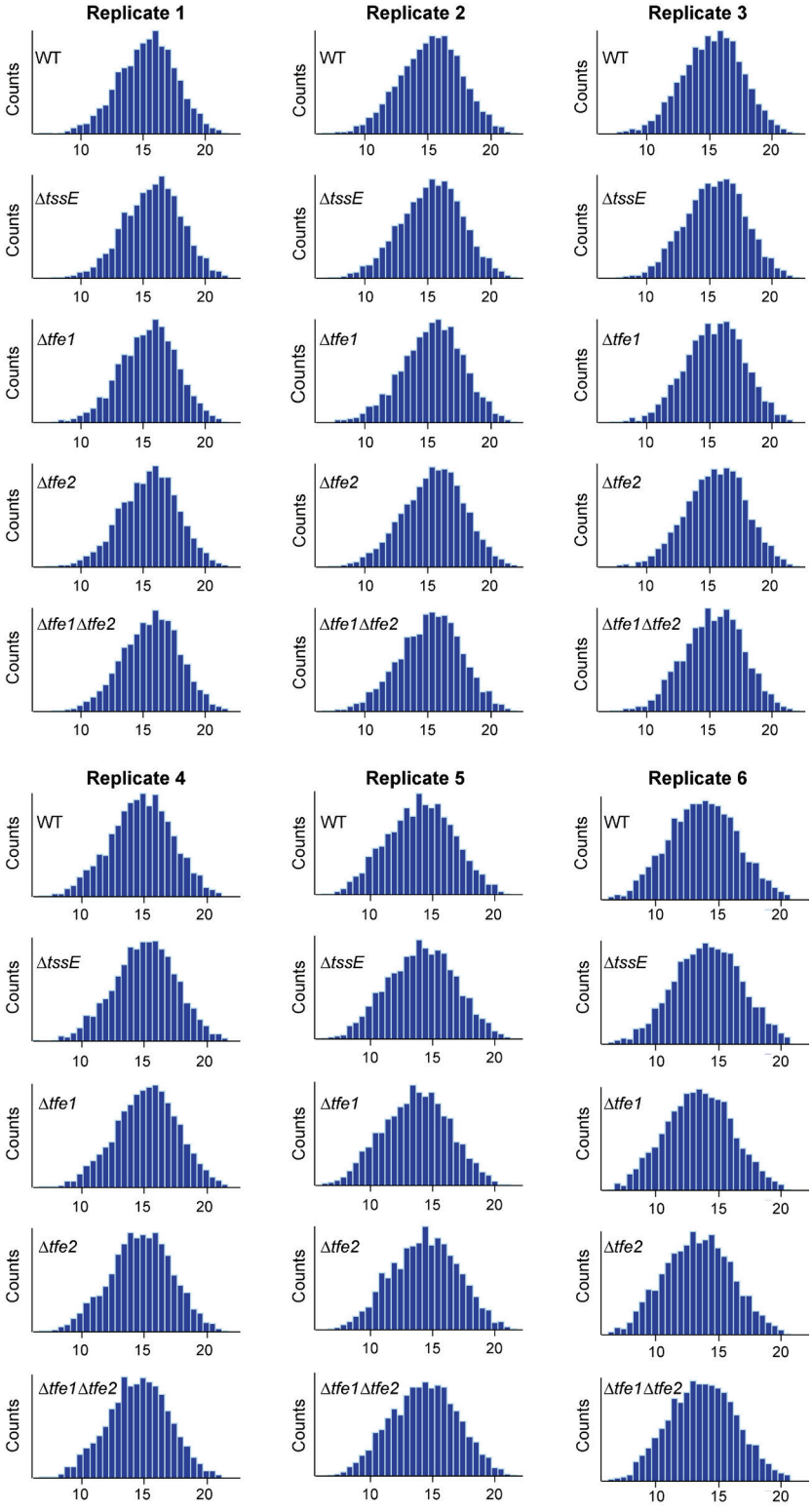
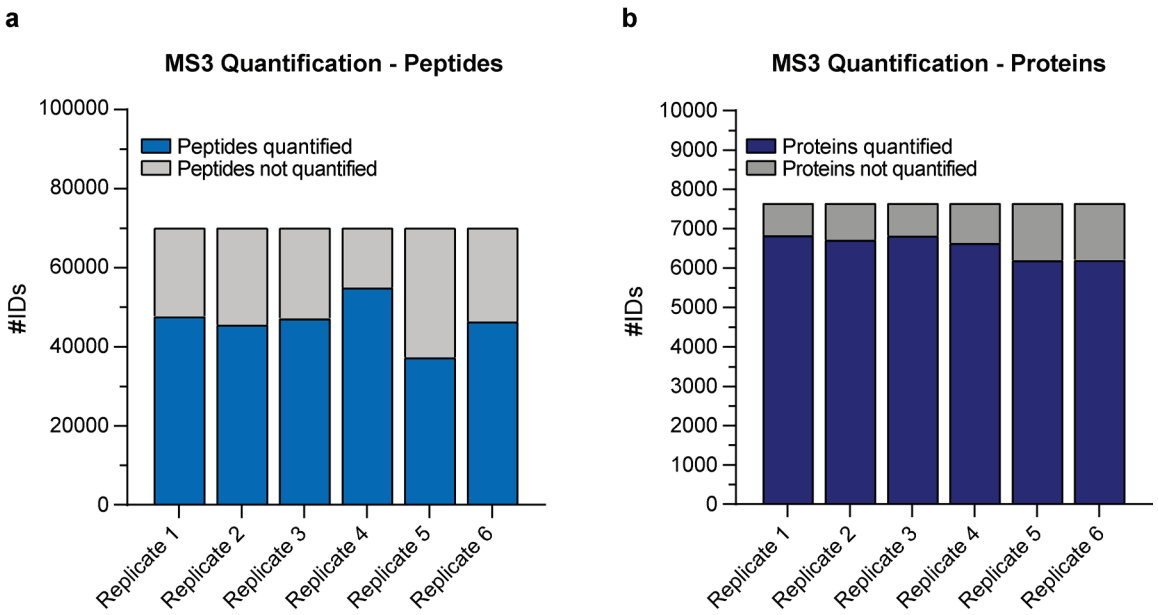


Supplementary Figure 12. Multi-scatter plots from 'in competition' proteomics experiment show high-reproducibility. Multi-scatter plots comparing the log2 intensities from the TMT-based MS3

