11th ADVANCES AGAINST
ASPERGILLOSIS AND MUCORMYCOSIS
25 - 27 JANUARY 2024
MILAN
HYBRID MEETING
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Fumagillin

Xabier Guruceaga, Ph.D.

Session 11

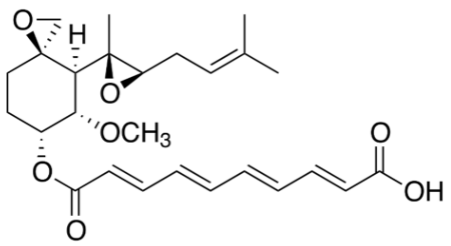
Quick Fire Summaries of *fumigatus* mycotoxins

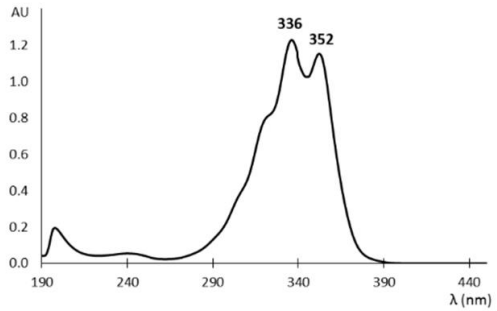
Milan, Italy

01/27/2024

FUMAGILLIN

What is fumagillin?





Is a bioactive molecule that is not required for *A. fumigatus* growth but helps the microorganism in its adaptation to different environmental conditions:

- Improves competitiveness against other microbes
- Improves competitiveness against immune responses

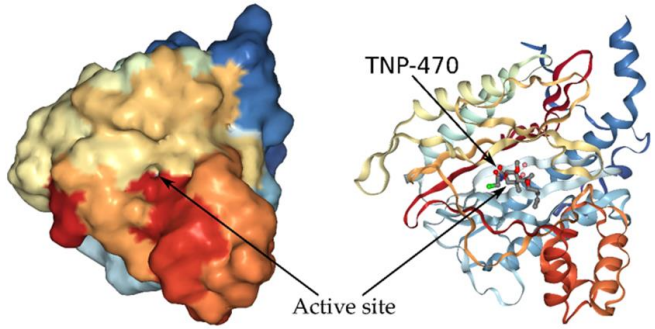
Bennet and Bentley 1989; Raffa and Keller 2019

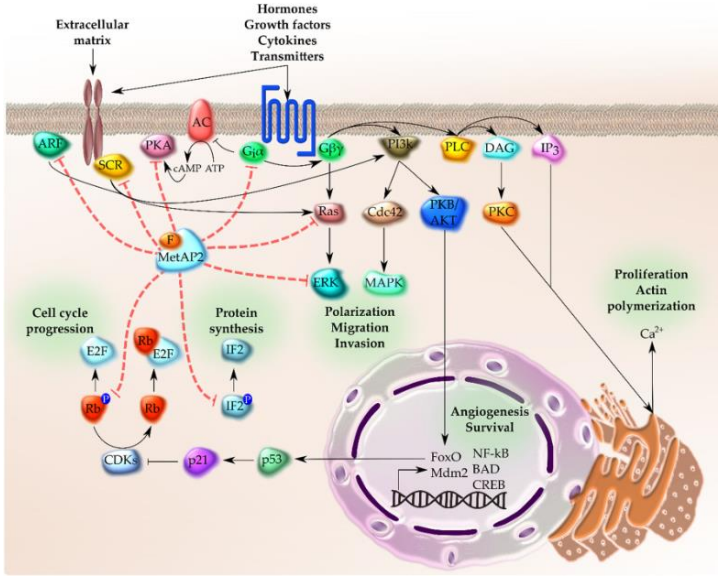
Small molecule — Detectable by UHPLC

Molecular weight 458.54 g/mol

Guruceaga *et al.*, 2020

How fumagillin fulfills its function?





Schematic diagram depicting MetAP2 implication in different pathways.

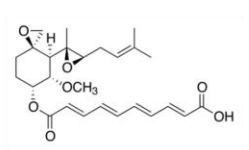
Fumagillin binds histidine 231 located on the active site of the Methionine aminopeptidase II (MetAP2) blocking the function of the protein

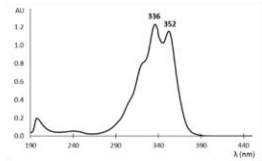
Impacts over the correct maintenance of cellular homeostasis, cell proliferation, cell cycle or new protein synthesis among others…

Guruceaga *et al.*, 2020

FUMAGILLIN APLICATIONS

What is fumagillin?





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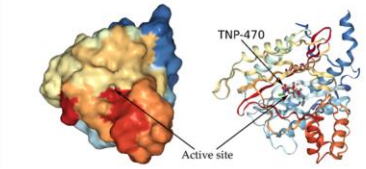
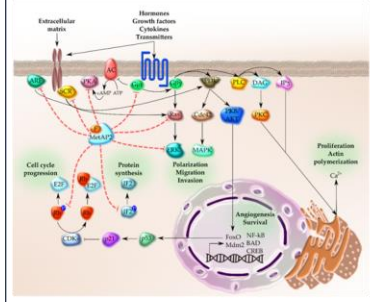


