Figure S1

A

Palbociclib IC50

T47D	**MCF7**	**TE14**	**OE21**	**KYSE410**	**KYSE510**
139 nM	164 nM	50 nM	86 nM	118 nM	122 nM
TE10	**TE9**	**TE15**	**KYSE180**	**KYSE140**	**TE11**
146 nM	186 nM	187 nM	582 nM	615 nM	698 nM
TE8	**KYSE70**	**TE1**	**GCIY**	**HARA**	**BICR6**
808 nM	972 nM	10020 nM	12460 nM	58 nM	500 nM

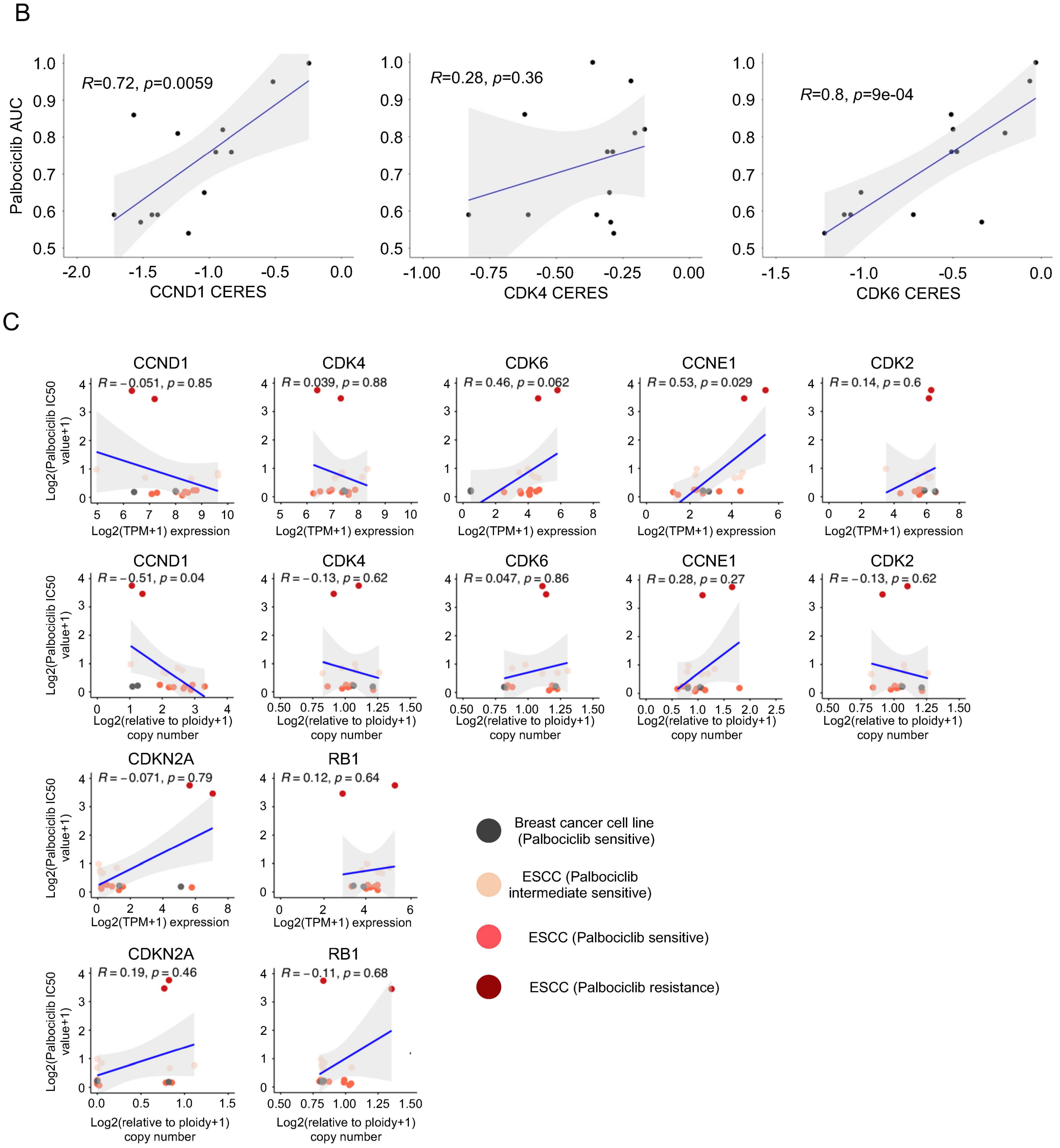
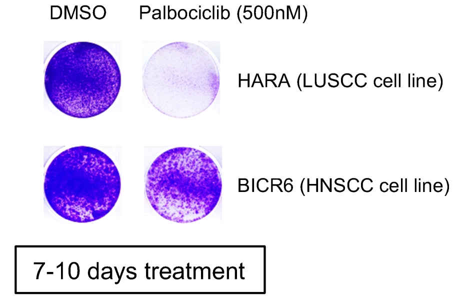
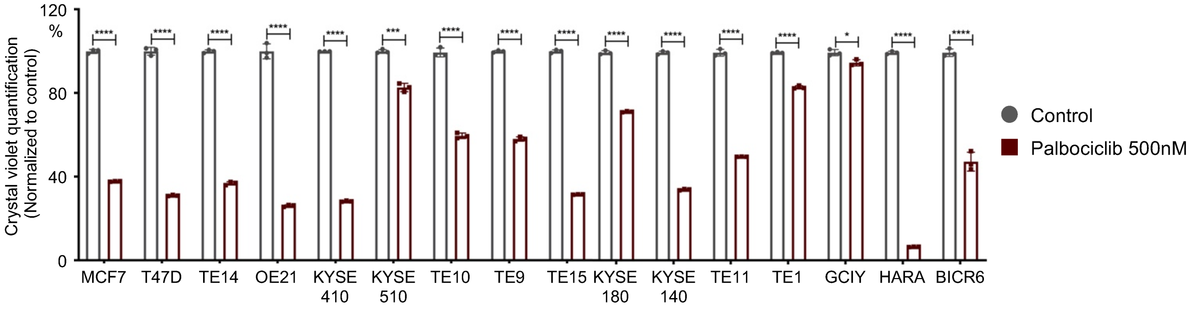


Figure S1 Continue

D



E



S1: **CDK4/6 inhibitors as a putative target for esophageal SCC**

A. Palbociclib IC50 value of cell lines shown in Figure 1b.

B. Pearson correlation of palbociclib drug sensitivity AUC and gene dependency scores of CCND1, CDK4, and CDK6 in ESCC cell lines.

