Syreradikal, vän eller fiende?

Rikard Holmdahl

Martin H:son Holmdahl föreläsningen

SFAI kongress

Stockholm 22 sept 2015

Karolinska Institutet

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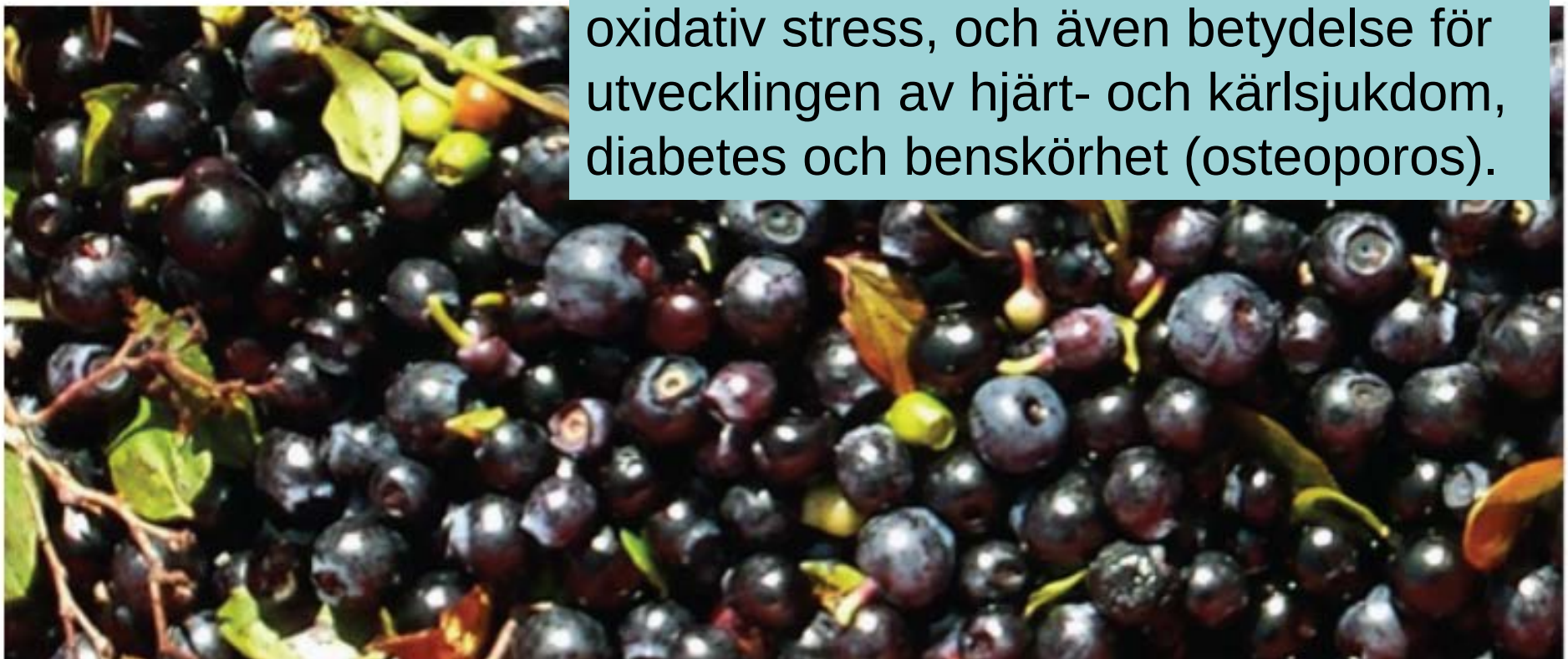
Dogm
(en sanning som är odiskutabel):

Syreradikaler orsakar inflammation
och anti-oxidanter skyddar mot det mesta som hotar oss

< Vitaminer och antioxidanter

Antioxidanter

Fria radikaler
Vi behöver syre för att andas men när syret omsätts bildas samtidigt fria radikaler. Fria radikaler kan ha skadande verkan på kroppen, så kallad oxidativ stress, och även betydelse för utvecklingen av hjärt- och kärlsjukdom, diabetes och benskörhet (osteoporos).



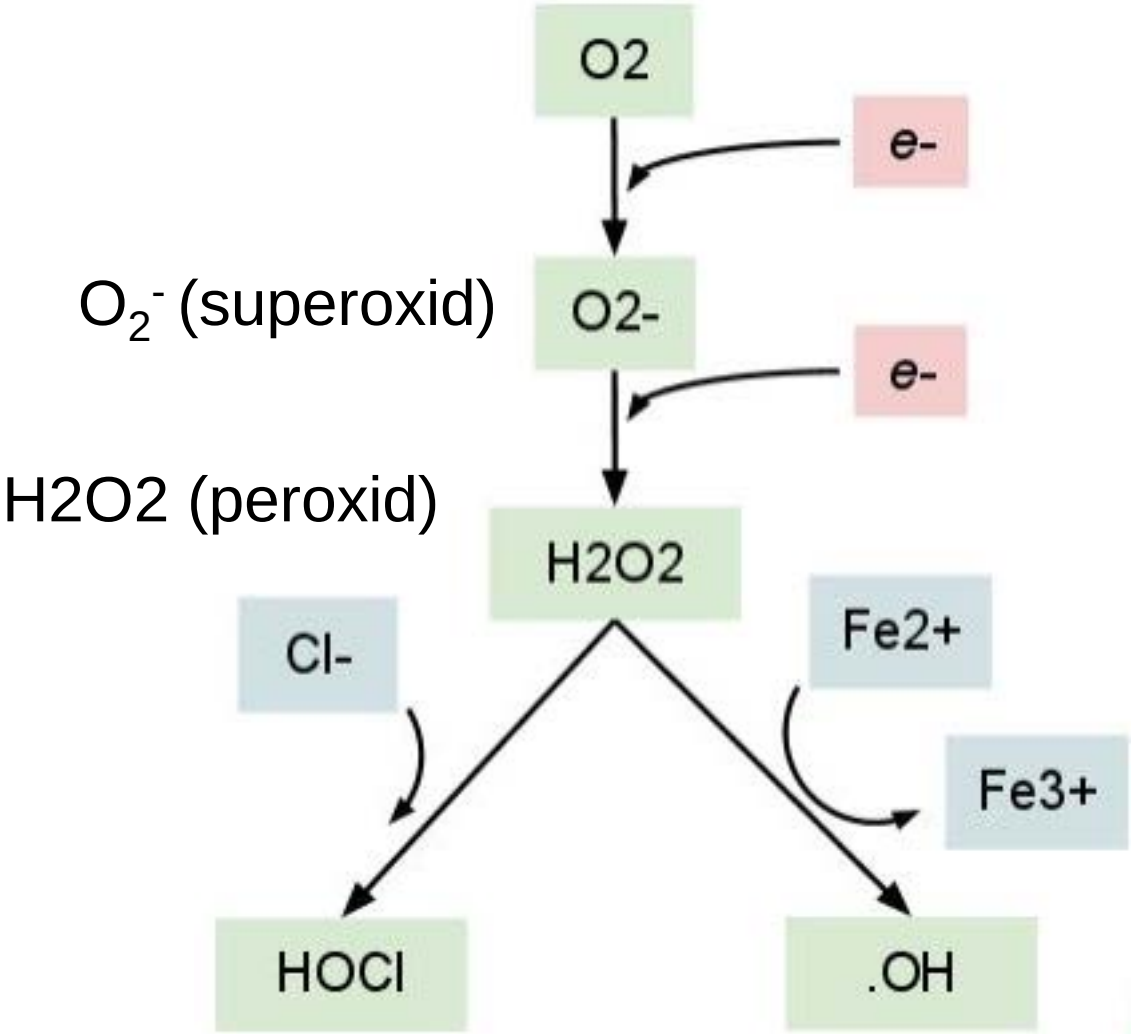
Klart fler antioxidanter i ekomat

Publicerad 2014-07-12 16:26

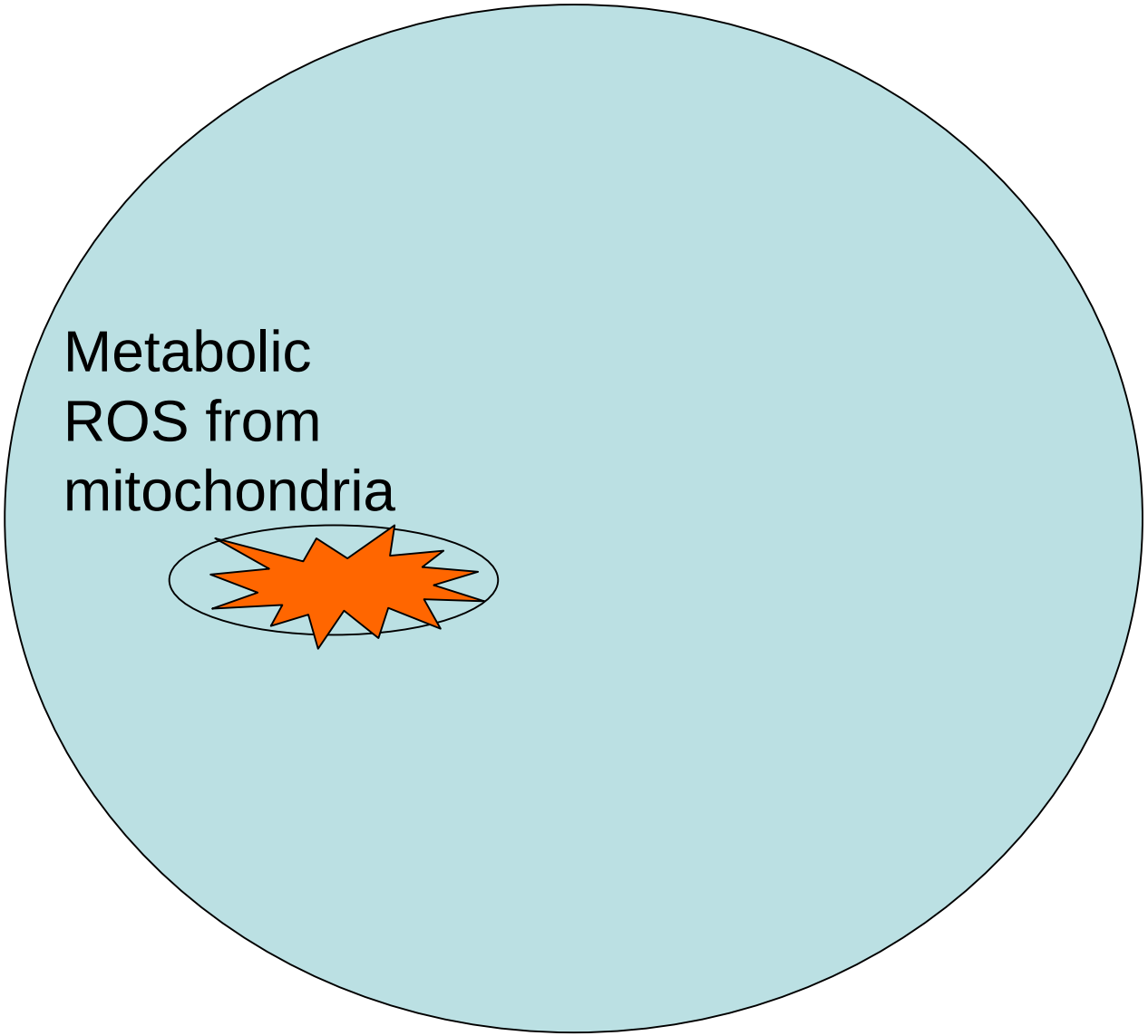
Leif R Jansson/TT Ekologiskt är klart nyttigare än konventionellt odlat, enligt brittiska forskare.

Vad är då syreradikaler?

Reaktiva syremetaboliter (reactive oxygen species/ROS) bildas av tillförsel av elektroner.



Where are ROS normally produced?



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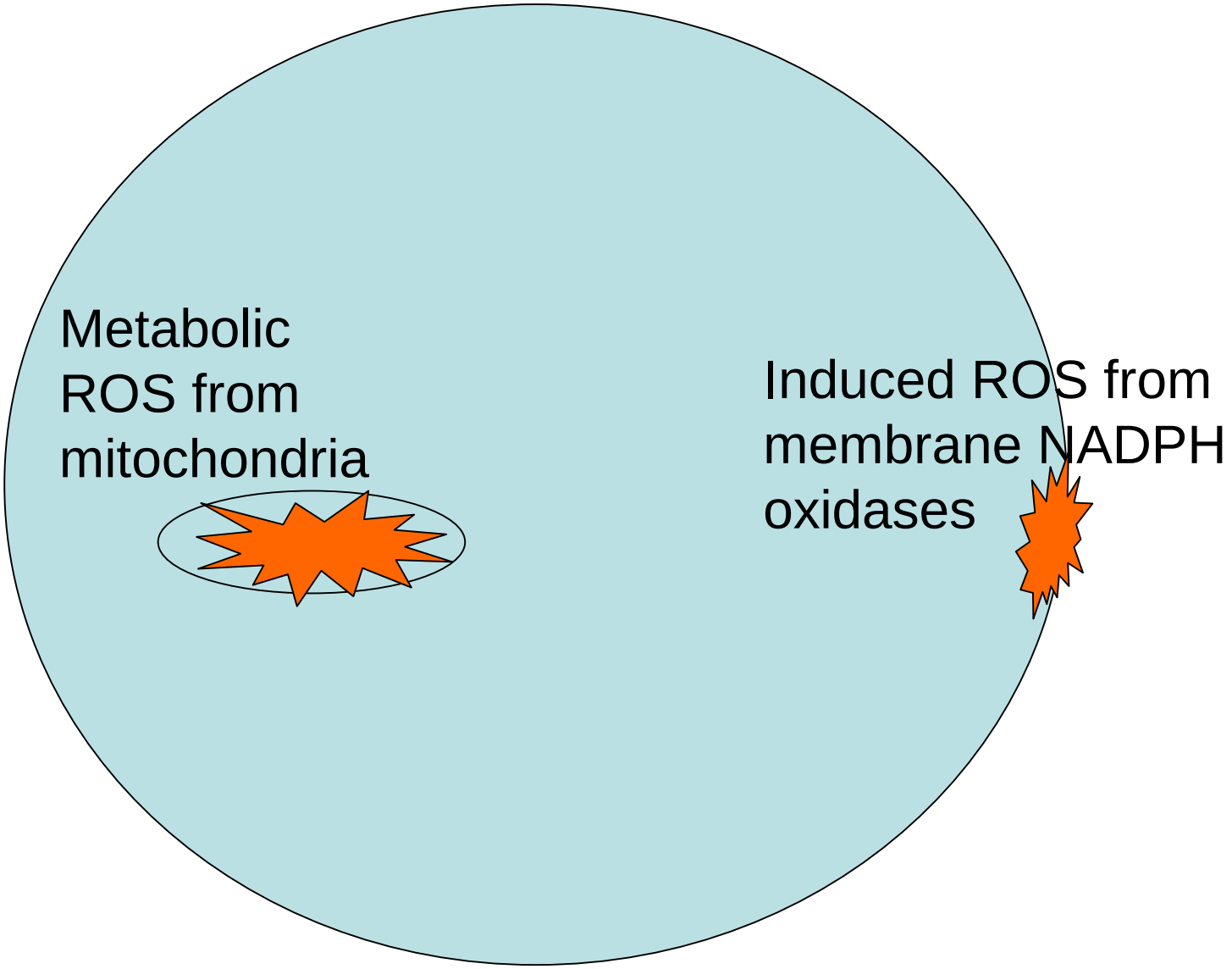

