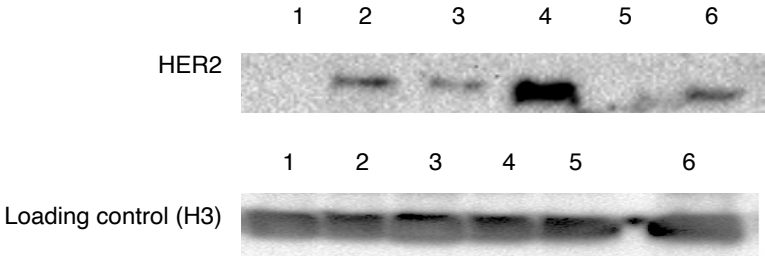


Figure Supplementary 1

A HER2 protein expression in paired samples of patients



B Quantification of HER2 signal vs. loading control

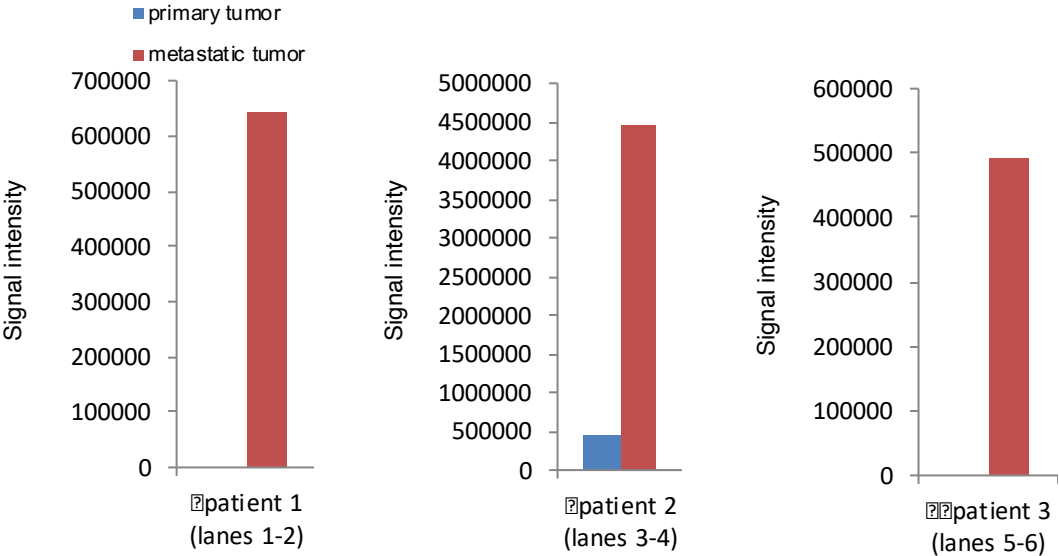
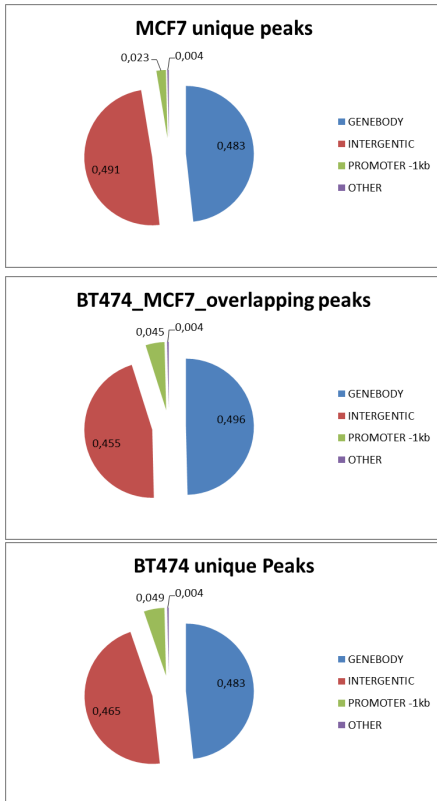
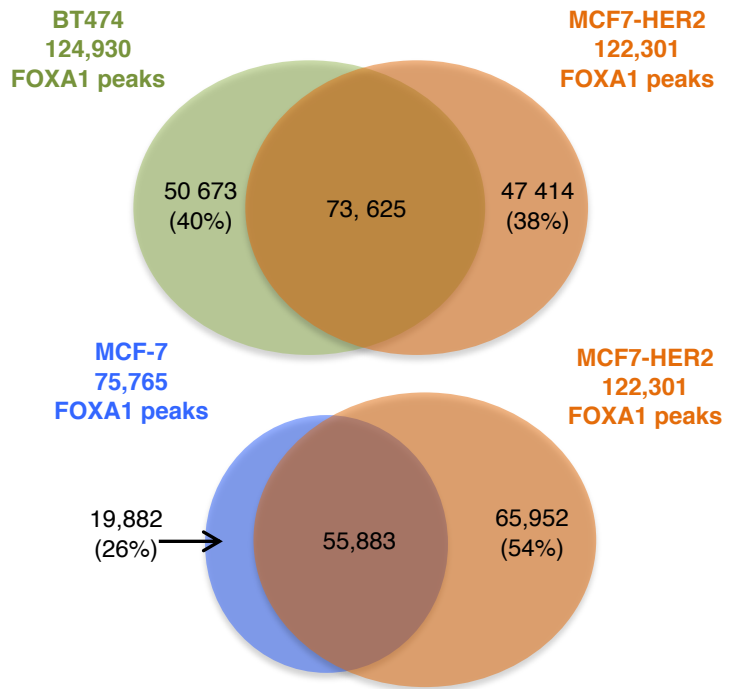


Figure Supplementary 2

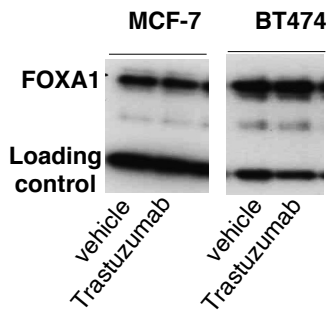
A Genomic distribution of FOXA1 binding sites



