Abstract 47P

SYROS

Epigenomic analysis of primary breast cancer tumors reveals novel tumor cell vulnerabilities and therapeutic targets

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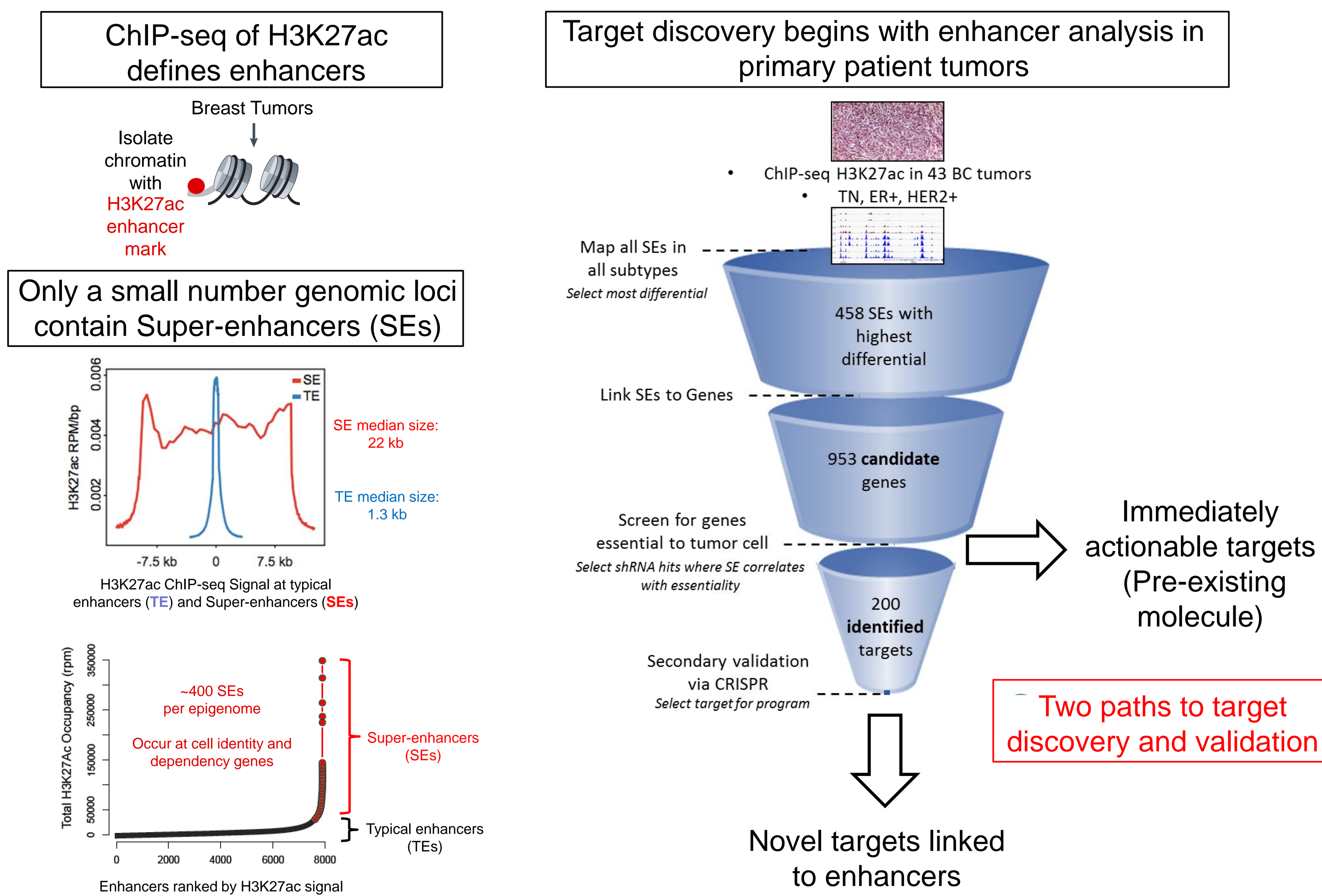
Abstract

