Epigenomic Characterization of ER Transcriptional Activation via Liquid Biopsy

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BACKGROUND

Endocrine therapies (ET) are a cornerstone treatment for estrogen receptor-positive (ER+) breast cancer (BC).

ET efficacy depends on the transcriptional addiction of the cancer cells to estrogen receptor signaling, for which there is no current clinical test.

ER IHC and ESR1 mutations are insufficient proxies for ER transcriptional dependency.

A blood-based biomarker for longitudinal measurement of the ER transcriptional dependency of a tumor could greatly improve the ability to identify patients who would most benefit from continued ET and expand the reach of emerging anti-estrogen therapies beyond patients with known ESR1 mutations.

METHODS

We profiled key epigenomic determinants of ER transcriptional activity in a broad array of ER+ BC cell lines, and then translated the resulting epigenomic signature into a form assayable in 1mL of plasma from BC patients.

First, we profiled the epigenome of 8 breast cancer cell lines (MCF7, T47D, BT483, CAMA1, HCC1428, ZR-75-1, MDA-MB-361) in estrogen depleted media (referred to as estrogen deprivation) and in the presence of physiologically relevant levels of estradiol (E2, 0.1nM, referred to as estrogen treatment).

Using ChIP-seq for histone modifications associated with active promoters and enhancers paired with enrichment-based DNA methylation we identified the epigenomic loci most associated with response to E2. The concurrent plating and treatment of these cell lines makes this a unique resource for the identification of enhancers, promoters, and DNA methylation marks associated with transcriptional response to estrogen signaling.

We then employed the Precede multi-analyte liquid biopsy assay, which profiles these same epigenomic features from 1mL of patient plasma and used proprietary computational methods to combine information across analytes to create a patient-specific ER activity quantification algorithm robust to varying levels of circulating tumor DNA fraction (ctDNA%).

We analyzed plasma from 23 patients with ER+ metastatic BC seen at the OHSU Knight Cancer Institute who had blood drawn within 1 week of tumor biopsy with ER and HER2 IHC.

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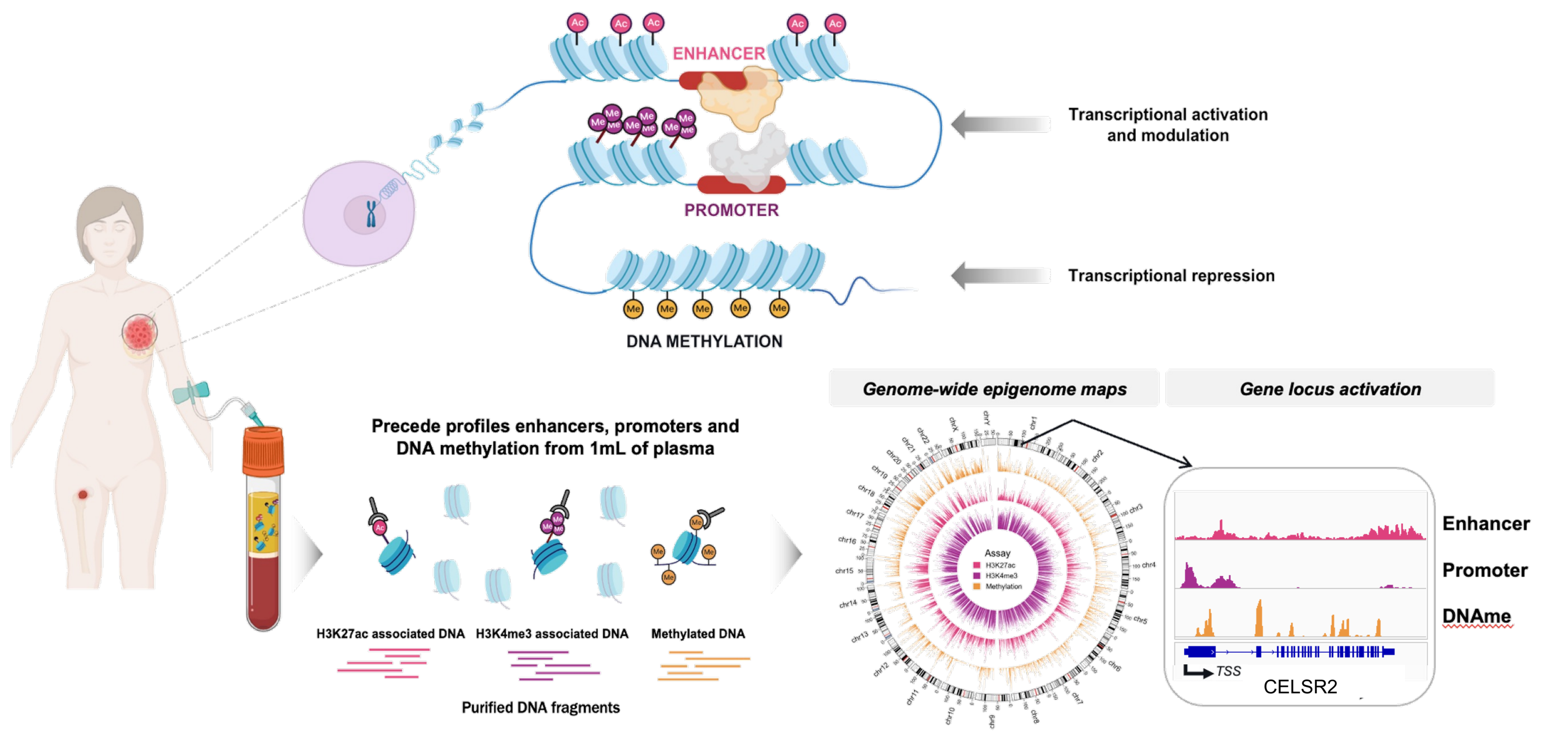
RESULTS

