The effects of breast cancer therapy on estrogen receptor signaling throughout the body

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Chapter 3

Comparative Cistromics Reveals Genomic Cross-talk between FOXA1 and ERα in Tamoxifen-Associated Endometrial Carcinomas

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Abstract

Tamoxifen, a small-molecule antagonist of the transcription factor estrogen receptor alpha (ERα) used to treat breast cancer, increases risks of endometrial cancer. However, no parallels of ERα transcriptional action in breast and endometrial tumors have been found that might explain this effect. In this study, we addressed this issue with a genome-wide assessment of ERα-chromatin interactions in surgical specimens obtained from patients with tamoxifen-associated endometrial cancer.

ERα was found at active enhancers in endometrial cancer cells as marked by the presence of RNA polymerase II and the histone marker H3K27Ac. These ERα binding sites were highly conserved between breast and endometrial cancer and enriched in binding motifs for the transcription factor FOXA1, which displayed substantial overlap with ERα binding sites proximal to genes involved in classical ERα target genes.

Multifactorial ChIP-seq data integration from the endometrial cancer cell line Ishikawa illustrated a functional genomic network involving ERα and FOXA1 together with the enhancer-enriched transcriptional regulators p300, FOXM1, TEAD4, FNFIC, CEBP8, and TCF12. Immunohistochemical analysis of 230 primary endometrial tumor specimens showed that lack of FOXA1 and ERα expression was associated with a longer interval between breast cancer and the emergence of endometrial cancer, exclusively in tamoxifen-treated patients.

Our results define conserved sites for a genomic interplay between FOXA1 and ERα in breast cancer and tamoxifen-associated endometrial cancer. In addition, FOXA1 and ERα are associated with the interval time between breast cancer and endometrial cancer only in tamoxifen-treated breast cancer patients.

Abbreviations

ChIP-seq, Chromatin Immunoprecipitation coupled with massive parallel sequencing; CEAS, cis-regulatory element annotation system; CPM, in counts per million; DEX, dexamethasone; E2, estradiol; ERα, estrogen receptor alpha; EtOH, ethanol; FOXA1, Forkhead box protein A1; FPKM, fragments per kilobase of exon per million fragments mapped; IPA, Ingenuity Pathway Analysis; NR3C1, Glucocorticoid Receptor; RNA Pol II, RNA polymerase II; TAMARISK, Tamoxifen Associated Malignancies: Aspects of RISK; TMA, tissue microarray.

Introduction

Of all breast tumors, roughly 75% depend on ERα for cell proliferation and tumor progression. Consequently, most breast cancer treatment modalities are aimed to inhibit ERα activity. Tamoxifen is the most widely applied hormonal therapy, which acts through competitive inhibition of ERα's natural ligand estrogen[1]. Although tamoxifen inhibits ERα in breast cells, it stimulates ERα in certain other tissues, including the endometrium, specifically in a low estrogen environment, i.e., postmenopausal women[2–6].

Due to tissue-selective action of tamoxifen, postmenopausal breast cancer patients on this drug have an increased risk of endometrial cancer development by 2- to 7-fold, depending on the duration of use[4,5,7,8]. This increased ERα activity was linked with an altered expression level of coactivator SRC1[9] and PAX2[10] in endometrial cancer cells. However, manipulating expression levels of either SRC1 or PAX2 in breast cancer cell line models did not support these findings[11,12], implying that these proteins are not the sole drivers of agonistic features of tamoxifen observed in endometrial tissue. In addition, ERα transcriptional regulation in endometrial cancer has exclusively been studied using cell lines[13] while ERα genomic behavior in primary human endometrial tumors remains unexplored.

For ERα to bind the chromatin in breast cancer cells, it requires FOXA1[14–16]. FOXA1 is a pioneer factor, regulating chromatin accessibility and thereby enables ERα-dependent gene activation and proliferation of breast cancer cells[14]. FOXA1 was previously identified as one of the luminal breast cancer–defining transcription factors[17,18], and its expression correlates with a favorable outcome in breast cancer[19–21]. In ERα-negative breast cancer cells, exogenous introduction of ERα and FOXA1 along with GATA3 was sufficient to reprogram these cells toward hormone responsiveness[22]. Cumulatively, these reports position FOXA1 as a crucial player in ERα functionality in breast cancer. In endometrial cancer, the pioneer factor for ERα is unknown, and the mechanisms that dictate ERα action in this tissue remain elusive.

To study the genomic features of ERα in endometrial tumors, we performed chromatin immunoprecipitation sequencing (ChIP-seq) in surgical samples from five endometrial tumors of patients who were previously treated with tamoxifen. ERα binding sites in endometrial tumors were enriched for RNA polymerase II as well as for H3K27Ac, the posttranslational histone modification that marks both active promoters and active enhancers. Overlap of ERα chromatin binding sites was observed between breast tumors and endometrial cancers. In addition, we identified a genomic functional network in the endometrial cancer cell line Ishikawa, implicating FOXA1 and ERα as part of a large multiprotein transcriptional network that enrolls FOXM1, TEAD4, and TCF12. The direct clinical implications of this coordinated action between ERα and FOXA1

in endometrial carcinogenesis was assessed for 230 endometrial cancer patients who were previously treated for breast cancer. In contrast to ERα, FOXA1 expression did not correlate with endometrial cancer patient survival. Interestingly, both ERα and FOXA1 expression did associate with short interval between breast cancer treatment and endometrial cancer diagnosis, exclusively for the patients who received tamoxifen.

With this, we present a genomic conservation of ERα action between breast and endometrial tumor specimens that highlights FOXA1 as a common player of hormonal response in both tissues.

Materials and Methods

Patient Series

Endometrial tumor tissue from 230 patients of the Tamoxifen Associated Malignancies: Aspects of RISK (TAMARISK) study was analyzed as described before[5,7,23]. Clinicopathologic parameters from endometrial cancers can be found in Supplementary Table S1. Tumor samples were analyzed anonymously, with coded leftover material that cannot be traced back to the patients that these materials originated from. This study was performed in accordance with the Code of Conduct of the Federation of Medical Scientific Societies in the Netherlands (http://www.fmwv.nl). The study has been approved by the local medical ethics committee of the Netherlands Cancer Institute.

ChIPs and Analyses

ChIPs were performed as described previously[24,25] on endometrioid andenocarcinoma tumors that are part of the TAMARISK study[5,7,23]. These endometrial tumors were collected from patients who were still on tamoxifen on the day of surgery or had stopped 1 to 2 months prior to surgery. Clinicopathologic parameters can be found in Supplementary Table S2. Tumor samples were cryosectioned, fixed in 1% formaldehyde for 20 minutes, and processed for sonication. For each ChIP, 10 μg of antibody, and 100 μL of Protein A (for ERα and H3K27ac) and Protein G (for FOXA1 and RNA polymerase II) magnetic beads (Invitrogen) were used. Antibodies raised to detect ERα (SC-543; Santa Cruz Biotechnology), RNA polymerase II (ab5408; Abcam), H3K27ac (39133; Active Motif), and FOXA1/2 (SC-6554; Santa Cruz Biotechnology) were used. The specificity of SC-6554 antibody to detect FOXA1 was verified using immunoprecipitation followed by Western blot using specific antibodies (Seven Hills, WMAB-2F83 and WRAB-1200; Supplementary Figure S1). Primer sequences used for ChIP-qPCR are listed in Supplementary Table S3. All Ishikawa ChIP-seq and RNA-seq datasets were generated by the ENCODE consortium, and

Supplementary Table S4 shows all accession numbers of the used datasets that also includes breast cancer ERα ChIP-seq data from Jansen and colleagues[25]. Patient characteristics of breast cancer tumors can be found in Supplementary Table S5.

Illumina Sequencing and Enrichment Analysis

ChIP DNA was amplified as described[26]. Sequences were generated by the Illumina Hiseq 2000 genome analyzer (using 50-bp reads) and aligned to the Human Reference Genome (assembly hg19, February 2009). Reads were filtered based on MAPQ quality (reads with MAPQ>20) to eliminate reads from repetitive elements. Peak calling was performed over input, using Dfilter[27] and MACS peak caller version 1.4[28]. Only peaks identified using both algorithms were considered. MACS was run with the default parameters, except $P = 10^{-7}$. DFilter was run using settings bs = 50, ks = 30, refine, nonzero. Details on the number of reads obtained and the percentage of reads aligned, and number of peaks called can be found in Supplementary Table S6. Details on bioinformatics analyses can be found in the Supplementary Methods.

RNA Isolation and mRNA Expression

RNA from tumors was isolated using the AllPrep DNA/RNA/miRNA Universal Kit (Qiagen). cDNA was synthesized with Superscript III Reverse Transcriptase (Invitrogen), using random hexamer primers. RT-PCR was performed using SYBR Green (GC Biotech) on a Roche Lightcycler, using 50 cycles of amplification for all genes tested. RT-PCR products were detected by agarose gel electrophoresis. Primer sequences are listed in Supplementary Table S7.

Immunohistochemistry and Tissue Microarray Analyses

For immunohistochemical analysis, an antibody for FOXA1 was applied (2F83, Seven Hills Bioreagents). This antibody was previously reported to be highly specific for FOXA1[29]. Antibody specificity was confirmed by Western blot (Supplementary Figure S1). Immunohistochemistry on the TAMARISK tissue microarray (TMA) was performed using a Ventana Benchmark Ultra system, applying standard protocols. A kappa of 0.78 was calculated from scoring of two independent observers. ERα staining was previously performed as described[7]. Patients were categorized based on ERα and FOXA1 expression levels (at 10% cutoff) and stratified over tamoxifen use. The mean time interval between breast and endometrial cancer diagnosis was compared between the groups and significance

of the differences was assessed using the t-test. For survival analysis, Kaplan–Meier survival plots were generated categorizing for ERα and FOXA1 expression levels and stratifying over tamoxifen use.

Data Access

All sequencing data can be found at GEO (GSE81213).

Results

ERα is Found at Active Enhancers in Human Endometrial Tumor Specimens

Tamoxifen exposure increases endometrial cancer risk[5]. The genomic features of ERα in relation to tamoxifen in endometrial tumor specimens remain unexplored. To investigate tamoxifen-associated endometrial cancer[5], five endometrial tumors were analyzed from postmenopausal patients who received tamoxifen to treat breast cancer (Figure 1; Supplementary Table S2 for patient details). Endometrioid adenocarcinomas were selected because these are generally ERα-positive, irrespective of whether the endometrial tumor was spontaneous or tamoxifen associated[7].

For all five tumors, ChIP-seq was performed for ERα as well as RNA polymerase II and H3K27Ac, which marks histones both at active promoters and active enhancers (Figure 1A). Only for tumor D, H3K27Ac ChIP-seq data were absent. Consistently between all tumors, ERα sites were co-occupied by H3K27Ac and RNA polymerase II (exemplified in Figure 1B, shown genome-wide in Figure 1C and quantified in Figure 1D). For tumors C and E, RNA polymerase II signal was relatively low at ERα sites (Figure 1B), but still quantifiably detected and enriched over background (Figure 1C and D). ChIP-seq peaks were successfully validated by ChIP-qPCR for all three factors (Supplementary Figures S2A–S2C). Experiments were technically reproducible, with considerable overlap between replicates for the same tumor (ERα: Pearson correlation coefficient = 0.68; H3K27Ac: Pearson correlation coefficient = 0.88; see Supplementary Figures S3A–S3C). Between two sections from the same tumor, RNA polymerase II ChIP-seq was more variable (Pearson correlation coefficient = 0.41), suggesting intratumor heterogeneity of this factor. Analogous to what was previously reported in breast cancer specimens[25,30], endometrial ERα is rarely found at promoters and mainly binds distal intergenic regions and introns (Figure 1E). Cumulatively, these data illustrate that ERα in tamoxifen-associated endometrial tumor specimens shows the same pattern as previously identified in breast cancer, mainly occupying active enhancer regions, positive for both H3K27Ac and RNA polymerase II (Figure 1F).

