A		В
ļ	2 3 –log(pValue)	
1	1.Signal transduction_ESR1- membrane pathway	LAMA3 (Epilipin)
2	2.Signal transduction_ESR1-nuclear	YB-1 p538P1 BAF47 p55
3	3.Proteolysis ECM remodeling	FIP200 HMGA2 PPLAPACY (ICCPT.40hp)
4	4.Apoptosis_Anti-Apoptosis	Caretoulin TRIP2
1	PI3K/AKT	SAFEZ 2 RIPHO
5	5.Reproduction_Progesterone signaling	NTA3 NCO46 (TRBP)
6	6.Signal transduction_ERBB-family	
7	7.Development_Hedgehog signaling	MOZ Planten XPD XTF.2 NG1 Bardt HBDA JAB1
8	8.Cardiac development_FGF_ErbB	NCOA3 (rdP)SRC3)
9	9.Transcription_Nuclear receptors	28ct N-CoR LMO4 CDK7
	transcriptional regulation	2 - Rela (665 NF-18 suturit) - 2 - 2 - 2 - 2 - 2 - 2 - 2 - 2 - 2 -
10	Cholecystokinin signaling	COBRA1 EZH2 VPHL NRIF3 p130 SMRT SAFB p14ARF Beta-caterin p16INK4
11	11.Proliferation_Positive regulation cell proliferation	
12	12.Reproduction_Gonadotropin	
13	13.Cytoskeleton_Regulation of	
	cytoskeleton rearrangement	NF-ATSINFATCA
14	pathway	
15	15.Apoptosis_Anti-Apoptosis mediated by external signals by	KLF9, ATBF1 ESR1 (nuclear) CHV0 CLUII PPAR-beladella)/KCR-alpha HM3B1 STAT1 En2 Androgen receptor
	Estrogen	
16	Transmission of nerve impulse	PEA3 PPARceta(delta) SIAIO ERRI TR-alpha ERI TR-alpha Telfuel) Fre-1 H0XA5 UHRP1 TWISTI VORIXIX-alpha ETS ATF-2/c-Jun Lef-1
17	17.Proteolysis_Connective tissue degradation	RAR-alphaRVR-alpha
18	18.Signal transduction_CREM pathway	RUNX2 CEBPalcha SREEPTIprecursor TCF8 CEBP
20	19.Muscle contraction 20.Signal transduction WNT signaling	
21	21.Development_Neurogenesis_	HEYI NF46 p65 p65 p73 X8P1 PPAR aphaRXx apha SMAD2 ESR2 HSF1 CTCF OL:34 TRPS1 NF482 (p52) GATA-3
22	Synaptogenesis 22.Cell adhesion Cadherins	N YE WASHE ON THE VESSES OF MIT IN THE TAX
23	23.Cell adhesion_Platelet	* * * * * * * * * * * * * * * * * *
24	24.Proliferation_Negative regulation	HMGIY RARbeta SP1 E2F1 ATF-1 STAT58 CIEBPdelta ETS2 WT1 PR (nuclear) p53 NF-kB1 (p50) SMAD4 MYOD MTA1 HIF1A
	of cell proliferation	C
25	microtubules	0
26	26.Inflammation_IL_6 signaling	
27	regulation	2 2
28	28.Development_EMT_Regulation of epithelial-to-mesenchymal	SDP1 NCOA4 (ARA70) Neuropulin 1 IGF-1 EGF Estradiol cytoplasm
-	29 Cell adhesion Platelet-	NRIF3 MCOLE (TRBD)
29	endothelium-leucocyte interactions	LCoR (MLR2) ErbB2 ErbB3 ErbB4 (GF-1 recentor EGFR Adventish purples
30	30.Development_Blood vessel	MIAT NCOA2 (GRIP1/TIF2) 2 PI OS1 P38a(phia (MAPK14)
31	31.Cytoskeleton Actin filaments	MTA2 NCOA3 (pCIPISRC3) DDX5 AT P cytoplasm
32	32.Apoptosis_Anti-Apoptosis	TRIP2 Paret SAFB2 COCC58
-	MAPK and JAK/STAT	LUBE202 PI3K reg class IA Curtin AMP intravellifar CDK7
33	33.Signal transduction_Neuropeptide signaling pathways	PELP1 NCOA1 (SRC1)
34	34.Apoptosis_Anti-apoptosis	GRP78 RNPC4 PI3K cat class IA PKA-reg (cAUP-dependent)
-	NF-kB	RIPI40 H-Ras PAK5
35	35.Development_Neurogenesis in general	PRMT2 PKA-cat (AMP-dependent)
36	36.Reproduction_GnRH signaling	SET PIAST CA
37	37.Development_Regulation of	Gamma-synuction HEXIMI MRF-1 Pitonsi(3.4,5)P3 intracellular FDXM1 STAT5A AP-2A
-	angiogenesis 38 Reproduction Spermatogenesis	
-	motility and copulation	FKBP2 SRA1 LINU DUY SHO HINGT,2 STATOD
39	39.Transport_Synaptic vesicle exocytosis	DSCAM ERR-10 MLL2 ID R C PDK (PDPK1) FKHR
40	40.Blood coagulation	Zend LUCZDA SP1 ERK12 PAK1 SUTTEN DV1 Brid res wind
41	41.Cell adhesion_Synaptic contact 42.Immune response. Th17-derived	Zaci COBRA1 Pour CYP19 CORP.1 Pour Pour Pour Pour Pour Pour Pour Pour
-	cytokines	HUEL MSH2 Tp60 JunB PAINSA NF-AT3INFATCAI RXRA XBP1 SMAD4 SPBP
43	4.3.Reproduction_Feeding and Neurohormone signaling	SMRT C021 JAB1 Cum p300 2 A reference A
44	44.Signal Transduction_TGF-beta, GDF and Activin signaling	2 2 HSP27 KCRB ESR1 (nuclear) P21 cMyc III-140na REX04 NF-AB1 (p105
45	45.Neurophysiological process_	CAPER ING1 MTA3 Islet-1 p53 RB82 PPAR-gamma WT1
46	46.Reproduction_Male sex	RBM9 DLC1 (Dynein LC8a) DDX54 SREAP1 (randear)
	differentiation	X C X X X X X X X X X X X X X X X X X X
47	4/JIIIammauon_Kallikrein-kinin system	PR (nudear) ZNF217 HSD1783 CYP7B1 CYP7B1 CYF7B1 CYP7A1 CRF-BP CYP1A1 Caveolin-1 RAMP3 SOD1 BCAS3 TFF1 SMAD3 MPG RARalpha HSD17B1
48	48.Cell adhesion_Integrin-mediated cell-matrix adhesion	Adrenomedulin Cyclin G2 CYP881 TYRP2 SHP E88P SOCS3 E8AG9 oFos LRH1 FAP-1 APJ CYP181 Cyclin D1 MAO8 CTIP1 R88P8 (CIIP)
49	49.Neurophysiological process_	Y X < T T X X T < T T X 2 2 2 X 4
50	50.Inflammation_IL-2 signaling	Prolactin receptor Lactoferm CYP17 Urocortin CG alpha MEA AP-2C SDF-1 COMT Galanin BMP6 VEGFR-2 Keratin 19 LRP16 C3 ARID4A Cathepsin D
* INCLWORKS		

