

Protein markers for *Candida albicans* EVs include claudin-like Sur7 family proteins and GTPases

Charlotte Dawson^a, Donovan Garcia Ceron^a, Harinda Rajapaksha^b, Pierre Faou^b,
Marilyn Anderson^a, Mark Bleackley^a

^a*Department of Biochemistry and Genetics, La Trobe Institute for Molecular Science, La Trobe University,
Melbourne, 3086, Victoria, Australia*

^b*La Trobe Comprehensive Proteomics Platform, La Trobe Institute for Molecular Science, La Trobe University,
Melbourne, 3086, Victoria, Australia*

Corresponding author: Mark Bleackley, m.bleackley@latrobe.edu.au

1 Tables

Table 1: Candidate positive protein markers for *C. albicans* EVs. This list of proteins consists of those that were found to be exclusive to EVs or significantly enriched in EVs 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) (ref:footnote1) unless otherwise indicated. The \log_2 ratio of the abundance (mean MaxQuant LFQ intensity) of each protein in EVs compared to whole cell lysate (WCL) for each strain is listed. “ex” indicates where a protein was only quantified in the EV fraction and not the WCL for that strain. The “TM” column indicates the number of transmembrane domains for each protein as annotated in UniProt. “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 vesicle-depleted culture media (i.e. the proteins may also be in the soluble secretome) (31). Underlined proteins are those identified as the best candidates for positive EV markers according to the criteria depicted in Supplementary Figure S1.

Name	Function	\log_2 (fold change) EV vs WCL						
		DAY Y	A9	A1	DAY B	TM	SP	VDM
Plasma membrane								
<u>ARF3</u>	<u>Arf family GTPase^a</u>	<u>ex</u>	<u>1.69</u>	<u>ex</u>	<u>3.45</u>			
<u>CDC42</u>	<u>Rho family GTPase</u>	<u>ex</u>	<u>ex</u>	<u>ex</u>	<u>ex</u>			
CDR1;CDR2	Multidrug transporter of ABC superfamily	ex	ex	ex	11.19	12;12		
CHS3	Major chitin synthase of yeast and hyphae	ex	4.12	ex	ex	5		
ENA21	Predicted P-type ATPase sodium pump ^a	ex	ex	ex	ex	9		
<u>FAA4</u>	<u>Long-chain fatty acid-CoA ligase^a</u>	<u>2.89</u>	<u>2.24</u>	<u>2.28</u>	<u>1.45</u>			
<u>FET34</u>	<u>Multicopper feroxidase</u>	<u>ex</u>	<u>ex</u>	<u>ex</u>	<u>6.18</u>	<u>1</u>	<u>Y</u>	
GAP4	High-affinity S-adenosylmethionine permease	ex	ex	ex	4.28	12		
GSC1	1,3-beta-glucan synthase	2.57	3.45	5.97	8.01	15		
HGT1	High-affinity MFS glucose transporter	ex	ex	ex	ex	12		
HGT6	Putative high-affinity MFS glucose transporter	4.04	4.78	6.53	5.68	11		
HGT7	Putative MFS glucose transporter	ex	5.95	ex	ex	11		
<u>MTS1</u>	<u>Sphingolipid C9-methyltransferase</u>	<u>2.20</u>	<u>1.86</u>	<u>1.41</u>	<u>2.13</u>	<u>2</u>		
<u>NCE102</u>	<u>Non-classical protein export protein</u>	<u>3.34</u>	<u>2.20</u>	<u>ex</u>	<u>5.32</u>	<u>4</u>		
<u>EVP1^d</u>	<u><i>S. cerevisiae</i> ortholog is Pun1, plasma membrane protein^a</u>	<u>ex</u>	<u>ex</u>	<u>ex</u>	<u>ex</u>	<u>3</u>		<u>Y</u>
PHM7	Putative ion transporter	ex	ex	ex	ex	11		
PMA1	Plasma membrane ATPase	5.65	4.38	5.60	6.46	8		
<u>RAC1</u>	<u>G-protein of RAC subfamily</u>	<u>ex</u>	<u>ex</u>	<u>ex</u>	<u>3.92</u>			
<u>RHO1</u>	<u>Rho family GTPase</u>	<u>2.81</u>	<u>3.68</u>	<u>3.74</u>	<u>4.03</u>			
<u>RHO3</u>	<u>Rho family GTPase</u>	<u>ex</u>	<u>ex</u>	<u>ex</u>	<u>ex</u>			
<u>SSO2</u>	<u>Plasma membrane t-SNARE^a</u>	<u>ex</u>	<u>1.88</u>	<u>3.57</u>	<u>ex</u>	<u>1</u>		

