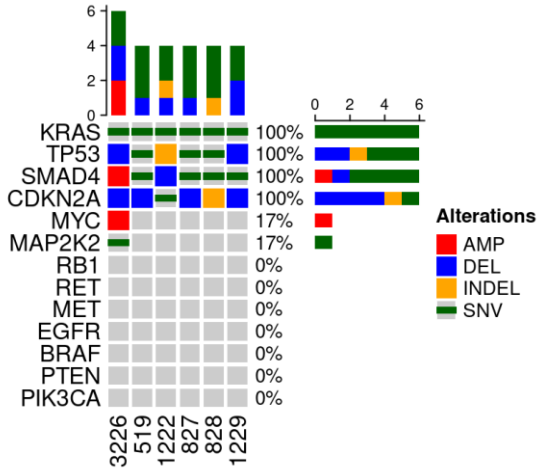
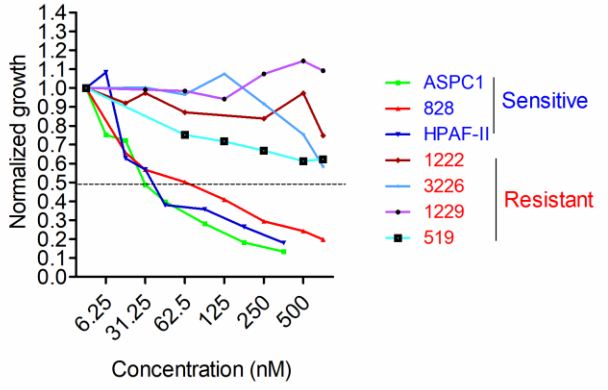
A



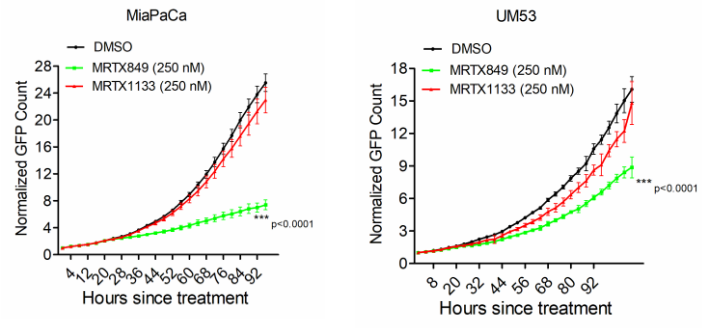
B

Cell line	Gene	VAF%
828	KRAS	81.8
1229	KRAS	100
519	KRAS	52.3
1222	KRAS	72.3
3226	KRAS	62
827	KRAS	50

C



D

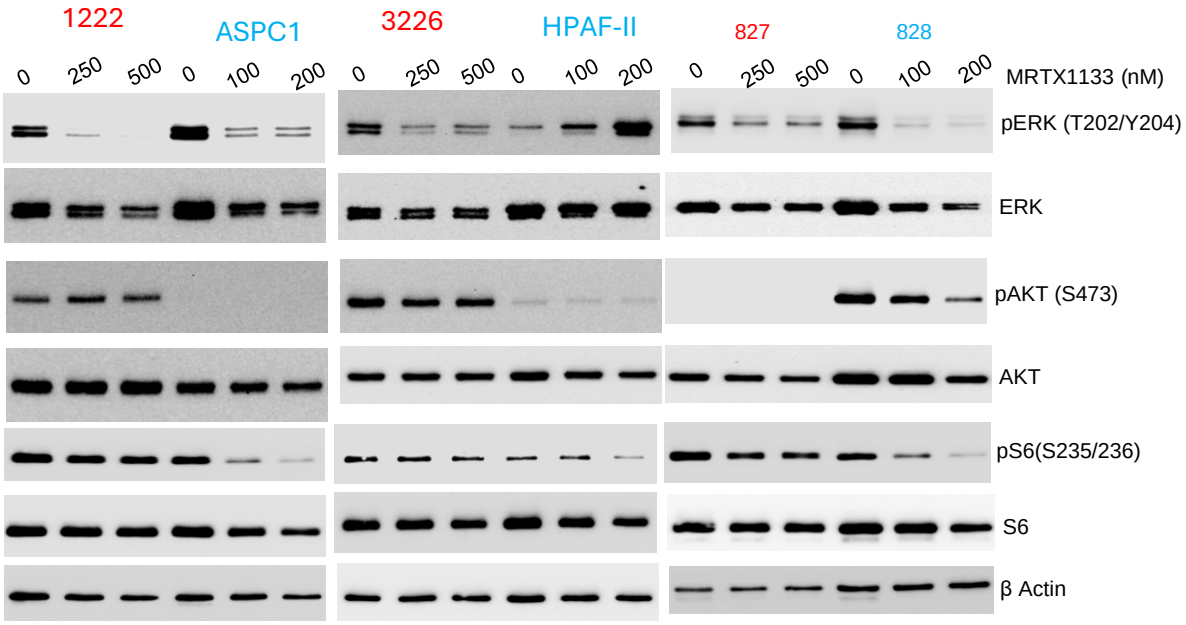


(A) Oncoprint depicting the mutations on the indicated genes from 6 different patient derived PDX cell lines. **(B)** Variant allele frequency in *KRAS* from the indicated cell lines. **(C)** Dose response curve illustrating the normalized growth of different PDAC cells in the presence of increasing concentrations of MRTX1133 following 5 days of treatment. Normalized growth is determined based on the fold change in cell number at an indicated dose after 5 days of treatment normalized to untreated condition. Based on the EC50 values of MRTX1133, ASPC1, HPAF-II and 828 were considered as sensitive lines while 1222, 3226, 1229 and 519 were considered as resistant models. **(D)** Differential effect of MRTX1133 and MRTX849 on MiaPaCa and UM53 cell lines. Mean and SD were shown from triplicates. Experiments were done at two independent times. (***p<0.0001 as determined by two-way ANOVA)

