Supplementary material

Decreased neurotensin induces ovulatory dysfunction via the NTSR1/ERK/EGR1 axis in polycystic ovary syndrome

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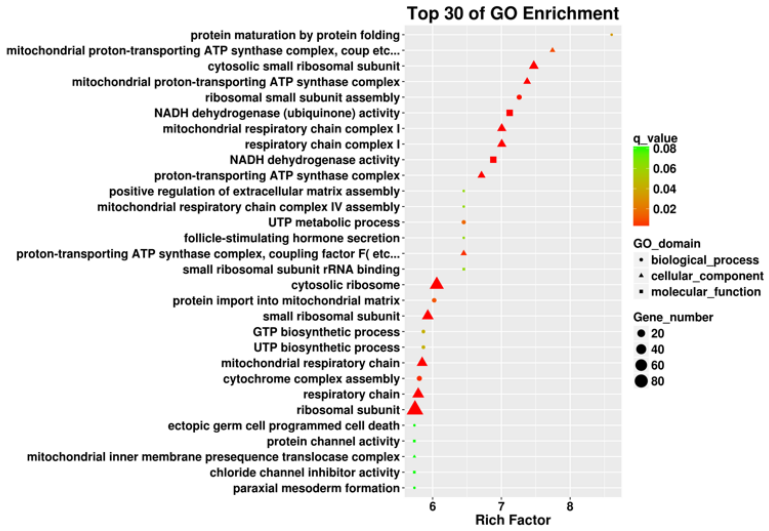

Fig. S1 Gene ontology (GO) terms of different expressed genes (DEGs) enrichment between control and SR48692-treated oocytes.

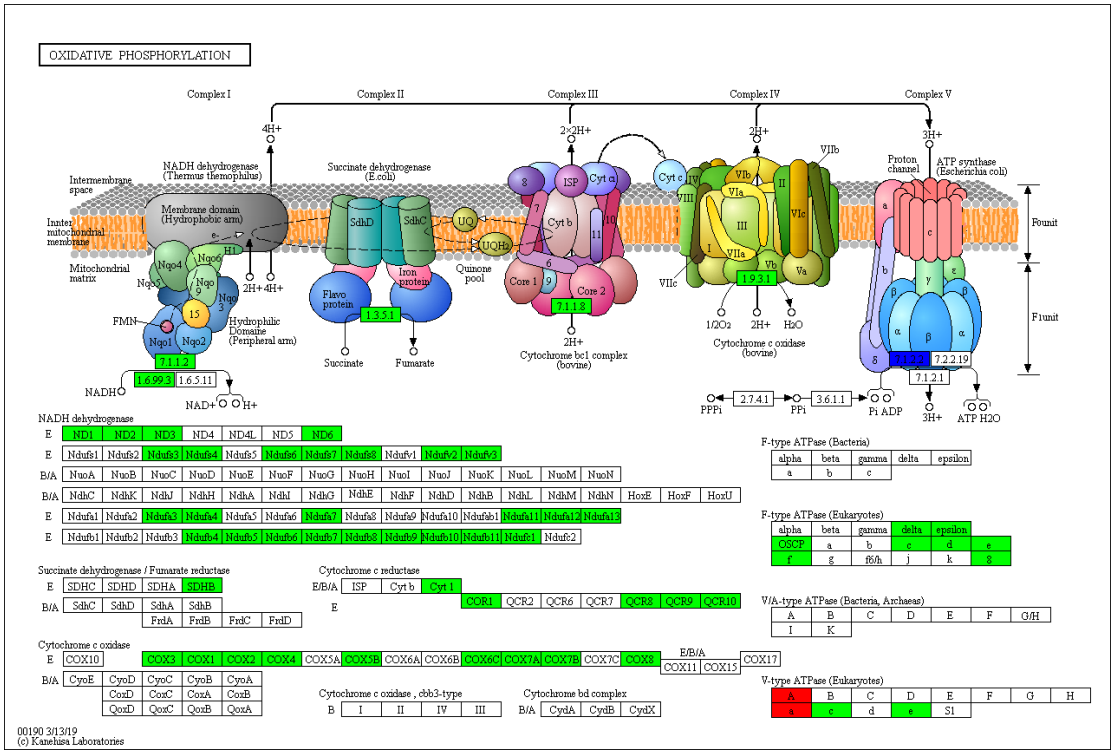

Fig. S2 The KEGG pathway of the oxidative phosphorylation responds to SR48692-treated oocytes. Gene expression levels were indicated as significantly higher (red) or lower (green) in the SR48692-treated group compared to the control group.

