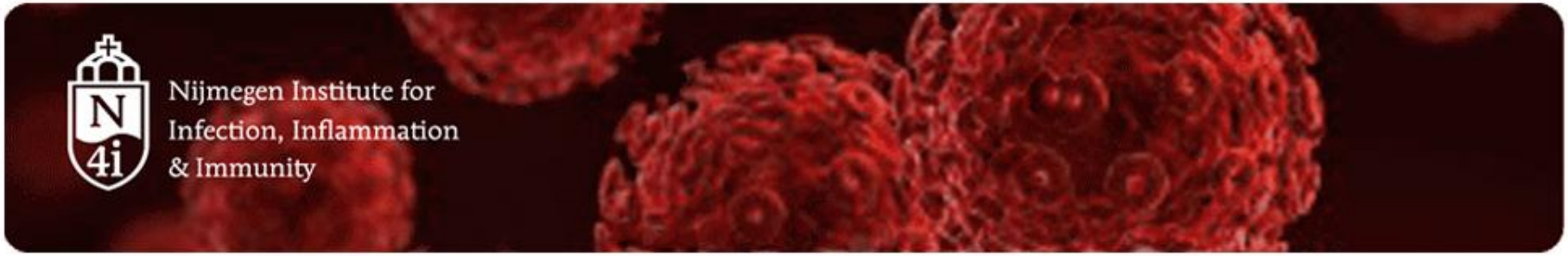


# A short history of immunology: from phagocytosis to the human genome

Mihai G. Netea

UMC St Radboud

Nijmegen Institute for Infection, Inflammation & Immunity

# The Toll of infectious diseases

## MEDIEVAL MAGAZINE

Monday, August 5, 1352

# Black Death kills 3000 in one week

This week more than 3,000 people in Britain have died from the Black Death.

This brings the total dying across Europe to 15 million dying from the bubonic plague and 10 million dying from the terrible pneumonic plague. Will this ever stop? Will anyone survive?

Already 17 of our best writers have died and 20 are at home sick.

Whose fault is it? In Strasbourg, it is said that the Jews are to blame, in Italy, God has supposedly brought this upon them. However the real reason is that our roads are simply not clean enough.

The Lord Mayor of London has ordered the streets to be cleaned and others are following this ingenious order.

With doctors still not being able to find a cure we can only hope that this disease simply goes away.

The Black Death is usually associated with Europe but it neither began nor ended then. The earliest records of this pestilence are in China.

In 46 AD an epidemic in Mongolia killed two-thirds of the population.

In 312 northern and central China became a wasteland and in the province of Shensi, only one or two out of 100 taxpayers survived. In 468, 140,000 people died in the Chinese cities of Honan, Hopei, Shantung and others.

**Estimated population of Europe from 1000 to 1352.**

- 1000 38 million
- 1100 48 million
- 1200 59 million
- 1300 70 million
- 1347 75 million
- 1352 50 million

**25 million people died in just under five years between 1347 and 1352.**

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# Miasmae vs germs

A representation of the cholera epidemic of the 19th century depicts the spread of the disease in the form of poisonous air

-The theory of miasma disease made sense to the English sanitary reformers of the mid-19th century.
- Miasma explained why cholera and other disease were epidemic in places where water was undrained and foul-smelling.
- the theory led to improvements in water drainage and sanitation, which decrease cholera outbreaks, leading increase support for the theory.

- Florence Nightingale was a strong supporter of the theory

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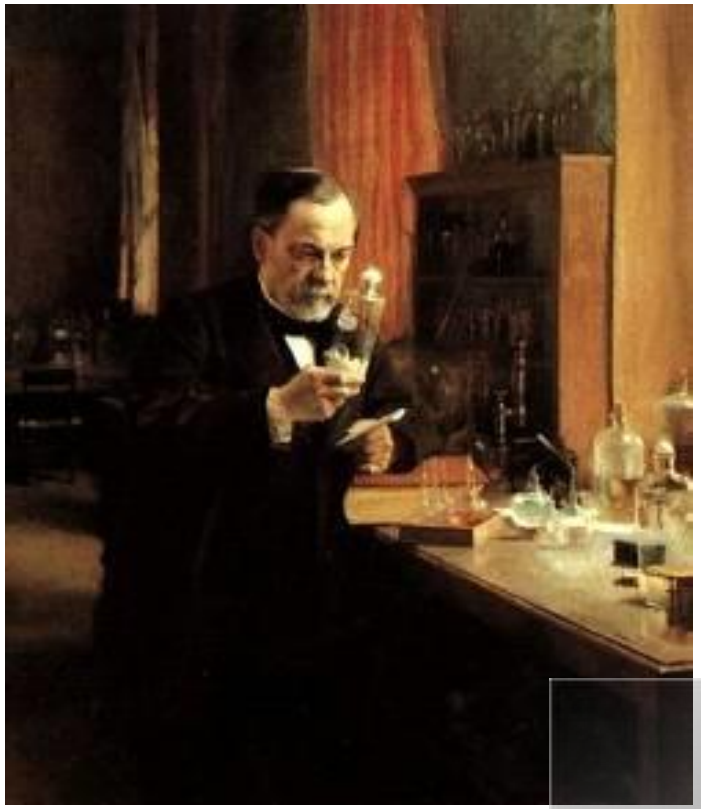
# The golden age of microbiology



Louis Pasteur

Robert Koch

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# How do we kill microorganisms ?

By eating them:

the phagocytosis

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# Ilya Metchnikov

1905 Nobel speech:

“eventually molecules will be identified on the surface of leukocytes which could identify microorganisms”

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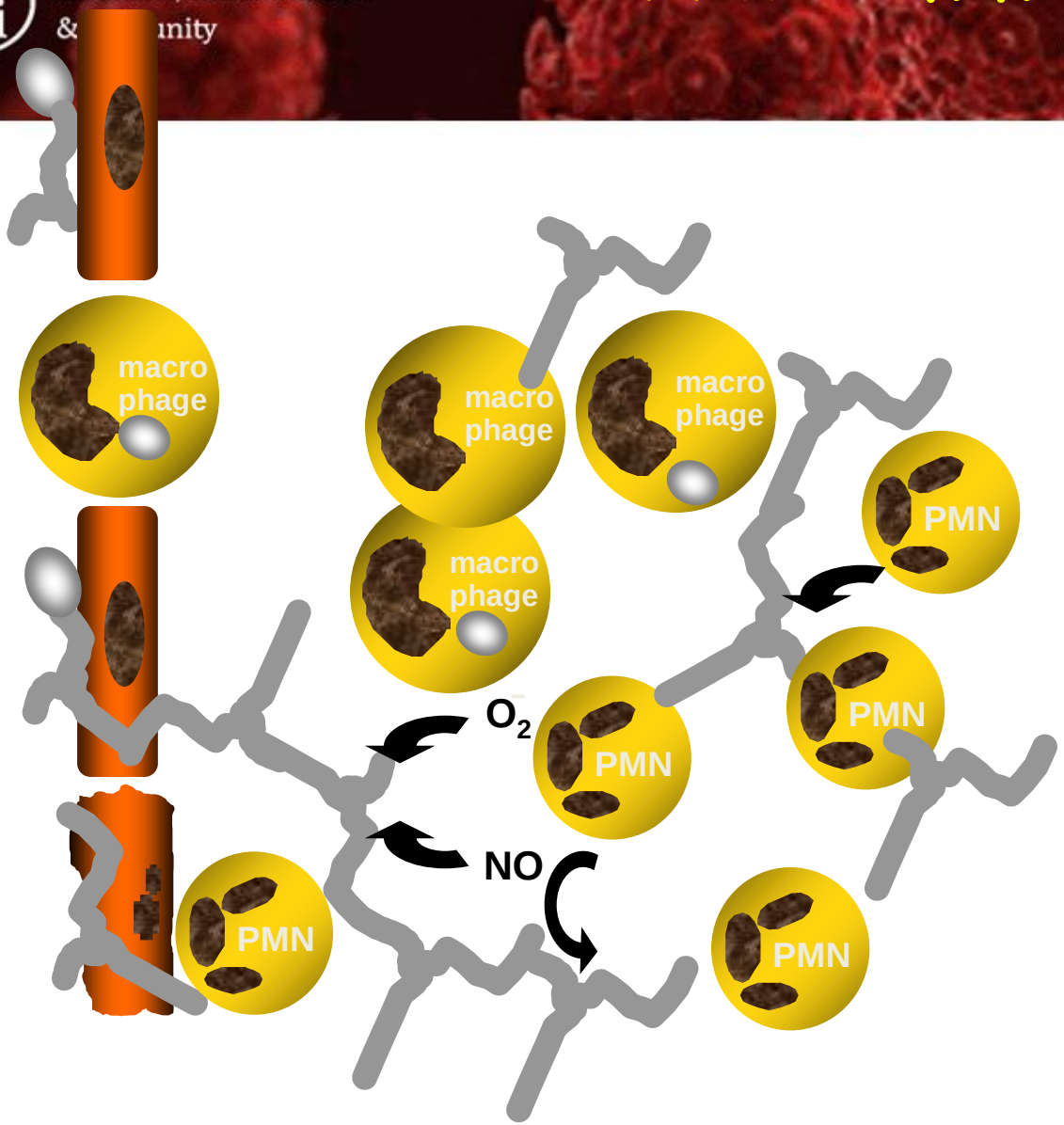
# Innate immunity

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# Humoral immunity

Emile von Behring

Paul Ehrlich

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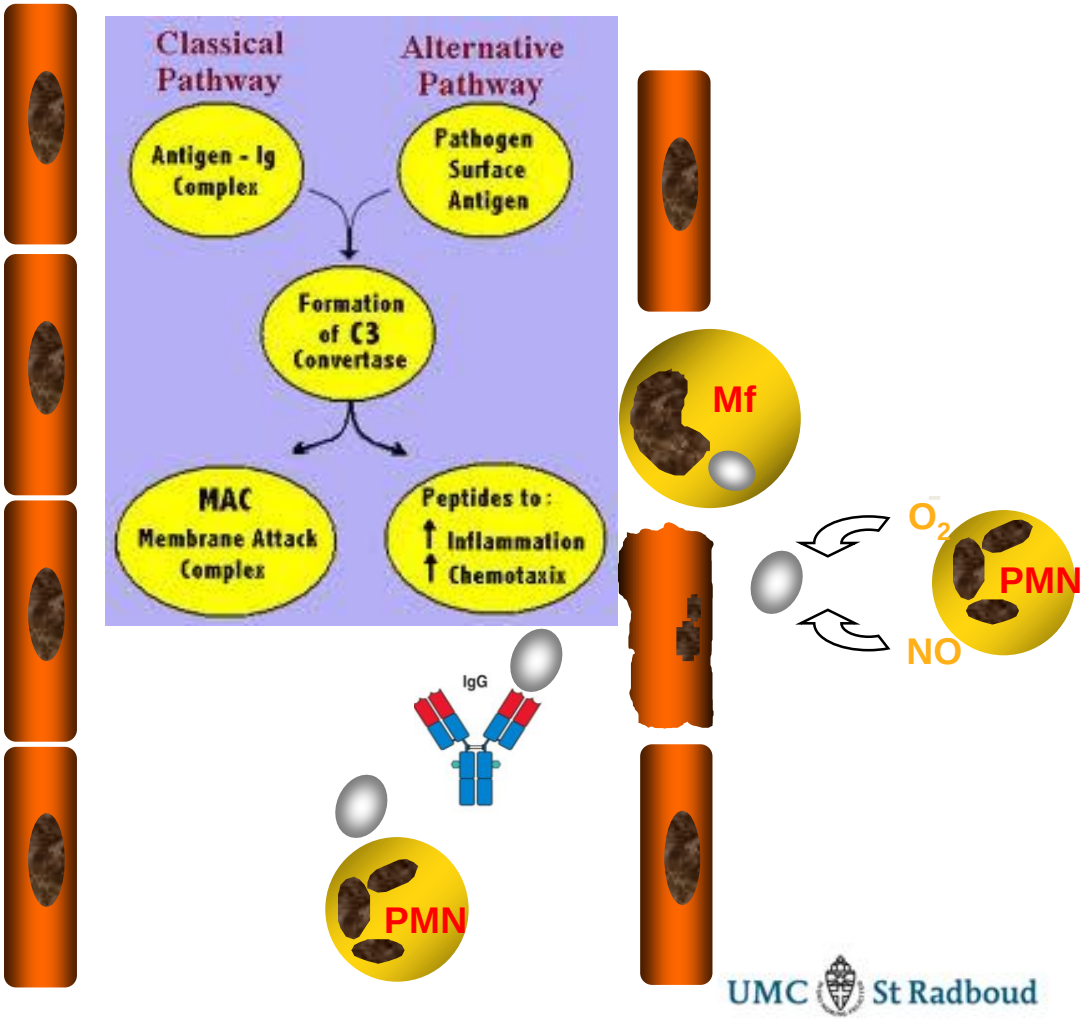
# Complement

Jules Bordet

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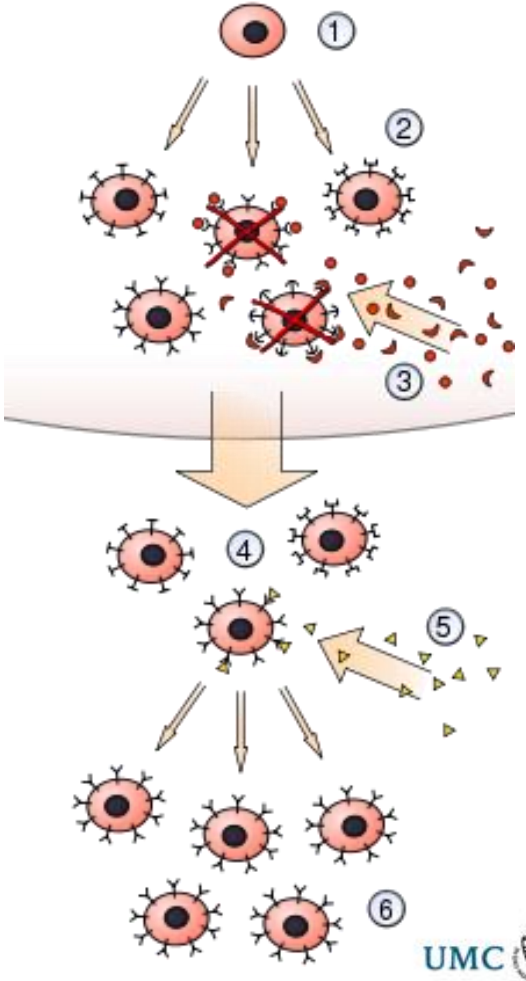
# Clonal selection

Frank Macfarlane Burnet

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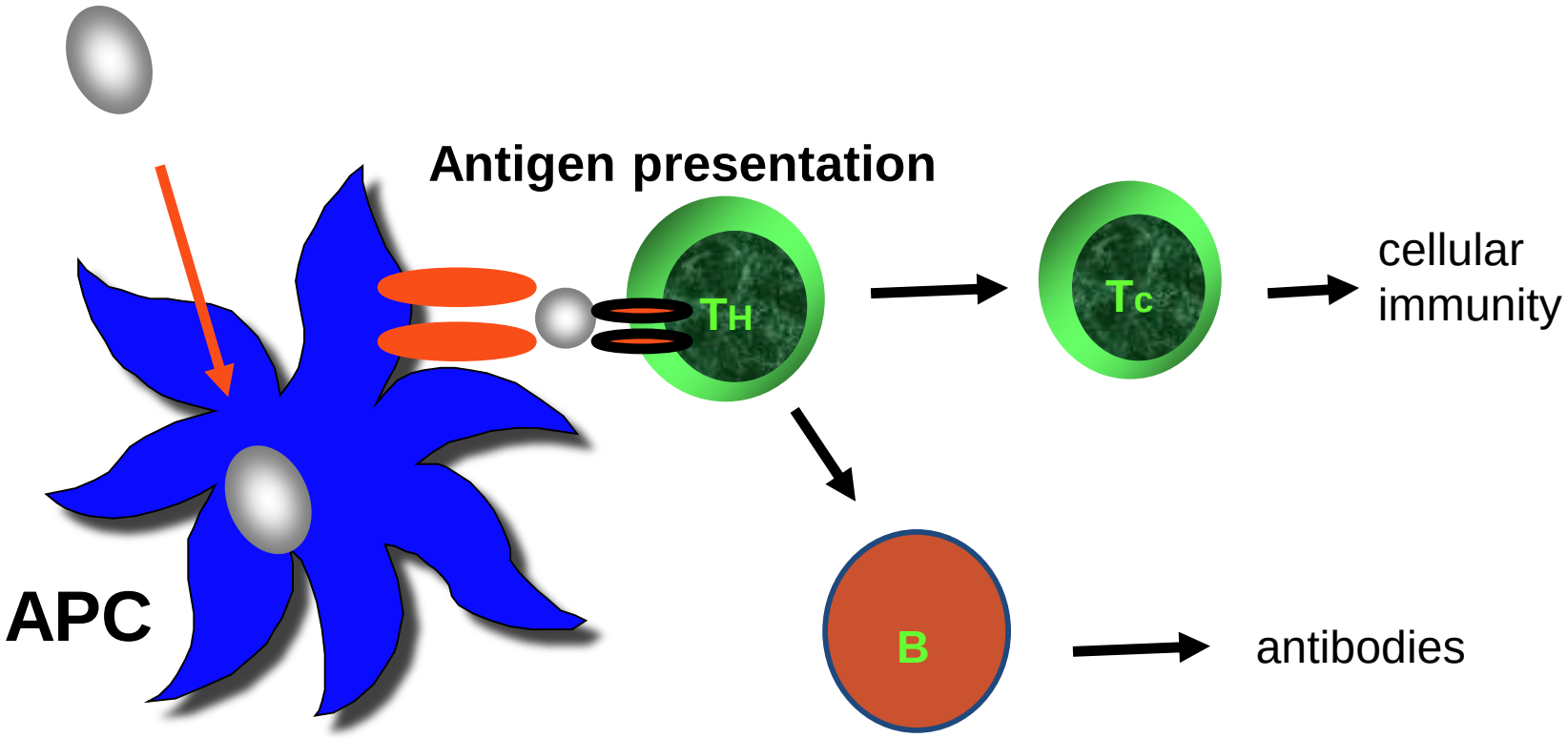
# Adaptive immunity

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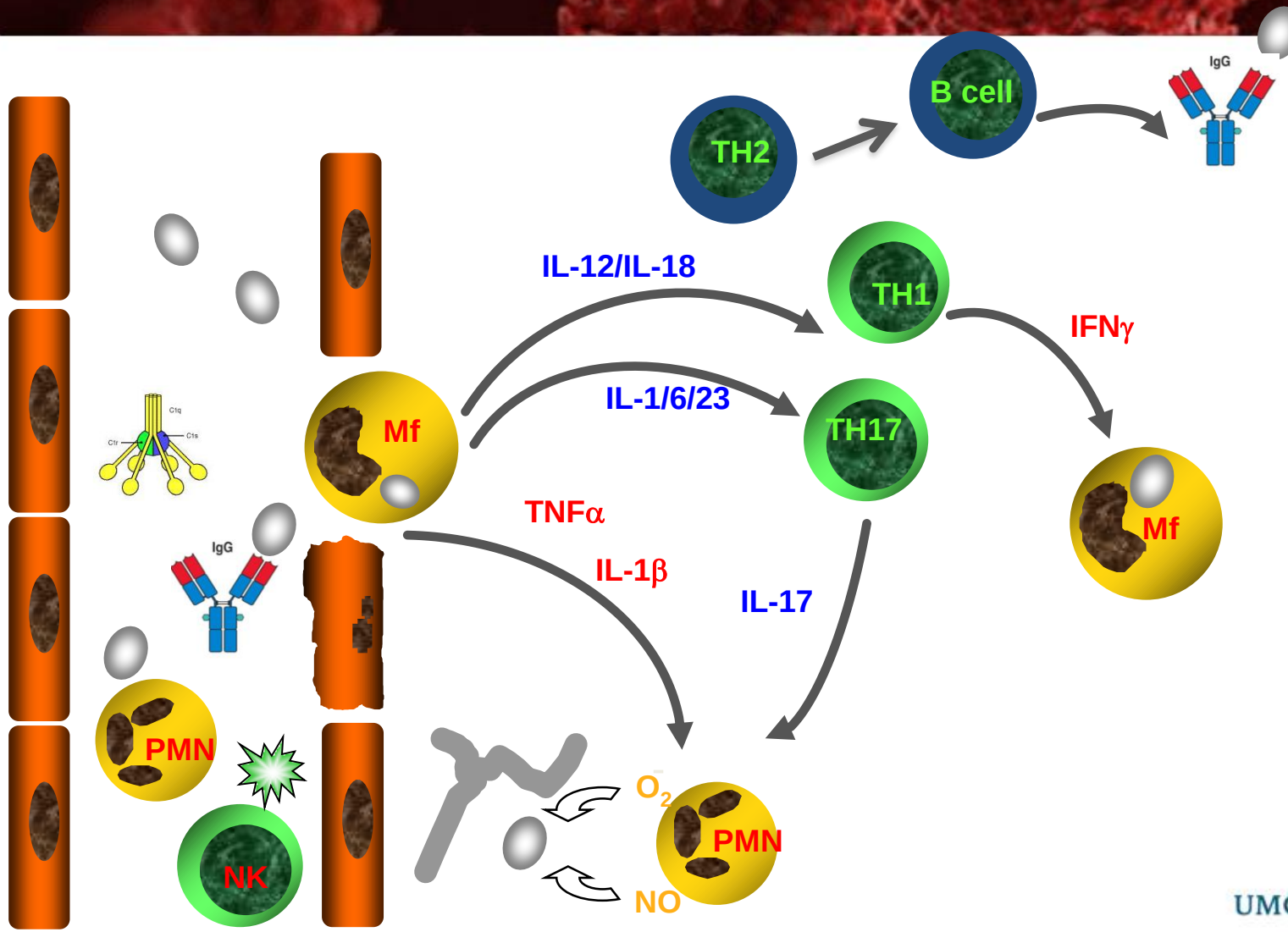
# The host defense

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# Innate versus adaptive immunity

- **Innate immunity:**
- STRONG but STUPID
- - rapid
- effective
- - not-specific, indiscriminate

- **Specific immunity:**
- LAZY but SMART
- - needs 10-14 days
- - a specific activation against a particular microorganism, enhancing the effectivity of the response

