

Supplementary information for:

Protein kinase A Mediated Effects of Protein kinase C Partial Agonist HMI-1a3 in Colorectal Cancer Cells

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Conflict of interest disclosure statement: The authors declare no potential conflicts of interest.

Supplemental Data

LDH viability data

Table S1. Necrotic cell death in Caco2 cells after 24-h exposure to the test compounds, measured by LDH assay. Results are presented as mean (%) of cytotoxicity (N=3).

	DMSO	1a3						
		0,1 μ M	0,5 μ M	1 μ M	2 μ M	4 μ M	10 μ M	20 μ M
Mean (%)	0.00	-0.14	1.88	2.07	2.79	1.93	1.56	4.16
stdev	2.13	2.95	1.86	1.39	1.44	1.31	2.02	3.07

Table S2. Necrotic cell death in Colo205 cells after 24-h exposure to the test compounds, measured by LDH assay. Results are presented as mean (%) of cytotoxicity (N=3).

	DMSO	1a3						
		0,1 μ M	0,5 μ M	1 μ M	2 μ M	4 μ M	10 μ M	20 μ M
Mean (%)	0.00	1.83	0.06	0.12	1.52	1.38	1.39	1.57
stdev	1.40	5.43	3.38	2.00	1.29645	2.04	1.58	2.79

Table S3. Necrotic cell death in HT29 cells after 24-h exposure to the test compounds, measured by LDH assay. Results are presented as mean (%) of cytotoxicity (N=3).

	DMSO	1a3						
		0,1 μ M	0,5 μ M	1 μ M	2 μ M	4 μ M	10 μ M	20 μ M
mean (%)	0.00	-2.89	-1.48	-1.52	-1.05	-1.89	-2.32	-1.73
stdev	5.10	5.13	3.34	3.87	2.29	4.92	5.67	6.64

Immunoblotting

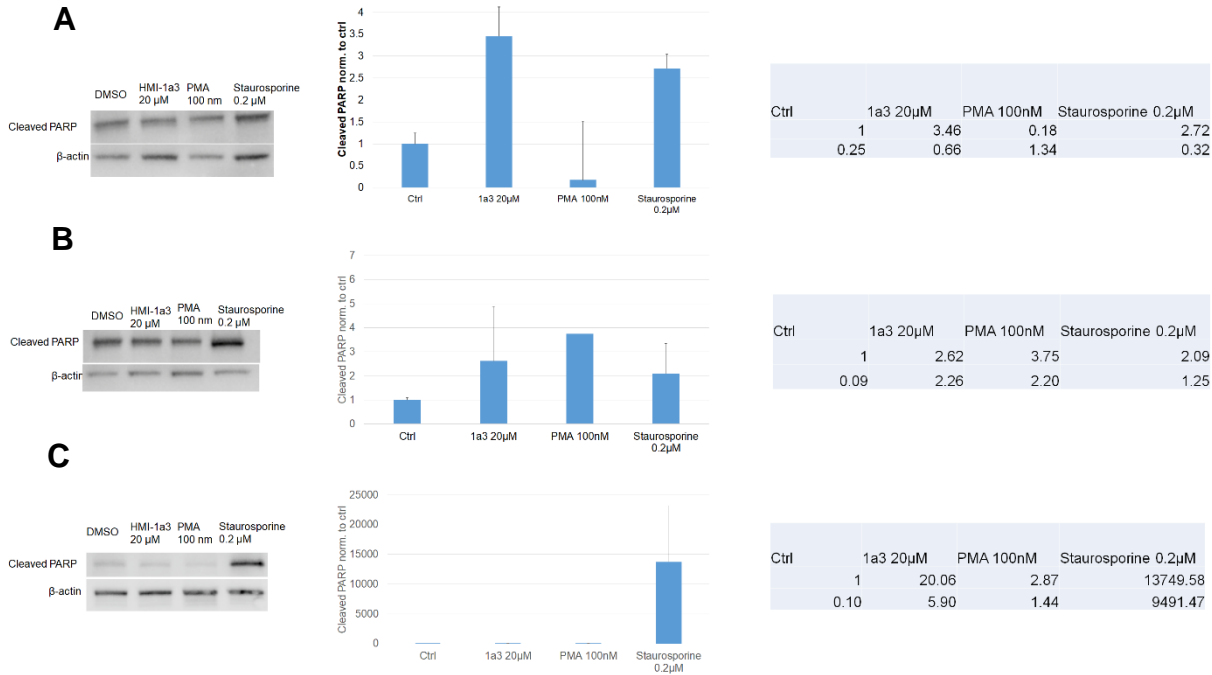


Figure S1. HMI-1a3 induces apoptosis in all tested CRC lines. HMI-1a3 increases the cleaved PARP apoptosis marker expression in Caco2 (A), Colo205 (B) and HT29 (C) cell lines.

