PD53-01: Development of a Whole-Urine, Multiplexed, Next Generation RNA-Sequencing Assay for Aggressive Prostate Cancer Early Detection

Andi K. Cani[1,2,3,4], Kevin Hu[1,5], Javed Siddiqui[1,3], Yingye Zheng[6], Sumin Han[1,3*], Chia-Jen Liu[1,3,4], Trinh Pham[7], Daniel H. Hovelson[1,3,5**], Srinavas Nallandhighal[7], Lanbo Xiao[1,3,4], Heng Zheng[1,3,4], Jeffrey J. Tosoian[7], Ganesh S. Palapattu[7], Todd M. Morgan[7], Aaron Udager[1,3,4], Arul M. Chinnaiyan[1,2,3,4,7], John T. Wei[1,7], Scott A. Tomlins[1,2,3,7], **Simpa S. Salami[1,4,7]**

[1]Michigan Center for Translational Pathology, University of Michigan Medical School, Ann Arbor, Michigan, USA;
[2]Molecular and Cellular Pathology Graduate Program, University of Michigan Medical School, Ann Arbor, Michigan, USA.
[3]Department of Pathology, University of Michigan Medical School, Ann Arbor, Michigan, USA.
[4]Rogel Cancer Center, University of Michigan, Ann Arbor, Michigan, USA.
[5]Department of Computational Medicine and Bioinformatics, University of Michigan Medical School, Ann Arbor, Michigan, USA.
[6]Public Health Sciences Division, Fred Hutchinson Cancer Center, Seattle, Washington, USA
[7]Department of Urology, University of Michigan Medical School, Ann Arbor, Michigan, USA.

*Current affiliation: Bristol-Myers Squibb, Redwood City, CA, USA
**Current affiliation: Strata Oncology, Ann Arbor, MI, USA

Disclosures

- None

Background

- Early detection of aggressive prostate cancer [PCa; Grade Group (GG) 3-5] continues to pose significant clinical challenge.

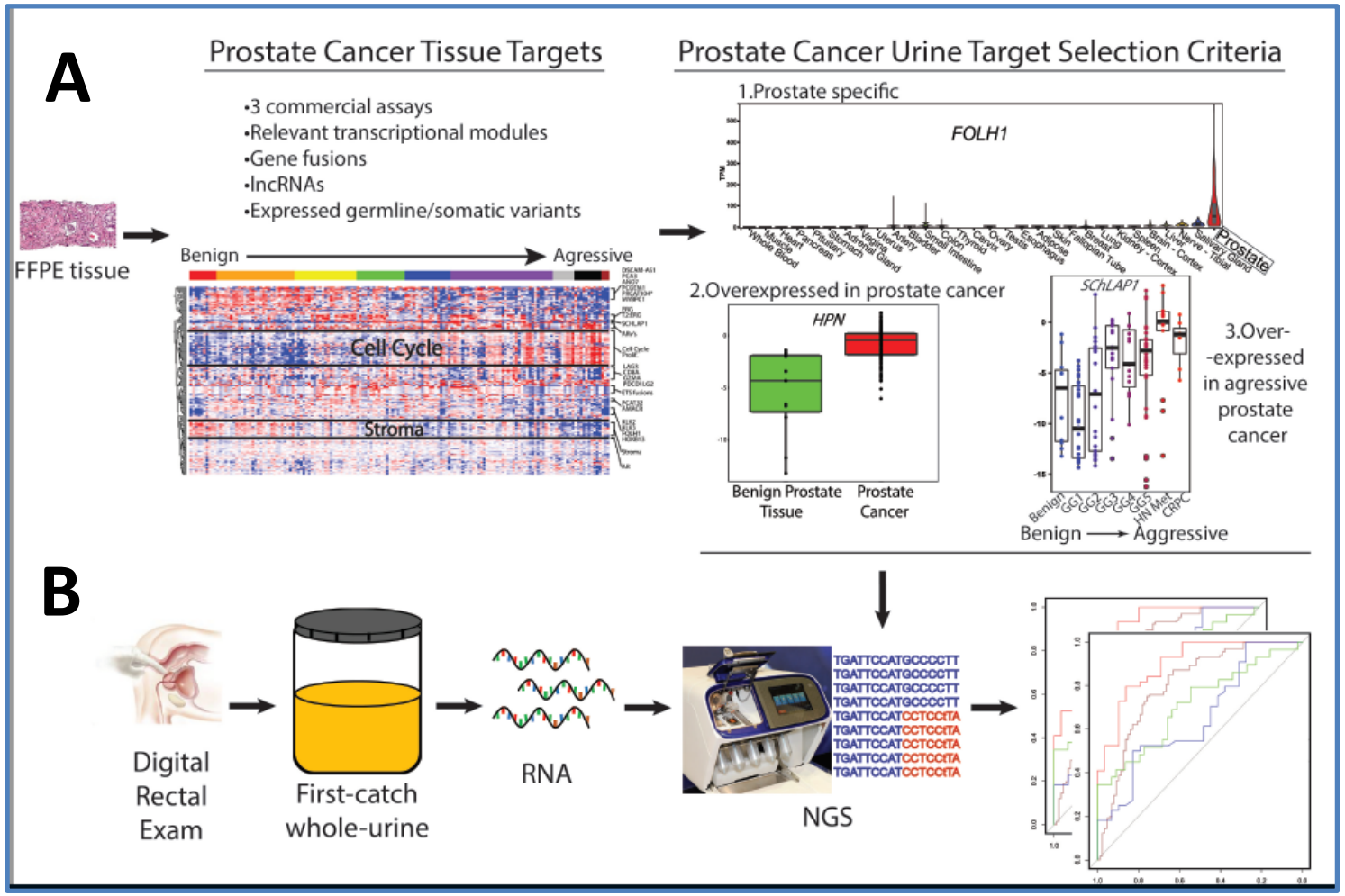
- We previously developed the Michigan Prostate Score (MiPS) for predicting aggressive PCa, which is available for clinical use.

