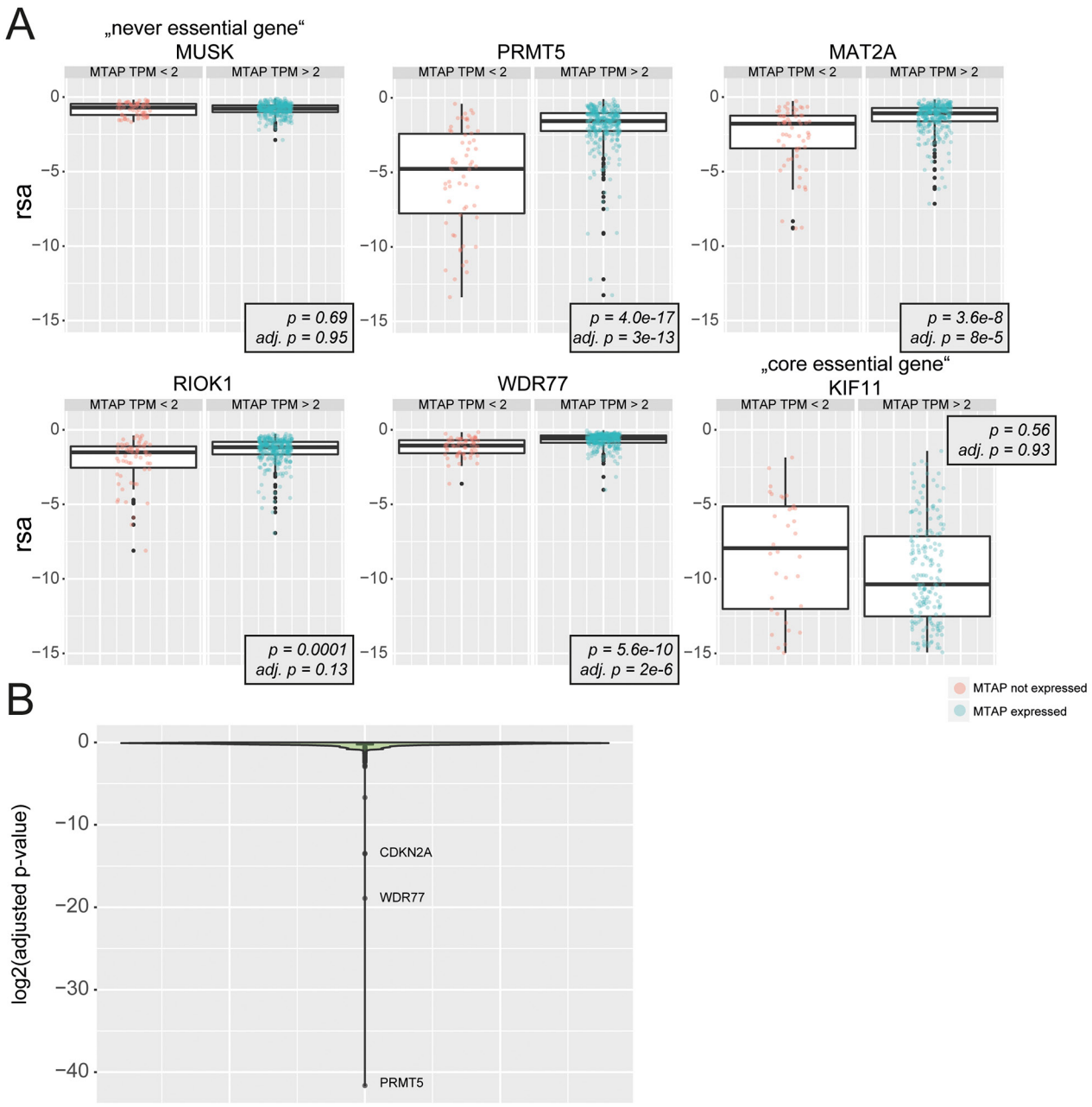
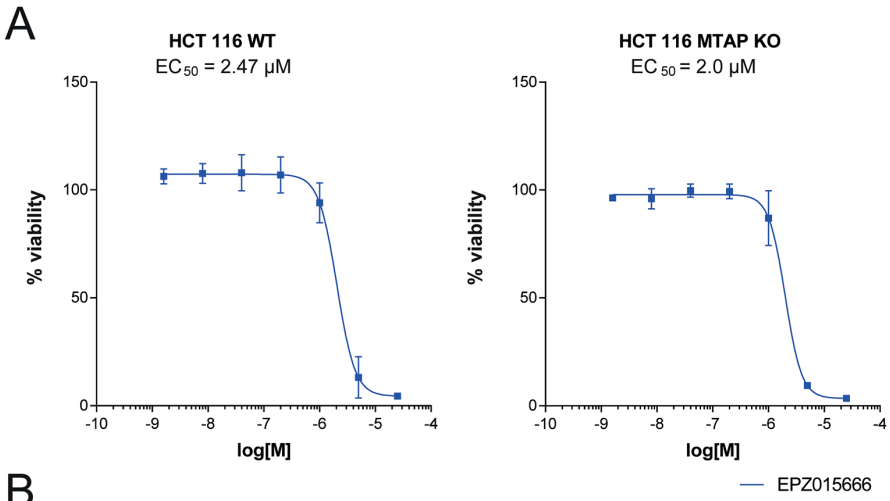
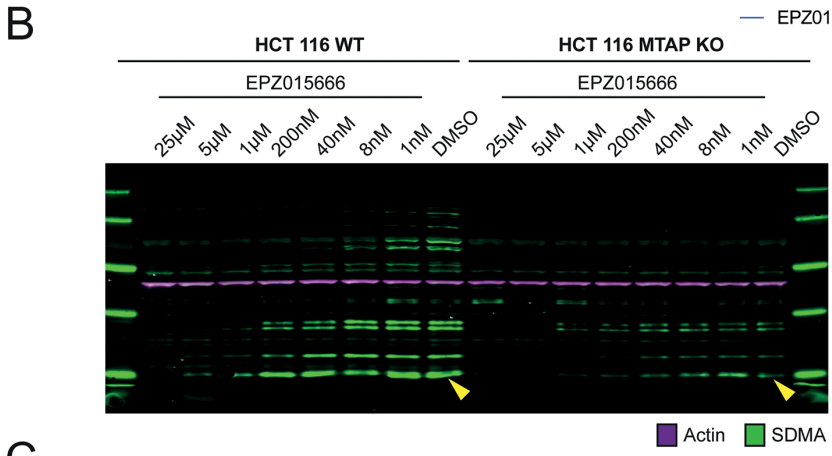
RIOK1 kinase activity is required for cell survival irrespective of *MTAP* status

