ISTANBUL KÜLTÜR UNIVERSITY

OMICS International

International Conference on
Prostate Cancer
June 22-24, 2015 Florida, USA

Epibrassinolide Induced Apoptosis via Endoplasmic Reticulum Stress Regardless of p53 Expression in Prostate Cancer Cells

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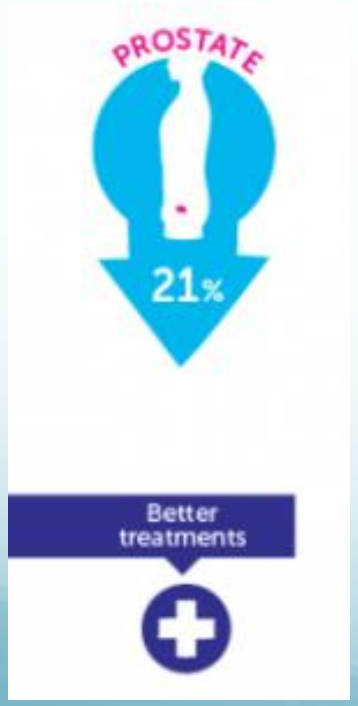
Home > Cancer Types > Prostate Canc

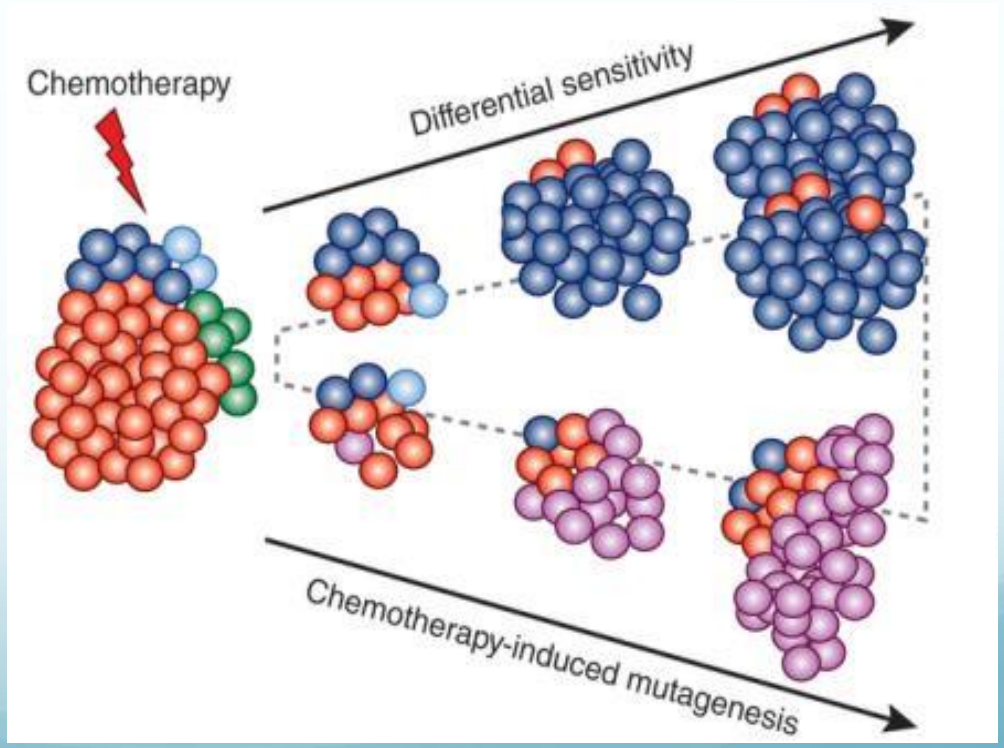
PROSTATE CANCER

Incidence and Mortality

Estimated new cases and deaths from prostate cancer in the United States in 2015:[15][A Snapshot of Prostate Cancer]

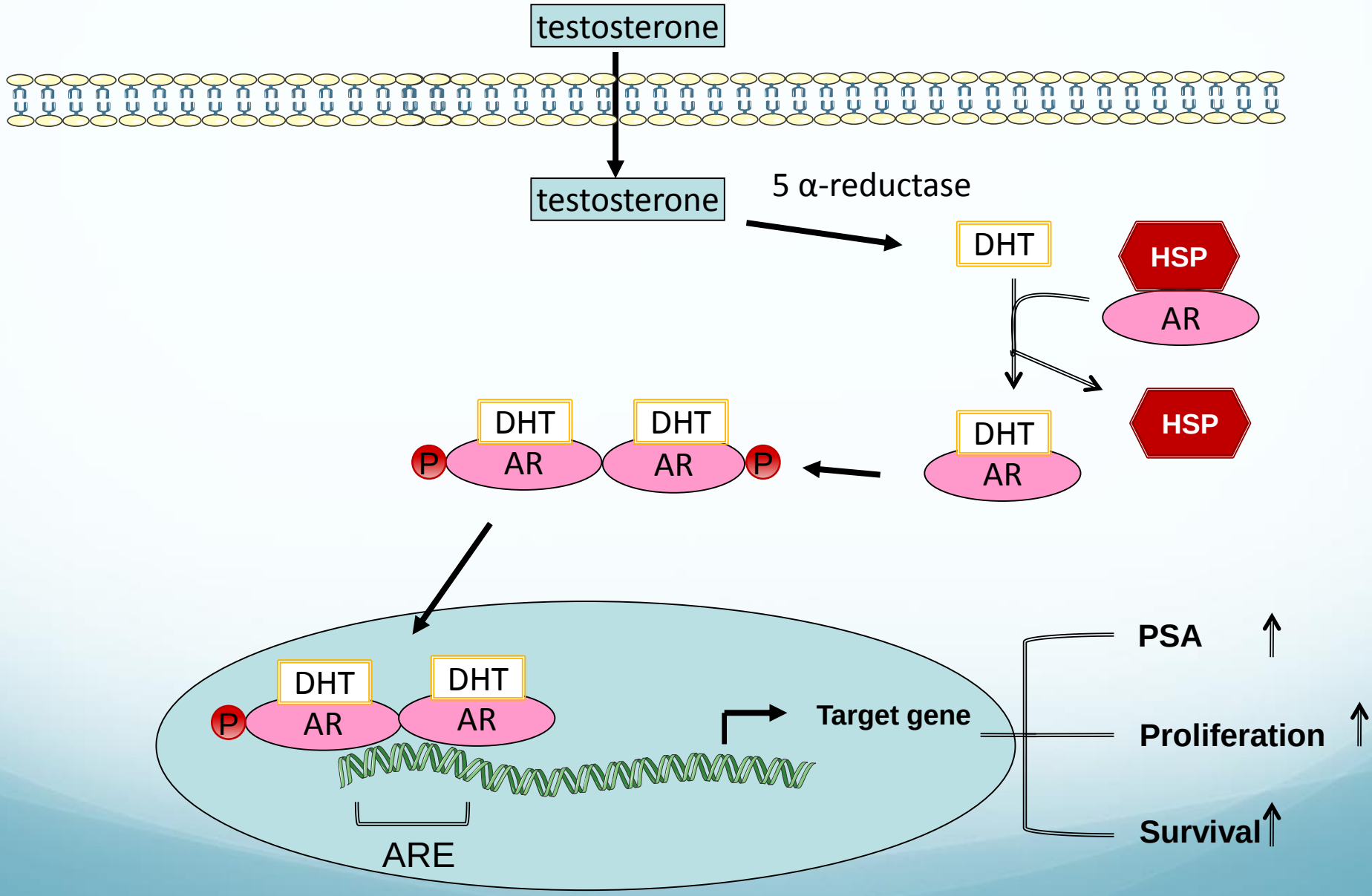
- New cases: 220,800.
- Deaths: 27,540.





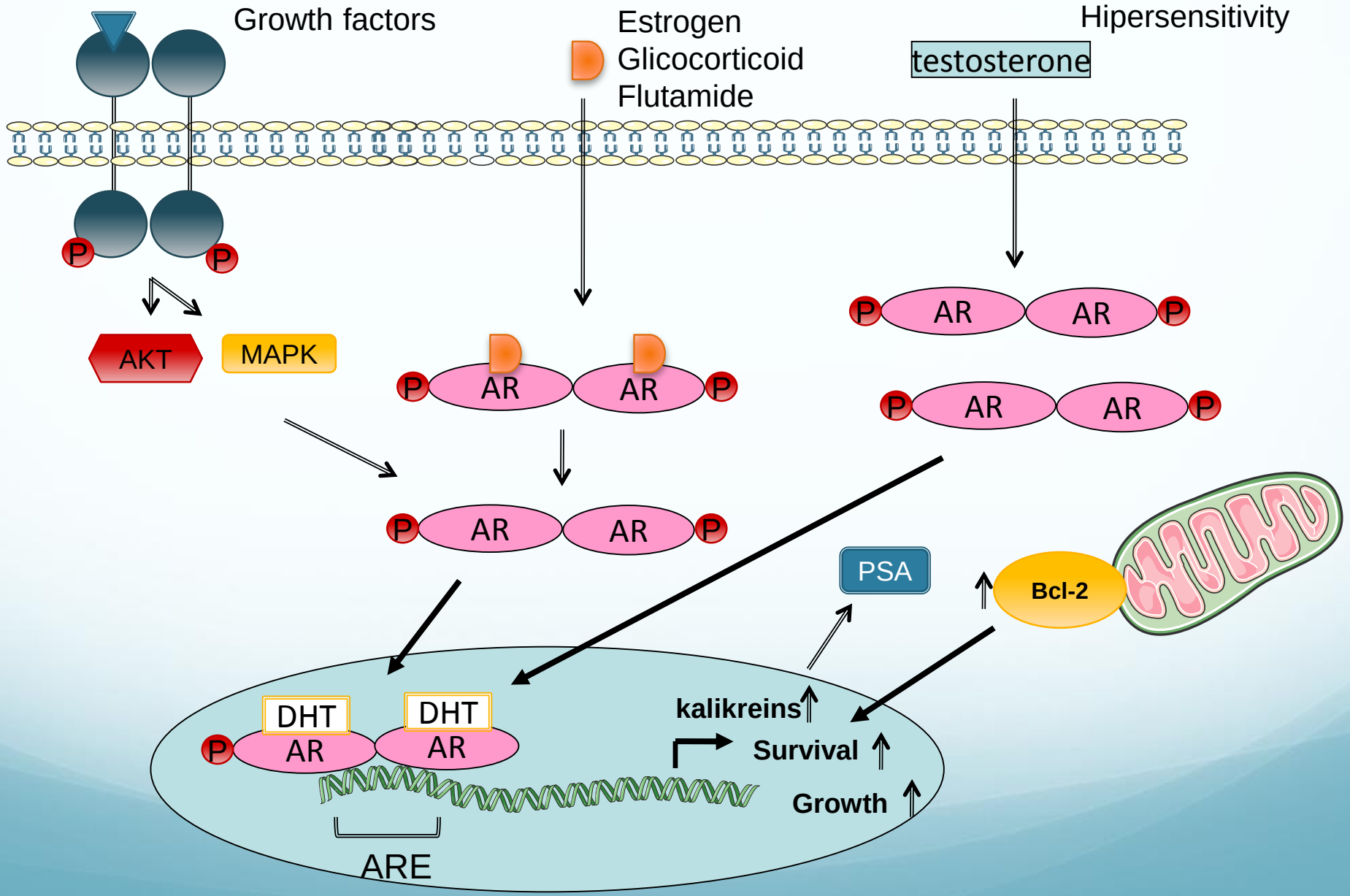
http://www.cancer.gov/types/prostate/hp/prostate-treatment-pdq#link/_1679_toc

ROLE OF ANDROGEN RECEPTOR (AR)



DHT: Dihydrotestosterone
PSA: Prostate spesific antigen

OTHER FACTORS



Molecular markers

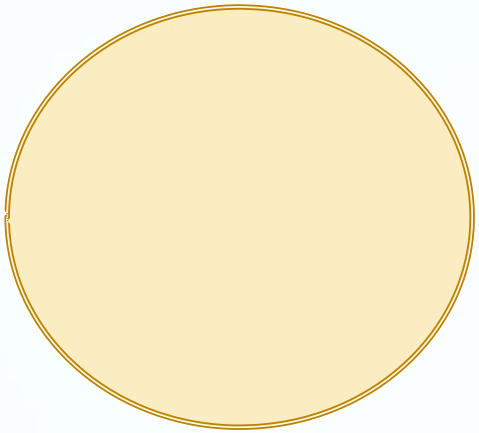
A number of tumor markers have been reported to be associated with the outcome of prostate cancer patients.[21,22] These include:

- Markers of apoptosis including Bcl-2, Bax.
- Markers of proliferation rate, such as Ki67.
- p53 mutation or expression.
- p27.
- E-cadherin.
- Microvessel density.
- DNA ploidy.
- p16.
- PTEN gene hypermethylation and allelic losses.

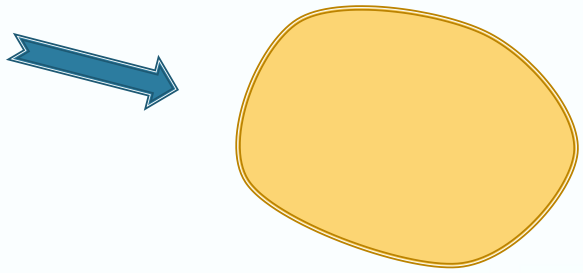
Related to APOPTOSIS

http://www.cancer.gov/types/prostate/hp/prostate-treatment-pdq#link/_1679_toc

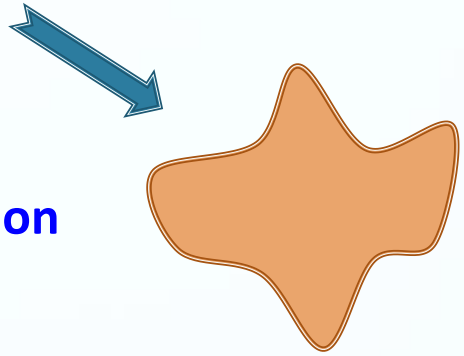
APOPTOSIS



Normal Cell



Cell shrinkage
Chromatin condensation

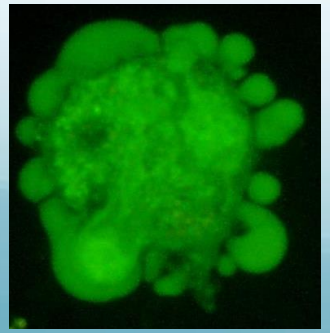


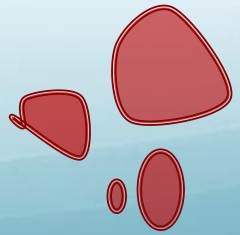
Membrane blebbing

Mitochondrial membrane potential loss
Activation of caspases
Protein degradation
DNA fragmentation



