

Supplemental information

Characterization of RAS/RAF/ERK Signal Cascade as a Novel Regulating Factor in Alpha-Amanitin Induced Cytotoxicity in Huh-7 cells

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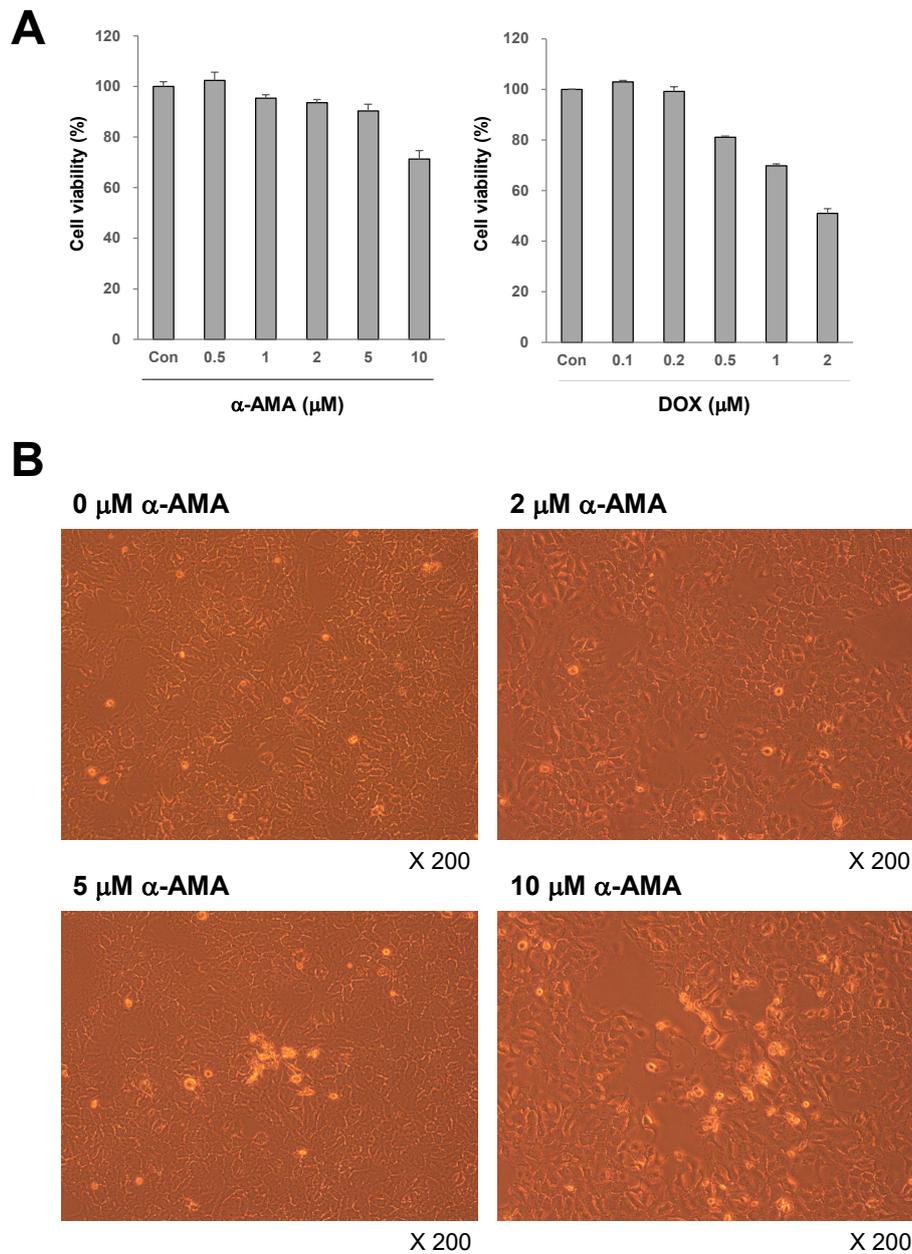


Figure S1. Alpha-amanitin (α-AMA) induced cytotoxicity in Huh-7 cells. A. Cell viability assay after α-AMA treatment. Cell density was 5×10^3 cells/well in 96-well plate.

The CCK-8 assay kit was used for checking cell viability. Doxorubicin (DOX) was treated as an experiment control. Data are showed by the mean \pm SD. **B.** Cell morphology changes. Microscopical magnification was set to 200 X.