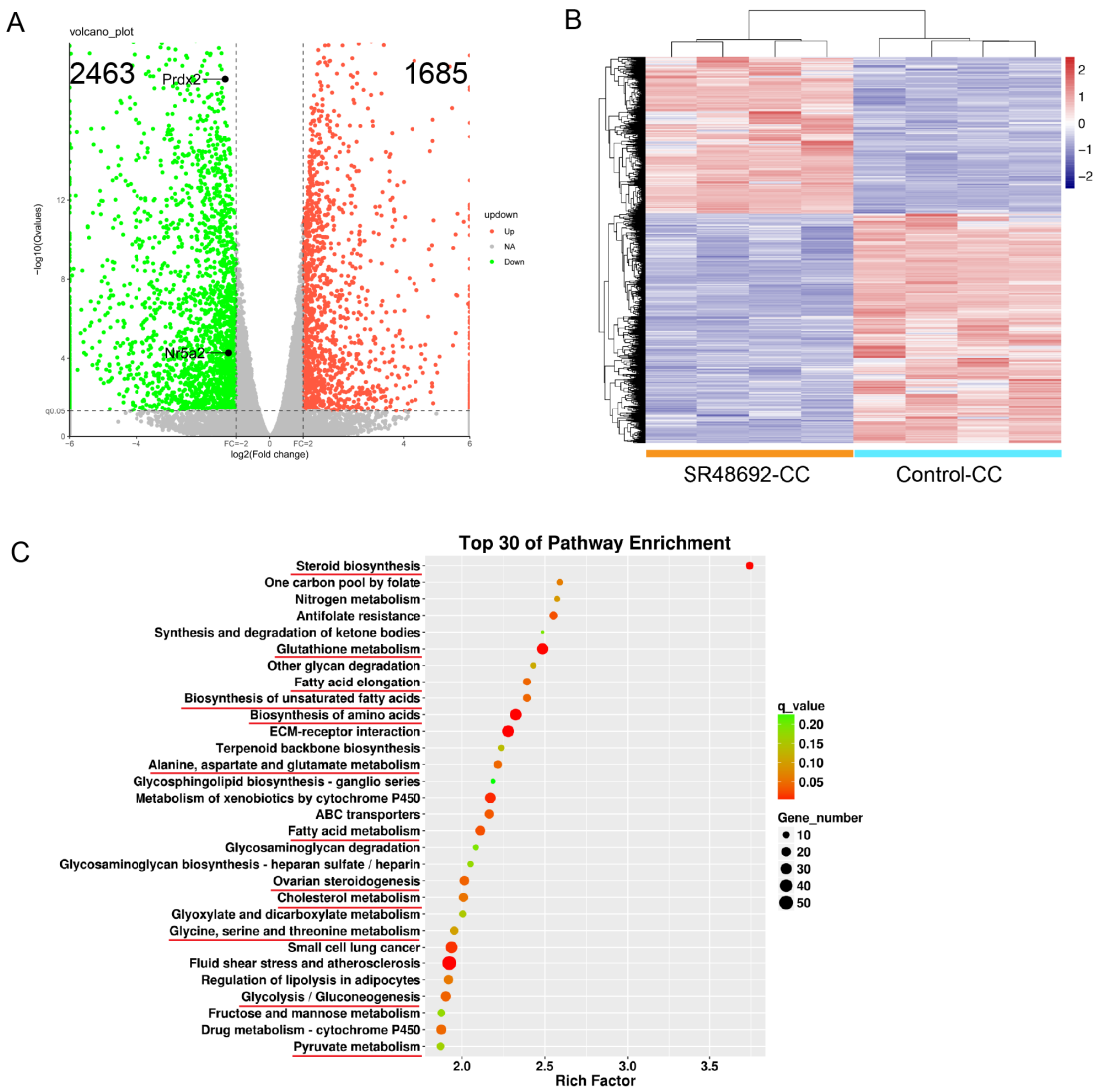

Fig. S3 Transcriptome changes in SR48692-treated cumulus cells (CCs). (A) The corresponding volcano plot of SR48692-treated CCs versus controls showed 1685 upregulated (fold change > 2, q-value < 0.05, red) and 2463 downregulated (fold change < −2, q-value < 0.05, green) genes. (B) Expression heatmap of DEGs in SR48692-treated CCs compared to controls by RNA-seq. (C) Top 30 signaling pathways of the DEGs in KEGG enrichment analysis between control and SR48692-treated CCs.

