Epigenetic therapy suppresses endocrine-resistant breast tumour growth by re-wiring ER-mediated 3D chromatin interactions

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One line summary: Epigenetic therapy suppresses tumour growth in metastatic ER+ breast cancer via DNA hypomethylation-induced rewiring of long-range ER-mediated enhancer-promoter chromatin interactions to activate ER target genes involved in repression of proliferation and the cell cycle.

Keywords
3D genome architecture; DNA methylation; Decitabine, Hi-C; epigenetic therapy; ER+ breast cancer; endocrine-resistance; transcription factors; enhancers
Abstract

Three-dimensional (3D) epigenome remodelling is emerging as an important mechanism of gene deregulation in cancer. However, its potential as a target to overcome cancer therapy resistance remains largely unaddressed.

Here we show that treatment of endocrine-resistant estrogen receptor positive (ER+) breast cancer with an FDA-approved epigenetic therapy Decitabine (5-Aza-mC), results in genome-wide DNA hypomethylation and suppression of tumour growth in preclinical metastatic ER+ breast tumour xenograft models. Systematic integration of matched chromatin conformation capture (Hi-C), Promoter Capture Hi-C, RNA-seq and ER ChIP-seq data revealed widespread effects on epigenome deregulation, including de-compaction of higher order chromatin structure and loss of topologically associating domains (TAD) boundary insulation. Key enhancer ER binding sites were demethylated and re-activated after Decitabine treatment, resulting in new ER-mediated enhancer-promoter interactions and concordant activation of tumour-suppressive gene pathways. Importantly, we show that the activated ER target genes were also predictive of good outcome in multiple ER+ breast cancer clinical cohorts.

Together our study reveals a previously undescribed mechanism of Decitabine in rewiring DNA methylation-dependent 3D genome architecture resulting in suppression of tumour growth, and highlights the potential of epigenetic therapy in targeting ER+ endocrine-resistant breast cancer.
Main

Around 70% of breast cancers are driven by the Estrogen Receptor-alpha (ER). ER is a critical ligand-activated transcription factor that controls breast cancer cell proliferation and tumour growth upon exposure to estrogenic hormones\(^1\). Drugs that target ER pathways are highly effective in the treatment of ER+ breast cancer\(^2,3\), however de novo or acquired resistance to these agents (endocrine resistance) affects a large proportion (>30%) of patients and is the major cause of breast cancer mortality. Therefore, it is critical to understand mechanisms associated with endocrine resistance to provide new therapeutic interventions that prevent or overcome the resistant phenotype for ER+ breast cancer patients.

Genome-wide ER chromatin binding patterns, called cistromes, are associated with ER-mediated transcriptional activity in endocrine-sensitive and endocrine-resistant breast cancer contexts\(^1\). ER chromatin binding is primarily located at distal regulatory elements and putative enhancer regions\(^4-8\). Previous studies carried out in endocrine-resistant cell lines have demonstrated that differential ER binding is a key mechanism that drives clinical response to endocrine therapies\(^4,9\) and importantly, this endocrine resistance can be characterised by epigenetic reprogramming of enhancer elements occupied by ER in the endocrine sensitive state\(^10\). Epigenetic reprogramming involves altered DNA methylation of enhancers that regulate ER-responsive genes and represents a complementary mechanism that may drive primary and acquired endocrine resistance. Enhancer hypermethylation associated with endocrine resistance occludes ER recruitment, which results in transcriptional reprogramming of ER signalling and estrogen independence\(^10\).

Gene expression is highly regulated by (3D) genome architecture, from the local level of chromatin compaction to the higher level organisation of topological associated domains (TADs) and chromosome compartments\(^11\). Alterations to the 3D genome architecture have been described in a number of different cancers, including prostate cancer\(^12,13\), breast cancer\(^14-18\), gliomas\(^19\), and several hematologic cancers\(^20,21\). Although cancer cells maintain the general pattern of 3D genome folding, distinctive structural changes have been described in cancer genomes at all of levels of 3D organisation\(^22\). We and others recently reported that 3D genome structure is also disrupted in endocrine-resistant ER+ breast cancer cells\(^17,18,23\), notably through long-
range chromatin changes at ER-enhancer binding sites that are DNA hypermethylated in resistant cells\textsuperscript{17}. These key findings suggest that DNA hypermethylation promotes both reprogramming of the ER cistrome and 3D genome disorganisation, leading to altered ER transcriptional activity in ER+ endocrine resistant breast cancer.

DNA demethylating agents such as Decitabine (5-aza-2’-deoxycytidine, DAC) have emerged as a promising therapeutic strategy for treating various cancers\textsuperscript{24}. Decitabine is approved by many international regulatory agencies, including the US FDA and the European Commission (EC), for treating haematological cancers\textsuperscript{24}. In solid cancers (including colorectal and ovarian cancer), Decitabine has been shown to demethylate regulatory regions that result in re-activation of tumour suppressor genes\textsuperscript{25,26}. Additionally, treatment with DNA de-methylating agents has been shown to stimulate immune response pathways in cancer cells through increased transcription of DNA repeat elements, which induces a viral mimicry response\textsuperscript{27-29}. However, the direct effect of these epigenetic drugs on the tumour cells, including epigenome and 3D genome structure, remains largely unexplored, especially in clinically relevant patient-derived model systems or clinical samples.

To test the therapeutic efficacy of epigenetic therapy with Decitabine in endocrine-resistant ER+ patient-derived xenograft (PDX) models we assessed the molecular consequences of treatment on DNA methylation, 3D genome architecture and transcriptional pathways. Our data revealed that Decitabine treatment caused an inhibition of endocrine-resistant tumour growth due to 3D epigenome remodelling and re-activation of ER-driven tumour-suppressive gene pathways, highlighting the potential of epigenetic therapy in treatment of ER+ endocrine-resistant breast cancer.
Results

Low-dose Decitabine inhibits tumour growth and decreases cell proliferation

To study the efficacy of epigenetic therapy in the context of endocrine-resistant ER+ breast cancer and to establish its impact on the 3D genome and epigenome, we used patient-derived xenograft (PDX) models (Gar15-13, HCI-005) (Fig. 1a). Gar15-13 and HCI-005 PDXs were derived from the metastases of two ER+ patients who had disease progression following one or more lines of endocrine therapy and have been used for several pre-clinical studies. Using a low, well-tolerated and non-cytotoxic dose (0.5mg/kg; Extended Data Fig. 1a) of Decitabine, we first interrogated the anti-cancer effect of epigenetic therapy on tumour growth. Following tumour implantation and initial period of growth (to a volume of 150-200 mm$^3$), mice were randomized to twice-weekly injections of PBS (Vehicle) or 0.5mg/kg Decitabine. Treatment continued with twice-weekly measurement of tumour volume for 35 days or until tumour volume exceeded 1000 mm$^3$. At endpoint mice were sacrificed and tumour material collected for analysis. In both Gar15-13 and HCI-005 PDX models, Decitabine treatment elicited a strong growth inhibitory response (Fig. 1b, c) and a significant reduction in the proliferative index as measured by Ki-67 positivity at endpoint (Fig. 1d).

Decitabine induces widespread DNA hypomethylation

To directly identify the epigenetic reprogramming events induced by Decitabine treatment, we first analysed genome-wide DNA methylation data generated using Infinium EPIC Methylation arrays. EPIC arrays were performed on four biological replicates of Vehicle and Decitabine-treated PDX tumours at end-point. All Decitabine-treated tumours exhibited genome-wide DNA methylation loss (Fig. 1e, f and Extended Data Fig. 1b, c, $P$ value < 0.0001, two-tailed Mann-Whitney test), with Gar15-13 showing more hypomethylation compared to HCI-005 (Fig. 1e and Extended Data Fig. 1d) (average 14.55% and 8.74%, respectively). To quantify the extent of DNA methylation loss, we identified differentially methylated probes (DMPs) and differentially methylated regions (DMRs) between Vehicle and Decitabine-treated tumours (Supplementary Table 1) and found that in Gar15-13, Decitabine treatment resulted in 279,908 hypomethylated DMPs, which were encompassed by 71,677 DMRs (total size >65Mb, mean size = 898.8, SD = 918.4).
Hypomethylated DMPs were mainly located at non-coding genomic regions (introns and intergenic) (Extended Data Fig. 1e, f) and significantly enriched at putative enhancer regions (Fig. 1g and Extended Data Fig. 1g) ($P < 0.001$, permutation test). In agreement, we observed extensive DNA hypomethylation at enhancers in Gar15-13 tumours (approx. 21.58%; Extended Data Fig. 1h, $P$ value $< 0.0001$, two-tailed Mann-Whitney test), while promoters were less demethylated (approx. 10.12%; Extended Data Fig. 1i, $P$ value $< 0.0001$, two-tailed Mann-Whitney test).

We also evaluated genome-wide DNA methylation levels at different classes of transposable elements (TEs) using the REMP package\textsuperscript{35}, including LTRs, LINE1 elements and Alu elements. We observed genome-wide loss of DNA methylation at all repeat element sub-groups (Extended Data Fig. 1j and Extended Data Fig. 1k) with ~12% loss of DNA methylation in Decitabine treated tumours. Additionally, we observed TE expression alterations and activation of anti-viral signalling (Supplementary Note), as previously observed with Decitabine treatment in other cancers\textsuperscript{27,28}. Notably, although all TE classes showed some degree of DNA methylation loss, the extent of DNA hypomethylation measured at repeat elements was less than those observed genome-wide and significantly less compared to DNA hypomethylation at enhancer regions (Extended Data Fig. 1h).

**Decitabine-induced DNA hypomethylation reprograms the ER cistrome**

To examine the transcriptional consequences of Decitabine treatment in PDX tumours (Gar15-13 and HCI-005), we next analysed RNA-seq data, followed by gene set enrichment analysis (GSEA)\textsuperscript{36} of differentially expressed genes. We found in the ER+ endocrine-resistant PDX models that Decitabine treatment also negatively correlated with gene signatures of cell proliferation and cell cycle (E2F targets, G2M checkpoint and Myc targets; for example $SMC3$, $TK1$ and $KIF20A$) (Extended Data Fig. 2a, b), consistent with suppressed tumour growth and decreased cytological proliferation markers observed in both models. In Gar15-13 tumours, Decitabine treatment also enriched for multiple hallmark genesets related to hormone signalling (estrogen response early and late, androgen response) (Fig. 2a, Extended Data Fig. 2c). Notably, Decitabine treatment resulted in up-regulation of a large proportion of genes belonging to the Hallmark Estrogen Response genesets (Extended Data Fig. 2c).
2d), including multiple co-regulators of ER (for example CD44, PGR, KRT8 and KRT18).

