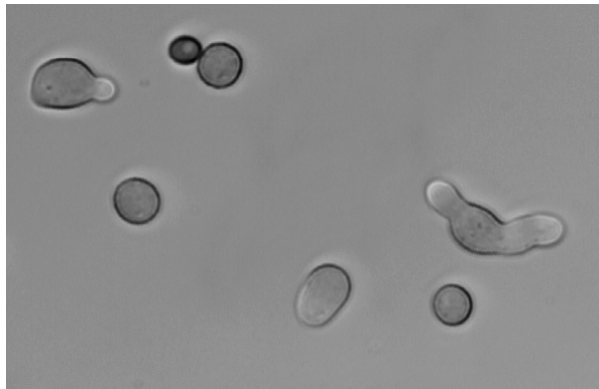
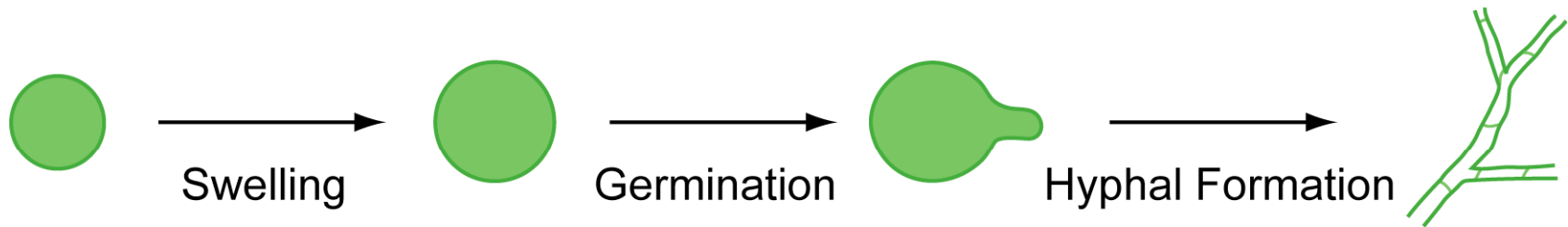


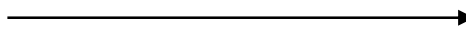
***Cracking the Fungal Armor -  
Studies on Host Defense Mechanisms  
against A. fumigatus***

**Tobias M. Hohl, MD, PhD  
Memorial Sloan-Kettering Cancer Center  
hohlt@mskcc.org**

# *A. fumigatus* Germination

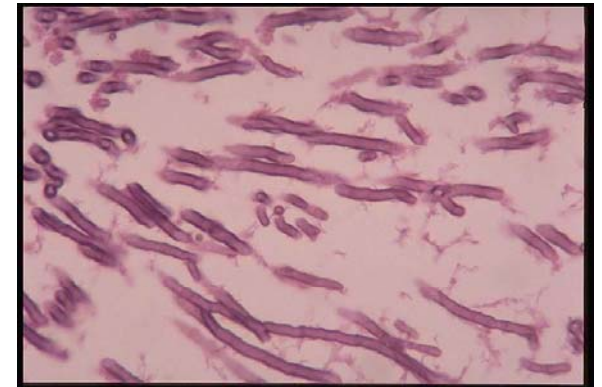


**Intact pulmonary immune defense**



**Conidial clearance**

**Defective pulmonary immune defense**

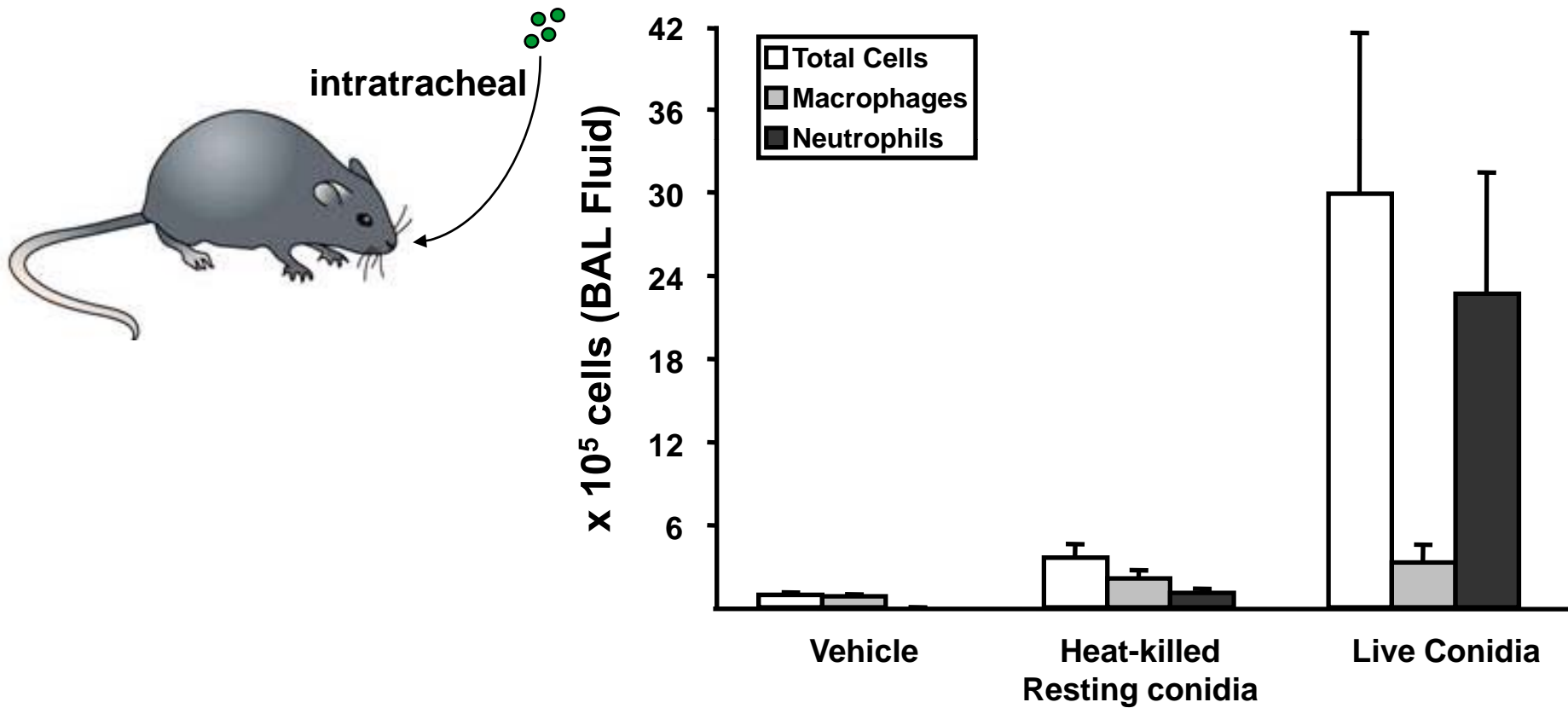


**Tissue-invasive hyphae**

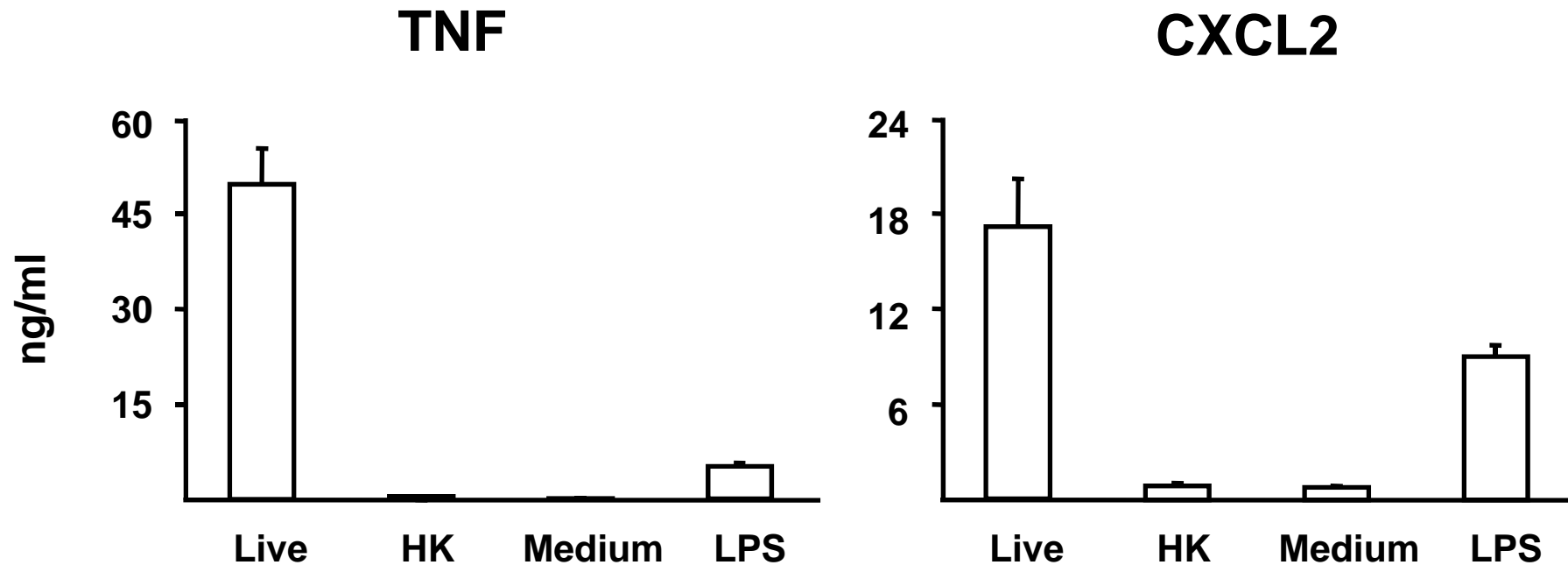
## **Host Immune Defense against *A. fumigatus***

- **Recognition of inhaled spores by the innate immune system**
- **Modulation of inflammatory responses by antifungal therapy**
- **Monocytes and the initiation of CD4 T cell responses**

