Figure S1 Palbociclib resistant breast cancer cells are cross-resistant to abemaciclib and ribociclib. a Dose response curves showed that acquired palbociclib resistant cells MCF7-palR and T47D-palR and their parental cells MCF7-pa and T47D-pa were treated with the other CDK4/6 inhibitors ribociclib and abemaciclib for 5 days and the proportions of viable cells were analyzed by cell viability assay. **b** Colony formation showed that the effect of 0.5 μM ribociclib and 0.1 μM abemaciclib for 14 days on resistant cells and their parental cells. **c** Results of EdU incorporation assay in resistant cells and their parental cells treated with 0.5 μM ribociclib and 0.1 μM abemaciclib. For graph **a-c**, data showed means ± s.d. (n=3), and two-tailed Student's t test determines the p-values. N.S., not significant or p >0.05.

Figure S2 CDKs and Cyclins promoted palbociclib resistance in T47D-palR breast cancer cells.
a,f Colony formation assay showing siRNA-transfected T47D-palR cells treated with control, 1μM palbociclib for 14 days. EdU incorporation assay (**b, g**) and cell cycle analysis (**c, h**) of T47D-palR cells transfected with negative control siRNA (NC), Cyclin D1, CKD4, Cyclin E1 or CDK2 siRNAs for 48h and then treated with control, 1μM palbociclib for 24h. **d, e and i, j** Western blot showing the key regulators profile of T47D-palR cells transfected with Cyclin D1 and CDK4 siRNAs (**d, e**) or Cyclin E1 and CDK2 siRNA (**i, j**) for 48h and then treated with control, 1μM palbociclib for 24h. For graph **a-c, f-h** data showed means ± s.d. (n=3), and two-tailed Student's t test determines the p-values. N.S., not significant or p >0.05.

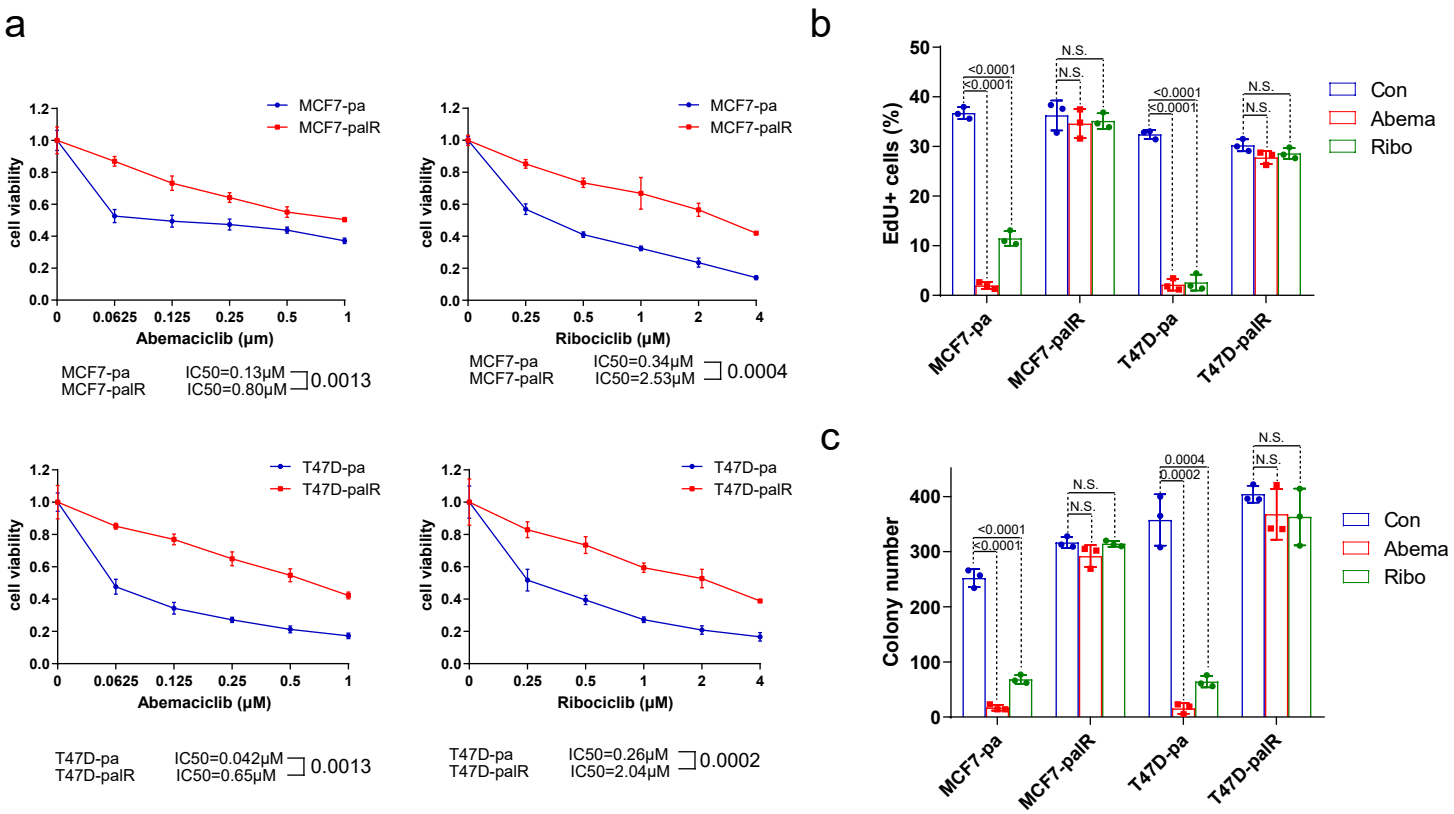
Figure S3 Differentially expressed genes (DEGs) of between MCF7-pa and MCF7-palR breast cancer cells. a The green plot in volcano plot represented down-regulated genes (Log2 Fold change ≤ −1 and p <0.05). The red plot in volcano plot represented up-regulated genes (Log2 Fold change ≥ 1 and p <0.05).

