## Summary of Supplementary Data

| Sheet | Title | Description |
|---|---|---|
| 1 | Summary | Summary of Supplementary Data |
| 2 | Ubiquitination Targets | List of ubiquitination targets in *C. albicans* |
| 3 | MET3p-UBI4 control | Effects of stress upon *C. albicans MET3p-UBI4/ubi4* in the absence of methionine and cysteine |
| 4 | Ponceau S Stain | Ponceau S stain of *UBI4/UBI4, UBI4/ubi4 and ubi4/ubi4* to control loading |
| 5 | Primers | List of PCR primers used in this study |

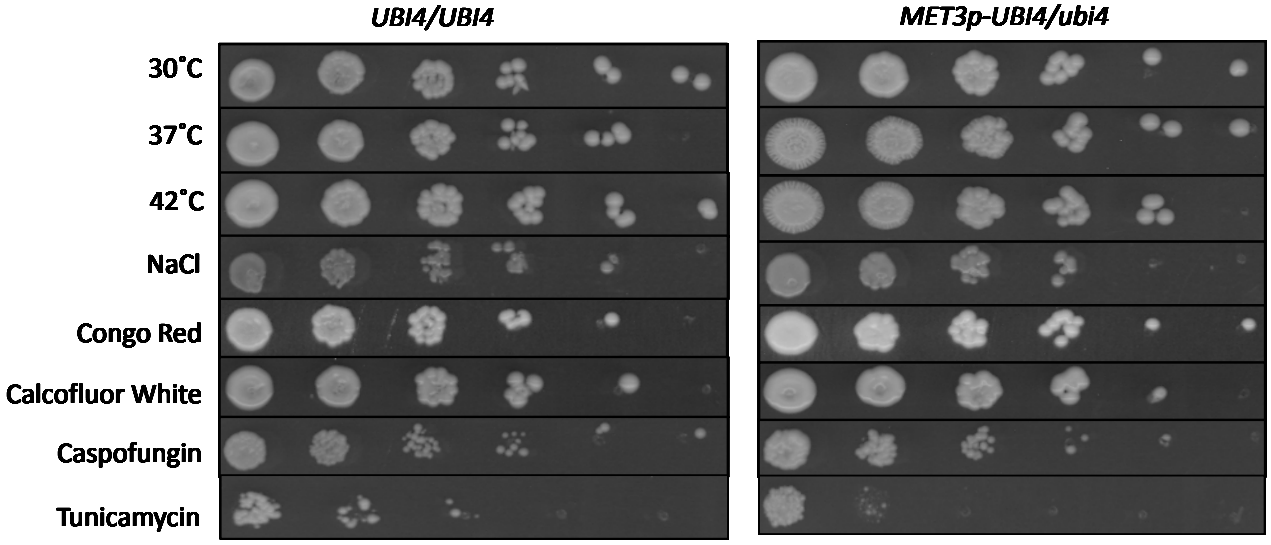
## Identification of probable ubiquitination targets in *C. albicans*