Conservation of ERα/Chromatin Binding between Surgical Specimens of Breast and Endometrial Cancer Patients

ERα binds active enhancer regions in endometrial tumors, analogous to what was found in breast cancer. To assess any potential overlap in the chromatin binding distributions of ERα between both tumor types, ChIP-seq data from the five endometrial cancers (Figure 1) were compared with publicly available ERα ChIP-seq datasets we previously generated from five breast tumors (Figure 2A; ref.25). As exemplified, robust ERα ChIP-seq signal was detected for both tumor types in all 10 tumors (Figure 2B). Consistent with previous reports in breast cancer[25,30], a number of ERα binding sites greatly deviated between tumor samples, as we now also show in endometrial tumors (Figure 2C). In total, 9,507 ERα interaction sites were found in at least 2 out of 5 breast cancers, while 12,771 ERα sites were found shared in at least 2 out of 5 endometrial cancers. Between the two tumor types, 3,074 ERα sites were shared (Figure 2C).

ERα sites shared between breast cancer and endometrial cancer were robust as illustrated in a heatmap (Figure 2D, for quantification see Supplementary Figure S4). To identify any potential transcriptional regulators that interact with ERα at these sites, conserved chromatin binding events between endometrial and breast tumors were mined for enriched motifs (Figure 2E). As expected, ERα binding sites in both endometrial and breast tumors were strongly enriched for ESR1 motifs ($-\log_{10}P = 690.776$). Interestingly, FOXA1 motifs were also found highly enriched at ERα binding sites in both tumor types ($-\log_{10}P = 342.067$). FOXA1 is classically known to facilitate ERα/chromatin interactions in breast cancer[15,16], but our findings suggest that FOXA1 may play a role for ERα functioning in tamoxifen-associated endometrial cancer as well.

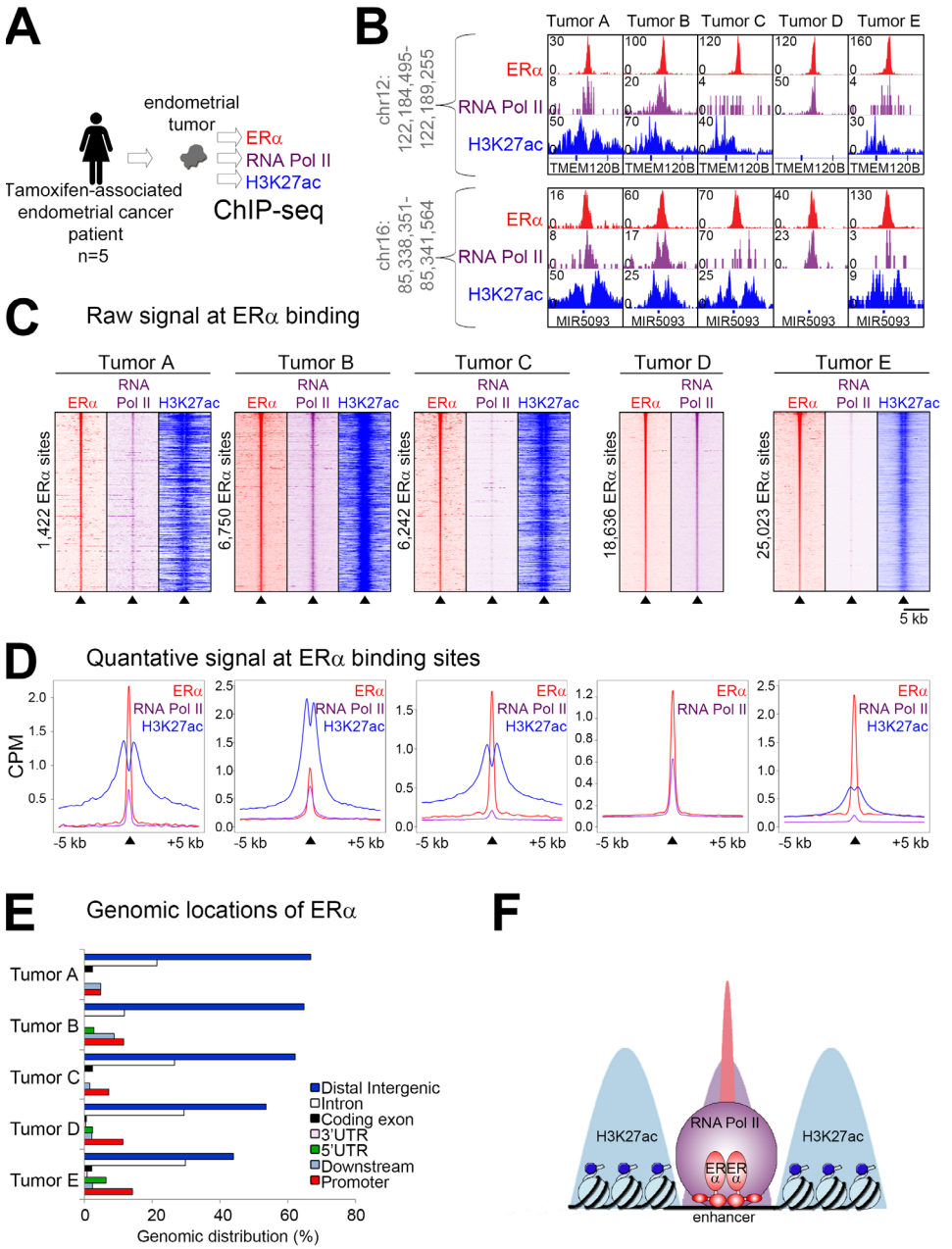


Figure 1. ERα binds to active enhancers in tamoxifen-associated endometrial tumor specimens.

A) Experimental overview. Tumor samples from five endometrial cancer patients were used to ChIP-seq ERα, RNA polymerase II (RNA Pol II), and H3K27ac.

B) Snapshots depicting ChIP-seq data for ERα (red), RNA polymerase II (purple) and H3K27Ac (blue) from five different endometrial tumor specimens. Genomic locations and read counts are shown.

Genomic Interactions of FOXA1 and ERα in Endometrial Tumor Specimens

FOXA1 motifs were enriched at ERα binding sites present in both breast and endometrial cancer samples. Immunohistochemistry analysis validated ERα and FOXA1 expression in both tumor types (Figure 3A). This was previously reported by others for breast cancer[30, 31] as well as endometrial cancer[32–34]. Next, we performed ChIP-seq for FOXA1 in the five endometrioid adenocarcinoma specimens that were previously used to ChIP ERα, RNA polymerase II, and H3K27ac. We directly compared these FOXA1 binding patterns with ERα profiles within the same tumor (Figure 3B).

For tumor D, FOXA1 ChIP-seq signal was not observed (tested in two biologic replicates), nor did we detect its mRNA expression (Supplementary Figure S5). FOXA1 binding sites as identified by ChIP-seq could successfully be validated by ChIP-qPCR (Supplementary Figure S2D). Furthermore, FOXA1 ChIP-seq was technically robust (Pearson correlation coefficient = 0.67; Supplementary Figure S3D). ERα and FOXA1 were found at overlapping genomic locations in all tested tamoxifen-associated endometrioid adenocarcinomas, which were also shared with the endometrioid adenocarcinoma cancer cell line Ishikawa (Figure 3B and C, quantified in D).

For FOXA1, 549 sites were shared between two out of four endometrial tumors. For ERα, 4,623 sites were shared in at least three out of five tumors (Figure 3C and D). However, raw data analyses illustrated that at ERα binding sites, FOXA1 signal was generally observed at the same genomic regions. This suggests that false negativity was observed due to the peak-calling threshold. Analogous to these findings, FOXA1 sites were commonly positive for ERα (Figure 3C and quantified in Figure 3D). Next, potential interacting transcriptional regulators were identified by motif enrichment analysis. As expected, forkhead motifs and ESR motifs were found for regions bound by ERα as well as FOXA1 (Figure 3E). Furthermore, additional motifs were identified for transcription factors previously described to be involved in endometrial cancer, including androgen receptor[35], progesterone receptor[36], RORB[37], as well as SOX proteins[38]. Cumulatively, these data implicate that FOXA1 binds to ERα sites in tamoxifen-associated endometrial cancer that are potentially co-occupied by other transcription factors.

C) Heatmap depicting raw reads of ChIP-seq data for ERα (red), RNA polymerase II (purple), and H3K27Ac (blue). ERα peaks are selected and sorted on intensity. Heatmaps are centered on the peak, showing reads within a 5 kb window around the peak.
D) Normalized average signal (in counts per million, CPM) of data shown in C.
E) Genomic distribution of ERα ChIP-seq peaks in five endometrial cancers.
F) Model for the genomic landscape of ERα in tamoxifen-associated endometrial tumors.

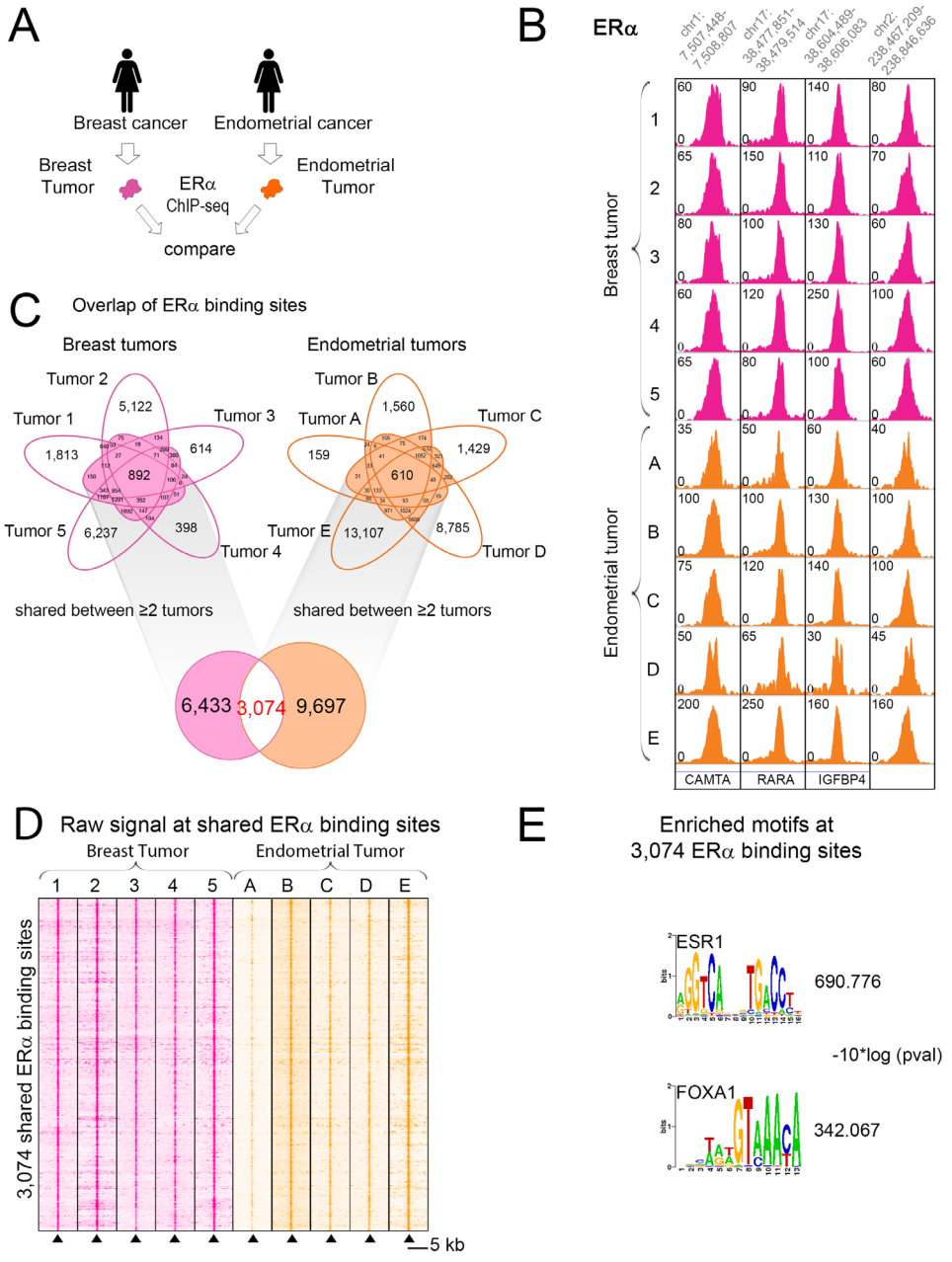


Figure 2. ERα chromatin binding landscape in tamoxifen-associated endometrial cancers and breast tumor samples.