B FOXA1 binding in breast cancer cell lines



C FOXA1 protein upon HER2 inhibition



D HER2 protein levels in MCF-7 and MCF7-HER2

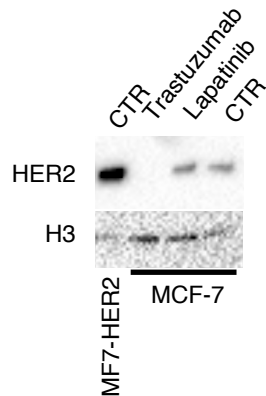
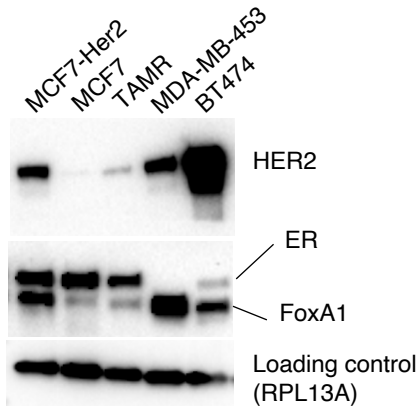
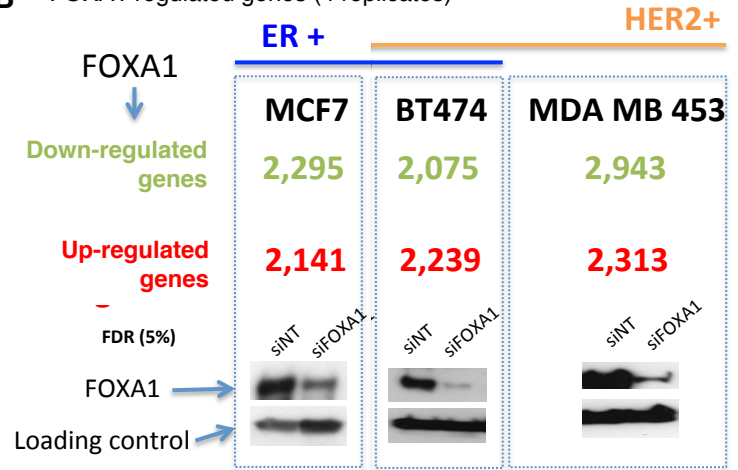


Figure Supplementary 3

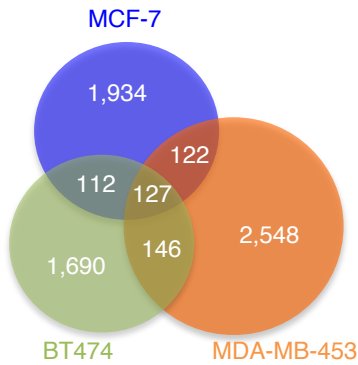
A HER2, ER and FOXA1 protein levels in breast cancer cell lines



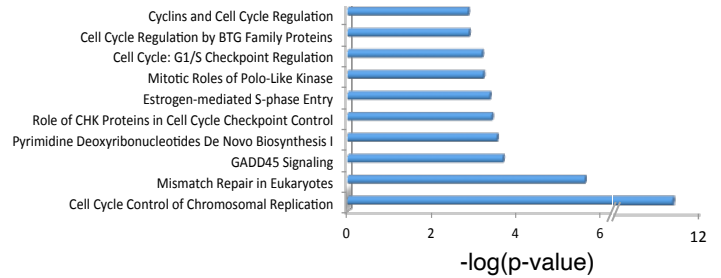
B FOXA1 regulated genes (4 replicates)



C Down-regulated genes in FOXA1 depleted cells

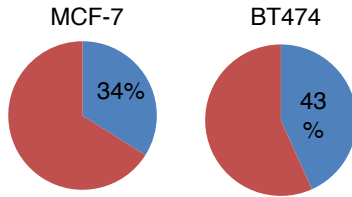


D Integrated Pathway Analysis of the common down-regulated genes

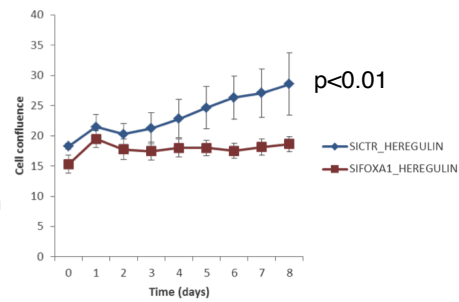
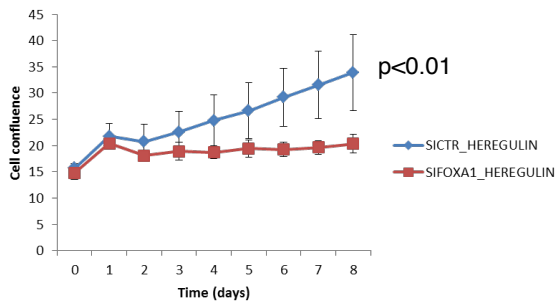
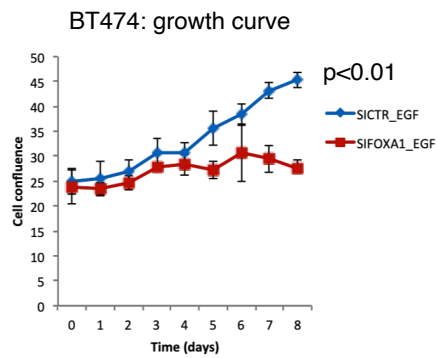
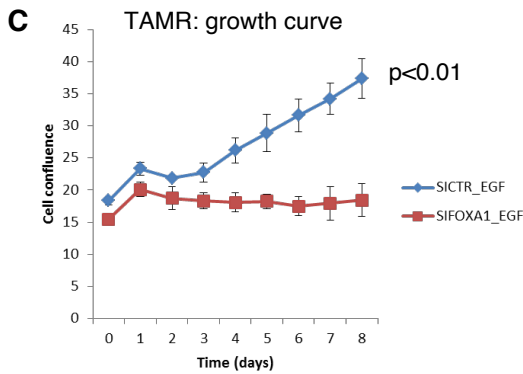
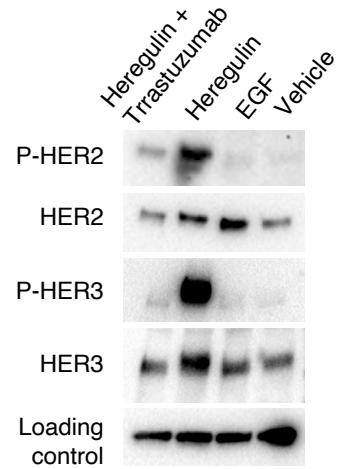


