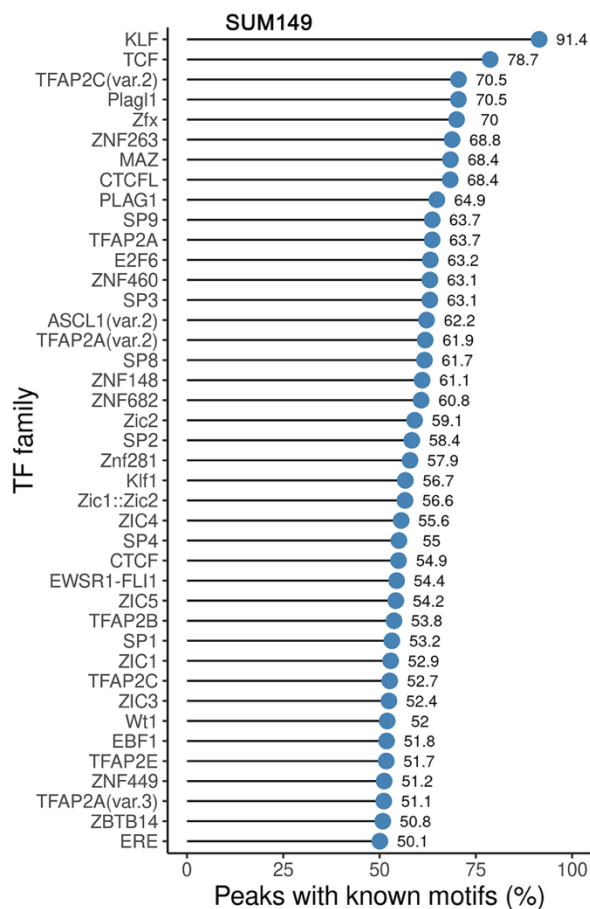
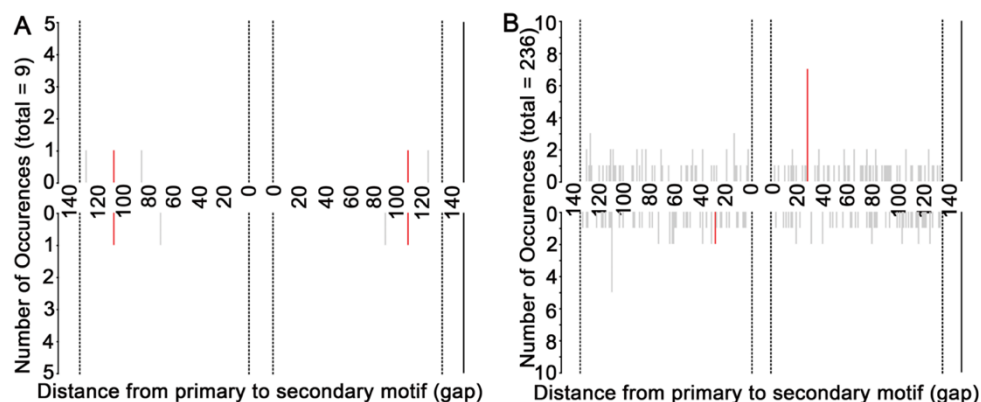


Supplementary figures and tables



Supplementary Figure 1. Transcription factor motifs in ER β DNA binding sites

Top significantly enriched transcription factor binding motifs that occur in ER β -binding sites of SUM149 cells using FIMO analysis ($p < 0.0001$). ERE is among the significant motifs within the ER β -binding sites.

