Unit in Biomedical Research in Urology

In search for good therapeutic targets to annihilate the resistance in aggressive prostate cancer

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Group Leader
Cell Signaling and Cancer Progression

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Generalitat de Catalunya
Departament d'Economia i Coneixement

Major aims of our research

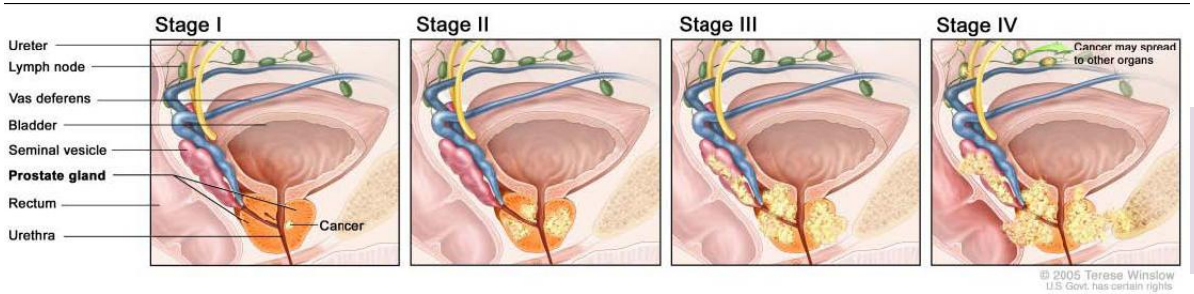
- To identify PCa markers helpful for the diagnosis and prognosis of the aggressive disease.
- To understand the mechanisms implicated in PCa progression to a resistant disease. This may lead to discover more efficient therapeutic targets to eliminate the recurrent metastatic cancer.

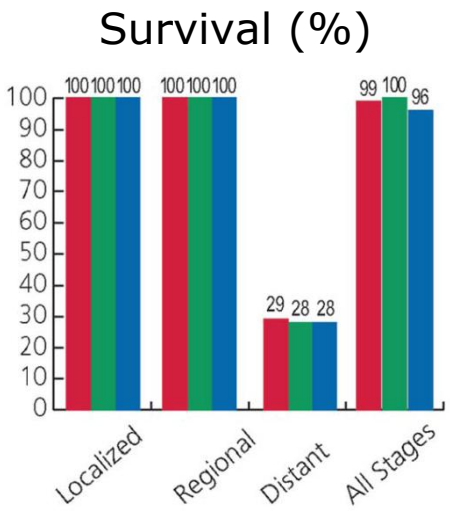
- Discovery of the oncoprotein PTOV1, hints about its function and mechanisms of action in aggressive PCa, and in the resistance to chemotherapy.

- Models systems to study in vitro the biology of PCa and their response to treatments.

PROSTATE CANCER

- Most common neoplasia among males in the western Countries.
- Second leading cause of death from cancer in men.





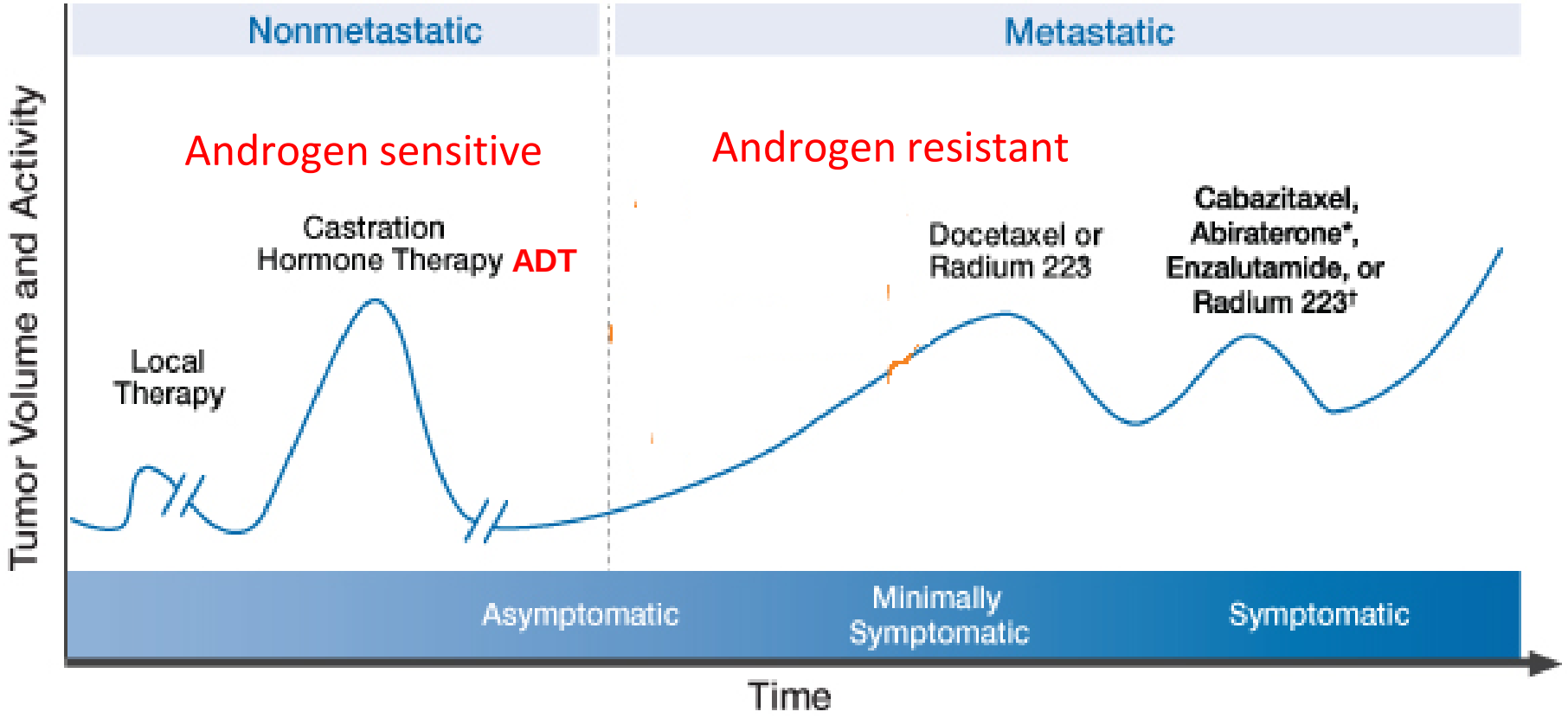
Estimated New Cases*

			Males
Prostate	241,740	29%	
Lung & bronchus	116,470	14%	
Colon & rectum	73,420	9%	
Urinary bladder	55,600	7%	
Melanoma of the skin	44,250	5%	
Kidney & renal pelvis	40,250	5%	
Non-Hodgkin lymphoma	38,160	4%	
Oral cavity & pharynx	28,540	3%	
Leukemia	26,830	3%	
Pancreas	22,090	3%	
All Sites	**848,170**	**100%**	

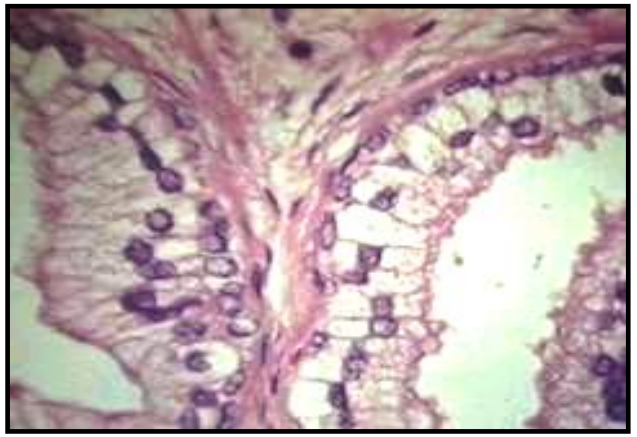
Estimated Deaths

			Males
Lung & bronchus	87,750	29%	
Prostate	28,170	9%	
Colon & rectum	26,470	9%	
Pancreas	18,850	6%	
Liver & intrahepatic bile duct	13,980	5%	
Leukemia	13,500	4%	
Esophagus	12,040	4%	
Urinary bladder	10,510	3%	
Non-Hodgkin lymphoma	10,320	3%	
Kidney & renal pelvis	8,650	3%	
All Sites	**301,820**	**100%**	

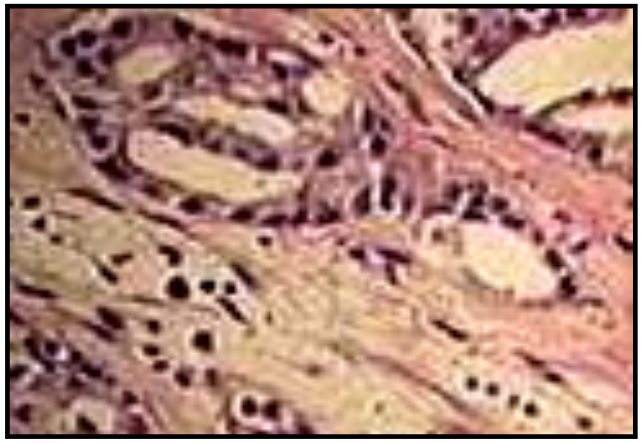
Therapeutic approaches for Prostate Cancer progression



Progression of human Prostate Cancer

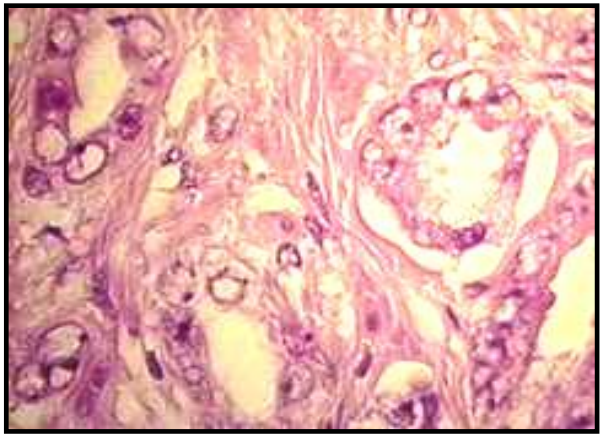


Normal Prostate



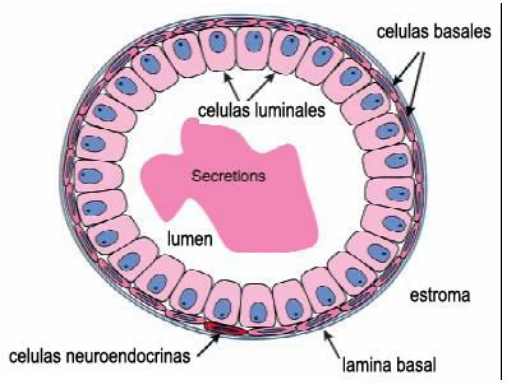
Pre-malignant

(HG-PIN)



Localized Tumor
Androgen-independent

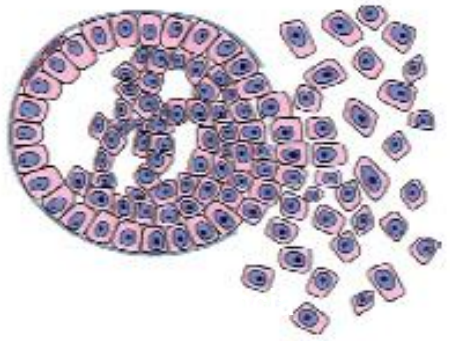
Normal Epithelia



HG-PIN

Localized Tumor

Metastasis



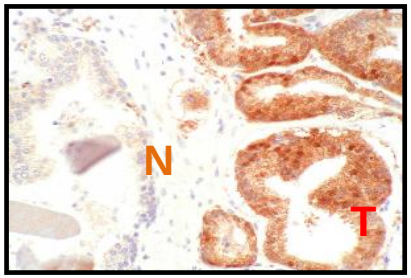
Discovery of the oncoprotein PTOV1

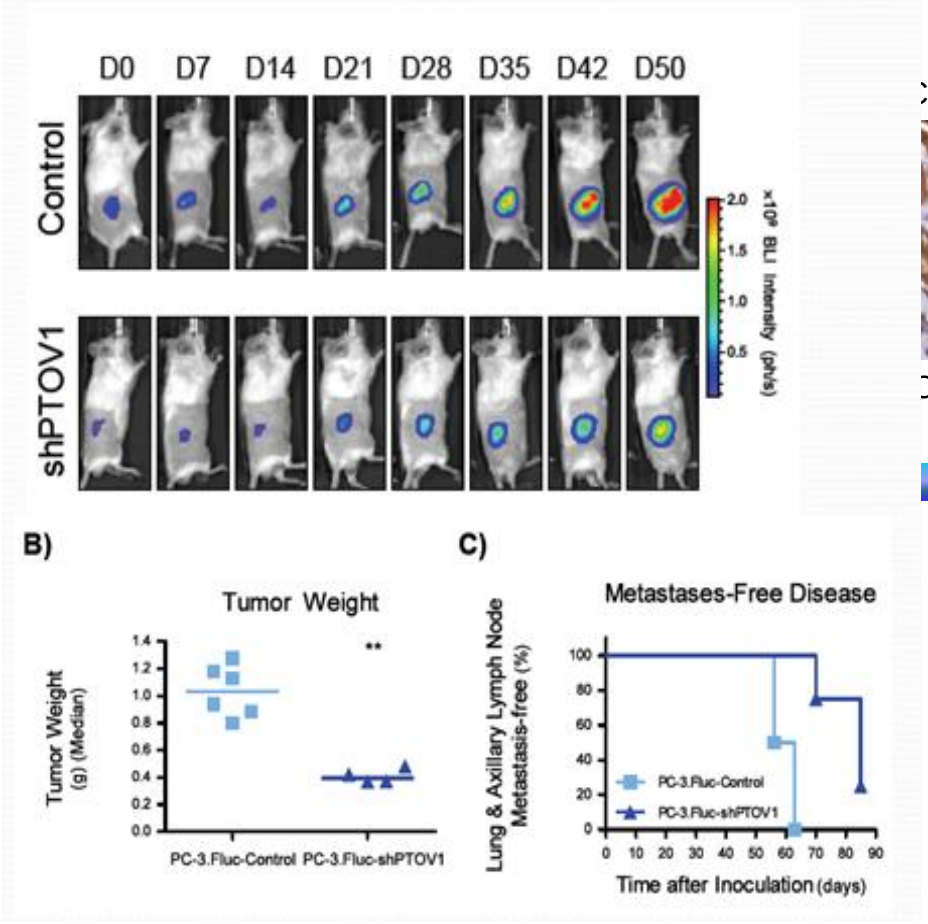
Function and mechanisms of action in aggressive PCa, and in the resistance to chemotherapy

Discovery of the oncoprotein Prostate Tumor Overexpressed-1, PTOV1

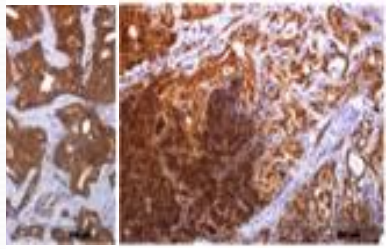
PTOV1 is required for full tumor growth and metastasis of PC3 cells

Human Prostate Cancer





Cancer Progression



Ca Met

Metastasis

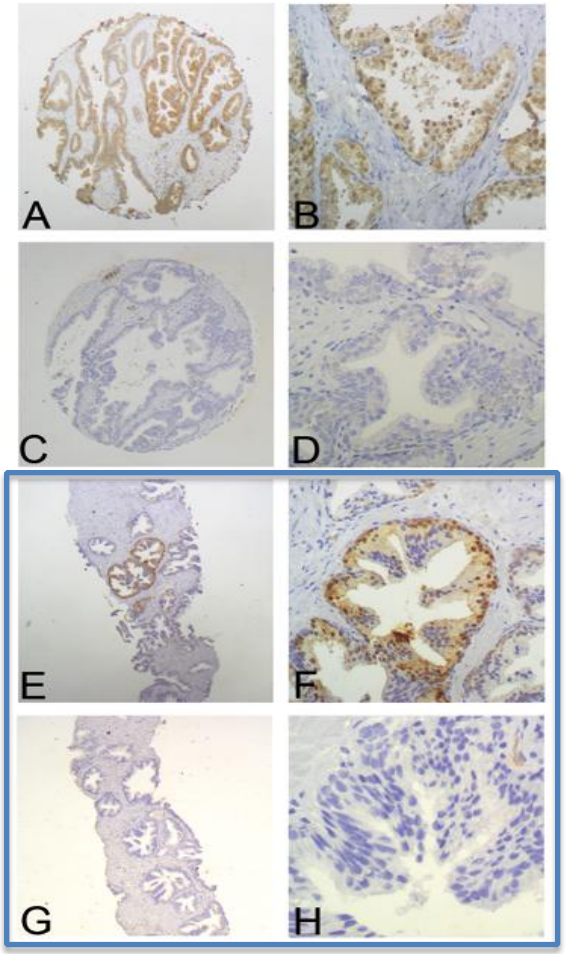
The expression of PTOV1 in pre-neoplastic lesions (HGPIN) found in needle biopsy is associated to the presence of cancer.

