Cell Reports, Volume *34*

Supplemental Information

Aspergillus fumigatus tryptophan metabolic route differently affects host immunity

Teresa Zelante, Tsokyi Choera, Anne Beauvais, Francesca Fallarino, Giuseppe Paolicelli, Giuseppe Pieraccini, Marco Pieroni, Claudia Galosi, Claudia Beato, Antonella De Luca, Francesca Boscaro, Riccardo Romoli, Xin Liu, Adilia Warris, Paul E. Verweij, Eloise Ballard, Monica Borghi, Marilena Pariano, Gabriele Costantino, Mario Calvitti, Carmine Vacca, Vasilis Oikonomou, Marco Gargaro, Alicia Yoke Wei Wong, Louis Boon, Marcel den Hartog, Zdeněk Spáčil, Paolo Puccetti, Jean-Paul Latgè, Nancy P. Keller, and Luigina Romani

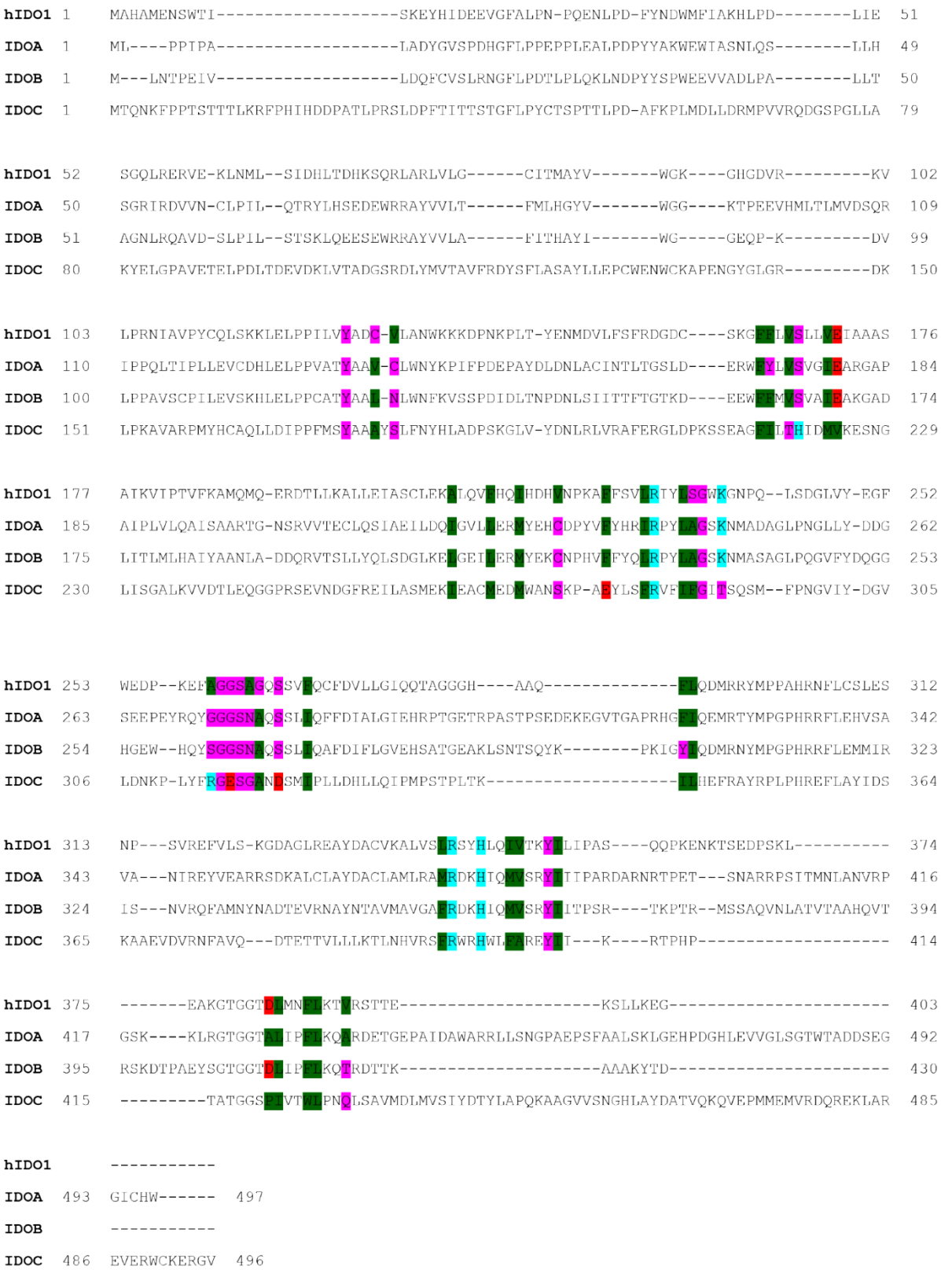

hIDO1 1 MAHAMENSWTI----------SKEYHIDEEVGFALPN-PQENLPD-FYNDWMFIAKHLPD--------LIE 51
IDOA 1 ML----PPIPA----------LADYGVSPDHGFLPPEPPLEALPDPYYAKWEWIASNLQS--------LLH 49
IDOB 1 M---LNTPEIV----------LDQFCVSLRNGFLPDTLPLQKLNDPYYSPWEEVVADLPA--------LLT 50
IDOC 1 MTQNKFPPTSTTTLKRFPHIHDDPATLPRSLDPFTITTSTGFLPYCTSPTTLPD-AFKPLMDLLDRMPVVRQDGSPGLLA 79
hIDO1 52 SGQLRERVE-KLNML--SIDHLTDHKSQRLARLVLG------CITMAYV-------WGK----GHGDVR---------KV 102
IDOA 50 SGRIRDVVN-CLPIL--QTRYLHSEDEWRRAYVVLT------FMLHGYV-------WGG----KTPEEVHMLTLMVDSQR 109
IDOB 51 AGNLRQAVD-SLPIL--STSKLQEESEWRRAYVVLA------FITHAYI-------WG-----GEQP-K---------DV 99
IDOC 80 KYELGPAVETELPDLTDEVDKLVTADGSRDLYMVTAVFRDYSFLASAYLLEPCWENWCKAPENGYGLGR---------DK 150
hIDO1 103 LPRNIAVPYCQLSKKLELPPILVYADC-VLANWKKKDPNKPLT-YENMDVLFSFRDGDC----SKGFFLVSLLVEIAAAS 176
IDOA 110 IPPQLTIPLLEVCDHLELPPVATYAAV-CLWNYKPIFPDEPAYDLDNLACINTLTGSLD----ERWFYLVSVGIEARGAP 184
IDOB 100 LPPAVSCPILEVSKHLELPPCATYAAL-NLWNFKVSSPDIDLTNPDNLSIITTFTGTKD----EEWFFMVSVAIEAKGAD 174
IDOC 151 LPKAVARPMYHCAQLLDIPPFMSYAAAYSLFNYHLADPSKGLV-YDNLRLVRAFERGLDPKSSEAGFILTHIDMVKESNG 229
hIDO1 177 AIKVIPTVFKAMQMQ-ERDTLLKALLEIASCLEKALQVFHQIHDHVNPKAFFSVIRIYLSGWKGNPQ--LSDGLVY-EGF 252
IDOA 185 AIPLVLQAISAARTG-NSRVVTECLQSIAEILDQVGVLERMYEHCDPYVIYHRIRPYLAGSKNMADAGLPNGLLY-DDG 262
IDOB 175 LITLMLHAIYAANLA-DDQRVTSLLYQLSDGLKELGEIFERMYEKCNPHVFFYQIRPYLAGSKNMASAGLPQGVFYDQGG 253
IDOC 230 LISGALKVVDTLEQGGPRSEVNDGFREILASMEKIEACTEDMWANSKP-AFYLSIRVFIEGIITSQSM--FPNGVIY-DGV 305
hIDO1 253 WEDP--KEFAGGSAGQSSVFQCFDVLLGIQQTAGGGH----AAQ----------FLQDMRRYMPPAHRNFLCSLES 312
IDOA 263 SEEPEYRQYGGGSNAQSSLLQFFDIALGIEHRPTGETRPASTPSEDEKEGVTGAPRHGFLQEMRTYMPGPHRRFLEHVSA 342