Supplementary Figure 4

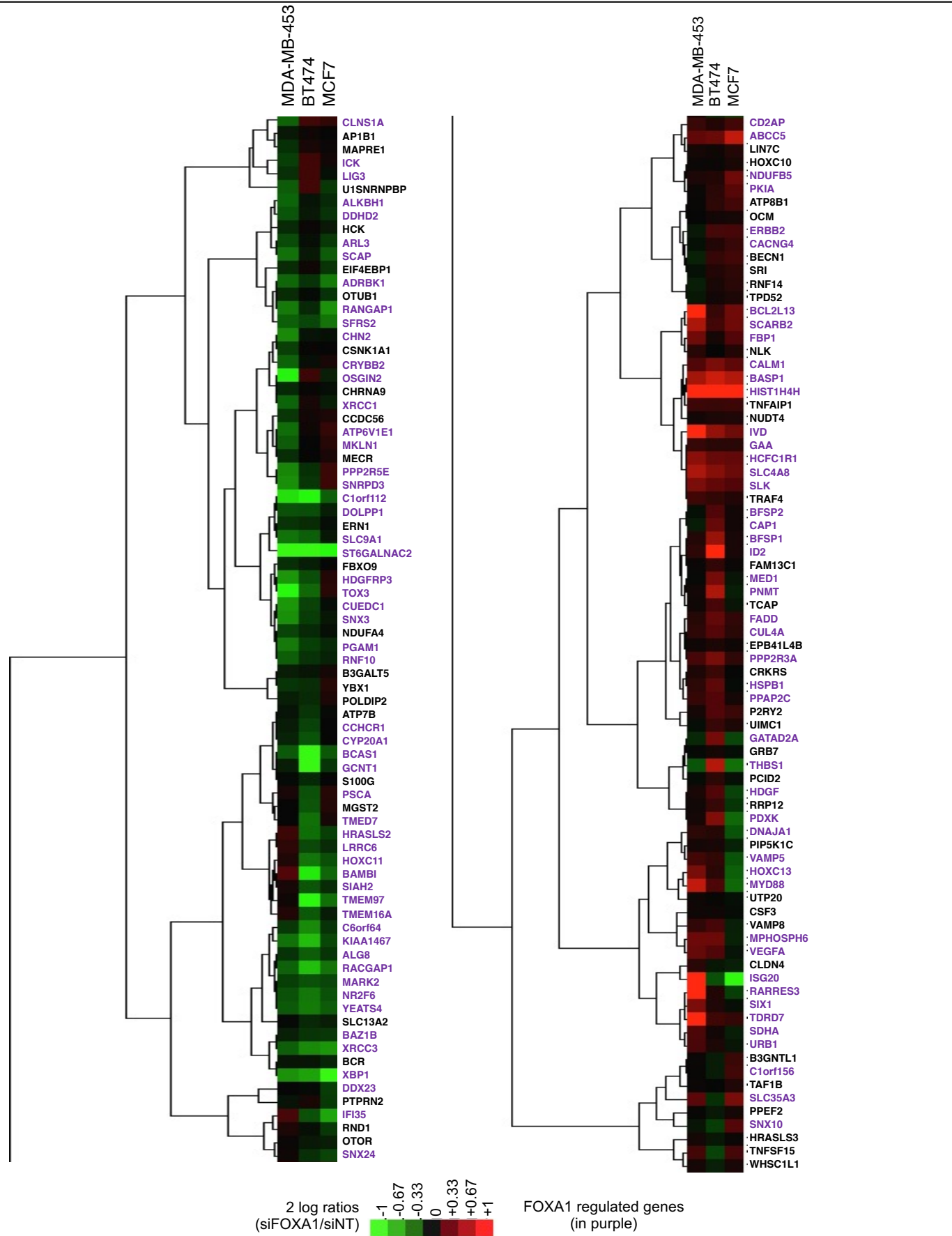
A Genes significantly regulated by FOXA1 and with HER2 regulated FOXA1 site (+/-20kb)



