

Detection of non-small cell lung and bladder cancer signatures in peripheral blood using a novel active chromatin capture assay

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Introduction

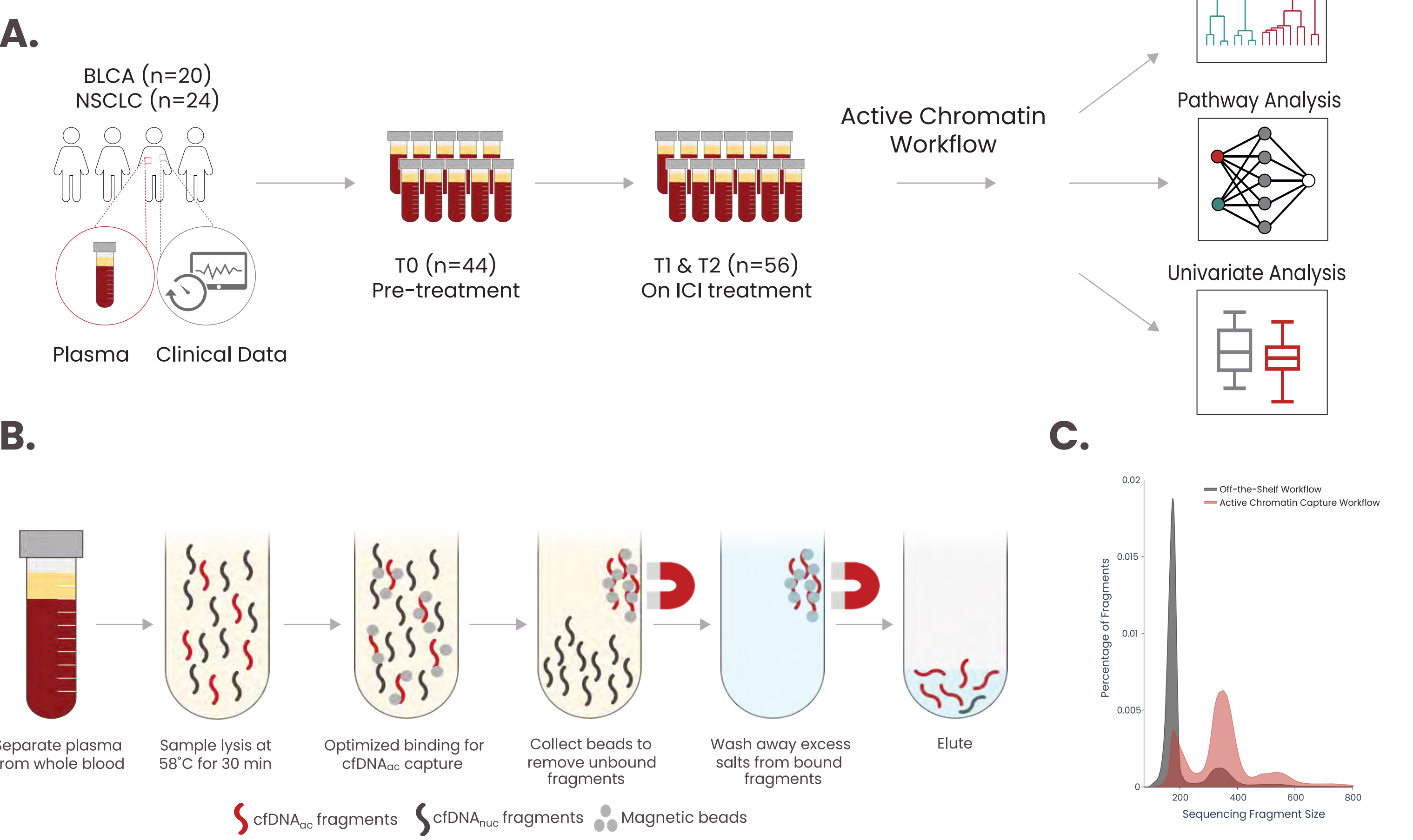
Immune checkpoint inhibition (ICI) targeting the programmed cell death protein 1 (PD-1) and its ligand (PD-L1) has transformed cancer treatment, significantly improving clinical outcomes in bladder cancer (BLCA) and non-small cell lung cancer (NSCLC). However, the efficacy of ICI therapies is hampered by low response rates, highlighting a need to identify who will benefit. While PD-L1 expression, tumor mutational burden, and micro-satellite instability have all been important biomarkers for predicting favorable ICI response, there are limitations to their clinical utility, most notably being the requirement for tumor tissue, which can be challenging, costly, and timely to obtain.

