

## **Supplementary Data**

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**Table S3.** Genes regulated 2.5 fold or greater in *C. dubliniensis* following 1h in H<sub>2</sub>O plus 10% calf serum

**Table S4.** Genes regulated 2.5 fold or greater in *C. dubliniensis* following 3h in H<sub>2</sub>O plus 10% calf serum

**Table S5.** *Candida albicans* genes expressed during hyphal growth not significantly regulated in *C. dubliniensis* or with no reciprocal orthologue in *C. dubliniensis*

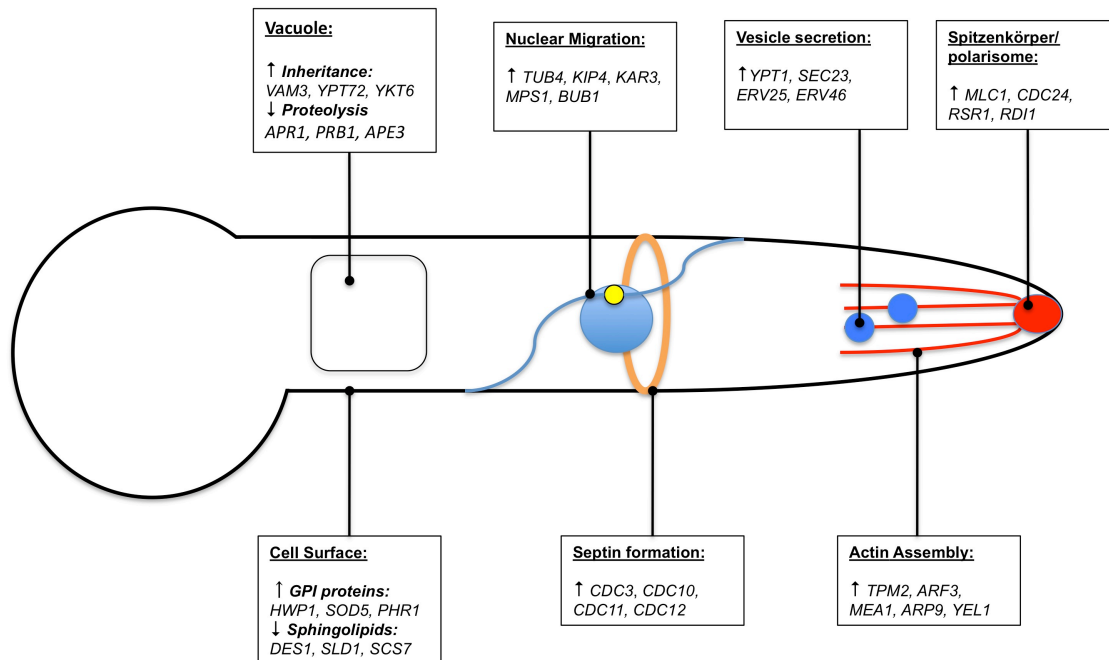
**Table S1.** Genotypes of strains used in this study

Strain	Parent	Genotype	ITS genotype	Reference
<i>C. albicans</i>				
SC5314	-	WT	NA	(4)
CaEGFP	SC5314	<i>CDR1/cdr1::P<sub>ECE1</sub>-GFP</i>	NA	This study
CAI4	SC5314	<i>ura3::imm434/ura3::imm434</i>	NA	(1)
MMC3	CAI4	<i>ura3::imm434/ura3::imm434, nrg1::hisG-URA3-hisG/nrg1::hisG</i>	NA	(9)
132A	-	WT	NA	(2)
NCPF3153	-	WT	NA	(5)
KJ	-	WT	NA	(7)
JP10	-	WT	NA	(3)
529		WT	NA	(10)
<i>C. dubliniensis</i>				
Wü284	-	WT	genotype I	(8)
WüEGFP	Wü284	<i>CDR1/cdr1::P<sub>ECE1</sub>-GFP</i>	genotype I	This study
WüUME6	Wü284	<i>ADH1/adh1::pCaUME6</i>	genotype I	This study
WüNRG1	Wü284	<i>ADH1/adh1::pNRG1</i>	genotype I	This study
CDM10	Wü284	<i>nrg1::FRT/nrg1::FRT</i>	genotype I	(7)
M10EGFP	CDM10	<i>nrg1::FRT/nrg1::FRT, CDR1/cdr1::P<sub>ECE1</sub>-GFP</i>	genotype I	This study
M10UME6	CDM10	<i>ADH1/adh1::pCaUME6</i>	genotype I	This study
M10NRG1	CDM10	<i>ADH1/adh1::pNRG1</i>	genotype I	This study
CDM11	CDM10	<i>nrg1::FRT/nrg1::FRT, CDR1/cdr1::pCaNRG1</i>	genotype I	(7)

