What do we know about the role of gliotoxin in pathobiology of *Aspergillus*

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NIAID/NIH

Fungal secondary metabolites

Non-ribosomal peptides (NRPs)

β-lactam antibiotics (e.g. penicillin)

Cyclosporin- immunosuppressant

Echinocandin- antifungal drug

Gliotoxin **(Epipolythiodioxopiperazin, ETP)**

Polyketides (PKS)

Lovastatin- cholesterol lowering agent

Aflatoxin

Indole alkaloids

Ergotamine- migraine treatment

- Control of post partum bleeding

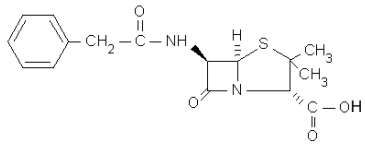
Terpenes

Trichodiene- toxin

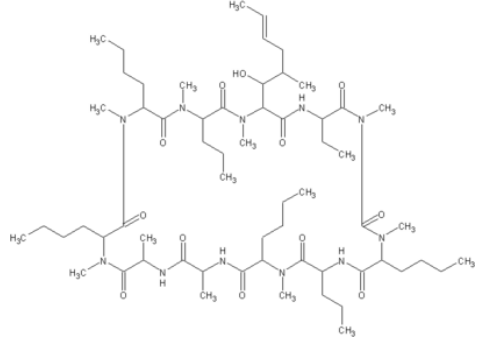
Aristolochene- toxin

A. fumigatus

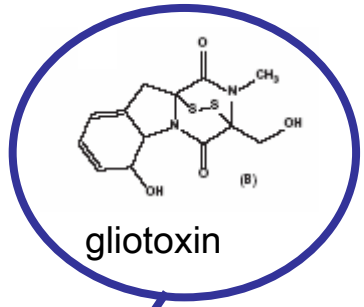
Peptides



penicillin G

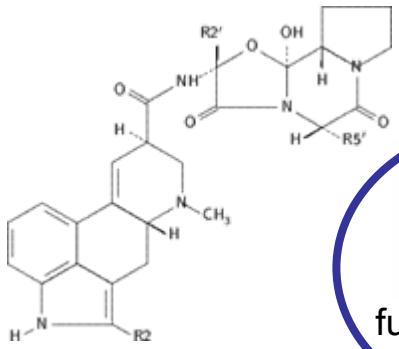


cyclosporin

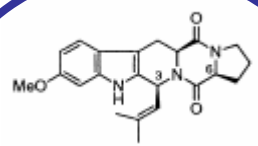


gliotoxin

Alkaloids

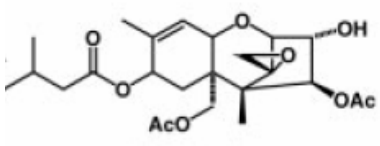


ergopeptides

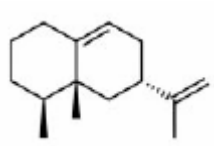


fumitremorgin C

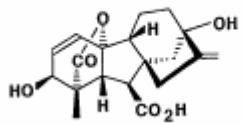
Terpenes



trichothecene T2 toxin

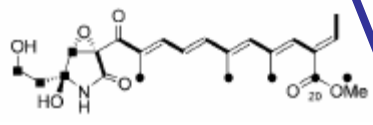


aristolochene

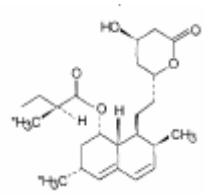


gibberellin GA3

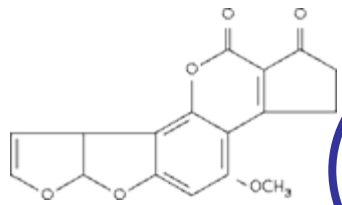
Polyketides



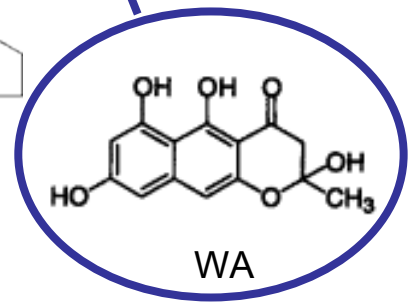
fusarin C



lovastatin



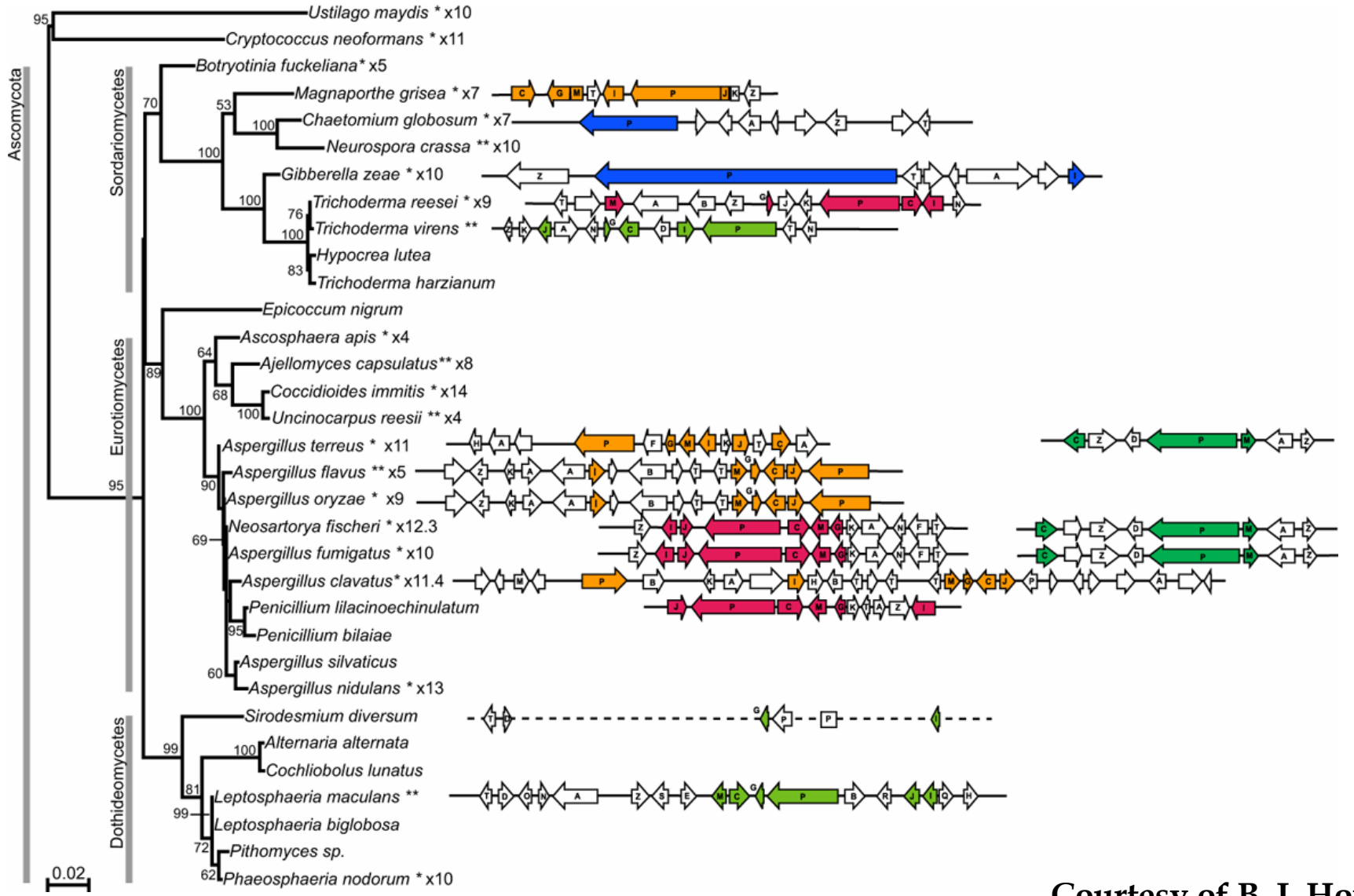
aflatoxin B1



WA

Modified from Geoffrey Turner

ETP producing fungal phylogeny based on 18S rDNA sequences

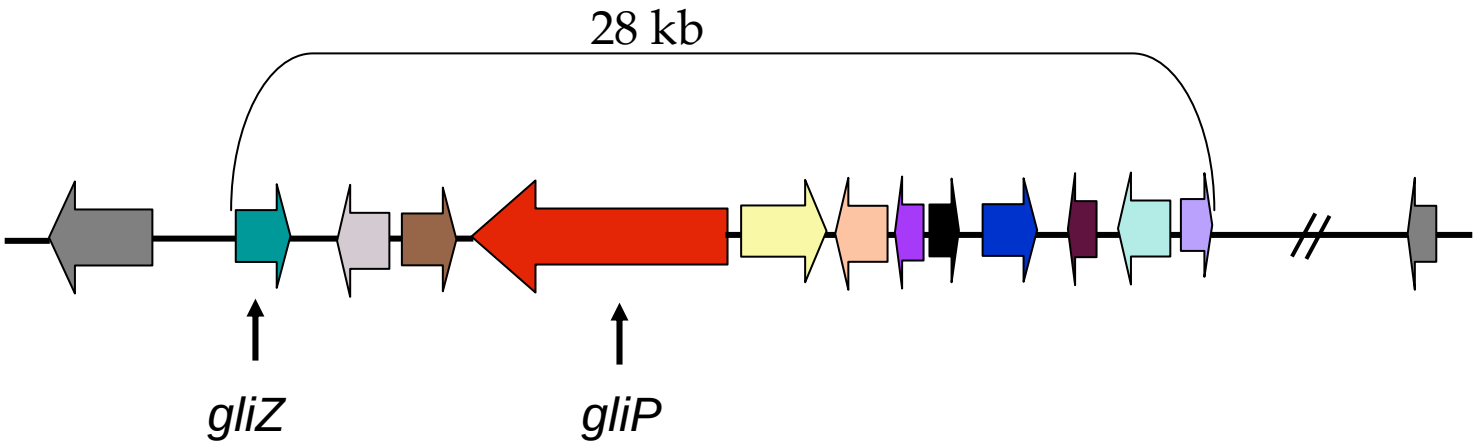


Courtesy of B. J. Howlett

Why is gliotoxin the best candidate-toxin for the study of pathogenic importance ?