<u>SUR7</u>	Protein required for normal cell wall, plasma membrane ^c	<u>ex</u>	<u>ex</u>	<u>ex</u>	<u>7.04</u>	<u>4</u>
<u>YCK2</u>	<i>S. cerevisiae</i> ortholog is Yck2, casein kinase	<u>ex</u>	<u>4.42</u>	<u>ex</u>	<u>ex</u>	
Cell wall, cell surface						
BGL2	1,3-beta-glucanoyltransferase	7.26	9.44	11.08	ex	Y Y
CRH11	GPI-anchored cell wall transglycosylase	ex	ex	ex	ex	Y Y
ECM33	GPI-anchored cell wall protein	4.39	5.28	8.31	7.03	Y Y
<u>GPD2</u>	<u>Glycerol-3-phosphate dehydrogenase</u>	<u>ex</u>	<u>3.48</u>	<u>2.39</u>	<u>1.30</u>	
MP65	Cell surface mannoprotein	ex	ex	ex	ex	Y Y
MSB2	Mucin family adhesin-like protein	ex	ex	ex	ex	1 Y Y
PGA4	1,3-beta-glucanoyltransferase	ex	ex	ex	ex	Y Y
PGA52	GPI-anchored cell surface protein of unknown function	ex	ex	ex	ex	Y Y
<u>PHR1</u>	<u>Cell surface glycosidase</u>	<u>ex</u>	<u>ex</u>	<u>ex</u>	<u>5.65</u>	<u>Y</u>
PHR2	Glycosidase	ex	3.51	6.44	ex	Y Y
PLB4.5	Phospholipase B	ex	ex	ex	ex	Y Y
SAP9	Secreted aspartyl protease	ex	ex	ex	ex	1 Y Y
YWP1	Secreted yeast cell wall protein	2.37	3.51	6.17	ex	Y Y
Endoplasmic reticulum						
ERO1	ER oxidoreductin	ex	ex	2.32	1.68	Y Y
<u>orf19.1054</u>	<u><i>S. cerevisiae</i> ortholog is Pom33, transmembrane nucleoporin^b</u>	<u>5.66</u>	<u>ex</u>	<u>ex</u>	<u>2.51</u>	<u>4</u>
<u>orf19.2168.3</u>	<u><i>S. cerevisiae</i> ortholog is Yop1, reticulon-interacting protein^a</u>	<u>4.77</u>	<u>1.98</u>	<u>2.29</u>	<u>1.81</u>	<u>4</u>
<u>orf19.3799</u>	<u><i>S. cerevisiae</i> ortholog is Rtn1, reticulon protein^b</u>	<u>2.24</u>	<u>1.40</u>	<u>3.00</u>	<u>1.29</u>	<u>2</u>
SEC61	ER protein-translocation complex subunit	2.53	2.16	2.10	1.46	8
Endosome, Golgi, transport vesicle						
<u>SEC4</u>	<u>Rab family GTPase^a</u>	<u>1.88</u>	<u>1.20</u>	<u>1.86</u>	<u>2.90</u>	
<u>YKT6</u>	<u>Palmitoyltransferase, putative vacuolar SNARE complex protein^a</u>	<u>ex</u>	<u>4.01</u>	<u>5.46</u>	<u>3.20</u>	
<u>YPT31</u>	<u>Rab family GTPase^a</u>	<u>ex</u>	<u>1.57</u>	<u>1.33</u>	<u>3.33</u>	
Vacuole						
<u>VAC8</u>	<u>Protein involved in vacuolar inheritance^a</u>	<u>2.79</u>	<u>ex</u>	<u>ex</u>	<u>3.83</u>	
Mitochondrion						
MIR1	Putative mitochondrial phosphate transporter ^a	3.68	4.38	4.24	1.60	
POR1	Mitochondrial outer membrane porin ^a	3.44	2.51	3.69	1.38	

^a Protein localisation was inferred from sequence similarity with *S. cerevisiae* homolog as annotated in the Candida Genome Database (48, 49).