To date, a large portion of cancer research has focused on somatic mutations in protein coding regions to identify putative oncogenic drivers. Here, we have investigated the roles of genomic non-coding regions in defining oncogenic cell state drivers and pinpointing novel druggable targets. Abnormally large clusters of cis-acting enhancers, called super-enhancers (SEs), have emerged as regulatory features of oncogenes and other key tumor drivers in cancer cells. Mapping these features through H3K27ac ChIP-seq in primary patient samples and linking them to protein-coding genes provides an inroad to identify novel dependencies and new therapeutic targets in cancer.

We have analyzed 43 primary breast cancer patient samples using H3K27ac ChIP-seq to map enhancers and SEs genome-wide. We find that these SE maps pinpoint known oncogenic drivers and recapitulate established clinical subgroups: Most samples classified as HER2+ contain a SE at the *HER2* locus, most samples classified as ER+ contain a SE at the *ESR1* locus while neither tend to appear in TNBC samples. These findings strengthen the hypothesis that SE analysis can be used to discover breast cancer dependencies *de novo*, independent of somatic mutations. In order to validate novel targets that were revealed by SEs in primary patient samples, we used both CRISPR-mediated gene ablation as well as chemical validation in a panel of cell lines that either exhibit or do not exhibit the gene-associated SEs. Using the chemical validation approach, we identified a SE at the *RARA* locus that predicts sensitivity to a potent RARα agonist (SY-1425) in a panel of breast cancer cell lines. The sensitivity of these cell lines to SY-1425 is correlated with enhancer size, identifying RARα as an enhancer-correlated vulnerability in breast cancer. We show that this correlation extends to *in vivo* xenograft models. Using the CRISPR-mediated validation approach, we discovered a number of novel targets that were identified by their association with SEs in primary samples and then validated in a panel of breast cancer cell lines.

Together, these studies indicate that super-enhancer analysis in primary patient samples can be used to define new biomarker-linked breast cancer vulnerabilities for therapeutic intervention.

