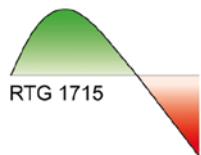


# Molecular Signatures of Adaptive Stress Responses

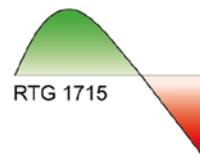


Reinhard Wetzker  
Research Training Group (RTG 1715)  
Friedrich-Schiller-University Jena



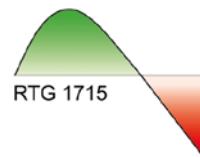
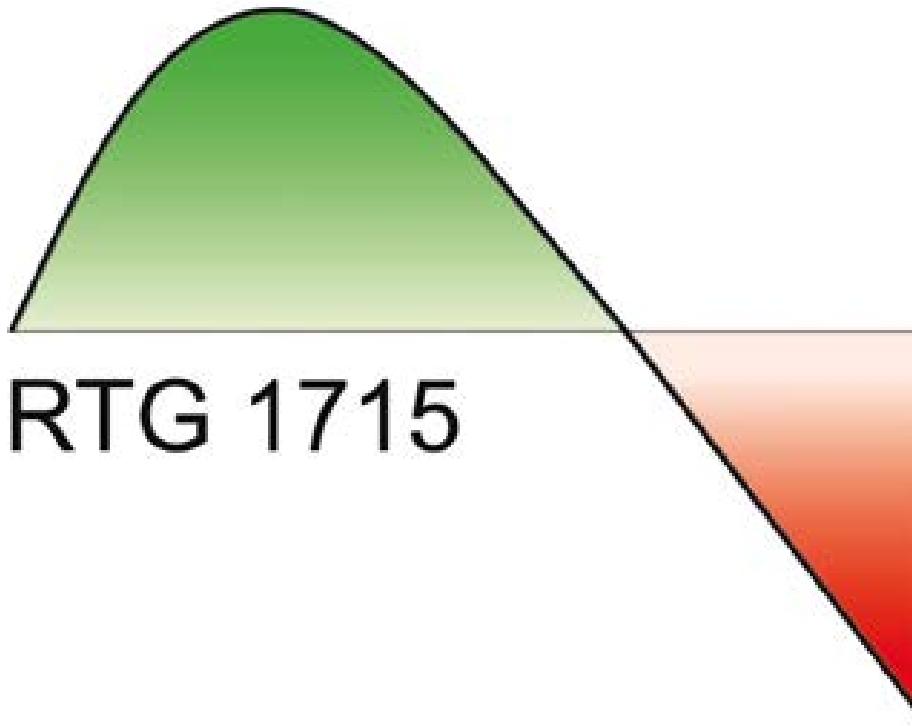
# **Central project idea**

## **RTG 1715**



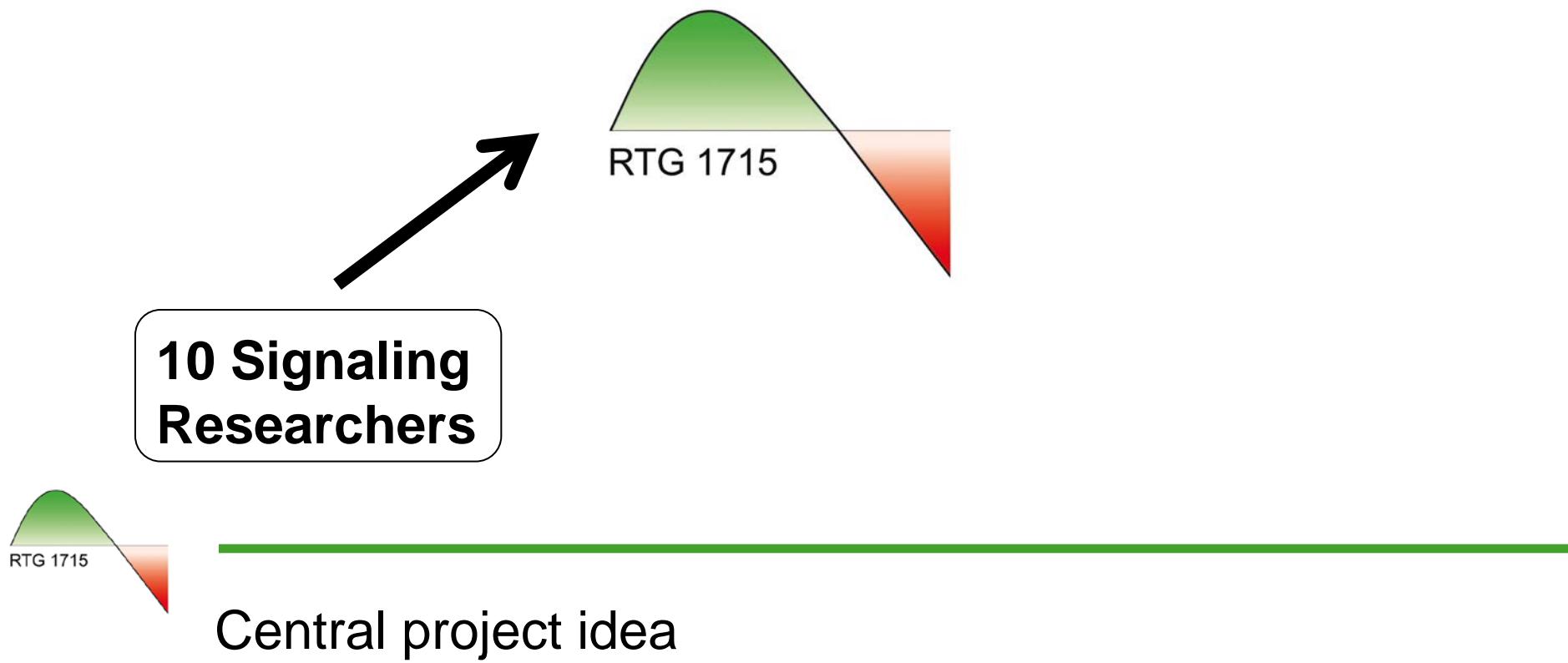
Central project idea

# Creation of the Research Center RTG 1715

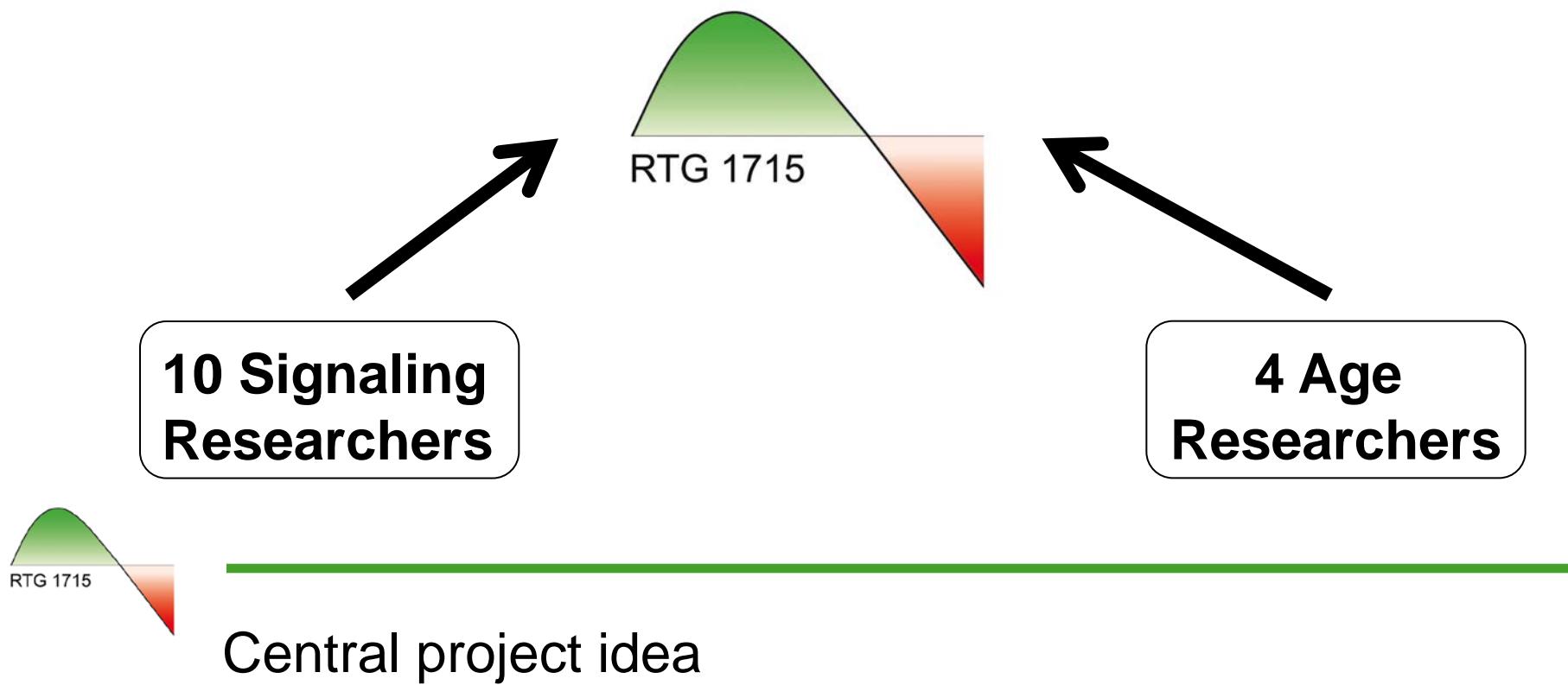


Central project idea

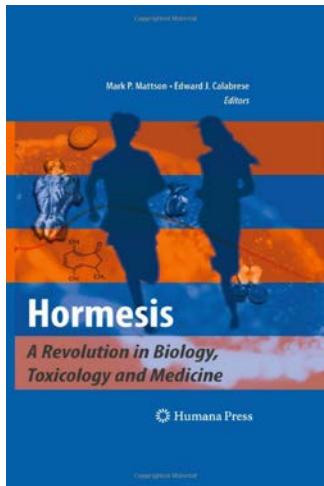
# Creation of the Research Center RTG 1715



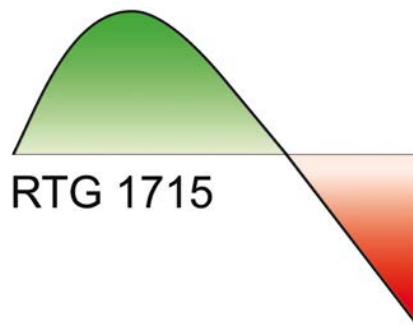
# Creation of the Research Center RTG 1715



# Creation of the Research Center RTG 1715



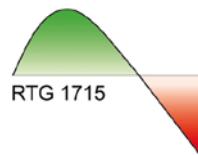
Inspiring  
ideas



10 Signaling  
Researchers

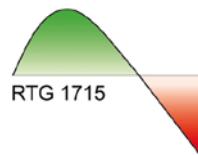
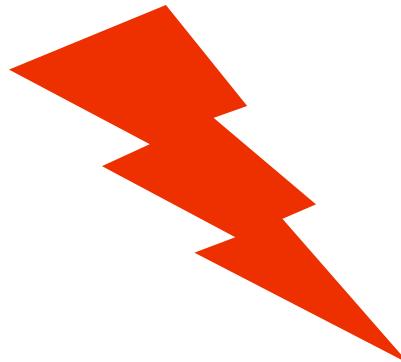
10 Signaling  
Researchers

4 Age  
Researchers



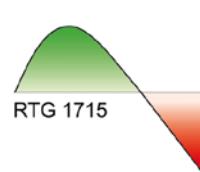
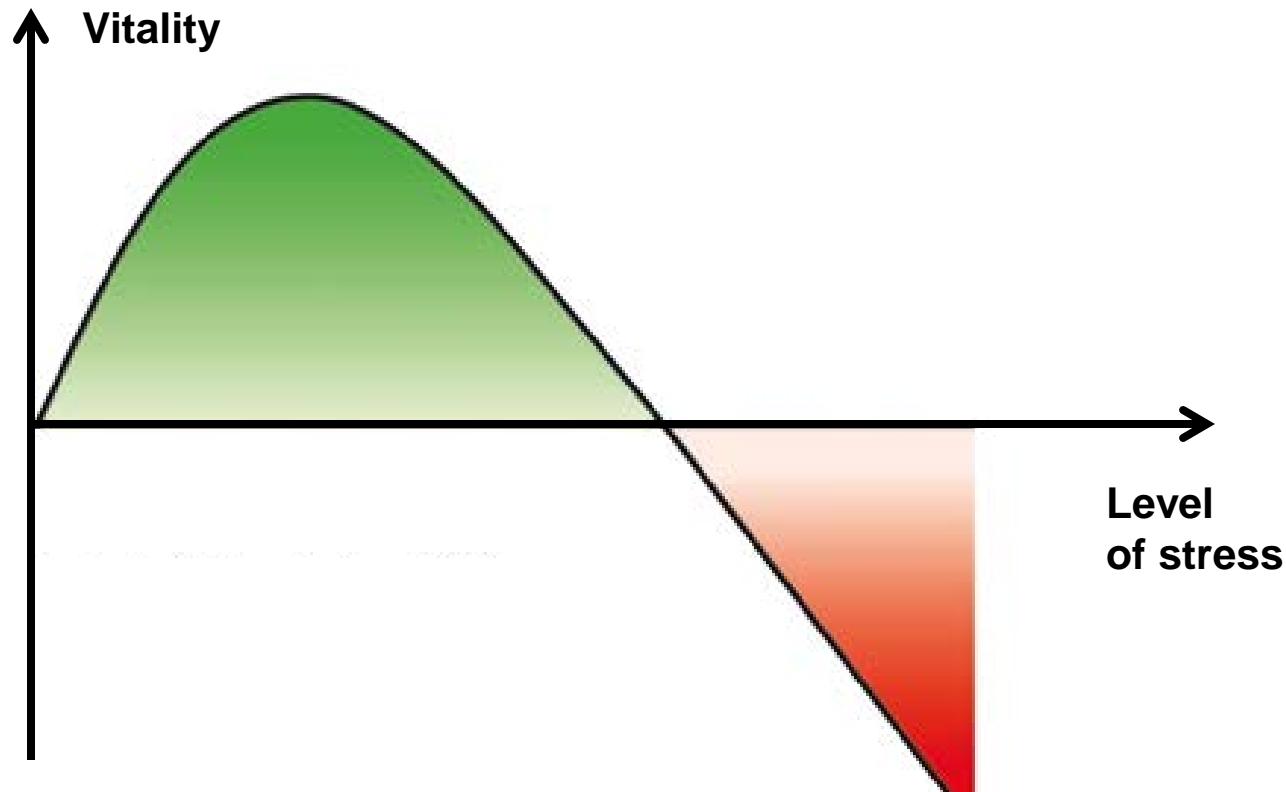
Central project idea

# Stress



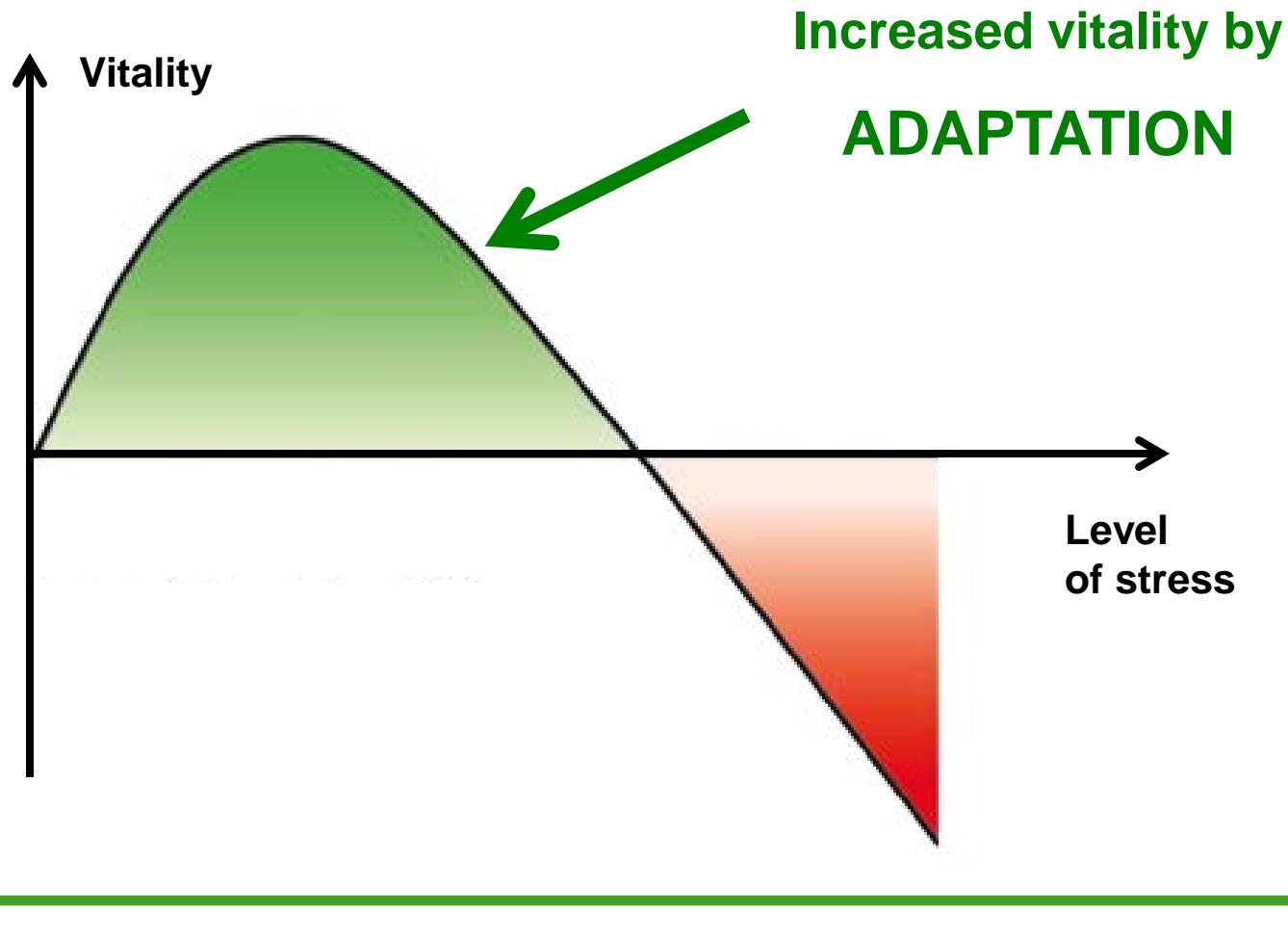
Central project idea

# Working hypothesis Biphasic stress response

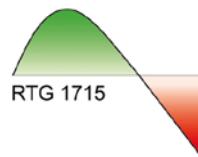
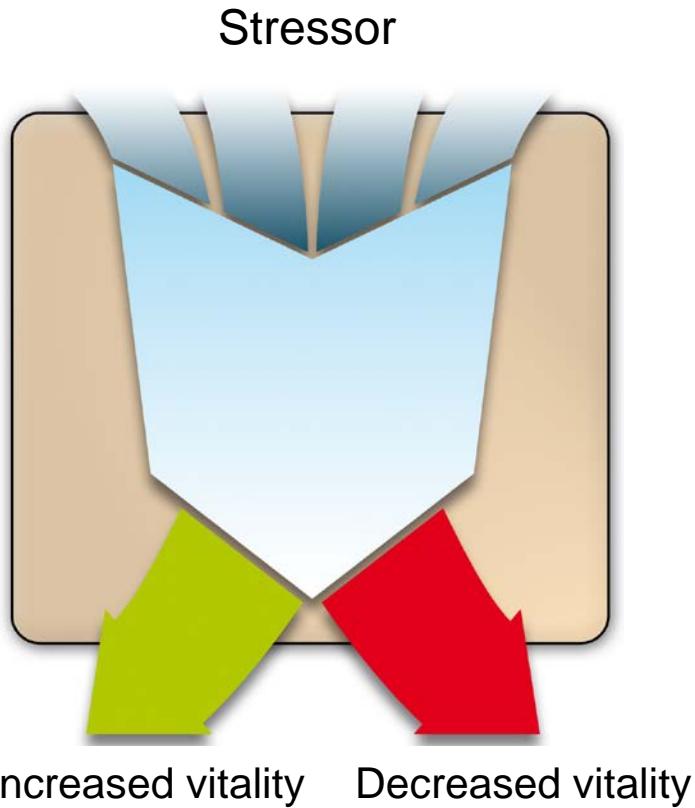


