SUPPORTING INFORMATION

SUPPLEMENTAL MATERIALS AND METHODS

Strain and DNA Constructions (detailed description)

The *ume6Δ/+* strain (DT208) was constructed as follows: approximately 500 bp of 5' and 3' flanking sequences were generated using primer pairs 1/2 and 3/4 (see Table S1 for a complete listing of primers used in this study), respectively. The 5' flank is located immediately upstream of the region -19.3 kb from the *UME6* start codon and the 3' flank is immediately downstream of the *UME6* coding sequence. The 5' and 3' flanks were next digested with BamHI-HindIII and NotI-ApaI, respectively, and cloned into plasmid pSN52 [1]. The *ume6Δ::HIS1* construct was generated by PCR from the resulting plasmid using primer pair 5/6 and transformed into strain SN152 to generate *ume6Δ/+* (DT208). *ume6Δ/+* was next used as a parent strain to generate *UME6* upstream intergenic region deletion strains DT297, DT290, DT281, DT315, DT299 and DT341 using the *SAT1* flipper technique [2]. Briefly, a 5' flanking sequence immediately adjacent to the *UME6* -19.3 kb region was generated using primer pair 7/8, digested with KpnI and XhoI, and cloned into pSFS2 to generate plasmid 5' *ume6Δ/+* pSFS2. 3' flanking sequences adjacent to the *UME6* -9.1 kb, -8.0 kb, -7.0 kb, -6.0 kb, -5.0 kb and -4.2 kb regions were generated using primers 9-20, digested with NotI and SacII, and cloned into 5' *ume6Δ/+* pSFS2. The resulting constructs were digested with KpnI and SacII to release each respective *SAT1*-marked deletion construct, which, in turn, was used to transform the *ume6Δ/+* strain. The *SAT1* marker was then looped out of each resulting *UME6* upstream intergenic region deletion strain as described previously [2]. To generate the *UME6*$_{-6kb\ UR}$-*lacZ* strain, *UME6* upstream intergenic regions from -1801 to -6011 and -1 to -2541 were amplified using primer pairs 21/22 and 23/24, respectively. The resulting PCR products were digested with PstI-HindIII and HindIII-PstI, respectively, and cloned separately into plasmid pBS [3]. The *UME6* -1 to -2077 bp fragment was released from pBS by digesting with SphI and

HindIII and the *UME6* ~ -2077 bp to -6011 bp fragment was released from pBS by digesting with HindIII and PstI. A 3-piece ligation was used to clone both fragments into plasmid placbasal digested with PstI and SphI, generating plasmid pUME6-6kb UR-lac. placbasal was also digested with XhoI and SalI, filled-in using Klenow DNA polymerase, and religated to generate plasmid plac. Both plasmids pUME6-6 kb UR-lac and plac were linearized with StuI at *RPS1* and transformed into CAI4 to generate the final *UME6-6 kb UR-lacZ* and *lacZ* strains, respectively. The *UME6 5' utrΔ/Δ* strain was generated using the *SAT1* flipper method [2]. Briefly, 5' and allele-specific 3' flanks immediately adjacent to the *UME6* 5' UTR were amplified using primer pairs 25/26, 27 /28 and 27 /29, respectively. The 5' flank was digested with KpnI and XhoI and each 3' flank was digested with NotI and SacII. The digested flanks were cloned into pSFS2 and the resulting plasmids were digested with KpnI and SacII to release the *UME6 5' utrΔ::SAT1* fragments, which were sequentially used to transform wild-type strain DK318. Following deletion of each *UME6 5' UTR* allele, the *SAT1* marker was looped out, ultimately generating the final *UME6 5' utrΔ/Δ* strain. To construct the *5' utrΔ-UME6-MYC* strain (DT640), *UME6-MYC* (HLY4078) was transformed with the *UME6 5' utrΔ::SAT1* fragment described above to delete the 5' UTR of the Myc-tagged *UME6* allele. The *SAT1* marker was then looped out to generate the final *5' utrΔ-UME6-MYC* strain. To construct *tetO-5'UTR-UME6* strain (DT553), 5' and 3' flanking sequences adjacent to the *UME6 5' UTR* were amplified using primer pairs 30/31 and 32/33, respectively. The 5' flank spans -3134 bp to -3696 bp and the 3' flank spans -2514 bp to -3072 bp relative to the *UME6* start codon (+1). The 5' flank was digested with KpnI and XhoI and the 3' flank was digested with SpeI and SacII. Both flanks were cloned into p97CAU1 to generate plasmid p97CAU1-5'UTR-UME6-OEx. This plasmid was digested with KpnI and SacII to release a fragment containing the *URA3* marker, *tetO* operator and *UME6* 5' UTR flanks, which was used to transform strain THE1, ultimately generating the *tetO-5'UTR-UME6* strain (DT553). To construct the $ACT1_{pr}$- GFP plasmid, *RPS1* was amplified from

genomic DNA using primer pair 34/35, digested with SacI and SpeI, and cloned into plasmid pFA GFPγ URA3 [4]. The resulting plasmid was digested with PstI and SpeI to release a fragment containing *GFPγ, URA3* and *RPS1*, which, in turn was cloned into pBS [3] to generate pBS-GFP. The *ACT1* promoter was next amplified from plasmid CIp-LexA [5] using primer pair 36 /37, digested with KpnI and XhoI and cloned into pBS-GFP. To generate plasmid $ACT1_{pr}$-UME6-5'UTR-GFP, the *UME6* 5' UTR plus *UME6* start codon was amplified from genomic DNA using primer pair 38/39, digested with XhoI and PstI , and cloned into $ACT1_{pr}$- GFP cut with the same restriction enzymes. To generate plasmid $ACT1_{pr}$-UME6-5'UTR uorf1Δ uorf2Δ -GFP, PCR site-directed mutagenesis was performed on the $ACT1_{pr}$-UME6-5'UTR-GFP plasmid to first delete uORF1. Briefly, complementary primers (52/53), were designed incorporating flanking regions for uORF1 and the resulting PCR product was digested with DpnI and transformed into *E. coli* Z-DH5α. The recovered plasmid was then sequenced to confirm the deletion. Next, a similar strategy was used on this plasmid in order to delete uORF2 with primer pairs 54/55 and 56/57. $ACT1_{pr}$- GFP, $ACT1_{pr}$-UME6-5'UTR-GFP and $ACT1_{pr}$-UME6-5'UTR uorf1Δ uorf2Δ -GFP plasmids were linearized at *RPS1* by digestion with StuI and transformed into strain CAI4 to generate the *$ACT1_{pr}$- GFP*, *$ACT1_{pr}$-UME6-5'UTR-GFP* and *$ACT1_{pr}$-UME6-5'UTR uorf1Δ uorf2Δ -GFP* final strains. All integration events were confirmed by whole cell PCR using primers flanking the integration sites.

