Appendix for

Gemcitabine and ATR inhibitors synergize to kill PDAC cells by blocking DNA damage response

Stefanie Höfer[1], Larissa Frasch[1], Sarah Brajkovic[1], Kerstin Putzker[2], Joe Lewis[2], Hendrik Schürmann[3,4,5], Valentina Leone[6], Amirhossein Sakhteman[1], Matthew The[1], Florian P. Bayer[1], Julian Müller[1], Firas Hamood[1], Jens T. Siveke[3,4], Maximilian Reichert[6,7], and Bernhard Kuster[1,7*]

[1]Chair of Proteomics and Bioanalytics, Technical University of Munich, Freising, Germany

[2]Chemical Biology Core Facility, EMBL Heidelberg, Heidelberg, Germany

[3]Bridge Institute of Experimental Tumor Therapy (BIT) and Division of Solid Tumor Translational Oncology (DKTK), West German Cancer Center, University Hospital Essen, University of Duisburg-Essen, Essen, Germany

[4]German Cancer Consortium (DKTK), partner site Essen, a partnership between German Cancer Research Center (DKFZ) and University Hospital Essen, Germany

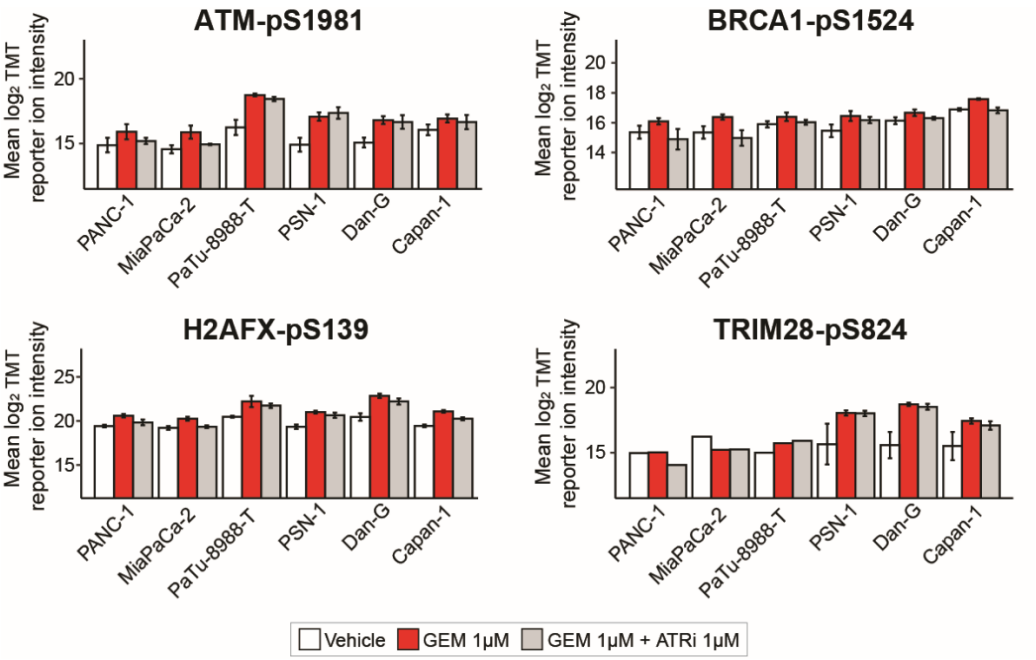
[5]Department of Medical Oncology, West German Cancer Center, University Hospital Essen, Essen, Germany

[6]Department of Internal Medicine II, University Hospital rechts der Isar, Technical University Munich, Munich, Germany