C. Pearson correlation of palbociclib drug sensitivity (IC50) and relative gene expression and copy number profiles of cell-cycle genes (CCND1, CDK4, CDK6, CCNE1, CDK2, CDKN2A, RB1) across a panel of cell lines. color coded as follows: black: Palbo-sensitivie ER positive breast cancer lines; red: Palbociclib sensitive ESCC lines; beige: Palbociclib intermediate sensitive ESCC lines; dark red: Palbociclib resistant ESCC lines.

D. Images showing crystal violet staining of HARA and BICR6 after 500nM palbociclib treatment for 7–10 days. Data from one representative experiment are presented (n=3).

E. Quantification of the crystal violet staining from Figure 1E. Data are shown as mean ± SD and *P<0.05, ***p<0.001 and ****p<0.0001 as calculated by *t*-tests (n=3).

Figure S2

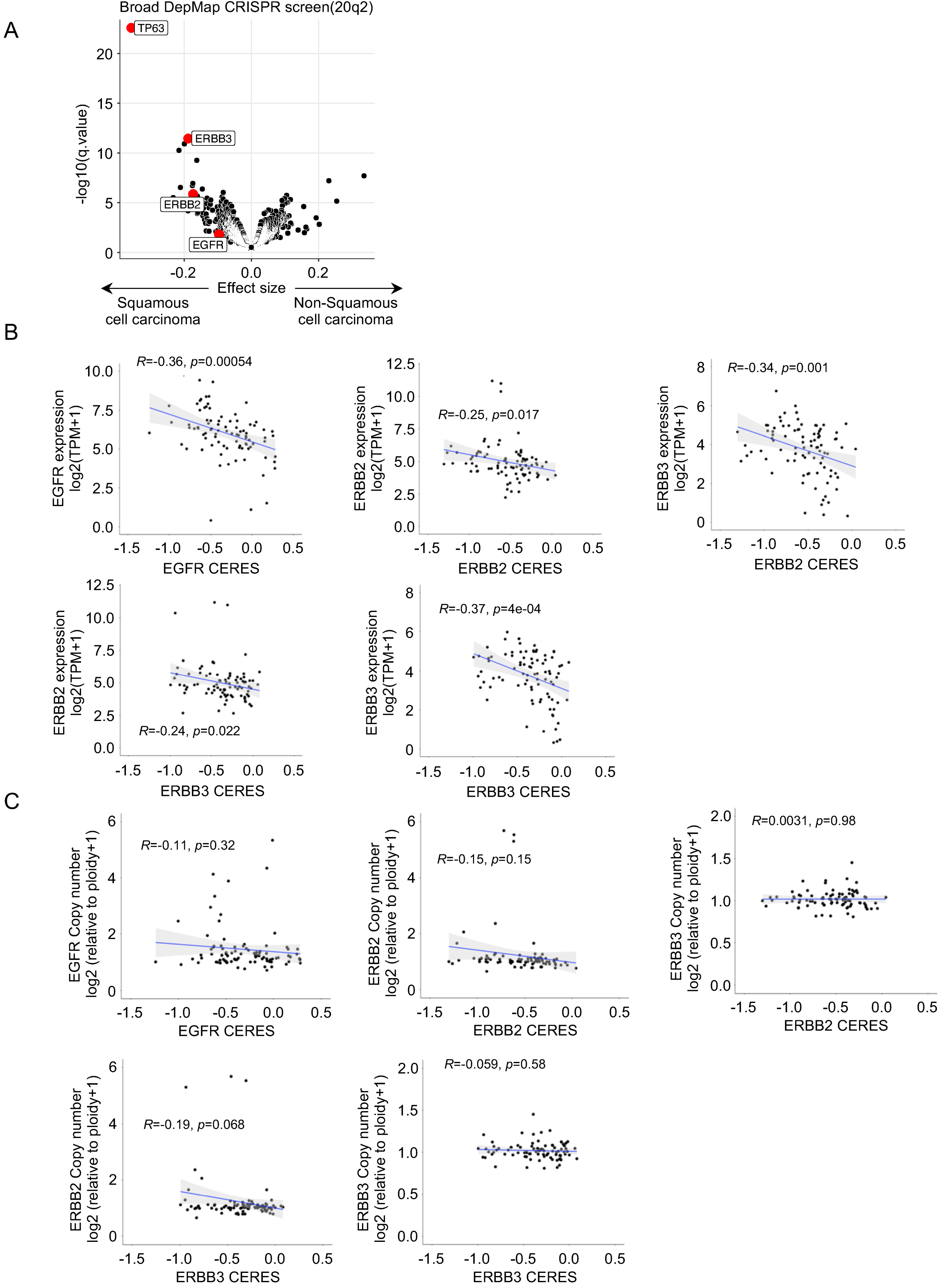

A
Broad DepMap CRISPR screen(20q2)
TP63
ERBB3
ERBB2
EGFR
-log10(q.value)
0
5
10
15
20
-0.2
0.0
0.2
Effect size
Squamous cell carcinoma
Non-Squamous cell carcinoma
B
R=-0.36, p=0.00054
EGFR expression log2(TPM+1)
EGFR CERES
R=-0.25, p=0.017
ERBB2 expression log2(TPM+1)
ERBB2 CERES
R=-0.34, p=0.001
ERBB3 expression log2(TPM+1)
ERBB2 CERES
R=-0.24, p=0.022
ERBB2 expression log2(TPM+1)
ERBB3 CERES
R=-0.37, p=4e-04
ERBB3 expression log2(TPM+1)
ERBB3 CERES
C
R=-0.11, p=0.32
EGFR Copy number log2 (relative to ploidy+1)
EGFR CERES
R=-0.15, p=0.15
ERBB2 Copy number log2 (relative to ploidy+1)
ERBB2 CERES
R=0.0031, p=0.98
ERBB3 Copy number log2 (relative to ploidy+1)
ERBB2 CERES
R=-0.19, p=0.068
ERBB2 Copy number log2 (relative to ploidy+1)
ERBB3 CERES
R=-0.059, p=0.58
ERBB3 Copy number log2 (relative to ploidy+1)
ERBB3 CERES
-1.5
-1.0
-0.5
0.0
0.5

