

**Supplemental Table 2:** Primers used in this study for construction of mutant strains.

Primer name*	Sequence
Afu3g08520 5F	5'-gtaacgccagggtttccactcagacgcCTTGCTTGCCTGCTT-3'
Afu3g08520 5R	5'- <u>gcatcagtgcctctcagacagaattccGCAGTAAACAAGGAACACCACG</u> -3'
pyrG FW	5'-GGAATTCTGCTTGAGAGGAGGC-3'
pyrG REV	5'-GATATCGAATTGCCTCAAAC-3'
Afu3g0852 3F	5'- <u>aagagcattgttgaggcgaattcgat</u> tcACTTTCTTGAATATTGCGATGG-3'
Afu3g0852 3R	5'-gcggataacaattcacacaggaacacgcTCAGCGTCCGGTAAGTTG-3'
rlmA 1000 FW	5'-CGTGTGGTTGCCAGCCTGACCAGG-3'
rlmA 1000 REV	5'-TCGTCTACCAGAACGACCTCGCCG-3'
rlmA ST SC 5F	5' - gtaacgccagggtttccactcagacgcATGGGTGAAAGAAAGATCGA-3'
rlmA ORF REV	5'- <u>agttcttcccttactcattcccggttccCGTCTGGATTTCCTGCC</u> -3'
rlmA 600 ups	5'-GAATGAGAAGAAAGGAGGAATGA-3'
Spacer GFP FW	5'- <b>GGAACACGGGGATGAGTAAAGGAGAAGAACTTTCA</b> -3'
GFP REV pyrG	5'- <u>GCATCAGTGCCTCCTCTCAGACAGAA</u> TTCCTATTGTATAGTTCATCCATGCCATG-3'
MpkA_5'_For <sup>§</sup>	5'-CTCATTCCCTGTTCTGATGCG-3'
MpkA_3'_Rev <sup>§</sup>	5'-GACTGTCGCAGAAATCCGCTT-3'
mpkA 600 ups	5'-GAGCCCTGACTTCAGTGCA-3'
cpkcA FW <sup>¶</sup>	5'-TACAACATACCTGGCTGGATG-3'

Small letters indicate homology to the pRS426 flanking sequence

Small underlined letters indicate homology to a fragment in the cassette

Bold letters indicate the Gly-Thr-Arg-Gly linker separating *rlmA* C-terminus and GFP start codon.

\* For primers location refer to Figure S1.

<sup>§</sup> Sequence from Valiante *et al.*, 2009

<sup>¶</sup> Sequence from Rocha *et al.*, 2015

## References

- Rocha, M.C., Godoy, K.F., de Castro, P.A., Hori, J.I., Bom, V.L., Brown, N.A., *et al.*, (2015) The *Aspergillus fumigatus* pkcAG579R Mutant Is Defective in the Activation of the Cell Wall Integrity Pathway but Is Dispensable for Virulence in a Neutropenic Mouse Infection Model. *PLoS One* **10**: e0135195.
- Valiante, V., Jain, R., Heinekamp, T. and Brakhage, A.A., (2009) The MpkA MAP kinase module regulates cell wall integrity signaling and pyomelanin formation in *Aspergillus fumigatus*. *Fungal Genet Biol* **46**: 909-918.