IDOB 254 HGEW--HQYSGGSNAQSSLLQAFDIFLGVEHSATGEAKLSNTSQYK--------PKIGYLQDMRNYMPGPHRRFLEMMIR 323
IDOC 306 LDNKP-LYFRGESGANDSMTPLLDHLLQIPMPSTPLTK----------LLHEFRAYRPLPHREFLAYIDS 364
hIDO1 313 NP---SVREFVLS-KGDAGLREAYDACVKALVSLRSYHLQIVTKYTLIPAS----QQPKENKTSEDPSKL---------- 374
IDOA 343 VA---NIREYVEARRSDKALCLAYDACLAMLRAMRDKHTQMVSRYTIIPARDARNRTPET---SNARRPSITMNLANVRP 416
IDOB 324 IS---NVRQFAMNYNADTEVRNAYNTAVMAVGAFRDKHIQMVSRYTLTPSR----TKPTR--MSSAQVNLATVTAAHQVT 394
IDOC 365 KAAEVDVRNFAVQ---DTETTVLLLKTLNHVRSFRWRHWLPAREYTT---K----RTPHP---------- 414
hIDO1 375 -------EAKGTGGTDLMNFLKTWRSTTE----------KSLLKEG---------- 403
IDOA 417 GSK----KLRGTGGTALIPFLKQWRDETGEPAIDAWARRLLSNGPAEPSFAALSKLGEHPDGHLEVVGLSGTWTADDSEG 492
IDOB 395 RSKDTPAEYSGTGGTDLIPFLKQTRDTTK----------AAAKYTD---------- 430
IDOC 415 ---------TATGGSPIVTWLPNQLSAVMDLMVSIYDTYLAPQKAAGVVSNGHLAYDATVQKQVEPMMEMVRDQREKLAR 485
hIDO1 ----------
IDOA 493 GICHW------ 497
IDOB ----------
IDOC 486 EVERWCKERGV 496

Figure S1. (Sequence alignment of *A. fumigatus* IdoA, IdoB, IdoC and human IDO1), Related to Figure 1. Protein sequence alignment between *Aspergillus* IdoA, IdoB, IdoC and human IDO1 protein sequences used to build the homology models. Binding site residues are colored according to their properties: polar positive cyan, polar negative red, polar neutral magenta and non-polar green.

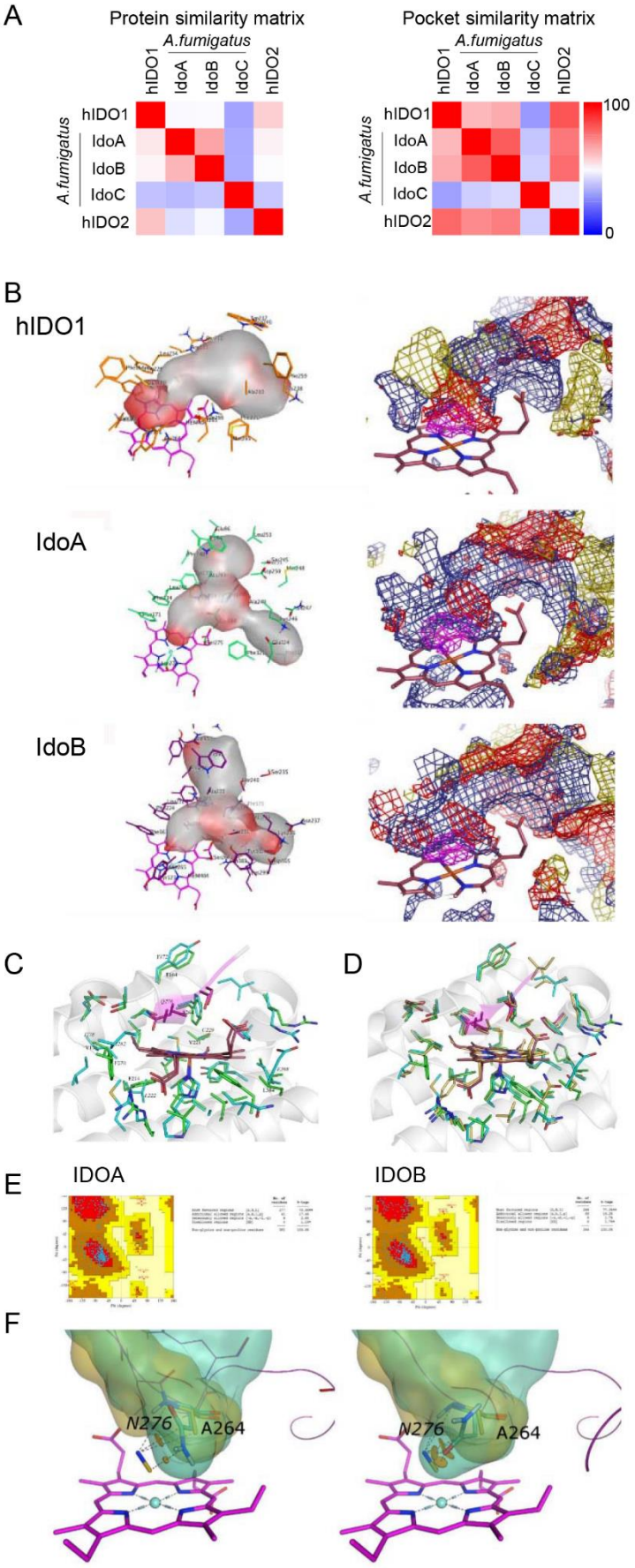

A
Protein similarity matrix
A.fumigatus
hIDO1
IdoA
IdoB
IdoC
hIDO2
A.fumigatus
Pocket similarity matrix
100
0
B
hIDO1
IdoA
IdoB
C
D
E
IDOA
IDOB
F
N276
A264
N276
A264

Figure S2. (Homology and computational model), Related to Figure 1

(**A**) Protein and pocket similarity matrices of human IDOs and *Aspergillus* Idos.

(**B**) Computational model showing binding sites shapes and properties for human IDO (2D0T), *A. fumigatus* IdoA and IdoB. In red are indicated the polar regions (left panel). On the right panel SiteMap analysis of the enzyme binding sites: metal binding areas (magenta), Hbond donor areas (blue), H bond acceptor areas (red) and lipophilic areas (yellow).

(**C**) Binding site residues of human IDO (2D0T,green) and *A. fumigatus* IdoA (cyan). Differences in residues are highlighted by labels (regular for human, in italics for *A. fumigatus*). The important differences on the top of the heme Fe atom are colored in magenta for both species. Hydrogen atoms are omitted for clarity.

