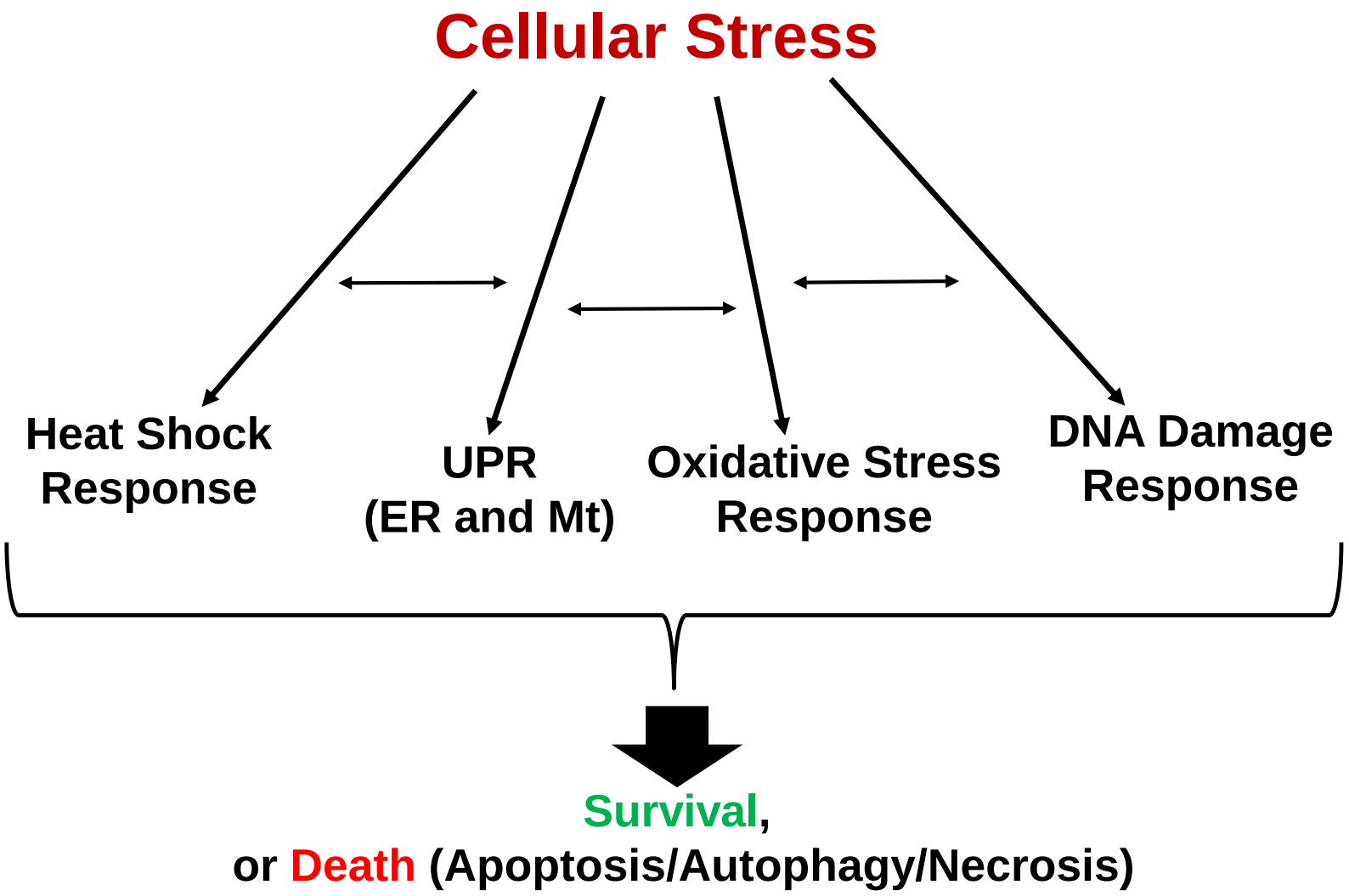
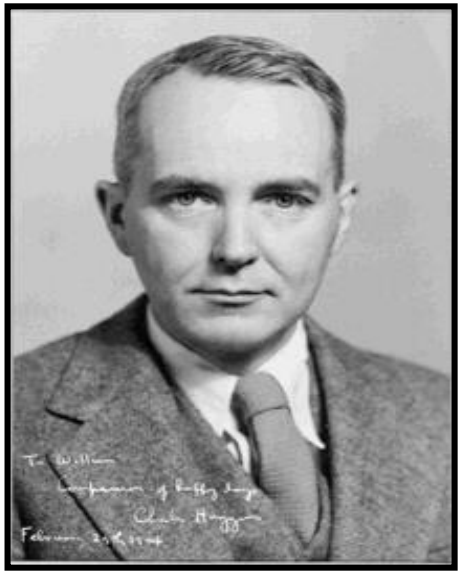
Androgens, cellular stress, and prostate cancer

Fahri Saatçıoğlu
Dept. of Biosciences
University of Oslo
Institute for Medical Genetics and Informatics
Oslo University Hospital, Oslo


Cellular Stress
Heat Shock Response
UPR (ER and Mt)
Oxidative Stress Response
DNA Damage Response
Survival,
or Death (Apoptosis/Autophagy/Necrosis)

History of Androgen Deprivation Therapy: Charles B. Huggins

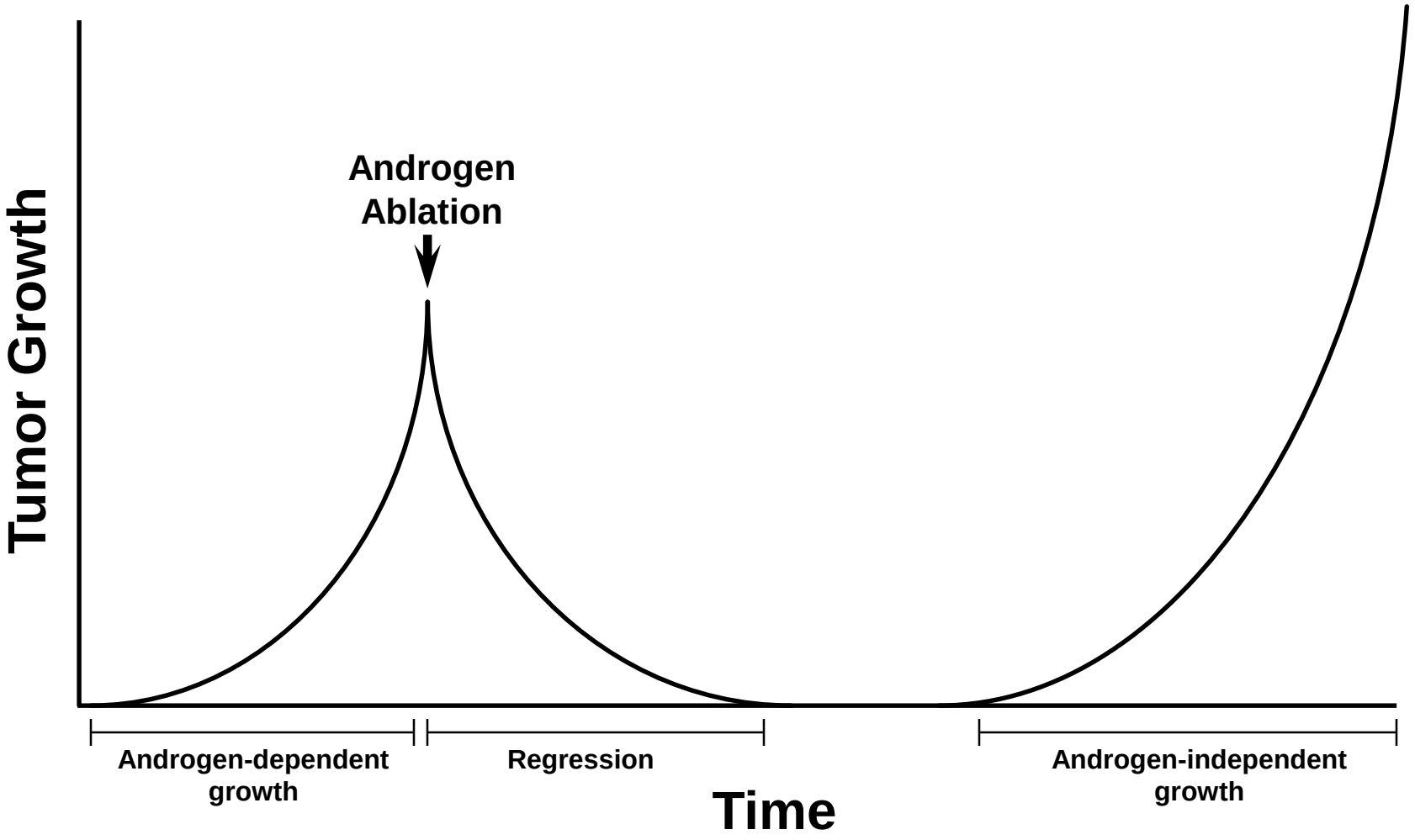
- In 1940 Huggins reported that testosterone removal (castration) resulted in rapid shrinkage of the enlarged prostate of older dogs.
- In 1941 Huggins and Hodges reported that androgen removal greatly aided patients with advanced prostate cancer.
- " Prostatic cancer is influenced by androgenic activity in the body."