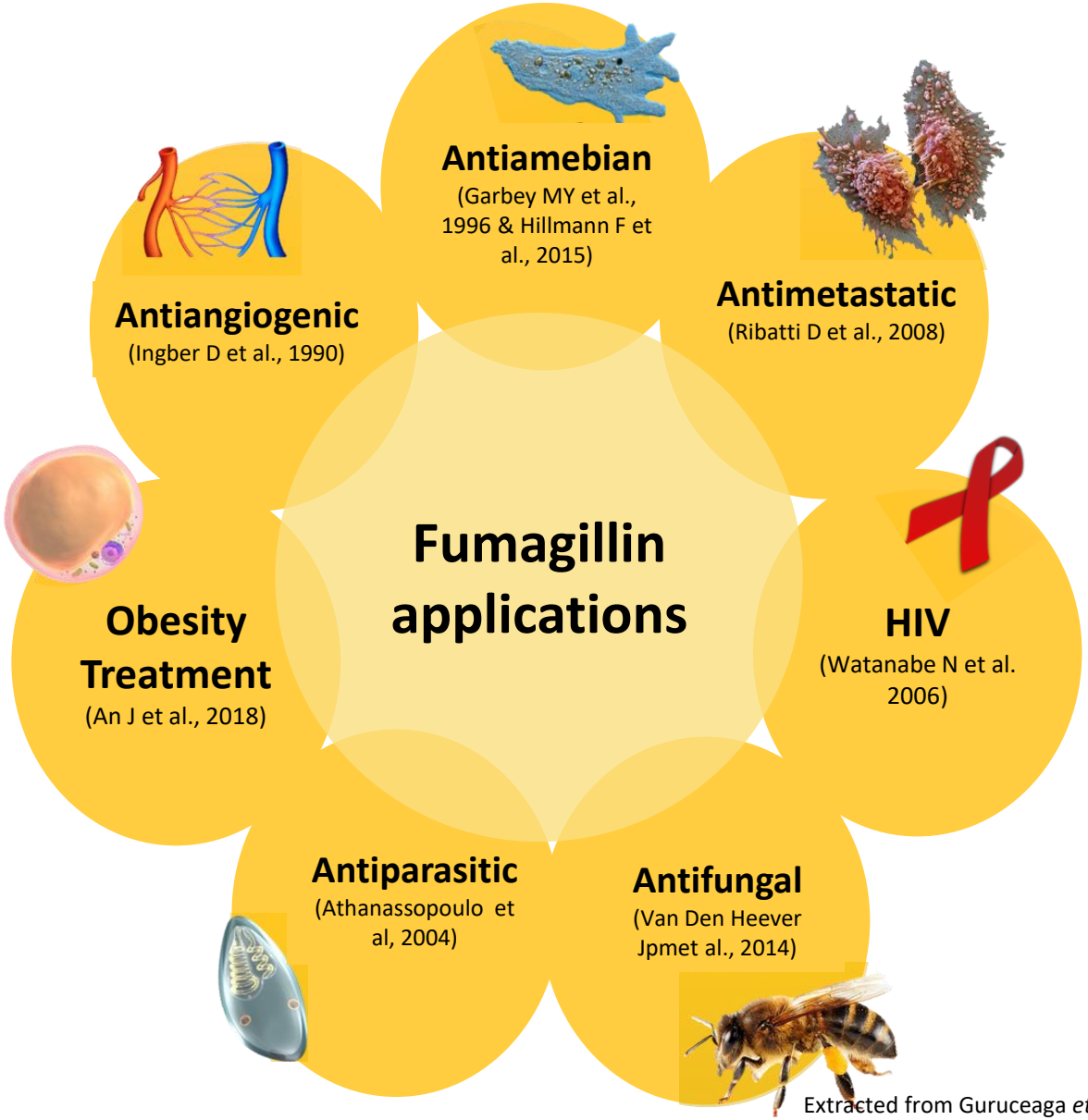


Fumagillin blocks Methionine aminopeptidase II (MetAP2) function

Blocks hydrolyzation of *iMet* of the new proteins

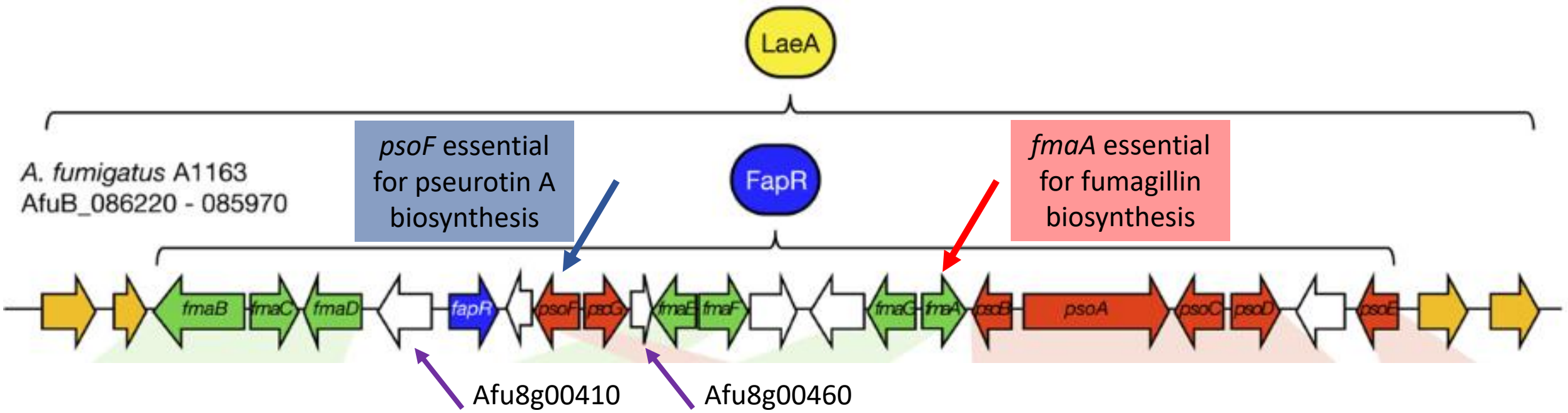
Impacts over the correct maintenance of cellular homeostasis

Extracted from Guruceaga *et al.*, 2020



Extracted from Guruceaga *et al.*, 2020

FUMAGILLIN BIOSYNTHETIC CLUSTER



The fumagillin biosynthetic gene cluster:

- Contains 21 genes
- From Afu8g00370 to Afu8g00520
- Named the *fma* cluster
- Contains genes for Fumagillin and Pseurotin A

Green arrows: Fumagillin biosynthesis

Red arrows: Pseurotin A biosynthesis

Orange arrows: Supercluster borders (LaeA but no FapR)

White arrows: Not involve in biosynthesis (LaeA and FapR)

Wiemann *et al.*, 2013

Dr. Aitor Rementeria

Microbiomics group in 2017

VIRULENCE
2018, VOL. 9, NO. 1, 1548–1561
https://doi.org/10.1080/21505594.2018.1526528

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RESEARCH PAPER

OPEN ACCESS Check for updates

A possible role for fumagillin in cellular damage during host infection by *Aspergillus fumigatus*

Xabier Guruceaga [a], Guillermo Ezpeleta [b,c], Emilio Mayayo[d], Monica Sueiro-Olivares[a], Ana Abad-Diaz-De-Cerio [a], José Manuel Aguirre Urízar [e], Hong G. Liu[f,g], Philipp Wiemann [h], Jin Woo Bok[h], Scott G. Filler [f,g], Nancy P. Keller [h,i], Fernando L. Hernando[a], Andoni Ramirez-Garcia [a], and Aitor Rementeria [a]

[a]Fungal and Bacterial Biomics Research Group, Department of Immunology, Microbiology and Parasitology, Faculty of Science and Technology, University of the Basque Country (UPV/EHU), Leioa, Spain; [b]Preventive Medicine and Hospital Hygiene Service, Complejo Hospitalario de Navarra, Pamplona, Spain; [c]Department of Preventive Medicine and Public Health, Faculty of Medicine and Nursing, University of the Basque Country (UPV/EHU), Leioa, Spain; [d]Pathology Unit, Medicine and Health Science Faculty, University of Rovira i Virgili, Reus, Tarragona, Spain; [e]Department of Stomatology II, Faculty of Medicine and Nursing, University of the Basque Country (UPV/EHU), Leioa, Spain; [f]Division of Infectious Diseases, Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, Torrance, CA, USA; [g]Department of Medicine, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA; [h]Department of Medical Microbiology and Immunology, University of Wisconsin, Madison, WI, USA; [i]Department of Bacteriology, University of Wisconsin, Madison, WI, USA

ABSTRACT

Virulence mechanisms of the pathogenic fungus *Aspergillus fumigatus* are multifactorial and depend on the immune state of the host, but little is known about the fungal mechanism that develops during the process of lung invasion. In this study, microarray technology was combined with a histopathology evaluation of infected lungs so that the invasion strategy followed by the fungus could be described. To achieve this, an intranasal mice infection was performed to extract daily fungal samples from the infected lungs over four days post-infection. The pathological study revealed a heavy fungal progression throughout the lung, reaching the blood vessels on the third day after exposure and causing tissue necrosis. One percent of the fungal genome followed a differential expression pattern during this process. Strikingly, most of the genes of the intertwined fumagillin/pseurotin biosynthetic gene cluster were upregulated as were genes encoding lytic enzymes such as lipases, proteases (DppIV, DppV, Asp f 1 or Asp f 5) and chitinase (chiB1) as well as three genes related with pyomelanin biosynthesis process. Furthermore, we demonstrate that fumagillin is produced in an *in vitro* pneumocyte cell line infection model and that loss of fumagillin synthesis reduces epithelial cell damage. These results suggest that fumagillin contributes to tissue damage during invasive aspergillosis. Therefore, it is probable that *A. fumigatus* progresses through the lungs via the production of the mycotoxin fumagillin combined with the secretion of lytic enzymes that allow fungal growth, angioinvasion and the disruption of the lung parenchymal structure.

ARTICLE HISTORY
Received 28 March 2018
Accepted 10 September 2018

KEYWORDS
Aspergillus; fumagillin; intranasal infection; AWAFUGE; epithelial cells; fumagillin; cytotoxicity; virulence

Journal of *Fungi*

MDPI

Article

Aspergillus fumigatus Fumagillin Contributes to Host Cell Damage

Xabier Guruceaga [1], Uxue Perez-Cuesta [1], Aize Pellon [2], Saioa Cendon-Sanchez [1], Eduardo Pelegri-Martinez [1], Oskar Gonzalez [3], Fernando Luis Hernando [1], Emilio Mayayo [4], Juan Anguita [2,5], Rosa M. Alonso [3], Nancy P. Keller [6,7], Andoni Ramirez-Garcia [1,*] and Aitor Rementeria [1,*]

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6 Department of Medical Microbiology and Immunology, University of Wisconsin, Madison, WI 53706, USA; npkeller@wisc.edu
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* Correspondence: andoni.ramirez@ehu.eus (A.R.-G.); aitor.rementeria@ehu.eus (A.R.)

