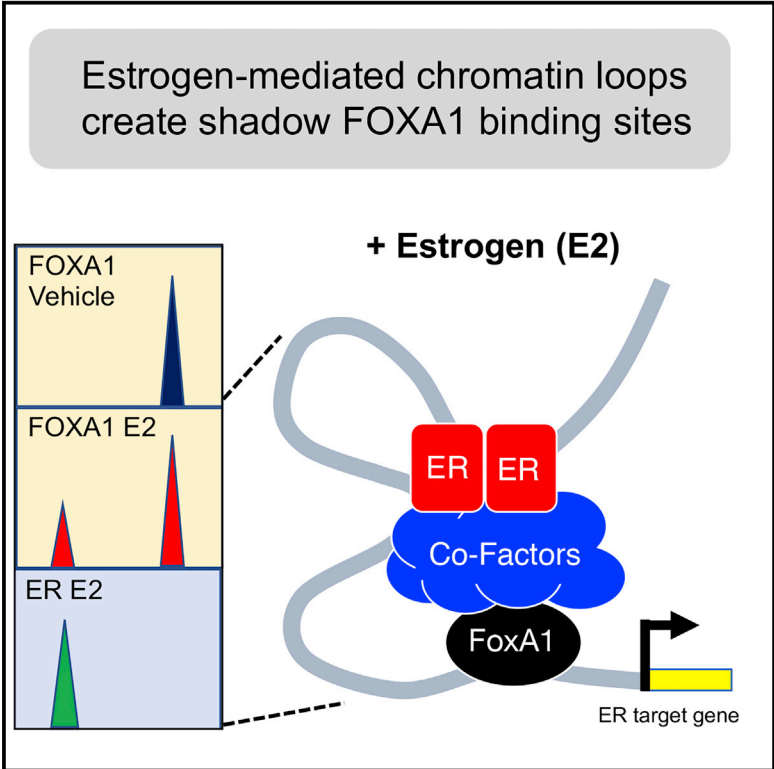


## Comprehensive Genomic Analysis Reveals that the Pioneering Function of FOXA1 Is Independent of Hormonal Signaling

### Graphical Abstract



### Authors

Silvia-E. Glont, Igor Chernukhin, Jason S. Carroll

### Correspondence

jason.carroll@cruk.cam.ac.uk

### In Brief

Glont et al. show that FOXA1 binding sites are not regulated by hormones. A small number (<1%) of FOXA1 binding events appear to be estrogen regulated, but these are shadow peaks that are created via pre-existing binding sites that form chromatin loops.

### Highlights

- FOXA1 binding events are not regulated by hormones
- Chromatin loops create shadow FOXA1 binding sites
- FOXA1 is a bone fide pioneer factor independent of hormone stimuli



# Comprehensive Genomic Analysis Reveals that the Pioneering Function of FOXA1 Is Independent of Hormonal Signaling

Silvia-E. Glont,<sup>1</sup> Igor Chernukhin,<sup>1</sup> and Jason S. Carroll<sup>1,2,\*</sup>

<sup>1</sup>Cancer Research UK Cambridge Institute, University of Cambridge, Robinson Way, Cambridge, CB2 0RE, UK

<sup>2</sup>Lead Contact

\*Correspondence: [jason.carroll@cruk.cam.ac.uk](mailto:jason.carroll@cruk.cam.ac.uk)

<https://doi.org/10.1016/j.celrep.2019.02.036>

## SUMMARY

Considerable work has linked hormone receptors, such as estrogen receptor- $\alpha$  (ER), with the pioneer factor FOXA1. Altered FOXA1 levels contribute to endocrine-resistant breast cancer, where it maintains ER-chromatin interactions, even in contexts in which cells are refractory to ER-targeted drugs. A recent study controversially suggests that FOXA1 binding can be induced by hormonal pathways, including the estrogen-ER complex. We now show that the vast majority (>99%) of FOXA1 binding events are unaffected by steroid activation. A small number (<1%) of FOXA1 binding sites appear to be induced by estrogen, but these are created from chromatin interactions between ER binding sites and adjacent FOXA1 binding sites and do not represent genuine new FOXA1-pioneering elements. FOXA1 is therefore not regulated by estrogen and remains a bone fide pioneer factor that is entirely upstream of the ER complex.

## INTRODUCTION

Although the term “pioneer factor” has been used recently for any transcription factor that can mediate binding of another transcription factor to chromatin, a bone fide pioneer can associate with condensed chromatin, independently of other factors, to initiate chromatin opening and creation of a *cis*-regulatory element (Zaret and Carroll, 2011). FOXA1 is the archetypal pioneer factor, capable of binding to compact chromatin independently of other proteins and creating a localized euchromatic environment (Cirillo et al., 1998, 2002). It can mediate estrogen receptor (ER) binding events in breast cancer cell lines (Carroll et al., 2005; Hurtado et al., 2011; Laganière et al., 2005), it is required for growth of drug-resistant cancer models (Hurtado et al., 2011), and it has been shown to directly contribute to endocrine resistance (Fu et al., 2016).

FOXA1 has been shown to be important for other nuclear receptors (NRs), such as androgen receptor (AR) in prostate cancer (Lupien et al., 2008), in which elevated levels can contribute to disease outcome (Jain et al., 2011; Robinson et al., 2014). A role for FOXA1 in castrate-resistant prostate cancer (CRPC) is

exemplified by the fact that models of CRPC, driven by AR splice variants, are still dependent on FOXA1 for cell growth (He et al., 2018; Jones et al., 2015).