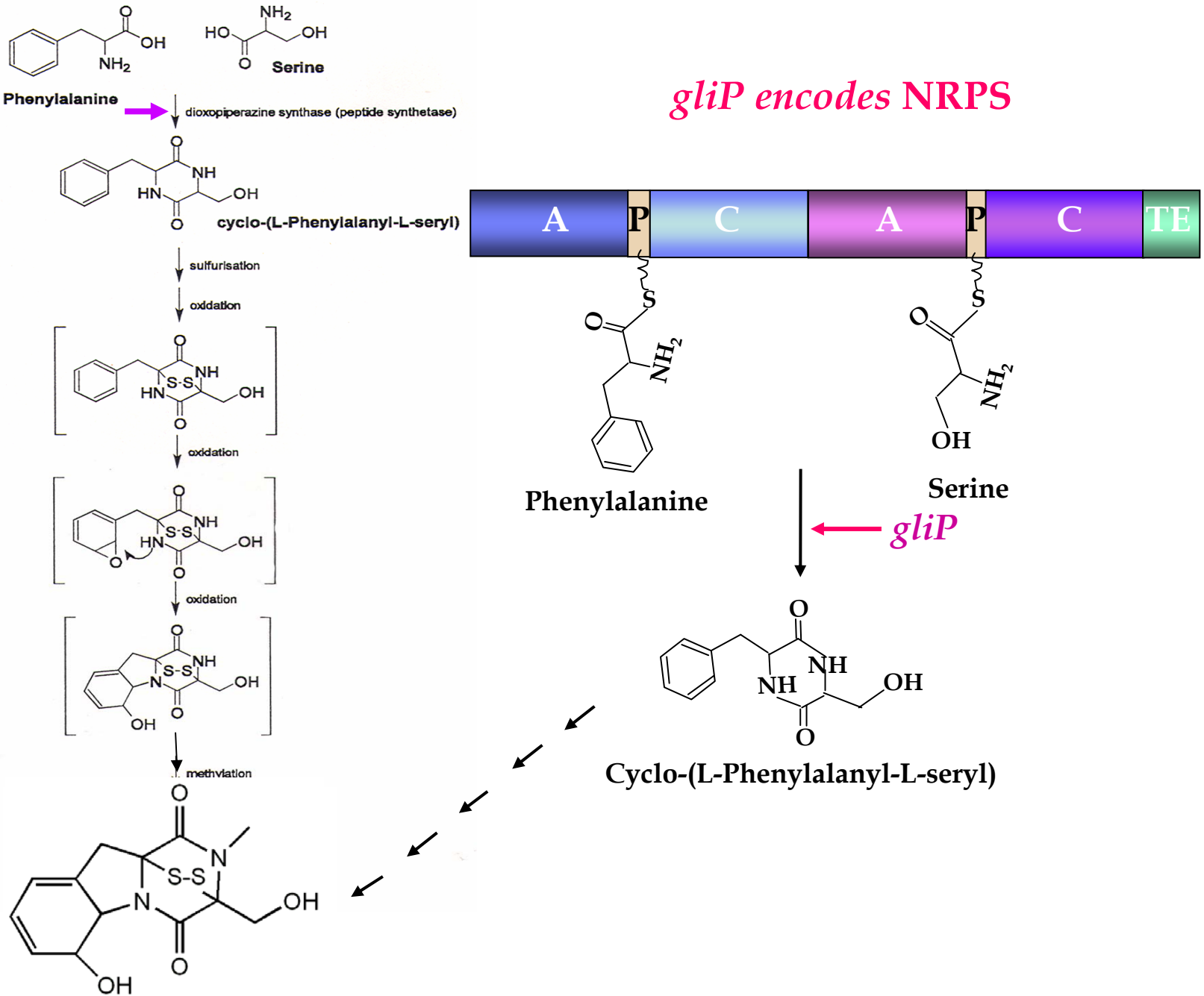
- **The most abundant as well as most potent toxin of all the secondary metabolites produced by *A. fumigatus***
- **Gliotoxin has immunosuppressivie properties and is detected in the serum and lung tissues in experimental and human invasive aspergillosis**
- **Ex-vivo experiments with mammalian cells (immune as well as non-immune cell lines) showed GT causes both apoptosis and necrotic cell death**
- **Gliotoxin inhibits assembly of the human respiratory burst NADPH oxidase**
- **Direct activation of the proapoptotic Bak followed by ROS generation**
- **It is known to exacerbate invasive aspergillosis in animals**

Genomic organization of the 12 *gli* gene cluster responsible for gliotoxin biosynthesis in *A. fumigatus*



- Zinc Finger transcription factor
- Amin. Cycloprop.Carboxyl. Synth
- Dipeptidase
- NRPS
- Cy. P450 monooxygenase
- Methyl transferases
- Glutathione S-transferase
- Transporter
- Hypo. ?
- Thioredoxin reductase

Putative Gliotoxin biosynthetic pathway



The 12 gene cluster is responsible for the synthesis of gliotoxin (ETP)

- **Bioinformatic approach allowed the discovery of a 12-gene cluster homologous to the gene cluster responsible for synthesis of sirodesmin (ETP) in *L. maculans***
- **Disruption of *gliZ* or *gliP* abolished the production of gliotoxin which recovered upon reconstitution of the mutants**
- **Over expression of *gliZ* resulted in higher production of gliotoxin**
- **Deletion of *gliZ* resulted in loss of gene expression of other *gli* cluster genes**