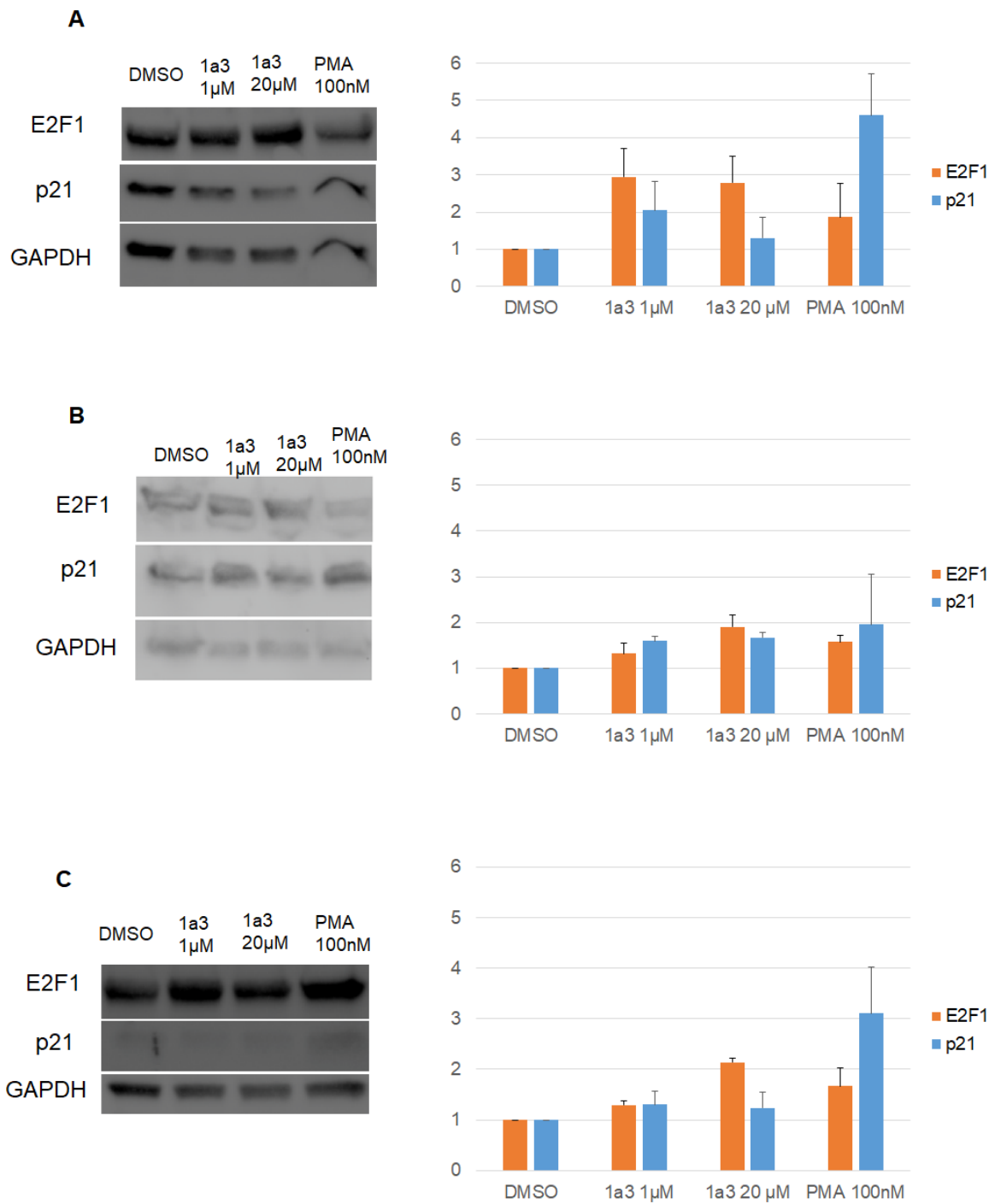


Figure S2. E2F1 and p21 expression in Caco2 (A), Colo205 (B) and HT29 (C) cells after 48-h exposure.