- MiPS uses TMA to quantify the expression of *TMPRSS2:ERG* (*T2:ERG*) and *PCA3* from whole urine obtained after a digital rectal exam (DRE), combined with serum PSA.

Objective

- To improve upon MiPS, develop and validate a targeted, multiplex RNA next generation sequencing urine assay (NGS-MiPS) for the detection of aggressive PCa.

Urine NGS-MiPS Panel Design and Workflow



A. NGS-MiPS Panel Design

~90 PCa transcriptomic biomarkers: including T2:ERG, PCA3, isoforms of common PCa gene fusions, mRNAs, lncRNAs, and expressed mutations.

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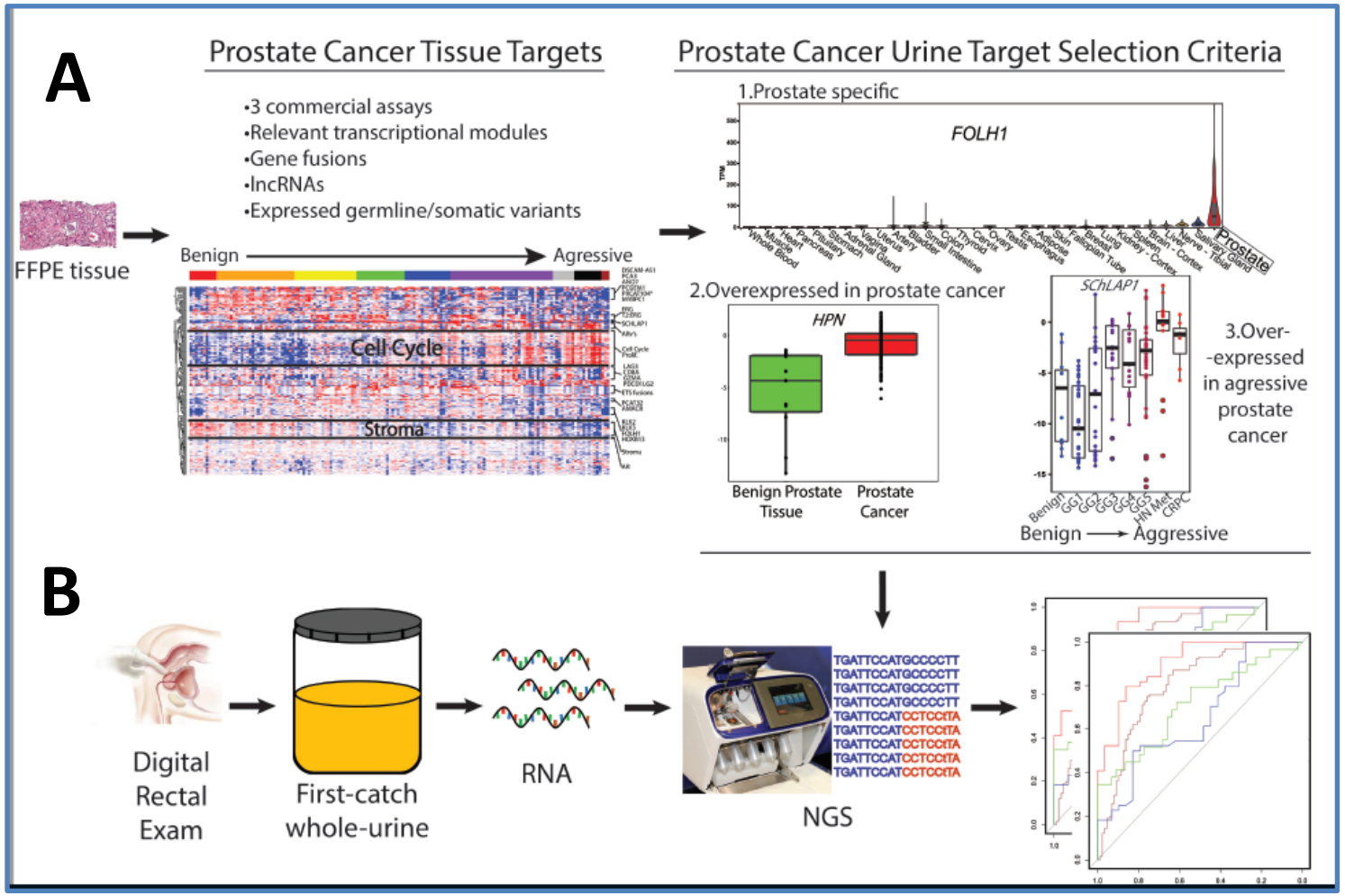
Urine NGS-MiPS Panel Design and Workflow