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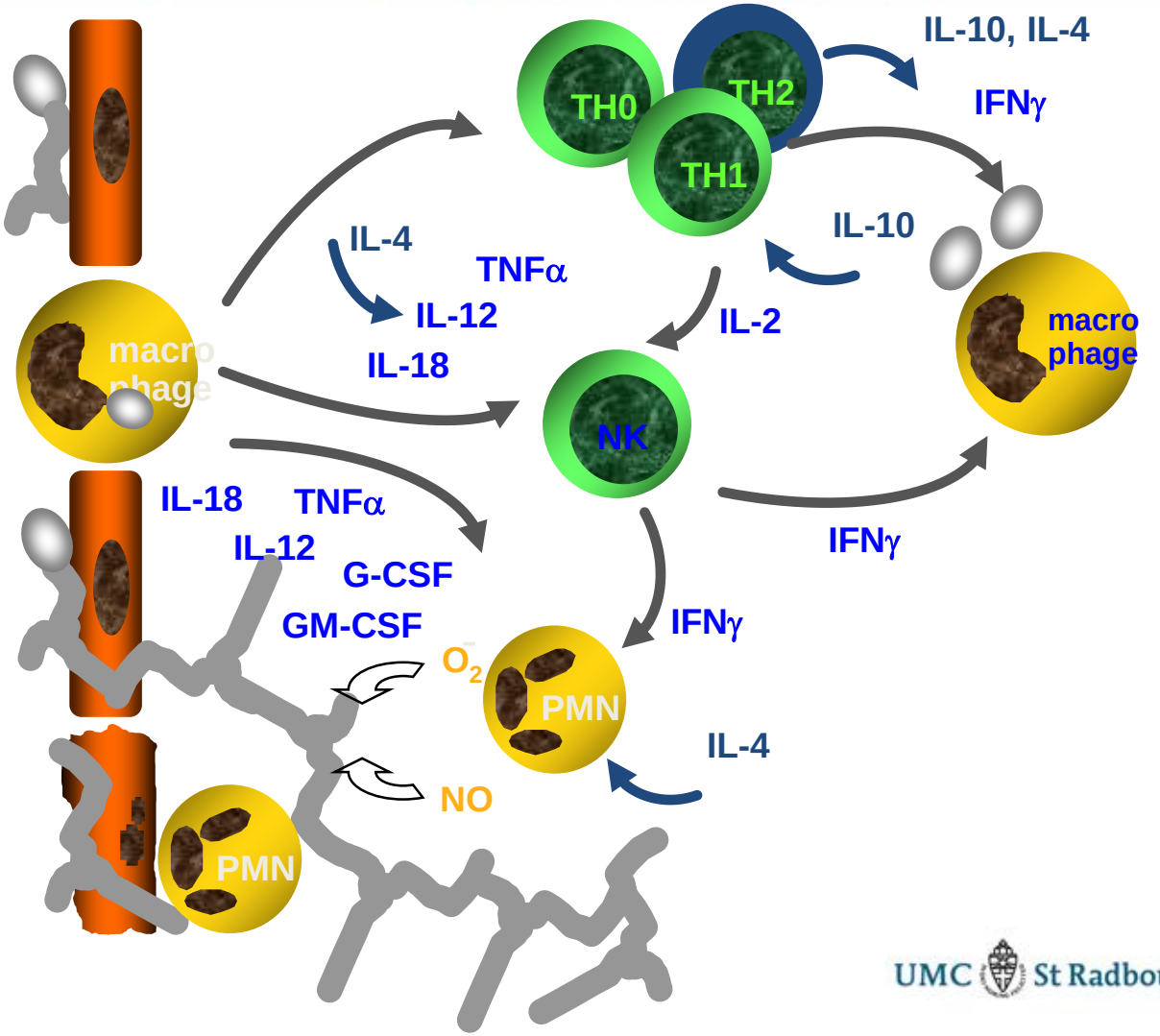
# Cytokines: the information network of innate immunity

Charles Dinarello

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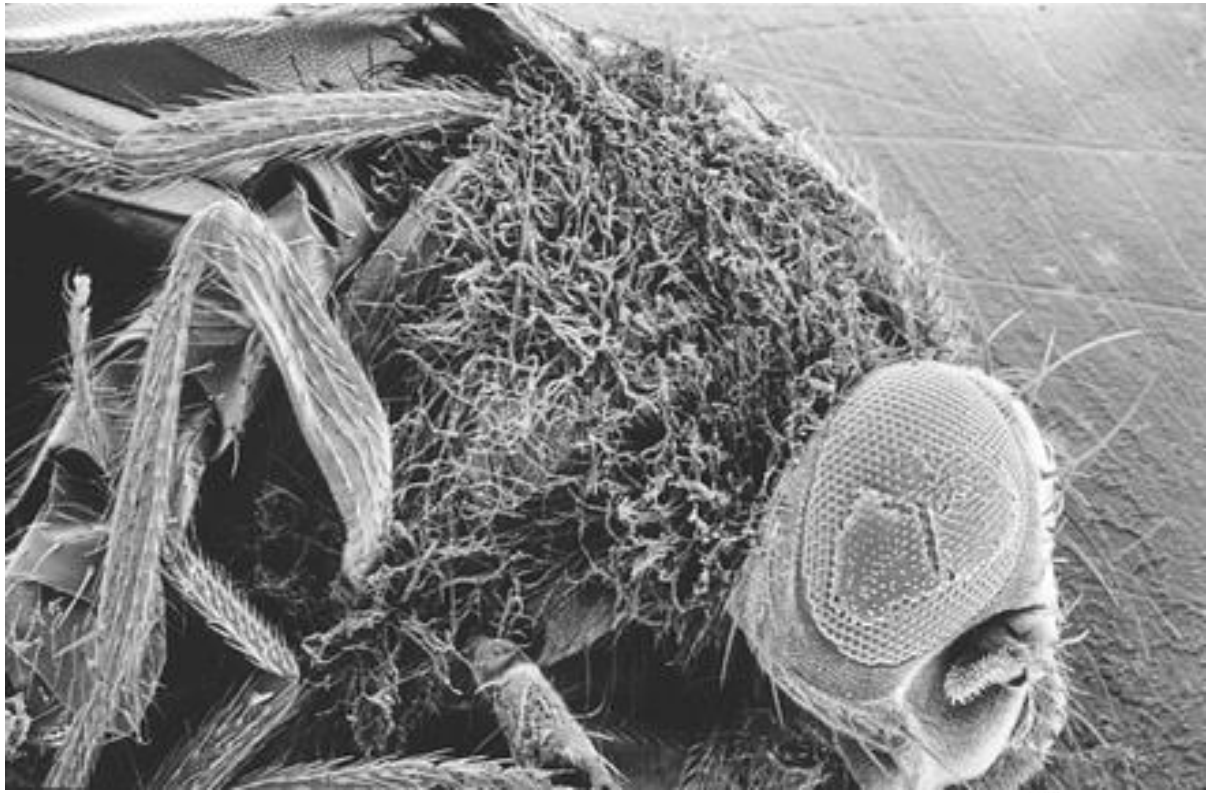
**Cell. 1996 Sep 20;86(6):973-83.**

**The dorsoventral regulatory gene cassette spatzle/Toll/cactus controls the potent antifungal response in Drosophila adults.**

**Lemaitre B, Nicolas E, Michaut L, Reichhart JM, Hoffmann JA.**



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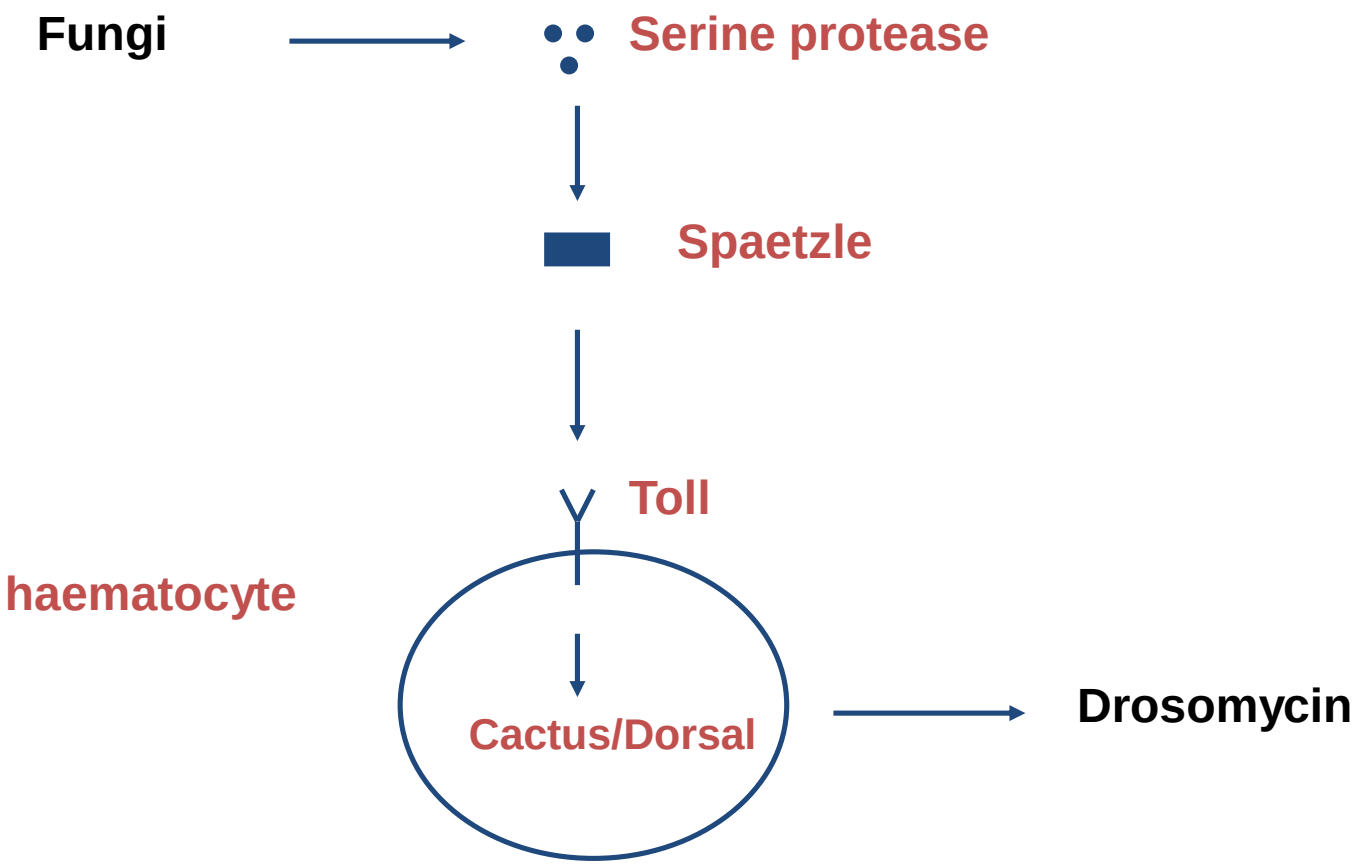
# Drosophila Toll receptor

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# The Toll-like receptor signal pathway

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| | Drosophila | Human |
|---|---|---|
| Ligands | Spaetzle | IL-1 |
| Receptors | Toll | IL-1R |
| Signaling proteins | Tube | MyD88 |
| | Pelle | IRAK, Traf6 |
| | Cactus | ikB |
| | Dorsal, Dif | NFkB |
| Target proteins | Drosomycin | IL-6, TNF |

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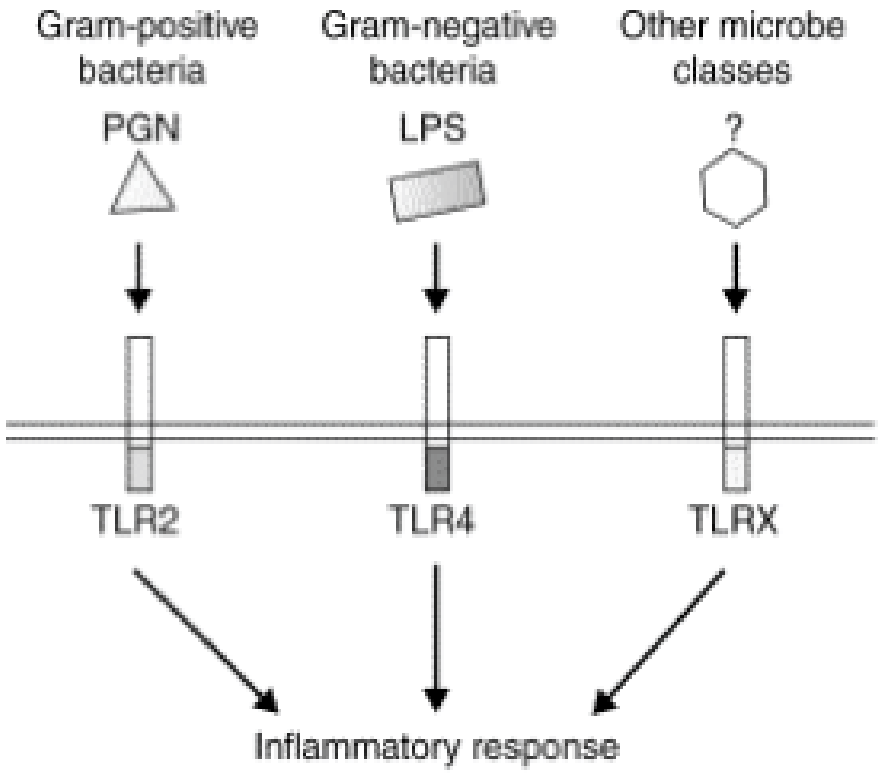
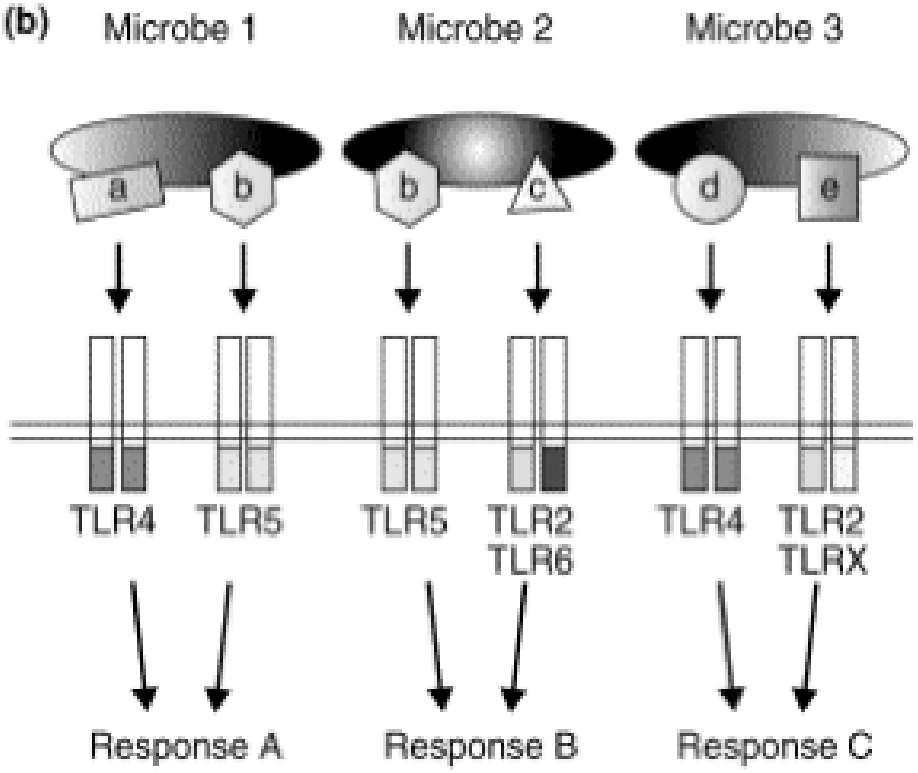
# Pattern recognition receptors

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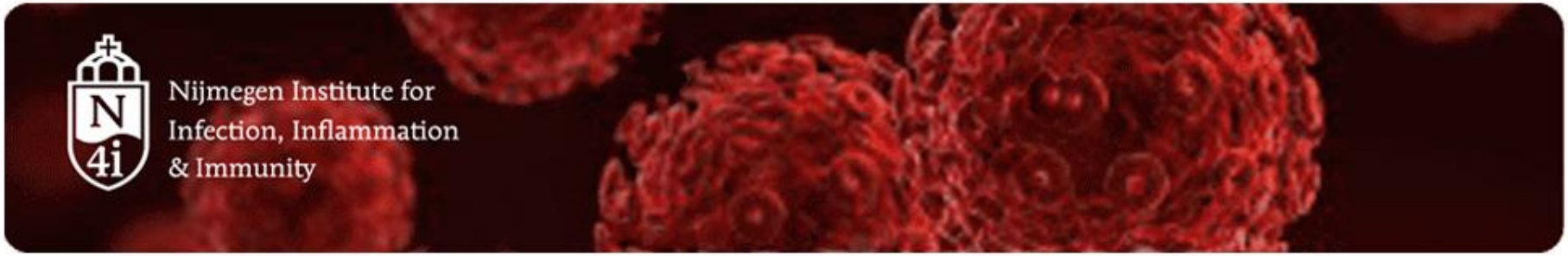


# Trained Immunity:
# a memory for innate host defense

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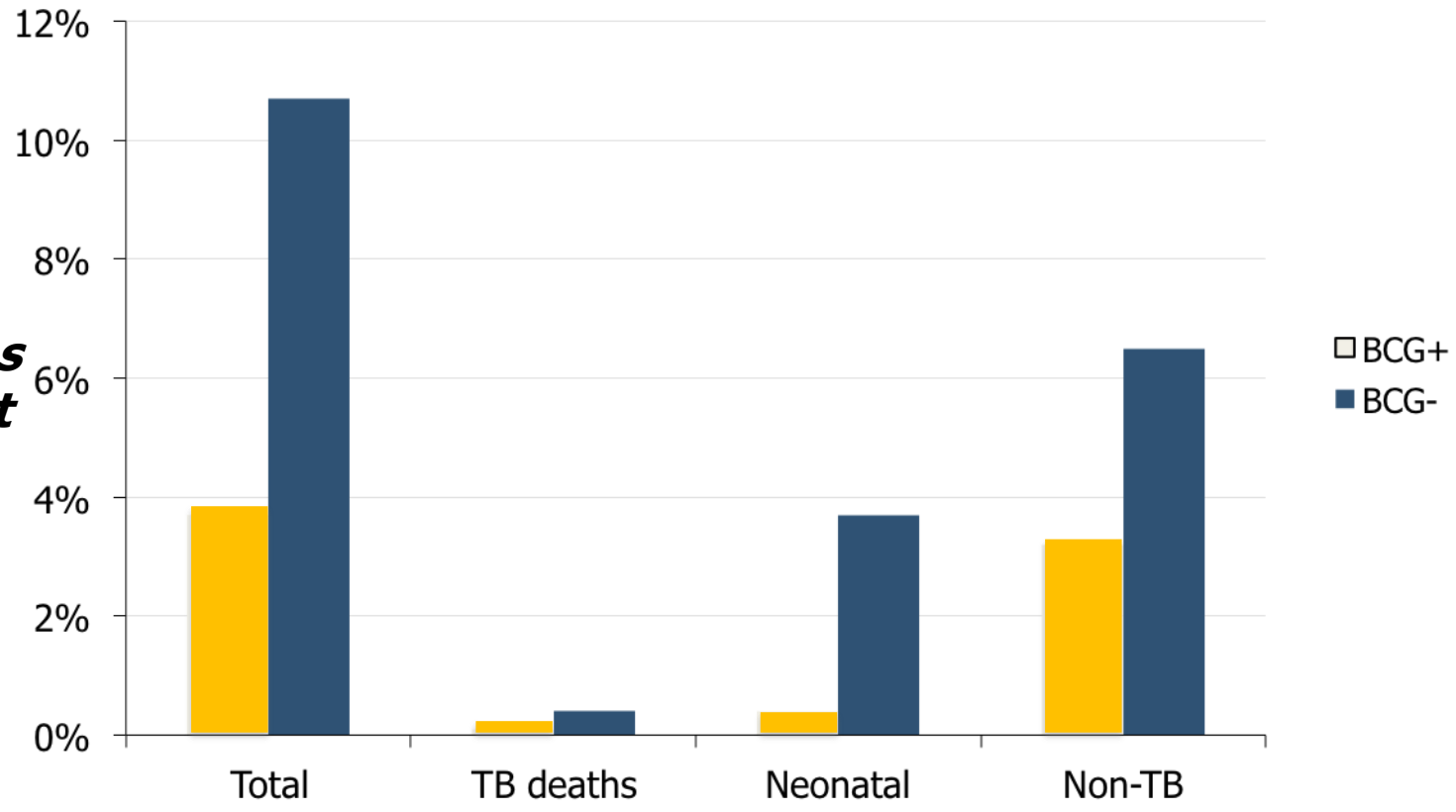
# Introducing BCG in Norrbotten, Sweden, 1927-31: Mortality at 0-4 years - 20,000 children

Coverage highest in families with TB

***Reduction was in infancy, but TB deaths occur later***

***This made little sense***




"One could evidently be tempted to find an explanation for this much lower mortality among vaccinated children in the idea that BCG provokes *a* ***non-specific immunity...***"

Carl Naeslund 1932

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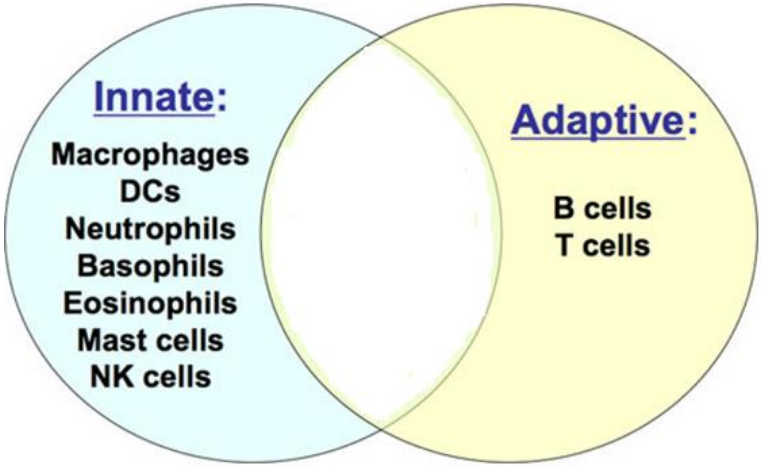
# Innate versus specific immunity




