

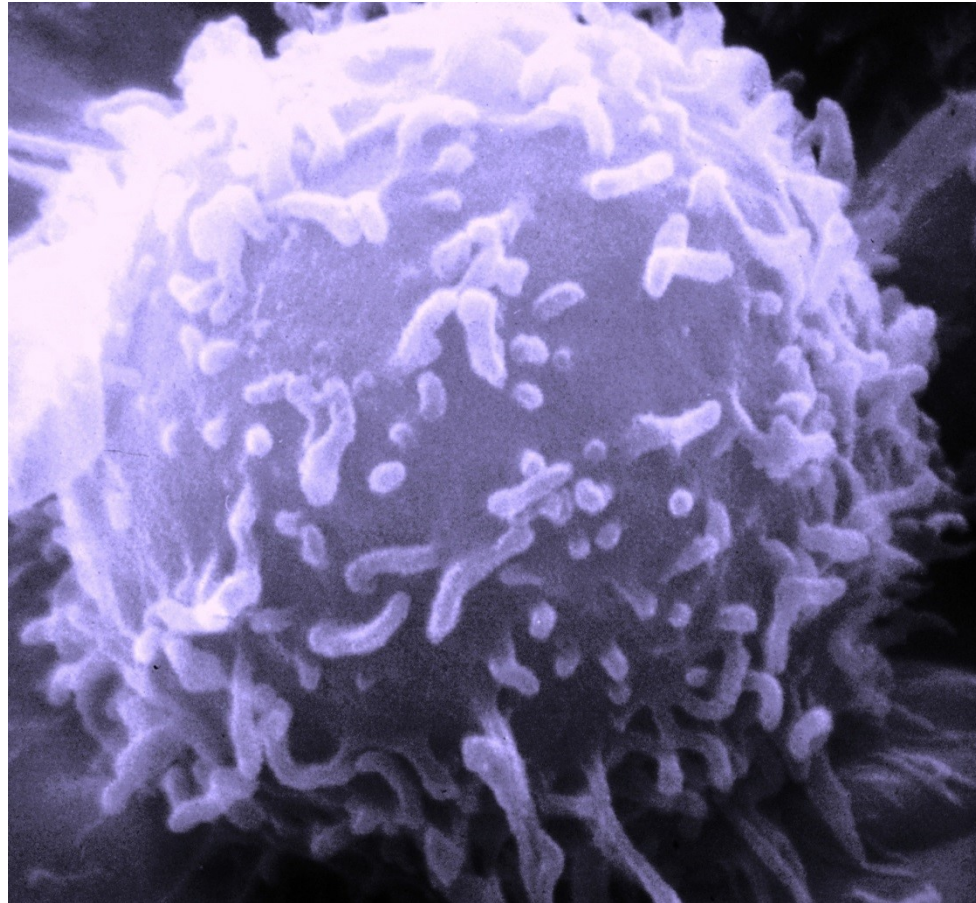
# The effects of high dose ionizing radiation on transcriptional regulation and paracrine signaling in human peripheral blood mononuclear cells

**L Beer**

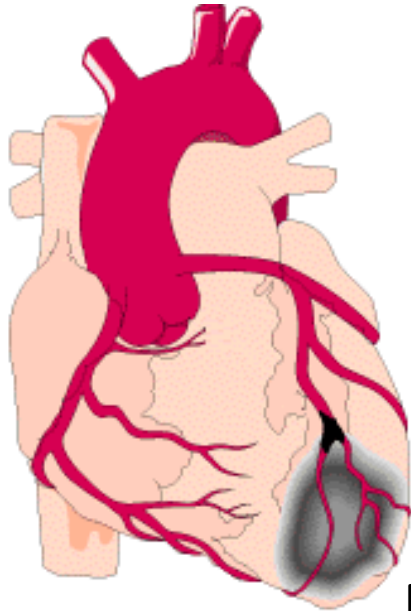
Department of Biomedical Imaging and Image-guided Therapy  
**CHRISTIAN DOPPLER LABORATORY**  
for the Diagnosis & Regeneration of Cardiac and Thoracic Diseases  
Medical University of Vienna  
[www.meduniwien.ac.at/applied-immunology](http://www.meduniwien.ac.at/applied-immunology)

## PBMCs

Peripheral blood mononuclear cells



## Myocardial Infarction



**Necrosis**

Attraction of immune cells

Secretion of pro-inflammatory cytokines

IL-1 IL-6 TNF- $\alpha$

Amplification of inflammation

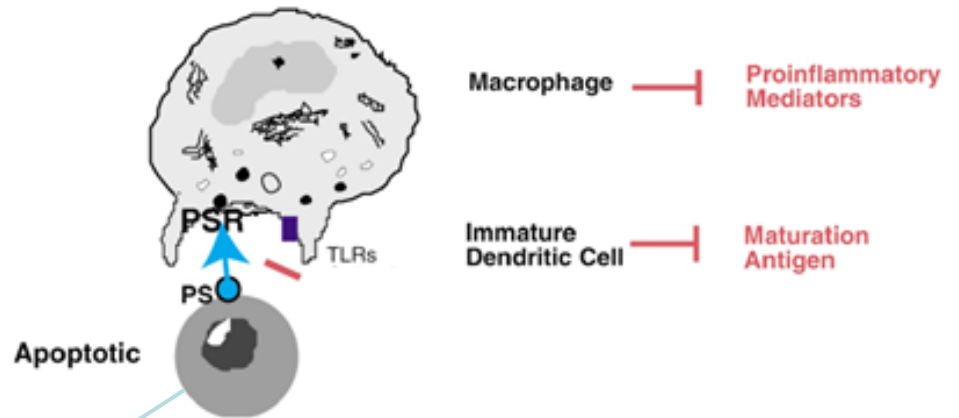
Inhibition of  
pro-inflammatory  
Signals

## The Dying Stem Cell Hypothesis

by Anker *et al.*

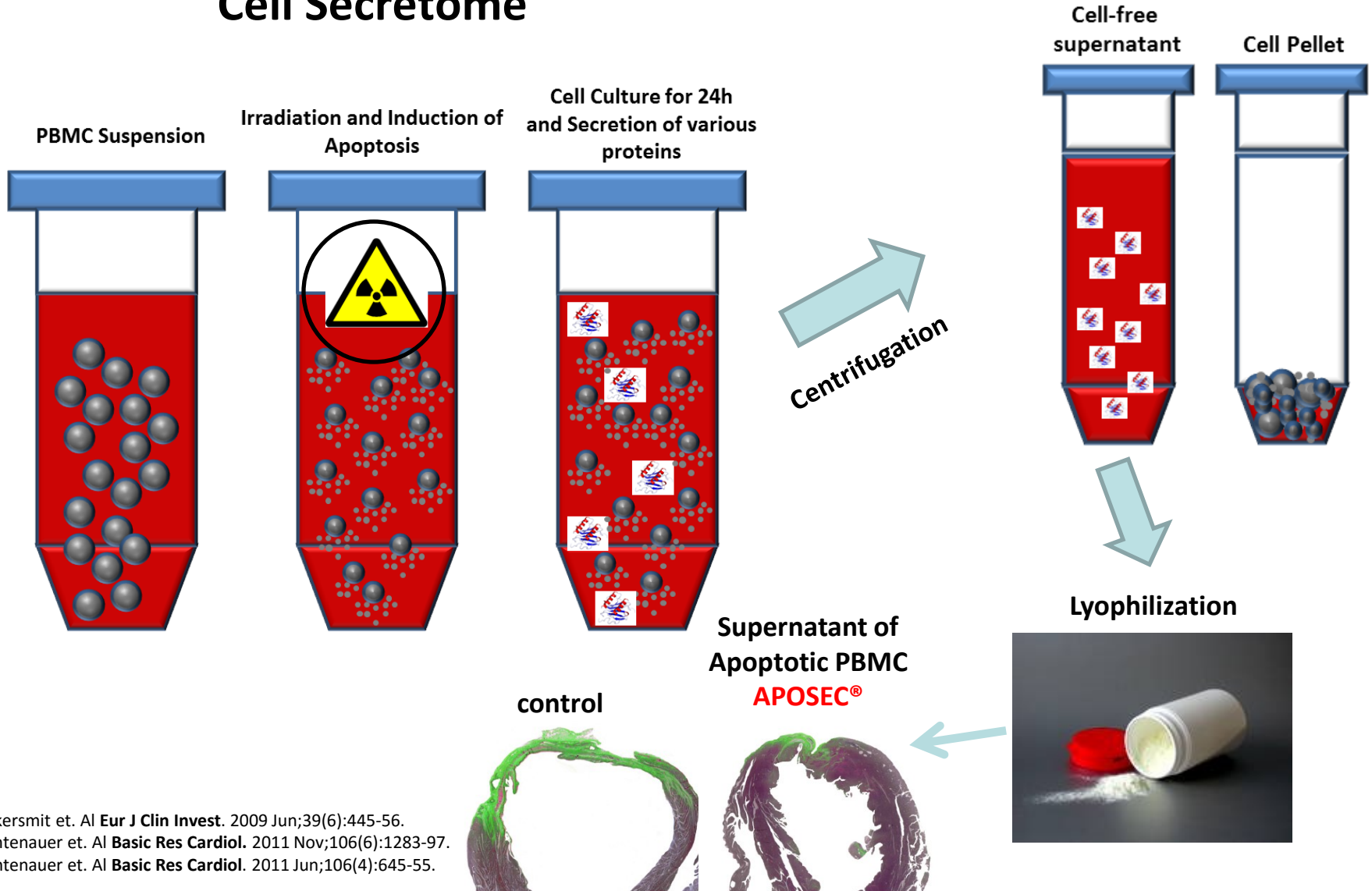
up to 25% of all transplanted cells are  
in the state of apoptosis

apoptotic cells induce transient immunosuppression



J Am Coll Cardiol. 2005 Nov 15;46(10):1799-802.  
J Clin Invest. 2001 Oct;108(7):957-62.

# Cell Secretome



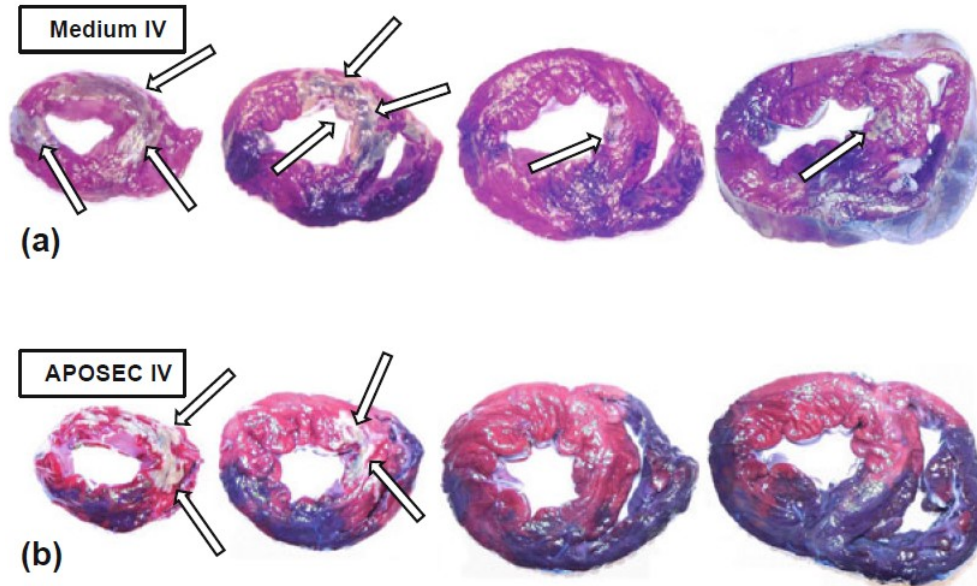
Ankersmit et. Al *Eur J Clin Invest.* 2009 Jun;39(6):445-56.  
Lichtenauer et. Al *Basic Res Cardiol.* 2011 Nov;106(6):1283-97.  
Lichtenauer et. Al *Basic Res Cardiol.* 2011 Jun;106(4):645-55.

## Preclinical Experiments

- Attenuation of acute myocardial infarction

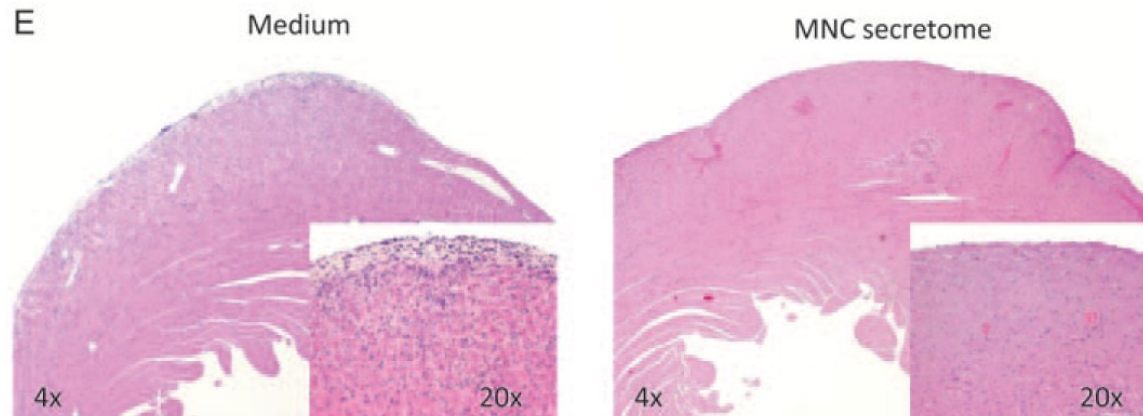
(Lichtenauer et al; Basic Res Cardiol. 2011)

Macroscopic analysis after 24 hours



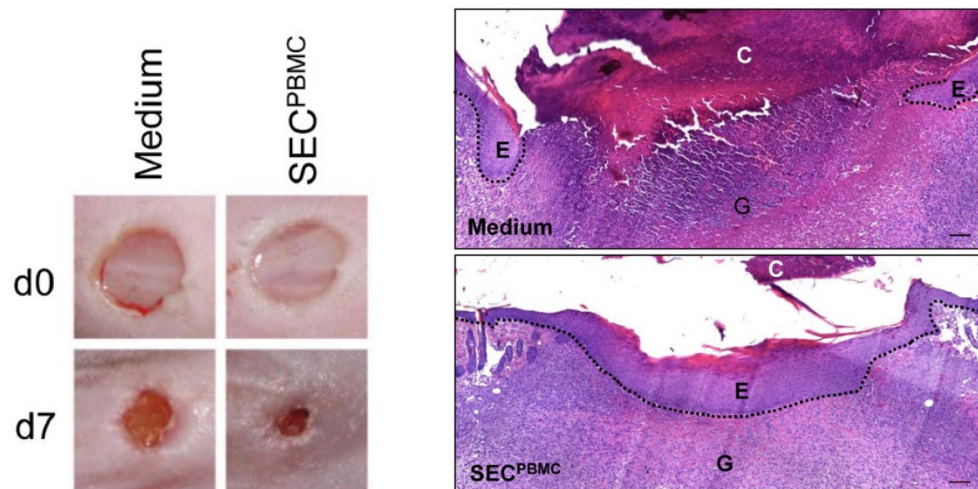
## Preclinical Experiments

- Attenuation of acute myocardial infarction  
(Lichtenauer et al; Basic Res Cardiol. 2011)
- Immunosuppression in an experimental myocarditis model  
(Hoetzencker et al; Eur Heart J. 2013)



## Preclinical Experiments

- Attenuation of acute myocardial infarction  
(Lichtenauer et al; Basic Res Cardiol. 2011)
- Immunosuppression in an experimental myocarditis model  
(Hoetzencker et al; Eur Heart J. 2013)
- Wound healing in a murine and porcine wound model  
(Mildner et al; PLoS One. 2013)



## Preclinical Experiments

- Attenuation of acute myocardial infarction  
(Lichtenauer et al; Basic Res Cardiol. 2011)
- Immunosuppression in an experimental myocarditis model  
(Hoetzencker et al; Eur Heart J. 2013)
- Wound healing in a murine and porcine wound model  
(Mildner et al; PLoS One. 2013)
- **Experimental stroke model**  
(Altmann et al; F1000Res. 2014 Jun 19 [revised 2014 Oct 28])