(**D**) Binding site residues of human IDO (2D0T,green) and *A. fumigatus* IdoA (cyan) and *A. fumigatus* IdoB (yellow).

(**E**) Ramachandran plot of IdoA and IdoB and related statistics produced with PROCHECK.

(**F**) Comparison between Ala264 in human Ido (yellow crystal 2D0u) and Asn276 in *A. fumigatus* IdoA (cyan). The two figures represent two possible conformations of Asn: left) the conformation of Asn276 allowed the superoxide binding, represented by cyanide of 2D0U; right) possible scenario where the carbonyl oxygen of Asn276 is in the fine position to coordinate the Fe atom, making it unnecessary (or more difficult) for the superoxide to bind.

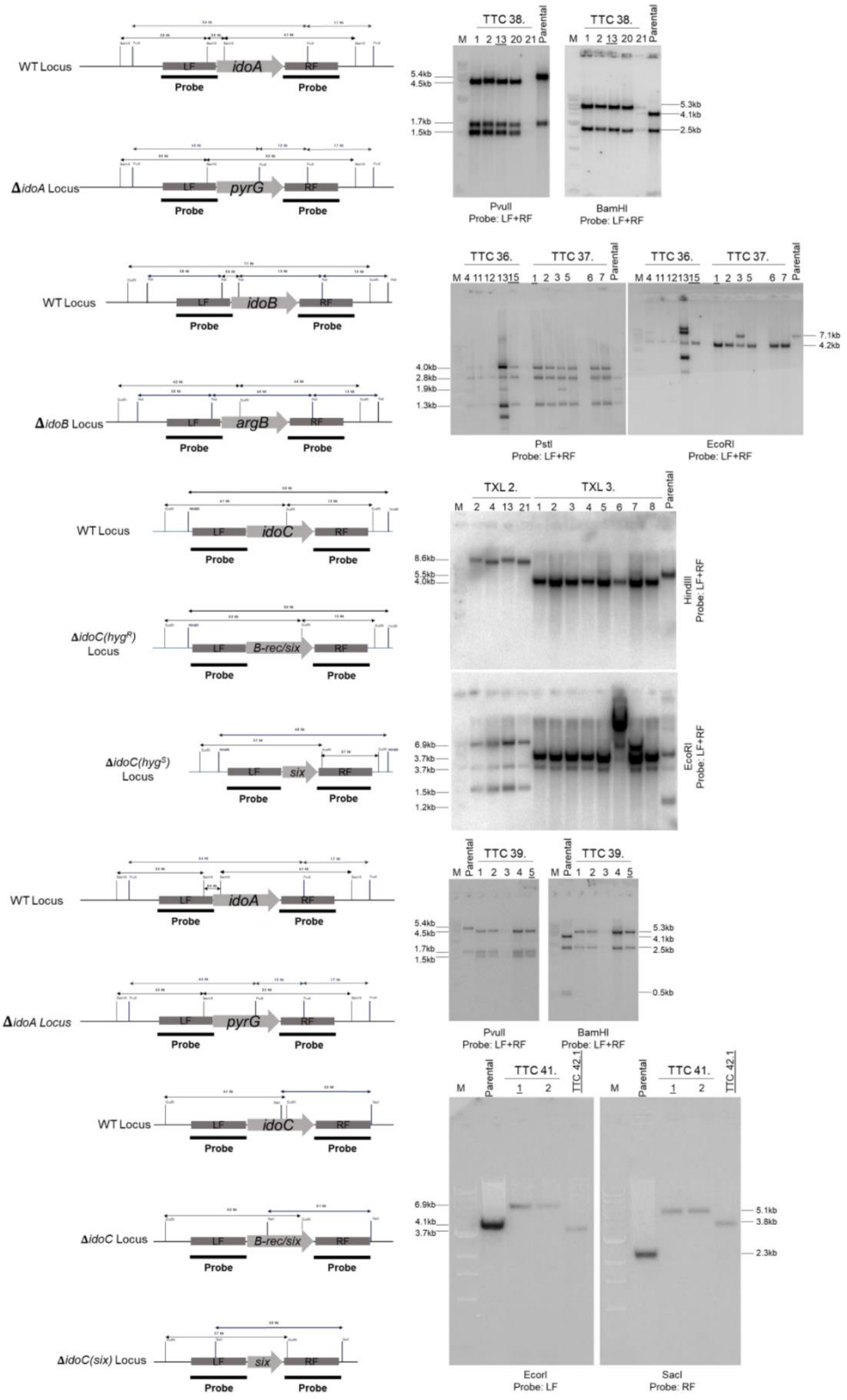


Figure S3. (Southern confirmation of deletion of *A. fumigatus ido* mutants), **Related to Figure 1.** Presented is the schematic representation of the *ido* genes, the markers used to delete the gene and the predicted sizes of DNA fragments of a correct gene deletion, also shown by radiolabeling on the right.

