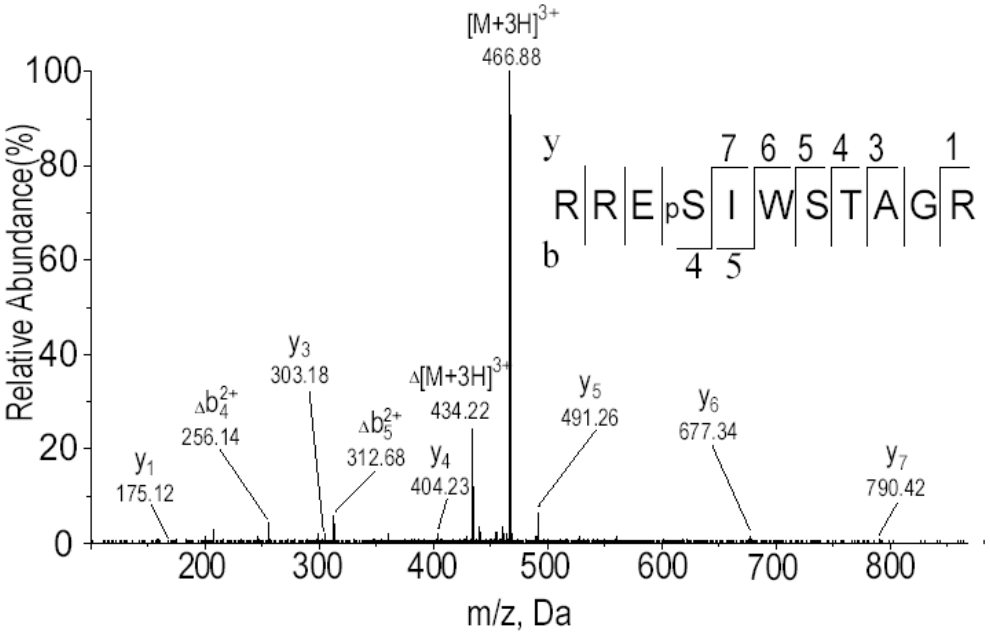


Figure S1. Identification of a phosphorylated form of Ser189 within the N-terminal region of COT1:

The collision-induced dissociation (CID) spectrum of the triply-charged phosphopeptide RREpSIWSTAGR (m/z at 466.88) is shown. The major b- and y- type ions are indicated in the graph. Δ represents neutral loss of a phosphoric acid.

