Supplementary data:

A genome-scale CRISPR screen identifies the ERRB and MTOR signalling networks as key determinants of response to PI3K inhibition in pancreatic cancer

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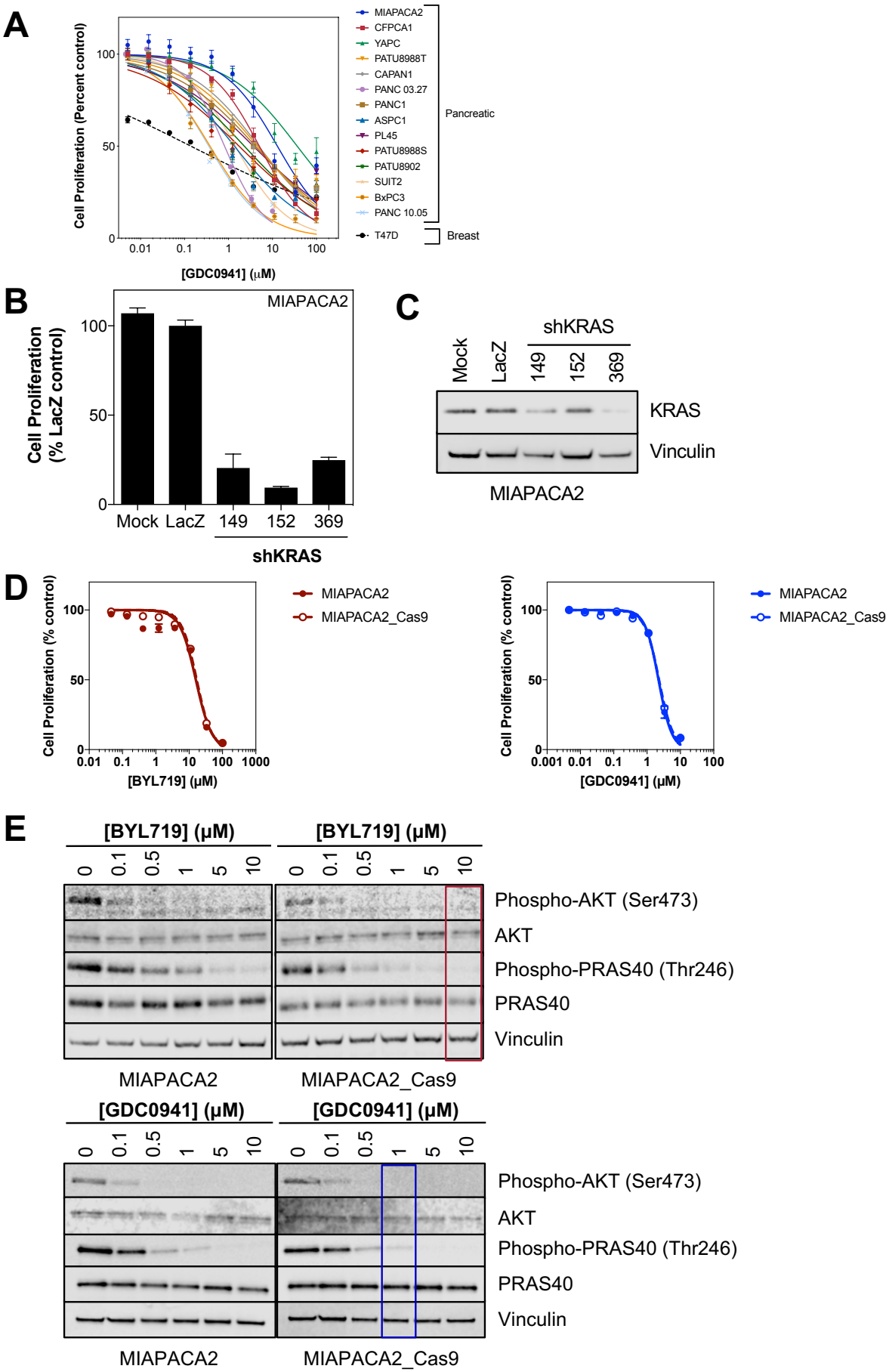
A
Cell Proliferation (Percent control)
100
50
0
0.01
0.1
1
10
100
[GDC0941] (μM)
MIAPACA2
CFPCA1
YAPC
PATU8988T
CAPAN1
PANC 03.27
PANC1
ASPC1
PL45
PATU8988S
PATU8902
SUIT2
BxPC3
PANC 10.05
Pancreatic
T47D
Breast
B
MIAPACA2
Cell Proliferation (% LacZ control)
100
50
0
Mock
LacZ
149
152
369
shKRAS
C
shKRAS
Mock
LacZ
149
152
369
KRAS
Vinculin
MIAPACA2
D
Cell Proliferation (% control)
100
50
0
0.01
0.1
1
10
100
1000
[BYL719] (μM)
MIAPACA2
MIAPACA2_Cas9
Cell Proliferation (% control)
100
50
0
0.001
0.01
0.1
1
10
100
[GDC0941] (μM)
MIAPACA2
MIAPACA2_Cas9
E
[BYL719] (μM)
0
0.1
0.5
1
5
10
[BYL719] (μM)
0
0.1
0.5
1
5
10
Phospho-AKT (Ser473)
AKT
Phospho-PRAS40 (Thr246)
PRAS40
Vinculin
MIAPACA2
MIAPACA2_Cas9
[GDC0941] (μM)
0
0.1
0.5
1
5
10
[GDC0941] (μM)
0
0.1
0.5
1
5
10
Phospho-AKT (Ser473)
AKT
Phospho-PRAS40 (Thr246)
PRAS40
Vinculin
MIAPACA2
MIAPACA2_Cas9