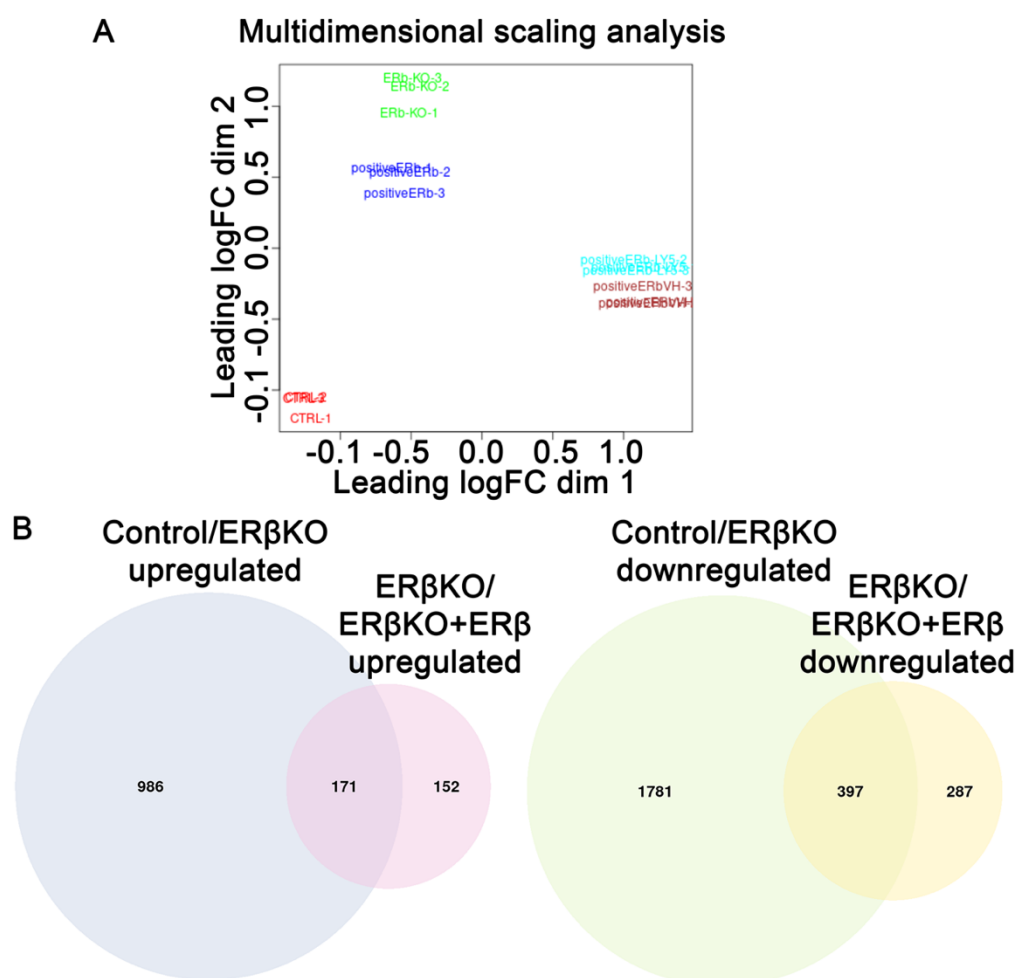


C

Primary TF	Secondary TF	Secondary motif	Gap	p-Value
KPL4 ERβKO/+ERβ				
ESR2	KLF13	RTGMCACGCCCTTTTG	106	<0,0001
ESR2	ZNF652	BAAAGRGTTAAN	99	<0,0001
ESR2	NEUROD1	NRACAGATGGYNN	91	<0,0001
KPL4 +LY500307				
ESR2	RPN4	GGTGGCG	7	<0,0001
ESR2	MET31	RNTGTGGCG	7	<0,0001
ESR2	RAV2	GCAACATA	28	<0,0001
ESR2	ZNF460	GCCTCMGCCTCCCRAG	28	<0,0001
ESR2	DOF5.8	WHTTTTTTHYTTTTTAC	43	<0,0001
SUM149+ERβ				
ESR2	RPN4	GGTGGCG	7	<0,0001
ESR2	MET31	RNTGTGGCG	7	<0,0001
ESR2	RAV2	GCAACATA	28	<0,0001
ESR2	ZNF460	GCCTCMGCCTCCCRAG	28	<0,0001
ESR2	DOF5.8	WHTTTTTTHYTTTTTAC	43	<0,0001
ESR2	FOXM	HAAAMAHAACAHAHA	52	<0,0001
ESR2	AP1	MYAAAAAARGAAA	63	<0,0001
ESR2	FOXO	NNHHAHAATGTTTDYNN	52	<0,0001
ESR2	ATF4	DDATGATGCAATMN	3	<0,0001
ESR2	STAT1/2	THAGTTTCRKTTTCY	51	<0,0001
ESR2	HOXB13	WDCCAATAAAWWWW	52	<0,0001