A

48 H

Fig. S2



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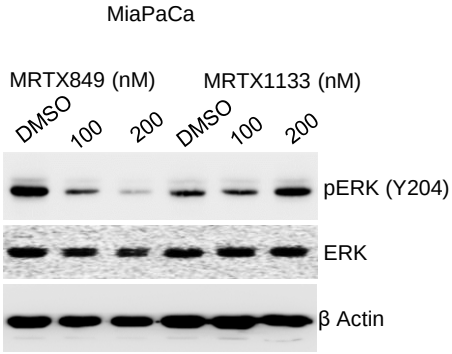
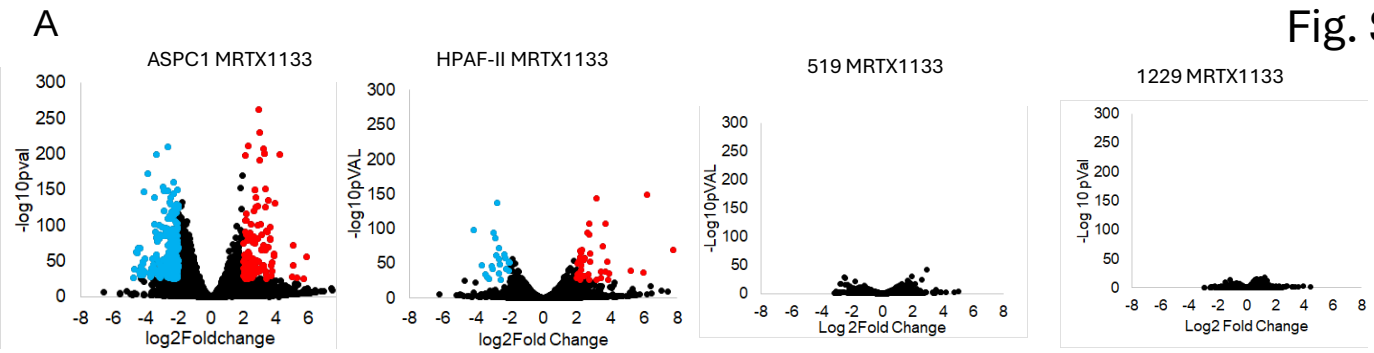
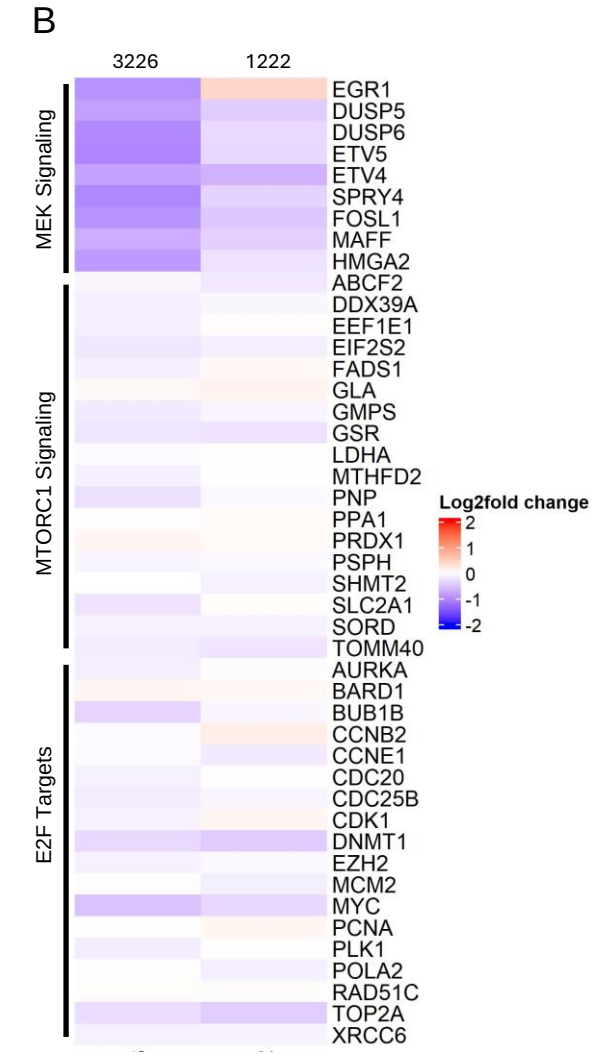
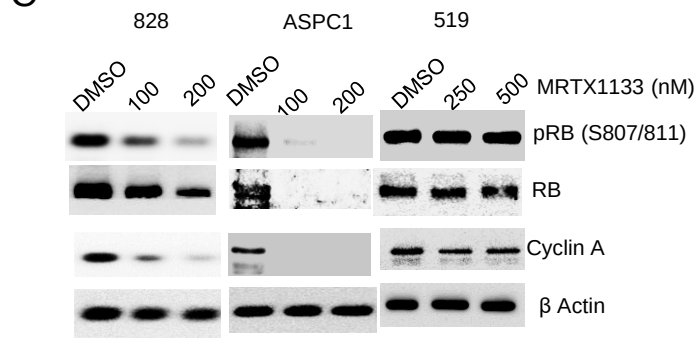


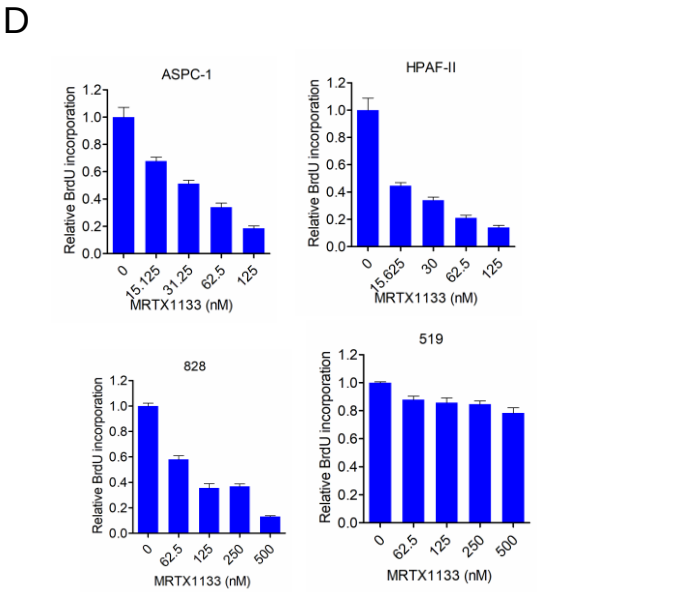
(A) Western blot analysis from 1222, ASPC1, 3226, HPAF-II, 827 and 828 on the indicated proteins following 48 hours treatment with MRTX1133 at the indicated concentrations. **(B)** Western blotting to evaluate the phosphorylation of ERK1/2 in MiaPaCa cell line treated with MRTX849 and MRTX1133 at two different concentrations up to 48 hours.

Fig. S3



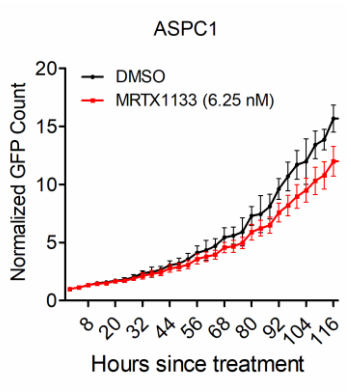


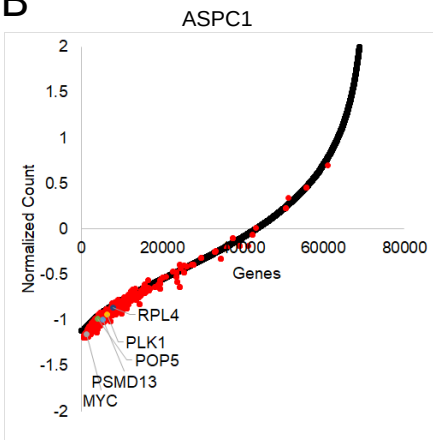


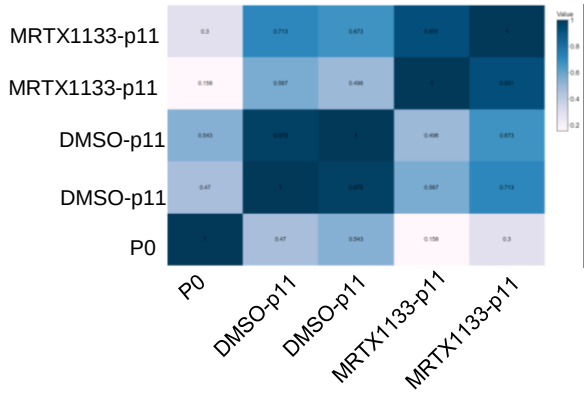


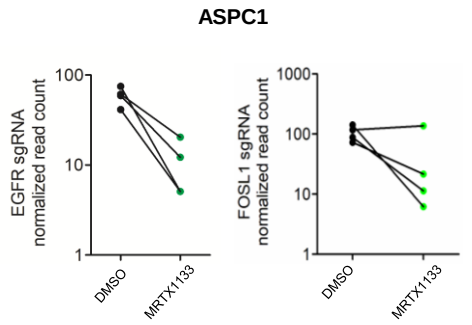
(A) Volcano plots indicating the differentially expressed genes on the indicated cell lines treated with MRTX1133 (100 nM) up to 48 H based on Transcriptome analysis. Blue: represents genes that are significantly downregulated (log_2Fold change<-1.2, -log_{10}pVal >2). Red represents genes that are significantly upregulated (log_2Fold change>1.2, -log_{10}pVal >2). **(B)** Heat map depicting the differential expression of the indicated genes that are involved in MEK signaling, MTOR signaling and E2F-regulated cell cycle genes from 1222 and 3226 cell lines treated with MRTX1133 (100 nM) for 48 hours based on bul RNA sequencing. **(C)** Biochemical analysis on 828, ASPC1 and 519 cells on the indicated proteins following 48 hour exposure with MRTX1133 at two different concentrations. **(D)** Effect of MRTX1133 on BrdU incorporation on the indicated cell lines following the treatment with different concentrations of MRTX1133 up to 72 hours. Error bars represent mean and SD from triplicates.