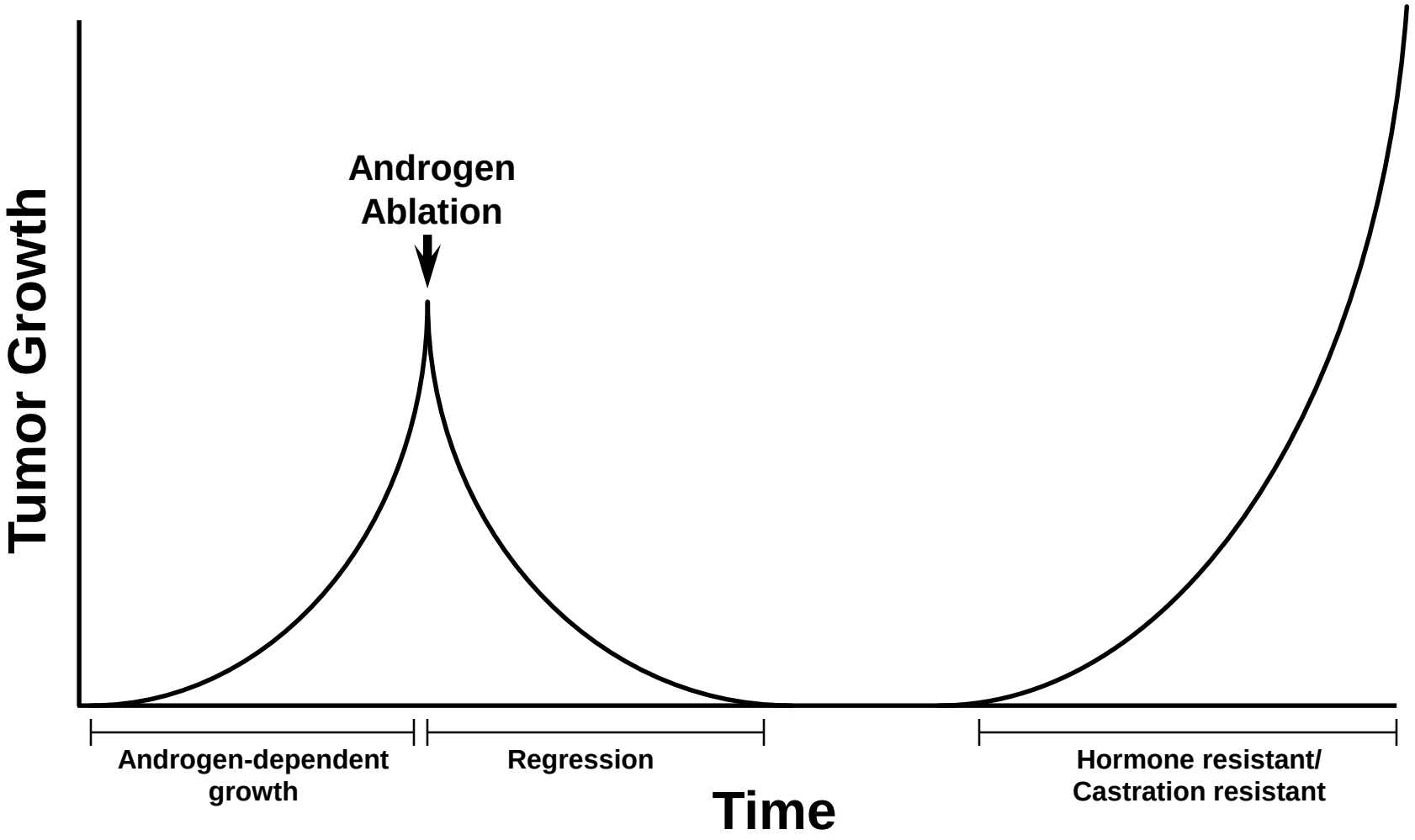
Charles Huggins

Nobel Laureate, 1966

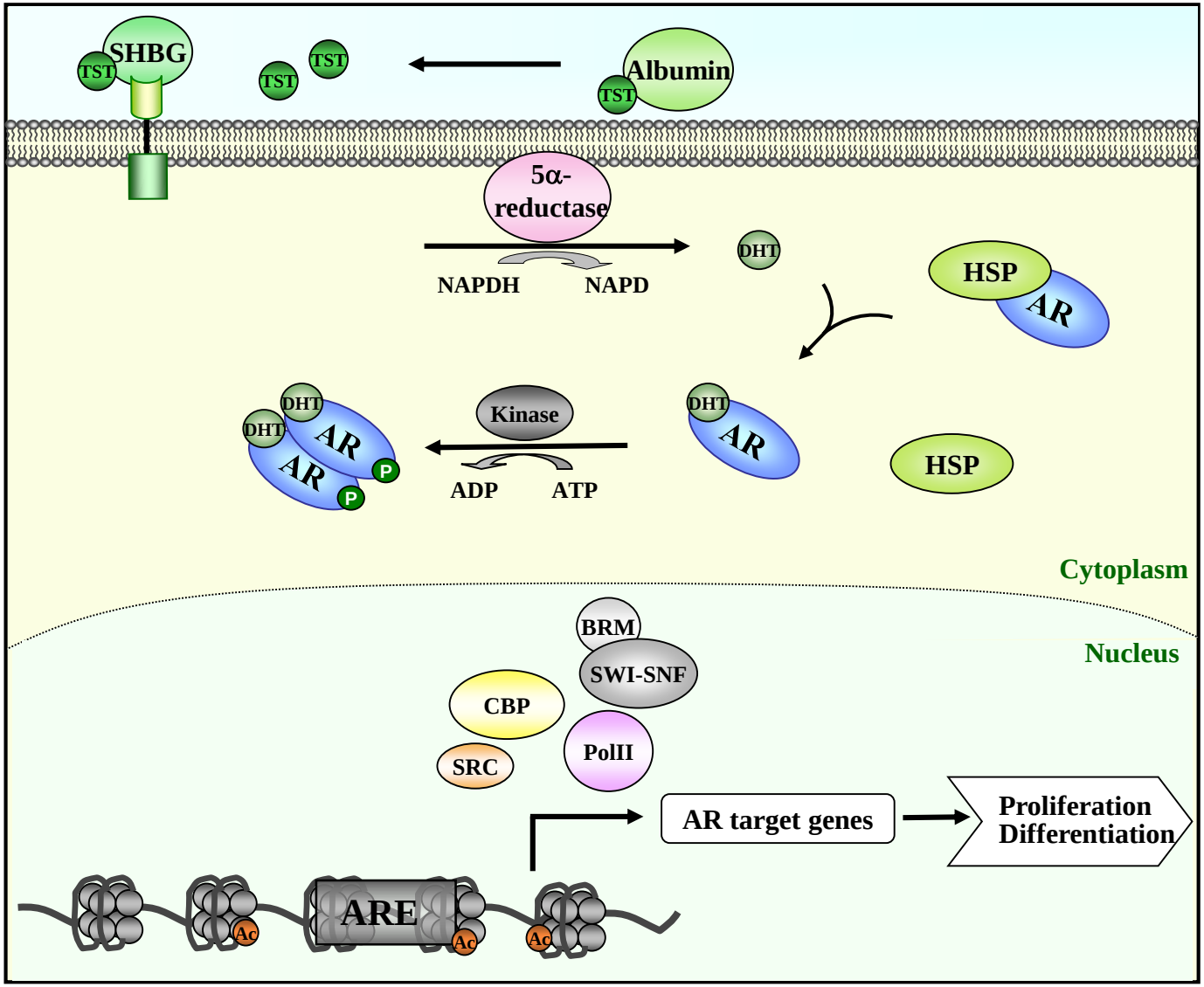
Prostate Cancer Progression



Prostate Cancer Progression

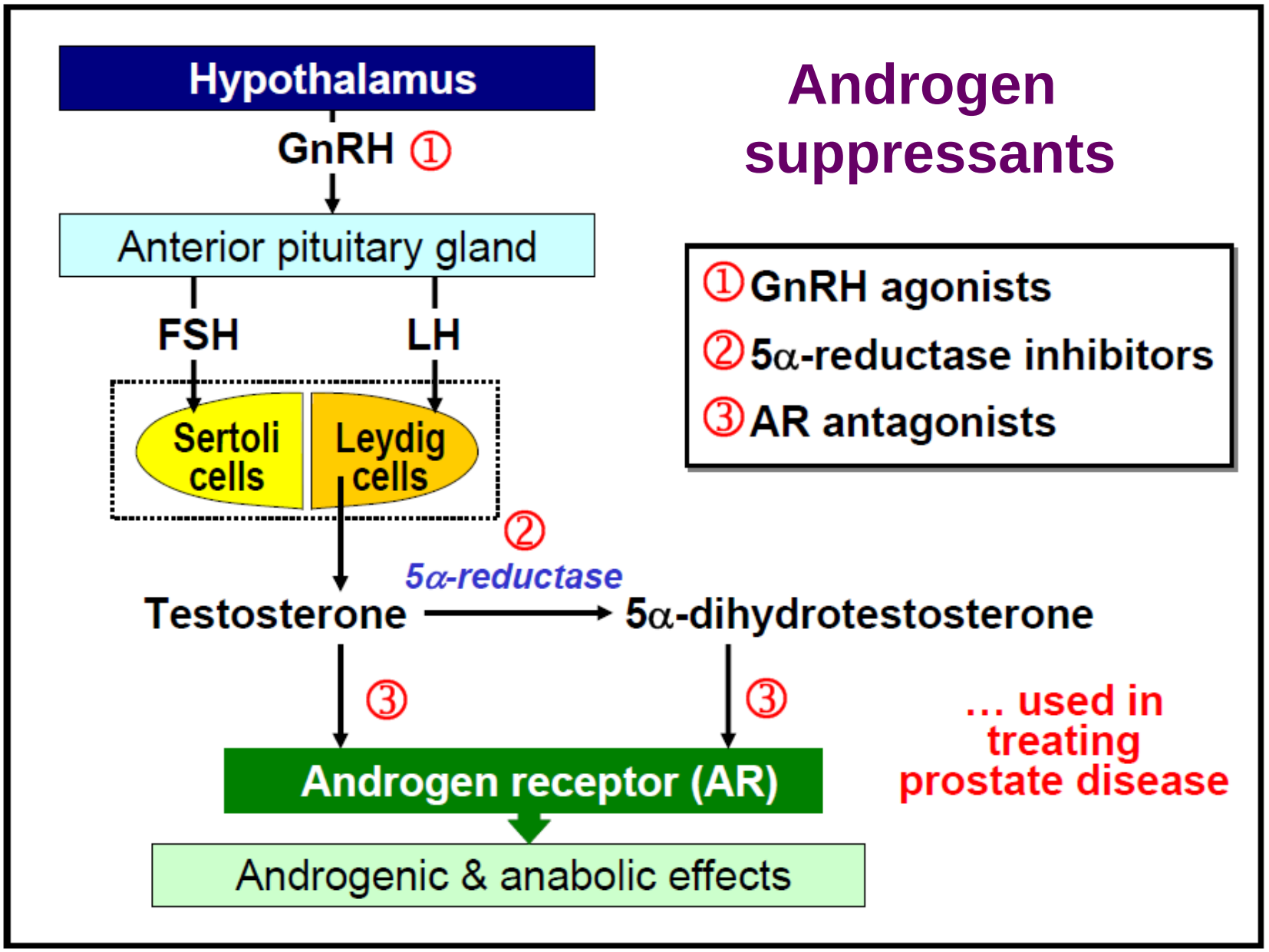


Androgen Receptor Activation



Importance of AR in Prostate Cancer Progression

- Vast majority of CRPC patients have marked increase in AR
- Forced AR expression increases PCa cell growth whereas AR knockdown empedes CRPC
- A large number of somatic mutations in AR in CRPC
- AR stimulates expression of TMPRSS2-ERG fusions associated with PCa
- High AR levels turn antagonists into agonists
- Expression of ligand-independent AR splice variants is enhanced in CRPC


Hypothalamus
GnRH ①
Anterior pituitary gland
FSH
LH
Sertoli cells
Leydig cells
②
5α-reductase
Testosterone
5α-dihydrotestosterone
③
③
Androgen receptor (AR)
Androgenic & anabolic effects
Androgen suppressants
① GnRH agonists
② 5α-reductase inhibitors
③ AR antagonists
… used in treating prostate disease

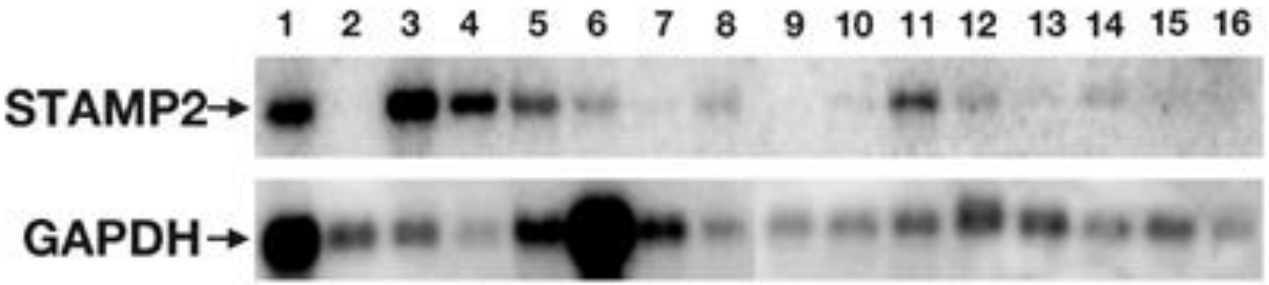
Three Components of Androgen Action

- Androgens
- AR and its cofactors
- AR target genes

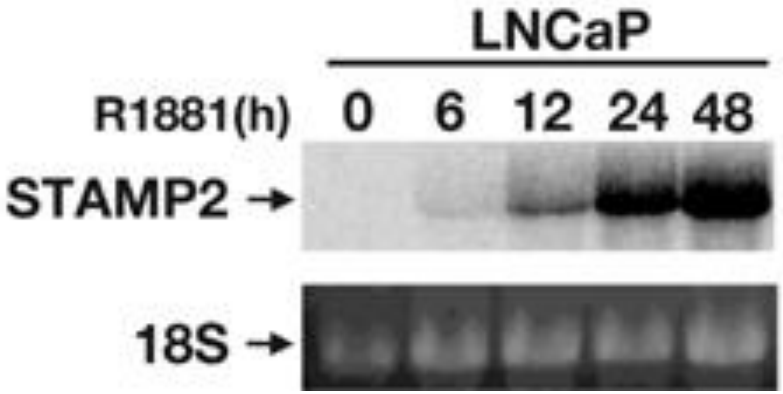
Three Components of Androgen Action

- Androgens
- AR and its cofactors
- AR target genes

STAMP2 is an androgen regulated gene in prostate cancer cells



1. heart, 2. brain, 3. placenta, 4. lung, 5. liver, 6. skeletal muscle, 7. kidney, 8. pancreas, 9. spleen, 10. thymus, 11. prostate, 12. testis, 13. ovary, 14. small intestine, 15. colon, 16. peripheral blood leukocyte

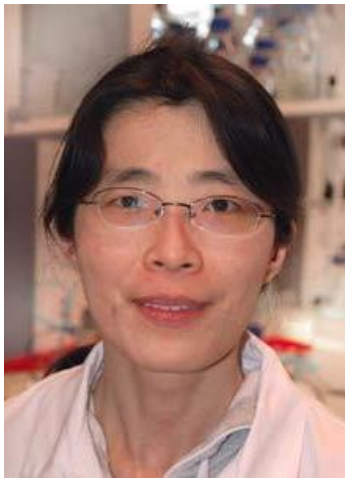


Korkmaz et al., Oncogene 2005

STAMP2 team



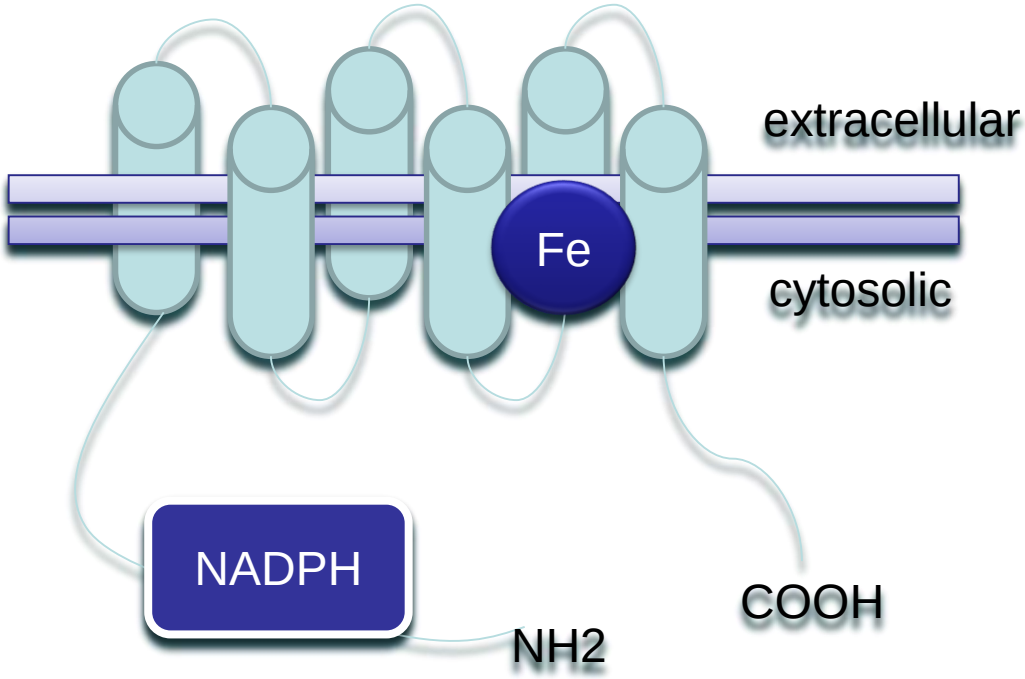
Yang Jin



Ling Wang

Jin et al., EMBO Mol Med 2015

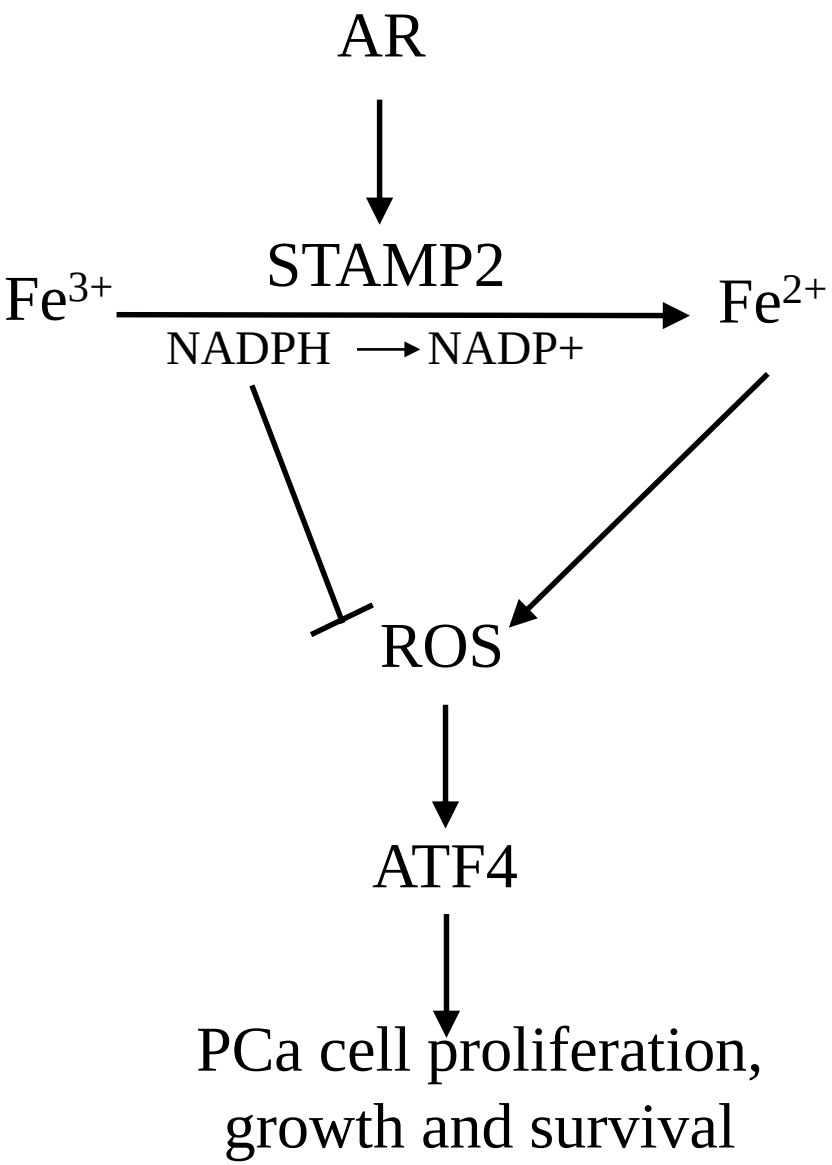
STAMP2 is a member of transmembrane ferrireductase family



- Regulates energy metabolism and inflammatory output
- Atherosclerosis in Stamp2 knockout mice
- Metalloreductase activity
- Regulates uptake of iron and copper

Ohgami et al., Nature Genetics. 2005
Wellen et al., Cell. 2007
Ten Freyhaus, et.al. Cell Metabolism. 2012.