Figure S2 Continue

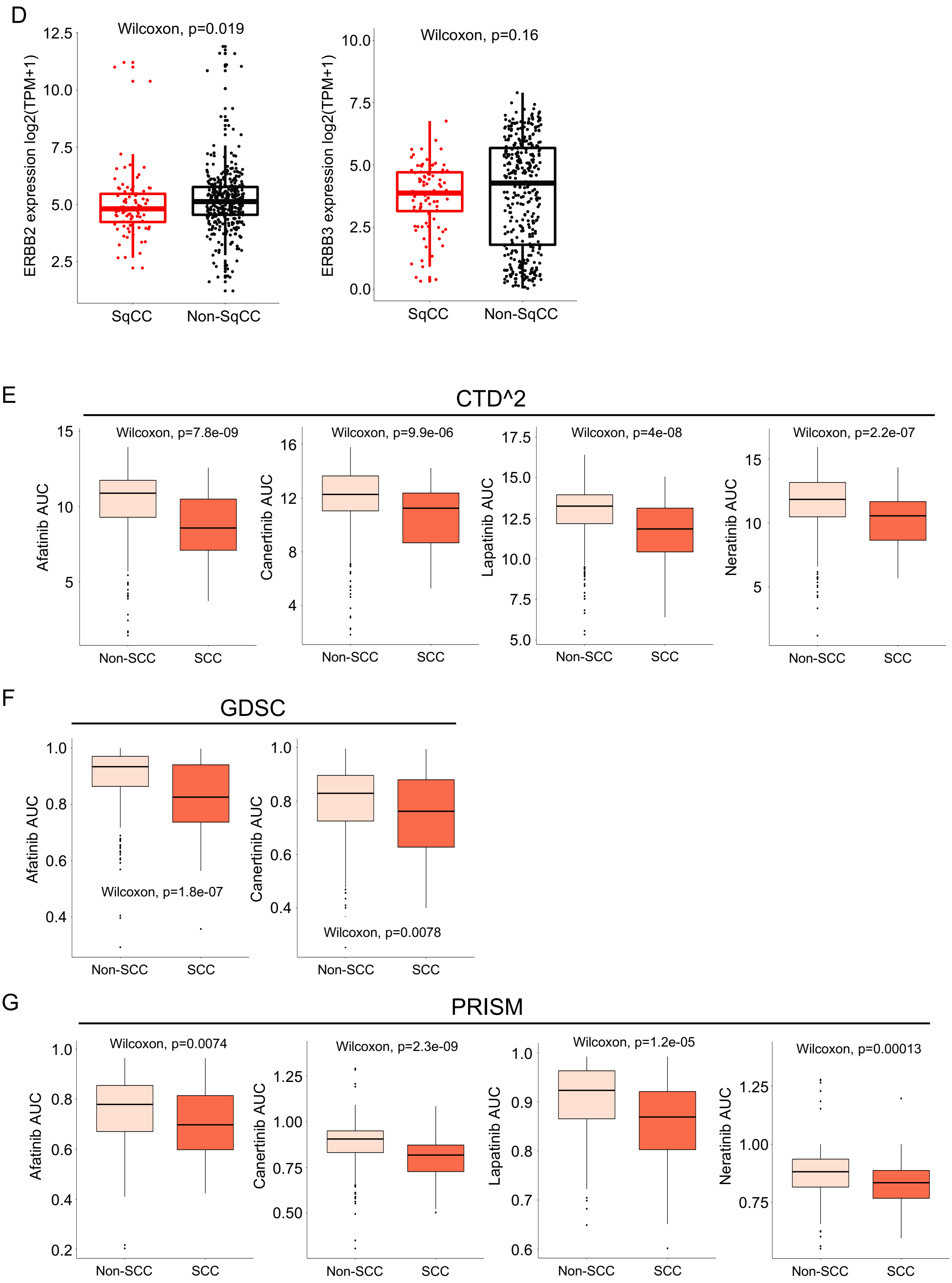


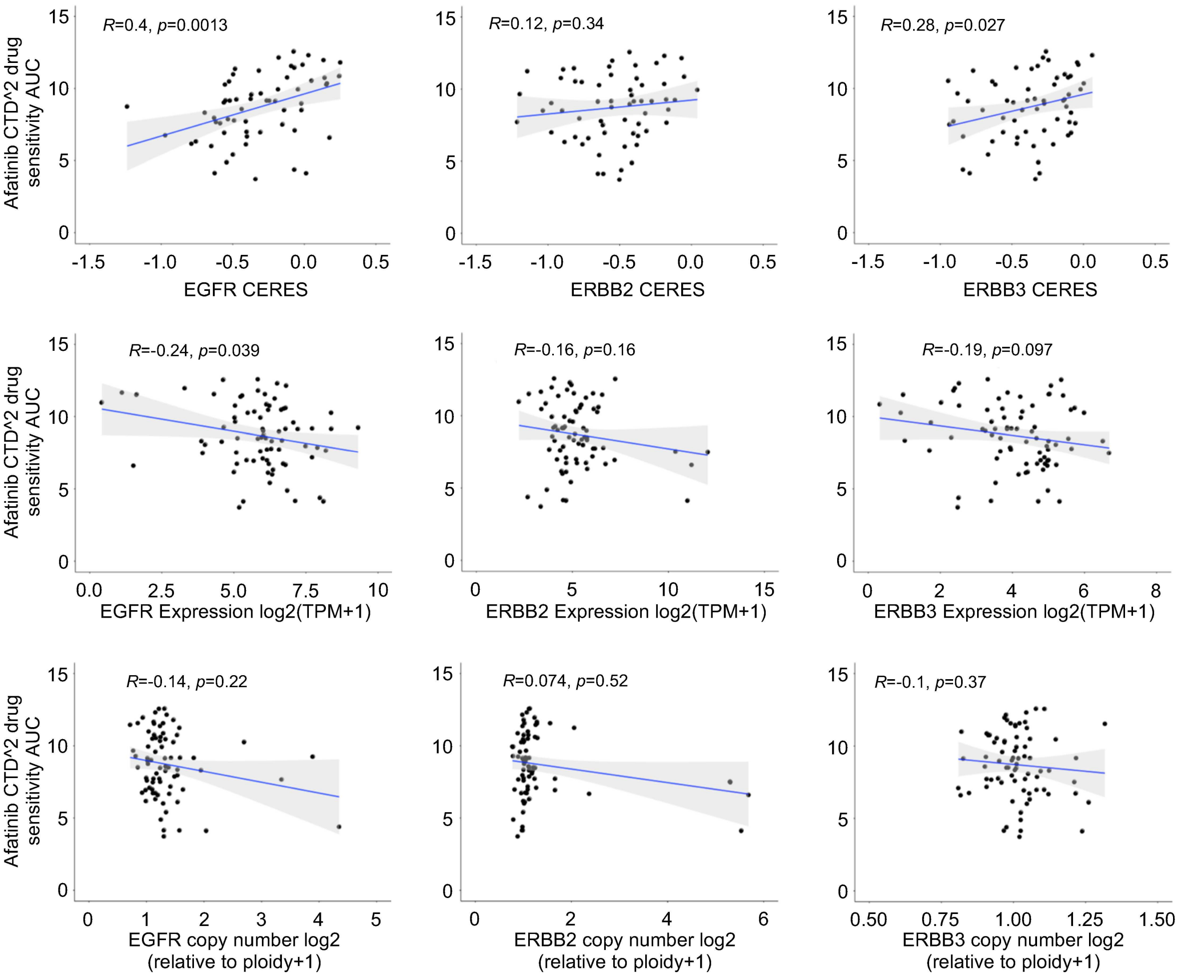
Figure S2 Continue

H

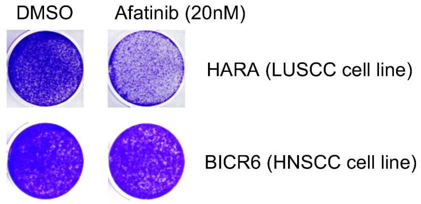
Afatinib IC50

T47D	**MCF7**	**TE14**	**OE21**	**KYSE410**	**KYSE510**
2296 nM	1184 nM	30 nM	8 nM	82 nM	2600 nM
TE10	**TE9**	**TE15**	**KYSE180**	**KYSE140**	**TE11**
395 nM	179 nM	40 nM	27 nM	22 nM	91 nM
TE8	**KYSE70**	**TE1**	**GCIY**	**HARA**	**BICR6**
1947 nM	400 nM	1854 nM	23 nM	182 nM	908nM

I



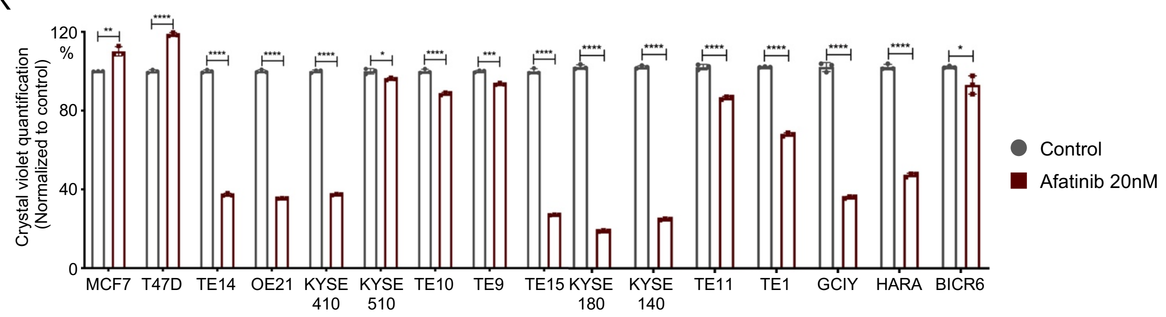
J



7-10 days treatment

Figure S2 Continue

K



S2: **ERBB family of kinases emerge as strong dependencies across ESCC**

A. Broad Institute Achilles CRISPR screen data (DepMap 20q2 public) analysis showing selective dependency genes in squamous carcinoma cell lines and non-squamous carcinoma cell lines, as illustrated in volcano plot. Each dot represents a gene, and the effect size explains the mean difference of gene dependency score between the two groups.

B. Pearson correlation of ERBBs gene expression and ERBBs gene dependency in squamous carcinoma cell lines.

C. Pearson correlation of ERBBs gene relative copy number and ERBBs gene dependency in squamous carcinoma cell lines.

D. mRNA expression of ERBB2(Left) and ERBB3(Right) in squamous cell carcinoma lines and non-squamous cell carcinoma lines based on Broad Institute Cancer Cell Line Encyclopedia data (DepMap 20Q2 public). Wilcoxon test was performed for two group comparison.