To further characterise the effect of Decitabine on estrogen signalling, we profiled ER binding site (ERBS) patterns genome-wide in Vehicle (n = 4) and Decitabine-treated Gar15-13 (n = 4) tumours via ER ChIP-seq. Differential binding analyses (Diffbind\(^4\)) revealed reprogramming of the ER cistrome characterised by 1095 gained ERBS and 279 lost ERBS following Decitabine treatment as compared to Vehicle (FDR < 5%) (Fig. 2b). Since FOXA1 is a critical ER pioneer factor required for ER to bind DNA\(^37,38\), we also assessed the FOXA1 cistrome and found stronger FOXA1 binding at ERBS in Vehicle samples that were lost upon Decitabine treatment, while Decitabine induced ERBS had weaker FOXA1 binding (Fig. 2b). We then mapped the ER binding intensity at ERBS reprogrammed by Decitabine. As expected, gained ERBS had a stronger average ER ChIP-seq signal in Decitabine samples as compared to Vehicle, while lost sites showed a moderate decrease in binding intensity genome-wide (Fig. 2c).

We next investigated the genomic distribution of gained and lost ER binding sites by analysing the RefSeq annotation and ChromHMM state. Remarkably, over 75% of gained ERBS were located at distal regions associated with active and poised enhancers (Fig. 2d and Extended Data Fig. 2e), and these sites were enriched for the estrogen response element (ERE) DNA motif (Fig. 2d). Lost ERBS were most frequently positioned close to a TSS (> 40% less than 1Kb from TSS) (Extended Data Fig. 2e), were associated with active promoters (Fig. 2e) and were enriched for Sp1 and NFY DNA response elements at promoter DNA motifs (Fig. 2e). Both lost and gained ERBS were highly enriched for the FOXA1 motif.

Notably, we found that Decitabine-induced ERBS were significantly enriched for ERBS found in endocrine-sensitive MCF7 cells (Extended Data Fig. 2f, h). This suggests that Decitabine treatment of endocrine-resistant tumours may lead to reprogramming of the ER cistrome toward an endocrine-sensitive ER binding pattern of the primary tumour. To further elucidate this hypothesis, we interrogated ER ChIP-seq data from two public clinical ER-ChIP-seq datasets of ER+ breast tumours. These included 15 primary ER+ breast cancer patient tumours ((GSE32222)\(^4\),

\[a\] \[b\] \[c\] \[d\] \[e\] \[f\] \[g\] \[h\] \[i\] \[j\] \[k\] \[l\] \[m\] \[n\] \[o\] \[p\] \[q\] \[r\] \[s\] \[t\] \[u\] \[v\] \[w\] \[x\] \[y\] \[z\]
We found that ER binding in primary tumours was significantly enriched for Decitabine gained ERBS ($P$ value < 0.001; permutation test) and not enriched for Decitabine lost ERBS (Extended Data Fig. 2g, h).

Finally, we investigated whether the DNA hypomethylation observed with Decitabine treatment was directly associated with reprogramming of the ER cistrome. We examined the DNA methylation levels of EPIC probes overlapping gained and lost ERBS. Gained ERBS showed an overall decrease (approx. 44.4%) in DNA methylation (Fig. 2f and Extended Data Fig. 2i). Figure 2g shows a representative example at the ANKRD2 gene enhancer regions where a gain in ER binding in Decitabine-treated tumours is associated with loss of DNA methylation (Fig. 2g). A further example of the BTBG9 gene locus is included in Extended Data Fig. 2j. In contrast the small proportion of lost ERBS remained unmethylated in both Vehicle and Decitabine treated samples (approx. 6.92%; Extended Data Fig. 2k), suggesting that this subset of ERBS were lost independently of a direct change in DNA methylation.

Together, these results indicate that Decitabine-induced DNA hypomethylation stimulates expression of estrogen response genes via ER cistrome and subsequent transcriptional reprogramming as a putative mechanism of response.

**Loss of DNA methylation results in de-compaction of chromatin and alters TAD boundary insulation**

Distinctive structural changes have been described in cancer genomes at all levels of 3D organisation. We were therefore motivated to study if genome-wide DNA hypomethylation induced by Decitabine may also lead to global changes in 3D genome architecture. We analysed *in situ* Hi-C data corresponding to three biological replicates of Vehicle and Decitabine-treated Gar15-13 PDX tumours (Supplementary Note). First, to detect open (active) and closed (silent) genomic compartments (A and B, respectively) we performed PCA analysis of the Hi-C data as described. Comparison of eigenvalues between samples revealed differences between Vehicle and Decitabine tumours as shown by reduced correlation (Extended Data Fig. 3a). To further understand these differences, we directly compared the eigenvalues between Vehicle and Decitabine-treated tumours and observed that while most bins retained
the same compartment status between the samples (either A to A or B to B), a large number of bins became more A-type in Decitabine as compared to Vehicle (B to A switch) (Fig. 3a). We next quantified this switching (see Methods) and identified 643 compartments (i.e. 2.28% of all compartments) that switched their assignment between A and B (Extended Data Fig. 3b). Around 64% of the changes induced by Decitabine involved compartment activation (B-type to A-type) (Fig. 3b). Notably, we observed significant DNA hypomethylation at B to A switches, while A to B switching regions maintained similar DNA methylation levels to Vehicle samples (Fig. 3c). Furthermore, in agreement with B-compartments becoming more de-methylated and active with Decitabine, we detected an overall increase in expression of genes located at regions that switched their assignment from B to A in Decitabine-treated tumours, while genes located at A to B switching compartments did not significantly change expression (Extended Data Fig. 3c). The newly activated compartments hosted 21 genes that decreased in expression and 87 genes that increased in expression with Decitabine (Extended Data Fig. 3d, e). Furthermore, we quantified A–A and B–B interaction frequencies in Decitabine and Vehicle-treated tumours and found decreased interaction strength between closed compartments (B-B interactions; \( P = 0.025 \), two-tailed Students t-test), increased contacts between active compartments (A-A interactions; \( P = 0.26 \), two-tailed Students t-test) and increased contacts between A-B compartments (\( P = 0.011 \), two-tailed Students t-test) (Fig. 3d, e and Extended Data Fig. 3f, g).

Given that we observed DNA hypomethylation to be functionally associated with changes to higher-order 3D genome organisation, we next investigated the impact of Decitabine treatment on the organisation of topologically associated domains (TADs). We observed a significant decrease in the average insulation score in Decitabine as compared to Vehicle-treated tumours (~36.53 in Decitabine and ~46.74 in Vehicle) (Fig. 3f and Extended Data Fig. 3h, \( P \) value < 0.0001, two-tailed Mann-Whitney test), suggesting that loss of DNA methylation is associated with reduced segmentation into TADs. Consistent with loss of TAD boundaries and potential merging of TADs, the total number of TADs was decreased in Decitabine-treated samples (Fig. 3g) and their corresponding average domain size increased (Extended Data Fig. 3i; \( P = 0.0289 \), t-test). Analysis of differential TAD boundaries revealed a large percentage (43.2%) of Vehicle-specific boundaries, which were lost in Decitabine tumours (Fig. 3h) and
characterised by decreased average insulation score in Decitabine tumours (Extended Data Fig. 3j). We exemplify one such region, where a TAD was lost in Decitabine-treated tumours, concomitant with loss of boundary insulation (Fig. 3i; further examples in Extended Data Fig. 3k-l).

Together, these results indicate that DNA hypomethylation induced by Decitabine treatment in vivo leads to significant de-compaction of chromatin, with reduced B-type compartments, increased interactions within A-type compartments and concomitant increase in regional gene expression. While most TADs maintain their structure after Decitabine treatment, their boundaries become less insulated suggesting increased intra-tumour heterogeneity in TAD structure and loss of some TAD boundaries at the regions of chromosomal compartment de-compaction.

Loss of DNA methylation rewires long-range promoter-anchored enhancer interactions

Since we observed that regulatory elements were significantly hypomethylated with Decitabine treatment (Extended Data Fig. 1h), we next asked if there were alterations in finer-scale 3D long-range interactions. To gain insights into chromatin interactions at the level of individual promoters and enhancers, we investigated genome-wide promoter-anchored contacts in three Decitabine and three Vehicle-treated tumours using Promoter Capture Hi-C (PCHi-C) (Supplementary Note). Hi-C libraries were hybridised to custom-designed genomic restriction fragments covering 23,711 annotated gene promoters, achieving a clear increase in the coverage of promoter-anchored interactions as compared to Hi-C (Fig. 4a). To identify statistically significant interactions between promoters and other regulatory elements from the PCHi-C data, we used the CHiCAGO pipeline⁴¹. Promoter (bait) regions were accordingly significantly enriched for active promoters, poised promoters and active enhancers ChromHMM states in both Vehicle and Decitabine-treated tumours (Fig. 4b). Notably, putative enhancer other-end interacting regions (i.e. enhancer OEs; exemplified in Fig. 4a) showed significant differential enrichment whereby active promoters were preferentially enriched in Vehicle tumours and enhancers were preferentially enriched in Decitabine-treated tumours, especially active enhancers (Fig. 4b). This suggests that long-range interactions are rewired from promoter-promoter to promoter-enhancer interaction after Decitabine treatment.
In order to directly identify differential promoter-anchored interactions, we integrated the results generated using Chicdiff pipeline with methods to intersect the promoter bait and enhancer OE regions for each interaction (see Methods). In total, we found 13,088 stable (no change) and 4,111 dynamic (gained or lost) contacts for promoters and 55,186 stable and 26,912 dynamic contacts for enhancer OEs (Fig. 4c). The majority of promoter regions were common between the Decitabine and Vehicle tumours. However, Decitabine treatment resulted in a large gain in the number of dynamic enhancer OEs, while only a small number of enhancer OEs were lost (24,694 gained and 2,218 lost in Decitabine) (Fig. 4c). Notably, interactions at gained enhancer OEs with Decitabine treatment were associated with longer interaction distances as compared to these that were at maintained or lost (Fig. 4d), consistent with an increased number of long-range interacting enhancers connecting to these promoters.