Mechanisms of STAMP2 function in PCa cells



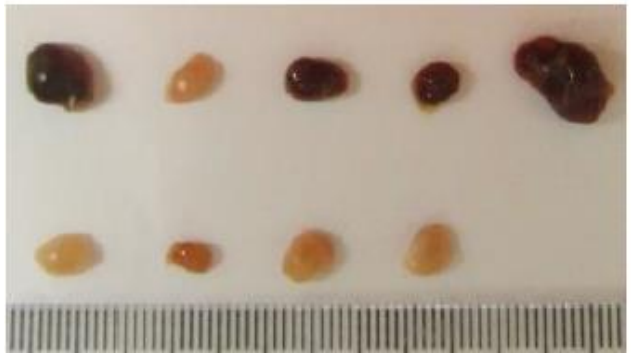
Jin et al., EMBO Mol Med 2015

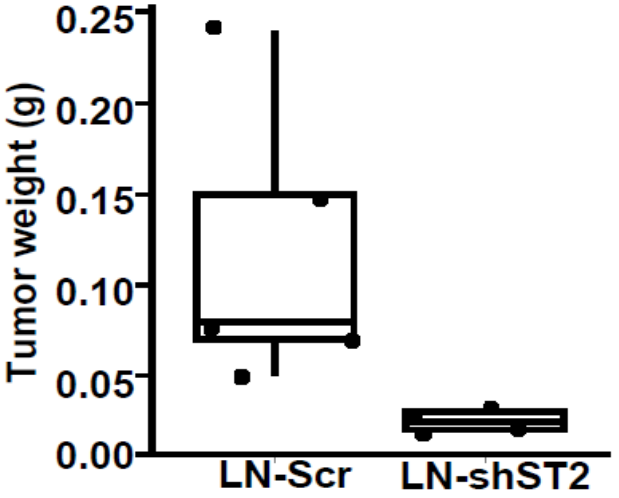
STAMP2 loss inhibits prostate cancer cells growth in vivo

LNCaP

Scr

shST2



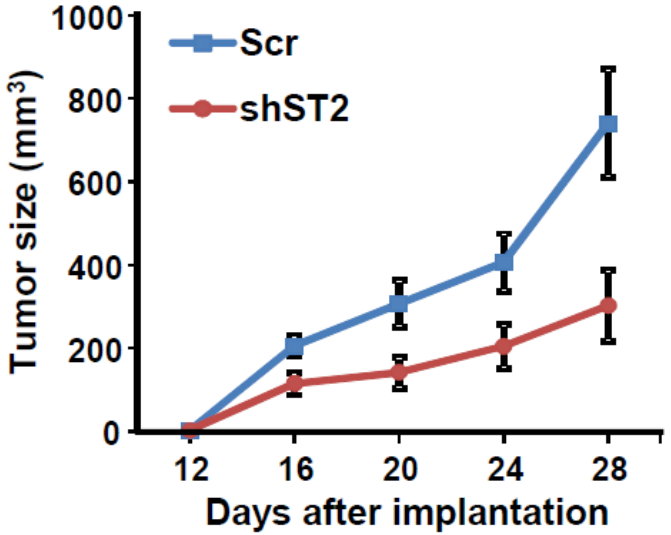


22RV1

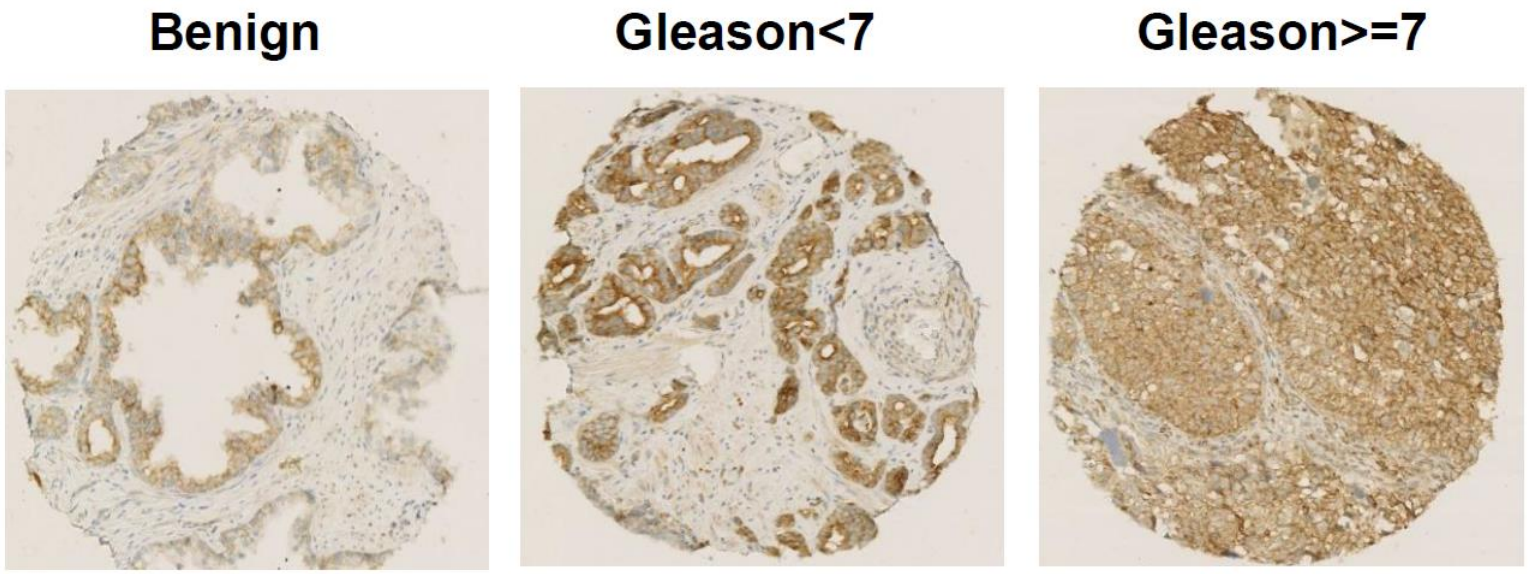
Scr

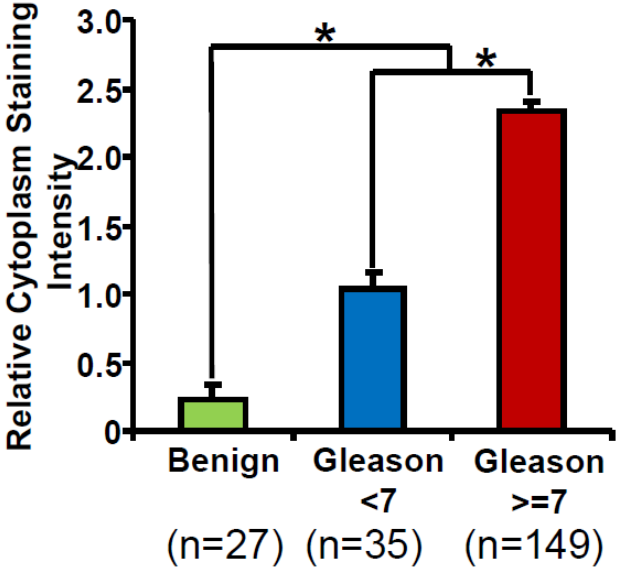
shST2



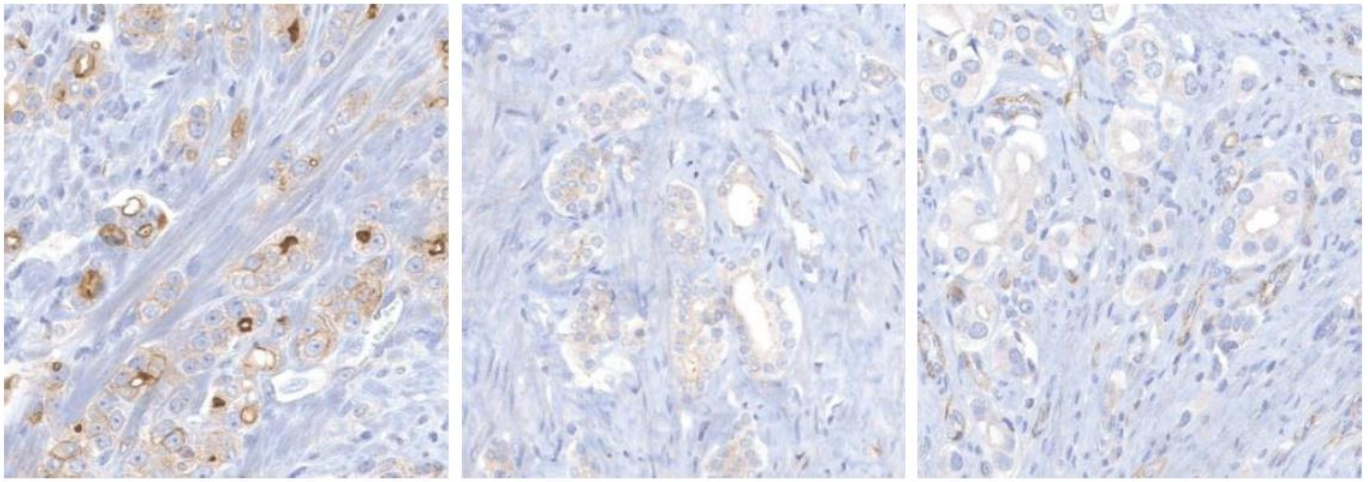


STAMP2 expression is up-regulated in human PCa specimens

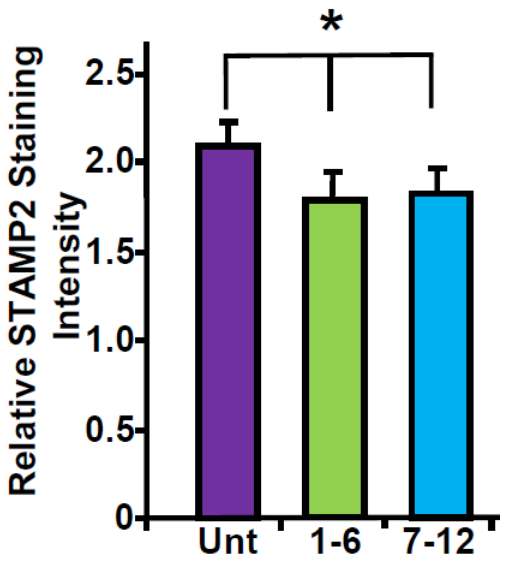




STAMP2 is associated with neoadjuvant hormone therapy

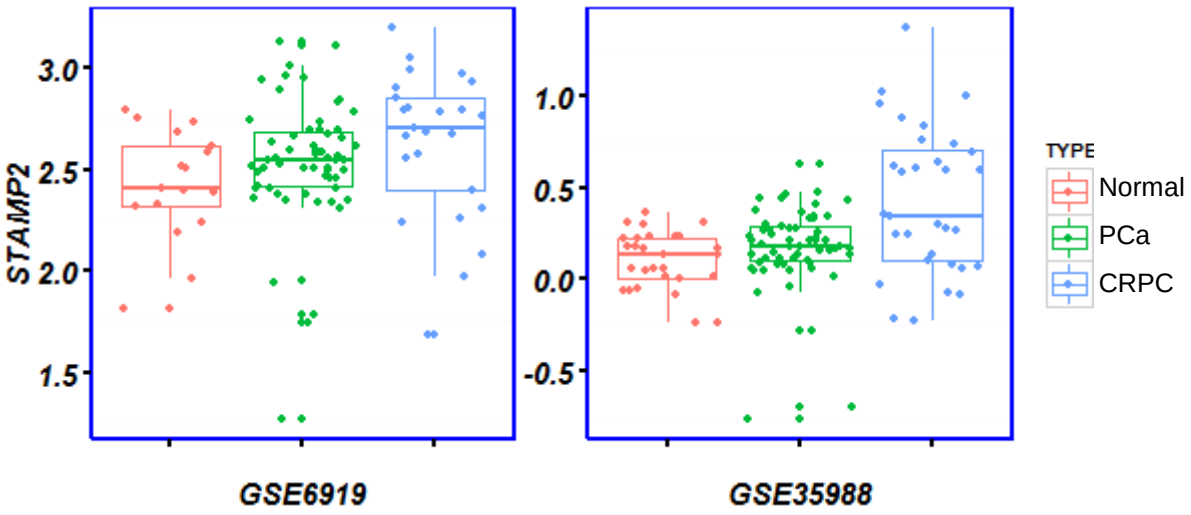


Untreated | 1-6 month NHT | 7-12 month NHT



STAMP2 is associated with CRPC

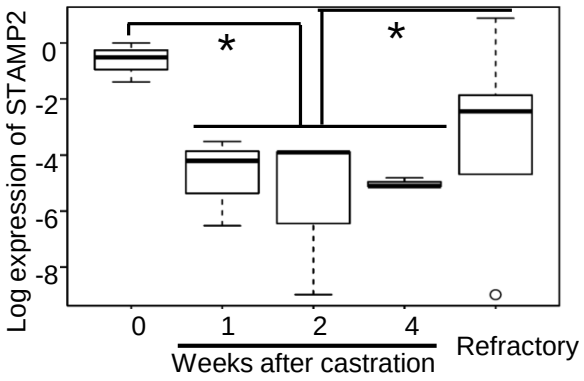
A



	PCa	CRPC
Normal	0.0607	0.0079
PCa		0.0350

	PCa	CRPC
Normal	0.0754	8.3E-05
PCa		9.8E-05

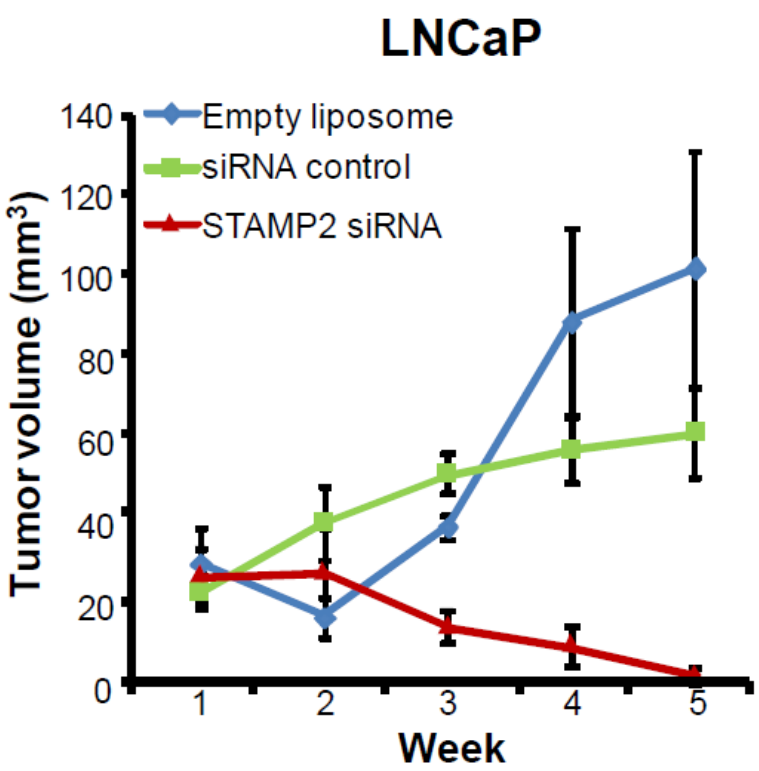
B

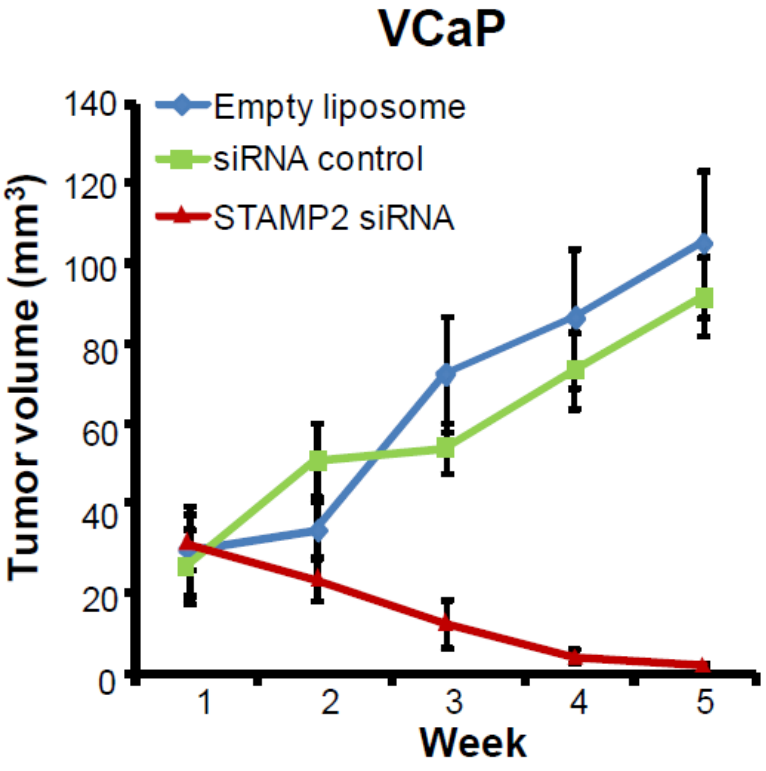


Nanoliposomal siRNA delivery to tumors

- **Efficiency:** DOPC based nanoliposomes can deliver siRNA *in vivo* into tumour cells 10- and 30-fold more effectively than cationic liposomes (e.g. DOTAP) and naked siRNA, respectively (Ozpolat et al, 2009)**.**
- **Efficacy:** Twice weekly intravenous or intraperitoneal injections of DOPC-nanoliposomes incorporating siRNA, at a dosing schedule of 150 μg kg^{-1} day^{-1}, resulted in substantial reduction in the expression of target genes (e.g., FAK, neuropilin-2, IL-8 or Bcl-2) and tumour size in mice with different human cancers (e.g. Landen et al., 2005; Halder et al., 2006)
- **Safety:** DOPC-based nanoliposomes did not cause any detectable distress or toxicity and were found to be safe in mice and in nonhuman primates.