E. Comparison of ERBB inhibitors afatinib, canertinib, lapatinib and neratinib drug sensitivity AUC data from CTRP CTD^2 dataset in SCC (squamous cell carcinoma) and Non-SCC (non-squamous cell carcinoma). Wilcoxon test was performed for two group comparison.

F. Comparison of ERBB inhibitors afatinib and canertinib drug sensitivity AUC data from Sanger GDSC dataset in SCC (squamous cell carcinoma) and Non-SCC (non-squamous cell carcinoma). Wilcoxon test was performed for two group comparison.

G. Comparison of ERBB inhibitors afatinib, canertinib, lapatinib and neratinib drug sensitivity AUC data from Broad Institute PRISM dataset in SCC (squamous cell carcinoma) and Non-SCC (non-squamous cell carcinoma). Wilcoxon test was performed for two group comparison.

H. Afatinib IC50 value of cell lines shown in Figure 2I.

I. Pearson correlation of afatinib drug sensitivity AUC from CTD^2 dataset and ERBBs gene dependency score (top), gene expression (middle), and relative copy number (bottom) in squamous cell carcinoma cell lines.

J. Images showing crystal violet staining of HARA and BICR6 after 20nM afatinib treatment for 7–10 days. Data from one representative experiment are presented (n=3).

K. Quantification of the crystal violet staining from Figure 2K. Data are shown as mean ± SD and $*P<0.05$, $***p<0.001$ and $****p<0.0001$ as calculated by *t*-tests (n=3).

Figure S3

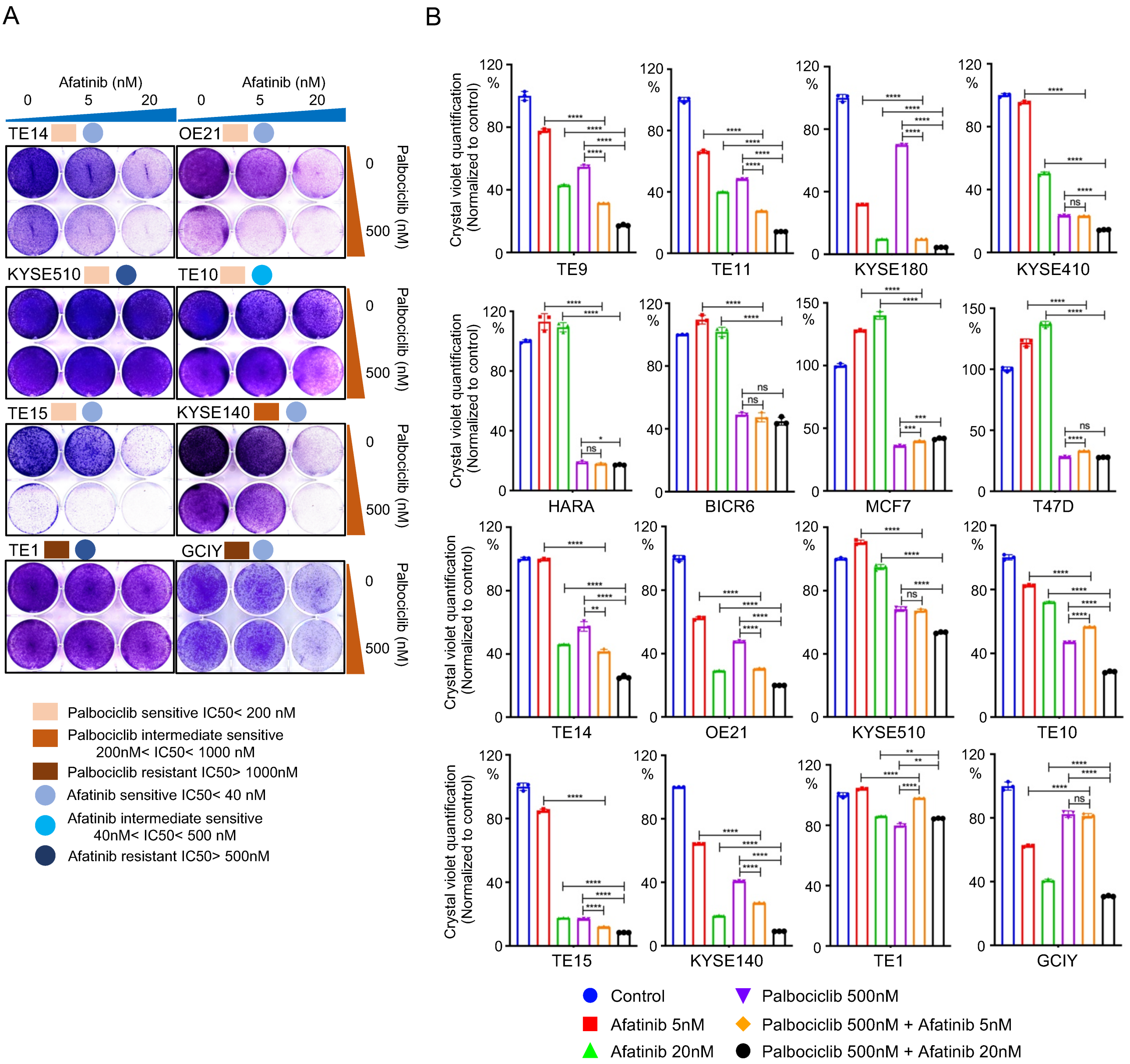

A
B
Afatinib (nM)
0
5
20
Palbociclib (nM)
0
500
TE14
OE21
KYSE510
TE10
TE15
KYSE140
TE1
GCIY
Palbociclib sensitive IC50< 200 nM
Palbociclib intermediate sensitive 200nM< IC50< 1000 nM
Palbociclib resistant IC50> 1000nM
Afatinib sensitive IC50< 40 nM
Afatinib intermediate sensitive 40nM< IC50< 500 nM
Afatinib resistant IC50> 500nM
Crystal violet quantification (Normalized to control)
%
TE9
TE11
KYSE180
KYSE410
HARA
BICR6
MCF7
T47D
TE14
OE21
KYSE510
TE10
TE15
KYSE140
TE1
GCIY
ns
Control
Afatinib 5nM
Afatinib 20nM
Palbociclib 500nM
Palbociclib 500nM + Afatinib 5nM
Palbociclib 500nM + Afatinib 20nM

