Fig. S1

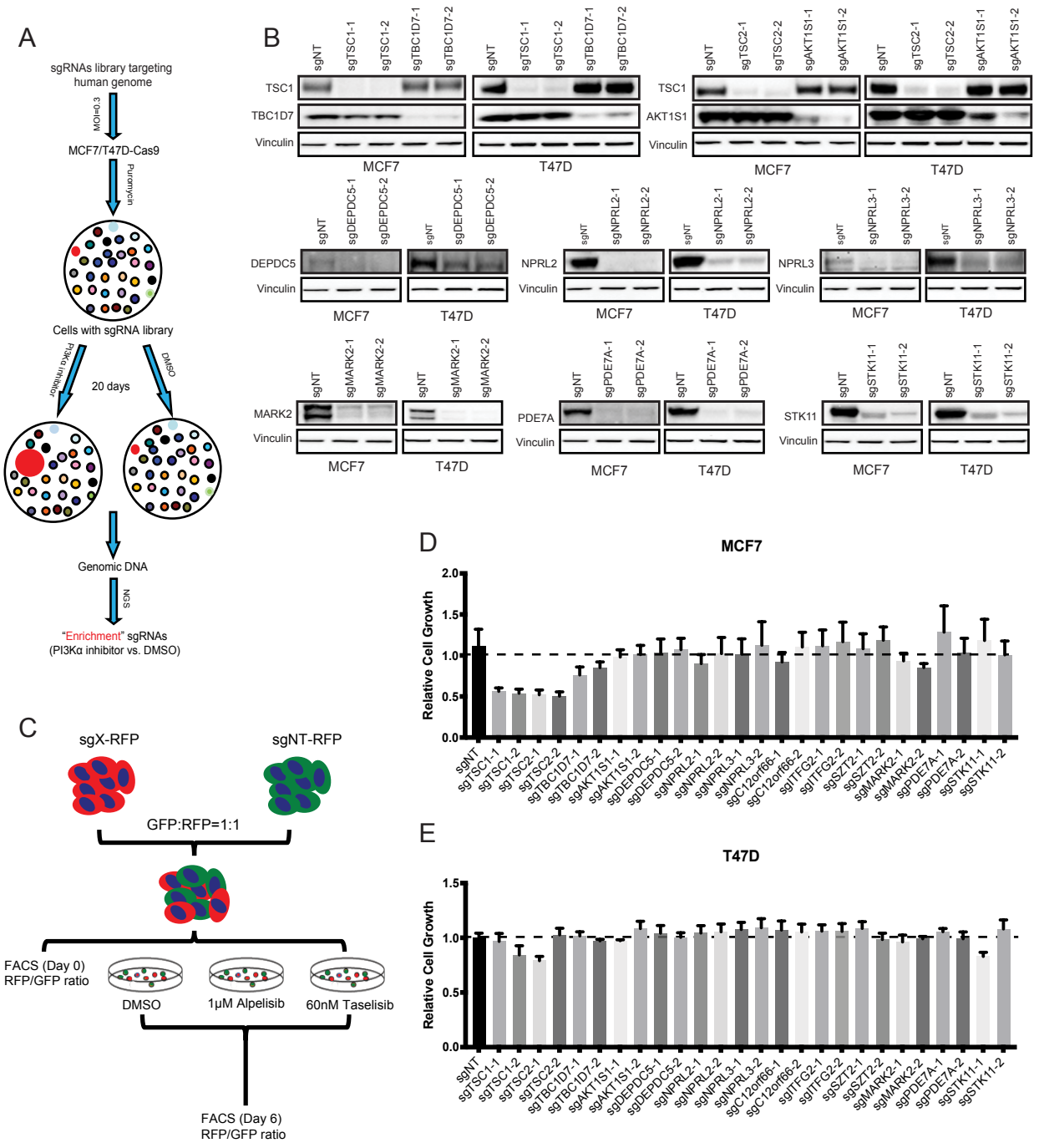

Figure S1. Loss of most individual candidate genes does not affect cell growth.

A. Scheme of whole genome CRISPR-Cas9 knockout screen.

B. Knock-out efficacy of individual sgRNA targeting the top candidate genes in MCF7 cells and T47D cells evaluated by immunoblotting.

