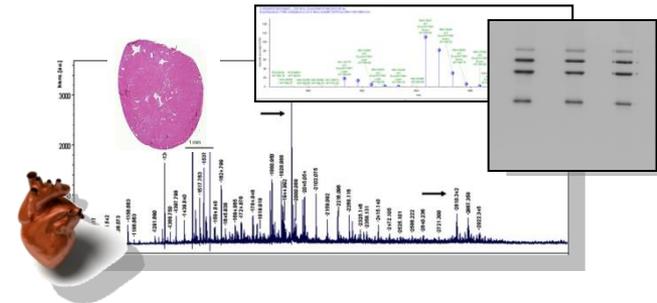


HelmholtzZentrum münchen

German Research Center for Environmental Health

Institute of Radiation Biology



PPAR alpha is a novel radiation target in exposed human and murine cardiac tissue

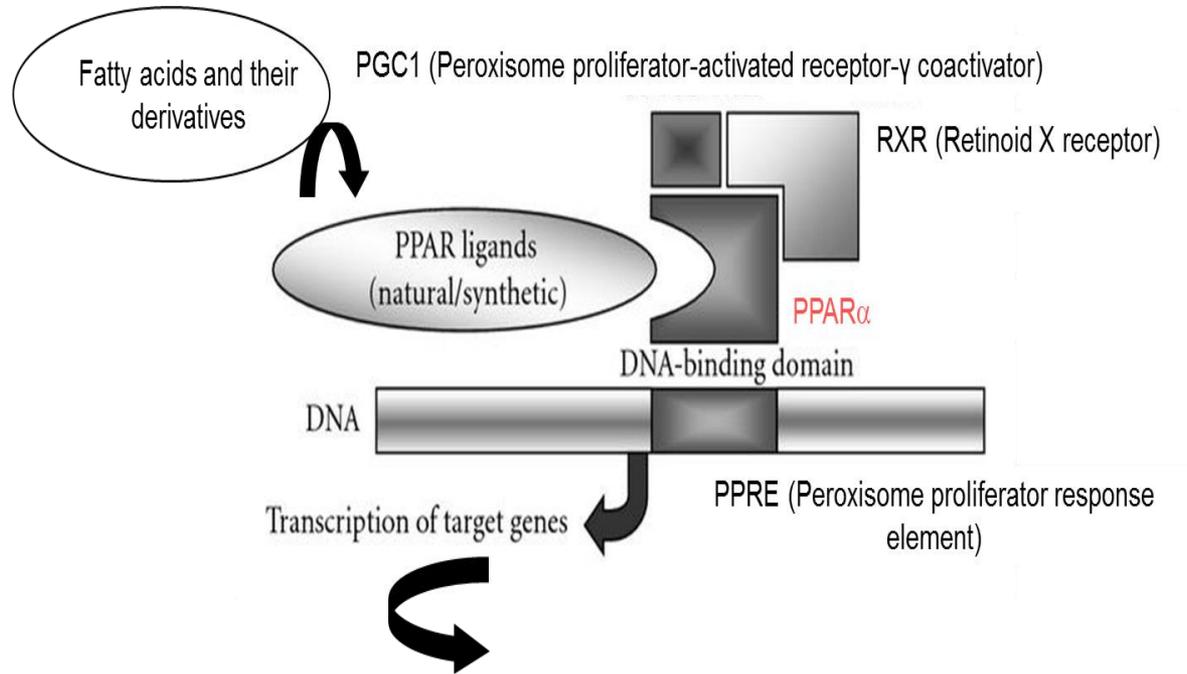
# What is PPAR alpha?



# Peroxisome proliferator-activated receptor alpha

Nuclear receptor protein that functions as transcription factor

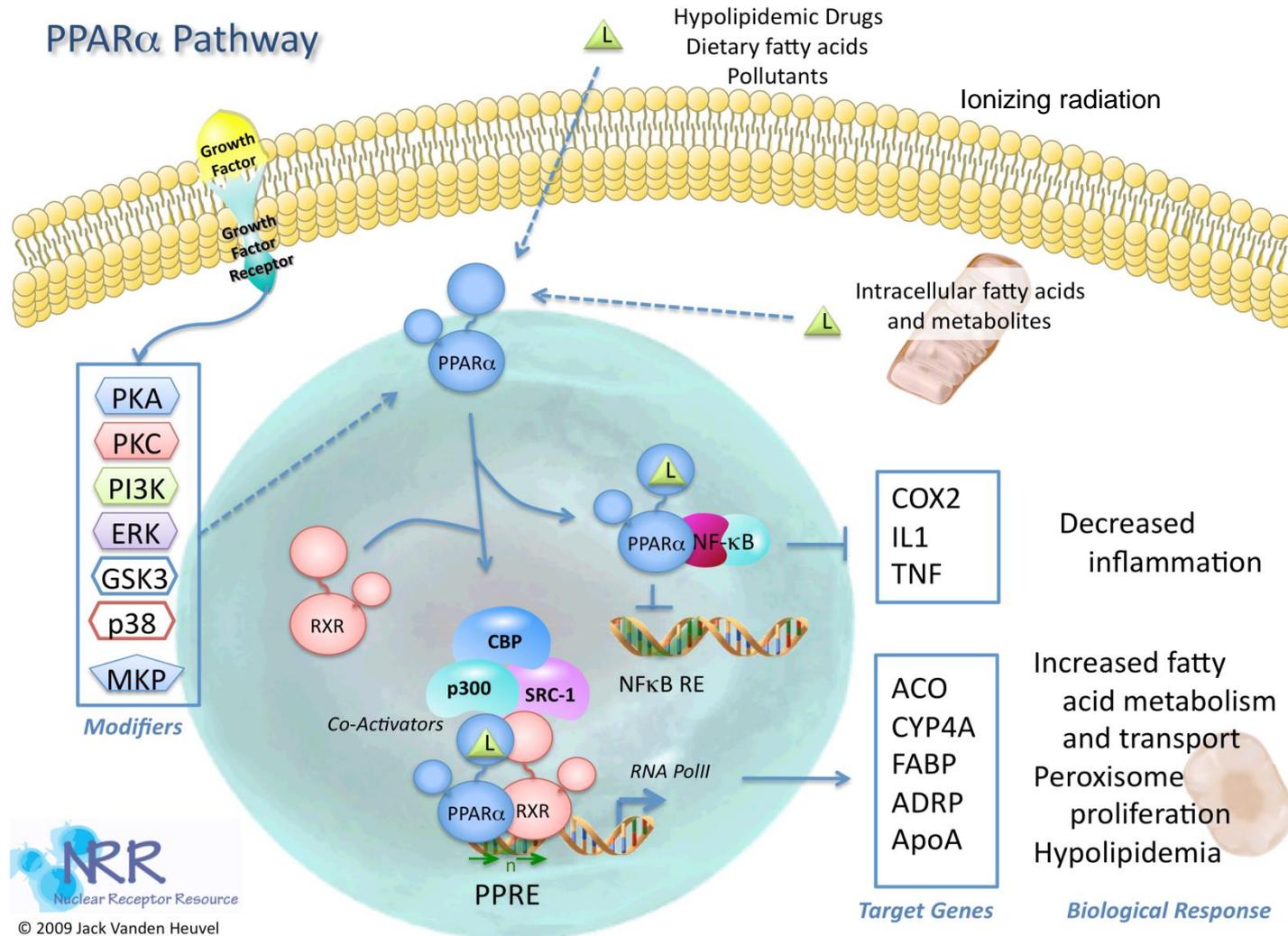
Expressed in heart, liver, kidney, muscle, adipose tissue, and others



Fatty acid metabolism and transport, mitochondrial oxidative stress and membrane potential

The activity of the PPAR alpha complex is regulated at transcriptional, translational and posttranslational level.

# PPAR alpha: Biological functions



# How is PPAR alpha responding to IR?



# How is PPAR alpha responding to IR?

## Low-dose study

Neonatal (PND 10) NMRI mice irradiated with TBI with doses of 0.02, 0.1, 0.5 and 1.0 Gy; analysis after 7 months



*Bakshi et al. Long-term effects of acute low-dose ionizing radiation on the neonatal mouse heart: a proteomic study. Radiat Environ Biophys. 2013 Jul 24.*

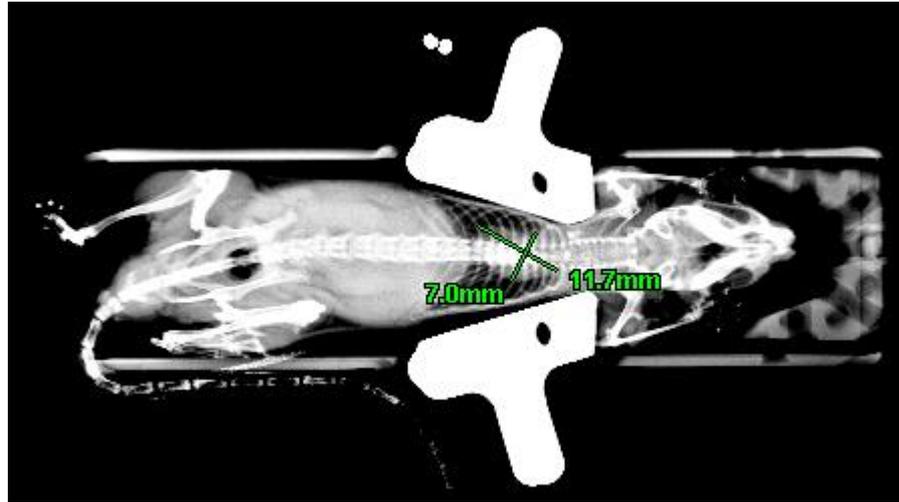
## High-dose study

8-week old C57Bl/6 mice were irradiated locally on the heart with doses of 8 and 16 Gy; analysis after 16 weeks