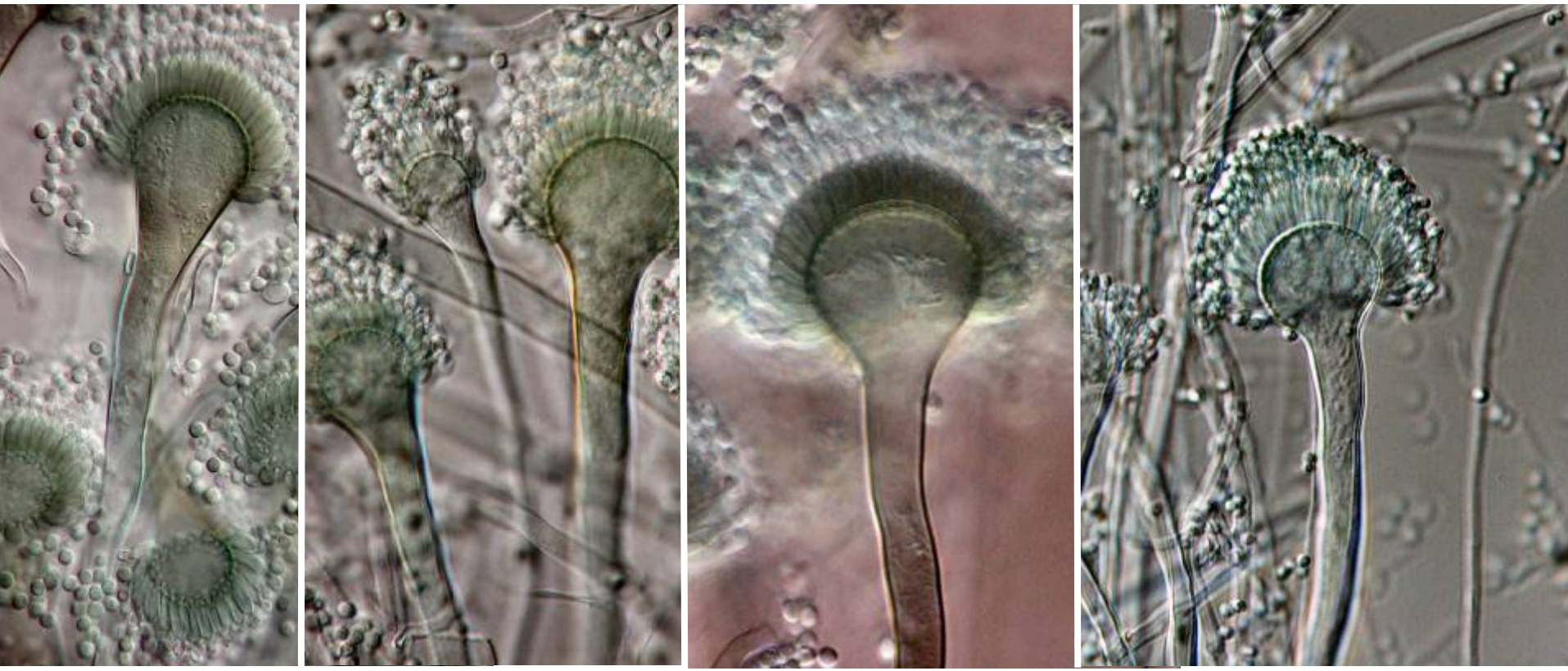
A. fumigatus strains (WT) used for functional studies of the genes involved in gliotoxin biosynthesis

Af 293 : Clinical isolate, Genomic strain, *MAT-2*

B-5233 : Clinical isolate from a case of fatal aspergillosis, *MAT-1*

CEA 10 : Clinical isolate from a case of aspergillosis, *MAT-1*

Gliotoxin production in *A. fumigatus* but not in morphologically similar species *A. lentulus, A. novofumigatus* and *A. fumigatiaffinis*



fumigatus *lentulus* *novofumigatus* *fumigatiaffinis*

Gliotoxin + **Gliotoxin -**

Coutesy of Samson, R.

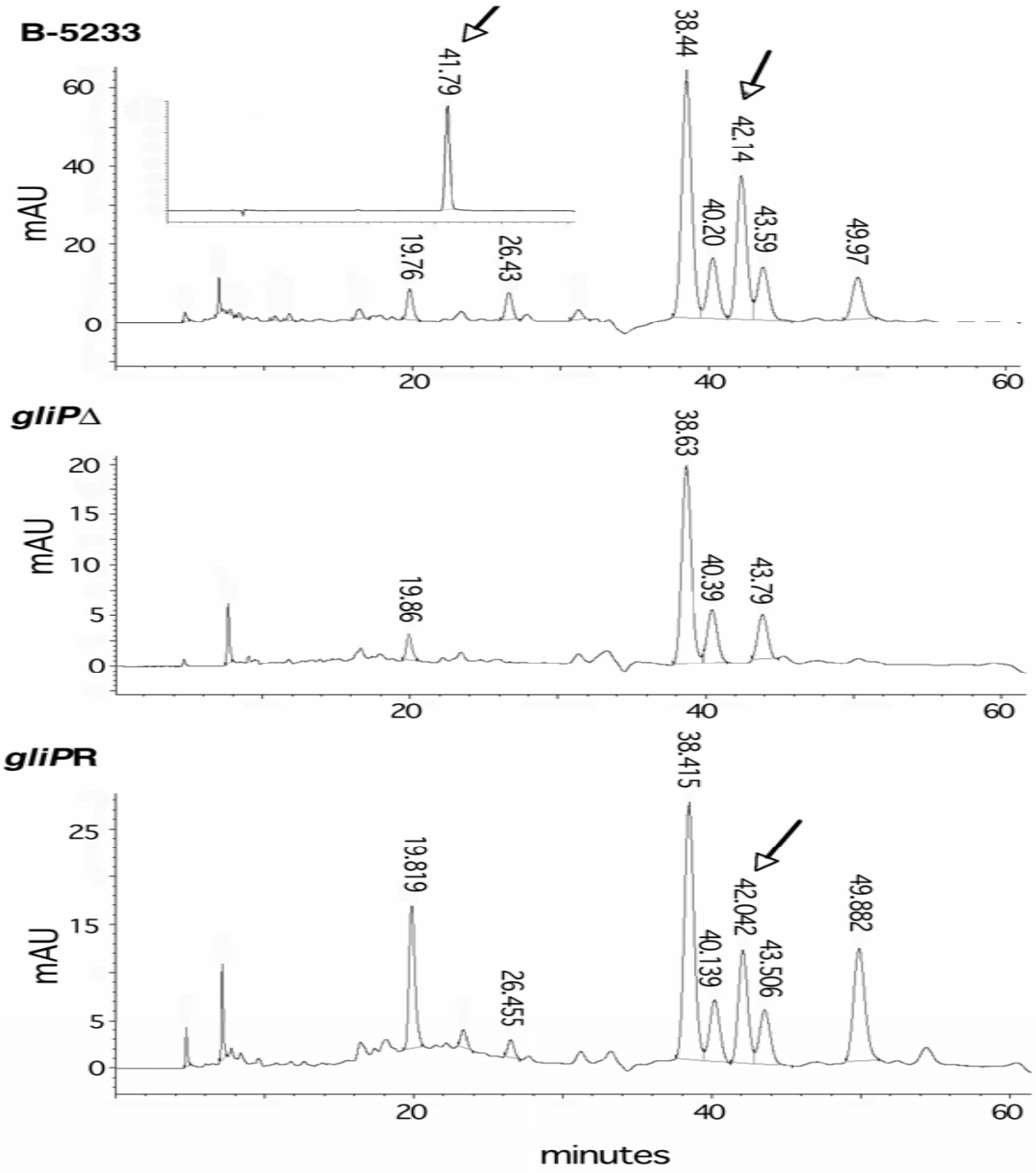
Contradictory results on the Role of gliotoxin in pathobiology of *A. fumigatus*