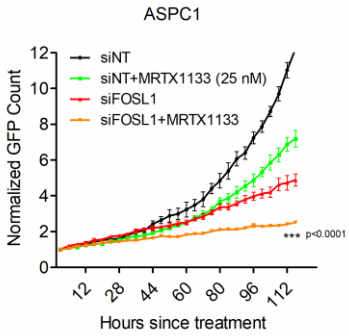
Fig. S4

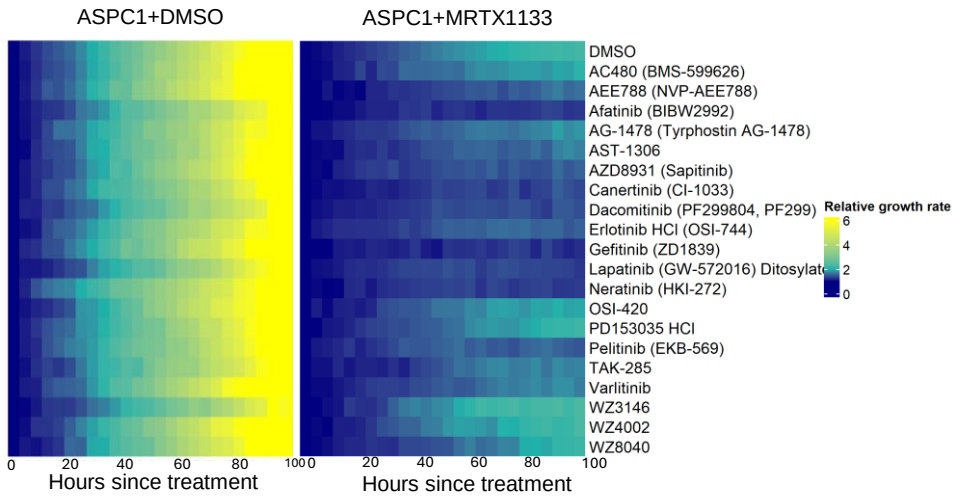


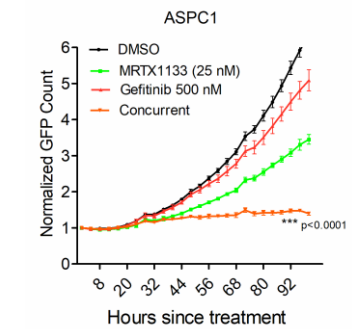




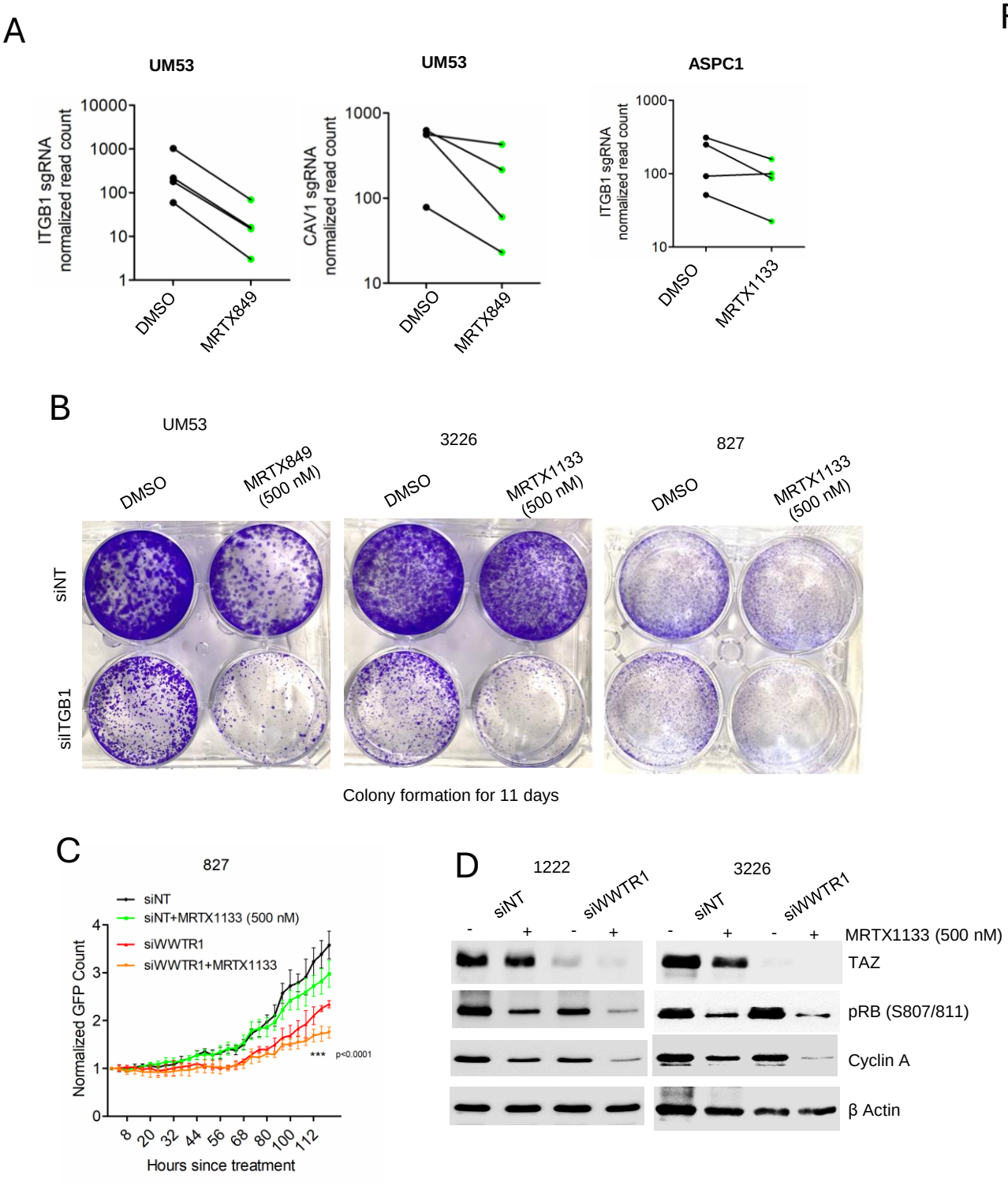








(A) Live cell imaging to determine the effect of MRTX1133 on the proliferation of ASPC1 cells after treating for 5 days at a low dose of 6.25 nM. **(B)** Scatter plot indicating the Normalized count of the sgRNAs from the ASPC1 cells that were selected in DMSO after 11 passages. The essential genes were highlighted. **(C)** Correlation analysis between the replicates from the CRISPR screen on ASPC1 cells that were selected in the presence and absence of MRTX1133. **(D)** Effect of MRTX1133 (6.25 nM) on the individual guides targeting, *EGFR* and *FOSL1* in ASPC1 cells. **(E)** Effect of *FOSL1* KD on the proliferation of ASPC1 cells in combination with MRTX1133 (25 nM). Error bars represent mean and SD from triplicates. **(F)** Heat map depicting the relative growth rate of ASPC1 cells that were treated with the indicated EGFR inhibitors (100 nM) in combination with DMSO and MRTX1133 (100 nM). **(G)** Live cell imaging to monitor the growth of ASPC1 cells, treated with MRTX1133 (25 nM) in combination with Gefitinib (500 nM). Mean and SD were shown. (*** p<0.0001 as determined by two-way ANOVA).



(A) Effect of MRTX849 (500 nM) on the individual guides targeting, *ITGB1, and CAV1* in UM53 cells. Effect of MRTX1133 (6.25 nM) on the depletion of individual guides targeting ITGB1 in ASPC1 cells. **(B)** Crystal violet staining in UM53, 3226 and 827 cell lines, that were treated with indicated KRAS inhibitors at a concentration of 500 nM following the deletion of ITGB1. **(C)** Effect of *WWTR1* deletion in the absence and presence of MRTX1133 (500 nM) on the proliferation of 827 cells based on live cell imaging. Error bars represent mean and SD from triplicates. (***p<0.0001 as determined by two-way ANOVA). **(D)** Biochemical analysis to validate the *WWTR1* gene deletion based on the expression of TAZ. The cellular response to TAZ deletion in the absence and presence of MRTX1133 (500 nM) up to 48 hours was determined based on RB phosphorylation and cyclin A expression.

