Fred Hutch Cancer Center

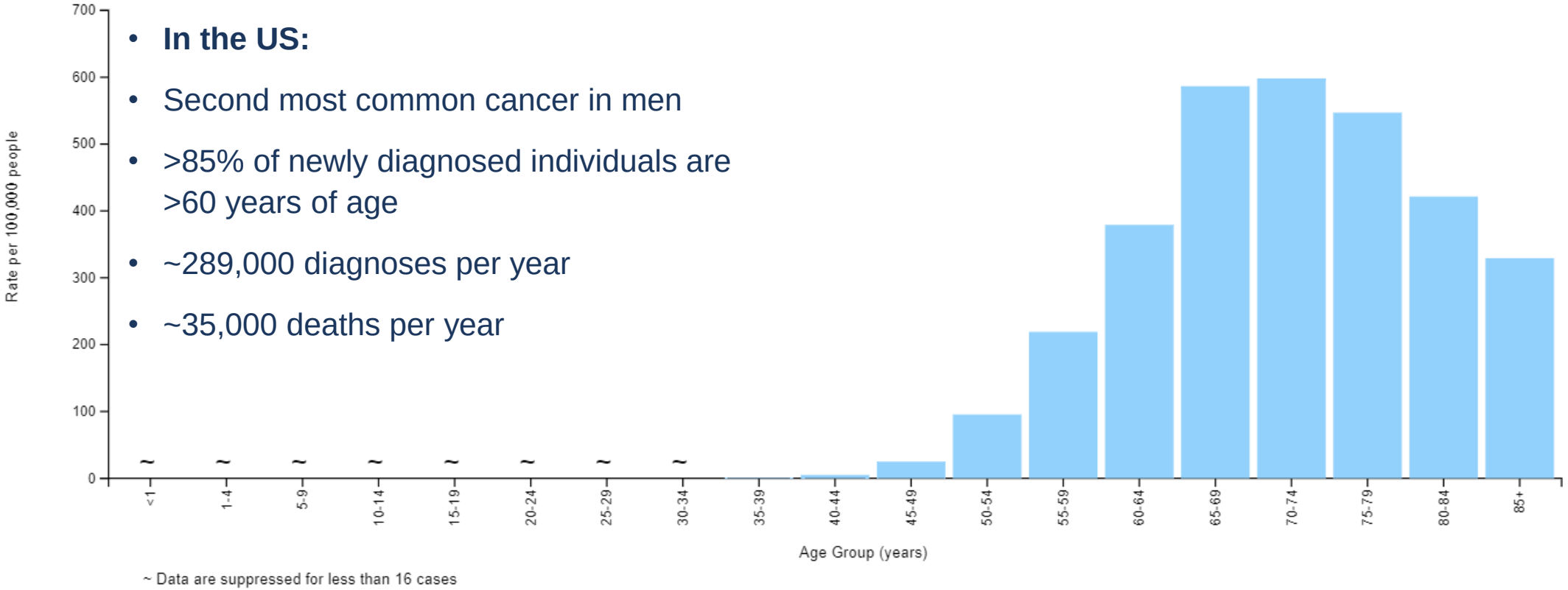
Disrupting the CXCL12-CD26/CXCR4,7 Axis Through Supraphysiological Levels of R1881

UW Medicine

Prostate Cancer Overview

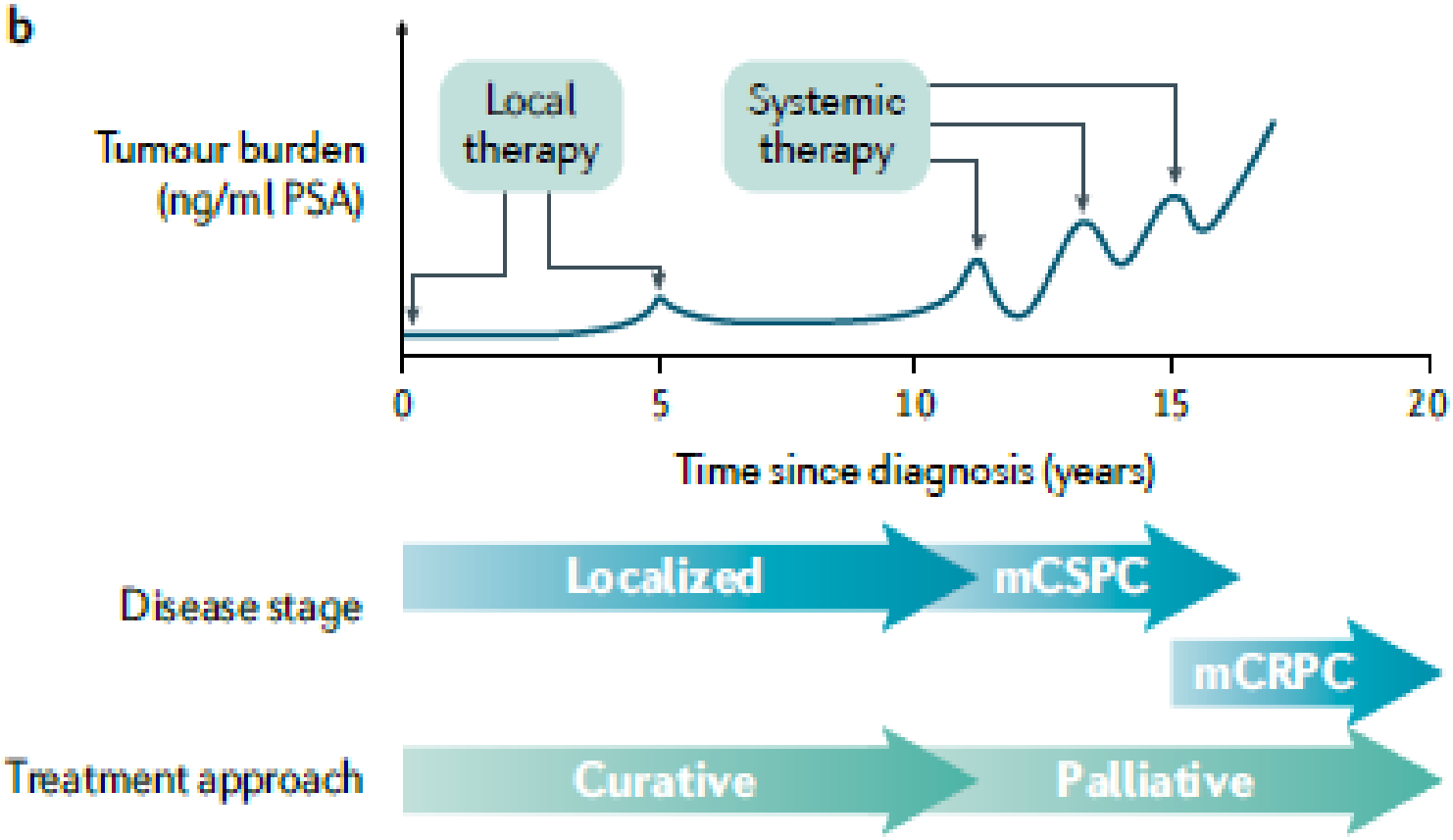
Demographic

- **In the US:**
- Second most common cancer in men
- >85% of newly diagnosed individuals are >60 years of age
- ~289,000 diagnoses per year
- ~35,000 deaths per year



Source - U.S. Cancer Statistics Working Group. U.S. Cancer Statistics Data Visualizations Tool, based on 2022 submission data (1999-2020): U.S. Department of Health and Human Services, Centers for Disease Control and Prevention and National Cancer Institute; https://www.cdc.gov/cancer/dataviz, released in June 2023.

Prostate Cancer Progression

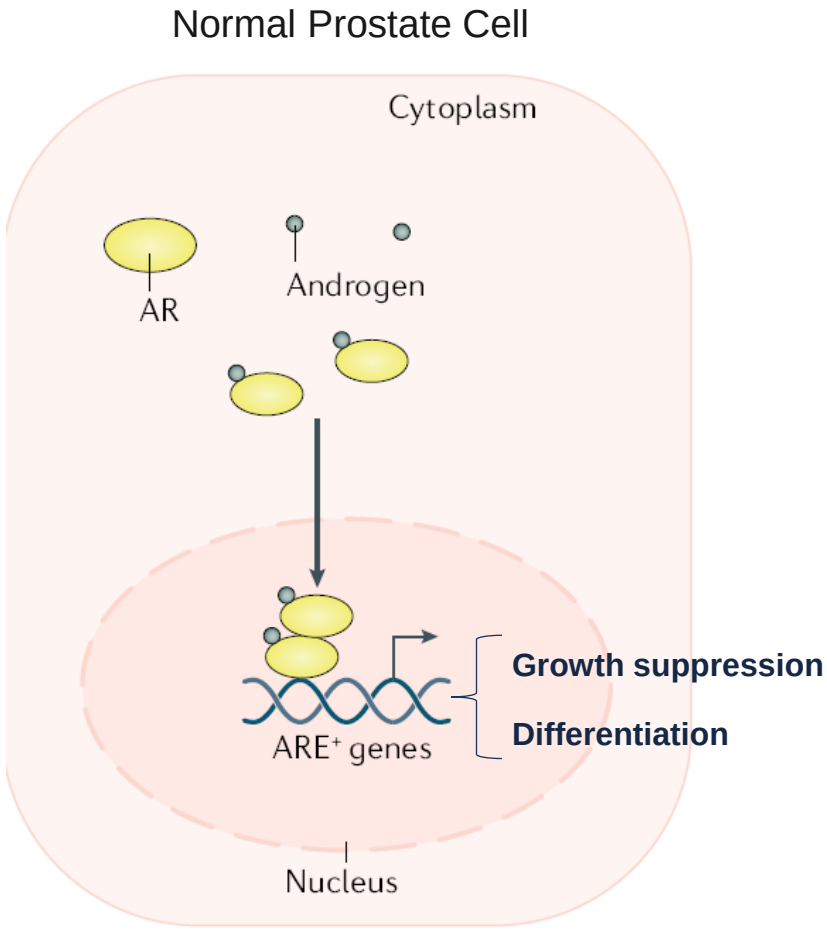


- **In the US:**
- 80% of men diagnosed with PC have localized PC
  - Survival rate as high as 99%
  - 20-30% of men advance to mPC within 5-10 yrs
- 15% of men diagnosed with PC have locoregional metastasis
- 5% of men diagnosed with PC have distant metastasis