A) Experimental overview. Tumor samples from five tamoxifen-associated endometrial cancer patients (orange) and five breast cancer patients (pink) were processed for ERα ChIP-seq and profiles were directly compared.

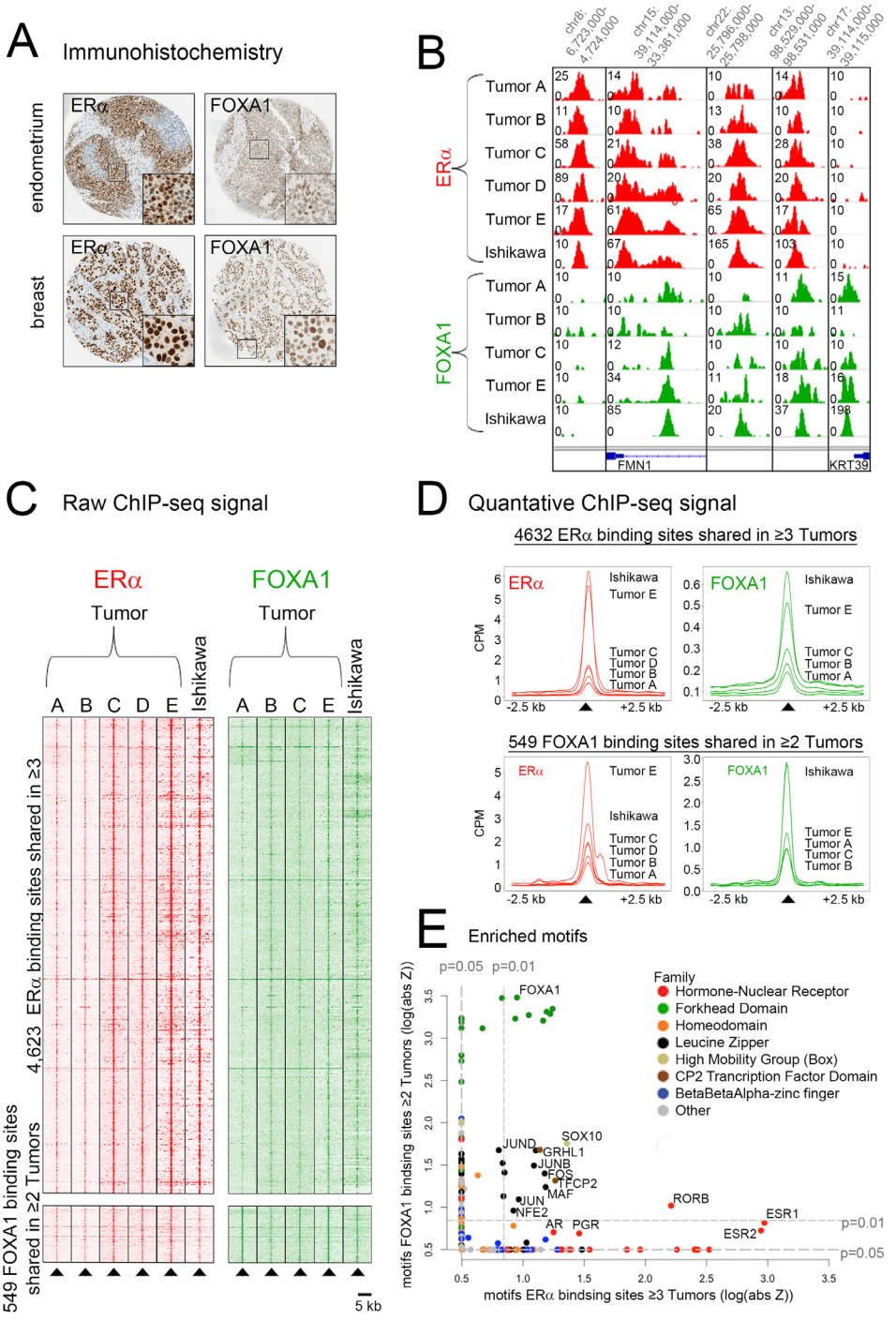
Endometrial FOXA1/ERα Sites Mark a Regulatory Transcription Factor Hub at Enhancers

Endometrial tumors express ERα as well as FOXA1, which share substantial overlap in binding sites. Importantly, ERα and FOXA1 chromatin binding profiles of Ishikawa cells are shared with primary endometrial tumor tissue samples, suggesting applicability of this cell line model to analyze the correlation between FOXA1 and ERα binding sites and to identify transcriptomic regulation at these sites (Figure 3). In Ishikawa, approximately 20% of FOXA1 binding sites overlap with ERα binding sites (Figure 4A), yielding a concise list of 599 shared ERα/FOXA1 binding sites in this cell line.

Compared to ERα unique binding sites and sites shared between ERα and FOXA1, the genomic regions uniquely bound by FOXA1 were markedly higher enriched at promoter regions (Figure 4B). Next, we analyzed DNA motifs in genomic regions bound exclusively by ERα, FOXA1, or shared by both transcription factors (Figure 4C). Sites shared by ERα and FOXA1 showed enrichment for ESR1 motifs as well as Forkhead transcription factors. As expected, ERα unique binding sites were devoid of Forkhead motifs, while FOXA1 unique sites lacked ESR1 motifs, suggesting no false negativity for the unique binding site subsets.

Apart from ERα and FOXA1 motifs, other transcription factors were found selectively enriched between the peak subsets. Substantial overlap was found between these motifs and those found in tumors (Figure 3E). Out of 106 motifs, 85 transcription factors were expressed in Ishikawa cells, based on publicly available RNA-seq data, which was generated as part of the ENCODE consortium (for accession numbers of used datasets see Supplementary Table S4). To provide experimental validation of the identified motifs, publicly available ChIP-seq datasets where used from Ishikawa cells (for GEO accession numbers see Supplementary Table S4). In accordance with the motif analyses, CTCF chromatin binding was found at FOXA1 sites, but not at ERα sites. Most other transcriptional regulators (TEAD4, EP300, FOXM1, MAX, NFIC, RAD21, SRF, TCF12, USF1) showed signal for unique regions of ERα and FOXA1, but was strongest at

B) Snapshots of ERα ChIP-seq signal in endometrial tumor tissue (orange) and breast tumor tissue (pink). Genomic locations and read count are shown.
C) Venn diagram visualizing shared and unique ERα chromatin binding events in five breast tumor samples (pink) and five endometrial tumor samples (orange). For each tumor type, the ERα peaks shared between at least two tumors were used. The number of overlapping peaks between the two tumor types is shown in red.
D) Heatmap visualization of the shared ERα binding sites between breast (pink) and endometrial (orange) tumor samples, as defined in C.
E) ERα binding sites shared between endometrial and breast tumor samples are enriched for ESR1 and FOXA1 motifs. Motifs sequence logos and P values are shown.


A
Immunohistochemistry
endometrium
breast
ERα
FOXA1
B
ERα
FOXA1
Tumor A
Tumor B
Tumor C
Tumor D
Tumor E
Ishikawa
FMN1
KRT39
C
Raw ChIP-seq signal
Tumor
Ishikawa
4,623 ERα binding sites shared in ≥3
549 FOXA1 binding sites shared in ≥2 Tumors
5 kb
D
Quantative ChIP-seq signal
4632 ERα binding sites shared in ≥3 Tumors
549 FOXA1 binding sites shared in ≥2 Tumors
CPM
-2.5 kb
+2.5 kb
E
Enriched motifs
p=0.05
p=0.01
Family
Hormone-Nuclear Receptor
Forkhead Domain
Homeodomain
Leucine Zipper
High Mobility Group (Box)
CP2 Trancription Factor Domain
BetaBetaAlpha-zinc finger
Other
FOXA1
SOX10
GRHL1
JUNB
FOS
TFCP2
MAF
JUN
NFE2
AR
PGR
RORB
ESR1
ESR2
motifs FOXA1 bindsing sites ≥2 Tumors (log(abs Z))
motifs ERα bindsing sites ≥3 Tumors (log(abs Z))

regions co-occupied by both FOXA1 and ERα (Figure 4D; Supplementary Figure S6 for quantifications). Furthermore, these transcription factors were tested for expression analysis in tamoxifen-associated cancers and Ishikawa cells (Supplementary Figure S5). All tested transcription factors were expressed in at least 3 out of 4 tumors and found expressed in Ishikawa cells. Genes that correspond to genomic regions bound by ERα and FOXA1 were identified (<20 kb upstream of transcriptional start site or within gene body; see Materials and Methods) and used for Ingenuity Pathway Analysis to reveal the network in which these proteins operate. As expected, ESR1 (P = 8.71E−08) was identified as a top upstream regulator. Accordingly, the top enriched network was centered around ESR1 (Figure 4E; Supplementary Table S8). All genes in this pathway were under control of ERα signaling, as identified by RNA-seq data integration from Ishikawa cells, treated for 4 hours with estradiol or vehicle control (Figure 4E; Supplementary Table S4).

To investigate the transcription factor context in which ERα and FOXA1 operate, we performed unsupervised hierarchical clustering based on the identified binding patterns for all analyzed transcriptional regulators, which resulted in the identification of distinct functional genomic clusters (Figure 4F). Cluster 1 was enriched for transcriptional regulators found at promoter regions (Figure 4G), such as RNA polymerase II, TAF1, YY1, MAX, EGR1, ZBTB7A, USF1, SRF, and CREB1 (Figure 4F). Cluster 2, which contained ERα and FOXA1 binding regions, was enhancer enriched (Figure 4G) and showed clustering among p300, FOXM1, NR3C1

Figure 3. Chromatin binding patterns of ERα and FOXA1 in tamoxifen-associated endometrial tumor specimens.

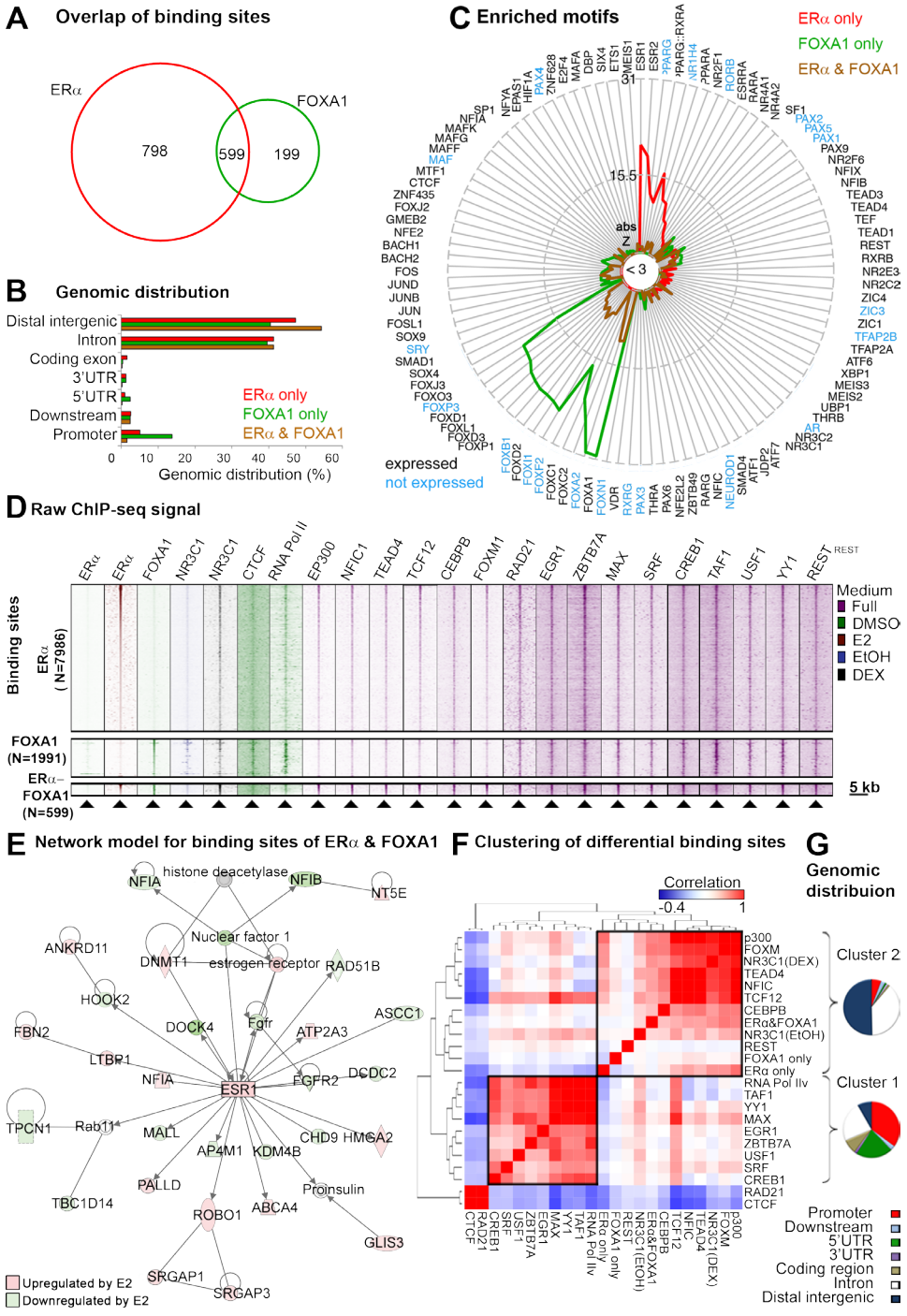
A) Breast and endometrial tissues were stained by immunohistochemistry for ERα and FOXA1. Example of ERα and FOXA1 expression in breast and endometrial tumor tissues. Magnification, ×200.