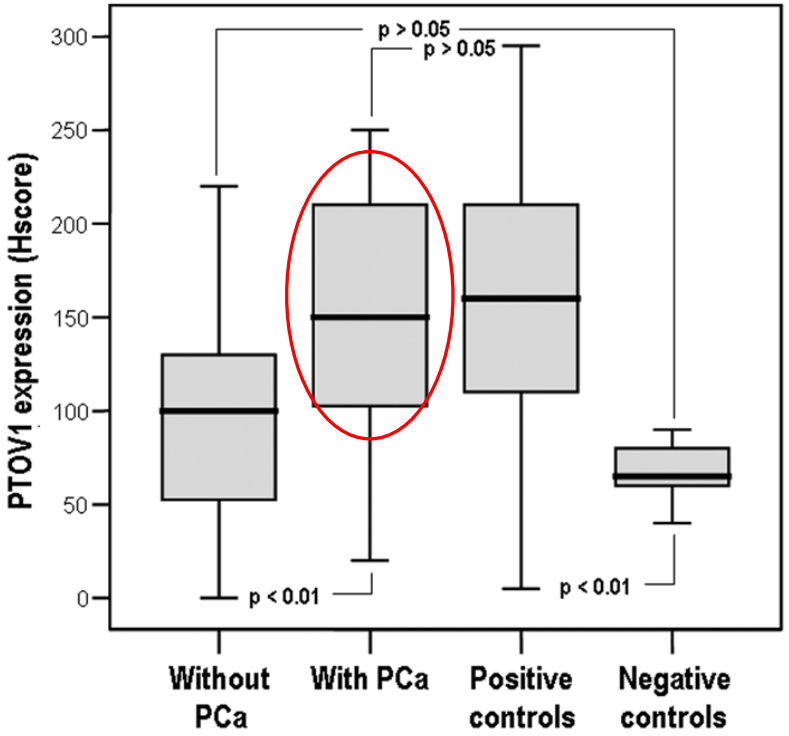
Positive control
Prostate cancer
HG-PIN

Negative control
Normal prostate
HG-PIN

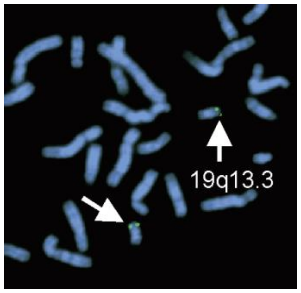
Cancer

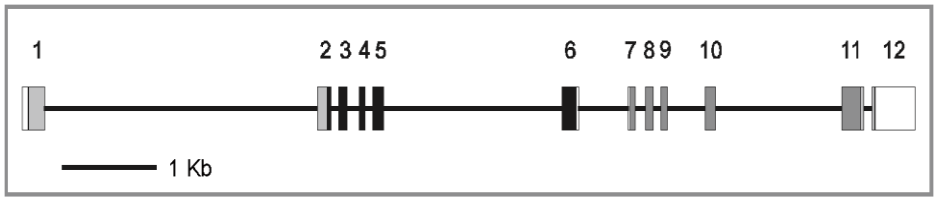
No cancer

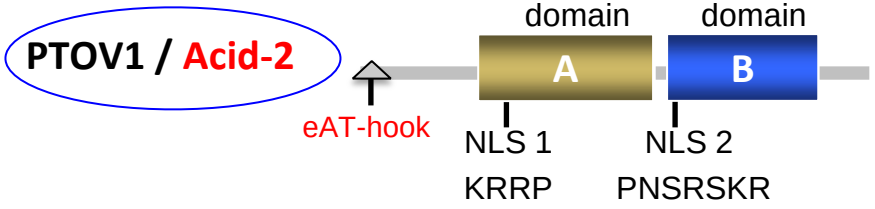




PTOV1: A NEW GENE FAMILY





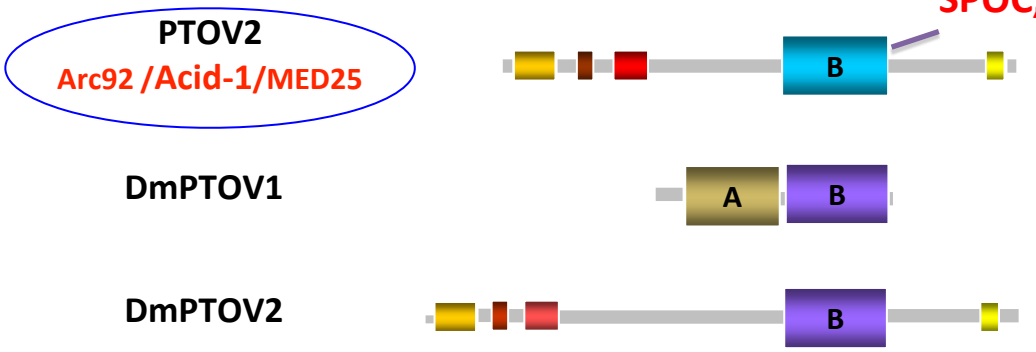


Benedit et al., Oncogene, 2001

Wang et al., BBRC 2001

Lee et al., EMBO J. 2007

SPOC, Ku70/Ku80 2011



Näär et al., Nature, 1999

Mittler et al., EMBO J. 2003

(Acc. AC013074) predicted

Adams et al., Science, 2000

PTOV1 Interacts with the Receptor of ACtivated protein Kinase C, RACK1

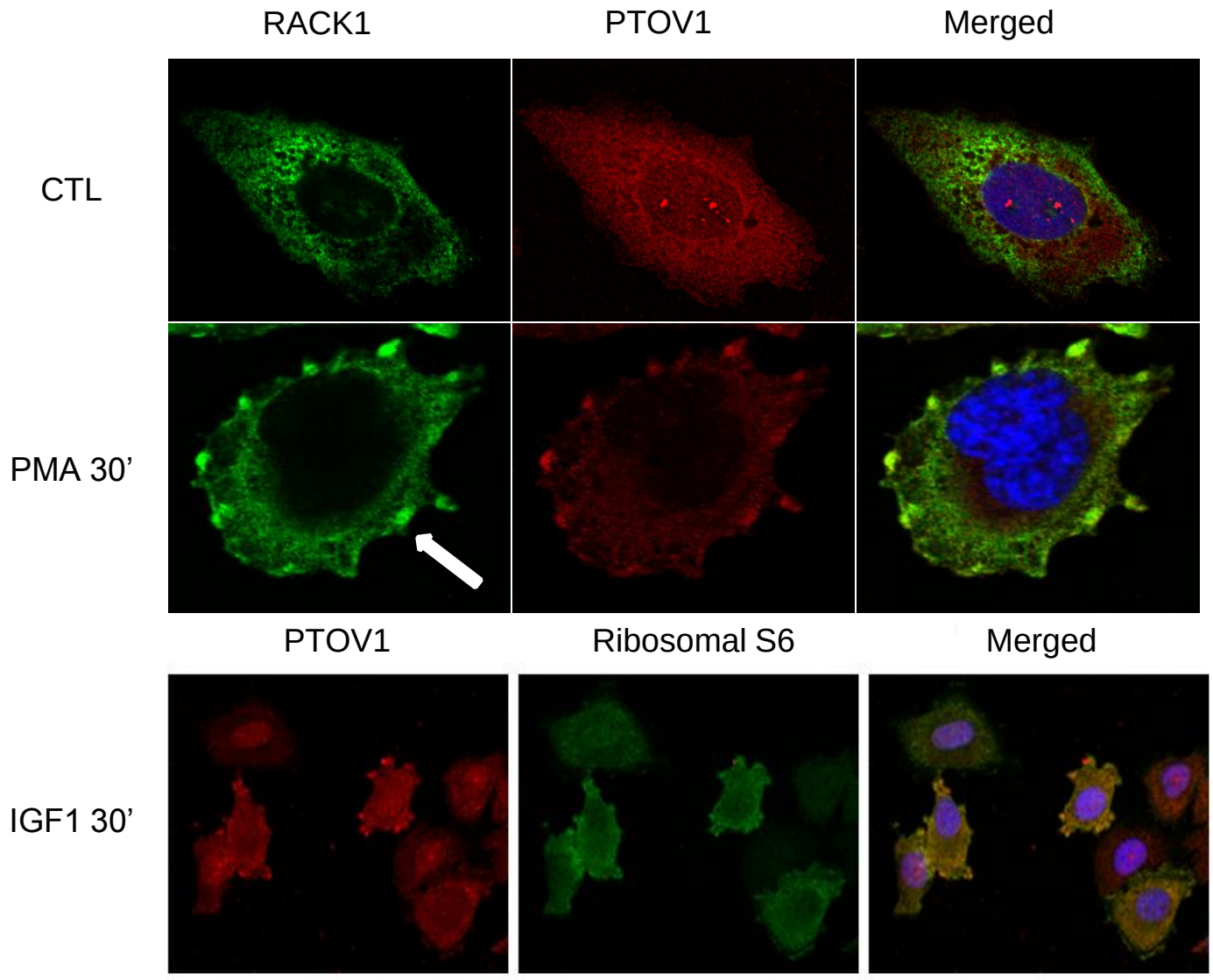
Receptor of ACtivated protein Kinase C,

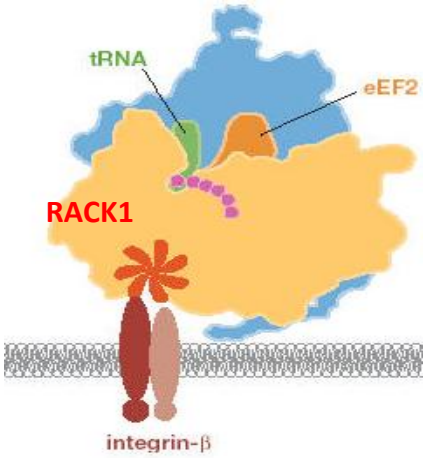
PKCβ, JNK, c-Jun, β-Integrin receptor..

- **Very conserved,** homologous to β-subunits of heterotrimeric G proteins.
- **7 WD repeats,** protein interaction domains**.**
- Regulates cell spreading, focal adhesions and cell-cell contacts
- **Binds to β-subunit of integrin receptor**: important for focal adhesion

- **Subunit of 40S ribosome**
- **Regulates translation initiation:** its recruitment of PKC allows the formation of the 80S.

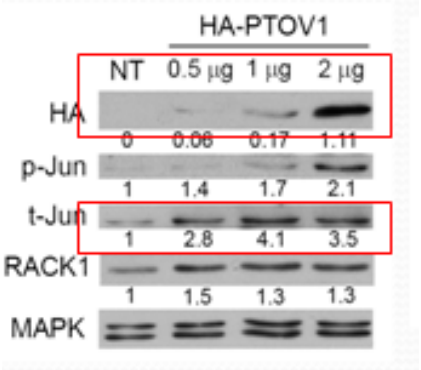
PTOV1 associate with RACK1 and ribosomes after activation with growth factors



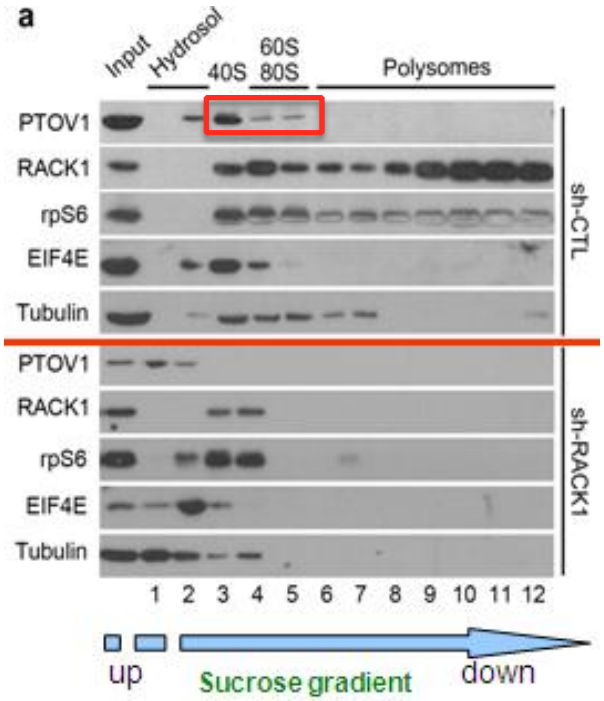


PTOV1 binds to ribosomes through RACK1 and promotes the translation of subsets of mRNAs, including *c-Jun*

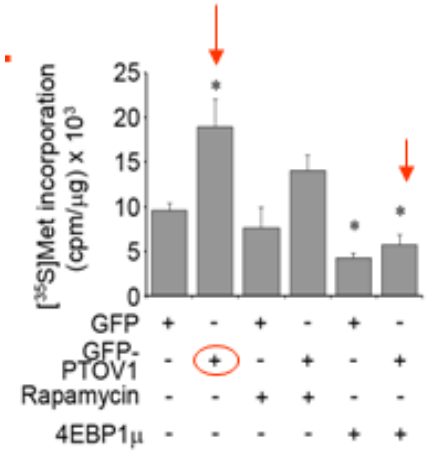
Increased PTOV1 produced increased translation of c-Jun protein



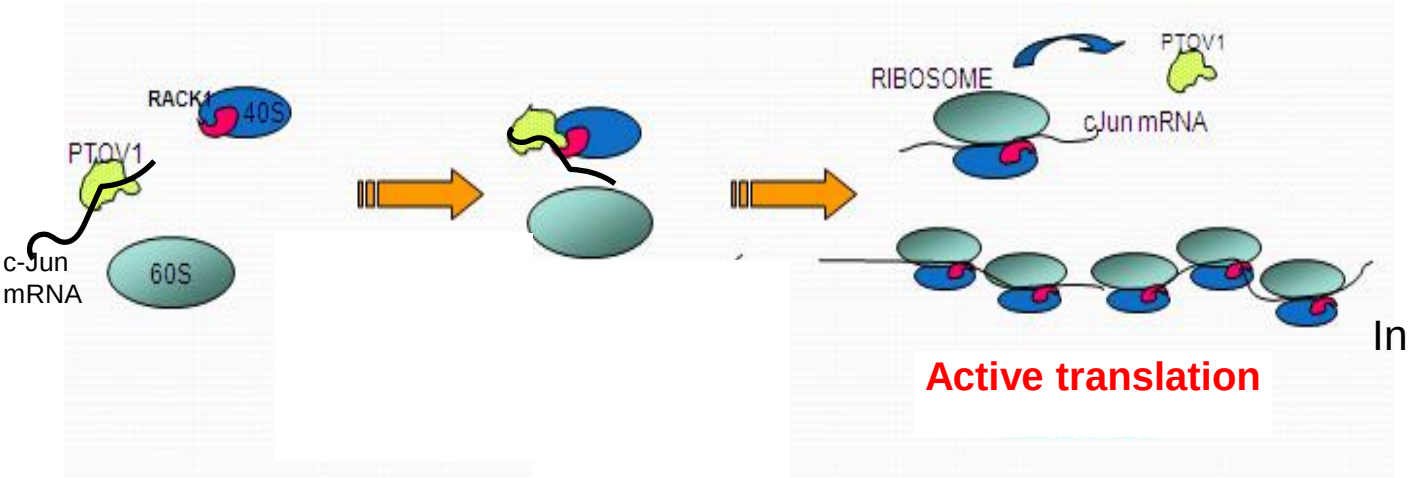
The protein binds to RACK1 on 40S ribosome, but not to polysomes

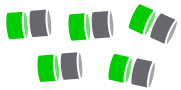


PTOV1 overexpression increased global protein synthesis



The complex PTOV1 /*c-Jun* RNA binds the 40S ribosomes and promotes translation of subsets of mRNAs, including *c-Jun*





