

3605: A Novel Active Chromatin Cell-free DNA (cfDNA_{ac}) Assay for Early Detection of Colorectal Cancer

Yue Wendy Zhang, Hsin-Ta Wu, Rich Rava, Diana Abdueva, Maggie C. Louie Aqtual Inc, Hayward, California, USA.

Background

- Colorectal cancer (CRC) is the third most diagnosed cancer and the second leading cause of cancer-related deaths worldwide (Xi et al., 2021). In the U.S. alone, 154,270 new cases and 52,900 deaths are projected in 2025, with incidence rising among younger adults.¹
- CRC typically progresses from normal epithelial tissue to adenocarcinoma through a well-defined sequence of molecular events. While current screening methods have reduced mortality, low compliance remains a critical barrier to early detection.
- To address this, we present a proof-of-concept machine learning (ML) classifier trained on active chromatin cell-free DNA (cfDNA_{ac}) signals from blood to differentiate CRC patients from healthy individuals.

Platform

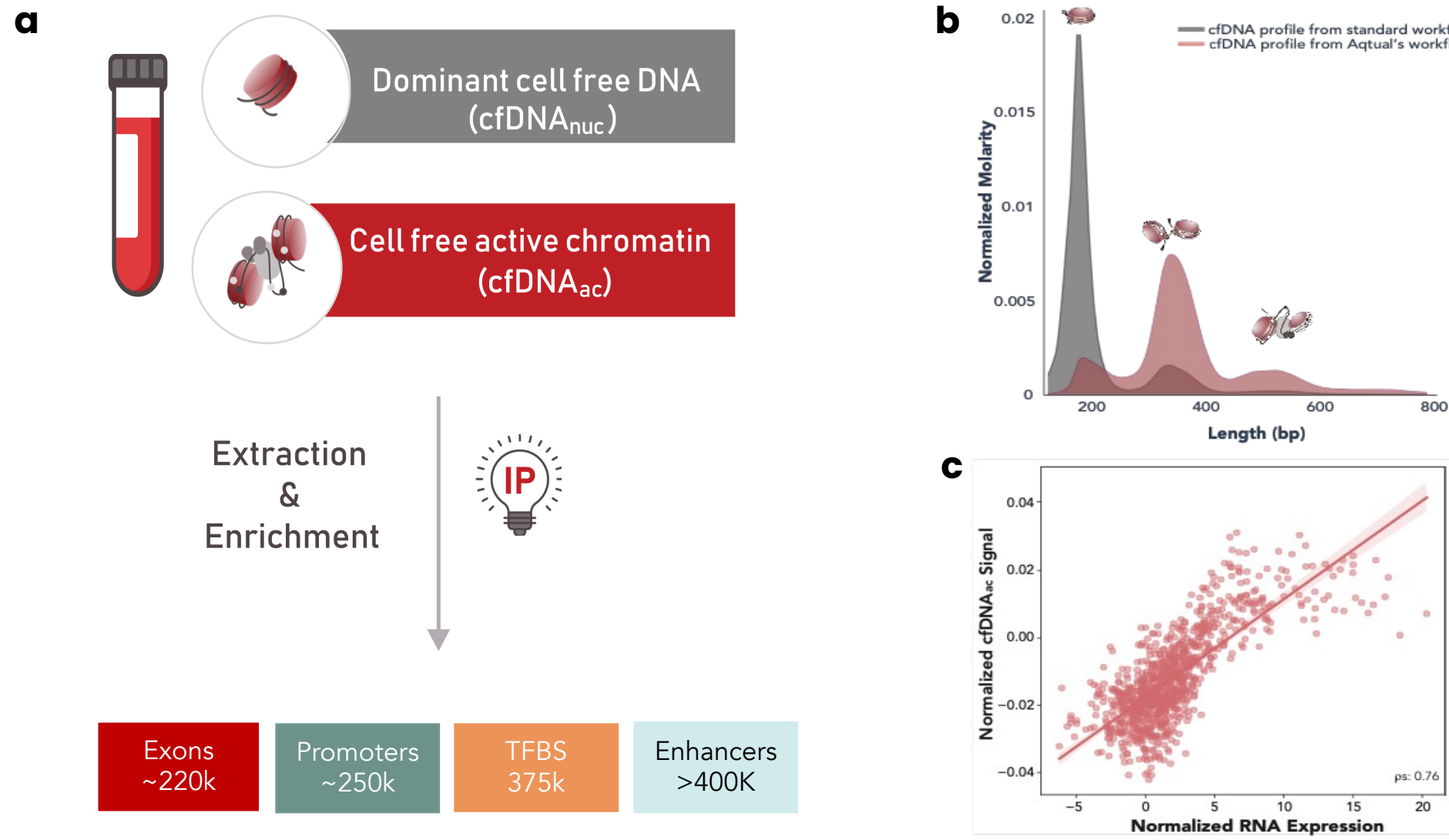


Figure 1: (a) cfDNA_{ac} workflow. (b) cfDNA profile of active chromatin.² (c) cfDNA_{ac} signal was correlated with the corresponding gene expression in matched tissue-derived RNA-seq from stage IV CRC patients (n=5).