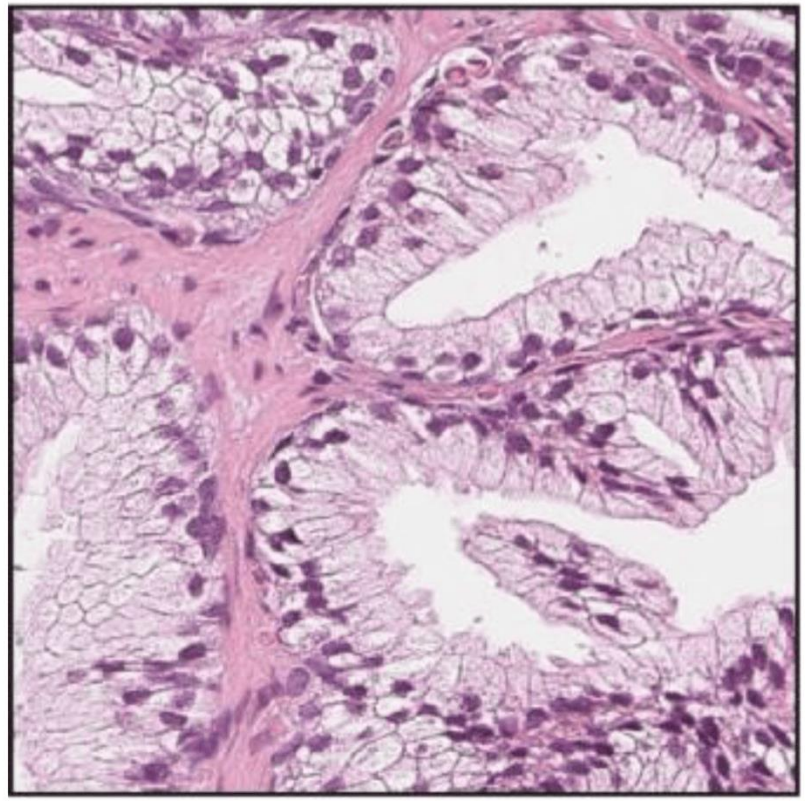
Modified from Rebello RJ et al., 2021

AR as Tumor Suppressor in Normal Prostate Cells

- **Androgen**: Male sex hormone
  - Testosterone (T)
  - Dihydrotestosterone (DHT)
- **Androgen Receptor** (AR): Transcription Factor
  - Regulates proliferation and apoptosis through gene transcription



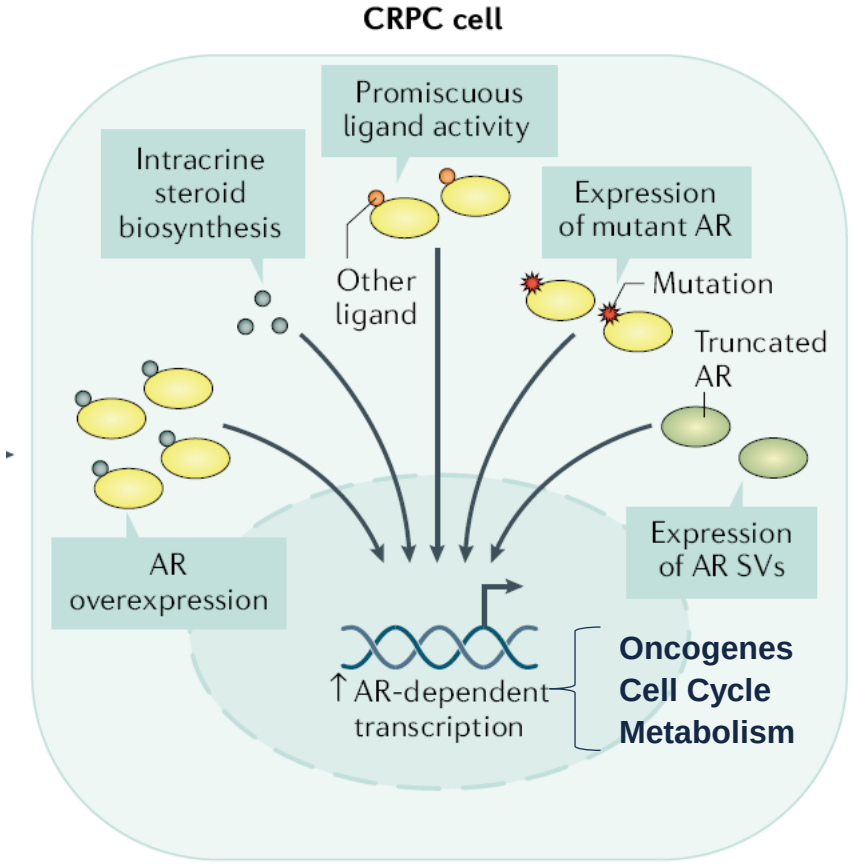
Normal Prostate Tissue



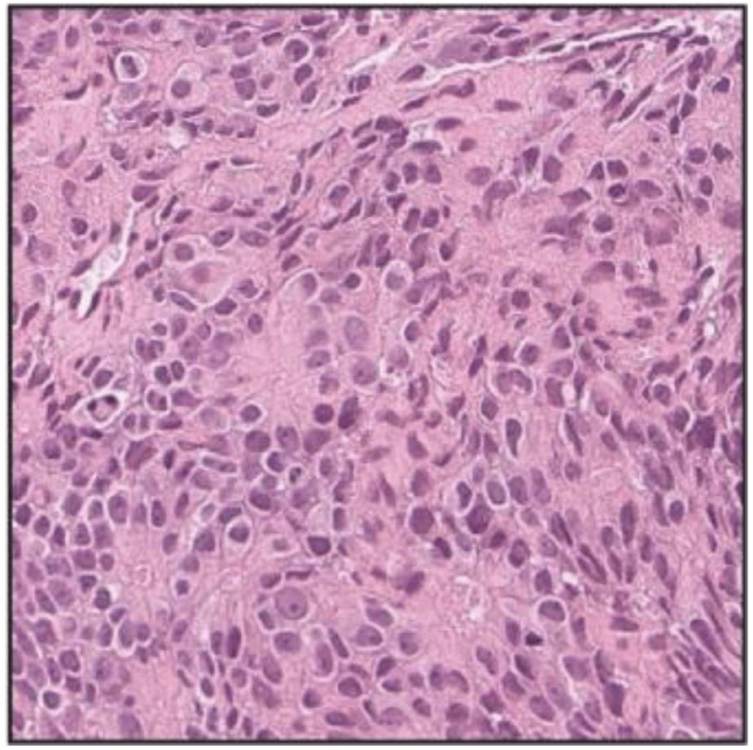
Modified from Rebello RJ et al., 2021

Massimo Salvi et al.,

AR in Castration-Resistant Prostate Cells



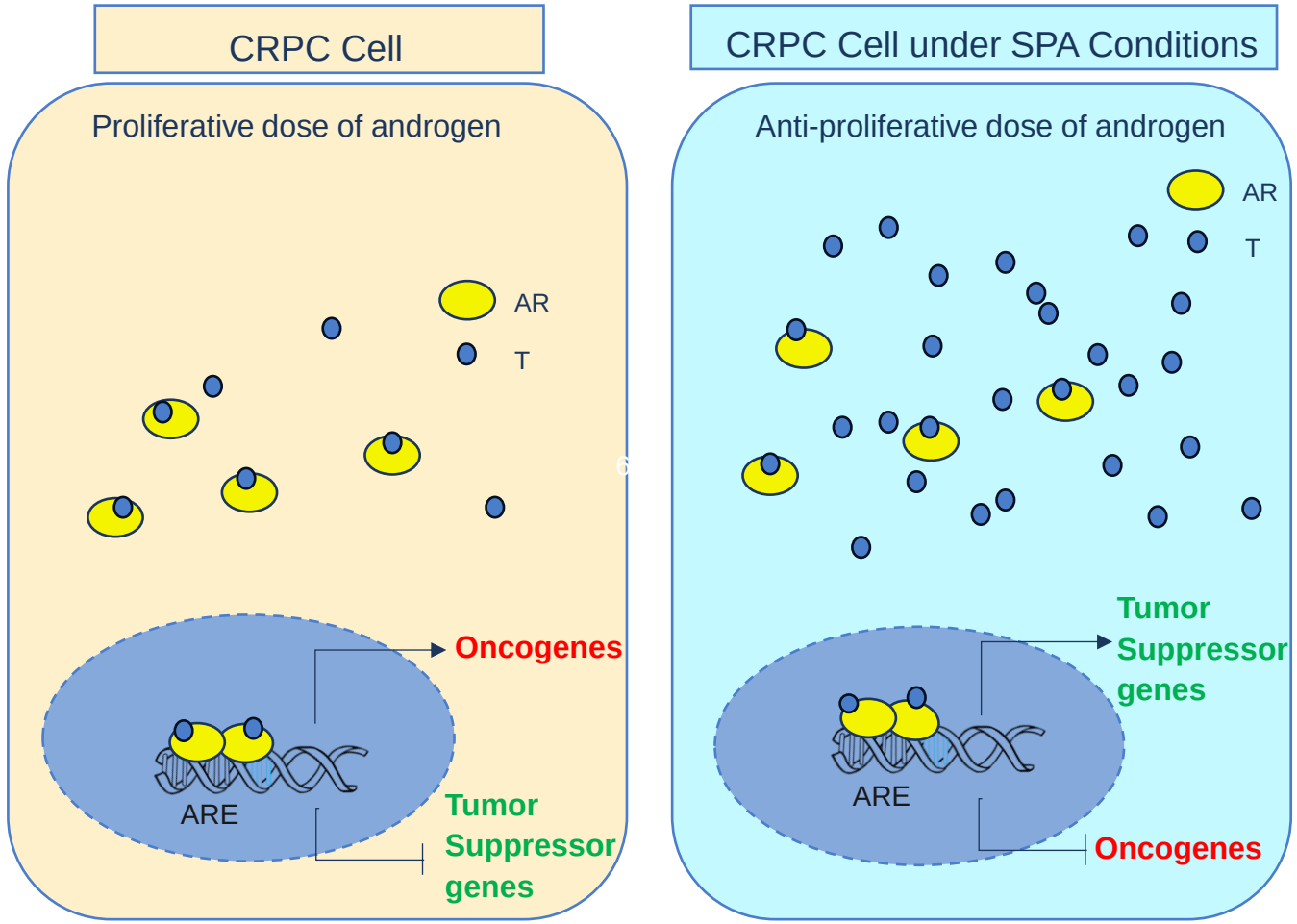
Cancer tissue



Modified from Rebello RJ et al., 2021

Massimo Salvi et al.,

Supraphysiological Androgen Suppresses Tumor Growth



J Clin Invest. 2019 Jul 16;129(10):4245-4260. doi: 10.1172/JCI127613.