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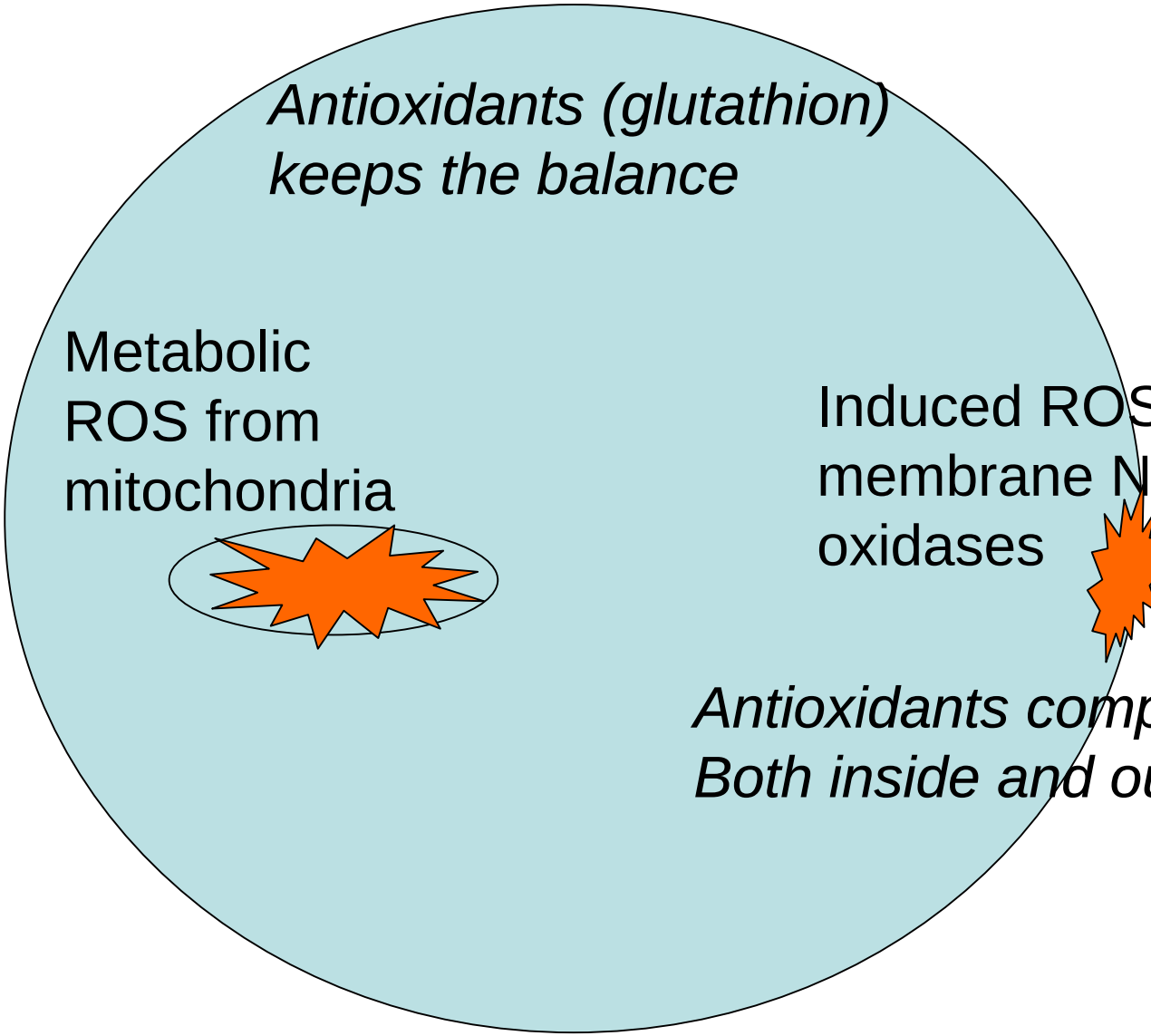


ROS are well controlled!

In vivo ROS are well controlled and balanced by anti-oxidant systems and participates in most biological processes

It is a biologic regulator in vivo!

If in excessive amounts and without balanced anti-oxidants ROS are toxic

Which often happens in vitro!

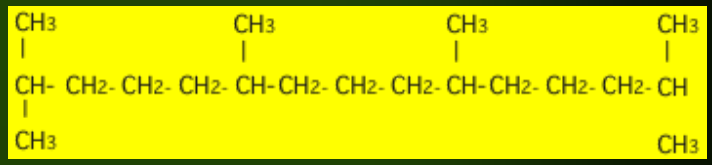
The starting question

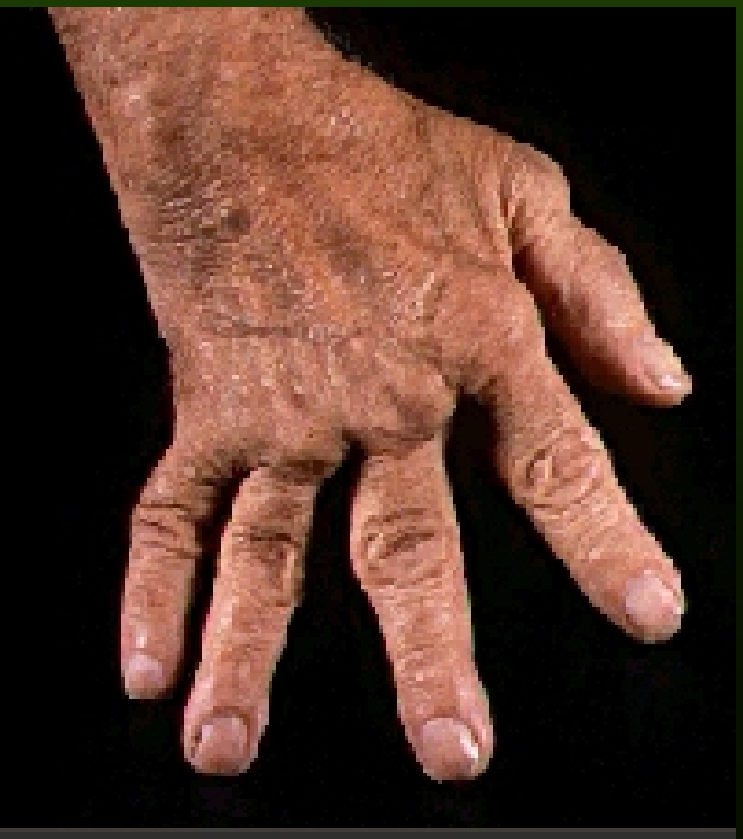
Which gene/s controls chronic inflammatory disease?

Vingsbo et al Am J Pathol 96

Pristane induced arthritis (PIA) - a model for rheumatoid arthritis (RA)

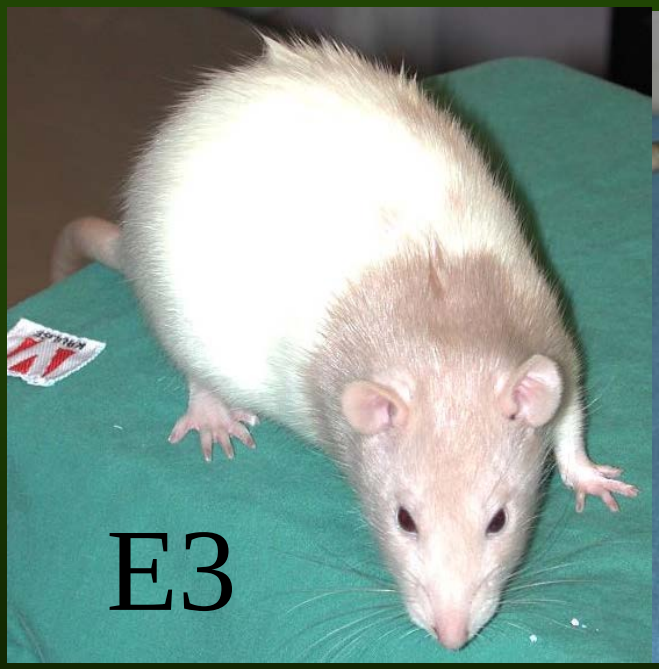
Induced with 150 µl pristane subcutaneously
2-4 weeks later: onset of severe polyarthritis



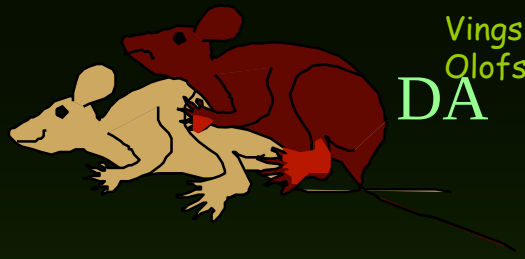


RA criteria	PIA
Morning stiffness	nd
Arthritis >3 areas, > 6 weeks	+
Arthritis hand, > 6 weeks	+
Symmetric arthritis	+
Rheumatoid noduli	nd
Serum rheumatoid factors	+
Radigraphic changes	+

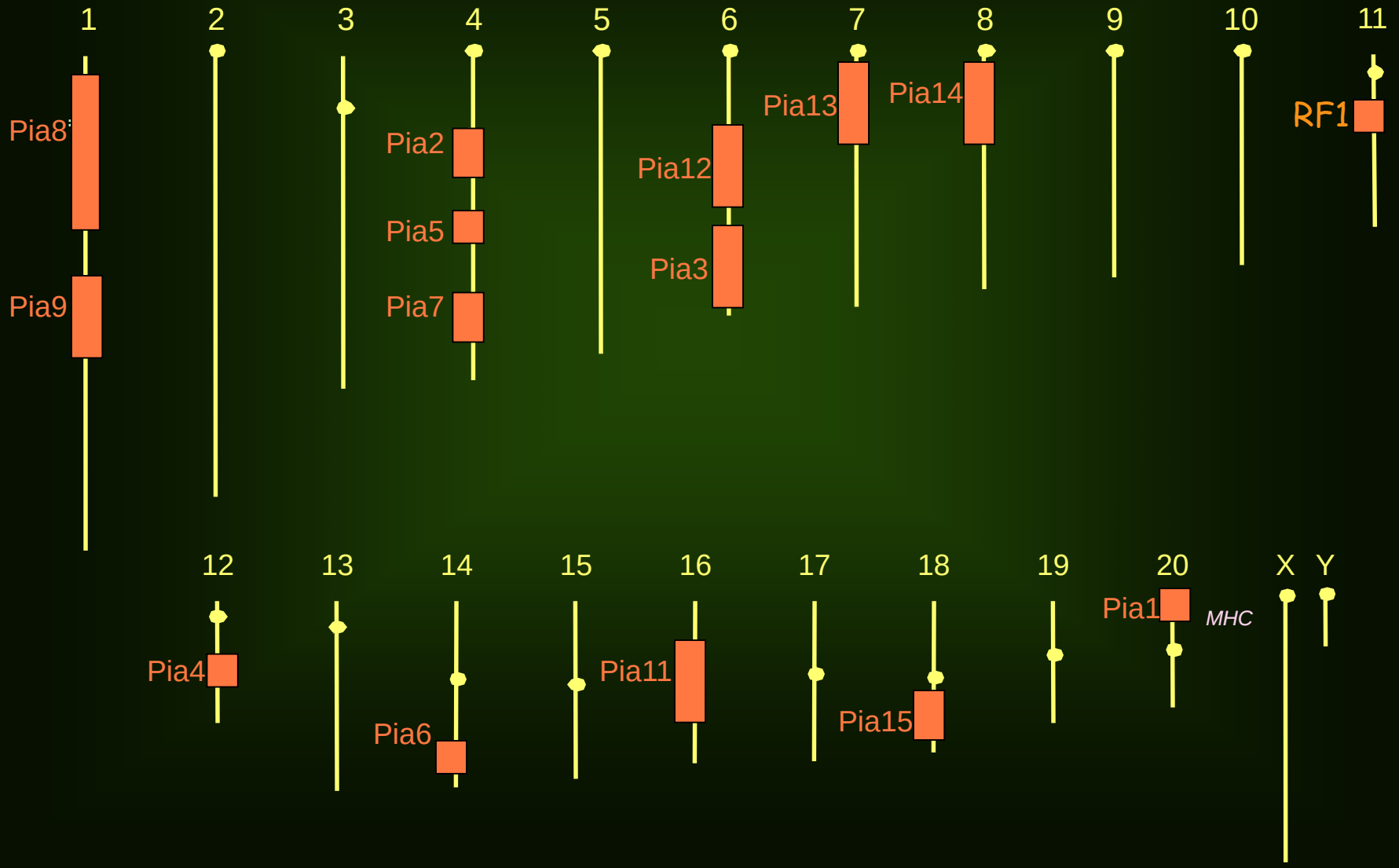
A rat cross with segregating genes



Loci associated with PIA based on >1000 rats in E3xDA crosses

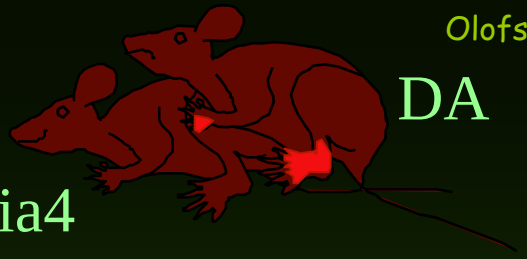


Vingsbo et al Nat Gen 98
Olofsson et al J Immunol 02



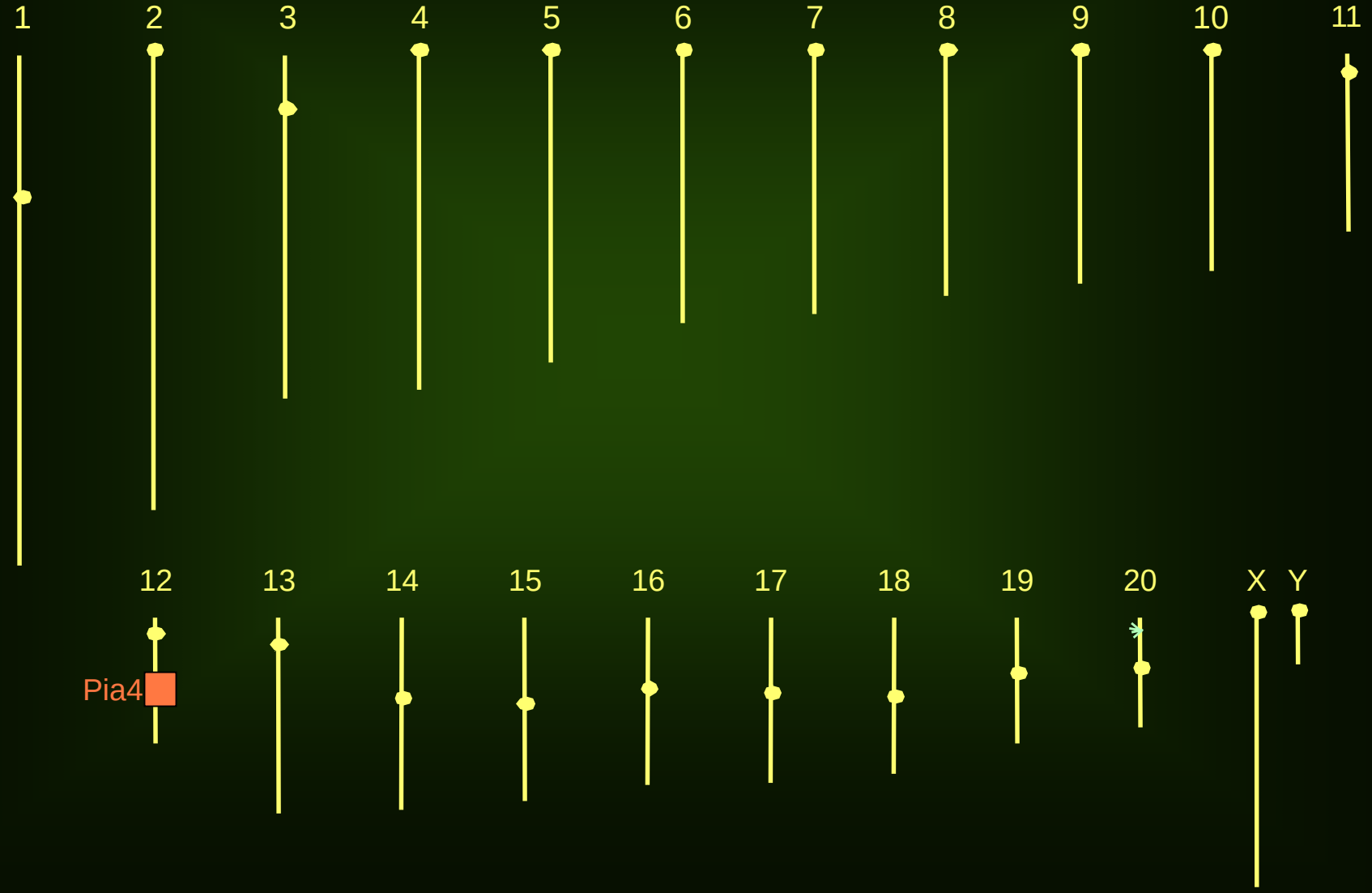
A DA congenic rat with the Pia4 derived from E3