SUPPLEMENTARY MATERIALS



Supplementary Figure 1: (A) Boxplots (overlaid: individual data points) depict depletion scores between MTAP non-expressing cells (MTAP TPM < 2) and MTAP expressing cells (MTAP TPM > 2) for the "never essential gene" *MUSK, PRMT5, MAT2A, RIOK1, WRD77* and the "core essential gene" *KIF11*. P-values and adjusted p-values for Wilcoxon test statistics are indicated in boxes. Y- axis depicts the *rsa* score as reported in [21]. **(B)** Explorative analysis for differentially required genes between MTAP expressing and non-expressing cells. Violin- and boxplot of log2 adjusted p-values (Wilcoxon test).



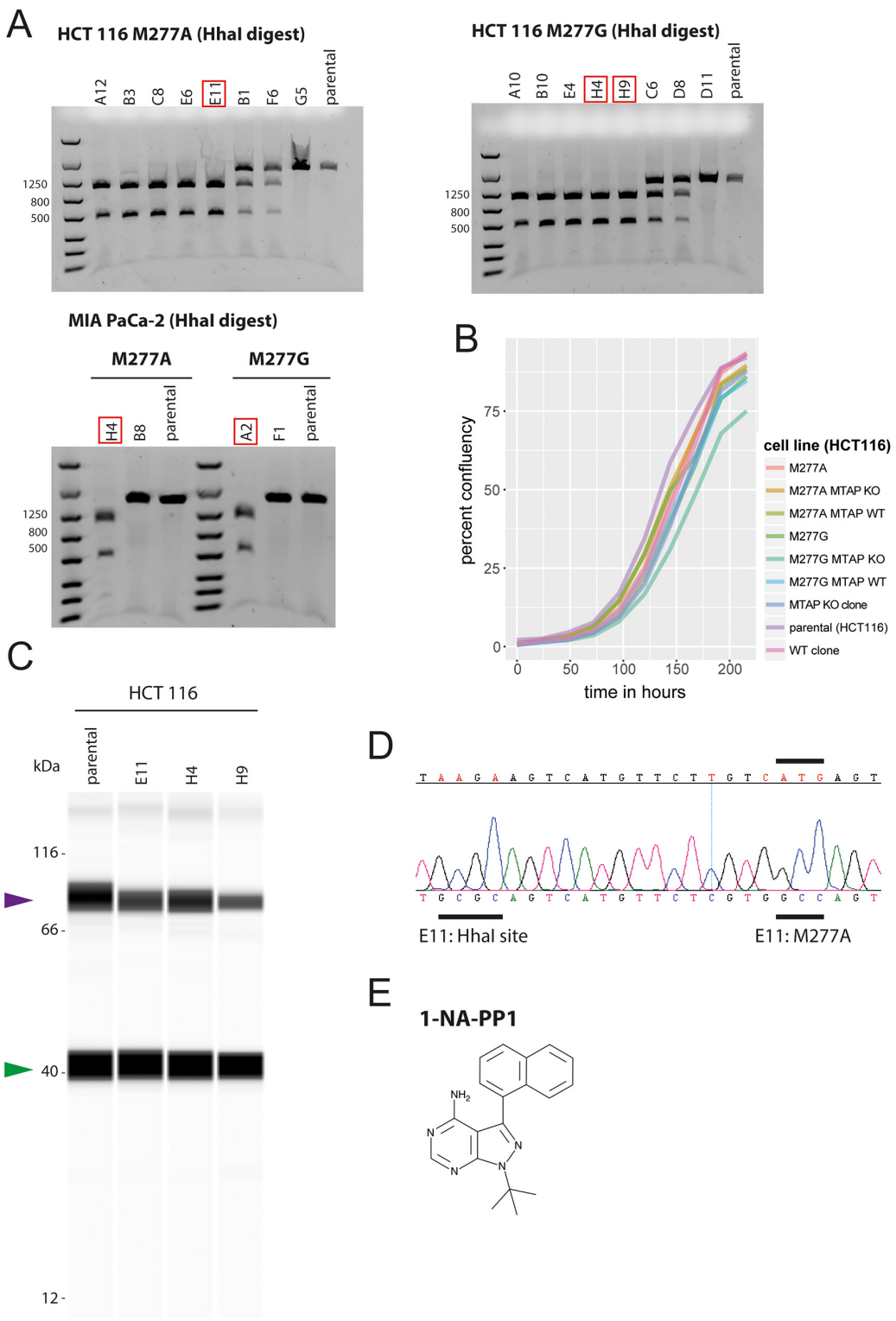


C

	MTAP TPM	EC_{50} [nM]
Pancreatic cancer cell lines		
Capan-1	1	4272
MIA PaCa-2	1	3955
DAN-G	1	6917
KP-4	1	4834
BxPC-3	1	>40000
SU.86.86	1	>40000
QGP-1	27	12060
Panc 04.03	61	11680
KP-3	78	>40000
HPAF-II	241	6643
MIA PaCa-2 OE	N.A.	12740
Immortalized/non-transformed cell lines		
MCF 10A	N.A.	1038
h-TERT RPE-1	50	>25000*

*Note: highest concentration measured: 25000 nM

Supplementary Figure 2: **(A)** Dose response curves of EPZ015666 in HCT 116 *MTAP* WT and *MTAP* KO cells. **(B)** Western Blot for SDMA (green) and Actin (loading control, magenta) upon 96h treatment with different EPZ015666 concentrations. Yellow arrowhead points out comparable band at same size. **(C)** Cell viability assays performed after 8 days of incubation with EPZ015666. MTAP expression values are