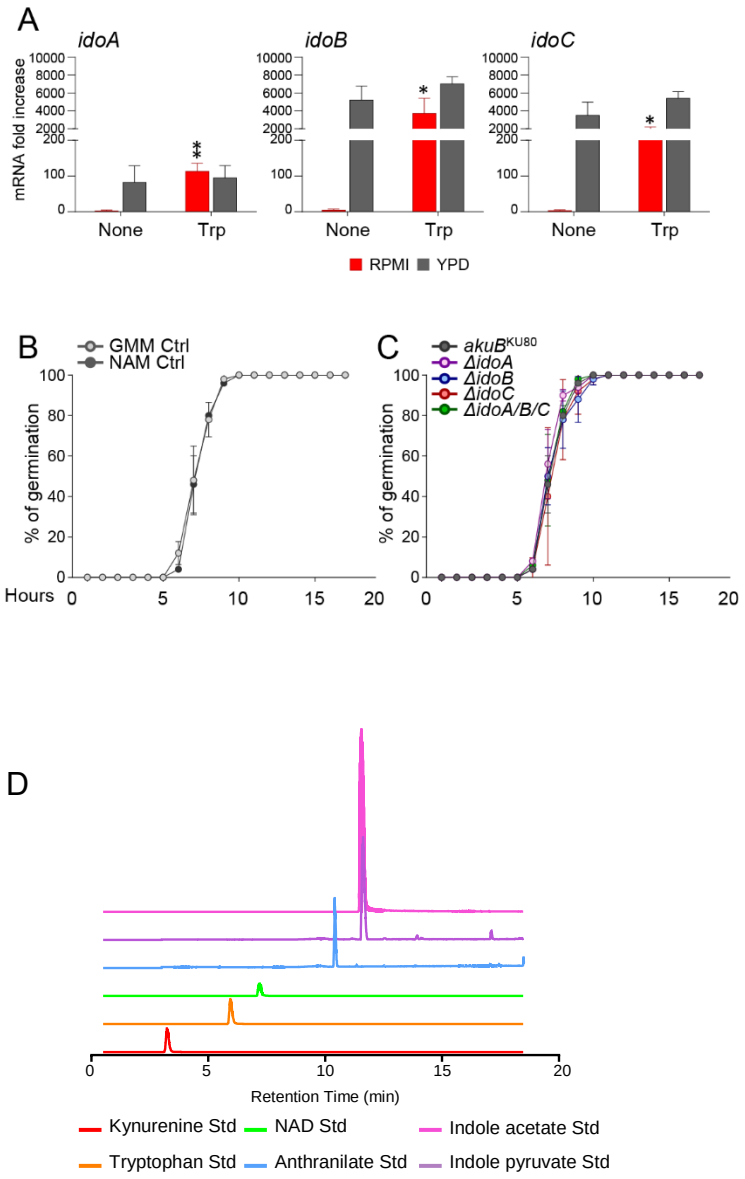


Figure S4. (*Aspergillus* mutants exposure to different media), Related to Figure 1 and Figure 2.
(A) mRNA expression (fold increase) of *idoA*, *idoB* and *idoC* in fungi grown in RPMI and YPD for 24 h with (60 μM) or without (none) L-tryptophan (Trp). Statistical significance (**P*<0.01,***P*<0.001) was determined against untreated (None) sample (Two-way ANOVA - Bonferroni post-hoc test).
(**B**) Germination assessment of *A. fumigatus* wild type in unsupplemented (GMM) medium or supplemented with nicotinamide medium (NAM).
(**C**) Germination assessment of *A. fumigatus* mutant strains in NAM medium.
(**D**) Traces of standards measured on LCMS.

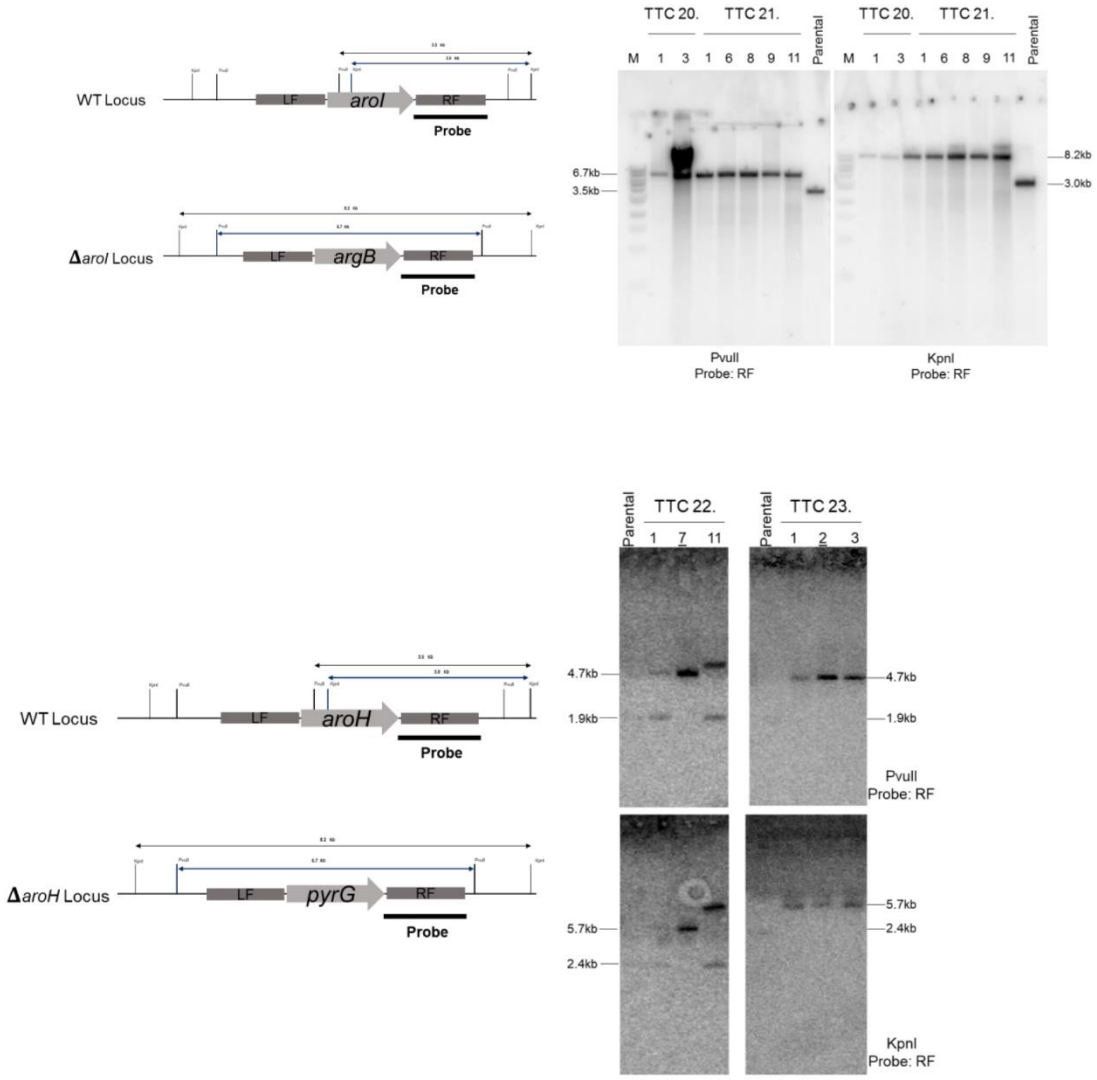


Figure S5. *A. fumigatus* aro mutants, Related to Figure 2.
(**A**) Southern confirmation of deletion of *A. fumigatus aro* mutants. Presented is schematic representation of the *aro* genes, the markers used to delete the gene and the predicted sizes of DNA fragments of a correct gene deletion, also shown by radiolabeling on the right.