Figure 1. Comprehensive Epigenomic Profiling of Breast Cancer Cell Lines and Patient Plasma

Comprehensive Epigenomic Platform Offers Dynamic Resolution into Target & Pathway Biology from 1mL of Plasma



Cell free DNA derived from tumors exist in circulation as chromatin fragments that faithfully maintain tumor-associated epigenetic modifications on the histones and DNA. Antibodies against active enhancers, active promoters and DNA methylation are used to enrich for associated DNA fragments from 1mL plasma and sequenced to capture the underlying transcriptional state of the tumor cells.[1]

Overview of Profiled Cell Lines

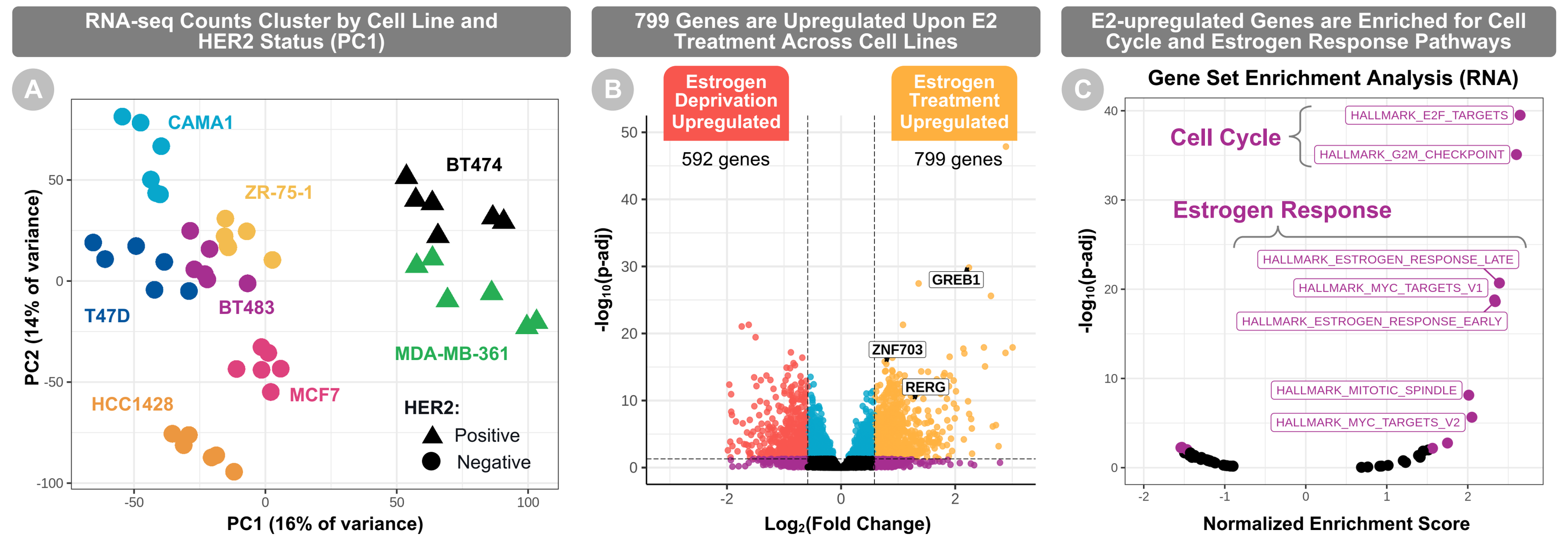
Cell Line Name	ER	PR	HER2	Gene (Function)
MCF7	+	+	-	PIK3CA
T47D	+	+	-	PIK3CA (GoF) TP53 (Likely LoF)
BT483	+	+	-	PIK3CA (GoF)
CAMA1	+	+	-	PTEN (LoF) TP53 (Likely LoF)
HCC1428	+	+	-	
ZR-75-1	+	-	-	PTEN (Likely LoF)
BT474	+	+	+	RIT1(Likely GoF) TP53 (Likely LoF) RHOA (Likely LoF) PIK3CA (Likely GoF)
MDA-MB-361	+	+	+	PIK3CA (GoF)

Assays performed in triplicate: RNA-seq, ChIP-seq for H3K27ac (enhancers) and H3K4me3 (promoters), DNA hypermethylation

Metastatic Breast Cancer Patient Cohort

Total Evaluated (23 Pts)		N=40 samples
ESR1 Mutation	mESR1 wtESR1	9 31
Sample Timepoint	trial enrollment undergoing treatment progression	17 11 12
ER Status	ER+ ER+ (previous biopsy)	37 3
HER2 Status	HER2+ HER2- Not Reported	3 27 10
Median Number of Lines of Metastatic Therapy at Time of Blood Draw (Range)		1 (0-12)
Median Number of Lines of Endocrine Therapy at Time of Blood Draw (Range)		1 (0-7)

Figure 2. RNA-seq Confirms Activation of ER Signaling Pathway upon Estrogen Deprivation/Exposure

