SUPPLEMENTAL MATERIAL

Table S1. Relative morphological distribution of genes induced by UME6 during the yeast-pseudohyphal-hyphal transition (time course experiment).

Table S2. Primers used in this study.

Supplemental Reference.

Figure S1. Cell images of pseudohyphal populations. Aliquots of cells from the 0.1 μg/mL Dox concentration of the UME6 steady state culture (dosage) experiment (A) or – Dox 3 hr. time point of the forward yeast-pseudohyphal-hyphal transition time course experiment (B) were fixed in 4.5% formaldehyde, washed twice in 1X PBS and visualized using DIC microscopy. Bar = 10 μm.

Figure S2. Large cluster diagrams of genes showing expression changes in the UME6 steady state culture (dosage) and forward transition time course experiments. (A) Cluster diagram of all genes showing ≥ 2-fold change in expression in at least one data point of the UME6 steady state culture (dosage) experiment with greater than 80% of data present. Each data point represents fold change in gene expression relative to the 20 μg/mL Dox culture. (B) Cluster diagram of all genes showing ≥ 2-fold change in expression in at least one data point of the forward transition time course experiment with greater than 80% of data present. Each data point represents fold change in gene expression relative to the 0 hr. time point. For both (A) and (B), data represents mean expression values based on two independent DNA microarray experiments (n= 2 biological replicates). Blue, increased expression; yellow, reduced expression; gray, no data.

Figure S3. Similar gene classes are represented in the sets of genes induced in pseudohyphae and hyphae generated by UME6 expression levels in a steady state culture. Genes induced in hyphae at least 2-fold in response to constitutive high-level UME6 expression in the absence of Dox (as defined in Table 1 and described in Dataset S1) and genes induced in pseudohyphae at least 2-fold in response to intermediate levels of UME6 expression in the presence of 0.1 μg/mL Dox (as defined in Table 1 and described in Dataset S2) were categorized by biological process and represented as a percentage of the entire hyphal-induced and pseudohyphal-induced gene sets, respectively. The GO Slim Mapper tool, available at the Candida Genome Database, was used to classify genes based on process ontology.

Figure S4. Similar gene classes are represented in the sets of genes induced in pseudohyphae and hyphae generated by UME6 expression over a time course. Genes induced in hyphae at least 2-fold at the 10 hr. time point in the absence of Dox relative to the 0 hr. time point (as defined in Table S1 and listed in Dataset S3) and genes induced in pseudohyphae at least 2-fold at the 3 hr. time point in the absence of Dox relative to the 0 hr. time point (as defined in Table S1 and listed in Dataset S4) were categorized by biological process and represented as a percentage of the entire hyphal-induced and pseudohyphal-induced gene sets, respectively. The GO Slim Mapper tool, available at the Candida Genome Database, was used to classify genes based on process ontology.
Figure S5. Correlation of gene expression values obtained using DNA microarray versus real time quantitative RT-PCR data for the reverse hyphal-pseudohyphal-yeast transition time course. For each gene, graphs represent mean change in gene expression (n=2) as determined by DNA microarray (x-coordinate) plotted against mean gene expression changes (n=3) determined using real time quantitative RT-PCR (y-coordinate) (values in log2). Pearson’s Correlation Coefficient (r-value) was determined for each graph and statistical significance was determined using the Student’s t-test (p ≤ 0.01).

Figure S6. Representation of gene classes in the sets of genes showing reduced expression in pseudohyphae and yeast as C. albicans undergoes the reverse hyphal-pseudohyphal-yeast transition. Genes reduced in yeast at least 2-fold at the 10 hr. time point in the presence of Dox, relative to the 0 hr. time point (as defined in Table 3 and described in Dataset S5) and genes reduced in pseudohyphae at least 2-fold at the 3 hr. time point in the presence of Dox, relative to the 0 hr. time point (as defined in Table 3 and described in Dataset S6), were categorized by biological process and represented as a percentage of the entire yeast-reduced and pseudohyphal-reduced gene sets, respectively. The GO Slim Mapper tool, available at the Candida Genome Database, was used to classify genes based on process ontology.

Figure S7. Gene classes overrepresented, compared to their representation in the genome as a whole, in the set of genes showing reduced expression as C. albicans undergoes the reverse hyphal-pseudohyphal-yeast transition in response to depletion of UME6. This histogram represents a continuation of the histogram shown in Figure 7. Only data for gene classes that represent < 7% of the gene set as a whole are shown.

