

Defense response

TLR4

TLR2

TLR5

TLR8

IRAK4

IRAK3

CD163

CD55

CD59

IL18R1

IL18RAP

IL1R1

IL1R2

IL1RAP

CARD12

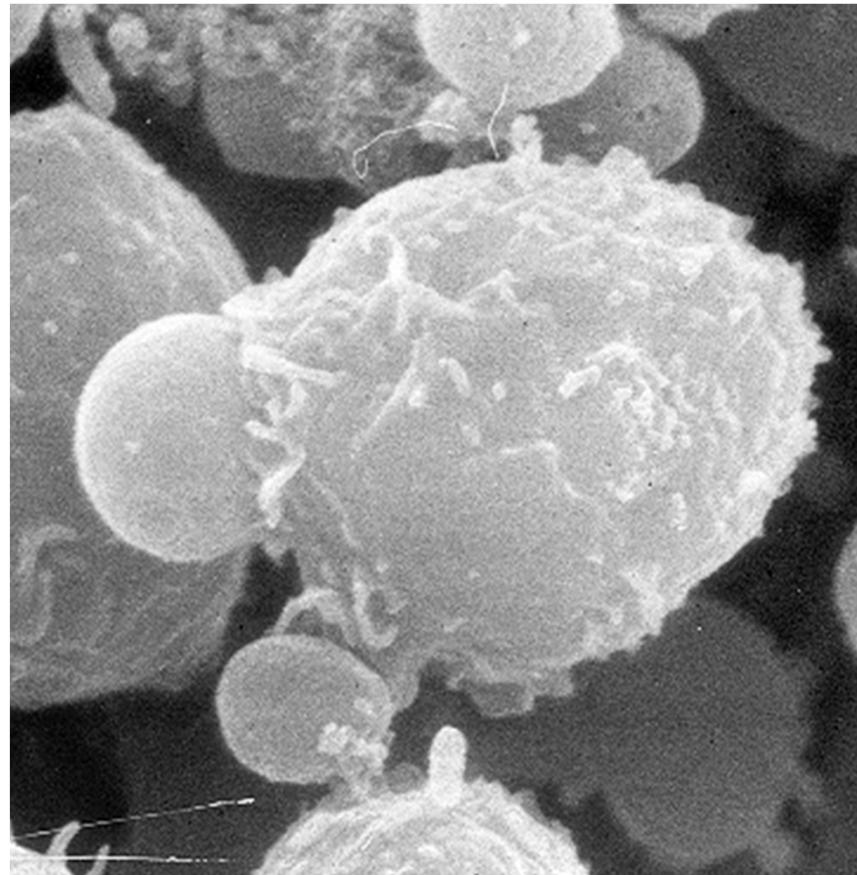
NALP3

NAIP

FCAR

FCGR1A

FCGR3B



CARD9

ALOX15

NCR3

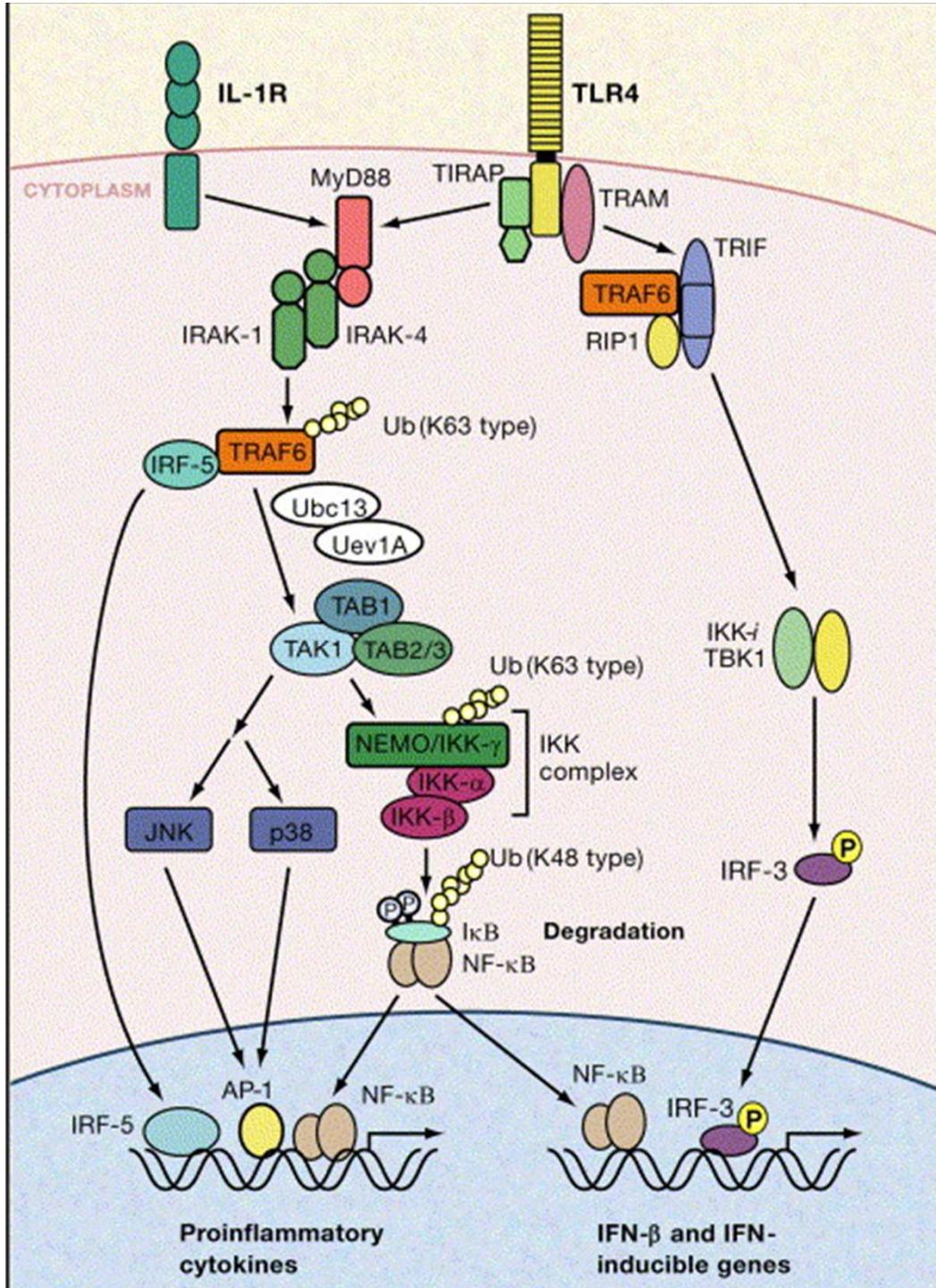
CXCL1

LY75

CD46

AZU1

FAIM3

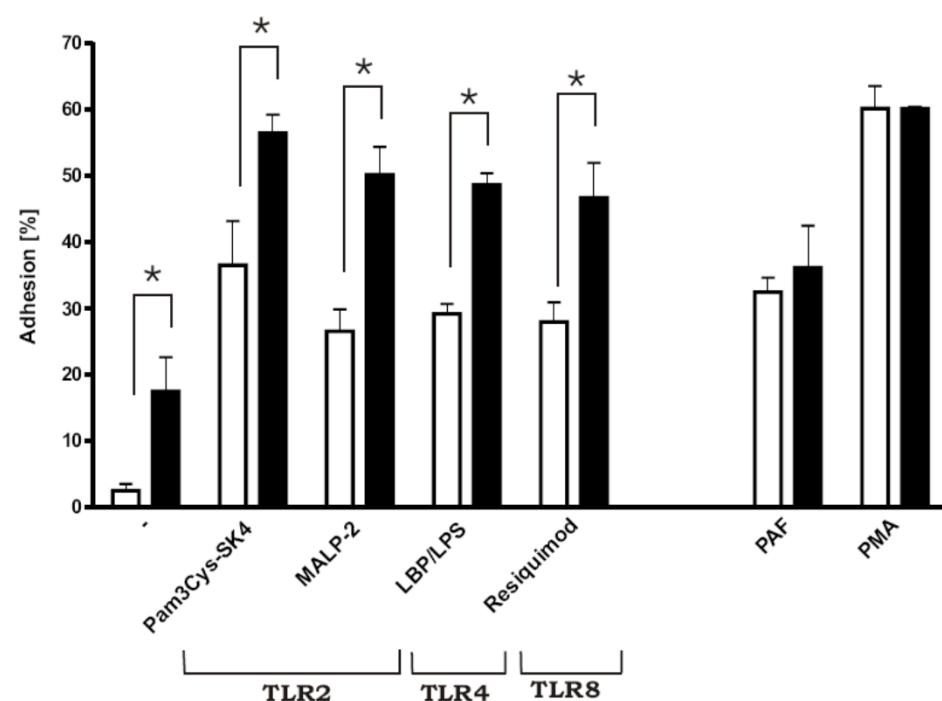
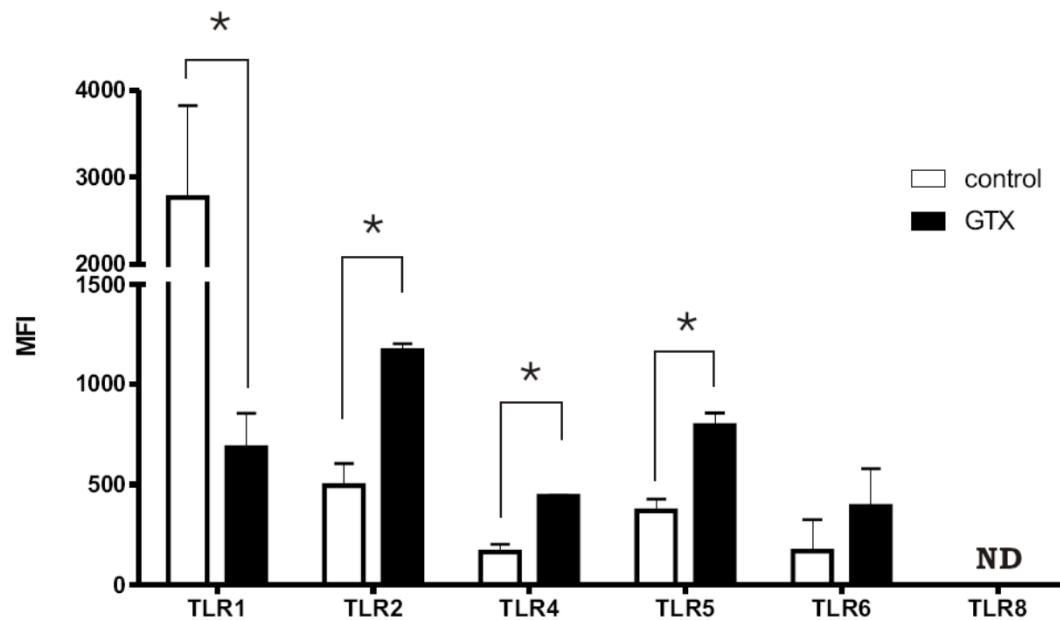