**Innate immunity:**

- rapid
- effective
- not-specific, indiscriminate
- lacks immunological memory

**Adaptive immunity:**

- needs 10-14 days
- a specific activation against a particular microorganism, enhancing the effectivity of the response
- builds immunological memory

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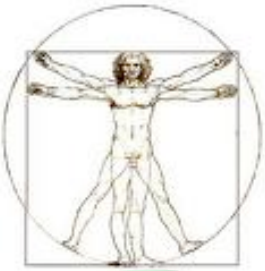
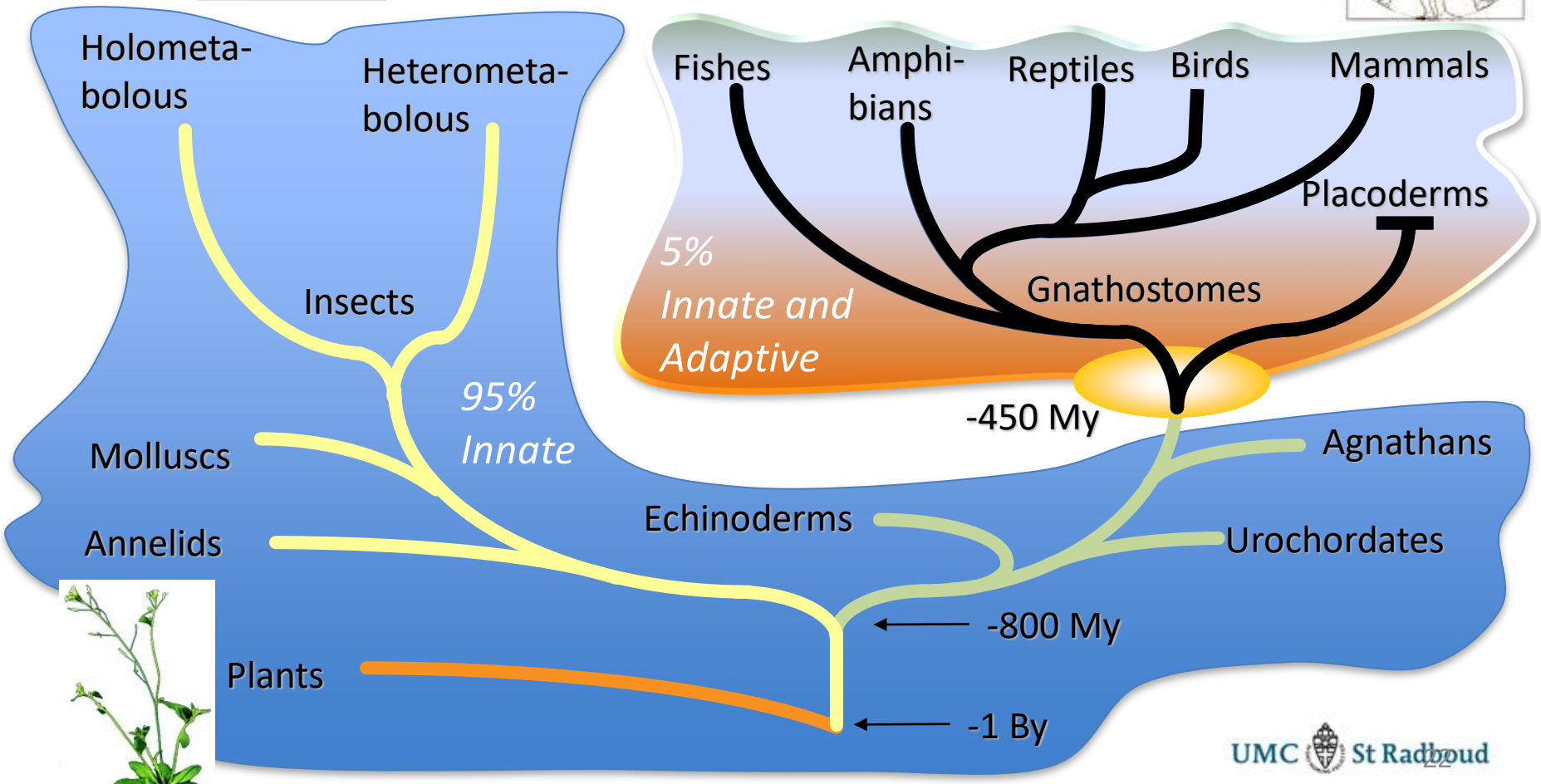
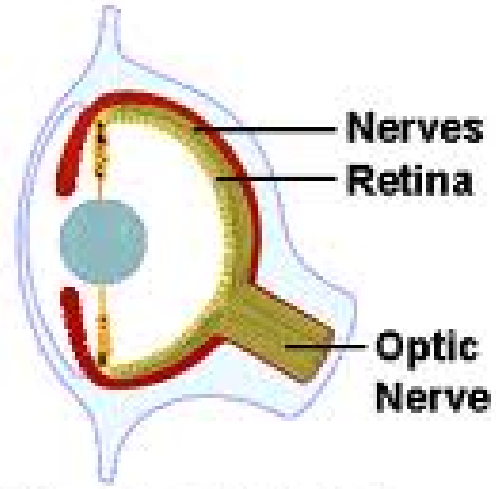
"Memory: the ability of a system to store and recall information on previously encountered characteristics"

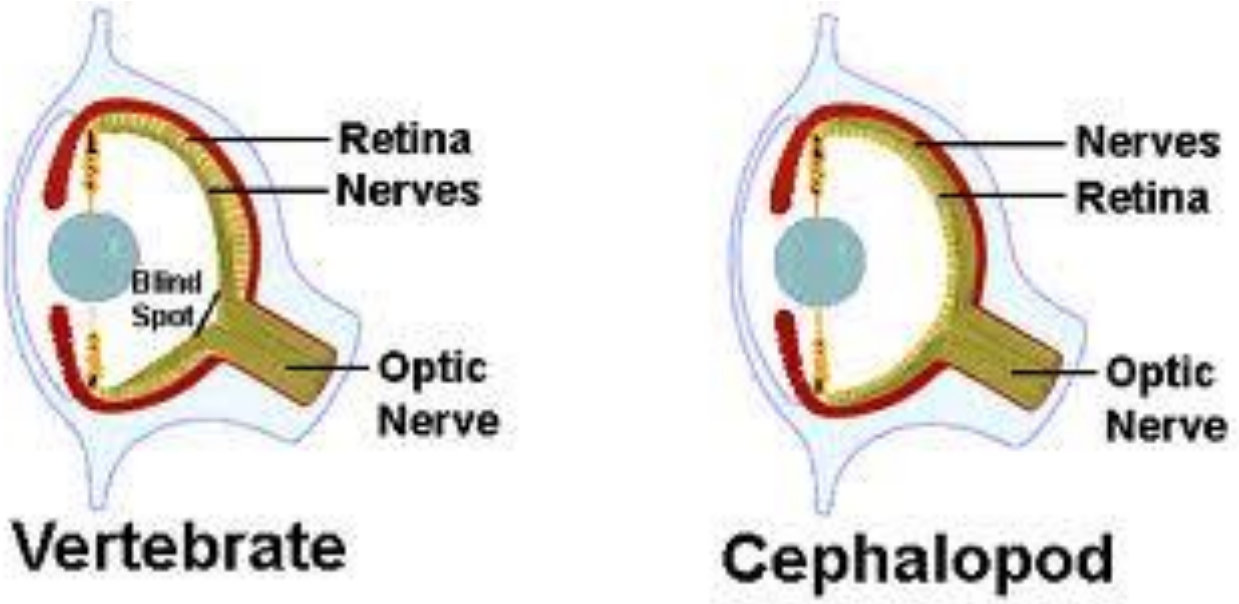


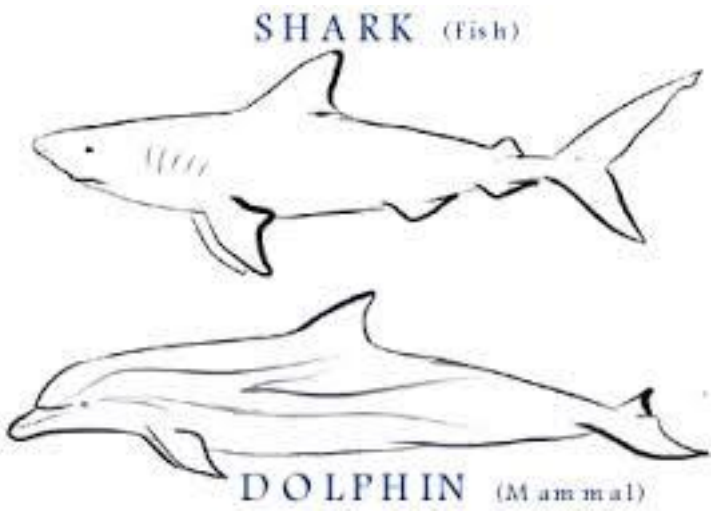


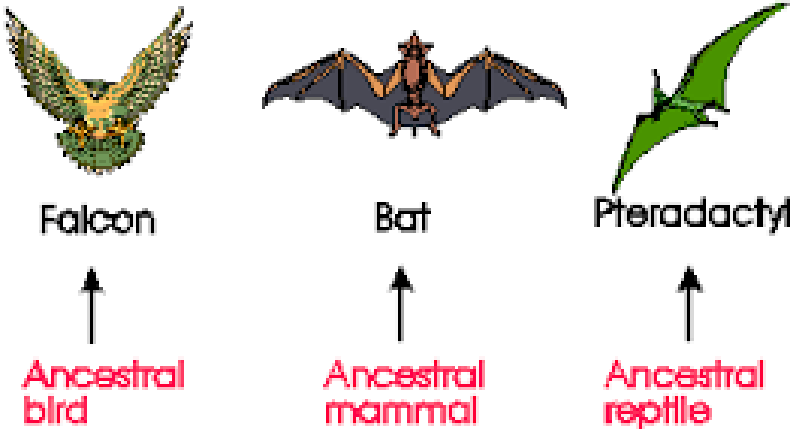
- **Convergent evolution**: development of the same property in independent group of organisms



- **Convergent evolution**: development of the same property in independent group of organisms




SHARK (Fish)
DOLPHIN (Mammal)


Falcon
Bat
Pteradactyl
Ancestral bird
Ancestral mammal
Ancestral reptile


Giant Armadillo
North America
Giant Pangolin
Africa
Giant Anteater
South America
Spiny Anteater
Oceania


OMG we have such similar beaks!!!! Are we related? :o
No...I think it's just because we live in the same area...:D

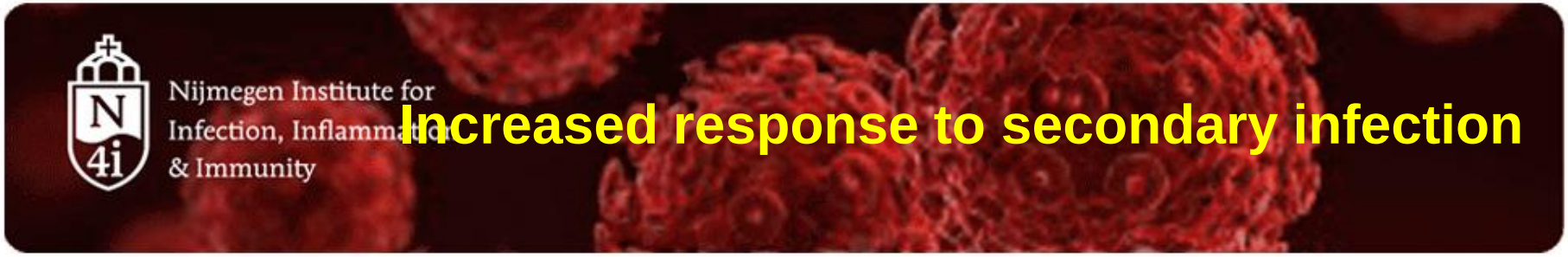



**Table 1. Selected Experimental Models in which Biological Activity Compatible with the Concept of Trained Innate Immunity Has Been Reported**

| Organism | Experimental Model | Biological Effect | Specificity | References |
|---|---|---|---|---|
| Plants—Systemic Acquired Resistance | | | | |
| Large variety of plants | Viruses, bacteria, fungi | Protection against reinfection | Variable | Durrant and Dong, 2004; Sticher et al., 1997 |
| Nonvertebrates | | | | |
| Mealworm beetle | LPS, or bacterial prechallenge | Protection against secondary infection | No | Moret and Siva-Jothy, 2003 |
| *Drosophila* | *S. pneumoniae* prechallenge | Protection against *S. pneumoniae* | Uncertain | Pham et al., 2007 |
| *Anopheles gambiae* | *Plasmodium* prechallenge | Protection against *Plasmodium* | No | Rodrigues et al., 2010 |
| Sponges | Transplantation | Rejection | Yes | Hildemann et al., 1979 |
| Corals | Transplantation | Rejection | Yes | Hildemann et al., 1977 |
| Vertebrates | | | | |
| Mice | BCG | Protection against candidiasis | No | Van 't Wout et al., 1992 |
| Mice | *Candida* vaccination | T/B cell-independent protection | No | Bistoni et al., 1986, 1988 |
| Mice | Murine CMV infection | NK-dependent protection | No | Sun et al., 2009 |
| Humans | BCG | Nonspecific protection to secondary infections | No | Garly et al., 2003 |

Netea et al: Cell Host and Microbe 2011

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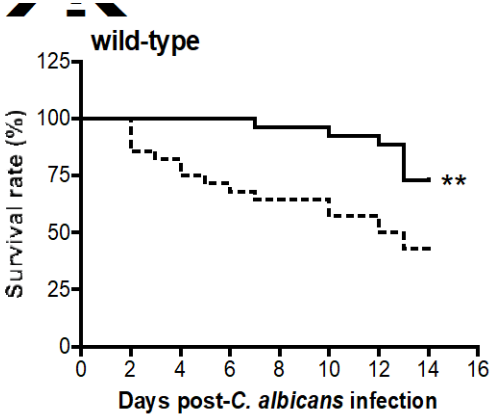
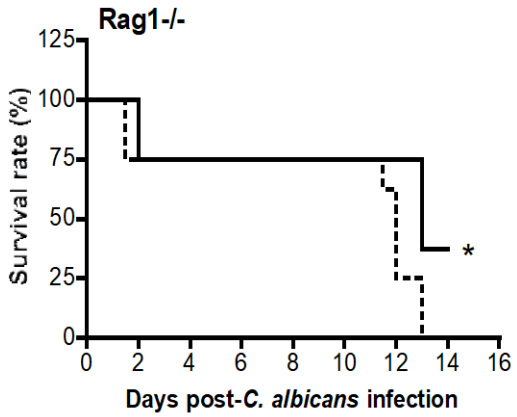
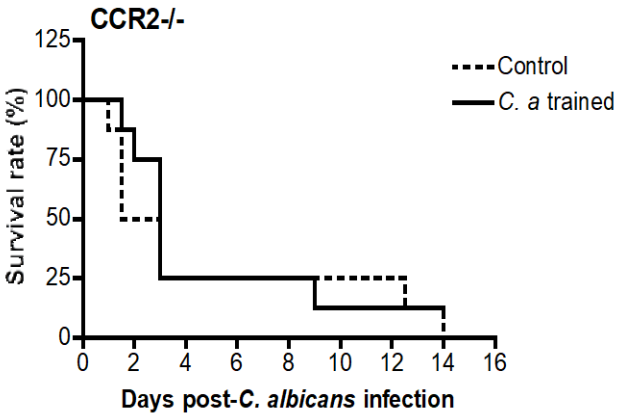
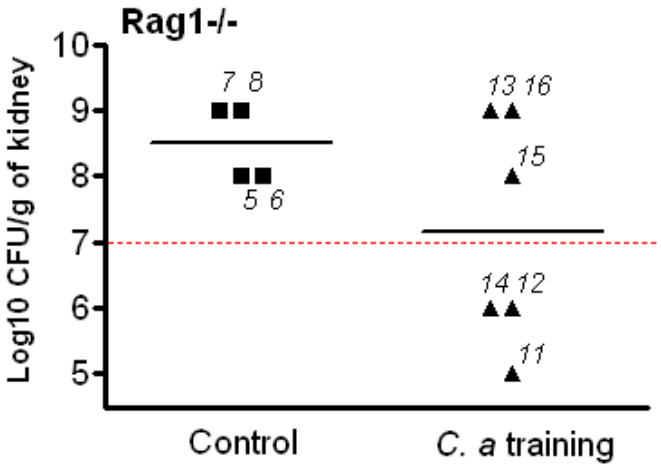
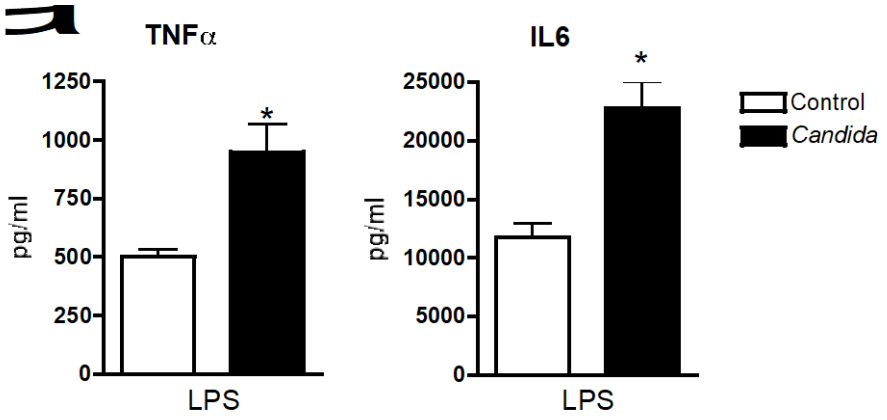
# Innate immunity-dependent protection in mice




Quintin et al, Cell H&M, 2012

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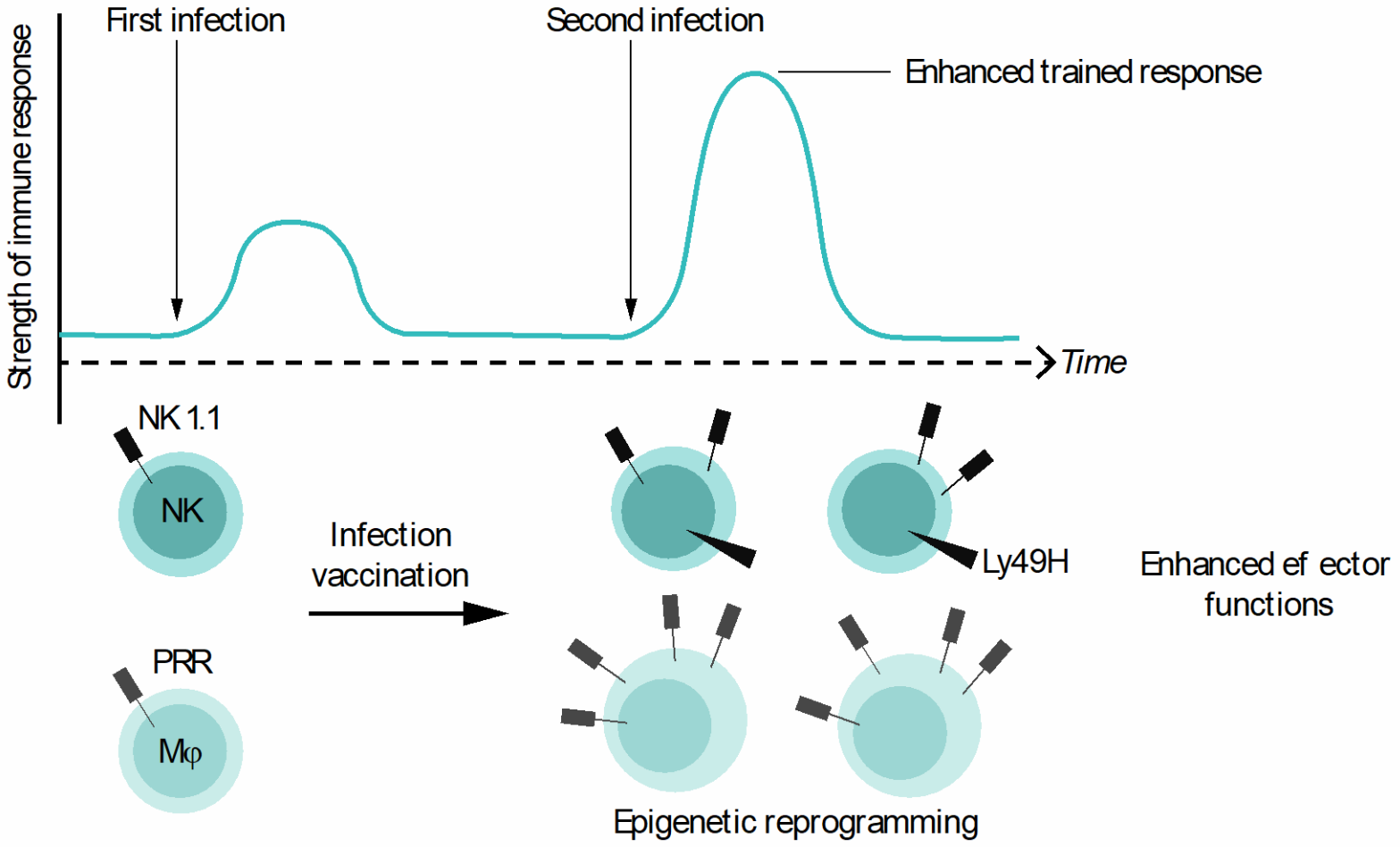
# Trained immunity