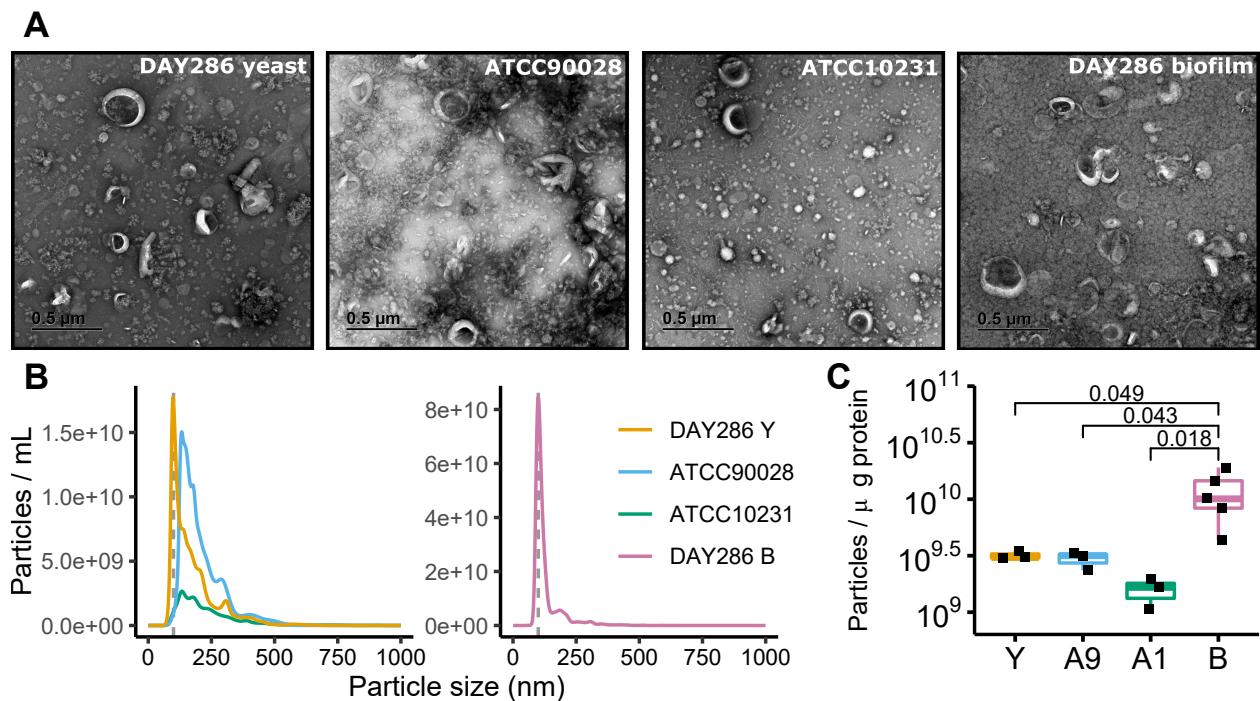
^b Protein localisation was obtained from the GO Cellular Component annotation in the *C. albicans* UniProt reference proteome UP000000559 (58).

^c Presence of transmembrane domains and absence of a signal peptide was predicted using TOPCONS2 (59).