TLR1-11
surface receptors

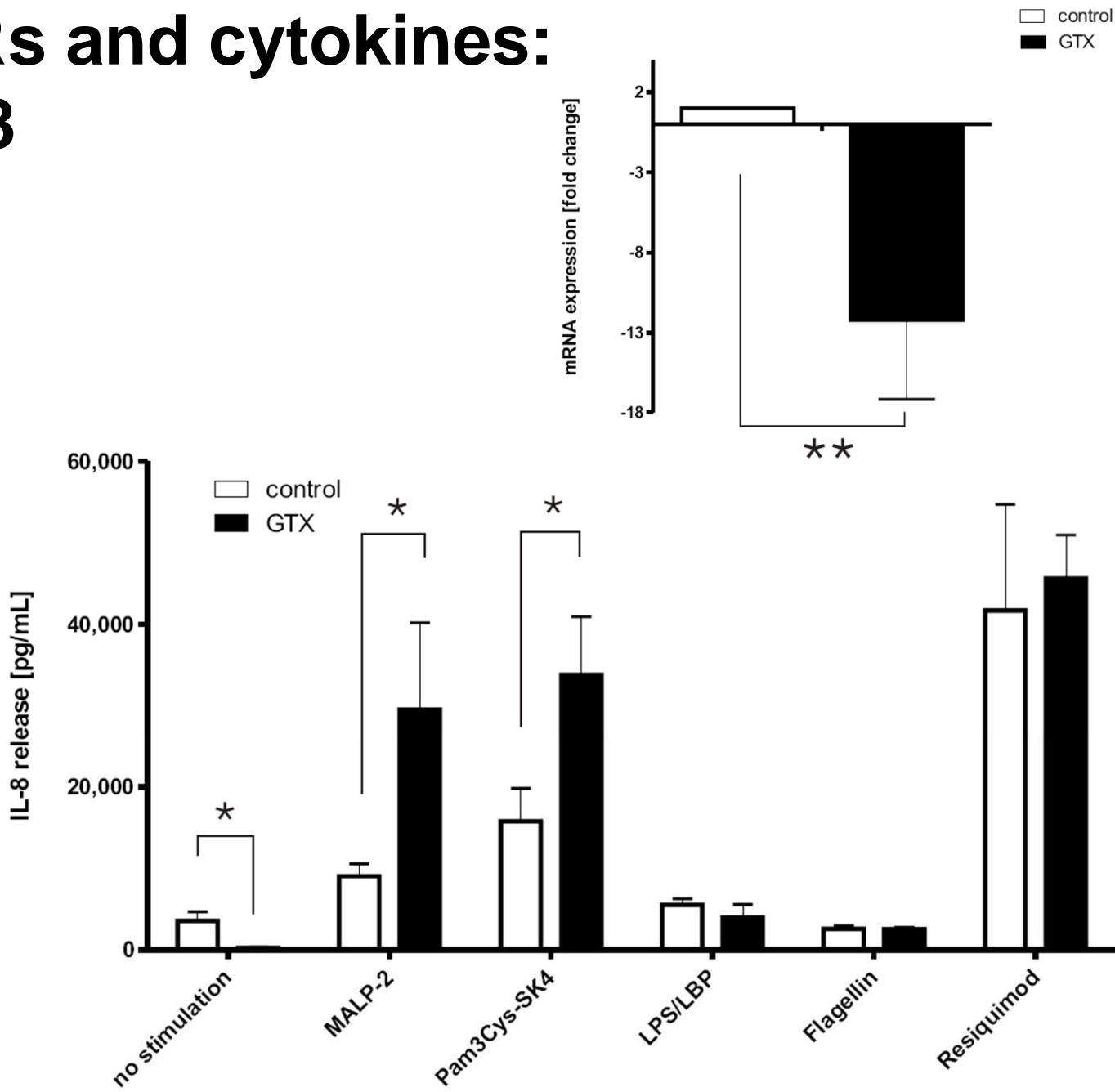
endosomal
receptors

**TLR-specific
recognition
& signaling**

Bacteria		
LPS	Gram-negative bacteria	TLR4
Diacyl lipopeptides	<i>Mycoplasma</i>	TLR6/TLR2
Triacyl lipopeptides	Bacteria and mycobacteria	TLR1/TLR2
LTA	Group B <i>Streptococcus</i>	TLR6/TLR2
PG	Gram-positive bacteria	TLR2
Porins	<i>Neisseria</i>	TLR2
Lipoarabinomannan	Mycobacteria	TLR2
Flagellin	Flagellated bacteria	TLR5
CpG-DNA	Bacteria (viruses) and mycobacteria	TLR9
ND	Uropathogenic bacteria	TLR11
Fungus		
Zymosan	<i>Saccharomyces cerevisiae</i>	TLR6/TLR2
Phospholipomannan	<i>Candida albicans</i>	TLR2
Mannan	<i>Candida albicans</i>	TLR4
Glucuronoxylosemannan	<i>Cryptococcus neoformans</i>	TLR2 & TLR4
Parasites		
Glycoinositolphospholipids	<i>Trypanosoma</i>	TLR2 & TLR4
Hemozoin	<i>Plasmodium</i>	TLR9
Profilin-like molecule	<i>Toxoplasma gondii</i>	TLR11
Viruses		
DNA	Viruses	TLR9
dsRNA	Viruses	TLR3
ssRNA	RNA viruses	TLR7 and TLR8
Envelope proteins	RSV, MMTV	TLR4
Hemagglutinin protein	Measles virus	TLR2
ND	HCMV, HSV1	TLR2



TLRs and cytokines: IL-8



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IL18RAP

IL1R1

IL1R2

IL1RAP

CARD12

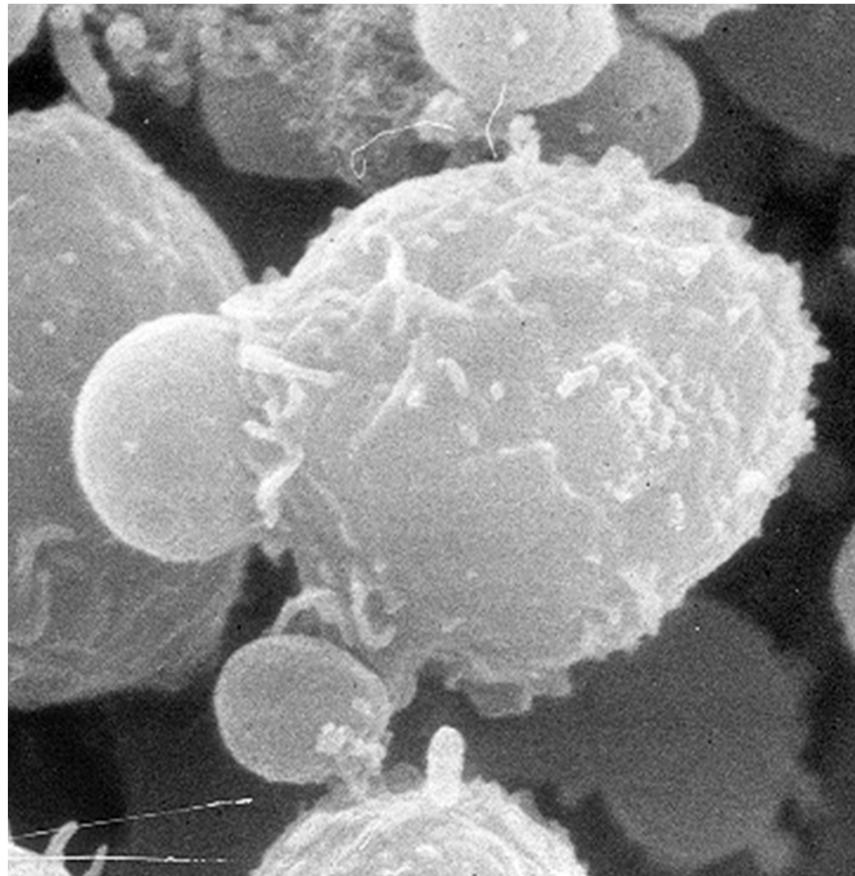
NALP3

NAIP5

FCAR

FCGR1A

FCGR3B



CARD9

ALOX15

NCR3

CXCL1

LY75

CD46

AZU1

FAIM3

Pyrin



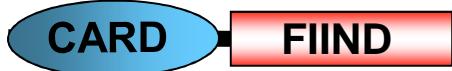
FMF



Caspase-1



CARD 8



NALP 3



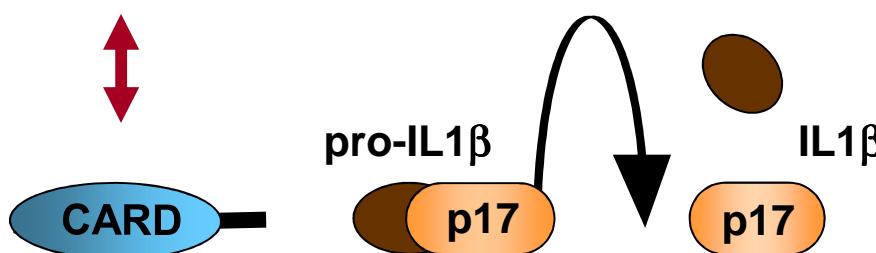
ASC



Caspase-1

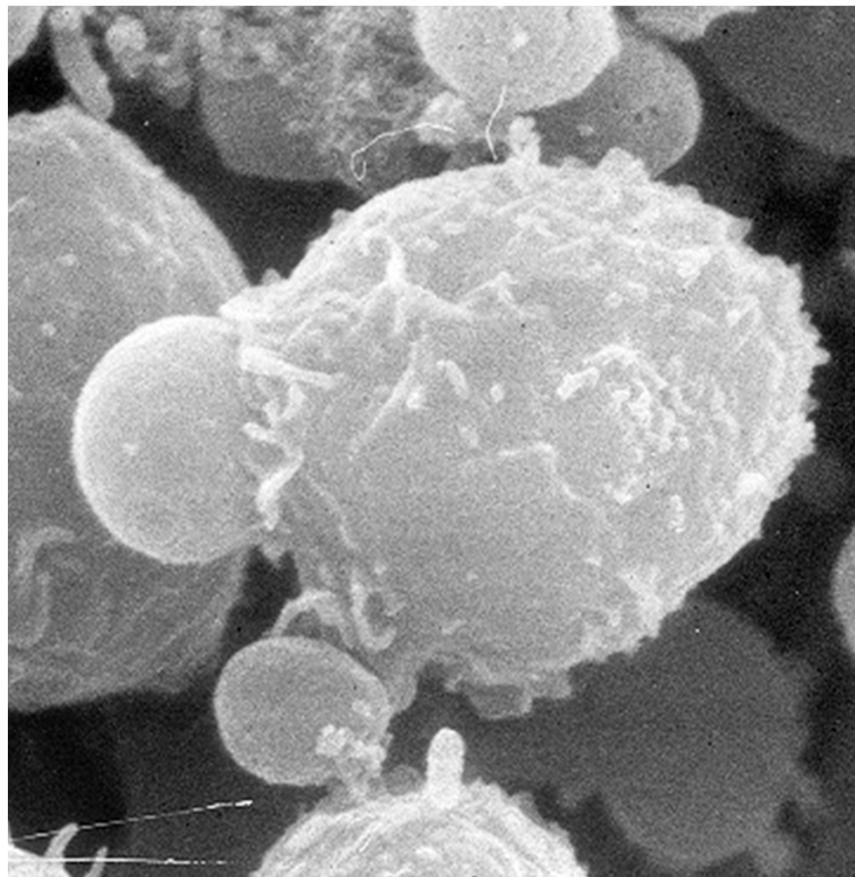


ICEBERG
pseudoICE
INCA



Defense response

TLR4
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CD163
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IL18R1
IL18RAP
IL1R1
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CARD12
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FCGR1A
FCGR3B