Increased levels c-Jun/AP1 oncoproteins

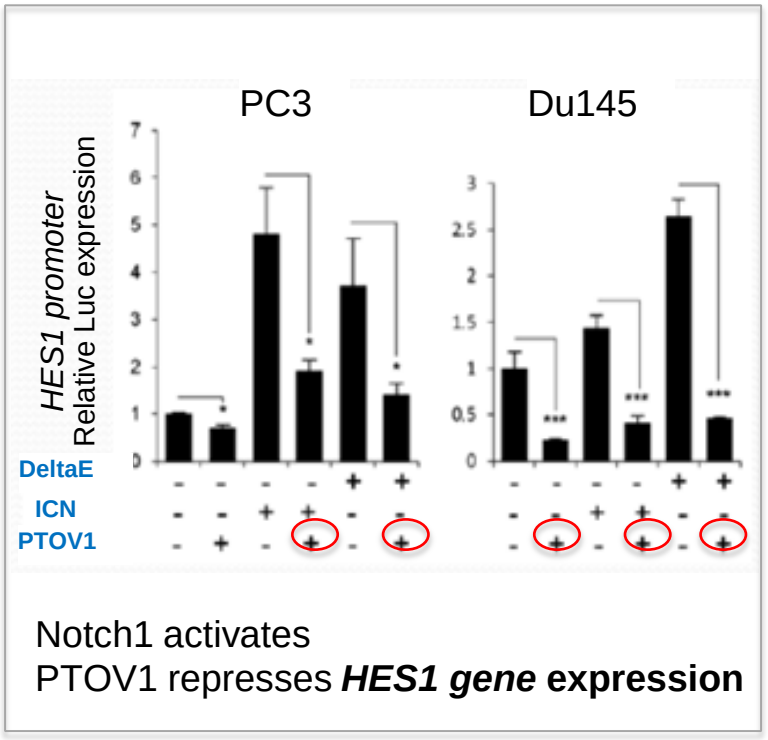
Mesenchymal gene program
(↑ Snail1, N-Cad, Vim... ↓ E-Cad)

Invasion and metastasis

IN THE NUCLEUS:

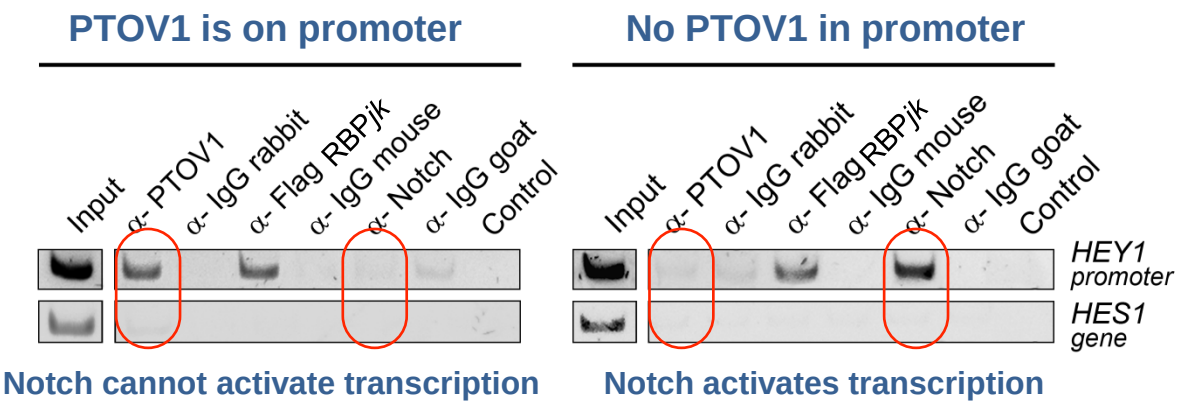
Oncogenic PTOV1 represses transcription downstream of the Notch receptor

HES1 promoter activity



Notch1 activates
PTOV1 represses ***HES1 gene*** **expression**

Chromatin Imm. Precipitation

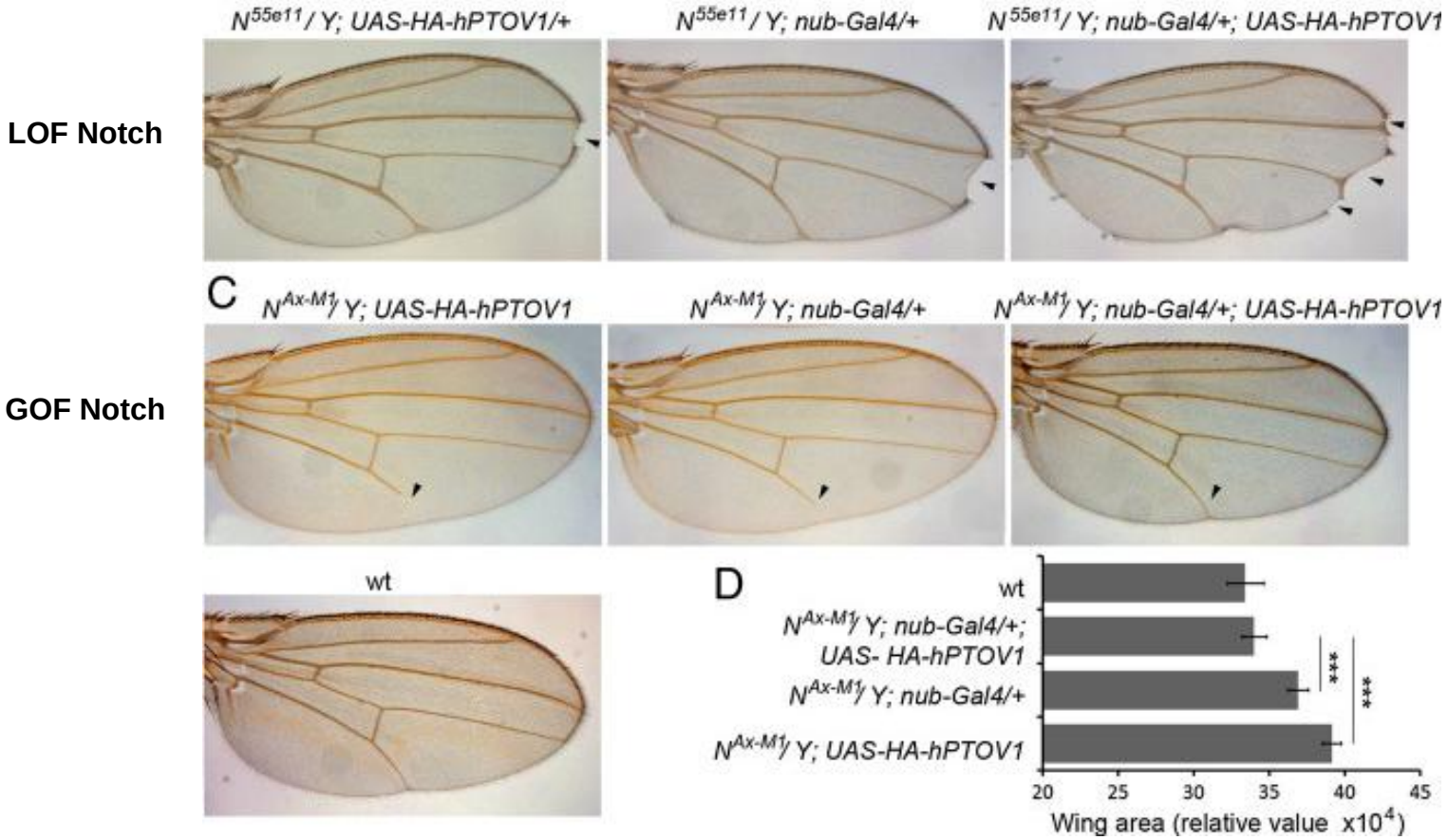


Notch cannot activate transcription

Notch activates transcription

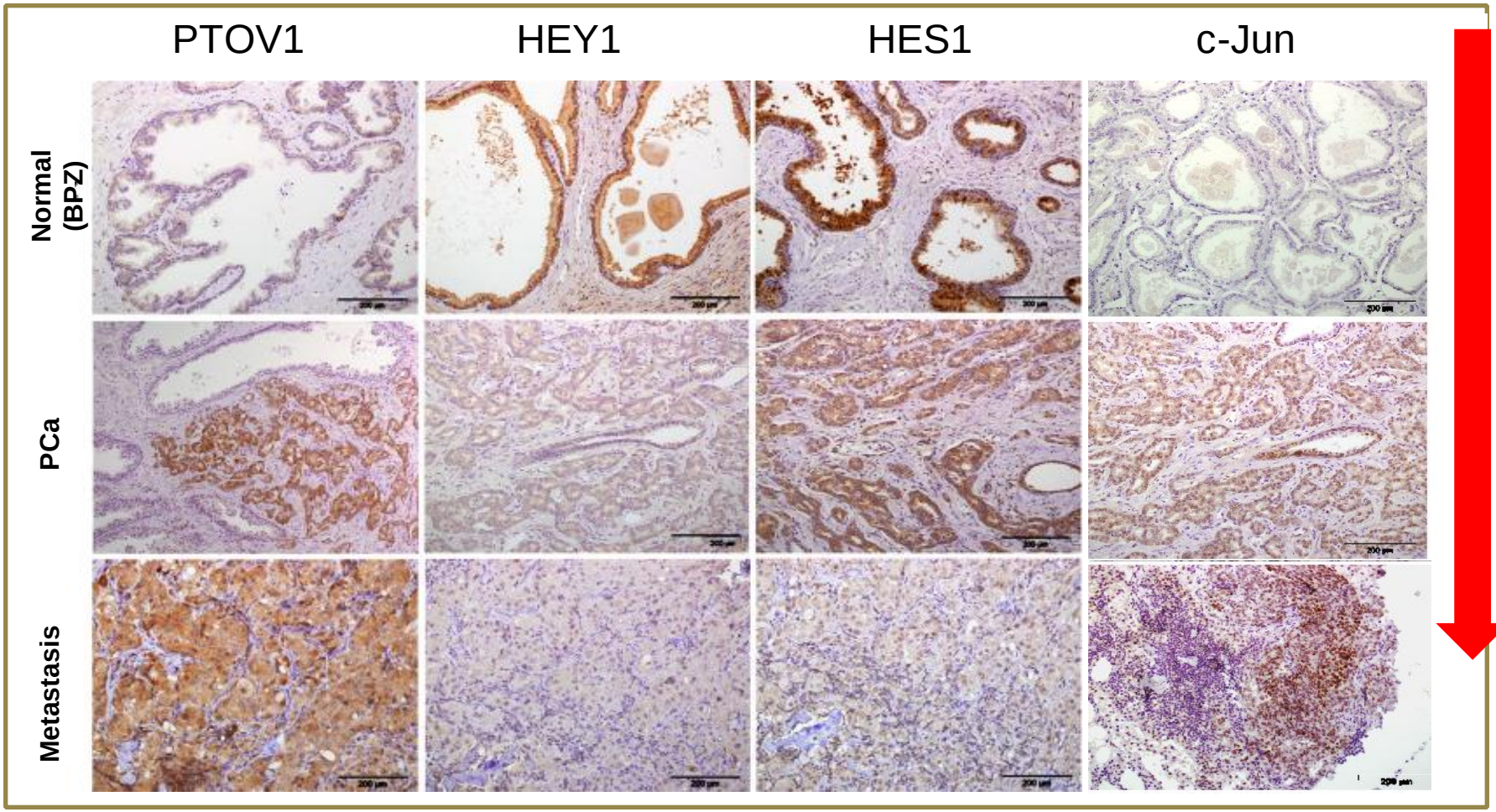
In the prostate
PTOV1 is oncogenic and
counteracts with
NOTCH tumor-suppressor functions

hPTOV1 antagonizes Notch activity in the Drosophila wing model

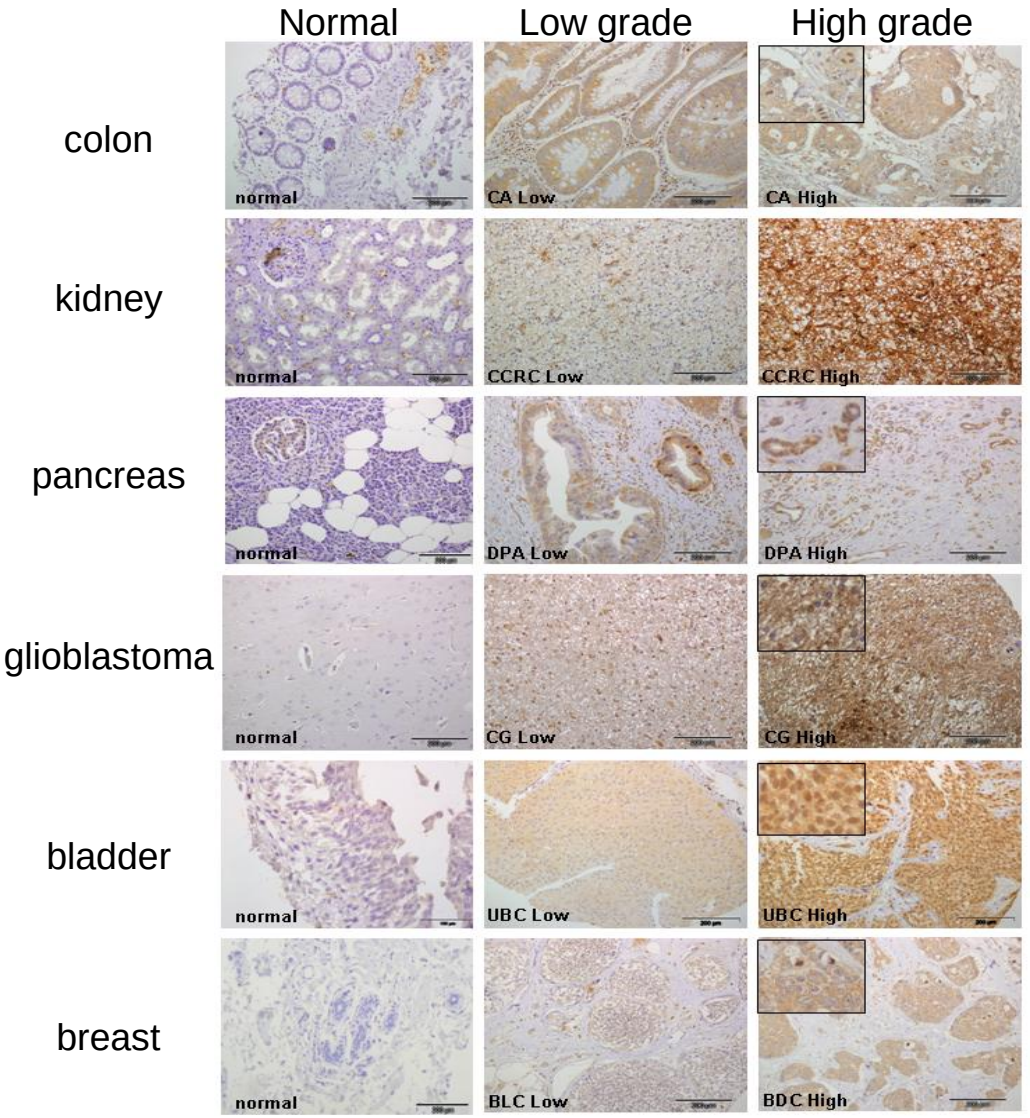


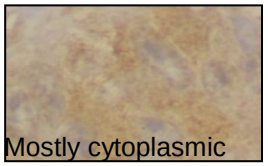
The expression of hPTOV1 increases the effects of Notch loss-of-function (LOF) and suppresses the effects of Notch gain-of-function (GOF).

