Stage-specific Innate Immune Recognition of *Aspergillus fumigatus* and Modulation by Echinocandin Drugs

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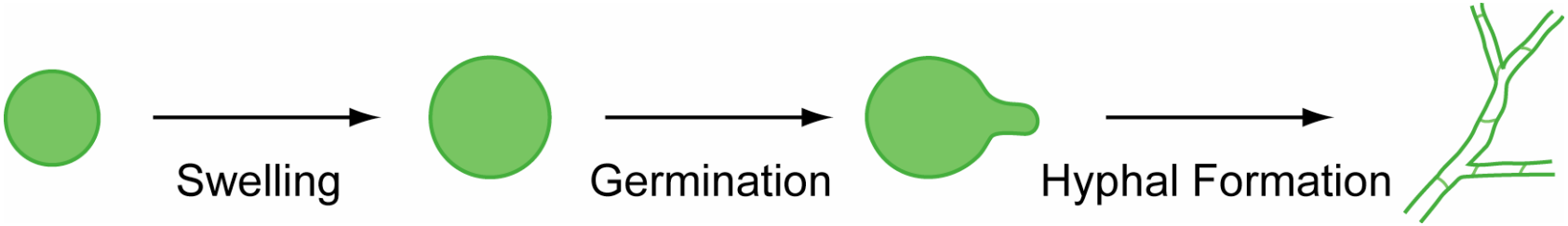
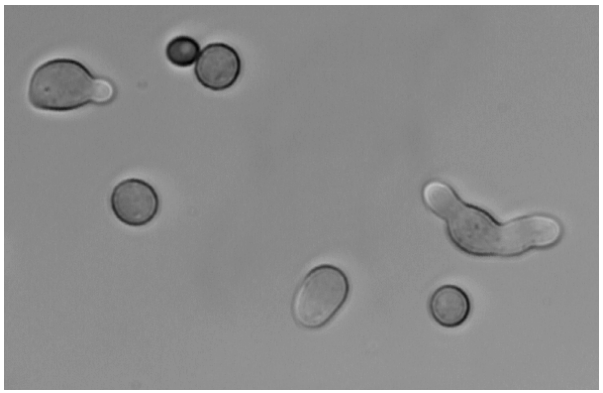
A. fumigatus Germination

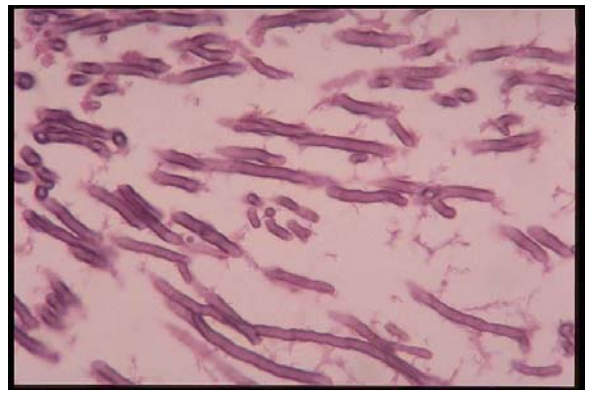




Intact pulmonary immune defense

Conidial clearance

Defective pulmonary immune defense



Tissue-invasive hyphae

Alveolar Macrophages in Host Defense

- **Sentinels at the portal of entry**
- **Conidial Phagocytosis**
- **Trigger effector cell recruitment through release of chemokines (CXCL1, CXCL2)**
- **Release inflammatory mediators**
- **Kill conidia in a phagocyte oxidase-dependent manner in vitro (Philippe et al., *Infect. Immun*, 2003)**

QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

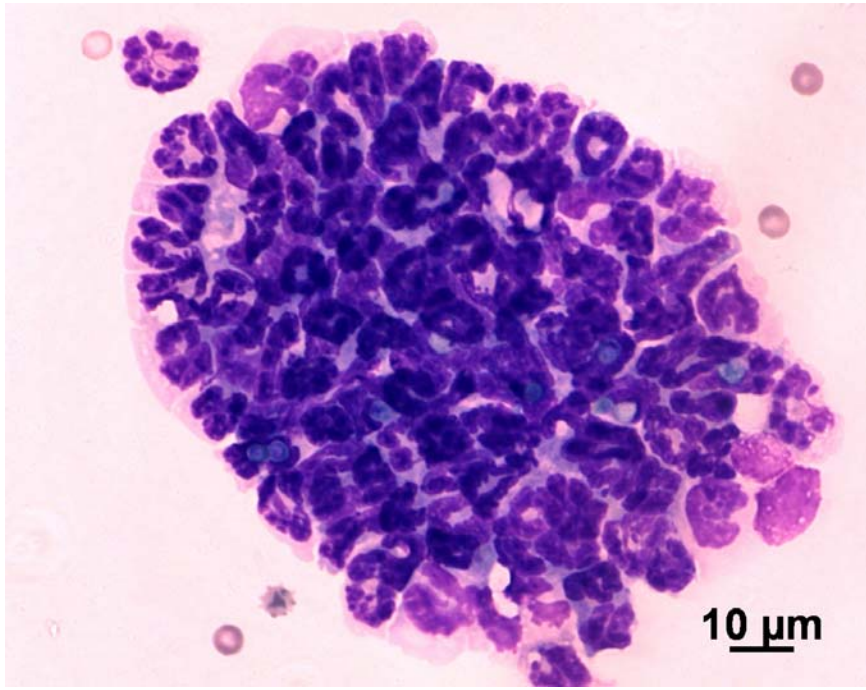
Image from Behnsen et al., *PLoS Pathog.* 3:e13, 2007.

Downloaded 1/09/08 at: http://upload.wikimedia.org/wikipedia/commons/thumb/4/43/S3-Alveolar_Macrophages_with_Conidia_in_Liquid_Medium.ogg/mid-S3-Alveolar_Macrophages_with_Conidia_in_Liquid_Medium.ogg.jpg

Neutrophils in Host Defense

- **Mice depleted of neutrophils or with defective neutrophil trafficking are highly susceptible to invasive apergillosis** (Mehrad et al., JI, 1999; Bonnett et al., Infect. Immun., 2006)

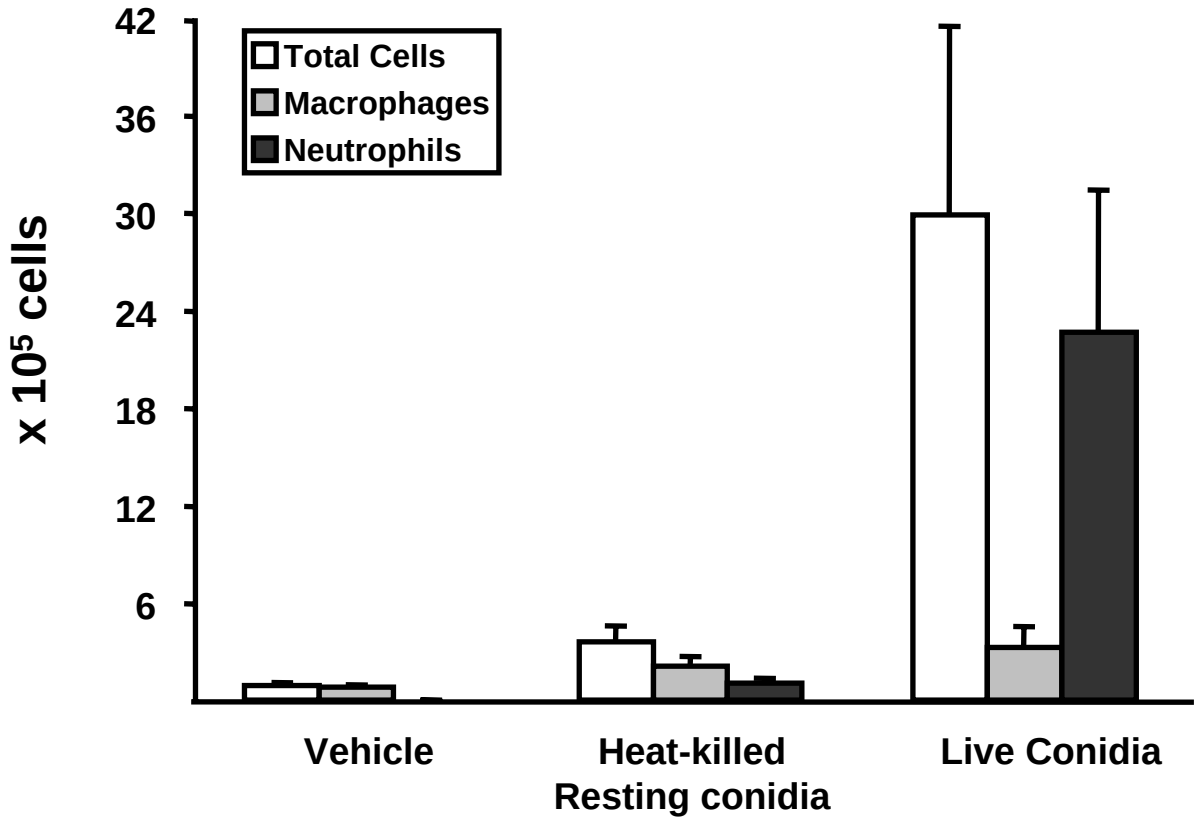
- **Antifungal effector functions**
  - **NADPH oxidase** (Morgenstern et al., *JEM*, 1997)
  - **Granule proteins**
    - **Neutrophil Elastase, Cathepsin G** (Tkalcevic et al., *Immunity*, 2000)
    - **Lactoferrin** (Zarember et al., *J. Immunol.*, 2007)
    - **Pentraxin-3** (Garlanda et al., *Nature*, 2002)
  - **Neutrophil BAL Aggregates** (Bonnett et al., *Infect. Immun*., 2006)
  - **Neutrophil Extracellular Traps** (Jaillon et al., *JEM*, 2007)



