

2<sup>nd</sup> eSI-WORKSHOP

29th-30th September 2006, Alicante



MEDIZINISCHE UNIVERSITÄT  
INNSBRUCK

# IFN $\alpha$ induced nephrotoxicity involves an apoptotic cell death pathway



*Judith Lechner\*, Nadia Malloth\*, Thomas Seppi\*\*, Walter Pfaller\**

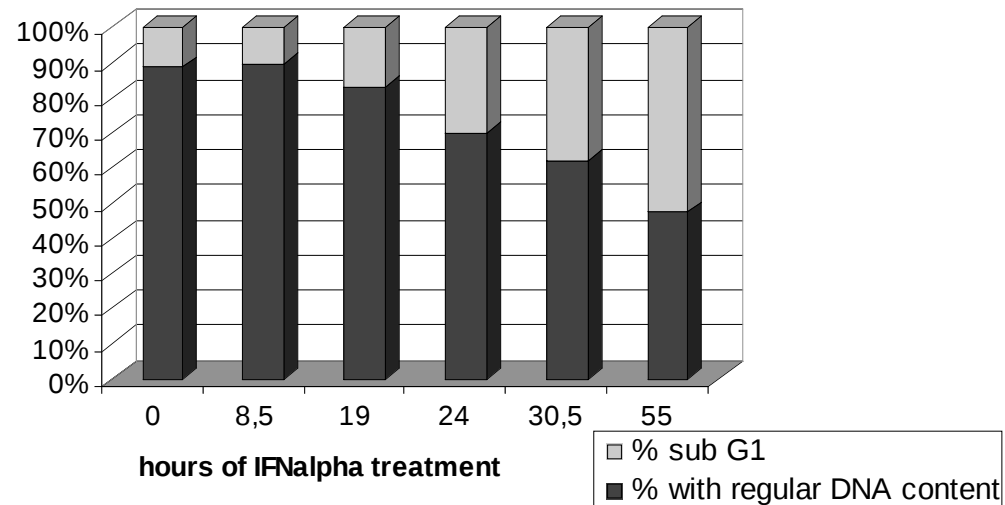
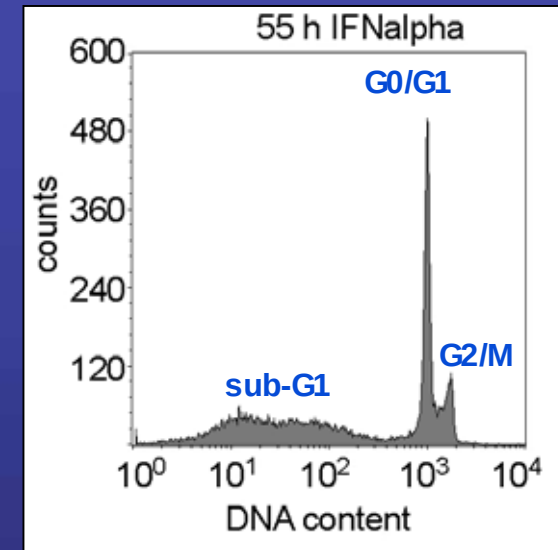
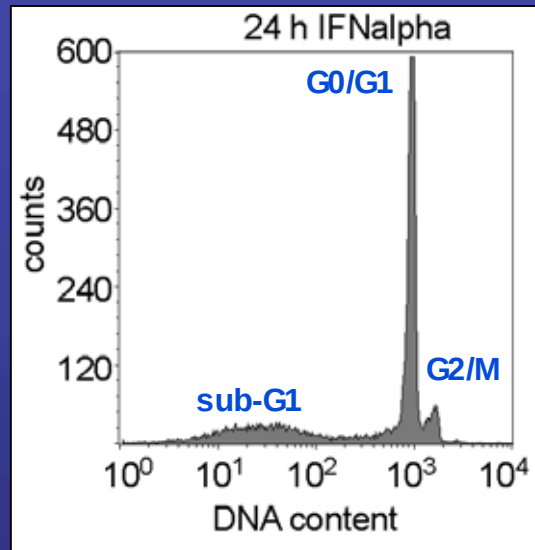
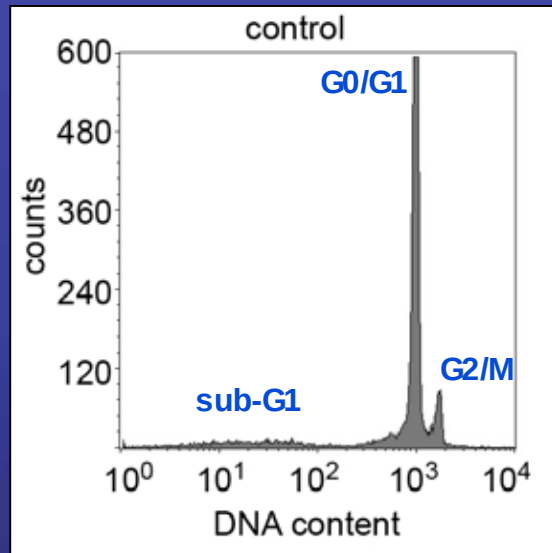
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Funded by  
Fonds zur Förderung der wissenschaftlichen Forschung  
and  
Tiroler Wissenschaftsfonds

# Interferons

- major components of the innate immune system
- discovered in 1957
- 2 groups:
  - Type I interferons ( $\text{IFN}\alpha$ ,  $\text{IFN}\beta$ ...)
  - Type II interferons ( $\text{IFN}\gamma$ )
- Therapy:
  - therapy of cancer
  - therapy of virus diseases

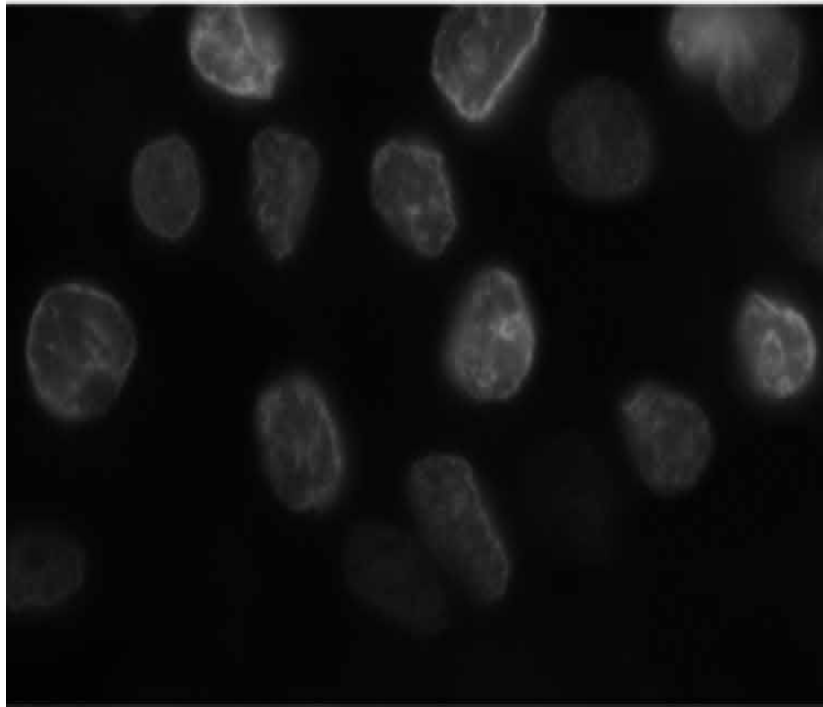
# IFN $\alpha$ treatment of LLC-PK<sub>1</sub> monolayers induces a sub-G1 peak indicative of DNA fragmentation



