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DNA methylation in prostate cancer

Overarching theme

- Epigenetic regulation allows the genome to be responsive to the environment
  - Sets the tone for transcriptional response to signals

- Epigenetic derangement provides an exceptional route for cancer cell "evolution" as cancer progresses to advanced phenotypes

  - Environmental stresses drive "evolution" through malignant progression

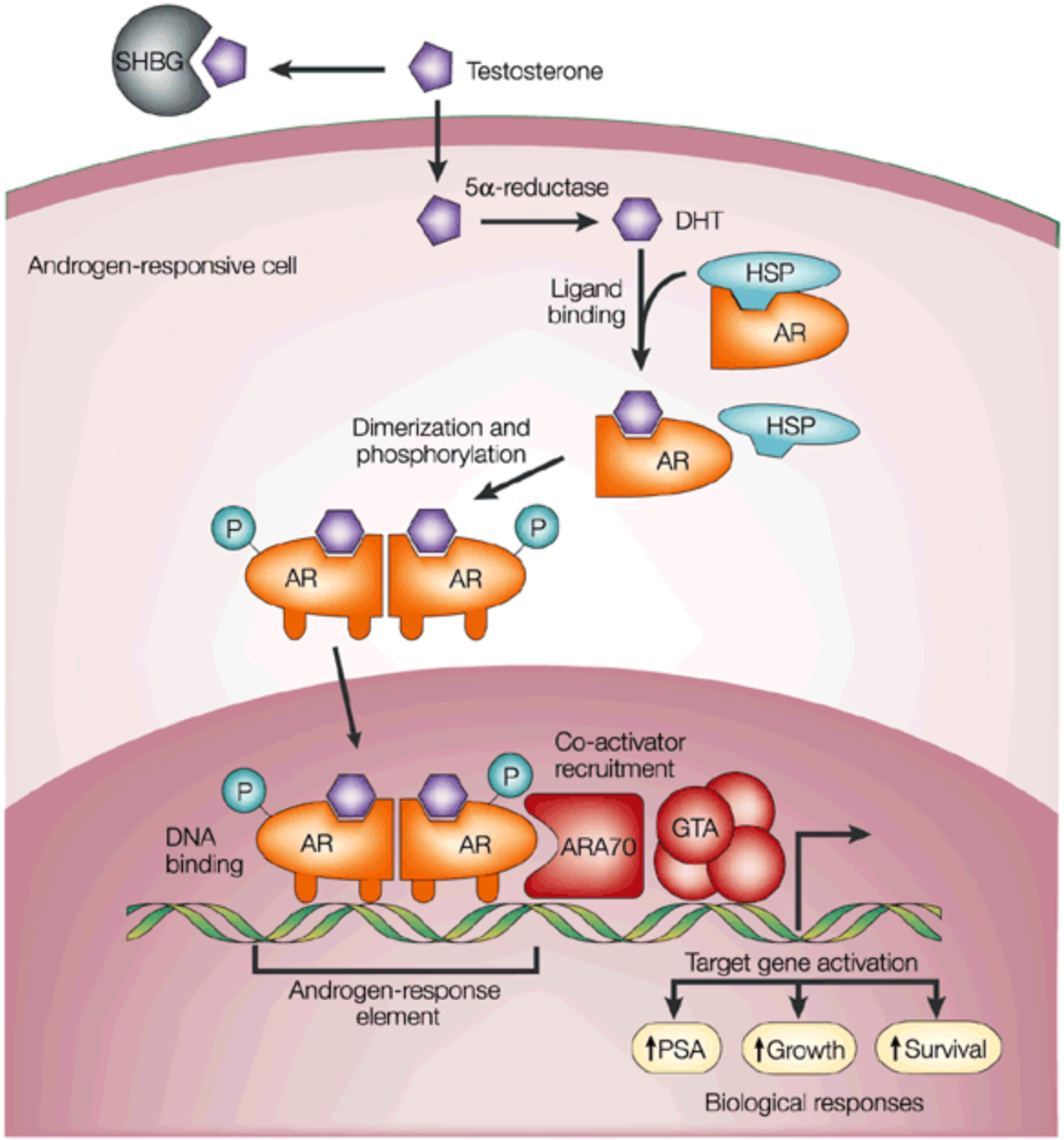
    - Impaired mitochondrial function – DNA methylation changes associated with loss of mtDNA content

    - Inflammation – Involution of breast ducts post pregnancy; association with breast cancer risk; epigenetic contribution

    - Hormone signaling – Dynamic changes in DNA methylation related to normal AR signaling; distortion in malignancy

    - Hormone signaling – Sudden loss of AR signaling; CpG island methylation and progression to castration recurrence

    - Micronutrients – Folate metabolism and prostate cancer
      - Therapeutic potential; population genetics potential



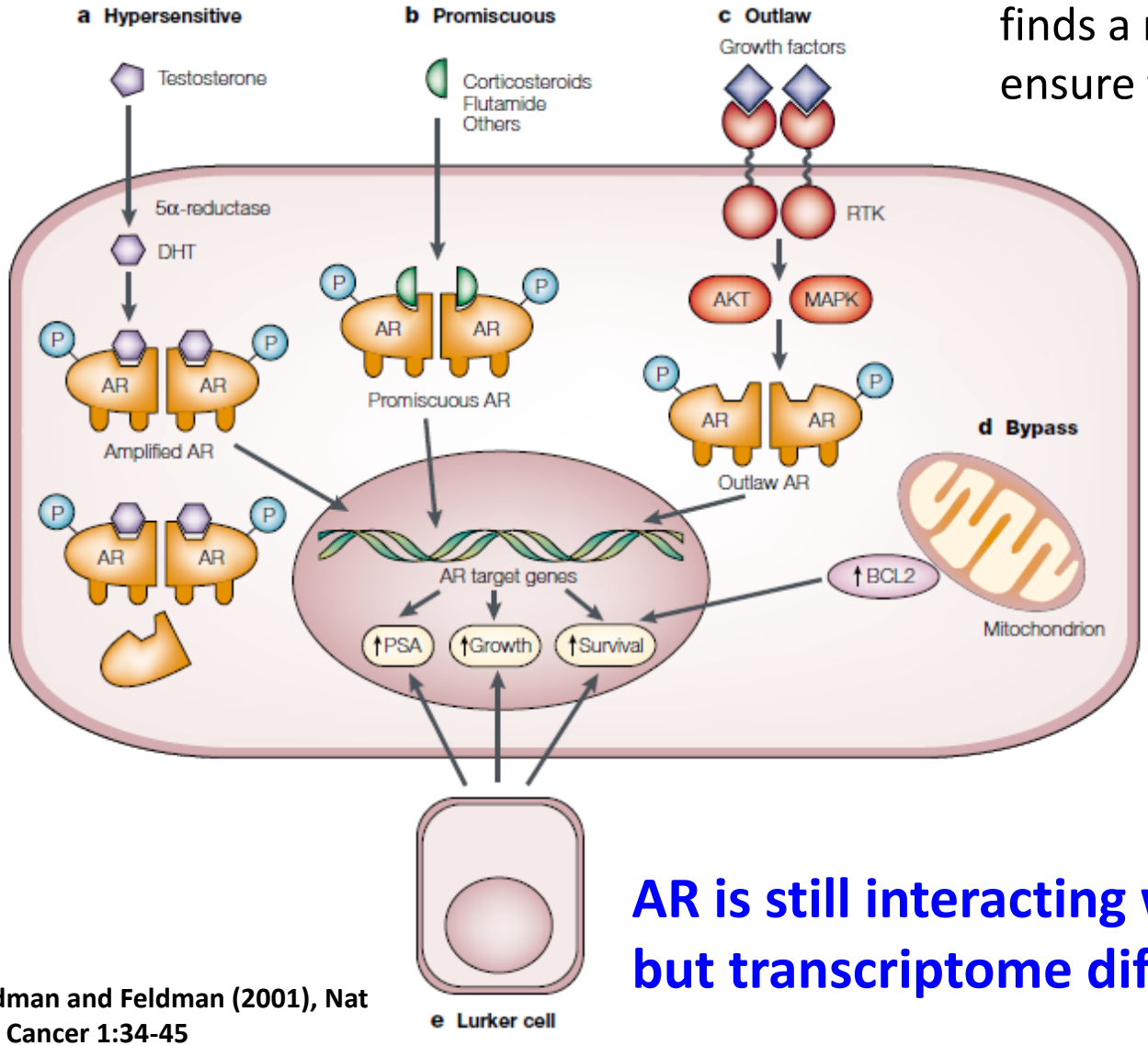
Feldman and Feldman (2001), Nat Rev Cancer 1:34-45

Nature Reviews | Cancer

- Ligand bound AR regulates 100's of genes
  - Activation and suppression
  - "The AR transcriptome"
- Directs terminal differentiation, blocks growth in normal prostate
- Directs a different transcriptome in primary CaP
  - Promotes growth and survival
- Yet again a different transcriptome in ADT-RCaP
  - Promotes growth and survival despite castrate levels of androgens

Onset of Androgen Independence

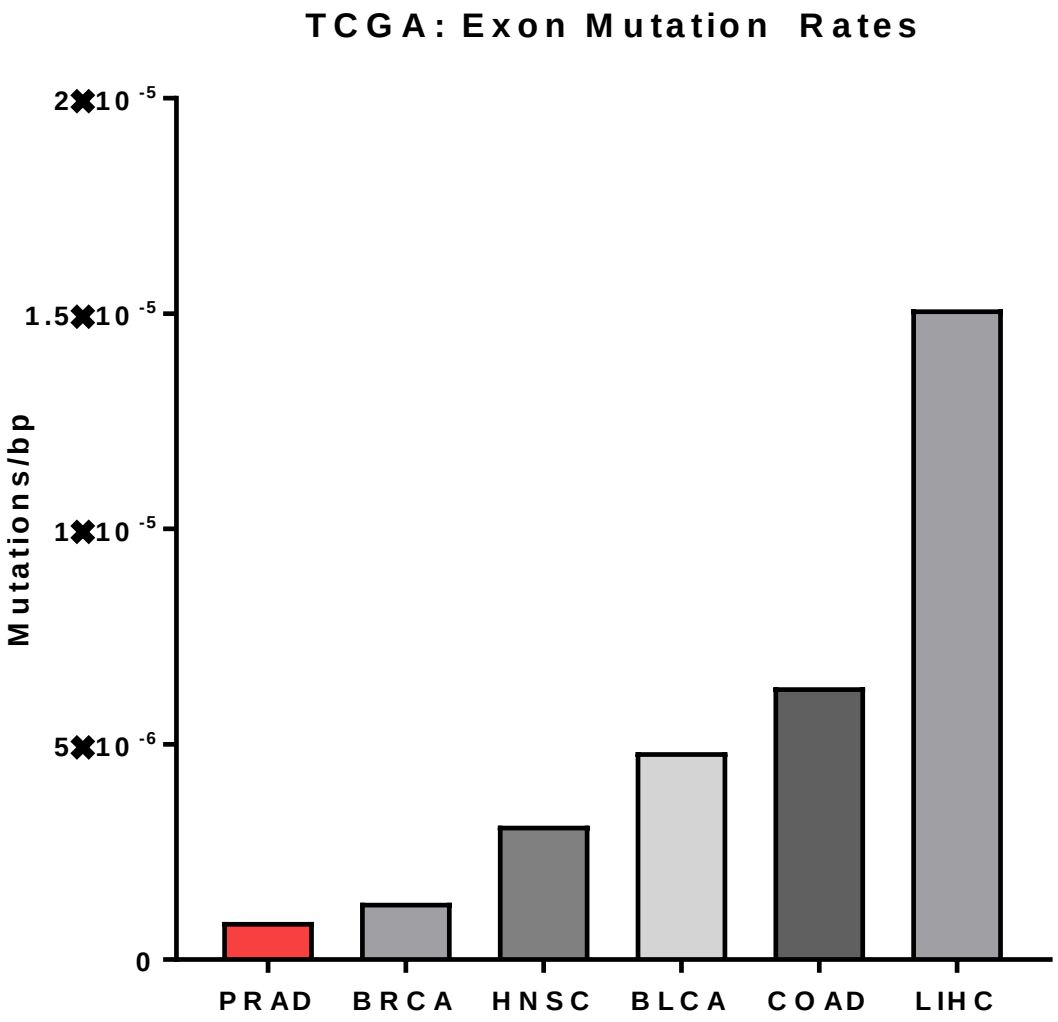
- "androgen-independent" CaP finds a myriad of ways to ensure there is AR signaling



AR is still interacting with the genome – but transcriptome differs.