## Preclinical Experiments

- Attenuation of acute myocardial infarction

(Lichtenauer et al; Basic Res Cardiol. 2011)

- Immunosuppression in an experimental myocarditis model

(Hoetzencker et al; Eur Heart J. 2013)

- Wound healing in a murine and porcine wound

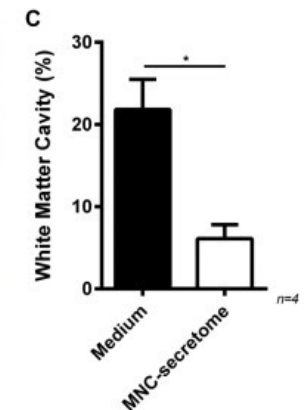
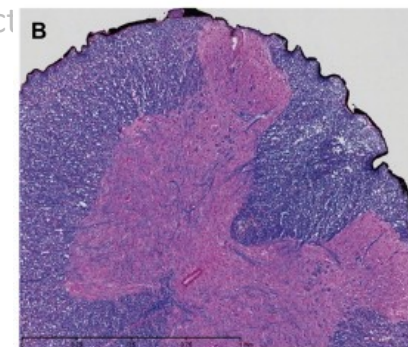
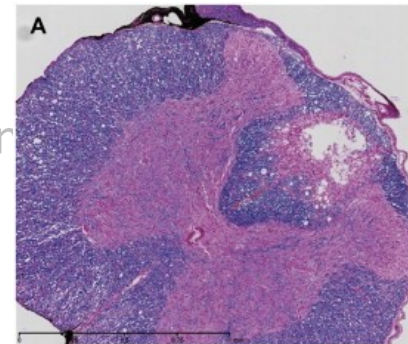
(Mildner et al; PLoS One. 2013)

- Experimental stroke model

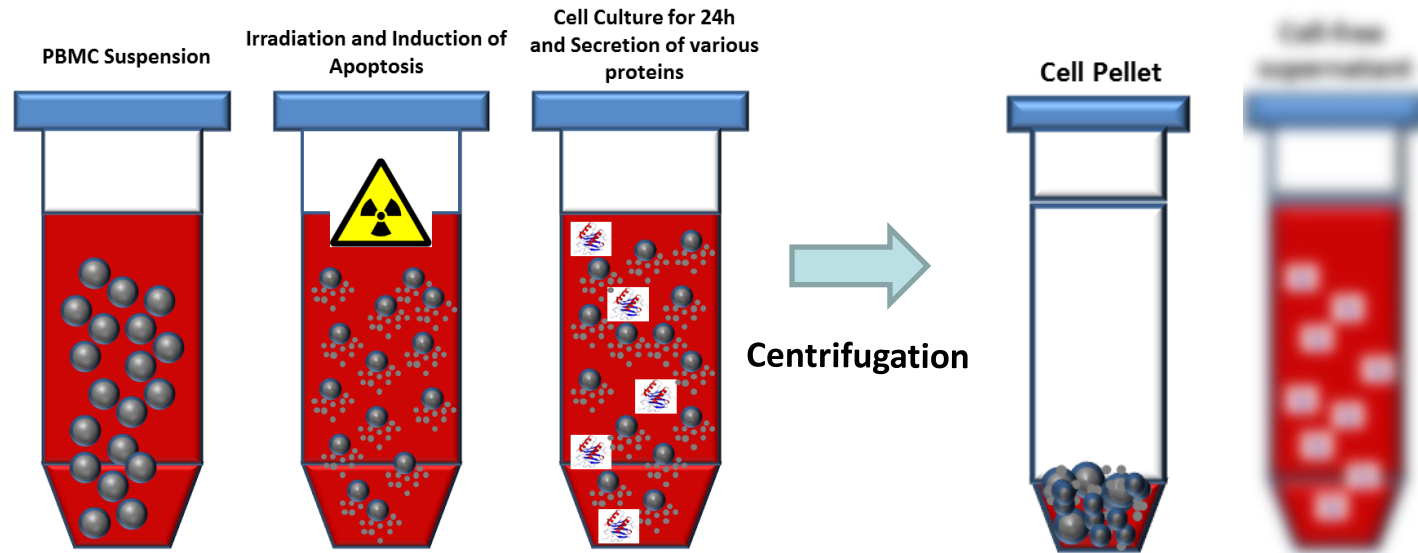
(Altmann et al; F1000Res. 2014 Jun 19 [revised 2014 Oct 15])

- Spinal Cord Injury

(Haider et al; Exp Neurol 2015)

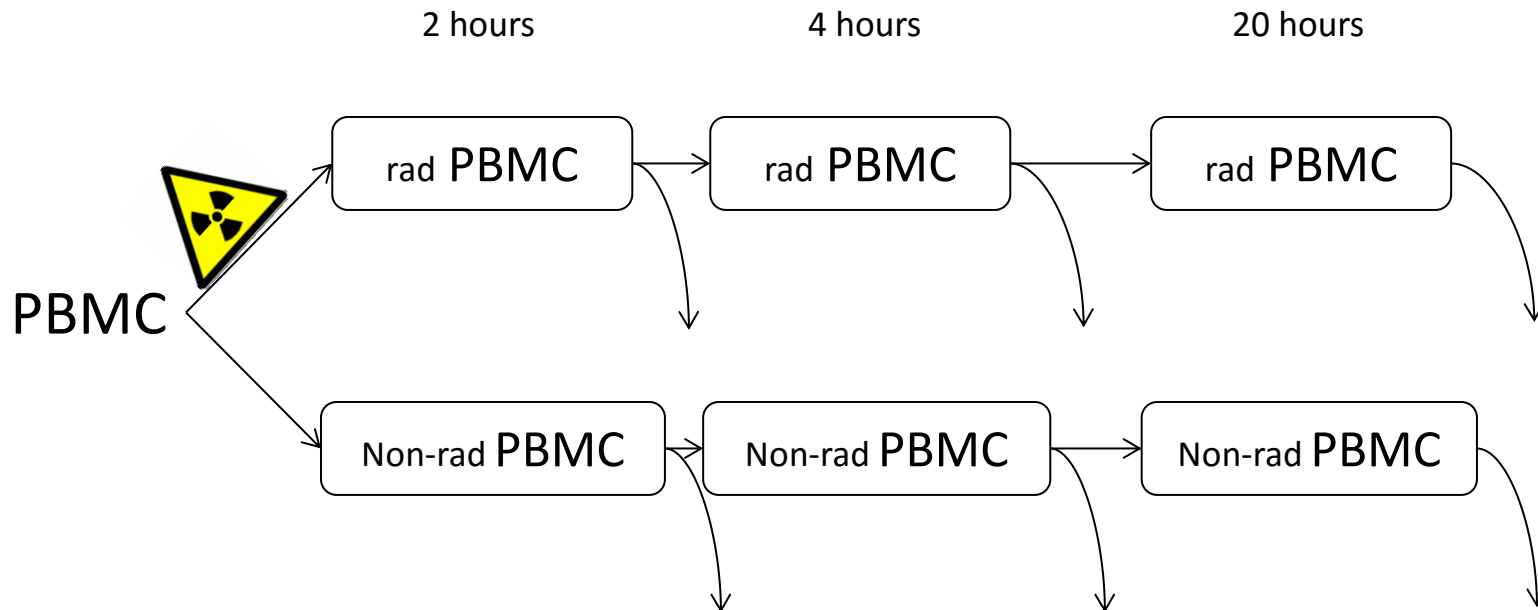


## Study aims



1. Identification of **biological processes** activated or repressed in irradiated PBMCs using microarray analysis
2. Identification and functional characterization of secreted factors released from irradiated PBMCs

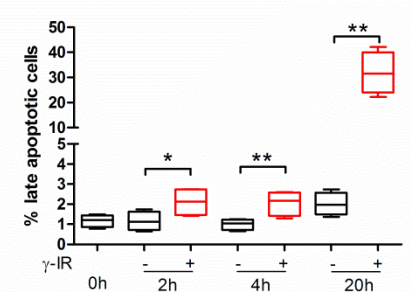
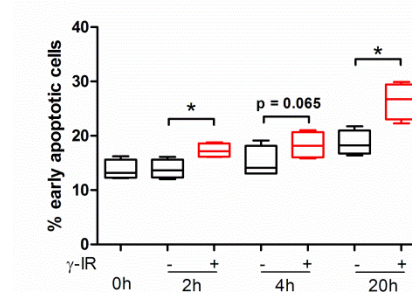
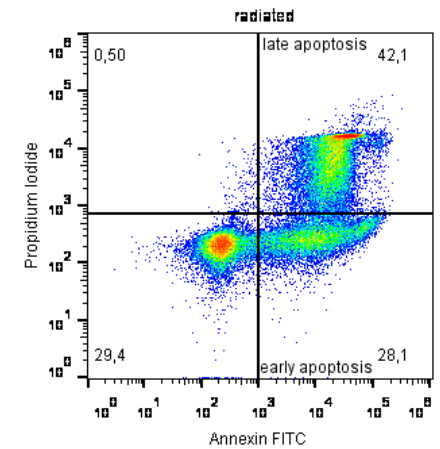
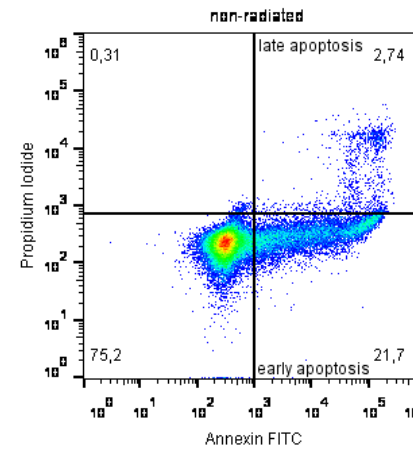
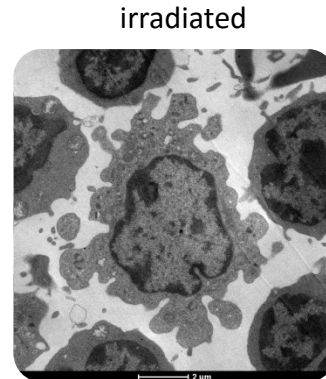
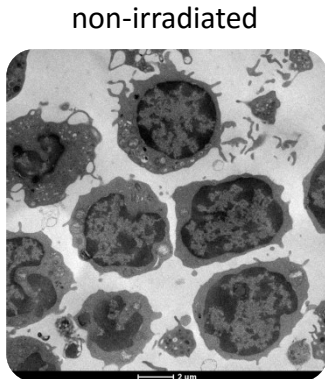
## Study protocol



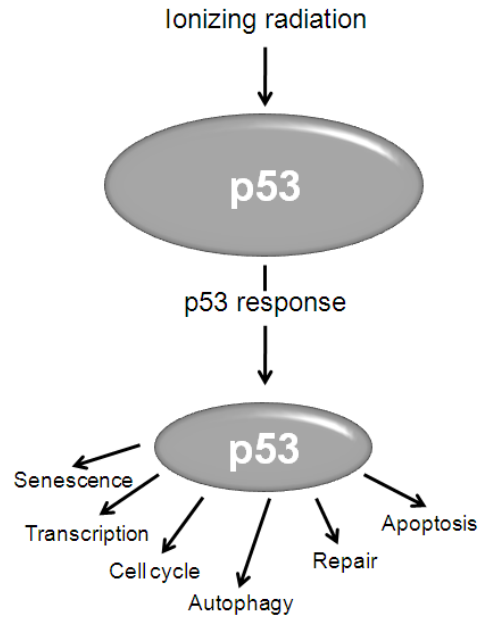
**mRNA , microRNA, protein  
analysis**

n= 4  
4 time points  
0h one condition  
28 mRNA samples

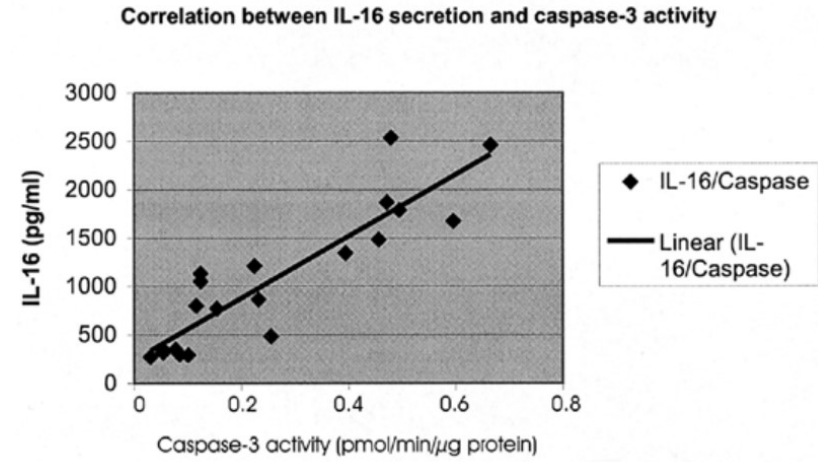
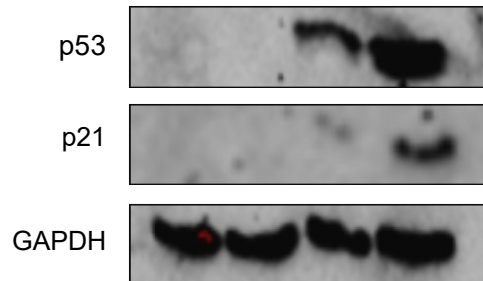
# Ionizing radiation induces Apoptosis



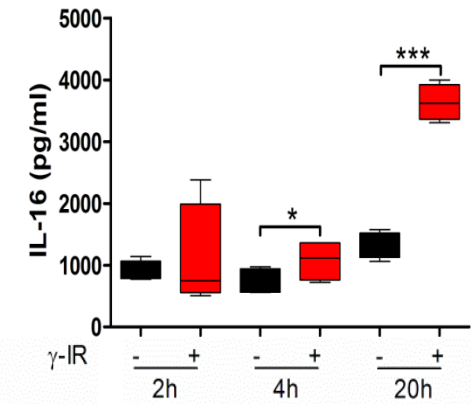
# Ionizing radiation induces Apoptosis



γ-IR    -    -    +    +



Ludwiczek et al. Eur Cytokine Netw. 2001

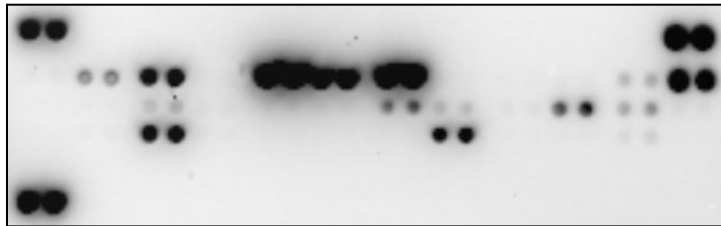


N=4

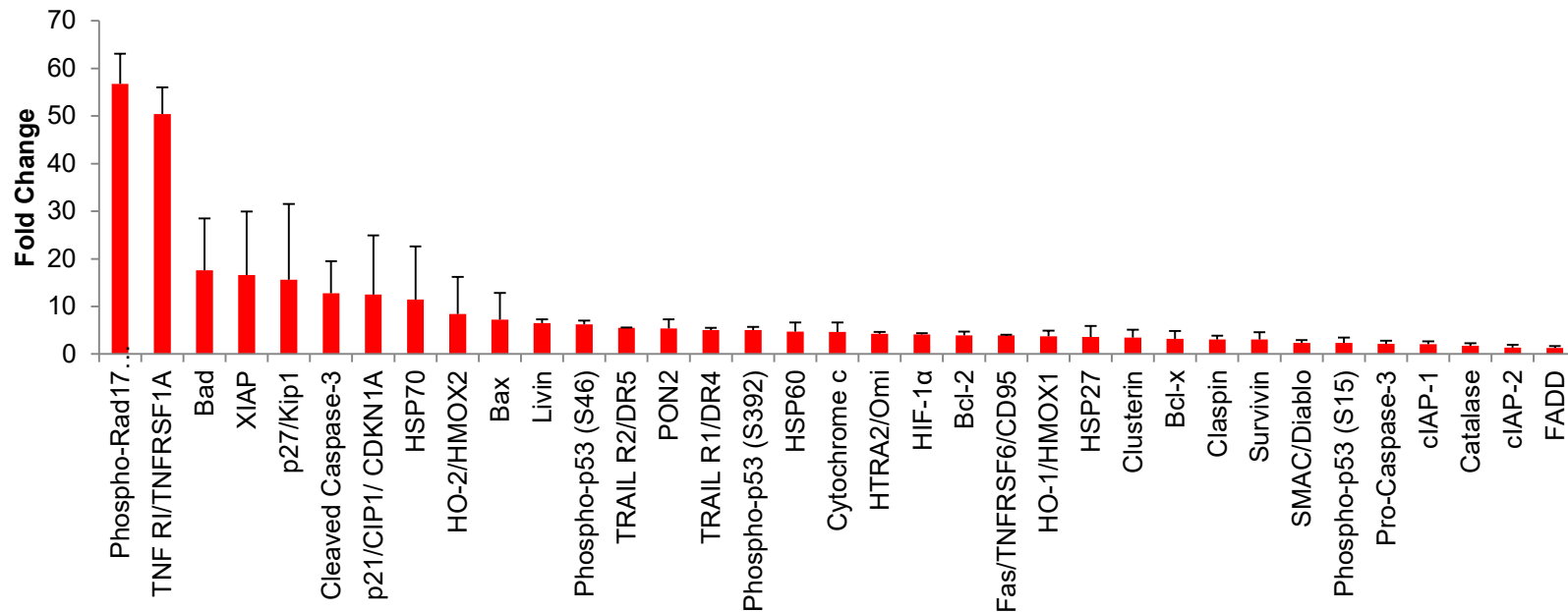
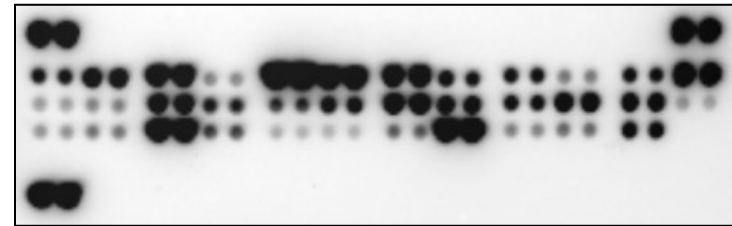
Beer et al. BMC Genomics 2014

