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ERα K303R mutation reorganizes its binding to FoxA1 regions and induces chromatin opening.

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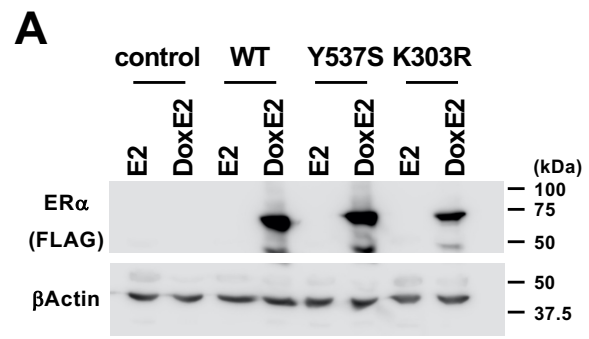
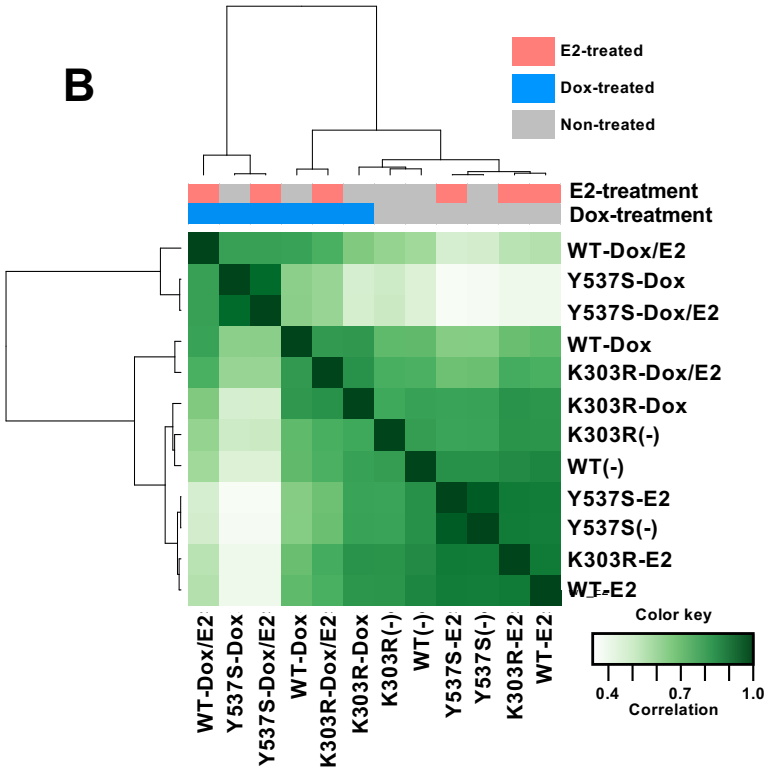
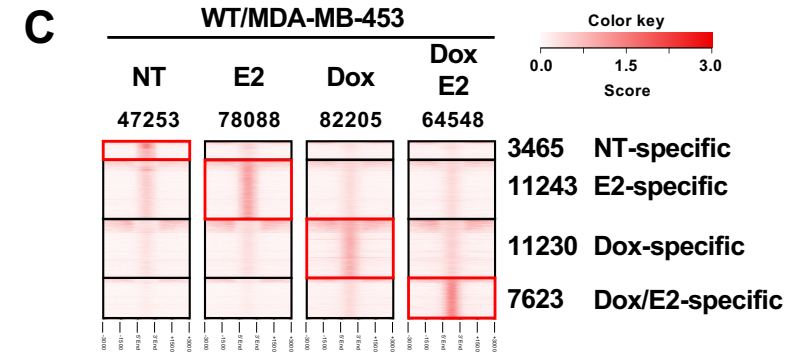
Supplemental figures
Fig. S1