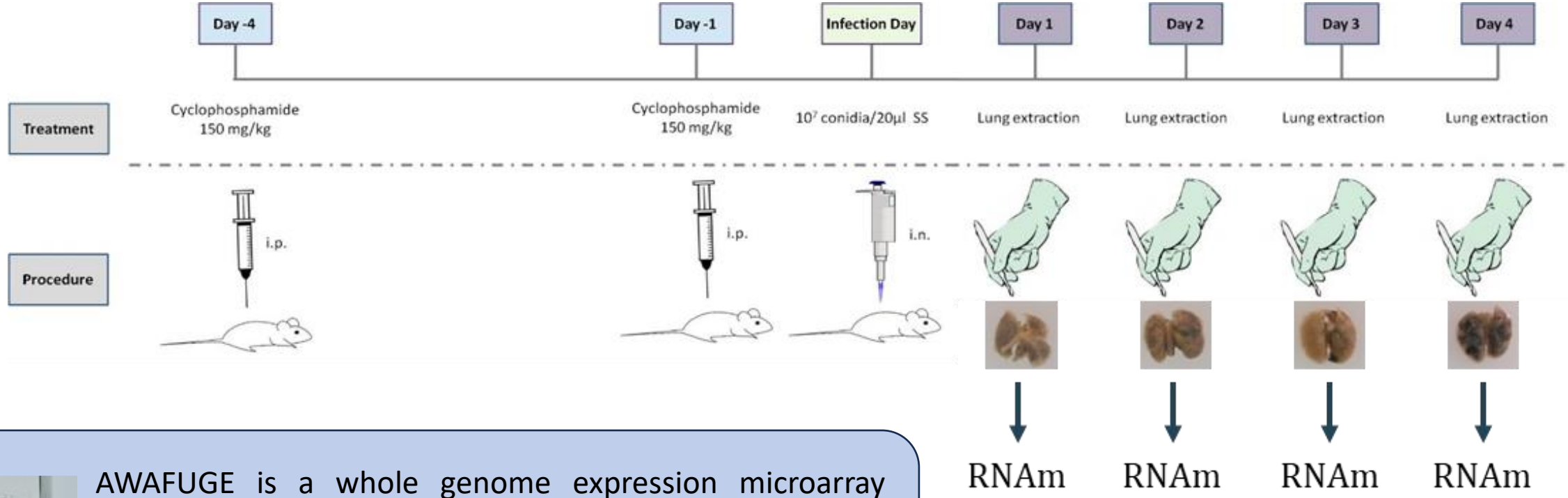
check for updates

Citation: Guruceaga, X.; Perez-Cuesta, U.; Pellon, A.; Cendon-Sanchez, S.; Pelegri-Martinez, E.; Gonzalez, O.; Hernando, F.L.; Mayayo, E.; Anguita, J.; Alonso, R.M.; et al. *Aspergillus fumigatus* Fumagillin Contributes to Host Cell Damage. *J. Fungi* **2021**, *7*, 936. https://doi.org/10.3390/jof7110936

Received: 7 September 2021
Accepted: 1 November 2021
Published: 3 November 2021

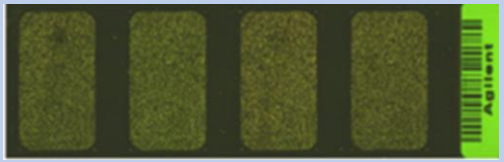
Abstract: The activity of fumagillin, a mycotoxin produced by *Aspergillus fumigatus*, has not been studied in depth. In this study, we used a commercial fumagillin on cultures of two cell types (A549 pneumocytes and RAW 264.7 macrophages). This toxin joins its target, MetAP2 protein, inside cells and, as a result, significantly reduces the electron chain activity, the migration, and the proliferation ability on the A549 cells, or affects the viability and proliferation ability of the RAW 264.7 macrophages. However, the toxin stimulates the germination and double branch hypha production of fungal cultures, pointing out an intrinsic resistant mechanism to fumagillin of fungal strains. In this study, we also used a fumagillin non-producer *A. fumigatus* strain (Δ*fmaA*) as well as its complemented strain (Δ*fmaA::fmaA*) and we tested the fumagillin secretion of the fungal strains using an Ultra High-Performance Liquid Chromatography (UHPLC) method. Furthermore, fumagillin seems to protect the fungus against phagocytosis in vitro, and during in vivo studies using infection of immunosuppressed mice, a lower fungal burden in the lungs of mice infected with the Δ*fmaA* mutant was demonstrated.

IN VIVO MODEL OF IA



AWAFUGE is a whole genome expression microarray developed by Aitor Rementeria's group designed to study *A. fumigatus* transcriptome during infections:

- Mice infections (Guruceaga et al., 2018)
- Cell culture infections (Guruceaga et al., 2024)



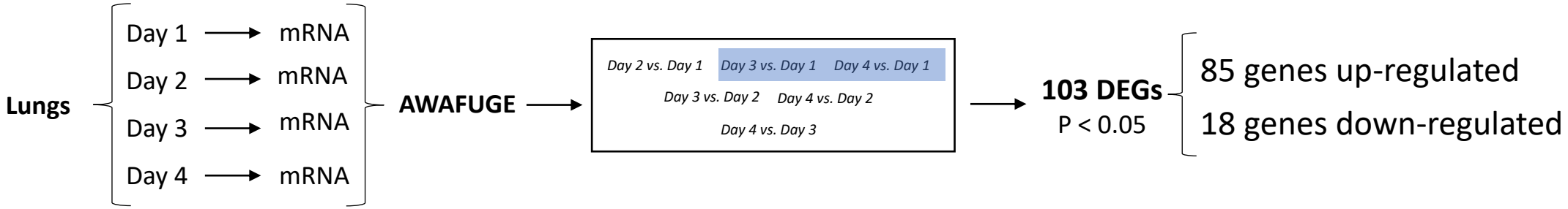
- 28.890 *A. fumigatus* probes (3/gene)
- 16.330 control probes

(Human, Mouse, *A.fumigatus*, Positive and Negative)

AWAFUGE

Guruceaga *et al.*, 2018

A. FUMIGATUS GENES DIFFERENTIALLY EXPRESSED



			Fold Change			
			Day3 vs Day1		Day4 vs Day1	
Product description	**Systematic Name**	**Standard Name**	**AWAFUGE**	**RT-qPCR**	**AWAFUGE**	**RT-qPCR**
Fumagillin/Pseurotin pathway						
Polyketide synthase	Afu8g00370	*fma-PKS / fmaB*		6.28	5.66	7.10
O-methyltransferase	Afu8g00390	*fmaD*	6.37	7.97	5.71	8.46
Hypothetical protein	Afu8g00400		7.8	15.90	6.84	16.23
Methionine aminopeptidase. type II	Afu8g00410	*metAP / fpaII*	4.53	5.03	4.09	4.88
C6 finger transcription factor	Afu8g00420	*fumR / fapR*	4.28	5.48		5.52
Hypothetical protein	Afu8g00430		7.21	9.52	6.97	9.61
Steroid monooxygenase	Afu8g00440	*psoF*	5.22	8.63	4.89	9.05
Phytanoyl-CoA dioxygenase family protein	Afu8g00480	*fmaF*	4.63	11.24	4.65	11.56
Acetate-CoA ligase	Afu8g00500		5.03	9.95	4.68	10.36
Cytochrome P450 oxidoreductase OrdA-like	Afu8g00510	*fmaG*	5.87	11.07	5.02	10.92
α/β hydrolase	Afu8g00530	*psoB*	5.41	8.30	5.29	8.47
Methyltransferase	Afu8g00550	*psoC*	7.09	10.12	6.90	10.70
Cytochrome P450 oxidoreductase	Afu8g00560		4.61	ND	4.46	ND
Glutathione S-transferase like	Afu8g00580	*elfB / psoE*	5.06	9.48	4.96	9.87

