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Interactome Analysis of the ER Stress Sensor Perk Uncovers Key Components of ER-Mitochondria Contact Sites and Ca^{2+} Signalling

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Abstract

We recently reported that the ER stress kinase PERK regulates ER-mitochondria appositions and ER– plasma membrane (ER-PM) contact sites, independent of its canonical role in the unfolded protein response. PERK regulation of ER-PM contacts was revealed by a proximity biotinylation (BioID) approach and involved a dynamic PERK-Filamin A interaction supporting the formation of ER-PM contacts by actin-cytoskeleton remodeling in response to depletion of ER-Ca^{2+} stores. In this report, we further interrogated the PERK BioID interactome by validating through co-IP experiments the interaction between PERK and two proteins involved in Ca^{2+} handling and ER-mitochondria contact sites. These included the vesicle associated membrane (VAMP)-associated proteins (VAPA/B) and the main ER Ca^{2+} pump sarcoplasmic/endoplasmic reticulum Ca ATPase 2 (SERCA2). These data identify new putative PERK interacting proteins with a crucial role in membrane contact sites and Ca^{2+} signaling further supporting the uncanonical role of PERK in Ca^{2+} signaling through membrane contact sites (MCSs).

Keywords

cell biology, endoplasmic reticulum, ER stress, sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA), mitochondrial associated membranes (MAM)

Introduction

The endoplasmic reticulum (ER) is a major site of protein folding in the cell, handling roughly one-third of all cellular proteins and all proteins destined for transport towards the plasma membrane (PM) or extracellular matrix (Brodsky & Wojcikiewicz, 2009). To cope with this demand, the ER has evolved to possess an intricate folding machinery and an ideal protein folding environment. However, when the ER can no longer match cellular protein folding demand, the resulting accumulation of unfolded proteins causes ER stress (Ron & Walter, 2007). The unfolded protein response (UPR) consists of the activation of a conserved signal transduction pathway, ultimately eliciting a transcriptional program that operates as a principal safeguard against the loss of ER homeostasis caused by ER stress. The UPR is launched by the activation of three ER membrane proteins. The ER stress kinase PKR-like endoplasmic reticulum kinase (PERK) is one of these three mediators and is

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activated upon ER stress (Ron & Walter, 2007). In homeostatic conditions, PERK is kept inactive through the binding of the ER chaperone BiP to its luminal domain, and is activated upon its release, resulting in the oligomerization of PERK, followed by its autophosphorylation. Activated PERK is then able to phosphorylate eukaryotic initiation factor 2 alpha (eIF2α), leading to a protein translation pause and giving the ER folding machinery time to deal with its protein burden (Ron & Walter, 2007).

In our previous studies (van Vliet et al., 2017; Verfaillie et al., 2012), we uncovered that independent of its UPR function, PERK moonlights at the ER-mitochondria contacts and aids apoptotic cell death by the transfer of ROS signals from the ER to the mitochondria. More recently, we showed that the activation of PERK can occur independently of its luminal domain and canonical ER stress, instead of being activated by a rise in cytosolic Ca^{2+}.

To uncover the roles of PERK we carried out an unbiased proximity biotinylation (BioID) screen, using the promiscuous biotinylation enzyme BirA* tagged to PERK on its cytosolic side (Roux et al., 2012; van Vliet et al., 2017). Using this technique, we discovered the cytoskeletal protein Filamin A (FLNA) as a novel PERK interactor. The Ca^{2+}-mediated PERK-FLNA axis was found to be required to support the formation of ER-PM contacts and store-operated Ca^{2+} entry (SOCE) (van Vliet et al., 2017). These findings together hint at a broader cross-talk between ER stress proteins and the regulation of membrane contact sites (MCSs). However, many hits uncovered through our BioID experiment warrant further investigation in order to get a better picture of the potential additional roles of PERK. In this report, we reveal the full dataset of PERK interacting proteins identified through BioID analysis. We further confirmed by IP/Co-IP analysis, the physical interaction between PERK and sarcoplasmic/endoplasmic reticulum Ca ATPase 2 (SERCA2) and VAMP-associated protein A / B (VAPA/B) and show that for these interactions, PERK kinase activity is dispensable.