B. Workflow

- Urine was collected after DRE.
- RNA was extracted from 2.5 mL of urine.
- Targeted RNA NGS was performed using the NGS-MiPS panel.

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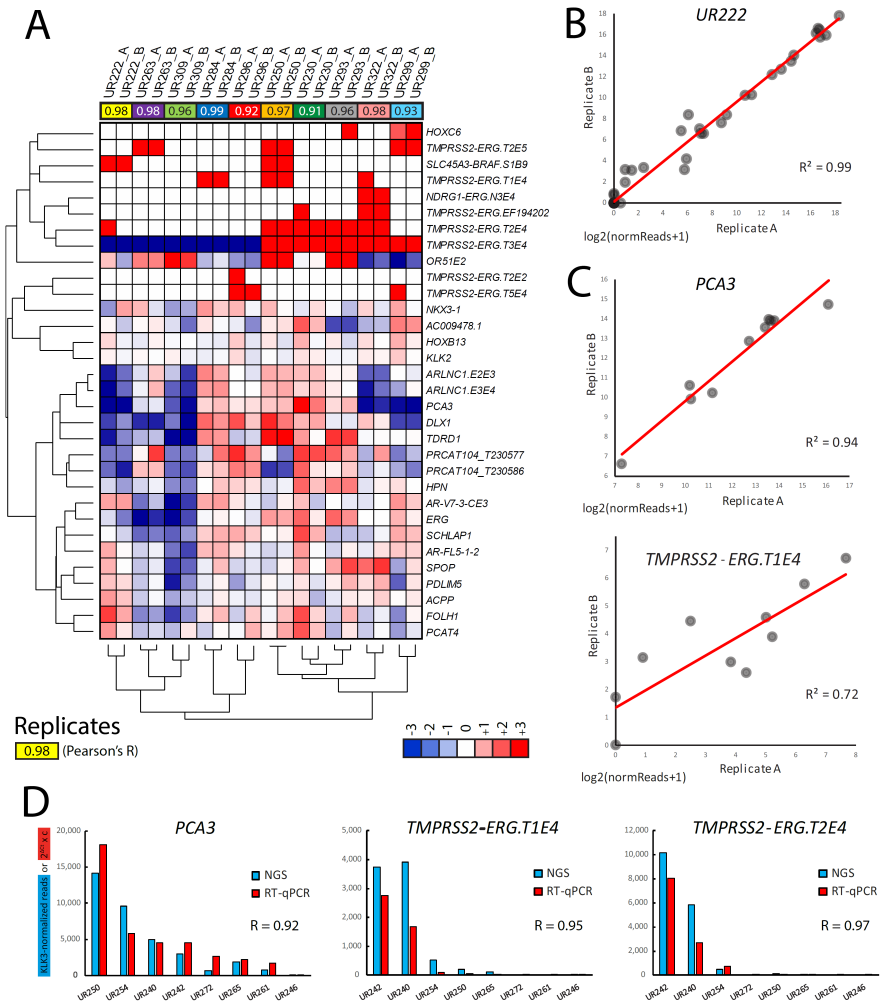
Cohort Description

- **Primary Analytic Cohort:**
  - *Extreme case design*: Grade Group (GG) 3-5 vs. Benign/GG1.
  - Training (n = 73).
  - Testing (n = 36).

- **Secondary Analytic Cohort:**
  - Active Surveillance Cohort (n = 45).
  - Comparison: NCCN Intermediate/Low risk vs. Benign/very low risk.

NGS-MiPS Technical Validity I



NGS-MiPS showed a 98% informative sample rate, high technical reproducibility, robustness and concordance with orthogonal methods (TMA and RT-qPCR).

