

Estrogen receptor α confers Nab-paclitaxel resistance in breast cancer by promoting miR199a-5p maturation to inhibit Caveolin 1 translation

Supplementary Information

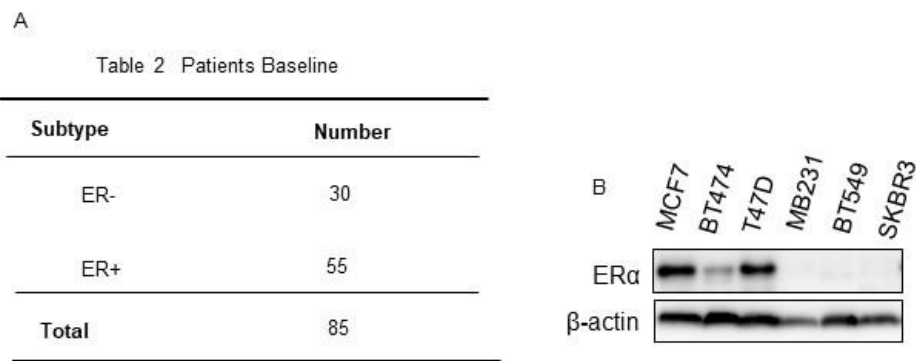
Table S1 RNA oligonucleotide sequences.

Primers	
Name	Sequence
Actin	F: CACCAACTGGGACGACAT
	R: ACAGCCTGGATAGCAACG
CAV1	F: ATGTCTGGGGGCAAATACGTA
	R: GTTGAGGTGTTTAGGGTCGC
miR199a-5p	F: CCCAGTGTTTCAGACTACCTGTTC
	R: universal primer
miR199 primary	F: AGTGGTGGTTTCCTTGGCT
	R: GGGGCCGGCTATCCATCC
miR199 precursor	F: GCCAACCCAGTGTTTCAGACT
	R: universal primer
pri-miR199 transcript	F: AGTGGTGGTTTCCTTGGCTG
	R: TTCTATGCGAGGCTCTGCCA
miRNAs mimics and inhibitors	
NC mimic	S: UUCUCCGAACGUGUCACGUTT
	AS: ACGUGACACGUUCGGAGAATT
miR199a-5p mimic	S: CCCAGUGUUCAGACUACCUGUUC
	AS: ACAGGUAGUCUGAACACUGGGUU
Inhibitor NC	S: CAGUACUUUUGUGUAGUACAA
miR-493-3p inhibitor	S: CCUGGCACACAGUAGACCUUCA
miR-520a-3p inhibitor	S: ACAGUCCAAAGGGAAGCACUUU
miR-124-3p inhibitor	S: UUGGCAUUCACCGCGUGCCUUA
miR-302b-3p inhibitor	S: CUACUAAAACAUGGAAGCACUUA
miR-384 inhibitor	S: UAUGAACAAUUUCUAGGAAU

miR-199a-5p inhibitor	S: GAACAGGUAGUCUGAACACUGGG
siRNAs	
Control siRNA	S: UUCUCCGAACGUGUCACGUTT
	AS: ACGUGACACGUUCGGAGAATT
CAV1-1	S: GCCGUGUCUAUUCCAUCUATT
	AS: UAGAUGGAAUAGACACGGCTT
CAV1-2	S: GCGACCCUAAACACCUCAATT
	AS: UUGAGGUGUUUAGGGUCGCTT
ESR1-1	S: GAGGGAGAAUGUUGAAACATT
	AS: UGUUUCAACAUUCUCCCUCTT
ESR1-2	S: GGUUCCGCAUGAUGAAUCUTT
	AS: AGAUUCAUCAUGCGGAACCTT
METTL3-1	S: GCAAGAAUUCUGUGACUAUTT
	AS: AUAGUCACAGAAUUCUUGCTT
METTL3-2	S: GCUGCACUUCAGACGAAUUTT
	AS: AAUUCGUCUGAAGUGCAGCTT

Supplementary Figures:

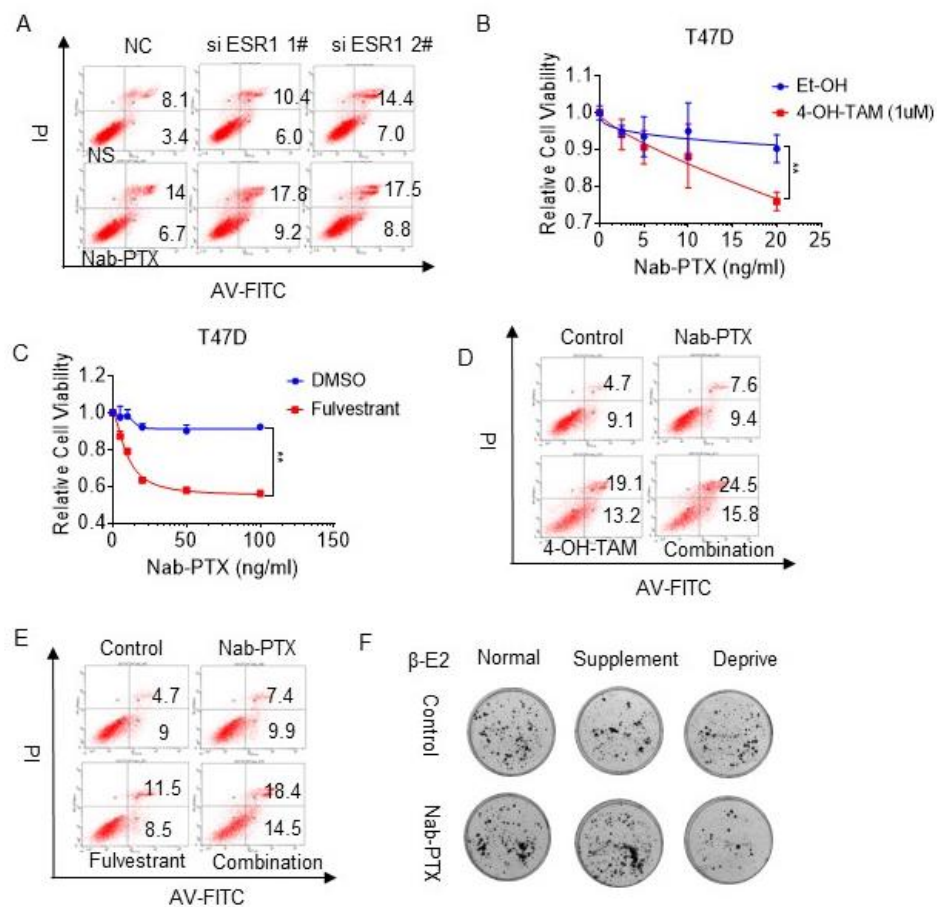
Fig.S1



Supplementary Figure 1. Lower response to Nab-paclitaxel in ER+BC than ER-BC.

