# Supplementary Fig 1: Schematic representation of the experimental design for iTRAQ labeling



(A) Two replicates from Sm25%-Ca75% protein mix of the single-species biofilm and six replicates of the proteins from the mixed-species biofilm were labeled with iTRAQ labeling reagents 113, 114 and 115, 116, 117, 118, 119, 121 respectively. (B) Protein expression of each species in mixed-species biofilm compared with respective single-species biofilms was determined by taking the average of all cross comparisons between each replicate of the mixed-species biofilm vs single-species biofilm protein mix.

Supplementary Fig 2. Flow chart illustrating the different filtering methodology used to analyze the abundance changed proteins in *S. mutans* mixed-species biofilms and single-species biofilms



Supplementary Fig 3. Flow chart illustrating the different filtering methodology used to analyze the abundance changed proteins in *C. albicans* mixed-species biofilms and single-species biofilms



Supplementary Fig 4: Normalization of proteins in the mixed-species and single-species biofilms

	All blue ladder	Sm 100%	Ca 100%	Sm 25% Ca 75%	Sm 50% Ca 50%	Sm 75% Ca 25%	Sm-Ca Mix1	Sm-Ca Mix2	Sm-Ca Mix3	Sm-Ca Mix4
kD	And show	-	-		-	THE OWNER OF		-		
250										
150	-									
100	-		-							
75	-	And and a second						-		and and a
50	-									
37	-									
25	-									
20	-									
15										
10	-	-	-						-	

Sm-Streptococcus mutans Ca-Candida albicans

The mixed-species biofilm had different amount of proteins derived from *S. mutans* and *C. albicans* counterparts. We compared the amount of proteins from each organism in the mixed-species biofilm (Sm-Ca Mix 1,2,3,4) against the protein amounts derived from *S. mutans* and *C. albicans* single-species biofilms, mixed according to specific ratios (Sm100%-Ca0%; Sm0%-Ca100%; Sm25%-Ca75%; Sm50%-Ca50%; Sm75%-Ca25%). We identified that a protein mixture of 25% of *S. mutans* proteins and 75% of *C. albicans* proteins single-species biofilms mixed together corresponds to the amount of *S. mutans* and *C. albicans* proteins detected in the mixed-species biofilm.

**Supplementary Fig 5.** Percentage variation in iTRAQ ratios of the same proteins found in various replicates. The primary vertical axis represents the corresponding number of proteins (bars) having different % co-efficient of variation (%CV) that was plotted in the horizontal axis. The secondary vertical axis represents the cumulative % of the counted proteins (lines). Variation against 88% coverage was taken into account for determining the fold cut-off, considering the population outside 88% as significantly altered.

*C. albicans* - 37% variation corresponding to >1.37 for increased abundance and <0.729927 (1/1.37) for decreased abundance



*II. S. mutans* - 31% variation corresponding to >1.312 for increased abundance and <0.762195 (1/1.312) for decreased abundance



Supplementary table 1: Some important Candida albicans genes affected in Sm-Ca mixed-species biofilms

## 1. Genes involved in hyphal formation

## I. Genes directly involved in hyphal formation

gene/genes	function	reference
CEK2	encoding a MAPK protein important for filamentous growth	
(W5Q_06054)		
BNI1 (W5Q_01255)	required for the maintenance of polarized hyphal growth was upregulated. BNI1-mediated	(1), *
	actin cables are necessary for positioning the golgi complex to a putative site of germ tube	
	emergence and for coordinating the transport and deposition of membrane and cell wall material to a growing hypha. A study using <i>in vivo</i> time-lanse microscopy showed that	
	deletion of the <i>C. albicans BNII</i> results in polarity defects during yeast growth and hyphal	
	stages	
SHO1 (W5Q_00881)	a gene involved in invasive filamentation into semi-solid medium that plays a role in	*
	morphological dimorphic transition was upregulated	
<i>FGR27</i> (W5Q_01867),	involved in hypha formation	*
<i>SUV3</i> (W5Q_03522),		
HSP21 (W5Q_01833),		
SLN1 (W5Q_05559),		
CAS5 (W5Q_03421),		
SSU1 (W5Q_06368),		
TPS1 (W5Q_06032)		
TPS1 (W5Q_06032)	encoding an $\alpha,\alpha$ -trehalose-phosphate synthase was upregulated. Disruption of the <i>C</i> . <i>albicans TPSI</i> gene has shown to impair the formation of hyphae and decreased infectivity	(2),*