We next compared the total number of unique promoter and enhancer OE regions involved in interactions between Vehicle and Decitabine-treated tumours and found a significant increase in the number of enhancer OEs in Decitabine tumours while the number of interacting promoters stayed the same (Extended Data Fig. 4a, b). On average we detected 3.73 unique enhancer OEs per promoter in Vehicle samples and 7.06 unique enhancer OEs per promoter in Decitabine samples (Fig. 4e). To directly assess the gain in the number of interacting enhancers with Decitabine, we calculated the number of interacting enhancer OEs per each individual promoter and compared this number between Vehicle and Decitabine-treated tumours (Fig. 4f). We found that the majority of bait promoters involved in interactions in Vehicle tumours showed a large gain in the number of enhancer OEs they connect to in Decitabine-treated tumours, suggesting reprogramming of one-to-many promoter-enhancer interactions. Furthermore, we identified gained multi-way interactions that had, on average, significantly higher CHiCAGO scores in Decitabine-treated tumours compared to Vehicle (Wilcoxon P value < 0.0001) (Extended Data Fig. 4c), consistent with an overall increase in the total number of interactions with Decitabine treatment.

To directly address if the identified gained enhancer-promoter interactions impact regional transcription, we identified genes connected to newly gained enhancer OEs and compared their average expression between Vehicle and Decitabine tumours. We
identified a total of 4025 genes associated with the new enhancer-promoter interactions, of which 417 were upregulated after Decitabine treatment ($P$ value < 0.05; logFC > 1) (Extended Data Fig. 4d). This suggests that the dynamic increase in the number of enhancer OEs connected to a promoter results in an overall increase in expression of genes and is in agreement with the current models of transcriptional control via enhancer-promoter interactions.\textsuperscript{43,44}

Given that the frequency of interactions is directly linked with higher-order compartment assignment, we next measured the overlap between dynamic promoter-anchored interactions and A/B compartment flipping. A gain of interactions was associated with a shift from B compartment assignment toward compartment A in Decitabine-treated tumours (~76%) (Fig. 4g and Extended Data Fig. 4e), while a loss of interactions was associated with switch from A-type to B-type assignment (~80%). This was particularly pronounced at lost interactions involving promoter bait regions (>90% switched A to B) (Fig. 4g).

Together, these results suggest that promoter interactions can be rewired following DNA methylation loss associated with Decitabine treatment, leading to increased promoter-anchored interactions involving multiple enhancers connecting to gene promoters.

**Rewired promoter-anchored interactions regulate ER target genes**

In addition to 3D genome changes, our analyses revealed ER cistrome and transcriptional reprogramming in tumour cells in response to Decitabine. To explore the mechanistic role of rewired promoter-anchored interactions in the ER transcriptional co-regulatory program, we evaluated whether specific transcription factor motifs were related to gain in promoter-anchored interactions in Decitabine-treated tumours. Dynamic promoter regions occurred at motifs associated with TSSs, including El4 (ETS), NRF and Sp1 (Zf) (Extended Data Fig. 5a). Several TF binding motifs were enriched at gained enhancer OEs, including CTCF, ERE, ELF5 and ZNF165 (Fig. 5a). Consistent with motifs enriched at gained enhancer OEs, we found significant enrichment for gained ER binding sites (Fig. 5b) and a genome-wide increase in ER binding density at enhancer OEs induced by Decitabine treatment (Fig.
We propose that these ER-associated promoter-enhancer interactions are mediated by a change in ER binding at enhancer OEs (“ER-mediated interactions”).

We next investigated gained ER-mediated promoter-enhancer interactions in detail by identifying connected genes and comparing their expression between Vehicle and Decitabine tumours. The majority (~74%) of genes involved in gained ER-mediated interactions showed an overall increase in expression following Decitabine treatment (Fig. 5d) and included established ER target genes (for example KRT8, MYO3B, SEMA3G) as well as genes associated with a good clinical outcome in ER+ breast cancer (for example SPATA18, GALNT5, IGFBP4). We examined one such exemplary region (SPATA18), where multiple ER-mediated promoter-enhancer interactions were gained with Decitabine treatment, concomitant with gain in ER binding, loss of DNA methylation and activation of ER target genes (Extended Data Fig. 5b). An ER binding site was gained following Decitabine treatment at the enhancer region of the SPATA18 gene, with multiple ER-mediated enhancer-promoter interactions gained at this locus (Fig. 5e). Notably, this ERBS is also present in the ER ChIP-seq data representing primary ER+ breast tumours from Ross-Innes et al., 2012 and Severson et al., 2018 as well as in the MCF7 ER+ breast cancer cell line (Fig. 5e). Finally, we found that high expression of SPATA18 gene is associated with good prognosis in ER+ breast cancer (Extended Data Fig. 5c). We also examined two additional regions at the KRT8 (Fig. 5f and Extended Data Fig. 5c, f) and MYOB3 (Extended Data Fig. 5d) ER target genes, which exemplify the relationship between Decitabine-induced gain of multiple ER-mediated enhancer-promoter interactions and activation of ER target genes that are associated with good prognosis in ER+ breast cancer. At both genes we found that ER binding sites gained with Decitabine treatment were also present in primary ER+ breast tumours (Fig. 5f and Extended Data Fig. 5d).

Next, we generated a gene signature by integrating RNA-seq, ER ChIP-seq, and enhancer-promoter interactions. The resulting signature consisted of 17 genes that were induced with Decitabine treatment (P value < 0.05 and logFC > 0) and located at gained ER-mediated enhancer-promoter interactions (highlighted in bold in Fig. 5d). We showed in the METABRIC and TCGA cohorts of ER+ breast cancer patients that high expression of genes belonging to the signature was associated with better patient
prognosis and a higher proportion of luminal A tumour subtypes (Fig. 6a, b). Accordingly, genes in the signature were expressed at higher levels in tumours from patients with a better survival outcome (Fig. 6c, d). Taken together, these results highlight the relationship between Decitabine-induced rewiring of ER-mediated enhancer-promoter interactions and reveals candidate genes with prognostic potential for good outcome and endocrine treatment response in ER+ breast cancer.

Our study provides extensive pre-clinical evidence that Decitabine treatment induced DNA hypomethylation, which reprograms ER binding and re-wires long-range 3D enhancer-promoter interactions leading to re-expression of ER target genes (Fig. 6e). Activated ER target genes are involved in repression of proliferation and cell cycle genes, consistent with Decitabine-induced suppression of tumour growth and significant anti-proliferative effect as assessed by Ki67 expression.

**Discussion**

Three-dimensional (3D) epigenome remodelling, including widespread changes to DNA methylation and 3D chromatin structure, is an emerging mechanism of gene deregulation in cancer. Our previous work demonstrated that DNA hypermethylation and concomitant loss of ER binding at enhancers was a key event associated with alterations in 3D chromatin interactions in ER+ endocrine-resistant breast cancer. Therefore, we were intrigued to determine if these 3D chromatin alterations could be switched with epigenetic therapies that induce DNA hypomethylation, leading to a reversal of the endocrine resistant state and potentially associated with a beneficial clinical outcome in ER+ breast cancer patients. To date, most studies investigating the use of epigenetic therapies have focused on assessing efficacy in blood cancers and in activating the immune system response. While some studies demonstrated that Decitabine has anti-tumour properties in solid cancers (reviewed in), the exact mechanism that underpins this response was unclear and evidence for therapeutic activity was not convincing.

Here, using patient-derived xenograft models of ER+ endocrine-resistant breast cancer, we show that treatment with Decitabine induced DNA hypomethylation and had potent anti-tumour activity via suppression of tumour growth and cell proliferation gene pathways. To assess the broader genomic impact of DNA
hypomethylation, we analysed multiple layers of 3D genome organisation, including chromosomal compartments, TADs and long-range promoter-enhancer chromatin interactions and integrated the generated data with DNA methylation profiles, transcriptome profiles and transcription factor (ER, FOXA1) cistrome profiles in Decitabine and Vehicle-treated PDX tumours. Collectively we found that Decitabine treatment results in enhancer DNA hypomethylation, reprogramming of ER chromatin binding, rewiring of ER-mediated enhancer-promoter interactions, leading to activation of tumour suppressive ER signalling pathways (Fig. 6e). Importantly, rewired ER-mediated chromatin interactions connect enhancers to specific target genes, which included ER target genes involved in cell cycle inhibition and tumor suppressors that may directly inhibit tumor growth either in co-operation with or independently of ER signalling. Such molecular mechanisms likely underpin the Decitabine-induced tumour growth inhibition.

Decitabine has been previously demonstrated to have some therapeutic efficacy in multiple subtypes of breast cancer and in overcoming drug resistance47,48. Transient low-dose treatment with Decitabine resulted in decrease in promoter DNA methylation, gene re-expression, and had antitumor effect in vivo in breast cancer cells49. Low-dose Decitabine has also been shown to prevent cancer recurrence by disrupting the pre-metastatic environment through its effect on myeloid-derived suppressor cells in breast and other cancers50. In triple negative breast cancer (TNBC) PDX organoids, Decitabine sensitivity was positively correlated with protein levels of DNMTs, suggesting DNMT levels as potential biomarkers of response51. A recent study of Decitabine in a panel of breast cancer cell lines observed that Decitabine also induced genes within apoptosis, cell cycle, stress, and immune pathways52. However, knockdown of key effectors of the immune pathway did not affect Decitabine sensitivity suggesting that breast cancer growth suppression by Decitabine is independent of viral mimicry.

We found that the low dose Decitabine treatment we used resulted in minimal DNA hypomethylation at repetitive elements (Δ ~12%). Despite this, we observed a relatively large number of TEs becoming activated with treatment, consistent with previous studies in cancer cell lines53. Loss of DNA methylation at repetitive elements and expression of TEs has been shown to drive viral mimicry response in tumours
treated with epigenetic therapies\textsuperscript{27-29}. In agreement with studies in colorectal tumour cells\textsuperscript{27,54}, our results indicate that treatment with Decitabine results in up-regulation of multiple immune pathways (Interferon and Inflammatory Response pathways), which could promote anti-tumour immunity. However, immunodeficient NOD-scid IL2r\textsuperscript{null} (NSG) mice required for PDX experiments in our study largely lack mature immune cells and therefore the potential immune response could not solely account for the tumour inhibitory effects of Decitabine treatment observed in our study. This highlights the need to study both the immune-based and tumour-based mechanisms that underpin response to epigenetic therapies.