Strain back ground	Gene deleted	Mouse Strains, Ino.rout	Immunosuppressive regimen	Virulence	Ref.
Af293	*gliP*	Outbred ICR, inhal.	Cyclophosphamide + Cortisone acetate	No effect	Cramer *et al*, 2006
Af293	*gliP*	Balb/C, inhal.	Cyclophosphamide + Cortisone acetate	No effect	Spikes *et al*, 2008
Af293	*gliP*	Balb/C, inhal.	Cortisone acetate	Attenuated	Spikes *et al*, 2008
CEA10	*gliP*	Balb/C, IN	Cyclophosphamide + Cortisone acetate	No effect	Kupfahl et al, 2006
B-5223	*gliP*	Balb/C, IN	Cortisone acetate	Attenuated	Sugui et al, 2007
B-5233	*gliP*	129/Sv	Cortisone acetate	Attenuated	Sugui et al, 2007
Af 293	*gliZ*	Outbred ICR, IN	Cyclophosphamide + Cortisone acetate	No effect	Bok et al, 2006

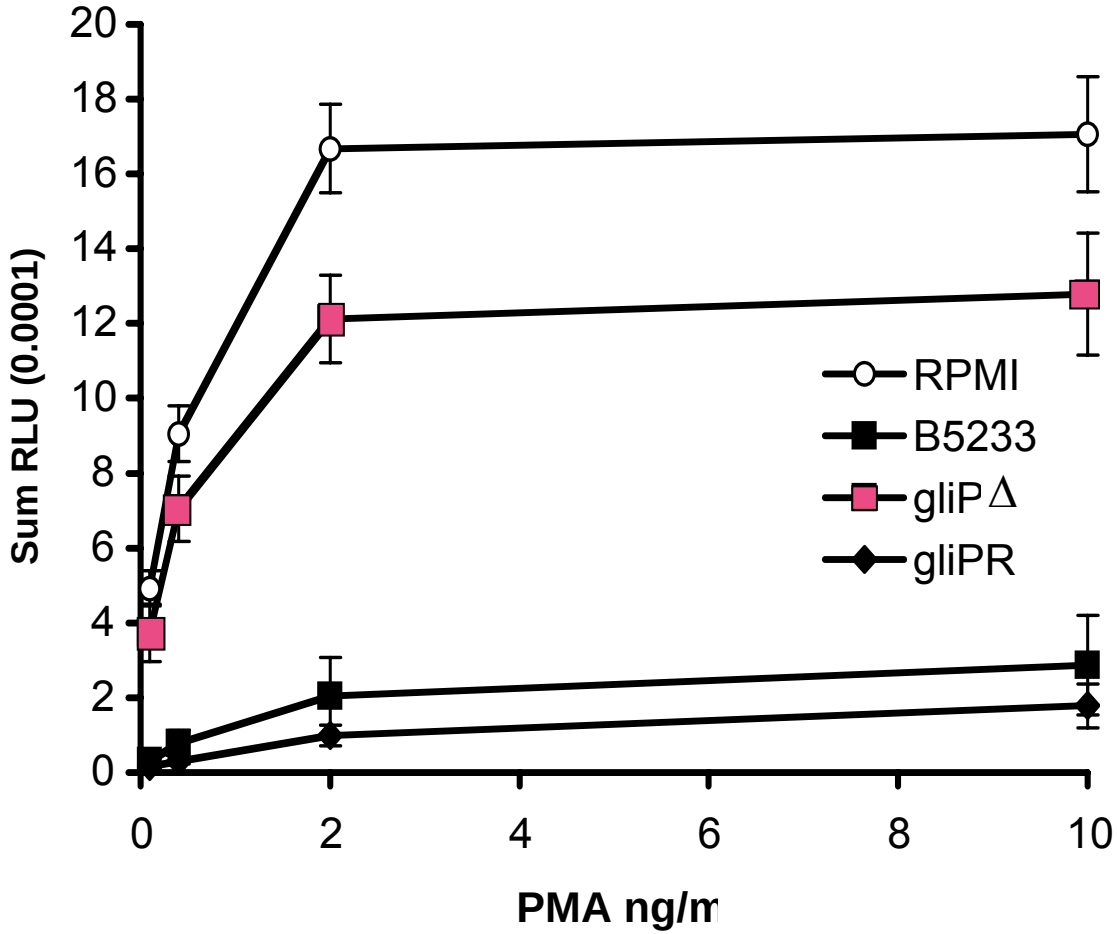
Functional studies of *gliP* that showed a positive effect of gliotoxin in the pathobiology of *A. fumigatus*

B-5233 : Sugui et al., 2007 (Eukaryot. Cell)

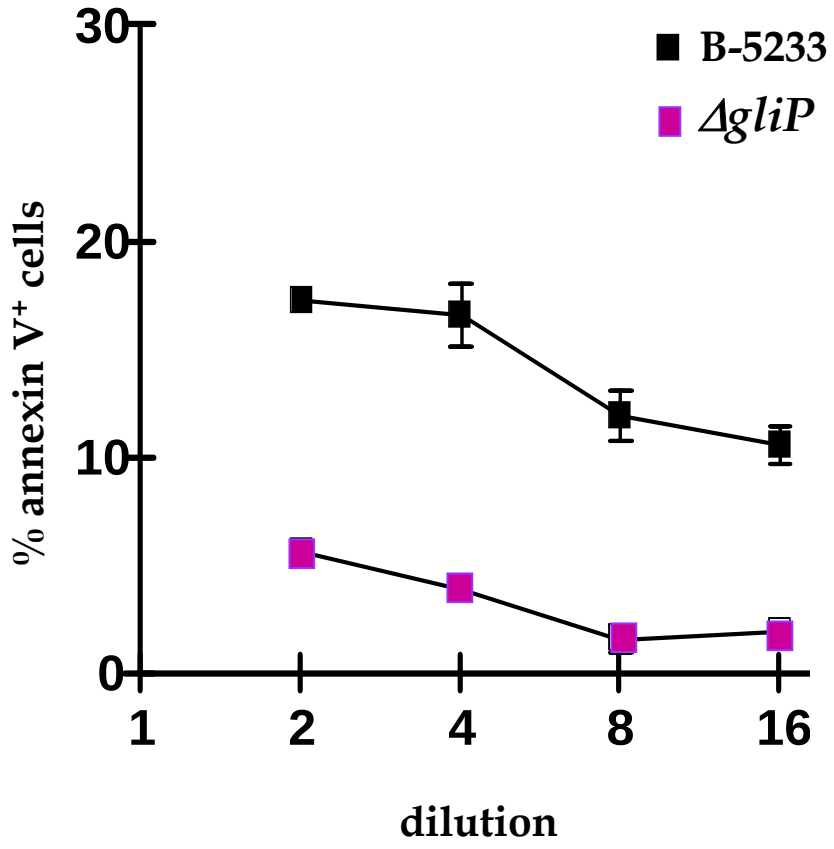
HPLC analysis of gliotoxin in the culture filtrates