Figure S1. KRAS-dependency and effect of Cas9 expression on response to PI3K inhibition.

A. Proliferation of 14 human pancreatic cell lines and the T47D breast cancer cell line was measured after incubation with increasing concentrations of GDC0941 for 72 h. Proliferation was quantified using CellTiter-Blue and compound GI_{50} values were calculated using GraphPad Prism. Mean cell proliferation, relative to DMSO control, is plotted ± standard error (n=3).

B. MIAPACA2 cells were transduced with shRNAs directed against KRAS (149, 152 and 369) or a negative control (LacZ). Successfully transduced cells were selected for by incubation with 2 µg/ml puromycin for 3 d. Cell proliferation was measured by CellTiter-Blue, 7 d after transduction. Mean cell proliferation, relative to the LacZ control is plotted ± SE (n=3).

C. Cells were harvested 3 d after transduction as in B and lysates analyzed for expression of KRAS and vinculin by Western blotting.

D. Proliferation of MIAPACA2 and MIAPACA2_Cas9 cells was measured after incubation with increasing concentrations of BYL719 or GDC0941 for 72 h. Proliferation was quantified using CellTiter-Blue and analyzed using GraphPad Prism. Mean cell proliferation is plotted relative to DMSO control ± SE (n=3). Data shown is representative of three independent experiments.

E. MIAPACA2 and MIAPACA2_Cas9 cells were treated with the increasing concentrations of BYL719 or GDC0941 for 2 h and cell lysates analysed for the indicated proteins by Western blotting.

