Supplementary Information

Pooled screening for anti-proliferative inhibitors of protein-protein interactions

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Supplementary Figures. 1 to 11

Supplementary Datasets. 1, 2, and 3

Supplementary Tables

Supplementary Table 1. Summary of high-throughput screen.

	RWP1			HEK293T		
Time	Number of reads*	Number of peptides**	Number of reads/peptide***	Number of reads*	Number of peptides**	Number of reads/peptide***
T0	9596819	50290	71.04	11874786	50330	87.56
T1	9147277	50069	68.03	9446511	50099	70.20
T2	11219646	50148	83.36	10215684	48530	78.33
T3	7892054	49763	59.02	9053584	48771	69.10

* Total number of reads identified from three replicates

** Average number of peptides that are identified in each replicates

*** Average number of reads per peptide that are identified in each replicate

Supplementary Table 2. Experimental validation of effect of peptides on cell viability.

Peptide	Peptide Sequence	Type	Structural region		Dropout Score (RWP1)	Dropout Score (HEK293T)	Cell viability at Day 3					
							Estimation***		Infection (Average cell viability, %)			
			Disordered regions*	Linear motif**			RWP1	HEK293T	RWP1	MiaPaCa	Panc0203	HEK293T
ILF3	SGNSVCL	Cancer-specific	VCL are disordered	LIG_BIR_II_1, LIG_PDZ_Class_2	-10.01	0.32	69.16	97.26	83.79	100.08	84.25	88.03
P53/MDM2	ETFSDLW	Cancer-specific	Disordered region, ETF are disordered	-	-11.61	0.40	64.71	108.15	83.29	75.30	79.97	86.58
INCENP	AYSLKKH	Cancer-specific	-	LIG_BIR_II_1	-6.78	0.21	81.53	102.85	65.96	85.08	81.41	91.71
MUS81	CSYGPLT	Cancer-specific	LT are disordered	-	-4.85	0.52	87.53	104.94	64.53	82.95	81.01	89.41
p53/SFN	TEGPDSD	Cancer-specific	Disordered region, TEGPDSD are disordered	-	-5.74	0.95	87.81	108.63	73.14	74.85	79.96	94.51
GNG4	KFFCTIL	Cancer-specific	-	LIG_PDZ_Class_1	-6.40	-0.05	79.01	104.88	83.09	93.86	85.64	97.99
SLCO1A2	DELKTKL	Cancer-specific	Disordered region, DELKTKL are disordered	LIG_PDZ_Class_1	-9.34	0.38	72.43	102.12	94.57	81.24	81.97	100.02
DEFB136	AKDPWVH	Cancer-specific	AKDPWVH are disordered	LIG_AP2alpha_2, LIG_BIR_II_1	-10.40	0.10	68.92	97.13	83.27	98.34	92.23	99.80
PREP	DNFEGEY	Cancer-specific	-	LIG_AP2alpha_2, LIG_BIR_II_1	-9.62	0.33	72.68	109.25	70.91	82.10	82.30	81.28
OTOA	SSSRSPA	Cancer-specific	Disordered region, SSSRSPA are disordered	LIG_PDZ_Class_1, LIG_BIR_II_1	-11.09	-0.54	64.86	103.40	79.57	73.95	85.76	84.81
DPY19L2	YRVLKVN	Normal-specific	-	DOC_CYCLIN_1	-0.98	-3.43	93.63	82.91	93.97	88.40	94.43	81.48
EIF4B	PKLNLKP	Normal-specific	Disordered region, PKLNLKP are disordered	-	-0.98	-3.15	90.65	86.81	96.61	99.83	94.20	90.90
TMEM25	SSDEIWL	Normal-specific	SDEIW are disordered	LIG_BIR_II_1, LIG_PDZ_Class_2	0.95	-3.18	105.94	82.62	94.21	82.30	76.10	85.52
ATL1	SEEEEPV	Normal-specific	Disordered region, SEEEEPV are disordered	LIG_BIR_II_1, LIG_PDZ_Class_3, LIG_TRAF2_1	0.60	-2.77	105.42	86.81	91.25	96.78	96.70	85.92
MTMR2	TSSSERA	Normal-specific	Disordered region, TSSSERA are disordered	LIG_PDZ_Class_3	0.93	-2.66	99.94	90.85	95.52	100.38	93.05	78.42

* Disordered regions are defined as segments of at least 10 disordered residues. Disordered residues are identified using DISOPRED2 (http://bioinf.cs.ucl.ac.uk/psipred/?disopred=1)
** Elm names (http://elm.eu.org) are shown as linear motifs (probability < 0.005)
*** Cell viability at day 3 is estimated using polynomial regression

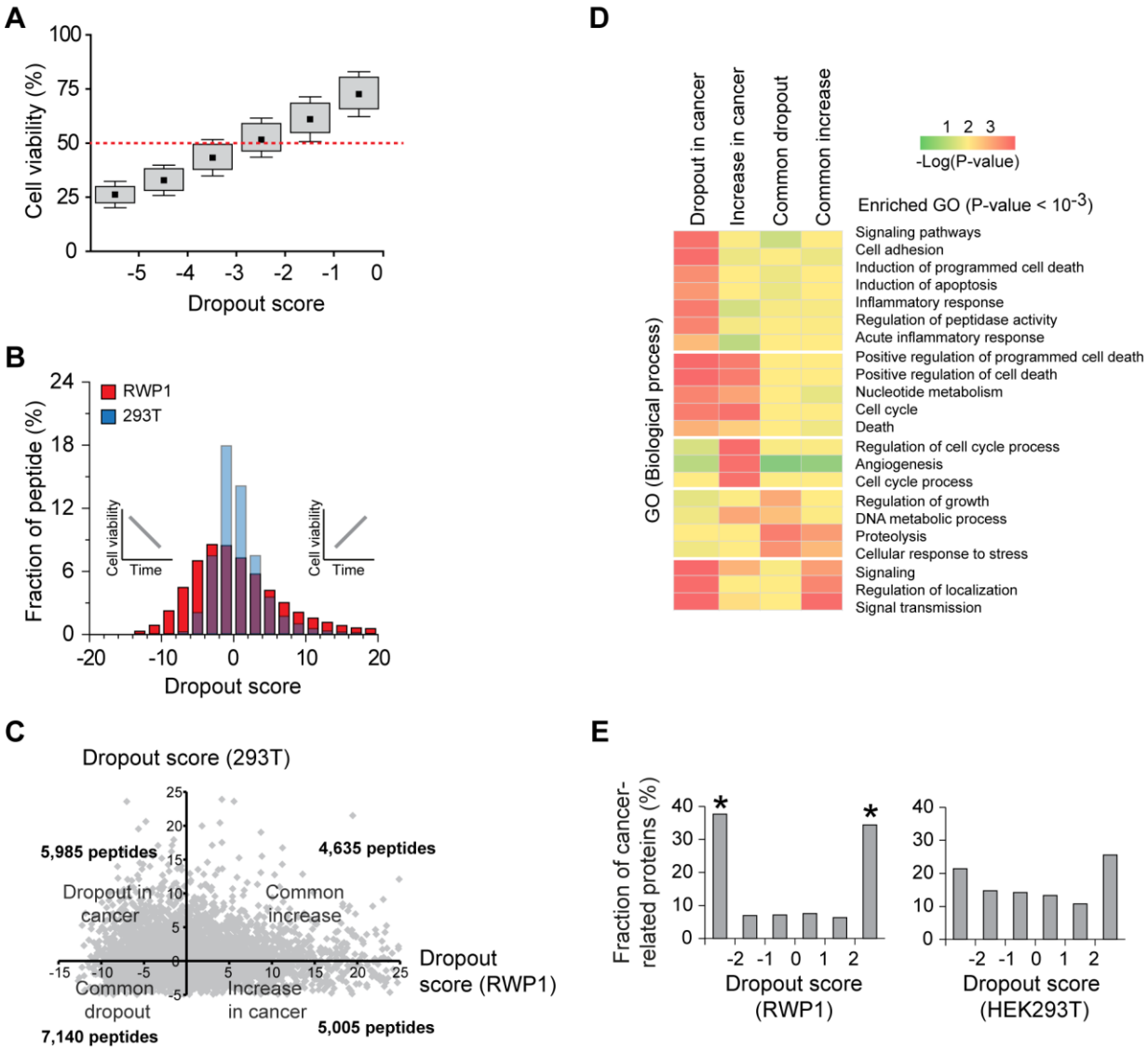
Supplementary Table 3. Structures and analytical data of peptides used in the studies.