There have been limited studies to date on the role of DNA methylation in shaping the 3D genome organisation. Simultaneous profiling of DNA methylation and 3D genome in single cells revealed pervasive interactions between these two epigenetic layers in regulating gene expression\textsuperscript{55}. Additionally, binding of CCCTC-binding factor (CTCF), an insulator protein involved in creating chromatin loops and domain boundaries has been shown to be methylation-sensitive\textsuperscript{56-59}. DNA hypermethylation at CTCF binding site in IDH mutant gliomas results in its reduced CTCF binding, loss of insulation between topological domains and aberrant gene activation\textsuperscript{19}. More recently, DNA hypomethylation has been shown to result in de-compaction of chromatin and loss of compartmental organisation\textsuperscript{54,60,61}. This is consistent with our data showing closed (B-type) to active (A-type) compartment shifting with Decitabine-induced DNA hypomethylation and reduced interactions between B-type compartments.

Importantly, no studies have examined the effect of epigenetic therapy and DNA hypomethylation on ER-mediated chromatin interactions and transcriptional programs. The ER chromatin binding profiles (cistromes) are distinct in breast cancers that have poor versus favourable patient outcomes and the ER cistromes of poor outcome tumours is more like endocrine resistant versus endocrine sensitive breast cancer cell lines\textsuperscript{4}. A number of studies have suggested an important role of DNA methylation in altering ER binding at regulatory elements\textsuperscript{10,62}. We have previously shown that DNA methylation differences underpin differential ER binding events associated with endocrine resistance\textsuperscript{10}. Furthermore, recent studies demonstrated that ER-mediated 3D chromatin interactions are altered in endocrine-
resistant cells\textsuperscript{17,18}. In this study, our 3D genome analyses following Decitabine treatment indicated that rewiring of enhancer-promoter interactions and ER binding change coincidentally in response to Decitabine-induced DNA hypomethylation. The investigation of transcriptomic datasets demonstrated a strong association between gain in ER-mediated enhancer-promoter interactions and activation of connected ER target genes. These results add to the current research, showing that enhancer reprogramming through transcription factor binding or chromatin remodelling can promote transcriptional programs that are associated with endocrine response in breast cancer\textsuperscript{63,64}. This suggests an important role of ER-mediated enhancer-promoter interactions in controlling tumor growth during Decitabine treatment.

Our high-resolution promoter interactions data also revealed an increase in the number of interacting enhancers connecting to gene promoters induced by Decitabine. We hypothesise that the overall increase in enhancer connectivity results in creation of active transcription hubs\textsuperscript{44} or frequently interacting regions\textsuperscript{65} at activated genes, which will be investigated in future studies. This is in agreement with recent reports of transcriptional activation occurring in non-membrane bound nuclear compartments that harbour multiway enhancer-promoter interacting hubs\textsuperscript{66} as well as additive effect of multiway enhancer interactions on gene expression\textsuperscript{67}.

In summary, our work highlights a novel molecular mechanism of epigenetic therapies in endocrine-resistant ER+ breast cancer. We provide mechanistic insights into how Decitabine-induced 3D epigenome remodelling contributes to its tumour suppressive properties and position these 3D changes as potential targets for the treatment of endocrine-resistant ER+ breast cancer.
Methods

Patient-derived xenograft (PDX) models of ER+ breast cancer

All in vivo experiments, procedures and endpoints were approved by the Garvan Institute of Medical Research Animal Ethics Committee (HREC #14/35, #15/25) and performed at the Garvan Institute of Medical Research using standard techniques as described previously in accordance with relevant national and international guidelines. The Gar15-13 model was generated in-house at the St Vincent’s Hospital under the Human Research Ethics protocol (HREC/16/SVH/29) and the HCI-005 model was developed by the Welm laboratory at the Huntsman Cancer Institute (University of Utah). Gar15-13 was derived from a resected breast cancer liver metastasis of ER-positive, PR-negative, HER2-negative metastatic breast cancer. HCI-005 was derived from a pleural effusion of ER-positive (ERmutL536P), PR-positive, HER2-positive metastatic breast cancer. Growth of HCI-005 was supported by estrogen supplementation in the form of a 60 day 17-β-estradiol pellet implanted simultaneously with the tumour chunks. Mice implanted with Gar15-13 did not receive estrogen supplementation as this model does not require additional estrogen for growth.

At surgery, 4 mm³ sections of tumour tissue were implanted into the 4th inguinal mammary gland of 6–8-week-old female NOD-scid IL2Rγnull (NSG) mice, obtained from Australian BioResources (Sydney, Australia). For HCI-005, tumour growth was supported by implantation of an E2 pellet inserted subcutaneously via the incision site before it was sealed with an Autoclip wound clip. When tumours became palpable, tumour growth was assessed twice weekly by calliper measurement (using the formula: width² × length/2) and mice were randomised to treatment arms when tumours reached 200 mm³ using an online randomisation tool (https://www.graphpad.com/quickcalcs/randomize1.cfm) (n = 6 - 8 mice per group for therapeutic studies, exact numbers specified in figure legends).

Pharmacological treatments

The DNA methyltransferase inhibitor, Decitabine (5-Aza-2’-deoxycytidine: Sigma, Cat #3656) was reconstituted in PBS and stored at -80°C.
Decitabine was administrated in 0.5mg/kg/mouse dose in PBS 100ul IP 2 times weekly. Vehicle mice were treated with 100ul PBS IP. Mice were treated for 60 days or until tumour volume reached 1000 mm^3. Upon reaching ethical or pre-defined experimental endpoints, mice were euthanized, and the primary tumour collected. After weighing, the tumour was cut into pieces that were allocated to be snap-frozen, fixed overnight at 4°C in 10% neutral-buffered formalin, or embedded in cryo-protective optimal cutting temperature (OCT) compound before being snap frozen. Frozen samples were kept at -80°C. Formalin-fixed samples were sent to the Garvan Institute Histology Core Facility for paraffin embedding. Tumour growth curves were analysed in GraphPad Prism (GraphPad Software) by two-tailed, unpaired t-test. Tumour mass at end-point was analysed by two-tailed Mann-Whitney t-test as per figure legends unless otherwise specified.

**Immunohistochemistry (IHC) and quantification**

Tumour tissue was harvested and immediately fixed in 10% neutral buffered formalin at 4°C overnight before dehydration and paraffin embedding. Antibodies used for IHC were anti-ER (M7047, 1:300, Agilent) and anti-Ki67 (M7240, 1:400, Agilent). Primary antibodies were detected using biotinylated IgG secondary antibodies (Agilent, 1:400), using streptavidin-HRP (Agilent) for amplification of signal followed by the addition of 3,3′-diaminobenzidine (Sigma) substrate. Images were scanned using Leica Aperio Slide Scanner (Leica Biosystems) and analysed using QuPath software to differentiate tumour tissue from stroma and necrosis, and to quantify Ki-67 positivity in tumour tissue.

**Low input in situ Hi-C in snap-frozen PDX tumour samples**

Tumour tissue samples were flash frozen and pulverized in liquid nitrogen before formaldehyde cross-linking in TC buffer. Hi-C was then conducted using the Arima-HiC kit, according to the manufacturer’s protocols (Cat. #A510008) with minor modifications. Briefly, for each Hi-C reaction between ~100,000 - 500,000 cells were cross-linked with 2% formaldehyde and nuclei were isolated by incubating cross-linked cells in Lysis Buffer at 4°C for 30 minutes. The Arima kit uses two restriction enzymes recognizing the following sequence motifs ^GATC and G^ANTC (N can be either of the 4 genomic bases), which after ligation of DNA ends generates 4 possible ligation junctions in the chimeric reads: GATC-GATC, GANT-GATC, GANT-
ANTC, GATC-ANTC. Hi-C libraries were prepared using the Swift Biosciences Accel-NGS 2S Plus DNA Library Kit with a modified protocol provided by Arima with 8 PCR cycles for library amplification as required. Hi-C libraries were sequenced on Illumina HiSeq X10 in 150bp paired-end mode.

**Promoter Capture Hi-C**

To perform promoter Capture Hi-C (PCHi-C), we computationally designed RNA probes that capture promoter regions of previously annotated human protein-coding genes. Promoter Capture was performed as previously described\(^{69-71}\). First, to identify promoter capture targets, 23,711 unique Ensembl annotated genes were extracted from the GRCh38 gene annotation file in Ensembl database, version 95. These compromised of protein-coding (18,741), antisense (84), lincRNA (170), miRNA (1,878), snoRNA (938), snRNA (1,898) or multiple (2) transcripts. We then located transcription start sites (TSS) of each gene and mapped the TSS coordinates to the in silico digested genome (^GATC and G^ANTC) and extracted the restriction fragment containing the TSS, as well as one restriction fragment upstream and one restriction fragment downstream for each TSS. The final target list of TSS mapped to 3 consecutive restriction fragments. The average length of the 3 consecutive restriction fragments for each TSS is 786bp and the median is 927bp, with a range of 54-4174bp. Moreover, for the individual restriction fragments smaller than 700bp, all nucleotides within these fragments are less than or equal to 350bp from the nearest cut site, and therefore the entire restriction fragment was defined as a target region for subsequent probe design. This scenario represents the vast majority of cases, because the mean length of an individual restriction fragment is 263bp, with a median of 431bp. However, if an individual restriction fragment was greater than 700bp, then the 350bp on each inward facing edge of the restriction fragment was defined as a target region for probe design, and the centre most portion of the restriction fragment was excluded from the probe design. After this final processing, a final BED file of target bait regions was input into the Agilent SureDesign tool, and probes were designed using a 1X tiling approach, with moderate repeat masking and balanced boosting. Promoter Capture was carried out using Hi-C libraries from three Vehicle-treated tumour samples and three Decitabine-treated tumour samples with the SureSelect target enrichment system and RNA bait library according to manufacturer’s instructions (Agilent Technologies kit), using 12 post-capture PCR cycles as required. PCHi-C
libraries were sequenced on the Illumina HiSeq X10 platform in 150bp paired-end mode.