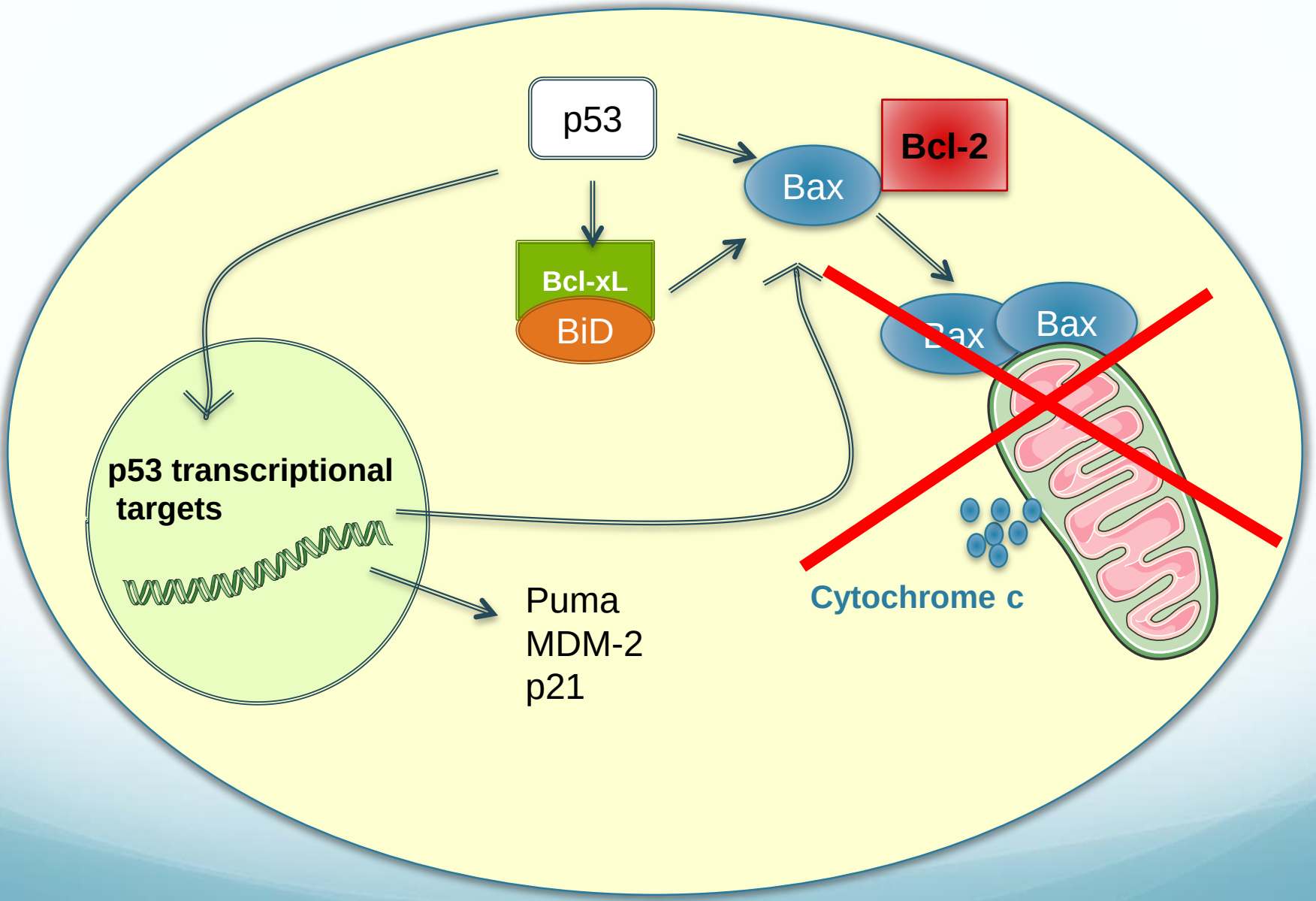
Nuclear collapse

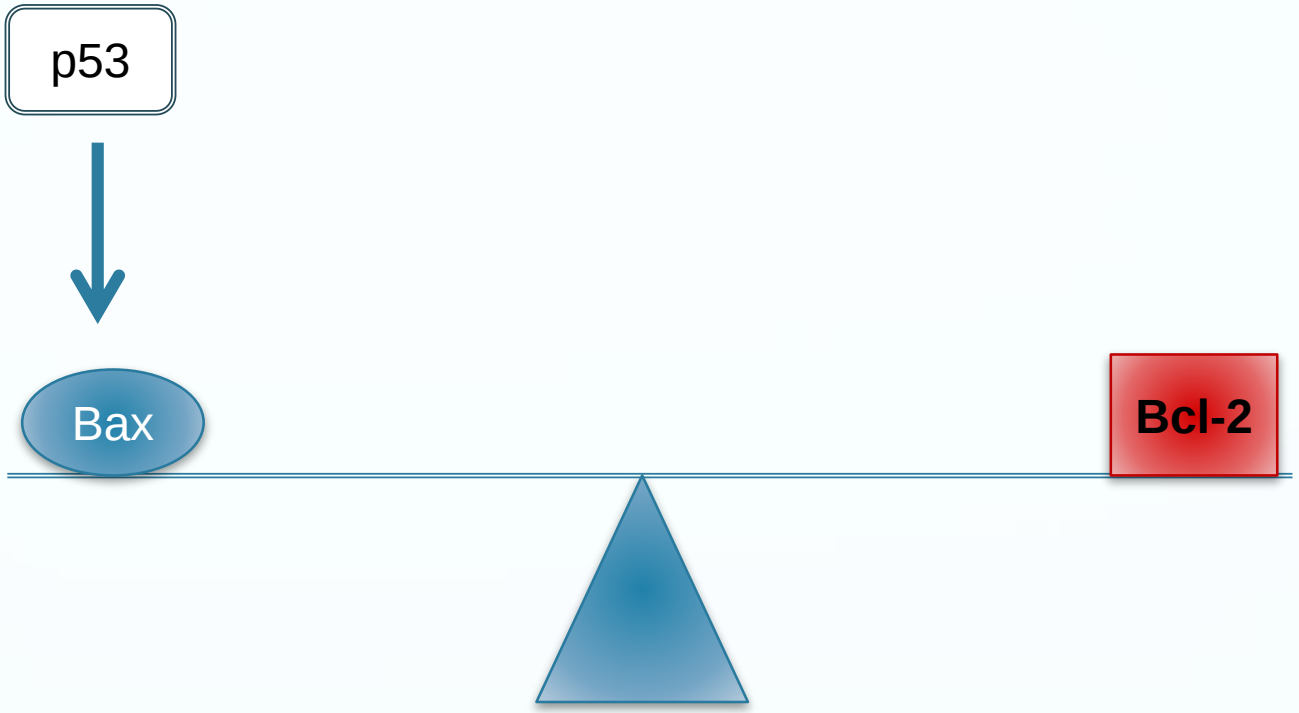


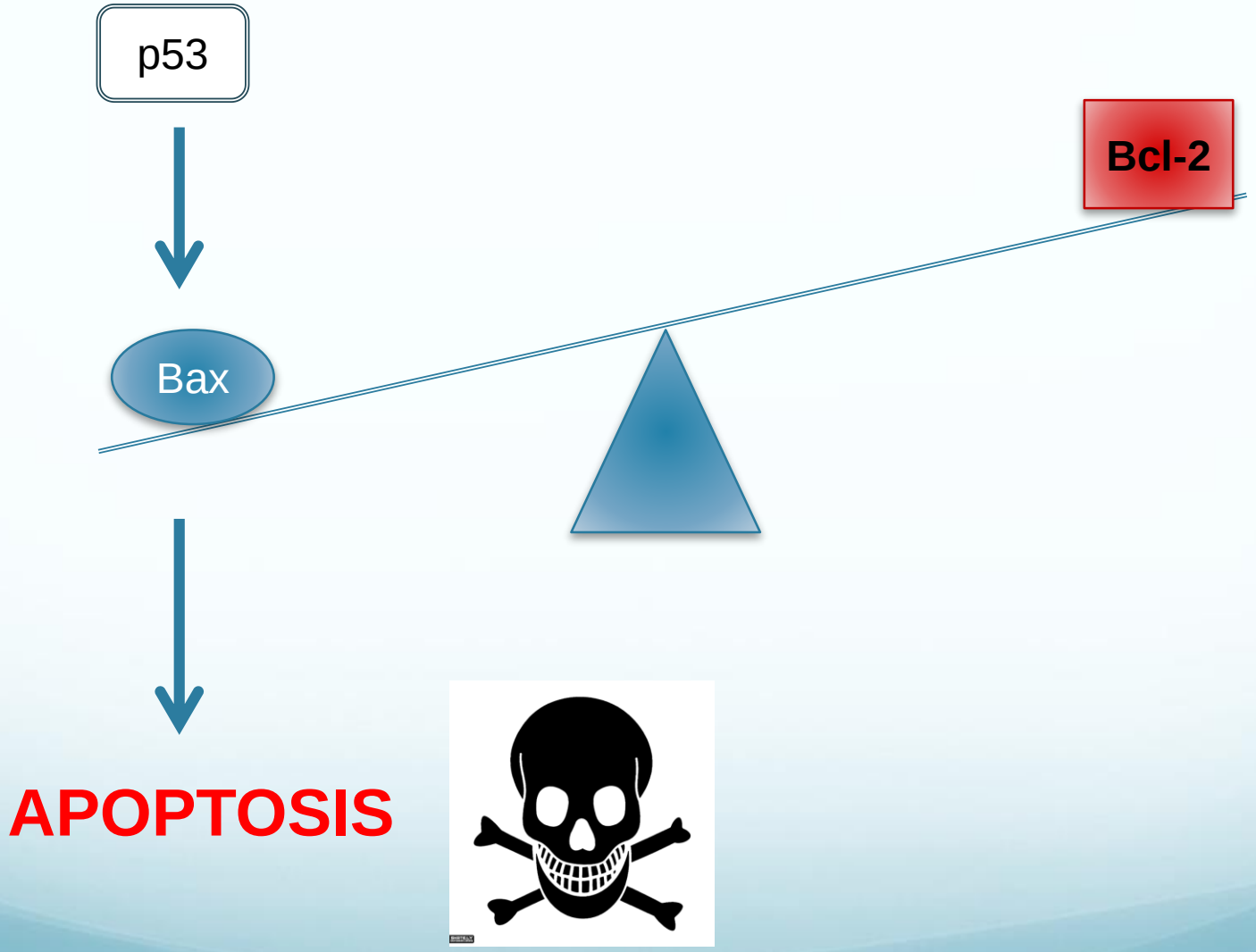


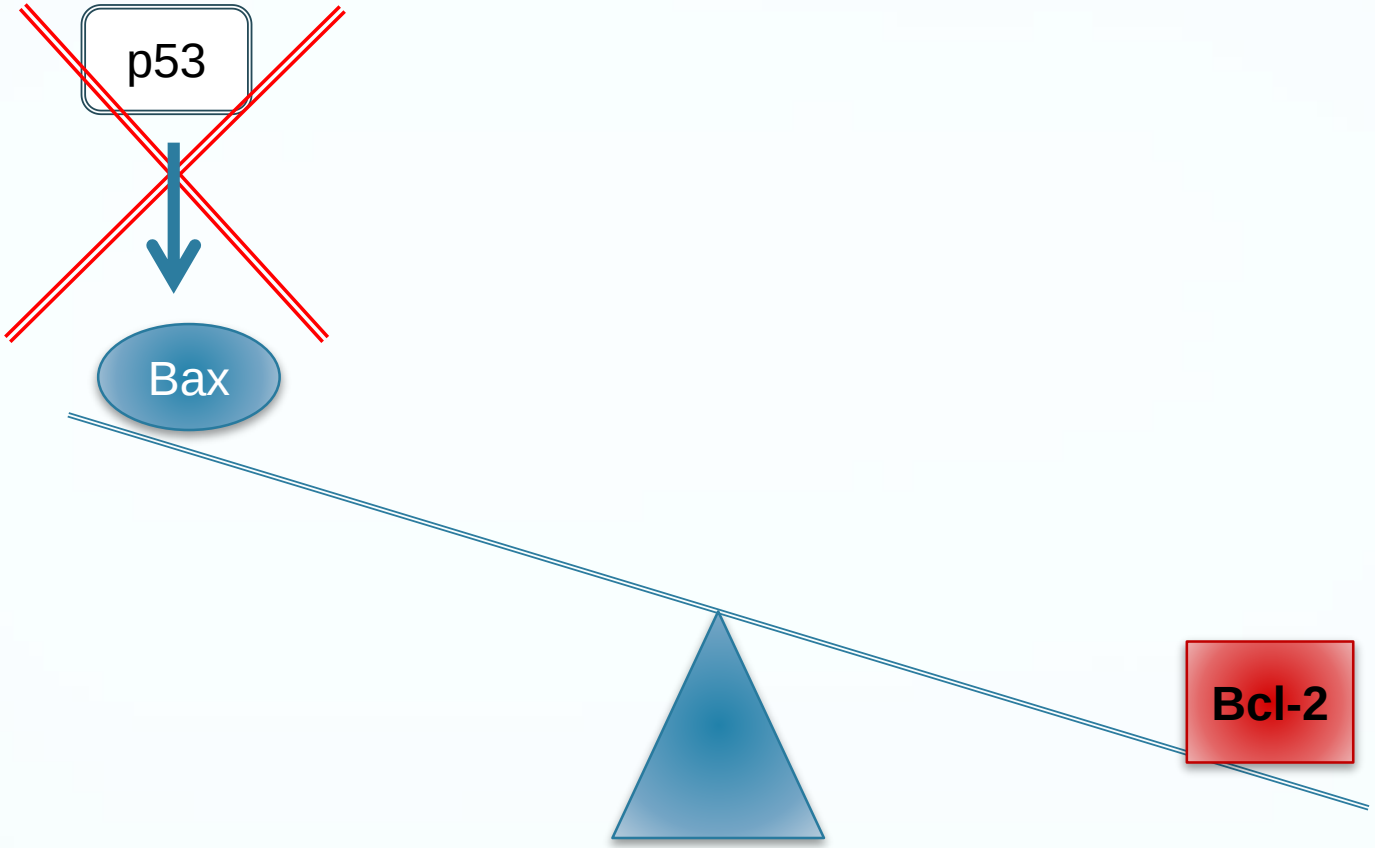
Apoptotic bodies

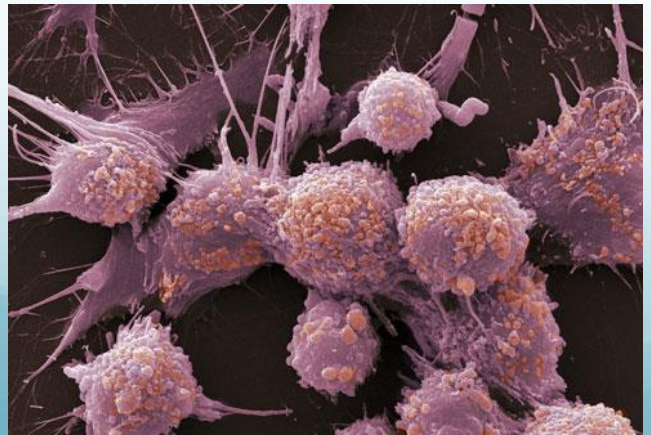
p53 ROLE DURING APOPTOSIS




p53
Bax
Bcl-2


p53
Bcl-2
Bax
APOPTOSIS





CANCER CELL SURVIVAL
CHEMO-RESISTANCE

The search for a compound for the cure of advanced prostate cancer:

- **without toxic effect on normal epithelial cells**
- **effective on both androgen receptor (+) and (-) cells**
- **miminum side effects**
- **easy to deliver**

A HUMAN STEROID-LİKE NATURAL AGENT



EPIBRASSINOLIDE

Epibrassinolide (EBR):

- A member of **brassinosteroids** (plant growth factor family)
- Anti-proliferative activity in cancer cells (MCF-7) *(Malikova et al., Phytochemistry 2008)*
- Cell cycle arrest, mitotic arrest

(Steigerova et al., Steroids, 2013)

Nuclear hormone receptor blocker?

