

β -Lapachone, a 'Kiss of death' therapy for cancer

David A. Boothman, Ph.D.

Laboratory of Molecular Stress Responses

Program in Cell Stress and Cancer Nanomedicine

***Simmons Comprehensive Cancer Center
University of Texas Southwestern Medical
Center at Dallas***

September 14, 2008

Cellular Damage



New Gene Expression



**Exploitable Target: Expressed
in Human Tumors, not (or low)
in Normal Tissues**



**Bioactivate Drug For
Tumor-Selective Killing**

IR



xips



NQO1



β -Lapachone

- Not cell cycle regulated
- Not dependent on p53 status
- Not dependent on caspases

- Not affected by drug resistance
- Not affected by hypoxia
- Selective for tumors

β -Lapachone (β -Lap)

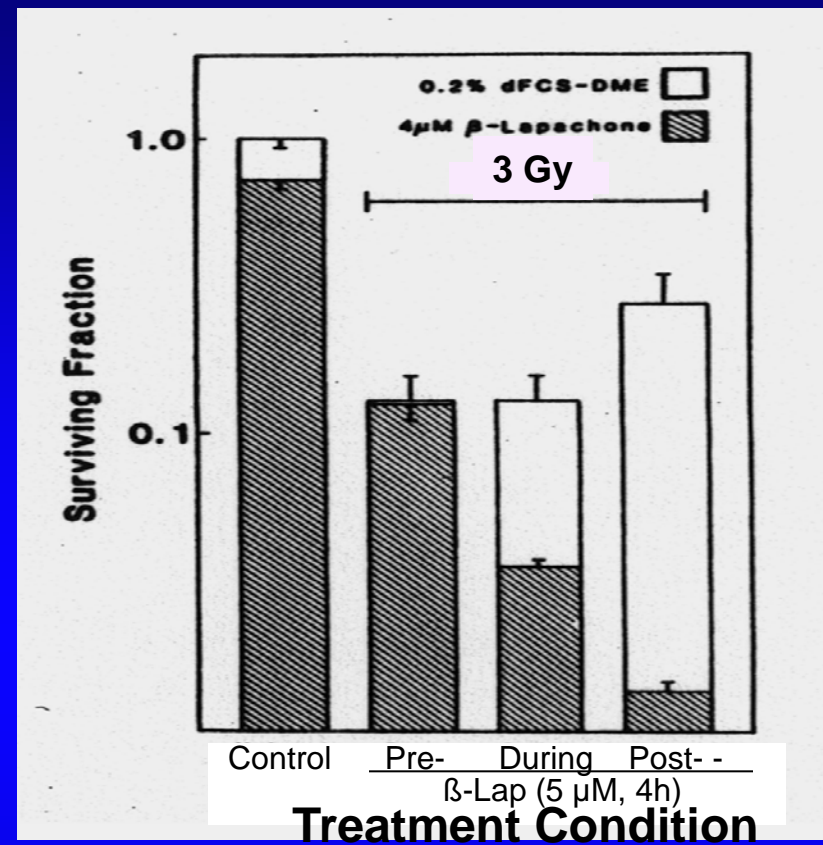
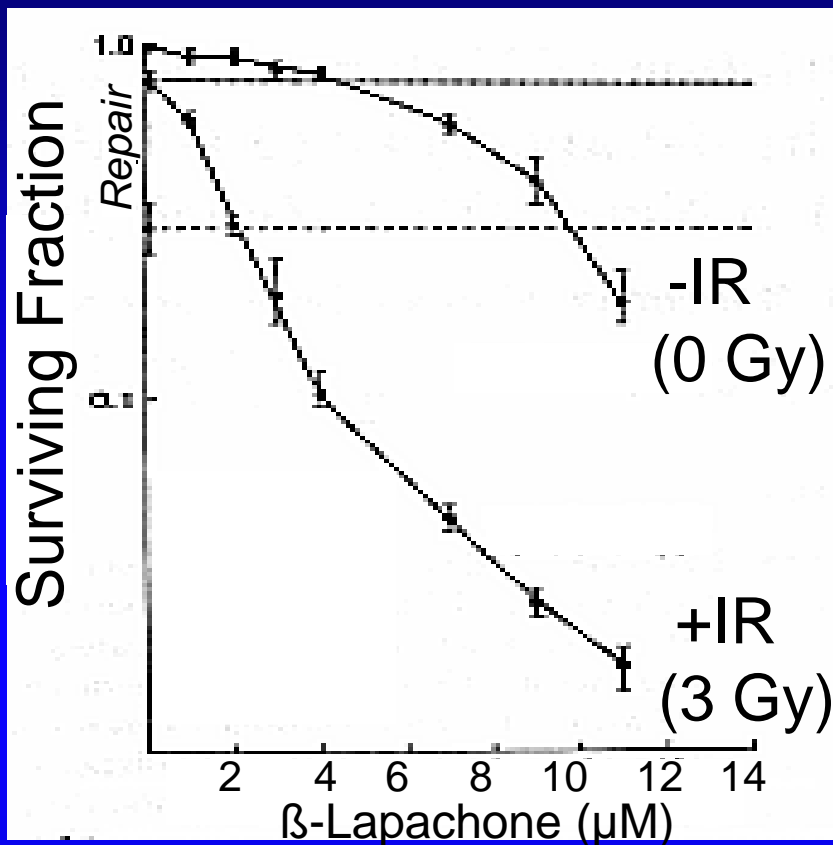


The Lapacho Tree

Tabebuia avellanedae

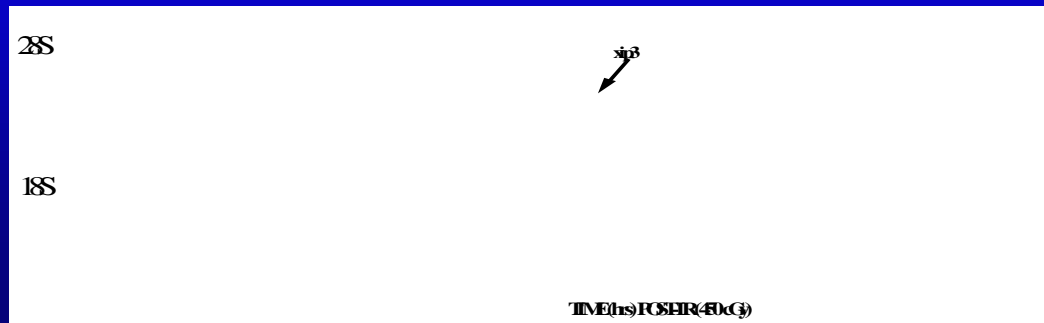


β -Lapachone radiosensitized human cancer, but not normal, cells



Radiosensitization Mechanism:

IR induces NQO1(xip3), the principal determinant of β -lap cytotoxicity (Pink, et al., JBC, 2000).



Boothman et al., Cancer Res., 1989

Boothman et al., PNAS, 1990. 1993

NQO1: An Important Target for Cancer Therapy

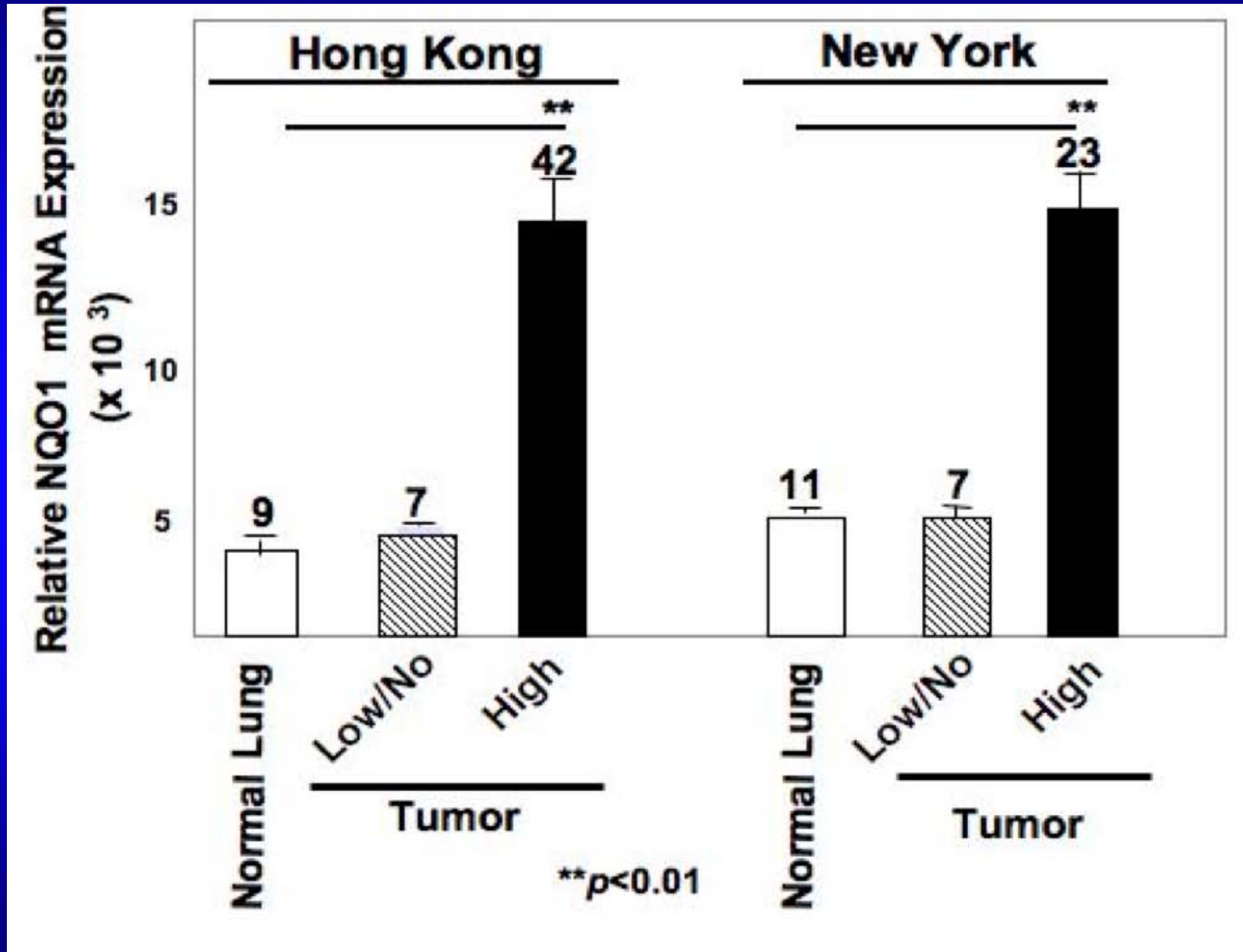
- Early marker of carcinogenesis
 - Up-regulated after carcinogenic cell stress
 - Over-expressed in many cancers, as well as in angiogenic endothelial cells
-

Tumor-selective NQO1 Elevation

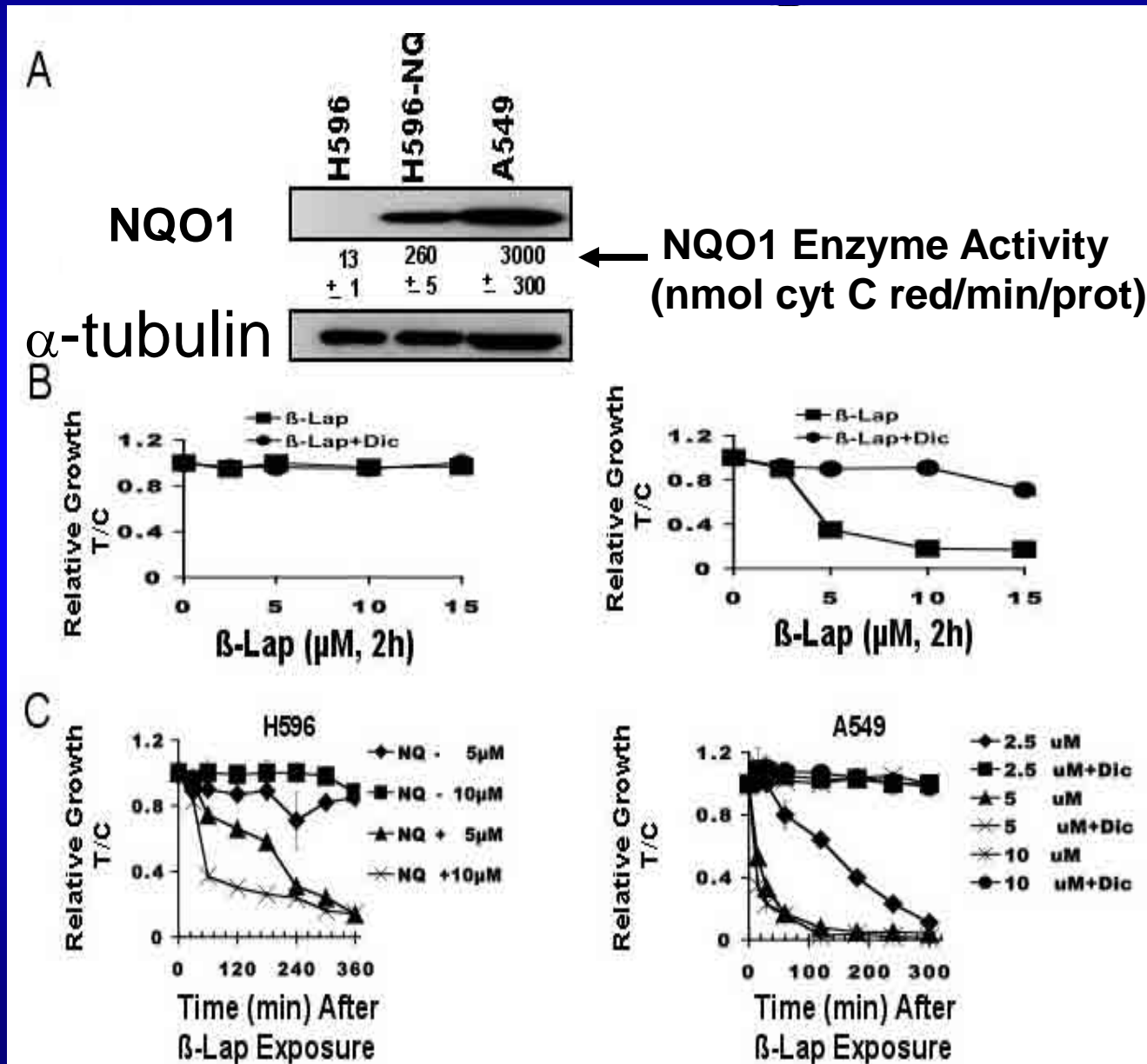
- 80% Breast Cancers, 10- to 20-fold **
- 70% Prostate Cancers, 10- to 20-fold **
- 60% Colon Cancers, 5- to 10-fold
- 90% Pancreatic Cancers,
(J. Cullen, U. Iowa)
- 70% NSCLC (not SCLC), 20- to 40-fold **

** Will discuss isogenic models +NQO1

NQO1 Is Elevated In Nonsmall Cell Lung Cancer (NSCLC)



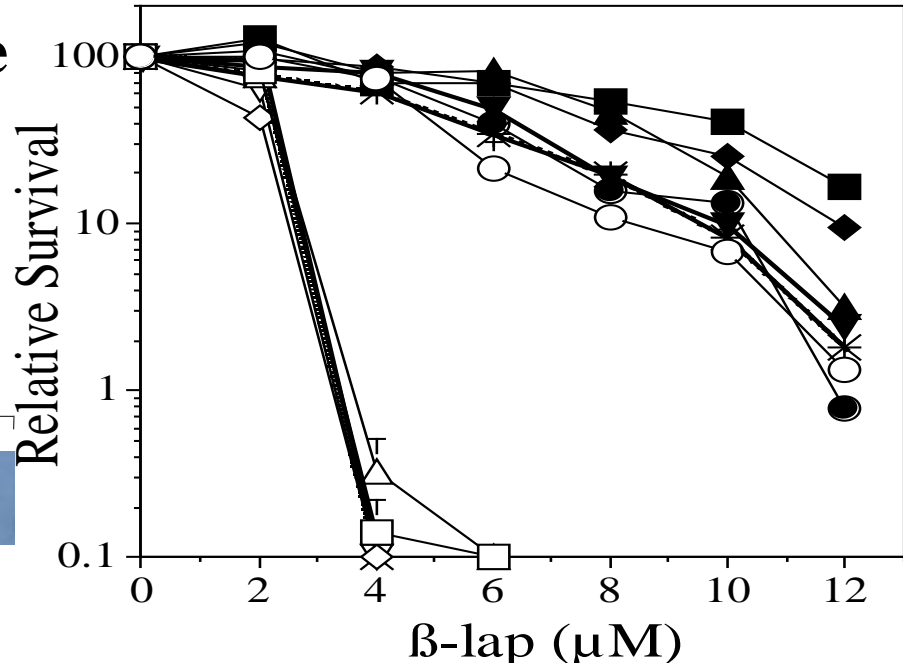
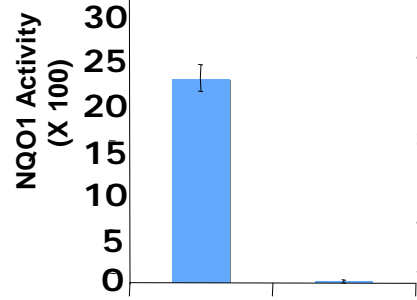
NQO1 Expression Confers Cytotoxicity to H596 NSCLC Cells



*Bey et al.,
PNAS, 2007*

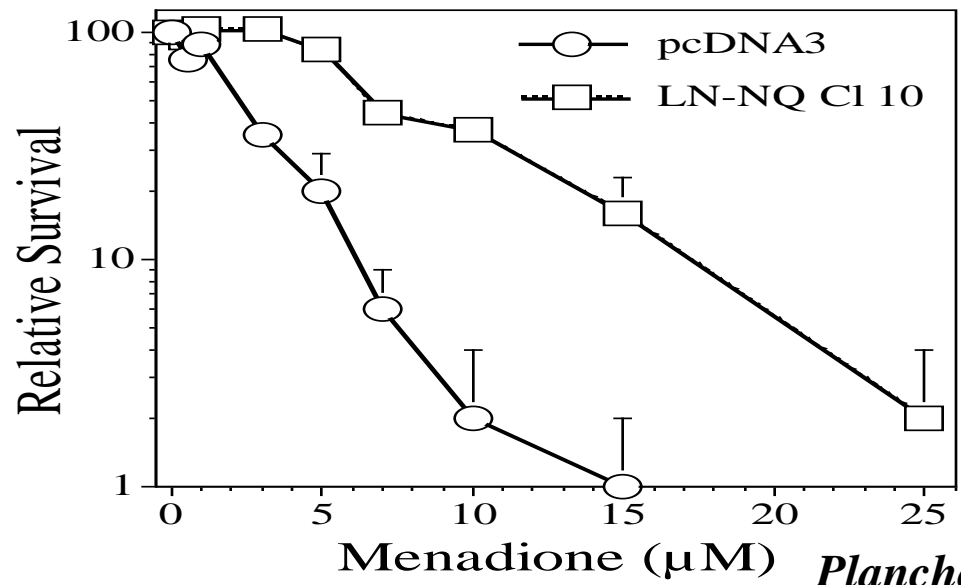
NQO1-dependent lethality in human prostate cancer cells

β -Lapachone



- LN-pcDNA3
- LN-pcDNA3 + Dic
- LN-NQ CI 1
- LN-NQ CI 1 + Dic
- ◇ LN-NQ CI 2
- ◆ LN-NQ CI 2 + Dic
- △ LN-NQ CI 3
- ▲ LN-NQ CI 3 + Dic
- ▽ LN-NQ CI 4
- ▼ LN-NQ CI 4 + Dic
- × LN-NQ CI 10
- ※ LN-NQ CI 10 + Dic