A

Fig. S6

3226

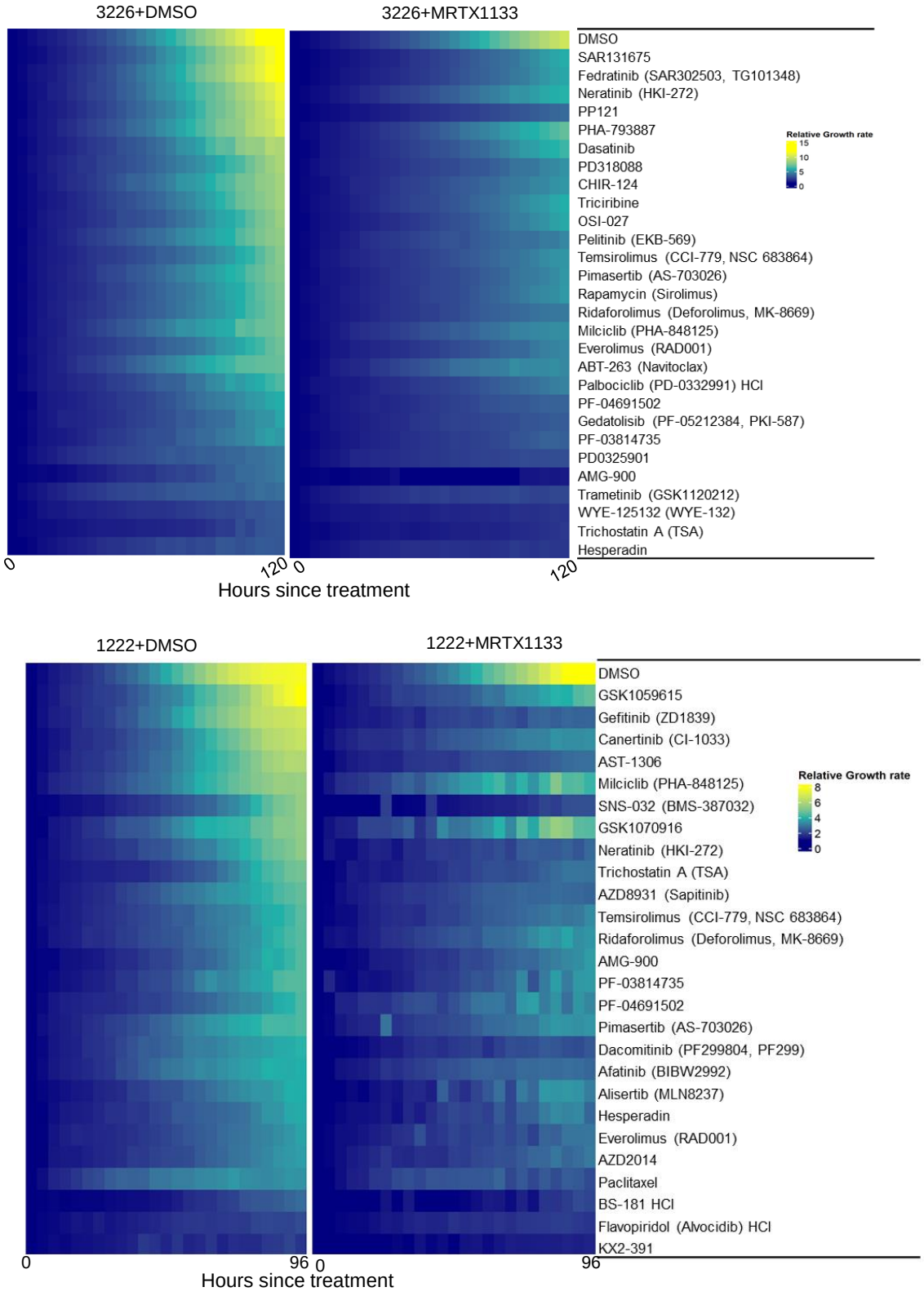
Drugs	Targets
AMG-900	Aurora Kinase
PP121	PDGFR,mTOR,DNA-PK
Triciribine	Akt
PF-04691502	Akt,mTOR,PI3K
Hesperadin	Aurora Kinase
ABT-263 (Navitoclax)	Bcl-2
PHA-793887	CDK
Milciclib (PHA-848125)	CDK
Palbociclib (PD-0332991) HCl	CDK
CHIR-124	Chk
Pelitinib (EKB-569)	EGFR
Neratinib (HKI-272)	EGFR,HER2
PF-03814735	FAK,Aurora Kinase
Trichostatin A (TSA)	HDAC
Fedratinib (SAR302503, TG101348)	JAK
PD318088	MEK
Pimasertib (AS-703026)	MEK
PD0325901	MEK
Trametinib (GSK1120212)	MEK
Ridaforolimus (Deforolimus, MK-8669)	mTOR
Temsirolimus (CCI-779, NSC 683864)	mTOR
WYE-125132 (WYE-132)	mTOR
Everolimus (RAD001)	mTOR
OSI-027	mTOR
Rapamycin (Sirolimus)	mTOR,Autophagy
Gedatolisib (PF-05212384, PKI-587)	PI3K,mTOR
Dasatinib	Src,Bcr-Abl,c-Kit
SAR131675	VEGFR

1222

Drugs	Targets
PF-04691502	Akt,mTOR,PI3K
Hesperadin	Aurora Kinase
Alisertib (MLN8237)	Aurora Kinase
GSK1070916	Aurora Kinase
AMG-900	Aurora Kinase
SNS-032 (BMS-387032)	CDK
BS-181 HCl	CDK
Milciclib (PHA-848125)	CDK
Flavopiridol (Alvocidib) HCl	CDK
Gefitinib (ZD1839)	EGFR
AST-1306	EGFR
Dacomitinib (PF299804, PF299)	EGFR
Neratinib (HKI-272)	EGFR,HER2
Canertinib (CI-1033)	EGFR,HER2
AZD8931 (Sapitinib)	EGFR,HER2
Afatinib (BIBW2992)	EGFR,HER2
PF-03814735	FAK,Aurora Kinase
Trichostatin A (TSA)	HDAC
Pimasertib (AS-703026)	MEK
Paclitaxel	Microtubule Associated,Autophagy
Temsirolimus (CCI-779, NSC 683864)	mTOR
Everolimus (RAD001)	mTOR
Ridaforolimus (Deforolimus, MK-8669)	mTOR
AZD2014	mTOR
GSK1059615	PI3K,mTOR
KX2-391	Src

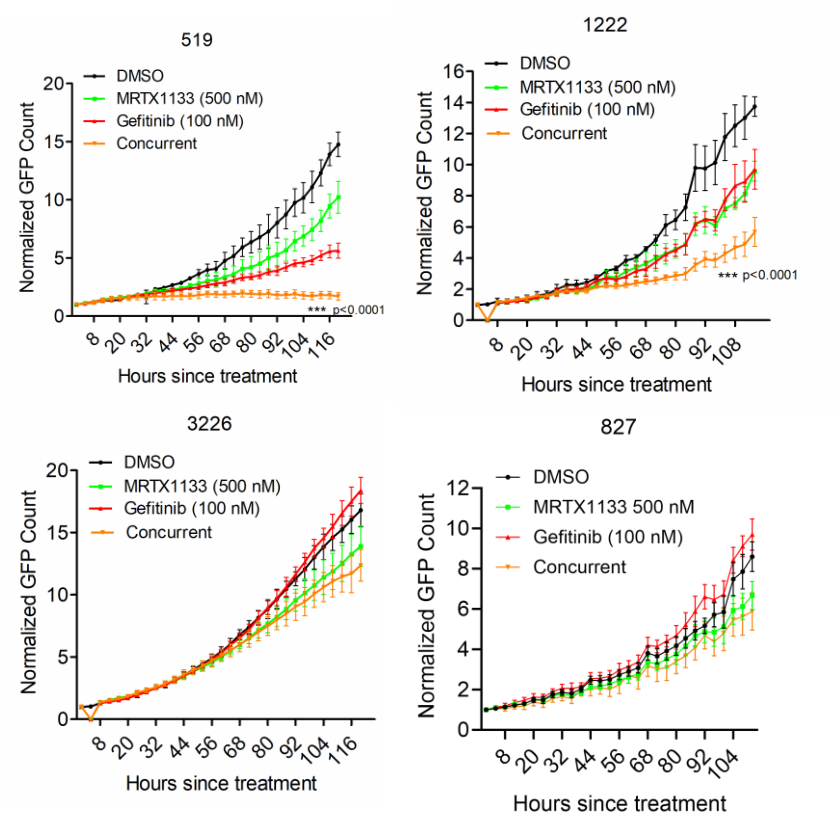
(A) List of drugs and their associated targets identified based on drug screen analysis in 3226 and 1222 cell lines in combination with MRTX1133 (500 nM).