## II. Genes involved in transcriptional regulation related to the filamentous growth of the fungi

FKH2 (W5Q_02558)	a potential forkhead-like transcription factor similar to <i>S. cerevisiae FKH2</i> (YNL068C) involved in pseudohyphal growth. A study showed that Fkh2 during hyphal growth	(3), *
	regulates C. albicans pathogenesis	
ADR1 (W5Q_03549)	upregulated gene that encodes transcriptional regulation of hyphal growth	*
EFG1 (W5Q_04510)	the key transcription factors in C. albicans involved in the yeast to hypha transition	*
<i>RFG1</i> (W5Q_05709)	encodes a transcription regulator that functions in both the positive and negative regulation of filamentous growth, depending upon the environmental conditions. Rfg1p regulates genes encoding cell wall components that are specifically expressed in the filamentous forms such as <i>HWP1</i> , <i>RBT1</i> , <i>HYR1</i> , <i>ECE1</i> , <i>ALS1</i> , <i>RBT4</i> and <i>RBT5</i>	*
<i>FGR23</i> (W5Q_02736) and <i>FGR27</i> (W5Q_03522)	encoding transcription factors involved in filamentous growth was upregulated	*

# III. Other genes indirectly involved in hyphal growth

VPS34 (W5Q_00648)	a gene mainly involved in vacuolar sorting and segregation encoding a Phosphatidylinositol 3-kinase in <i>C. albicans</i> which has been shown to be important for filamentous growth was upregulated. Expression studies have indicated that the vps34 mutant reacts to the phenotypical defects with an up-regulation of genes involved in filament formation such as <i>ALS1</i> and <i>HWP1</i>	(4), *
MYO2 (W5Q_01331)	encoding myosin, a protein that modulate the structure of the actin cytoskeleton shown to be essential for polarized growth and hyphal transition	(5), *
SIR2 (W5Q_01578)	shown to be involved in the phenotypic switching	(6), *
MDS3 (W5Q_03241)	identified as a new member of the TOR pathway that contributes to the morphogenesis in <i>C. albicans</i>	(7), *
ECM7 (W5Q_03320)	involved in cell wall maintenance, oxidative stress response and hyphal development	(8), *
HWP2 (W5Q_03621)	encoding Hwp2p, a cell wall GPI-anchored cell wall protein that was previously shown to be necessary for hyphal and invasive growth on solid media was upregulated	(9), *

### 2. Genes associated with fungal vacuolar development genes were upregulated

VPS8 (W5Q_02560) and	proteins associated with vacuolar development were upregulated	*
VPS13 (W5Q_03896)		
VPS15 (W5Q_04709)	a protein kinase involved in vacuolar protein sorting	*
VAC7 (W5Q_03740)	encoding a vacuolar segregation protein were also upregulated	*
AVT42 (W5Q_03055)	a potential transmembrane amino acid transporter that controls efflux of large neutral amino	*
	acids in vacuolar-like organelles	

### 3. Genes associated with biofilm dispersal was upregulated in Candida albicans

SET3 (W5Q_01366) and SNT1 (W5Q_01183)	genes were upregulated which are two parts of the four core members of a conserved histone deacetylase complex (Set3, Hos2, Snt1, and Sif2). Histone deacetylase complex members have been shown to play a vital role in biofilm formation, including dispersal of cells from biofilms	(10), *
YWP1 (W5Q_02272)	encoding a potential yeast form cell wall protein was upregulated, which plays an anti- adhesive role and promotes dispersal of yeast forms allowing the organism to seek new sites for colonization.	*
DIT1 (W5Q_05894)	required for biosynthesis of dityrosine in the outer layer of the spore wall	*
DTR1 (W5Q_05892)	encoding a dityrosine transporter essential for spore wall maturation was among the upregulated genes	*

### 4. Candida albicans genes in cation uptake and transport were upregulated

FTR1 (W5Q_01365)	encoding one of two plasma membrane iron permeases was upregulated. Microbial pathogens must compete with the iron-withholding defense systems of their host to acquire this essential nutrient. A study showed that out of two high-affinity iron permease genes <i>FTR1</i> and <i>FTR2</i> , <i>FTR1</i> expression was induced under iron-limited conditions and repressed when iron supply was sufficient	(11), *
FET3 (W5Q_04636)	was upregulated encoding an iron transport multicopper ferroxidase required for $Fe^{2+}$ high affinity uptake, which oxidizes $Fe^{2+}$ and release it from the transporter	*
CCH1 (W5Q_00104)	encoding a likely iron transport protein	*
ALR1 (W5Q_02751)	encoding a magnesium transporter	*
SMF12 (W5Q_03402)	encoding a manganese transporter	*