B) Snapshots of ChIP-seq data for ERα (red; five samples) and FOXA1 (green; four samples) in independent tamoxifen-associated endometrial tumors and the endometrial cancer cell line Ishikawa. Read count and genomic locations are shown.

C) Heatmap visualization of ERα (red) and FOXA1 (green) binding sites in tumors and the endometrial cancer cell line Ishikawa. For ERα, all 4,623 sites found in at least 3 out of 5 tumors were selected. For FOXA1, 549 sites were identified in at least 2 out of 4 tumors. All peaks identified for ERα (top) and FOXA1 (bottom) were analyzed separately, where raw data for ERα and FOXA1 ChIP-seq are shown. Raw read count of all peaks is vertically aligned on the center of the peak region.

D) Normalized average read count (CPM) of ChIP-seq data visualized in C. Data were centered on the peak regions and include a 2.5-kb window around the peak. The y-axis shows normalized read count.

E) Scatter plot showing motif enrichment analysis of ERα and FOXA1 ChIP-seq sites depicted in C and D. The individual dots represent the absolute Z-score of enriched motif in log-scale. Two vertical and horizontal dashed gray lines indicate the absolute Z-score corresponding to P value of 0.05 and 0.01 for ERα and FOXA1 binding sites, respectively.


A
Overlap of binding sites
ERα
FOXA1
798
599
199
B
Genomic distribution
Distal intergenic
Intron
Coding exon
3'UTR
5'UTR
Downstream
Promoter
ERα only
FOXA1 only
ERα & FOXA1
0 10 20 30 40 50 60
Genomic distribution (%)
C
Enriched motifs
ERα only
FOXA1 only
ERα & FOXA1
15.5
abs Z
< 3
expressed
not expressed
D
Raw ChIP-seq signal
ERα
ERα
FOXA1
NR3C1
NR3C1
CTCF
RNA Pol II
EP300
NFIC
TEAD4
TCF12
CEBPB
FOXM1
RAD21
EGR1
ZBTB7A
MAX
SRF
CREB1
TAF1
USF1
YY1
REST
REST
Binding sites
ERα (N=7986)
FOXA1 (N=1991)
ERα– FOXA1 (N=599)
Medium
Full
DMSO
E2
EtOH
DEX
5 kb
E
Network model for binding sites of ERα & FOXA1
histone deacetylase
NFIA
NFIB
NT5E
Nuclear factor 1
ANKRD11
DNMT1
estrogen receptor
RAD51B
HOOK2
FBN2
DOCK4
Fgfr
ATP2A3
ASCC1
LTBR1
NFIA
ESR1
FGFR2
DCDC2
TPCN1
Rab11
MALL
AP4M1
KDM4B
CHD9
HMGA2
PALLD
TBC1D14
ROBO1
ABCA4
Proinsulin
GLIS3
SRGAP1
SRGAP3
Upregulated by E2
Downregulated by E2
F
Clustering of differential binding sites
Correlation
-0.4
1
p300
FOXM
NR3C1(DEX)
TEAD4
NFIC
TCF12
CEBPB
ERα&FOXA1
NR3C1(EtOH)
REST
FOXA1 only
ERα only
RNA Pol IIv
TAF1
YY1
MAX
EGR1
ZBTB7A
USF1
SRF
CREB1
RAD21
CTCF
Cluster 2
Cluster 1
G
Genomic distribuion
Promoter
Downstream
5'UTR
3'UTR
Coding region
Intron
Distal intergenic

(Glucocorticoid Receptor), TEAD4, NFIC, TCF12, and CEBPB. These cell line data are in agreement with primary tumor data where ERα was found to bind active enhancers (Figure 1). Cumulatively, these data implicate genomic locations shared by ERα and FOXA1 as central genomic hubs for enhancer action, where multiple transcriptional regulators bind proximal to genes involved in classical estrogen-regulated processes.

Interplay of FOXA1 and ERα in Tamoxifen-associated Endometrial Cancer

ERα's functional activity is linked with FOXA1 in tamoxifen-associated endometrial cancer. We next determined the potential clinical implications of the interplay of FOXA1 and ERα in this context (Figure 5), using TMAs from the TAMARISK study[7]. The TAMARISK study involves samples from 230 endometrial tumors that were collected from patients who were previously diagnosed with breast cancer, half of whom received tamoxifen (Figure 5A; Supplementary Table S1). We assessed the interval time between breast cancer diagnosis and endometrial cancer diagnosis as well as endometrial cancer-related survival. These clinical variables were

Figure 4. Comparative genomics between ERα and FOXA1 in endometrial cancer cells Ishikawa.

A) Venn diagram visualizing overlap between chromatin binding events for ERα (red) and FOXA1 (green).

B) Genomic distribution of sites bound by either ERα alone (red), FOXA1 alone (green), or shared by ERα and FOXA1 (brown).

C) Radar plot, visualizing DNA motif enrichment for genomic sites bound by either ERα (red) or FOXA1 (green) alone, or by both (brown). The radial data points represent the absolute value of Z-score. The lower bound of the abs Z-score is 3, which corresponds to a P value of 0.00135. Genes expressed in Ishikawa [genes with fragments per kilobase of exon per million fragments mapped (FPKM) > 1] are in black, while genes not expressed are in blue.

D) Heatmap visualization of ChIP-seq ENCODE data for several transcriptional regulators at sites bound by ERα and/or FOXA1. Raw read count of all peaks was vertically aligned, and data were centered on the center of the peak region. Colors correspond to different treatments; cells were either grown in full medium (purple) or hormone deprived, followed by a 1-hour incubation of either DMSO (green), 10 nM estradiol (E2, brown), ethanol (EtOH, blue), or 100 nM dexamethasone (DEX, black).

E) Ingenuity Pathway Analysis depicts the top-network model of genes with a shared ERα/FOXA1 site within the gene body or within 20k upstream of the transcriptional transcription start site. The node color indicates the FPKM fold change in Ishikawa cells upon a 4-hour estradiol (E2) stimulation, being either upregulated (red) or downregulated (green) related to control.

F) Unsupervised clustering of binding sites of all available transcriptional regulators. FOXA1 unique, ERα unique, and shared ERα/FOXA1 sites are analyzed separately. Red, positive correlation; blue, negative correlations.

G) Genomic distribution of peaks shared between at least four out of nine proteins in either cluster 1 or cluster 2.

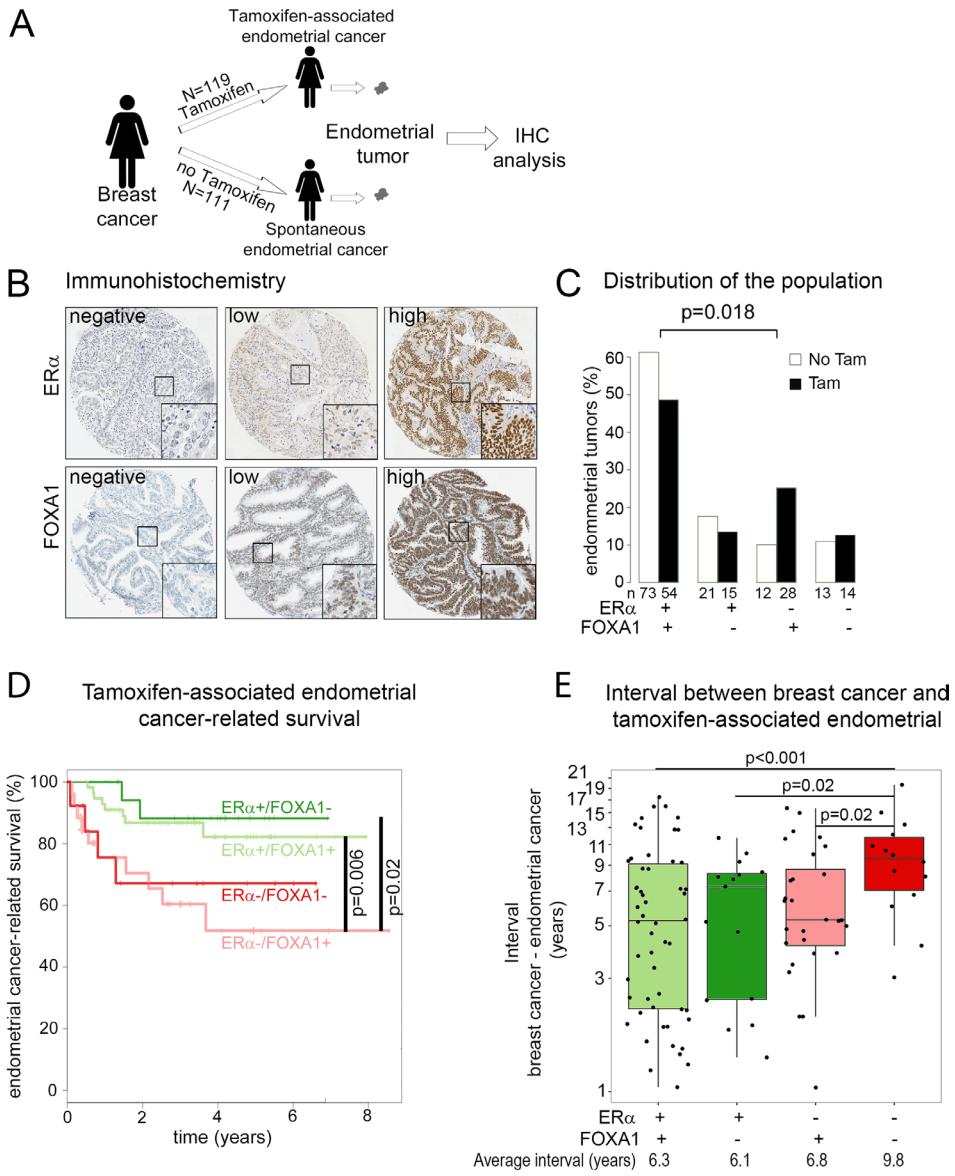


Figure 5. Immunohistochemistry analysis of FOXA1 and ERα in tamoxifen-associated endometrial cancer.

A) Overview of the study design. Endometrial tumor tissue was analyzed from patients who were previously treated for breast cancer. Half of these patients received tamoxifen for breast cancer treatment, while the other half did not receive any endocrine treatment.

B) Immunohistochemistry for ERα and FOXA1 showing no (left), low (middle), or high (right) protein expression.

C) Bar graph representing percentage and absolute numbers of patients that were categorized as ERα^{+}/FOXA1^{+}, ERα^{+}/FOXA1^{-}, ERα^{-}/FOXA1^{+}, or ERα^{-}/FOXA1^{-} and

tested for association with ERα and FOXA1 levels, as determined by immunohistochemistry (Figure 5B for example staining).