Peptide	Peptide structure	Theoretical molecular mass	Molecular mass determined by LC/MS*
ILF3-1	Pal-LRVSGNSVCL-OH	1285.74	1285.8
CTLA4-1	Pal- KQFQPYFIPIN-NH_2	1632.14	1632
GCHFR-1	Pal-VGQTLVWCLHKE-NH_2	1650.17	1649.4
TGFBR3-1	Pal-TQSTPCSSSSTA-OH	1394.69	1394.7
GNG4-1	Pal-NPFREKKFFCTIL-NH_2	1880.49	1880.6
GNG4-2	Pal-CFLFNERPKIKFT-NH_2	1880.49	1879.9
MUS81-1	Pal-RTLSQLYCSYGPLT-OH	1840.35	1840
MUS81-2	Pal-CLRGTYLPQYTSLS-OH	1840.35	1840
MUS81-3	Ac-RTLSQLYCSYGPLT-OH	1643.85	1643.2
MUS81-4	Ac-CLRGSYLPQYTSLT-OH	1643.85	1643.2
TRAF2-1	Pal-IFIKAIVDLTGL-NH_2	1540.12	1539.6
INCENP-1	Pal-PSSLAYSLKKH-OH	1468.93	1468.2
INCENP-2	Pal-SHKLSPKSYLA-OH	1468.93	1468.2
INCENP-3	Ac-PSSLAYSLKKH-OH	1272.43	1272
INCENP-4	Ac-SHKLSPKAYLS-OH	1272.43	1272

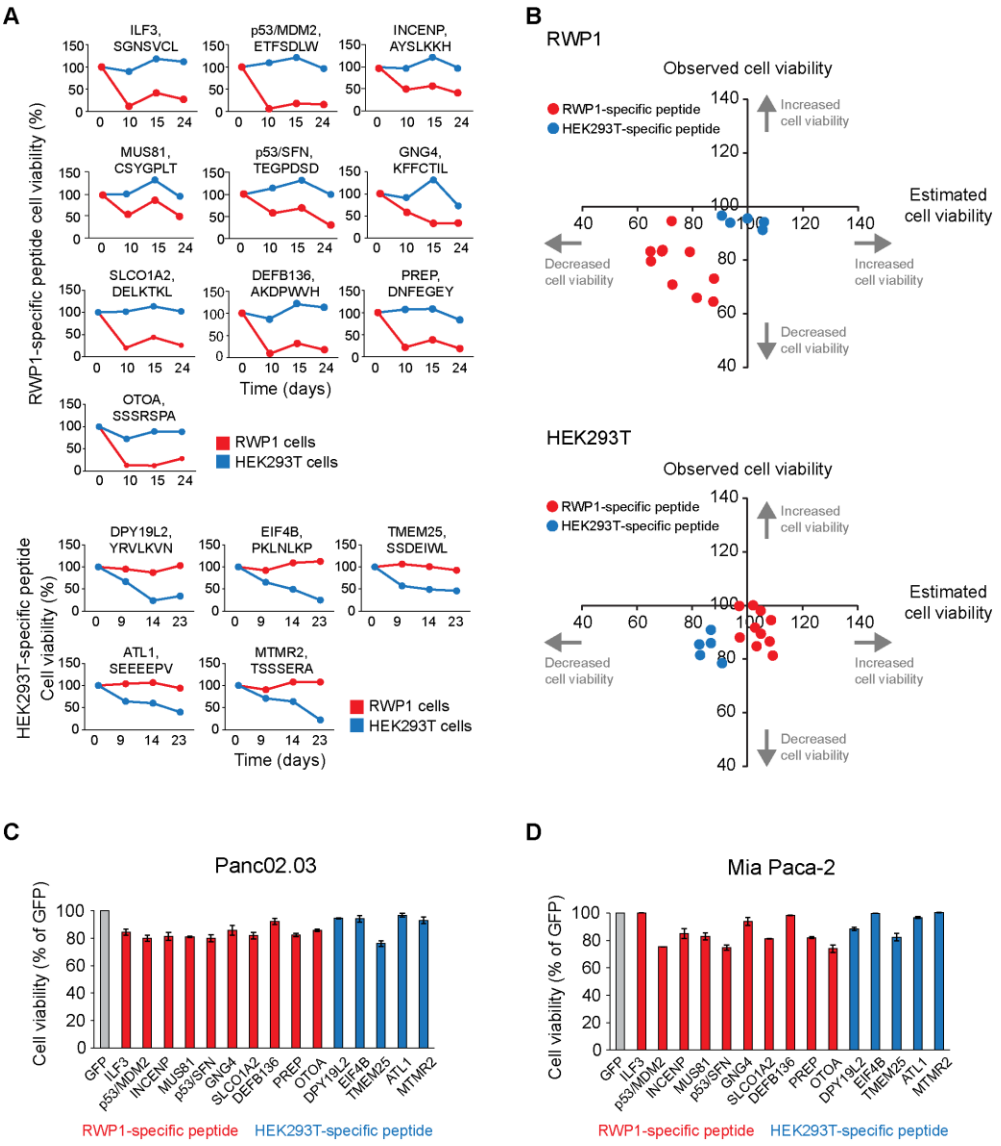
*Molecular masses were determined using Agilent 6100 LC/MS spectrometer.

Scrambled peptides of GNG4, MUS81 and INCENP are represented as GNG4-2, MUS81-2/ MUS81-4 and INCENP-2/INCENP-4.

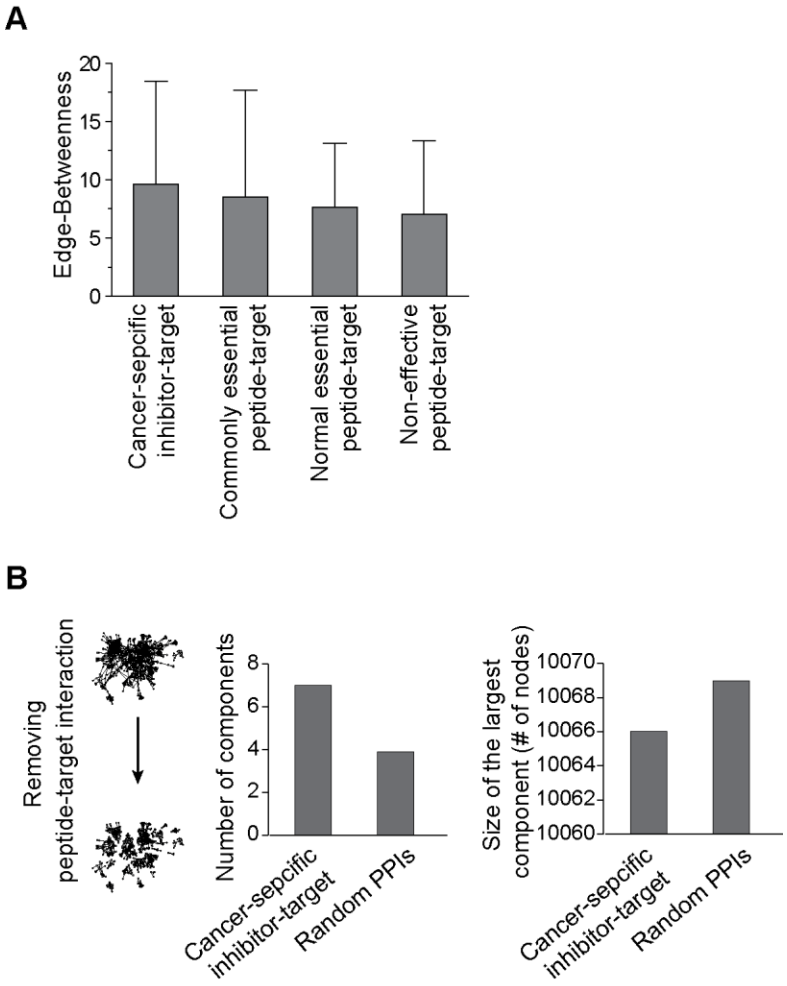
Supplementary Figures



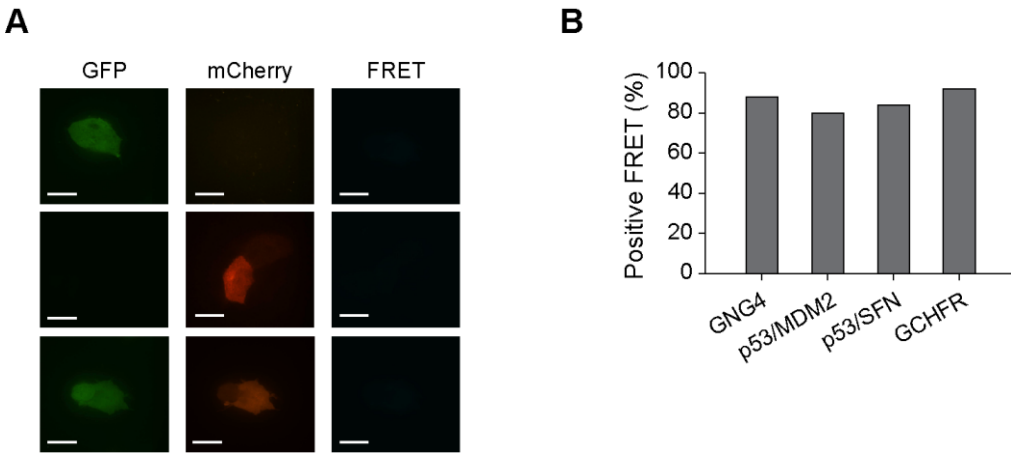
Supplementary Fig. 1. Biological properties of c-terminal peptides. (A) The change of cell viability depending on dropout score. Data represent mean values ± s.d. (B) Dropout score distribution in RWP1 and HEK293T cells. (C) Comparison of dropout scores in RWP1 and HEK293T cells. (D) Functional enrichment of proteins hosting the peptides that lead to specific regulation of cell viability. (E) Cancer association of peptides. * P-value < 0.0001.