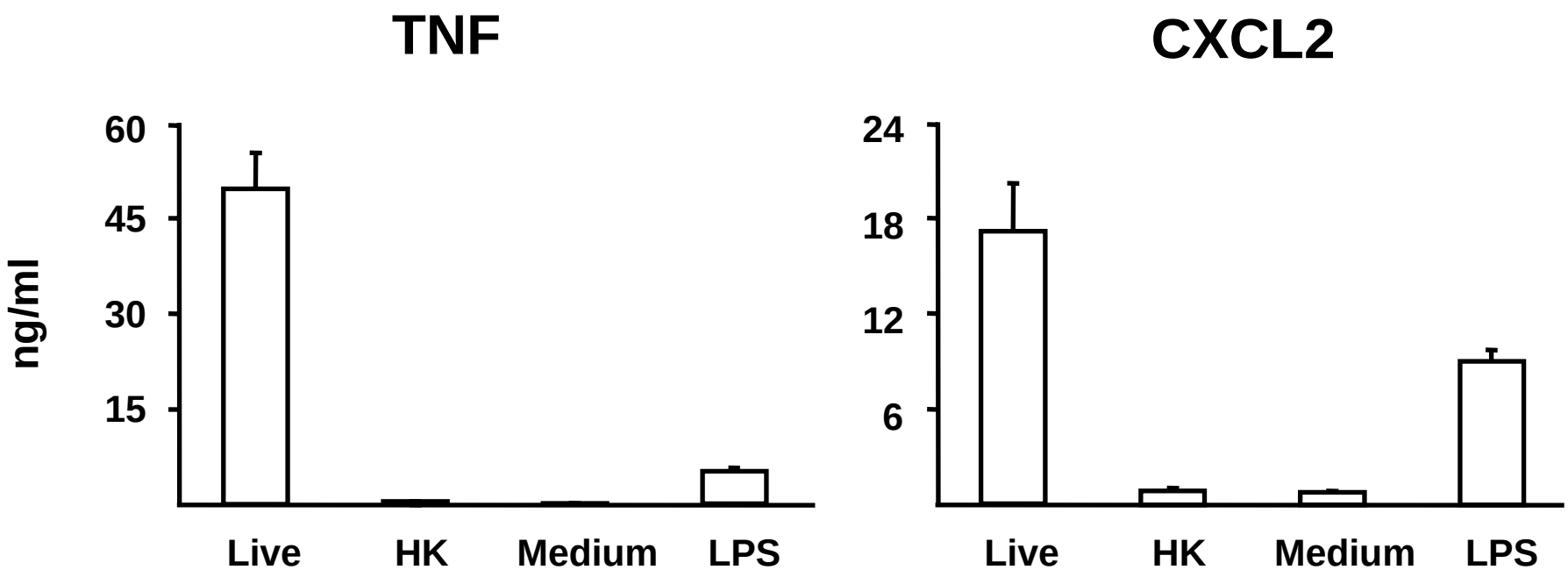
Micrograph courtesy of J. Burritt (Montana State University)

How do conidia trigger host inflammatory responses?

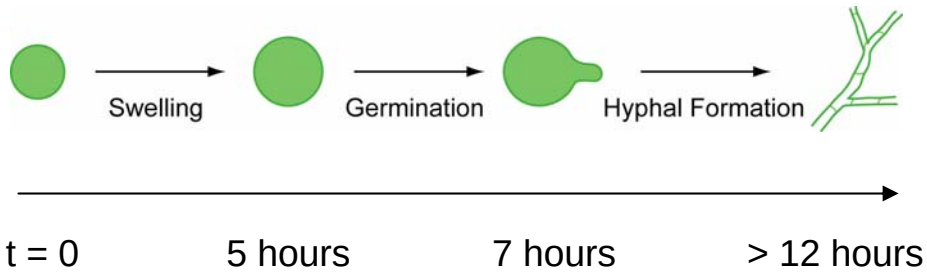
Live Conidia induce Neutrophil Recruitment into the BAL fluid at 24 hours *p.i.*

