

# Modelling ageing to enhance a healthy lifespan

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**AXA**  
Research Fund

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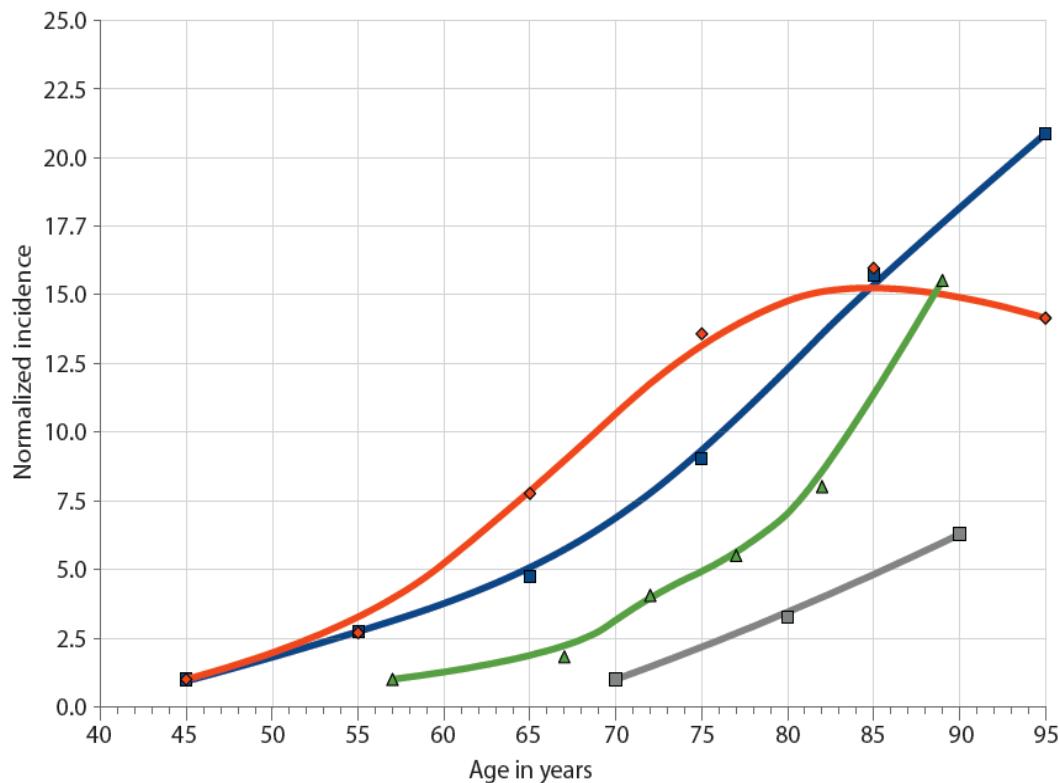
**MRC** | Medical  
Research  
Council



# Age is a major risk factor for chronic disease

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Chronic diseases are the major cause of death and disability in the world  
– estimated at around 70% WHO 2005

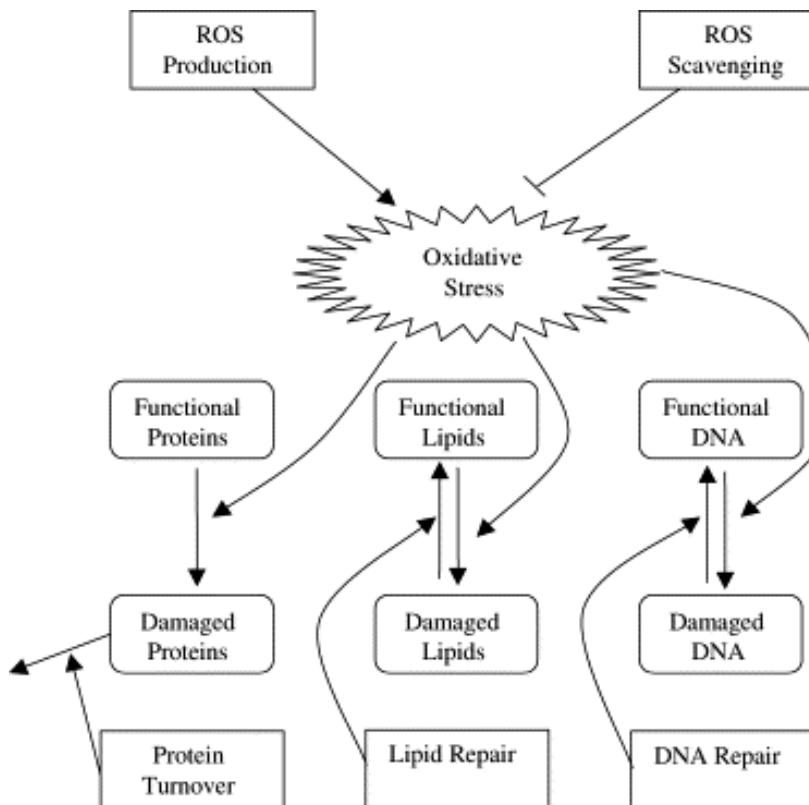


Cancer – DNA damage  
CVD – Oxidative stress and lipid peroxidation  
AD – Protein aggregation  
Infections

# Ageing results from the accumulation of molecular damage

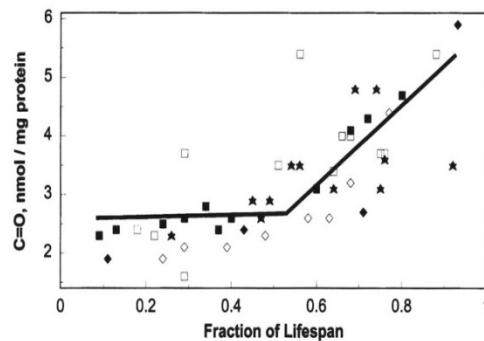
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Irreversible accumulation of macromolecular damage. Free radical/Oxidative stress theory of ageing (Harman 1956, 1976), protein and DNA copy errors, other sources of damage including glucose, toxins, UV.



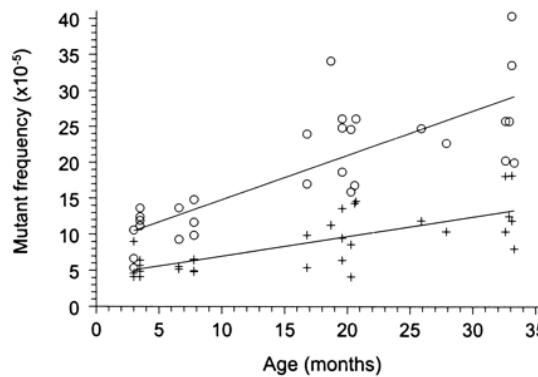
# Levels of molecular damage increase with age

Levels of  
oxidised Protein



Sohal et al 1996

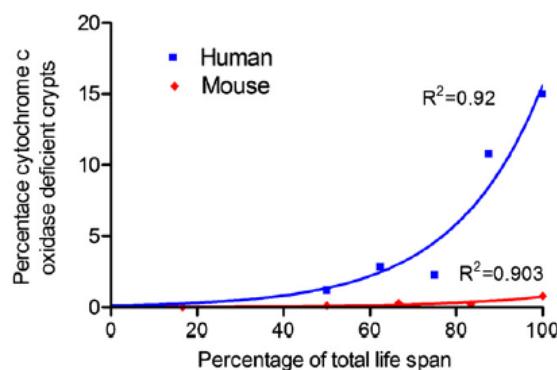
Mutational frequency  
In nuclear DNA



Small intestine  
heart

Dolle et al 2000

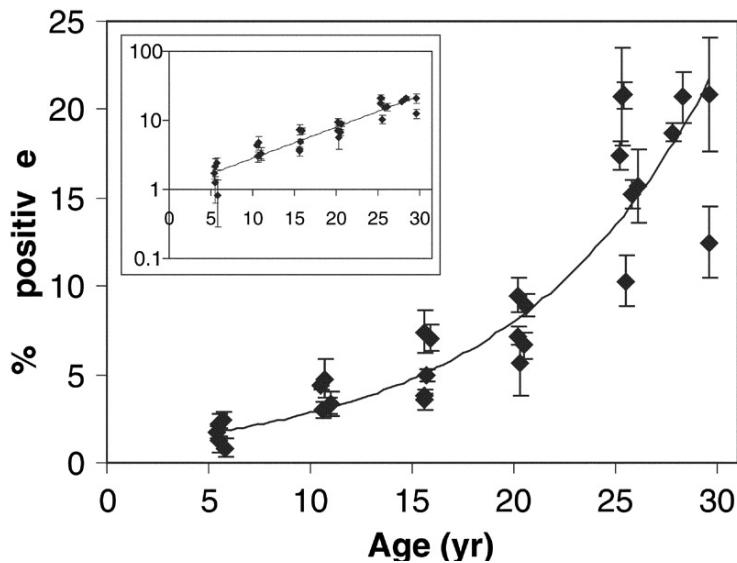
Mutational frequency  
In mitochondrial DNA