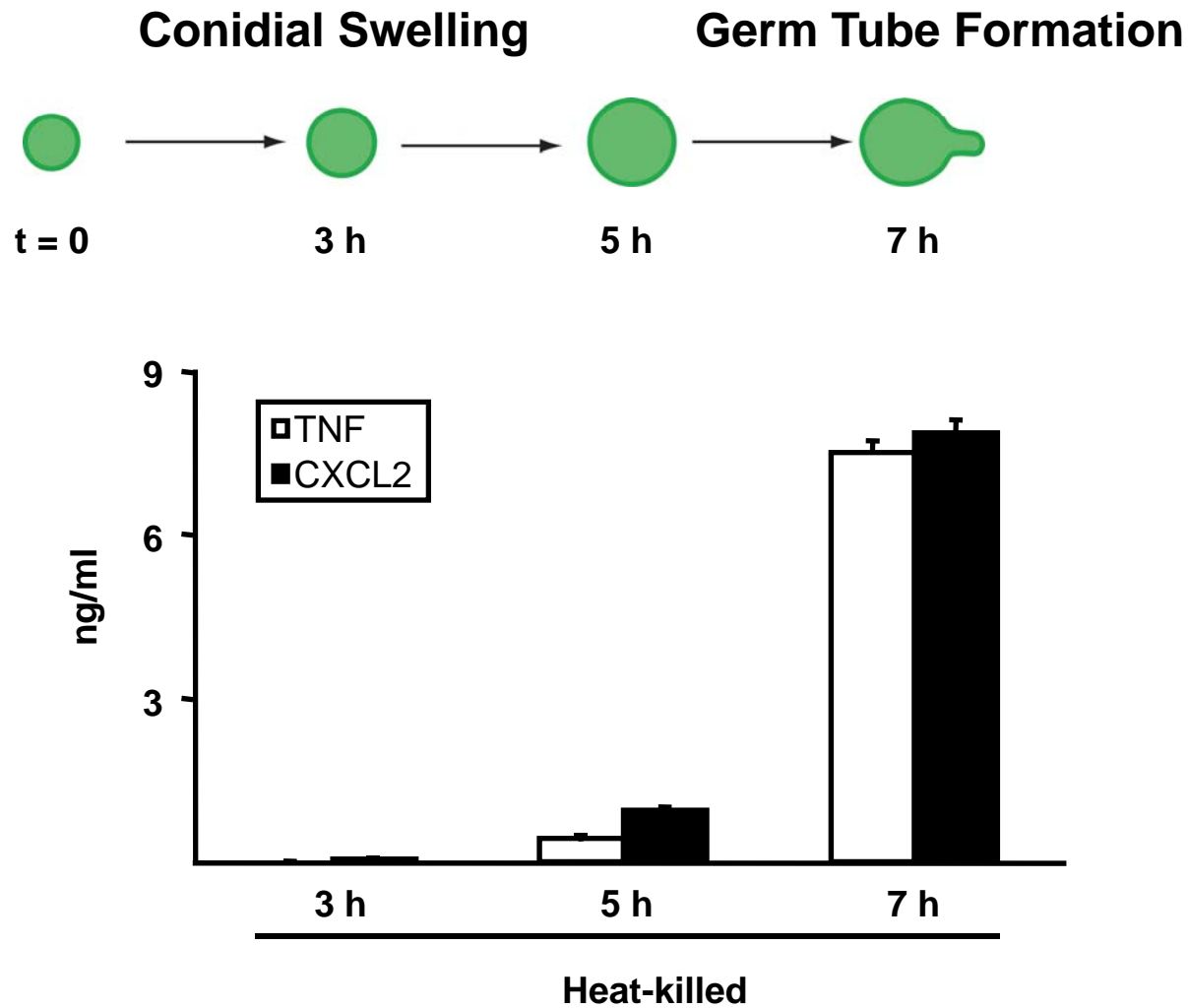
# Live Conidia induce Airway Neutrophil Recruitment



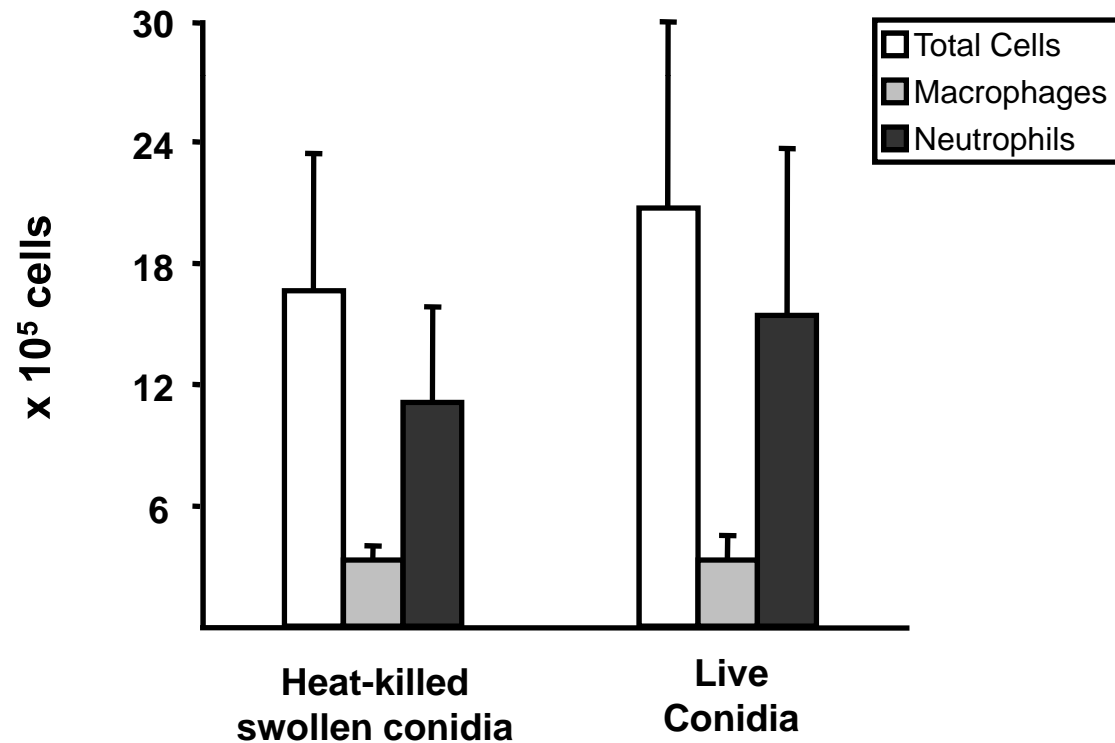
# Live Conidia induce TNF/ CXCL2 Secretion by Alveolar Macrophages



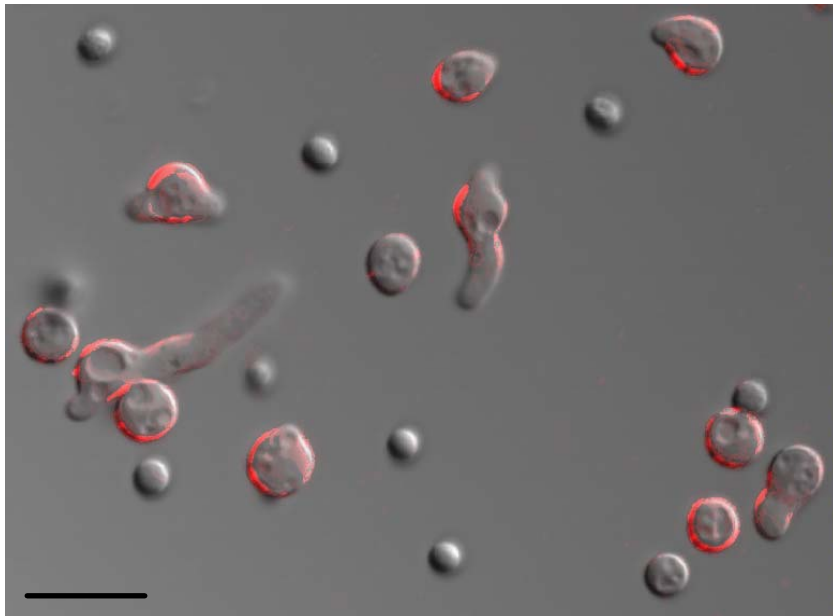
# Killed Germinating Conidia are highly Inflammatory



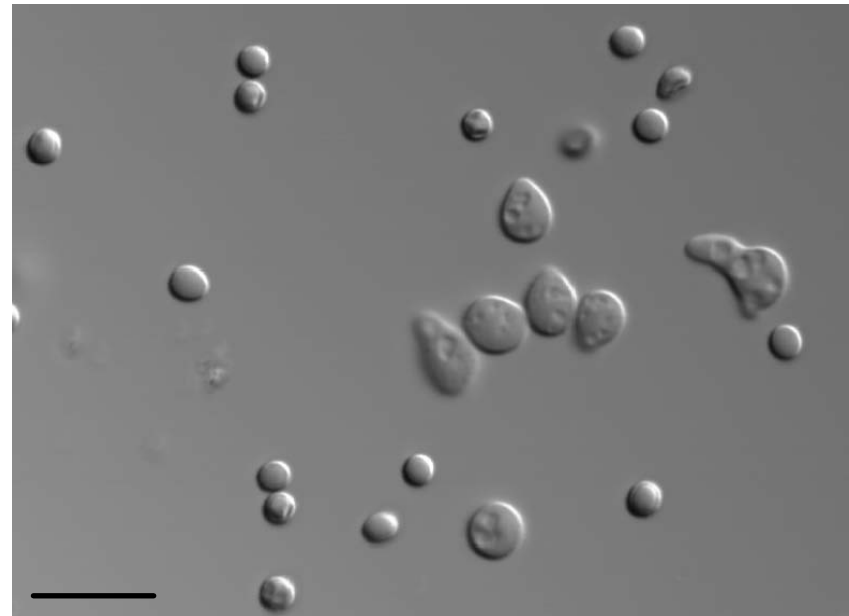
# Killed swollen Conidia induce Neutrophil Influx into the BAL fluid