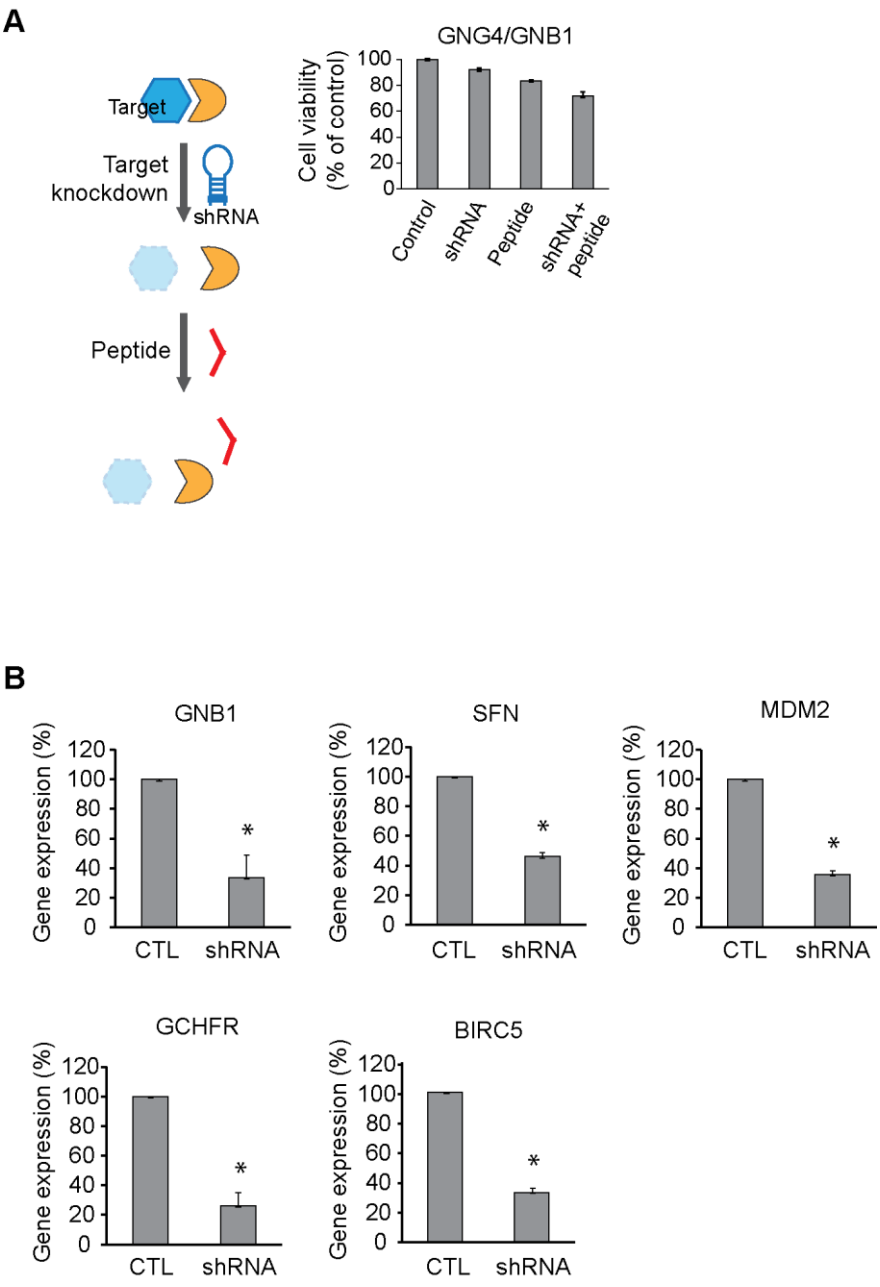
Supplementary Fig. 2. Experimental validation of the effect of peptides on cell viability. (A) Time-dependent changes of cell viabilities. Cell viabilities are measured based on pooled screens. Source proteins and peptide sequences are shown. (B) Scatter plot showing correlations of the effects on cell viability of the pooled (estimated cell viability) and single peptide assays (observed cell viability). The correlations of the quantitative cell viability effects between the pooled and single peptide assays are 0.33for HEK293T and 0.37 for RWP1. Experimental validation of the effect of peptides on cell viability in single-lentivirus infection experiments in Panc02.03 (C) and Mia Paca-2 (D) cell lines. Red bars represent cancer (RWP1)-specific peptides and blue bars represents normal (HEK293T)-specific peptides. Experiments were done in triplicate. Data represent mean values ± s.d.



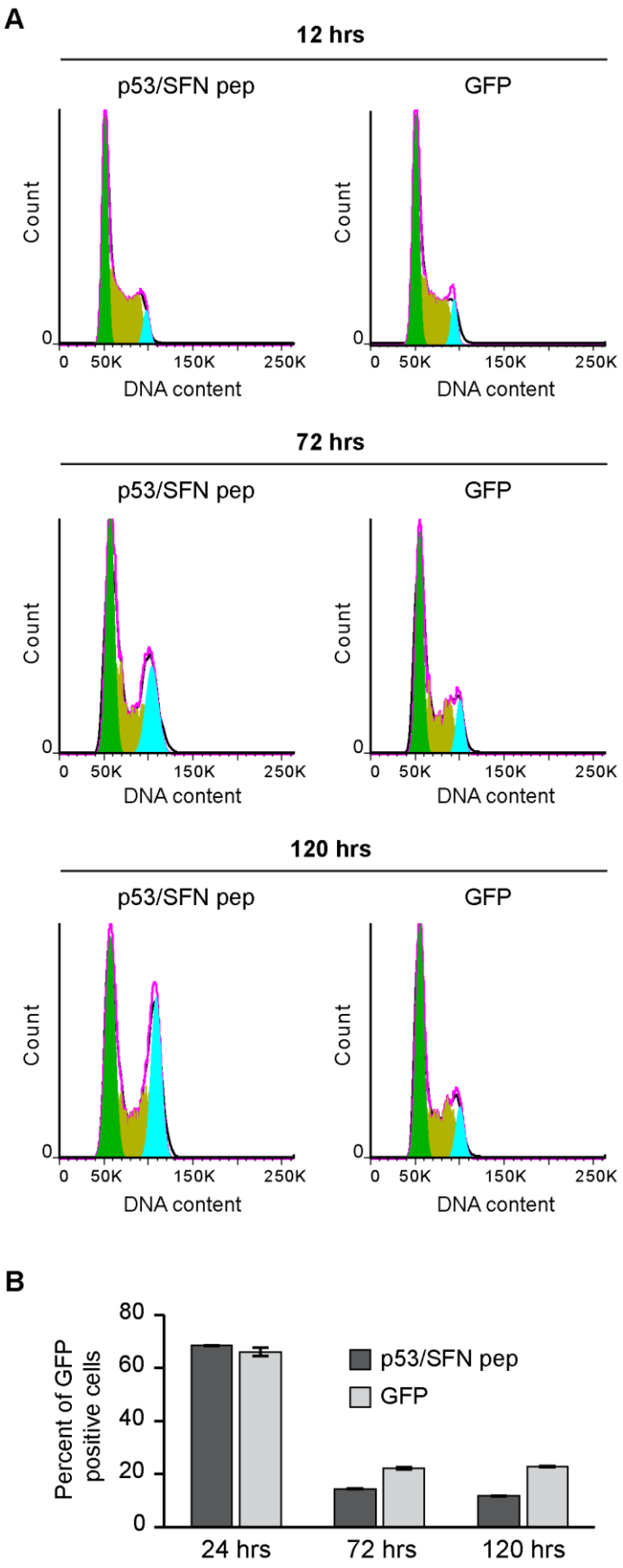
Supplementary Fig. 3. Network topological properties of peptide-target interactions. (A) Edge-betweenness of cancer-specific inhibitor-target interactions and other types of interactions. Data represent mean values ± s.d. (B) Change of number of components and size of the largest component in network by removing cancer-specific inhibitor-target interactions.