Methodology: Super-enhancers (SEs) define key cancer drivers



Super-enhancer (SE) analysis of breast cancer tumors

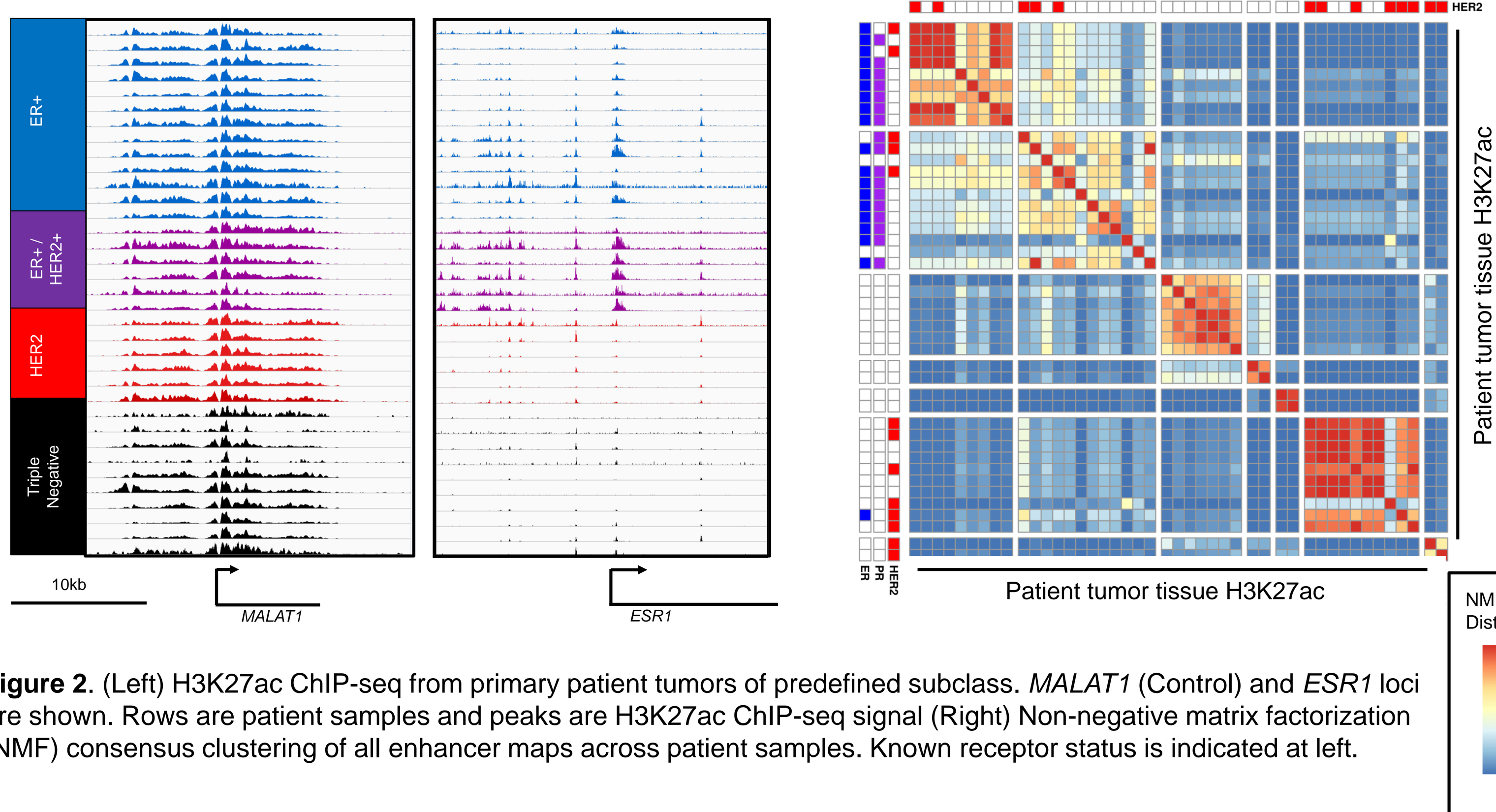


Figure 2. (Left) H3K27ac ChIP-seq from primary tumors of predefined subclass. *MALAT1* (Control) and *ESR1* loci are shown. Rows are patient samples and peaks are H3K27ac ChIP-seq signal (Right) Non-negative matrix factorization (NMF) consensus clustering of all enhancer maps across patient samples. Known receptor status is indicated at left.