Olofsson et al Nat Gen 03



DA

DA.Pia4

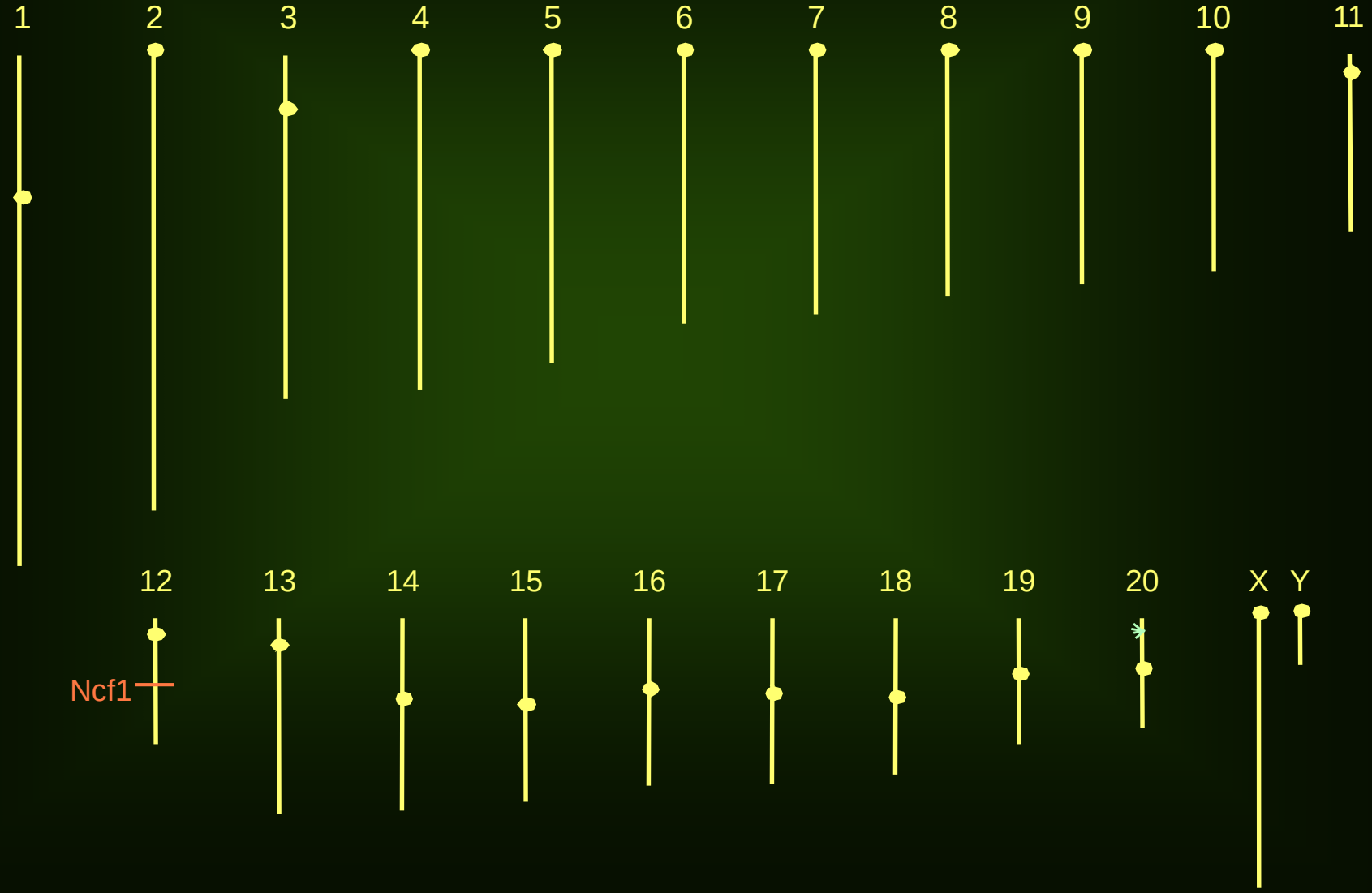


Positional cloning of Ncf1

(coding for p47phox)

Olofsson et al Nat Gen 03

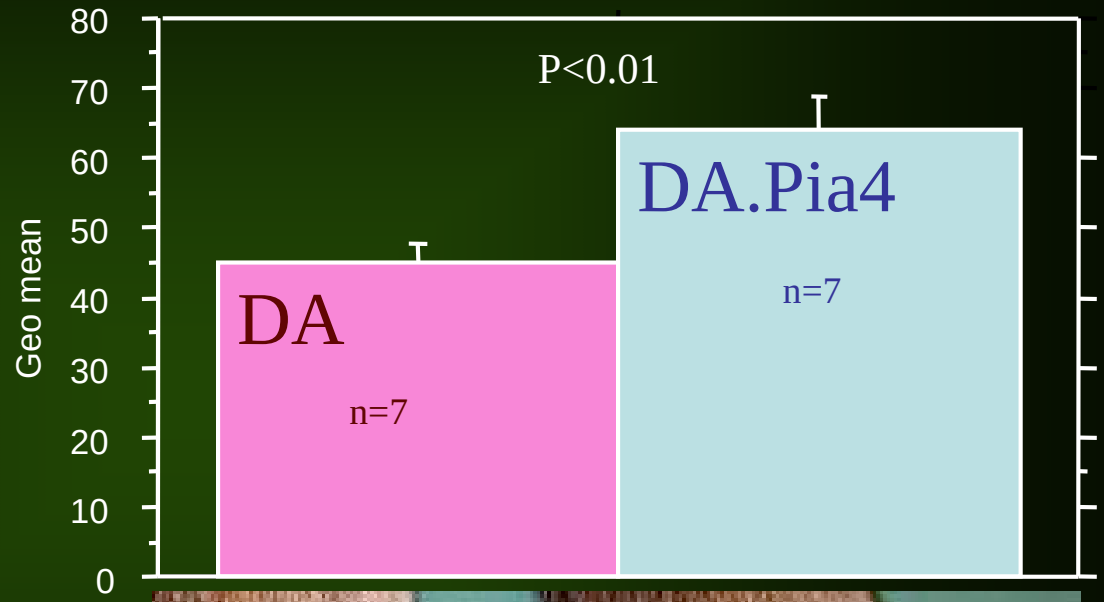
DA.Ncf1^{E3}



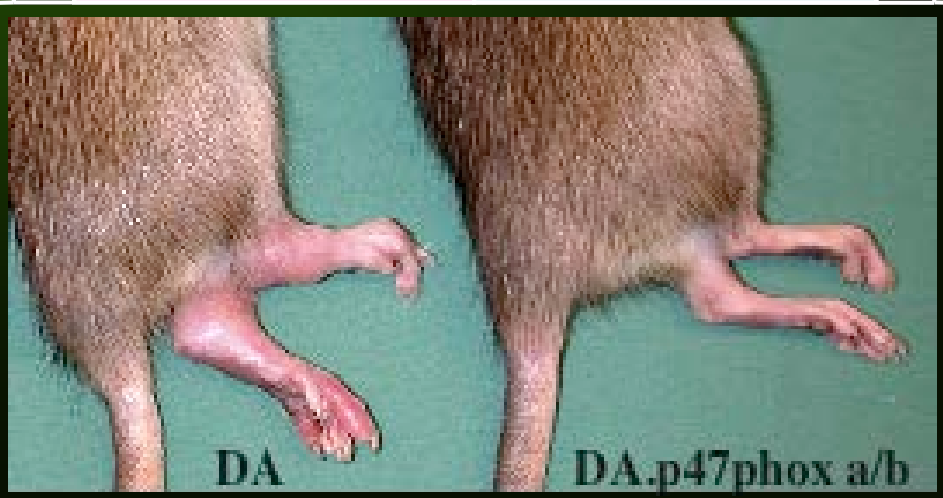
Olofsson et al Nat Gen 03

The DA.Pia4 rat has a higher degree of burst and is protected from disease, while the DA rat has a lower oxidative burst and is susceptible

Oxidative burst in blood cells from naïve rats

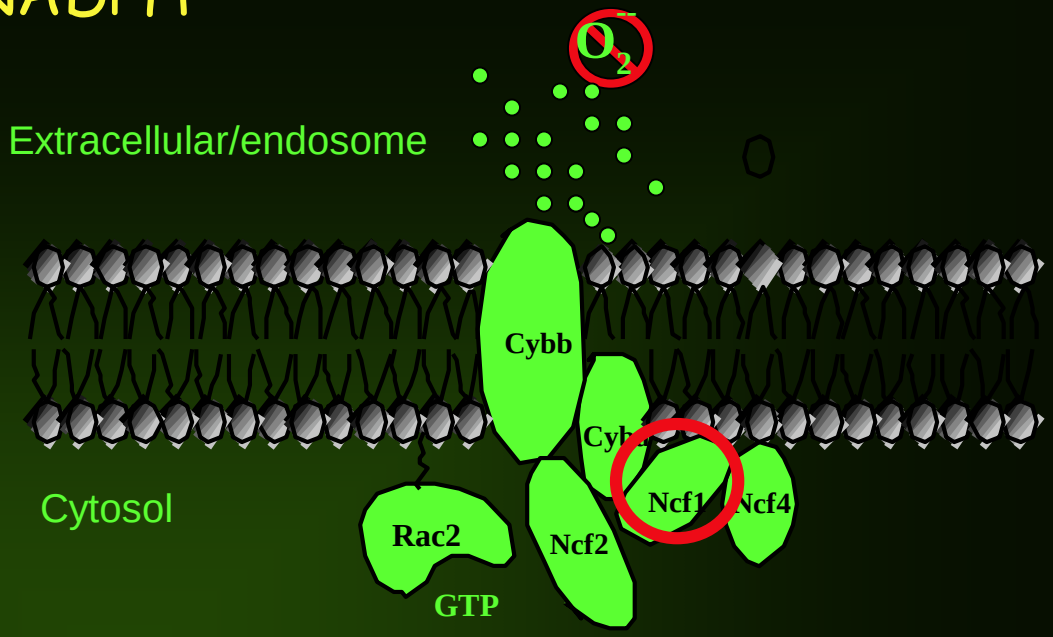


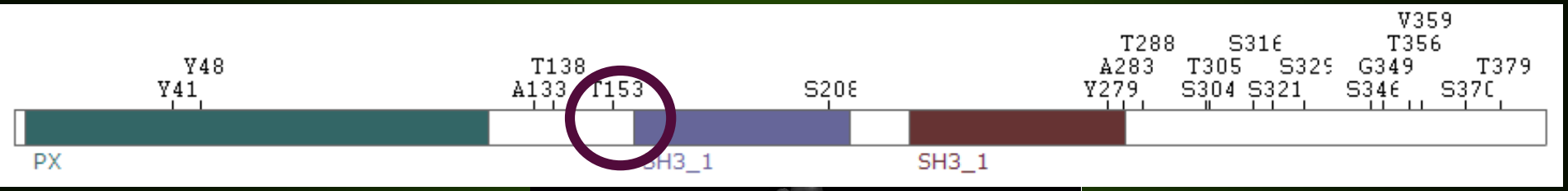
Development of arthritis after pristane injection



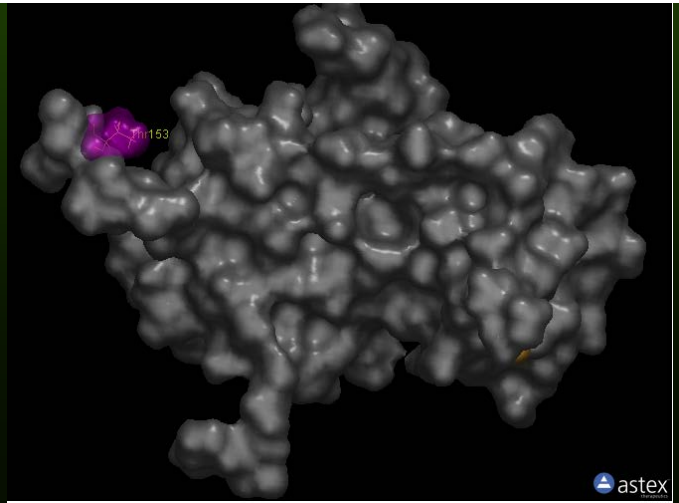
Ncf1 (p47phox) and the NADPH oxidase (NOX2) complex

Ncf1 was cloned as an arthritis gene from an E3 derived locus in DA rats. M153T is associated with arthritis and oxidative burst function, confirmed both in vitro (transfections) and in vivo (natural recombinants).



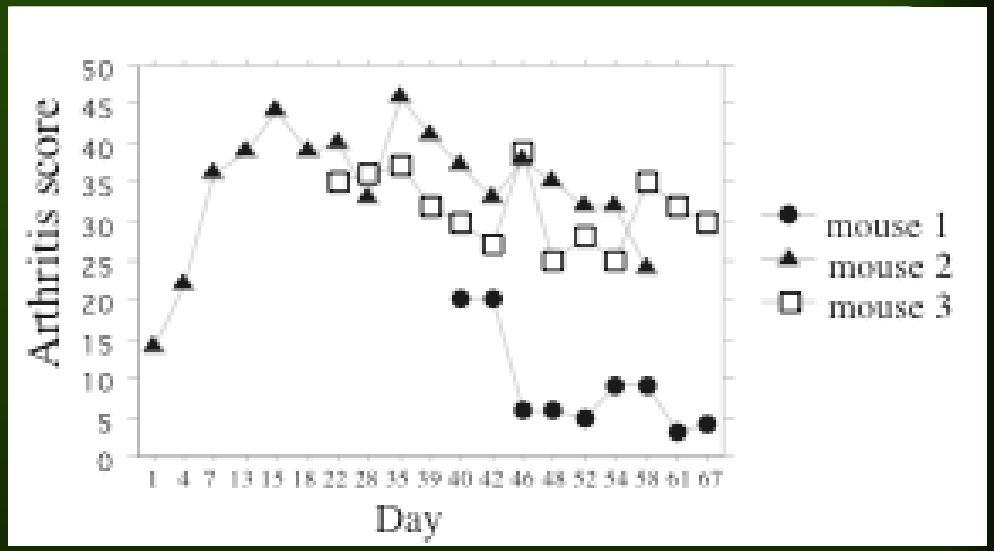


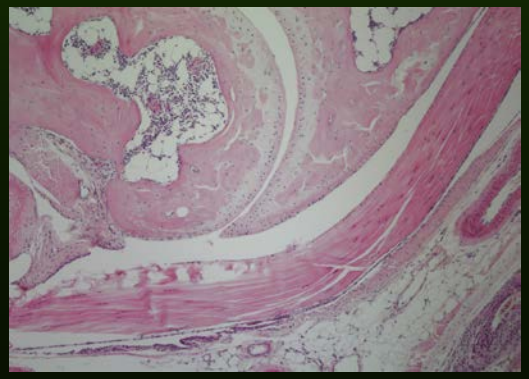
The mutation M153T is located in the hinge region between the SH domain region and the Px region