- EBR induces apoptosis regardless of AR expression in prostate cancer cells without affecting normal prostate epithelial cells ***(Obakan et. al, Aminoacids, 2014)***
- EBR induces p53-independent apoptosis in prostate cancer cells ***(Obakan et. al, The Prostate, 2014)***

EPIBRASSİNOLİDE AS AN APOPTOTIC INDUCER IN BOTH AR+ AND AR- PROSTATE CANCER CELLS W/O AFFECTING NORMAL PROSTATE EPITHELIAL CELLS

Amino Acids
DOI 10.1007/s00726-013-1574-1

ORIGINAL ARTICLE

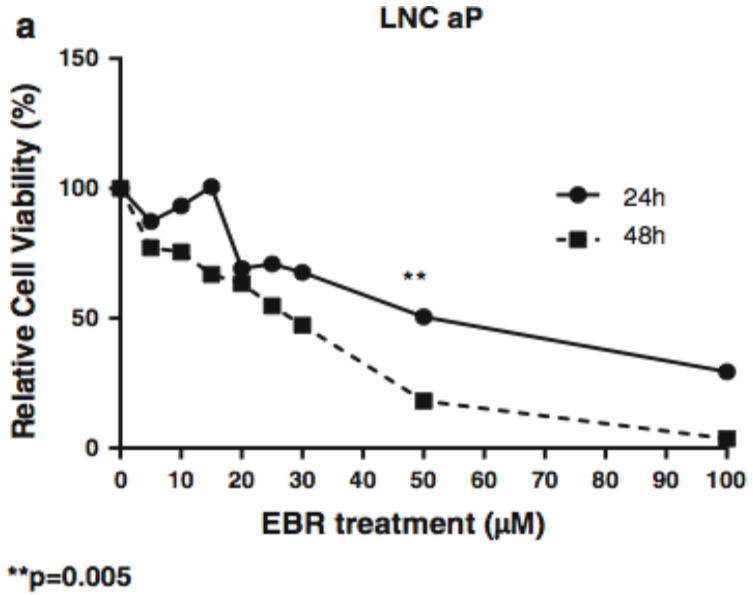
Activation of polyamine catabolic enzymes involved in diverse responses against epibrassinolide-induced apoptosis in LNCaP and DU145 prostate cancer cell lines

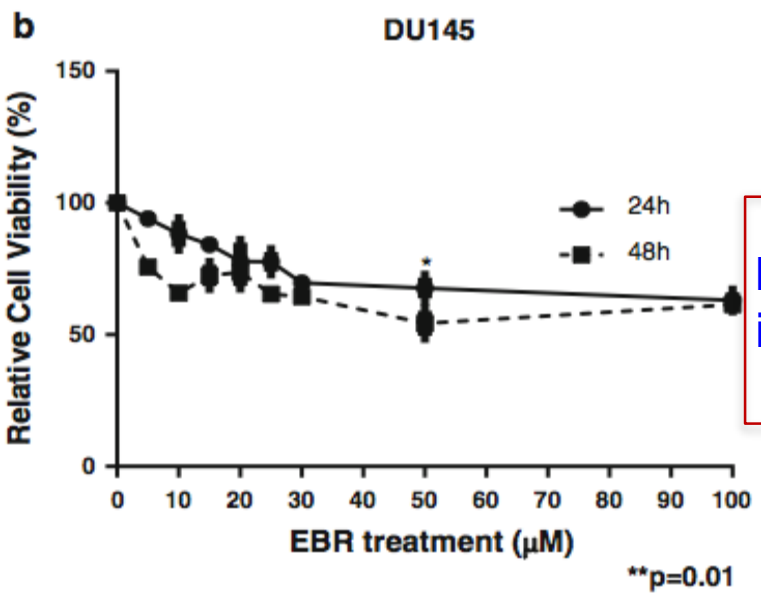
AR –
p53 -

AR +
p53 +

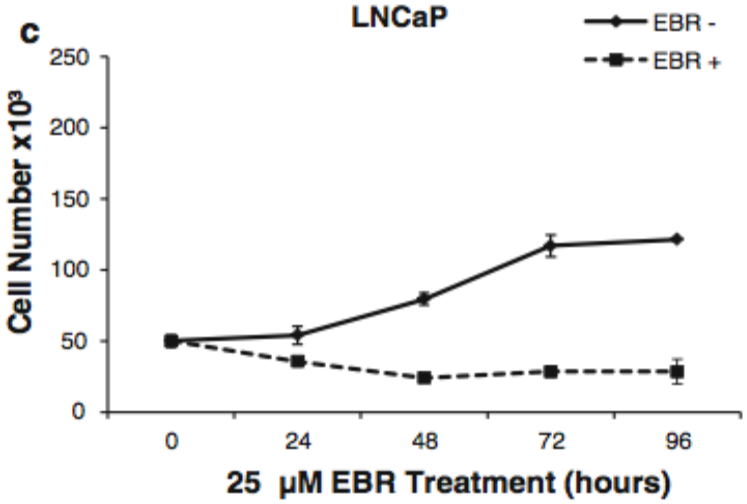
Pinar Obakan · Elif Damla Arisan · Annarica Calcabrini · Enzo Agostinelli · Şehnaz Bolkent · Narçin Palavan-Unsal

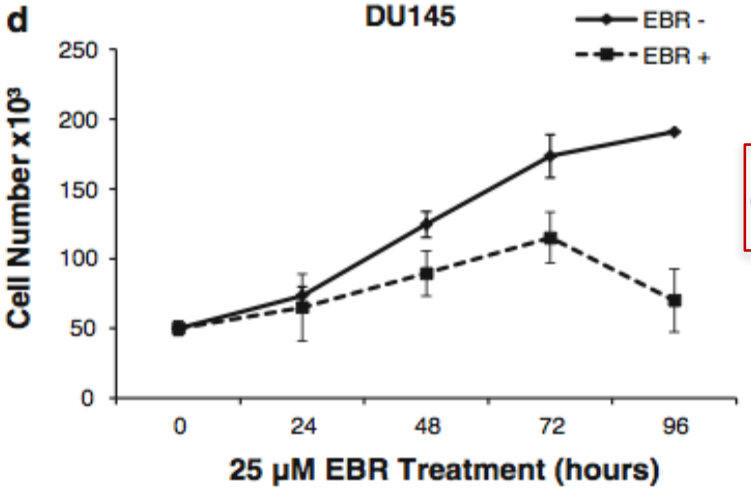
Received: 22 May 2013 / Accepted: 31 July 2013
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Decrease in cell viability





Cytotoxic

Fig. 1 EBR decreased cell viability in dose- and time-dependent manner in LNCaP and DU145 prostate cancer cells. **a**, **b** 1×10^4 cells were seeded into a 96-well plate and treated with EBR (0–100 μM) for 24 and 48 h. Cell viability loss was determined by MTT assay. Values represent the mean ± SD of three different experiments with at least five replicates. **c**, **d** Cells were seeded into a 96-well plate and treated with EBR (25 μM) for 24, 48, 72 and 96 h. Cell proliferation was determined by trypan blue dye exclusion method. Values represent the mean ± SD of three different experiments with at least five replicates

Obakan et. al, *Amino acids*, 2013

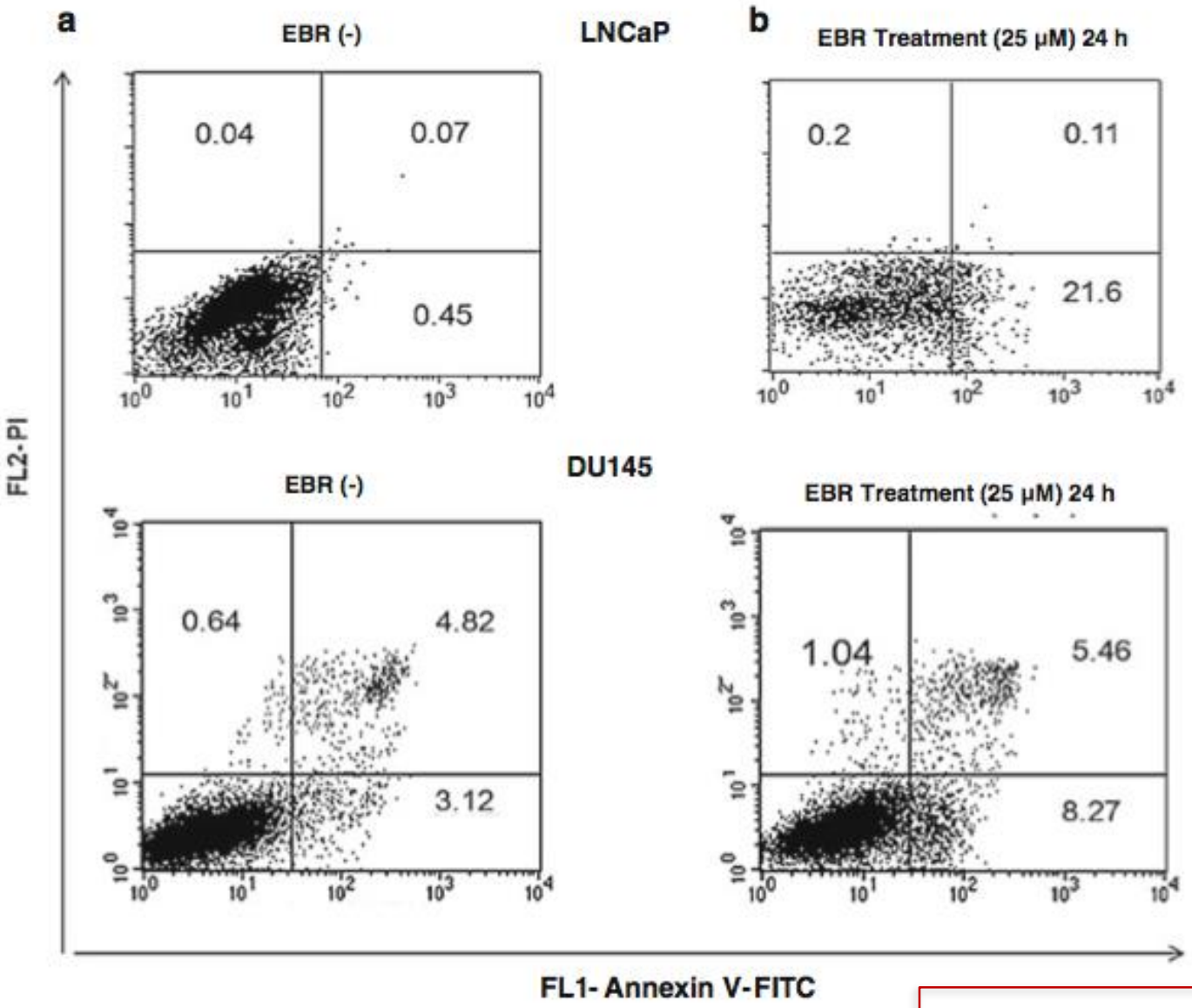


Fig. 3 EBR induced apoptosis in LNCaP and DU145 prostate cancer cells. 3×10^5 cells were seeded into a six-well plate and treated with EBR for 24 h. Annexin V–PI staining was performed to determine apoptotic cell populations. **a** Untreated cells, **b** EBR treated (25 µM). Fluorescence signals from Annexin V–FITC and from PI are reported on the *x*-axis and *y*-axis, respectively. Numbers shown in the four quadrants represent the percentage of viable (*lower left*), necrotic (*upper left*), early apoptotic (*lower right*) and late apoptotic (*upper right*) cells. Data shown are obtained from a representative experiment

Increase in apoptotic population

Obakan et. al, *Amino acids*, 2013

Apoptosis induction

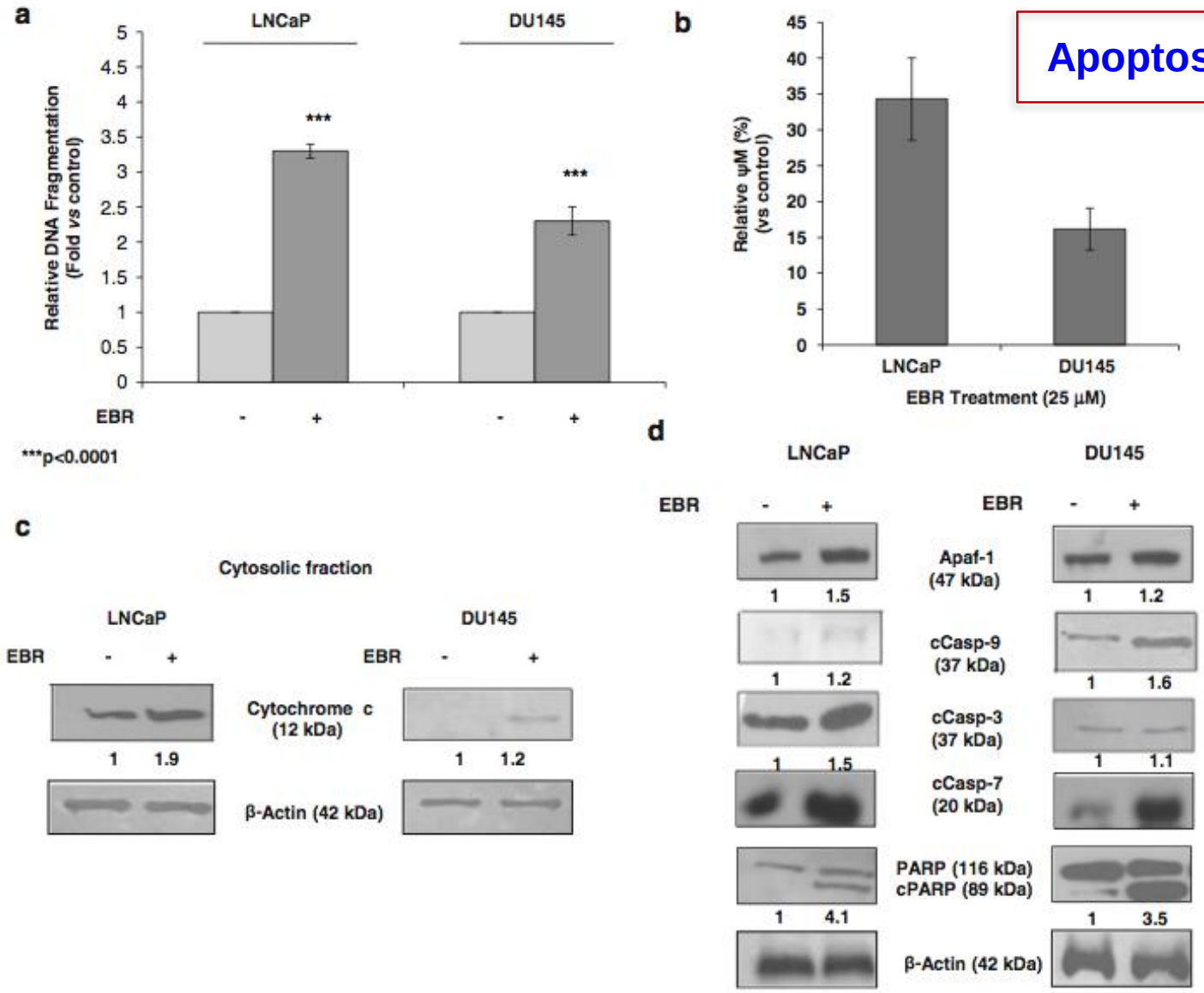
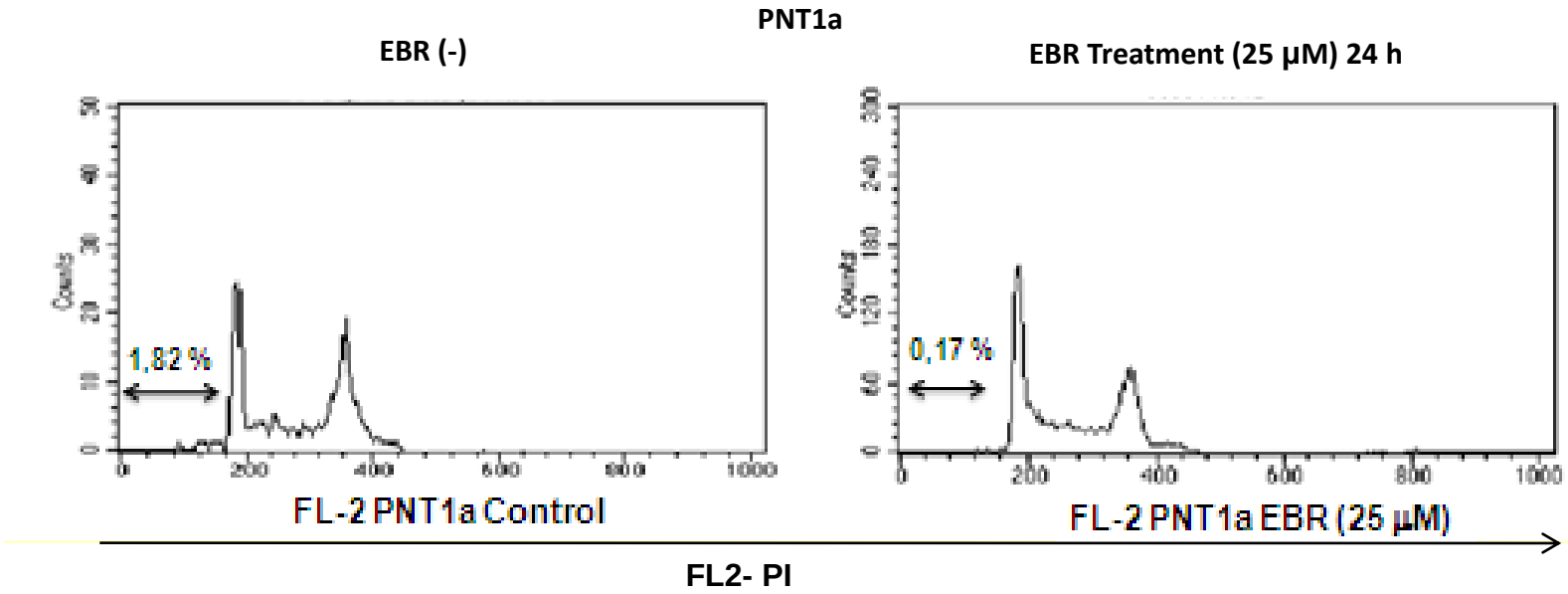
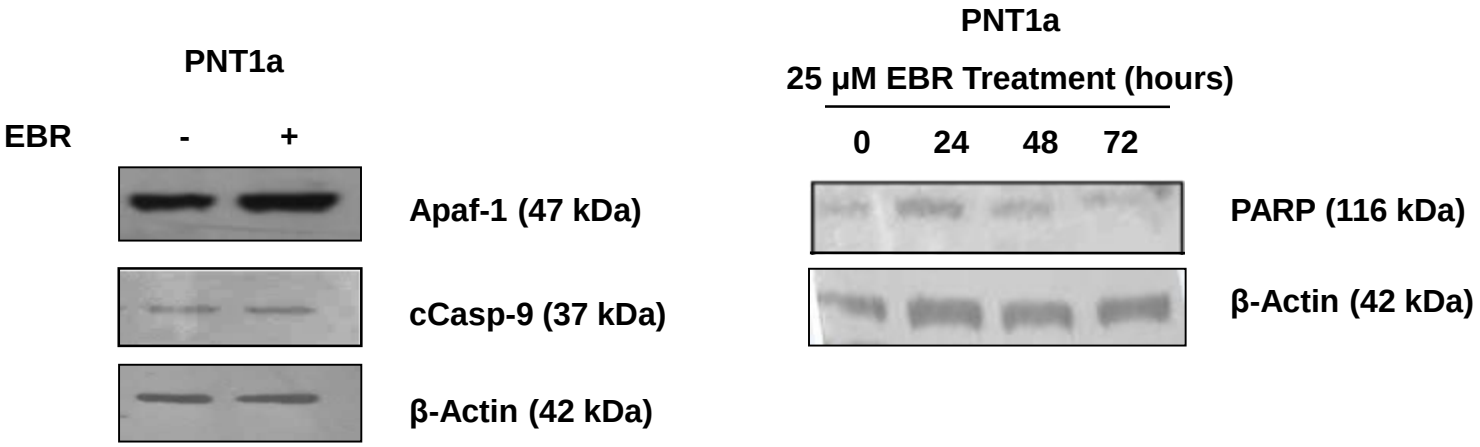