References

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Table S1 **Strains used in this study**

Strain	Genotype	Source
SC5314 (WT)	Prototroph	(1)
CAF2-1 (WT)	*ura3Δ::imm^{434}/ URA3 iro1Δ::imm^{434}/IRO1*	(2)
DK318 (WT)	*arg4Δ::ARG4 /arg4Δ his1Δ::HIS1/his1Δ ura3Δ::imm^{434}/URA3 iro1Δ::imm^{434}/IRO1*	(3)
CAI4	*ura3Δ::imm^{434}/ ura3Δ::imm^{434} iro1Δ::imm^{434}/iro1Δ::imm^{434}*	(2)
DK312 (*ume6Δ/Δ*)	*ume6Δ::C.m.LEU2/ume6Δ::C.d.HIS1 arg4Δ::ARG4/arg4Δ leu2Δ/leu2Δ his1Δ/his1Δ ura3Δ::imm^{434}/URA3 iro1Δ::imm^{434}/IRO1*	(3)
DT553 (*UME6 5' utrΔ/Δ*)	*arg4Δ::ARG4 /arg4Δ his1Δ::HIS1/his1Δ UME6 5' utrΔ::frt/UME6 5' utrΔ::frt ura3Δ::imm^{434}/URA3 iro1Δ::imm^{434}/IRO1*	This study
MBY38 (*tetO-UME6*)	*ade2*::*hisG/ade2*::*hisG ura3*::*imm^{434}/ura3*::*imm^{434} ENO1/eno1*::*ENO1-tetR-ScHAP4AD-3×HA-ADE2 tetO-UME6::URA3/UME6*	(4)
DT515 (*tetO-5'UTR-UME6)*	*ade2*::*hisG/ade2*::*hisG ura3*::*imm^{434}/ura3*::*imm^{434} ENO1/eno1*::*ENO1-tetR-ScHAP4AD-3×HA-ADE2 tetO-5' UTR-UME6::URA3/UME6*	This study
SN152	*arg4Δ/arg4Δ leu2Δ/leu2Δ his1Δ/his1Δ ura3Δ::imm^{434}/URA3 iro1Δ::imm^{434}/IRO1*	(5)
DT208 (*ume6Δ/+*)	*ume6$_{-19.3\ kb\ through\ ORF}$Δ::C.m.HIS1/UME6 leu2Δ /leu2Δ arg4Δ /arg4Δ ura3Δ::imm^{434}/URA3 iro1Δ::imm^{434}/IRO1*	This study

DT297 (*ume6*$_{-9.1\ kb\ to\ -19.3\ kb}$*Δ/Δ)*	*ume6*$_{-19.3\ kb\ through\ ORF}$*Δ::C.m.HIS1/ ume6*$_{-9.1kb\ to\ -19.3kb}$*Δ::frt leu2Δ /leu2Δ arg4Δ /arg4Δ ura3Δ::imm*^{434}*/URA3 iro1Δ::imm*^{434}*/IRO1*	This study
DT290 (*ume6*$_{-8.0\ kb\ to\ -19.3\ kb}$*Δ/Δ*)	*ume6*$_{-19.3\ kb\ through\ ORF}$*Δ::C.m.HIS1/ ume6*$_{-8.0\ kb\ to\ -19.3\ kb}$*Δ::frt leu2Δ /leu2Δ arg4Δ /arg4Δ ura3Δ::imm*^{434}*/URA3 iro1Δ::imm*^{434}*/IRO1*	This study
DT281 (*ume6*$_{-7.0\ kb\ to\ -19.3\ kb}$*Δ/Δ*)	*ume6*$_{-19.3\ kb\ through\ ORF}$*Δ::C.m.HIS1/ ume6*$_{-7.0\ kb\ to\ -19.3\ kb}$*Δ::frt leu2Δ /leu2Δ arg4Δ /arg4Δ ura3Δ::imm*^{434}*/URA3 iro1Δ::imm*^{434}*/IRO1*	This study
DT315 (*ume6*$_{-6.0\ kb\ to\ -19.3\ kb}$*Δ/Δ*)	*ume6*$_{-19.3\ kb\ through\ ORF}$*Δ::C.m.HIS1/ ume6*$_{-6.0\ kb\ to\ -19.3\ kb}$*Δ::frt leu2Δ /leu2Δ arg4Δ /arg4Δ ura3Δ::imm*^{434}*/URA3 iro1Δ::imm*^{434}*/IRO1*	This study
DT299 (*ume6*$_{-5.0\ kb\ to\ -19.3\ kb}$*Δ/Δ*)	*ume6*$_{-19.3\ kb\ through\ ORF}$*Δ::C.m.HIS1/ ume6*$_{-5.0\ kb\ to\ -19.3\ kb}$*Δ::frt leu2Δ /leu2Δ arg4Δ /arg4Δ ura3Δ::imm*^{434}*/URA3 iro1Δ::imm*^{434}*/IRO1*	This study
DT341 (*ume6*$_{-4.2\ kb\ to\ -19.3\ kb}$*Δ/Δ*)	*ume6*$_{-19.3\ kb\ through\ ORF}$*Δ::C.m.HIS1/ ume6*$_{-4.2\ kb\ to\ -19.3\ kb}$*Δ::frt leu2Δ /leu2Δ arg4Δ /arg4Δ ura3Δ::imm*^{434}*/URA3 iro1Δ::imm*^{434}*/IRO1*	This study
DT133 (*lacZ*)	*ura3Δ::imm*^{434}*/ ura3Δ::imm*^{434} *iro1Δ::imm*^{434}*/IRO1 rps1::S.t. lacZ - URA3/RPS1*	This study
DT389 (*UME6*$_{-6\ kb\ UR}$ *- lacZ*)	*ura3Δ::imm*^{434}*/ ura3Δ::imm*^{434} *iro1Δ::imm*^{434}*/IRO1 rps1:: UME6*$_{-6\ kb\ ur}$ *- S.t. lacZ - URA3/RPS1*	This study