PamGene data

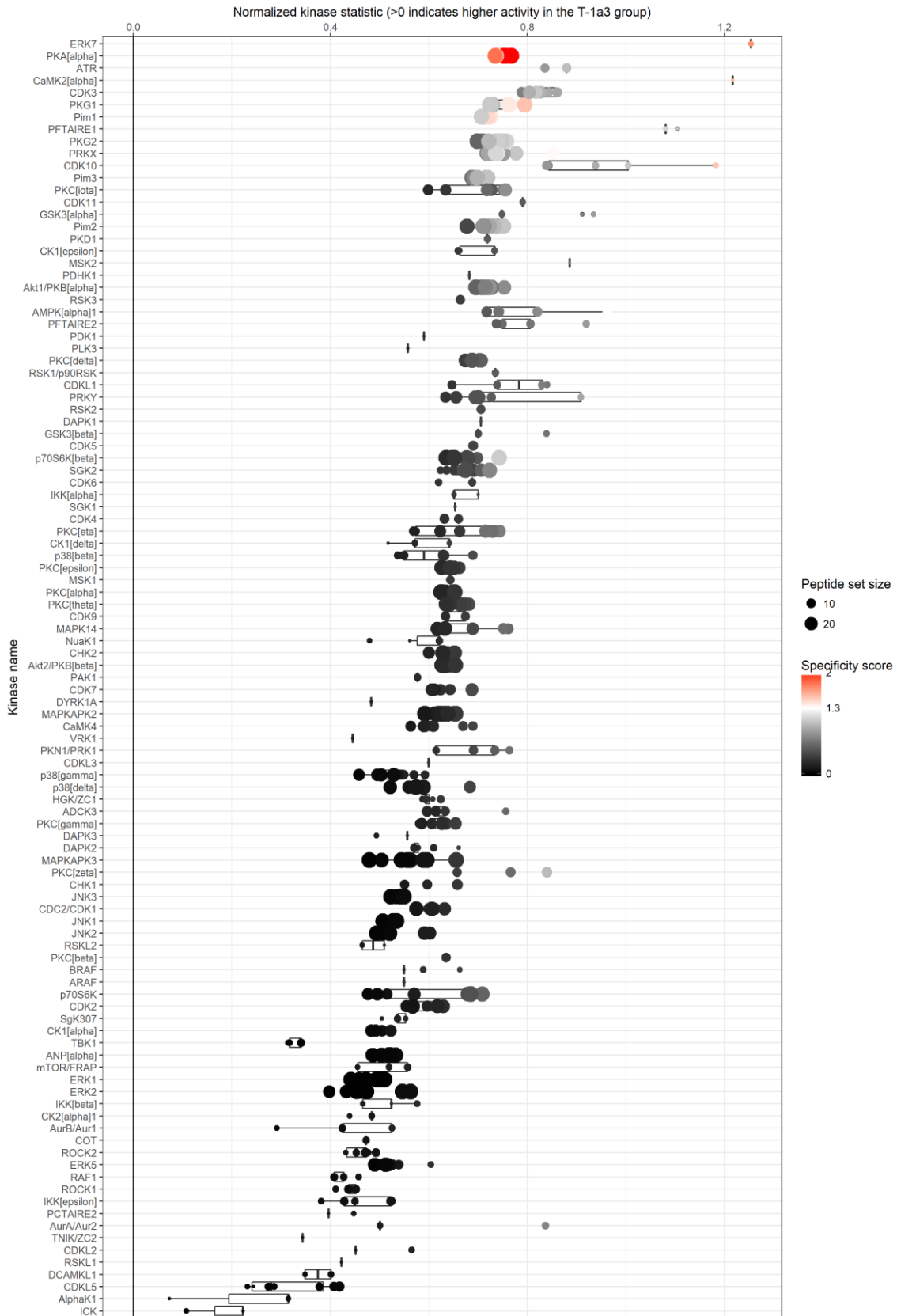


Figure S3. Kinase tree after 30-minute HMI-1a3 20 μ M treatment on Colo205 cell lysates versus DMSO treated cell lysates. The normalized kinase statistic is shown in the X-axis, and values >0 indicate activation compared to DMSO control. The colour of the point represents the specificity score from the kinase analysis, and a higher specificity score indicates a higher likelihood of the corresponding kinase to contributing to the observed phosphorylation changes. The size of the point indicates the peptide set size used for analysis.