Circulating tumor DNA from blood is a promising non-invasive biomarker; however, it falls short in delineating the transcriptional programs that dictate cancer phenotypes and their evolution over the disease trajectory. To address these unmet needs, we have developed a plasma-based active chromatin capture method that enables the comprehensive epigenetic and transcriptomic interrogation of both tumor and immune components from a simple blood collection¹. Given that the half-life of cfDNA is in hours², analysis of active chromatin cell free DNA (cfDNA_{ac}) offers a unique insight into the dynamic landscape of tumor development and immune responses.

We analyzed plasma samples of both BLCA and NSCLC patients undergoing ICI therapy and of healthy individuals. Cancer samples exhibited higher levels of total cfDNA, and significantly higher levels of cfDNA_{ac} fragments. We identified features that differentiated ICI responders from non-responders pre-treatment. Notably, both BLCA and NSCLC displayed common enrichments of cancer and immune-specific signatures across response classes, indicating shared mechanisms of response and resistance. Longitudinal analysis revealed potential pharmacodynamic signatures that could provide insights into mechanisms of action and potential pathways of resistance. In summary, our study highlights the use of cfDNA_{ac} analysis as a non-invasive approach to understanding dynamic changes at both the gene and pathway levels, underscoring its potential for a wide range of clinical applications in precision oncology.

Methods

Study design and active chromatin workflow



A. Plasma samples (n=100) of individuals with BLCA (n=20) and NSCLC (n=24) undergoing ICI therapy, along with samples (n=70) from healthy individuals (n=5), were processed using our **B.** novel active chromatin workflow¹ to extract (cfDNA_{ac}) and nucleosomal fragments (cfDNA_{nuc}). 30% (n=13) of individuals were responders (complete or partial responders) according to the RECIST 1.1 criteria, while 70% (n=31) were non-responders (progressive disease). **C.** Size distributions of sequenced cfDNA fragments extracted using traditional cfDNA workflow (gray) and the active-chromatin capture workflow (red).

Table 1. Cancer plasma samples have higher total cfDNA and cfDNA_{ac} compared to healthy plasma samples

	Individuals (N)	Samples (N)	Total cfDNA (ng) mean±SD	P-value	cfDNA _{ac} (ng) mean±SD	P-value
Healthy	5	70	5.70±2.62	-	4.25±2.02	-
BLCA	20	52	12.24±6.68	1.57E-11	10.78±5.89	3.20E-14
NSCLC	24	48	15.56±13.41	2.45E-09	14.10±12.54	2.32E-08

Results

Figure 1. BLCA and NSCLC have common enrichment in immune and cancer pathways compared to healthy individuals

