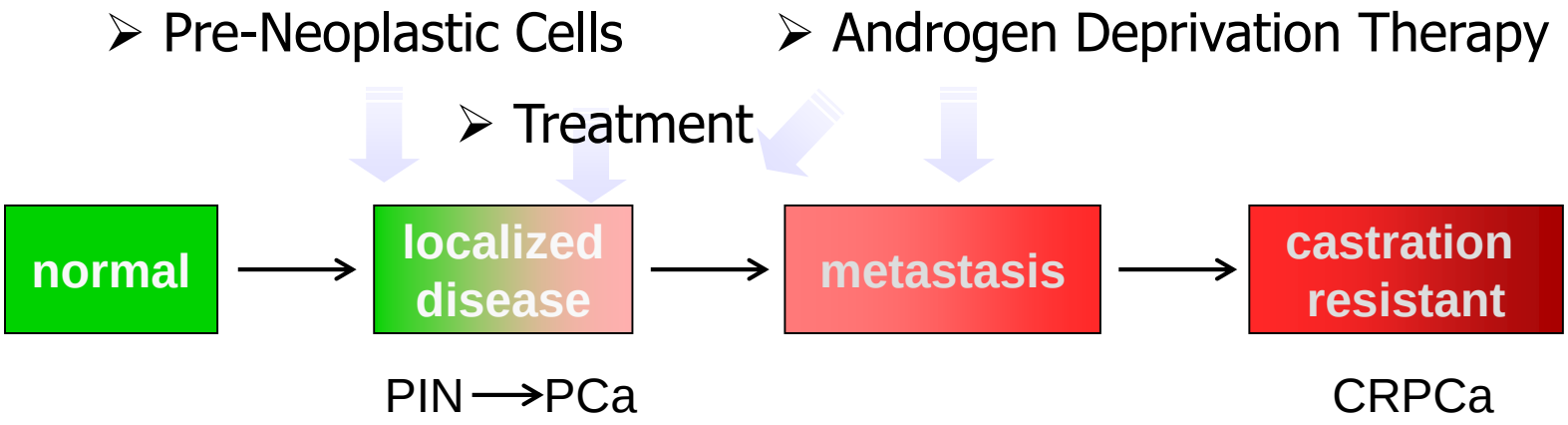
Activation of Wnt/beta-catenin Signaling Promotes Castrate-Resistant PCa Progression



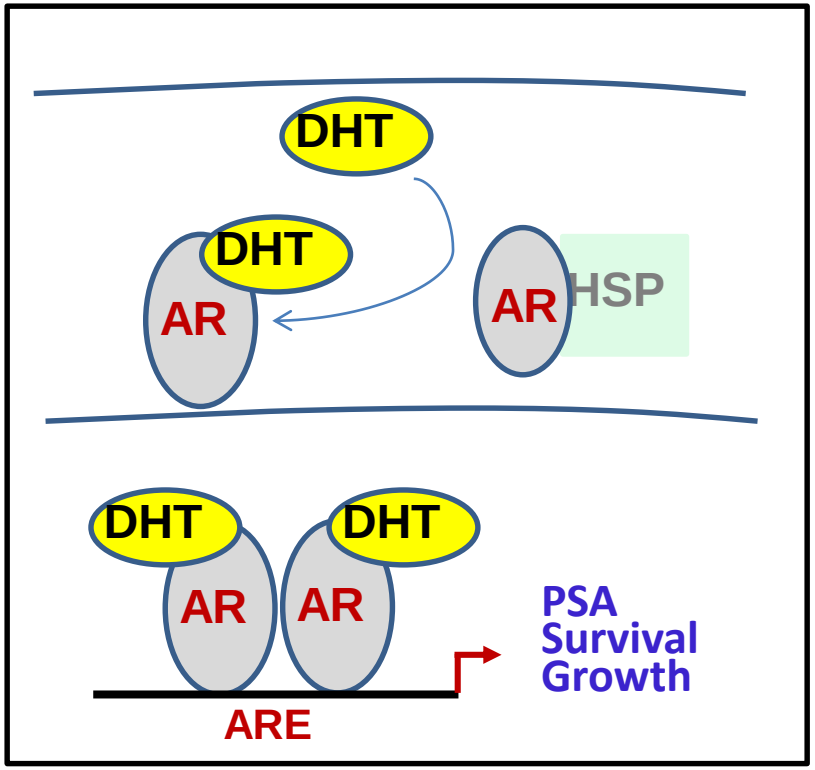
Xiuping Yu, Ph.D.
Biochemistry & Molecular Biology
LSUHSC Shreveport

Prostate cancer progression



(PIN: Prostate Intraepithelial Neoplasia)

Mechanisms for CRPC

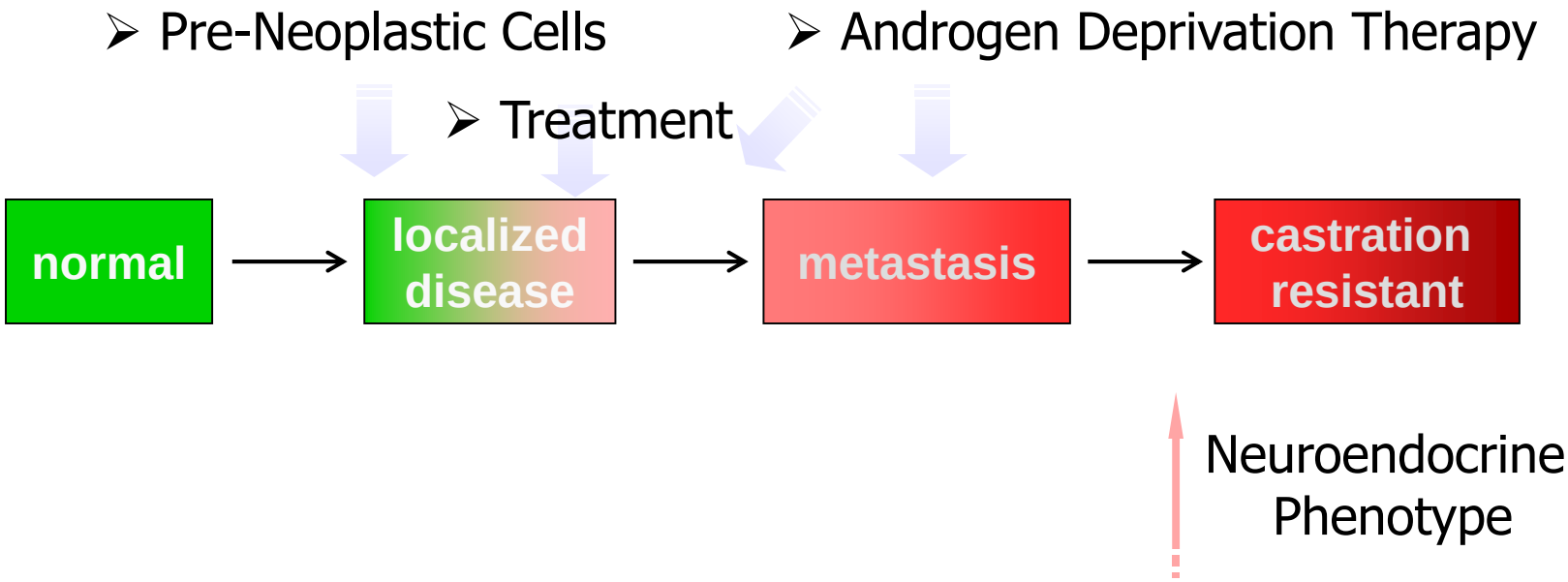


- AR-centered mechanisms
  - AR amplification
  - AR mutations
  - AR activation by growth factors
  - AR variants
  - Intra-prostate synthesis of androgens
- Pathways that bypass AR: NED

DHT: Dihydrotestosterone
AR: Androgen Receptor
ARE: Androgen Response Element

NED: Neuroendocrine Differentiation
PSA: Prostate Specific Antigen
HSP: Heat Shock Protein

NE phenotype is increased in CRPCa



Many patients that fail androgen deprivation therapy and/or chemotherapy develop NE PCa (Mosquera JM, *Neoplasia*. 2013; Sun Y, *Am J Transl. Res.* 2009)

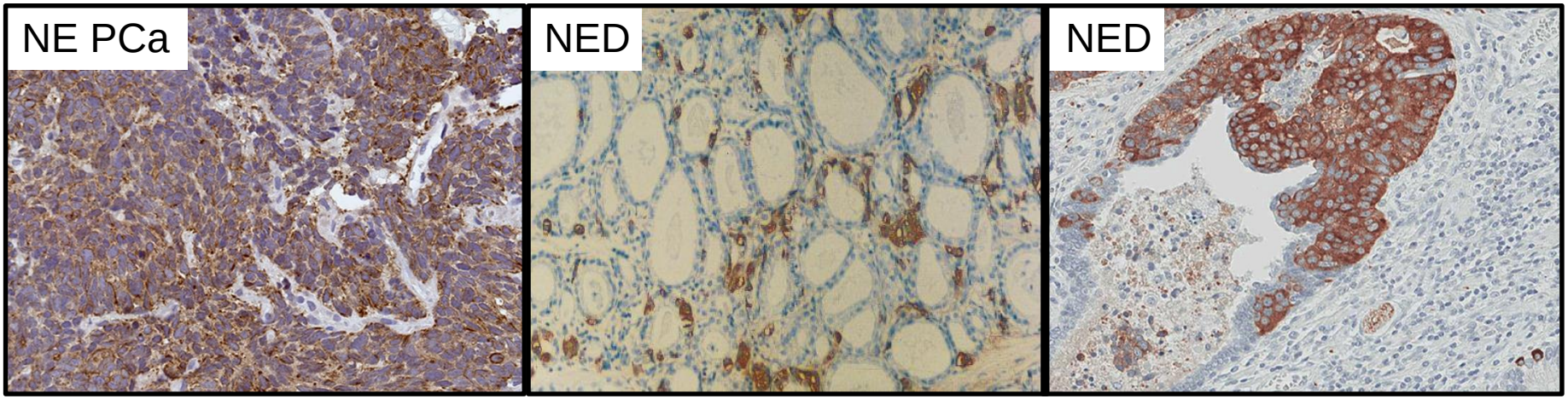
Our own research has found about 40% mCRPC has NED and studies from other labs found 25% heavily treated PCa have small cell carcinoma.