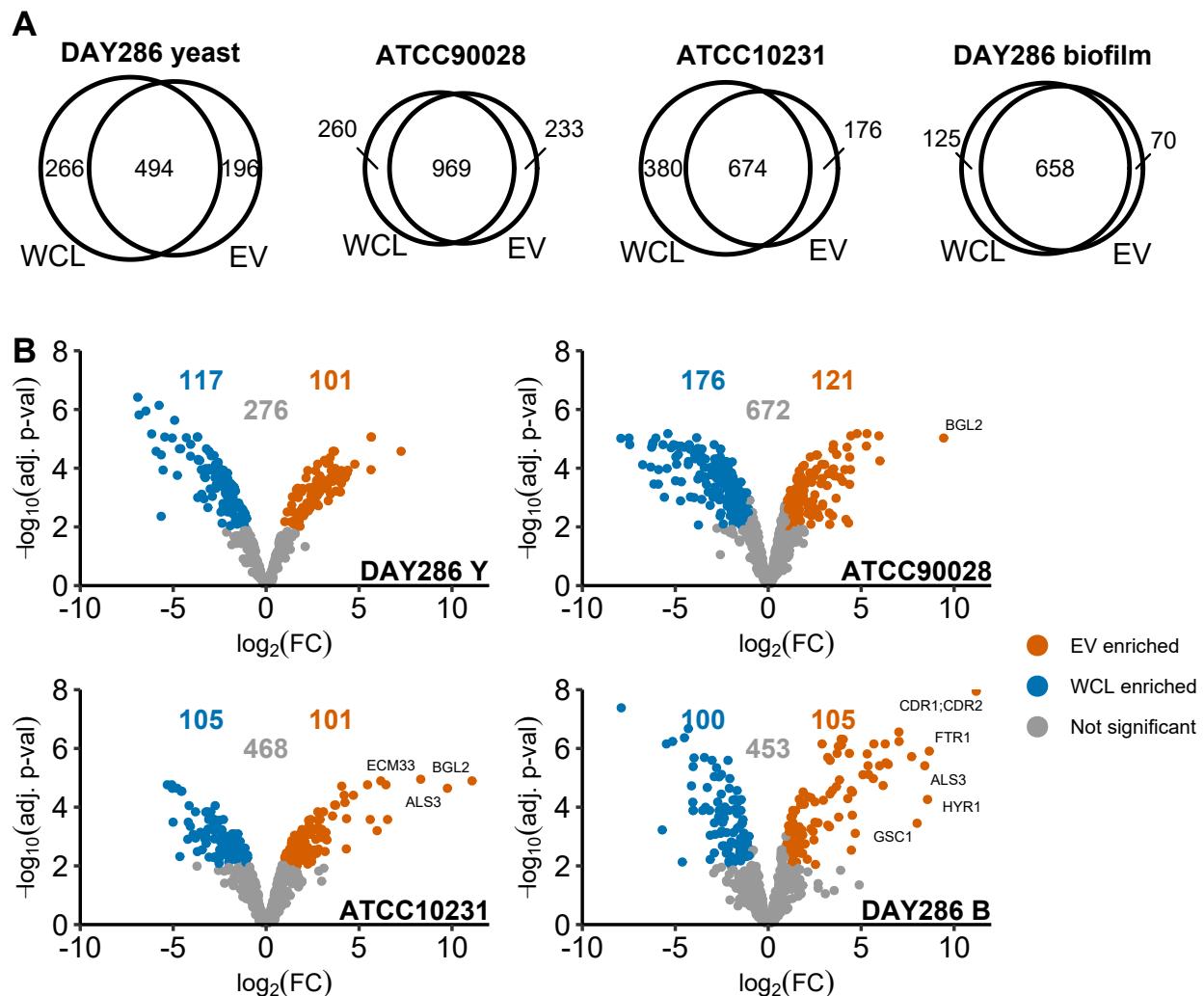
^d The name Evp1 for the protein encoded by orf19.6741 was proposed in the present study.