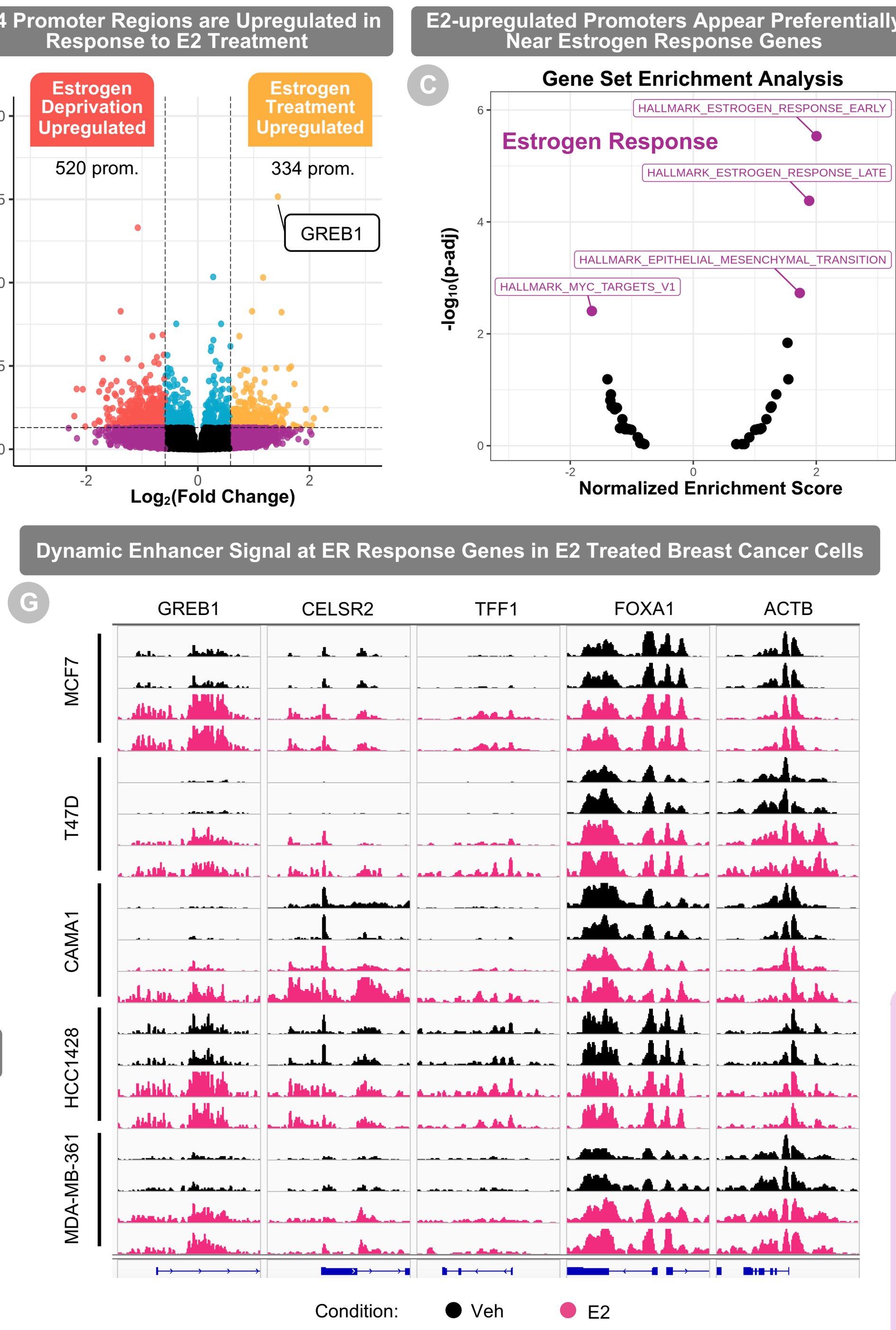


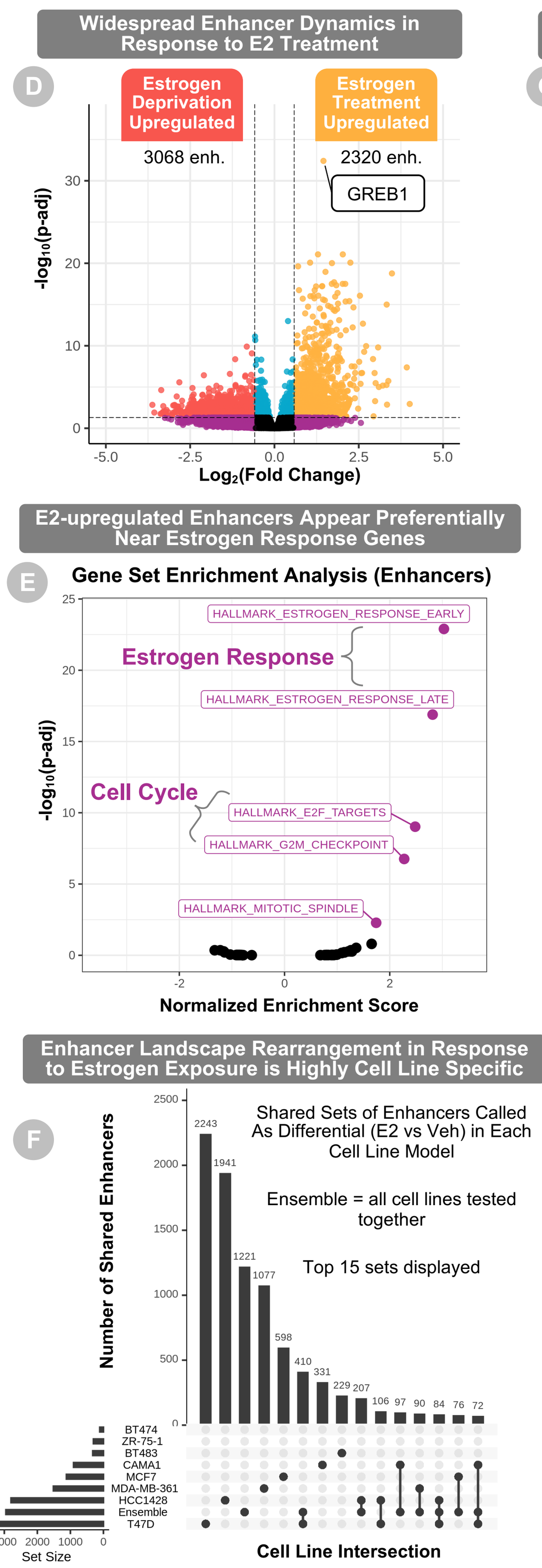
A. Principal component analysis of cell line RNA-seq clusters replicates by cell line and stratifies samples by HER2 status on PC1 axis.