Netea et al. Science 2016

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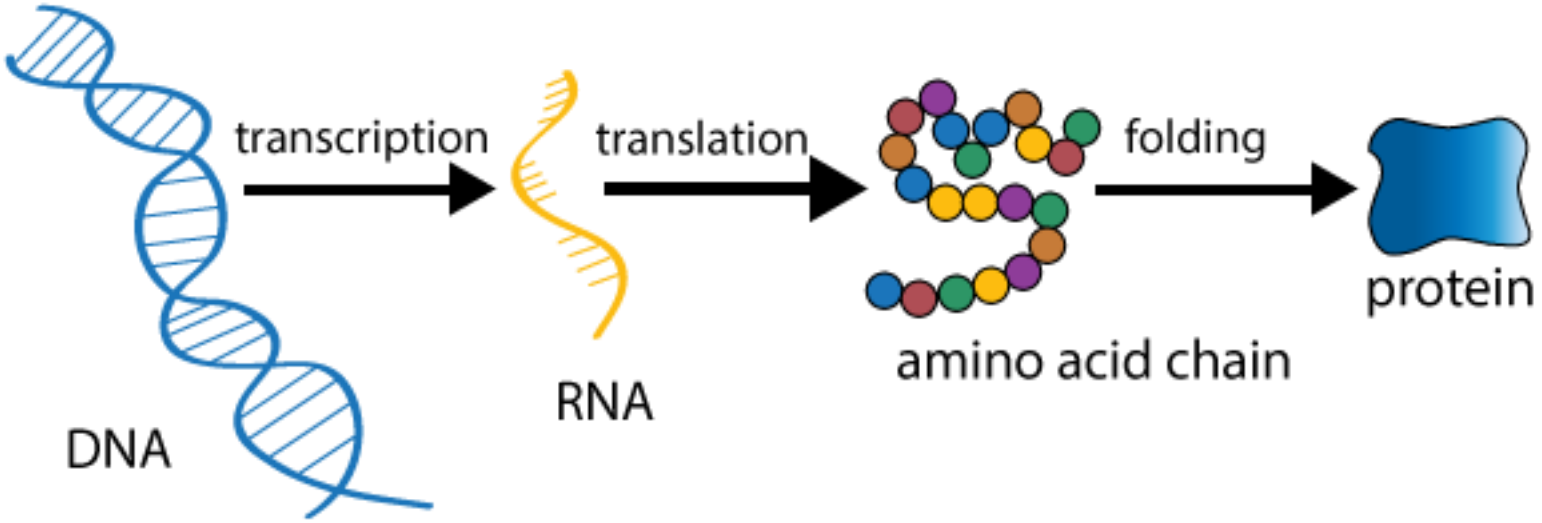
# From DNA to protein




Netea et al. Science 2016

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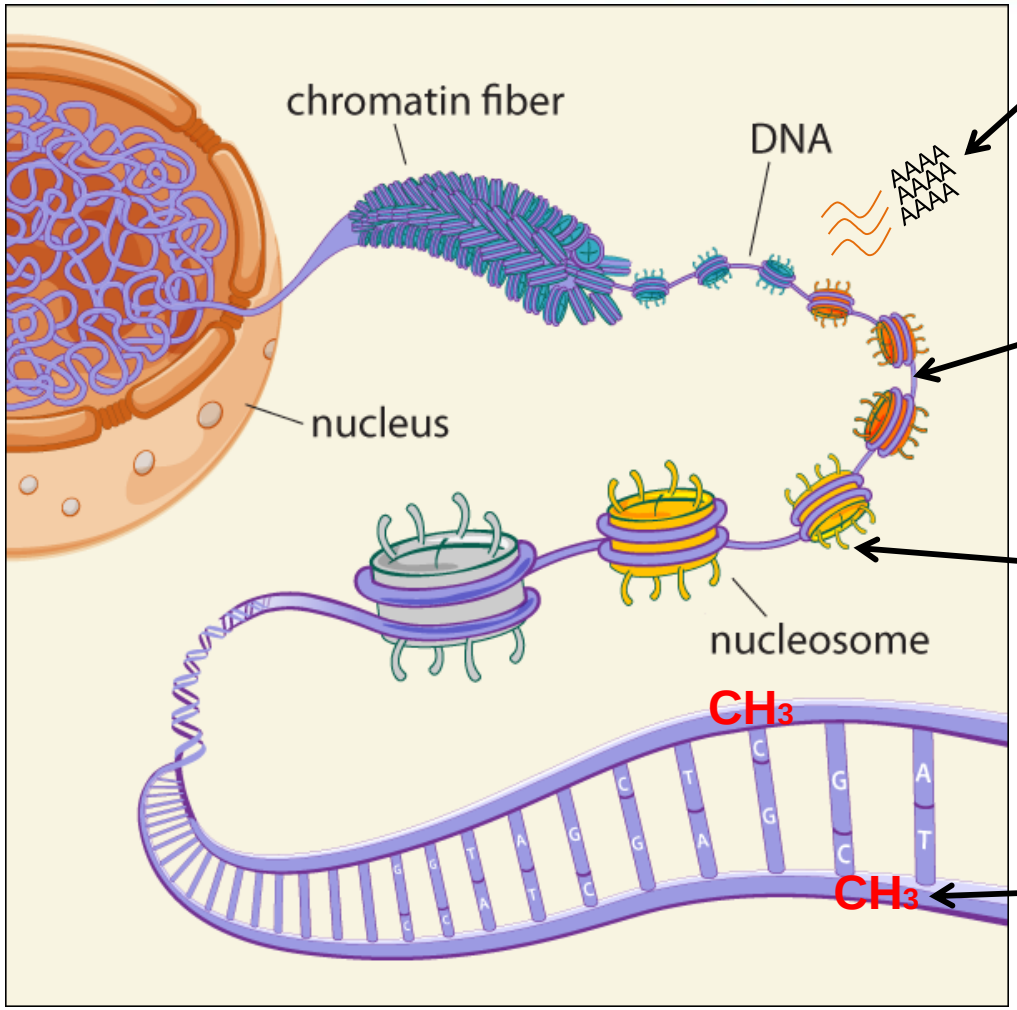
# Long-term epigenetic reprogramming in myeloid cells




**RNA-seq**
Gene expression
Non-coding regulatory RNAs

**ATAC-seq**
Open chromatin (i.e. nucleosome-free regions) can be bound by TFs, which can be identified by motif sequence

**ChIP-seq**
Histone tail modifications determine 'activity' by attracting TFs
(we use 5 histone modifications)

**WGBS – whole genome bisulfite sequencing**
DNA methylation maintains DNA in a closed state

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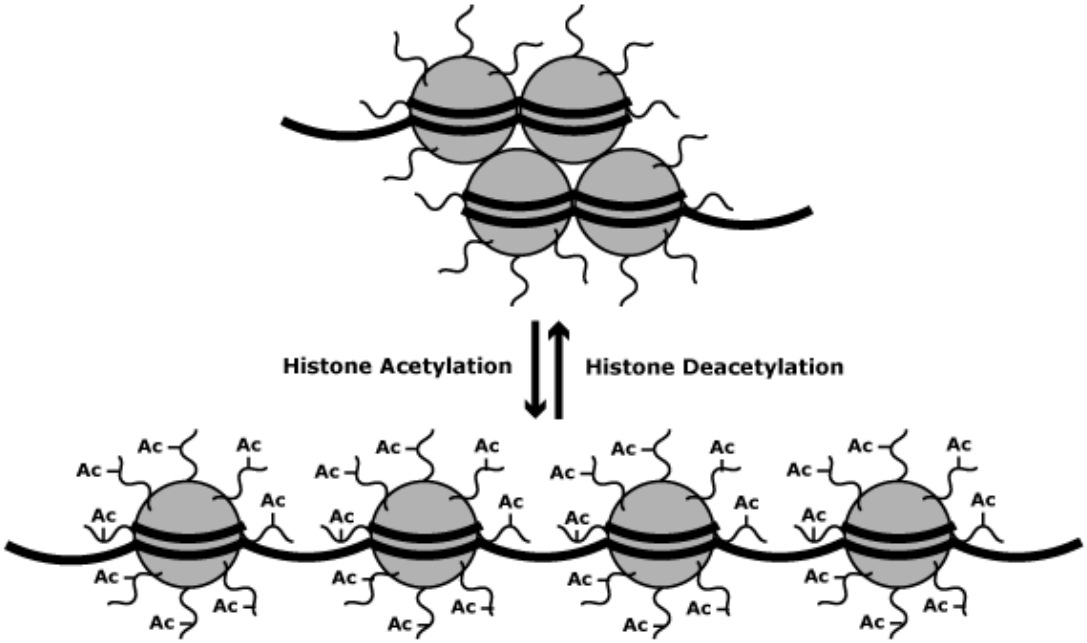
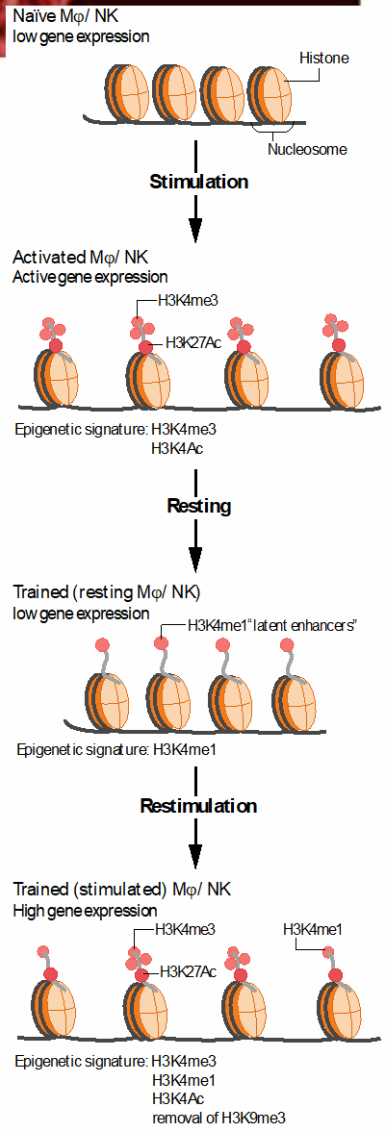
# Chromatin architecture




**DNA in the nucleus**



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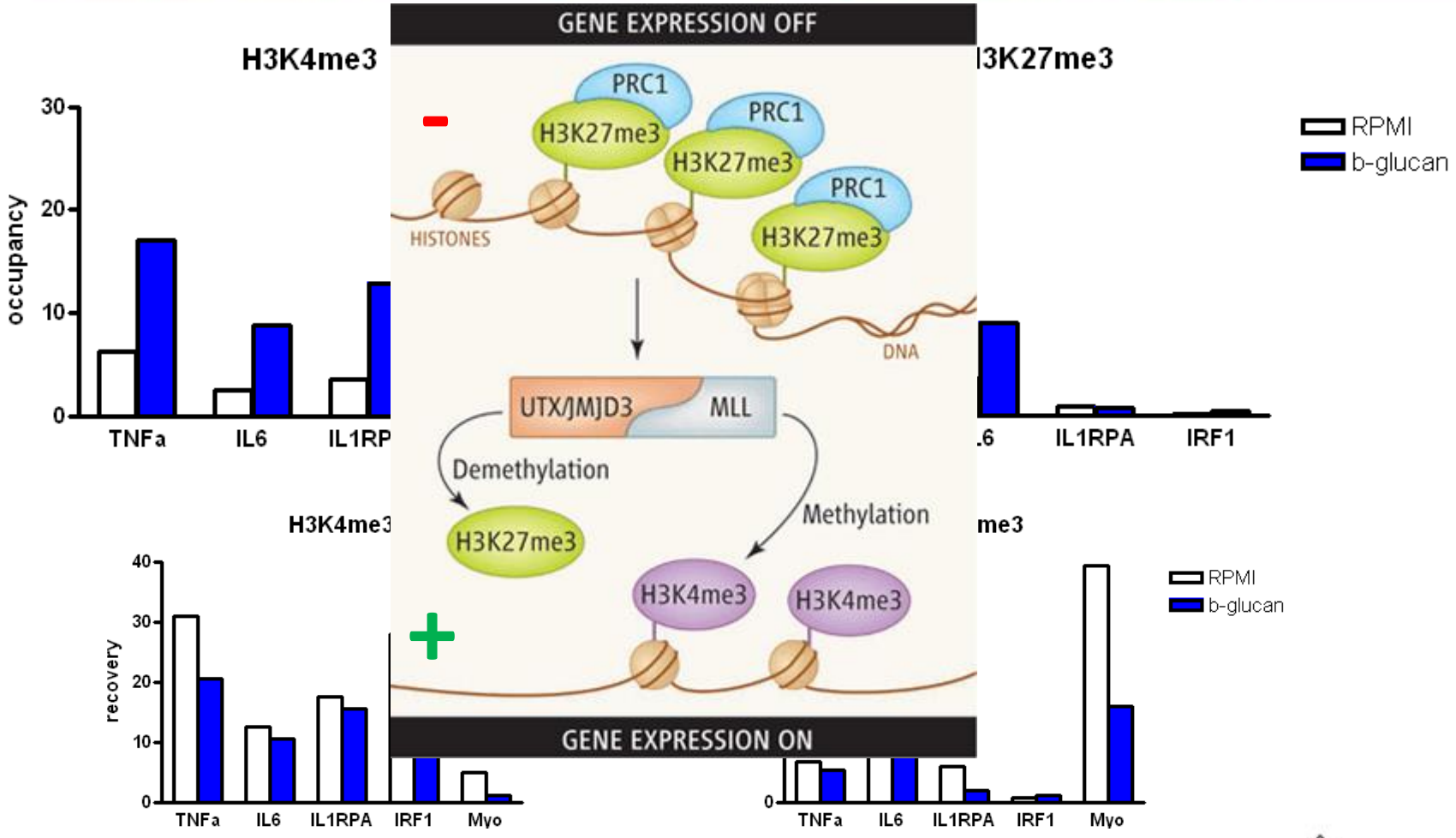
# Methylation status of H3




Quintin et al, Cell H&M, 2012

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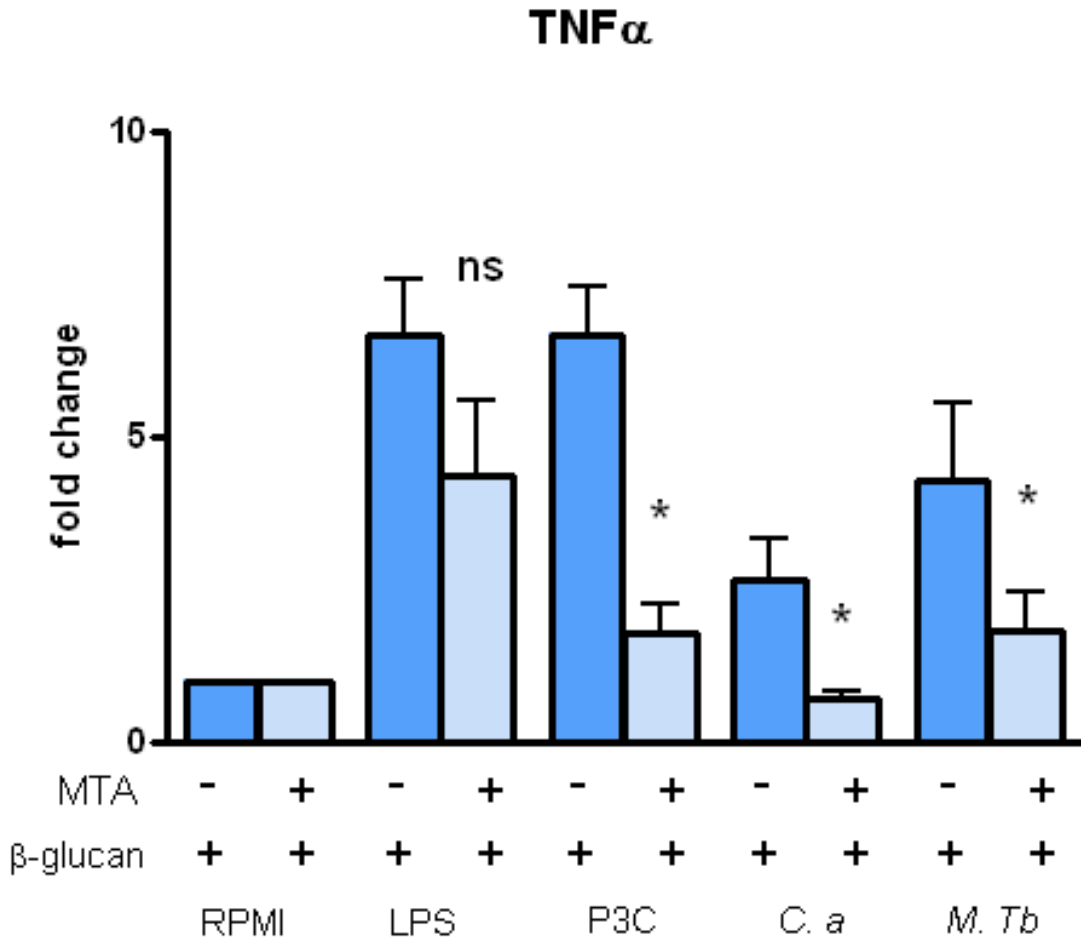
# MTA: Histone methyltransferase inhibitor

## Less Methylation

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Quintin et al, Cell H&M, 2012

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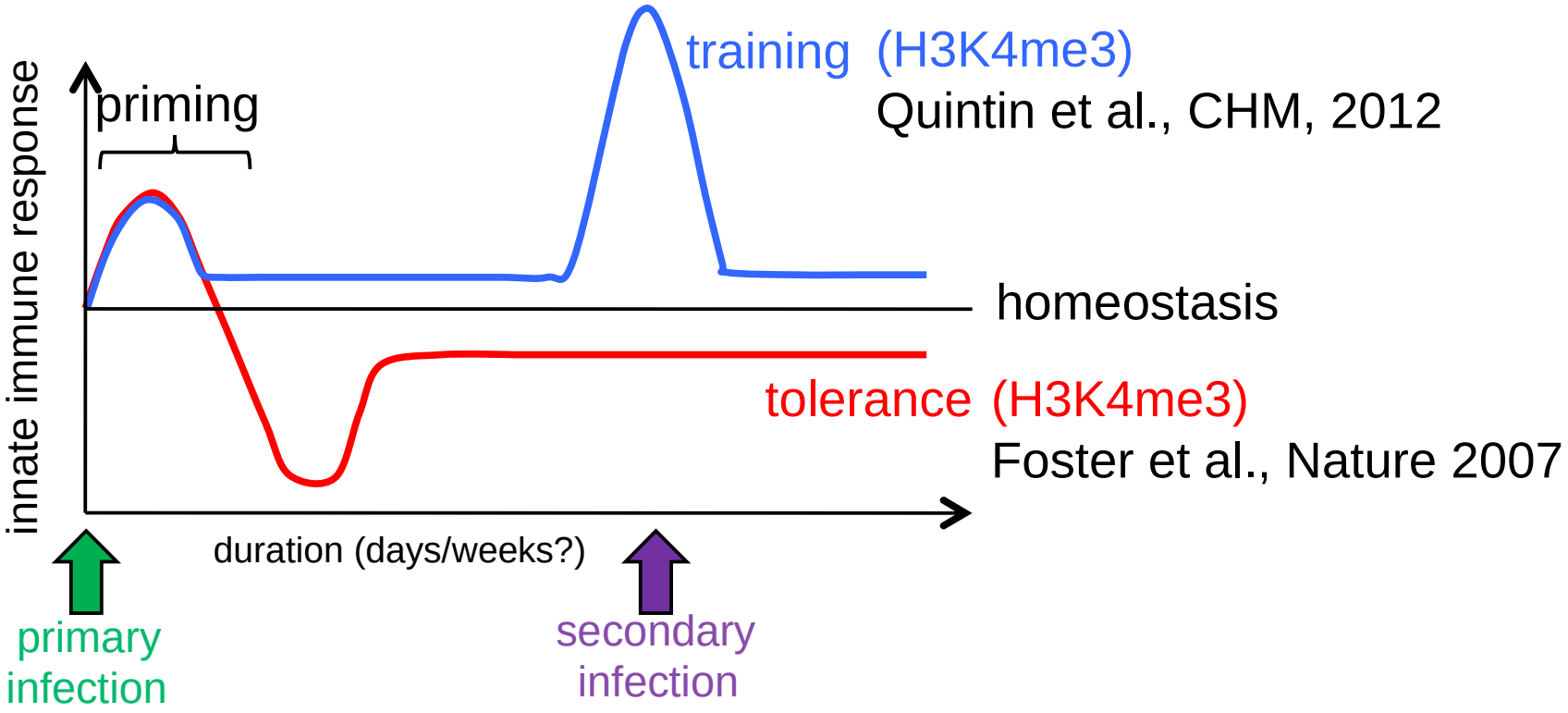
# Trained immunity versus tolerance