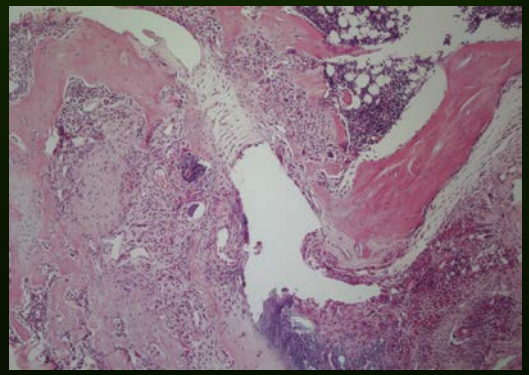
Hultqvist et al PNAS 04

Spontaneous arthritis in *Ncf1* mutated mice after partus



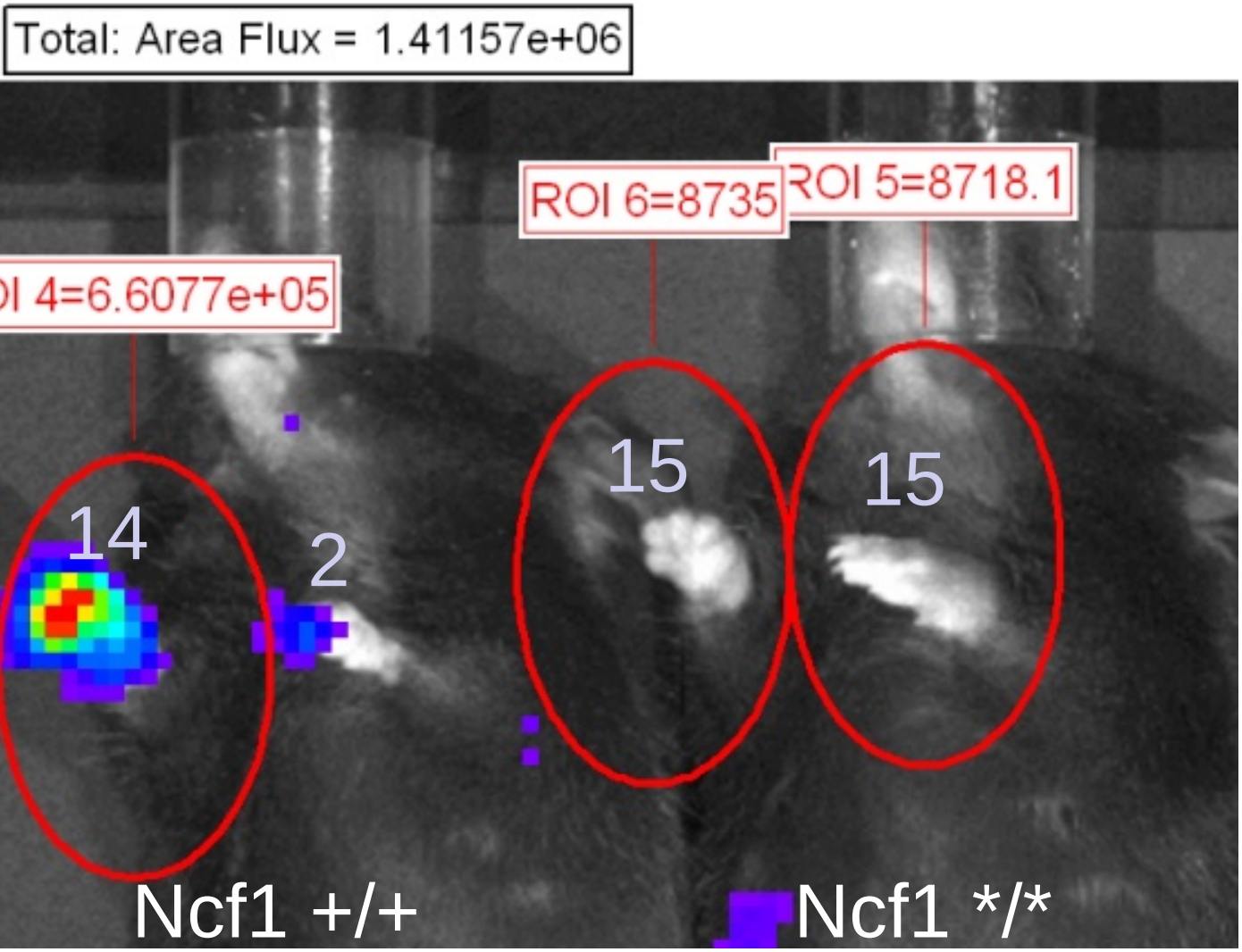


B10Q.*Ncf1**/* healthy



B10Q.*Ncf1**/* with spontaneous arthritis

Ncf1 controls oxidative burst in vivo



All paws surrounded by red circle have severe arthritis

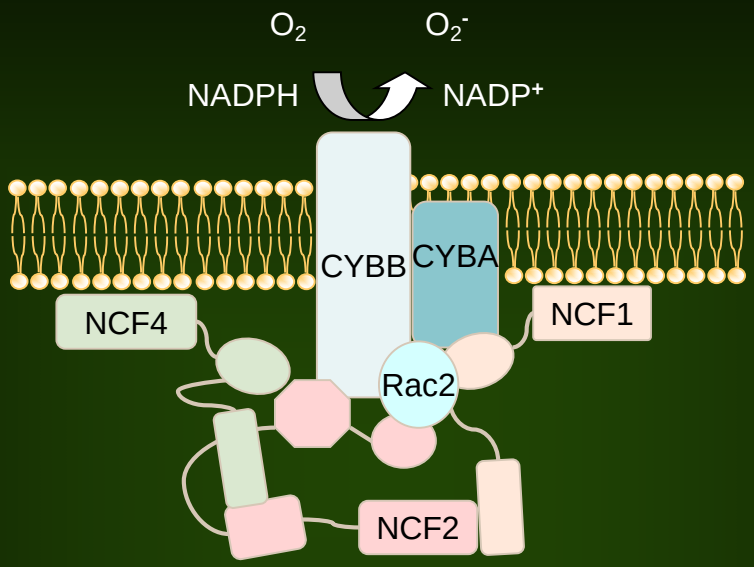
Numbers indicate scores

White boxes indicate the amount of light produced by the dye within the circle

Ncf1wt (but not Ncf1*) show ROS production in joints with arthritis

Collagen induced arthritis (CIA). L012 injected to visualize oxidative burst through luminescens

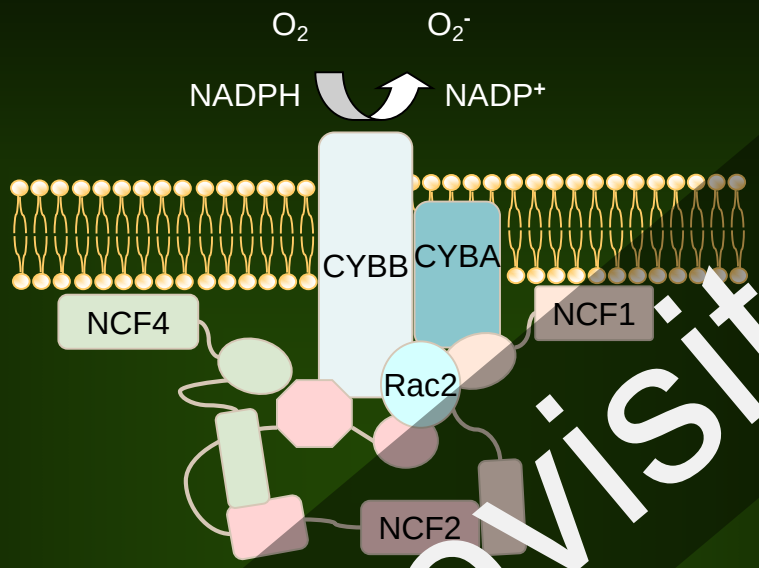
To analyse complexity we need a tool with the most minimal change affecting ROS in a controlled organism!



A single nucleotide polymorphism in the Ncf1 gene leads to blockage of electron transport over membrane induced by various inflammatory stimuli.
And is associated with complex disease. Against dogma.

…and we expect involvement in most inflammatory and biological settings!

To analyse complexity we need a tool with the most minimal change affecting ROS in a controlled organism!



Redox revisited

A single nucleotide polymorphism in the Ncf1 gene leads to blockage of electron transport over membrane induced by various inflammatory stimuli.
And is associated with complex disease. Against dogma.

...and we expect involvement in most inflammatory and biological settings!

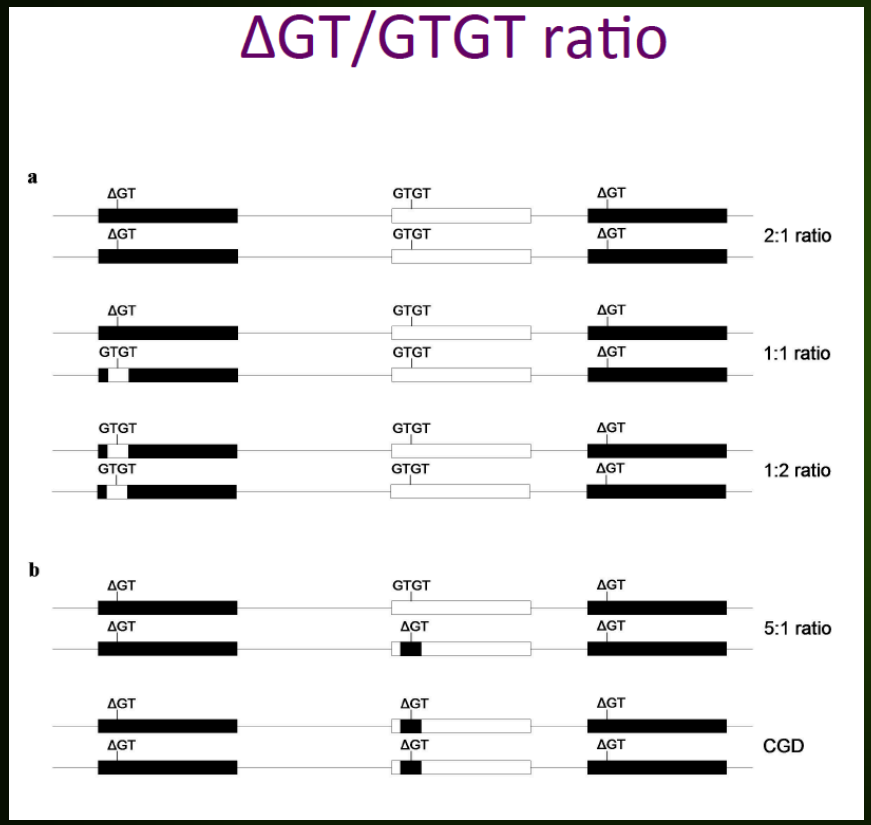
So what? Who cares about mice and rats?

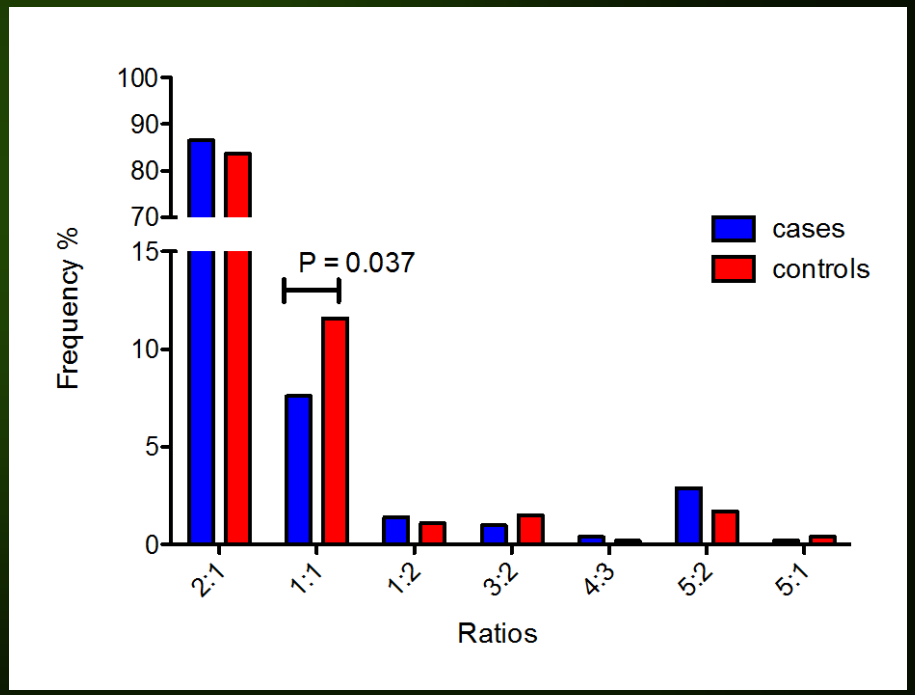
- Mutations in Ncf1 (or other NOX2 genes) cause CGD (chronic granulomatous disease) in humans

- Polymorphism in NOX2 genes are associated with autoimmune disease

NCF1 gene association with Rheumatoid Arthritis

Not included in any GWAS
Copy number variation (affected have less copies 7% vs 11 %, p=0.037)

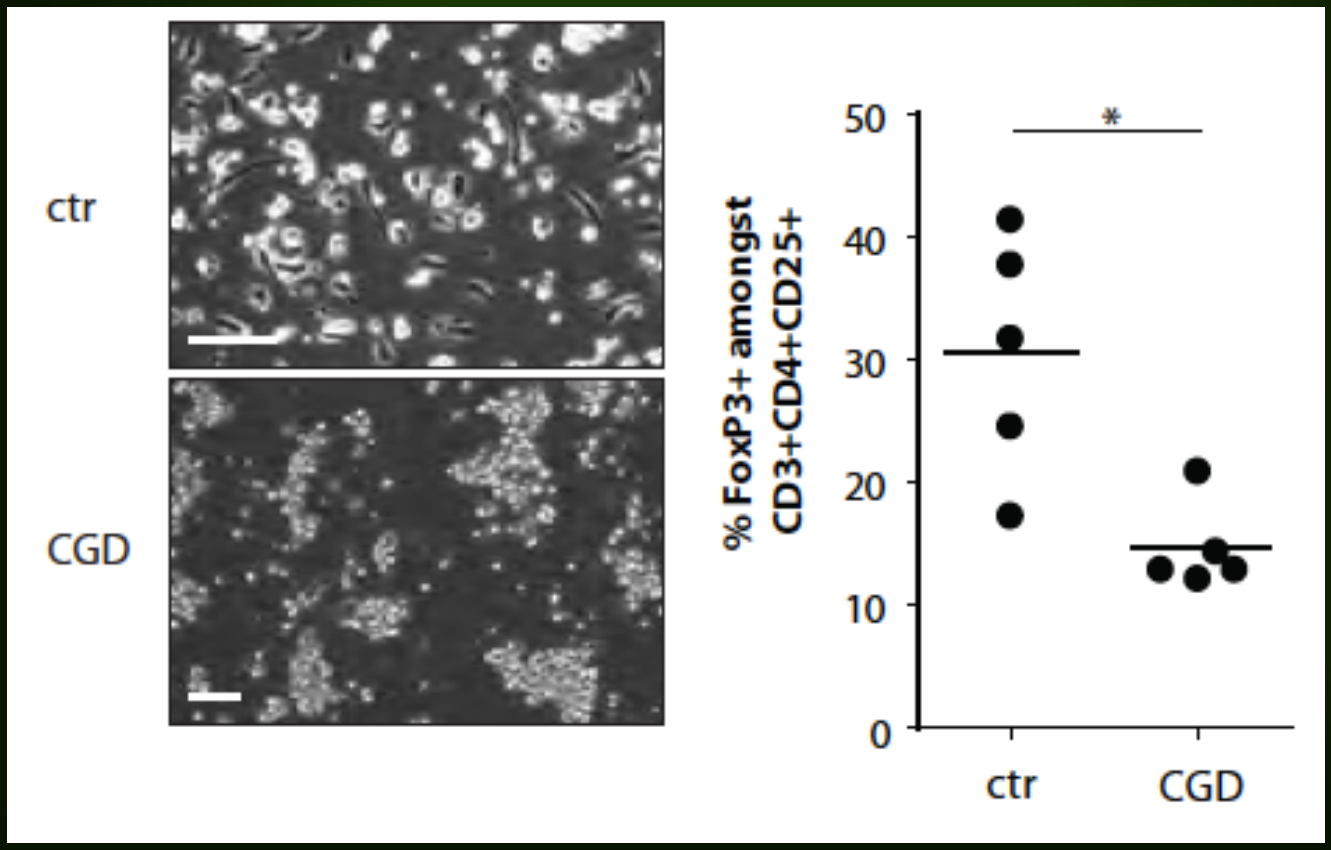




Olsson et al ARS 2011

Human chronic granulomatous diseases (CGD) show the same effect on inflammation and T cell activity

Activated T cells (decreased Treg function) due to lack of ROS in CGD
In both humans, mice and rats



Kraaij/Gelderman et al PNAS 2010

A comparative analysis of downstream effects of NOX2 activation in humans, rats and mice

1) Mice with mutation in Ncf1, before and after arthritis
2) Rats with mutation in Ncf1, before and after arthritis
3) Humans with mutation in a NOX2 component (CGD)

mRNA expression of cells from blood and spleen.

Which are the common downstream pathways?