B. Differential expression analysis reveals 799 genes upregulated in estrogen (charcoal stripped media + E2) treatment relative to estrogen deprivation (CSM + vehicle), and 592 genes upregulated in estrogen deprivation relative to estrogen treatment. Genes upregulated in the E2-treated condition include known ER response genes, such as GREB1, ZNF703, and RERG.

C. Gene set enrichment analysis of RNA-seq data reveals expected enrichment of estrogen response gene sets in estrogen exposure conditions. MSigDB Hallmark gene sets tested for enrichment.

Figure 3. Widespread Enhancer Rearrangement Upon Estrogen Deprivation/Exposure





A. No significantly differential DNA methylation sites are evident across 48 hours of estrogen exposure/deprivation conditions.

B. Differential analysis of promoters across estrogen exposure states reveals several hundred differentially modulated promoter regions, including known ER response genes such as GREB1.

C. Gene set enrichment analysis of promoters with differential activity across estrogen exposure states reveals expected enrichment of estrogen response terms in E2 treated condition, in addition to upregulation of EMT pathway genes.

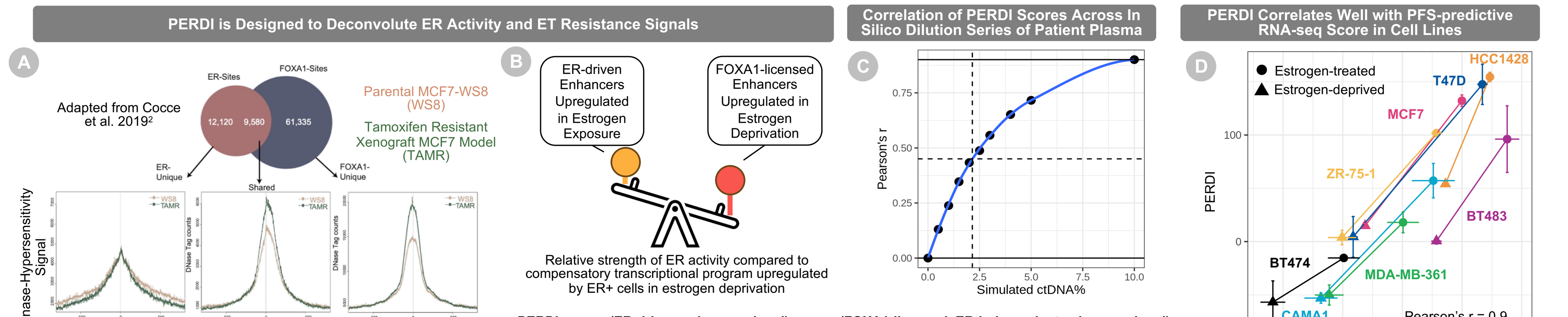
D. Thousands of genome-wide enhancers are rearranged in response to exogenous estrogen exposure including enhancers near known ER response genes like GREB1.

