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IRE1 a dynamic molecular platform linking diverse cellular functions: A quantitative proteomic analysis.

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ABSTRACT

IRE1 branch of unfolded protein response (UPR) signalling network represents the highly conserved pathway of all the three and provides a major platform for deciding the fate of the cells under stress. IRE1 is a type I transmembrane protein with dual enzyme activity, kinase and endoribonuclease localized to ER membrane. Upon activation, IRE1 catalyse the non-canonical splicing of hac1 mRNA in yeast and xbp1 mRNA in humans. In addition, IRE1 degrades a subset of mRNAs localized to ER by process, termed as Regulated IRE1 Dependent Decay (RIDD). IRE1 acts as a platform in order to orchestrate with other signalling pathways. A number of proteins either acting as inhibitors or cofactors of IRE1 have been found to interact and modulate the IRE1 signalling pathway, the concept which introduced 'UPRosome' as a signalling platform. We performed immuno pull down of IRE1 associated factors, followed by Mass Spectrometric analysis. Proteins belonging to diverse cellular functions were found to interact with IRE1. These proteins were divided into 19 different functional clads. In addition we found that these proteins interact with IRE1 in a stress dependent manner. Our results thus imply that IRE1 is at the centre of intricate cellular networks.

Keywords:ER stress, Proteomic analysis, IRE1, UPR, UPRosome

I.INTRODUCTION

Endoplasmic reticulum is the largest single membrane bound intracellular compartment is associated with proper maturation and secretion of proteins, which enter the lumen. ER stress, induced by external or internal cues, activates a well-orchestrated process aimed at either restoring cellular homeostasis or committing to cell death and is mediated by engagement of an integrated signal transduction-transcriptional network known as unfolded protein response (UPR) [1-3].

The IRE1 (Inositol requiring enzyme 1) arm of UPR is the most evolutionarily conserved and ancient of the three and it aims to increase cells protein folding ability by inducing chaperone synthesis and facilitating removal of misfolded proteins. IRE1 is a dual function enzyme with both kinase and nuclease activities.

Activation of UPR results in IRE1 dimerization and trans auto-phosphorylation of its kinase domains, which in turn activates its RNAse activity and catalyses splicing of XBP1mRNA. Splicing of XBP 1 results in formation of an active transcriptional factor XBP 1protein which induces a plethora of genes involved in protein folding and lipid biosynthesis [4]. Besides XBP 1 splicing, IRE1 also cleaves ER associated mRNAs leading to their decay an activity termed as Regulated IRE1 Dependent Decay (RIDD) [5]. RIDD degrades `ER localized mRNAs preferentially by cleaving them at XBP1 like consensus site [6]. WhereasXBP1 splicing is cytoprotective in response to ER stress RIDD has revealed many unexpected features. For instance RIDD has an activity divergent from XBP1 splicing and can either preserve ER homeostasis or induce cell death [7].

Apart from its endoribonuclease activity, IRE1 also perform diverse cellular functions by binding directly with adaptor proteins. TNFR-associated factor 2 (TRAF2), an adaptor protein binds to the cytosolic region of IRE1 and initiate the process of apoptosis by activation of downstream signalling molecules like apoptosis signal-regulating kinase 1 (ASK1) and cJun-N terminal kinase (JNK) [8-10]. Ire1 also initiates autophagy by binding to other adaptor molecules and JNK [11]. IRE1 communicates to ERAD pathway through binding with ubiquitin specific protease (USP) 14, DERLIN-1, DERLIN-3, SEL1, and HRD1, [12]. IRE1 represents a huge platform for diverse protein-protein interactions, allowing the assembly of a protein complex referred as UPRosome [13, 14]. This model would be useful in addressing how IRE1 performs distinct and diverse cellular functions. Dynamic association and dissociation of different proteins would result in different cellular outputs.

In this study we performed proteomic analysis to understand the multitude of cellular functions performed by IRE1. Our results revealed that IRE1 interacts with proteins involved in multitude of cellular processes like apoptosis, autophagy, redox response, mitochondrial functions, cytoskeleton and RNA binding. These results suggest that IRE1 might aid in various cellular functions by directly binding to factors involved in respective pathways.

II.MATERIALS AND METHODS

2.1 Cell Culture, Reagents and Antibodies

HEK-T cells were maintained in Dulbecco's modified Eagles medium (Gibco by Life Technologies) supplemented with 10% fetal bovine serum (Gibco by Life Technologies) and 1 × penicillin-streptomycin solution (Gibco by Life Technologies) at 37°C in a humidified incubator with 5% CO2. The protease inhibitor cocktail was purchased from Calbiochem and used according to the manufacturer's instructions. Tunicamycin (UPR inducer) was purchased from Calbiochem. Protein G Resin was purchased from GeneScript. Anti-IRE1 (Phospho S724) was purchased from Abcam.