Microarray genome-wide DNA methylation
DNA from four Decitabine and four Vehicle-treated tumours from two PDX models (Gar15-13 and HCl-005) was isolated from snap-frozen tumour samples using the Qiagen QIAamp DNA Mini Kit. DNA (500ng) was treated with sodium bisulphite using EZ-96 DNA methylation kit (Zymo Research CA, USA). DNA methylation was quantified using the Illumina Infinium MethylationEPIC (EPIC) BeadChip (Illumina, CA, USA) run on the HiScan System (Illumina, CA, USA) using manufacturer’s standard protocol.

ChIP-seq
Tumour samples were snap-frozen in Optimal Cutting Temperature compound (Tissue-Tek) and used for ER and FOXA1 ChIP-seq experiments. Using a cryostat (Leica, #CM3050-S), a minimum of 50 x 30 μm sections were cut from each tumour at -20°C and subjected to double cross-linking with DSG and formaldehyde as previously described. ER ChIP-seq was performed with an anti-ER antibody (Santa Cruz, SC-543X) and FOXA1 ChIP-seq was performed with an anti-FOXA1 antibody (Abcam, Ab23738). 5μg of antibody was used to ChIP each tumour sample and 10ng of immunoprecipitated DNA was submitted to the David R. Gunn Genomics Facility at the South Australian Health and Medical Research Institute (SAHMRI) for sequencing. Conversion of the DNA into sequencing libraries was performed using the Ultralow Input Library Kit (Qiagen, #180495) and sequenced on the Illumina NextSeq 500 (Illumina) in 75bp single-end mode to achieve a minimum of 20 million reads per sample.

RNA-seq
RNA was extracted from snap-frozen tumour PDX tissue samples using the RNeasy Mini Kit (QIAGEN) and quality of purified RNA was confirmed with RNA ScreenTape TapeStation (Agilent). All samples processed for RNA-seq had a RIN equivalent (RIN⁵) quality score ≥ 8.0. Total RNA was supplied to the genomics core facility (Kinghorn Centre for Clinical Genomics) for library preparation and sequencing. RNA was prepared for sequencing using the TruSeq Stranded mRNA
Library Prep kit (Illumina) and libraries were sequenced on Illumina NovaSeq 6000 S4 in paired-end mode.

**EPIC DNA methylation analyses**

Raw intensity data (IDAT) files were imported and quality controlled using minfi package (v.1.34.0)\(^2\). Data was then normalised with background correction. To reduce the risk of false discoveries, we removed probes affected for cross-hybridization to multiple locations on the genome or overlapped SNPs, as previously described\(^3\). Beta (β) values were calculated from unmethylated (U) and methylated (M) signal \([M/(U + M + 100)]\) and ranged from 0 to 1 (0 to 100% methylation). β values of loci whose detection P values were > 0.01 were assigned NA in the output file. To map EPIC arrays to hg38/GRCh38 assembly, all probes were annotated with the EPIC.hg38.manifest.tsv.gz files as described in\(^4\).

For initial visualisation of the EPIC data, multidimensional scaling plots were generated using the ‘mdsPlot’ function in the minfi Bioconductor package (v.1.34.0)\(^2\). Differential analyses were then performed between treatment arm with Decitabine versus Vehicle samples. For each comparison, β values were transformed using logit transformation: \(M = \log2(\beta/(1-\beta))\). We used the limma package (v.3.46)\(^5\) to identify DMPs between sample groups with adjusted \(P\) value cut-off of < 0.01. The R package DMRcate (v.2.2.3)\(^6\) was used to identify DMRs, with DMP \(P\) value cut-offs of FDR < 0.01. DMRs were defined as regions with a maximum of 1000 nucleotides between consecutive probes and a minimum of 2 CpG sites, a methylation change > 30% and we applied Benjamini-Hochberg correction for multiple testing.

ChromHMM data downloaded from GEO (GSE73783) for tamoxifen-resistant (TAMR) MCF7 cells was used to annotate DMPs to chromatin states.

REMP R package (v.1.14.0)\(^7\) was used to assess genome-wide locus-specific DNA methylation of repeat elements (LTR, LINE1 and Alu) from EPIC data with IlluminaHumanMethylationEPICanno.ilm10b5.hg38 annotation (GitHub).

**Hi-C analyses**

Hi-C sequenced reads (150bp paired-end) were quality checked with FastQ Screen v.0.14.1\(^8\) for mouse host reads contamination. Reads were then processed with Xenome (v.1.0.1)\(^9\) as described in\(^10\). Remaining reads were aligned to the human genome (hg38/GRCh38) using HiC-Pro\(^11\) (v.2.11.4). Initially, to generate Hi-C
contact matrices, the aligned Hi-C reads were filtered and corrected using the ICE “correction” algorithm\textsuperscript{81} built into HiC-Pro, which corrects for the CNV-related variability in the tumours\textsuperscript{82}. Inter-chromosomal interactions were excluded from further analyses to control for the effect of inter-chromosomal translocations in the tumours. Contact matrices for 3D genome feature annotation and visualisation were created and Knight-Ruiz (KR) normalized using Juicer tools\textsuperscript{83} using contact matrices in .hic format generated by hicpro2juiciebox script in HiC-Pro as input (hic file version 8). Hi-C matrices were visualised using JuiceBox\textsuperscript{84} and WashU Epigenome Browser\textsuperscript{85}. We confirmed data quality by assessing the proportion of cis/trans interactions and percentage of valid fragments for each library. Overall, we obtained an average of 100 million unique, valid contacts per replicate (~310 million per treatment arm), for an average resolution of 10kb. Statistics for each library can be found in Supplemental Table 2. These data was used to derive loops, TAD boundaries and chromosomal compartment structures.

**Insulation score and identification of TAD boundaries**

Topological domain boundary calling was performed by calculating insulation scores in ICE normalised contact matrices at 20kb resolution using TADtool\textsuperscript{86}. To identify appropriate parameters, we called TADs across chromosome 1 using contact matrices at 20kb and threshold values: 10, 50 and 100. The final TADs were called for all chromosomes at window 102353 and cut-off value 50. Boundaries that were found overlapping by at least 1 genomic bin were merged. Boundaries separated by at least one genomic bin were considered different between datasets. Pyramid-like heatmap plots were generated with GENOVA\textsuperscript{87}.

**Identification of compartments A and B**

For each chromosome in each sample, compartments where called using the standard PCA method\textsuperscript{40} in Homer package (version 4.8). The resolution was set to 50kb and the window size to 100 kb. Compartments were defined as regions of continuous positive or negative PC1 values using the findHiCCompartments.pl tool in Homer. To detect which compartment is the open “A-type” and which is the closed “B-type,” the genome-wide gene density was calculated to assign the “A-type” and “B-type” compartmentalisation. To identify genomic regions that switch between two compartment types, we used correlation difference script (getHiCcorrDiff.pl) with
findHiCCompartments.pl tool in Homer. Compartments were considered common if they had the same compartment definition within the same genomic bin. Compartment changes between treatment arms were computed after considering compartments that were overlapping between biological replicates, unless otherwise indicated.

To directly quantify the tendency of each region to interact with the other regions in either A or B compartments, we calculated the “A/B interaction ratio”, defined for each 100kb genomic window as the ratio of interaction frequency with A versus B compartments using O/E matrix with GENOVA\(^87\) (v1.0.0, [https://github.com/robinweide/GENOVA](https://github.com/robinweide/GENOVA)). Log\(_2\) contact enrichments were plotted as a heat saddle plot. Summarised A-A, B-B and B-A compartment strengths were calculated as the mean log\(_2\) contact enrichment between the top (A) or bottom (B) 20% of PC1 percentiles. The compartment strength ratio was calculated as log\(_2\)(A-A/B-B).

**PCHi-C analyses**

PCHi-C sequenced reads were mapped and filtered using HiCUP (v.0.7.4)\(^88\) with hg38/GRCh38 genome digested with --arima flag and minimum di-tag length set to 20. Statistics for each library can be found in Supplementary Table 3. On target rate was calculated by counting number of valid, unique reads overlapping bait fragments (min. overlap > 0.6). Unique, valid mapped reads from HiCUP were converted into .chinput files using bam2chicago.sh utility and obtained chinput files were further filtered and processed with CHiCAGO (v.1.14.0)\(^41\). CHiCAGO design files were created with following parameters to account for multiple restriction enzymes used in the Arima HiC kit and the Arima-specific design of the bait fragments: MaxLBrowndist = 75000; binsize = 1500; minFragLen = 25; maxFragLen = 1200. Significant interactions were called with CHiCAGO using score cut-off of 5. All bait-to-bait interactions were discarded. Chicdiff package\(^42\) (v.0.6) was used to compare PCHi-C data from Vehicle and Decitabine tumours and the difference in the mean asinh-transformed CHiCAGO scores between conditions above 1 was used to prioritise the potential differential promoter-anchored interactions. Only interactions that have CHiCAGO score of more than 5 in at least 2 replicates were included for downstream analysis. Volcano plots were generated using EnhancedVolcano R package (v.1.8.0)\(^89\). For downstream analysis of merged replicate data and for
visualisation of interactions in WashU Epigenome Browser\textsuperscript{85} replicates were merged with CHiCAGO. We defined reprogrammed promoter-enhancer interactions by constructing a consensus, gained and lost subset of promoter-anchors (baits) and other end anchors (OEs) based on CHiCAGO promoter interactions, Chicdiff analysis and setdiff R function across the replicates. The following criteria was used to obtain these regions: CHiCAGO score > 5 in 2 out of 3 replicates in either condition, Chicdiff generated asinh-transformed CHiCAGO scores between conditions above 1 and no overlap between regions, allowing for 10Kb maximal gap in 3 out of 3 replicates. ChromHMM data downloaded from GEO (GSE73783) for tamoxifen-resistant (TAMR) MCF7 cells was used to annotate promoter-anchored interactions to chromatin states.