| Sample Ref | Accession Number (CandidaDB) | Accession Number (CGD) | Protein Name | Mol. Wt. (Da) | pI | Mascot Score | Peptides Matched | Sequence Coverage | ELDP | Function | Ubiquitinated in *S. cerevisiae*? | Predicted Ubiquitination Site(s) | Ubiquitination detected in absence of stress? | Ubiquitination upregulated in response to stress? |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ***Constitutively ubiquitinated*** | | | | | | | | | | | | | | |
| 1 | Ca2937 | orf19.3325.3 | RPS21 B | 8807 | 8.85 | 372 | 4 | 48% | 2 | Similar to ribosomal protein S21 | No | Medium confidence, position 5 | Yes | Downregulated |
| 2 | Ca1691 | orf19.3651 | PGK1 | 45266 | 6.07 | 2080 | 37 | 84% | 12 | Phosphoglycerate kinase | Yes | Medium confidence, position 6; low confidence, position 296 | Yes | No |
| 3 | Ca5180 | orf19.4618 | FBA1 | 39362 | 5.69 | 2000 | 24 | 79% | 9 | Putative fructose-bisphosphate aldolase | Yes | Medium confidence, position 72 and 192; low confidence, position 77 | Yes | No |
| 4 | Ca2939 | orf19.3324 | TIF1 | 44742 | 5.22 | 1397 | 26 | 72% | 15 | Translation initiation factor | Yes | Low confidence, position 24 | Yes | No |
| | Ca0824 | orf19.691 | GPD2 | 41174 | 5.21 | 313 | 9 | 33% | 7 | Glycerol-3-phosphate dehydrogenase | Yes | Low confidence, position 274 and 318 | Yes | No |
| ***Increased ubiquitination in response to heat*** | | | | | | | | | | | | | | |
| 5 | Ca3874 | orf19.395 | ENO1 | 47202 | 5.54 | 204 | 7 | 23% | 7 | Enolase | Yes | High confidence, position 273; medium confidence, position 265; low confidence, position 432 | Yes | Heat |
| 6 | Ca4389 | orf19.3123 | RPT5 | 47814 | 5.25 | 1059 | 21 | 60% | 13 | Regulatory particle triphosphatase | Yes | Medium confidence, position 55, 63, 163, 170, 284 and 290; low confidence, position 35 and 92 | Yes | Heat |
| 7 | Ca3924 | orf19.5112 | TKL1 | 73841 | 5.48 | 1036 | 23 | 45% | 10 | Putative transketolase | Yes | Low confidence, position 351 | No | Heat |
| 8 | Ca2895 | orf19.2461 | PRN4 | 37072 | 5.31 | 496 | 16 | 47% | 3 | Putative pirin by homology | No | High confidence, position 315; medium confidence, position 234; low confidence, position 240, 320 and 327 | No | Heat |
| 9 | Ca5239 | orf19.5024 | GND1 | 57159 | 6.14 | 478 | 19 | 38% | 13 | Putative 6-phosphogluconate dehydrogenase | Yes | Medium confidence, position 6; low confidence, position 151 and 388 | No | Heat |
| | Ca2011 | orf19.3087 | UBI3 | 17485 | 9.86 | 42 | 1 | 14% | 0 | Functional homologue of *S. cerevisiae* RPS31 | No | Medium confidence, position 105 | No | Heat |
| 10 | Ca0210 | orf19.5525 | IPF4328 | 37783 | 5.53 | 417 | 11 | 42% | 9 | Putative NADP(H) oxidoreductase | No | Medium confidence, position 285; low confidence, position 268 and 344 | No | Heat |
| ***Increased ubiquitination in response to $H_2O_2$*** | | | | | | | | | | | | | | |
| 11 | Ca4261 | orf19.550 | PDX3 | 28773 | 6.02 | 356 | 13 | 68% | 1 | Pyridoxine (pyridoxamine) phosphate oxidase | Yes | Medium confidence, position 22 and 58 | No | $H_2O_2$ |
| 12 | Ca2675 | orf19.5493 | GSP1 | 24470 | 6.53 | 146 | 5 | 35% | 5 | Small RAN G-protein | Yes | Medium confidence, position 193 | No | $H_2O_2$ |
| | Ca5932 | orf19.6771 | UBI4 | 25776 | 7.76 | 53 | 1 | 10% | 1 | Ubiquitin precursor (polyubiquitin) | Yes | Low confidence, postion 33, 48, 63, 139, 179 and 215 | No | $H_2O_2$ |
| 13 | Ca1673 | orf19.3612 | PST2 | 21714 | 6.51 | 206 | 7 | 52% | 7 | Putative NADH:quinone oxidoreductase | Yes | None | No | $H_2O_2$ |