## Swollen Conidia and Germlings expose $\beta$ -glucan on their surface

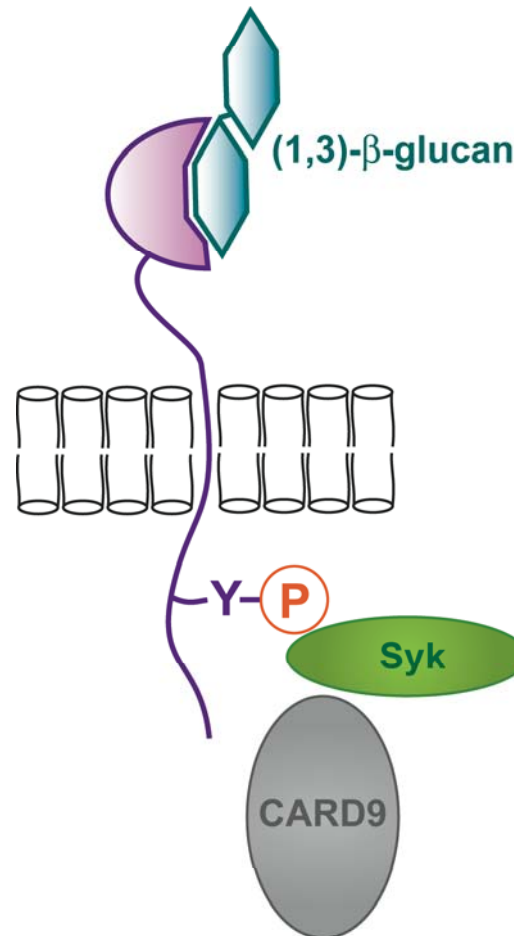


Anti  $\beta$ -glucan



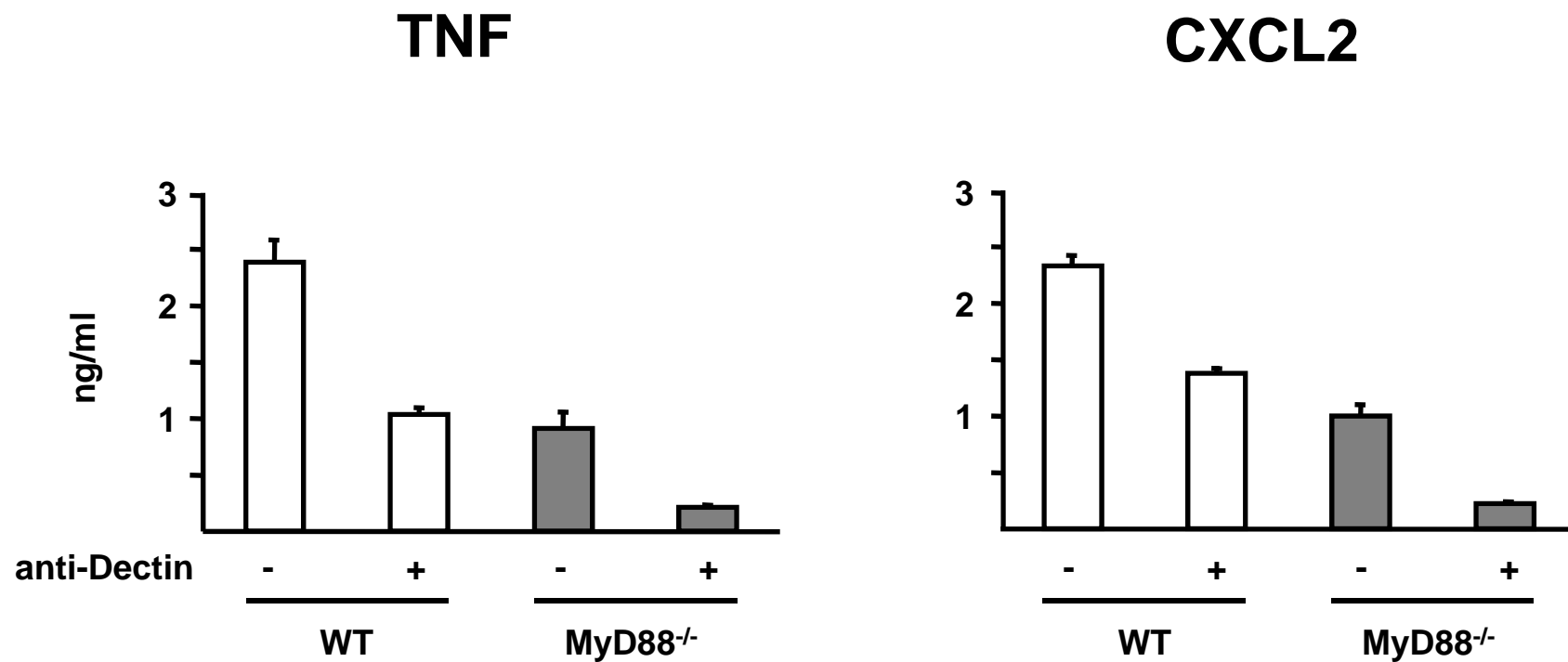
Isotype Control Ab

# Dectin-1 binds and signals in response to $\beta$ -(1,3) glucan



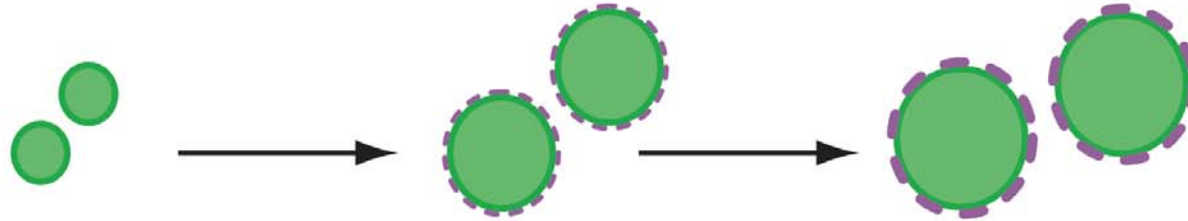
Brown, G. D., *Nat Rev Immunol* 6:33-43, 2006.

# Conidia Stimulate Dectin-1- and MyD88-dependent Pathways



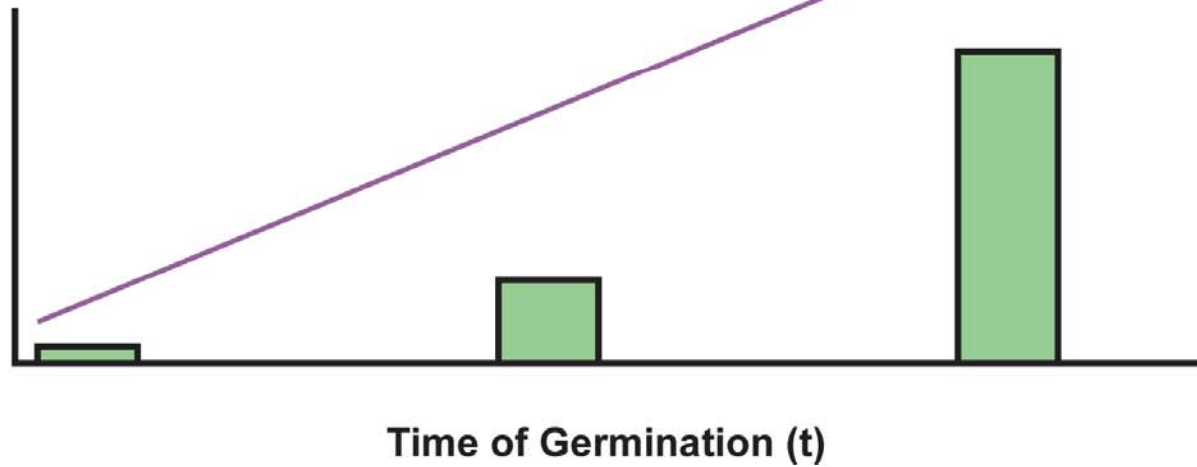
Inhaled Resting Conidia

Swollen Conidia



Alveolar M $\phi$  Inflammatory Responses  
(TNF and CXCL2 Production)

Surface  $\beta$ -glucan Exposure

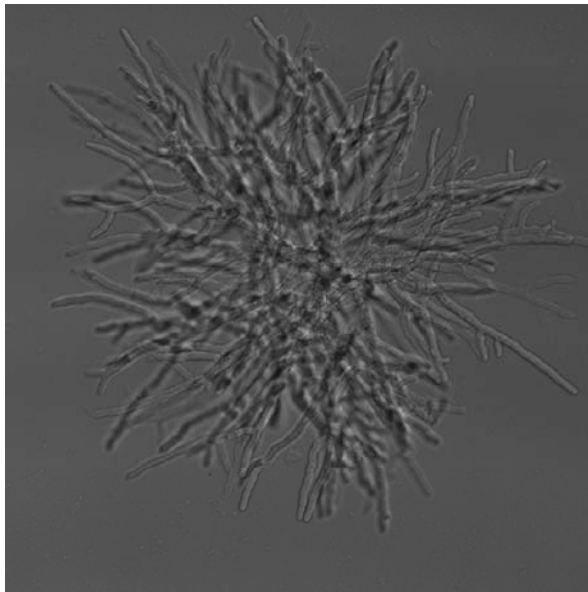


## **Host Immune Defense against *A. fumigatus***

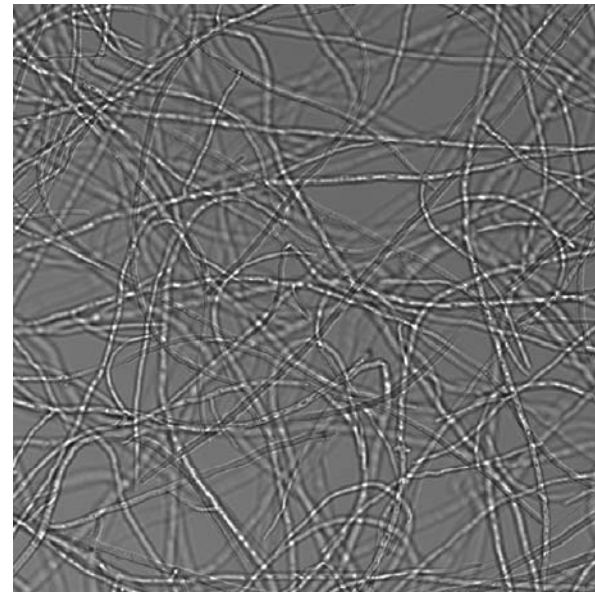
- **Recognition of inhaled spores by the innate immune system**
- **Modulation of inflammatory responses by antifungal therapy**
- **Monocytes and the initiation of CD4 T cell responses**

# Modulation of Host Inflammatory Responses by Antifungal Therapy

- Echinocandins target fungal- $\beta$ -D-glucan synthase
- Echinocandins reduce *A. fumigatus* bulk  $\beta$ -glucan levels  
(Kahn, J. et al., *Antimicrob Agents Chemother* 50:2214-2216, 2006)
- Echinocandins do not fully inhibit *A. fumigatus* growth, yet induce prominent morphologic changes at or above the MEC