CD36	-	WT	genotype I	(11)
CD57	-	WT	genotype I	(7)
CD38	-	WT	genotype I	(11)
CBS8500	-	WT	genotype I	(6)
CM1	-	WT	genotype I	(11)
CAN6	-	WT	genotype II	(3)
CD506	-	WT	genotype II	(3)
CD539	-	WT	genotype II	(3)
CD41	-	WT	genotype II	(7)
CD519	-	WT	genotype III	(3)
p7718	-	WT	genotype IV	(3)

**Table S2.** Oligonucleotide primers used for real-time PCR

Oligo Name	Sequence 5'-3'
NRG1a	GTCTGCAAAGTGTGTTTCGAG
NRG1b	GACGAGCAAAACGGGCTTCA
QRTTEF1R	CCACTGAAGTCAAGTCCGTTGA
QRTTEF1F	CACCTTCAGCCAATTGTTTCG
UME6F2	ACCACCACTACCACCACCAC
UME6R1	TATCCCCATTTCCAAGTCCA

**Figure S1.**

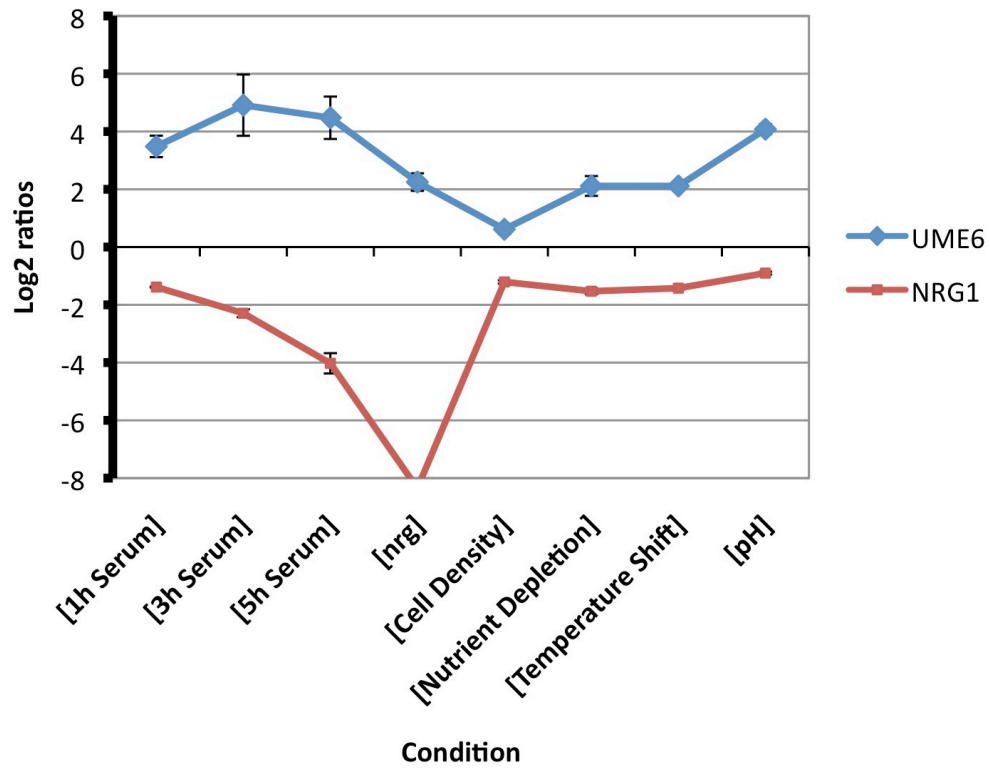
**Figure S1.** Graphic representation of processes regulated during the early stages of filamentation in *Candida dubliniensis*. Polarised growth in *C. dubliniensis* was associated with changes in the expression of genes encoding components of the cytoskeleton including genes involved in the assembly of actin cables (*TPM2*, *ARF3*, *MEA1*, *ARP9* and *YEL1*) along with increased transcription of the Spitzenkörper associated protein Mlc1. Upregulation of several genes with roles in regulating polarised growth was also observed including *CDC24*, encoding a GDP-GTP exchange factor for Cdc42p and several other GTPases with roles in actin organisation including *RSR1*, *RAC1*, *RDI1* and *RHO3*. We also observed increased expression of a significant number of genes annotated with the GO terms ‘cellular bud neck’ (n=18, p 0.07; Table S1) and the ‘septin ring’