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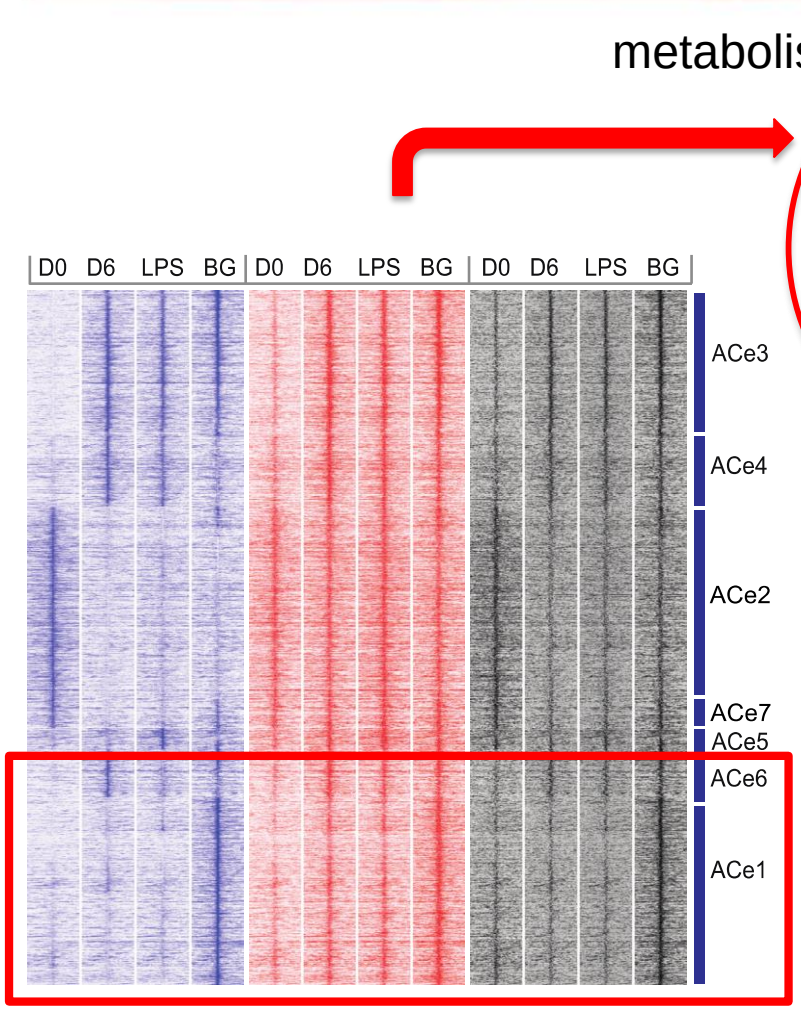
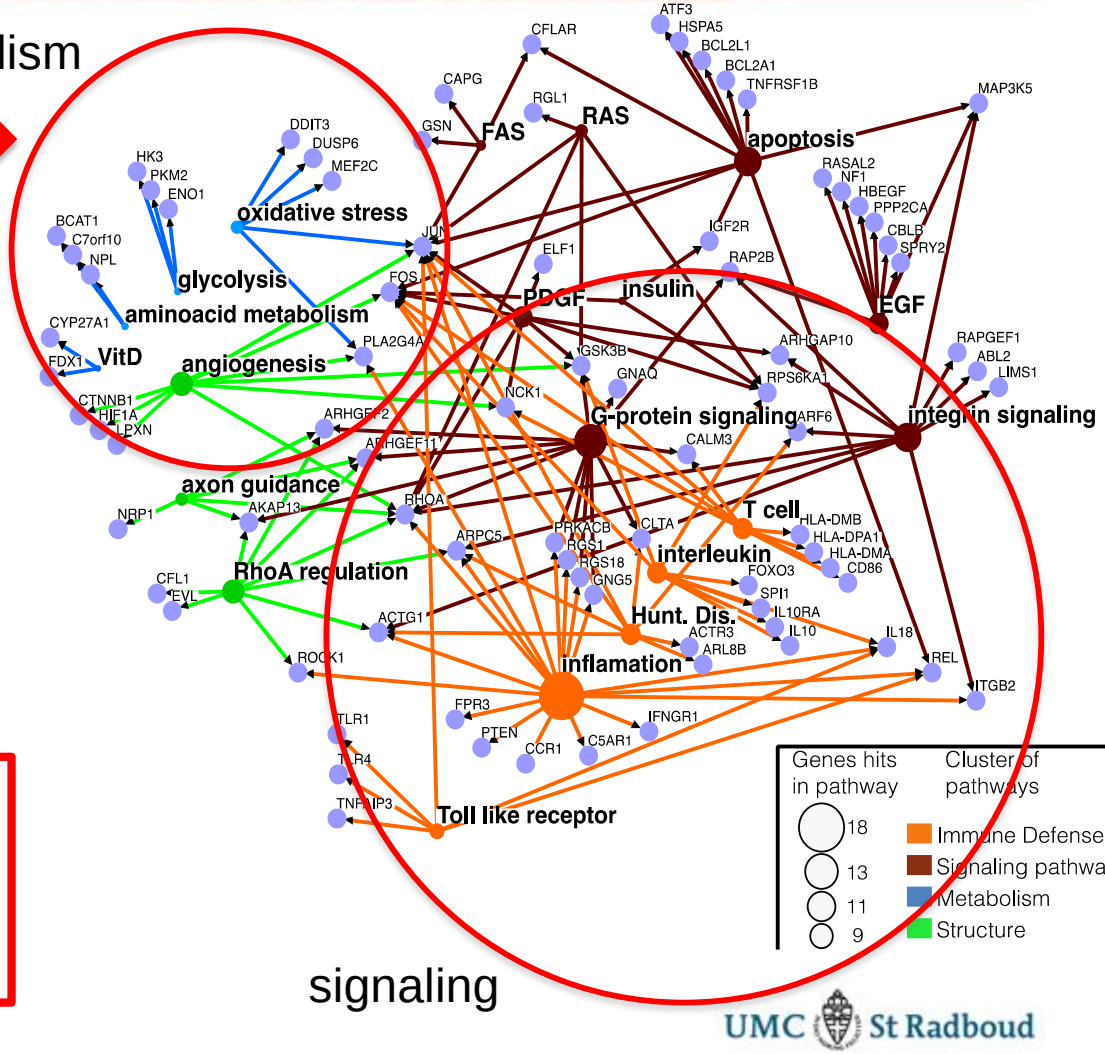
# What are the pathways distinguishing Training vs Tolerance ?

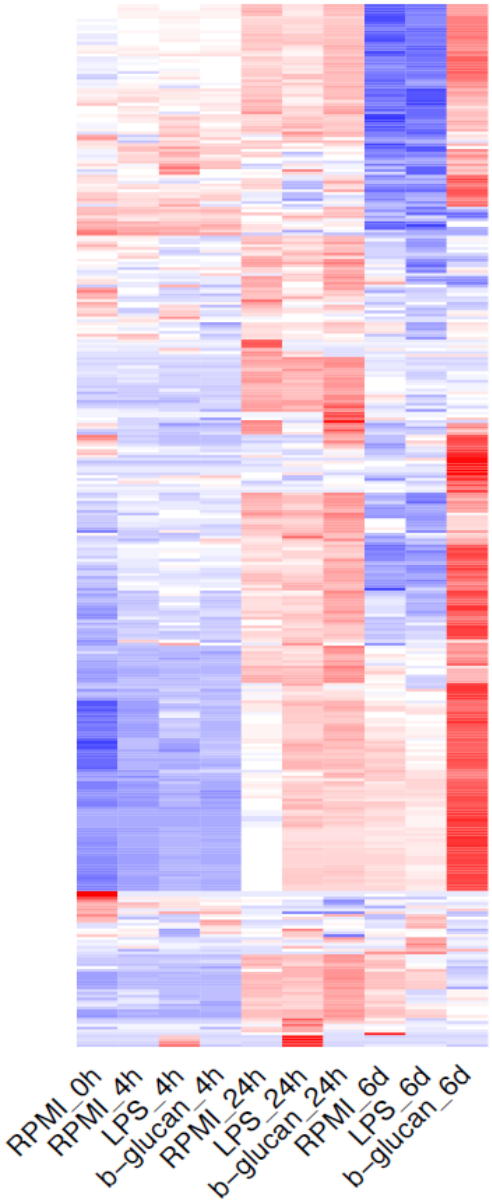


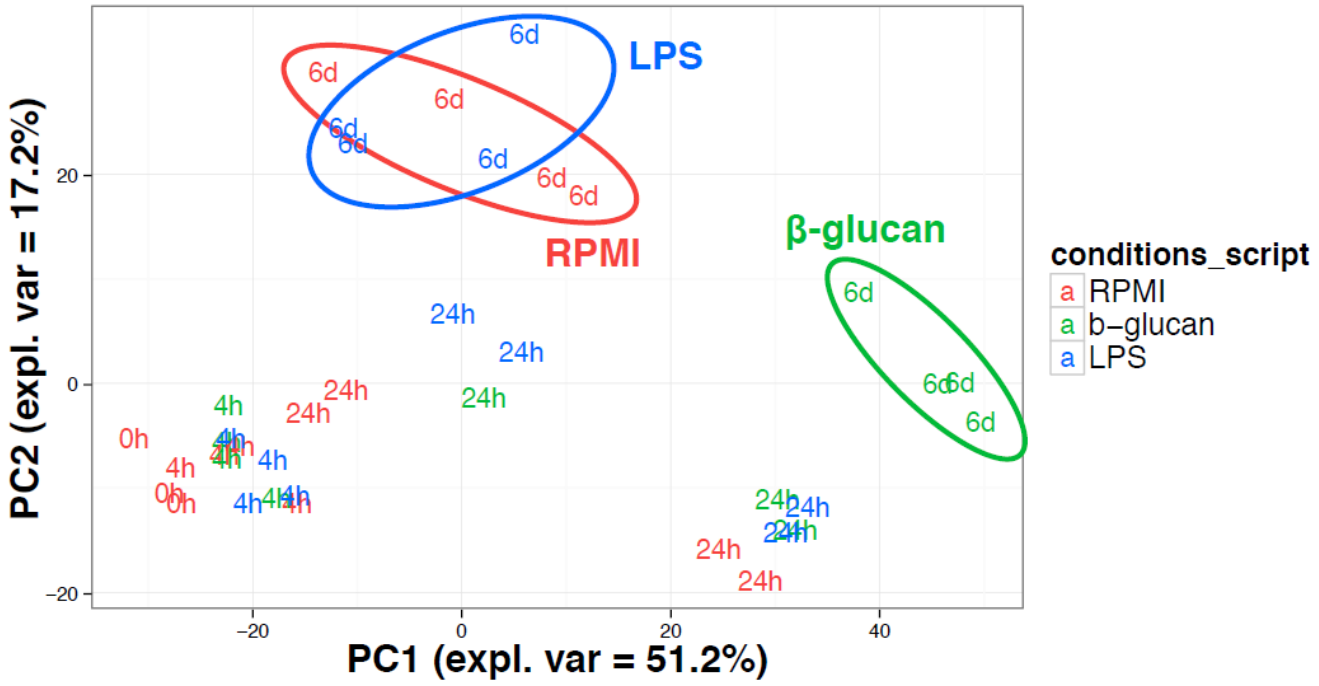




Saeed et al, Science, 2014

UMC St Radboud

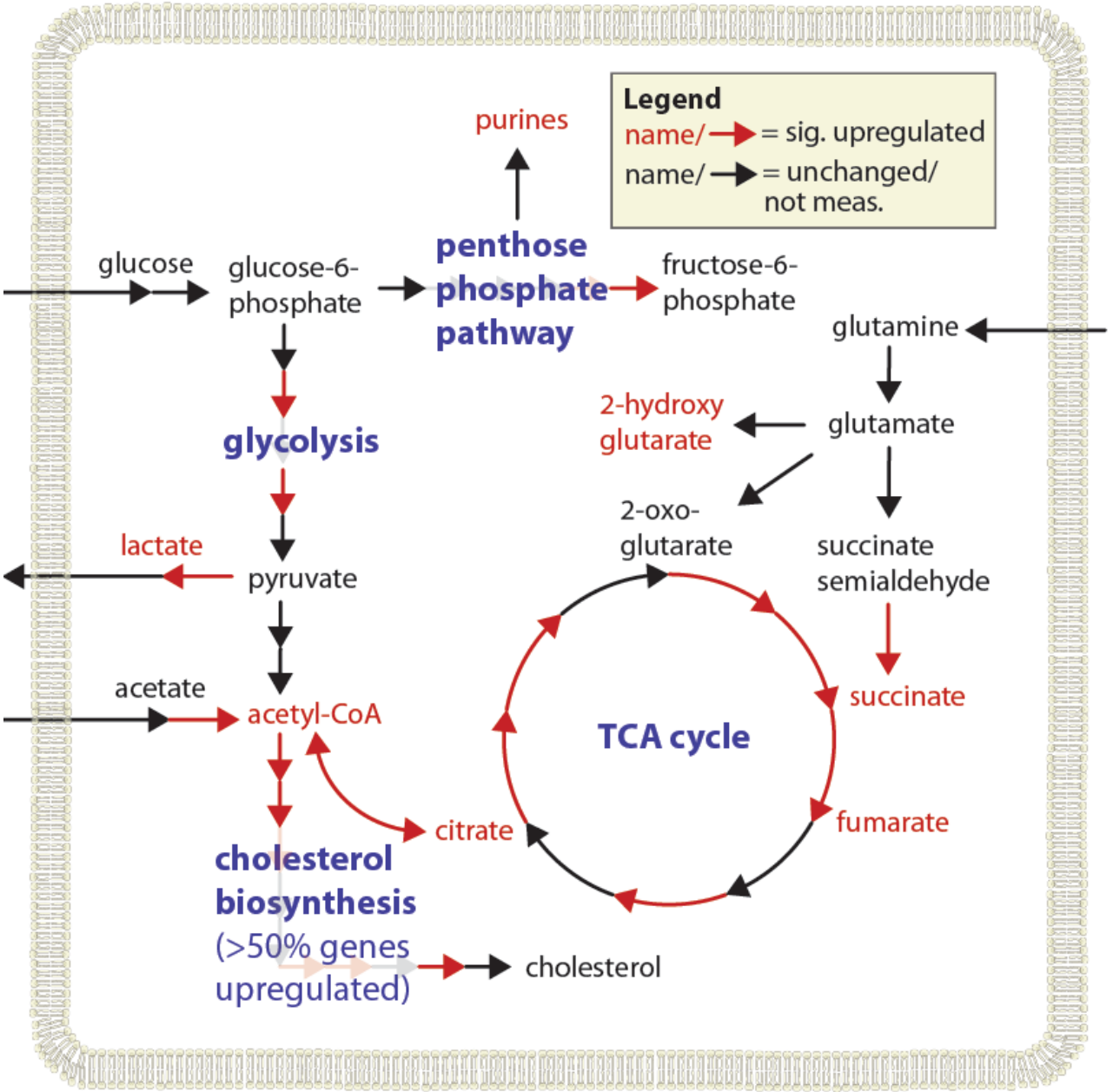
# Metabolic status in trained monocytes



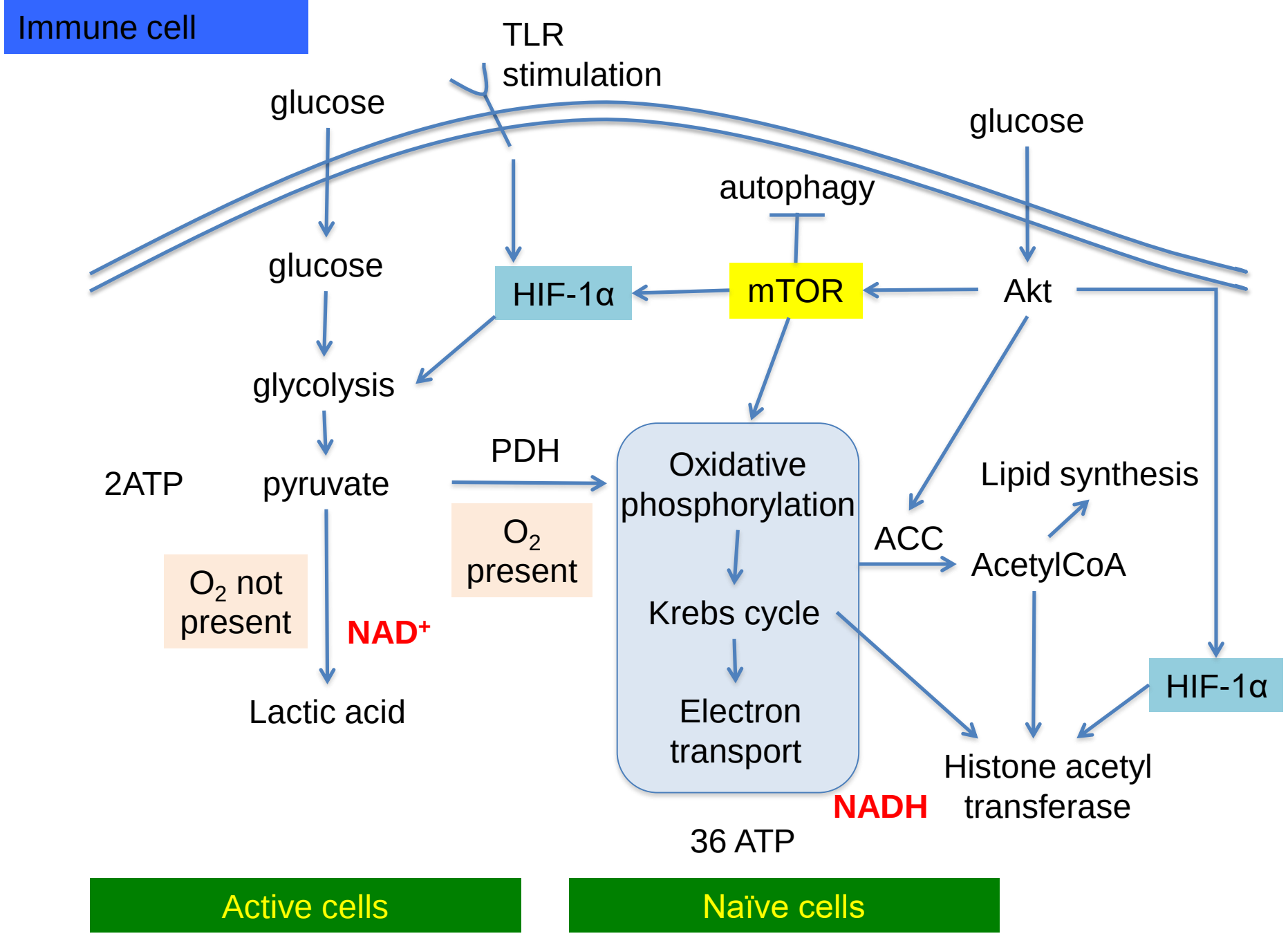




Arts et al, Cell Metabolism 2016

# Metabolic status in trained monocytes

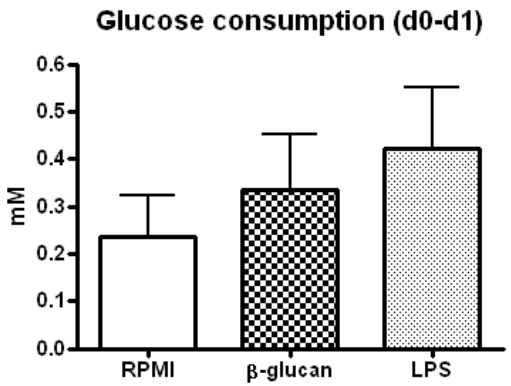


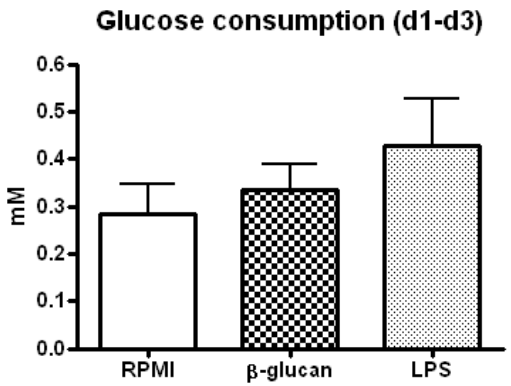

Arts et al, Cell Metabolism 2016

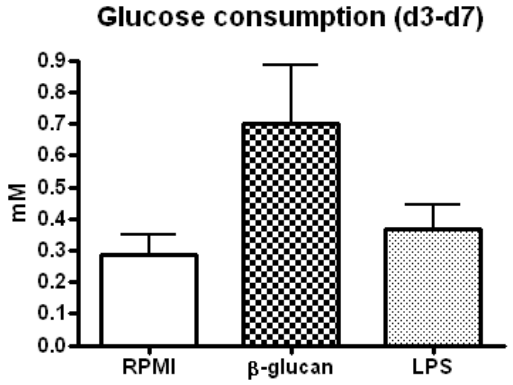

Immune cell
TLR stimulation
glucose
glucose
glucose
autophagy
HIF-1α
mTOR
Akt
glycolysis
2ATP
pyruvate
PDH
Oxidative phosphorylation
Lipid synthesis
ACC
AcetylCoA
O2 not present
NAD+
O2 present
Krebs cycle
Electron transport
HIF-1α
Lactic acid
Histone acetyl transferase
NADH
36 ATP
Active cells
Naïve cells