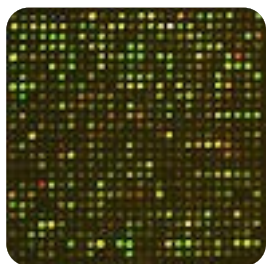
## Ionizing radiation induces Apoptosis

non-irradiated

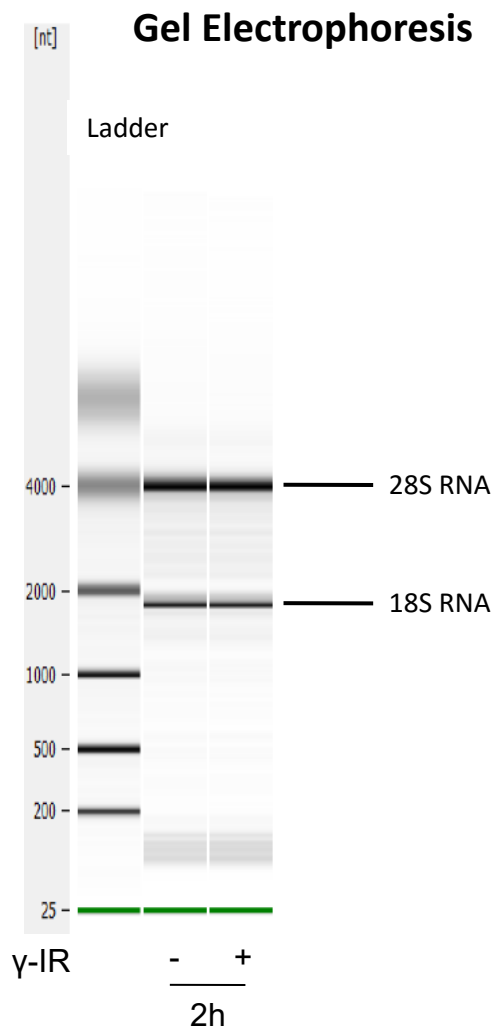


irradiated

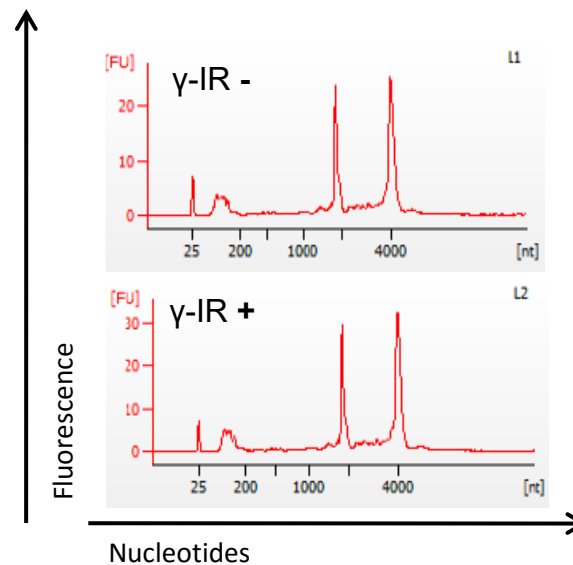




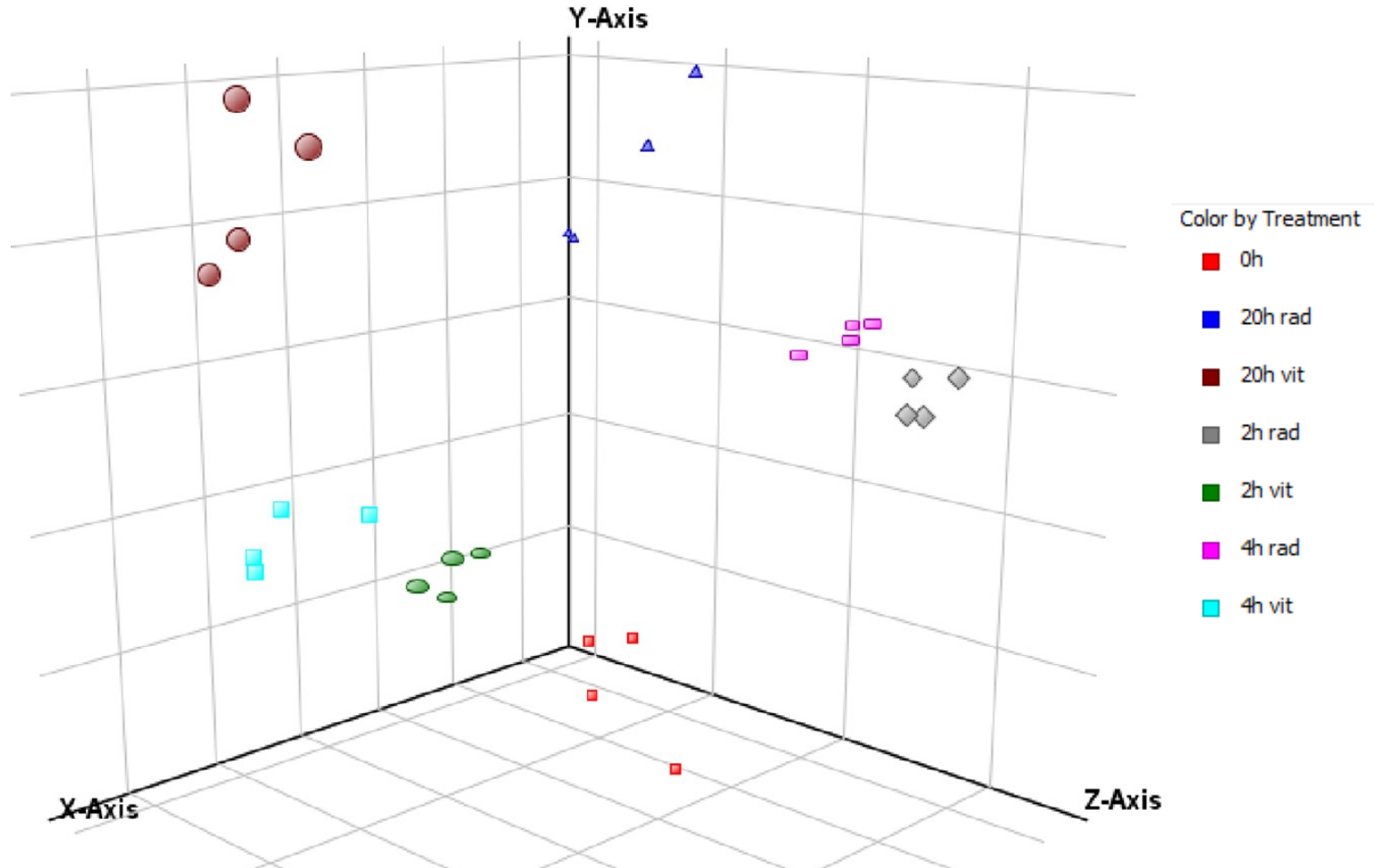
# Quality Control



# Electropherograms



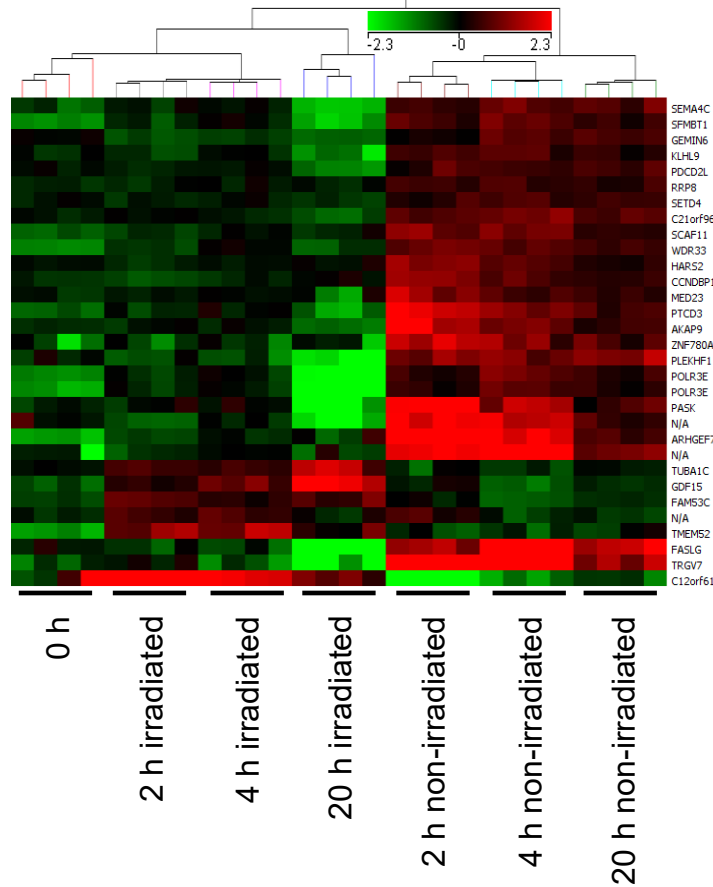
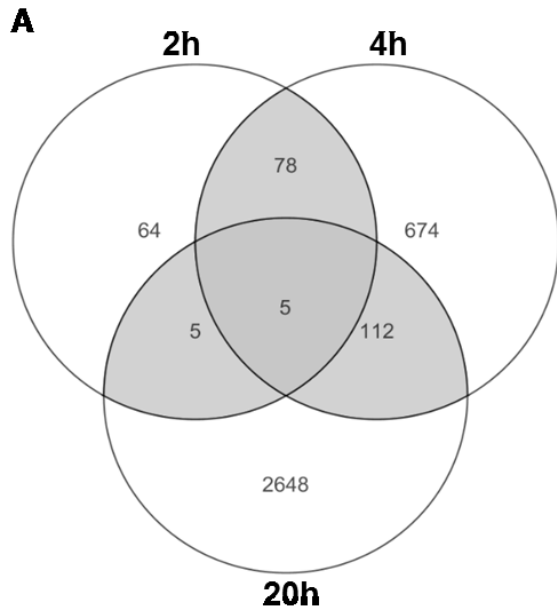
# Principal Component Analysis



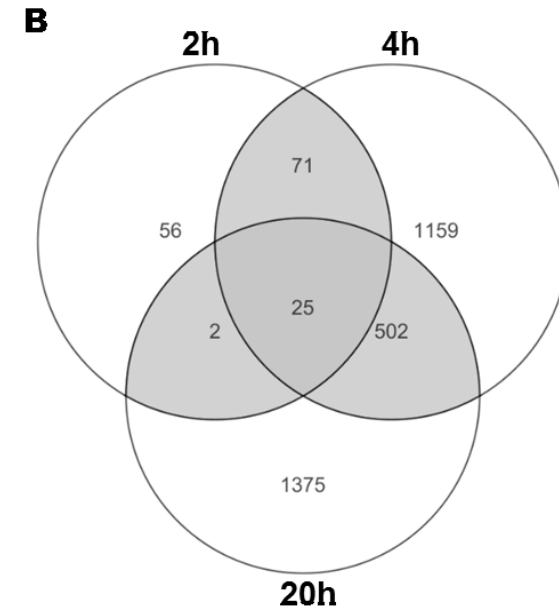


# mRNA – microarray

UP regulated genes

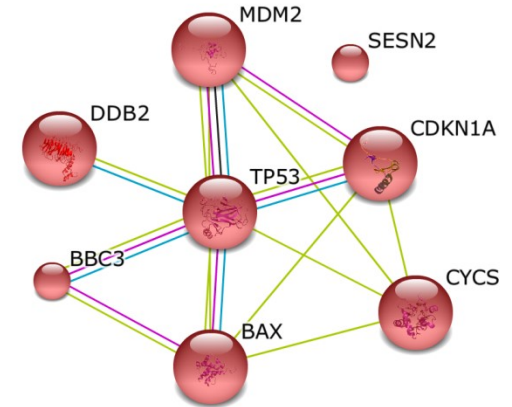


DOWN regulated genes



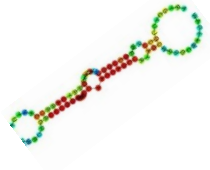
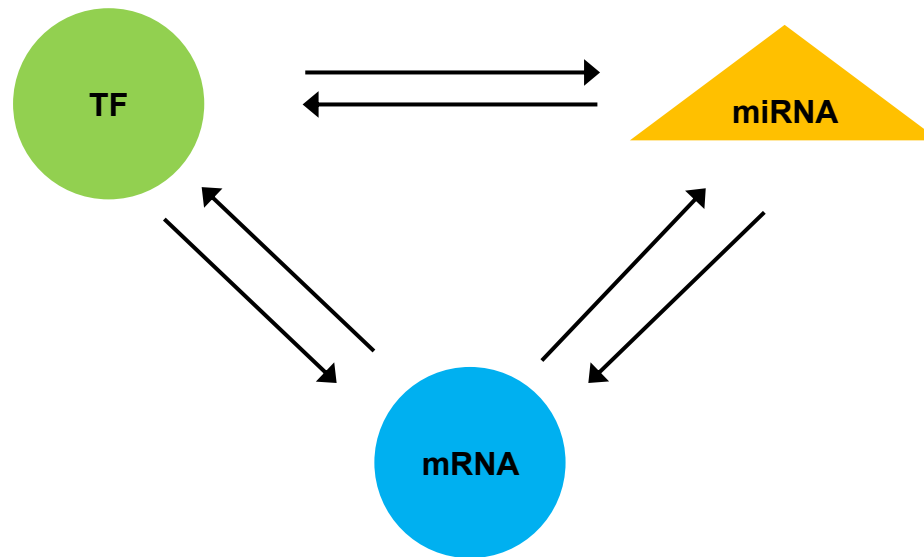
## Canonical Pathway Analysis

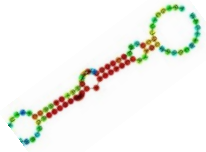
KEGG Pathways up-regulated in irradiated PCMBs  
(BH Corrected p-value <0.05, FC ≥1.5 in 2 of 4 samples).