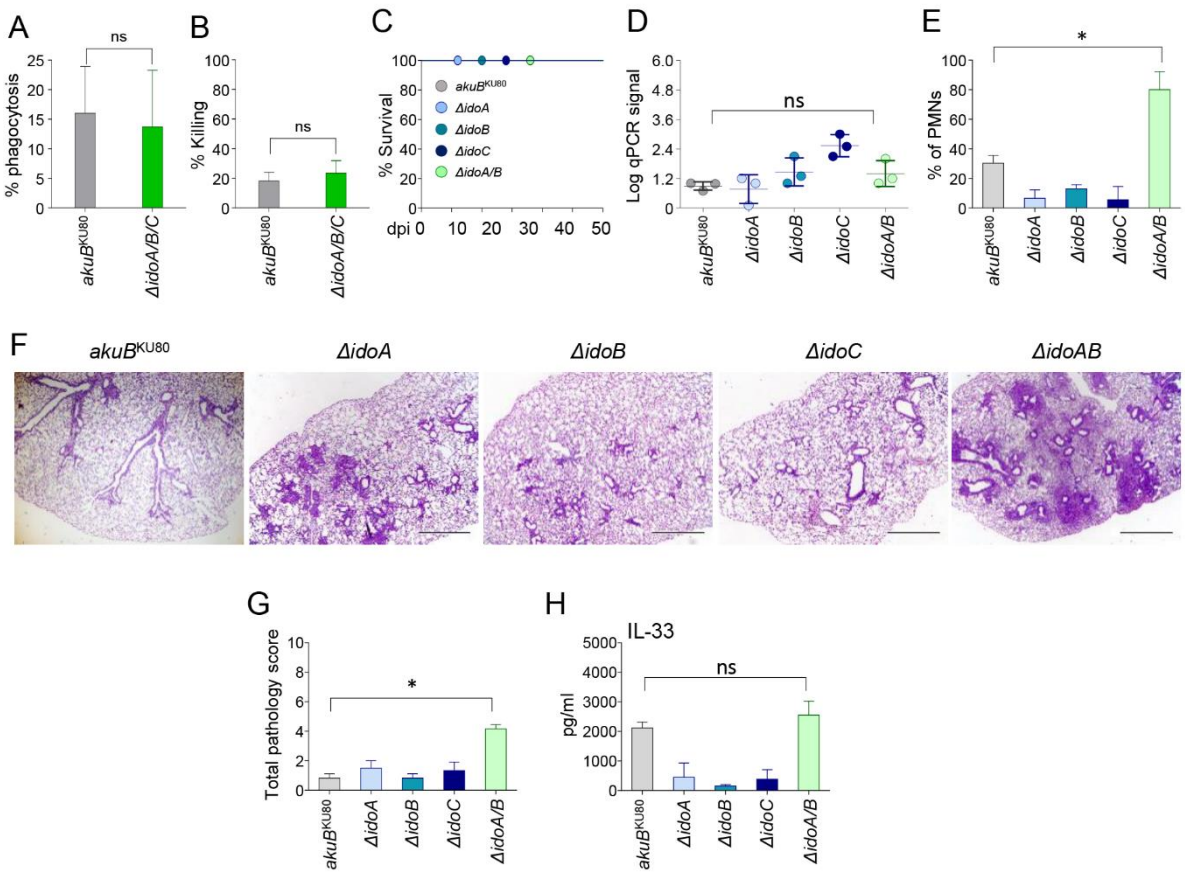


Figure S6. (Pathogenicity *of Aspergillus* mutants *in vivo*), Related to Figure 3.

(**A**) Percentage of phagocytosis (mean ± sd), determined as the percentage of neutrophils containing at least 1 conidium. The experiments were repeated twice for each fungal species with two different primary cultures.

(**B**) Percentage of killing (mean ± sd), determined as: percentage of colony formation inhibition = 100 − (CFU experimental group/CFU control cultures) × 100. For **B**, killing assay was done with five wells per condition in more than three independent experiments**.**

(**C**) Survival rates of infected mice. For **C**-**H**, C57BL/6 mice (n = 12) were infected intranasally with 2 × 10^7*A. fumigatus* strains and sacrificed at 7 days post infections (dpi).

(**D**) 18S rRNA expression measured by qPCR from C57BL/6 lungs.

(**E**) Flow cytometry analysis of lung cells showing the percentage of pulmonary PMNs ($CD11b^+$ (FITC) and $GR1^+$ cells (V450)).

(**F**) Histopathological analyses (PAS staining) on lungs at 7 dpi, scale bars, 10 μm.

(G) Total pathology score on lung histology at 7 dpi.

(H) Cytokine levels by ELISA. Data are represented as mean ± s.d. of triplicate measurements. (**G, H**) Statistical significance (*P<0.01,) was determined against mice infected with the *Aspergillus akuB*^{KU80} strain (One-way ANOVA - Bonferroni post-hoc test).

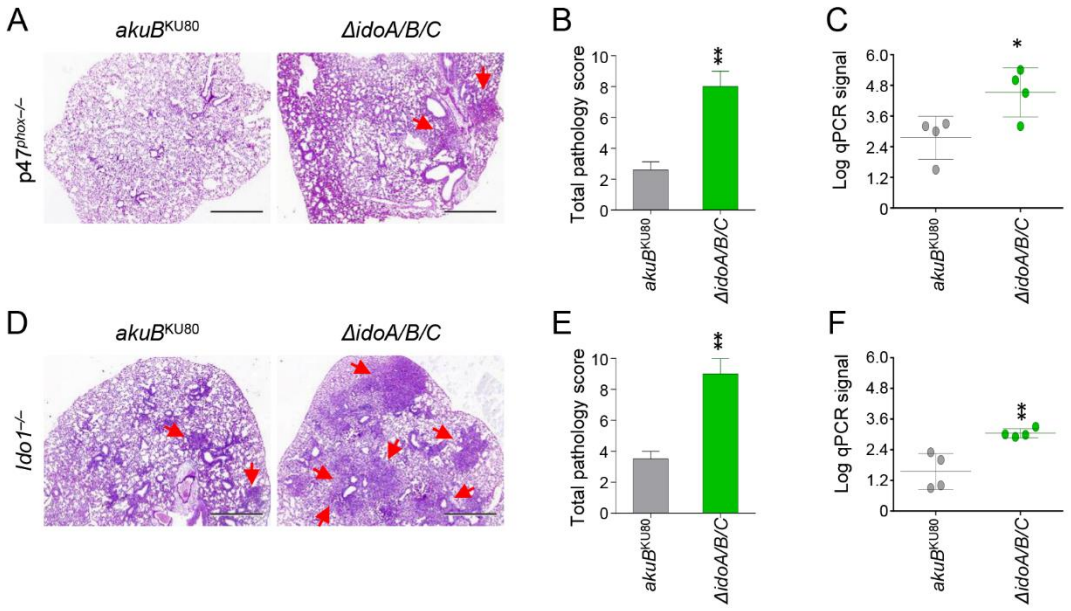
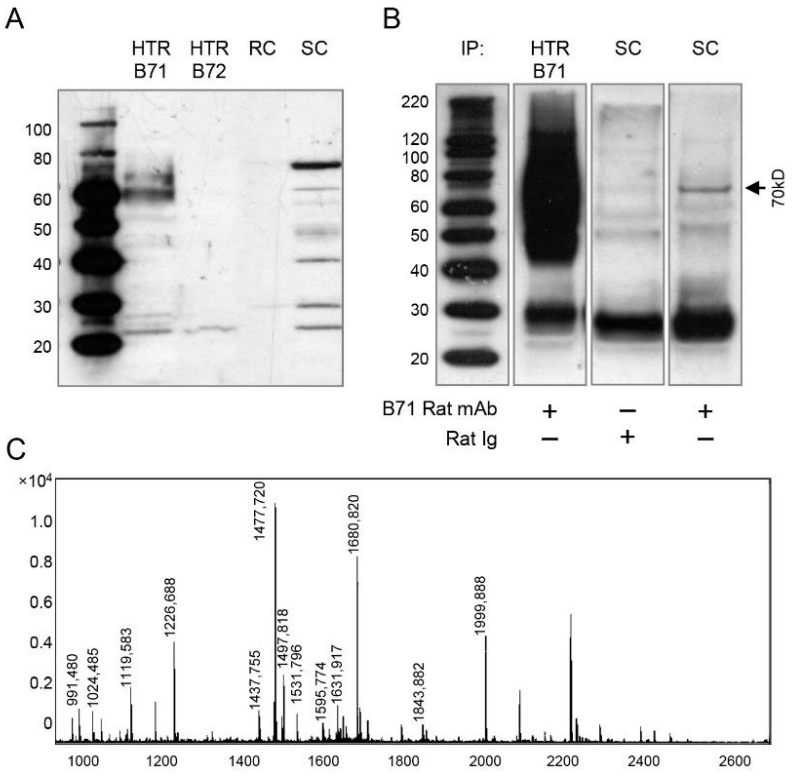


Figure S7. (Mice susceptibility *of Aspergillus* mutants for *idos*), Related to Figure 3. The indicated strain of mice (n = 12) were infected intranasally with 2 × 10^7 *A. fumigatus* strains and sacrificed at 7 days post infections (dpi).
(**A** and **D**) Representative histopathological analyses (PAS staining) on lungs, scale bars, 10 μm.
(**B** and **E**) Total pathology score on lung histology.
(**C** and **F**) 18S rRNA expression measured by qPCR from C57BL/6 lungs. Data are represented as mean ± s.d. of triplicate measurements. Statistical significance (*P<0.01, **P<0.001) was determined against mice infected with the *Aspergillus akuB*^{KU80} strain (Two tailed Student's t Test unpaired parametric).