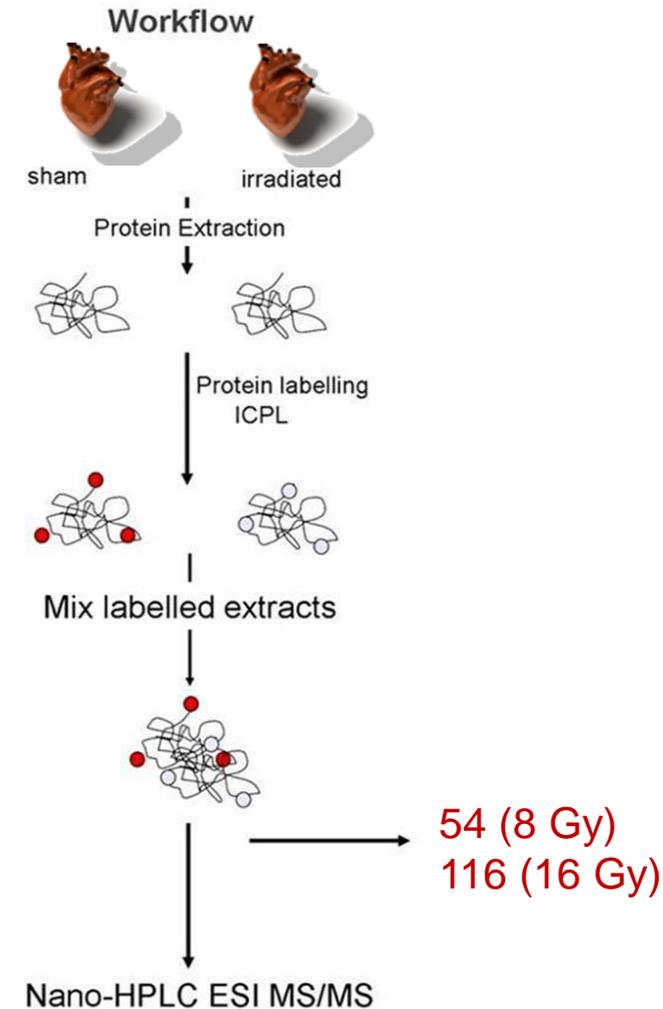
*Azimzadeh et al. PPAR alpha: a novel radiation target in locally exposed Mus musculus heart revealed by quantitative proteomics. J Proteome Res. 2013 Jun 7;12(6):2700-14.*

# How is PPAR alpha responding to high doses?

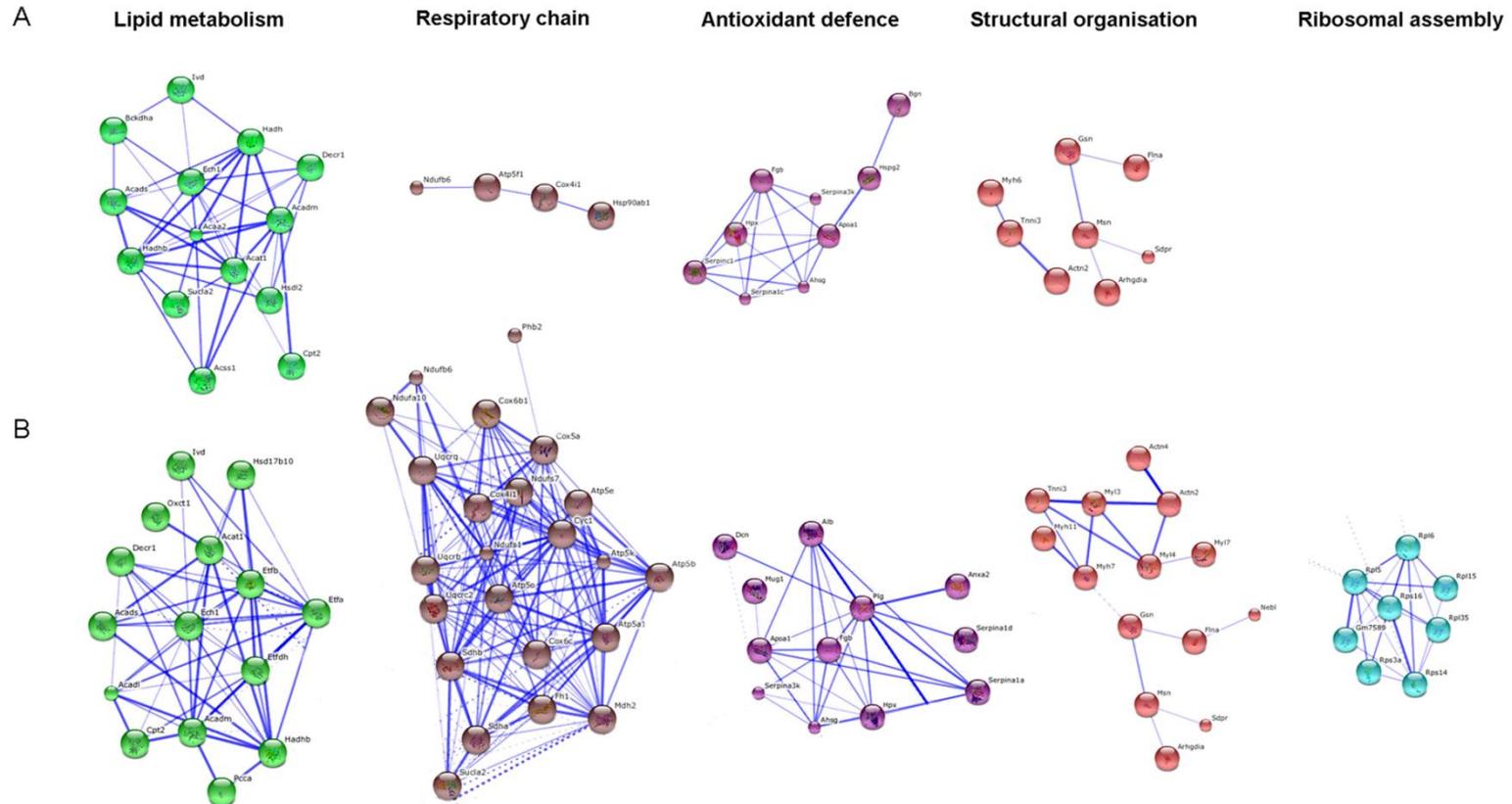


Allowing for margins and individual anatomical variation: 10.6 x 15.0 mm field  
33% lung in field

(With the courtesy of F. Stewart)



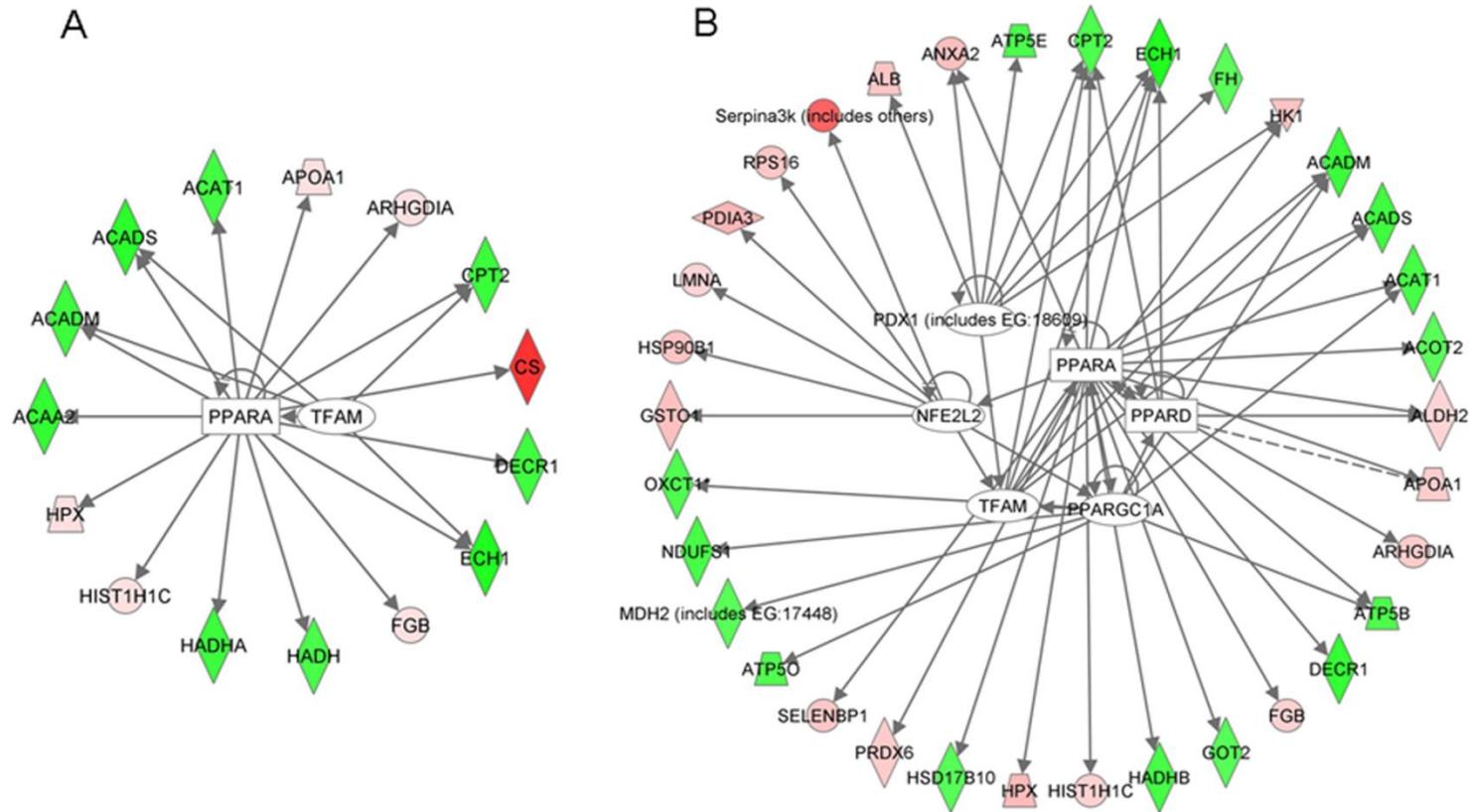
# Most affected biological networks



Protein-protein interaction analysis of the significantly differentially expressed proteins.