Neuroendocrine (NE) PCa and NE differentiation (NED) in PCa

- Primary NE PCa is rare but aggressive
- NE PCa is androgen independent: AR negative

Neuroendocrine (NE) PCa and NE differentiation (NED) in PCa

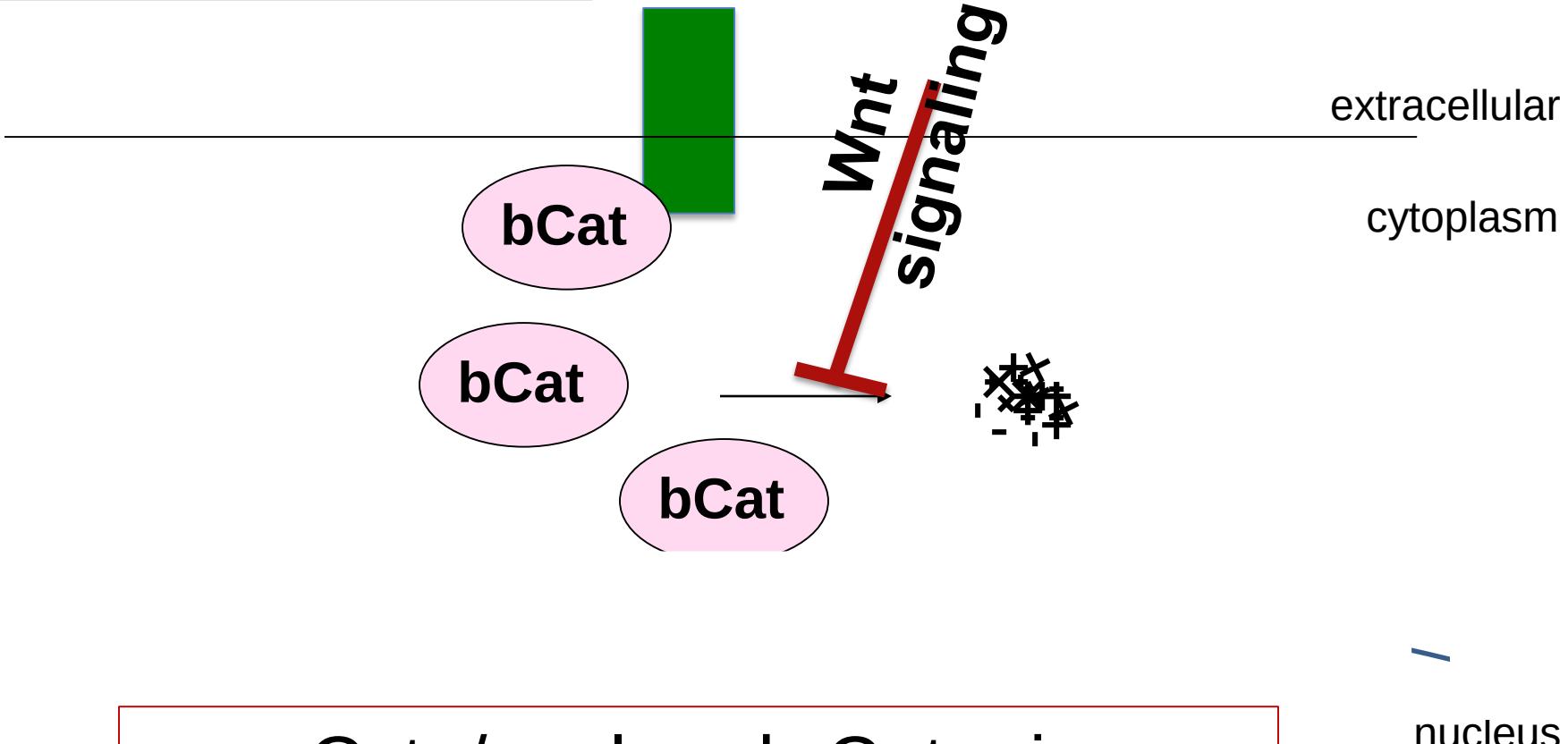
- NE PCa is rare but aggressive
- NE PCa is androgen independent: AR negative
- NE Differentiation of adenocarcinoma occurs in 40% to 100% of advance PCa
- NED is associated with poor prognosis
- NE secretions promote the androgen independent growth of a non-NE PCa



Jiborn *et al. Adult Urology*. 1997
Grabowska *et al. Cancer Metastasis Rev*. 2014

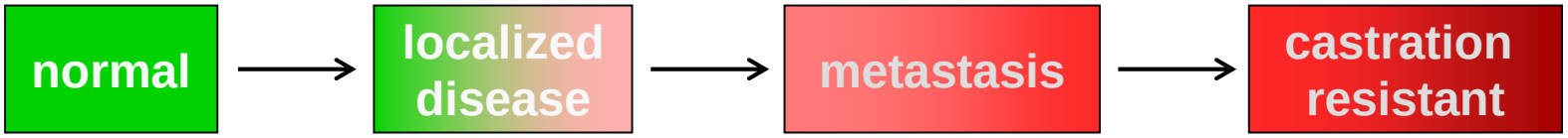
Wnt/beta-Catenin signaling is involved in PCa initiation and progression

Wnt/b-Catenin Signaling



Cyto/nuclear b-Catenin
= active Wnt/b-Catenin

Active Wnt/b-Catenin is associated with advanced stage PCa



0 to 5%

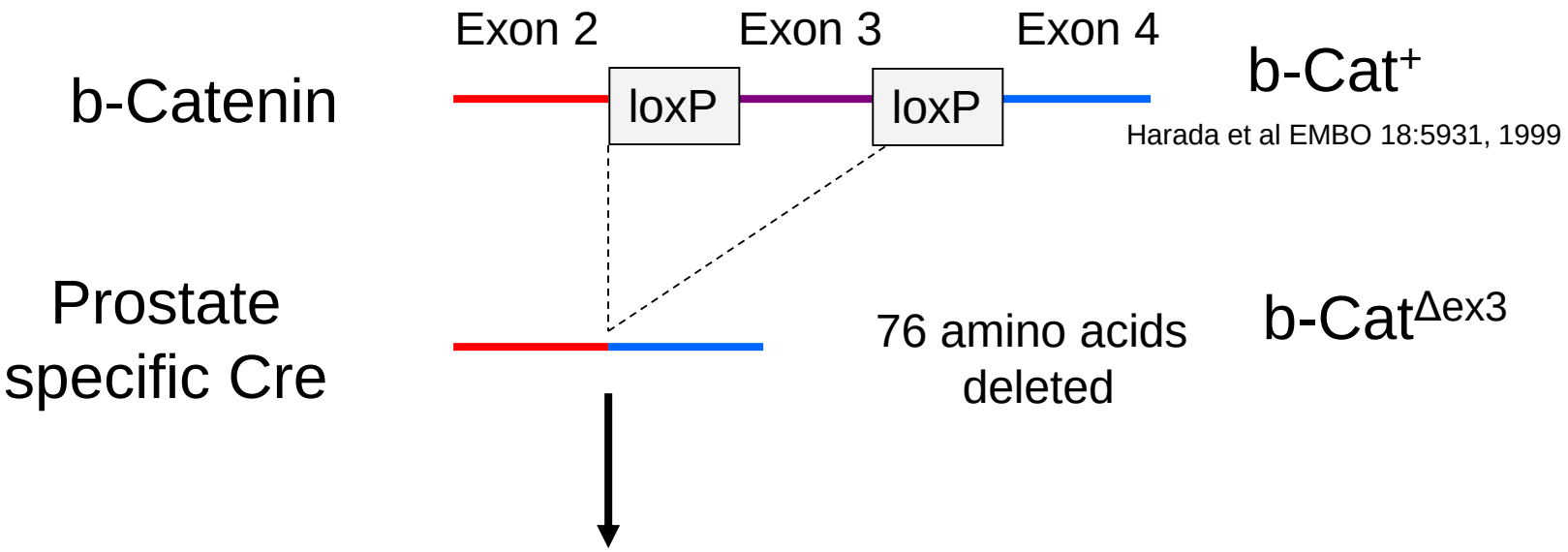
Activation Mutations in Wnt/b-Catenin

30 to 85%

Nuclear b-Catenin

- Mutations in Wnt/b-Catenin signaling were enriched in CRPC (Grasso, *et al. Nature,* 2012)
- Nuclear b-Catenin was detected in CRPC specimens (Kumar, *et al. PNAS*, 2011)
- Wnt1 and Nuclear b-Catenin was detected in 77% of lymph node metastases and in 85% of skeletal metastases. (Chen, *et al. Cancer*, 2004)

Constitutively activate Wnt/b-Catenin signaling in mouse prostate

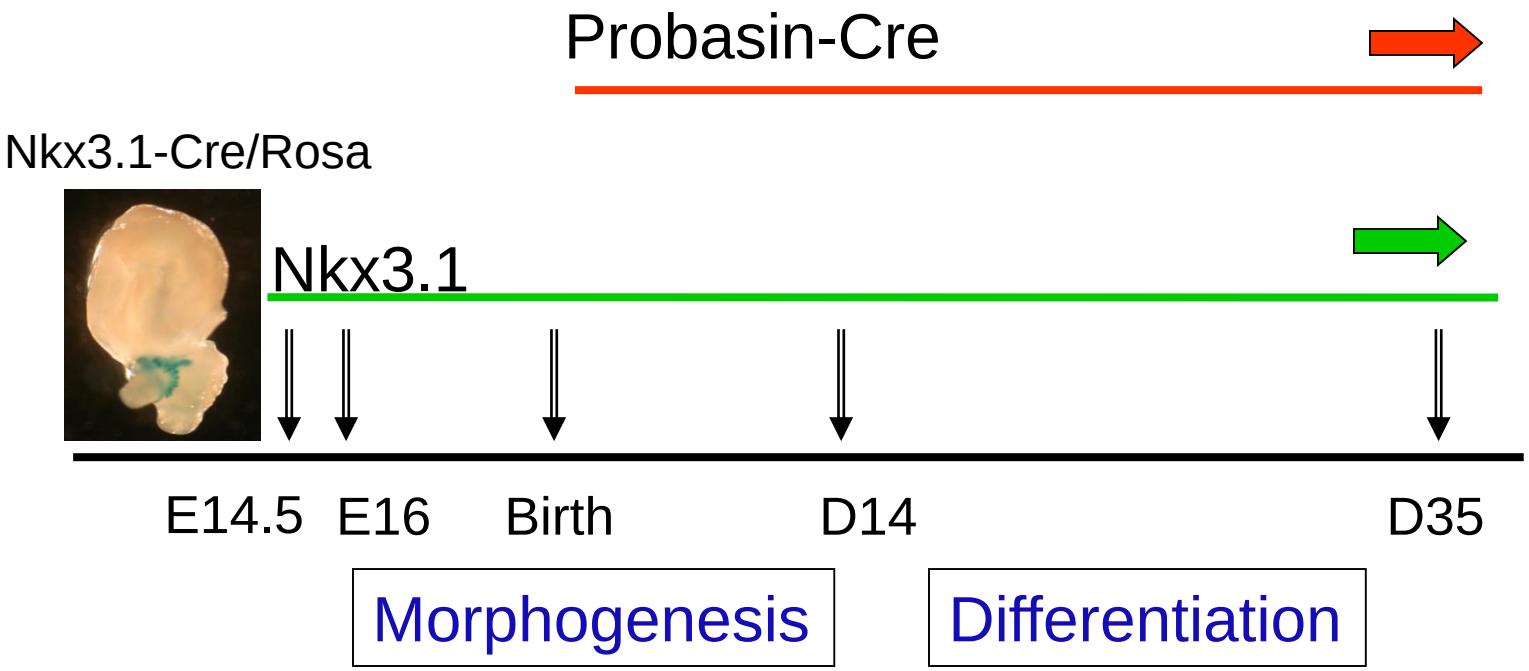


Deletion of Exon 3 results in constitutive appearance of nuclear b-Catenin in prostatic epithelium

Dominant Active (DA) b-Catenin

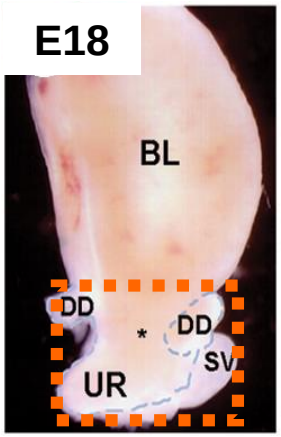
Yu *et al. Prostate* 2009

Selected promoters target Cre transgene at different times in prostate development