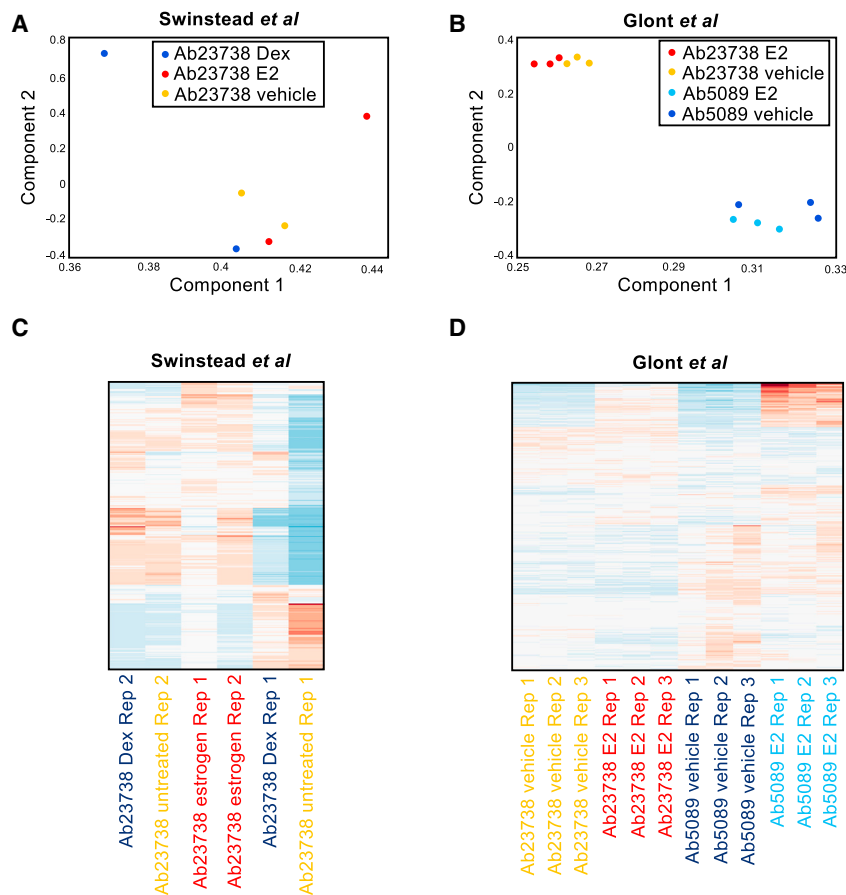
FOXA1 binding has been consistently implicated as an event that happens upstream of NR association with *cis*-regulatory elements, and experimental data to date show no change in FOXA1 binding when ER is modulated (Hurtado et al., 2011), and FOXA1 chromatin interaction does not require ER when exogenously expressed (Sérandour et al., 2011). The dependence on a single catalytic transcription factor for hormone receptor signaling represents an attractive therapeutic target (Jozwik and Carroll, 2012; Nakshatri and Badve, 2007). Importantly, an inhibitor targeting FOXA1 would circumvent many of the known mechanisms of resistance, including changes in NR fidelity, growth factor activation, changes in the occupancy of co-factors, and additional mechanisms that alter the binding potential or ligand dependency of the NR.

The aforementioned paradigms have recently been challenged, with a study suggesting that FOXA1 binding can be influenced by steroid activation of the cognate NR (Swinstead et al., 2016). This suggests that FOXA1 binding potential can be dictated partly by hormones, including estrogen and glucocorticoids. This questions the concept of transcription factor hierarchies, in which specialized transcription factors can function as biological pathway-determining catalysts. We have repeated the key genomic transcription factor mapping experiments that lead to the paradigm-challenging conclusions. We find that the estrogen-induced FOXA1 binding sites, which were described before (Swinstead et al., 2016), result from a lack of robust replicates and are not observable when additional, technically similar, chromatin immunoprecipitation sequencing (ChIP-seq) biological replicates are conducted. Any altered FOXA1 binding sites represent a tiny fraction of the overall FOXA1 binding sites (less than 1%) that result from chromatin loops that occur between *cis*-regulatory elements at estrogen-regulated gene regions, creating shadow binding events that do not represent new *cis*-regulatory elements.

## RESULTS

By mapping FOXA1 binding using ChIP-seq in ER+ breast cancer cells, Swinstead et al. (2016) concluded that FOXA1 binding could be substantially altered by hormonal steroid treatment. The primary conclusion that FOXA1 binding was hormonally regulated was based largely on the results from their ChIP-seq





**Figure 1. PCA and Unbiased Clustering of the Different ChIP-Seq Datasets**

Read densities from aligned libraries of equal size of 20 million reads were measured on corresponding FOXA1 binding sites from Swinstead et al. (2016) (GEO: GSE72249).

(A) The peaks for all treatments were merged in a single set prior to the measurement for each study, and obtained data were subjected to PCA. The PCA plots illustrate degree of similarity between the replicates. Spearman rank correlation between ER-mediated chromatin interactions (ChIA-PET) and the 357 estrogen-induced FOXA1 binding sites (ab5089). (B) Hierarchical clustering of the Swinstead et al. (2016) binding sites. For hierarchical clustering of the Swinstead et al. (2016) binding sites, the yielded read densities were normalized using median absolute deviation and clustered in MATLAB framework using the “ward” method with the linkage function. The duplicate samples from Swinstead et al. (2016) did not cluster on the basis of treatment condition. (C) PCA of our FOXA1 ChIP-seq generated with two different FOXA1 antibodies (ab23738 and ab5089). (D) Hierarchical clustering of our FOXA1 binding sites, showing clustering on the basis of replicates.

experiments. We downloaded their FOXA1 ChIP-seq data, obtained in breast cancer cell lines, but could not reproduce the binding numbers described in the publication, because of insufficient information about peak calling and how input DNA was integrated into the analyses. We used the peak coordinates described by Swinstead et al. (2016) and compared read densities of their duplicate libraries mapped to those coordinates using both principal-component analysis (PCA) and hierarchical clustering. Their samples did not cluster by treatment condition when assessed using PCA, and samples from the same treatment condition showed substantial variability (Figure 1A), suggesting that the replicate samples were not similar. This lack of consistency between duplicates is a potential source of false-positive “differential” binding sites. As expected, differential peak patterns showed little consistency between replicates (Figure 1B), implying that any differential binding sites might be due to technical variability between replicates. Given this replicate-to-replicate variability (even between samples of the same treatment conditions), the lack of any ChIP-qPCR validation, and the significant implication of the conclusions, we sought to repeat the key ChIP-seq experiments to determine if FOXA1 binding was in fact modulated by hormonal stimulation, as claimed (Swinstead et al., 2016).