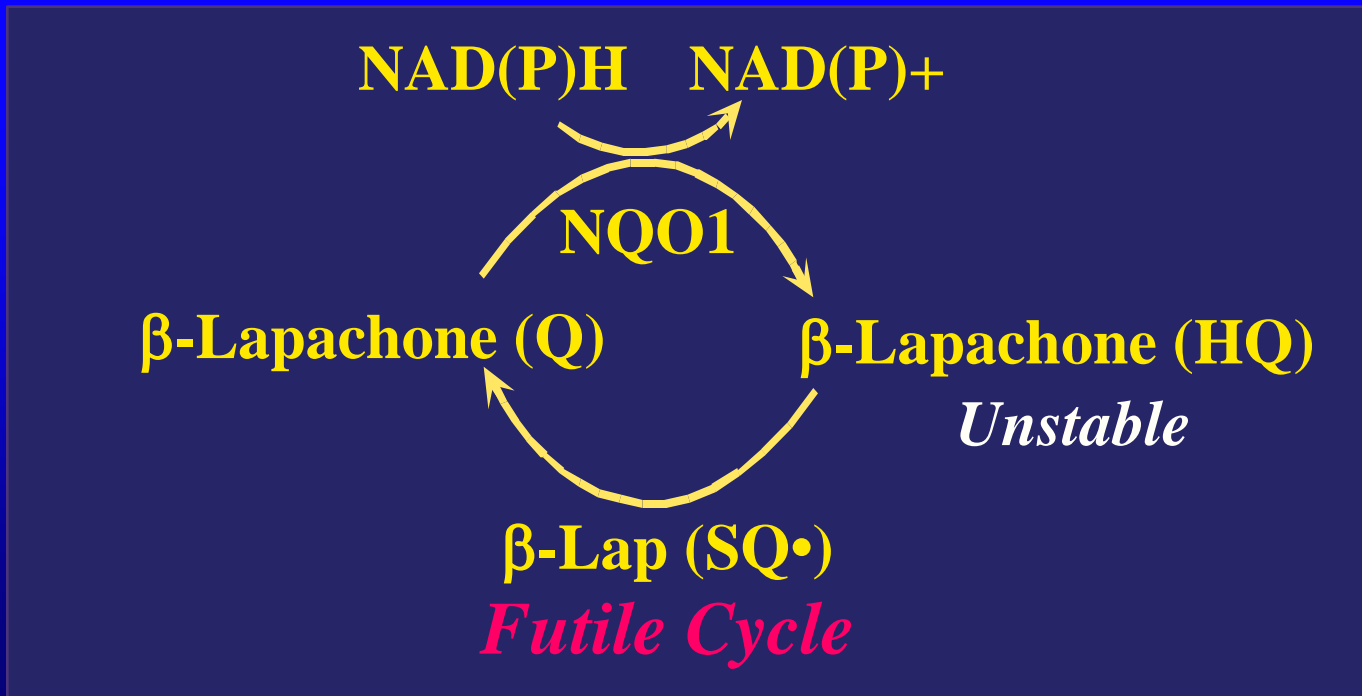
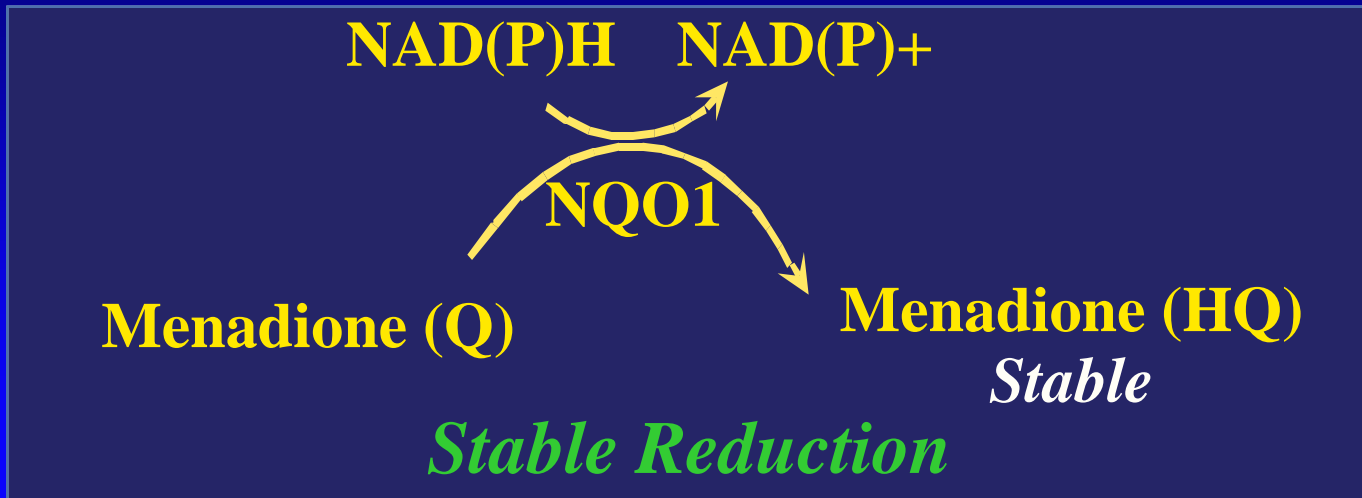
Menadione



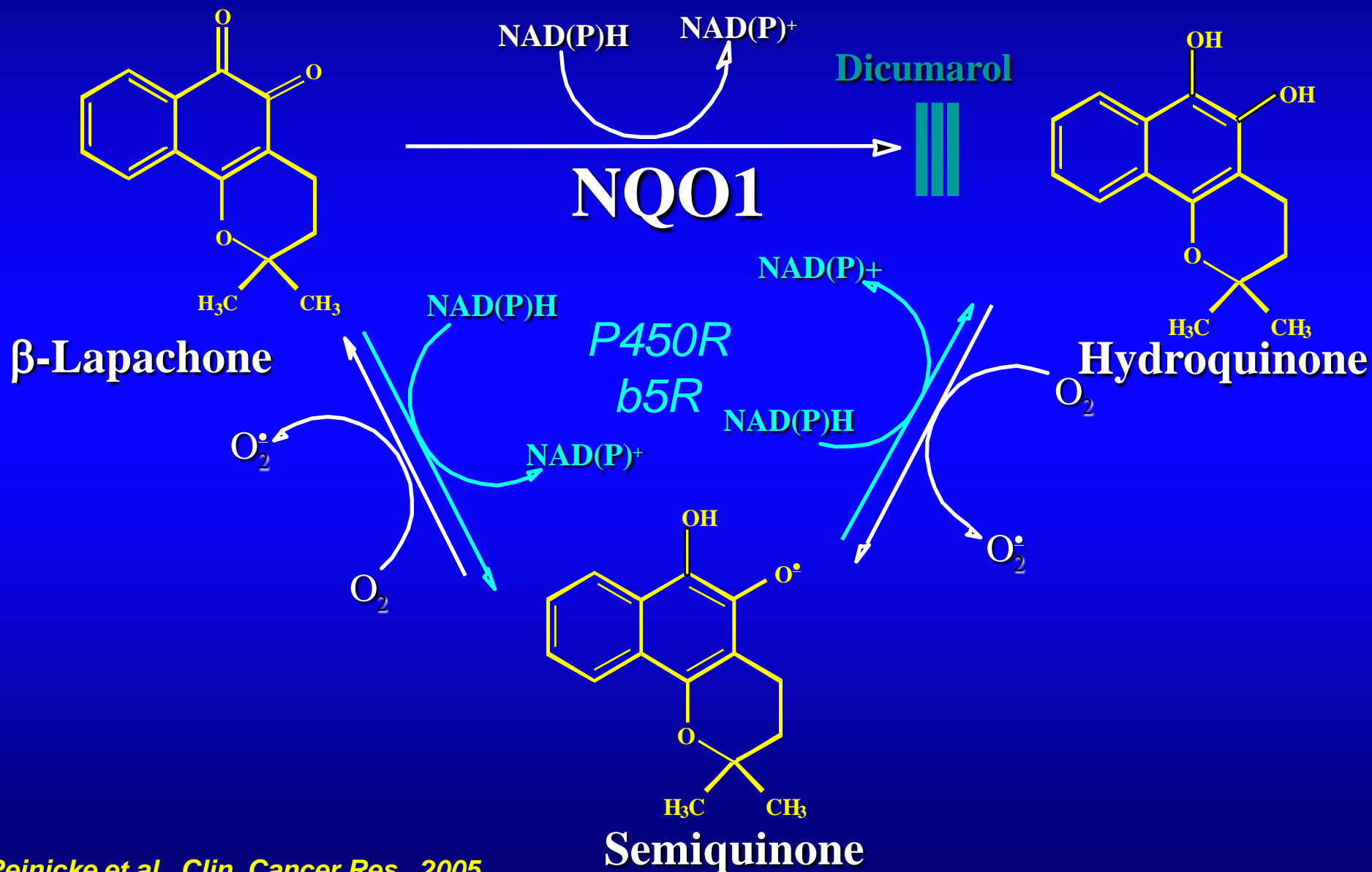
Conclusion

NQO1 “bioactivates” β -Lap
NQO1 “inactivates” Menadione

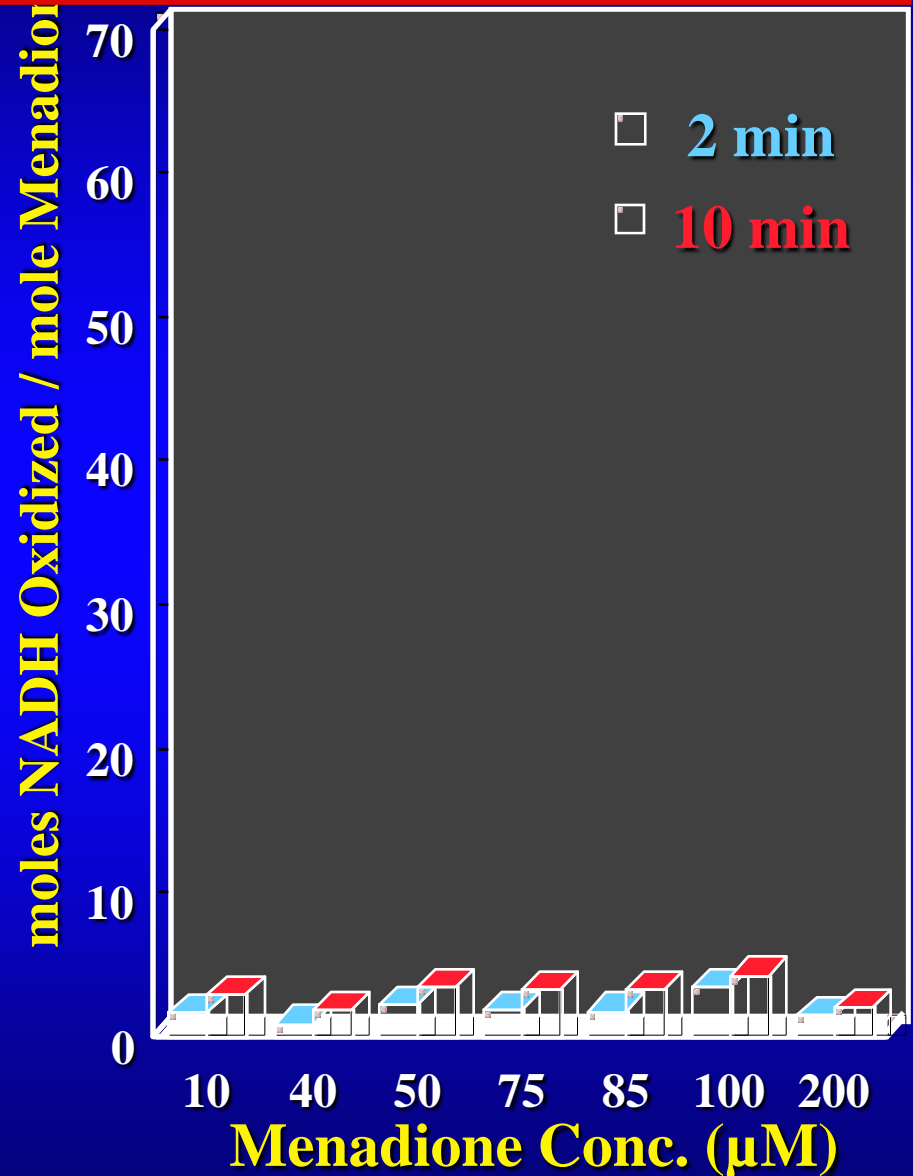
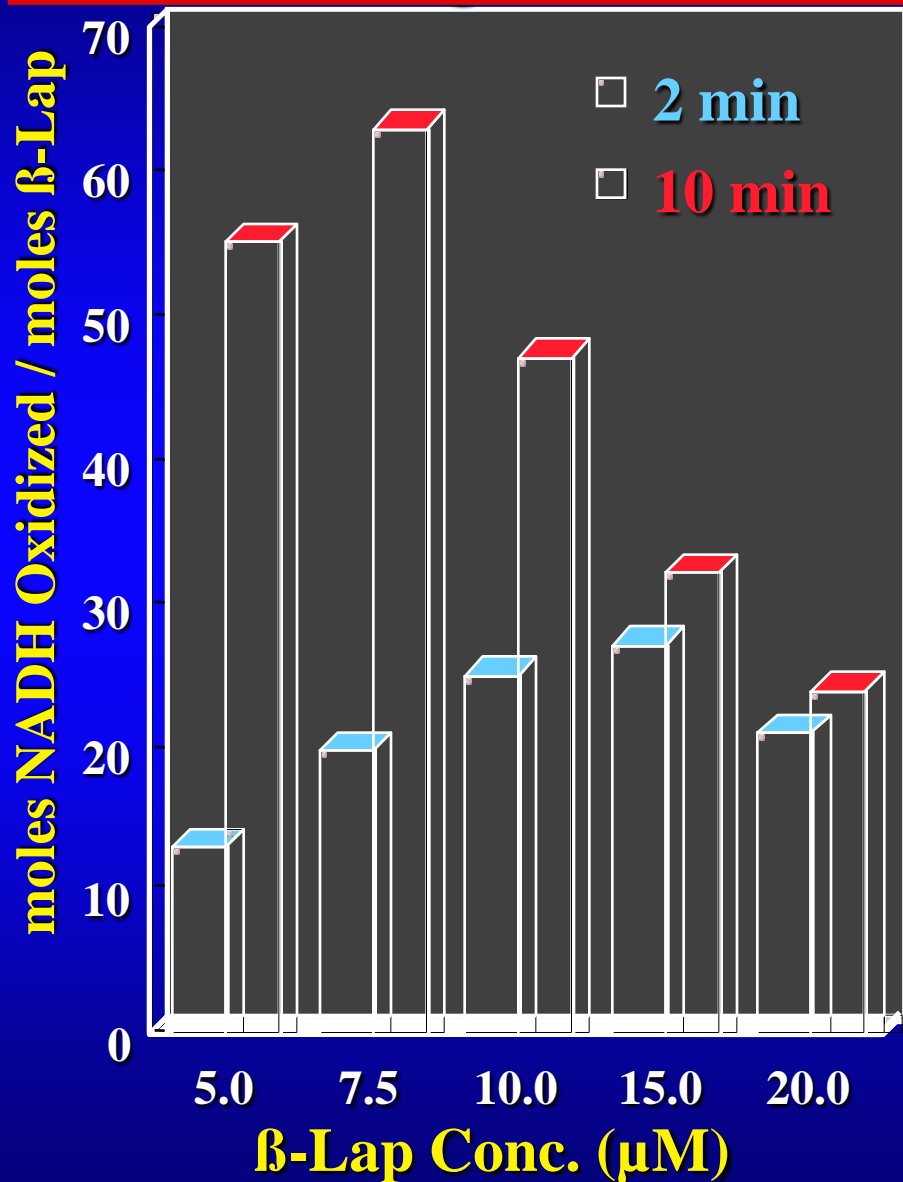
NQO1-Mediated Reduction of β -Lap and Menadione



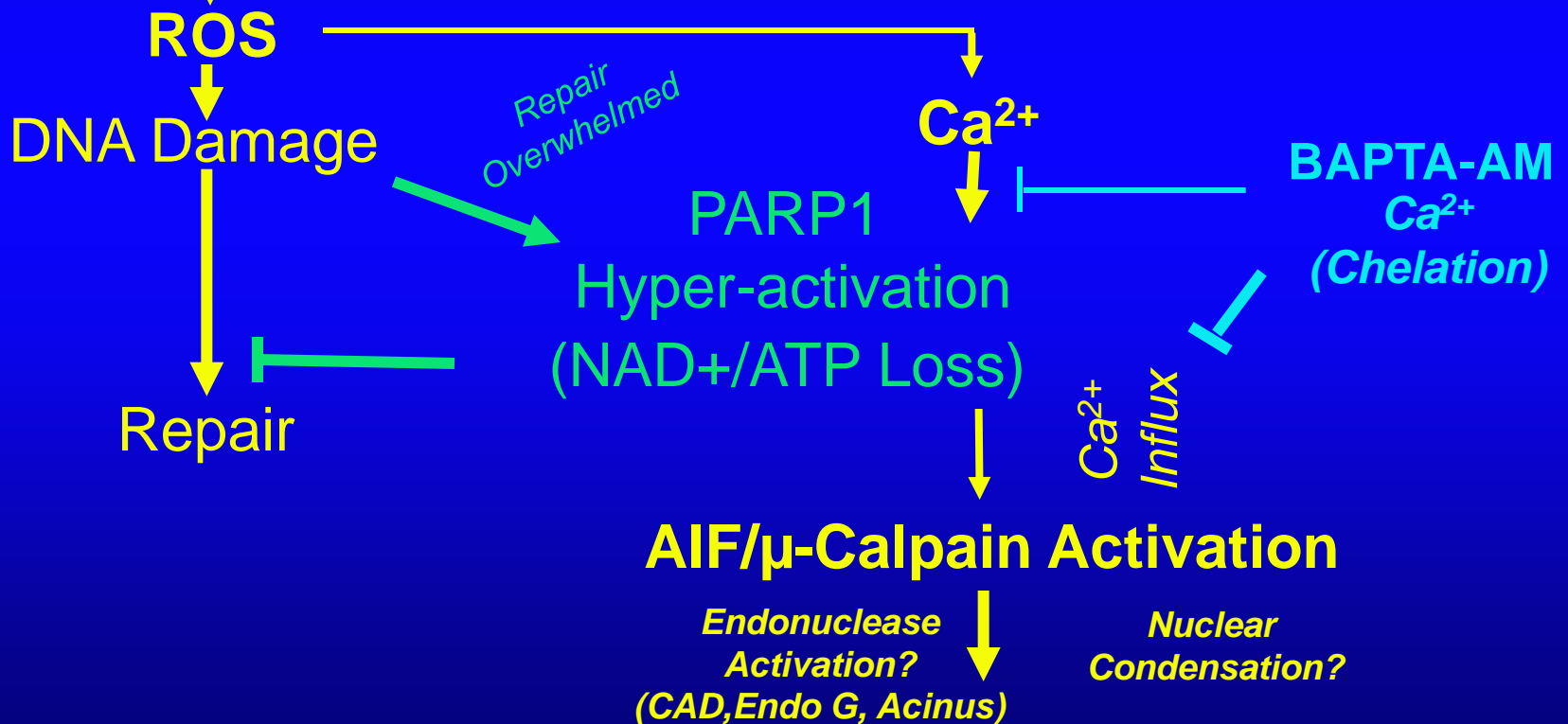
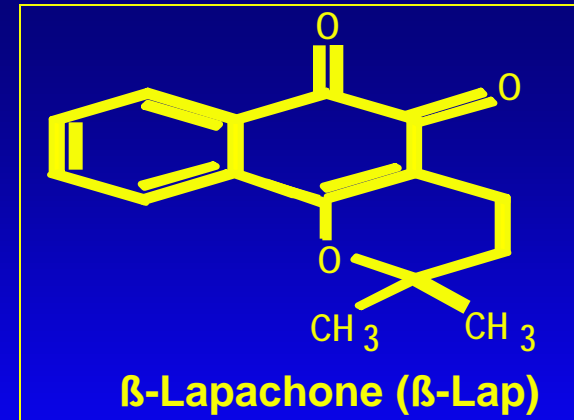
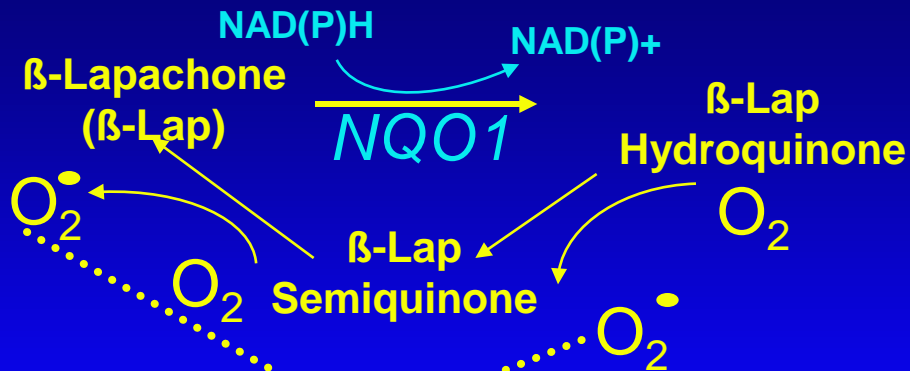
β -Lapachone Redox Cycling



