Supplementary information

A critical role of calcineurin in stress responses, hyphal formation, and virulence of the pathogenic fungus *Trichosporon asahii*

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### Supplementary table 1 Primers used in this study

| Primers                                      | Nucleic acid sequence                                                                 |
|----------------------------------------------|---------------------------------------------------------------------------------------|
| pAg1-cna1(5’UTR)-NAT1-cna1(3’UTR) for cloning |                                                                                        |
| **pAg1-cna1 (5’UTR)-NAT1 (1st cloning)**     |                                                                                        |
| F pAg1 for cna1(5’UTR)                       | CTCCGCTCATGATCAGATTGTGTTTCCCAG                                                       |
| R NAT1 for cna1(5’UTR)                       | GCTGCGAGGATGTGAGCTGGAGAGC                                                            |
| F cna1(5’UTR)                                | ATCTGATCATGAGCGAGCGAGTTGGCAAAACTGCTGGGAAGCTTGG                                       |
| R cna1(5’UTR)                                | AGCTCACAATCTCTCGAGCCCTTGGTGAGCTGGCAATGAGAGCAGTGTCTGC                                 |
| **pAg1-cna1(5’UTR)-NAT1-cna1(3’UTR) (2nd cloning)** |                                                                                        |
| F pAg1 for cna1(3’UTR)                       | GAAAAACCTGGCGTTACCAACTTAAATCG                                                        |
| R NAT1 for cna1(3’UTR)                       | GAAGAGATGTAGAAACTAGCTCTGCTGGTTTCAGAG                                                |
| F cna1(3’UTR)                                | TAGTTTCTACATCTCTCCGGCTCGTGGTTGGCAAAATGTGGTGTC                                        |
| R cna1(3’UTR)                                | GGTAACCGCCAGGTTTCTCCCTTGCGATGCGGCTGCCCTCTCAAGACC                                     |
| Amplification of cna1 cassette for electroproportion |                                                                                   |
| F cna1-cassette                             | ATCTGATCATGAGCGAGCGAGTTGGCAAAACTGCTGGGAAGCTTGG                                       |
| R cna1-cassette                             | GGTAACCGCCAGGTTTCTCCCTTGCGATGCGGCTGCCCTCTCAAGACC                                     |
| pAg1-cna1(5’UTR)-cna1-hph-cna1(3’UTR) for cloning |                                                                                        |
| **pAg1-cna1(5’UTR)-hph-cna1(3’UTR) (1st cloning)** |                                                                                        |
| F 3’UTR-pAg1-5’UTR for hph                  | CGGCTCGTGGTTGGCAAAATGTGGTGTC                                                        |
| R 3’UTR-pAg1-5’UTR for hph                  | CCTGCGTCGCTCGTGGCGATGTTGTC                                                          |
| F hph                                       | ACCGACACGACGACAAAGGGGGGCCCCCTCGGAGGATG                                              |
| R hph                                       | GCAAACACGACGAGCGGGGGAATCGAGATGGAAGC                                                 |
| **pAg1-cna1(5’UTR)-cna1-hph-cna1(3’UTR) (2nd cloning)** |                                                                                        |
| F hph-3’UTR-pAg1-5’UTR for cna1             | GGGCCCCCTCGGAGGATG                                                                 |
| R hph-3’UTR-pAg1-5’UTR for cna1             | CTTGCGTCGCTCGTGAGTGTTGTC                                                          |
| F cna1                                      | ACCGACACGACGACAAAGGGGGGCCCCCTCGGAGGATG                                              |
| R cna1                                      | CATCGTCGACGGGGGCCCTTTAGGGAGGAGGATGGAAGC                                               |
| Amplification of cna1 revertant cassette for |                                                                                        |
| Primers-1 for cna1 genotyping | Primers-2 for cna1 genotyping | Primers-3 for cna1 genotyping | Primers-4 for cna1 genotyping | Primers-5 for cna1 genotyping | Primers-6 for cna1 genotyping |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| F cna1 gene locus | R cna1 gene locus | F cna1 gene locus | R cna1 gene locus | F cna1 gene locus | R cna1 gene locus |
| ATCTGATCATGAGCGGAGCGAGTTGCCAAACT | GCGTATGCACGCACGCACCTTCTGGG | GCGTATGCACGCACGCACCTTCTGGG | GCGTATGCACGCACGCACCTTCTGGG | GCGTATGCACGCACGCACCTTCTGGG | GCGTATGCACGCACGCACCTTCTGGG |
| GCTGGCGAAGCTTGGGG | AGTCTCCCCCTCCAAACCATCCGGACCCAC | AGTCTCCCCCTCCAAACCATCCGGACCCAC | AGTCTCCCCCTCCAAACCATCCGGACCCAC | AGTCTCCCCCTCCAAACCATCCGGACCCAC | AGTCTCCCCCTCCAAACCATCCGGACCCAC |
| GGTAACGCCAGGGTTTTCGCCTTCTGGCTCGGATGCCTCTCCAAGACC | GACGACACTCGTGGTTGGCTGACTCAAGCGC | GACGACACTCGTGGTTGGCTGACTCAAGCGC | GACGACACTCGTGGTTGGCTGACTCAAGCGC | GACGACACTCGTGGTTGGCTGACTCAAGCGC | GACGACACTCGTGGTTGGCTGACTCAAGCGC |
| Primers-1 for cna1 genotyping | Primers-2 for cna1 genotyping | Primers-3 for cna1 genotyping | Primers-4 for cna1 genotyping | Primers-5 for cna1 genotyping | Primers-6 for cna1 genotyping |
| F cnb1 (5'UTR) | R cnb1 (5'UTR) | F cnb1 (3'UTR) | R cnb1 (3'UTR) | Amplification of cna1 cassette for electroporation |
| ATCTGATCATGAGCGGAGCGAGTTGCCAAACT | GCTGGCGAAGCTTGGGG | GCTGGCGAAGCTTGGGG | GCTGGCGAAGCTTGGGG | CGCGATGCTGGCGAACTCCGACGAGTTGGG |
| GCTGGCGAAGCTTGGGG | AGTCTCCCCCTCCAAACCATCCGGACCCAC | AGTCTCCCCCTCCAAACCATCCGGACCCAC | AGTCTCCCCCTCCAAACCATCCGGACCCAC | AGTCTCCCCCTCCAAACCATCCGGACCCAC | AGTCTCCCCCTCCAAACCATCCGGACCCAC |
| GGTAACGCCAGGGTTTTCGCCTTCTGGCTCGGATGCCTCTCCAAGACC | GACGACACTCGTGGTTGGCTGACTCAAGCGC | GACGACACTCGTGGTTGGCTGACTCAAGCGC | GACGACACTCGTGGTTGGCTGACTCAAGCGC | GACGACACTCGTGGTTGGCTGACTCAAGCGC | GACGACACTCGTGGTTGGCTGACTCAAGCGC |