DT590 (*$ACT1_{pr}$-GFP*)	*ura3Δ::imm^{434}/ ura3Δ::imm^{434} iro1Δ::imm^{434}/IRO1 rps1::$ACT1_{pr}$-GFP-URA3/RPS1*	This study
DT585 (*$ACT1_{pr}$-UME6 5' UTR-GFP*)	*ura3Δ::imm^{434}/ ura3Δ::imm^{434} iro1Δ::imm^{434}/IRO1 rps1::$ACT1_{pr}$-UME6-5'UTR-GFP-URA3/RPS1*	This study
DT625 (*$ACT1_{pr}$-UME6-5'UTR uorf1Δ uorf2Δ –GFP*)	*ura3Δ::imm^{434}/ ura3Δ::imm^{434} iro1Δ::imm^{434}/IRO1 rps1:: $ACT1_{pr}$-UME6-5'UTR uorf1Δ uorf2Δ -GFP-URA3/RPS1*	This study
HLY4078 (*UME6-MYC*)	*ura3::1 imm434/ura3::1 imm434 UME6/UME6-13MYC-URA3*	(6)
DT640 (*5' utrΔ-UME6-MYC*)	*ura3::1 imm434/ura3::1 imm434 UME6/5' utrΔ::frt-UME6-13MYC-URA3*	This study

C.d. = Candida dubliniensis
C.m. = Candida maltosa
S.t. = Streptococcus thermophilus

References

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Table S2 **Primers used in this study**

#	Primer Name	Sequence	Description
1	DTO202	agtactgaaggatccCTGTGTGTGTGTGGGTGG	5' *UME6* -19.3 kb flank upstream primer for cloning into pSN52
2	DTO203	agtactgaaaagcttCAGCAACAGCAACAGTGCC	3' *UME6* -19.3 kb flank upstream primer for cloning into pSN52
3	DTO204	agtactgaagcggccgcCAGGTTGACGGTTGACGGTGG	5' *UME6* -19.3 kb flank downstream primer for cloning into pSN52
4	DTO205	agtactgaagggcccGGGAATGAGTTACAGTTTATCGGG	3' *UME6* -19.3 kb flank downstream primer for cloning into pSN52
5	DTO186	CTGTGTGTGTGTGGGTGG	5' primer to generate *ume6Δ::HIS1*
6	DTO189	GGGAATGAGTTACAGTTTATCGGG	3' primer to generate *ume6Δ::HIS1*
7	DTO193	agtactgaaggtaccCTGTGTGTGTGTGGGTGG	5' *UME6* -19.3 kb flank upstream primer for cloning into pSFS2
8	DTO194	agtactgaactcgagCAGGTTGACGGTTGACGGTGG	3' *UME6* -19.3 kb flank upstream primer for cloning into pSFS2
9	DTO226	agtactgaagcggccgcGATGTAGTTTCCACAGCACG	5' *UME6* -9.1 kb flank downstream primer for cloning into pSFS2
10	DTO227	agtactgaaccgcggCTTGACTCTGTTTGAAGGG	3' *UME6* -9.1 kb flank downstream primer for cloning into pSFS2
11	DTO224	agtactgaagcggccgcGTATTCTATCTGGAAGTGG	5' *UME6* -8.0 kb flank downstream primer for cloning into pSFS2
12	DTO225	agtactgaaccgcggGTGTGATGGAGTGATGGC	3' *UME6* -8.0 kb flank downstream primer for cloning into pSFS2
13	DTO222	agtactgaagcggccgcGAGGCAAATGGCAAGCACC	5' *UME6* -7.0 kb flank downstream primer for cloning into pSFS2
14	DTO223	agtactgaaccgcggGTTGATGGTTATGCACCC	3' *UME6* -7.0 kb flank downstream primer for cloning into pSFS2
15	DTO220	agtactgaagcggccgcCTAGACCTAAACTACTACG	5' *UME6* -6.0 kb flank downstream primer for cloning into pSFS2
16	DTO221	agtactgaaccgcggCAACATTATCACCTAGACCC	3' *UME6* -6.0 kb flank downstream primer for cloning into pSFS2
17	DTO218	agtactgaagcggccgcGCAAATTACTTGCTGCATGTCGG	5' *UME6* -5.0 kb flank downstream primer for cloning into pSFS2