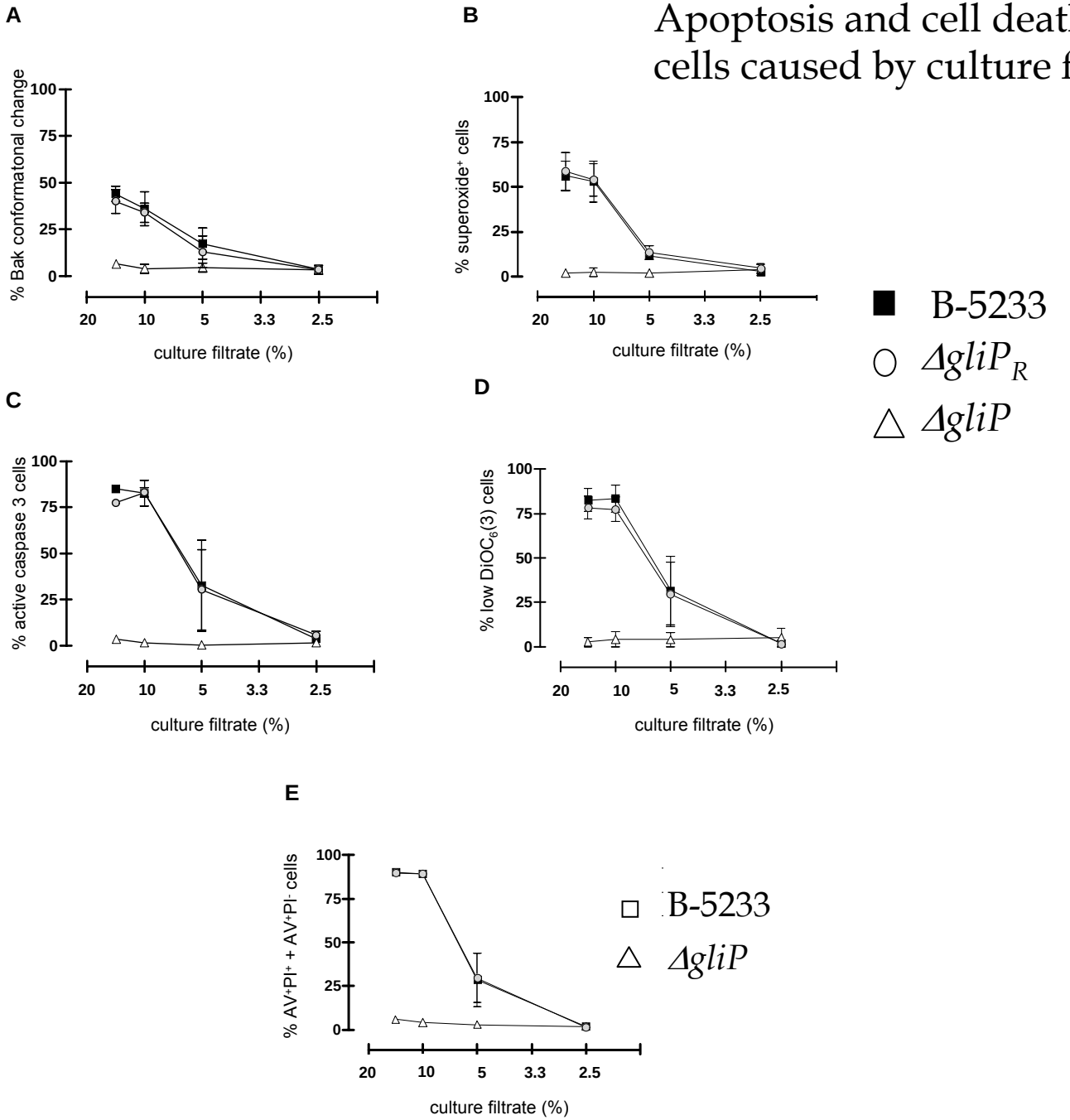
Chemiluminescence of neutrophils incubated with culture filtrates of *A. fumigatus*, B-5233, *gliP* and reconstituted strains



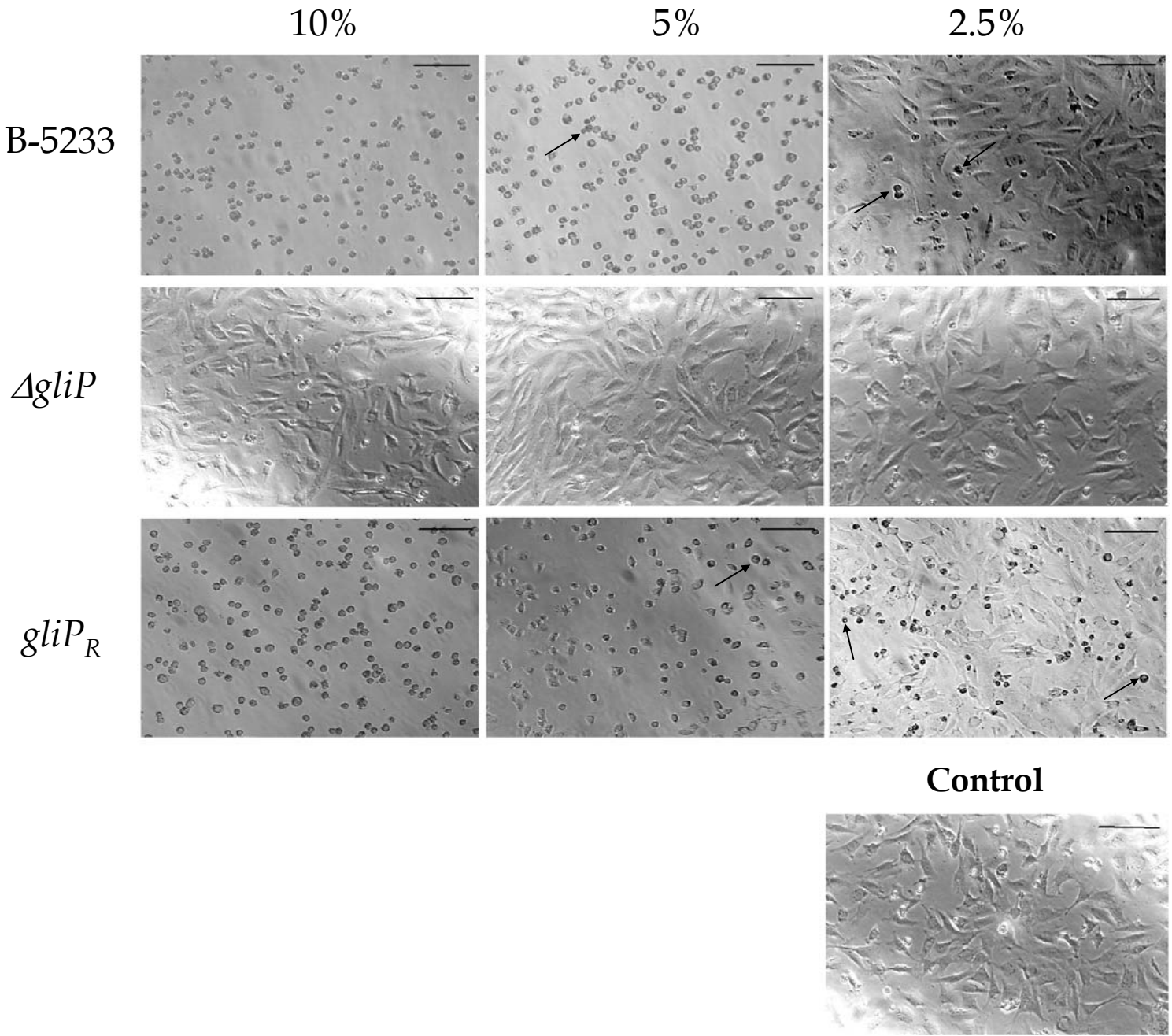
Phosphatidylserine translocation induced by CF of *A. fumigatus* B-5233 in bone marrow-derived neutrophils of 129/sV mice