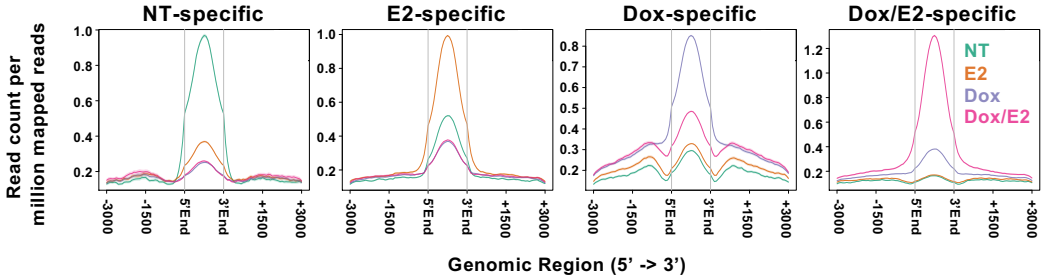
Cell Line	Sourse	Type	Molecular Subtypes						Data from DepMap												Absolute mRNA (log2((ymol/2ul cDNA)+1) (this study)			Luciferase assay results		
										Presense of mutation						Relative mRNA (log2(TPM+1))			Relative protein (log2(RPPA signal))								
			Neve et al., 2006	Charafe-Jauffret et al., 2006	Sieuwerts et al., 2009	Prat et al., 2010	Subik et al., 2010	Dai et al., 2017	Cell line ID	ESR1 (ERa)	ERBB2 (HER2)	PGR (PR)	TP53	BRCA1	BRCA2	ESR1 (ERa)	ERBB2 (HER2)	PGR (PR)	ERa	HER2	PR	ESR1 (ERa)	ERBB2 (HER2)	PGR (PR)	Ectopic ERa-dependency	E2 responsiveness	Type
MCF7	PE	IDC	Lu	Lu	Lu	-	Lu A	Lu A	ACH-000019	0	0	0	0	0	0	5.4	5.5	2.4	3.3	0.4	0.2	12.5	11.8	11.1	X	O	A
T-47D	PE	IDC	Lu	Lu	Lu	-	NA	Lu A	ACH-000147	0	0	0	1	0	0	5.5	6.3	6.3	3.4	0.7	0.8	12.2	12.8	15.3	X	O	A
MDA-MB-453	PeE	Ad	Lu	Lu	HER	-	-	HER	ACH-000910	0	1	0	0	0	0	0.0	8.0	0.0	0.6	1.7	-0.1	0.8	15.2	6.2	O	O	B
YMB-1	PB	IDC	NA	NA	NA	NA	NA	NA	ACH-001249	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	5.5	14.3	3.3	O	O	B
BT-20	PB	IDC	BaA	Ba	Ba	-	Ba	BaA	ACH-000536	0	0	0	1	0	0	2.0	6.1	0.0	0.8	0.5	-0.1	8.3	12.5	0.0	X	O	A
HCC1143	PB	DC	BaA	NA	NA	-	NA	BaA	ACH-000374	0	0	0	1	0	0	2.6	5.1	0.0	0.3	-0.4	0.0	6.8	12.2	0.0	No ERE activity		
HCC1806	PB	ASC	NA	NA	NA	NA	NA	BaA	ACH-000624	0	0	0	1	0	0	1.2	5.0	0.0	NA	NA	NA	8.9	12.3	0.0	O	X	C
HCC1937	PB	DC	BaA	Ba	Ba	-	NA	BaA	ACH-000223	0	0	0	1	0	0	2.2	5.6	0.0	0.4	0.0	0.1	9.3	12.3	0.0	No ERE activity		
HCC70	PB	DC	BaA	NA	NA	-	Ba	BaA	ACH-000668	0	0	0	1	0	0	1.4	5.4	0.0	-0.3	0.4	-0.2	0.0	12.8	0.0	No ERE activity		
MDA-MB-468	PE	Ad	BaA	NA	Ba	-	NA	BaA	ACH-000849	0	1	0	1	0	1	1.2	4.6	0.0	0.0	-2.2	-0.1	7.2	10.6	0.0	No ERE activity		
HCC1395	PB	DC	NA	NA	NA	NA	NA	BaB	ACH-000699	0	0	0	1	1	1	0.0	4.2	0.0	-0.4	0.6	-0.5	6.3	13.8	1.5	No ERE activity		
HCC38	PB	DC	BaB	Ba	NA	-	NA	BaB	ACH-000276	0	0	0	1	0	1	0.0	7.1	0.7	-0.2	1.2	-0.2	0.2	13.5	1.9	No ERE activity		
BT549	PB	IDC	BaB	NA	Nor	CL	NA	BaB	ACH-000288	0	0	0	1	0	0	0.0	4.1	0.1	NA	NA	NA	0.1	12.9	0.0	No ERE activity		
SUM159PT	PB	AC	BaB	NA	Nor	CL	NA	BaB	ACH-001391	0	0	1	1	0	0	0.0	4.2	0.1	NA	NA	NA	0.8	11.8	0.7	O	X	C
Hs578T	PB	IDC	BaB	Me	Nor	CL	NA	BaB	ACH-000148	0	0	0	1	0	0	0.1	4.7	0.0	0.2	-0.7	0.4	1.8	11.4	2.5	O	O	B
MDA-MB-157	PE	MC	BaB	Me	Nor	CL	NA	BaB	ACH-000621	0	0	0	1	0	0	0.2	4.5	0.2	-0.4	0.5	-0.1	2.2	11.4	6.9	No ERE activity		
MDA-MB-231	PE	Ad	BaB	Me	Nor	CL	Ba	BaB	ACH-000768	0	0	0	1	0	0	0.1	4.7	0.0	0.1	0.0	-0.1	0.9	11.3	0.0	No ERE activity		
MDA-MB-436	PE	Ad	BaB	NA	Nor	CL	HER	BaA	ACH-000573	0	0	0	1	1	0	0.1	4.0	0.0	0.1	-0.6	0.0	1.5	9.5	2.3	No ERE activity		
MCF 10A	PB	FD	BaB	NA	NA	NA	NA	NA	ACH-001357	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.9	10.3	0.0	No ERE activity		