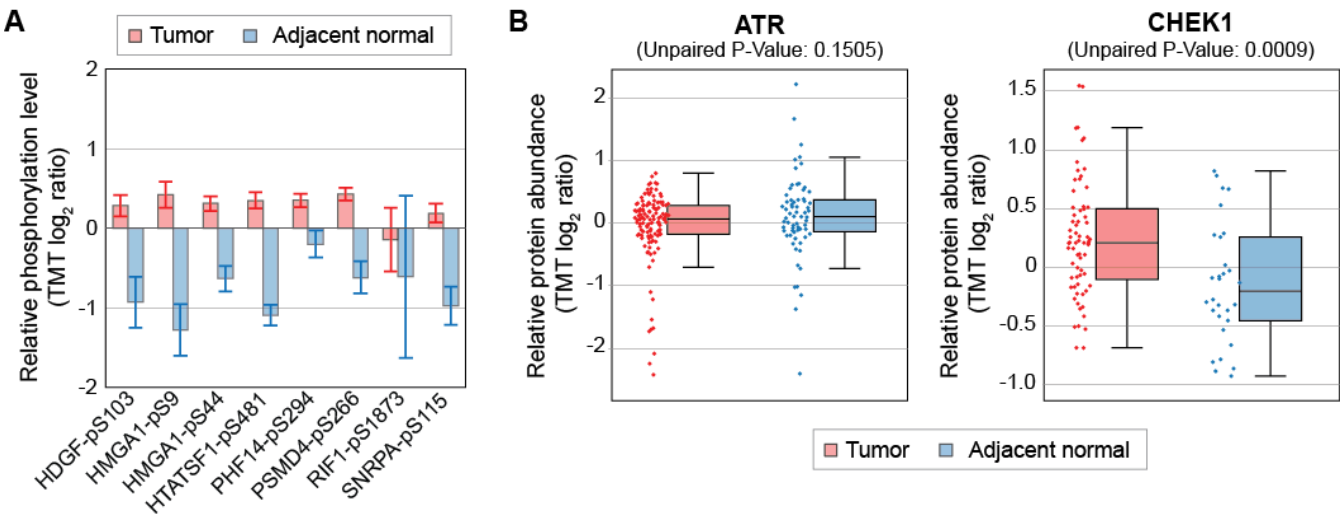
[7]German Cancer Consortium (DKTK), Partner Site Munich, Munich, Germany

*Corresponding author. Email: kuster@tum.de

Table of contents



Appendix Figure S1. Substrates of ATM are not affected in phosphorylation upon ATRi.
TMT reporter ion intensity (log_2) of selected ATM substrates in six PDAC cell lines treated with vehicle, 1 µM GEM, or 1 µM GEM plus 1 µM Elimusertib. Data are presented as mean values, with error bars representing the ± s.d. of quadruplicates (n = 4). Information on kinase-substrate-relationship based on *PhosphoSitePlus* (Hornbeck *et al*, 2015).

