Supplementary Materials

Supplementary Figure 1: PCR analysis for double mutant confirmation. The Δ*trm-9*Δ*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, Δ*trm-9*, Δ*nca-2*, and Δ*trm-9*Δ*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 Δ*trm-9*Δ*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* (SM), *Spathaspora passalidarum* (SP), *Scheffersomyce sstipitis* (SS), *Trichophyton equinum* (TE), *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 Δ*trm-9* mutant and Δ*trm-9*Δ*nca-2* double mutant strains. Profile of the sterols extracted from the wild-type, Δ*nca-2*, Δ*trm-9*, Δ*trm-9*Δ*nca-2*, and *erg-3* mutant strains were analysed by UV spectrophotometer.

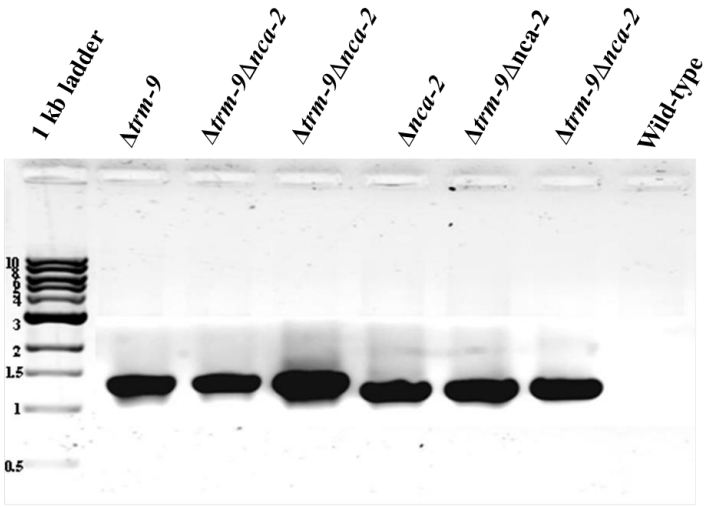
Supplementary Figure 8: (A) Calcium sensitivity, (B) development of aerial hyphae, and (C) UV survival. (A) Ca^{2+}sensitivity analysis of wild-type, Δ*trm-9*, Δ*nca-2*, and Δ*trm-9*Δ*nca-2* double mutant strains. Colony diameter (cm h^{-1}) were measured at regular intervals and plotted against various concentrations of $CaCl_2$. Standard errors calculated from the data for three independent experiments are shown using error bars. (B) Aerial hyphae development of the wild-type, Δ*trm-9*, Δ*nca-2*, and Δ*trm-9*Δ*nca-2* double mutant strains on VSM agar media in test tube. The aerial hyphae growth of Δ*trm-9*Δ*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, Δ*trm-9*, Δ*nca-2*, and Δ*trm-9*Δ*nca-2* double mutant and *upr-1* 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:

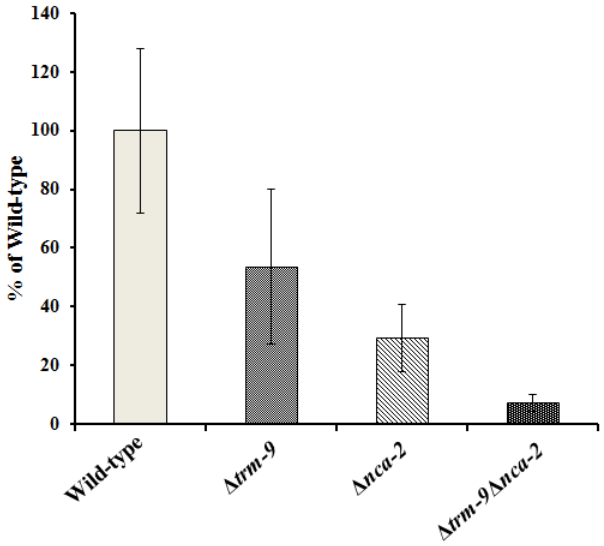
Colot H.V., Park G., Turner G.E., Ringelberg C., Crew C.M., Litvinkova L., Weiss R.L., Borkovich K.A., Dunlap J.C., 2006, A high-throughput gene knockout procedure for *Neurospora* reveals functions for multiple transcription factors, Proc Natl Acad Sci U S A 103:10352-10357
http://dx.doi.org/10.1073/pnas.0601456103