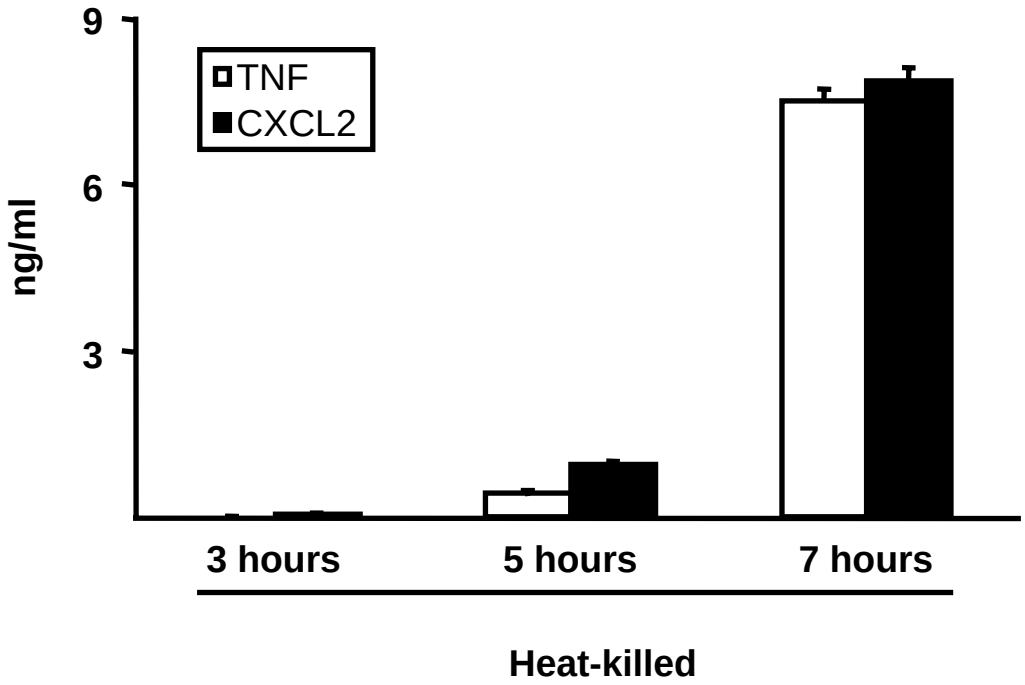


Live Conidia induce TNF/ CXCL2 Secretion by Alveolar Macrophages

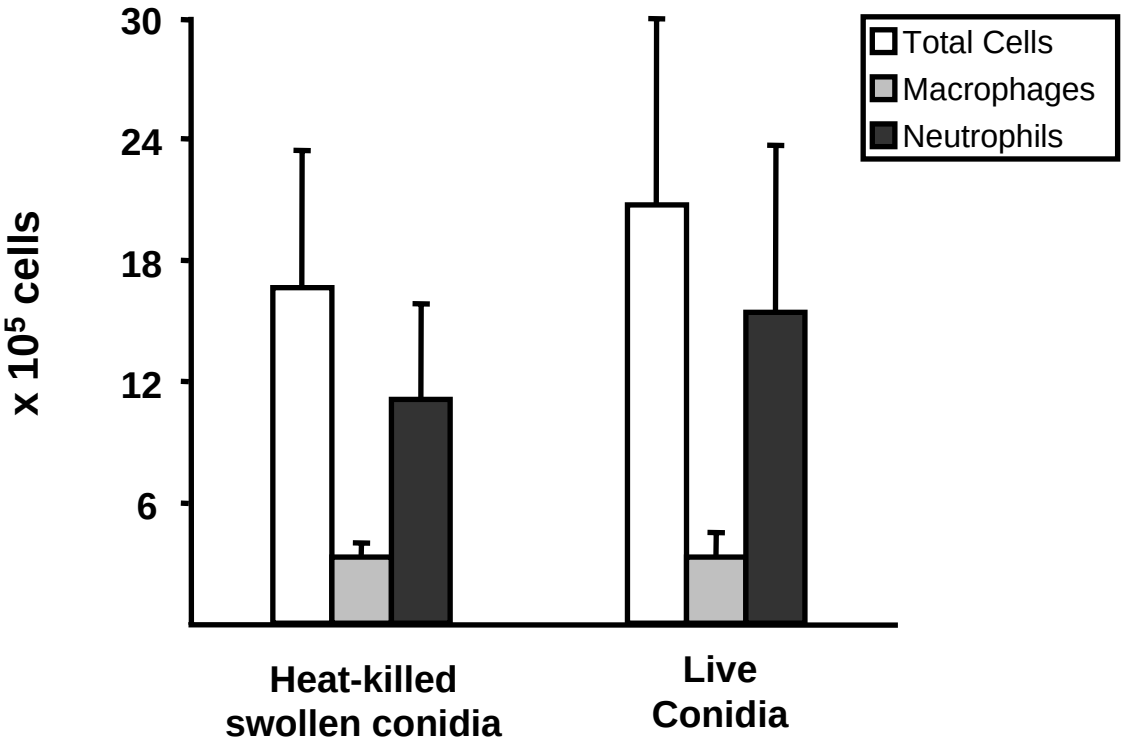


Killed germinating Conidia are highly inflammatory

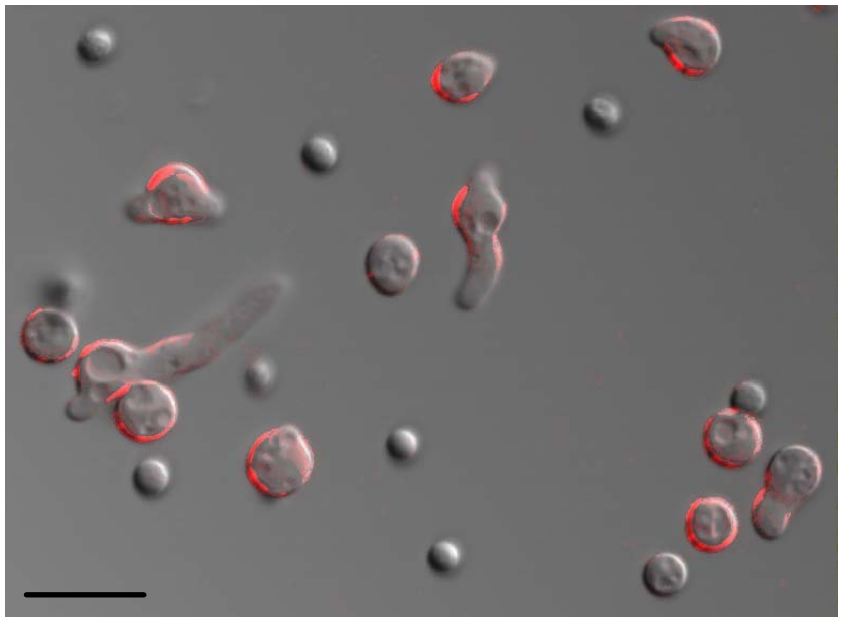




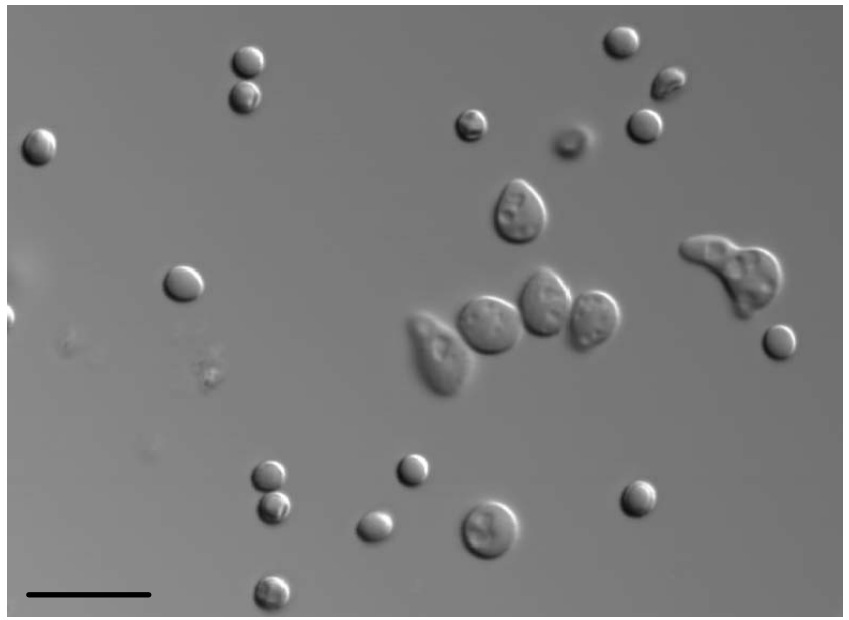
Killed swollen Conidia induce Neutrophil Influx into the BAL fluid