Supraphysiological androgens suppress prostate cancer growth through androgen receptor–mediated DNA damage

Payel Chatterjee [1], Michael T Schweizer [2 3], Jared M Lucas [1], Ilsa Coleman [1], Michael D Nyquist [1], Sander B Frank [1], Robin Tharakan [1], Elahe Mostaghel [2 3], Jun Luo [4], Colin C Pritchard [5], Hung-Ming Lam [6], Eva Corey [6], Emmanuel S Antonarakis [7], Samuel R Denmeade [7], Peter S Nelson [1 2 3]

Affiliations + expand

PMID: 31310591 PMCID: PMC6763228 DOI: 10.1172/JCI127613

Free PMC article

Cancer Res. 2021 Dec 1;81(23):5948-5962. doi: 10.1158/0008-5472.CAN-20-3607. Epub 2021 Oct 13.

Supraphysiologic Testosterone Induces Ferroptosis and Activates Immune Pathways through Nucleophagy in Prostate Cancer

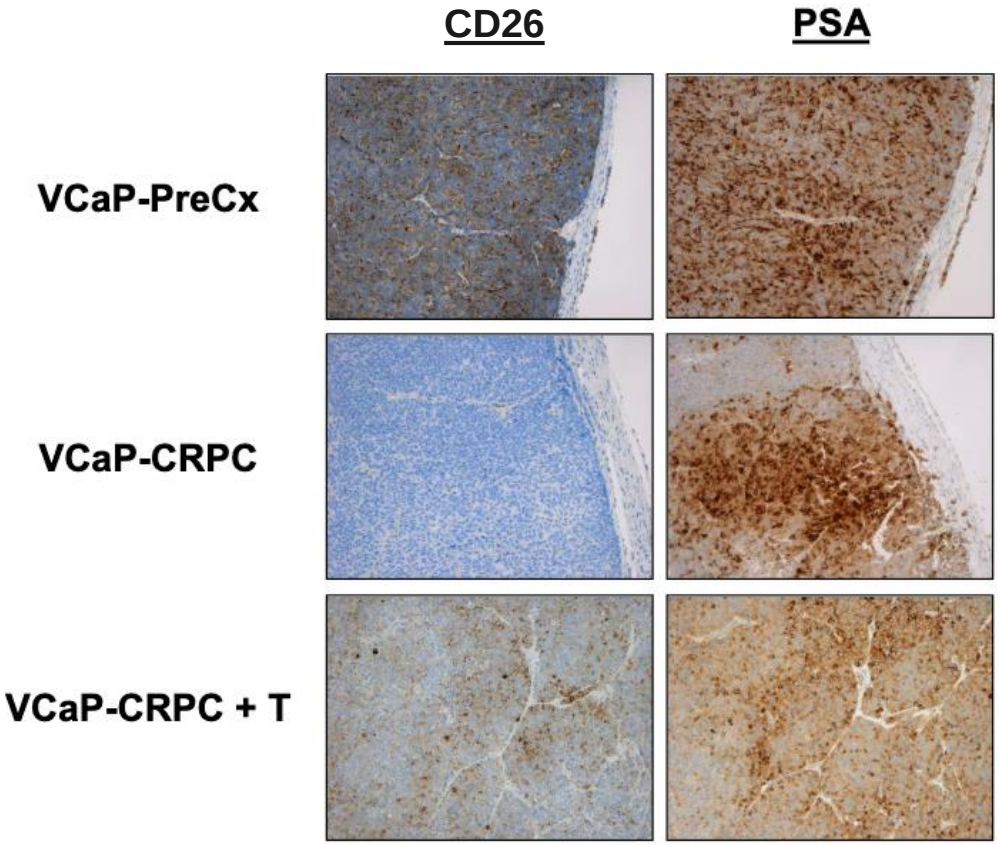
Rajendra Kumar [# 1], Janet Mendonca [# 1], Olutosin Owoyemi [1], Kavya Boyapati [1], Naiju Thomas [1], Suthicha Kanacharoen [1], Max Coffey [1], Deven Topiwala [1], Carolina Gomes [1], Busra Ozbek [1], Tracy Jones [1], Marc Rosen [1], Liang Dong [1], Sadie Wiens [2], W Nathaniel Brennen [1], John T Isaacs [1], Angelo M De Marzo [1], Mark C Markowski [1], Emmanuel S Antonarakis [1], David Z Qian [2], Kenneth J Pienta [1], Drew M Pardoll [1], Michael A Carducci [1], Samuel R Denmeade [1], Sushant K Kachhap [3]

Affiliations + expand

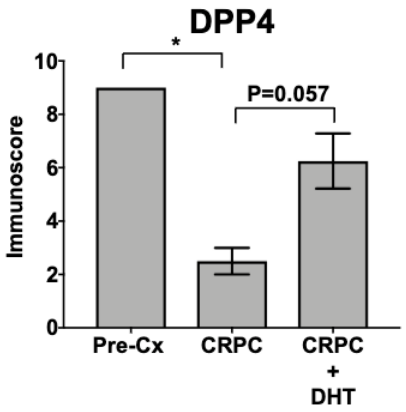
PMID: 34645612 PMCID: PMC8639619 DOI: 10.1158/0008-5472.CAN-20-3607

Free PMC article

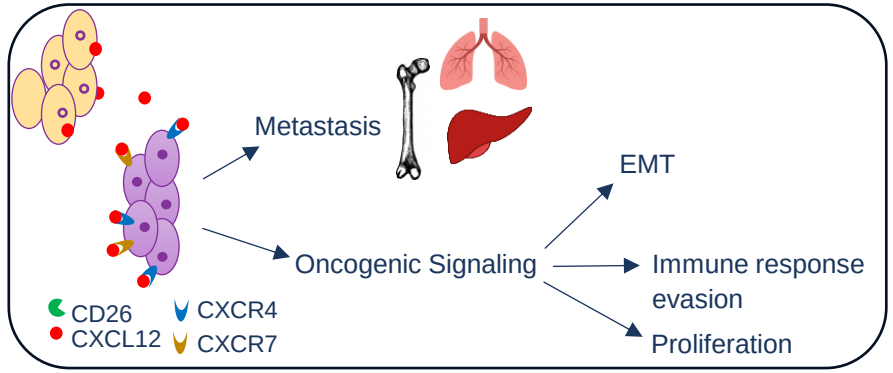
Supraphysiologic androgens can modulate the impact of the tumor microenvironment by restoring *DPP4*/CD26 expression.



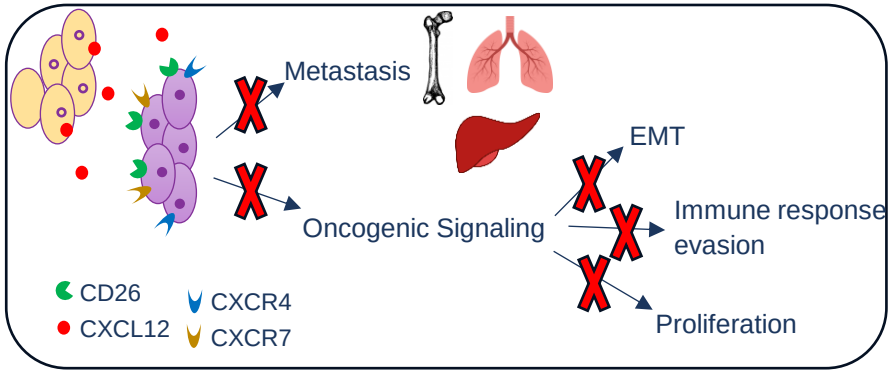
(200mg/kg)
for three days



DPP4/CD26 is repressed in advanced PC:



If *DPP4*/CD26 is restored under SPA

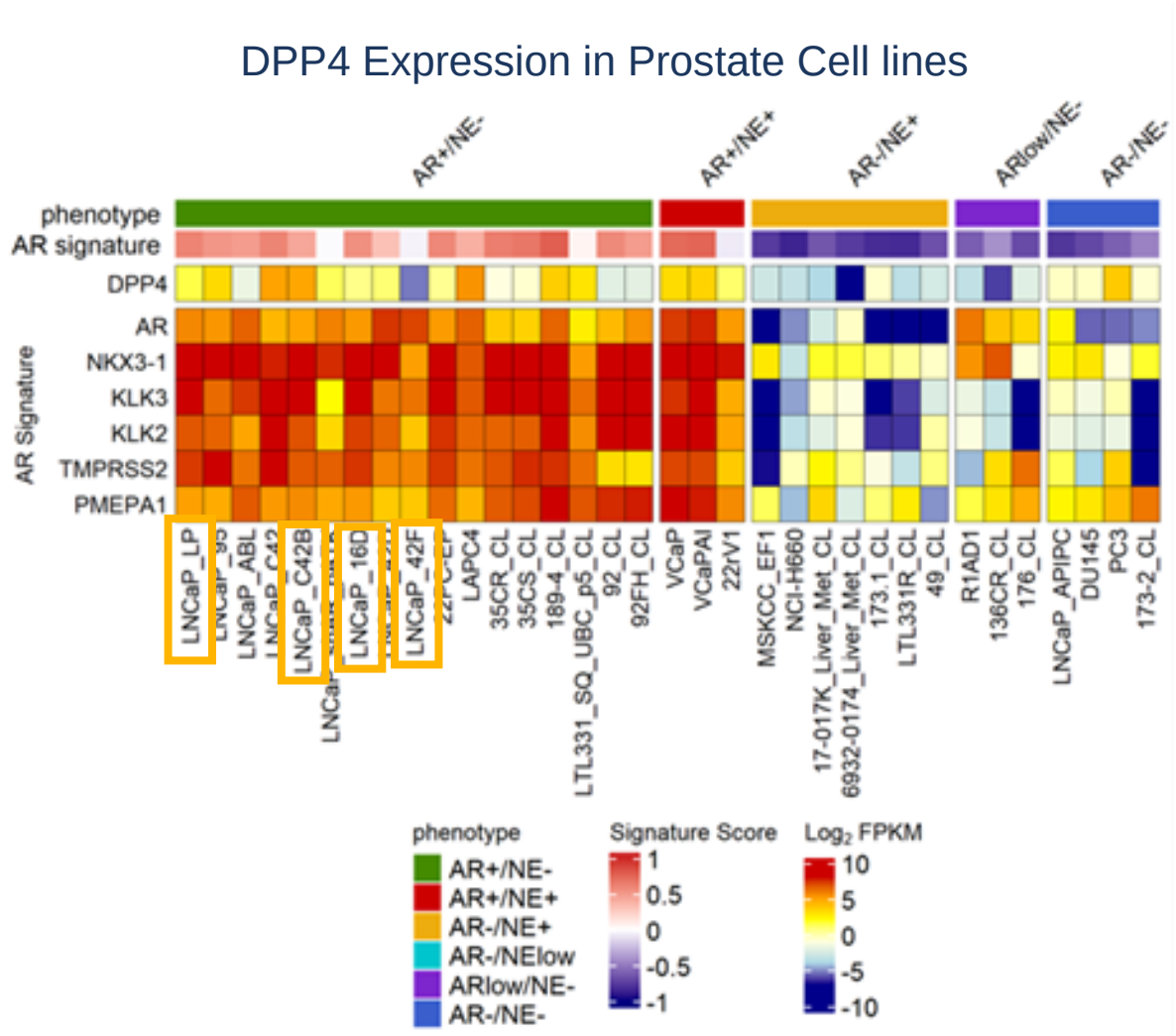


Russo JW... Nelson, PS et al., 2018

Hypothesis:

Restoring CD26 expression through SPA treatment can alter the oncogenic activity of the CXCL12-CXCR4,7 axis in prostate cancer

Experiment Model Cell Lines



Following PC Progression

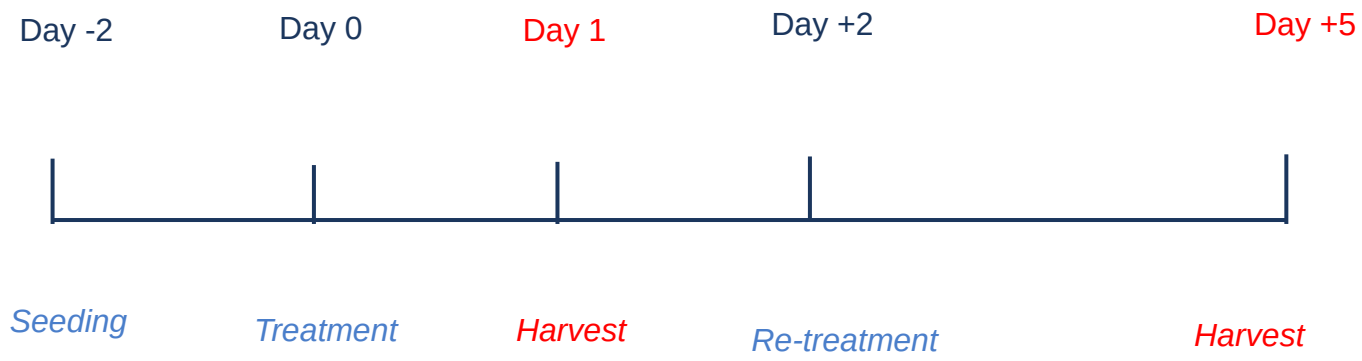
- **LNCaP**
  Castration sensitive
- **LNCaP_16D**
  Castration resistant
  Enzalutamide sensitive
- **LNCaP_49F**
  Castration resistant
  Enzalutamide resistant

Metastasis Model

- **LNCaP_C42B**
  Castration resistant
  High levels of CXCL12

Ilsa Coleman

Experimental Design

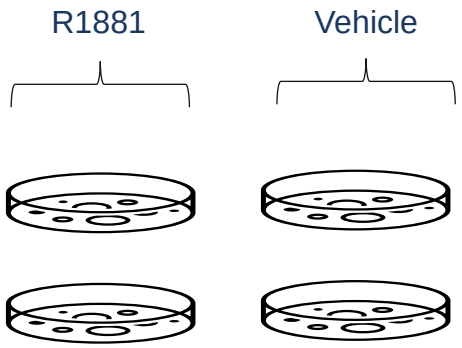


Cell Seeding Density:

Day 1: $4x10^6$ cells/10mL dish

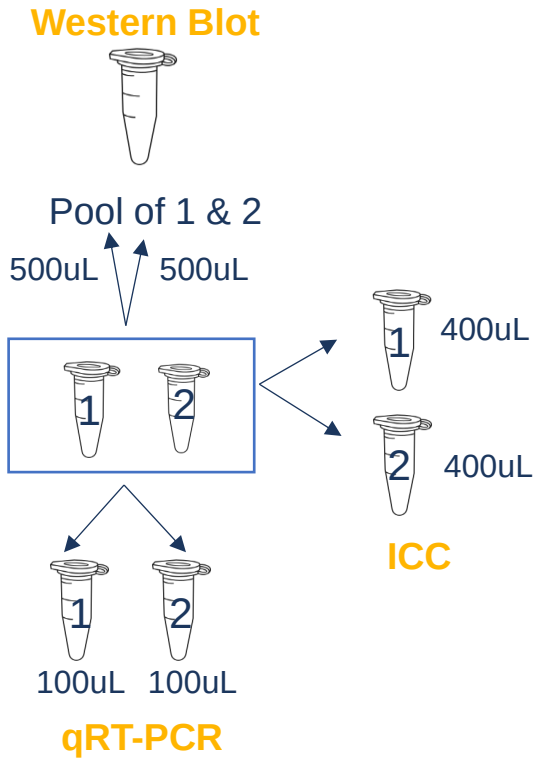
Day 6: $3x10^6$ cells/10mL dish

Experimental Plan



Treatment = 100nM R1881

➡ Dissociate ➡ Wash In PBS ➡ Pellet ➡ Re-suspend in PBS ➡

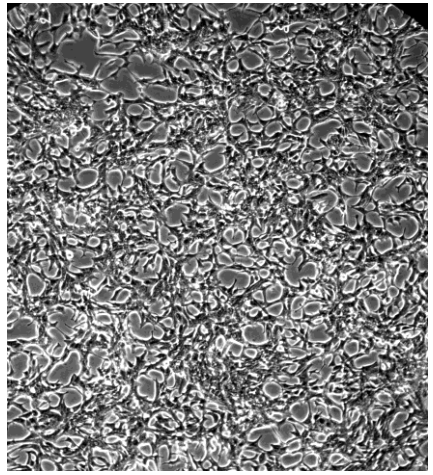
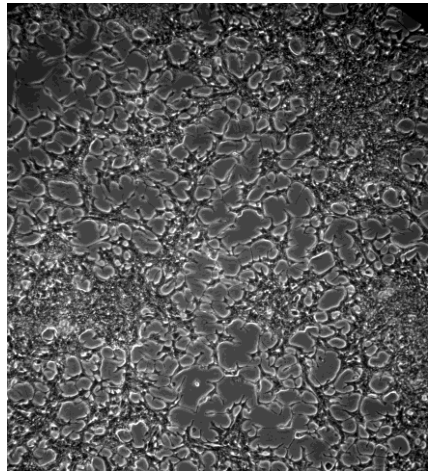
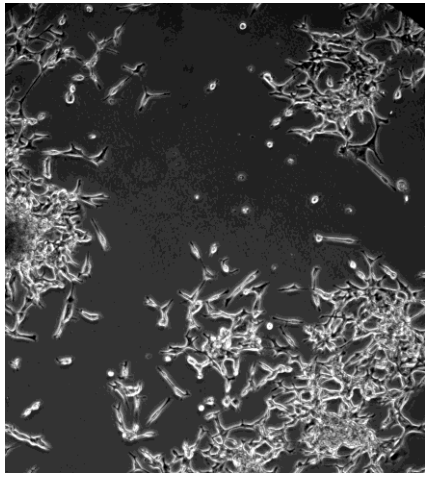


Genes/Proteins to Assay:

- **qRT-PCR**: AR, FKBP5, NKX3.1, PSA, cMYC, CD26, CXCR4, CXCR7,
- **WB**: AR, NKX3.1, PSA, cMYC CD26,, CXCR7
- **ICC**: AR, NKX3.1, PSA, CD26, CXCR4

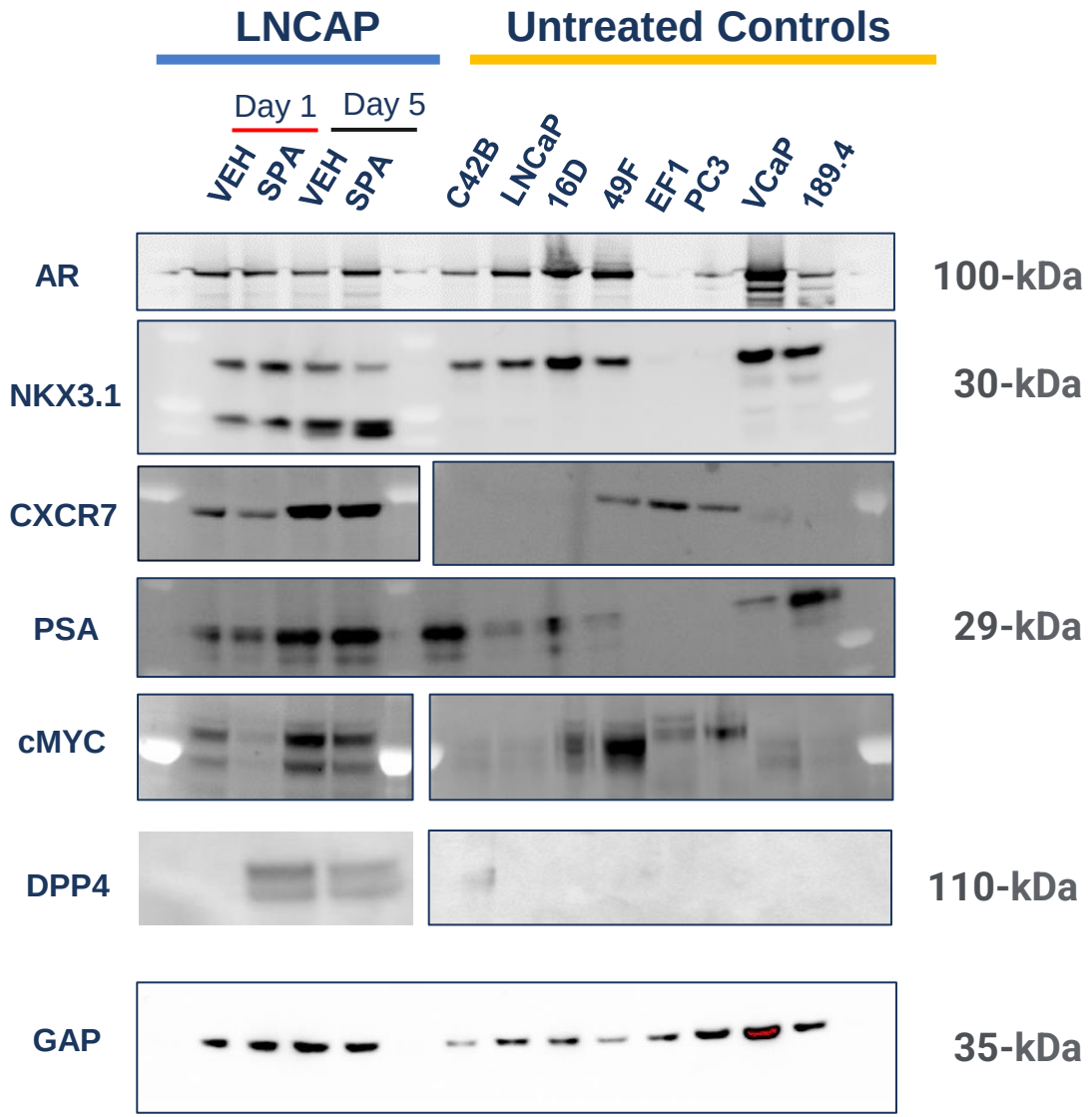
LNCaP Cell Confluency Before and After Treatment