Figure S4 PI3K inhibitor and mTOR inhibitor reduced CDK4 and Cyclin D1 expression and restored CDK4/6i sensitivity in T47D-palR breast cancer cells.
a Western blot showing PI3K/AKT/mTOR pathways and their downstream in T47D-pa and T47D-palR treated with control, 1μM palbociclib for 24h. **b** The mRNA expression of Cyclin D1, CDK4, Cyclin E1 and CDK2 in MCF7-palR and MCF7-pa cells treated with 1μM everolimus and 1μM BYL719 for 24h. **c** Edu incorporation assay of T47D-palR cells treat with vehicle, 1μM everolimus, 1μM BYL719, 1μM palbociclib and their combination for 24h. **d** Colonies formation assay of T47D-palR cells treated with control, 1μM everolimus, 1μM BYL719, 1μM palbociclib and their combination for 14 days. **e** Combination index analysis of T47D-palR treated with combination of palbociclib and everolimus or BYL719 for 5 days based on the viability inhibition using CalcuSyn software. Data are means, n=3. **f** Western blot analysis of T47D-palR cells treated with vehicle, 1μM everolimus, 1μM BYL719, 1μM palbociclib and their combination for 24 hours. For graph **b-d**, data showed means ± s.d. (n=3), and two-tailed Student's t test determines the p-values. N.S., not significant or p >0.05.

Figure S5 Protein stability of Cyclin D1 and CDK4 in MCF7-pa and MCF7-palR breast cancer cells.
a,b Western blot of the expression kinetics of Cyclin D1 (**a**) and CDK4 (**b**) in MCF7-pa and MCF7-palR cells treated with the transcription inhibitor CHX.

Figure S6 PI3K inhibitor and mTOR inhibitor had little influence on body weight of mice.
a. Body weight of mice in different groups during the treatment.

Figure S1


a
MCF7-pa
MCF7-palR
cell viability
Abemaciclib (μm)
MCF7-pa IC50=0.13μM
MCF7-palR IC50=0.80μM
0.0013
Ribociclib (μM)
MCF7-pa IC50=0.34μM
MCF7-palR IC50=2.53μM
0.0004
T47D-pa
T47D-palR
Abemaciclib (μM)
T47D-pa IC50=0.042μM
T47D-palR IC50=0.65μM
0.0013
Ribociclib (μM)
T47D-pa IC50=0.26μM
T47D-palR IC50=2.04μM
0.0002
b
EdU+ cells (%)
<0.0001
N.S.
Con
Abema
Ribo
MCF7-pa
MCF7-palR
T47D-pa
T47D-palR
c
Colony number
0.0004
0.0002