A



(A) Heat map depicting the list of drugs that cooperatively inhibit the proliferation 3226 and 1222 cells in combination with MRTX1133 (500 nM) as determined by live cell imaging.

A



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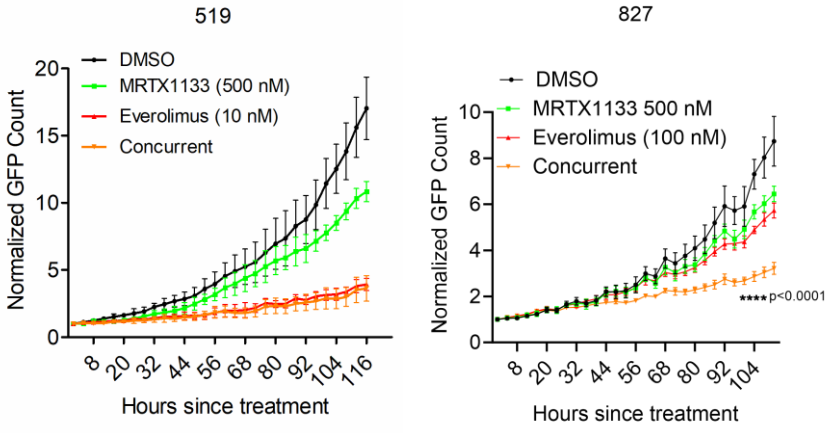
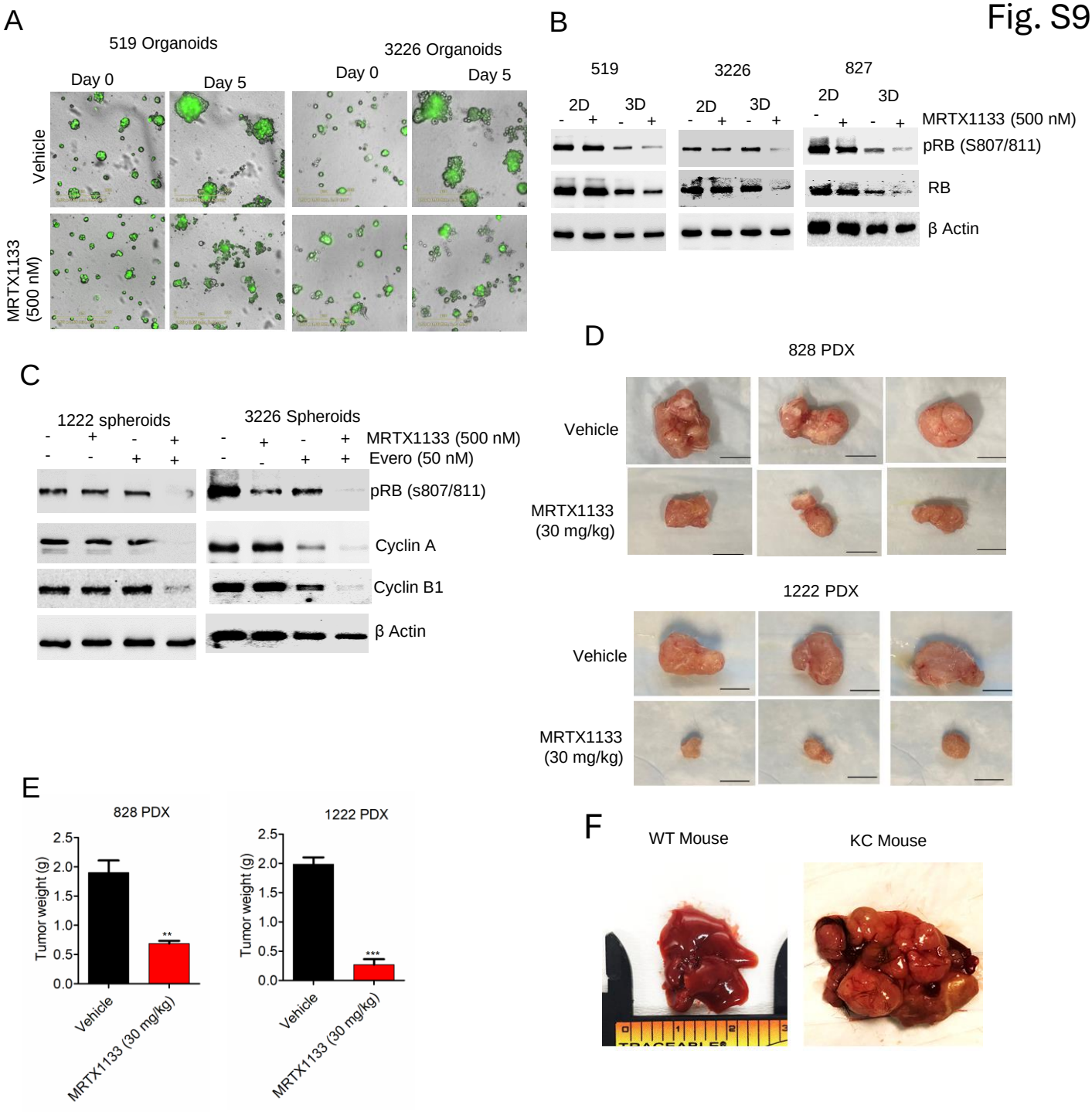


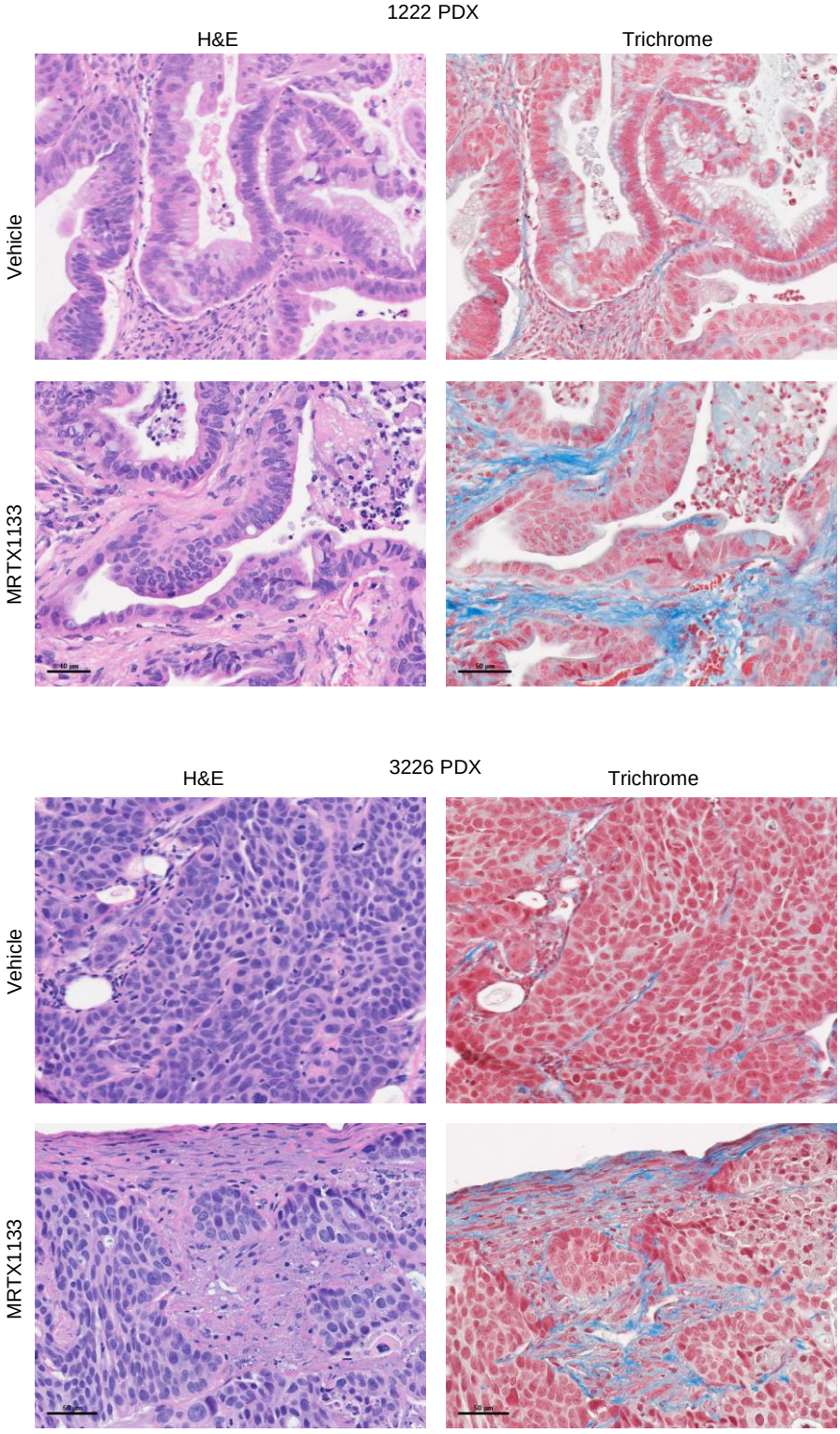
(A) Live cell imaging to examine the effect of Gefitinib (100 nM) in combination with MRTX1133 (500 nM) on the proliferation of 519, 1222, 3226 and 827 cell lines. **(B)** Effect of everolimus at the indicated concentration in combination with MRTX1133 (500 nM) on the proliferation of 519 and 827 cells. Error bars were determined based on mean and SD from triplicates. Experiment was done at 2 independent times. (***p<0.0001 as determined by two-way ANOVA).