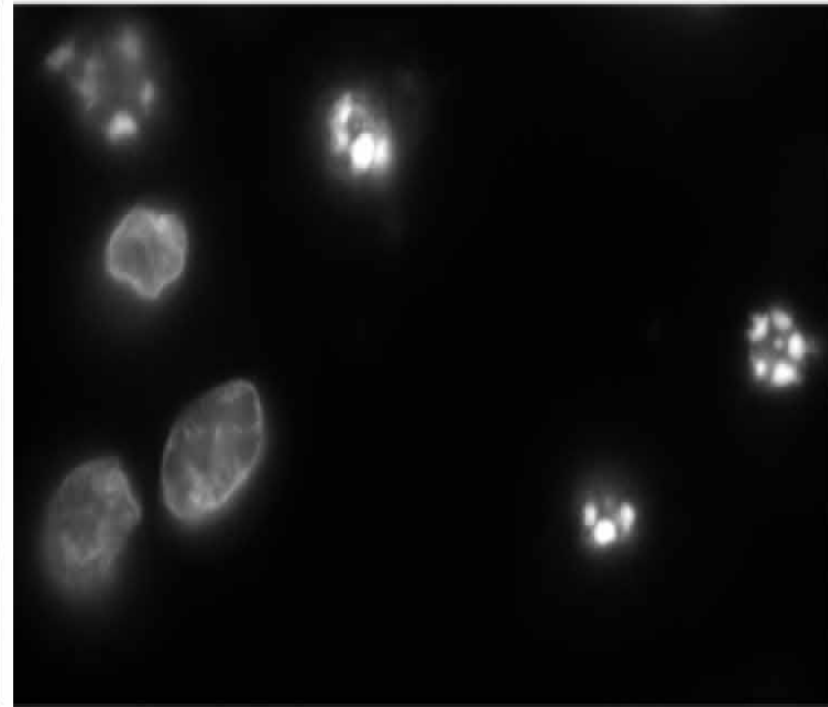
# IFN $\alpha$ treatment of LLC-PK1 cells induces DNA condensation

A

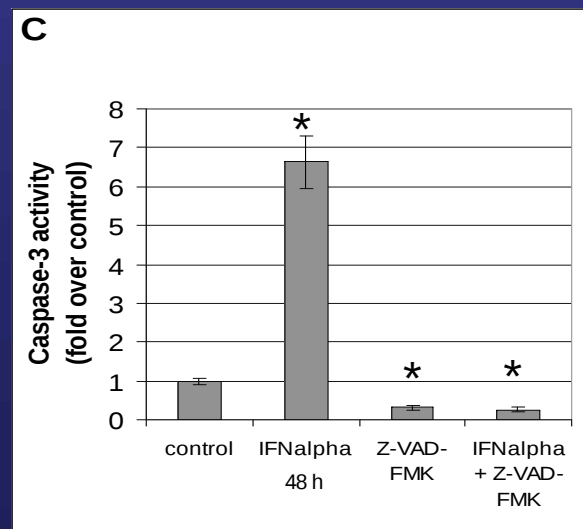
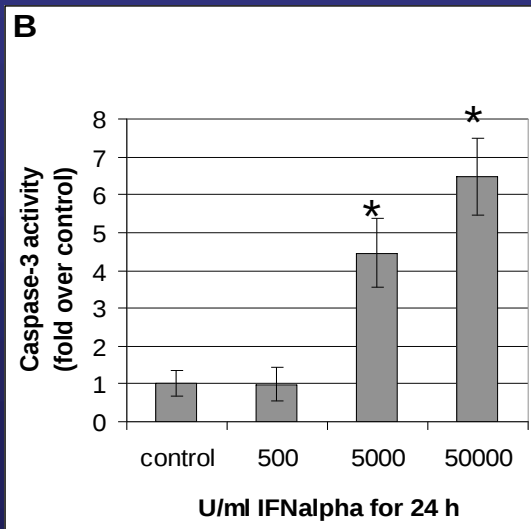
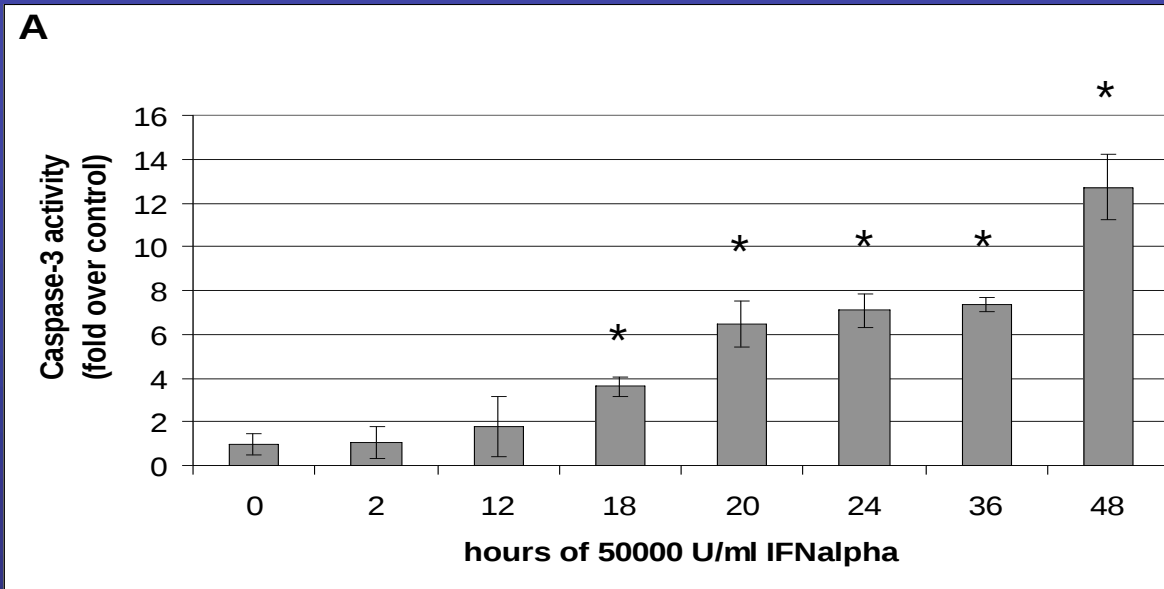
control