Scatter plot visualizations illustrated that FOXA1 and ERα positive cells in endometrial tumor specimens do not associate (Supplementary Figure S7), suggesting that ERα and FOXA1 expression levels may identify different patient groups. Therefore, tumors were categorized in groups that express only ERα, only FOXA1, both ERα and FOXA1, or neither. Most endometrial tumors are positive for both ERα and FOXA1, irrespective of treatment (Figure 5C). $FOXA1^{+}/ER\alpha^{-}$ tumors were enriched among tamoxifen-treated patients at the expense of the tumors that are positive for both ERα and FOXA1 (Fisher exact test: P = 0.018). ERα expression strongly associated with endometrial cancer patient survival, in agreement with previous immunohistochemical reports[39,40].

In contrast to previous reports[33,34], FOXA1 expression did not associate with survival of endometrial cancer patients, irrespective whether they did (Figure 5D) or did not receive tamoxifen (Supplementary Figure S8). Interestingly, tamoxifen-treated patients who developed an endometrial tumor had a shorter interval time between breast cancer and endometrial cancer when their endometrial tumor expressed ERα and/or FOXA1 (ERα: P = 0.02, FOXA1: P = 0.02; ERα/FOXA1: P < 0.001; Figure 5E). For patients who did not receive tamoxifen treatment for breast cancer, no significant differences in interval time between breast cancer and endometrial cancer diagnosis were found, based on ERα and FOXA1 levels in the endometrial tumor (Supplementary Figure S9).

Our data implicate a coordinated action between ERα and FOXA1, associated with a short interval time between breast cancer and endometrial cancer development in the tamoxifen-treated cases, without affecting endometrial cancer-related survival.

Discussion

Tamoxifen is a highly successful drug in the treatment of breast cancer. However, tamoxifen increases the risk of endometrial cancer. This direct link between breast cancer treatment and endometrial cancer development suggests a crucial role for ERα in both tumor types. Tamoxifen

grouped in tamoxifen-treated patients (white) and non–tamoxifen-treated patients (black). For statistics, Fisher exact test was used.

D) Endometrial cancer–specific survival of tamoxifen-treated patients, categorized as $ER\alpha^{+}/FOXA1^{+}$, $ER\alpha^{+}/FOXA1^{-}$, $ER\alpha^{-}/FOXA1^{+}$, or $ER\alpha^{-}/FOXA1^{-}$.

E) Box plot depicting interval time (years) between breast cancer diagnosis and endometrial cancer diagnosis for patients who received tamoxifen. P values were not corrected for multiple testing. Average interval time in years is shown.

has been reported in endometrial cells to stimulate the recruitment of coactivators to a subset of genes, similar to estrogen[9]. Between a breast cancer cell line and an endometrial cancer cell line, limited overlap of ERα sites was observed[13], giving rise to differential gene expression between those cell lines following ligand treatment. More recently, the same lab reported shared ERα sites between these cell lines to be associated with strong EREs, inaccessible chromatin regions and lack of DNA methylation[29]. In contrast, non–ERE-mediated ERα action was found dominant in tissue-type specific interactions, which co-occurred with other transcription factors and cell-type specific chromatin accessibility[29]. This previous report was based on a single endometrial cancer cell line, while our study is the first to assess genomic behavior of ERα in several surgical specimens of endometrial cancer patients. We show a significant level of ERα overlap between breast cancer samples and endometrial cancer samples, being enriched for ESR1 and FOXA1 motifs. With this, our data illustrate that functional characterization of ERα genomic action can be accelerated by a joined analysis on cell line studied in conjunction with primary human tissue specimens.

Analogous to previous observations in breast cancer, endometrial ERα preferably binds enhancers, which were marked by H3K27ac and RNA polymerase II, suggesting that these enhancers are active. Furthermore, ERα binding events in endometrial tumor specimens are enriched for motifs of well-established transcriptional regulators in the field of breast cancer, including FOXA1[15]. FOXA1 was previously reported to be expressed in endometrial tissue, and downregulated in poorly differentiated endometrial cancer[32] where low FOXA1 is associated with poor outcome[33,34]. In line with these previous reports, we show that endometrial tumor cells express FOXA1, even though we do not observe an association of FOXA1 with clinical outcome in endometrial cancer in our cohort. Because patients in our cohort developed both breast cancer as well as endometrial cancer[7], we may have enriched for patients with a genetic predisposition to developing both tumors types. Therefore, our study population may be intrinsically different from sporadic cases that only developed endometrial cancer[33]. Here, we illustrate that FOXA1 chromatin binding sites are shared with ERα in tamoxifen-associated endometrial cancer. These sites are not exclusively bound by ERα or FOXA1, but are in fact regulated through a large multiprotein transcriptional network, jointly mediating ERα-driven gene profiles in endometrial cancer.

Multiple other proteins found to associate with breast cancer outcome have been implicated in endometrial cancer development, including PAX2[10,12] and SRC1[9,41]. Here, we show that FOXA1 can be added to this list, exposing a bivalent role in response to tamoxifen treatment, dependent on tissue type; while high FOXA1 expression is a favorable prognostic factor in breast cancer[21], its expression levels associate with short interval

time between breast cancer diagnosis and tamoxifen-associated endometrial cancer development without affecting outcome in this cohort. This closely follows parallel observations made for PAX2, and validates inverse relations of transcriptional regulators between breast and endometrial cancer with respect to tumorigenesis and response to endocrine agents.

To conclude, we show that endometrial tumor cells express FOXA1, serving the classical ERα-pioneer factor role as was originally identified in breast cancer[15]. Yet, instead of facilitating the inhibitory potential of tamoxifen on ERα activity, FOXA1 may enable receptor activation through tamoxifen in endometrial tissue in postmenopausal women. This function of FOXA1 has far-reaching consequences, where it may dictate the stimulatory effects of tamoxifen treatment on endometrial cancer development.

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Supplementary Methods

Data Visualization, Motif Analysis and Genomic Distributions of Binding Events

ChIP-seq data snapshots were generated using the Integrative Genome Viewer IGV 2.1 (www.broadinstitute.org/igv/). The genomic distributions of binding sites were analyzed using the cis-regulatory element annotation system (CEAS)[1]. The genes closest to the binding site on both strands were analyzed. If the binding region is within a gene, CEAS software indicates whether it is in a 5'UTR, 3'UTR, coding exon, or intron. Promoter is defined as up to and including 3kb upstream from RefSeq 5' start. If a binding site is >3kb away from the RefSeq transcription start site, it is considered distal intergenic. Motif analyses were performed through the Cistrome (cistrome.org), applying the SeqPos motif tool[2]. For motif analyses, an equal number of genomic regions were analyzed for the different conditions, and enriched motifs were identified for each of the peak subsets according to p-value with cutoffs indicated in the figure legends. RNA-seq data for Ishikawa cells, with and without estradiol treatment, is obtained from Encyclopedia of DNA Elements[3] (for GEO accession number, see Supplementary Table S4). The cut-off used for gene expression was set at Fragments Per Kilo-base of exon per Million fragments mapped (FPKM) > 1 in two replicates. To compare binding sites of FOXA1 and ERα with other transcription factors in Ishikawa cell line, we obtained 22 ChIP-seq datasets for Ishikawa from the Encyclopedia of DNA Elements[3] (Supplementary Table S4). To identify ERα and FOXA1 binding sites, only peaks conserved between both replicates were considered. For overlap between factors, Pearson correlation coefficients were measured using the dba.overlap function of the DiffBind package[4]. A network model was constructed using Ingenuity Pathway Analysis (IPA) to delineate cooperative and differential gene regulation, where all genes with a binding event of ERα and FOXA1 in the gene body or within 20kb upstream of the transcription start site were considered. The FPKM fold-change upon estradiol treatment of the genes is taken into account for constructing the network model. Given a set of genes, IPA infers 1) network models that maximally include the genes in the set and known interactions between them; 2) canonical pathways significantly associated to the set of genes; and 3) potential upstream regulators of the genes.

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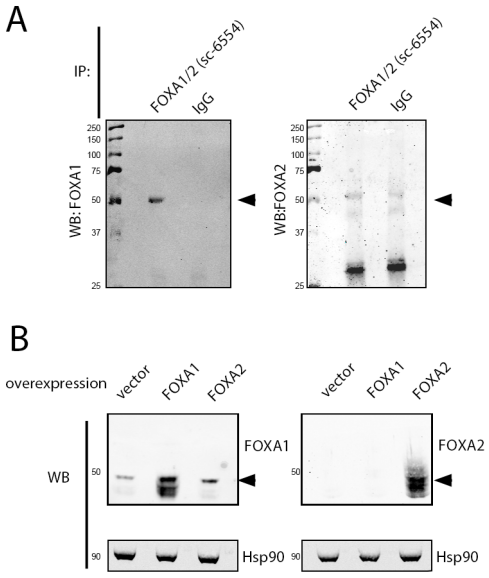
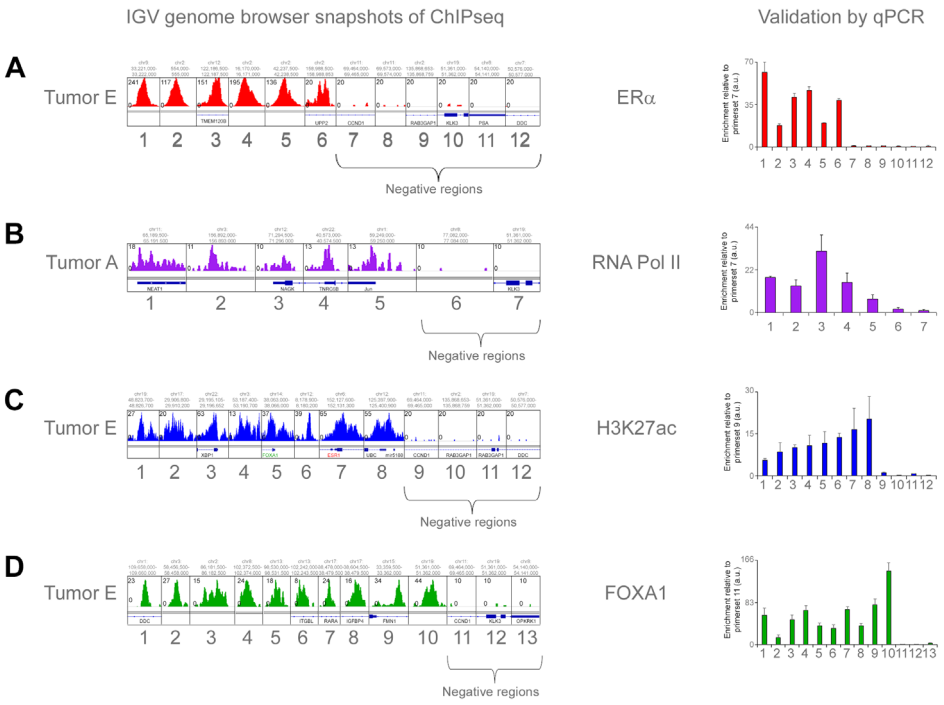
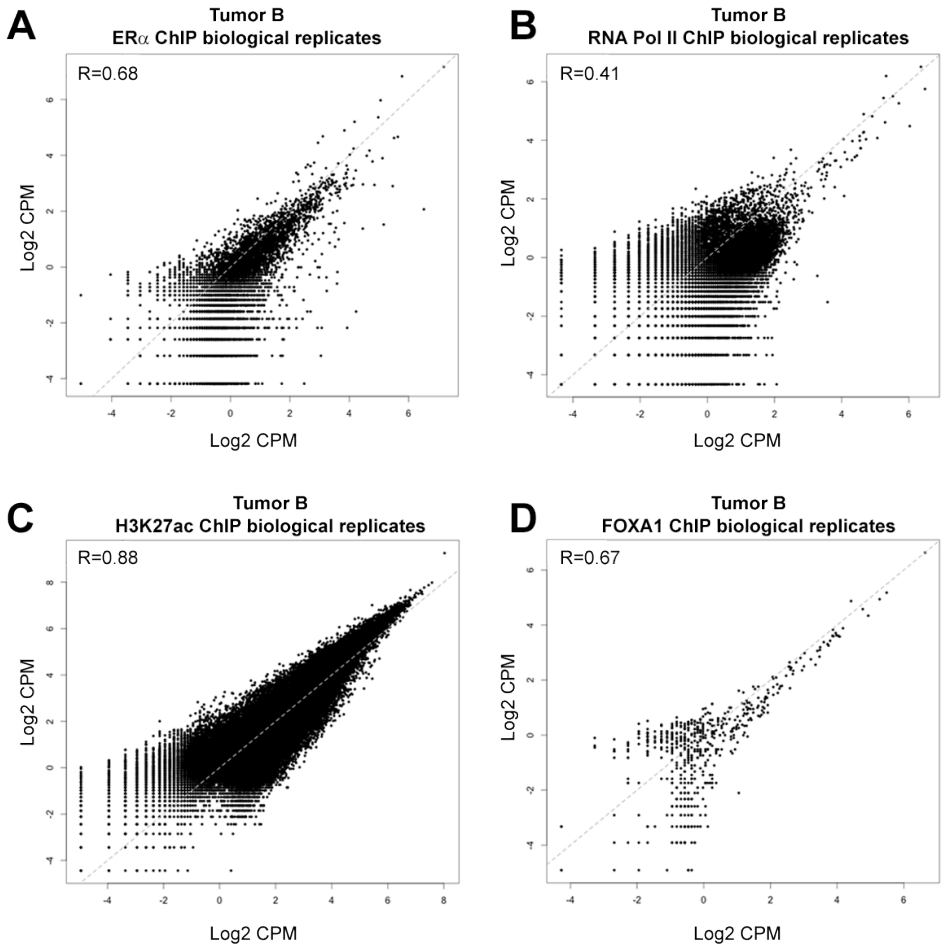


Supplementary Figure S1.
Validation of two FOXA1 antibodies that were used for either ChIP or Western blot. A, FOXA1/2(sc-6554) and the control IgG antibodies were used for an immunoprecipitation in MCF-7 cells. Western blot depicts immunoprecipitated fractions with specific antibodies raised for either FOXA1 (WMAB-2F83) or FOXA2 (WMAB-1200). B, Western blots, depicting transient overexpression of an empty vector, FOXA1 and FOXA2 in MCF-7 cells. The left blot was stained for FOXA1 (WMAB-2F83) and the right blot (WMAB-1200) for FOXA2. HSP90 was used as a loading control.