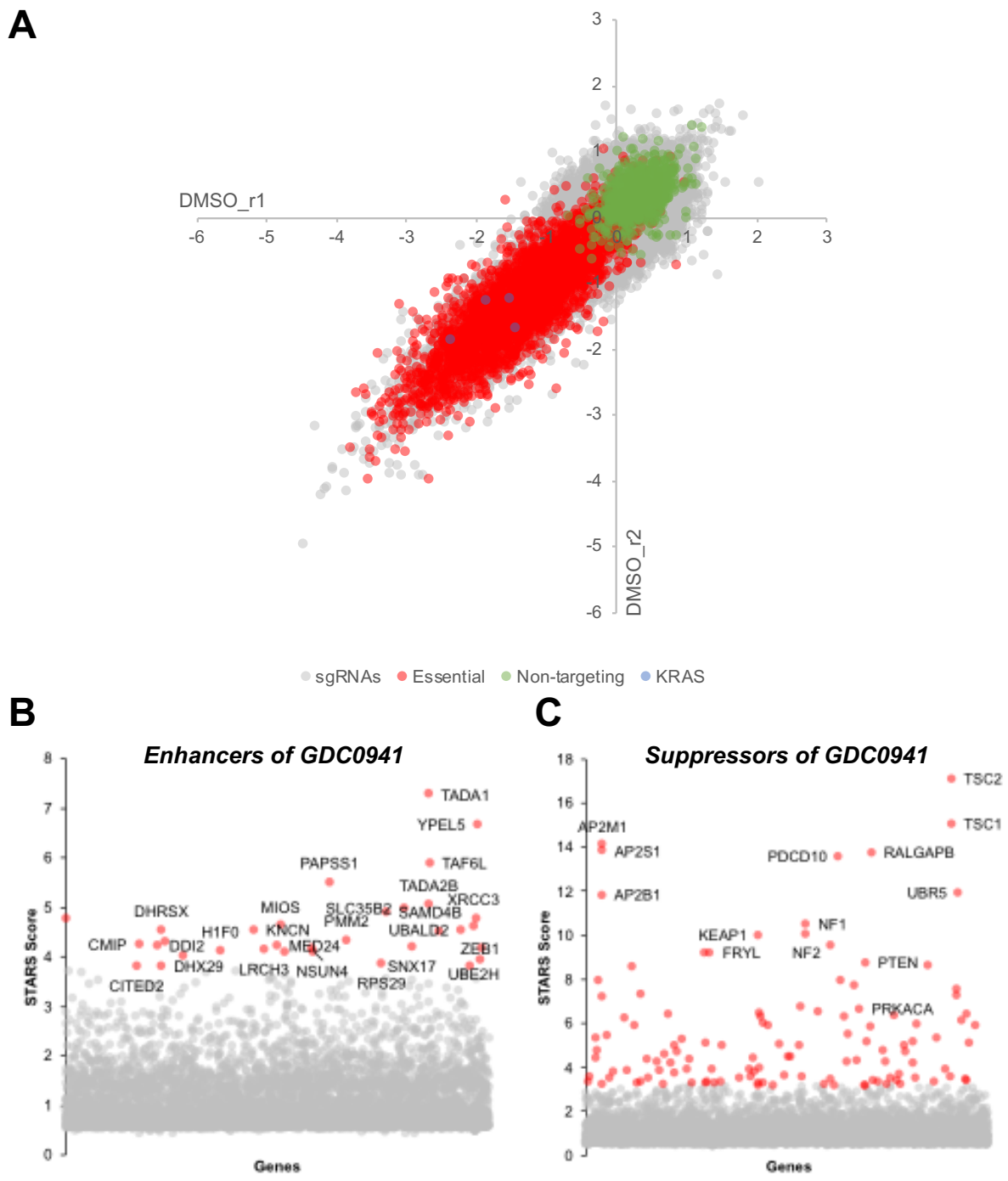

Figure S2. Whole-genome CRISPR screen shows good replicate correlation and identifies enhancers and suppressors of GDC0941 antiproliferative activity.

A. LFC in abundance of each of the 74,687 sgRNAs in two DMSO-treated replicates after 8 population doublings, normalised to their abundance in the pDNA reference. 1000 non-targeting sgRNAs are highlighted in green, 3536 sgRNAs targeting essential genes are in red and 4 sgRNAs targeting KRAS are in blue.

B. STARS analysis identifies enhancers of GDC0941 antiproliferative activity, hits with an FDR<0.3 are indicated in red.

C. STARS analysis identifies suppressors of GDC0941 antiproliferative activity, hits with an FDR<0.3 are indicated in red.