We hormone deprived MCF-7 and ZR-75-1 breast cancer cells and treated with vehicle or estrogen for 45 min, a duration shown

to result in maximal ER binding and enhancer activity (Shang et al., 2000). ER ChIP-qPCR was conducted at known binding loci (Figure S1; Table S1) in order to confirm the estrogen response. We subsequently conducted FOXA1 ChIP-seq experiments using two different antibodies in both cell line models with three biological replicates from independent passages. Importantly, these were collected from matched experiments used to confirm estrogen responsiveness (Figure S1). One of the antibodies used in our study was the same antibody (ab23738) used by Swinstead et al. (2016). Matched input samples were included for each experiment. Peaks were called using MACS2 (Ross-Innes et al., 2012; Stark and Brown, 2011). In MCF-7 cells, this resulted in 64,823 FOXA1 peaks in vehicle-treated and 62,000 peaks in estrogen-treated conditions using the same antibody as Swinstead et al. (2016) and 37,318 vehicle and 35,925 estrogen FOXA1 peaks with the second independent antibody ab5089 (Table S2). PCA of our samples showed that the samples clustered tightly on the basis of replicates (Figure 1C), providing confidence when comparing peaks (Figure 1D). The samples clustered on the basis of the antibody used for ChIP-seq and showed minimal difference between vehicle or estrogen conditions. In ZR-75-1 cells, the ab23738 antibody generated 70,602 FOXA1 peaks in vehicle conditions and 66,604 peaks in estrogen conditions. The second antibody (ab5089) generated 35,763 FOXA1 peaks in vehicle conditions and 31,361 peaks in estrogen conditions (Table S2). As such, estrogen treatment did not result in a global increase in FOXA1 binding events, with either antibody or in either cell line assessed.

One possibility is that FOXA1 binding could be redistributed, resulting in similar binding numbers, but at different locations

in the genome. We therefore performed DiffBind analyses (Ross-Innes et al., 2012) (Table S3) and observed no FOXA1 redistribution. In MCF-7 cells, there were 14 estrogen-induced peaks with the ab23738 antibody and 2 peaks enriched in vehicle conditions, representing 0.02% of all FOXA1 peaks that are estrogen induced (Figures 2A and 2C). This is in contrast to the results obtained using the exact same antibody and cell line in Swinstead et al. (2016), attesting to the potential problems that result from lack of sufficient replicates. The biggest change observed in any of the ChIP-seq experiments we undertook was in MCF-7 cells using the ab5089 FOXA1 antibody (which was not used by Swinstead et al., 2016) (Figures 2A–2C), which revealed a total of 357 FOXA1 peaks enriched in estrogen conditions (representing less than 1% of all peaks called) and 5 peaks enriched in vehicle conditions (Figure 2B).

To establish the degree of variability in this ChIP-seq experiment conducted with sufficient biological replicates, we purposely mixed up the samples from the ab23738 antibody-based ChIP-seq in different combinations and subsequently called peaks. Following DiffBind analysis, we found between 121 and 180 peaks that were considered differential, even in samples that were randomly mixed up with the incorrect treatment samples, representing ~0.5% of all peaks.

In the ZR-75-1 cell line, we observed 23 estrogen-enriched and 2 vehicle-enriched FOXA1 binding sites using the same FOXA1 antibody used by Swinstead et al. (2016) (Figures 2D and 2F). This small number of estrogen-induced FOXA1 binding sites represents less than 0.03% of all peaks. When using the second FOXA1 antibody (ab5089) in ZR-75-1 cells, we found 109 estrogen-induced FOXA1 binding sites (0.03% of total FOXA1 binding sites) and 1 vehicle-enriched site (Figure 2E).

Our ChIP-seq data with two different FOXA1 antibodies, conducted in two independent cell line models, revealed that 0.02%–1% of the FOXA1 binding sites were induced by estrogen. This is in contrast to Swinstead et al. (2016), who claimed that there is an appreciable number of FOXA1 binding events that can be hormonally regulated. Importantly, the same antibody that was used by Swinstead et al. (2016) revealed no significant changes in FOXA1 binding in either cell line model in our ChIP-seq analysis.

The second FOXA1 antibody (ab5089) that we used produced a small number of estrogen-induced FOXA1 binding sites (357 sites), although it is important to note that these differential binding events constitute less than 1% of total FOXA1 binding events in the ChIP-seq dataset. Only 28 common FOXA1 binding events were identified in both MCF-7 and ZR-75-1 cell lines, implying that these differential sites are not reproducible between different cancer models (Figure 2G).

Further analysis of the estrogen-induced FOXA1 binding sites in MCF-7 and ZR-75-1 revealed the estrogen responsive element (ERE) motif ( $p = 1 \times 10^{-42}$ ), but no forkhead motifs (Figure 2H), suggesting that FOXA1 is not directly interacting with the chromatin at these regions. On the basis of the motif analysis, we hypothesized that the small number of estrogen-induced FOXA1 binding sites might be indirect FOXA1 binding events, potentially mediated via chromatin loops connecting estrogen-induced genes and their enhancers.