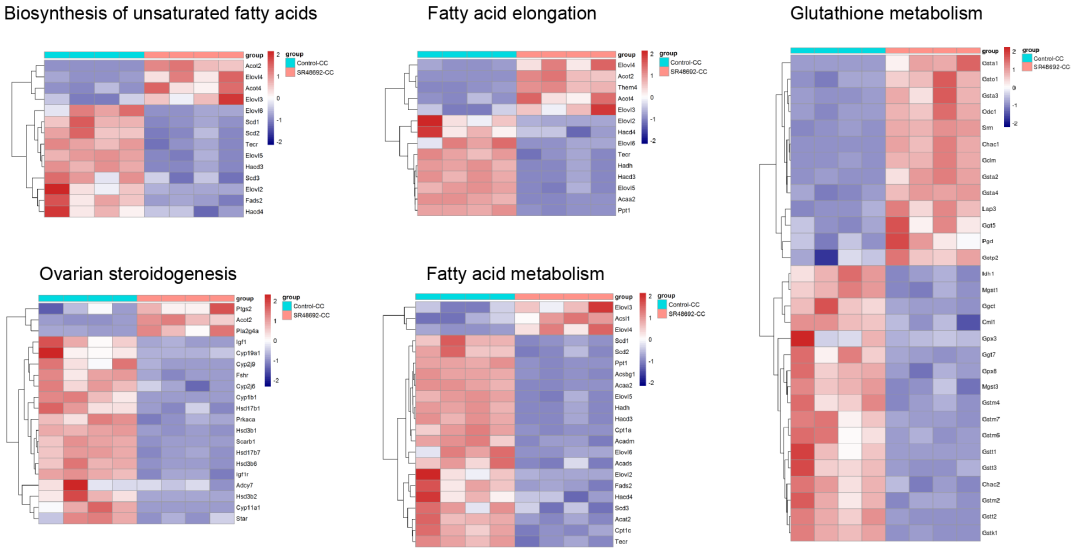

Fig. S4 Heat maps of relative expressions of the indicated genes in distinct metabolic and steroidogenic pathways in control and SR48692-treated CCs. Each box corresponds to a different sample.