Swollen Conidia and Germlings expose β-glucan on their surface

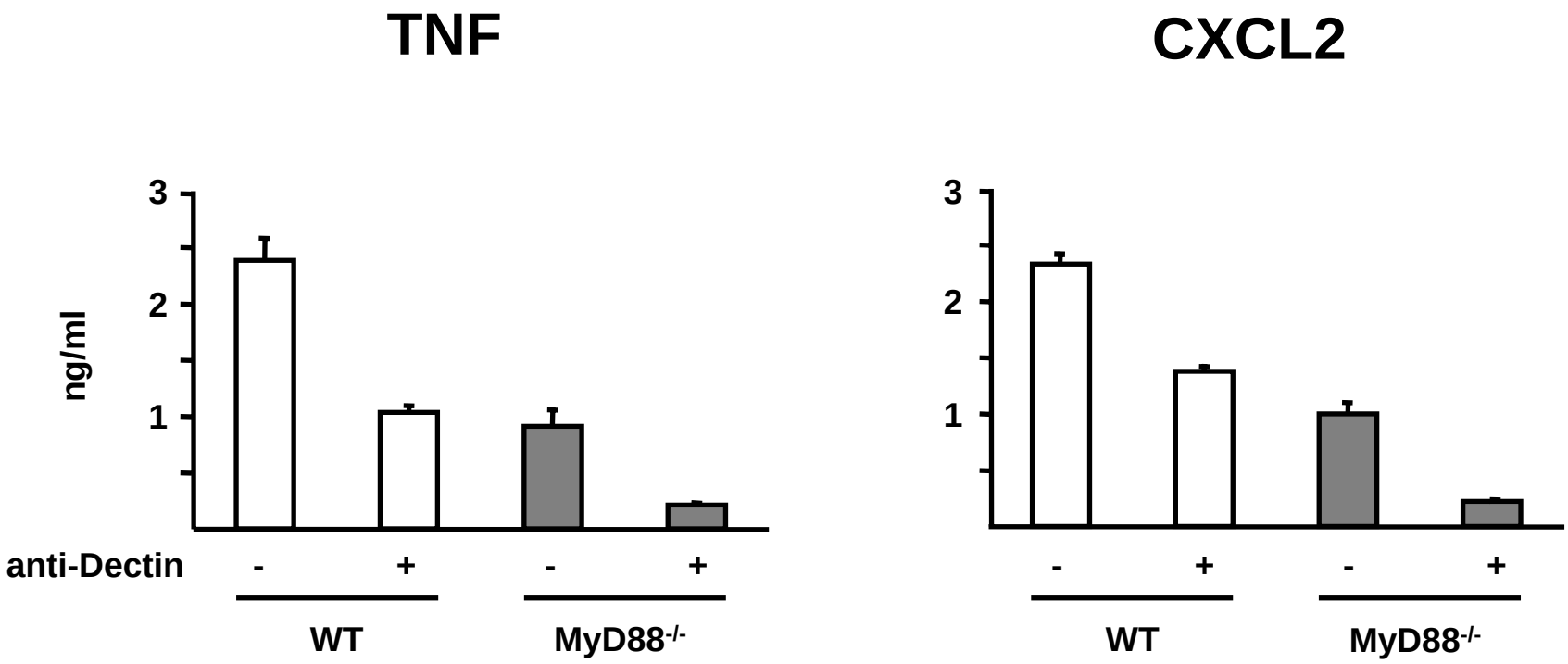


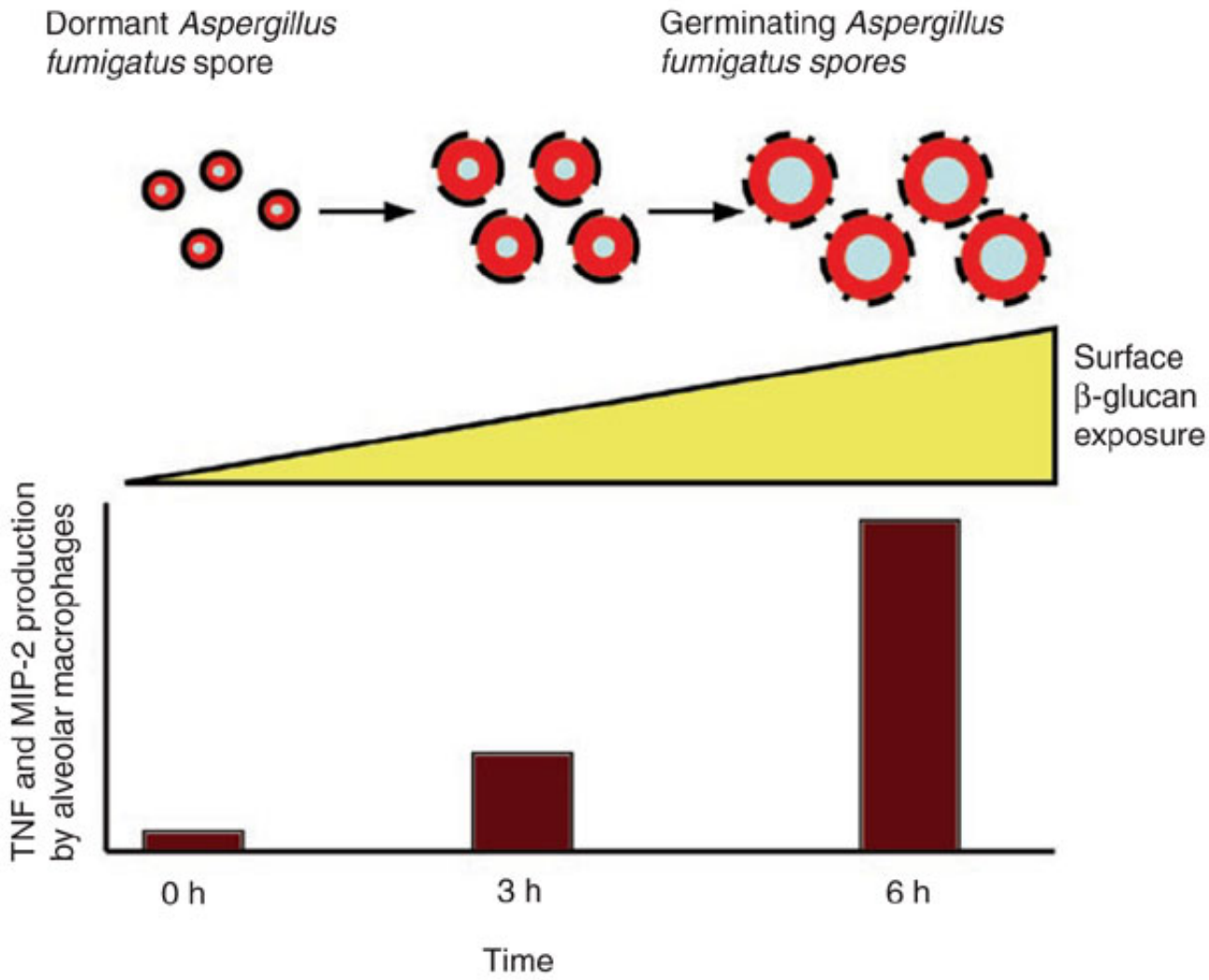
Anti β-glucan



Isotype Control Ab

Conidia Stimulate Dectin-1- and MyD88-dependent Pathways

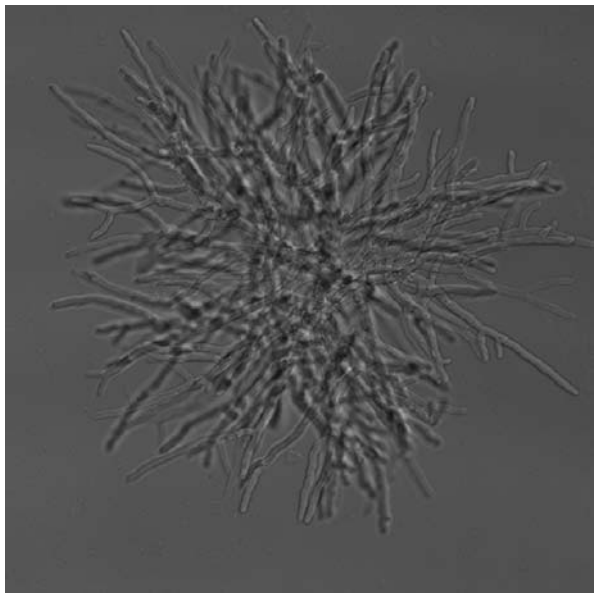




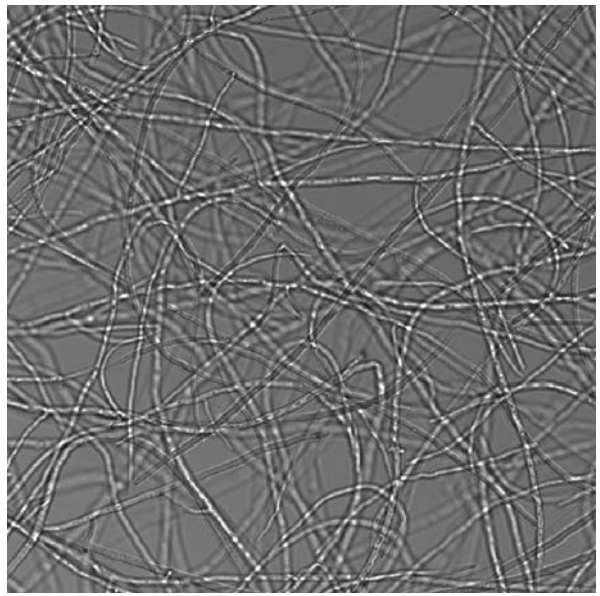
Pamer, E. G. *Nat. Immunol*. 8: 1173-78, 2007.