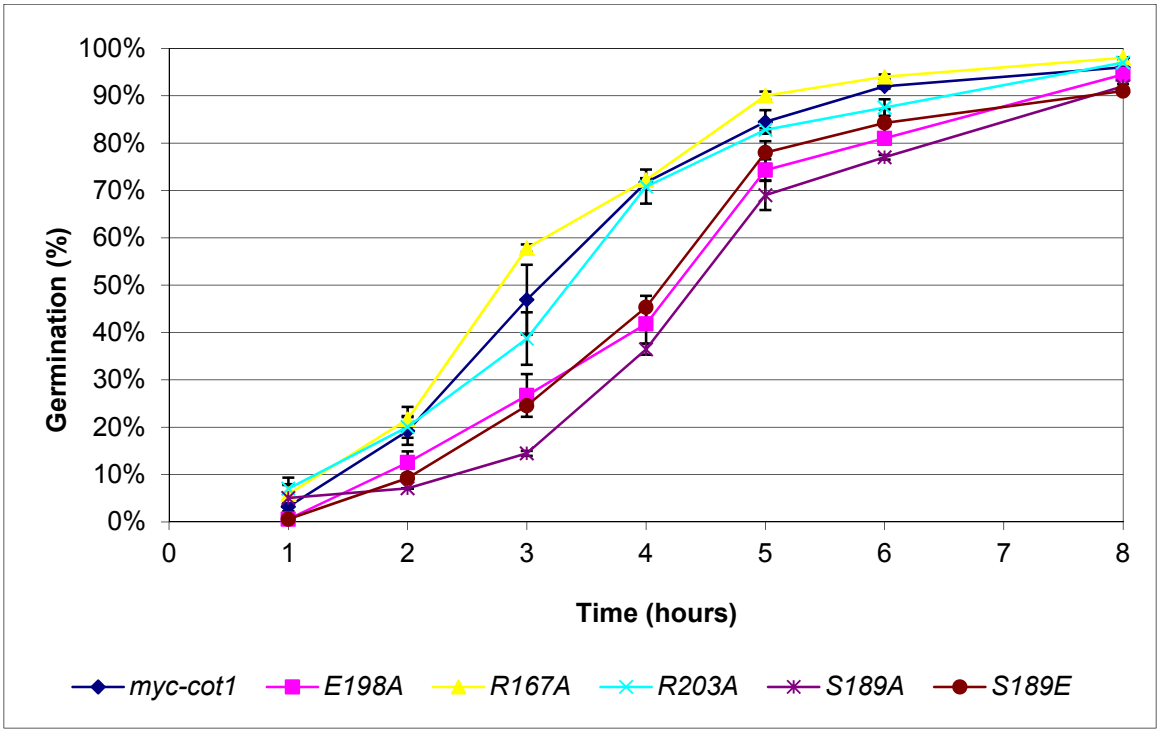


Figure S2. Mutations in Ser189 of COT1 result in delayed conidial germination:

Conidia of *myc-cot1* and the different COT1 NTR mutants were incubated in Vogel's sucrose minimal medium at 34°C. The amount of germinating conidia (% of total conidia) was determined every 1-2 hours. Standard deviations are shown.