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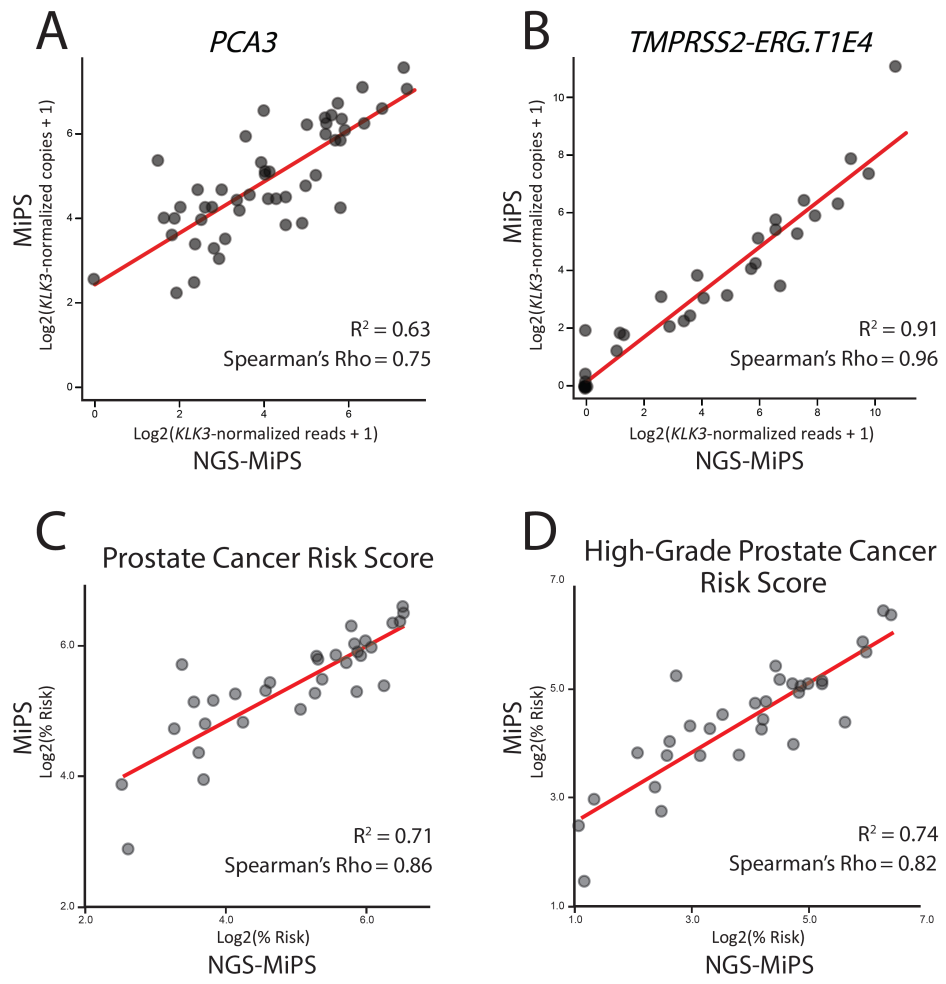
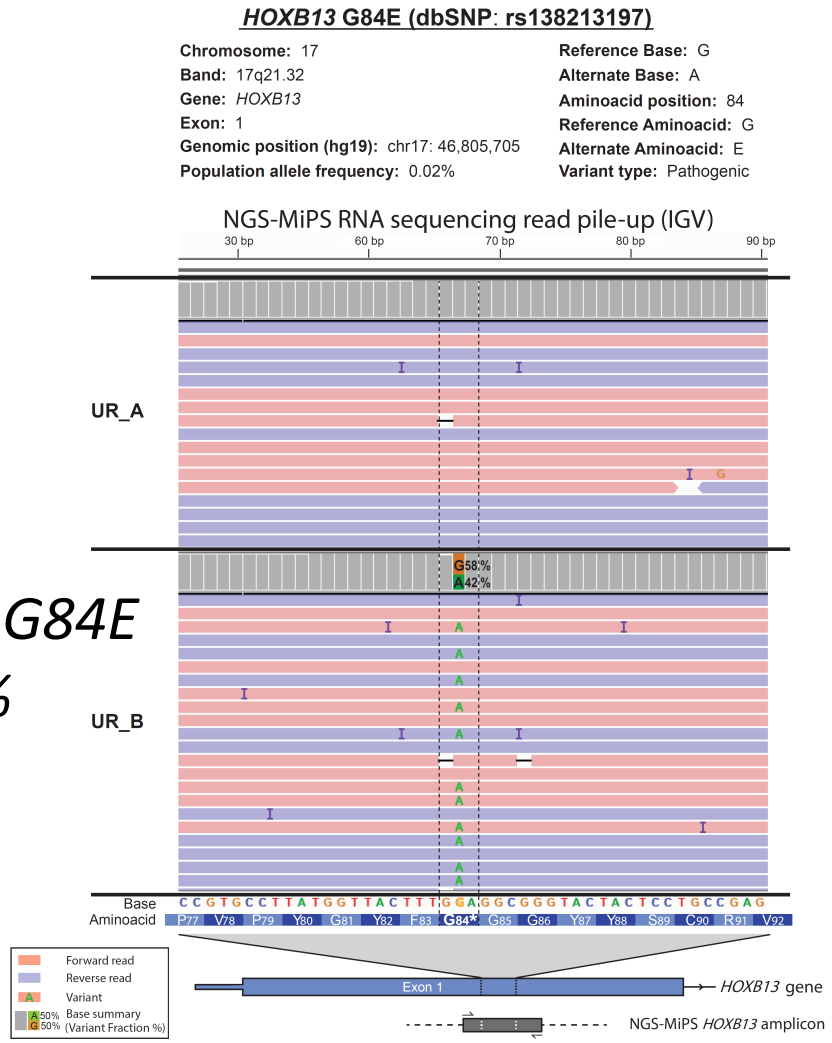
NGS-MiPS Technical Validity II