### 5. Genes associated with heat shock proteins were upregulated

<i>CDC37</i> (W5Q_04837),	encoding Hsp90 co-chaperones in C. albicans	*
AHA1 (W5Q_06467) and		
CNS1 (W5Q_00050)		
HSP21 (W5Q_01833)	upregulated gene required for invasive growth and filament formation under various	*
	filament inducing conditions	
HSP60 (W5Q_06099),	Encoding heat shock proteins	*
HSP70 (W5Q_01302),		
HSP104 (W5Q_06286)		
and STI1 (W5Q_04212)		
HSP78 (W5Q_01774)	Hsp78 acts in concert with mitochondrial Hsp70, for the dissociation, resolubilization and	*
	refolding of aggregates of damaged proteins in the mitochondrial matrix after heat stress	
HCH1 (W5Q_04765)	encodes a co-chaperone that binds to the molecular chaperone HSP82 and stimulates its	*
	ATPase activity	

# 6. Streptococcus mutans enhances Candida albicans genes associated with drug transport

MTE4 (W5Q_00077)	potential Multi Antimicrobial Extrusion (MATE) family drug/sodium antiporter	*
QDR3 (W5Q_04716)	multidrug resistance transporter	*
YHK8 (W5Q_04766)	encoding a probable drug/proton antiporter YHK8	*
CDR1 (W5Q_02918)	encoding the multidrug resistance protein CDR1 shown to be resistant against	*
	cycloheximide	
CDR3 (W5Q_03632)	which encodes the opaque-specific ABC transporter CDR3 involved in drug export	*
MDR1(W5Q_04891)	encoding a multidrug resistance protein was also upregulated	*

# 7. Streptococcus mutans enhances Candida albicans carbohydrate metabolism

	-	
ITR1 (W5Q_02514)	important for the acquisition of inositol	(12), *
ITR2 (W5Q_01924)	associated with potential sugar transporter activity was upregulated	*
SNF3 (W5Q_03002)	expression was enhanced encoding the Snf3p in S. cerevisiae analogues to the Hgt4 protein	*
	of C. albicans (orf19.5962) which acts as glucose sensors and govern sugar acquisition by	
	regulating the expression of genes encoding hexose transporters	
GAL1 (W5Q_00201)	encodes galactokinase similar to S. cerevisiae GAL3 associated with galactose metabolism	*
ARA1 (W5Q_02234D)	encoding arabinose dehydrogenase involved in arabinose metabolism	*
TPS3 (W5Q_02484)	encoding regulatory subunit of trehalose-6-P synthase involved in trehalose metabolism	*
GPM1 (W5Q_00421)	encoding the protein phosphoglycerate mutase which is involved in the third step of the	*
	subpathway that synthesizes pyruvate from D-glyceraldehyde 3-phosphate in glycolysis	
FUMH (W5Q_03272)	encodes a fumarate hydratase were also upregulated in tri-carboxylic acid cycle	*
CYC2 (W5Q_04301)	encoding a cytochrome c mitochondrial import factor similar to CYC2 in S. cerevisiae	*
COX15 (W5Q_04661)	encodes cytochrome c oxidase assembly protein COX15 involved in biosynthesis of heme	*
	A during cellular respiration	
PDP1 (W5Q_06274)	encodes a pyruvate dehydrogenase phosphatase	*
PDC2 (W5Q_00962)	essential for the synthesis of pyruvate decarboxylase and encodes a transcriptional regulator	*
	for the pyruvate decarboxylase gene	
YAT1 (W5Q_00164)	contributes to the transport of acetyl-CoA from the cytosol during growth on ethanol or	*
	acetate	
LPG20 (W5Q_00403)	encoding a putative aryl alcohol dehydrogenase	*
SAD3 (W5Q_01879)	encoding one of a tandem pair of alcohol dehydrogenase	*
ADH2 (W5Q_00801)	encoding an alcohol dehydrogenase 2 which catalyzes the conversion of ethanol to	*
	acetaldehyde	
CYB2 (W5Q_01316)	encoding Cvb2, a heme-containing dehydrogenase (L-lactate cvtochrome- <i>C</i> oxidoreductase)	*
	essential for the utilization of L-lactate as a carbon source	
DLD1 (W5Q 01734)	a putative D-lactate dehydrogenase	*