B P-Protein levels in MCF-7 cells

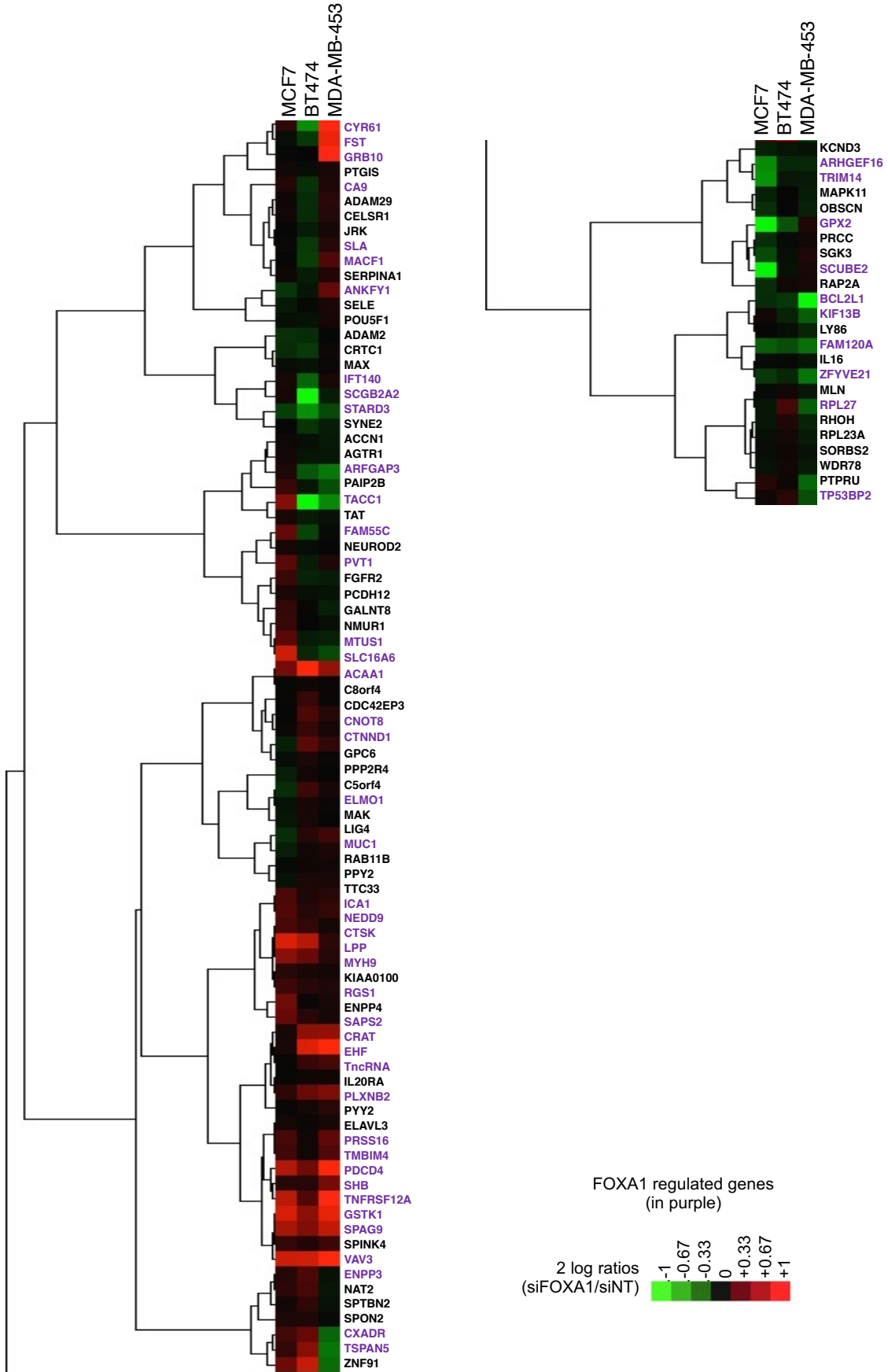


High expression (157 genes)



Continuation of Figure supplementary 5

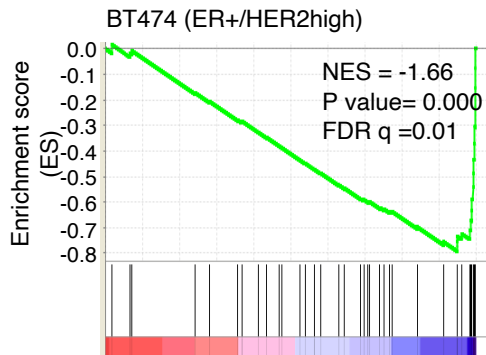
Low expression (107 genes)



A The optimal set of 55 up-regulated genes predicting poor clinical outcome in breast cancer patients (defined as developing metastases with 5 years) from van't Veer *et al*)

ALDH4A1	MCM6
AP2B1	MELK
BBC3	MMP9
C16orf61	MS4A7
C17orf109	MTDH
C20orf46	NDC80
CCNE2	NMU
CDC42BPA	NUSAP1
CENPA	ORC6
COL4A2	OXCT1
DCK	PALM2-AKAP2
DIAPH3	PITRM1
DTL	PRC1
EBF4	QSOX2
ECI2	RAB6B
ESM1	RFC4
EXT1	RTN4RL1
FGF18	RUNDC1
FLT1	SCUBE2
GMPS	SERF1A
GNAZ	SLC2A3
GPR126	STK32B
GPR180	TGFB3
GSTM3	TSPYL5
HRASLS	UCLH5
IGFBP5	WISP1
LOC286052	ZNF385B
LPCAT1	

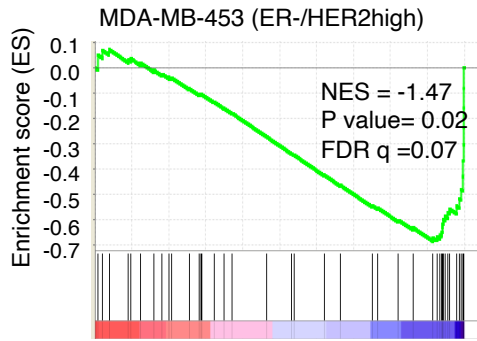
B GSEA of FOXA1 up-regulated genes



17/55 genes (37%)

QSOX2	CCNE2
UCLH5	MS4A7
DCK	CENPA
DIAPH3	NUSAP1
MTDH	RFC4
TGFB3	MELK
RUNDC1	NDC80
TDL	MCM6
	PRC1

C GSEA of FOXA1 down-regulated genes



20/55 genes (43%)

HRASLS	IGFBP5
DCK	RUNDC1
DIAPH3	PRC1
OXCT1	MELK
UCLH5	RFC4
TGFB3	CCNE2
PITRM1	DTL
CENPA	MCM6
AP2B1	STK32B
NUSAP1	MS4A7

Shared genes between both HER2high cell lines → 14/55 genes (25%)