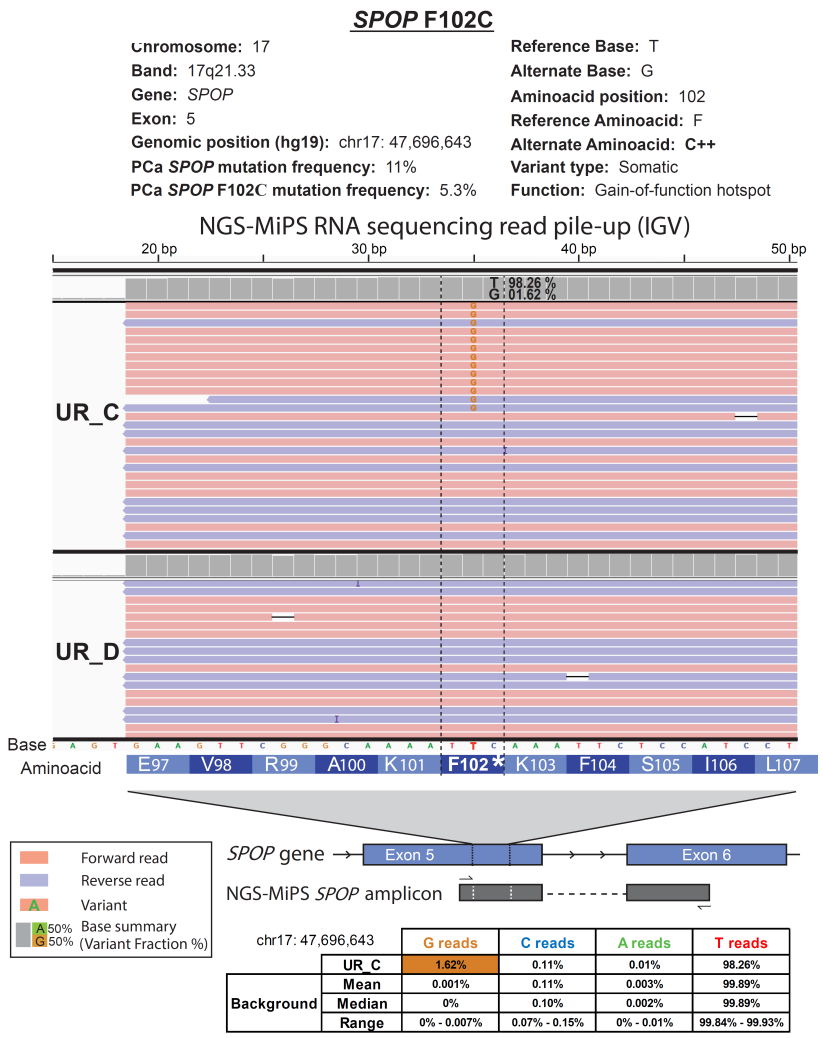
NGS-MiPS accurately recapitulated clinical MiPS-measured risk scores for presence of PCa or high-grade PCa (GG >1) on biopsy as determined by clinical MiPS vs. the same model but with NGS-MiPS data.