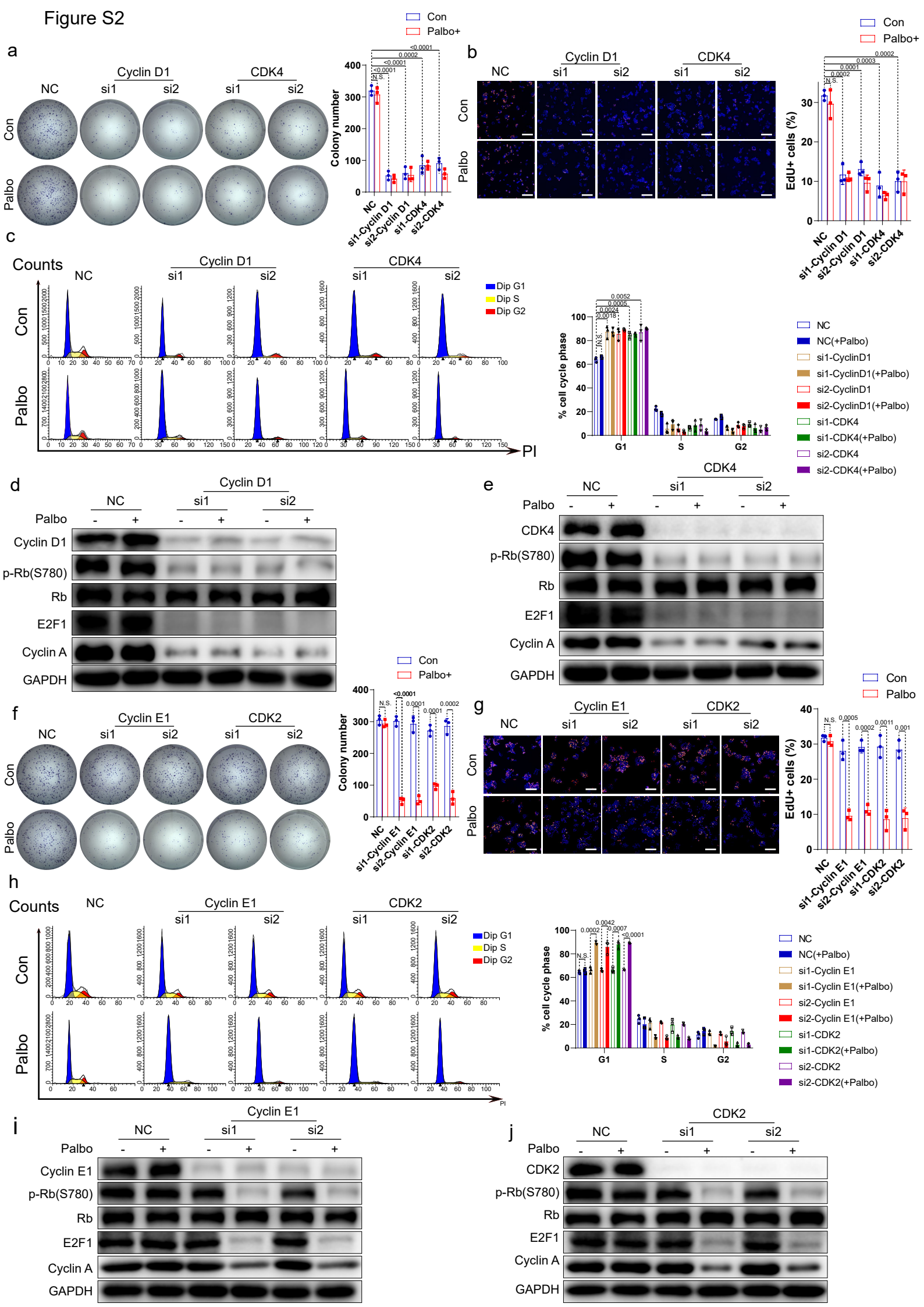


Figure S2
a
Cyclin D1
CDK4
NC
si1
si2
si1
si2
Con
Palbo
Con
Palbo+
Colony number
NC
si1-Cyclin D1
si2-Cyclin D1
si1-CDK4
si2-CDK4
b
Cyclin D1
CDK4
EdU+ cells (%)
c
Counts
Dip G1
Dip S
Dip G2
PI
% cell cycle phase
G1
S
G2
NC
NC(+Palbo)
si1-CyclinD1
si1-CyclinD1(+Palbo)
si2-CyclinD1
si2-CyclinD1(+Palbo)
si1-CDK4
si1-CDK4(+Palbo)
si2-CDK4
si2-CDK4(+Palbo)
d
Palbo
Cyclin D1
p-Rb(S780)
Rb
E2F1
Cyclin A
GAPDH
e
CDK4
f
Cyclin E1
CDK2
g
h
i
j