β -Lapachone Induces a *Futile Cycle* of NQO1-Mediated NADH Oxidation

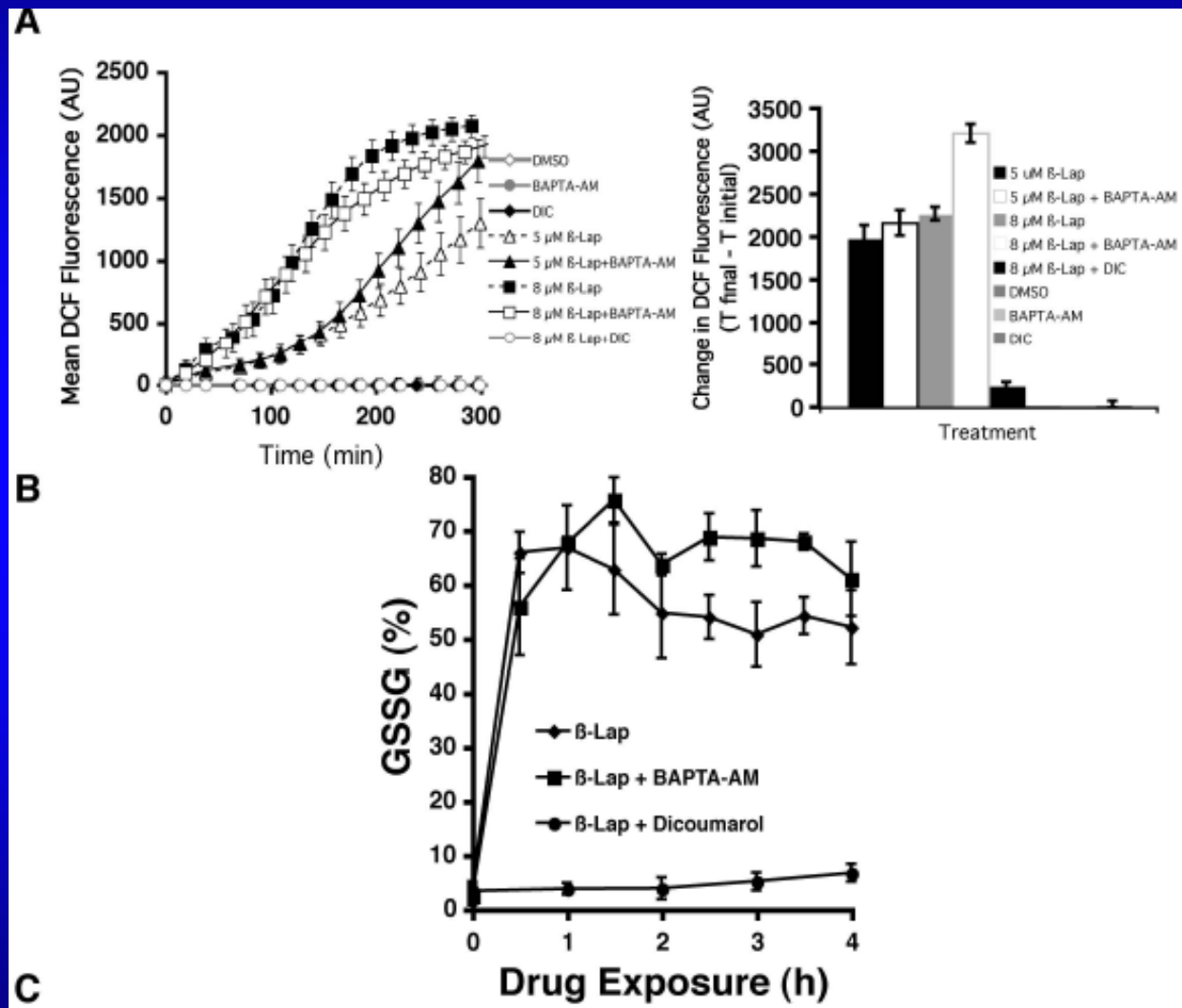
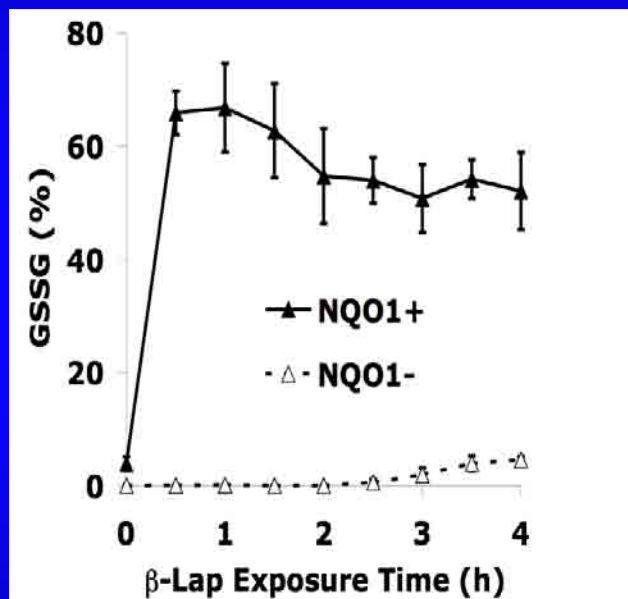


β -Lap Cytotoxicity: "Noncaspase-mediated Cell Death"



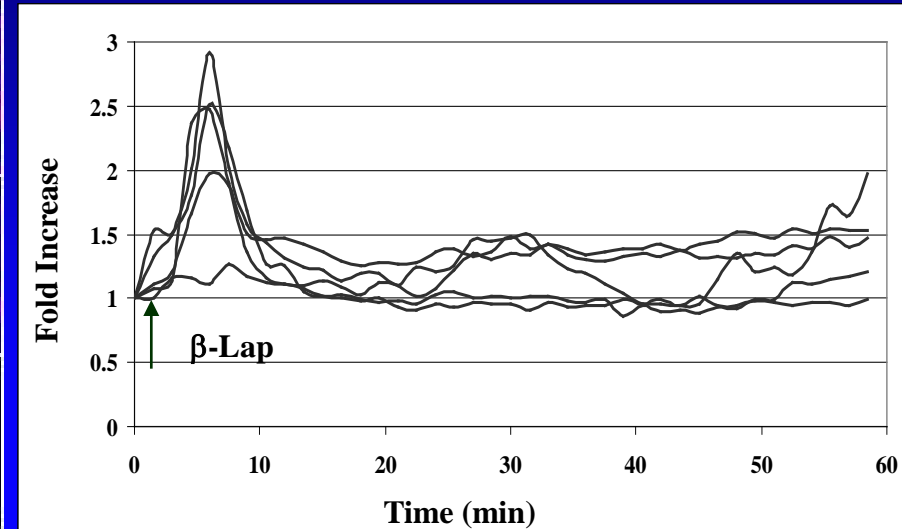
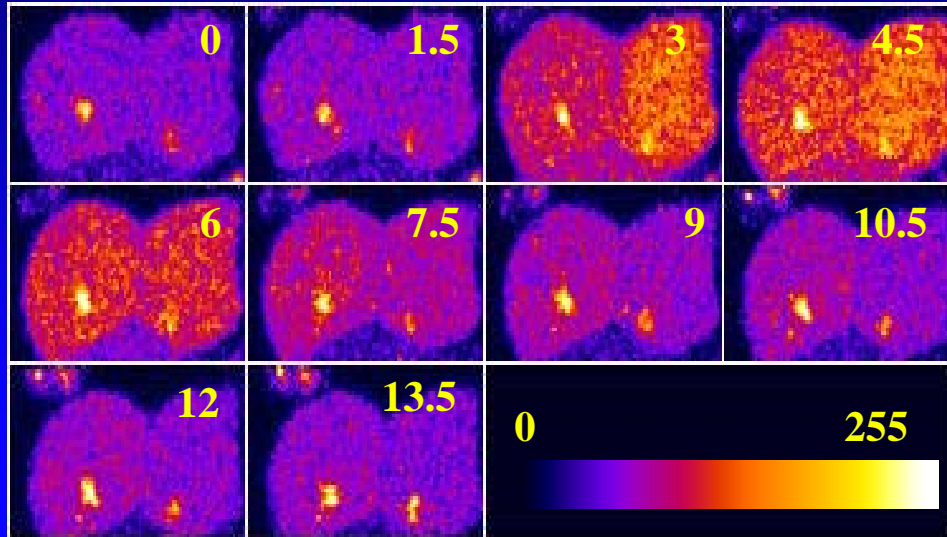
Caspase-Independent Apoptosis

NQO1-dependent ROS formation



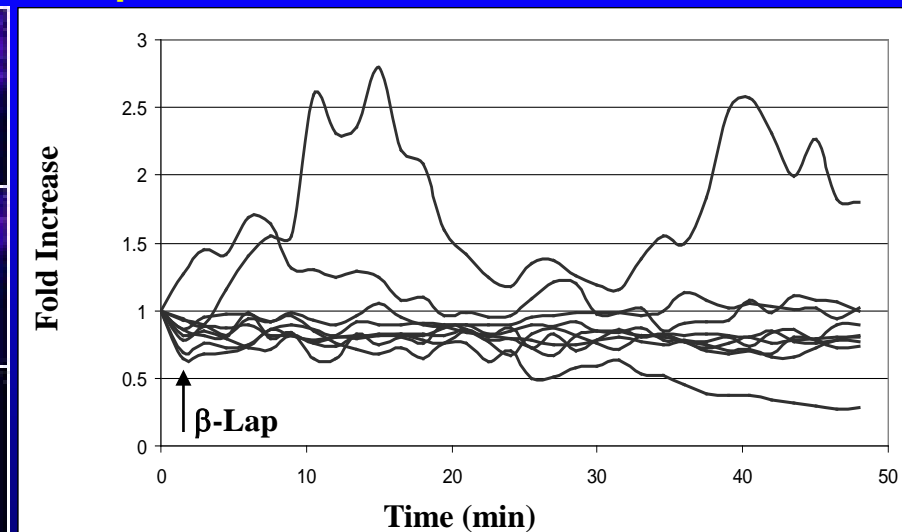
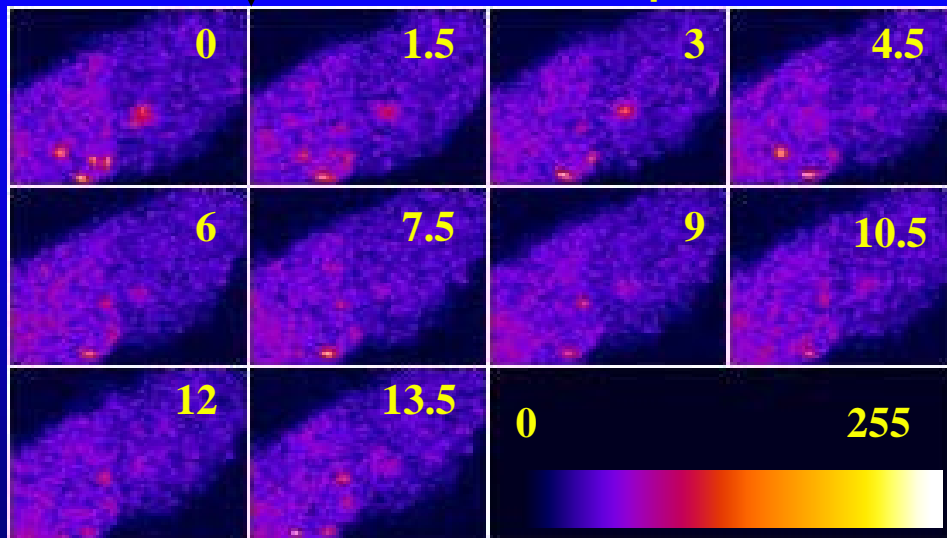
Calcium Release After β -Lap

β -lap



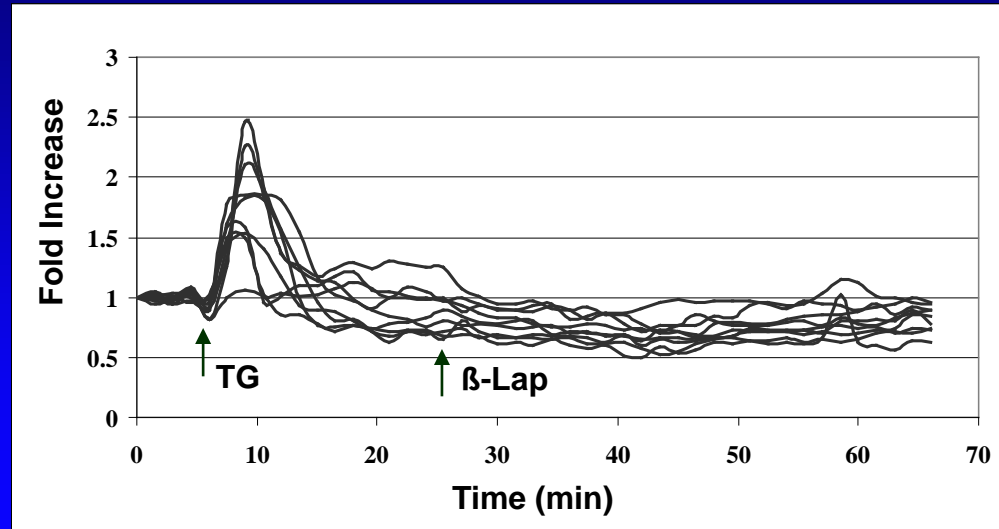
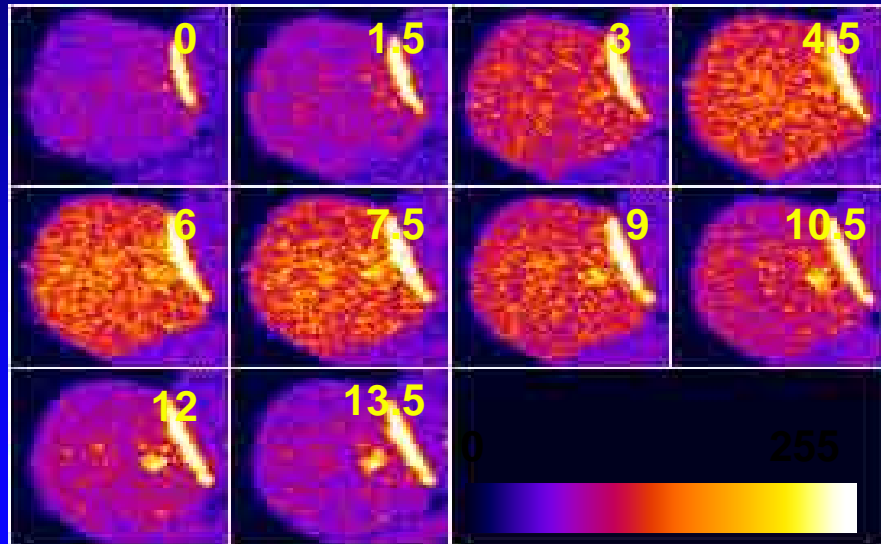
β -lap

20 μ M BAPTA-AM or 40 μ M dicoumarol

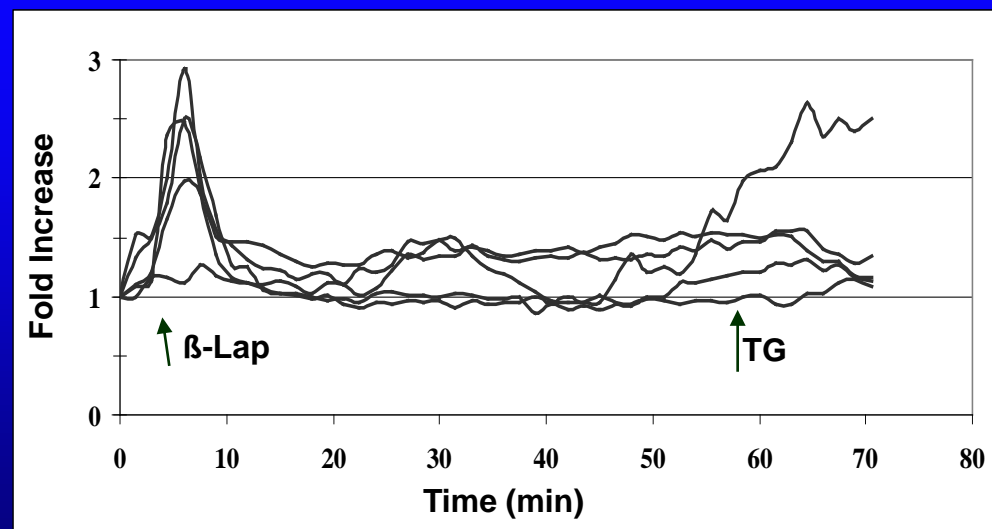
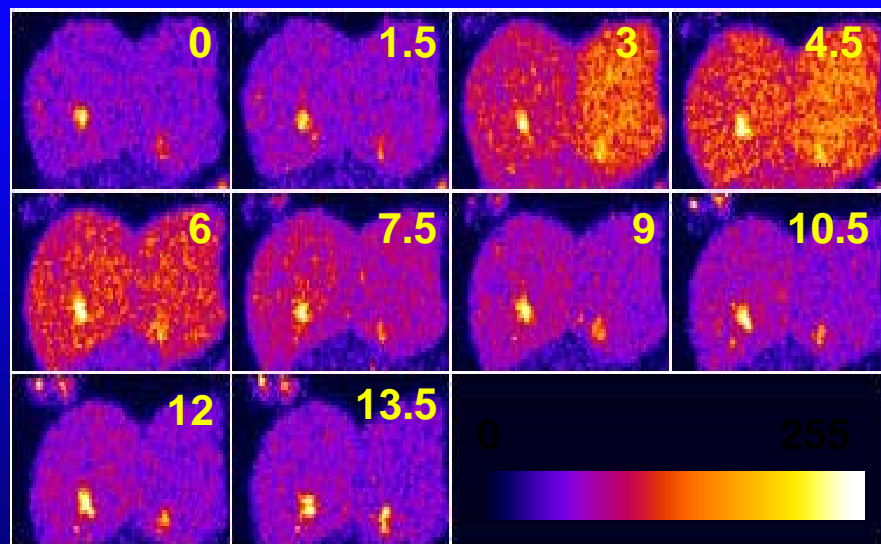