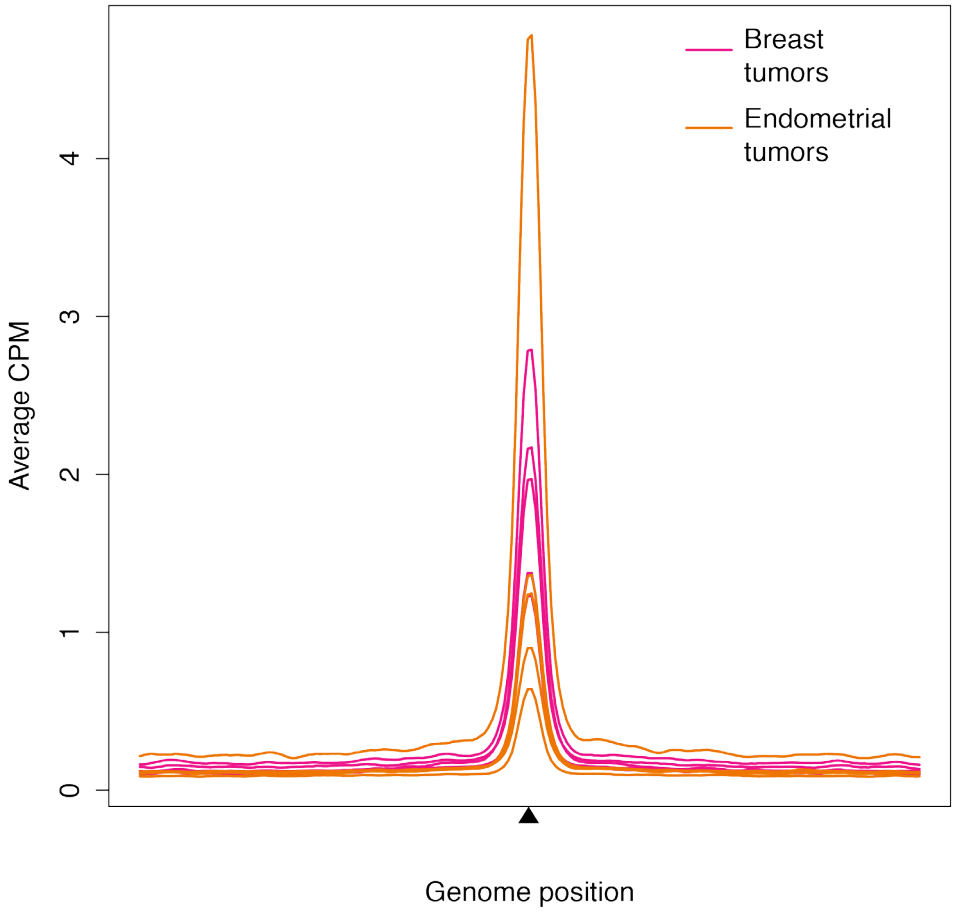


Supplementary Figure S2.
ChIP-QPCR validation of ChIP-seq peaks found in endometrial cancer specimens. ChIP-QPCR analyses were performed for both positive and negative regions, as identified through ChIP-seq, to validate binding sites identified for ERα (A), RNA Polymerase II (B), H3K27Ac (C) and FOXA1 (D). Left: Genome browser snapshots for the positive and negative regions. Tag count and genomic coordinates are indicated. Right: ChIP-QPCR analyses for the same binding sites. Data are normalized over negative region as indicated on the y-axis. Error bars indicate SD values from triplicate measurements. Corresponding primers can be found in supplementary table S3.

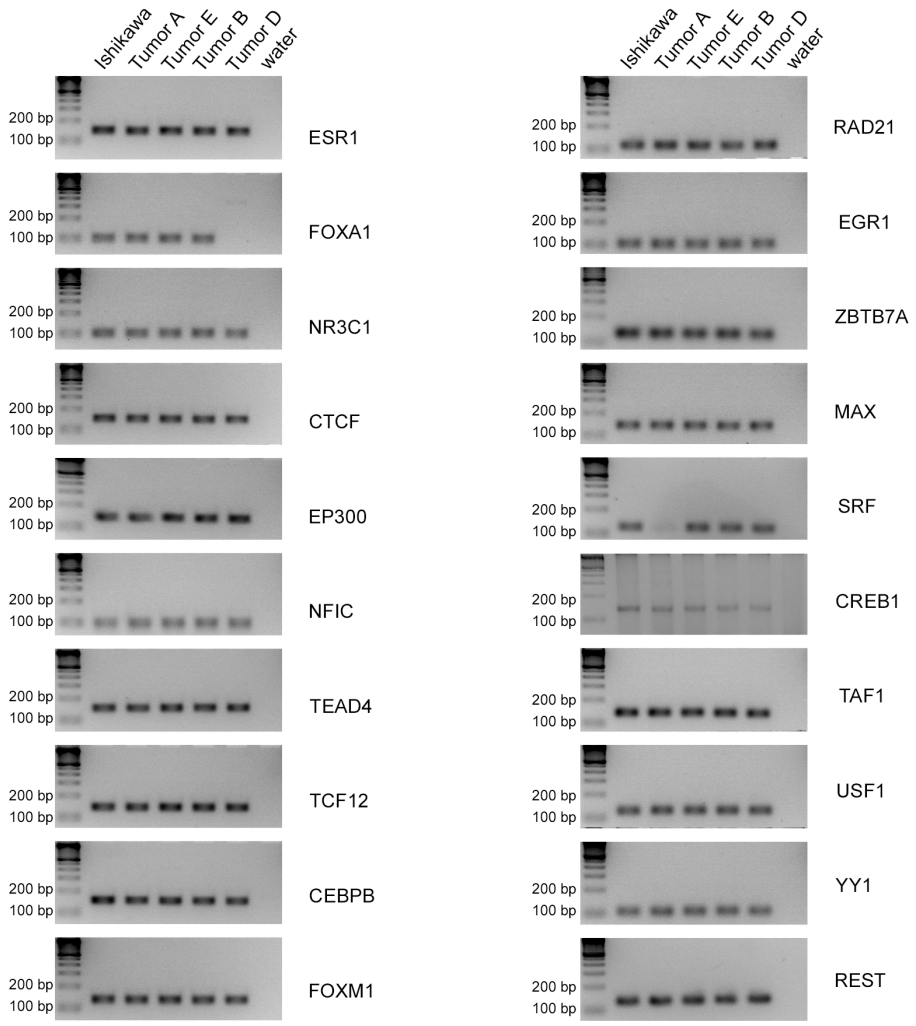
Scatter plots of the reads in the union of peaks between the replicates (log-normalized values)



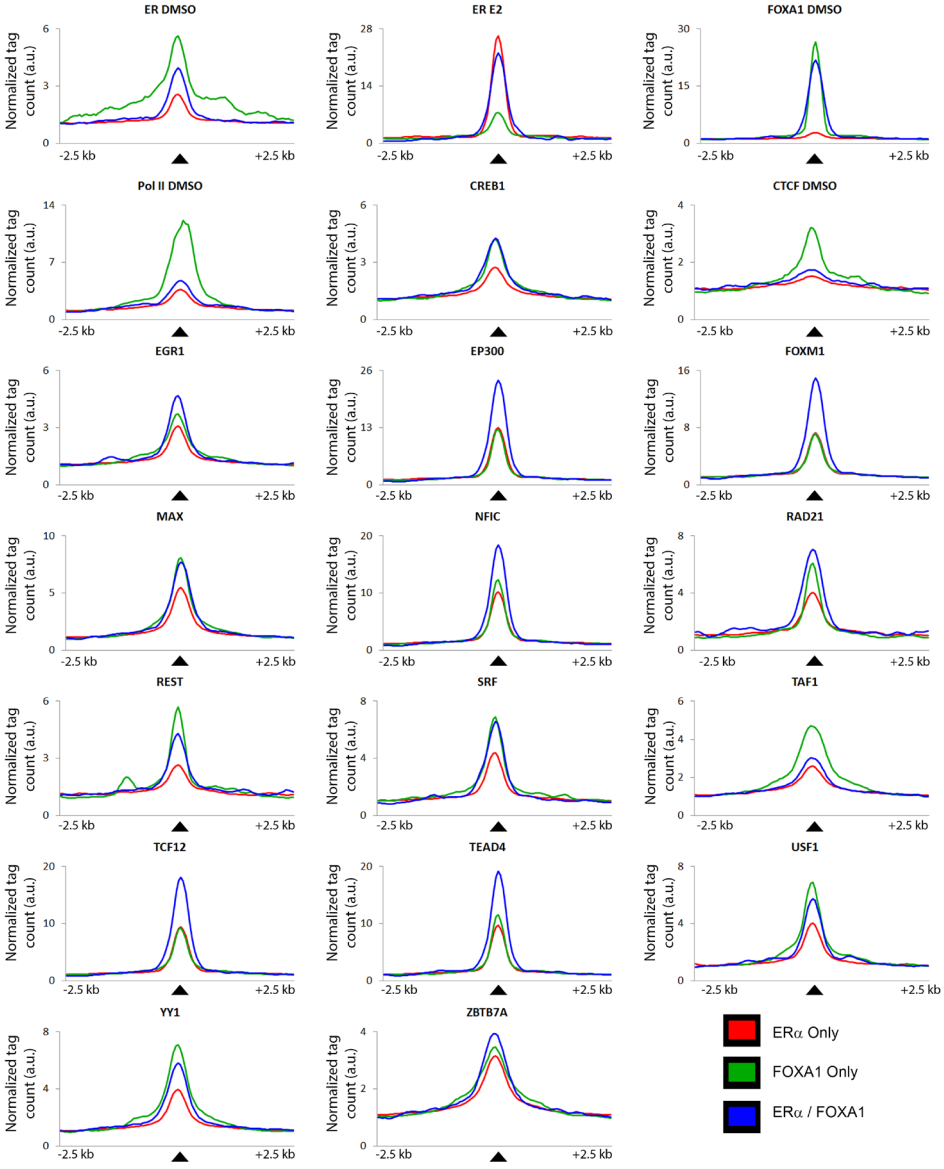
Supplementary Figure S3.
Biological replicates of ChIP-seq analyses for ERα (A), RNA Polymerase II (B), H3K27Ac (C) and FOXA1 (D). Scatterplot indicates correlation of the read counts for the identified peaks in the two replicates for each factor. Pearson correlation coefficients are shown.