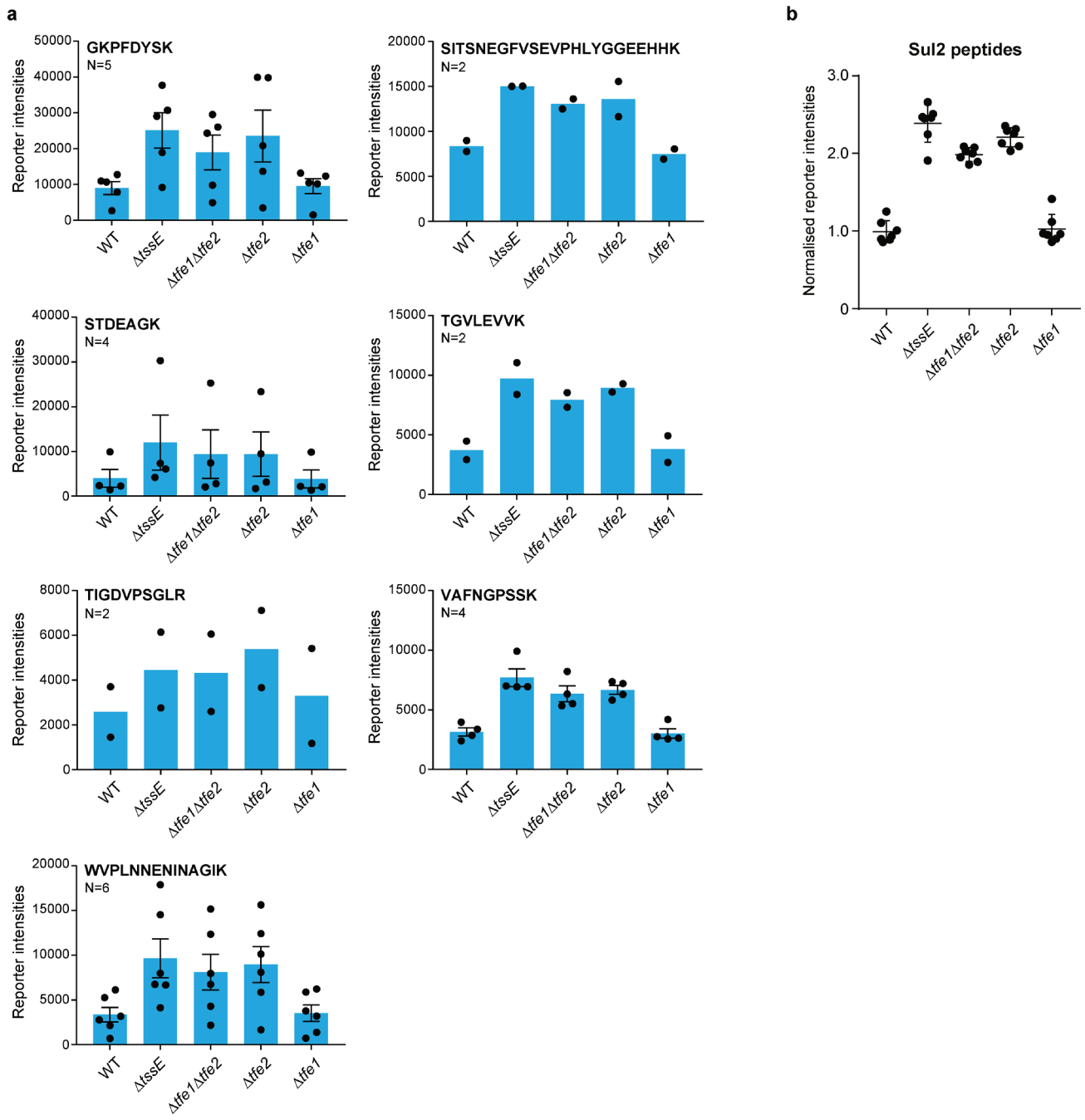
quantification of the *C. albicans* proteome after co-culture with wild type *S. marcescens* Db10 (WT) and with mutants Δ*tssE,* Δ*tfe1,* Δ*tfe2* or Δ*tfe1*Δ*tfe2* for all six biological replicates. The calculated Pearson correlation is displayed in blue, showing an excellent reproducibility between the different types of strains and replicates (Pearson correlation >0.95).



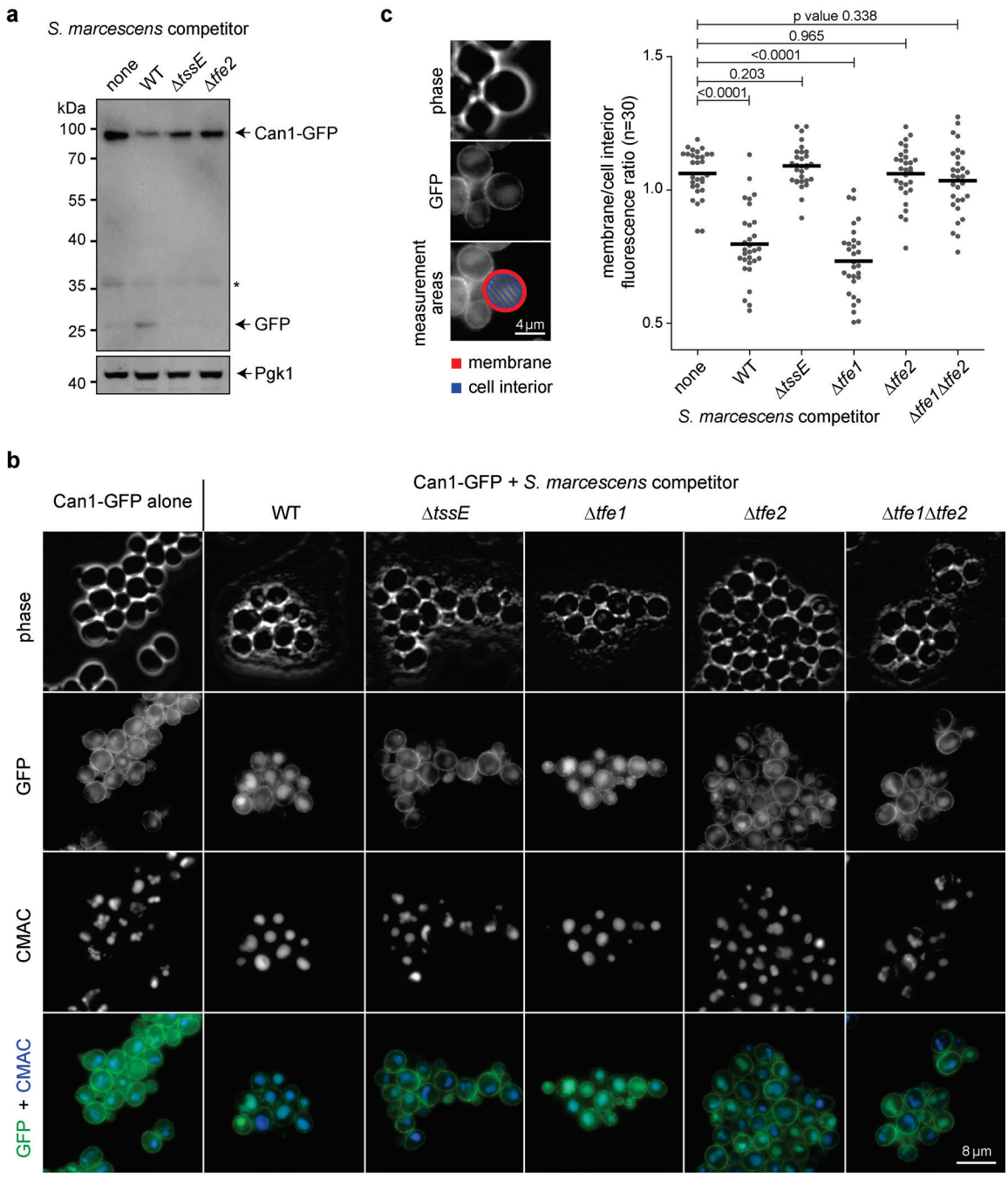
Supplementary Figure 13. Intensity distribution histograms of *Candida albicans* proteins show the reproducibility of the 'in competition' proteomics experiments. Intensity distribution histograms of *C. albicans* proteins following co-culture with wild type *S. marcescens* Db10 (WT), Δ*tssE*, Δ*tfe1*, Δ*tfe2* or Δ*tfe1*Δ*tfe2* in six TMT replicates show log-normal distributions of the non-normalised data and equal loading of samples.