Dataset S1. Genes induced in hyphae generated by constitutive high-level UME6 expression in a steady state culture. All genes show a > 2-fold mean induction (n=2) in the absence vs. presence of 20 μg/mL Dox and are defined as described in Table 1.

Dataset S2. Genes induced in pseudohyphae generated by constitutive intermediate-level UME6 expression in a steady state culture. All genes show a > 2-fold mean induction (n=2) in the presence of 0.1 μg/mL vs. 20 μg/mL Dox and are defined in Table 1.

Dataset S3. Genes induced in hyphae generated by UME6 expression over a time course. All genes show a mean induction > 2-fold at the 10 hr. time point in the absence of Dox relative to the 0 hr. time point (n=2) as defined in Table S1.

Dataset S4. Genes induced in pseudohyphae generated by UME6 expression over a time course. All genes show a mean induction > 2-fold at the 3 hr. time point in the absence of Dox relative to the 0 hr. time point (n=2) as defined in Table S1.

Dataset S5. Genes showing reduced expression in yeast as C. albicans undergoes the reverse hyphal-pseudohyphal-yeast transition in response to UME6 depletion over a time course. All genes show a mean reduction > 2-fold at the 10 hr. time point in the presence of Dox relative to the 0 hr. time point (n=2) as defined in Table 3.
**Dataset S6.** Genes showing reduced expression in pseudohyphae as *C. albicans* undergoes the reverse hyphal-pseudohyphal-yeast transition in response to *UME6* depletion over a time course. All genes show a mean reduction > 2-fold at the 3 hr. time point in the presence of Dox relative to the 0 hr. time point (n=2) as defined in Table 3.

**Dataset S7.** Complete set of gene expression values for the forward yeast-pseudohyphal-hyphal transition in response to *UME6* dosage in a steady state culture.

**Dataset S8.** Complete set of gene expression values for the forward yeast-pseudohyphal-hyphal transition in response to *UME6* expression over a time course.

**Dataset S9.** Complete set of gene expression values for the reverse hyphal-pseudohyphal-yeast transition in response to *UME6* depletion over a time course.

**Dataset S10.** Complete set of gene expression values for the *tetR-HAP4* control strain grown in the absence vs. presence of 20 µg/mL Dox.

**Dataset S11.** Genes showing reduced expression in hyphae generated by constitutive high-level *UME6* expression in a steady state culture. All genes show a > 2-fold mean reduction (n=2) and are reduced at least 2-fold in two independent experiments (n=2) in the absence vs. presence of 20 µg/mL Dox.

**Dataset S12.** Genes showing reduced expression in hyphae generated by *UME6* expression over a time course. All genes show a mean reduction > 2-fold (n=2) and are reduced at least 2-fold in two independent experiments (n=2) at the 10 hr. time point in the absence of Dox relative to the 0 hr. time point. Data excludes genes showing at least 2-fold reduced expression in two independent experiments (n=2) at the 10 hr. time point + 20 µg/mL Dox relative to the 0 hr. time point.

**Dataset S13.** Genes induced in yeast as *C. albicans* undergoes the reverse hyphal-pseudohyphal-yeast transition in response to *UME6* depletion over a time course. All genes show a mean induction > 2-fold (n=2) and at least 2-fold induction in two independent experiments (n=2) at the 10 hr. time point in the presence of Dox relative to the 0 hr. time point. Data excludes genes showing at least 2-fold induced expression in two independent experiments (n=2) at the 10 hr. time point in the absence of Dox relative to the 0 hr. time point.

**Dataset S14.** Genes showing reduced expression in pseudohyphae generated by constitutive intermediate-level *UME6* expression in a steady state culture. All genes show a > 2-fold mean reduction (n=2) and at least 2-fold reduced expression in two independent experiments (n=2) in 0.1 µg/mL vs. 20 µg/mL Dox.

**Dataset S15.** Genes showing reduced expression in pseudohyphae generated by *UME6* expression over a time course. All genes show a mean reduction > 2-fold (n=2) and at least 2-fold reduced expression in two independent experiments (n=2) at the 3 hr. time point in the absence of Dox relative to the 0 hr. time point. Data excludes genes showing at least 2-fold reduced
expression in two independent experiments (n=2) at the 3 hr. time point + 20 µg/mL Dox relative to the 0 hr. time point.
Table S1. Relative morphological distribution of genes induced by *UME6* during the yeast-pseudohyphal-hyphal transition (time course experiment).