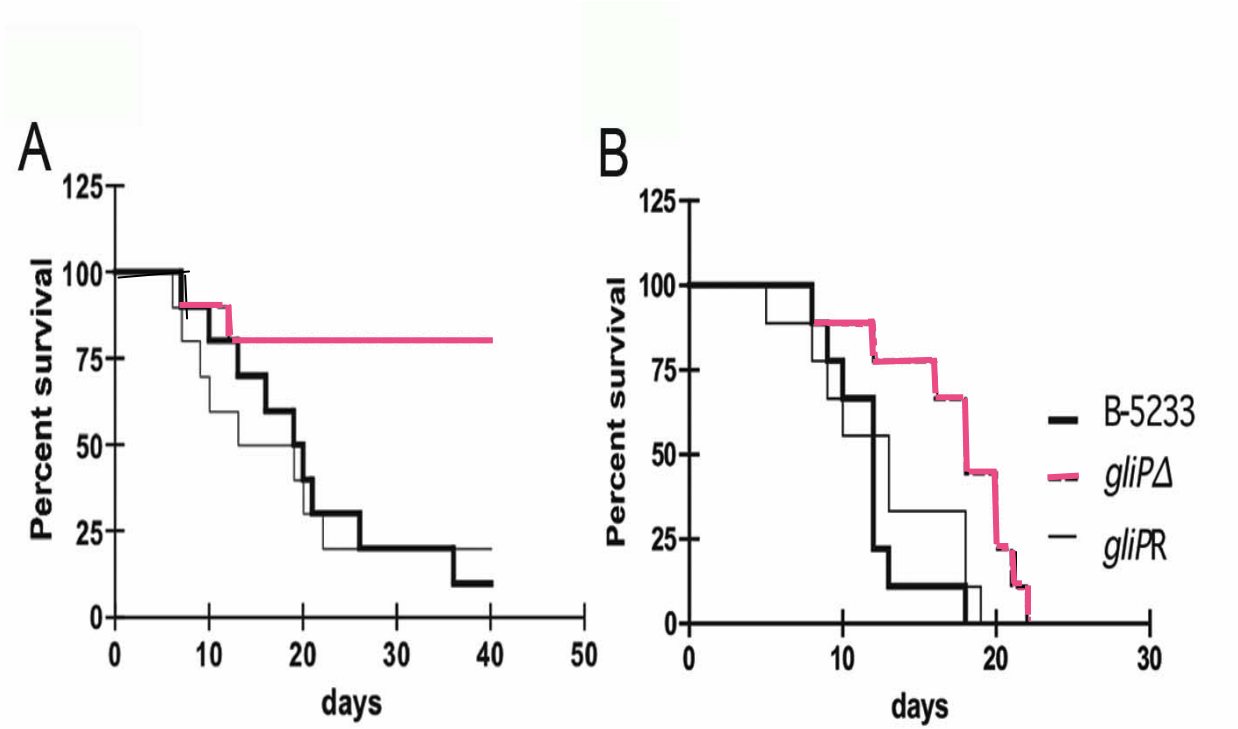
Apoptosis and cell death of EL4 thymoma cells caused by culture filtrates of *A. fumigatus*



Cell detachment of Mouse Embryonic Fibroblast cell line by CF



Virulence of *A. fumigatus*, B-5233, *glipΔ* and reconstituted strains in two different mouse strains

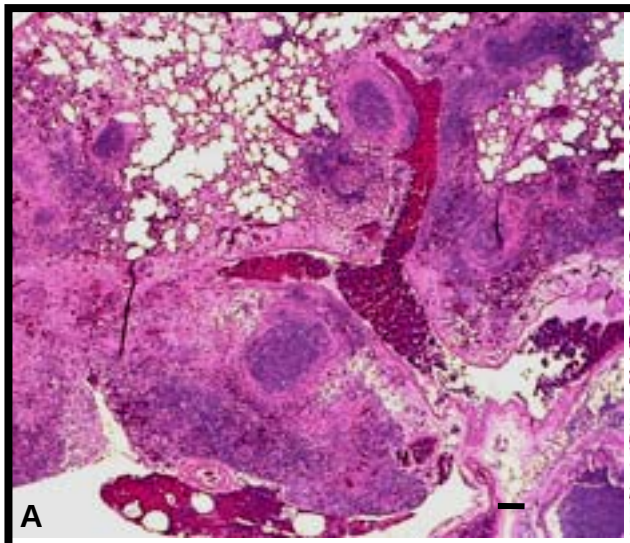


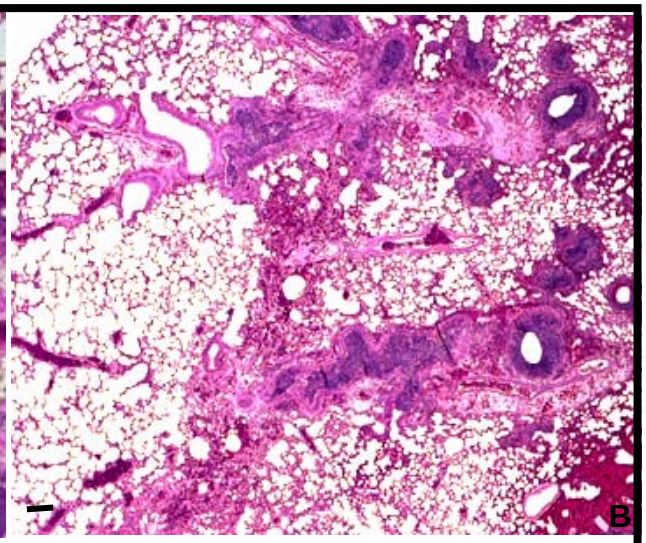
Cortisone acetate treated mice: **129/Sv** **Balb/C**