Deka R., Kumar R., Tamuli R., 2011, *Neurospora crassa* homologue of Neuronal Calcium Sensor-1 has a role in growth, calcium stress tolerance, and ultraviolet survival, Genetica 139:885-894
http://dx.doi.org/10.1007/s10709-011-9592-y

Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3

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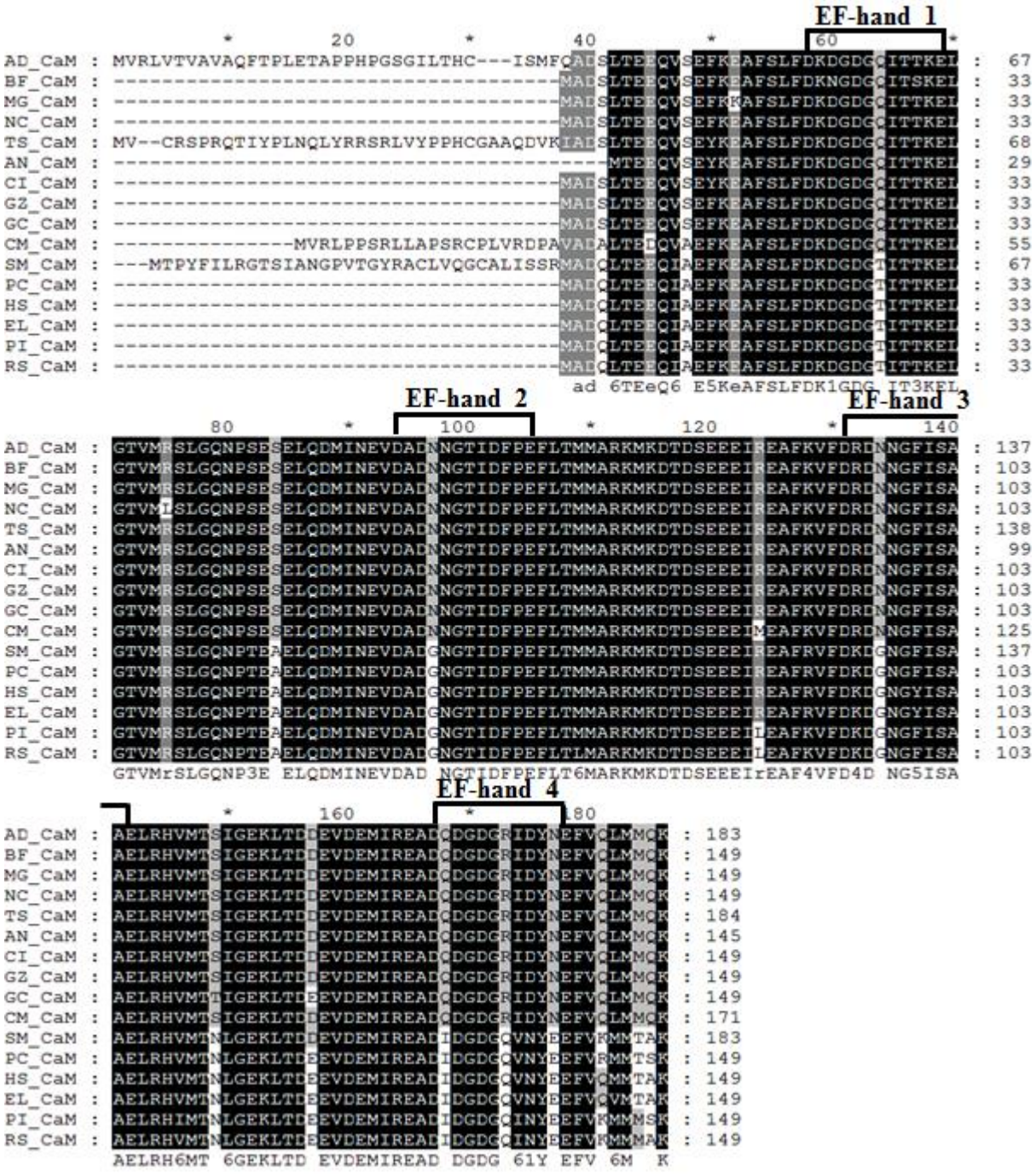