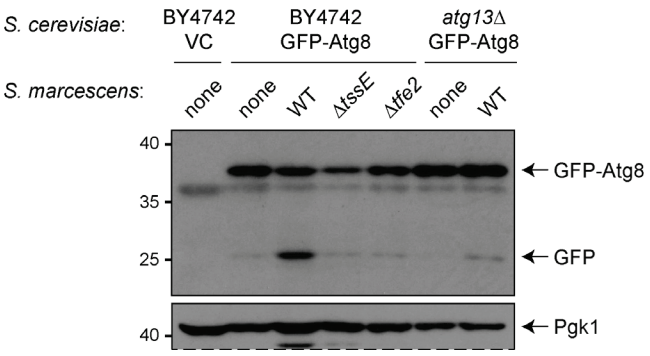
Supplementary Figure 14. Peptides and proteins quantified in each replicate of the 'in competition' proteomics experiments. Number of unique peptides (a) and proteins (b) identified and quantified over the six replicates.



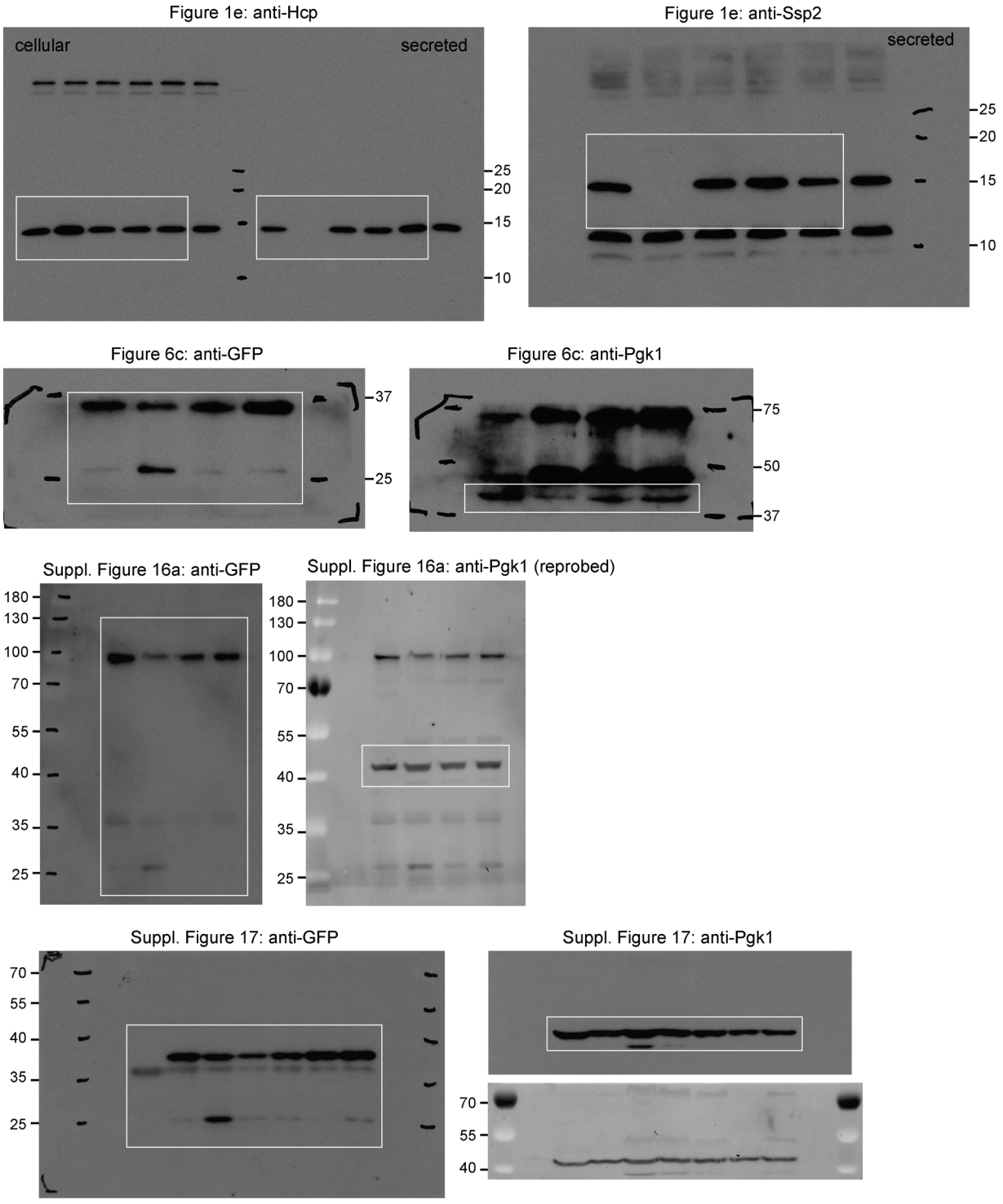
Supplementary Figure 15. Quantification data for Sul2, an example of a *C. albicans* transporter protein downregulated by Tfe2-mediated intoxication. (a) Extracted TMT intensities for unique individual peptides of Sul2 from total cellular protein samples from co-cultures of *C. albicans* with *S. marcescens* Db10 (WT), Δ*tssE,* Δ*tfe1,* Δ*tfe2* or Δ*tfe1*Δ*tfe2*. Blue bars show the mean intensity values, error bars represent ± SEM with individual data points overlaid, and "N" states the number of quantifications of the peptide over the six injected TMT replicates. (b) Normalised extracted TMT intensity ratios from the seven Sul2 unique peptides in (a). Data are shown as mean ± SEM.