18	DTO219	agtactgaaccgcggGTAGCCTCAATTAATCTTGACC	3' *UME6* -5.0 kb flank downstream primer for cloning into pSFS2
19	DTO239	agtactgaagcggccgcCCATTCATTCAGTCATTCG	5' *UME6* -4.2 kb flank downstream primer for cloning into pSFS2
20	DTO240	agtactgaaccgcggCTTGATCTAGTTGTGGATGTGG	3' *UME6* -4.2 kb flank downstream primer for cloning into pSFS2
21	DTO243	agtactgaactgcagGTCGACCTAGACCTAAACTACTACG	5' *UME6*$_{-6\ kb\ UR}$ upstream primer for cloning into placbasal
22	DTO90	CTGTTAAGTTGTTGTGGTAGG	3' *UME6*$_{-6\ kb\ UR}$ primer downstream of native HindIII site for cloning into placbasal
23	DTO89	GGTCCAGTCGACCAAGAACAATCCATTCTCG	5' *UME6*$_{-6\ kb\ UR}$ primer upstream of native HindIII site for cloning into placbasal
24	DTO250	agtactgaactgcaggcatgcTTATACAGTGGATTAAAAAATAAAACTAATTGGAAGTAAATTGAGG	3' *UME6*$_{-6\ kb\ UR}$ downstream primer for cloning into placbasal
25	DTO262	agtactgaaggtaccCTACAACCACATCCACATCC	5' *UME6* 5' UTR upstream primer for cloning into pSFS2
26	DTO263	agtactgaactcgagCCTAAATGTCACCTCAAATACCC	3' *UME6* 5' UTR upstream primer for cloning into pSFS2
27	PCO32	gatcataccgaacagagcggccgcCCTCAATTTACTTCCAATTAG	5' *UME6* 5' UTR downstream primer for cloning into pSFS2
28	DTO272	agtactgaaccgcggGCATGATCATGACCATGACC	3' *UME6* 5' UTR orf19.1822 downstream primer for cloning into pSFS2
29	DTO271	agtactgaaccgcggCATCATCATGATCATGACC	3' *UME6* 5' UTR orf19.9381 downstream primer for cloning into pSFS2
30	DTO264	agtactgaaggtaccGTCGTAGCTGCAACAACTAGTTACC	5' *UME6* 5' UTR upstream primer for cloning into p97CAU1
31	DTO265	agtactgaactcgagCTTCTTTCGTGAGGAGGG	3' *UME6* 5' UTR upstream primer for cloning into p97CAU1
32	DTO266	agtactgaaactagtGGAACATCAATTTACATTTCC	5' *UME6* 5' UTR downstream primer for cloning into p97CAU1
33	DTO267	agtactgaaccgcggTGTAATAAACGAGAATGG	3' *UME6* 5' UTR downstream primer for

			cloning into p97CAU1
34	DTO293	agtactgaagagctcATGGCTGTCGGTAAAAACAAG	5’ *RPS1* primer for cloning into pFA-GFPγ-URA3
35	DTO294	agtactgaaactagtTTAAACAGATTCTAAAACAACATC	3’ *RPS1* primer for cloning into pFA-GFPγ-URA3
36	DTO298	agtactgaaggtaccCTATTAAGATCACCAGCC	5’ *ACT1* promoter primer for cloning into pBS
37	DTO299	agtactgaactcgagTTTAAGCTTTTTGAATGA	3’ *ACT1* promoter primer for cloning into pBS
38	DTO300	agtactgaactcgagGGAACATCAATTTACATTTCC	5’ *UME6 5’* UTR primer for cloning into pBS
39	DTO289	agtactgaactgcagCATTTATACAGTGGATTAAAAAATAAAACTAATTGGAAGTAAATTGAGG	3’ *UME6* 5’ UTR primer for cloning into pBS
40	DTO169	CGTCTTTGCAGATCGTACCC	5’ *lacZ* primer for qRT-PCR
41	DTO170	CTGGGAATGTTGCTTCTTCG	3’ *lacZ* primer for qRT-PCR
42	DTO85	TTGCTCCAGAAGAACATCCAG	5’ *ACT1* primer for qRT-PCR
43	DTO86	AGTAACACCATCACCAGAATCC	3’ *ACT1* primer for qRT-PCR
44	DTO167	GAACAATGGTGGTGGTAGTGG	5’ *UME6* primer for qRT-PCR
45	DTO168	AATTCGACAAATCCAACATCC	3’ *UME6* primer for qRT-PCR
46	DTO314	CTCCAATTGGTGATGGTCC	5’ *GFP* primer for qRT-PCR
47	DTO316	GTAACAAATTCTAACAAGACC	3’ *GFP* primer for qRT-PCR
48	DK239	GTTGGGACTAGGATTGGTAAAGC	5’ *UME6* primer for Northern probe
49	DK240	GATGTGGAGTAGACTTGGATAATGG	3’ *UME6* primer for Northern probe
50	DK523	GTTGACCGAAGCTCCAATGAATCC	5’ *ACT1* primer for Northern probe
51	DK526	CAGCAATACCTGGGAACATGG	3’ *ACT1* primer for Northern probe
52	DTO312	GAGAAATCTTTTTTTTTTTTTTTTTATCGTTGCCCCACTTTTTTCTTAC	5’ primer for *UME6* 5’ UTR uORF1 deletion
53	DTO313	GTAAGAAAAAAGTGGGGCAACGATAAAAAAAAAAAAAAAAGATTTCTC	3’ primer for *UME6* 5’ UTR uORF1 deletion
54	DTO324	GGTTTTTTCTATTGTTTAATAAGAAGGTTCTATTTGGGGAGTTGCTTATTATTGATATTAATAATTC	5’ primer for *UME6* 5’ UTR uORF2$_{1\text{-}46\ bp}$ deletion
55	DTO323	GAATTATTAATATCAATAATAAGCAACTCCCCAAATAGAACCTTCTTATTAAACAATAGAAAAAACC	3’ primer for *UME6* 5’ UTR uORF2$_{1\text{-}46\ bp}$ deletion

56	DTO325	GGTTTTTTCTATTGTTTAATAAGAAGGTTCTATTTGAGGGAAAAAAGAATTCTTCG	5’ primer for *UME6* 5’ UTR uORF2$_{47\text{-}102\ bp}$ deletion
57	DTO326	CGAAGAATTCTTTTTTCCCTCAAATAGAACCTTCTTATTAAACAATAGAAAAAACC	3’ primer for *UME6* 5’ UTR uORF2$_{47\text{-}102\ bp}$ deletion
58	DTO332	TTTTTGCGCAAGTTAAAATCC	5' *UME6* 5' UTR primer for qRT-PCR
59	DTO333	CGAGATTTTCTGATTGATTTATGC	3' *UME6* 5' UTR primer for qRT-PCR
60	DTO76	CTCTTGGTATCTCTTTACCC	3' *UME6* 5' UTR 5' RACE primer (for-3041 transcript start site identification)
61	DTO258	CAACGATACTAACGACGAGGG	3' *UME6* 5' UTR 5' RACE primer (for -1923 transcript start site identification)
62	DTO259	CTGGAGATAATGAATAGTGGG	3' *UME6* 5' UTR 5' RACE primer (for -2126 transcript start site identification)
63	DTO302	TGCAAAGCCAATTCCCGAAG	5' Solaris spike-in primer for qRT-PCR
64	DTO303	CCATTGTAGTGAACAGTAGGAC	3' Solaris spike-in primer for qRT-PCR

Note: Lowercase bases indicate flanking sequences within primers that contain restriction sites for cloning.