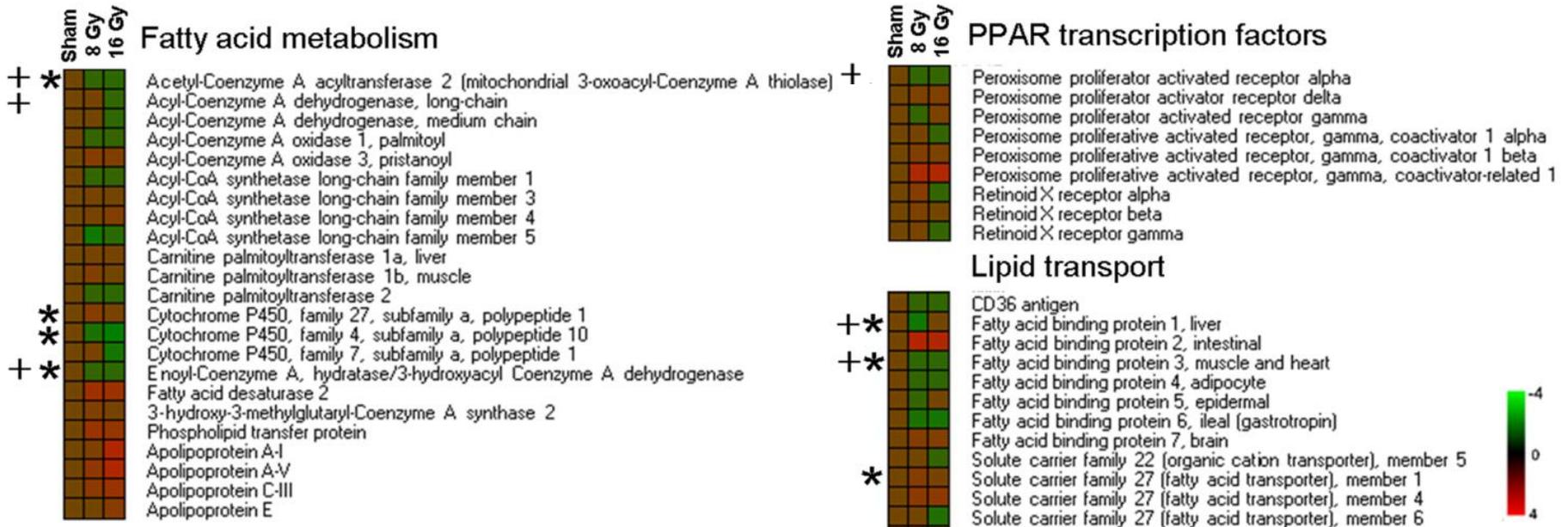
Association networks were analyzed by the STRING software tool after 8 Gy (A) and 16 Gy (B) (<http://string-db.org>).

# PPARA is a central transcriptional regulator



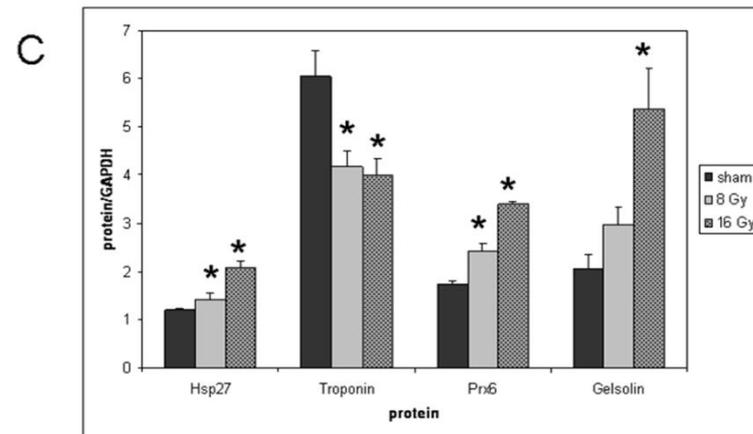
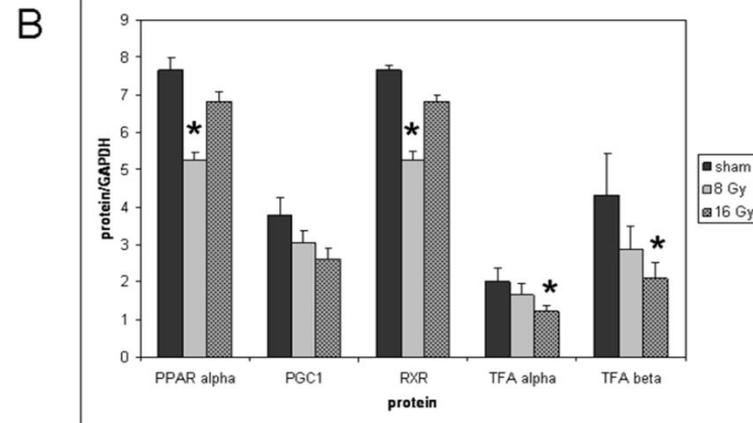
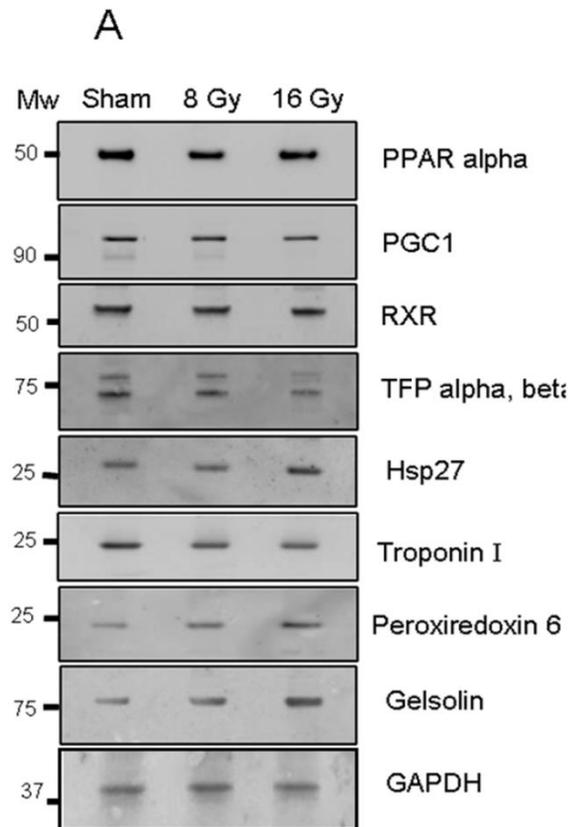
**Peroxisome proliferator-activated receptor (PPARA) is an upstream transcriptional regulator of the deregulated protein networks after 8 Gy (A) and 16 Gy (B).** The up-regulated proteins are marked in red and the down-regulated in green. The nodes represent transcription factors. (<http://www.INGENUITY.com>).

# PPAR alpha -related transcriptome

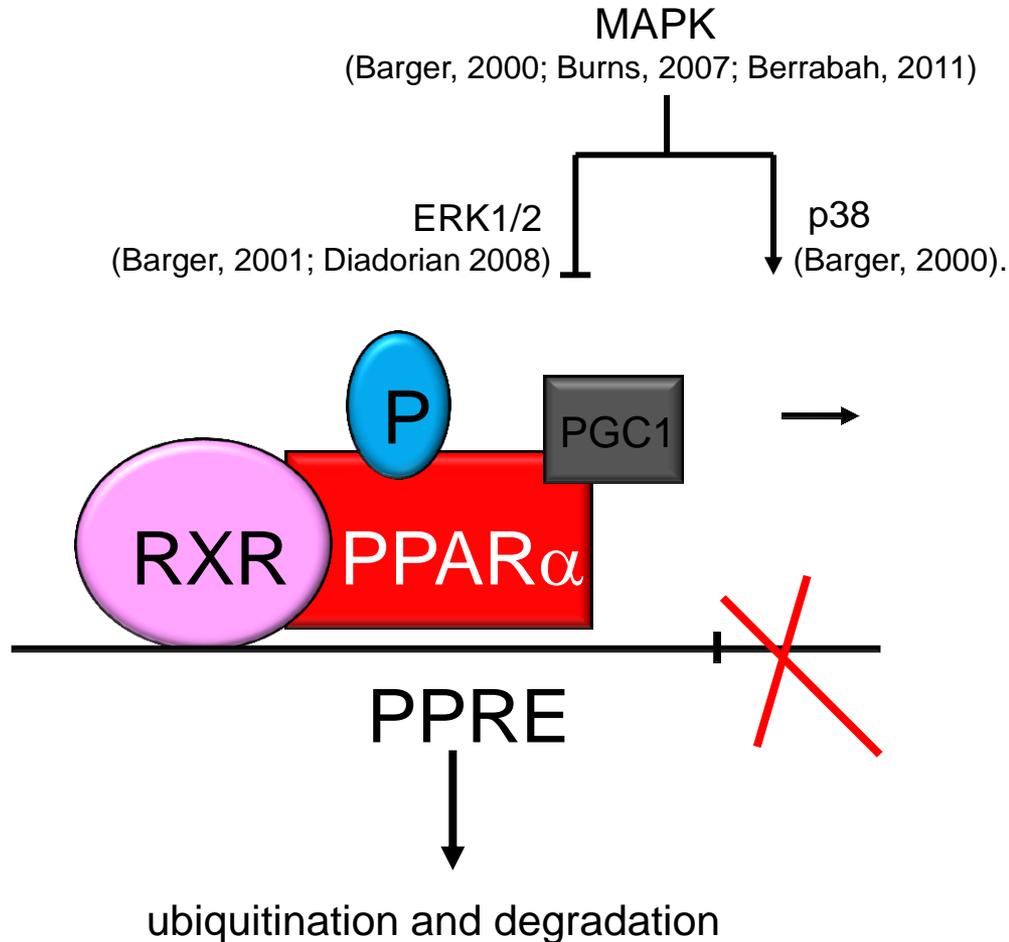


The + and \* show the significantly changed gene levels after 8 and 16 Gy, respectively (t test; \*p < 0.05; n = 3).