### 8. Streptococcus mutans enhances peroxisomal assembly and fatty acid oxidation in Candida albicans

<i>PEX1</i> (W5Q_05290),	encoding peroxisomal membrane proteins	*
<i>PEX6</i> (W5Q_01973),		
PEX11 (W5Q_05007 and		
W5Q_05034) and PEX29		
(W5Q_04167)		
FOX2 (W5Q_02570)	involved in fatty acid $\beta$ -oxidation. A study showed that a $fox 2\Delta/fox 2\Delta$ mutant,	(13), *
	displayed strong growth defects on nonfermentable carbon sources other than fatty acids	
	(e.g., acetate, ethanol or lactate)	
SCT1 (W5Q_02571),	genes involved with peroxisomal fatty acid beta-oxidation	
<i>POT2</i> (W5Q_02635),		
POX102 (W5Q_02683),		
POX104 (W50 02686),		
FAA21 (W5Q_02795)		
and ADR1 (W5Q_03549)		
MLS1 (W5Q_00935)	encoding malate synthase 1, a key enzyme in the glyoxalate pathway	*
SAK1 (W5Q_03749)	SNF1-activating kinase 1 protein in the glyoxalate cycle. A study using sak1 mutants	(14), *
· - /	showed that Sak1p ensures basal expression of glyoxylate cycle and gluconeogenesis genes	
	even in glucose-rich media and thereby contributes to the metabolic plasticity of C.	
	albicans	

# 9. Streptococcus mutans alters Candida albicans cell wall and cell membrane properties

# I. Mannan production

MNN1 (W5Q_04290)	encoding a putative alpha-1,3-mannosyltransferase	*
MNN11 (W5Q_00231)	encoding alpha-1,6-mannosyltransferase	*
MNN26 (W5Q_05390)	encoding alpha-1,2-mannosyltransferase	*
ALG11 (W5Q_04805)	encoding an alpha-1,2-mannosyltransferase	*
BMT7 (W5Q_02844)	encoding beta-mannosyltransferase	*
MNN4 (W5Q_04865),	involved in transfer of mannosylphosphate to cell wall mannans were similarly upregulated	*
MNN42 (W5Q_01801)		
and MNN41		
(W5Q_01803)		
WRY7 (W5Q_00489)	encoding a putative beta-mannosyltransferase required for the addition of beta-mannose to	*
	the acid-labile fraction of cell wall phosphopeptidomannan	

# II. Glucan synthesis

GSC1 (W5Q_00233)	encoding beta-1,3-glucan synthase	*
IFF11 (W5Q_02548 and	encoding a protein similar to HYR1p. Analysis of the C. albicans genome has	(15), *
W5Q_02550		
	identified the <i>IT T</i> gene family as encoding the fargest family of cen wan-related proteins	
	which is conserved in a wide range of <i>Candida</i> species. <i>IFF11</i> is important for	
	maintaining the cell wall structure	
ALS2 (W5Q_05013),	Agglutinin-like proteins are important cell wall proteins in C. albicans required for	(16), *
ALS5 (W5Q_05030),	adhesion and biofilm formation	
ALS6 (W5Q_03142) and		
ALS7 (W5Q_03128 and		
W5Q_03143)		
<i>PGA25</i> (W5Q_04597)	encode a probable GPI-anchored cell wall protein involved in cell wall organization, hyphal	*
and PGA50	growth as well as virulence	
(W5Q_00609)		
HYR4 (W5Q_05538)	encoding a hyphally regulated cell wall protein 4	*
GCA1 (W5Q_00993) and	genes encoding a glycosidase-like protein in the cell wall	*
GCA12 (W5Q_01020)		
CCW12 (W5Q_00875)	also known as PGA6 Predicted GPI-anchored protein 6. The expression of probable cell	*
	wall proteins that participate in adhesive cell-cell interactions	

## III. Ergosterol synthesis

<i>ERG3</i> (W5Q_00467)	encoding a sterol C5,6-desaturase which is essential for synthesis of ergosterol	*
ERG6 (W5Q_02705 and	encoding a methyltransferase	*
W5Q_02721)		
ERG252 (W5Q_03456)	encoding C-4 sterol methyl oxidase	*