LNCaP (10x)

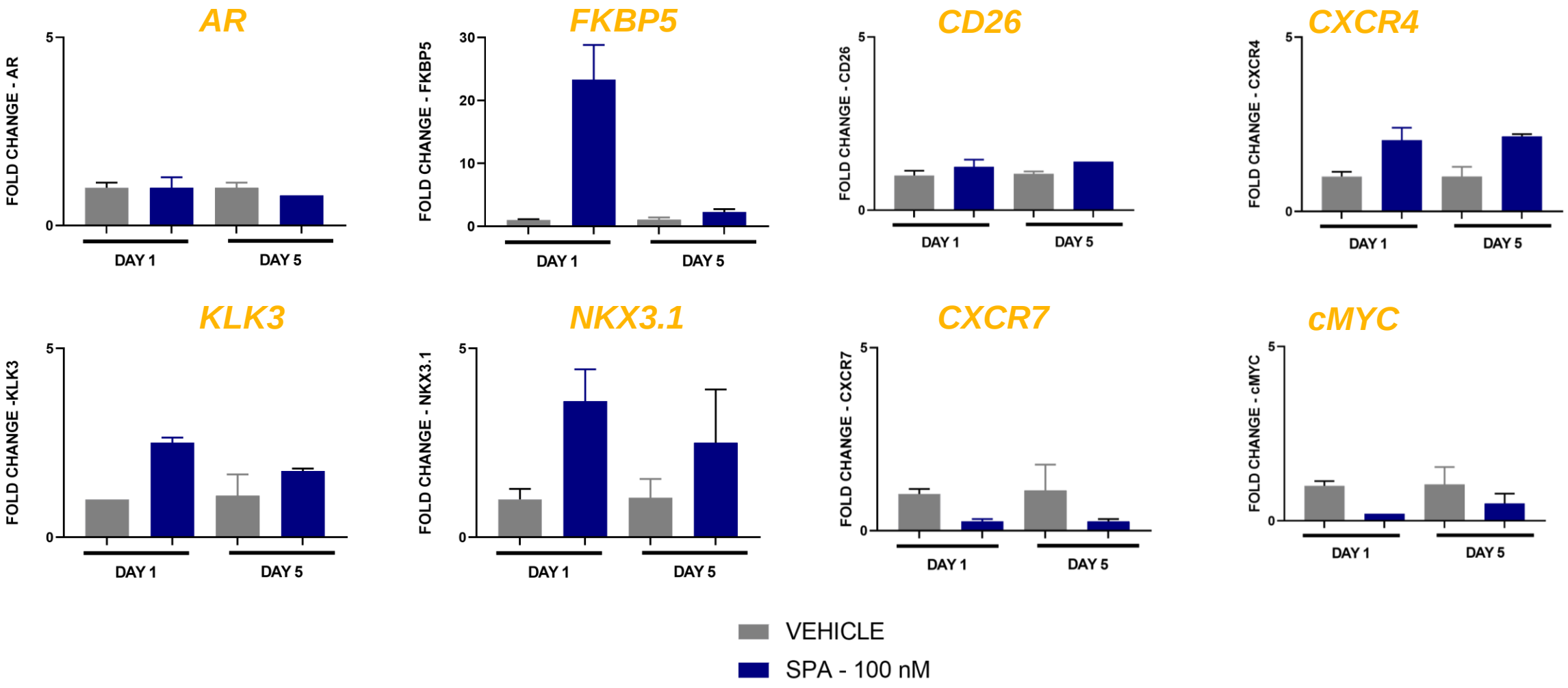
	Vehicle	SPA
Day 1		
Day 6		

***SPA = 100nM R1881**

Western Blot Analysis of Treated LNCaP Samples

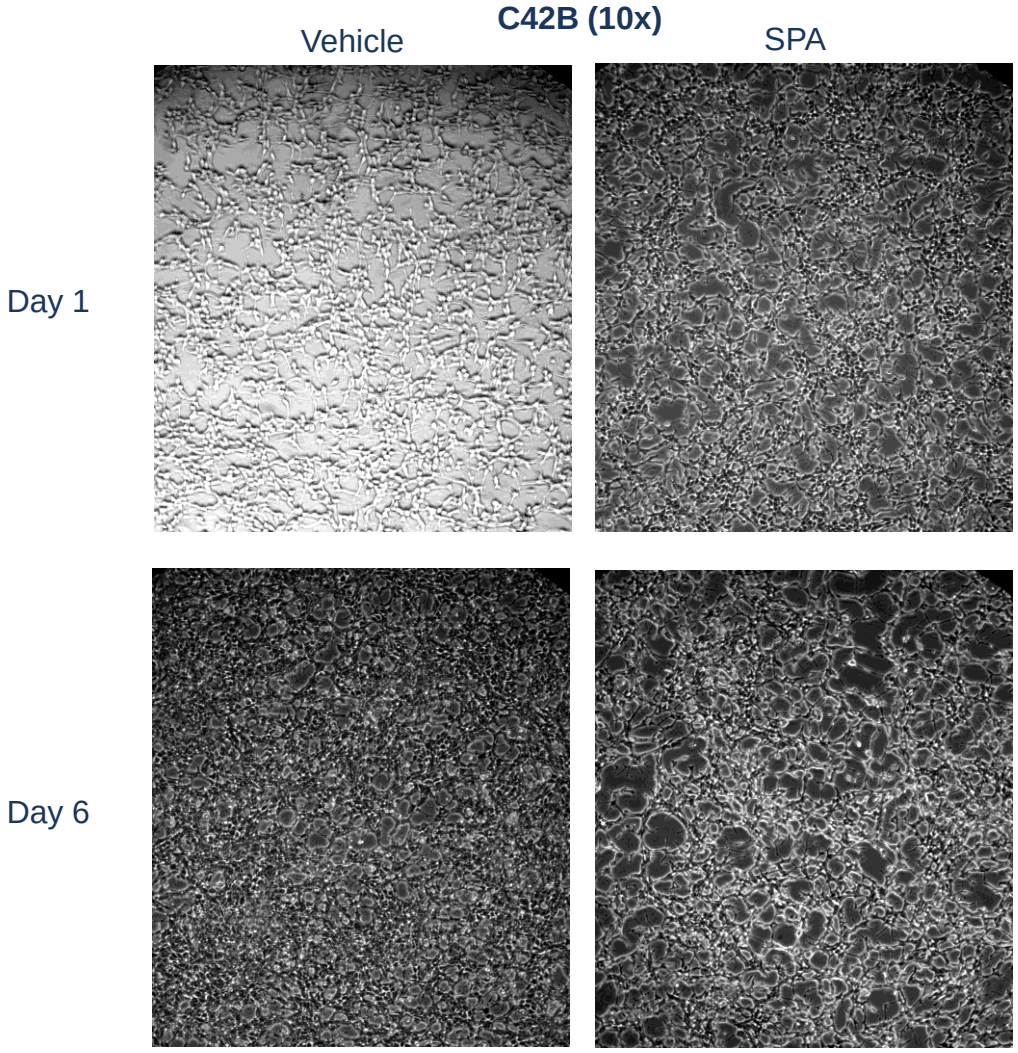


qRT-PCR Analysis of LNCaP

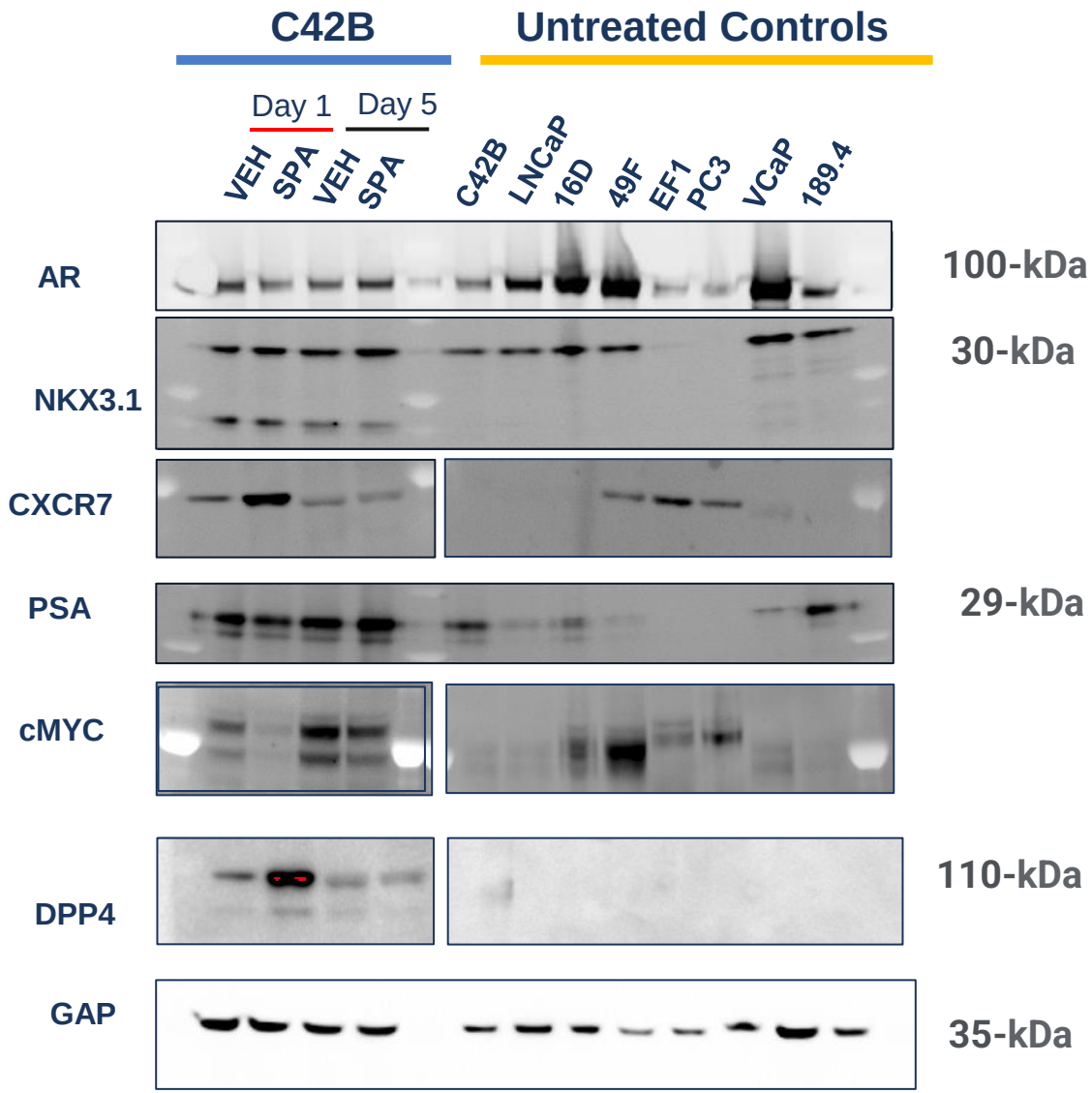


C42B Cell Confluency Before and After Treatment

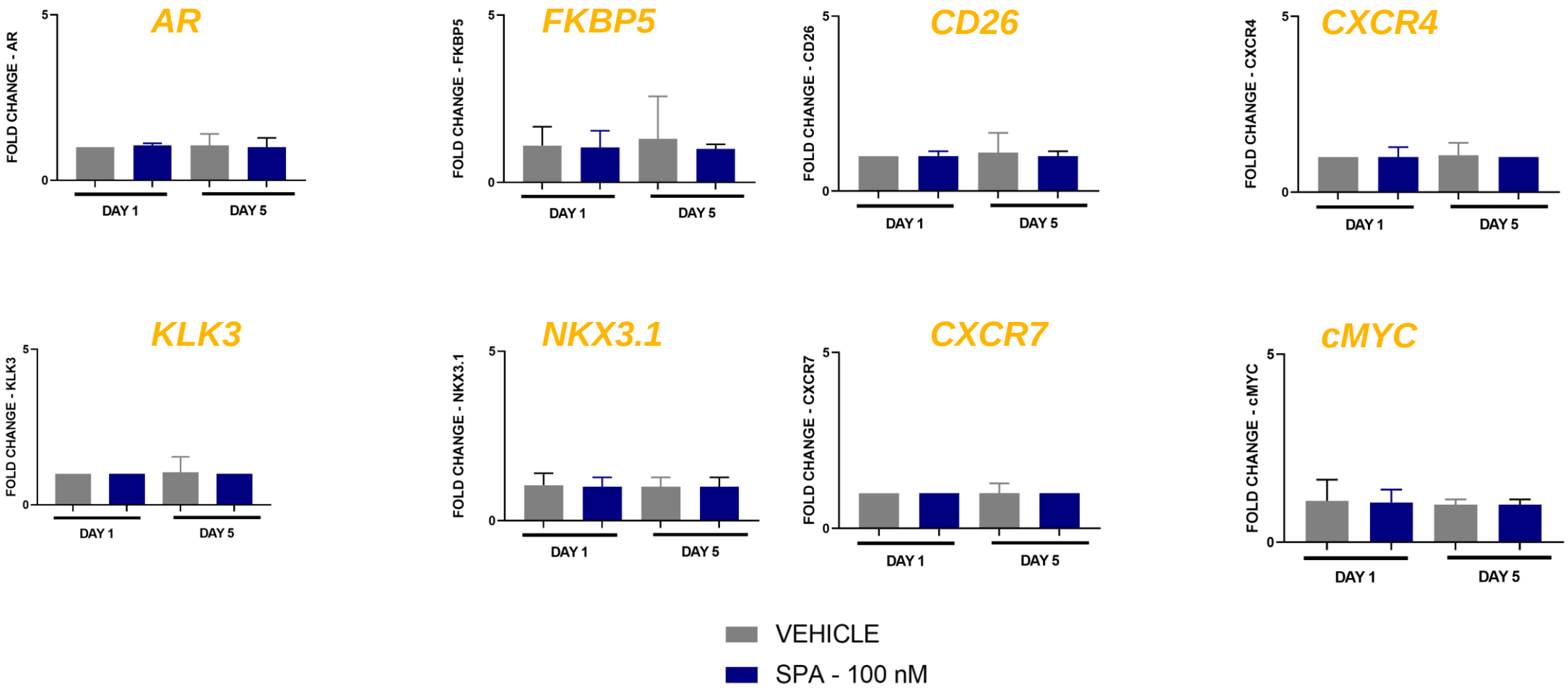
C42B (10x)



