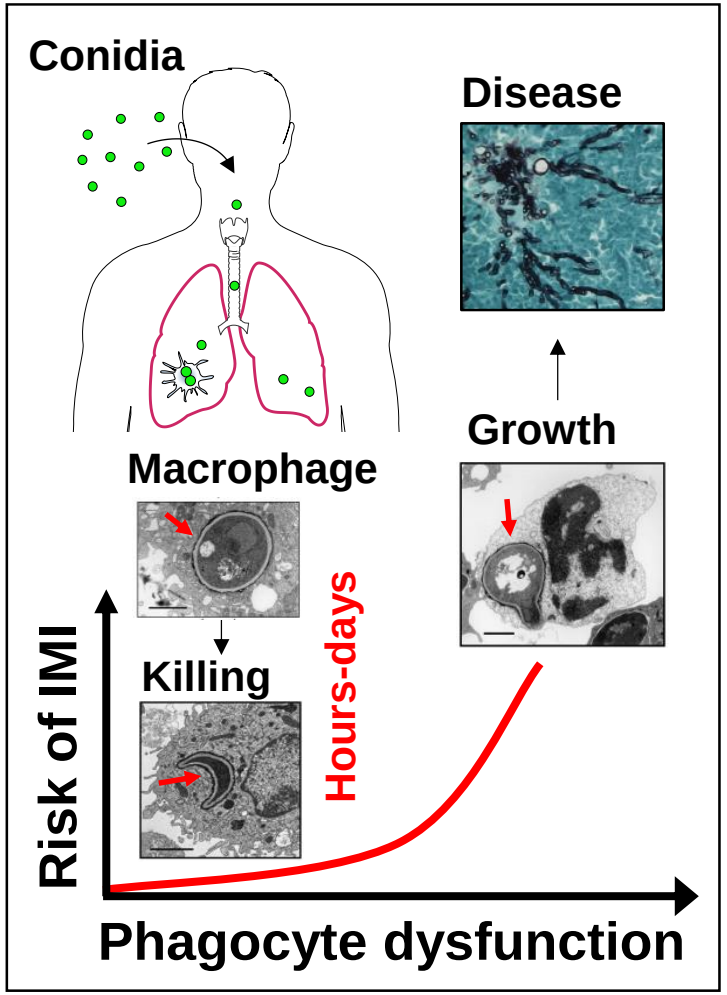
Molecular mechanisms regulating fungal phagosome biogenesis in health and disease

Giorgos Chamilos, MD
hamilos@imbb.forth.gr

Institute of Molecular Biology and Biotechnology (IMBB), Foundation of Research and Technology (FORTH), Greece

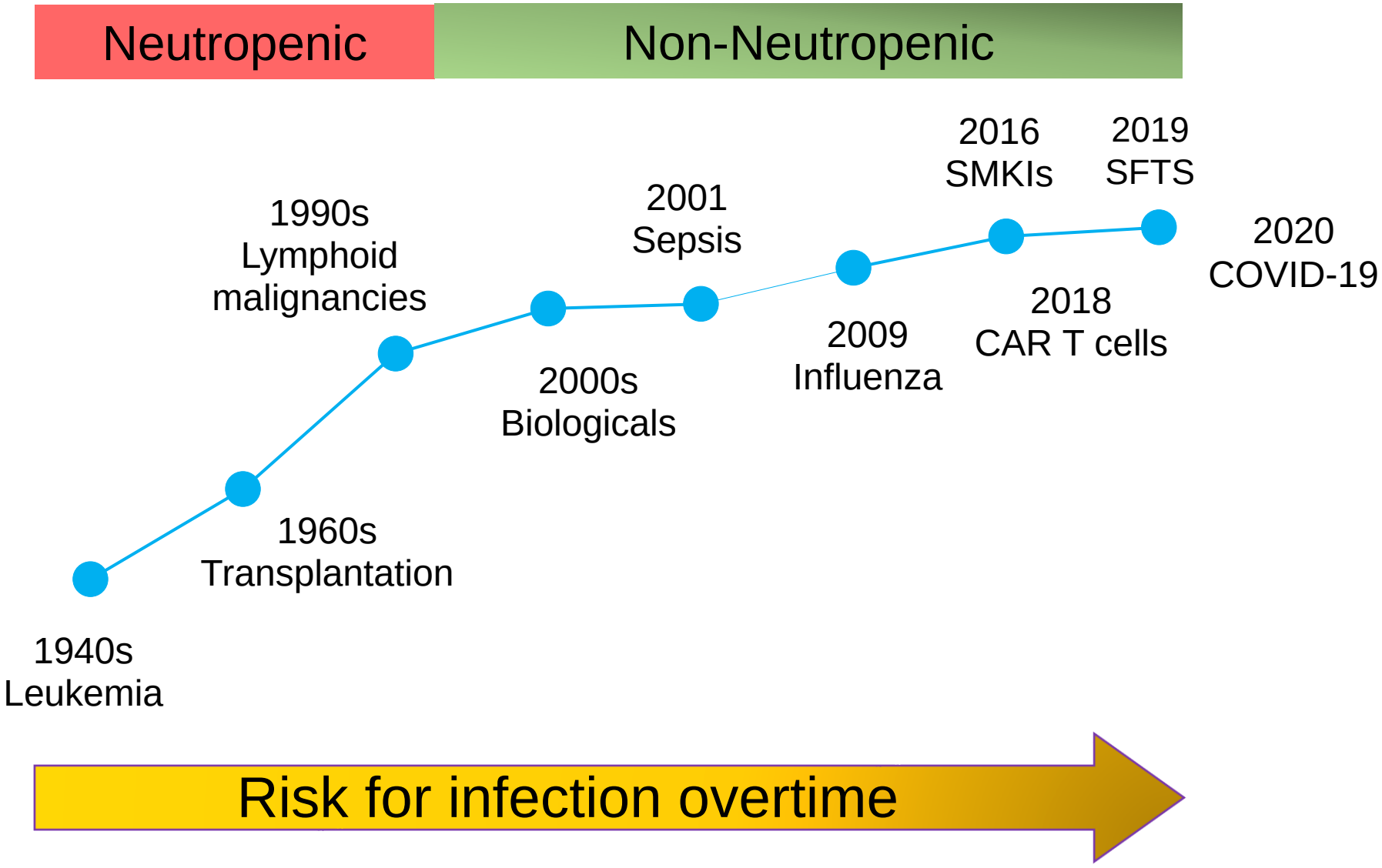
Invasive mold infections (IMI): **emerging** and **underestimated** human diseases



- ***Aspergillus*** and **Mucorales** fungi
- **400,000** pts/year, > **50%** mortality**, $2 billion** annual cost (US), **evolving** epidemiology
- Patients with mold infection display complex i**mmunometabolic defects**

The pathobiology *of invasive mold infections is incompletely understood*

The evolving epidemiology of IMIs

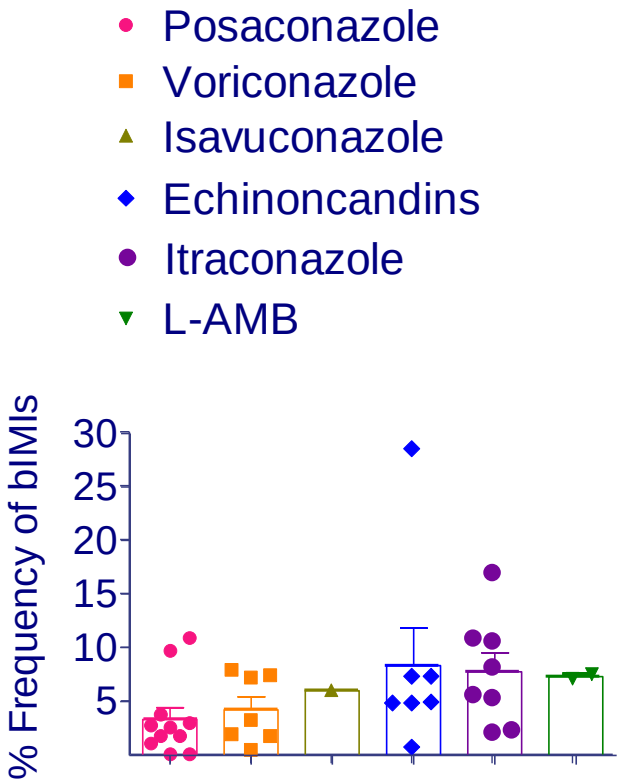


Latge & Chamilos Clin Microbiol Rev

Do we need immunotherapy for IMIs?

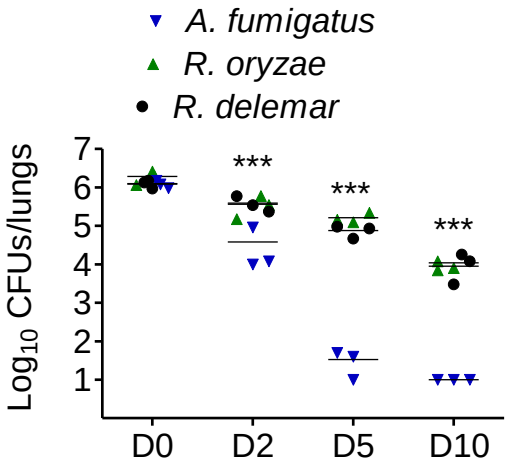
Proven or probable breakthrough (b)-IMIs in hematological malignancy patients on mold-active antifungal prophylaxis

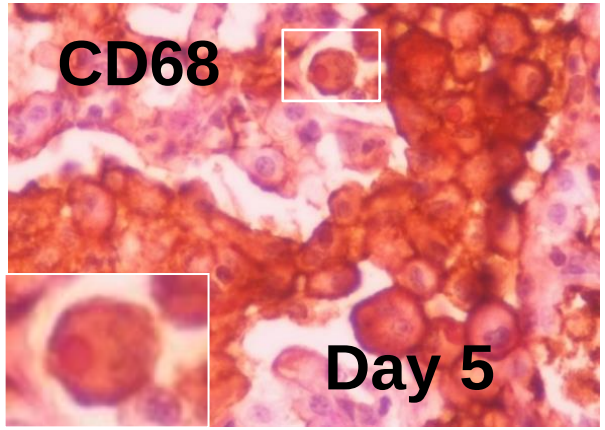
- ***No antifungal prophylactic therapy is perfect*** *in patients with severe underlying immune defects!*

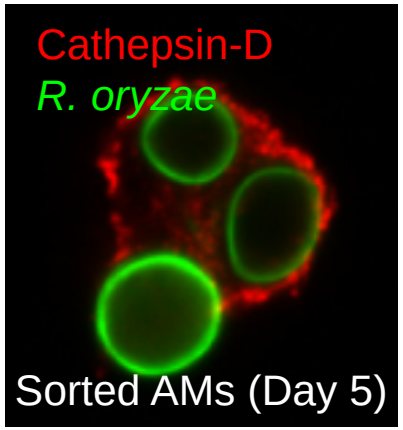


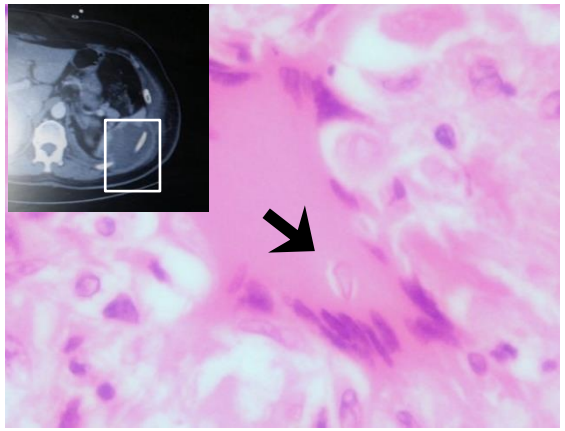
Data from Lionakis MS, Lewis RE, Kontoyiannis DP, Clin Infect Dis 2018

Lack of activity of antifungals against “persister” fungal conidia





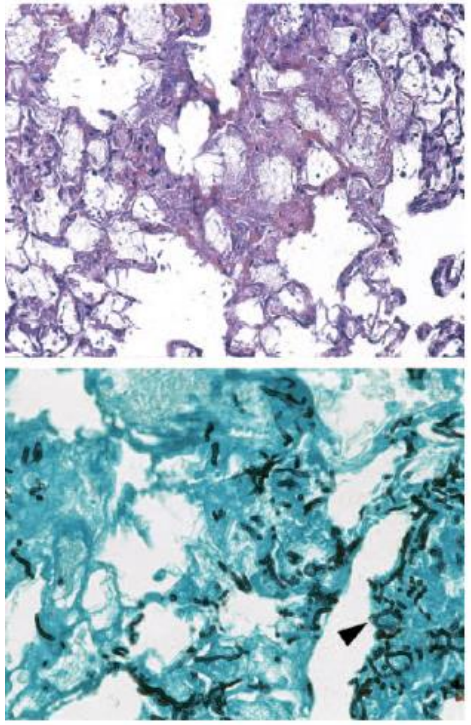




Andrianaki et al., Nat Commun 2018; unpublished data Kyrmizi I et al.

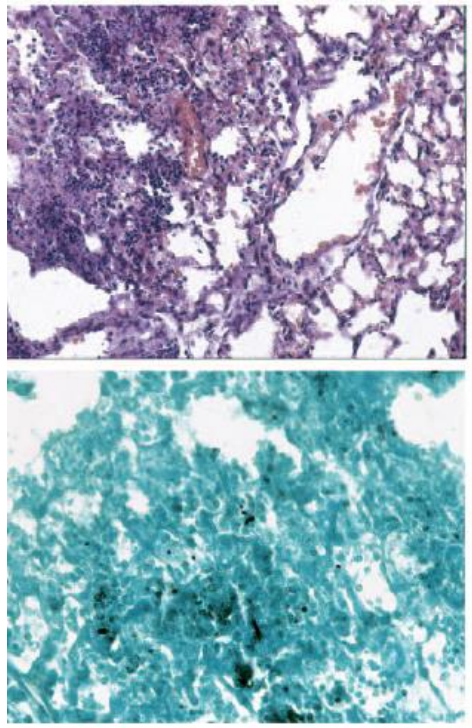
Impact of IMI pathogenesis on the activity of antifungal therapy

Neutropenia



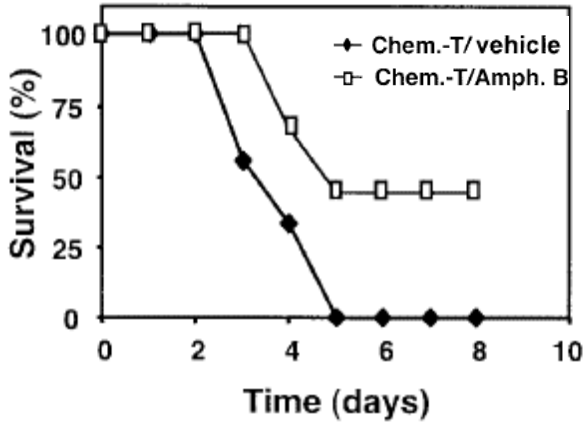
↑ fungal burden, lack of inflammation

Corticosteroids

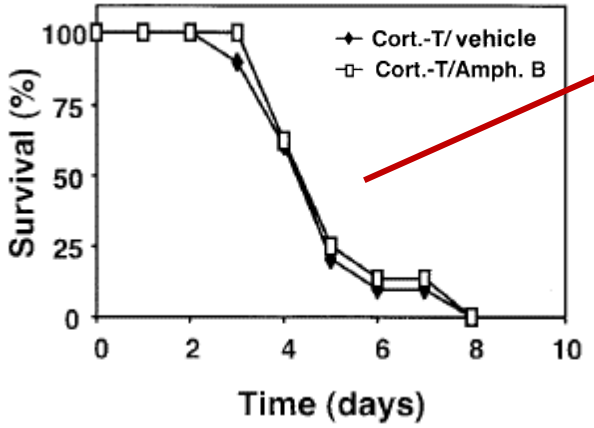


Inflammatory immunopathology

Neutropenia



Corticosteroids



Antifungal therapy has no activity!