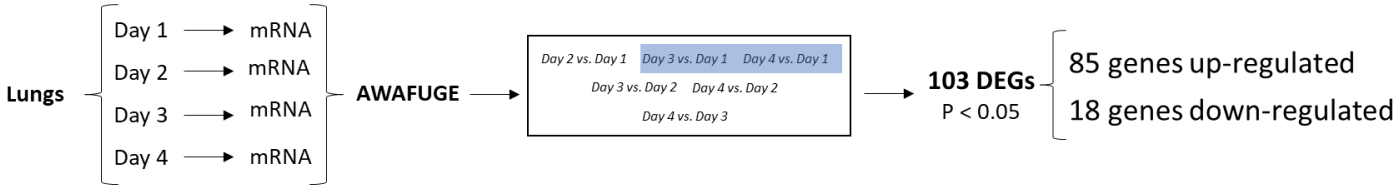
***Cluster* 21 genes**

67 %

14 DEGs up-regulated

Guruceaga *et al.*, 2018

A. FUMIGATUS GENES DIFFERENTIALLY EXPRESSED



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Hypothetical protein	Afu8g00430		7.21	9.52	6.97	9.61
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Phytanoyl-CoA dioxygenase family protein	Afu8g00480	*fmaF*	4.63	11.24	4.65	11.56
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α/β hydrolase	Afu8g00530	*psoB*	5.41	8.30	5.29	8.47
Methyltransferase	Afu8g00550	*psoC*	7.09	10.12	6.90	10.70
Cytochrome P450 oxidoreductase	Afu8g00560		4.61	ND	4.46	ND
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***Cluster* 21 genes**
67 %
14 DEGs
up-regulated

Conclusions:

1. Clear up-regulation of the fumagillin cluster
2. The first study to propose fumagillin as an important virulence factor during early stages of infection

Guruceaga *et al.*, 2018

What is the biological significance of the overexpression?
Can *A. fumigatus* synthesize the toxin *in vitro*?
Does *A. fumigatus* derive benefits from producing the toxin?

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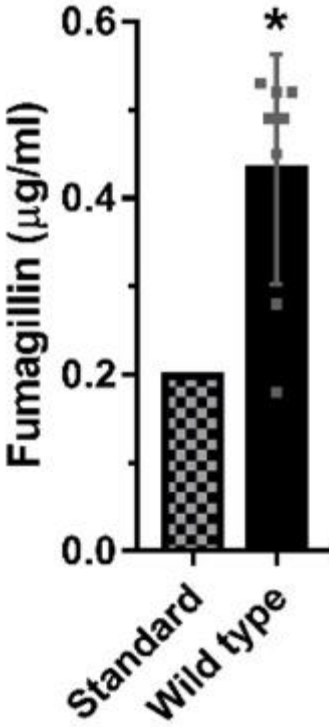
Microchemical Journal

journal homepage: www.elsevier.com/locate/microc

A novel SPE-UHPLC-DAD method for the determination of fumagillin produced by *Aspergillus fumigatus* in cell culture media

Oskar González [a,*], Ane Yaldebere [a], Xabier Guruceaga [b], Andoni Ramírez-García [b], Aitor Rementeria [b], Rosa María Alonso [a]

[a] FARMARTEM Group, Department of Analytical Chemistry, Faculty of Science and Technology, University of the Basque Country (UPV/EHU), Barrio Sarriena s/n, 48940 Leioa, Spain
[b] Fungal and Bacterial Biomics Research Group, Department of Immunology, Microbiology and Parasitology, Faculty of Science and Technology, University of the Basque Country (UPV/EHU), Barrio Sarriena s/n, 48940 Leioa, Spain



1. We standardized a UHPLC method to detect fumagillin.
2. We studied the ability of the Wt strain to produce fumagillin *in vitro* (0.5 µg/ml)
3. Set of in vitro assays using A549 (pneumocytes) and RAW 264.7 (macrophages)
    - Treated with 0.5, 1 and 2 µg/ml of commercial fumagillin
4. *In vivo* assays using the Δ*fmaA* strain (no fumagillin)

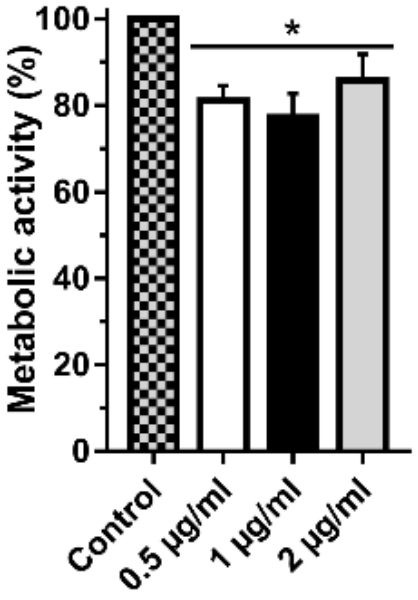
5 x 10^6 conidia incubated in RPMI for 24 hours at 37˚C, 5% CO_2 and 95% humidity

Gonzalez et al., 2021, Guruceaga *et al.,* 2021

FUMAGILLIN EFFECT OVER DIFFERENT CELL TYPES (A549 & RAW264.7)

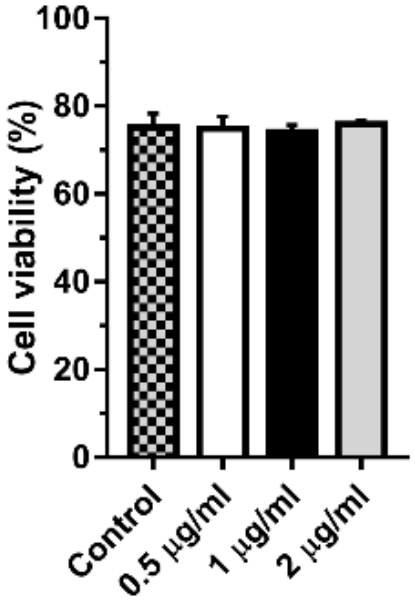
A549

MTT assay



Significant reduction of the electron transporter chain activity (ETC)

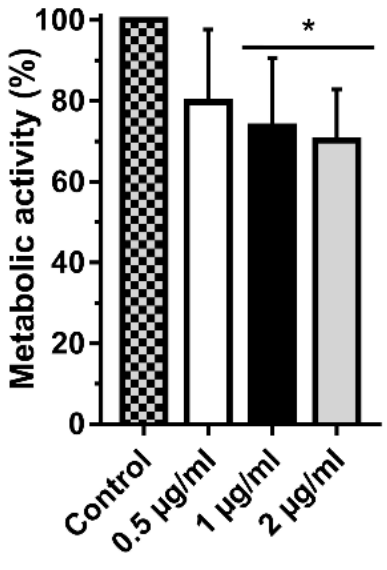
LDH assay



No affect cell viability

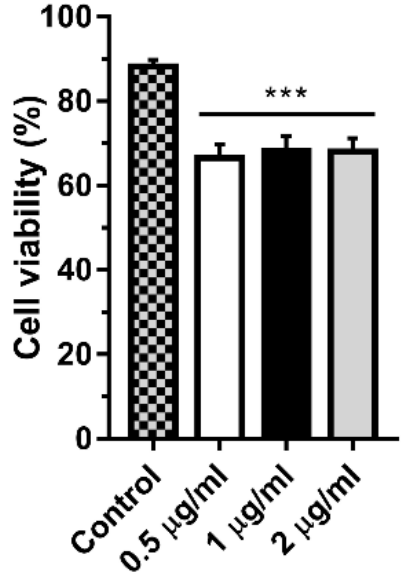
RAW 264.7

MTT assay



Significant reduction ETC (1 µg/ml y 2 µg/ml)