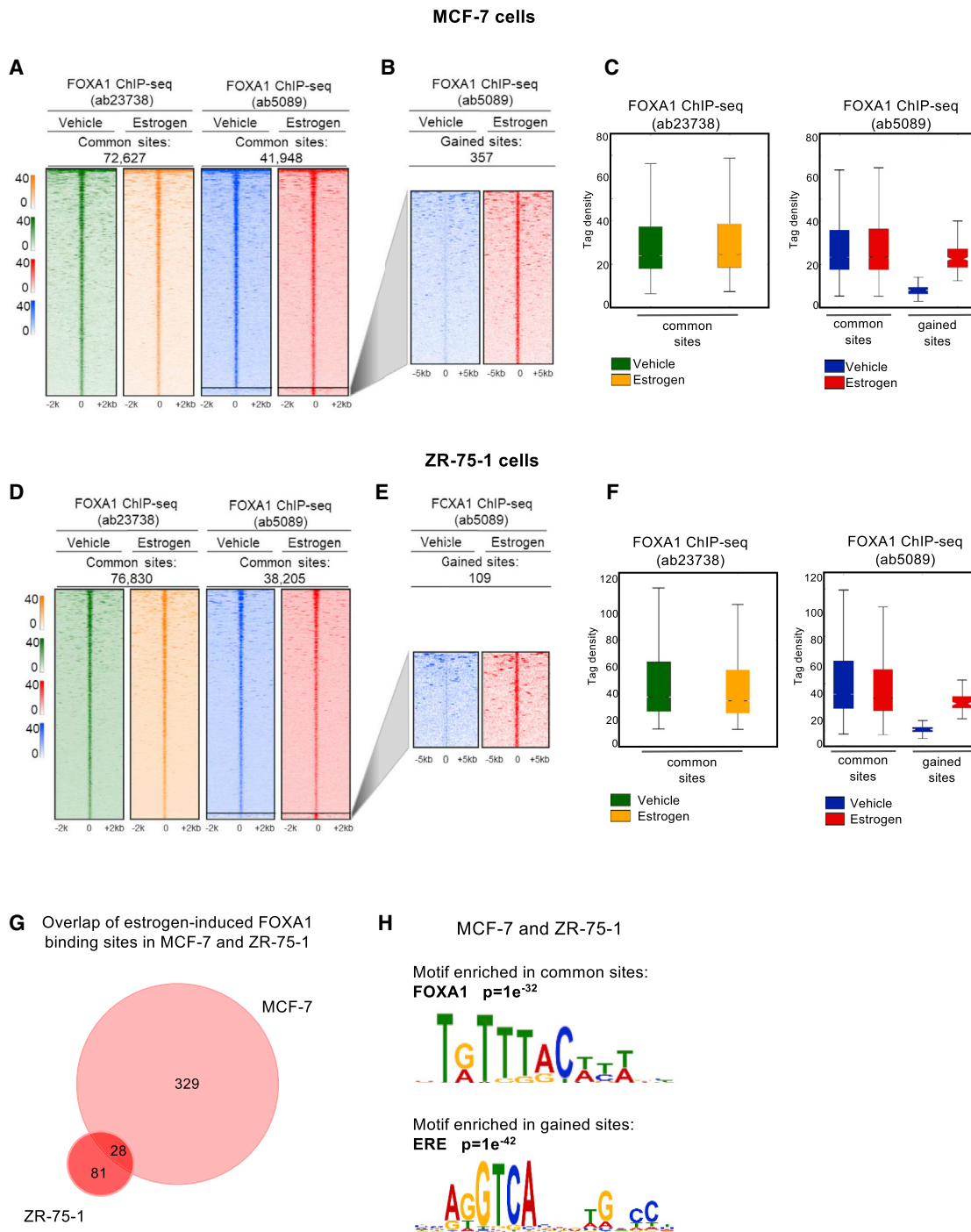
Given the wealth of genomic, transcriptomic, and chromatin looping data in the MCF-7 cell line model, we investigated the underlying properties of the 357 estrogen-induced FOXA1 binding sites. We used published RNA-seq data following estrogen treatment of MCF-7 cells (Figure 3A) and observed that the 357 estrogen-induced FOXA1 binding sites were significantly biased toward the most estrogen-regulated genes (Figure 3B) with almost all of the binding sites within *cis*-regulatory domains adjacent to ER target genes.

It is well established that lineage-specific genes tend to be regulated by clusters of transcription factor binding sites (Hnisz et al., 2013; Whyte et al., 2013). This is true for estrogen-regulated genes, in which the classic estrogen-induced genes (i.e., those with the greatest estrogen response) are regulated by clusters of closely associated *cis*-regulatory domains (Carroll et al., 2006). Several well-characterized ER target genes are shown in Figure 3C as examples. As typified by the examples shown, there are FOXA1 and ER co-bound regions, but importantly, there are sites at which one transcription factor binds but the other one does not. The 357 estrogen-induced FOXA1 binding sites are all adjacent to an independent ER binding event and other FOXA1 binding sites (Figures 3D and 3E), indicating their presence in regions of enriched transcription factor binding.

Following estrogen-mediated stimulation, physical associations between *cis*-regulatory elements occur (Fullwood et al., 2009; Pan et al., 2008), and we postulated that FOXA1 could associate with adjacent ER binding sites through chromatin looping. Because of the cross-linking in the ChIP-seq protocol, these indirect chromatin loops create FOXA1 binding sites that are not direct *cis*-regulatory elements and therefore represent “shadow peaks.” At these regions, FOXA1 does not function as a pioneer factor, and new regulatory elements are not created. Our hypothesis is that the small fraction (<1%) of FOXA1 binding events that appear to be induced by estrogen are in fact simply indirect peaks mediated via ER at those genomic regions. To assess this possibility, we used the ChIA-PET (chromatin interaction analysis by paired-end tag sequencing) data that provide an unbiased map of the ER-mediated chromatin interactions that occur, in the presence of estrogen, in MCF-7 cells (Fullwood et al., 2009). Of the 357 estrogen-induced FOXA1 peaks in MCF-7 cells, 89% were detected in experimentally identified ER ChIA-PET chromatin loops (Figure 4A). Examples of estrogen-induced FOXA1 binding sites existing within ChIA-PET chromatin loops are shown in Figure 4B. This finding confirms that the limited number of estrogen-induced FOXA1 binding events are in fact created by clusters of *cis*-regulatory elements brought into proximity during gene expression. Therefore, FOXA1 is a bone fide pioneer factor that binds upstream of NRs, and direct FOXA1-chromatin binding is not influenced by steroid hormones.