Infect and Immun, Jan. 2005, p. 494–503

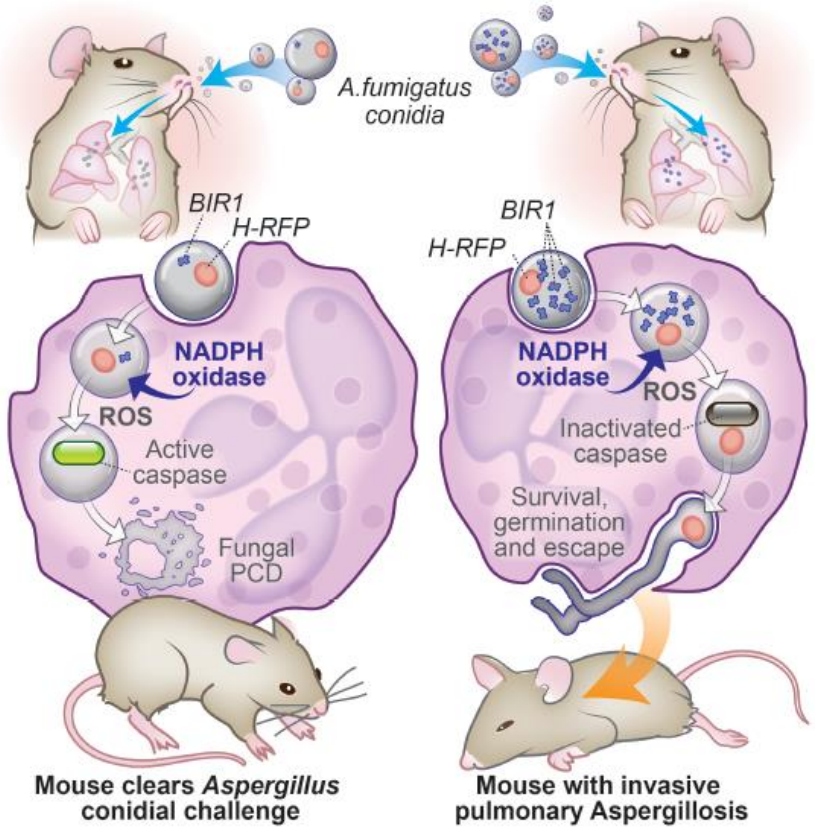
Invasive asspergillosis: lessons from primary immunodeficiencies

Defects in **myeloid cell numbers and function** mediate susceptibility to invasive mold infections

- **Functional defects**
  - **Chronic granulomatous disease (CGD):** impaired NADPH oxidase mediated **ROS production**
  - Pulmonary alveolar proteinosis (**GM-CSF** signaling)
- **Defects in numbers**
  - MonoMAC syndrome (GATA2)-**numbers**
  - Severe congenital neutropenia (ELA2, HAX1)-**numbers**
  - Leucocyte adhesion deficiency (CD18)-**trafficking**
  - CARD9 deficiency-**chemotaxis**

Lionakis MS et al., JCI Insight. 2017; Lionakis MS Nat Rev Immunol 2023

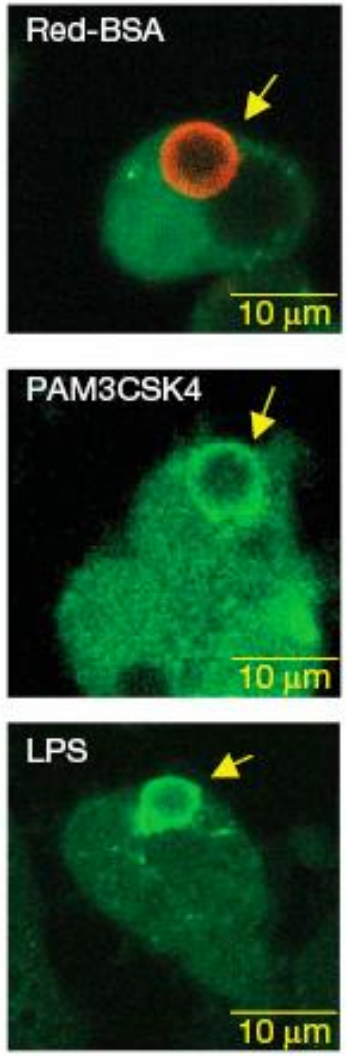
ROS are major **antifungal effectors** in neutrophils



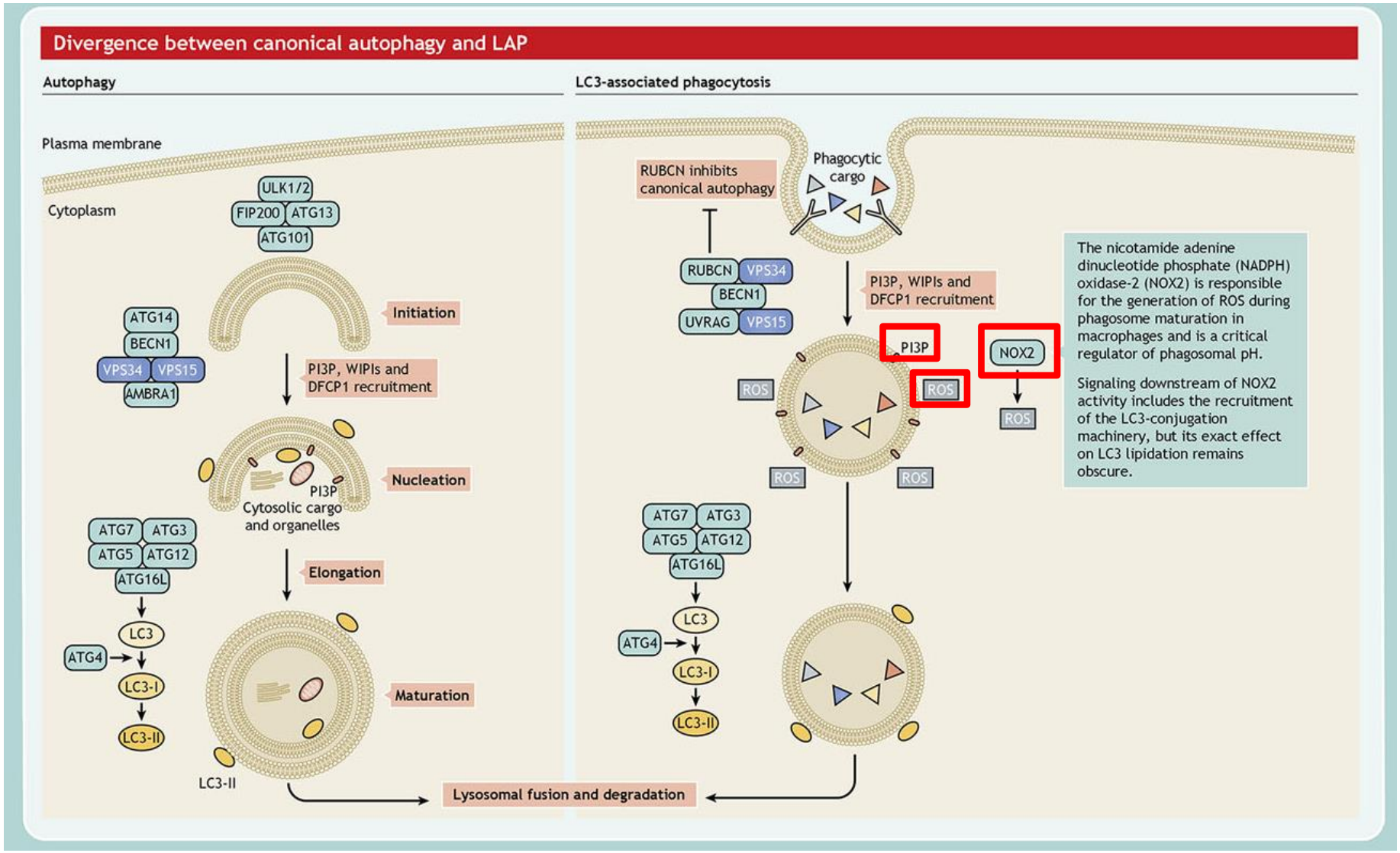
ROS-dependent induction of an **apoptosis-like** *Aspergillus* cell death by neutrophils

Shlezinger N, et al., Science. 2017

LC3 associated phagocytosis (LAP): a specialized autophagy pathway regulated by **ROS**

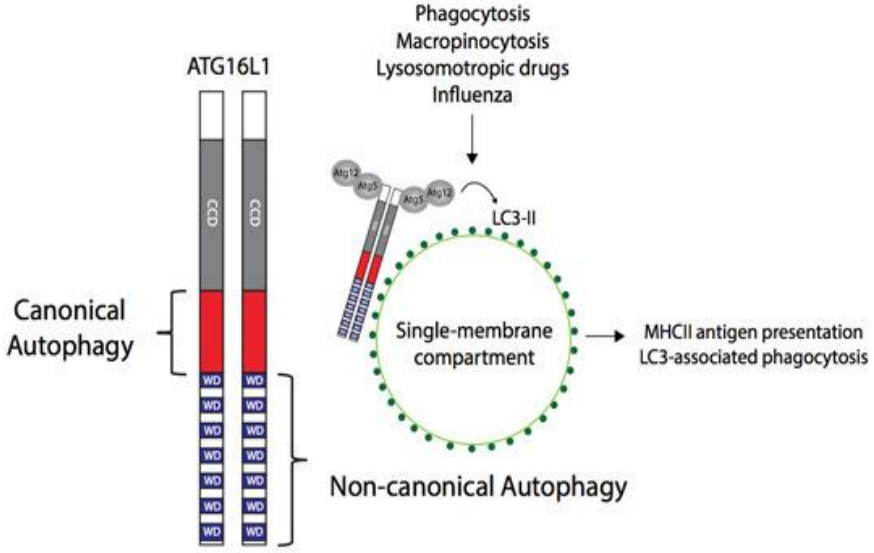


Nature 405, 1253-1257 (2007)