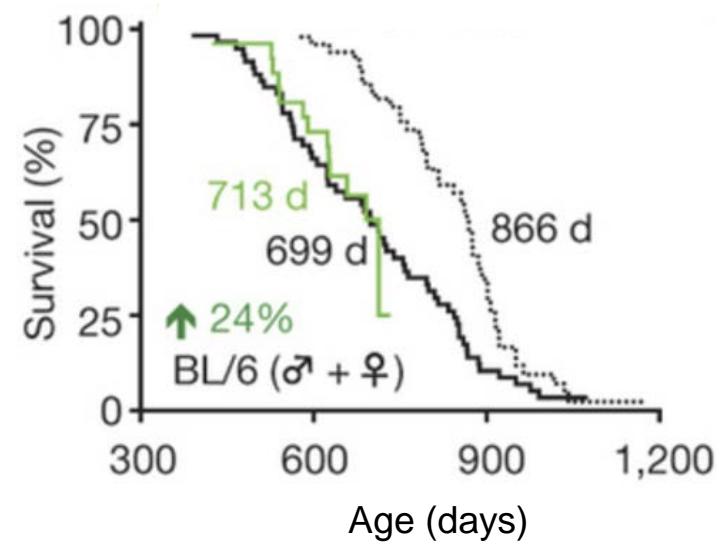
Greaves et al 2011

# Senescent cell frequencies increase with age and removal has been shown to affect survival

% Senescent cell (TIF) in skin with age (Baboons)



Survival benefit of removing senescent cells beginning 1 year (mouse)

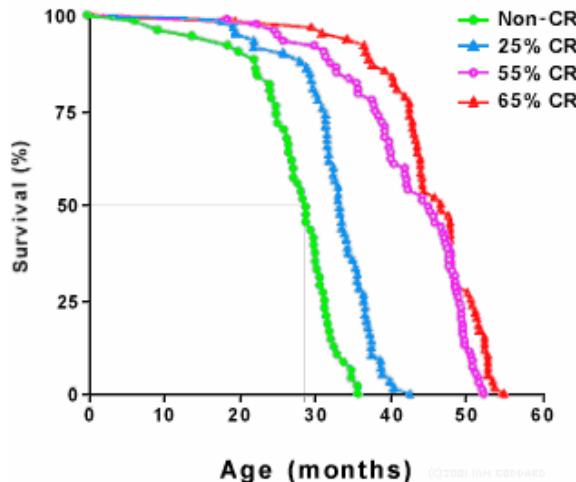


Herbig et al Science 2006

Baker et al Nature 2016

# Plasticity in ageing

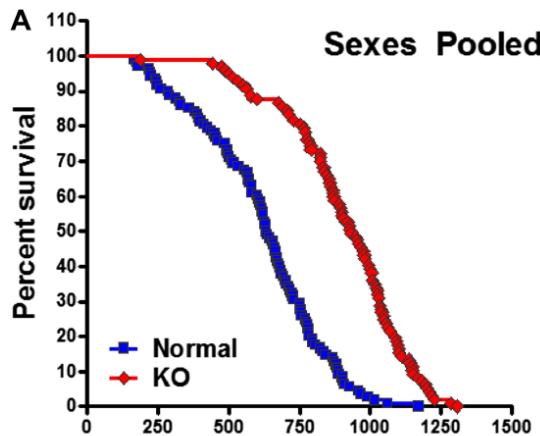
## Nutrition e.g. Caloric restriction



Weindruch et al J Nutr 1986

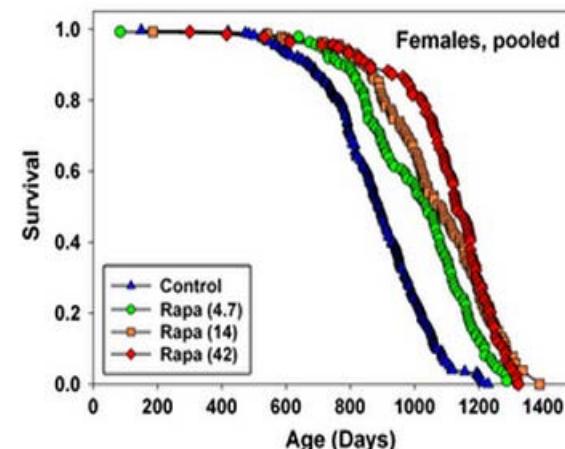


## Genetic e.g. GHRHKO dwarf mice



Sun et al eLife 2013

## Drugs e.g. Rapamycin - dose response



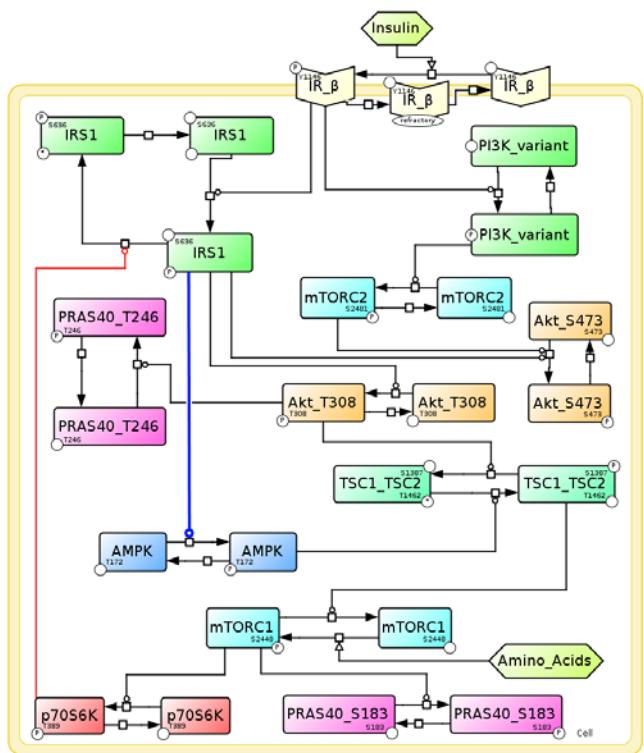
Miller et al Aging Cell 2014

# What are we trying to achieve with computational modelling in ageing research

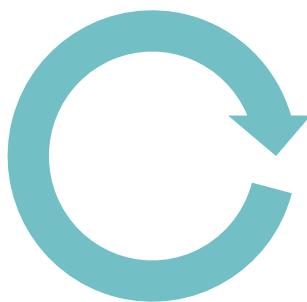
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- With known mechanisms we can explore interventions
- With known interventions we can explore mechanisms
- With known interventions and mechanisms we can use the models to optimise synergy/antagonism, dose and timing
- We know there are multiple mechanisms in damage accumulation which can be modulated together through targeting high level regulatory processes
- Most damage accumulation and indeed mortality level increase exponentially (positive feedback) – can we slow or break the cycle?