(A). Patients Baseline; (B). ER status test by Western blot

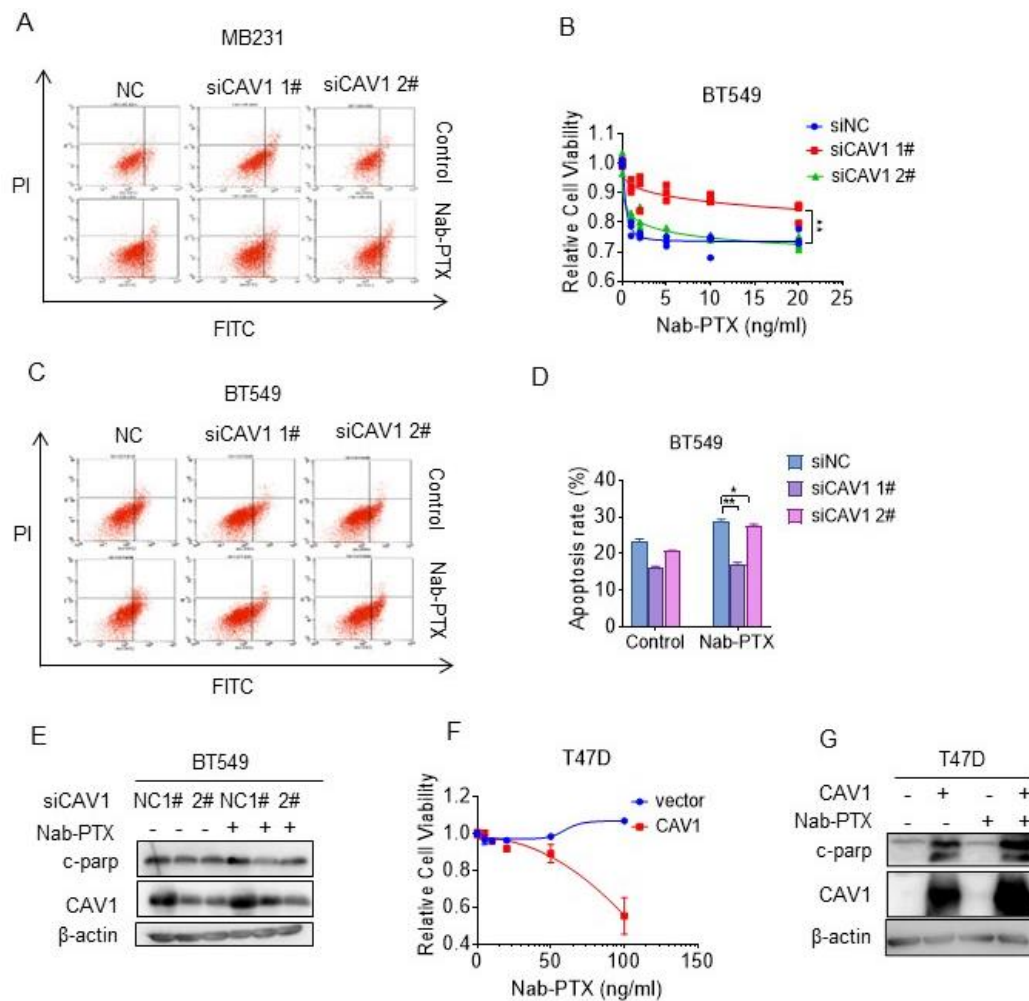
Fig.S2



Supplementary Figure 2. inhibition of ER α enhance the sensitivity to Nab-paclitaxel in vitro.

(A) Analysis of apoptosis after siESR1 with Nab-PTX (10ng/ml) in MCF7 by flow cytometry. (B) Analysis of cell viability after combine 4-OH-TAM (1uM) with Nab-PTX in T47D by MTS assays. (C) Analysis of cell viability after combine Fulvestrant (0.5uM) with Nab-PTX in T47D by MTS assays. (D) Analysis of apoptosis after combine 4-OH-TAM (5uM) with Nab-PTX (10ng/ml) in MCF7 by flow cytometry. (E) Analysis of apoptosis after combine Fulvestrant (1uM) with Nab-PTX (10ng/ml) in MCF7 by flow cytometry. (F) Colony formation assay before and after β -E2 with Nab-PTX (10ng/ml) in MCF7.