Supplementary Figure 16. Intoxication of *S. cerevisiae* by T6SS-delivered Tfe2 results in a decrease in total levels of Can1 and its redistribution from the plasma membrane to the vacuole. (a) Immunoblot detection of Can1-GFP in *S. cerevisiae* BY4741 *CAN1-GFP*, when cultured alone or with wild type (WT) or mutant (Δ*tssE*, Δ*tfe2*) strains of *S. marcescens* Db10. Pgk1 is included as a loading control; *, non-specific band; the full, uncropped blots can be found in Supplementary Fig. 18. Representative of three independent experiments. (b) Fluorescence microscopy of *S. cerevisiae* BY4741 *CAN1-GFP* following culture alone or with strains of *S. marcescens* Db10 as indicated, and staining with the vacuolar dye CMAC. From top to bottom, panels show phase contrast, GFP fluorescence, CMAC fluorescence, and a merge of the GFP and CMAC channels. Scale bar, 8 µm. Representative of two independent experiments. (c) Quantification of the relative distribution of Can1-GFP fluorescence signal between an exterior (plasma membrane) location and an interior location (including the vacuole) for the co-cultures shown in part b. Left, schematic of manually defined cell areas (scale bar, 4 µm); right, quantitation for 30 randomly chosen yeast cells in each condition. Points represent individual cells and bars show mean (p values are indicated; unpaired, two-sided t-test).



Supplementary Figure 17. Processing of GFP-Atg8 in response to Tfe2 is dependent on Atg13. Immunoblot detection of GFP-Atg8 processing in *S. cerevisiae* BY4742 or its mutant derivative *atg13*Δ, following co-culture with wild type (WT) or mutant (Δ*tssE* and Δ*tfe2*) strains of *S. marcescens* Db10. Yeast cells carry either the reporter plasmid GFP-ATG8(416) directing expression of GFP-Atg8 from the endogenous Atg8 promoter, or the vector control (pRS316, VC). GFP-Atg8 has a MW of 41 kDa and cleaved GFP is 27 kDa. Pgk1 was used as control cellular protein and a dashed line indicates where the blot membrane was cut prior to detection with Pgk1 antibody; the full, uncropped blots can be found in Supplementary Fig. 18. Representative of two independent experiments.



Supplementary Figure 18. Uncropped western blot images. MW markers are indicated (kDa).

Supplementary Table 1. *C. albicans* proteins with altered abundance in response to T6SS-mediated delivery of Tfe2

Protein ID	Relative abundance (log2 WT/mutant)				Protein name		Function
	Δ*tfe1*	Δ*tfe2*	Δ*tfe1*Δ*tfe2*	Δ*tssE*			
orf19.4654	**-0.79**	**4.09**	**3.11**	**3.40**	-		Protein of unknown function
orf19.1358	-0.14	**2.47**	**2.78**	**2.25**	Gcn4		bZIP transcription factor, amino acid control response
orf19.6222.1	**-0.51**	**1.48**	**1.33**	**1.28**	-		Protein of unknown function
orf19.5620	-0.35	**0.83**	**0.71**	**0.64**	Phz1	(Yhi9)	Protein of unknown function
orf19.1340	**-0.45**	**0.82**	**0.81**	**0.79**	-	(Ydl124w)	Putative aldose reductase
orf19.6689	-0.20	**0.78**	**0.91**	**0.77**	Arg4		Argininosuccinate lyase
orf19.3841	0.37	**0.72**	**0.61**	**0.67**	Atg1		Putative protein Ser/Thr kinase, predicted role in autophagy and Cvt-pathway
orf19.6514	-0.21	**0.62**	**0.67**	**0.59**	Cup9	(Tos8)	Transcription factor
orf19.3142	-0.23	**0.56**	**0.44**	**0.43**	-		Protein of unknown function
orf19.6245	-0.24	**0.55**	**0.66**	**0.58**	-		Protein of unknown function
orf19.5720	-0.05	**0.55**	**0.60**	**0.59**	-	(Mch4)	Predicted membrane transporter, member of the monocarboxylate porter family
orf19.6994	-0.23	**0.54**	**0.42**	**0.46**	Bat22	(Bat2)	Putative branched chain amino acid aminotransferase
orf19.7469	-0.11	**0.52**	**0.70**	**0.52**	Arg1		Argininosuccinate synthase
orf19.3770	-0.33	**0.51**	**0.57**	**0.49**	Arg8		Putative acetylornithine aminotransferase
orf19.5645	-0.07	**-0.39**	**-0.50**	**-0.39**	Met15	(Met17)	O-acetylhomoserine O-acetylserine sulfhydrylase
orf19.4099	0.00	**-0.43**	**-0.63**	**-0.45**	Ecm17	(Met5)	Putative beta subunit of assimilatory sulphite reductase
orf19.2451	0.15	**-0.46**	**-0.89**	**-0.58**	Pga45	(-)	Putative GPI-anchored cell wall protein
orf19.6939	0.01	**-0.47**	**-0.48**	**-0.46**	-		Protein of unknown function
orf19.6937	0.25	**-0.50**	**-0.57**	**-0.52**	Ptr22	(Ptr2)	Oligopeptide transporter
orf19.4076	0.00	**-0.50**	**-0.70**	**-0.46**	Met10		Putative alpha subunit of assimilatory sulphite reductase
orf19.2661	-0.20	**-0.50**	**-0.71**	**-0.68**	Tlo34	(-)	Protein of unknown function
orf19.4077	-0.18	**-0.56**	**-0.53**	**-0.58**	Mit1	(Sur1)	Mannosylinositol phosphorylceramide synthase catalytic subunit
orf19.5642	-0.30	**-0.63**	**-0.58**	**-0.81**	-	(Dif1)	Putative protein regulating nuclear localization of Rnr2 and Rnr4
orf19.5811	0.00	**-0.68**	**-0.77**	**-0.61**	Met1		Putative urophorphyrin-3 C-methyltransferase
orf19.3642	0.33	**-0.70**	**-0.45**	**-0.43**	Sun41	(Sun4)	Cell wall glycosidase
orf19.6600	-0.10	**-0.72**	**-1.11**	**-0.75**	-	(Ups1)	Putative phosphatidic acid transporter activity
orf19.2738	0.04	**-1.08**	**-0.95**	**-1.09**	Sul2	(Sul1)	Sulfate transporter
orf19.668	-0.20	**-1.11**	**-0.96**	**-0.74**	Tos4		Putative fork-head transcription factor
orf19.97	-0.11	**-1.34**	**-1.43**	**-0.97**	Can1		Basic amino acid permease
orf19.6993	0.08	**-1.83**	**-1.62**	**-0.64**	Gap2	(Gap1)	General broad specificity amino acid permease