Fig. 4 EBR-induced apoptosis was mitochondria-mediated and caspase dependent. a Cells were treated for 24 h with EBR, and DNA fragmentation was determined with cell death ELİSA. b Relative $\Delta\psi m$ was evaluated after 24 h of EBR treatment with DiOC6 staining by fluorometric analysis (ex: 488 nm, em: 525 nm). c Following 24 h of EBR treatment, cytosolic proteins were isolated and separated in 12 % SDS gel, transferred onto PVDF membrane and blotted with anti-cytochrome c antibody. d Total proteins were isolated after EBR treatment and the expression profiles of Apaf-1, cleaved caspase-9, cleaved caspase-3, cleaved capase-7 and PARP were determined by immunoblotting using appropriate antibodies. β-actin was used as a loading control

Obakan et. al, *Amino acids*, 2013

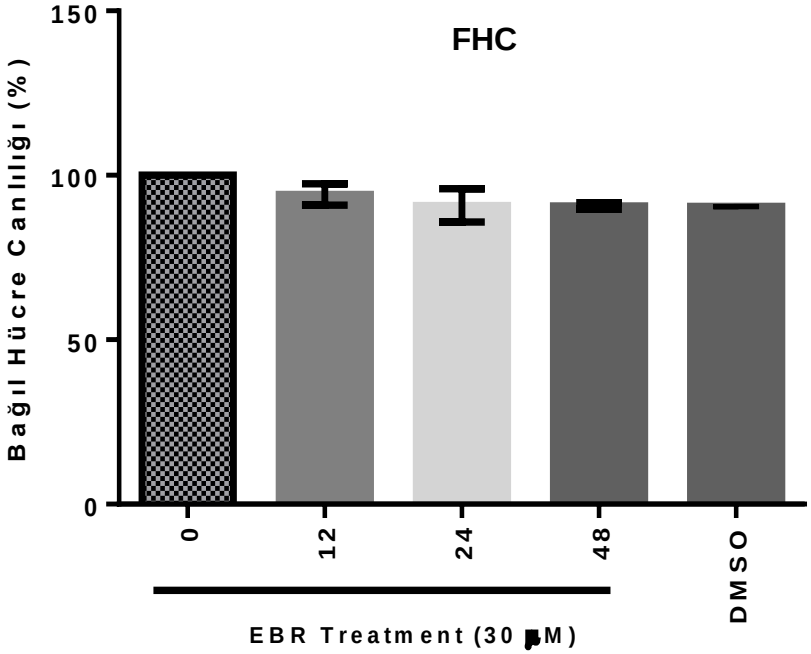
No apoptotic effect in normal prostatic epithelial cells

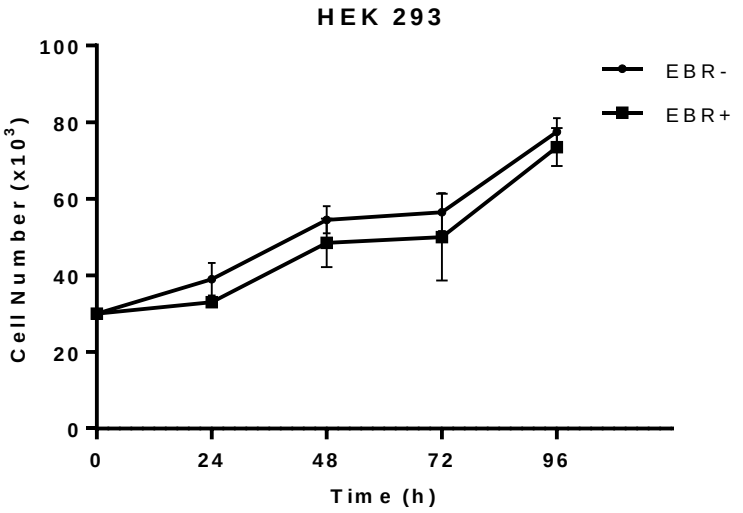




Obakan et. al, *Amino acids*, 2013

Normal epithelial cells are not affected by EBR treatment





The Prostate 74:1622–1633 (2014)

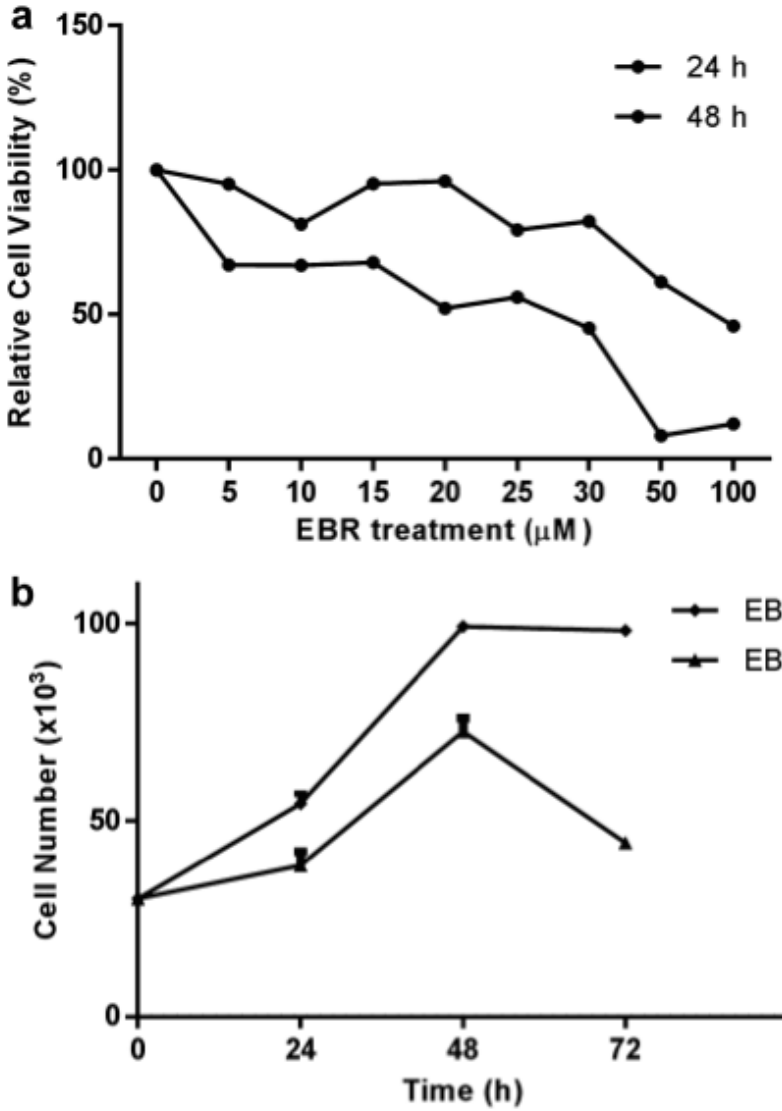
Epibrassinolide-Induced Apoptosis Regardless of p53 Expression via Activating Polyamine Catabolic Machinery, a Common Target for Androgen Sensitive and Insensitive Prostate Cancer Cells

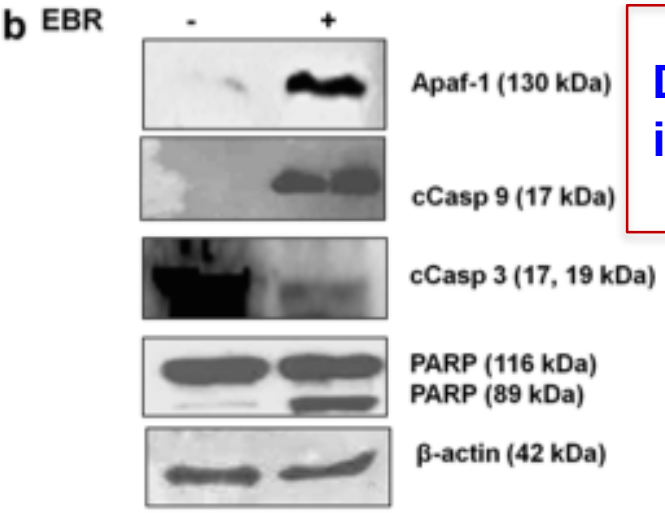
Pinar Obakan,* Elif Damla Arisan, Ajda Coker-Gurkan, and Narcin Palavan-Unsal

Department of Molecular Biology and Genetics, Istanbul Kultur University, Atakoy Campus, Bakirkoy, Istanbul, Turkey

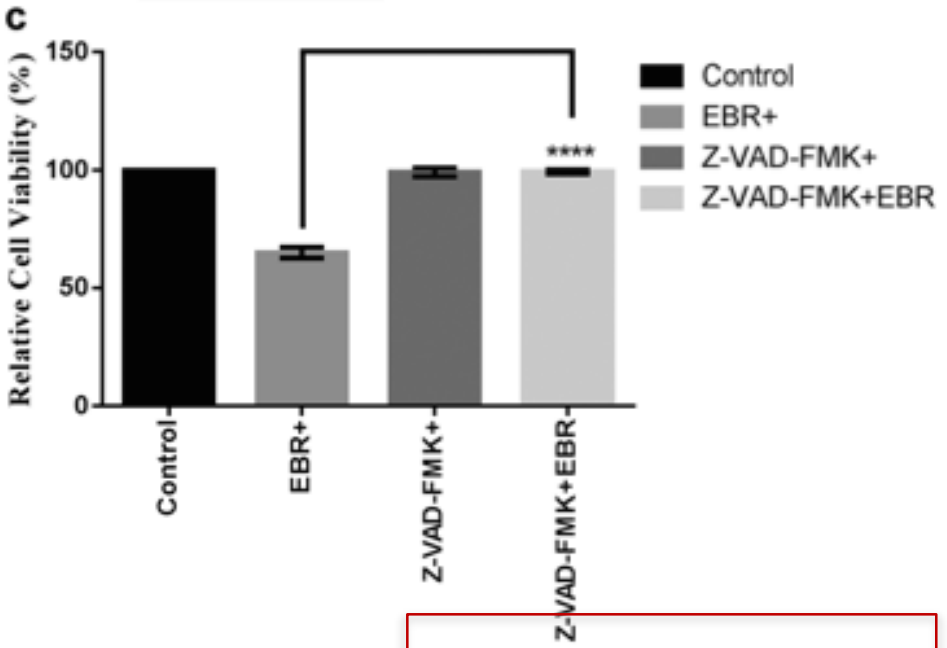
PC3 prostate cancer cells and PC3 p53+
AR –
p53 -

Fig. 1. EBR decreased cell viability and survival in PC3 prostate cancer cells. **a**: Cells were seeded into a 96-well plate and treated with EBR (0–100 μM) for 24 and 48 hr. Cell viability loss was determined by MTT assay. **b**: Cells were seeded into a six-well plate and treated with EBR (25 μM) for 24, 48, 72 and 96 hr. Cell proliferation was determined by trypan blue dye exclusion method.