Histopathology of mouse lungs

WT at 72h

H & E



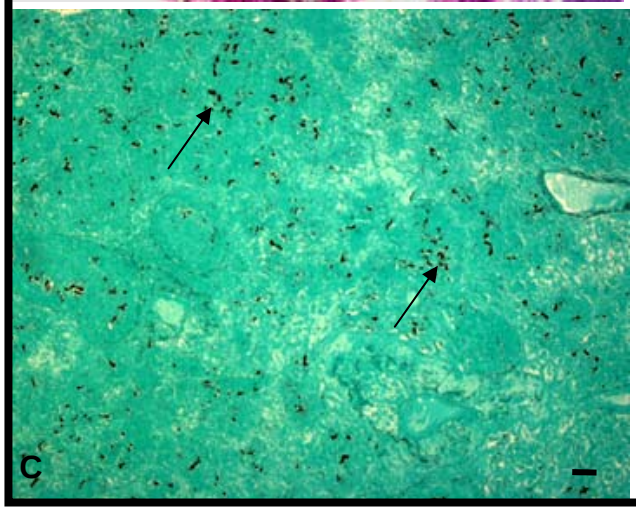


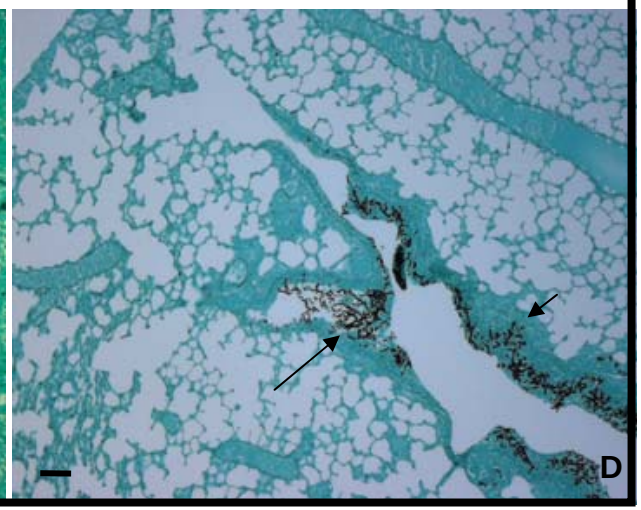
***glipΔ* at 72h**

H & E

WT at 96h

GMS





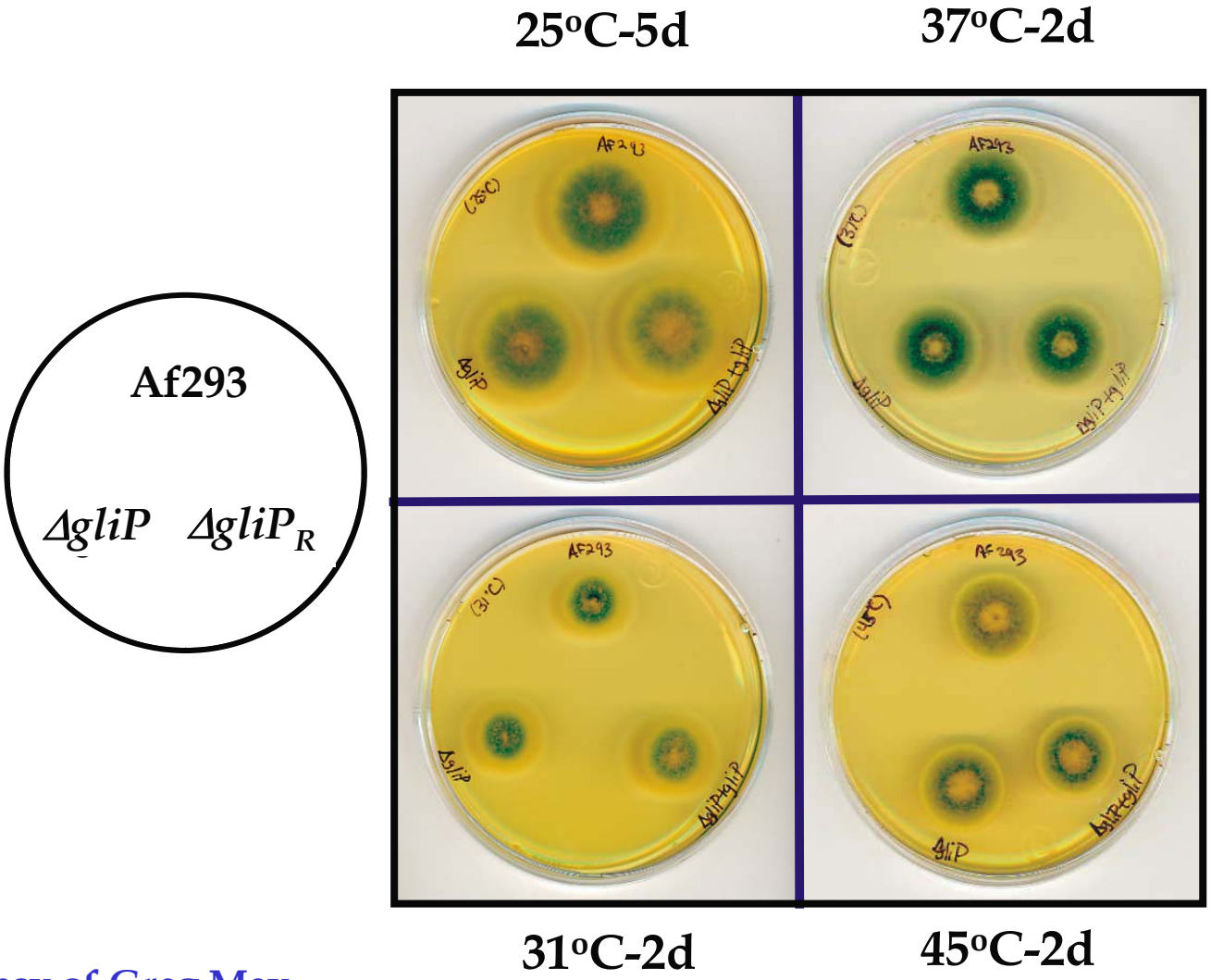
***glipΔ* at 96h**

GMS

Functional studies of *gliP* that showed a positive effect of gliotoxin in the pathobiology of *A. fumigatus*

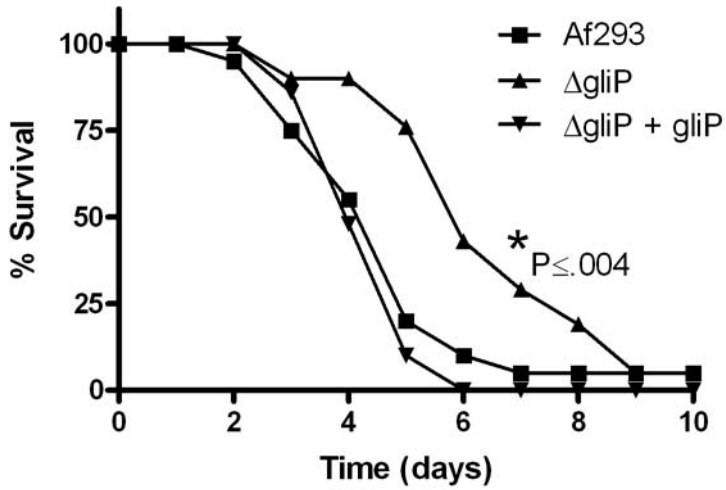
Af 293 : Spikes et al., 2008 (JID in Press)

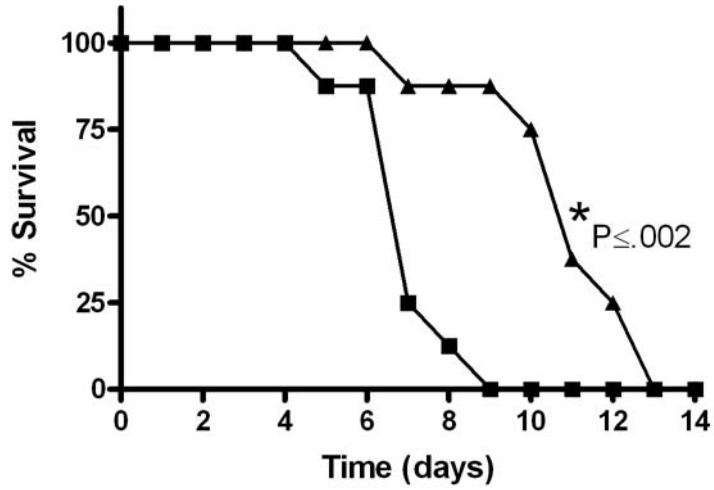
Deletion of *gliP* does not affect growth or sporulation



Courtesy of Greg May

Survival of Balb/C mice infected with Af293 strains immunosuppressed with cortisone-acetate