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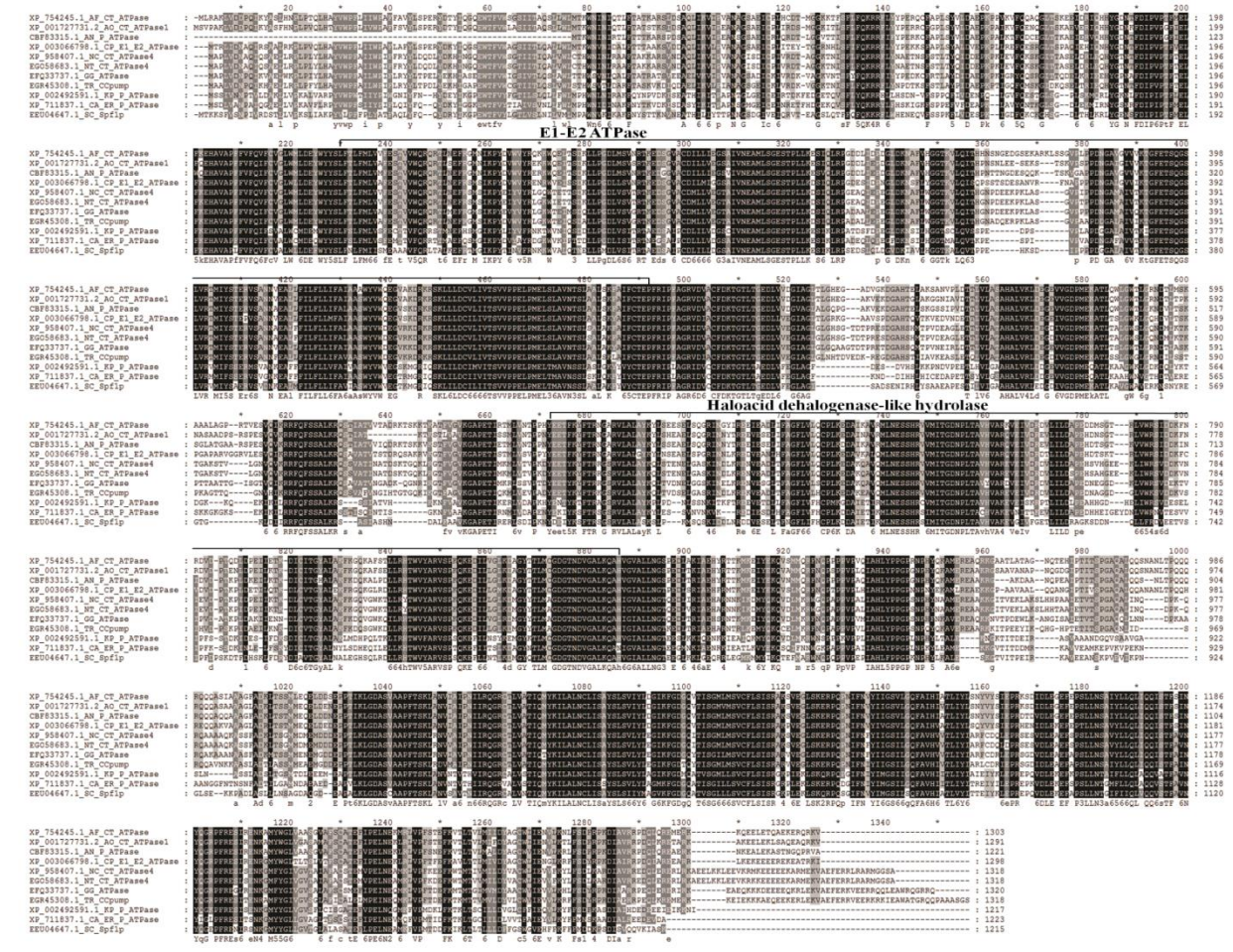
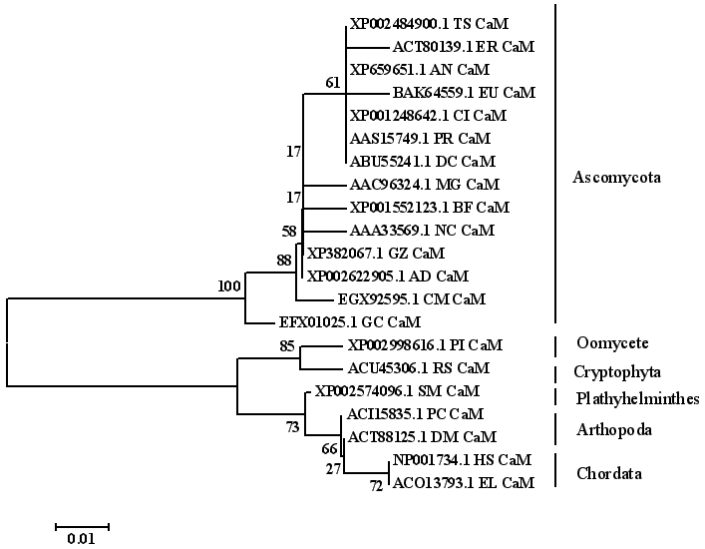


