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Supplementary Information for
"Dual-purpose isocyanides produced by Aspergillus fumigatus contribute to cellular copper sufficiency and exhibit antimicrobial activity"

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Supplementary Information Text

Materials and Methods

Mutant construction

Double-joint PCR was used to generate DNA constructs for transformation described previously (1). DNA constructs containing the *pyrG* or *argB* selectable marker fused to 1 kB homologous regions flanking the gene of interest that insert via homologous recombination. Flanks were amplified with 20 bp overlaps using primers designed using SeqBuilder (DNASTAR, Madison, WI). Selectable markers were amplified from either the pJW24 (*pyrG*) (2) or pJMP4 (*argB*) (3) plasmids. Deletion mutants were constructed by whole-gene deletion and overexpression mutants were created by inserting a constitutively active *A. nidulans gpdA* promoter upstream of the ATG translation start site of a gene. The DNA constructs were transformed into the *pyrG* auxotroph TFYL 80.1 via PEG transformation described previously (1). For double mutants, the double auxotroph TFYL 44.1 (*pyrG*$^{-}$ and *argB*$^{-}$) was first transformed with either the overexpression or deletion *xanC* construct and upon successful transformation, further transformed with a DNA deletion construct containing the *argB* marker. Resulting transformants were then screened via PCR using the 5' forward flank and the marker reverse primer. Positive mutants were further screened via southern blot analysis using P-32 labelled 1 kB flanking regions described previously to confirm single integrations (Figure S8).

Fungal Growth Conditions and Physiological Assays

Unless otherwise specified, *Aspergillus* strains were grown on glucose minimal medium (GMM)(4). *Aspergillus* strains were activated by streaking out on a plate of GMM from a glycerol stock. Spore stocks were generated by harvesting spores using 0.01% Tween 20 in ddH_2O and gentle scrubbing using a L-shaped spreader. Spores were then filtered through sterile MiraCloth (Calbiochem) to remove debris and washed twice with Millipore-filtered water. Spore stocks were counted using a hemocytometer and diluted to the desired concentration. *Cryptococcus neformans* H99 was grown on minimal media (15 mM D-glucose, 10 mM $MgSO_4$, 29.4 mM KH_2PO_4, 13 mM glycine, 3 μM thiamine, and 1.5% (w/v) agar, pH 5.5) with 1 mM L-DOPA to induce melanization (Cayman Chemical) where

indicated. For testing response to varying levels of copper, GMM was prepared as described previously (5) without copper or EDTA. After autoclaving, copper or BCS was supplemented. Plates were inoculated with 2 µl of spore suspension and the plates grown at 37 °C for 72 hours. For liquid shake cultures, 50 ml of GMM was inoculated with $1x10^6$ spores/ml and incubated at 37 °C, shaking at 250 rpm for 48 hours. Lyophilized mycelia were obtained by filtering cultures through MiraCloth, freezing in liquid nitrogen, and lyophilizing overnight. For sporulation assay, strains were suspended in 10 ml molten GMM and overlayed onto a plate of GMM. An agar plug was transferred to a solution of 0.01% Tween and homogenized. Samples were then diluted and spores were enumerated using a hemocytometer.

RNA Extraction and Quantitative RT-PCR

Liquid shake cultures were inoculated with $1x10^6$ spores/ml of wild type *A. fumigatus* and allowed to incubate at 37°C for 24 hours shaking at 250 rpm. Hydrogen peroxide was added to a final concentration of 6 mM and were allowed to continue incubate for an additional 3 hours. Total culture mycelia was then flash-frozen in liquid nitrogen and lyophilized. RNA was extracted using the QIAzol Lysis Reagent (Qiagen). RNA was then subjected to a DNase I digestion (New England Biolabs) and reverse-transcribed using iScript cDNA synthesis kit (Biorad). Semi-quantitative PCR was performed by amplifying the gene of interest via designed primers (Table S5) and using *A. fumigatus* actin as a loading control.

Chrome Azurol S Assay

Chrome azurol S (CAS) assay plates were made by adding 5 mL of sterile phosphate buffer (26 g/L KH_2PO_4 and 62 g/L $Na_2HPO_4 - 7H_2O$) and 100 ml of 10x CAS (0.5 mM $CuSO_4$, 0.5025 mM Chrome Azurol S (Sigma-Aldrich), 1.05 mM HDTMA) to 1 L of molten GMM. Upon solidifying, a sterile razor was used to remove half of the CAS media. 10 ml of warm molten GMM was then inoculated with $1x10^7$ spores and poured into the vacant space. The plates were then allowed to solidify and incubated for 5 days at 37 °C in the dark. The zone of activity was quantified by measuring the distance between the growing mycelia and the edge of the Cu-CAS complex with a ruler.

Laccase Activity Assay

Approximately $1x10^7$ spores were inoculated on small plates of GMM for 48 hours at 37°C in the dark. 2,2'-azino-bis(3-ethylbenzothiazoline)-6-sulphonic acid (ABTS) (Sigma-Aldrich) stock solution was prepared by dissolving ABTS in 0.1 M sodium acetate buffer (pH 4.5). Plates were flushed with 10 ml of 1 mM ABTS and incubated at ambient temperature for 24 hours. 200 µl aliquots of supernatant were transferred to a 96-well plate and measured the absorbance at 420 nm. Data were quantified and normalized to the negative control, uninoculated plates with the appropriate level of copper, flushed, and incubated with 1 mM ABTS.

Metabolite Extraction

Strains were grown as an overlay culture, inoculating 10 ml of warm, molten GMM (containing 8 g/L agar) with $1x10^6$ spores and pouring onto a plate containing 20 ml of solidified GMM. Upon solidification, the plates were then incubated for 5 days at 37 °C. The plates were frozen using liquid nitrogen and lyophilized. The lyophilized samples were extracted with 20 ml of ethyl acetate-methanol (9:1) for 1.5 h with vigorous stirring. Extracts were filtered over cotton, evaporated to dryness, and stored in 4-ml vials. Crude extracts were suspended in 0.5 ml of methanol and centrifuged to remove insoluble materials, and the supernatant was analyzed by UHPLC-HRMS.

HPLC-HRMS Analytical Methods and Equipment Overview

High-resolution HPLC-MS (HPLC-HRMS) was performed on a ThermoScientific-Dionex Ultimate 3000 UHPLC system equipped with a diode array detector and connected to a ThermoScientific Q Exactive Orbitrap mass spectrometer operated in electrospray positive (ESI^+) or electrospray negative (ESI^-) ionization mode. An Agilent Zorbax RRHD Eclipse XDB-C18 column (2.1 × 100 mm, 1.8 µm particle diameter) was used with acetonitrile (organic phase) and 0.1% formic acid in water (aqueous phase) as solvents at a flow rate of 0.5 mL/min. A solvent gradient scheme was used, starting at 2% organic for 1 min, followed by a linear increase to 100% organic over 14 min, holding at 100% organic for 2.5 min, decreasing back to 2% organic for 0.1 min and holding at 2% organic for the final 1.4 min, for a total of 18 min.

ICP-MS

The spores and mycelia were analyzed by ICP-MS after acid digestion.100 µl of concentrated trace metal grade nitric acid, 50 µl of 18 MΩ water and 25 ul of 500 ng/ml of scandium used as internal standard solution were added to the samples in a metal free 5 ml conical vial. After the digestion was carried out in a heating block at 90 ^{o}C for 2 hours with venting every 20 minutes. The samples were brought to 2.5 ml with 18 MΩ water. The samples were then analyzed for total Cu content in an Agilent 7500 ICP-MS system with a Cetac ASX-520 auto sampler contained in an acrylic box. The ICP-MS system was configured with a Micromist nebulizer, a double pass Scott spray chamber held at 2 ^{o}C, a 2.5 mm torch with platinum shield torch, and nickel sample and skimmer cones. The instrument was run with 3.5 ml/min of helium in energy discrimination mode. The external calibration method was used with a calibration range of 0.05 to 25 ng/ml. The mass of the samples (200-800 µg) needed for the quantification of Cu was calculated by measuring the phosphorous content in the digested samples according to (6).