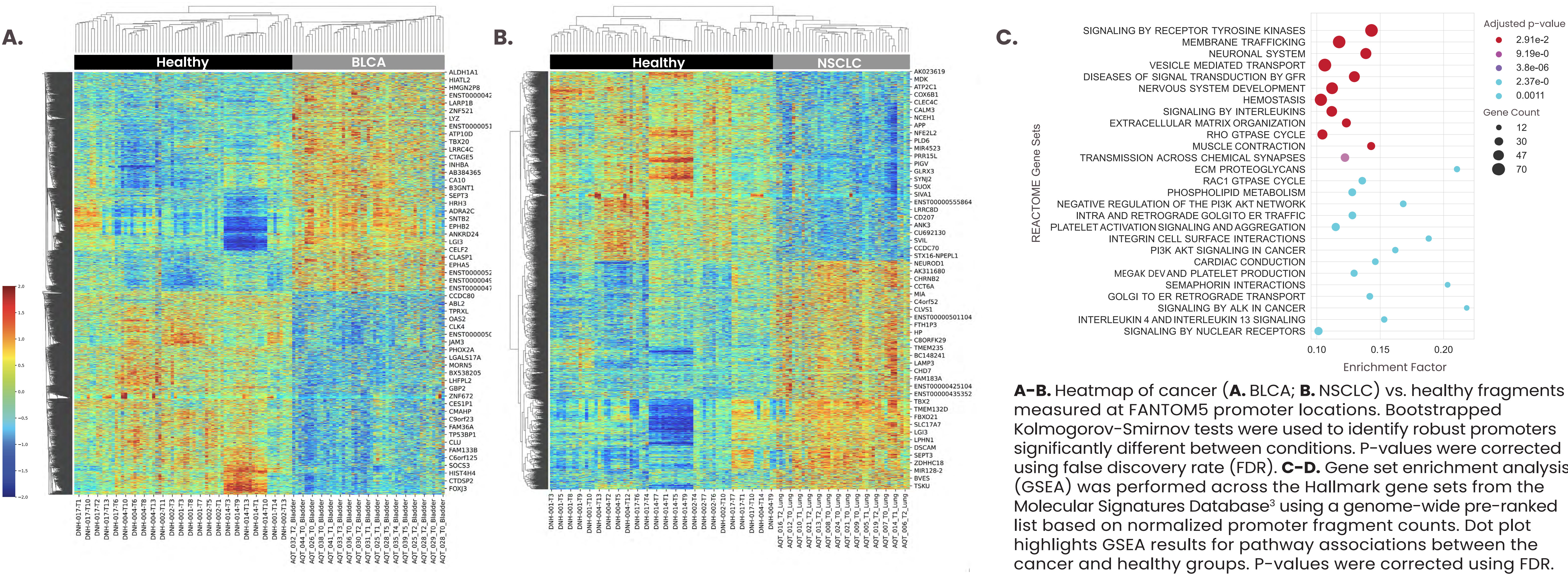


Figure 2. Responders and non-responders exhibit significant differences in cfDNA_{ac} signatures at pre-treatment time point (T0)