CARD9

ALOX15

NCR3

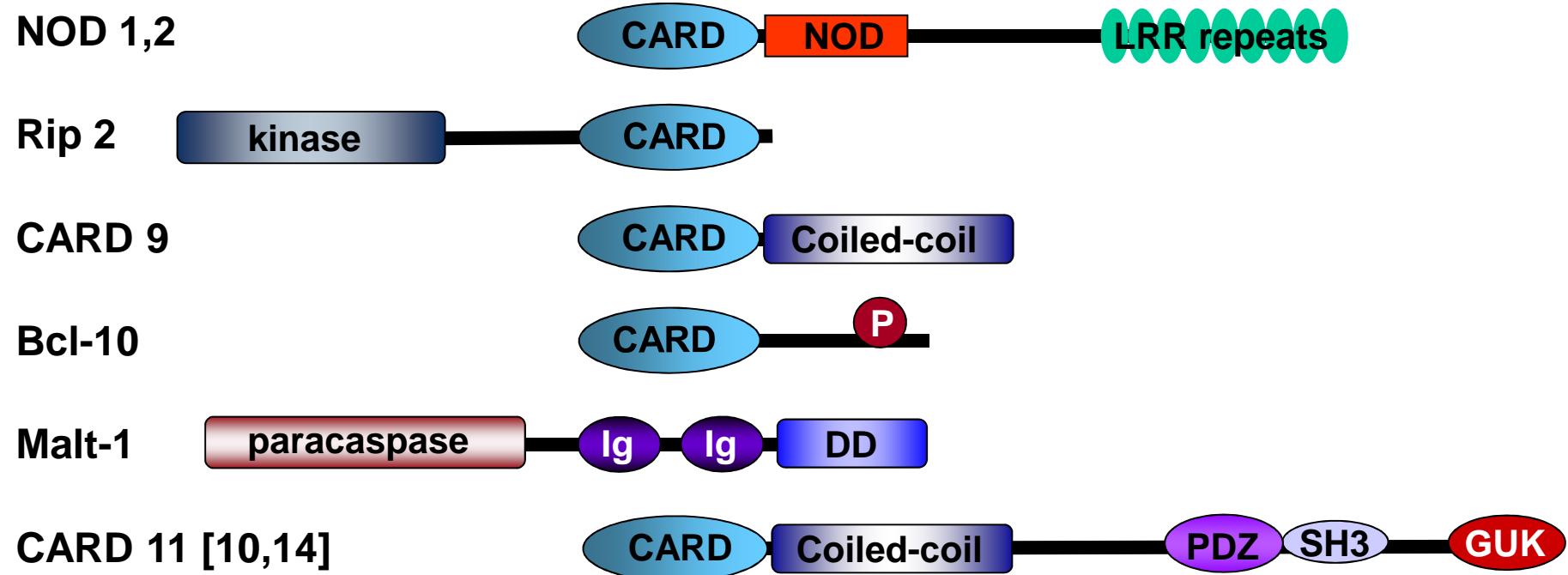
CXCL1

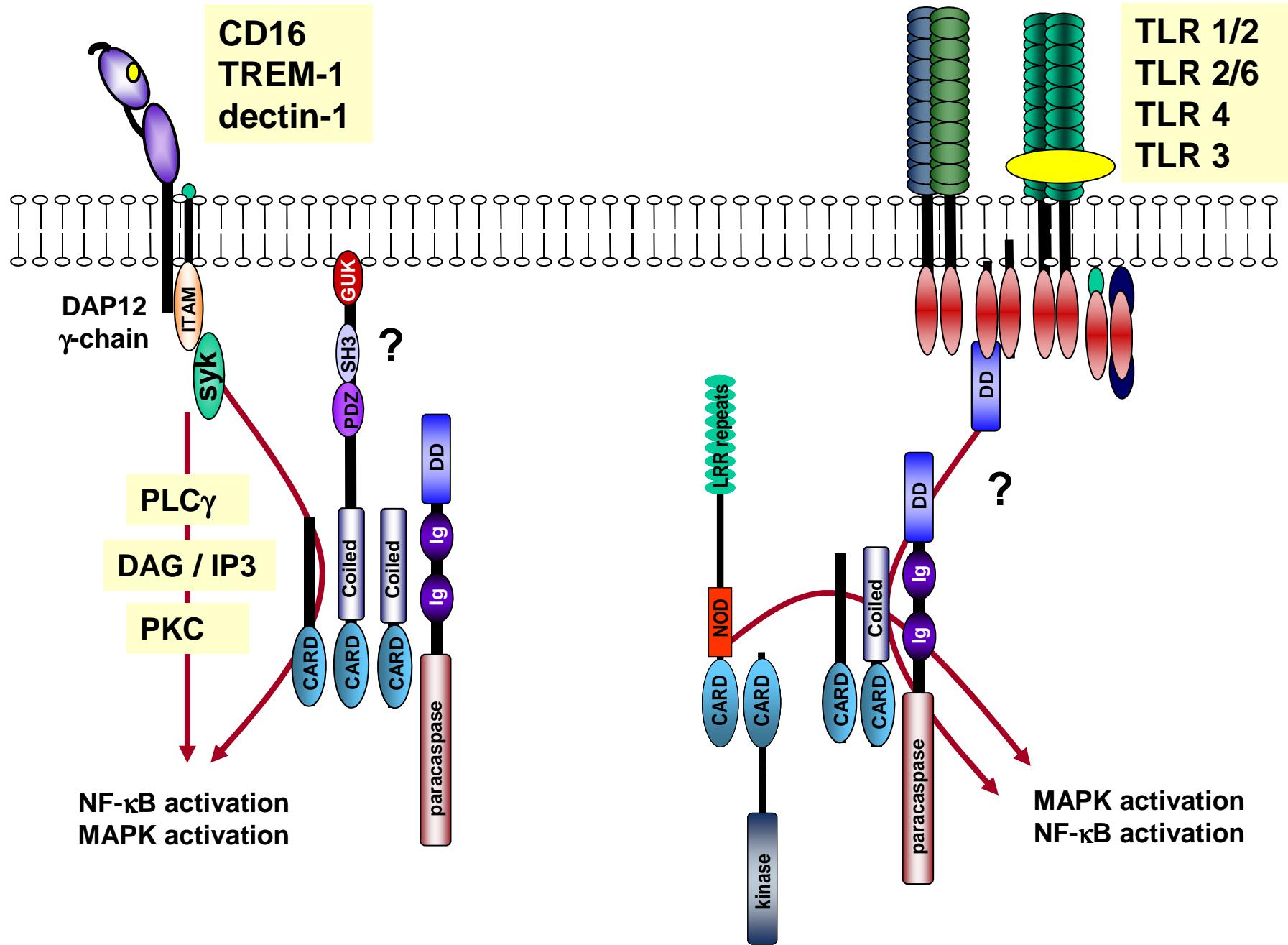
LY75

CD46

AZU1

FAIM3





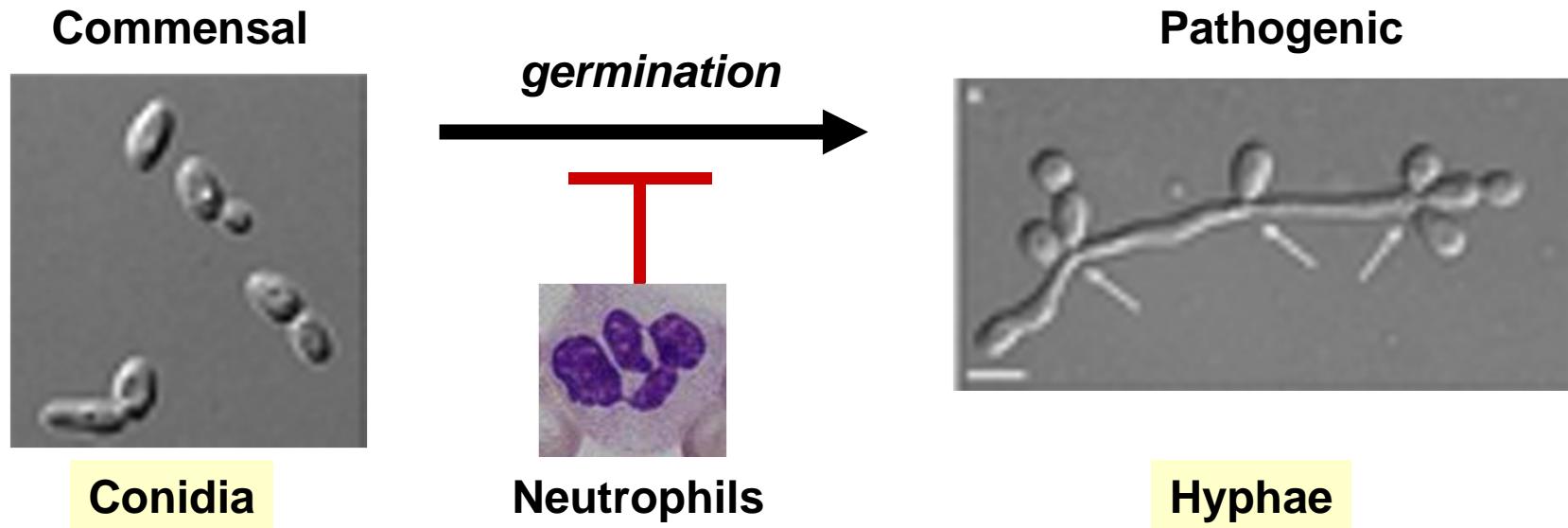
blood

Prepublished online January 18, 2013;
doi:10.1182/blood-2012-08-450551

Invasive fungal infection and impaired neutrophil killing in human CARD9 deficiency

Agata A. Drewniak, Roel P. Gazendam, Anton T.J. Tool, Michel van Houdt, Machiel H. Jansen, John L. van Hamme, Ester M.M. van Leeuwen, Dirk Roos, Emmanuel Scalais, Carine de Beaufort, Hans Janssen, Timo K. van den Berg and Taco W. Kuijpers

Neutrophil inhibition of outgrowth of *Candida* and *Aspergillus* species



unopsonized
serum opsonized

- conidia outgrowth after 2 hrs
- germination of hyphae at 20 hrs
(number of hyphae [GFP])
- hyphae killing (MTT staining)

Granulocyte Transfusions

Granulocyte concentrates & fungal infections after 1995?