2 Figures

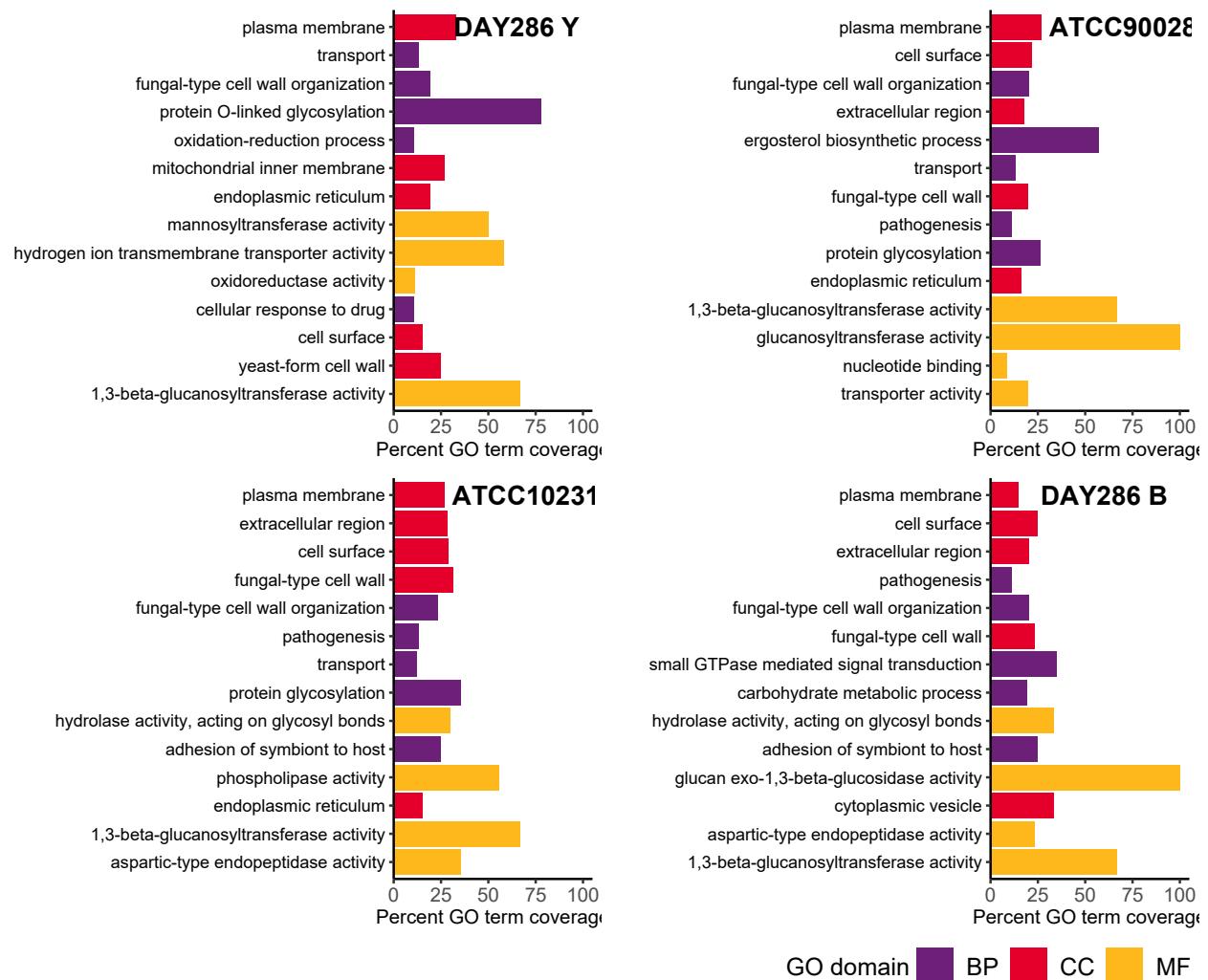
2.1 Figure 1



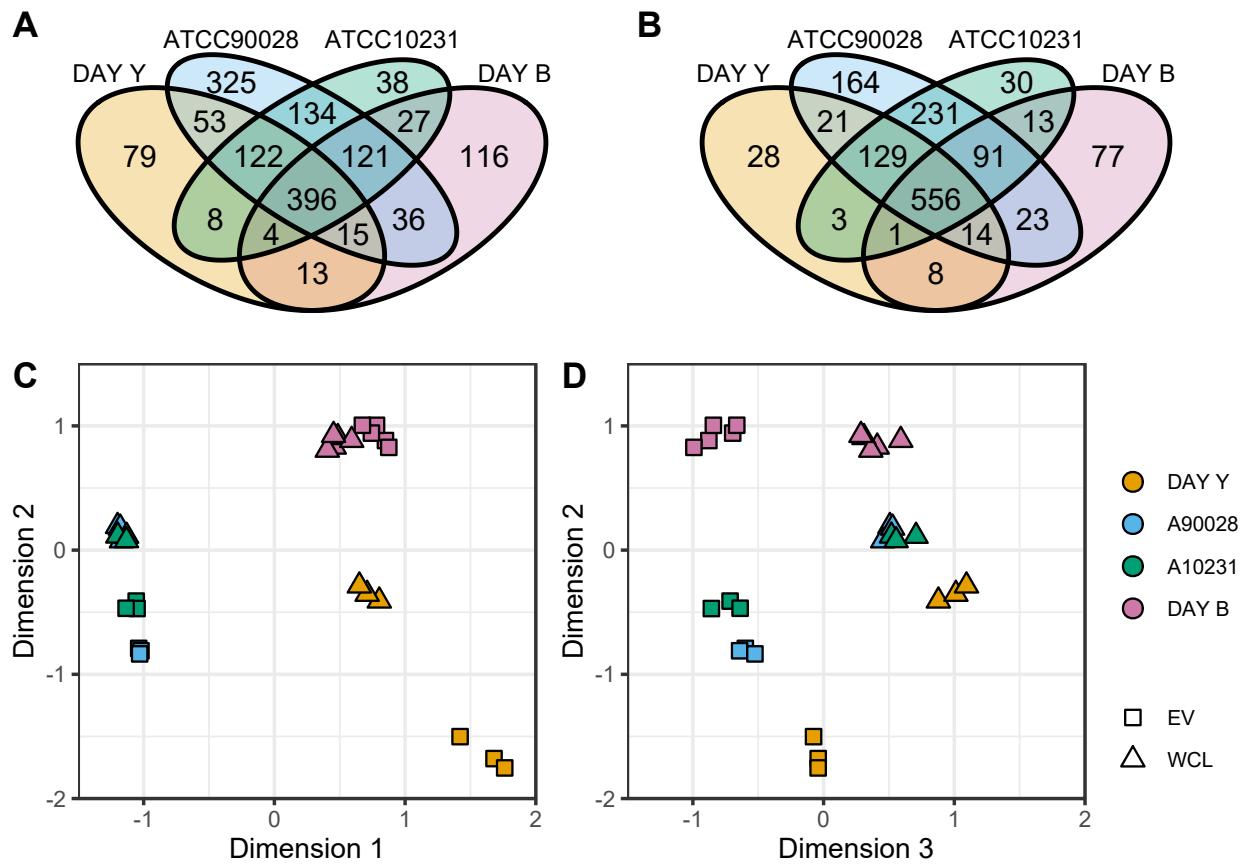