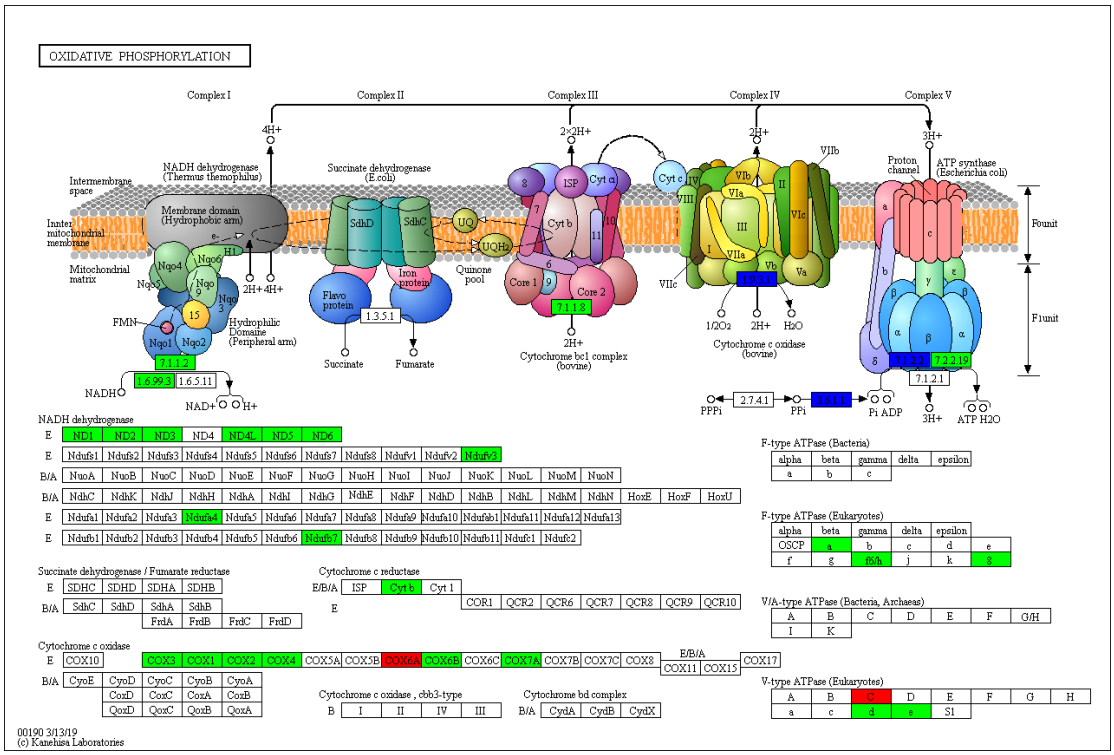

Fig. S5 The KEGG pathway of the oxidative phosphorylation responds to SR48692-treated CCs. Gene expression levels were indicated as significantly higher (red) or lower (green) in the SR48692-treated group compared to the control group.

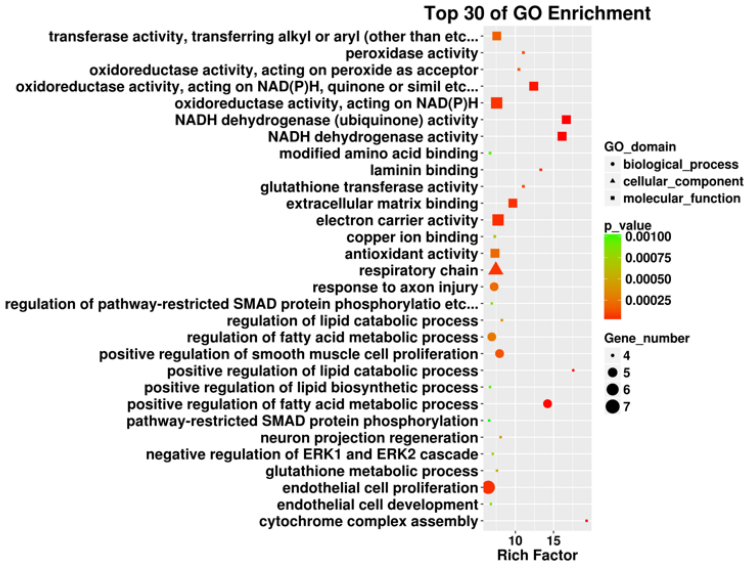

Fig. S6 GO analyses of the overlap in transcripts that were downregulated in SR48692-treated oocytes and CCs.