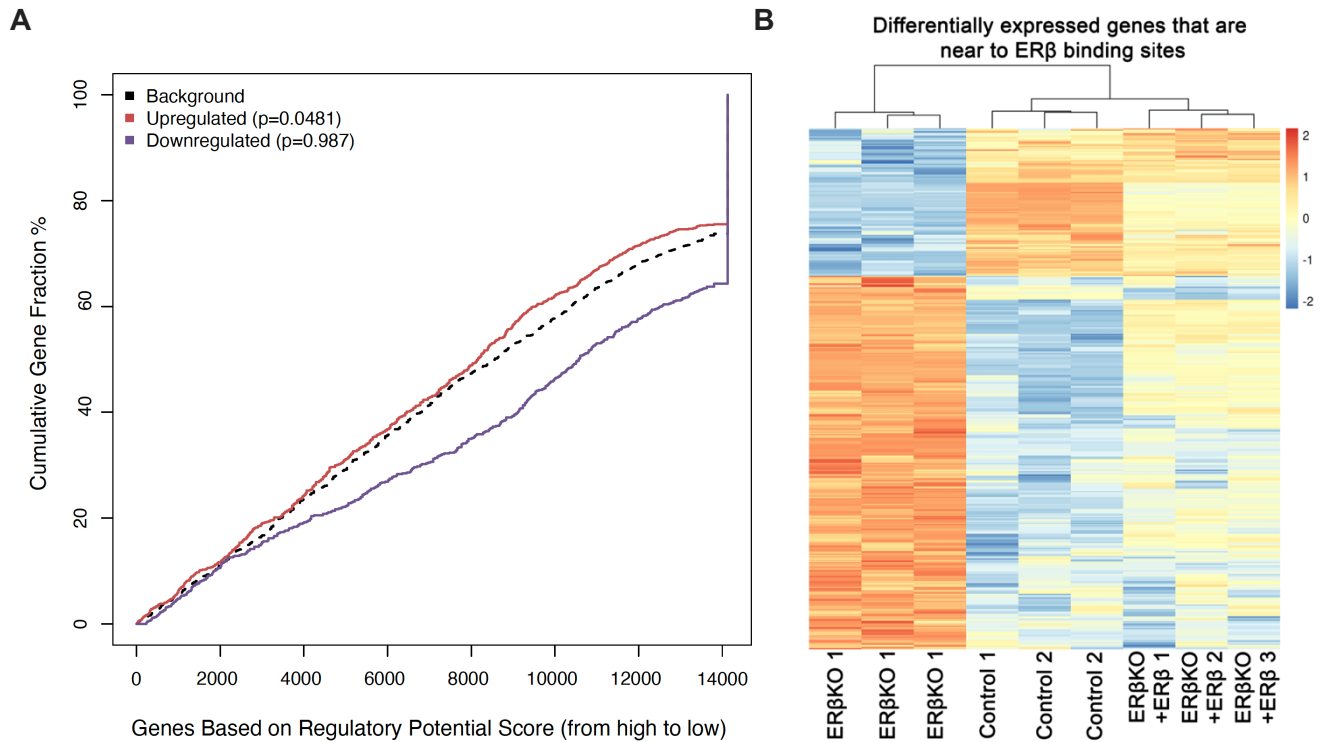
Supplementary Figure 2. Enriched motif spacing patterns of ERβ in inflammatory breast cancer cells

Enriched motif spacing patterns indicative of specific complexes between the focal (ERβ) and secondary transcription factors. **(A, B)** Graphs depicting two of the significant motif hits within provided peaks showing enrichment of ERβ and KLF13 (A) or ZNF460 (B) motifs at specific distance from each other. **(C)** Table listing significant co-occurrences of focal (ERβ) and secondary transcription factor motifs in the same binding site, the distance between the primary and secondary motif and statistical significance in ERβ binding sites that were identified in KPL4 and SUM149 cells with different ERβ levels and after treatment with the ERβ agonist LY500307. Certain motifs (RPN4, MET31, RAV2, ZNF460, DOF5.8) exhibit hits with same distance from the ERβ motif in KPL4 and SUM149 cells, suggesting the formation of specific transcription factor complexes during the binding of ERβ in IBC cells.



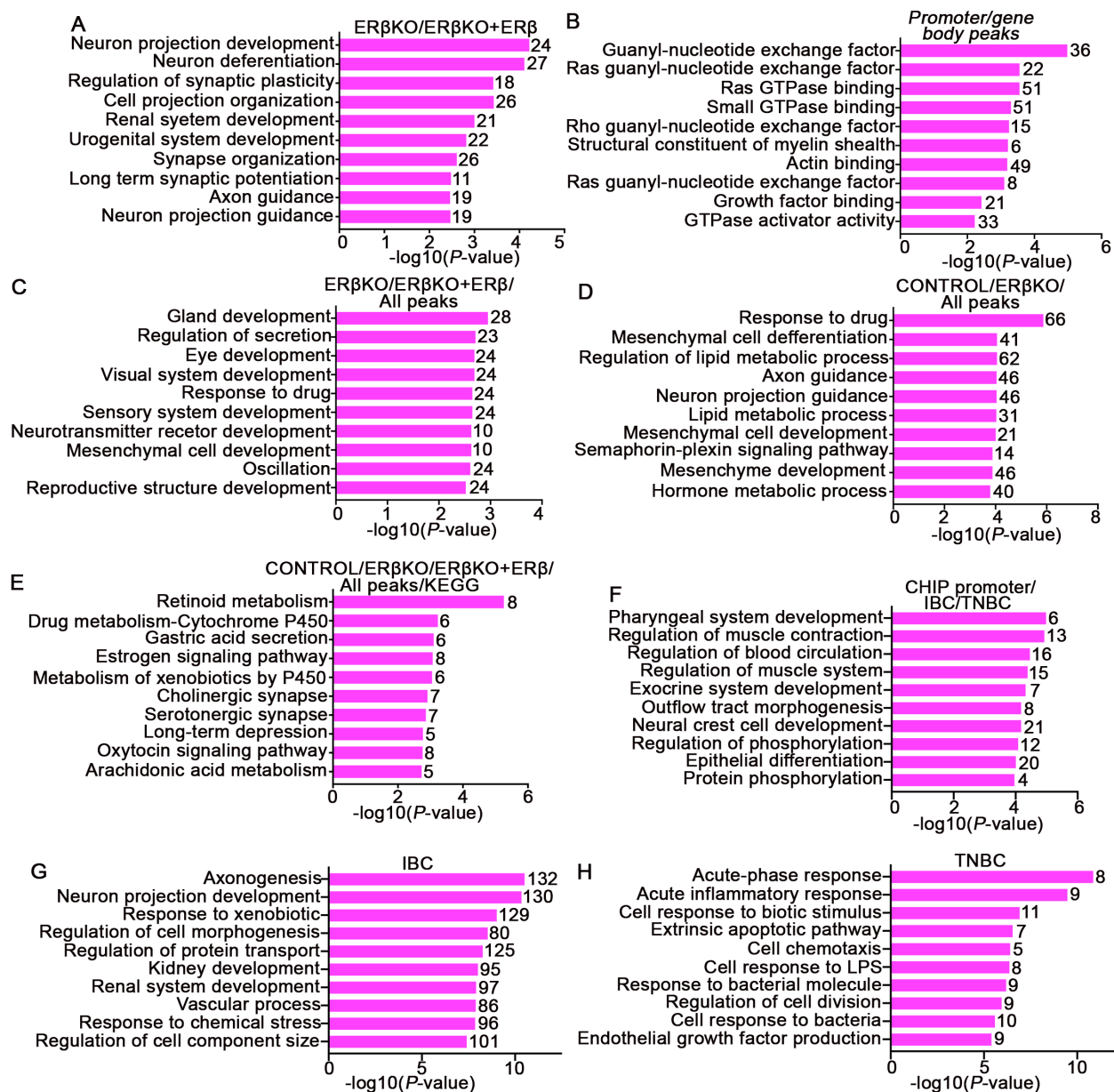
Supplementary Figure 3. Evaluation of RNA-Seq data analysis