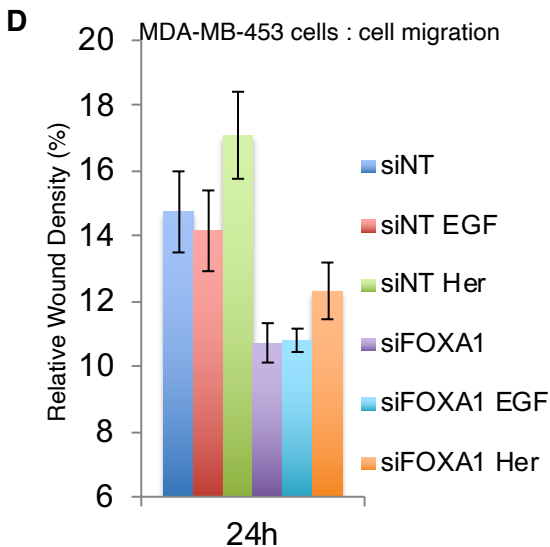


Figure Supplementary 7

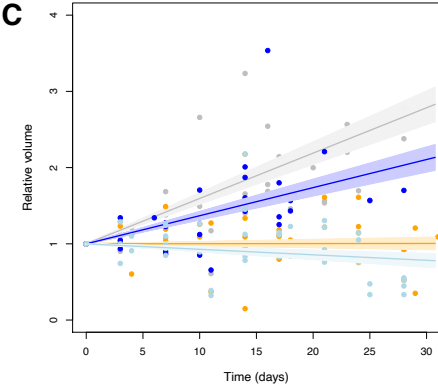
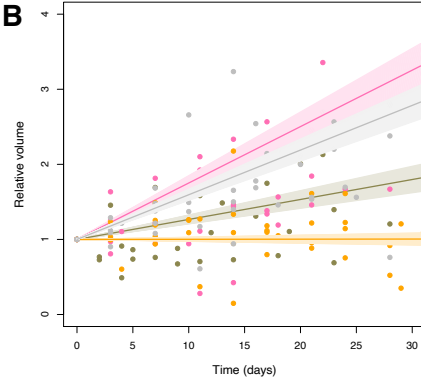
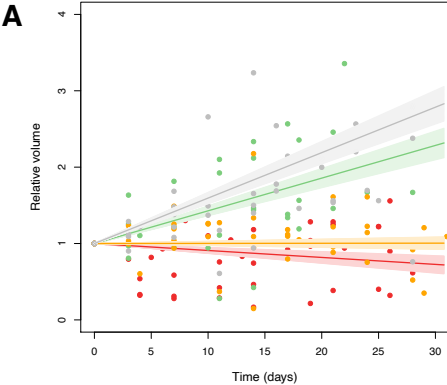
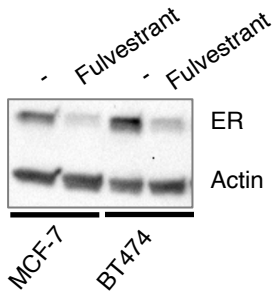
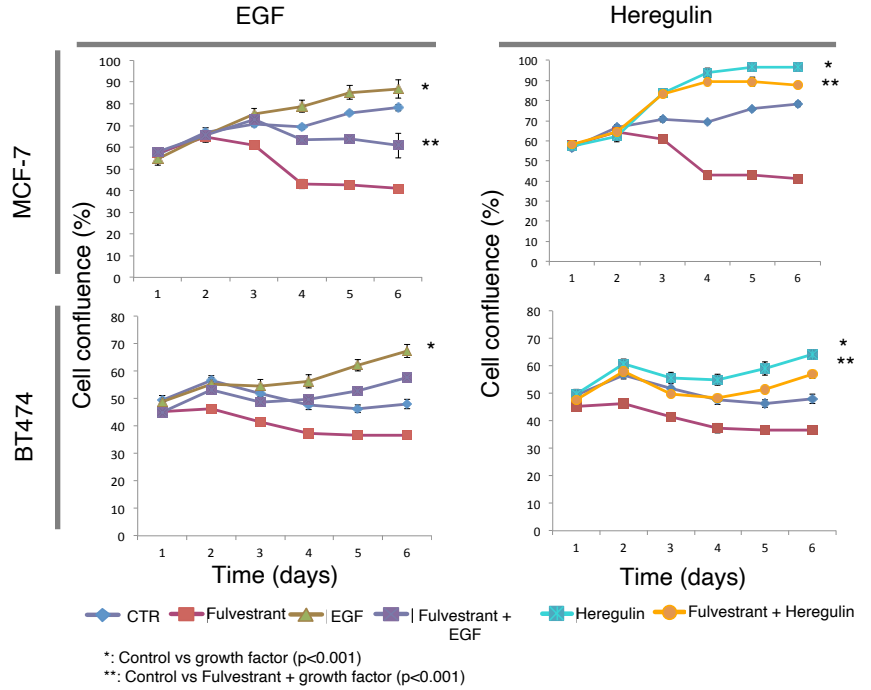


Figure Supplementary 8

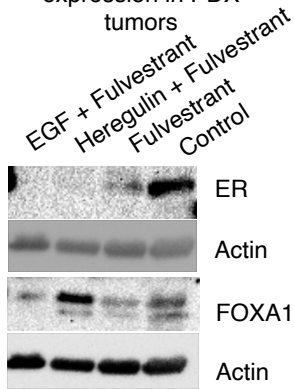
A ER levels in breast cancer cells



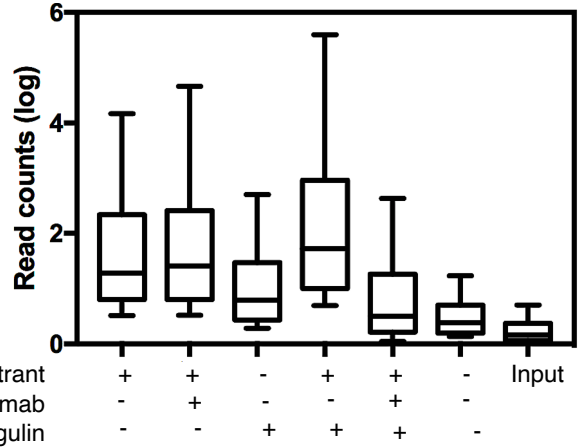
Cell growth: breast cancer cells



B ER and FOXA1 protein expression in PDX tumors



C FOXA1 ChIP-seq in PDX tumors (FOXA1 sites associated with poor prognosis)



D Genomic distribution of FOXA1 sites at PDX tumors

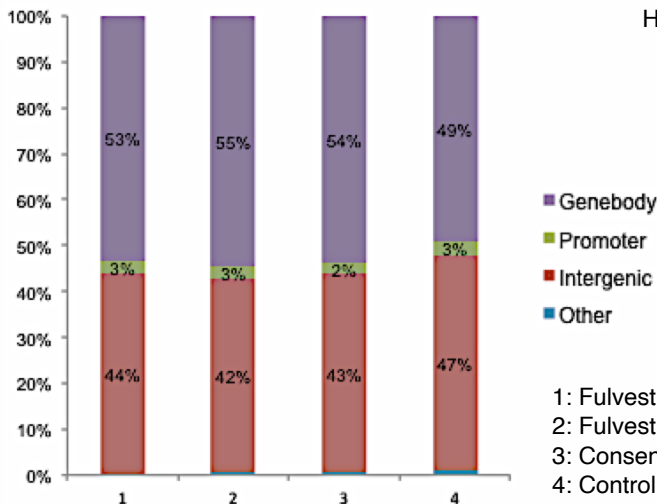
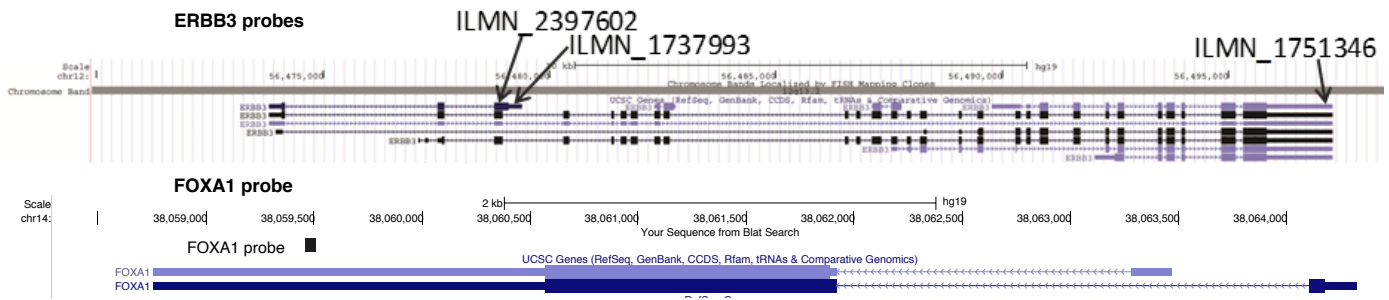