# PPAR alpha: targeted protein expression

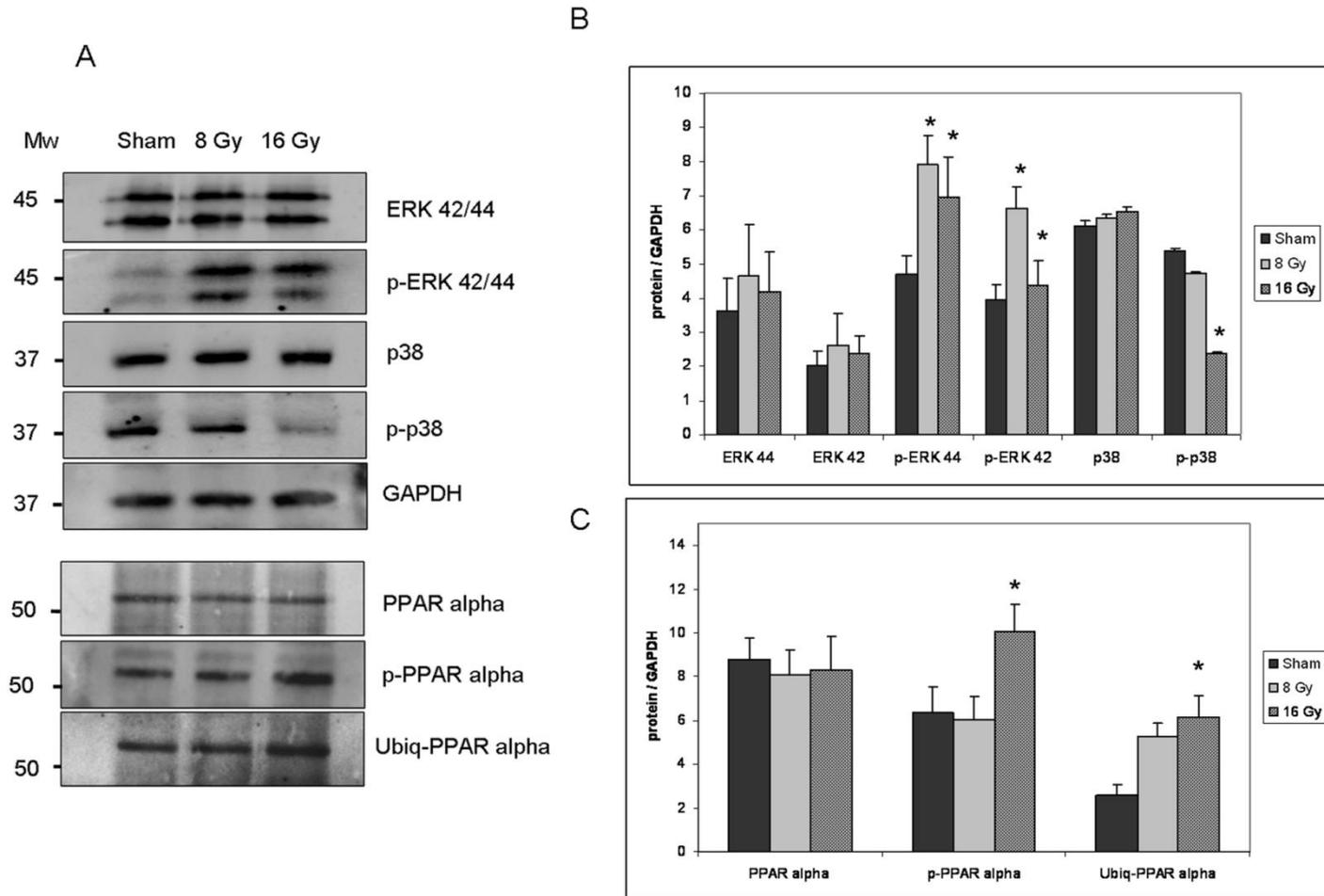


# Alteration of PPAR alpha activity following the phosphorylation

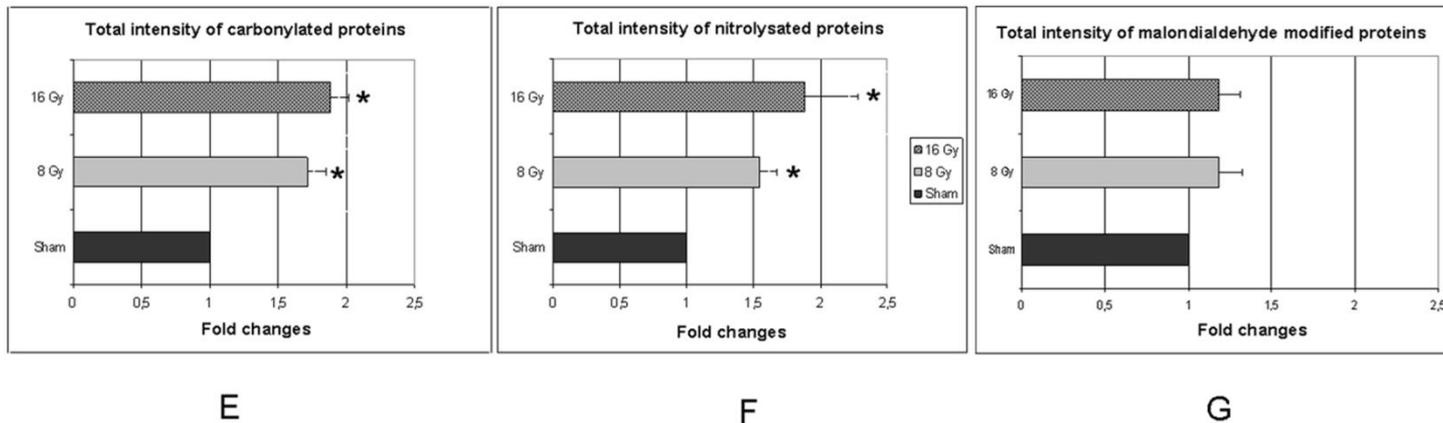
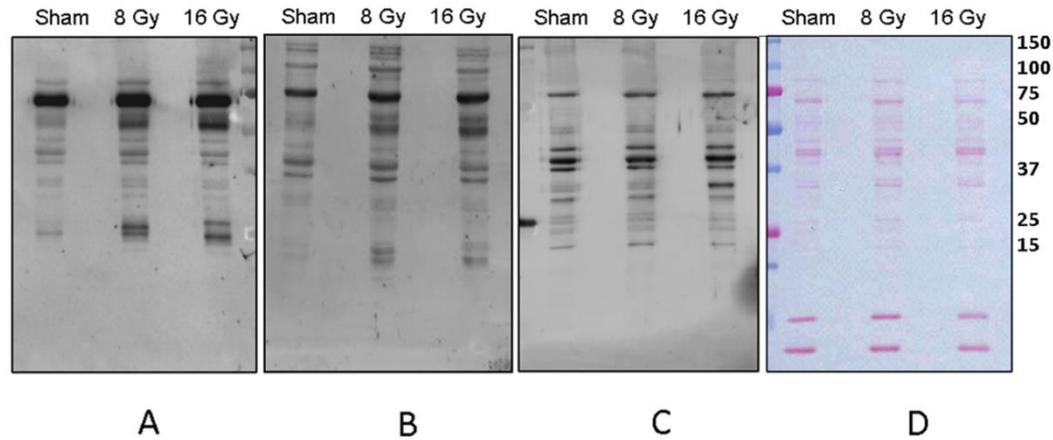


(Tansey, 2001; Lange, 2000).