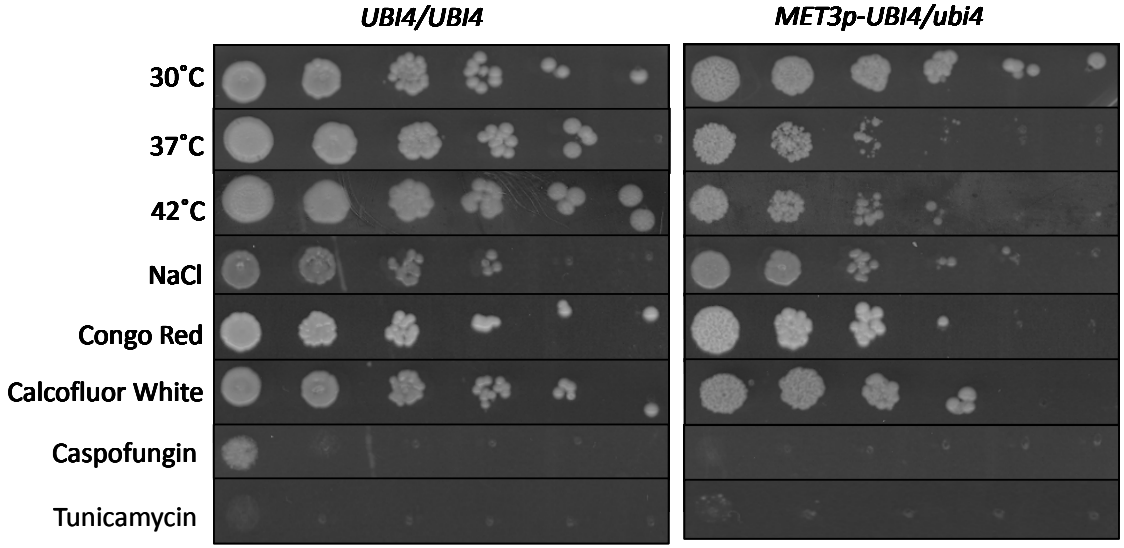
| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Ca5037 | orf19.6991 | PRE3 | 23629 | 6.42 | 155 | 7 | 32% | 3 | **B**eta-1 subunit of proteasome | No | Low confidence, postion 12 | No | $H_2O_2$ |
| 14 | Ca4791 | orf19.4025 | PRE1 | 22119 | 6.43 | 273 | 11 | 38% | 4 | Beta 4 subunit of the 20S proteasome (*S. cerevisiae* ) | No | Low confidence, position 19 | No | $H_2O_2$ |
| | Ca4589 | orf19.6906 | ASC1 | 23619 | 6.3 | 85 | 5 | 27% | 3 | 40S ribosomal subunit protein | Yes | None | No | $H_2O_2$ |
| | Ca5932 | orf19.6771 | UBI4 | 25776 | 7.76 | 76 | 3 | 24% | 1 | Ubiquitin precursor (polyubiquitin) | Yes | | No | $H_2O_2$ |
| ***Increased ubiquitination in response to heat and $H_2O_2$*** | | | | | | | | | | | | | | |
| 15 | CA3534 | orf19.6367 | SSB1 | 66580 | 5.25 | 2786 | 35 | 67% | 22 | Putative HSP70 family heat shock protein | Yes | High confidence, position 528 and 545; medium confidence, position 521, 530, 543 and 597; low confidence, position 191, 314 and 393 | No | Heat and $H_2O_2$ |
| 16 | Ca1246 | orf19.18 | IMH3 | 40055 | 6.67 | 1879 | 25 | 74% | 8 | Inosine monophosphate (IMP) dehydrogenase | No | Medium confidence, position 7; low confidence, position 24, 175, 230 and 517 | No | Heat and $H_2O_2$ |
| 17 | Ca3546 | orf19.6385 | ACO1 | 84625 | 5.96 | 1669 | 38 | 52% | 22 | Aconitase | No | Medium confidence, position 25. | No | Heat and $H_2O_2$ |
| 18 | Ca2858 | orf19.1064 | ACS2 | 74215 | 5.73 | 1300 | 32 | 54% | 20 | Probable acetyl-CoA synthetase | Yes | Medium confidence, position 32 and 596; low confidence, position 17 and 583 | No | Heat and $H_2O_2$ |
| 19 | Ca2470 | orf19.2871 | SDH12 | 70930 | 6.03 | 790 | 21 | 47% | 11 | Flavoprotein subunit of succinate dehydrogenase | No | Medium confidence, position 480; low confidence, position 417, 492 and 500 | No | Heat and $H_2O_2$ |