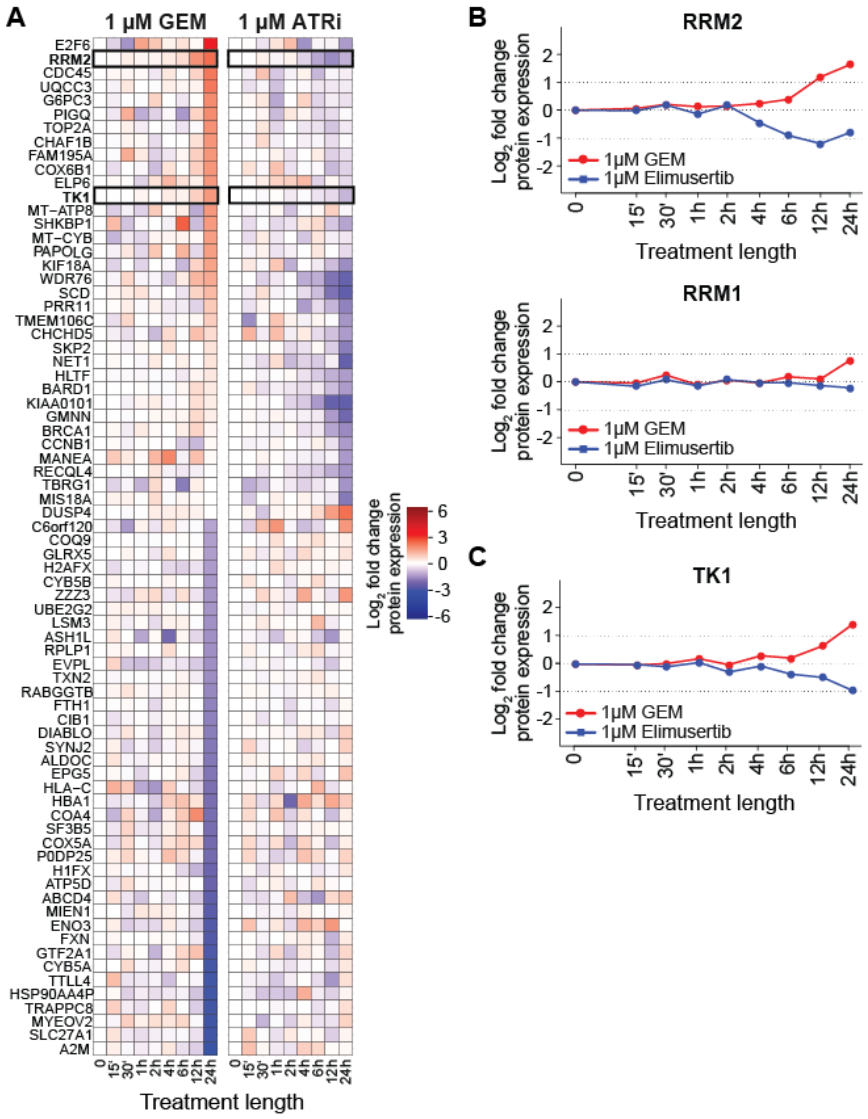


Appendix Figure S2. Clinical data implies increased DNA damage response activity in PDAC cells.

A. Relative phosphorylation levels of 8 of the 36 pSQ/pTQ sites in PDAC tumor (red) vs. adjacent normal cells (blue). The counts of tumor and adjacent normal cells are: 38 and 19 (HDGF-pS103), 11 and 5 (HMGA1-pS9), 85 and 40 (HMGA1-pS44), 46 and 25 (HTATSF1-pS481), 15 and 8 (PHF14-pS294), 26 and 11 (PSMD4-pS266), 5 and 2 (RIF1-pS1873, or 36 and 16 (SNRPA-pS115), respectively. Data are presented as mean values, and error bars indicate the standard error.

B. Relative protein abundance of ATR and CHEK1 in PDAC tumor (red) vs. adjacent normal cells (blue). Unpaired p-values were calculated using the Mann-Whitney U-test. Tumor counts: 124 (ATR) and 69 (CHEK1); adjacent normal cells counts: 59 (ATR) and 30 (CHEK1). The boxplots display the median (center), the interquartile range (bounds of the box), and the upper and lower fences (whiskers) of relative protein abundances.

Data information: The data were generated by the National Cancer Institute Clinical Proteomic Tumor Analysis Consortium (CPTAC) and accessed through Proteomic Data Commons (Thangudu *et al*, 2020). The analysis and plots were generated using the embedded web tool *cProSite* (https://cprosite.ccr.cancer.gov/) (Wang *et al*, 2023).



Appendix Figure S3. GEM and ATRi affect expression of nucleotide metabolism enzymes over time. A. Log_2 fold changes in protein expression in AsPC-1 cells treated with 1 µM GEM or 1 µM Elimusertib over time relative to untreated cells. **B-C.** Log_2 fold changes in protein expression over time upon treatment with 1 µM GEM (red) or 1 µM Elimusertib (blue) of RRM2 (B, upper), RRM1 (B, lower), and TK1 (C).