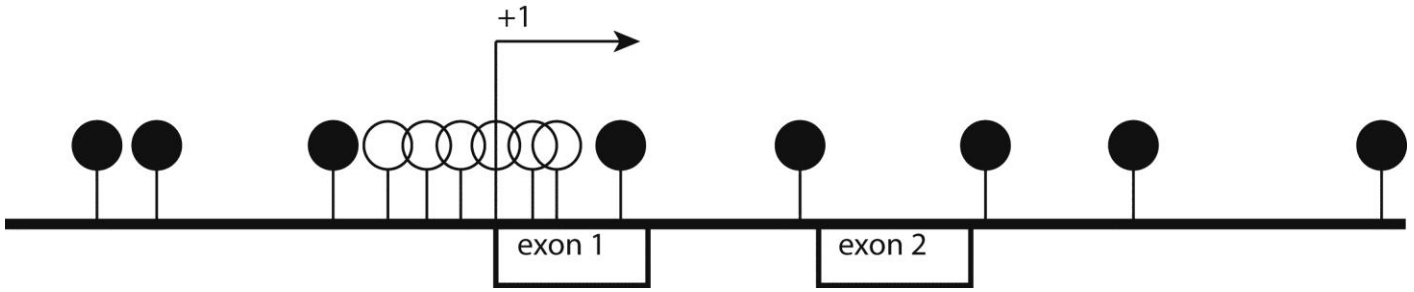
Feldman and Feldman (2001), Nat Rev Cancer 1:34-45

The mutational load in prostate cancer is relatively low



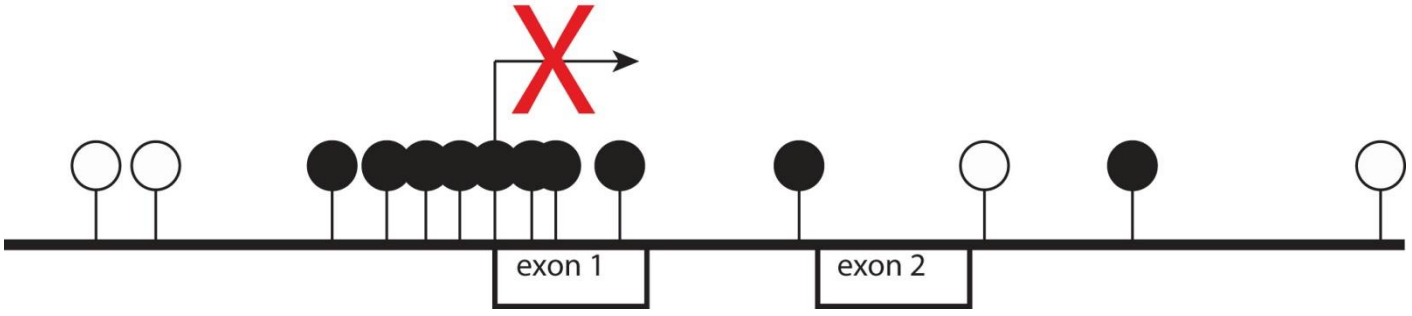
Argues that epigenetics must play a large role is shaping the prostate cancer phenotype

DNA methylation patterns



Carcinogenesis

- Occurs at between 0% and 10% of CpG islands
  - ~1% to 3% is typical
- CpG island hypermethylation associated with gene silencing
- Why THIS gene, not THAT gene?
  - Selection can only partially explain; susceptibility must play a role



Could epigenetic *mechanisms contribute to* a different transcriptome being directed by the same nuclear receptor?

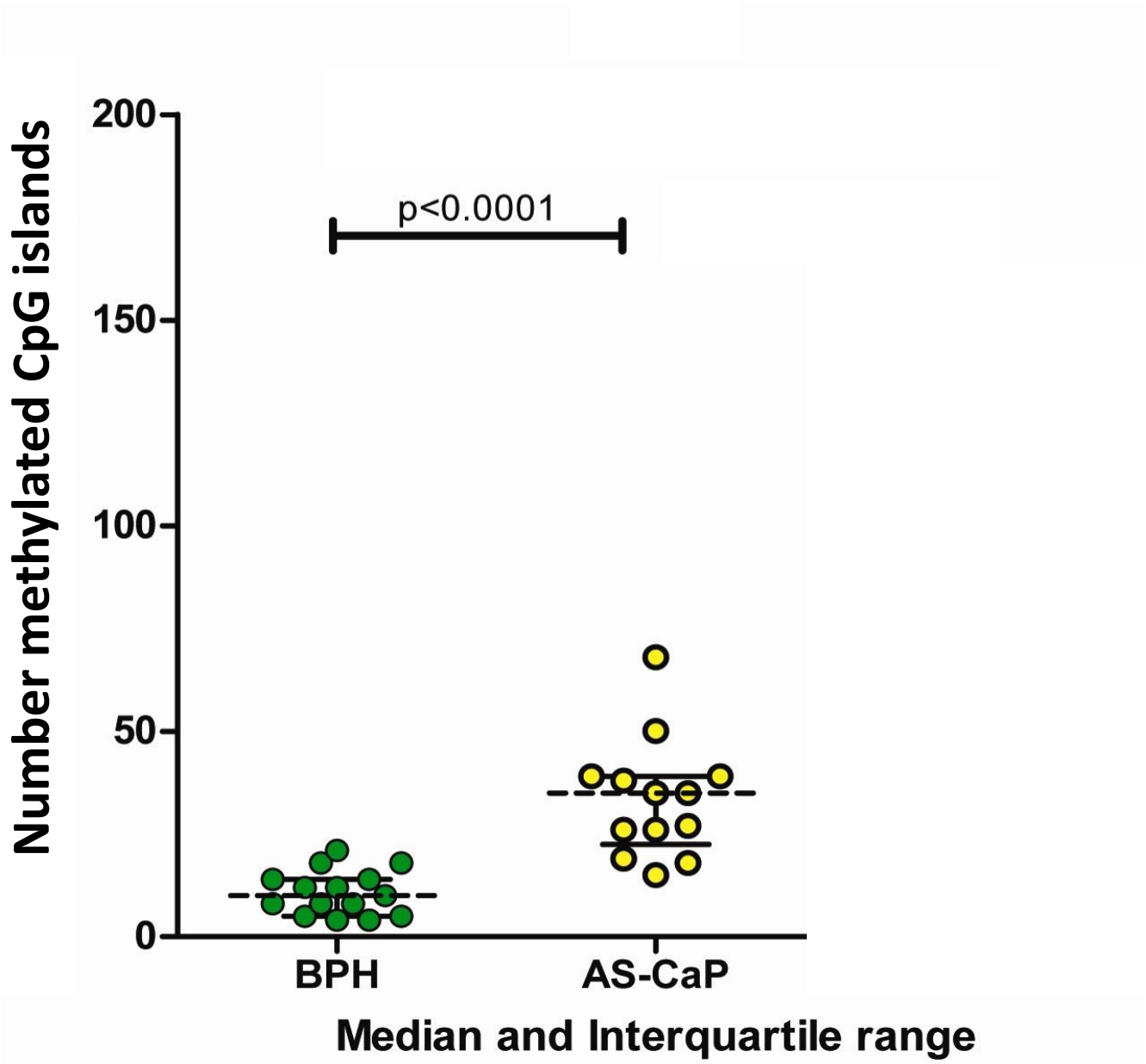
Could epigenetic *marks reflect* a different transcriptome being directed by the same nuclear receptor?

- 15 – benign prostatic hyperplasia (approximate 'normal' prostate)
- 13 – androgen stimulated CaP (AS-CaP) – enriched to >70% carcinoma
- 12 – androgen deprivation therapy recurrent CaP (ADT-RCaP)
  - extremely rare samples – collected by TURP to relieve urinary symptoms during ADT

Perform restriction landmark genomic scanning (RLGS) analysis to measure CpG island methylation

- Assess methylation state of ~1200 CpG islands by methylation sensitive restriction enzyme

Greatly increased methylation phenotype in ADT-RCAP



Perspective

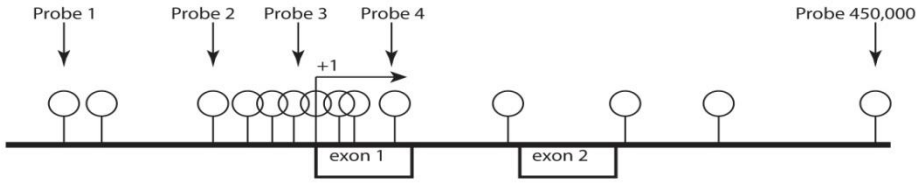
Table 1. Survey of RLGS methylation detected in various cancer types.

Cancer Type	Samples (n=234 total tumors)	Mean RLGS Methylation (n=1197 spots)
Recurrent Prostate	**12**	**7.4%**
Glioblastoma	14	3.3%
Chronic Lymphocytic Leukemia	10	2.9%
Androgen Stimulated Prostate	**13**	**2.8%**
Acute Myelogenous Leukemia	33	2.0%
Colon	26	1.9%
Cervical	17	1.2%
Non-Small Cell Lung	16	1.2%
Head and Neck LN Mets	13	0.6%
Medulloblastoma	25	0.5%
Primative Neuroectoderm	8	0.5%
Nonseminomatous Testicular	9	0.4%
Breast	14	0.3%
Head and Neack Primary	17	0.2%
Seminomatous Testicular	7	0.0%
Total	234	1.7%

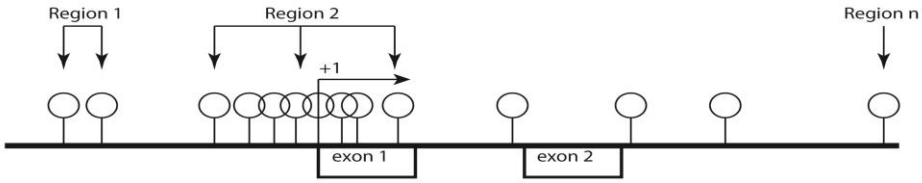
ADT-RCaP has more than double the CpG island hypermethylation on the next most methylating cancer type