CRISPR-mediated validation of target genes

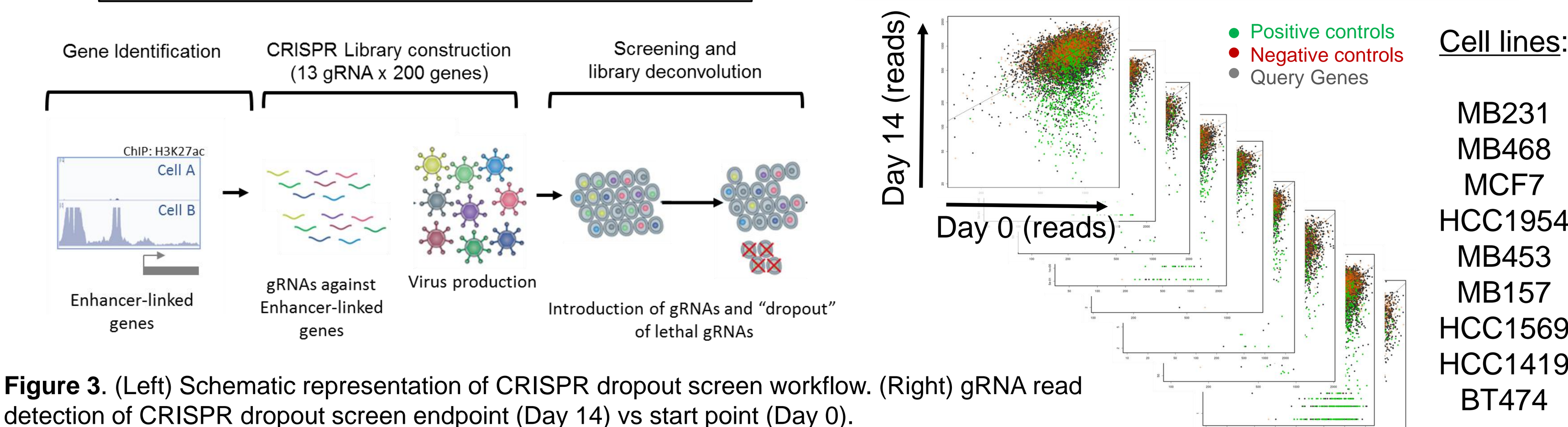


Figure 3. (Left) Schematic representation of CRISPR dropout screen workflow. (Right) gRNA read detection of CRISPR dropout screen endpoint (Day 14) vs start point (Day 0).