| Fold change relative to yeast expression | > 3-fold | > 4-fold | > 10-fold |
|-----------------------------------------|----------|----------|-----------|
| # of genes up in hyphal cells*          | 163      | 104      | 33        |
| % of genes also up > 2-fold in pseudohyphal cells† | 15%      | 20%      | 27%       |
| % of genes also up > 4-fold in pseudohyphal cells§ | 8%       | 12%      | 18%       |
| % of genes also up > 10-fold in pseudohyphal cells§ | 2%       | 4%       | 9%        |

| # of genes up in pseudohyphal cells* | > 2-fold | > 3-fold | > 4-fold |
|-------------------------------------|----------|----------|----------|
| 44                                  | 27       | 15       |
| % of genes also up >2-fold in hyphal cells† | 84%      | 100%     | 100%     |
| % of genes also up >4-fold in hyphal cells§ | 68%      | 89%      | 93%      |
| % of genes also up >10-fold in hyphal cells§ | 34%      | 48%      | 47%      |

* Fold changes are based on mean gene expression values from two independent experiments (n=2). All genes were induced at least 2-fold in both experiments.

† Percentage of genes showing an induction of at least 2-fold in two independent experiments (n=2) in the indicated cell morphology.

§ Percentage of genes showing the indicated mean fold induction in the indicated morphology based on two independent experiments (n=2). All genes were induced at least 2-fold in both experiments.

Data excludes genes with expression values affected in a control strain by Dox alone (as determined by the tetR-*HAP4* control experiment) and genes induced at least 2-fold in two independent experiments (n=2) in the *tetO-UME6* strain time course control culture (for genes up in pseudohyphal cells, control sample is 3 hour time point +Dox; for genes up in hyphal cells, control sample is 10 hour time point +Dox). The *tetO-UME6* strain was grown overnight in YEPD + 1 μg/mL Dox at 30°C to OD₆₀₀ ~ 0.5, cells were washed 1X with YEPD, diluted into prewarmed YEPD–Dox at 30°C and grown over a 10 hour time course. Yeast cells = 0 hr. time point prior to removal of Dox, pseudohyphal cells = 3 hr. time point and hyphal cells = 10 hr. time point.
| #  | Primer Name | Sequence                  | Description                   |
|----|-------------|---------------------------|-------------------------------|
| 1  | DTO85       | TTGCTCCAGAAGAACATCCAG     | 5’ *ACT1* primer for RT-qPCR  |
| 2  | DTO86       | AGTAACACCACATCACCAGAATCC  | 3’ *ACT1* primer for RT-qPCR  |
| 3  | DTO167      | GAACAAATGGTGTTGGTAGTGG    | 5’ *UME*6 primer for RT-qPCR  |
| 4  | DTO168      | AATTCGACAAATCACCACATCC    | 3’ *UME*6 primer for RT-qPCR  |
| 5  | PCO91       | GATTGCTCGGCTATTTCTGC      | 5’ *PHR1* primer for RT-qPCR  |
| 6  | PCO92       | CTTCACCAGAGGAAGATGC       | 3’ *PHR1* primer for RT-qPCR  |
| 7  | PCO94       | GGTTCCTGCTCTCAACTGG       | 5’ *HYR1* primer for RT-qPCR  |
| 8  | PCO95       | CCTGAACCTTCGTTGATCC       | 3’ *HYR1* primer for RT-qPCR  |
| 9  | PCO96       | CCAGAAATTGTCTCGTTGTG*      | 5’ *ECE1* primer for RT-qPCR  |
| 10 | PCO97       | CAGGACGCCTCAAAAAACG*      | 3’ *ECE1* primer for RT-qPCR  |
| 11 | PCO106      | GTATCGCTGTTCTCGTGC        | 5’ *HGC1* primer for RT-qPCR  |
| 12 | PCO107      | GACTCCACTCATAACACTACC     | 3’ *HGC1* primer for RT-qPCR  |
| 13 | PCO108      | CTCCAGCCTGAAACACC         | 5’ *HWP1* primer for RT-qPCR  |
| 14 | PCO109      | TCCATAGGGATGGAAAGGC       | 3’ *HWP1* primer for RT-qPCR  |

*Primer sequences obtained from Cleary et al., 2010 (1).
Supplemental Reference

1. Cleary IA, Mulabagal P, Reinhard SM, Yadev NP, Murdoch, C, et al. (2010) Pseudohyphal regulation by the transcription factor Rfg1p in *Candida albicans*. Eukaryot. Cell 9:1363-1373.
Figure S3
Figure S5
Figure S6