E. Dynamic enhancers appear preferentially near genes involved in estrogen response and cell cycle pathways, which closely resembles the RNA-seq gene set enrichment results performed on the same cells (Figure 2c).

F. Enhancers were tested for differential signal (E2 vs veh) in each cell line individually or an ensemble model capturing all cell lines. Differential enhancers identified were highly cell type specific, highlighting the benefit of analyzing all cell lines to identify the core ER responsive enhancer set likely to translate to patient samples.

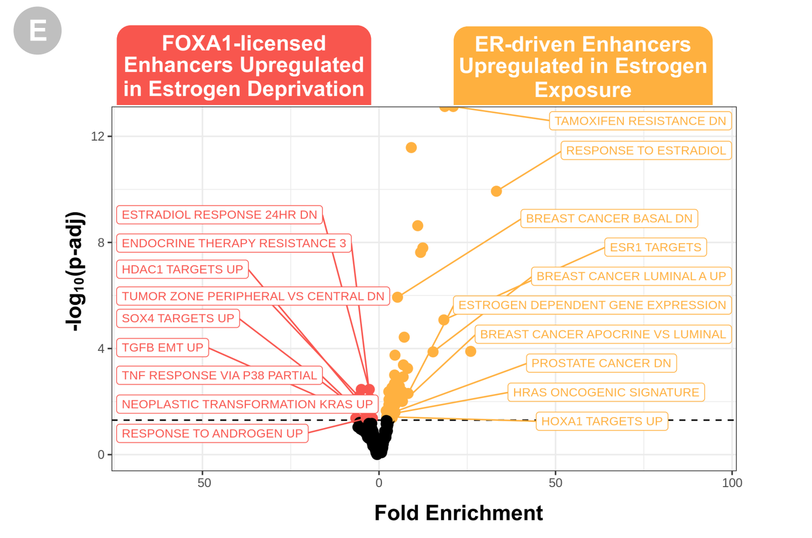
G. Genome browser visualization of dynamic enhancers near known near ER response genes (GREB1, CELSR2, TFF1), a known breast cancer subtype marker gene (FOXA1) and housekeeping gene (ACTB). A subset of cell lines, replicates profiled are displayed.

Figure 4. Precede ER Dependence Index (PERDI): Assessing the Relative Intensity of ER-dependent/independent Signaling via Liquid Biopsy



PERDI = mean(ER-driven enhancer signal) – mean(FOXA1-licensed, ER-independent enhancer signal)

PERDI construction: Subset of dynamic cell line enhancers selected and refined for regions that exhibit tumor-associated signal in ER+ mBC patient plasma and low signal in healthy volunteers.



A. FOXA1-specific binding sites display increased epigenomic signal in tamoxifen resistance (TAMR) model(s). Figure adapted from *Cocci et al*[2].