(n=7, p 0.039; Table S1), including the septins *CDC3*, *CDC10*, *CDC11*, *CDC12* (Fig. S3). Increased expression of *GIN4*, involved in the formation of the barrier septum was observed later at 3h. Transport functions were largely associated with intracellular localisation (65 genes; Table S1), in particular, golgi-vesicle transport and secretion (27 genes and 7 genes respectively). These proteins are likely involved in the movement of secretory vesicles along actin cables to the growing hyphal tip (Fig. S2). We observed increases in expression of genes involved in nuclear migration and division including proteins of the mitotic spindle (*TUB4*, *KIP4*) and of the spindle pole body (*KAR3*, *MPS1* and *BUB1*; Fig. S2). Upregulated genes annotated with the GO term 'Cell cycle' (n=44; Table S1) were largely associated with DNA replication (n=19, p 0.009; Table S1) and mitotic sister chromatid segregation (15, p 0.006; Table S1). We identified down regulation of 27 genes annotated as a component of the vacuole (GO component term 'vacuole'). In particular, we noted significant down regulation of genes associated with vacuolar protein catabolysis (8/10 annotated genes: *CPY1*, *LAP41*, *APR1*, *PRB1*, *APE3*, *PRC2*, *VPS13*, orf19.7196), suggesting a shut down in autophagic processes. However, increased expression of genes with roles in vacuolar biogenesis and inheritance was also observed (*VAM3*, *YPT7*, *YPT72* and *YKT6*).

Reorganisation of the plasma membrane and cell surface was also evident with an increase in transcription of genes involved in the early stages of fatty acid synthesis (*ACC1*, *FAD3*, *FAS1*, orf19.6343) and a significant decrease in sphingolipid metabolism (9/25 annotated genes; *SLD1*, *YDC1*, *SCS7*, orf19.399, *DES1*, *HSX11*, orf19.5257, orf19.6951, *NCRI*; Table S1). Major reorganisation of the protein components of the cell surface was also evident with increased expression of 20 genes encoding cell wall

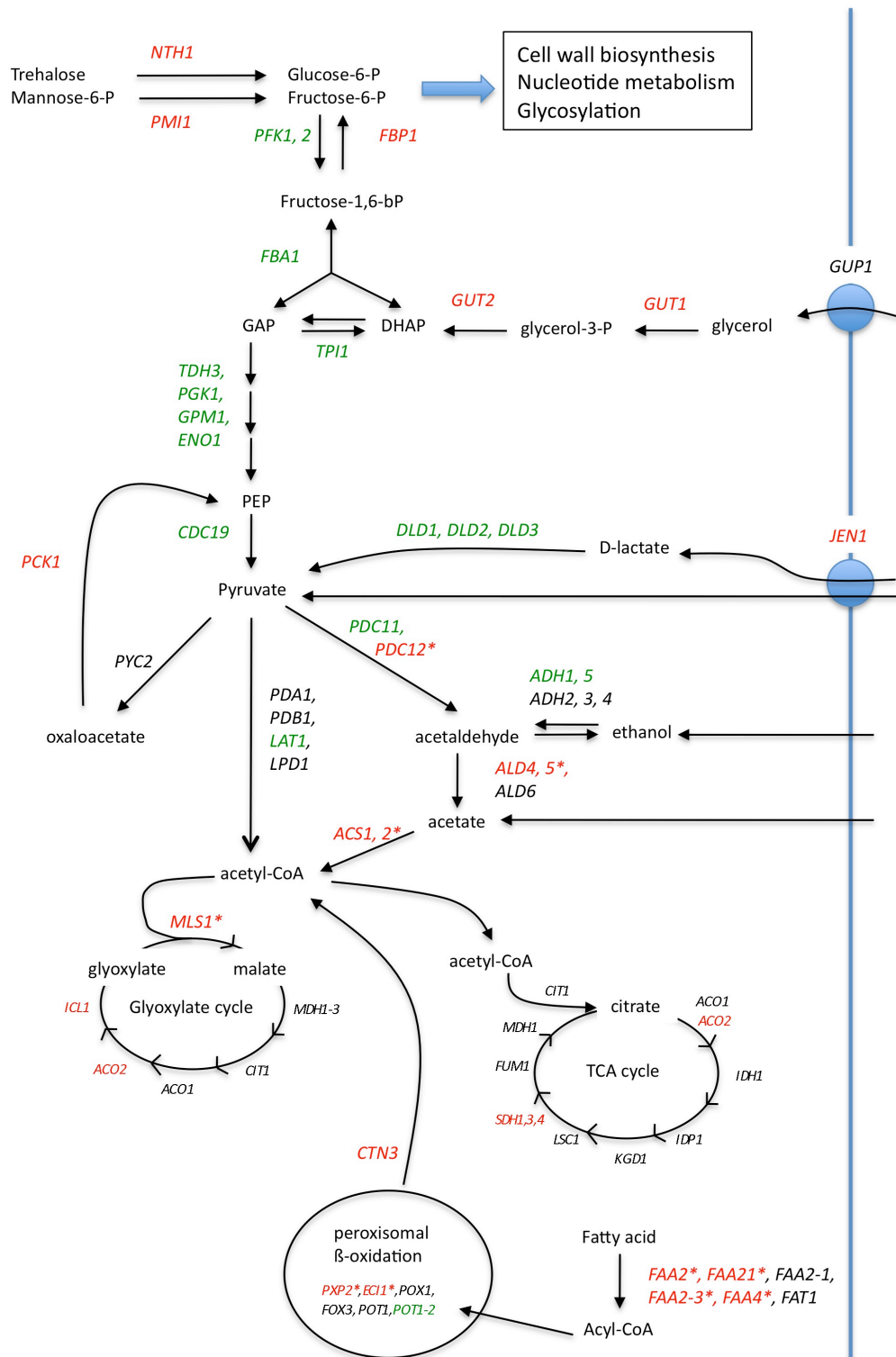
proteins and down-regulation of a further 37 cell wall protein encoding genes (Table S1). This was associated with an increase in expression of genes associated GPI anchor biosynthesis (*DPM1*, *MCD4*, *orf19.538*) and glycosylation (*PMII*, *GFA1*, *ALG6*, *ALG7*, *orf19.2298*, *ALG5*, *DPM1*, *PMT2*, *orf19.7426*, *PMT5*).