**Effects of stress upon *C. albicans MET3p-UBI4/ubi4* in the absence of methionine and cysteine**

**NO Met/Cys control**

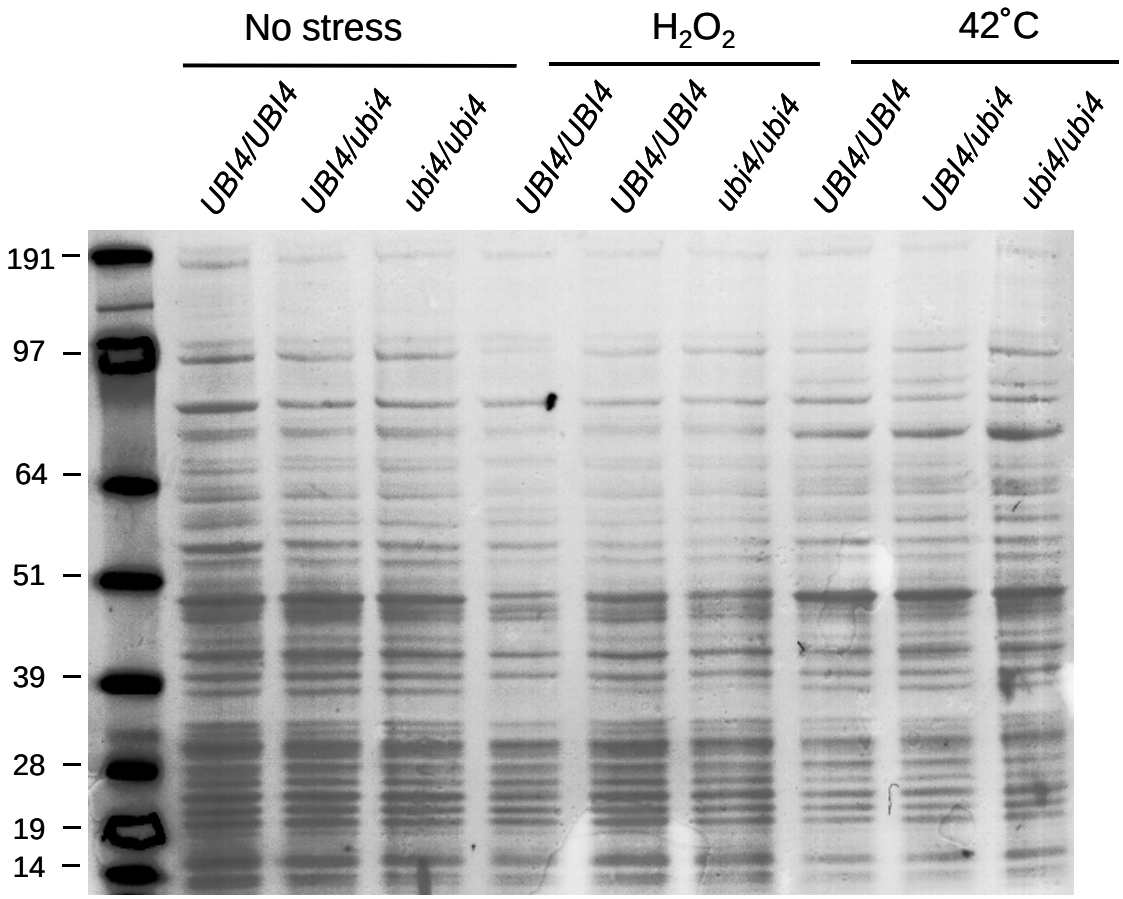



**PLUS Met/Cys (Figure 4B)**




**Sensitivity of a MET3p-UBI4/ubi4 conditional mutant to stresses. Serial dilutions were spotted onto SC plates containing the appropriate stress and containing 2.5 mM methionine and cysteine: UBI4/UBI4 (BWP17); MET3p-UBI4/ubi4 (MLC05). These strains grew at similar rates on control plates that lacked methionine and cysteine (Above).**

