Supplementary Materials

Supplementary Figure 1: PCR analysis for double mutant confirmation. The $\Delta trm-9\Delta nca-2$ double mutants were verified by using the forward primers NCU04898-5F 5' GGTTAGTGAGCTTTGAGTCG 3' and NCU04736 -5F 5' TACACTGGTAATGGACCACG 3' specific for upstream of the open reading frame of genes *trm-9* and *nca-2*, respectively, and with the common reverse primer 5HPHR 5' ATCCACTTAACGTTACTGAAATC 3' that is specific for the *hph* cassette used to generate the knockout mutants (Colot et al., 2006; Deka et al., 2011). Amplification of PCR products of size ~1.2 and ~1.018 kb indicate the presence *trm-9* and *nca-2* knockout alleles respectively. The wild-type was used as negative controls for the knockout alleles (indicated in the parenthesis) using the allele specific primer pairs. PCR products were visualized in a 0.8% agarose gel with 1 kb DNA ladder.

Supplementary Figure 2: Conidial cell count of wild-type, $\Delta trm-9$, $\Delta nca-2$, and $\Delta trm-9\Delta nca-2$ double mutant strains grown in Vogel's glucose medium. Conidia count of wild-type and mutant strains are plotted with relative counting with respect to wild-type. Error bars indicate the standard errors calculated from the data for three independent experiments. Conidial cell count of $\Delta trm-9\Delta nca-2$ double mutant strain was less than the parental single knockout mutant strains and wild-type.

Supplementary Figure 3: Sequence alignment of *cmd* **and** *trm9* **homologues.** (A) Sequence alignment of the *Neurospora crassa* CaM homologues. (B) Sequence alignment of the *trm-9* homologues. Conserved amino acids are indicated in black (100%), dark gray (>80%) and light gray (>60%). The homologue sequences used for the sequence analysis are from *Ajellomyces capsulatus* (AC), *Ajellomyces dermatitidis SLH14081* (AD), *Aspergillus fumigatus* (AF), *Aspergillus nidulans* (AN), *Botryotinia fuckeliana B05.10* (BF), *Candida albicans* (CA), *Coccidioides immitis* (CI), *Cordyceps militaris CM01* (CM), *Coccidioides posadasii* (CP), *Dichotomomy cescejpii* (DC), *Esox lucius* (EL), *Grosmannia clavigera kw1407* (GC), *Glomerella graminicola* (GG), *Gibberella zeae PH-1* (GZ), *Homo sapiens* (HS), *Komagataella pastoris* (KP), *Magnaporthe grisea* (MG), *Neurospora crassa* (NC), *Neurospora tetrasperma* (NT), *Procambarus clarkii* (PC), *Phytophthora infestans T30-4* (PI), *Rhodomonas sp. CCMP768* (RS), *Saccharomyces cerevisiae* (SC), *Schistosoma mansoni* (SM), *Spathaspora passalidarum* (SP), *Trichoderma reesei* (TR), and *Talaromyces stipitatus ATCC 10500* (TS).

Supplementary Figure 4: Phylogenetic analysis of the (A) CaM and (B) TRM-9 proteins. Protein sequences are described using GenBank accession numbers, phylum is indicated at major clades, and bar indicates scale of genetic distances. The homologue sequences used for the sequence analysis are from Ajellomyces capsulatus (AC), Ajellomyces dermatitidis SLH14081 (AD), Aspergillus fumigatus (AF), Arthroderma gypseum (AG), Aspergillus kawachii (AK), Aspergillus nidulans (AN), Aspergillus oryzae (AO), Botryotinia fuckeliana B05.10 (BF), Candida albicans (CA), Coccidioides immitis (CI), Cordyceps militaris CM01 (CM), Coccidioides posadasii (CP), Dichotomomy cescejpii (DC), Drosophila melanogaster (DM), Exophiala dermatitidis (ED), Esox lucius (EL), Eurotium rubrum (ER), Emericella unguis (EU), Grosmannia clavigera kw1407 (GC), Glomerella graminicola (GG), Gibberella zeae PH-1 (GZ), Homo sapiens (HS), Komagataella pastoris (KP), Metarhizium anisopliae (MA), Magnaporthe grisea (MG), Neurospora crassa (NC), Neurospora tetrasperma (NT), Ogataeapara polymorpha (OP), Paracoccidioides brasiliensis (PB), Procambarus clarkii (PC), Puccinia graminis f. sp. Tritici (PG), Phytophthora infestans T30-4 (PI), Penicillium rolfsii (PR), Pyrenophora tritici-repentis (PT), Rhodomonas sp. CCMP768 (RS), Saccharomyces cerevisiae (SC), Saccharomyces cerevisiae RM11-1a (SC RM11), Saccharomyces cerevisiae x Saccharomyces kudriavzevii VIN7 (SCSK), Sordaria macrospora Spathaspora passalidarum (SP), Scheffersomyce sstipitis (SS), Trichophyton equinum (TE), (SM). Trichoderma reesei (TR), Talaromyces stipitatus ATCC 10500 (TS), and Verticillium albo-atrum (VA).