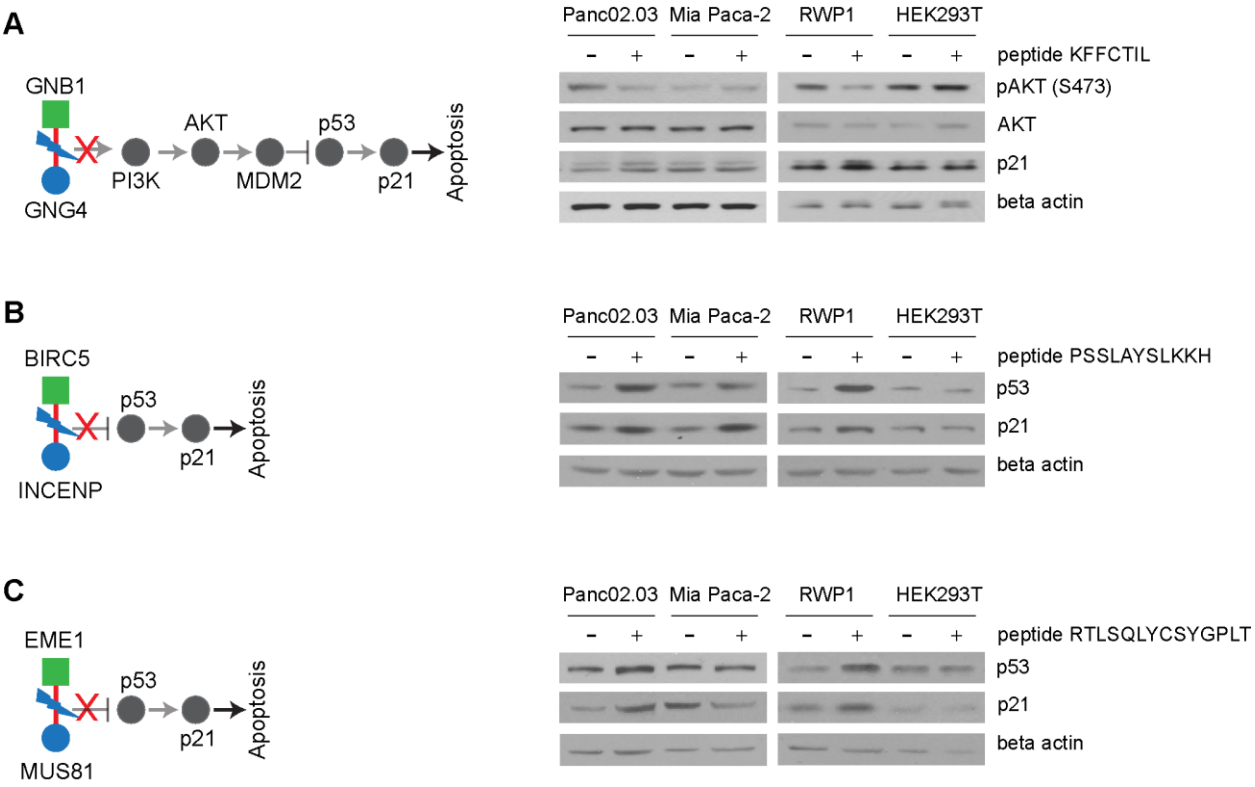
Supplementary Fig. 4. Characterization of peptide-target interactions using FRET. (A) FRET signal of controls. Upper images represent GFP alone, middle images represent mCherry alone, and bottom images represent GFP and mCherry in the same cell. White scale bars represent 15 µm. (B) Quantification of positive FRET signal. Counting 25 individual cells in each microscopic image, we observe positive signal between the peptides and their putative target in over 80% of cells.



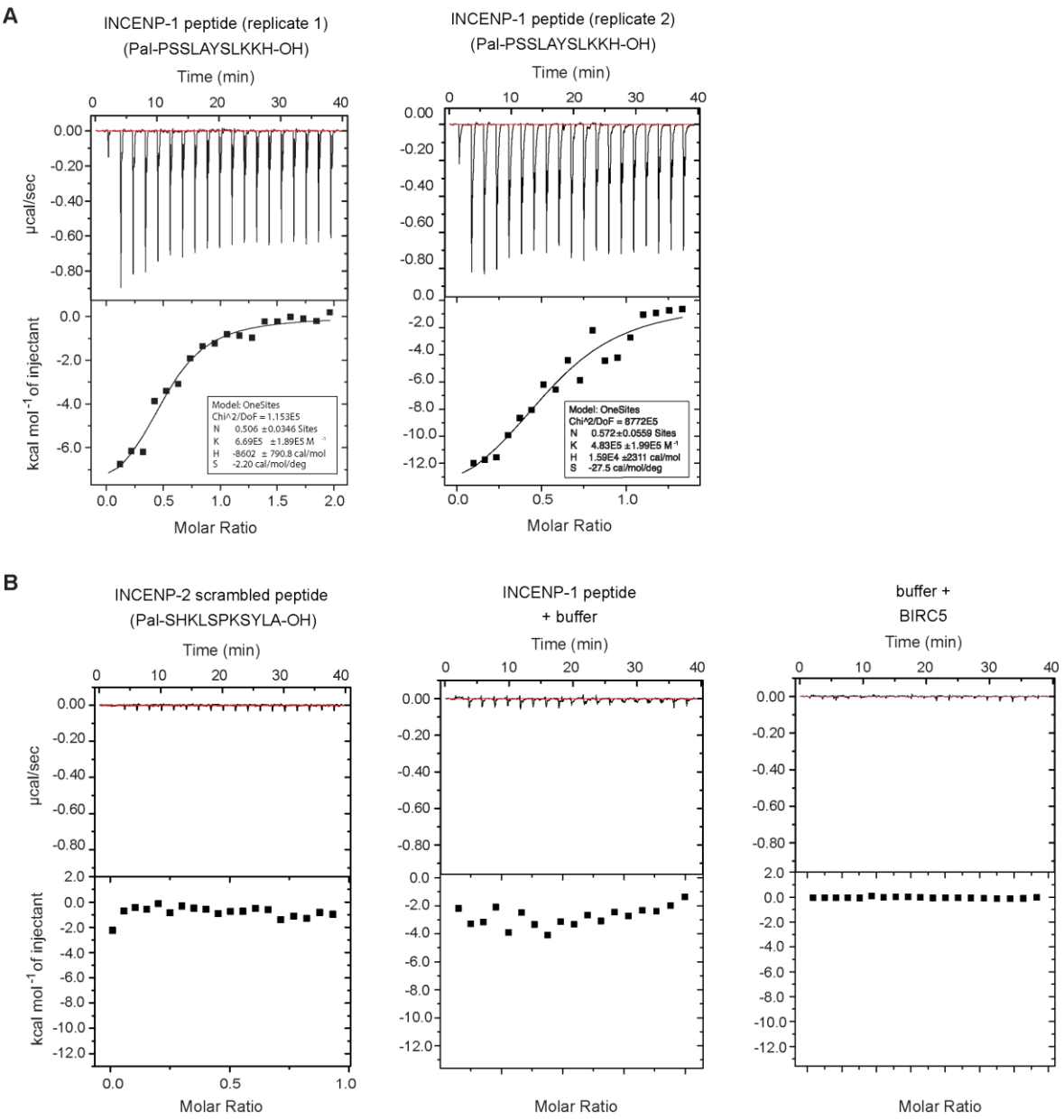
Supplementary Fig. 5. Experimental validation of cancer-specific peptide-target interactions. (A) shRNA-induced cell viability rescue experiment. Cell viabilities are measured with cells stably expressing shRNA or transfected with the peptide. For the rescue experiment, cells stably expressing shRNA are transfected with the peptide. Experiments were done in triplicate. Data represent mean values ± s.d. (B) Change of gene expression level after shRNA knockdown. Gene expression of target protein is measured with cells stably expressing shRNA. * P-value < 0.05. Experiments were done in triplicate. Data represent mean values ± s.d.



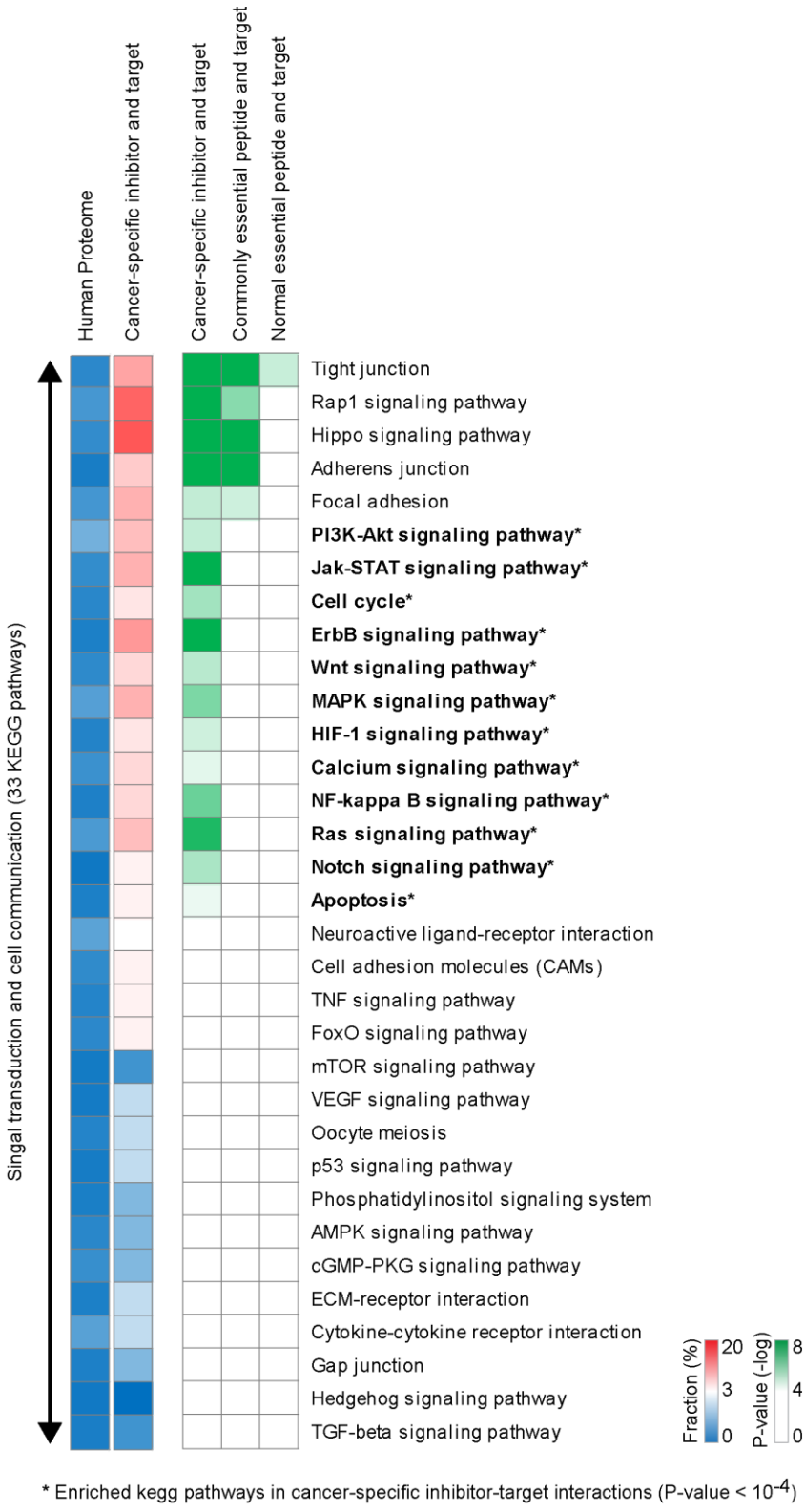
Supplementary Fig. 6. Time-dependent effect of SFN peptide on cell cycle. (A) Cells are transfected with the peptide and stained with DAPI for FACS analysis of cell cycle. The effect of 24 hours, 72 hours and 120 hours are shown. (B) Percent of GFP positive cells at different time points. Experiments were done in triplicate. Data represent mean values ± s.d.