2.2 Cloning

IRE1 alpha-pcDNA3.1-EGFP clone (#13009) was purchased from Addgene Inc., and used as a template for Ire1 cloning. Ire1 insert (2.9) kb was excised from this clone as HindIII and XhoI fragment and cloned at pcDNA 3.1(+) vector (5.4 kb) at HindIII and XhoI site using the standard cloning methods.

2.3 Immuno-precipitation

HEK T cells were transfected with pcDNA3.1 (+) clone using PEI transfection reagent. Cells with Ire1 clone transfected and without transfection were treated with 6μM of tunicamycin for 5 hrs. As a control, similar experiments were performed on cells without tunicamycin treatment. Cells were harvested in cold Tris-buffered saline. Then 120ul of lysis buffer (20 mM Tris-Cl, pH 8.0, 137 mM NaCl, 0.01% NP-40, and 2mM EDTA, 0.1% glycerol, NaF 10mM, β-glycerolphosphate, 1X protein inhibitor cocktail) was added and incubate on Ice for 1hour. After centrifugation supernatant was collected. Protein G Resin was incubated with 2ug of antibody overnight and next day protein lysate was added and again incubated overnight. Beads were then washed with 1X lysis buffer and eluted with 2X loading dye. Samples were then run on 12% SDS page. Stained with Coomassie blue and destained gel to a clear background so that bands were easily seen. Gel slices were prepared corresponding to each sample. For immunoprecipitation assay, Anti-IRE1 (phospho S724) antibody was used.

2.4 Sample preparation and mass spectrometry analysis

For Mass spectrometry analysis, proteins were eluted from gel slices followed by reduction and alkylation of cysteine residues. The proteins were then treated with TPCK-trypsin (Promega, Madison, WI) for 16 h at 37 °C. The peptides were resuspended in 0.1% formic acid, desalted using C18 StageTips and analyzed by nano LC-MS on a hybrid quadrupole-orbitrap mass spectrometer (Orbitrap Fusion Tribrid mass spectrometer, Thermo Scietific). The raw data obtained after analysis was searched using Proteome Discoverer suite (Thermo scientific). Mascot and Sequest search engines were used to search the LCMS/ MS data against human RefSeq protein databases. Carbamidomethylation of cysteine was used as a fixed modification and oxidation of methionine as a variable modification. FDR was calculated by employing decoy database searches to avoid false positive identifications. Only proteins that were absent in the control condition were considered as potential interaction partners.

III.RESULTS

3.1 Cloning of Ire1 and pull-down of IRE1 associated factors

Ire1 gene was cloned in a mammalian expression vector pcDNA3.1 (+). The clone was confirmed by digesting the extracted plasmids with HindIII and XhoI restriction enzymes. Digested products were checked on agarose gel. We could see a release of 2.9 kb insert and 5.4 kb vector backbone (Fig1). The clone was transiently transfected in HEKT cell line and checked for its expression. Different experiments sets viz untreated (Mock-T), Tm treated (Mock), Ire1 transfected without treatment (Ire1 -T) and Ire1 transfected and Tm treated (Ire1+T) were subjected to immune-pull down using anti-IRE1 (phospho S724) antibody (Fig2). Tunicamycin (Tm) is an N-glycosylation inhibitor, which induces UPR. (Mock -T) represents a control, whereas (Mock +T), (Ire1 -T), (Ire1+T) represent different levels of ER stress and Ire1 activation.

3.2 Proteomic analysis of Ire1 interactors

To identify interacting partners of IRE1 proteomic analysis of the four different IP experimental sets was performed using mass spectrometry. More than 150 proteins were found to potentially interact with Ire1 in different experimental sets in comparison to control (Mock –T). These proteins were differentially present in different experimental sets. There was a set of proteins found in all the three experimental sets (Table1). Another set of proteins was only present in tunicamycin treated and Ire1 overexpressed sample (Ire1 +T) (Table2). Surprisingly there were some proteins exclusively present in Ire1 overexpressed sample without any treatment (Mock –T) (Table3). Next we divided these proteins based on their functions using NCBI web search. There were around 19 functional groups of these proteins (Fig3). These functional groups include Metabolism (16%), RNA binding proteins (14%), Transport Proteins (12%), Cytoskeleton (9%), Protein Modification system (7%), Protein folding (6%), Mitochondrial biology (5%), DNA Repair (5%), Translation (5%), Cell cycle (5%), Cell signalling (4%), Cell proliferation (3%), Gene expression (2%), Apoptosis (3%), Membrane component (2%), Autophagy (1%), Nucleosome (1%), Oxidative pathway (1%), and immune Response (1%).