**RNA-seq data analyses**

For canonical gene expression, RNA-seq raw reads were quality checked (FastQ Screen v.0.14.1\textsuperscript{77}). Sequence adaptors were trimmed using Trim Galore (v.0.11.2), reads were processed with Xenome v.10.1\textsuperscript{78} to remove mouse sequences and remaining reads were mapped with STAR (v.2.7.7a)\textsuperscript{90} to the hg38/GRCh38 human genome build with GENCODE v33 used as a reference transcriptome (parameter settings: --quantMode TranscriptomeSAM--outFilterMatchNmin 101 --outFilterMultimapNmax 20). Statistics for each library can be found in Supplementary Table 4. TMM normalisation was applied to normalise for RNA composition\textsuperscript{91} and differential expression was performed with edgeR 3.18.1\textsuperscript{92} using the generalised linear model (GLM). RNA-seq tracks were generated using bedtools v.2.22 genomeCoverageBed to create normalised.bedGraph files and bedGraphToBigWig (USCS utils) to create.bigwig files.

For analysis of TE expression, adapter-trimmed, human-only RNA-seq reads were mapped with STAR (v.2.7.7a), allowing for multimapping alignments (flags: --outFilterMultimapNmax 100 --outFilterMismatchNmax 100). Annotation GTF files for canonical genes were downloaded from Ensembl genome browser (v.102, GRCh38.p13 assembly) and TE annotation GTF file (GRCm38_Ensembl_rmsk_TE.gtf) was downloaded from TEtranscripts \textsuperscript{93} website (http://hammelllab.labsites.cshl.edu/software/#TEtranscripts). Normalisation and differential expression was performed for all genes and TEs using DESeq2\textsuperscript{94}. TEs with and FDR < 0.1 and logFC > 0.7 were considered differentially expressed in pair-
wise comparisons. Volcano plots were generated using EnhancedVolcano R package
(v.1.8.0)\textsuperscript{89}.

**ChIP-seq data analyses**

ChIP-seq reads were aligned against human genome (hg38/GRCh38) using bowtie2
with default parameters\textsuperscript{95}. Non-uniquely mapped, low quality (MAPQ < 15) and PCR
duplicate reads were removed. Peak calling of individual ChIP–seq experiments was
performed with MACS2 with default parameters\textsuperscript{96,97}. Statistics for each library can be
found in Supplementary Table 5. Consensus peaks were identified by intersecting
MACS2 peaks obtained from each sample using bedtools intersect (v.2.25.0) with
min. overlap > 0.6. Differential binding analyses were performed using DiffBind
(v.3.0.9)\textsuperscript{4} and DESeq2 (v.1.3.0)\textsuperscript{94} with FDR < 5%. Enrichment analyses were
performed using GAT\textsuperscript{98}, ChIPseeker (v.1.26.0)\textsuperscript{99} and normalised to library size.
Merged bigwig tracks for visualisation were created from merged bam files from all
replicates using the bamCoverage function with scaling factor normalisation and
heatmaps and average profiles were plotted with deepTools2\textsuperscript{100}. ChromHMM data
downloaded from GEO (GSE73783) for tamoxifen-resistant (TAMR) MCF7 cells
was used to annotate ER binding sites to chromatin states. The Homer motif
discovery suite\textsuperscript{101} was used for motif analysis using the bed regions from differential
peaks detected by DiffBind with default parameters, using random, matched regions
as background. ER ChIP-seq data from primary breast tumours were obtained from
GSE32222\textsuperscript{4} and GSE104399\textsuperscript{39}, and re-processed as described above. Merged bigwig
tracks for visualisation were created from merged bam files from all replicates using
the bamCoverage function with scaling factor normalisation and heatmaps and
average profiles were plotted with deepTools2\textsuperscript{100}.

**Gene ontology analysis**

Gene ontology enrichment analysis and pathway enrichment were done using GSEA
(v.4.1.0) and MSigDB 7.2\textsuperscript{36}. All significant biological processes and pathways had an
adj. P value < 0.001.

**Clinical samples and survival analyses**

We used the METABRIC (Molecular Taxonomy of Breast Cancer International
Consortium)\textsuperscript{102,103} dataset and the TCGA (The Cancer Genome Atlas) dataset\textsuperscript{104,105}
for patient survival analysis. These datasets contain RNA-seq and microarray data as well as detailed clinical information from breast cancer patients. Data was accessed using cBioPortal (https://www.cbioportal.org/). Patient survival analysis was performed using the survminer (v.0.4.9) R package. Survival curves were drawn using ggplot2. Cohorts were split by mean gene expression and disease-specific survival was compared by Cox proportional hazards analysis.

Statistical analyses
The Mann-Whitney-Wilcoxon test was used for 2-group non-parametric comparisons. Unless otherwise stated, statistical tests were two-sided. Permutation test was used to calculate empirical P values, which does not make any assumptions on the underlying distribution of the data. Tumor growth curve data was analysed at ethical end point using a two-tailed unpaired Student’s t-test. IHC data were analysed by a two-tailed, unpaired Student’s t-test.

Public datasets
ChIP-seq data sets were downloaded from GSE32222 by Ross-Innes et al., 2012 and GSE104399 by Severson et al., 2018. ChromHMM data was downloaded from GSE73783 by Achinger-Kawecka et al., 2020.

Data availability
All sequencing data created in this study have been uploaded to the Gene Expression Omnibus (GEO; https://www.ncbi.nlm.nih.gov/geo/) and are available under primary accession code GSE171074. Biological material used in this study can be obtained from the authors upon request.

Code availability
Python script language (v.2.7.8 and v.3.9.1) and R (v.3.6.3 and v.4.0.3) were used to develop the bioinformatics methods and algorithms in this work. All code for Hi-C and PCHi-C analyses is available within the GitHub repository https://github.com/JoannaAch/PDX_Decitabine_3DEpigenome.

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**Author Contributions**


**Ethics declarations**

A.S. is an employee of Arima Genomics, Inc. Other authors declare no competing interests.

**References**


60. Du, Q. et al. DNA methylation is required to maintain DNA replication timing precision and 3D genome integrity. *bioRxiv* (2020).


Fig. 1

a) Patient-derived metastatic breast cancer xenograft (PDX) models

HCl-005 (pleural effusion) Gar15-13 (liver)
Breast cancer metastases

Treatments

NO TREATMENT
(Vehicle; 100nM PBS; N = 8 Gar15-13
N = 7 HCl-005)

EPIGENETIC THERAPY
(Decitabine; 0.5mg/kg;
N = 8 Gar15-13
N = 7 HCl-005)

b) Observations of Tumour Volume (mm³) for Gar15-13 and HCl-005 over 40 days.

Gar15-13
ER⁺, PR⁺, HER2⁺

Vehicle
Decitabine

HCl-005

ERmut⁺, PR⁺, HER2⁺

Vehicle
Decitabine

Observed / Expected Fold change

1
0
2

**

Hypo

Hyper

DNA methylation

β-value

0.00
0.25
0.50
0.75
1.00

Veh1, Veh2, Veh3, Veh4, Dec1, Dec2, Dec3, Dec4

Top 50,000 most variable probes

β-value

0.00
0.25
0.50
0.75

Vehicle
Decitabine

Observed / Expected Fold change

Promoter
Polycomb
Active Promoter
Active Enhancer
Poised Enhancer
Enhancer

1332
21
773
13
811
1460
5
9
3
6

*
Fig. 1 Decitabine suppresses tumour growth and proliferation markers in PDX models of endocrine-resistant ER+ breast cancer.

(a) Schematic of models and treatments used in the study and summary of genome-wide assays applied to PDX tumours at end-point.

(b) Growth of treatment naïve (Vehicle) (100nM PBS, n = 7) and Decitabine-treated (0.5mg/kg, n = 7) Gar15-13 PDX tumours for 35 days or until ethical endpoint. All data are mean ± SEM. *** P value < 0.001.

(c) Growth of treatment naïve (Vehicle) (100nM PBS, n = 8), and Decitabine-treated (0.5mg/kg, n = 7) HCI-005 PDX tumours for 35 days or until ethical endpoint. All data are mean ± SEM. *** P value < 0.001.

(d) Quantification of the proliferation marker Ki-67 at endpoint in Gar15-13 and HCI-005 tumours. Ki-67 proportions from all replicates per treatment were compared by two-tailed T test. *P value < 0.001.

(e) Boxplots showing the distribution of DNA methylation profiles for Gar15-13 Vehicle and Decitabine-treated tumours for all EPIC probes across the genome. Black line indicates mean ± SD.

(f) Boxplots showing DNA methylation distribution for the 50,000 most variably methylated probes in Vehicle compared to Decitabine for Gar15-13 tumours. Black line indicates mean ± SD.

(g) Bar plot showing the association of differentially methylated probes in Gar15-13 Decitabine treatment compared to Vehicle across different regulatory regions of the genome as determined by TAMR ChromHMM annotation. Observed over expected fold change enrichment shown. * P value < 0.001. The numbers of hypo/hypermethylated probes located within each specific region are presented in the respective column.
Fig. 2

**a**

<table>
<thead>
<tr>
<th>Pathway</th>
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<th>Decitabine</th>
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<tbody>
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**b**

Decitabine Vehicle

**c**

Gained ER binding sites

Lost ER binding sites

**d**

ERBS gained in Decitabine

**e**

ERBS lost in Decitabine

**f**

DNA methylation (EPIC)

**g**

Chromosome 10

ERE motif

Sp1(Zf) Promoter

NFY(CCAAT) Promoter
**Fig. 2** Decitabine-induced DNA hypomethylation reprograms the ER cistrome.

(a) Normalized enrichment scores (NES) for signature gene sets representing differentially expressed genes in RNA-seq data from Gar15-13 PDX tumours treated with Decitabine compared to Vehicle (n = 4; FDR < 0.05)

(b) Overlap of consensus ER cistromes in Vehicle and Decitabine-treated Gar15-13 PDX tumours (n = 4 each). Heatmaps indicate ER ChIP-seq signal intensity at ER binding sites gained and lost in Decitabine compared to Vehicle-treated tumours and FOXA1 ChIP-seq signal intensity in Vehicle tumours.

(c) Average signal intensity of ER ChIP-seq binding (Gar15-13 Vehicle and Decitabine tumours) at gained and lost ER binding sites with Decitabine treatment.

(d) ChromHMM (TAMR) annotation (*P value < 0.001) and motifs enriched at ER binding sites gained with Decitabine treatment compared to matched random regions generated from ERE binding motifs across the genome. Size of the overlap is presented in the respective column.