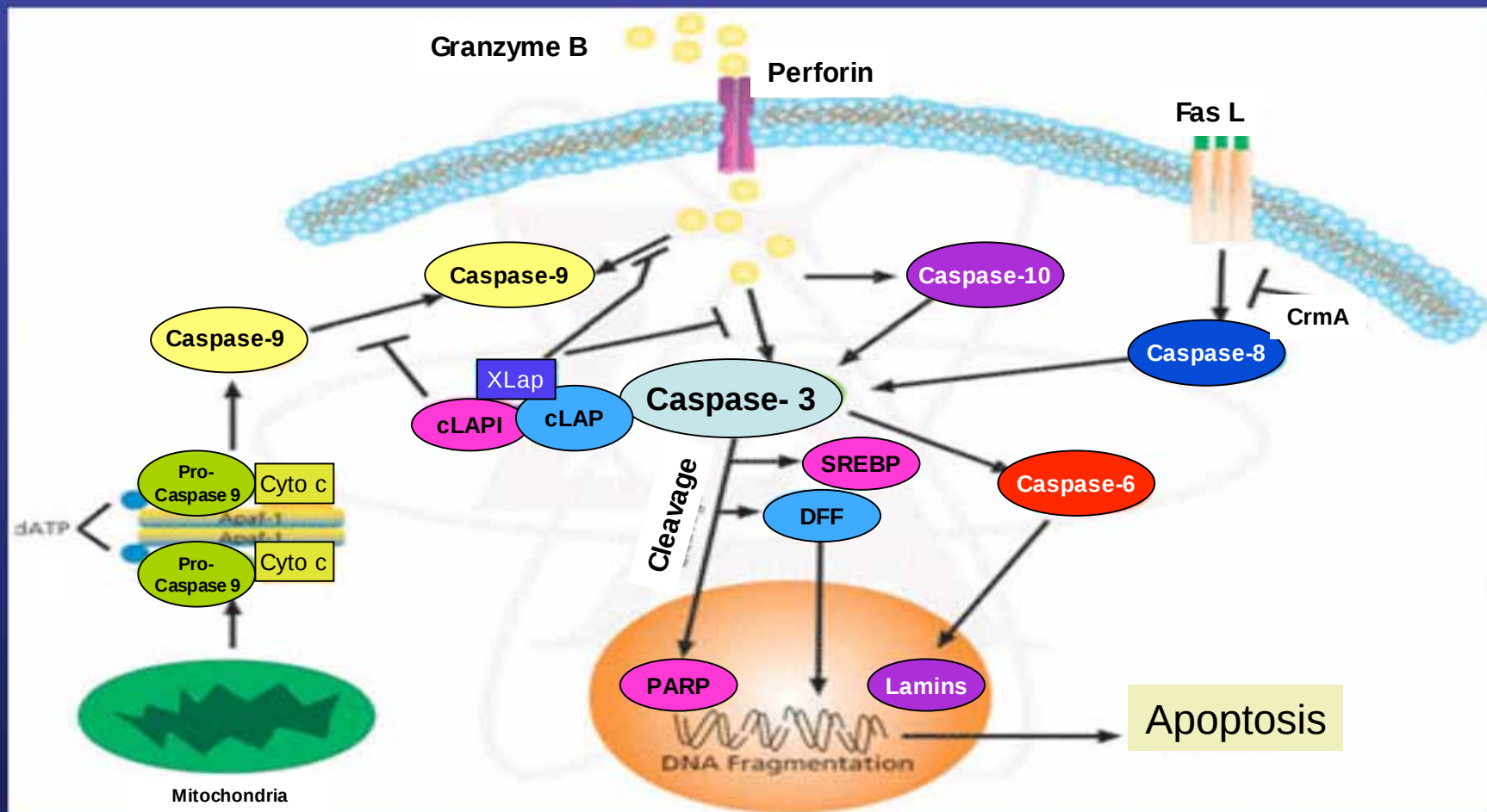
48 h IFN $\alpha$



# IFN $\alpha$ induces Caspase-3 activation in LLC-PK1 monolayers



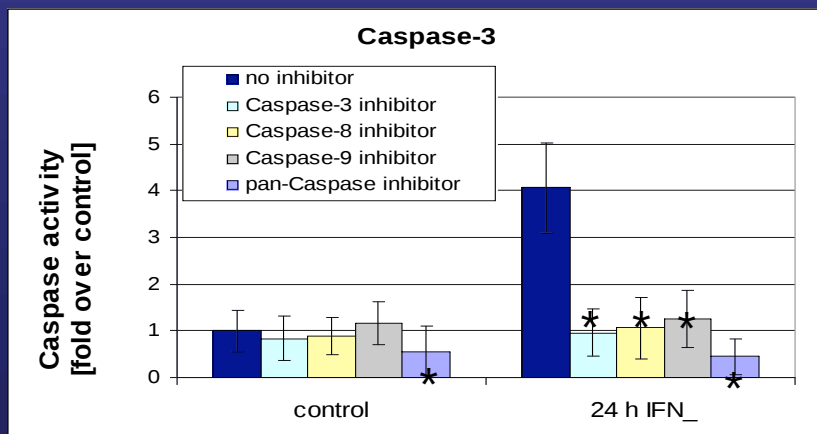
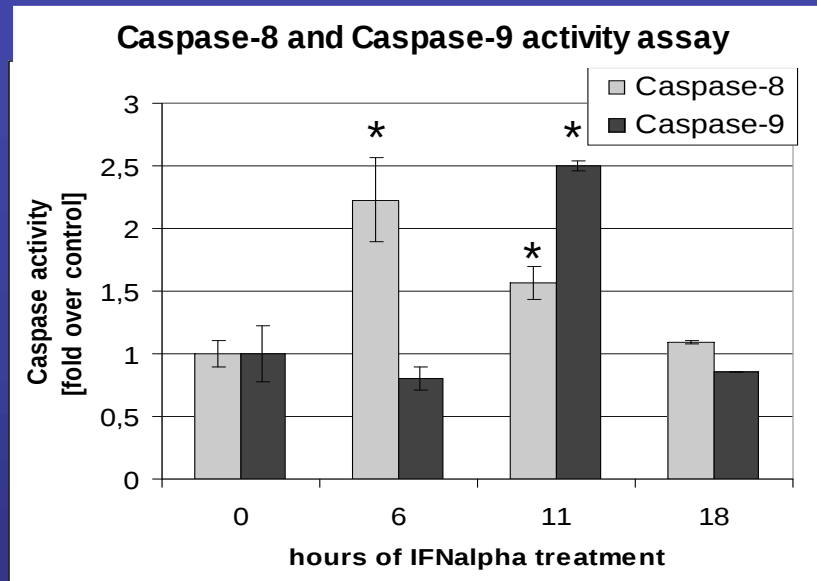
# Caspase cascade



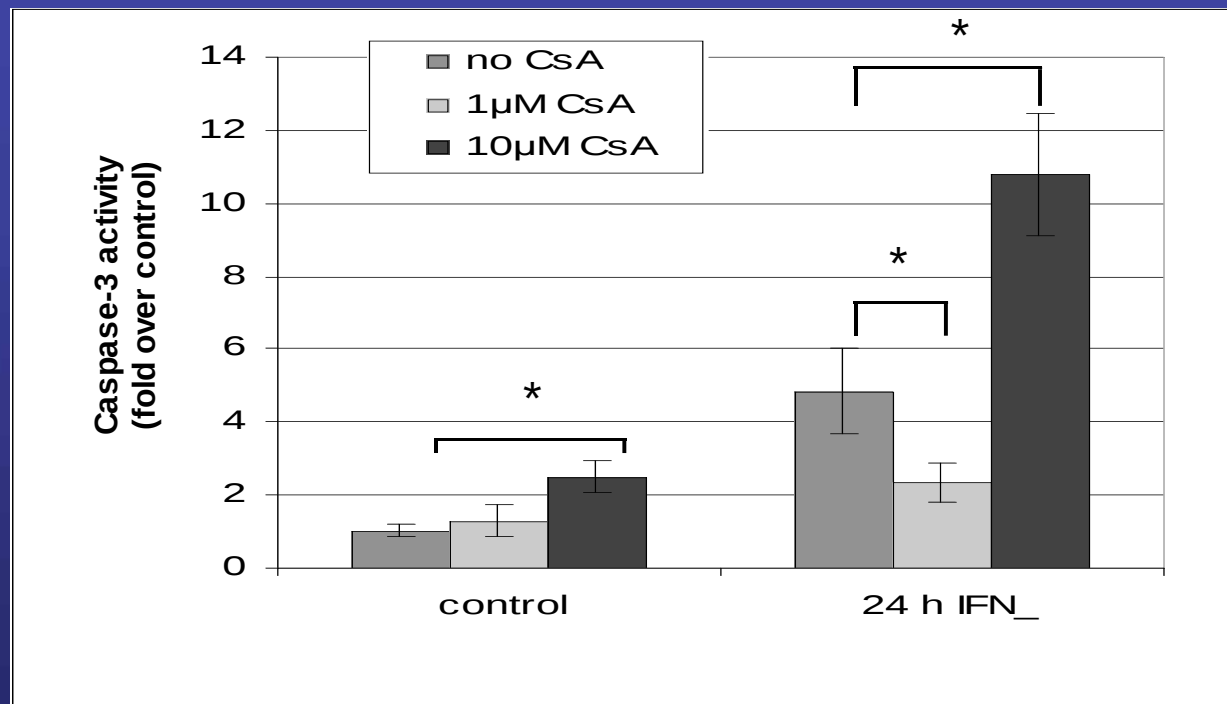
from:

<http://www.sigmaaldrich.com>

# IFN $\alpha$ induces Caspase-8 and Caspase-9 in LLC-PK1 monolayers

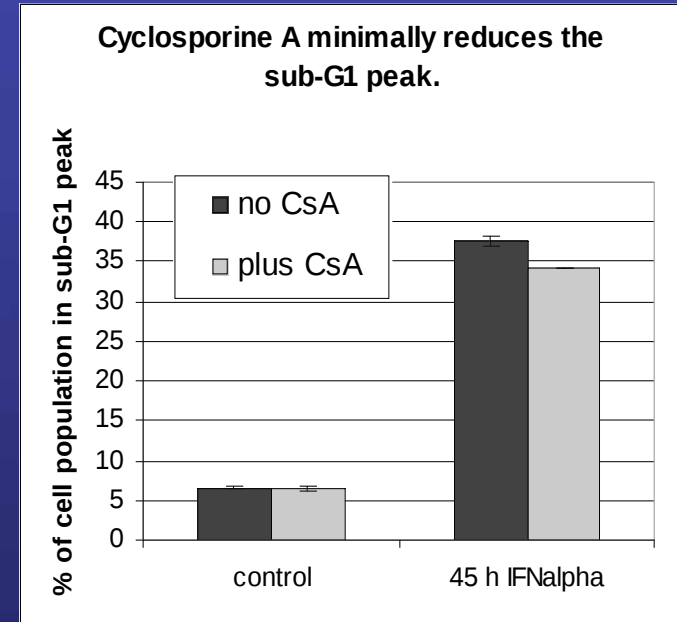
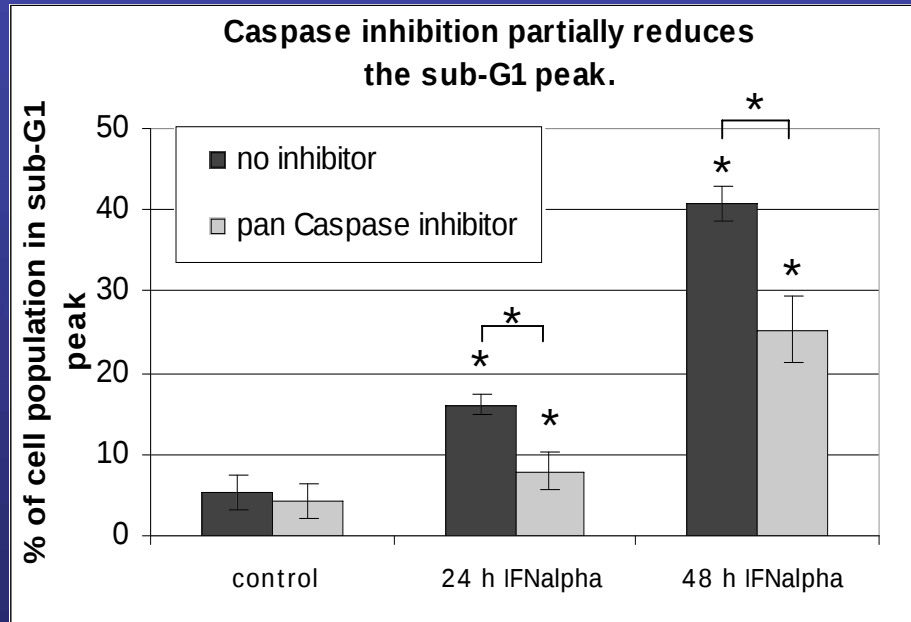


# Mitochondrial megapore block by low dose Cyclosporine A partially prevents IFN $\alpha$ induced Caspase-3 activation



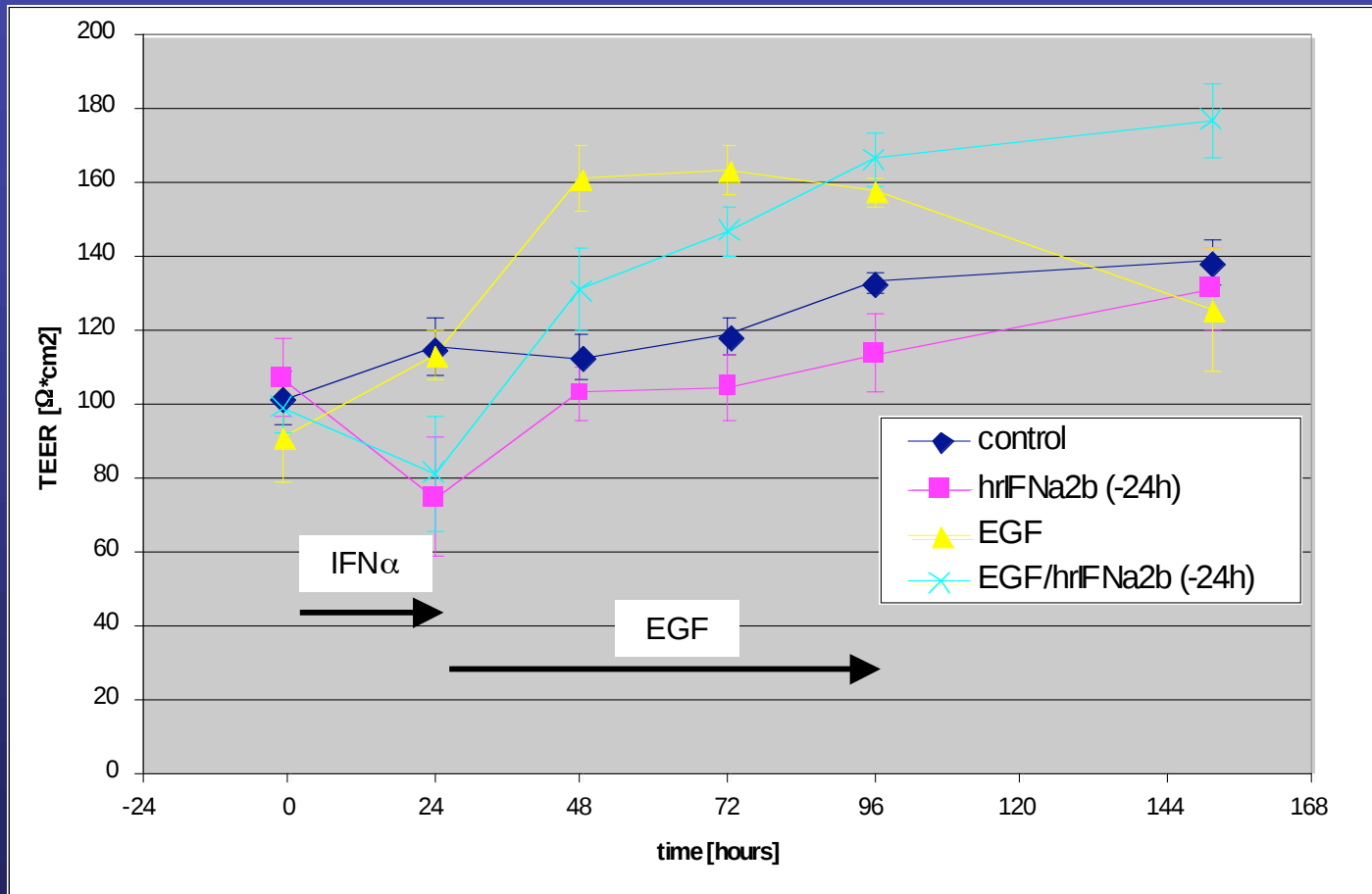
\* P<0,005 versus control or as indicated by 