Therapeutic efficacy of STAMP2 silencing in preclinical models of prostate cancer

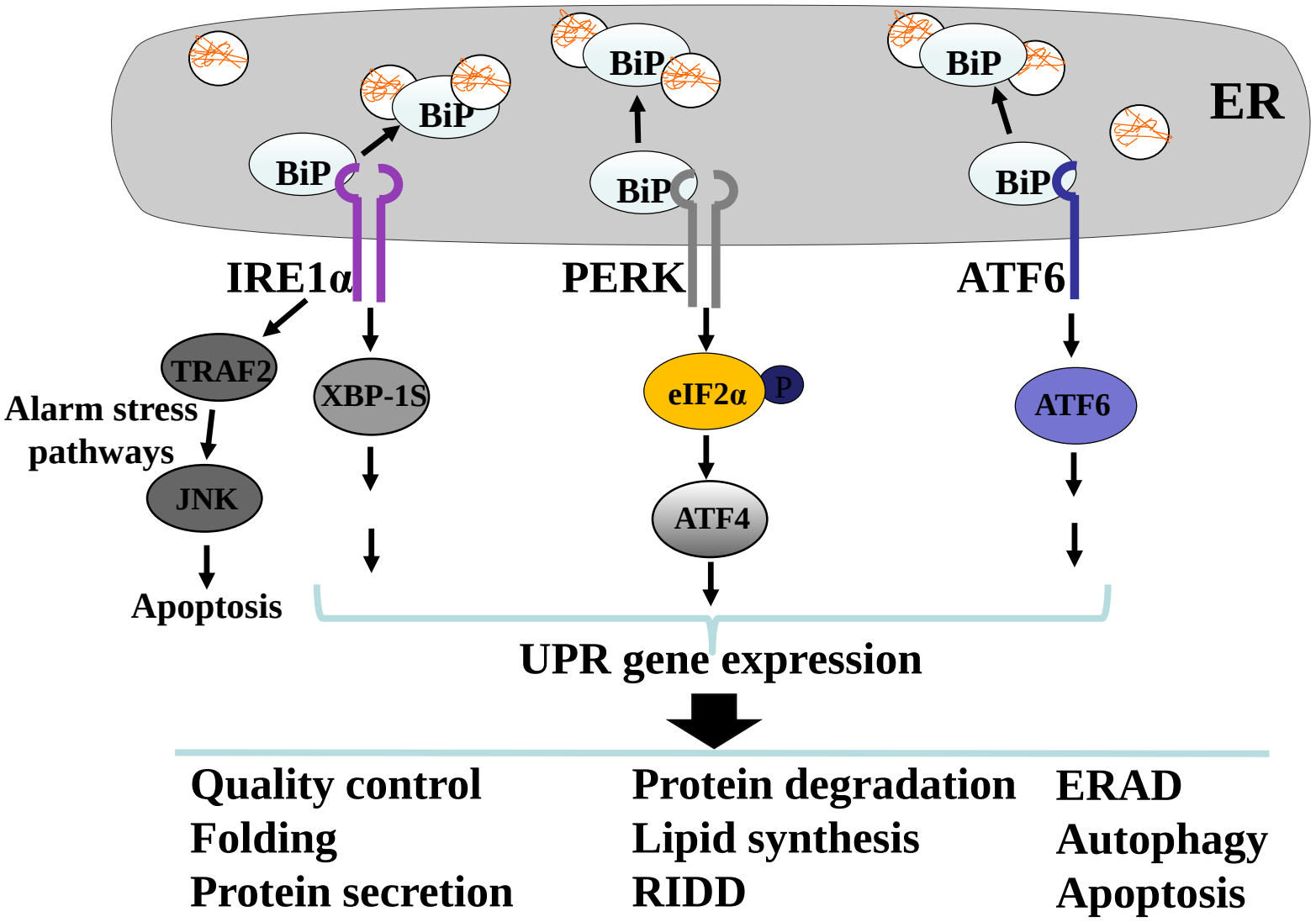




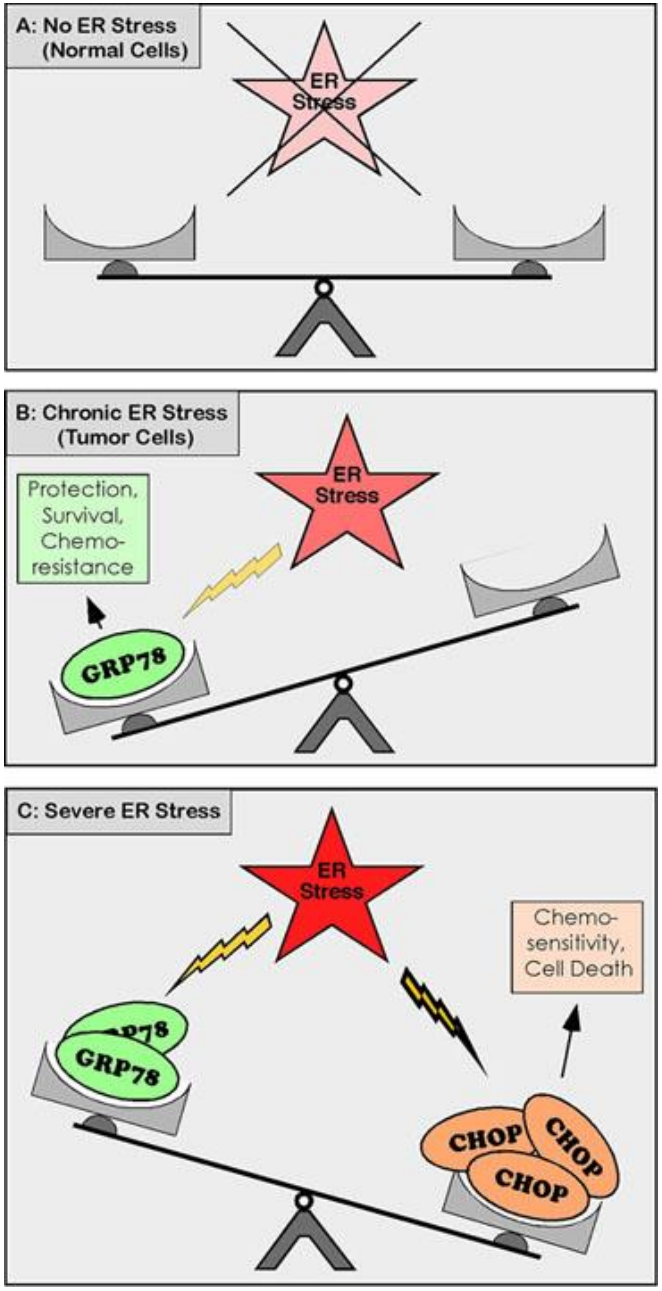
Future Perspectives, STAMP2

- Further molecular characterization of STAMP2-ATF4 axis
- Explore whether STAMP2 knockdown can improve efficacy of hormone and chemotherapy
- Targeted nanoliposomal siRNA mediated knockdown/optimization
- Clinical studies

The Unfolded Protein Response



ER stress in cancer



(Schonthal et al., 2012)

Androgens and ER stress?

The hypothesis:

A) induction of prostate secretory cells by androgens activates the UPR to induce ER expansion to accommodate increased protein expression.

B) activation of the UPR may be part of the differentiation program induced by androgens that occurs prior to induction of high-level protein synthesis.

Thus, the UPR may contribute to a programmed response to androgen that increases the prostate cell's protein-secretory demand/cell survival. This may be deregulated in prostate cancer.



Yke Arnoldussen



Xia Sheng



Margrethe Storm

Androgens and ER stress



Martina Tesikova



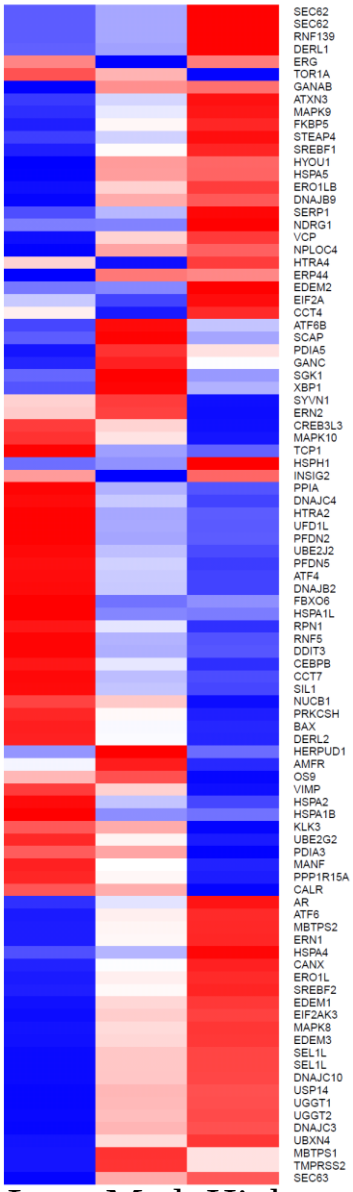
Yang Jin



Zeynep Kirli

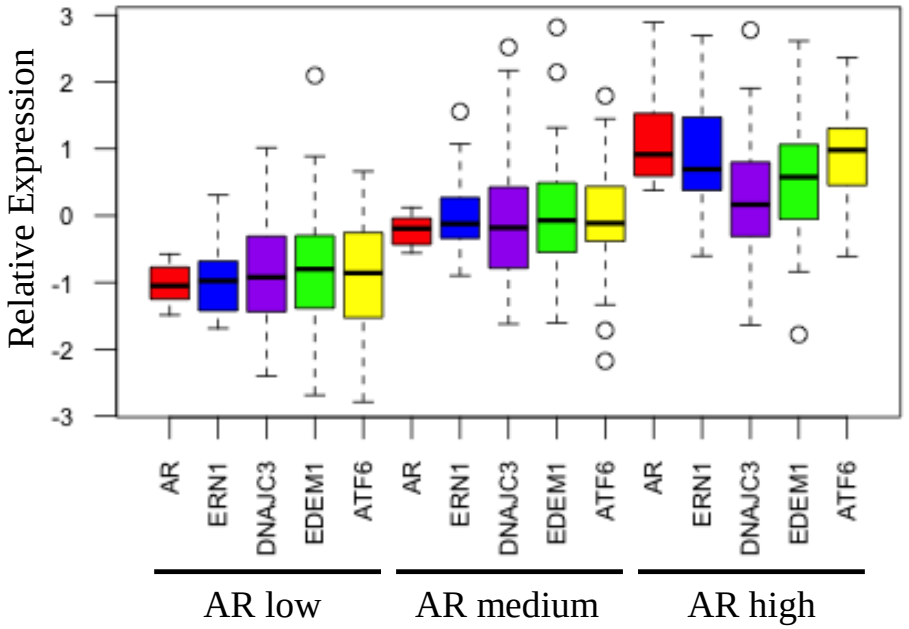
AR and UPR gene expression are correlated in human prostate cancer