NGS-MiPS detected expressed *HOXB13 p.G84E* variant and *SPOP* mutations

HOXB13 G84E
42%



SPOP F102C
1.6%



chr17: 47,696,643		G reads	C reads	A reads	T reads
	UR_C	1.62%	0.11%	0.01%	98.26%
Background	Mean	0.001%	0.11%	0.003%	99.89%
	Median	0%	0.10%	0.002%	99.89%
	Range	0% - 0.007%	0.07% - 0.15%	0% - 0.01%	99.84% - 99.93%

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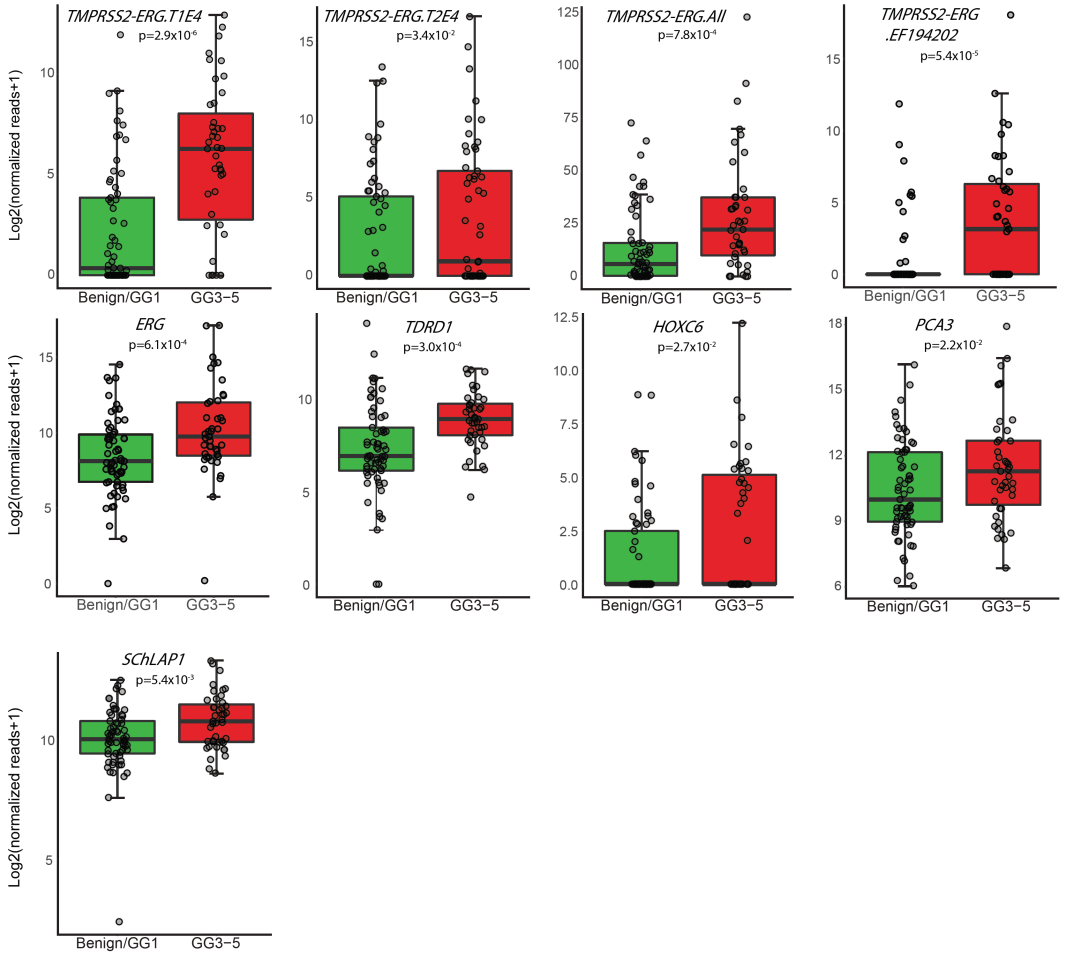
Significant differential expression between high vs. benign/low grade PCa



In an extreme case design, (GG ≥3 vs. Benign/GG1) NGS-MiPS showed expected differences in the levels of *T2:ERG* T1E4 ($p<0.00001$) and *PCA3* ($p=0.02$), with additional *T2:ERG* splice isoforms and other biomarkers also showing significantly different expression between low vs. high grade disease.