C. Scheme of cell competition assay. Cells transduced with sgNT-RFP or sgRNA-RFP targeting individual candidate genes are mixed with cells transduced with sgNT-GFP at ratio of 1:1 which will be confirmed by flow cytometry as T0. The mixed cells are seeded for DMSO, 1 μM alpelisib or 60nM taselisib treatment. The percentages of GFP and RFP labeled cells will be analyzed by flow cytometry after 6 days of treatment (T7).

D and E. Relative cell growth of MCF7 cells (B) and T47D cells (C) with target gene loss are shown as the relative ratios of RFP+/GFP+ labeled cells compared to Day 0. Mean and SEM are shown; n=4 or 3 experiments. P-values were calculated using ordinary one-way ANOVA with Dunnett's post-hoc tests.

Fig. S2

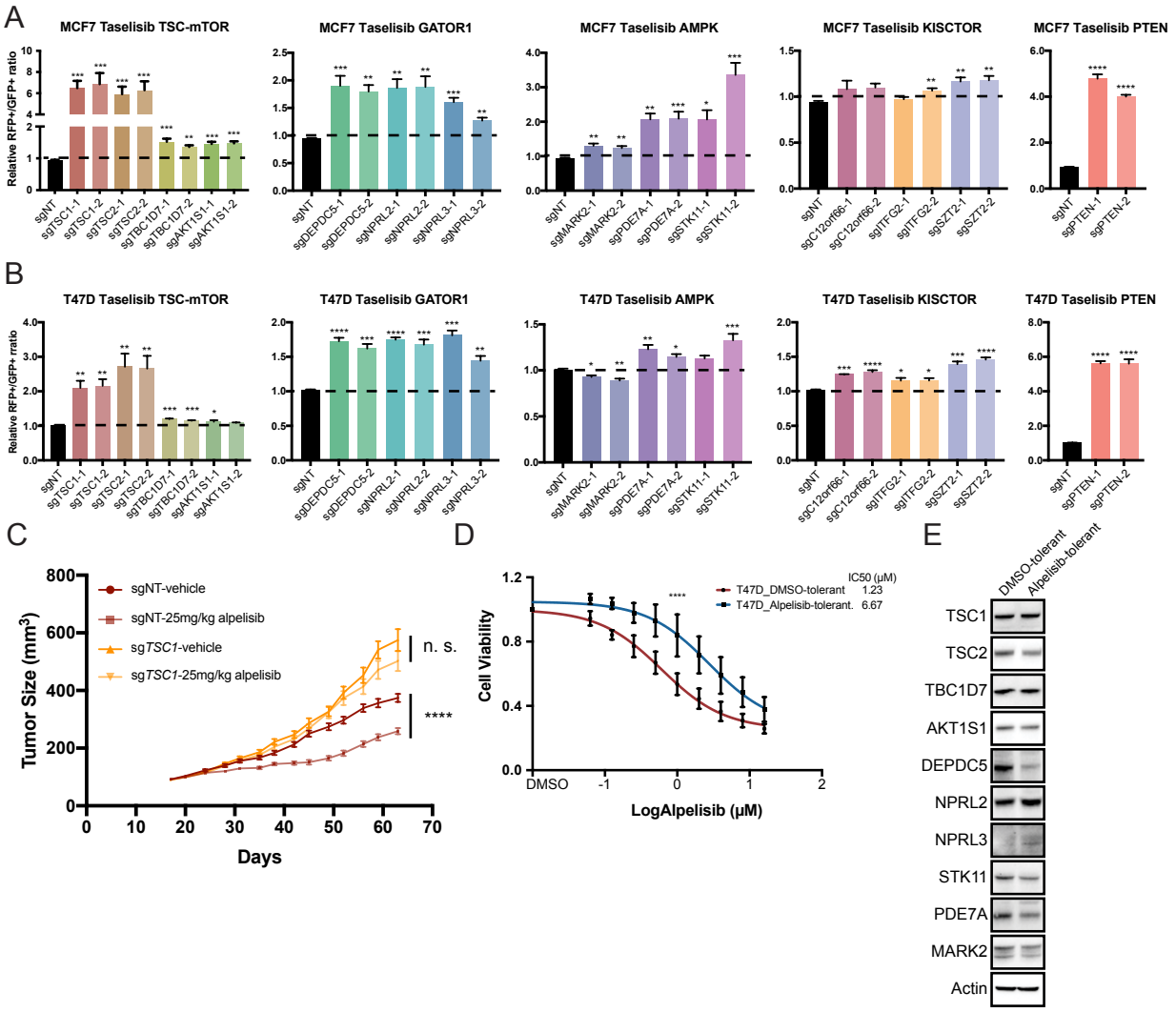

Figure S2. Loss of the candidates individually reduced the drug sensitivity of ER+ breast cancer cell lines.

Drug responses are shown as the fold changes of RFP-labeled MCF7 (A) and T47D (B) after 6 days of 60nM taselisib treatment compared to DMSO treatment. Mean and SEM are shown; n=4 or 3 experiments. P-values were calculated using one-way ANOVA with Dunnett's post-hoc tests. *, $p<0.05$; **, $p<0.005$; ***, $p<0.0005$; ****, $p<0.0001$.

C. T47D cells transduced with sgNT or sgTSC1 *in vivo* xenografts treated with vehicle or alpelisib (25mg/kg per day). P-values were calculated using two-way ANOVA with Tukey's post-hoc tests and statistical significance for the selected dose was shown the end time point.

D. Dose responses of DMSO-tolerant or alpelisib-tolerant T47D cells to alpelisib measured by CellTiter-Glo. Mean and SEM are shown; n=3 experiments. IC50 were shown. P-values were calculated using two-way ANOVA with Tukey's post-hoc tests and statistical significance for the selected doses were shown.