A

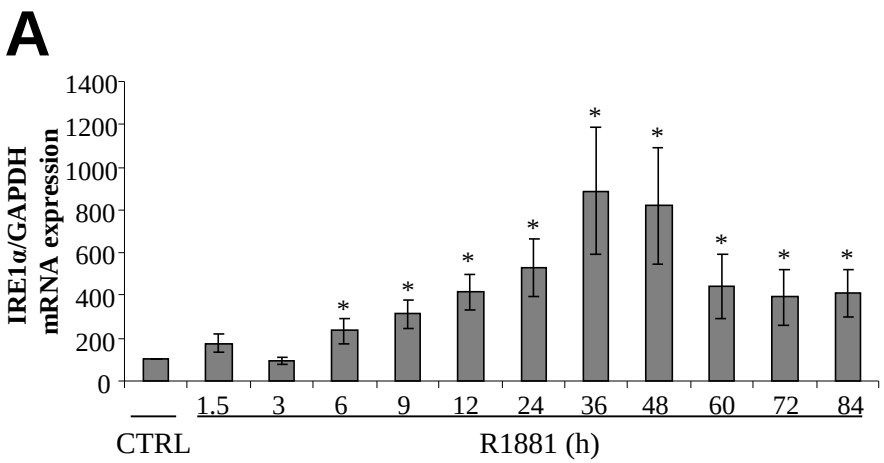


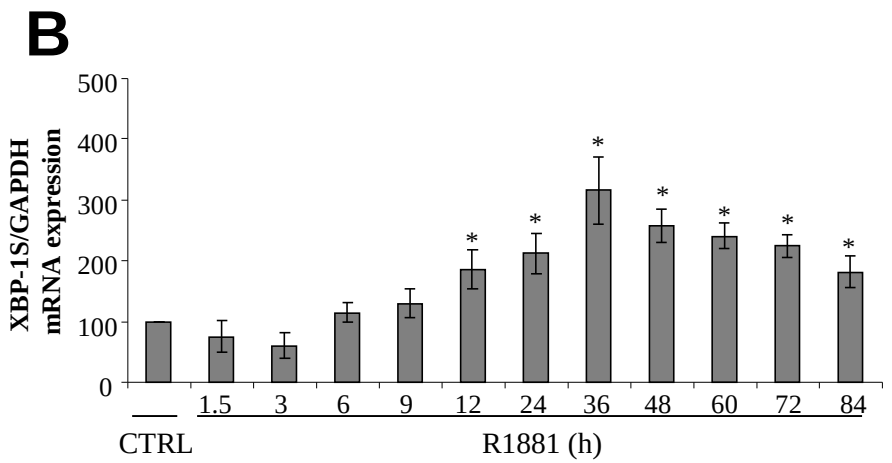
AR: Low Med High

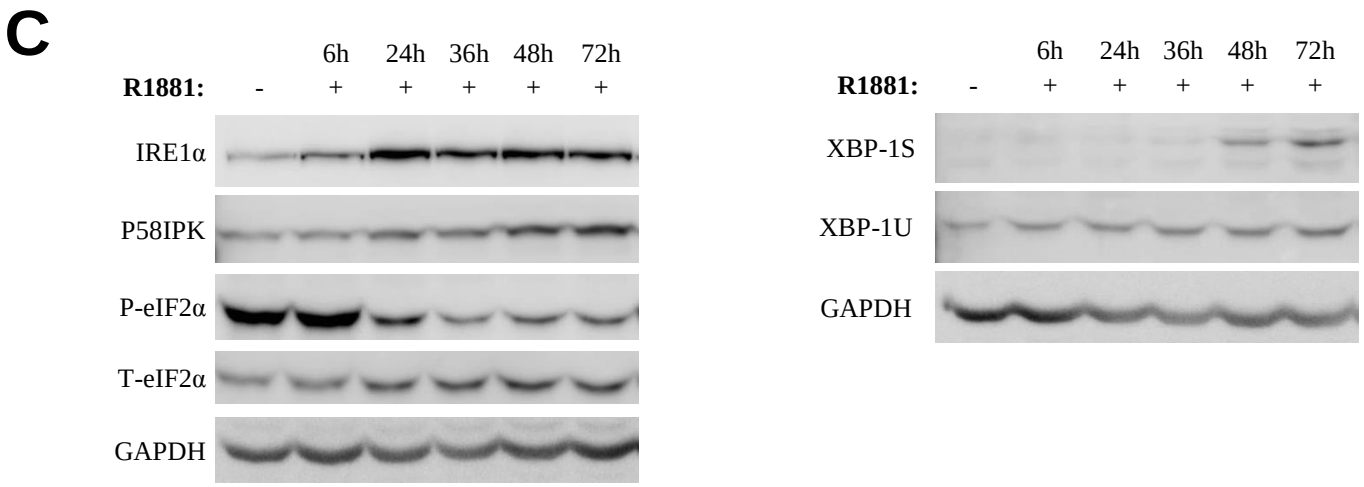
B



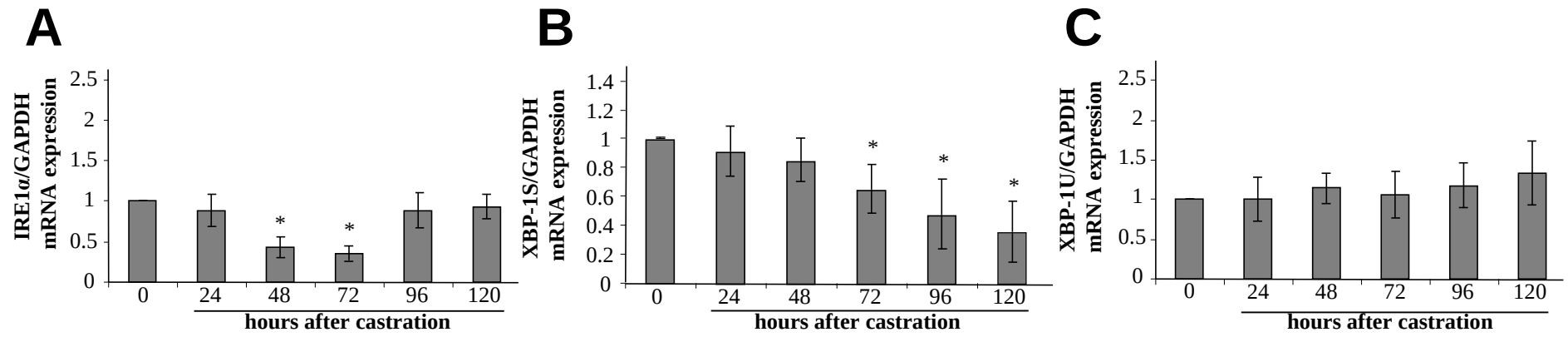
Androgens activate IRE1, but inhibit PERK signaling in prostate cancer cells



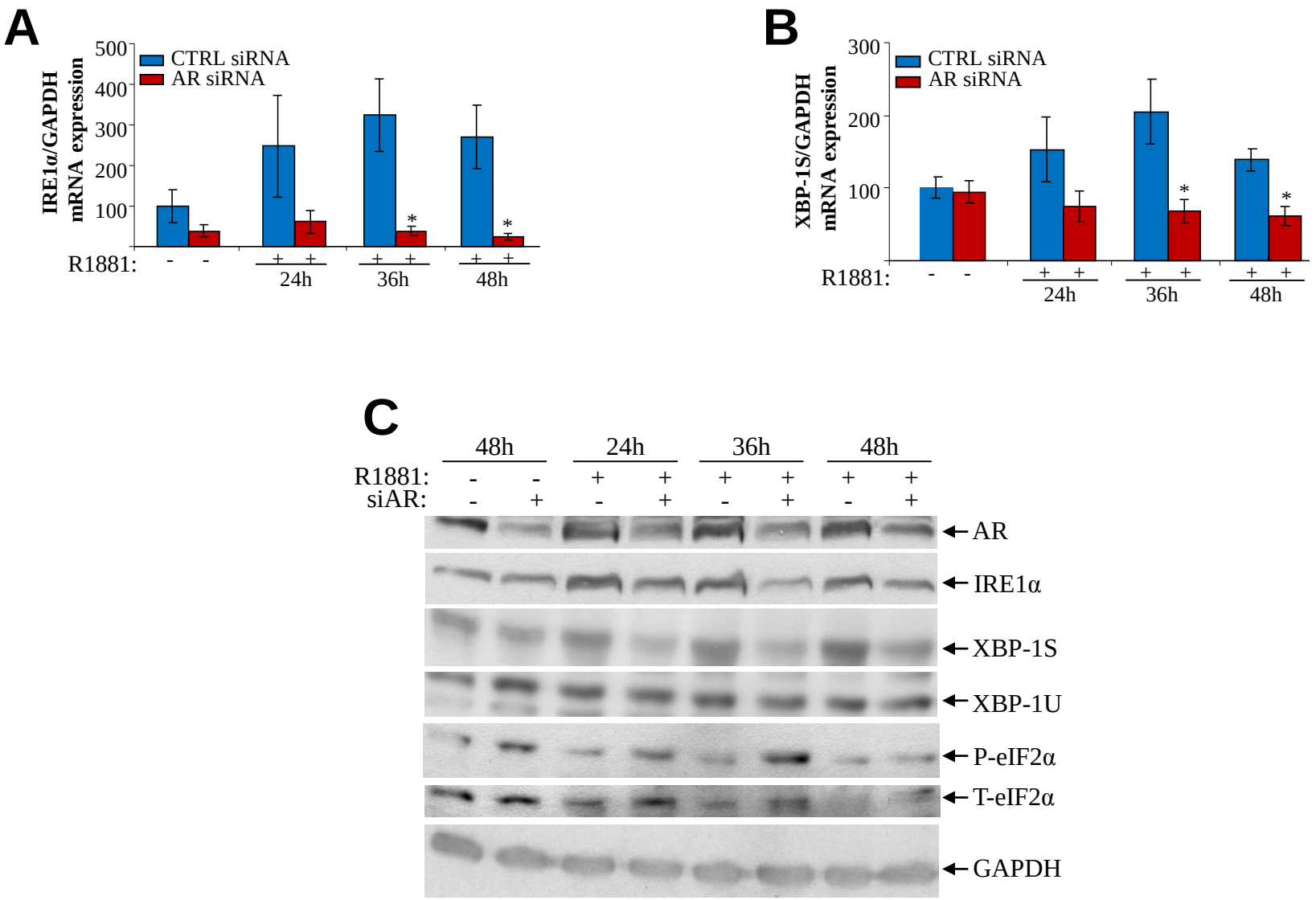




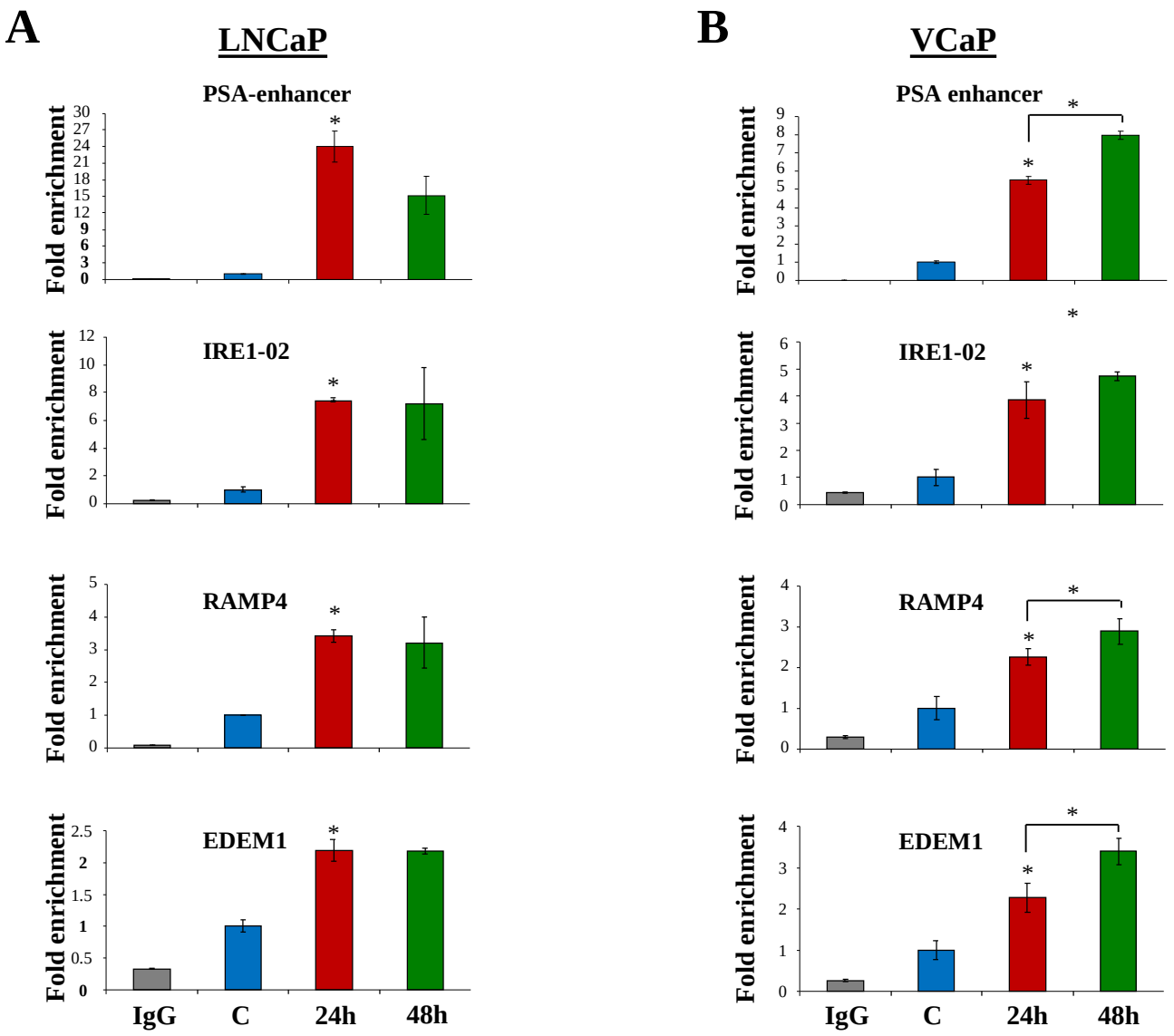
Androgens activate the IRE1 pathway in prostate cancer xenografts



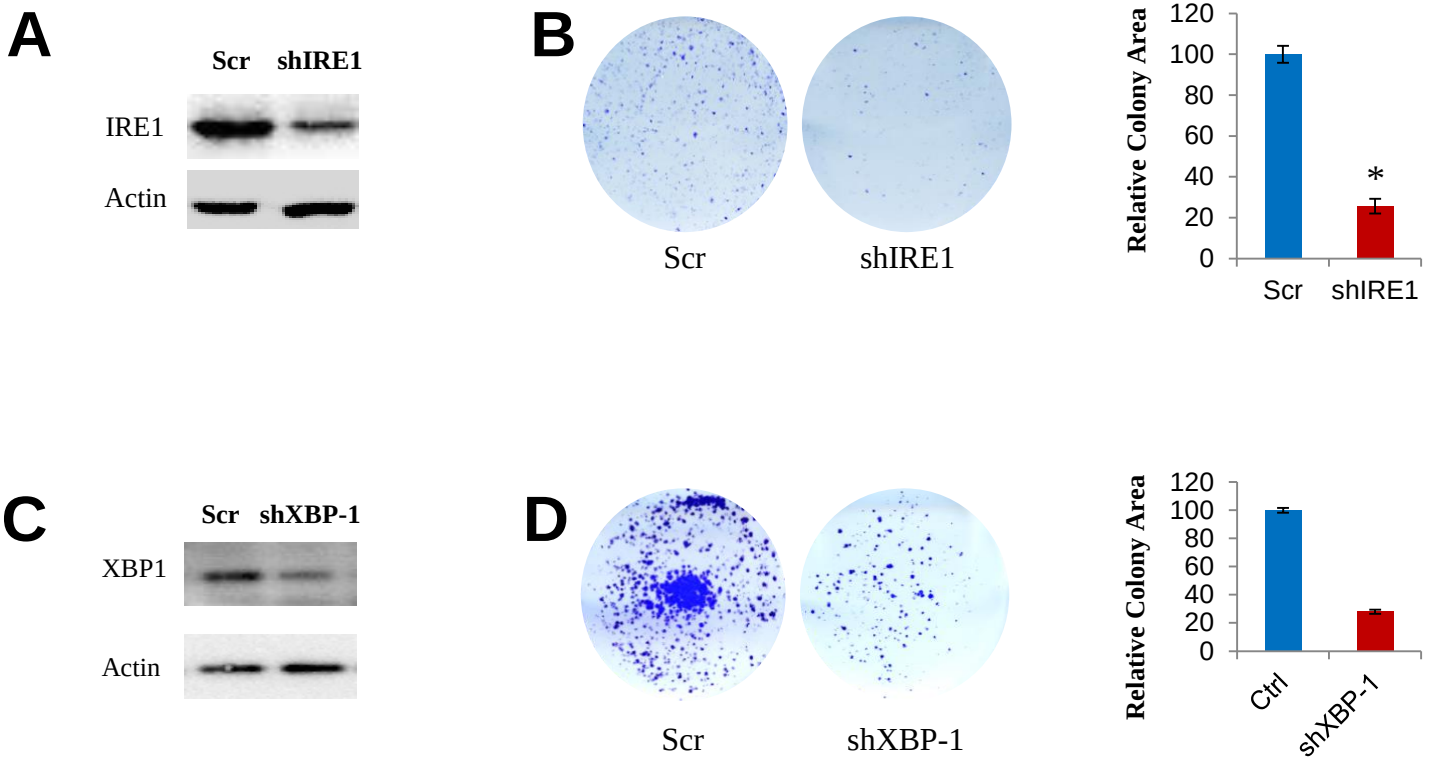
AR is required for androgen regulation of ER stress in prostate cancer cells