Calcium Release After β -Lap is from ER stores

↓ TG



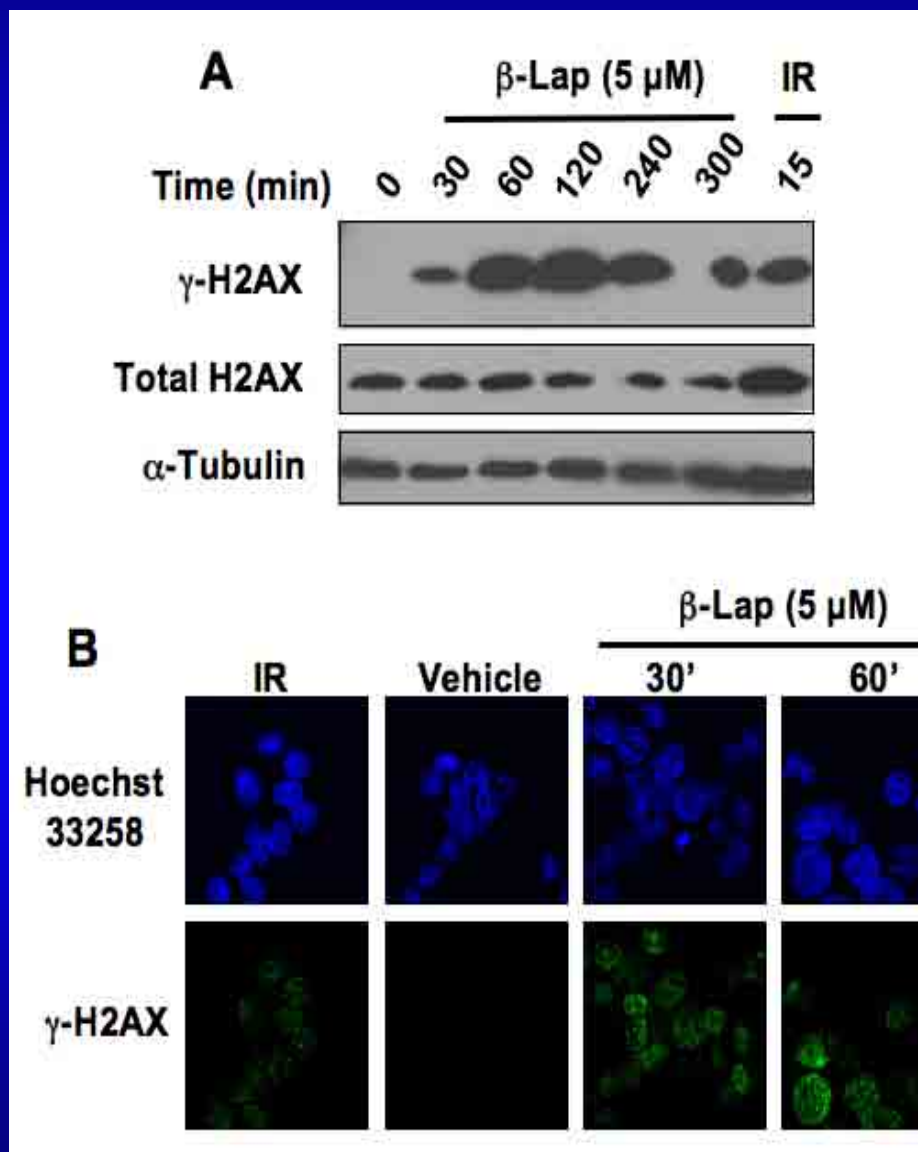
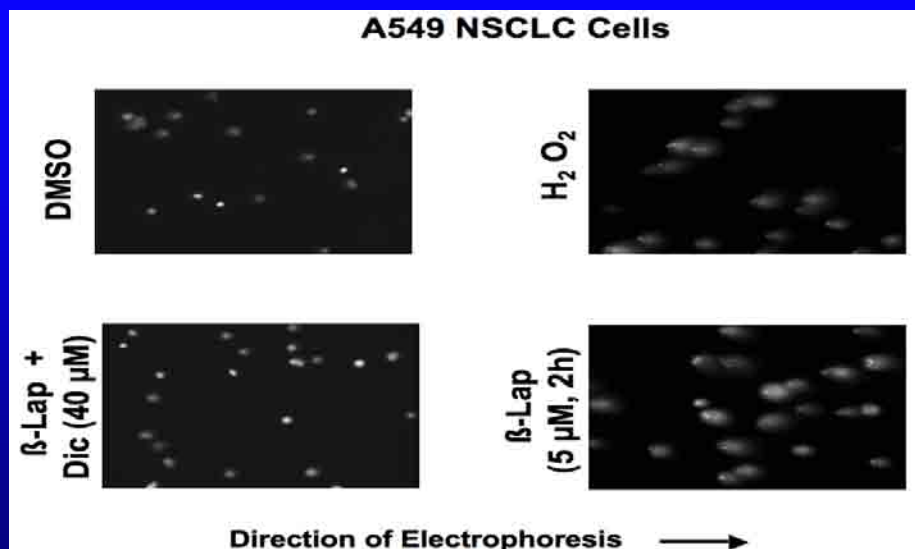
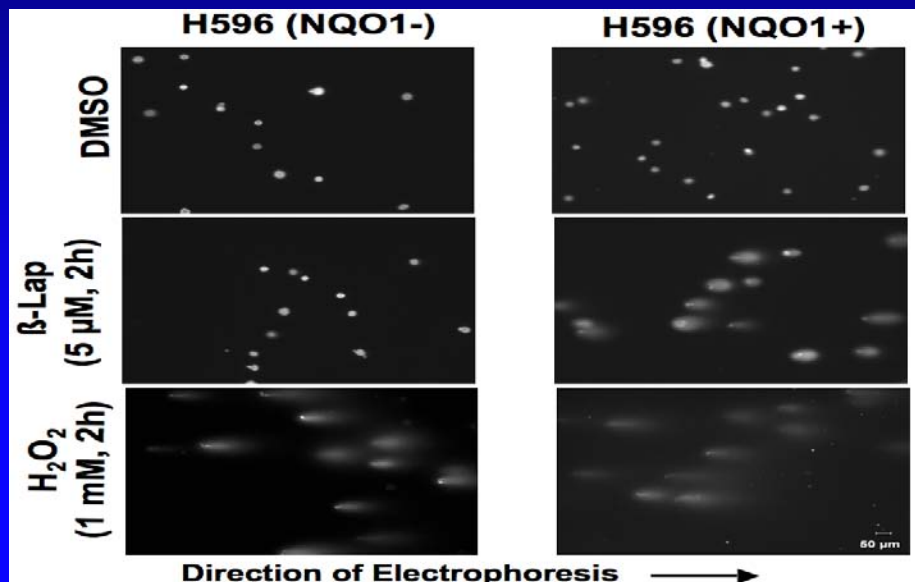
↓ β -lap



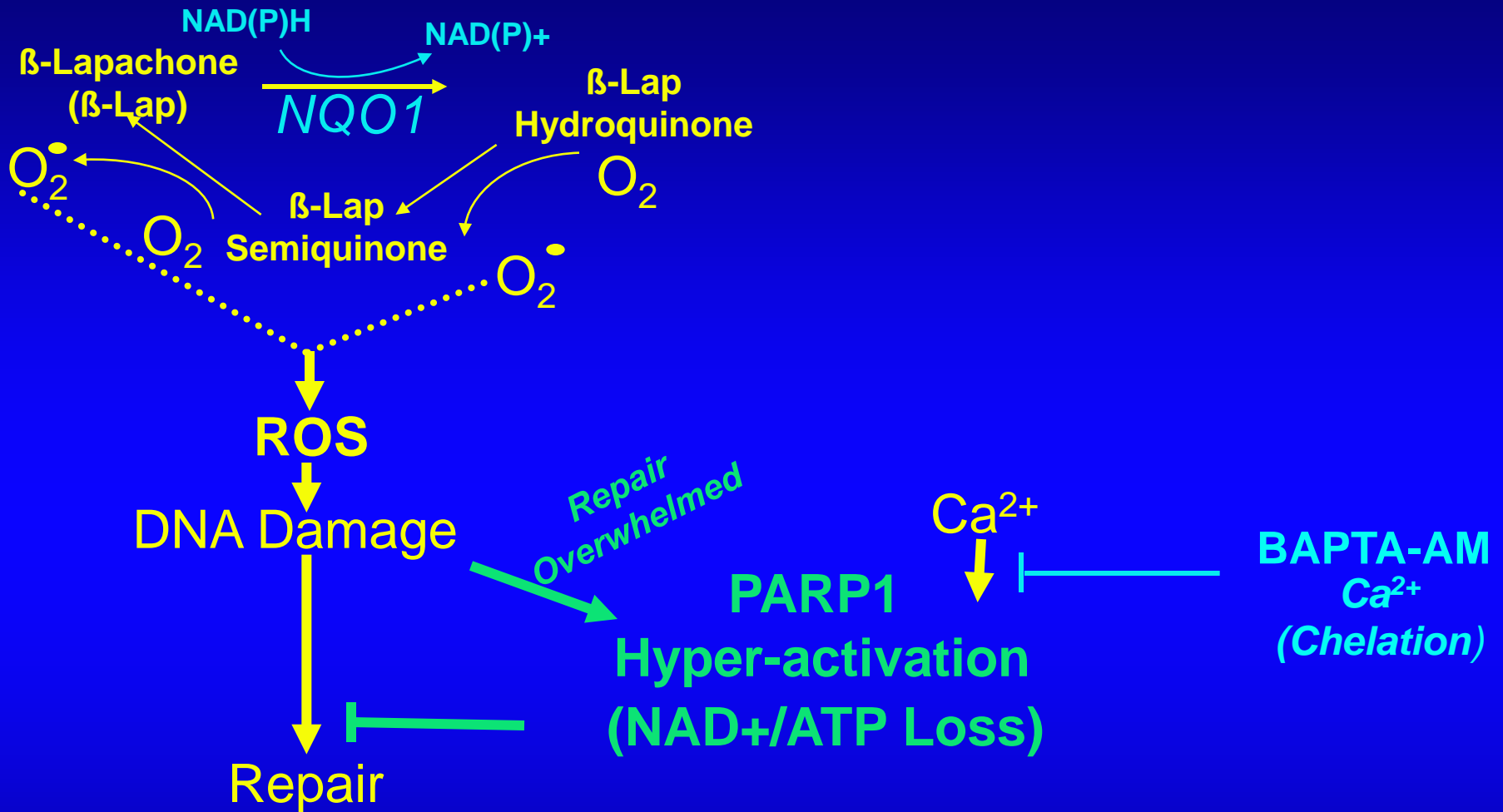
NQO1-dependent, β -Lap-induced DNA damage

Comet Assays

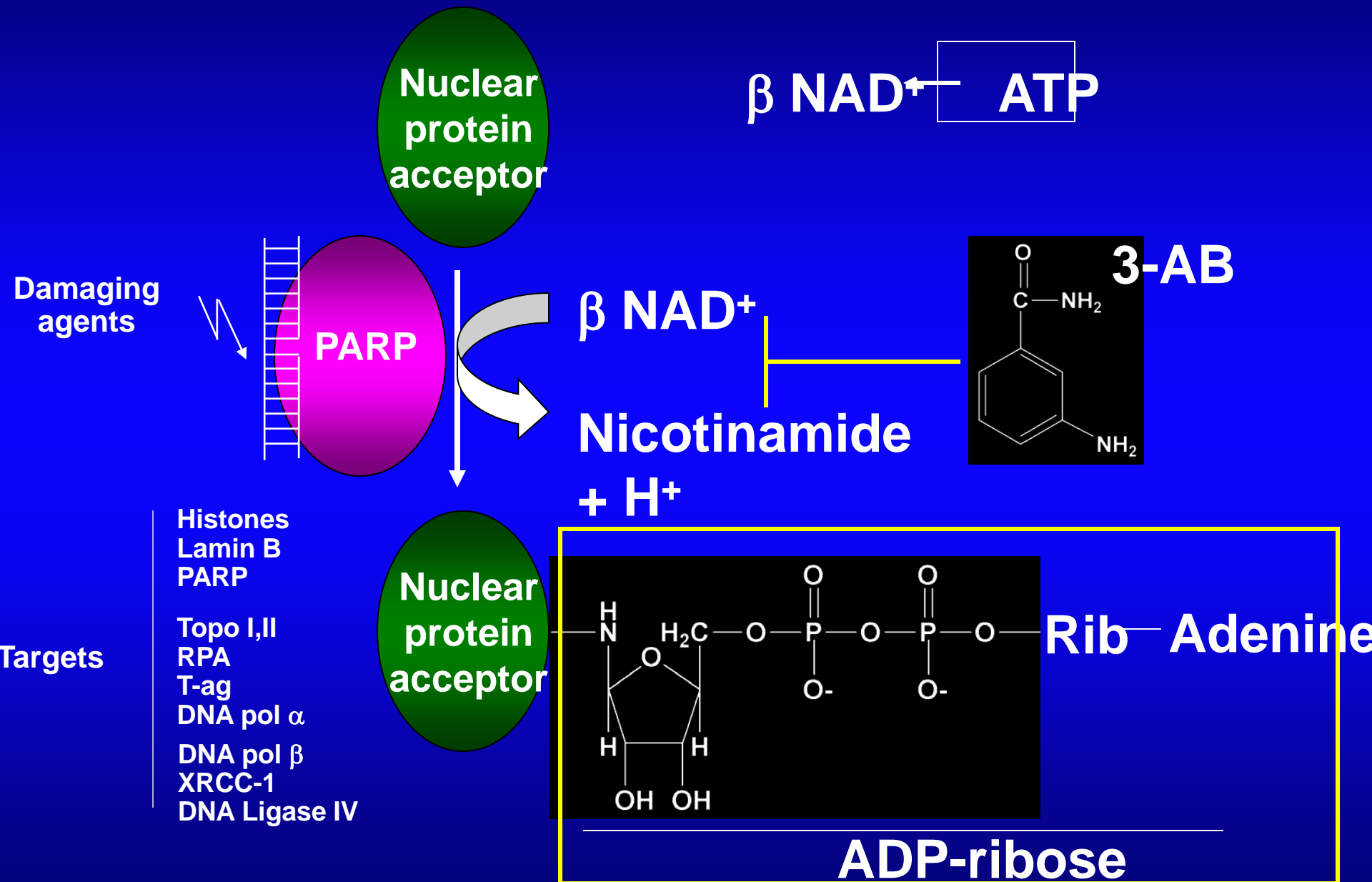
γ -H2AX



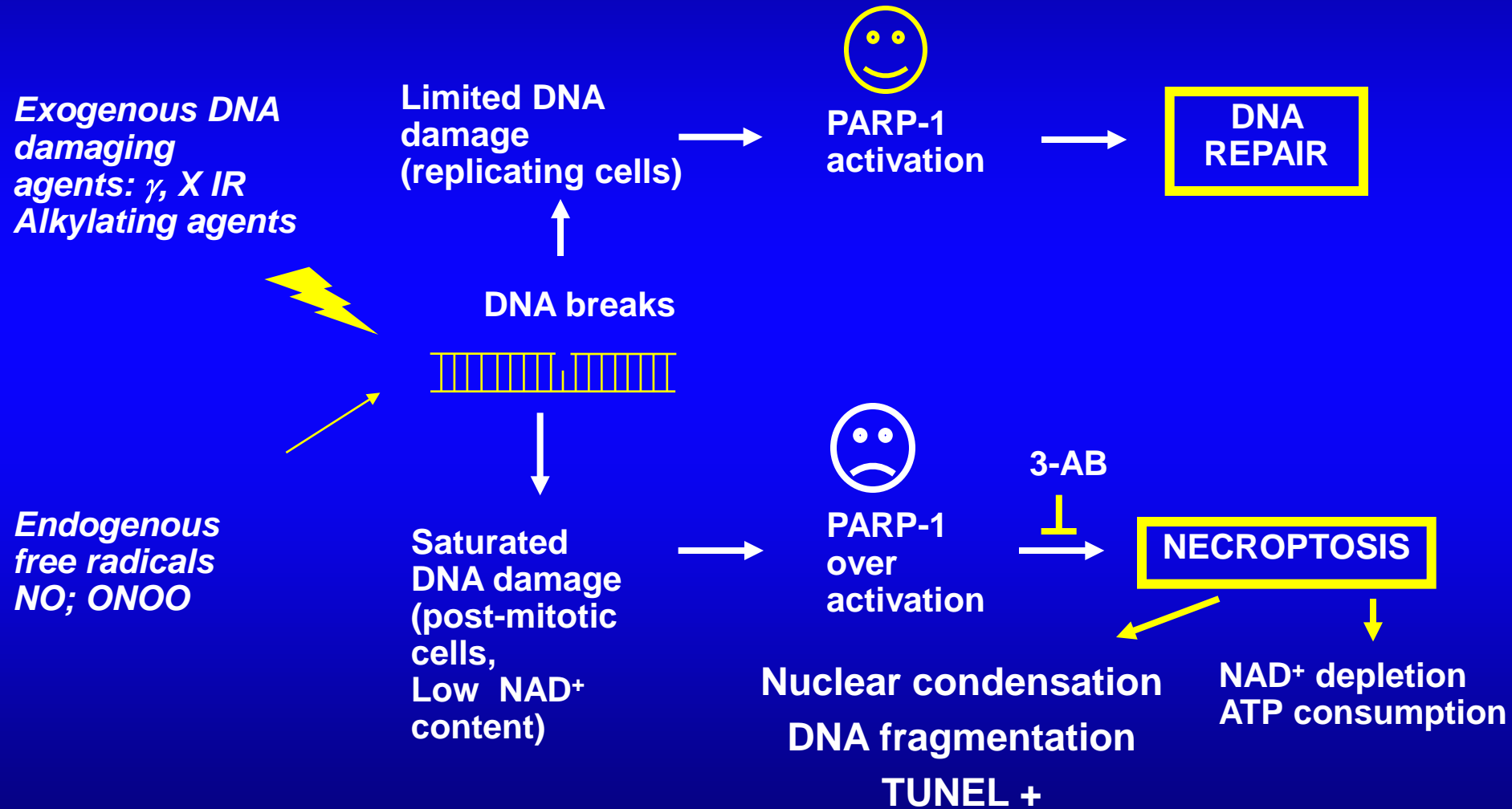
β -Lap Cytotoxicity: "Noncaspase-mediated Cell Death"