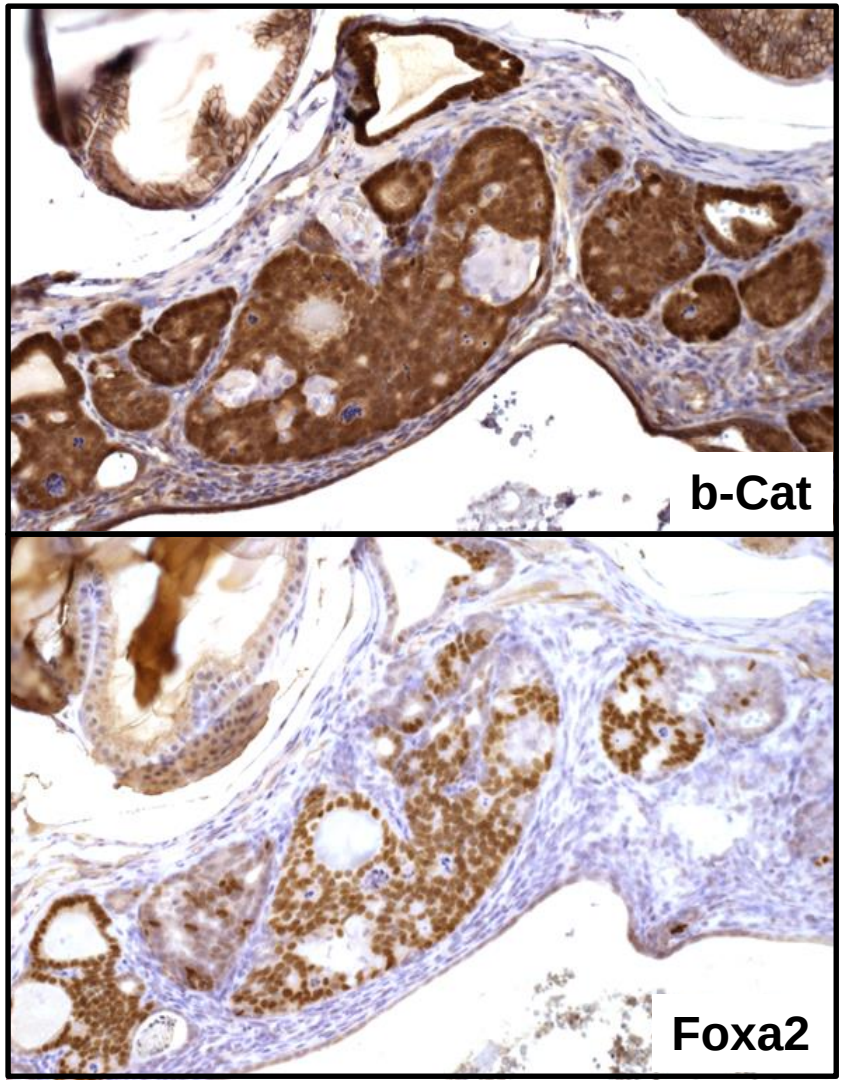


Embryonic activation of Wnt/b-Catenin by Nkx3.1-Cre

Organ Rescue: Nkx3.1-Cre/b-Catenin prostates develop PIN

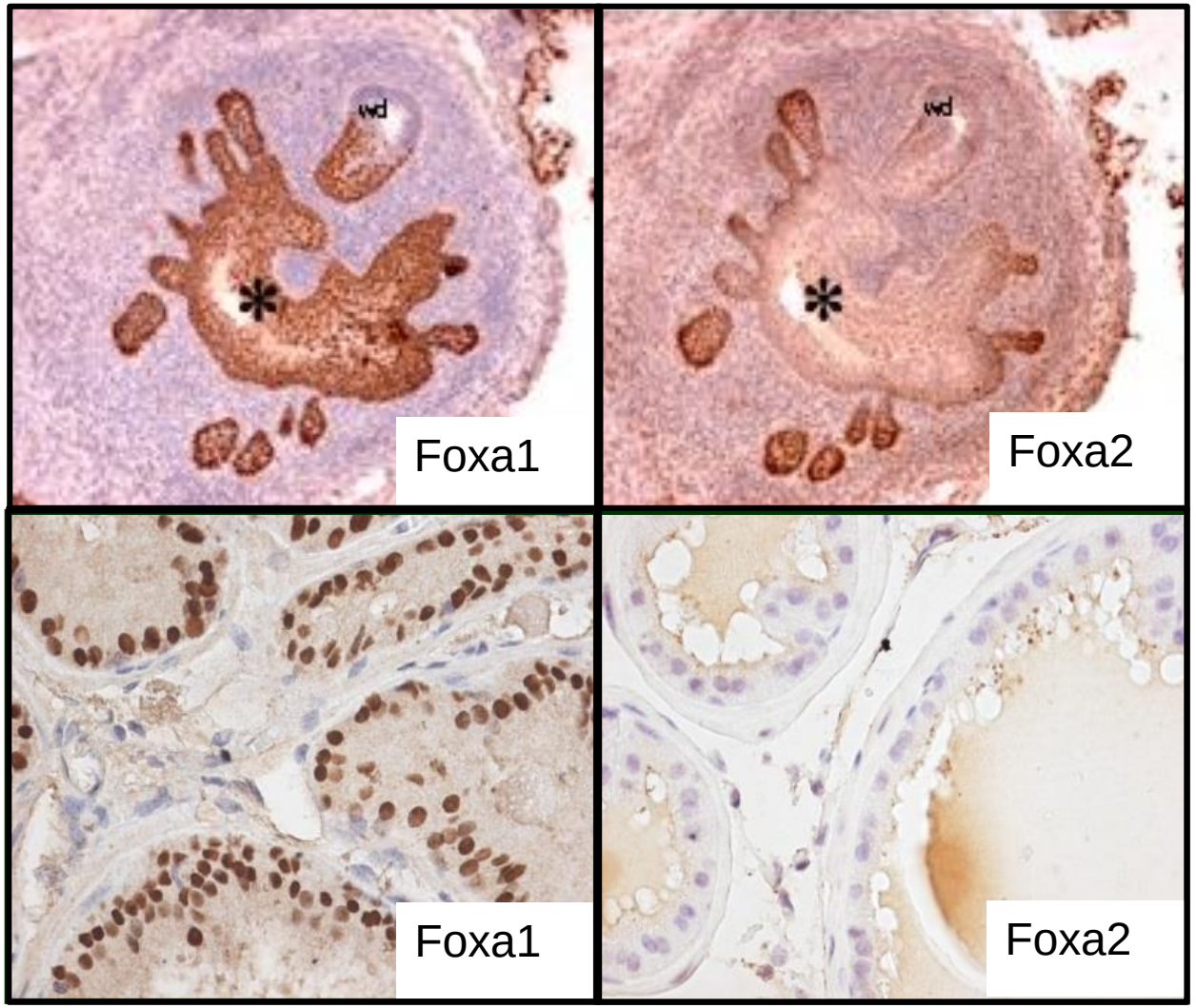






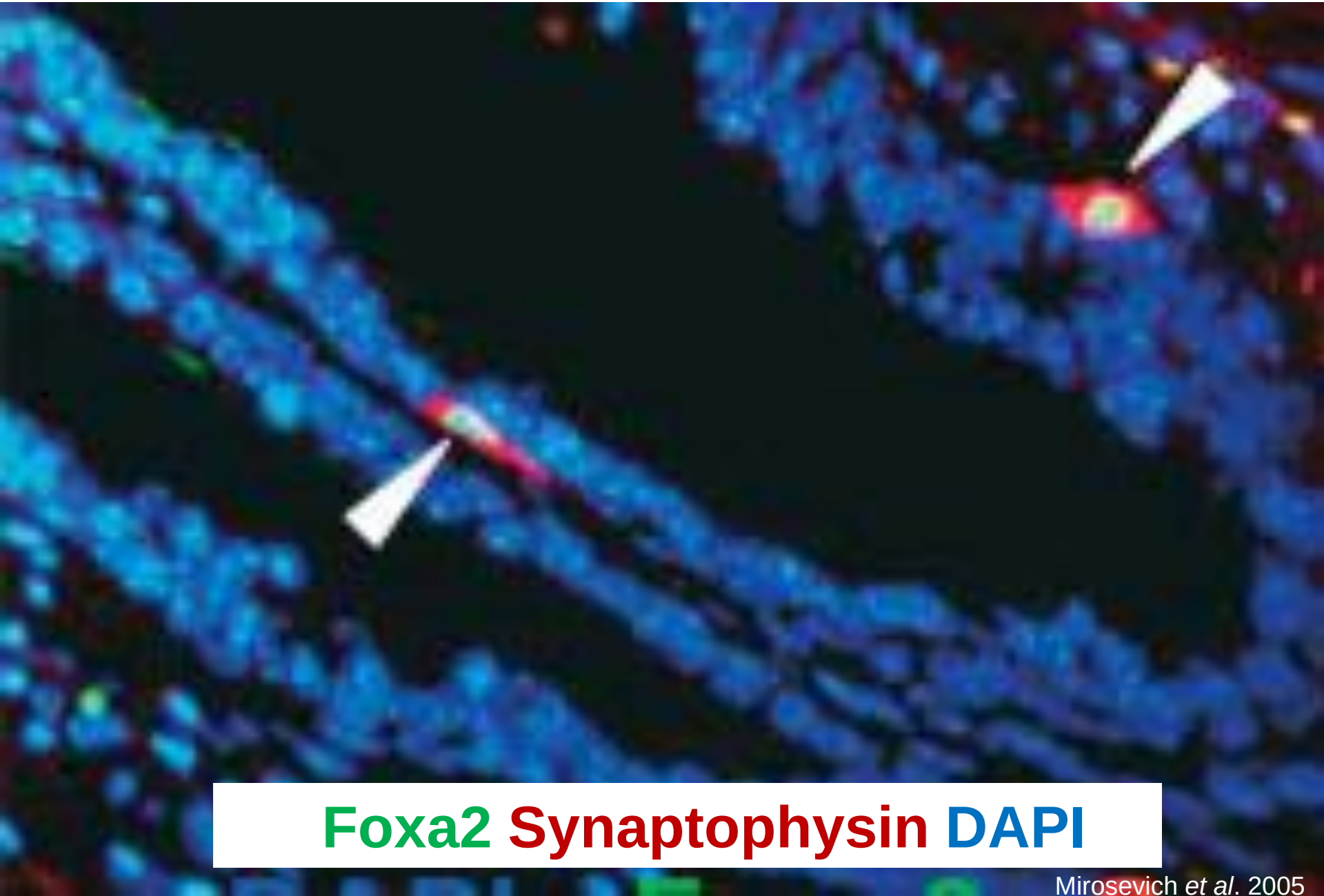
Yu *et al. Prostate* 2009

Foxa1 and Foxa2 are expressed in prostate



Yu *et al*. *Ann N Y Acad Sci.* 2005
Yu et al. Mol Endocrinol. 2006
Mirosevich *et al*. *Prostate* 2006

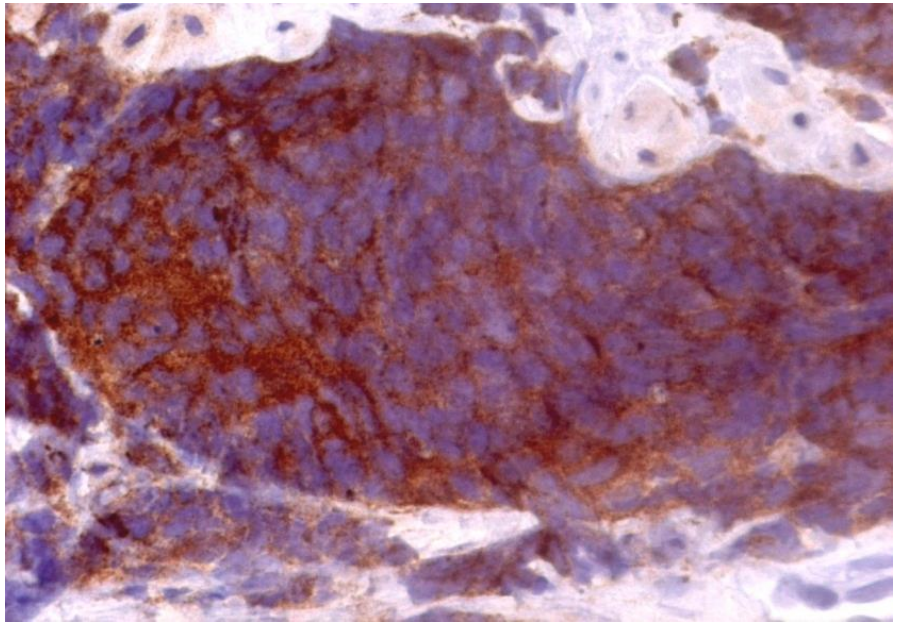
Foxa2 is expressed in rare NE cells in adult prostate