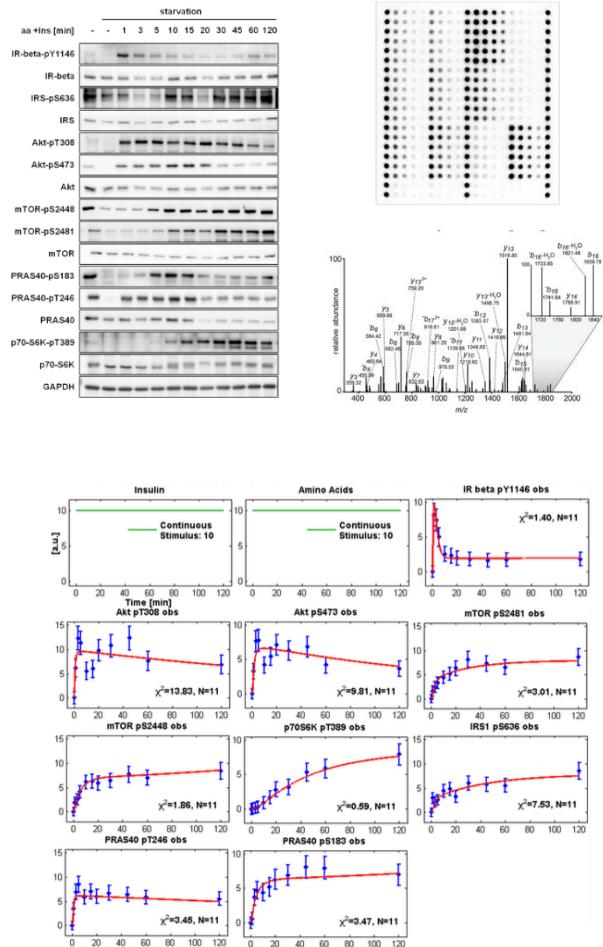
# Iterative modelling process



Data  
Network

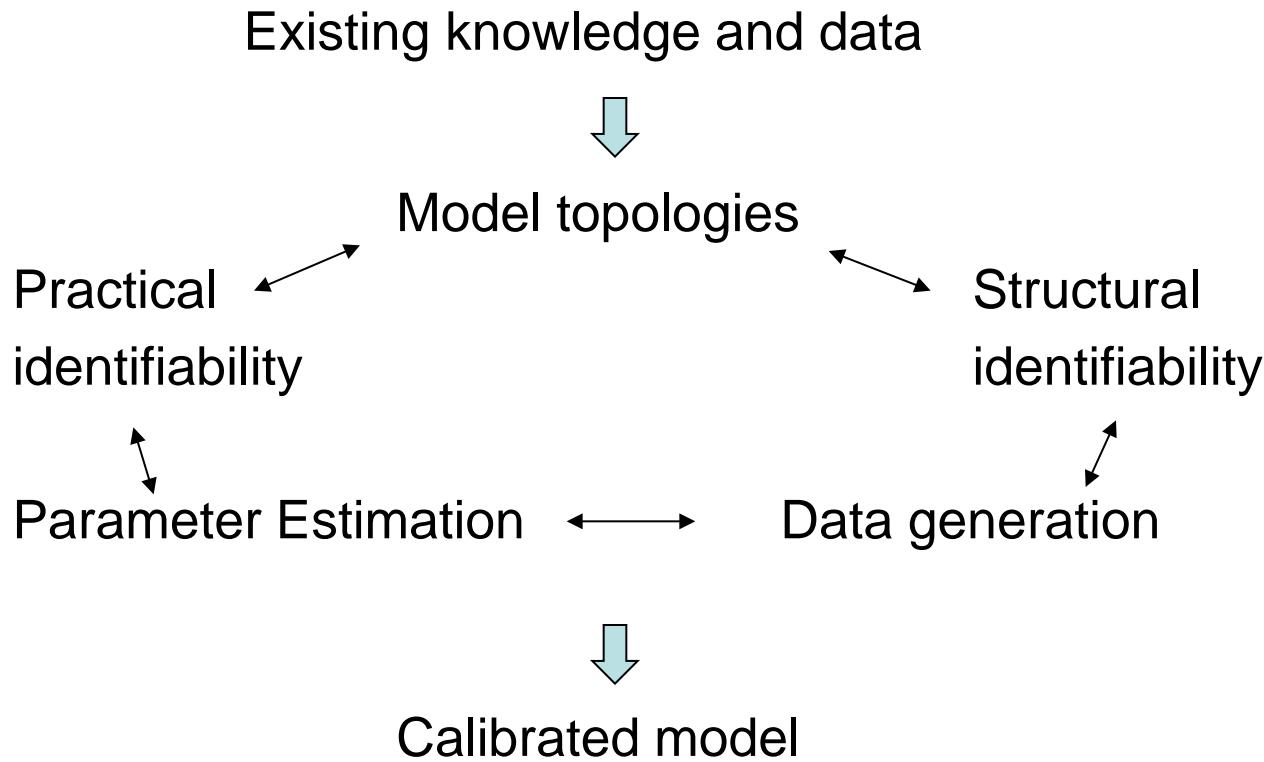


Model



# Computational model development

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Sensitivity analysis

Validate model simulations with selected perturbations

Predictions

# Overview of wet-laboratory and computational methods

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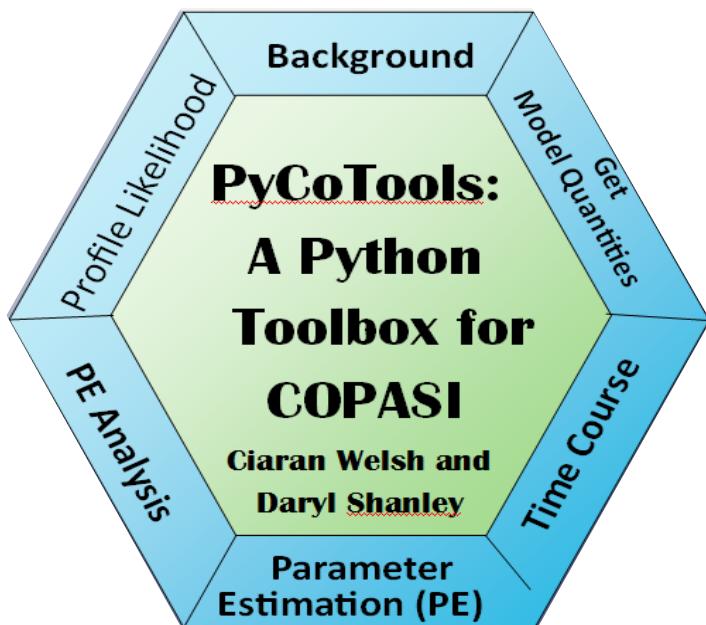
## Wet-laboratory

- Mostly cellular level using cell lines (MRC5, HeLa, c2c12...)
- Population – Western blotting, RPPA, Mass Spec  
*quantification e.g. normalization and multiplexing*
- Single cell – FACs, microscopy

## Computational

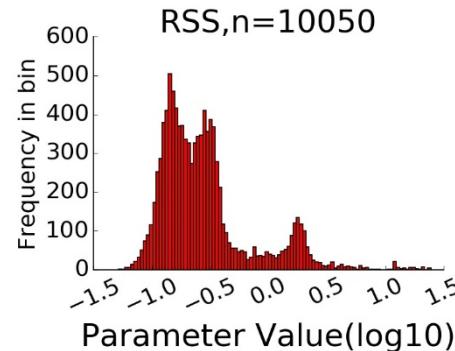
- Cytoscape, CellDesigner
- COPASI \*, Matlab (PottersWheel, Data2Dynamics)

# Software development for automating computational tasks



Available from github and can be installed with `$pip install PyCoTools`

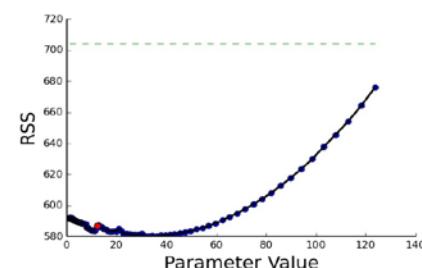
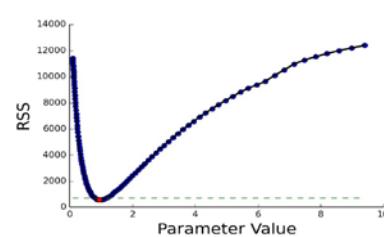
## Analysis of output from parameter estimation



## Profile likelihood for identifiability

Identifiable parameter

Non-identifiable

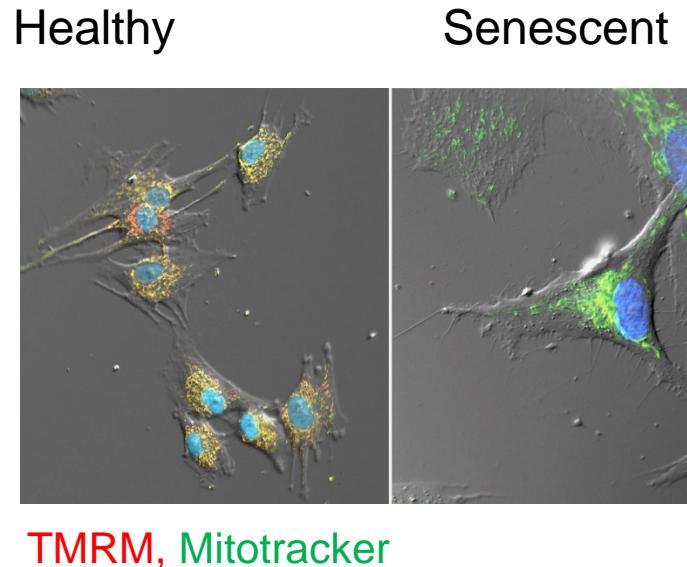


(Raue 2009; Schaber 2012)

# Modelling cellular senescence

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- A permanent and irreversible arrest of cell proliferation due to uncapped telomeres or DNA damage via a DNA Damage Response (DDR)
- Senescent cells alter tissue function
- Proportion of senescent cells increases with age (*Herbig et al Science 2006*)
- Clearance of senescent cells delays age-related disorders and enhances survival (*Baker et al Nature 2014; 2016*)
- Cell signaling, mitochondrial dysfunction, chromatin structural changes, changed morphology, transcriptional reprogramming including secreted bioactive peptides (SASP), ...