# PPAR alpha is phosphorylated by ERK

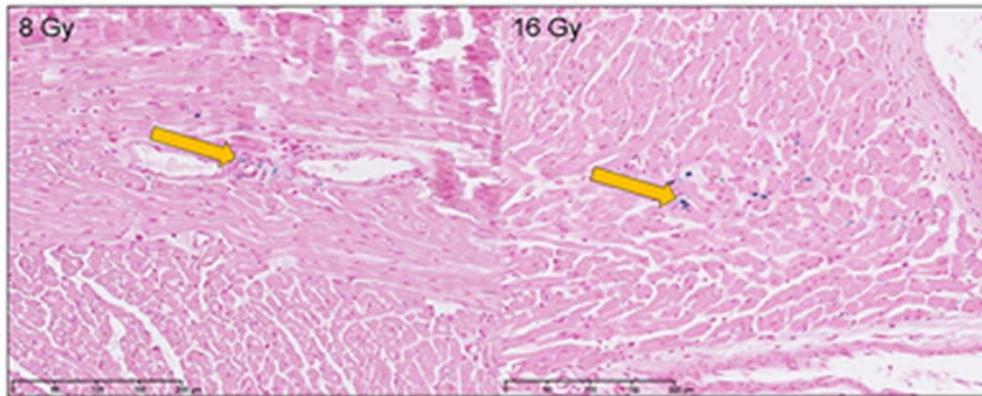


# Protein modifications are increased

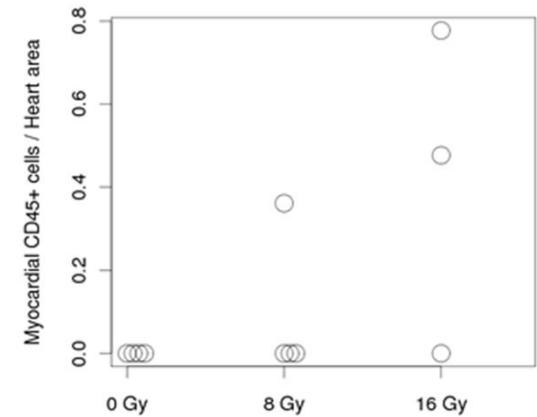


# Inflammation is increased

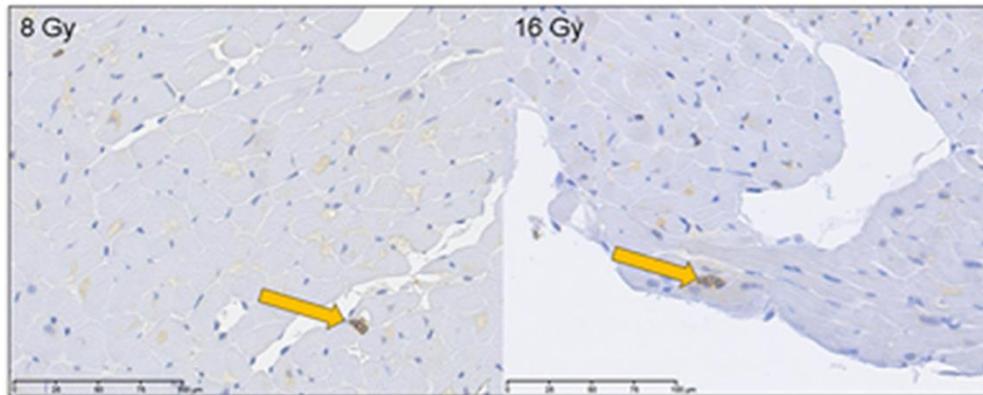
**A**



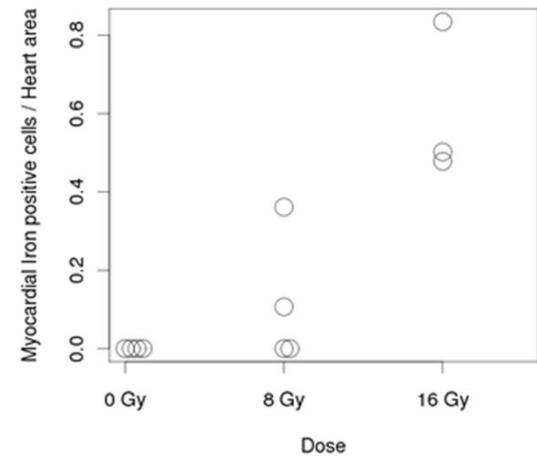
**B**



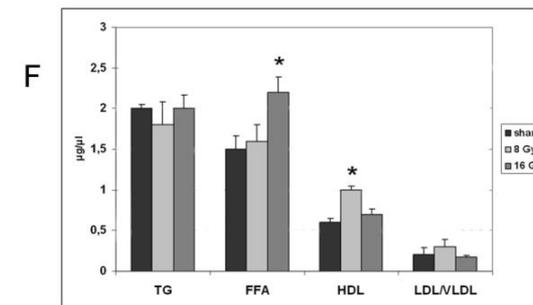
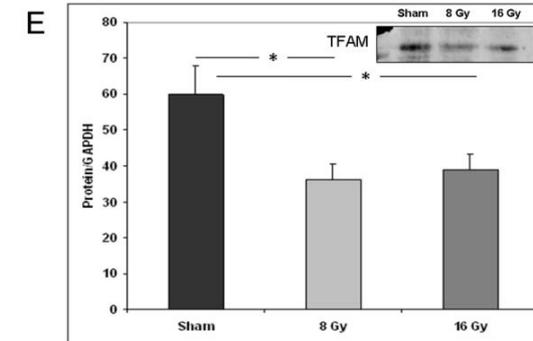
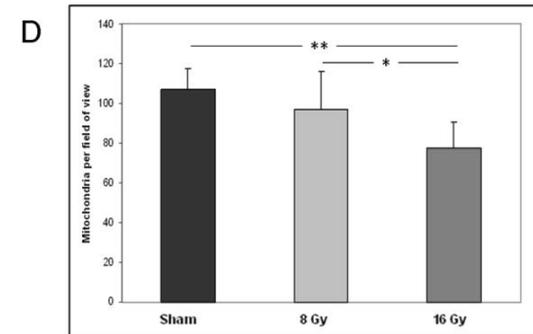
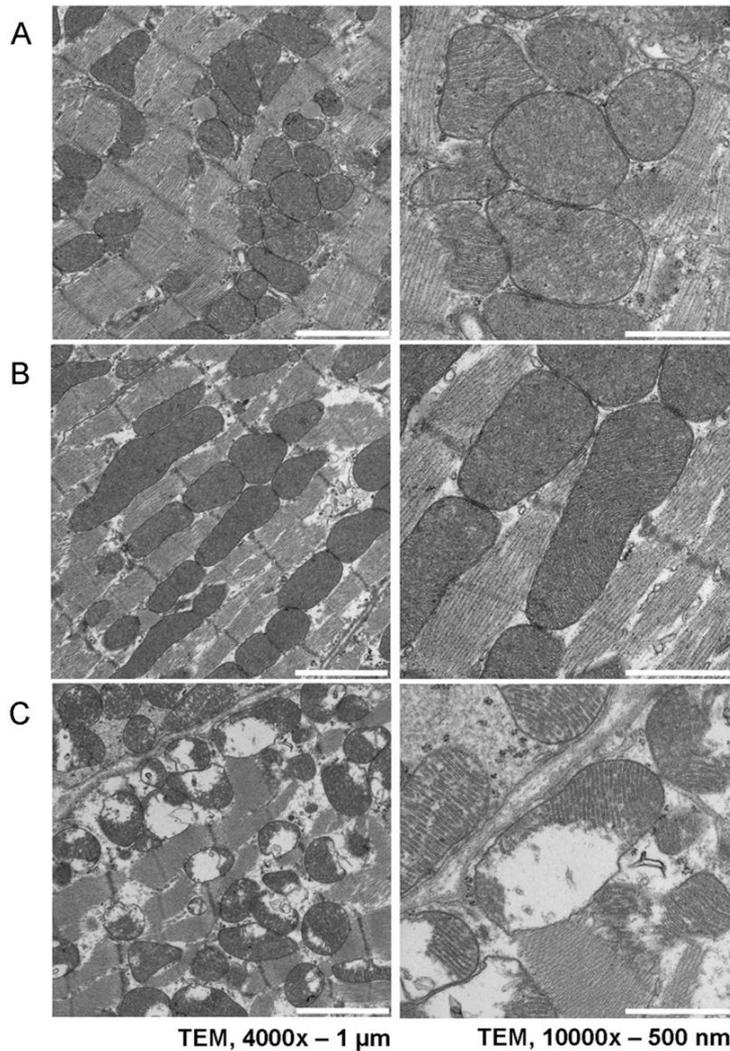
**C**



**D**

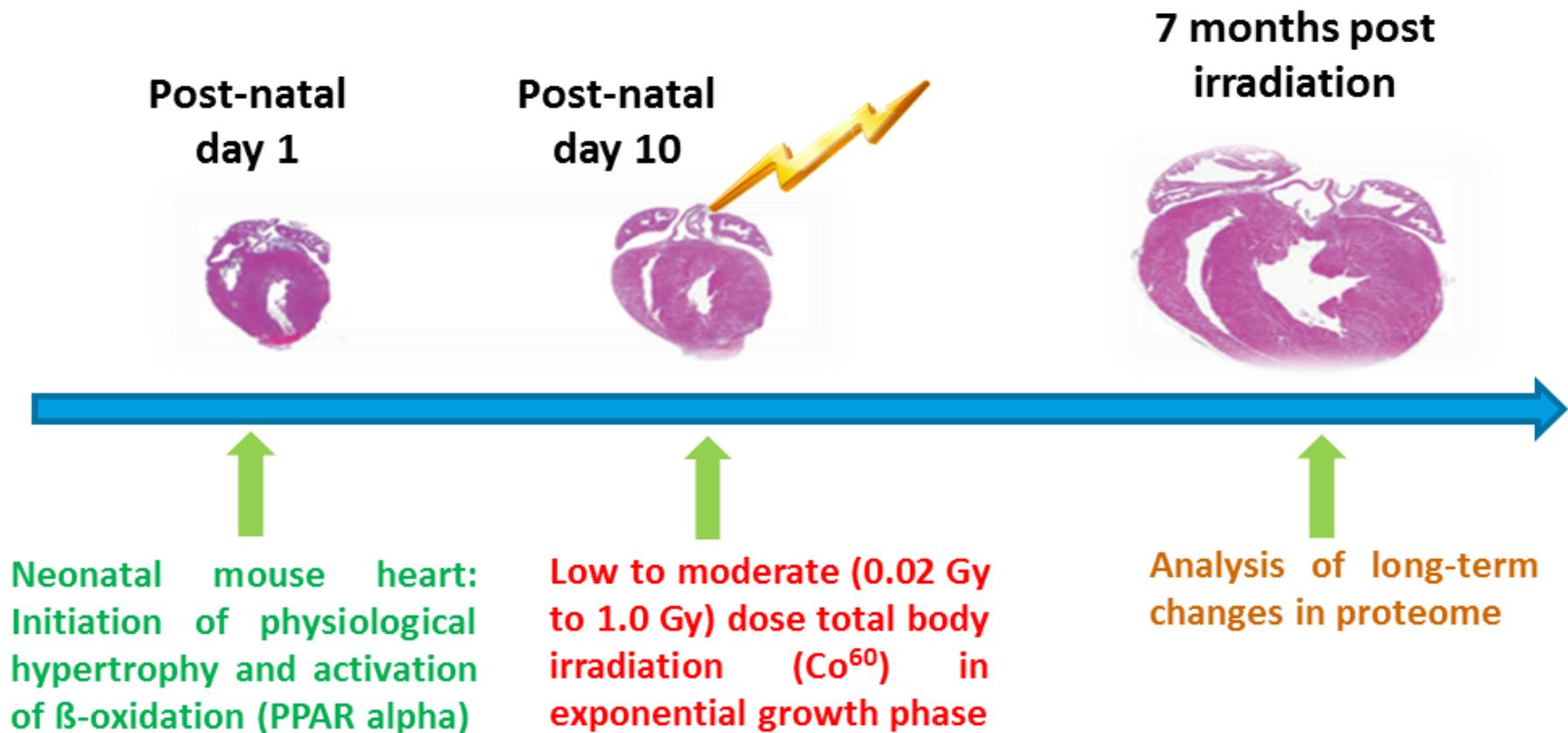


# Mitochondrial structure is damaged and the number is decreased

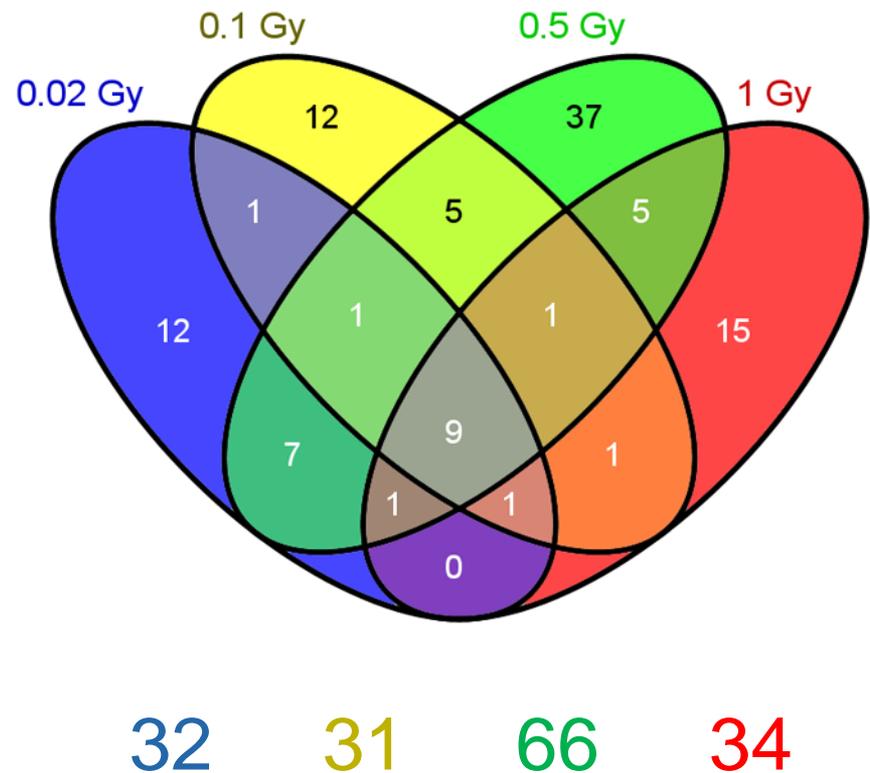
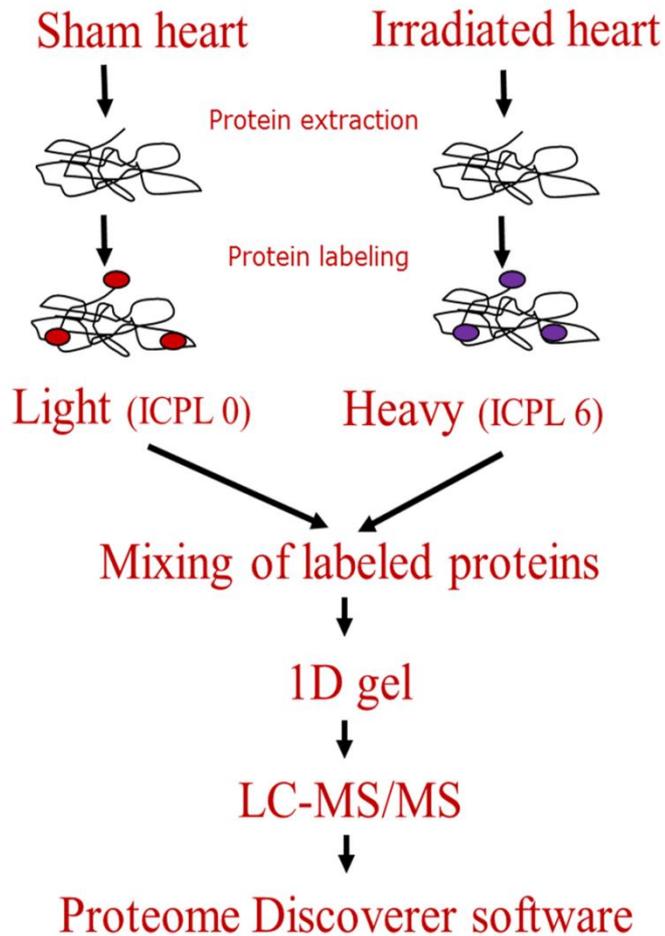




