**Supplement to: Protein markers for *Candida albicans* EVs include claudin-like Sur7 family proteins**

**Charlotte S Dawson1,3, Donovan Garcia-Ceron1, Harinda Rajapaksha2, Pierre Faou2, Mark R Bleackley1$,Marilyn A Anderson1$#**

**1 Department of Biochemistry and Genetics, La Trobe Institute for Molecular Science. La Trobe University, Kingsbury Drive. Bundoora. VIC. Australia. 3086**

**2 La Trobe Comprehensive Proteomics Platform, La Trobe Institute for Molecular Science. La Trobe University, Kingsbury Drive. Bundoora. VIC. Australia. 3086**

**3 Cambridge Centre for Proteomics, Milner Therapeutics Institute, Jeffrey Cheah Biomedical Centre, University of Cambridge, Puddicombe Way, Cambridge CB2 0AW, United Kingdom**

**# To whom correspondence should be addressed.** m.anderson@latrobe.edu.au

**$ These authors contributed equally to this work**

**ORCID:**

**Charlotte Dawson** csd51@cam.ac.uk https://orcid.org/0000-0002-7151-5971

**Donovan Garcia Ceron** 18538197@students.latrobe.edu.au https://orcid.org/0000-0003-4718-4233

**Harinda Rajapaksha** k.rajapaksha@latrobe.edu.auhttps://orcid.org/0000-0001-7521-0991

**Pierre Faou** p.faou@latrobe.edu.au https://orcid.org/0000-0002-3755-7502

**Marilyn Anderson** m.anderson@latrobe.edu.auhttps://orcid.org/0000-0002-8257-5128

**Mark Bleackley** m.bleackley@latrobe.edu.auhttps://orcid.org/0000-0002-9717-7560

**Table captions**

**Table S1: Metadata for each EV isolation analysed in this study.** The optical density (at 600 nm) and volume of each culture from which the EVs were isolated is given. The protein and particle concentration of the EVs along with the NTA size distribution results for each biological replicate are also shown.

**Table S2: Candidate negative protein markers for C. albicans EVs.** This list of proteins consists of those that were found to be exclusive to whole cell lysate (WCL) or significantly enriched in WCL across the four C. albicans strains examined in this study. Proteins are grouped according to their subcellular localisation as annotated in the Candida Genome Database (candidagenome.org) [48, 49] unless otherwise indicated. The log2 ratio of the abundance (mean MaxQuant LFQ intensity) of each protein in EVs compared to WCL for each strain is listed. A negative value indicates that a protein was enriched in WCL compared to EV. “ex” indicates where a protein was only quantified in the WCL fraction and not in EVs for that strain. The “TM” column indicates the number of transmembrane domains for each protein as annotated in UniProtKB. “SP” indicates whether a protein is annotated as having a signal peptide according to UniProt. “VDM” shows whether a protein has previously been detected in vesicle­depleted culture media (i.e. the proteins may also be in the soluble secretome) [31]. Underlined proteins are those identified as the best candidates for negative EV markers according to the criteria depicted in Supplementary Figure S1.

**Table S2 footnotes:**

a Protein localisation was inferred from sequence similarity with S. cerevisiae homolog as annotated in the Candida Genome Database [48, 49].

b Protein localisation was obtained from the GO Cellular Component annotation in the C. albicans UniProt reference proteome UP000000559 [58].

c Protein and has no Cellular Component annotation in the Candida Genome Database or UniProt reference proteome.

**Figure captions**

**Figure S1: Schematic of the workflow used for the isolation and proteomic analysis of *C. albicans* EVs and the selection of EV protein markers.** Steps 1 to 6 were performed for each of the four strains examined in this study: DAY286 yeast, ATCC90028 yeast, ATCC10231 yeast, and DAY286 biofilm. The results from the four separate analyses were combined (step 7) to identify proteins which commonly appeared as EV marker candidates. The MISEV2018 criteria for protein content­based EV characterisation and other considerations were used to select the best *C. albicans* EV marker proteins.

**Figure S2: Size distribution of *C. albicans* EVs as determined by nanoparticle tracking analysis (NTA).** A line plot for each EV biological replicate from each strain is shown. Each line plots represents the average of three technical replicates and the dashed grey lines indicate 100 nm.

**Figure S3: Statistical comparisons of *C. albicans* EV isolations.** (A) Boxplot showing EV mode diameter measured using NTA. Differences in average EV mode diameters were compared using One­way ANOVA followed by Tukey’s HSD *post­hoc* test. Adjusted p­values indicating significant differences are shown. (B) Boxplot showing total particle concentration for each EV preparation as measured by NTA. Differences in average total particle concentration were compared by Kruskal­Wallis test followed by Dunn’s Test. Adjusted p­values indicating significant differences are shown.

**Figure S4: Differential abundance analysis of proteins identified in EVs isolated from two different morphologies of the DAY286 *C. albicans* strain.** (A) Venn diagram comparing the biofilm EV and yeast EV proteome data sets shown in Figure 2A. (B) Volcano plot depicting significantly enriched biofilm EV or yeast EV proteins. Differential abundance analysis was performed by comparing the mean normalised LFQ intensities of common DAY286 EV proteins (i.e. proteins in the Venn overlap) using the package *limma* [52, 54]. Significantly enriched proteins were identified using a Benjamini­Hochberg adjusted p­value cut­off of 0.01 and a log2(FC) cut­off of 1. Counts of significant and non­significant proteins are indicated. Proteins with a log2(FC) greater than 5 or less than ­5 are labelled. Data underlying this plot are provided in Supplementary Data S6.

**Figure S5: Functional enrichment analyses of EVs from two DAY286 morphologies.** Top 10 significantly enriched biological process (BP), cellular component (CC), and molecular function (MF) GO terms for the (A) yeast EV enriched and exclusive proteins or (B) biofilm EV enriched or exclusive proteins shown in Supplementary Figure S4. GO analyses were performed using the online tool FungiFun2 (elbe.hki­jena.de/fungifun) (Hypergeometric distribution, Benjamini­Hochberg adjusted p­value < 0.01) [7]. The GO terms are presented top to bottom in order of increasing adjusted p­value. Full lists of enriched GO terms can be found in Supplementary Data S7.