Figure S3

a

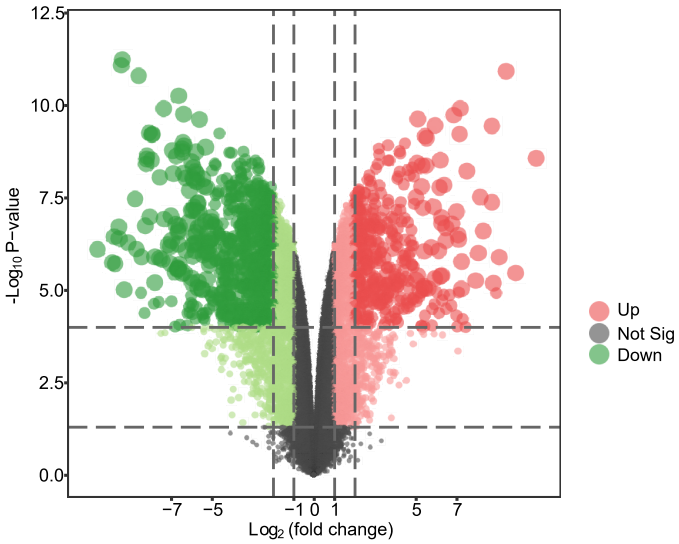

12.5
10.0
7.5
5.0
2.5
0.0
-Log10 P-value
−7
−5
−1
0
1
5
7
Log2 (fold change)
Up
Not Sig
Down