Cell line	Supplier or provider	Supplier order #	Medium	Medium supplements**	Cell density (384-well)
AsPC-1	ATCC	CRL-1682	DMEM	10% FBS, 1% non-essential amino acids	1000
PANC-1	ATCC	CRL-1469	DMEM	10% FBS	2000
PSN-1	ATCC	CRL-3211	RPMI	10% FBS	1000
BxPC-3	ATCC	CRL-1687	RPMI	10% FBS	1000
MiaPaCa-2	ATCC	CRL-1420	DMEM	10% FBS	1000
Capan-1	CLS	300143	RPMI	10% FBS, 1% HEPES	2000
FAMPAC	CLS	300309	RPMI	10% FBS, 1% HEPES	1000
HuP-T4	creative-bioarray	CSC-C0333	DMEM/Hams-F12 (1:1)	10% FBS, 15 mM HEPES	1000
Dan-G	DSMZ	ACC-249	RPMI	10% FBS	1000
PaTu-8988-T	DSMZ	ACC-162	DMEM	10% FBS	1000
Colo-357	Molecular Oncology Department of Radiation, LMU*	-	DMEM	10% FBS	2000
HPDE	Molecular Oncology Department of Radiation, LMU*	-	RPMI/keratinocyte serum-free medium (1:1)	10% FBS, 5 ng/ml human epidermal growth factor, 50 µg/ml bovine pituitary extract	1000
Suit2-07	Molecular Oncology Department of Radiation, LMU*	-	RPMI	10% FBS	1000

*Cell lines received from Molecular Oncology Department of Radiation, LMU were kindly provided by Prof Kisten Lauber

**For high-throughput drug screening, all cell culture medium was supplemented with 1% 100 U/mL penicillin and 100 ug/mL streptomycin.

Appendix Table S1. Information on 13 PDAC cell lines used for drug screening. Information on supplier and order number (if applicable) of screened cell lines is given, as well as cell culture medium, supplements and cell densities seeded per well of a 384-well plate.

PDO set	PDO	Age at diagnosis/ operation	Sex	Site	Sample	KRAS Mutation
1	B250	71	male	pancreas	surgical	G12D
1	B535	44	male	pancreas	surgical	G12D
1	B678	54	male	pancreas	endosonography	G12V
2	UME008	57	male	pancreas	surgical	G12D
2	UME012	65	male	ascites	ascites	G12D
2	UME051	53	male	pancreas	endosonography	G12D
2	UME053	56	female	metastasis (abdominal wall)	surgical	WT
2	UME060	65	male	metastasis (liver)	endosonography	G12V
2	UME061	70	female	ascites	ascites	G12R

Appendix Table S2. Information on nine pancreatic PDOs tested in viability assays. Information on the age and sex of the donor is given, as well the site and procedure of retrieval, and the KRAS mutational state.