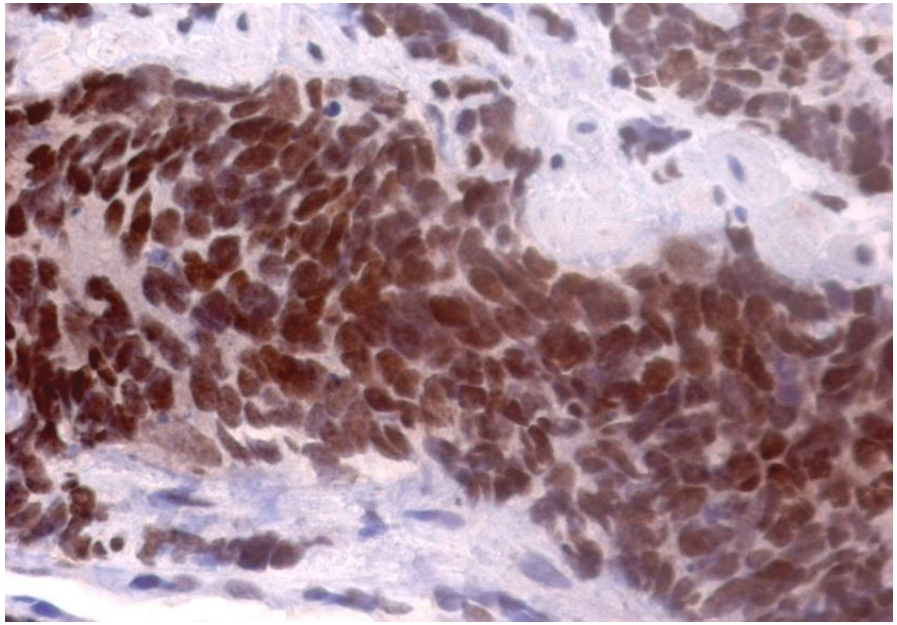
Mirosevich *et al*. 2005

Foxa2 is expressed in human NE PCa

Synaptophysin

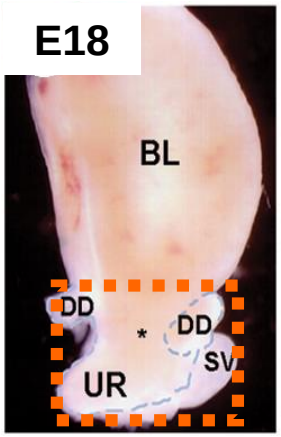


Foxa2

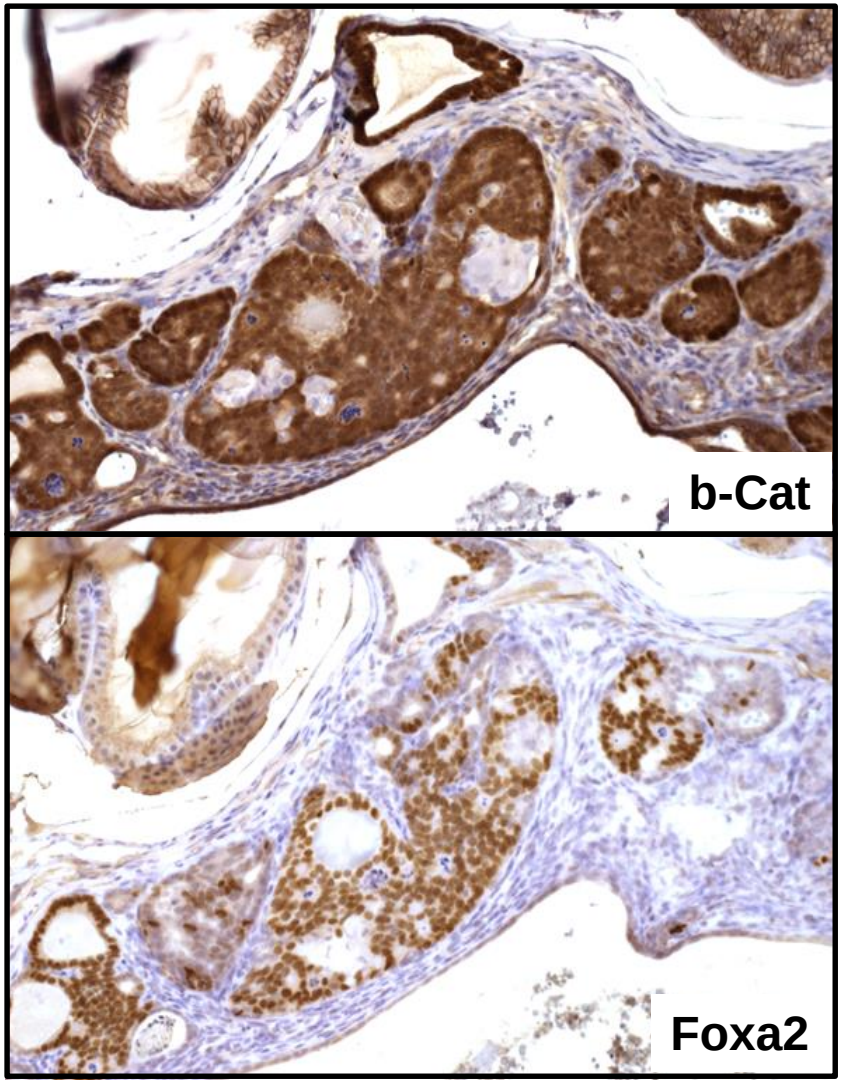


Yu *et al. Mol Endocrinol.* 2006

Activation of Wnt/b-Catenin induces the expression of Foxa2



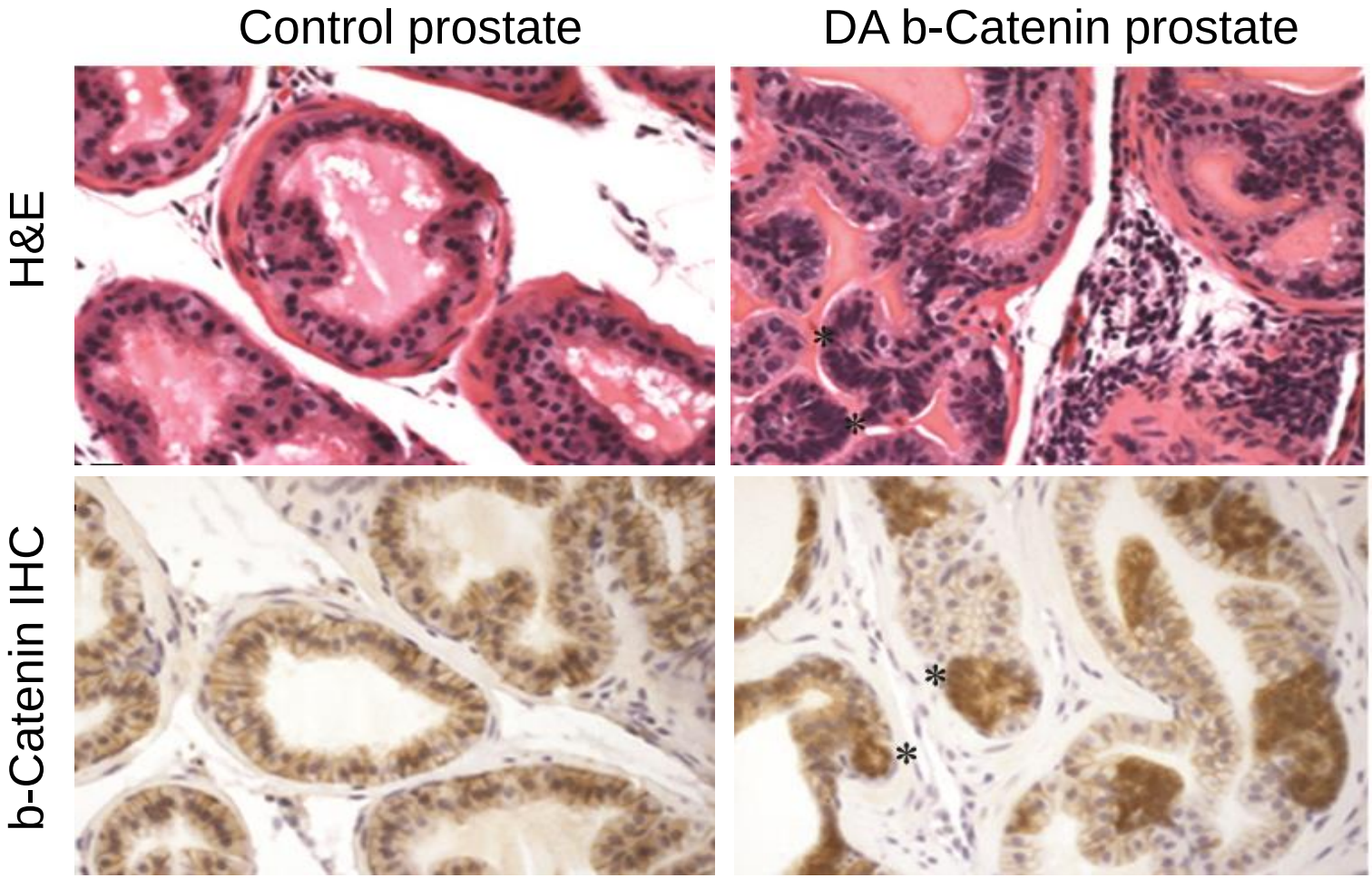




Yu *et al. Prostate* 2009

Post-natal activation of Wnt/b-Catenin by PB-Cre

Activation of b-Catenin in prostate by PB-Cre causes hyperplasia and PIN at 12 weeks

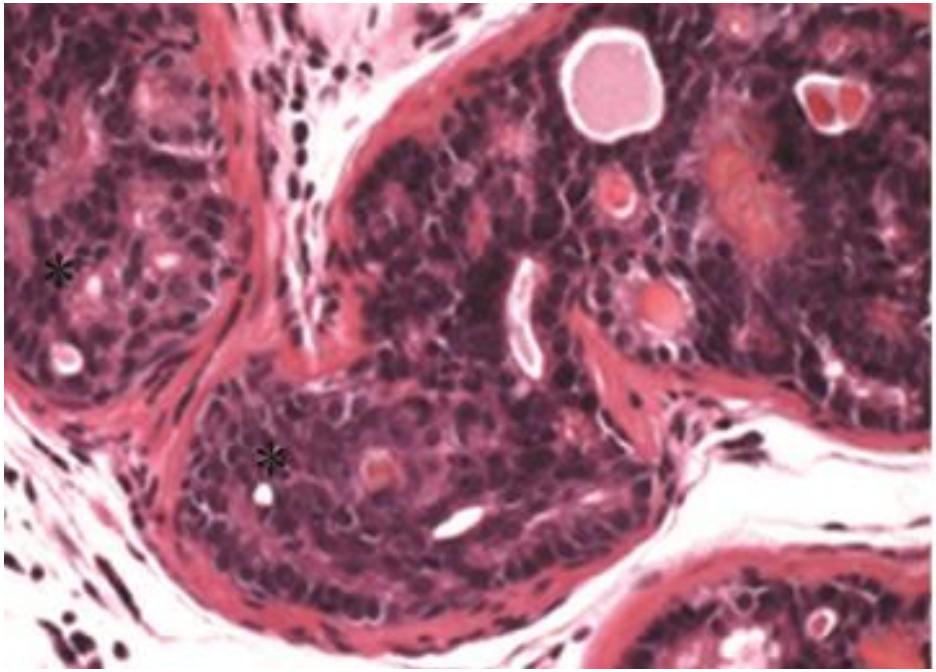


PIN: Prostate Intraepithelial Neoplasia
DA b-Catenin: Dominant Active b-Catenin

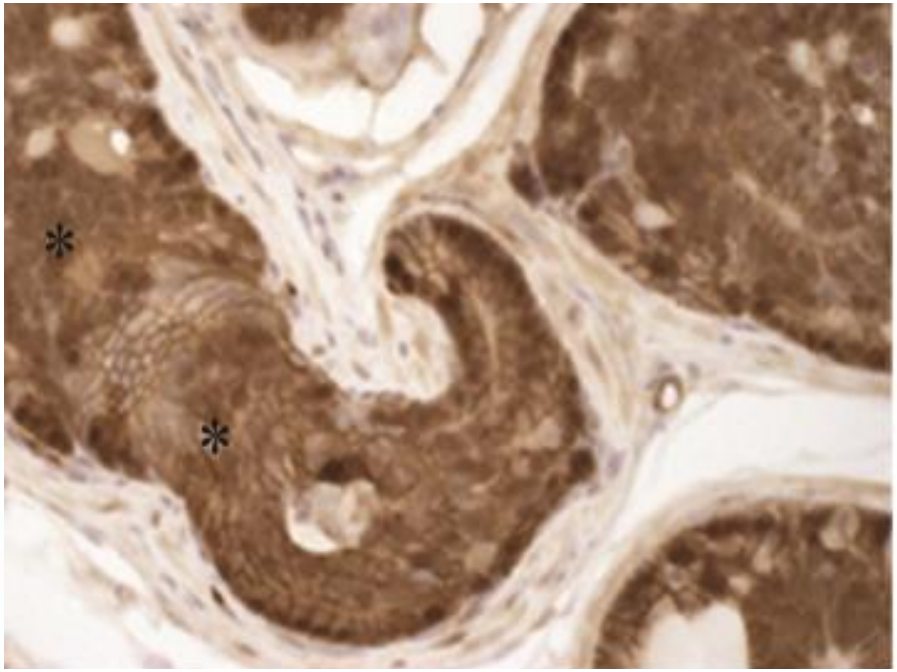
Yu *et al. Prostate* 2009

Activation of b-Catenin in prostate by PB-Cre causes HGPIN at later age (9 Months)