# How is PPAR alpha responding to low doses?

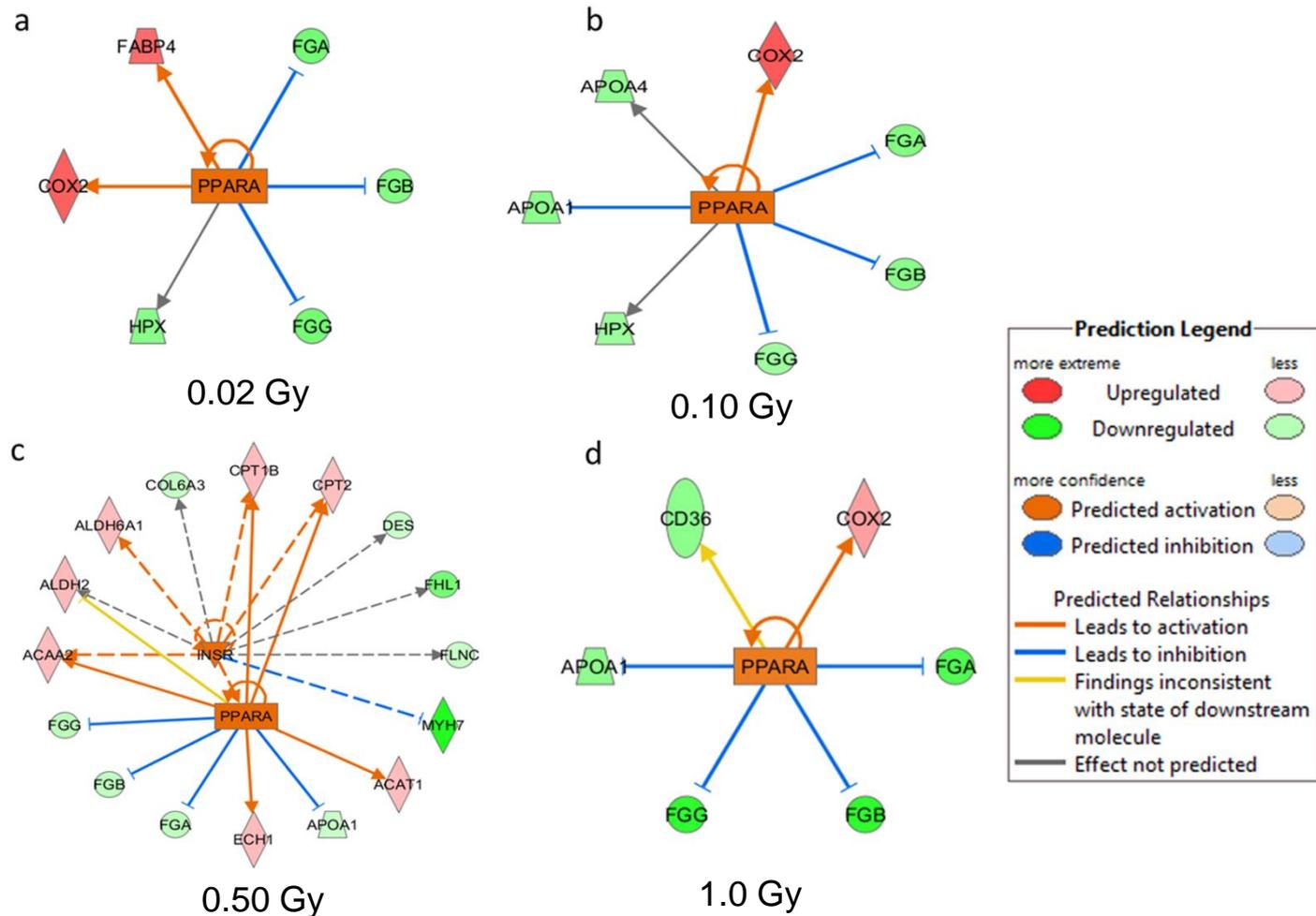


# Proteomic analysis



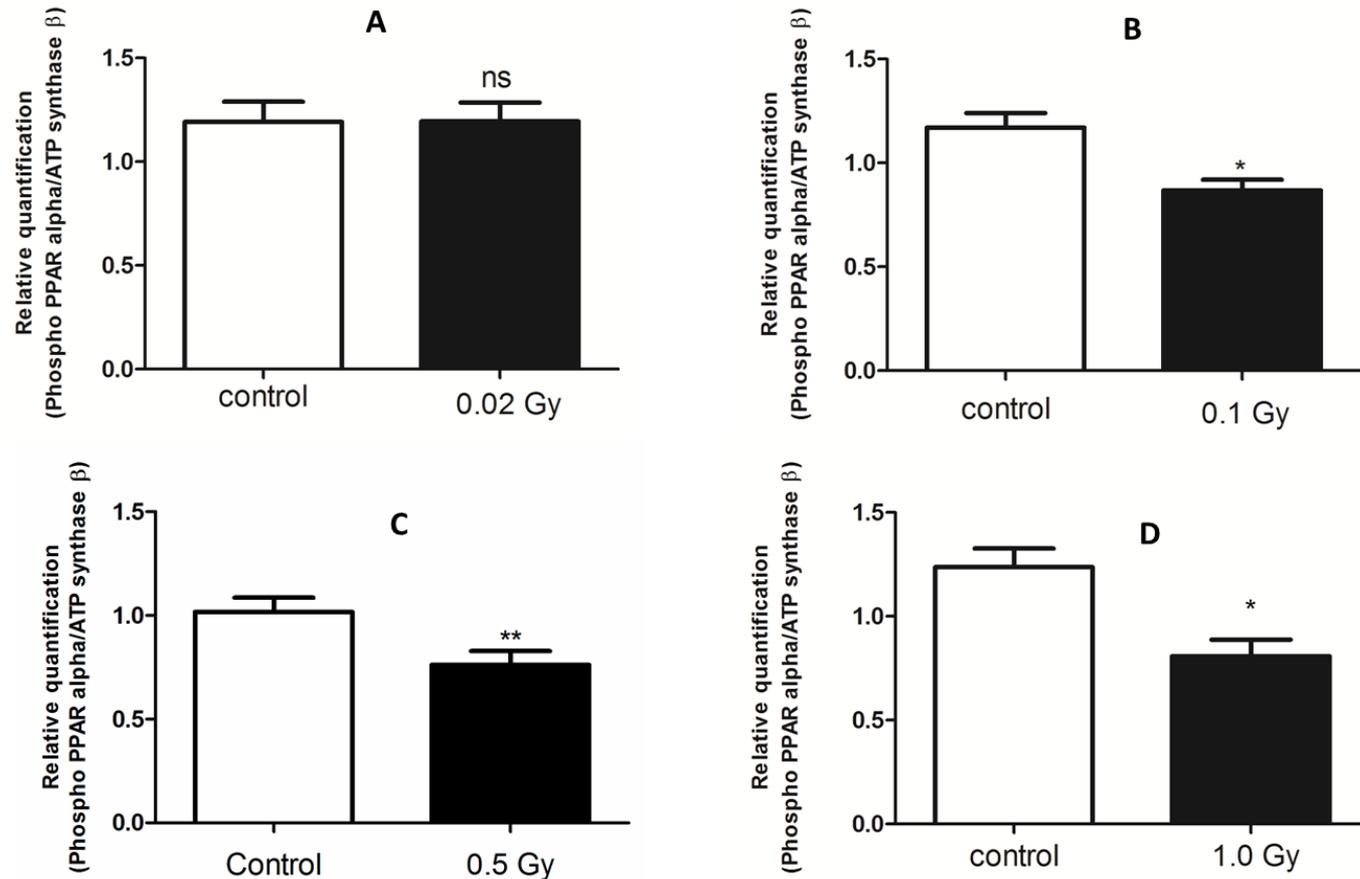


# PPAR alpha is activated by low doses

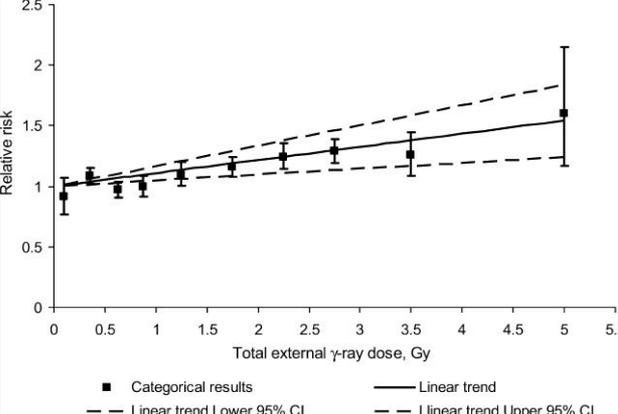
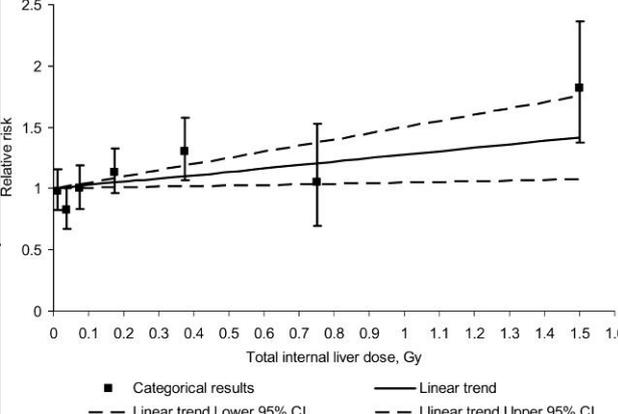