Proteins are included on the basis of their abundance in the total cellular proteome of *C. albicans* being significantly altered upon co-culture with wild type *S. marcescens* Db10 (WT) compared with the Δ*tfe2* mutant (fold change >1.3, significant t-test for the pairwise comparison (two-sided, $p<0.05$) and significant ANOVA score ($p<0.05$, multiple sample test) across all conditions), and also showing an equivalent change (>1.3-fold) when comparing the wild type with the Δ*tfe1*Δ*tfe2* double mutant and the T6SS-inactive mutant Δ*tssE* (n=6). The table summarises the data from six independent biological replicates, analysed using six-plex TMT mass spectrometry, and changes in abundance are presented as log2 of WT/mutant. Protein names are according to the *Candida* Genome Database (CGD), with the *S. cerevisiae* homologue, where different, given in brackets. Fold change values greater than 1.3 are shown in purple and orange (increase or decrease, respectively) and entries are ordered according to changes in Δ*tfe2*. It should be noted that TMT experiments suffer from ratio compression[1] and the ratios are in reality probably higher than reported.

Supplementary Table 2. Additional Gene Ontology (GO) analysis of Tfe2-dependent *Candida albicans* proteins.

GOID	GO_term	Count	P-value	Gene(s) annotated to the term
Cellular Component				
5886	Plasma membrane	17	0.0047	C2_00940W_A, ZRT2, ITR1, RTA2, PTR22, GAP2, MET15, SUL2, RHD3, TNA1, CAG1, CTR1, CAN1, CAN2, C7_03350C_A, TPO4, SLP3
9337	Sulfite reductase complex (NADPH)	2	0.0120	ECM17, MET10
324	Fungal-type vacuole	12	0.0174	NCR1, ITR1, ECM17, RTA2, MIT1, PTR22, GAP2, ATG1, TNA1, VTC3, TPO4, SLP3
Molecular Function				
5215	Transporter activity	15	0.0031	NCR1, ZRT2, ITR1, RTA2, PTR22, GAP2, SUL2, TNA1, GNP1, CTR1, CAN1, CAN2, C6_03540W_A, TPO4, CR_09620C_A
→ 46943	Carboxylic acid transmembrane transporter activity	6	0.0060	GAP2, TNA1, GNP1, CAN1, CAN2, C6_03540W_A
→ 15181	Arginine transmembrane transporter activity	3	0.0031	GAP2, CAN1, CAN2
→ 15193	L-proline transmembrane transporter activity	2	0.0137	GAP2, GNP1
4783	Sulfite reductase (NADPH) activity	2	0.0137	ECM17, MET10
Biological Process				
44281	Small molecule metabolic process	21	0.0029	SNZ1, C1_02970W_A, DUR1,2, MET1, RNR21, LYS22, ECM17, BAT22, MET15, SPS20, ARG8, LYS1, C5_00800C_A, HAM1, CAR1, CAG1, ILS1, C7_03350C_A, ARG4, ARG1, VTC3
→ 6082	Organic acid metabolic process	17	0.0003	SNZ1, C1_02970W_A, MET1, LYS22, ECM17, BAT22, MET15, SPS20, ARG8, LYS1, C5_00800C_A, CAR1, CAG1, ILS1, ARG4, ARG1, VTC3
→ 6520	Cellular amino acid metabolic process	14	0.0000	SNZ1, C1_02970W_A, MET1, LYS22, ECM17, BAT22, MET15, ARG8, LYS1, C5_00800C_A, CAR1, ILS1, ARG4, ARG1
→ 6525	Arginine metabolic process	4	0.0020	ARG8, CAR1, ARG4, ARG1
→ 6591	Ornithine metabolic process	3	0.0192	ARG8, CAR1, ARG4
0097	Sulfur amino acid biosynthetic process	5	0.0057	SNZ1, C1_02970W_A, MET1, ECM17, MET15

GO-Term analysis of the 76 proteins found to be differentially abundant (± 1.3-fold) in the total proteome of *C. albicans* when co-cultured with wild type *S. marcescens* compared with the Δ*tfe2* mutant. Proteins were included based on a single pairwise comparison, WT/Δ*tfe2* (p-value < 0.05, unpaired two-sided t-test; n=6 biological replicates). Shown are enrichments in the three ontologies 'cellular component', 'molecular function' and 'biological process' (p-value ≤ 0.02), generated using the GO Term Finder tool at the Candida gene database (http://www.candidagenome.org/). Arrows represent parent-child relationships and redundancies were curated manually. This analysis includes an extended set of Tfe2-dependent proteins compared with that depicted in Fig. 5b but identifies very similar areas of metabolism and cellular transport.

Supplementary Table 3. Strains and Plasmids used in this study.