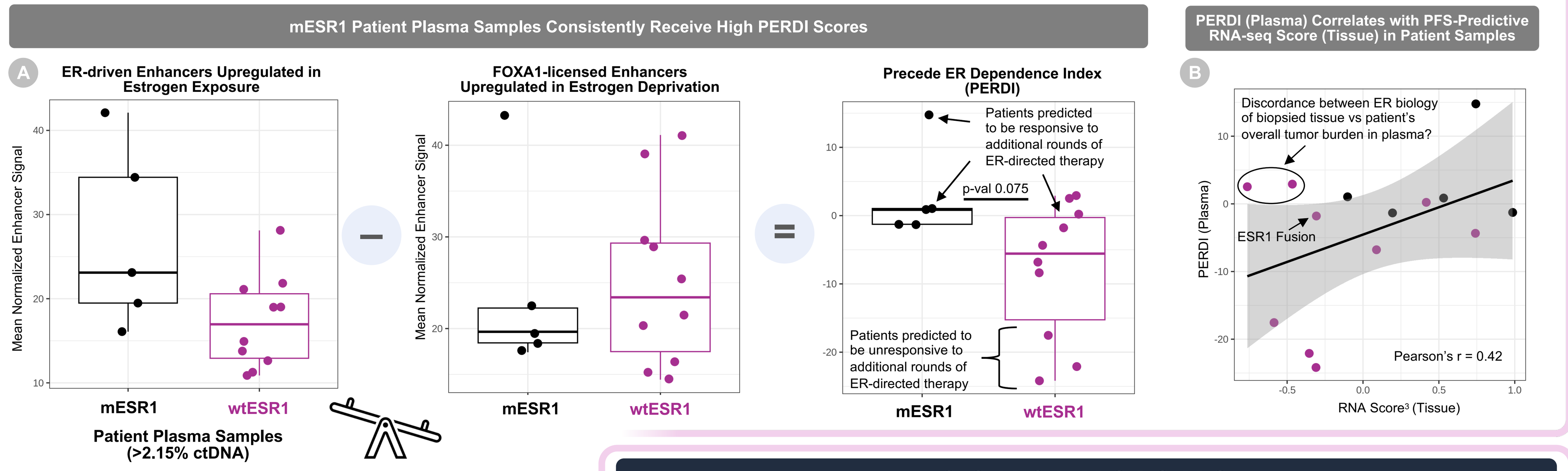
B. Epigenomic liquid biopsy assay offers the ability to segregate ER specific transcriptional activity from FOXA1 dependent activity associated with loss of addiction to endocrine pathway. The Precede ER Degrader Index (PERDI) is informed using ER positive breast cancer patient plasma and cell line experiments, along with previously annotated ER binding sites, FOXA1 binding sites, and genes associated with endocrine therapy resistance.

C. PERDI has a preliminary estimated limit of quantification (LOQ) of 2.15% ctDNA as assessed by concordance of score along *in silico* dilution series of patient plasma samples. Additional score refinement will aim to (1) aggregate signal from additional analytes / analytical methods to lower LOQ, and (2) determine appropriate LOQ threshold by quantifying enrichment of patients which respond to ER-directed therapies.

D. PERDI correlates well with RNA-seq based score for ER activity adapted from *Guan et al*[3], which is predictive of PFS response to Giridescent. Each point represents one of 8 ER+ cell lines grown in E2 supplemented (circle) and charcoal stripped (triangle) media.

E. Enhancers quantified by PERDI appear preferentially near genes of pathways relating to ER response and various ET resistance mechanisms. Select gene sets enriched near the two classes of enhancers constituting PERDI are labeled.

Figure 5. PERDI correlates with Clinically Relevant Biomarkers of ER Activity in Patient Plasma Samples



A. Mutant ESR1 patient plasma samples consistently display elevated ER-dependent transcriptional activity and lower FOXA1-dependent (ER-independent) transcriptional activity, resulting in consistently elevated PERDI scores.

B. PERDI trends with the PFS predictive RNA-seq score from *Guan et al*[3] in patient plasma samples. Samples where the two measures disagree merit further investigation into whether discordance is a result of score construction or difference between biology of biopsy site vs patient's overall tumor burden.

CONCLUSIONS

Epigenomic re-programming of ER transcriptional pathway activation is detectable by the Precede comprehensive epigenomic liquid biopsy assay, enabling a measurement of breast cancer dependence on endocrine signaling from 1mL of plasma through an ER dependence index. This ER dependence index is biologically interpretable and correlates with previous RNA-seq based predictors of ER activity. While ESR1 mutant patients score consistently high, a subset of ESR1 wildtype patients also demonstrate high ER dependence indices, implying a potentially expanded patient population for treatment with novel anti-endocrine agents such as SERDs.

References

1. Baca, S.C., Seo, JH., Davidsohn, M.P. *et al.* Liquid biopsy epigenomic profiling for cancer subtyping. *Nat Med* **29**, 2737–2741 (2023).
2. Cocce KJ *et al*. The Lineage Determining Factor GRHL2 Collaborates with FOXA1 to Establish a Targetable Pathway in Endocrine Therapy-Resistant Breast Cancer. *Cell Rep*. **29**, (2019)
3. Guan J, *et al*. Therapeutic Ligands Antagonize Estrogen Receptor Function by Impairing Its Mobility. *Cell*. **178** (2019)