H&E



b-Catenin IHC



PIN: Prostate Intraepithelial Neoplasia
DA b-Catenin: Dominant Active b-Catenin

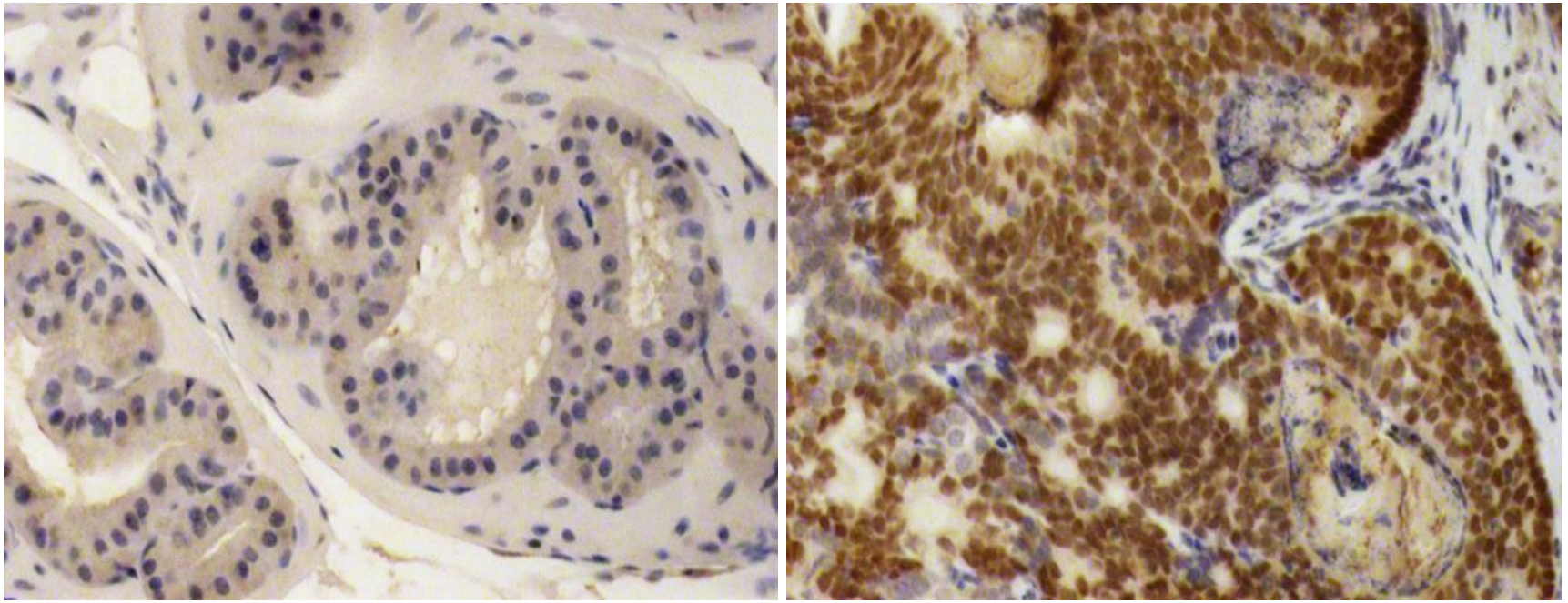
Yu *et al*. *Prostate* 2009

Foxa2 was re-expressed in DA b-Catenin mouse prostates

Control prostate

DA b-Catenin prostate

Foxa2 IHC



DA b-Catenin: Dominant Active b-Catenin

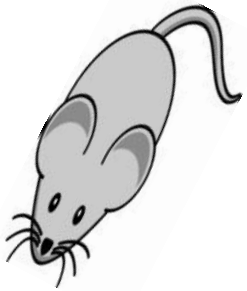
Yu *et al. Prostate* 2009

T-antigen/DA b-Catenin mice

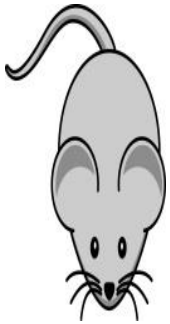


Lady LPB-Tag
12T-7f
Develops PIN

X



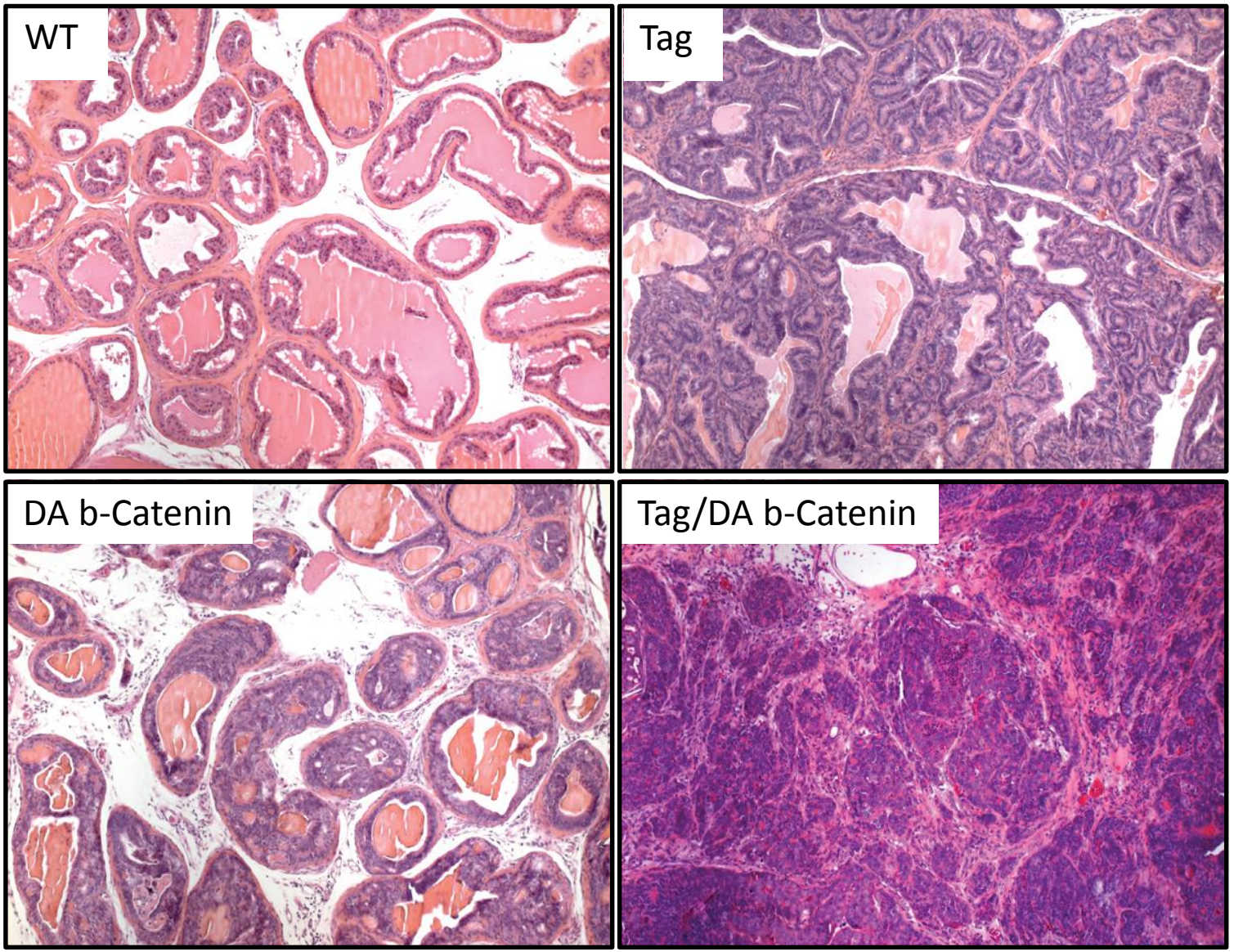
Activated b-Catenin
b-Cat$^{\Delta ex3}$
Develops PIN



