# **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 mixedspecies 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 singlespecies biofilms**



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



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* singlespecies 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.

*I. C. albicans* **-** 37% variation corresponding to >1.37 for increased abundance and  $\langle 0.729927 \, (1/1.37)$  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**



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



# **III. Other genes indirectly involved in hyphal growth**



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



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



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



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



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



### **7.** *Streptococcus mutans* **enhances** *Candida albicans* **carbohydrate metabolism**



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



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

### **I. Mannan production**



# **II. Glucan synthesis**



### **III.** *Ergosterol synthesis*



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



# **11.** *Streptococcus mutans* **stimulated** *Candida albicans* **cellular reproduction**

#### **I. Mitotic cell division**



#### **II. Meiotic cell division**



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

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



#### **II. Mitochondrial membrane associated genes**



# **III. Vacuolar membrane proteins**



\* Information from UniprotKB for *Candida albicans* database

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