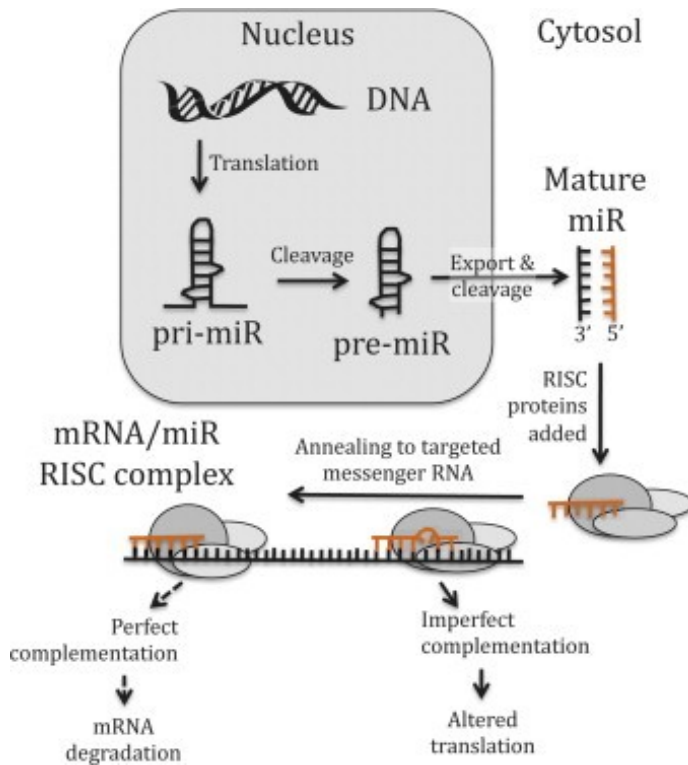
Pathway	p = value	Enrichment Score	Number of Genes
<b>p53 signaling pathway</b>	p<0.0001	15,52	16
<b>Pathways in cancer</b>	p<0.0001	4,34	46
<b>Lysosome</b>	p<0.0001	7,26	41
<b>Phagosome</b>	p<0.0001	5,88	42
<b>Chemokine signaling pathway</b>	p<0.0001	5,77	28
<b>Adherens junction</b>	p<0.0001	4,4	15

## Up-stream regulators / Network analysis

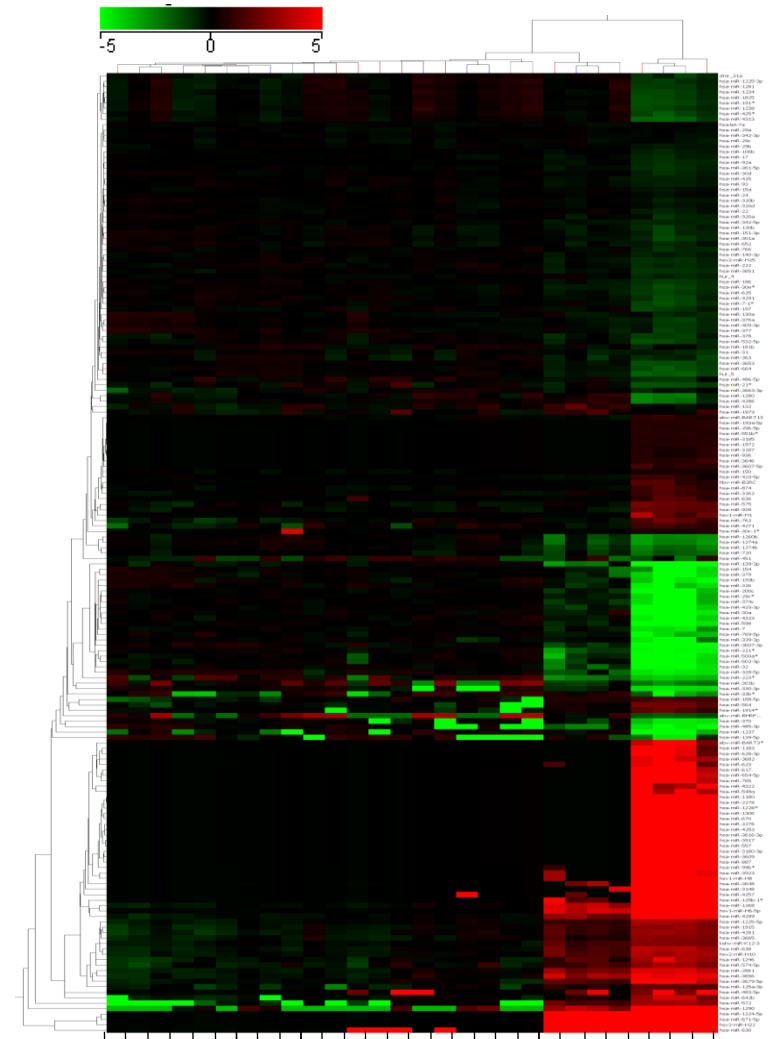




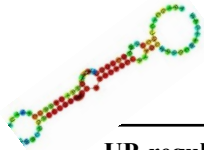
# microRNA



Catto et al. European Urology 2011

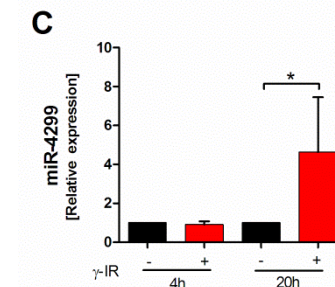
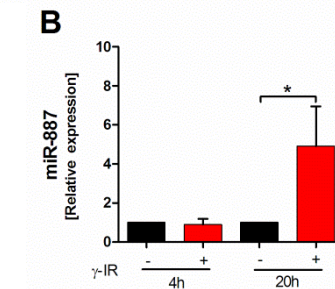
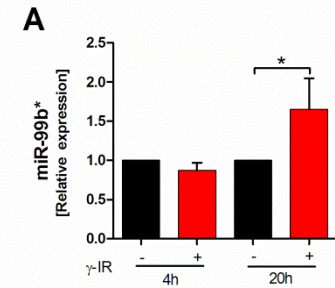


## IR alters microRNA expression

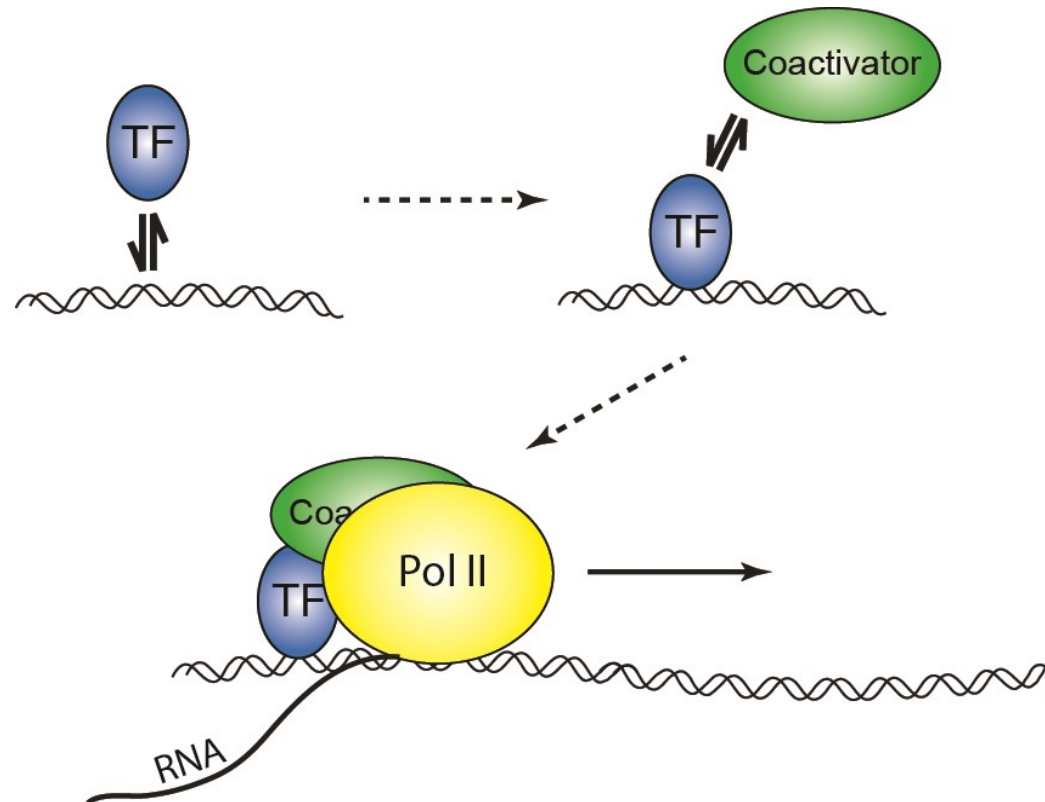


UP-regulated miRNA in PBCMs 20 h after irradiation with a adj.P value < 0.5

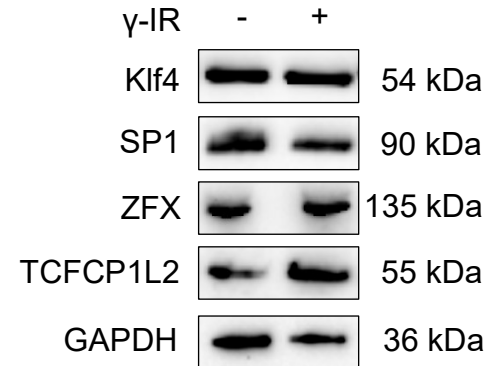
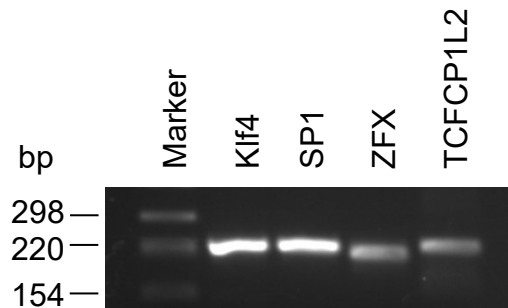
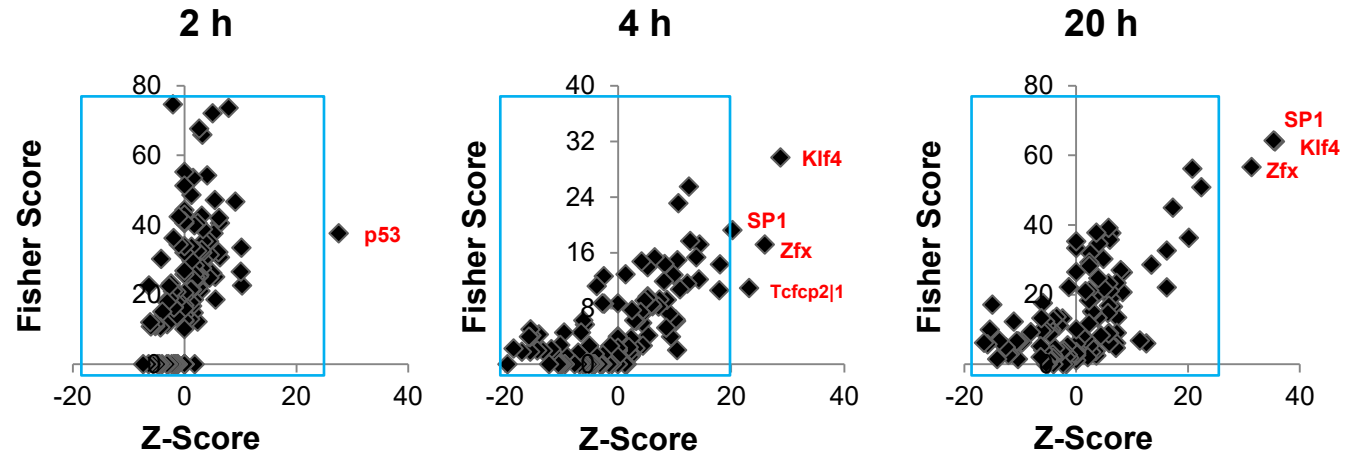
miRNA	adj.P	Donor 1	Donor 2	Donor 3	Donor 4
hsa-miR-3609	7.7E-06	7.63	7.88	7.32	7.61
hsa-miR-1306	4.1E-04	7.02	8.29	6.37	7.79
hsa-miR-3616-3p	4.5E-05	7.44	7.17	6.52	7.03
hsa-miR-4253	4.3E-05	7.16	7.06	6.35	6.70
hsa-miR-1180	4.3E-04	6.36	7.68	5.92	7.12
hsa-miR-1228*	7.5E-04	5.95	7.52	5.54	6.97
hsa-miR-99b*	2.1E-05	5.85	6.16	6.48	6.09

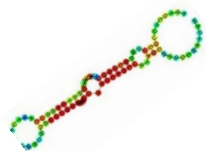


## Transcription Factor Analysis

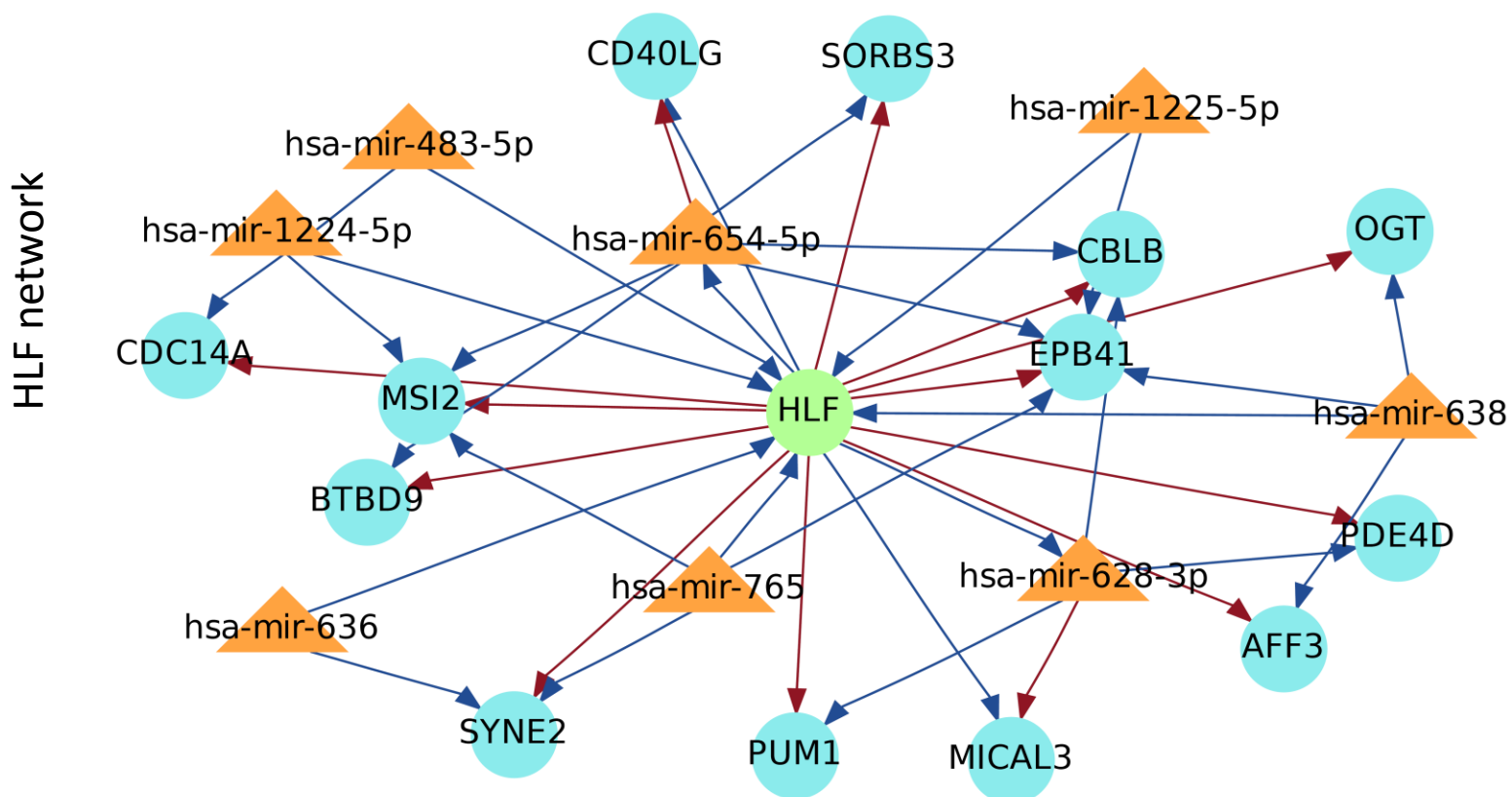


# Transcription Factor





## miRNA-mRNA correlation

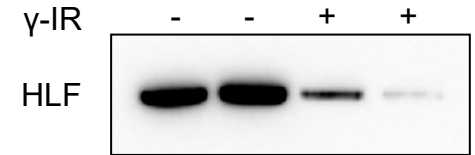
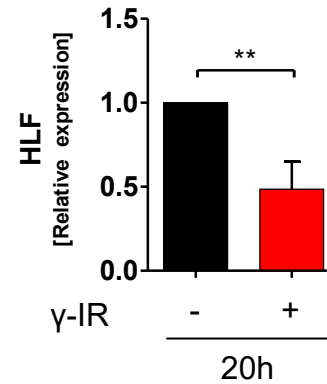
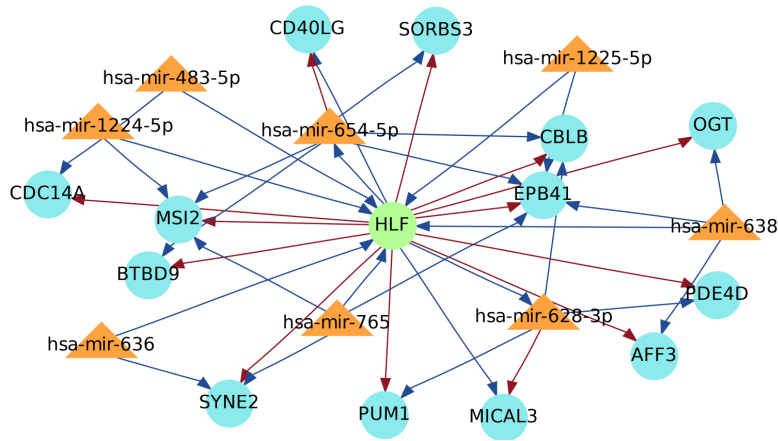