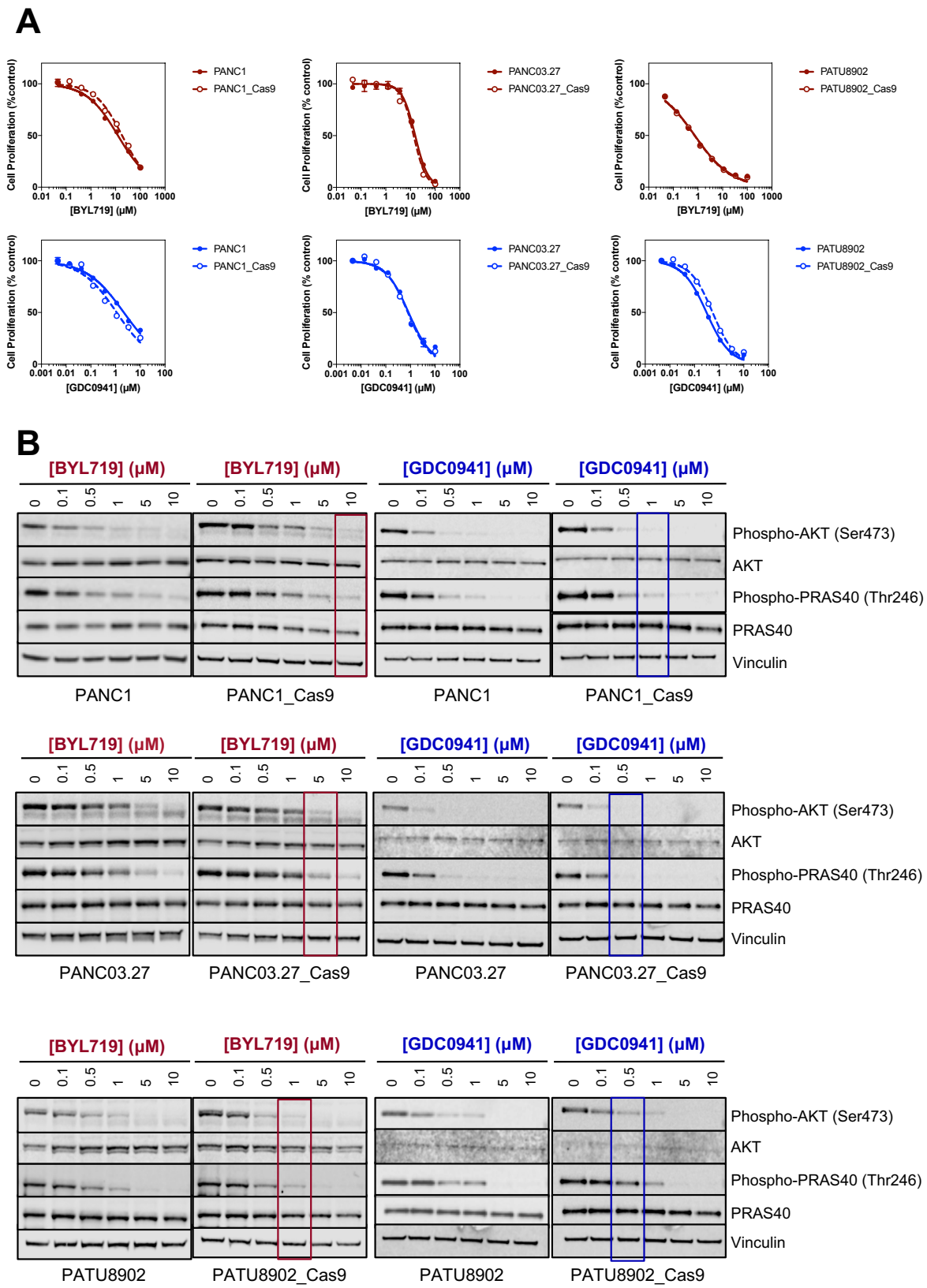

Figure S3. Expression of Cas9 does not alter response to BYL719 or GDC0941.

A. Proliferation of parental cell lines and their Cas9 expressing counterparts was measured after incubation with increasing concentrations of BYL719 or GDC0941 for 72 h. Proliferation was quantified using CellTiter-Blue and compound GI_{50} values were calculated using GraphPad Prism. Mean cell proliferation is plotted relative to DMSO control ± SE (n=3). Data shown is representative of three independent experiments.

B. Phosphorylation of AKT and PRAS40 was assessed by Western blotting after incubation with increasing concentrations of BYL719 or GDC0941 for 2 h, in the indicated cell lines (n=2). Coloured boxes indicate the concentrations of each compound selected for the minipool validation screen.