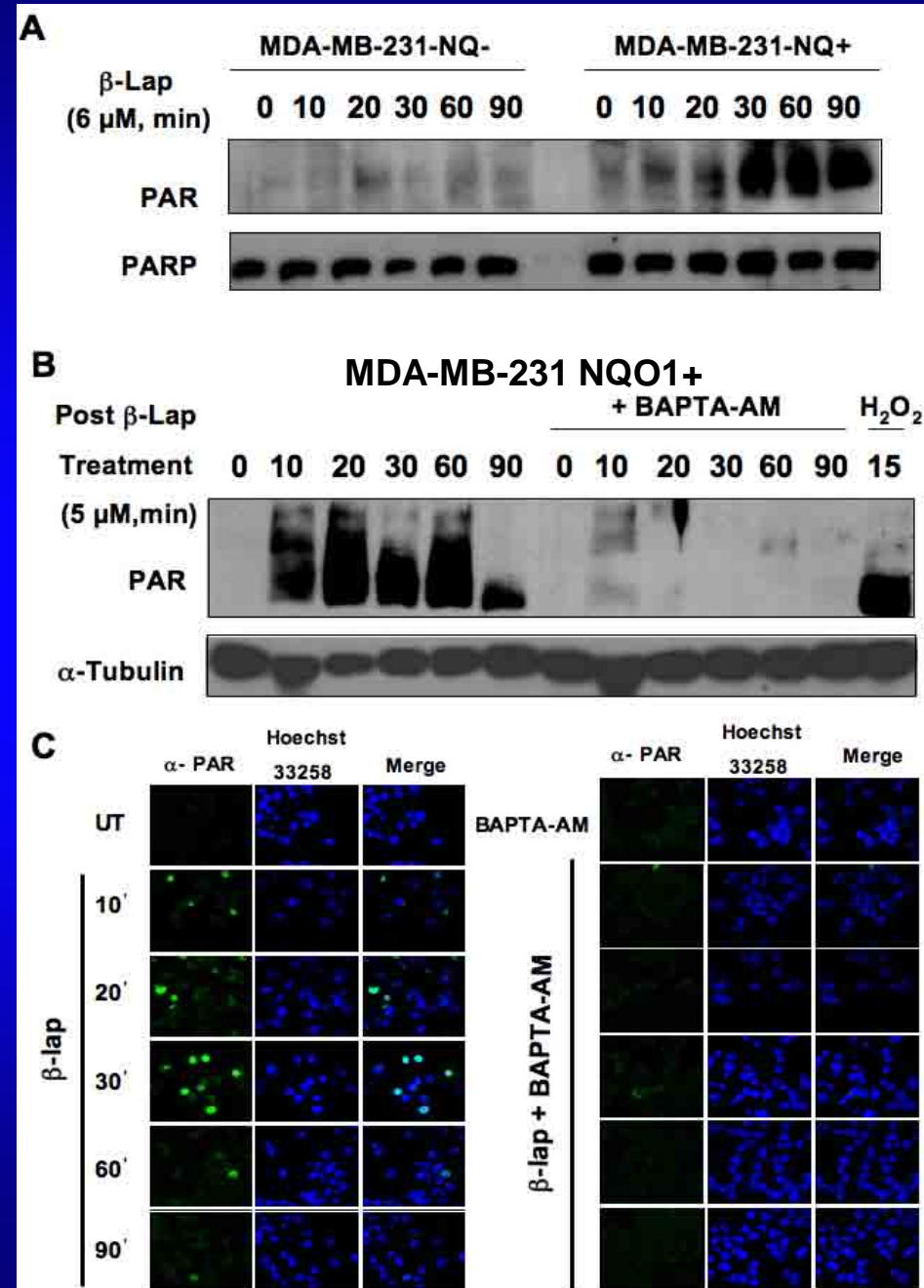
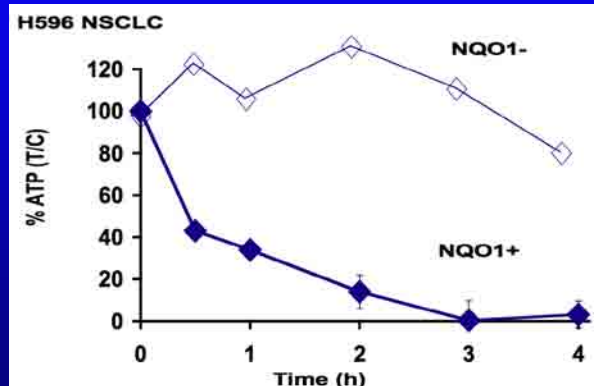
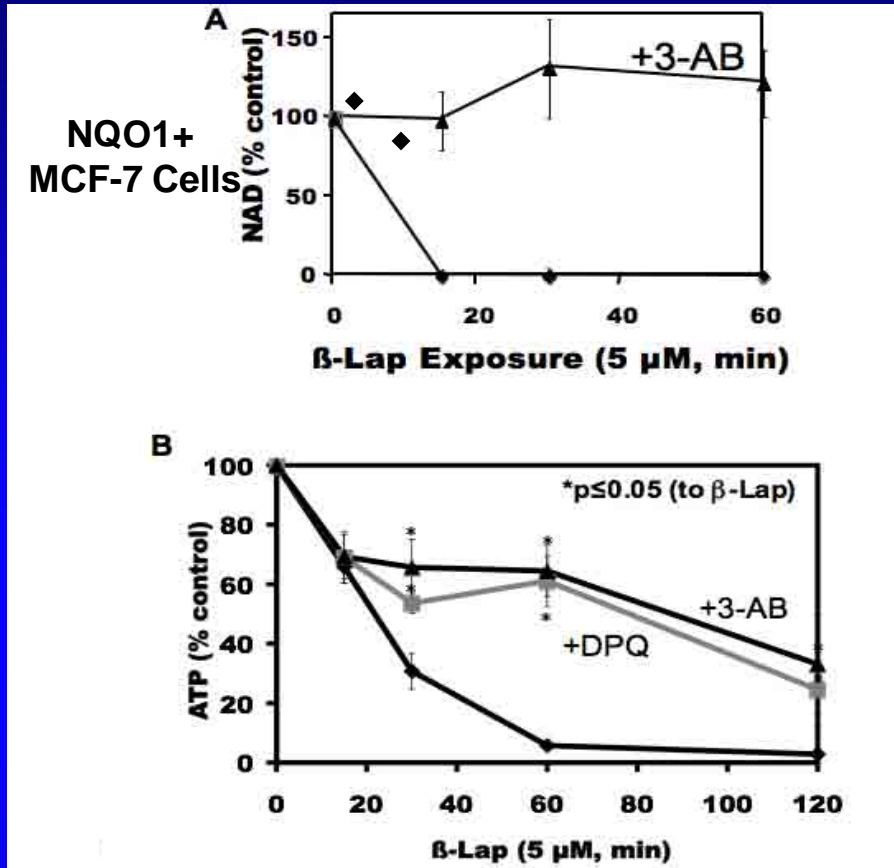
PARP in Action



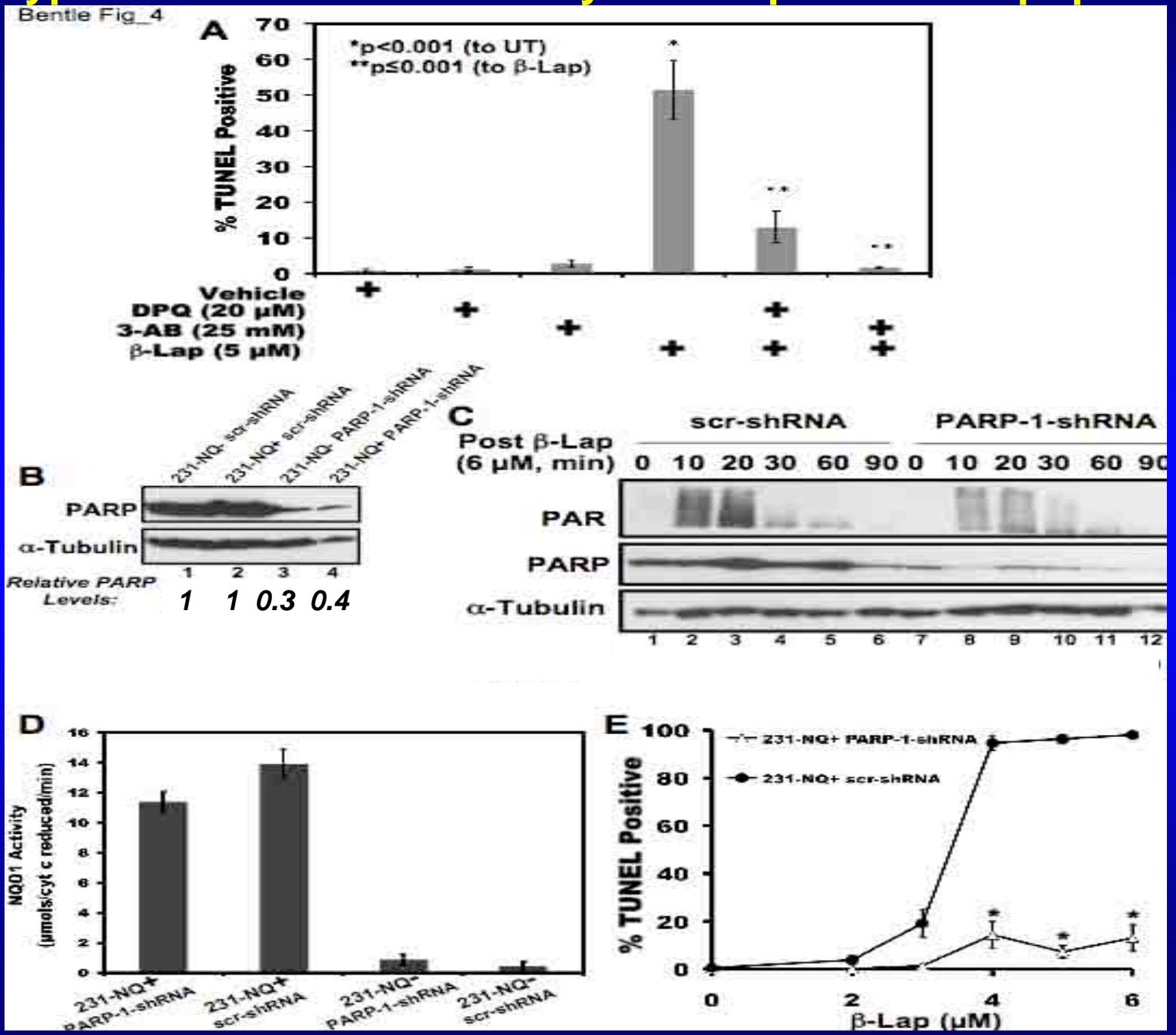
The Two Facets of PARP-1 Activation



NQO1-dependent, PARP1 hyperactivation & nucleotide loss

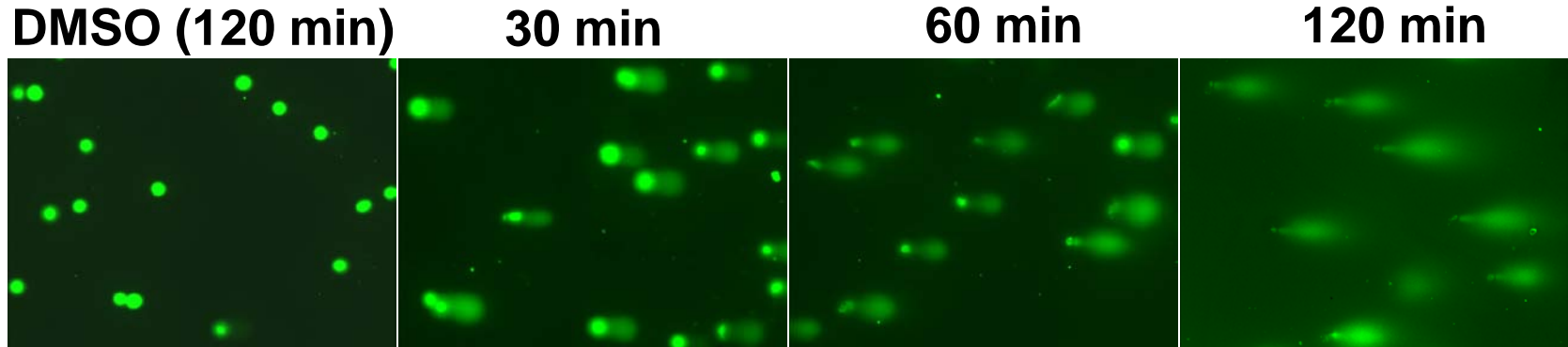


PARP hyperactivation is necessary for β -lap-induced apoptosis

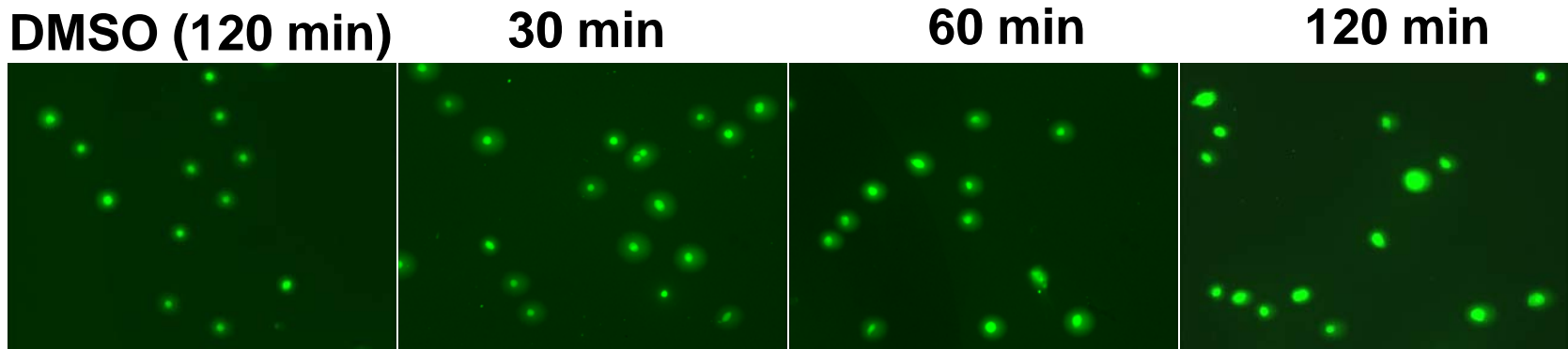


β -Lap-induces DNA single strand breaks (SSBs)

Alkaline Comet Assay (Measures Total Breaks)

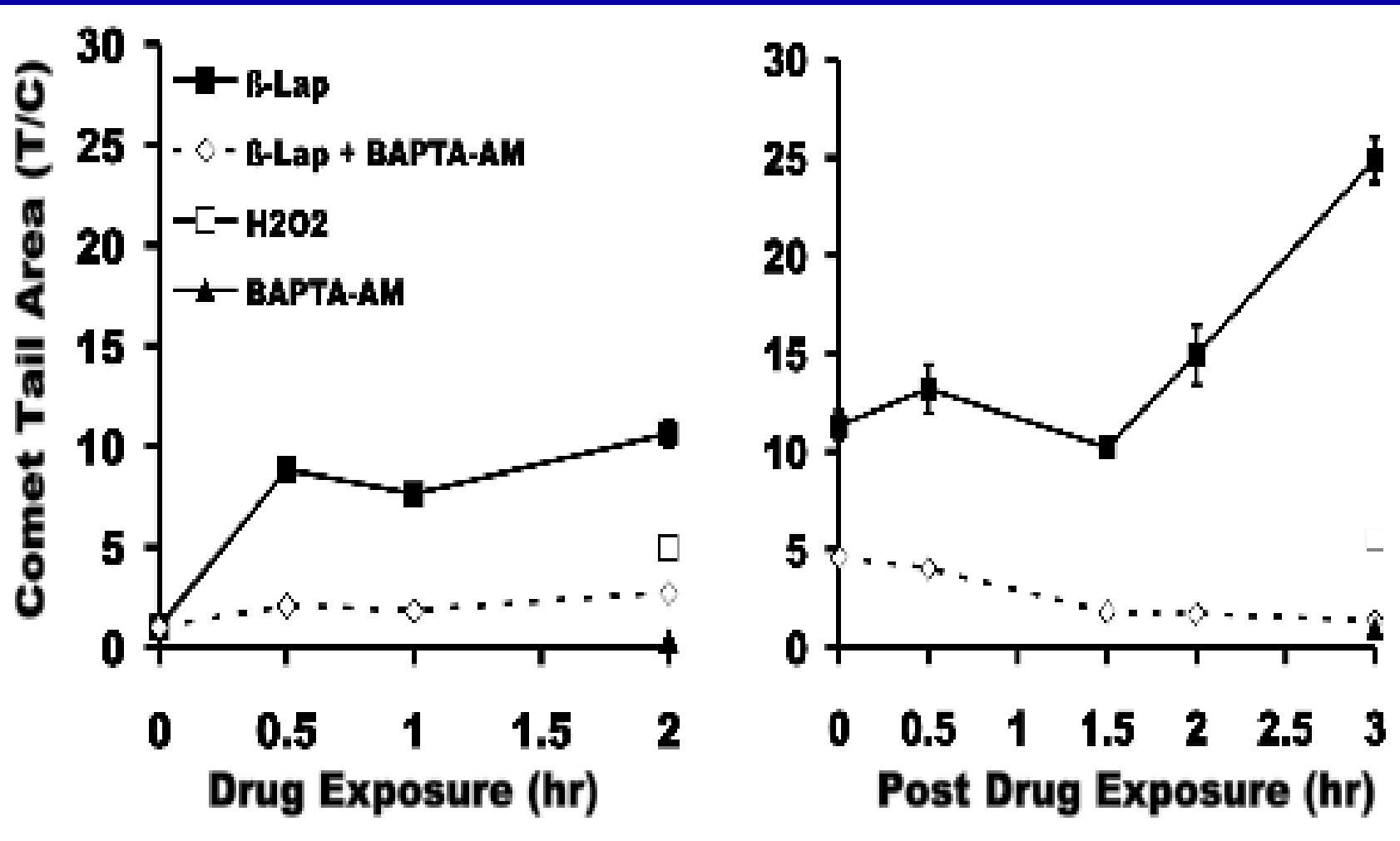


Neutral Comet Assay (Measures DSBs)

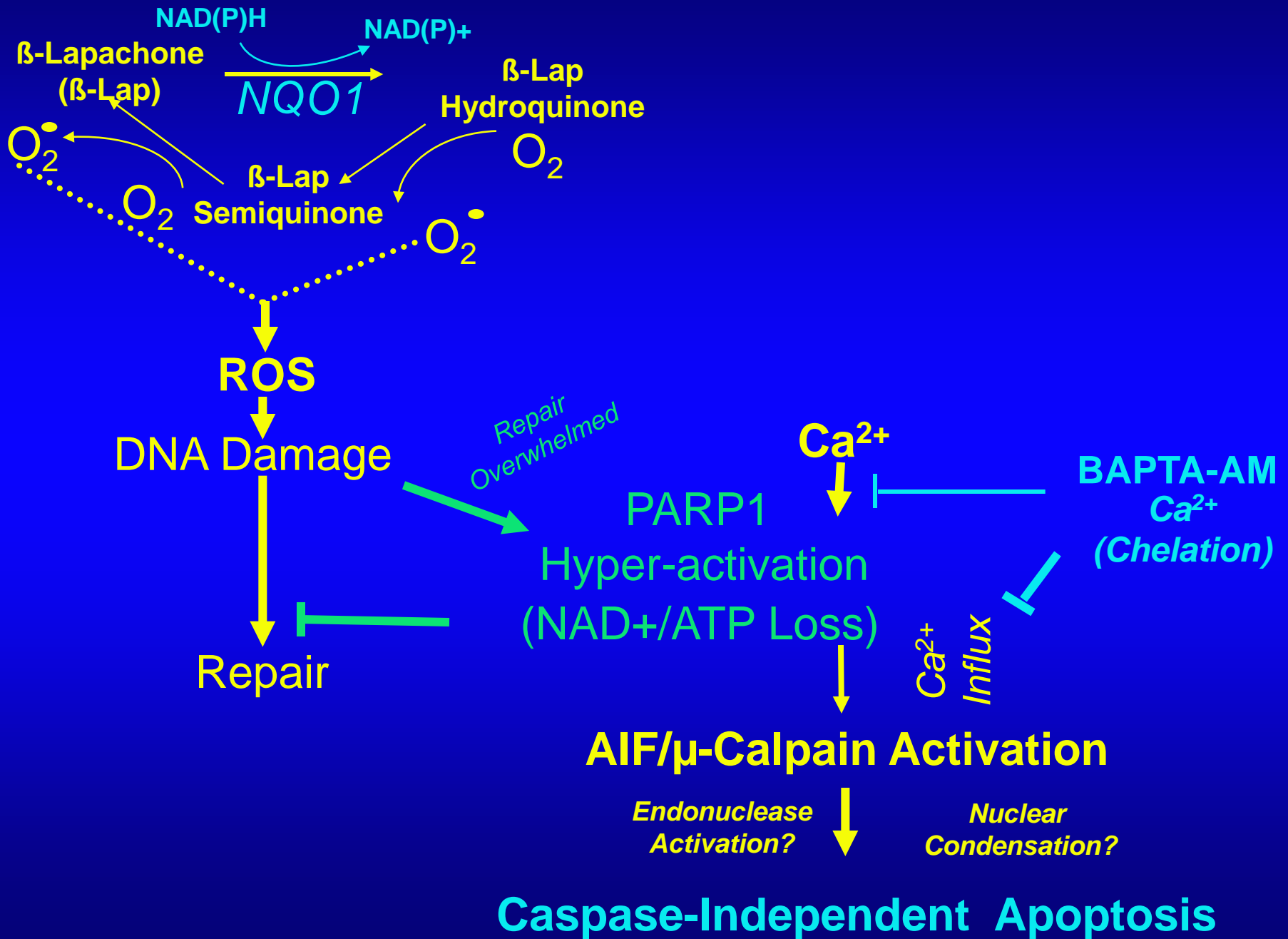


(4 μ M β -lap treatment)

Ca²⁺ chelation allows repair and recovery after β -lap

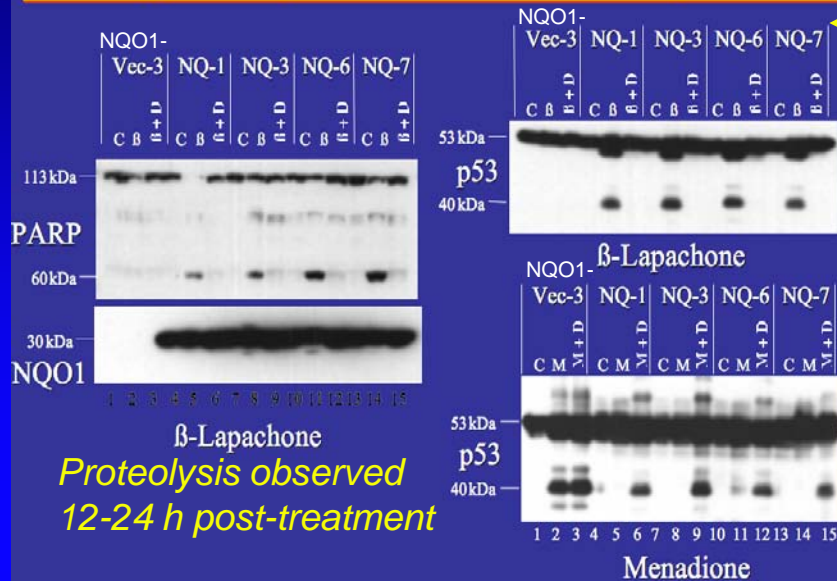


β -Lap Cytotoxicity: "Noncaspase-mediated Cell Death"