Figure S3 Continue

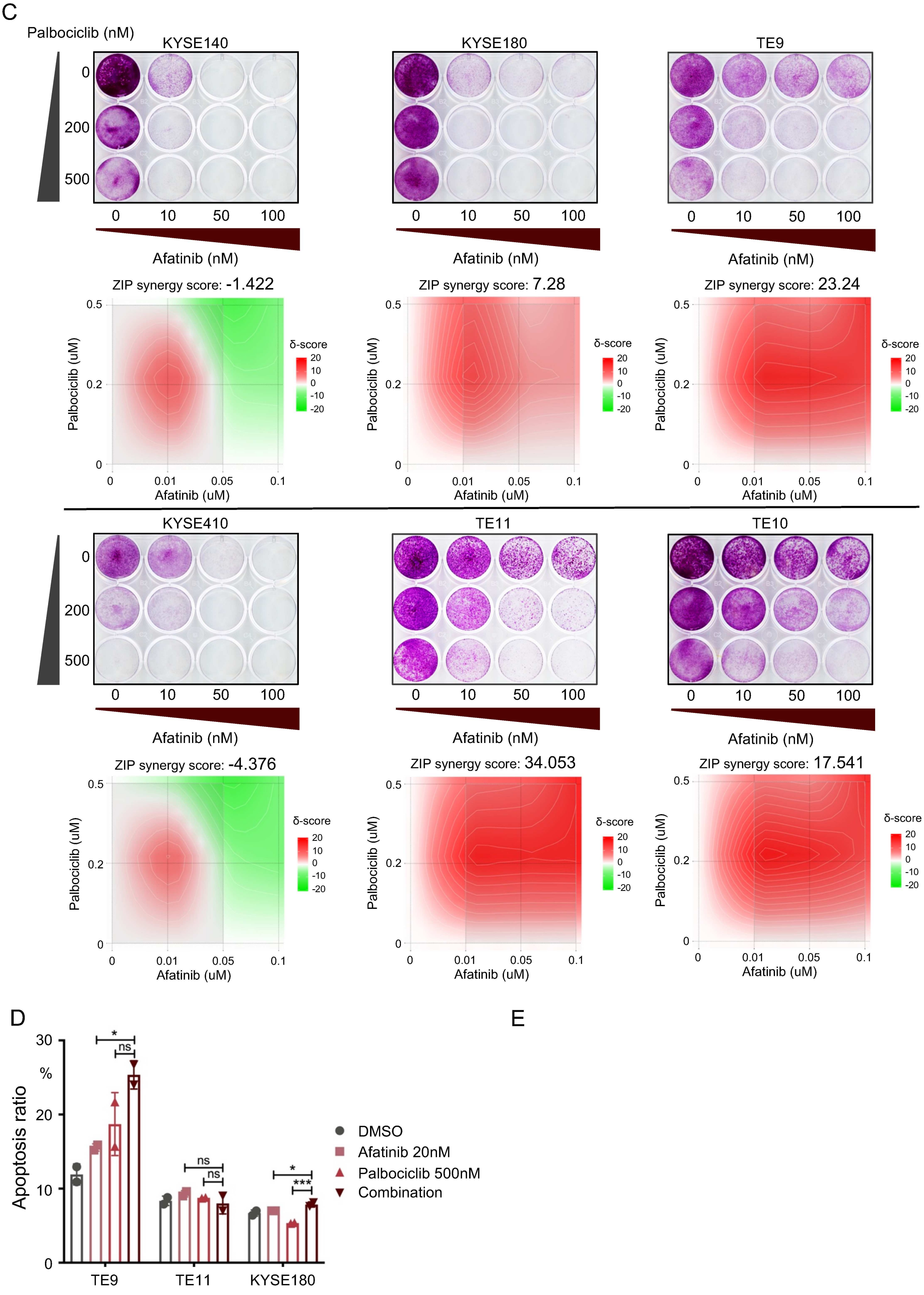
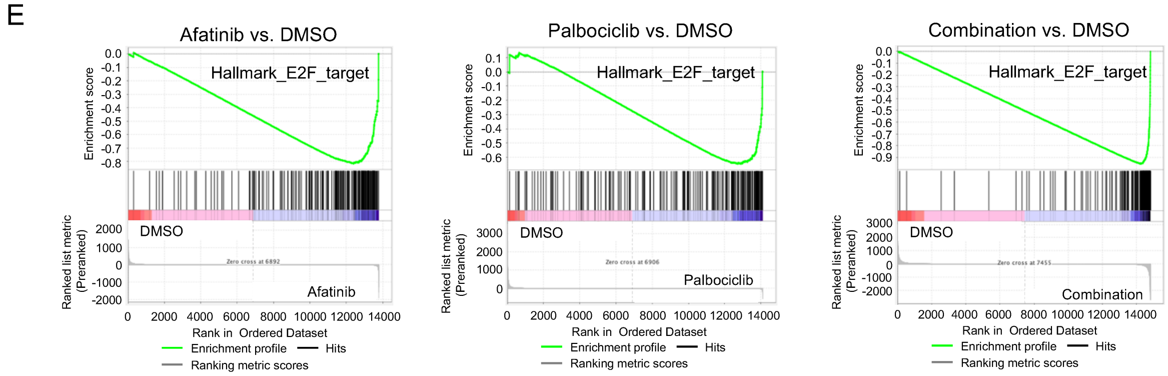


Figure S3 Continue



NES=-1.67; P<0.001; FDR=0.0059

NES=-1.42; P=0.019; FDR=0.33

NES=-1.75; P<0.001; FDR<0.001

S3: **pan ERBB and CDK4/6 pathway dual inhibition demonstrated efficacy in ESCC**

A. Images showing crystal violet staining of additional ESCC cell lines upon treatment with afatinib (20nM), palbociclib (500nM), the combination or with DMSO control for 7-10 days. Data from one representative experiment is presented (n=2).