# Caspase inhibition and Cyclosporine A partially prevent DNA fragmentation (sub-G1 peak) by IFN $\alpha$

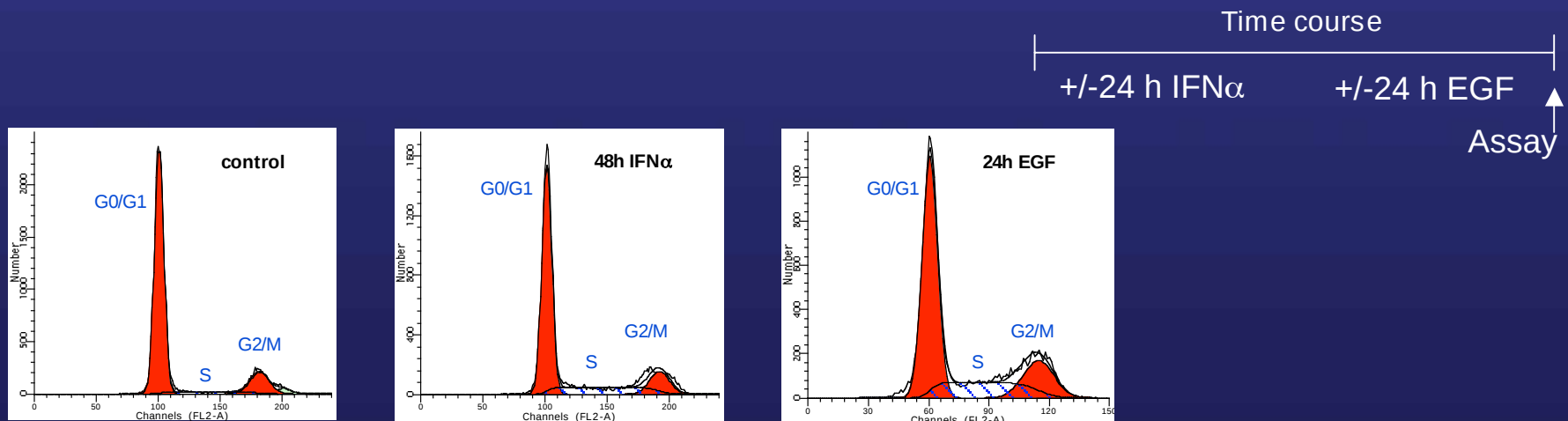
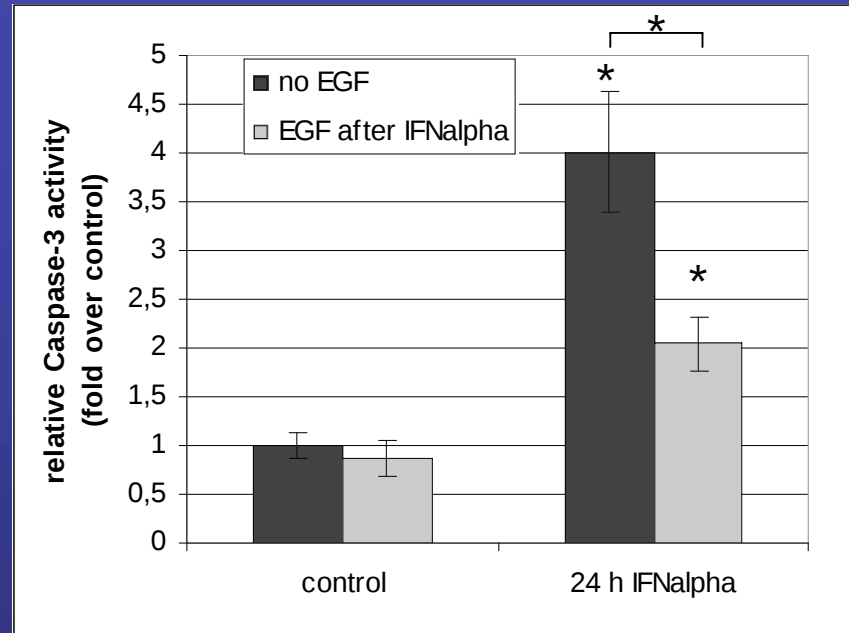
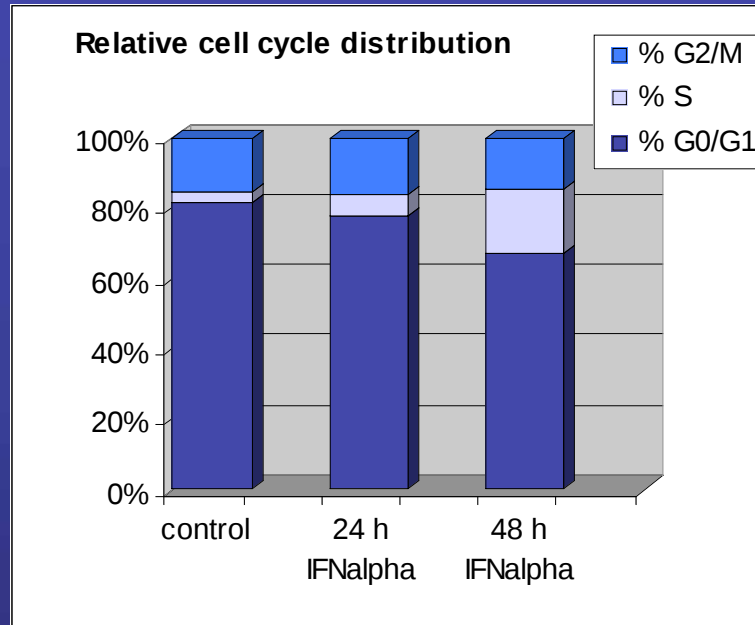


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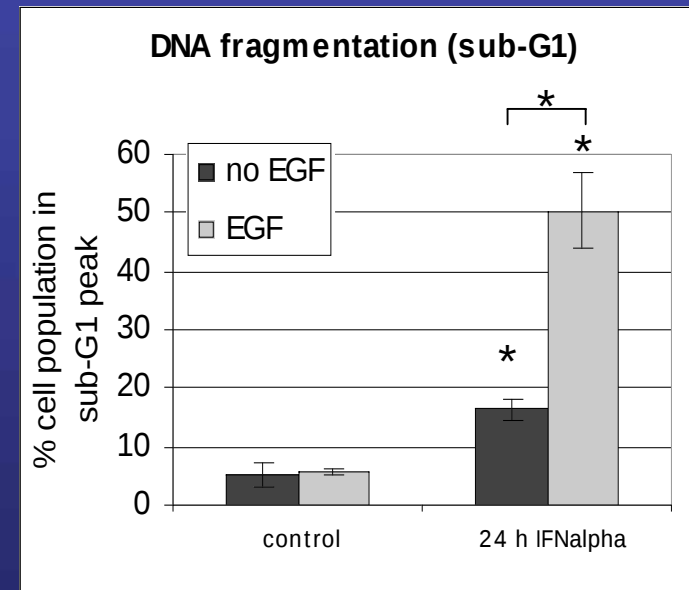
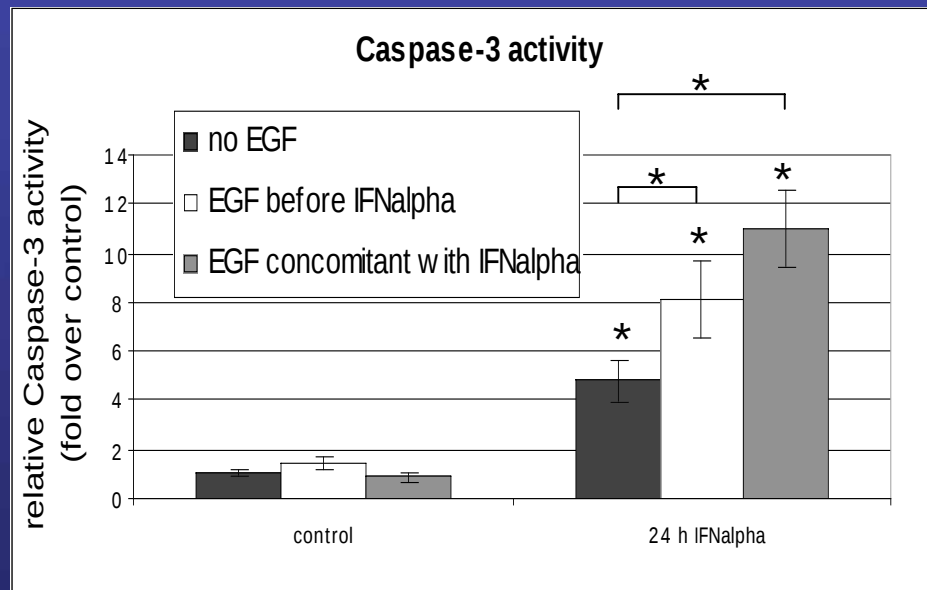
# Transepithelial electrical resistance (TEER) recovery after IFN $\alpha$ treatment