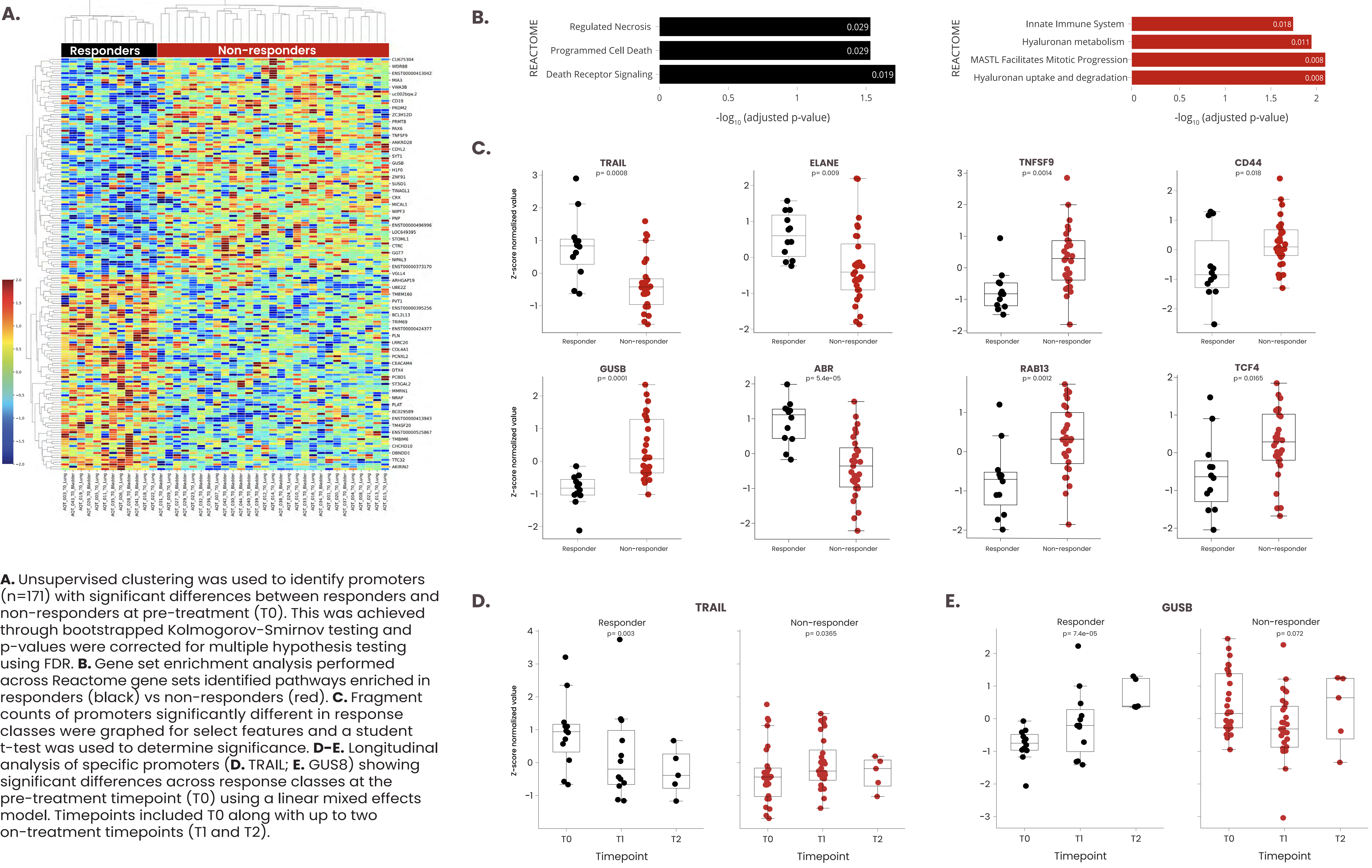
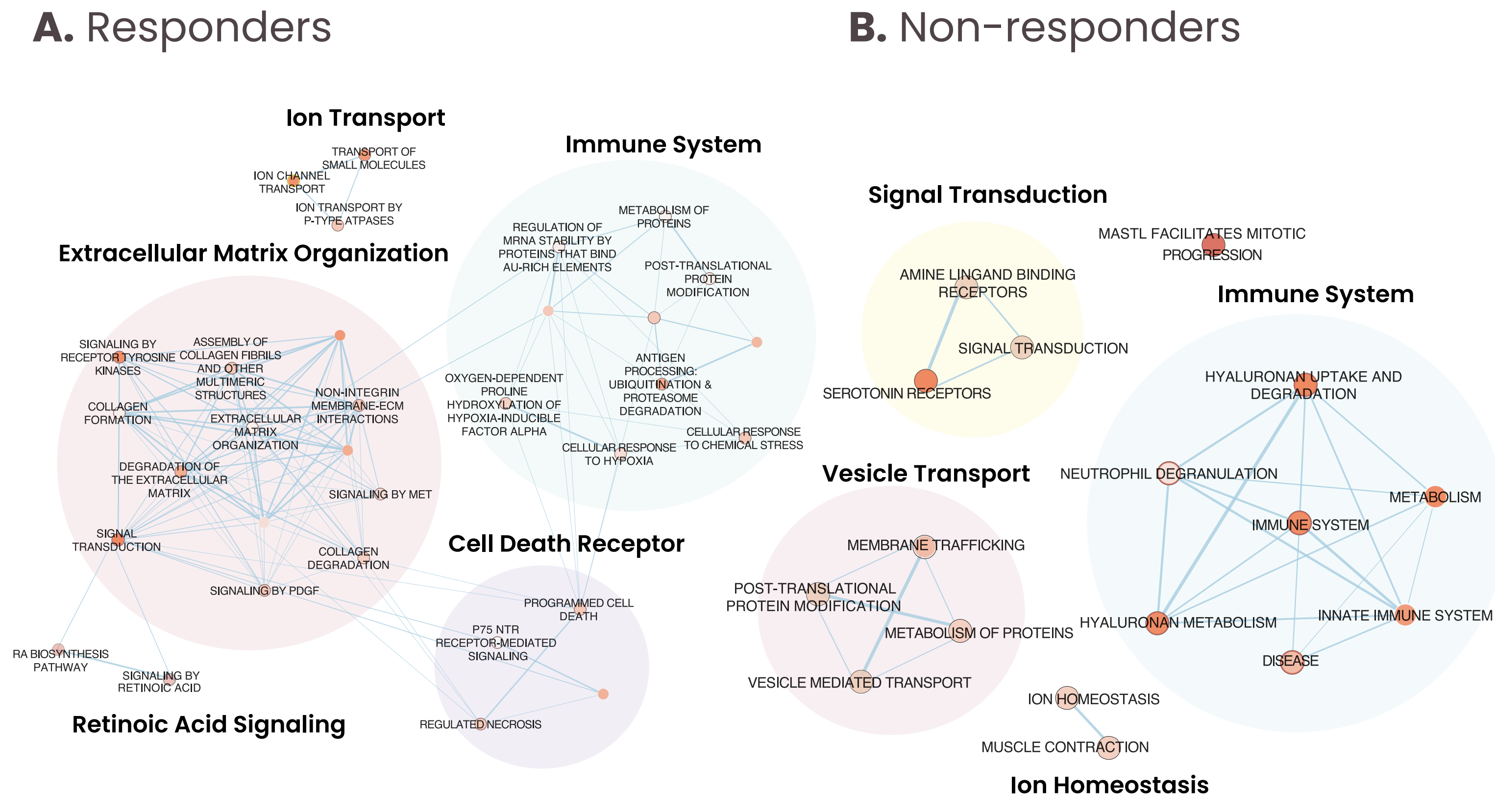