Demographics & Methods

Table 1	Training set		Hold-out set		
	Healthy	CRC Stage I-III	Healthy	Advanced Adenoma	CRC Stage I-IV
Samples (N)	19	31	21	14	23
Age: Mean (SD)	58.7 (7.3)	69.7 (9.1)	55.9 (9.5)	64.9 (10.1)	67.8 (12.9)
BMI: Mean (SD)	26.4 (3.5)	28.0 (7.0)	28.2 (3.7)	27.4 (6.0)	27.8 (5.7)
Gender: N (%)					
Female	7 (36.8)	16 (51.6)	9 (42.9)	10 (71.4)	15 (65.2)
Male	12 (63.2)	15 (48.4)	12 (57.1)	4 (28.6)	8 (34.8)
Ethnicity: N (%)					
Caucasian	15 (78.9)	27 (87.1)	19 (90.5)	5 (35.7)	22 (95.7)

- Samples were divided into a training set and a hold-out test set. A machine learning classifier was trained using 5 repeat, 5-fold cross-validation on the training set.
- Feature selection was performed using the Mann-Whitney U test ($p \leq 0.05$) on normalized cfDNA_{ac} fragment counts. Selected features were then reduced via partial least squares dimensionality reduction, applied separately for each feature type.
- Base models were trained independently for each feature type and combined using a logistic regression stacking approach.

