Supplementary Materials

Supplementary Table S1. qPCR primers.

Supplementary Table S2. Normalized sgRNA read counts.

Supplementary Table S3. The gene level sgRNA depletion evaluated by the MAGeCK algorithm.

Supplementary Table S4. ED_{50} and the maximum inhibition of colony formation in soft agar of small molecule compounds tested in four Wnt-addicted cancer cell lines.

Supplementary Figure S1. RNAi knockdown of glucose metabolism genes can be well tolerated by pancreatic cancer (PDAC) and colorectal cancer (CRC) cell lines *in vitro*. The fitness scores (RSA scores) of indicated genes were retrieved from a published *in vitro* RNAi screen dataset (Ref. 27). RSA score of -3 indicates essential genes with statistical significance. The more negative the RSA score, the more essential the gene is for that cell line. The cross (×) means missing value.

Supplementary Figure S2. Genetic alterations in cancer cell lines used in this study. A, Summary of the common genetic alterations in the Wnt-addicted pancreatic cancer and cholangiocarcinoma lines used in this study. **B,** The *PTPRK*(exon1)-*RSPO3*(exon2) fusion in EGI-1 cell line. The cDNA of EGI-1 was PCR amplified using the *PTPRK*(exon1) and *RSPO3*(exon2) specific primers. The amplicon was purified and subject to Sanger sequencing.

Supplementary Figure S3. ETC-159 had no effect on the sensitivity to PI3K/mTOR inhibitors in Wnt-independent pancreatic cancer cell lines. MIA PaCa-2 or PANC-1 cells were seeded in soft agar, and treated with increasing concentrations of pan-PI3K inhibitor

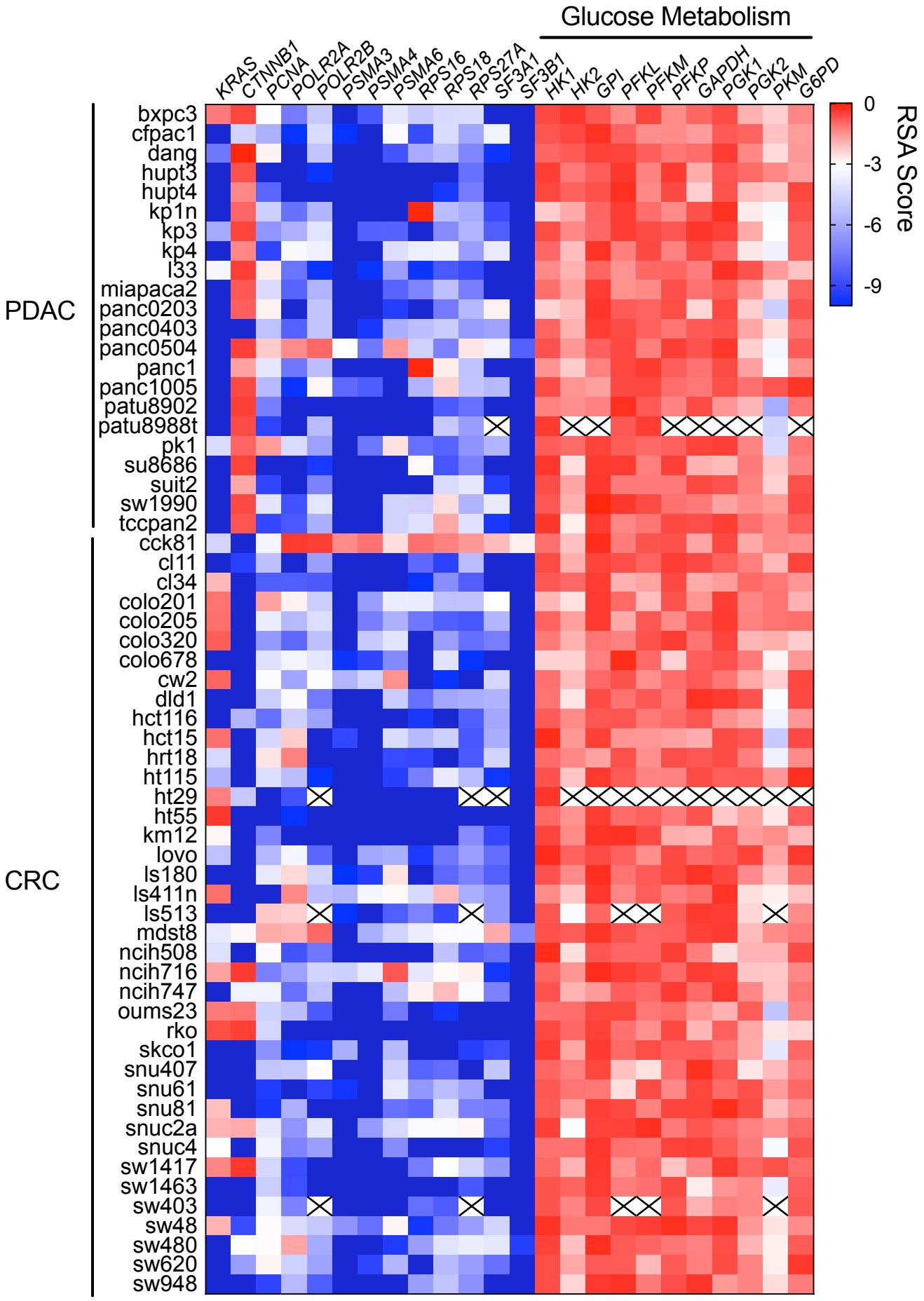
GDC-0941 or mTOR inhibitor Rapamycin in the absence or presence of 250 nM ETC-159 for around two weeks. Colonies were quantified and the relative colonies count is shown here.