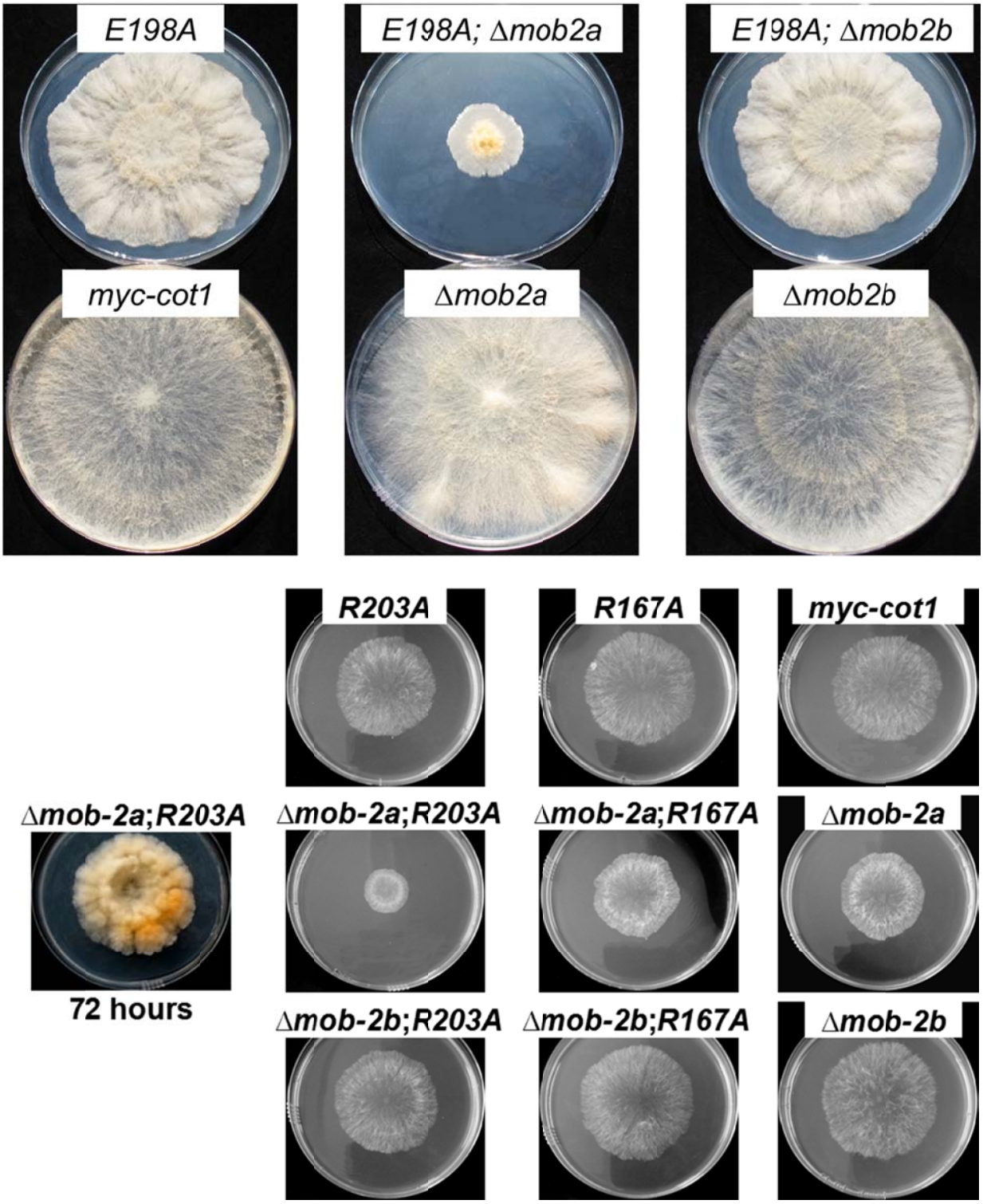


Figure S3. Synergistic interaction between *cot-1*(E198A) and *cot-1*(R203A) mutants with Δ*mob-2a*:

Strains were grown on Vogel's sucrose minimal medium at 34°C, for 2-3 days.

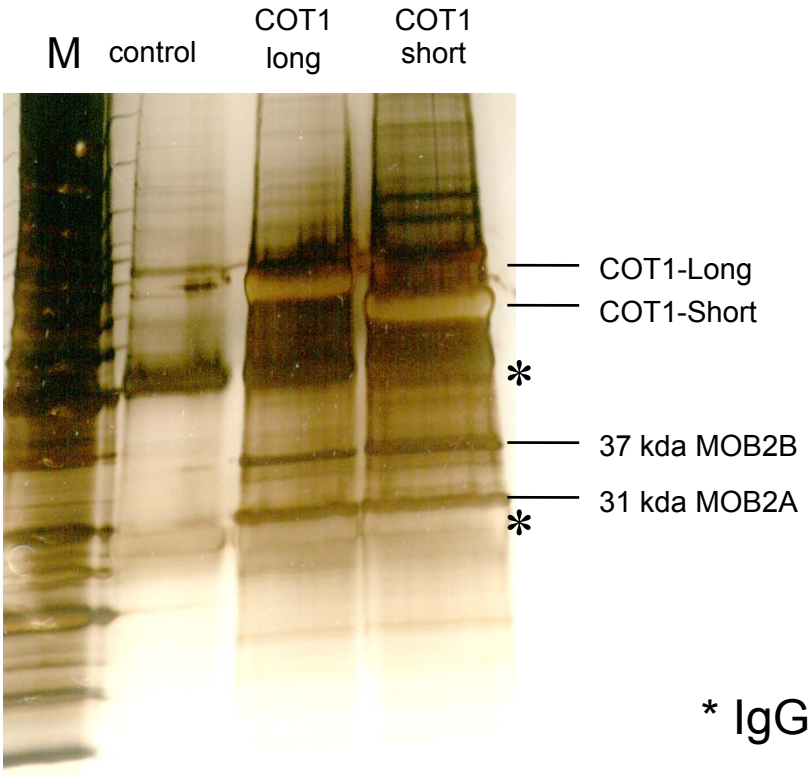


Figure S4. Affinity purification with the two MYC::HIS::COT1 isoforms:

Pull-down of both COT1 isoforms resulted in the co-purification of MOB2A (NCU03314) and MOB2B (NCU07460). Asterisk indicates IgG.