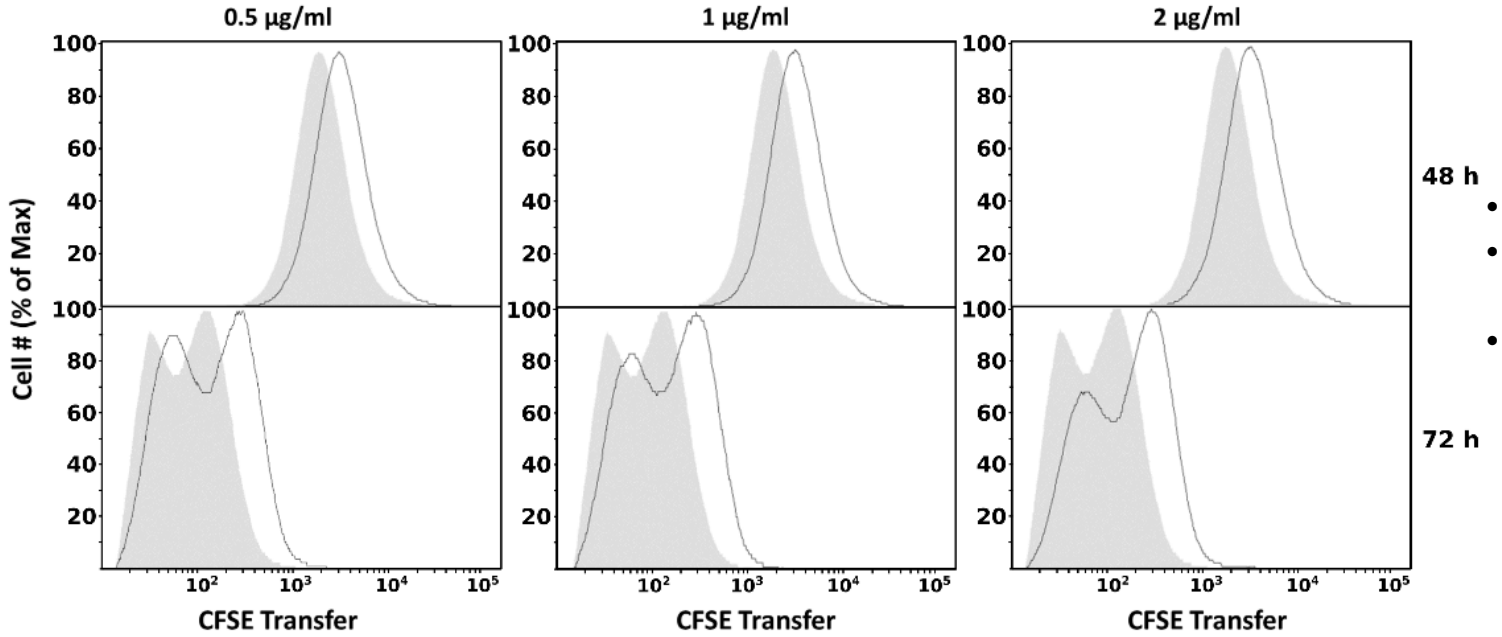
LDH assay



Significant reduction cell viability

Guruceaga *et al.*, 2021

DOES FUMAGILLIN AFFECT A549 PROLIFERATION?

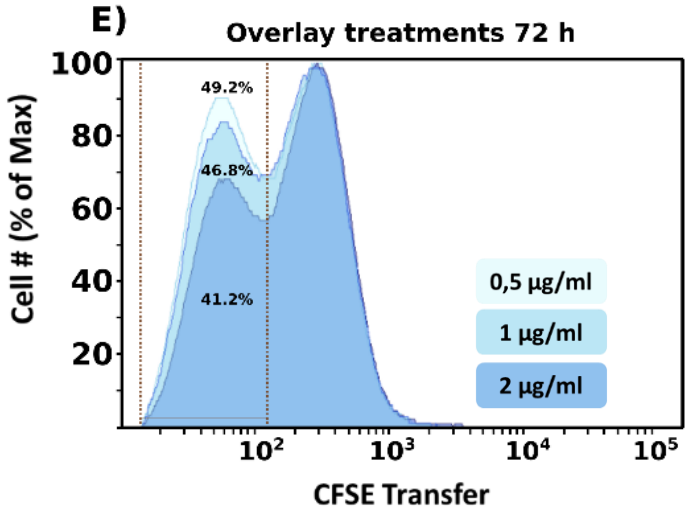


No Drug

Drug

CFSE proliferation assay

- Penetrates cell nuclei
- After every cell division, the compound's intensity is reduced by half
- This technique allows to study cell proliferation



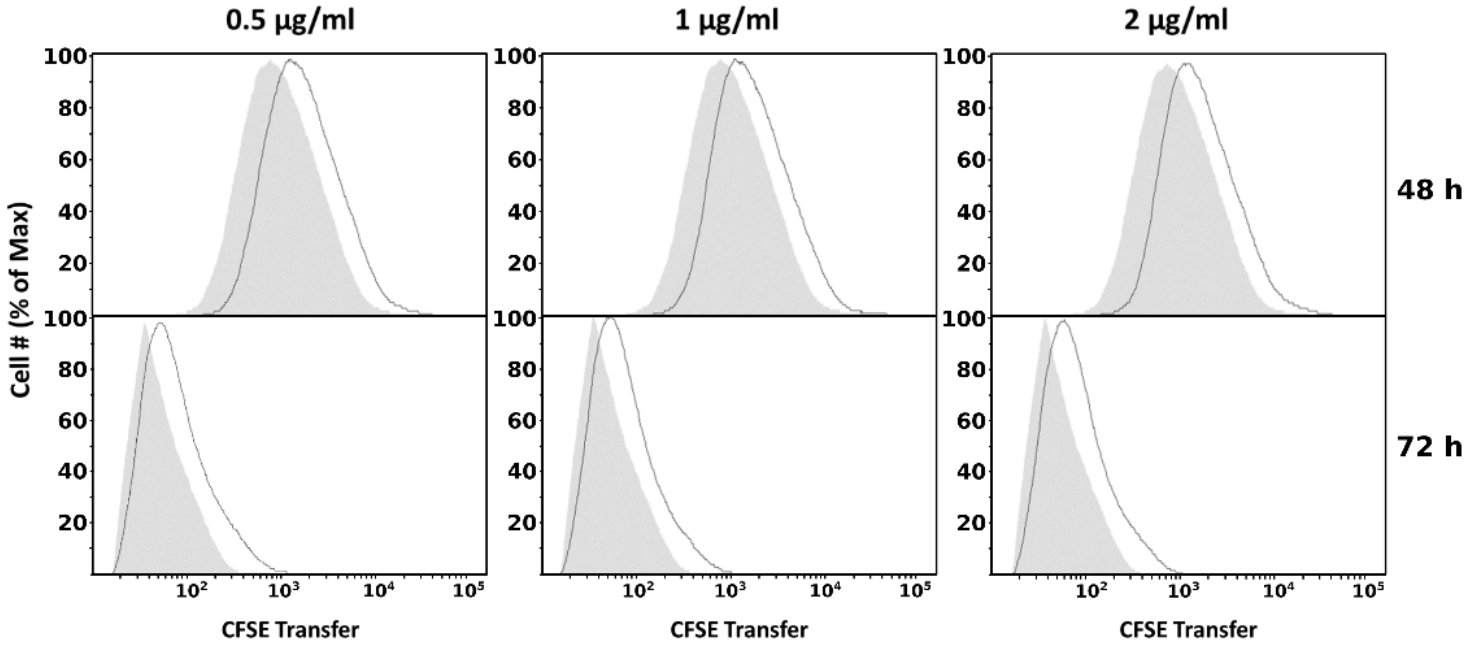
Cell proliferation inhibition

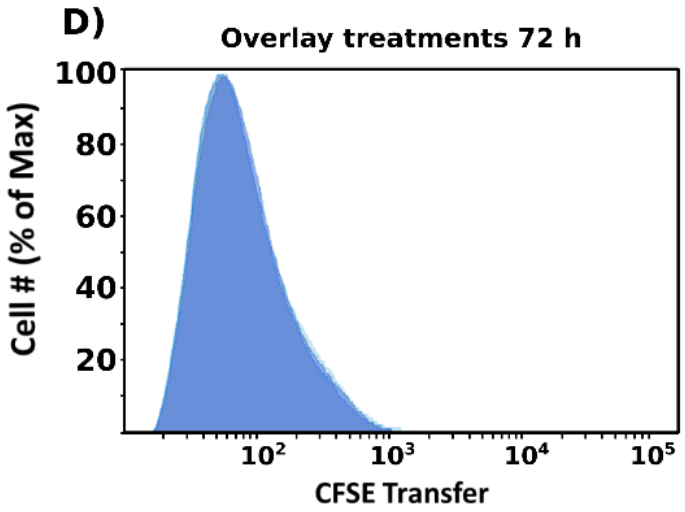
Dose – Inhibition relationship

Further on the left the peak is
↓
More cell proliferation
↓
Older cell population

Guruceaga *et al.*, 2021

DOES FUMAGILLIN AFFECT RAW264.7 PROLIFERATION?





