

## SUPPORTING INFORMATION

### **Inhibition of the flavin-dependent monooxygenase Siderophore A (SidA) blocks siderophore biosynthesis and *Aspergillus fumigatus* growth**

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**Supplemental Table 1.** Complete list of hits obtained in the high throughput screening of the library against SidA.

**Supplemental Table 2.** Melting temperatures of SidA in the presence of different ligands.

**Supplemental Table 3.** Observed and calculated masses of purified siderophores.

**Supplemental Figure 1.** Melting curves of SidA in the presence of L-Orn, NADP<sup>+</sup> and celastrol.

**Supplemental Figure 2.** Picture of the AMM-Fe agar plate with different concentrations of celastrol used to determine MIC.

**Supplemental Figure 3.** HPLC separation of siderophore extracts.

**Supplemental Figure 4.** Effect of addition of the celastrol in the production of siderophores in developed fungal cultures.

## Tables

Table S1. Resulting hits from HTS against SidA using the probe ADP-TAMRA. Reported anisotropy corresponds to percent of normalized values.

Name	Anisotropy	Name	Anisotropy
Celastrol	60	Baicalin	60
Dihydrocelastrol	62	Clofazimine	27
4,4'-Diisothiocyanostilbene-2,2'-sulfonic acid sodium salt	64	Nadide	50
Obtusaquinone	57	Daunorubicin	58
Hematein	57	Flurandrenolide	57
Plumbagin	49	Methylprednisolone	42
Gossypetin	33	Laccaic acid A	17
Pyrogallin	48	Haematoporphyrin	56
Tannic acid	57	Calcein	44
Theaflavin	48	Mesalamine	56
Theaflavin monogallates	40	Meclofenamate sodium	55
Irigenol	48	Fast green FCF	20
Aurin tricarboxylic acid	57	Adenosine phosphate	63
7-Deshydroxypyrogallin-4-carboxylic acid	50	Quinalizarin	60
Brazilin	33	Chicago sky blue	64
Tanshinone IIA sulfonate sodium	59	Koparin	65

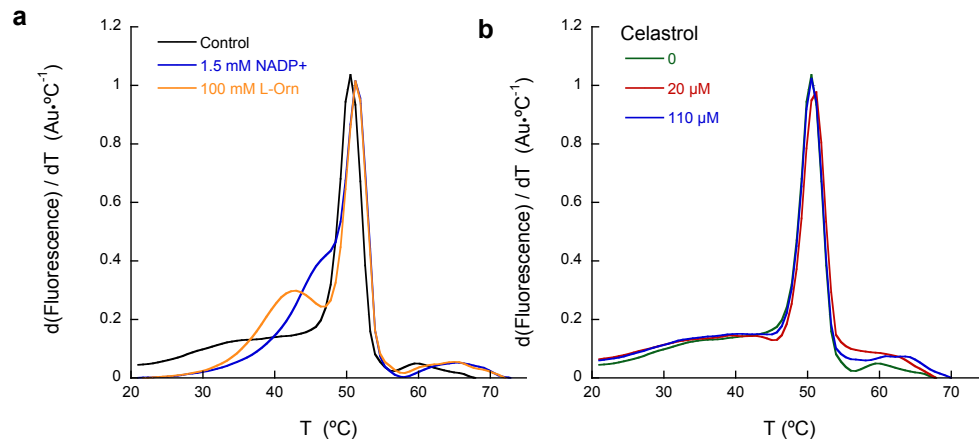
**Table S2.** Melting temperatures of SidA in the presence of different ligands and 2 % DMSO. Values obtained using ThermoFAD method. Samples were done in triplicate.