Ingenuity Pathways Analysis: PPAR alpha as a dose-independent upstream regulator

# PPAR alpha phosphorylation is decreased



The inactive (phosphorylated) form of PPAR alpha is decreased with doses  $\geq 0.1$  Gy  $\rightarrow$  activation

Data	Average heart/brain dose (range) Sv	Numbers in cohort (person years follow-up)	Endpoint	Excess relative risk Sv <sup>-1</sup> (and 95%CI)
<b>Mayak workers</b> <sup>a</sup> External gamma dose (Gy)	0.83 (0 - 5.92) <sup>a</sup>	12,210 (205,249)	Ischaemic heart disease morbidity (ICD9 410- 414)	0.109 (0.049, 0.168)
<sup>b</sup> Internal alpha -particle dose (Sv), applying a relative biological effectiveness (RE)				

“A new finding was a statistically significant decrease in IHD incidence among workers exposed to external gamma-rays doses of 0.2 Gy - 0.5 Gy in relation to the external doses below 0.2 Gy. A gender-specific analysis showed that this significantly decreased risk is only present for female workers.”  
Azizova et al. Health Phys 2012

# How about humans (Mayak study)?

## Preliminary data!

Dose groups: (i) < 50 mGy (ii) 50 mGy – 500 mGy (iii) > 500 mGy

Cause of death: myocardial infarction

Tissue analyzed: left ventricular wall

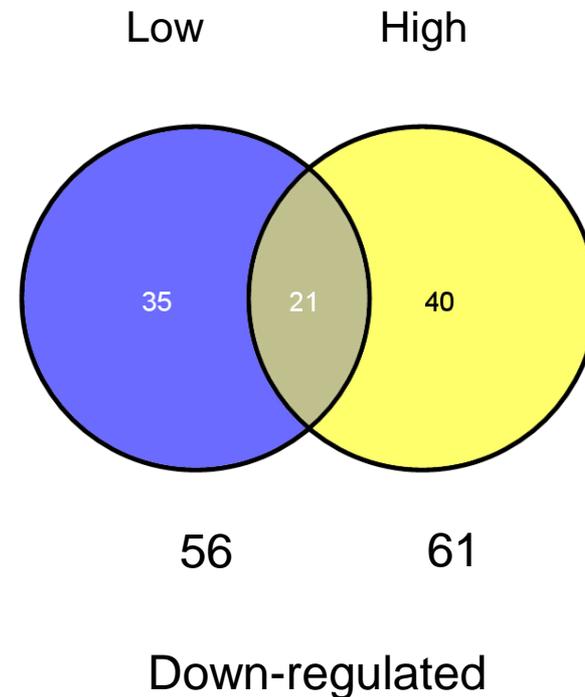
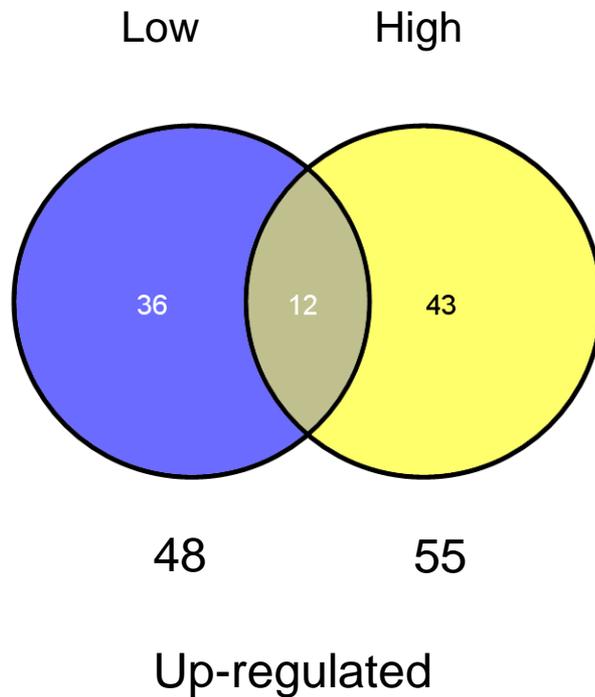
Data on samples from 3 individuals sent in November 2012														
ID	Case Number on vial	Gender	Date of birth	Date of death	Ethnicity	Smoking status	Alcohol consumption status	Year of started employment at Mayak PA	Year of stopped employment at Mayak PA	Plant	Year of first external exposure	Year of last external exposure	Total whole body dose from external gamma rays by the death (Gy)	Total liver dose from internal alpha-particle exposure (Gy)
36498	case No. 2	male	04.03.1952	28.02.2012	Russian	smoker	drinker	1972	1982	auxiliary plant	1973	1982	0,043687874	-
3877	case No. 3	male	09.03.1958	02.03.2012	Bashkir	smoker	drinker	1976	2007	reactor	1976	2007	0,114535221	-
22130	case No. 4	male	29.05.1929	17.03.2012	Russian	ex-smoker	drinker	1948	1983	reactor, plutonium plant, auxiliary plant	1949	1983	1,772490786	0,0325