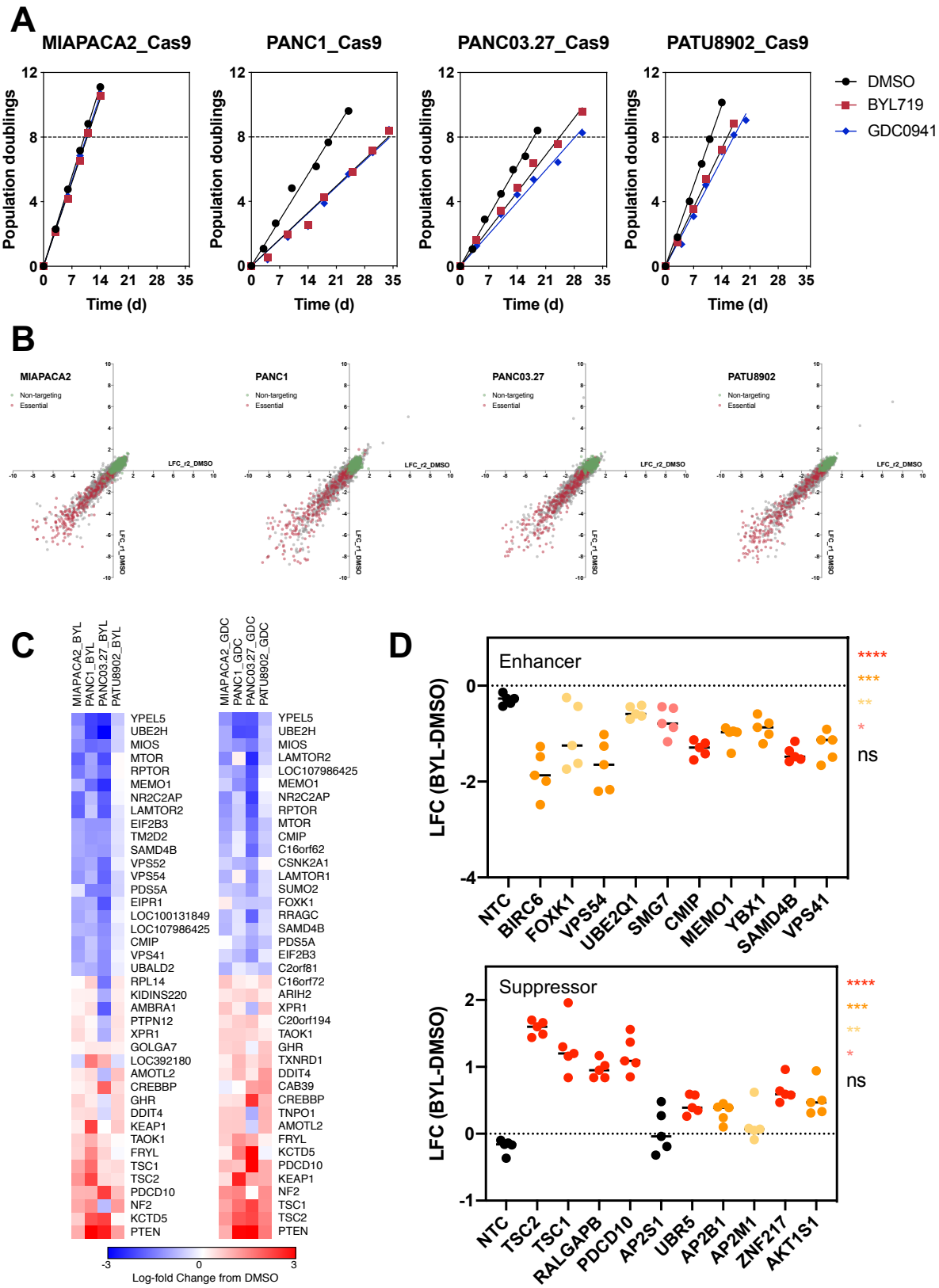

Figure S4. Population doublings, replicate correlation and alternative analysis of the minipool screen.

A. Cas9-expressing MIAPACA2, PANC1, PANC03.27 and PATU8902 cells were infected with the secondary minipool of 3067 sgRNAs and cultured in the presence of DMSO, BYL719 or GDC0941 for at least 8 population doublings. gDNA was extracted and sgRNAs PCR-amplified and barcoded for sequencing.

B. Correlation of sgRNA LFC relative to the pDNA reference sample, for DMSO-treated replicates for each cell line. Non-targeting control sgRNAs and sgRNAs targeting essential genes are indicated.

C. Orthogonal analysis of the minipool screen. Ranking of enhancer and suppressor genes based on the average LFC of sgRNAs, averaged across the four cell lines. The top 20 genes that either enhance or suppress the activity of BYL719 or GDC0941 are shown.

D. Confirmation of hits from the MIAPACA2 primary screen in the MIAPACA2 minipool screen. The average LFC of sgRNAs for the top 10 enhancer or suppressor genes was compared to a set of 5 randomly selected, non-targeting control sgRNAs. Significant depletion (enhancers) or enrichment (suppressors) was determined by T-test, the level of significance is indicated by the colour coding (**** $p<0.0001$, *** $p<0.001$, ** $p<0.01$, * $p<0.05$). The top 5 sgRNAs per gene were included to reduce the effect of inactive sgRNAs.

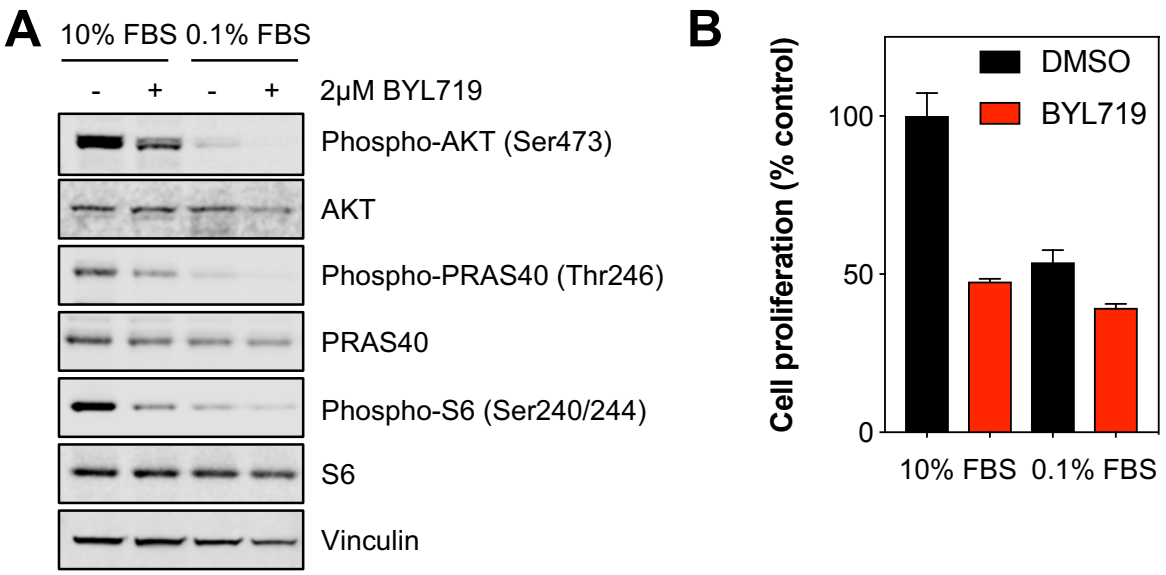

Figure S5. PI3K signalling is reduced following serum starvation.

A. PATU8988S cells were cultured in either 10% FBS or 0.1% FBS in the presence of either DMSO or 2 μM BYL719 for 72 h. Cell lysates were analysed by Western blotting for the indicated proteins.

