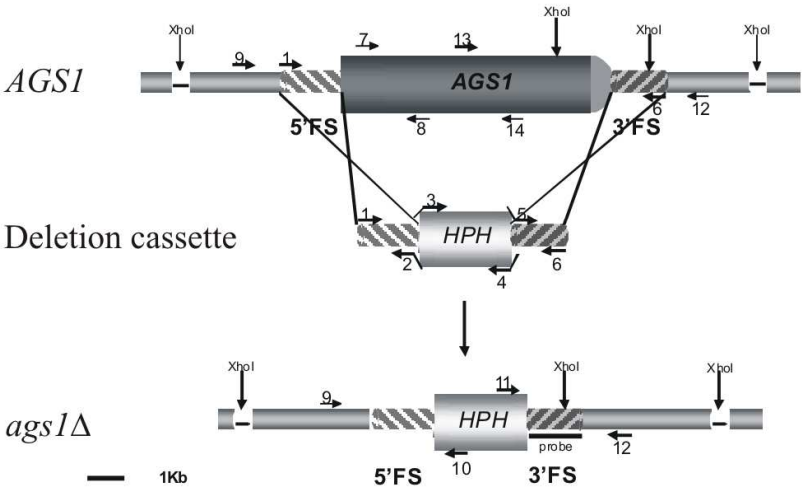
# Supplementary Material File

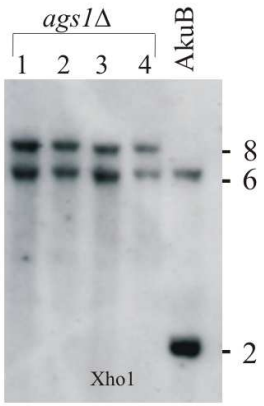
## Supplementary Method

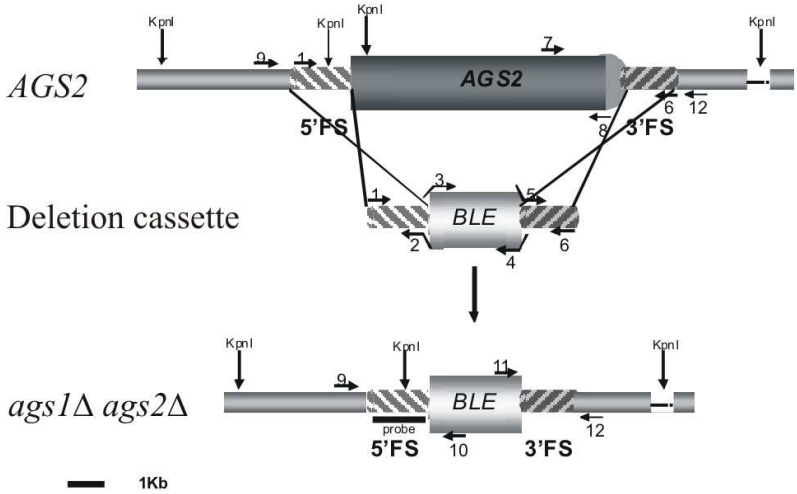
Four complementation strategies were attempted to complement the triple *AGS* mutant. These strategies were based on the complementation of the *AGS1* and *AGS3* genes. The *ags1*Δ showed a reduction of 50% α1,3 glucans and *ags3*Δ produced 3 times more melanin than the wild type, which make easier the identification of the complemented strain (2, 21). Strategies 1 and 2 used the *AGS3* construct which was obtained by PCR and contained 2kb of the 5' end, the full *AGS3* gene and 0.5kb of the 3'end. In strategy 1, co-transformations were undertaken with the *AGS3* construct and the *pTRI* plasmid containing the Pyrithiamine resistance gene (17), and the transformants were selected on 2.5 µg/ml pyrithiamine. In strategy 2 the transformants were selected on 1mg/ml 5 Fluoro-Orotic acid to pop-out the deletion cassette and replace it by homologous recombination with the *AGS3* construct (1). In Strategies 3 and 4 the cosmid containing the *AGS1* gene was used as previously described for the complementation of the single *ags1*Δ mutant (2): co-transformations were undertaken with a cosmid containing the *AGS1* gene (2) and either the *pSK-pTRI* plasmid containing the Pyrithiamine resistance marker (strategy 3), or the *pCB1635* plasmid containing the Sulfonylurea resistance gene, and the transformants were selected on Chlorimuron (Strategy 4). The presence of the WT copy of the genes was investigated by PCR.