# Glucose consumption & lactate secretion

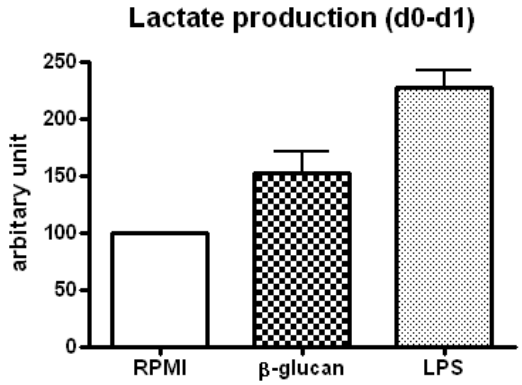
## Glucose consumption

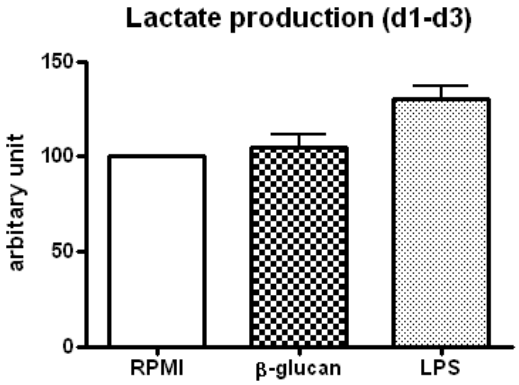


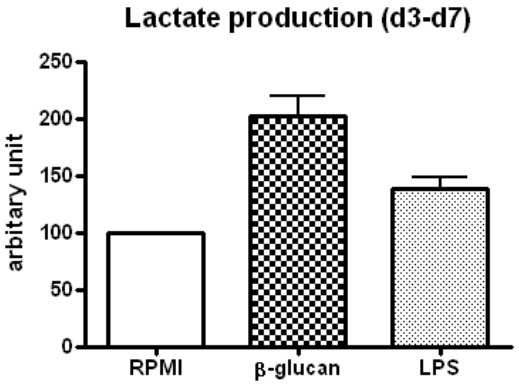




## Lactate secretion




Cheng et al, Science, 2014

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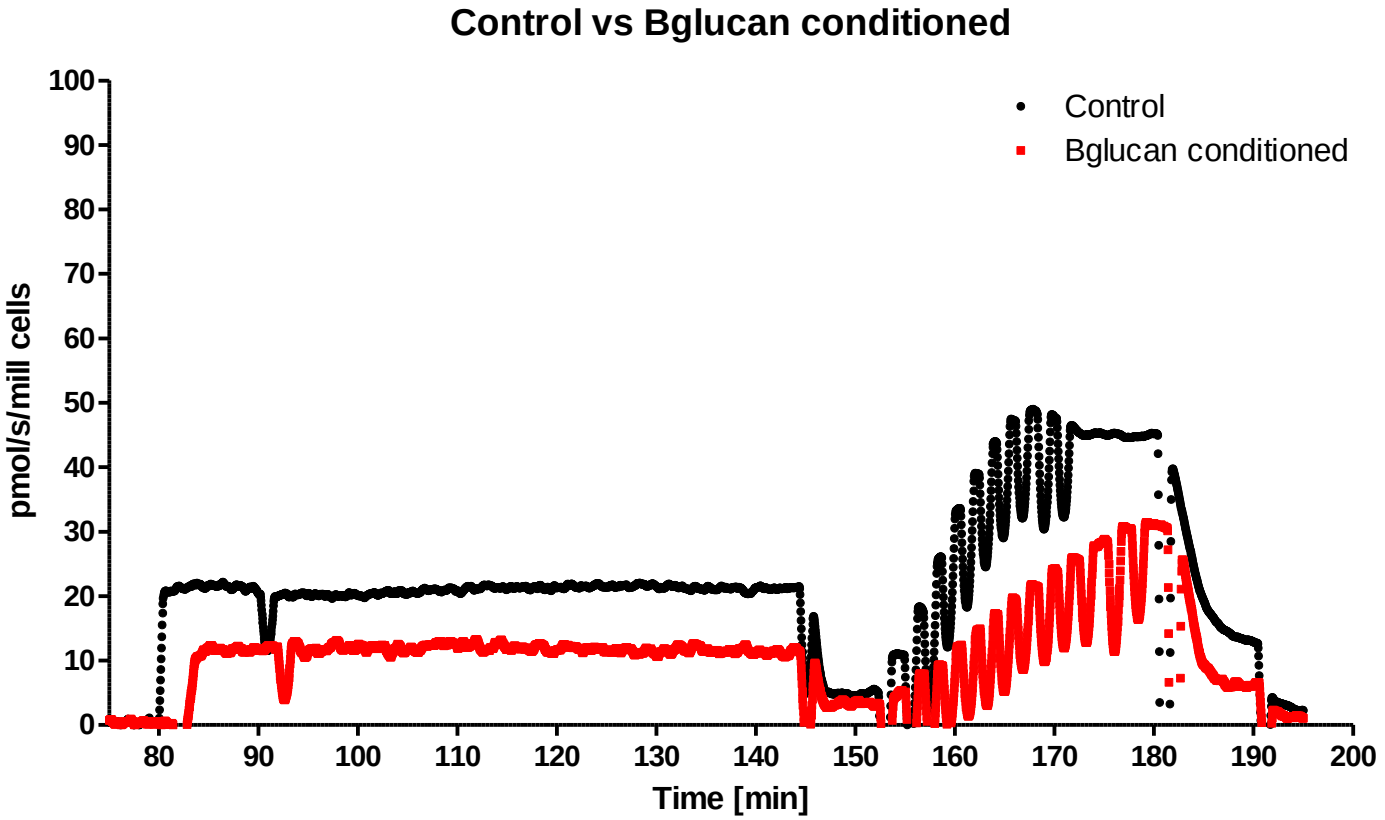
# Reduced ATP-induced respiration




| Cells | Basal | LPS 50 ng/ml | Oligomycin sensitive | Oli + FCCP | Rot + AA |
|---|---|---|---|---|---|
| Control | 21.58 | 21.27 | 3.7 | 48.7 | 2.36 |
| Bglucan conditioned | 11.77 | 11.54 | 3.7 | 31.06 | 2.36 |

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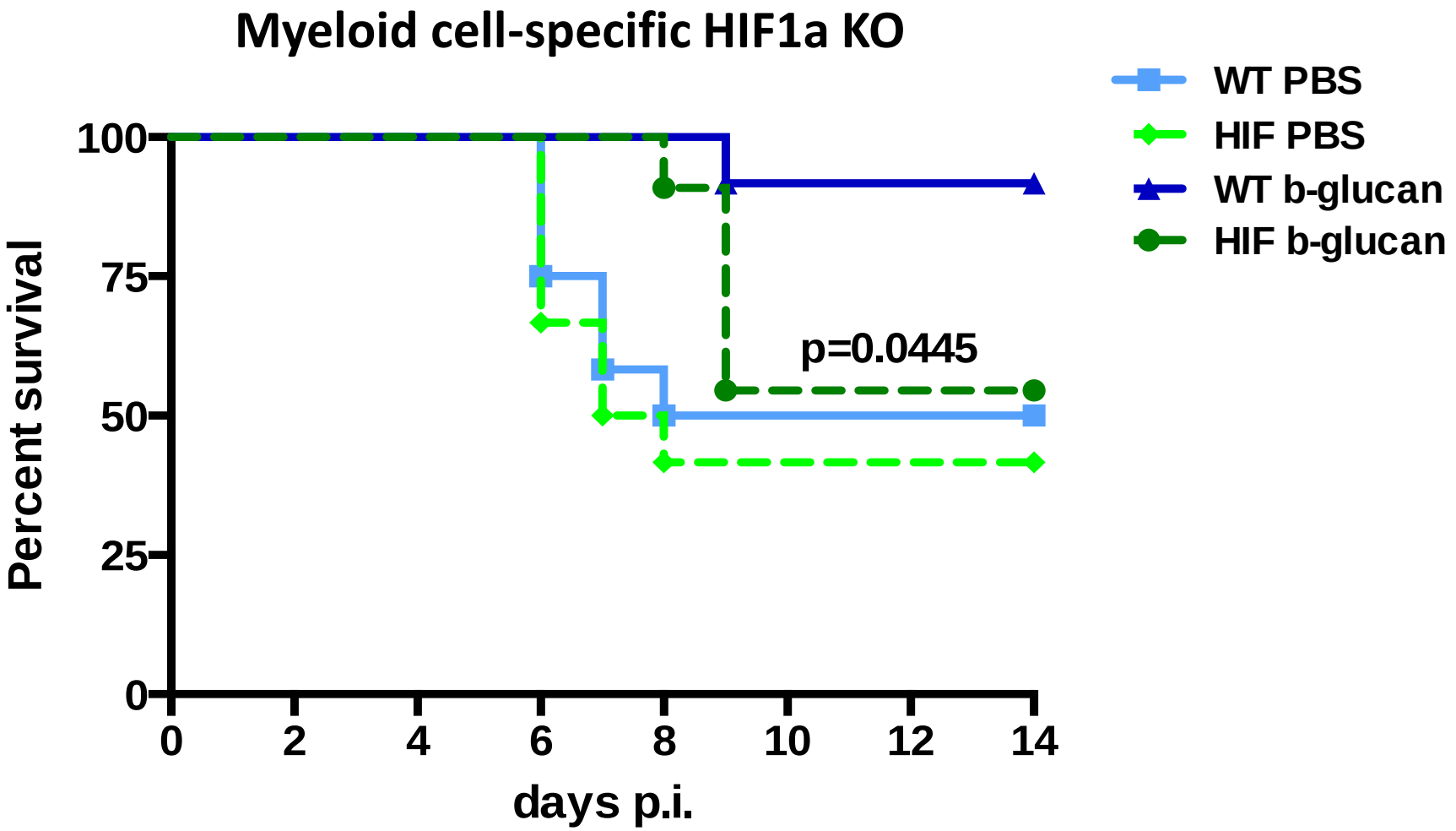
**Cheng et al, Science, 2014**

# Blocking glucose consumption in-vivo inhibits trained immunity




Cheng et al, Science, 2014

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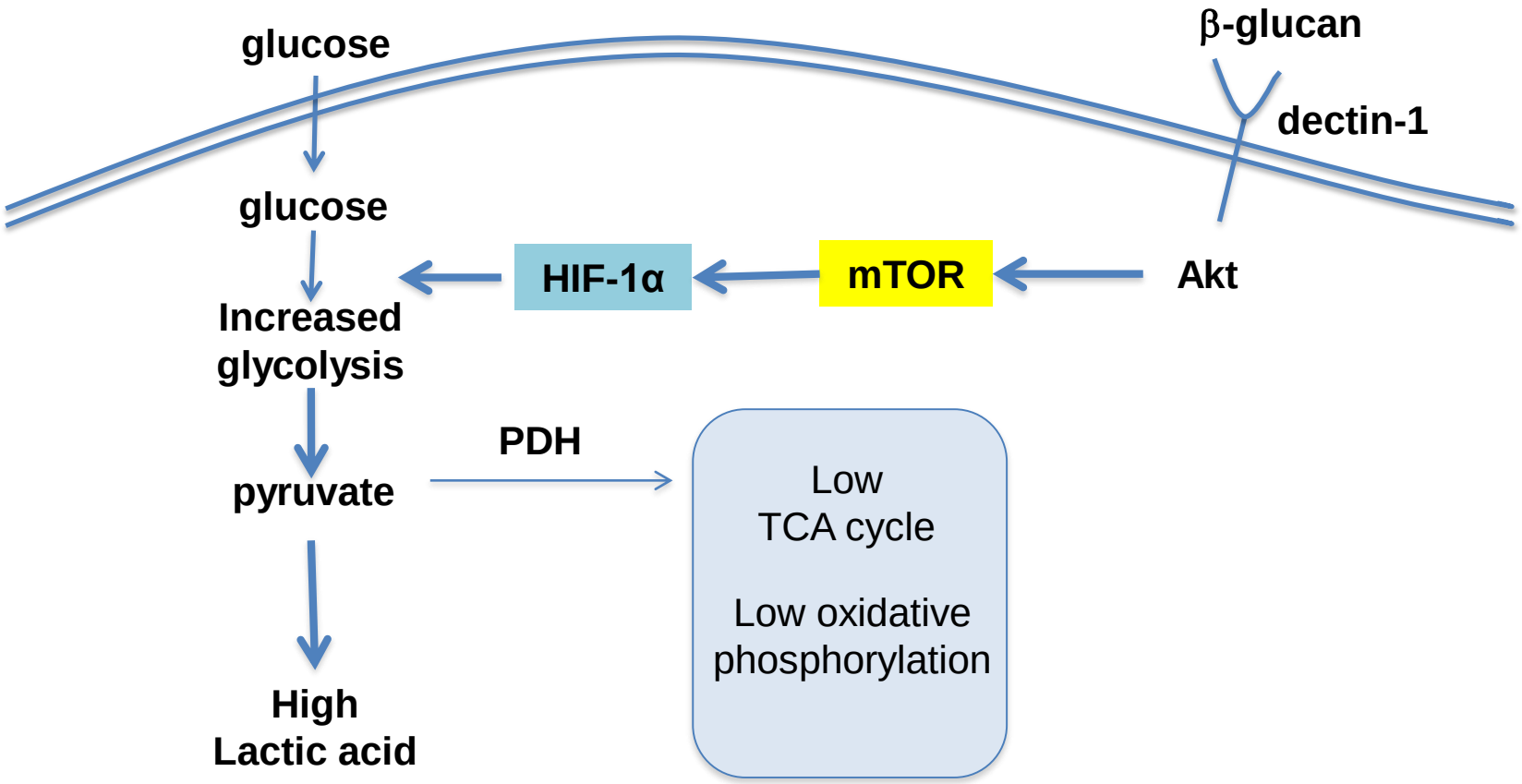
# Model of metabolic activation of trained monocytes




Cheng et al, Science, 2014

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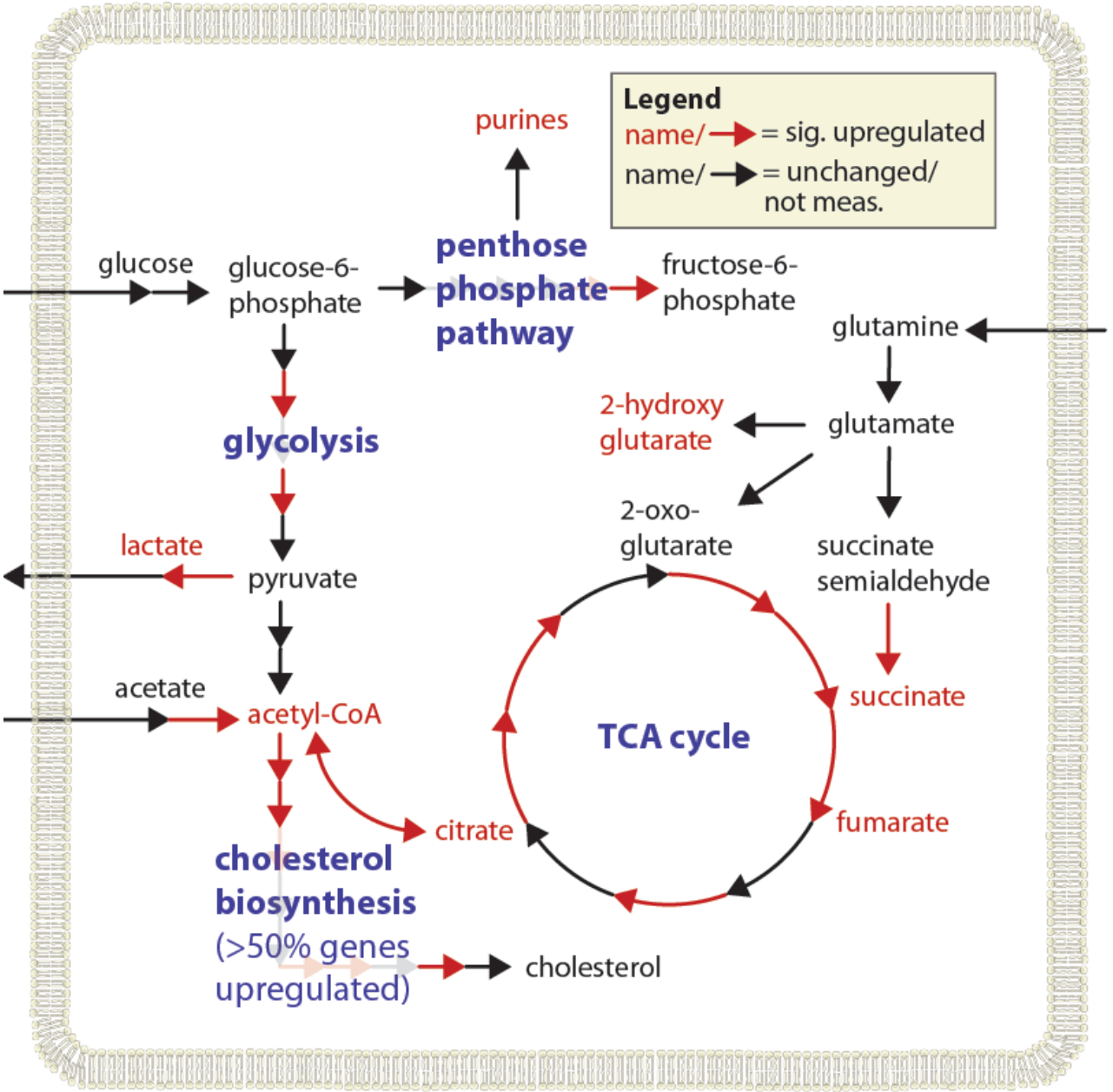
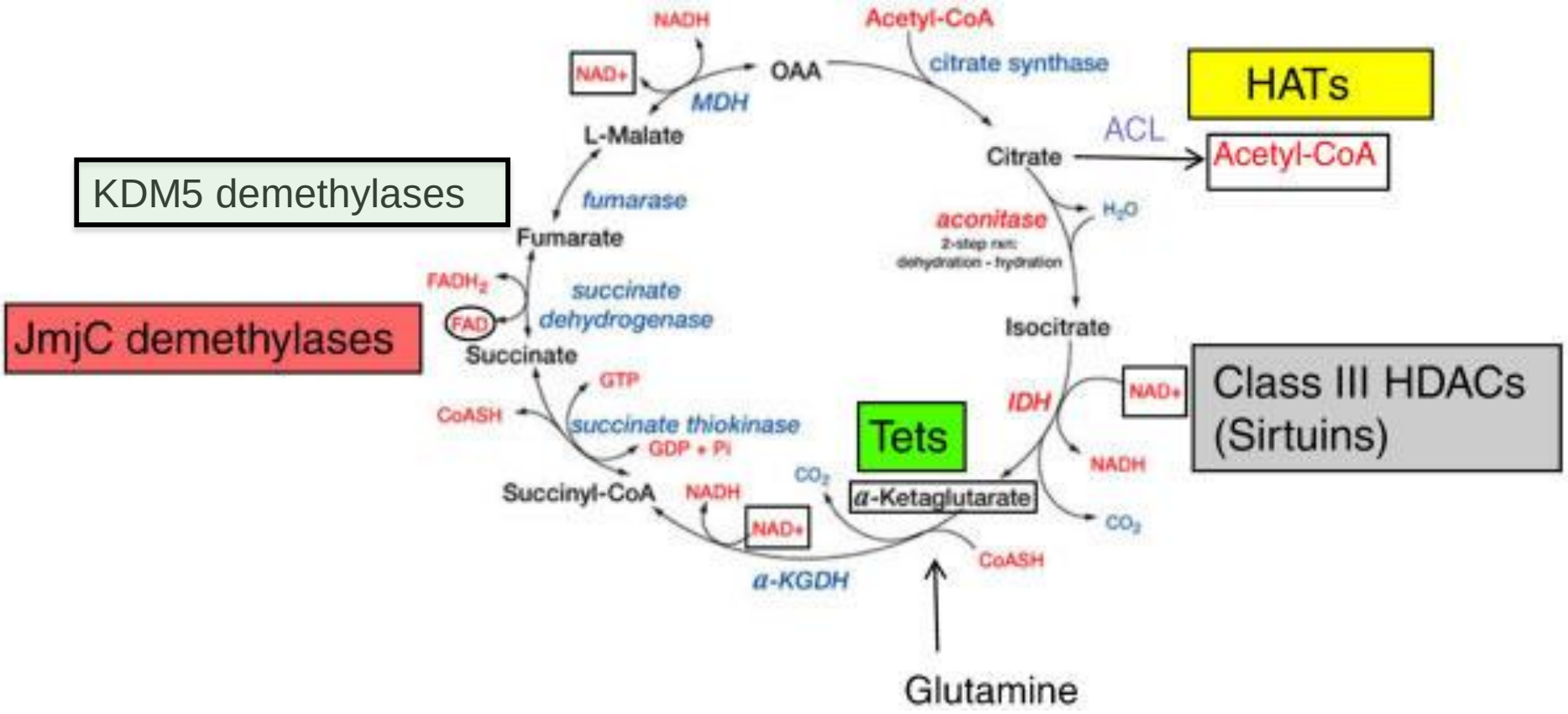
# Metabolic status in trained monocytes



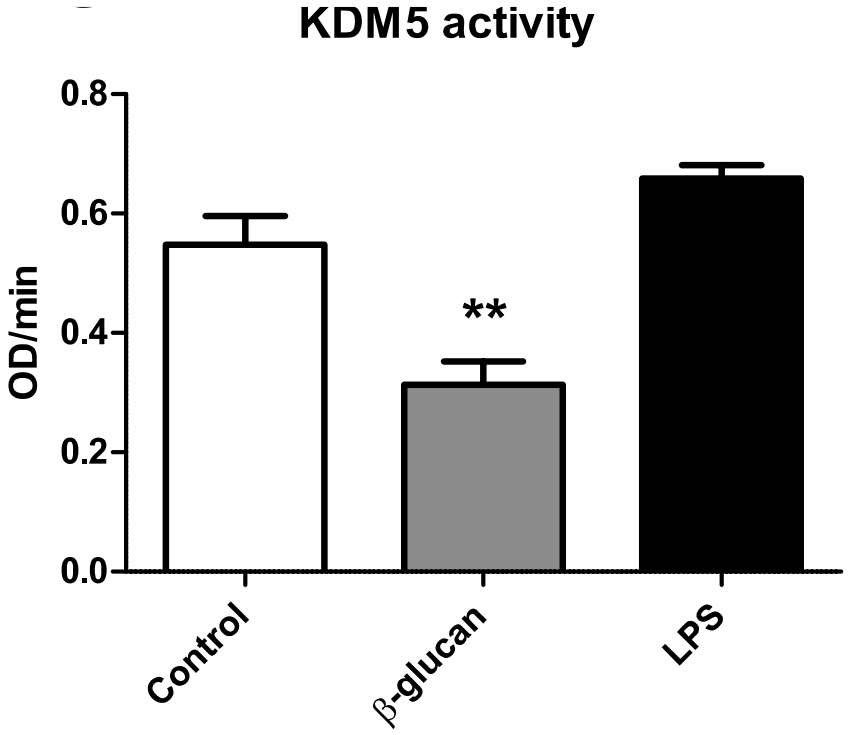

Arts et al, Cell Metabolism 2016

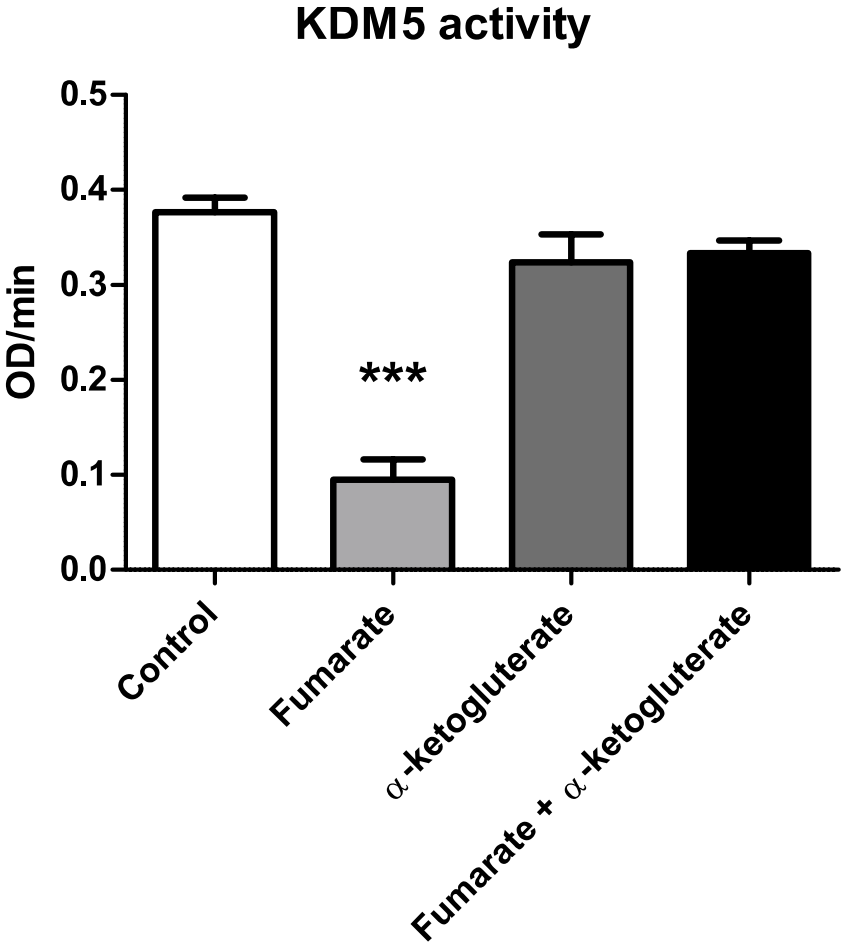
# Metabolites are co-factors for epigenetic enzymes