B. PATU8988S cells were cultured as in A. Cell proliferation was assessed by CellTiter-Blue assay. Mean cell proliferation, relative to the 10% FBS, DMSO treated control is plotted ± SE (n=3).

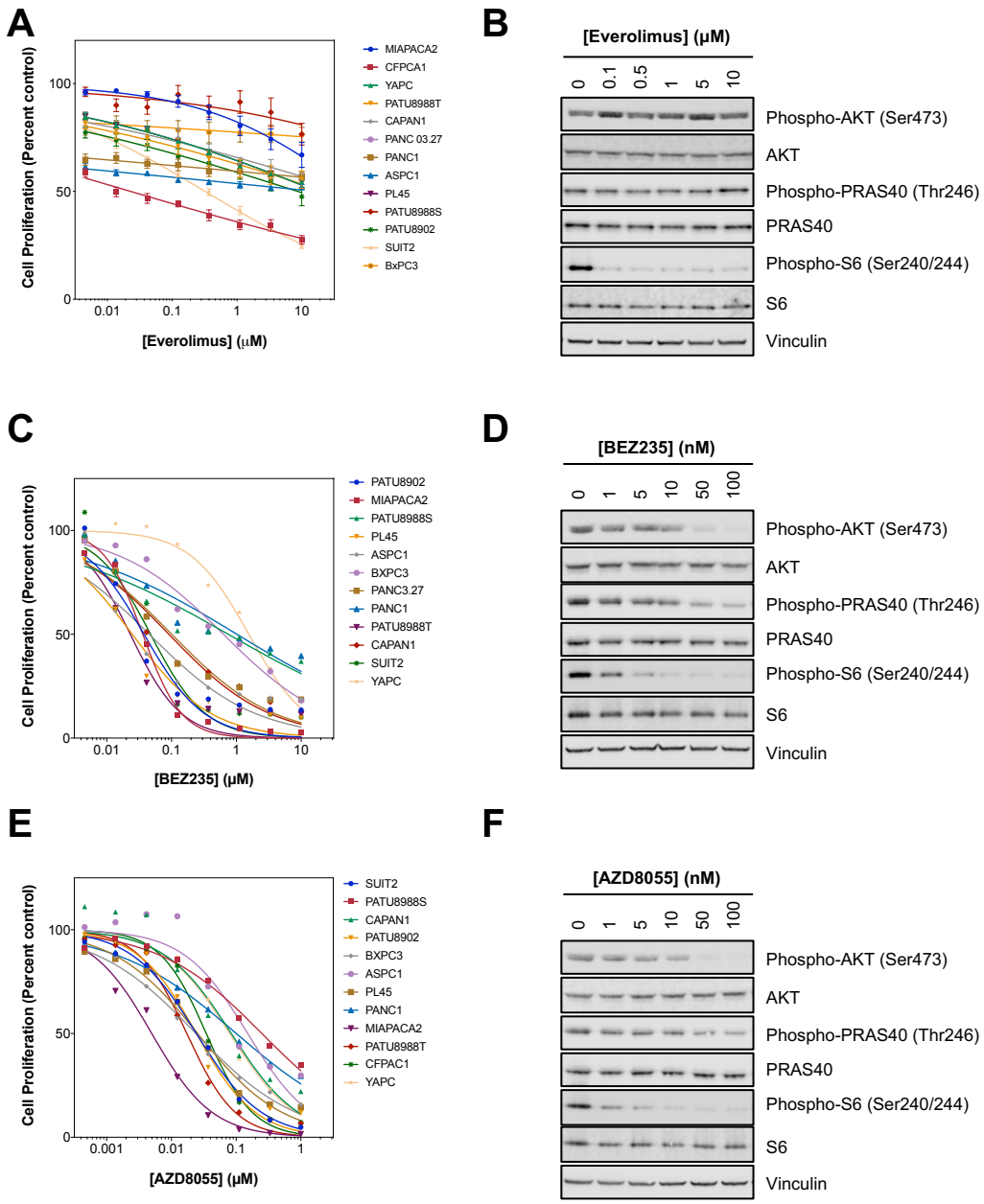

Figure S6. Combined inhibition of PI3K and mTOR results in potent antiproliferative activity in pancreatic cancer cell lines.

A. Compound GI_{50} values were determined for a panel of 12 pancreatic cancer cell lines. Cells were incubated with increasing concentrations of everolimus (RAD001) for 72 h and cell proliferation was measured using CellTiter-Blue. Mean cell proliferation is plotted relative to DMSO treated control ±SE (n=3).

B. MIAPACA2 cells were incubated with the indicated concentrations of everolimus for 2 h. Cells lysates were analysed by Western blotting.

C. Compound GI_{50} values were determined for a panel of 12 pancreatic cancer cell lines. Cells were incubated with increasing concentrations of BEZ235 for 72 h and cell proliferation was

measured using CellTiter-Blue. Mean cell proliferation is plotted relative to DMSO treated control ±SE (n=3).