NQO1-dependent μ -calpain activation

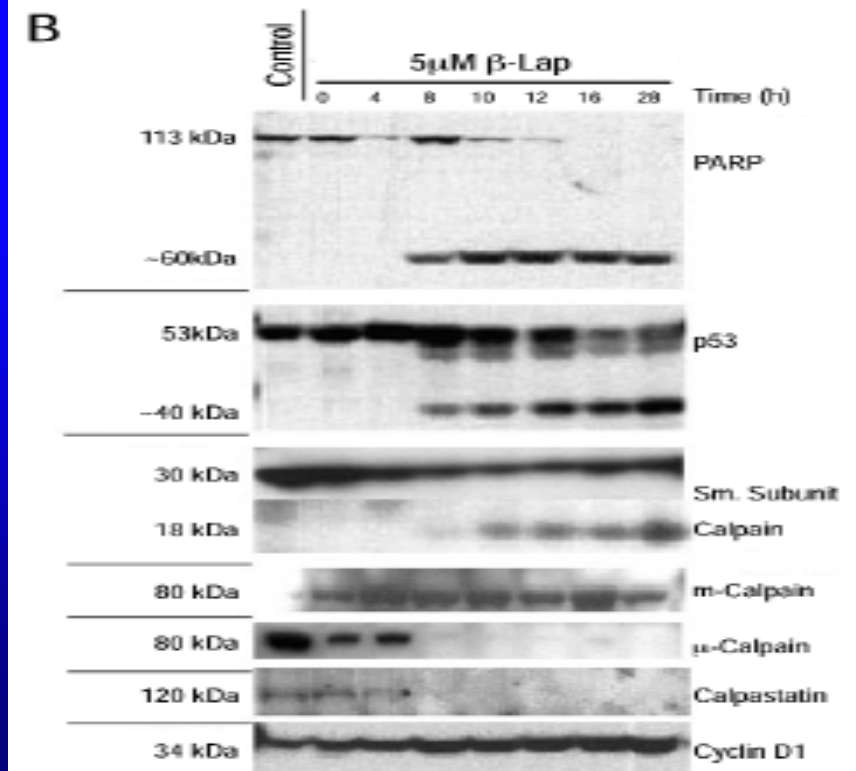
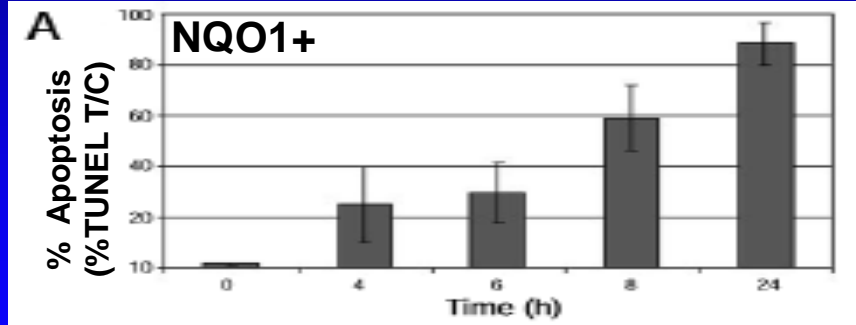
Exogenous NQO1 Sensitizes Cells to β -Lapachone



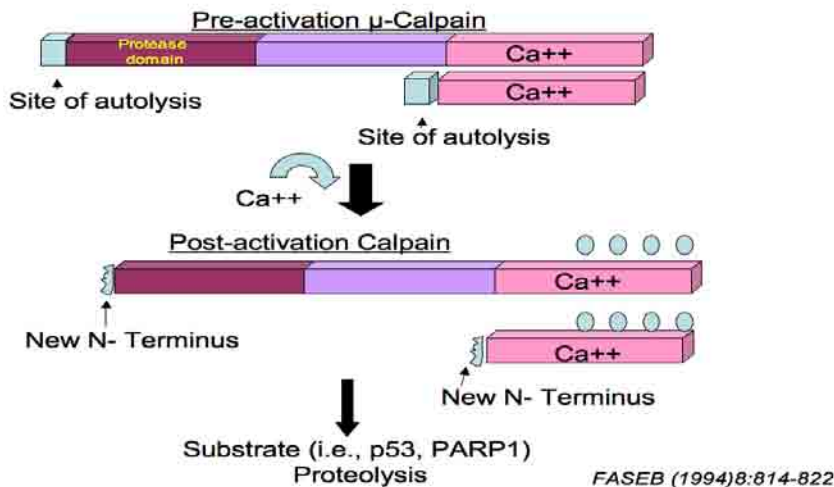
p53 and atypical PARP1 cleavage were hallmarks of β -lap cell death

Proteolysis observed 12-24 h post-treatment

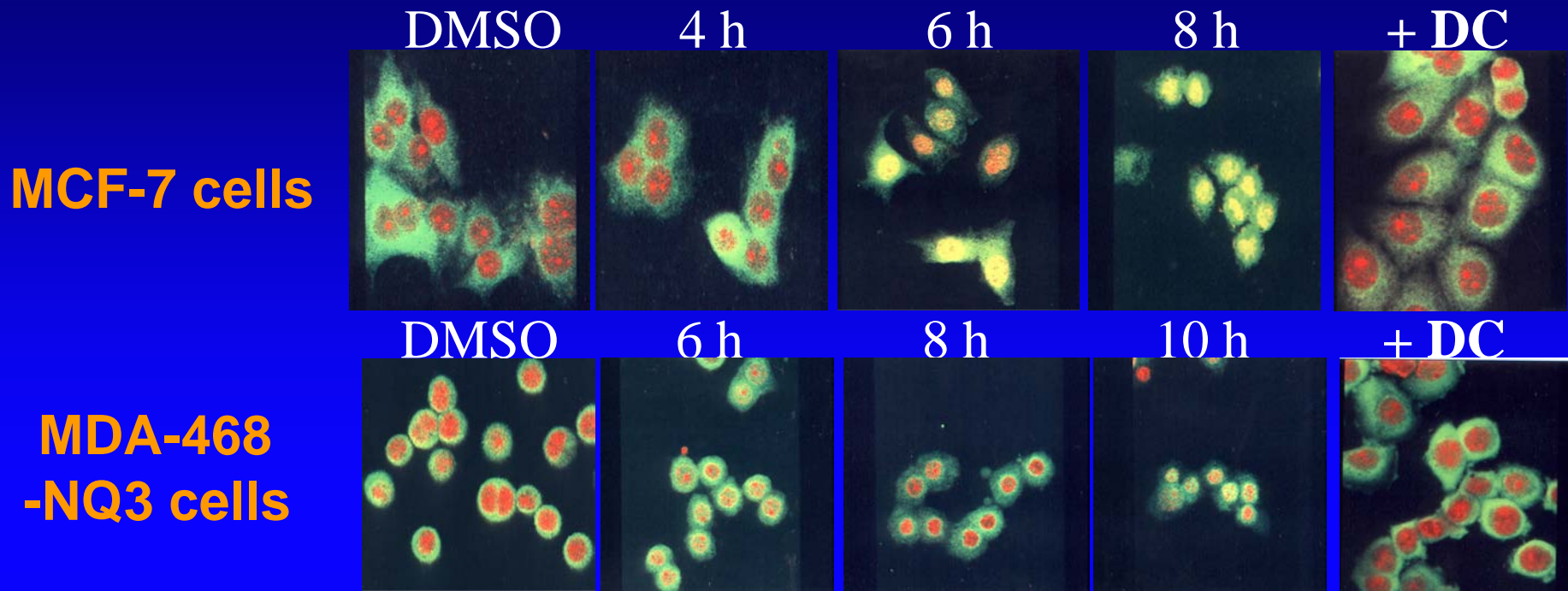
Pink et al., JBC, 2000



μ -Calpain activation



μ -Calpain Translocation to the Nucleus



Anti-NQO1/PI

MCF-7 (8 h)

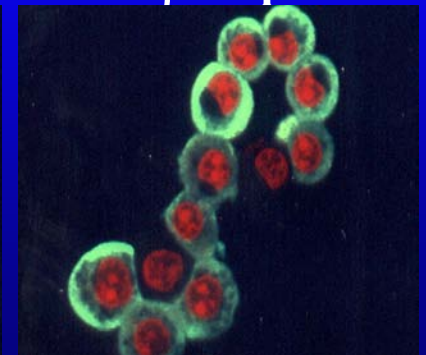
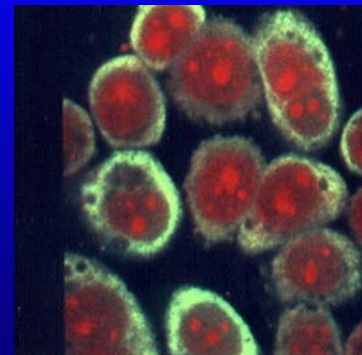
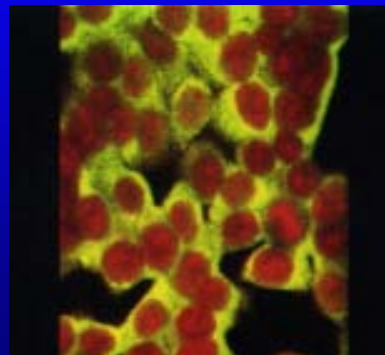
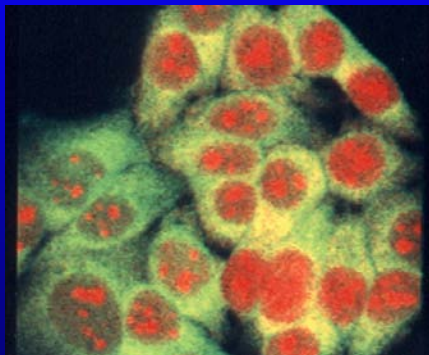
MDA-468-NQ3 (10 h)

Control

β -Lap

Control

β -Lap



NQO1-dependent, Ca²⁺-regulated apoptosis inducing factor (AIF) activation

NQO1+ MCF-7

β -lapachone (5 μ M, 2 h)

Time (h) Post Treatment

DMSO

4

8

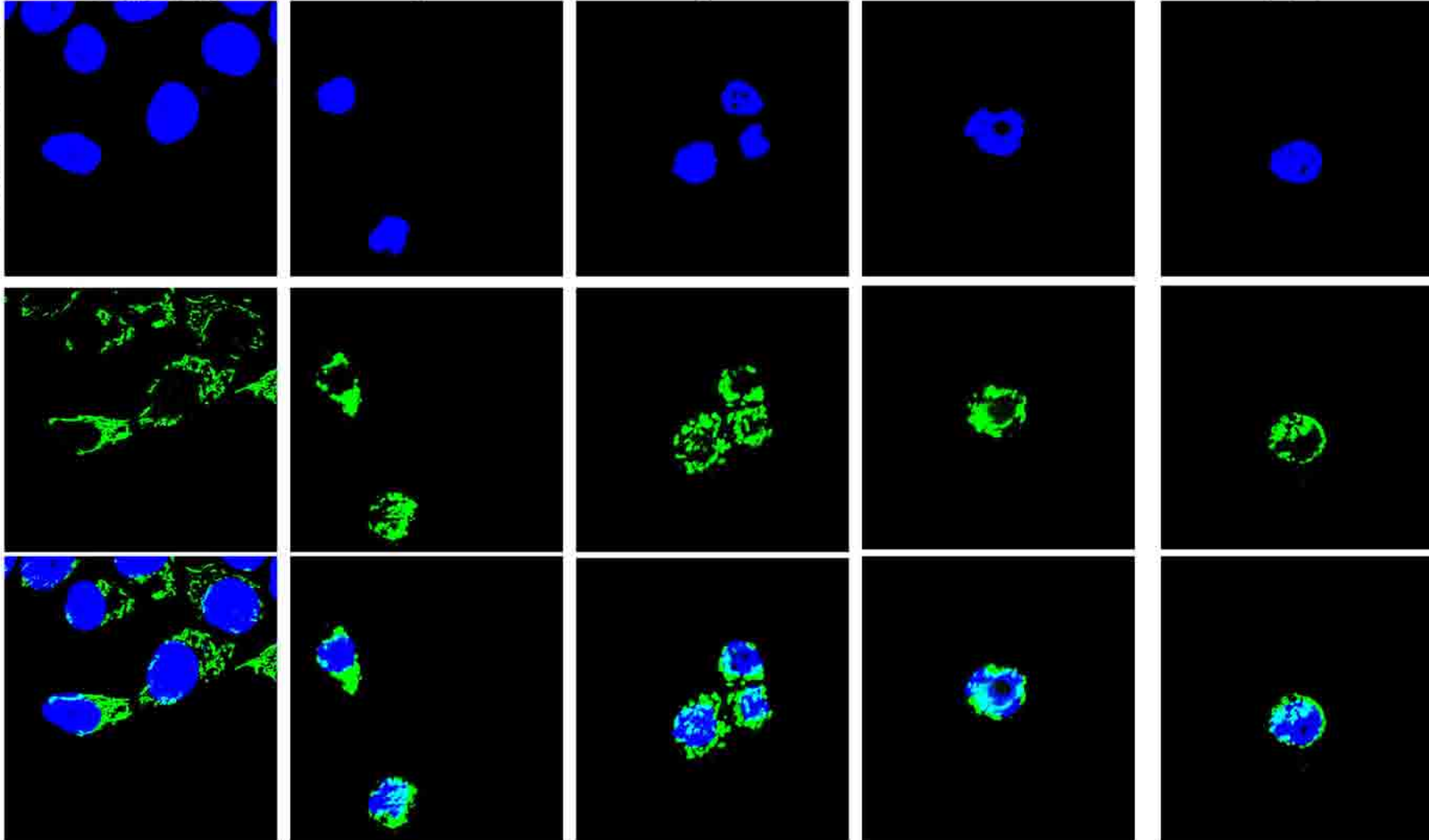
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24