Donohoe & Bultman, J Cell Physiol 2012

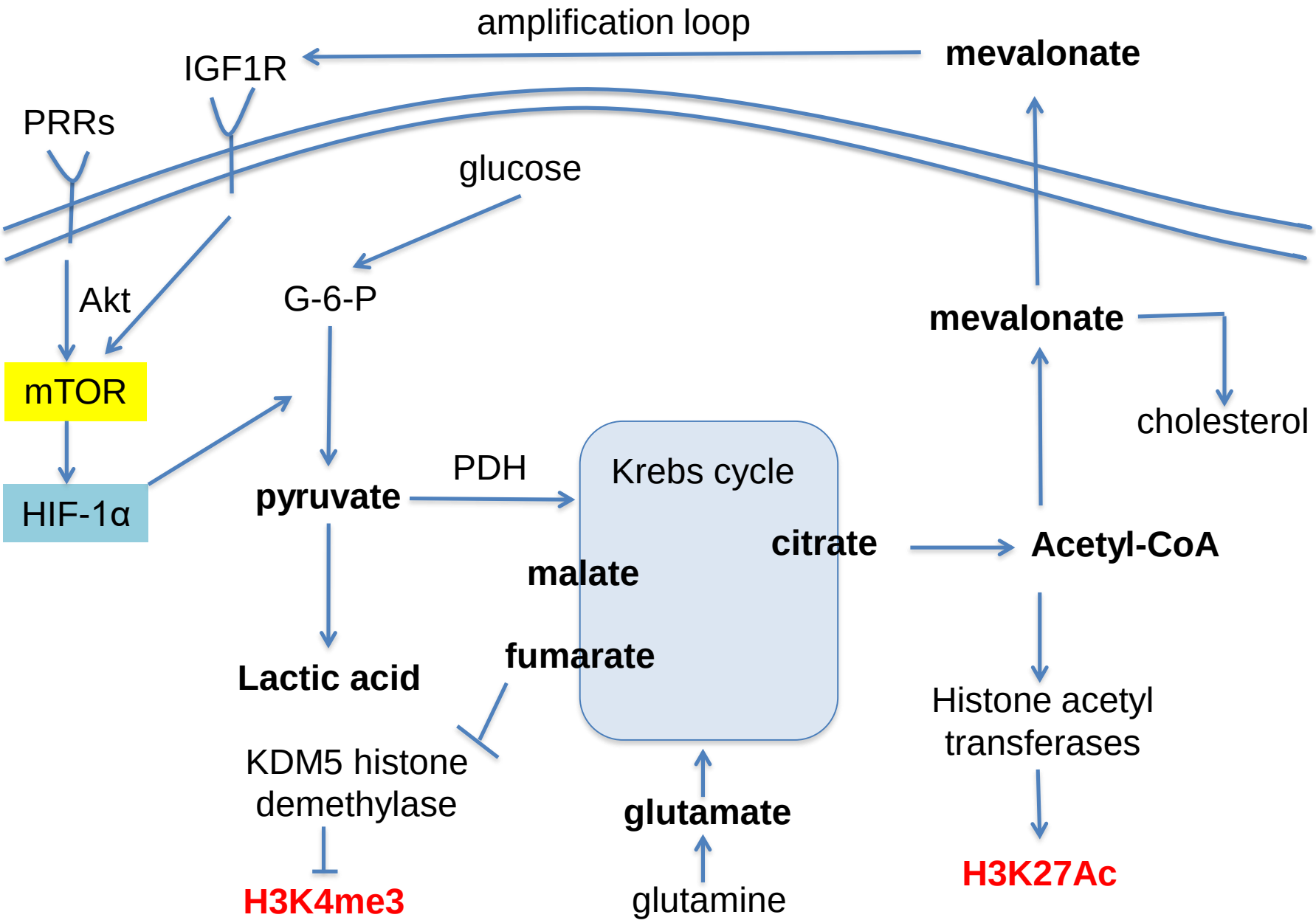
# Fumarate induces trained immunity




Arts et al, Cell Metabolism 2016

# Metabolic status in trained monocytes



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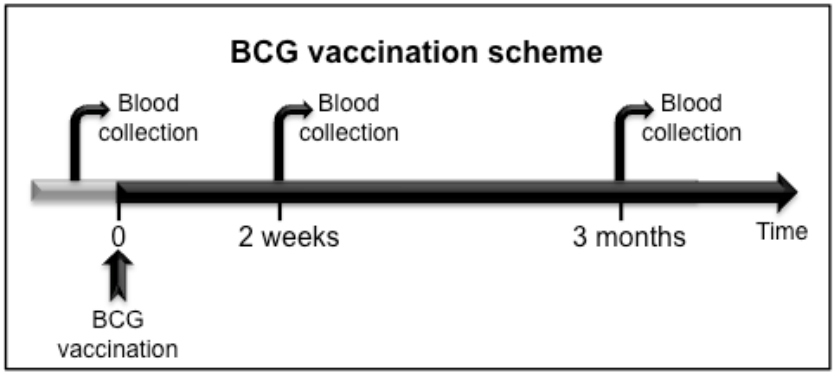
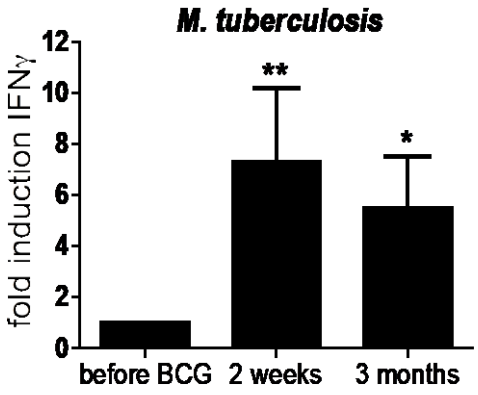
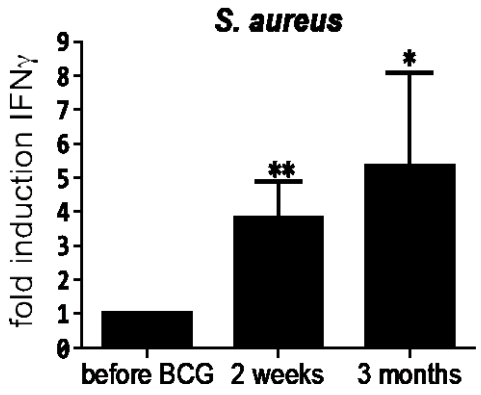
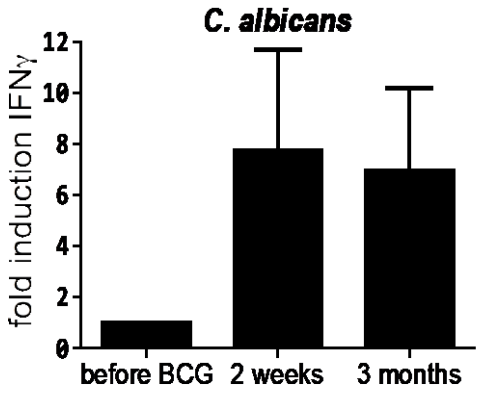
# Does this happen in vivo in humans?




Kleinnijenhuis et al, PNAS, 2012

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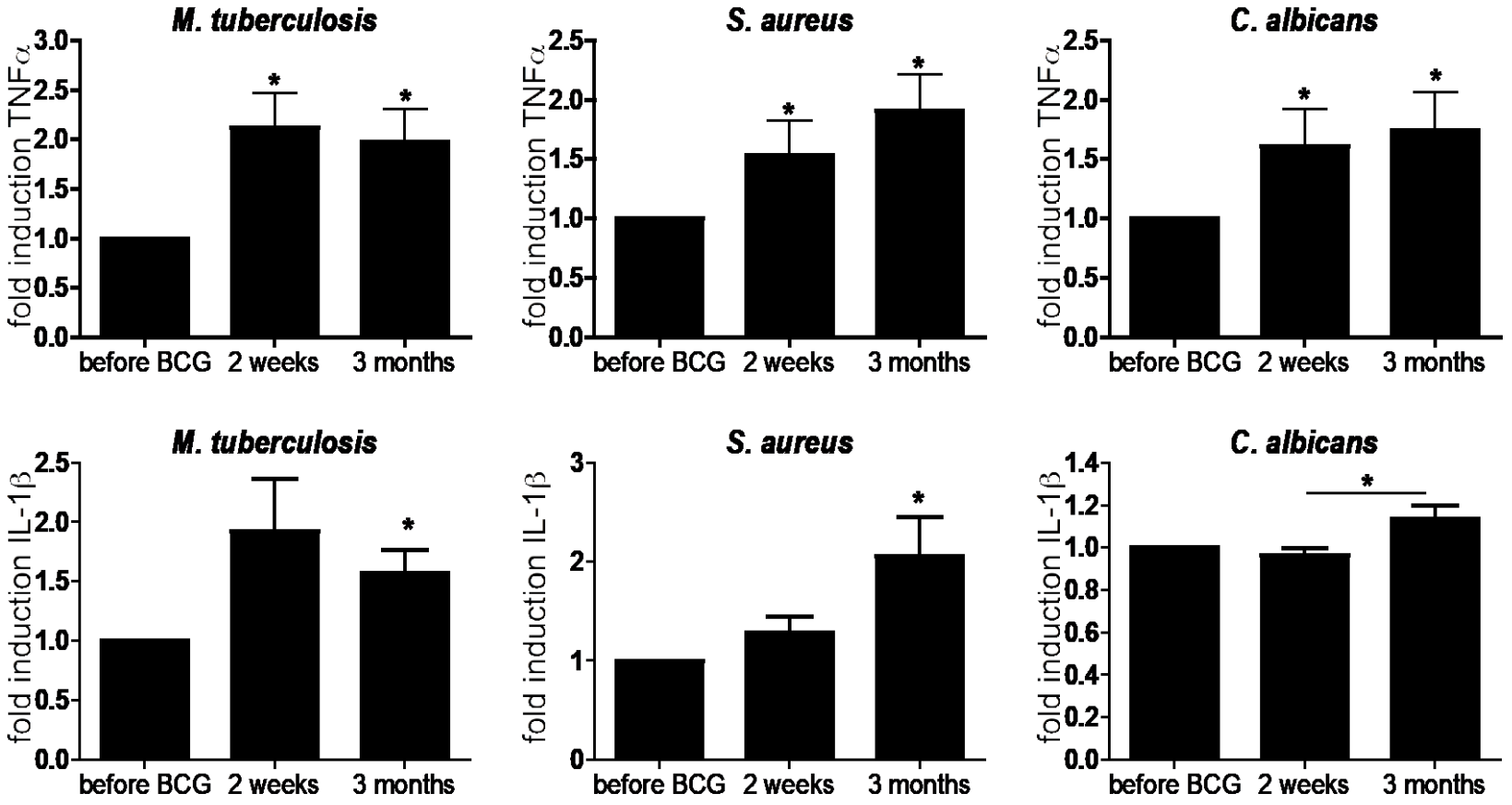
# BCG enhances monocyte-derived cytokines




Kleinnijenhuis et al, PNAS, 2012

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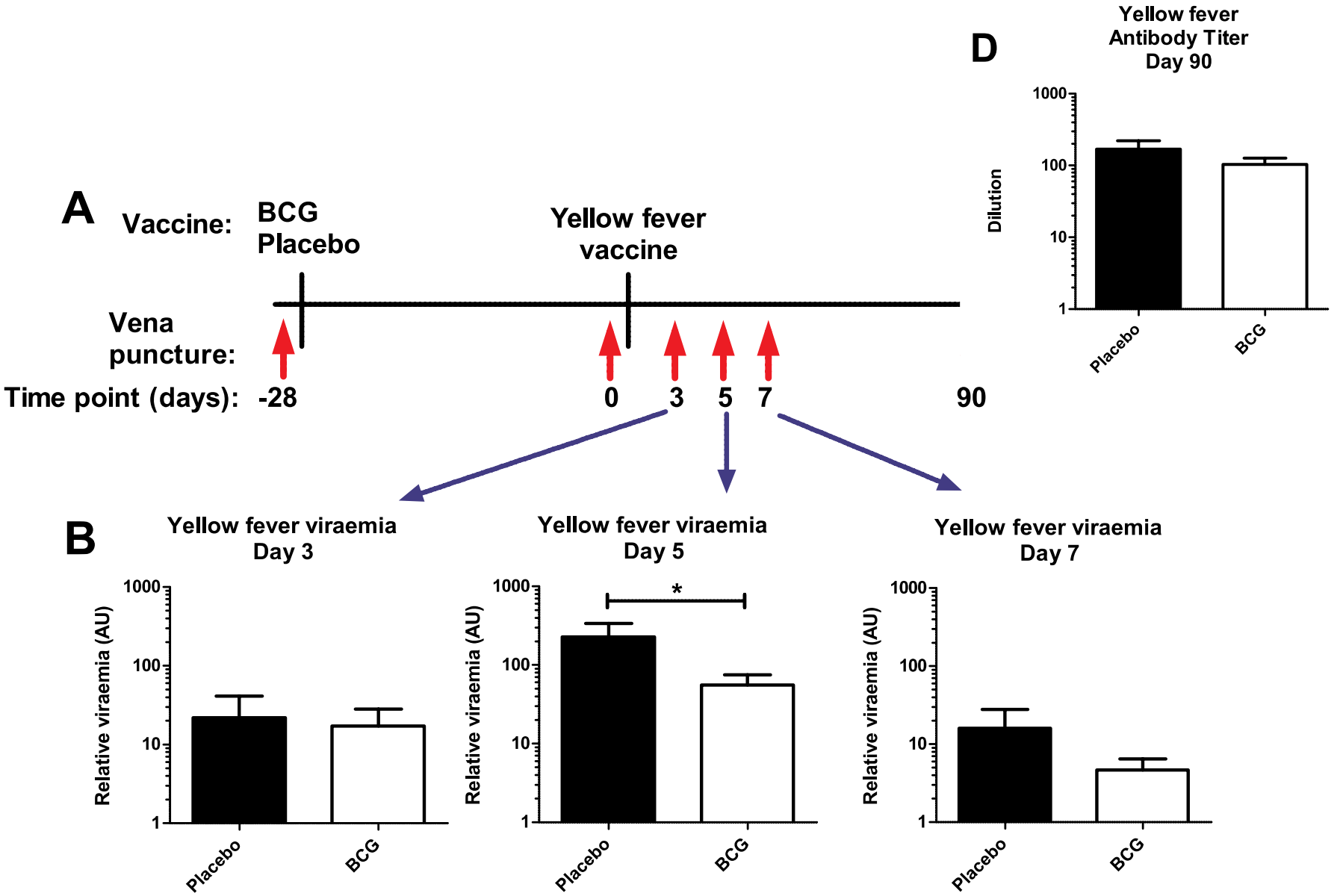
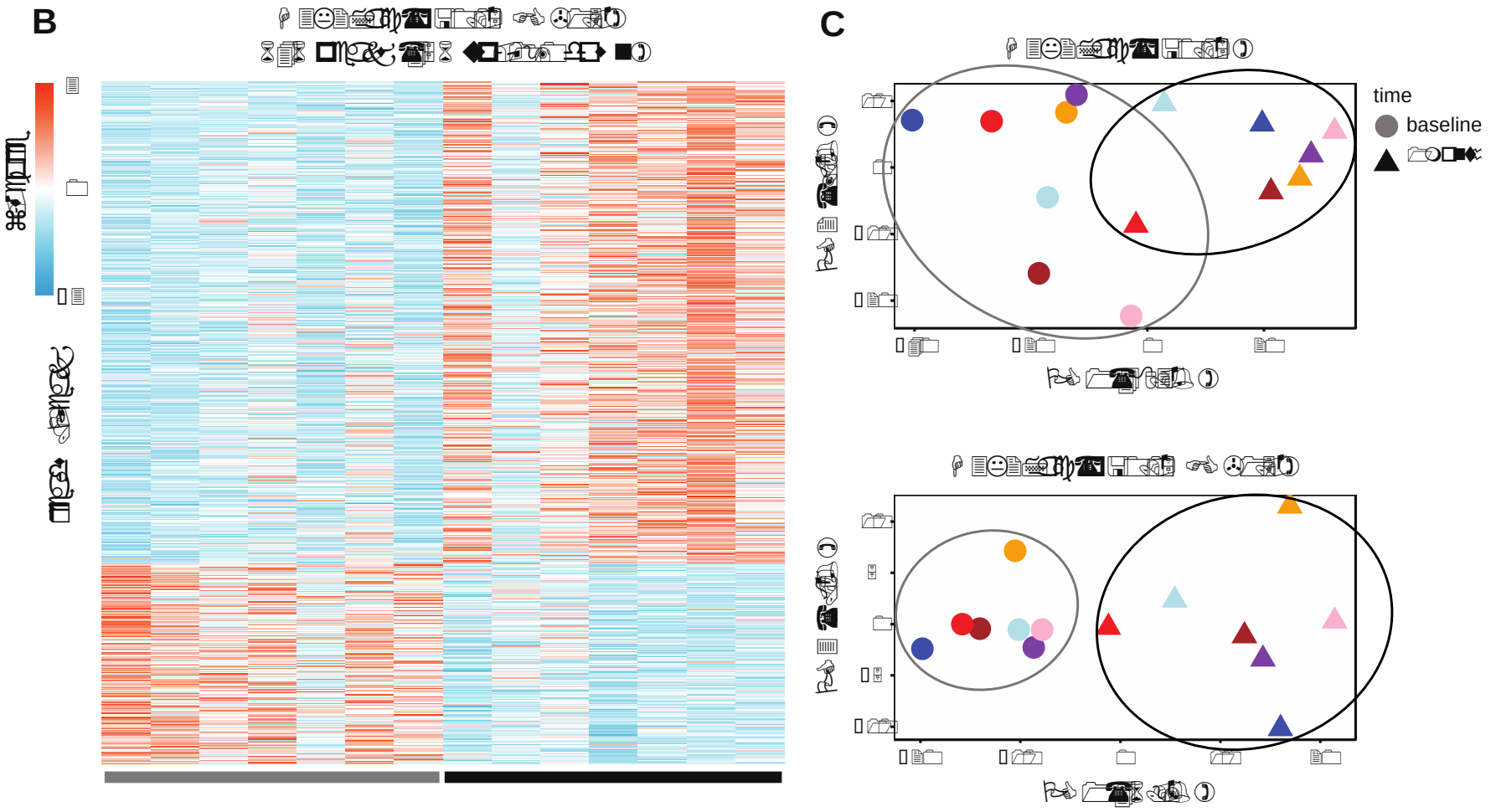
# BCG vaccination in vivo yellow fever vaccine



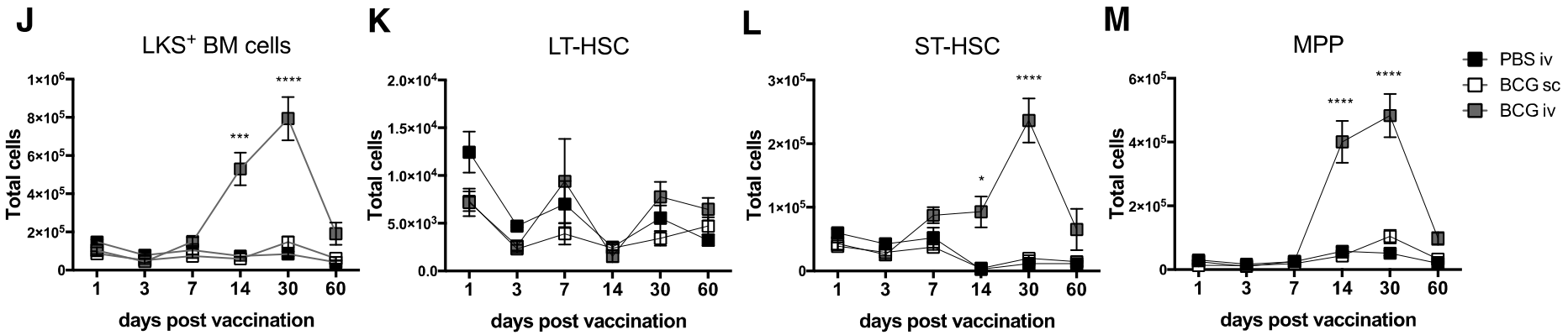

Arts et al, Cell Host Microbe, 2018 in press

# BCG vaccination induces epigenetic reprogramming




Arts et al, Cell Host Microbe, 2018

# BCG acts on myeloid cell progenitors




Kaufmann et al, Cell 2018

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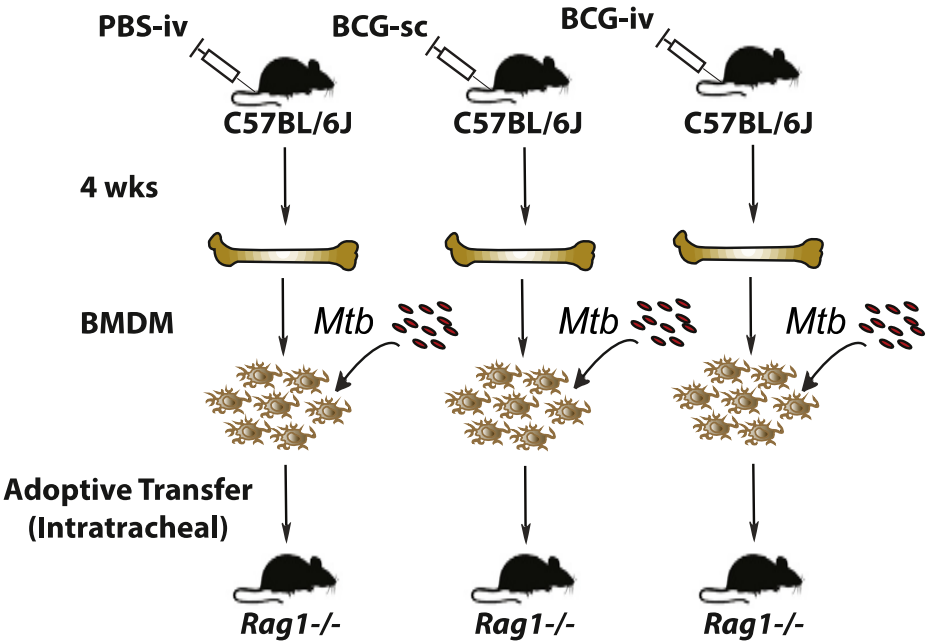
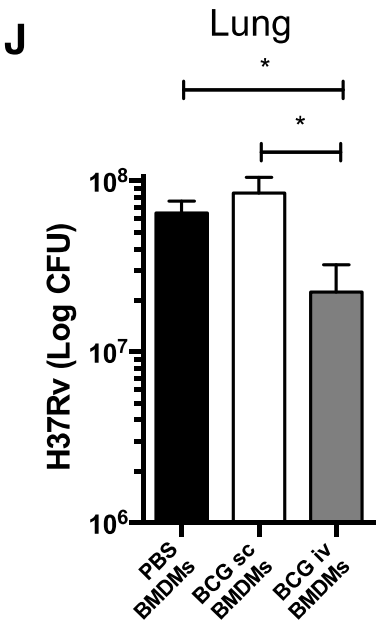
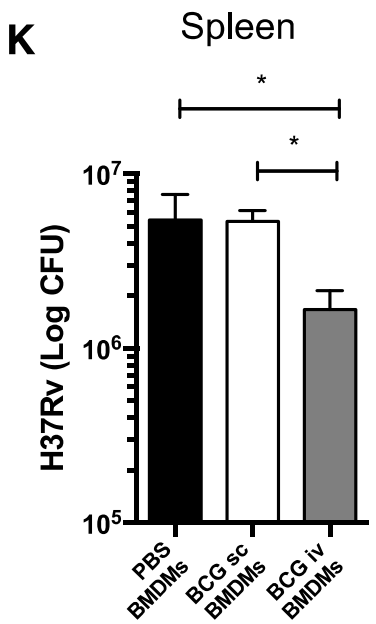
# BCG trained effects are sustainable in vivo