(A) Multi-dimensional scaling analysis of RNA-seq data indicates very similar replicates but distinct values in different conditions. Notably, libraries generated after estrogen depletion and LY500307 treatment are substantially distinct from the remaining libraries and more similar to each other. Control libraries are also clustered apart from other datasets. **(B)** Venn diagrams depicting overlapping altered genes in contrasts `Control vs. ER $\beta$ KO` (endogenous ER β) and `ER $\beta$ KO vs. ER $\beta$ KO+ER $\beta$ ` (transfected ER β) that exhibit high numbers of differentially expressed genes.



Supplementary Figure 4. ER β target gene expression in inflammatory breast cancer

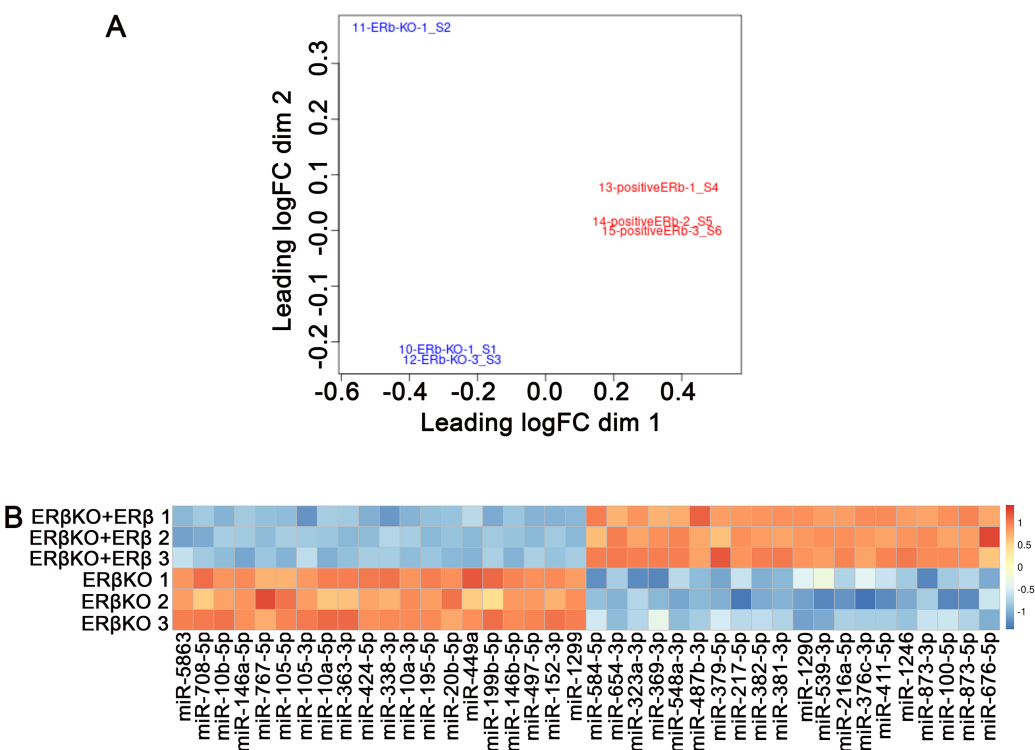
(A) Integration of ER β ChIP-seq peaks with differentially expressed genes with the Binding and Expression Target Analysis (BETA) method using 3,000bp proximity window from TSS, <0.01 as Rank Product Threshold and <0.05 as FDR threshold for differentially expressed genes. This analysis identified 1635 high-confidence ER β target genes (944 upregulated and 692 downregulated) including most of the direct targets listed in Fig. 3E-G. The cumulative distribution plot shows the regulatory potential rank for upregulated (red), downregulated (purple) and (black dashed) background genes. Kolmogorov-Sminrov test shows significant enrichment of upregulated genes for proximal ER β binding ($p=0.048$) while downregulated genes show no significant enrichment relative to background ($p=0.987$), indicating the potential that ER β functions as direct transcriptional activator through proximal DNA binding and repressor via either distal DNA binding or DNA binding-independent mechanisms. **(B)** Heatmap illustrating significantly altered genes that contain ER β binding sites within promoter, gene body and upstream of the promoters and downstream of gene end in KPL4 cells with endogenous (Control) and transfected ER β (ER β KO+ER β) compared with the ER β knockout cells (ER β KO).