E. Expression of the indicated proteins in DMSO-tolerant or alpelisib-tolerant T47D cells measured by immunoblotting.

Fig. S3

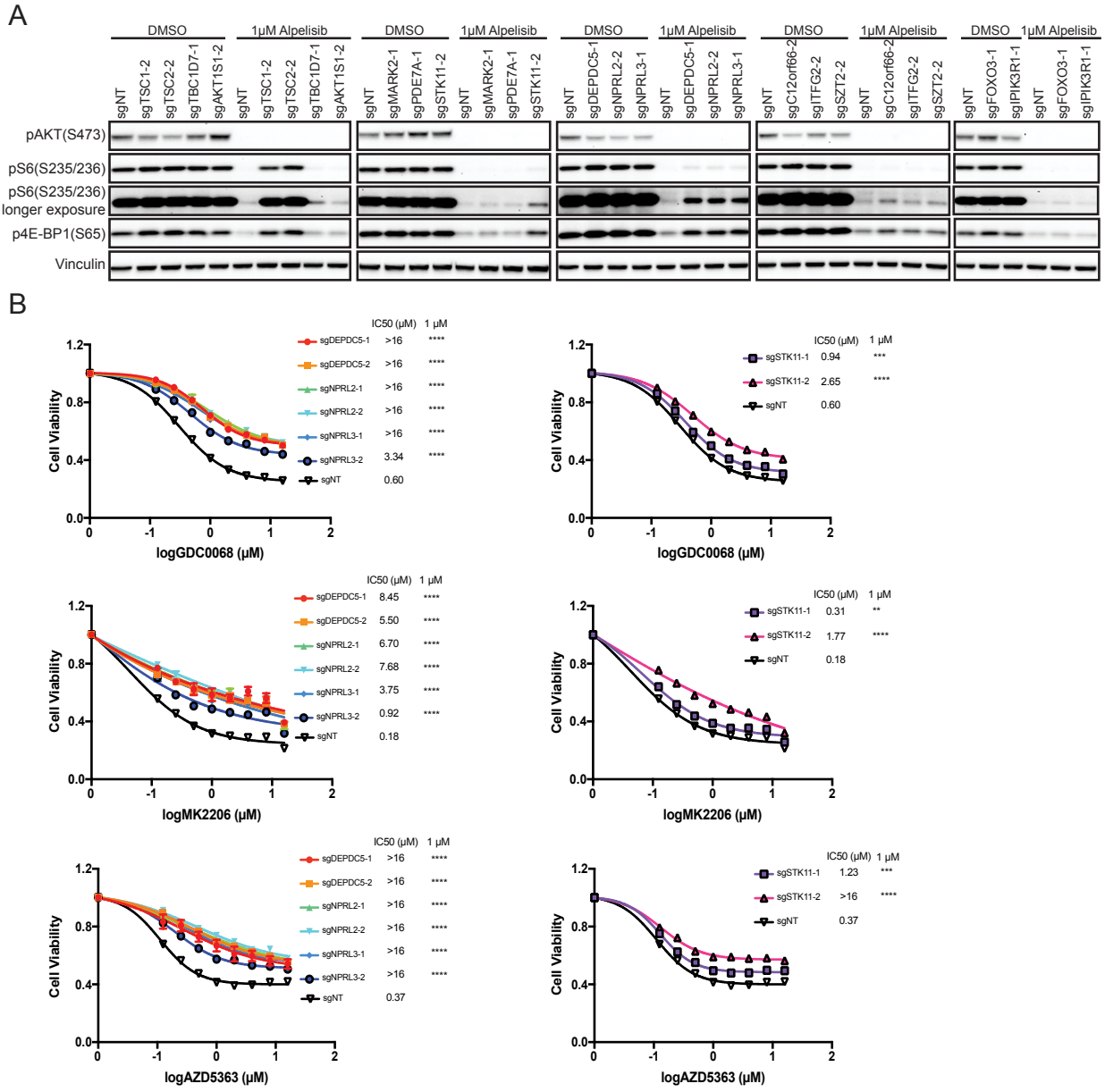

Figure S3. Loss of each candidate maintains mTOR activity of T47D cells in response to PI3Kα inhibition.

A. T47D cells stably expressing sgNT or sgRNA targeting individual candidate genes were treated with DMSO or 1μM alpelisib for 4hrs. Cell lysates were analyzed by immunoblotting for the indicated proteins.

B. T47D cells stably expressing sgNT or sgRNAs targeting *DEPDC5*, *NPRL2*, *NPRL3* or *STK11* were treated with various doses of AKT inhibitors (GDC0068, MK2206 and AZD5363) for 4 days. Cell proliferation was determined by CellTiter-Glo assay and IC50 were shown. Mean and SEM are shown; n=3 experiments. P-values were calculated using two-way ANOVA with Tukey's post-hoc tests and statistical significance for the selected doses were shown next to IC50 values.