B. Quantification of the crystal violet staining from Figure 3A and Supplemental Figure 3A. Data are shown as mean ± SD and *P<0.05, ***p<0.001 and ****p<0.0001 as calculated by *t*-tests (n=3).

C. Images showing crystal violet staining of representative squamous carcinoma cell lines upon treatment with afatinib (10nM, 50nM and 100nM), palbociclib (200nM and 500nM), the combination or with DMSO control for 7-10 days. Data from one representative experiment are presented (n=2). (bottom) Analysis of drug combination by SynergyFinder using the Zero Interaction Potency (ZIP) model using the absorbance readings of solubilized stain (normalized to no drug treatment). The combination treatment to be additive in KYSE140, KYSE180 and KYSE410 while synergistic in TE9, TE10, and TE11 (a score of less than -10 is considered antagonistic, between -10 and 10 is considered additive, and above 10 is considered synergistic; data displayed in synergy distribution maps)

D. The percentage of apoptotic cells of representative ESCC cell lines upon treatment with afatinib (20nM), palbociclib (500nM), the combination or with DMSO control for 72h. Following treatment, the cells were harvested, stained with annexin V and PI and assayed with flow cytometry. Data are shown as mean ± SD and *P<0.05,**p<0.01, ***p<0.001 and ****p<0.0001 as calculated by the one-way ANOVA test followed by Benjamini-Hochberg correction.

E. Gene-Set Enrichment Analysis (GSEA) curves of HALLMARK E2F_TARGETS gene signature in three treatments (left: 20nM afatinib, middle: 500nM palbociclib, right: combination) vs. DMSO