Supplementary Figure 5. ERβ-associated biological functions in inflammatory breast cancer cells

(A) Significantly enriched biological processes associated with ERβ repressed genes in ERβKO KPL4 cells following upregulation of ERβ (ERβKO+ERβ). **(B)** Significantly enriched GO molecular functions associated with genes located near ERβ binding sites in the gene body region. **(C, D)**

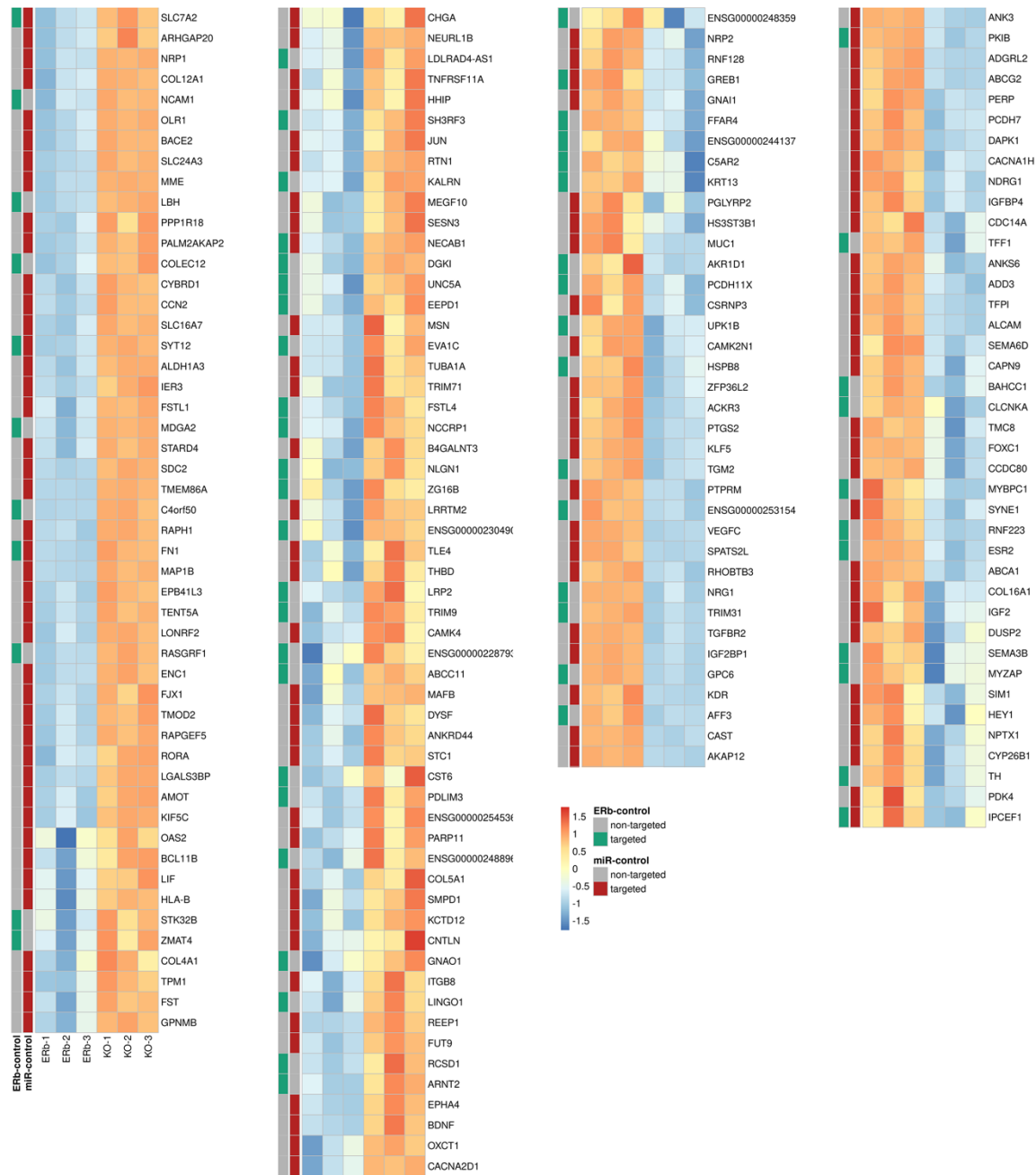
Significantly enriched GO biological processes associated with differentially expressed genes in KPL4 cells with transfected (ER β KO+ER β , C) or endogenous (Control, D) ER β compared to ER β KO cells that are located near ER β binding sites in all genomic regions. **(E)** Significantly enriched KEGG pathways associated with the common differentially expressed genes in KPL4 cells with transfected and endogenous ER β that are located near ER β binding sites in all genomic regions ($p < 0.05$). **(F)** Significantly enriched GO biological functions that are associated with genes that contain overlapping ER β binding sites in their promoter in IBC and TNBC cells. **(G-H)** GO biological processes associated with differentially expressed genes observed specifically in IBC KPL4 (G) and TNBC (H) cells.