IV. DISCUSSION

IRE1, a transmembrane signalling protein that represents the start point of one of the UPR signalling cascades, is highly conserved and a major platform deciding the cell fate, is endowed with kinase and endoribonuclease activities. Upon activation the ribonuclease activity of IRE1 results in xbp 1 splicing, and in addition cleaves ER associated mRNAs leading to their decay, an activity termed as Regulated IRE1 Dependent Decay (RIDD). RIDD & XBP-1 splicing can be activated differentially, suggesting an unanticipated complexity in UPR. [4-7]

Many studies have described various UPR binding partners with particular reference to IRE1, giving rise to the concept of ‘UPRsome’- a dynamic signalling platform in which many regulatory and adaptor proteins assemble to activate and modulate downstream cascade of UPR [13]. The list of proteins interacting physically with IRE1 and modulating its activity are many which include proteins with wide range of functions from cytosolic chaperones like HSP 72, pro-apoptotic proteins BAX and BAK, MAPK related proteins like ASK1-interacting protein1 (AIP1), JNK inhibitory kinase (JIK), JUN activation domain binding protein1 (JAB1) to protein Tyr phosphatase1B (PTP1B) [15-20]. This implies that UPRosome acts as a molecular platform in order to orchestrate with other signalling pathways.

Our proteomic studies revealed that IRE1 interacts with multitude of protein factors spanning over diverse cellular pathways. Around 19 clads of proteins based on their functional types were found to interact with IRE1. Largest functional clad among them is metabolic proteins, which were found to interact with IRE1. These proteins mostly include those involved in glucose metabolism. In a previous study it was found that inhibition of glucose metabolism affected the phosphorylation status of IRE1 [21]. This finding corroborates our data for having the connecting link of IRE1 with glucose metabolism. The next richest clad involved the RNA binding proteins. These proteins include mostly ones which are involved RNA processing and transport. The other group covered the cytoskeleton proteins, which are already known to have role in in stabilizing the IRE1 structure and

activation [22]. Proteins involved in cell proliferation and cell cycle regulation were also found to interact with IRE1 possibly indicating the role of IRE1 in cell division, which highlights the important function of Ire1 as a cell fate executor.

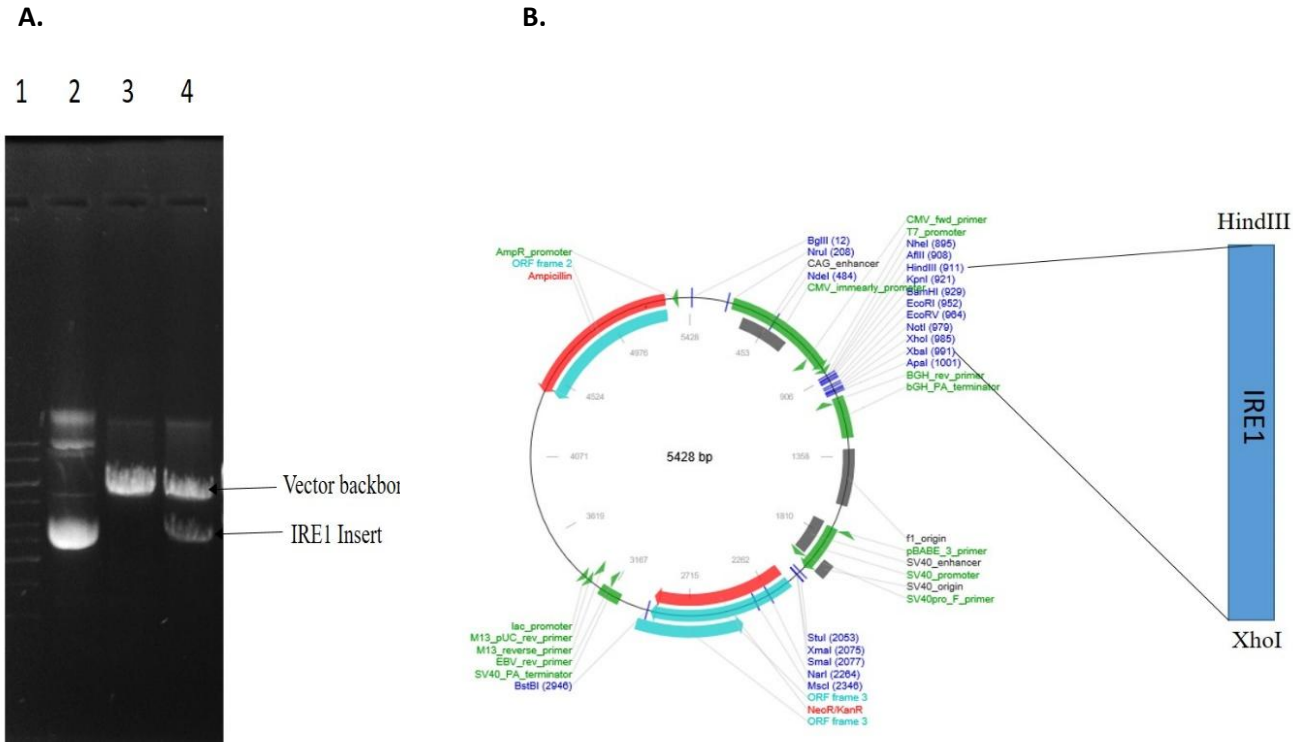
Our study revealed that IRE1 interacts with proteins involved in apoptosis that which is reinforced by many other studies. These studies included the interaction between key apoptotic transducers like TRAF2 (8, 9), BCL2 family proteins, such as BAX and BAK, and the BI-1 BCL2 regulatory protein [23]. Thus these protein-protein interactions serve as the convergent points of different pathways, which cumulatively send pro-death signals leading to apoptosis. Previous studies have confirmed the existing link between autophagy and UPR with IRE1 as a central molecule [24]. This supports our data that demonstrates a physical connection between proteins involved in autophagy and IRE1.The link between ER and Mitochondrion commonly known as mitochondria-associated ER membranes (MAMs) unravelled a crucial role for ER in cell death regulation [25, 26]. MAMS are predominately presided by IRE1 [27, 28], which supports our data where mitochondrial proteins were found to interact with IRE1.