For cloning

| Primers-1 for cna1 genotyping | Primers-2 for cna1 genotyping | Primers-3 for cna1 genotyping | Primers-4 for cna1 genotyping | Primers-5 for cna1 genotyping | Primers-6 for cna1 genotyping |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| F cnb1 (5'UTR) | R cnb1 (5'UTR) | F cnb1 (3'UTR) | R cnb1 (3'UTR) | Amplification of cna1 cassette for electroporation |
| ATCTGATCATGAGCGGAGCGAGTTGCCAAACT | GCTGGCGAAGCTTGGGG | GCTGGCGAAGCTTGGGG | GCTGGCGAAGCTTGGGG | CGCGATGCTGGCGAACTCCGACGAGTTGGG |
| GCTGGCGAAGCTTGGGG | AGTCTCCCCCTCCAAACCATCCGGACCCAC | AGTCTCCCCCTCCAAACCATCCGGACCCAC | AGTCTCCCCCTCCAAACCATCCGGACCCAC | AGTCTCCCCCTCCAAACCATCCGGACCCAC | AGTCTCCCCCTCCAAACCATCCGGACCCAC |
| GGTAACGCCAGGGTTTTCGCCTTCTGGCTCGGATGCCTCTCCAAGACC | GACGACACTCGTGGTTGGCTGACTCAAGCGC | GACGACACTCGTGGTTGGCTGACTCAAGCGC | GACGACACTCGTGGTTGGCTGACTCAAGCGC | GACGACACTCGTGGTTGGCTGACTCAAGCGC | GACGACACTCGTGGTTGGCTGACTCAAGCGC |

For cloning

| Primers-1 for cna1 genotyping | Primers-2 for cna1 genotyping | Primers-3 for cna1 genotyping | Primers-4 for cna1 genotyping | Primers-5 for cna1 genotyping | Primers-6 for cna1 genotyping |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| F cnb1 (5'UTR) | R cnb1 (5'UTR) | F cnb1 (3'UTR) | R cnb1 (3'UTR) | Amplification of cna1 cassette for electroporation |
| ATCTGATCATGAGCGGAGCGAGTTGCCAAACT | GCTGGCGAAGCTTGGGG | GCTGGCGAAGCTTGGGG | GCTGGCGAAGCTTGGGG | CGCGATGCTGGCGAACTCCGACGAGTTGGG |
| GCTGGCGAAGCTTGGGG | AGTCTCCCCCTCCAAACCATCCGGACCCAC | AGTCTCCCCCTCCAAACCATCCGGACCCAC | AGTCTCCCCCTCCAAACCATCCGGACCCAC | AGTCTCCCCCTCCAAACCATCCGGACCCAC | AGTCTCCCCCTCCAAACCATCCGGACCCAC |
| GGTAACGCCAGGGTTTTCGCCTTCTGGCTCGGATGCCTCTCCAAGACC | GACGACACTCGTGGTTGGCTGACTCAAGCGC | GACGACACTCGTGGTTGGCTGACTCAAGCGC | GACGACACTCGTGGTTGGCTGACTCAAGCGC | GACGACACTCGTGGTTGGCTGACTCAAGCGC | GACGACACTCGTGGTTGGCTGACTCAAGCGC |