# 10. Streptococcus mutans enhances Candida albicans cellular stress related gene responses

CTA1 (W5Q_00661)	encoding Catalase A was upregulated, protecting cells from the toxic effects of hydrogen peroxide stress	*
CCP1 (W5Q_02762)	encoding cytochrome-c peroxidase was upregulated which destroys radicals which are normally produced within the cells and which are toxic to biological systems	*
<i>GPX2</i> (W5Q_00712)	encoding Gpx2p capable of reducing $H_2O_2$ and <i>tert</i> -butyl hydroperoxide in the presence of thioredoxin, thioredoxin reductase and NADPH were among the upregulated proteins	(17), *
POS5 (W5Q_03211)	encoding a mitochondrial NADH kinase involved in oxidative stress	*

# 11. Streptococcus mutans stimulated Candida albicans cellular reproduction

#### I. Mitotic cell division

STU1 (W5Q_00713)	encoding a microtubule binding protein that promotes the stabilization of dynamic	*
	microtubules and required for mitotic spindle formation	
MAD1 (W5Q_01226)	encoding a spindle assembly checkpoint protein	*
CSE1 (W5Q_00745)	involved in chromosome segregation were among the overexpressed genes. CSE1 and	(18), *
	CSE2 were identifies as two new genes required for accurate mitotic chromosome	
	segregation in S. cerevisiae	
ASE1 (W5Q_03103)	anaphase spindle elongation protein	*
APC5 (W5Q_03823)	encoding an anaphase-promoting complex subunit 5	*
CDC27 (W5Q_05582)	encoding an anaphase-promoting complex subunit	*
SWI6 (W5Q_00835)	encoding a putative protein component of the transcription complexes termed MBF and	*
	SBF involved in G1/S cell-cycle progression	
CAC2 (W5Q_04378)	encoding a chromatin assembly factor 1 subunit p60	*
CDC37 (W5Q_04837)	encoding cell cycle proteins necessary for passage in the cell division cycle	*
<i>CDC4</i> (W5Q_05627),	Proteins important for cell division control	*
BUB1 (W5Q_03598),		
BUB31 (W5Q_04346)		
MEC1 (W5Q_04425)	proteins associated with cell cycle checkpoints	*

### II. Meiotic cell division

MEK1 (W5Q_02173)	encoding a meiosis-specific serine/threonine-protein kinase	*
MEI5 (W5Q_06018)	encoding a protein showing weak similarity to S. cerevisiae protein required for synapsis	*
	and meiotic recombination	
MND1 (W5Q_06176)	encoding a meiotic nuclear division protein 1	*
MSC1 (W5Q_06365)	encoding a meiotic sister chromatid recombination protein 1	*
RIM4 (W5Q_03616)	a positive regulator of sporulation-specific genes and of sporulation and is required for	*
	premeiotic DNA synthesis and meiotic chromosomal segregation	

#### 12. Several Candida albicans genes downregulated in the presence of Streptococcus mutans

### I. Genes associated with cell membrane structure and function

POM33 (W5Q_00188)	encoding a pore membrane protein associated with cell membrane transport	*
TOM20 (W5Q_00254)	encoding an outer membrane translocase	*
FTR2 (W5Q_01374)	a plasma membrane iron permease gene	*
ERP3 (W5Q_01960) and	encoding membrane trafficking proteins	*
LSB5 (W5Q_02390)		
SNL1 (W5Q_01017),	genes encoding integral membrane proteins	*
SUR7 (W5Q_04751)		
YHU0 (W5Q_04051)	a C. albicans pH regulated GPI-anchored membrane protein	*
YL326 (W5Q_00305),	several uncharacterized membrane proteins	*
<i>YG11</i> (W5Q_06078),		
YO073 (W5Q_03020) and		
YD090 (W5Q_03275)		

#### II. Mitochondrial membrane associated genes

YMC1 (W5Q_00699)	encoding a likely mitochondrial carrier protein	*
TOM37 (W5Q_01661)	encoding a translocase of the outer mitochondrial membrane	*
SHH3 (W5Q_01613) and	encoding mitochondrial membrane proteins	*
FMP42 (W5Q_05906)		

# III. Vacuolar membrane proteins

PFF1 (W5Q_00001)	a vacuolar membrane protease	*
VCX1 (W5Q_00826)	a calcium transport protein	*
PHM7 (W5Q_02235)	a potential transmembrane protein	*
YO292 (W5Q_06403)	encoding a vacuolar membrane protein	*
TVP18 (W5Q_00946),	Membrane proteins in the golgi apparatus	*
TVP15 (W5Q_04011)		
SEC61 (W5Q_03289),	coding for an ER transmembrane protein	*
PBN1 (W5Q_04724) and		
YET1 (W5Q_03342)		

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