Coordinated expression in prostate cancer progression



PTOV1 is overexpressed in several tumor types and significantly associated to high grade malignant tumors





Low *vs* High
p = 0.045,
Cramer's coeff. = 0.25

For bladder
Low *vs* High *p* = **0.026**,
Cramer's coeff. = **0.84**

VHIR

Vall d'Hebron Institut de Recerca

PTOV1 is an independent prognostic factor associated with cancer progression and poor survival in several neoplasias

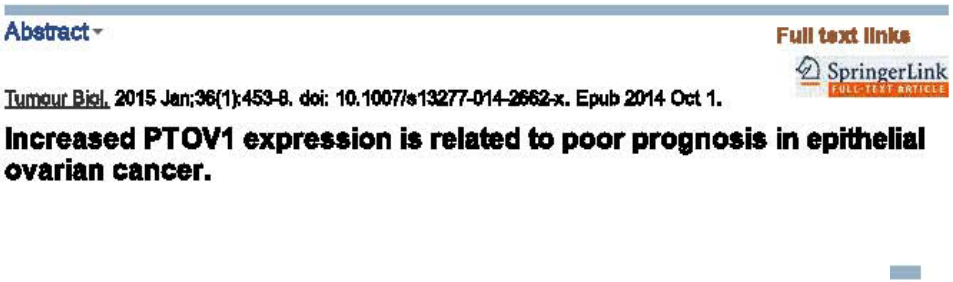
Lei et al. BMC Cancer 2014, 14:457
http://www.biomedcentral.com/1471-2407/14/457

BMC Cancer

RESEARCH ARTICLE Open Access

Overexpression of prostate tumor overexpressed 1 correlates with tumor progression and predicts poor prognosis in breast cancer

Fang



Abstract

Full text links

SpringerLink FULL-TEXT ARTICLE

Tumour Biol. 2015 Jan;36(1):453-8. doi: 10.1007/s13277-014-2662-x. Epub 2014 Oct 1.

Increased PTOV1 expression is related to poor prognosis in epithelial ovarian cancer.

Does PTOV1 have a role in the metastatic resistant prostate cancer?

THE JOURNAL OF Pathology

A Journal of The Pathological Society Understanding Disease

Original Paper

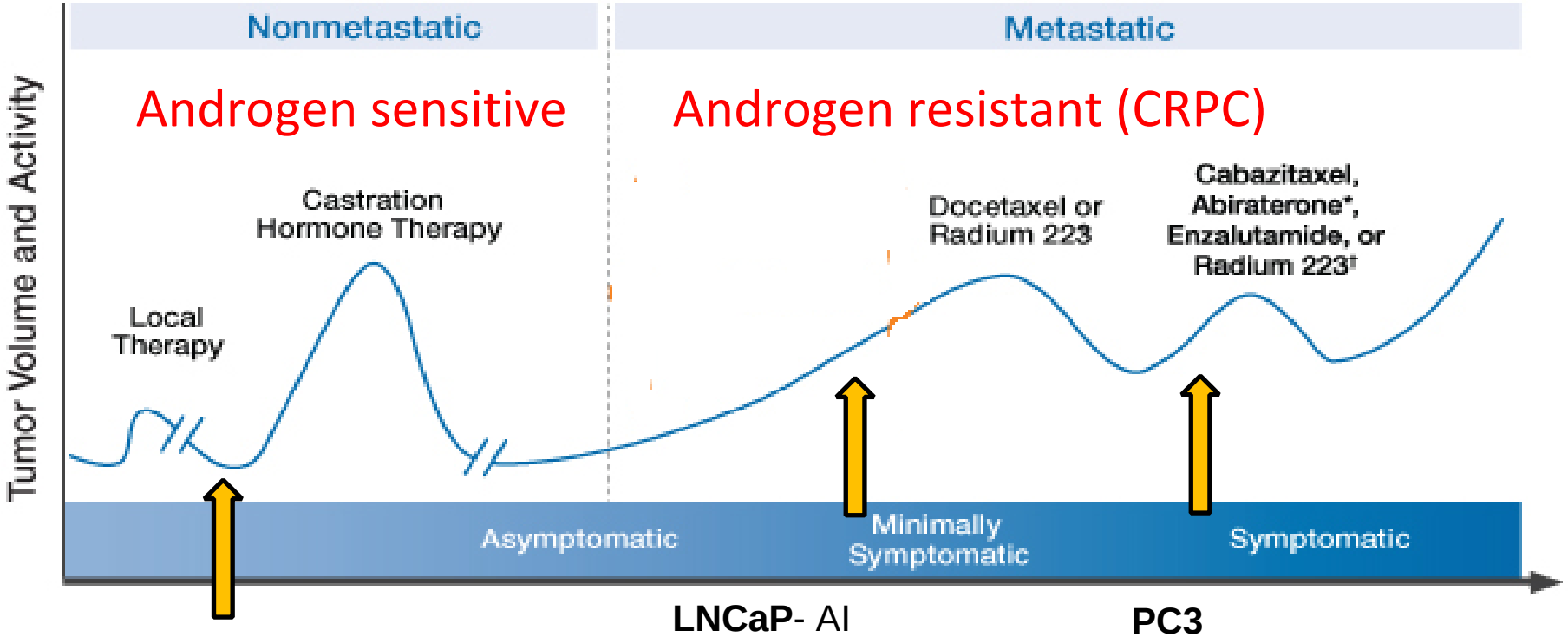
Prostate tumour overexpressed-1 promotes tumourigenicity in human breast cancer via activation of Wnt/β-catenin signalling

Yanmei Cui,Weifeng Ma,Fangyong Lei,Qingyuan Li,Yanhong Su,Xi Lin,Chuyong Lin,Xin Zhang,Liping Ye,ShuZhongyu Yuan,Libing Song

•**First published:** Full publication history 30 May 2016
•**DOI:** 10.1002/path.4725

Cancer cell models used to study recurrent

Castration Resistant Prostate Cancer (CRPC)



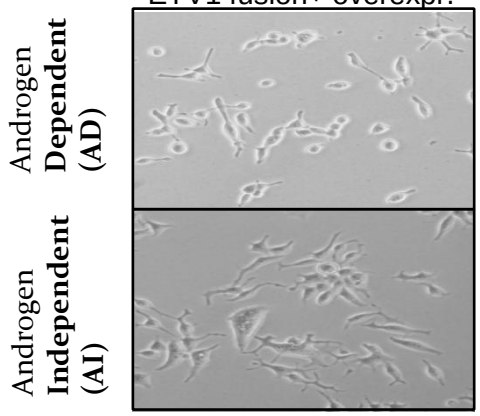
LNCaP- AD
Androgen-dependent AD

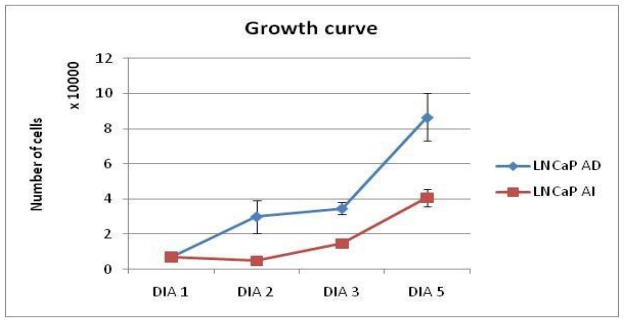
LNCaP- AI
PC3, Du145
docetaxel sensitive DS

PC3
Du145
docetaxel resistant DR

LNCAP

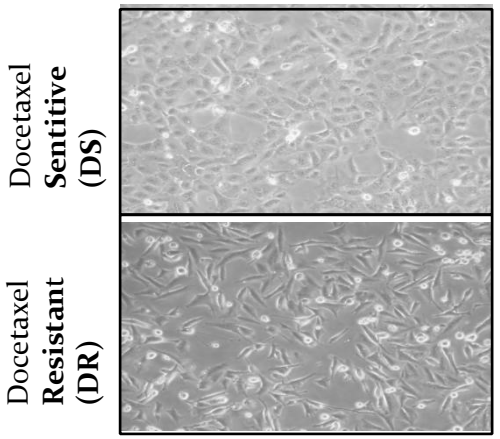
- From metastatic site: supraclavicular lymph node
- **Androgen-sensitive**
- PTEN *null*
- p53 WT / RB WT
- ***AR* positive**
- PSA positive
- ETV1 fusion+ overexpr.

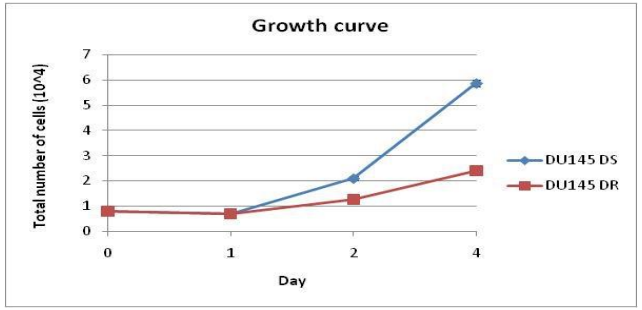




DU145

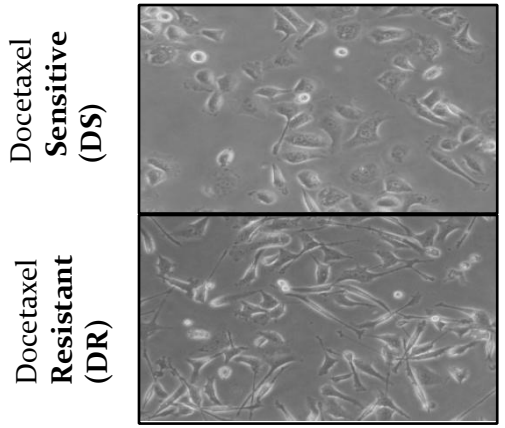
-From metastatic site: brain
- **Androgen-resistant**
- PTEN +/-
- p53 mutant/ RB *null*
- ***AR* negative**
- PSA negative

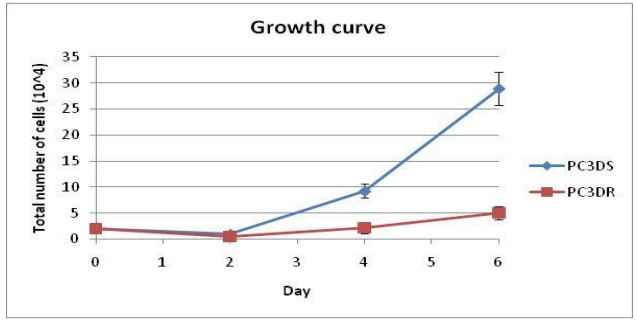




PC3

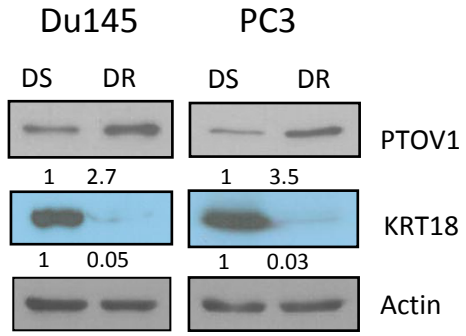
- From metastatic site: bone
- **Androgen-resistant**
- PTEN *null*
- p53 *null*/ RB WT
- ***AR* negative**
- PSA negative
- ETV4+ overexpr.





'mutation status' from Barbieri et al, Eur Urology 2013

PTOV1 overexpressed in CRPC cells sensitive to docetaxel promotes their resistance to chemotherapy