Fig. S2









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Motif Name	Motif	Gene Symbol	Family	Log P-value			
				no treat	E2	Dox	Dox/E2
ERE(NR),IR3/MCF7-ERa-ChIP-Seq		ESR1	NHR	-1	0	-246	-1797
COUP-TFII(NR)/Artia-Nr2f2-ChIP-Seq		NR2F2	NHR	0	0	-48	-1110
EAR2(NR)/K562-NR2F6-ChIP-Seq		NR2F6	NHR	0	0	-14	-828
THRb(NR)/Liver-NR1A2-ChIP-Seq		THRB	NHR	-1	0	-81	-815
COUP-TFII(NR)/K562-NR2F1-ChIP-Seq		NR2F1	NHR	0	0	-20	-703
Fra1(bZIP)/BT549-Fra1-ChIP-Seq		FOSL1	AP1	-7	-97	-1614	-231
Fra2(bZIP)/Striatum-Fra2-ChIP-Seq		FOSL2	AP1	-6	-98	-1548	-228
JunB(bZIP)/DendriticCells-Junb-ChIP-Seq		JUNB	AP1	-8	-85	-1550	-225
BATF(bZIP)/Th17-BATF-ChIP-Seq		BATF	AP1	-9	-77	-1508	-224
Atf3(bZIP)/GBM-ATF3-ChIP-Seq		ATF3	CREB	-8	-88	-1471	-215