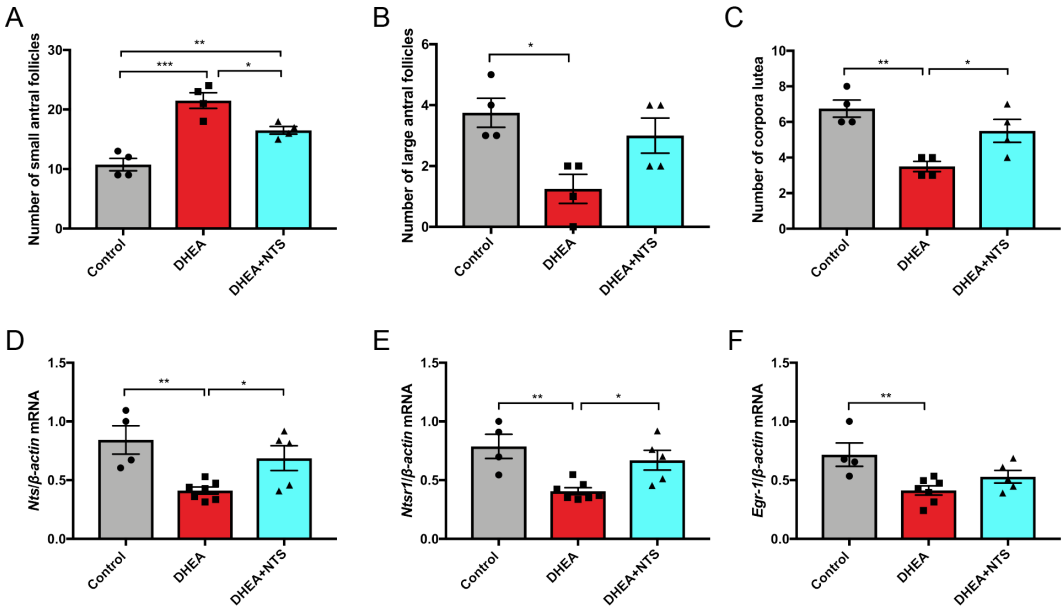

Fig. S7 Ovarian histological and molecular examinations in polycystic ovary syndrome (PCOS) mice. (A-C) The number of small antral follicles, large antral follicles, and corpora lutea in the half ovaries of mice from each group. (D-F) Quantitative real-time PCR (qRT-PCR) detection of *Nts*, *Ntsr1*, and *Egr1* expression in the ovaries of mice from each group. Data are shown as means ± SEM. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

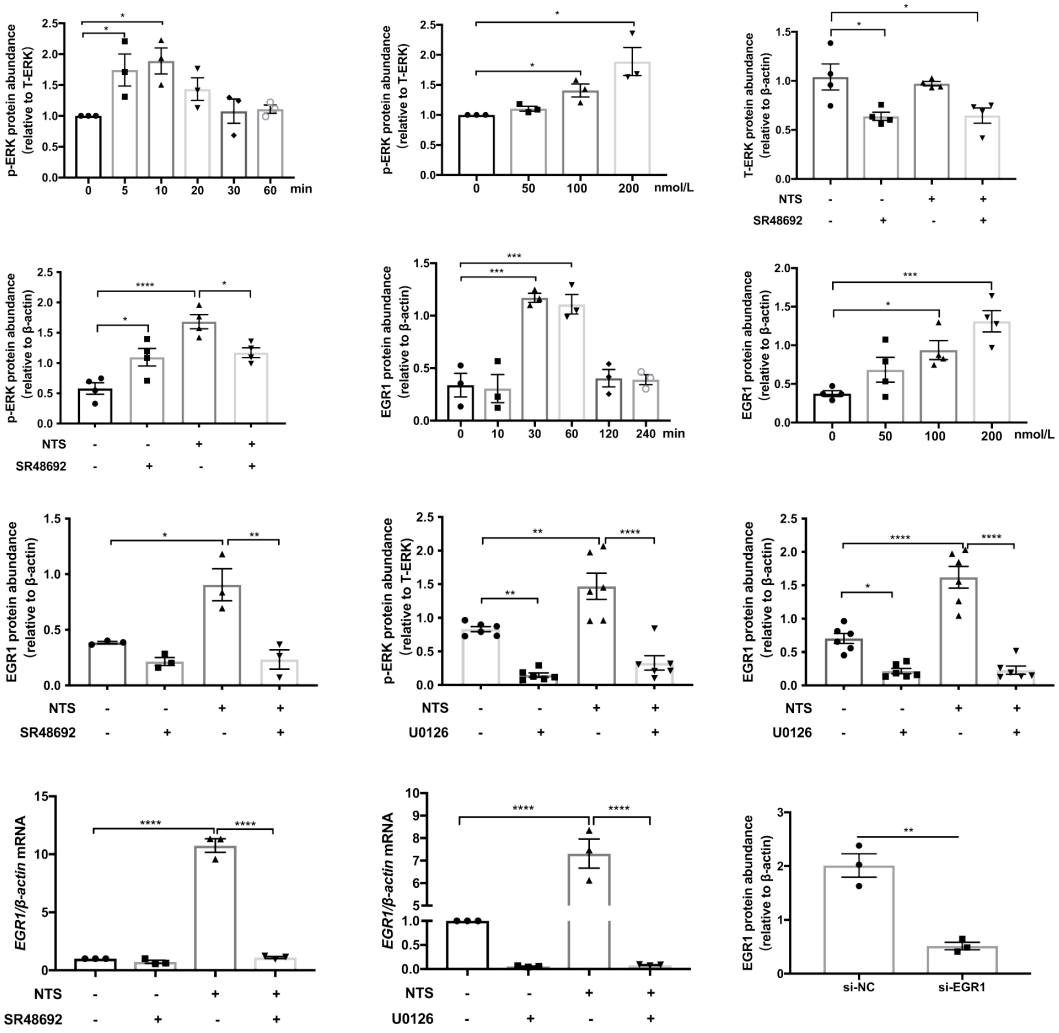

Fig. S8 Quantifications of western blot analysis and results of qRT-PCR in Fig. 8. Data are shown as means ± SEM. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001.