LPB-Tag + b-Cat$^{\Delta ex3}$

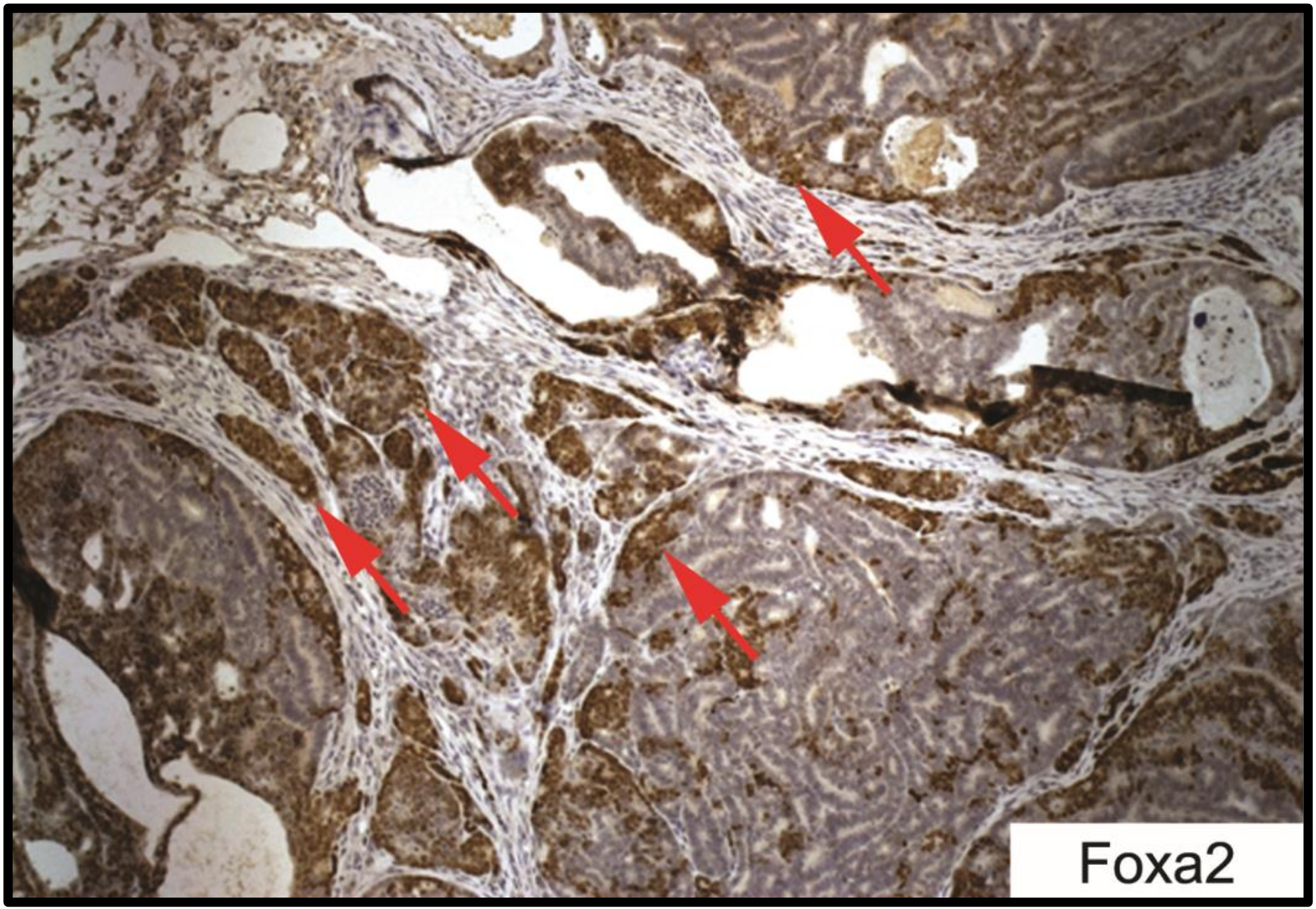
Yu *et al. Oncogene*, 2011

Activation of Wnt/b-Catenin and T-antigen results in invasive prostate cancer

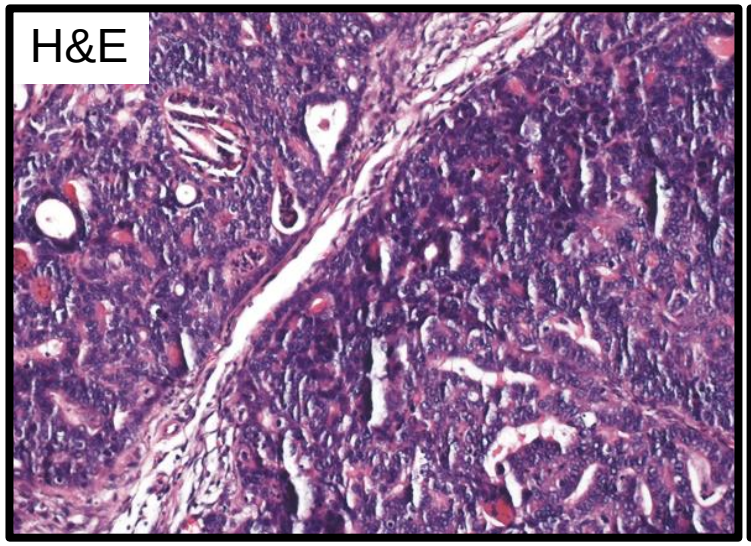


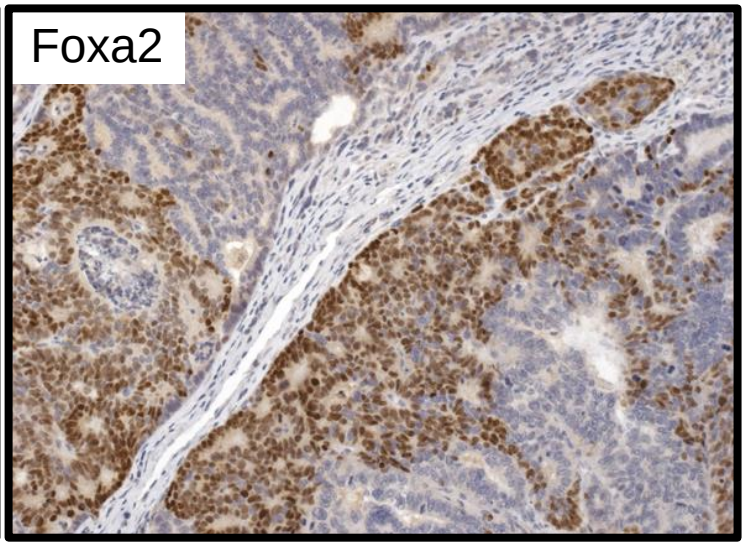
Yu *et al. Oncogene*, 2011

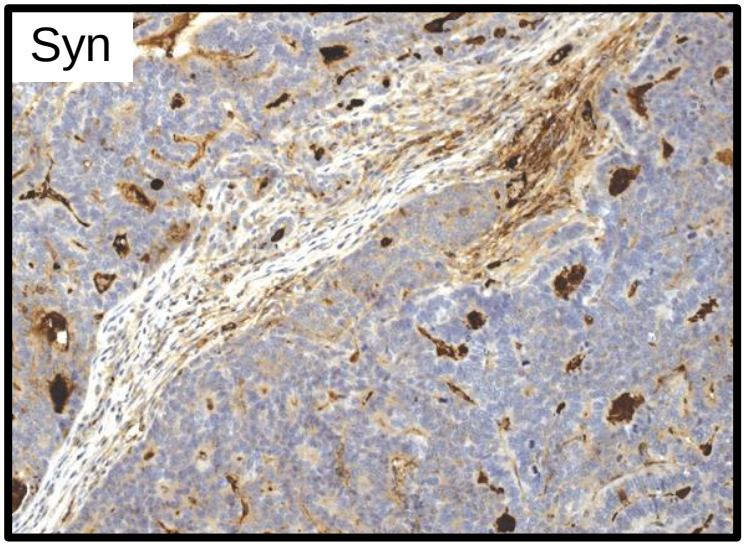
Foxa2 is expressed at the invasive front of PCa

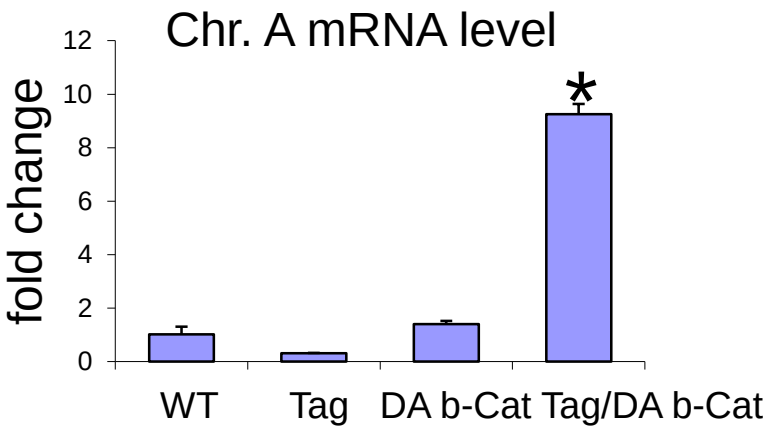


DA b-Catenin/T-antigen mouse prostates display increased NED





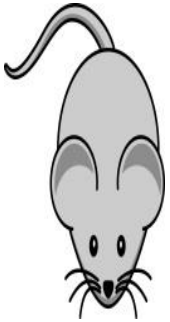




Yu *et al. Oncogene*, 2011

Question:
Can active Wnt/b-Catenin signaling promote castrate-resistant growth?

Control mice



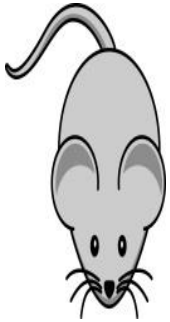
Castration

↓

prostate regress

2 weeks later

DA b-Catenin mice

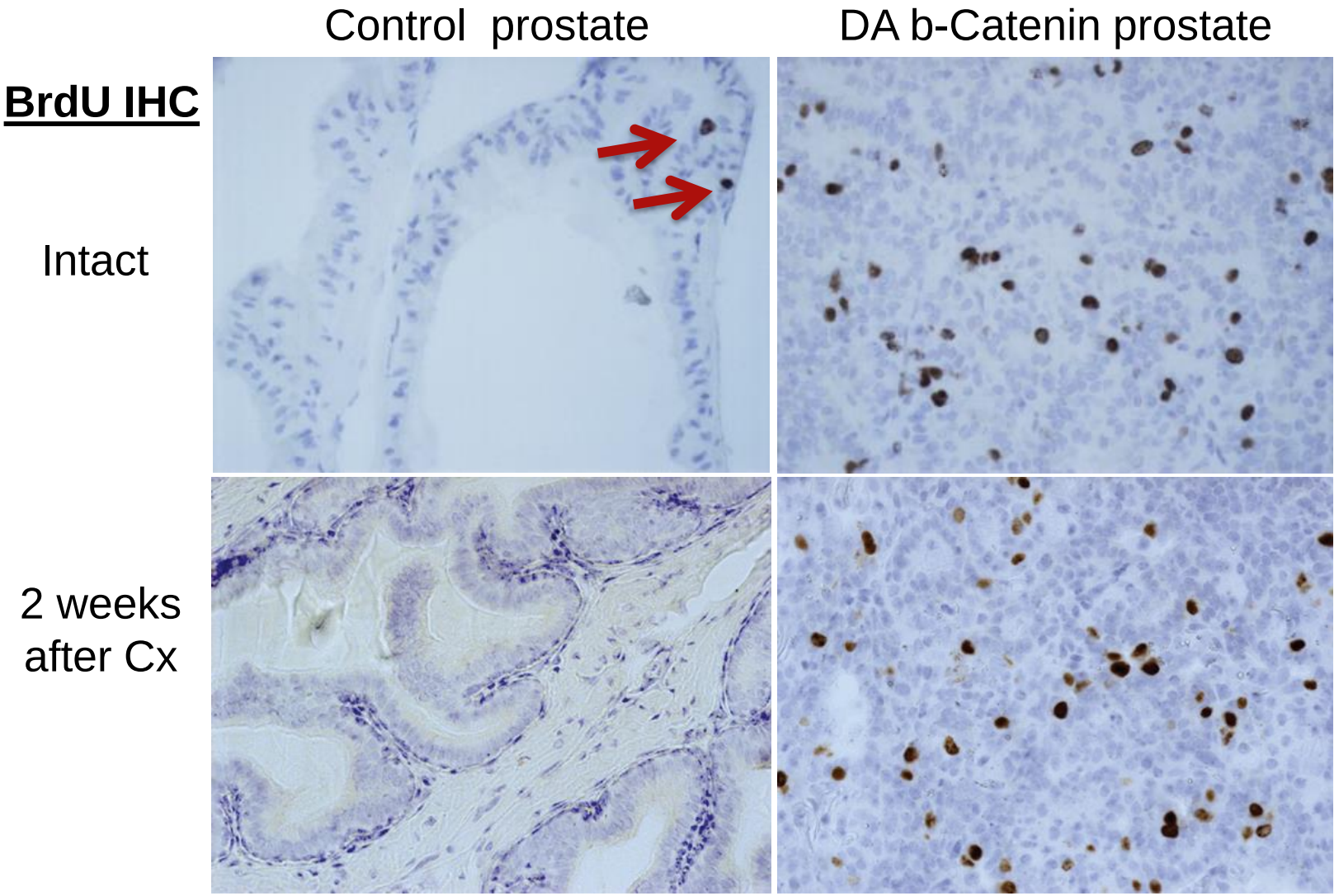


Castration

↓

prostate continue growing?