Ligand	T <sub>m</sub> / °C
Reference (no ligand)	47.9 ± 0.8
100 mM L-Orn	48.29 ± 0.03
12.5 mM NADP <sup>+</sup>	46.68 ± 0.04
Celastrol 20 µM	48.1 ± 0.1
Celastrol 110 µM	47.6 ± 0.2

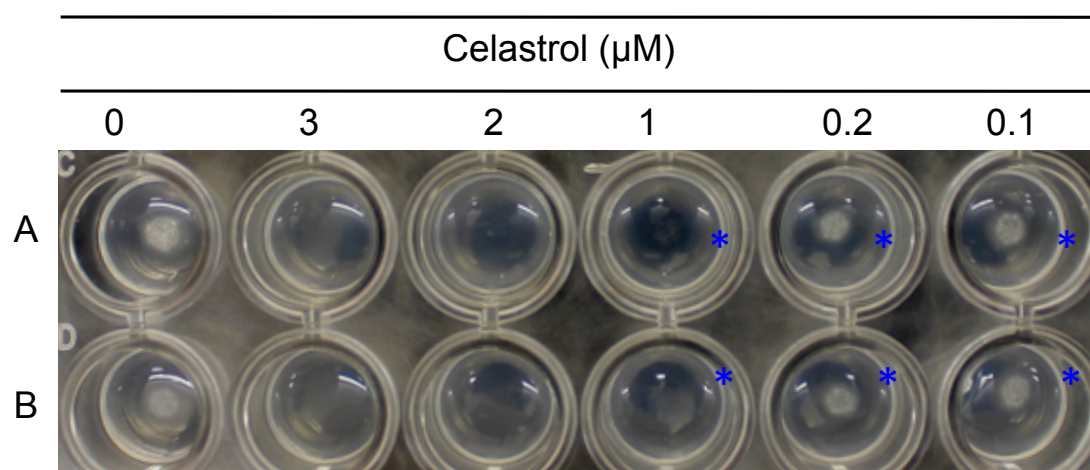
**Table S3.** Calculated (Calc.) and observed (Obs.) mass data of ferrated siderophores extracted from *A. fumigatus* cultures and used as rescue compounds.

Ionic Form	Fe(III)-TAFC		Fe(III)-FC		Fe(III)FsC	
	Calc.	Obs.	Calc.	Obs.	Calc.	Obs.
H <sup>+</sup>	906.7	906.2	770.5	771.2	779.6	779.2
Na <sup>+</sup>	928.7	928.3	793.5	793.3	802.5	-