Novel breast cancer targets linked to patient enhancers

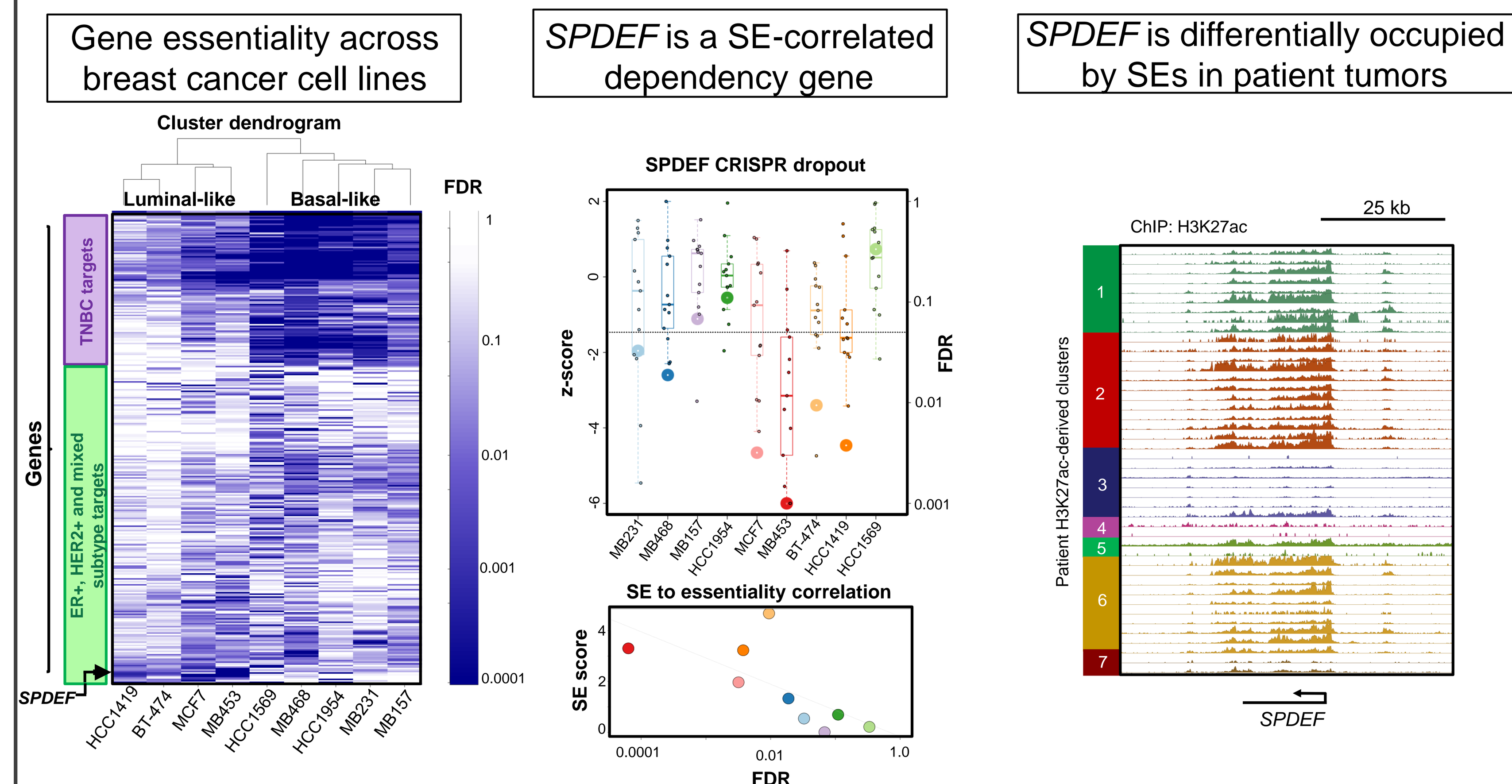


Figure 4. (Left) Heat map of gene essentiality in breast cancer cell lines. Gene essentiality expressed as False Discovery Rate (FDR). (Center, Top) 14-day CRISPR gRNA dropout of *SPDEF*-targeted gRNAs in breast cancer cells. (Center, Bottom), Enhancer correlation with *SPDEF* essentiality across breast cancer cells. (Right) H3K27ac ChIP-seq profiles at the *SPDEF* locus in primary patient tissue.

An immediately actionable enhancer-linked target: RARα

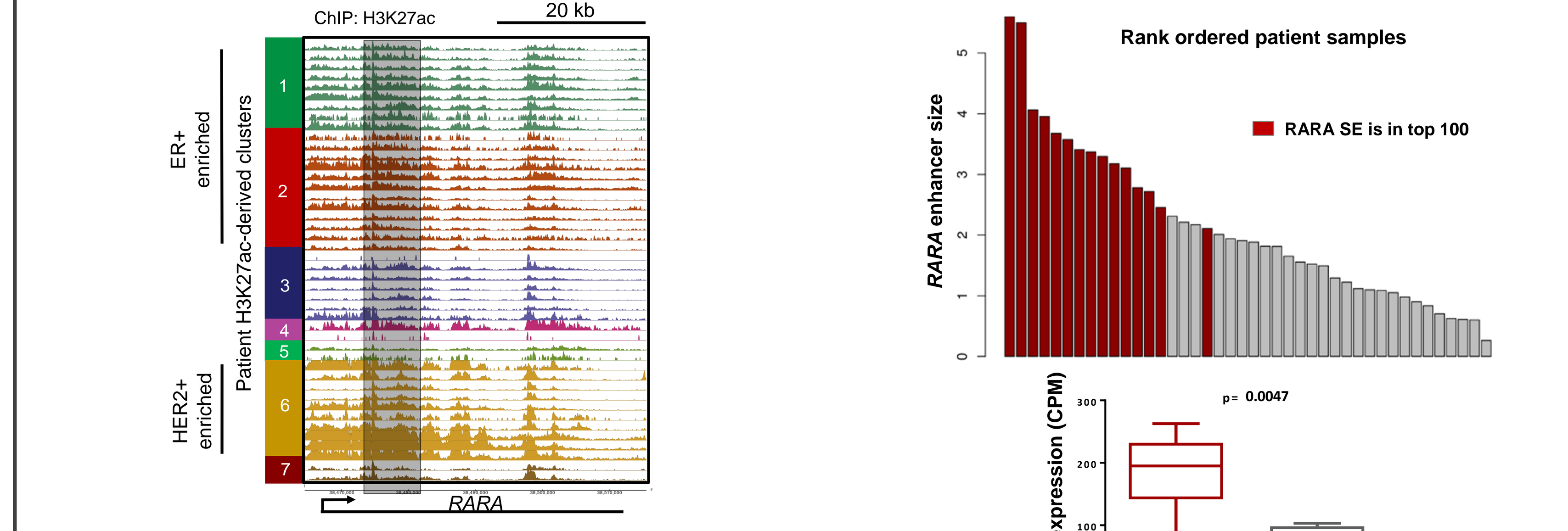


Figure 5. (Left) H3K27ac ChIP-seq profiles at the RARA locus in primary patient tissue. Shaded area is patented biomarker. (Right) RARA enhancer size across patient tissues and relationship to RNA expression