Samples	Medium name	Component	Final concentration
PDO set 1	Anti-Anti solution	PBS	1x
PDO set 1	Anti-Anti solution	Anti-Anti (Merck, A5955)	1x
PDO set 1	Anti-Anti solution	Gentamicin (Merck, G1272)	100µg/mL
PDO set 1	Washing medium	Advanced DMEM/F12 (Thermo Fisher , 12634-010)	1X
PDO set 1	Washing medium	Glutamax (Thermo Fisher, 35050038)	1X
PDO set 1	Washing medium	HEPES (Thermo Fisher, 15630056)	1X
PDO set 1	Washing medium	Primocin (InvivoGen)	1X
PDO set 1	Digestion buffer	Washing medium	-
PDO set 1	Digestion buffer	Collagenase Type II (Thermo Fisher, 17101015)	6 mg/ml
PDO set 1	Digestion buffer	Dispase II (Merck, 4942078001)	100 µg/ml
PDO set 1	PDAC PDO medium	Advanced DMEM/F12 (Thermo Fisher, 12634-010)	1X
PDO set 1	PDAC PDO medium	Glutamax (Thermo Fisher, 35050038)	1X
PDO set 1	PDAC PDO medium	HEPES (Thermo Fisher, 15630056)	1X
PDO set 1	PDAC PDO medium	Penicillin/Streptomicin (Thermo Fisher, 15140122)	1%
PDO set 1	PDAC PDO medium	R-spondin conditioned medium (home made)	10% (v/v)
PDO set 1	PDAC PDO medium	B-27 Supplement	1X
PDO set 1	PDAC PDO medium	N-2 Supplement	1X
PDO set 1	PDAC PDO medium	N-acetylcysteine	1 mM
PDO set 1	PDAC PDO medium	Recombinant human EGF	50 ng/ml
PDO set 1	PDAC PDO medium	A83-01	0.5 µM
PDO set 1	PDAC PDO medium	Primocin	100 µg/ml
PDO set 1	PDAC PDO medium	Recombinant human Noggin	25 ng/ml
PDO set 1	PDAC PDO medium	Recombinant human Wnt3A	50 ng/ml
PDO set 1	PDAC PDO medium	Nicotinamide	10 mM
PDO set 1	PDAC PDO medium	Recombinant human FGF-10	100 ng/ml
PDO set 1	PDAC PDO medium	human Gastrin-I	1 nM
PDO set 1	PDAC PDO medium	Prostaglandin-2	1 µM
PDO set 2	Anti-Anti solution	Washing medium	-
PDO set 2	Anti-Anti solution	Anti-Anti (Thermo Fisher, 15240-062)	1x
PDO set 2	Washing medium	Advanced DMEM/F12 (Gibco)	1X
PDO set 2	Washing medium	GlutaMAX (Gibco, 35050061)	1X
PDO set 2	Washing medium	HEPES (Gibco, 15-630-080)	1X
PDO set 2	Washing medium	Primocin (InvivoGen)	125 µg/ml
PDO set 2	Digestion buffer	Washing medium	-
PDO set 2	Digestion buffer	Collagenase Type II (Thermo Fisher, 17101015)	5 mg/ml
PDO set 2	Digestion buffer	Dispase II (Life Technologies, 17105041)	1.25 mg/ml
PDO set 2	Digestion buffer	Trypsin inhibitor (Thermo Fisher, 17075029)	1 mg/ml
PDO set 2	PDAC PDO medium	Advanced DMEM/F12 (Gibco)	1X
PDO set 2	PDAC PDO medium	GlutaMAX (Gibco, 35050061)	1X
PDO set 2	PDAC PDO medium	HEPES (Gibco, 15-630-080)	1X
PDO set 2	PDAC PDO medium	R-spondin conditioned medium (home made)	10% (v/v)
PDO set 2	PDAC PDO medium	B-27 Supplement	1X
PDO set 2	PDAC PDO medium	N-acetylcysteine	1 mM
PDO set 2	PDAC PDO medium	Recombinant human EGF	50 ng/ml
PDO set 2	PDAC PDO medium	A83-01	0.5 µM
PDO set 2	PDAC PDO medium	Primocin	125 µg/mL
PDO set 2	PDAC PDO medium	Recombinant human Noggin	100 ng/ml
PDO set 2	PDAC PDO medium	Wnt3a-conditioned medium (home made)	50% (v/v)
PDO set 2	PDAC PDO medium	Nicotinamide	10 mM
PDO set 2	PDAC PDO medium	Recombinant human FGF-10	100 ng/ml
PDO set 2	PDAC PDO medium	Human Gastrin-I	10 nM

Appendix Table S3. Information on media and buffers used handling pancreatic PDOs. PDO set 1: B250, B535, B678; PDO set 2: UME008, UME012, UME051, UME053, UME060, UME061.

TMT Channel	Cell line	GEM [nM]	ATRi [nM]
TMT11: 11-plex 131H	AsPC-1	0	0
TMT10: 10-plex 131L	AsPC-1	1000	0
TMT10: 10-plex 130H	AsPC-1	1000	1
TMT10: 10-plex 130L	AsPC-1	1000	3
TMT10: 10-plex 129H	AsPC-1	1000	10
TMT10: 10-plex 129L	AsPC-1	1000	30
TMT10: 10-plex 128H	AsPC-1	1000	100
TMT10: 10-plex 128L	AsPC-1	1000	300
TMT10: 10-plex 127H	AsPC-1	1000	1000
TMT10: 10-plex 127L	AsPC-1	1000	3000
TMT10: 10-plex 126	AsPC-1	1000	10000

Appendix Table S4. TMT-mapping for phospho-experiments in AsPC-1 cells (decrypM). The tested ATRi were Elimusertib, Gartisertib, Berzosertib, and Ceralasertib.