Fig. S3

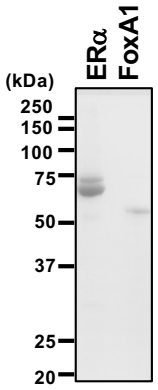

Fig. S4

	Luciferase assay results			TPM of each gene																									
							ERα Co-activator													ERα Co-repressor									
							LBD-binding co-activator											Co-activator binding to other than LBD		LBD-binding co-repressor							Co-repressor binding to other than LBD	
	Ectopic ERα-dependency	E2 responsiveness	Type	ESR1	GATA3	FOXA1	MED1	NCOA1	NCOA2	NCOA3	PPARGC1A	PPARGC1B	PRC1	SRA1	DDX5	ERBB4	CITED	BAF57	CCND1	NCOR1	NCOR2	NRIP1	NR0B1	NR0B2	PHB2	BRCA1	UBE3A
MCF7	X	O	A	56	876	182	18	14	33	586	0	3	175	47	864	1	36	18	541	32	37	40	0	0	228	17	20
T-47D	X	O	A	52	691	235	71	18	25	48	1	3	159	55	269	9	49	38	212	20	22	54	0	0	84	34	34
BT-20	X	O	A	3	68	56	44	29	27	137	0	2	242	30	208	0	25	15	191	40	49	90	0	0	342	21	32
YMB-1	O	O	B	40	123	207	47	20	42	71	0	7	172	26	510	23	65	30	614	34	97	17	NA	NA	710	22	57
Hs578T	O	O	B	0.1	5	2	50	18	31	55	0	1	14	61	342	0	185	3	322	48	97	13	0	0	211	23	27
MDA-MB-453	O	O	B	0	690	384	48	15	33	44	0	4	95	33	567	1	53	14	451	39	30	69	0	NA	301	12	51
HCC1806	O	X	C	1	44	24	31	5	9	67	0	4	50	15	291	0	63	10	381	41	81	33	3	0	220	18	55
SUM 159PT	O	X	C	N/A	N/A	N/A	68	13	16	46	NA	8	240	41	361	NA	88	31	442	32	103	16	NA	NA	448	46	38
HCC1143	No ERE activity			5	16	19	36	8	28	56	0	3	193	55	263	0	79	12	1644	32	26	172	0	NA	178	22	33
HCC1937	No ERE activity			4	26	25	58	14	32	73	0	7	77	61	336	0	85	11	488	39	57	59	0	2	309	40	26
HCC70	No ERE activity			2	69	50	34	18	61	42	1	1	113	35	221	0	24	8	121	26	55	40	0	1	168	25	30
MDA-MB-468	No ERE activity			2	44	33	37	33	23	48	0	5	115	49	233	0	173	18	253	24	67	103	0	NA	165	25	22
MDA-MB-157	No ERE activity			0.1	0.3	0.2	40	31	25	27	3	5	148	102	426	0	17	28	108	26	49	38	1	NA	174	21	33
MDA-MB-231	No ERE activity			0.1	10	0.1	46	12	7	23	1	1	160	43	265	0	78	23	543	36	38	7	0	NA	214	19	20
HCC1395	No ERE activity			0	0.7	0.2	41	8	18	66	1	6	133	46	258	0	35	14	216	32	56	34	37	0	560	8	24
HCC38	No ERE activity			0	35	10	94	21	33	55	0	16	146	48	469	0	104	24	618	34	74	107	3	1	175	9	45
BT549	No ERE activity			0	14	0	56	11	37	55	1	4	7	54	239	0	247	1	18	59	42	7	0	0	373	27	19
MDA-MB-436	No ERE activity			0	0.6	4	29	11	18	17	1	10	146	4	180	0	28	14	177	20	85	49	19	0	750	14	36
MCF 10A	No ERE activity			N/A	N/A	N/A	48	10	15	104	2	9	158	42	267	NA	28	26	171	44	23	19	NA	NA	397	32	36

Expression level (TPM)