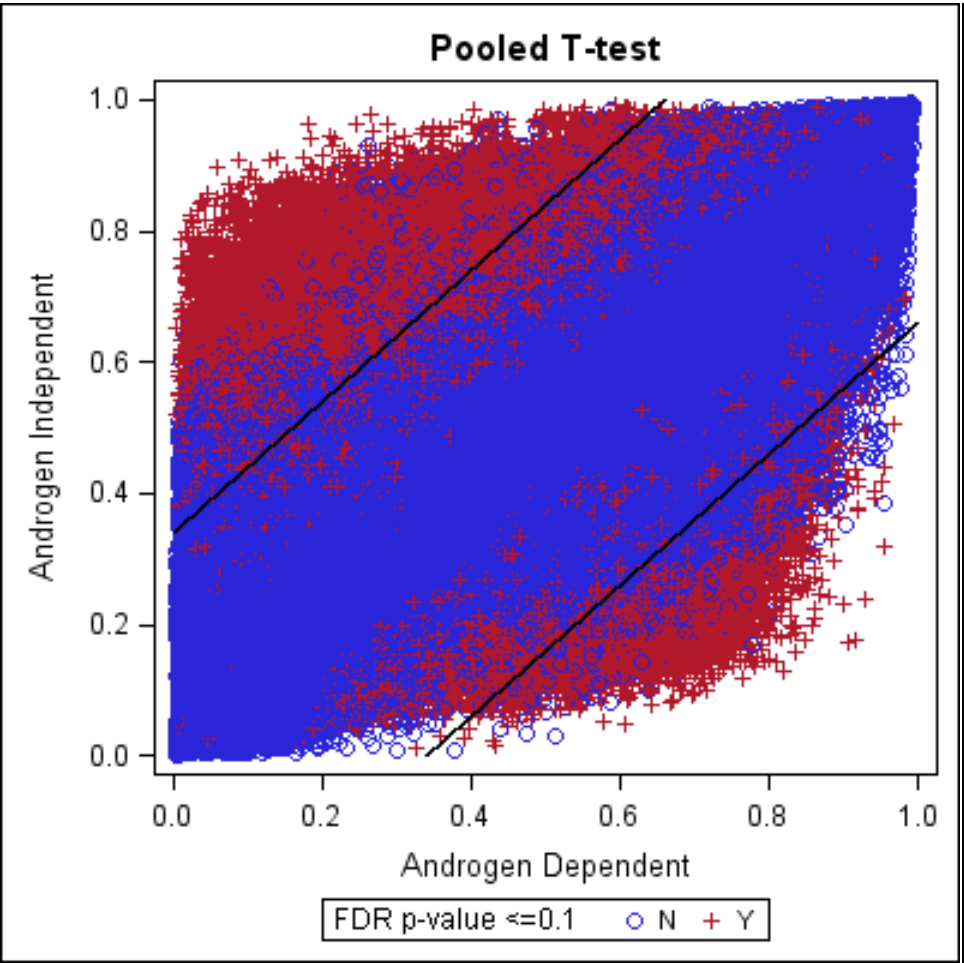
Global confirmation of increased CpG island hypermethylation phenotype in ADT-RCaP Illumina 450K Bead Array