Figure S4

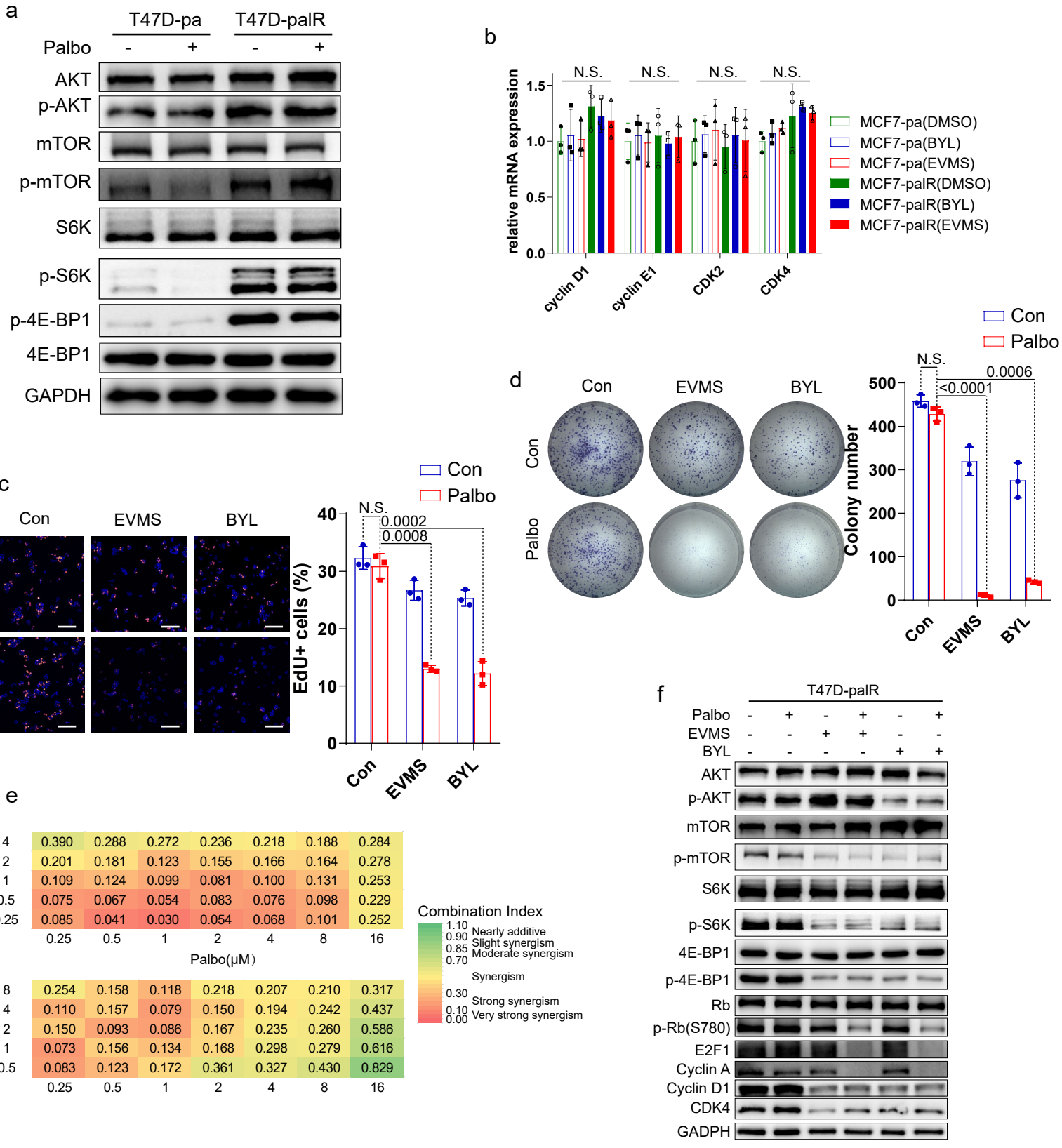

a
T47D-pa
T47D-palR
Palbo
AKT
p-AKT
mTOR
p-mTOR
S6K
p-S6K
p-4E-BP1
4E-BP1
GAPDH
b
relative mRNA expression
N.S.
cyclin D1
cyclin E1
CDK2
CDK4
MCF7-pa(DMSO)
MCF7-pa(BYL)
MCF7-pa(EVMS)
MCF7-palR(DMSO)
MCF7-palR(BYL)
MCF7-palR(EVMS)
c
Con
EVMS
BYL
Palbo
EdU+ cells (%)
0.0002
0.0008
d
Colony number
<0.0001
0.0006
e
EVMS(μM)
Palbo(μM)
BYL(μM)
Combination Index
Nearly additive
Slight synergism
Moderate synergism
Synergism
Strong synergism
Very strong synergism
f
T47D-palR
Rb
p-Rb(S780)
E2F1
Cyclin A
Cyclin D1
CDK4
GADPH