Besides the functional crosstalk of IRE1 our study indicated that proteins interacting with IRE1 showed differential association under varied experimental conditions. One set of proteins showed ubiquitous association with IRE1 irrespective of the experimental conditions [table1]. It highlights that the presence of these proteins in UPRosome might be independent of the levels of IRE1 activation and reasonably the oligomerization state. While the other two sets of proteins showed varied association depending on the presence of UPR inducer [table 2, table 3]. The UPR induction and IRE1 over-expression individually or in combination imitates the different levels of IRE1 activation and, therefore suggest the reason that binding partners of IRE1 are dependent on its activation/oligomerization state. In fact this argument is supported by previous studies from other groups [7, 29]. Therefore it could be put forth that the extent of ER stress and IRE1 oligomerization regulates the association of different proteins in the UPRosome in a dynamic manner.

Our study captured an overall picture that presents IRE1 as a central molecule orchestrating the diverse cellular pathways. This cumulatively sends a downstream signal regulating cell's important functions including cell fate determination. However these interactions need to be individually validated and functionally characterized, in order to get detailed mechanistic insights of the IRE1's communicating partners and the factors, which govern them.

V.FIGURES AND TABLES

5.1 Figures



B

Figure1. *Cloning of Ire1 gene in Mammalian Expression Vector* **A.)** Agarose gel electrophoresis showing restriction digestion pattern of Ire1 clone. Lane 1 1kb DNA, lane 2 undigested clone, lane 3 vector digested with HindIII and XhoI enzyme, and lane 4 Ire1 clone digested with HindIII and XhoI enzyme. **B.)** Shows a vector map of pcDNA3.1 (+) IRE1 clone.

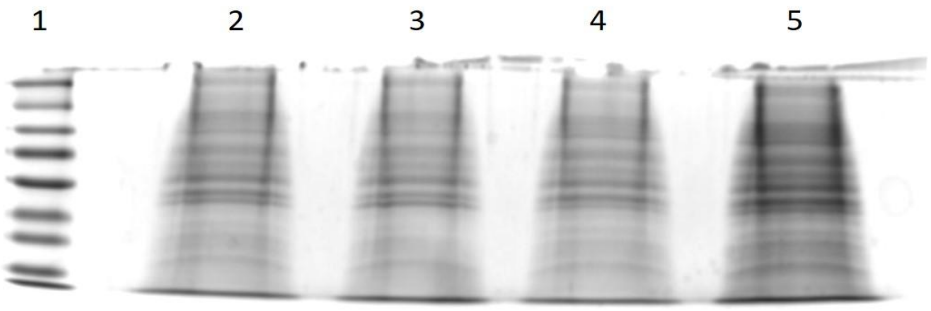


Figure2. *IRE1 pull down*: Immuno-precipitated proteins were run on 12% SDS PAGE stained with Coomassie blue. Lane 1, pre-stained protein marker, lane2 Mock –T, lane3 Mock +T, lane4 Ire1 –T, and lane5 Ire1 +T.