- **Increased survival in patients unresponsive to standard therapy:
9 of 15 patients (60%) showed objective improvement**
(Hester *et al.* J Clin Apheresis 1995;10:188-93)
- **Impressive responses in 11 of 15 patients (80%) with invasive
fungal diseases resistant to amphotericin B** (Dignani *et al.* Leukemia
1997;11:1621-30)
- **Survival to day 100 of 14 of 23 patients (60%) with severe fungal
infection** (Peters *et al.* Br J Haematol 1999;106:689-96)

Granulocyte Transfusions

- How to select patients-at-risk for interventional GTx ?
- RCT studies required for further confirmation of the clinical effects observed in GTx studies are missing

Safety and Effectiveness of Granulocyte Transfusions in Resolving Infection in People With Neutropenia (The RING Study)

ClinicalTrials.gov

A service of the U.S. National Institutes of Health

This study is currently recruiting participants.

Sponsor:

[National Heart, Lung, and Blood Institute \(NHLBI\)](#)

Information provided by (Responsible Party):

National Heart, Lung, and Blood Institute (NHLBI)

ClinicalTrials.gov Identifier:

NCT00627393

Estimated Enrollment:236

Study Start Date:April 2008

Estimated Study Completion Date:December 2013

Sanquin Research, Amsterdam

Dept Blood Cell Research

Timo van den Berg

Martin de Boer

Willemijn Breunis

Robin van Bruggen

Agata Drewniak

Roel Gazendam

Judy Geissler

Michel van Houdt

Dirk Roos

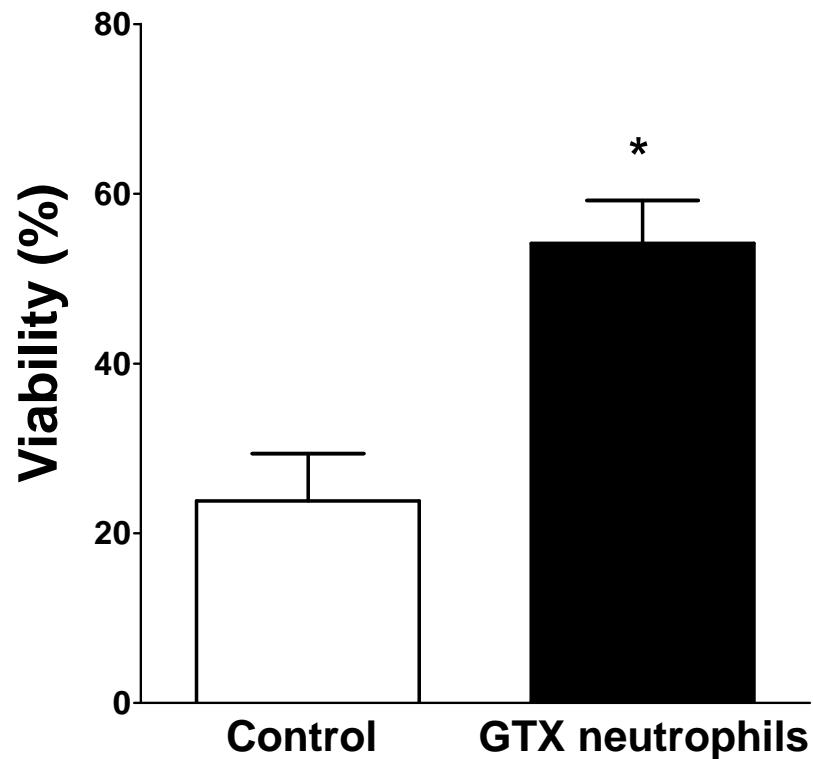
Anton Tool

Paul Verkuijlen



Neutrophil killing of *Candida albicans*

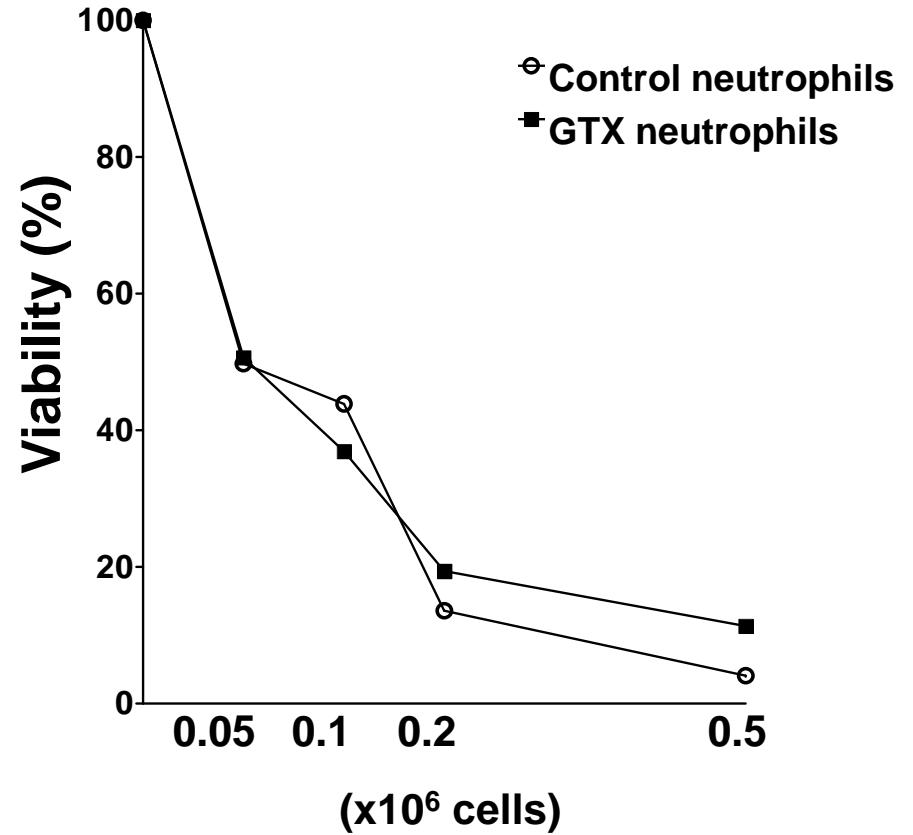
Candida conidia



2 hours

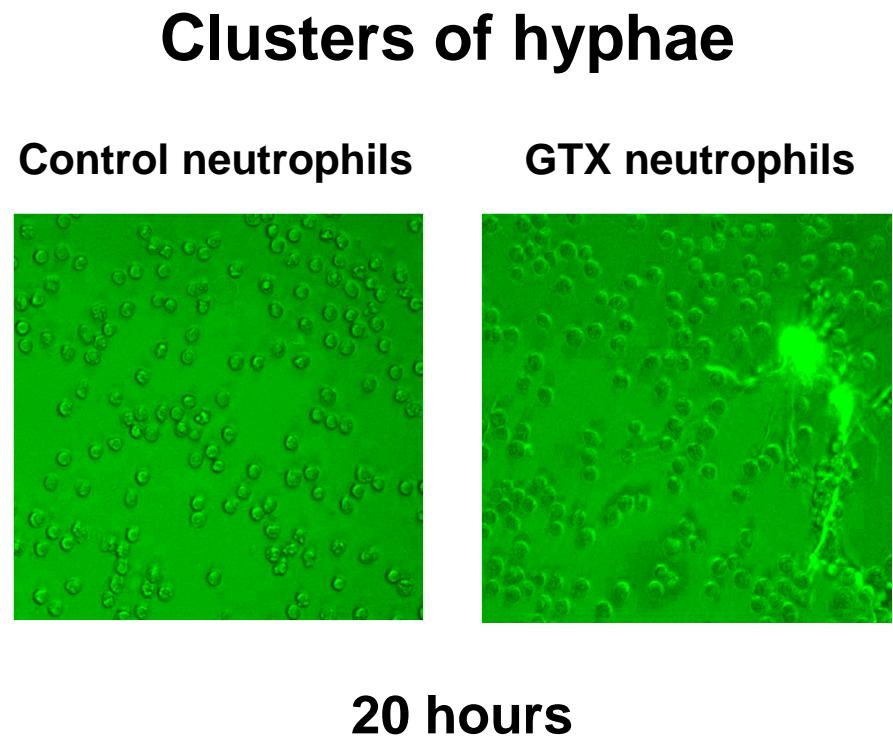
N = 5

Candida hyphae

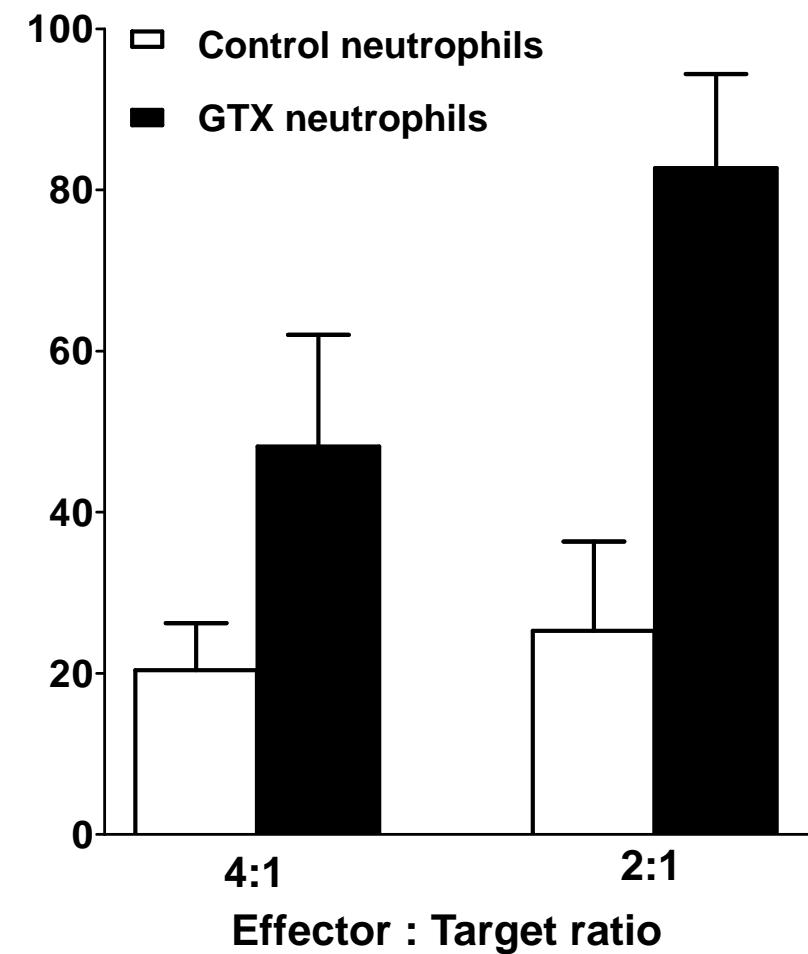


2 hours

Inhibition of *Candida albicans* germination

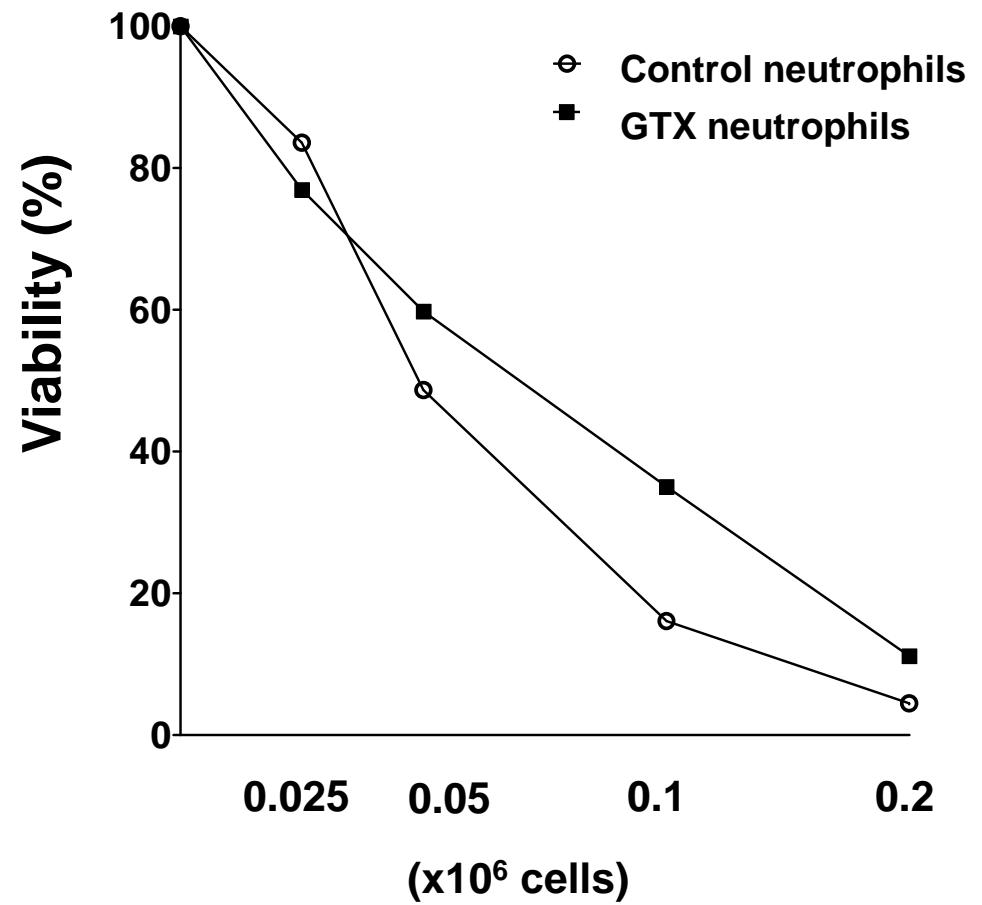
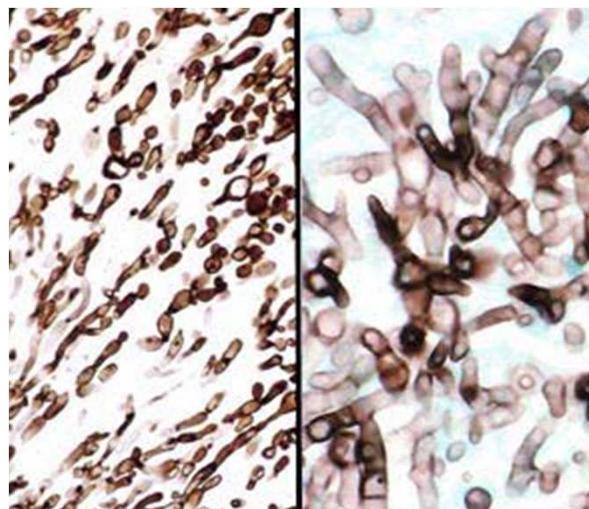


N=5



Killing of *Aspergillus fumigatus* hyphae

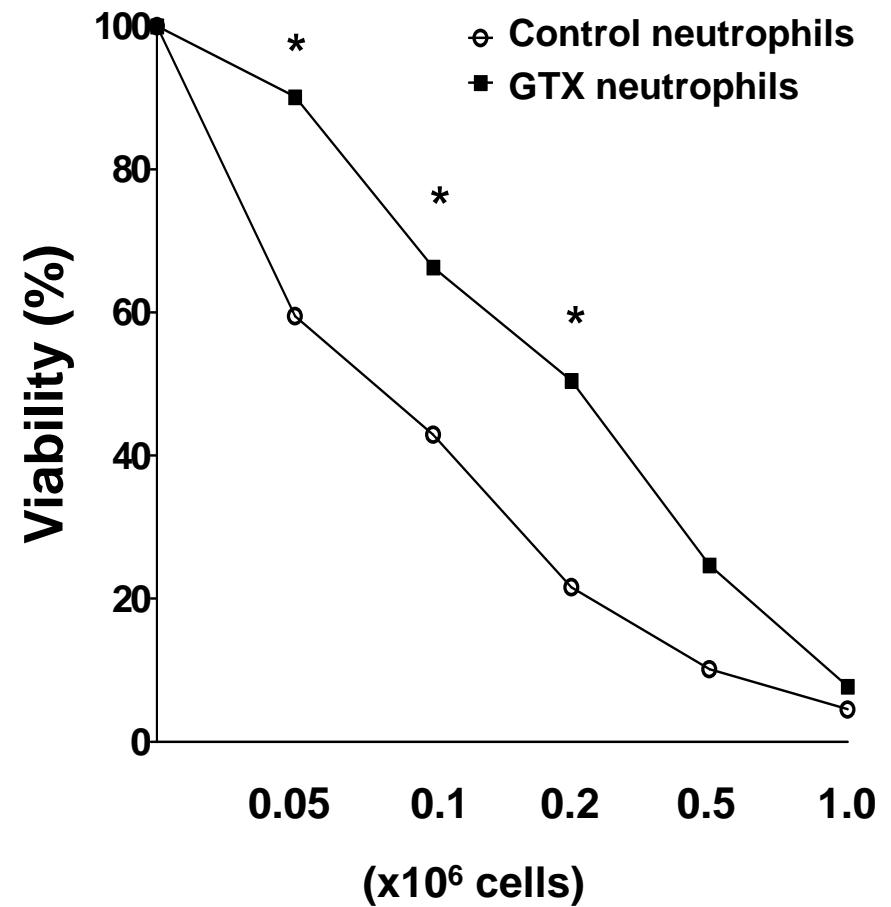
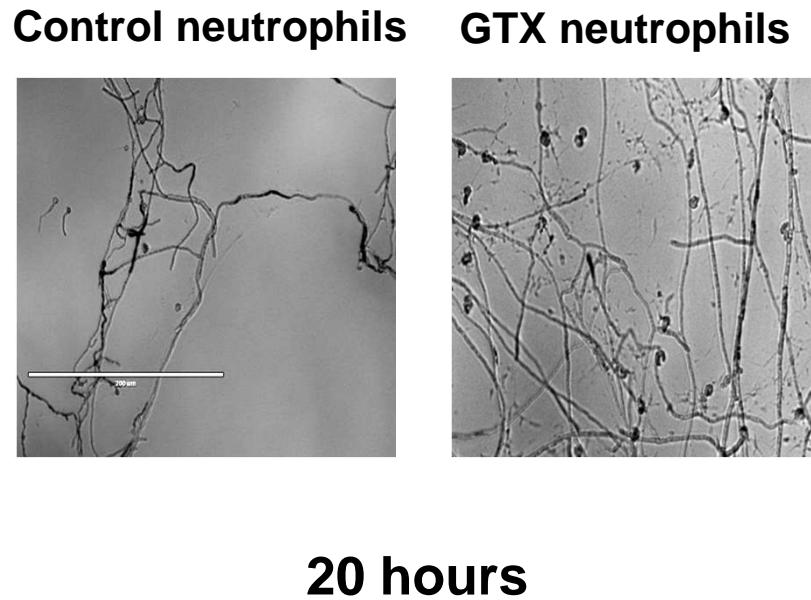
2 hours