**Ponceau S staining to demonstrate even loading**



**Oligonucleotides used in this study**

| Primer | Sequence (5' to 3') | Application |
|---|---|---|
| UBI4d-F | CCTAGTTGCCTTAACGACAG | Diagnosis of *UBI4* allele |
| UBI4d2-F | GCGACATTAAAATAGGAGAGGC | Diagnosis of *UBI4* , *ARG4* and *URA3* alleles |
| UBI4d-R | GTCAGACAAGGTTCTGCC | Diagnosis of *UBI4* and *URA3-MET3-UBI4* alleles |
| UBI4d2-R | CTTGAGAAGACATAAGTAAGG | Diagnosis of *UBI4, ARG4, URA3* and *URA3-MET3-UBI4* alleles |
| MET3p-F | ATTGCTGTGGATCACGTGC | Diagnosis of *URA3-MET3-UBI4* allele |
| LALd-R | GCCCATCTAATAGGTTGAGC | Diagnosis of *UBI4::ARG4* allele |
| LULd-R | GTATGGGGTTGTTGCTCAGG | Diagnosis of *UBI4::URA3* allele |
| MetUS-F | CCTCGTTCATTCTAATTCAATACAAGTTTGTATTGTTTATACCATGTTTAT ACAAACGGGTTTAATTTATAATTACGGTAGCCGGCCGACTTGGCCAAG CCTAGATC | Amplification of *URA3-MET3-UBI4* cassette |
| MetUS-R | GGATTTGACGTTATCGATGGTGTCAGAAGATTCGACTTCTAAGGTAATG GTTTTACCAGTCAAAGTTTTAACGAAAATTTGCATTGGGGAGGGTATTT ACTTTTAAATA | Amplification of *URA3-MET3-UBI4* cassette |
| LAL-F | CCTTACGTACAGCACACACATACCACTGTCGTGCACTGACCAACAACA ATGCGGTGTTAATCGATAACCAAAAGATTATAAATAGGGGGTGGAAGG TCGCCCCAGGGTTTTCCCAGTCACG | Amplification of *UBI4::ARG4* cassette |
| LAL-R | GCAGCAATGATTATAAAAAATTAGAAACCACCTCTCAATCTCAAGACCA AGTGCAAGGTAGATTCTTTTTGGATGTTGTAGTCAGACAAGGTTCTGCC ACTAAAGGGAACAAAAGC | Amplification of *UBI4::ARG4* cassette |
| LUL-F | CATACATTGGGTAGGTCATTCATAACAATTGATAGATGCAAGCTAATTG GAATGAAAAATCCATCTTGTATCAAAACCCTTTGTTCCTCATAGTTAATC CGCCAGGGTTTTCCCAGTCACG | Amplification of *UBI4::URA3* cassette |
| LUL-R | CTTTATAGAGTTTATAAATGTCAGTTTAAGCTGAAAAAAAATTATTATAG TACAAATGTAAGATACTCTCATAATCAATGGGACCTGAATTGGACCCAC TAAAGGGAACAAAAGC | Amplification of *UBI4::URA3* cassette |
| UBI4S-F | CGTCAACTAACATATATATAC | Amplification of *UBI4* probe for Southern analysis |
| UBI4S-R | GCCTAGTCTATAAAACAAAAC | Amplification of *UBI4* probe for Southern analysis |
| LALS-F | GGTAGATCAAGAAATGATC | Amplification of *ARG4* probe for Southern analysis |
| LALS-R | CAATCAGTAAAGTAGAAATC | Amplification of *ARG4* probe for Southern analysis |
| LULS-F | CCTATAGTGAGAGAGCAG | Amplification of *URA3* probe for Southern analysis |
| LULS-R | GGATCTCTTCCTTTACC | Amplification of *URA3* probe for Southern analysis |
| ACT1-F | ACCACCGGTATTGTTTTGGA | Real time RT-PCR of *ACT1* transcript levels |
| ACT1-R | AGCGTAAATTGGAACAACGTG | Real time RT-PCR of A*CT1* transcript levels |
| ACT1-P | ACCACCGGTATTGTTTTGGATTCTGGTGATGGTGTTACTCACGTTGTTC CAATTTACGCT | Probe for real time RT-PCR of *ACT1* transcript levels |
| UBI4q-F | CGTCAAATCCAAGATCCAAGA | Real time RT-PCR of *UBI4* transcript levels |
| UBI4q-R | CGGCGAAAATCAATCTTTGT | Real time RT-PCR of *UBI4* transcript levels |
| UBI4q-P | CCATCGATAACGTCAAATCCAAGATCCAAGACAAAGAAGGTATTCCACC AGACCAACAAAGATTGATTTTCGCCGGTAAACAATT | Probe for real time RT-PCR of *UBI4* transcript levels |
| UBI4-F | GAACAGATATGTTGGTTCCAGGATTGAAAAG | Amplification of *UBI4* |
| UBI4-R | CCTTATTTGTAGACTCGGGTAATAGC | Amplification of *UBI4* |
| UBI4C-F | TATCGAATTCACGCGTGACTCAGATCGATAAGCGAACATCACAGC | Amplification of *UBI4* for cloning |
| UBI4C-R | GGTACCTGGAGTCGACCAATGTGAGGACTGGCTAACTTTAATAGG | Amplification of *UBI4* for cloning |