(A) Representative images of organoids at day 0 and day 5 in the absence and presence of MRTX1133 (500 nM). Scale bar represents 300 µm. **(B)** Western blot analysis to compare the effect of MRTX113 (500 nM) on RB phosphorylation between cell grown in 2D monolayer and 3D culture following 48 hour exposure. **(C)** Biochemical analysis to determine the effect of everolimus (50 nM) as single agent and in combination with MRTX1133 (500 nM) on cell cycle proteins in spheroids derived from 1222 and 3226 cells. **(D)** Representative images of tumors excised from 828 and 1222 PDX that were treated with vehicle and MRTX1133 (30 mg/kg) for 21 days. **(E)** Column graphs illustrating the tumor weights from 828 and 1222 PDX in the presence and absence of MRTX113 (30 mg/kg). Error bars represent mean and SEM. (*** $p<0.0001$ and ** $p<0.001$ as determined by student t test). **(F)** Pancreas images from a healthy mouse and the genetically modified KC mouse, bearing the tumor.

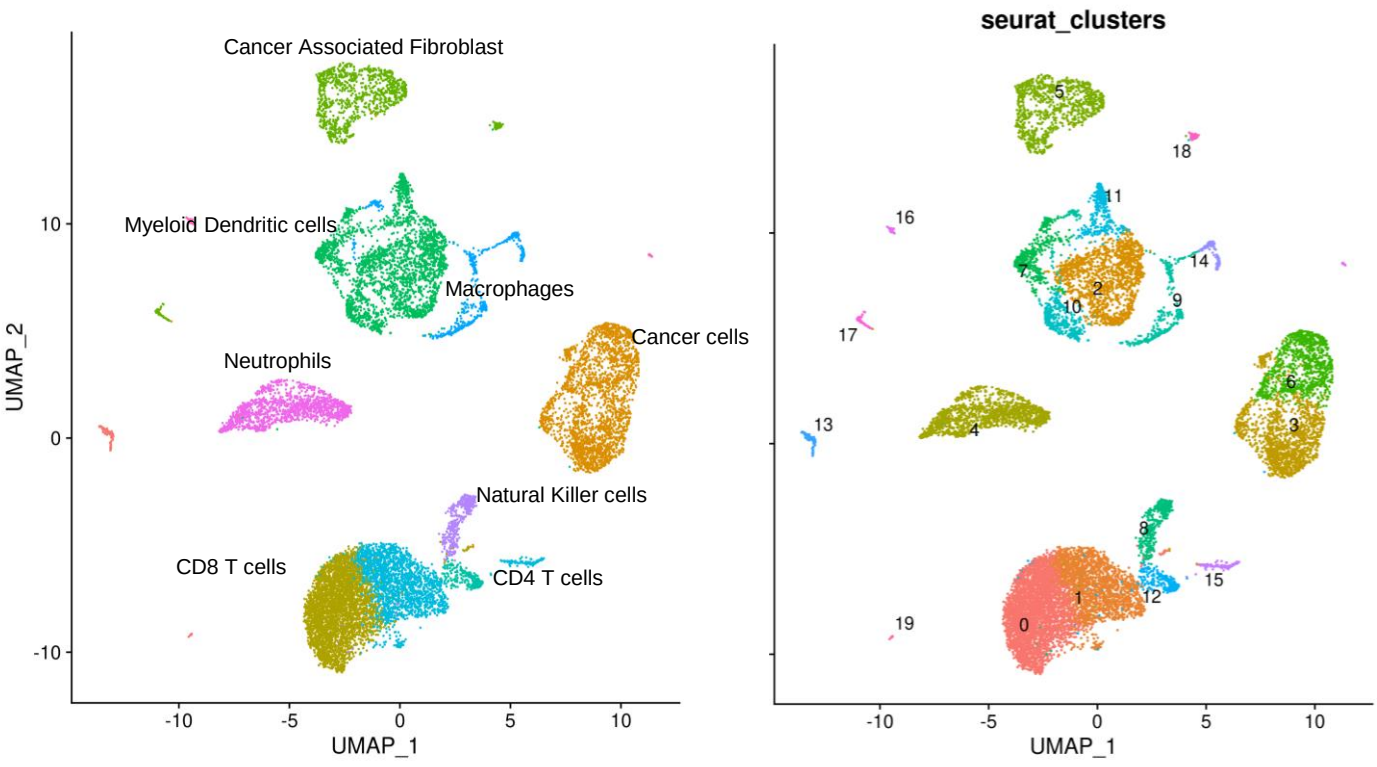
A

Fig. S10



(A) Representative images of H&E and Masson-Trichrome staining from the tumor tissues derived from 3226 and 1222 PDX treated with vehicle and MRTX1133 (30 mg/kg). Scale bar represents 50 µm.

