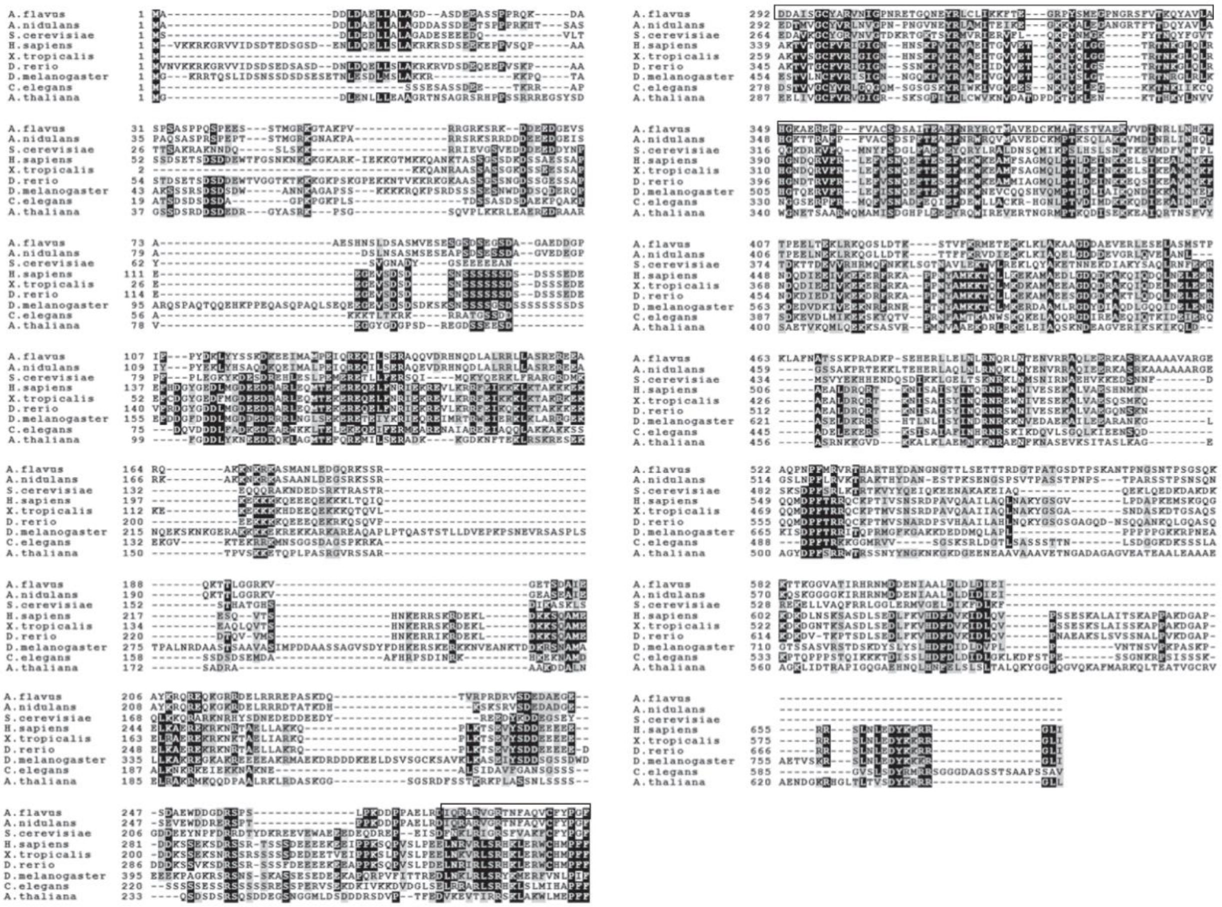

Figure S1. The *A. flavus* RtfA amino acid sequence presents low conservation with respect to putative homologs in higher eukaryotes. The deduced amino acid sequence corresponding to L morphotype NRRL 3357 *A. flavus* RtfA (XP_002377748.1). Since CA14 genetic background was used in this study, the *rtfA* nucleotide sequence was verified by DNA sequencing, compared to the NRRL 3357 *rtfA* sequence, and modified accordingly. Plus3 domain in *A. flavus* RtfA sequence is indicated with a black box. Accession numbers for amino acid sequences in other eukaryotic organisms are provided in **Table S1** and obtained from NCBI.