**Systems approach to model the development of the senescent phenotype with a view to find interventions to prevent progression and reverse the phenotype**

# Key processes in cellular senescence

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Cellular senescence involves complex interplay of:

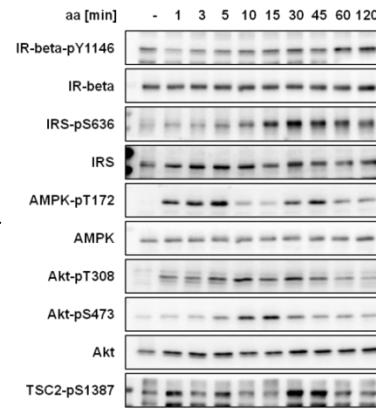
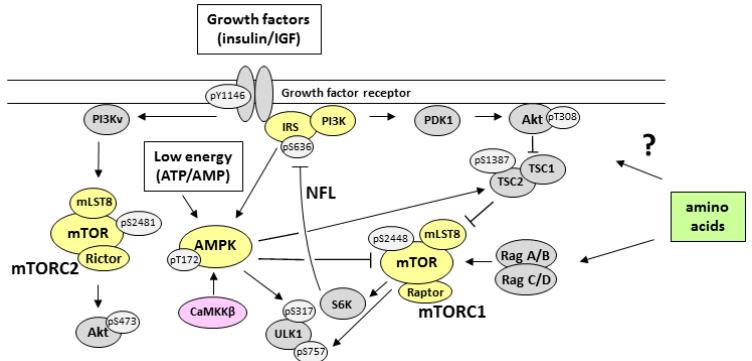
1. Insulin-TOR-AMPK signalling
2. Stress response e.g. FoxO and JNK
3. DNA damage repair and DDR signalling
4. Mitochondrial dynamics and Reactive Oxygen Species
5. Metabolism
6. SASP

# Key processes in cellular senescence

## 1. Insulin-TOR-AMPK signalling

(Dalle Pezze et al 2012, 2014, 2016; Sonntag et al 2012; Smith & Shanley 2013)

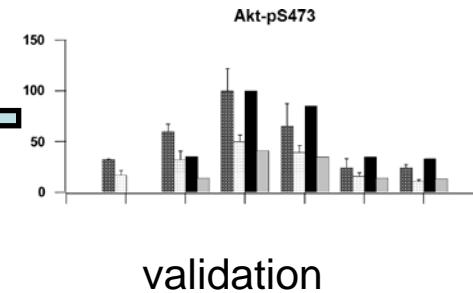
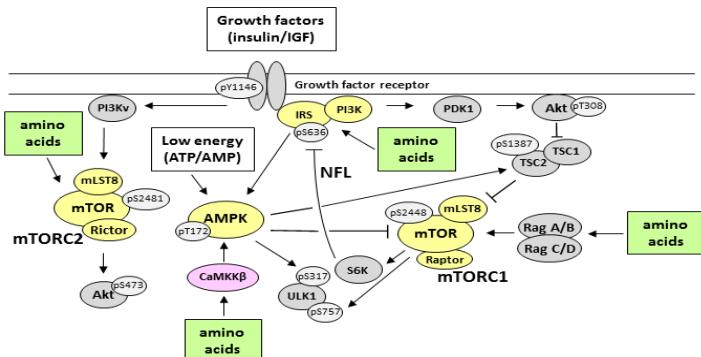
How do amino acids stimulate mTOR network? (Dalle Pezze et al 2016)



time course data

Amino Acid Inputs in addition to mTORC1	AIC
IRS-p, AMPK-pT172, mTORC2	680.23
IRS-p, AMPK-pT172, PI3K_Variant-p	723.26
IRS-p, AKT-pT308, mTORC2	794.55
IRS-p, AKT-pS473, mTORC2	798.72
IRS-p, mTORC2, PI3K_Variant-p	799.24
IRS-p, AKT-pT308, PI3K_Variant-p	862.10
IRS-p, AMPK-pT172, AKT-pS473	862.72
IRS-p, AKT-pS473, PI3K_Variant-p	885.01
IRS-p, AKT-pT308, AKT-pS473	901.91
IRS-p, AMPK-pT172, AKT-pT308	916.40
AMPK-pT172, AKT-pT308, mTORC2	834.65
AMPK-pT172, AKT-pT308, PI3K_Variant-p	880.76
AMPK-pT172, AKT-pT308, AKT-pS473	884.79
AMPK-pT172, AKT-pS473, mTORC2	906.65
AMPK-pT172, mTORC2, PI3K_Variant-p	918.46
AMPK-pT172, AKT-pS473, PI3K_Variant-p	944.06
AKT-pT308, AKT-pS473, mTORC2	1004.40
AKT-pT308, mTORC2, PI3K_Variant-p	1017.12
AKT-pT308, AKT-pS473, PI3K_Variant-p	1047.02
AKT-pS473, mTORC2, PI3K_Variant-p	1213.74

identify network topology



validation



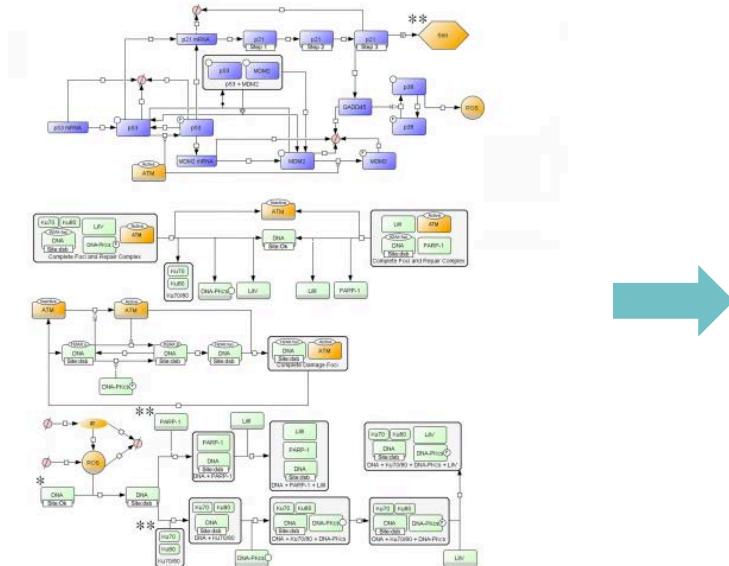
Akt pS473

$\chi^2=63.10$ , N=10

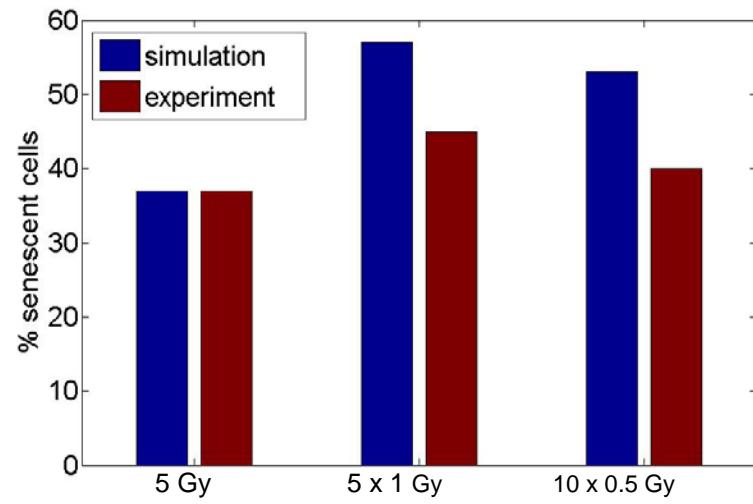
calibrated model

# Key processes in cellular senescence

1. Insulin-TOR-AMPK signalling
2. Stress response e.g. FoxO and JNK  
(Smith & Shanley 2010, 2013)
3. DNA damage repair and DDR signalling  
(Dolan et al 2013, 2015)