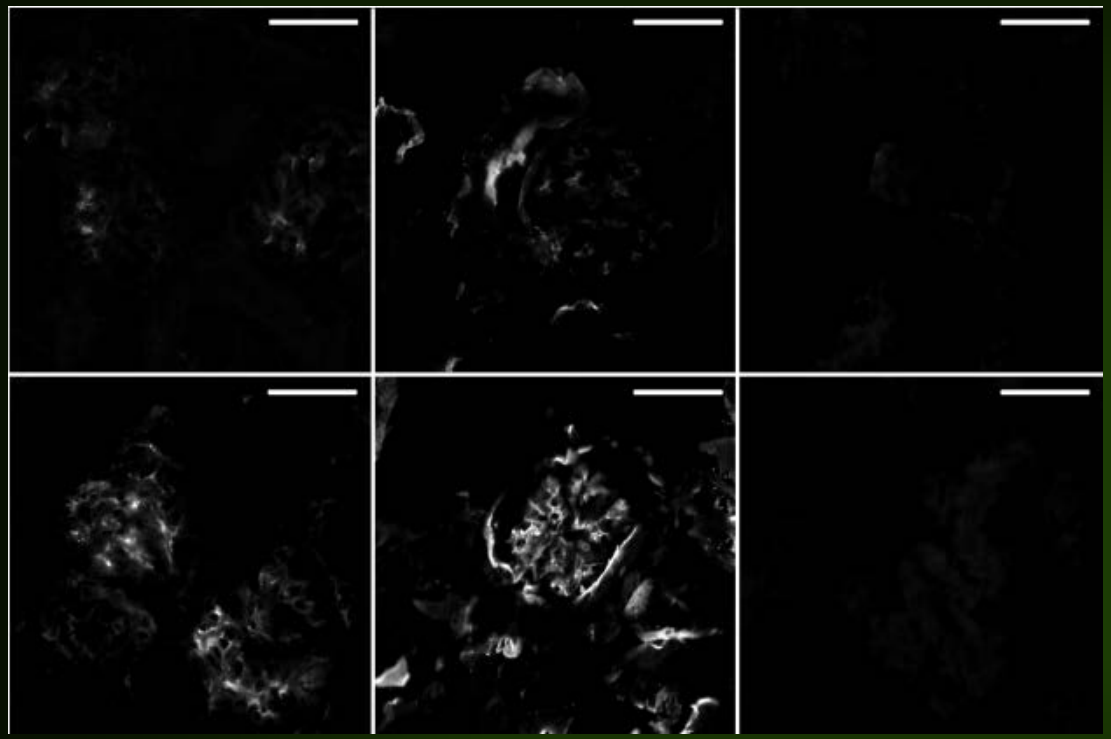
Summary, NOX2 downstream pathways

	Naive	Priming	Disease
Mice	STAT1	BCR/TCR STAT1	BCR/TCR STAT1
Rats	STAT1	TCR STAT1	TCR STAT1
Humans	STAT1 BCR		

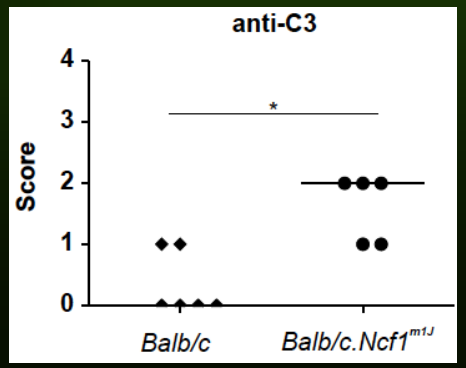
Kelkka et al ARS 2014

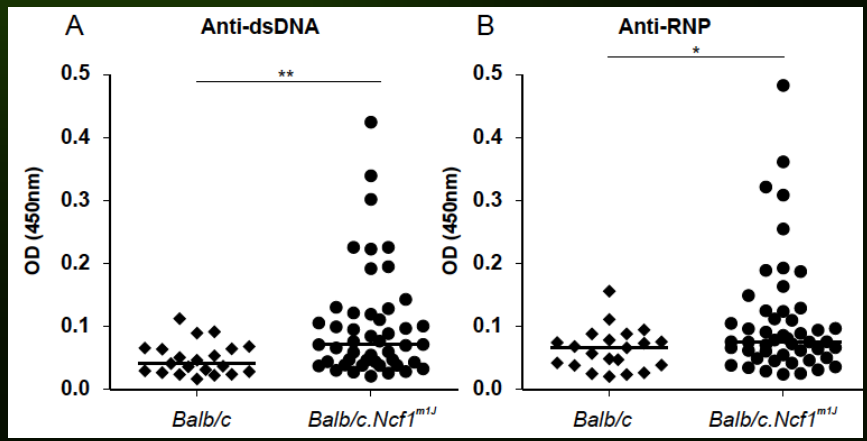
Spontaneous development of lupus in Balb/c Ncf1* mice - effect of the STAT1 pathway

Glomerulonephritis



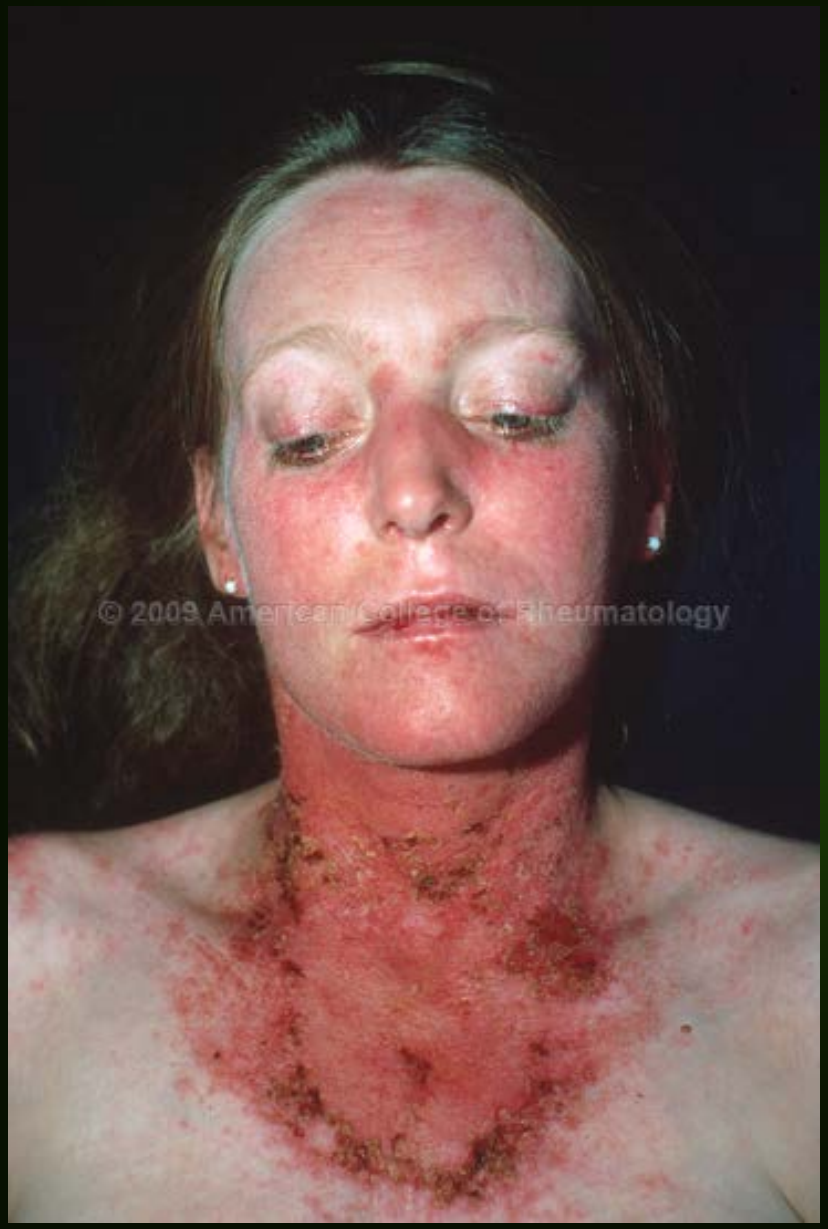
Serum autoantibodies

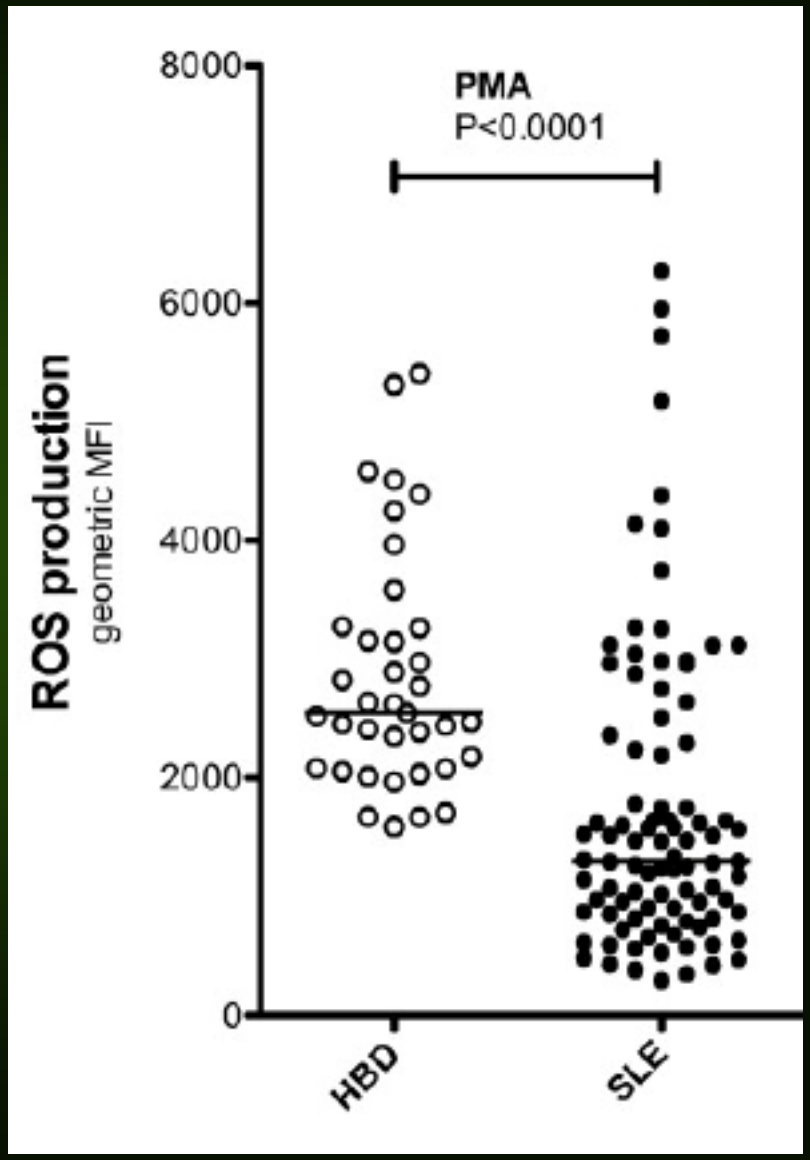




Kelkka et al ARS 2014

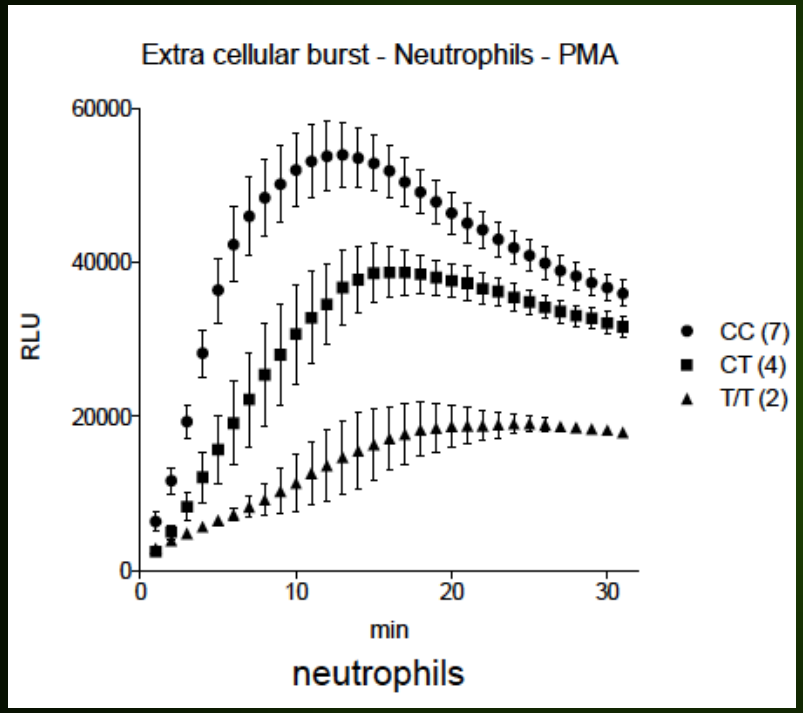
SLE patients blood cells produce less ROS than controls



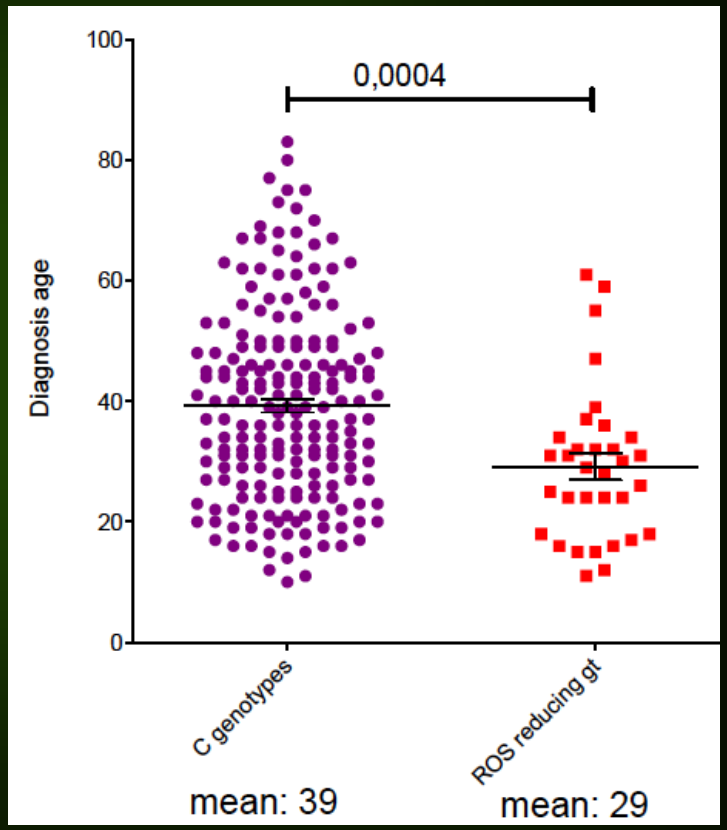


Bengtsson et al ART 2014

Carriers of an Ncf1 allele giving low ROS develops more severe and earlier (10 years) SLE



A Ncf1 SNP associated with low ROS



Olsson et al unpublished

CGD can get severe infections

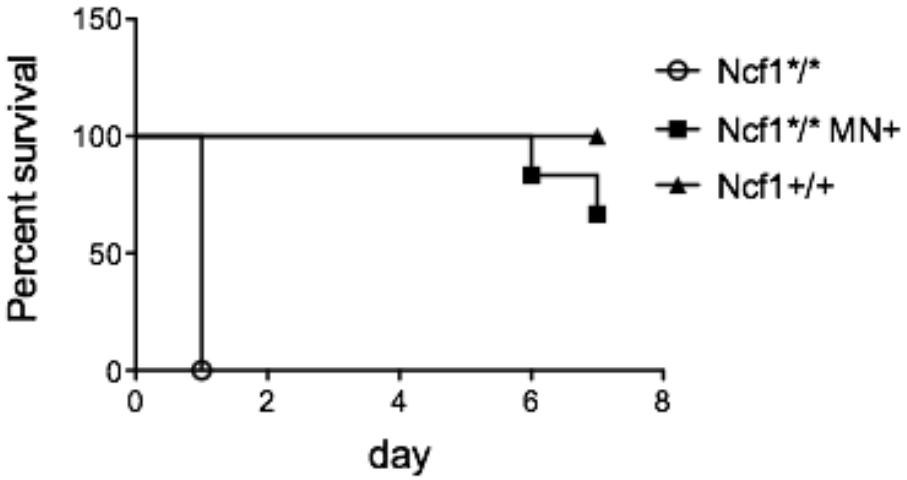
Is the observed chronic inflammation secondary to infections?