Du145

IC_{50} : 2.0 nM DS-GFP

IC_{50} : 3.9 nM DS-HAPTOV1

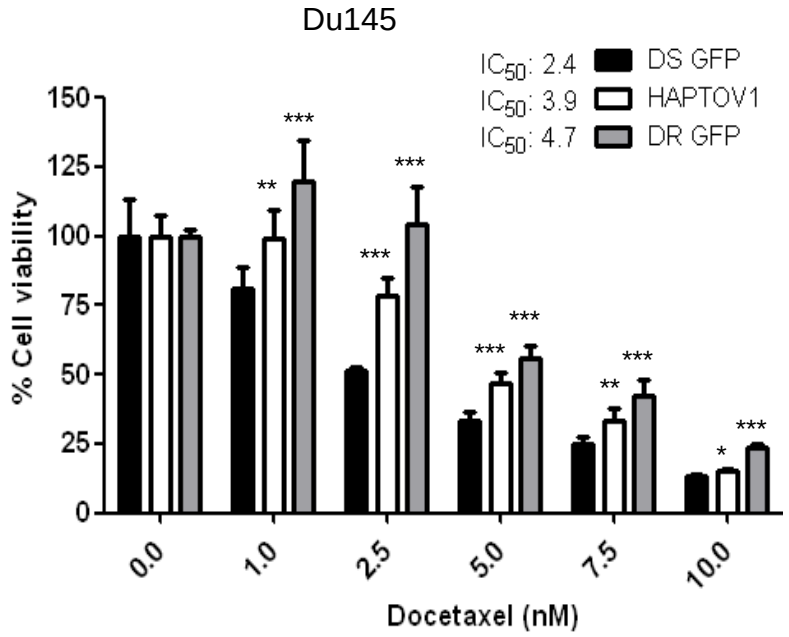
IC_{50} : 5.3 nM DR

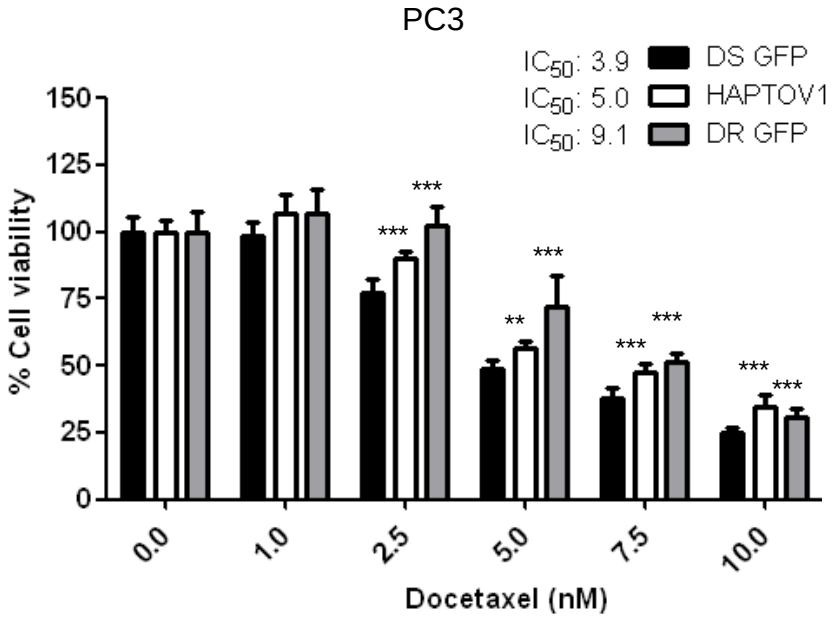
PC3

IC_{50} : 4.8 nM DS-GFP

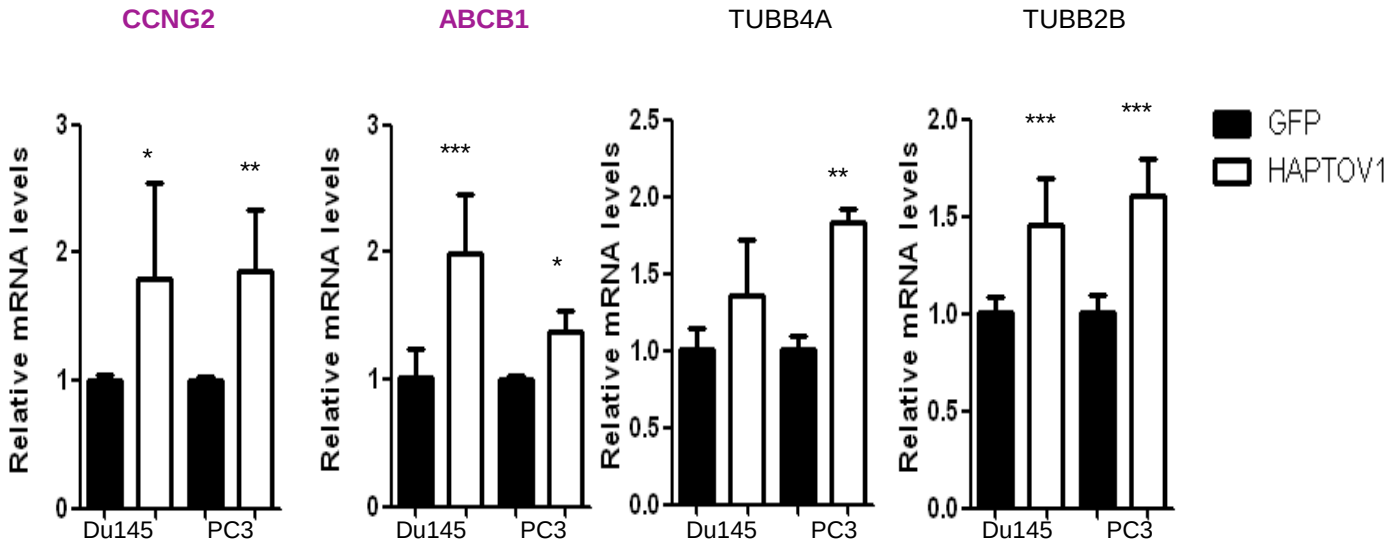
IC_{50} : 5.6 nM DS-HAPTOV1

IC_{50} : 7.2 nM DR

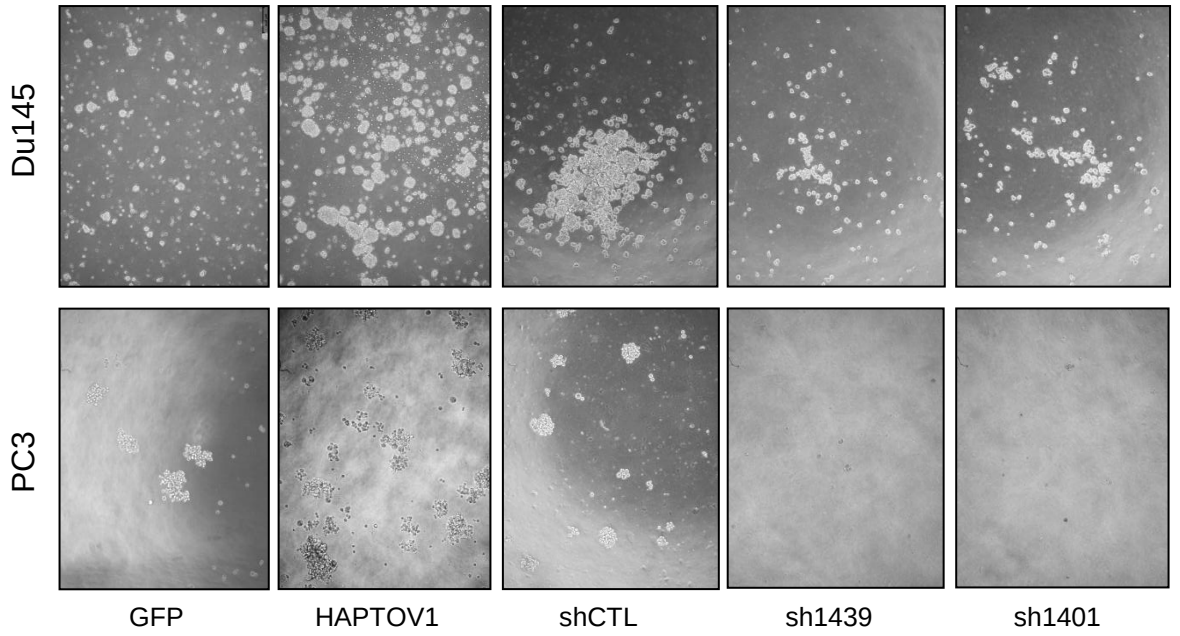


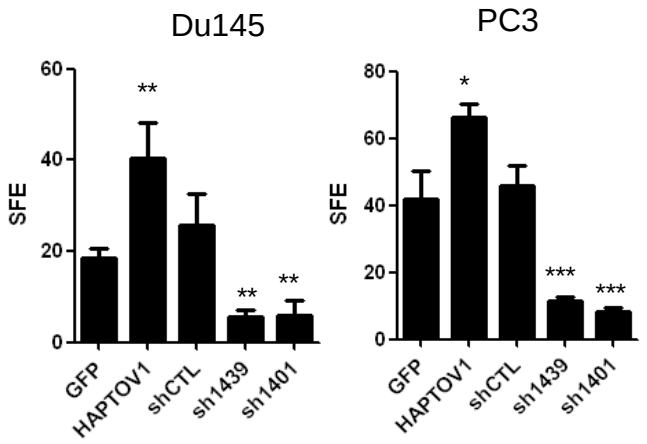


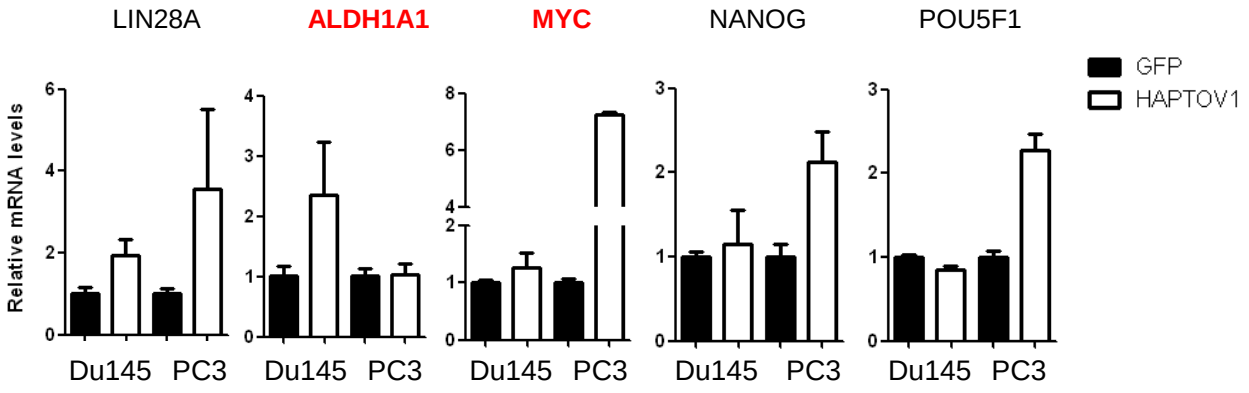
The overexpression of PTOV1 induces an increase of expression of genes associated to resistance to chemotherapy



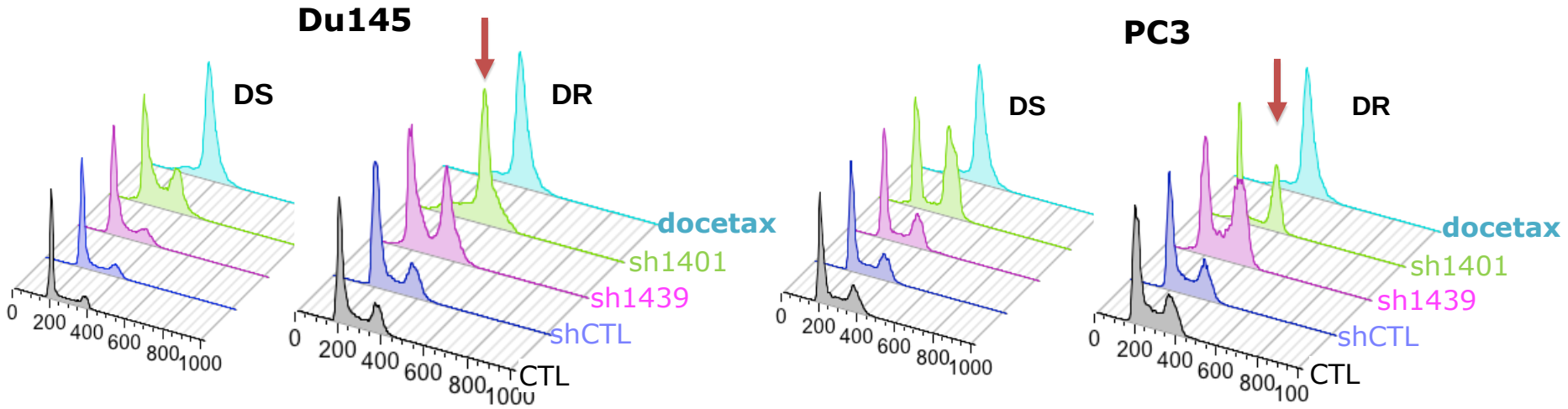
Higher levels of PTOV1 increase the spheroid forming capacity and expression of stemness genes

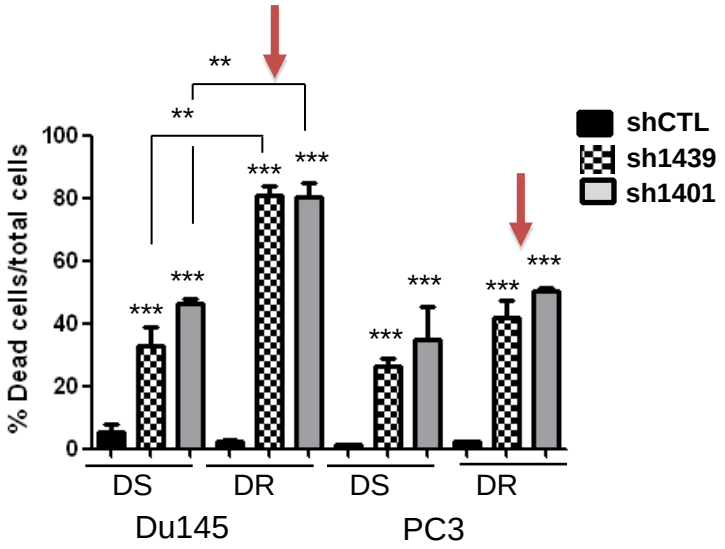




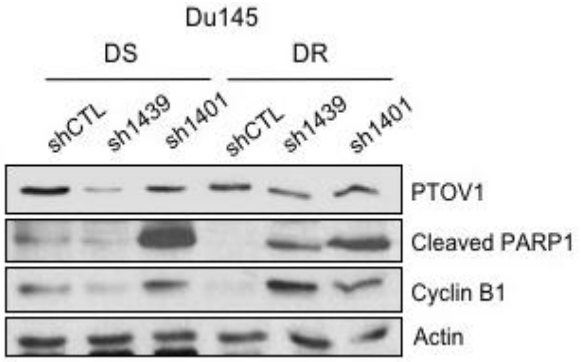


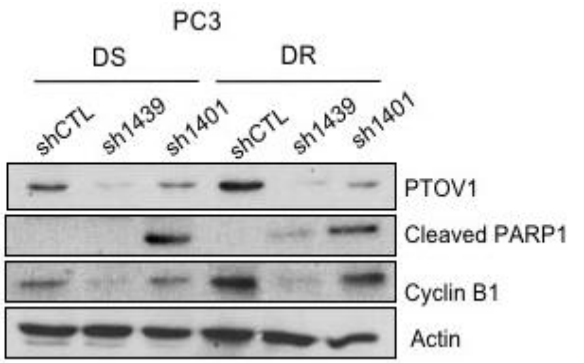
The KD of PTOV1 arrested cells at the G2-M phase. Apoptosis is observed. DR cells are more sensitive



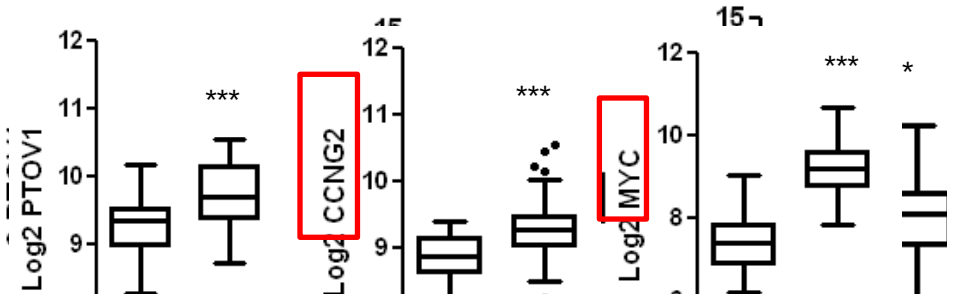


Cells arrest at G2-M phase and show a significant increase in apoptosis.



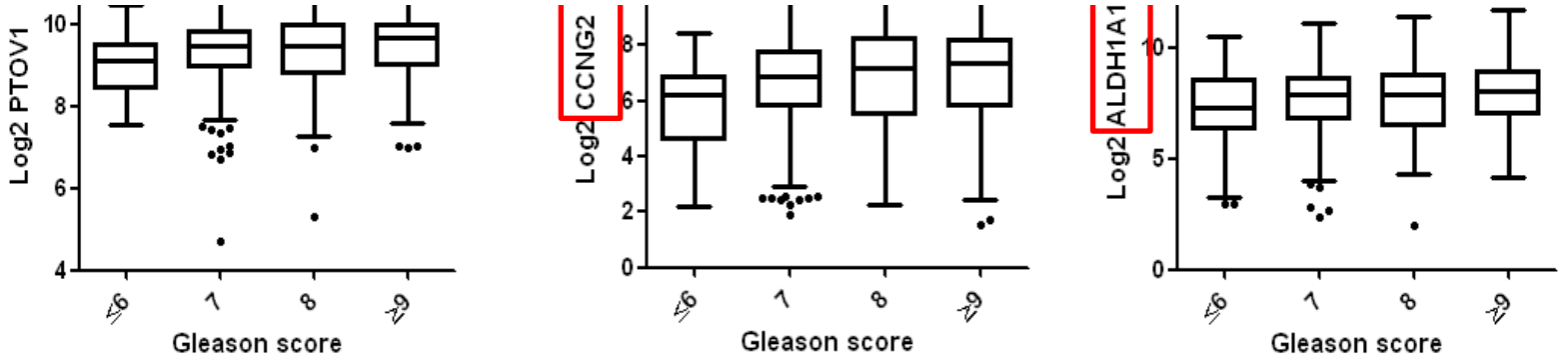


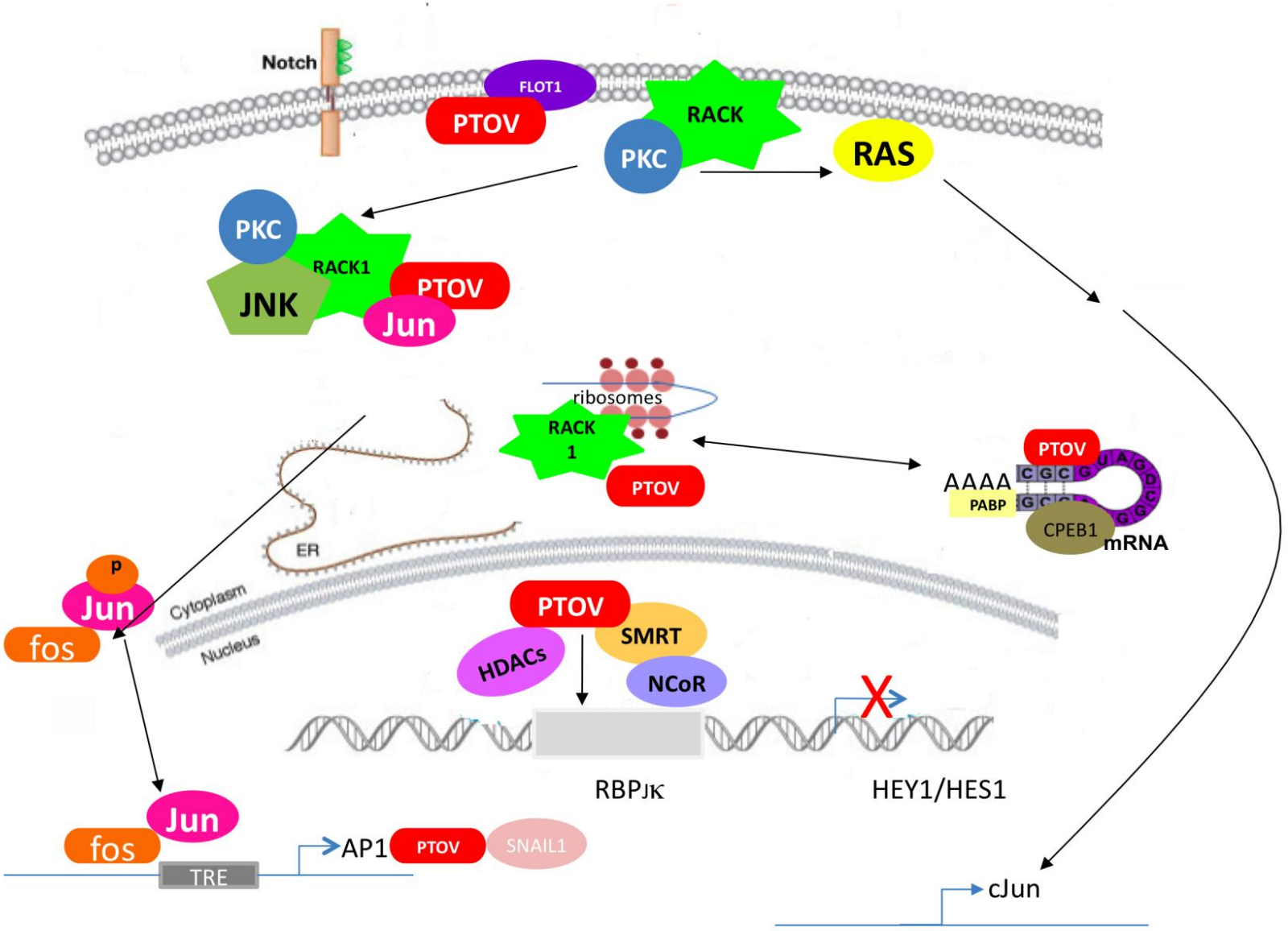
The expression of *PTOV1, ALDH1A1, CCNG2* and *MYC* is significantly increased in human prostate tumors