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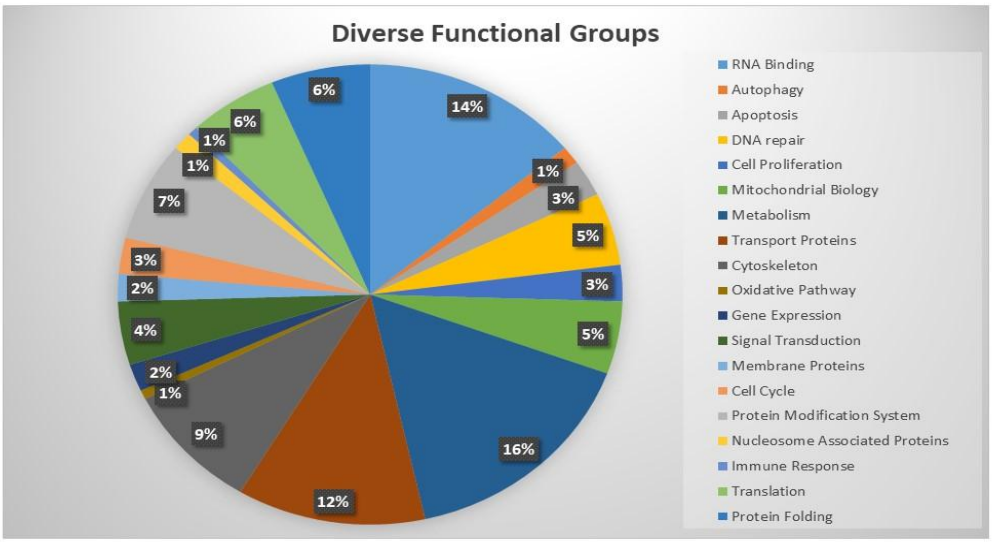

Figure3: IRE1 interacting partners divided according to their functional clads.

5.2 Tables

Table1 IRE1 interacting partners present in all experimental sets viz [Mock +T], [Ire1 –T], [Ire1 +T]

Gene symbol	Description	Coverage	MW [kDa]	calc. pI	Score Mascot
AARS	alanine--tRNA ligase, cytoplasmic	4.64876	106.743	5.53	442.34
ADSL	adenylosuccinate lyase isoform a	4.132231	54.854	7.11	186.5651
ANKHD1-EIF4EBP3	ANKHD1-EIF4EBP3 protein	0.840657	277.004	5.86	117.71
ARCN1	coatomer subunit delta isoform 1	3.913894	57.174	6.21	170.7514
ARL6IP5	PRA1 family protein 3	4.787234	21.6	9.77	85.06
ATAD3A	ATPase family AAA domain-containing protein 3A isoform 1	14.51104	71.325	8.98	636.2529
ATP2A2	sarcoplasmic/endoplasmic reticulum calcium ATPase 2 isoform b	4.894434	114.683	5.34	121.1921
CANX	calnexin precursor	6.756757	67.526	4.6	191.4423
CBR1	carbonyl reductase [NADPH] 1 isoform 1	3.249097	30.356	8.32	0
CCZ1	vacuolar fusion protein CCZ1 homolog	3.526971	55.83	6.48	112.98
CDC37	hsp90 co-chaperone Cdc37	2.380952	44.44	5.25	24.86
CKAP4	cytoskeleton-associated protein 4	3.156146	65.983	5.92	49.4371
DARS	aspartate--tRNA ligase, cytoplasmic isoform 1	11.57685	57.1	6.55	170.3874
DDB1	DNA damage-binding protein 1	5.614035	126.887	5.26	36.52333
DDX46	probable ATP-dependent RNA helicase DDX46 isoform 1	1.744186	117.389	9.29	38.94
DDX5	probable ATP-dependent RNA helicase DDX5 isoform b	17.58958	69.044	8.85	465.4743