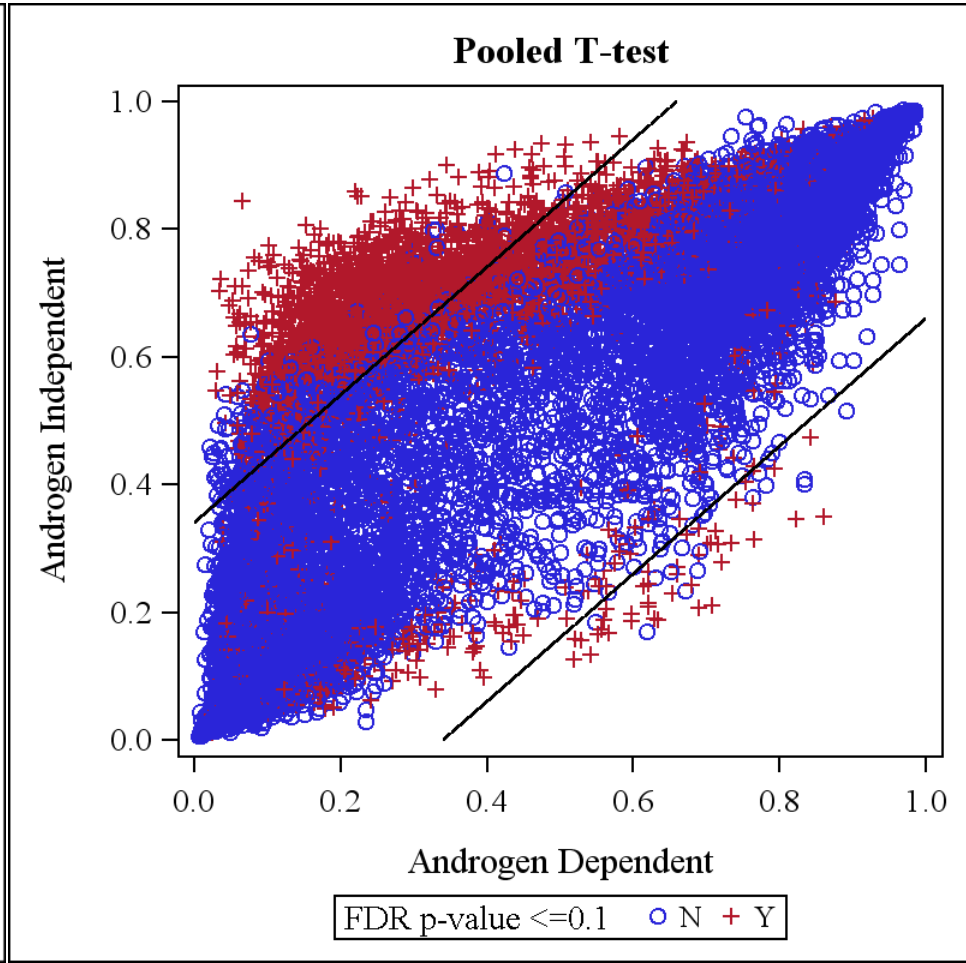
Probe level analysis



Region level analysis

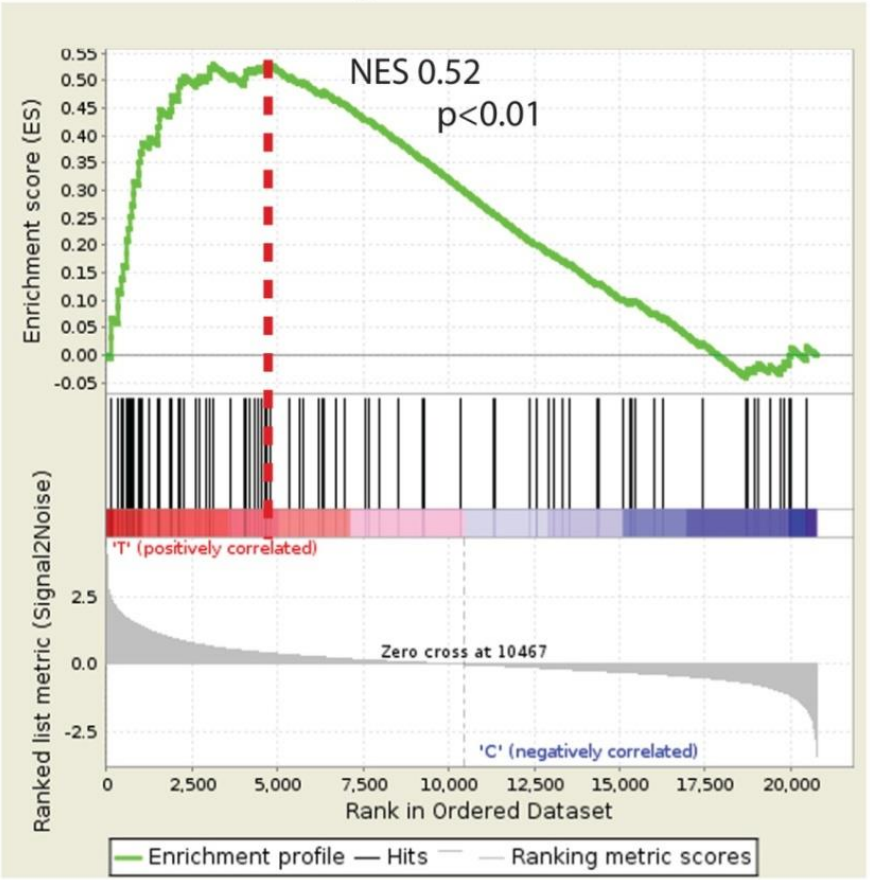




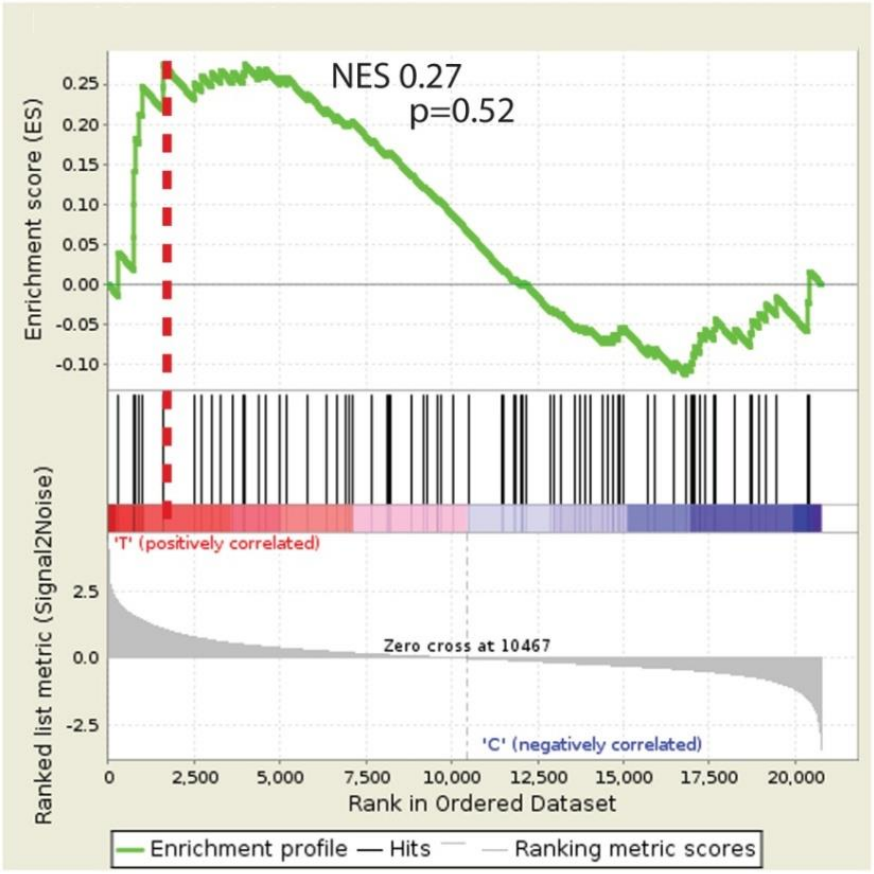


Gene Set Enrichment Analysis

Genes down regulated in metastatic CaP

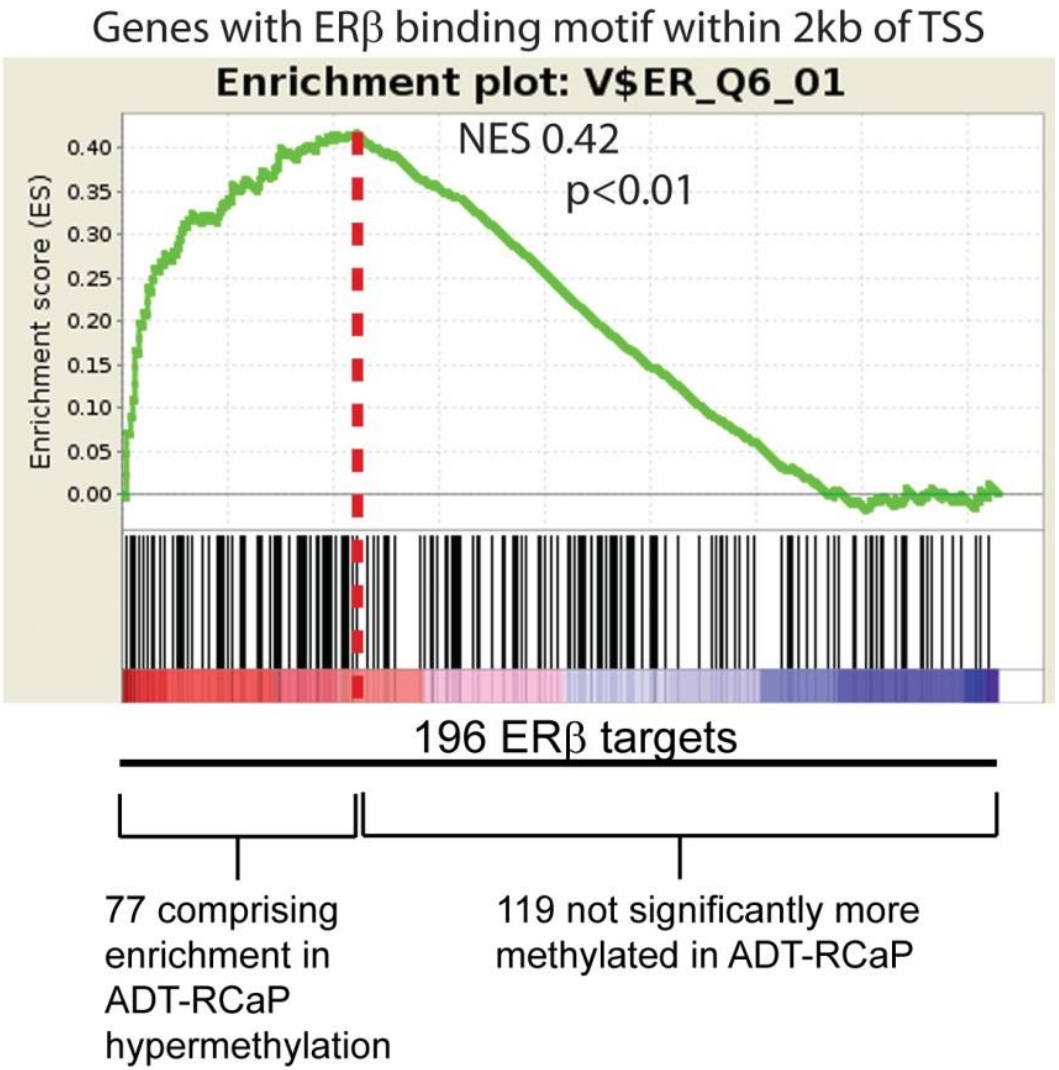


Genes up regulated in metastatic CaP



Regions hypermethylated specifically in ADT-RCaP are enriched genes found to be downregulated in metastatic CaP from patients who failed ADT

Hypermethylation restricts promoter choice away from differentiation



- ERβ is methylated in early-stage disease – ablates downstream estrogen signaling
- ERβ is re-expressed in late-stage CaP – re-establishing downstream estrogen signaling
  - But a *SKEWED* estrogen signaling

ERβ targets that are hypermethylated in ADT RCaP enrich for genes involved in *differentiation*, *cell fate decisions*, and *gland development*

The non-methylated targets do not show any enrichments

Summary

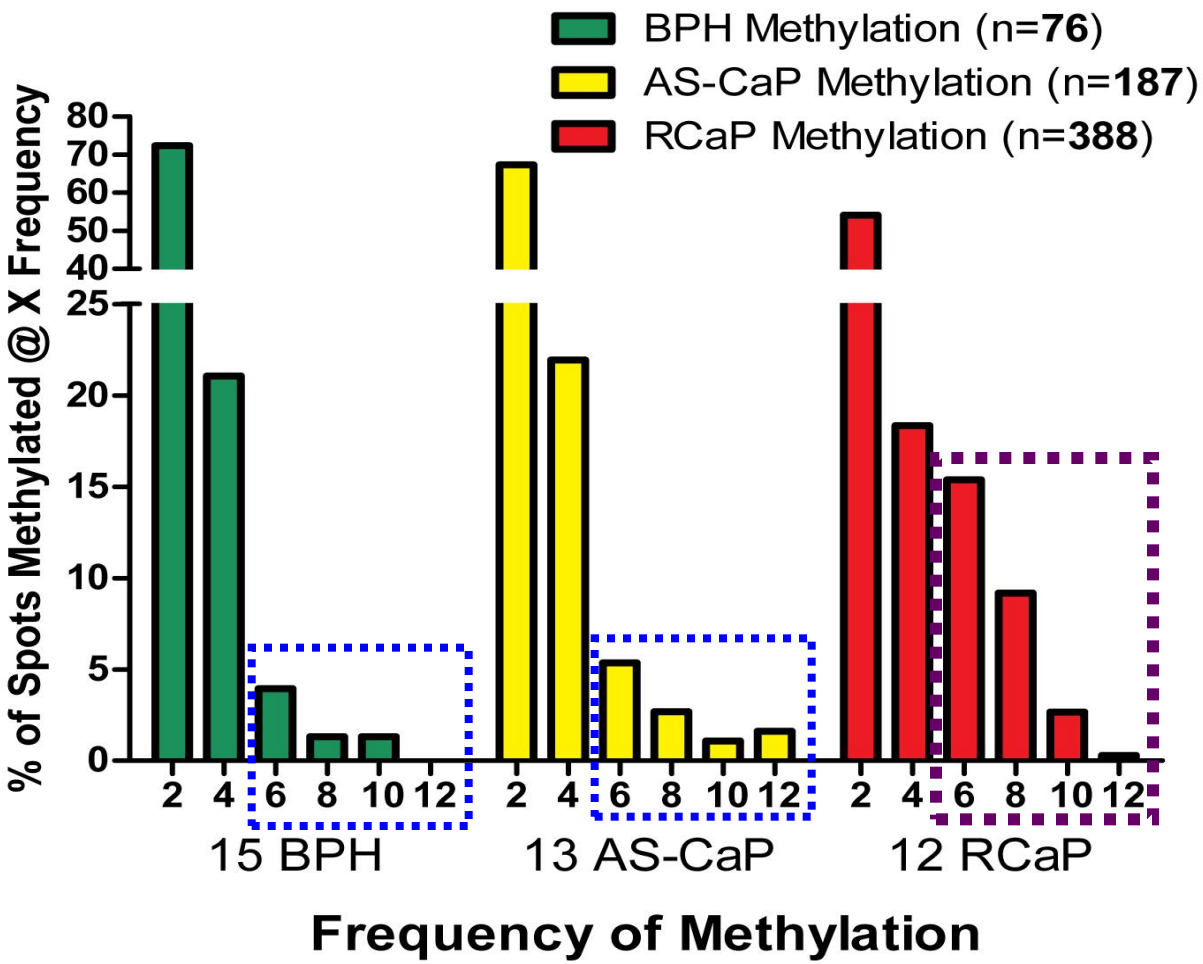
- CpG island hypermethylation is common in AS-CaP
  - Level of CpG island methylation is similar to that seen in colorectal cancer

- CpG island hypermethylation is **dramatically** increased in RCaP
  - Level of CpG island methylation is more than double any other cancer

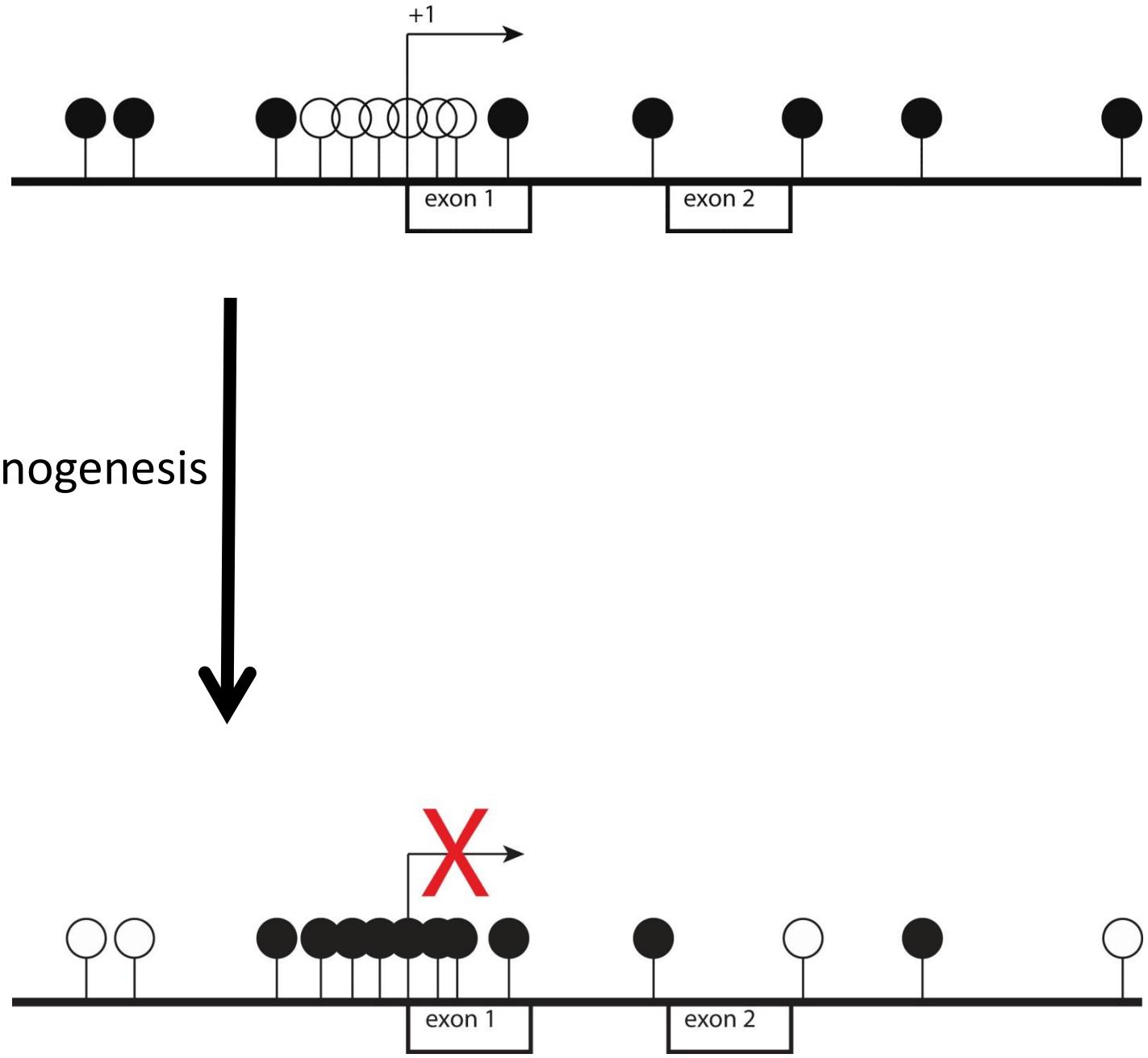
- Dramatically different CpG island methylation landscape in RCaP suggests that the way in which AR interacts with the genome may also be dramatically different.

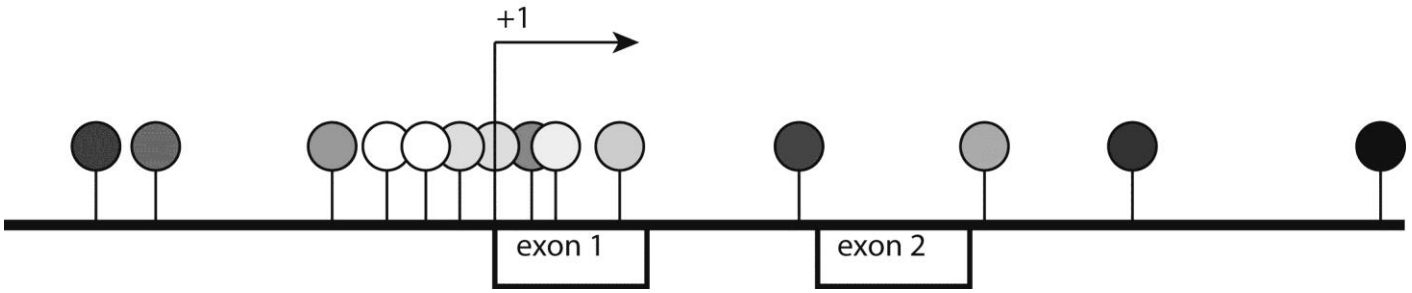
What is the biological significance of these observations?