Central project idea

# Working hypothesis Biphasic stress response



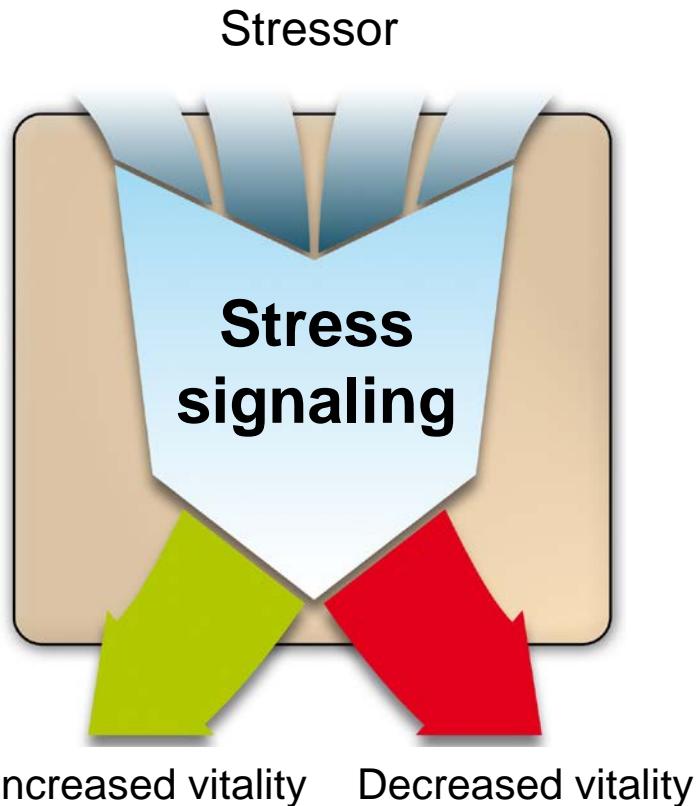
# Adaptive stress response



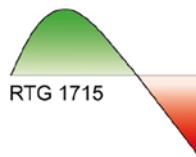
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Central project idea

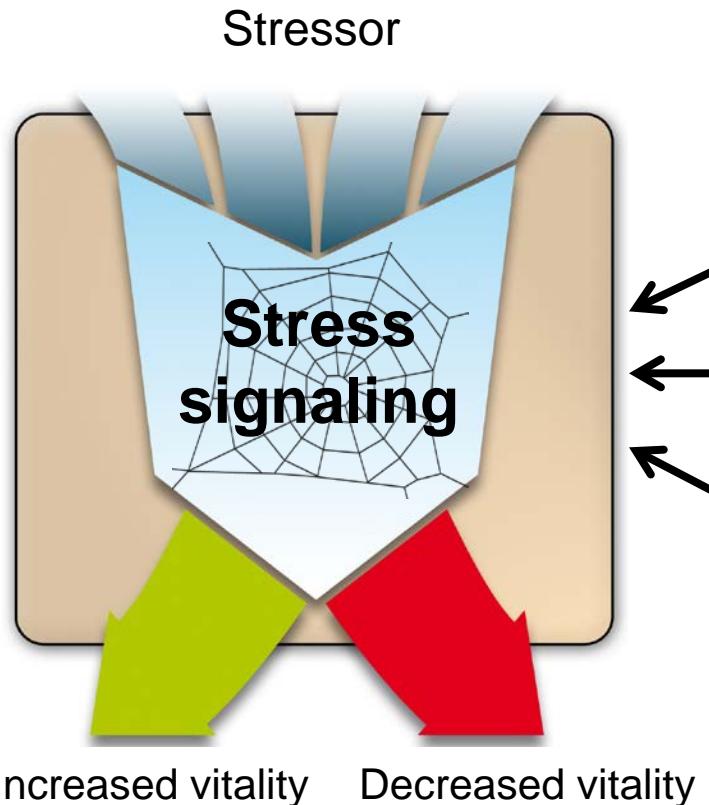
# Adaptive stress response



Central project idea



# Adaptive stress response



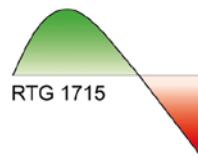
**Identification  
of mediators**



**Dissection**

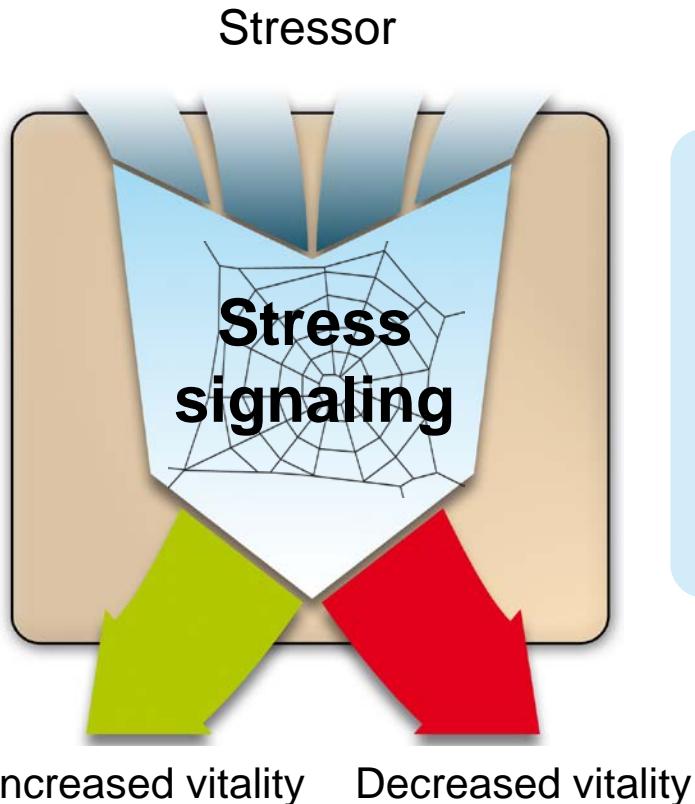


**Integrated  
analysis**

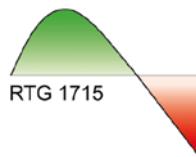


Central project idea

# Adaptive stress response

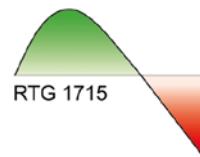


**How do  
signaling networks  
mediate adaptive  
stress responses?**



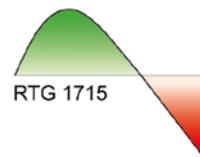
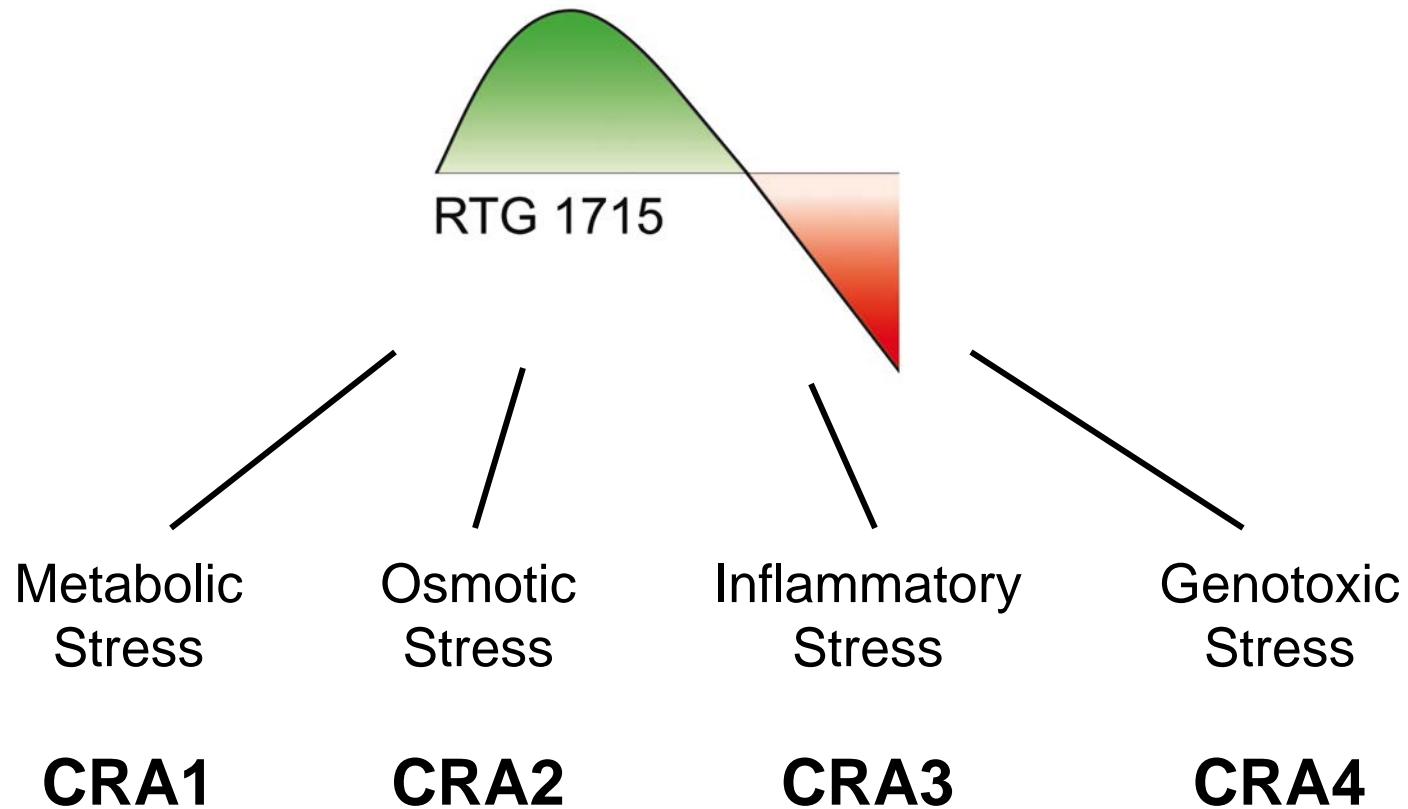
Central project idea

# **Research program & Cooperation**



Research program & Cooperation

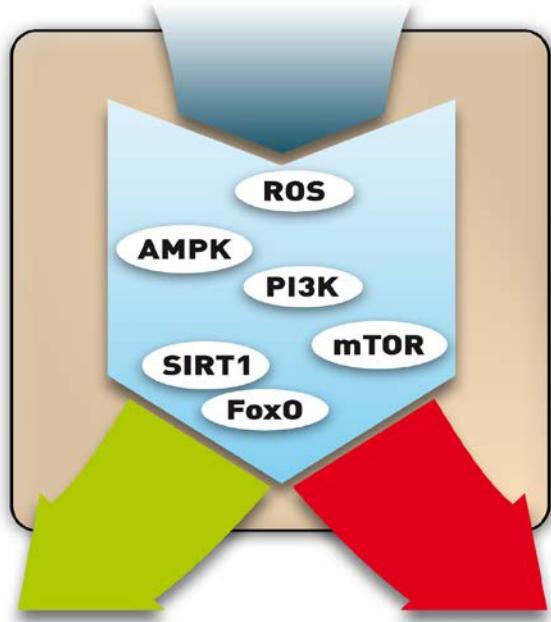
# Exploring adaptive stress responses



Research program & Cooperation

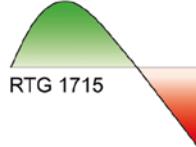
# Exploring adaptive stress responses

Metabolic Stress



Increased vitality      Decreased vitality

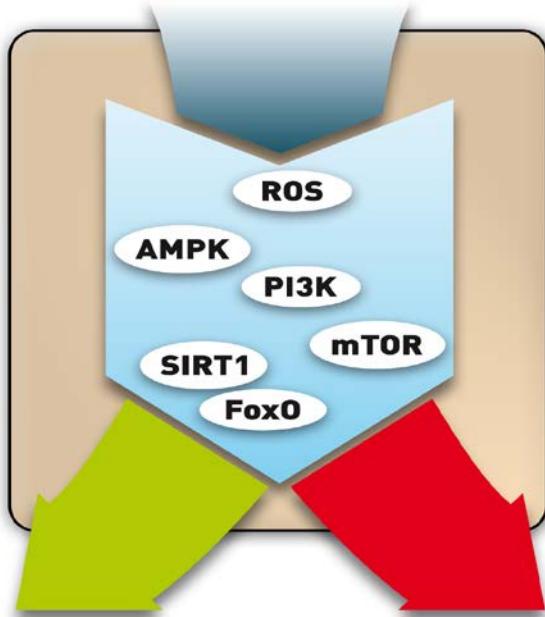
**CRA1**



Research program & Cooperation

# Exploring adaptive stress responses

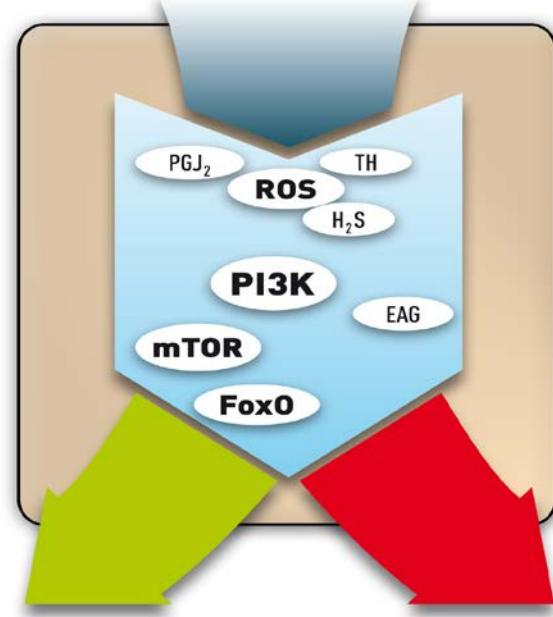
Metabolic Stress



Increased vitality      Decreased vitality

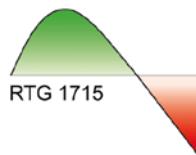
**CRA1**

Inflammatory Stress



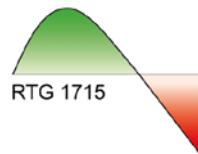
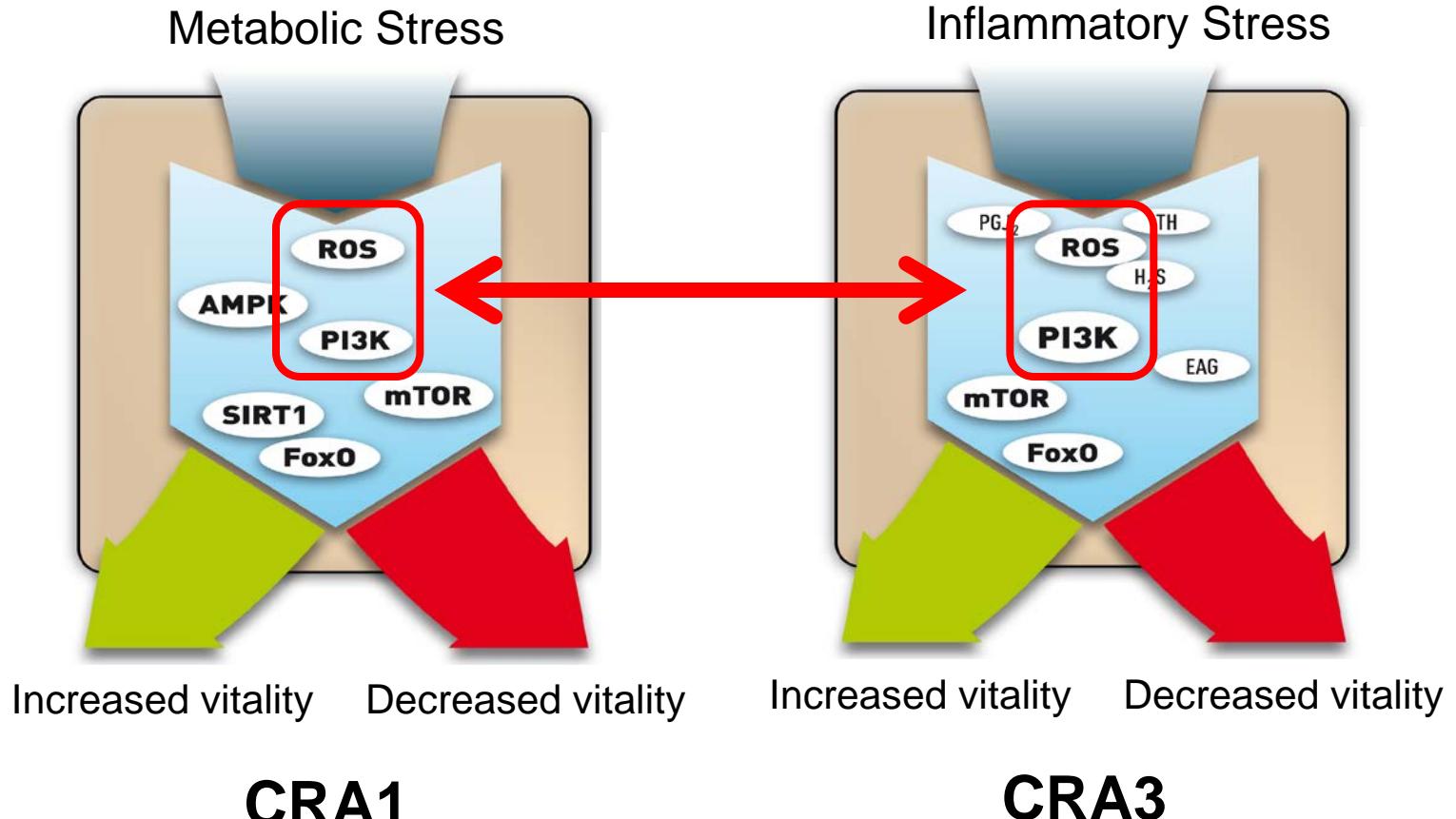
Increased vitality      Decreased vitality

**CRA3**



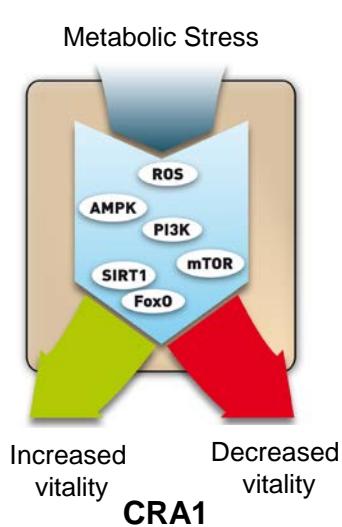
Research program & Cooperation

# Exploring adaptive stress responses



Research program & Cooperation

# First data of the RTG



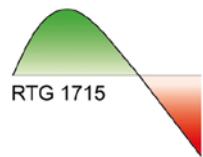
Increased vitality      Decreased vitality

CRA1



Increased vitality      Decreased vitality

CRA3



Research program & Cooperation

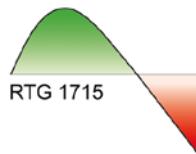
# Mitochondrial ROS signaling

## Aging Cell

(accepted March 2013)