Name	Description/ genotype	Source / Reference
Strains		
Serratia marcescens		
Db10	Wild type	[2]
Db11	Spontaneous Sm-resistant derivative of Db10	[2]
SJC11	Db10 Δ*tssE* (Δ*SMDB11_2271*)	[3]
SJC3	Db10 Δ*tssH* (Δ*SMDB11_2274*)	[3]
KT65	Db10 Δ*tfe1* (Δ*SMDB11_1112*)	[4]
KT148	Db10 Δ*tfe2* (Δ*SMDB11_1083*)	This study
KT149	Db10 Δ*tfe1*Δ*tfe2* (Δ*SMDB11_1112,* Δ*SMDB11_1083*)	This study
KT64	Db10 Δ*sip3* (Δ*SMDB11_1111*)	This study
KT69	Db10 Δ*sip3*, Sm-resistant, derived from KT64[a]	This study
KT66	Db10 Δ*sip3*Δ*tfe1* (Δ*SMDB11_1111-1112*)	This study
KT98	Db10 Δ*sip3*Δ*tfe1*, Sm-resistant, derived from KT66[a]	This study
MLM01	Db10 Δ*ssp1-ssp6* (Δ*SMDB11_2261,* Δ*SMDB11_2264,* Δ*SMDB11_1112,* Δ*SMDB11_3980,* Δ*SMDB11_4628,* Δ*SMDB11_4673*)	This study
21C4	Db11 *shlB*::miniTn*5*-Cm	[5]
YL23	Db10 *shlB*::miniTn*5*-Cm (*shl*)	This study
YL24	Db10 Δ*tssE shlB*::miniTn*5*-Cm (*shl* Δ*tssE*)	This study
Pseudomonas fluorescens		
KT02	Sm-resistant derivative of *P. fluorescens* 55	[3]
Escherichia coli		
CC118λ*pir*	Cloning host and donor for pKNG101-based allelic exchange plasmids (λ*pir*)	[6]
HH26 pNJ5000	Mobilizing strain for conjugal transfer	[7]
Saccharomyces cerevisiae		
K699	*MAT***a** *ade2-1 trp1-1 leu2-3,112 his3-11,15 ura3 can1-100*	[8]
BH01	K699 *his3*::P_{GAL1}[b]	This study
KT164	K699 *his3*::P_{GAL1}-*GFP*[b]	This study
KT165	K699 *his3*::P_{GAL1}-*SMDB11_1083*[b]	This study
KT167	K699 *his3*::P_{GAL1}-*SMDB11_1112*[b]	This study
BY4742	*MAT*α *his3*Δ*1 leu2*Δ*0 lys2*Δ*0 ura3*Δ*0*	[9]
*atg13*Δ	BY4742 *atg13*Δ::kanMX6	SGDC
BY4741	*MAT***a** *his3*Δ*1 leu2*Δ*0 met15*Δ*0 ura3*Δ*0*	[9]
Can1-GFP	BY4741 *CAN1-GFP::his5*$^{+}$	[10]
Candida albicans		
SC5314	Wild type clinical isolate	[11]
KT158	SC5314 *ADH1*/*adh1*::P_{tet}-*caGFP*[c]	This study
KT159	SC5314 *ADH1*/*adh1*::P_{tet}-*caSMDB11_1112*[c]	This study
KT160	SC5314 *ADH1*/*adh1*::P_{tet}-*caSMDB11_1083*[c]	This study
*tup1*Δ	*arg4*Δ/*arg4*Δ *leu2*Δ/*leu2*Δ *his1*Δ/*his1*Δ *URA3*/*ura3*Δ::*imm*^{434} *IRO1*/*iro1*Δ::*imm*^{434} *tup1*Δ::*HIS1*/*tup1*Δ::*LEU2*	[12]
Candida glabrata		
ATCC2001	Wild type	ATCC

Plasmids

pKNG101	Suicide vector for allelic exchange (Sm^R *sacBR mobRK2 oriR6K*)	[13]
pSC1249	pKNG101-derived allelic exchange plasmid for the generation of chromosomal in-frame Δ*SMDB11_1112* (Δ*tfe1*) deletion	[4]
pSC1339	pKNG101-derived allelic exchange plasmid for the generation of chromosomal in-frame Δ*SMDB11_1083* (Δ*tfe2*) deletion	This study
pSC1248	pKNG101-derived allelic exchange plasmid for the generation of chromosomal in-frame Δ*SMDB11_1111* (Δ*sip3*) deletion	This study
pSC1250	pKNG101-derived allelic exchange plasmid for the generation of chromosomal in-frame Δ*SMDB11_1111-1112* (Δ*sip3*Δ*tfe1*) deletion	This study
pSC123	pKNG101-derived allelic exchange plasmid for the generation of chromosomal in-frame Δ*SMDB11_2261* (Δ*ssp1*) deletion	[14]
pSC125	pKNG101-derived allelic exchange plasmid for the generation of chromosomal in-frame Δ*SMDB11_2264* (Δ*ssp2*) deletion	[14]
pSC828	pKNG101-derived allelic exchange plasmid for the generation of chromosomal in-frame Δ*SMDB11_3980* (Δ*ssp4*) deletion	[4]
pSC829	pKNG101-derived allelic exchange plasmid for the generation of chromosomal in-frame Δ*SMDB11_4628* (Δ*ssp5*) deletion	[4]
pSC1241	pKNG101-derived allelic exchange plasmid for the generation of chromosomal in-frame Δ*SMDB11_4673* (Δ*ssp6*) deletion	[4]
pSUPROM	Vector for constitutive expression of cloned genes under the control of the *E. coli tat* promoter (Kn^R)	[4]
pSC1332	pSUPROM-derived plasmid for constitutive expression of *SMDB11_1111-1112* (*sip3-tfe1*)	This study
pSC1343	pSUPROM-derived plasmid for constitutive expression of *SMDB11_1083* (*tfe2*)	This study
pBAD18-Kn	Vector for arabinose-inducible expression of cloned genes under the control of the P_{ara} promoter	[15]
pSC1231	pBAD18-Kn-derived plasmid for arabinose-inducible expression of *SMDB11_1112* (*tfe1*)	[4]
pSC1232	pBAD18-Kn-derived plasmid for arabinose-inducible expression of *SMDB11_1111-1112* (*sip3-tfe1*)	[4]
pNIM1	Tetracycline-inducible expression vector for integration into *C. albicans* (*ADH1* locus) carrying the *caGFP* gene (Nat^R, Amp^R)	[16]
pSC1352	pNIM-derived plasmid for Tetracycline-inducible expression of ca*SMDB11_1112*^{d} (*caTFE1*)	This study
pSC1354	pNIM-derived plasmid for Tetracycline-inducible expression of ca*SMDB11_1083*^{d} (*caTFE2*)	This study
pRB1438	Yeast expression vector carrying the bi-directional Gal1,10 promoter element (*URA3*, CEN, Amp^R)	D. Botstein
pUG23	Yeast expression vector carrying *yEGFP3* under *MET25* promoter (*HIS3*, CEN, Amp^R)	[17]
pSC1356	pRB1438-derived plasmid for galactose-inducible expression (P_{GAL1}) of *yEGFP3* derived from pUG23	This study
pSC1296	pRB1438-derived plasmid for galactose-inducible expression (P_{GAL1}) of *SMDB11_1112* (*TFE1*)	This study
pSC1357	pRB1438-derived plasmid for galactose-inducible expression (P_{GAL1}) of *SMDB11_1083* (*TFE2*)	This study
pSC1834	pRB1438-derived plasmid for galactose-inducible expression (P_{GAL1}) of *SMDB11_2261* (*SSP1*)	This study
pSC1832	pRB1438-derived plasmid for galactose-inducible expression (P_{GAL1}) of *SMDB11_2264* (*SSP2*)	This study