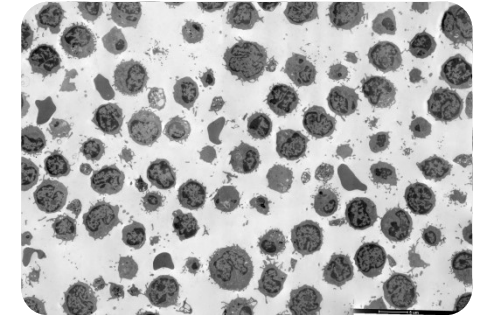
# HLF network

novel regulator of apoptosis



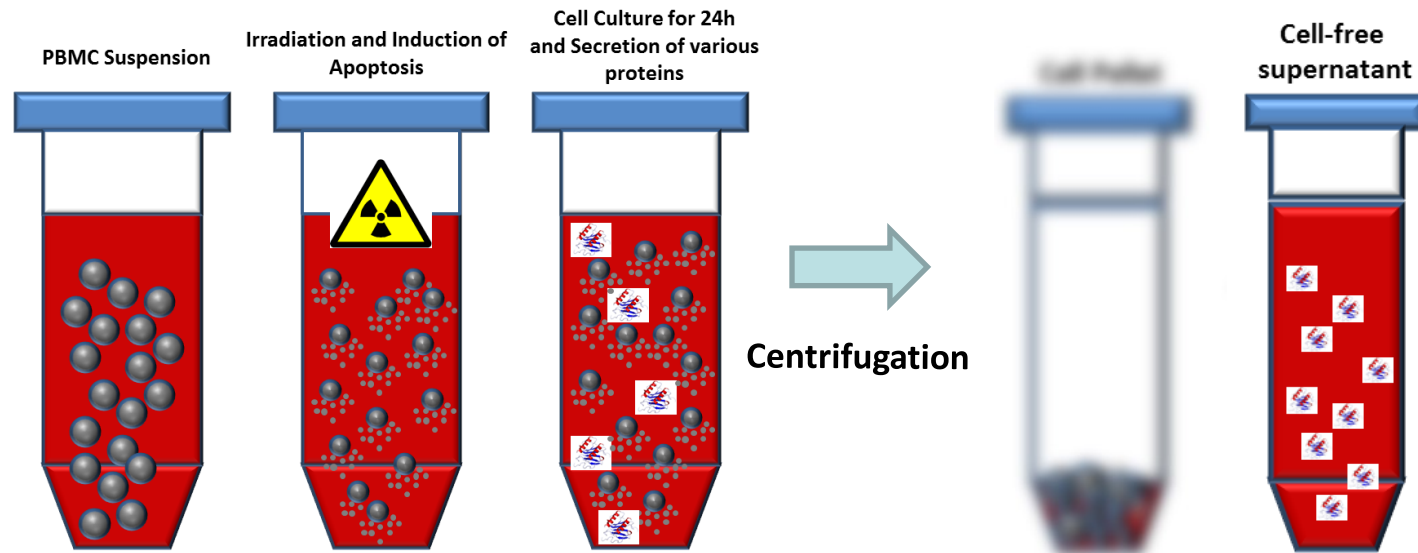
## Summary

In this study we were able to show that



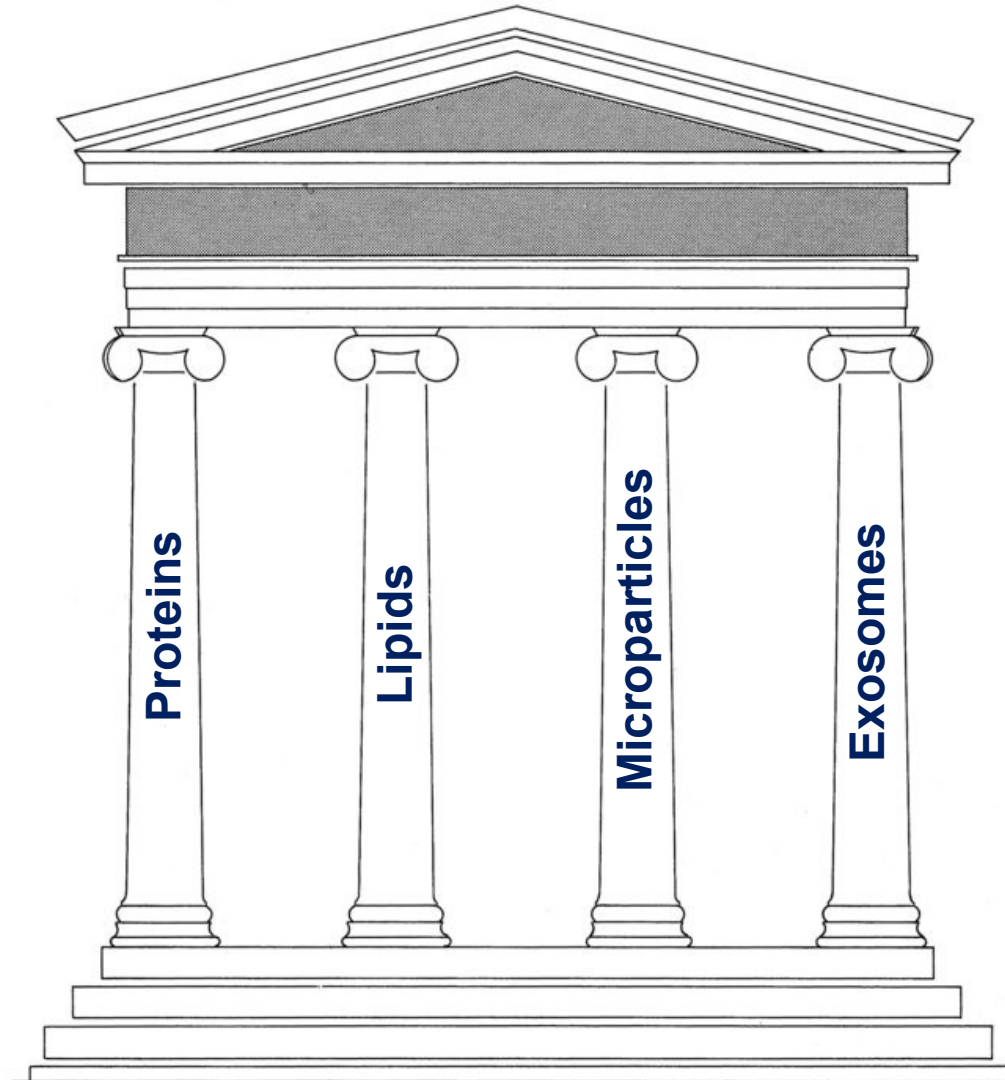
1. IR induces **apoptosis** in human PBMCs
2. IR alters expression of both **mRNAs** and **miRNAs**
3. greatest mRNA and miRNA expression alterations are **detectable 20 hours** after IR
4. **Klf4**, **SP1**, **ZFX** and **TCFCP1L2** are TF present in response to IR
5. **HLF** is a central node build up by miRNA-mRNA and transcription-factor interaction networks

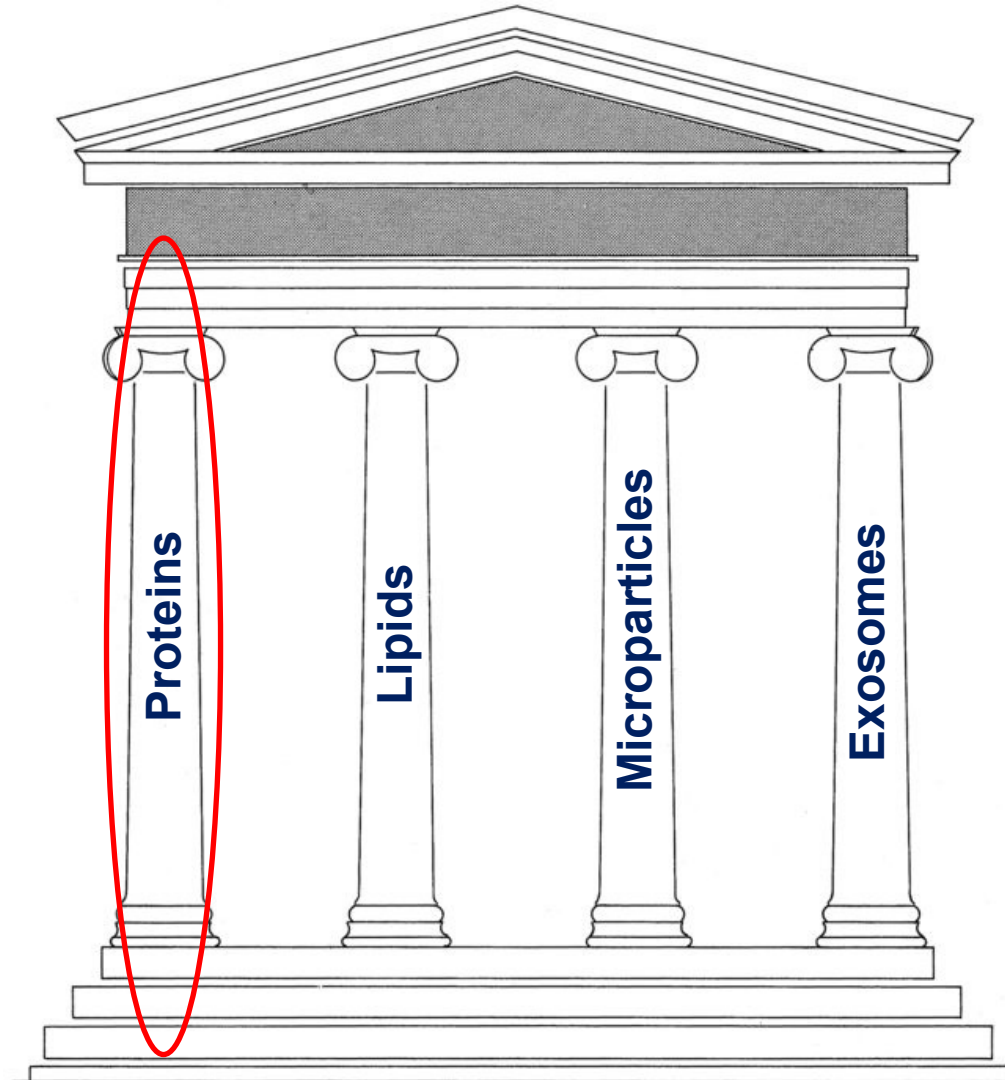
## Study aims

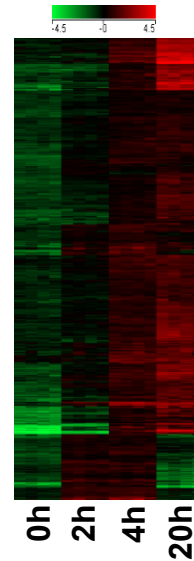
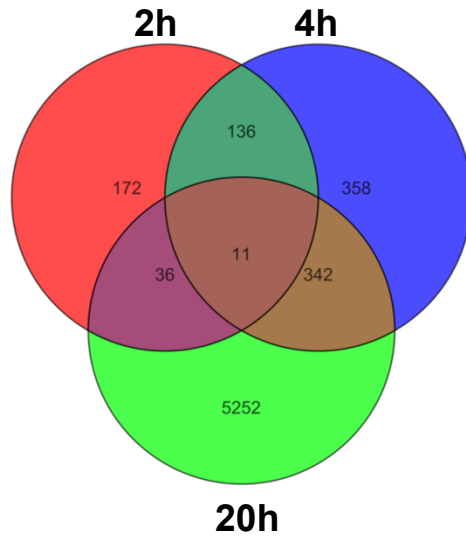


1. Identification of biological processes activated or repressed in irradiated PBMCs using microarray analysis
2. **Identification and functional characterization of secreted factors released from irradiated PBMCs**





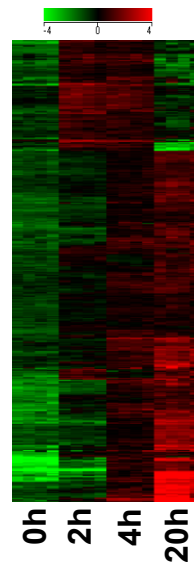
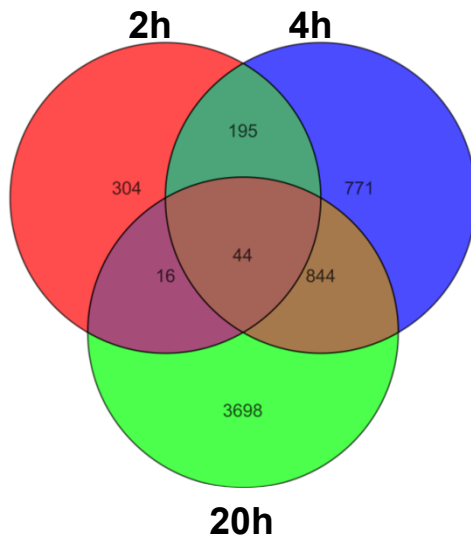