Fig. 4S

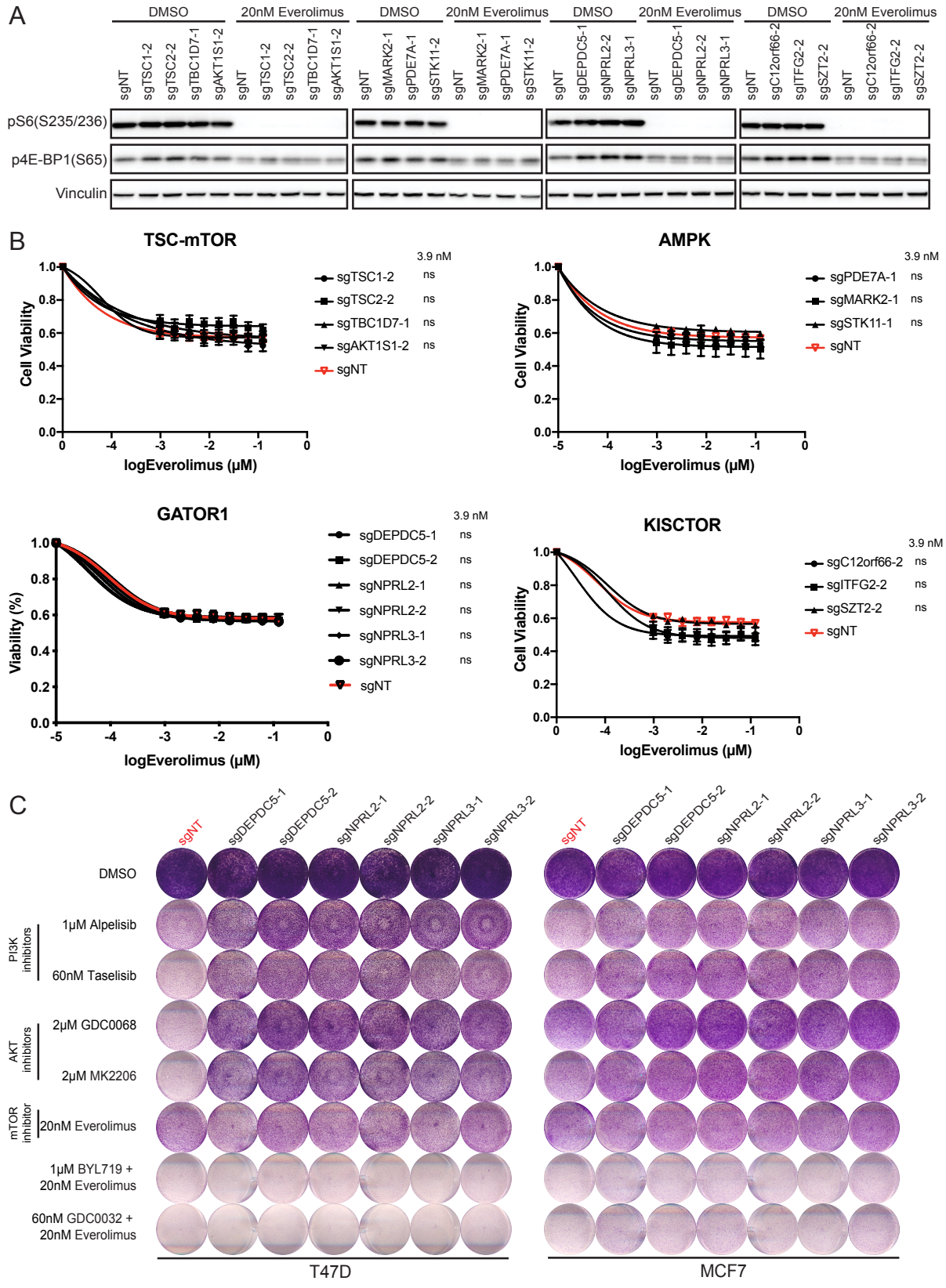
A
DMSO
20nM Everolimus
sgNT
sgTSC1-2
sgTSC2-2
sgTBC1D7-1
sgAKT1S1-2
sgMARK2-1
sgPDE7A-1
sgSTK11-2
sgDEPDC5-1
sgNPRL2-2
sgNPRL3-1
sgC12orf66-2
sgITFG2-2
sgSZT2-2
pS6(S235/236)
p4E-BP1(S65)
Vinculin
B
TSC-mTOR
AMPK
GATOR1
KISCTOR
Cell Viability
Viability (%)
logEverolimus (μM)
3.9 nM
ns
C
DMSO
1μM Alpelisib
60nM Taselisib
2μM GDC0068
2μM MK2206
20nM Everolimus
1μM BYL719 + 20nM Everolimus
60nM GDC0032 + 20nM Everolimus
PI3K inhibitors
AKT inhibitors
mTOR inhibitor
T47D
MCF7

Figure S4. mTOR inhibitor everolimus efficiently inhibits mTORC1 downstream signaling.

A. T47D cells stably expressing sgNT or sgRNA targeting individual candidate genes were treated with DMSO or 20nM everolimus for 4hrs. Cell lysates were analyzed by immunoblotting for the indicated proteins.

B. T47D cells stably expressing sgNT or sgRNAs targeting the individual candidate genes were treated with various doses of everolimus for 4 days. Cell viability was determined by CellTiter-Glo assay. Mean and SEM are shown; n=3 experiments. P-values were calculated using two-way ANOVA with Tukey's post-hoc tests and statistical significance for the selected doses were shown. ns, not significant.

C. MCF7 and T47D cells with knockout of GATOR1 component, DEPDC5, NPRL2 or NPRL3 were treated with DMSO, PI3Kα inhibitors (1μM alpelisib, 60nM taselisib), AKT inhibitors (2μM GDC0068, 2μM MK2206), mTOR inhibitor (20nM everolimus) or 20nM everolimus combined with 1μM alpelisib or 60nM taselisib for 6 days followed by staining with crystal violet. Representative results were shown, and three independent experiments were repeated.