SUPPLEMENTAL FIGURES

Figure S1. 5' RACE analysis of the *UME6* transcript. Total RNA and cDNA were prepared from cells of a wild-type (SC5314) strain following induction in YEPD + 10% serum at 37°C. (A) 5' RACE analysis was performed using the indicated primers (reverse arrows) within the *UME6* 5' UTR to yield the indicated PCR products (red bars). +1 indicates the *UME6* translation start site. (B and C) The 5' RACE PCR products described in (A) were run on 0.8% agarose gels to determine their size. A DNA ladder is shown at left (sizes in kb). "-" indicates minus reverse transcriptase (RT) control. The precise size and identity of these PCR products was also determined more accurately by sequencing.

Figure S2. *UME6* transcript is expressed at equivalent levels in wild-type and *UME6 5' utr Δ/Δ* strains upon induction in Spider medium at 37°C. The indicated strains were grown overnight in YEPD at 30°C, washed, diluted into pre-warmed YEPD at 30°C or Spider medium at 37°C, grown for 36 hours and harvested for total RNA preparation and cDNA synthesis. *UME6* transcript levels were determined by qRT-PCR analysis and normalized to those of an *ACT1* internal control. Fold induction for each strain was determined by dividing normalized *UME6* expression values at the 36-hr. time point by those at the 0 hr. time point. Data shown represents the average of three biological replicates run in technical duplicate (mean ± SEM).

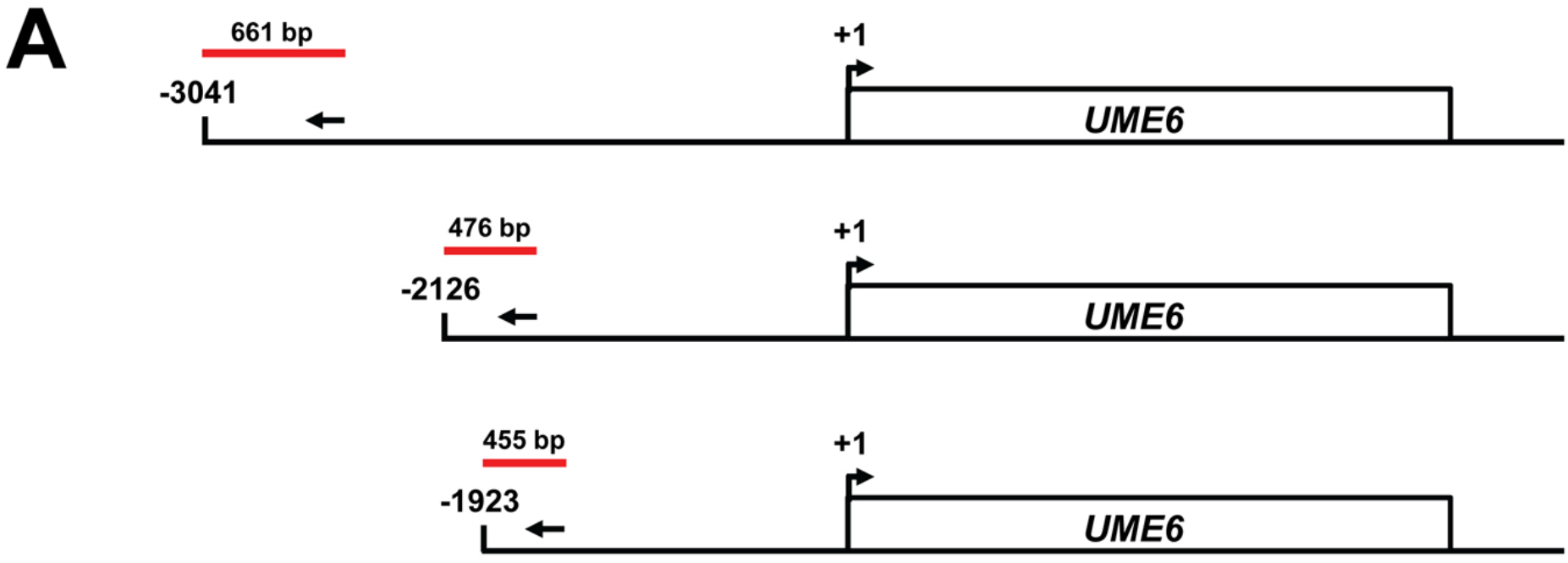
Figure S3. The *UME6* 5' UTR inhibits *UME6*-driven hyphal growth under non-filament-inducing conditions in an overnight culture. The indicated strains were grown as described in Figure 4A. Cell aliquots were harvested at the 24 hr. time point, fixed with 4.5% formaldehyde, washed twice with 1X PBS and visualized by DIC microscopy. Bar = 10 μm.

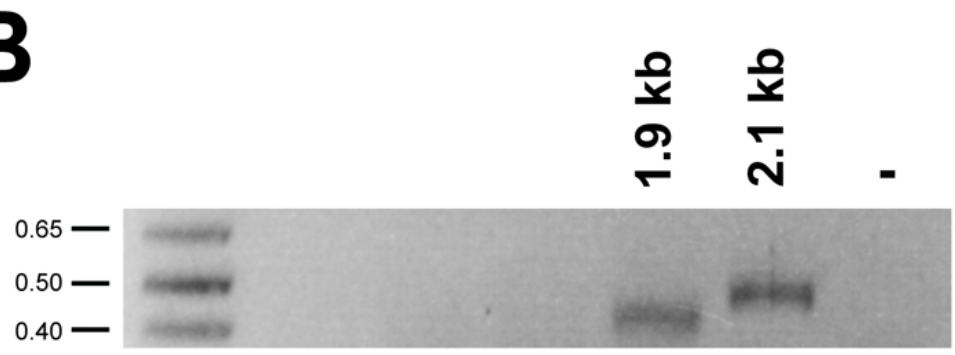
Figure S4. *ACT1* transcript abundance in polysome fractions. Using primers specific to the *ACT1* transcript, qRT-PCR analysis was performed with RNA from the samples grown in YEPD + 10% serum at 37°C (no EDTA treatment) described in Figure 5. Data shown represents normalized mean *ACT1* transcript levels based on two independent experiments (± SEM).