IR induces expression of  
secretory proteins



**179** secreted proteins in  
**non-irradiated** PBMCs

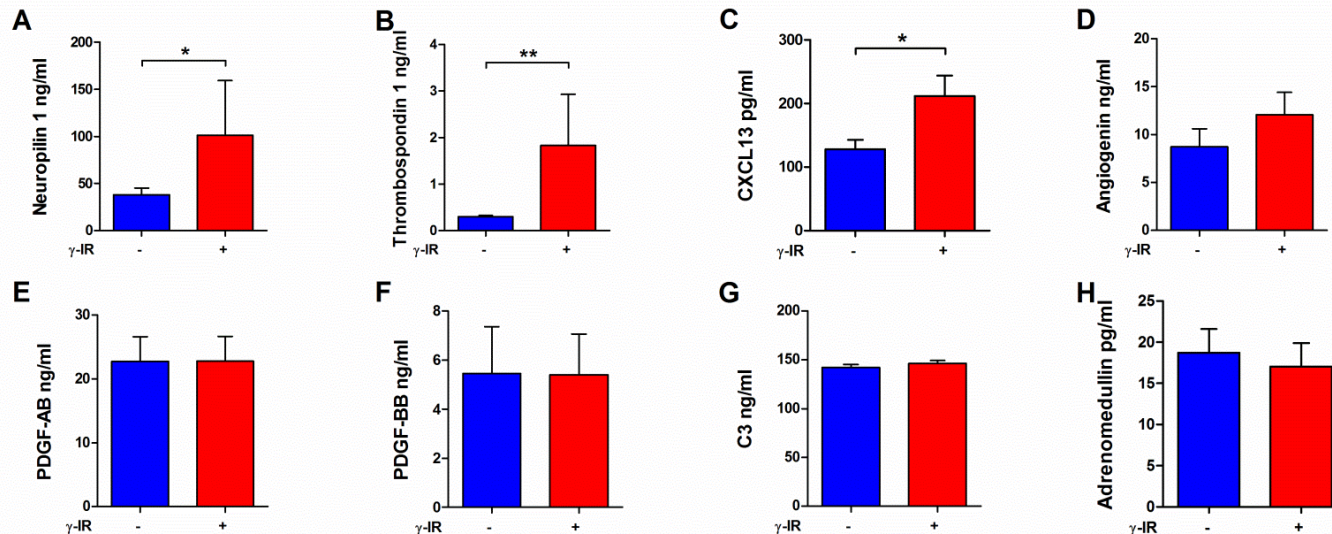


**213** secreted proteins in  
**irradiated** PBMCs

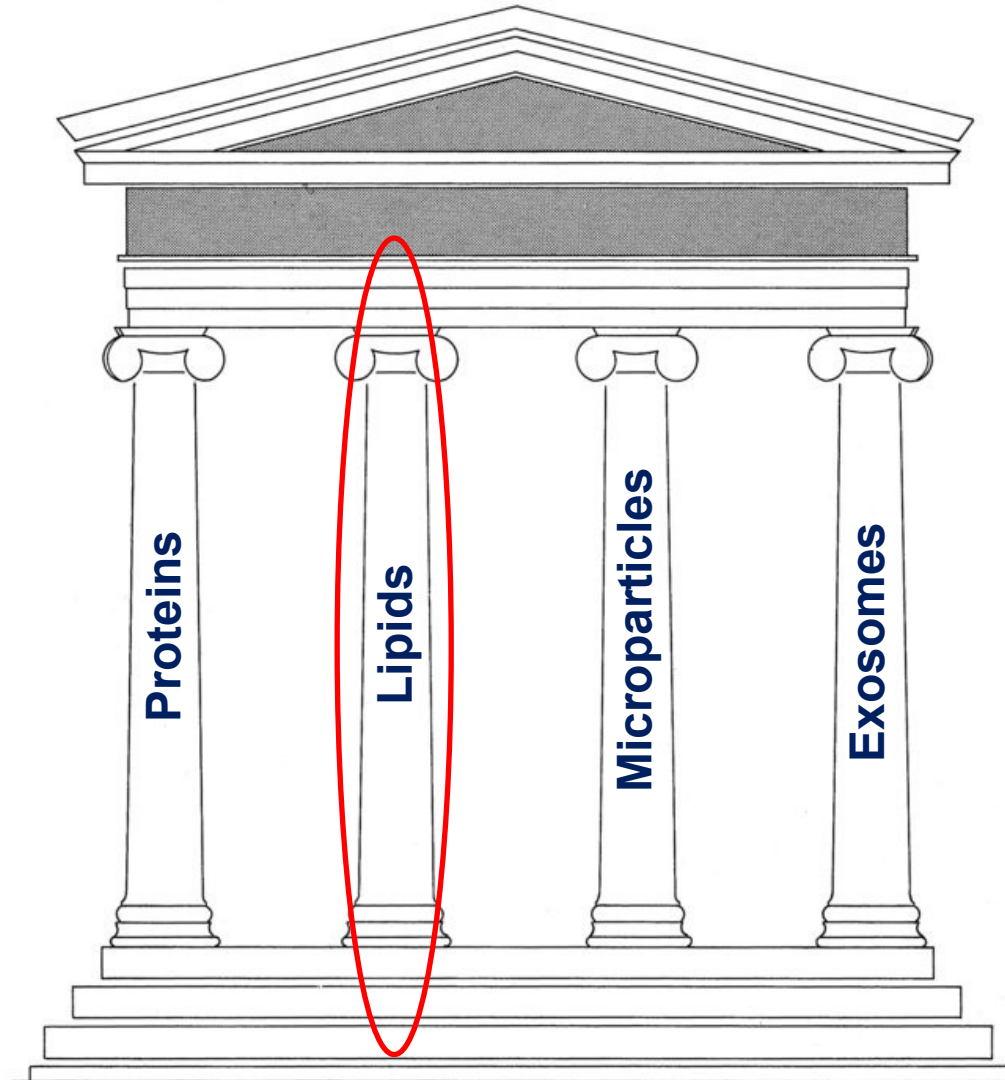
## Related biological processes of secreted proteins

Enrichment of GO-terms in up-regulated genes coding for secretory proteins in irradiated PBMCs.

GO-Term	Enriched terms	p-value	Enrichment Score	Number of Genes
GO:0001568	<b>blood vessel development</b>	1.53e-07	5.32	22
GO:0045766	positive regulation of <b>angiogenesis</b>	3.00e-05	6.34	10
GO:0042060	<b>wound healing</b>	0.0012	2.74	21
GO:0032502	<b>developmental</b> processes	0.0007	1.65	83
GO:0007599	<b>hemostasis</b>	0.0007	3.08	19
GO:0050819	negative regulation of <b>coagulation</b>	0.0007	11.68	6
GO:0050900	<b>leukocyte migration</b>	0.0007	4.28	13







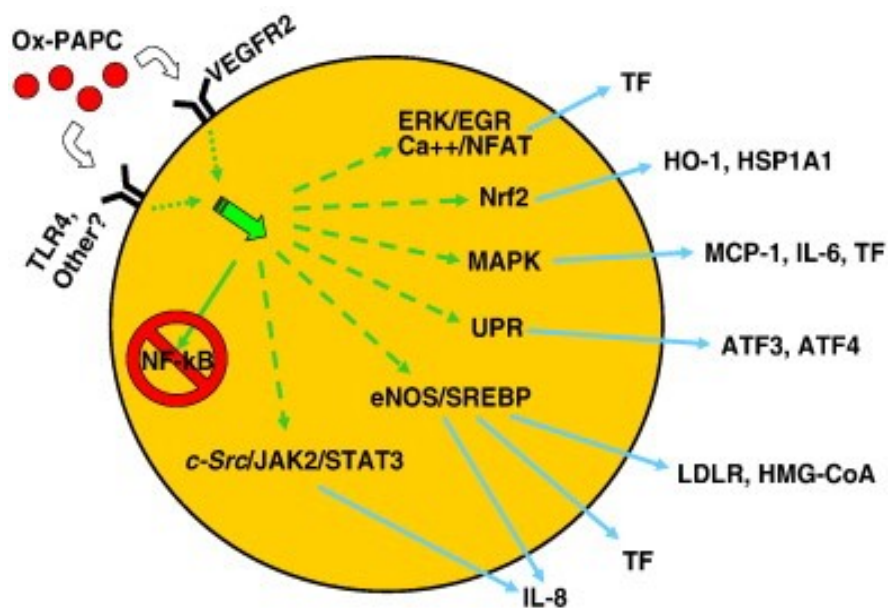
## IR modulates lipid content of PBMC supernatant

IR - +



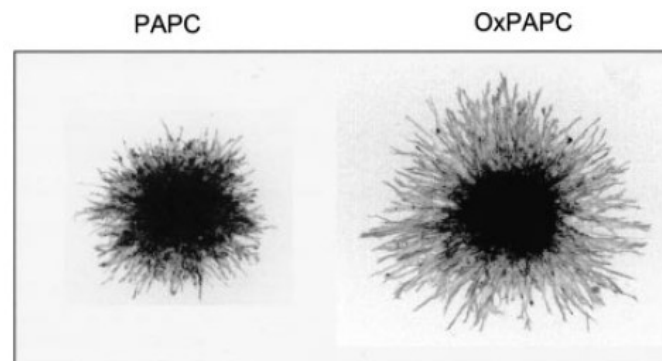
Thin Layer Chromatography

## Background: oxLipids stimulate angiogenesis



Berlinger J. Free Radical Biology and Medicine 2008

### HUVEC sprouting assay



Bochkov V. Circulation Research. 2006;

# IR induces generation of Ox-PC

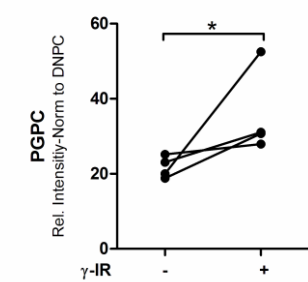
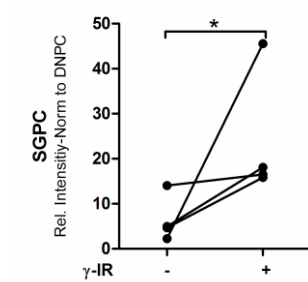
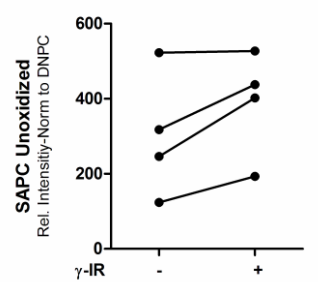
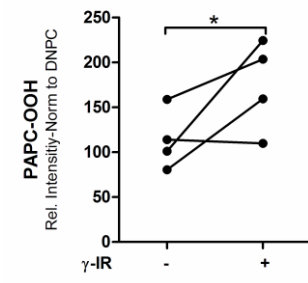
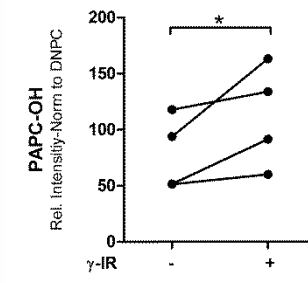
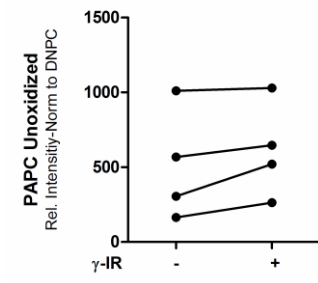
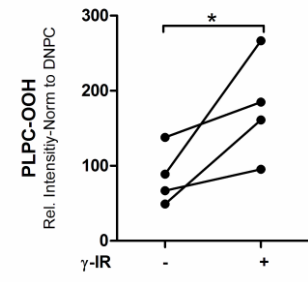
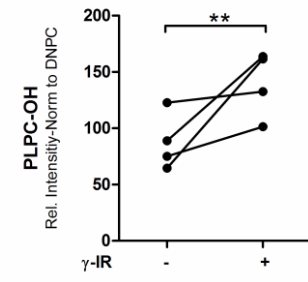
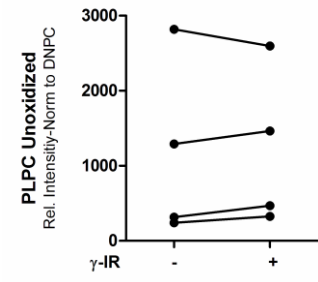
## Non-oxdyzed

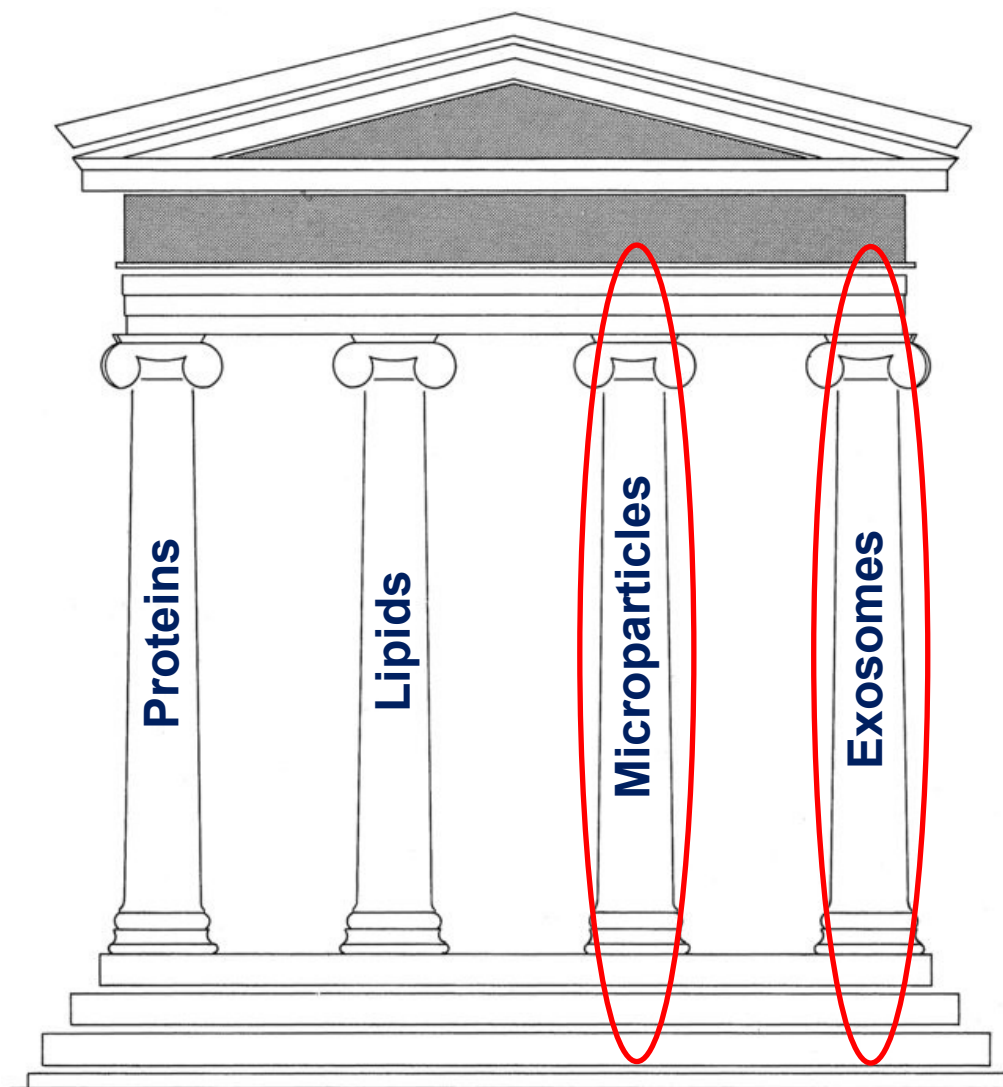
## Oxydized

**PLPC** = 1-palmitoyl-2-linoleoyl-sn-glycero-3-phosphocholine

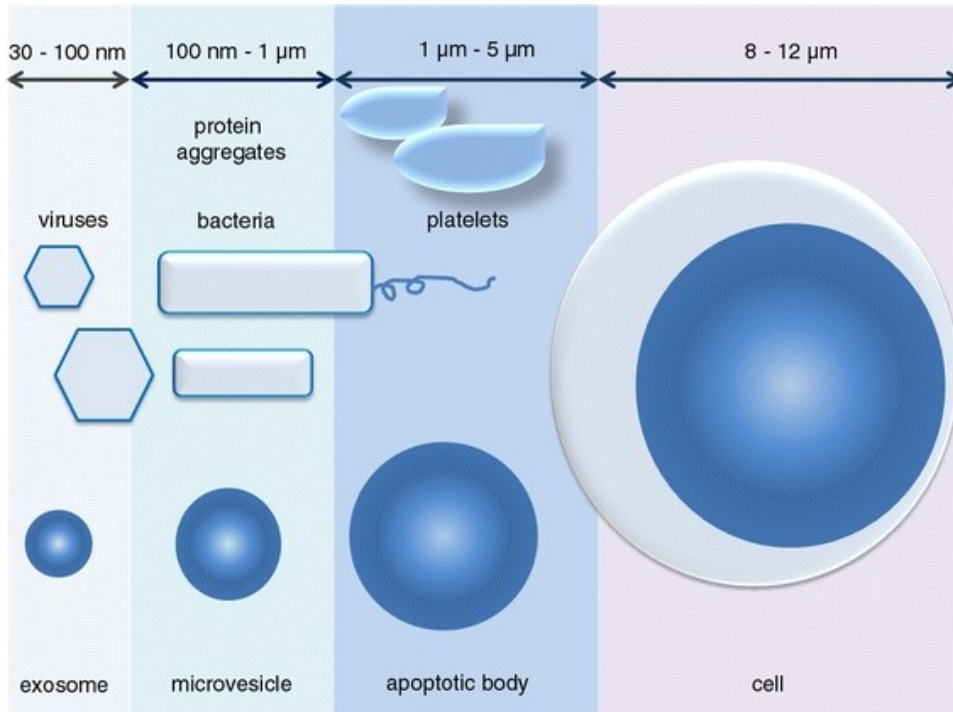
**PAPC** = 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphocholine

**SAPC** = 1-stearoyl-2-arachidonoyl-sn-glycero-3-phosphocholine

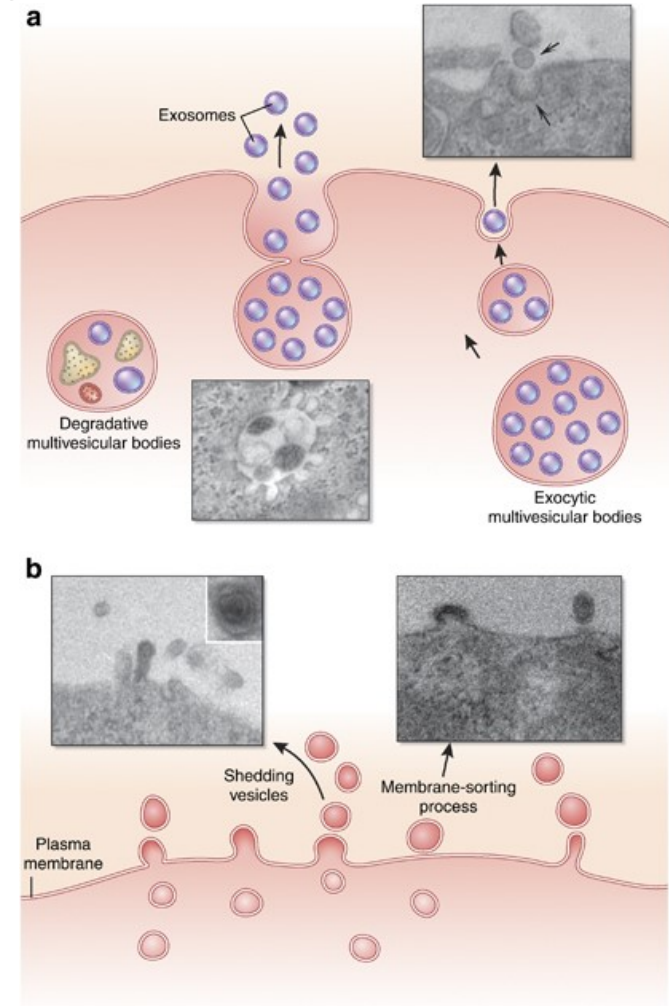




# Extracellular Vesicles

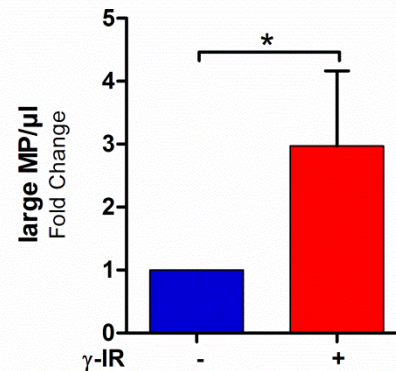
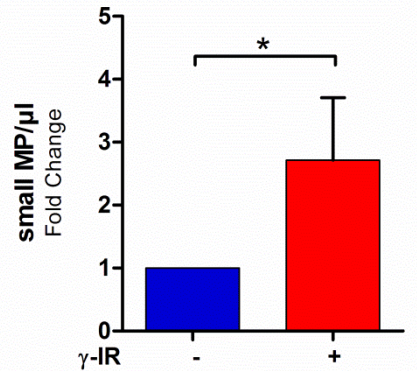
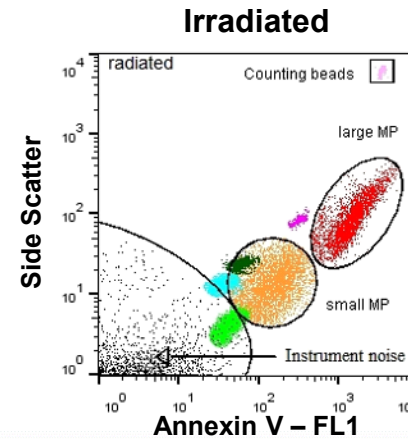
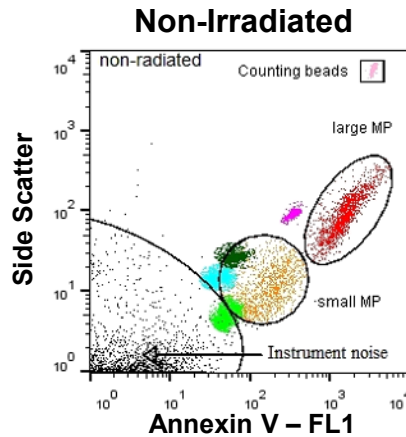


György et al. 2010



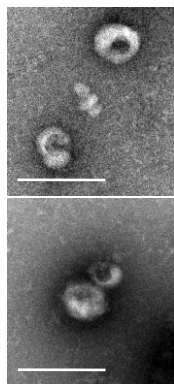
Giovanni et al. Nature 2010

# PBMC secretome contains Microparticles

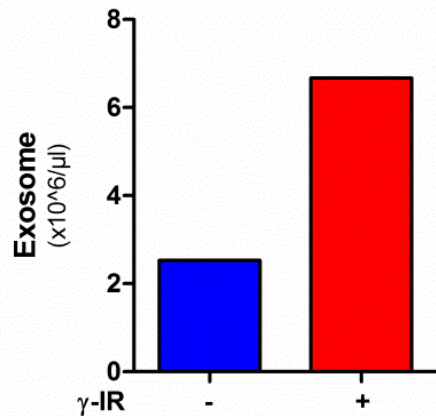


## PBMC secreome contains exosomes

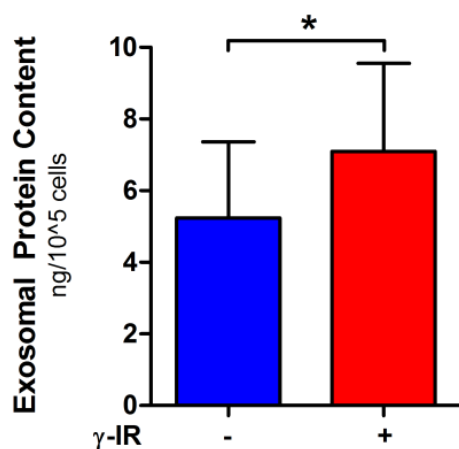
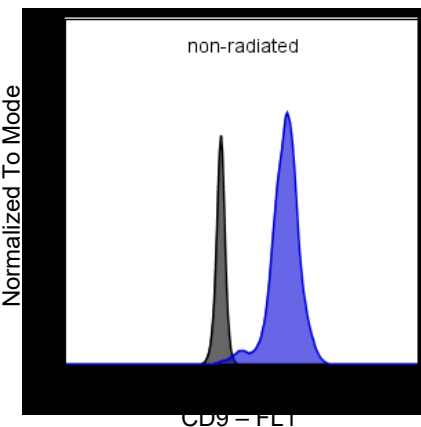
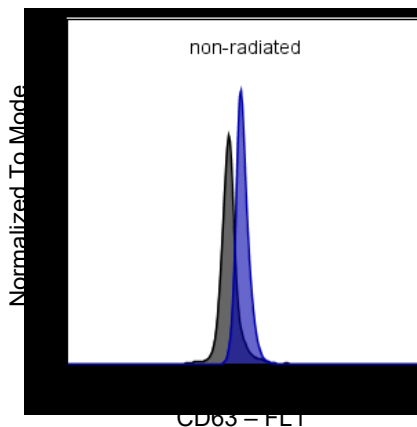
**ELMI**



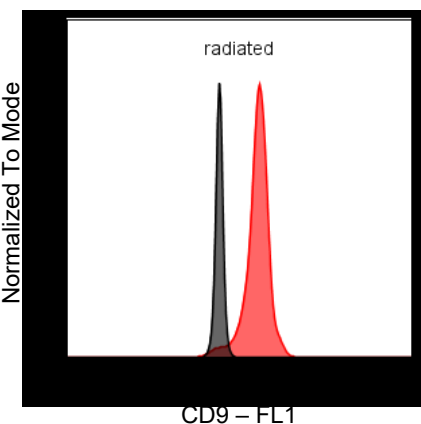
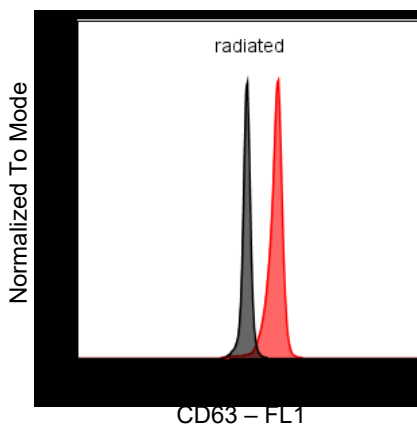
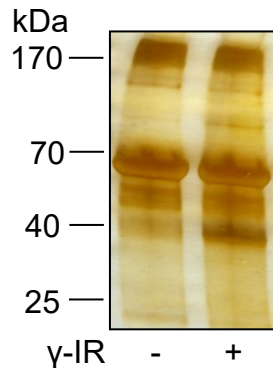
**Quantification**



**FACS**

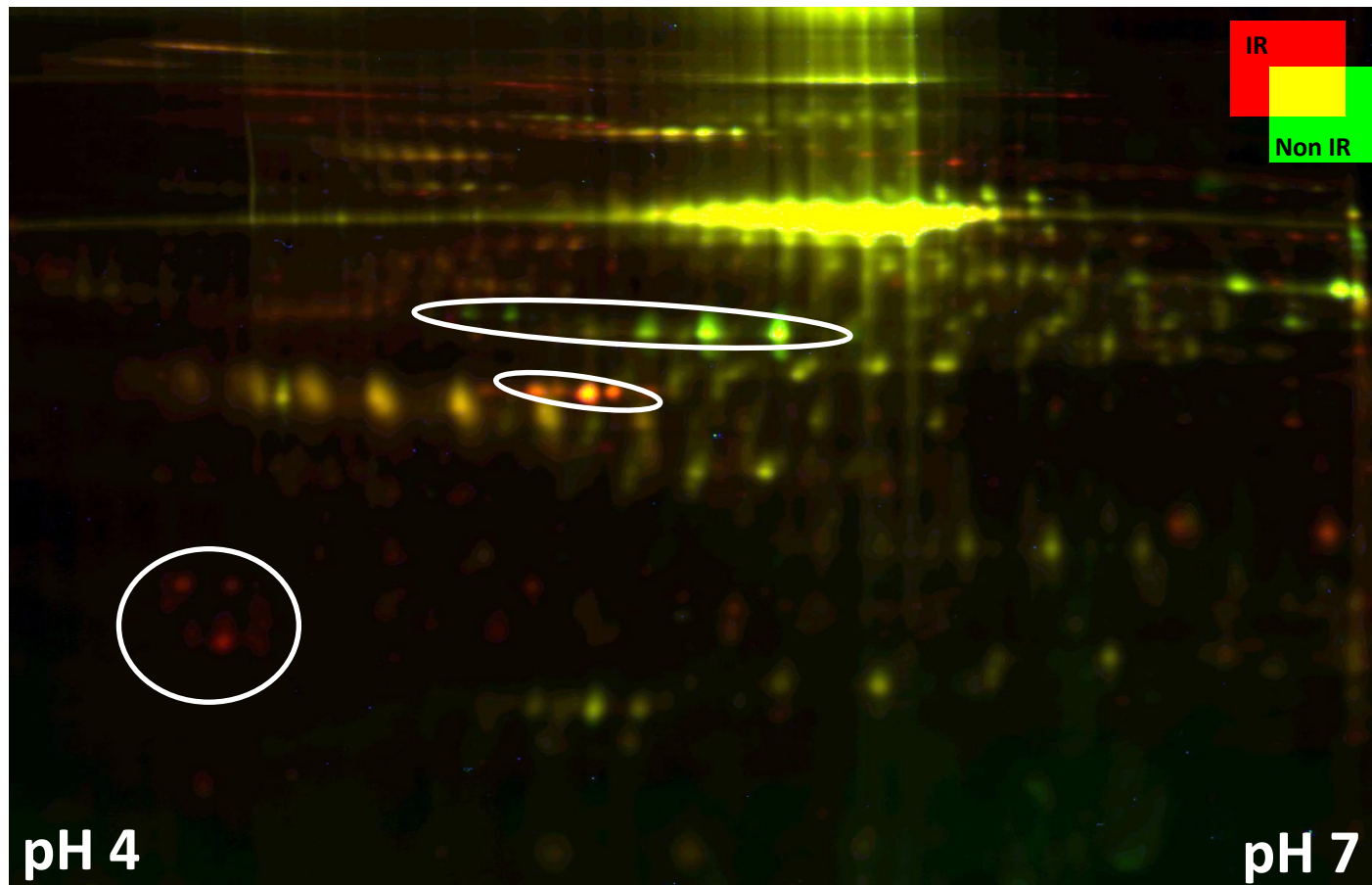


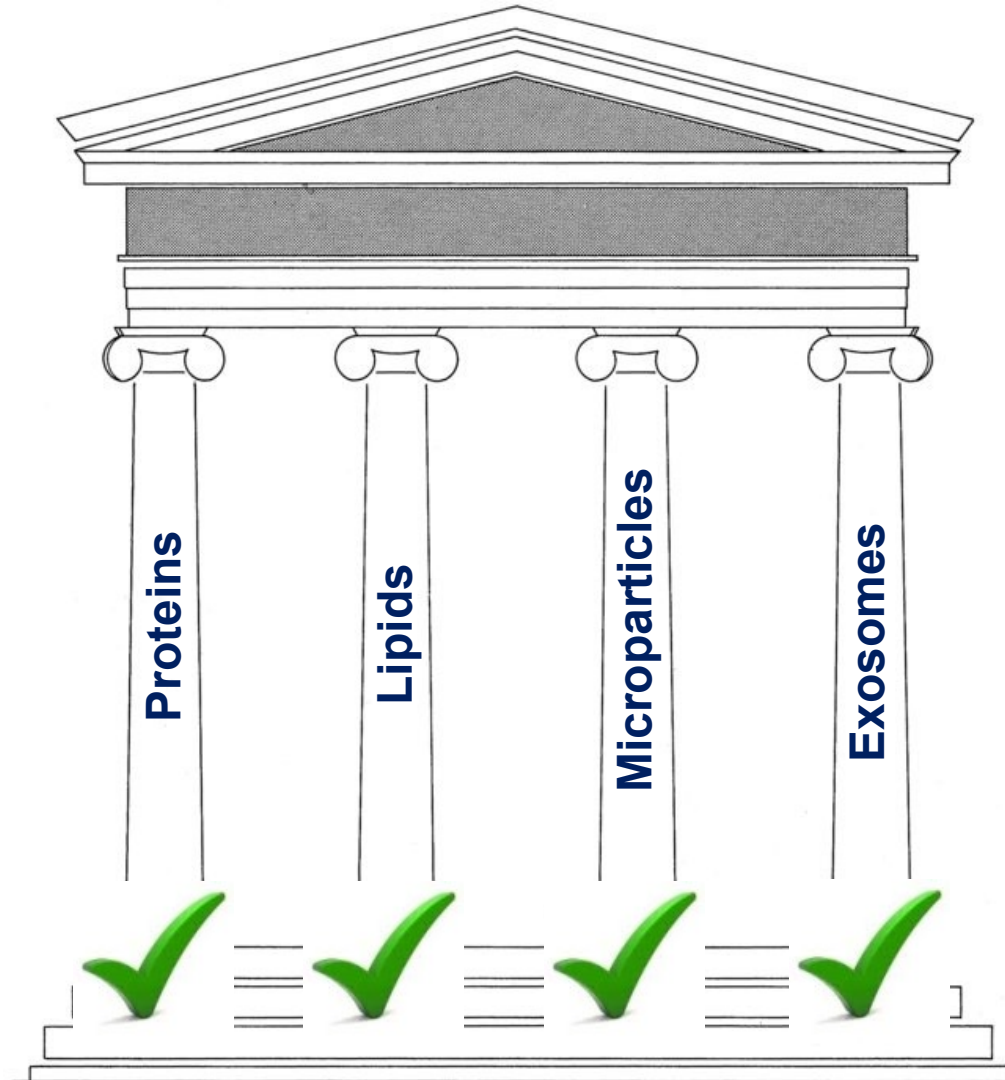
**Protein Content**





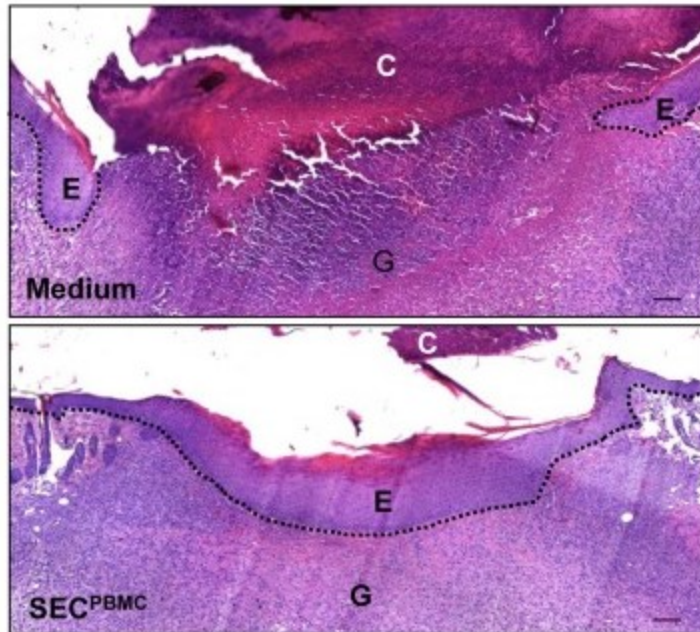
## IR modulates exosome proteome content