Supplementary Figure 5: Promoter analysis of (A) cmd and (B) trm-9 gene of N. crassa. Gray boxes

showed the important regulatory sequences of gene and transcription start site (TSS) are indicated by using arrows.

Supplementary Figure 6: Expression studies of *cmd* gene in the presence of inhibitors. Fold change in expression was calculated by $2^{-\Delta\Delta Ct}$ method, using wild-type and β -tubulin as calibrator and endogenous control respectively. Standard errors calculated from the data for two independent experiments are shown using error bars.

Supplementary Figure 7: Ergosterol is present in the $\Delta trm-9$ mutant and $\Delta trm-9\Delta nca-2$ double mutant strains. Profile of the sterols extracted from the wild-type, $\Delta nca-2$, $\Delta trm-9$, $\Delta trm-9\Delta nca-2$, and *erg-3* mutant strains were analysed by UV spectrophotometer.

Supplementary Figure 8: (A) Calcium sensitivity, (B) development of aerial hyphae, and (C) UV survival. (A) Ca²⁺sensitivity analysis of wild-type, $\Delta trm-9$, $\Delta nca-2$, and $\Delta trm-9\Delta nca-2$ double mutant strains. Colony diameter (cm h⁻¹) were measured at regular intervals and plotted against various concentrations of CaCl₂. Standard errors calculated from the data for three independent experiments are shown using error bars. (B) Aerial hyphae development of the wild-type, $\Delta trm-9$, $\Delta nca-2$, and $\Delta trm-9\Delta nca-2$ double mutant strains on VSM agar media in test tube. The aerial hyphae growth of $\Delta trm-9\Delta nca-2$ double mutant strain was less as compared to parental single mutants and wild-type strain. (C) UV survival. Spot-test analysis of wild-type, $\Delta trm-9$, $\Delta nca-2$, and $\Delta trm-9\Delta nca-2$ double mutant strain grown on VG agar at 30°C for 48 h in dark then illuminated for 24 h. UV survival assay was done essentially as described previously (Deka et al., 2011).

Supplementary references:

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A.



B.

XF_754245.1_AF_CT_ATFase XF_001727731.2_A0_CT_ATFase XF_00066798.1_CF_HIZ_ATFase XF_00066798.1_CF_HIZ_ATFase XF_00066798.1_CF_ATFase XF_00040737.1_CF_ATFase XF_000405551.1_FF_P_ATFase XF_000405551.1_FF_P_ATFase XF_0104647.1_SC_Spfp		198 199 123 196 196 196 196 196 190 191
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XP_754245.1_AF_CT_ATPase XP_01727731.2_AO_CT_ATPase1 ERF3335.1_AAF_AFPase XP_00066798.1_CP_11E2_ATPase4 XP_00066798.1_CP_ATPase4 EF031737.1_CO_ATPase EF031737.1_CP_ATPase EF03081.7FC_CPump XP_002492551.1_FP_P_ATPase EEU04647.1_BC_Epfp		186 174 181 177 177 178 169 116 128 120
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B. EEQ90898.1 AD PATPase 100 EER38625.1 AC CTATPase — XP 002792040.1 PB CTATPase XP 003066798.1 CP E1 E2ATPase 87 XP 003176597.1 AG CTATPase 4 100 XP 003238565.1 TR CTATPase 100 EGE02335.1 TE CTATPase 100 Eurotiomycetes XP 754245.1 AF CTATPase XP 001727731.2 AO CTATPase 1 84 GAA84582.1 AK CTATPase 100 97 CBF83315.1 AN PATPase 81 EHY61112.1 ED CTATPase - XP 001935452.1 PT CTATPase 4 - EFX02155.1 GC CTprotein Dothideomycetes 100 64 100 EGR45308.1 TR CCpump EFY99478.1 MA CTATPase 4 100 EGX91104.1 CM CTATPase 4 - XP 003001543.1 VA CTATPase Sordariomycetes 36 EFQ33737.1 GG ATPase XP 003344921.1 SM CTATPase 4 100 XP 958407.1 NC CTATPase 4 100 EGO58683.1 NT CTATPase 4 100 EEU04647.1 SC Spflp EDV08801.1 SCRM11 CTATPase 4 100 – EHN02853.1 SCSK Spflp XP 002492591.1 KP PATPase 5 100 Saccharomycetes - EFW96493.1 OP PATPase - XP 001385220.2 SS PATPase - XP 711837.1 CA potPATPase 100 EGW33133.1 SP PATPase EFP84205.2 PG CLTATPase Pucciniomycetes 0.05

