Decrease in cell viability

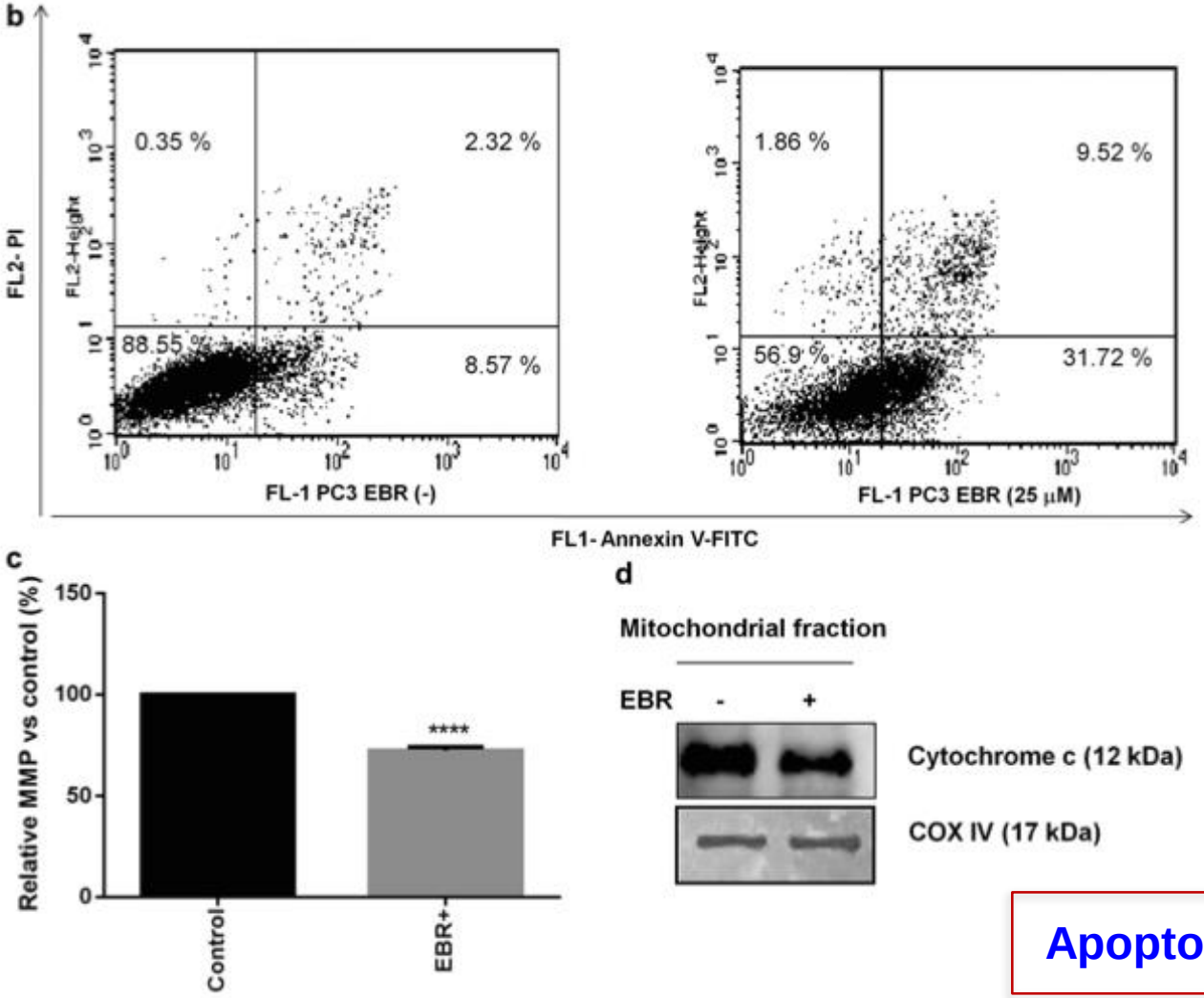


Apoptosis induction

Fig. 3. EBR modulated Bcl-2 family proteins expressions and induced caspase-dependent apoptosis. **a**: Following 24 hr EBR treatment, total proteins were isolated and separated in 12% SDS gel, transferred onto PVDF membranes and blotted with Puma, Bak, Bax, Bim, Bax, Bcl-2, Mcl-1 antibodies. β-actin was used as a loading control. **b**: Total proteins were isolated after EBR treatment and the expression profiles of Apaf-1, cleaved caspase-9, cleaved caspase-3 and PARP were determined by immunoblotting using appropriate antibodies. β-actin was used as a loading control. **c**: Cells were treated with EBR (25 μM) for 24 hr. MTT cell viability assay was used to evaluate the caspase-dependent apoptotic induction with genereal caspase inhibitor (z-VAD-FMK).

Obakan et. al, *The Prostate*, 2014

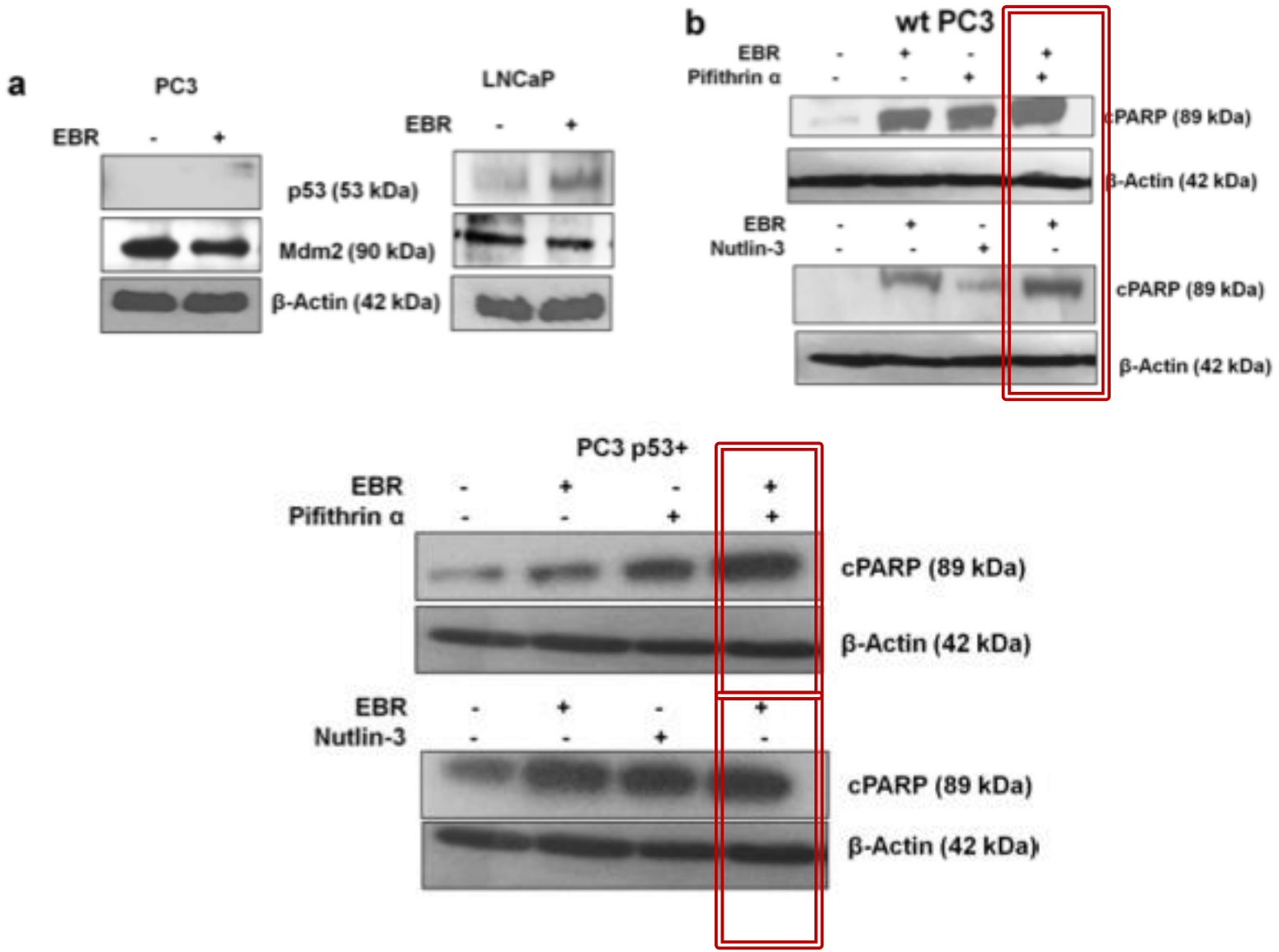
Obakan et. al, *The Prostate*, 2014



Apoptosis induction

Fig. 2. EBR induced apoptosis in PC3 cells. **a**: PC3 cells were seeded into a six-well plate and treated with EBR (25 μM) for 24, 48 and 72 hr. After ethanol fixation and PI (propidium iodide) staining, samples were analyzed by flow cytometry. Cell cycle distributions of cells were determined by FlowJosoftware. **b**: 3×10^5 cells were seeded into a six-well plate and treated with EBR for 24 hr. Annexin V–PI staining was performed to determine apoptotic cell populations. Fluorescence signals from Annexin V–FITC and from PI are reported on the x-axis and y-axis, respectively. Numbers shown in the four quadrants represent the percentage of viable (lower left), necrotic (upper left), early apoptotic (lower right) and late apoptotic (upper right) cells. Data shown are obtained from a representative experiment. **c**: Relative $\Delta\psi m$ was evaluated after 24 hr of EBR treatment with DiOC6 staining by fluorometric analysis (ex: 488 nm, em: 525 nm). **d**: Following 24 hr of EBR treatment, cytosolic and mitochondrial proteins were isolated and separated in 12% SDS gel, transferred onto PVDF membrane and blotted with anti-cytochrome c antibody. β-actin and COXIV were used as loading controls. C: cytoplasmic, M: mitochondrial.

No p53 effect in EBR-induced apoptosis



Obakan et. al, *The Prostate*, 2014

RESULTS:

- EBR induces apoptosis in AR expressing or non expressing prostate cancer cells.

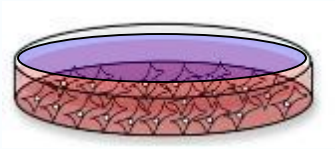
- EBR induces p53-independent apoptosis

WHAT IS THE EXACT MECHANISM OF EBR?

Stable isotope labelling by aminoacids in cell culture (SILAC)

Non-radioactive isotopes containing aminoacids are incorporated in newly synthesized proteins

EBR (-)



LIGHT Media

EBR (+)