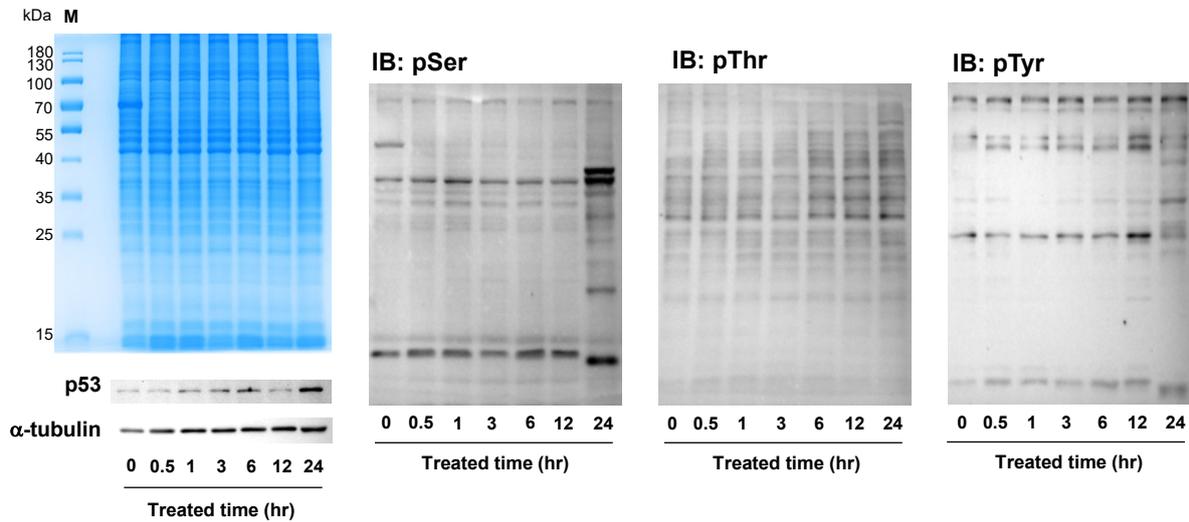


Figure S2. Monitoring of phosphorylation expression patterns by Western blots in Huh-7 cells after alpha-amanitin (α -AMA) treatment. Phosphorylation changes were observed up to 24 hr after treatment with 5 μ M AMA in Huh-7 cells by Western blotting analysis. Phosphorylation patterns for the phospho-serine (pSer), phospho-threonine (pThr) and phospho-tyrosine (pTyr) were detected using corresponding antibodies.

Table S1. All identified phosphosites after treatment to α -AMA (5 μ M) up to 12 hr to Huh-7 cells.

Identified as excel file

Table S2. Phosphorylation information classified in Spliceosome of KEGG category in Cluster 8

Proteins	Protein names	Gene	Position
S4R3H4	Apoptotic chromatin condensation inducer in the nucleus	ACIN1	S158, S780, S652, S330, S599, S328, S946
F8W1J5	Probable ATP-dependent RNA helicase DDX23	DDX23	S107, S109
J3KTA4	DEAD box protein 5	DDX5	S498
A2AB15	Pre-mRNA-splicing factor ATP-dependent RNA helicase DHX16	DHX16	S100 S47 S46 S43
K7END7	ATP-dependent RNA helicase DHX8	DHX8	H58
F8W6I7	Helix-destabilizing protein	HNRNPA1	S6
H7C1J8	Heterogeneous nuclear ribonucleoprotein A3	HNRNPA3	S92, S95
P61978	Heterogeneous nuclear ribonucleoprotein K	HNRNPK	S216, S284
Q15365	Poly(rC)-binding protein 1	PCBP1	S190, S173
A0A0A0MRN0	Pre-mRNA-splicing factor 38B	PRPF38B	S416, S418, S209
O75400	Pre-mRNA-processing factor 40 homolog A	PRPF40A	S883, S885, S888
Q5W011	Splicing factor 45	RBM17	S155
P49756	RNA-binding protein 25	RBM25	S677
Q9Y5S9	RNA-binding protein 8A	RBM8A	S46, S56
H3BUY5	RNA-binding motif protein, X chromosome	RBMX	S80, S198
O75533	Splicing factor 3B subunit 1	SF3B1	T248
E9PJ04	Splicing factor 3B subunit 2	SF3B2	S305, S307
O95391	Pre-mRNA-splicing factor SLU7	SLU7	S215
O75643	U5 small nuclear ribonucleoprotein 200 kDa helicase	SNRNP200	S225
P08621	U1 small nuclear ribonucleoprotein 70 kDa	SNRNP70	S226
G3V4X8	SNW domain-containing protein 1	SNW1	S62
J3KSR8	Serine/arginine-rich-splicing factor 1	SRSF1	S129, Y132, S94, S100
O75494	Serine/arginine-rich splicing factor 10	SRSF10	S156, S158, S160, S107, S133, S131, S129
Q01130	Serine/arginine-rich splicing factor 2	SRSF2	T25, S26, S189, S191
Q13243	Serine/arginine-rich splicing factor 5	SRSF5	S253, S250
Q13247	Serine/arginine-rich splicing factor 6	SRSF6	S303, S314, S316
Q13242	Serine/arginine-rich splicing factor 9	SRSF9	S211, S216, H213 Y221, Y214
Q13595	Transformer-2 protein homolog alpha	TRA2A	T202
P62995	Transformer-2 protein homolog beta	TRA2B	S85, Y86, S87
P26368	Splicing factor U2AF 65 kDa subunit	U2AF2	S79
H7C4V2	U2 snRNP-associated SURP motif-containing protein	U2SURP	T239
F8WC91	U4/U6.U5 tri-snRNP-associated protein 2	USP39	S82