DNAJC7	dnaJ homolog subfamily C member 7 isoform 1	7.08502	56.405	6.96	192.684
EZR	ezrin	2.901024	69.37	6.27	38.10566
FECH	ferrochelatase, mitochondrial isoform a precursor	4.895105	48.594	8.72	44.11
FKBP4	peptidyl-prolyl cis-trans isomerase FKBP4	6.753813	51.772	5.43	82.08657
GANAB	neutral alpha-glucosidase AB isoform 3 precursor	2.691511	109.369	6.24	167.9868
GARS	glycine--tRNA ligase isoform 1 precursor	5.142084	83.113	7.03	307.5718
GART	trifunctional purine biosynthetic protein adenosine-3 isoform 1	4.950495	107.699	6.7	44.72667
GLG1	Golgi apparatus protein 1 isoform 1 precursor	1.745636	137.132	6.9	191.1414
GLUL	glutamine synthetase	2.680965	42.037	6.89	157.21
GOLGA2	golgin subfamily A member 2	3.293413	113.017	5.02	150.1353
HNRNPA1	heterogeneous nuclear ribonucleoprotein A1 isoform b	4.83871	38.723	9.13	512.6586
HNRNPR	heterogeneous nuclear ribonucleoprotein R isoform 1	3.144654	71.17	8.13	137.9293
HNRNPUL1	heterogeneous nuclear ribonucleoprotein U-like protein 1 isoform a	7.71028	95.679	6.92	120.099
HSPA4L	heat shock 70 kDa protein 4L isoform 2	5.057471	97.597	5.95	106.0278
KARS	lysine--tRNA ligase isoform 2	5.862647	68.005	6.35	233.6875
KTN1	kinectin isoform a	2.800295	156.179	5.64	189.3934
LAP3	cytosol aminopeptidase	5.202312	56.131	7.93	324.0894
LETM1	LETM1 and EF-hand domain-containing protein 1, mitochondrial precursor	2.70636	83.302	6.7	371.1954
LMNB1	lamin-B1 isoform 1	5.972696	66.368	5.16	71.1
LONP1	lon protease homolog, mitochondrial isoform 1 precursor	0.938478	106.422	6.39	174.72
LRRFIP1	leucine-rich repeat flightless-interacting protein 1 isoform 3	5.074257	89.199	4.65	252.64
LUC7L3	luc7-like protein 3	7.407407	51.435	9.79	214.8757
MARCKSL1	MARCKS-related protein	14.35897	19.517	4.67	174.97
MSH2	DNA mismatch repair protein Msh2 isoform 1	0.963597	104.677	5.77	26.78
MSH6	DNA mismatch repair protein Msh6 isoform 1	2.867647	152.689	6.9	34.54667
NOMO2	nodal modulator 2 isoform 1 precursor	2.052092	139.351	5.76	40.82333
OGT	UDP-N-acetylglucosamine--peptide N-acetylglucosaminyltransferase 110 kDa subunit isoform 1	2.390057	116.85	6.7	123.6044
P4HB	protein disulfide-isomerase precursor	7.086614	57.081	4.87	573.9591
PCBP1	poly(rC)-binding protein 1	20.22472	37.474	7.09	336.6625
PGM1	phosphoglucomutase-1 isoform 2	1.206897	63.75	5.83	38.98
POP1	ribonucleases P/MRP protein subunit POP1	2.246094	114.636	9.22	0
PRKCSH	glucosidase 2 subunit beta isoform 3 precursor	15.70093	60.154	4.41	170.36
PSMA1	proteasome subunit alpha type-1 isoform 1	2.60223	30.22	6.99	35
PSMC5	26S protease regulatory subunit 8 isoform 1	6.650246	45.597	7.55	740.2789
PSMD11	26S proteasome non-ATPase regulatory subunit 11	5.450237	47.434	6.48	158.1071
RARS	arginine--tRNA ligase, cytoplasmic	8.787879	75.331	6.68	77.51286
RBM25	RNA-binding protein 25	3.795967	100.124	6.32	55.34
RBM26	RNA-binding protein 26 isoform 2	4.170905	110.956	9.07	467.1438
SCFD1	sec1 family domain-containing protein 1 isoform a	1.401869	72.334	6.27	71.25667
SEC63	translocation protein SEC63 homolog	1.052632	87.942	5.31	36.82774
SLC25A6	ADP/ATP translocase 3	16.10738	32.845	9.74	377.7405

SPRR1B	CF	8.988764	9.881	8.48	71.15
SPRR2G	small proline-rich protein 2G	30.13699	8.152	7.96	128.49
SPTBN2	spectrin beta chain, non-erythrocytic 2	0.878661	271.127	6.11	60.0514
SRP72	signal recognition particle subunit SRP72 isoform 1	6.85544	74.56	9.26	379.3827
SUCLG2	succinyl-CoA ligase [GDP-forming] subunit beta, mitochondrial isoform 2 precursor	4.62963	46.481	6.39	107.13
TFRC	transferrin receptor protein 1 isoform 1	3.684211	84.818	6.61	149.2543
TPI1	triosephosphate isomerase isoform 2	16.43357	30.772	5.92	290.4018
TXLNA	alpha-taxilin	10.25641	61.853	6.52	243.5214
USP10	ubiquitin carboxyl-terminal hydrolase 10 isoform 1	2.244389	87.479	5.27	57.32
USP5	ubiquitin carboxyl-terminal hydrolase 5 isoform 1	2.564103	95.725	5.03	79.17
ZYX	zyxin	4.895105	61.238	6.67	166.2738

Table 2. IRE1 interacting partners present in only [Ire1 +T] experimental set.