Total Synthesis of Xanthocillin

Synthetic xanthocillin was prepared in 10 steps from commercially available 4-hydroxybenzaldehyde by following the approach developed by Tatsuta and co-workers (7). The NMR and HRMS data of the synthetic sample (Figure S7) match with the reported ones. Pure synthetic xanthocillin sample was obtained after column chromatorgraphy for the corresponding biological evaluations.

Xanthocillin synthesis

To a solution of xanthocillin silly ether (prepared using the reported approach;[7] 12 mg, 0.023 mmol) in THF (1 mL) at 0 ºC under an atmosphere of argon was added TBAF/AcOH (90 uL, 1: 1/mol: mol, 0.09 mmol, premixed) dropwise. The reaction mixture was stirred at 0 ºC for 30 min and then warm up to room temperature for 10 min. The reaction mixture was purified as it was by column chromatography (silica gel, 10-35% EtOAc in hexane) to provide xanthocillin (5 mg, 76% yield) as a yellow solid. R_f= 0.27 (33% EtOAc in hexane); IR (film) 3275, 2131, 1593, 1515, 1434, cm^{-1}; ^{1}H NMR (500 MHz, CD_3OD) δ 7.73 (d,

J=8.5, 4H), 7.00 (s, 2H), 6.88 (d, *J*=8.5, 4H); ^{13}C NMR (125 MHz, CD_3OD) δ 174.6, 161.0, 133.0, 128.7, 125.0, 116.9, 116.7; HRMS (ESI): calcd for $C_{18}H_{13}N_2O_2$ $(M+H)^+$ 289.0977, found 289.0972. The ^{1}H and ^{13}C NMR spectra of synthetic xanthocillin were recorded on a 500 MHz NMR spectrometer in CD_3OD, with residual CH_3OH signal(s) as the internal reference. Chemical shifts are reported as δ values (ppm). The solvent peaks are set as follows: CH_3OH at δ 3.31 and δ 49.0 ppm for ^{1}H and ^{13}C NMR. Chemical shifts are reported as follows: s = singlet, d = doublet. IR spectra were taken on an FT-IR spectrophotometer. High-resolution mass spectra (HRMS) were measured by the ESI method. Silica gel was used for flash column chromatography with mixed ethyl acetate (EtOAc) and hexane as the eluting solvents. Yields refer to chromatographically and spectroscopically (^{1}H NMR) homogeneous materials. Anhydrous THF was freshly distilled from sodium benzophenone ketyl under argon. Other reagents were obtained commercially and used as received.

Cocultures and Growth Conditions

Candida albicans, Staphylococcus aureus, Salmonella enterica, Pseudomonas aeruginosa (PAK), and *Pseudomonas aeruginosa* (PAO1) were activated from glycerol stock onto plates of lysogeny broth (LB) or yeast peptone dextrose (YPD), streaking for single colonies. A single colony was then selected and inoculated into 5 ml of liquid LB or YPD, shaking at 37 °C overnight. Coculture experiments were performed by transferring GMM agar plugs of an actively growing overlay fungal culture to solid LB containing 16 g/L agar. An overlay of the bacteria/fungi was generated by suspending microorganisms to a final $O.D._{600}$ of 0.05 in 10 ml of molten top agar and dispensing onto the LB containing the agar plugs. The plates were then incubated overnight at either 37 °C in the case of the bacterial strains or 30 °C in the case of the *Candida albicans*. Zones of inhibition were visually evaluated to determine inhibition of growth. For cocultures involving *Aspergillus nidulans*, GMM containing various levels of copper was inoculated with $1x10^7$ spores of *A. nidulans* and preincubated for one day at 37 °C. The plates were then inoculated with $1x10^7$ spores of the *xan* mutant strains and incubated for an additional three days at 37 °C. For 96-well plate assays, overnight cultures were diluted to an $O.D._{600}$ of 0.05 in liquid LB and 190 µl was dispensed into a well containing 10 µl of the corresponding

extract/xanthocillin. The plate was then incubated at 37 °C. Growth was tracked by checking the $O.D._{600}$ at 48 hours. For assays involving the addition of metals, filter-sterilized 1000x stocks were diluted in LB prior to dispensing into 96-well plates.

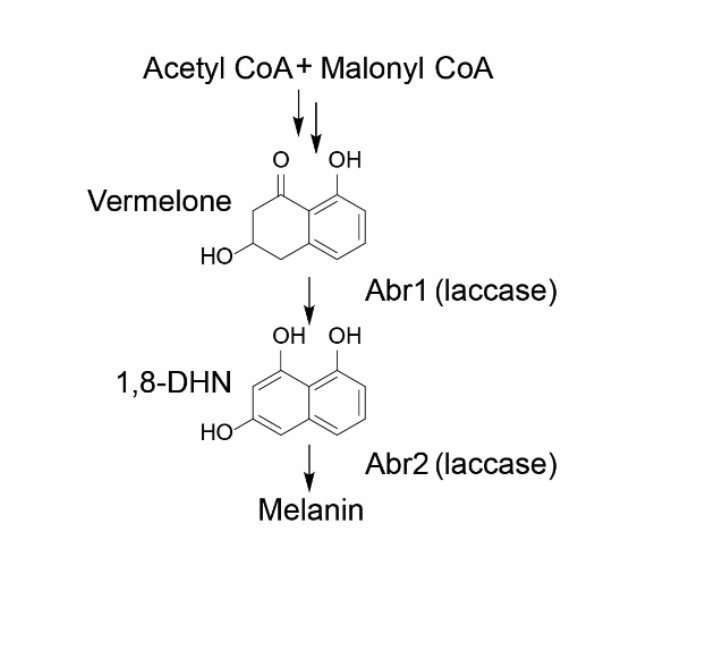

Figure S1 – Biosynthetic pathways of DHN-melanin in *A. fumigatus*.