Supplementary Figure 6. ER β associated miRNA expression in inflammatory breast cancer

(A) Multi-dimensional scaling analysis of small RNA-seq data sets the conditions ER β KO and ER β KO+ER β apart with the exception of one ER β KO replicate (library 11-ER β -KO-1_S2) that deviates from its replicates on the second dimension. **(B)** Heatmap illustrating the top 20

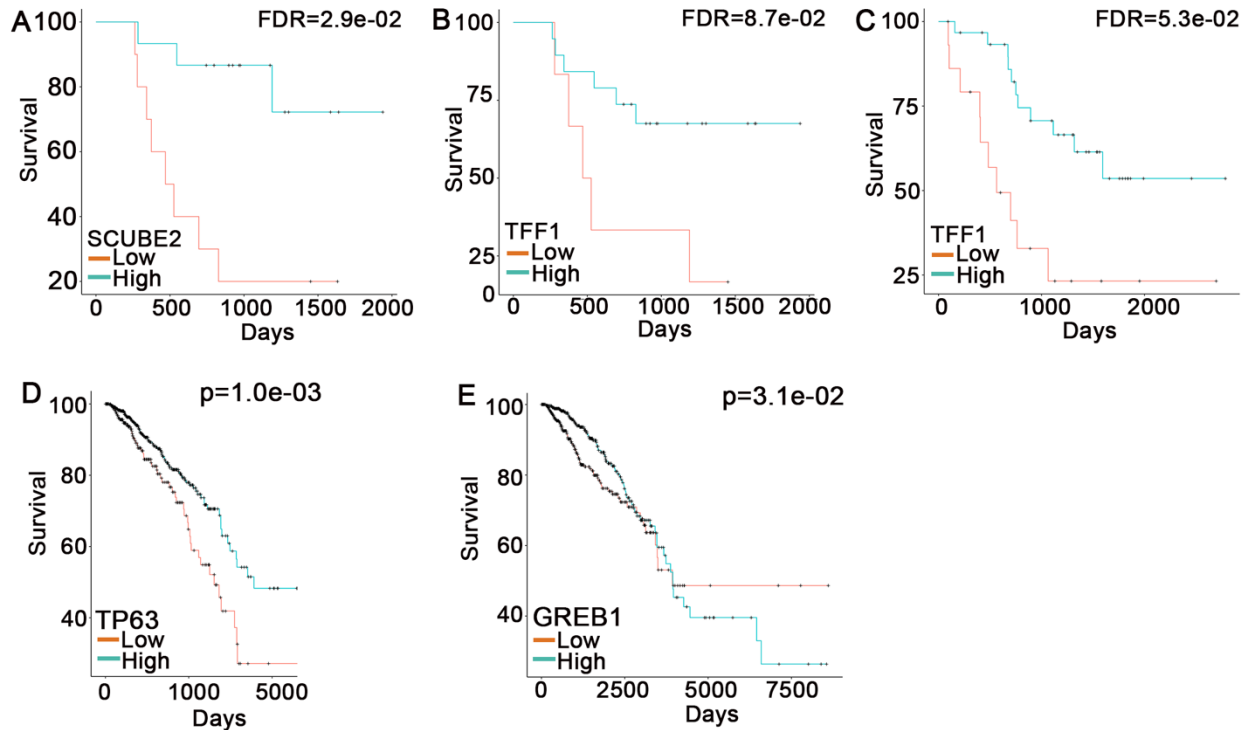
significantly altered miRNAs in ERβ knockout (ERβKO) and ERβKO with transfected ERβ (ERβKO+ERβ) KPL4 cells.



