

Nc GQDVTMIDTLREVMALFEDAQNAVFKLMASDSVPKFLRNPKEYEQLRNYDFDSITPVH-- 750
An GDDDSMLKSLQEVVQLFEMAQTSVFKLMSSDSVPKFLRDPKYSAILQEHDVDDLIGGRS 700
: :*:.:*:**:* ***.:*****:*****:***. *::*.*.:

Nc ---GQGRMVERSQRSNRT 766
An YSPTPGNVPERSMSRSQRS 719
*.: *** ***:**:

Figure S3. Sequence alignment of *flbA* proteins. The amino acid sequence of *Neurospora crassa* (Nc) *flbA* (NCU08319) and *Aspergillus nidulans* (An) *flbA* (AN5893) were aligned. Highlighted in red is the codon where a C in the coding sequence has been deleted in the *flbA* gene of *N. crassa* strains expressing high levels of *aod-1* mRNA. This deletion leads to the formation of forty codons of frame-shifted information followed by a premature stop codon and truncation of the protein. Highlighted in yellow are two DEP (Dishevelled, Egl-10 and Pleckstrin) domains which may be involved in targeting the protein to the appropriate sites on the cell membrane. Highlighted in cyan is the RGS (Regulator of G-protein signaling) domain which is necessary for interaction with the G α protein. Both domains are necessary for normal protein function and both are well conserved in the *N. crassa* and *A. nidulans* proteins. The frameshift mutation and truncation of the protein is predicted to render the mutated NCU08319 non-functional in high-expressing *aod-1* strains due to the loss of these domains. “*” (asterisk) indicates positions which have a single, fully conserved amino acid residue; “:” (colon) indicates conservation between residues with strongly similar properties - scoring > 0.5 in the Gonnet PAM 250 matrix. “.” (period) indicates conservation between residues with weakly similar properties scoring \leq 0.5 in the Gonnet PAM 250 matrix. Alignment was done using clustal omega (Goujon et al. 2010; Sievers et al. 2011; McWilliam et al. 2013). Identification of protein domains was achieved using the Inter-Pro protein sequence analysis and classification software at the European Bioinformatics Institute (GB) (<https://www.ebi.ac.uk/interpro/>).

Goujon, M., H. McWilliam, W. Li, F. Valentin, S. Squizzato *et al.*, 2010 A new bioinformatics analysis tools framework at EMBL-EBI. *Nucleic Acids Res* 38 (Web Server issue):W695-699.

McWilliam, H., W. Li, M. Uludag, S. Squizzato, Y.M. Park *et al.*, 2013 Analysis Tool Web Services from the EMBL-EBI. *Nucleic Acids Res* 41 (Web Server issue):W597-600.

Sievers, F., A. Wilm, D. Dineen, T.J. Gibson, K. Karplus *et al.*, 2011 Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. *Mol Syst Biol* 7:539.