Modulation of Host Inflammatory Responses by Antifungal Therapy

- **Echinocandins target fungal-β-D-glucan synthase**
- **Echinocandins reduce *A. fumigatus* bulk β-glucan levels**
  (Kahn et al., *Antimicrob. Agents Chemother.* 50:2214, 2006)
- **Echinocandins do not fully inhibit *A. fumigatus* growth, yet induce prominent morphologic changes at or above the MEC**

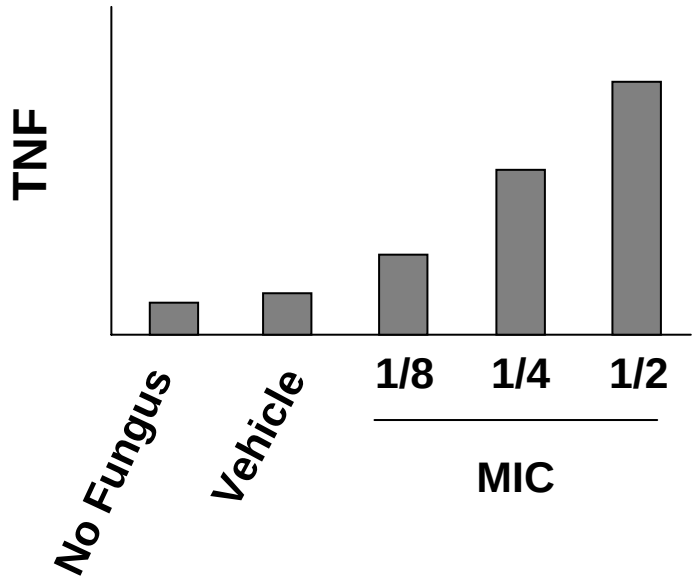


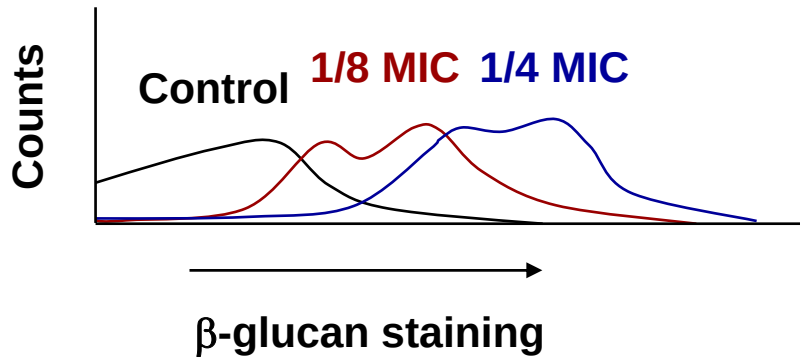
1 x MEC Caspofungin



No Caspofungin

Echinocandins Alter the *C. albicans* β-glucan Surface Content at sub-MIC Concentrations

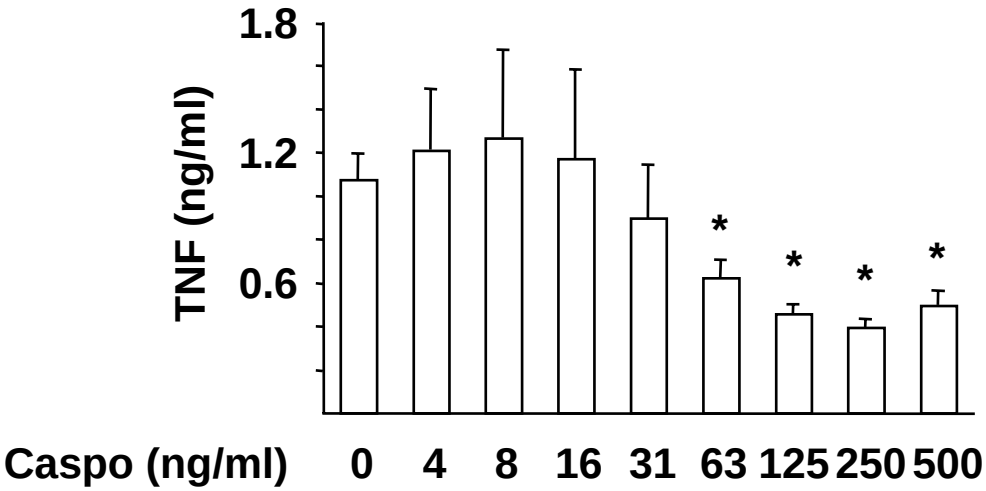


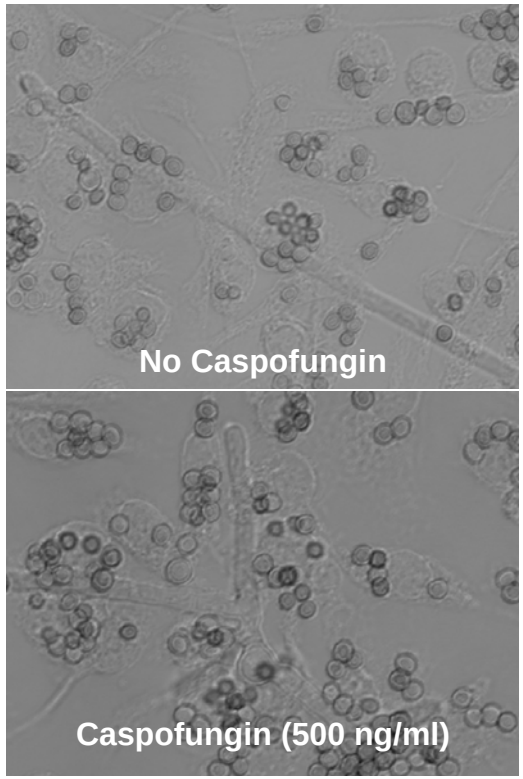


C. albicans **Caspofungin MIC_{50} 2.5 ng/ml**

Wheeler and Fink. *Plos Pathog.* 2:e35, 2006.

Caspofungin Exposure Decreases Macrophage Inflammatory Responses to *A. fumigatus* Conidia

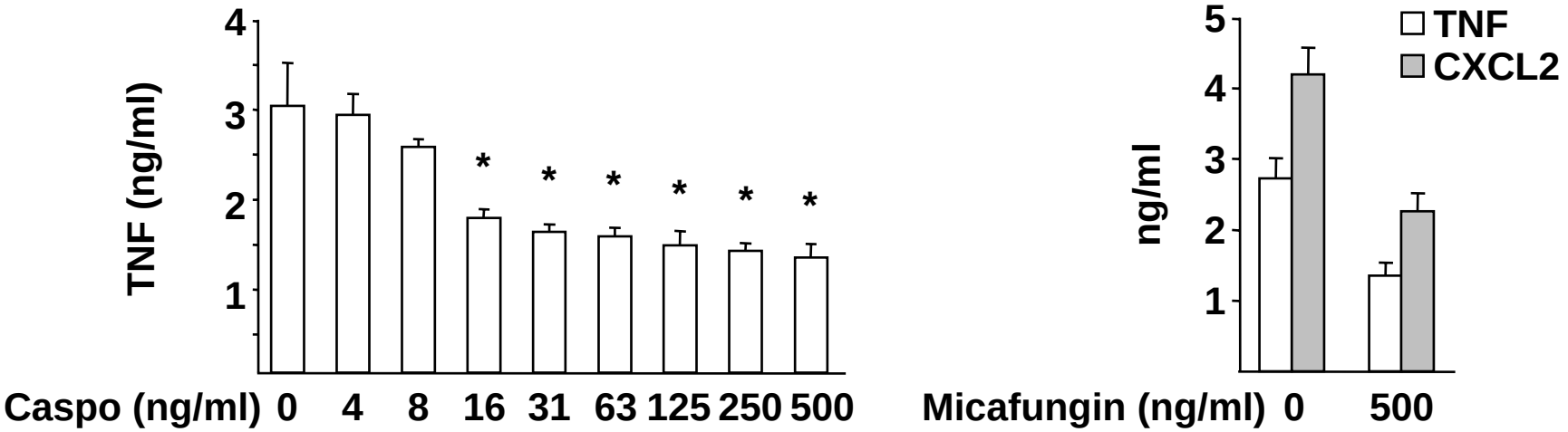




BMMϕ TNF/CXCL2 release (500 ng/ml caspfungin vs. no drug exposure):