Figure 3. Responders and non-responders exhibit significant differences in cfDNA_{ac} signatures



A-B. Enrichment map of **A.** responder- and **B.** non-responder-specific gene hubs involved in distinct biological processes. Connectivity networks highlight pathway overlaps of genes identified through bootstrapped Kolmogorov-Smirnov tests (171 total genes). Node size represents magnitude of the summary effect size for each gene and is annotated for response association.

Summary

Using a novel active chromatin capture workflow, we identified molecular signatures specific for bladder (BLCA) and non-small cell lung cancer (NSCLC) patients in plasma.

BLCA and NSCLC cancers exhibit a common enrichment of cancer and immune-specific signatures.

Using only the pre-treatment timepoint (T0), 171 features were identified as significantly different between responders vs non-responders.

- In responders, promoters mapped to genes associated with programmed cell death and death receptor response, consistent with the roles of these pathways in immune checkpoint inhibition.^{4,5}
- In non-responders, the promoters mapped largely to genes involved in the immune system and dysregulated hyaluronic acid metabolism, which has been shown to be important in maintain tumor microenvironment and could drive cancer inflammation and immune suppression.^{6,7}
- Longitudinal analysis of a subset of features that exhibited significant differences across response classes revealed potential pharmacodynamic signatures that could offer insights into the underlying mechanisms of action and potential pathways of resistance.

Understanding these changes could provide insights into novel therapeutic approaches that target the tumor microenvironment and the immune interactions and improve diagnostic approaches to guide therapy selection.

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Disclosures

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