Frequency Distribution of RGLS Methylation Events



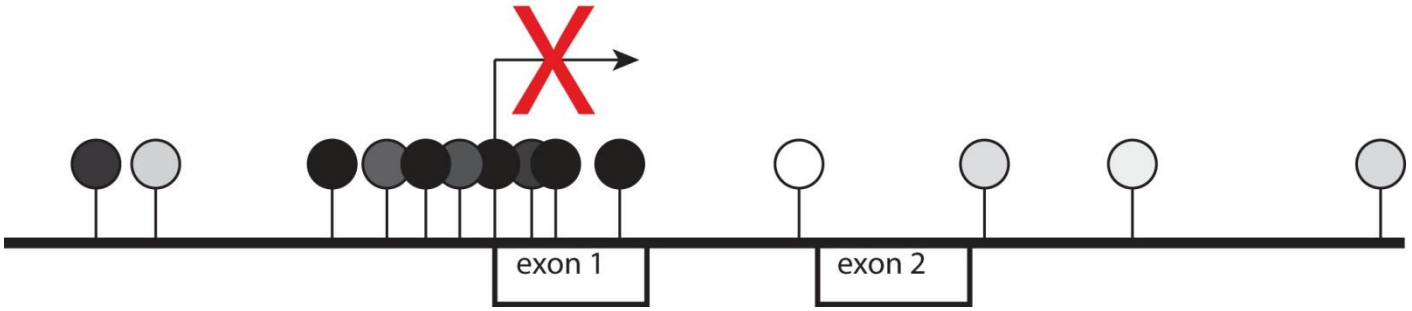
- More loci are methylated, and also they are methylated more frequently
  - Suggests there is some level of selection of the targets
    - Functional? – methylation of the locus helps in acquisition of the phenotype?
    - Locus susceptibility? – loci become preferred targets of broken methylation machinery as the cancer acquires androgen “independence”?

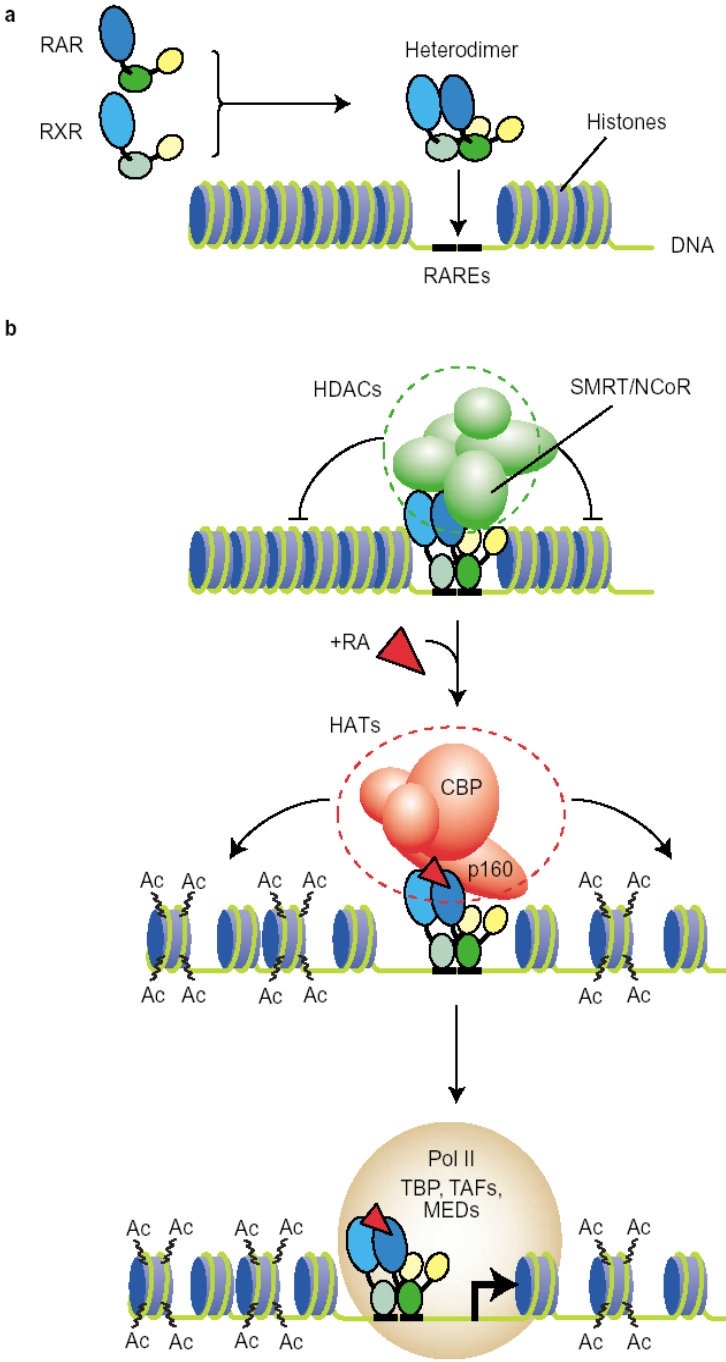

+1
exon 1
exon 2
Carcinogenesis
exon 1
exon 2



Carcinogenesis

- Meaningful difference, or nitpicking?
  - Nitpicking in terms of gene “on” or “off”
  - Perhaps meaningful in terms of process
- Why should 10% of cells have methylation at a CpG?
  - Do the same cells have methylation in the next CpG, in which 20% of cells are methylated?
  - Does this shed any light on how we get from normal to cancer state?





Stable silencing | Transient silencing | Primed for activity | Strong transcription

Deacetylated — **acetylated**

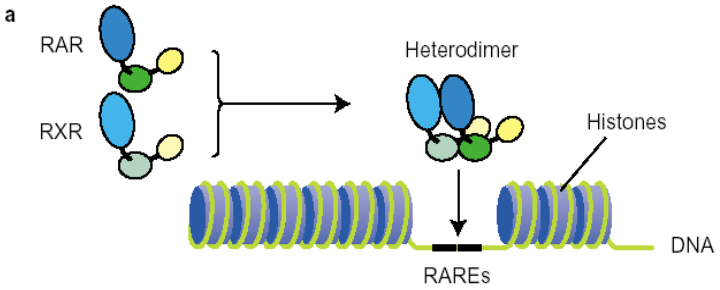
Dynamic equilibrium of histone modifications

RARα attracts complexes shifting local equilibrium

RA interaction changes the complexes shifting the equilibrium towards activation

Assembly of transcriptional machinery drives the equilibrium into the red

Clarke N. et. al. Expert Rev Mol Med. 2004 6(25):1-23.



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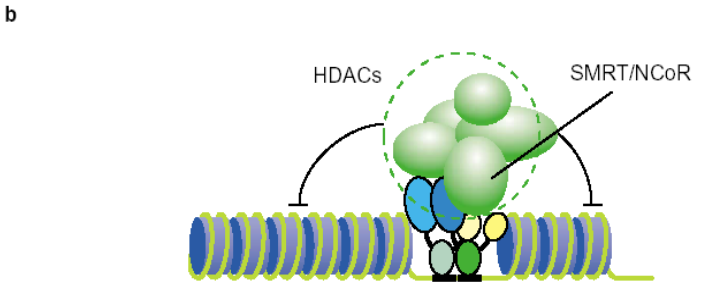


RARα attracts complexes shifting local equilibrium

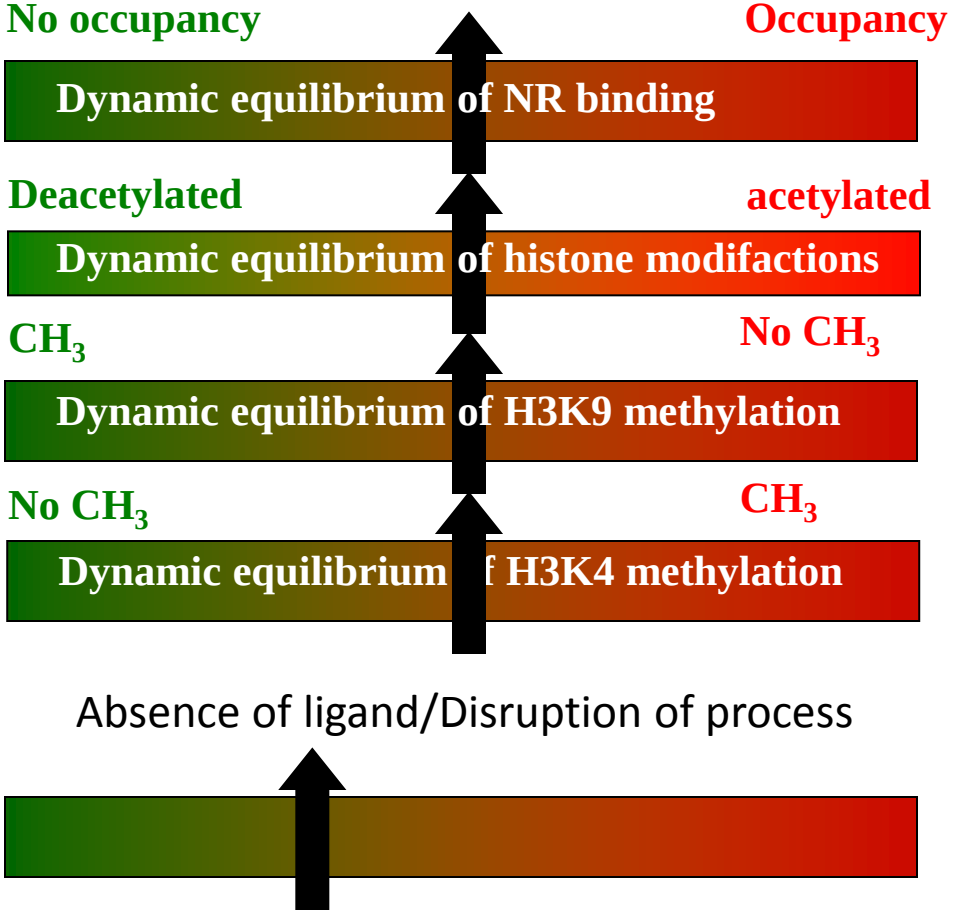
Repression

- If RARα is altered, absent, or blocked up with an antagonist:
  - perhaps a histone H3K9 de-methylase is lost from the region
  - Allows for acquisition of H3K9 methylation
  - This signals for DNA methylation

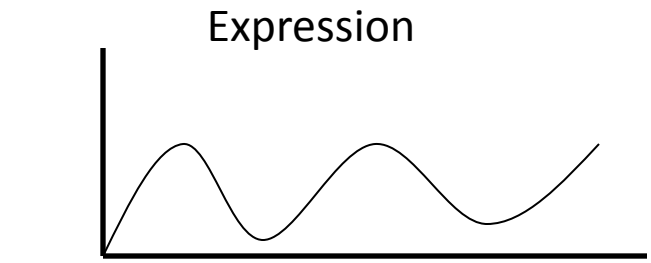
Aberrant RARa function pushes equilibrium towards stable silencing with histone methylation and DNA methylation