For cloning
### cnb1 5'-UTR/ORF fragment

#### 1st PCR
- TaCNA-F1/SpeI
- TaCNB-R2(-KpnI)
- TaCNB-F2(-KpnI)
- TaCNB-R6/ApaI

#### 2nd PCR (overlapping PCR)
- TaCNA-F1/SpeI
- TaCNB-R6/ApaI

### hph cassette

#### 1st PCR
- CnPactin(F)ApaI
- CnPact-hph(R)
- CnPact-hph(F)
- hph-Ttrp1(R)

#### 2nd PCR (overlapping PCR)
- CnPactin(F)ApaI
- Ttrp1(R)BamHI

### Primers-1 for cnb1 genotyping

#### F cnb1 gene locus
- GGAGTGAAGAAGGGCAGAGAGCAACAACACAGCGGT

#### R cnb1 gene locus
- CCGTGATCGCATGGGGCGTGCACAAAGTG

### Primers-2 for cnb1 genotyping

#### F cnb1 gene ORF
- CGGCTCGGGGTACGGTAGACTTCCAGGAGTTTGTGCG

#### R cnb1 gene ORF
- AACAGGTCTCAGCACGTGTCATCTGCTTGACGATGT

### Primers-3 for cnb1 genotyping

#### F cnb1 gene outside 1
- CATATCCCTCACGGTGAGGTCAGGCGCC

#### R cnb1 gene outside 1
- CTGGTGCGGTACCGGTAAGCCGTTGTCGTAAG
| Primers-4 for cnb1 genotyping |  |
|-----------------------------|--|
| F \( cnb1 \) gene outside 2 | GGACGGCGAGCAGGCAGCGCTCTACATGAGC |
| R \( cnb1 \) gene outside 2 | CTGAGTCCCATCGCCCTTGCTTTCAAGCTACC |

| Primers-5 for cnb1 genotyping |  |
|-----------------------------|--|
| F \( cnb1 \) gene outside 1 | CATATCCCTCACGTTGCGGTCAGGCAGCC |
| R \( cnb1 \) gene outside 3 | CCGAGAGCTGATCAGGTCGAGAGCG |

| Primers-6 for cnb1 genotyping |  |
|-----------------------------|--|
| F \( cnb1 \) gene outside 3 | CAGGTCGATGCGACGCAATCGTCCGTAC |
| R \( cnb1 \) gene outside 2 | CTGAGTCCCATCGCCCTTGCTTTCAAGCTACC |

| RT-primers-1 for RT-PCR |  |
|-------------------------|--|
| \( FHXL1 \) (intact) | AACGCTCACCTCGCTCGGCG |
| \( RHXL1 \) (Splicing) | GGGGGCAGCGAGATGGAGTG |

| RT-primers-2 for RT-PCR |  |
|-------------------------|--|
| \( FHXL1 \) | AACGCTCACCTCGCTCGGCG |
| \( RHXL1 \) (Splicing) | GCTGGCAAGTCCAGTTGCTCTTG |

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Supplementary Fig. S1 Effect of DTT on the HXL1 mRNA splicing in T. asahii.

(a) The HXL1 mRNA splicing by Ire1-mediated UPR signaling in T. asahii. (b) Location of the primers for RT-PCR. (c) The T. asahii parent strain was incubated on Sabouraud dextrose medium with or without DTT (12 mM) at 37°C for 1 h. Total RNA was extracted according to the general method (NucleoSpin RNA, Takara, Shiga, Japan). Reverse transcription was performed using a kit (ReverTra Ace qPCR RT Master Mix, TOYOBO Co., Ltd., Osaka, Japan). Primers used in the experiments were listed in Supplementary table 1. Control: without DTT. DTT: with DTT (12 mM). Two samples per group were shown. (d) The T. asahii parent strain (Parent), the cnal gene-deficient mutant (∆cnal), the revertant of ∆cnal (CNA1), the cnb1 gene-deficient mutant (∆cnb1), and the revertant of ∆cnb1 (CNB1) was incubated on Sabouraud dextrose medium with or without DTT (12 mM) at 37°C for 1 h. Control: without DTT. DTT: with DTT (12 mM).
Supplementary Fig. S2 The representative cells to determine the cell types.
Supplementary Fig. S3 Full-length blots of Figure 1d and 1h. Marker 1: OneSTEP Marker 1(λ/HindIII digest) (Nippon Gene Co., Ltd., Tokyo, Japan), Maker 2: Gene Ladder 100 (Nippon Gene Co., Ltd., Tokyo, Japan).