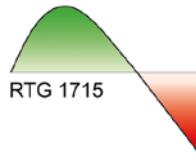
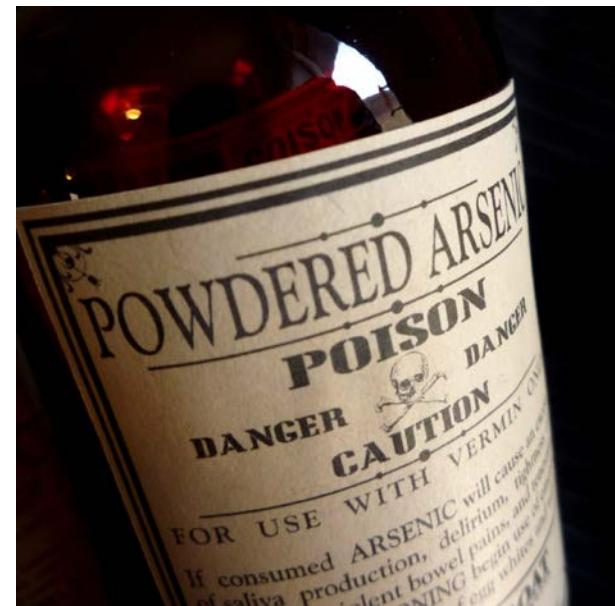
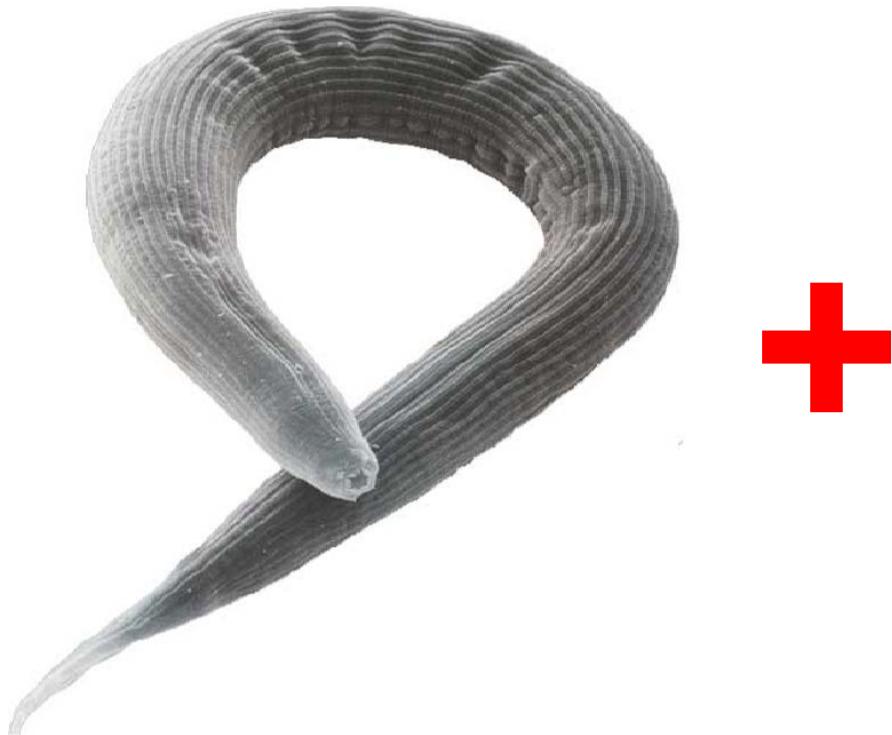
**Mitochondrial hormesis links low-dose arsenite exposure to lifespan extension**

Sebastian Schmeisser, Kathrin Schmeisser, Sandra Weimer, Marco Groth, Steffen Priebe, Eugen Fazius, Doreen Kuhlow, Denis Pick, Jürgen W. Einax, Reinhard Guthke, Matthias Platzer, Kim Zarse and Michael Ristow



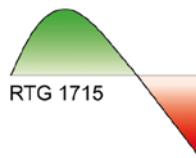
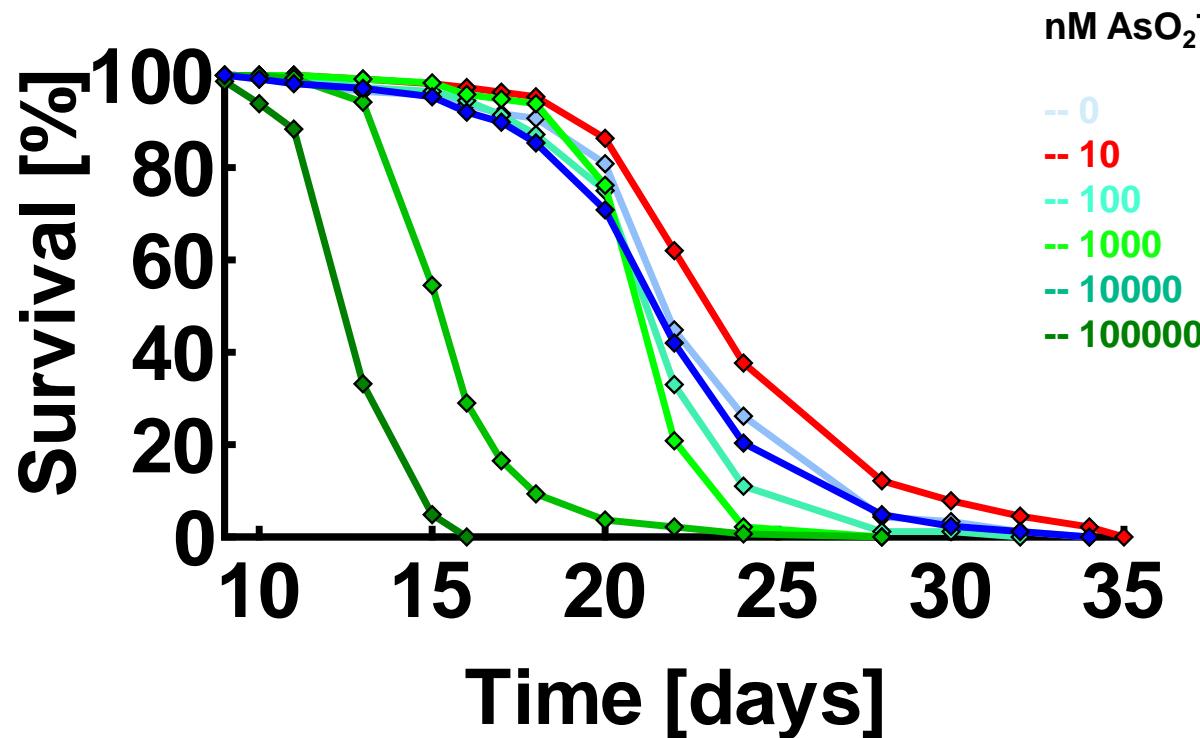
Mitochondrial ROS signaling

# Effects of arsenite on life span of the round worm *C. elegans*



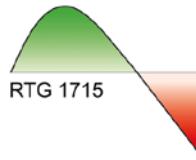
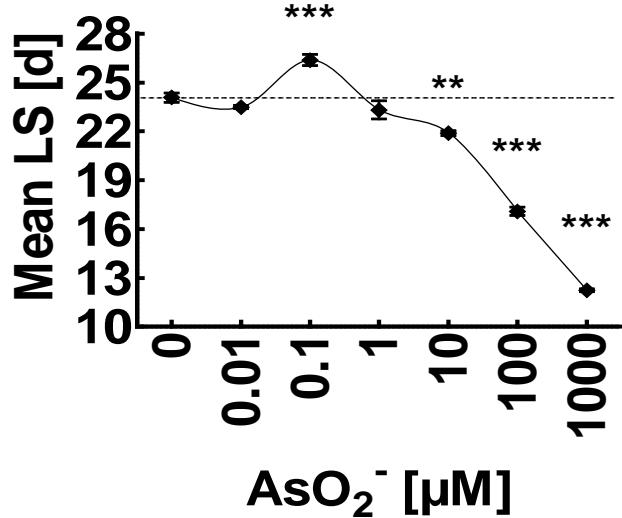
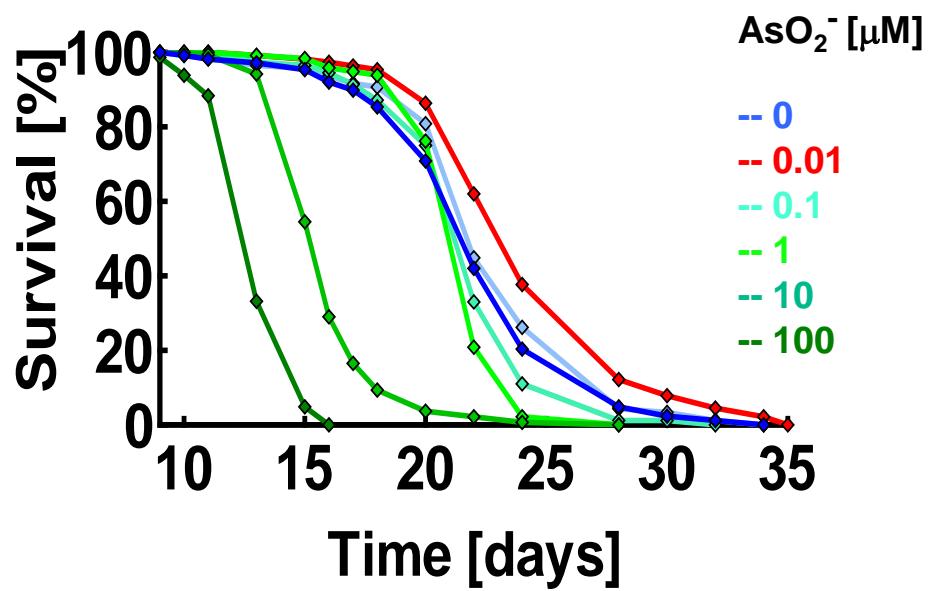
Mitochondrial ROS signaling

# Low dose arsenite promote life span of *C. elegans*



Mitochondrial ROS signaling

# Low dose arsenite promotes life span of *C. elegans*

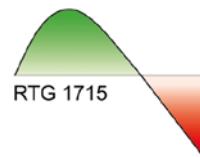


Mitochondrial ROS signaling

# Motility of *C. elegans*

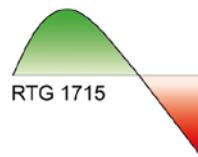
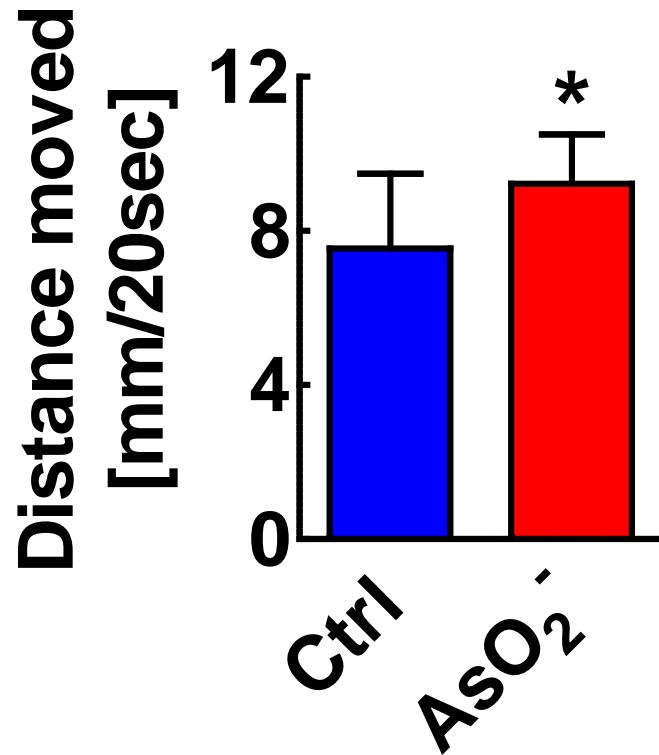


Wikipedia



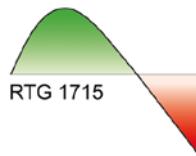
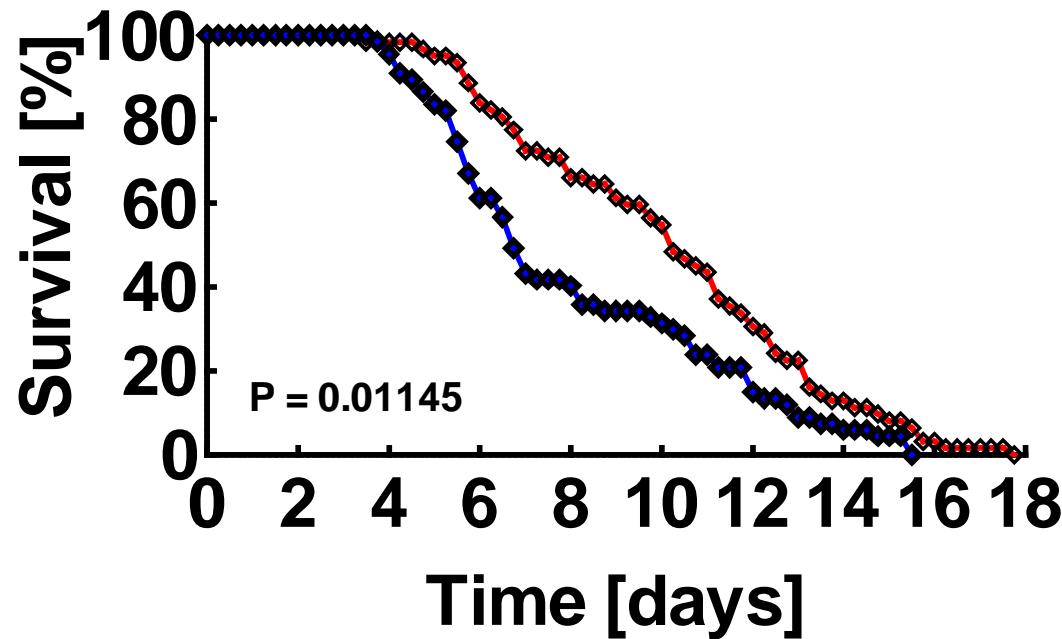
Mitochondrial ROS signaling

# Low dose arsenite promotes motility of *C. elegans*



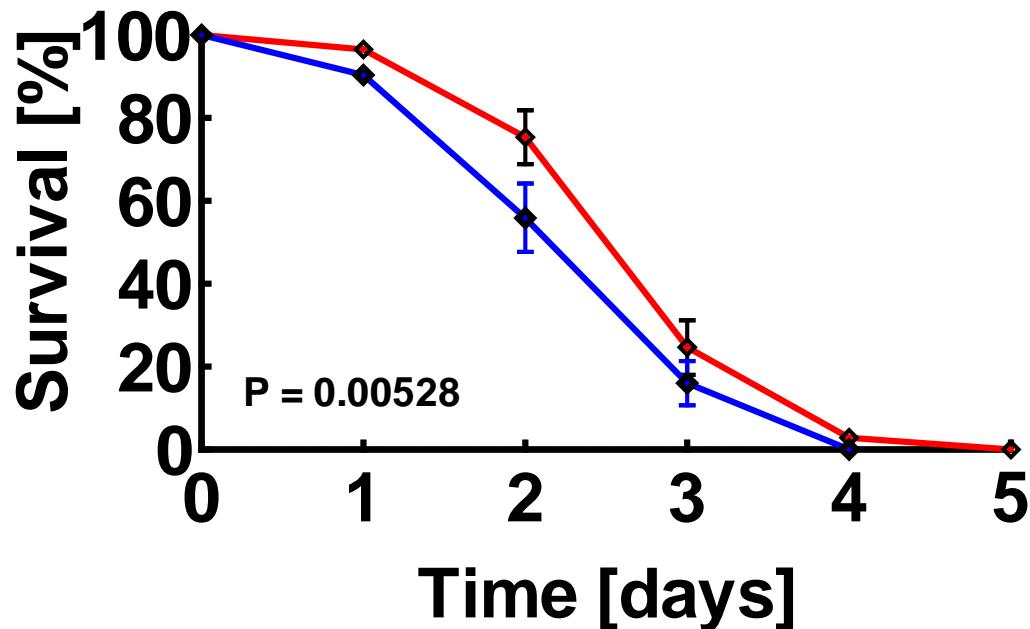
Mitochondrial ROS signaling

# Low dose arsenite promotes resistance of *C. elegans* against subsequent lethal heat stress

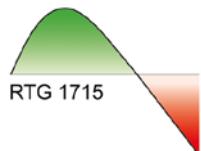


Mitochondrial ROS signaling

# Low dose arsenite promotes resistance of *C. elegans* against subsequent letal oxidant stress

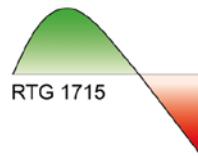
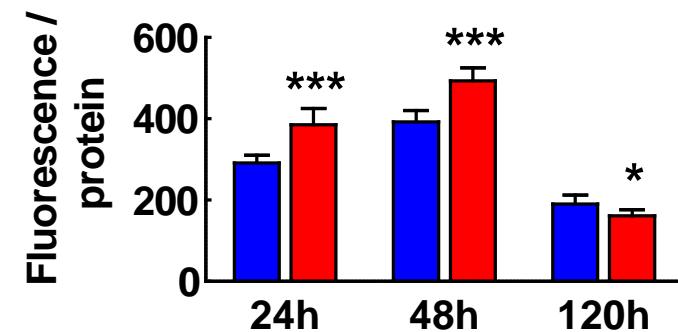


Oxidant stressor: Paraquat



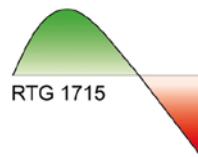
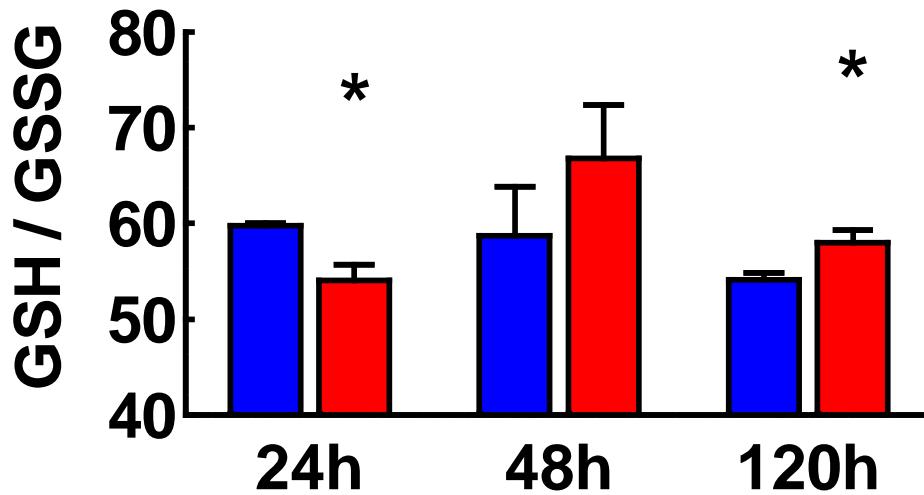
Mitochondrial ROS signaling