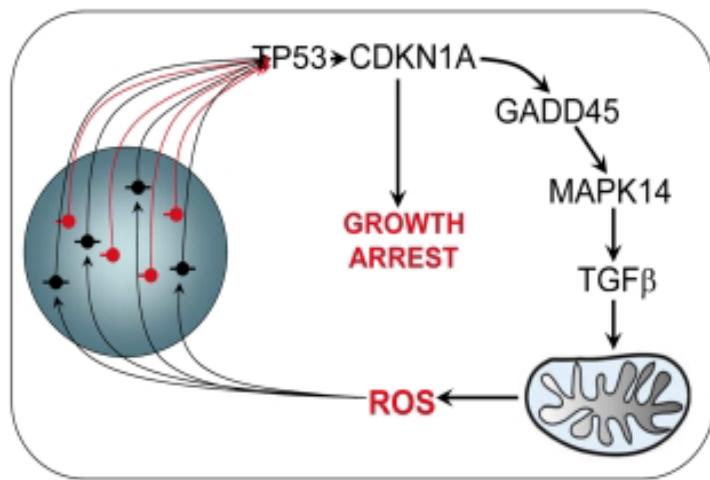
Integrative model of NHEJ and DDR



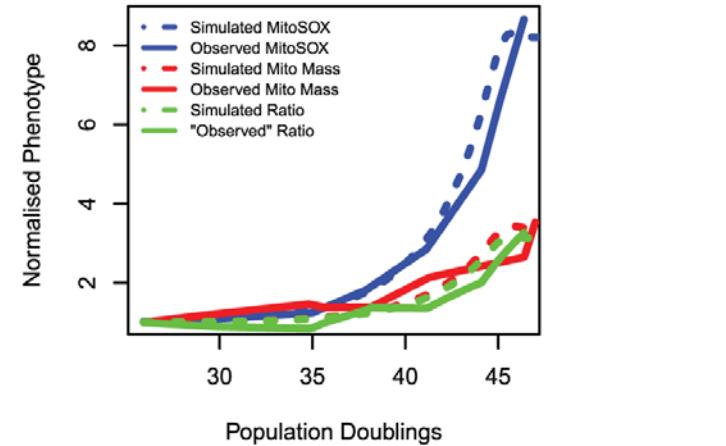
Repeat low dose irradiation as effective has single high dose

# Key processes in cellular senescence

1. Insulin-TOR-AMPK signalling
2. Stress response e.g. FoxO and JNK
3. DNA damage repair and DDR signalling
4. Mitochondrial dynamics and Reactive Oxygen Species  
(Passos *et al* 2007, 2010; Lawless *et al* 2012; Tomalin *et al* 2016)



Positive feedback of DNA damage and mitochondria/ROS



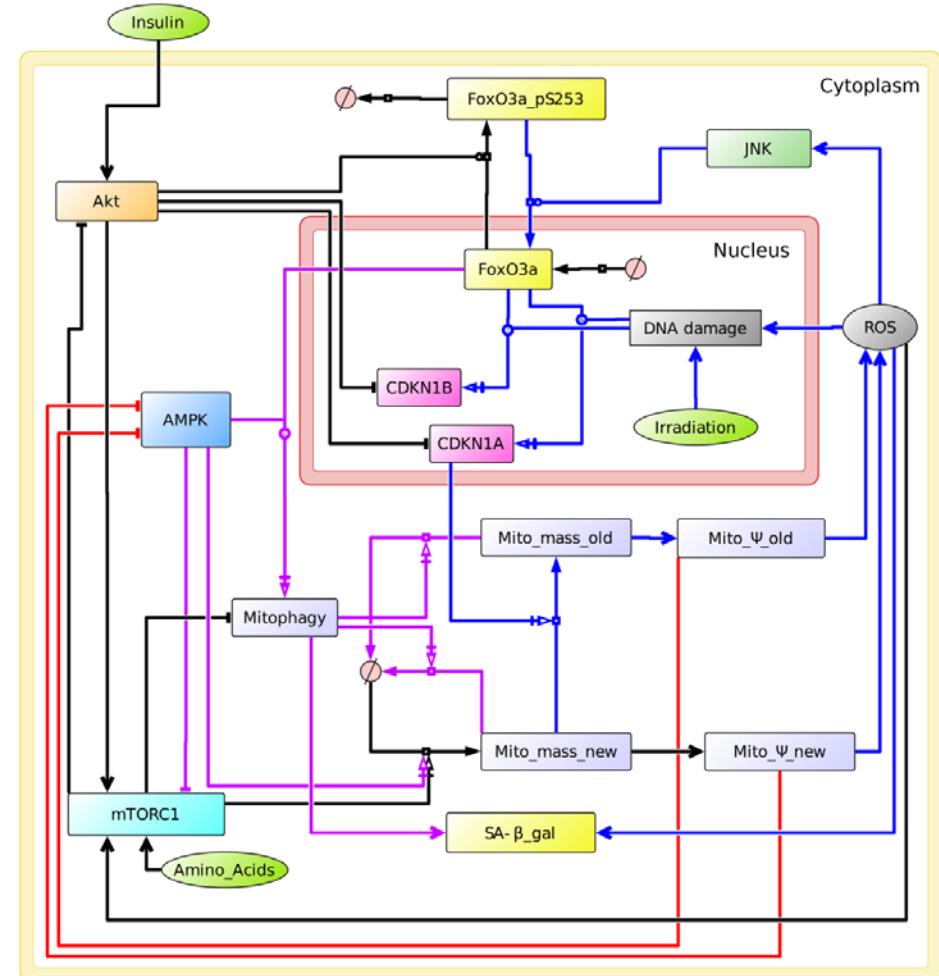
Simple model supports role of this feedback