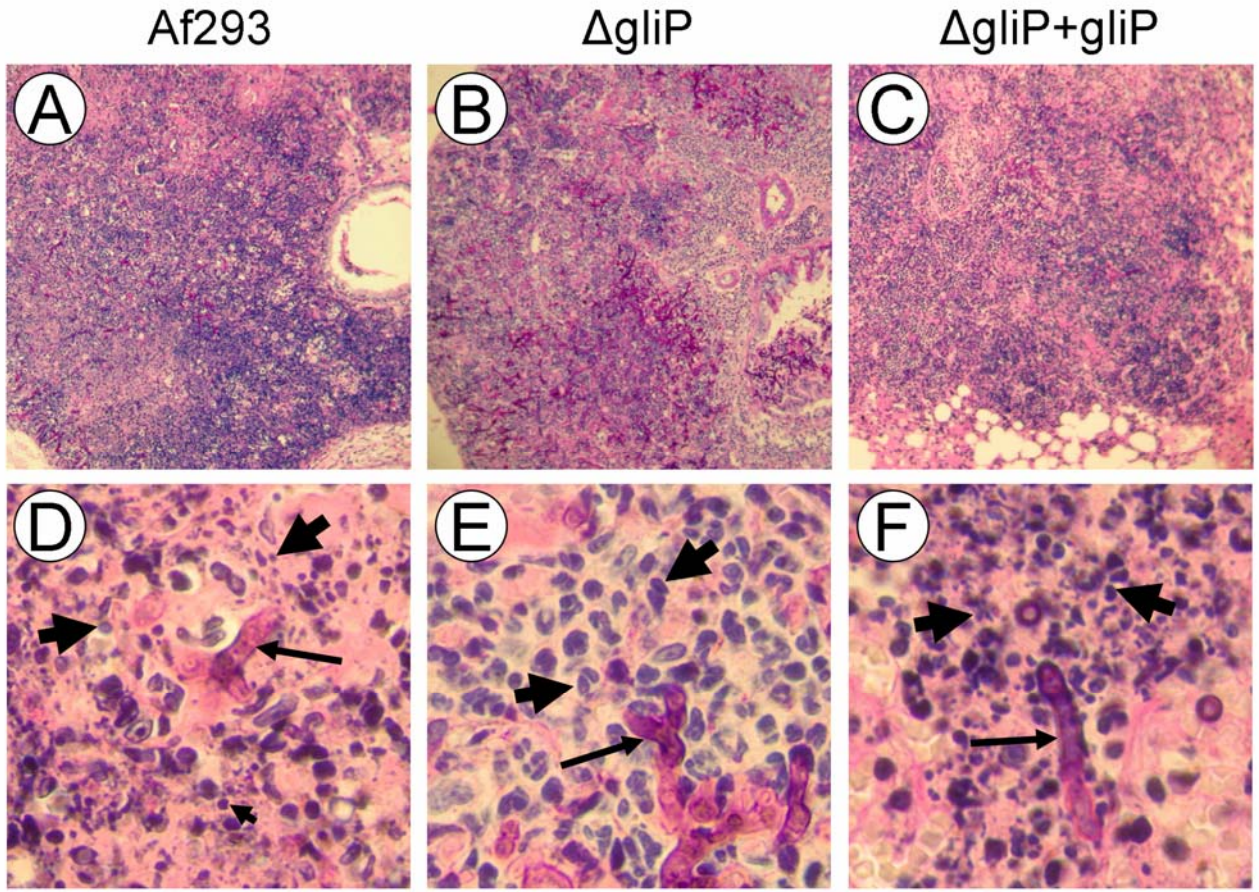




Intranasal inoculation
5 million spores

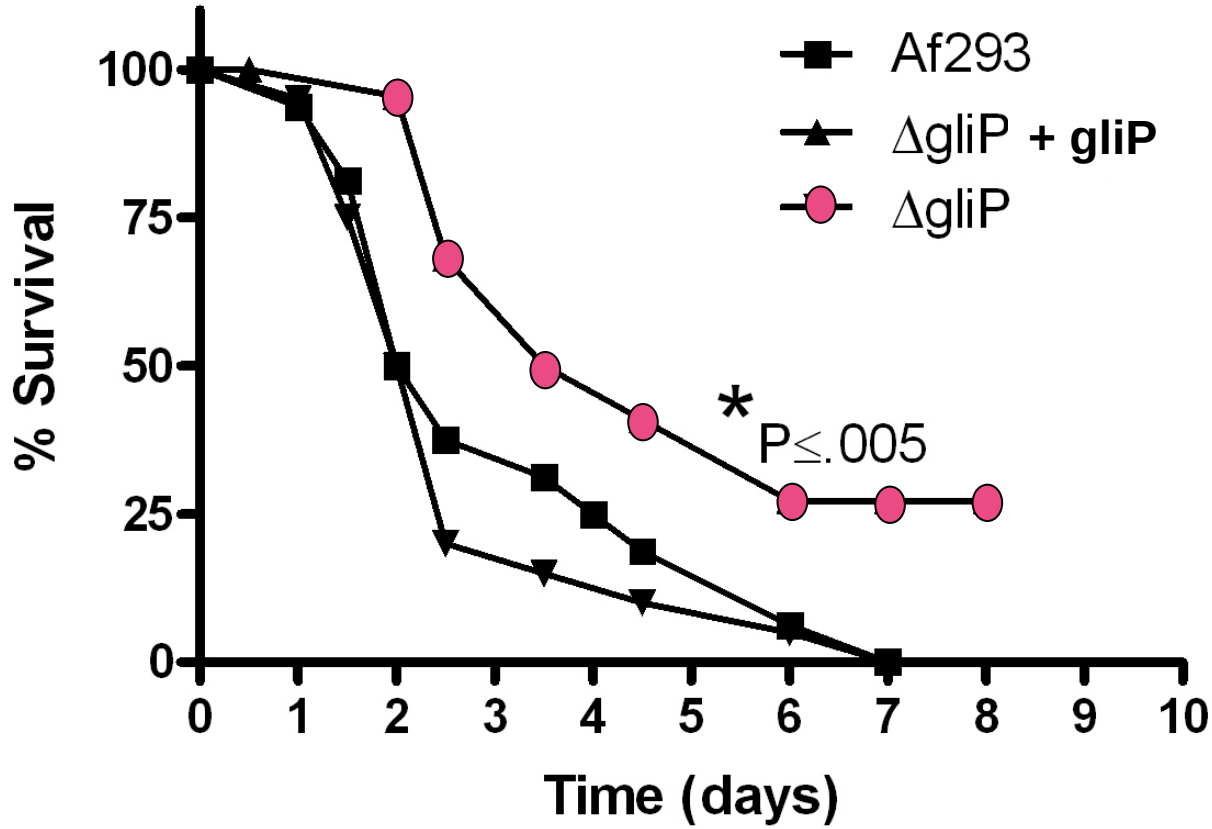
Inhalation: 1h exposure
to 12 ml suspension (10^9 spore/ml)

Histological sections stained with PAS strain showing neutrophil destruction in the lung by the wild type, *glipΔ* and reconstituted strains (Af293)



Spikes et al, JID. 2008

Virulence of the wild type, *gliPΔ* and reconstituted strains in Toll-deficient *Drosophila melanogaster*

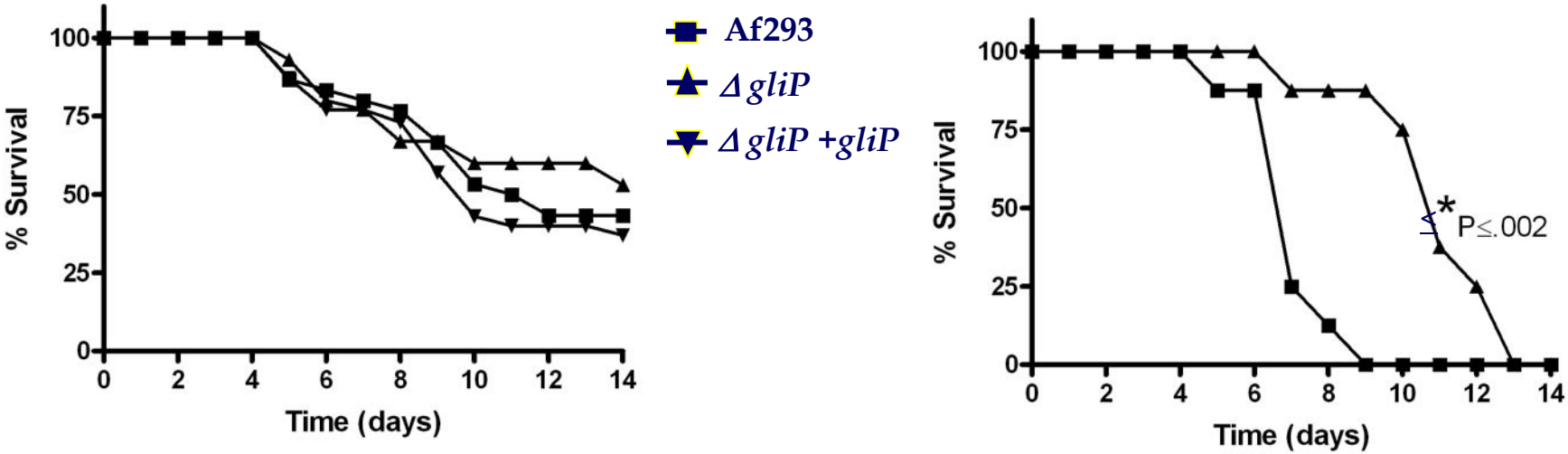


Spikes et al, JID in press

Reasons for contradictory results of the pathobiological role of *ΔgliP*

- Mouse strain : No
- Fungal strain : No
- Route of infection : No
- Immunosuppressive regimens: Yes

Virulence of Af293, *gliPΔ* and reconstituted strains in Balb/C mice



Treated with: Cyclophosphamide + cortisone acetate — Cortisone acetate

Infected via: Inhalation — Inhalation

Spikes *et al*, JID. 2008

Conclusions

- **Gliotoxin plays an important role in the pathobiology of *A. fumigatus***
- **Contribution of gliotoxin to *A. fumigatus* virulence can be detected only in non-neutropenic mice**
- **Drosophila is a useful model to assess the toxic effect of gliotoxin and may also be for other secondary metabolites**
- **These results suggest that the major target of gliotoxin is neutrophils/ phagocytes**
- **Neutropenic mouse model widely used for *A. fumigatus* virulence study may be inappropriate for the assessment of the importance of other secondary metabolites in aspergillosis**

Acknowledgements

LCID/NIH	*LHD/NIAID*	*Max-Planck-Institut für Immunbiologie*
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Yun C. Chang	**Kol Zarember**	**Julian Pardo**

Greg May and his co-workers for the gliotoxin project, in press