- **TNF** 0.49 ± 0.04* (range 0.46-0.54; n=4)
- **CXCL2** 0.55 ± 0.10* (range 0.43-0.62; n=4)

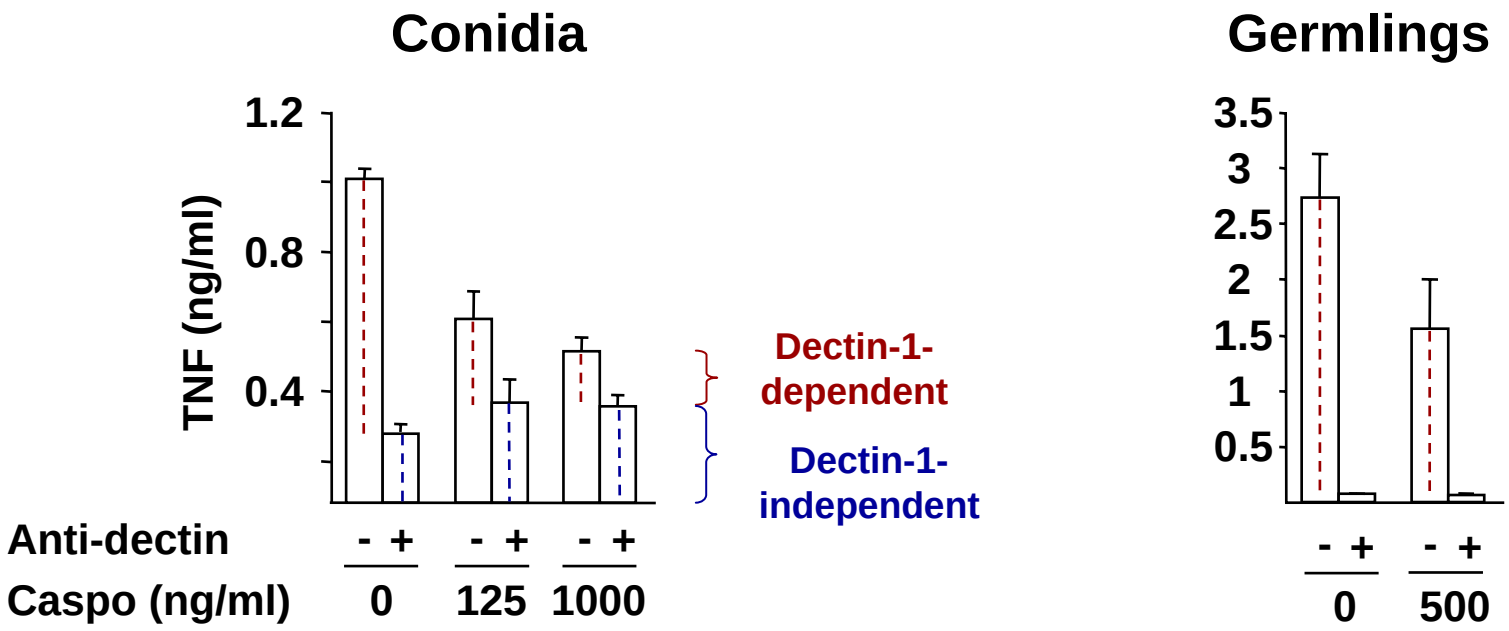
Caspofungin Exposure Decreases Macrophage Inflammatory Responses to *A. fumigatus* germlings



BMMϕ TNF/CXCL2 release (500 ng/ml caspfungin vs. no drug exposure)

- **TNF** 0.51 ± 0.07* (range 0.43-0.64; n=7)
- **CXCL2** 0.61 ± 0.08* (range 0.53-0.74; n=7)

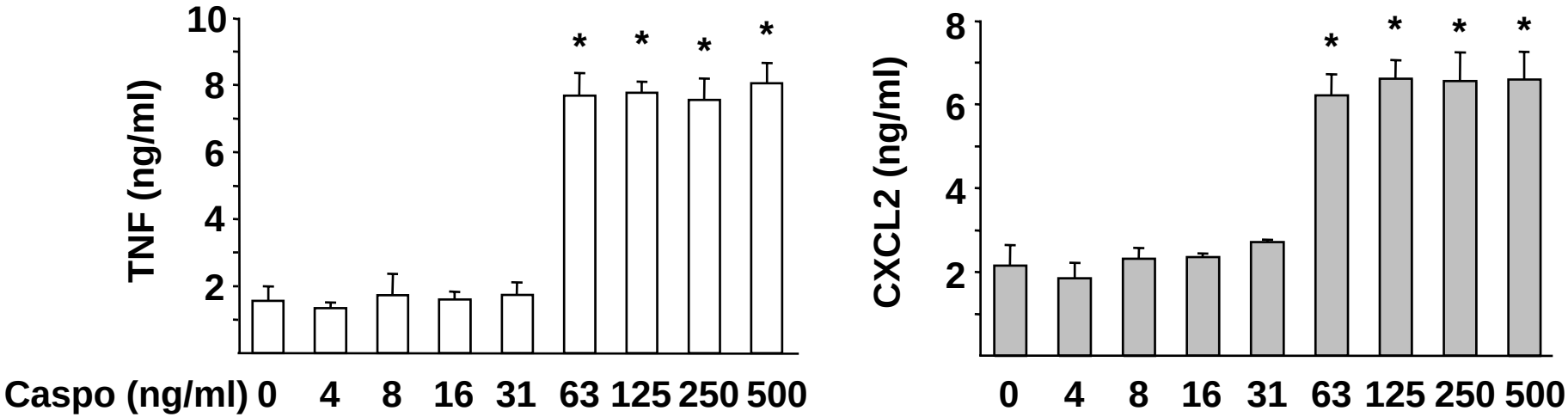
Reduced Inflammatory Responses to Conidia and Germlings Reflect Diminished Dectin-1 Signaling



----- Dectin-1-dependent TNF release

----- Dectin-1-independent TNF release

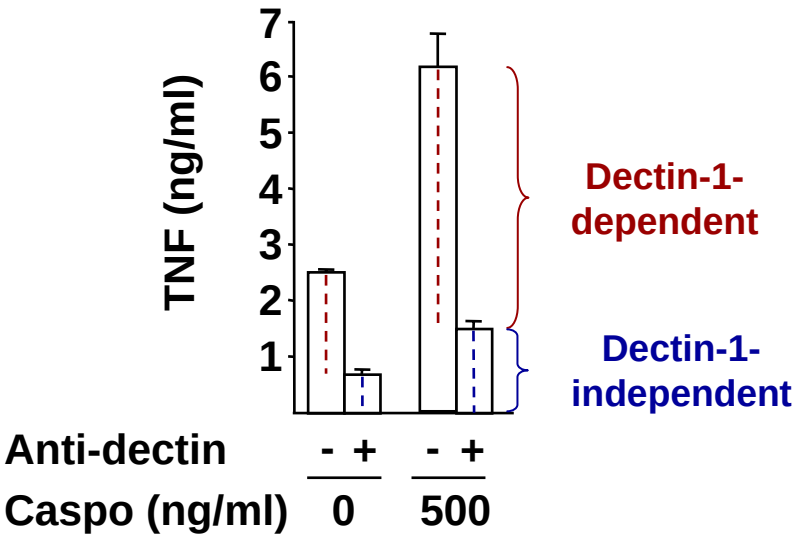
Caspofungin Exposure enhances Inflammatory Responses to *A. fumigatus* Hyphae

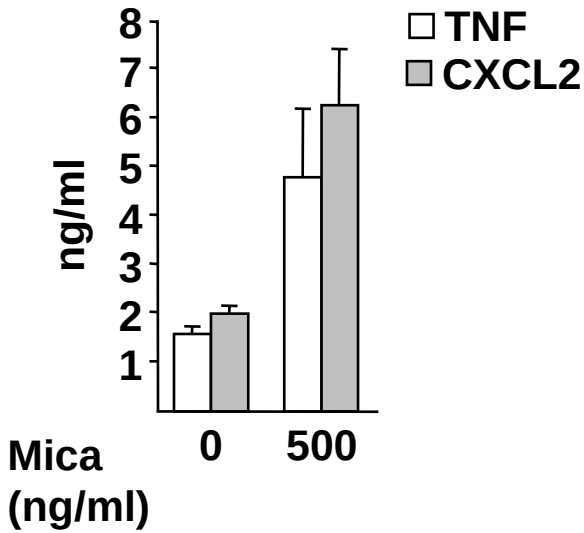


BMMϕ TNF/CXCL2 release (500 ng/ml caspfungin vs. no drug exposure)