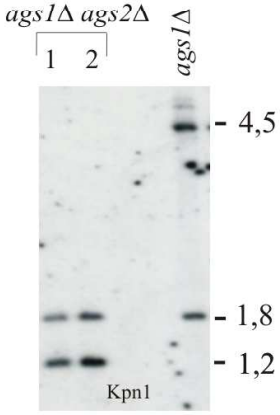
## Supplementary Figure S1

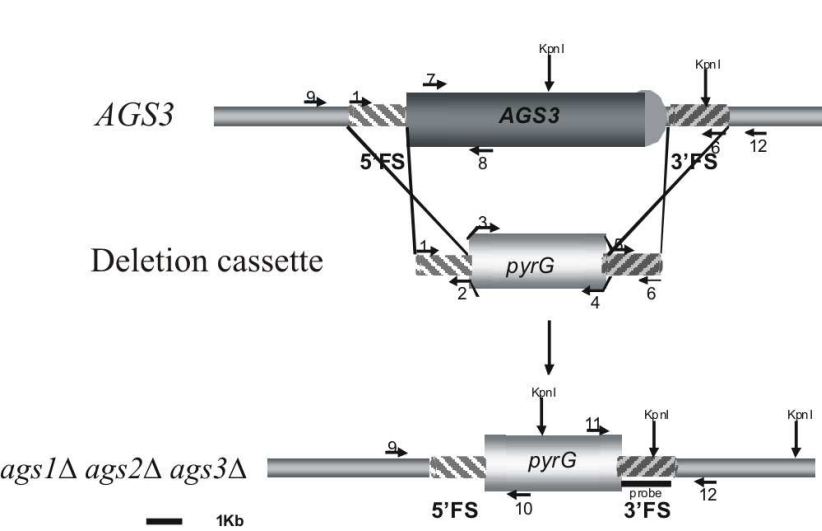
Figure S1: Construction of a triple *AGS* mutant in A. fumigatus and Southern blots showing the correct integration of the resistance marker. (A) Deletion of *AGS*1. In the left panel is the restriction map of the *AGS1* locus before (upper section) and after (lower section) gene replacement using deletion cassette emcompassing the *HPH* gene (middle section). Southern hybridation of genomic DNA with XhoI restriction enzyme digests of AkuB$^{ku80}$*pyrG*Δ (AkuB) and *AGS1* transformants (*ags1*Δ). The band size correlated with the predicted size from the left panel. (B) Deletion of *AGS2* using *BLE* gene (description as in 1A). (C) Deletion of *AGS3* using *PYRG* gene (description as in 1A). For the double and triple mutants, it was verified that the successive deletion did not result in any perturbation of the successive mutations (right panel in C).