## DISCUSSION

It is well established that many NRs and other transcription factors regulate genes from significant distances (Carroll et al., 2005; Lin et al., 2007). However, additional factors are required for NR to work (Glass and Rosenfeld, 2000; Shang et al., 2000). Recent observations have revealed that cells containing

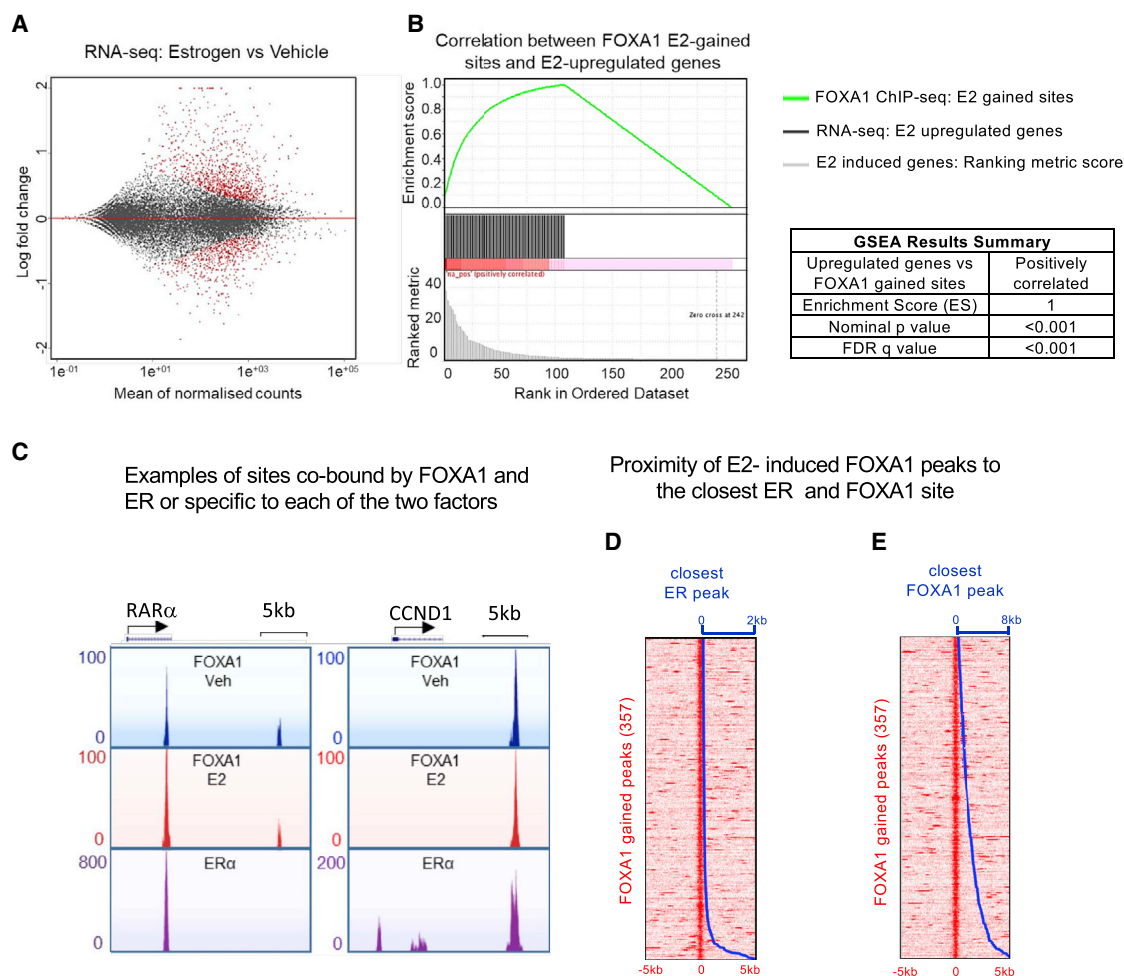


**Figure 2. Analysis of FOXA1 ChIP-Seq Binding with Two Separate Antibodies in Response to Estrogen Treatment in MCF-7 and ZR-75-1 Cells** (A, C, D, and F) ChIP-seq tag densities visualized at FOXA1-occupied genomic locations in control and estrogen-treated MCF-7 (A and C) and ZR-75-1 (D and F) cells, using antibodies ab23738 and ab5089.

(B and E) Zoomed heatmap shows differential binding of FOXA1 specific to ab5089 in MCF-7 cells (B) and ZR-75-1 (E), respectively.

(G) Overlap of estrogen-enriched FOXA1 binding sites between MCF-7 and ZR-75-1 cells.

(H) Transcription factor motifs found overrepresented in the common and estrogen-induced FOXA1 sites.



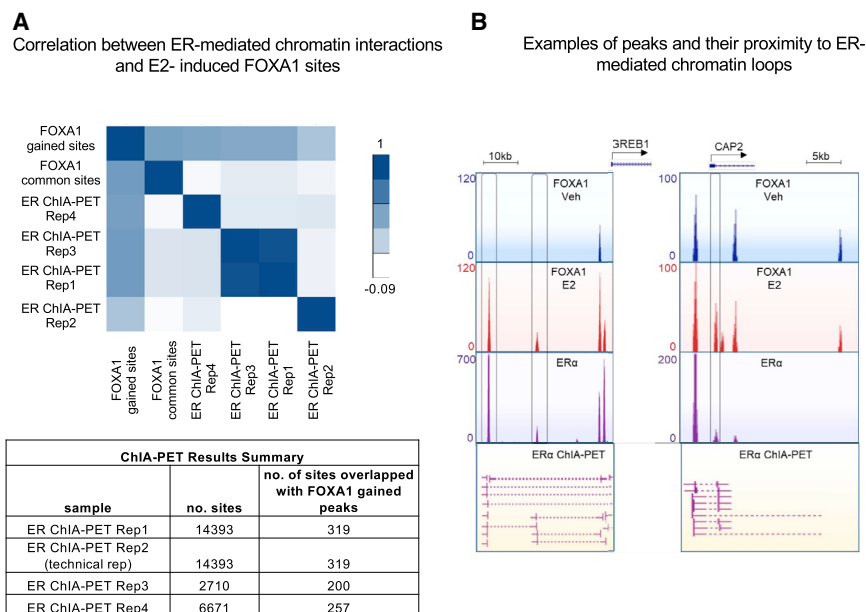
**Figure 3. Integration of the Estrogen-Enriched FOXA1 Binding Events with Estrogen-Mediated Gene Expression Events**