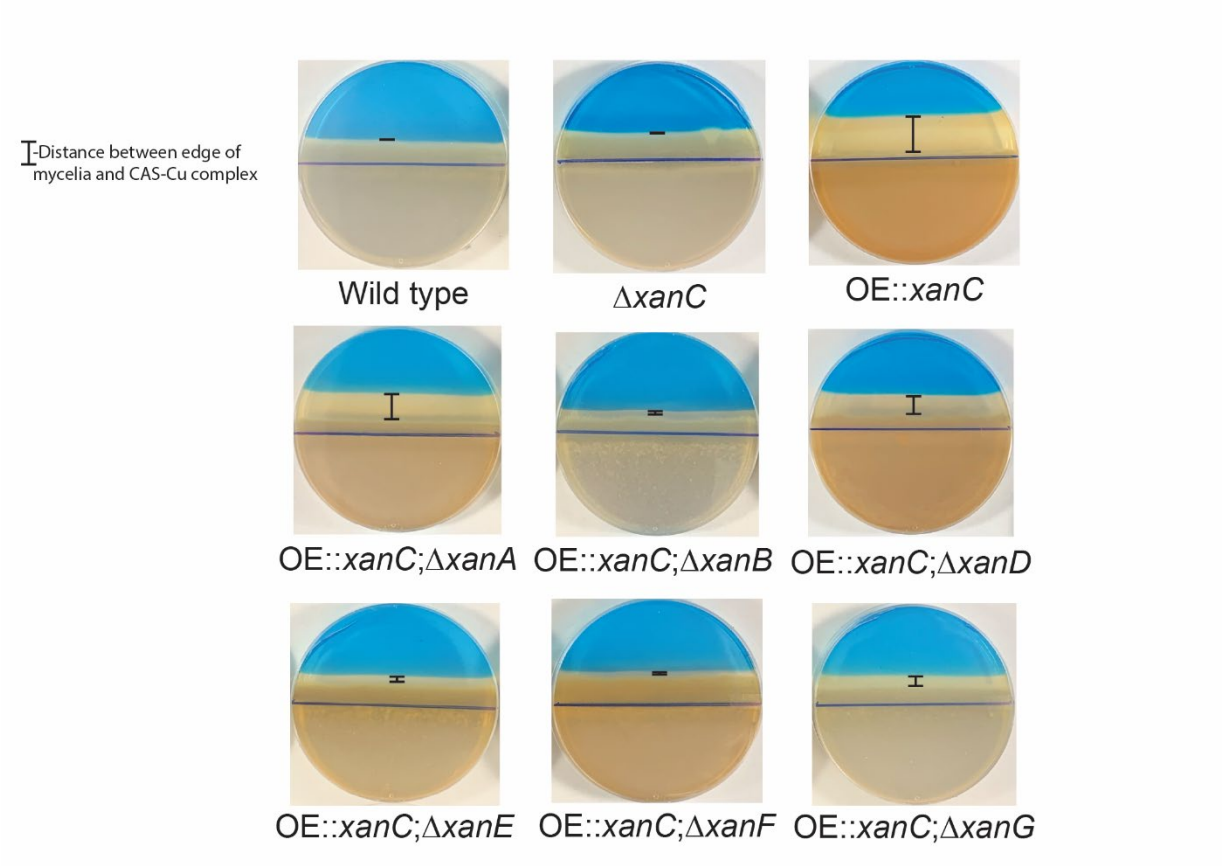

Figure S2- CAS assay for detection of secreted copper-binding molecules. Displayed are representative images of the CAS assay results. Measured distances of the zone of activity are indicated. Zone of activity is defined as the distance between the edge of the growing mycelia and the CAS-Cu complex.

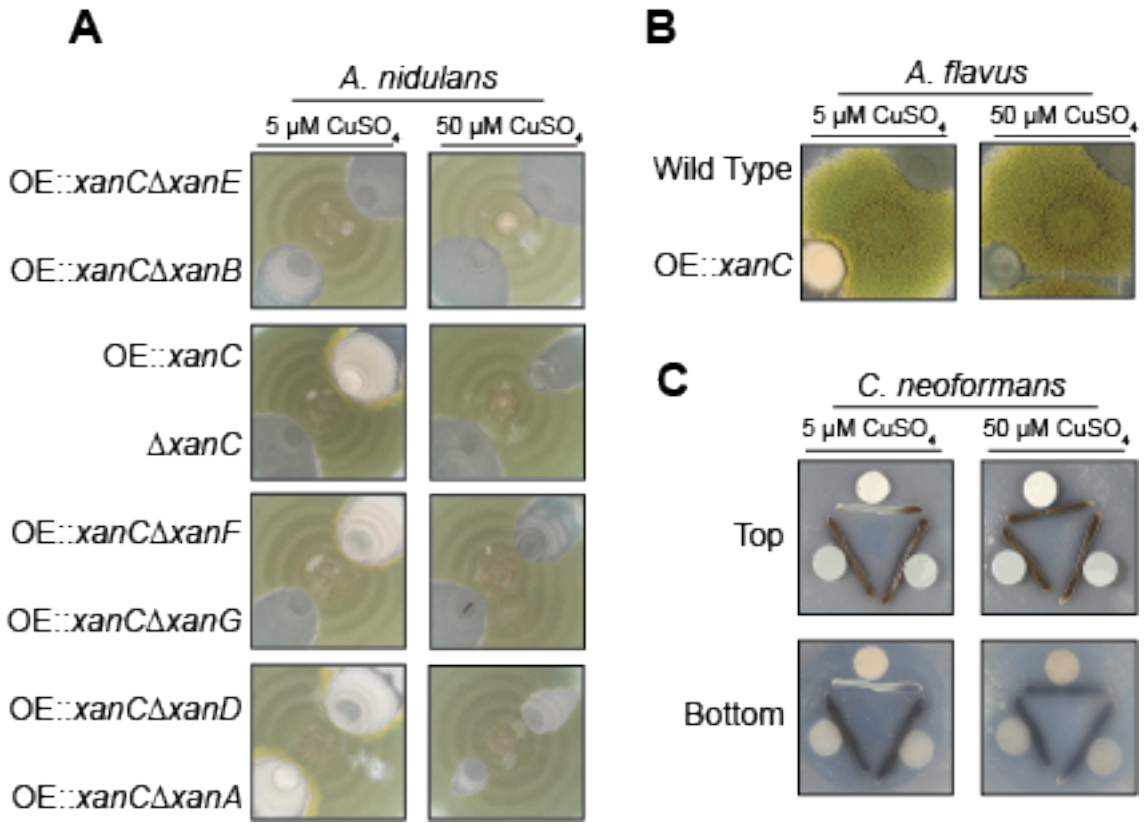

Figure S3- Cocultures of *xan* double mutants with A.) *Aspergillus nidulans*, B.) *Aspergillus flavus,* and C.) *Cryptococcus neoformans* H99.

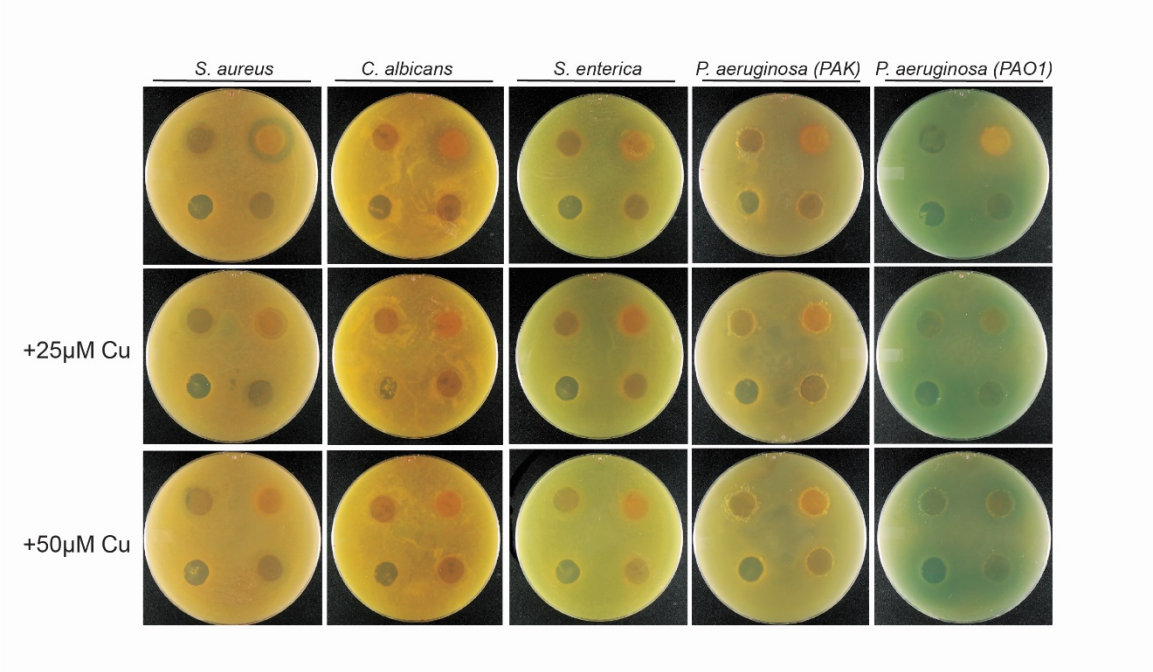

Figure S4 – *xan* mutant antimicrobial assay. Agar plugs of actively growing *xanC* mutants were transferred to a plate of lysogeny agar containing the indicated microbe (Top of figure) and supplemented with copper (Left of figure). Per-plate: Top left- WT, top right - OE::*xanC*, bottom left Δ*xanC*, bottom right- negative control (GMM).