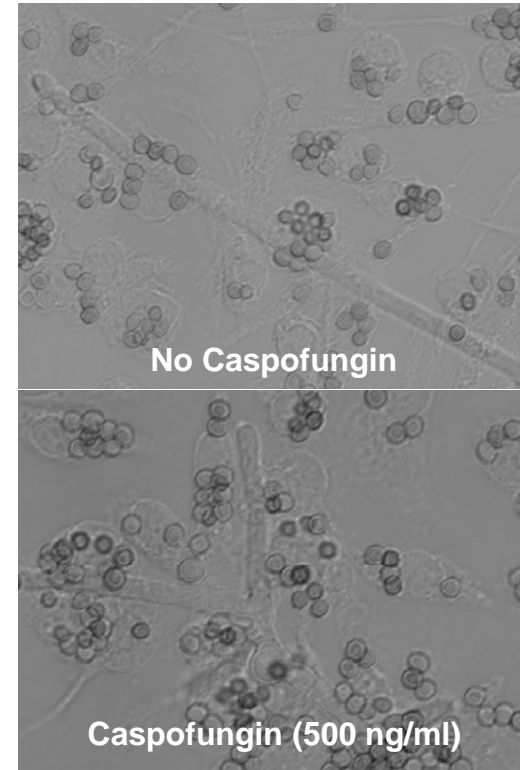
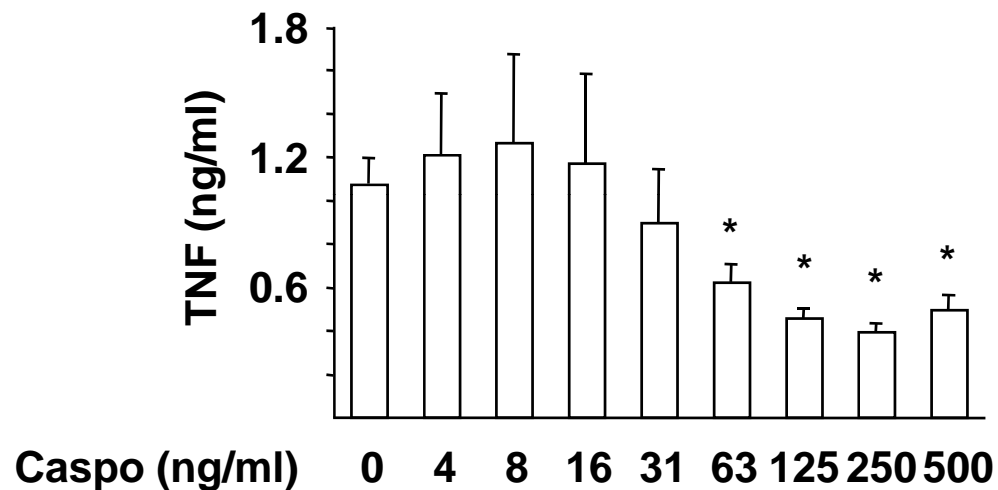


1 x MEC Caspofungin (63 ng/ml)



No Caspofungin

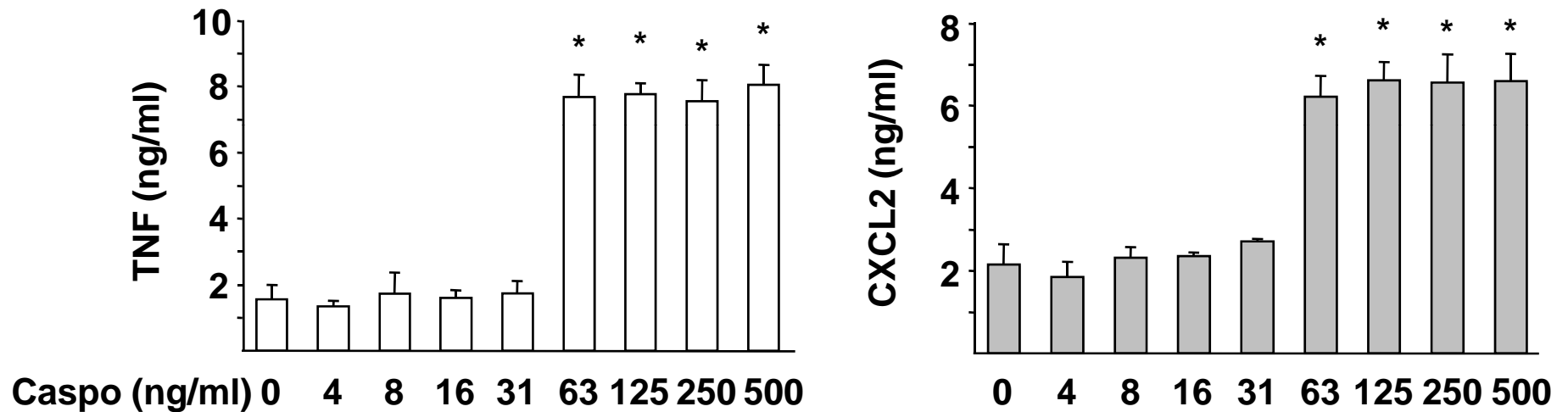
# Caspofungin Decreases Macrophage Inflammatory Responses to Conidia



## BMM $\phi$ TNF/CXCL2 release (500 ng/ml caspofungin vs. no drug exposure):

- TNF  $0.49 \pm 0.04^*$  (range 0.46-0.54; n=4)
- CXCL2  $0.55 \pm 0.10^*$  (range 0.43-0.62; n=4)

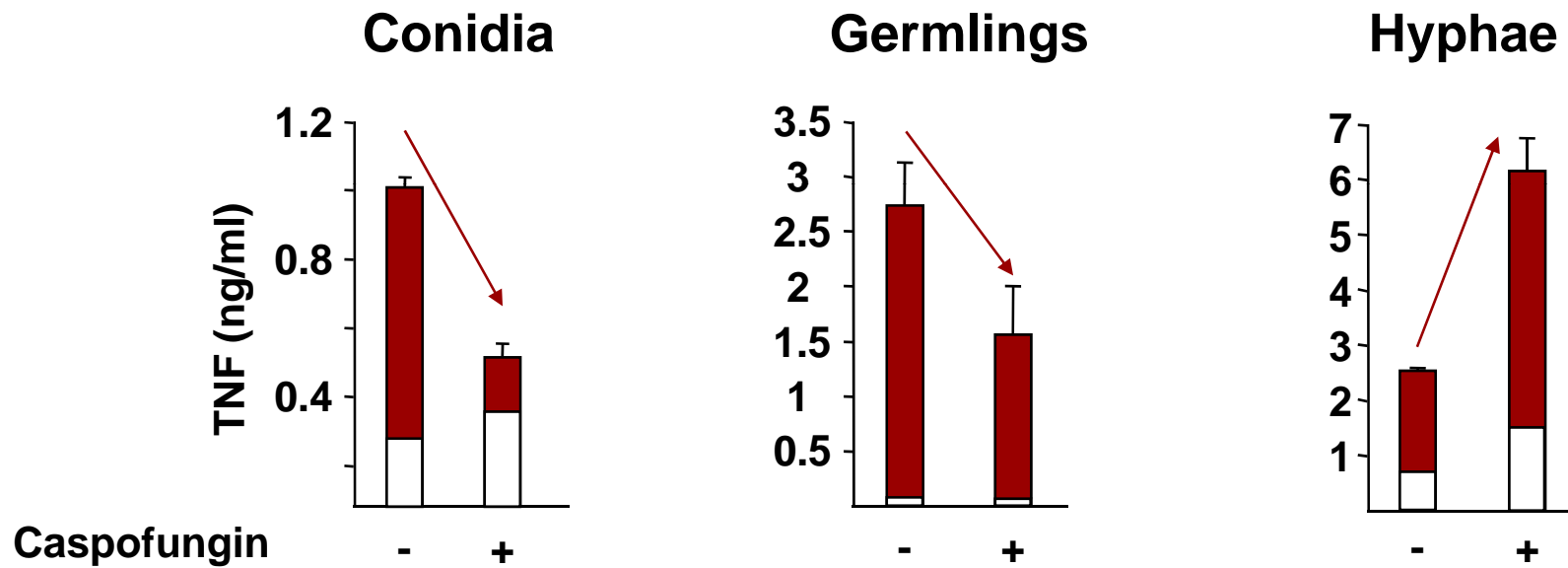
## Caspofungin Enhances Macrophage Inflammatory Responses to Hyphae



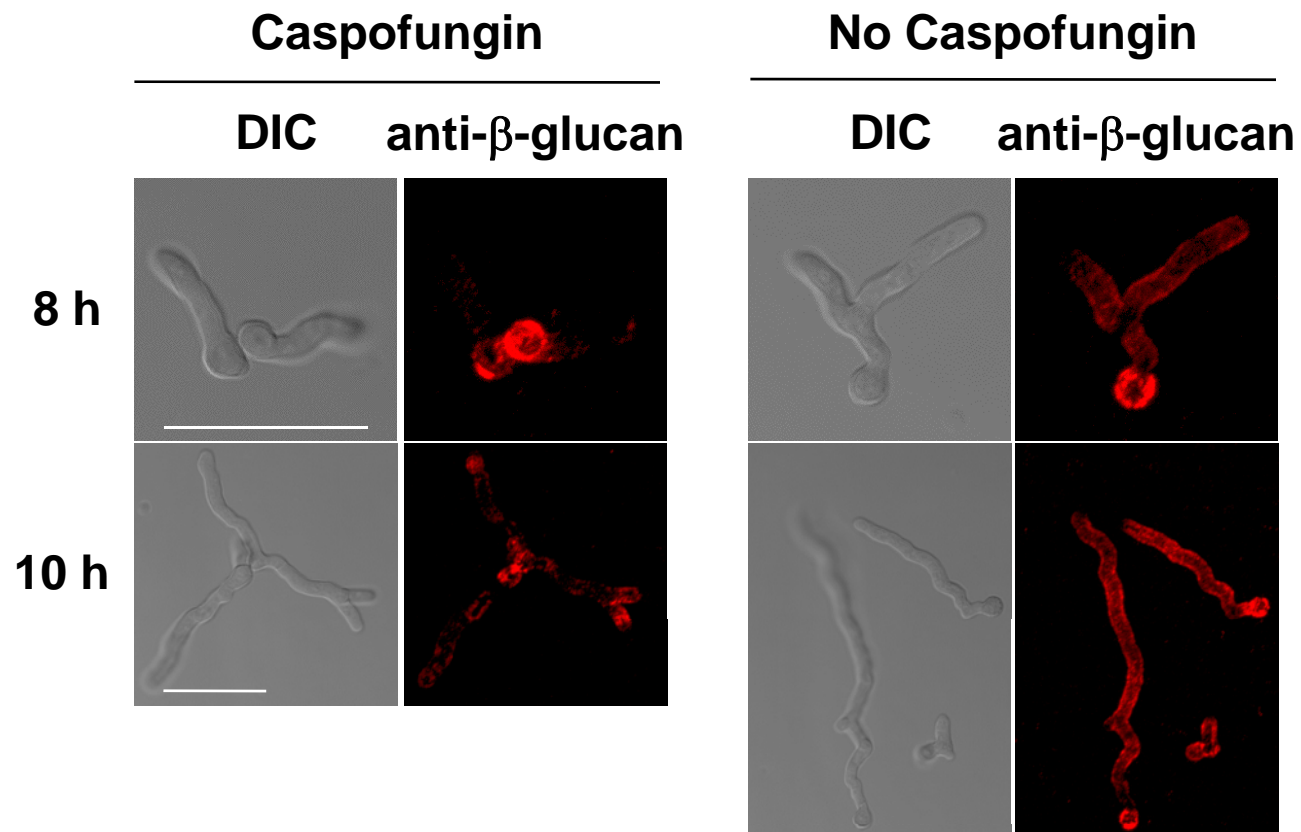
**BMM $\phi$  TNF/CXCL2 release (500 ng/ml caspofungin vs. no drug exposure)**