Results

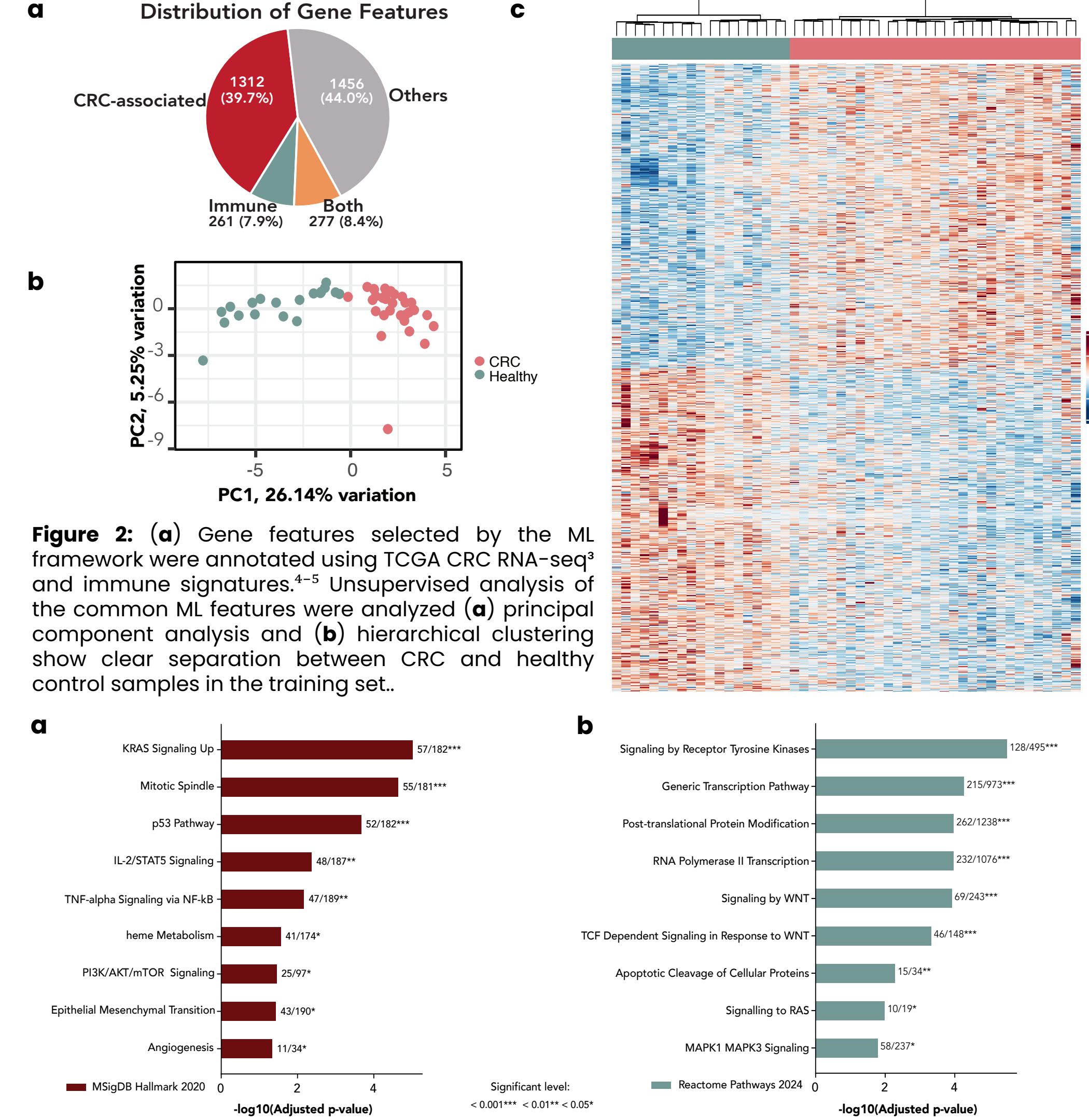
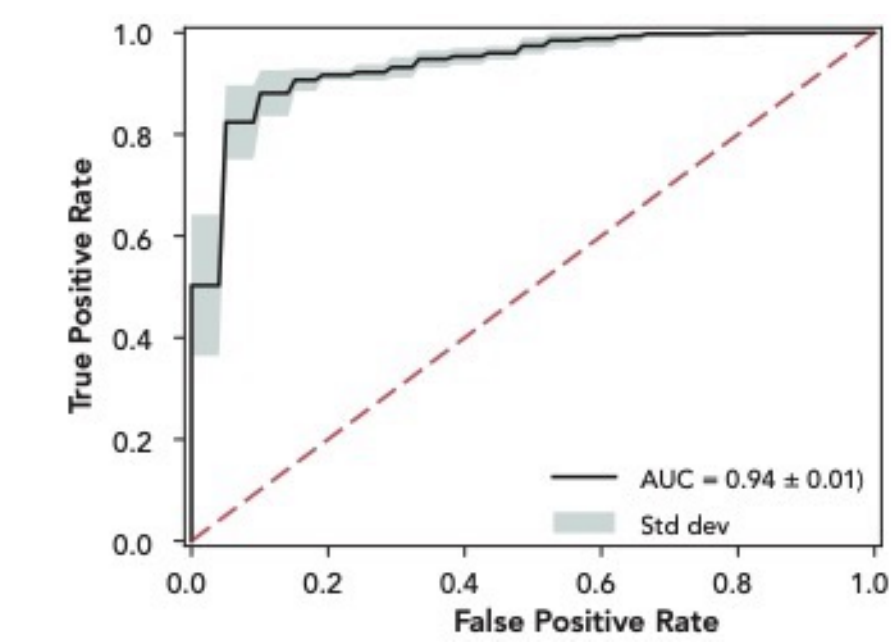


Figure 2: (a) Gene features selected by the ML framework were annotated using TCGA CRC RNA-seq³ and immune signatures.⁴⁻⁵ Unsupervised analysis of the common ML features were analyzed (a) principal component analysis and (b) hierarchical clustering show clear separation between CRC and healthy control samples in the training set.

Final Performance



Sensitivity	Advance Adenoma (n=14)	Total CRC (n=23)	Stage I-III (n=12)	Stage IV (n=11)
@90% Specificity	81%	93%	94%	91%

Figure 5: Receiver operator curve (ROC) and performance of the classifier on hold-out set.