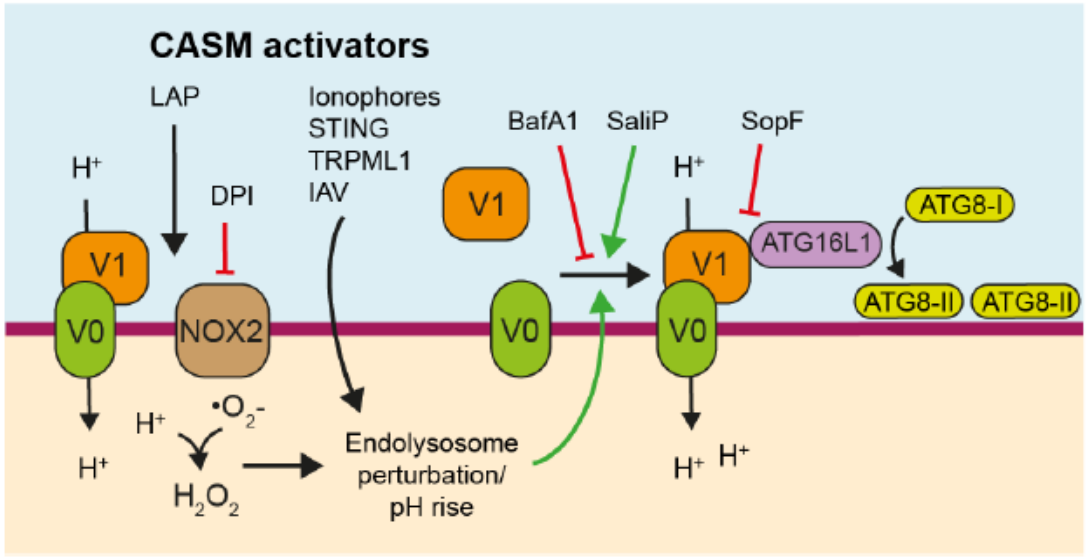


Heckmann and Green, J. Cell Sci. ,2019

Mechanism of non-canonical conjugation of LC3 on Single Membranes

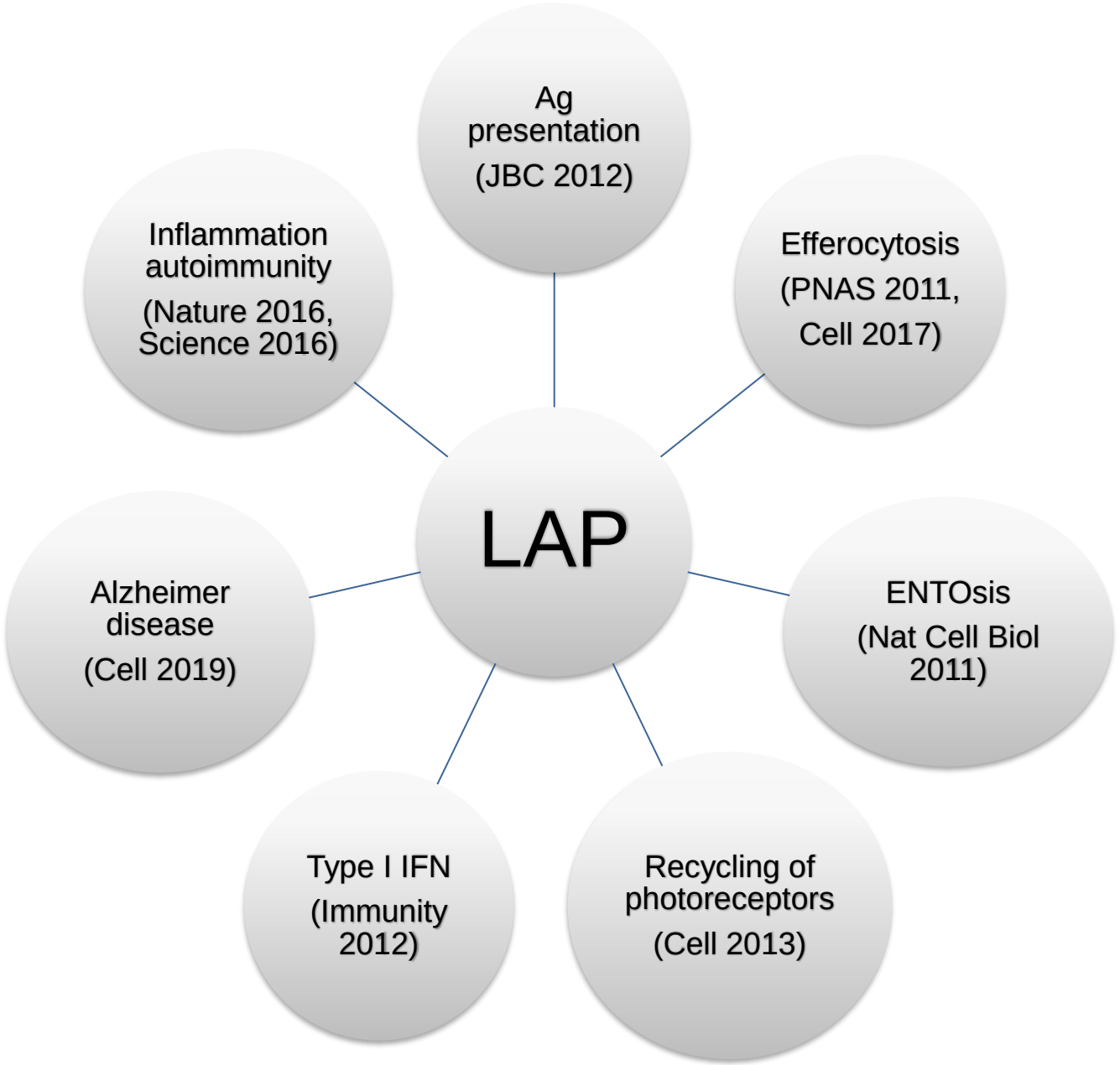


Fletcher et al. The EMBO journal, 2017

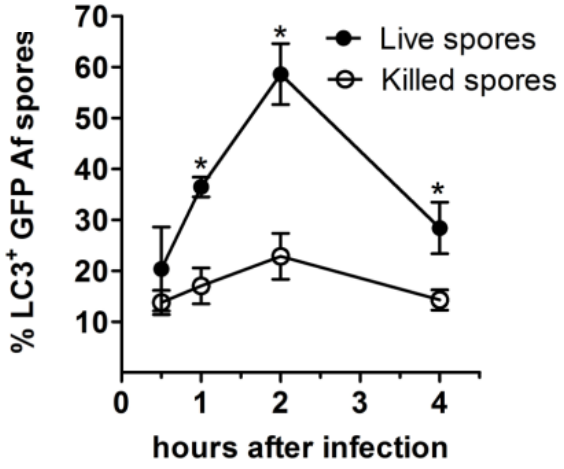


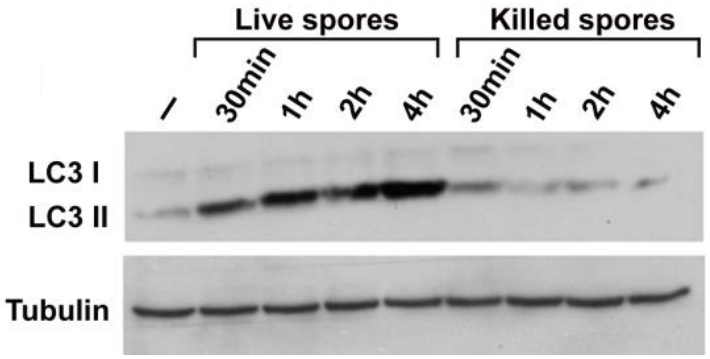
Hooper et al, J. Cell Biol. 2022

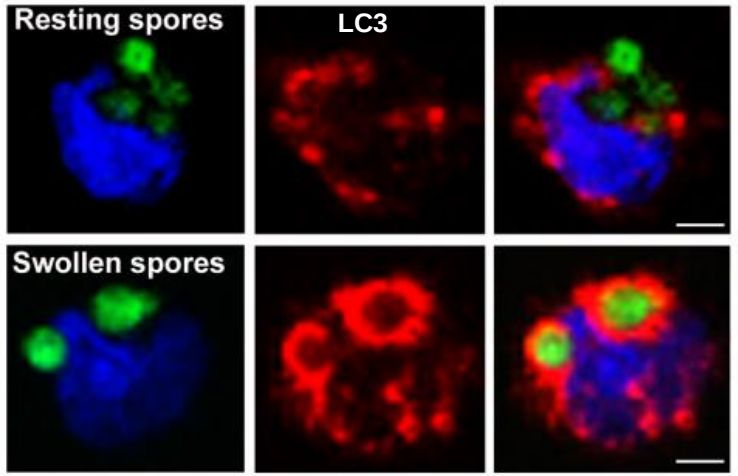
LAP regulates diverse physiological responses apart from pathogen killing



Selective LC3 recruitment in *Aspergillus* phagosomes during fungal cell wall swelling



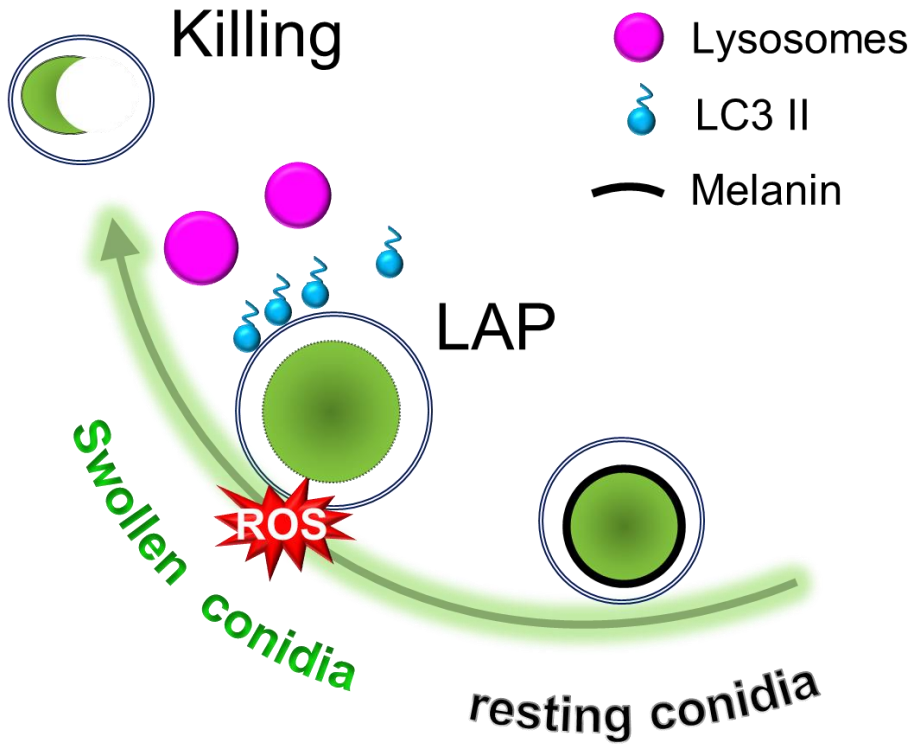




Kyrmizi et al., J Immunol, 2013

ROS-dependent activation of **LC3-associated phagocytosis (LAP)** orchestrates anti-*Aspergillus* host defense

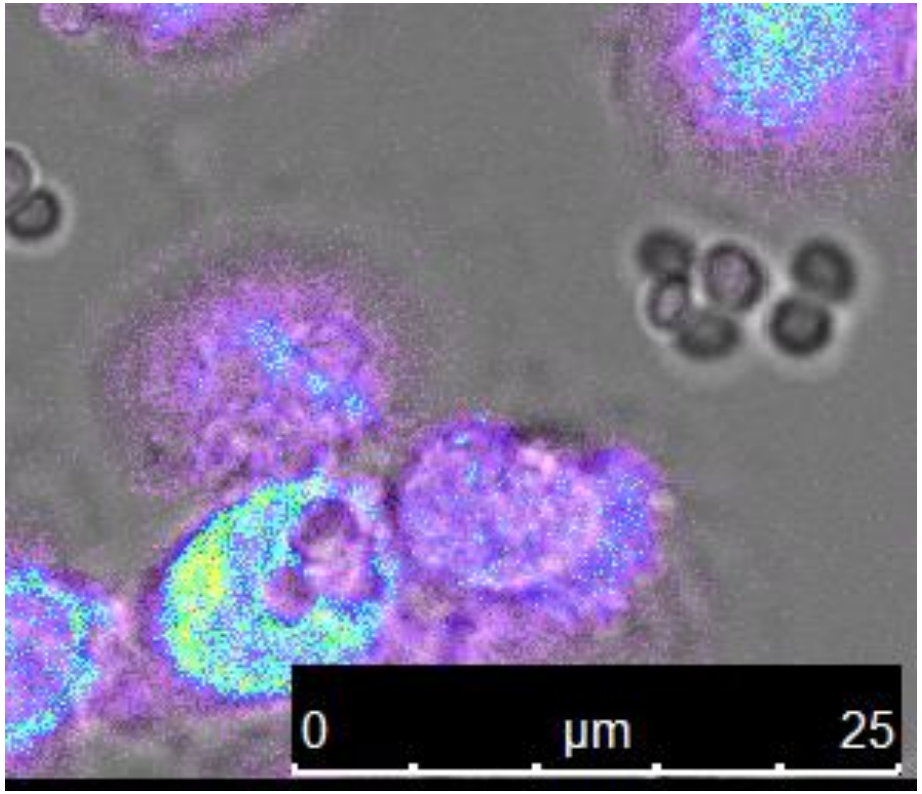
Balanced inflammation



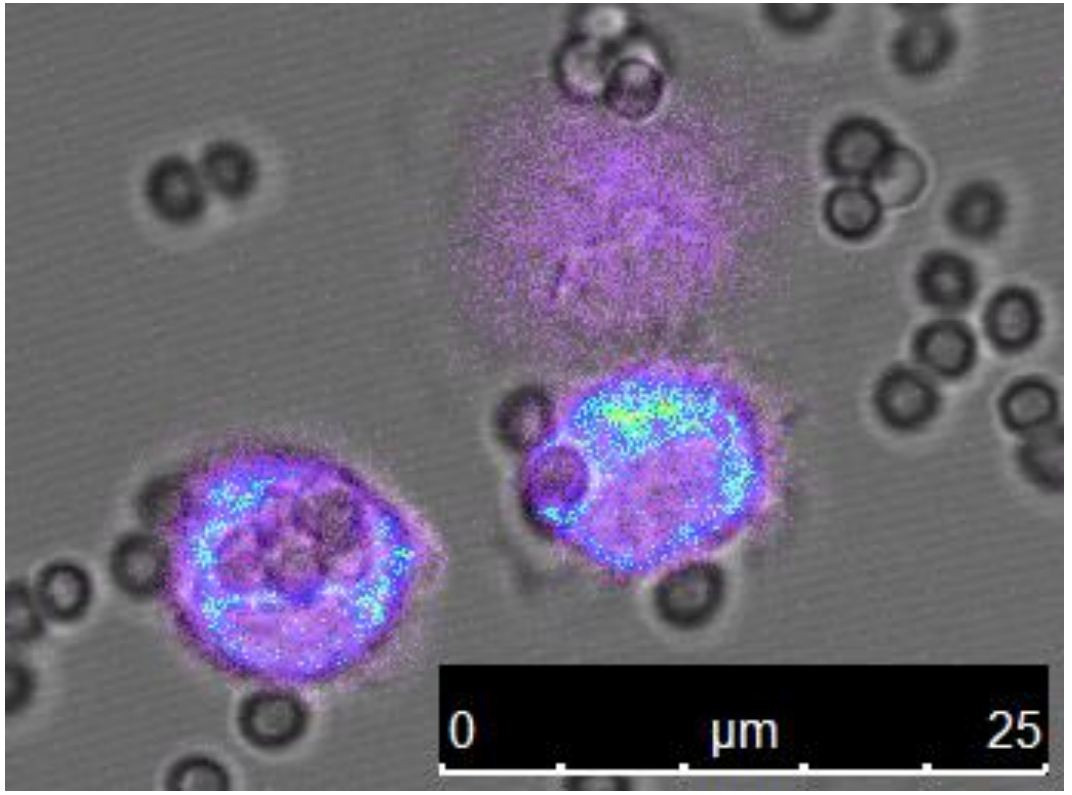
Kyrmizi et al., *J Immunol* **2013;** Akoumianaki et al., *Cell Host Microbe* **2016**; Kyrmizi et al., *Nat Microbiol* **2018**; Andrianaki et al., *Nat Commun* **2018;** Akoumianaki et al., *Cell Host Microbe* **2021**

Fungal melanin inhibits Ca^{2+} signaling during phagocytosis

Melanin deficient (Δ*pksP* strain)

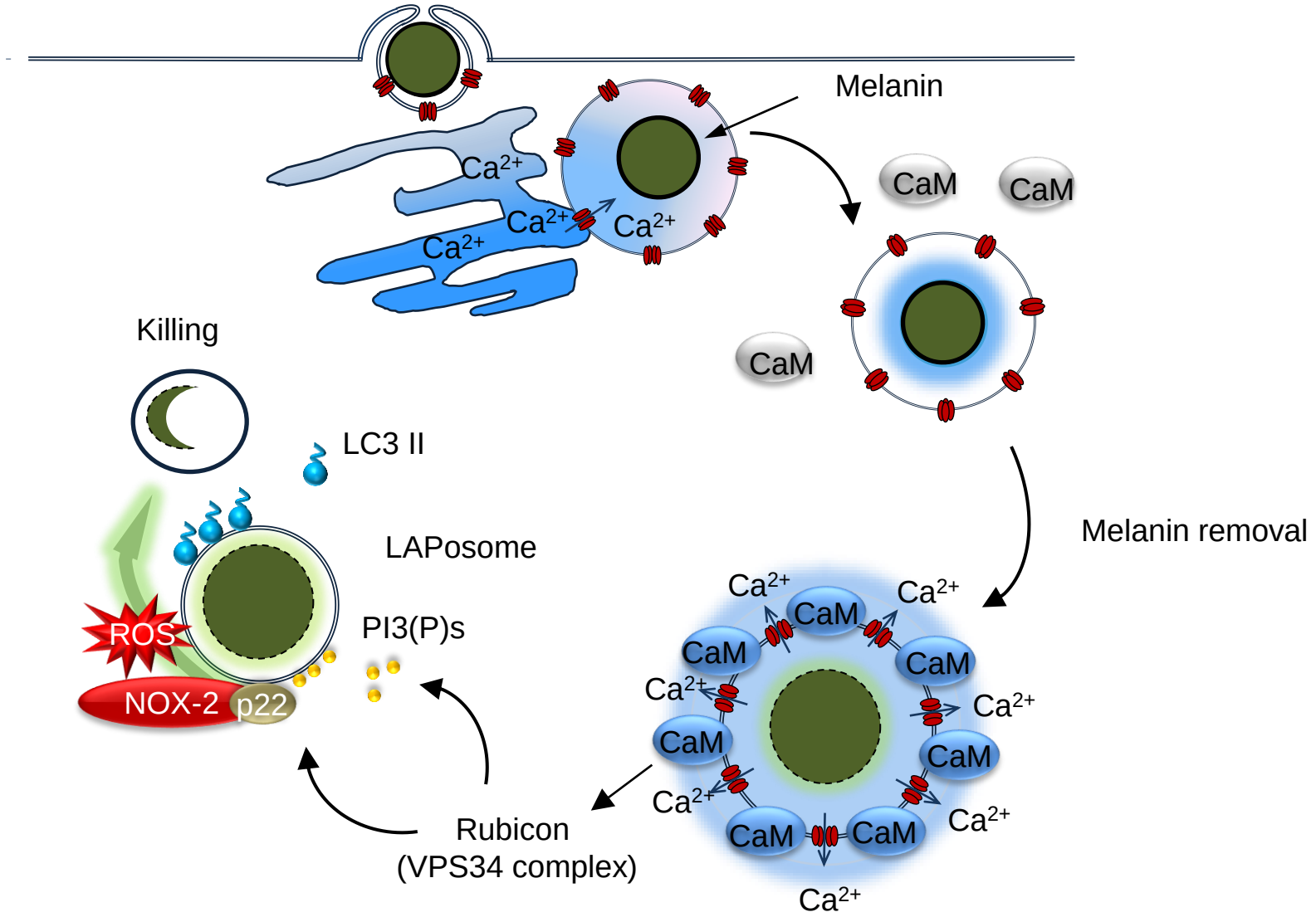


Isogenic wild type strain (*ku80*)