2.2 Figure 2



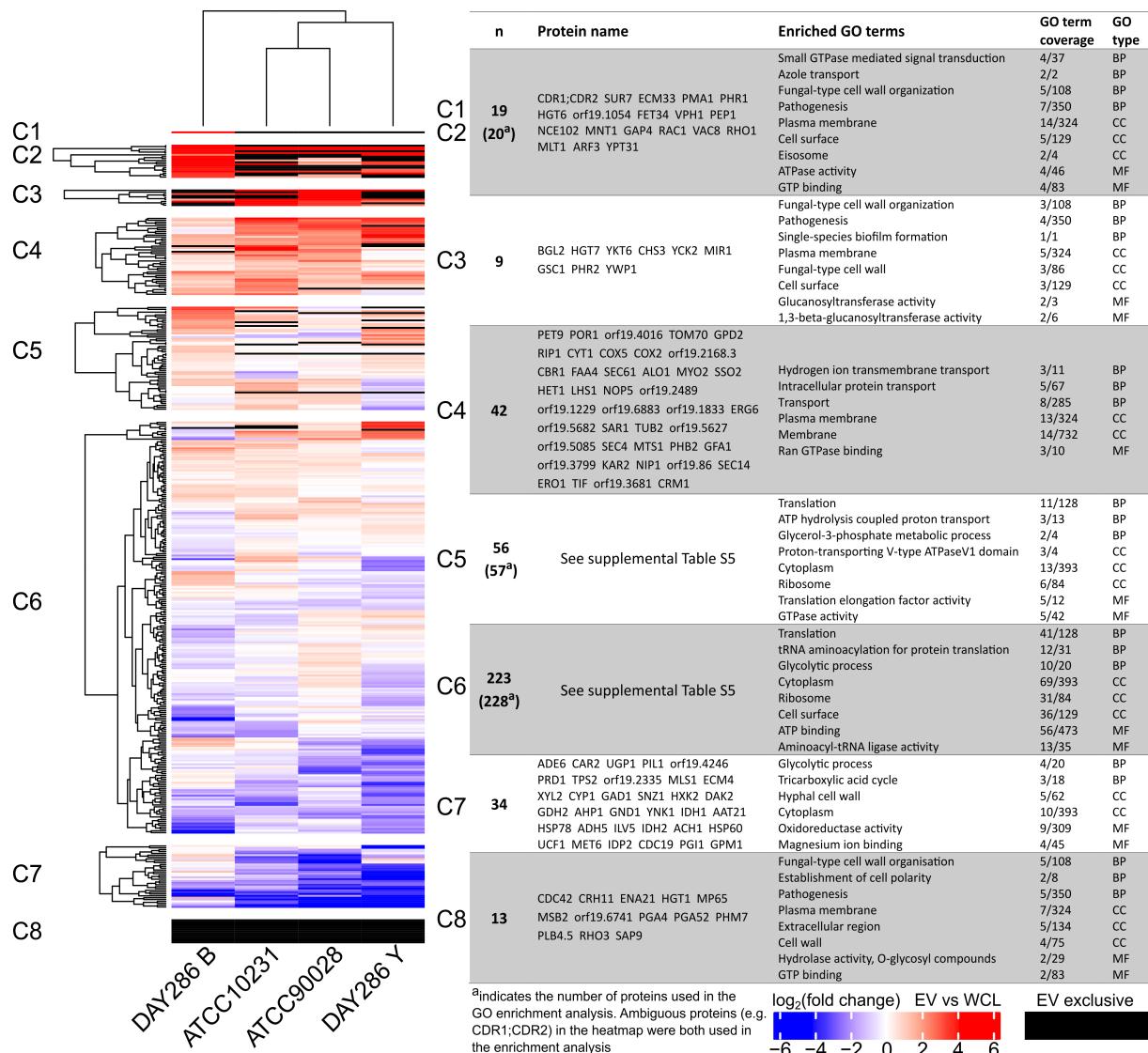
2.3 Figure 3



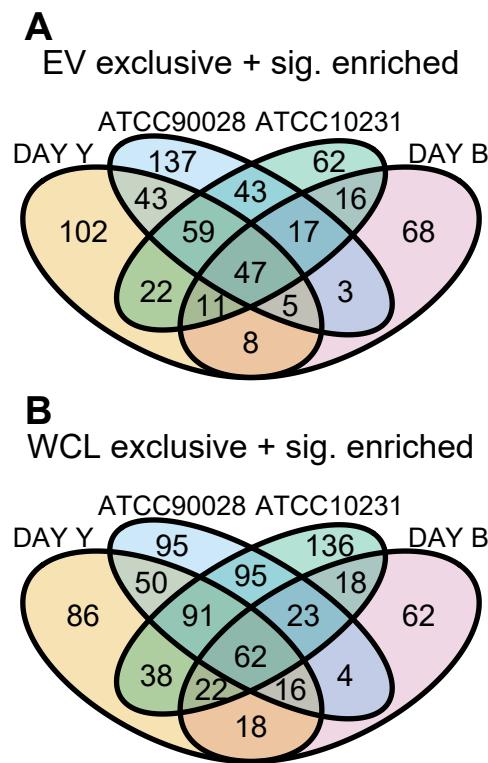