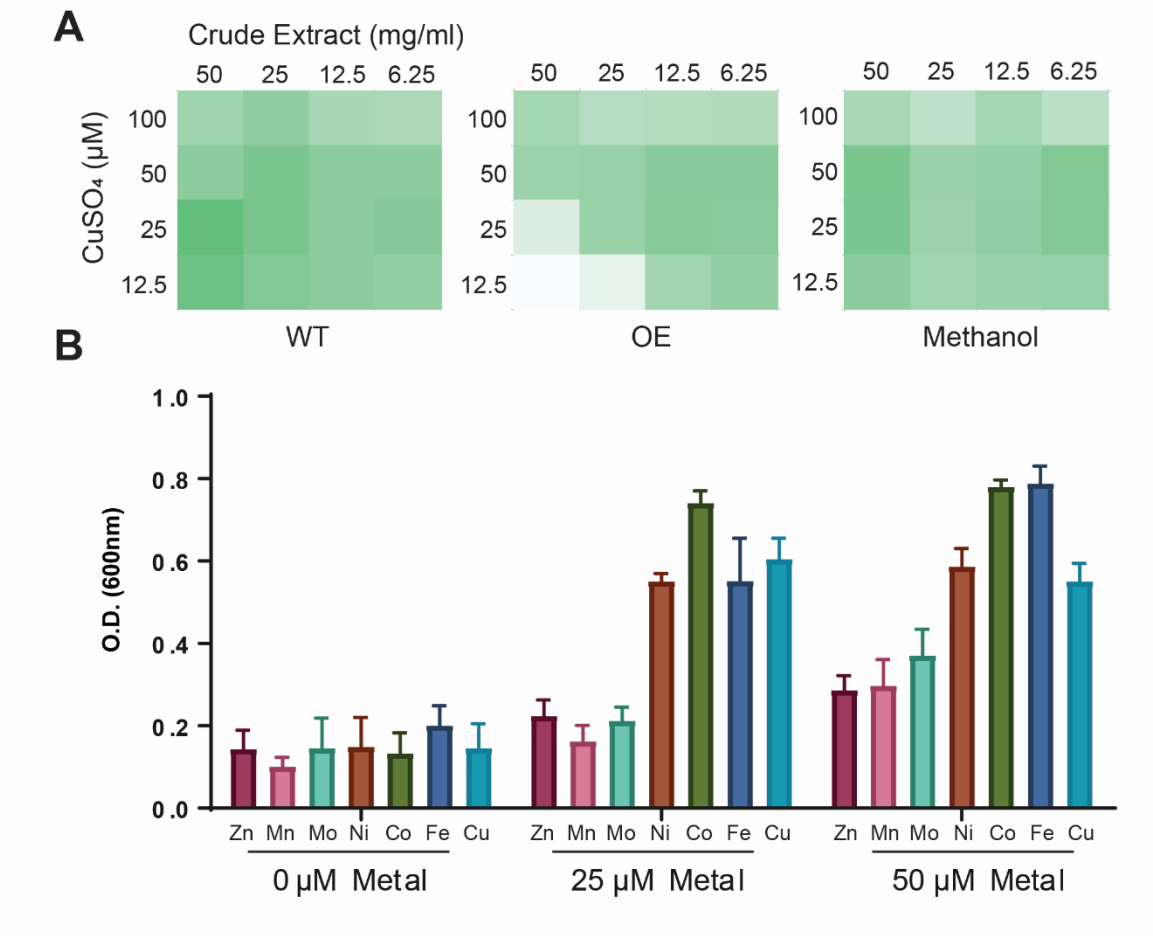

Figure S5 – *Pseudomonas aeruginosa* PAO1challenged with *xan* mutant extracts. A.) Heat map indicating the relative growth of *P. aeruginosa* when challenged with crude extract (x-axis) and supplemented with copper (y-axis). Wild type, OE::*xanC* extract, or negative control is indicated at the bottom of the map. B.) Response of *P. aeruginosa* challenged with OE::*xanC* extract and supplemented with metals. Shown is the mean of three replicates with the SEM indicated by the error bars.

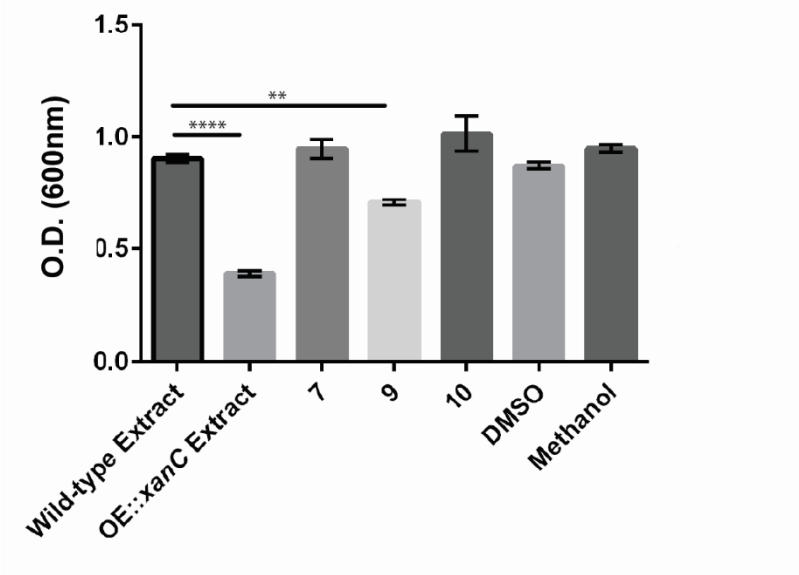

Figure S6- *Pseudomonas aeruginosa* PAO1 challenged with xanthocillin derivatives (256 µg/ml). Numbers are associated with the compounds in Figure 2. Shown is the mean of three technical replicates with the SEM indicated by the error bars. Statistical analysis was performed using an ANOVA. **P<0.01, ****P<0.0001.

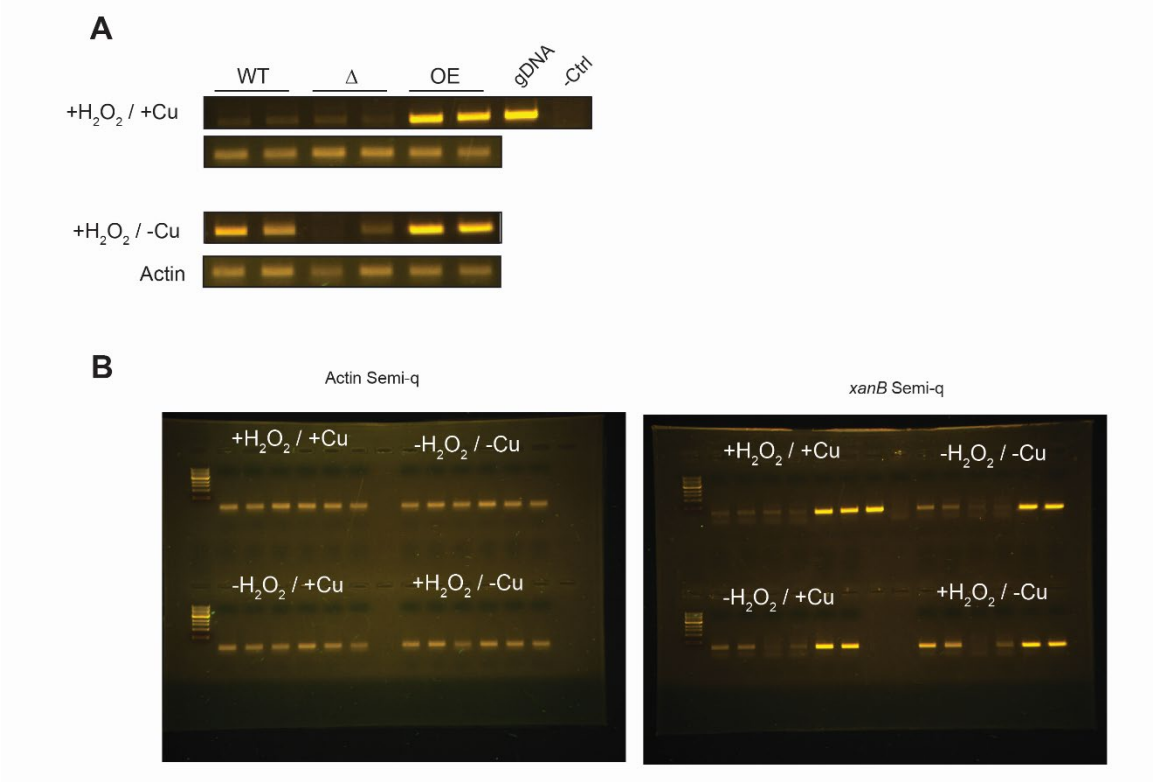

Figure S7- Semi-quantitative PCR of actin control (*act1*) (left) and *xanB* (right) in the indicated conditions. From left to right, samples were performed in duplicate wild type, Δ*xanC*, OE::*xanC*. The *xanB* +H_2O_2/+Cu condition contains an additional genomic DNA positive control and negative control.