Figure S2



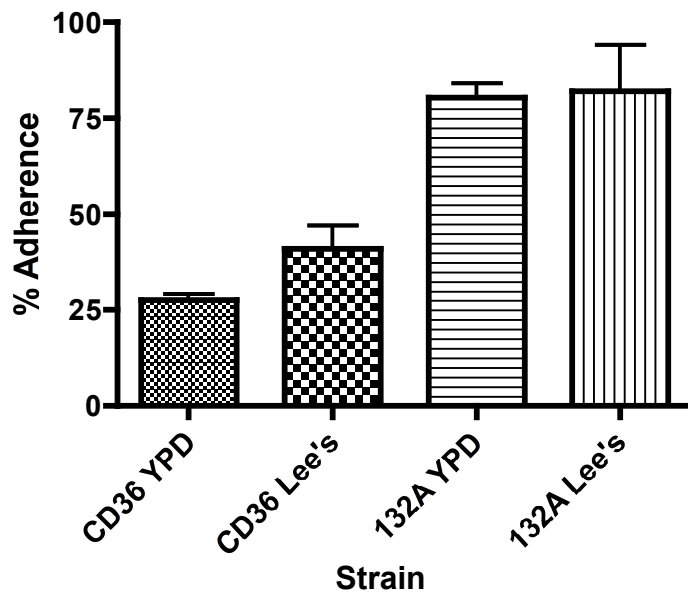
**Figure S2.** Regulation of *UME6* and *NRG1* in *C. dubliniensis* during microarray analysis. Expression levels shown are the average data from two unique oligonucleotide probes spotted in quadruplicate on each array. Each array experiment was performed with four biological replicates. The average expression from both oligonucleotides is displayed with standard deviations.

Figure S3.





**Figure S3.** Metabolic map showing the changes in metabolic gene expression in *Candida dubliniensis* during hypha formation in water plus 10% calf serum. Genes in red exhibited a 2.5-fold or greater increase in expression relative to preculture cells. Genes in green exhibited a 2.5-fold or greater reduction in expression. Genes exhibited a significant change in expression at 1 h ( $p < 0.015$ ) unless marked with an asterisk, indicating a significant change detected at 3h. Notable metabolic changes highlighted here include down regulation of genes encoding glycolytic enzymes by 1h (including *PGK1*, *ENO1*, *PFK1*, *FBA1*, *PFK2* and *TPI*). Alternative carbon source utilisation was evident due to the increased expression of genes involved in glycerol fermentation (*GUT1*, *GUT2*) and the glyoxylate cycle (*ICL1*, *ACO2*; Fig. S4). At 3H, fatty acid utilization was evident with increased expression of genes involved in the fatty acid beta-oxidation (*ECI1*, *PXP2*, *FAA21*, *FAA23*, *FAA24* and *FAA4*). The change in nutrient availability was also reflected by increased expression of genes involved in carbohydrate uptake (*HGT2*, *MAL31*, *HGT1*, *HGT10*, *HUT1*, *HGT12*) and amino acid uptake (*CAN2*, *HIP1*, *GPT1*, *CAN3*).

**Figure S4.**

**Figure S4.** Adherence of *C. dubliniensis* CD36 and *C. albicans* 132A to TR146 cell monolayers. Cells were precultured in Lee's medium (pH 4.5) at 30°C or YPD at 37°C as indicated. Adherence was measured following 90 min incubation with TR146 monolayers (see materials and methods).

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