Supplementary Figure S4. The ETC-159 and GDC-0941 combinational treatment was tolerated by mice. A, Only a minor decrease of the mice body weights was observed in the combo treatment group. The body weight change of mice from the experiment of Figure 4A is shown here. **B,** Mice in the combo treatment groups showed normal gut architecture. The intestine of mice in Figure 4A was harvested at the end of the experiment (19 days drug treatment). Haematoxylin and Eosin (H&E) staining was performed on the intestinal sections.

Supplementary Figure S5. ETC-159 treatment up-regulated mucin expression in HPAF-II tumors. The Alcian Blue staining for mucins was performed on sections of HPAF-II tumors from Figure 4A harvested at the end of the experiment.

Supplementary Figure S6. The protein abundances of glucose metabolic enzymes were down-regulated by ETC-159 treatment. HPAF-II tumors from Figure 4A harvested at the end of the experiment were analyzed by western blot for GPI, ENO1, and PGD. Each lane represents an independent tumor. The band density of GPI, ENO1, and PGD was quantified and normalized to the loading control β-tubulin.

Supplementary Figure S1



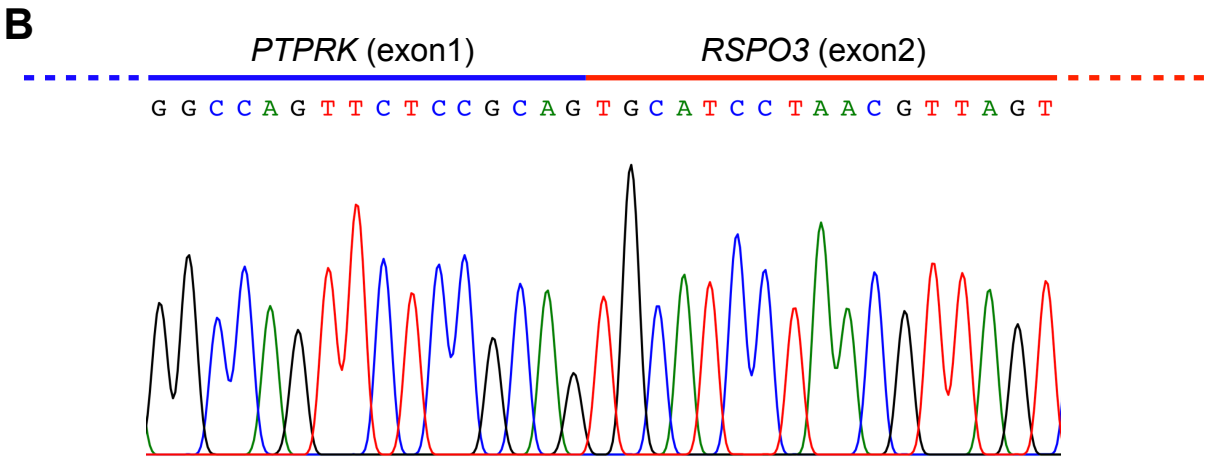
Supplementary Figure S2

A

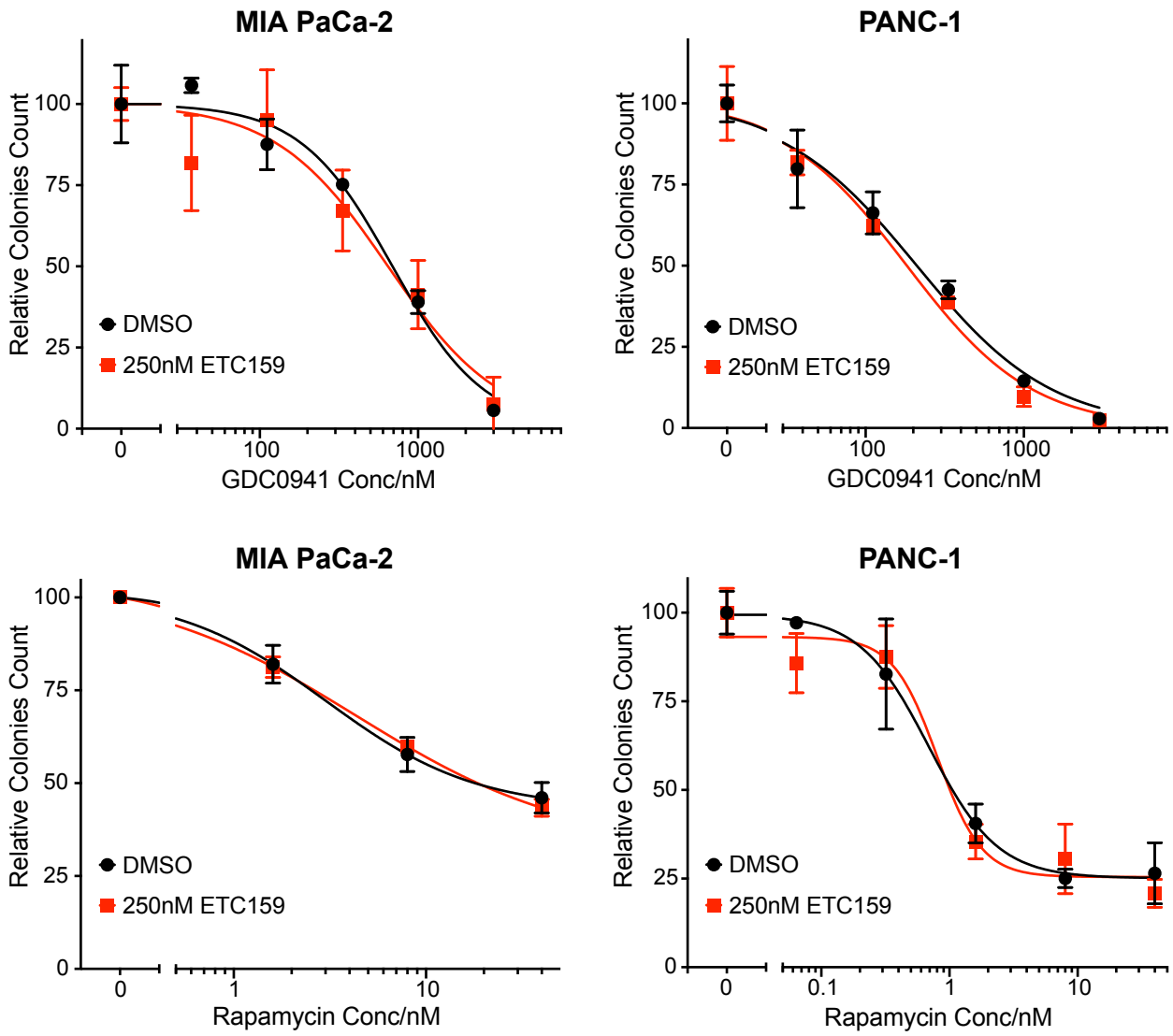
Cell Line	Cancer Type	*RNF43*	*RSPO3*	*KRAS*	*TP53*
HPAF-II	Pancreatic ductal adenocarcinoma	p.E174X [a]		p.G12D [c]	p.P151S [c]
AsPC-1	Pancreatic ductal adenocarcinoma	p.S720X [b]		p.G12D [c]	p.C135fs*35 [c]
PaTu8988S	Pancreatic ductal adenocarcinoma	p.F69C [a]		p.G12V [d]	p.R282W [d]
CFPAC-1	Pancreatic ductal adenocarcinoma	Wild type [a]		p.G12V [c]	p.C242R [c]
EGI-1	Cholangiocarcinoma		PTPRK(e1)–RSPO3(e2) fusion	p.G12D [c]	p.R273H [c]