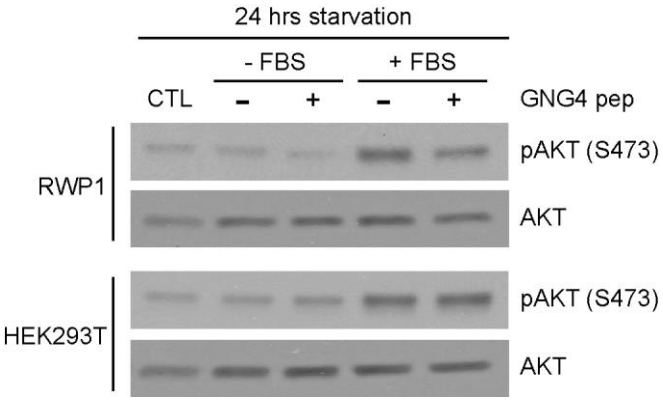
Supplementary Fig. 7. Mechanism of action of cancer-specific peptides (GNG4, INCENP and MUS81)-target (GNB1, BIRC5 and EME1) interaction. (A) KFFCTIL (inhibiting GNG4/GNB1). Expression of this peptide leads to a decrease in AKT activity (as measured by AKT phosphorylation at S473) and subsequently an increase in P21 levels, suggesting it triggers apoptosis. (B) PSSLAYSLKKH (inhibiting INCENP/BIRC5) and (C) RTLSQLYCSYGPLT (inhibiting MUS81/EME1) induce an increase in P53 and P21 levels, suggesting they lead to apoptosis. All experiments were done in triplicate. Full gel images in Supplementary Fig. 11.



Supplementary Fig. 8. ITC validation of INCENP peptide binding to BIRC5. (A) The binding of the INCENP peptide can be fit to a Kd≈1.49±0.42 µM (replicate 1) and Kd≈2.07±1.03 µM (replicate 2). Experiments were done in quadruplicate. (B) The scrambled peptide displays no detectable binding. Titration of the peptide into buffer and buffer titration into the protein (BIRC5) are shown.

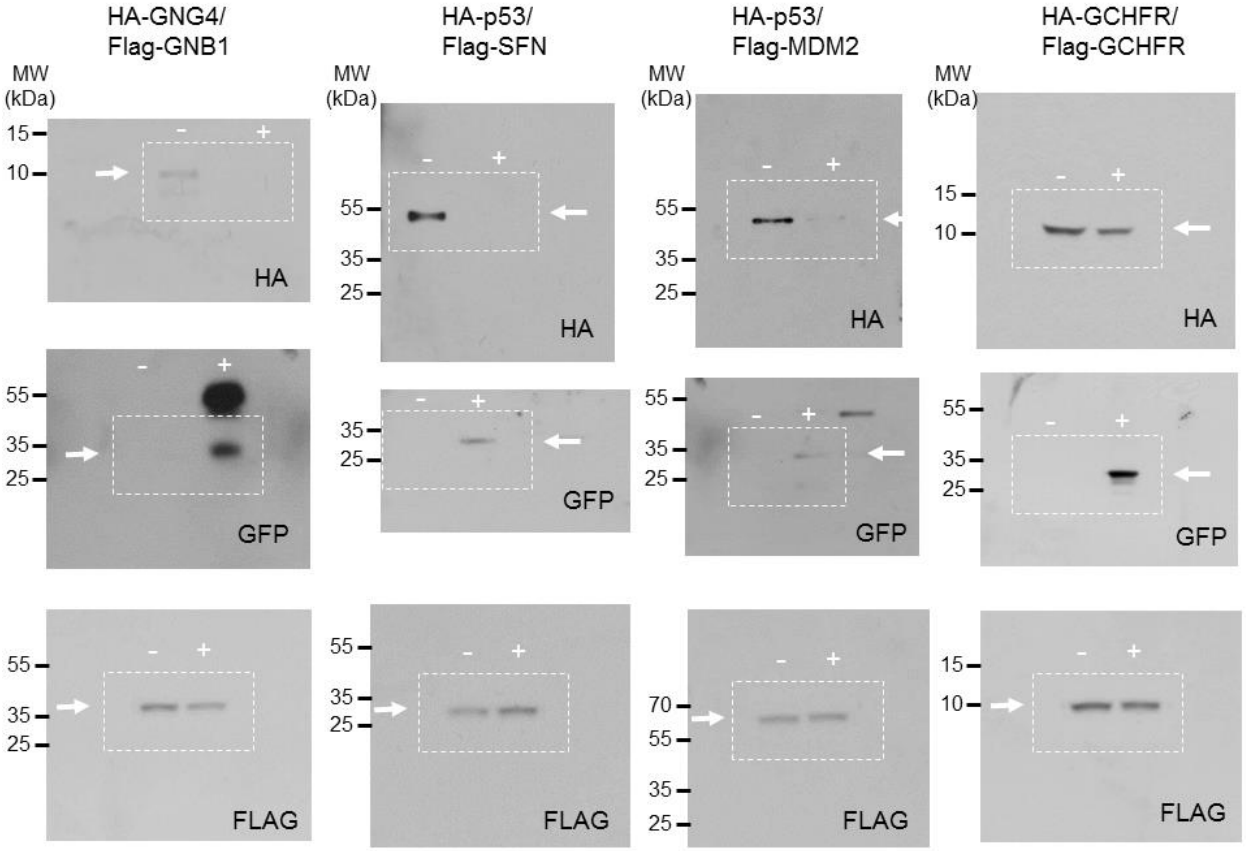


Supplementary Fig. 9. Enriched pathways of cancer-specific inhibitors and their interacting targets. Proportion of cancer-specific inhibitors and their targets in each pathway is graded from red (high) to blue (low). Enrichment of pathways in cancer-related inhibitors/targets, common dropout peptide/targets and normal-specific peptide/targets are graded from green (enriched) to white (not enriched).



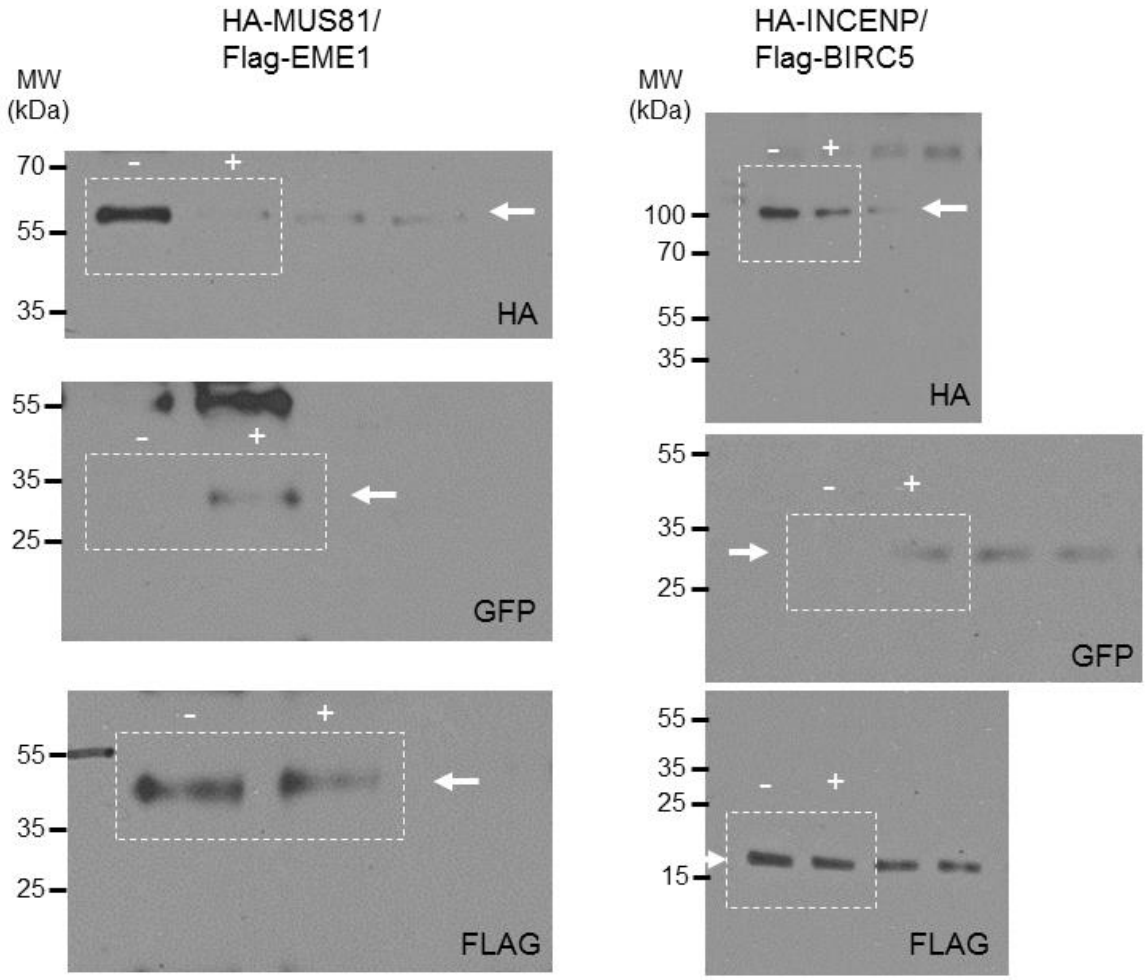
Supplementary Fig. 10. GNG4 peptide inhibits AKT phosphorylation induced by serum stimulation in RWP1. Cells are starved for 24 hours and 10% FBS is added for 20 min for induction of AKT phosphorylation. Experiments were done in duplicate. Full gel images in Supplementary Fig. 11.