pGED1	Constitutive expression vector for integration into *S. cerevisiae HIS3* locus (*LEU2*, Amp^R)	G. Eitzen
pSC1384	pGED1 derived plasmid carrying empty P_{GAL1} promoter construct (integrative)	This study
pSC1379	pGED1 derived plasmid for galactose-inducible expression (P_{GAL1}) of *yEFGP3* (integrative)	This study
pSC1382	pGED1 derived plasmid for galactose-inducible expression (P_{GAL1}) of *SMDB11_1112* (*TFE1*) (integrative)	This study
pSC1380	pGED1 derived plasmid for galactose-inducible expression (P_{GAL1}) of *SMDB11_1083* (*TFE2*) (integrative)	This study
GFP-ATG8(416)	pRS416-derived plasmid for expression of N-terminal *GFP*-tagged *ATG8* under the control of the endogenous promoter (*URA3*, CEN, Amp^R), Addgene # 49425	[18]

[a] Streptomycin-resistant derivatives were generated by phage ϕIF3-mediated transduction of the resistance allele from *S. marcescens* Db11, as described previously[14]
[b] The whole cassette containing the indicated target gene under control of the *GAL1* promoter and the LEU2 selection marker was inserted into the *HIS3* allele.
[c]The whole cassette containing rtTA under control of the *ADH1* promoter, the *caSAT1* selection marker, and the indicated target gene under control of the Tet-inducible promoter was inserted into one of the *ADH1* alleles.
[d] CDS was codon optimised for expression in *C. albicans*

Supplementary Table 4. Oligonucleotide primers and additional details for plasmid construction, primers for Southern blot probe generation and primers for qPCR.

Plasmid	Sequence of relevant primers (5'-3')[1]	Description
pSC1339	TATATCTAGAGTGCCGATCGATATTGAAGAGG	Forward primer to clone upstream region of SMDB11_1083 in pKNG101 (*Xba*I)
	TATAAAGCTTTGGCATAGCTGCCCCTC	Reverse primer to clone upstream region of SMDB11_1083 in pKNG101 (*Hind*III)
	TATAAAGCTTCAGGCTGGATAAGCCTTTCCC	Forward primer to clone downstream region of SMDB11_1083 in pKNG101 (*Hind*III)
	TATAGTCGACGTTTGGCACCAACCCCATC	Reverse primer to clone downstream region of SMDB11_1083 in pKNG101 (*Sal*I)
pSC1248	TATAGGATCCGACAAAAATGACGG	Forward primer to clone upstream region of SMDB11_1111 in pKNG101 (*Bam*HI)
	TATAAAGCTTCGGCTGCATAACCACCC	Reverse primer to clone upstream region of SMDB11_1111 in pKNG101 (*Hind*III)
	TATAAAGCTTCTAGAGGGTGAAGAGGACAGTCCC	Forward primer to clone downstream region of SMDB11_1111 in pKNG101 (*Hind*III)
	TATAGTCGACGCAACGGCGGGTTTAAATAAGG	Reverse primer to clone downstream region of SMDB11_1111 in pKNG101 (*Sal*I)
pSC1250	TATAGGATCCGACAAAAATGACGG	Forward primer to clone upstream region of SMDB11_1111 in pKNG101 (*Bam*HI)
	TATAAAGCTTCGGCTGCATAACCACCC	Reverse primer to clone upstream region of SMDB11_1111 in pKNG101 (*Hind*III)
	TATAAAGCTTGCCTGAGCCGGTCCG	Forward primer to clone downstream region of SMDB11_1112 in pKNG101 (*Hind*III)
	TATAGTCGACCTTGTGGCCACCGTCACC	Reverse primer to clone downstream region of SMDB11_1112 in pKNG101 (*Sal*I)
pSC1332	TATAGGATCCGTGGTTATGCAGCCGTATAAAGAGG	Forward primer to clone SMDB11_1111-1112 in pSUPROM (*Bam*HI)
	TATAAAGCTTCAGGCGGGGAAAGGCC	Reverse primer to clone SMDB11_1111-1112 in pSUPROM (*Hind*III)
pSC1343	TATAGGATCCATGCCATTAGAAGTCGATGGG	Forward primer to clone SMDB11_1083 in pSUPROM (*Bam*HI)
	TATAAAGCTTTTATCCAGCCTGAGCCAGATC	Reverse primer to clone SMDB11_1083 in pSUPROM (*Hind*III)
pSC1309	GCCAGTTCCATGGCCTACCTTAGTCACTAC	Forward primer for QC to delete *Msc*I site in yEGFP3 (pUG23)
	GTAGTGACTAAGGTAGGCCATGGAACTGGC	Reverse primer for QC to delete *Msc*I site in yEGFP3 (pUG23)
pSC1356	TATAGGATCCATGTCTAAAGGTGAAGAATTATTCACTGG	Forward primer to clone yEGFP3 (pSC1309) in pRB1438 (*Bam*HI)
	TATATCTAGATTATTTGTACAATTCATCCATACCATGG	Reverse primer to clone yEGFP3 (pSC1309) in pRB1438 (*Xba*I)
pSC1296	TATAGGATCCATGCATTGGTTGAATTTCAAG	Forward primer to clone SMDB11_1112 in pRB1438 (*Bam*HI)
	TATAGAGCTCAGGCGGGGAAAGG	Reverse primer to clone SMDB11_1112 in pRB1438 (*Sac*I)
pSC1357	TATAGGATCCATGCCATTAGAAGTCGATGGG	Forward primer to clone SMDB11_1083 in pRB1438 (*Bam*HI)
	TATATCTAGATTATCCAGCCTGAGCCAGATC	Reverse primer to clone SMDB11_1083 in pRB1438 (*Xba*I)
pSC1834	TATAGGATCCATGAAACCTTTATATCGTCAGCTAAAAAGC	Forward primer to clone SMDB11_2261 in pRB1438 (*Bam*HI)
	TATATCTAGACTAGCTCAACTCCCAAAACCAGAC	Reverse primer to clone SMDB11_2261 in pRB1438 (*Xba*I)
pSC1832	TATAGGATCCATGAGTCGCCCTTCATTCCAAC	Forward primer to clone SMDB11_2264 in pRB1438 (*Bam*HI)
	TATATCTAGATTATTTTAGTAACCATATAGATGCCTCGTTG	Reverse primer to clone SMDB11_2264 in pRB1438 (*Xba*I)
pSC1384	TATAAGATCTCCTTGAATTTTCAAAAATTCTTAC	Forward primer to clone P_{GAL1} in pGED1 (*Bgl*II)
	TATAGCATGCTTAGGATCCGGGGTTTTTTCTC	Reverse primer to clone P_{GAL1} in pGED1 (*Sph*I)