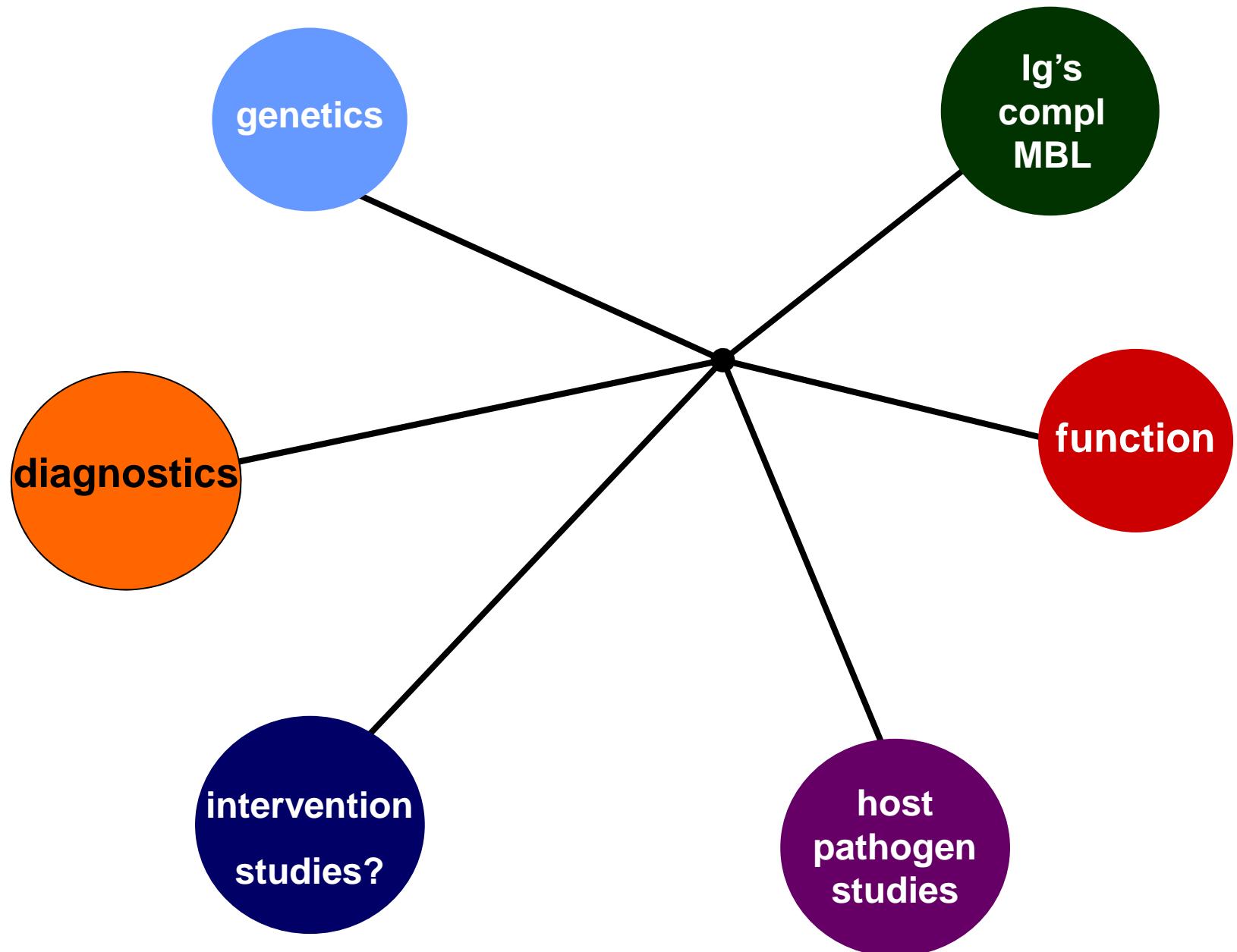
N=3

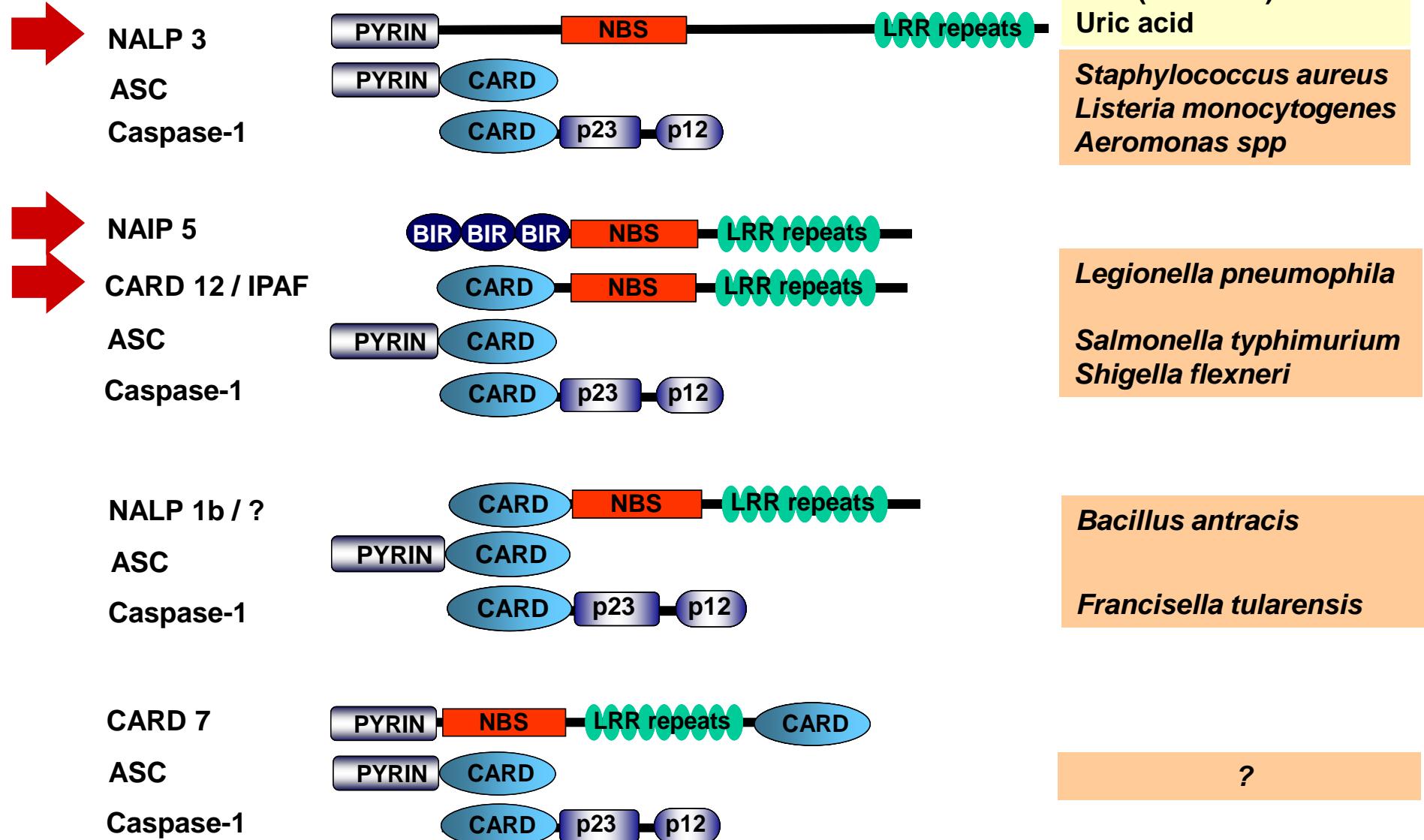
Inhibition *Aspergillus fumigatus* germination

Clusters of hyphae



N=4





**TLRs
CLRs
TREMs**

**NBS-LRRs
NLRs
IRFs**

**fraticide
factors
?**

**PRRs
opsonins**

**inflammasome
signalosome**

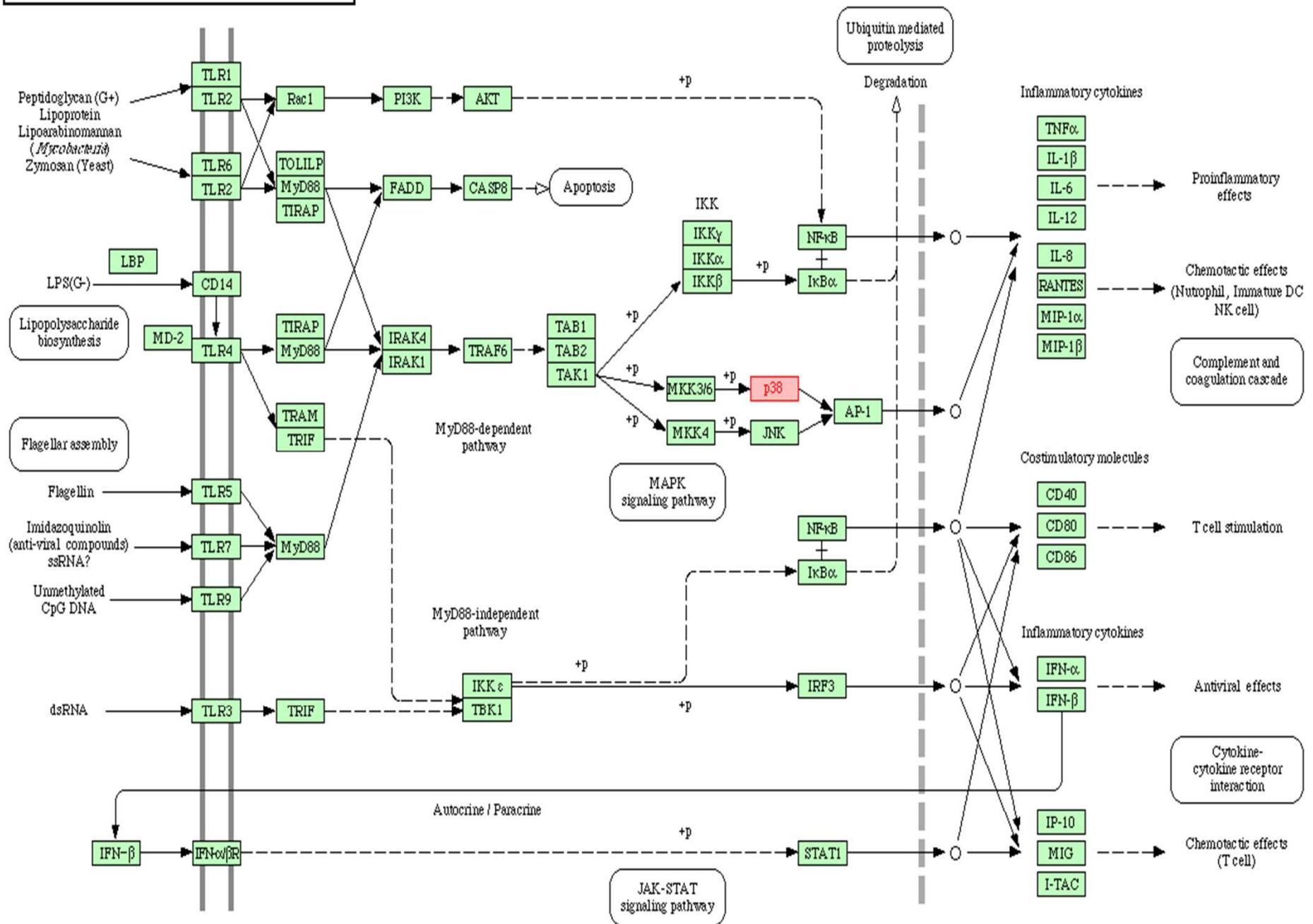
**apoptosome
cell death**

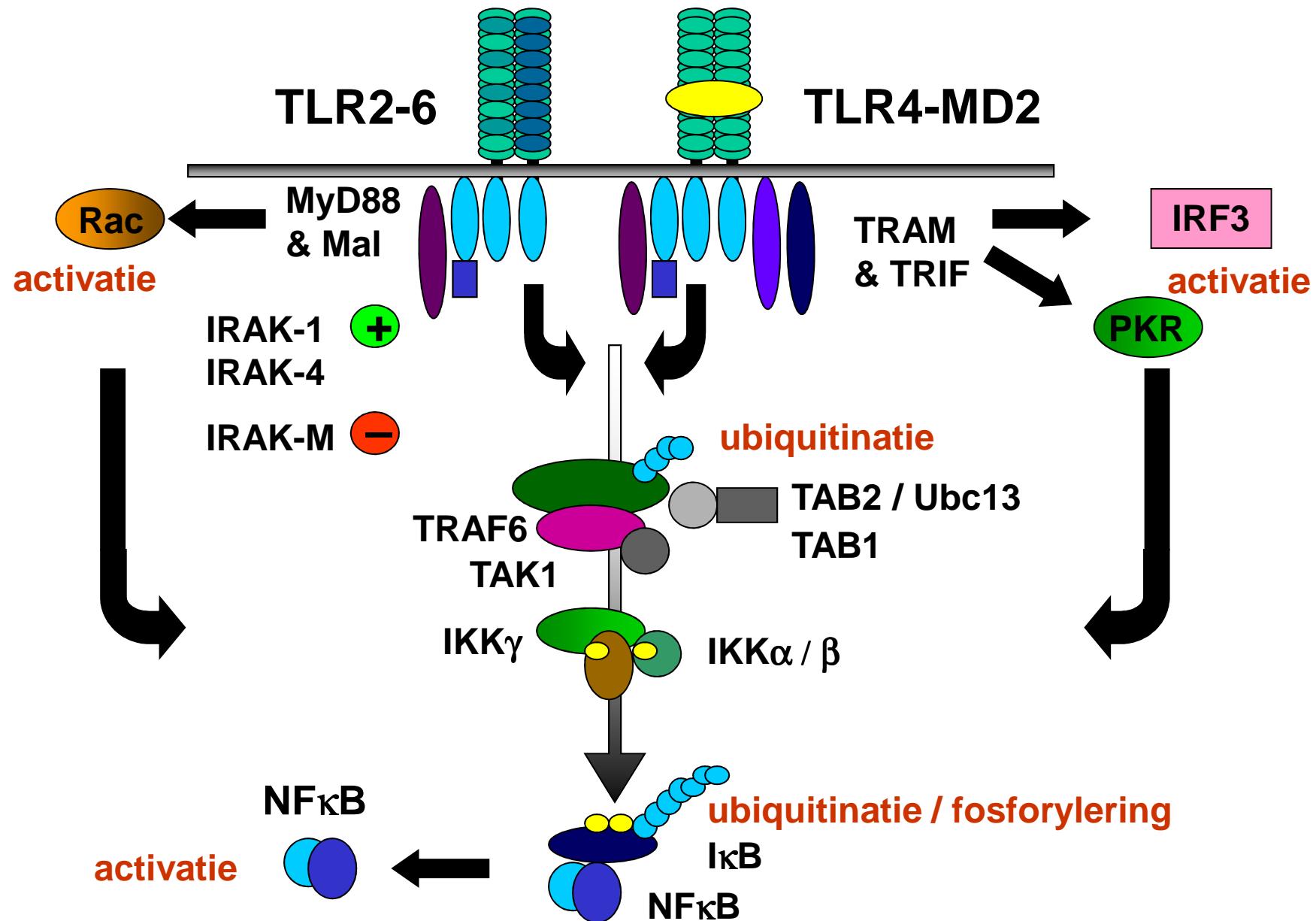
**antibodies
complement
soluble lectins
bacterial products**