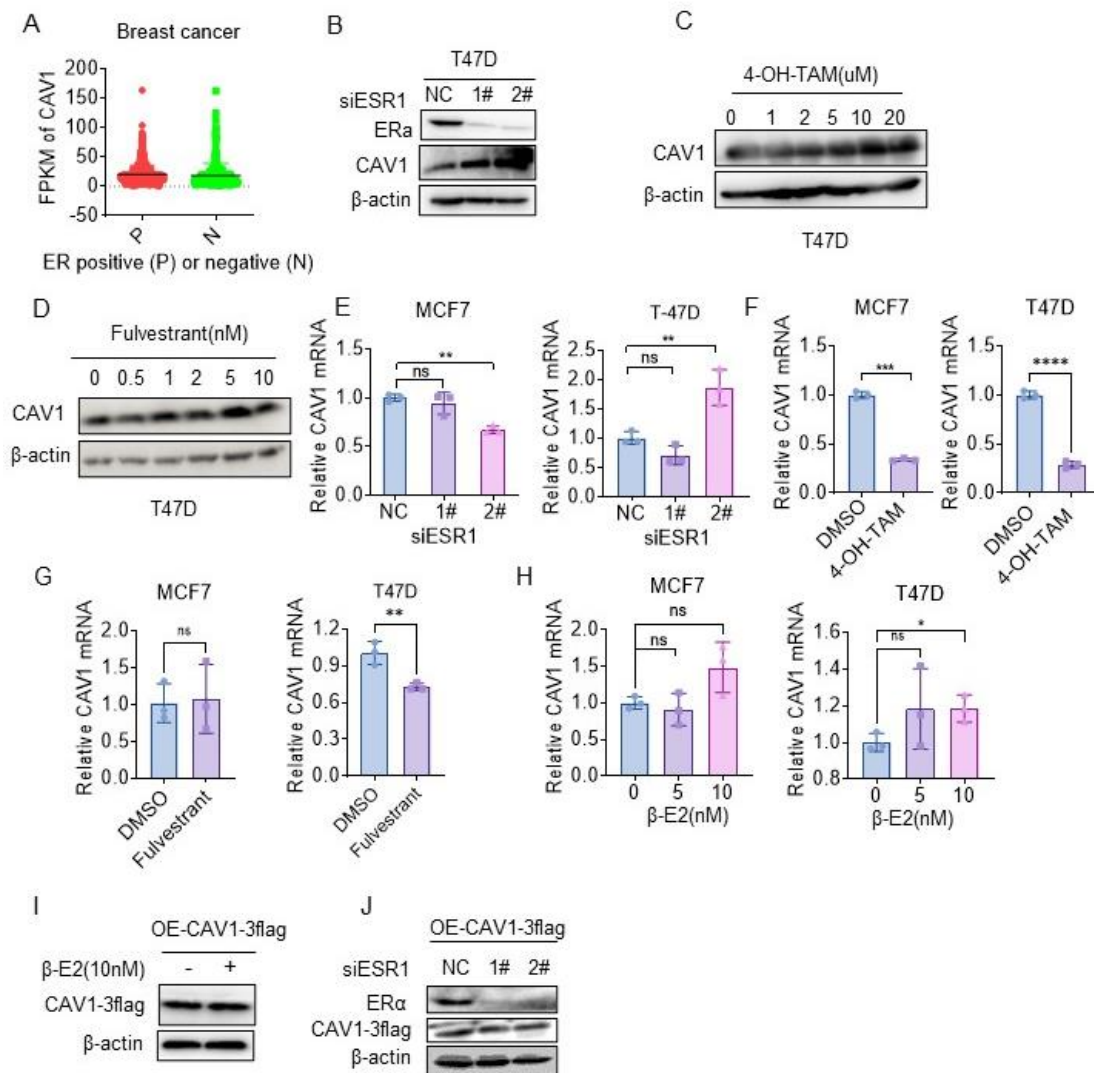
Fig.S3



Supplementary Figure 3. CAV1 is relevant to the sensitivity of breast cancer cells to Nab-paclitaxel.

(A) Analysis of apoptosis after siCAV1 with Nab-PTX (10ng/ml) in MB231 by flow cytometry. (B) Analysis of cell viability after siCAV1 with Nab-PTX in BT549 by MTS assays. (C) and (D) Analysis of apoptosis after siCAV1 with Nab-PTX (10ng/ml) in BT549 by flow cytometry. (E) Analysis of apoptosis after siCAV1 with Nab-PTX (10ng/ml) in BT549 by Western blot. (F) Analysis of cell viability after CAV1 overexpress with Nab-PTX in T47D by MTS. (G) Analysis of apoptosis after CAV1 overexpress with Nab-PTX (10ng/ml) in T47D by Western blot.