Supplementary tables

Table S1. Clinical and biochemical indicators of women with and without PCOS in qRT-PCR.

	Control (n=38)	PCOS (n=39)	P value
Age (years)	27.63±3.43	27.64±2.68	0.989
BMI (kg/m^2)	21.82±2.89	22.46±3.36	0.375
Basal FSH (mIU/ml)	6.21±1.32	5.75±1.54	0.176
Basal LH (mIU/ml)	5.47±3.01	9.70±7.29	0.002
LH/FSH	0.91±0.59	1.81±1.88	0.006
Basal E2 (pg/ml)	35.44±21.22	41.44±15.66	0.168
Basal T (mIU/ml)	0.95±0.37	1.32±0.54	0.007
AMH (ng/ml)	4.47±1.63	10.63±3.69	<0.0001

Data are presented as mean ± SD, BMI: body mass index, LH: luteinizing hormone, FSH: follicle stimulating hormone, T: testosterone, and AMH: anti-Müllerian hormone.

Table S2. Clinical and biochemical indicators of women with and without PCOS in ELISA.

	Control (n=26)	PCOS (n=27)	P value
Age (years)	30.39±3.40	30.85±3.67	0.633
BMI (kg/m^2)	21.17±2.66	22.55±3.79	0.133
Basal FSH (mIU/ml)	6.27±1.24	5.99±1.58	0.476
Basal LH (mIU/ml)	5.47±1.69	8.45±4.46	0.003
LH/FSH	0.89±0.28	1.43±0.74	0.001
Basal E2 (pg/ml)	37.53±18.73	39.70±12.41	0.622
Basal T (mIU/ml)	1.01±0.33	1.38±0.60	0.029
AMH (ng/ml)	4.67±2.21	9.00±4.26	<0.0001

Data are presented as mean ± SD, BMI: body mass index, LH: luteinizing hormone, FSH: follicle stimulating hormone, T: testosterone, and AMH: anti-Müllerian hormone.

Table S3. Primer sequences used in the qRT-PCR analysis.

Target Genes	Species		Primer sequences (5'-3')
ACTB	Human	Forward	GGGAAATCGTGCGTGACATTAAG
		Reverse	TGTGTTGGCGTACAGGTCTTTG
NTS	Human	Forward	TGCTTTAGATGGCTTTAGCTTGG
		Reverse	TTCCTGGATTAACTCCCAGTGT
EGR1	Human	Forward	GGTCAGTGGCCTAGTGAGC
		Reverse	GTGCCGCTGAGTAAATGGGA
Actb	Mouse	Forward	GTGACGTTGACATCCGTAAAGA
		Reverse	GCCGGACTCATCGTACTCC
Nts	Mouse	Forward	GCAAGTCCTCCGTCTTGGAAA
		Reverse	TGCCAACAAGGTCGTCATCAT
Egr1	Mouse	Forward	CAGTCCCATCTACTCGGCTG
		Reverse	TGTGGAAACAGATAGTCAGGGAT

Table S4. Primary antibodies used in the Western blot analysis.

Protein Name	Host	Dilution	Manufacturer
β-Actin	Mouse monoclonal	WB：1:3000	Proteintech
p44/42 MAPK (Erk1/2)	Rabbit monoclonal	WB：1:1000	CST
Phospho-p44/42 MAPK (Erk1/2)	Rabbit monoclonal	WB：1:1000 IF：1:200	CST
EGR1	Rabbit monoclonal	WB：1:1000 IF：1:200	CST
NTS	Rabbit polyclonal	WB：1:500	Abcam

Table S5. siRNA sequences used in the cell knockdown assay.

Oligo Name	Species	Sequence	
		sense（5'-3'）	antisense（5'-3'）
si-*Egr1*	Mouse	GGACAAGAAAGCAGACAAATT	UUUGUCUGCUUUCUUGUCCTT
si-*EGR1*	Human	GGCAUACCAAGAUCCACUUTT	AAGUGGAUCUUGGUAUGCCTT