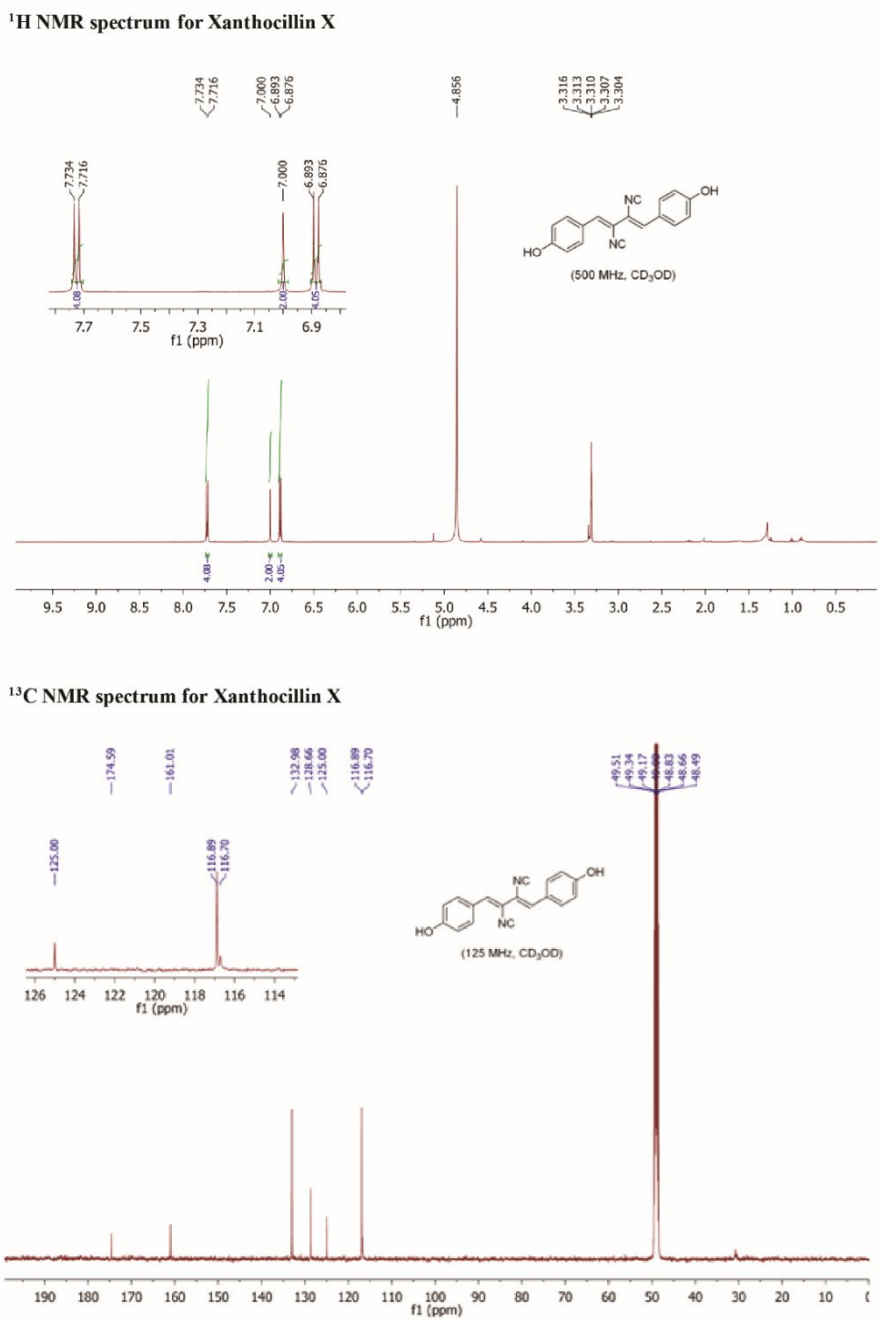

Figure S8- 1H and 13C NMR spectra of synthetic xanthocillin.