D

```
CLUSTAL 2.1 multiple sequence alignment

XP_750490.1     MAPAVGIDLGTTYSCVGVFRDDRIEIIANDQGNRTTPSFVAFTDTERLIGDAAKNQVAMN
AAI45844.1      ----------------------------------------------------MACNCQLMQD
                                                                     *.: *:  :

XP_750490.1     PHNTVFDAKRLIGRRFQDAEVQSDMKHWPFKVVEKGGKPIIEVEFKGETKQFTPEEISSM
AAI45844.1      TPLLKFPCPRLILLFVLLIRLS-----QVSSDVDEQLSKSVKDKVLLPCRYNSPHEDESE
                .    * . ***   .  .:.       . *::  .  :: :.    :  :*.* .*

XP_750490.1     VLTKMRETAEAYLGGTVNNAVITVPAYFNDSQRQATKDAGLIAGLNVLRIINEPTAAAIA
AAI45844.1      DRIYWQKHDKVVLS-VIAGKLKVWPEYKNRTLYDNTTYS------------------LII
                     ::  :. *. .: . : . * * * :  : *. :                   *

XP_750490.1     YGLDKKAEGERNVLIFDLGGGTFDVSLLTIEEG-IFEVKATAGDTHLGGEDFDNRLVNHF
AAI45844.1      LGLVLSDRGTYSCVVQKKERGTYEVKHLALVKLSIKADFSTPNITESGNPSADTKRITCF
                 **  . .*  . :: .   **::*. *:: :  *    :*.. *. *. . *.: :. *

XP_750490.1     VNEFKRKHKKDLTTNARALRRLRTACERAKRTLSSAAQTSIEIDSLFEGIDFYTSITRAR
AAI45844.1      ASGGFPKPRFSWLENGRELPGINTTISQDP---------ESELYTISSQLDFNTTRN---
                ..    * : .   *.* *  :.*: .:          . *: :: . .:** *: .

XP_750490.1     FEELCQDLFRSTMEPVERVLRDAKLDKSSVHEIVLVGGSTRIPKIQRLVADFFNKEANKS
AAI45844.1      ---------------------------HTIKCLIKYG----------------------
                                           ::: ::  *

XP_750490.1     INPDEAVAYGAAVQAAILSGDTSSKSTNEILLLDVAPLSLGIETAGGVMTPLIKRNTTIP
AAI45844.1      ------------------------------------------------------------


XP_750490.1     TKKSETFSTYSDNQPGVLIQVYEGERARTKDNNLLGKFELTGIPPAPRGVPQIEVTFDVD
AAI45844.1      ------------------------------DAHVSEDFTWEKPPEDPPDSKNTLVLFGAG
                                             * ::  .*     *  * .  :  * *...

XP_750490.1     ANGIMNVSAVEKGTGKTNKITITNDKGRLSKEEIERMLADAEKYKEEDEAEAARIQAKNG
AAI45844.1      FGAVITVVVIVVIIKCFCKHRSCFRR----RNEASRETNNSLTFGPEEALAEQTVFL---
                 ..::.* .:        *      :    ::* .*   :: .:  *:      :

XP_750490.1     LESYAYSLKNTISEGKLNISDADKEKVSSKVEEIISWLDNNQTATKDEYESQQKELESVA
AAI45844.1      ------------------------------------------------------------


XP_750490.1     NPIISAAYGGAAGAAPGGAAPGGATRTADEVEERPEELD
AAI45844.1      ---------------------------------------
```

Figure S8. (Identification of CTLA-4 binding molecule by mass spectrometry), Related to Figure 4.
(**A**) Levels of B71-like protein molecule detected by western blot analysis.
(**B**) Silver-stained SDS-PAGE gel of anti-B71 immunoprecipitate. Bands were excised from the gel, trypsin-digested, and analyzed by MALDI-TOF. Identified protein is indicated by arrow at 70kD.
(**C**) MALDI-TOF spectrum of peptides corresponding to HSP70 (ID: XP_750490.1) protein.
(**D**) Multiple sequence alignment of the *A. fumigatus* HSP70 (ID: XP_750490.1) and mouse protein sequence of B71 (ID: AAI45844.1) using Clustal 2.1. (*) = identical aa; (:) = highly conserved aa substitution; (.) = conserved substitution. Alignment identified a similar region of identity between the two molecules at G115 of B71 protein, which is a peculiar binding region for B71 binding to CTLA-4 in mammalians in the IgV-like domain (red box).

ID	Strain	Genotype	Reference
Control	CEA17 *pyrG+* KU80	*pyrG1*, Δ*akuB*::*pyrG*	*Silva Ferreira et al., 2006*
CEA17	CEA17 *pyrG-* KU80	*pyrG1*, Δ*akuB*::*pyrG*, *pyrG1*	*Silva Ferreira et al., 2006*
TJG1.6	Δ*argB*	*pyrG1*, Δ*akuB*::*pyrG*, *pyrG1*, Δ*argB*::*pyrG*	*Wiemann et al., 2017*
TMN2.1	ΔΔ*argB/pyrG*	*pyrG1*, Δ*akuB*::*pyrG*, *pyrG1*, Δ*argB*::*pyrG*, Δ*pyrG*	*Wiemann et al., 2017*
TTC38.13	Δ*idoA*	*pyrG1*, Δ*akuB*::*pyrG*, *pyrG1*, *argB*::*pyrG*, Δ*AFUB_066940*::*argB*	This study
TTC37.1	Δ*idoB*	*pyrG1*, Δ*akuB*::*pyrG*, *pyrG1*, Δ*AFUB_034980*::*pyrG*	This study
TXL3.2	Δ*idoC*	*pyrG1*, Δ*akuB*::*pyrG*, *pyrG1*, Δ*argB*::*pyrG*, Δ*pyrG*, Δ*AFUB_088580*::*six*, *pyrG*, *argB*	This study
TTC39.5	ΔΔ*idoA/B*	*pyrG1*, Δ*akuB*::*pyrG*, *pyrG1*, Δ*argB*::*pyrG*, Δ*pyrG*, Δ*AFUB_034980*::*pyrG*, Δ*AFUB_066940*::*argB*	This study
TTC42.1	ΔΔΔ*idoA/B/C*	*pyrG1*, Δ*akuB*::*pyrG*, *pyrG1*, Δ*argB*::*pyrG*, Δ*pyrG*, Δ*AFUB_034980*::*pyrG*, Δ*AFUB_066940*::*argB*, Δ*AFUB_088580*::*six*	This study
TTC 21.6	Δ*aroI*	*pyrG1*, Δ*akuB*::*pyrG*, *pyrG1*, Δ*argB*::*pyrG*, Δ*akuB*::*pyrG*, Δ*AFUB_051500*::*argB*	This study
TTC 22.7	Δ*aroH*	*pyrG1*, Δ*akuB*::*pyrG*, *pyrG1*, Δ*AFUB_029280*::*pyrG*	This study
TTC 23.1	ΔΔ*aroH/I*	*pyrG1*, Δ*akuB*::*pyrG*, *pyrG1*, Δ*argB*::*pyrG*, Δ*pyrG*, Δ*AFUB_051500*::*argB*, Δ*AFUB_029280*::*pyrG*	This study