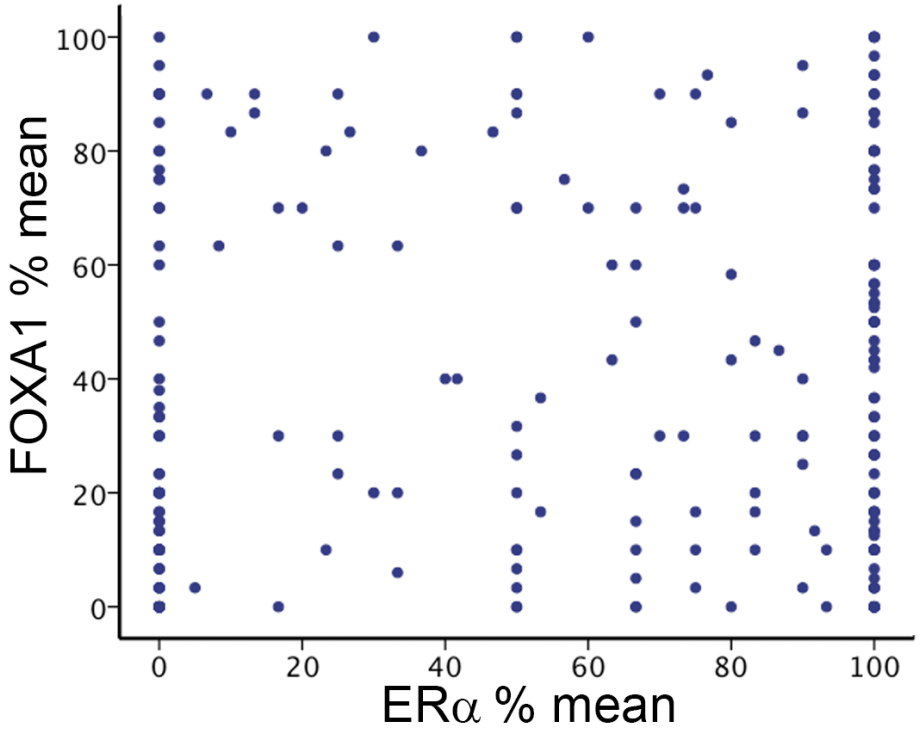
Supplementary Figure S4.
Average normalized ERα ChIP-seq signal of endometrial and breast cancer specimens. Counts per Million (CPM)-normalized average read count of ChIP-seq data visualized in Figure 2D. Data was centered on the peak regions and includes a 5 kb window around the peak. Y-axis shows normalized read count.



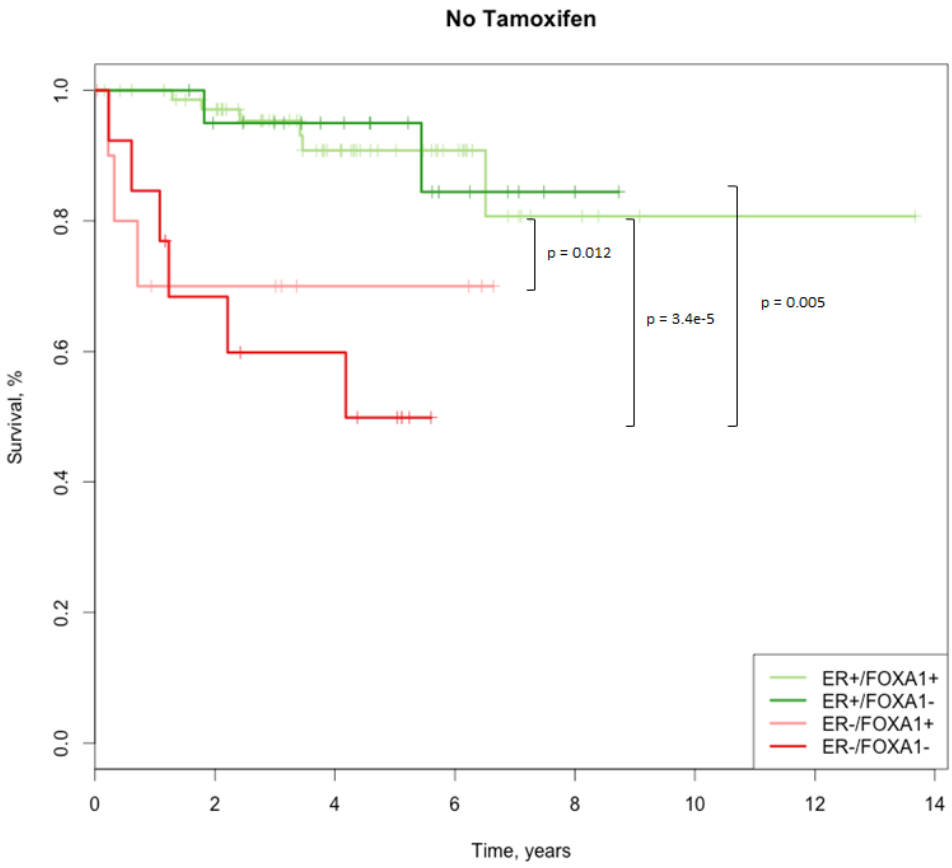
Supplementary Figure S5.
RT-PCR analysis of 20 transcription factors in four tamoxifen-associated endometrial tumors and Ishikawa cells. Corresponding primers can be found in supplementary table S6.



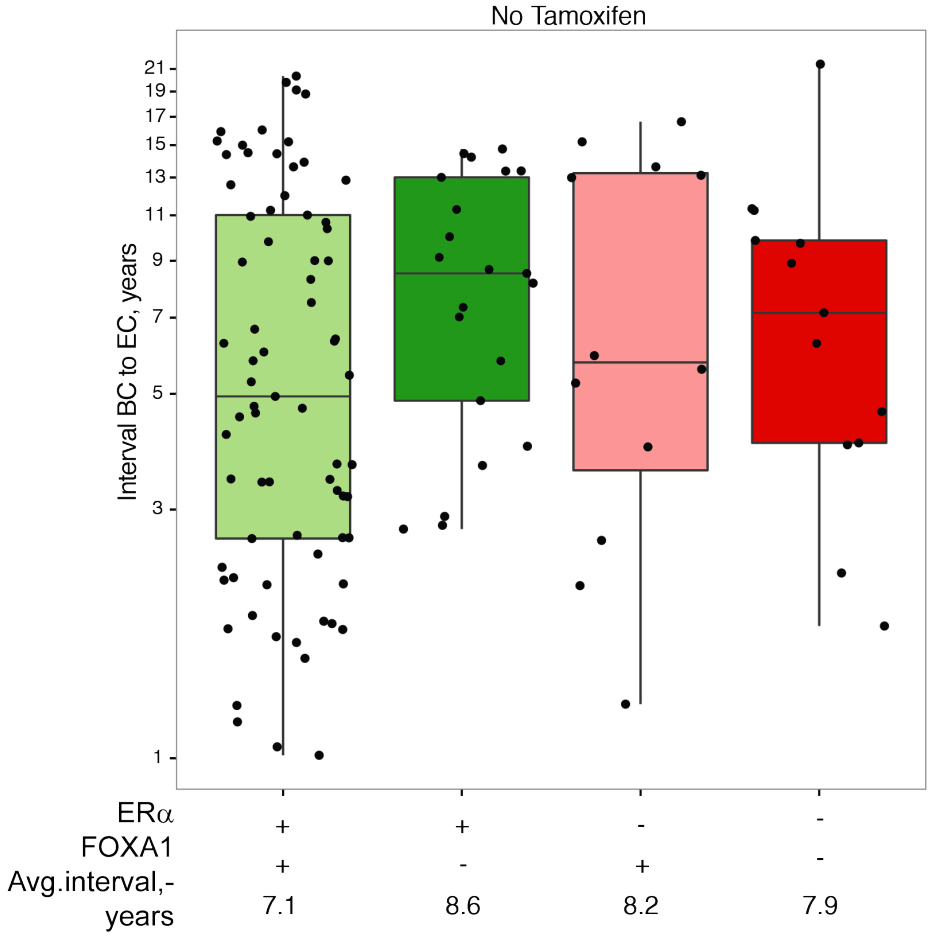
Supplementary Figure S6.
2D graph, showing normalized read count of the shared binding sites for ERα and FOXA1 (blue), ERα only (red) and FOXA1 only (green) in ChIP-seq datasets of all indicated transcriptional regulators in the endometrial cancer cell line Ishikawa (ENCODE, see Supplementary table S4). Data are centered on the top of the peak regions, depicting a 5 kb window around the peak. Y-axis shows read count.



Supplementary Figure S7.
Scatter plot showing the relationship between ERα (X-axis) and FOXA1 (Y-axis) staining. Mean % of positive tumor cells is shown, where each dot indicates one tumor sample. Pearson correlation coefficient is 0.081, p=0.308.



Supplementary Figure S8.
Kaplan-Meier survival plot of endometrial cancer patients who were not treated with tamoxifen for their breast cancer, categorized as ERα+/FOXA1+, ERα+/FOXA1-, ERα-/FOXA1+ or ERα-/FOXA1-.



Supplementary Figure S9.
Box plot, depicting interval time (years) between breast cancer diagnosis and endometrial cancer diagnosis for patients who did not receive tamoxifen. P values were not corrected for multiple testing. Average interval time in years is shown. MC-UC = interval between breast cancer and endometrial cancer.

Supplementary Table S1: Clinicopathological parameters.

	tamoxifen (n=111)		No tamoxifen (n = 119)	
Interval between diagnosis of breast and uterine cancer [months]				
<24	14	12.6%	14	11.8%
24-36	13	11.7%	16	13.4%
36-60	18	16.2%	22	18.5%
60-120	42	37.8%	29	24.4%
>120	24	21.6%	38	31.9%
Age at diagnosis of breast cancer [years]				
<55	22	19.8%	34	28.6%
55-64	38	34.2%	34	28.6%
65-74	30	27.0%	39	32.8%
>75	21	18.9%	12	10.1%
Age at diagnosis of endometrial cancer				
<65	33	29.7%	38	31.9%
65-74	34	30.6%	45	37.8%
>75	44	39.6%	36	30.3%
Histological type				
Endometrioid adenocarcinoma	83	74.8%	100	84.0%
clear cell and serous adenocarcinoma	14	12.6%	9	7.6%
Carcinosarcoma	6	5.4%	6	5.0%
Sarcoma	8	7.2%	4	3.4%
Grade (endometrioid adenocarcinomas only)				
1	51	45.9%	63	52.9%
2	18	16.2%	23	19.3%
3	12	10.8%	14	11.8%
missing	2	1.8%	0	0.0%
FIGO stage				
I	81	73.0%	93	78.2%
II	15	13.5%	14	11.8%
III	10	9.0%	5	4.2%
IV	4	3.6%	6	5.0%
missing	1	0.9%	1	0.8%

Supplementary Table S2: Patient characteristics of endometrioid adenocarcinomas used for ChIP-seq.

					Number of peaks per ChIP				Tamoxifen status	
Tumor	Figo Stage	Tamoxifen (yrs)	MC-UC (yrs)	Age UC (yrs)	ERα	RNA Pol II	H3K27ac	FOXA1	at surgery	Tumor (%)
A	I	5.59	6.37	76.44	1422	1780	36469	706	User	70
B	I	3.16	3.18	69.20	6750	13720	30619	384	User	60
C	I	5.26	5.03	60.35	6242	159	31461	352	Stopped 1-2 months	75
D	I	2.70	3.43	54.46	18636	7143	N/A	N/A	User	70
E	I	4.18	4.65	55.78	25023	198	28272	6811	User	60
Biological replicate B					2242	7376	40791	292		

Supplementary Table S3: Primer sequences for ChIP-qPCR
Corresponds with Supplementary Figure S2.