Kyrmizi I et al., Nat Microbiol 2018

Ca^{2+} scavenging by melanin inside the phagosome inhibits LAP

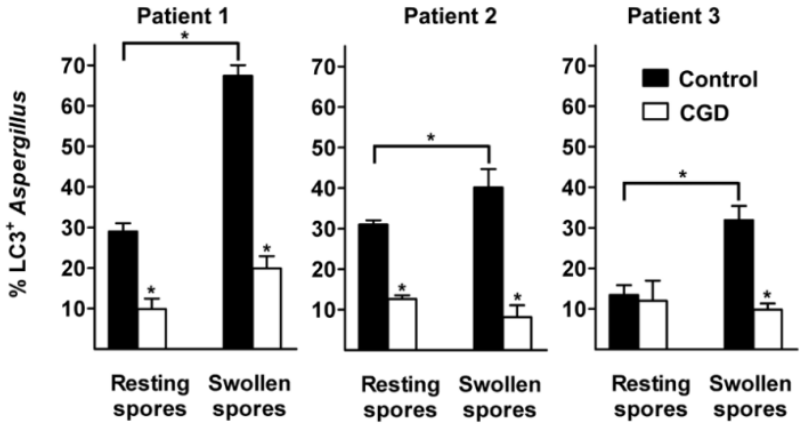


Kyrmizi I et al., Nat Microbiol 2018

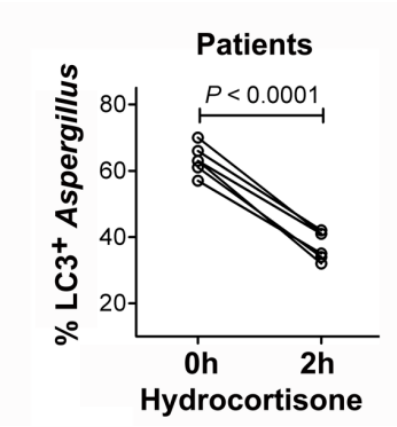
Role of LAP defects in **pathogenesis of invasive aspergillosis?**

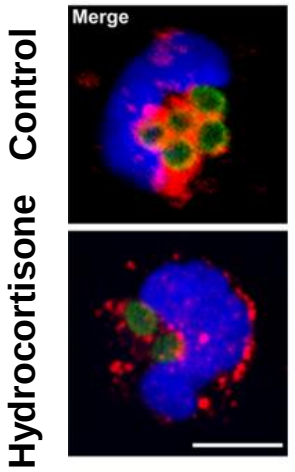
Defective activation of LAP in monocytes of CGD and corticosteroid-treated patients

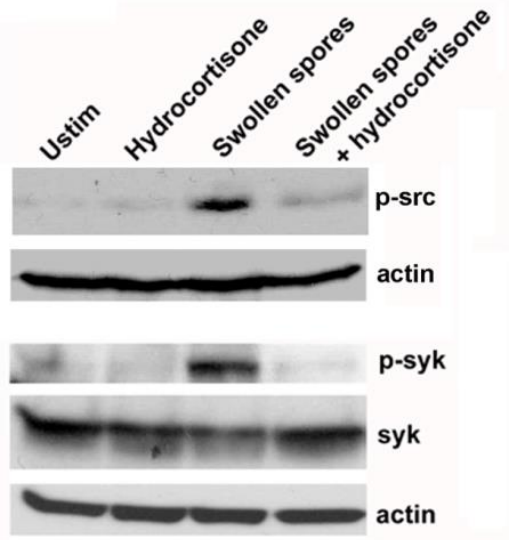
Monocytes of CGD (p47phox-/-) patients



Monocytes of patients receiving corticosteroids

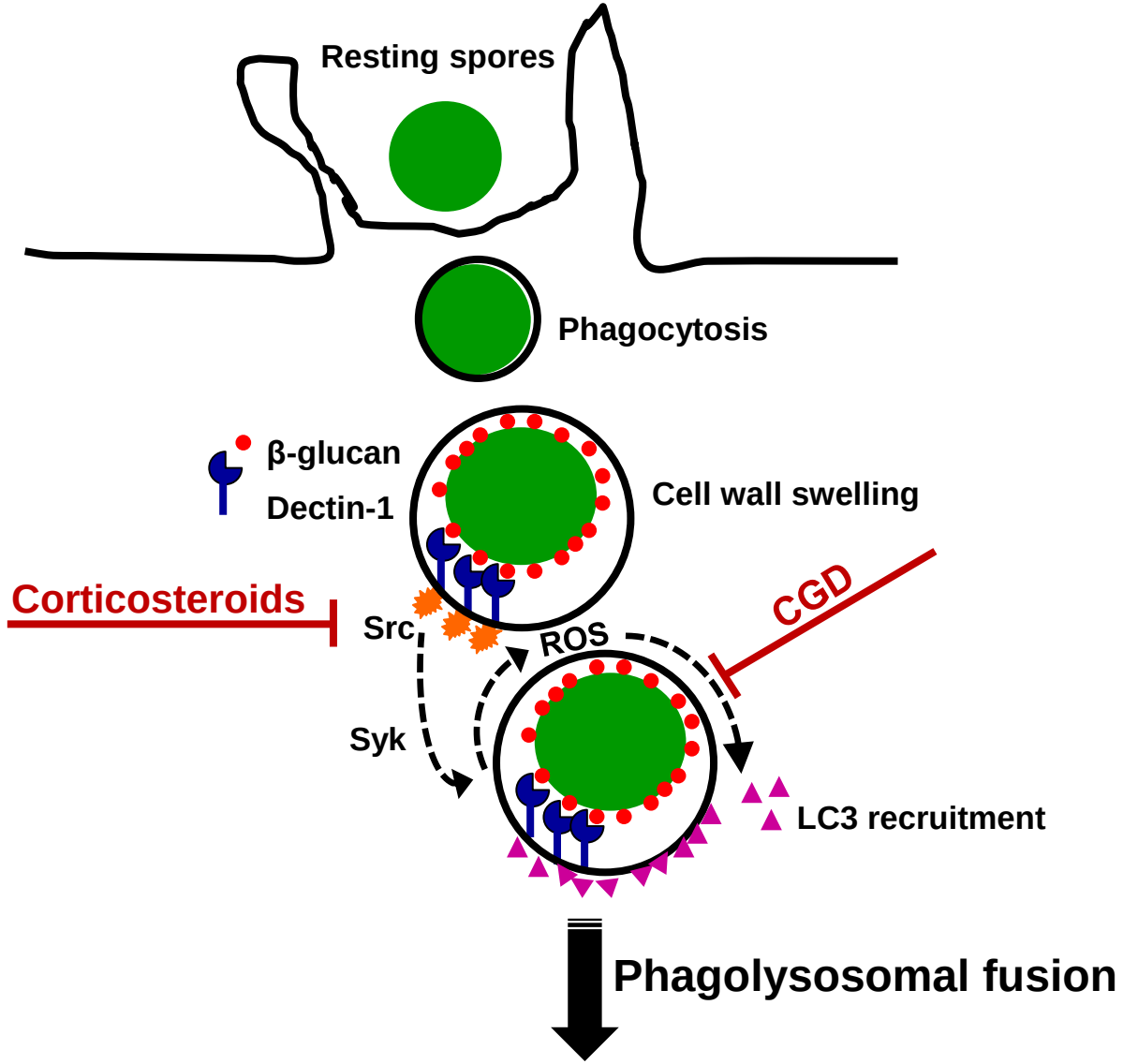






Kyrmizi et al., J Immunol, 2013

Model of IMI pathogenesis induced by LAP blockade

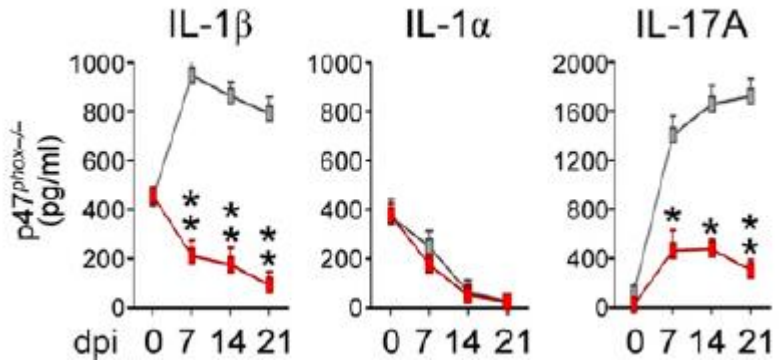


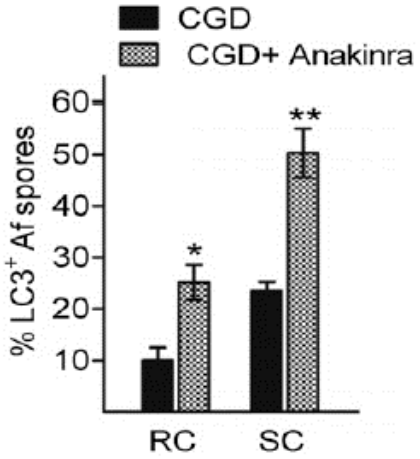
Mechanisms of cross-talk of LAP pathway with cytokine signaling in macrophages?

IL-1 receptor blockade restores autophagy and reduces inflammation in chronic granulomatous disease in mice and in humans

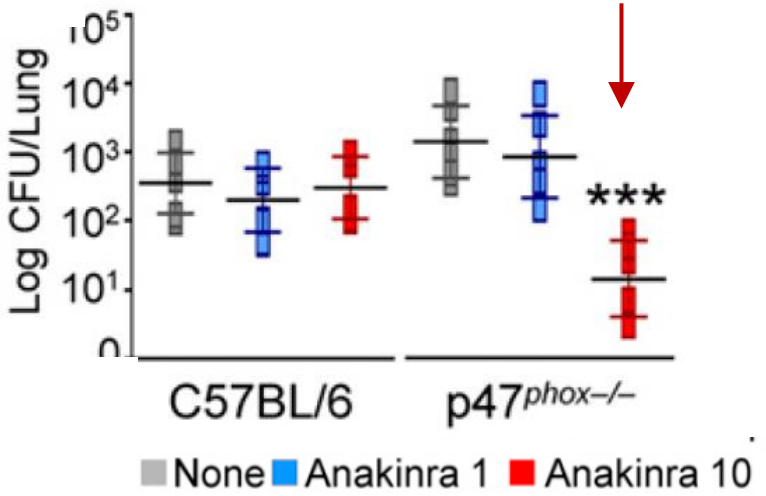
3526–3531 | PNAS | March 4, 2014 | vol. 111 | no. 9

Antonella de Luca[a,1], Sanne P. Smeekens[b,c,1], Andrea Casagrande[a], Rossana Iannitti[a], Kara L. Conway[d], Mark. S. Gresnigt[b,c], Jakob Begun[d], Theo S. Plantinga[b,c], Leo A. B. Joosten[b,c], Jos W. M. van der Meer[b,c], Georgios Chamilos[e], Mihai G. Netea[b,c], Ramnik J. Xavier[d,f], Charles A. Dinarello[b,c,f,2], Luigina Romani[a,3], and Frank L. van de Veerdonk[b,c,g,2,3]





CGD-specific effect!

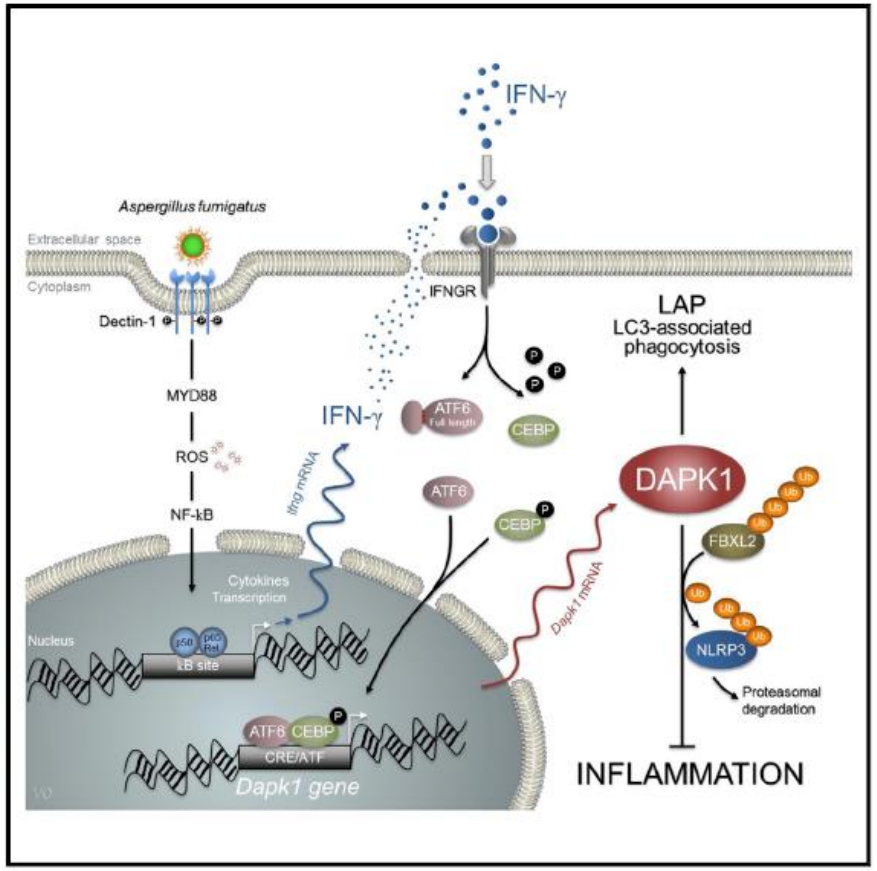


Improved clinical symptoms of CGD (colitis, abscesses, inflammatory markers)

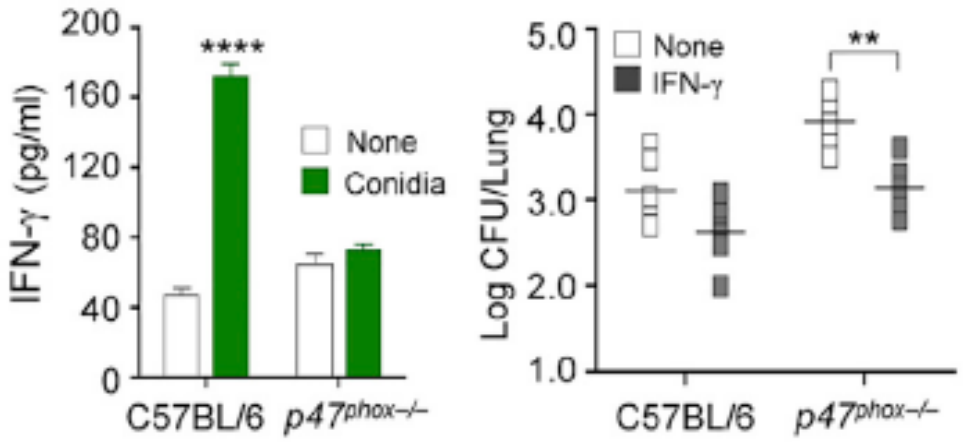
Cell Host & Microbe

Noncanonical Fungal Autophagy Inhibits Inflammation in Response to IFN-γ via DAPK1