AR binds to regulatory sites in ER stress genes *in vivo*

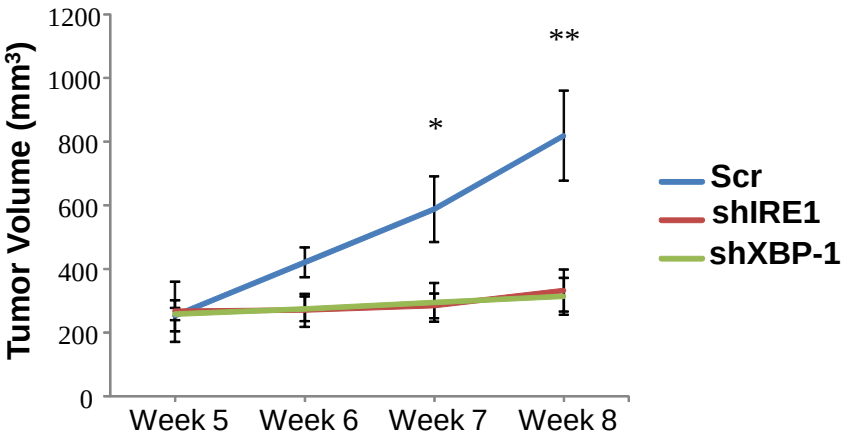


IRE1 signaling is necessary for prostate cancer growth *in vitro*

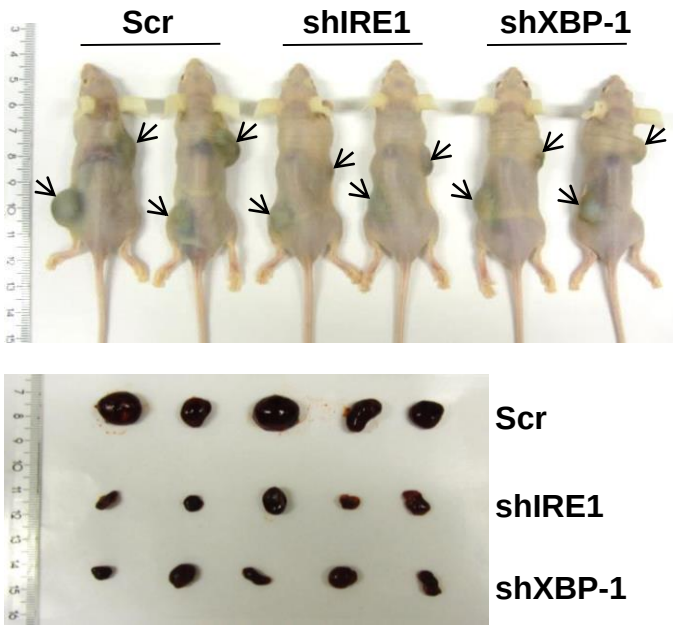


IRE1 signaling is necessary for prostate cancer growth *in vivo*

A

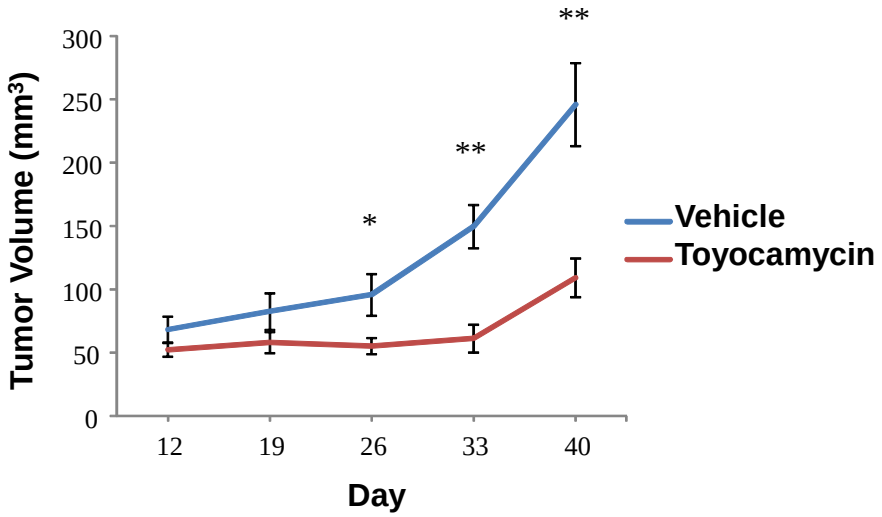


B

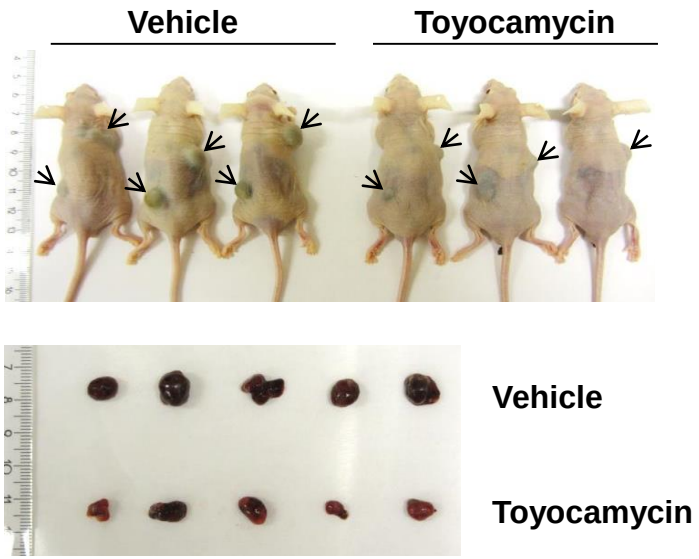


A small molecule inhibitor of IRE1 inhibits prostate cancer growth *in vivo*

A



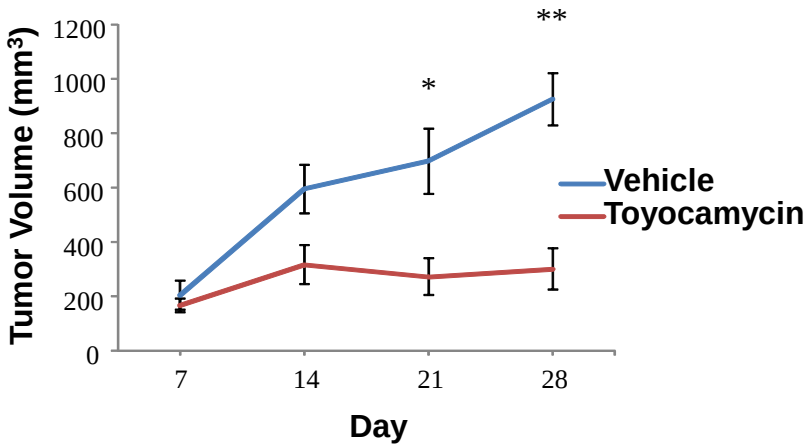
B



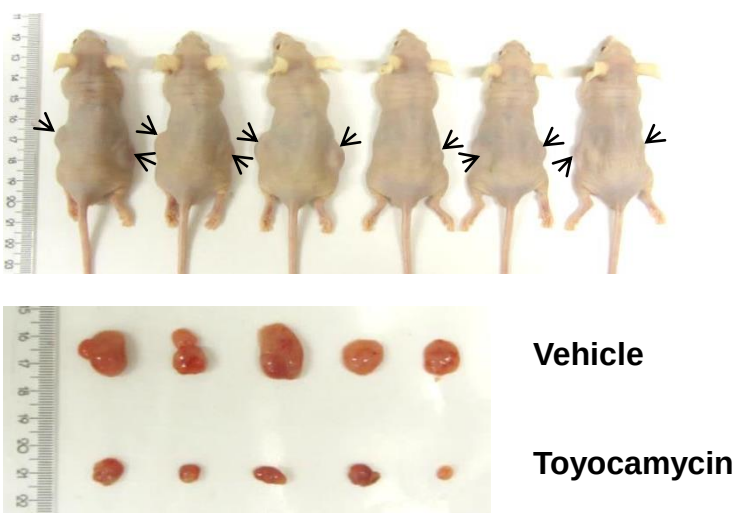
Toyocamycin: An adenosine analogue isolated from a Streptomyces species

A small molecule inhibitor of IRE1 inhibits prostate cancer growth *in vivo*

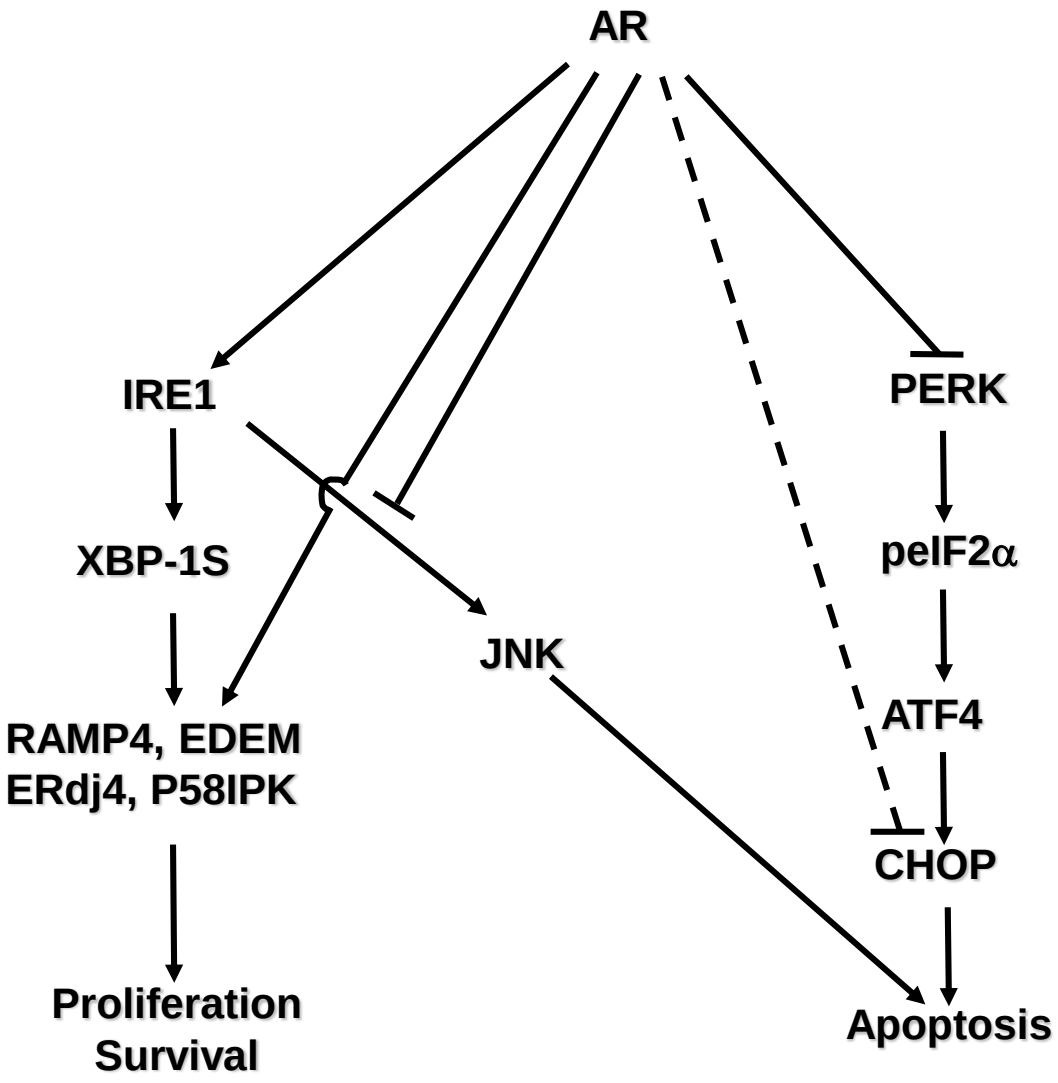
A



B

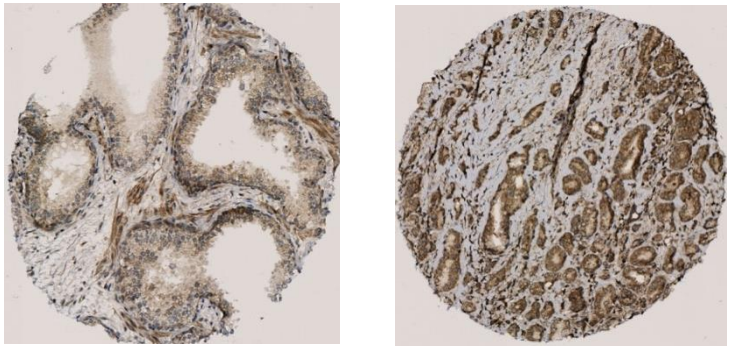


Divergent androgen regulation of ER signaling in prostate cancer

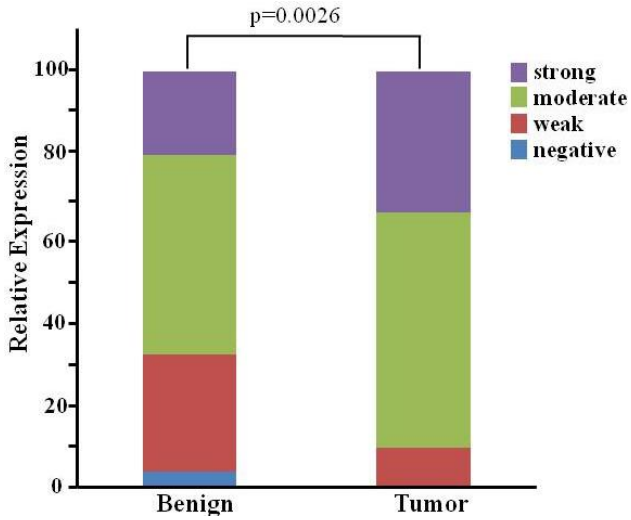


IRE1 signaling is deregulated in human prostate cancer

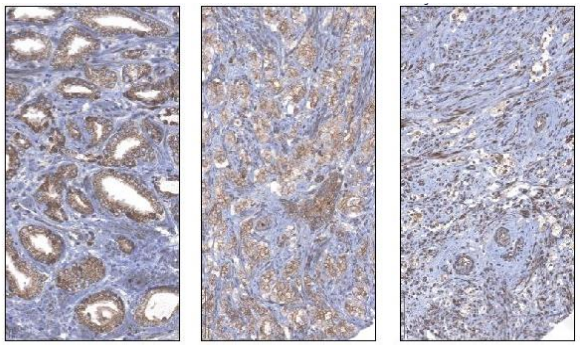
A



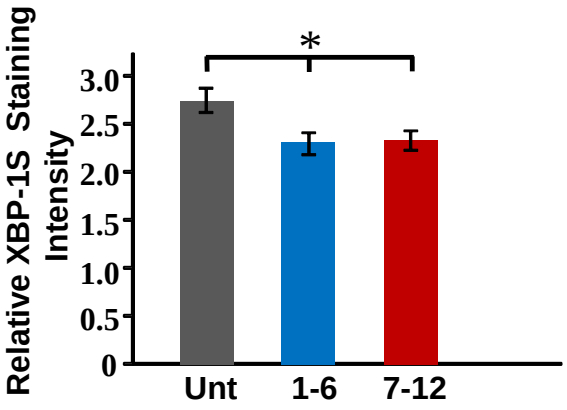
B



C



D



Future Perspectives, androgens and ER stress

- Molecular details of how IRE1 and PERK signaling are divergently regulated by AR
- Could simultaneous IRE1 pathway inhibition and PERK activation have added effect?
- Targeted nanoliposomal siRNA mediated knockdown of IRE1 signaling
- Further development of small molecule inhibitors of IRE1 signaling and clinical testing