Fig. 3B

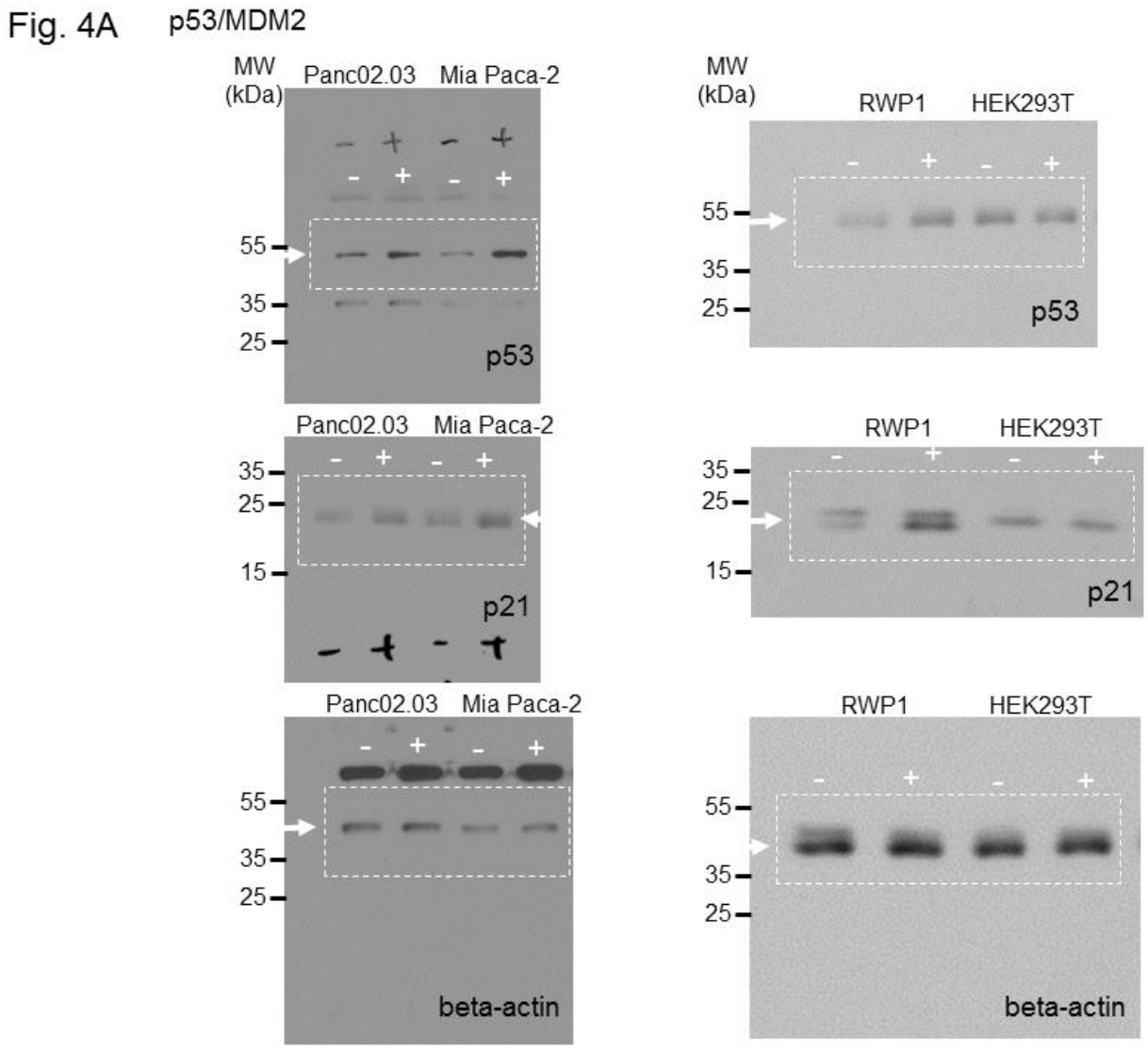


Supplementary Fig. 11. Full-size images of blots and gels (continue to next page)

Fig. 3B

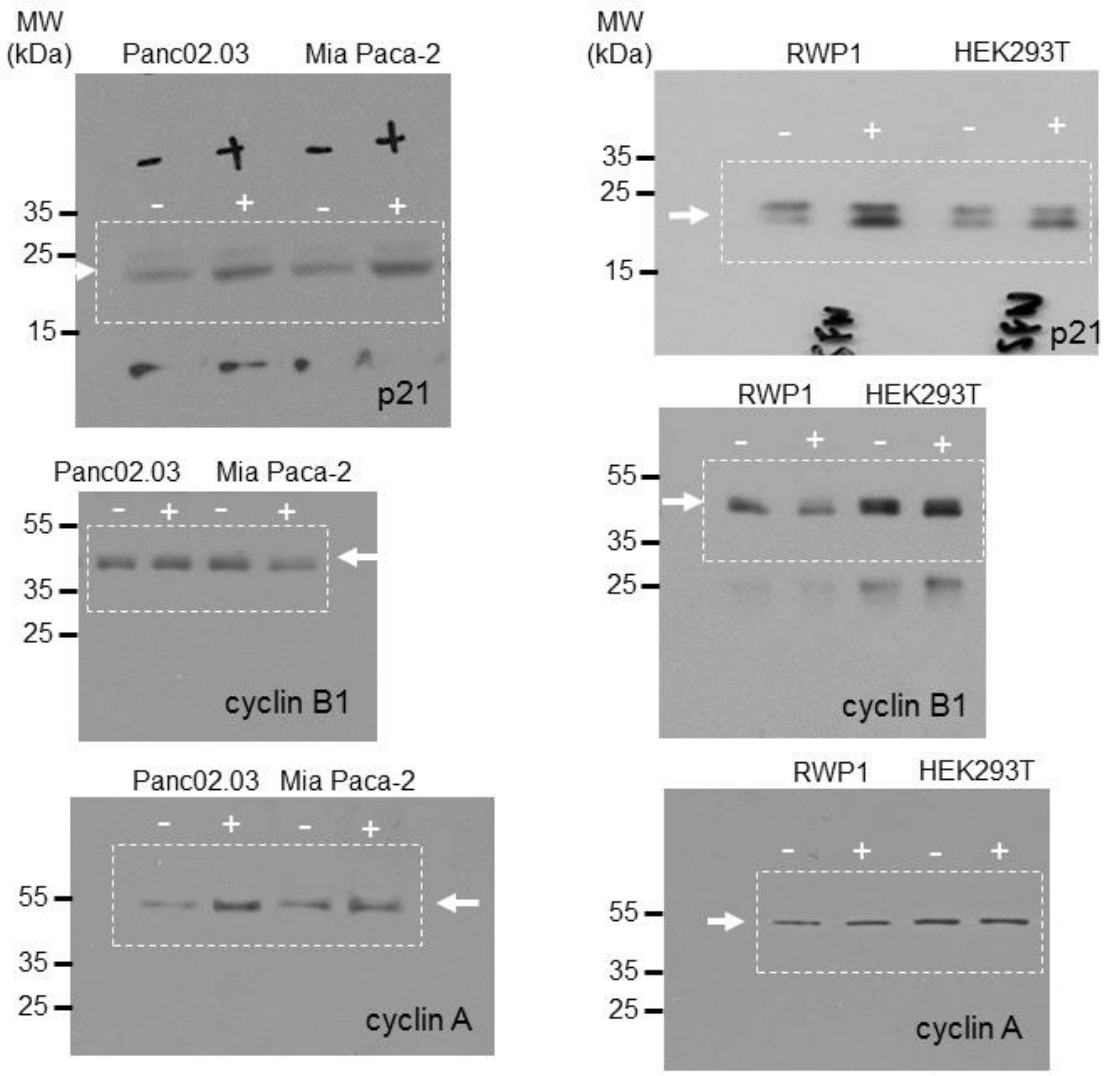


Supplementary Fig. 11. Full-size images of blots and gels (continue to next page)



Supplementary Fig. 11. Full-size images of blots and gels (continue to next page)