**microbial products
 K^+ changes
RIG1 / MDA**

**acidification
caspases
other?**

TOLL-LIKE RECEPTOR SIGNALING PATHWAY





Protein representation of micro-array data

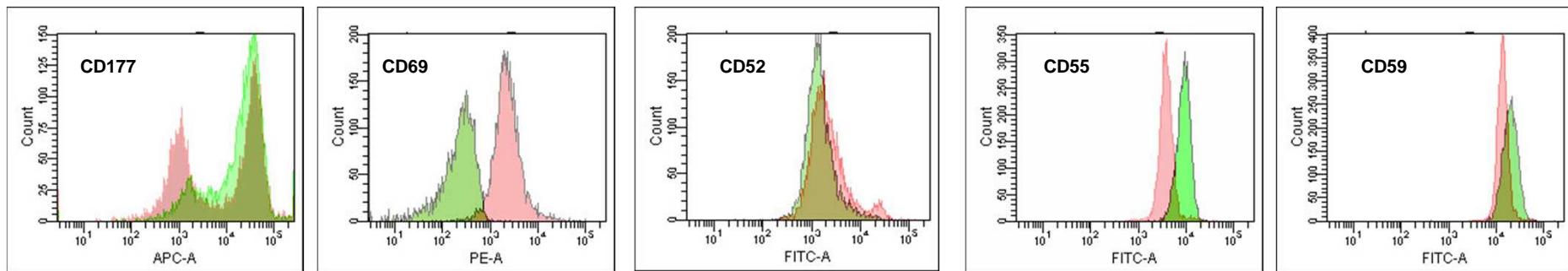
Gene name	Fold change (after G-CSF/dexa)	Protein	Expression (MFI; control vs. sample)
NB1, HNA2A	(+) 20.3	CD177	25265 vs. 31745
CD69,CLEC2C	(-) 28.6	CD69	2980 vs. 222
CD52	(-) 15	CD 52	3654 vs. 1957
CD55, DAF	(+) 3.4	CD55	4095 vs. 9356
CD59	(+) 5	CD59, Protectin	14512 vs. 21245



control



G-CSF/dexamethasone
(*in vivo*)



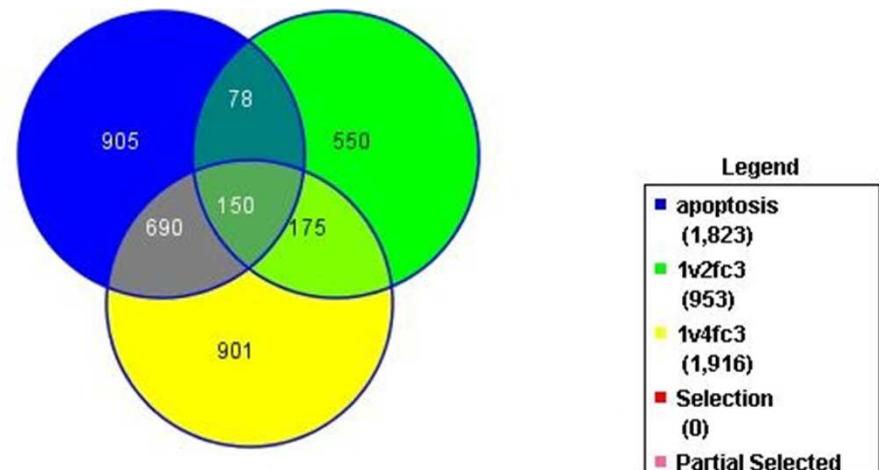
Graphs are representative for
3 separate experiments

Gene ontology analysis

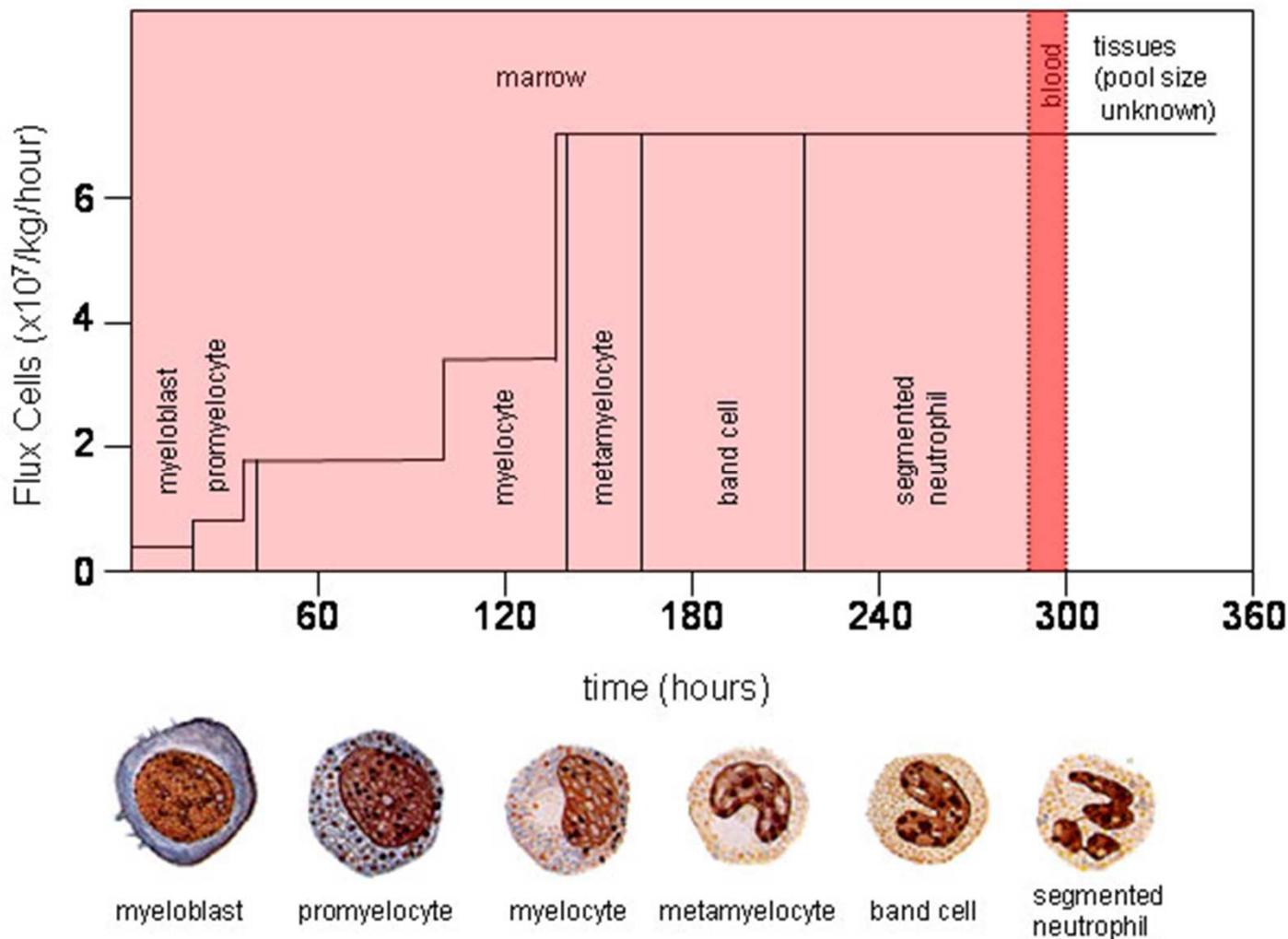
Spotfire (AMC)

- On line programs:**
- GO Tree machine
 - Onto express
 - GO Stats

Venn Diagram: 3 Sequence Set(apoptosis, 1v2fc3, 1v4fc3)



Neutrophil Differentiation



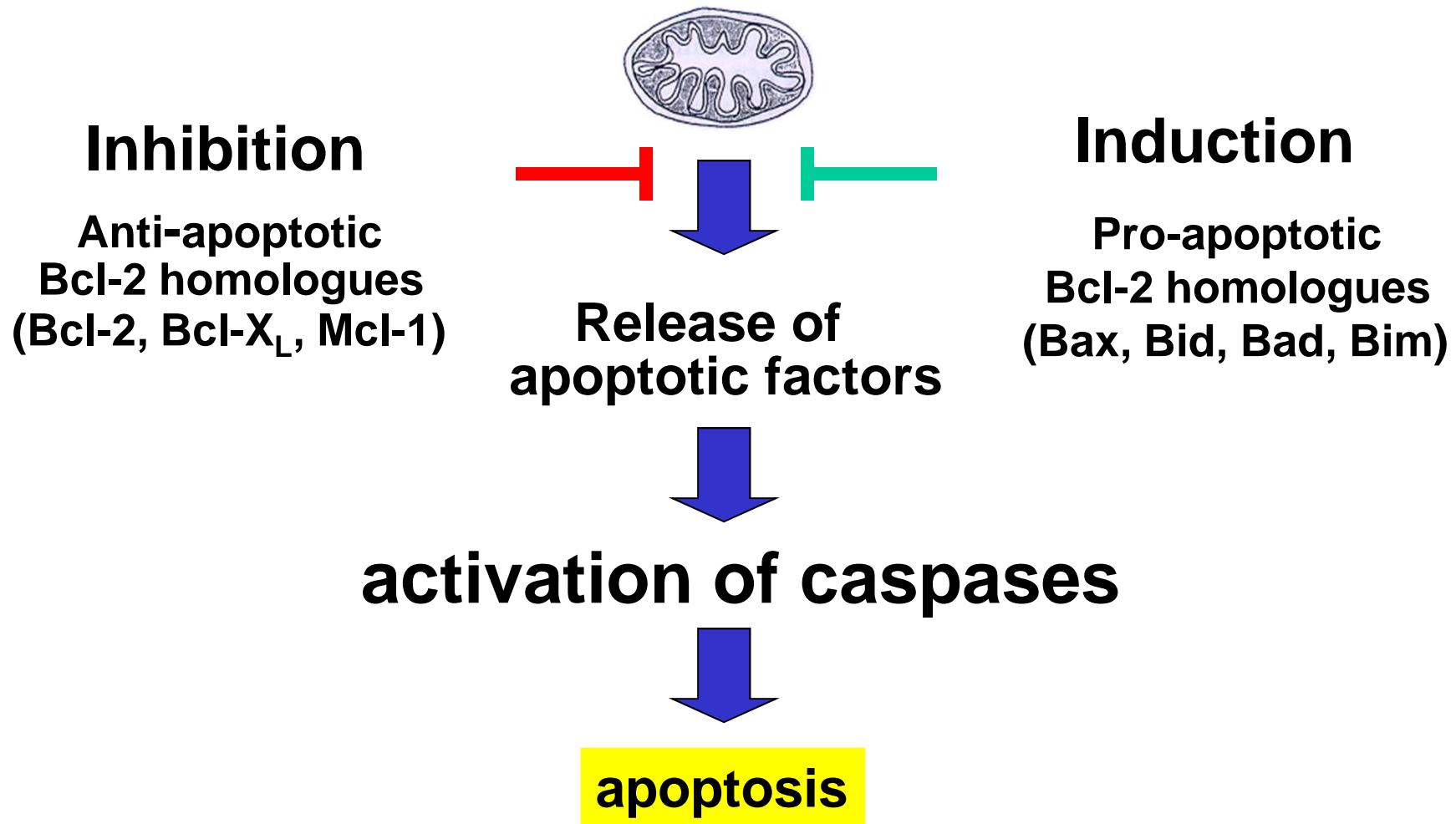
In the bone marrow billions of neutrophils are produced every day