D. MIAPACA2 cells were incubated with the indicated concentrations of BEZ235 for 2 h. Cells lysates were analysed by Western blotting.

E. Compound GI_{50} values were determined for a panel of 12 pancreatic cancer cell lines. Cells were incubated with increasing concentrations of AZD8055 for 72 h and cell proliferation was measured using CellTiter-Blue. Mean cell proliferation is plotted relative to DMSO treated control ±SE (n=3).

F. MIAPACA2 cells were incubated with the indicated concentrations of AZD8055 for 2 h. Cells lysates were analysed by Western blotting.

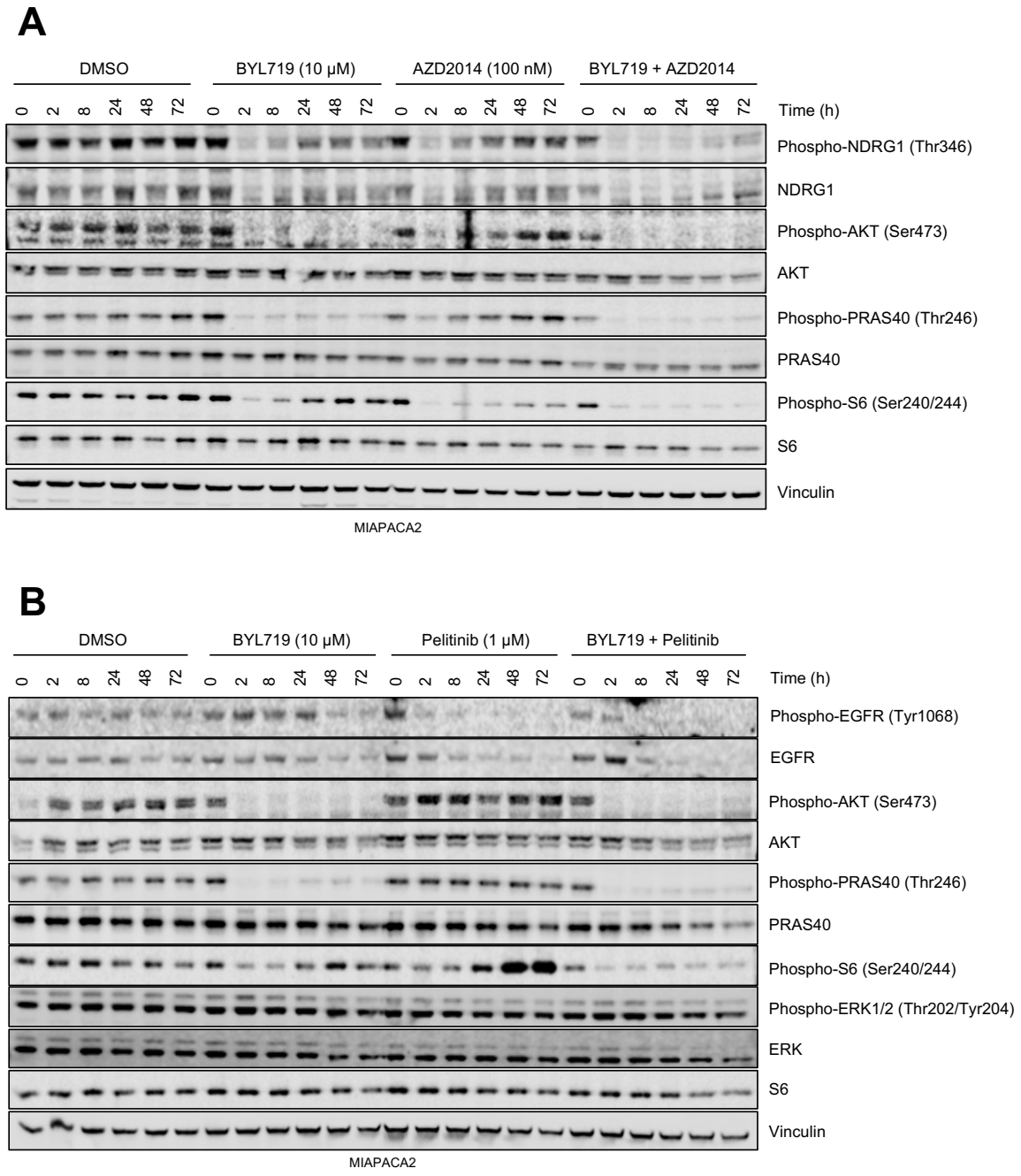

Figure S7. Combined p110α and mTOR or ERBB-family inhibition leads to sustained suppression of S6 phosphorylation.

A. MIAPACA2 cells were treated with BYL719 and AZD2014 as indicated for up to 72 h. Cell lysates were analysed by Western blotting for the indicated proteins.