Supplementary Figure 7. ERβ associated genes that are regulated by miRNAs

TarBase identifies many ERβ-regulated mRNAs (protein coding genes) that are targeted by the differentially expressed miRNAs that were identified in IBC cells with different ERβ levels.

According to Spearman's correlation analysis, the identified ER β -target mRNAs follow an inverse expression pattern with that of miRNAs ($r < -0.8$) upon upregulation of ER β .



Supplementary Figure 8. ER β target genes are associated with clinical outcome in inflammatory breast cancer

(A-C) Kaplan-Meier analysis identifies associations between the expression of the ER β target gene SCUBE2 (A) and overall survival in patients with IBC (n=25) as well as TFF1 in patients with IBC (B) and non-IBC (n=58) (C). Patients were divided into subgroups of “low” and “high” expression based on optimal cutoff and significance was considered at FDR < 0.1. **(D, E)** Kaplan-Meier estimates of overall survival in patients with breast cancer from TCGA database. Comparison was made between the group of patients with high and low tumor TP63 (D) and GREB1 (E) expression based on optimal cutoff. *p*-value was generated by two-sided log-rank test.

A

Gene	Cell line	Correlation	FDR
SEMA3B	KPL4	Better OS*	3.7e-02
UBTD1	KPL4	Worse OS	6.4e-02
TFF3	KPL4	Better OS	2.9e-02
SLC26A1	KPL4	Worse OS	9.3e-02
IGFBP4	KPL4	Better OS	4.7e-02
HSPB7	KPL4	Better OS	7.8e-02
RET	KPL4	Better OS	7.8e-02
RARA	KPL4	Better OS	7.8e-02
SLC7A5	KPL4	Worse OS	3.6e-02
APC2	KPL4	Worse OS	9.4e-02
DAPK2	KPL4	Worse OS	9.4e-02
CUEDC1	KPL4	Better OS	3.5e-02
PDLIM3	KPL4	Better OS	9.8e-02
CA12	KPL4	Better OS	9.1e-02
CNTNAP2	SUM149	Better OS	4.9e-02