2.4 Figure 4



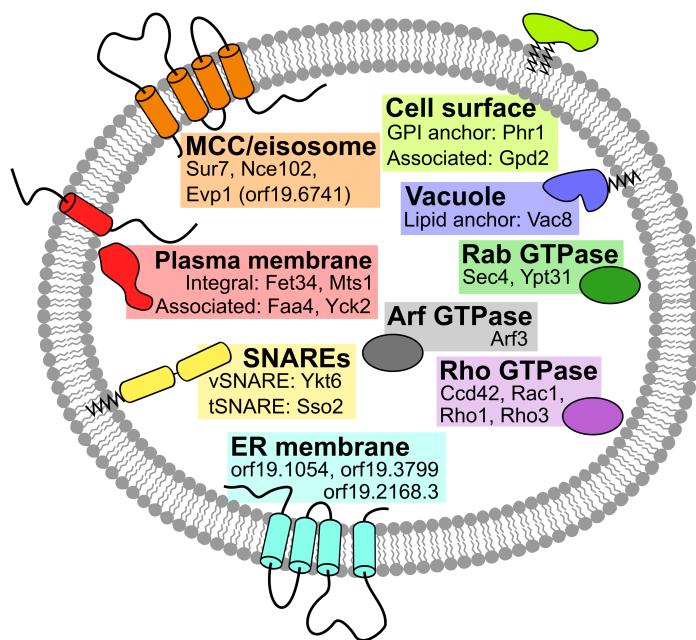
2.5 Figure 5



2.6 Figure 6



2.7 Figure 7



2.8 Figure 8