Kaufmann et al, Cell 2018

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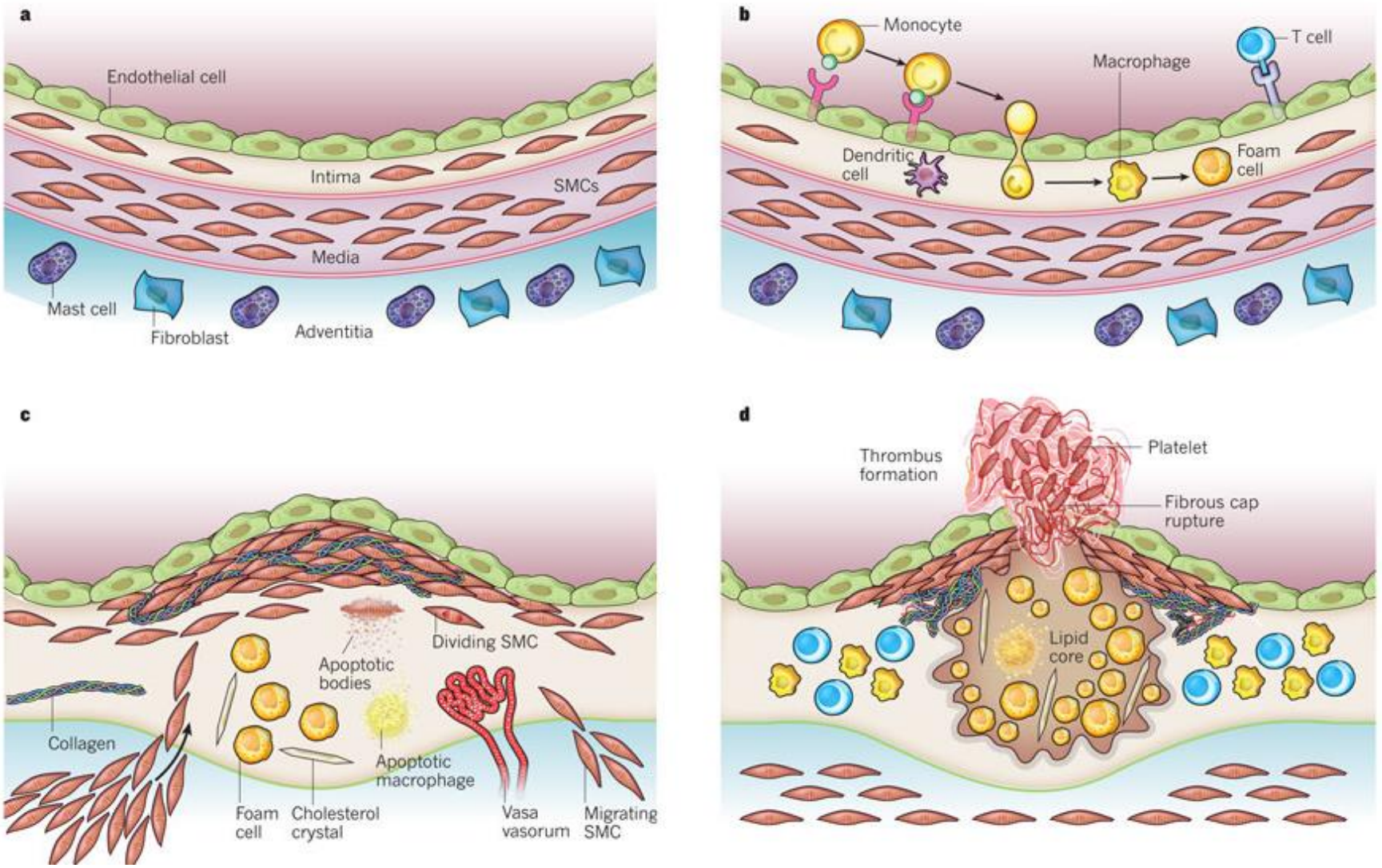
# Conclusions BCG vaccination

- BCG vaccination induces trained immunity in circulating monocytes
- BCG induces long-term reprogramming at the level of myeloid cell progenitors in the bone marrow
- BCG vaccination leads to non-specific protection against unrelated infections: e.g. yellow fever vaccine viremia, malaria
- Responses to other vaccines are influenced as well

# Atherosclerosis: non-resolving inflammation




*Libby et al, Nature 2011*

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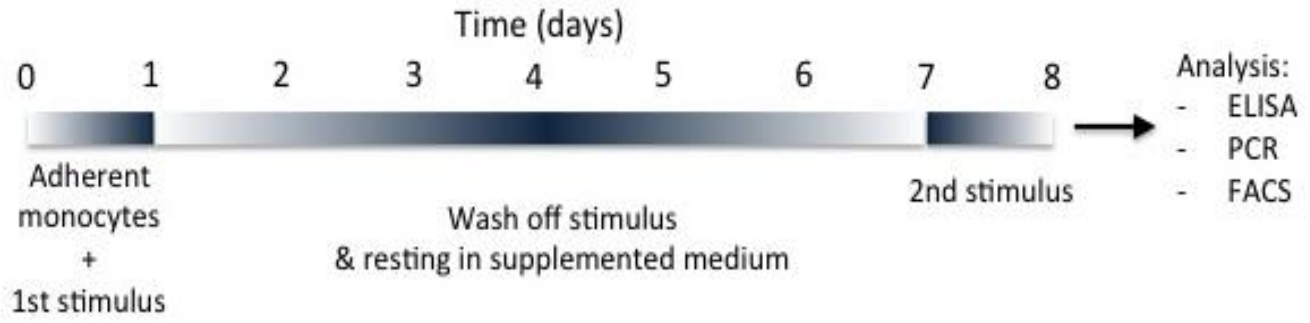
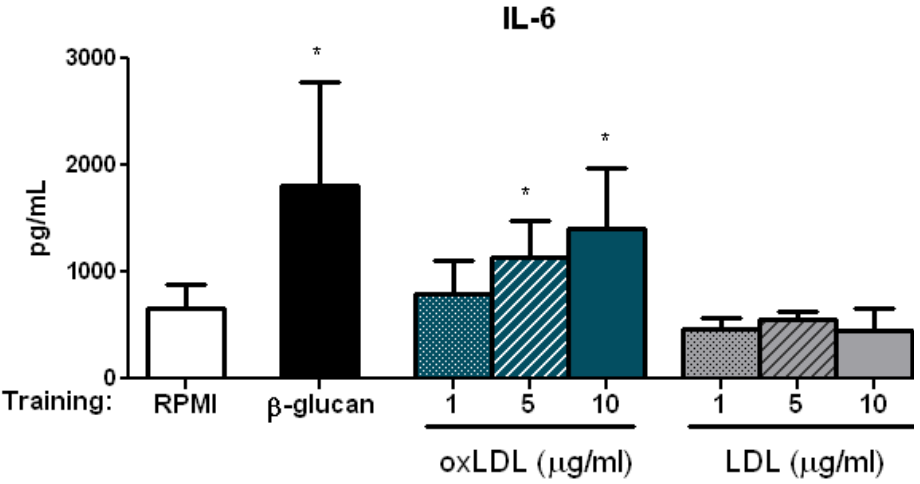
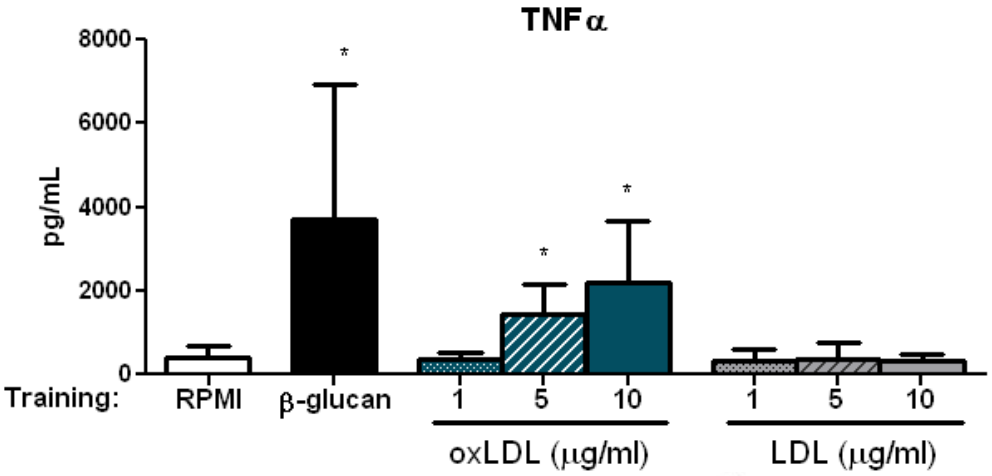
# oxLDL but not LDL can train monocytes *in vitro*

Methods:




Bekkering et al, ATVB 2014

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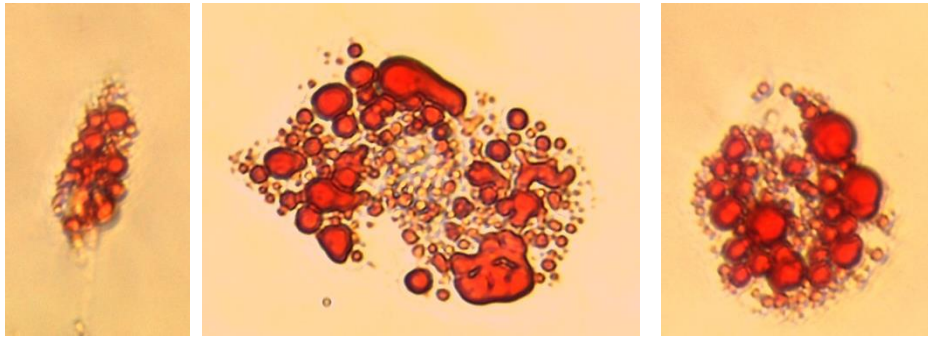
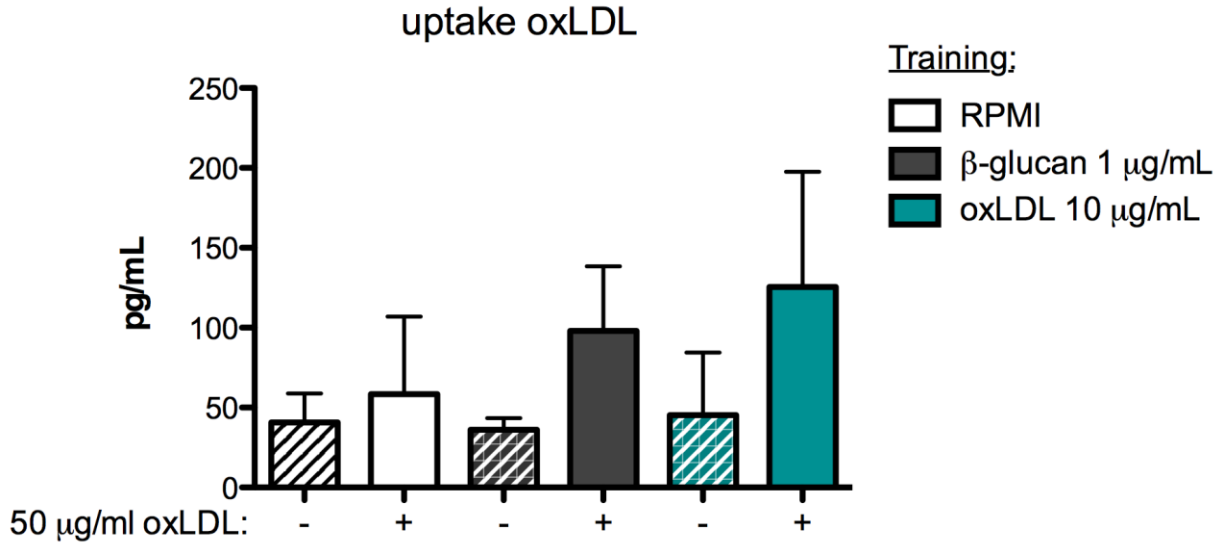
# Trained monocytes show increased foam cell formation



RPMI β-glucan oxLDL




Bekkering et al, ATVB 2014

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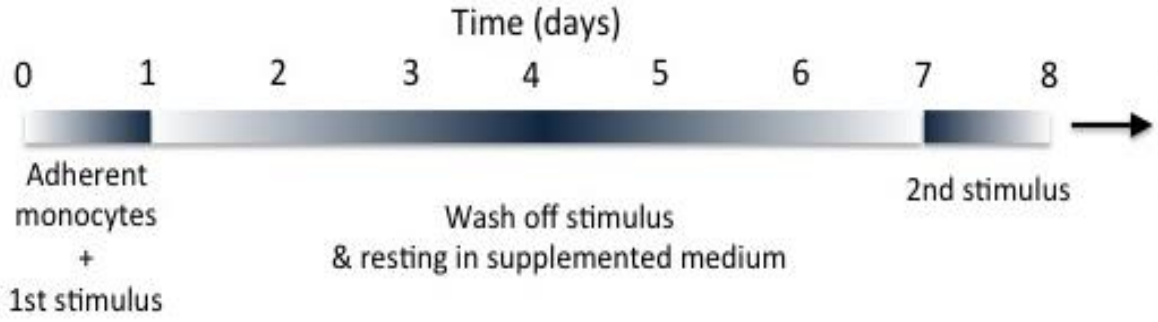
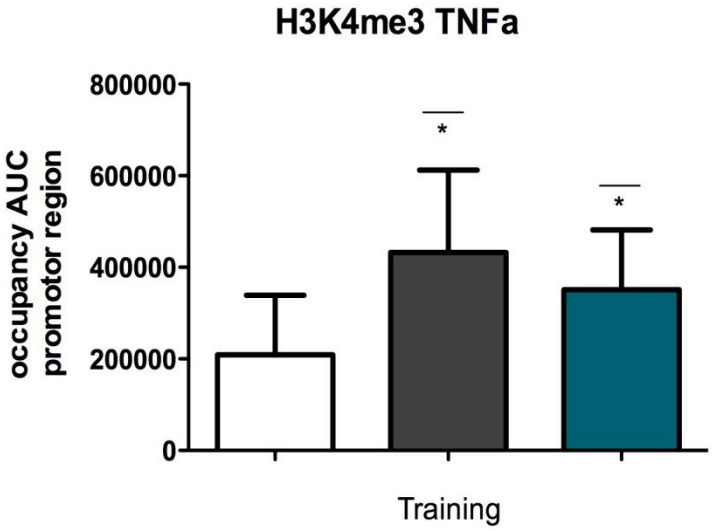
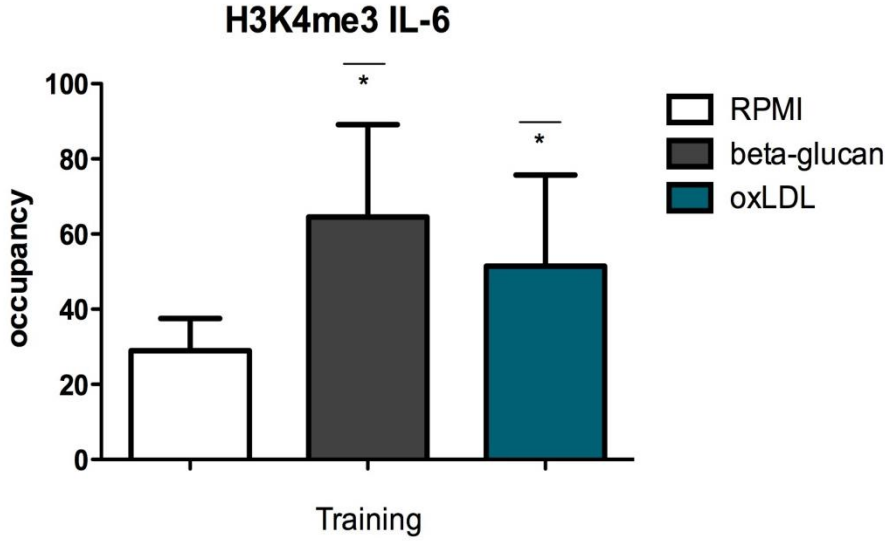
# oxLDL training induces upregulated H3K4me3 on the promotor of TNFα




Analysis:
ChIP
H3K4me3




Bekkering et al, ATVB 2014

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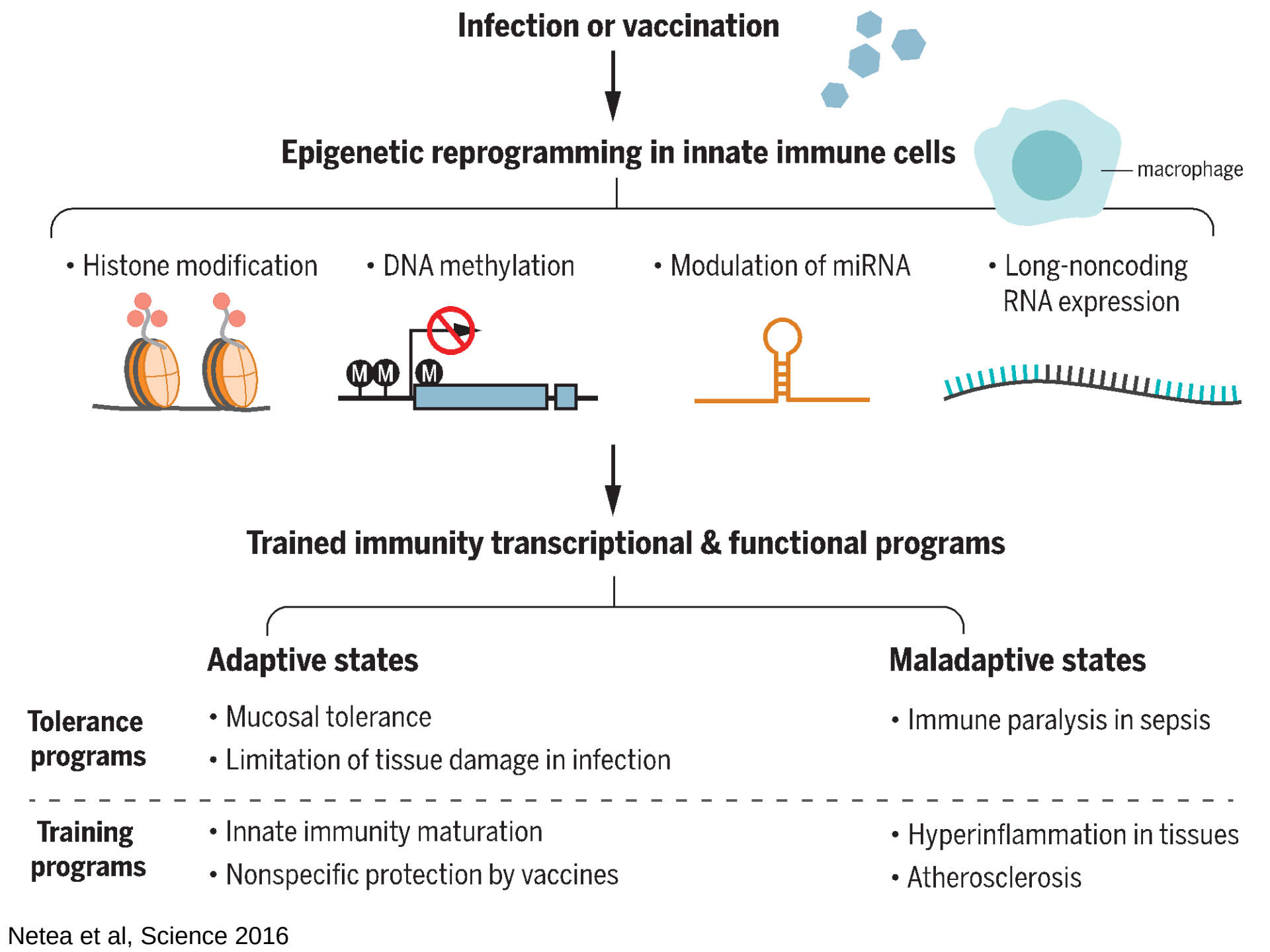


Netea et al, Science 2016