pSC1379	TATAAGATCTCCTTGAATTTTCAAAAATTCTTAC	Forward primer to clone P_{GAL1}-yEGFP3 (pSC13756 in pGED1 (*Bgl*II)
	TATAGCATGCTTATTTGTACAATTCATCCATACCATGG	Reverse primer to clone P_{GAL1}-yEGFP3 (pSC1356) in pGED1 (*Sph*I)
pSC1376	CAGCGGGGAGATGGCCTGGGATGCGGTG	Forward primer for QC to delete *Sph*I site in SMDB11_1112 (pSC1296)
	CACCGCATCCCAGGCCATCTCCCCGCTG	Reverse primer for QC to delete *Sph*I site in SMDB11_1112 (pSC1296)
pSC1382	TATAAGATCTCCTTGAATTTTCAAAAATTCTTAC	Forward primer to clone P_{GAL1}-SMDB11_1112 in pGED1 (*Bgl*II)(pSC1376)
	TATAGCATGCTCAGGCGGGGAAAGGCC	Reverse primer to clone P_{GAL1}-SMDB11_1112 in pGED1 (*SphI*)(pSC1376)
pSC1380	TATAAGATCTCCTTGAATTTTCAAAAATTCTTAC	Forward primer to clone P_{GAL1}-SMDB11_1083 in pGED1 (*Bgl*II)(pSC1357)
	TATAGCATGCTTATCCAGCCTGAGCCAGATC	Reverse primer to clone P_{GAL1}-SMDB11_1083 in pGED1 (*Sph*I)(pSC1357)
pSC1352	Synthetic insert produced by Life Technologies of the coding sequence of SMDB11_1112 with all CTG to TTG codon exchanges, cloned into pNIM1 (*Sal*I, *Bgl*II)	
pSC1354	Synthetic insert produced by Life Technologies of the coding sequence of SMDB11_1083 with all CTG to TTG codon exchanges, cloned into pNIM1 (*Sal*I, *Bgl*II)	
pGED1	P_{TEF}-*NAT1*-T_{TEF} (*Sal*I, *Eco*RI) cut from pAG25[19] was cloned into Ylplac128[20] with subsequent introduction of a synthetic *HIS3* fragment carrying residues 495-623 followed by residues 209-304 of the *HIS3* CDS (*Hind*III, *Sal*I). Both *HIS3* regions are joined by a unique *Msc*I site for linearization to facilitate homologous recombination.	

Southern Blot Probe generation

Probe1	TGATAGAGACCCAATGCAAAGCC	Forward primer to generate probe1, situated in homologues region 1 of pNIM1 (*ADH1* promoter)
	GGCACGAGACGGAAACTCTTTAGG	Reverse primer to generate probe1, situated in homologues region 1 of pNIM1 (*ADH1* promoter)
Probe2	GGTTTATCTGACTTGCCAGAAGTCTTC	Forward primer to generate probe2, situated downstream of homologues region 2 of pNIM1 (*ADH1* terminator)
	CAGGACCAATCAAGCAATTCAG	Reverse primer to generate probe2, situated downstream of homologues region 2 of pNIM1 (*ADH1* terminator)

qPCR		
ACT1	CAAGGTATCATGGTCGGTATGG	Forward primer for qPCR of *ACT1*
	CGTGTTCAATTGGGTAACGTAAAG	Reverse primer for qPCR of *ACT1*
AGP1	CGCCATATGTCATTGCTGTTG	Forward primer for qPCR of *AGP1*
	ACTGGAGTAGAAGGAGGAGTTAG	Reverse primer for qPCR of *AGP1*
GNP1	TTGCTAACGGTGCCTTCTATAC	Forward primer for qPCR of *GNP1*
	AGCAGGTCTACCTTCCCTATC	Reverse primer for qPCR of *GNP1*
BAP2	GAGGATGGCGTTGAGTCTATC	Forward primer for qPCR of *BAP2*
	GTCCCAATACCTGTCCCTAAAG	Reverse primer for qPCR of *BAP2*
BAP3	AGGAATCTCGCAATGGGTTAG	Forward primer for qPCR of *BAP3*
	ATTTGGTACCATCCTCCAAGTC	Reverse primer for qPCR of *BAP3*
DIP5	AACCCTGGTGTGTGGATTAC	Forward primer for qPCR of *DIP5*
	GACCCAACATTACCATGACTTTG	Reverse primer for qPCR of *DIP5*
CAN1	CGAGAGTAAATGGCGAGGATAC	Forward primer for qPCR of *CAN1*
	TACCACCAAGGGCAATCATAC	Reverse primer for qPCR of *CAN1*
PTR2	GGCTGTTGCCTGCTTTATTTC	Forward primer for qPCR of *PTR2*
	TCGTAGTCCATAGCGTTCATTTC	Reverse primer for qPCR of *PTR2*
SSY1	GTGGCGATCCAAGACTACTATC	Forward primer for qPCR of *SSY1*
	GTCCTAAGTCTCCAGTCCATTC	Reverse primer for qPCR of *SSY1*
STP1	GGCCATTCCAGGCAAGATATAC	Forward primer for qPCR of *STP1*
	TCTTTGCCTTCCTCCTTTGTC	Reverse primer for qPCR of *STP1*
STP2	CCAATGTGGAAGACGACAAATC	Forward primer for qPCR of *STP2*
	AGCAGTCTTTCTGTGGATGG	Reverse primer for qPCR of *STP2*

SUL1	GGGTTGGGTATACTGCGTTTAG	Forward primer for qPCR of *SUL1*
	CCATGAGAGCCGGAATTTGA	Reverse primer for qPCR of *SUL1*
SUL2	ACGGTGAAAGGAGCATGTAG	Forward primer for qPCR of *SUL2*
	CTGCAATAAGATCCGCAGTAAAC	Reverse primer for qPCR of *SUL2*
GCN4	CGATGTTTCATTGGCTGATAAGG	Forward primer for qPCR of *GCN4*
	TCTAGAACAGGAGTGGGTAAGA	Reverse primer for qPCR of *GCN4*

[1]Incorporated restriction sites for cloning into the respective vector are underlined.

Supplementary References

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