depicted in TPM (transcripts per million). EC_{50} values are in nM. Note: MIA PaCa-2 OE refers to MIA PaCa-2 cells overexpressing MTAP. TPM value for MIA PaCa-2 OE cell line has not been determined (N.A.). **(D)** Western blot assays comparing RIOK1 (green) and PRMT5 (green) protein levels in HCT 116 parental (par), HCT 116 wild type clone (WT), HCT 116 MTAP knockout (KO), MIA PaCa-2 parental (par) and MIA PaCa-2 MTAP overexpressing (OE) cells.



Supplementary Figure 3: **(A)** Restriction enzyme analysis of genome edited *RIOK1* alleles. Presence of double band indicates complete editing. Single uncut band indicates WT allele. Red boxes indicate selected clones. **(B)** Cell growth assay comparing the growth rate of different engineered and parental HCT 116 cell lines. **(C)** RIOK1 protein expression analysis using a capillary based immunoassay. Upper bands (magenta arrowhead) correspond to RIOK1; lower bands (green arrowhead) correspond to GAPDH. Cell lines and clone identifiers are indicated on top. **(D)** Representative chromatogram of a Sanger sequencing experiment to confirm the full editing of the RIOK1 gatekeeper residue of HCT 116 clone E11. **(E)** Structure of the 1-NA-PP1 analog.

A

MIA PaCa-2

	parental	MTAP OE
	MIA PaCa-2 parental	
1-NA-PP1	8576.48	7984.05
Panobinostat	4.74	7.43
	M277A (H4)	
1-NA-PP1	79.88	78.66
Panobinostat	3.82	4.19
	M277G (A2)	
1-NA-PP1	234.52	379.28
Panobinostat	1.55	3.66

EC50 96h Cell Viability Assay [nM]