The Ncf1 mutated mice develop infections typical of CGD

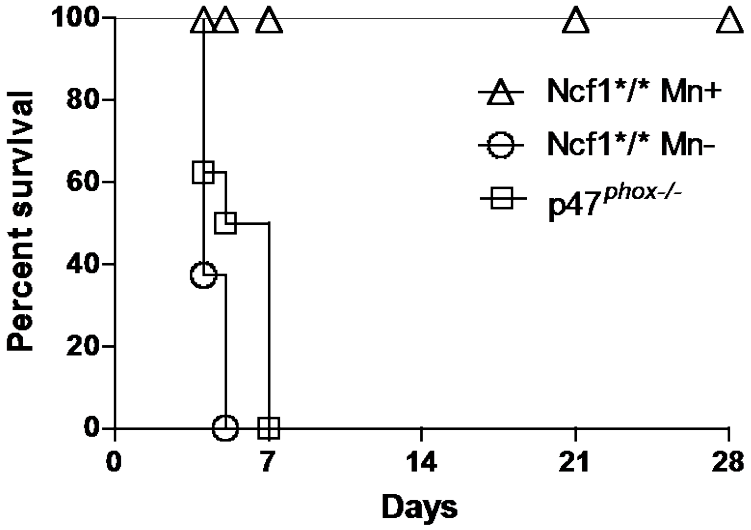
Interestingly, the protective effect is dependent of macrophages (not PMN)

ROS from macrophages protects against CGD associated infections

S.saprophyticus 0.8x10^8/mouse



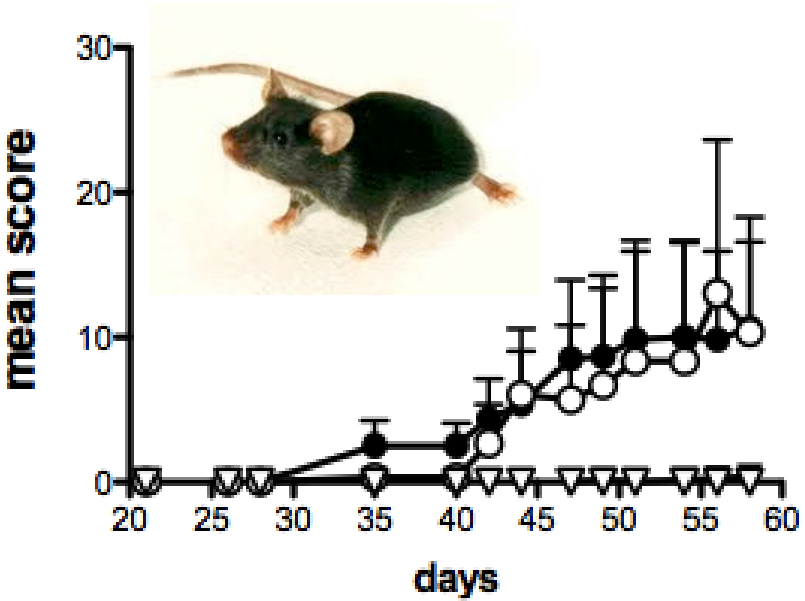
4x 10^5 CFU /mouse Burkholderia cepacia



Pizolla et al JI 2012

Is autoimmunity in CGD secondary to infections?

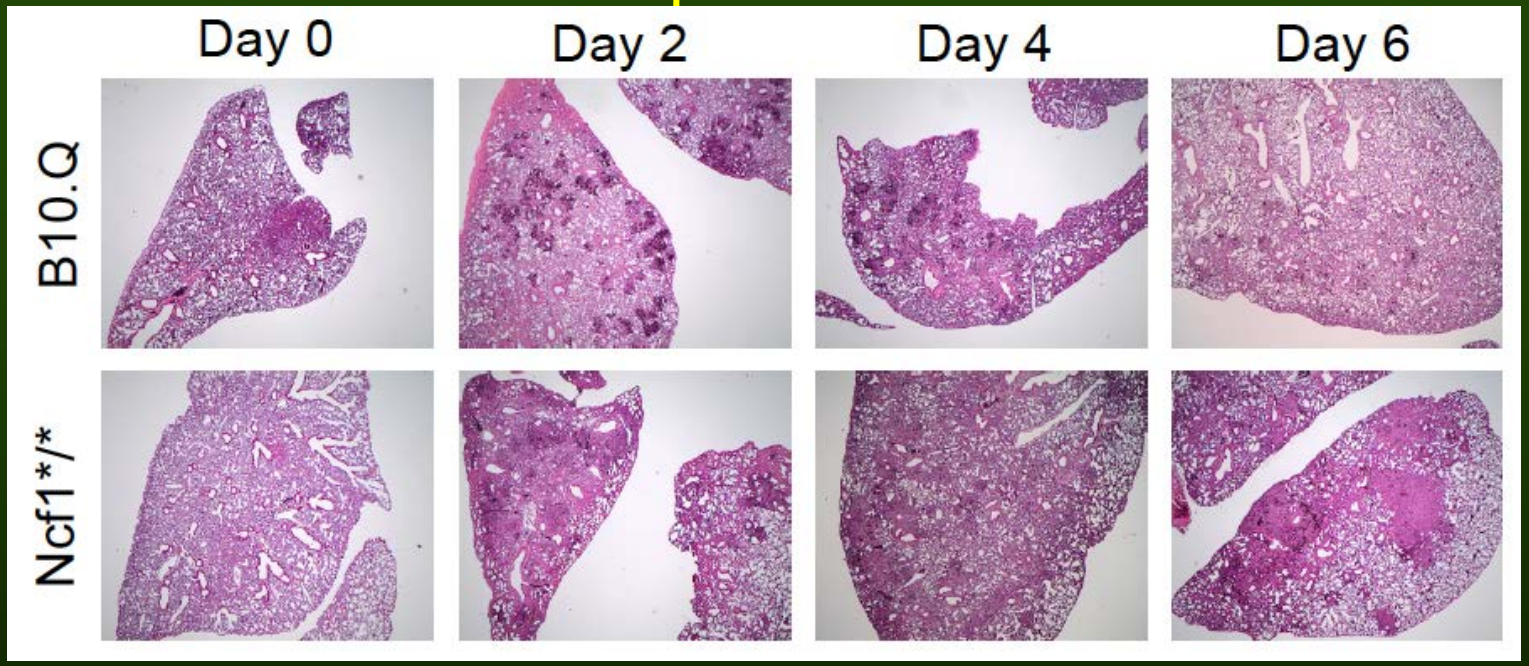
Germ free Ncf1 mutant mice are also susceptible to arthritis…

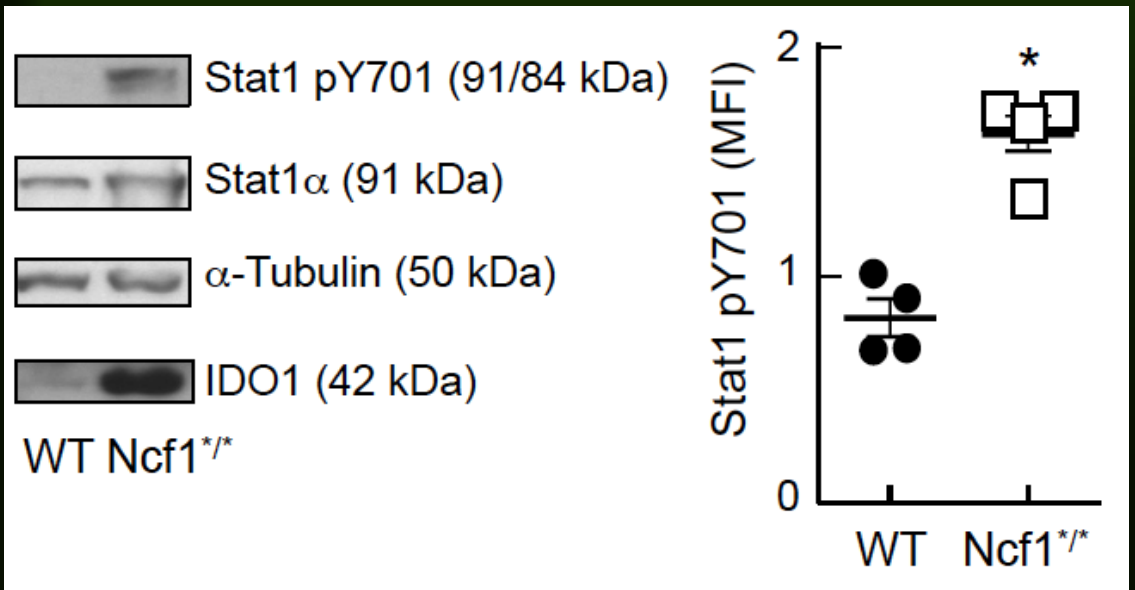


…and with the same pathways (STAT1, BCR/TCR) as conventional mice and humans

Wing et al EJI 2014

Nasal application of sterile aspergillosis antigen induces more severe pneumonitis in ncf1* mice





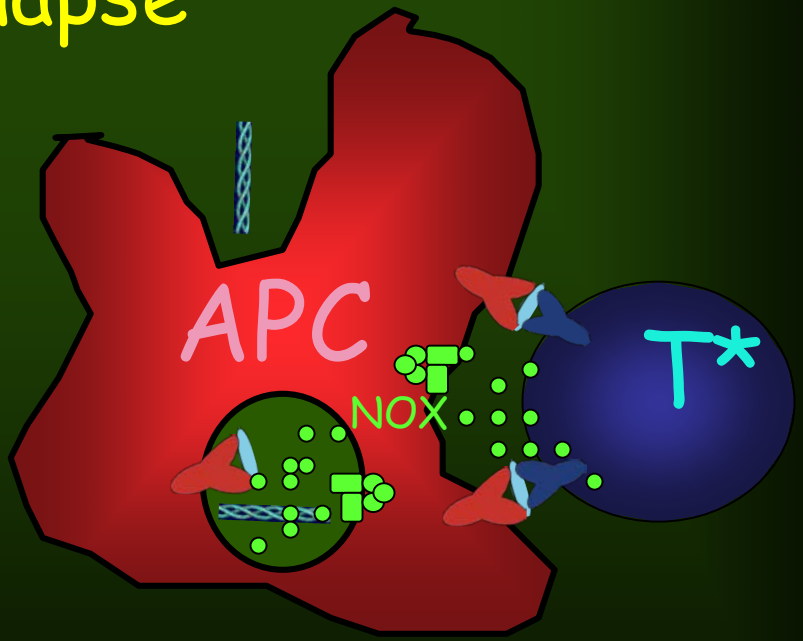
…and induction of STAT1 posphorylation

Maghzal et al Nature 2014

T cells:

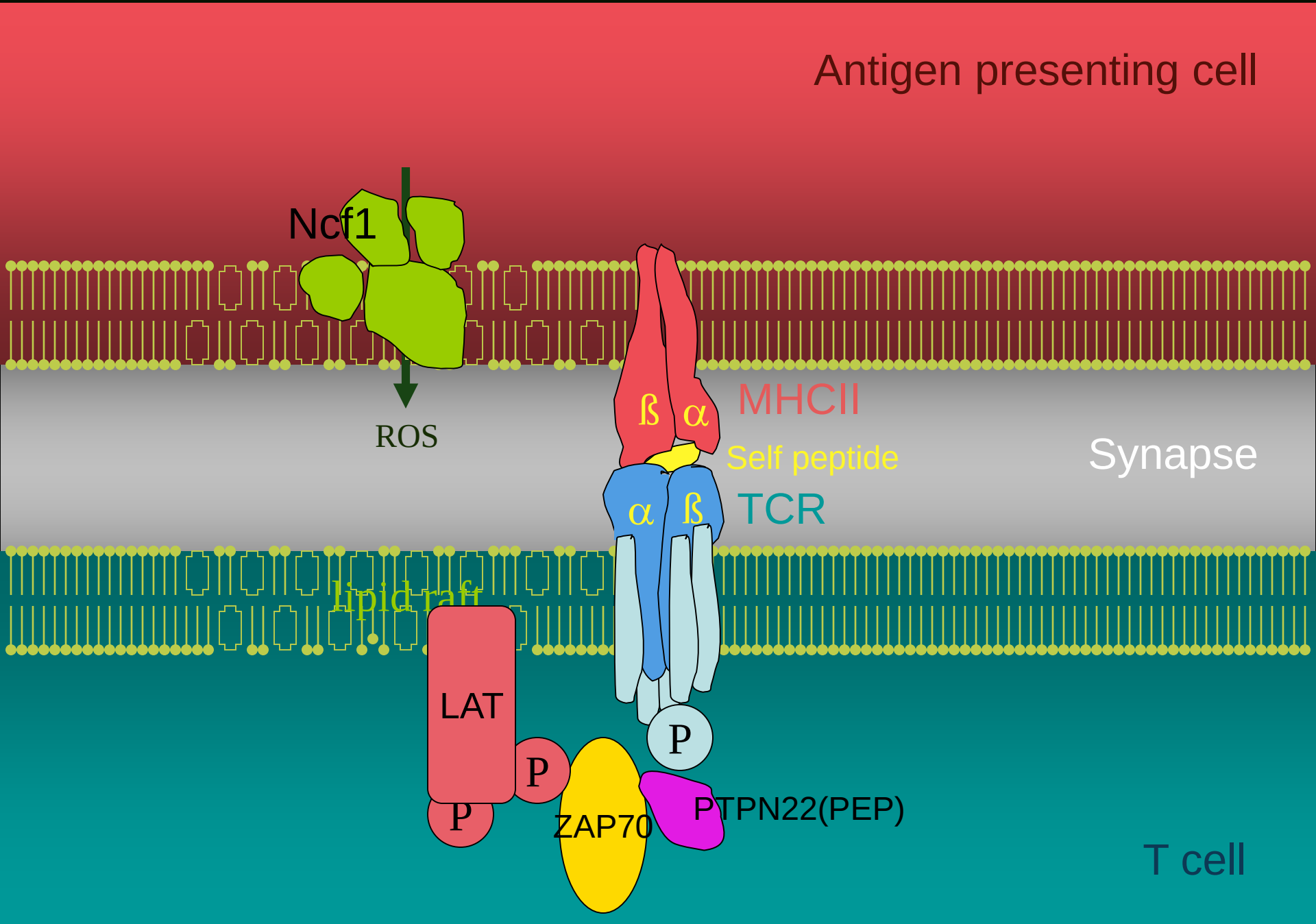
Ncf1 polymorphism controls ROS as a transmittor in the immunological synapse

Antigen presenting cells (APC) burst on T cells in the synapse during during antigen presentation