Acknowledgements

U. of Oslo
Haiying Kong
Martina Tesikova
Su Qu
Ling Wang
Jin Yang
Zeynep Kirli
Sen Zhao

NCMM, Oslo
Ian G. Mills

Vancouver Pros. Centre
Ladan Fazli
Paul Rennie

U. of Porto/Port. Onc. Inst
Carmen Jerónimo
Manuel R. Teixeira

DNR, Oslo
Håvard Danielsen
Alexander Kristian
Gunhild Mælendsmo
Bjørn Risberg
Håkon Wæhre

HSPH, Boston
Gokhan Hotamisligil
Katy Wellen

MD Andersen Cancer Center
Neslihan Alpay
Kivanc Gorgulu
Gabriel Lopez-Berestein
Bulent Ozpolat
Anil Sood
Ibrahim Tekedereli
Erkan Yuca













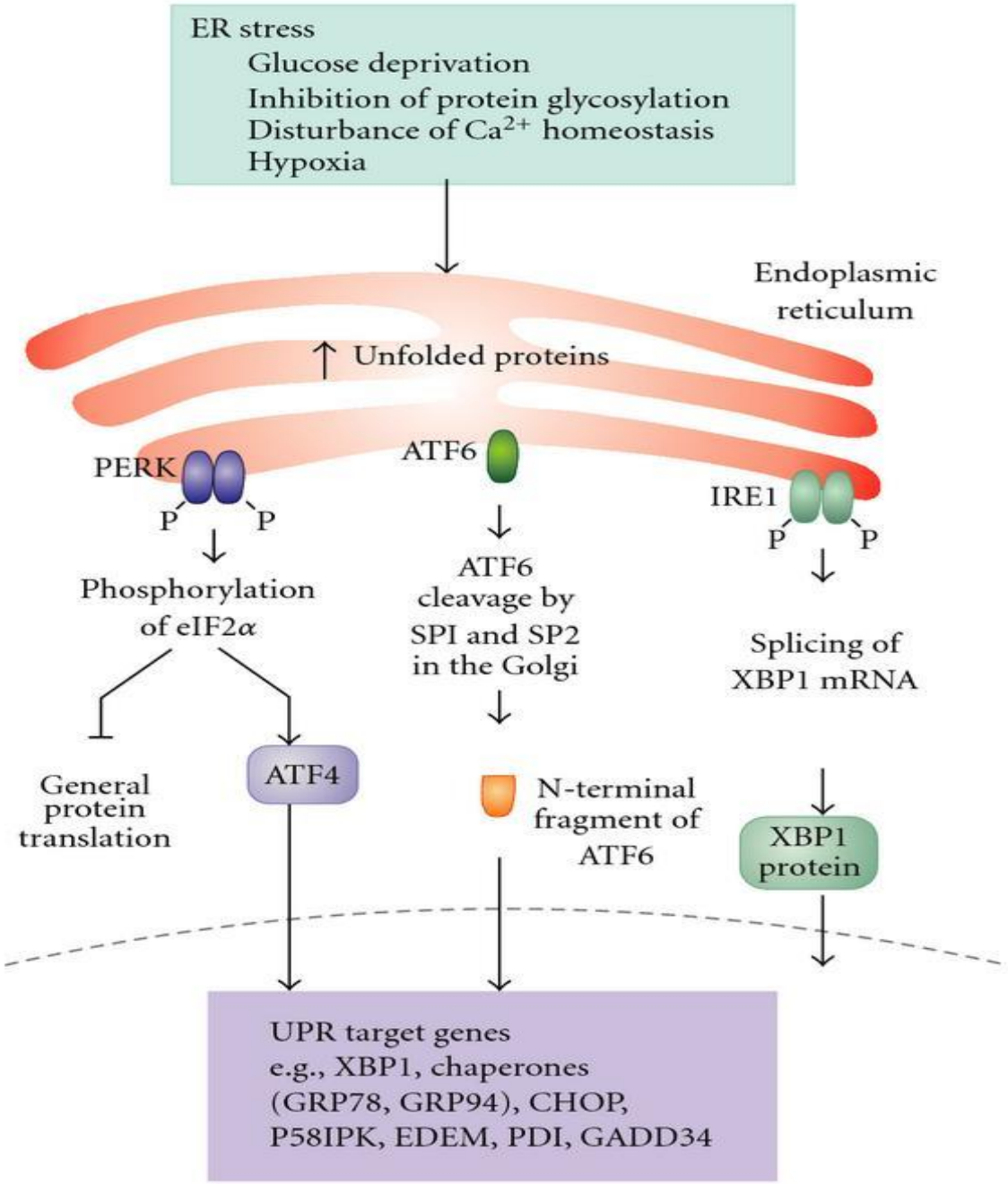
ER stress and cancer

- ER stress is increased in hypoxic tumors to help protein folding and to protect the tumor (Marx 2006).
- PERK-/- xenografts grow poorly (Bi *et al,* 2005).
- XBP1 mediates survival of cells under hypoxia and is essential for tumor growth (Koong *et al,* 2006).
- UPR contributes to resistance for some cancer drugs, but some studies have also shown that UPR increases the sensitivity to chemotherapeutic drugs (Backer *et al*., 2009).

ER stress in prostate cancer

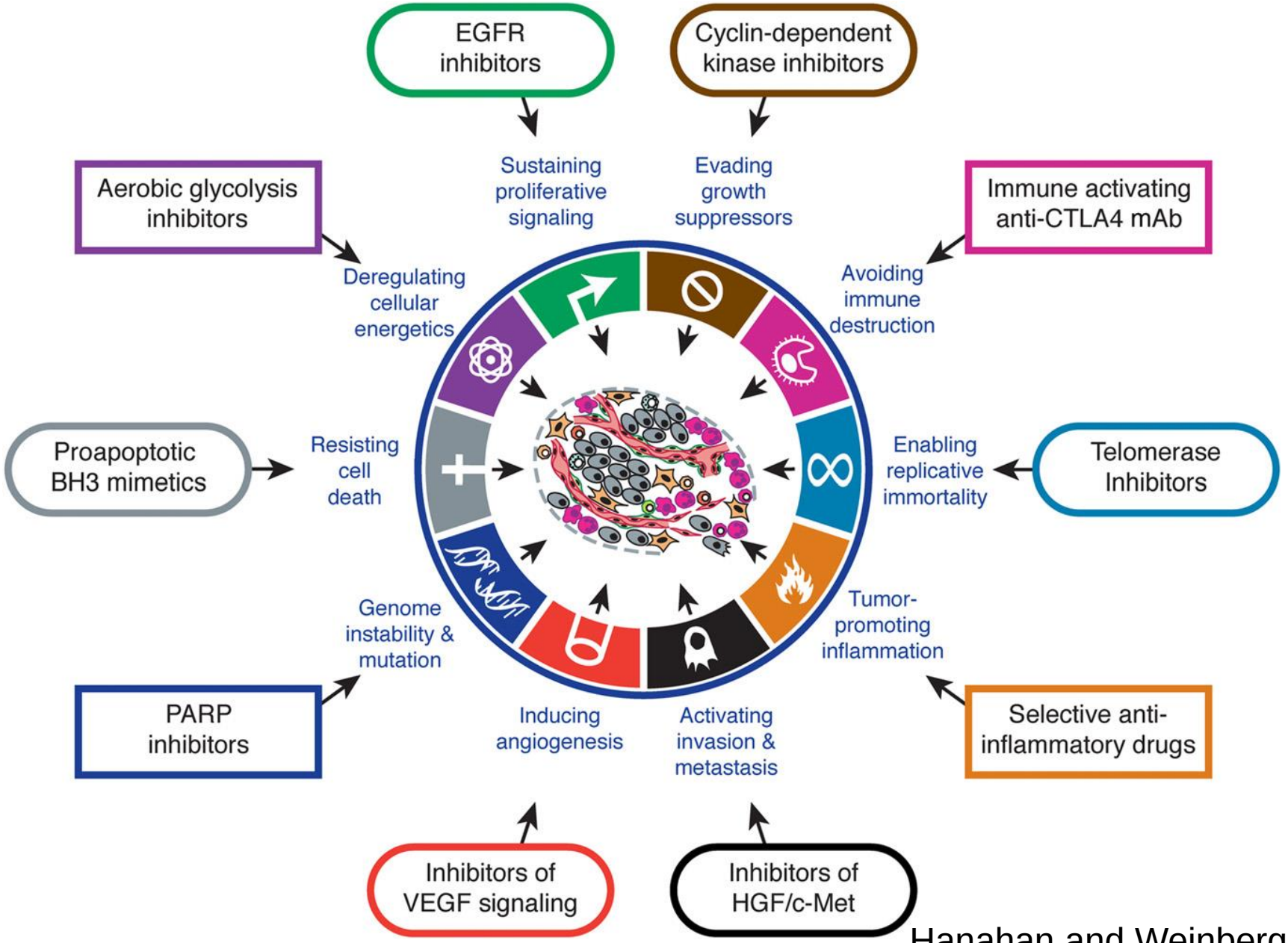
- Increased expression of BiP/GRP78 is associated with greater risk of PCa recurrence and worse survival (Pootrakul *et al.*, 2006).
- BiP/GRP78 is upegulated in PCa and correlates with recurrence and survival (Daneshmand *et al.*, 2007).
- Down-regulation of BiP/GRP78 sensitizes resistant prostate cancer cells to gene-therapeutic overexpression of REIC/Dkk-3 (Tanimoto *et al*., 2010).

Endoplasmic reticulum (ER) stress

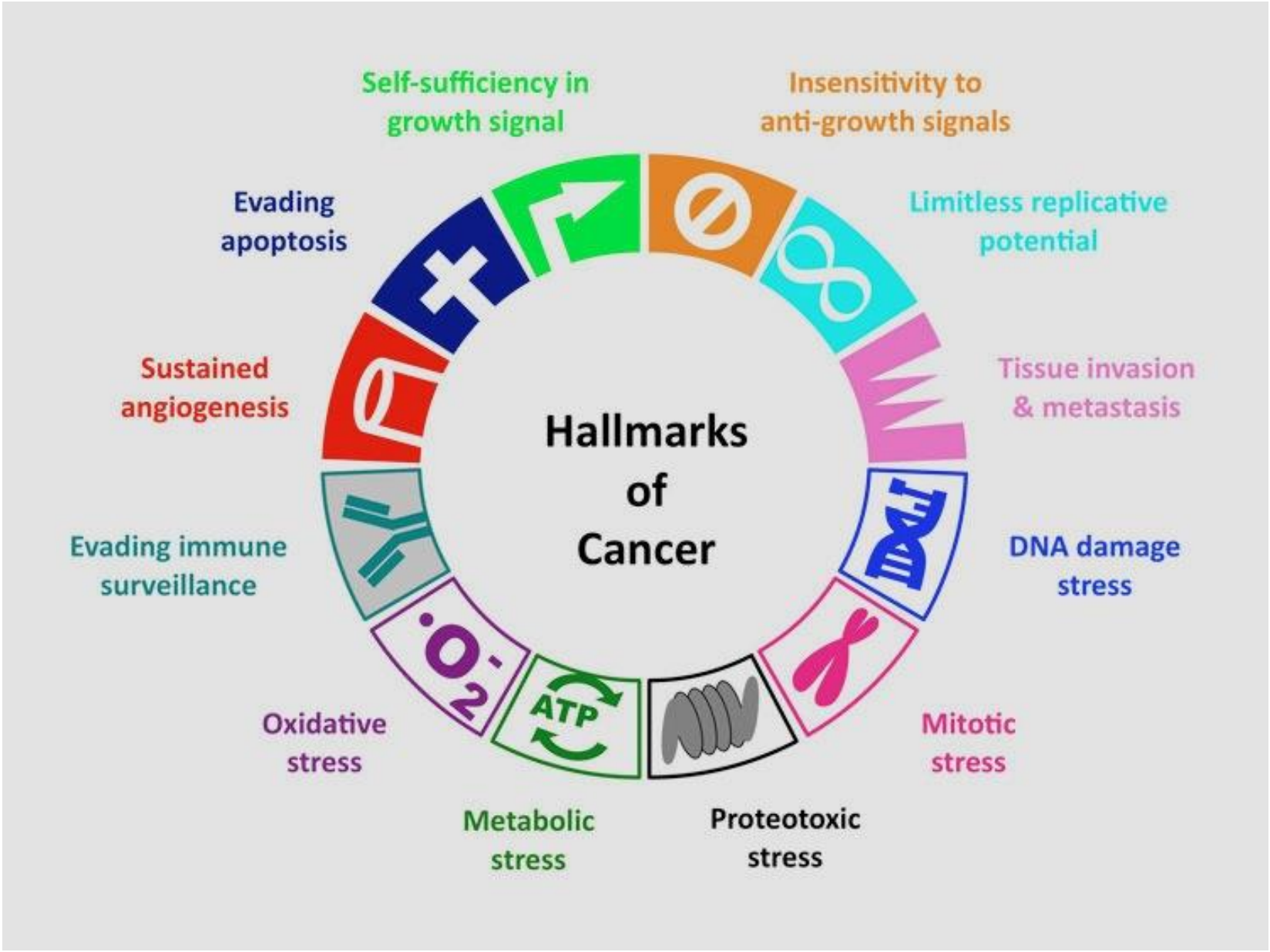


(Fulda et al. 2009)