Figure S4

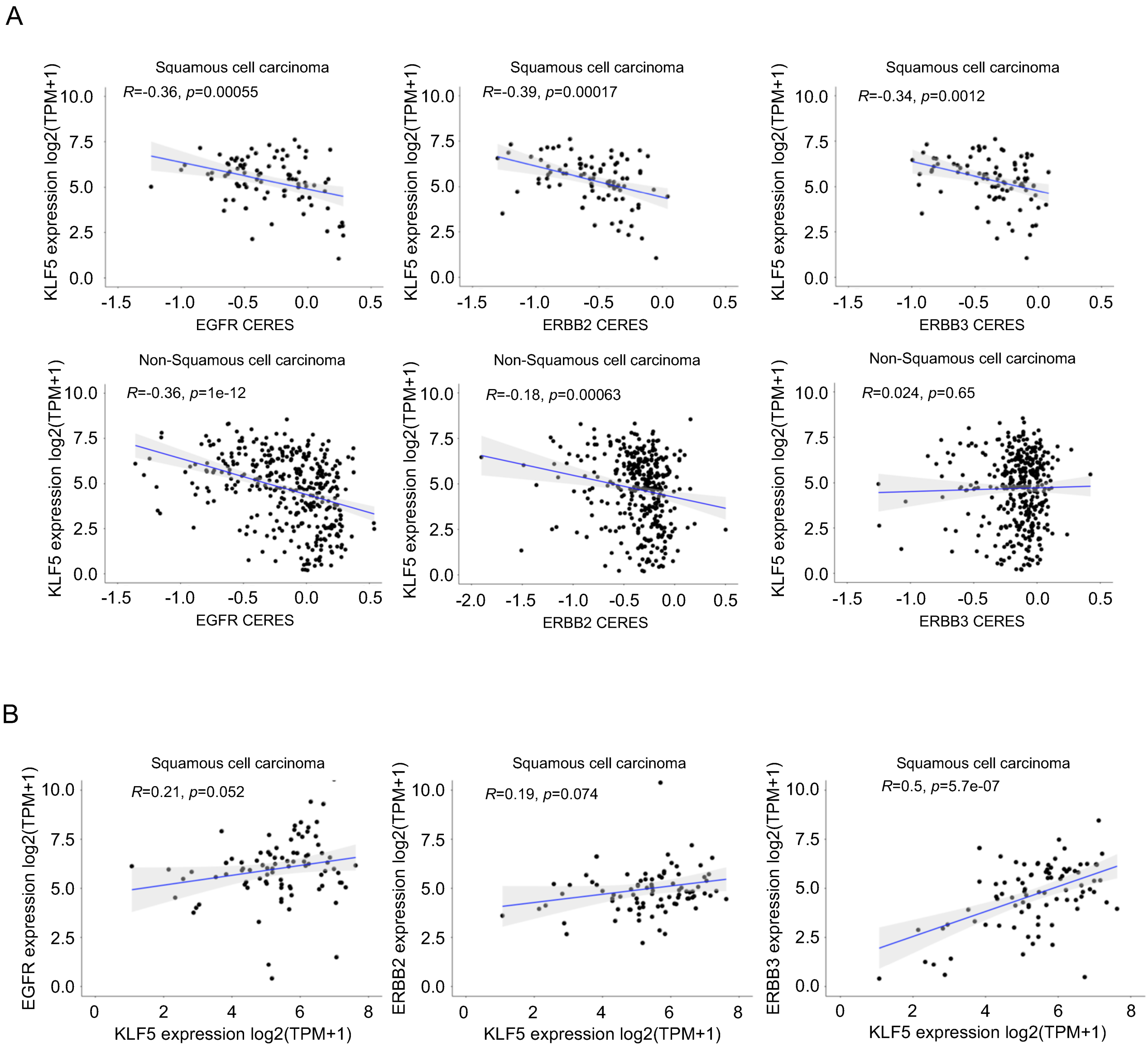
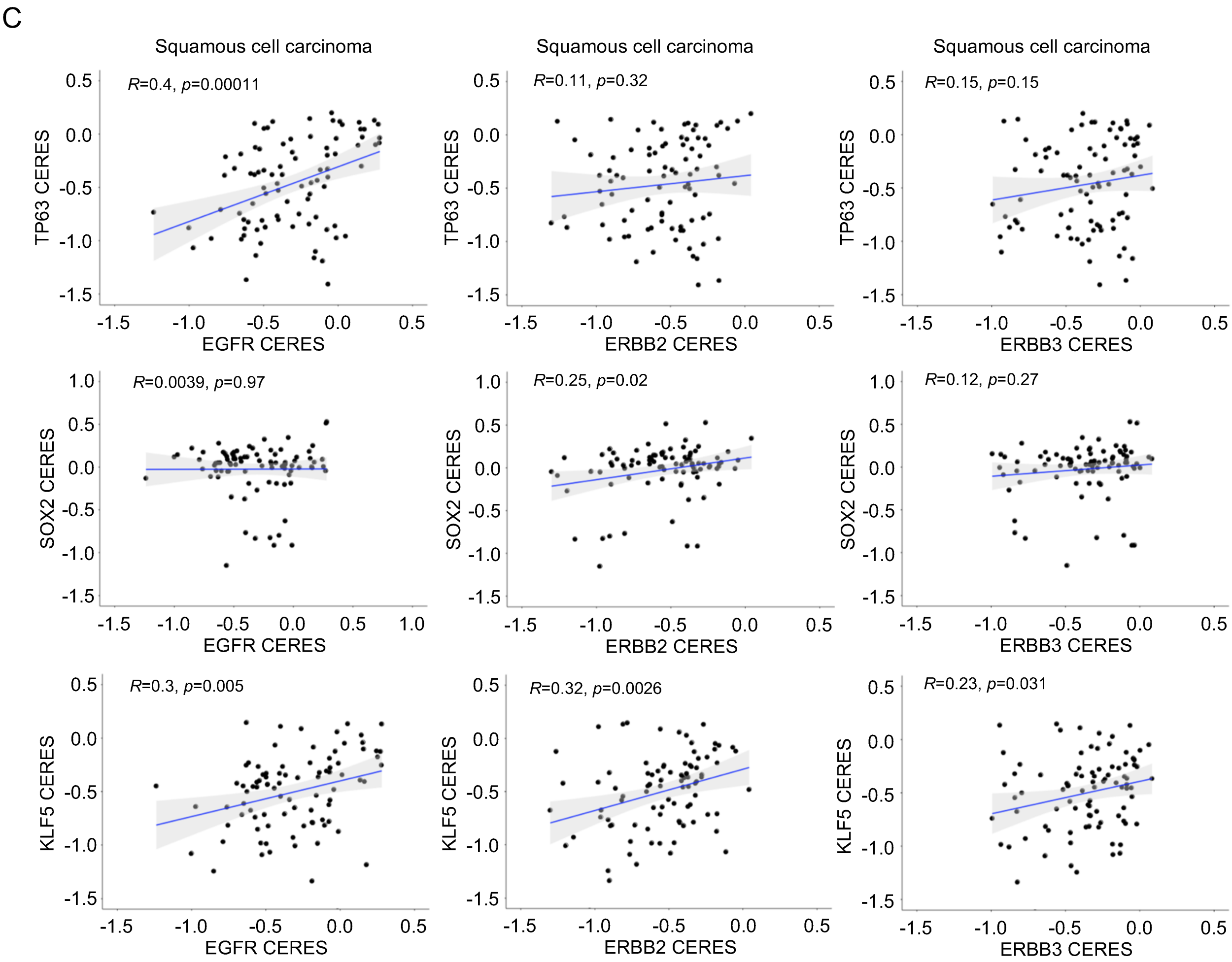

A
Squamous cell carcinoma
R=-0.36, p=0.00055
KLF5 expression log2(TPM+1)
EGFR CERES
Squamous cell carcinoma
R=-0.39, p=0.00017
KLF5 expression log2(TPM+1)
ERBB2 CERES
Squamous cell carcinoma
R=-0.34, p=0.0012
KLF5 expression log2(TPM+1)
ERBB3 CERES
Non-Squamous cell carcinoma
R=-0.36, p=1e-12
KLF5 expression log2(TPM+1)
EGFR CERES
Non-Squamous cell carcinoma
R=-0.18, p=0.00063
KLF5 expression log2(TPM+1)
ERBB2 CERES
Non-Squamous cell carcinoma
R=0.024, p=0.65
KLF5 expression log2(TPM+1)
ERBB3 CERES
0.0
2.5
5.0
7.5
10.0
-2.0
-1.5
-1.0
-0.5
0.0
0.5
B
Squamous cell carcinoma
R=0.21, p=0.052
EGFR expression log2(TPM+1)
KLF5 expression log2(TPM+1)
Squamous cell carcinoma
R=0.19, p=0.074
ERBB2 expression log2(TPM+1)
KLF5 expression log2(TPM+1)
Squamous cell carcinoma
R=0.5, p=5.7e-07
ERBB3 expression log2(TPM+1)
KLF5 expression log2(TPM+1)
0
2
4
6
8

Figure S4 Continue



S4: **KLF5 facilitated ERBBs activation and promoted ERBBs dependency**

A. Pearson correlation of ERBBs gene dependency score and KLF5 gene expression in squamous cell carcinoma cell lines(Top) and Non-squamous cell carcinoma cell lines(Bottom).

B. Pearson correlation of ERBBs gene expression and KLF5 gene expression in squamous cell carcinoma cell lines.

C. Pearson correlation of ERBBs gene dependency score and squamous lineage transcription factor TP63 (top), SOX2 (middle) and KLF5 (bottom) dependency score in squamous cell carcinoma cell lines.