Fig. S5

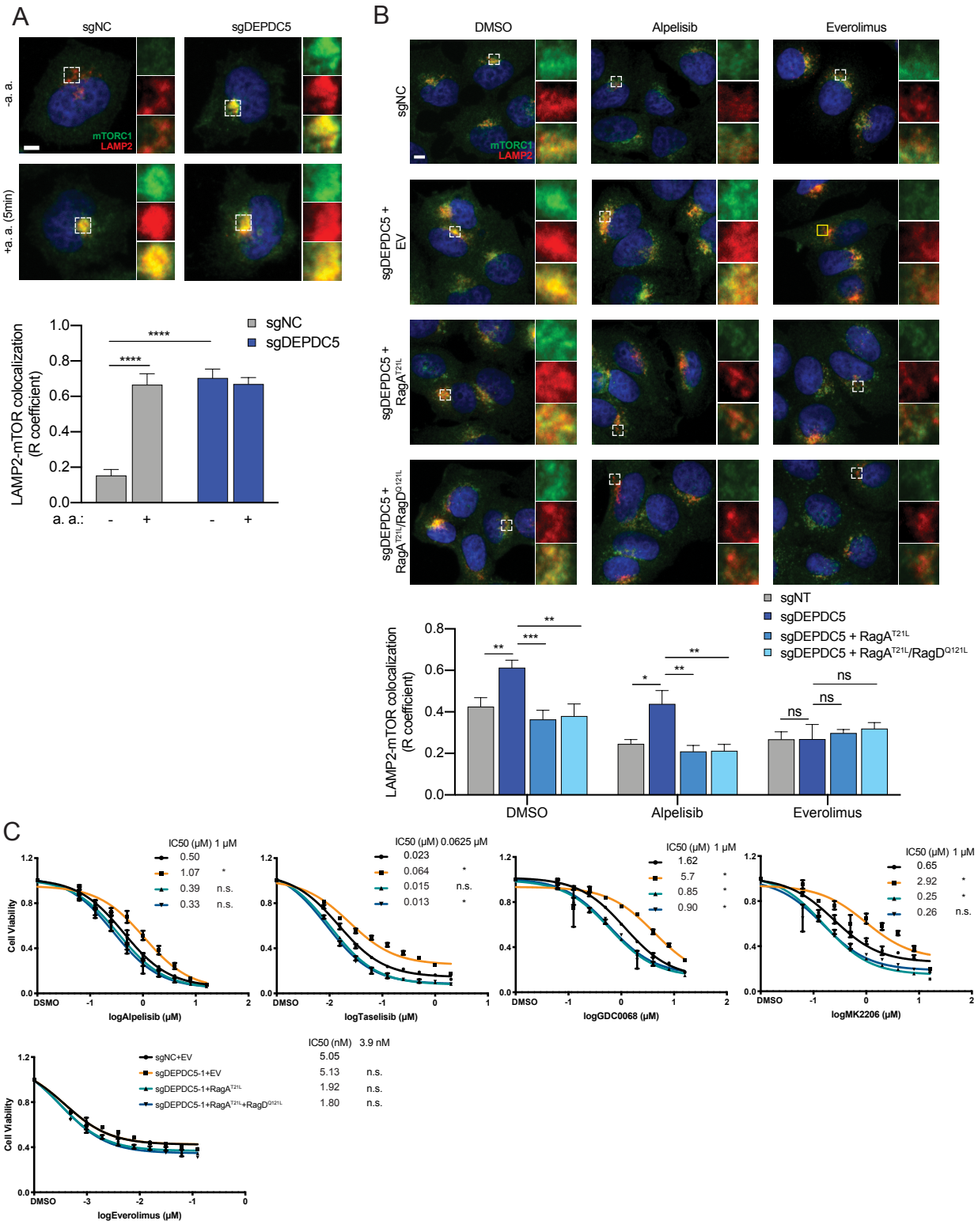
A
sgNC
sgDEPDC5
-a. a.
+a. a. (5min)
mTORC1
LAMP2
LAMP2-mTOR colocalization (R coefficient)
sgNC
sgDEPDC5
a. a.:
B
DMSO
Alpelisib
Everolimus
sgNC
sgDEPDC5 + EV
sgDEPDC5 + RagA^T21L
sgDEPDC5 + RagA^T21L/RagD^Q121L
sgNT
sgDEPDC5
sgDEPDC5 + RagA^T21L
sgDEPDC5 + RagA^T21L/RagD^Q121L
C
IC50 (μM) 1 μM
0.50
1.07 *
0.39 n.s.
0.33 n.s.
Cell Viability
logAlpelisib (μM)
IC50 (μM) 0.0625 μM
0.023
0.064 *
0.015 n.s.
0.013 *
logTaselisib (μM)
IC50 (μM) 1 μM
1.62
5.7 *
0.85 *
0.90 *
logGDC0068 (μM)
IC50 (μM) 1 μM
0.65
2.92 *
0.25 *
0.26 n.s.
logMK2206 (μM)
IC50 (nM) 3.9 nM
sgNC+EV 5.05
sgDEPDC5-1+EV 5.13 n.s.
sgDEPDC5-1+RagA^T21L 1.92 n.s.
sgDEPDC5-1+RagA^T21L+RagD^Q121L 1.80 n.s.
logEverolimus (μM)