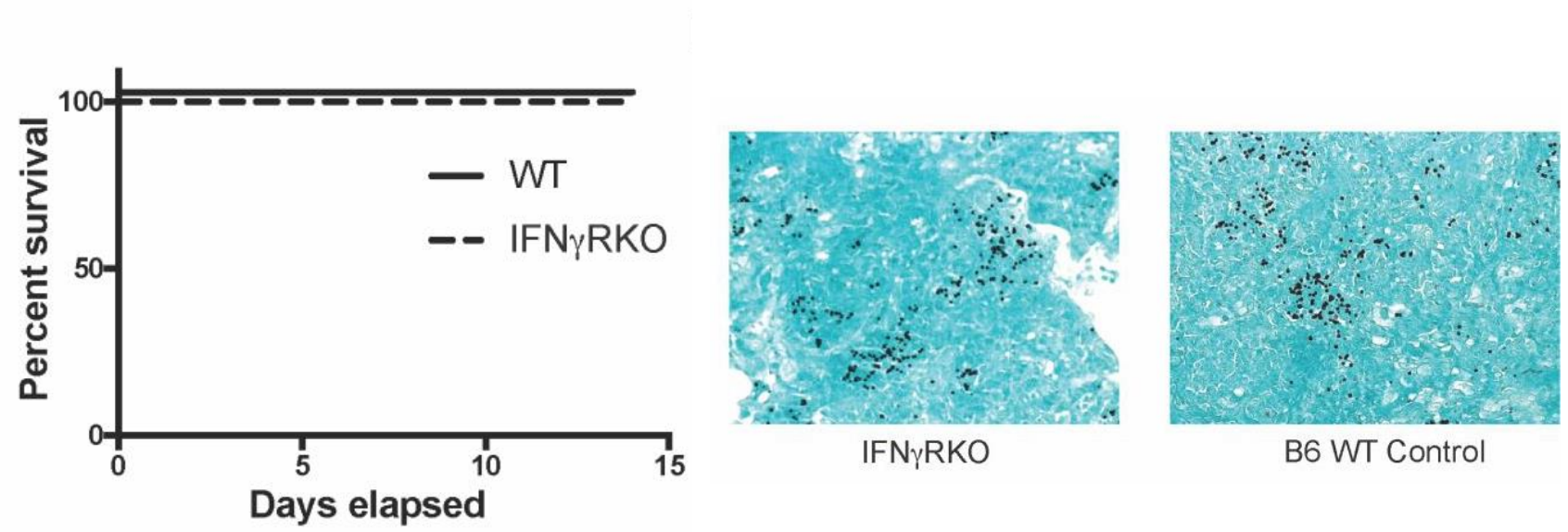
Vasilis Oikonomou,[1] Silvia Moretti,[1] Giorgia Renga,[1] Claudia Galosi,[1] Monica Borghi,[1] Marilena Pariano,[1] Matteo Puccetti,[1] Carlo A. Palmerini,[2] Lucia Amico,[3] Alessandra Carotti,[3] Lucia Prezioso,[4] Angelica Spolzino,[4] Andrea Finocchi,[5] Paolo Rossi,[5] Andrea Velardi,[3] Franco Aversa,[4] Valerio Napolioni,[1] and Luigina Romani[1,6,*]



CGD-specific effect of IFNγ



IFNγ signaling is redundant in physiological immune responses against *Aspergillus*



Espinosa V et al., Sci Immunol. 2017 Oct 6;2(16). pii: eaan5357

Do we study how cytokine administration affects **immune responses**?

Organ Specific Cytokine Therapy

Local Activation of Mononuclear Phagocytes by Delivery of an Aerosol of Recombinant Interferon-γ to the Human Lung

The Journal of Clinical Investigation, Inc.
Volume 88, July 1991, 297–302

H. Ari Jaffe,* Roland Buhl,* Andrea Mastrangeli,* Kenneth J. Holroyd,* Cesare Saltini,* Dorothy Czerski,* Howard S. Jaffe,‡ Susan Kramer,‡ Stephen Sherwin,‡ and Ronald G. Crystal*
**Pulmonary Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland 20892; and ‡Genentech, Inc., South San Francisco, California 94080*

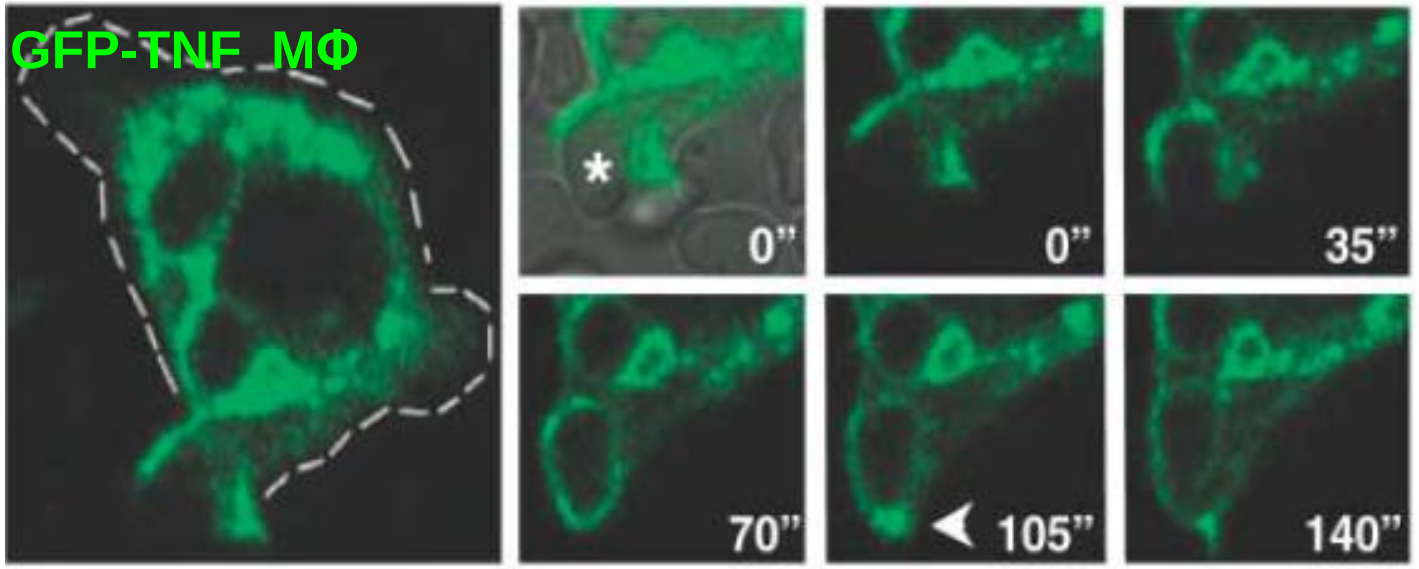
Inhaled IFN-γ for persistent nontuberculous mycobacterial pulmonary disease due to functional IFN-γ deficiency

T.S. Hallstrand*, H.D. Ochs#, Q. Zhu#, W.C. Liles*

Eur Respir J 2004; 24: 367–370.

Do we understand the **autocrine** functions of cytokines?

TNF rapidly accumulates in the phagocytic cup during *Candida* infection



TNF function in the phagocytic cup?

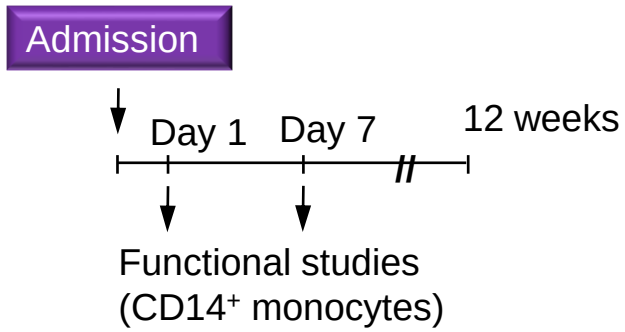
Science*. *2005 *Dec 2;310(5753):1492-5*

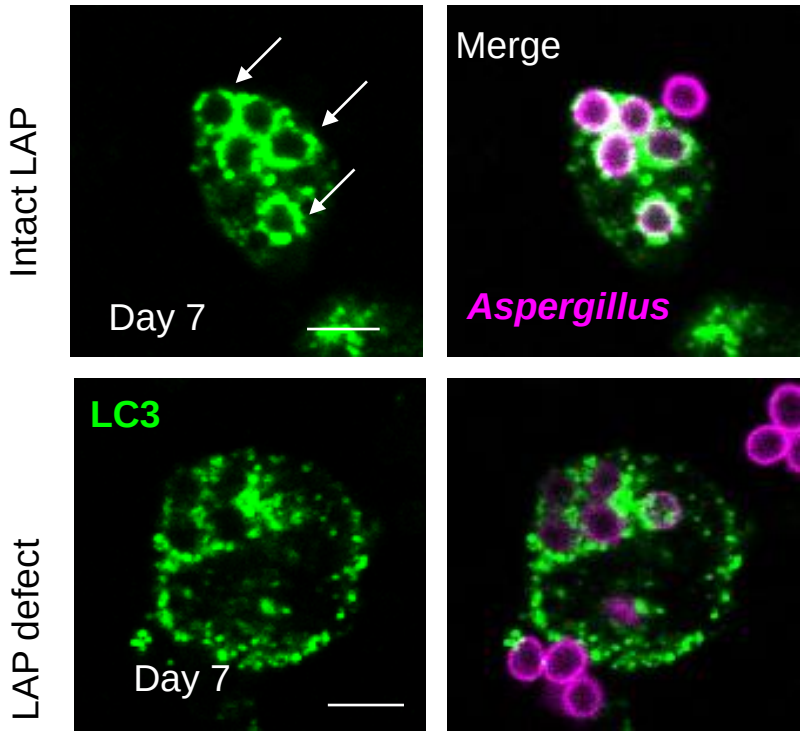
Role of LAP defects in sepsis immunoparalysis?

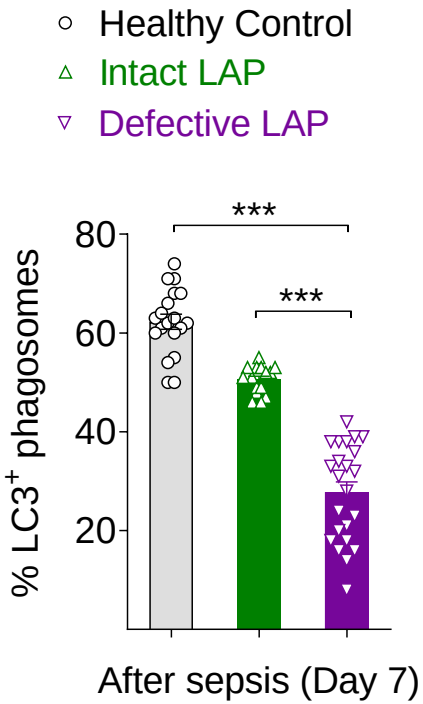
Immunoparalysis of myeloid phagocytes: sepsis and sepsis-like syndromes

- Incompletely characterized pathogenesis
- Characterized by **cytokine hypo-responsiveness**
- **Diminished microbicidal capacity** of phagocytes
- Susceptibility to **superinfections** by bacterial and fungal pathogens
- ***The molecular link between cytokine signaling and the phagosome is unknown***

Defective activation of LAP in monocytes of a selective group of patients with sepsis

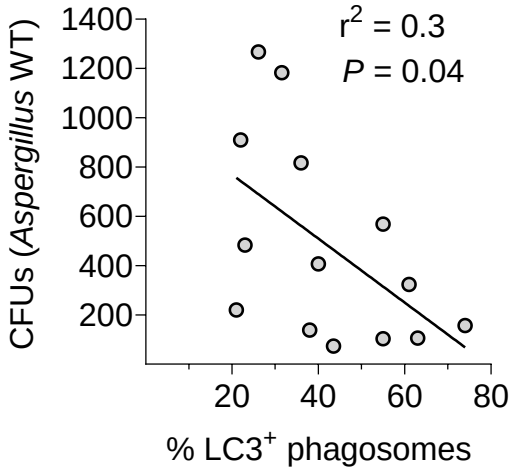


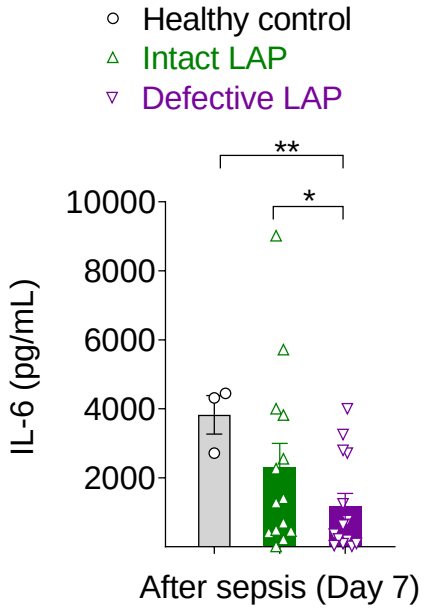


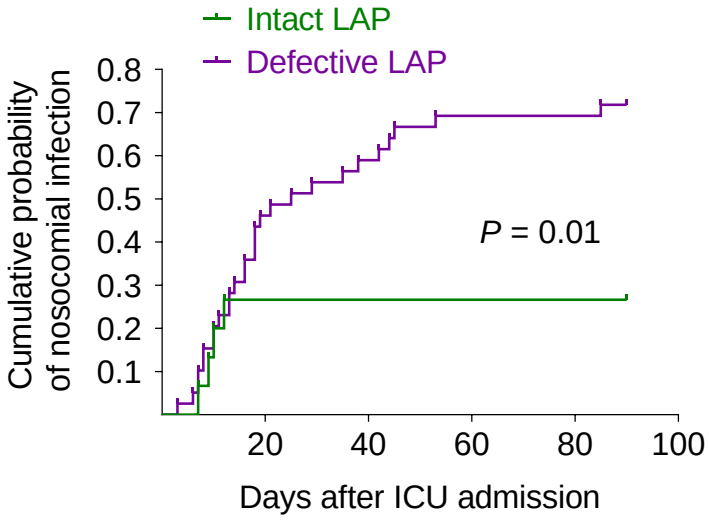


Akoumianaki et al., (Cell Host Microbe 2021)

Defective activation of LAP defects is a hallmark of immunoparalysis during sepsis