A



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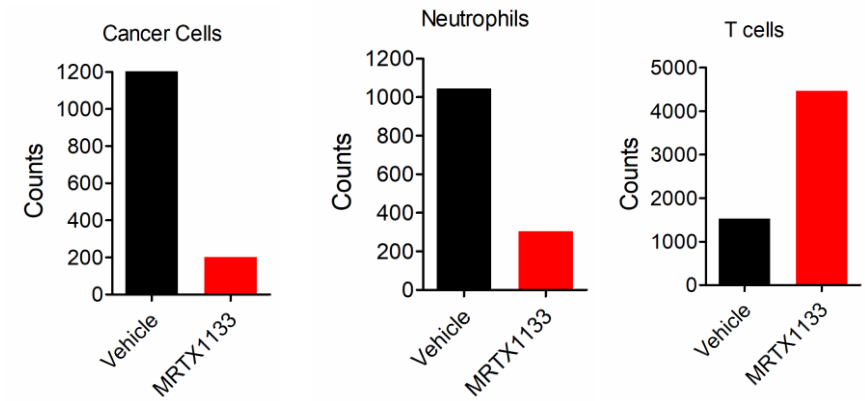
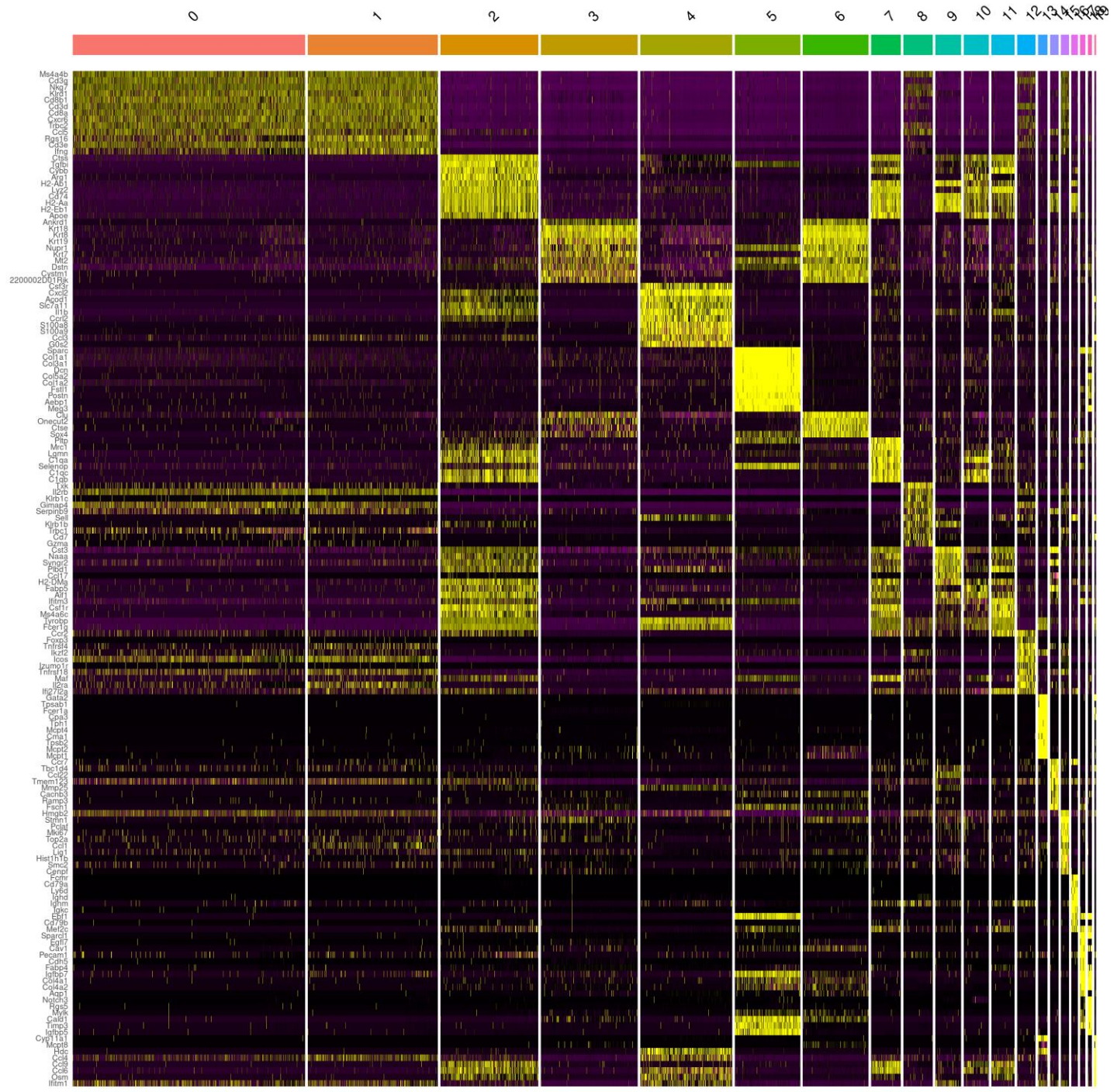


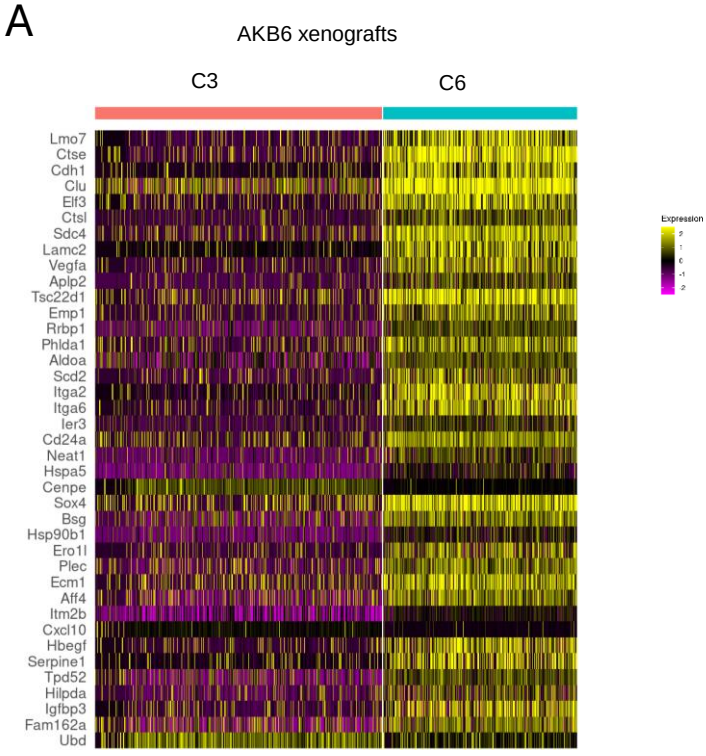
(A) Single-cell clustering of vehicle and MRTX1133-treated AKB6 tumors to selectively indicate different cellular components. (B) Changes on the indicated cellular population following MRTX1133 treatment. (***p<0.0001 as determined by Fisher Extract test).