RARα agonist SY-1425 inhibits tumor cell proliferation

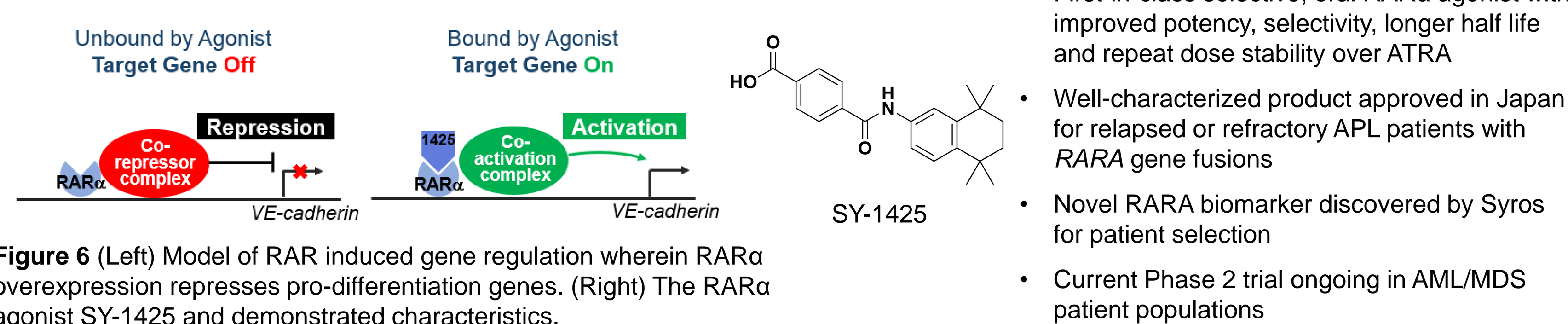


Figure 6 (Left) Model of RAR induced gene regulation wherein RARα overexpression represses pro-differentiation genes. (Right) The RARα agonist SY-1425 and demonstrated characteristics.

- First-in-class selective, oral RARα agonist with improved potency, selectivity, longer half life and repeat dose stability over ATRA
- Well-characterized product approved in Japan for relapsed or refractory APL patients with *RARA* gene fusions
- Novel RARA biomarker discovered by Syros for patient selection
- Current Phase 2 trial ongoing in AML/MDS patient populations

SY-1425 anti-proliferative effect is highly dependent on *RARA* enhancer strength