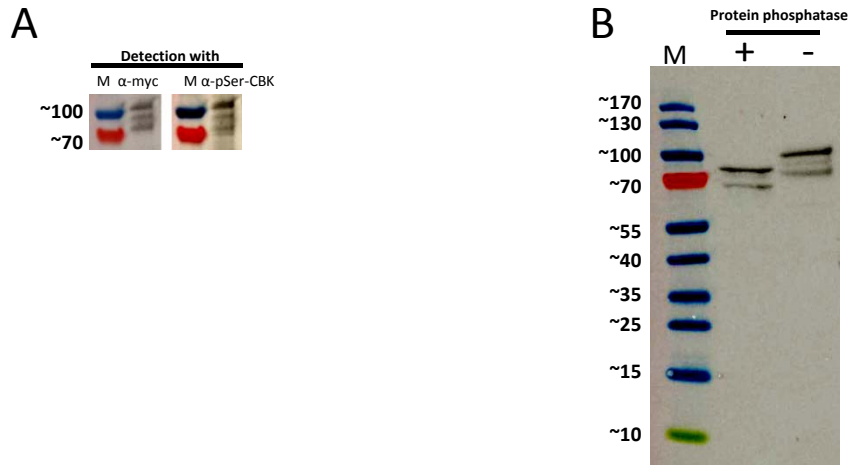
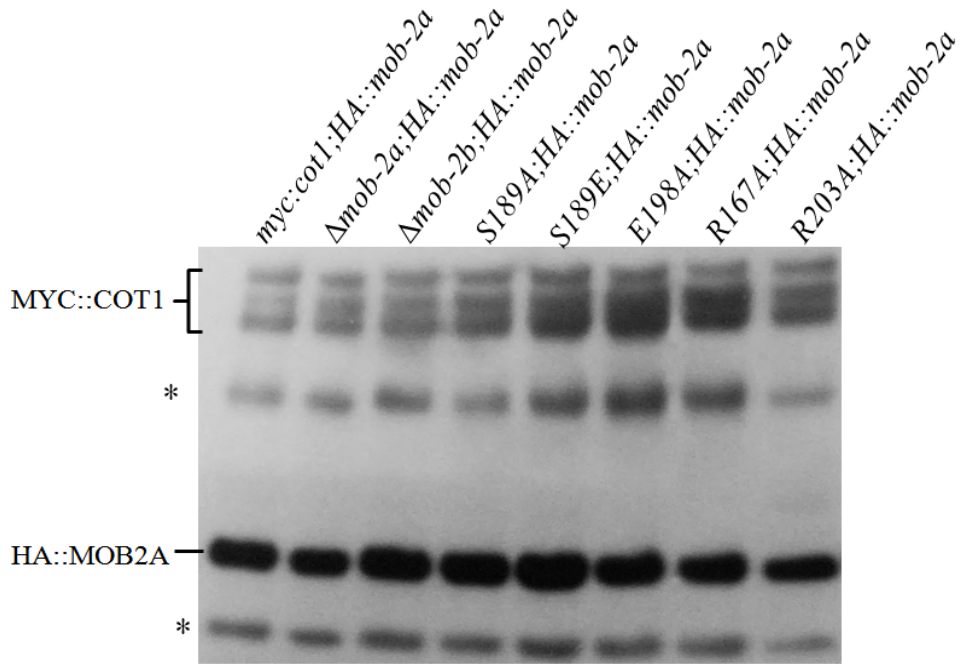


Figure S5. COT1 is expressed as three phosphorylated isoforms

(A) Western blot analysis of MYC::COT1 from wild-type that was immuno-precipitated with anti-myc antibodies and detected with anti-myc antibodies (left panel) or with anti-Ph-Ser-CBK1 antibodies (right panel). M: size marker (kDa)

(B) Western blot analysis of MYC::COT1 from wild-type after phosphatase treatment. Total proteins dephosphorylation resulted in a faster migration pattern of all three COT1 isoforms, indicating that all three COT1 isoforms are phosphorylated. M: size marker (kDa). Left lane (+): total protein extract after the phosphatase treatment. Right lane (-): same extract before the phosphatase treatment.