Fig. 4B p53/SFN



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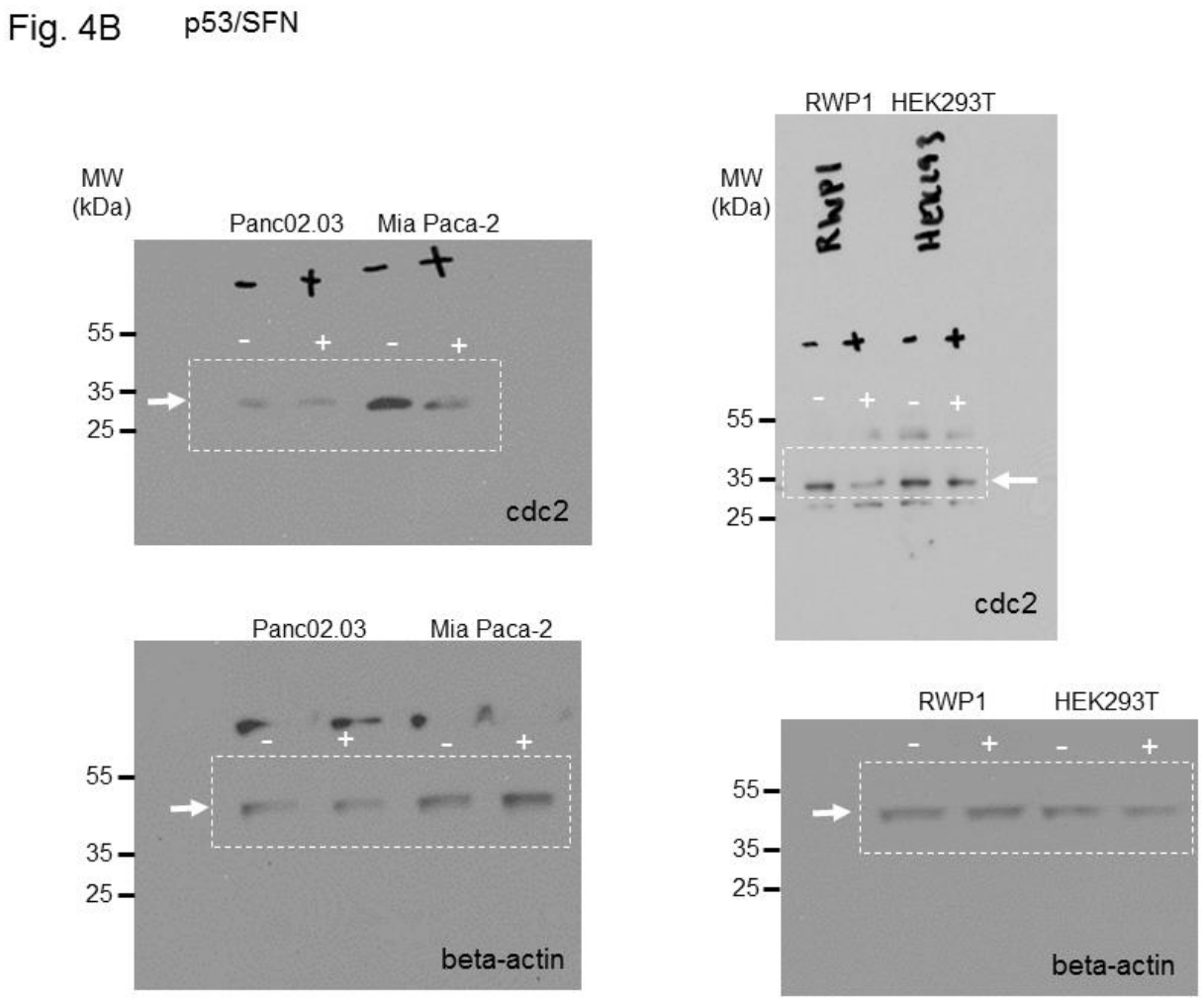
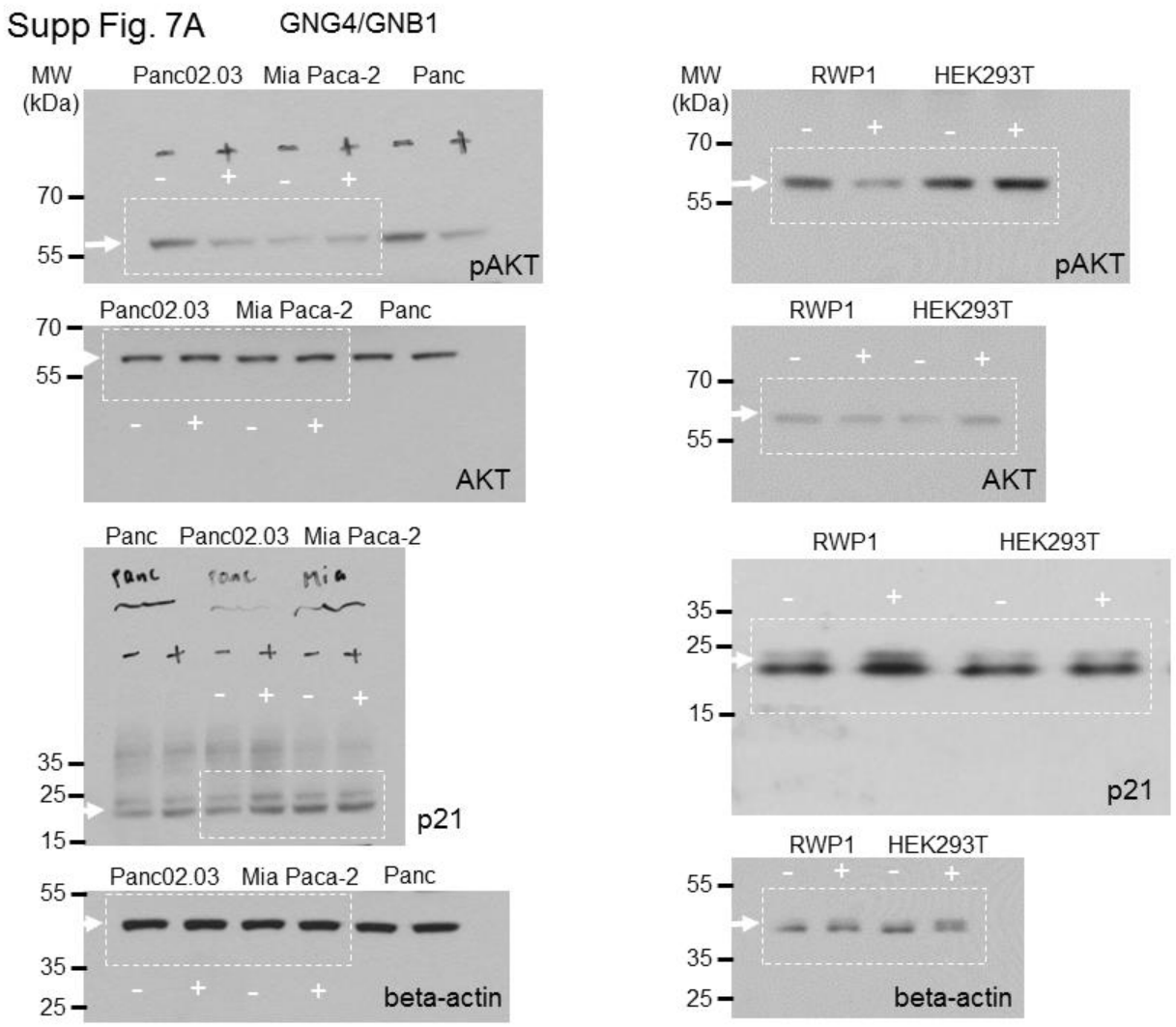
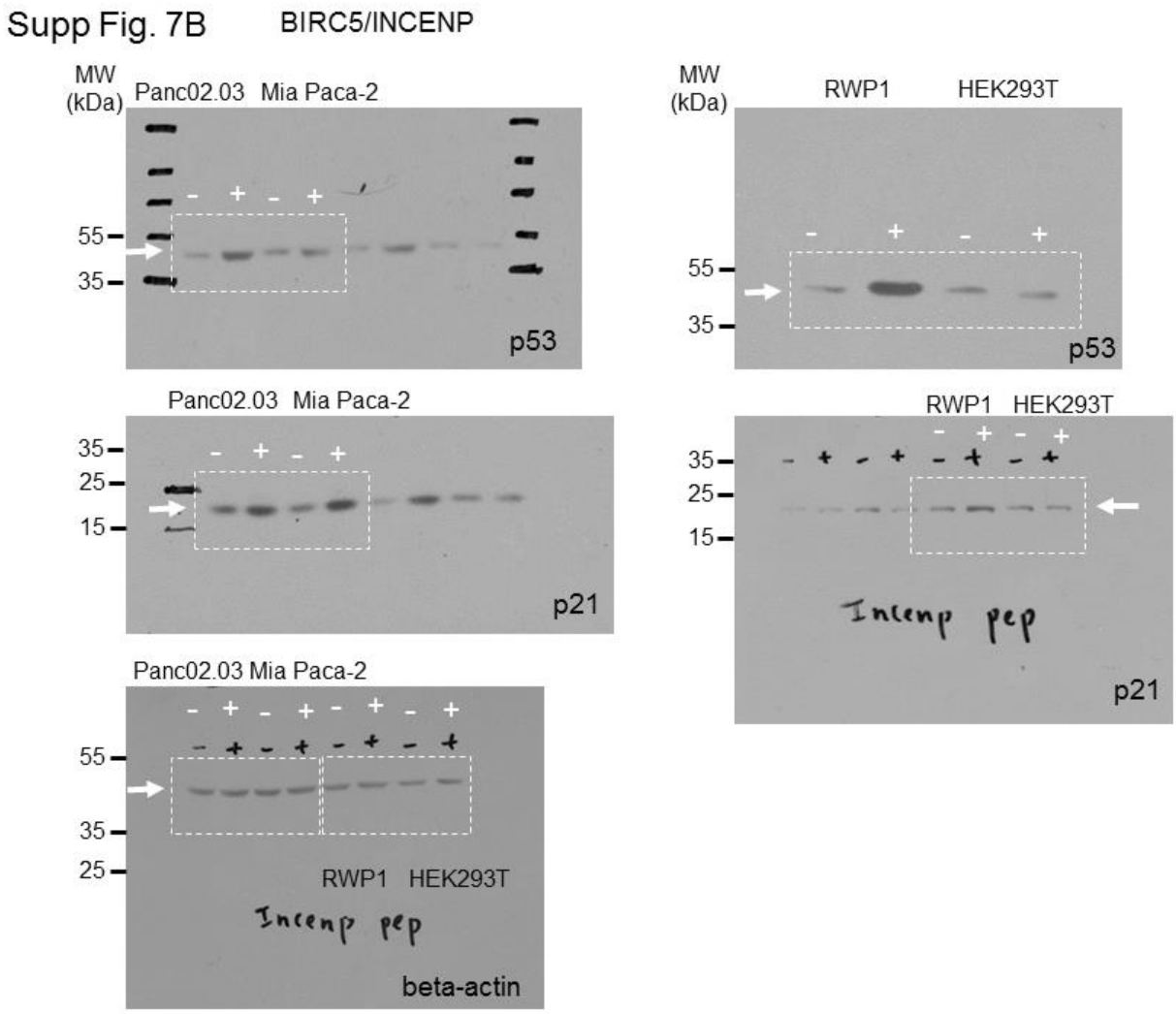