	TMT Channel	Cell line	GEM [nM]	Elimusertib [nM]
TMT batch 1	TMT10: 10-plex 131L	AsPC-1	1000	1000
	TMT10: 10-plex 130H	Panc-1	1000	1000
	TMT10: 10-plex 130L	Panc-1	1000	0
	TMT10: 10-plex 129H	Panc-1	0	0
	TMT10: 10-plex 129L	PaTu-8988-T	1000	1000
	TMT10: 10-plex 128H	PaTu-8988-T	1000	0
	TMT10: 10-plex 128L	PaTu-8988-T	0	0
	TMT10: 10-plex 127H	MiaPaCa-2	1000	1000
	TMT10: 10-plex 127L	MiaPaCa-2	1000	0
	TMT10: 10-plex 126	MiaPaCa-2	0	0
TMT batch 2	TMT10: 10-plex 131L	AsPC-1	1000	1000
	TMT10: 10-plex 130H	Dan-G	1000	1000
	TMT10: 10-plex 130L	Dan-G	1000	0
	TMT10: 10-plex 129H	Dan-G	0	0
	TMT10: 10-plex 129L	PSN-1	1000	1000
	TMT10: 10-plex 128H	PSN-1	1000	0
	TMT10: 10-plex 128L	PSN-1	0	0
	TMT10: 10-plex 127H	Capan-1	1000	1000
	TMT10: 10-plex 127L	Capan-1	1000	0
	TMT10: 10-plex 126	Capan-1	0	0

Appendix Table S5. TMT-mapping for phospho-experiments in six additional PDAC cell lines (non-decryptM). Each experiment was performed in quadruplicates. The channel containing AsPC-1 cells was used for inter-batch normalization.

TMT Channel	Cell line	Time point	GEM or Elimusertib [nM]	Description
TMTpro: 16-plex 126	AsPC-1	0	0	Vehicle for 15 min
TMTpro: 16-plex 127N	AsPC-1	30 min	1000	-
TMTpro: 16-plex 127C	AsPC-1	30 min	0	Vehicle for 30 min
TMTpro: 16-plex 128N	AsPC-1	1 h	1000	-
TMTpro: 16-plex 128C	AsPC-1	1 h	0	Vehicle for 1 h
TMTpro: 16-plex 129N	AsPC-1	2 h	1000	-
TMTpro: 16-plex 129C	AsPC-1	2 h	0	Vehicle for 2 h
TMTpro: 16-plex 130N	AsPC-1	4 h	1000	-
TMTpro: 16-plex 130C	AsPC-1	4 h	0	Vehicle for 3 h
TMTpro: 16-plex 131N	AsPC-1	6 h	1000	-
TMTpro: 16-plex 131C	AsPC-1	6 h	0	Vehicle for 6 h
TMTpro: 16-plex 132N	AsPC-1	12 h	1000	-
TMTpro: 16-plex 132C	AsPC-1	12 h	0	Vehicle for 12 h
TMTpro: 16-plex 133N	AsPC-1	24 h	1000	-
TMTpro: 16-plex 133C	AsPC-1	24 h	0	Vehicle for 24 h

Appendix Table S6. TMT-mapping for time-dependent full proteome experiment in AsPC-1 cells. Cells were either treated with 1,000 nM of GEM or with 1,000 nM Elimusertib (separate TMT experiments).

References

Hornbeck PV, Zhang B, Murray B, Kornhauser JM, Latham V, Skrzypek E (2015) PhosphoSitePlus, 2014: mutations, PTMs and recalibrations. *Nucleic Acids Res* 43: D512-520

Thangudu RR, Rudnick PA, Holck M, Singhal D, MacCoss MJ, Edwards NJ, Ketchum KA, Kinsinger CR, Kim E, Basu A (2020) Abstract LB-242: Proteomic Data Commons: A resource for proteogenomic analysis. *Cancer Research* 80: LB-242-LB-242

Wang D, Qian X, Du YN, Sanchez-Solana B, Chen K, Kanigicherla M, Jenkins LM, Luo J, Eng S, Park B *et al* (2023) cProSite: A web based interactive platform for online proteomics, phosphoproteomics, and genomics data analysis. *J Biotechnol Biomed* 6: 573-578