Supplementary Figure 4

A.



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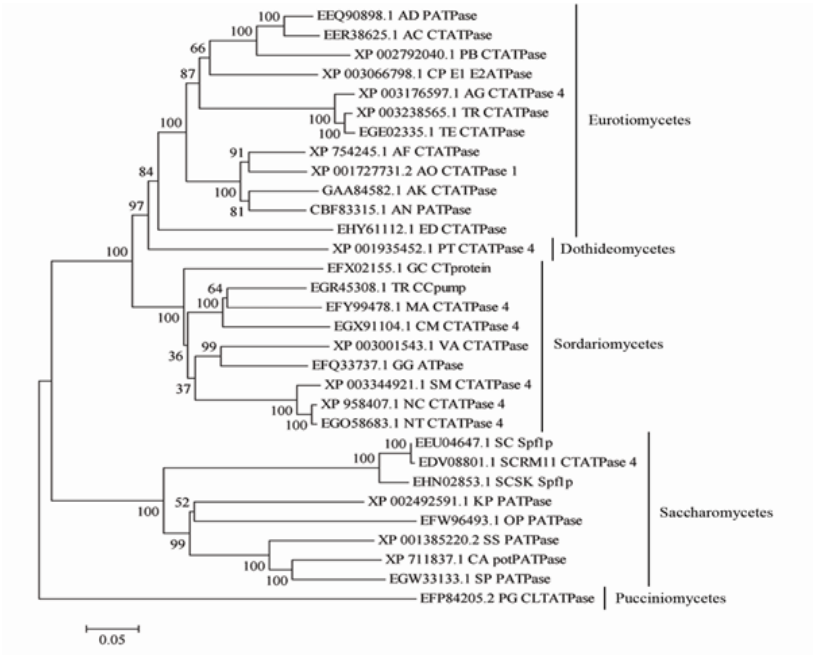
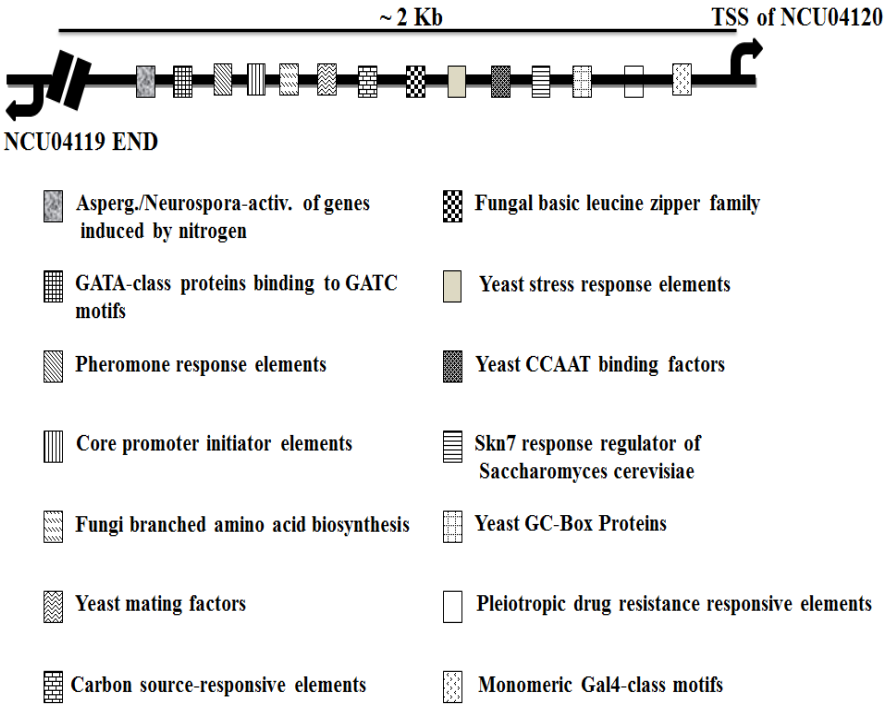