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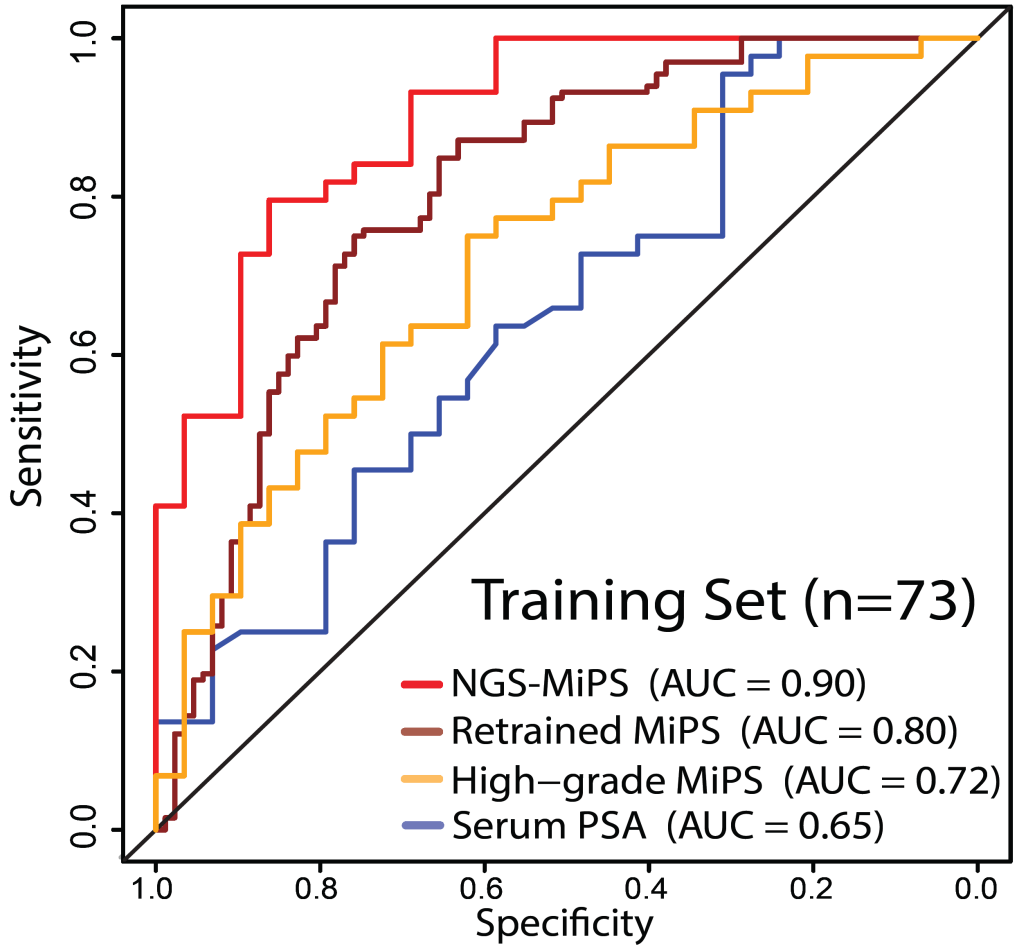
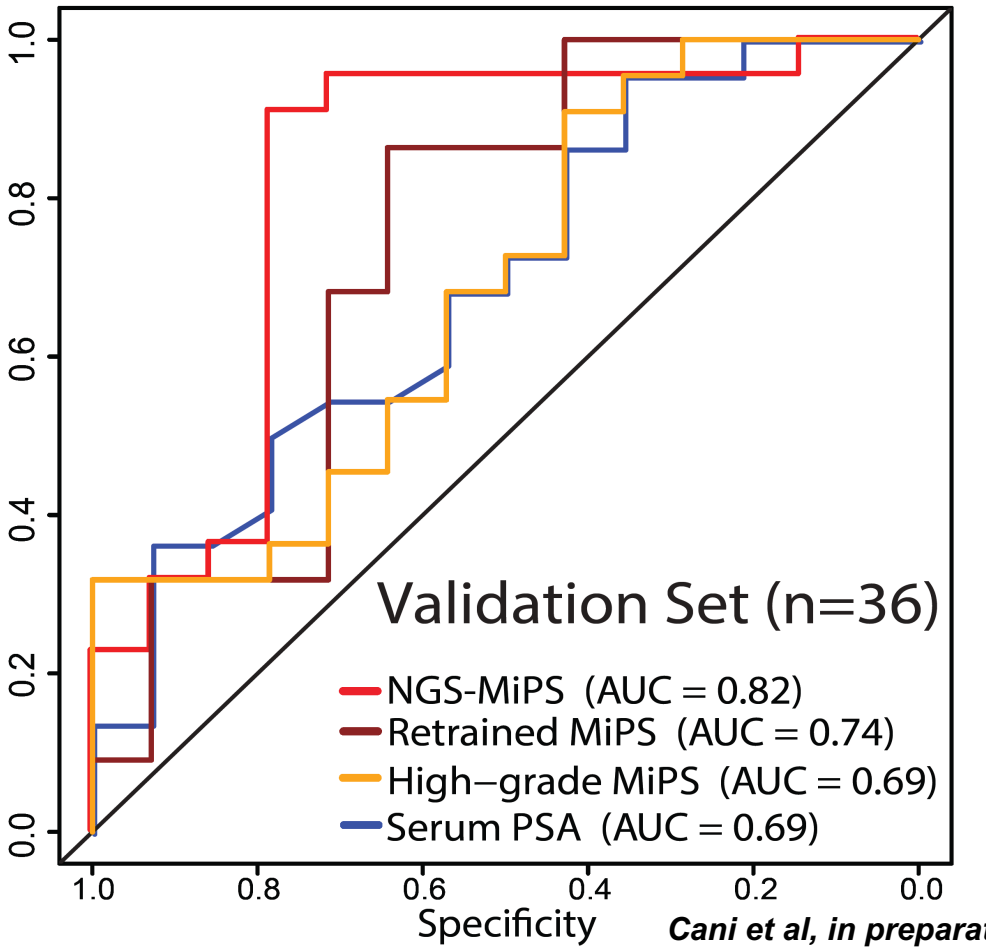
Training and Testing of the NGS-MiPS Model to Predict GG 3-5 PCa