Figure S5. *UME6* transcript levels under a variety of filament-inducing conditions are not significantly affected by deletion of the 5' UTR. Aliquots of cells grown for the Western analysis described in Figure 6 were used to prepare total RNA. Northern analysis was performed using 5 μg of RNA from each sample and the indicated probes. *ACT1* is included as a loading control. Please note that for the *5' utrΔ-UME6-MYC* strain, the upper band corresponds to the wild-type *UME6* allele and the lower band corresponds to the *MYC*-tagged *UME6* 5' *utrΔ* allele.

Figure S6. The *UME6* 5' UTR is sufficient to inhibit protein expression by a heterologous *GFP* reporter, as determined by microscopy. The indicated strains were grown overnight in YNB minimal medium at 30°C, diluted to OD_{600} ~1.0 in YNB minimal medium at 30°C and grown for 3 hours. Cells were visualized by DIC (left panels) and fluorescence (right panels) microscopy. Bar =10 μm.

Figure S7. *GFP* transcript levels are not significantly affected by addition of the *UME6* 5' UTR. Northern analysis was performed using 3 μg of RNA samples described in Figure 7 and the indicated probes. *ACT1* is included as a loading control.


A
661 bp
-3041
+1
UME6
476 bp
-2126
+1
UME6
455 bp
-1923
+1
UME6


B
1.9 kb
2.1 kb
-
0.65
0.50
0.40

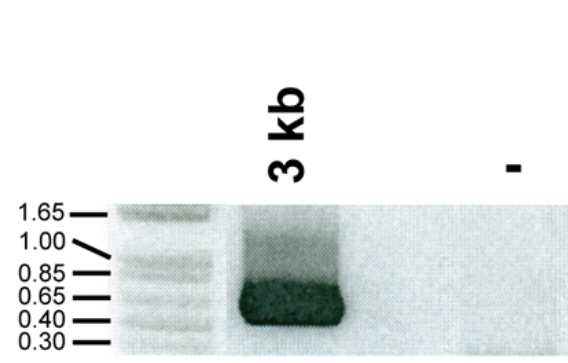

C
3 kb
-
1.65
1.00
0.85
0.65
0.40
0.30

Figure S1

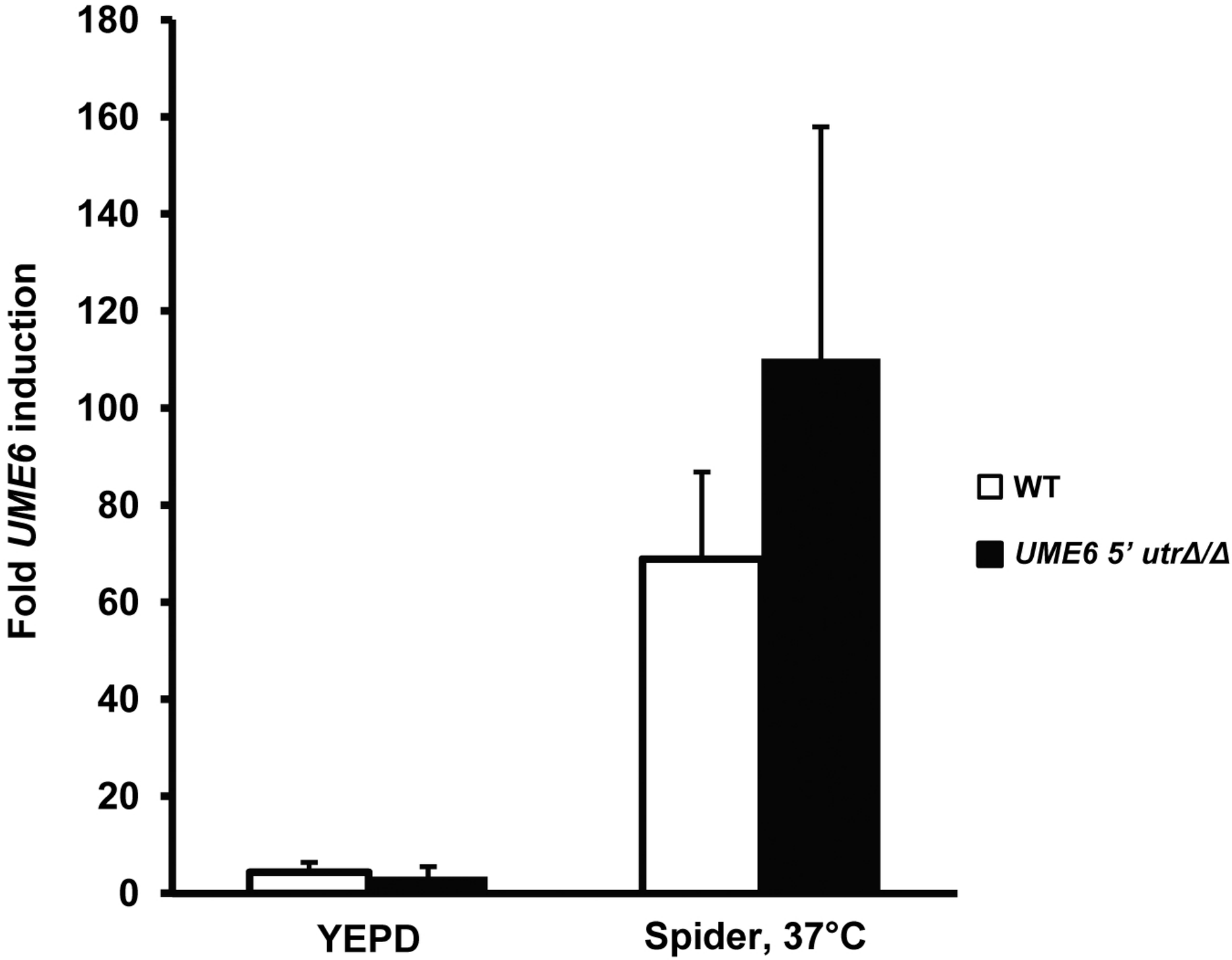

180
160
140
120
100
80
60
40
20
0
Fold UME6 induction
WT
UME6 5' utrΔ/Δ
YEPD
Spider, 37°C