# Recovery from IFN $\alpha$ treatment



# EGF treated LLC-PK1 cultures are more susceptible to apoptosis induction by IFN $\alpha$



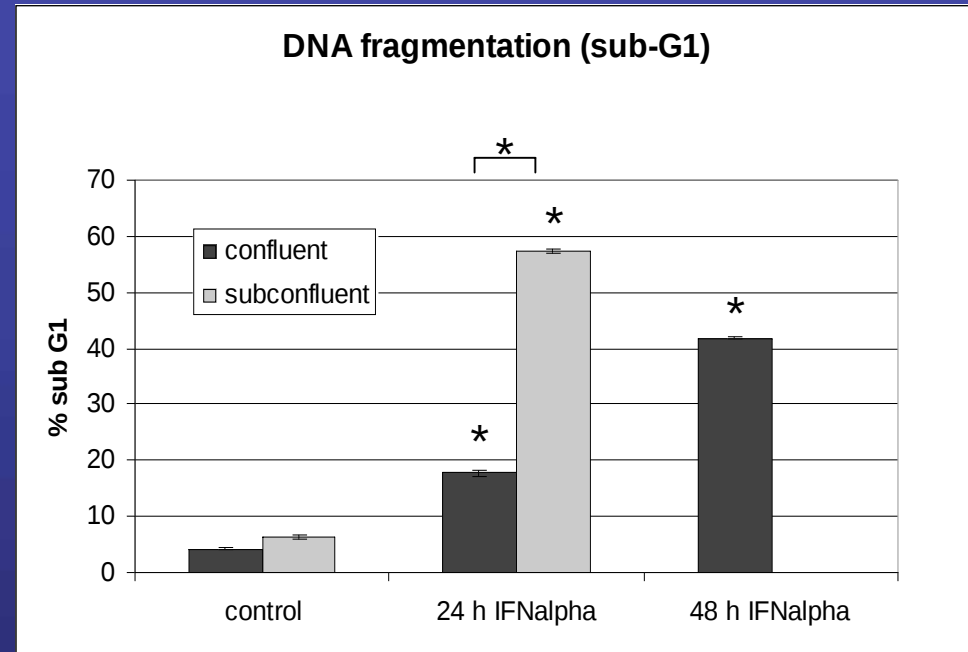
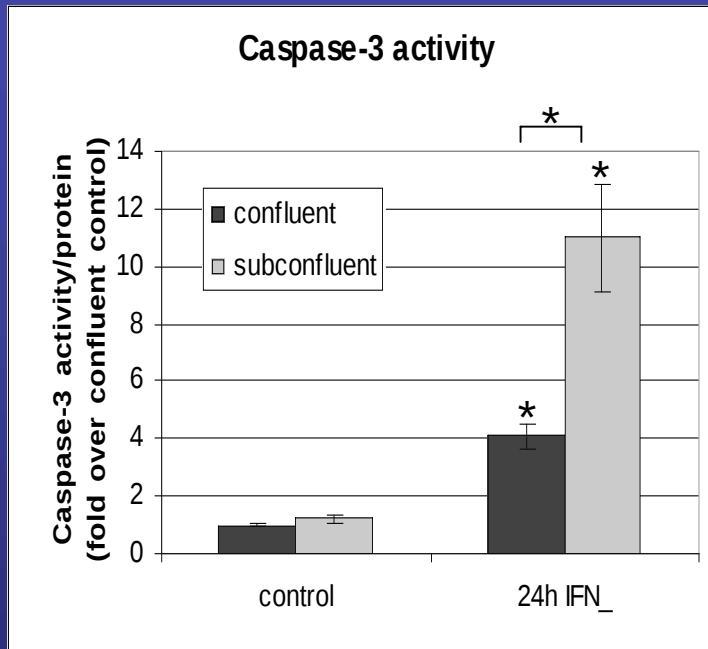
\* P<0,005 versus control or as indicated by 

Time course

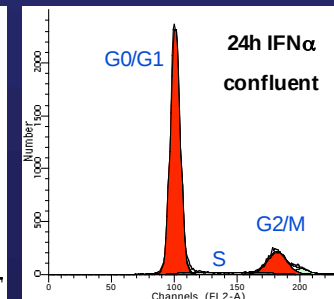
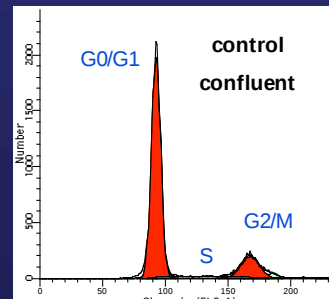
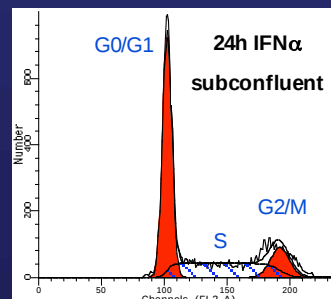
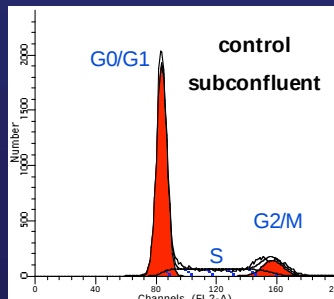
+/- 24 h EGF  
+/- 24 h IFN $\alpha$

Assay 

# Subconfluent LLC-PK1 cultures are more susceptible to apoptosis induction by IFN $\alpha$ than confluent monolayers



\* P<0,005 versus control or as indicated by [ ]



## Summary

- IFN $\alpha$ -induced nephrotoxicity involves a direct effect of IFN $\alpha$  on renal proximal tubular cells initiating an apoptotic cell death program
- Caspase-3, -8 and -9-like activities are involved in IFN $\alpha$ -induced apoptosis
- IFN $\alpha$ -induced apoptosis is exacerbated by Cyclosporine A co-treatment and a proliferative status of the cells

# Acknowledgment



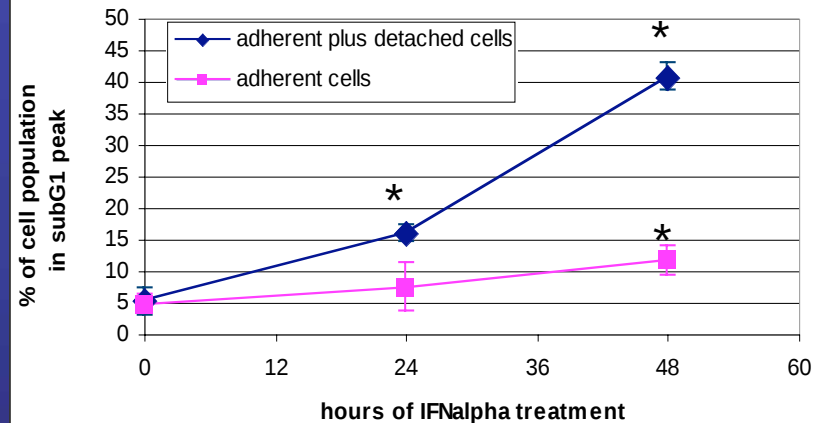
- Dr. Judith Lechner
- Prof. Dr. Walter Pfaller
- Dr. Paul Jennings
- Mag. Sonia Aydin
- Mag. Sara Signorelli
- Dr. Thomas Lechleitner
- Judy Schwabl
- Edna Nemati

## Summary

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- Caspase-3, -8 and -9-like activities are involved in IFN $\alpha$ -induced apoptosis

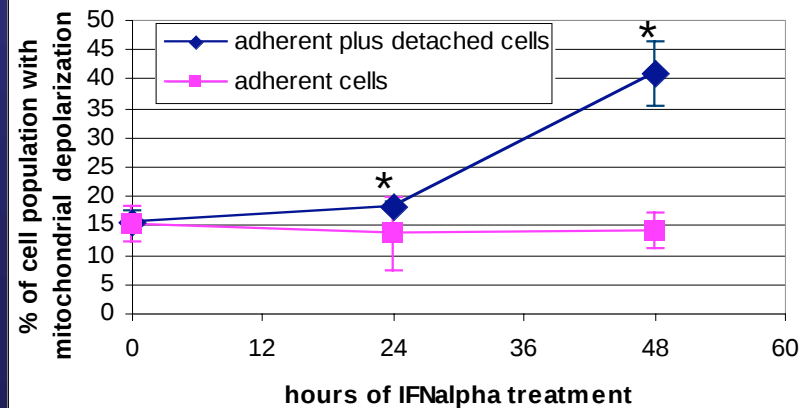
# DNA fragmentation and mitochondrial membrane depolarization mainly concern cells detached from the monolayer

DNA fragmentation by IFN $\alpha$  treatment shortly precedes cell detachment from the monolayer.