# Low dose arsenite promotes ROS formation in *C. elegans*



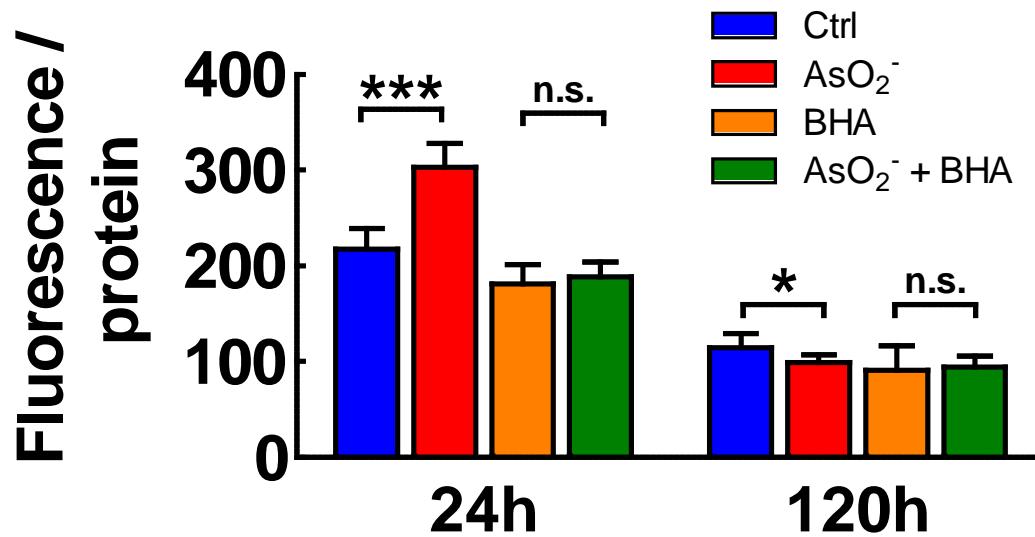
Mitochondrial ROS signaling

# Low dose arsenite promotes ROS defense in *C. elegans*

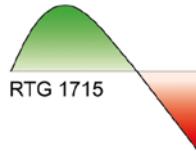


Mitochondrial ROS signaling

# ROS production induced by arsenite is suppressed by BHA in *C. elegans*

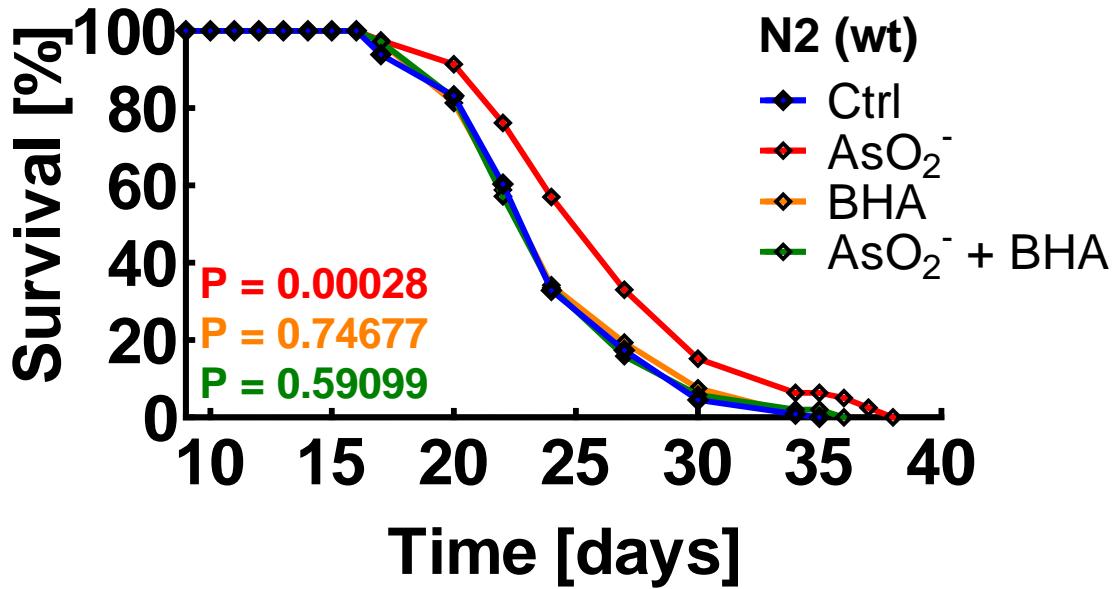


BHA = butylated hydroxyanisole

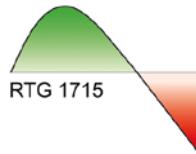


Mitochondrial ROS signaling

# Suppression of ROS production by BHA prevents the stimulatory effect of arsenite on *C. elegans* life span

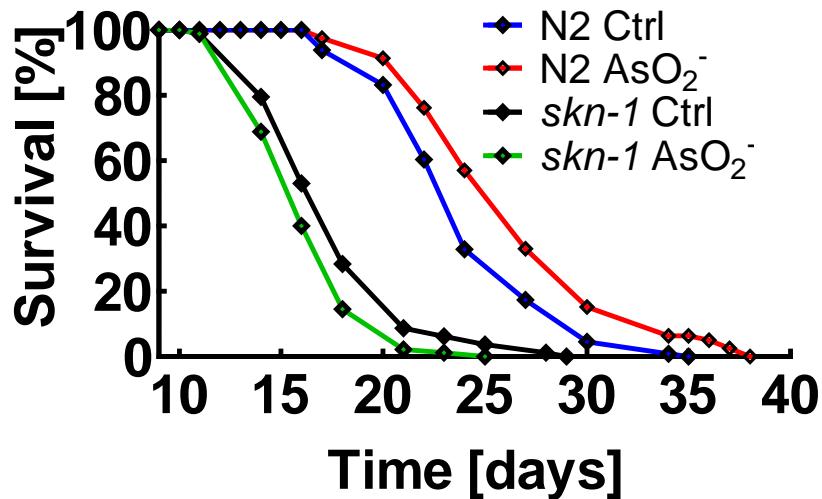


BHA = butylated hydroxyanisole

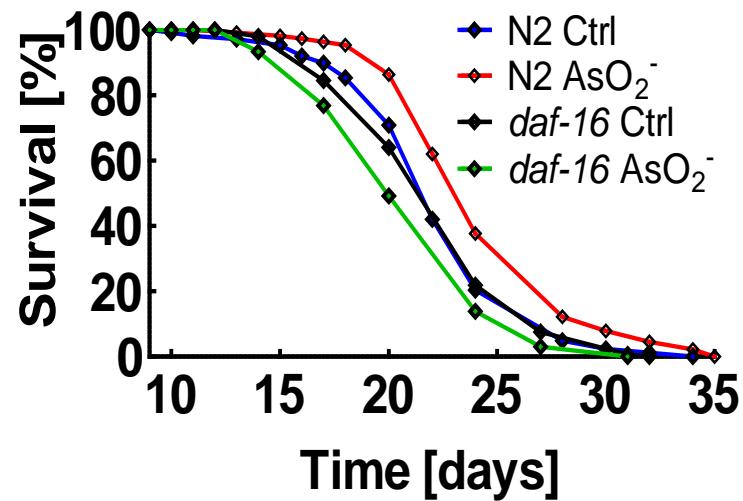


Mitochondrial ROS signaling

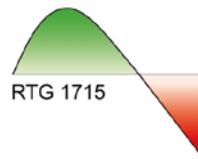
# Analysis of mutant *C. elegans* indicates *skn-1* and *daf-16* as mediators of life span extension induced by low dose arsenite



skn-1 / Nrf

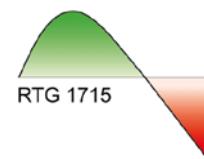
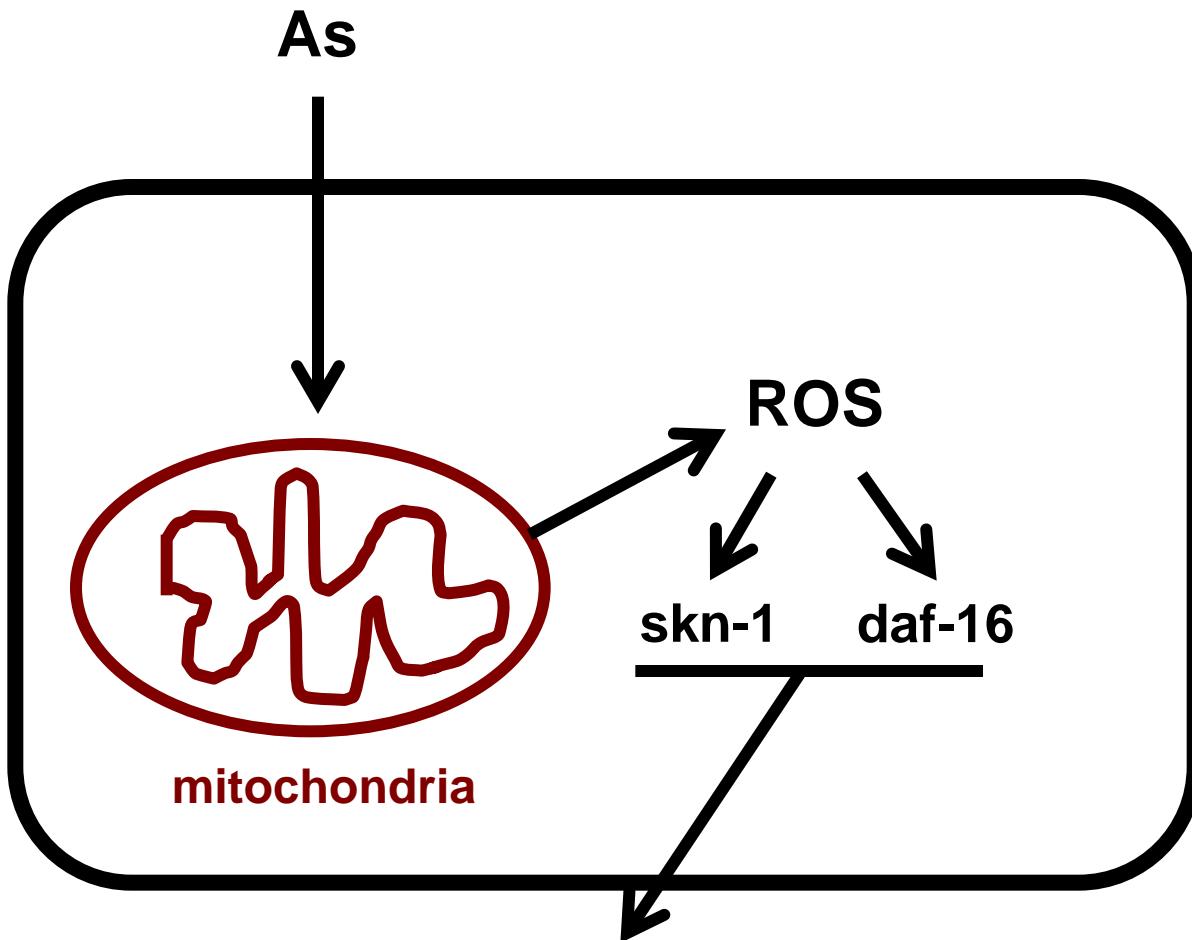


daf-16 / FoxO



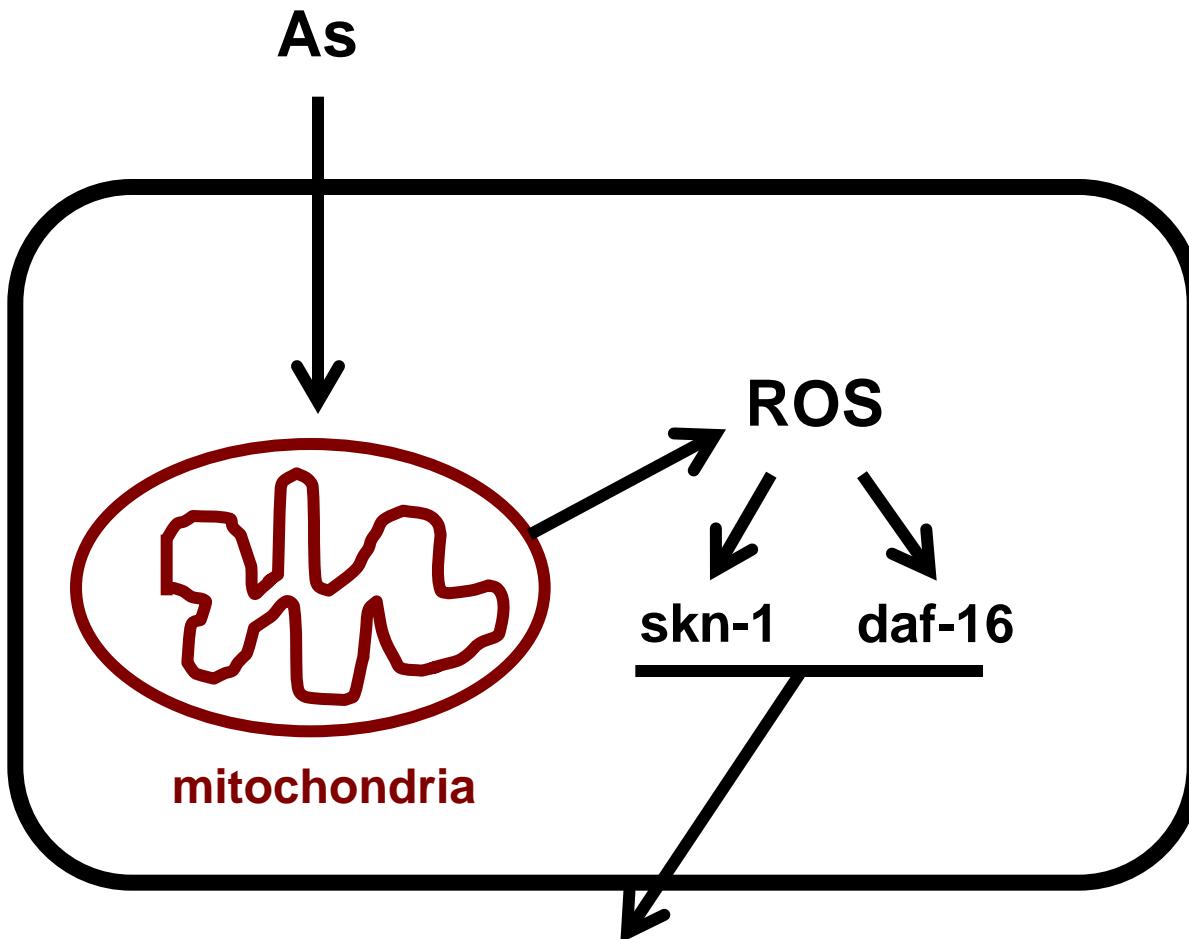
Mitochondrial ROS signaling

# ROS mediates Arsenic response of *C. elegans*

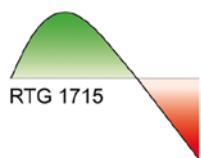


Mitochondrial ROS signaling

# ROS mediates Arsenic response of *C. elegans*

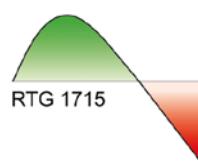
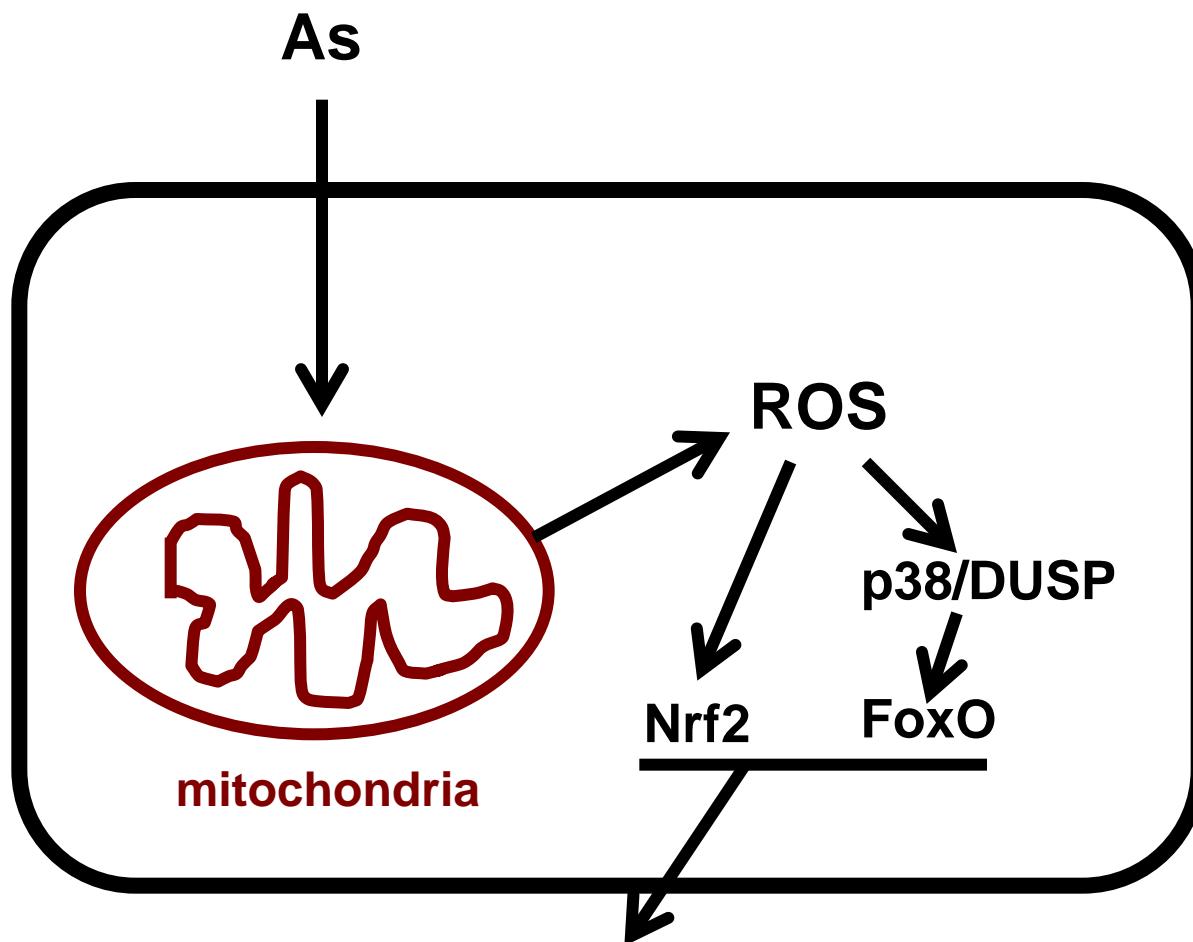


„Mitohormesis“



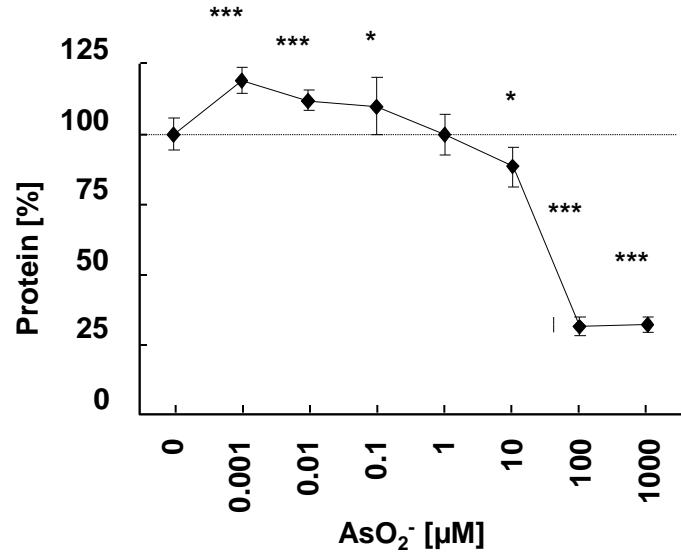
Mitochondrial ROS signaling

# ROS dependent Arsenic response in mammalian cells?

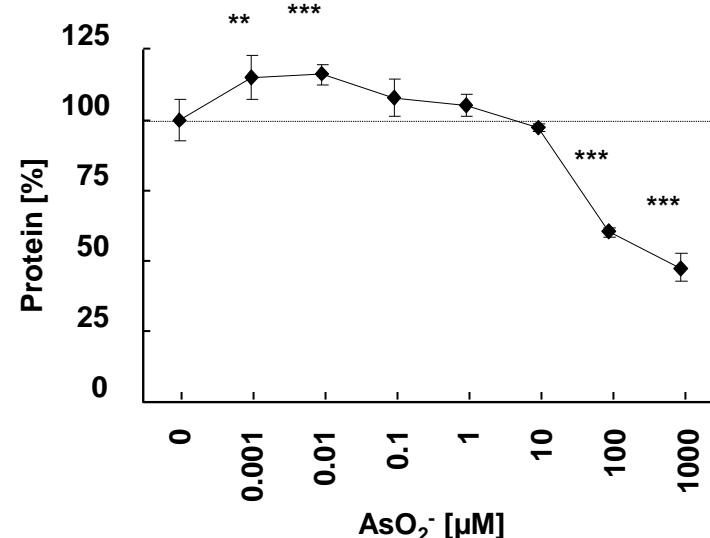


Mitochondrial ROS signaling

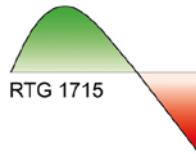
# Low dose Arsenite promotes growth of mammalian cells