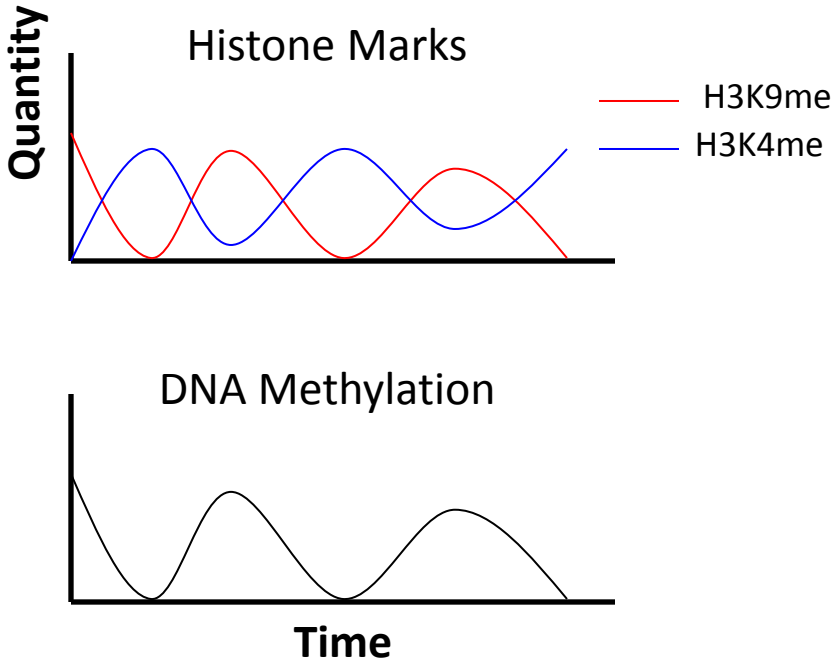
+Ligand → rapid, dynamic changes

Stable silencing	Transient silencing	Primed for activity	Strong transcription



Absence of ligand/Disruption of process





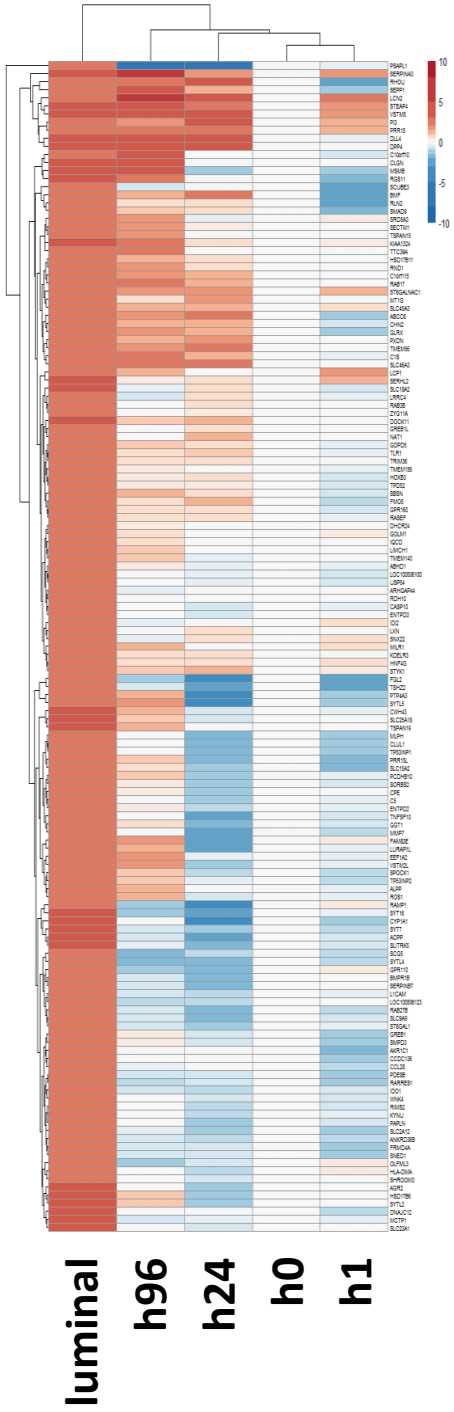
Recently shown for ER, VDR, and PPARγ regulated genes

Onset of cancer may interfere with regulation, the arrow may be pushed (permanently?) to one extreme.

- Hypothesis: Dynamic, cyclical changes in DNA methylation occur broadly across the genome in response to AR stimulation in non-malignant cells.

    - Such dynamic regions may be susceptible to aberrant DNA methylation and heterochromatinization in malignant cells

- HPr-1AR cell line
    - Immortalized normal prostate epithelial cells (HPV16 E6/E7)
    - Non-malignant
    - over expresses AR; translocates to nucleus after addition of ligand
        - Without ligand, cells grow well, semi-undifferentiated
        - With ligand, growth arrest; differentiation



Addition of DHT drives a differentiation program towards more luminal like phenotype

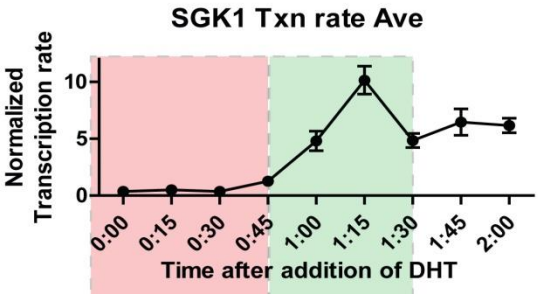
Is there a relationship between genes normally regulated by AR and aberrant methylation in CaP?

Top 10 androgen upregulated genes in HPr-1AR cells are hypermethylated in CaP

Table 1: Methylation and Expression status of candidate genes in prostate cancer			
Gene	**Methylated in AS-CaP (n=3)**	**Methylated in ADT-RCaP (n=3)**	**Downregulated in CaP; multiple Oncomine data sets**
KRT73	**80%**	**61%**	**X**
TIPARP	**76%**	**87%**	
S100P	**54%**	**69%**	
AQP3	**52%**	**81%**	**X**
TMEM37	45%	**75%**	**X**
SGK1	43%	**75%**	**X**
SLCO2A1	43%	**75%**	**X**
CXCR7	43%	**58%**	**X**
MGC16121	36%	**84%**	**X**
SEMA3G	N/A	NA	

- Treat cells with DHT and collect DNA and nascent RNA every 15 minutes
  - Measure transcription rate and DNA methylation at each time point
    - Ask if methylation changes in relation to changes in transcription rate

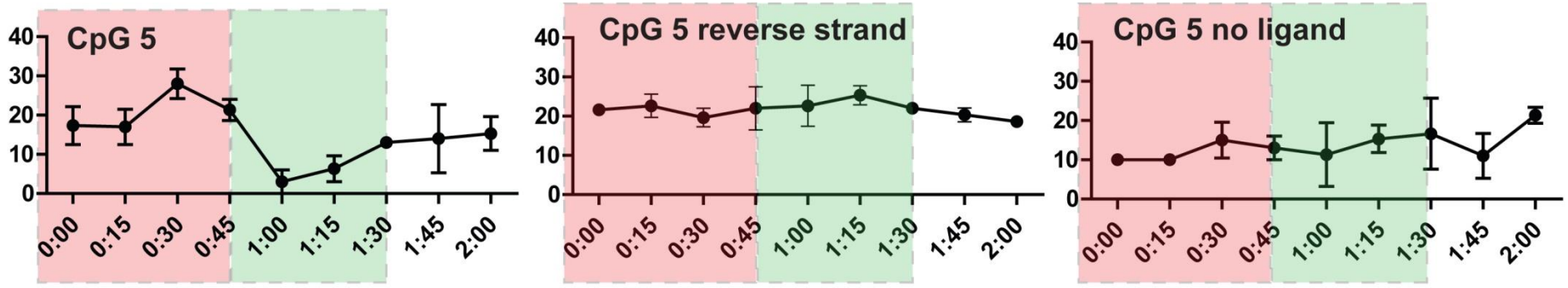
Dynamic methylation at SGK1 upstream androgen binding region



- Data represent biological triplicates
- No artificial synchronization other than addition of ligand

- **Some CpGs show dynamics in methylation level**

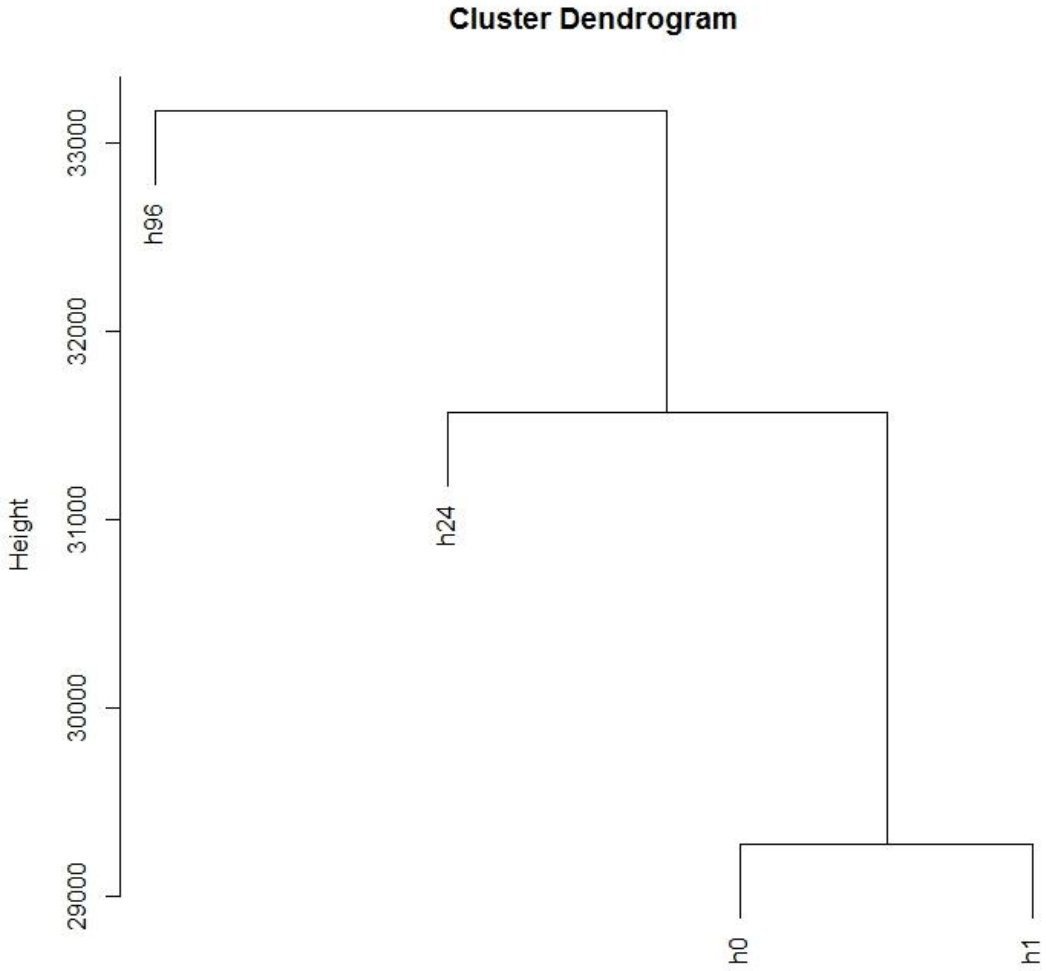
Strand and ligand specificity



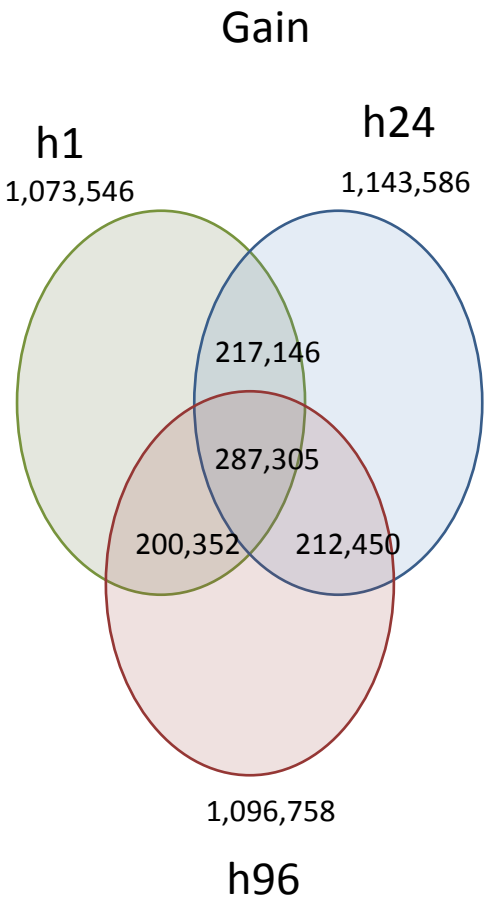
Can we apply this concept genome wide?