Table S1. Fungal Strains used in this study, Related to STAR Methods

Number	Name	Sequence 5'- 3'	Associated Gene
TC-1131	TC-DAfu3g14250 F1	ATTACCCTGGATGCGTCAACG	*idoA*
TC-1134	TC-DAfu3g14250 R1	TTCGATATCAAGCTATCGATACCTCGACTCTGTAGG CAGGATTCAATGCAC	*idoA*
TC-1132	TC-DAfu3g14250 F2	CTGTCGCTGCAGCCTCTCCGATTGTCGAATCCCGAT CAGGGTATTGAGAGT	*idoA*
TC-1135	TC-DAfu3g14250 R2	TCGTGAAGAAGACTTCGGAGC	*idoA*
TC-1133	TC-DAfu3g14250 F3	ATTGACTACGTCCTCAAGGCTG	*idoA*
TC-1136	TC-DAfu3g14250 R3	TGTTTGAGATGCAGGACTGC	*idoA*
TC-1137	TC-diAfu3g14250 F	ATTGGCCAGGATATATTGTCTCC	*idoA*
TC-1138	TC-diAfu3g14250 R	GATCCGAGAGCCCCCTTAG	*idoA*
TC-20	Arg F	GAACGCGGTCTGCATCCAAG	*argB*
TC-21	ArgR	GAAGGAGAGACCCATACATCC	*argB*
TC-1113	TC-DAfu4g09830 F1	ACCAAGCTGCAAAATCGACG	*idoB*
TC-1114	TC-DAfu4g09830 R1	TGTCTTGGATGCAGACCGCGTTCTGTCGAAGCCAGA ATATAAGGGGACG	*idoB*
TC-1115	TC-DAfu4g09830 F2	ATCAAATGGATGTATGGGTCTCTCCTTCACTCATTT GAGTGGAAACTCAGC	*idoB*
TC-1116	TC-DAfu4g09830 R2	TTCGAAATCCAAATCAAGC	*idoB*
TC-1117	TC-DAfu4g09830 F3	ACTGGATTCCCGGAAGCAGG	*idoB*
TC-1118	TC-DAfu4g09830 R3	AGCCCATGGAACCGTTCTACG	*idoB*
TC-1119	TC-diAfu4g09830 F1	TCGCTGATTTACCAGCTCTGC	*idoB*
TC-1120	TC-diAfu4g09830 R1	TGCTGGAGTGTCCTTCGAACG	*idoB*
TC-4	pyrG_prom_F	CGTAATACGACTCACTATAGGG	*pyrG*
TC-5	pyrG_term_R	ATTCGACAATCGGAGAGGCTGC	*pyrG*
XL-1	DAfu7g02010 F1	TCGAGTTCGTTGCTTGGATGC	*idoC*
XL-2	DAfu7g02010 R1	CTATTGACCTATAGGACCTGAGTGATGCGGTAGAA GTTGACAAGAAGAGAG	*idoC*
XL-3	DAfu7g02010 F2	TAAGTTGAGCATAATATGGTCCATCTAGTGCTTGTC CGTAGATTGTGTAGGTAG	*idoC*
XL-4	DAfu7g02010 R2	GCAGGCTCCTGCTCTTCATCTT	*idoC*
XL-5	diAfu7g02010R	TGGTGTCACCATCAAGACCCA	*idoC*
XL-6	DAfu7g02010F3	TAGTTGGTGAGCTGTACGGTG	*idoC*
XL-7	DAfu7g02010R3	GCGTCTTTCGACATGTCTTCC	*idoC*
TC-1104	TC- DAfu2g13630F1	CGATTGTGCTAAACAACAGGGGC	*aroH*
TC-1105	TC- DAfu2g13630R1	GTCGCTGCAGCCTCTCCGATTGTCGAATTCAGAATA CGATGCTCTCAAGTTCAGCCGG	*aroH*
TC-1106	TC-DAfu2g13630 F2	CGATATCAAGCTATCGATACCTCGACTCACAATACC AACGCCATTTTTGTCTCCGCCG	*aroH*
TC-1107	TC-DAfu2g13630 R2	ATTGGCTATGGATCGAGATGGCC	*aroH*
TC-1108	TC-diAfu2g13630 F1	ACGGCATTCAAGCAGATCAACG	*aroH*
TC-1109	TC-diAfu2g13630 R1	AGTCATGCACTAGCCACACG	*aroH*

TC-1110	TC-diAfu2g13630F1	GCAGTGATATGTTCAAGAGCCC	*aroH*
TC-1111	TC-diAfu2g13630R1	CACGCTAATCTCTTCGAAAGGG	*aroH*
TC-1141	TC-DAfu5g02990 F1	TGAGGATGCAGGTTTACTGACAC	*aroI*
TC-1142	TC-DAfu5g02990 R1	GAAAATTTGTCTTGGATGCAGACCGCGTTCCTTGAG CAATCAAGTCCTTTACAGC	*aroI*
TC-1143	TC-DAfu5g02990 R3	TACTGATTTGGCTGGCAAGG	*aroI*
TC-1144	TC-DAfu5g02990F2	ATCAAATGGATGTATGGGTCTCTCCTTCTTGTTTAT AATAGCATCGAGACG	*aroI*
TC-1145	TC-DAfu5g02990F3	AGGTTCAAAAGTGCCTGATCTTG	*aroI*
TC-1146	TC-DAfu5g02990R2	AGGTGGTCCCATAATCAAACG	*aroI*
TC-1147	TC-diAfu5g02990F	TGATTTCTATCGAAAAATGGTGG	*aroI*
TC-112	TC-Act 1 F2	CTTCCAGCCTAGCGTTCTG	*actA*
TC-113	TC-Act 1 R2	ATCCACATCTGCTGGAAGG	*actA*
TC-1206	IdoA F	ATGCTTCCTCCTATCCCCGCTC	*idoA*
TC-1207	IdoA R	GAGGGAGCTCTAGATGGTCGC	*idoA*
TC-1119	IdoB F	TCGCTGATTTACCAGCTCTGC	*idoB*
TC-1208	IdoB R	TGAAGAACCATTCTTCGTCCTTGG	*idoB*
TC-1210	IdoC F	AAAGTGGTCGATACCCTTGAGC	*idoC*
TC-1211	IdoC R	AACTCGTGTAGGATCTTGGTGAG	*idoC*
TC-1139	AroH F	TTCGTACCTGAGCCTAGATGTCG	*aroH*
TC-1140	AroH R	GATGCTTCTGCCAATCGATCTC	*aroH*
TC-1234	AroI F1	AACTGGGATTGCACCAAAGGACG	*aroI*
TC-1235	AroI R1	AGTCTCAGTAAGGCGGGTGATCC	*aroI*