Figure S5. Dominant negative RagA or RagA/D heterodimer reverses *DEPDC5* loss-induced mTORC1 lysosomal localization and drug resistance.

A. WT and *DEPDC5* KO MCF7 cells were starved for 6 hrs and stimulated with or without amino acids for 5 min. The localization of mTORC1 and LAMP2 was determined by immunostaining and confocal imaging. Representative images are shown (upper panel). Inset is a magnified view of the boxed region. a.a., amino acid. Scale bar, 5μm. Co-localization of mTORC1 and LAMP2 was quantified using ImageJ Coloc2 plugin and showed as mean R coefficient + SEM (lower panel). Approximately 50 cells were analyzed for each condition. P-values were calculated using two-way ANOVA with Tukey's post-hoc tests.

B. WT and *DEPDC5* KO MCF7 cells stably expressing the indicated constructs were treated with DMSO, 1μM alpelisib or 25nM everolimus for 4hrs in full media. The localization of mTORC1 and LAMP2 was determined by immunostaining and confocal imaging. Representative images are shown (upper panel). Inset is a magnified view of the boxed region. a.a., amino acid. Scale bar, 5μm. Co-localization of mTORC1 and LAMP2 was quantified using ImageJ Coloc2 plugin and showed as mean R coefficient + SEM (lower panel). Approximately 100 cells were analyzed for each condition. P-values were calculated using two-way ANOVA with Tukey's post-hoc tests.

C. WT and *DEPDC5* KO MCF7 cells stably expressing the indicated constructs were treated with DMSO or different doses of the indicated inhibitors for 4 days. Cell viabilities were determined by CellTiter-Glo assay and IC50 (μM) were shown below. Mean and SEM are shown; n=3 experiments. P-values were calculated using two-way ANOVA with Tukey's post-hoc tests and statistical significance for the selected doses were shown next to IC50 values.