Western Blot Analysis of Treated C42B Samples

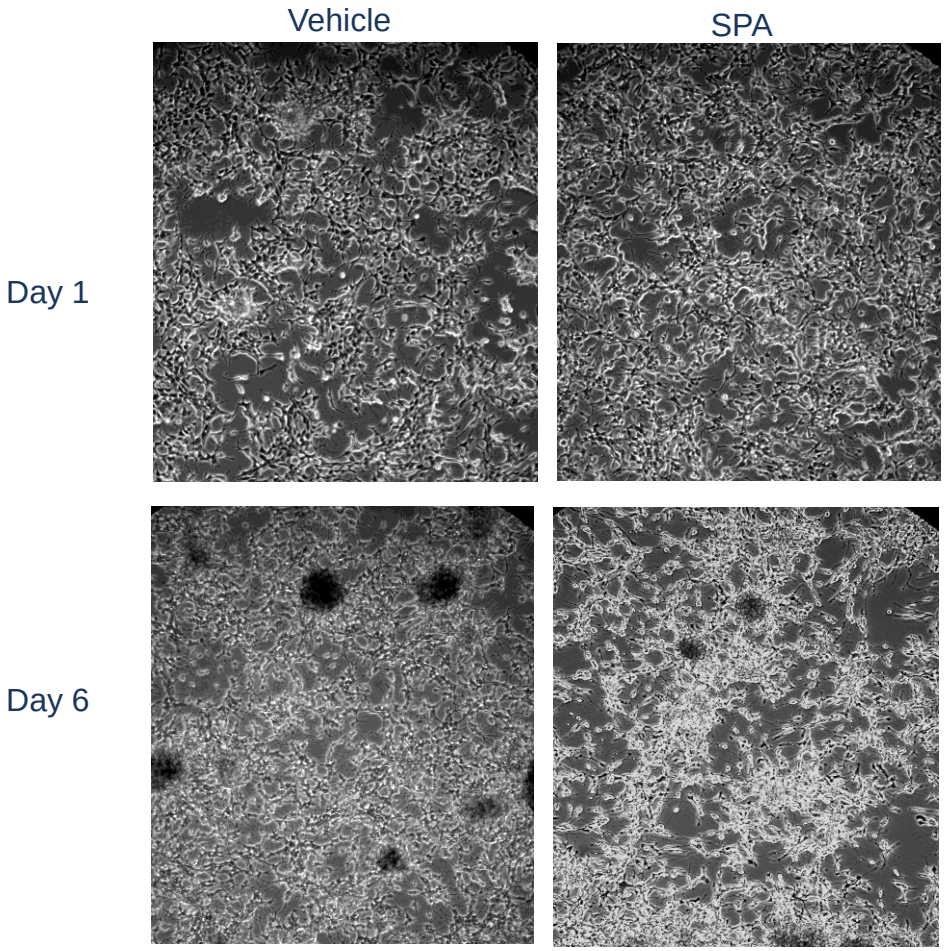


qRT-PCR Analysis of C42B

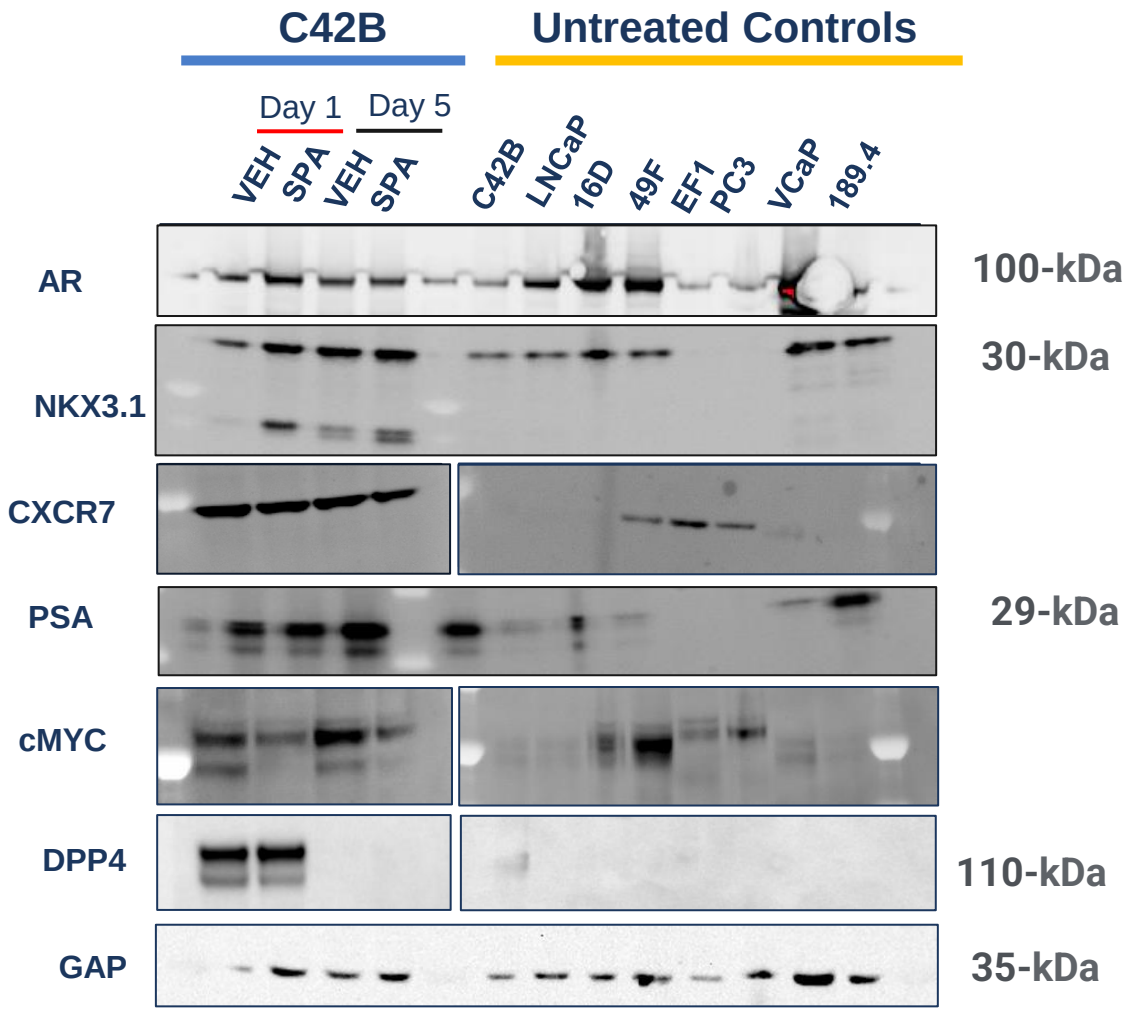


16D Cell Confluency Before and After Treatment

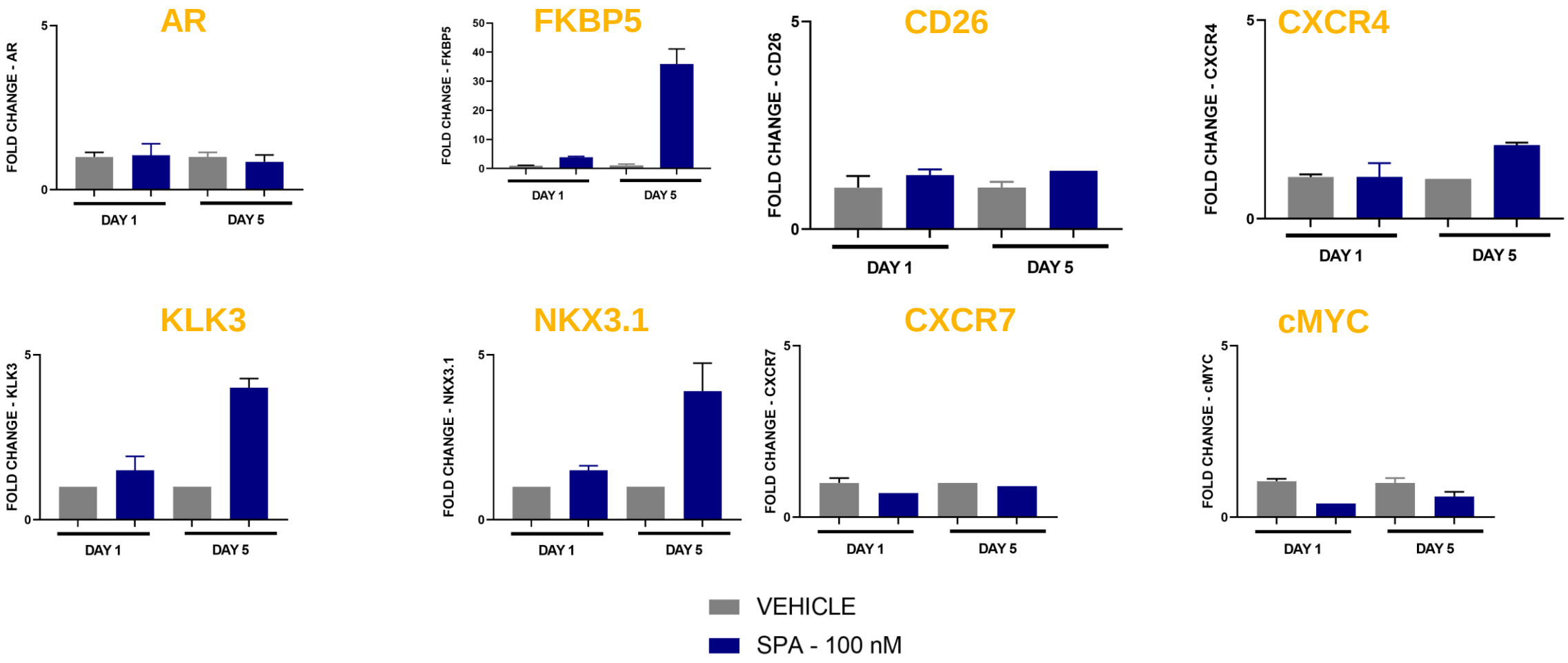
16D (10x)



Western Blot Analysis of Treated 16D Samples

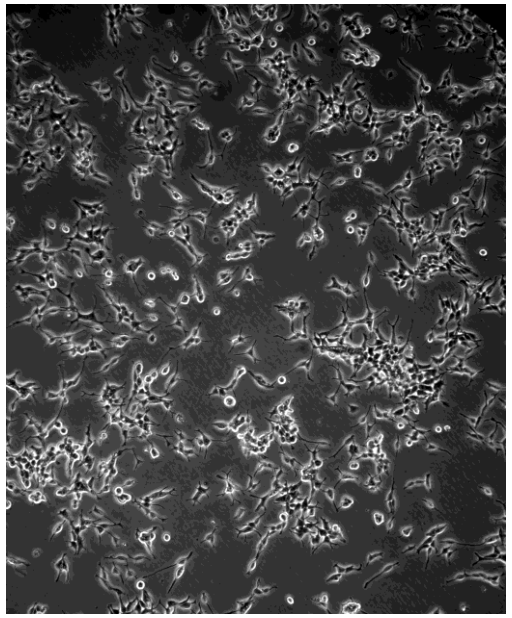
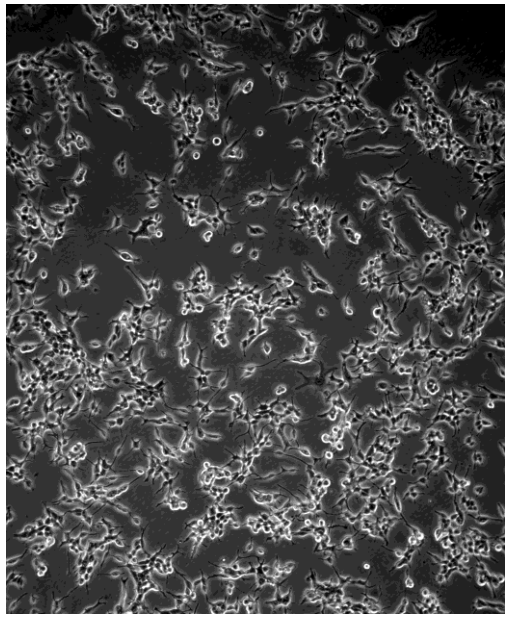