*Overall survival

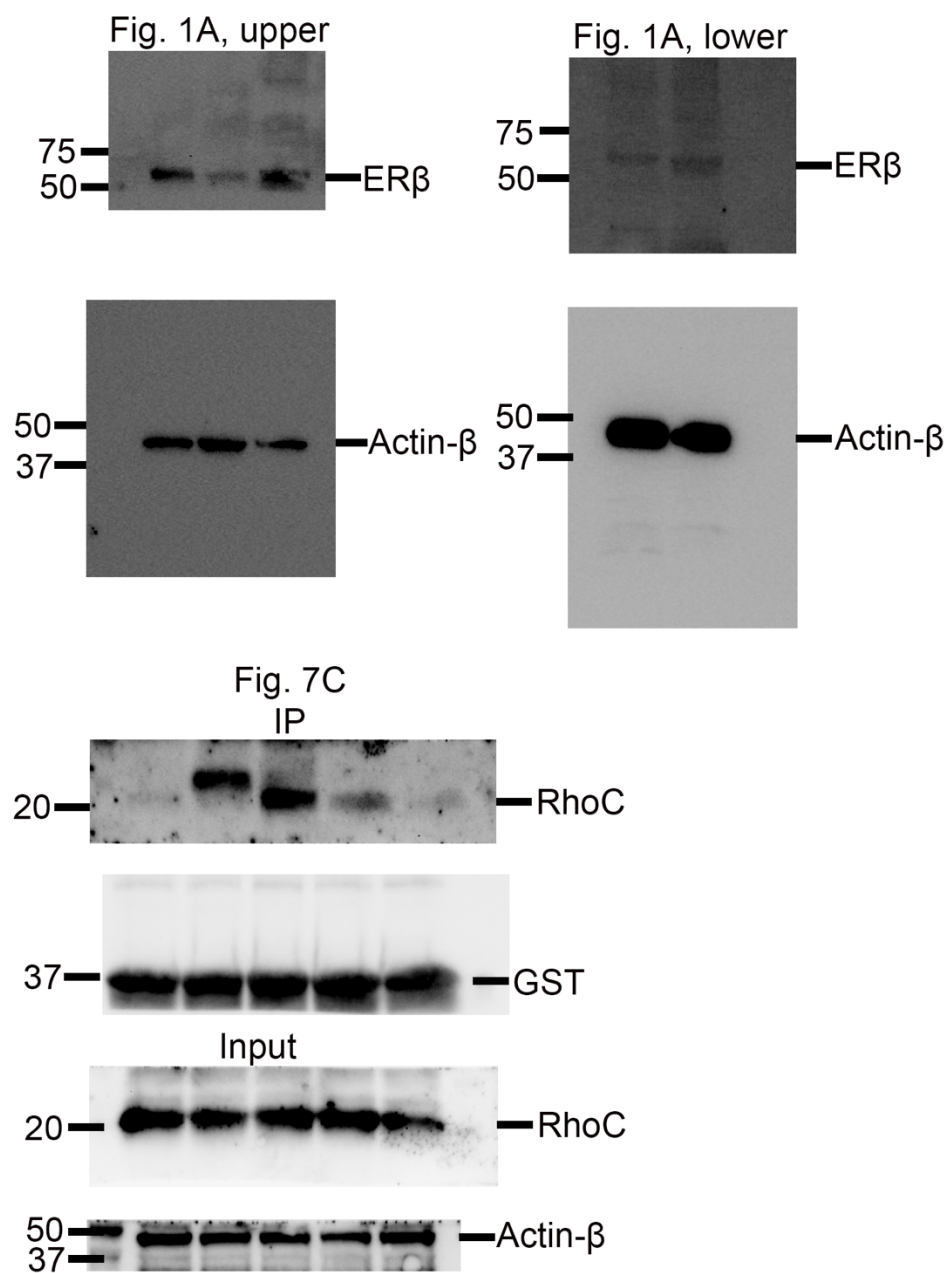
B

Breast cancer		
Gene	Correlation	p-Value
SEMA3B	Better OS	0.0001
TP63	Better OS	0.0010
AFF3	Worse OS	0.0065
C1orf116	Worse OS	0.0073
IL1R1	Worse OS	0.0140
SLC45A1	Better OS	0.0226
CCR3	Worse OS	0.0228
CACNA1D	Better OS	0.0308
GREB1	Worse OS	0.0309
UCA1	Better OS	0.0316
GAS7	Better OS	0.0387
UPK1B	Better OS	0.0395
TH	Worse OS	0.0429
CAPN8	Better OS	0.003
NCCRP1	Worse OS	0.034
SH3RF2	Better OS	0.037
Triple negative breast cancer		
Gene	Correlation	p-Value
MRAS	Better OS	0.0004

CREB5	Better OS	0.0055
CRYL1	Worse OS	0.0193
PC	Worse OS	0.0322
HSPB8	Worse OS	0.0378
LMO7	Better OS	0.0431
UCA1	Worse OS	0.0228
GAS7	Worse OS	0.0368
SLIT3	Worse OS	0.0252
UCA1	Worse OS	0.0474
STK32B	Worse OS	0.0108
TPRN	Worse OS	0.0201
OLIG1	Worse OS	0.0399
NCCRP1	Worse OS	0.0335

Supplementary Figure 9. ER β target genes are associated with clinical outcome in non-inflammatory breast cancer

(A) Associations between the expression of ER β target genes that were identified in IBC cells and clinical outcome in the cohort with 58 patients with non-IBC (FDR < 0.1). **(B)** Analysis of TCGA data identifies associations between the expression of ER β target genes that were identified in IBC cells and clinical outcome in patients with breast cancer including TNBC (P < 0.05).



Supplementary Figure 10. Uncropped western blot images

Supplementary Table 1

Cell Line	Treatment	Repl.	Sequenced reads (M)	Mapped reads (M)	Mapping rate (%)	Usable reads (M)	FRiP (%)
KPL4	ER β KO	1	27.96	26.33	94.16	19.85	0.2
KPL4	ER β KO	2	61.52	53.22	86.50	41.39	0.1
KPL4	ER β KO+ER β	1	26.23	24.73	94.29	18.81	1.3
KPL4	ER β KO+ER β	2	38.76	37.36	96.40	28.63	1.0
KPL4	ER β KO+ER β +Vehicle	1	59.50	25.61	43.05	18.59	4.6
KPL4	ER β KO+ER β +Vehicle	2	48.37	11.38	23.52	5.54	0.6
KPL4	ER β KO+ER β +Vehicle	3	43.88	14.91	33.98	10.79	0.4
KPL4	ER β KO+ER β +LY500307	1	72.49	29.32	40.44	22.06	16.8
KPL4	ER β KO+ER β +LY500307	2	60.31	33.60	55.72	25.74	5.8
KPL4	ER β KO+ER β +LY500307	3	107.69	74.19	68.89	55.88	4.1
SUM149	Empty vector	1	85.26	81.86	96.01	66.56	3.9
SUM149	Empty vector	2	63.35	59.78	94.37	47.54	0.3
SUM149	ER β -overexpressing	1	18.34	13.33	72.69	9.86	28.8
SUM149	ER β -overexpressing	2	46.49	39.41	84.78	30.38	0.7

Supplementary Table 1. Quality control metrics for ER β ChIP-seq data. Usable reads: uniquely mapped reads after filtering for ENCODE blacklisted regions; FRiP: Fraction of Reads in Peaks.