B. MIAPACA2 cells were treated with BYL719 and pelitinib as indicated for up to 72 h. Cell lysates were analysed by Western blotting for the indicated proteins.

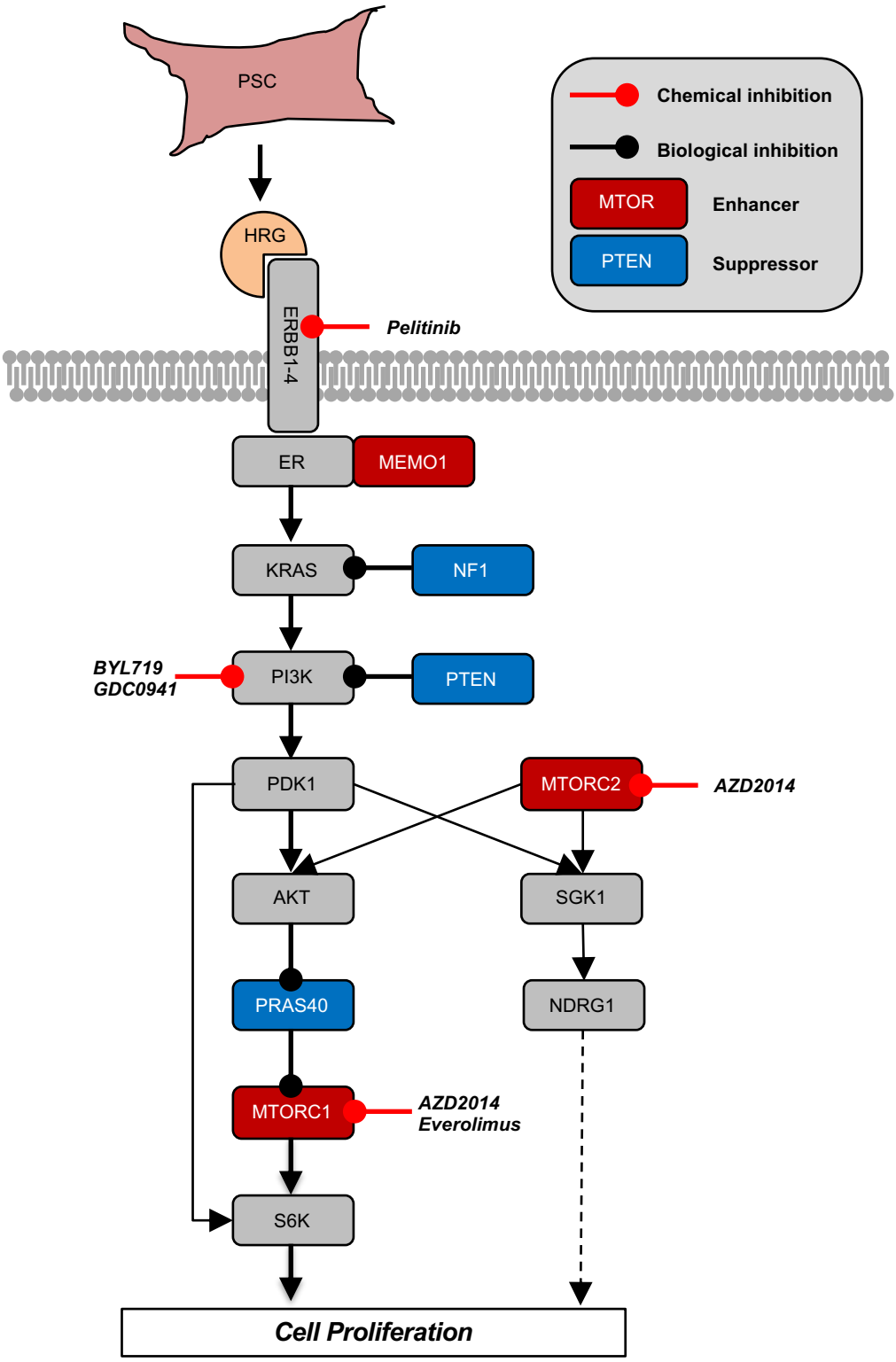

Figure S8. CRISPR and drug combination screens implicate the ERBB-PI3K-MTOR signalling axis as a key vulnerability of pancreatic cancer cells.

A simplified cartoon representation of signalling from the ERBB family through KRAS to the PI3K pathway. Loss-of-function CRISPR screening identified MEMO1 and MTOR complexes 1 and 2 as enhancers of PI3K inhibition. Loss of NF1, PTEN and PRAS40 drives resistance to PI3K inhibition. Small molecule inhibitors of ERBB and MTORC1/2 show synergy with PI3K

inhibition. HRG, possibly secreted from pancreatic stellate cells (PSCs), are sufficient to promote resistance to PI3K inhibition via sustained PI3K-MTOR-S6 signalling.