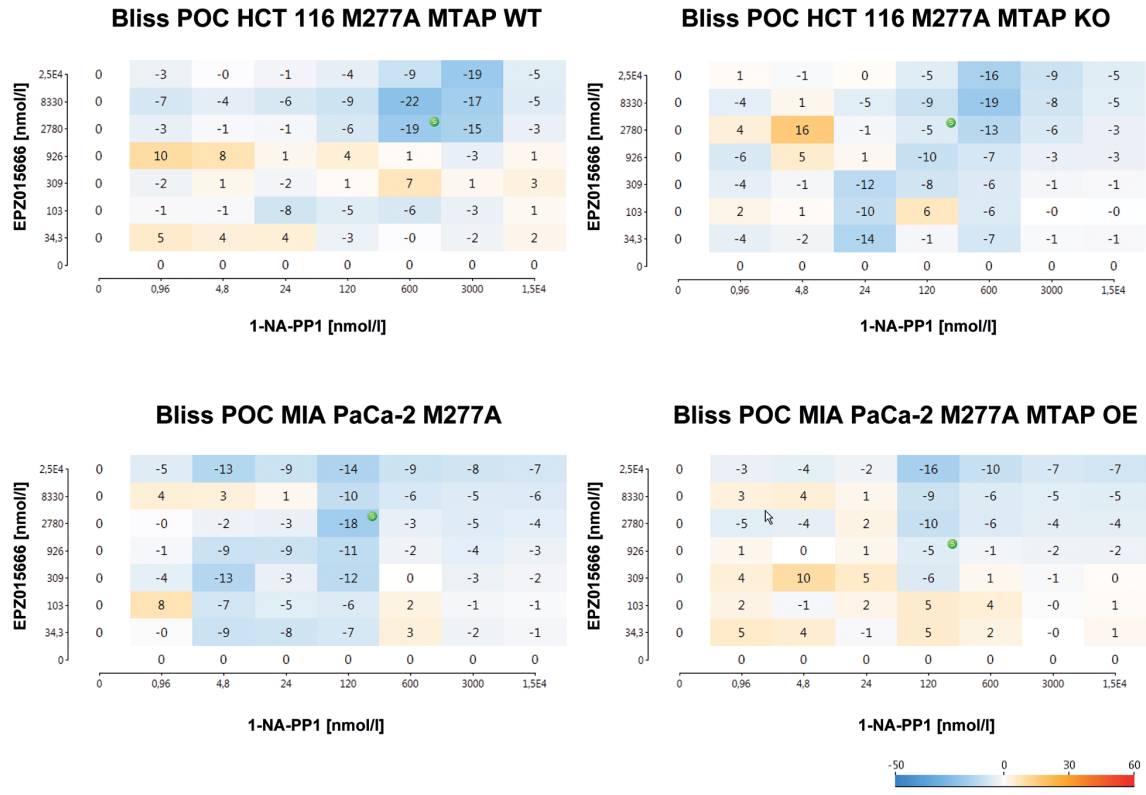
B

HCT 116

	parental	MTAP KO	MTAP WT
	HCT 116 parental		
1-NA-PP1	4742.3	11434.3	12282.8
Panobinostat	9.2	8.0	7.9
	M277A (E11)		
1-NA-PP1	461.89	270.2	373.0
Panobinostat	10.2	10.4	8.4
	M277G (H9)		
1-NA-PP1	364.8	114.9	262.0
Panobinostat	10.0	7.3	7.8
	M277G (H4)		
1-NA-PP1	579.3	360.3	568.2
Panobinostat	9.5	7.6	10.0

EC50 96h Cell Viability Assay [nM]

C



Supplementary Figure 4: **(A)** Table summarizing EC_{50} values of 1-NA-PP1 and Panobinostat (control) in multiple independently derived isogenic MIA PaCa-2 clones. **(B)** Table summarizing EC_{50} values of 1-NA-PP1 and Panobinostat (control) in multiple independently derived isogenic HCT 116 clones. **(C)** Combination drug matrix of percent of control (POC) CellTiter-Glo cell viability measurements of MIA PaCa-2 and HCT 116 *RIOK1* genome engineered cells treated with the indicated amounts of EPZ015666 and 1-NA-PP1. Values depict Bliss excess POC.

Supplementary Table 1: Sequences for gRNAs, primers and CRISPR genome engineering repair templates used in this study.

See Supplementary File 1

Supplementary Table 2: CRISPR screen results HCT 116 wild type cells - epigenome domain CRISPR library (gRNA information see supplementary table 1).

See Supplementary File 2

Supplementary Table 3: CRISPR screen results HCT 116 MTAP knock out cells - epigenome domain CRISPR library (gRNA information see supplementary table 1).

See Supplementary File 3

Supplementary Table 4: CRISPR screen results HCT 116 wild type cells - kinase domain CRISPR library (gRNA information see supplementary table 1).

See Supplementary File 4

Supplementary Table 5: CRISPR screen results HCT 116 MTAP knock out cells - kinase domain CRISPR library (gRNA information see supplementary table 1).

See Supplementary File 5