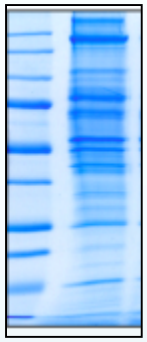


HEAVY Media

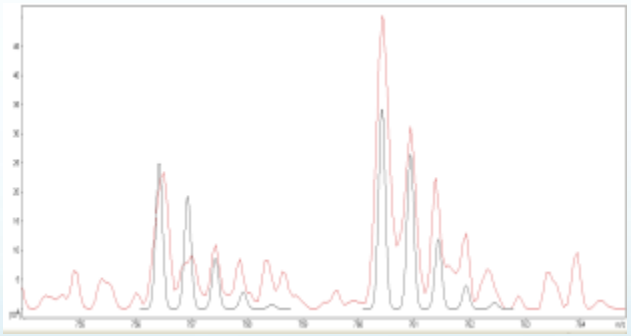
Protein Labelling



Protein Mix

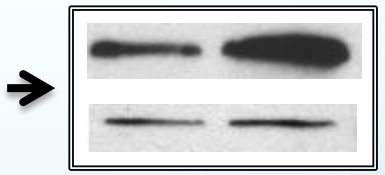


1D-SDS PAGE in gel digestion

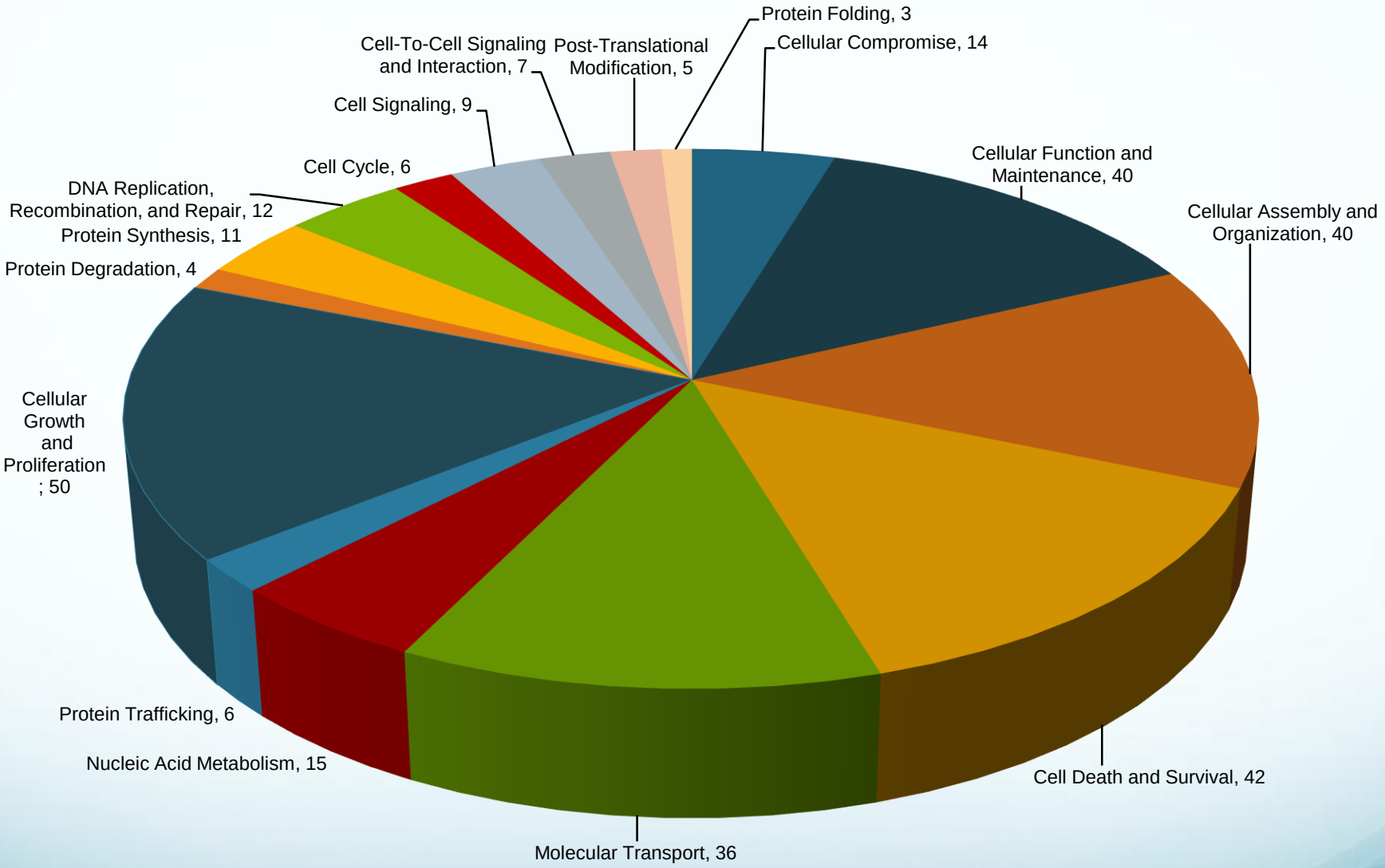


Protein quantification by LC-MS/MS

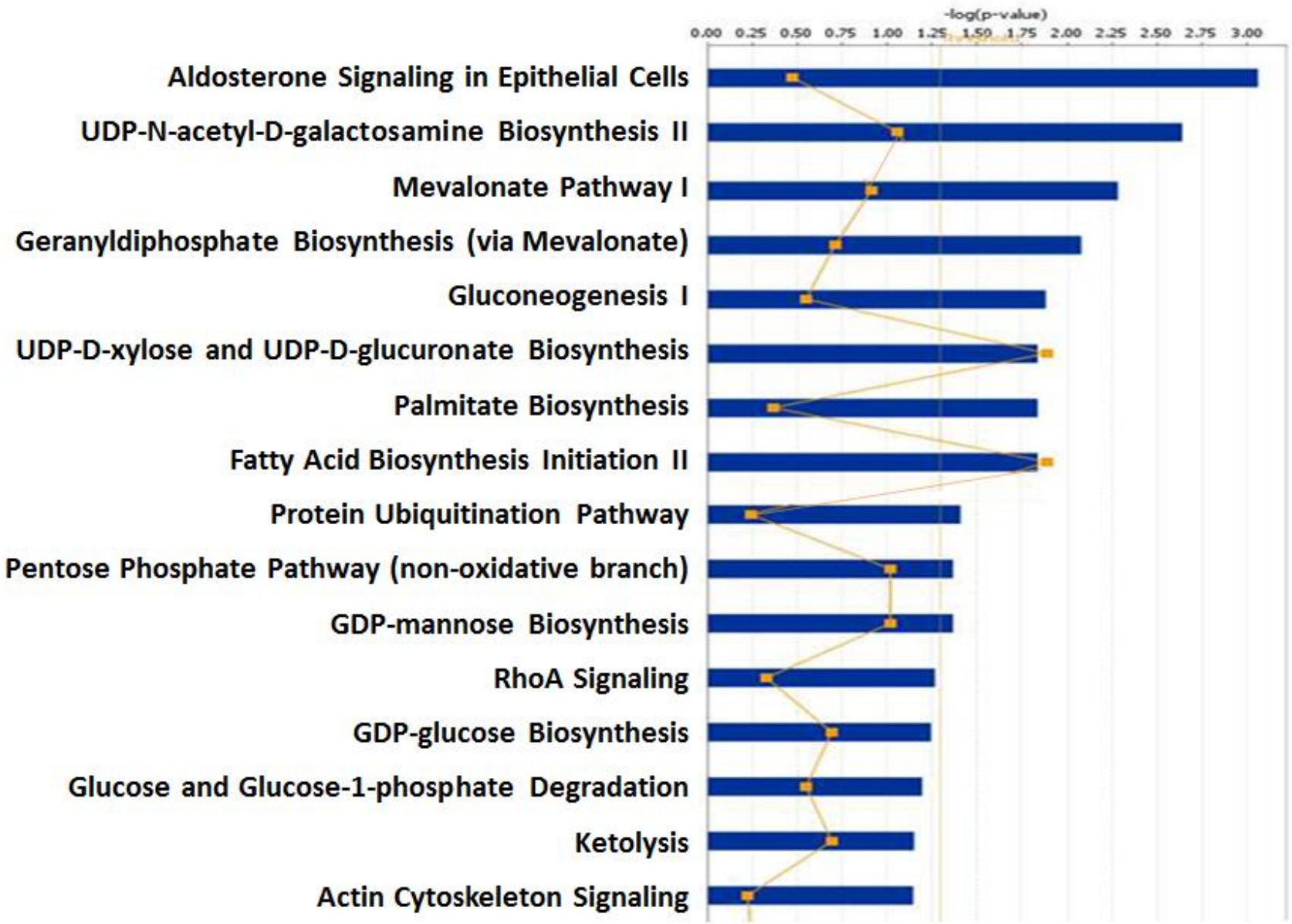
ER Stress



Validation by immunoblotting


Protein Folding, 3
Cellular Compromise, 14
Cell-To-Cell Signaling and Interaction, 7
Post-Translational Modification, 5
Cell Signaling, 9
Cellular Function and Maintenance, 40
Cell Cycle, 6
DNA Replication, Recombination, and Repair, 12
Cellular Assembly and Organization, 40
Protein Synthesis, 11
Protein Degradation, 4
Cellular Growth and Proliferation ; 50
Protein Trafficking, 6
Nucleic Acid Metabolism, 15
Cell Death and Survival, 42
Molecular Transport, 36

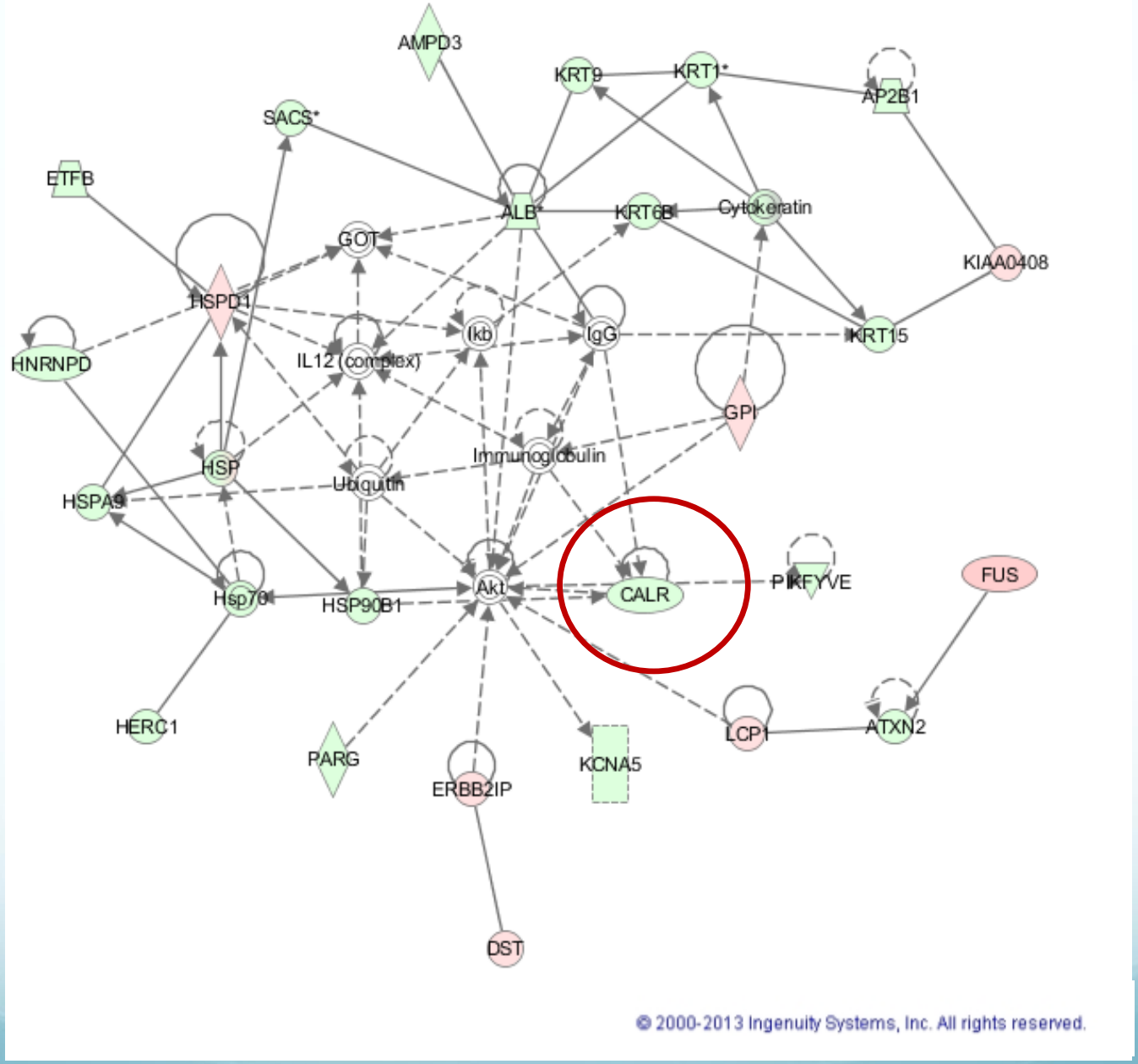
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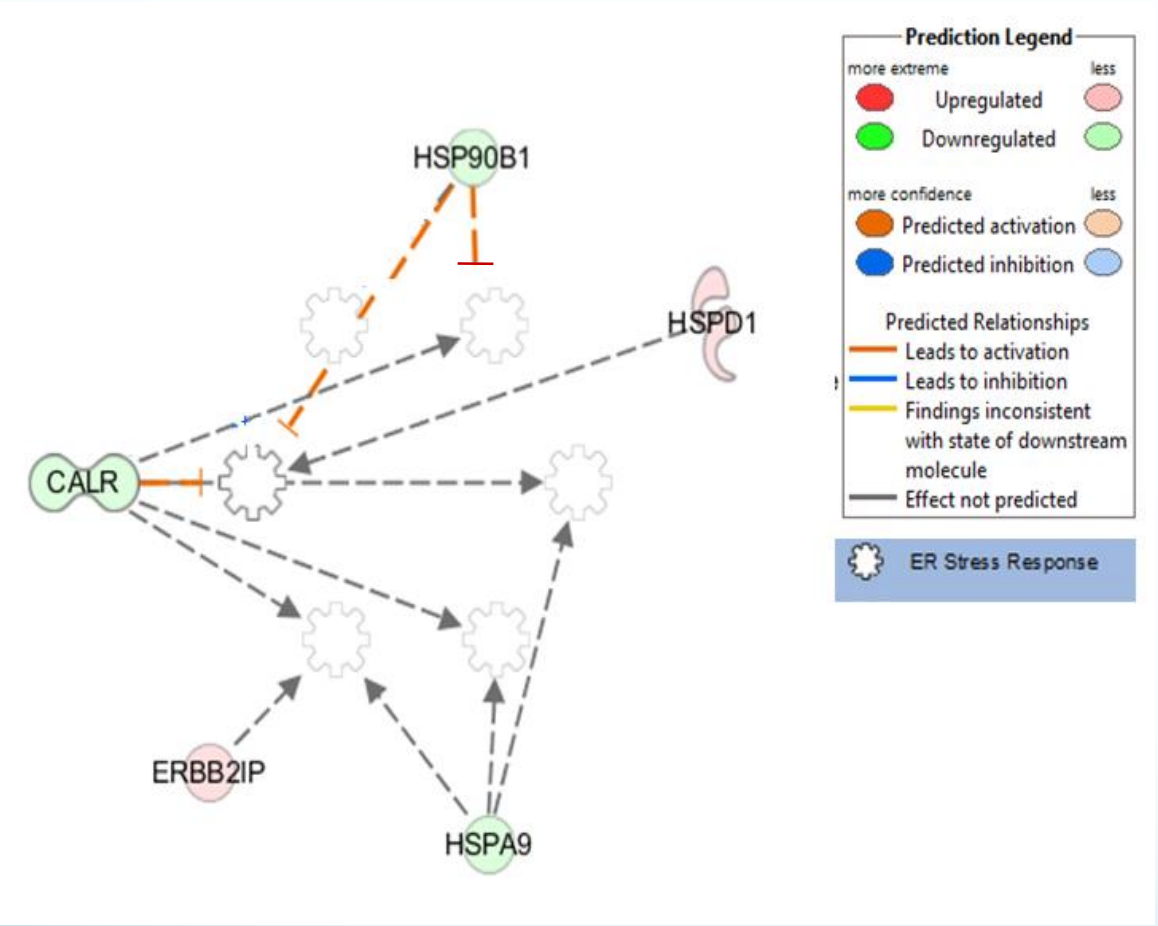


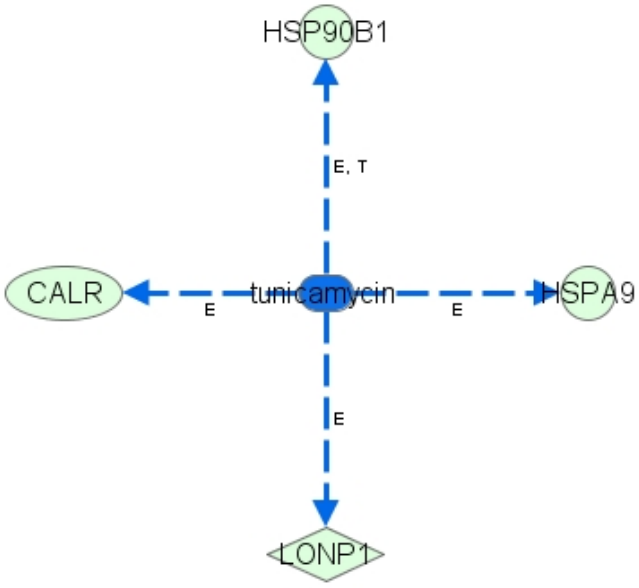
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Table. Fold changes in the molecules associated with the SILAC analysis

Symbol	Entrez Gene Name	Accession number GenPept/UniProt/Swiss-Prot	Fold Change
SCN11A	sodium channel, voltage-gated, type XI, alpha subunit	Q9UI33	7.059
PTDSS1	phosphatidylserine synthase 1	P48651	3.950
CACNA1S	calcium channel, voltage-dependent, L type, alpha 1S subunit	Q13698	3.395
HSPD1	heat shock 60kDa protein 1 (chaperonin)	P10809	2.855
RNASEL	ribonuclease L (2',5'-oligoisoadenylate synthetase-dependent)	Q05823	2.329
HIST3H2BB	histone cluster 3, H2bb	Q8N257	-2.017
HSP90B1	heat shock protein 90kDa beta (Grp94), member 1	P14625	-2.113
EIF5B	eukaryotic translation initiation factor 5B	O60841	-2.235
CALR	**calreticulin Score: 150 Match: 11 # Peptides: 4**	**P27797**	**-2.287**
TUBB	tubulin, beta class I	P07437	-2.295
HSPA9	heat shock 70kDa protein 9 (mortalin)	P38646	-2.300
GANAB	glucosidase, alpha; neutral AB	Q14697	-2.316
AMPD3	adenosine monophosphate deaminase 3	Q01432	-2.350
KCNA5	potassium voltage-gated channel, shaker-related subfamily, member 5	P22460	-2.353
VDAC1	voltage-dependent anion channel 1	P21796	-2.478
ACADVL	acyl-CoA dehydrogenase, very long chain	P49748	-2.511
PSME1	proteasome (prosome, macropain) activator subunit 1 (PA28 alpha)	Q06323	-2.662


AMPD3
KRT9
KRT1*
AP2B1
SACS*
ETFB
ALB*
KRT6B
Cytokeratin
GOT
KIAA0408
HSPD1
Ikb
IgG
KRT15
HNRNPD
IL12 (complex)
GPI
Immunoglobulin
HSP
Ubiquitin
HSPA9
Hsp70
HSP90B1
Akt
CALR
PIKFYVE
FUS
HERC1
PARG
ERBB2IP
KCNA5
LCP1
ATXN2
DST
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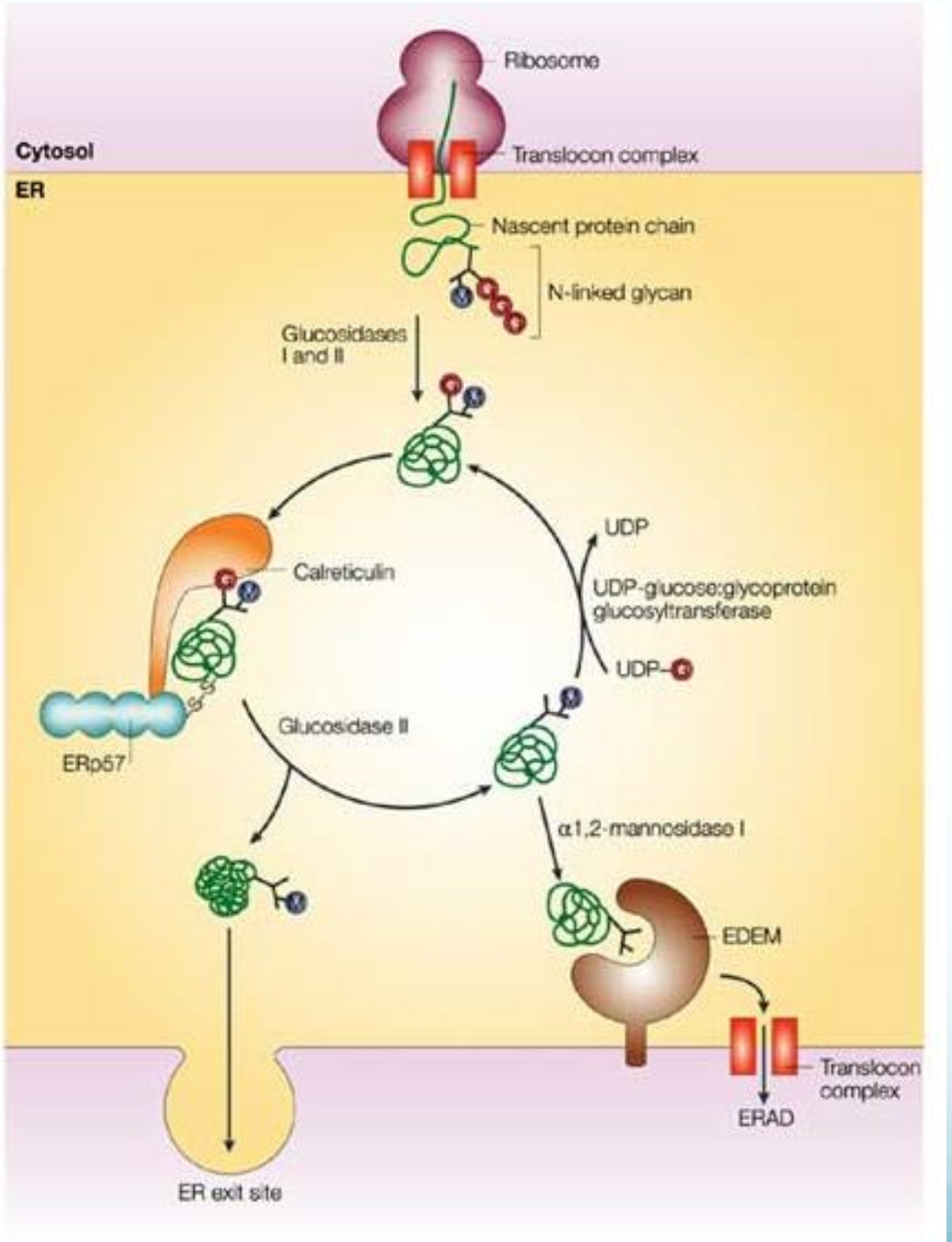