- TNF  $4.11 \pm 2.39^*$  (range 1.90-7.84; n=8)
- CXCL2  $2.90 \pm 1.40^*$  (range 1.53-5.41; n=8)

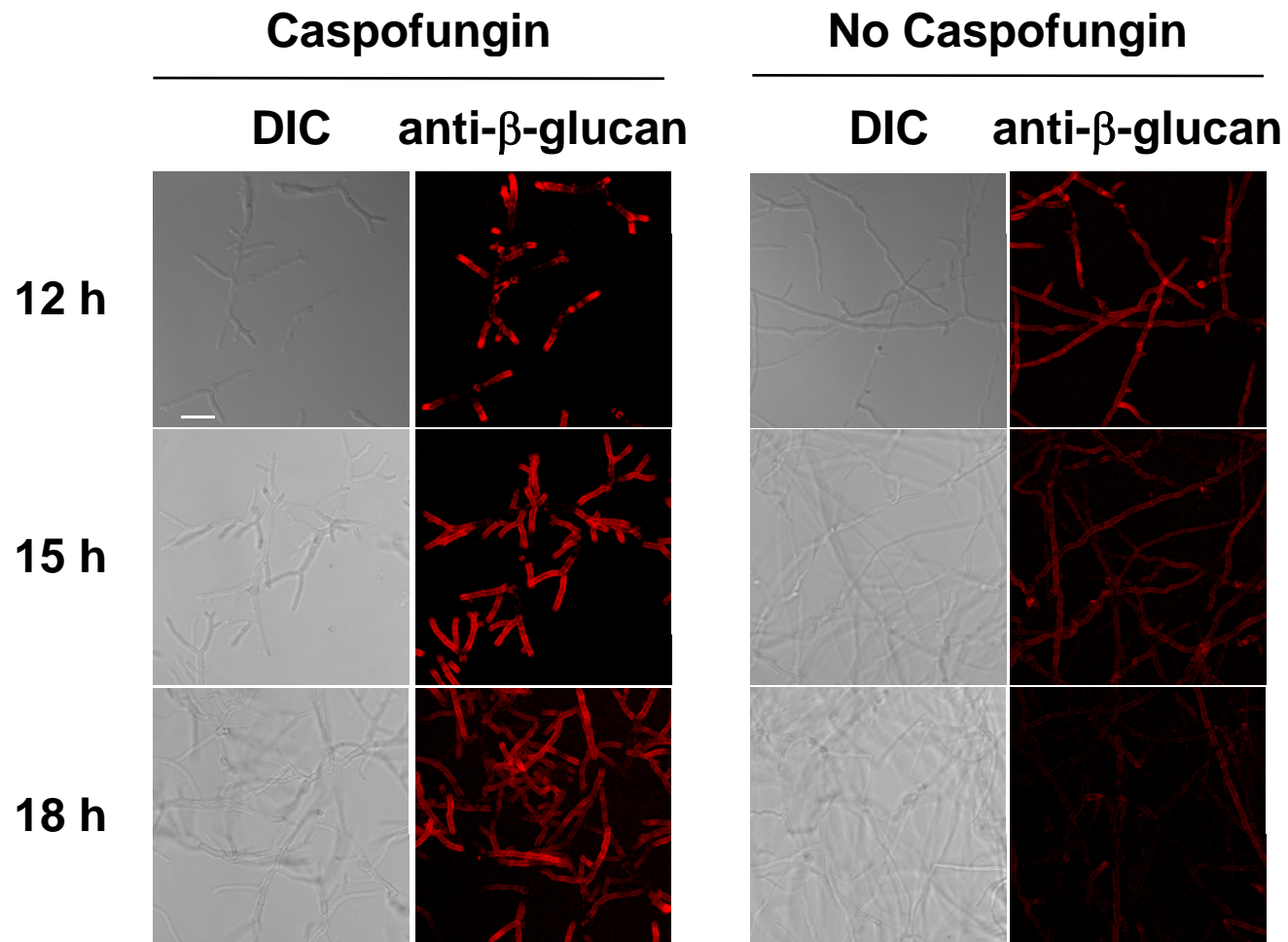
# Caspofungin Modulates Dectin-1-dependent Inflammatory Responses to Conidia, Germlings, and Hyphae



# Effects of Echinocandin Drugs on $\beta$ -glucan Exposure



# Effects of Echinocandin Drugs on $\beta$ -glucan Exposure



Hohl, T.M. et al. *J Infect Dis*, 2008.

## Quantitative Analysis of $\beta$ -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 $\pm$ 8.3*	1.83 $\pm$ 0.73
Expt. 2	43.7 $\pm$ 7.0*	2.96 $\pm$ 4.67

Each value represents the average ratio ( $\pm$  SD) of  $\beta$ -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).

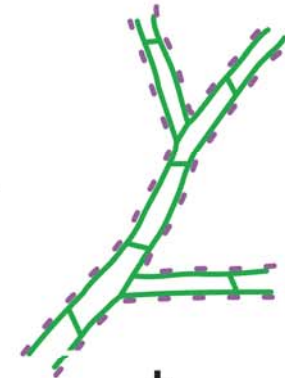
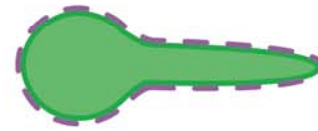
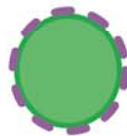
# Echinocandin Drugs have an Immunopharmacologic Mechanism of Action

Swollen Conidia

Germlings

Hyphae

No Drug



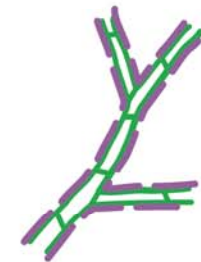
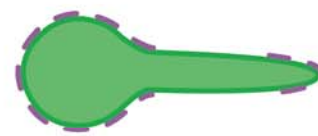
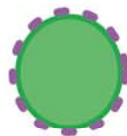
Inflammatory Response