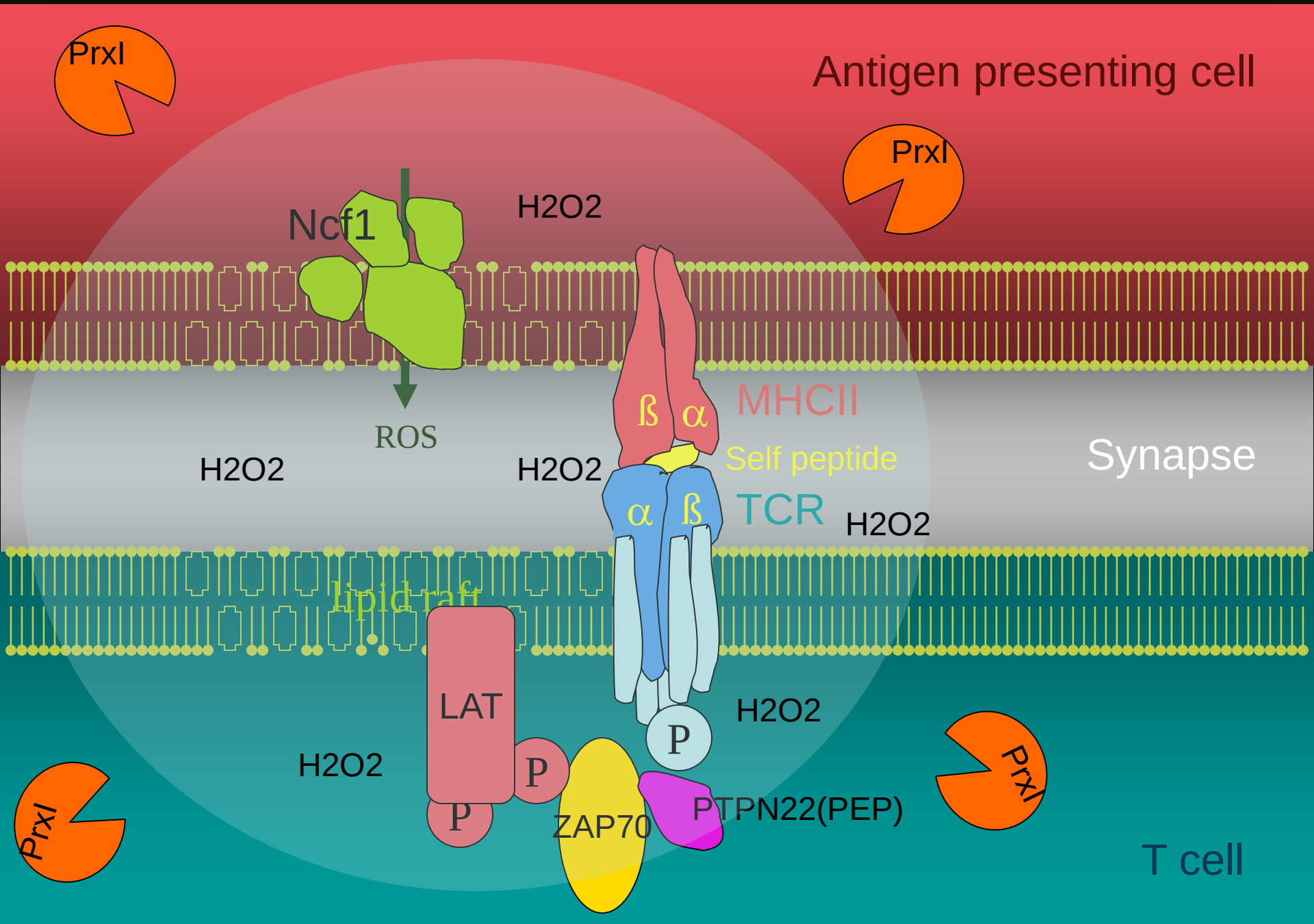


Holmdahl et al IR 2012

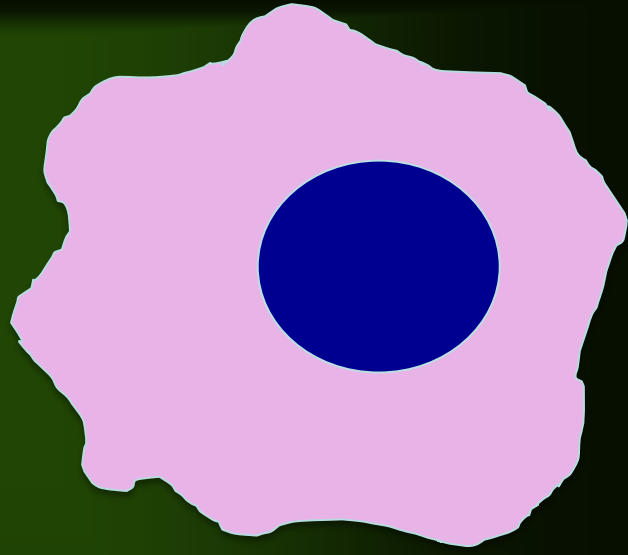
T cell membrane proteins, LAT and PTP are possible ROS targets



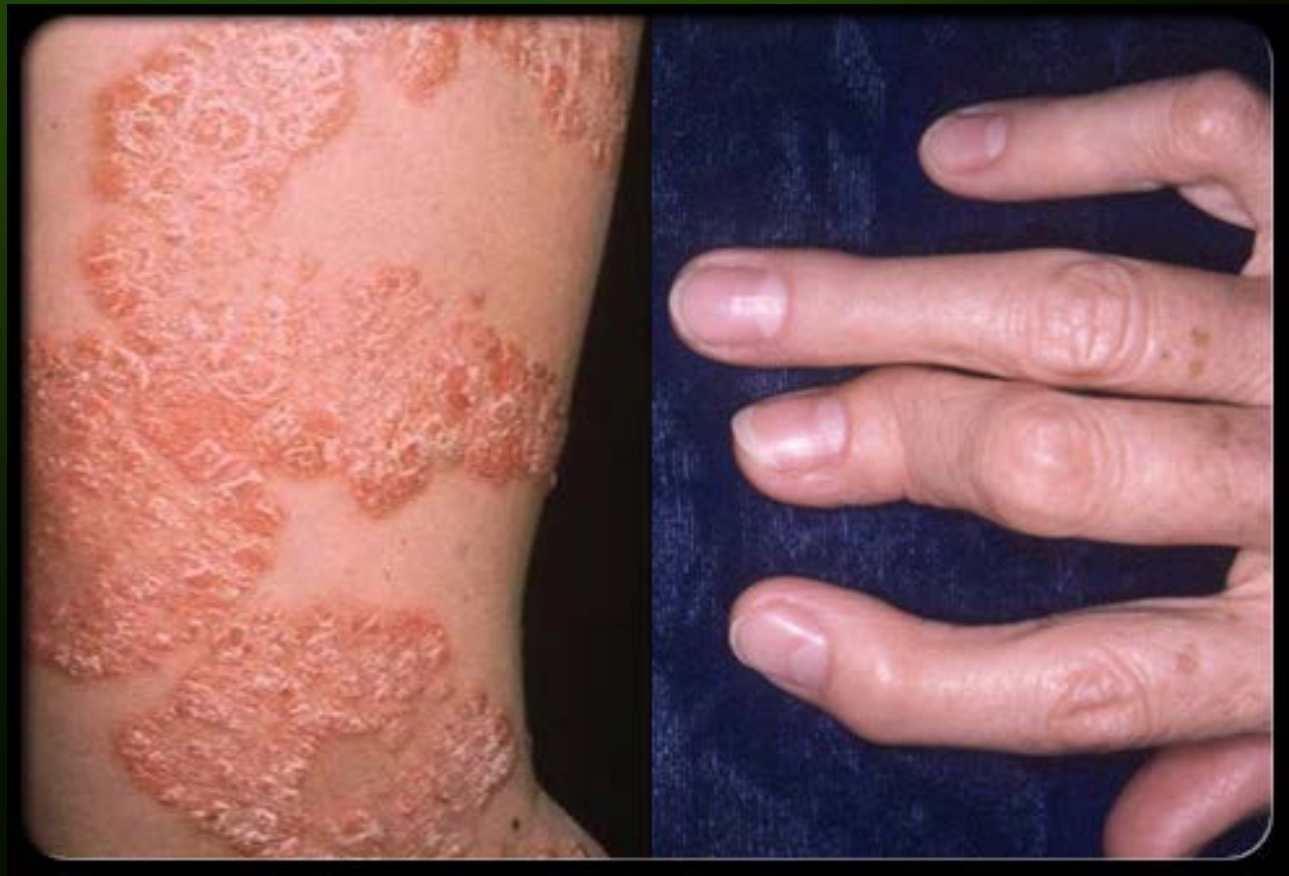
ROS as transmitters. Antioxidants compartmentalize the ROS



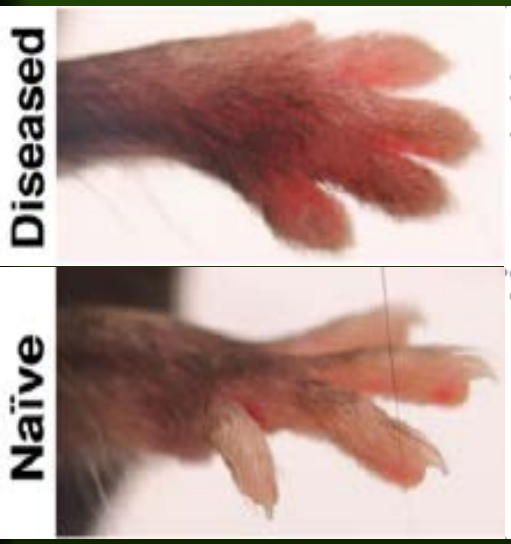
Macrophages:
ROS is an autocrine modifier of inflammatory macrophages

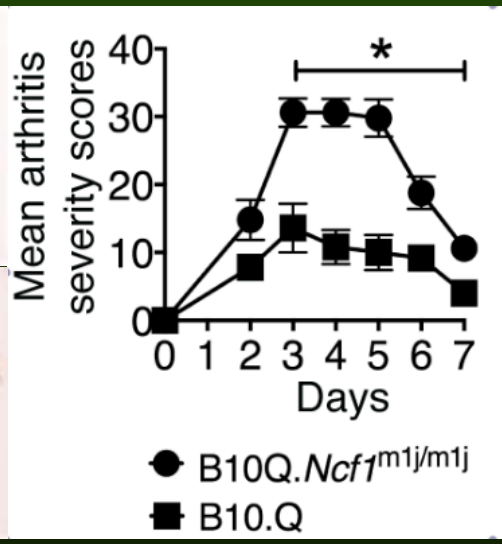


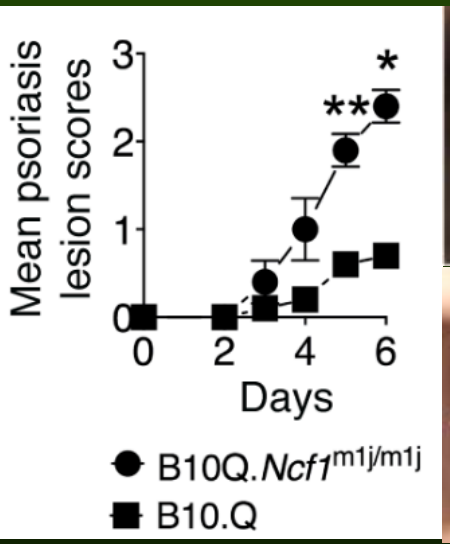
Example psoriasis and psoriasis arthritis



Mannan-induced psoriasis and psoriasis-arthritis (MIP) are enhanced in Ncf1* mice

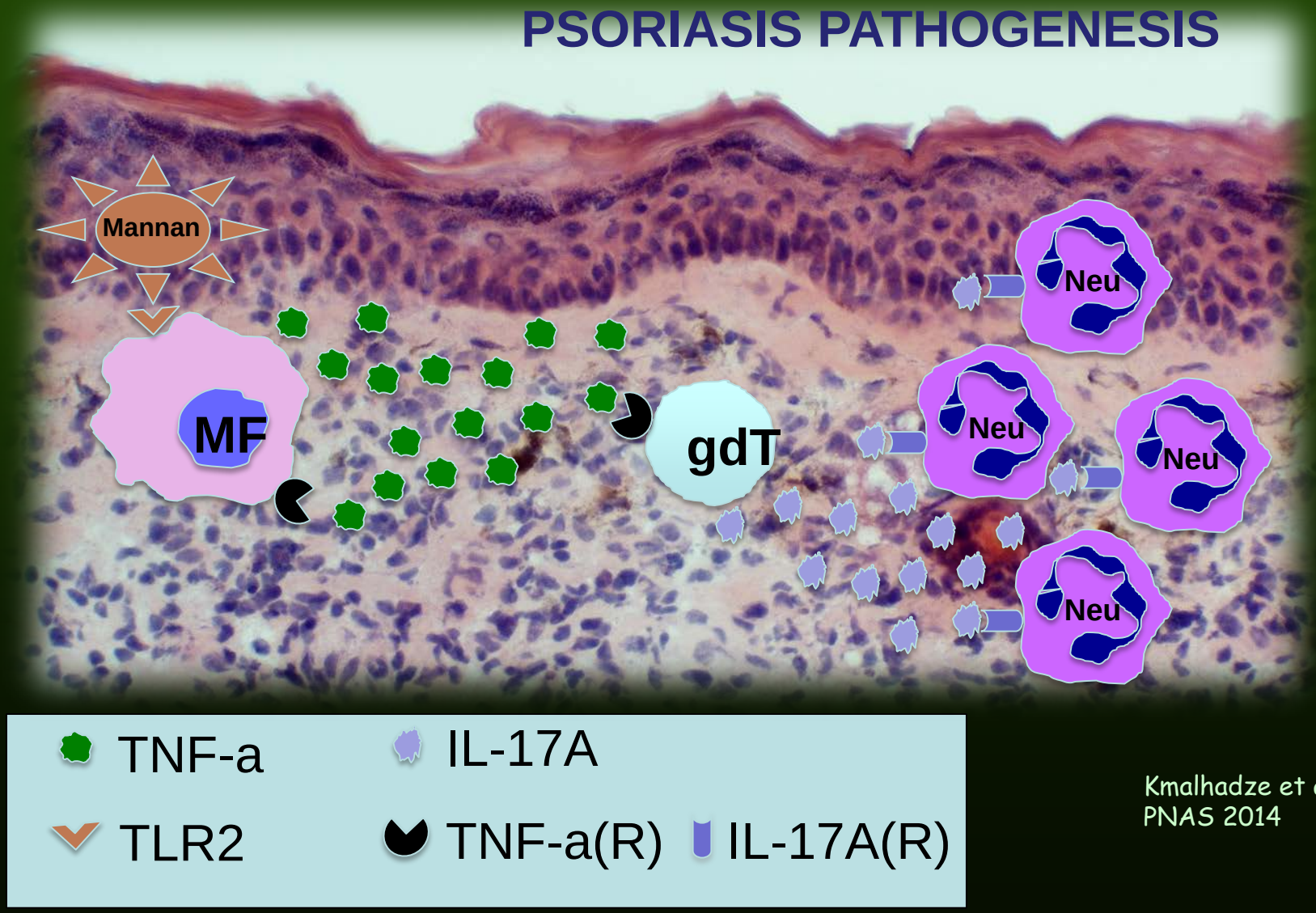






Khmaladze et al
PNAS 2014

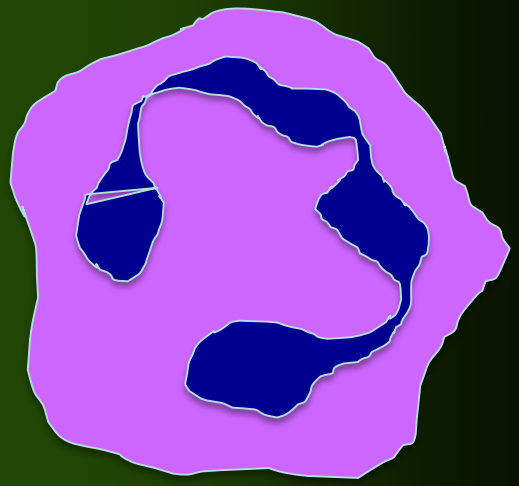
MIP is caused by mannan activation of ROS deficient MF, secreting TNF, activating gdT to secrete IL17 leading to recruitment of PMN



Kmalhadze et al
PNAS 2014

Neutrophilic granulocytes:

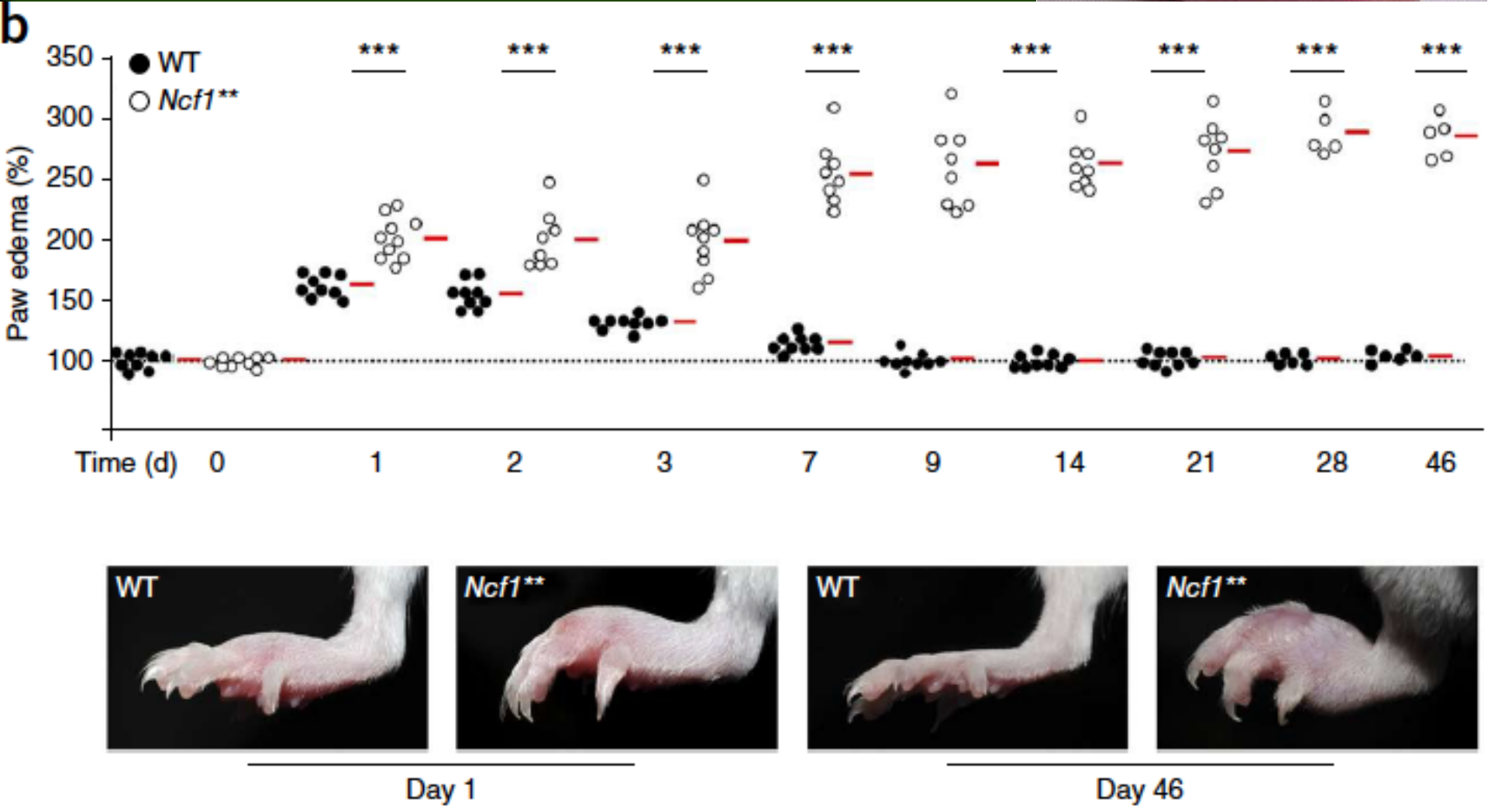
ROS as an inducer of NETS protects against inflammation by cytokine absorption