Gene symbol	Description	Coverage	MW [kDa]	calc. pI	Score Mascot
AASS	alpha-aminoadipic semialdehyde synthase, mitochondrial	1.079914	102.066	6.64	63.51
AHCYL2	adenosylhomocysteinase 3 isoform a	1.963993	66.678	7.36	54.7
AHSA1	activator of 90 kDa heat shock protein ATPase homolog 1	2.95858	38.25	5.53	0
AKAP1	A-kinase anchor protein 1, mitochondrial precursor	1.550388	97.281	4.94	0
AKAP12	A-kinase anchor protein 12 isoform 1	0.785634	191.367	4.41	0
ALDOC	fructose-bisphosphate aldolase C	8.241758	39.431	6.87	61.85
AP1B1	AP-1 complex subunit beta-1 isoform a	1.264489	104.54	5.06	42.64
ATP2B2	plasma membrane calcium-transporting ATPase 2 isoform 1	0.884956	136.789	5.91	0
ATXN10	ataxin-10 isoform 1	1.894737	53.455	5.25	0
BICD2	protein bicaudal D homolog 2 isoform 1	1.28655	96.746	5.44	39.65
CCDC129	coiled-coil domain-containing protein 129 isoform 3	4.613936	117.641	5.39	
CFL1	cofilin-1	7.228916	18.491	8.09	46.54
CLCC1	chloride channel CLIC-like protein 1 isoform 1 precursor	3.085299	61.983	5.55	18.52
DBNL	drebrin-like protein isoform b	2.790698	48.178	5.05	14.74
DPYSL2	dihydropyrimidinase-related protein 2 isoform 1	1.329394	73.457	6.35	0
ETFA	electron transfer flavoprotein subunit alpha, mitochondrial isoform a	5.405405	35.058	8.38	29.34
EXOC4	exocyst complex component 4 isoform a	1.232033	110.429	6.49	46.22
G6PD	glucose-6-phosphate 1-dehydrogenase isoform b	1.553398	59.219	6.84	23.23
GLA	alpha-galactosidase A precursor	1.631702	48.735	5.6	29.83
GOT2	aspartate aminotransferase, mitochondrial isoform 1 precursor	3.255814	47.487	9.01	34.31
GRIPAP1	GRIP1-associated protein 1	1.307967	95.931	5.11	0
HLA-C	HLA class I histocompatibility antigen, Cw-1 alpha chain precursor	2.459016	40.685	5.87	27.8
IGF2BP3	insulin-like growth factor 2 mRNA-binding protein 3	2.24525	63.666	8.87	0
IPO4	importin-4	0.647549	118.64	4.96	34.18