NIH 3T3  
(fibroblast cell line)



HepG2  
(hepatocyte cell line)

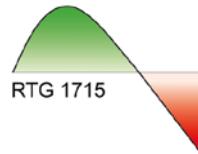


Mitochondrial ROS signaling

# **PI3K $\gamma$ mediates microglia stress response**

**PI3K $\gamma$  mediates hormetic effects of LPS on microglia stress response**

Caroline Schmidt, Nadine Schneble and Reinhard Wetzker

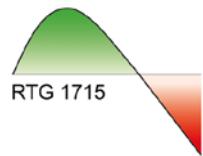


**PI3K $\gamma$  mediates microglia stress response**

# Crystal structure of PI3K $\gamma$

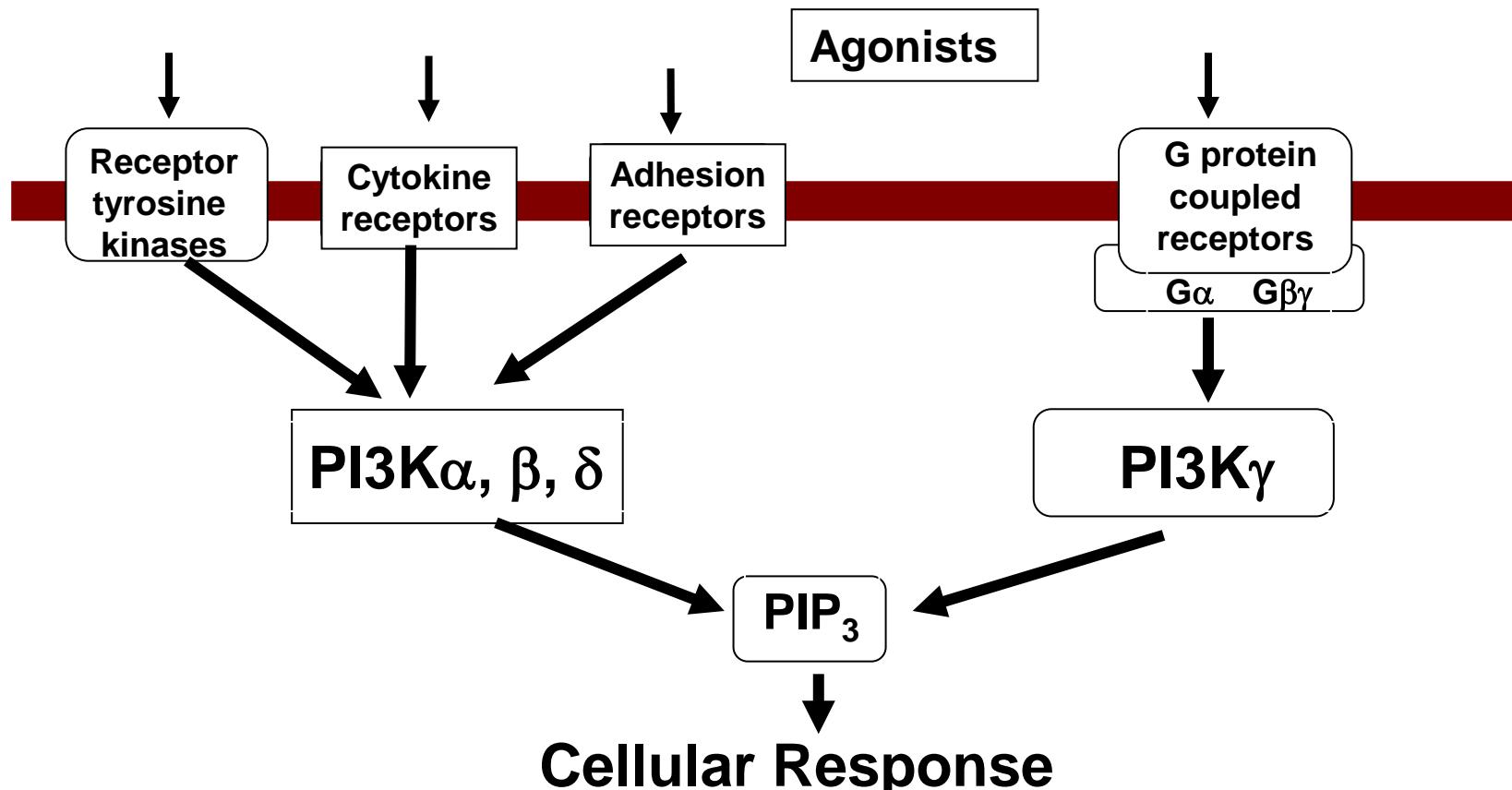


Walker et al. (1999) Nature 204, 313

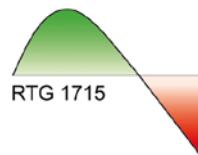


PI3K $\gamma$  mediates microglia stress response

# PI3K species and functions

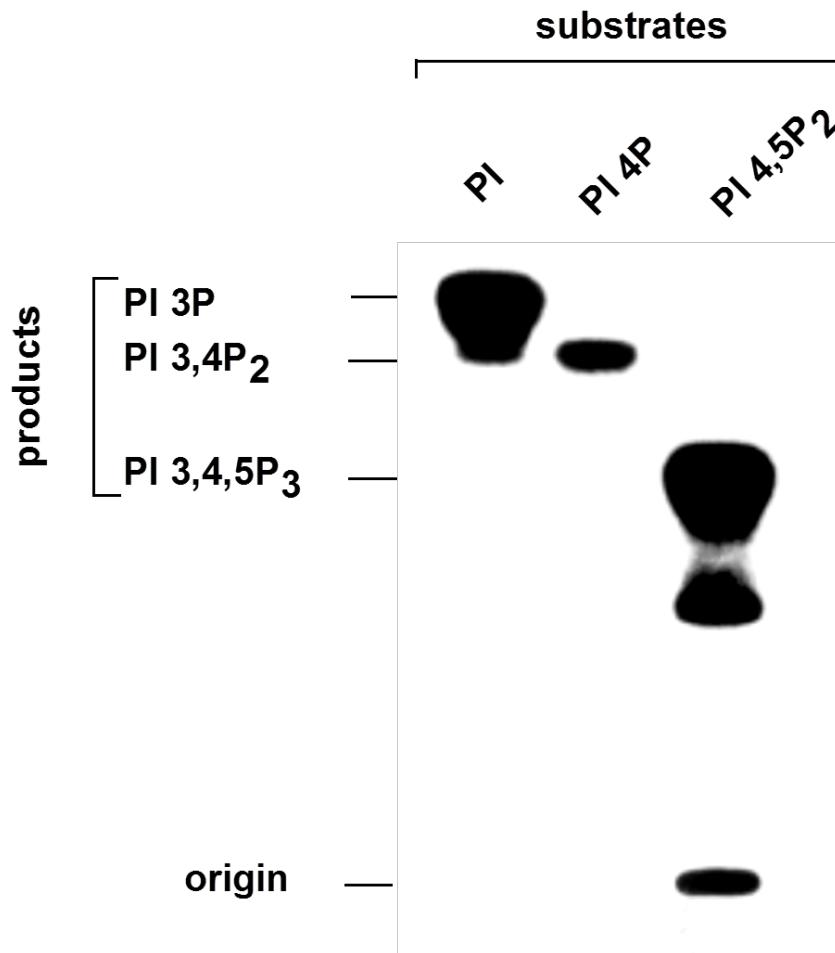


Differentiation  
Apoptosis  
Adhesion  
Proliferation  
Endocytosis  
Motility

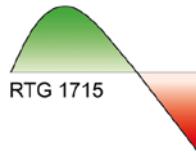


PI3K $\gamma$  mediates microglia stress response

# Lipid kinase activity of PI3K $\gamma$

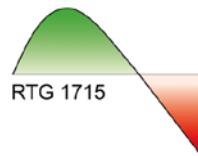
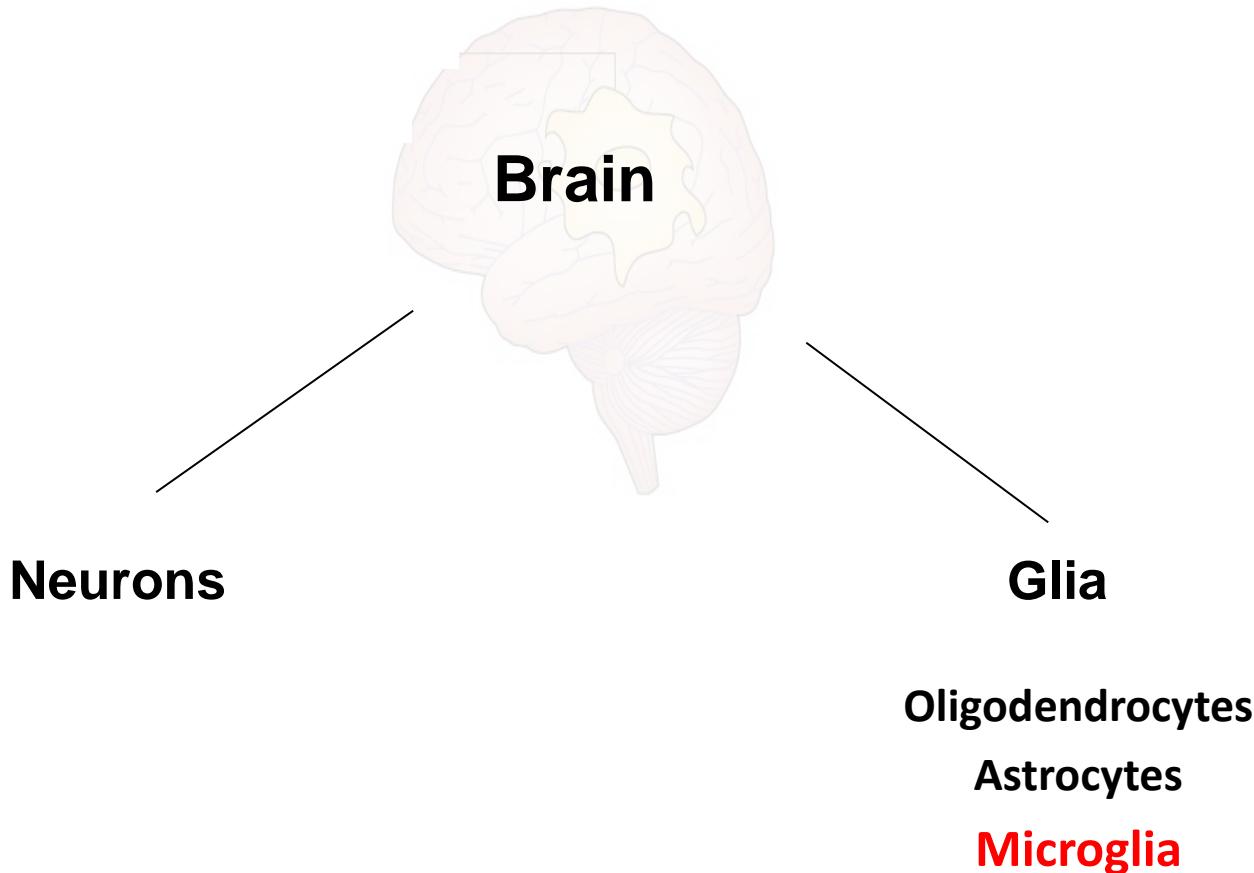


Stoyanov et al. Science 269, 690 (1995)



PI3K $\gamma$  mediates microglia stress response

# Microglia are macrophages of the brain



PI3K $\gamma$  mediates microglia stress response

# Activation of microglia

## Supporting Functions

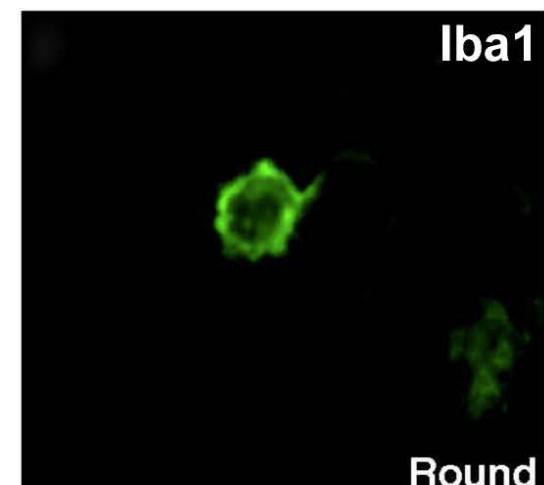
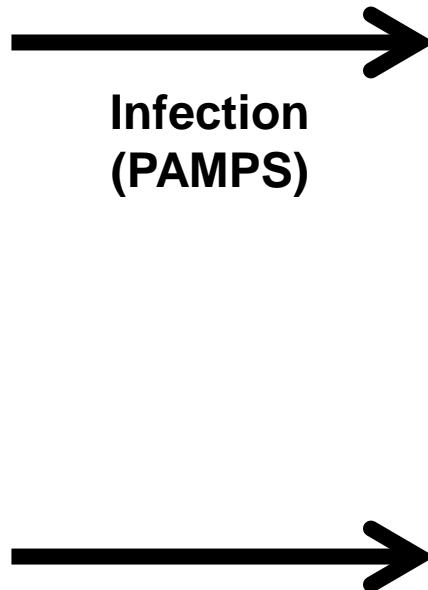
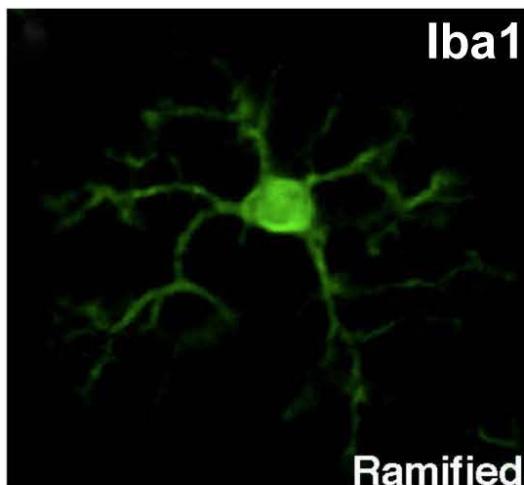
Generation of neurotrophic factors

Injury (DAMPs)

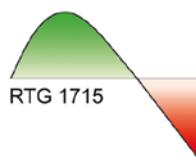
Infection (PAMPs)

## Activated Functions

Migration  
Phagocytosis  
Cytokine production  
Proliferation



(Saijo 2011, Ekdahl 2012)



PI3K $\gamma$  mediates microglia stress response

# Activation of microglia

## Supporting Functions

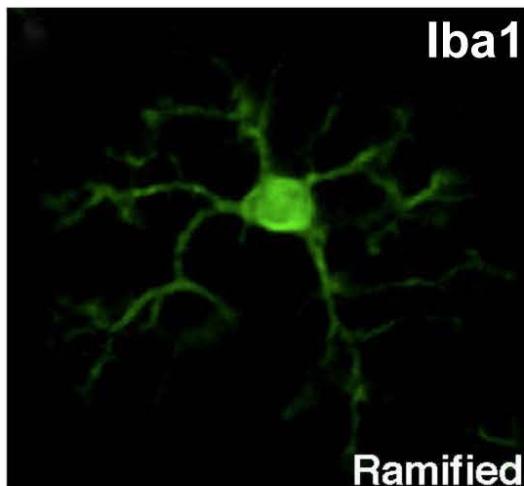
Generation of neurotrophic factors

Injury (DAMPS)

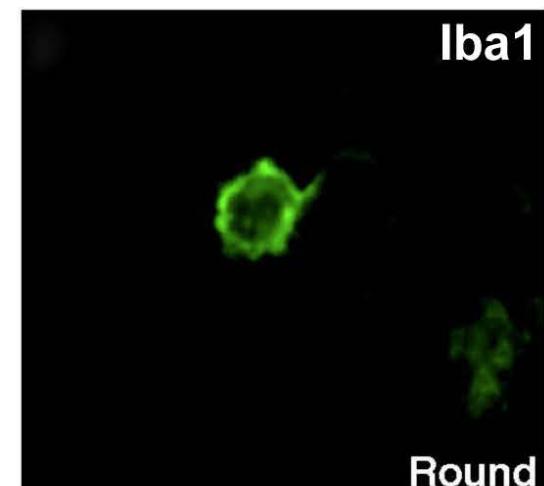
Infection (PAMPS)

## Damaging Functions

Migration  
Phagocytosis  
Cytokine production  
Proliferation  
Protease release  
High ROS, NOS or glutamate production

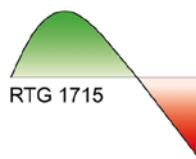


Ramified



Round

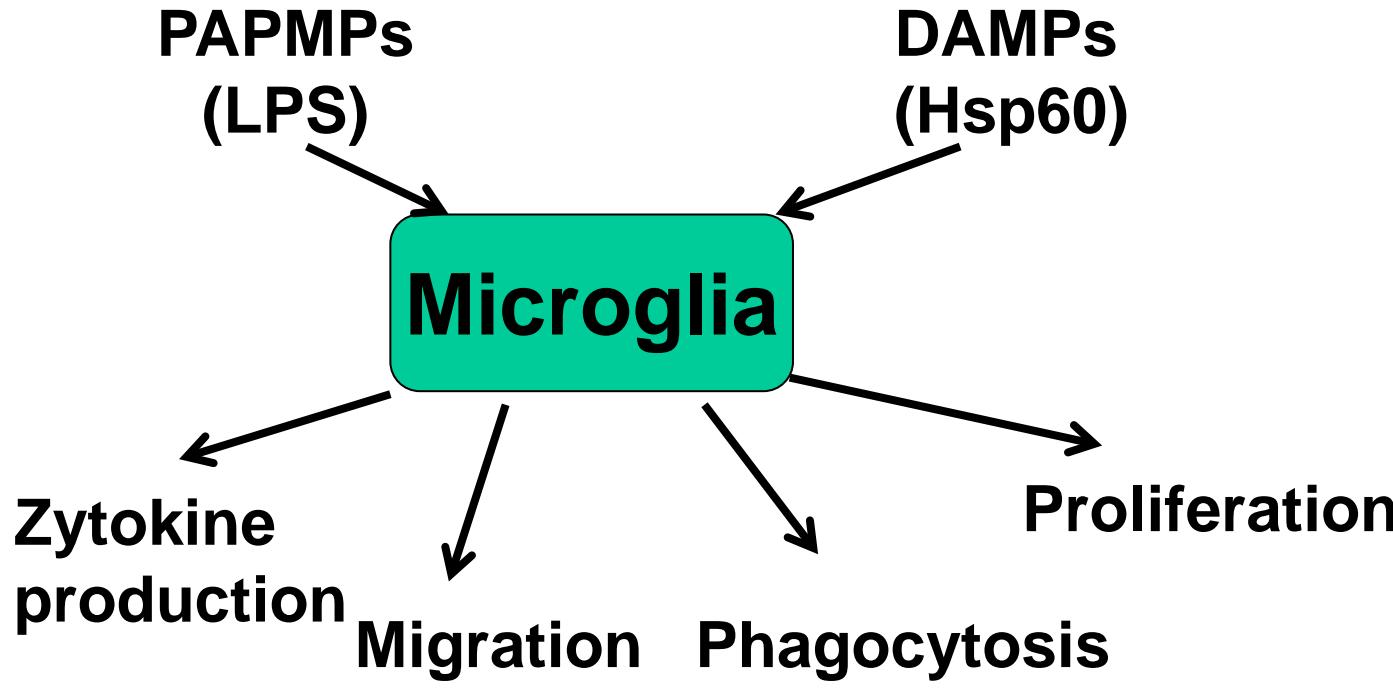
(Saijo 2011, Ekdahl 2012)