Neutrophils are short-lived cells (8-20 hours)

Their life-span can be increased up to several days if the cells leave the circulation and enter tissues, especially during inflammation or infection

Adapted from Bainton et al. 1980

Crossroads of Life and Death: role of mitochondria



Changes in gene expression of granulocytes during in vivo granulocyte colony-stimulating factor/dexamethasone mobilization for transfusion purposes

Agata Drewniak,^{1,2} Bram J. van Raam,^{1,2} Judy Geissler,¹ Anton T.J. Tool,¹ Olaf R.F. Mook,³ Timo K. van den Berg,¹ Frank Baas,³ and Taco W. Kuijpers^{1,2}

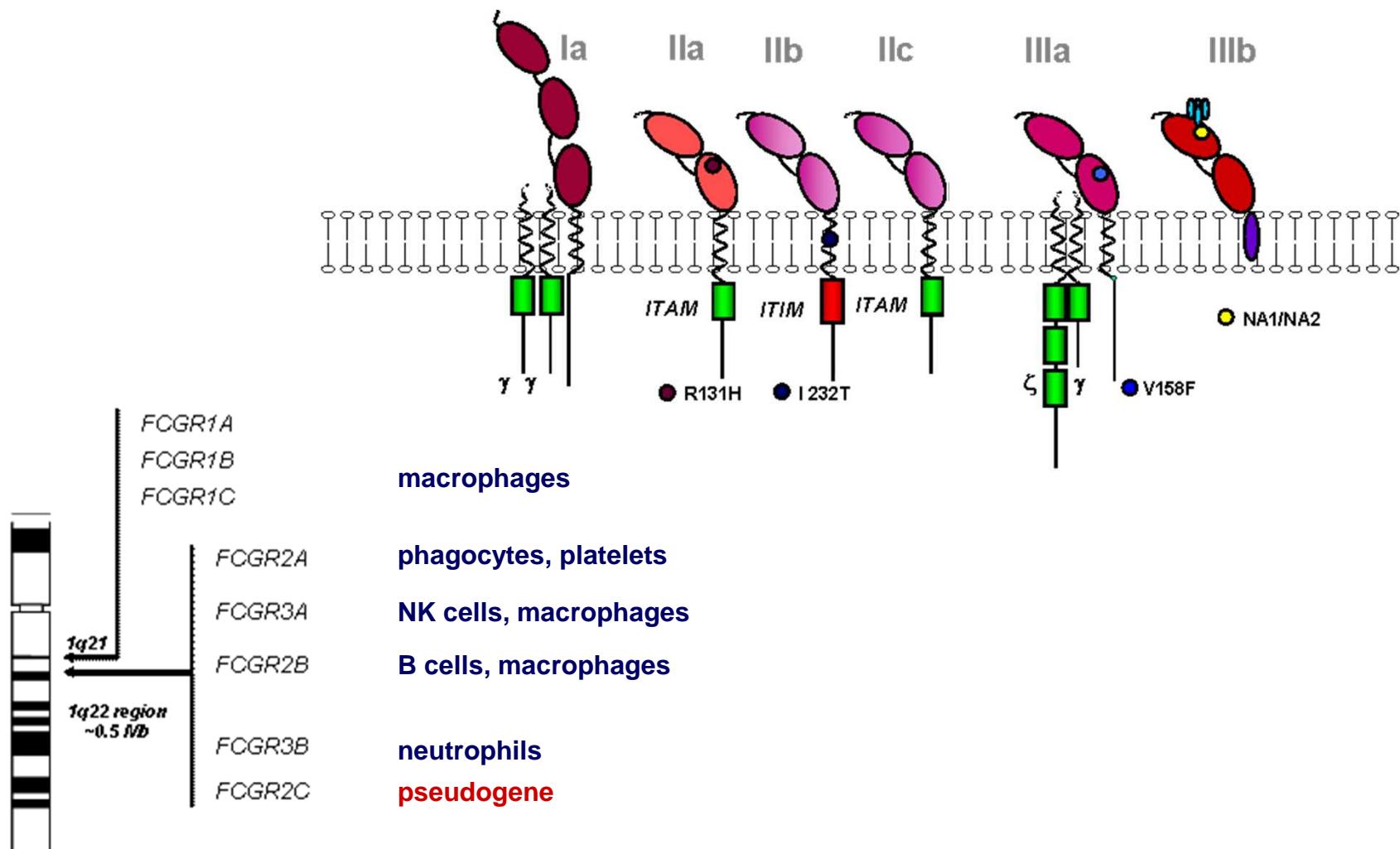
¹Department of Blood Cell Research, Sanquin Research and Landsteiner Laboratory, Amsterdam; ²Emma Children's Hospital, Academic Medical Center, University of Amsterdam, Amsterdam; and ³Department of Neurogenetics, Academic Medical Center, Amsterdam, The Netherlands

The treatment of healthy donors with granulocyte colony-stimulating factor (G-CSF) and dexamethasone results in sufficient numbers of circulating granulocytes to prepare granulocyte concentrates for clinical purposes. Granulocytes obtained in this way demonstrate relatively normal functional behavior combined with a prolonged life span. To study the influence of mobilizing agents on granulocytes, we used oligonucleotide microarrays to identify genes that are differentially expressed in mobilized granulocytes compared with control granulocytes. More

than 1000 genes displayed a differential expression pattern, with at least a 3-fold difference. Among these, a large number of genes was induced that encode proteins involved in inflammation and the immune response, such as C-type lectins and leukocyte immunoglobulin-like receptors. Because mobilized granulocytes have a prolonged life span, we focused on genes involved in the regulation of apoptosis. One of the most prominent among these was *CAST*, the gene encoding calpastatin. Calpastatins are the endogenous inhibitors of calpains, a family

of calcium-dependent cysteine proteases recently shown to be involved in neutrophil apoptosis. Transcriptional activity of the *CAST* gene was induced by G-CSF/dexamethasone treatment both in vivo and in vitro, whereas the protein expression of *CAST* was stabilized during culture. These studies provide new insight in the genotypic changes as well as in the regulation of the immunologic functions and viability of mobilized granulocytes used for clinical transfusion purposes. (Blood. 2009;113:5979-5998)

IgG receptors (Fc γ RI, Fc γ RII, Fc γ RIII)



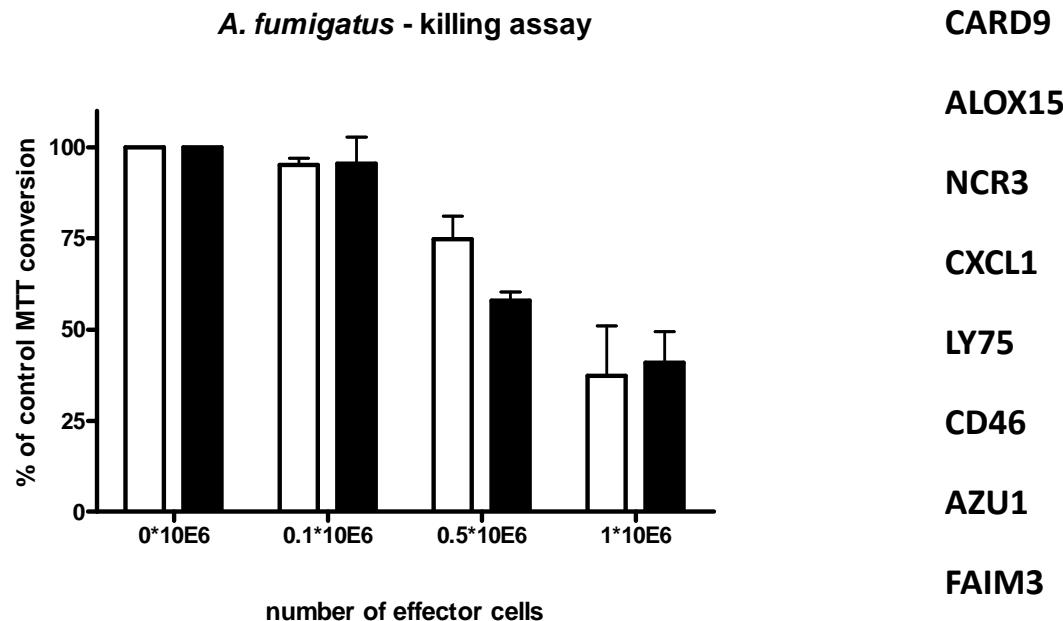
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FCGR1A
FCGR3B



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ALOX15
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LY75
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AZU1
FAIM3

FEVER syndromes

Hoffmann HM, Mueller JL, Boride DH, Wanderer AA, Koldner RD

Mutation of a new gene encoding a putative pyrin-like protein causes familial cold autoinflammatory syndrome and Muckle-Wells syndrome.

Nat Genet. 2001; 29: 301-5.

Feldmann J, Prieur A, Quartier P, Berquin P, Cortis E, Teillac-Hamel D, Fischer A, de Saint Basile G

Chronic infantile neurological cutaneous and articular syndrome is caused by mutations in CIAS1, a gene highly expressed in polymorphonuclear cells and chondrocytes.

Am J Hum Genet 2002; 70:198-203

Aganna E, Martinon F, Hawkins PN, Ross JB, Swan DC, Booth DR, Lachmann HJ, Gaudet R, Woo P, Feighery C, Cotter FE, Thome M, Hitman GA, Tschopp J, McDermott MF

Association of mutations in the NALP3/CIAS1/PYPAF1 gene with a broad phenotype including recurrent fever, cold sensitivity, sensorineural deafness, and AA amyloidosis.

Arthritis Rheum 2002; 46: 2445-52

Dodé C, Le Du N, Cuisset L, Letourneur F, Berthelot JM, Vaudour G, Meyrier A, Watts RA, Scott DG, Nicholls A, Granel B, Frances C, Garcier F, Edery P, Boulinguez S, Domergues JP, Delpech M, Grateau G

New mutations of CIAS1 that are responsible for Muckle-Wells syndrome and familial cold urticaria: a novel mutation underlies both syndromes.

Am J Hum Genet 2002; 70: 1498-506