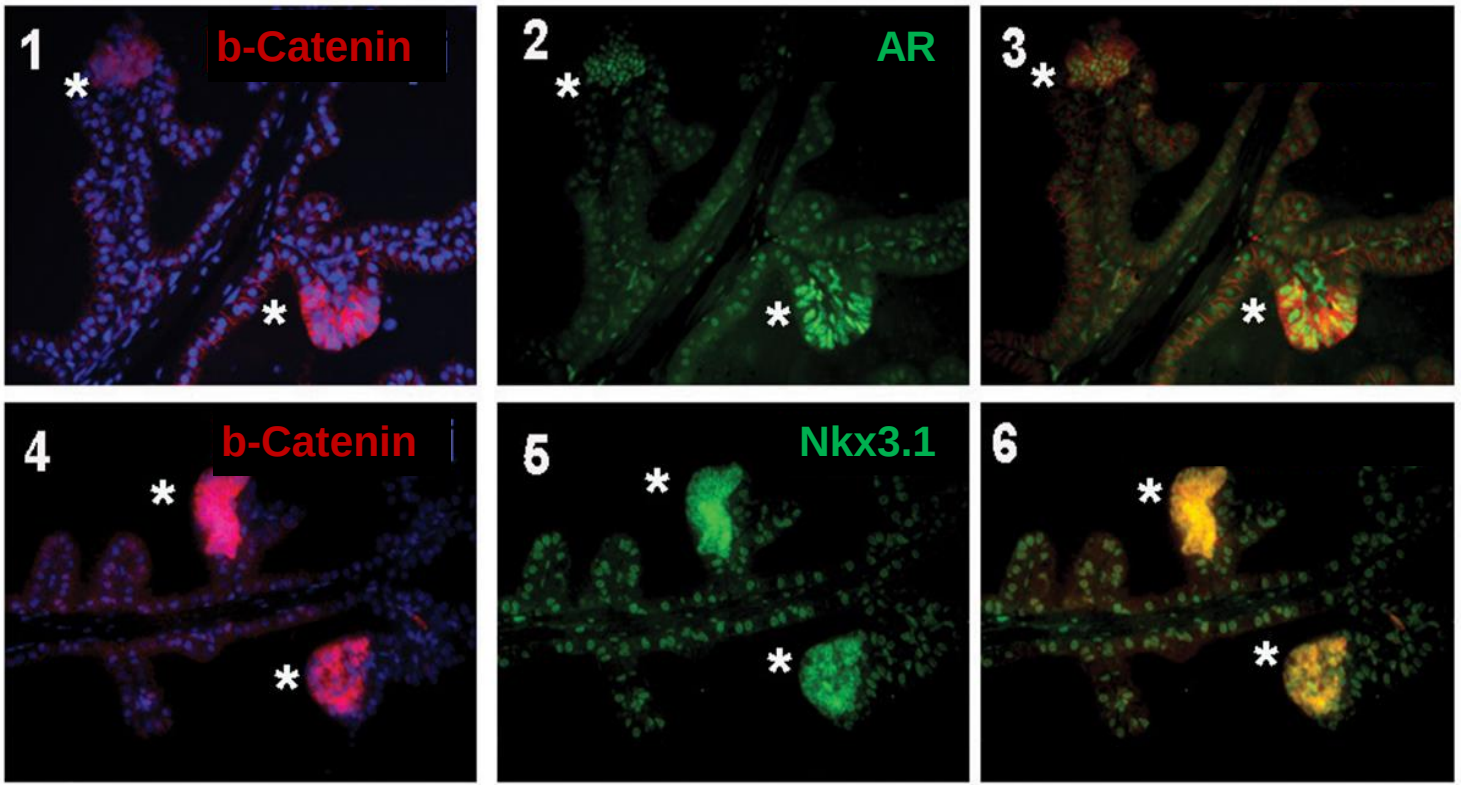
Activation of Wnt/b-Catenin results in castrate-resistant prostate growth



DA b-Catenin: Dominant Active b-Catenin

Yu *et al. Prostate* 2009

Activation of Wnt/b-Catenin regulates AR signaling differentially at early and late age

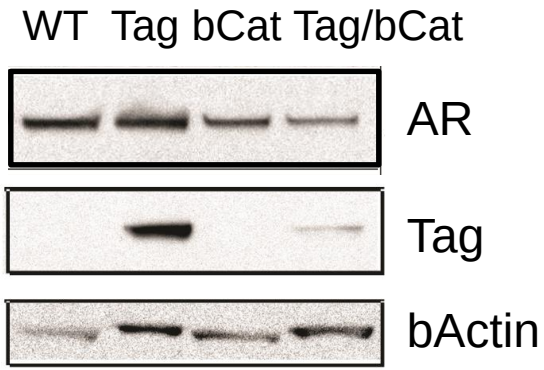


Nkx3.1: AR target gene



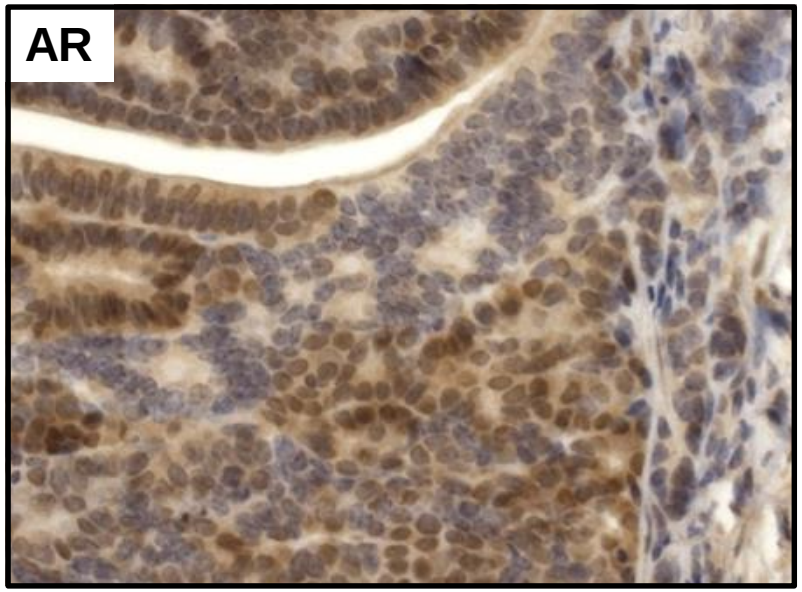
Yu *et al. Prostate*, 2009

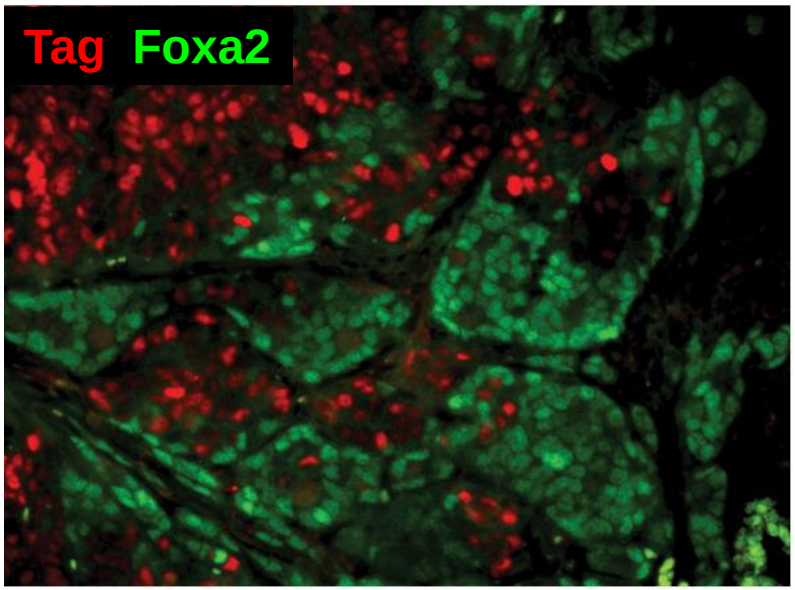
AR & AR signaling are down-regulated in T-antigen/DA b-Catenin mouse prostates



Tag: reporter of androgen signaling

Foxa2: target gene of Wnt/b-catenin

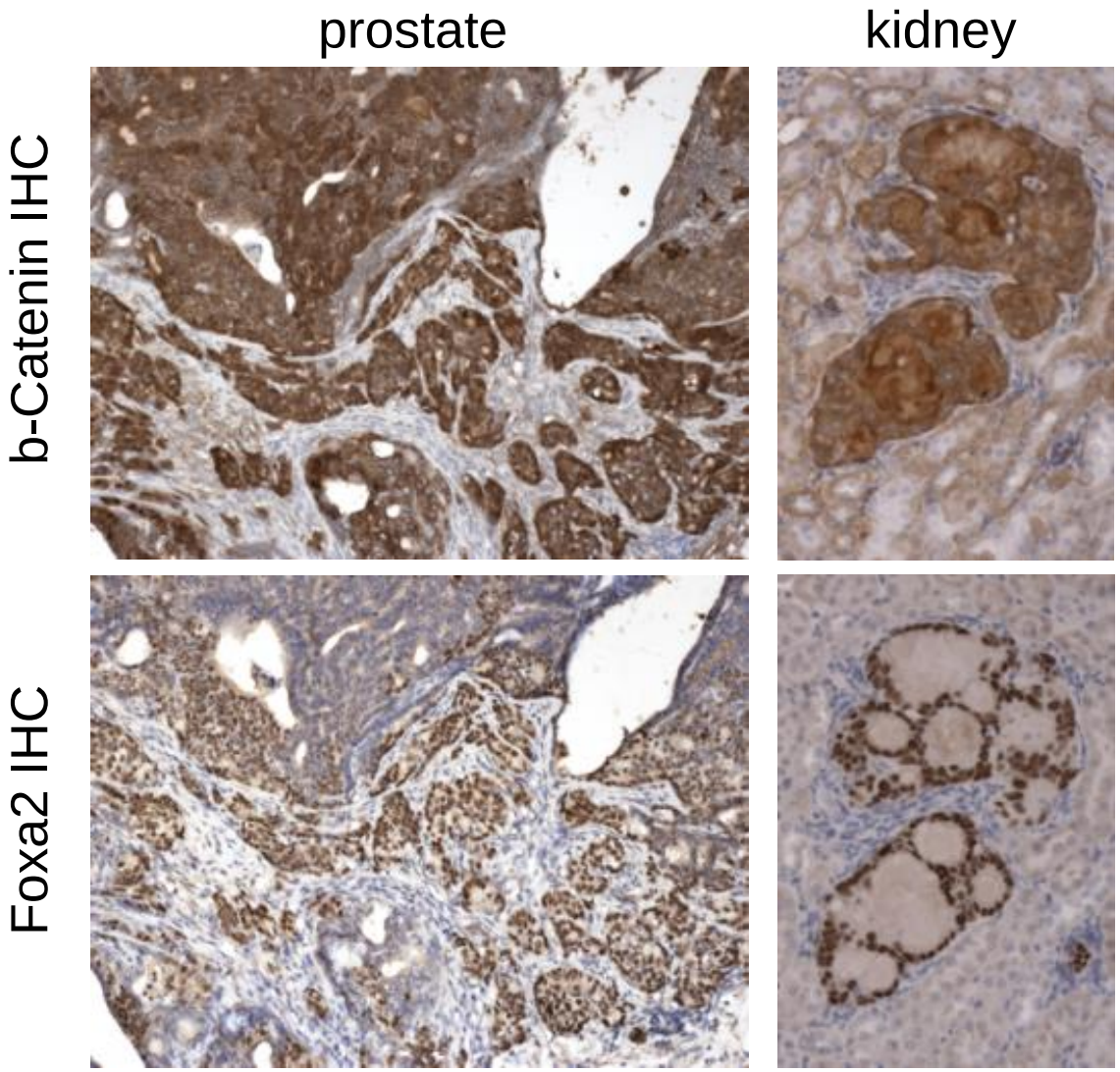




Yu *et al. Oncogene* 2011

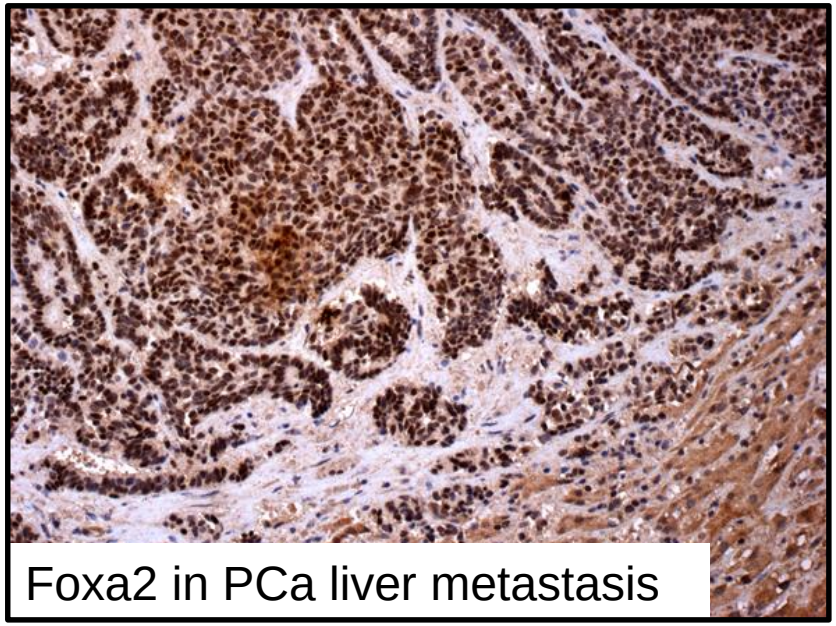
Candidate:
Foxa2 mediates Wnt/b-Catenin induced castration resistance