### Triplex ICPL

Case 2 : light labelled

Case 3 and case 4 : medium and heavy labelled

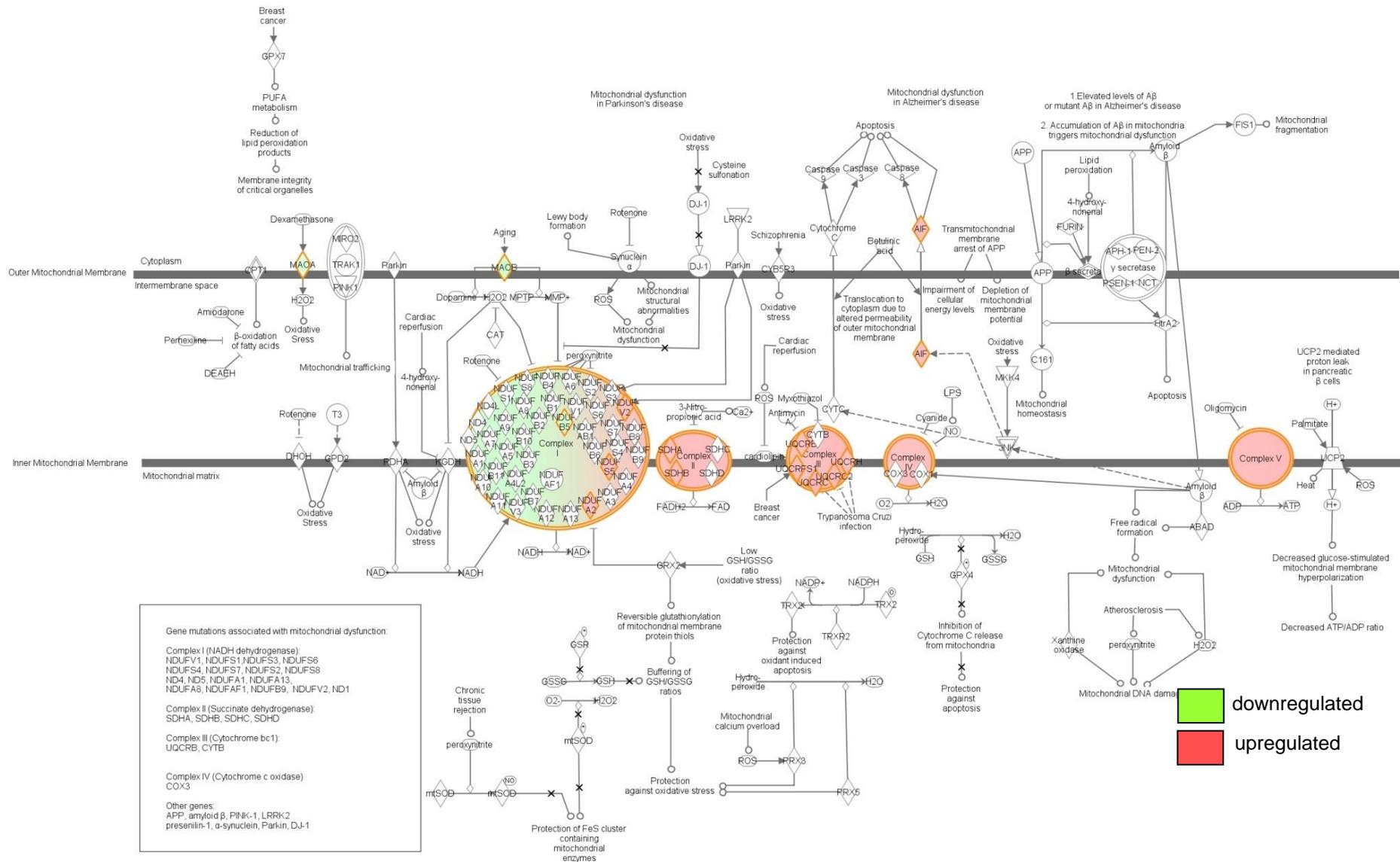
# Venn diagram of altered proteins





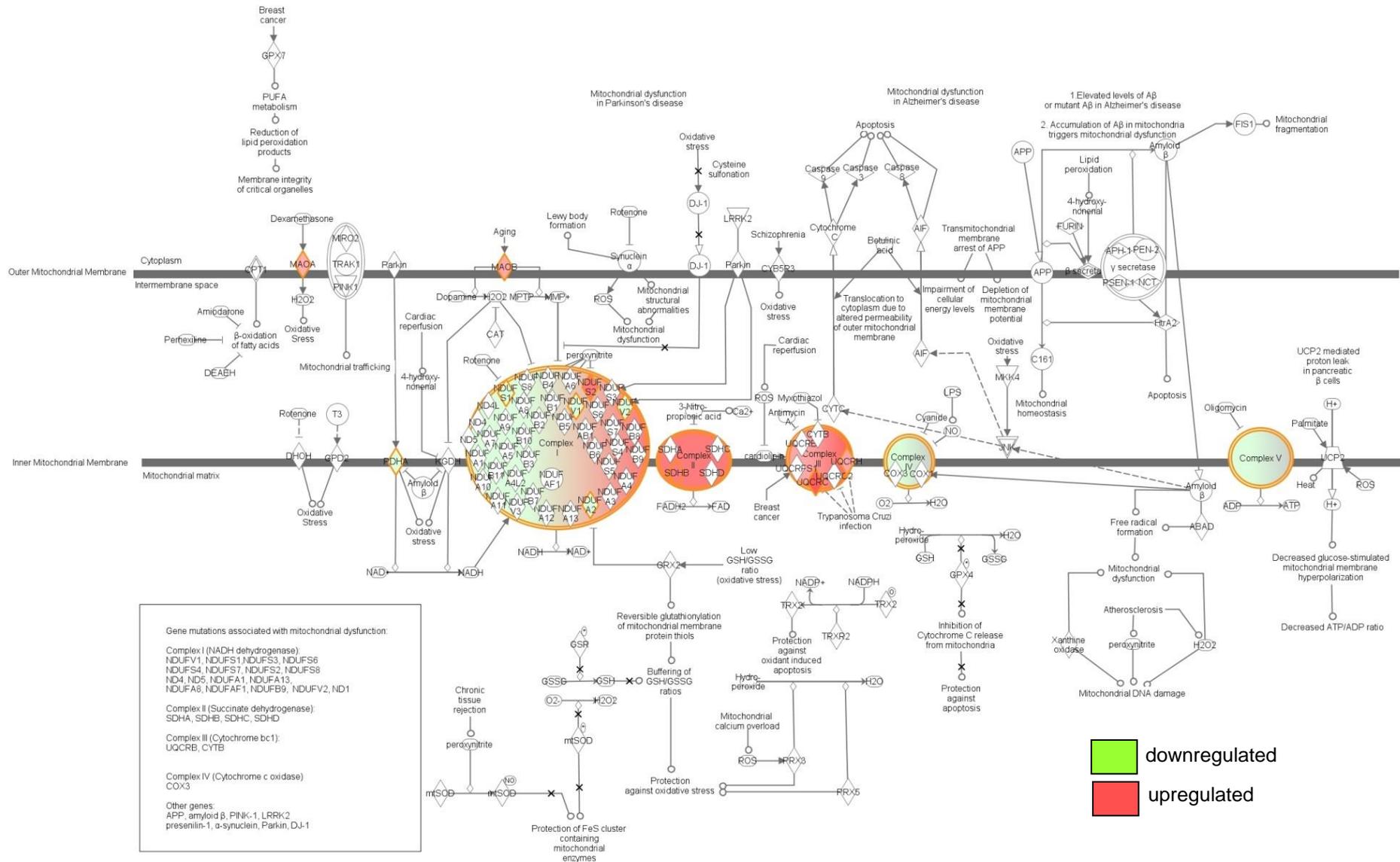
# Mitochondrial dysfunction in case 3 (low 0.115 Gy)

Mitochondrial Dysfunction

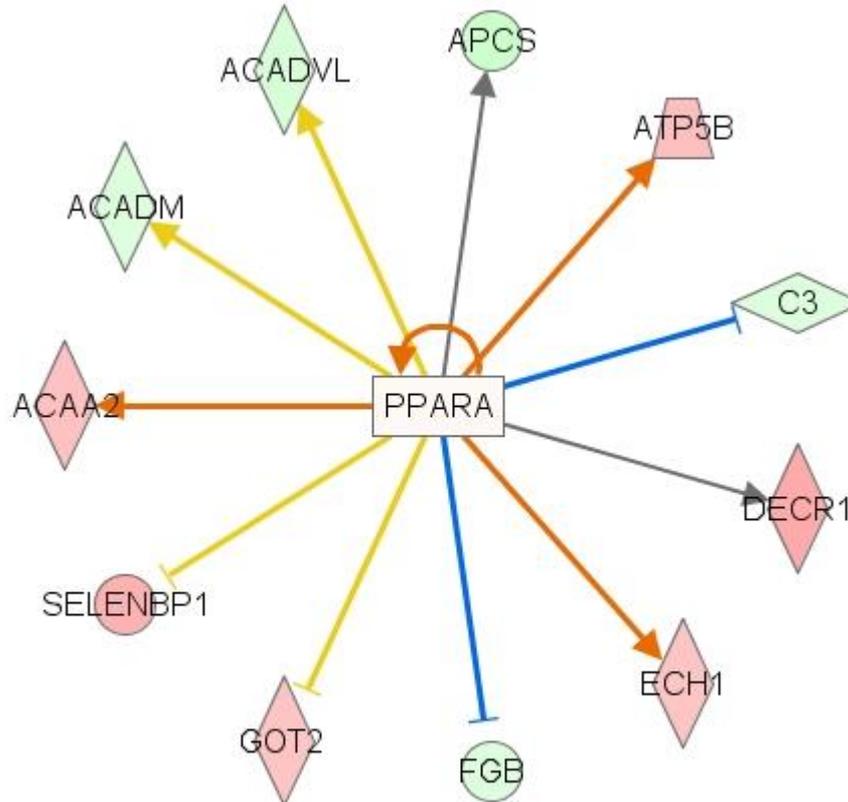


# Mitochondrial dysfunction in case 4 (high 1.77 Gy)

Mitochondrial Dysfunction



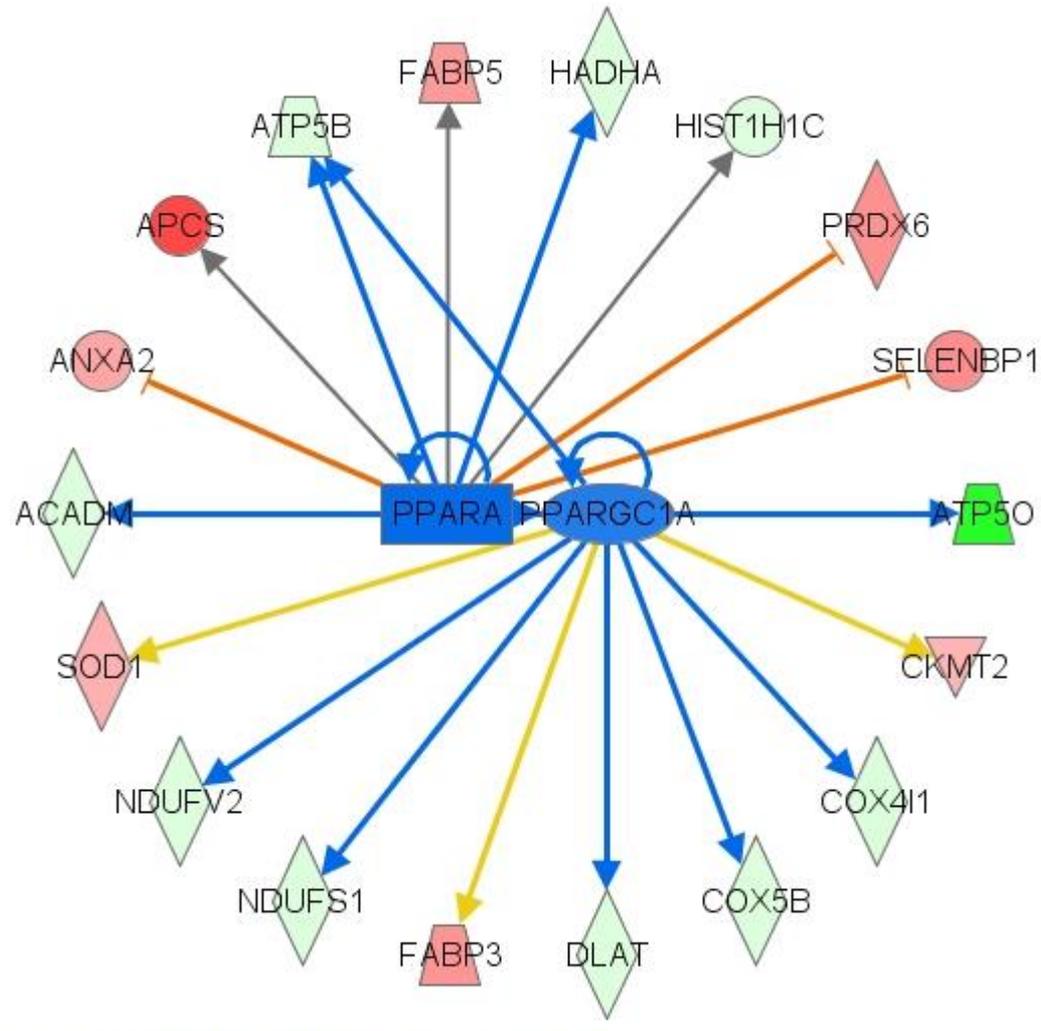
# Slight activation of PPAR alpha (low)



green square downregulated  
red square upregulated

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# Inactivation of PPAR alpha (high)



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# Conclusions

Ionizing radiation causes significant changes in **lipid metabolism** and **oxidative phosphorylation** in irradiated hearts as a **long-term effect**.

This is observed in both **acute** and **chronic** exposures and **local** or **total body** irradiation in **humans** and in **mice**.

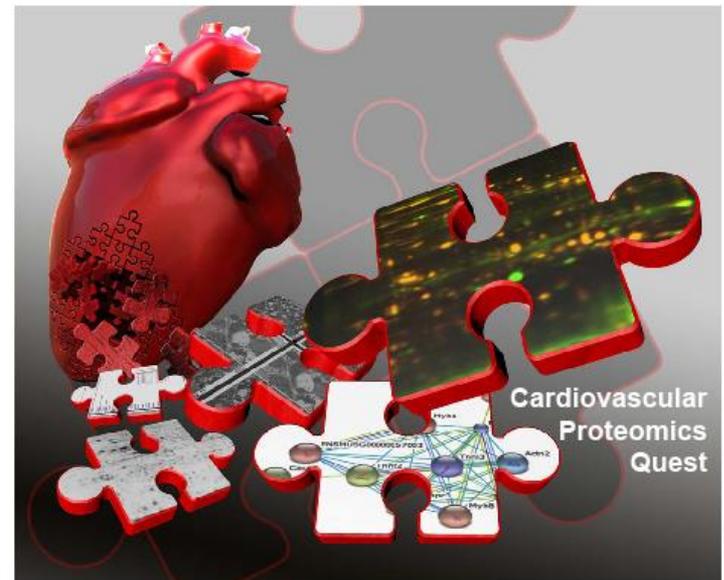
**PPAR alpha** seems to play a central regulatory role in these pathways. It seems to be **activated by low doses** and **inactivated by high doses**.

Whereas altered metabolism was originally considered to be a **byproduct** of the pathologic state, evidence is emerging that metabolic abnormalities **contribute** to the pathogenesis of cardiac disease.

# PROTEOMICS

Clinical Applications

[www.clinical.proteomics-journal.com](http://www.clinical.proteomics-journal.com)



Editor:  
Michael J. Dunn

Published in an  
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**HU**  
WILEY-BLACKWELL

# Thank you!



**Omid  
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Bakshi**

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**Per Eriksson**

**Marius Ueffing**