# An integrative dynamic model for cellular senescence

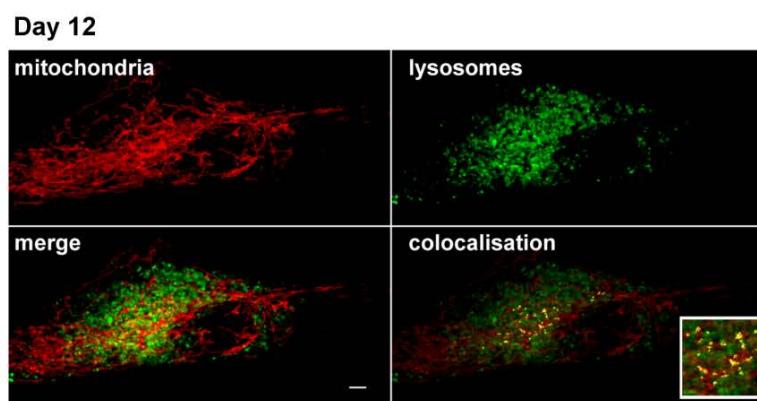
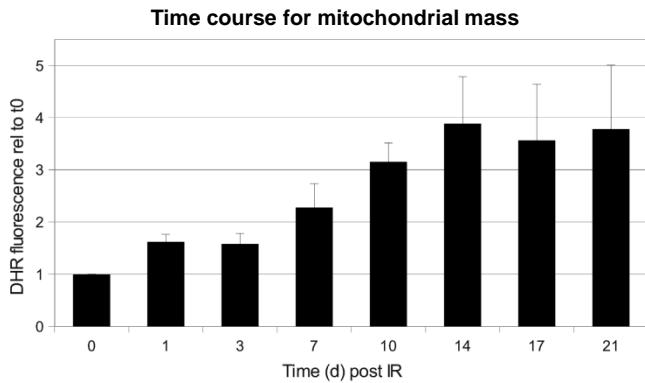
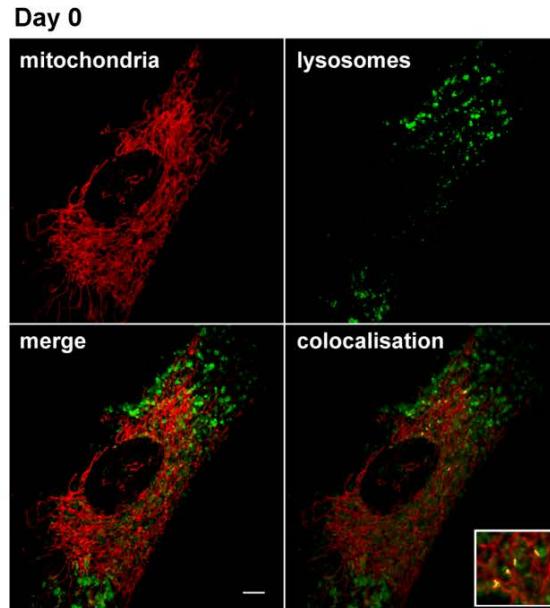
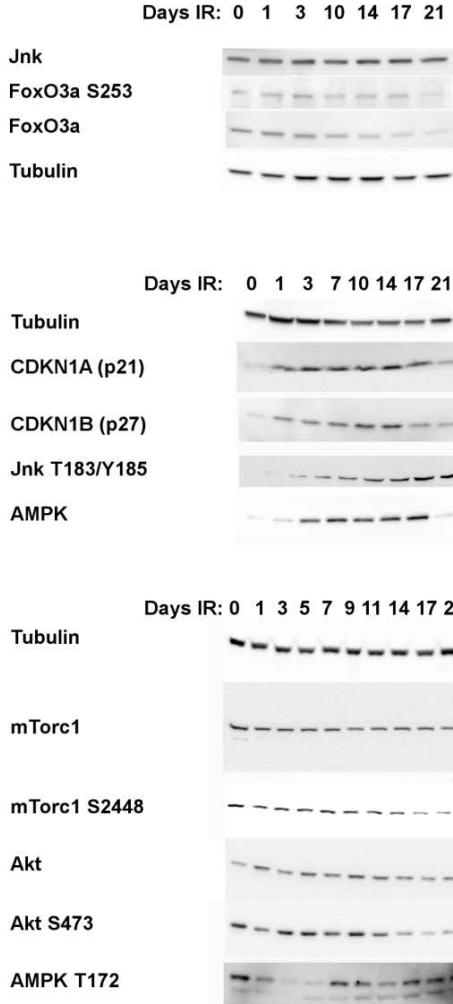
Integration of the 4 key processes of insulin-mTOR-AMPK, FOXO/JNK, DNA damage/DDR, and mitochondria/ROS into one dynamic model

How do these different process interact to generate the senescent state?

Can we find treatments to intervene?

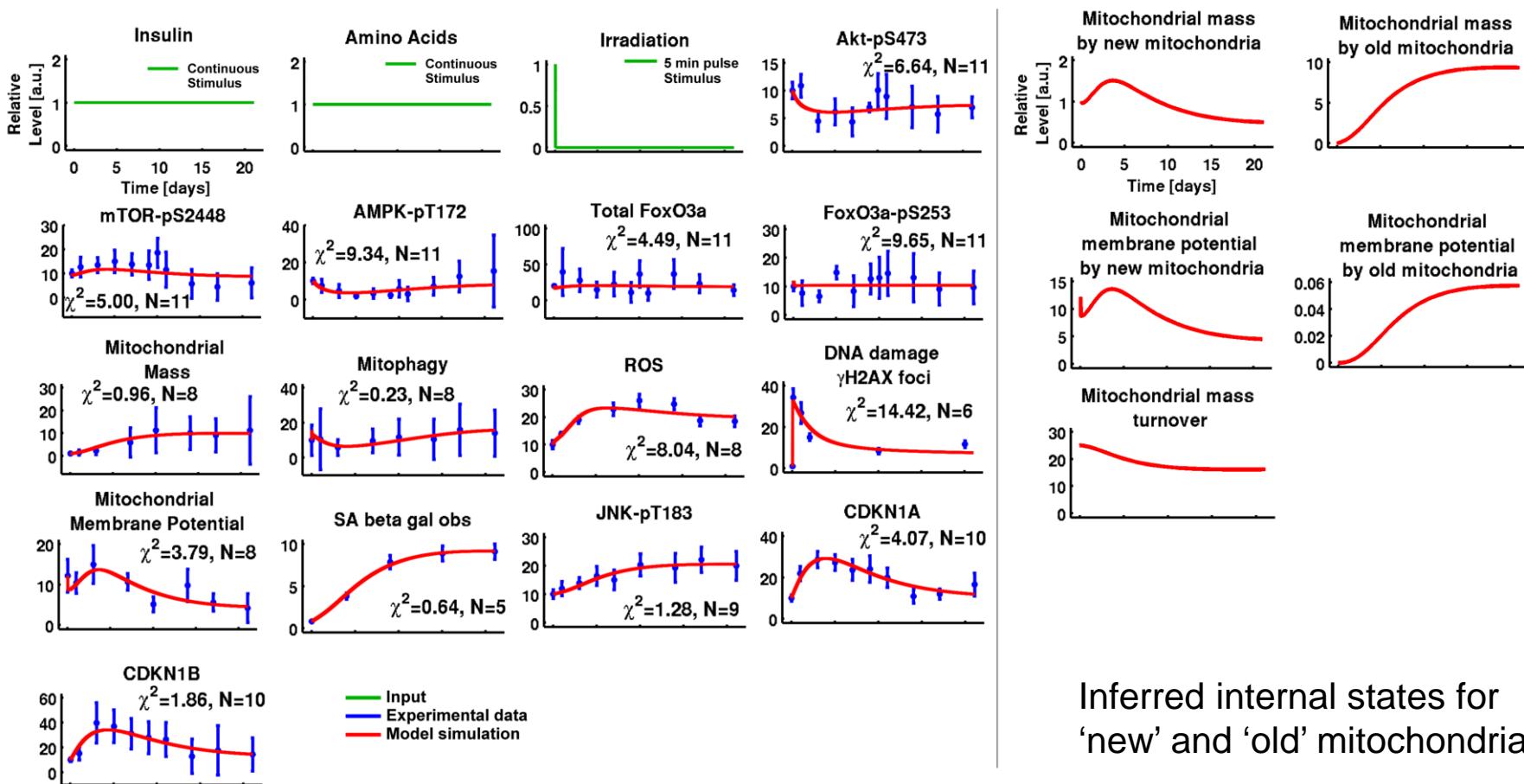


# *In vitro* time course data for estimating the model parameters



- Human Diploid fibroblast MRC5
- 20 Gy irradiation (5 min)
- 21 days post irradiation
- Minimum n=5

# *In silico* model fit to *in vitro* time course data



Inferred internal states for  
'new' and 'old' mitochondria

*In vitro* data are Mean  $\pm$  1 St Dev

# Applications for the model

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Interventions for improving mitochondrial function and phenotype

## *In silico* predictions

ROS inhibition



## *In vitro* testing

SOD + Catalase

mTOR inhibition



Torin1

Combined ROS-mTOR inhibition



SOD + Catalase + Torin1

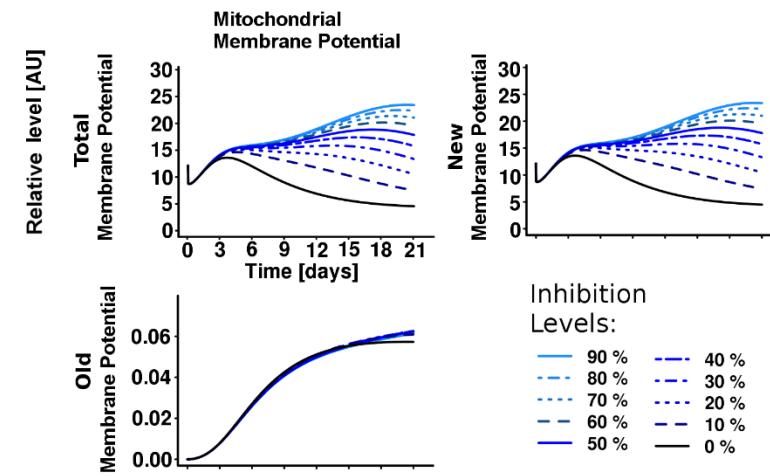
AMPK activation



Metform

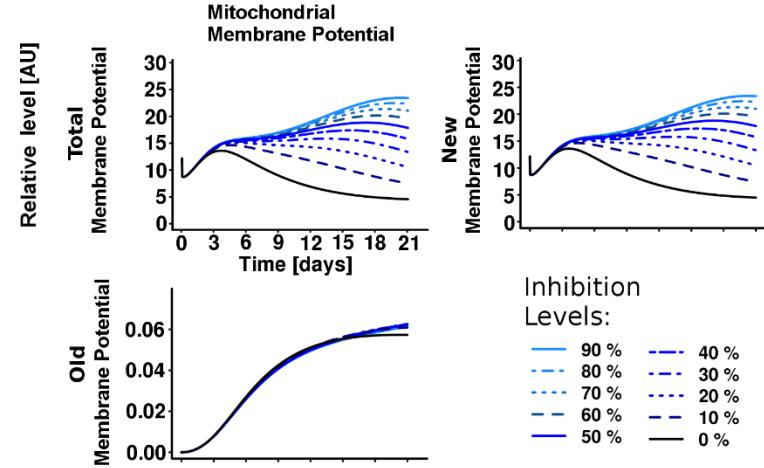
# Model prediction of increased mitochondrial membrane potential upon ROS inhibition