IRF2BP2	interferon regulatory factor 2-binding protein 2 isoform A	1.873935	60.987	8.69	59.42
LMF2	lipase maturation factor 2	1.131542	79.647	10.1	21.12
LUC7L	putative RNA-binding protein Luc7-like 1 isoform b	9.433962	43.701	9.92	139.8114
LYPLAL1	lysophospholipase-like protein 1 isoform a	4.219409	26.299	7.84	100.1867
MGME1	mitochondrial genome maintenance exonuclease 1 isoform 1 precursor	3.064067	41.198	7.68	
MKLN1	muskelin isoform 2	1.768707	84.713	6.34	77.97
NEK9	serine/threonine-protein kinase Nek9	0.81716	107.1	5.74	19.98
NOM1	nucleolar MIF4G domain-containing protein 1	1.27907	96.198	8.1	0
NRBP1	nuclear receptor-binding protein	2.990654	59.807	5.08	17.51
NUP88	nuclear pore complex protein Nup88 isoform 1	1.717305	85.451	5.81	48.07
PANK4	pantothenate kinase 4	1.792574	86.871	6.34	23.56
PCCB	propionyl-CoA carboxylase beta chain, mitochondrial isoform 2 precursor	2.862254	60.483	7.24	0
PGM2	phosphoglucomutase-2	2.124183	68.24	6.73	23.11
PPA1	inorganic pyrophosphatase	2.768166	32.639	5.86	69.33
PPAT	amidophosphoribosyltransferase precursor	3.675048	57.362	6.76	0
PPP4C	serine/threonine-protein phosphatase 4 catalytic subunit isoform 1	2.605863	35.057	5.06	55.63545
PPWD1	peptidylprolyl isomerase domain and WD repeat-containing protein 1 isoform 1	1.702786	73.528	7.15	42.01
PRDX6	peroxiredoxin-6	5.803571	25.019	6.38	17.92
PRKAR2A	cAMP-dependent protein kinase type II-alpha regulatory subunit	2.722772	45.49	5.07	0
PSMC6	26S protease regulatory subunit 10B	2.233251	45.768	7.78	0
PTPN11	tyrosine-protein phosphatase non-receptor type 11 isoform 1	1.854975	67.968	7.3	34.17
PYGL	glycogen phosphorylase, liver form isoform 1	2.479339	97.087	7.17	28.85
QTRT1	queuine tRNA-ribosyltransferase	3.473945	44.019	7.23	173.6234
RNASEH2B	ribonuclease H2 subunit B isoform 1	4.166667	35.116	9.13	22.07
SACM1L	phosphatidylinositide phosphatase SAC1 isoform 1	1.53322	66.924	7.12	26.93
SETD1A	histone-lysine N-methyltransferase SETD1A	0.820152	185.92	5.14	24.95
SLC19A1	folate transporter 1 isoform 1	1.692047	64.827	8.95	45.9
SRPRB	signal recognition particle receptor subunit beta	7.01107	29.684	9.04	44.93
SRRM1	serine/arginine repetitive matrix protein 1 isoform 1	1.091703	103.734	11.84	65.04
TBCD	tubulin-specific chaperone D	0.838926	132.515	6.19	26.59
TOR1AIP1	torsin-1A-interacting protein 1 isoform 1	2.226027	66.279	8.18	30.08
TOX3	TOX high mobility group box family member 3 isoform 1	10.9375	63.302	7.85	
TP53	cellular tumor antigen p53 isoform a	3.307888	43.625	6.79	89.36
TRIP12	E3 ubiquitin-protein ligase TRIP12 isoform a	0.686275	225.379	8.69	43.41
TRUB1	probable tRNA pseudouridine synthase 1	4.011461	37.229	8.25	67.39
UBIAD1	ubiA prenyltransferase domain-containing protein 1	2.662722	36.808	8.15	94.44406
USP15	ubiquitin carboxyl-terminal hydrolase 15 isoform 1	1.223242	112.348	5.22	116.93
VCPIP1	deubiquitinating protein VCIP135	1.309329	134.236	7.2	0
WDR48	WD repeat-containing protein 48 isoform 1	2.215657	76.162	7.03	64.56333
XPO6	exportin-6 isoform 2	0.711111	128.801	6.35	37.01

ZNF598	zinc finger protein 598	1.327434	98.605	8.4	0

Table 3. IRE1 interacting partners present in only [Ire1 –T] experimental set.

Gene symbol	Description	Coverage	MW [kDa]	calc. pI	Score Mascot
KIAA0368	proteasome-associated protein ECM29 homolog	0.34705	223.552	8.75	17.94
KRT77	keratin, type II cytoskeletal 1b	6.228374	61.864	5.99	2166.639
MRPL38	39S ribosomal protein L38, mitochondrial	1.842105	44.568	7.53	25.82
NPLOC4	nuclear protein localization protein 4 homolog	1.151316	68.077	6.38	55.12937
OR10H3	olfactory receptor 10H3	18.03797	35.696	8.92	
PABPN1	polyadenylate-binding protein 2	5.228758	32.729	5.06	108.4
PALLD	palladin isoform 1	0.979519	123.617	6.87	15.57
PDCD11	protein RRP5 homolog	0.481026	208.57	8.87	0
PFAS	phosphoribosylformylglycinamidine synthase	0.822123	144.643	5.76	40.34
PFKFB2	6-phosphofructo-2-kinase/fructose-2, 6-bisphosphatase 2 isoform a	1.782178	58.44	8.38	17.78
PLP1	myelin proteolipid protein isoform 1	2.888087	30.057	8.35	68.08721
PPP2CA	serine/threonine-protein phosphatase 2A catalytic subunit alpha isoform	2.588997	35.571	5.54	43.9527
PTBP3	polypyrimidine tract-binding protein 3 isoform 6	1.792115	60.379	9.19	88.8479
RAP1GDS1	rap1 GTPase-GDP dissociation stimulator 1 isoform 1	1.973684	66.346	5.31	36.59
TP53BP2	apoptosis-stimulating of p53 protein 2 isoform 1	1.851852	126.245	6.21	0
UBA6	ubiquitin-like modifier-activating enzyme 6	0.855513	117.895	6.14	86.92333
YTHDF1	YTH domain-containing family protein 1	9.660107	60.836	8.79	32.03

VI. CONCLUSION

Our study implicated that IRE1 acts as structural and functional platform serving as docking site for huge number of proteins, and thereby representing a connecting link between diverse cellular pathways. However, study is subjected to further validation.

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