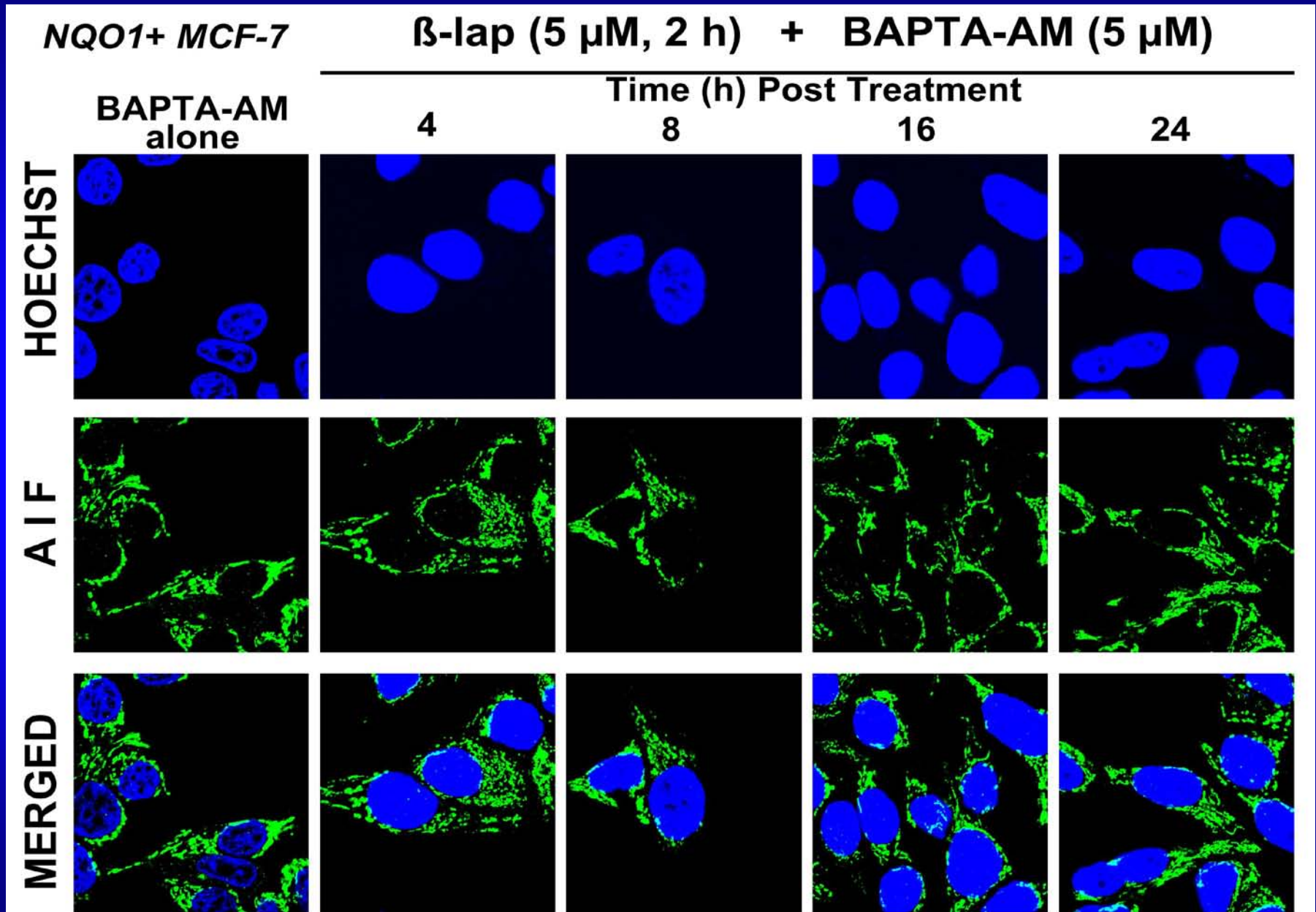
HOECHST

AIF

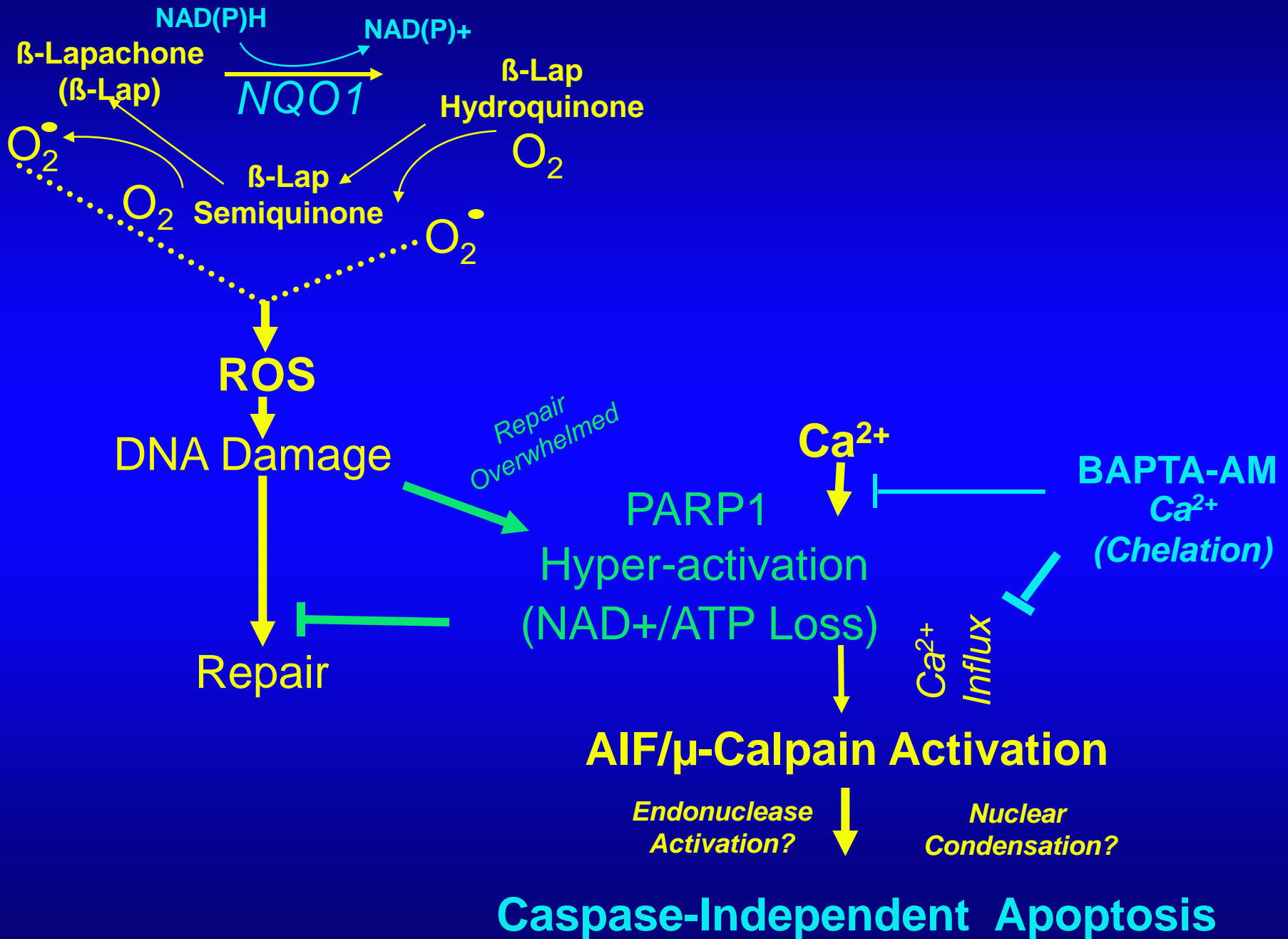
MERGED



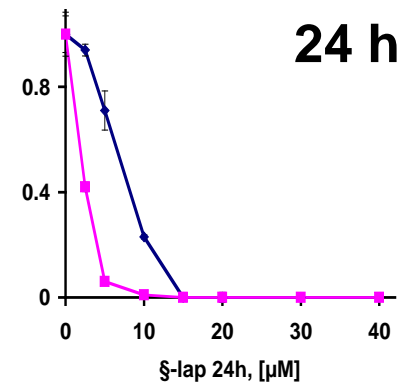
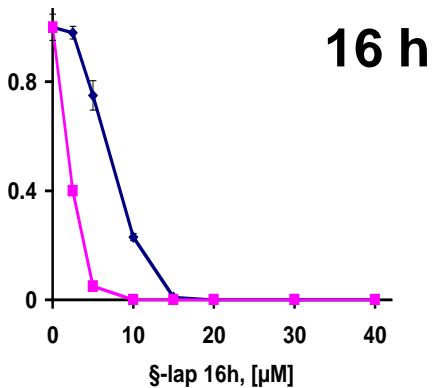
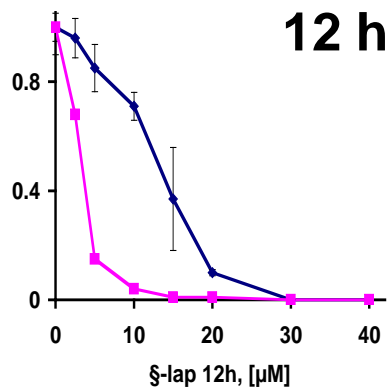
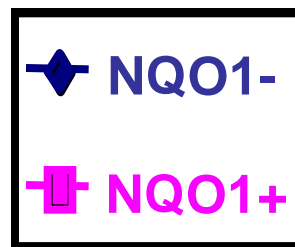
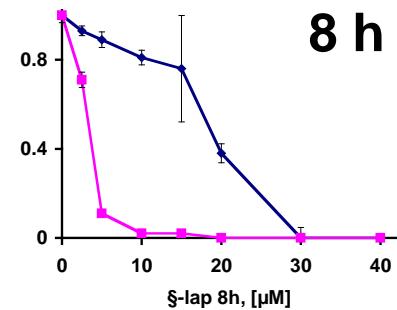
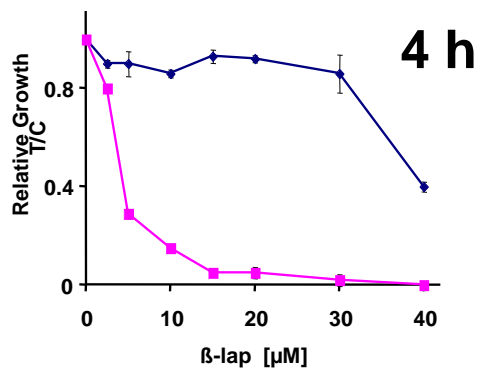
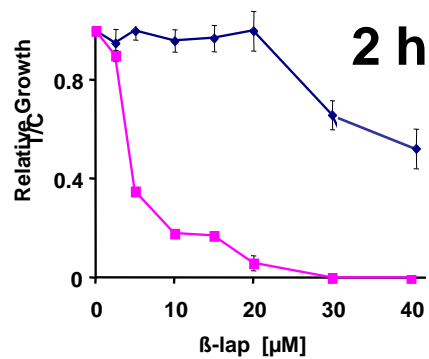
Ca²⁺ chelation by BAPTA-AM pre-loading blocks AIF activation



β -Lap Cytotoxicity: "Noncaspase-mediated Cell Death"

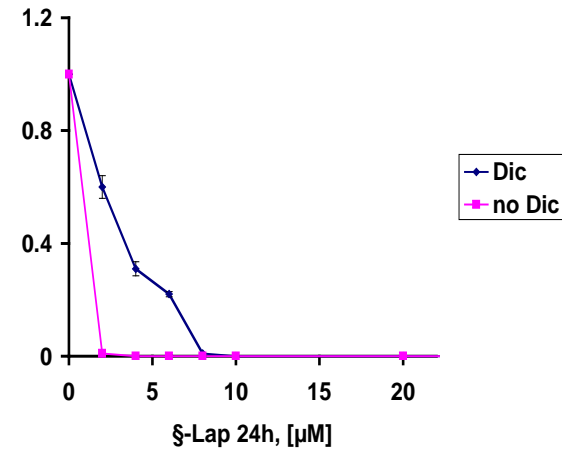
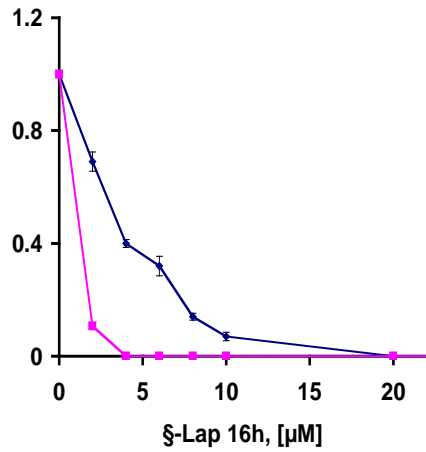
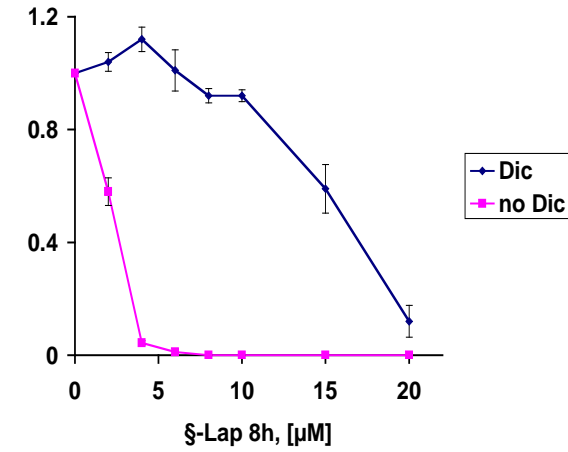
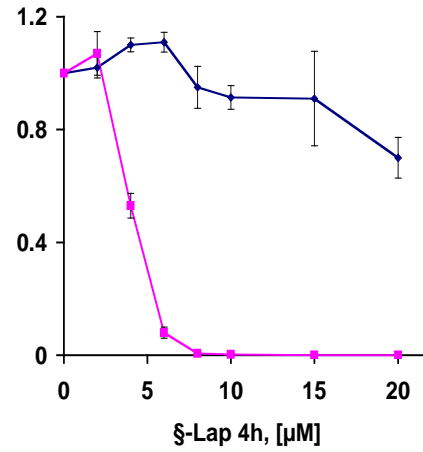
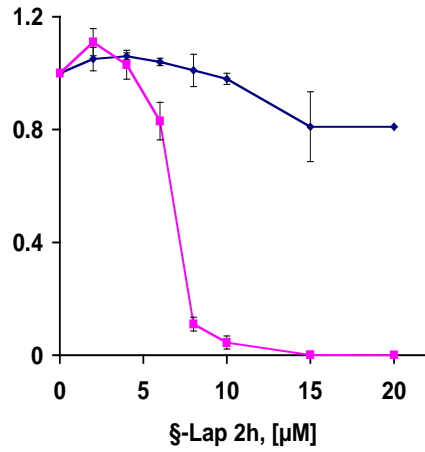


Maximum antitumor therapeutic window for treating NSCLC with β -lap



H596 NSCLC cells

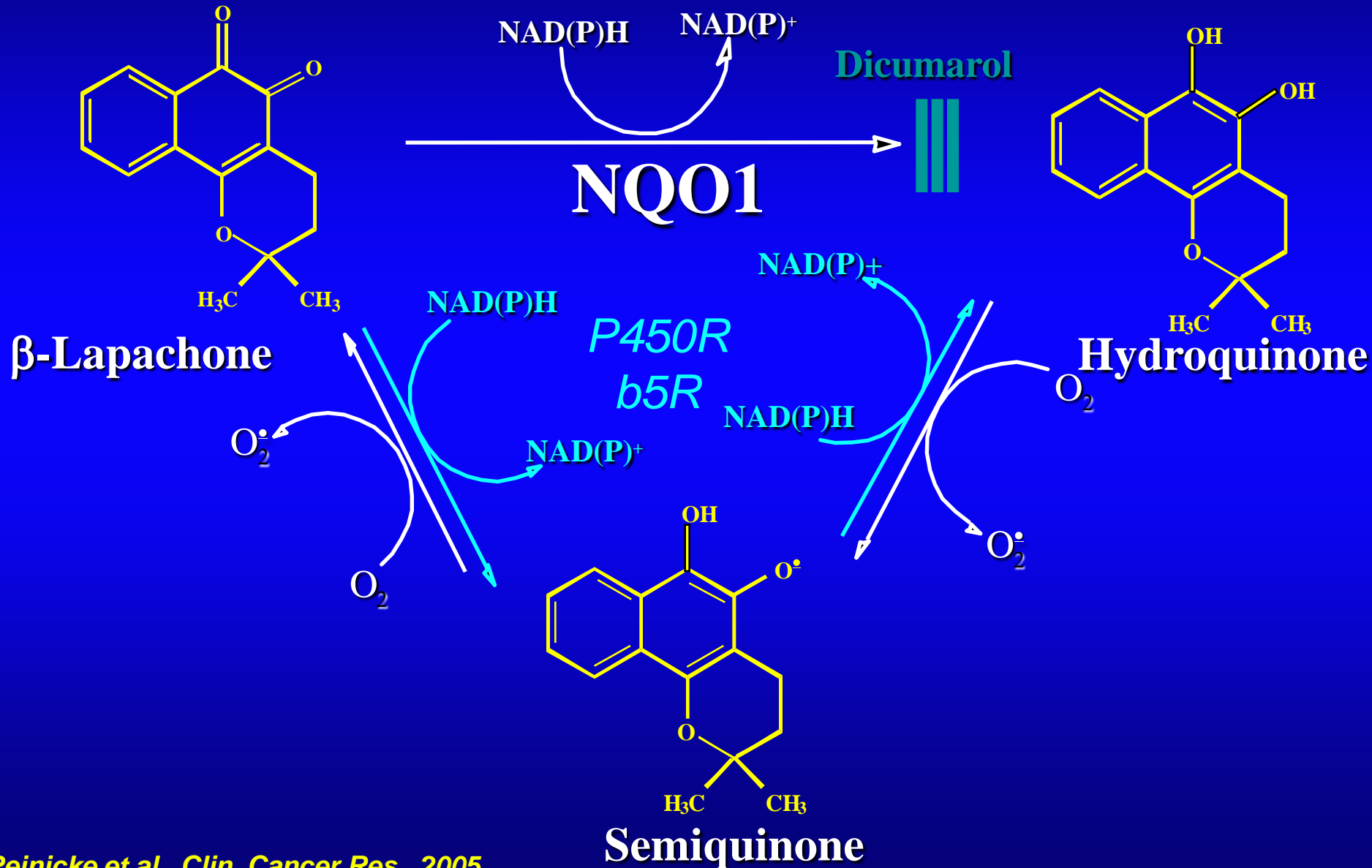
Short pulses of β -lap may increase its therapeutic index in treating NSCLC



A549 NSCLC cells

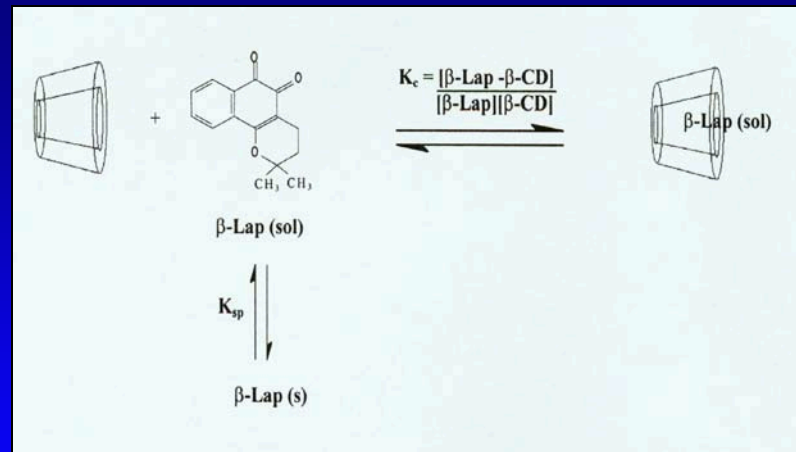
Bey et al., PNAS, 2007

β -Lapachone Redox Cycling



β -Lapachone delivery methodology development for specific cancer therapies

I. β -Cyclodextrin [Systemic administration]



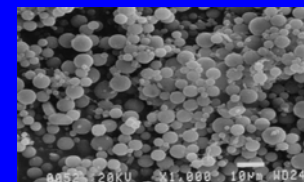
II. Millirods [Brachytherapy, prostate cancer]

Device Design: Double Layer Millirods

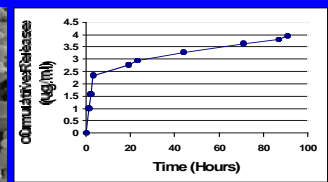


III. Nanoparticles (cRGD micelles) [Lung Cancer, antiangiogenesis] -Use Lung cancer-specific ligands (e.g., $\alpha_v\beta_6$) (Brown)

Polymer Microspheres

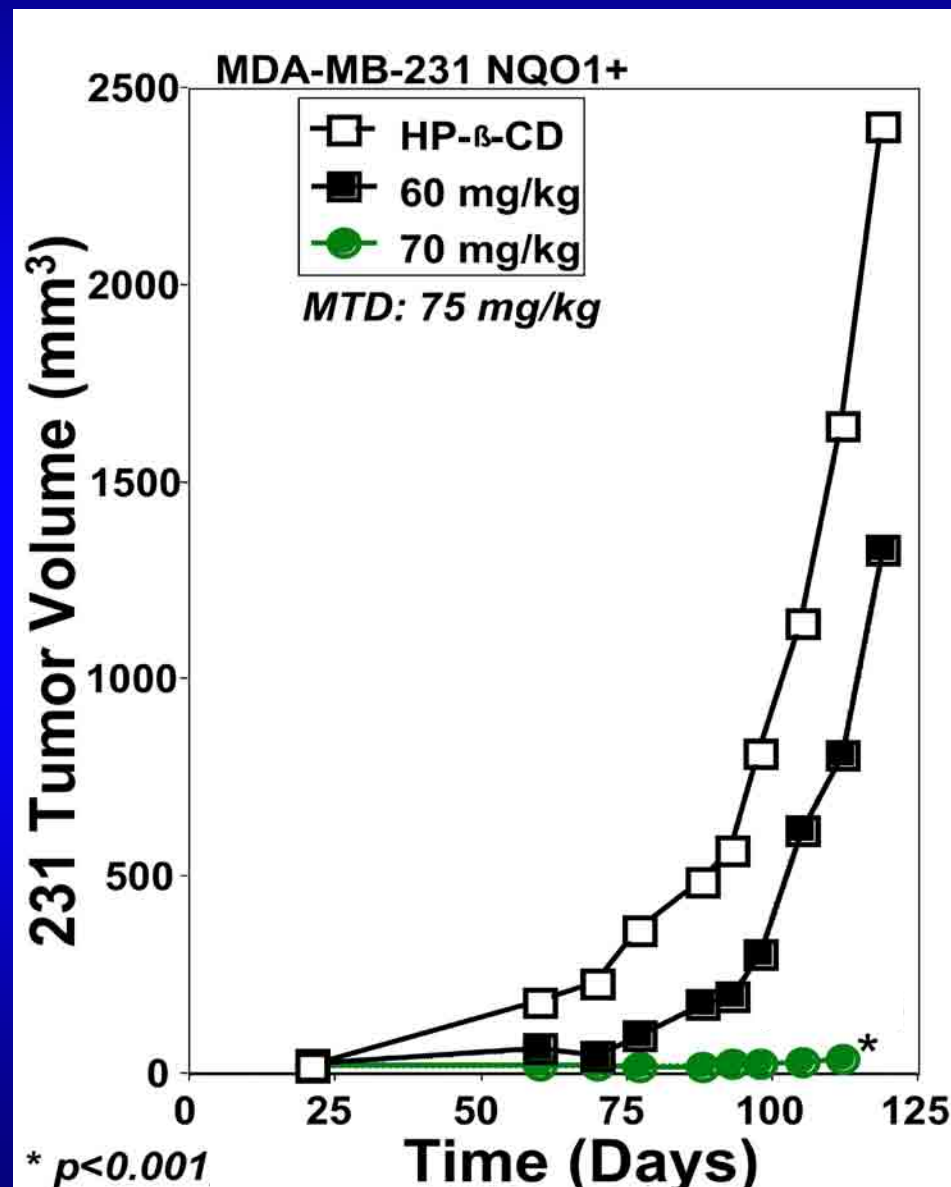
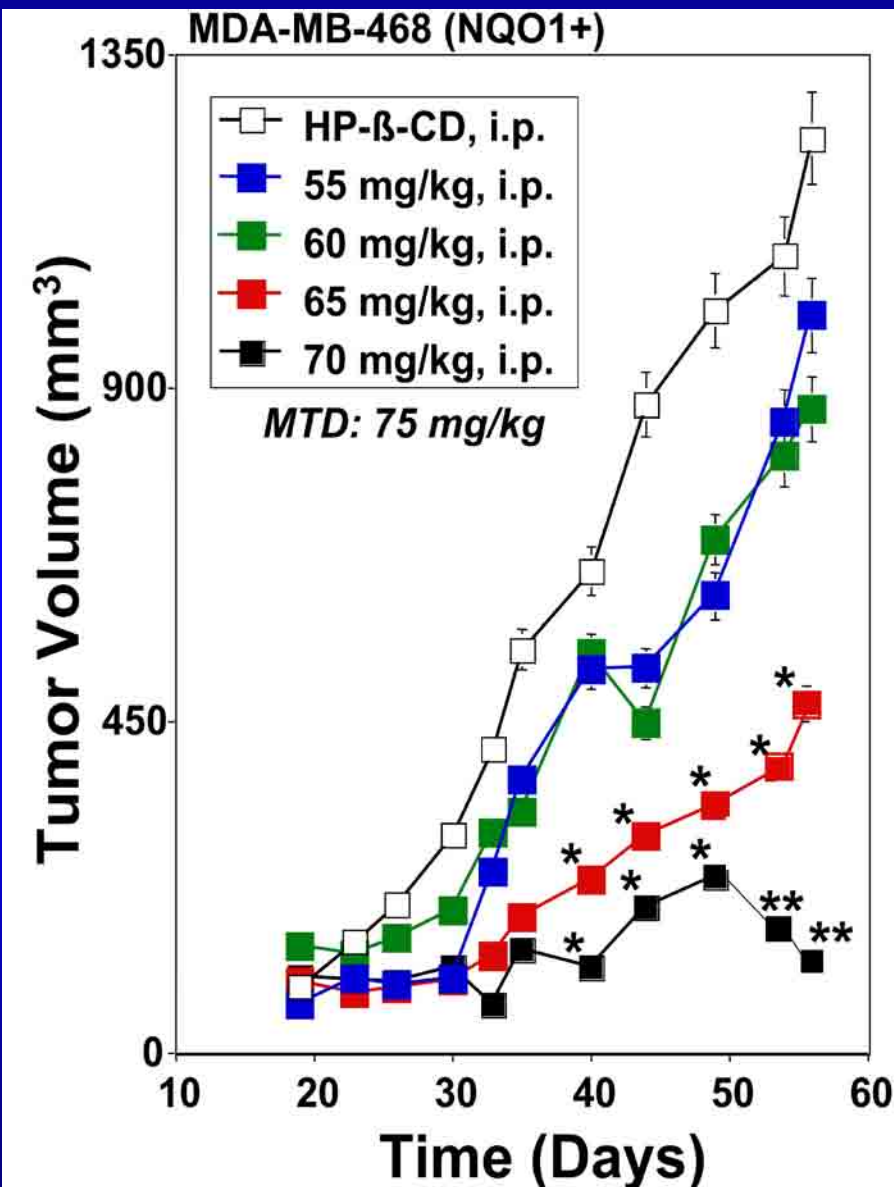