- **TNF** 4.11 ± 2.39* (range 1.90-7.84; n=8)
- **CXCL2** 2.90 ± 1.40* (range 1.53-5.41; n=8)

Increased Dectin-1 Signaling Accounts for Enhanced Responses to Drug-treated Hyphae

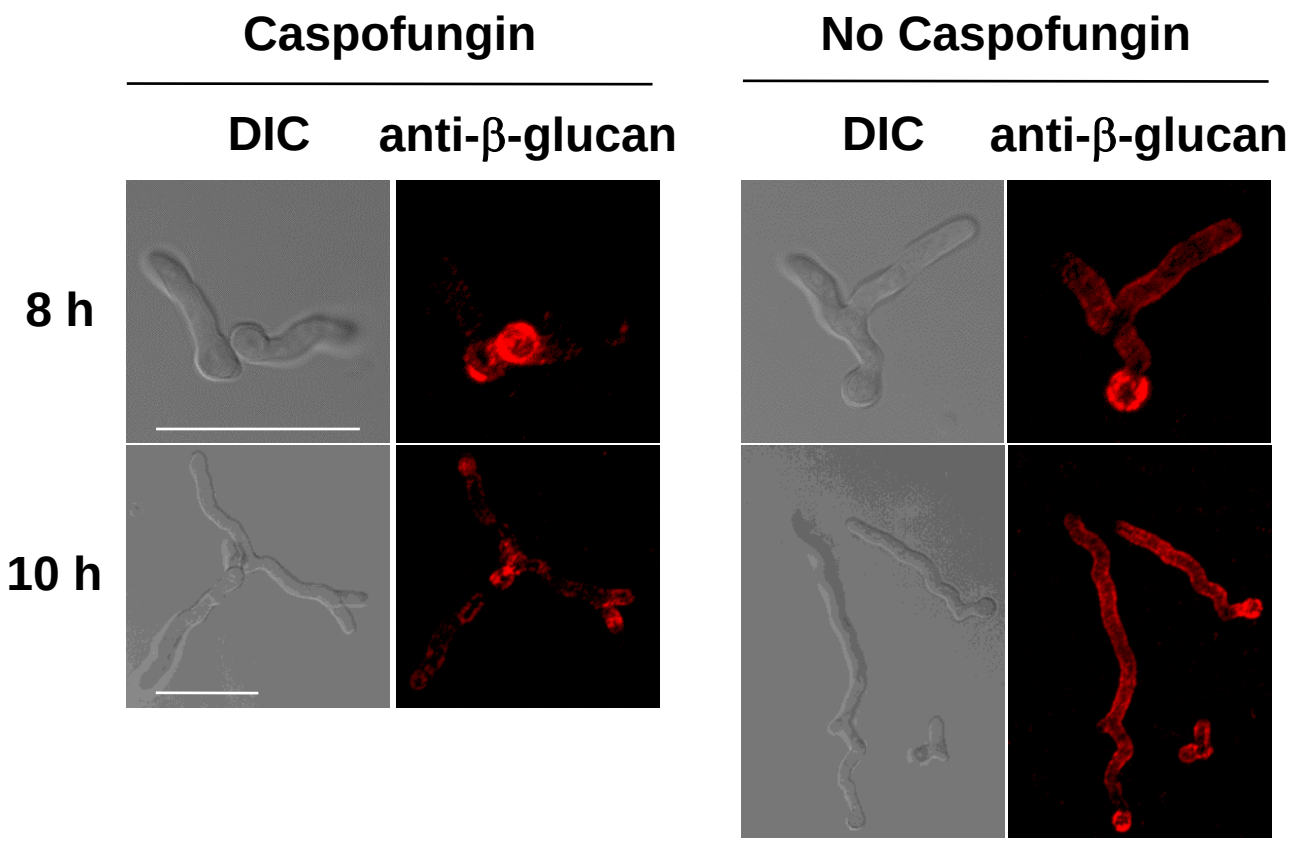




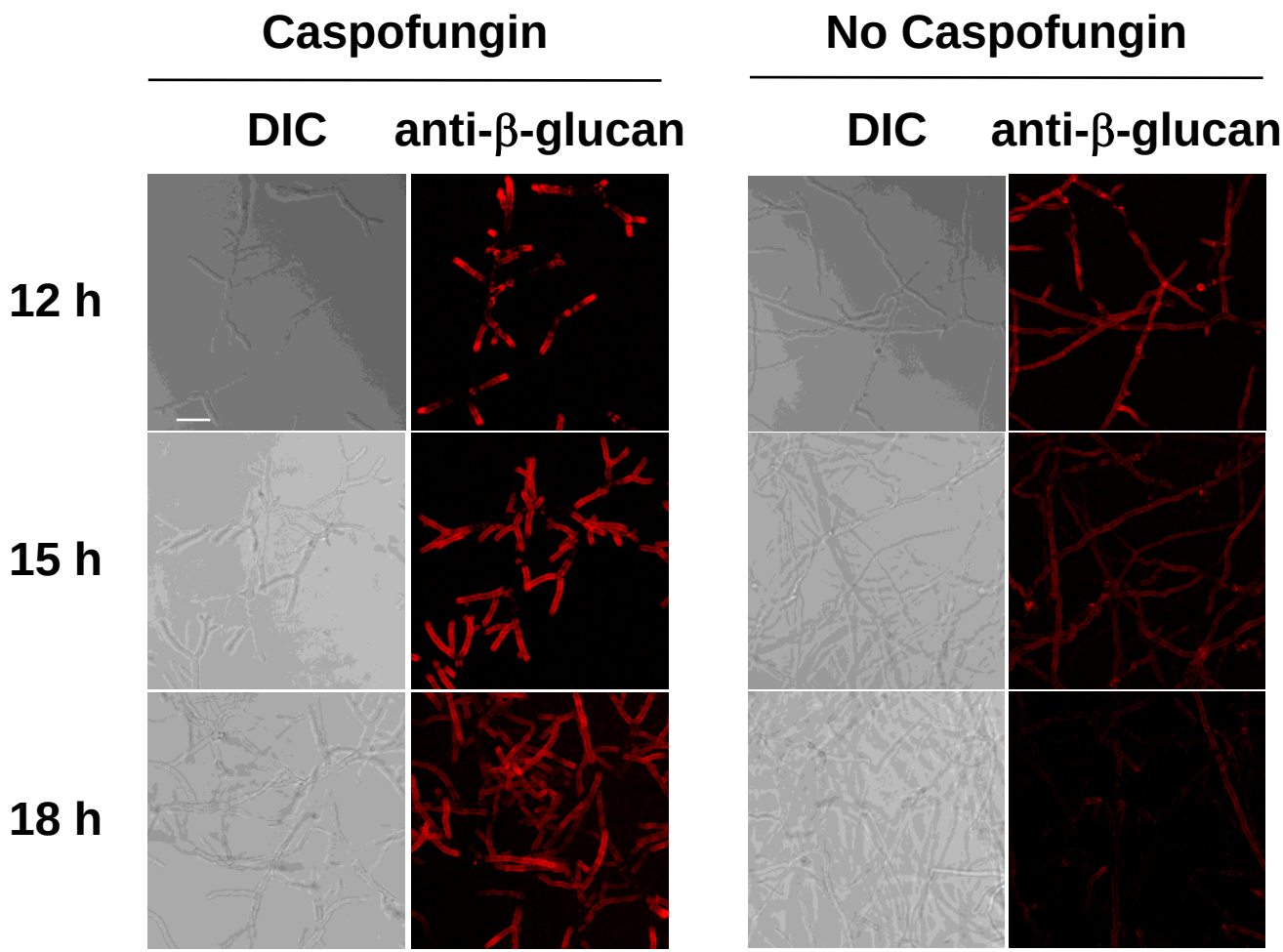
----- Dectin-1-dependent TNF release

----- Dectin-1-independent TNF release

Effects of Echinocandin Drugs on β-glucan Exposure

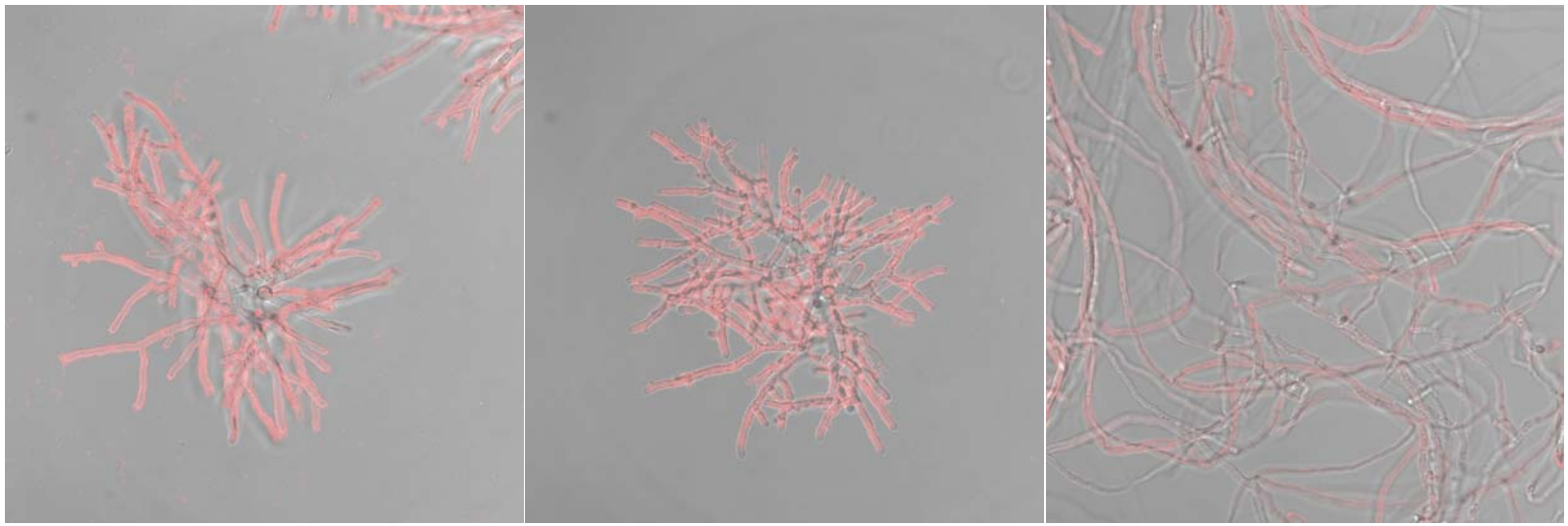


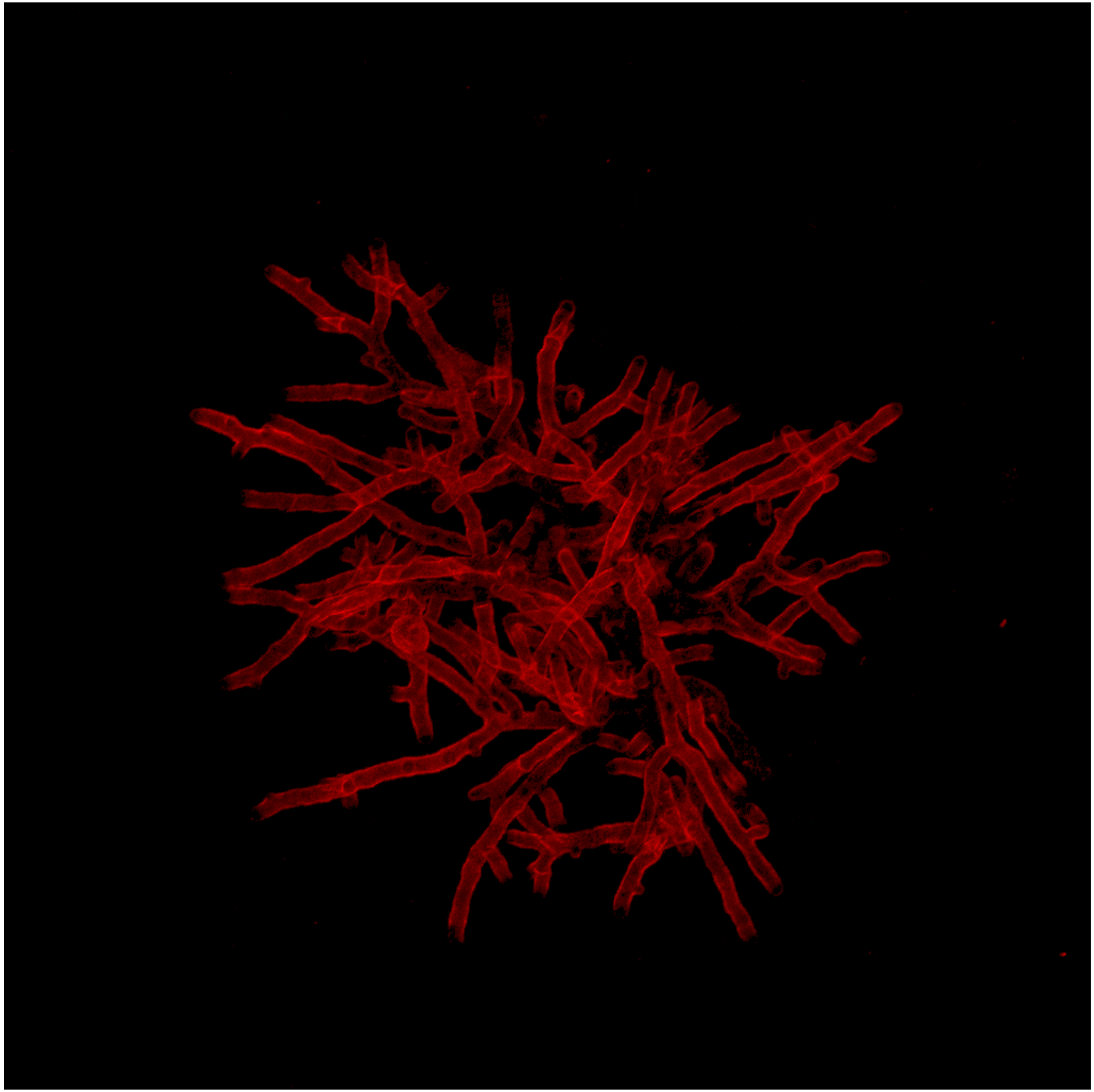
Effects of echinocandin drugs on fungal β-glucan exposure



Echinocandin drugs increase β-glucan surface immunoreactivity on hyphae

Caspofungin **Micafungin** **No Echinocandin**





Quantitative Analysis of β-glucan Immunoreactivity associated with Caspofungin-treated and Untreated Hyphae

	Integrated Fluorescence Intensity/Fungal Mass (Arbitrary Units)	
	Caspofungin-treated Hyphae	Untreated Hyphae
Expt. 1	21.4 ± 8.3*	1.83 ± 0.73
Expt. 2	43.7 ± 7.0*	2.96 ± 4.67

Each value represents the average ratio (± SD) of β-glucan immunofluorescence intensity normalized to hyphal mass as calculated from 4-5 fields of view per condition.

* $p < 0.02$ compared to control condition (untreated hyphae).

Summary (Part II)

- **Echinocandin drugs alter inflammatory responses to *A. fumigatus***
  - **by altering fungal surface β-glucan levels**
  - **and triggering dectin-1-dependent responses**

- **Enhanced inflammatory responses to drug-treated hyphae represents a novel mechanism of action that is independent of effects on fungal growth**

- **This immunopharmacologic mechanism of action may have implications for prophylactic and therapeutic strategies for invasive aspergillosis**

- **Similar results for Aspergillus and non-Aspergillus molds presented by Lamaris et al., 47th ICAAC, Chicago, IL, September 17-20th, 2007 (Abstract M-1857 and M-1858).**

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MSKCC

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