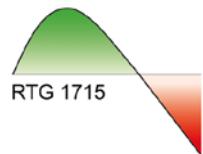
PI3K $\gamma$  mediates microglia stress response

Working hypothesis:

## Dose dependent stress reactions of microglia



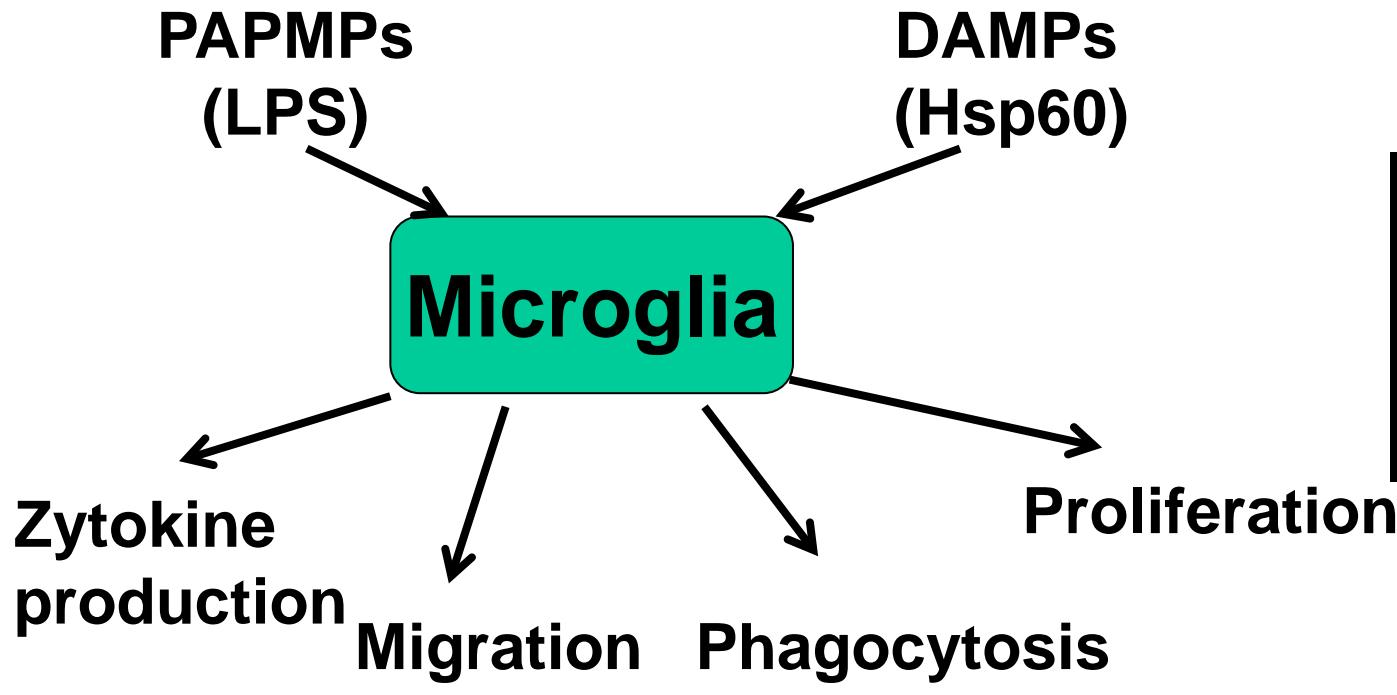
Regeneration or degeneration of neurons



PI3K $\gamma$  mediates microglia stress response

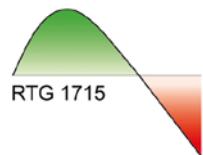
Working hypothesis:

## Dose dependent stress reactions of microglia



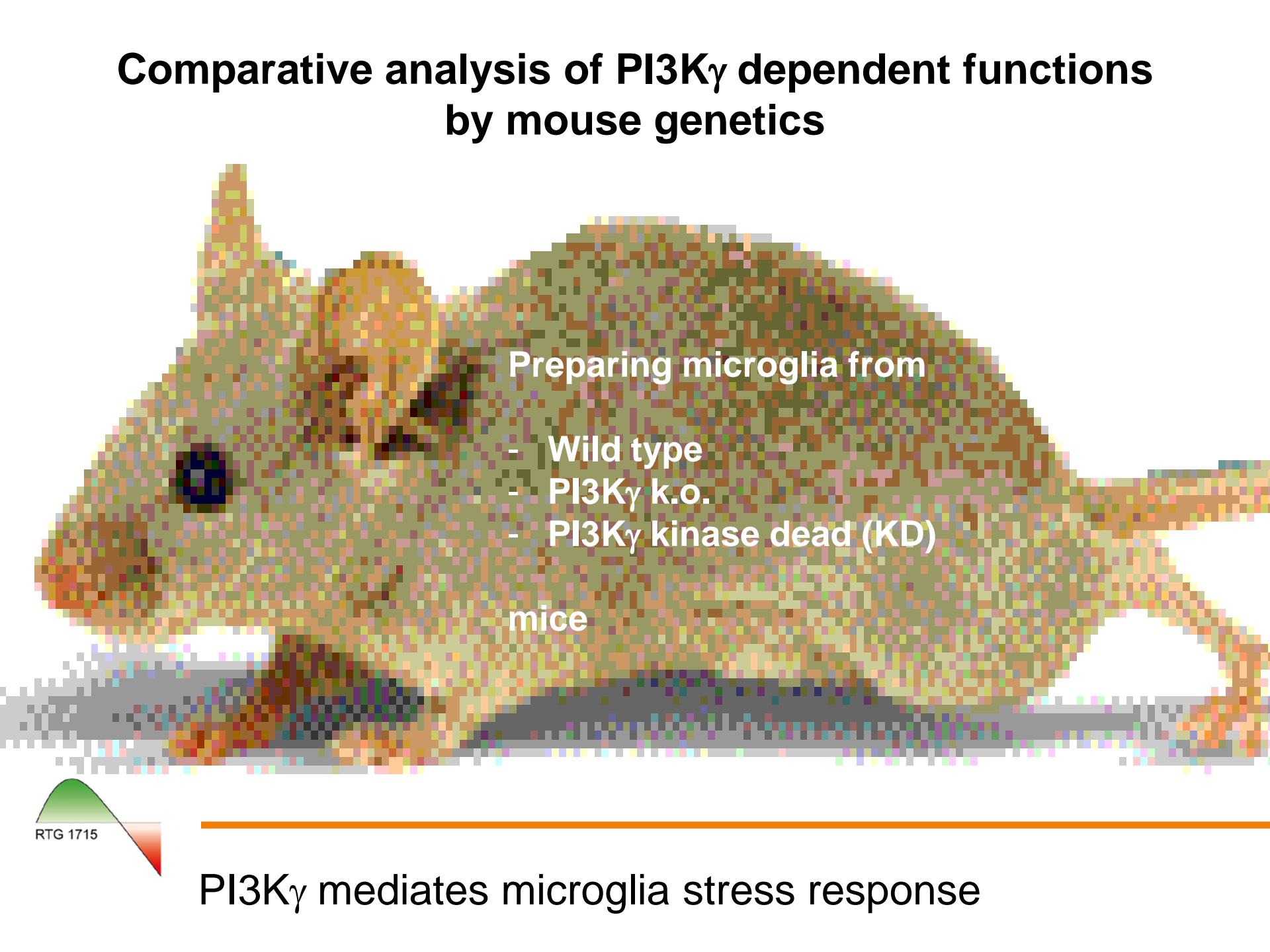
Role of  
PI3K $\gamma$ ?

Regeneration or degeneration of neurons



PI3K $\gamma$  mediates microglia stress response

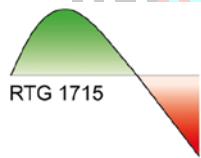
# Comparative analysis of PI3K $\gamma$ dependent functions by mouse genetics



Preparing microglia from

- Wild type
- PI3K $\gamma$  k.o.
- PI3K $\gamma$  kinase dead (KD)

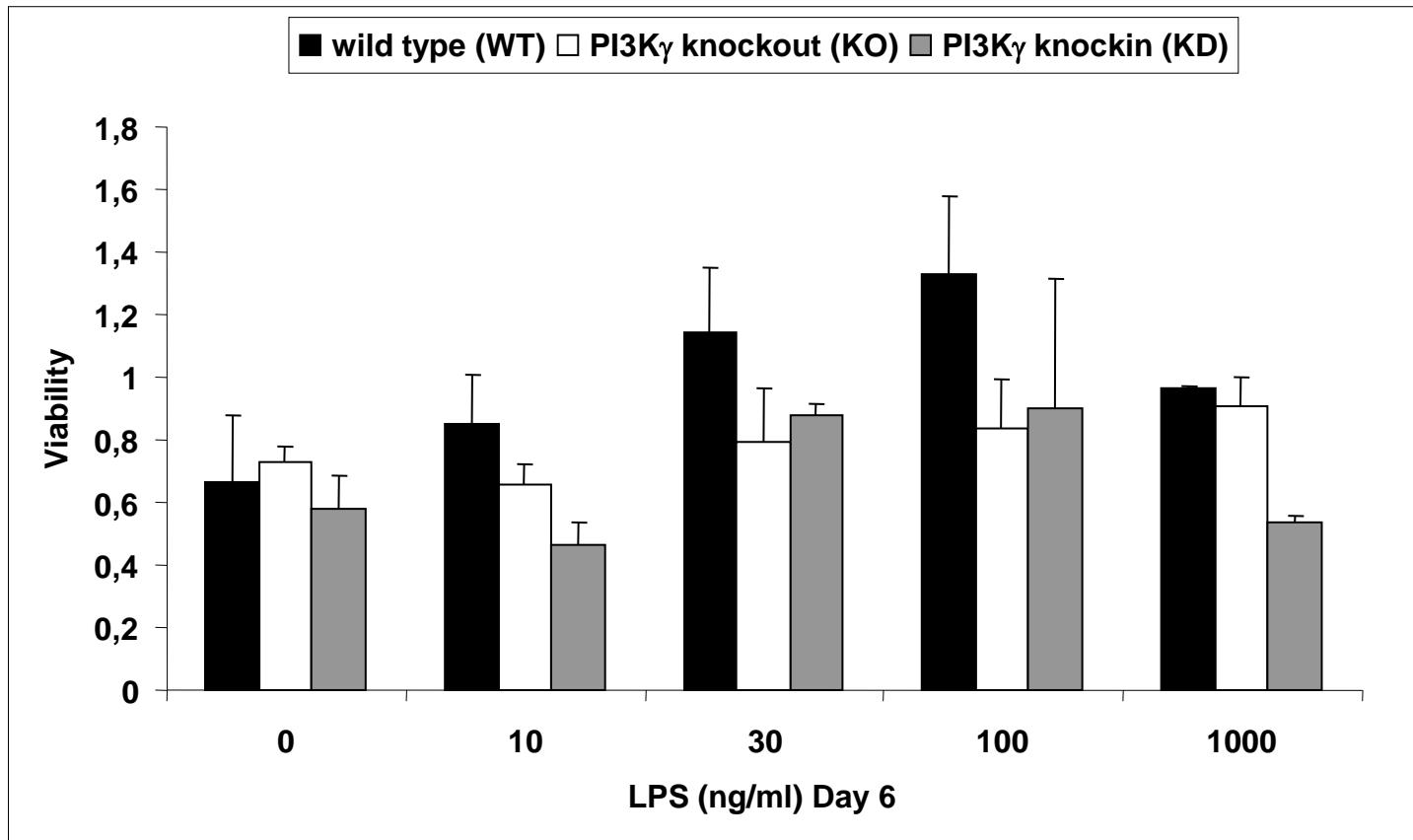
mice



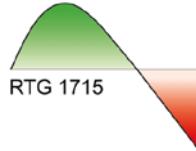
PI3K $\gamma$  mediates microglia stress response

# Effects of LPS on stress response of microglia

## Role of PI3K $\gamma$

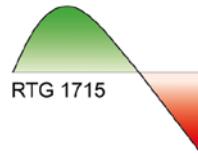
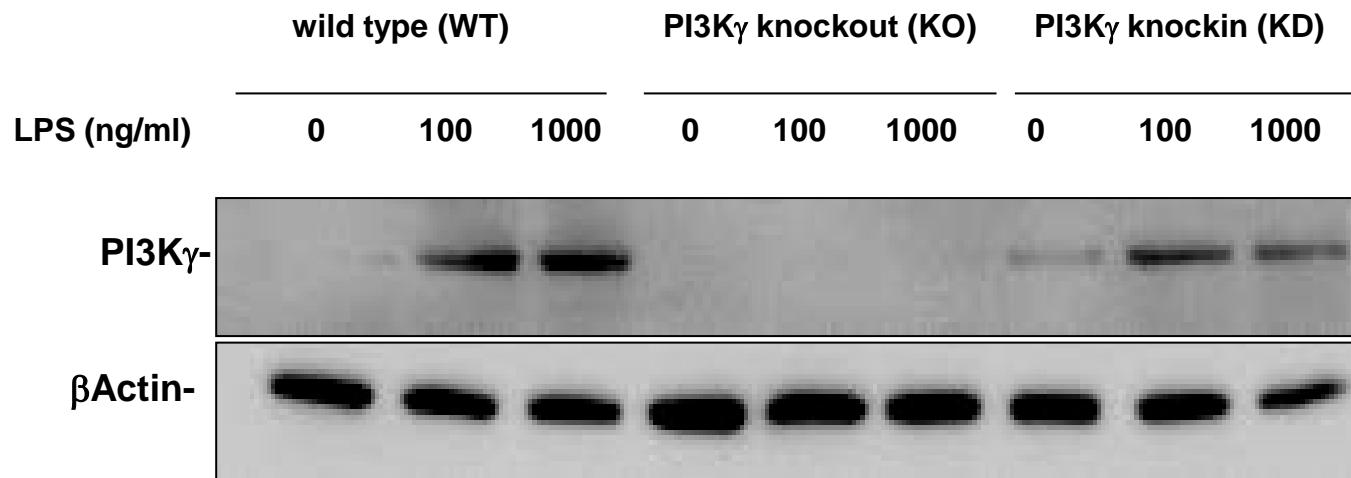


MTT assay (570 nm)



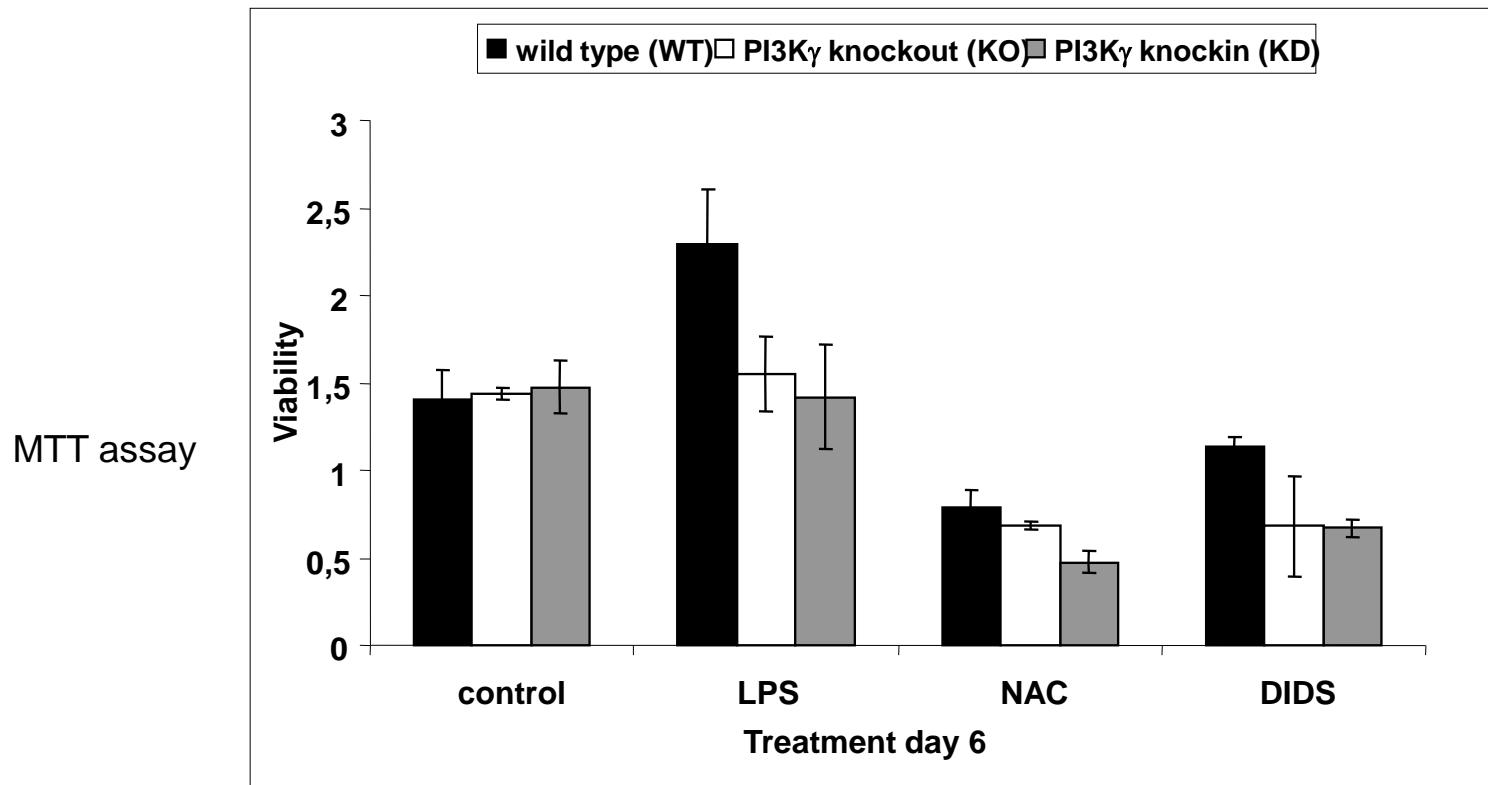
PI3K $\gamma$  mediates microglia stress response

# Effects of LPS on PI3K $\gamma$ expression



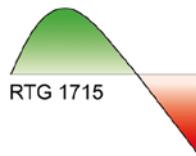
PI3K $\gamma$  mediates microglia stress response