Supplementary Figure 5

A.



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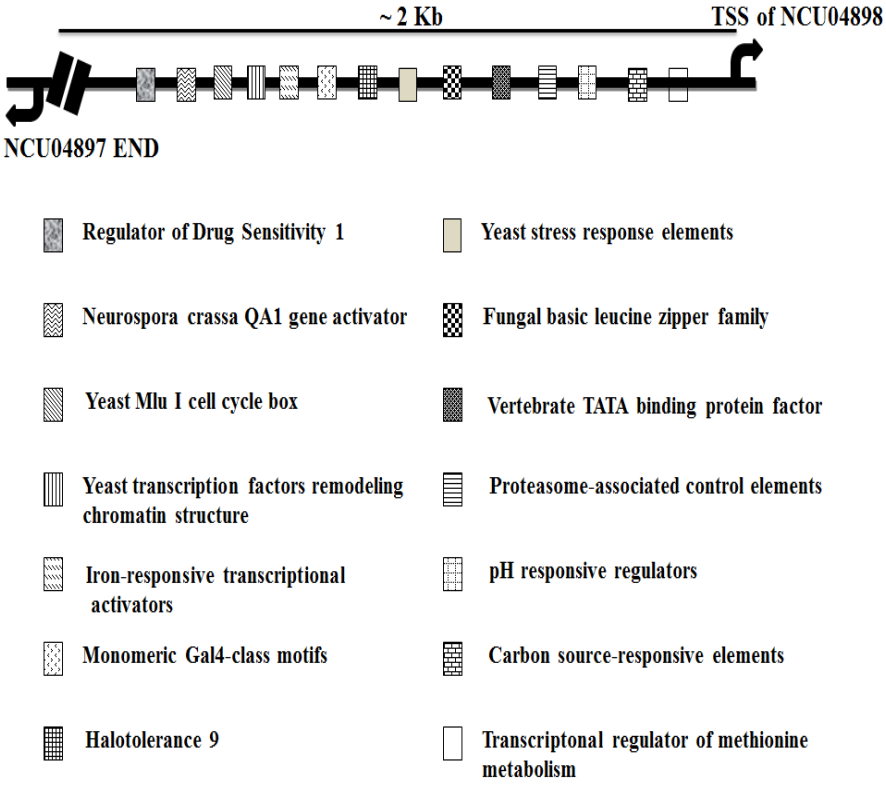