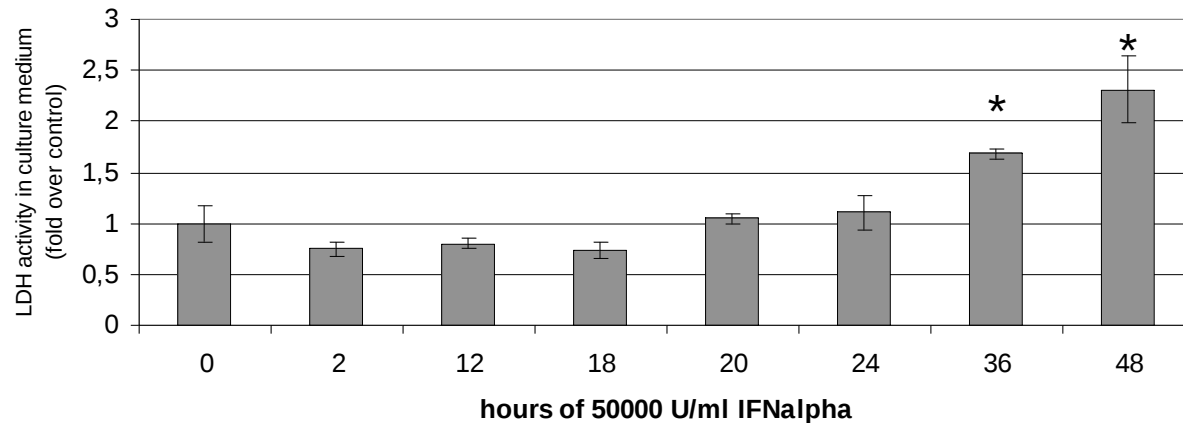
\* P<0,005 versus 0 h IFN $\alpha$

Mitochondrial depolarization occurs mainly after detachment of the cells from the monolayer.

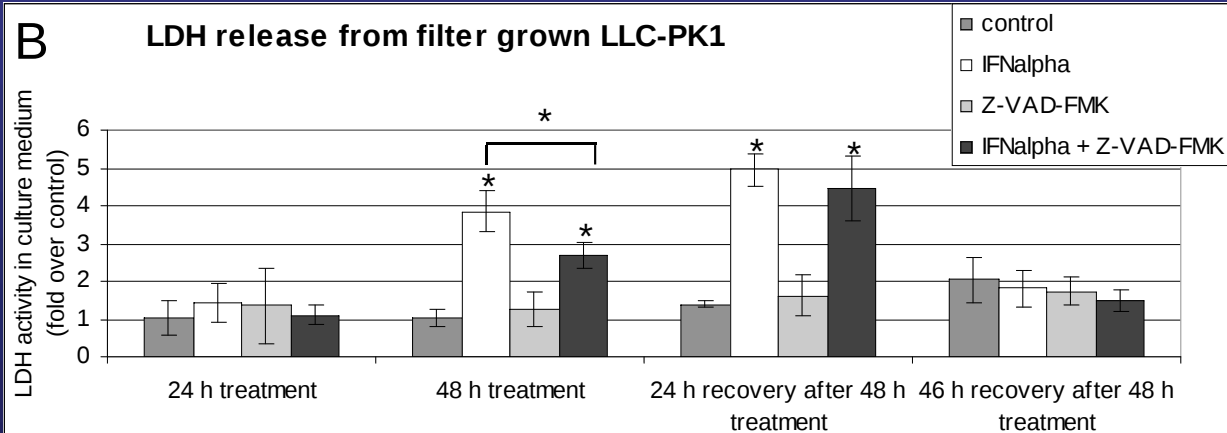


## Lactate dehydrogenase (LDH) release indicates loss of plasma membrane integrity after long term IFN $\alpha$ treatment

**A** LDH release (LLC-PK1 on standard plastic tissue culture plates)



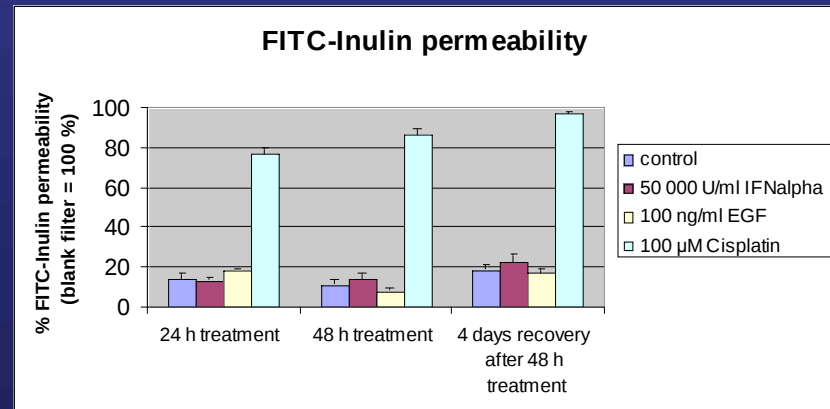
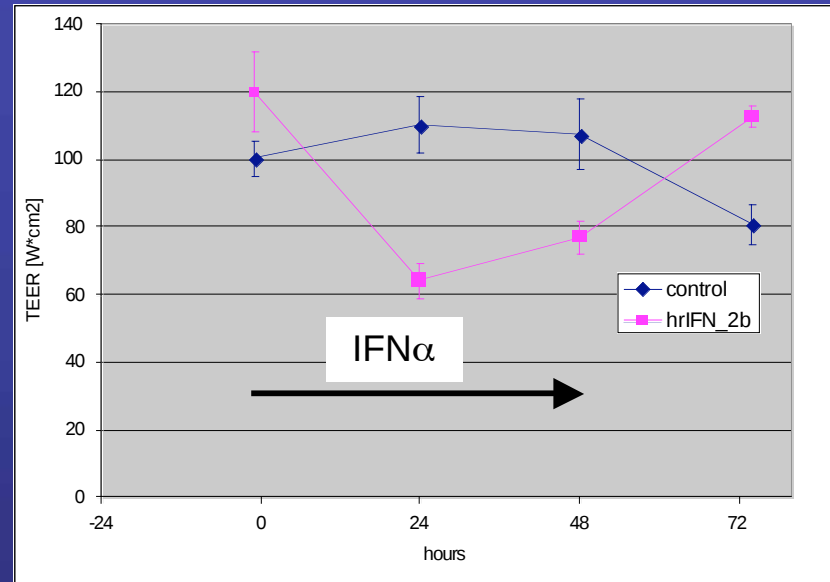
**B** LDH release from filter grown LLC-PK1