# Suppression of LPS induced microglia stress response by ROS scavengers NAC and DIDS



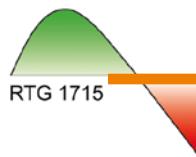
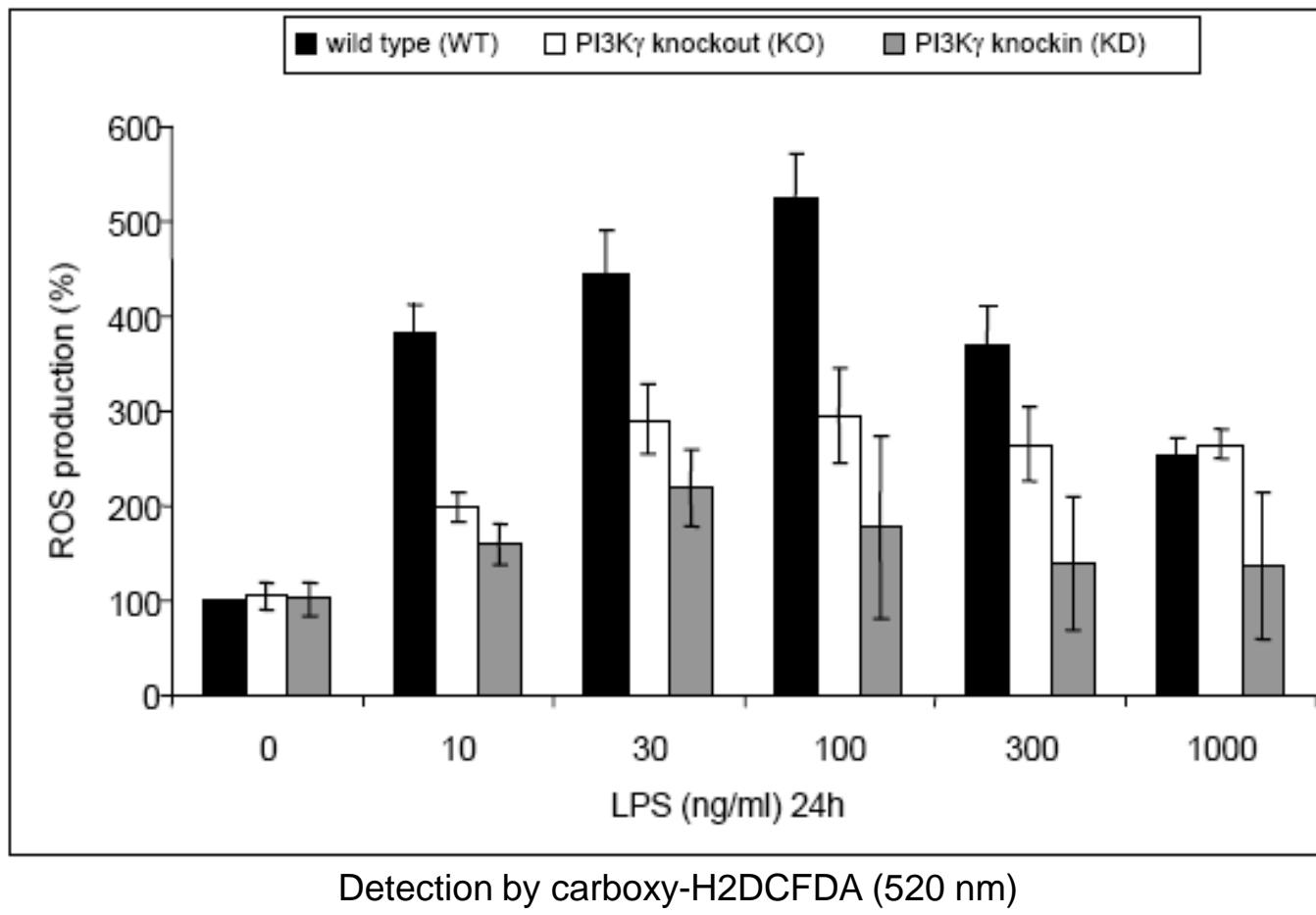
NAC (cyto ROS inhibitor) – N-acetylcysteine 20  $\mu$ M

DIDS (mito ROS inhibitor) - 4,4 diisothiocyanostilbene-2,2-disulfonic acid 10  $\mu$ M



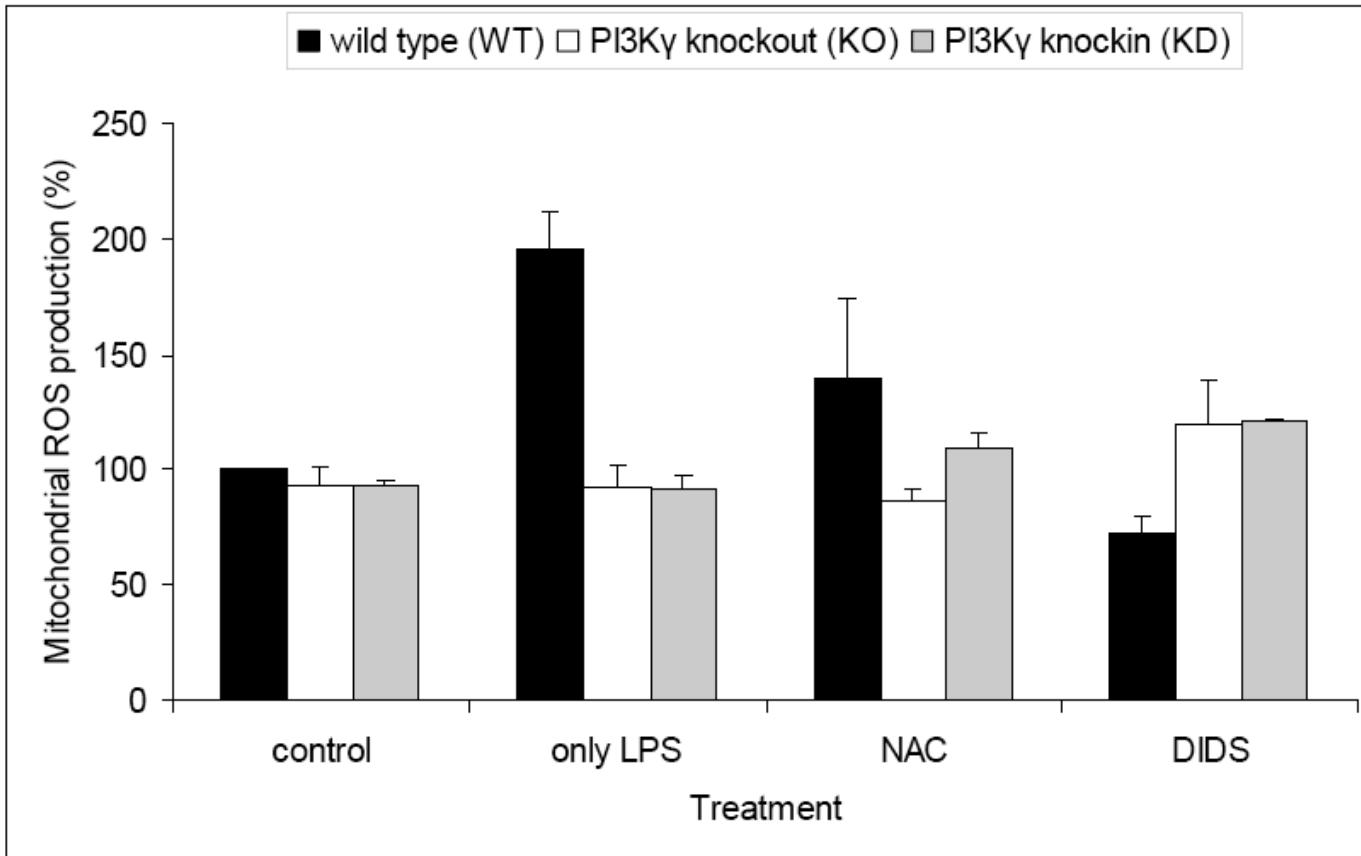
PI3K $\gamma$  mediates microglia stress response

# Dependence of LPS induced ROS production in microglia on PI3K $\gamma$ lipid kinase activity

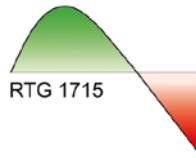


PI3K $\gamma$  mediates microglia stress response

# PI3K $\gamma$ is essential for LPS induced mitochondrial ROS production in microglia

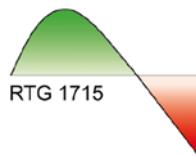
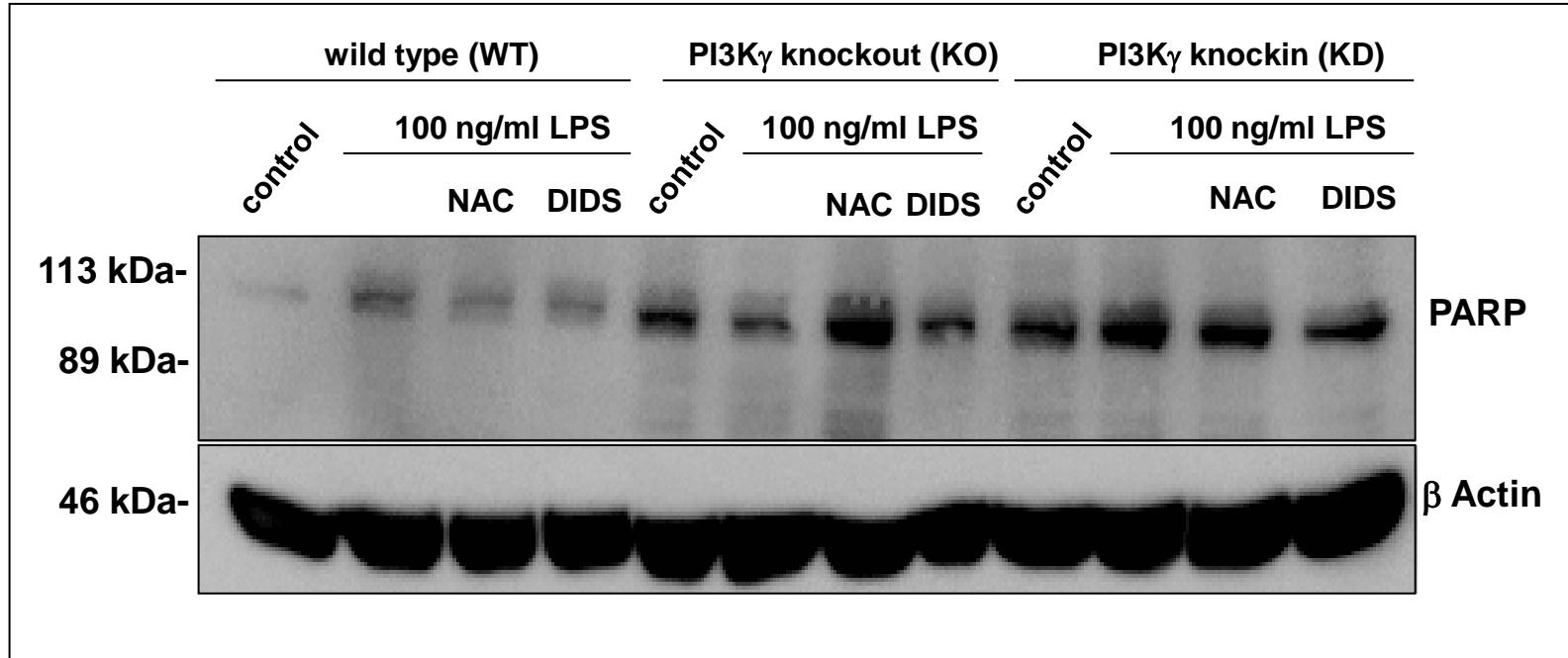


Detection by carboxy-H2DCFDA (520 nm)



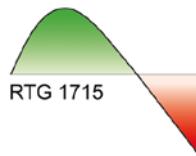
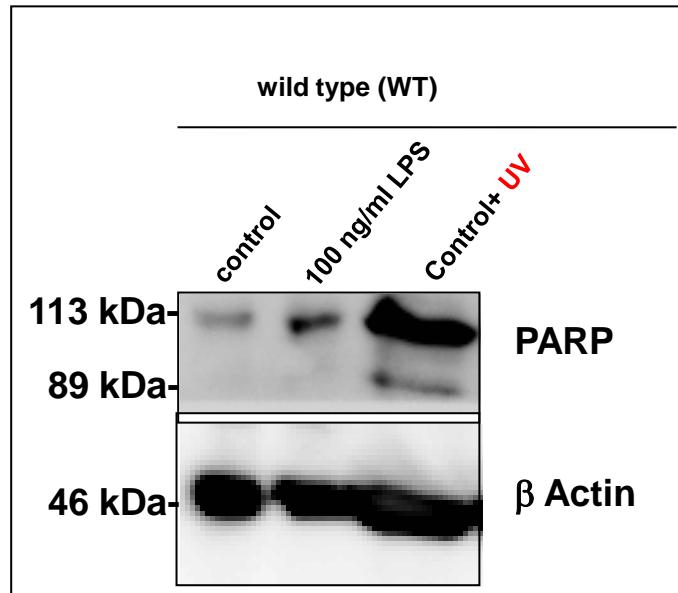
PI3K $\gamma$  mediates microglia stress response

# ROS inhibitors DIDS and NAC don't induce significant apoptosis after 6 days treatment



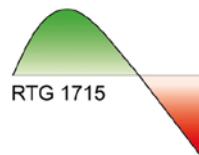
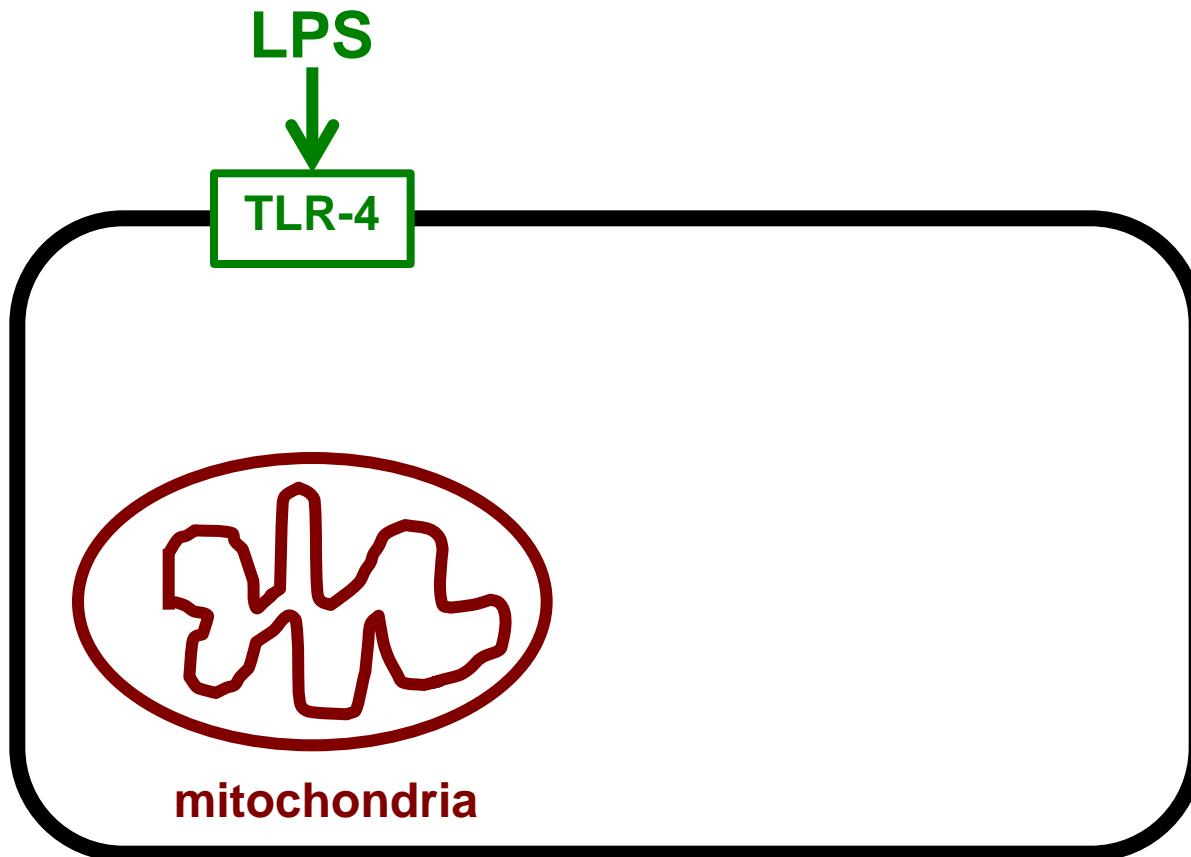
PI3K $\gamma$  mediates microglia stress response

# UV induced apoptosis after 6 days treatment



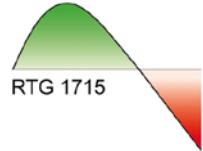
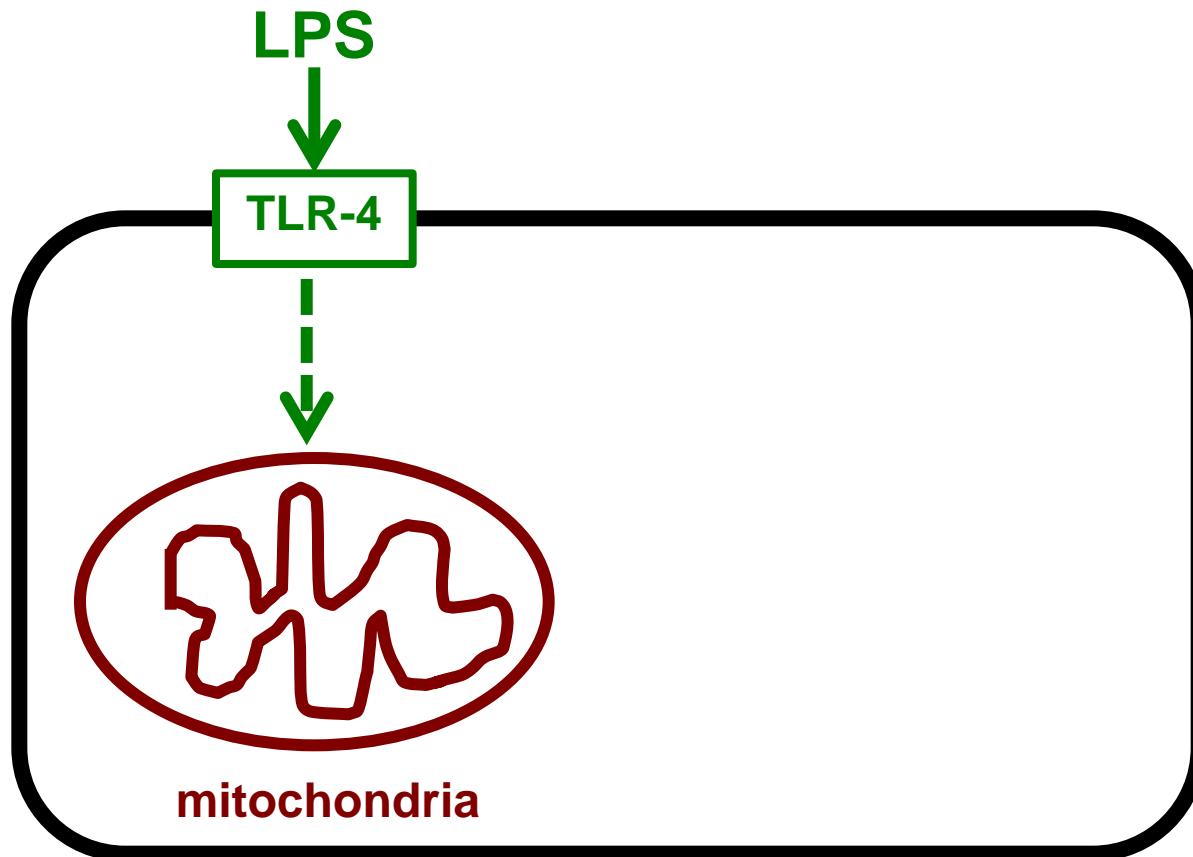
PI3K $\gamma$  mediates microglia stress response