++

++

+

Echinocandin Drug Exposure



Inflammatory Response

+

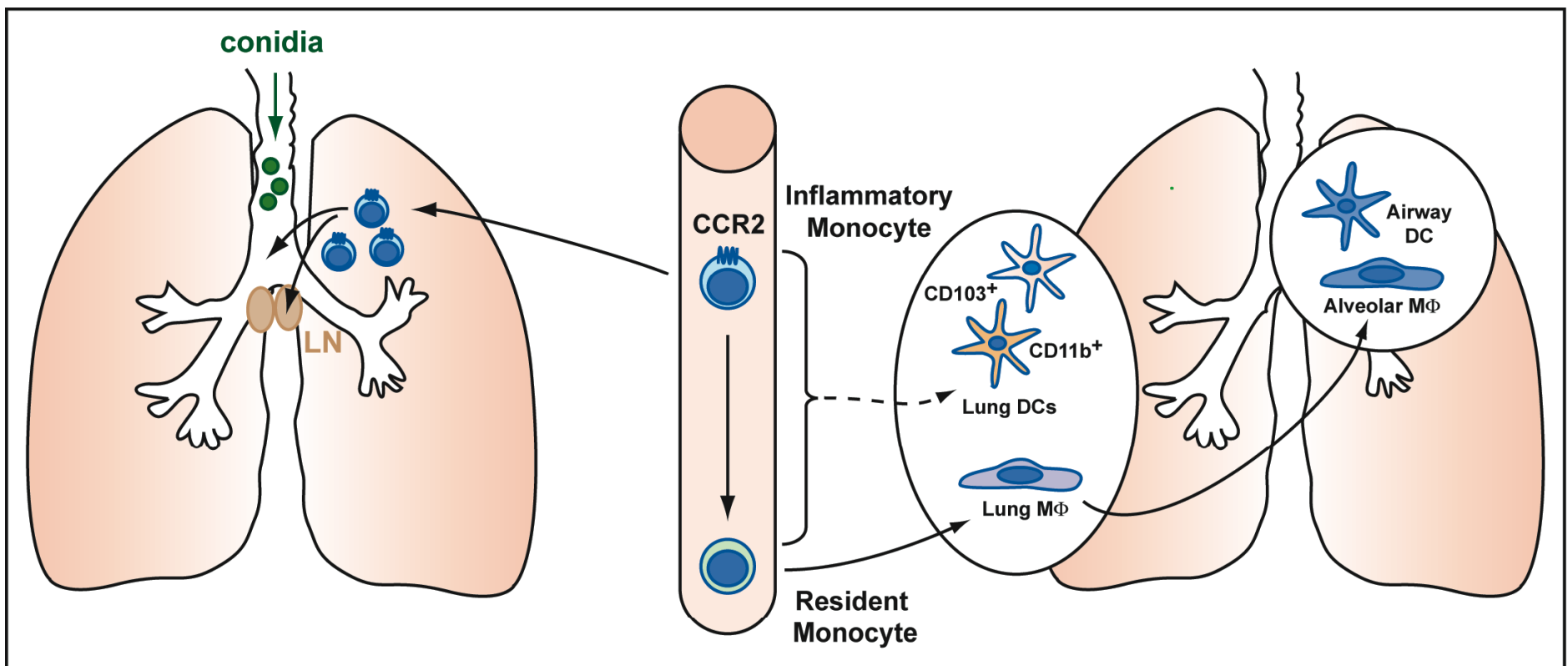
+

+++

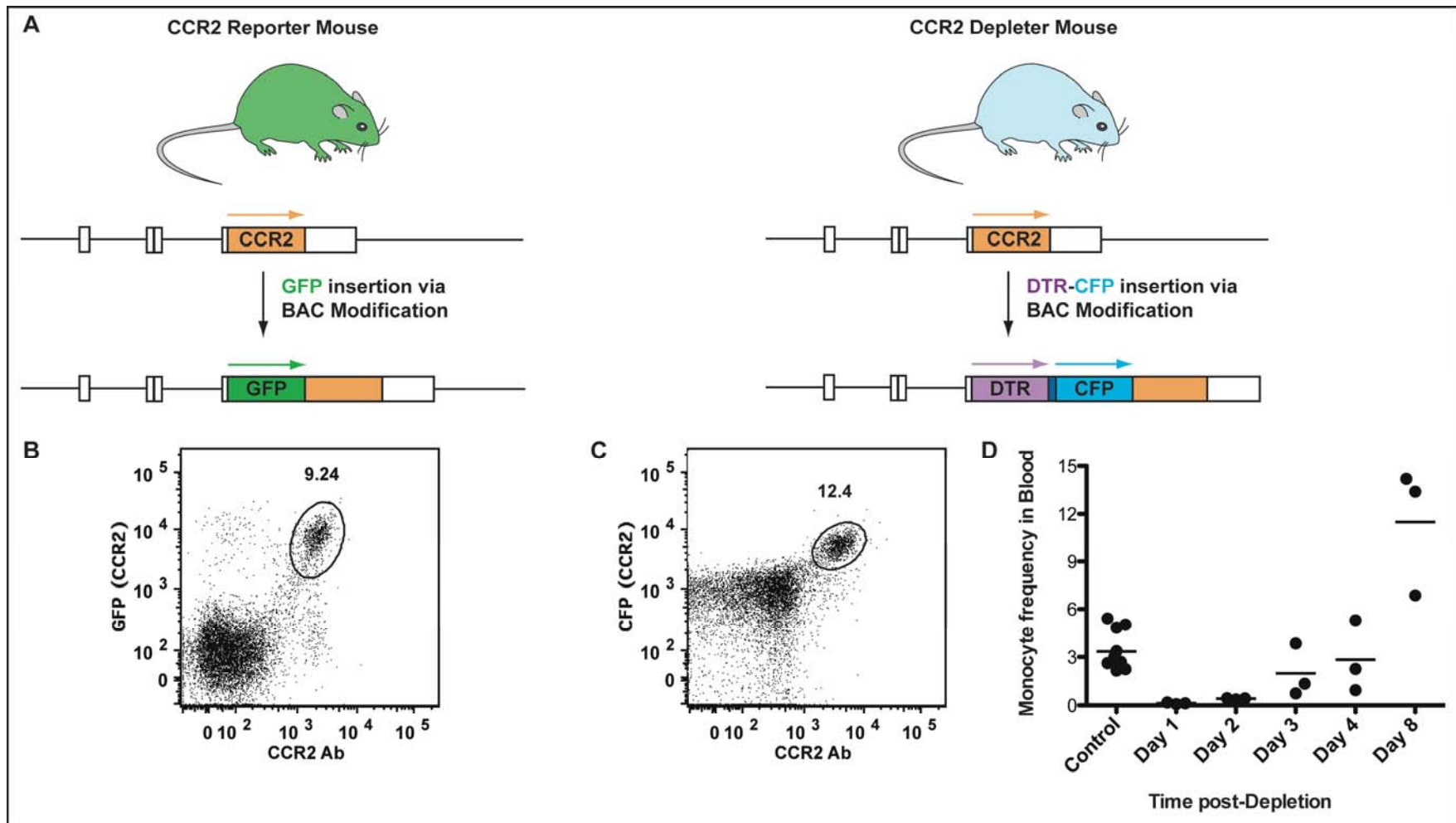
## **Host Immune Defense against *A. fumigatus***

- **Recognition of inhaled spores by the innate immune system**
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- **Monocytes and the initiation of CD4 T cell responses**

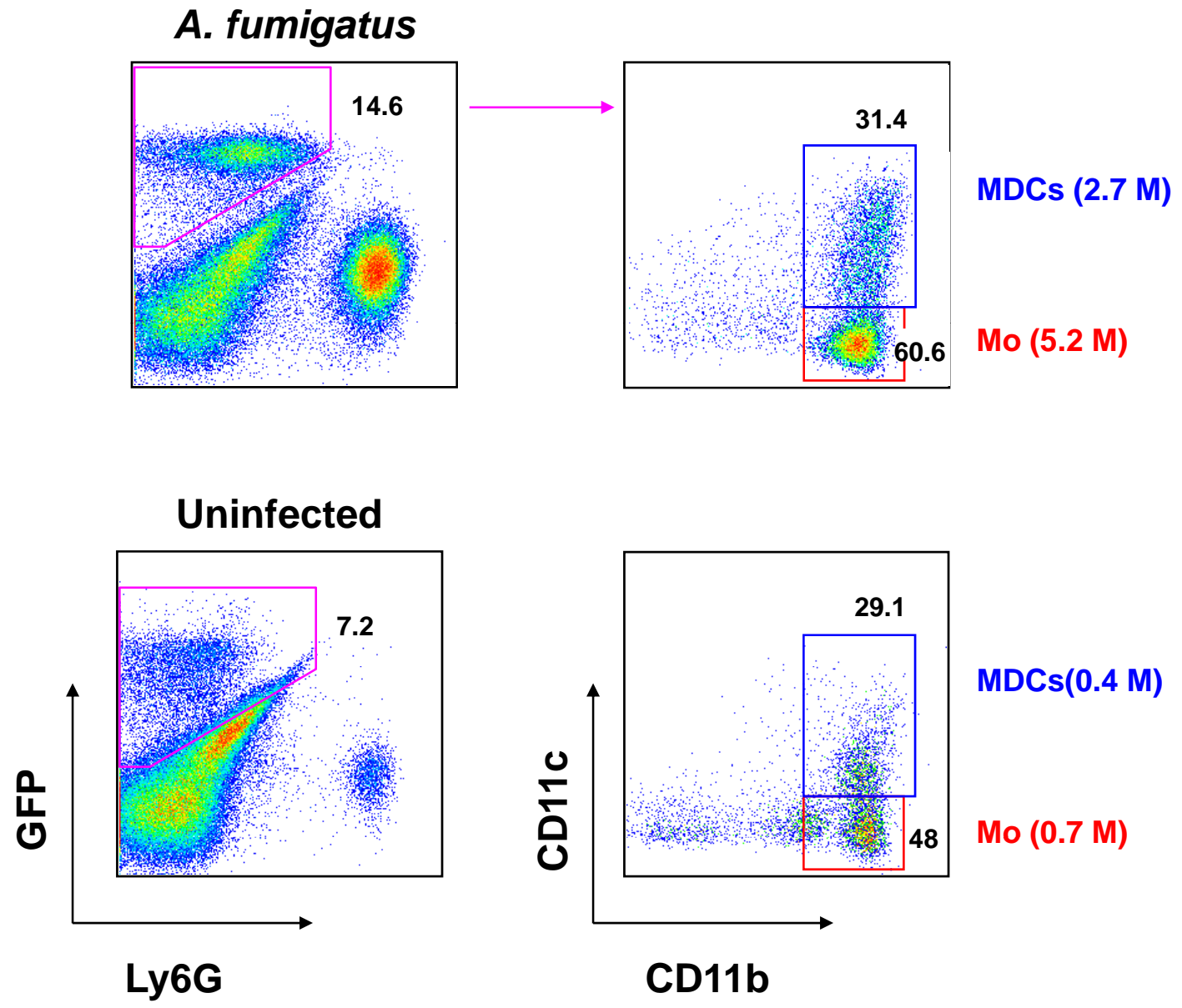
# Monocyte-derived Populations in Host Defense against *A. fumigatus*



# Development of an Experimental System to Track and Ablate Monocytes



# Recruitment of GFP<sup>+</sup> monocytes and myeloid DCs into the lungs of *A. fumigatus*-infected mice



# Cell Recruitment to mLN after intratracheal infection with *A. fumigatus* conidia

*A. fumigatus*

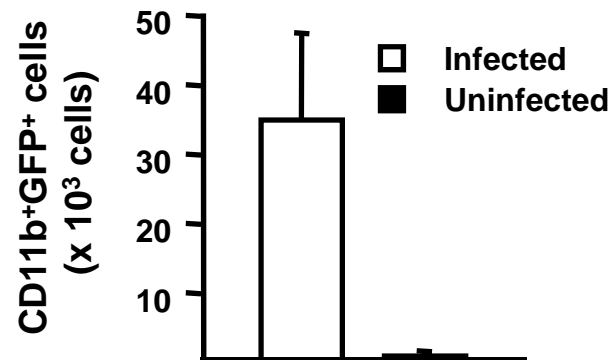
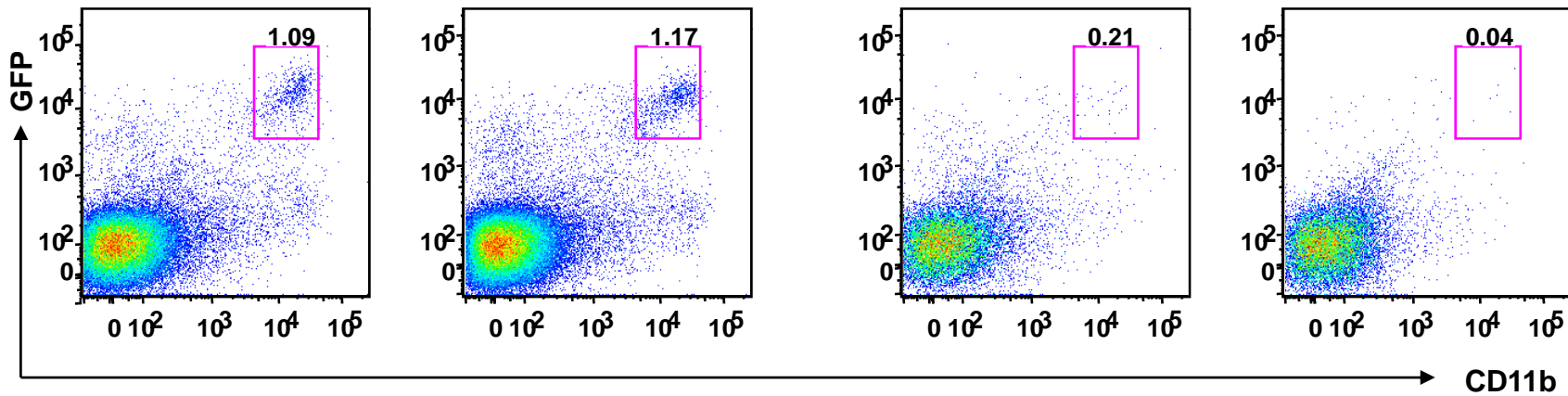
Uninfected