GSE29079;
benign n=48; tumor n=47

PTOV1 represents a good target to eliminate aggressive prostate cancer cells and potential CSCs-like subpopulations




Notch
FLOT1
PTOV
RACK
PKC
RAS
PKC
RACK1
JNK
PTOV
Jun
ribosomes
RACK
1
PTOV
PTOV
AAAA
PABP
CPEB1
mRNA
ER
P
Jun
fos
Cytoplasm
Nucleus
PTOV
SMRT
HDACs
NCoR
RBPJκ
HEY1/HES1
Jun
fos
TRE
AP1
PTOV
SNAIL1
cJun

Models systems to study in vitro the biology of human prostate tumors and their response to treatments

Generation of models from cell lines and human tumors

Cell line models
sensitive/resistant to treatments

Induction of
resistant tumors
in vitro by
androgen
deprivation (ADT)

CTL

ADT

Funcional selection
of CSCs-like
by
3D cultures
(Spheres)

Primary tumors,
Cell lines
and *spheres*

In vitro assays for specific inhibitors aimed to eliminate CSCs populations

Subcutaneous injection

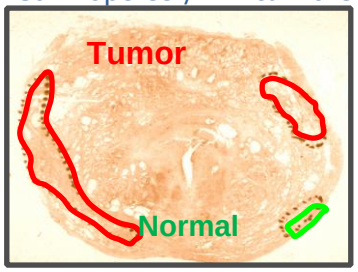
- Spheres
- Parental cells

Tumors

Omics

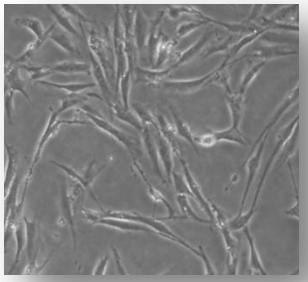
Screening for more specific targets to eliminate CSC populations

PCa Biopsies / RP tumors

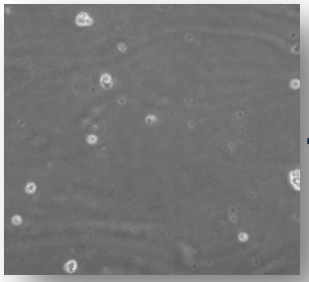


Enrequecimiento de células tumorales con capacidad de crecer no-adheridas formando esferas (CSC)

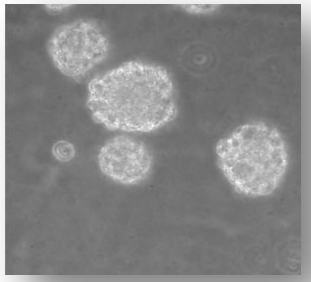
Cultivos Adherentes



Cultivos no-adherentes



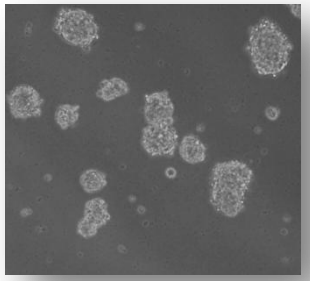
Día 1



Día 7: **G1**

Disociación 1 de las esferas

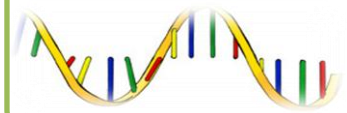
Cultivos no-adherentes



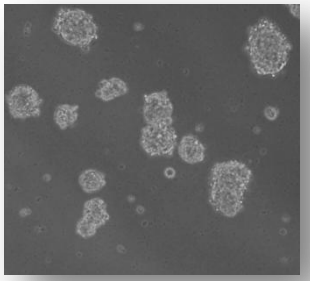
Día 7: **G2**

Disociación 2 de las esferas

RNA extraction

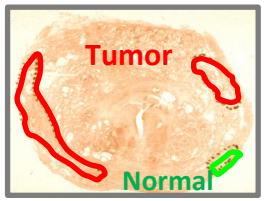


Cultivos no-adherentes



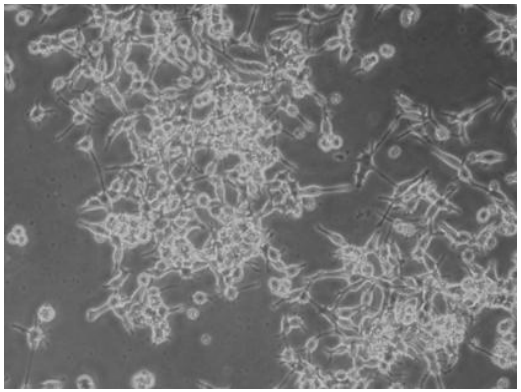
Día 7: **G3**

Etc..



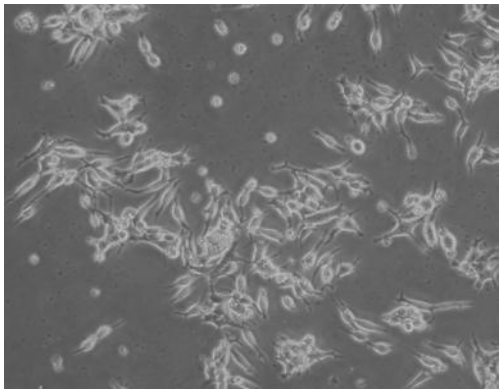
Modelo celular LNCaP: el estrés por la falta de andrógeno resulta en un enriquecimiento de CSC-like

Andrógeno Dependientes

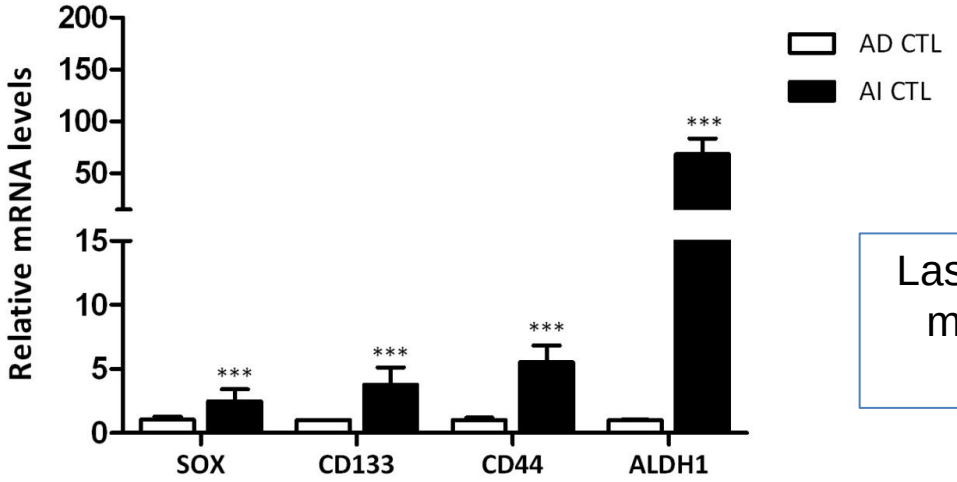


AD

Andrógeno Independ.



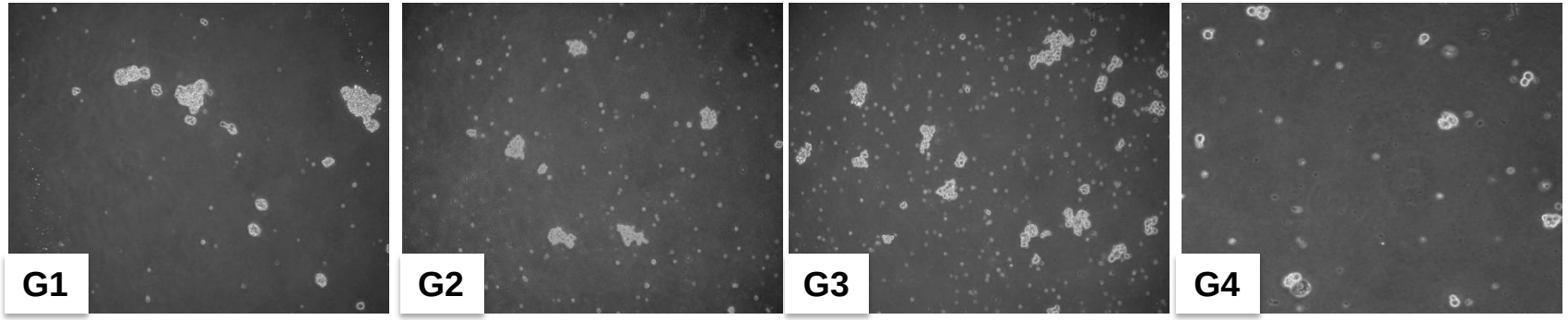
AI



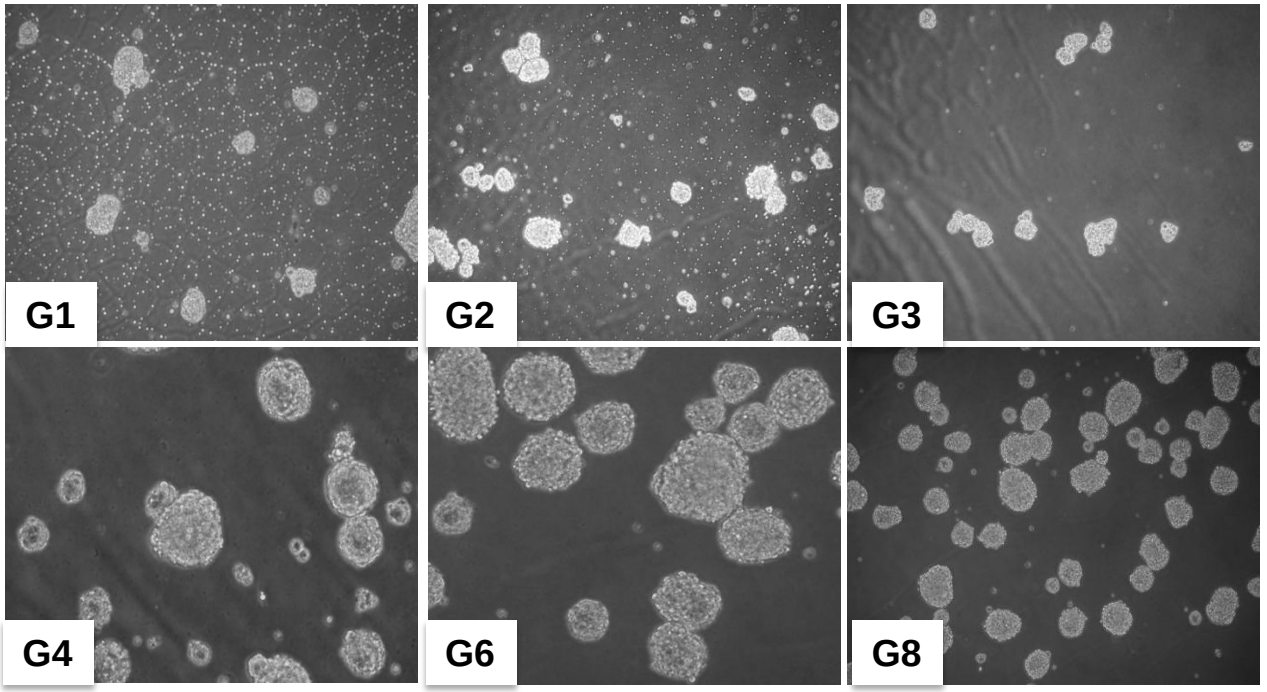
Las células AI expresan niveles más altos de Marcadores de CSC

Modelo celular LNCaP : Andrógeno Dependientes y Andrógeno Independiente formación de esferas

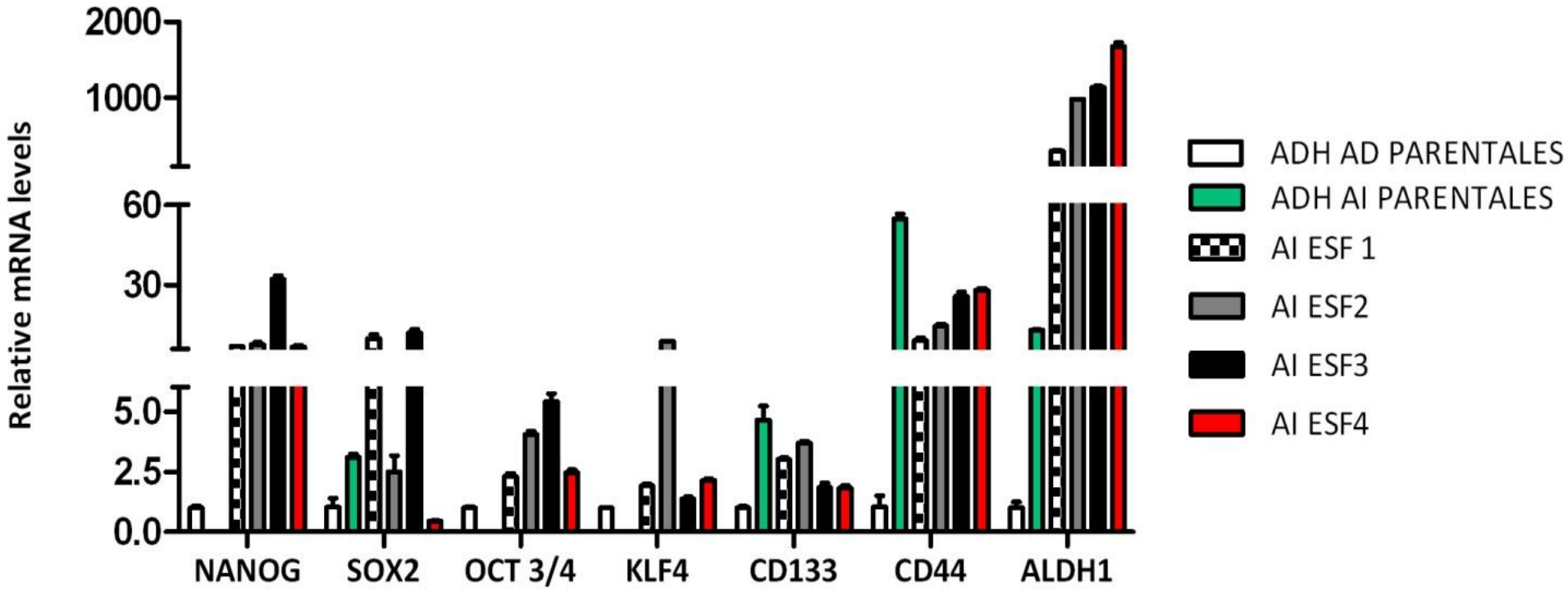
AD



AI



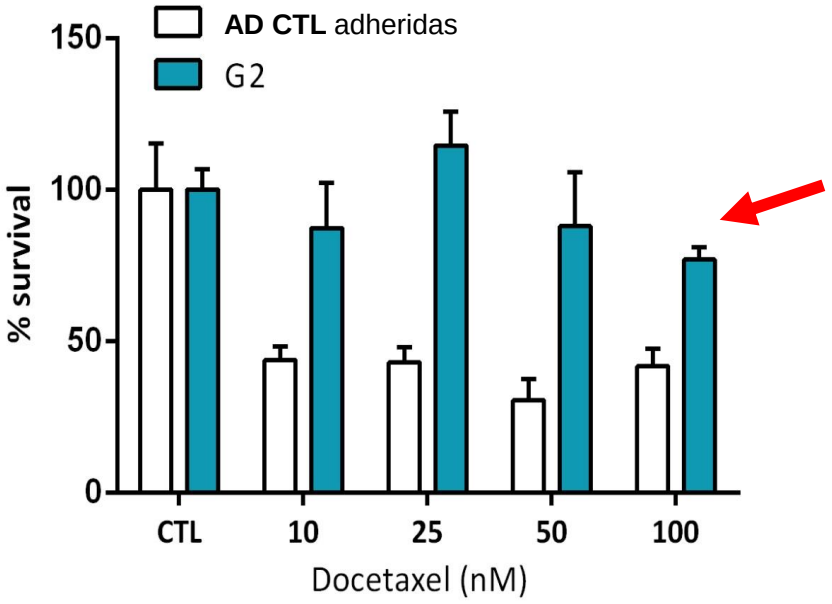
LNCaP AD y AI: expresión de marcadores de ‘stemness’