CFSE proliferation assay

- Penetrates cell nuclei
- After every cell division, the compound's intensity is reduced by half
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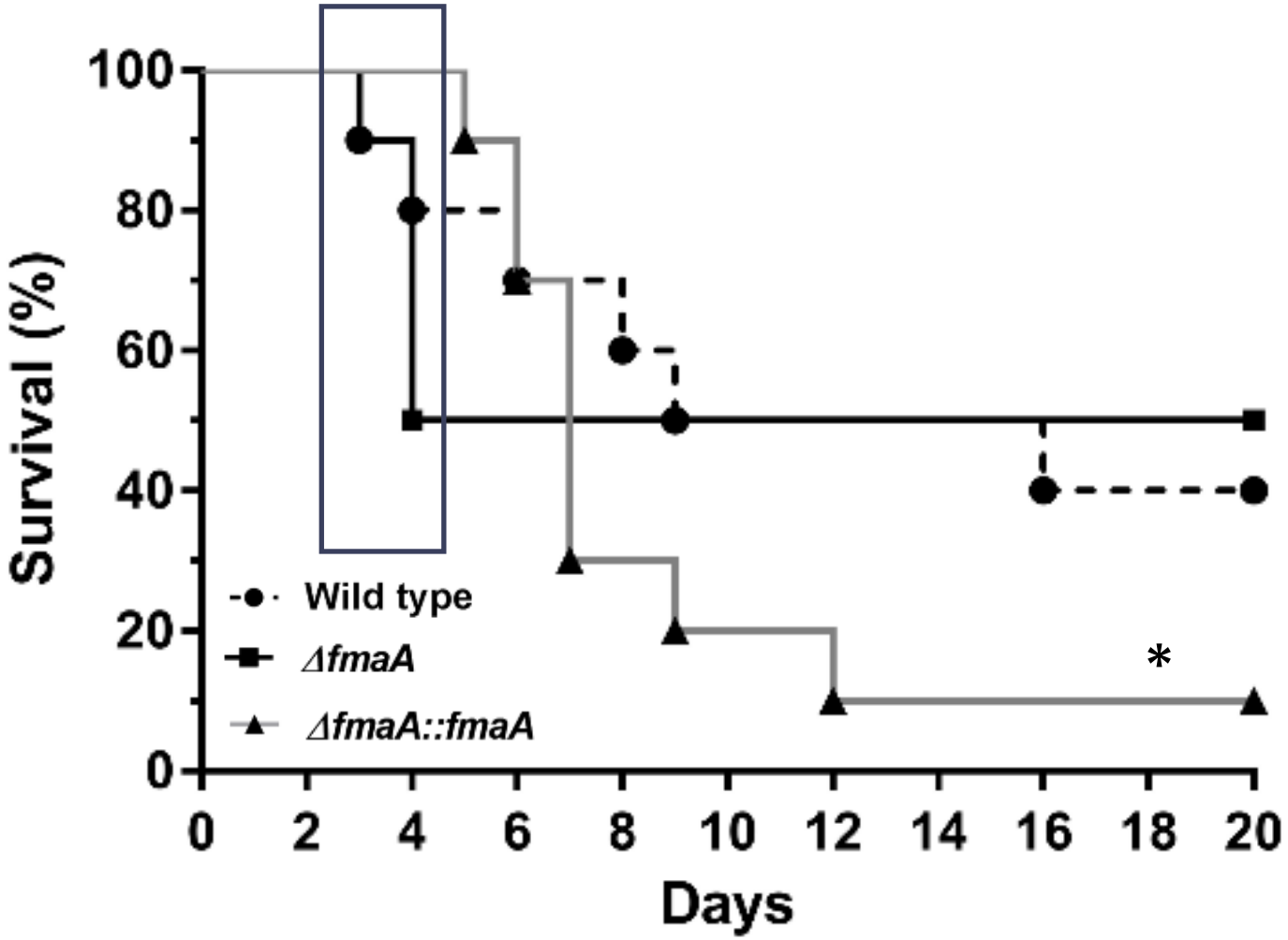
Cell proliferation inhibition