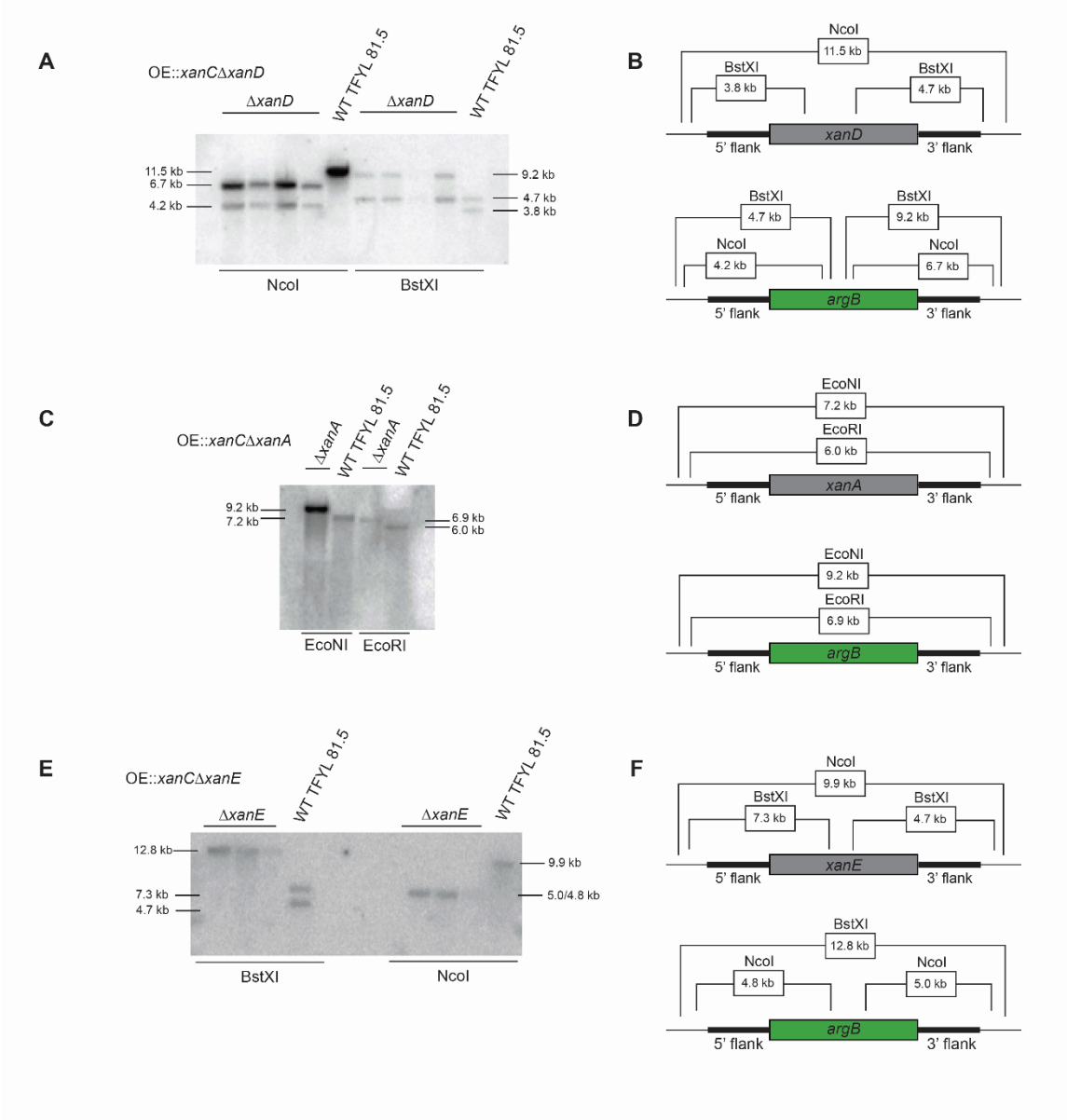

Figure S9- Southern Blot Confirmation analysis of the OE::*xanC*Δ*xanA*, OE::*xanC*Δ*xanD*, OE::*xanC*Δ*xanE* mutants used in this study. A.) Southern blot analysis of the OE::*xanC*Δ*xanD* mutant. B.) Digestion strategy of the OE::*xanC*Δ*xanD* mutant carried out with the designated restriction enzymes. C.) Southern blot analysis of the OE::*xanC*Δ*xanA* mutant. D.) Digestion strategy of the OE::*xanC*Δ*xanA* mutant carried out with the designated restriction enzymes. E.) Southern blot analysis of the OE::*xanC*Δ*xanE* mutant. B.) Digestion strategy of the OE::*xanC*Δ*xanE* mutant carried out with the designate restriction enzymes.

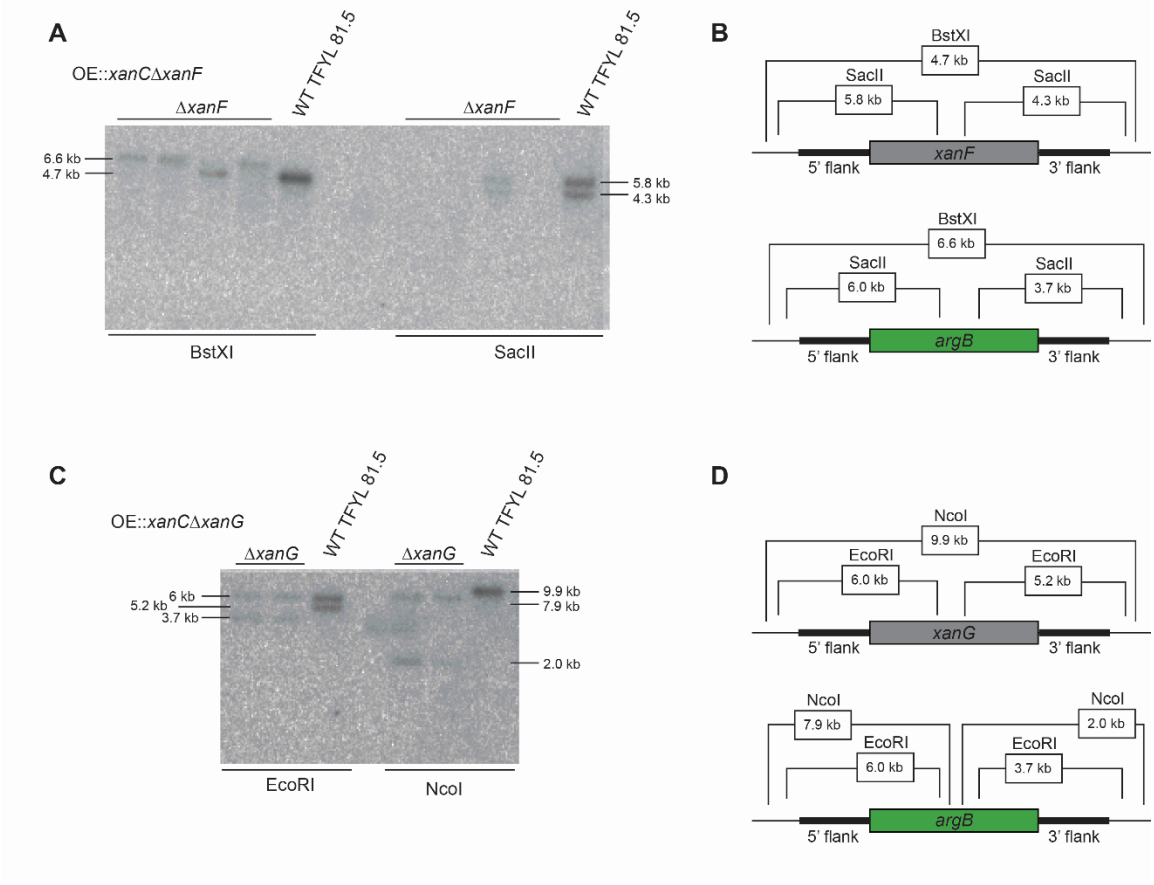

Figure S10- Southern Blot Confirmation analysis of the OE::*xanC*Δ*xanF* and OE::*xanC*Δ*xanG* mutants used in this study. A.) Southern blot analysis of the OE::*xanC*Δ*xanF* mutant. B.) Digestion strategy of the OE::*xanC*Δ*xanF* mutant carried out with the designated restriction enzymes. C.) Southern blot analysis of the OE::*xanC*Δ*xanG* mutant. D.) Digestion strategy of the OE::xanCΔxanG mutant carried out with the designated restriction enzymes.