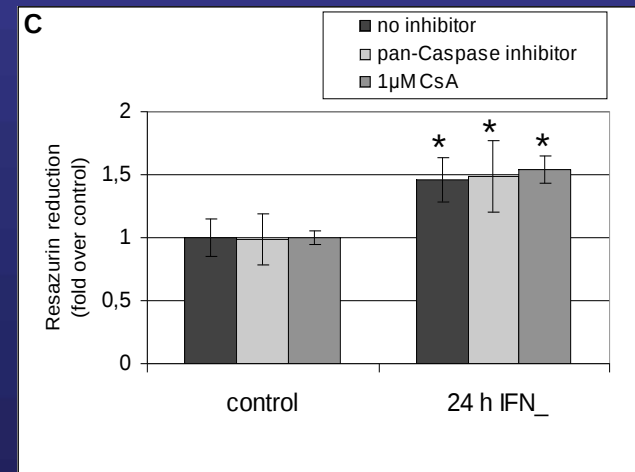
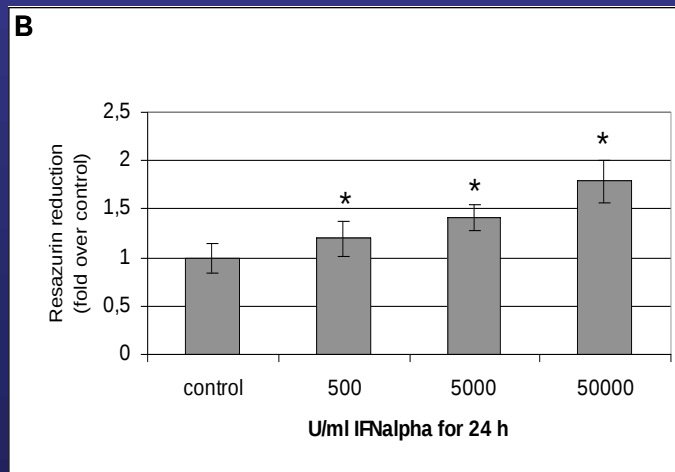
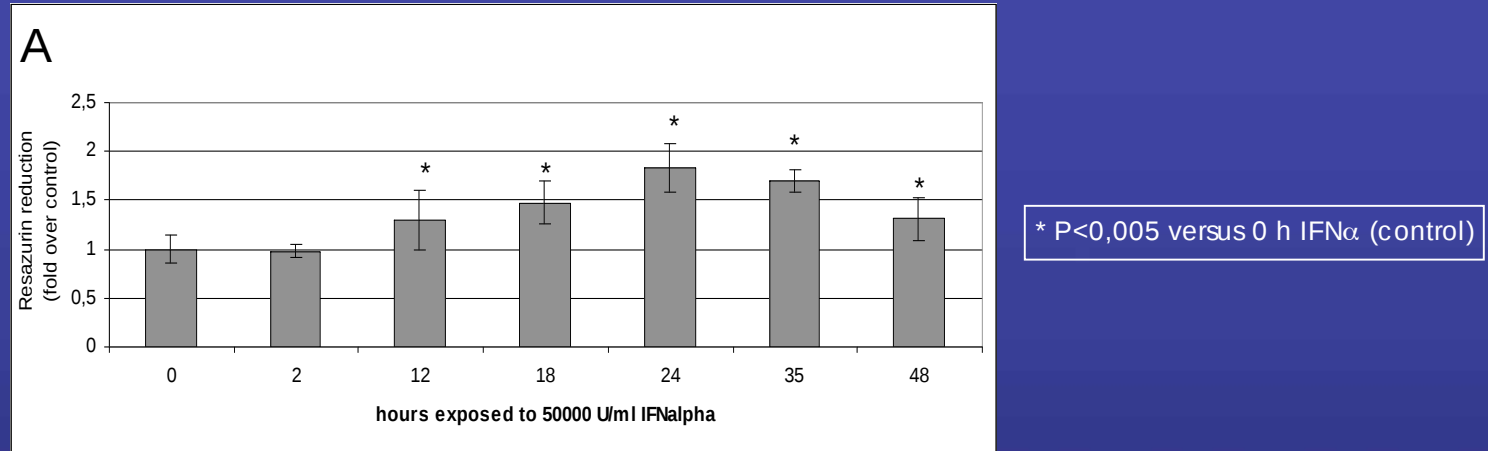
Z-VAD-FMK =  
pan Caspase inhibitor

\* P<0,005 versus control or as indicated by 

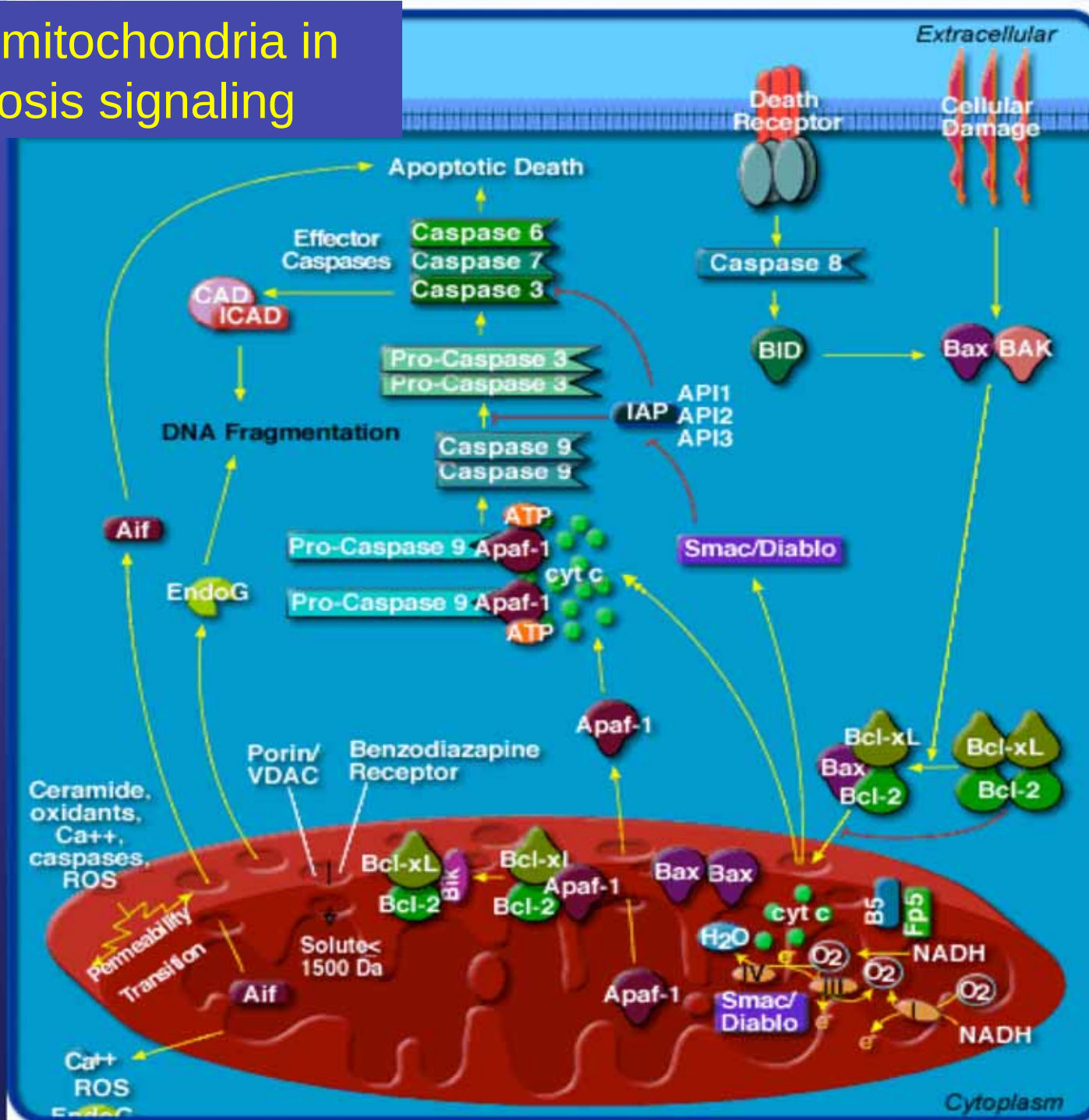
# Transepithelial electrical resistance (TEER) recovery after IFN $\alpha$ treatment



# Effect of IFN $\alpha$ treatment on a standard cell viability assay (Resazurin conversion)



# Role of mitochondria in apoptosis signaling



from:

<http://www.biocarta.com>