Model simulation upon ROS inhibition

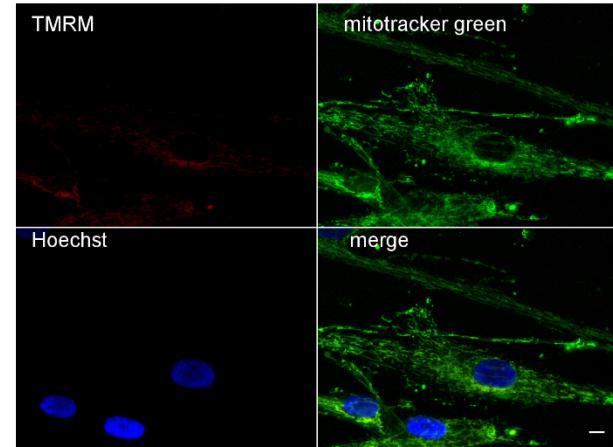


# In vitro confirmation of increased mitochondrial membrane potential by ROS inhibition

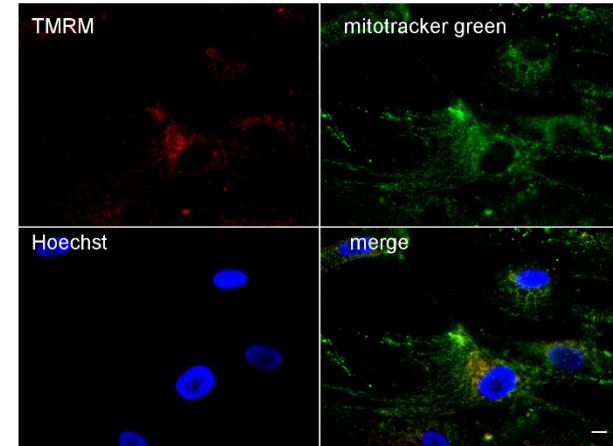
Model simulation upon ROS inhibition



15 day control

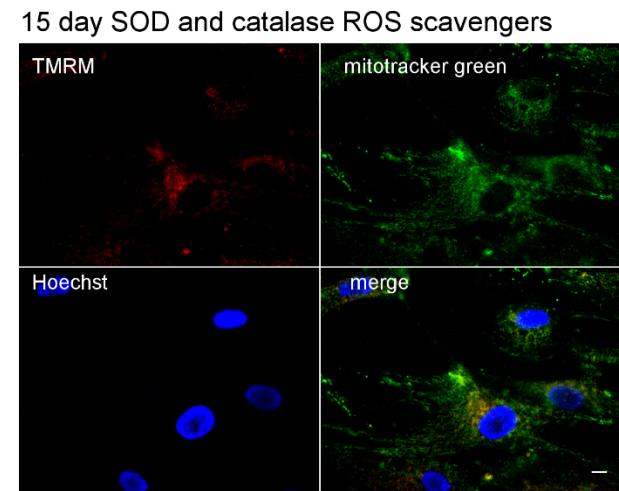
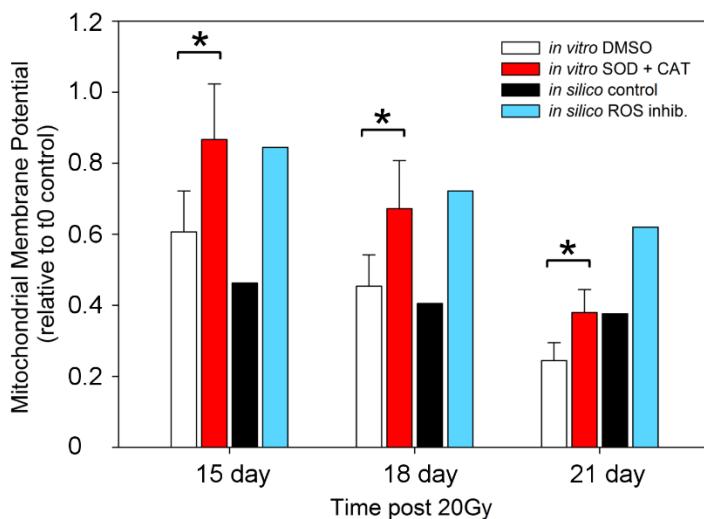
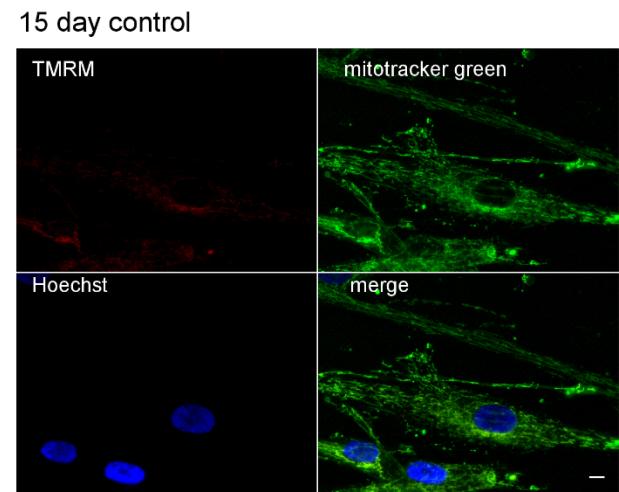
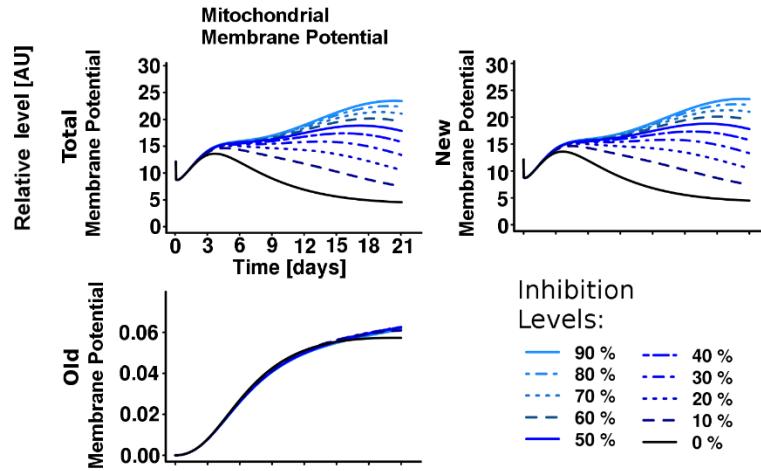


15 day SOD and catalase ROS scavengers



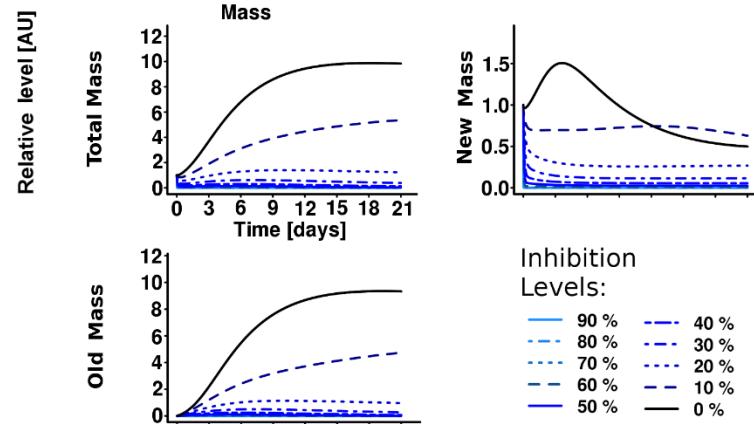
# In vitro confirmation of increased mitochondrial membrane potential by ROS inhibition