Figure S2

tetO-5'UTR-UME6

tetO-UME6

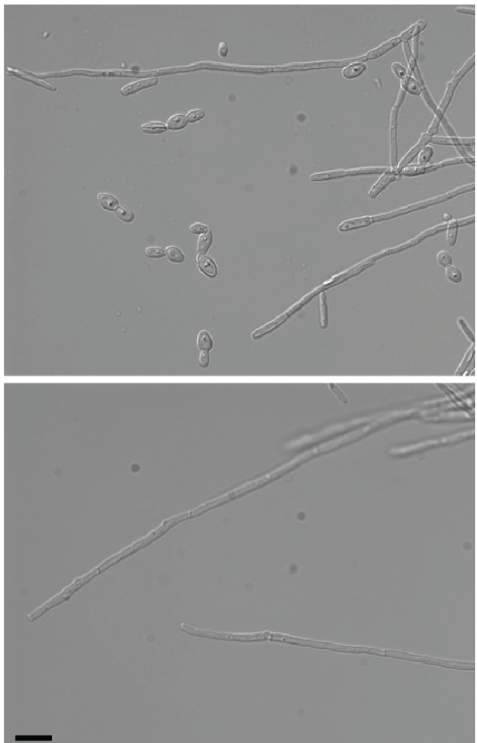


Figure S3

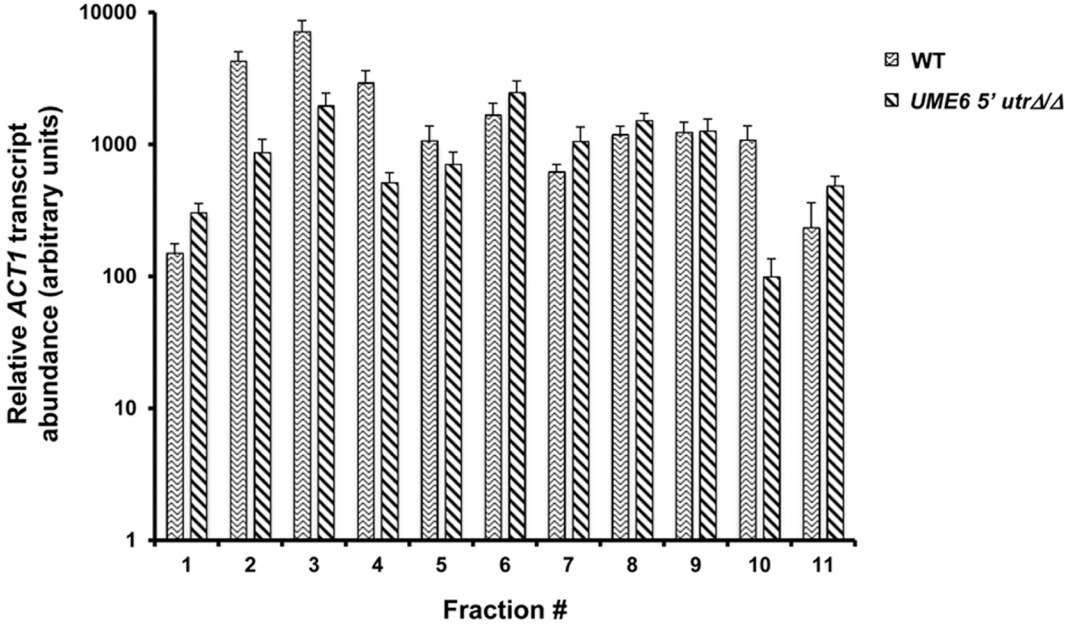

10000
1000
100
10
1
Relative ACT1 transcript abundance (arbitrary units)
1
2
3
4
5
6
7
8
9
10
11
Fraction #
WT
UME6 5' utrΔ/Δ