Figure Supplementary 9

A Genomic location of the three ERBB3 and the one FOXA1 mRNA probes analyzed for Metabarc Project (Curtis *et al*)



B Lineal regression of expression between FOXA1 and each of the 3 ERBB3 probes in Luminal A, Luminal B or HER2 breast cancer subtypes

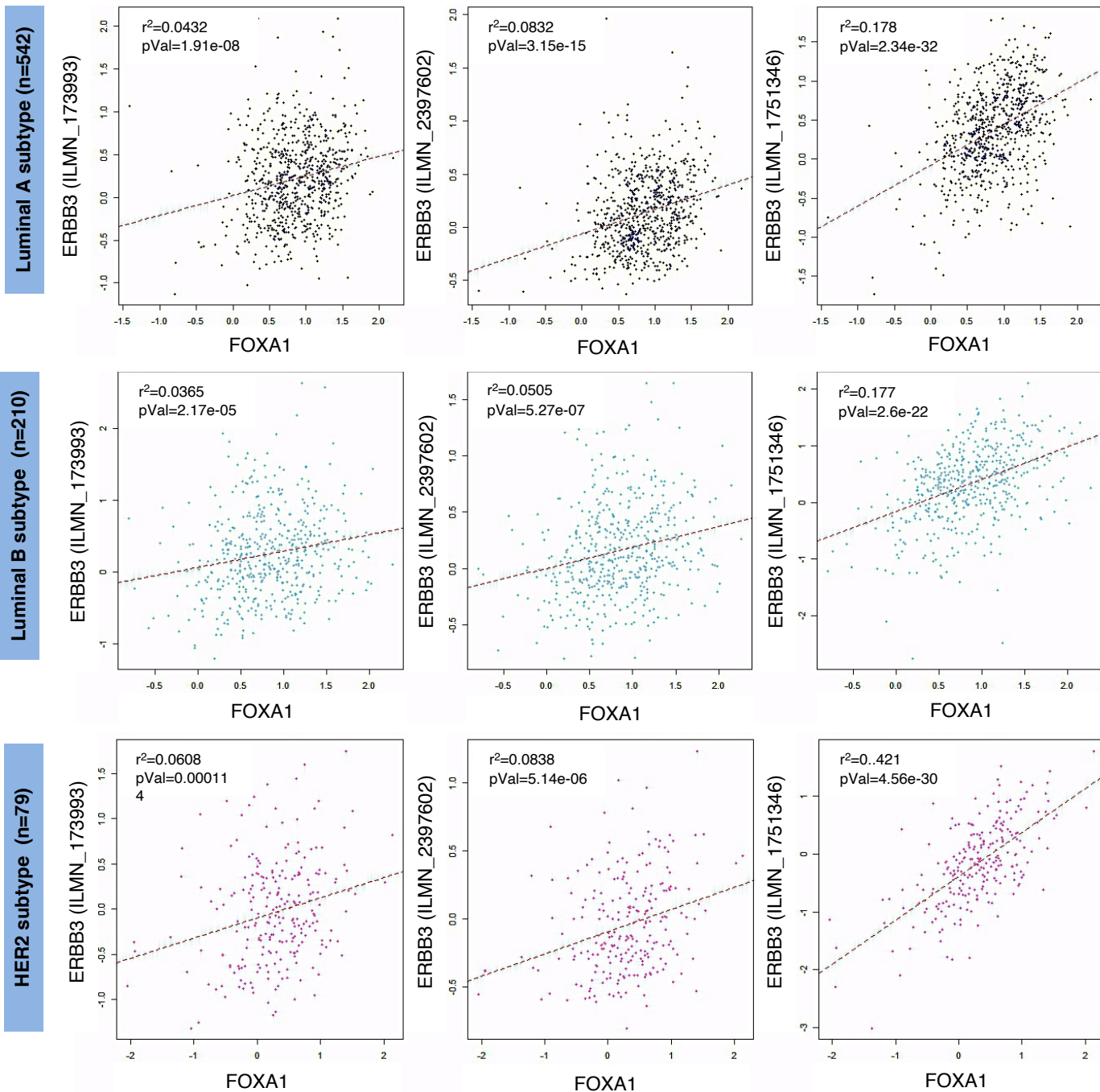
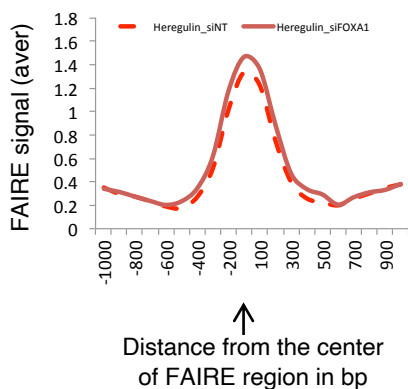