Model simulation upon ROS inhibition

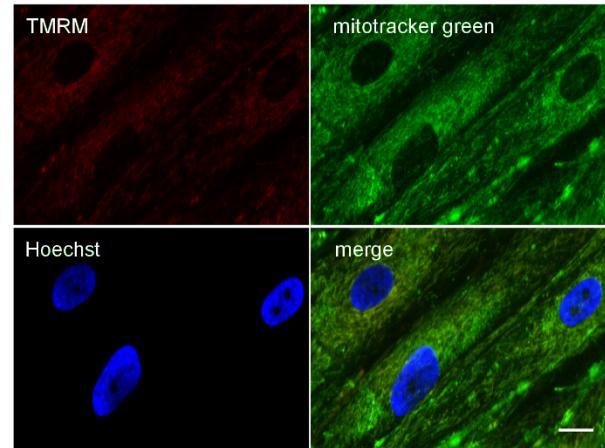


# mTOR inhibition by Torin1 decreased mitochondrial mass, but lost effectiveness over time

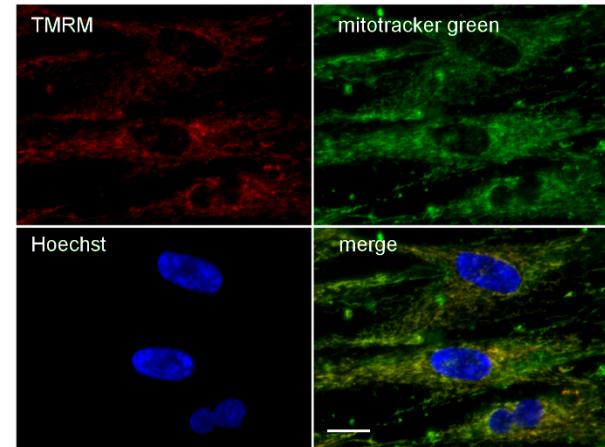
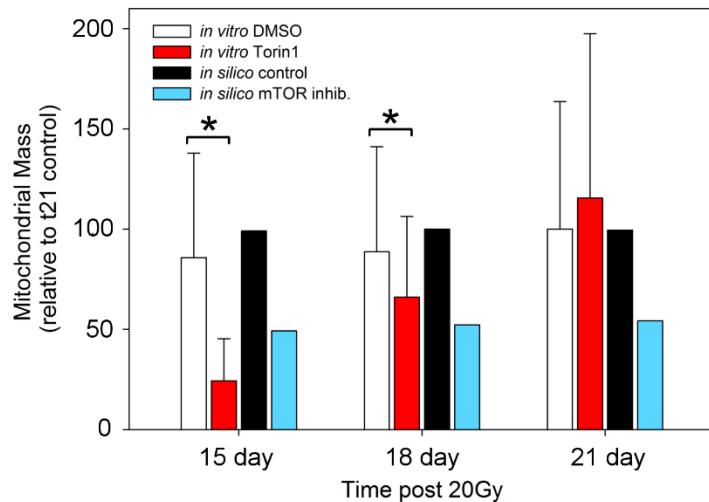
Model simulation upon mTOR inhibition



15 day control



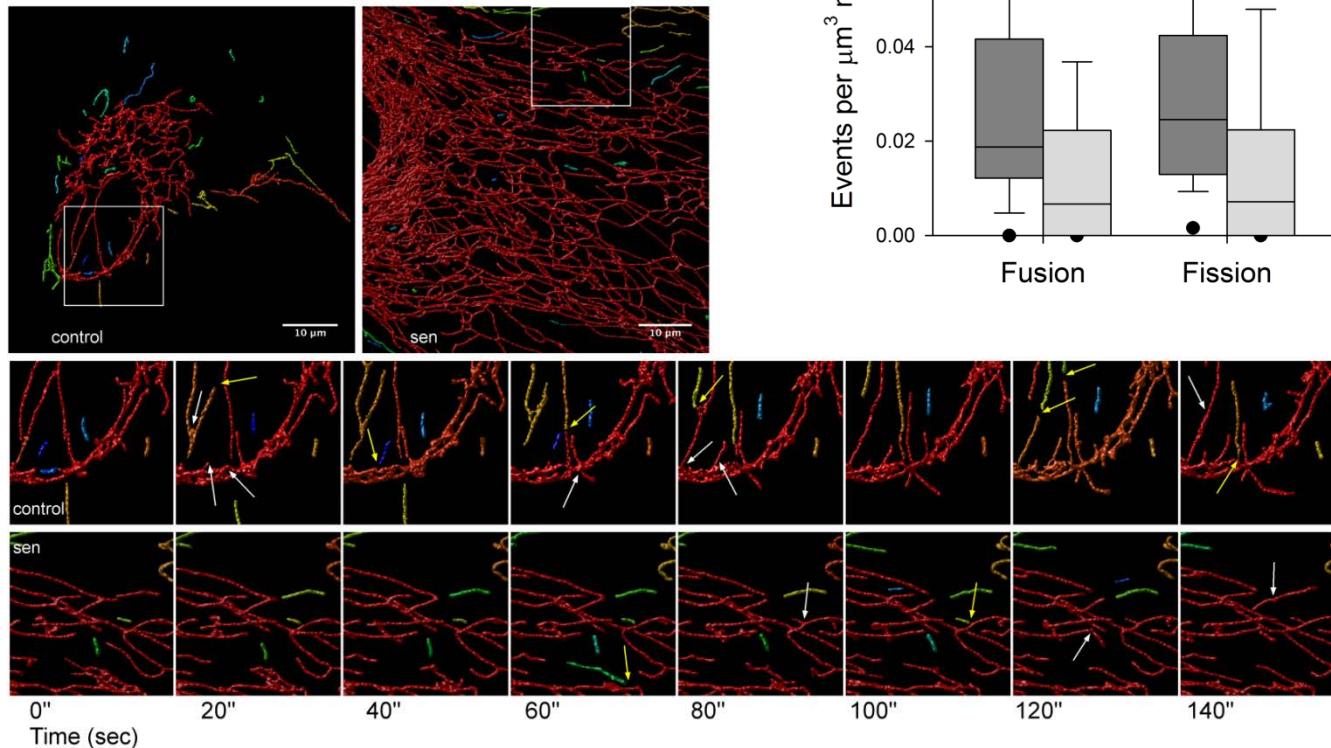
15 day Torin1



# Why were global mitochondria function and phenotype not restored?

Mitophagy is functional:

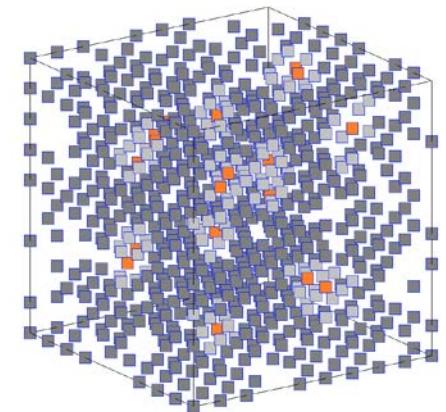
- Mitophagy is functional
- loss of turnover for the old mt population suggests fission events decrease
- A decrease in fusion and fission events confirmed in senescence.



# Conclusions and future directions

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- We know that ageing is caused by accumulation of molecular damage and we know a lot of mechanistic details. We also know it is plastic but the mechanisms are multiple and highly connected – requires systems biology.
- We are making progress in modelling several processes important in ageing.
- Developments in wet-lab and computational technologies are helping.
- A process such as cellular senescence is clearly a complex phenotype and a systems approach provides a means to probe its development and maintenance.
- A combination of mTOR specific inhibitor, antioxidants and AMPK activators improves mitochondrial health before entering deep senescence. Mitochondrial dynamics are reduced over time driven by inability to remove network of dysfunctional mitochondria.
- Increase the detail of model and extend to tissue and organ level.
- Use the model to study ageing-related diseases.



# Acknowledgements

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