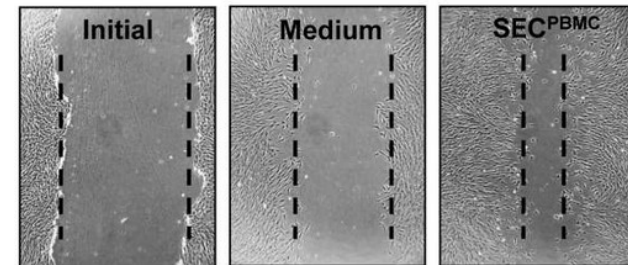


## What is known

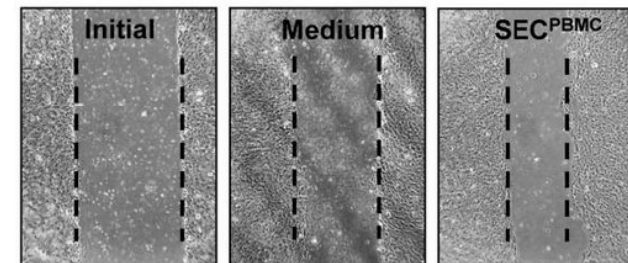
Secretome of PBMC  
enhances wound healing



Secretome of PBMC  
induces cell migration

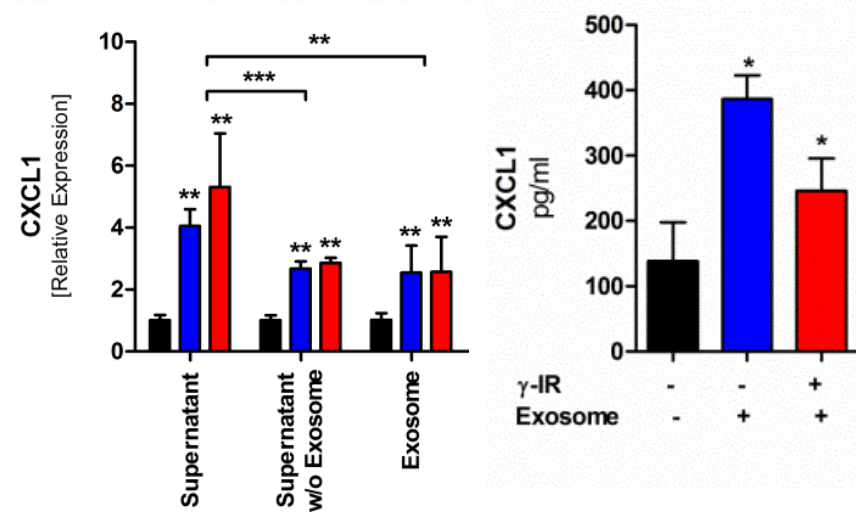
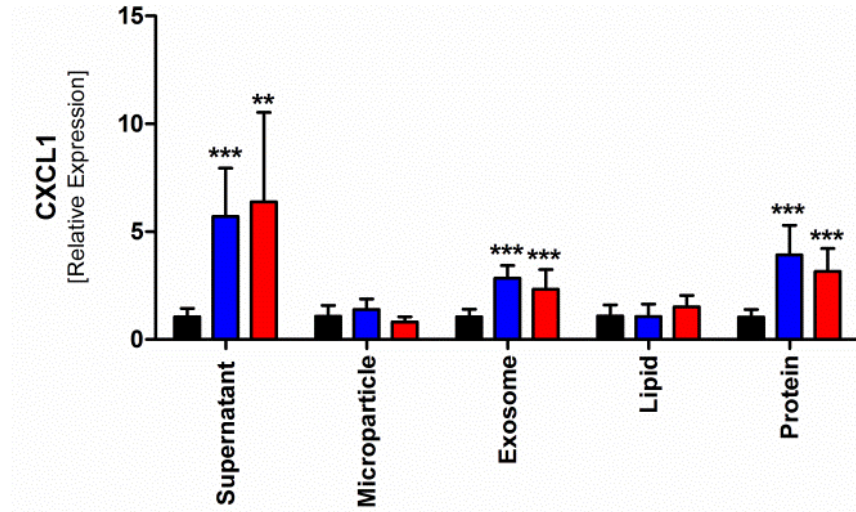


Fibroblast

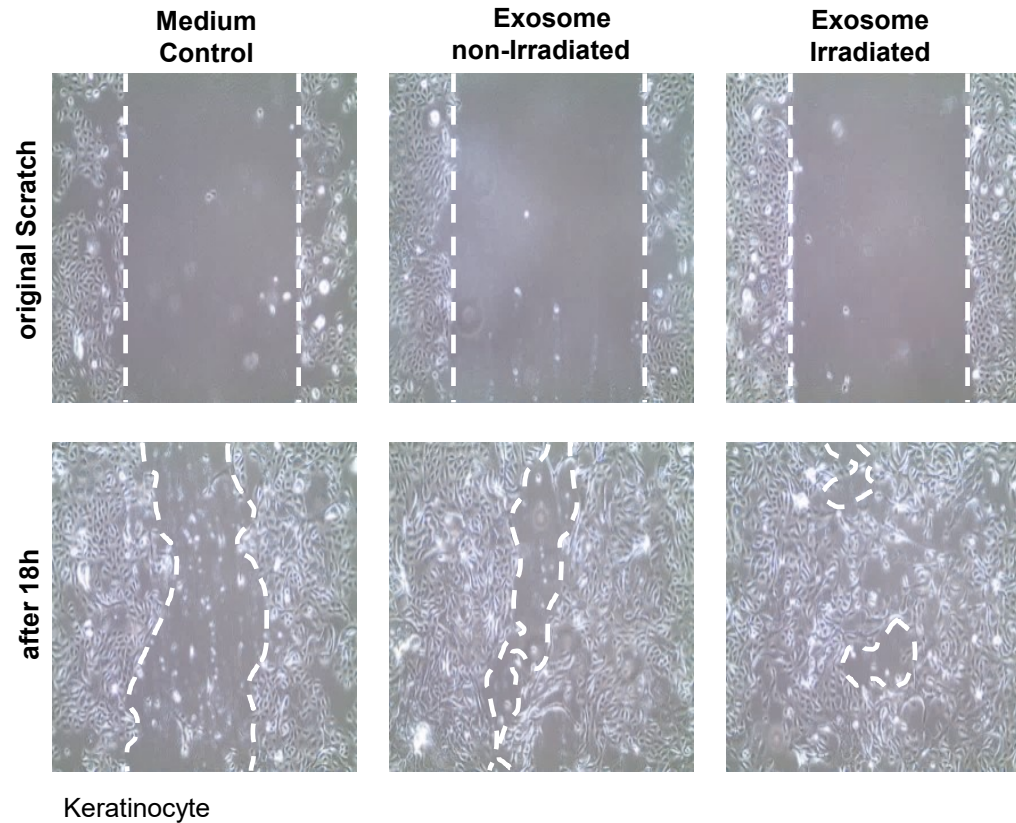


Keratinocyte

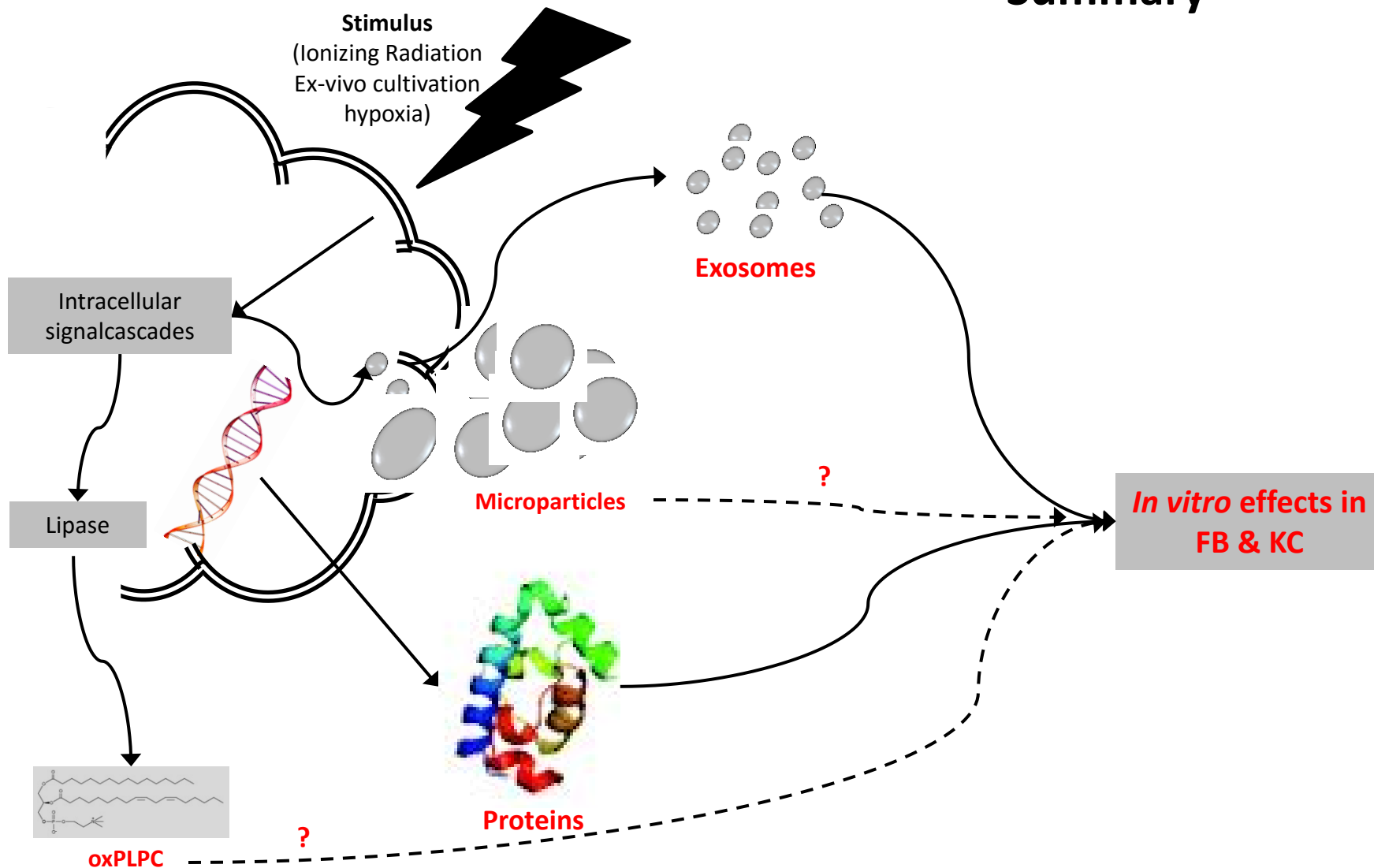
# Exosome and Proteins stimulate CXCL1 & CXCL8 secretion



## Exosome and Proteins stimulate FB und KC migration

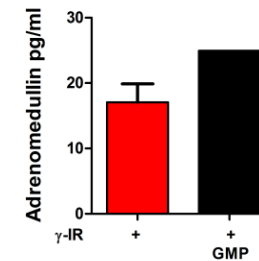
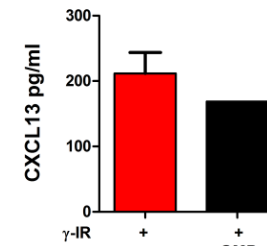
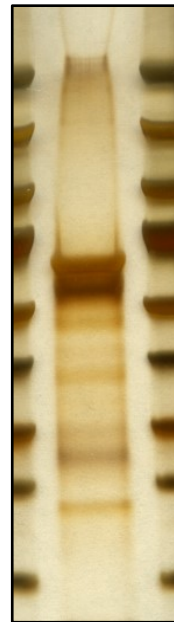
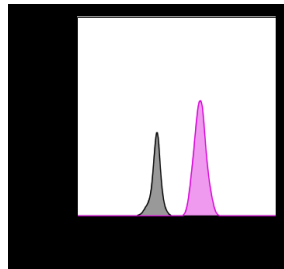
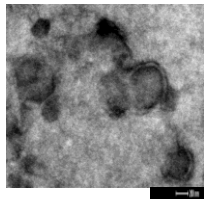
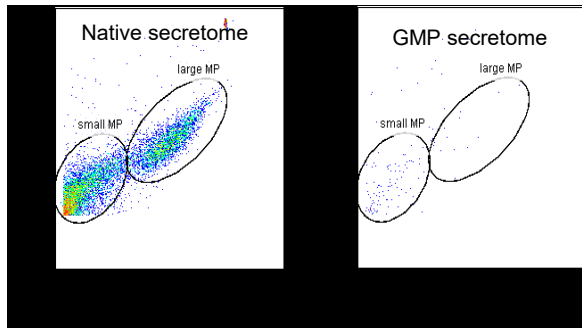


## Summary



## “From bench to bedside” - Viral cleared GMP-produced APOSEC

- Viral clearance: 60,000 Gy + methylene-blue inactivation
- Viral clearance mandatory for clinical research
- 1<sup>st</sup> clinical trial positively finalized (Phase I study)



Secretome irradiated  
Secretome non-irradiated  
GMP secretome irradiated

**Aposec GMP** = secretome of irradiated PBMC produced in accordance to the good manufacturing guidelines (GMP)

## Summary

- IR leads to enhanced release of
  - Secretory proteins
  - Oxidized phospholipids
  - Extracellular vesicles
- *APOSCE GMP* is biological active and contains comparable biological components except for microparticles.
- Exosomes and proteins are the biological active fractions in our *in vitro* assays used.



## Outlook

- Biological active components display promising targets for **cell-free therapies** in the field of **regenerative medicine**.
- Based on the data we speculate that **pleiotropic** biological effects might be evoked by **different biological components** of APOSCE.
- We are currently planning to investigate the effects of the **exosome** and **protein** fraction in further experimental setups to identify the underlying **molecular mechanisms**.

# Acknowledgment's

## Medical University Vienna

### **Christian Doppler Laboratory** for Cardiac and Thoracic Diagnosis and Regeneration

Hendrik Jan Ankersmit  
Matthias Zimmermann  
Andreas Mitterbauer  
Mohammad Mahdi Kasiri  
Elisabeth Simader  
Konrad Hoetzenecker  
Thomas Haider  
Denise Traxler-Weidenauer

### **Red Cross Blood Transfusion Service**

Christian Gabriel

### **Section for Medical Statistics**

Robin Ristl

### **University Hospital of Craniomaxillofacial**

Rudolf Seemann

### **Department of Histology**

Adolf Ellinger

### **Department of Physiology**

Maria Zellner

### **Department of Dermatology**

Michael Mildner  
Florian Gruber  
Marie Narzt

### **Department of Cardiology**

Mariann Gyöngyösi