(A) RNA sequencing (RNA-seq) expression profile following short-term (3 h) estrogen treatment of MCF-7, shown as a dispersion plot. (B) Gene set enrichment analysis (GSEA) pre-ranked test correlating estrogen-induced genes with the 357 estrogen-induced FOXA1 binding sites. (C) Examples of sites co-bound by FOXA1 and ER, as well as sites unique to each of the two transcription factors. (D and E) Proximity of estrogen-induced FOXA1 peaks and the closest ER (D) or FOXA1 (E) site. Heatmap represents FOXA1-gained sites in red.

mutations in ER (ESR1) can be enriched because of selective pressure imposed by specific ER-targeted drugs (Merenbakh-Lamin et al., 2013; Robinson et al., 2013; Toy et al., 2013), resulting in ligand-independent ER activity. As such, there is a significant interest in defining critical components of the ER complex that might constitute potential drug targets. One such protein is FOXA1, a pioneer factor, shown to facilitate chromatin “opening” independently of additional proteins, enabling binding and activity of other transcription factors. Importantly, this includes ER in breast cancer and AR in prostate cancer. Although additional modes of NR binding can occur, such as assisted loading, involving complexes of multiple ATP-dependent chromatin factors (Voss et al., 2011), an absolute dependence on a single functionally catalytic protein, such as FOXA1, holds promise for therapeutic exploitation.

FOXA1 has been shown to be required for growth of resistant cancers (Hurtado et al., 2011), it contributes to endocrine resis-

tance (Fu et al., 2016), and, importantly, it is essential for ER binding and activity, even in endocrine-resistant contexts (Hurtado et al., 2011). This places FOXA1 as a key driver of resistance and reveals a vulnerability in the ER pathway, where absolute dependence on a single upstream pioneer factor creates an opportunity for therapeutic intervention, potentially overcoming known mechanisms of resistance. Interest in FOXA1 as a therapeutic target for ER+ breast cancer (Jozwik and Carroll, 2012; Nakshatri and Badve, 2007, 2009) was compromised by recent claims that FOXA1 binding is estrogen regulated (Swinstead et al., 2016). The significance of this conclusion means that ER-targeted agents should, in theory, show effectiveness in inhibiting FOXA1 binding and transcriptional potential, reducing the need to develop direct FOXA1 inhibitors. Our comprehensive analysis of FOXA1 binding following estrogen stimulation reveals no appreciable estrogen regulation of FOXA1 binding. Different antibodies and different



**Figure 4. ER Binding Mediates Indirect FOXA1 Binding via Chromatin Looping at *cis*-Regulatory Elements**

(A) Correlation between ER-mediated chromatin interactions (ChIA-PET) and the 357 estrogen-induced FOXA1 binding sites (ab5089). The table shows the correlation values between ChIA-PET interactions and the 357 estrogen-induced FOXA1 binding sites.

(B) Examples of ER and FOXA1 peaks at regions that are involved in chromatin loops, as detected by ChIA-PET. The images of the ChIA-PET loops are taken from Fullwood et al. (2009).

ER+ breast cancer cell line models show that >99% of FOXA1 binding sites are impervious to hormonal context. The residual FOXA1 changes represent less than 1% of FOXA1 binding events and result from peaks formed within clusters of ER/FOXA1 binding sites at genes that are estrogen regulated. As such, these lack the hallmarks of genuine FOXA1 binding sites, they do not result in the creation of new regulatory elements, and they do not result in new gene expression events. The lack of robust, reproducible FOXA1 binding sites confirms that FOXA1 binding is not estrogen regulated and functions upstream of ER activity. In support of this conclusion, previous experimental data showed that the breast cancer treatment fulvestrant (ICI 182780), an ER degrader, does not alter FOXA1 binding (Hurtado et al., 2011).

The major distinction in conclusions between the work of Swinstead et al. (2016) and the present dataset results from technical differences that can be attributed to insufficient replicates in the previous study (Figure 1). A lack of biological and/or technical replicates is a source of problems in the reproducibility of ChIP-seq datasets, particularly when claiming treatment or condition-specific binding events. We conclude that recent claims of estrogen-mediated FOXA1 binding events are influenced, in large part, by a lack of independent biological ChIP-seq replicates and duplicate samples that show unacceptable variability between purportedly replicate samples (Figures 1A and 1B).

Swinstead et al. (2016) identified similar steroid hormone changes in FOXA1 binding in two distinct systems, namely, estrogen responsiveness and dexamethasone (dex) activation of glucocorticoid receptor (GR) (Swinstead et al., 2016). Although we have only focused on the estrogen-treated conditions, it is reasonable to assume that the majority of dex-mediated changes in FOXA1 are also false positives that result from a lack of independent biological replicates. This is based on the fact that the experimental approach was comparable, and the

same degree of differential FOXA1 binding was observed in both hormonal systems. The conclusion that steroids could change FOXA1 binding was suggested in large part by ChIP-seq analyses. In addition to these assays, Swinstead et al. (2016) also assessed FOXA1 chromatin dwell time using an exogenous, tagged FOXA1-based approach. Despite the caveat that exogenous FOXA1 alters levels and potentially the function of endogenous FOXA1, and the tagged protein might not faithfully recapitulate endogenous FOXA1, there was a minimal change in FOXA1 dwell time comparing the presence or absence of estrogen, suggesting that this non-ChIP-based method supports the conclusion that FOXA1 binding is not altered in an appreciable way by hormone status.