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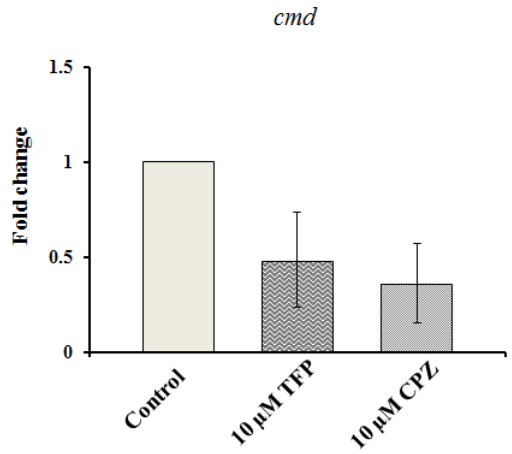

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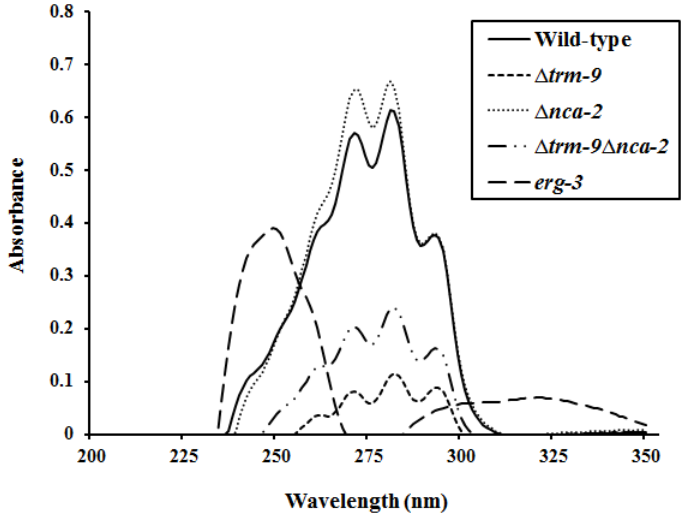
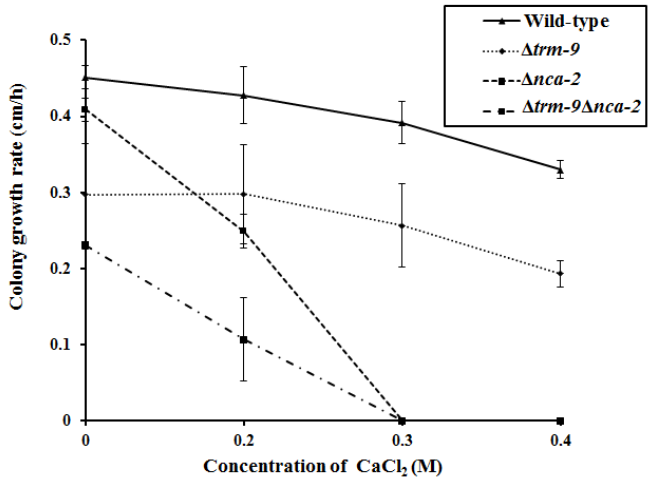


Supplementary Figure 8

A.



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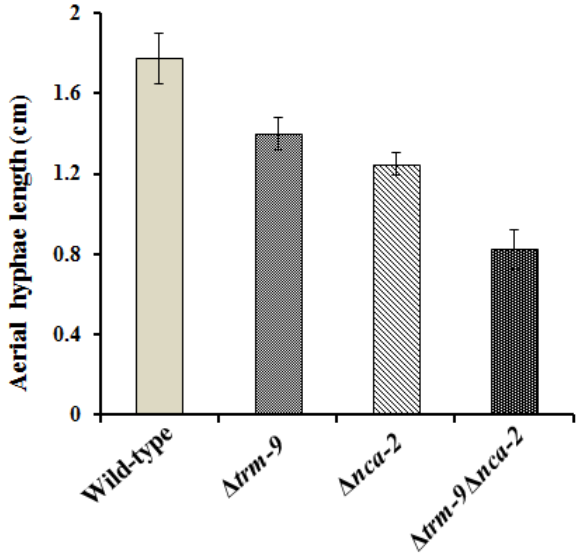

C.