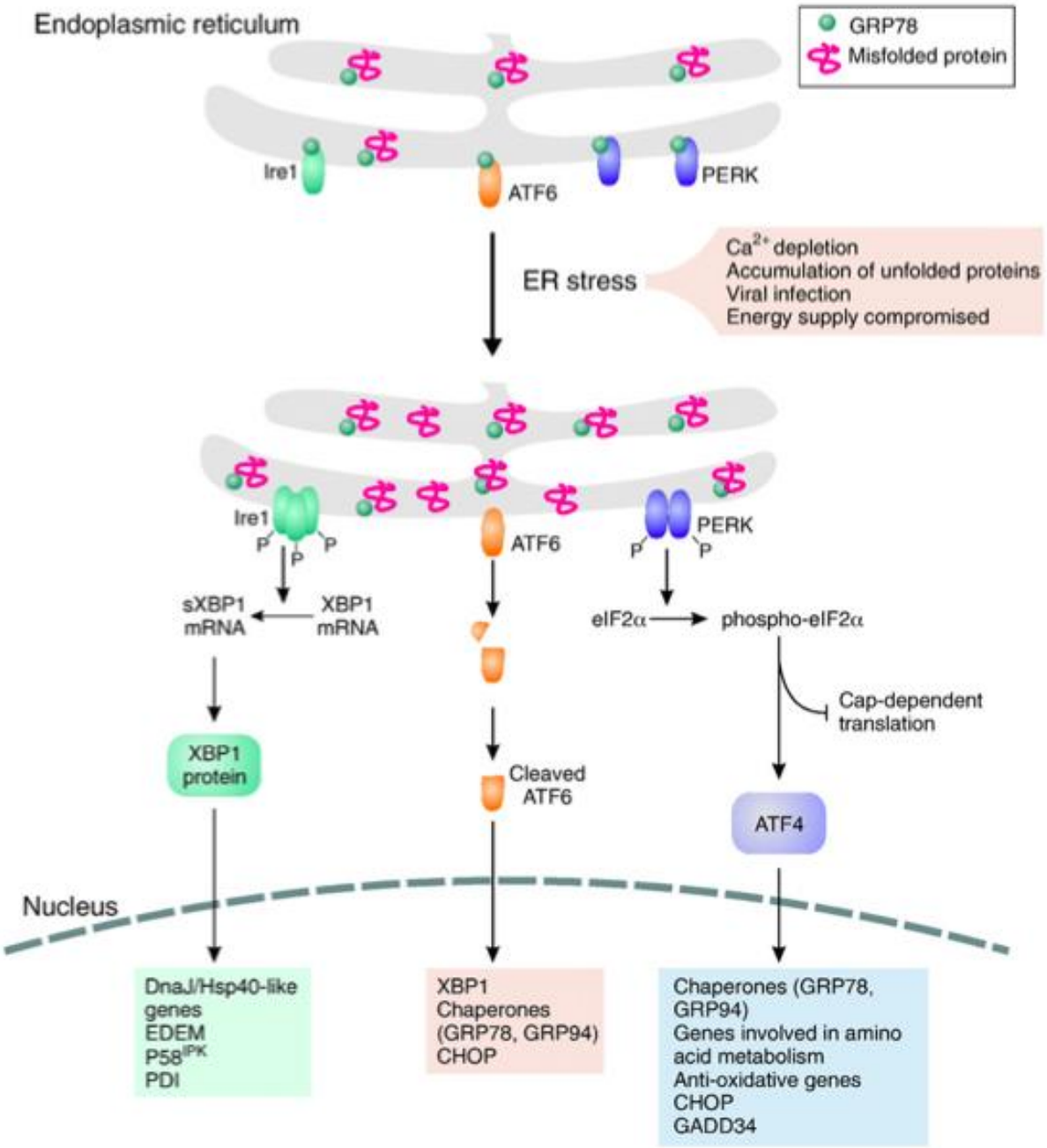
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CALR-ER stress network

Protein folding in ER lumen

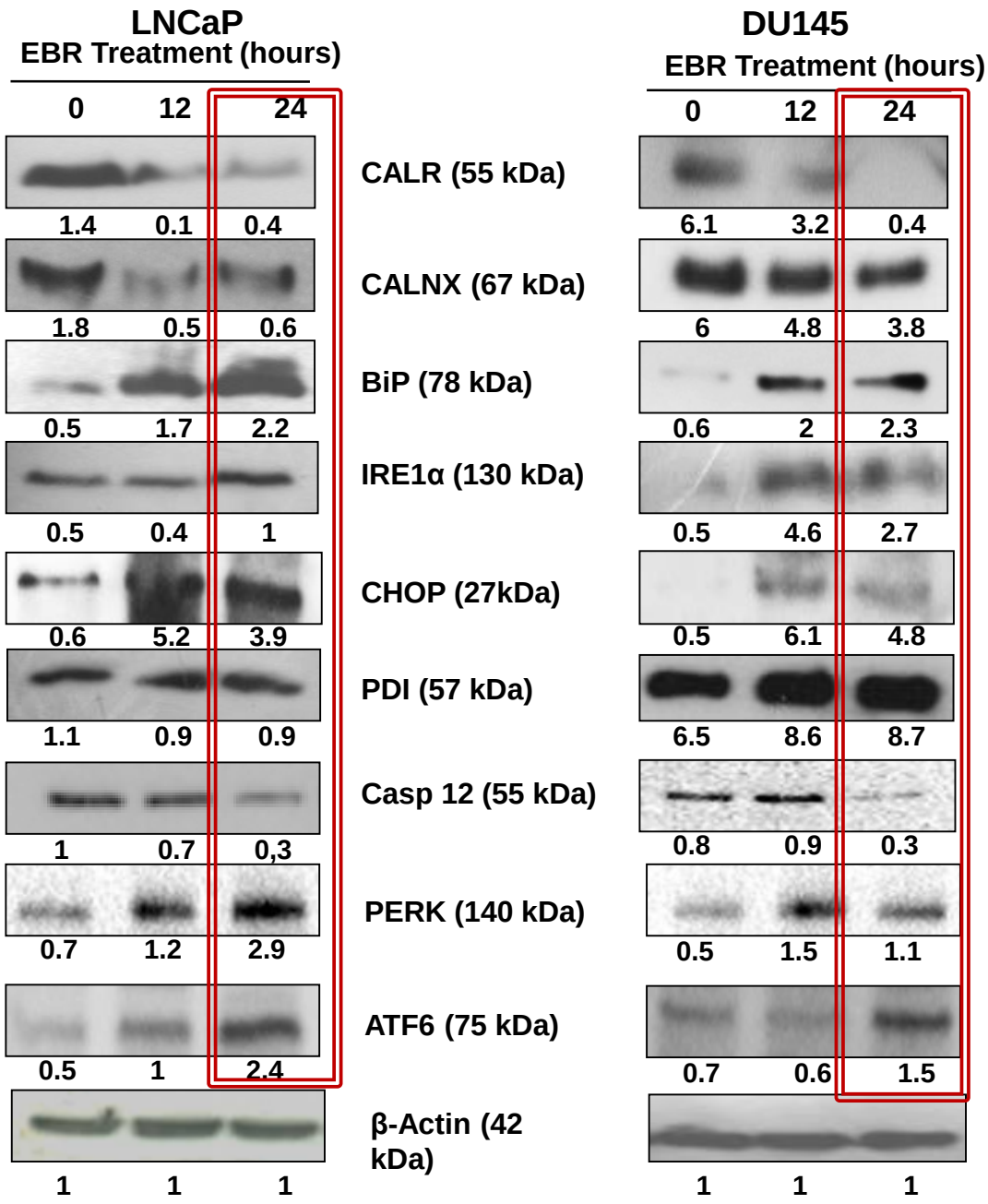


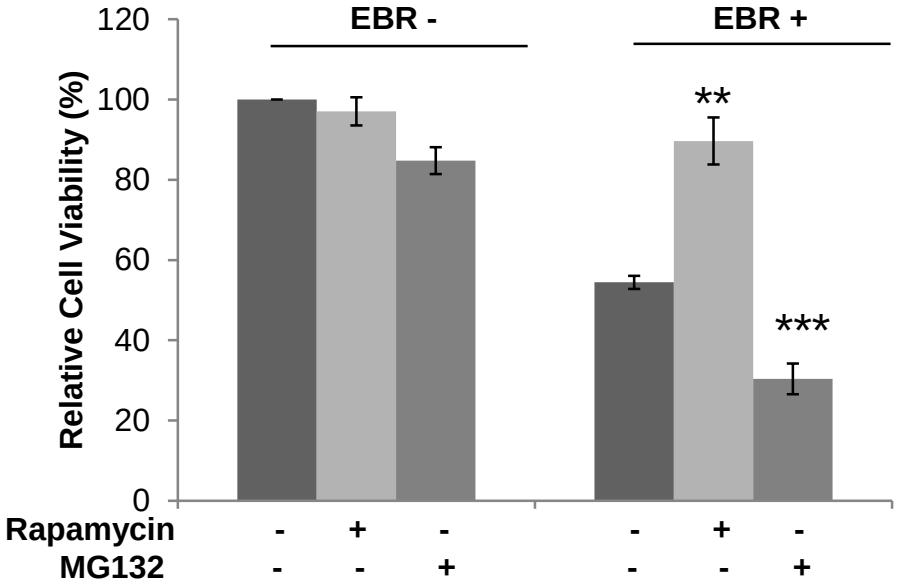
Ellgaard and Helenius, 2003

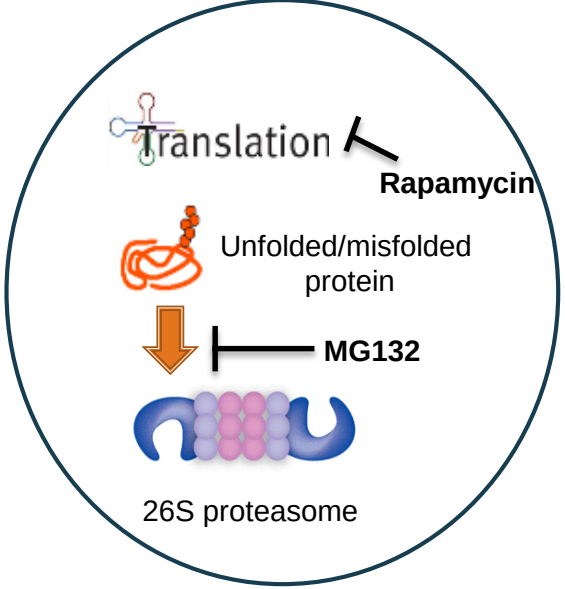


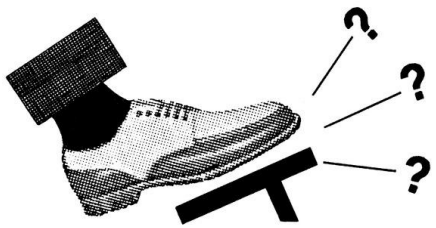
Healy et al., European Journal of Pharmacology, 2009

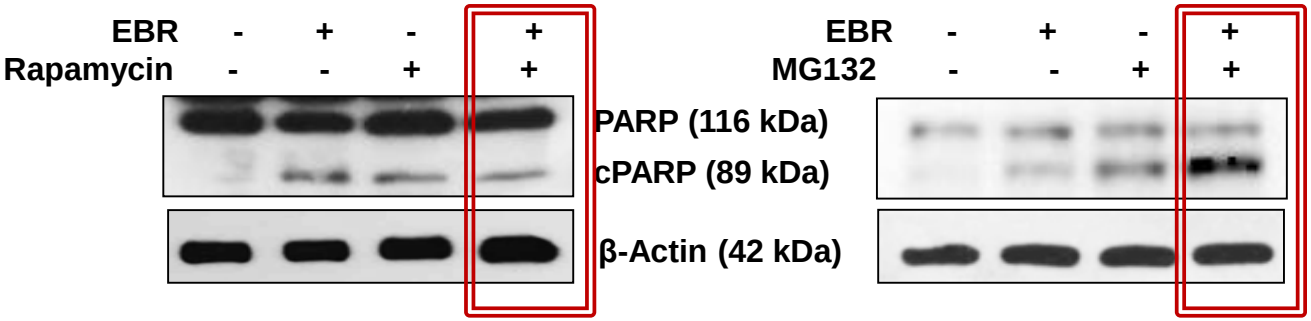
ER stress is induced after EBR treatment