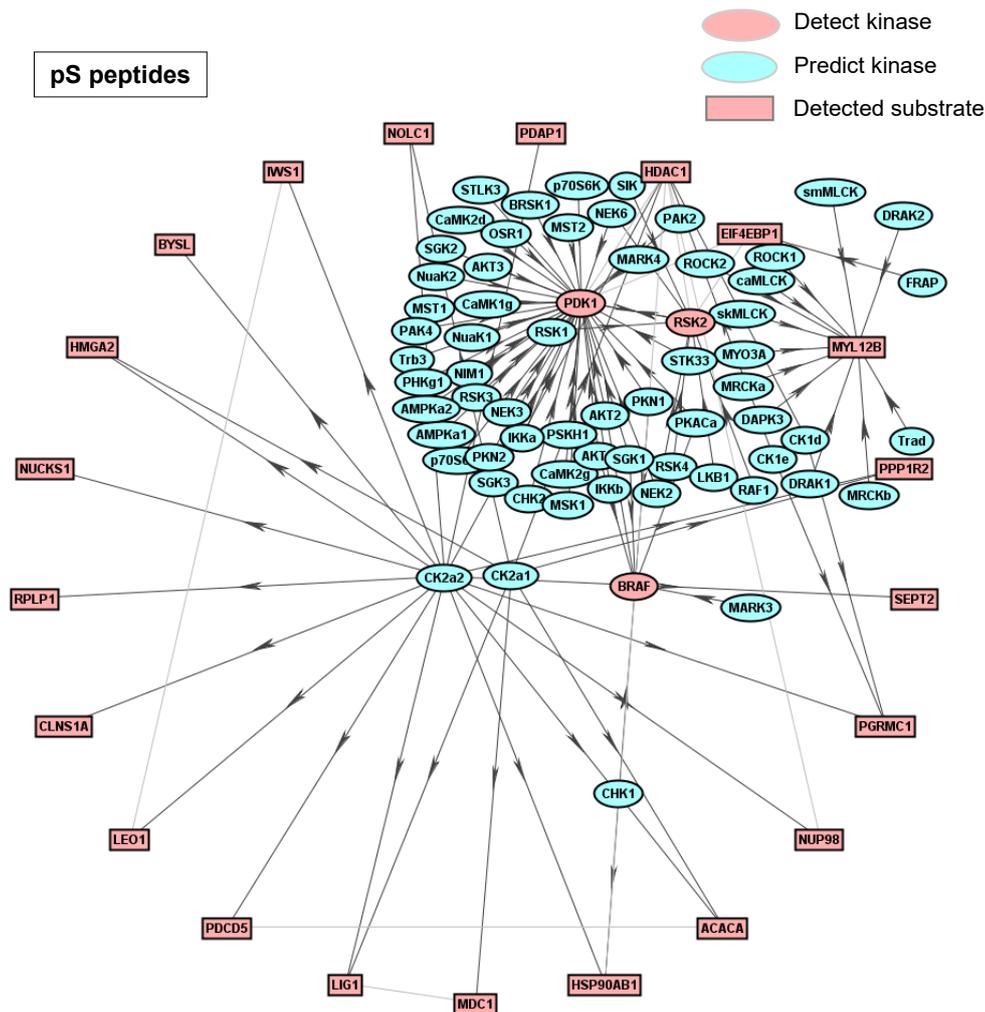


Figure S3. Kinase–substrate interaction analysis of phospho–serine proteins using iGPS