A: IP with α-MYC



B: IP with α-HA

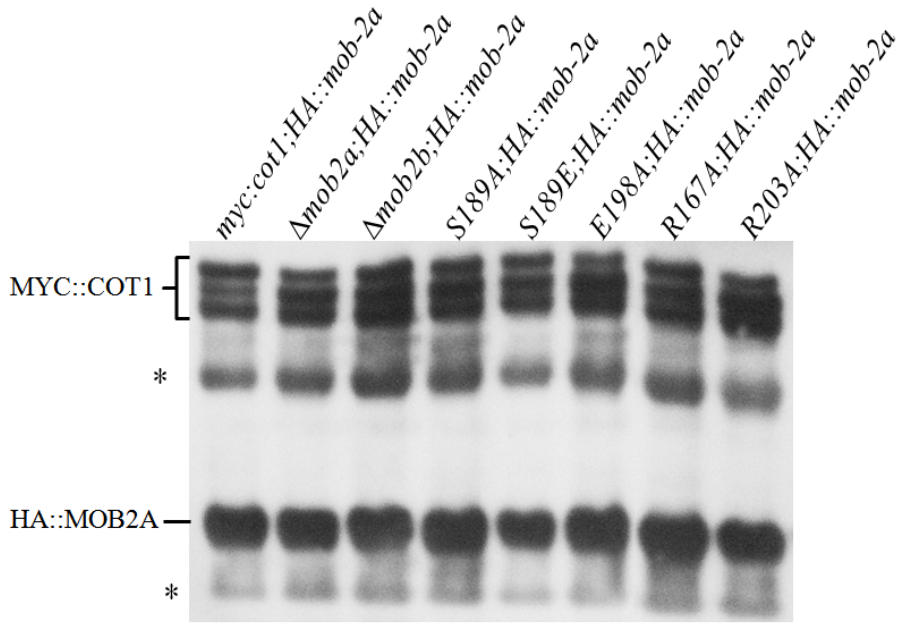


Figure S6. Co-IP of MYC-COT1 and HA-MOB2A detected with both antibodies.

(A) IP with anti-MYC antibodies. (B) IP with anti-HA antibodies. * marks the IgG.

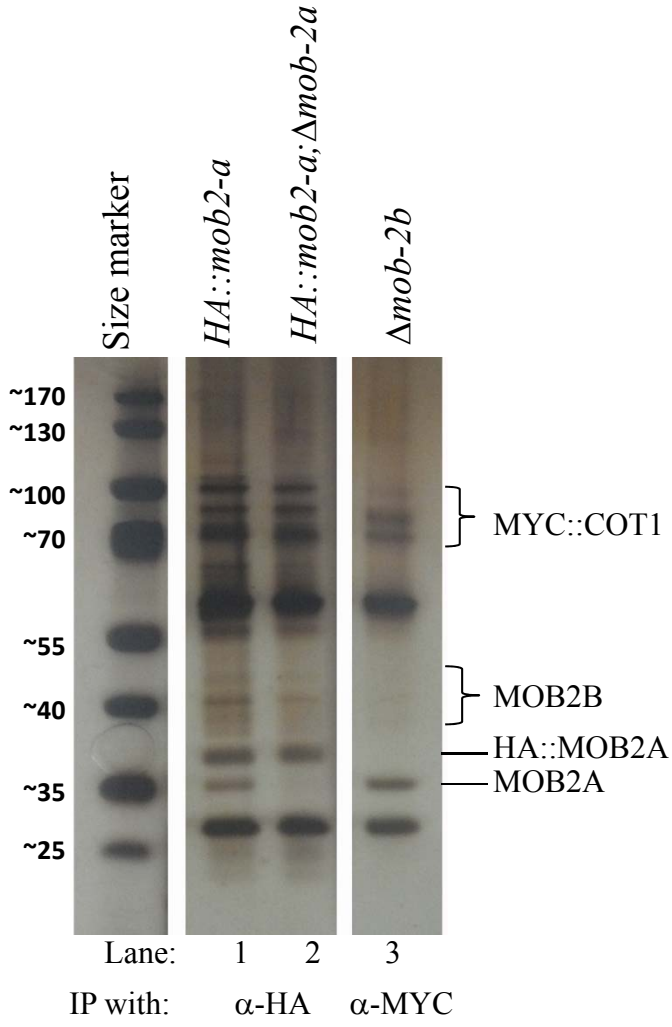


Figure S7. MOB2B is immune-precipitated by HA-MOB2A

Silver-stain of proteins obtained following IP performed with anti-HA antibodies (lanes 1 and 2) or with anti-MYC antibodies (lane 3), showing that MOB2B can be immuno-precipitated by MOB2A with COT1. The strains used for this assay were all in a myc-cot-1 background.