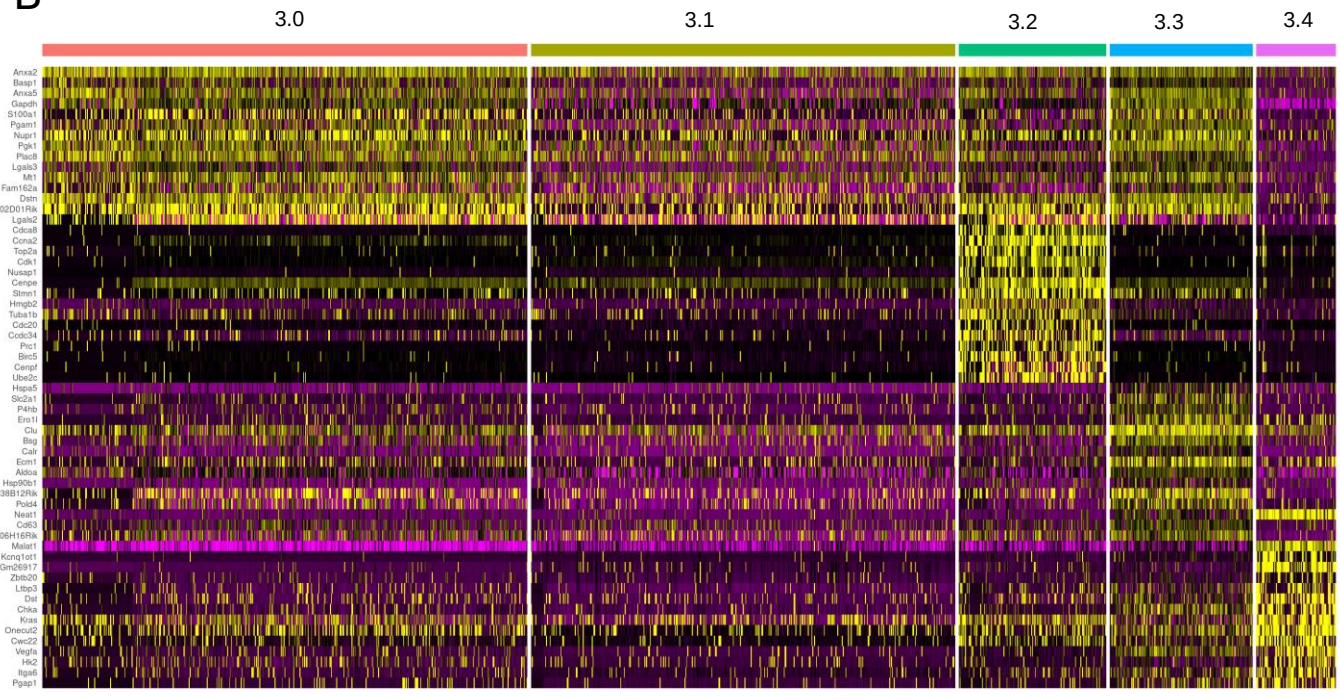
A



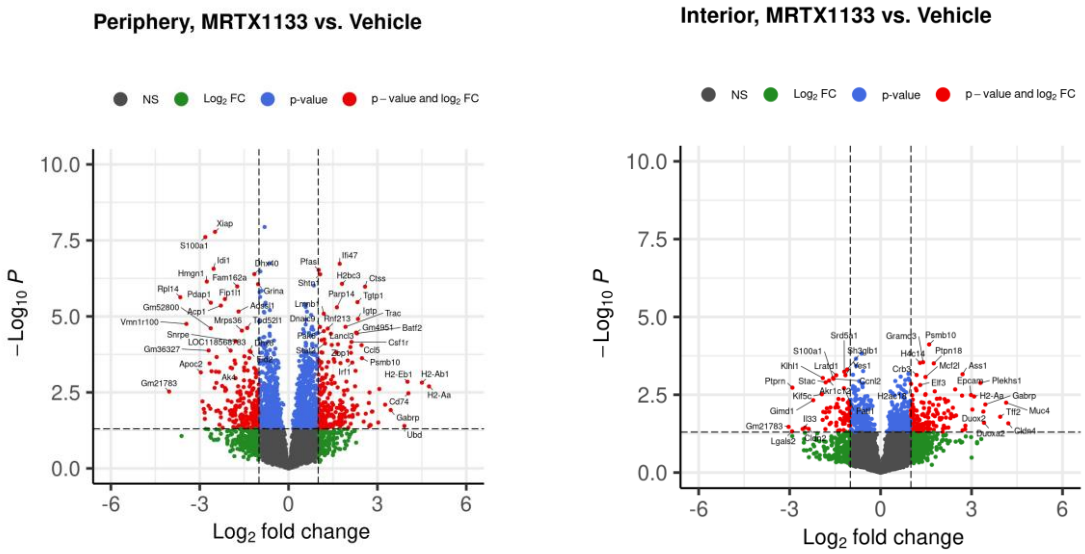
(A) Seurat heatmap depicting the expression of genes that distinguish different clusters that represent different cellular components.

Fig. S13





(A) Seurat heatmap depicting the expression of genes that distinguish two different tumor population. **(B)** Seurat heatmap to illustrate the differential expression of the indicated genes across the sub-clusters from the tumor cluster 3.



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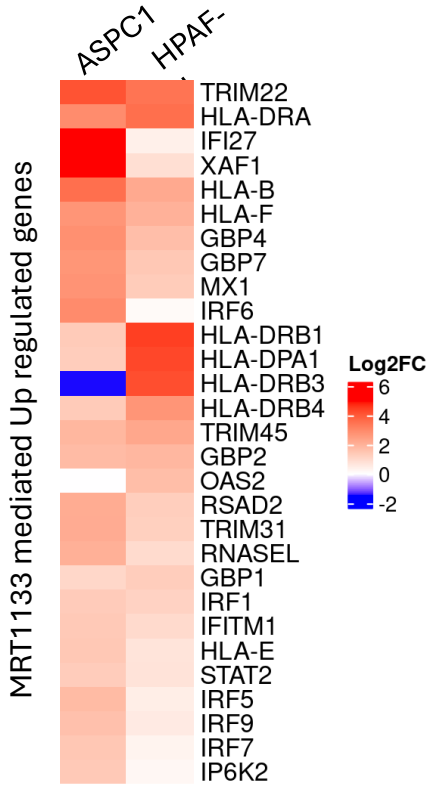
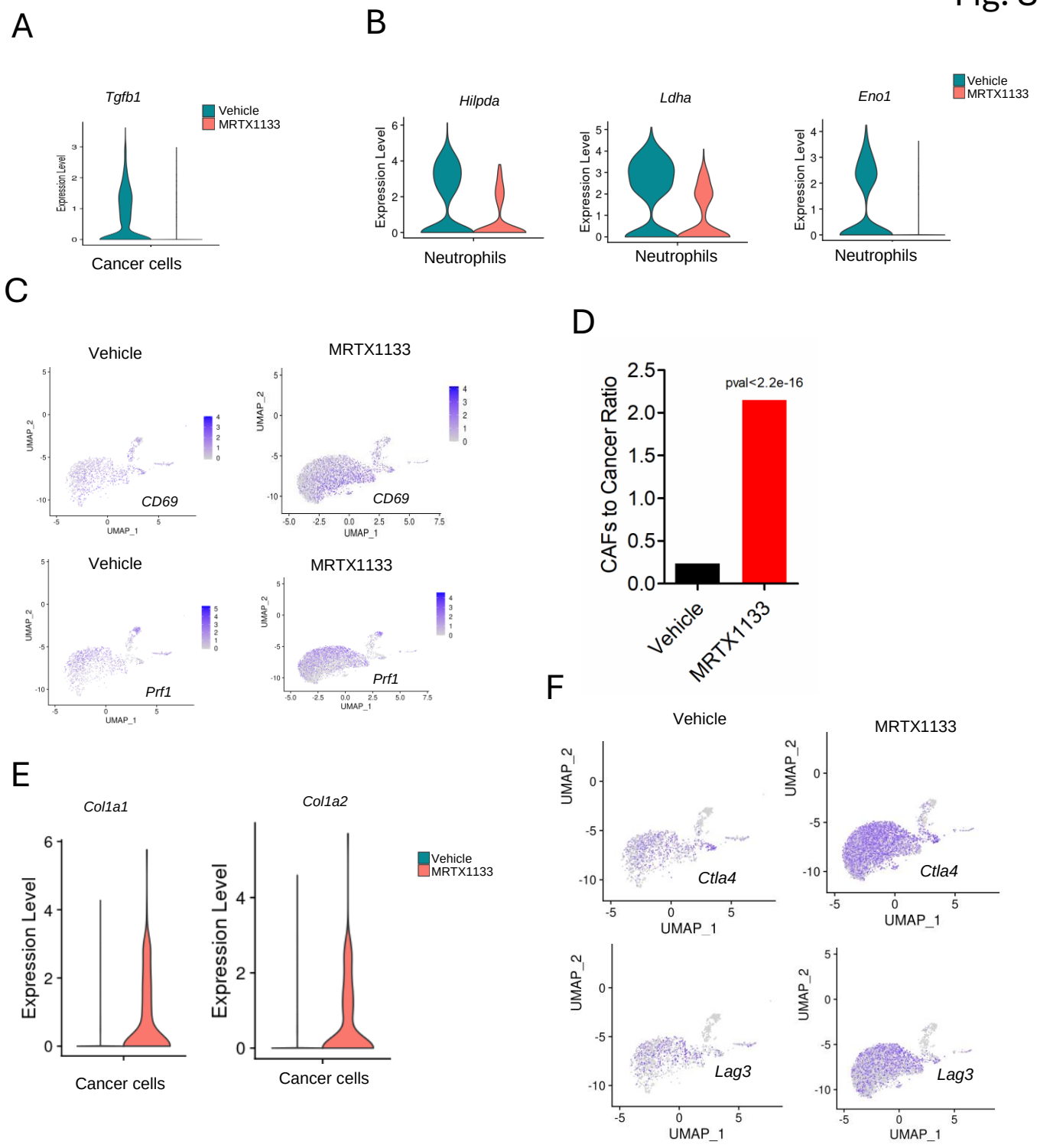


(A) Volcano plots illustrate the differentially expressed genes, on the PanCK+ cells following MRTX1133 treatment on the AKB6 xenografts. Blue: represents downregulated genes (log_2Fold change<-0.5). Red represents upregulated genes (log_2Fold change>0.5). **(B)** Heat map depicting the differential expression of a subset of genes associated with interferon γ signaling and antigen presentation from ASPC1 and HPAF-II cell lines following the treatment with MRTX1133 (100 nM) up to 48 hours based on transcriptome analysis.



(A) Violin plots indicating the expression of Tgfb1 within the tumor population population in the presence and absence of MRTX1133. **(B)** Violin plots indicating the expression of indicated genes within the neutrophil population in the presence and absence of MRTX1133**(C)** Seurat feature plots to illustrate the differential expression of representative T-cell markers from the vehicle and MRTX1133-treated groups. **(D)** Column graph illustrating the relative abundance of CAFs with respect to tumor population from the vehicle and MRX1133 (30 mg/kg) treated tumors. **(E)** Violin plots indicating the expression of *Col1a1 and Col1a2* within the cancer population in the absence and presence of MRTX1133 (30 mg/kg) **(F)** Seurat feature plots to illustrate the differential expression of exhaustion markers from the T cell population from the vehicle and MRTX1133-treated groups.