Understanding what enables FOXA1 binding is of importance, and recent suggestions that steroid hormones could function in this capacity to modulate FOXA1-DNA binding potential (Swinstead et al., 2016) present an attractive hypothesis. We show that the vast majority (>99%) of FOXA1 binding is not regulated by estrogen, and the small fraction of altered FOXA1 binding events are created via chromatin interactions during the course of ER-mediated gene expression. FOXA1 therefore exists entirely upstream of the NR, its chromatin binding capacity is not influenced by estrogen signaling, and it remains a relevant and important drug target in hormone-dependent cancers.

## STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- CONTACT FOR REAGENT AND RESOURCE SHARING
- EXPERIMENTAL MODEL AND SUBJECT DETAILS
  - Cell culture
- METHOD DETAILS
  - Chromatin Immunoprecipitation
  - Integration of RNA-seq and ChIP-seq data
- QUANTIFICATION AND STATISTICAL ANALYSIS
  - ChIP Sequencing Analysis
- DATA AND SOFTWARE AVAILABILITY

## SUPPLEMENTAL INFORMATION

Supplemental Information includes one figure and three tables and can be found with this article online at <https://doi.org/10.1016/j.celrep.2019.02.036>.

## ACKNOWLEDGMENTS

We would like to thank the genomics core and the Bioinformatics Core at Cancer Research UK (CRUK) Cambridge Institute. We thank Dr. Sankari Nagarajan and Dr. Rasmus Siersbæk from CRUK Cambridge Institute for critical reading of the manuscript. We thank Danya Cheeseman and Emily Archer Goode for help compiling information about the methods. We acknowledge the support of the University of Cambridge and Cancer Research UK. J.S.C. is supported by an ERC Consolidator award (project number 646876), CRUK funding, and a Susan G. Komen Breast Cancer Foundation scholarship.

## AUTHOR CONTRIBUTIONS

Experiments were designed by S.-E.G. and J.S.C., and all work was conducted by S.-E.G. All bioinformatic work was conducted by I.C. All authors wrote the paper.

## DECLARATION OF INTERESTS

J.C. is the founder and CSO of Azeria Therapeutics.

Received: April 12, 2018

Revised: October 17, 2018

Accepted: February 11, 2019

Published: March 5, 2019

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## STAR★METHODS

### KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
<b>Antibodies</b>		
Rabbit Anti- ER $\alpha$ (HC-20) polyclonal antibody	Santa Cruz	Cat# sc-543, RRID; AB_631471
Goat Anti-FOXA1 polyclonal antibody – ChIP grade	Abcam	Cat# ab5089, RRID; AB_304744
Rabbit Anti-FOXA1 polyclonal antibody – ChIP grade	Abcam	Cat# ab23738, RRID; AB_2104842
<b>Chemicals, Peptides, and Recombinant Proteins</b>		
Dynabeads Protein A	Invitrogen	Cat#10001D
Dynabeads Protein G	Invitrogen	Cat#10003D
Pierce 16% Formaldehyde (w/v), Methanol-free	Thermo Scientific	Cat# 28908
$\beta$ -Estradiol	Sigma-Aldrich	Cat# E8875
Dulbecco's Modified Eagle Medium (DMEM)	GIBCO	Cat# 41966029
RPMI 1640 Medium	GIBCO	Cat# 21875034
Fetal Bovine Serum, qualified, heat inactivated	GIBCO	Cat# 16140071
Fetal Bovine Serum, charcoal stripped	GIBCO	Cat# 12676029
Penicillin-Streptomycin	GIBCO	Cat#15070063
L-Glutamine (200 mM)	GIBCO	Cat# 25030081
Trypsin-EDTA (0.5%), no phenol red	GIBCO	Cat# 15400054
cOmplete EDTA-free Protease inhibitor cocktail	Sigma-Aldrich	Cat# 05056489 001
Phosphatase Inhibitor cocktail	Thermo Scientific	Cat#78427
<b>Critical Commercial Assays</b>		
ThruPlex DNA-seq kit	Rubicon Genomics	Cat# R400407
<b>Deposited Data</b>		
Gene Expression Omnibus (GEO)	<a href="https://www.ncbi.nlm.nih.gov/geo/">https://www.ncbi.nlm.nih.gov/geo/</a>	GSE112969; RRID:SCR_005012
<b>Experimental Models: Cell Lines</b>		
MCF-7	ATCC	Cat# HTB-22, RRID:CVCL_0031; ATCC HTB-22
ZR-75-1	ATCC	Cat# CRL-1500, RRID:CVCL_0588; ATCC CRL-1500
<b>Oligonucleotides</b>		
Primer for ChIP Forward: ER3 negative site (5'- GCCACCAGCCTGCTTTCTGT-3')	This study	n/a
Primer for ChIP Reverse: ER3 negative site (5'- CGTGGATGGGTCCGAGAAAC-3')	This study	n/a
Primer for ChIP Forward: XBP1 negative site (5'- ACCCTCCAAAATTCTTCTGC-3')	This study	n/a
Primer for ChIP Reverse: XBP1 negative site (5'- ATGAGCATCTGAGAGCAAGC-3')	This study	n/a
Primer for ChIP Forward: XBP1 target site (5'- ATACTTGGCAGCCTGTGACC-3')	This study	n/a
Primer for ChIP Reverse: XBP1 target site (5'- GGTCCACAAAGCAGGAAAAA-3')	This study	n/a

(Continued on next page)