[a] Ref. 8
[b] Ref. 19
[c] COSMIC
[d] CCLE

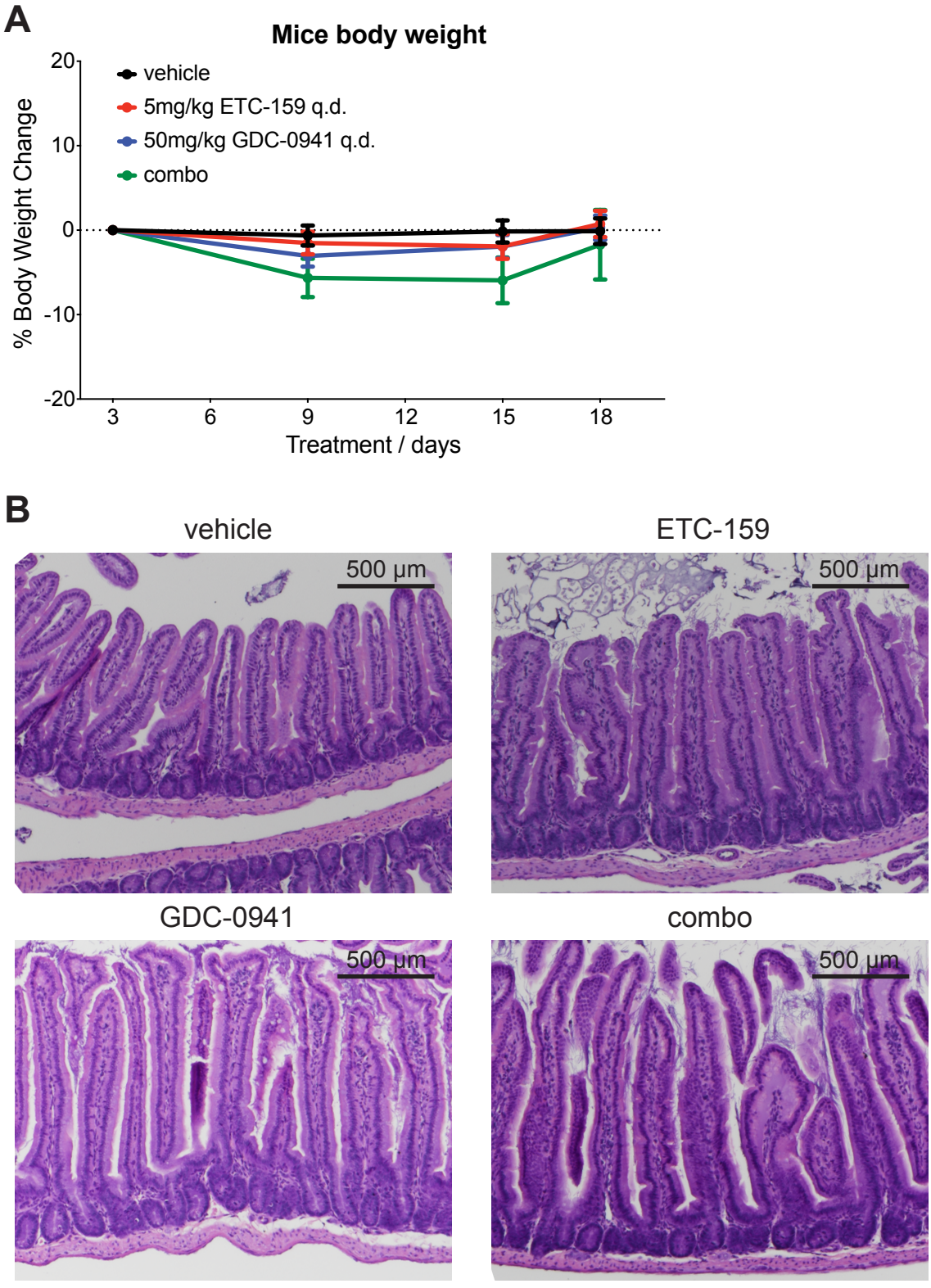
B



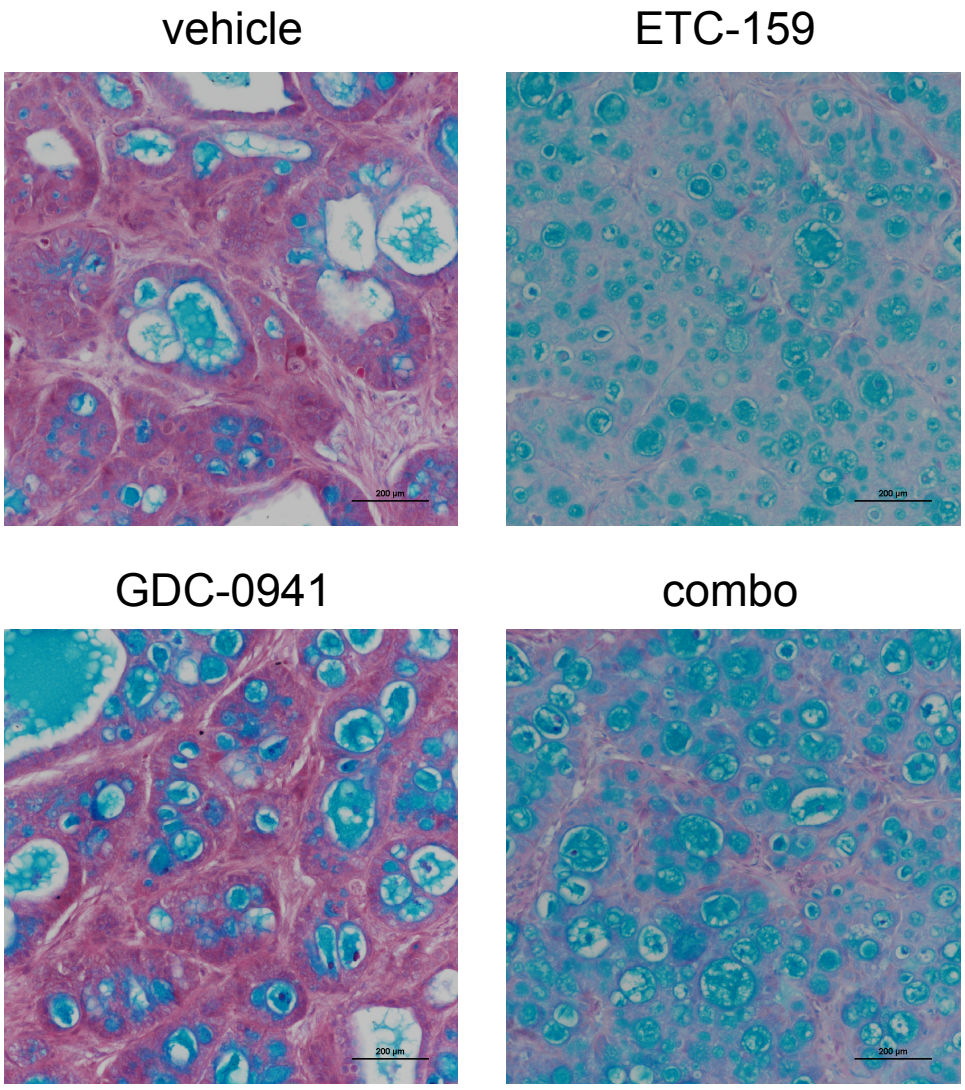
Supplementary Figure S3



Supplementary Figure S4



Supplementary Figure S5



Supplementary Figure S6