Results and Discussion

The PERK Proximity Interactome

We generated a C-terminally tagged PERK-BirA* construct that was well expressed in HEK293-T cells and showed the expected ER localization (van Vliet et al., 2017). Our BioID approach was designed and performed to closely match the original study reporting BioID (Roux et al., 2012). Similar to that study, our approach relied on the expression of PERK-BirA* in HEK293-T cells, using mock-transfected parental HEK293-T cells treated in the same way (50 μM biotin for 24 h), as a control. Only biotinylated protein hits identified by LC-MS/MS in the streptavidin pull-down from PERK-BirA* transfected cells and not from mock-transfected cells were taken into consideration as putative interaction partners. Table 1 shows a list of PERK proximity interactors showing no spectral counts in the pull-down from mock-transfected cells. Interestingly, while our previous research focused on one of the most prominent hits, Filamin A, which we further validated functionally, the BioID dataset showed several other potential interacting proteins (Table 1), ranging from more proteins involved in actin cytoskeleton maintenance (cofilin, profilin,...), proteins involved in ER trafficking (syntaxin 5, coatomer subunits), proteins involved in membrane contact sites (VAPA/B, E-Syt1, junctophilin) to proteins linked with metabolism (ATP-citrate synthase, acyl-protein thioesterase 2). Interestingly, PERK has been linked recently with metabolic regulatory networks (Balsa et al., 2019; Moncan et al., 2021; Sorge et al., 2020), although whether these potential interactors uncovered by our BioID are relevant remains to be tested. To gain more insight into the various groups of proteins in the dataset, we performed a gene ontology analysis using WEB-based GEne SeT AnaLysis Toolkit (http://www.webgestalt.org/, Figure 1). This analysis yielded a set of enriched biological processes in our dataset. As a validation of our approach, two of these groups are linked to PERK's main role in protein folding and ER stress response (Figure 1A and B).

Considering our previous and ongoing studies, we decided to focus on validating the interactions of PERK with proteins with a known tethering role and/or with a relevant function in membrane contact sites and ER Ca^{2+} homeostasis. Two hits that were interesting in this regard were SERCA1/2 and VAPA/B, found at positions 47 and 62, respectively, on our list (Table 1).

PERK Interaction With VAPB

The isoforms VAPA and B are members of a small VAP protein family and have broadly similar structures and functions (Murphy & Levine, 2016). They are tail-anchored ER membrane proteins that are central to the formation of MCSs. VAPs act as tethers for a growing group of proteins, including protein tyrosine phosphatase interacting protein 51 (PTPIP51), StAR Related Lipid Transfer Domain Containing 3 (STARD3), oxysterol-binding protein (OSBP), Nir2, and Sorting nexin 2 (SNX2), among others (Alpy et al., 2013; Amarilio et al., 2005; De Vos et al., 2012; Dong et al., 2016; Wyles et al., 2002). VAPB is an important mediator of both ER-mitochondria contact sites and ER-plasma membrane contact sites, where it can interact with various proteins responsible for tethering and lipid trafficking at these contact sites, including PTPIP51 on mitochondria and Nir2 at the ER-PM contacts. Given the role of PERK in modulating both ER-mitochondria and indirectly ER-PM contacts, we then explored the possibility that VAPA/B is a bona fide interacting partner of PERK.

In our dataset, we picked up unique peptides for both VAPA and VAPB, indicating that both proteins might interact with PERK. Because VAPB has traditionally been the

Table 1. List of identified proteins (Scaffold, FDR < 1%) resulting from the BioID interactome screen using PERK-BirA as bait. Protein hits were only detected using PERK-BirA as bait and not in control. Parental cells are shaded in yellow. Relative quantification of proteins is based on spectral counts ('Total spectra'). Only proteins with atleast 2 exclusive unique peptides per protein are listed.

Identified proteins (276)	Accession number	Alternate ID	Molecular weight	Quantitative value (total spectra) PERK-BirA	Exclusive unique peptide count PERK-BirA	Quantitative value (total spectra) Control	Exclusive unique peptide count Control	Protein identification probability PERK-BirA	Protein identification probability Control
Eukaryotic translation initiation factor 2-alpha kinase 3 OS = Homo sapiens OX = 9606 GN = EIF2AK3 PE = 1 SV = 3	Q9NZJ5	EIF2AK3	125 kDa	415	70	0	0	100%	0
Filamin-A OS = Homo sapiens OX = 9606 GN = FLNA PE = 1 SV = 4	P21333	FLNA	281 kDa	94	66	0	0	100%	0
Coatomer subunit gamma-2 OS = Homo sapiens OX = 9606 GN = COPG2 PE = 1 SV = 1	Q9UBF2	COPG2	98 kDa	40	26	0	0	100%	0
Lamina-associated polypeptide 2, isoforms beta/gamma OS = Homo sapiens OX = 9606 GN = TMPO PE = 1 SV = 2	P42167	TMPO	51 kDa	28	16	0	0	100%	0
Keratin, type I cytoskeletal 16 OS = Homo sapiens OX = 9606 GN = KRT16 PE = 1 SV = 4	P08779	KRT16	51 kDa	26	4	0	0	100%	0
Kinectin OS = Homo sapiens OX = 9606 GN = KTN1 PE = 1 SV = 1	Q86UP2	KTN1	156 kDa	24	23	0	0	100%	0
RuvB-like 1 OS = Homo sapiens OX = 9606 GN = RUVBL1 PE = 1 SV = 1	Q9Y265	RUVBL1	50 kDa	20	13	0	0	100%	0
E3 SUMO-protein ligase RanBP2 OS = Homo sapiens OX = 9606 GN = RANBP2 PE = 1 SV = 2	P49792	RANBP2	358 kDa	20	19	0	0	100%	0
78 kDa glucose-regulated protein OS = Homo sapiens GN = HSPA5 PE = 1 SV = 2	P11021| GRP78_HUMAN	HSPA5	72 kDa	16	13	0	0	100%	0
Double-strand break repair	P49959	MRE11	81 kDa	15	14	0	0	100%	0

(continued)

Table 1. Continued.