Scanning electron micrograph



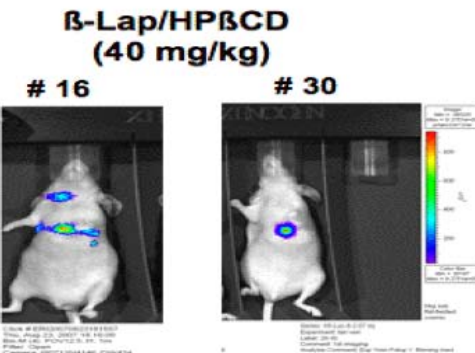
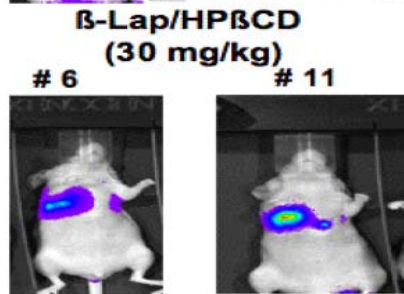
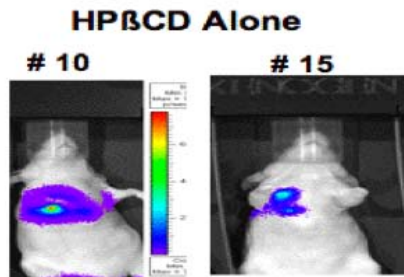
Release of Trypan blue over 4 days

HP- β -CD greatly improves β -lapachone antitumor efficacy



β -Lapachone antitumor responses using A549 cells improve using an orthotopic model

Day 0



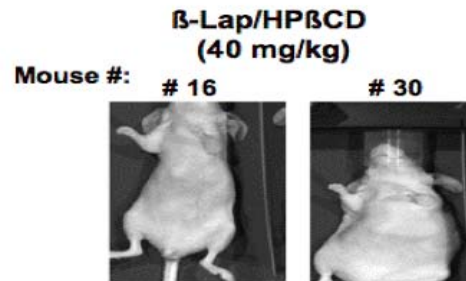
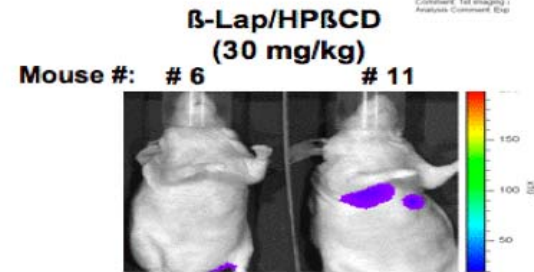
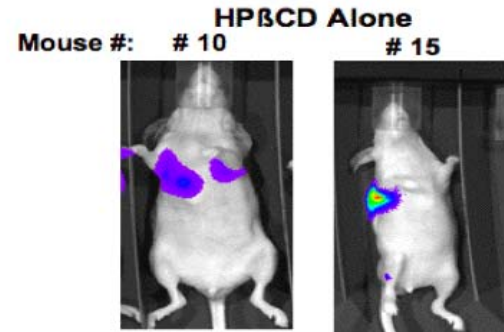
Avg RLU
(x 10⁷)

2.7

2.6

2.4

Day: 11



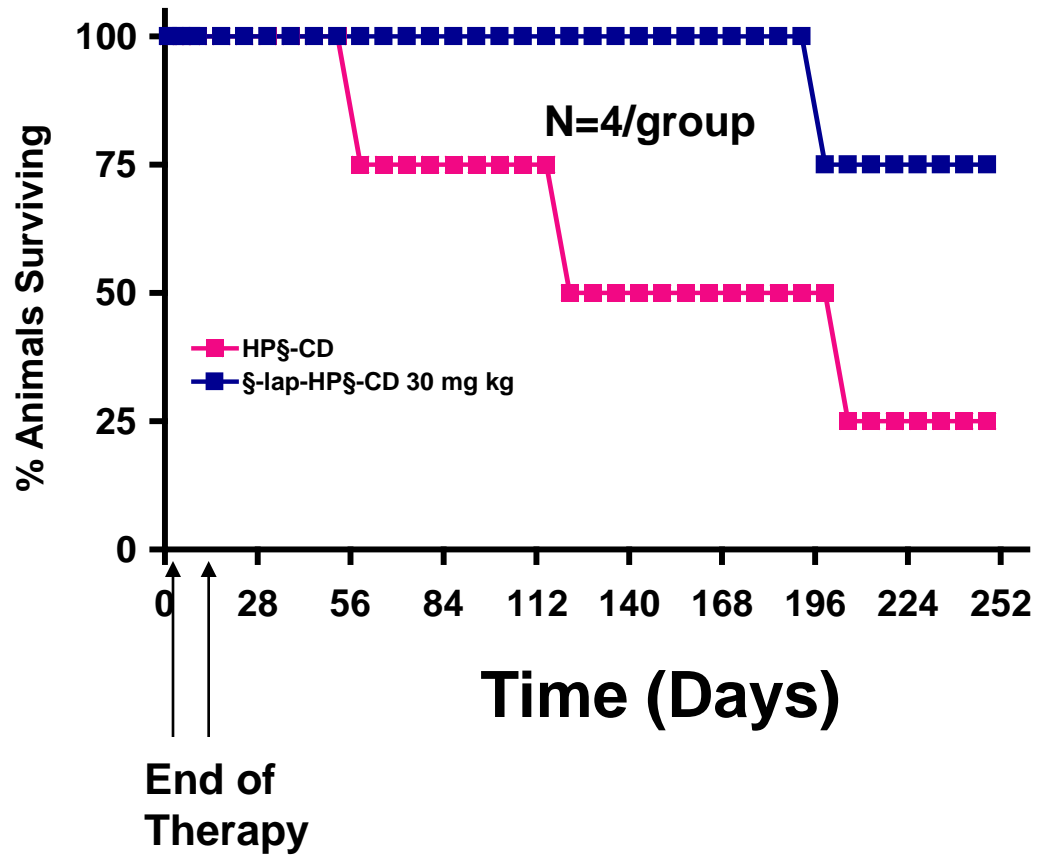
Avg. RLU
(x 10⁷)

10.0

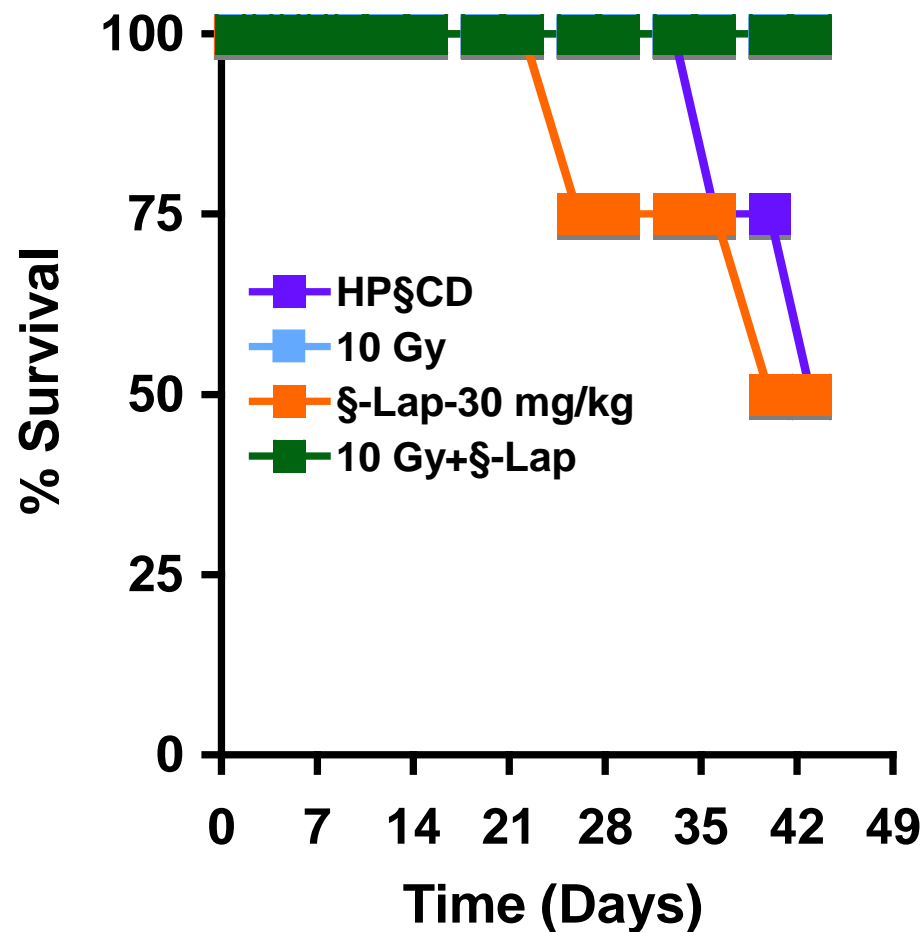
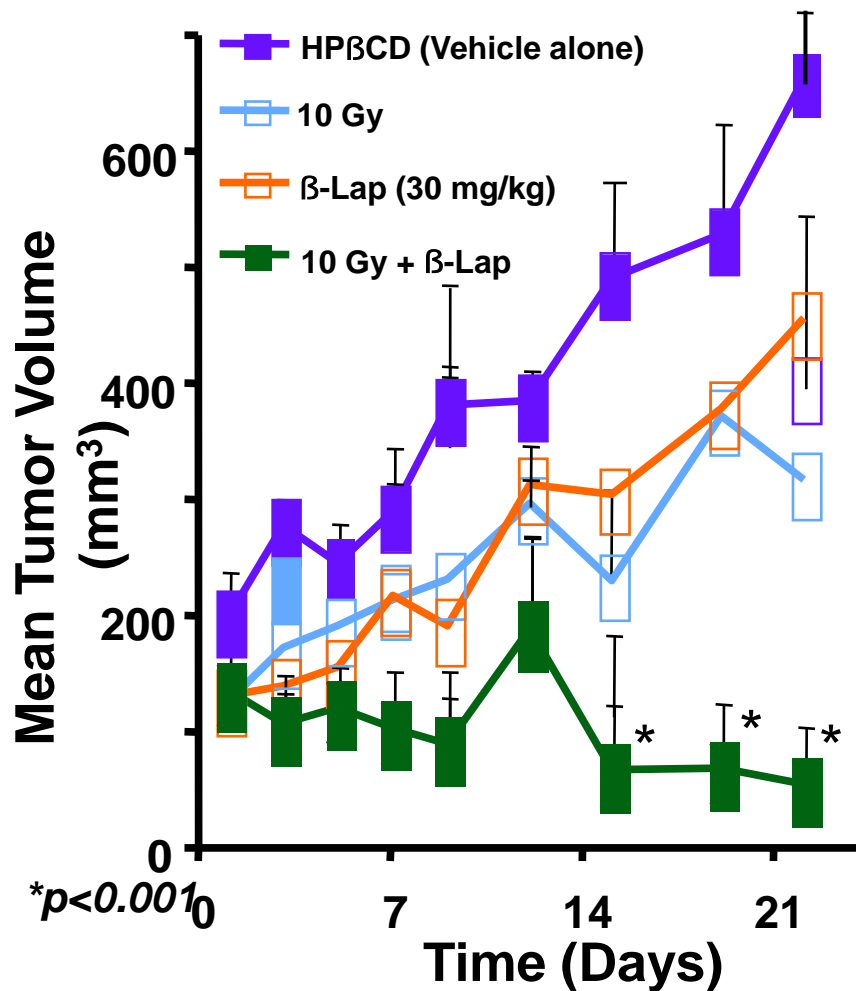
0.8

0.04

A549-Luc orthotopic model survival

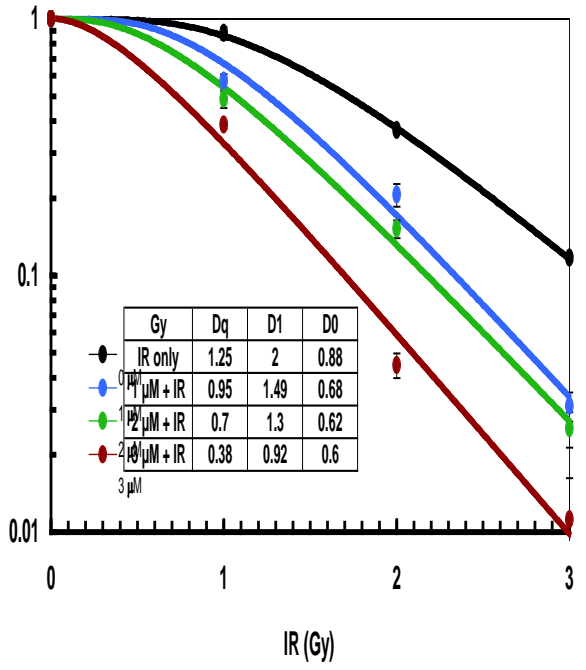


β -Lapachone is a potent radiosensitizer

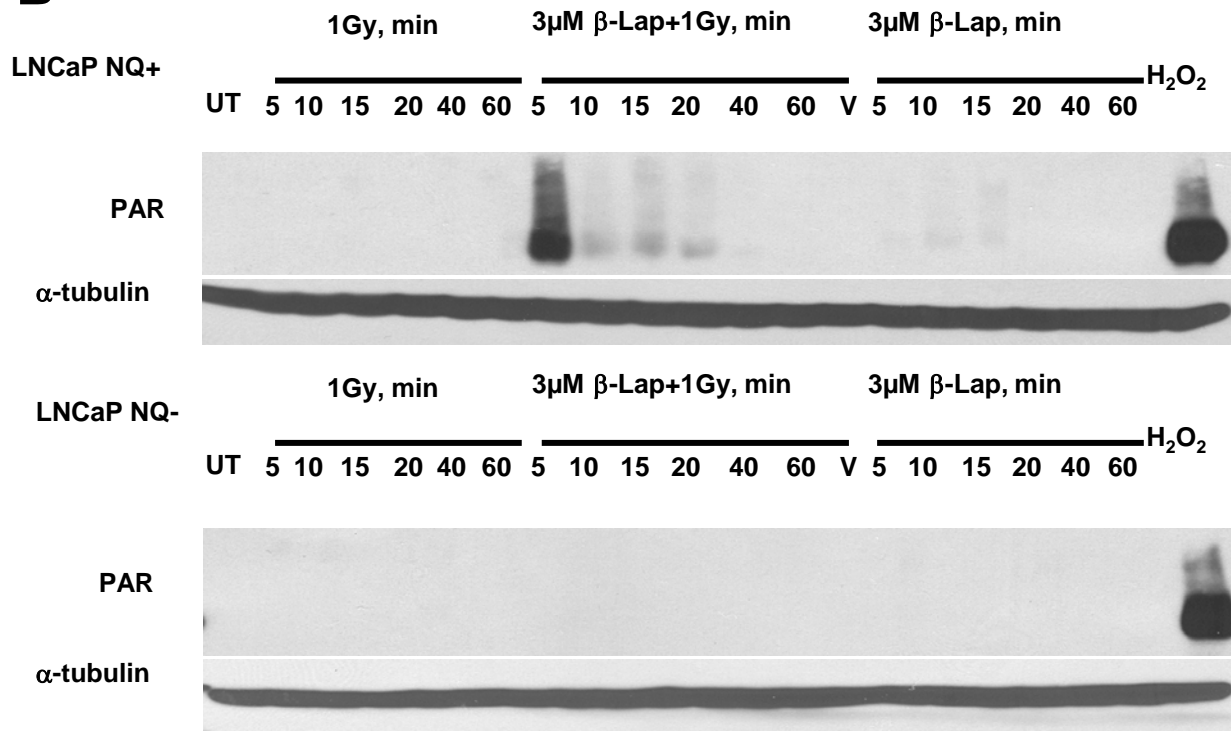


A549 s.c. xenografts

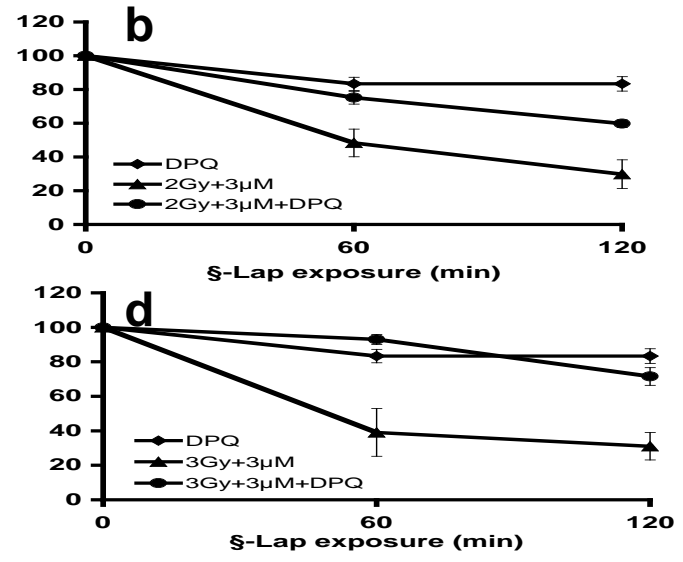
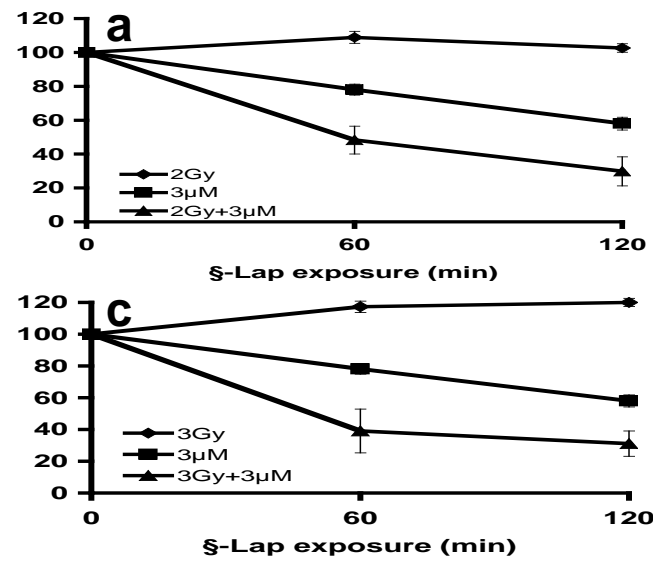
A



B



C



Acknowledgements

Collaborators

At UTSW:

J. Gao, PhD

J. Minna, Ph.D.

M. Peyton, Ph.D.

W. Bornmann, PhD

C. Thompson, MD, PhD

C. Distelhorst, MD

G. Dubyak, PhD

K. Brown, PhD

S. Ingalls



**ERIK A. BEY
MELISSA BENTLE
KATE REINICKE**