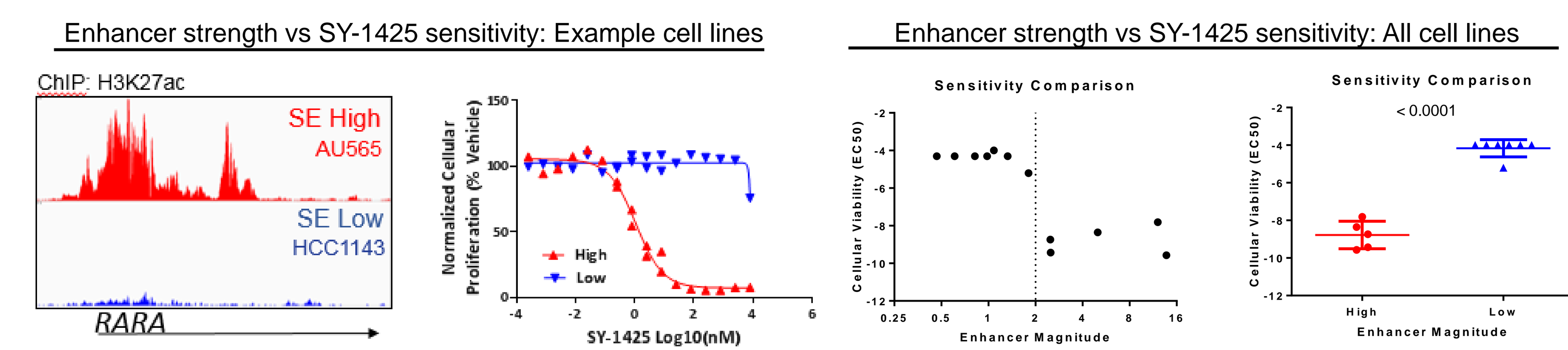


Figure 7. (Left half) H3K27ac ChIP-seq enrichment in breast cancer cells AU565 and HCC1143 and cell proliferation in response to SY-1425. (Right half). SY-1425 growth inhibition in 12 breast cancer cell lines containing High or Low SE at RARA locus.

SY-1425 inhibits tumor growth in *RARA*-high models resistant to SOC

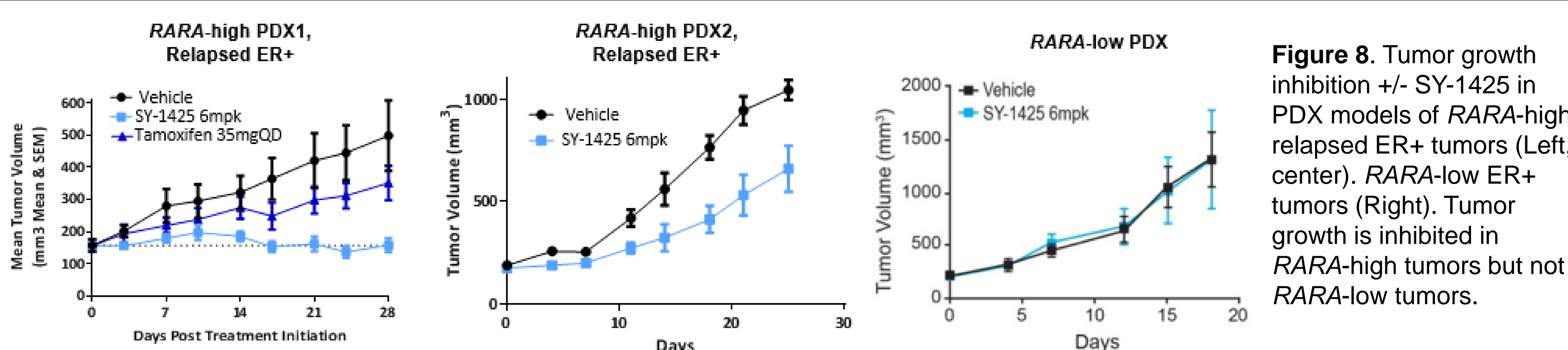


Figure 8. Tumor growth inhibition +/- SY-1425 in PDX models of *RARA*-high relapsed ER+ tumors (Left, center). *RARA*-low ER+ tumors (Right). Tumor growth is inhibited in *RARA*-high tumors but not *RARA*-low tumors.

Conclusions

- Super-enhancer analysis reveals both SE-linked novel drug targets and novel patient subsets for existing drugs in breast cancer
- SE analysis in primary breast cancer samples revealed at least 14 potential new drug targets in TNBC, including 8 enzymes, 2 surface receptors, 1 signaling protein and 1 metabolism protein
- The RARα agonist SY-1425 induces an anti-proliferative response in *RARA*-SE high breast cancer cell lines and slows tumor growth in *RARA*-high (not *RARA*-low) PDX models of breast cancer
- The use of a patient-selection biomarker, effectiveness of SY-1425 in preclinical models of breast cancer, and prior human experience of SY-1425 may provide a new therapeutic approach for breast cancer patients

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