Figure S4

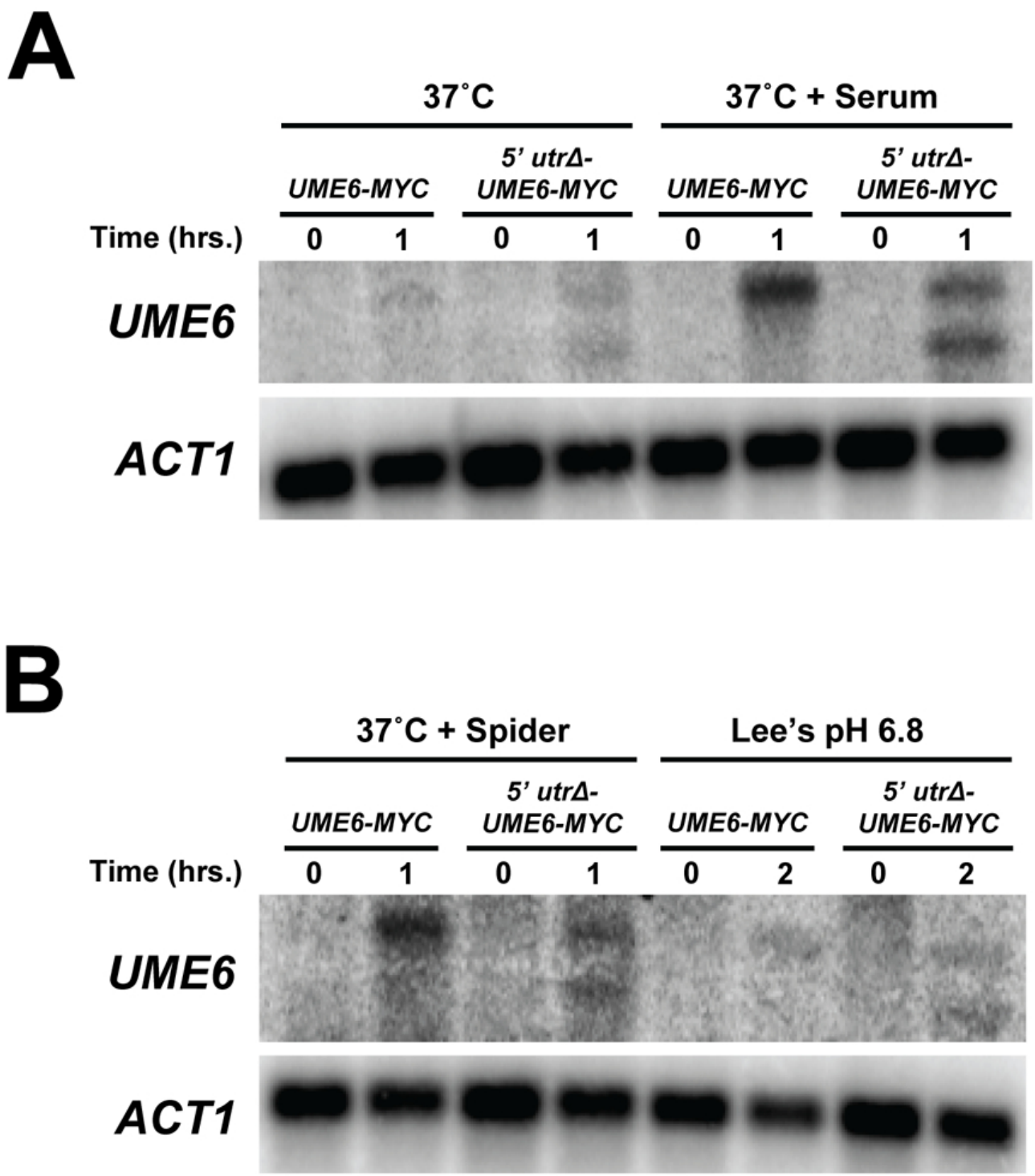

A
37˚C
37˚C + Serum
UME6-MYC
5' utrΔ-UME6-MYC
UME6-MYC
5' utrΔ-UME6-MYC
Time (hrs.)
0 1 0 1 0 1 0 1
UME6
ACT1
B
37˚C + Spider
Lee's pH 6.8
UME6-MYC
5' utrΔ-UME6-MYC
UME6-MYC
5' utrΔ-UME6-MYC
Time (hrs.)
0 1 0 1 0 2 0 2
UME6
ACT1

Figure S5

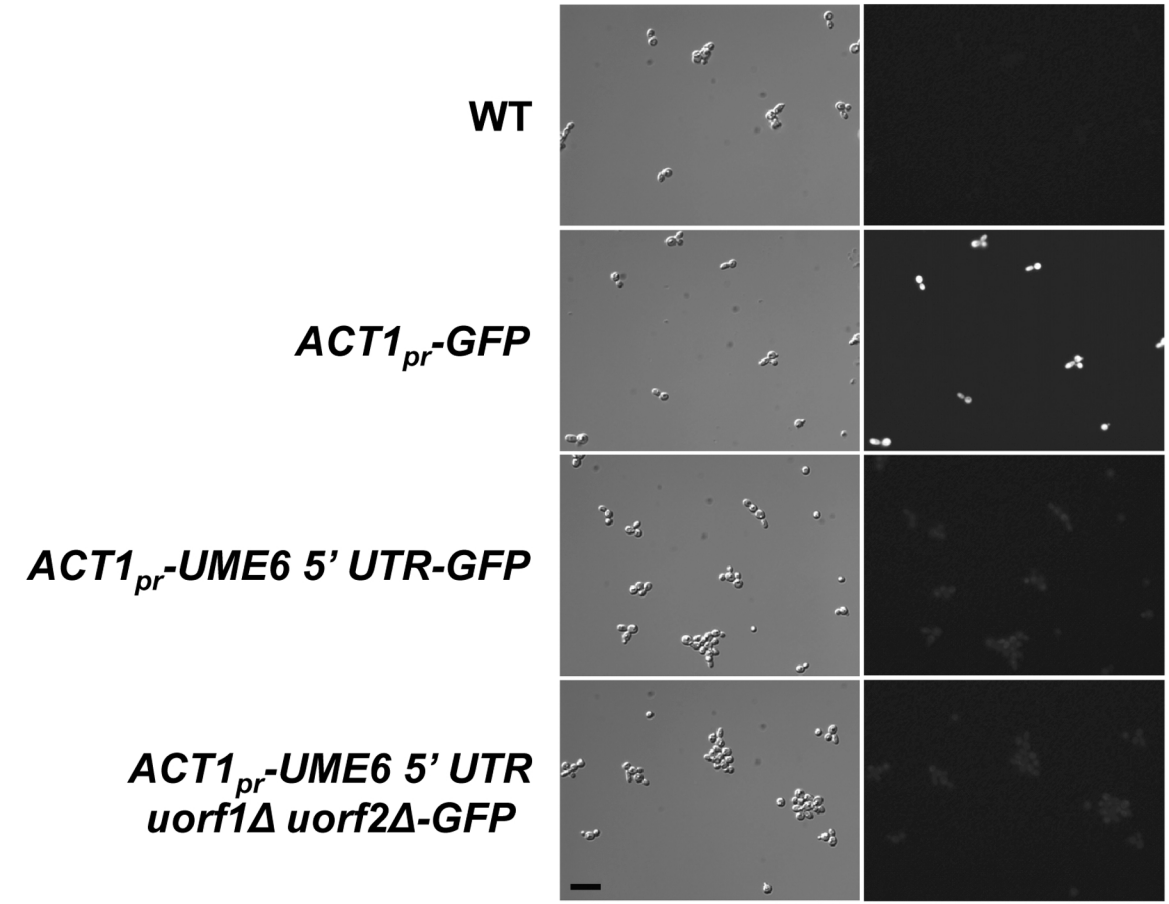

WT
ACT1pr-GFP
ACT1pr-UME6 5' UTR-GFP
ACT1pr-UME6 5' UTR
uorf1Δ uorf2Δ-GFP

Figure S6

ACT1pr-GFP
ACT1pr-UME6 5' UTR-GFP
ACT1pr-UME6 5' UTR uorf1Δ orf2Δ–GFP
WT
GFP
ACT1

Figure S7