Example with gout

Gout

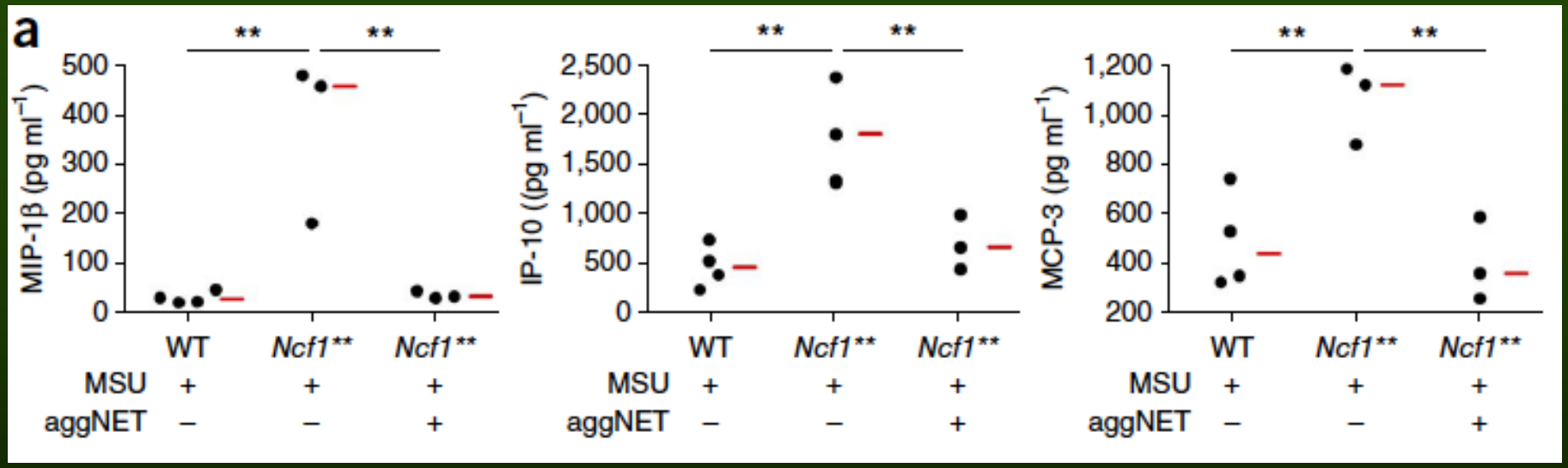
Induced gout is more severe in Ncf1* mice due to lack of NETs absorbing inflammatory cytokines



Sc injection of MSU crystals in paws

Schauer et al Nat Med 2014

Ncf1* mice do not develop NETs in gout lesion

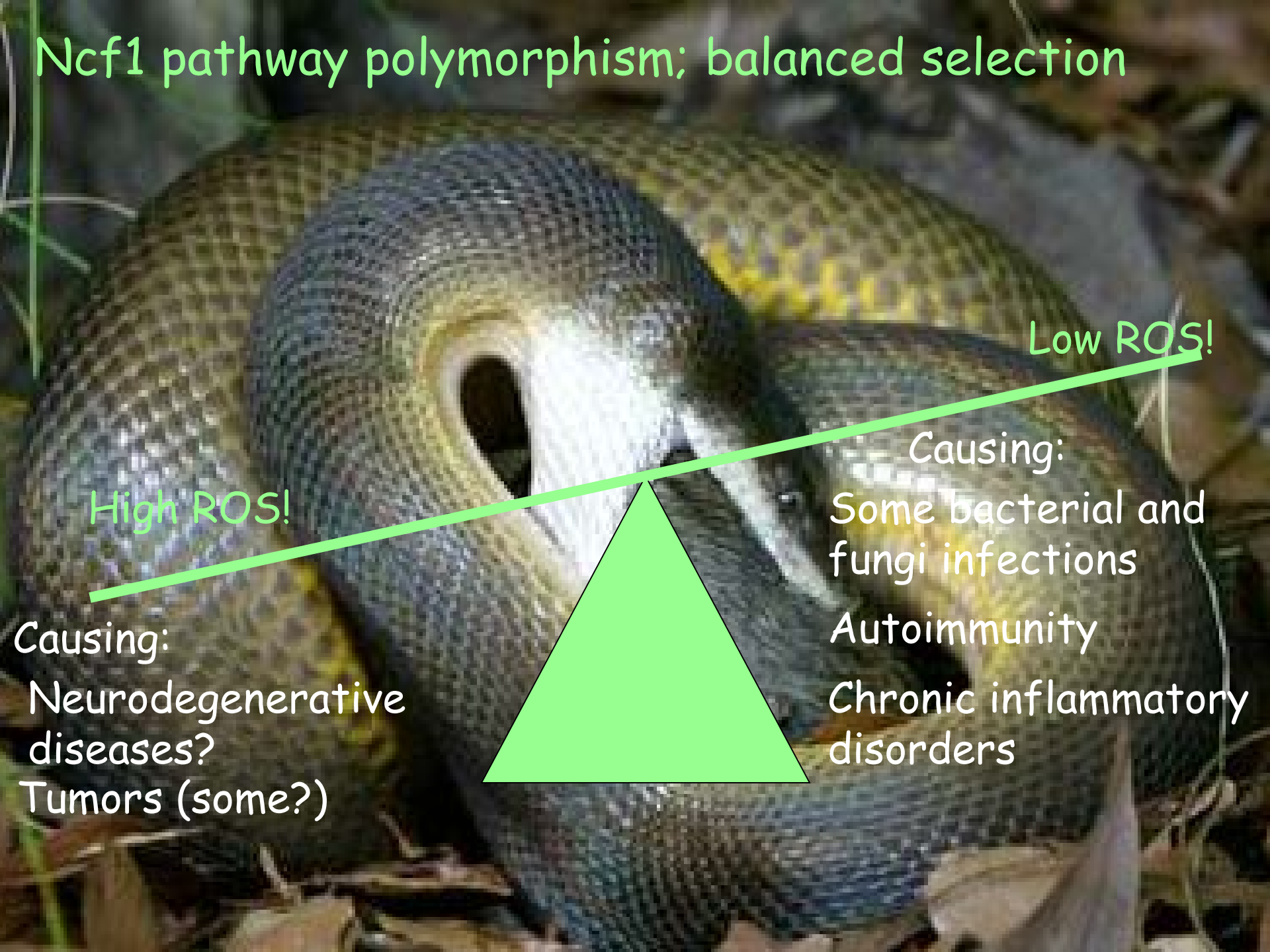


… and maintain higher inflammatory cytokine levels

Sc injection of MSU crystals in paws

Schauer et al Nat Med 2014

Ncf1 pathway polymorphism; balanced selection



Natural polymorphism in wild rats

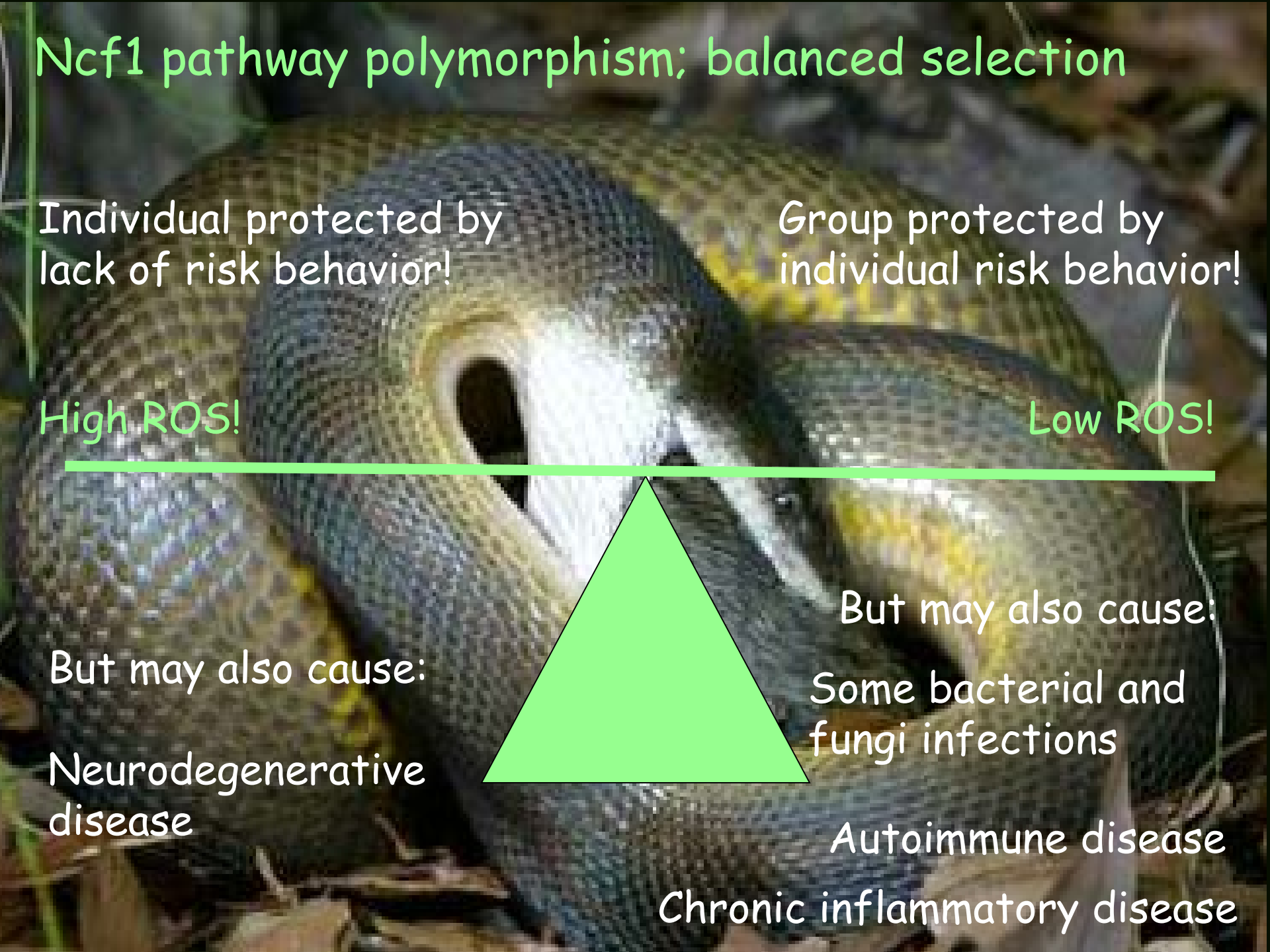
Wild rat	SNP-330 M/V 106	SNP-472 M/T 153	SNP-1166
1	E3	E3	DA/E3
2	E3	DA/E3	DA/E3
3	DA	DA	DA
4	DA	DA	DA
5	DA/E3	DA/E3	DA
6	DA/E3	DA	DA
7	DA/E3	DA	DA
8	E3	DA	DA
9	DA	DA	DA
10	E3	DA	E3
11	E3	DA	-
12	DA/E3	DA/E3	DA
13	E3	DA	DA
14	E3	DA	DA
15	E3	DA	DA/E3
16	E3	DA	DA/E3
17	E3	DA	DA
18	DA/E3	DA	DA/E3
19	DA	DA/E3	DA
20	DA	E3	DA
21	DA	E3	DA
22	E3	DA	E3

$Ncf1^{DA}$ (low ROS) allows risk behavior

DA strain	Exploratory Activity (sec)	Hypothalamus ROS (fold mRNA expression)	Adrenal gland ROS (fold mRNA expression)
$Ncf1^{E3}$	2.0 sec	5,5	3,0
$Ncf1^{DA}$	7.5 sec***	1,0***	1,0***

Schiavone et al Transl Psych 12

Ncf1 pathway polymorphism; balanced selection



So, what about antioxidants?

Melatonin is an anti-oxidant

Melatonin

5-hydroxy-N-Acetyl-tryptamine

↓

gonadal effects/sexual maturation,
analgesic, anti-oxidant,

↓

Immune activation

Arthritis enhancement

Melatonin regulate arthritis

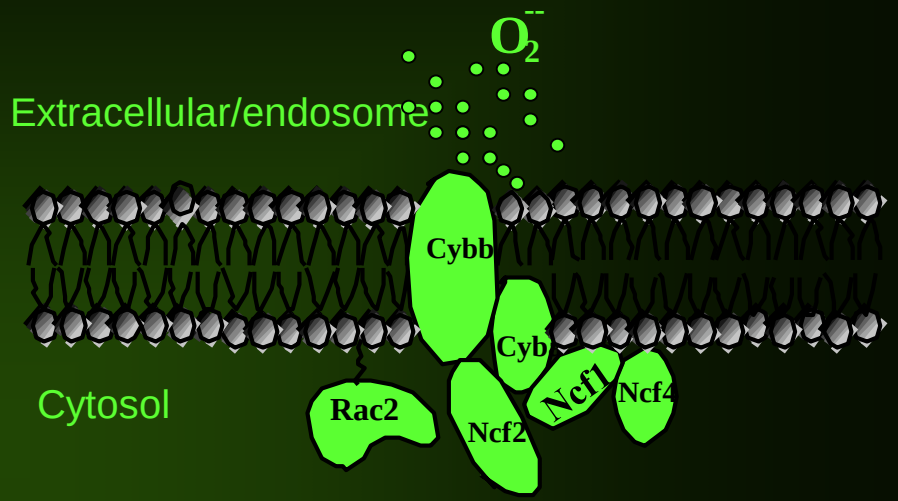
+ Darkness
+ Melatonin injections
- Light
- Epiphyseal ectomy

	Arthritis incidence	Arthritis severity	Anti-CII (μg/ml)
Darkness	95%*	8.8**	166±113**
Light	60%	4.5	91±27
Melatonin	85%*	5.5**	502
Control	38%	2.5	389

Conclusions

It is possible to find genes and pathways that Nature has selected (not scientists) through an hypothesis free approach

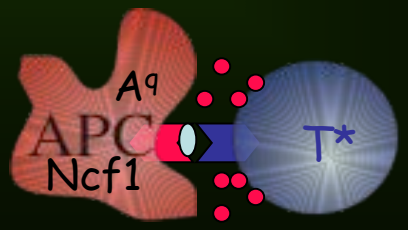
Low ROS by Ncf1 promote chronic inflammatory disease



ROS could be an autocrine modifier of macrophages to prohibit chronic inflammation!

ROS could enable NET formation by neutrophils protecting inflammation

Reactive oxidative species (ROS) could be an immune transmitter in the immunological synapse!



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mir
medical inflammation research

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