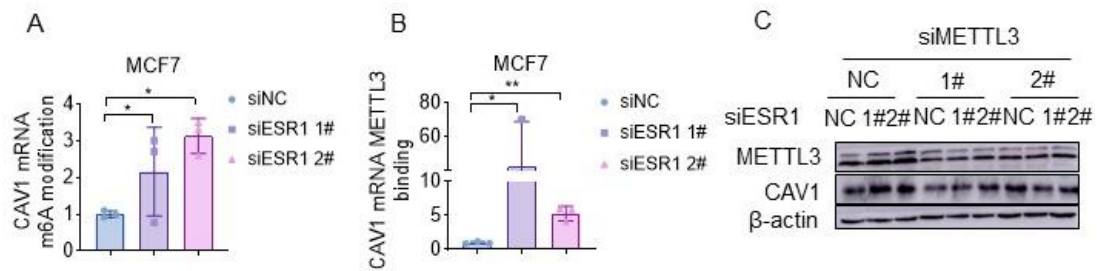
Fig.S4



Supplementary Figure 4. ERα downregulates CAV1 protein expression by inhibiting its translation

(A) Correlation between ERα and CAV1 mRNA levels in BC tissues from TCGA. (B) Western blot analysis CAV1 protein expression After siESR1 in T47D. (C) and (D) Western blot analysis CAV1 protein expression After 4-OH-TAM, Fulvestrant or β-E2 in T47D.(E)-(H) Real-time PCR analysis CAV1 mRNA expression After siESR1, 4-OH-TAM, Fulvestrant and β-E2. (I) and (J) Western blot analysis exogenous CAV1 protein expression after β-E2 and siESR1

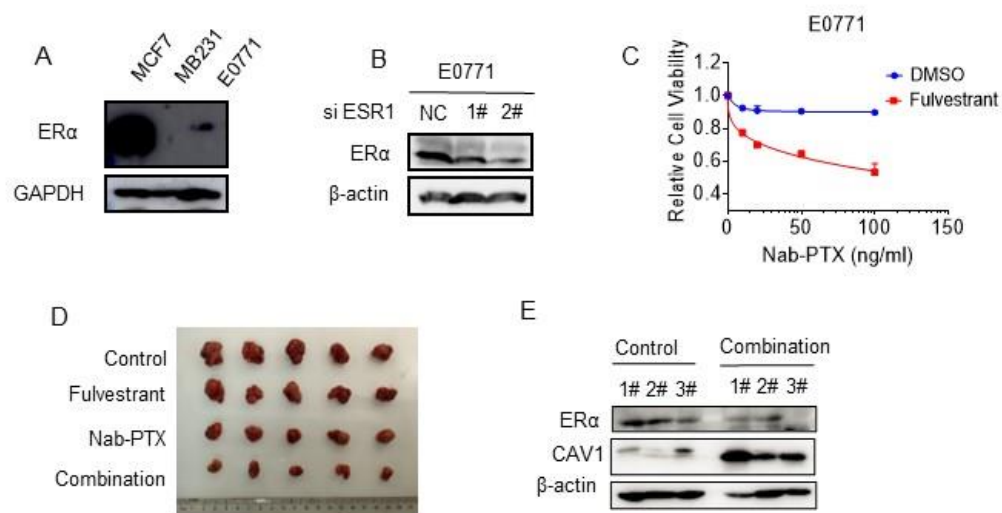
Fig.S5



Supplementary Figure 5. m6A modification interplay with miR199a-5p in CAV1 translation

(A) RIP analysis CAV1 m6A level after siESR1. (B) RIP analysis METTL3 binding ability to CAV1 mRNA after siESR1. (C) Western blot analysis CAV1 protein expression after siESR1 and rescued by siMETTL3

Fig.S6



Supplementary Figure 6. Fulvestrant has a synthetic lethal with Nab-Paclitaxel in vivo

(A) Western blot analysis ER α expression in Eo771. (B) Western blot analysis siESR1 knockdown efficacy. (C) Analysis of cell viability after combine Fulvestrant (100nM) with Nab-PTX in E0771 by MTS assays. (D) Morphology of tumor. (E) Western blot analysis

CAV1 expression in tumor after combination Fulvestrant and Nab-PTX