<b>Continued</b>		
REAGENT or RESOURCE	SOURCE	IDENTIFIER
Primer for ChIP Forward: GREB1 target site (5'- GAAGGGCAGAGCTGATAACG-3')	This study	n/a
Primer for ChIP Reverse: GREB1 target site (5'- GACCCAGTTGCCACACTTTT-3')	This study	n/a
Primer for ChIP Forward: MYC target site (5'- GCTCTGGGCACACACATTGG-3')	This study	n/a
Primer for ChIP Reverse: MYC target site (5'- GGCTACCCTTGCTGATGCT-3')	This study	n/a
<b>Software and Algorithms</b>		
Bowtie 2 v2.2.6	Langmead and Salzberg, 2012	<a href="https://sourceforge.net/projects/bowtie-bio/files/bowtie2/2.2.6/">https://sourceforge.net/projects/bowtie-bio/files/bowtie2/2.2.6/</a> ; RRID:SCR_016368
MEME tool FIMO v4.9.1	Bailey et al., 2009	<a href="http://meme-suite.org/doc/install.html?man_type=web">http://meme-suite.org/doc/install.html?man_type=web</a> ; RRID:SCR_001783
JASPAR CORE 2016 vertebrates	JASPAR	<a href="http://jaspar.genereg.net/matrix-clusters/vertebrates/">http://jaspar.genereg.net/matrix-clusters/vertebrates/</a> ; RRID:SCR_003030
MACS2 version 2.0.10.20131216	Zhang et al., 2008	<a href="https://pypi.org/project/MACS2/2.0.10.20131216/">https://pypi.org/project/MACS2/2.0.10.20131216/</a> ; RRID:SCR_013291
GSEAPreranked (18) analysis tool Gene Set Enrichment Analysis (GSEA) v2.2.3	Broad Institute, Massachusetts Institute of Technology	<a href="http://www.broadinstitute.org/gsea/">http://www.broadinstitute.org/gsea/</a> ; RRID:SCR_003199
Diffbind	Stark and Brown, 2011	<a href="https://bioconductor.org/packages/release/bioc/html/DiffBind.html">https://bioconductor.org/packages/release/bioc/html/DiffBind.html</a> ; RRID:SCR_012918
DESeq2	Love et al., 2014	<a href="https://bioconductor.org/packages/release/bioc/html/DESeq2.html">https://bioconductor.org/packages/release/bioc/html/DESeq2.html</a> ; RRID:SCR_015687
<b>Other</b>		
Bioruptor Plus sonicator	Diagenode	n/a

## CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Jason Carroll ([Jason.carroll@cruk.cam.ac.uk](mailto:Jason.carroll@cruk.cam.ac.uk)).

## EXPERIMENTAL MODEL AND SUBJECT DETAILS

### Cell culture

MCF-7 and ZR-75-1 cell lines were obtained from ATCC (Middlesex, UK) and represent female breast cancer cell line models. MCF-7 cells were cultured in Dulbecco's Modified Eagle Medium DMEM (GIBCO, Thermo Scientific, Leicestershire, UK, ref. 41966). ZR-75-1 cells were grown in RPMI-1640 medium (GIBCO, Thermo Scientific, Leicestershire, UK, ref. 21875-034). Both media were supplemented with fetal bovine serum (FBS), 50 U/ml penicillin, 50 µg/ml streptomycin and 2 mM L-glutamine.

MCF-7 and ZR-75-1 cells were seeded and treated either with ethanol or with 10nM Estrogen (Sigma) for 45 minutes previously described (Schmidt et al., 2009). All cell lines were regularly genotyped to ensure they were the correct cell lines.

## METHOD DETAILS

### Chromatin Immunoprecipitation

To validate the Estrogen induction, ER ChIP-qPCR was performed using the rabbit polyclonal sc-543 (Santa Cruz) antibody. FOXA1 ChIP-seq was performed using the goat polyclonal ab5089 (Abcam), and rabbit polyclonal ab23738 (Abcam) antibodies. Chromatin was prepared as previously described (Schmidt et al., 2009). DNA was isolated and purified using the phenol-chloroform-isoamyl DNA extraction method. ChIP-seq and the input libraries were prepared using the ThruPlex® DNA-seq kit (Rubicon Genomics, ref. R400407).

### Integration of RNA-seq and ChIP-seq data

Genes located around  $\pm 50$  kb from the peak regions were selected.  $-\log_{10}$  transformed p values from DESeq2 analyses of the RNA-Seq data were subsequently used for ranking and weighting of genes. GSEAPreranked (18) analysis tool from Gene Set Enrichment Analysis (GSEA) software, version 2.2.3, was used for the evaluation of statistically significant genes.

## QUANTIFICATION AND STATISTICAL ANALYSIS

### ChIP Sequencing Analysis

ER ChIP-qPCR and FOXA1 ChIP-seq were performed in biological triplicates, using cells from independent passages.

ChIP-seq reads were mapped to hg38 genome using bowtie2 2.2.6 (Langmead and Salzberg, 2012). Aligned reads with the mapping quality less than 5 were filtered out. The read alignments from three replicates were combined into a single library and peaks were called using MACS2 version 2.0.10.20131216 (Zhang et al., 2008) with sequences from MCF7 chromatin extracts as a background input control. The peaks yielded with MACS2 q value  $\leq 1e-3$  were selected for downstream analysis. MEME tool FIMO version 4.9.1 (Bailey et al., 2009) was used for searching all known TF motifs from JASPAR database (JASPAR CORE 2016 vertebrates) in the tag-enriched sequences. As a background control, peak size - matching sequences corresponding to known open chromatin regions in MCF7 cells were randomly selected from hg38. Motif frequency for both tag-enriched and control sequences calculated as sum of motif occurrences adjusted with MEM q-value. Motif enrichment analysis was performed by calculating odds of finding an overrepresented motif among MACS2-defined peaks by fitting Student's t-cumulative distribution to the ratios of motif frequencies between tag-enriched and background sequences. Yielded p values were further adjusted using Benjamini-Hochberg correction.

For visualizing tag density and signal distribution, heatmaps were generated with the read coverage in a window of  $\pm 2.5$  or 5 kb region flanking the tag midpoint using the bin size of 1/100 of the window length. Differential binding analysis (Diffbind) was performed as described previously (Stark and Brown, 2011).

## DATA AND SOFTWARE AVAILABILITY

All ChIP-seq data is deposited in GEO under the accession number: GSE112969. Data can be accessed using the password: gzmtegactlqtxwp