qRT-PCR Analysis of 16D

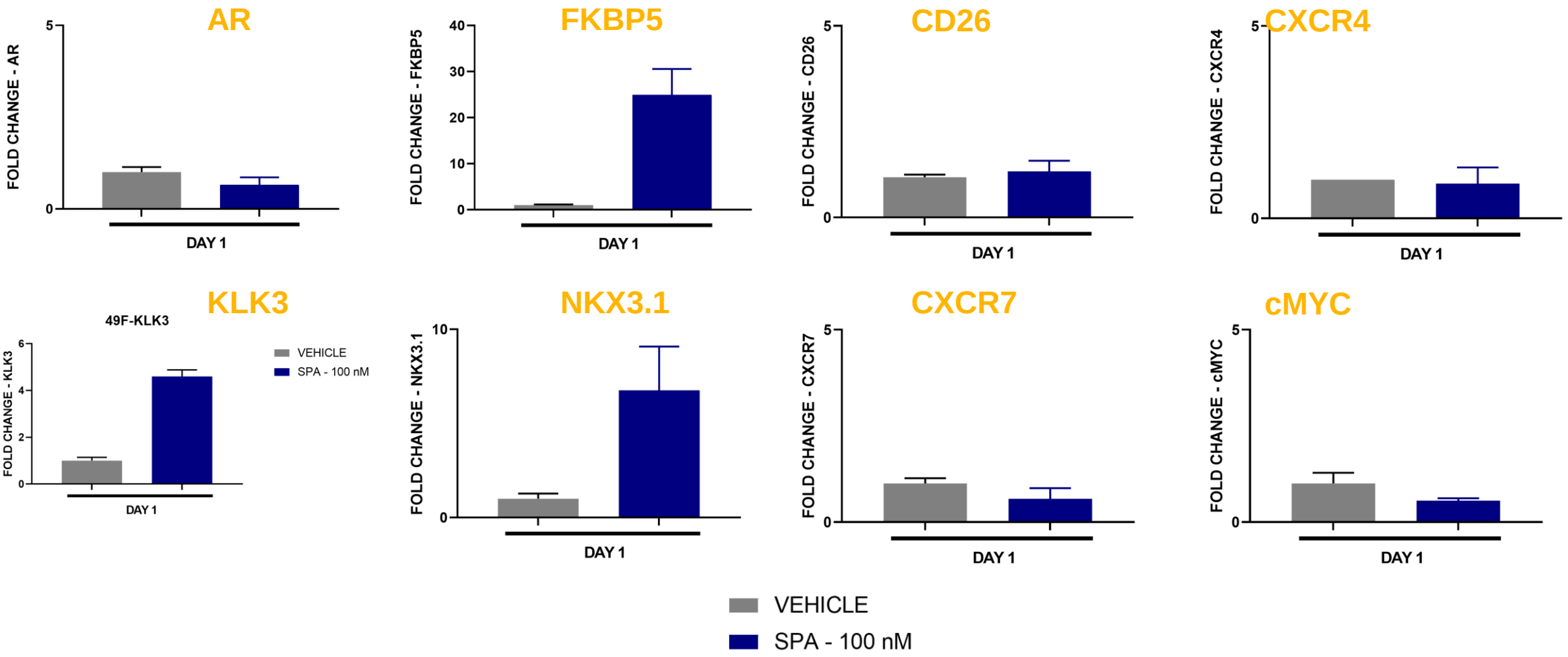


49F Cell Confluency Before and After Treatment

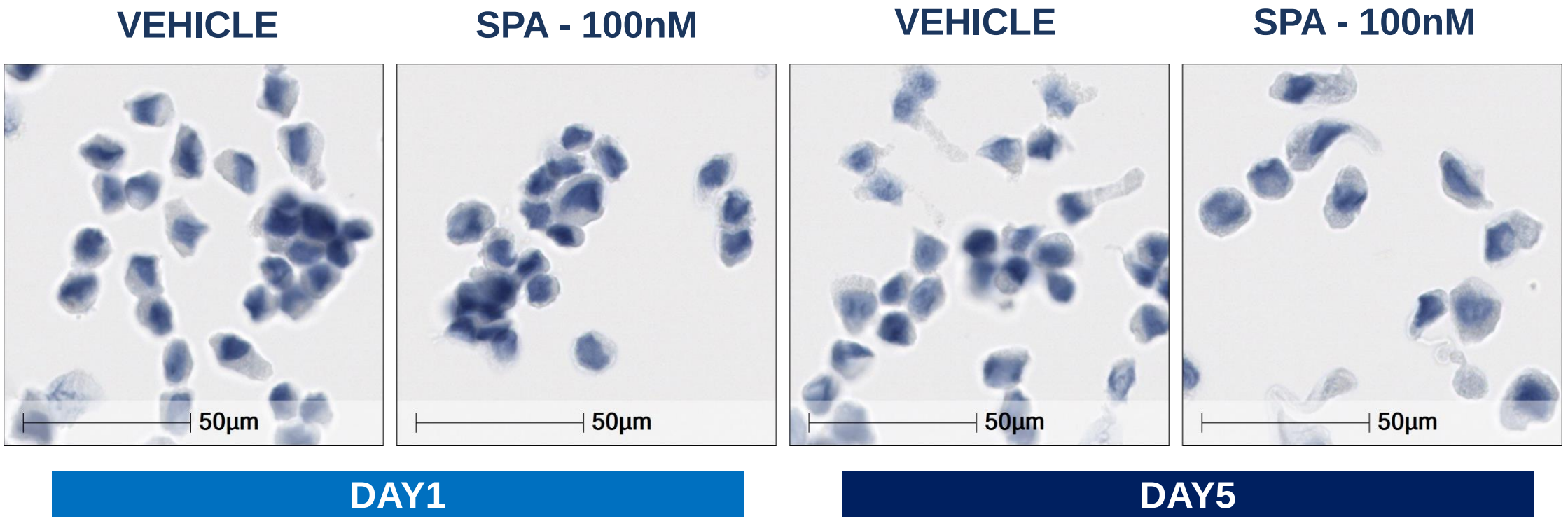
49F (10x)

	Vehicle	R1881
Day 1		

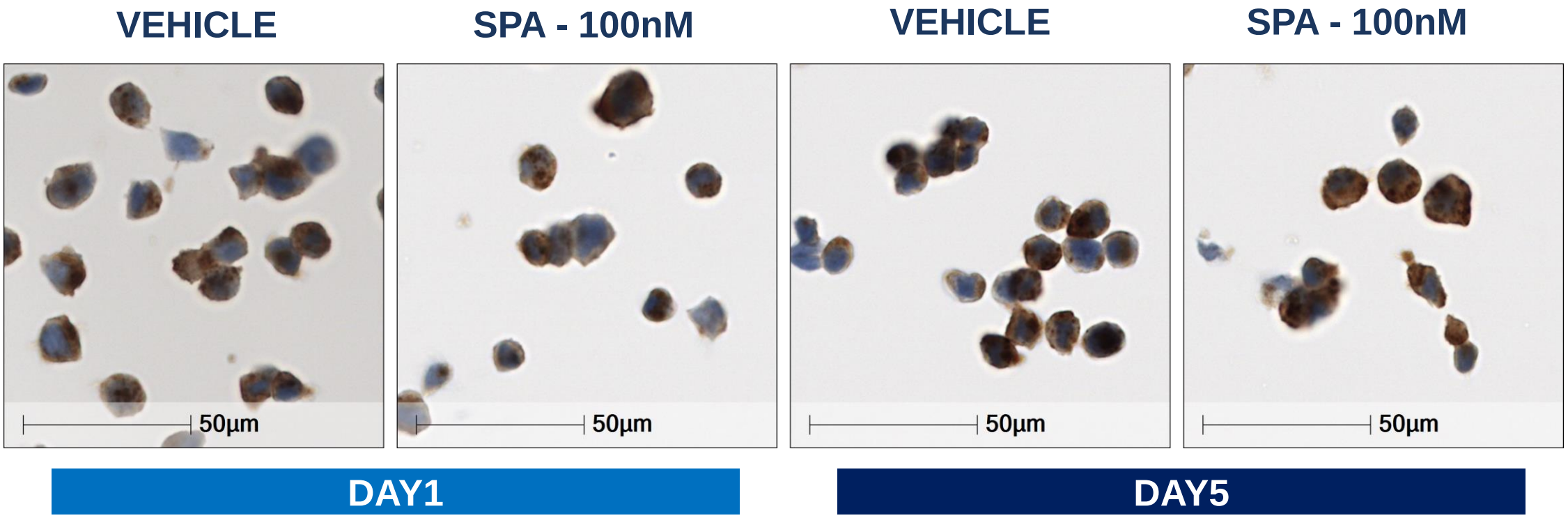
qRT-PCR Analysis of 49F



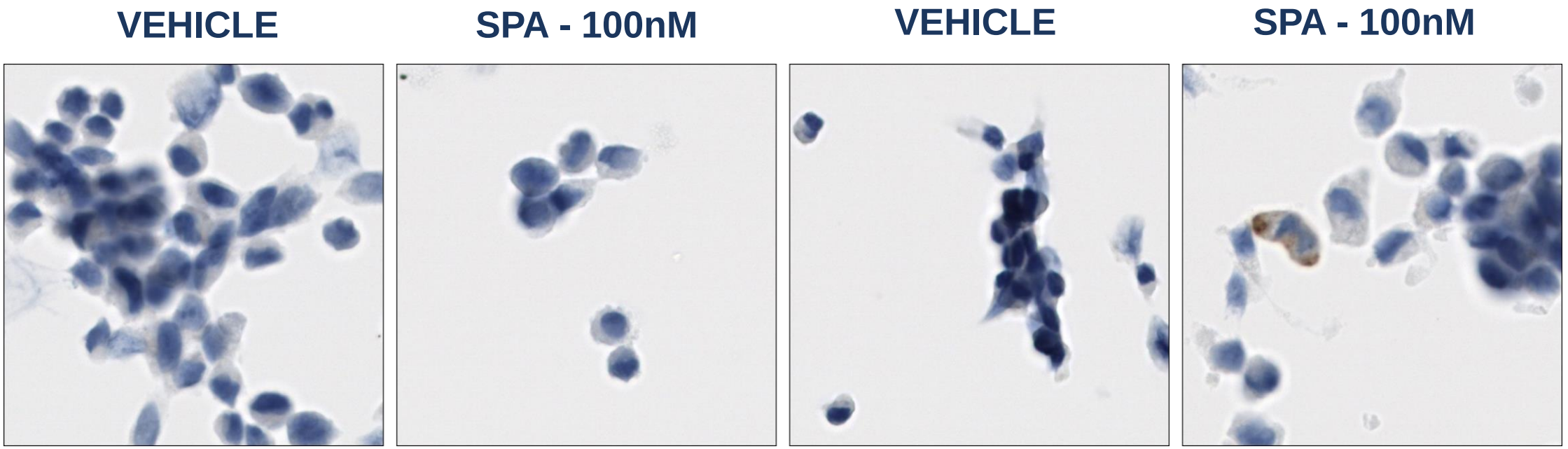
Immunocytochemistry of CD26 Expression in LnCap



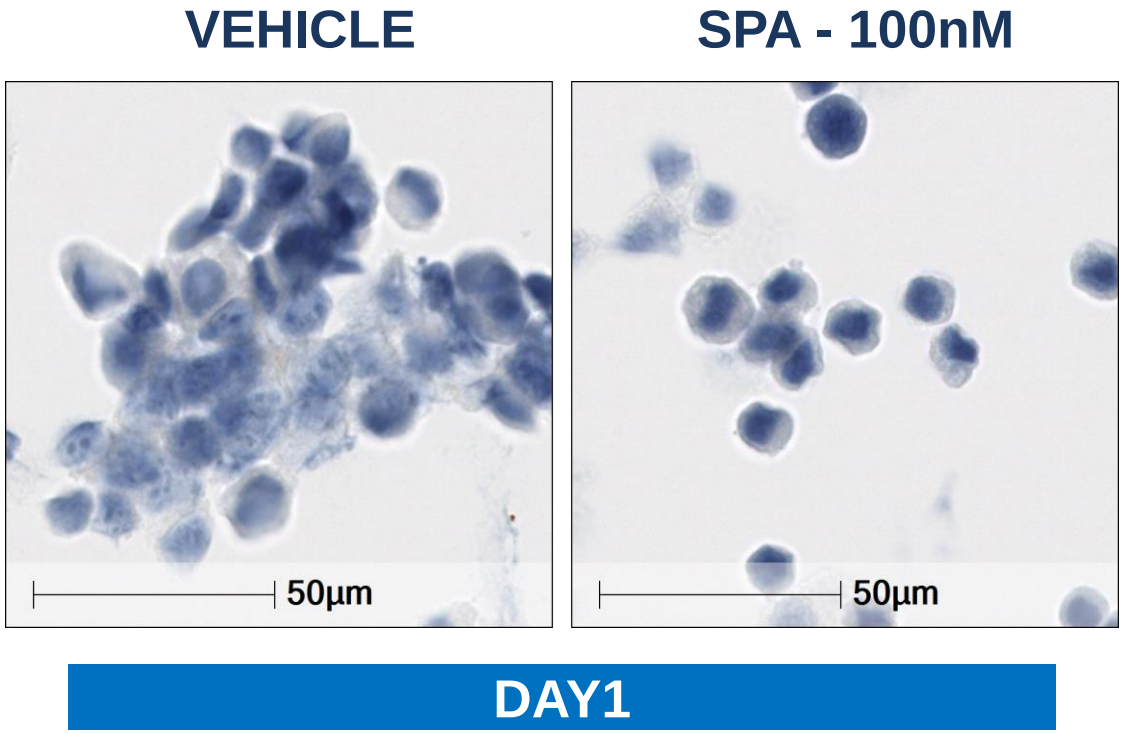
Immunocytochemistry of CD26 Expression in C42B



Immunocytochemistry of CD26 Expression in 16D



Immunocytochemistry of CD26 Expression in 49F



Future Direction

1. Finish the ICC analyses.

2. Repeat Western blot analysis for CD26 and CXCR4 using different antibodies.

3. Investigate the influence of CXCL12 on the growth of prostate cancer models treated with SPA.

Fred Hutch Cancer Center

Thank you

Dr. Peter Nelson

Our Mentors

Emery Boehnke

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Ruthy Dumpit

Nelson Lab

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Tarana Arman

Arnab Bose

Tony Chu

Ilsa Coleman

Canan Dirican

Sander Frank

Wanting Han

Brian Hanratty

Dapei Li

Jared Lucas

Galina Semenova

Saurabh Verma

Ryuta Watanabe

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Lee Lab

Gerardo Javier

Tina Wu

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