MIN 0 MAX

Fig. S5

Gene Symbol	Relative TPM in RNA-seq Data (GSE85870)	Relative average TPM in our scRNA-seq Data
FOXA1	100	100
FOXM1	8	12
FOXK2	7	84
FOXP1	5	34
FOXO3	3	50
FOXK1	3	82
FOXO1	2	38
FOXH1	0	0
FOXA2	0	0
FOXA3	0	0
FOXD3	0	0
FOXF1	0	0
FOXL2	0	0

Fig. S6

FOXA1(Forkhead)/LNCAP-FOXA1-ChIP-Seq(GSE27824)

FOXA1(Forkhead)/MCF7-FOXA1-ChIP-Seq(GSE26831)

FOXM1(Forkhead)/MCF7-FOXM1-ChIP-Seq(GSE72977)

FOXK2(Forkhead)/U2OS-FOXK2-ChIP-Seq(E-MTAB-2204)

Supplemental figure captions

Fig. S1 The BC cell lines tested for the luciferase assay, relates to Fig. 1. The BC cell lines tested for the luciferase assay was represented with various data and the summary of luciferase assay. The source, cell types, and the molecular subtypes are also shown (1-6). The information of gene mutations, and relative mRNA and protein levels were obtained from DepMap (https://depmap.org/portal/). The absolute mRNA quantities in the actually tested cell lines were measured by own RT-qPCR analysis (see Materials and Methods). The results of luciferase assay were also summarized as types or *No ERE activity* meaning no firefly Luciferase signal detected. Abbreviations are: PB, primary breast; PE, pleural effusion; PeE, pericardial effusion; IDC, invasive ductal carcinoma; AC, anaplastic carcinoma; DC, ductal carcinoma; ASC, Acantholytic Squamous Cell Carcinoma; MC, metaplastic carcinoma; Ad, adenocarcinoma; FD, fibrocystic disease; Lu: Luminal; LuA: Luminal A; Ba, Basal; BaA, Basal A; BaB, Basal B; HER, HER2 positive; Me, Mesenchymal; Nor, Normal; CL, Claudin-low. **B)**

Fig. S2 ATAC-seq analysis of Y537S/MDA-MB-453 and K303R/MDA-MB-453, relates to Fig. 2A. ERα expression in each clone. Whole-cell extracts of each clone were subjected to SDS-PAGE followed by western blotting to detect ERα or βActin with specific antibodies as indicated. **B)** DBA of ATAC-seq peak data. Treatments of each sample are also indicated. **C)** Heatmap showing the signal intensity of WT ERα ATAC-seq peaks in each condition-specific group. ATAC-seq peaks of WT/MDA-MB-453 were classified into each condition-specific group (red boxes) and those corresponding genomic regions in other groups also shown in parallel. Peak intensities are plotted in a ±3kb window from summits. **D)** Average peak density profile of WT ERα in each condition-specific group (panel C). **E)** Enrichment of TFBMs at ATAC-seq peaks of Dox(+)/E2(-) and Dox(+)/E2(+)-specific groups of WT/MDA-MB-453. Log(p-value) of top 5 enriched TFBMs in each group and those corresponding values in other groups are represented with gene symbol, belonging family, and motif logo.

Fig. S3 SDS-PAGE analysis of recombinant FoxA1 and ERa, relates to Fig. 5.

Fig. S4 Expression levels of ERα-related cofactors. Expression levels of ERα-related cofactors in the various BC cell lines were obtained from Expression Atlas

(https://www.ebi.ac.uk/gxa/home) and expression levels of each factor (TPM, transcripts per million) from multiple data sets were averaged and represented with a heatmap in parallel with the results of the luciferase assay. Because various ERα cofactors have been reported to contribute to E2-dependent and E2-independent ERα-driven transcription regulation (7-11).

Fig. S5 Relative expression levels of the Fox family members (TPM, transcripts per million) in MDA-MB-453 cells obtained from public RNA-seq (GSE85870) and own scRNA-seq analysis (unpublished data).

Fig. S6 Motif logos representing unique binding sequences of the various Fox family members stored in Homer software package.

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