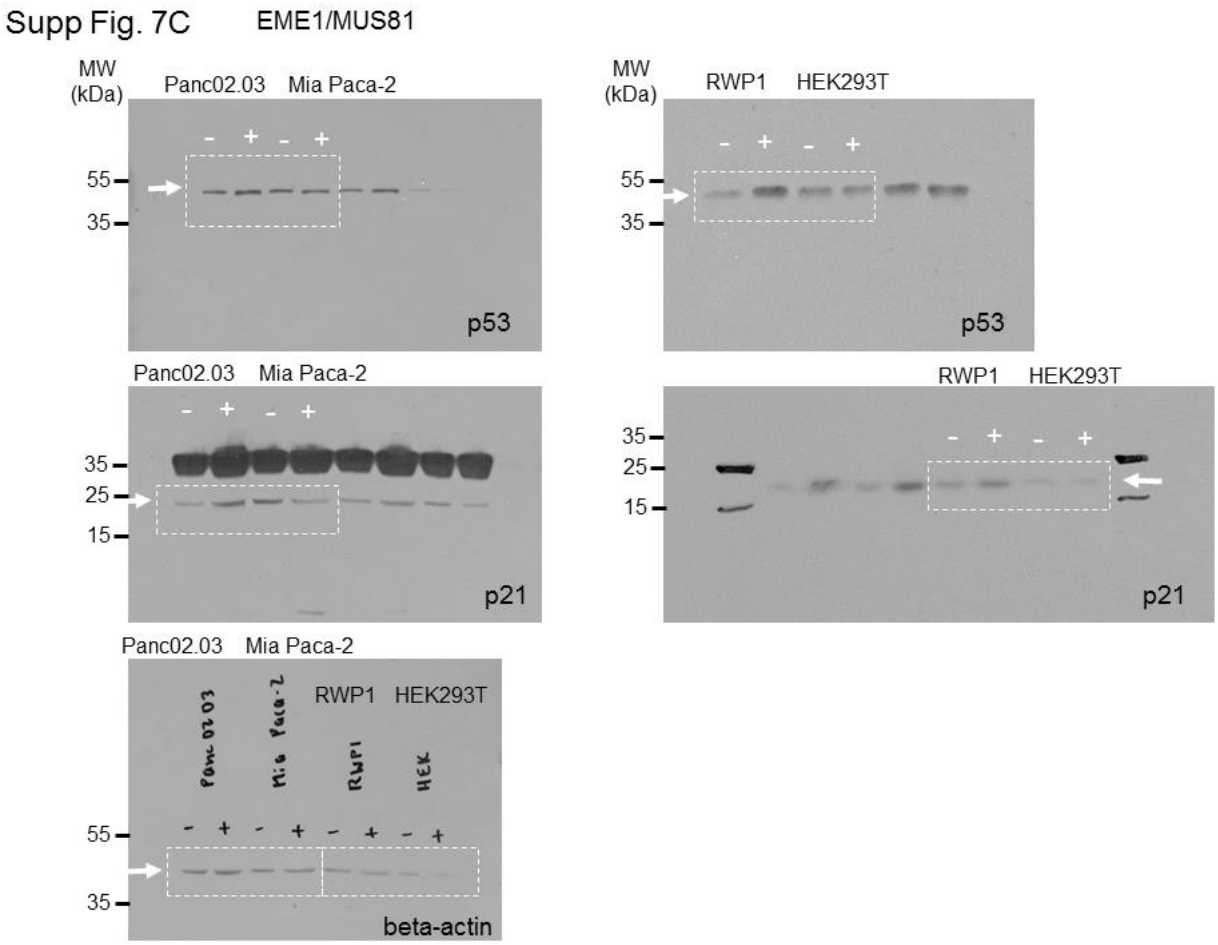
Supplementary Fig. 11. Full-size images of blots and gels (continue to next page)



Supplementary Fig. 11. Full-size images of blots and gels (continue to next page)

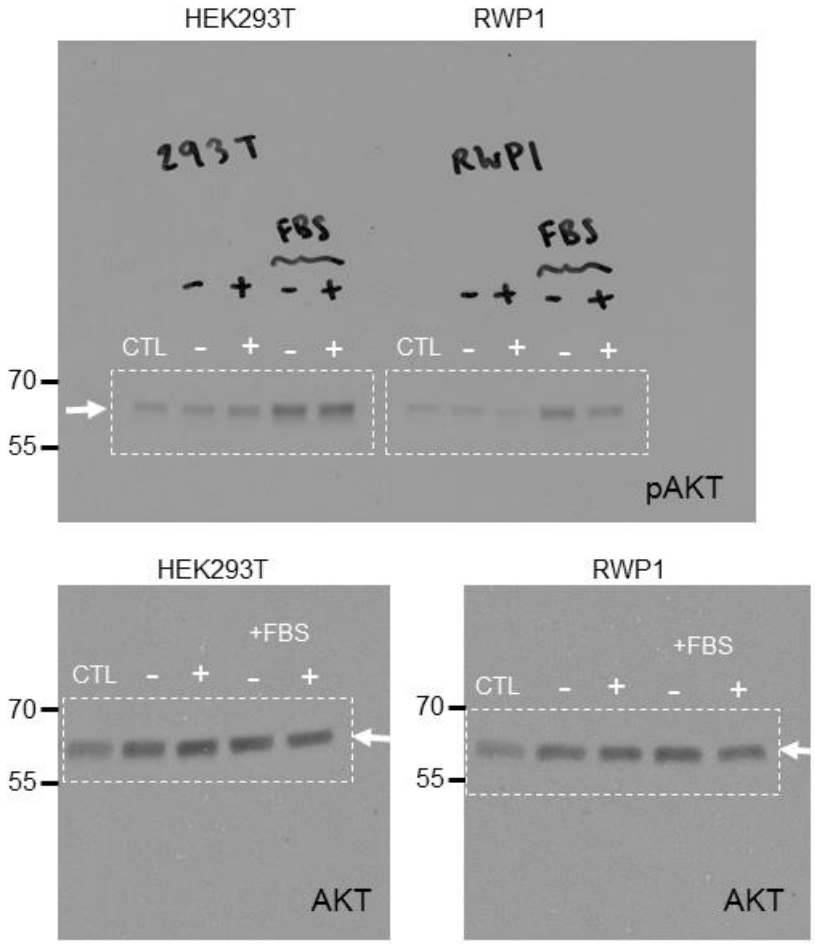


Supplementary Fig. 11. Full-size images of blots and gels (continue to next page)



Supplementary Fig. 11. Full-size images of blots and gels (continue to next page)

Supp Fig. 10 GNG4 pep



Supplementary Fig. 11. Full-size images of blots and gels.

Supplementary Datasets

Supplementary Dataset 1. The list of human peptides for dropout screen and their effect on cell viability. Dropout scores of 50,549 peptides in two cell lines and identified number of reads are presented.

Supplementary Dataset 2. A list of cancer-specific inhibitors. Peptides that show dropout (dropout score < -2.5) in RWP1 cells but do not show any change in HEK293T cells (-1 < dropout score < 1) are considered as cancer-specific inhibitors.

Supplementary Dataset 3. Peptide-target interactions. Potential targets of peptides are identified based on structural evidences and peptide linear motifs. Pubmed IDs are shown when targets have been reported their cancer-related function.