Figure S5

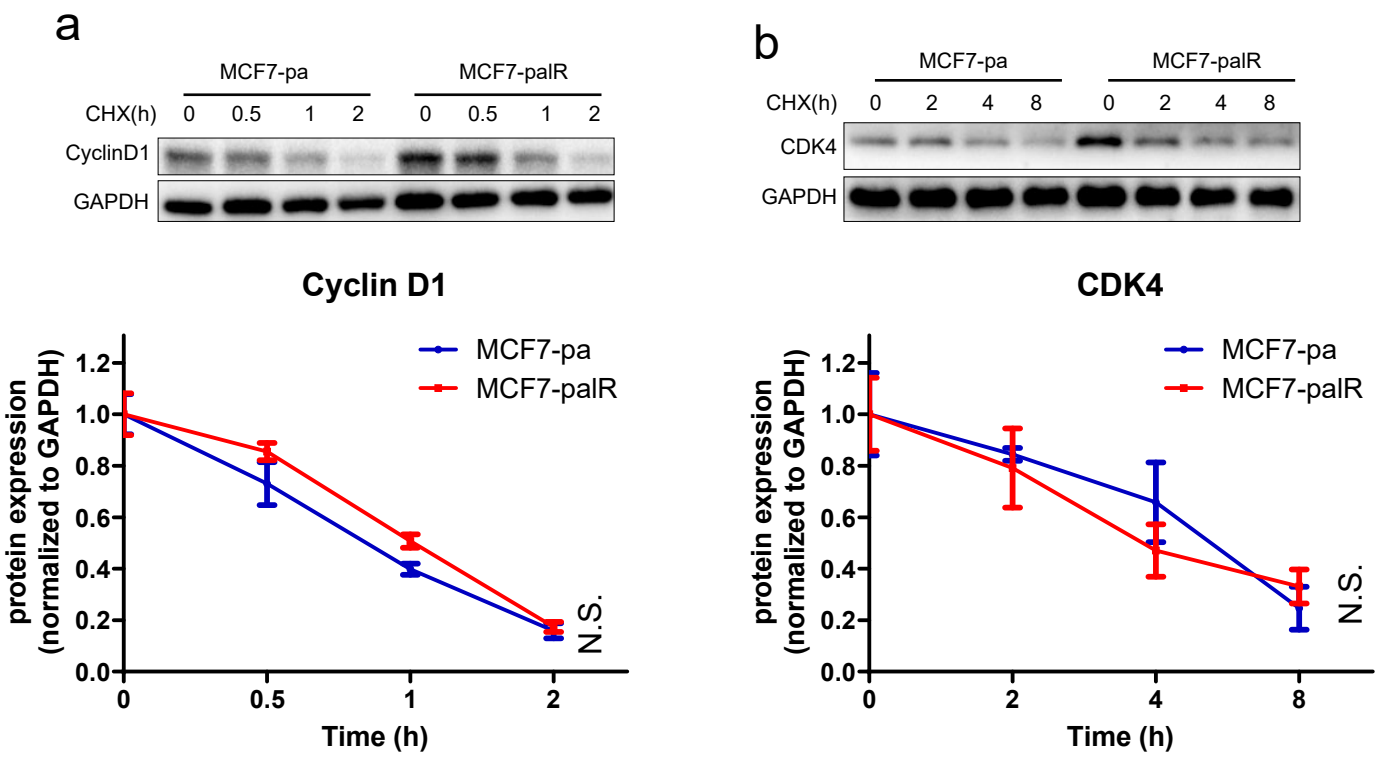

a
MCF7-pa
MCF7-palR
CHX(h) 0 0.5 1 2 0 0.5 1 2
CyclinD1
GAPDH
b
MCF7-pa
MCF7-palR
CHX(h) 0 2 4 8 0 2 4 8
CDK4
GAPDH
Cyclin D1
protein expression (normalized to GAPDH)
MCF7-pa
MCF7-palR
N.S.
Time (h)
CDK4
protein expression (normalized to GAPDH)
MCF7-pa
MCF7-palR
N.S.
Time (h)

Figure S6

a

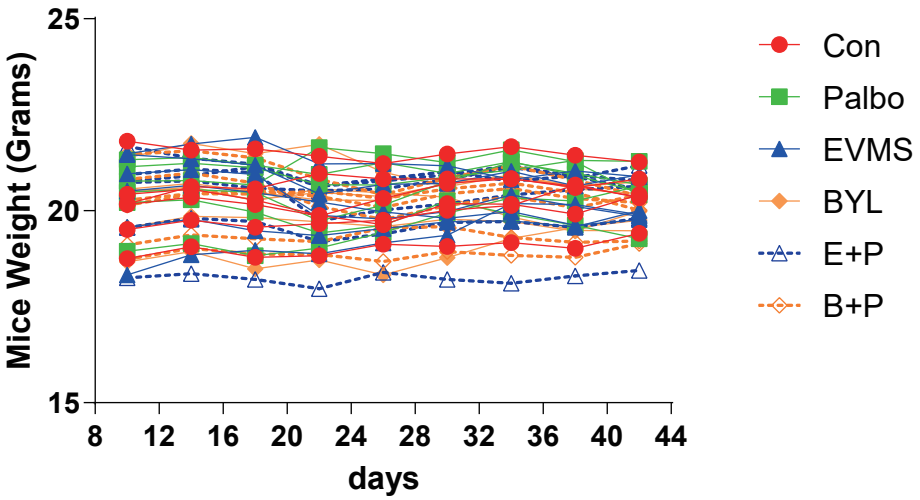

25
20
15
Mice Weight (Grams)
8
12
16
20
24
28
32
36
40
44
days
Con
Palbo
EVMS
BYL
E+P
B+P

Table S1 siRNA sequence

siRNA	gene	Sense
si1	Cyclin D1	CCAAUAGGUGUAGGAAAUAGCGCTG
si2	Cyclin D1	GCCCTCGGTGTCCTACTTCAA
si1	Cyclin E1	GGUGUGUGAAGUCUAUAAA
si2	Cyclin E1	GCAGGAUCCAGAUGAAGAA
si1	CDK2	GUACGGAGUUGUGUACAAA
si2	CDK2	CGGAGCUUGUUAUCGCAAA
si1	CDK4	GGAGUGUUGGCUGUAUCUU
si2	CDK4	GCAGCACUCUUAUCUACAU
si1	4EBP1	UCUAUGACCGGAAAUUCCUGAUGGA

Table S2 Primer sequence

qPCR-primer	Forward (5’ -> 3’)	Reverse(5’ -> 3’)
Cyclin D1	GCTGCGAAGTGGAAACCATC	CCTCCTTCTGCACACATTTGAA
CDK4	ATGGCTACCTCTCGATATGAGC	CATTGGGGACTCTCACACTCT
Cyclin E1	AAGGAGCGGGACACCATGA	ACGGTCACGTTTGCCTTCC
CDK2	CCAGGAGTTACTTCTATGCCTGA	TTCATCCAGGGGAGGTACAAC