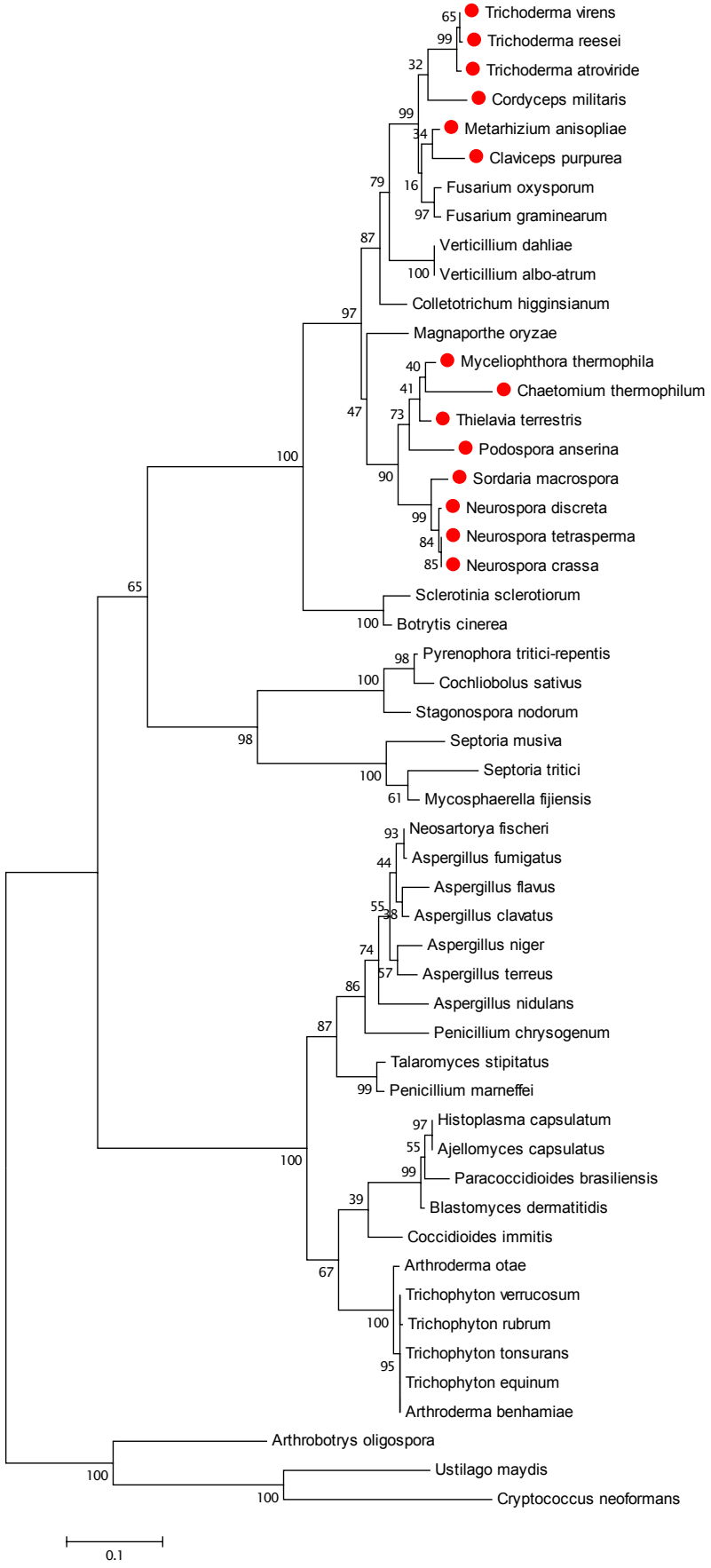


Figure S8. Phylogenetic tree of selected fungal COT1 homologs

Protein sequences of fungal COT1 homologs were used to construct a phylogenetic tree with the maximum-likelihood method.

The tree includes only fungi that were found to have homologs for *N. crassa* MOB2 and MOB1 proteins in NCBI.

Red dot indicates the presence of two different putative MOB2 proteins in the marked species.