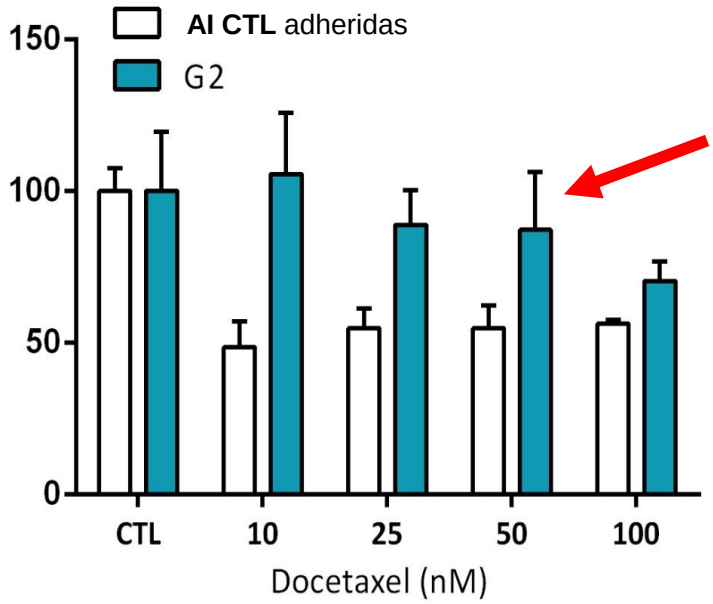
Embrionarios adultos

Modelo LNCaP: las células derivadas de esferas son resistentes a dosis altas de Docetaxel

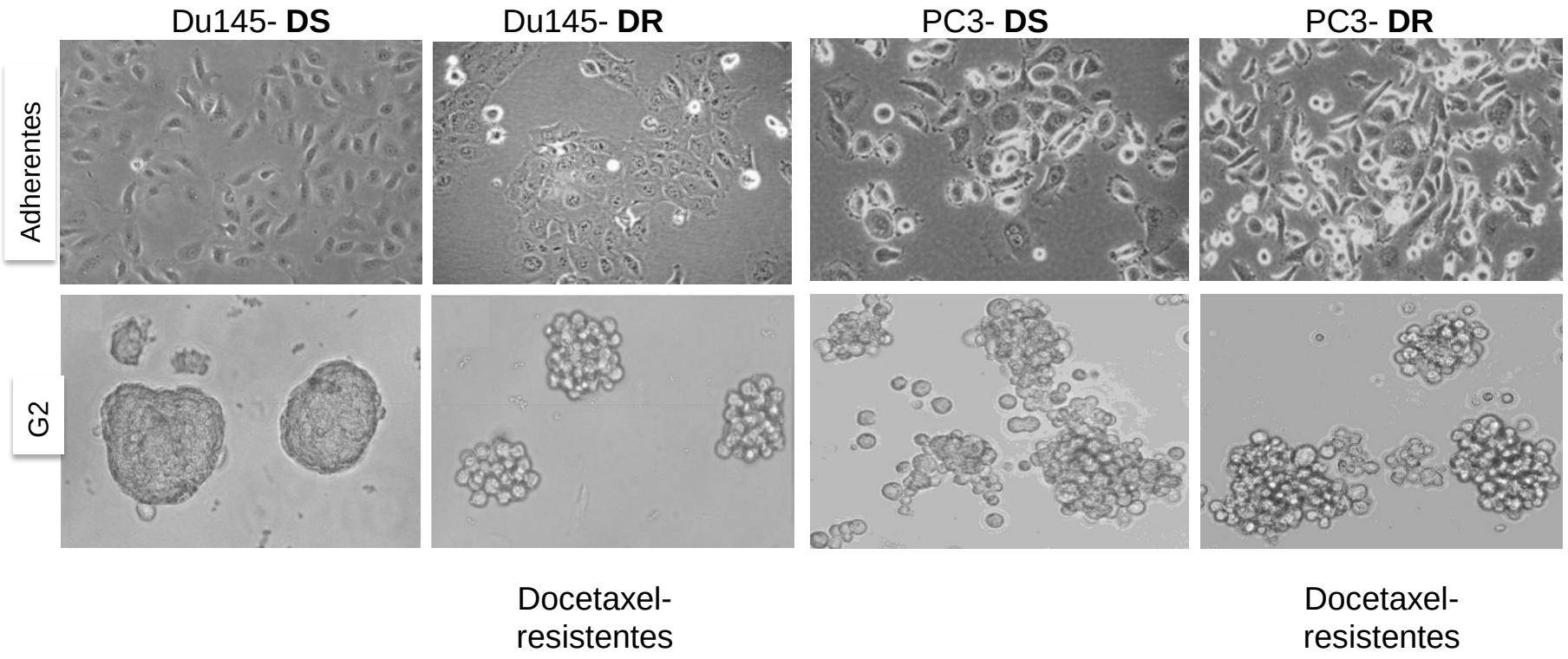
Esferas AD



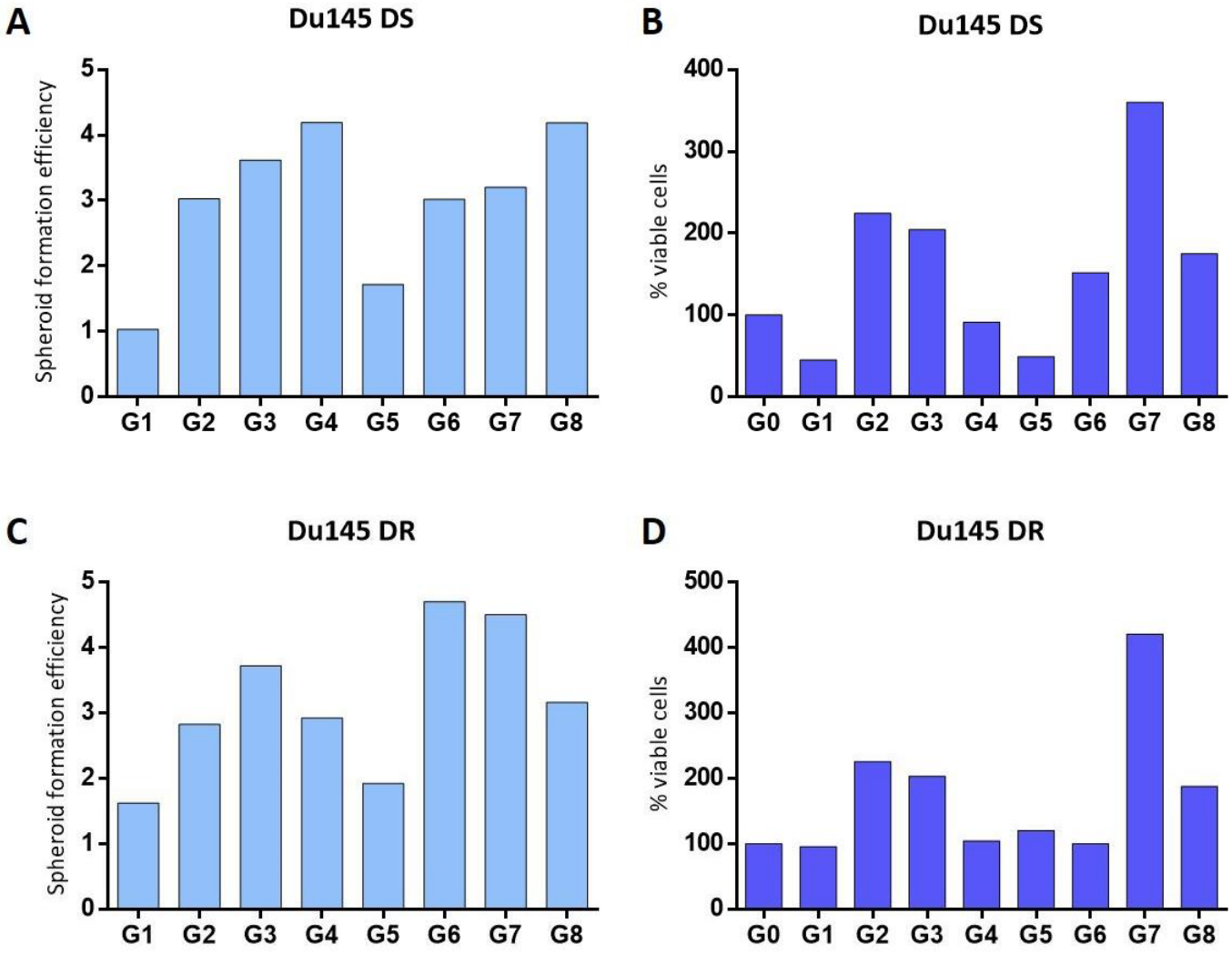
Esferas AI



Modelos celulares Du145 y PC3 (CRPC), sensibles y resistentes a docetaxel: formación de esferas

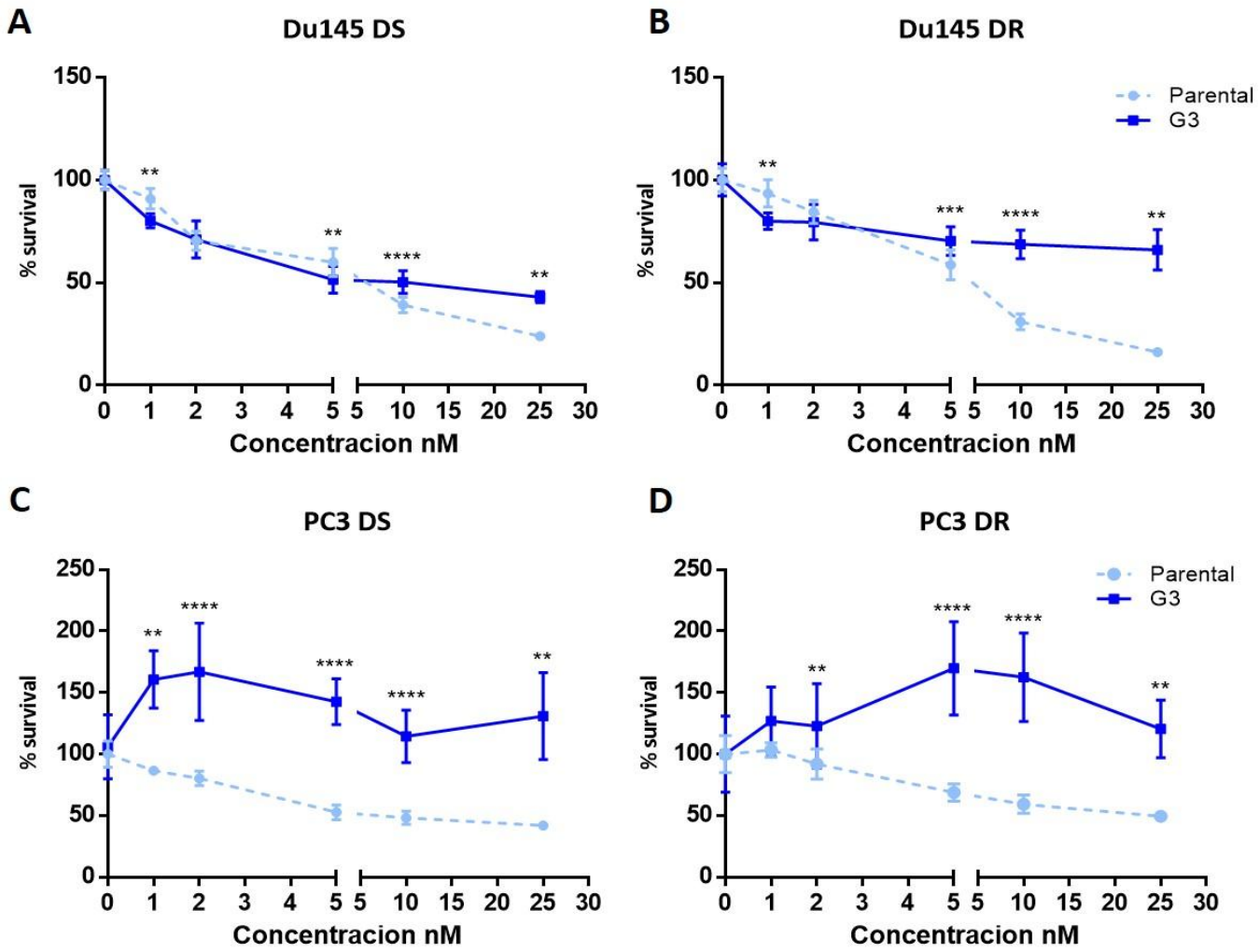


Enriquecimiento progresivo de la capacidad de formar esferas (capacidad tumorigenica)



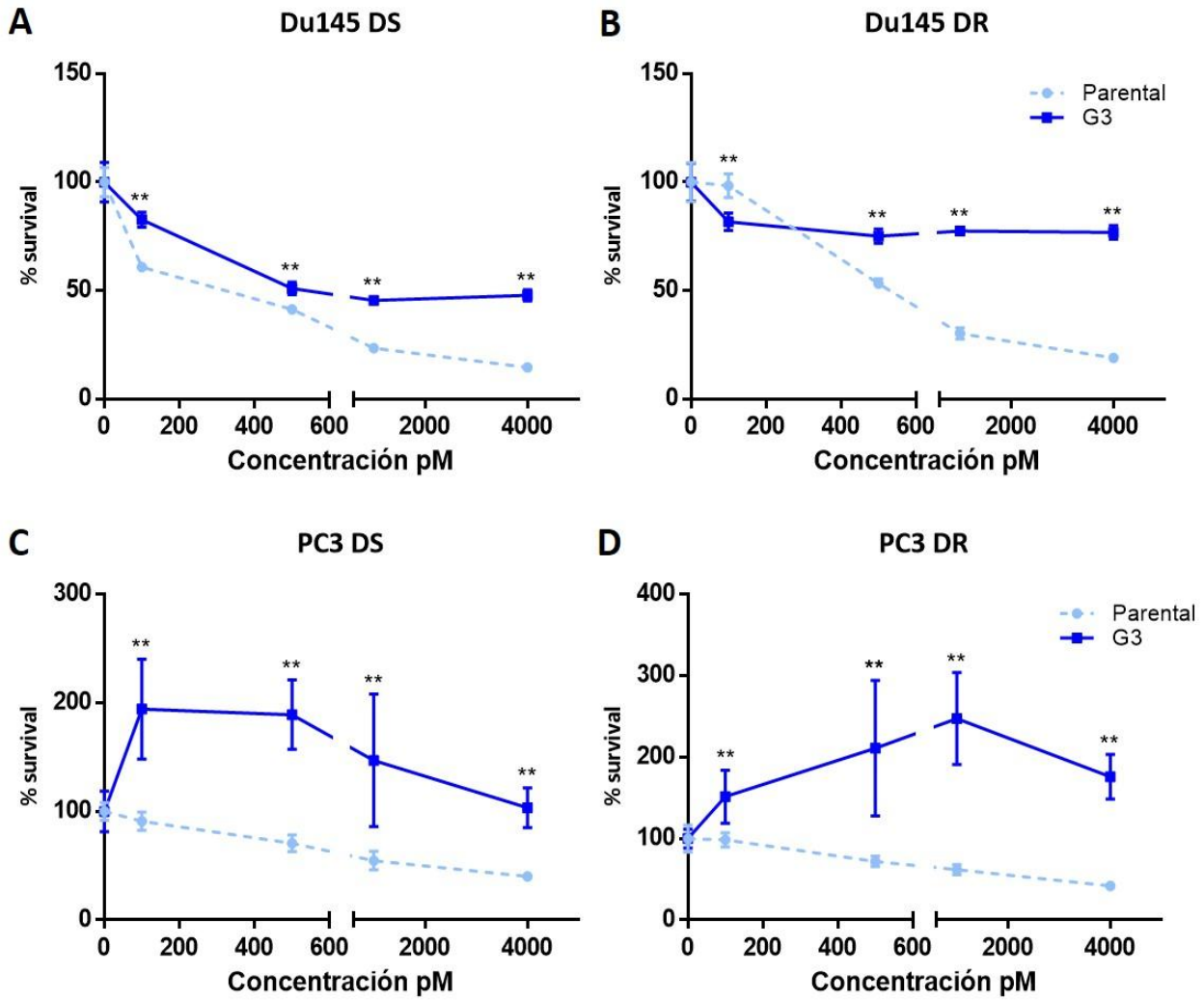
Prostatospheres cultures are more resistant to DOCETAXEL compared to parental cells grown in adherent conditions