Model Training



Model Testing



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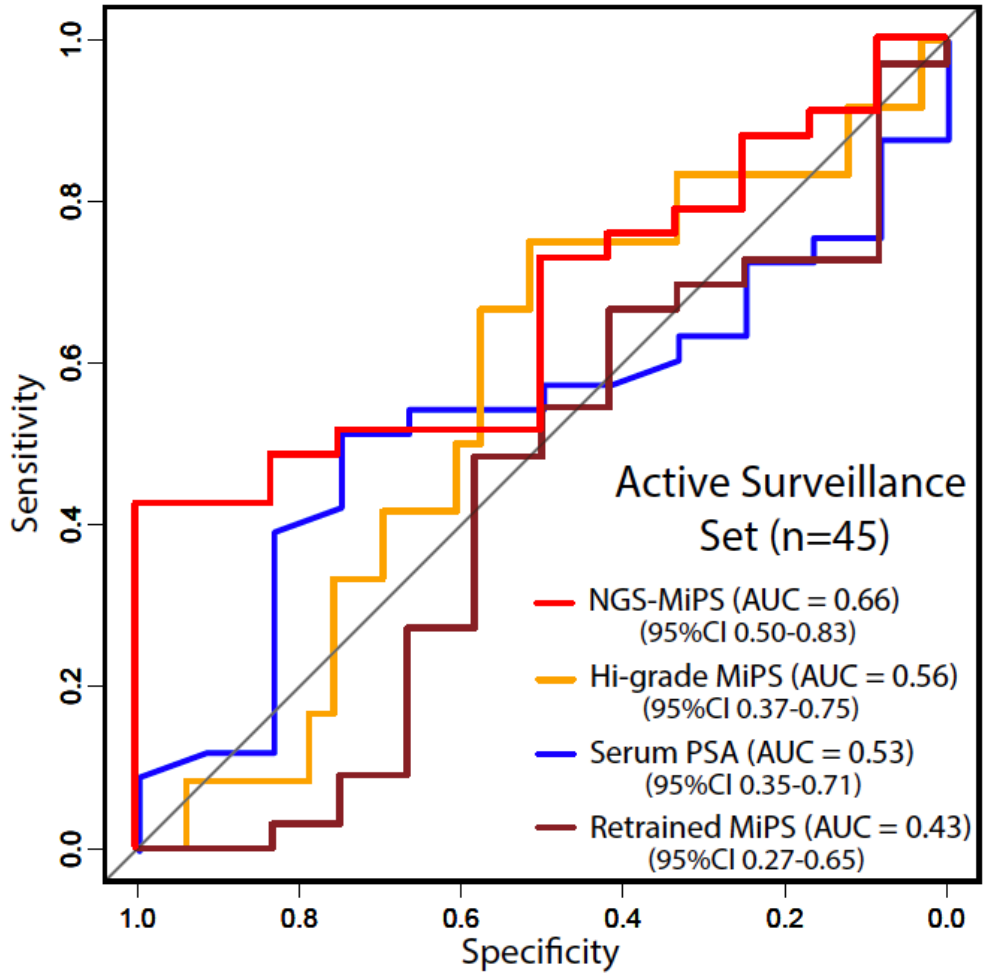
Testing of the NGS-MiPS Model in an Active Surveillance Cohort



In an exploratory analyses, the 29-transcript model also outperformed MiPS and serum PSA for classifying patients into NCCN intermediate/low risk vs. Benign/very low risk, as shown

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Limitations

- This is a selected cohort to represent the extremes of PCa grade groups. Further prospective development and validation in clinically practical settings is warranted.

- Whole urine collected after an attentive DRE is necessary for assay testing. Exploration of pre-DRE urine is ongoing.

Conclusion

- Our novel targeted RNA NGS-MiPS assay can detect transcriptomic and expressed genomic alterations in post-DRE urine

- These results support the potential utility and continued development of a urine-based targeted NGS assay to improve upon serum PSA for early detection of aggressive prostate cancer

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