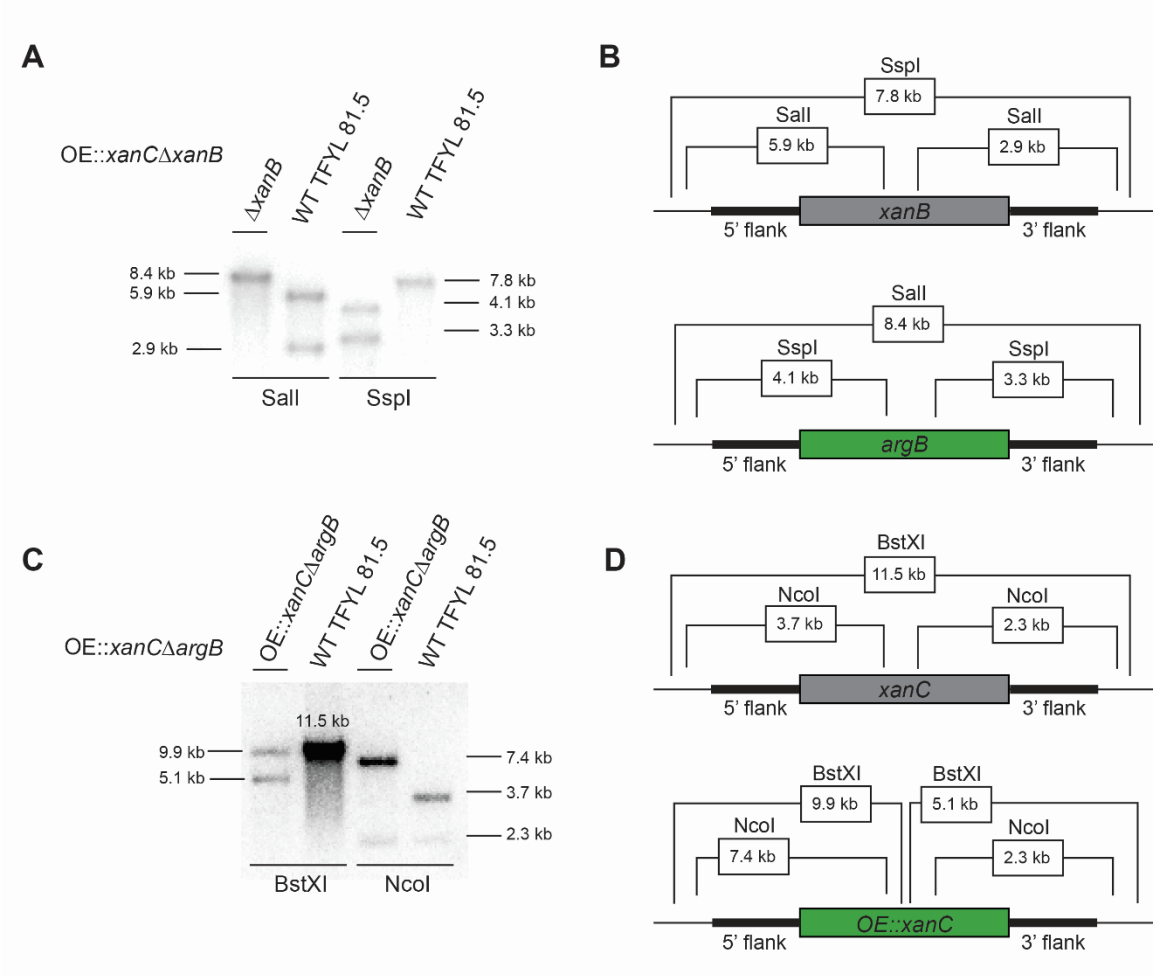

Figure S11- Southern Blot Confirmation analysis of the OE::*xanC*Δ*xanB* and OE::*xanC*Δ*argB* mutants used in this study. A.) Southern blot analysis of the OE::*xanC*Δ*xanB* mutant. B.) Digestion strategy of the OE::*xanC*Δ*xanB* mutant carried out with the designated restriction enzymes. C.) Southern blot analysis of the OE::*xanC*Δ*argB* mutant. D.) Digestion strategy of the OE::*xanC*Δ*argB* mutant carried out with the designated restriction enzymes.

Table S1- Production of xanthocillin derivatives comparing the OE::*xanC* mutants to deletion strains and WT.

strain	Dehydrogenated formyl tyrosine (**3**)	BU-4704[a] (**5**)	Xanthocillin X monomethyl ether[a] (**6**)	Fumiformamide (**7**)	*N,N*-((1Z,3Z)-1,4-bis(4-methoxyphenyl)buta-1,3-diene-2,3-diyl)diformamide (**8**)	Melanocin E (**9**)	Melanocin F (**10**)
WT	8.3E5 ± 1.3E5	2.4E9 ± 4.4E8	2.0E8 ± 4.5E7	3.2E6 ± 1.3E6	2.6E7 ± 8.5E6	5.3E5 ± 2.6E5	2.0E7 ± 1.5E6
OE::*xanC*	2.5E6 ± 2.3E5	1.3E10 ± 7.6E8	1.9E8 ± 1.3E7	5.0E9 ± 1.4E8	2.7E9 ± 1.3E8	4.1E8 ± 2.7E7	3.3E9 ± 6.5E7
OE::*xanC*□*xanA*	1.1E6 ± 1.4E5	5.0E9 ± 9.1E8	1.3E8 ± 3.5E7	5.2E9 ± 9.4E8	1.8E9 ± 1.8E8	5.7E8 ± 1.2E8	2.3E9 ± 1.1E8
OE::*xanC*□*xanB*	0	0	0	0	0	0	0
OE::*xanC*□*xanD*	1.0E7 ± 1.8E6	9.3E9 ± 9.0E8	2.2E8 ± 3.3E7	5.8E9 ± 7.8E8	2.3E9 ± 7.2E7	9.1E8 ± 1.7E8	2.6E9 ± 5.7E7
OE::*xanC*□*xanE*	1.8E8 ± 8.0E7	6.6E6 ± 3.1E6	8.7E4 ± 1.4E4	1.3E6 ± 1.7E5	2.6E7 ± 1.3E6	0	1.4E7 ± 1.0E6
OE::*xanC*□*xanF*	4.6E6 ± 5.5E5	2.2E9 ± 3.1E8	6.5E7 ± 6.3E6	1.5E9 ± 4.0E8	2.3E9 ± 2.2E8	3.0E7 ± 5.0E6	1.2E9 ± 9.9E7
OE::*xanC*□*xanG*	1.8E8 ± 6.2E7	0	0	0	0	0	0

[a]These compounds have an isocyanide functional group.

[b]The numbers indicate the average ± standard deviation for the peak intensity of each compound

Table S2- UHPLC-HRMS data for dehydrogenated formyl tyrosine.

Compound	HRESI(+/-) observed (*m/z*)	Ion	Calculated ion formula	Calculated *m/z*	Retention time (min)
Dehydrogenated formyl tyrosine (**3**)	230.04240	$[M+Na]^+$	$C_{10}H_9O_4NNa^+$	230.04238	3.7

Table S3. Production of various fungal secondary metabolites in the OE::*xanC* mutant compared to deletion strains and wild type.

Compounds	Ferricrocin	Hydroxyferricrocin	Hydroxyl-terezine D	Gliotoxin[a]	Dimethyl gliotoxin	Fumagillin
WT	6.8E7 ± 7.1E6	4.5E7 ± 5.2E6	1.9E8 ± 3.1E7	2.4E5 ± 1.7E5	1.1E7 ± 7.2E6	1.0E9 ± 2.0E8
OE::*xanC*	1.4E8 ± 6.4E6	5.8E7 ± 4.6E6	1.2E8 ± 6.8E6	0	0	3.5E8 ± 2.7E7
OE::*xanC* Δ*xanA*	1.5E8 ± 2.9E6	4.5E7 ± 2.3E6	1.6E8 ± 2.4E7	0	0	3.7E8 ± 3.3E7
OE::*xanC* Δ*xanB*	1.6E8 ± 1.3E7	3.2E7 ± 3.9E6	7.7E7 ± 1.0E7	0	0	5.0E8 ± 1.4E7
OE::*xanC* Δ*xanD*	1.6E8 ± 1.3E7	4.5E7 ± 9.0E8	1.4E8 ± 1.4E7	0	0	3.7E8 ± 5.2E7
OE::*xanC* Δ*xanE*	1.0E8 ± 5.0E6	4.0E7 ± 4.6E5	1.0E8 ± 3.2E6	0	0	2.9E8 ± 1.0E6
OE::*xanC* Δ*xanF*	1.2E8 ± 6.7E6	5.5E7 ± 2.0E6	7.9E7 ± 8.3E6	0	0	1.7E8 ± 2.0E7
OE::*xanC* Δ*xanG*	8.2E7 ± 5.1E6	2.2E7 ± 1.1E6	1.4E8 ± 8.3E6	0	0	3.4E8 ± 5.2E6

Compounds-continued	Pyripyropene A	Fumigaclavine A	Fumigaclavine C	Fumitremorgin C	Fumiquinazoline F	Fumiquinazoline H
WT	7.1E5 ± 2.5E5	5.2E9 ± 8.3E8	3.6E10 ± 6.6E9	2.7E7 ± 2.6E6	1.0E9 ± 1.7E8	1.1E6 ± 5.3E5
OE::*xanC*	1.1E6 ± 4.7E4	1.7E9 ± 9.8E7	6.7E10 ± 2.0E9	7.2E6 ± 4.6E5	2.8E9 ± 1.8E8	7.5E5 ± 8.1E4
OE::*xanC* Δ*xanA*	3.8E5 ± 5.8E4	3.0E9 ± 4.0E8	5.9E10 ± 7.1E9	8.0E6 ± 8.4E5	1.4E9 ± 1.2E8	1.5E6 ± 4.1E5
OE::*xanC* Δ*xanB*	2.0E5 ± 7.3E4	2.9E9 ± 1.0E8	2.2E10 ± 9.9E8	3.5E6 ± 4.5E5	7.8E8 ± 6.2E7	9.5E5 ± 1.3E5
OE::*xanC* Δ*xanD*	2.8E6 ± 8.8E5	2.7E9 ± 2.7E8	6.9E10 ± 8.3E9	5.4E6 ± 1.3E6	2.6E9 ± 4.5E8	9.2E4 ± 2.6E4
OE::*xanC* Δ*xanE*	8.2E4 ± 1.1E4	2.7E9 ± 1.5E8	2.6E10 ± 1.8E9	1.3E7 ± 6.6E5	3.5E8 ± 1.3E7	6.6E5 ± 9.1E4
OE::*xanC* Δ*xanF*	2.1E5 ± 1.2E4	2.2E9 ± 2.2E8	3.5E10 ± 3.9E9	6.9E6 ± 2.4E6	6.0E8 ± 5.7E7	4.4E5 ± 2.5E4
OE::*xanC* Δ*xanG*	2.2E4 ± 2.0E4	1.7E9 ± 1.3E8	1.7E10 ± 1.6E9	8.5E6 ± 5.4E5	2.7E8 ± 3.8E7	4.0E5 ± 7.4E4

[a] Although there was no gliotoxin in *xan* strains, the very low amount detected in the wild type makes it difficult to determine if this is a significant difference or if there is negative regulation by XanC.