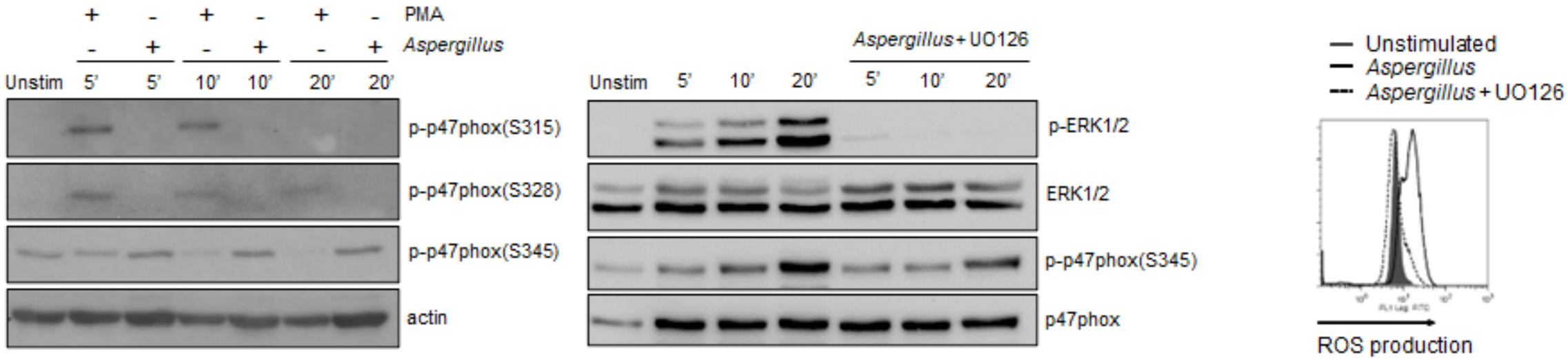




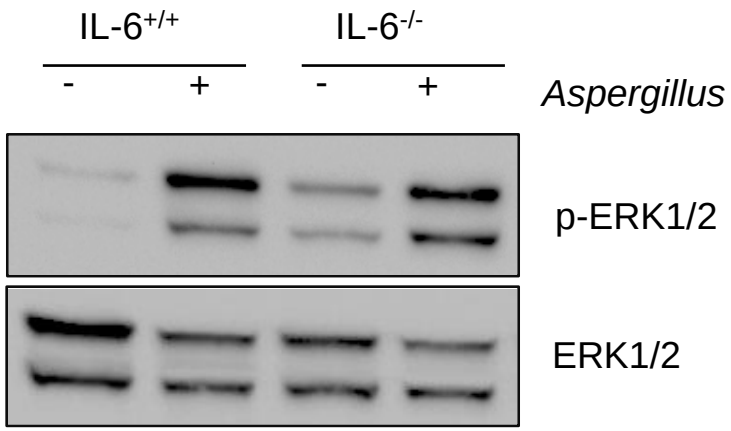


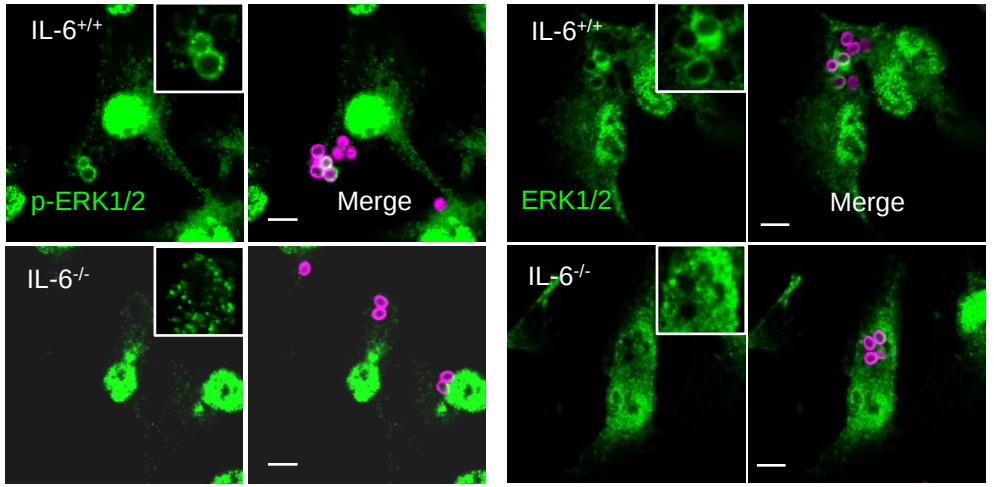
Akoumianaki et al., (Cell Host Microbe 2021)

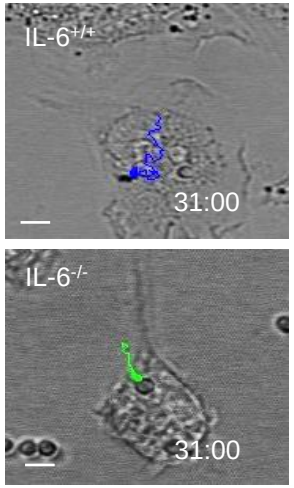
ERK signaling regulates NADPH oxidase-mediated ROS production by *Aspergillus fumigatus* in macrophages



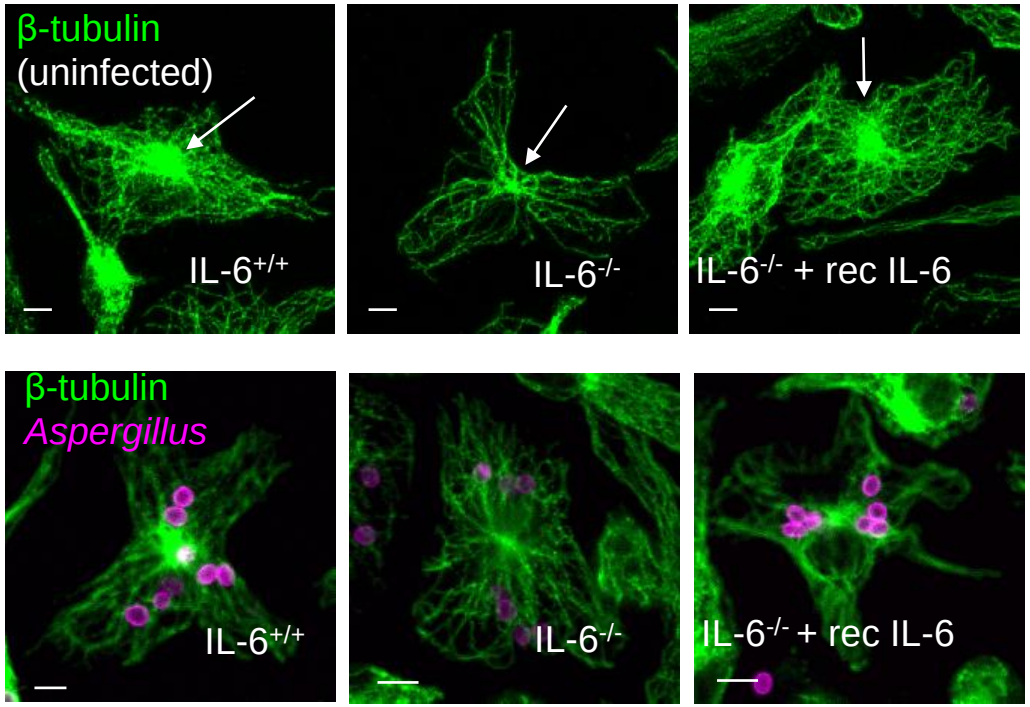
IL-6 regulates ERK trafficking on the phagosome

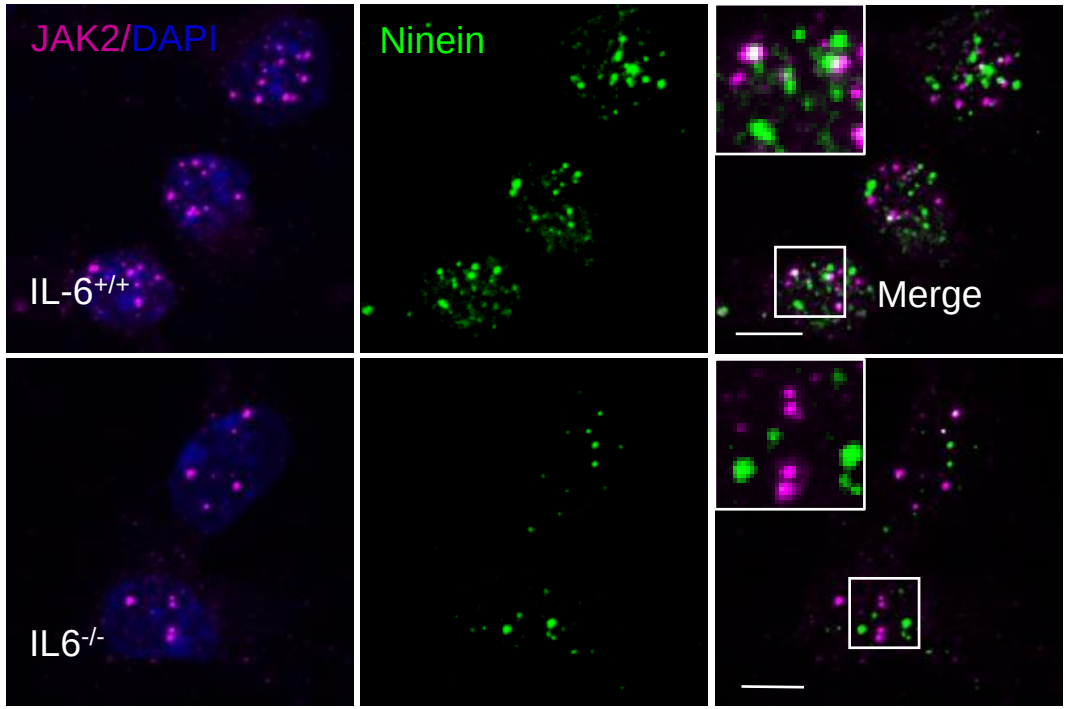






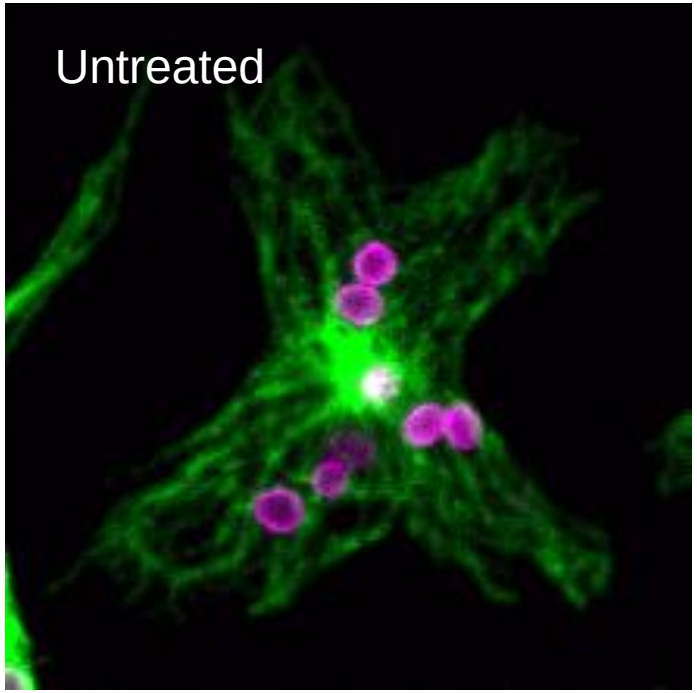
Uncoupling of IL-6 signaling from LAP drives immunoparalysis of macrophages

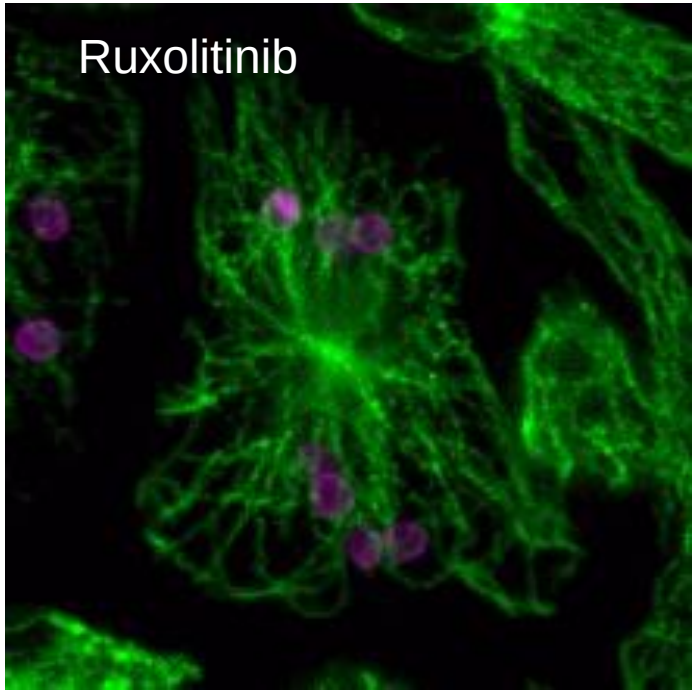


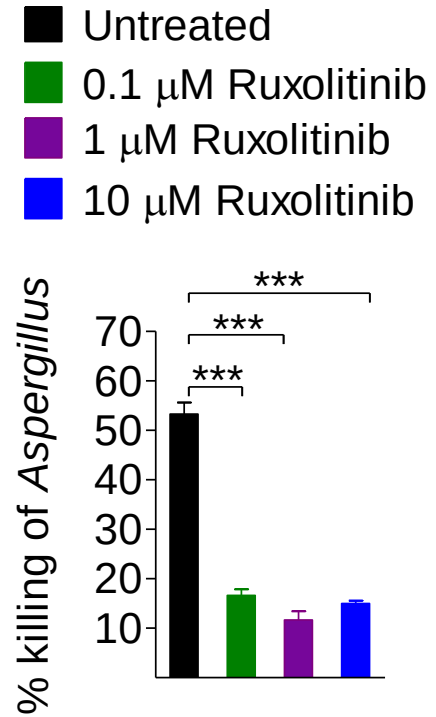


Akoumianaki et al., (Cell Host Microbe 2021)

JAK2 inhibitors block LAP-mediated killing of fungal pathogens by myeloid phagocytes