Foxa2 is a downstream target of Wnt/b-Catenin signaling

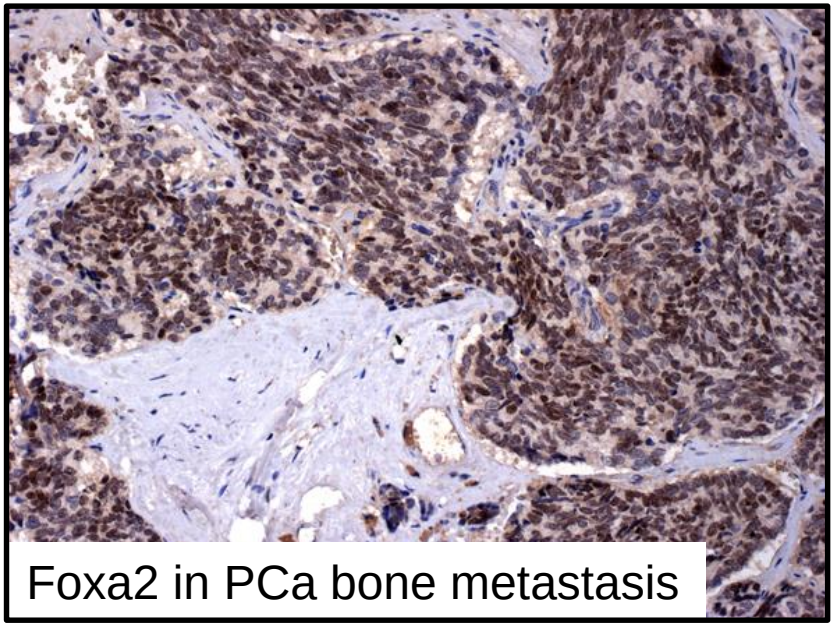


b-Cat$^{\Delta ex3}$ kidney tumor with Wilm's features

Foxa2 was detected in human metastatic CRPC



Foxa2 in PCa liver metastasis

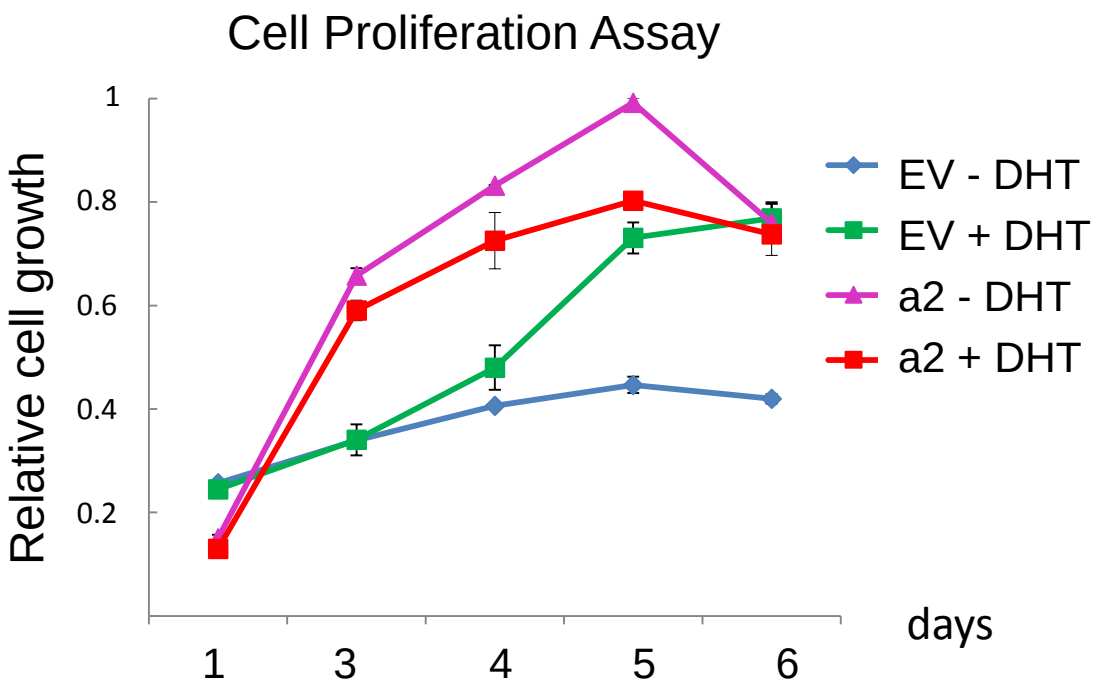


Foxa2 in PCa bone metastasis

Expression Pattern		Percentage
Foxa2+	Syn+	21%
	Syn-	8%
Foxa2-	Syn+	21%
	Syn-	50 %
Total		100%

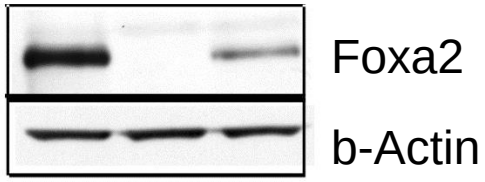
Expression of Foxa2 is sufficient to drive androgen-independent cell growth *in vitro*

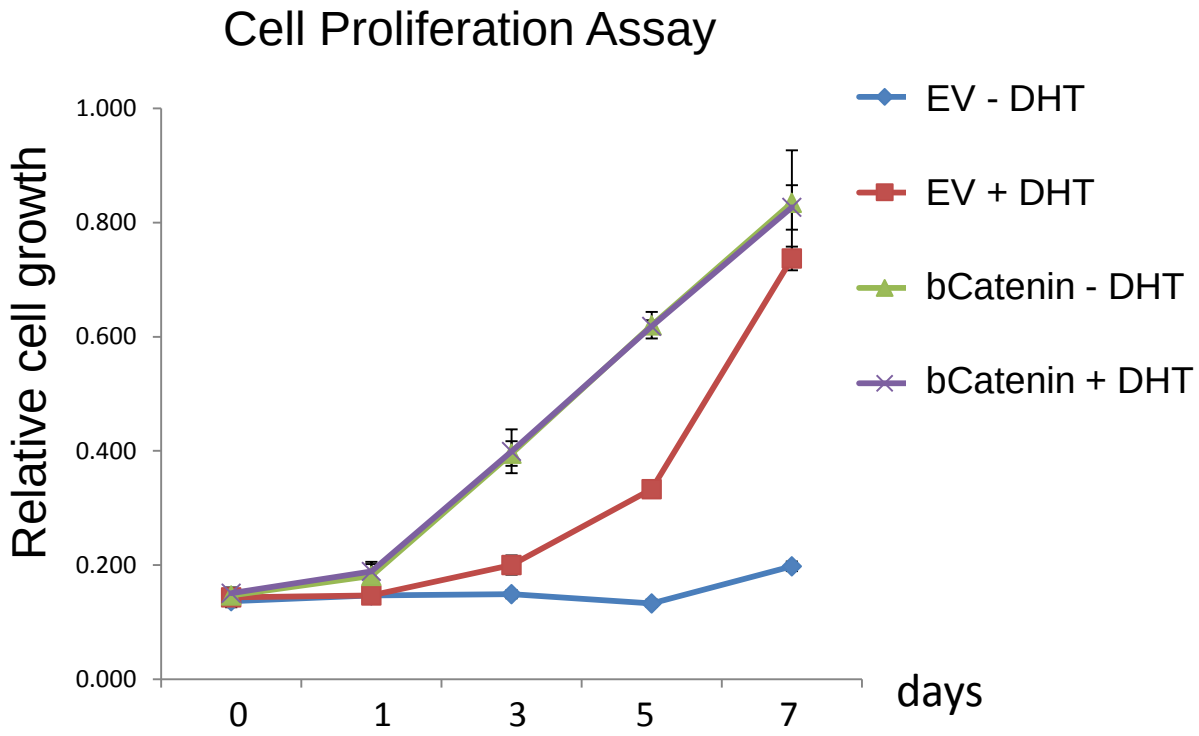




NT1: T antigen immortalized prostate epithelia
AR positive, androgen-dependent

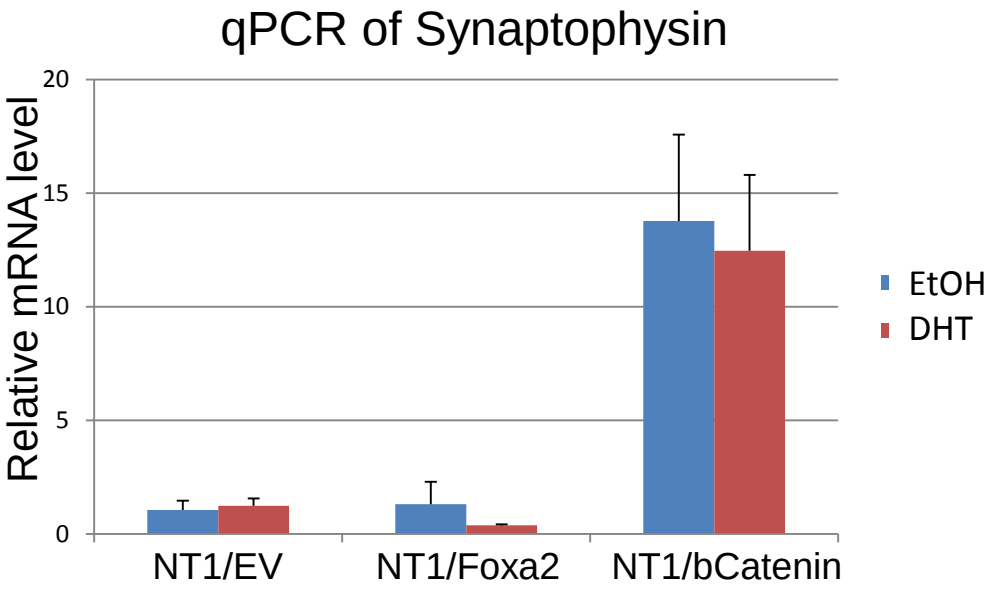
DA b-Catenin induced the expression of Foxa2 and enabled androgen-independent prostatic cell growth *in vitro*

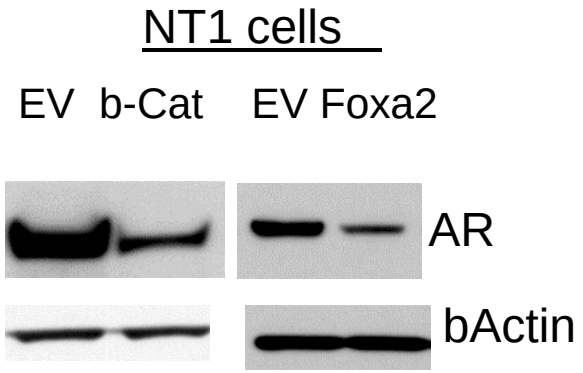




NT1: T antigen immortalized prostate epithelia
AR positive, androgen-dependent

DA b-Catenin but not Foxa2 induced NED in NT1 cells





- Foxa2 is not sufficient to induce NED
- Both the expression of DA b-Cat and Foxa2 down regulate AR
- A reduction in AR signaling fosters the emergence of NED in PCa (Kaplan-Lefko, *et al. Prostate,* 2003)
- ongoing: to study how b-Cat and Foxa2 down-regulates AR

Summary

- Activation of Wnt/b-Catenin promotes CRPCa progression
- Activation of Wnt/b-Catenin induces the expression of Foxa2 and down-regulate AR and AR signaling
- Activation of Wnt/b-Catenin induces NED in PCa

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University of Washington

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