Figure Supplementary 10

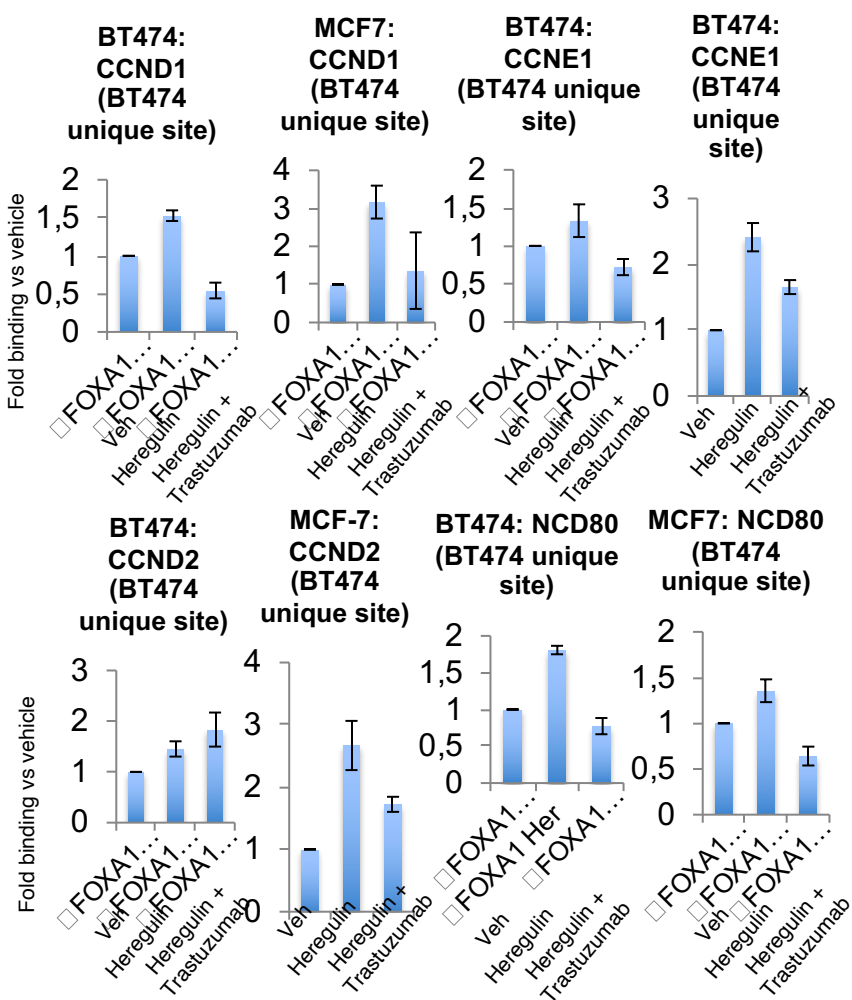
A Motif analysis

FOXA1 peaks	AP2Gamma	PBX1
MCF-specific	6.32% (5.09%) p-value 1e-10	0.55% (0.50%) p-value 1
MCF7-BT474 shared	11.60% (5.39%) p-value 1e-513	0.58% (0.40%) p-value 1e-7
BT474-specific	14.41% (5.47%) p-value 1e-1240	0.48% (0.37%) p-value 1e-4

B MCF-7 cells: FAIRE signal towards FOXA1 regulated genes (64% of the Heregulin induced regions and non-FOXA1 influenced)



D FOXA1 ChIP upon Heregulin treatment



C FOXA1 binding at chromatin fraction

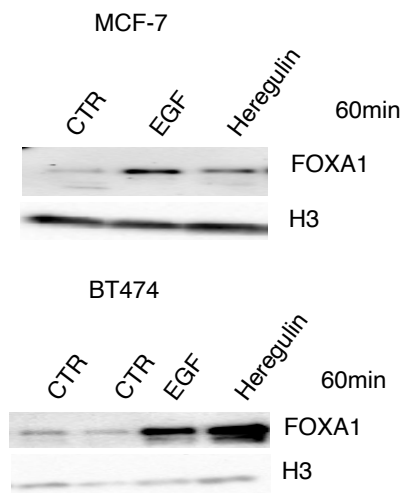
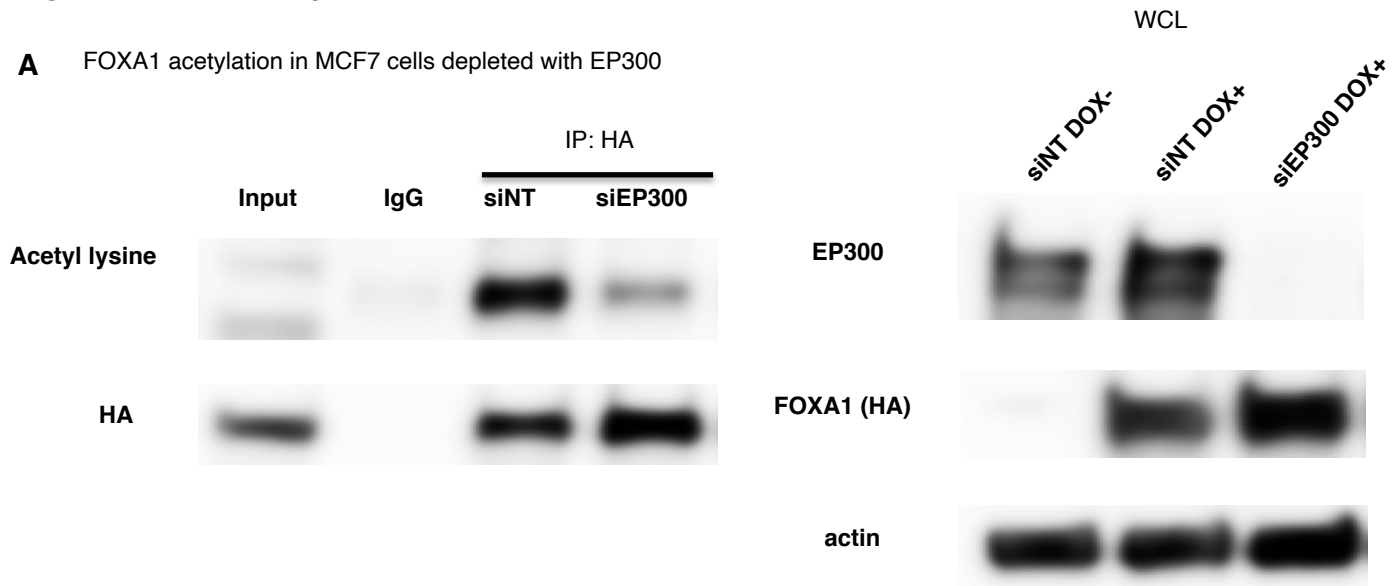
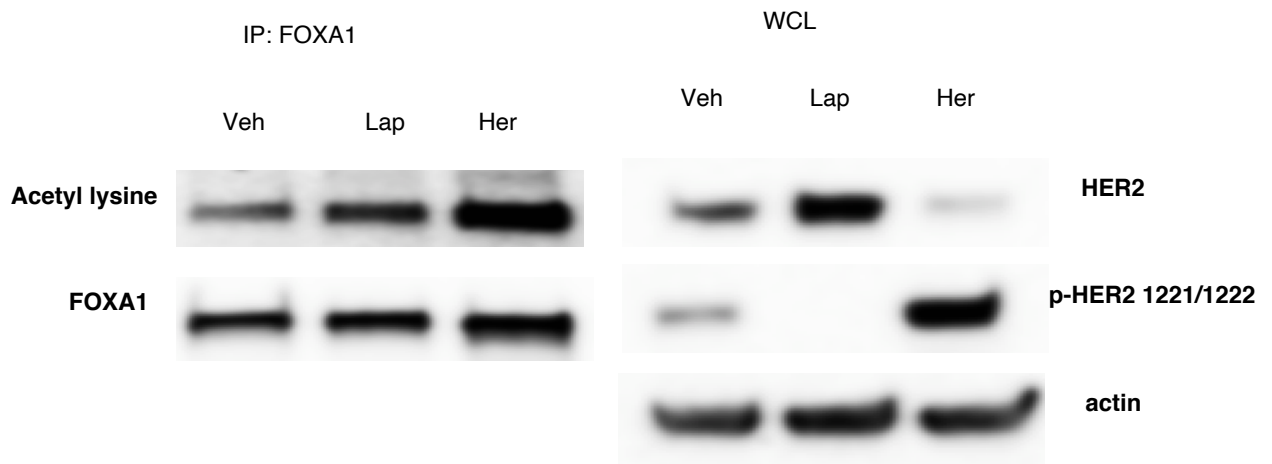