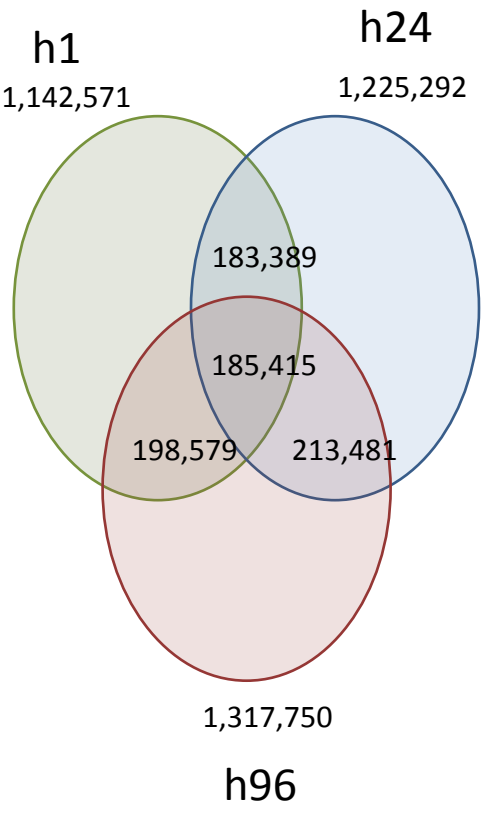
- Whole Genome Bisulfite Sequencing (WGBS) at 1, 24 and 96 hours
  - Do we see global variation in methylation over time?
  - Do these sites correlate with differential gene expression?
  - Are these sites susceptible to aberrant methylation in prostate cancer?

Whole-Genome Bisulfite Sequencing: Results

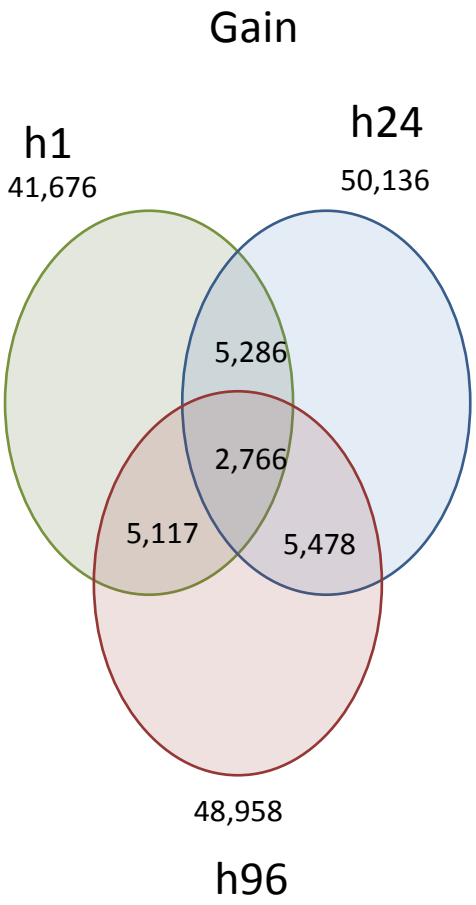


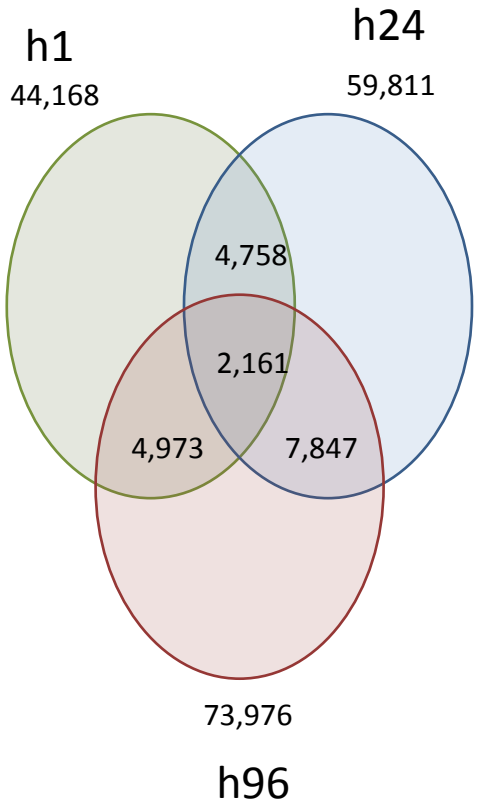
CpG Sites with changes in Methylation (+/- 10% relative to h0)





CpG Sites with changes in Methylation (+/- 30% relative to h0)





CpG Sites with progressive changes in Methylation

Gain

h96 > h24 > h1 > h0 = 297,311

h96 > h24 > h1 > h0 & h96 – h0 > 20= 54,654

h96 – h24 > 10 & h24 – h1 > 10 & h1 - h0 > 10 = 711

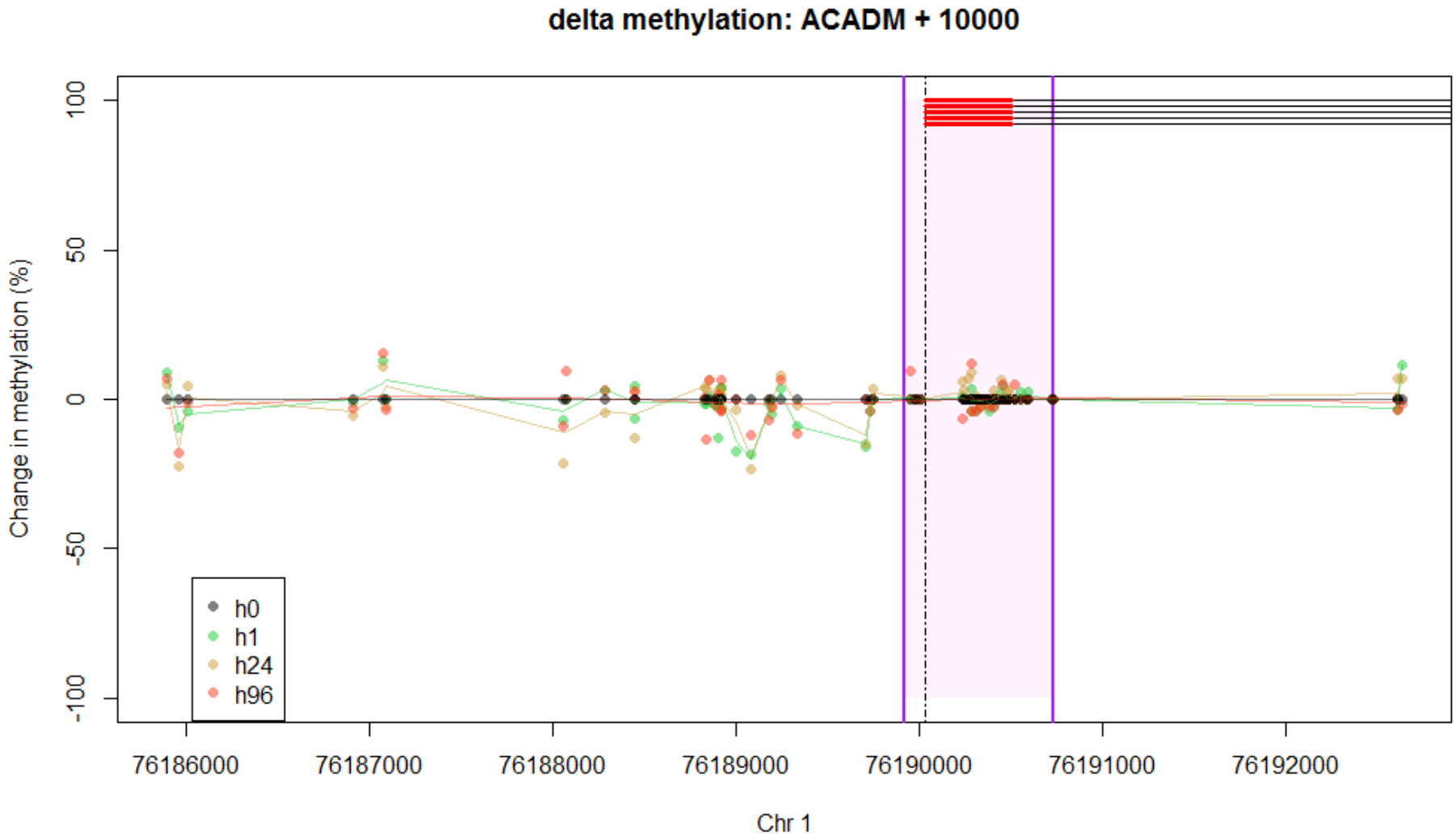
Loss

h96 < h24 < h1 < h0 = 338,636

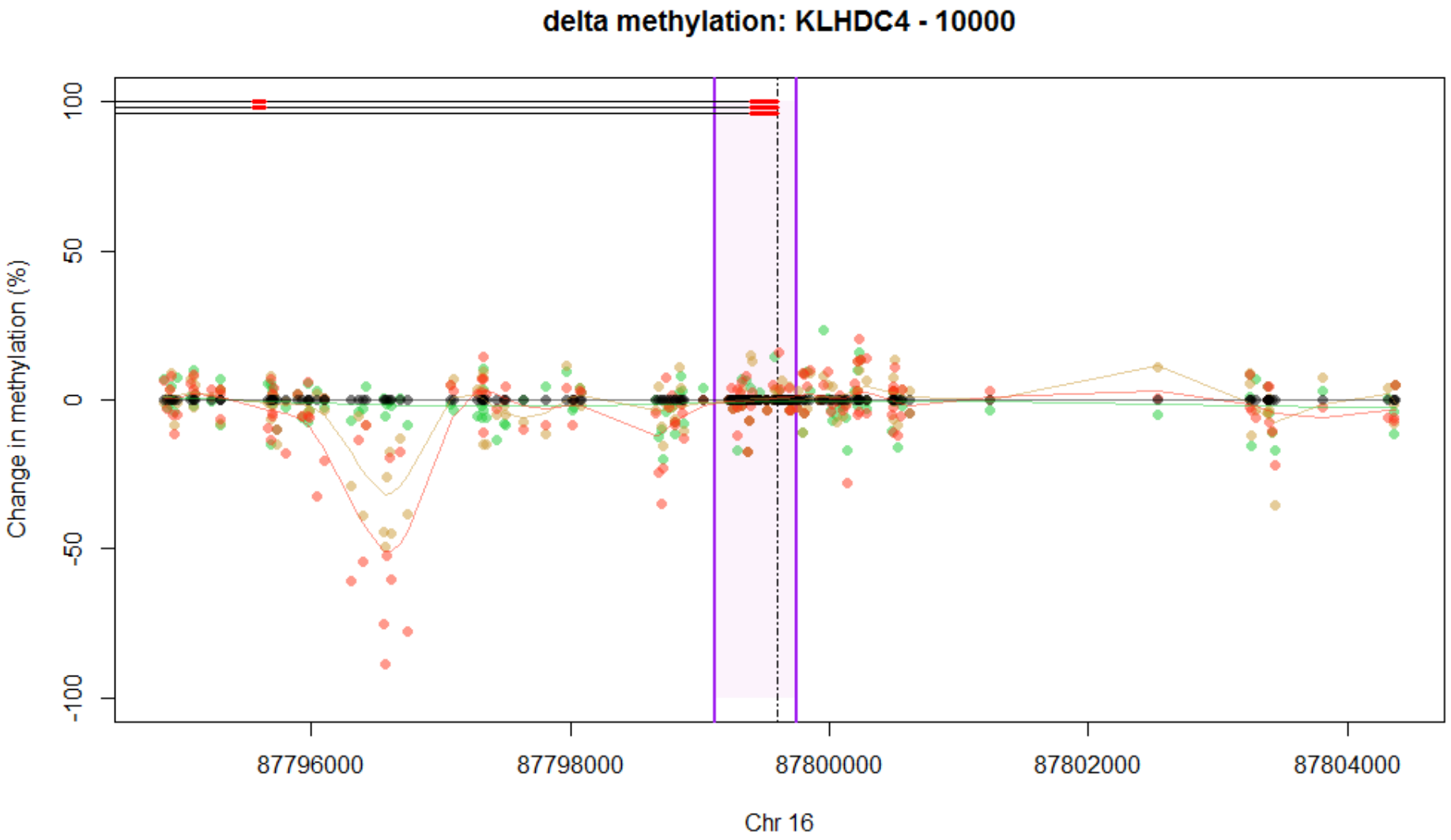
h96 < h24 < h1 < h0 & h96 – h0 < -20 = 74,281