Table S2: *Aspergillus* primers used in this study, Related to STAR Methods

Gene	Primers Sequence (5'-3')	Annealing Temperature (°C)
18S	Sense ➲ ATGGCCGTTCTTAGTTGGTG	58
	αSense ➲ GAGCCGATAGTCCCCCTAAG	
aroH	Sense➲AAAGTCCCGACAGCAATCTACA	60
	αSense➲TGGGACTTTCACGCTAATCTCT	
idoA	Sense ➲ ATGCCTGTCTCGCTATGC	55
	αSense ➲ CTCGGGTGTACGGTTTCG	
idoB	Sense➲ AGGAAGTTGTCGCTGATTTACC	54
	αSense ➲ ATGCTCGCCGCCATTCTG	
idoC	Sense ➲ TCAGCCAGGATGGCAGTC	55
	αSense ➲ TCGTCAGTCAGGTCAGGAAG	
β-actin	Sense ➲ AGCCATGTACGTAGCCATCC	59
	αSense ➲ CTCTCAGCTGTGGTGGTGAA	
Il33	Sense ➲ TCCTGCCTCCCTGAGTACAT	58
	αSense ➲ CACCTGGTCTTGCTCTTGGT	
Cyp1a1	Sense ➲ ACAGTGATTGGCAGAGATCG	60
	αSense ➲ GAAGGGGACGAAGGATGAAT	
pqsH	Sense ➲ TGATGTCGATGCCTTCCAGT	55
	αSense ➲CTCATCCAGCCCCTCCAGTA	
Il10	Sense ➲ CCCTTTGCTATGGTGTCCTT	56
	αSense ➲ TGGTTTCTCTTCCCAAGACC	
Foxp3	Sense ➲ CCCAGGAAAGACAGCAACCTTTT	59
	αSense ➲ TTCTCACAACCAGGCCACTTG	

Table S3: qPCR primers used in this study, Related to STAR Methods

NAME	SEQUENCE 5'→ 3'	USE
AB For LB *IDOA*	GCCAAGCTTGCATGCCCCCGGGAAACTTTTGGGTCCCTGCTT	Construction of the deletion cassette *IDOA*
AB Rev LB *IDOA*	GGACCTGAGTGATGCTAGGCAGGATTCAATGCACA	
AB For RB *IDOA*	TGGTCCATCTAGTGCATTTGCATTAGCGGTGCTTC	
AB RevRB *IDOA*	AATTCGAGCTCGGTACCCCGGGTGTACAAGCTCAAGCGGCTA	
AB For LB *IDOB*	GCCAAGCTTGCATGCCCCCGGGCACATGATCACTGGGAAACG	Construction of the deletion cassette *IDOB*
AB Rev LB *IDOB*	GGACCTGAGTGATGCGTCCATGTCGAAGCCAGAAT	
AB For RB *IDOB*	TGGTCCATCTAGTGCGTGGGAGCTTTCACCAGATG	
AB RevRB *IDOB*	AATTCGAGCTCGGTACCCCGGGAAACAATGCTCGCTTCGACT	
AB For LB *IDOC*	GCCAAGCTTGCATGCCCCCGGGCTTTGCTTCGTCGACACTGA	Construction of the deletion cassette *IDOC*
AB Rev LB *IDOC*	GGACCTGAGTGATGCAGAGAAACAGACGGGGGATT	
AB For RB *IDOC*	TGGTCCATCTAGTGCCGCTGTCCACTGCTACGAT	
AB RevRB *IDOC*	AATTCGAGCTCGGTACCCCGGGATTTGAGCCACCTTGTACGG	
AB Screen *IDOA* For	GAGTCAGCATTAGAATCTCCCT	*IDOA* primers for PCR analysis
AB Screen *IDOA* Rev	CCATTCCACATCTACACCCC	
AB Screen *IDOB* For	CAACACGCCTCCGTTATAGA	*IDOB* primers for PCR analysis
AB Screen *IDOB* Rev	CAGCTCAGCCAGCTTCTTTT	
AB Screen *IDOC* For	ACATCGGTCAATCCTCCAAG	*IDOC* primers for PCR analysis
AB Screen *IDOC* Rev	TTTCCCCACTTAATCCATGC	

Table S4: Primer list used in this study to generate fungal *ido* mutant strains by electroporation, Related to STAR Methods

Q1 (m/z)	Q3 (m/z)	RT (min)	ANALYTE	DP	FP	EP	CE	CXP
225.0	110	2.6	3-hydroxy-kynurenine	15	150	10	20	15
225.0	162	2.6	3-hydroxy-kynurenine	15	150	10	20	15
228.2	210	2.6	3-hydroxy-kynurenine-$^{13}C_2$-^{15}N	15	125	10	20	15
228.2	110	2.6	3-hydroxy-kynurenine-$^{13}C_2$-^{15}N	15	125	10	20	15
177.0	160	5.7	serotonine	24	258	10	15	8
177.0	115	5.7	serotonine	24	258	10	28	8
209.0	192	5.5	L -kynurenine	23	203	10	17	11
209.0	94	5.5	L -kynurenine	23	203	10	17	11
215.2	169	5.3	L -kynurenine D6	23	203	10	17	11
215.2	98	5.3	L -kynurenine D6	23	203	10	17	11
154.0	136	6.5	3-hydroxy-anthranilic-acid	15	140	10	15	8
154.0	80	6.5	3-hydroxy-anthranilic-acid	15	140	10	35	4
157.1	139	6.4	3-hydroxy-anthranilic-acid-D3	15	140	10	15	8
157.1	83	6.4	3-hydroxy-anthranilic-acid-D3	15	140	10	35	4
205.1	146	7.5	tryptophan	30	200	10	30	15
205.1	188	7.5	tryptophan	30	200	10	30	15
161.0	144	8.1	tryptamine	36	250	10	29	7
161.0	117	8.1	tryptamine	36	250	10	31	7
138.1	120	9.4	anthranilic-acid	15	100	10	16	7
138.1	92	9.4	anthranilic-acid	15	100	10	30	9
142.2	124	9.3	anthranilic-acid-D4	15	100	10	16	7
142.2	96	9.3	anthranilic-acid-D4	15	100	10	30	9
175.1	130	9.8	indol-3-acetamide	19	143	10	16	9
175.1	158	9.8	indol-3-acetamide	19	143	10	25	9
206.1	160	10.5	indol-3-lactic-acid	19	210	10	27	10
206.1	132	10.5	indol-3-lactic-acid	19	210	10	30	10

183.1	136	10.8	indol-3-acetic-acid D7	36	280	10	22	9
183.1	109	10.8	indol-3-acetic-acid D7	36	280	10	43	6
176.1	130	10.9	indol-3-acetic-acid	36	280	10	22	9
176.1	103	10.9	indol-3-acetic-acid	36	280	10	43	6
146.1	118	10.8	indole-3-carboxy-aldehyde	25	250	10	25	7
146.1	91	10.8	indole-3-carboxy-aldehyde	25	250	10	28	7
190.1	130	11.2	indol-3-propionic-acid	30	143	10	18	8
190.1	172	11.2	indol-3-propionic-acid	30	143	10	14	12
157.0	130	11.9	indol-3-acetonitrile	15	180	10	18	9
157.0	117	11.9	indol-3-acetonitrile	15	180	10	23	9

Table S5: List of chromatographic retention time (RT), selected MRM parameters, declustering potential (DP), focusing potential (FP), entrance potential (EP), collision energy (CE), cell exit potential (CXP) for each measured analyte, Related to STAR Methods

TIME (min)	FLOW (mL/min)	B (%)
0	0.300	0
11	0.300	40
13	0.300	95
21	0.300	95
22	0.300	0
33	0.300	0

Table S6: Chromatographic conditions, Related to STAR Methods