	Primer pair	Sequences
Positive control	ERα #1	TGGCCCTAAATGTGTCTGCT (FWD) TTTGGCCTCTTCCTCTTCCT (REV)
	ERα #2	CCTTCTTCCTGGGGGTGA (FWD) GCACCACCAACACTCACATT (REV)
	ERα #3	TGATGTCATTGCCACCTTGT (FWD) AACAGGGTCAGGGTGAACTG (REV)
	ERα #4	CTGGGGGAACCTTCAATTTT (FWD) GTGAGAACACTGCGAGGTCA (REV)
	ERα #5	CCCAGGTCACTGCAATCTTT (FWD) CCTGGTCCAGACCACAGAAT (REV)
	ERα #6	ATATTTGCCGCAGCTCAAGT (FWD) CCTGAGCTAGCACATGGTGA (REV)
	RNA Pol II #1	GGGACAGACAGGGAGAGATG (FWD) ACTGTGGTCCCCCTAGACCT (REV)
	RNA Pol II #2	TTGATTTCACAACGGTCGAG (FWD) GAGACTCGGATTTGCCAGTT (REV)
	RNA Pol II #3	GTCCATCTCTGGAAGCAGGA (FWD) CGTCCCTCAGGCTGTCTTC (REV)
	RNA Pol II #4	CCCCTTTCCTGATTGACAAA (FWD) GGCCATTTTGGCTTGAACTA (REV)
	RNA Pol II #5	CCCCTAAAAATAGCCCATGA (FWD) CATTACCTCATCCCGTGAGC (REV)
	H3K27ac #1	GTCGAGGCTGAGGTCTCACT (FWD) AGCTTAAGACCGGCACCTCT (REV)
	H3K27ac #2	GTACTCCTCCGCTCCTTCCT (FWD) TACAGCCATGCTGCTTTCTG (REV)
	H3K27ac #3	TCTCTGGGCTGGCACCAT (FWD) GCGGTGCGTAGTCTGGAG (REV)
	H3K27ac #4	TCAGAAATCCCAGCCTTCTC (FWD) GCAGTAATGGTGAGGCCTTG (REV)
	H3K27ac #5	GCGTGTCTGCGTAGTAGCTG (FWD) AGGGCTGGATGGTTGTATTG (REV)
	H3K27ac #6	GGACGTGTCCTTATCCCTGA (FWD) GACGCACTGAATGGAAAGGT (REV)
	H3K27ac #7	ATGACCATGACCCTCCACAC (FWD) TTGCTGCTGTCCAGGTACAC (REV)
	H3K27ac #8	GTAATGACGGGGCTTCCTTT (FWD) TGAGATCTGCCGAGTCATTG (REV)
	FOXA1 #1	ACCCACCCTCCTTCTCTGTT (FWD) TGTTCAATGGCCACTGTTTG (REV)
	FOXA1 #2	CCTAGCTGCAAACCCAAATC (FWD) GCAAATGAGGGAATGAAGGA (REV)
	FOXA1 #3	CATTTATGCCCTCCCCTTCT (FWD) GAAACCGATCTATGGGCTGA (REV)
	FOXA1 #4	CAGCCGTCTTGCAAAGAAA (FWD) GCCGAGGAACACTGAAAGAC (REV)
	FOXA1 #5	TGCCTGGGATACCAAGGTTA (FWD) CTGGTTGCTTCCTTGAGCTT (REV)
	FOXA1 #6	CGCAAATGTCAGCATGTTCT (FWD) TTGTTTCACAGGAGCCAATG (REV)
	FOXA1 #7	CTTGAGGTCAGGCAGTCTCC (FWD) AAGCCACTCCAAGGTAGGTG (REV)
	FOXA1 #8	GGCTCTTCAGTCTGCCAGTT (FWD) CACGCTGACTTCAACAGCTT (REV)
	FOXA1 #9	AGGGATAACCCACACGACTG (FWD) AGCCTGGGCTGTTTACTCTG (REV)
	FOXA1 #10	CACCAGTGTTAGGCTGCAAA (FWD) TCCTTCCCATTCATTTCCAA (REV)
Negative control	ERα #7 H3K27ac #9 FOXA1 #11	TGCCACACACCAGTGACTTT (FWD), ACAGCCAGAAGCTCCAAAAA (REV)
	ERα #8	TGGCCCTTGATACTGGAGTC (FWD), GACATCCAAGGCAAGATGGT (REV)
	ERα #9 H3K27ac #10	CTAGGAGGGTGGAGGTAGGG (FWD) GCCCCAAACAGGAGTAATGA (REV)
	ERα #10 Pol #7 H3K27ac #11 FOXA1 #12	CACACCCGCTCTACGATATGA (FWD) GAGCTCGGCAGGCTCTGA (REV)
	ERα #11 FOXA1 #13	CTGCTCCTGGCATTATCCTC (FWD) TGTGGCTCTCAGCAGGAAGT (REV)
	ERα #12 H3K27ac #12	AATCCTTTGGCTGCCAGTTA (FWD) AGGTACTTCTGGGCATGGTG (REV)
	Pol #6	AGACTCCAGACGCACCATCT (FWD) GGGTGACTTTGTGTCCGAAA (REV)

Supplementary Table S4: Accession numbers of Ishikawa RNA-seq and ChIP-seq datasets generated by the ENCODE consortium and publicly available ERα ChIP-seq data from breast tumors.

Cell line/tissue		**Accession number**
RNA-seq Ishikawa		GSE35584
Ishikawa	DMSO	GSM923423
Ishikawa	E2	GSM923427
ChIP-seq Ishikawa	Factor	GSE32465
Ishikawa	EGR1	GSM1010888
Ishikawa	USF1	GSM1010886
Ishikawa	TEAD4	GSM1010885
Ishikawa	CREB1	GSM1010857
Ishikawa	FOXM1	GSM1010856
Ishikawa	NFIC	GSM1010855
Ishikawa	TCF12	GSM1010842
Ishikawa	REST/NRSF	GSM1010841
Ishikawa	MAX	GSM1010807
Ishikawa	CEBPB	GSM1010802
Ishikawa	RAD21	GSM1010801
Ishikawa	CTCF	GSM1010774
Ishikawa	SRF	GSM1010762
Ishikawa	p300	GSM1010759
Ishikawa	YY1	GSM1010753
Ishikawa	ZBTB7A	GSM1010752
Ishikawa	TAF1	GSM1010733
Ishikawa	RNA Pol II	GSM803536
Ishikawa	GR (EtOH)	GSM803464
Ishikawa	GR (DEX)	GSM803340
Ishikawa	FOXA1	GSM803444
Ishikawa	ERα (E2)	GSM803422
Ishikawa	ERα (DMSO)	GSM803421
ChIP-seq Breast tumors	**Factor**	GSE40867
Breast tumor 1	ERα	GSM1003720
Breast tumor 2	ERα	GSM1003734
Breast tumor 3	ERα	GSM1003723
Breast tumor 4	ERα	GSM1003717
Breast tumor 5	ERα	GSM1003714

Supplementary Table S5: Patient characteristics of breast tumors used in Figure 2.

Sample	Age at diagnosis	Tumor type	Tumor Grade	PR.status	HER2. status
1	76	IDC	1	positive	N/A
2	58	IDC	N/A	positive	N/A
3	71	IDC	2	positive	negative
4	51	IDC	3	positive	negative
5	69	IDC	2	positive	negative

N/A = Not Available

Supplementary Table S6: ChIP-seq read count and mapped reads of endometrial tumors.

cell line/ tissue	ChIP Ab	read count	mapped reads	% mapped
Tumor A	ERα	15331544	14207826	92.7
Tumor A	RNA Pol II	23862573	21681488	90.9
Tumor A	H3K27ac	23175814	22016196	95.0
Tumor A	FOXA1	20609022	19267411	93.5
Tumor B	ERα	24549638	23652264	96.3
Tumor B	RNA Pol II	32192458	20334325	63.2
Tumor B	H3K27ac	23110704	22653542	98.0
Tumor B	FOXA1	21130957	19291797	91.3
Tumor C	ERα	21716690	20774510	95.7
Tumor C	RNA Pol II	16224209	15248669	94.0
Tumor C	H3K27ac	25380006	23402591	92.2
Tumor C	FOXA1	23378746	22458122	96.1
Tumor D	ERα	19603948	17976811	91.7
Tumor D	RNA Pol II	25018163	23111684	92.4
Tumor E	ERα	14930170	12101826	81.1
Tumor E	RNA Pol II	19557143	10884677	55.7
Tumor E	H3K27ac	20390107	19103140	93.7
Tumor E	FOXA1	15259727	13819149	90.6
Tumor input		22580567	21760992	96.4
Biological replicate B	ERα	19287976	18138678	94.0
Biological replicate B	RNA Pol II	21358572	20065872	94.0
Biological replicate B	H3K27ac	23442310	21669423	92.4
Biological replicate B	FOXA1	31834682	30078757	94.4

Supplementary Table S7: mRNA primer sequences
Primers correspond with Supplementary Figure S5.

	Primer Sequences
ESR1	TGGAGATCTTCGACATGCTG (FWD), TCCAGAGACTTCAGGGTGCT (REV)
FOXA1	GCCTGAGTTCATGTTGCTGA (FWD), AAAACGCGTATTGGAACTGC (REV)
NR3C1	CCCAGAGCAAATGCCATAAG (FWD), GAAATGGGCAAAGGCAATAC (REV)
CTCF	CACTTCAAGCGCTATCACGA (FWD), CCTCCATTTTCCCCCTCTAC (REV)
EP300	TTAAAAATGGCCGAGAATGTG (FWD), TCTGGTAAGTCGTGCTCCAA (REV)
NFIC	CTGAACCCAGCCAGCACT (FWD), GTGTCCACGTCTTCCTCCAT (REV)
TEAD4	TCCACGAAGGTCTGCTCTTT (FWD), GTGCTTGAGCTTGTGGATGA (REV)
TCF12	GCCTGCTGGTCACAGTGATA (FWD), GTCTTCCCGATGAGTTCCAA (REV)
CEBPB	CAGCGACGAGTACAAGATCC (FWD), AGCTGCTCCACCTTCTTCTG (REV)
FOXM1	GAAGCCACTGGATGTTGGAT (FWD), AACCTGCAGCTAGGGATGTG (REV)
RAD21	GTAGAACTTTGCGGCAGCTT (FWD), TCAGCAGATGCTTCATGGTC (REV)
EGR1	CAGCACCTTCAACCCTCAG (FWD), CAGCACCTTCTCGTTGTTCA (REV)
ZBTB7A	CATGTGCACCTTCAGCTTGT (FWD), ATCTGCGAGAAGGTCATCCA (REV)
MAX	TTGAAACCTCGGTTGCTCTT (FWD), TGTTGTTGTCGGTGACTTCC (REV)
SRF	GACAGCAGCACAGACCTCAC (FWD), ATGCGGGCTAGGGTACATC (REV)
CREB1	GCGAAGGGAAATTCTTTCAA (FWD), GCACCGTTACAGTGGTGATG (REV)
TAF1	AGACTGCCCAGGAGATTGTG (FWD), CATTGGGTCCAGGCTTTCTA (REV)
USF1	ACTGTCCCCTCTTCCGTTTC (FWD), AGCACTCAGGCCTGTGAATC (REV)
YY1	AAGAAGTGGGAGCAGAAGCA (FWD), CAACCACTGTCTCATGGTCAA (REV)
REST	CAGGAGAACGCCCATATAAA (FWD), GAGGCCACATAACTGCACTG (REV)

Supplementary Table S8:

Shared ERα/FOXA1			
Top Canonical Pathways	-log10(p-values)	Top Upstream Regulators	-log10 (p-value)
Molecular Mechanisms of Cancer	3.34	ESR1	7.06
Rac Signaling	3.28	TP63	5.52
HER-2 Signaling in Breast Cancer	3.25	TP53	4.49
Ephrin Receptor Signaling	3.22	ESR2	4.07
PPARα/ RXRα Activation	3.13	TEF	3.85
ERα only			
Top Canonical Pathways	-log10(p-values)	Top Upstream Regulators	-log10 (p-value)
Germ Cell-Sertoli Cell Junction Signaling	4.85	beta-estradiol	7.92
Epithelial Adherens Junction Signaling	3.70	TGFB1	6.49
Ceramide Signaling	3.32	trichostatin A	6.24
Sertoli Cell-Sertoli Cell Junction Signaling	3.25	ESR1	6.11
Wnt/β-catenin Signaling	3.02	FSH	5.92
FOXA1 only			
Top Canonical Pathways	-log10(p-values)	Top Upstream Regulators	-log10 (p-value)
TGF-β signaling	2.94	ESR1	5.82
CD27 Signaling in Lymphocytes	2.28	beta-estradiol	5.64
Wnt/β-catenin Signaling	2.09	dihydrotestosterone	4.48
Hereditary Breast Cancer Signaling	2.09	TP53	3.90
ATM signaling	2.09	NR3C1	3.65