Guruceaga *et al.*, 2021

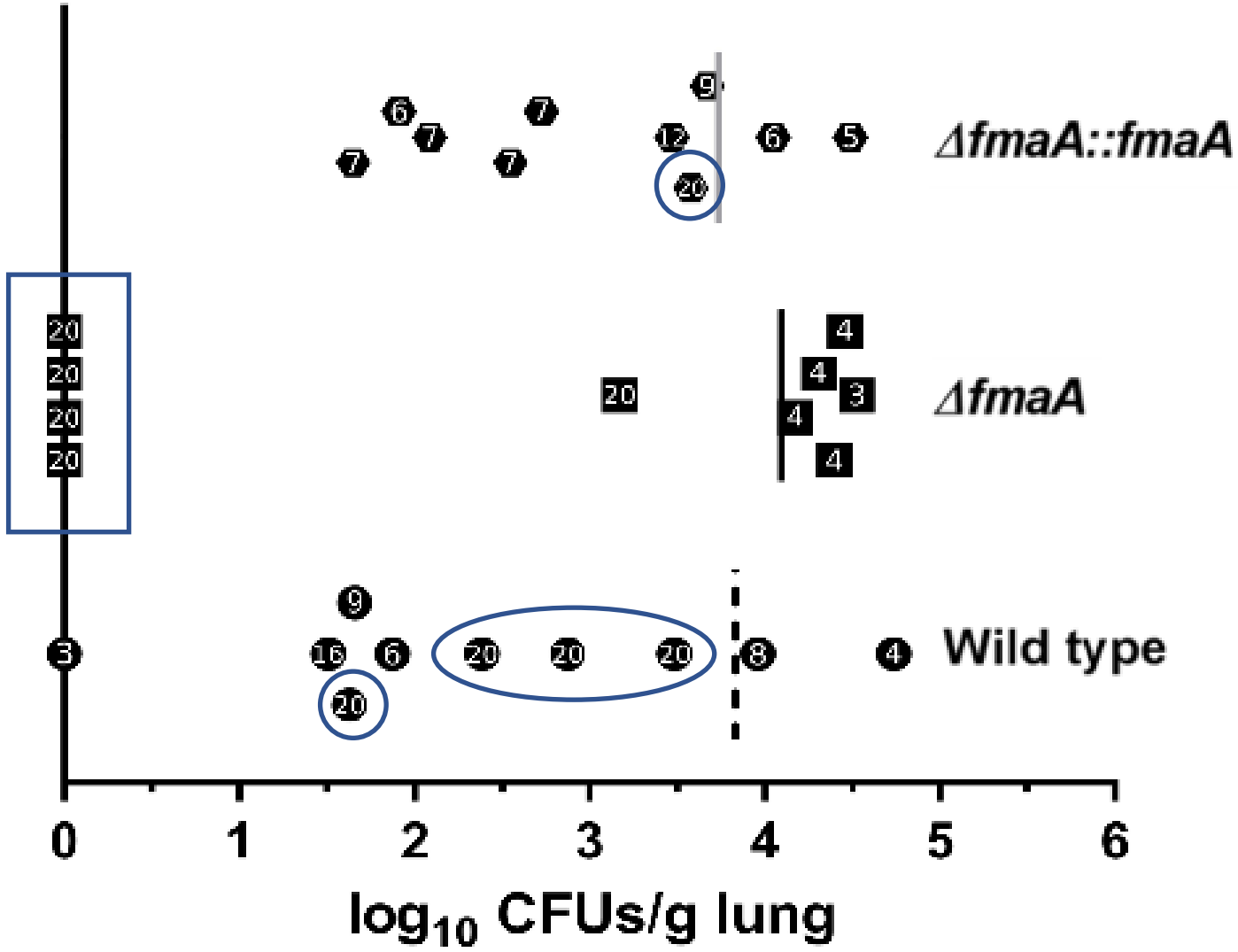
LACK OF FUMAGILLIN NOT IMPACT OVER *A. FUMIGATUS* VIRULENCE ABILITY



All mortality caused by *ΔfmaA* strain was during the first 4 days post-infection.

Guruceaga *et al.*, 2021

LACK OF FUMAGILLIN NOT IMPACT OVER *A. FUMIGATUS* VIRULENCE ABILITY

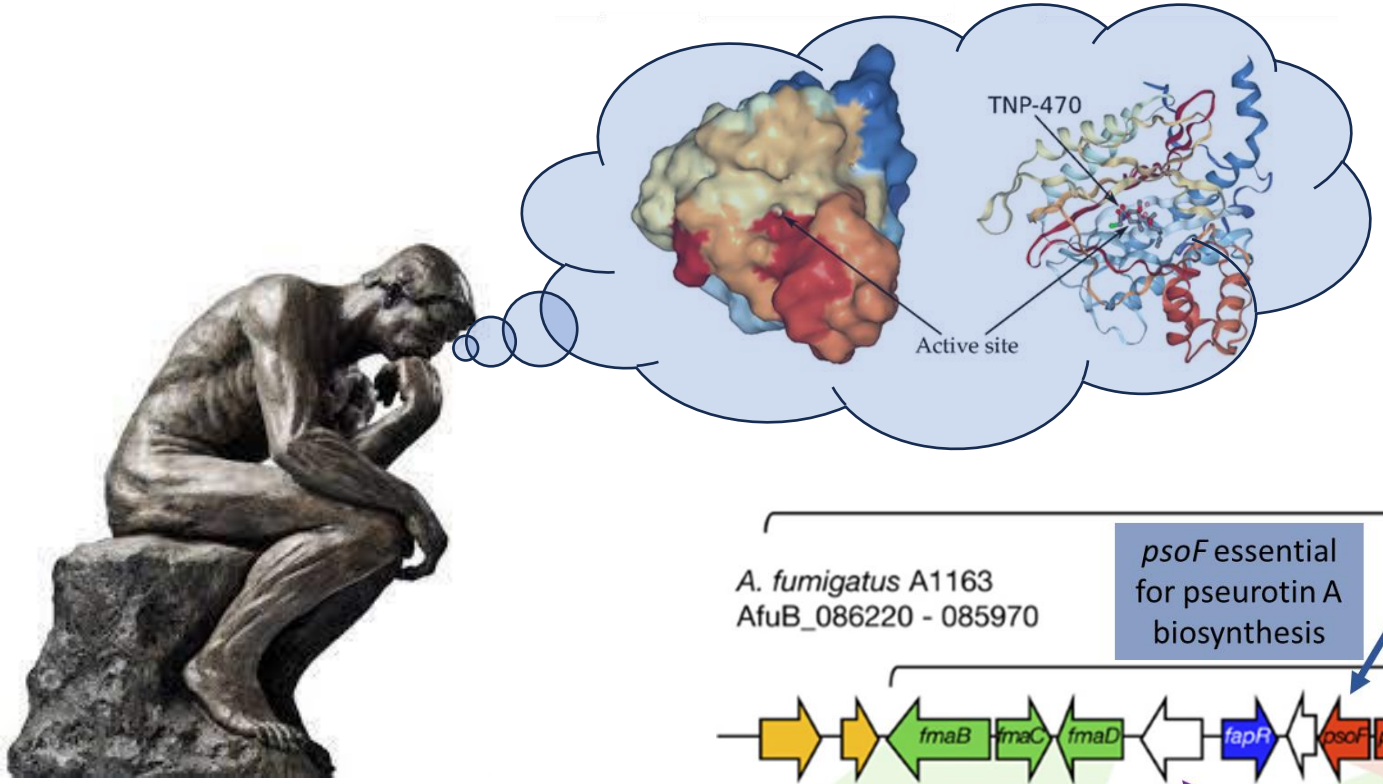


- Fungal burden analysis of all the lungs at final point
- Numbers mean the day each mouse died

Most of mice infected with the Δ*fmaA* strain survive until the end of the experiment without infection

Guruceaga *et al.*, 2021

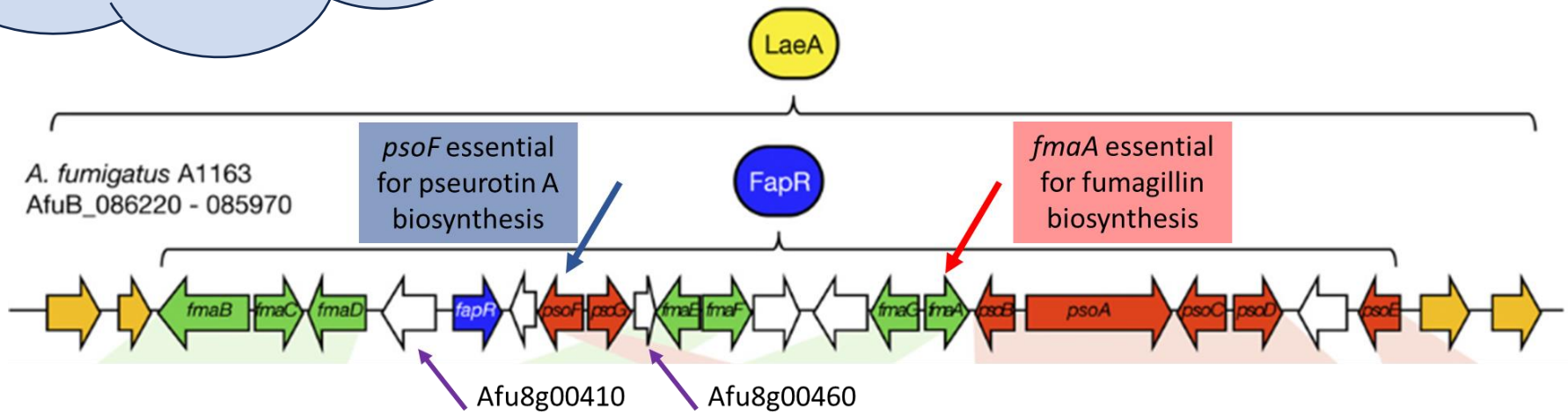
HOW A. FUMIGATUS RESISTS AGAINST ITS OWN FUMAGILLIN?



Le Penseur (Auguste Rodin)

Genomic redundancy

- More than 1 MetAP in the genome



Extra MetAPs not involved in mycotoxin biosynthesis but under FapR and LaeA control

The fumagillin biosynthetic gene cluster:

- Contains 21 genes
- From Afu8g00370 to Afu8g00520
- Named the *fma* cluster
- Contains genes for Fumagillin and Pseurotin A

Green arrows: Fumagillin biosynthesis

Red arrows: Pseurotin A biosynthesis

Orange arrows: Supercluster borders (LaeA but no FapR)

White arrows: Not involve in biosynthesis (LaeA and FapR)

Wiemann *et al.*, 2013

HOW A. FUMIGATUS RESISTS AGAINST ITS OWN FUMAGILLIN?