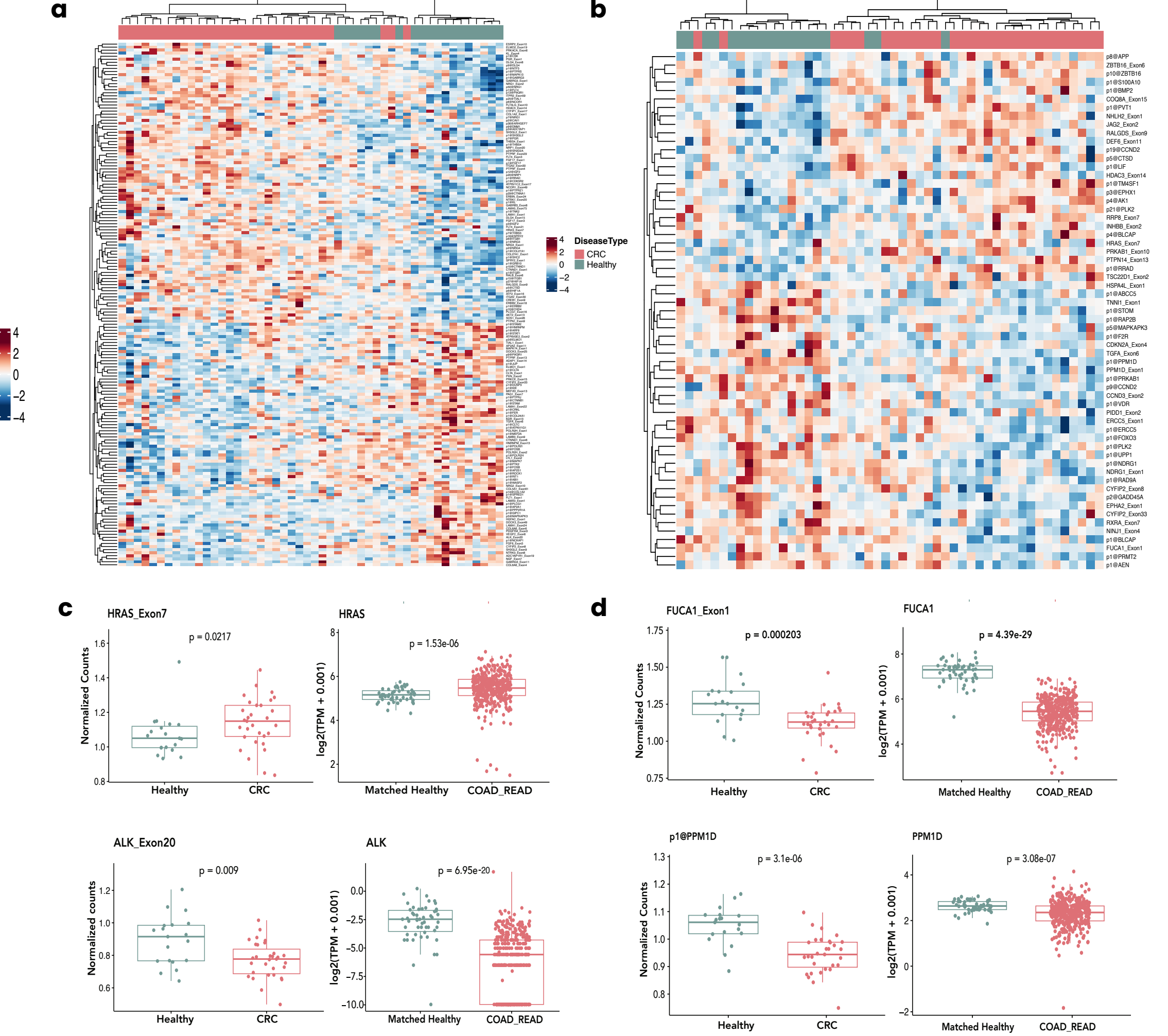


Figure 4: Heatmap of (a) receptor tyrosine kinase (RTK) signaling pathway- and (b) p53 pathway-enriched features identified in the over representation analysis. Unsupervised hierarchical clustering demonstrates the ability of each feature set to differentiate colorectal cancer (CRC) from healthy control samples. Boxplots of two representative features in the (c) RTK and (d) p53 pathways, comparing normalized cfDNA_{ac} counts in plasma with gene expression levels TCGA CRC tissue samples³.

Conclusion

- This proof-of-concept study demonstrates the potential of Aqtual's active chromatin (cfDNA_{ac}) capture assay to enrich and extract signals in plasma that can that differentiate colorectal cancer (CRC) and advanced adenomas from healthy control samples.
- Using a machine learning (ML) approach, the classifier shows promising potential for early cancer detection through a simple blood test.
- We are currently expanding the number of samples to help improve the model and confirm how well it works across a larger group of people.

Reference

- <https://www.cancer.org/cancer/types/colon-rectal-cancer/about/key-statistics.html>
- Lai, K., Dilger, K., Cunningham, R. et al. Extracting regulatory active chromatin footprint from cell-free DNA. Commun Biol 7, 1086 (2024).
- TCGA gene expression of CRC and matched normal tissue is downloaded from: https://toil-xena-hub.s3.us-east-1.amazonaws.com/download/TcgaTargetGtex_rsem_gene_tpm.gz
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- Lewis, M. J. et al. Cell reports 28, 2455 (2019).

Contact: diana.abdueva@aqtual.com