DOCETAXEL

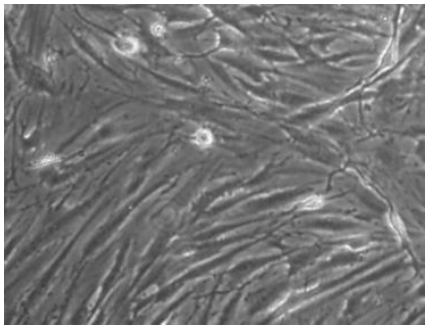


Prostatospheres cultures are more resistant to CABAZITAXEL compared to parental cells grown in adherent conditions

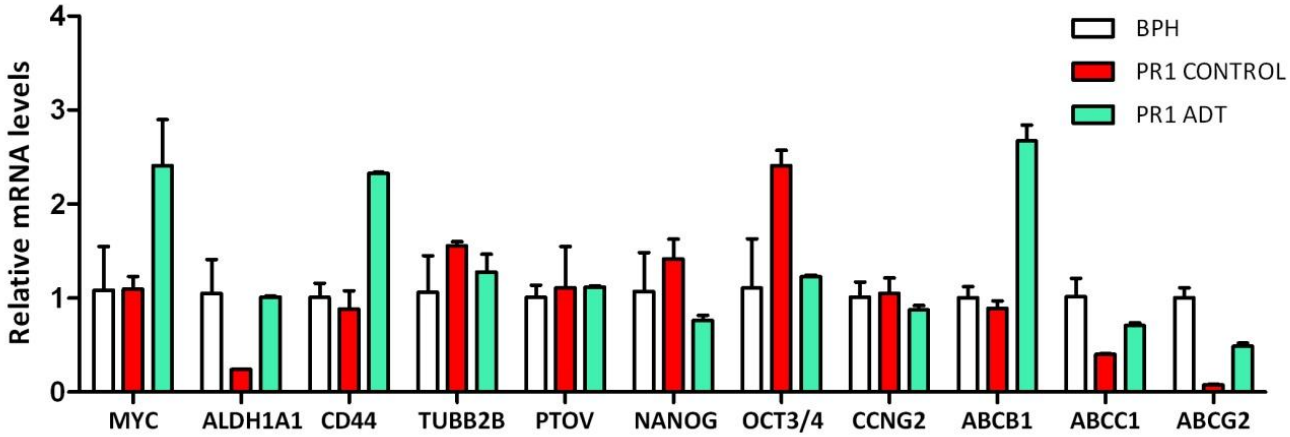
CABAZITAXEL

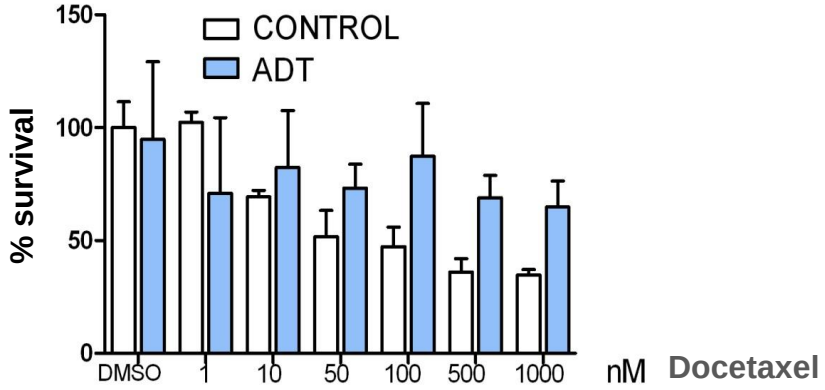


Prostate tumor explants cultured *in vitro* in adherent conditions: Androgen deprivation (ADT) induces a resistant culture with CSC-like phenotypes



PR1 – Radical Prostatectomy

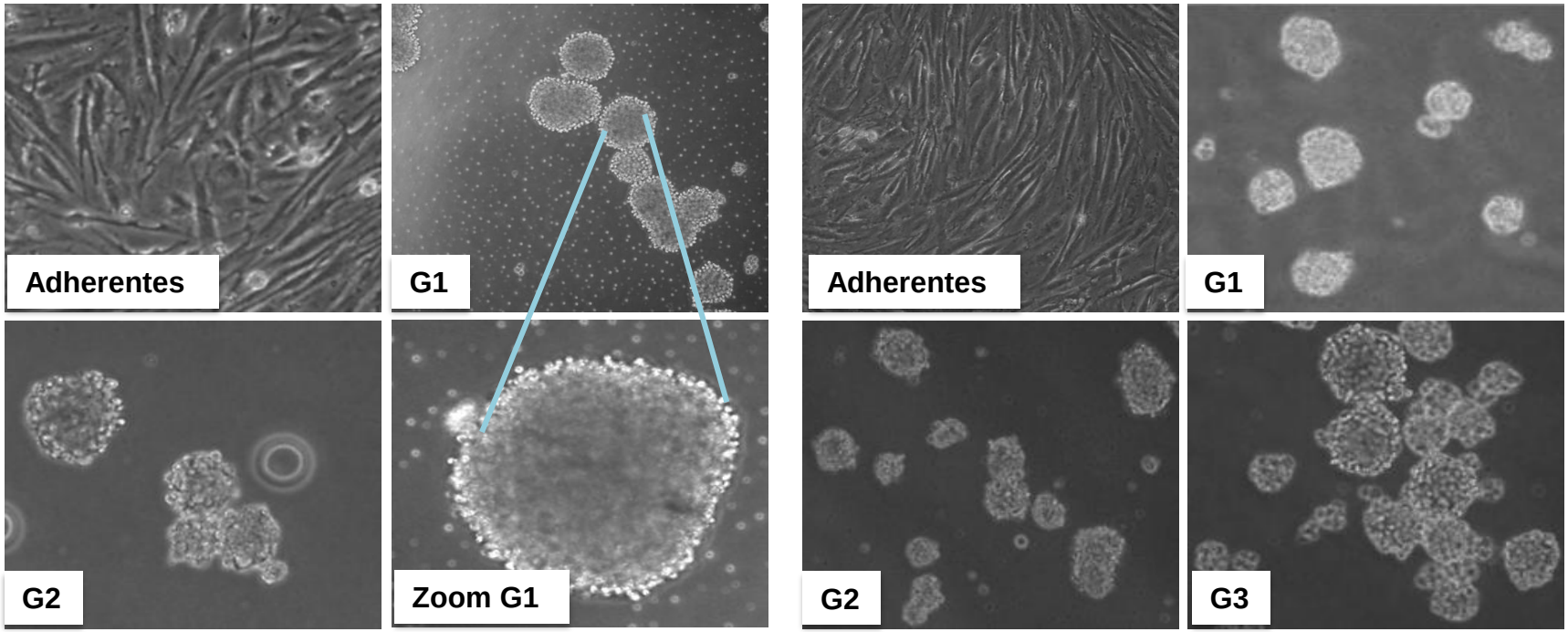




Prostate tumor explants cultured *in vitro* in **NON-adherent** conditions: spheres formation from androgen-dependent and -independent tumors

Andrógeno Dependientes

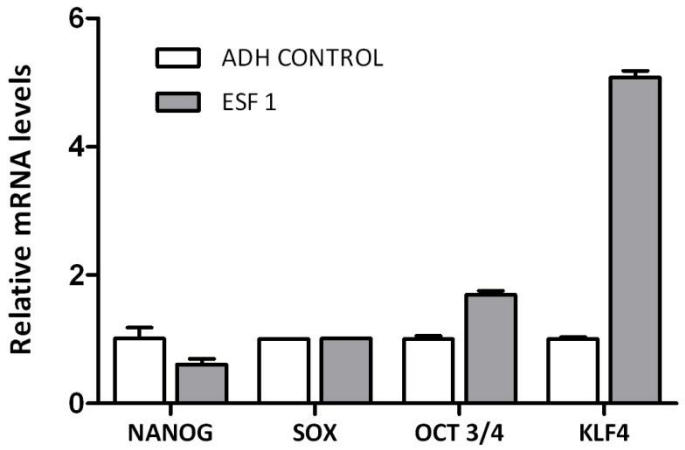
Andrógeno Independiente



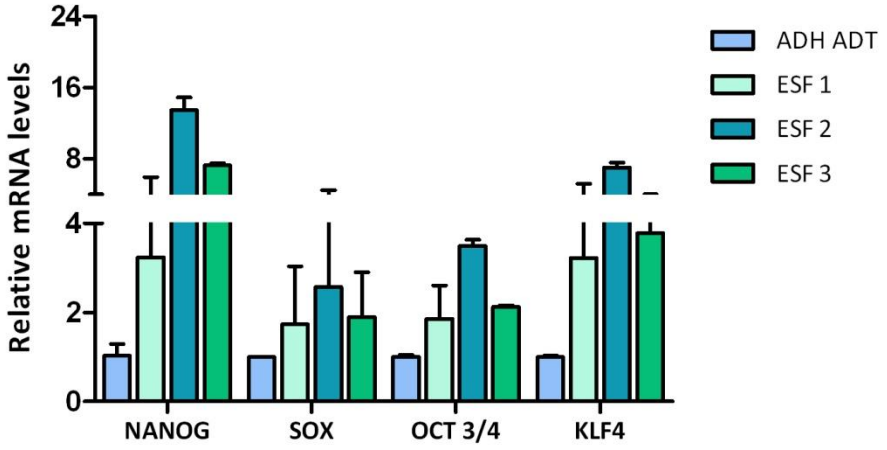
PR1 – Radical Prostatectomy

Prostate tumor explants cultured *in vitro* are selected for cells able to grow as spheres : Androgen Independent cells have a higher capacity to grow as spheres

Andrógeno Dependientes

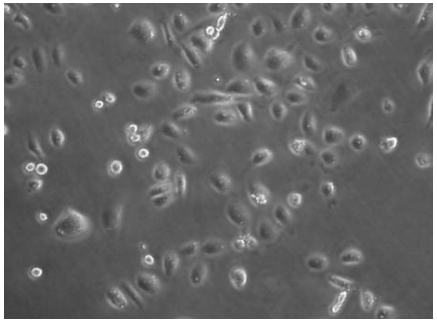


Andrógeno Independiente

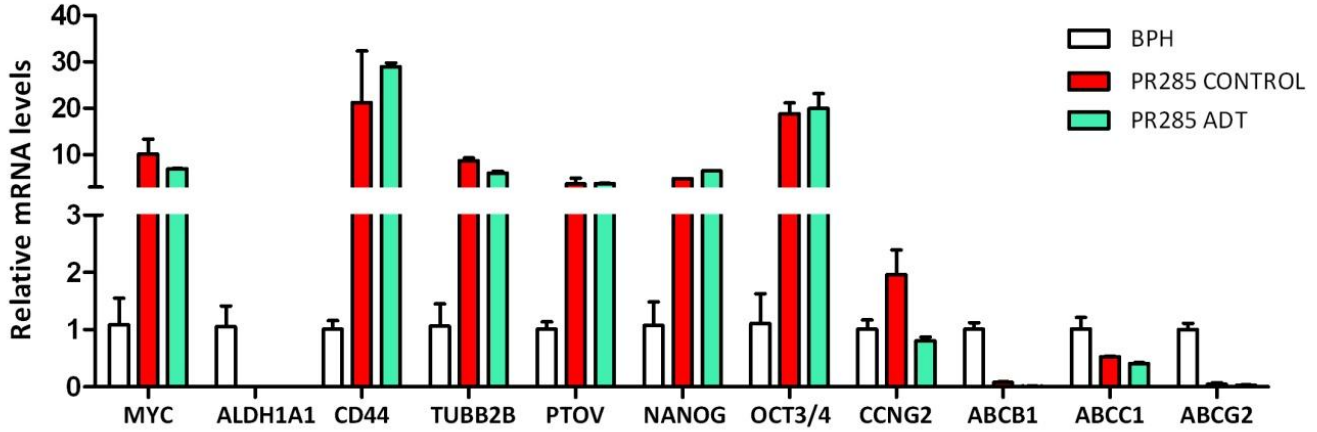


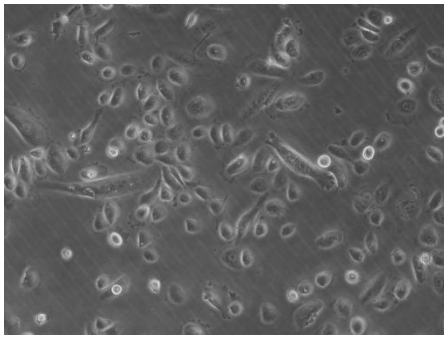
PR1 – Radical Prostatectomy

Prostate tumor explants cultured *in vitro* in adherent conditions: Androgen deprivation (ADT) induces a resistant culture with CSC-like phenotypes

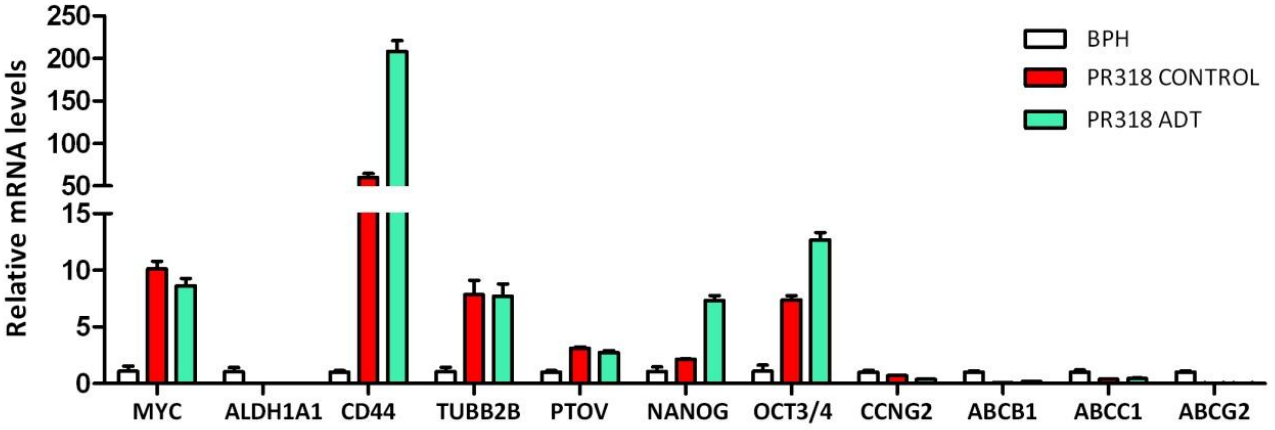


PR285 – Radical Prostatectomy

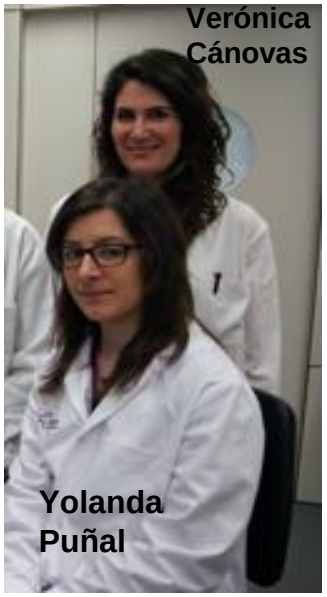


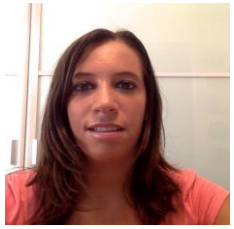


PR318 – Radical Prostatectomy



Thank you!





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