Hallmarks of cancer

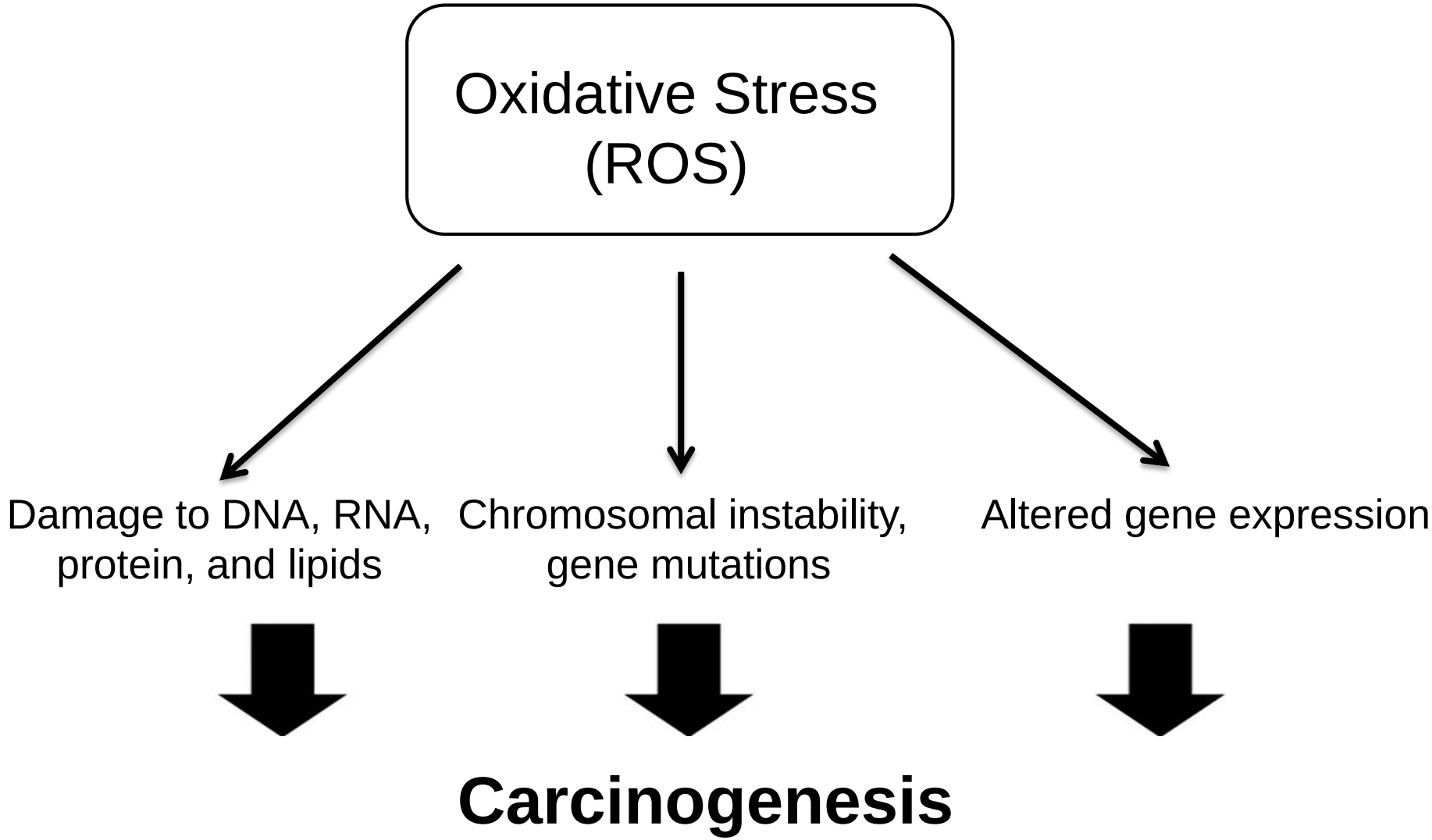


Hanahan and Weinberg, 2011



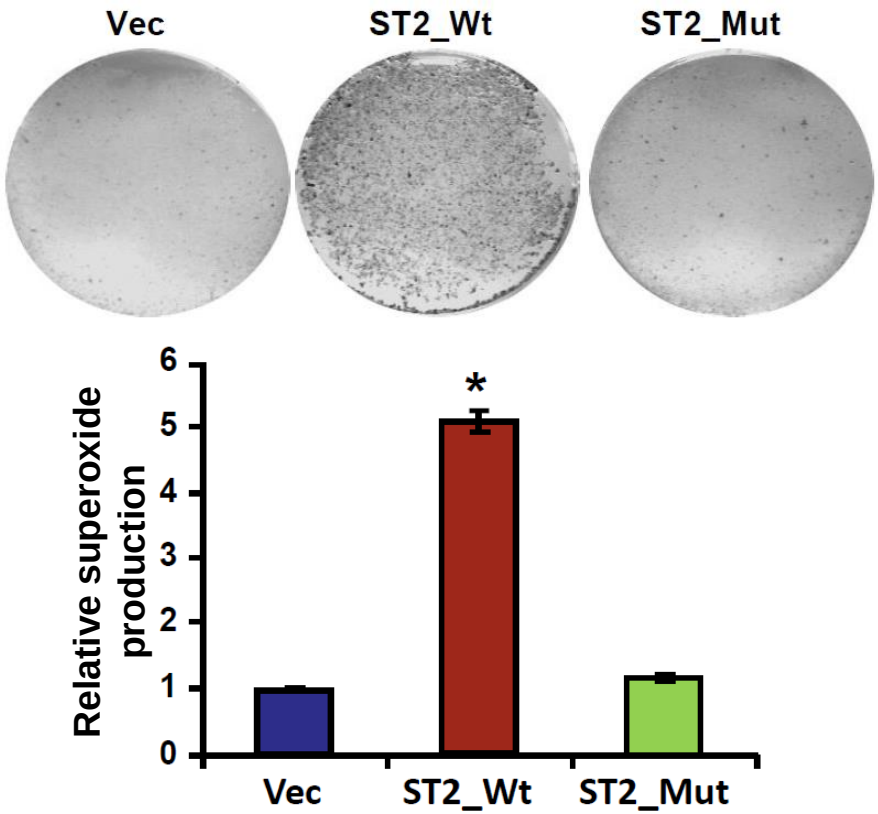
Hanahan and Weinberg, Cell. 2011.
Kroemer and Pouyssegur, Cancer Cell. 2008.
Stephen Elledge: Cancer Biology: Theoretical perspectives

Oxidative stress and its role in cancer development



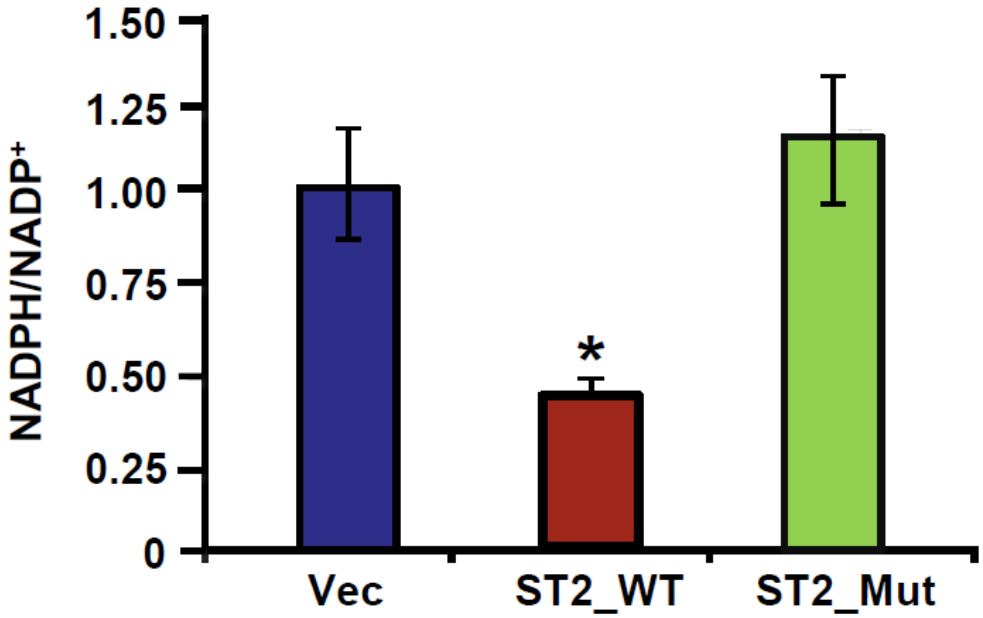
Klaunig J E et al. Toxicol Pathol. 2009

STAMP2 increases intracelular ROS levels

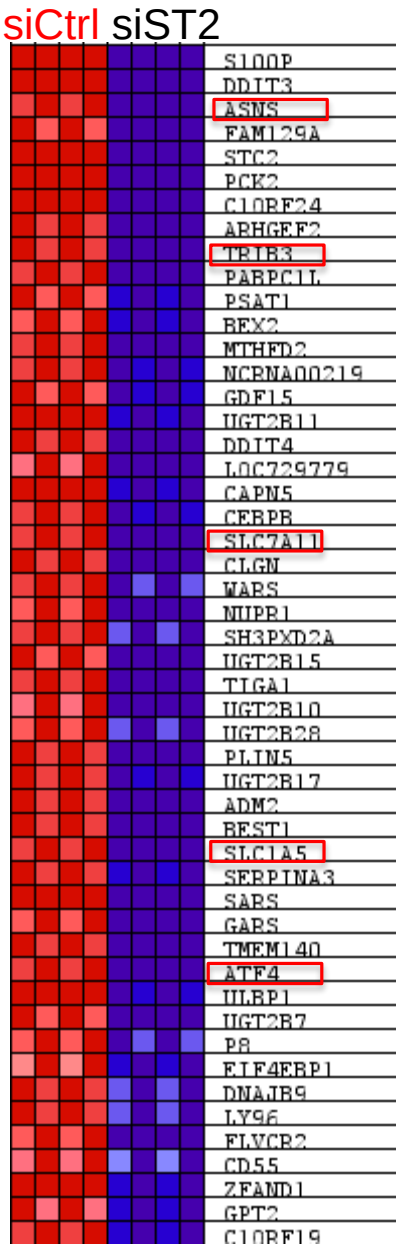


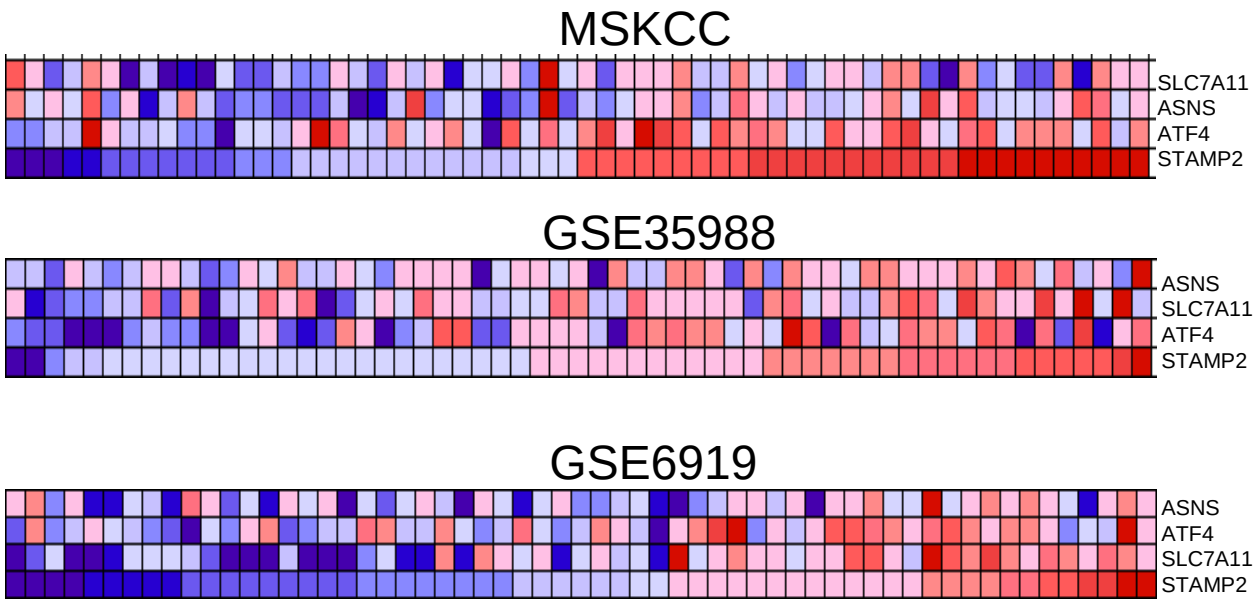
Yang et al. EMBO Mol Med, 2015

STAMP2 reduces NADPH/NADP$^+$ ratio

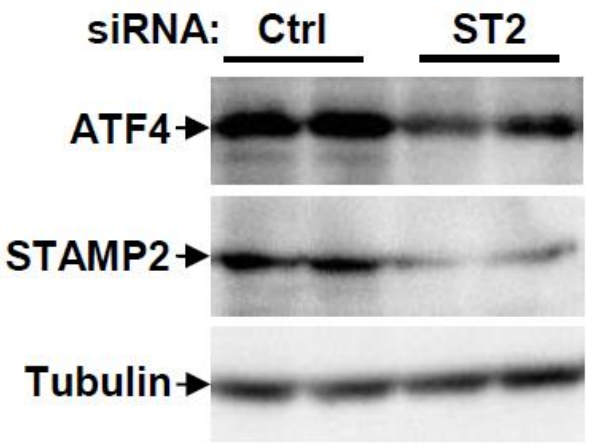


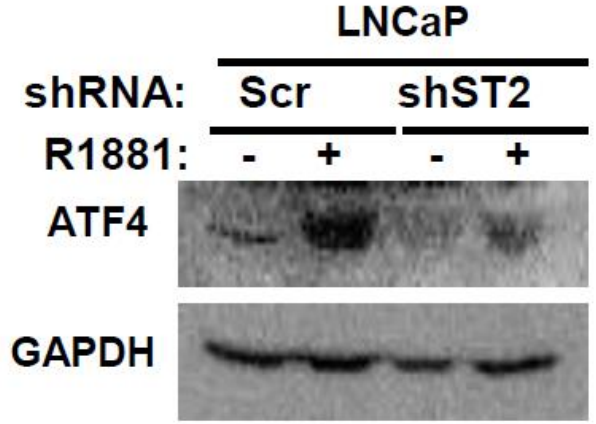
STAMP2 loss deregulates ATF4 signaling pathway

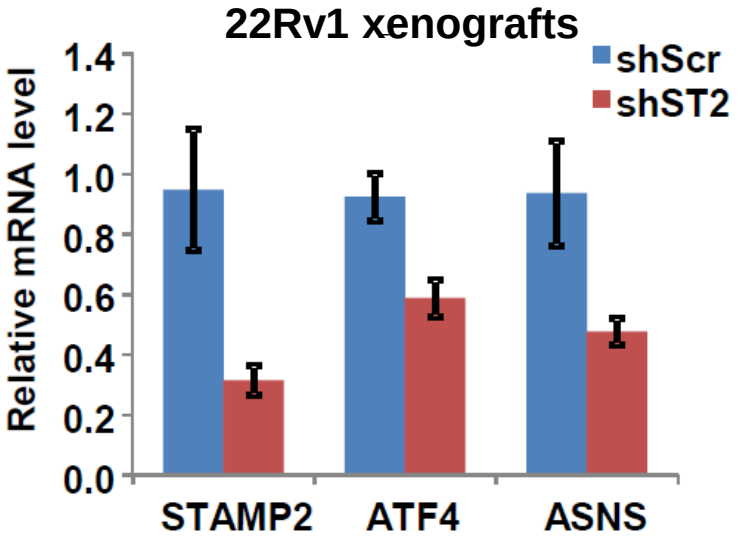




STAMP2 loss reduces ATF4 expression

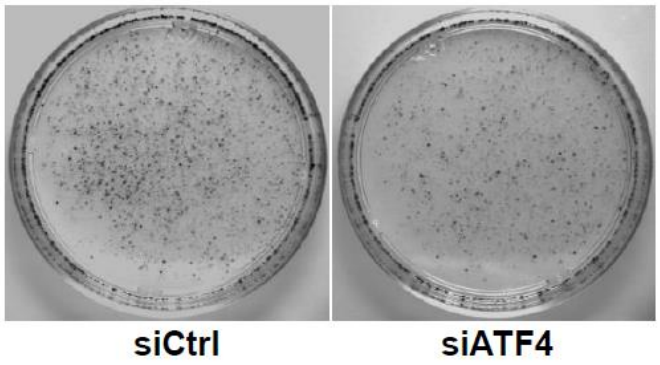


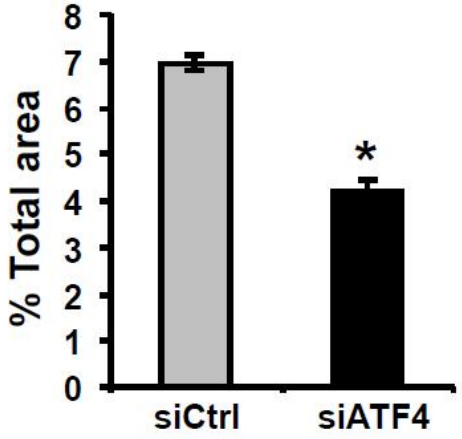




STAMP2 affects cell growth through regulating ATF4

A





B