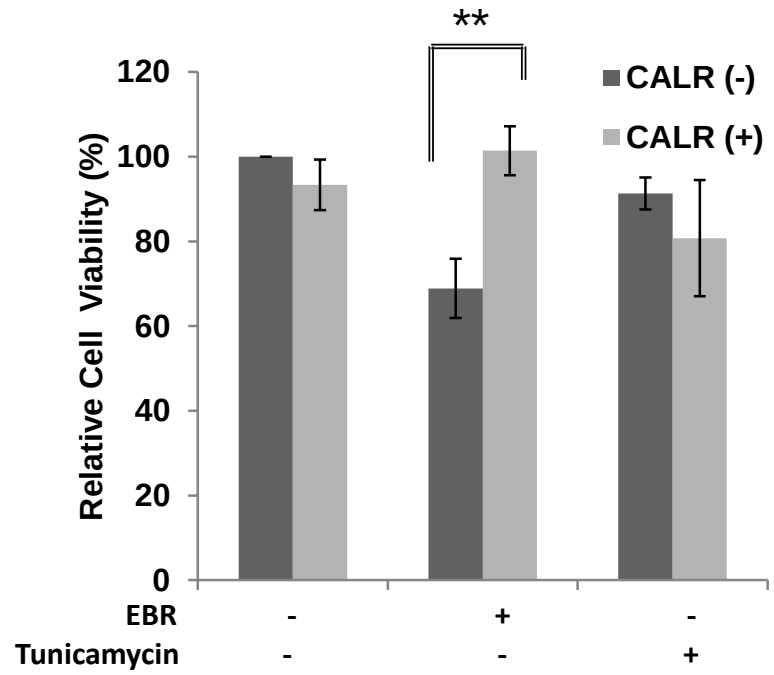


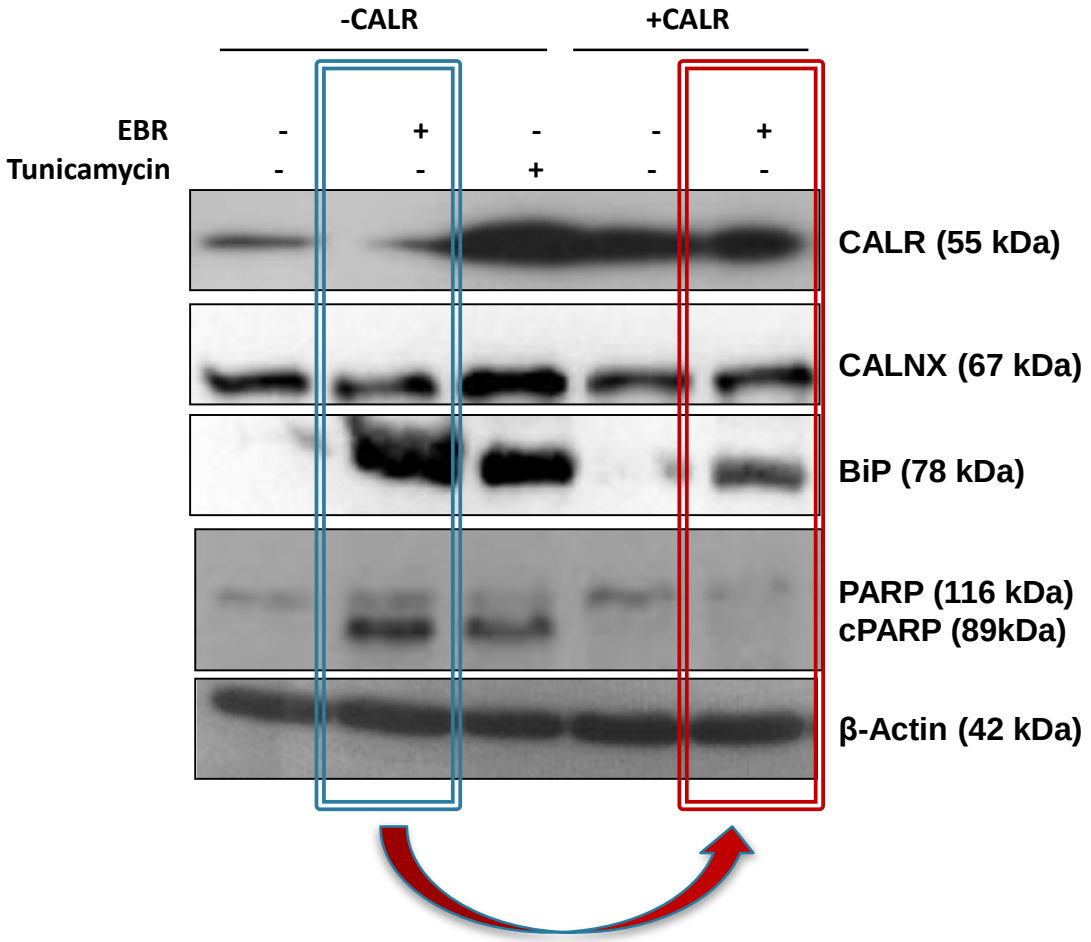




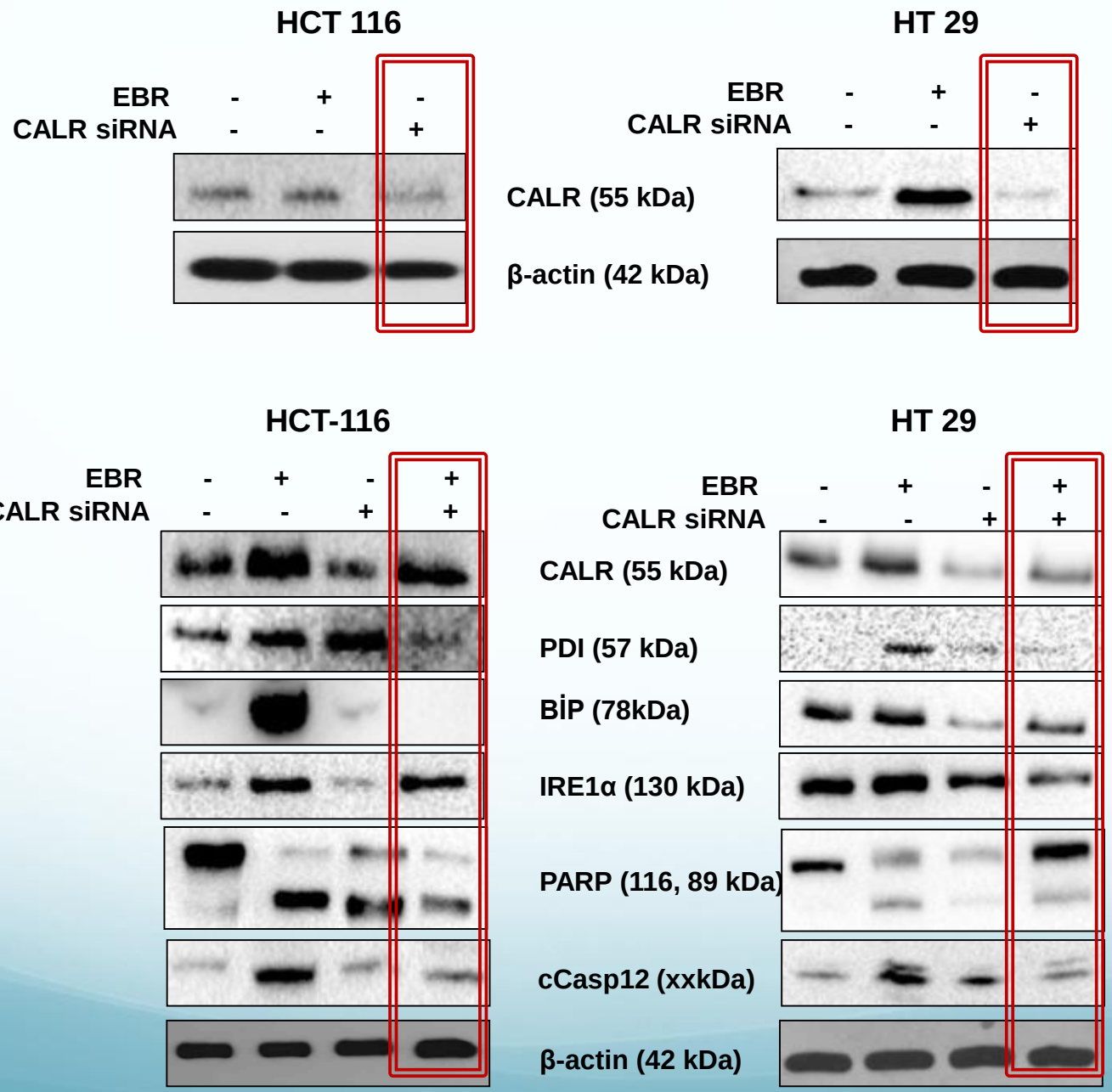
ER stress is responsible from EBR-induced apoptosis

CALR is an important molecule in EBR-induced ER stress



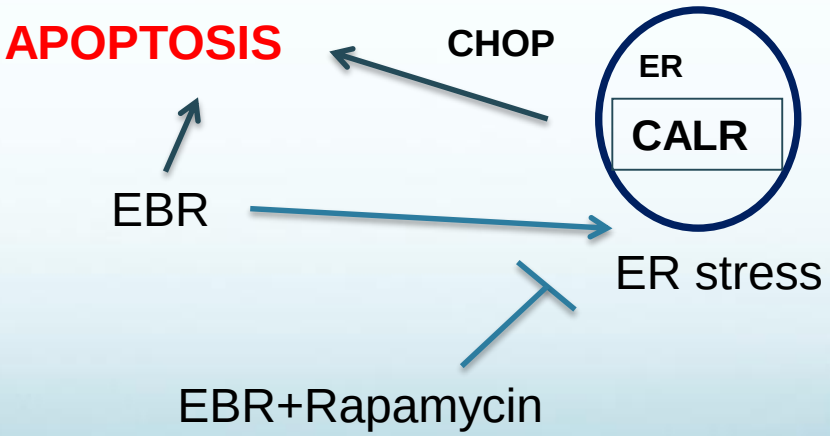


CALR is an important molecule in EBR-induced ER stress



RESULTS

- **EBR treatment induced apoptosis in prostate cancer cells as well as colon cancer cells**
- **CALR is a key molecule in EBR-induced apoptosis.**
- **EBR-induced apoptosis is due to ER stress induction.**



FUTURE WORK

- Molecular targets of EBR in animal models ?

  (COST project: 115Z037)

- The role of EBR in autophagy induction ?

ISTANBUL KÜLTÜR UNIVERSITY

Thank you...

TÜBİTAK

Project #: 113Z845

Narçın Palavan-Ünsal
Damla Arısan
Ajda Çoker-Gürkan
Pelin Özfiliz
Özge Berrak
Cagri Gumuskaptan
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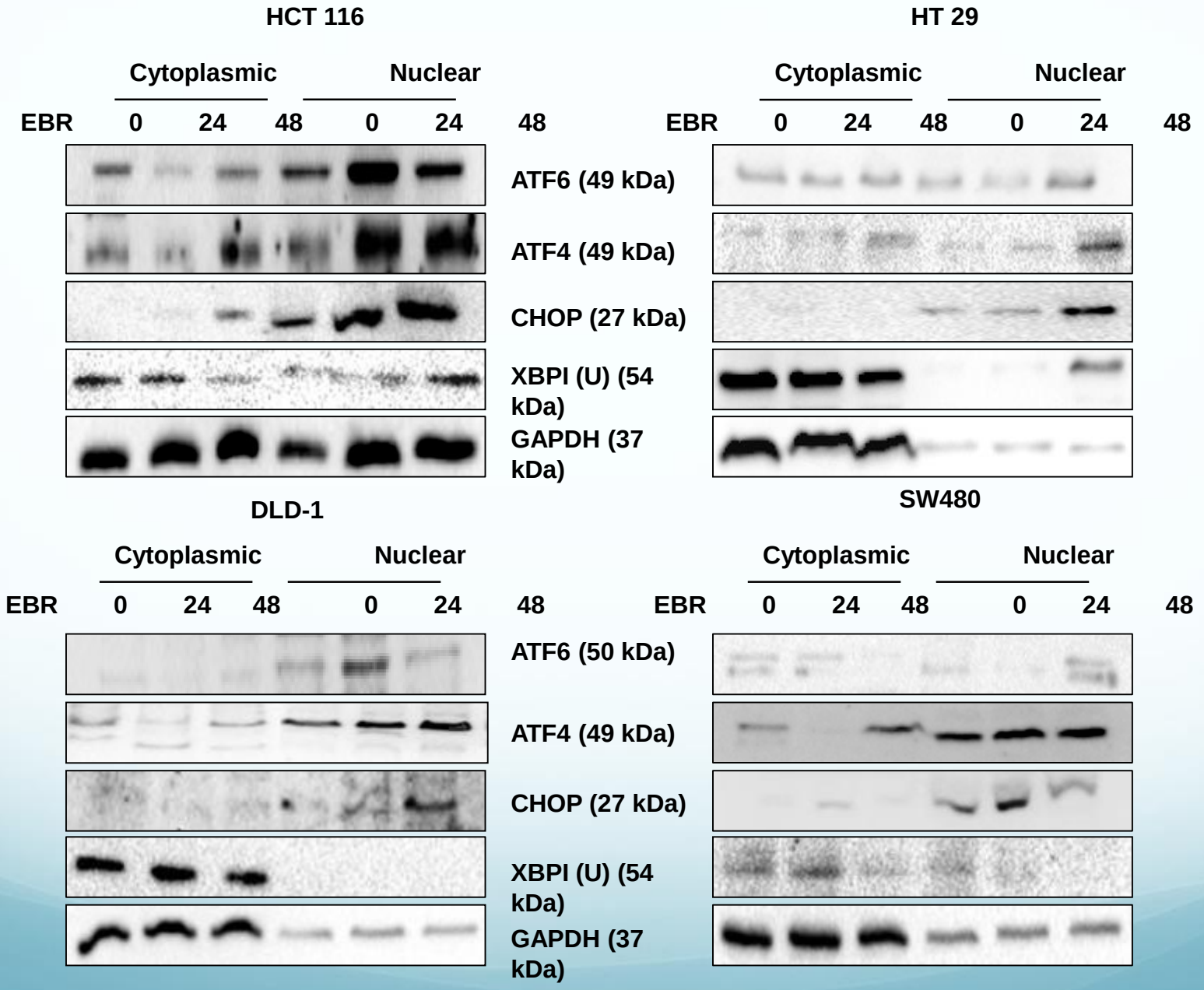
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For Cancer Research & Molecular Biology

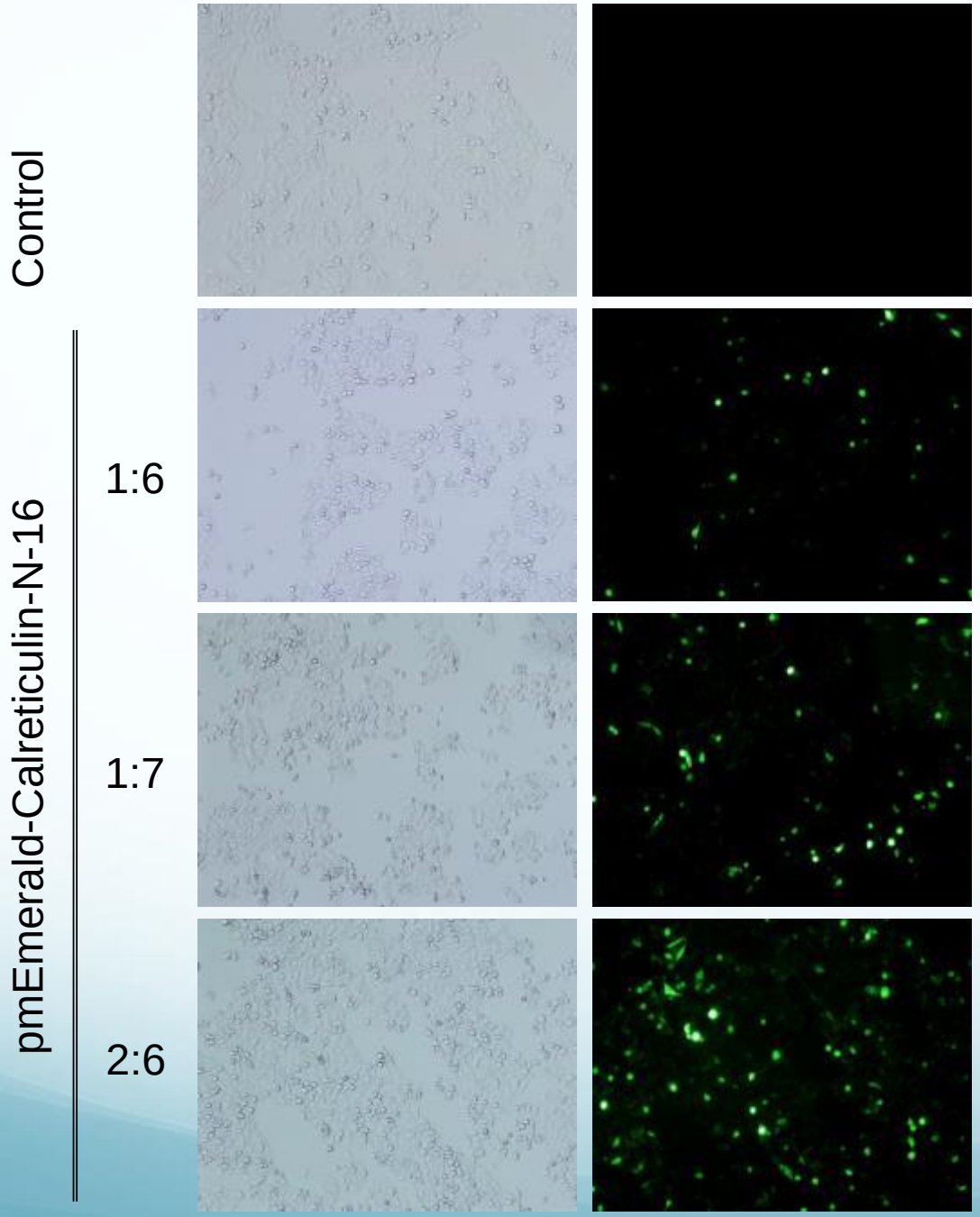
Salim Merali
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GENIE BM1408
Project #: 115Z037

ER stress is induced in response to EBR treatment in colon cancer cells





CALR is an important molecule in EBR-induced ER stress

SW480 cells