**Figure S1.** The METABRIC, TCGA, and GSE11352 databases were compared, and highly expressed genes from ER<sup>+</sup> patients were uploaded to the MetaCore database for disease and biomarker analysis. A. Significant "positive-regulation" genes (upregulation) among the TCGA and METABRIC clinical ER<sup>+</sup> patient databases and the GSE11352 (MCF7-Estradiol/MCF7-Con) database were uploaded to the MetaCore database for downstream pathway analysis. B. "Breast neoplasm transcription regulation", C. "Signal transduction ESR1-nuclear pathway" and other related genes were significantly upregulated in the ER<sup>+</sup> patient group and the MCF7-estradiol group.



**Figure S2.** Comparing the upregulated genes between the estradiol treatment and control groups and exploring the potential networks. (A) The GSE11352 database describes disease biomarker networks, where control MCF-7 groups (12C, 24C, and 48C) were compared with estradiol treatment for 12 (12E), 24 (24E), or 48 hour (48E) in MCF-7 cells, (B) "Breast neoplasm ESR1" and other related pathways are significantly upregulated in the estradiol group. (C) Novel downstream pathways identified in the estradiol treatment group, such as "breast neoplasm metabolism regulation".



## Amino acid metabolism in estrogen receptor-positive breast cancer

**Figure S3.** The mRNA expression levels of amino acid metabolism genes in the MCF-7 cell line. MCF-7 cells were seeded and treated with 10 nM estradiol for 16-18 hours. Total RNA was extracted, and amino acid metabolic genes were analyzed by RT-qPCR, using the mRNA of PGR, which is activated by the estrogen receptor, as a positive control. A. Argininosuccinate lyase/ASL. B. Tyrosine aminotransferase/TAT; phenylalanine hydroxylase/PAH. C. Phosphoglycerate dehydrogenase/PHGDH; serine hydroxymethyltransferase 1/SHMT1; serine hydroxymethyltransferase 2/SHMT2. D. Adenosylhomocysteinase/AHCY; methionine adenosyltransferase 2A/MAT2A. E. Methionine adenosyltransferase 2B/MAT2B; 5-methyltetrahydrofolate-homocysteine methyltransferase/MTR. F. Glutamate dehydrogenase 1/GLUD1; glutamate-ammonia ligase/GLUL; tryptophan 2,3-dioxygenase/TDO2; branched-chain amino acid transaminase 2/BCAT2. G. Cystathionine-beta-synthase/CBS; cystathionine gamma-lyase/CTH.