h96 – h24 < -10 & h24 – h1 < -10 & h1 - h0 < -10 = 1269

- Methylation variable positions (MVPs) defined as showing progressive change over time and totaling >20% methylation change
- 128,935 MVPs out of total of ~11 million CpGs
- ~26,000 associated with transcriptional start sites (TSS)
- ~6,500 TSS have 2 or more MVPs; ~5,700 have 1 MVP; ~14,000 have no MVP



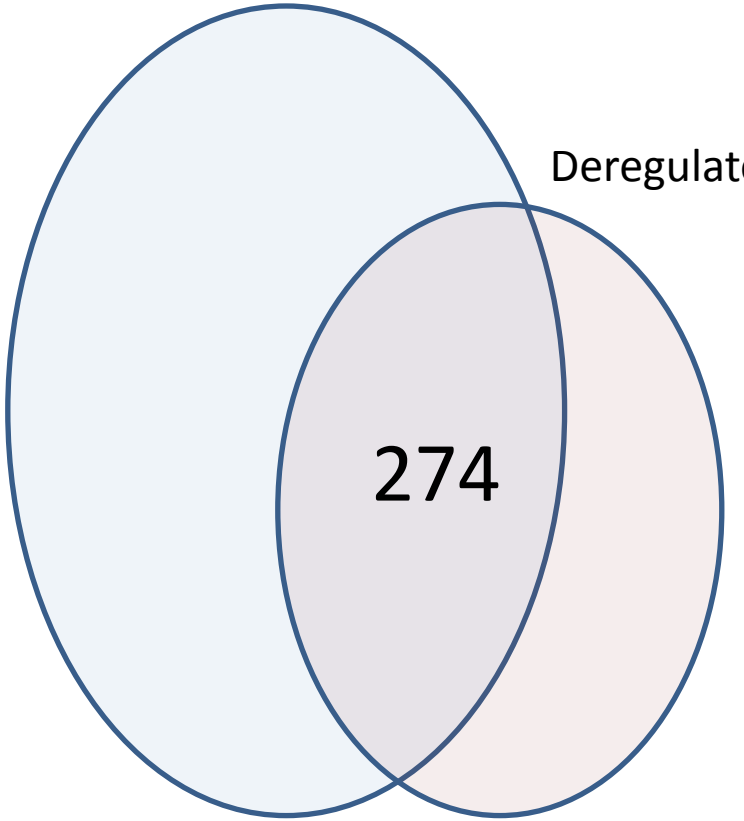
10kb window around TSS of a gene with no MVPs



10kb window around TSS of a gene with many MVPs

HPr1AR+10nM DHT
Deregulated Promoter Methylation
(2+ MVPs)

6536



TCGA-PRAD
Deregulated Promoter Methylation

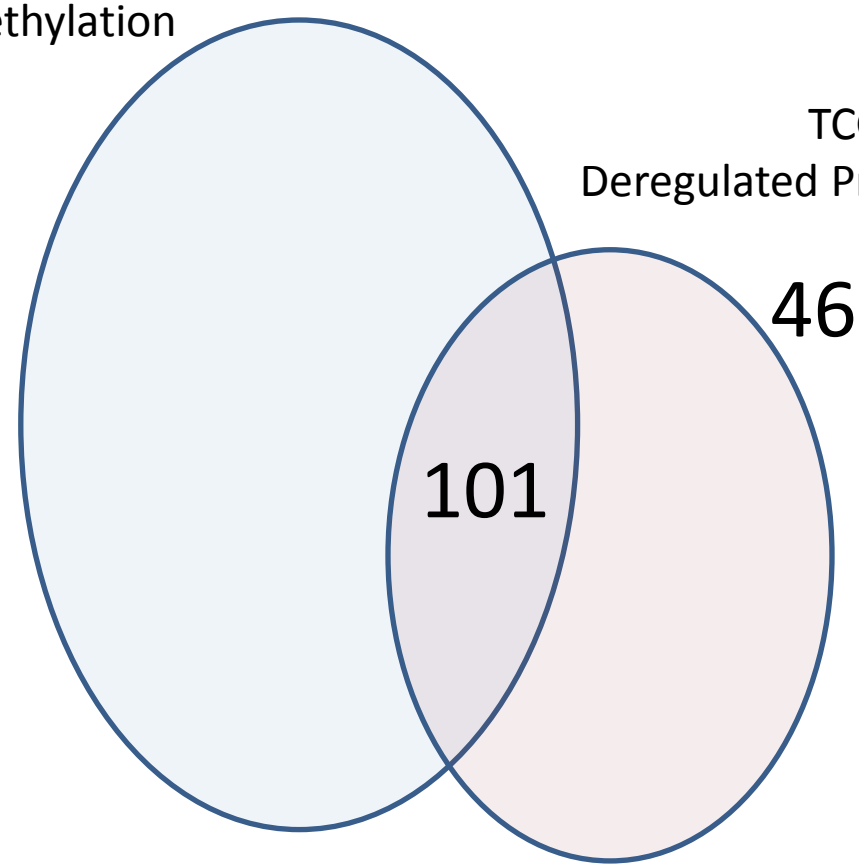
467

p = 3.05e-54

Genes containing >2 MVPs are **highly enriched** as targets of methylation in CaP.

HPr1AR+10nM DHT
Deregulated Promoter Methylation
(1 MVP)

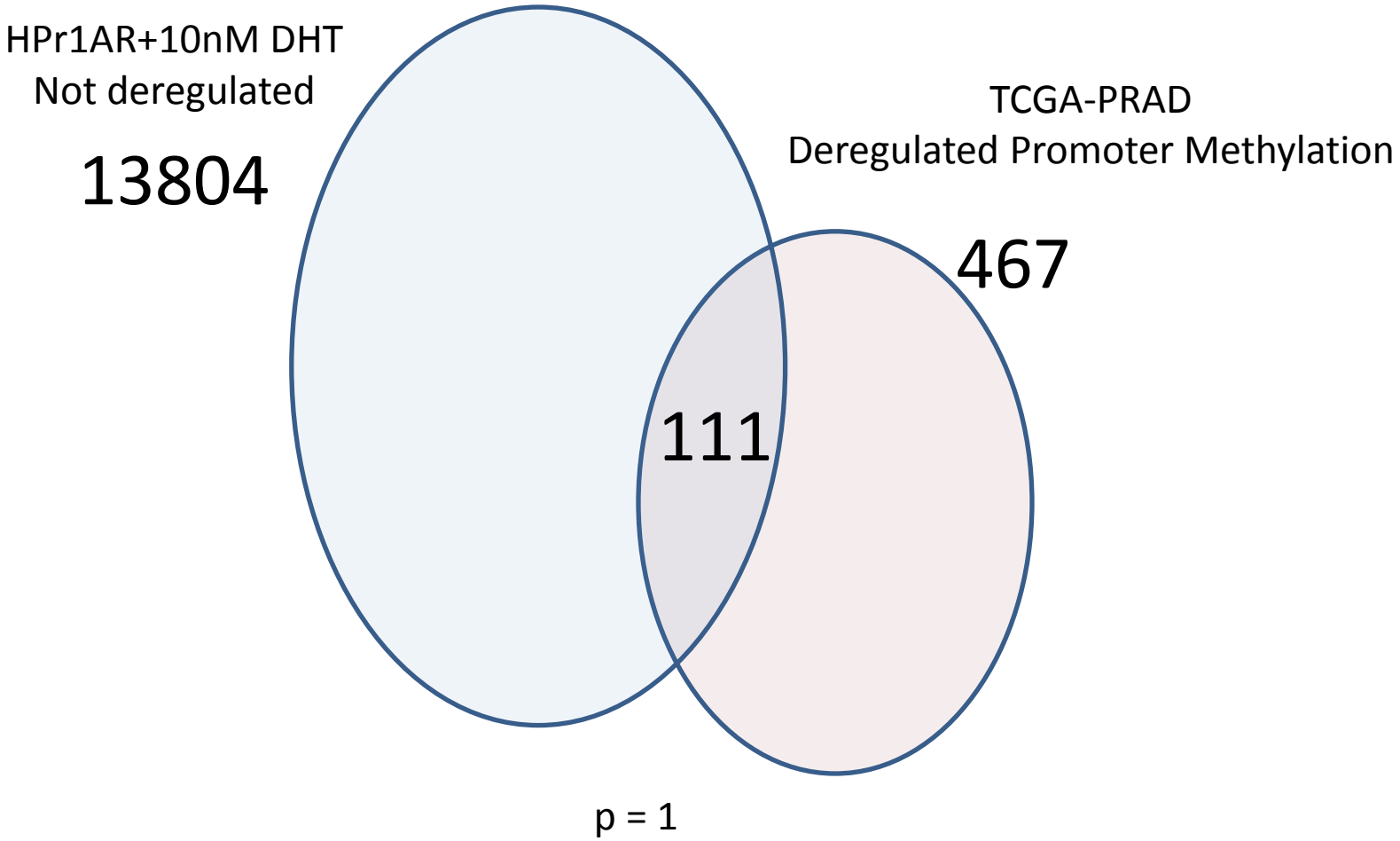
5771



TCGA-PRAD
Deregulated Promoter Methylation

467

p = 0.62



Non-MVP containing genes are **DE-ENRICHED** as targets of methylation in CaP.

- Whole Genome Bisulfite Sequencing (WGBS) at 1, 24 and 96 hours
  - Do we see global variation in methylation over time?
  - Do these sites correlate with differential gene expression?
  - Are these sites susceptible to aberrant methylation in prostate cancer?

Strong evidence that CpG regions that demonstrate dynamics in methylation levels during "quasi-normal" androgen driven basal to luminal epithelial cell differentiation are hyper-susceptible to aberrant methylation in prostate cancer.

Emphasizes the critical need for understanding normal epigenetic regulation, in order to gain insights into cancer specific epigenetic dysregulation.