2.24 M

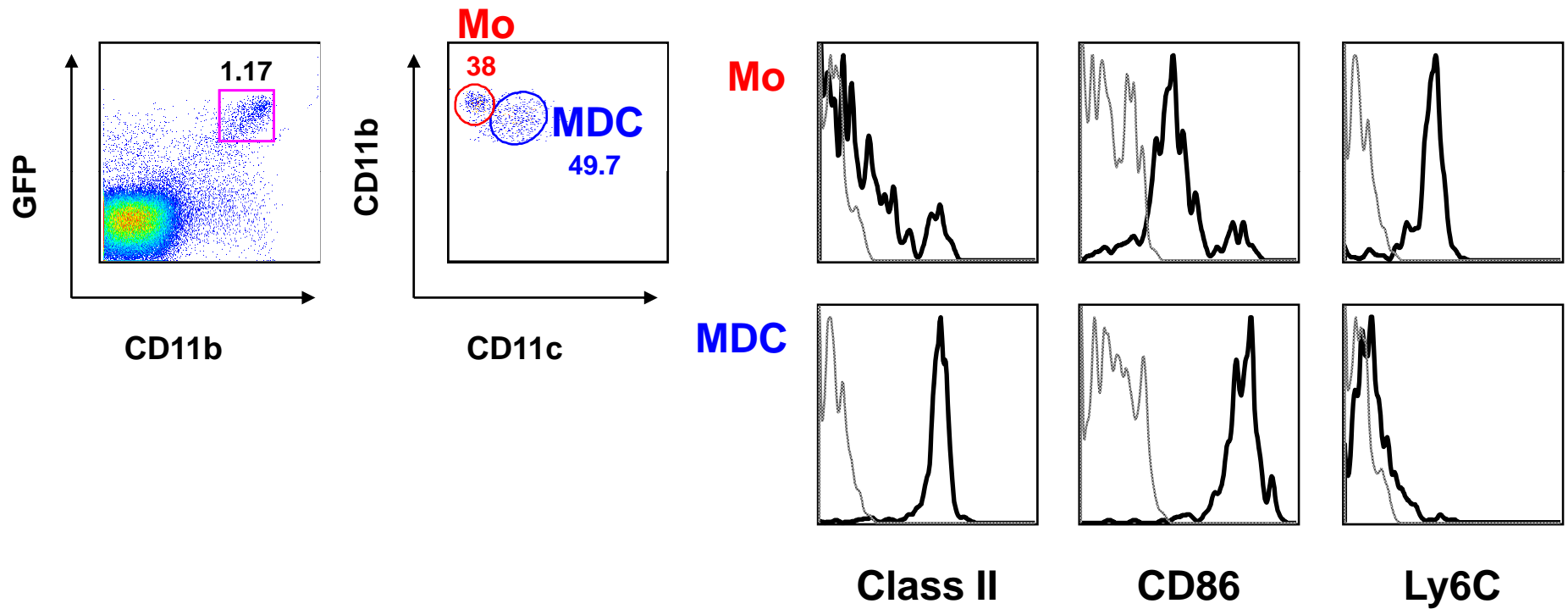
4.16 M

0.52 M

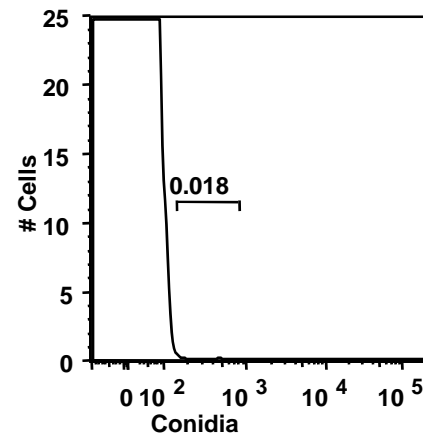
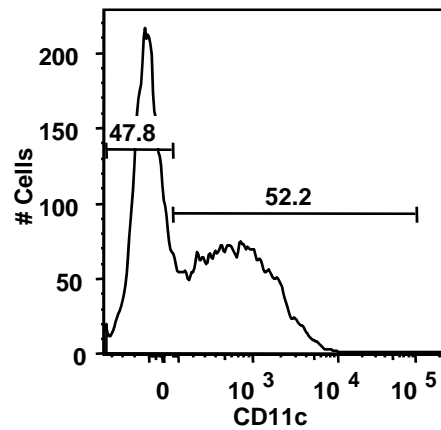
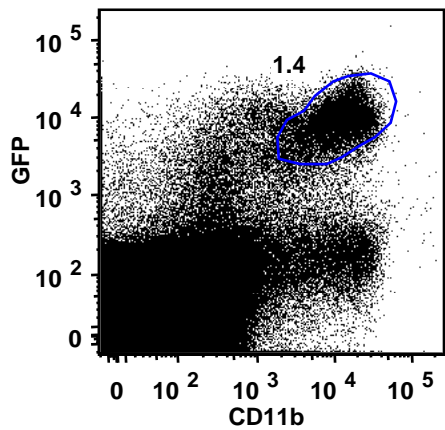
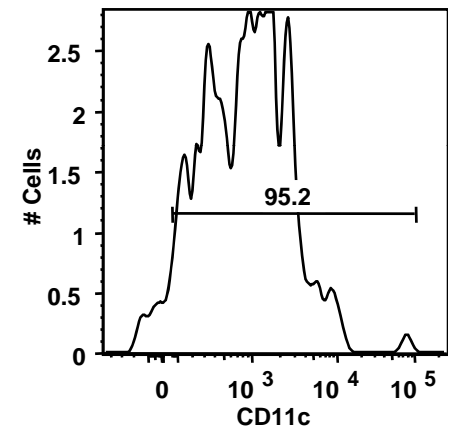
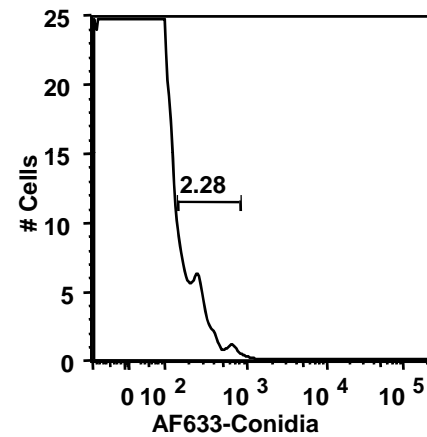
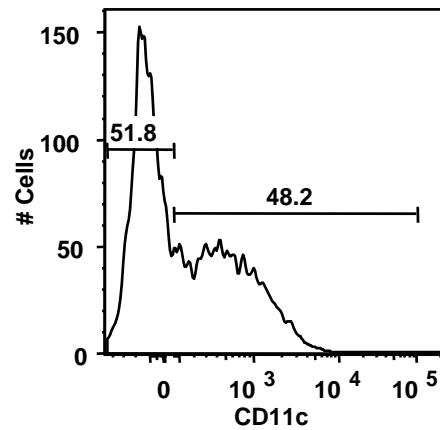
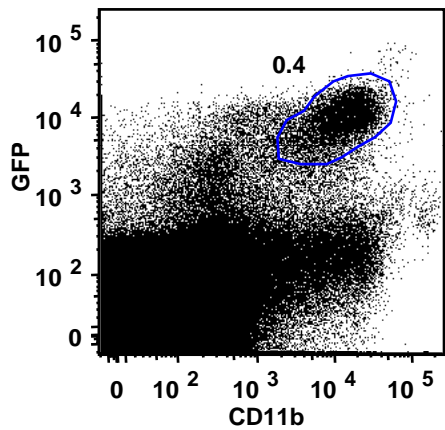
0.25 M