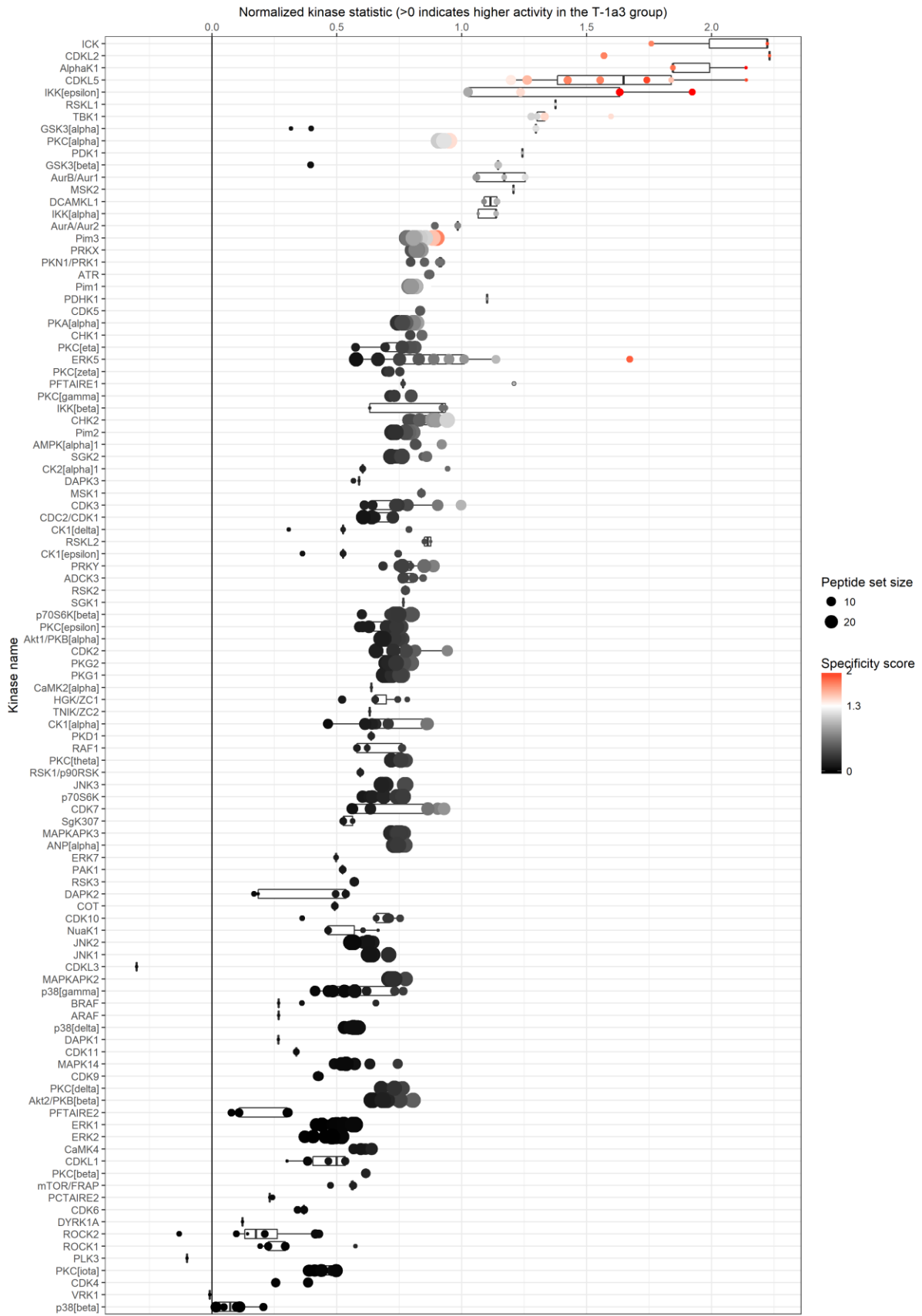


Figure S4. Kinase tree after 2-hour HMI-1a3 20 μ M treatment on Colo205 cell lysates versus DMSO treated cell lysates. The normalized kinase statistic is shown in the X-axis, and values >0 indicate activation compared to DMSO control. The colour of the point represents the specificity score from the kinase analysis, and a higher specificity score indicates a higher likelihood of the corresponding kinase to contributing to the observed phosphorylation changes. The size of the point indicates the peptide set size used for analysis.