Figure Supplementary 11

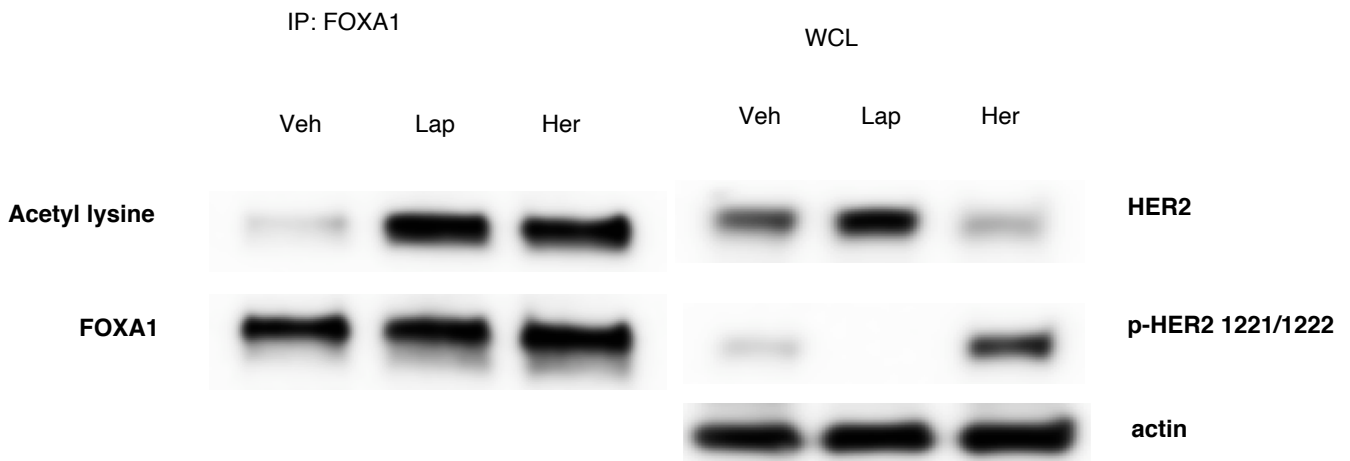
A FOXA1 acetylation in MCF7 cells depleted with EP300



B Inhibition of HER2/HER3 signaling increased FOXA1 acetylation in MCF-7 HER2 high cell line



C Inhibition of HER2/HER3 signaling increased FOXA1 acetylation in BT474 cell line



Supplementary Table 1

		N (%) of 15 patients
Age (median, range)	43 (27-74)	
Menopausal status	Premenopausal	10 (60)
	Postmenopausal	5 (33)
	Unknown	1 (7)
Pathologic tumor size	<2 cm	13 (86)
	2-5 cm	1 (7)
	>5 cm	0
	Unknown	1 (7)
Number of involved axillary nodes	0	8 (53)
	1-3	3 (20)
	≥4	1 (7)
	Unknown	3 (20)
Histology	IDC	12 (80)
	ILC	1 (6.66)
	Mixed (IDC+ILC)	1 (6.66)
	Unknown	1 (6.66)
Histologic grade	1	12 (80)
	2	1 (6.66)
	3	1 (6.66)
	Unknown	1 (6.66)
Hormone Receptor Status	ER+PR+	12 (75)
	ER+ PR-	1 (12.5)
	ER- PR+	1 (6.2)
	Unknown	1 (6.2)
Adjuvant chemotherapy	Yes	12 (80)
	No	2 (13)

20 tumor samples from 15 patients were analyzed. 5 patients have paired tumor samples, from primary tumors and from relapse (metastases from patients after exposure to endocrine adjuvant therapies). Only characteristics at diagnoses of first primary tumors are described. In all patients the initial treatment for their primary tumors comprised either mastectomy (with/without radiation therapy) or lumpectomy plus radiation therapy, together with sentinel node biopsy and/or axillary dissection as clinically indicated.

ER: Estrogen Receptor, PR: Progesterone Receptor, IDC: Invasive Ductal carcinoma, ILC: Invasive Lobular Carcinoma, AI: Aromatase Inhibitor.

ER and PR positivity was defined as >1% nuclei stained. HER2 positivity was defined according to 2007 American College of Pathologist Guidelines.

Supplementary Table 2

Linear regression parameters for each treatment group (1: active substance, 0: vehicle).

Fulvestrant	Heregulin	Trastuzumab	EGF	beta.pvalue	beta	beta.se	rvolume20.pred	rvolume20.se
1	1	1	0	0,020	-0,009	0,004	0,818	0,075
0	1	1	0	2,5E-09	0,029	0,004	1,587	0,076
1	0	1	0	0,311	0,003	0,003	1,062	0,061
0	0	1	0	6,1E-05	0,027	0,006	1,532	0,117
1	1	0	0	4,2E-08	0,043	0,006	1,856	0,125
0	1	0	0	7,5E-09	0,075	0,010	2,503	0,194
1	0	0	0	0,953	1,6E-04	0,003	1,003	0,053
0	0	0	0	2,5E-10	0,059	0,007	2,190	0,146
1	0	0	1	0,020	-0,007	0,003	0,856	0,060
0	0	0	1	8,0E-08	0,037	0,005	1,737	0,109