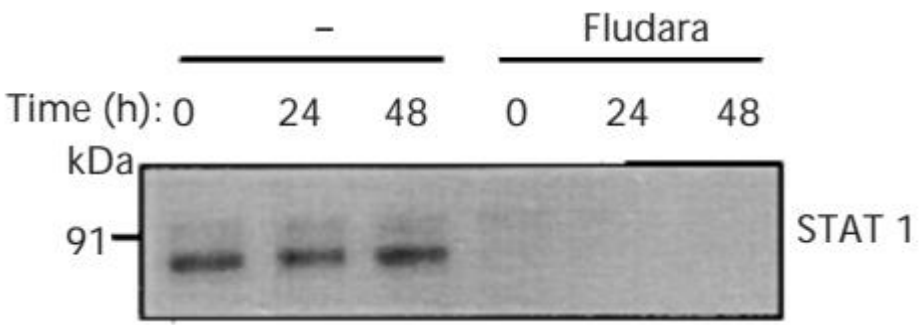


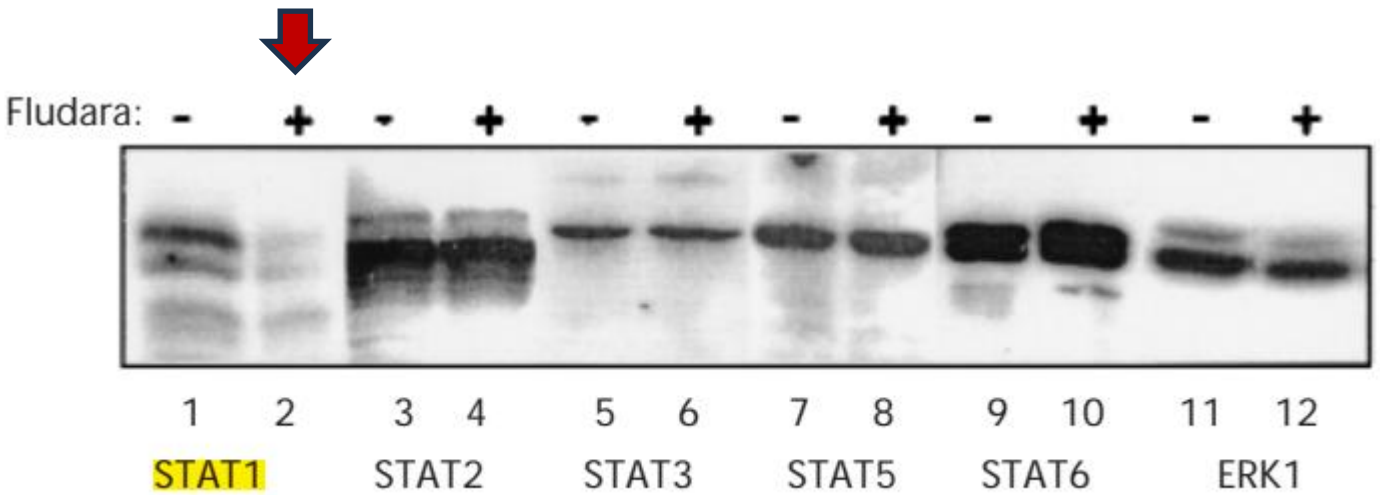
Akoumianaki et al., (Cell Host Microbe 2021)

Fludarabine-induced immunosuppression is associated with inhibition of STAT1 signaling

DAVID A. FRANK[1,2], SUDIPTA MAHAJAN[1] & JEROME RITZ[1,2]

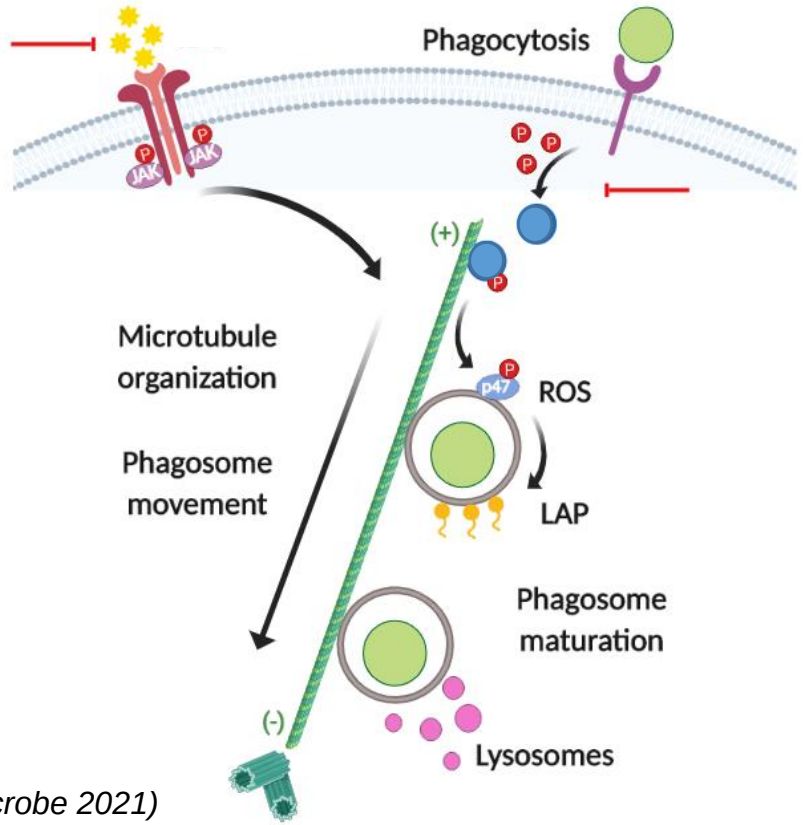
NATURE MEDICINE • VOLUME 5 • NUMBER 4 • APRIL 1999





A novel mechanism of regulation of LAP by IL-6

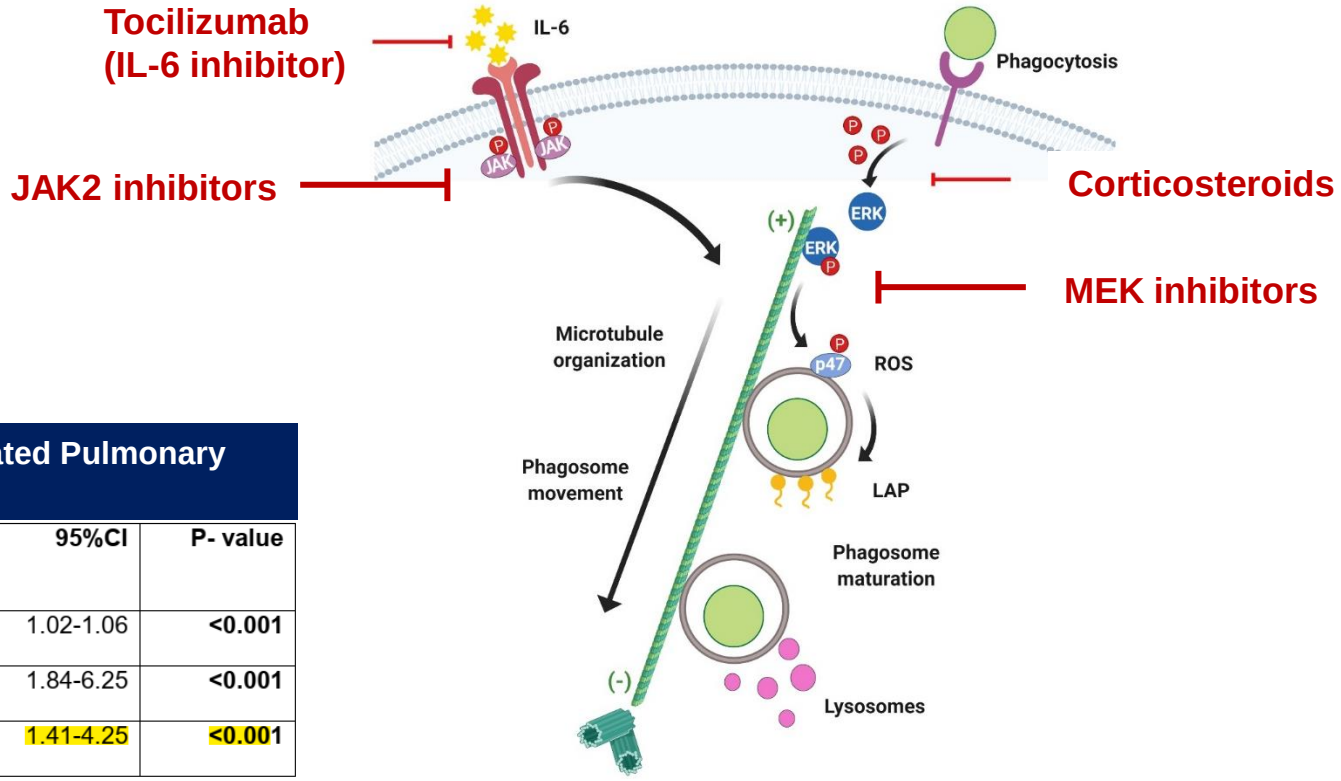
Trafficking



Signaling

Akoumianaki et al., (Cell Host Microbe 2021)

Synergistic inhibition of LAP by different immunosuppressive therapies

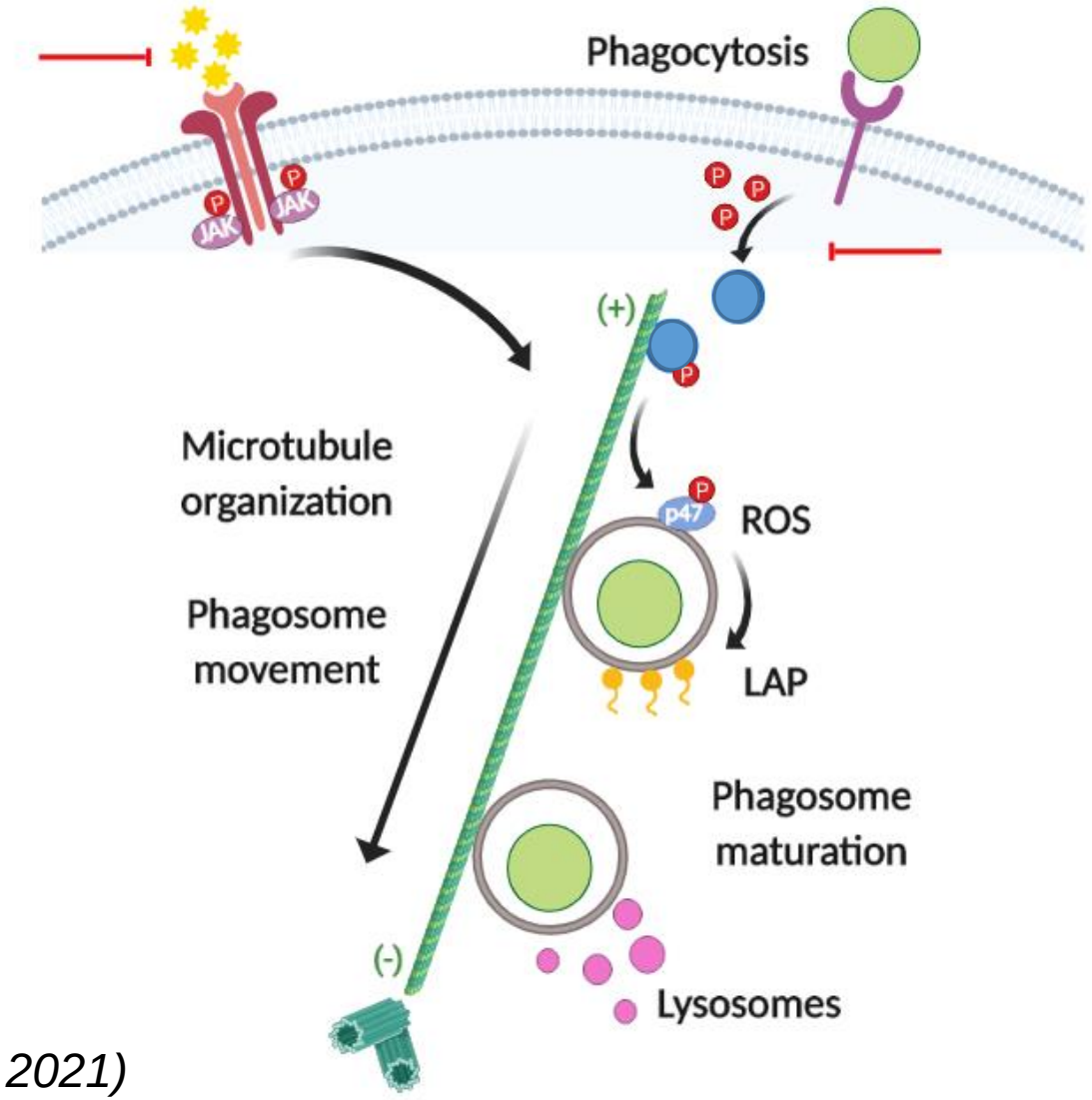


Clinical predictors of COVID-19 Associated Pulmonary Aspergillosis (CAPA)			
Variable	**Multivariable Hazard Ratio**	**95%CI**	**P- value**
Age per year	1.04	1.02-1.06	**<0.001**
Any invasive respiratory support	3.40	1.84-6.25	**<0.001**
Tocilizumab	2.45	1.41-4.25	**<0.001**

Prattes J et al., Clin Microbiol Infect 2021

Would you give cytokine therapy in patients with **signaling defects**?

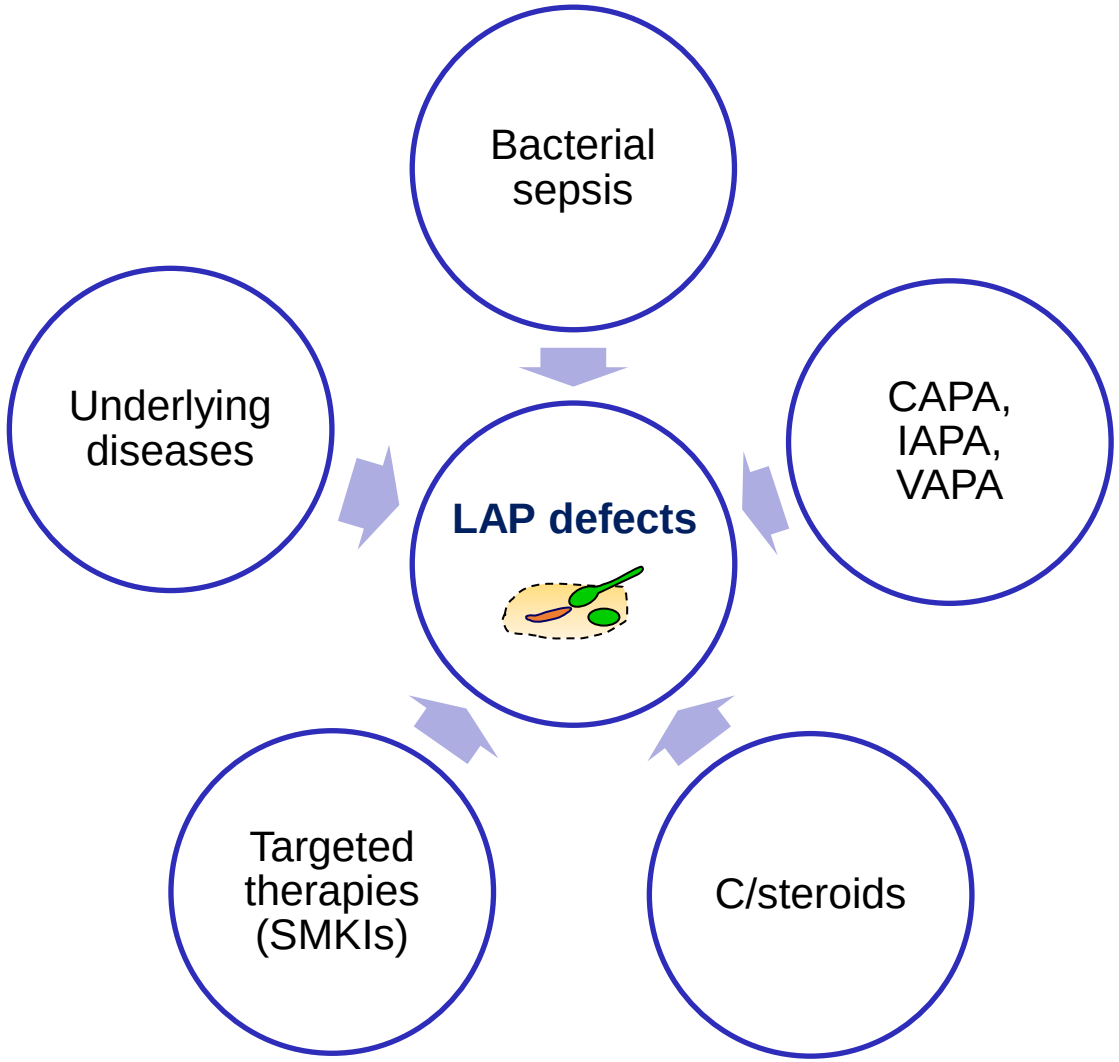
Trafficking



Signaling

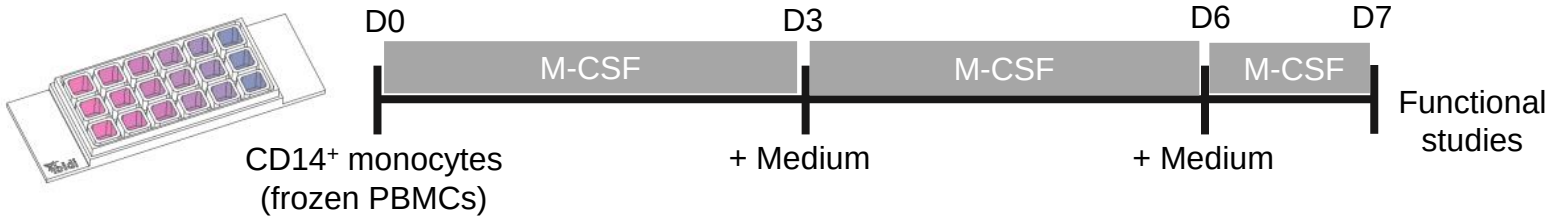
Akoumianaki et al., (CHM 2021)