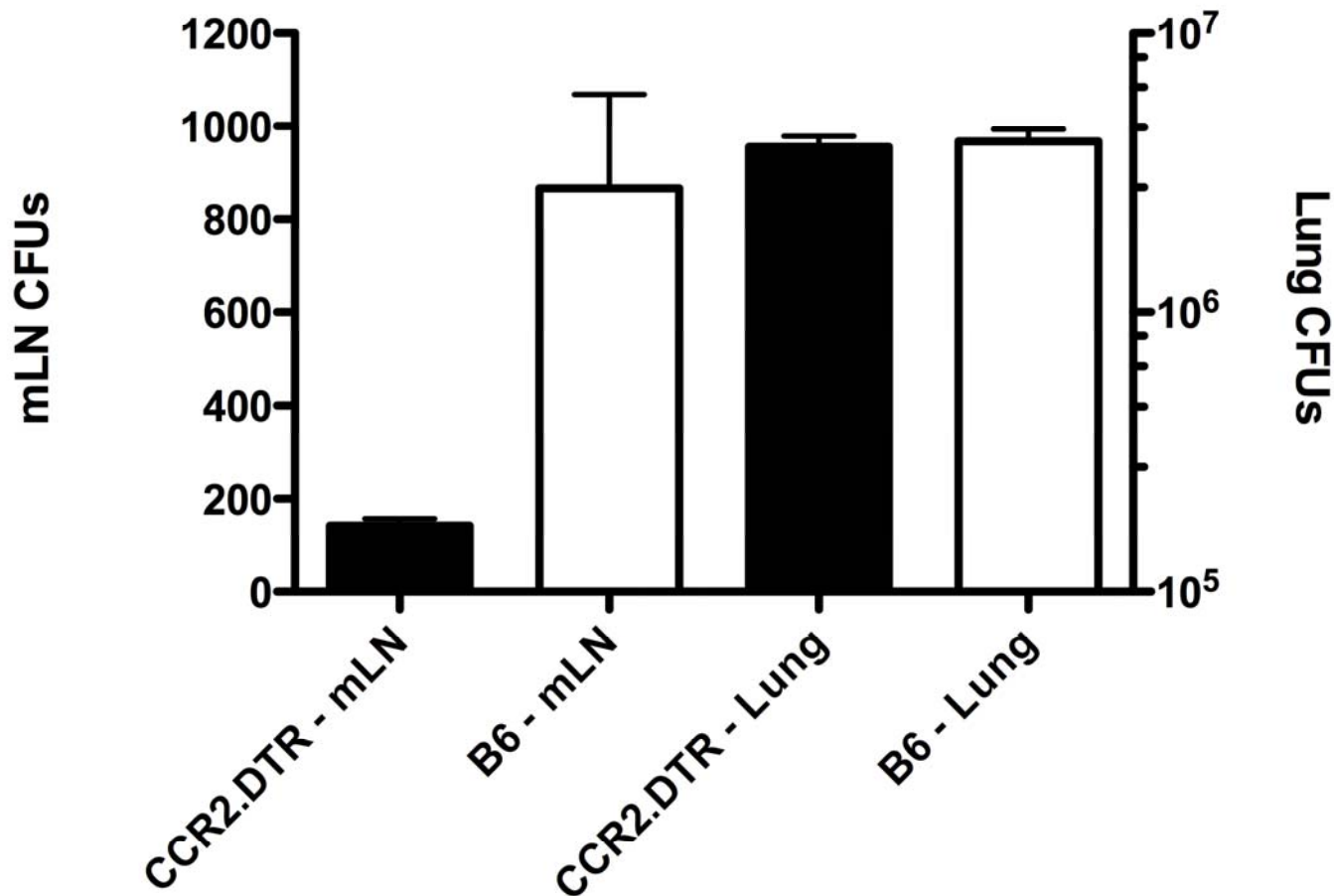
# Characterization of GFP<sup>+</sup> cells in the mLN 48 h post-infection with *A. fumigatus*



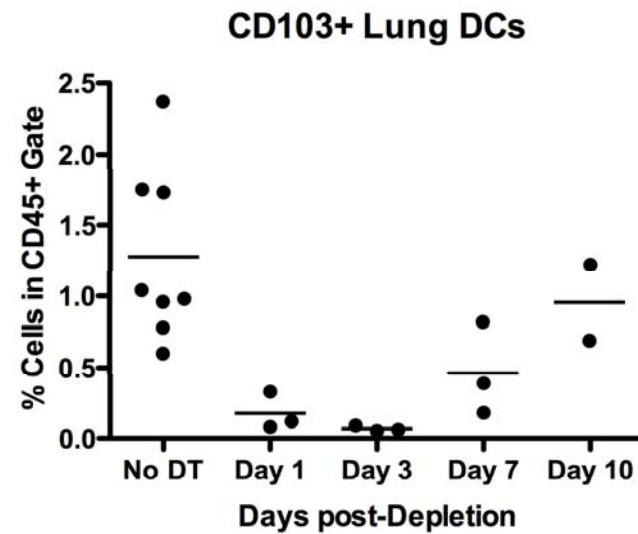
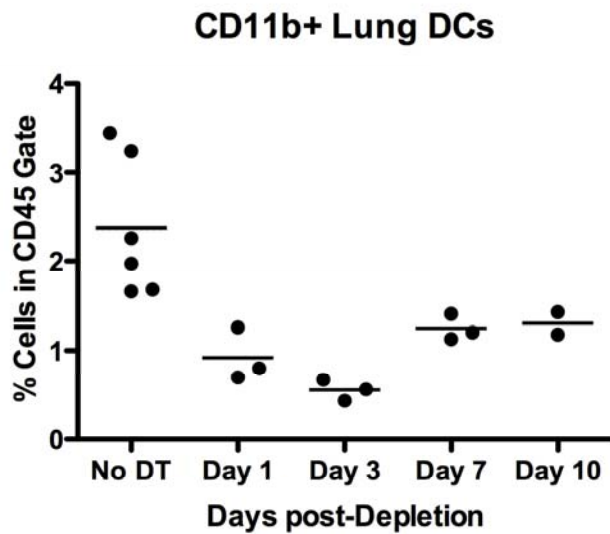
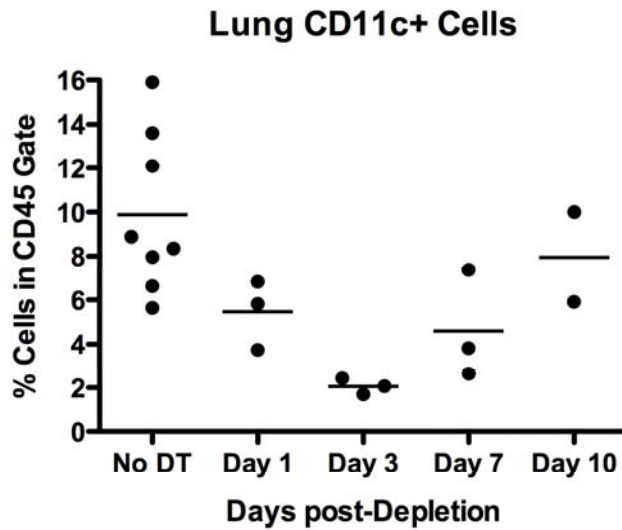
# CD11b<sup>+</sup>CD11c<sup>+</sup>GFP<sup>+</sup> cells transport labelled conidia to mLN



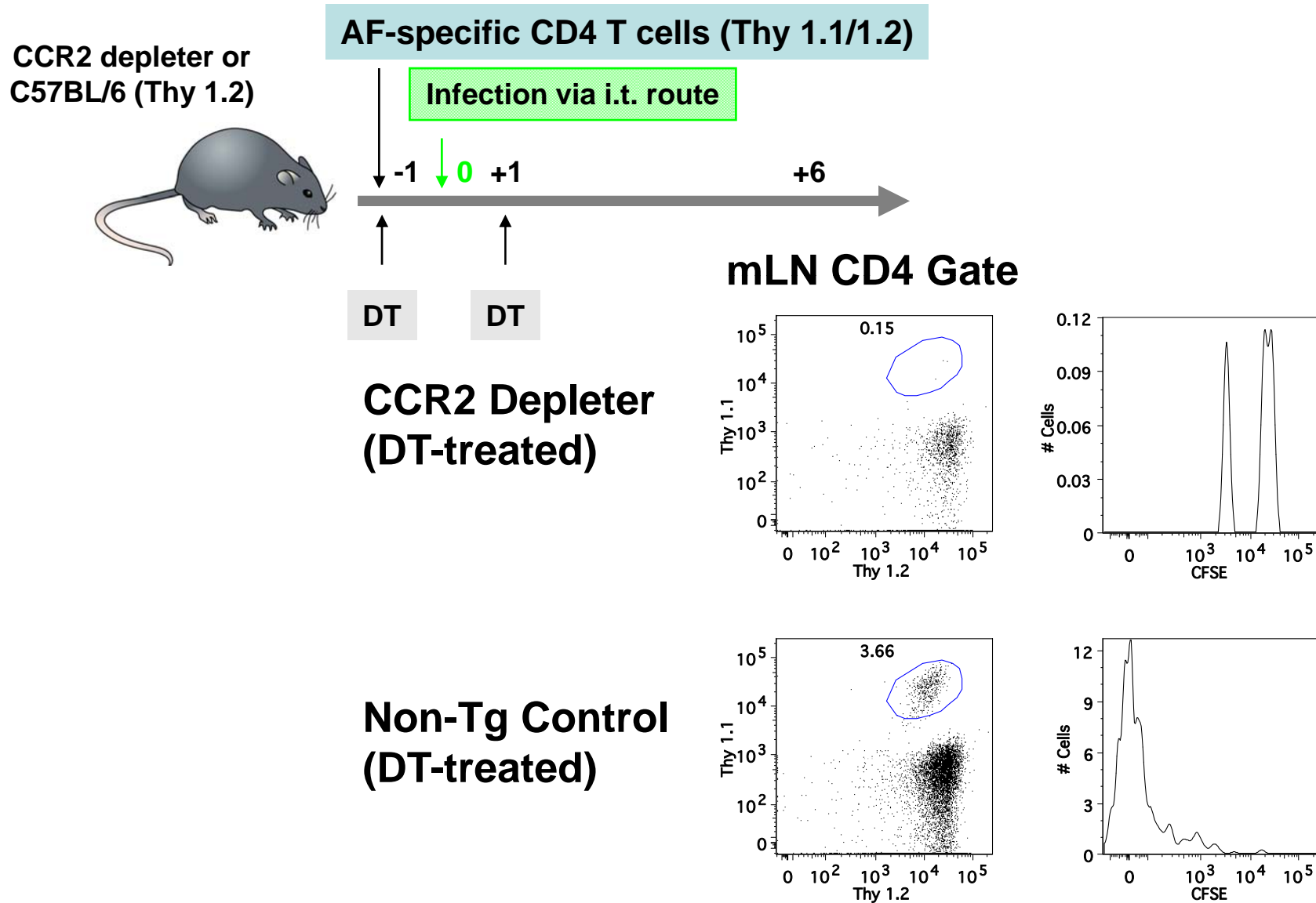
# Depletion of CCR2-expressing cells reduces conidial trafficking to the mLN



# Ablation of Lung DC subsets in CCR2 Depletor



# CCR2 Depletor mice cannot prime *A. fumigatus*-specific CD4 T cell responses



# Summary

- **CCR2 reporter and depleter mice represent valuable tools to dissect the role of monocytes and monocyte-derived cells in microbial defense**
- **Monocyte-derived lung DCs (CD11b<sup>+</sup>) transport conidia to draining lymph nodes**
- **Ablation results in loss of *A. fumigatus*-specific CD4 T cell priming**
- **Monocytopenia may contribute to impaired *A. fumigatus* CD4 T cell responses in patients undergoing HSCT**

# Acknowledgements

## *MSKCC*

- Eric Pamer
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- Alan Houghton
  
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## *University of Cape Town*

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