Fig. S6

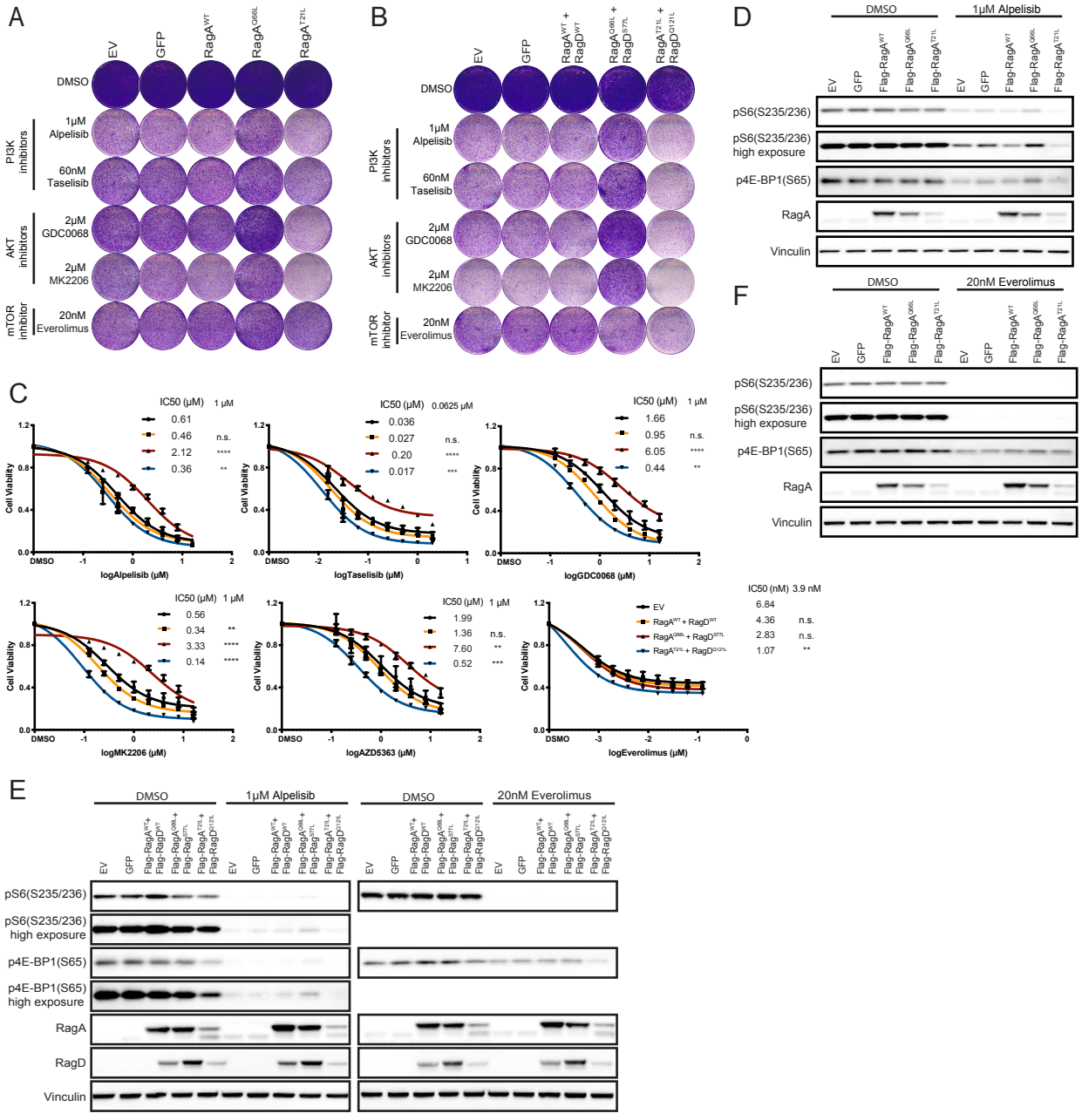

Figure S6. Over-expression of constitutively active RagA or RagA/D heterodimer mimics DEPDC5 loss-induced drug resistance and mTORC1 activation.

A and B. MCF7 cells expressing different forms of RagA (A) or RagA/D heterodimers (B) were treated with DMSO, 1μM alpelisib, 60nM taselisib, 2μM GDC0068, 2μM MK2206 or 20nM

everolimus for 6 days and stained with crystal violet. One representative result out of two biologically independent experiments is shown.

C. MCF7 cells expressing different forms of RagA (C) or RagA/D heterodimers (D) were treated with DMSO, or different doses of alpelisib, taselisib, GDC0068, MK2206, AZD5363 or everolimus for 4 days. Cell viability was determined by CellTiter-Glo assay and IC50 (μM) were shown. Mean and SEM are shown; n=3 experiments. P-values were calculated using two-way ANOVA with Tukey's post-hoc tests and statistical significance for the selected doses were shown next to IC50 values.

D and E. MCF7 cells expressing different forms of RagA were treated with DMSO, 1μM alpelisib (D) or 20nM everolimus (E) for 4 hrs. Cell lysates were prepared and immunoblotted for the indicated proteins.

F. MCF7 cells expressing different forms of RagA/D heterodimers were treated with DMSO, 1μM alpelisib or 20nM everolimus for 4 hrs. Cell lysates were prepared and immunoblotted for the indicated proteins.