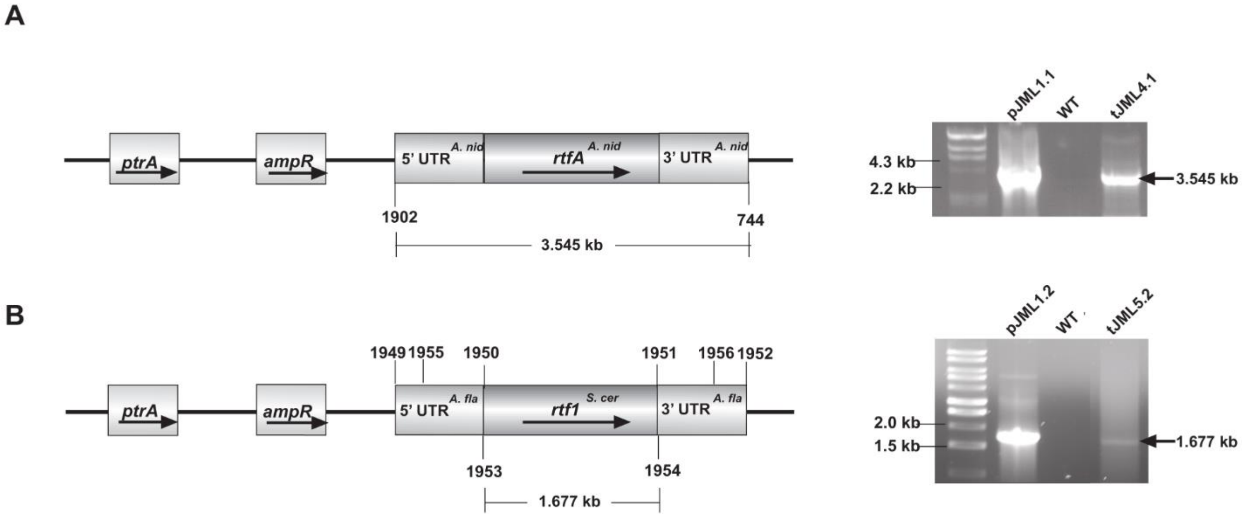

Figure S2. Confirmation of *rtfA* heterologous complementation strains. (A) Linear representation of *A. nidulans* heterologous complementation vector pJML1.1. Confirmation of integration of *A. nidulans rtfA* gene into the genome of the *A. flavus* Δ*rtfA* strain was carried out by diagnostic PCR using primers 1902 and 744, obtaining the expected 3.545 kb product. (B) Linear representation of *S. cerevisiae* heterologous complementation vector pJML1.2. Confirmation of integration of *S. cerevisiae rtf1* gene into the genome of the *A. flavus* Δ*rtfA* strain was carried out by diagnostic PCR using primers 1953 and 1954, obtaining the expected 1.577 kb product.

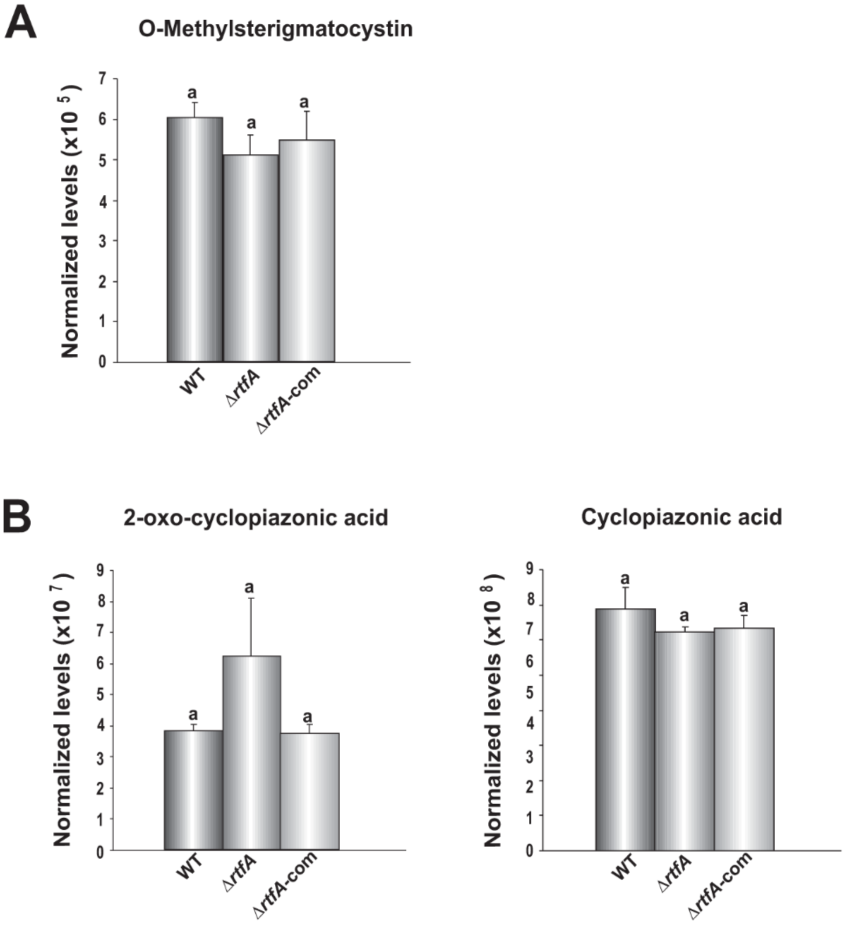

Figure S3. *rtfA* does not affect the production of O-methylsterigmatocystin and metabolites of the CPA biosynthetic pathway. *A. flavus* wild type (WT), Δ*rtfA*, and Δ*rtfA*-com strains were grown in YGT medium for 5 days at 30°C in the dark. Compounds in the culture supernatants were analyzed by LC-MS. (A) Analysis of O-methylsterigmatocystin. (B) Analysis of metabolites in the CPA pathway. Error bars represent standard error. Columns with different letters represent values that are statistically different ($p < 0.050$).

Table S1: *rtfA* putative homologs and amino acid sequence accession numbers utilized in the bioinformatic analysis

Species	**Accession Number**	**Emboss Needle-Pairwise Sequence Alignment (global alignment)**		
		Length	**% Identity**	**% Similarity**
Aspergillus flavus	XP_002377748.1	628	100.0	100.0
Aspergillus nidulans	XP_662174.1	600	70.4	82.1
Saccharomyces cerevisiae	NP_011270.1	558	25.0	44.0
Arabidopsis thaliana	NP_176299.1	643	21.3	37.7
Homo sapiens	NP_055953.3	670	20.8	37.9
Xenopus tropicalis	NP_001005068. 1	590	19.5	37.4
Danio rerio	NP_001077046.1	681	21.5	36.7
Drosophila melanogaster	NP_611665.1	775	19.5	33.9
Caenorhabditis elegans	NP_505473	613	20.1	36.1