Table S4- *A. fumigatus* strains used in this study.

Strain	Brief Genotype	Genotype	Reference
TFYL 81.5	WT	pyrG1, argB1, fumiargB, fumipyrG, ΔakuA::mluc	Throckmorton 2016
TFYL 84.1	ΔargB	pyrG1, argB1, fumipyrG, ΔakuA::mluc	Lim 2018
TFYL 80.1	ΔpyrG	pyrG1, argB1, fumiargB, ΔakuA::mluc	Lim 2018
TFYL 45.1	ΔpyrGΔargB	pyrG1, argB1, ΔakuA::mluc	Throckmorton 2016
TNLR 11.3	OE::xanCΔargB	pyrG1, argB1, parapyrG::gpdA(p)::AFUA_5G02655; ΔakuA::mluc	This Study
TNLR 9.1	ΔxanC	pyrG1, argB1, fumiargB, ΔAFUA_5G02655::parapyrG; ΔakuA::mluc	Lim 2018
TNLR 1.2	OE::xanC	pyrG1, argB1, fumiargB, parapyrG::gpdA(p)::AFUA_5G02655; ΔakuA::mluc	Lim 2018
TNLR 12.1	OE::xanCΔxanA	pyrG1, argB1, parapyrG::gpdA(p)::AFUA_5G02655; ΔAFUA_5G02670::fumiargB; ΔakuA::mluc	This Study
TNLR 13.2	OE::xanCΔxanB	pyrG1, argB1, parapyrG::gpdA(p)::AFUA_5G02655; ΔAFUA_5G02660::fumiargB; ΔakuA::mluc	This Study
TNLR 20.5	OE::xanCΔxanD	pyrG1, argB1, parapyrG::gpdA(p)::AFUA_5G02655; ΔAFUA_5G02650::fumiargB; ΔakuA::mluc	This Study
TNLR 17.5	OE::xanCΔxanE	pyrG1, argB1, parapyrG::gpdA(p)::AFUA_5G02655; ΔAFUA_5G02640::fumiargB; ΔakuA::mluc	This Study
TNLR 18.6	OE::xanCΔxanF	pyrG1, argB1, parapyrG::gpdA(p)::AFUA_5G02655; ΔAFUA_5G02630::fumiargB; ΔakuA::mluc	This Study
TNLR 19.2	OE::xanCΔxanG	pyrG1, argB1, parapyrG::gpdA(p)::AFUA_5G02655; ΔAFUA_5G02620::fumiargB; ΔakuA::mluc	This Study

Supplemental Table S5- Primers used to generate fungal strains.

Table S2. Primers used in this study

Strain/Gene	Sequence	Primer ID
TNLR 11.3 OE::*xanCΔargB*	GTGAGCCTCGTAGCCATACC	NR 655OE 5'F FOR
	TGCAGGATGCTTGTCTATGG	NR 655OE NEST FOR
	CGCCCTATAGTGAGTCGTATTACGCGCTGCTGATAATGGCCTTT	NR 655OE 5'F REV
	CGCTTGAGCAGACATCACCATGCATTCACAGACTACAAAGGACGAGC	NR 655OE 3'F FOR
	AACGATGACGACTGTGAAGG	NR 655OE NEST REV
	CTCAAAGCATCATGGACTGG	NR 655OE 3'F REV
TNLR 12.1 OE::*xanCΔxanA*	AATTTGTCTTGGATGCAGACCGCGTTCGGTGAACAATCTCTCGCCT	NR dxanA 5' REV
	CGGCTTCAAGCTTTCAGGAACG	NR dxanA 5' FOR
	TCAAATGGATGTATGGGTCTCTCCTTCGGGATCTCCCAAAGAGGCGATC	NR dxanA 3' FOR
	CCGAGGGTGACTTCGTCCTATC	NR dxanA 3' REV
	GCCTCCTAGCACATTGGGTTG	NR dxanA NEST FOR
	CCGTCGGTATGCACGGTTTT	NR dxanA NEST REV
TNLR 13.2 OE::*xanCΔxanB*	GGAGTGAGCAGCTGCTTGGA	NR dxanB 5' FOR
	CCCTTGATATGTCGGCATTCA	NR dxanB NEST FOR
	CTTGGATGCAGACCGCGTTCGAGGCCAGACATAGTGAGG	NR dxanB 5' REV
	CCTTCAAGGGCGAATTCTGCAGATGCCTTGTGCTCAGAGGGACT	NR dxanB 3' FOR
	CGTGCTGTAGTCTACTCAACCAG	NR dxanB NEST REV
	GGAACGTCTCCTCCAACC TC	NR dxanB 3' REV
TNLR 20.5 OE::*xanCΔxanD*	GGGGTATGGCTACGAGGCTC	NR dxanD 5' FOR
	GGCGCGGCTGGAAGAATATT	NR dxanD NEST FOR
	CTTGGATGCAGACCGCGTTCTGTGACTGCTTGGAGCCTGG	NR dxanD 5' REV
	AAGGGCGAATTCTGCAGATCGAATGGCTCAGCAACTGCG	NR dxanD 3' FOR
	CCGCTAGATGTGACGATGGA	NR dxanD NEST REV
	GCGTGGTCTTGTAAGGTGGAC	NR dxanD 5' REV
TNLR 17.5 OE::*xanCΔxanE*	CATCCCAAGTCAGCAAATCC	NR dxanE 5' FOR
	AAGTCATCGCGAAGTGTAGC	NR dxanE NEST FOR
	CTTGGATGCAGACCGCGTTCTCAGCATTGGTCGTAACTCG	NR dxanE 5' REV
	AAGGGCGAATTCTGCAGATGCAGTTGCTGAGACCATTCG	NR dxanE 3' FOR
	GTCTCGCCATCTCTGTCTGC	NR dxanE NEST REV
	GCAGCCATATTTGTCTGTGG	NR dxanE 3' REV
TNLR 18.6 OE::*xanCΔxanF*	CGGGAAAATTGAAGAACAGC	NR dxanF 5' FOR
	CACAGACTGGATGGCTTCG	NR dxanF NEST FOR
	CTTGGATGCAGACCGCGTTCAGACGTCGATTTGGTTACGG	NR dxanF 5' REV
	AAGGGCGAATTCTGCAGATCGCCGAGATTTTGATAAAGG	NR dxanF 3' FOR
	TCGCTCTGAGTGTTGTCAGC	NR dxanF NEST REV
	AGGAACTGCAACGACACTCC	NR dxanF 3' REV
TNLR 19.2 OE::*xanCΔxanG*	CTGTGGAATCCAATCCAACC	NR dxanG 5' FOR
	CTGCAGCTCTCTTTCCAACC	NR dxanG NEST FOR
	CTTGGATGCAGACCGCGTTCTCAGTGATGGGTGCAGTAGC	NR dxanG 5' REV
	AAGGGCGAATTCTGCAGATGAGCGAGGTCAAGGAGAGC	NR dxanG 3' FOR
	CATGCTCTGCTTCTTTGACC	NR dxanG NEST REV
	GCAGCGAATTAACGTCAGC	NR dxanG 3' REV
xanB	CCATTGAGCGAAGTTGATCG	NR xanB semiq FOR
	TCGATGACTTTCAGGACAGC	NR xanB semiq REV
act1	CTTCCAGCCTAGCGTTCT	MN_act1_FOR
	GTACATGGTGGTACCACCAG	MN_act1_REV

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