# PI3K $\gamma$ and ROS as mediators of LPS induced stress response of microglia



Mitochondrial ROS signaling

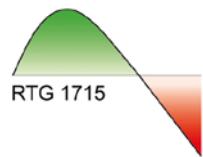
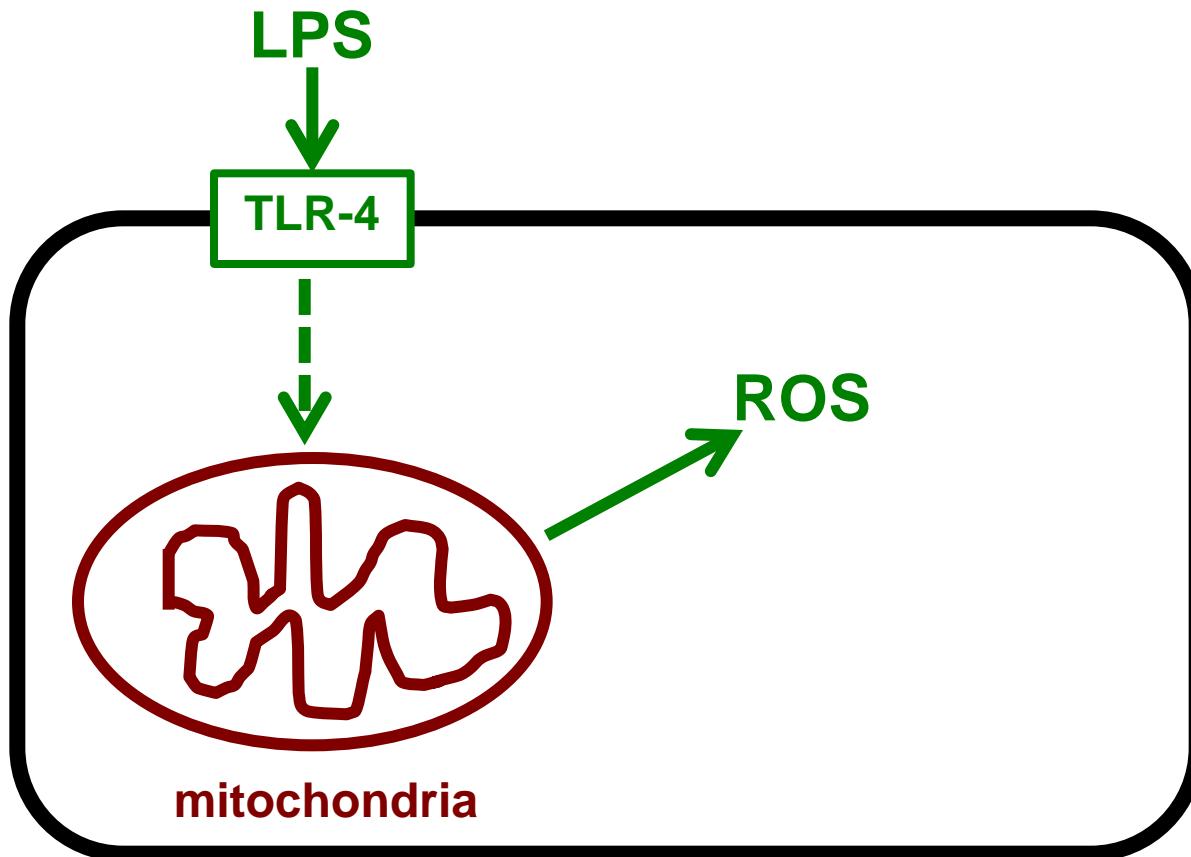
# PI3K $\gamma$ and ROS as mediators of LPS induced stress response of microglia



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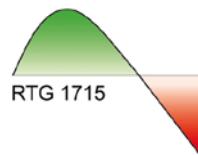
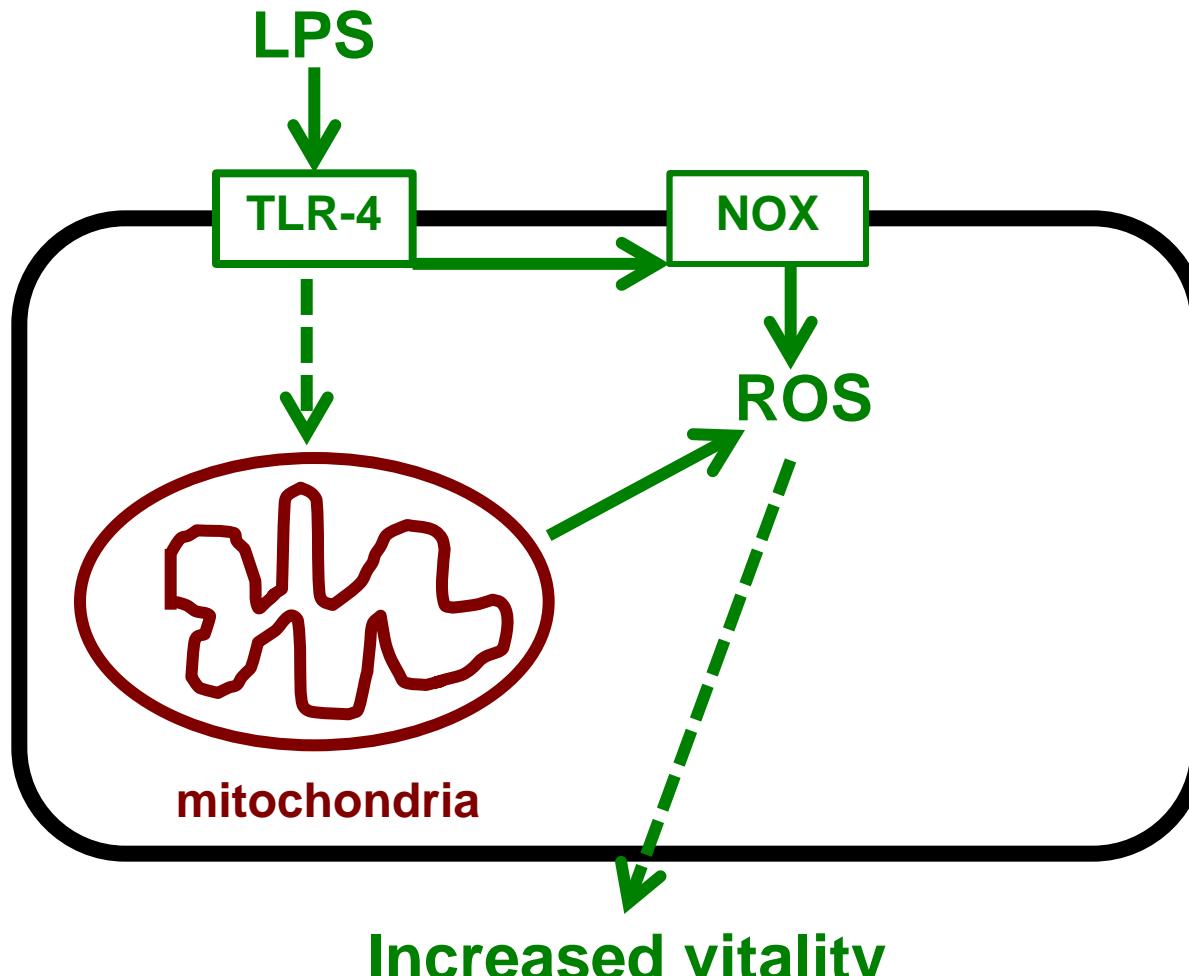
Mitochondrial ROS signaling

# PI3K $\gamma$ and ROS as mediators of LPS induced stress response of microglia



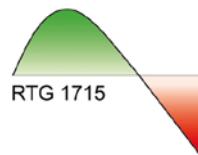
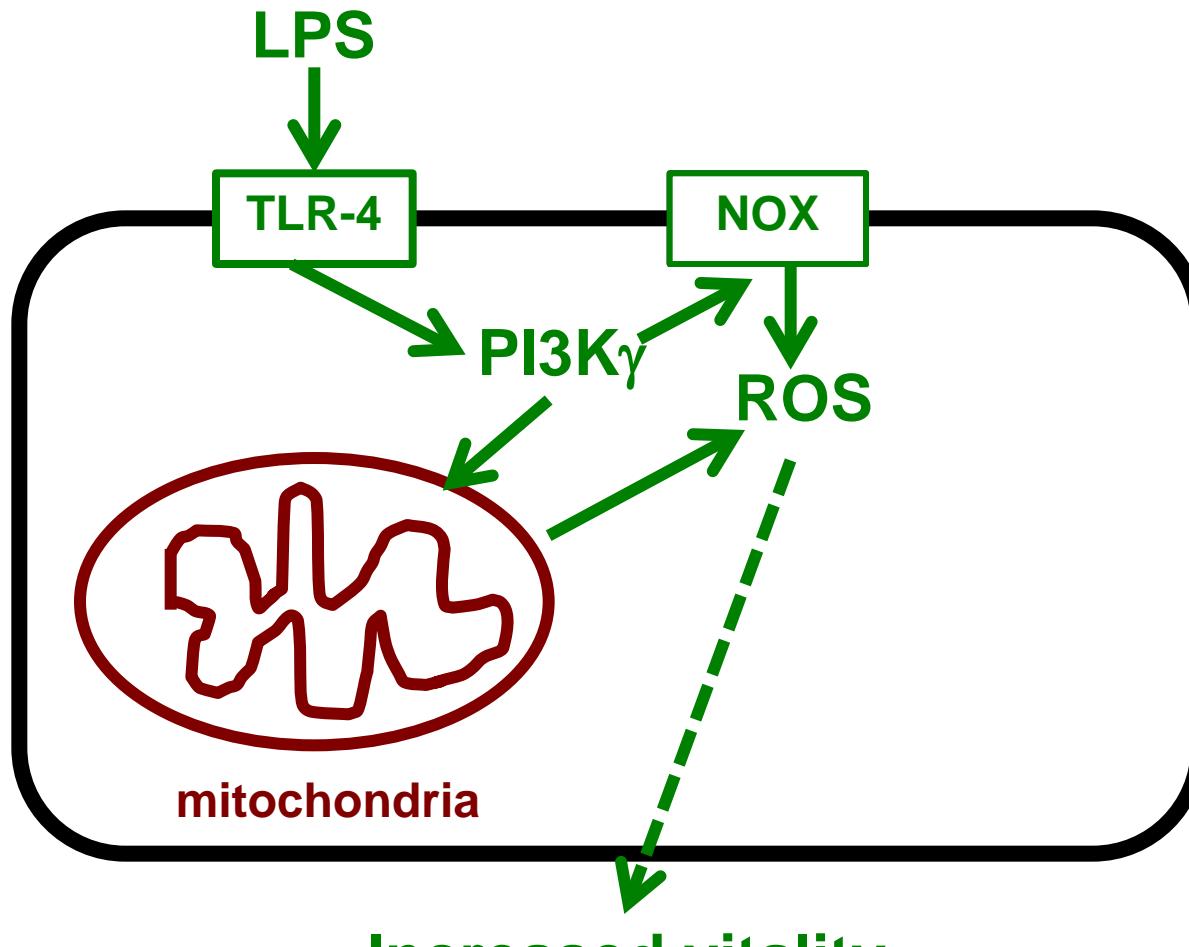
Mitochondrial ROS signaling

# PI3K $\gamma$ and ROS as mediators of LPS induced stress response of microglia



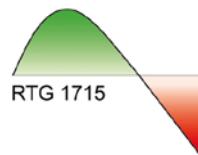
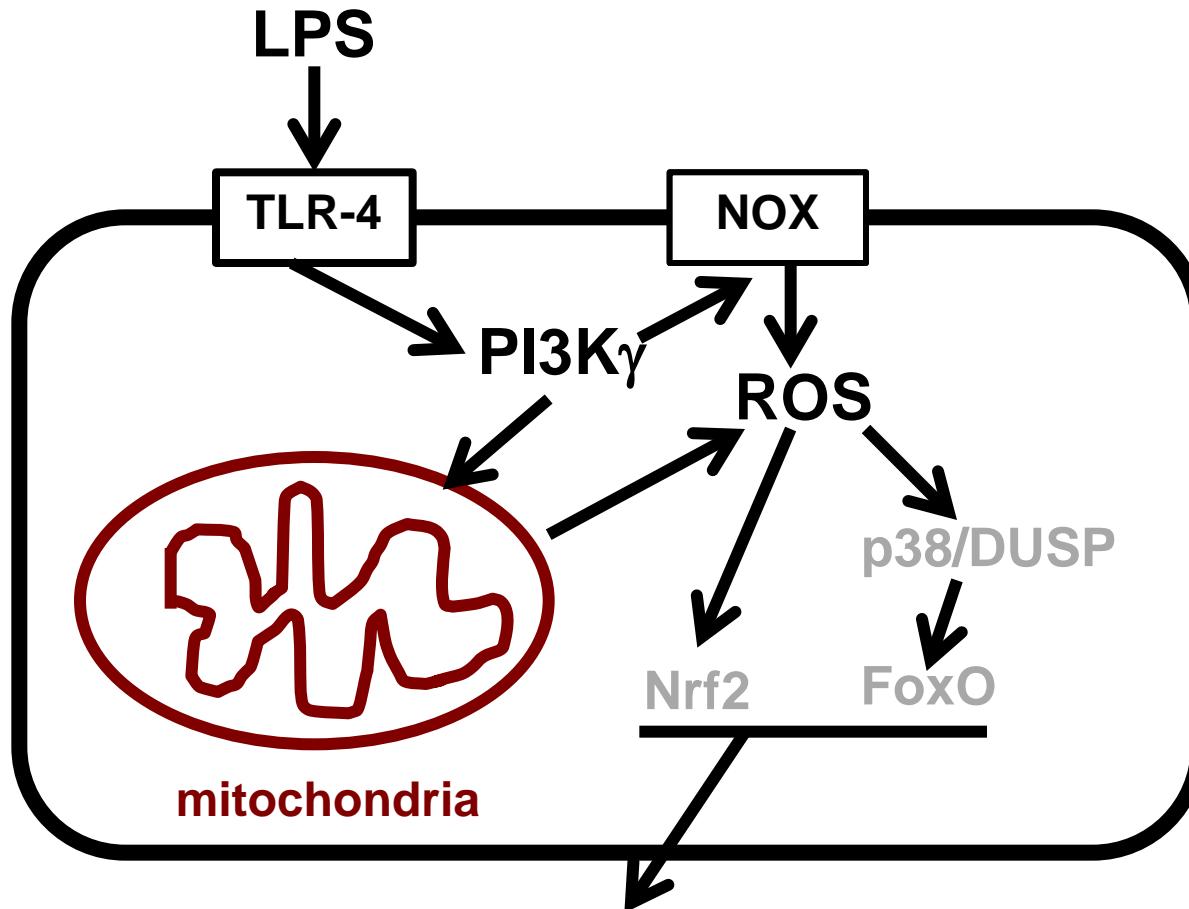
Mitochondrial ROS signaling

# PI3K $\gamma$ and ROS as mediators of LPS induced stress response of microglia



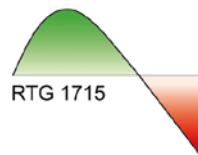
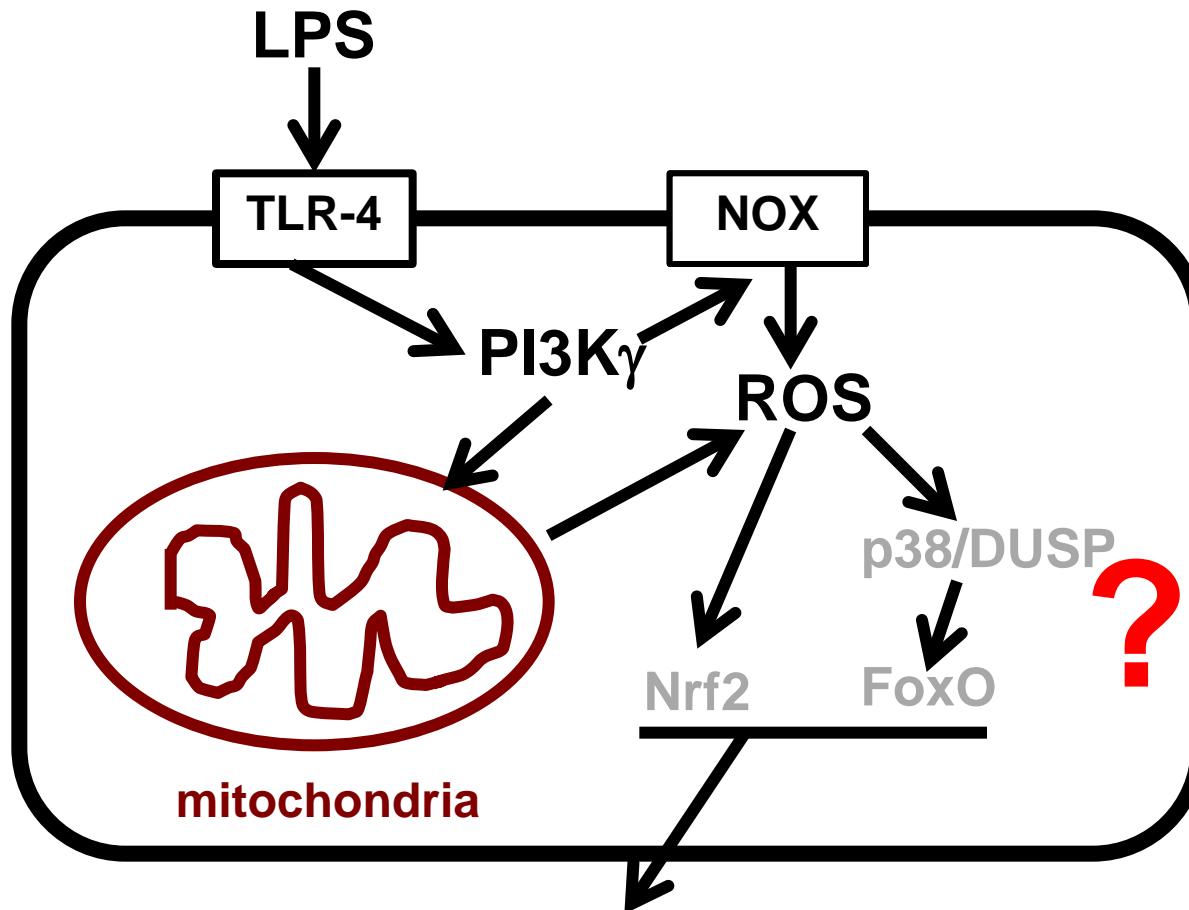
Mitochondrial ROS signaling

# PI3K $\gamma$ and ROS as mediators of LPS induced stress response of microglia



Mitochondrial ROS signaling

# PI3K $\gamma$ and ROS as mediators of LPS induced stress response of microglia

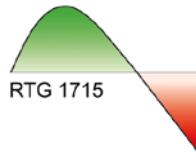


Mitochondrial ROS signaling

# **PI3K $\gamma$ and ROS as mediators of LPS induced stress response of microglia**

## ***Open Questions***

- **How PI3K $\gamma$  affects mitochondrial ROS production?**
- **Do cytosolic and mitochondrial ROS differentially affect LPS induced vitality?**
- **Mediators of ROS induced signaling.**

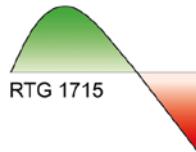


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Mitochondrial ROS signaling

# First general conclusions

- Adaptive (hormetic) stress responses of eucaryotic cells are reflected by specific signaling reactions.
- Adaptive stress responses are regulated by few specific mediators.
- Mitochondria may act specific intracellular stress sensors.





**JENA**