(e) ChromHMM (TAMR) annotation (*P value < 0.001) and motifs enriched at ER binding sites lost with Decitabine treatment compared to matched random regions generated from ERE binding motifs across the genome. Size of the overlap is presented in the respective column.

(f) Heatmap showing DNA methylation levels (β-values) at gained ER binding sites in Decitabine-treated (n = 4) and Vehicle (n = 4) PDX tumours.

(g) Browser snapshot of ER ChIP-seq together with EPIC DNA methylation (Vehicle and Decitabine treatment, n = 4 each) showing gain of ER binding and loss of DNA methylation at an enhancer region of ER target gene *ANKRD2*. 
Fig. 3

a) PC1 value Veh vs Decitabine
b) β-value B to B vs A to A

A to B

Total = 643
P < 0.0001

B to A

Vehicle - Decitabine

Veh - Dec

Vehicle - Decitabine

Vehicle

Decitabine

Vehicle-specific TAD boundaries

Decitabine-specific TAD boundaries

Common TAD boundaries

Vehicle

Decitabine

Vehicle

Decitabine

Vehicle

Decitabine

Vehicle

Decitabine

Vehicle

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Decitabine

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Vehicle
Fig. 3 Loss of DNA methylation results in de-compaction of chromatin.

(a) Scatterplot showing correlation between average eigenvalues per bin in Vehicle and Decitabine-treated Gar15-13 PDX tumours.

(b) Pie chart showing distribution of different types of switching compartments (A to B; B to A) in Decitabine-treated tumours as compared to Vehicle.

(c) Boxplot showing DNA methylation levels at compartment regions that switched their assignment from B to A and from A to B in Decitabine-treated (n = 4) and Vehicle (n = 4) PDX tumours. Black line indicates mean ± SD.

(d) Average contact enrichment (saddle plots) between pairs of 50Kb loci arranged by their PC1 eigenvector in Vehicle and Decitabine-treated tumours. Average data from n = 3 Hi-C replicates for Vehicle and Decitabine shown. The numbers at the centre of the heatmaps indicate compartment strength calculated as the log2 transformed ratio of \((A-A + B-B)/ (A-B + B-A)\) using the mean values.

(e) Difference in Hi-C data between Vehicle and Decitabine treatments. Saddle plots were calculated using the averaged PC1 obtained from Vehicle (n = 3) and Decitabine-treated (n = 3) tumours.

(f) Density plot of insulation scores calculated in Vehicle and Decitabine-treated tumours.

(g) Number of TADs identified in Vehicle (n = 3) and Decitabine-treated (n = 3) PDX tumours.

(h) Venn diagram showing overlap between TAD boundaries identified in Vehicle and Decitabine-treated tumours.

(i) Snapshot of region on chromosome 1, showing Vehicle (top panel) and Decitabine-treated tumour Hi-C matrixes (bottom panel), demonstrating loss of a TAD in Decitabine-treated samples (indicated with an arrow), concomitant with decreased insulation at that region. Merged Hi-C data from replicates (n = 3 each) shown at 10Kb resolution.
**Fig. 4**

(a) Chromosome 11

(b) Observed / Expected Fold change

(c) Promoter baits:

(d) Interaction distance log10(bp)

(e) Number of enhancer OEs per promoter

(f) Number of interacting enhancer OEs

(g) Percent of promoter-anchored interactions at switched compartments

---

*CHICAGO significant baits*  
*CHICAGO significant OEs*

---

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doi: bioRxiv preprint
Fig. 4 Loss of DNA methylation rewires long-range promoter-anchored enhancer interactions.

(a) Browser snapshot of interactions landscape at the PRR5L gene demonstrating increased coverage of promoter-anchored interactions in PCHi-C at 1.5Kb resolution compared to Hi-C at 10Kb resolution. Bait and other end (OE) regions marked for illustrative purposes.

(b) ChromHMM (TAMR) annotation of CHiCAGO significant interaction bait (promoter) and other end (OEs) regions (putative enhancers) in Decitabine-treated and Vehicle samples (* P value < 0.001)

(c) Overlap between promoter bait and other end (OE) enhancer regions for CHiCAGO significant interactions in Vehicle and Decitabine-treated tumours. Merged data across replicates shown.

(d) Violin plots showing the log10 genomic distance of promoter interactions whose enhancer OEs are gained, maintained or lost following Decitabine treatment. P value < 0.0001, Wilcoxon test. Merged data across replicates shown.

(e) Average number of enhancer OE interactions per promoter bait. Error bars indicate the interquartile range. P value Wilcoxon test.

(f) Scatterplot showing number of enhancer OE interactions per promoter bait for each CHiCAGO significant promoter-anchored interaction in Vehicle and Decitabine-treated tumours. Merged data across replicates shown.

(g) Percent of overlap of promoter baits and enhancer OEs that are either gained or lost in Decitabine with compartment that switch with Decitabine (A to B or B to A).
Fig. 5

(a) Gained enhancer OEs:

<table>
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<tr>
<th>Gene</th>
<th>ER, Decitabine</th>
<th>ER, Vehicle</th>
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(b) Fold enrichment (observed / expected)

(c) Normalized read density

(d) RNA-seq

(e) Chromosome 4

(f) Chromosome 12
**Fig. 5 Rewired promoter-anchored interactions connect ER-bound enhancers to ER target genes.**

(a) Transcription factor motifs significantly enriched at promoter-interacting enhancers (enhancer OEs) gained with Decitabine treatment. Only motifs with FDR < 0.05 are shown.

(b) Observed/expected fold change enrichment of gained enhancer OEs for ER binding gained and lost following Decitabine treatment. * P value < 0.0001.

(c) Average ER ChIP-seq signal intensity (Gar15-13 Vehicle and Decitabine-treated tumours) at ER binding sites located at DNA hypomethylation-induced enhancer OEs.

(d) Heatmap showing expression of genes connected to gained ER-mediated enhancer OEs in Vehicle and Decitabine-treated tumours. Genes in bold are significantly upregulated following Decitabine treatment and belong to a gene signature identified in our study.

(e) Browser snapshots showing the promoter-anchored interactions at the *SPATA18* gene, together with the average ER ChIP-seq signal, EPIC DNA methylation and ChromHMM track. Merged replicate data shown (n = 4 each). In Decitabine-treated tumours, the *SPATA18* promoter displays an increased number of interactions with an upstream enhancer region, which gains ER binding with Decitabine treatment, concomitant with loss of DNA methylation. This gained ER binding site is also present in ER ChIP-seq data representing primary ER+ breast tumours from Ross-Innes *et al.*, 2012 and Severson *et al.*, 2018. Expression of the *SPATA18* gene was significantly upregulated in Decitabine-treated tumours (shown in Data Fig. 5b).

(f) Browser snapshots showing promoter-anchored interactions at the *KRT8* ER target gene, together with ER ChIP-seq, EPIC DNA methylation and ChromHMM track. Merged replicate data shown (n = 4 each). In Decitabine-treated tumours, the *KRT8* promoter displays an increased number of interactions with multiple enhancers, one of which gains ER binding with Decitabine treatment. This gained ER binding site is also present in ER ChIP-seq data from primary ER+ breast tumours from Ross-Innes *et al.*, 2012 and Severson *et al.*, 2018. Expression of the *KRT8* gene was significantly upregulated in Decitabine-treated tumours (shown in Extended Data Fig. 5e).
**Fig. 6**

(a) METABRIC ER+ cohort

(b) TCGA ER+ cohort

(c) METABRIC ER+ cohort

(d) TCGA ER+ cohort

(e) Tumour growth and cell proliferation

Epigenetic therapy

Decitabine (DNMTi)

ER enhancer

ER-mediated interactions

Suppression of tumour growth and cell proliferation

Good outcome genes e.g. KRT8, SPATA18, MYO3B

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**Fig. 6 ER target gene signature is prognostic in ER+ breast cancer.**

**(a)** Kaplan–Meier survival plot showing ability of the gene signature identified in our study (n = 17 genes) derived from Decitabine-induced ER-mediated enhancer-promoter interactions to stratify ER+ breast cancer patients in the METABRIC cohort into good and poor outcome groups. Data were analysed using the log-rank test. *P* values indicated within the graph. Distribution of the PAM50 classification in the high and low gene signature-expressing patients in the ER+ breast cancer METABRIC cohort.

**(b)** Kaplan–Meier survival plot showing ability of the gene signature identified in our study (n = 17 genes) derived from Decitabine-induced ER-mediated enhancer-promoter interactions to stratify ER+ breast cancer patients in the TCGA cohort into good and poor outcome groups. Data were analysed using the log-rank test. *P* values indicated within the graph. Distribution of the PAM50 classification in the high and low gene signature-expressing patients in the ER+ breast cancer TCGA cohort.

**(c)** Boxplot of normalised log2 gene expression levels of genes belonging to the signature in non-malignant (n = 145), poor outcome (n = 342) and good outcome (n = 832) ER+ breast tumours from the METABRIC cohort. *P* value Mann-Whitney t test.

**(d)** Boxplot of normalised log2 gene expression levels of genes belonging to the signature in poor outcome (“Dead”, n = 109) and good outcome (“Alive”, n = 568) ER+ breast tumours from the TCGA cohort. *P* value Mann-Whitney t test.

**(e)** Proposed model of tumour growth suppression induced by epigenetic therapy with Decitabine *via* DNA hypomethylation and subsequent re-wiring of ER-mediated enhancer-promoter interactions resulting in activation of specific ER target genes.
Transposable Elements DNA methylation (Gar15-13)

**j**

- **LTR Elements**
  - Decitabine vs Vehicle
  - Methylation (β-value)
  - Significance: *P = 0.0009*

- **LINE1 Elements**
  - Decitabine vs Vehicle
  - Methylation (β-value)
  - Significance: *P = 0.0008*

- **Alu Elements**
  - Decitabine vs Vehicle
  - Methylation (β-value)
  - Significance: *P = 0.0019*

Transposable Elements DNA methylation (HCI-005)

**k**

- **LTR Elements**
  - Decitabine vs Vehicle
  - Methylation (β-value)
  - Significance: *P = 0.003*

- **LINE1 Elements**
  - Decitabine vs Vehicle
  - Methylation (β-value)
  - Significance: *P = 0.012*

- **Alu Elements**
  - Decitabine vs Vehicle
  - Methylation (β-value)
  - Significance: *P = 0.011*
**Extended Data Fig. 1**

(a) Mice were treated with indicated doses of Decitabine for 35 consecutive days and mice weight was assayed to determine the most appropriate Decitabine concentration.