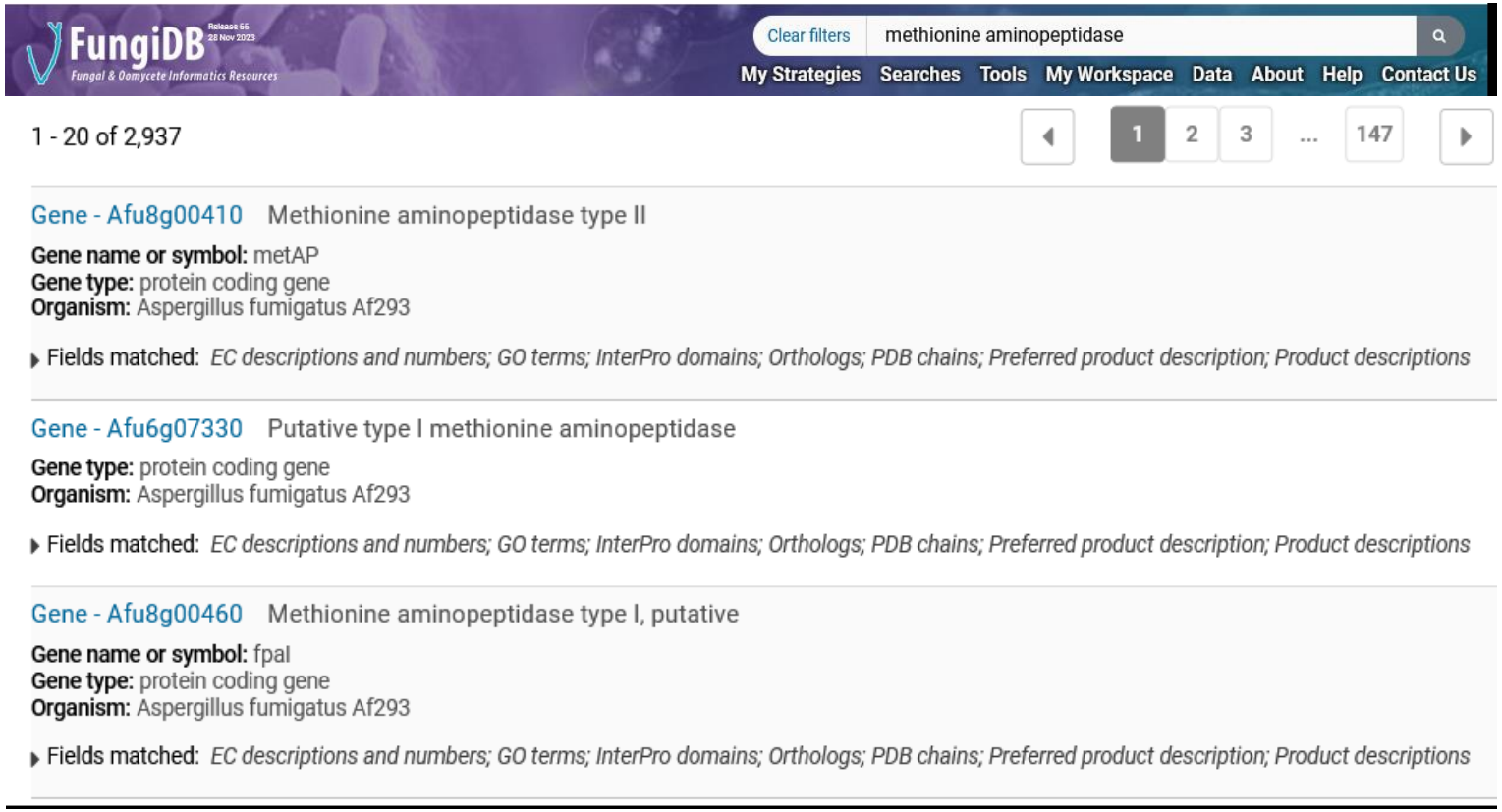
Saccharomyces cerevisiae

- 1 MetAP1 (*map1*) → Δ*map1* strain is sensible to fumagillin
- 1 MetAP2 (*map2*) → Δ*map2* strain is resistant to fumagillin

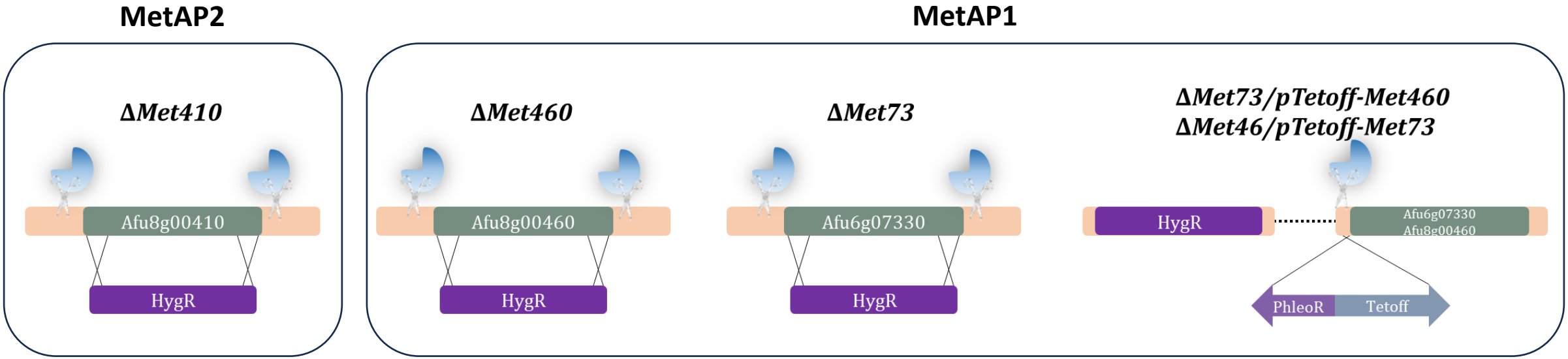
Sin *et al.*, 1997

Aspergillus fumigatus

- MetAP1 → 2 genes described → Afu6g07330, Afu8g00460
- MetAP2 → 1 gene described → Afu8g00410



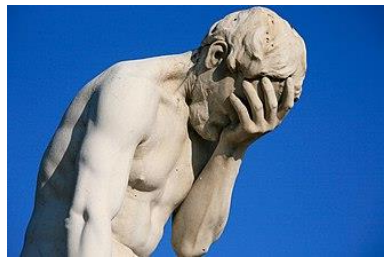
HOW A. FUMIGATUS RESISTS AGAINST ITS OWN FUMAGILLIN?



Should be resistant to fumagillin (No Target)

Should be resistant to fumagillin (Remain MetAP1 can compensate Inhibition MetAP2)

Should be susceptible to fumagillin (No MetAP1 to compensate inhibition of MetAP2)



Strain	Target silenced	MIC (Fumagillin)
Δ*akuB*^{ku80}		100 µg/ml
Δ*Met410*	MetAP2	100 µg/ml
Δ*Met460*	MetAP1 (1 de 2)	100 µg/ml
Δ*Met73*	MetAP1 (1 de 2)	100 µg/ml
Δ*Met73/pTetoff-Met460*	MetAP1 (2 de 2)	100 µg/ml
Δ*Met46/pTetoff-Met73*	MetAP1 (2 de 2)	100 µg/ml

Unpublished data

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Saioa Cendon-Sanchez **(PhD student)**

Candida spp. & Lomentospora spp.

Fernando L. Hernando **(PI)**

Andoni Ramirez-Garcia **(PI)**

Leire Martin-Souto **(PhD)**

Aitziber Antoran **(PhD)**

Idoia Buldain **(PhD)**

Leire Aparicio **(PhD student)**

Maialen Areitio **(PhD student)**

Former members

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MICRO BIOMICS EHU

eman ta zabal zazu

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Thank you!!

"Born to lose, live to win"
-Lemmy Kilmister-