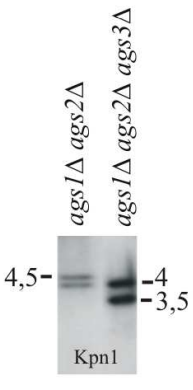


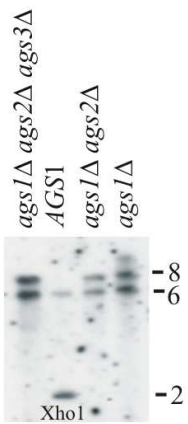



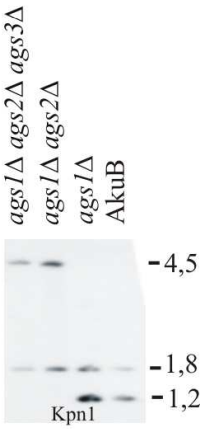




AGS3 AGS1 AGS2

## Supplementary Table S1

Table S1: Primers used in this study

| Name | Sequence (5' → 3') | Purpose of this study |
|---|---|---|
| *AGS1* (Afu3G00910) | | |
| 5Lags1 (1 in Fig. 1) | ATACAATCAATAATGACTGGGTATCTACAG | L-flanking amplification |
| 3Rags1 (6 in Fig. 1) | CAAACCCCAGACTCACCATTATAAAGAACC | R-flanking amplification |
| 5hph'-3Lags1 (2 in Fig. 1) | TCGTGAATCTTTTACCAGATCGGAAGCAATAATATGAATATCGTTATGTGCGTAATATG | *HPH* amplification and fusion |
| 3Lags1'-5hph (3 in Fig. 1) | CATATTACGCACATAACGATATTCATATTATTGCTTCCGATCTGGTAAAAGATTCACGA | L-flanking amplification and fusion |
| 5Rags1'-3hph (4 in Fig. 1) | GTCATTCCCTCGTTGAACTCCGGACATCAAATCAGAGCAGATTGTACTGAGAGTGCACC | R-flanking amplification and fusion |
| 3hph'-5Rags1 (5 in Fig. 1) | GGTGCACTCTCAGTACAATCTGCTCTGATTGATGTCCGGAGTTCAACGAGGGAATGAC | *HPH* amplification and fusion |
| ags1atg2 (7 in Fig. 1) | ATGAAGTGGGGATGGACC | transformants screening |
| ags1G1 (8 in Fig. 1) | TCGGAGTCAGCATCTCATTG | transformants screening |
| ags1S6 (9 in Fig. 1) | ATCCCCTCCGTCTCAACTCT | transformants screening |
| hph2 (10 in Fig. 1) | AAAGCAGGAGAGGCACGATA | transformants screening |
| TrpC4 (11 in Fig. 1) | GCACAGGTACACTTGTTTAGAGG | transformants screening |
| ags1S7 (12 in Fig. 1) | CCCTATCCGGTCAGAGTTCA | transformants screening |
| ags1a (13 in Fig. 1) | CGGCGTGGAATCAGTGCA | expression |
| agsE1 (14 in Fig. 1) | TCCTTGCTGGTGGACACG | expression |
| | | |
| *AGS2* (Afu2G11270) | | |
| 5Lags2 (1 in Fig. 1) | ATATAGTTGTTACTACGACAGTTCGATAG | L-flanking amplification |
| 3Rags2 (6 in Fig. 1) | GTCTTCACGGATGCTGGTTTTGAGT | R-flanking amplification |
| 5ble'-3Lags2 (2 in Fig. 1) | TCGTGAATCTTTTACCAGATCGGAAGCAATCCCTCTTTTTATCCTCTAGAAAAGTTATTA | *BLE* amplification and fusion |
| 3Lags2'-5ble (3 in Fig. 1) | TAATAACTTTTCTAGAGGATAAAAAGAGGGATTGCTTCCGATCTGGTAAAAGATTCACGA | L-flanking amplification and fusion |
| 5Rags2'-3ble (4 in Fig. 1) | AGTCAGAGCAAAAGTGCAAAAATATTACATCAGAGCAGATTGTACTGAGAGTGCACC | R-flanking amplification and fusion |
| 3ble'-5Rags2 (5 in Fig. 1) | GGTGCACTCTCAGTACAATCTGCTCTGATGTAATATTTTTGCACTTTTGCTCTGACT | *BLE* amplification and fusion |
| ags2 B2 (7 in Fig. 1) | CCTCAGTACCACACTGAT | transformants screening |
| ags2 B1 (8 in Fig. 1) | GTACGGTGACAAACATCG | transformants screening |
| ags2 S1 (9 in Fig. 1) | GCTATCACATGGAGGGAAGG | transformants screening |
| ble1 (10 in Fig. 1) | CAGAGCACCGGATGGGTCGAC | transformants screening |
| TrpC4 (11 in Fig. 1) | GCACAGGTACACTTGTTTAGAGG | transformants screening |
| ags2 S2 (12 in Fig. 1) | AGAAGGTTGCCGCCAATATC | transformants screening |
| | | |
| *AGS3 (*Afu1G15440) | | |
| 5Lags3 (1 in Fig. 1) | GTTAATAAGGCAGGGGGTGGCCATACTAAG | L-flanking amplification |
| 3Rags3 (6 in Fig. 1) | CCCTGTCTCTCAACCCTACTAGCATCACCT | R-flanking amplification |
| 5pyrG'-3Lags3 (2 in Fig. 1) | GGAAAACCCTGGCGTTACCCAACTTAATCTCGGTGTCTCAATAATCAAACGACATGAGA | *PYRG* amplification and fusion |
| 3Lags3'-5pyrg (3 in Fig. 1) | TCTCATGTCGTTTGATTATTGAGACACCGAGATTAAGTTGGGTAACGCCAGGGTTTTCC | L-flanking amplification and fusion |
| 5Rags3'-3pyrg (4 in Fig. 1) | AACCAAAGGCTGGTCCTATCACCATTACAACACAGGAAACAGCTATGACCATGATTACGC | R-flanking amplification and fusion |
| 3pyrG'-5Rags3 (5 in Fig. 1) | GCGTAATCATGGTCATAGCTGTTTCCTGTGTTGTAATGGTGATAGGACCAGCCTTTGGTT | *PYRG* amplification and fusion |
| ags3a (7 in Fig. 1) | AACTGGCGTTTCCCTGTCTA | transformants screening |
| ags3b (8 in Fig. 1) | GGACAATACGCTGGCAATCTG | transformants screening |
| ags3 S8 (9 in Fig. 1) | GGCCGTTAGCAATGAAAGAG | transformants screening |
| pyrG 7 (10 in Fig. 1) | TACGGTCGCATAGCAGTGAG | transformants screening |
| pyrG 9 (11 in Fig. 1) | GGTTCGTGTGCCTGGATACT | transformants screening |
| ags3 S9 (12 in Fig. 1) | GGAGGAGATTGGTCGATTCA | transformants screening |