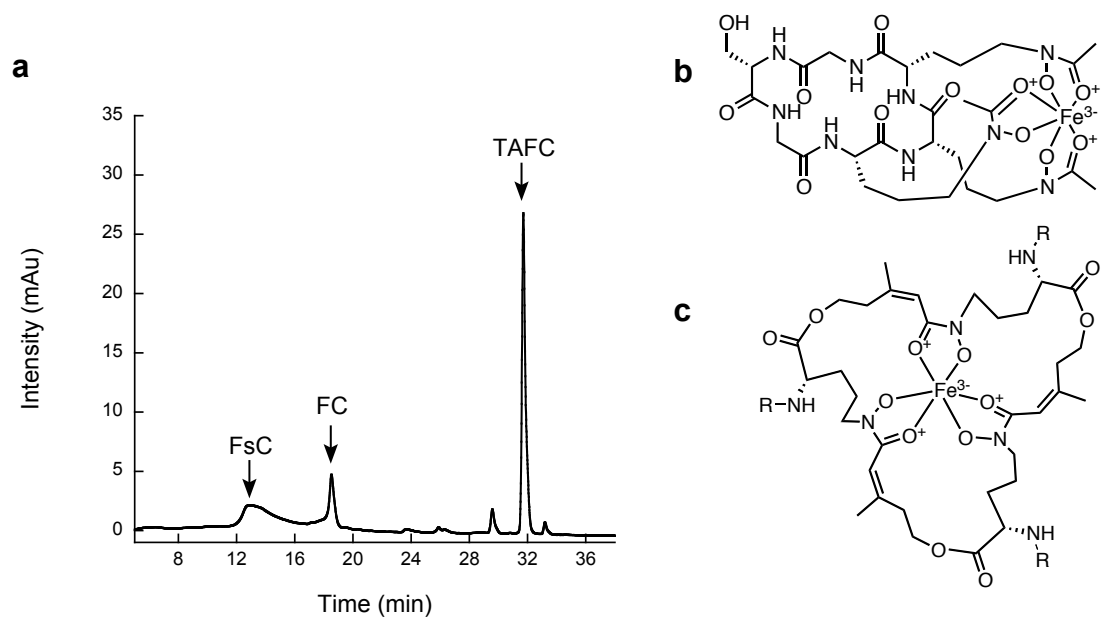
## Figures



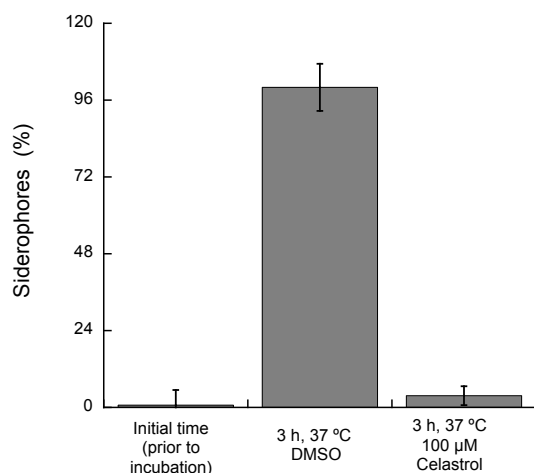
**Figure S1.** Melting curve of SidA obtained using FAD as the fluorescence probe (ThermoFAD method (1)). Total protein concentration was 3 mg/mL and the final DMSO concentration was 2%. (a) Saturating concentration of NADP<sup>+</sup> and L-Orn did not induced changes in the melting temperature. (b) Melting curve of SidA with different celastrol concentration, celastrol does not reduce the melting temperature.



**Figure S2.** Determination of the minimum inhibitory concentration (MIC) of Celastrol in AMM-Fe after 24 h of incubation at 37 °C. A and B are duplicate samples. The blue mark (\*) indicates the presence of growth. At a celastrol concentration of 2  $\mu\text{M}$  no visible growth was observed and this concentration was determined to be the MIC.



**Figure S3.** (a) HPLC traces at 435 nm of Fe-siderophores extracted from *Aspergillus fumigatus* cultures. Ferric form of the identified siderophores, (b) Ferricrocin, (c) fusarinine C (FsC) R=H; in triacetylfusarinine C (TAFC) R=acetyl.



**Figure S4.** Quantification of extracellular production of siderophores from *A. fumigatus* cultures (grown for 30 h at 37 °C) produced during three hours incubation (37 °C) in the presence and absence of 100 μM celastrol. 200 μL agar drops of AMM-Fe were spot-inoculated with  $7 \times 10^6$  spores and incubated at 37 °C for 30 h. Agar drops with grown cells were transferred to 2 mL fresh AMM-Fe and incubated for 10 min with continuous stirring. After this time, siderophores in wash solution were quantified with the Chrome Azurol S assay (CAS). A total of three washes were required to remove the siderophores in the agar drop. The first column labeled as initial time corresponds to the residual siderophores in the agar prior to the addition of celastrol (100 μM, 0.5 % DMSO) or DMSO (0.5%). After the third wash the agar drop was transferred into fresh AMM-Fe containing 100 μM celastrol or DMSO and incubated at 37 °C for 3 h. After this time, siderophores in the liquid phase were quantified using the CAS assay. Siderophore concentration was normalized to the DMSO control. Each sample was done in duplicate.

## References

1. Forneris, F., Orru, R., Bonivento, D., Chiarelli, L. R., and Mattevi, A. (2009) ThermoFAD, a ThermoFluor®-adapted flavin ad hoc detection system for protein folding and ligand binding. *FEBS J.* **276**, 2833-2840
2. Schwyn, B., and Neilands, J. B. (1987) Universal chemical assay for the detection and determination of siderophores. *Anal. Biochem.* **160**, 47-56