(b) Density plot showing DNA methylation distribution in four Vehicle and four Decitabine-treated Gar15-13 tumours.

(c) Density plot showing DNA methylation distribution in four Vehicle and four Decitabine-treated HCI-005 tumours.

(d) Boxplots showing the distribution of DNA methylation profiles for HCI-005 Vehicle and Decitabine-treated tumours for all DNA methylation EPIC probes across the genome.

(e) RefSeq annotation of Vehicle vs. Decitabine hypomethylated probes (Gar15-13)

(f) RefSeq annotation of Vehicle vs. Decitabine hypomethylated probes (HCI-005)

(g) Bar plot showing the association of differentially methylated probes in HCI-005 Decitabine treatment compared to Vehicle across regulatory regions of the genome as determined by TAMR ChromHMM annotation. Observed over expected fold change enrichment shown. * P value < 0.001. The numbers of hypo/hypermethylated probes located within each specific regions are presented in the respective column.

(h) Boxplots showing the distribution of DNA methylation profiles for Gar15-13 Vehicle and Decitabine-treated tumours for EPIC probes located at TAMR ChromHMM enhancer regions. Black line indicates mean ± SD.

(i) Boxplots showing the distribution of DNA methylation profiles for Gar15-13 Vehicle and Decitabine-treated tumours for EPIC probes located at TAMR ChromHMM promoter regions. Black line indicates mean ± SD.

(j) Boxplots showing the distribution of DNA methylation for Gar15-13 Vehicle and Decitabine-treated tumours for EPIC probes mapping to LTR, LINE1 and Alu repetitive elements (REMP annotation). Black line indicates mean ± SD.

(k) Boxplots showing the distribution of DNA methylation profiles for HCI-005 Vehicle and Decitabine-treated tumours for EPIC probes mapping to LTR, LINE1 and Alu repetitive elements (REMP annotation). Black line indicates mean ± SD.
Extended Data Fig. 2

(a) Normalized enrichment scores (NES) for signature gene sets representing differentially expressed genes in RNA-seq data from HCI-005 PDX tumours treated with Decitabine compared to Vehicle (n = 4; hallmarks with FDR < 0.05 are shown)

(b) Protein-protein interaction analyses of down-regulated genes belonging to the E2F Targets and G2M Checkpoint (GSEA) Hallmark. Interaction confidence indicated on the graph.

(c) Protein-protein interaction analyses of up-regulated genes belonging to the Estrogen Response (GSEA) Hallmark. Interaction confidence indicated on the graph.

(d) RNA-seq heatmap of Decitabine-induced changes in expression of genes belonging to the Estrogen Response (GSEA) Hallmarks. Top differentially expressed genes plotted (FDR < 0.05).

(e) RefSeq annotation of Vehicle vs. Decitabine lost and gained ER binding sites in Gar15-13.

(f) Average signal intensity of MCF7 ER ChIP-seq binding at ER binding sites gained and lost with Decitabine treatment. Merged data from n = 3 replicates shown.

(g) Average signal intensity of ER ChIP-seq binding in primary ER+ breast tumours from patients at ER binding sites gained and lost with Decitabine treatment. ChIP-seq data obtained from Ross-Innes et al., 2012 (left panel) and Severson et al., 2018 (right panel). Merged data from n = 9 (left panel) and n = 6 (right panel) shown.

(h) Observed/expected log2 enrichment of consensus ER binding sites in MCF7 cell line and primary ER+ breast tumours from Ross-Innes et al., 2012 and Severson et al., 2018 at ER binding sites gained and lost with Decitabine treatment. * P value < 0.001; permutation test.

(i) Violin plot showing DNA methylation levels at gained ER binding sites in Decitabine-treated (n = 4) and Vehicle (n = 4) PDX Gar15-13 tumours.

(j) Browser snapshot of ER ChIP-seq and EPIC DNA methylation (Vehicle and Decitabine-treated tumours, n = 4 each), showing concomitant gain of ER binding and loss of DNA methylation at enhancer of ER target gene BTBD9.

(k) Violin plot showing DNA methylation levels at lost ER binding sites in Decitabine-treated (n = 4) and Vehicle (n = 4) Gar15-13 tumours.
Extended Data Fig. 3

(a) Heatmap showing Spearman pairwise correlations between the eigenvectors (PC1) in three Decitabine-treated and three Vehicle PDX Gar15-13 tumours. Samples are ordered according to complete linkage hierarchal clustering.

(b) Pie chart showing distributing of stable (A to A; B to B) and switching (A to B; B to A) compartments in Decitabine-treated tumours compared to Vehicle

(c) Barplot showing logFC expression between Vehicle and Decitabine-treated tumours of genes located either at A to B or B to A switching compartments. *P value: Wilcox rank-sum test.

(d) Volcano plot showing Decitabine vs. Vehicle differential expression of genes located at compartment that switched from B to A assignment in Decitabine-treated tumours.

(e) Browser snapshot of Hi-C eigenvectors and RNA-seq in Vehicle and Decitabine-treated tumours (n = 3 Hi-C and n = 4 RNA-seq each), showing demarcation of open (A-type; positive values) and closed (B-type; negative values) compartment changes across a region on chromosome 5, which is associated with increased expression of genes located within this region.

(f) Barplot showing log2 observed over expected A–A, B–B and B–A compartment interactions in Vehicle and Decitabine tumours. * P value two-tailed Students t-test < 0.05

(g) Average contact enrichment between pairs of 50Kb loci arranged by their PC1 eigenvector in Vehicle and Decitabine-treated tumours. The numbers at the center of the heatmaps indicate compartment strength calculated as the log2 ratio of (A–A + B–B)/(A–B + B–A) using the mean values.

(h) Boxplot of average insulation score calculated by TADtool at 50Kb resolution in three replicates of Decitabine-treated and Vehicle PDX tumours. * P value Wilcox rank-sum test.

(i) Violin plot showing the distribution in TAD sizes for Vehicle and Decitabine treated Gar15-13 tumours (n = 3 each).

(j) Average insulation score at differential and common TADs. Lines show mean values, while light shading represents SEM.

(k) Snapshot of region on chromosome 3, showing insulation score calculated in Vehicle and Decitabine-treated tumour Hi-C matrixes, demonstrating loss of TAD
boundary insulation is Decitabine-treated samples (indicated with a red box). Merged Hi-C data from replicates shown at 10Kb resolution. (I) Snapshot of region on chromosome 4, showing insulation score calculated in Vehicle and Decitabine-treated tumour Hi-C matrixes, demonstrating loss of TAD boundary insulation is Decitabine-treated samples (indicated with a red box). Merged Hi-C data from replicates shown at 10Kb resolution.
Extended Data Fig. 4

(a) Number of CHiCAGO significant interactions.

(b) Number of CHiCAGO significant interactions with P value = 0.0023.

(c) CHiCAGO Score.

(d) Genes at gained OE interactions.

(e) Percent of promoter-anchored interactions.

- Stable A compartments
- Stable B compartments
- A to B switching
- B to A switching

Gained OE: 37.65% 33.23% 48.66% 8.74%
Lost OE: 58.61% 61.14% 48.66% 8.12%
Gained bait: 37.65% 33.23% 48.66% 8.74%
Lost bait: 58.61% 61.14% 48.66% 8.12%
Extended Data Fig. 4

(a) Number of promoter baits and enhancer OEs involved in significant CHiCAGO interactions for each of the PCHi-C maps from Vehicle and Decitabine-treated Gar15-13 tumours (n = 3 each).

(b) Average number of promoter baits and enhancer OEs involved in significant CHiCAGO interactions across the three Vehicle and three Decitabine-treated PCHi-C replicates. Error bars indicate SD. P value Wilcoxon rank-sum test.

(c) Boxplot of ChICAGO scores of promoter-anchored interactions identified from PCHi-C data in Decitabine and Vehicle tumours. Data from n = 3 tumours shown.

(d) Volcano plot showing Decitabine vs. Vehicle differential expression of genes that are located at enhancer-promoter interactions gained with Decitabine treatment.

(e) Bar plot showing the percentage of promoter-anchored interactions located in chromosomal compartments that switched assignments (A to B or B to A) and stable A or B compartments.
Extended Data Fig. 5

(a) Transcription factor binding motifs enriched at promoter-anchored interactions involving dynamic promoter bait regions. Only motifs with FDR < 0.05 shown.

(b) The relative mRNA expression levels of *SPATA18* gene from RNA-seq (two-tailed t-test *P* < 0.05 derived from four replicates). Error bars indicate standard deviation from four samples.

(c) Kaplan–Meier survival plot showing the ability of *SPATA18* gene to stratify ER+ breast cancer patients in the METABRIC cohort into good and poor outcome groups. Data were analysed using the log-rank test. *P* values indicated within the graph.

(d) Browser snapshots showing the promoter-anchored interactions at the *MYO3B* ER target gene, together with ER ChIP-seq, EPIC DNA methylation and ChromHMM track. Merged replicate data shown (*n* = 4 each). In Decitabine-treated tumours, the *MYO3B* promoter displays increased number of interactions with an enhancer, which gains ER binding with Decitabine treatment. This gained ER binding site is also present in ER ChIP-seq data from primary ER+ breast tumours from Ross-Innes *et al.*, 2012 and Severson *et al.*, 2018. The relative expression of the *MYO3B* gene was significantly upregulated in Decitabine-treated tumours (two-tailed t-test *P* < 0.05 derived from four replicates) and associated with good outcome in ER+ breast cancer patients in the METABRIC cohort. Data were analysed using the log-rank test. *P* values indicated within the graph.

(e) The relative mRNA expression levels of *KRT8* gene from RNA-seq (two-tailed t-test *P* < 0.05 derived from four replicates). Error bars indicate standard deviation from four samples.

(f) Kaplan–Meier survival plot showing the ability of *KRT8* gene to stratify ER+ breast cancer patients in the METABRIC cohort into good and poor outcome groups. Data were analysed using the log-rank test. *P* values indicated within the graph.