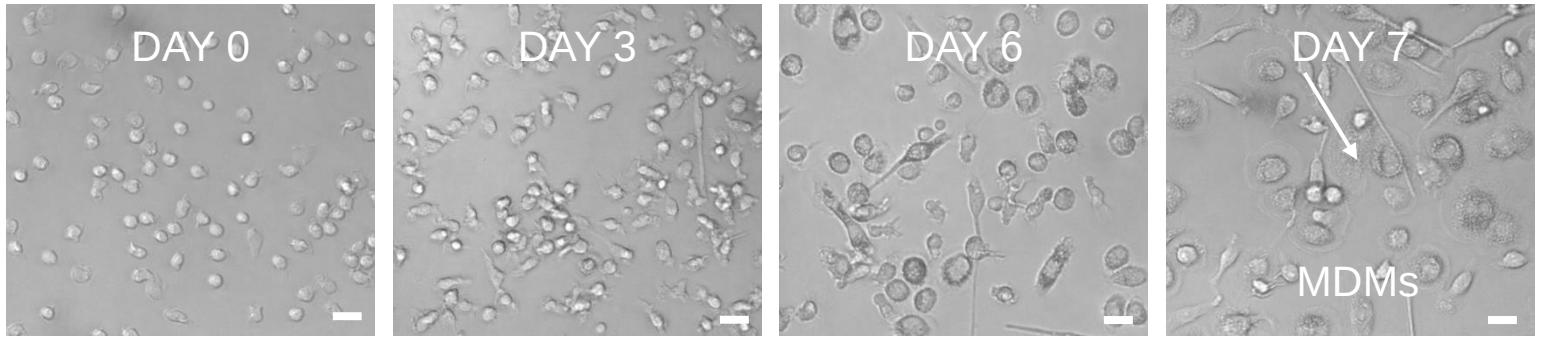
Need for **profiling of the mechanism of LAP blockade** for design of **personalized host-directed therapies**

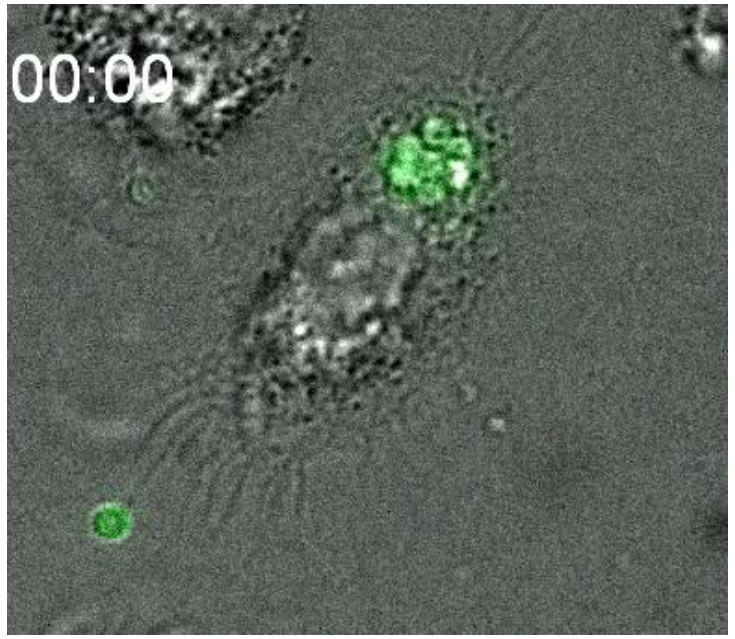


Strategies to assess phagosome defects in the clinical practice

Protocol for **high-content analysis** of phagosome responses in **human MDMs** differentiated from **cryopreserved** PBMCs

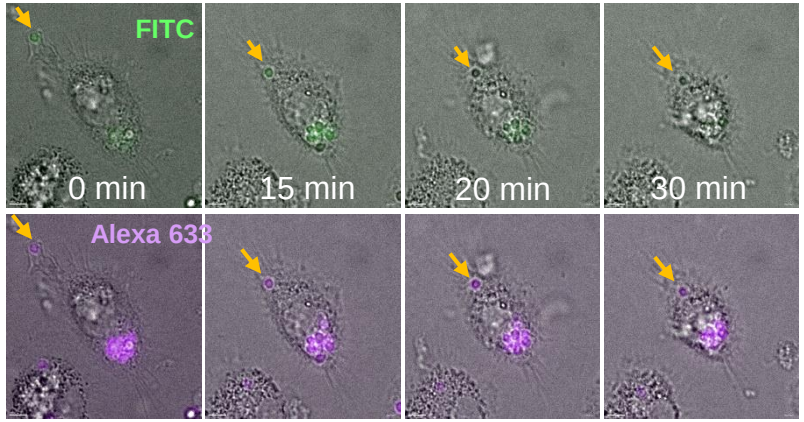


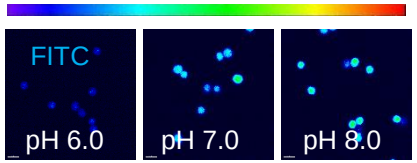


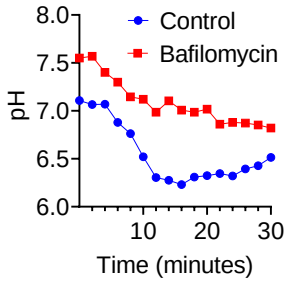


Dynamic analysis of phagosome responses in MDMs

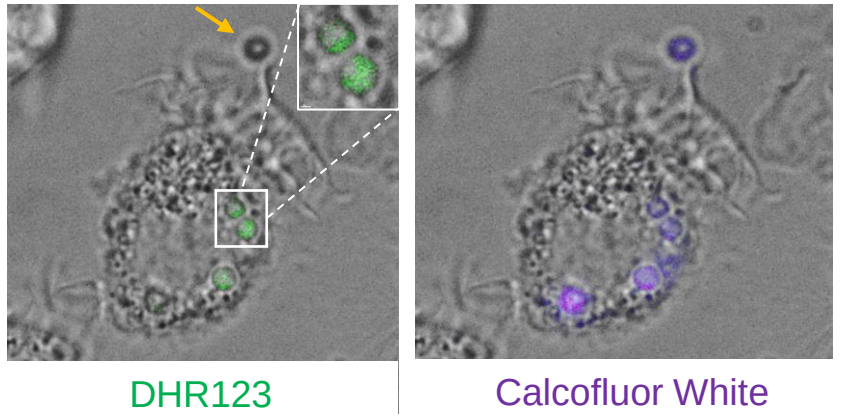
A



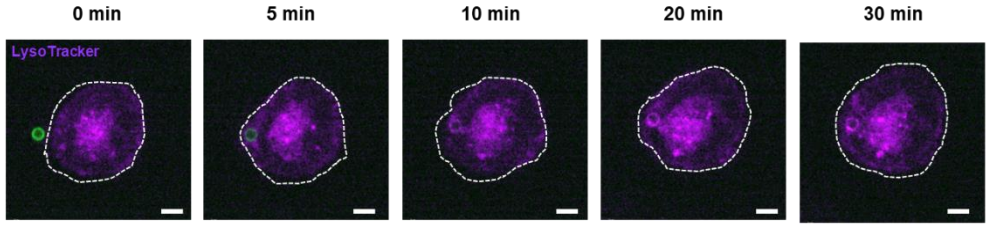


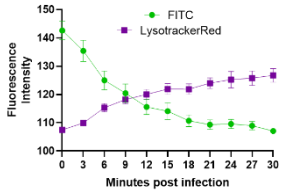


B

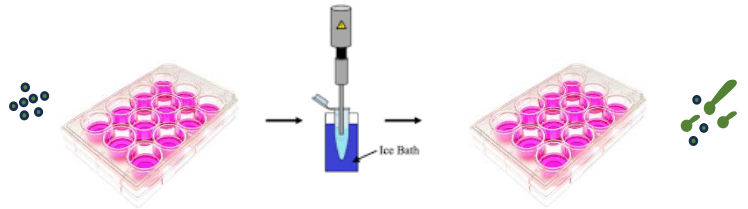


C





D



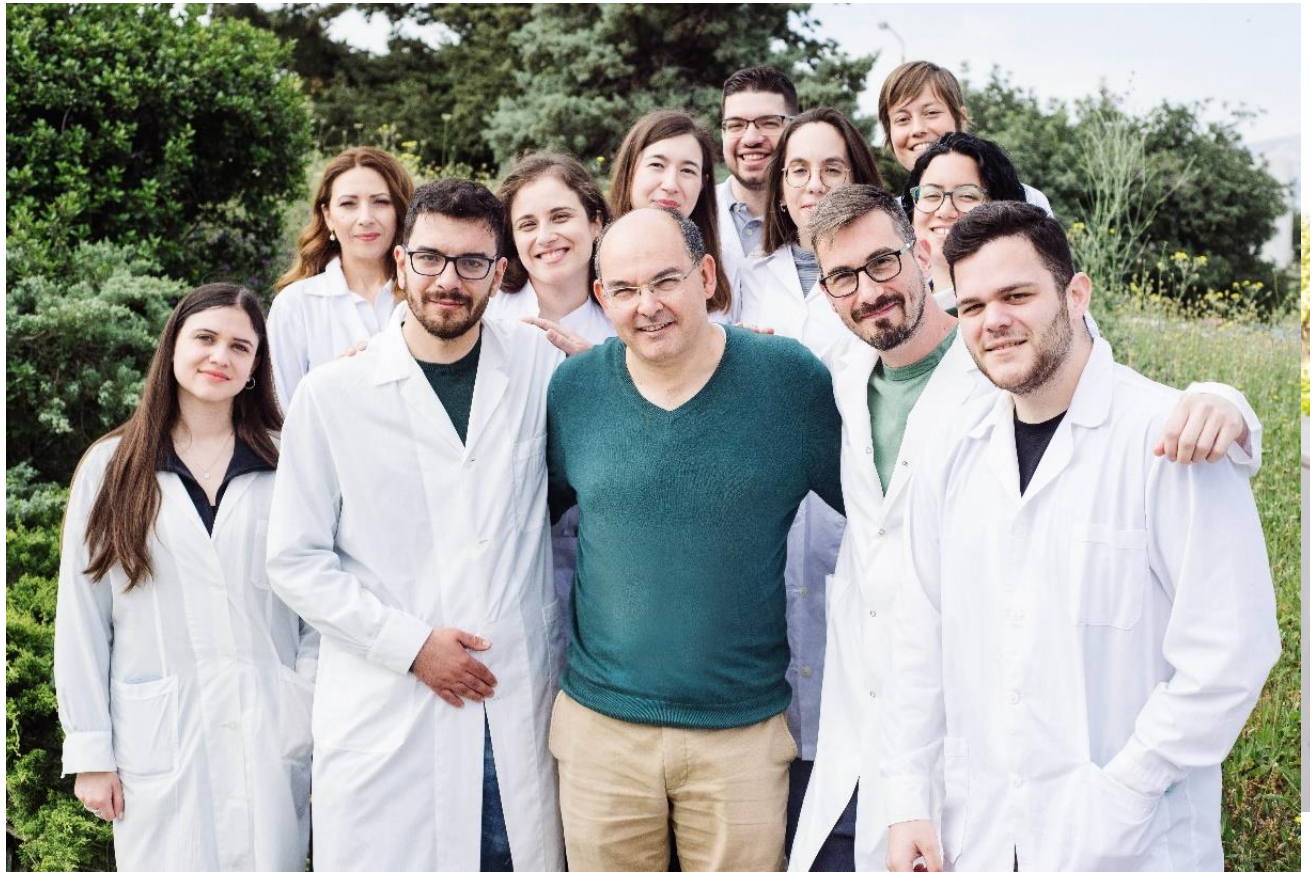
Infection with FITC-labeled Af

Sonication (1h, 6h p.i.)

Assessment of germination

Future perspectives on immunotherapy

- **Molecular understanding** of mechanisms of immunodeficiency (e.g. **LAP defects, signaling** vs. **trafficking**)
- **Host biomarkers** of immunodeficiency (LAP, cytokine response, other effectors)
- Need for **personalized cytokine therapy** (neutropenia, inflammatory immunopathology, immunoparalysis)
- **Clinical studies** with **translational immunology design** (immunophenotyping before and after therapy)
- Need to identify **different measures of outcome** (immunological criteria, effector functions)



INSTITUT MERIEUX

"la Caixa" Foundation

MARIE CURIE ACTIONS

Horizon 2020 Programme

European Research Council

erc

Collaborating Labs

School of Medicine, University of Crete
Elias Drakos, MD, PhD
Zoi Veneti, PhD
George Samonis, MD
Katerina Vaporidi, MD, PhD

BRFAA, Athens
Vagelis Andreakos, PhD
Kalliopi Thanopoulou, PhD

Institute of Microbiology, HKI, Jena, Germany
Axel A. Brakhage, PhD

University of Crete, Greece
Jean-Paul Latge, PhD

INSERM-U1149, CNRS-ERL8252, Paris, France
Jamel El-Benna, PhD

Fungal Pathogenesis Unit, NIH, USA
Michalis Lionakis, MD

University of Cincinnati, USA
Julio A Landero, PhD
George Deepe, MD

Radboud University, Netherlands
Frank L van de Veerdonk, MD
Mihai Netea, MD

University of Groningen, Netherlands
Vinod Kumar, PhD

School of Health Sciences, University of Minho, Portugal
Agostinho Carvalho, PhD
Cristina Cunha, PhD

3B´s Research Group, University of Minho, Portugal
Nuno M. Neves, PhD
Helena Ferreira, PhD

The MDACC, University of Houston, Houston,
Dimitrios P Kontoyiannis, MD

University of Maryland, Baltimore
Vincent Bruno, PhD

Harbor-UCLA Medical Center
Ashraf S Ibrahim, PhD

Thank you!