1.0. Circle (Kinase), Square (Substrate), Pink (Detected), Blue (Predicted).

Table S3. All identified phosphosites after treatment to α -AMA (10 μ M) with ERK 1/2 inhibitor (5 μ M) to Huh-7 cells

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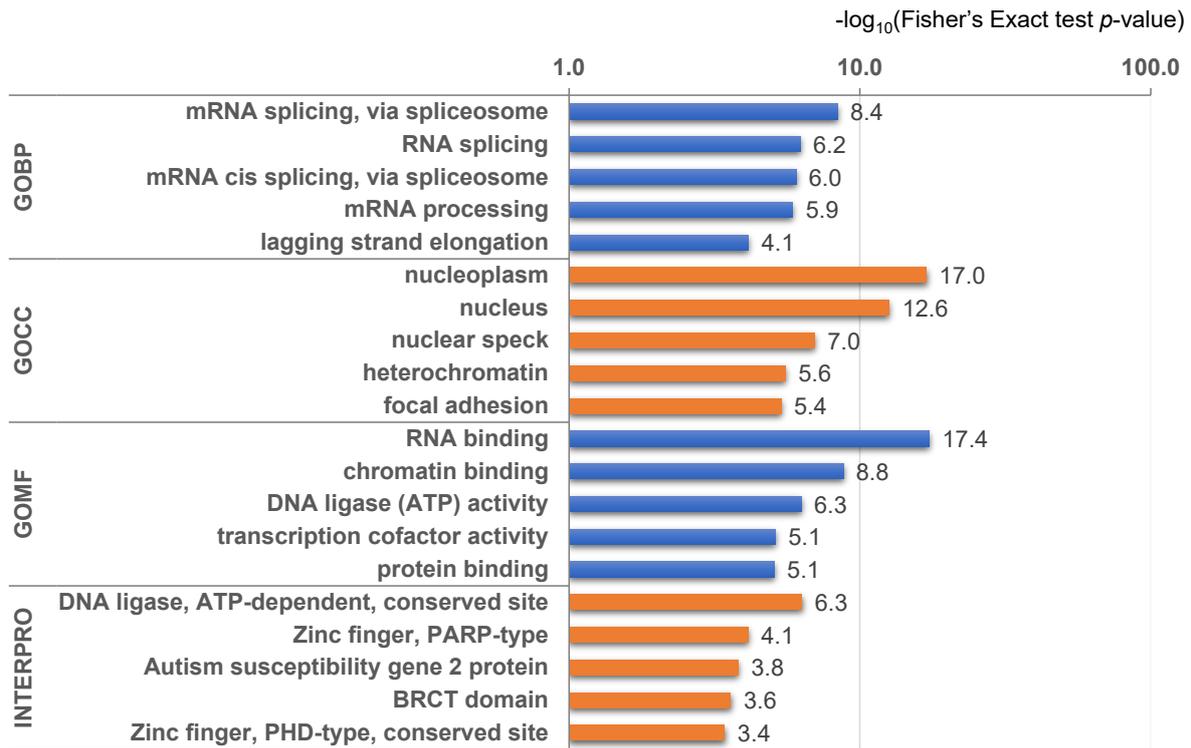


Figure S4. DAVID-generated Gene Ontology (GO) enrichment and Interpro of Cluster

5. Huh-7 cells were treated by α -AMA (10 μ M) and/or ERK1/2 inhibitor (FR180204, 5 μ M) for 24 hr.

Table S4. Phosphorylation information classified in Spliceosome of KEGG category in Cluster 5 after treatment to α -AMA (10 μ M) with ERK 1/2 inhibitor (5 μ M) to Huh-7 cells

Protein ID	Name	Gene	Position
F8WC91	U4/U6.U5 tri-snRNP-associated protein 2	USP39	S154
Q5W011	Splicing factor 45 (Fragment)	RBM17	S212
S4R3H4	Apoptotic chromatin condensation inducer in the nucleus	ACIN1	S182, S185, S432