Identified proteins (276)	Accession number	Alternate ID	Molecular weight	Quantitative value (total spectra) PERK-BirA	Exclusive unique peptide count PERK-BirA	Quantitative value (total spectra) Control	Exclusive unique peptide count Control	Protein identification probability PERK-BirA	Protein identification probability Control
protein MRE11 OS = Homo sapiens OX = 9606 GN = MRE11 PE = 1 SV = 3									
Zinc finger CCCH-type antiviral protein 1 OS = Homo sapiens OX = 9606 GN = ZC3HAV1 PE = 1 SV = 3	Q7Z2W4	ZC3HAV1	101 kDa	14	10	0	0	100%	0
Src substrate cortactin OS = Homo sapiens OX = 9606 GN = CTTN PE = 1 SV = 2	Q14247	CTTN	62 kDa	14	12	0	0	100%	0
Bifunctional glutamate/ proline--tRNA ligase OS = Homo sapiens OX = 9606 GN = EPRS PE = 1 SV = 5	P07814	EPRS	171 kDa	12	11	0	0	100%	0
Lamin-B receptor OS = Homo sapiens OX = 9606 GN = LBR PE = 1 SV = 2	Q14739	LBR	71 kDa	11	7	0	0	100%	0
Transgelin-2 OS = Homo sapiens OX = 9606 GN = TAGLN2 PE = 1 SV = 3	P37802	TAGLN2	22 kDa	10	7	0	0	100%	0
eIF-2-alpha kinase activator GCN1 OS = Homo sapiens OX = 9606 GN = GCN1 PE = 1 SV = 6	Q92616	GCN1	293 kDa	10	10	0	0	100%	0
UBX domain-containing protein 4 OS = Homo sapiens OX = 9606 GN = UBXN4 PE = 1 SV = 2	Q92575	UBXN4	57 kDa	10	6	0	0	100%	0
Staphylococcal nuclease domain-containing protein 1 OS = Homo sapiens OX = 9606 GN = SND1 PE = 1 SV = 1	Q7KZF4	SND1	102 kDa	9	9	0	0	100%	0
Vesicle-associated membrane protein-associated protein A OS = Homo sapiens OX =	Q9P0L0	VAPA	28 kDa	8	4	0	0	100%	0

(continued)

Table 1. Continued.

Identified proteins (276)	Accession number	Alternate ID	Molecular weight	Quantitative value (total spectra) PERK-BirA	Exclusive unique peptide count PERK-BirA	Quantitative value (total spectra) Control	Exclusive unique peptide count Control	Protein identification probability PERK-BirA	Protein identification probability Control
9606 GN = VAPA PE = 1 SV = 3									
Histone H1.5 OS = Homo sapiens OX = 9606 GN = HIST1H1B PE = 1 SV = 3	P16401	HIST1H1B	23 kDa	7	3	0	0	100%	0
Sodium/potassium-transporting ATPase subunit alpha-1 OS = Homo sapiens OX = 9606 GN = ATP1A1 PE = 1 SV = 1	P05023	ATP1A1	113 kDa	7	7	0	0	100%	0
Eukaryotic translation initiation factor 5 OS = Homo sapiens OX = 9606 GN = EIF5 PE = 1 SV = 2	P55010	EIF5	49 kDa	7	6	0	0	100%	0
Cytoskeleton-associated protein 4 OS = Homo sapiens OX = 9606 GN = CKAP4 PE = 1 SV = 2	Q07065	CKAP4	66 kDa	7	7	0	0	100%	0
Protein ELYS OS = Homo sapiens OX = 9606 GN = AHCTF1 PE = 1 SV = 3	Q8WYP5	AHCTF1	253 kDa	7	7	0	0	100%	0
MKL/myocardin-like protein 2 OS = Homo sapiens OX = 9606 GN = MKL2 PE = 1 SV = 3	Q9ULH7	MKL2	118 kDa	7	7	0	0	100%	0
Neuroblast differentiation-associated protein AHNAK OS = Homo sapiens OX = 9606 GN = AHNAK PE = 1 SV = 2	Q09666	AHNAK	629 kDa	7	7	0	0	93%	0
Protein disulfide-isomerase A4 OS = Homo sapiens OX = 9606 GN = PDIA4 PE = 1 SV = 2	P13667	PDIA4	73 kDa	6	5	0	0	100%	0
Coronin-1B OS = Homo sapiens OX = 9606 GN = CORO1B PE = 1 SV = 1	Q9BR76	CORO1B	54 kDa	6	6	0	0	100%	0
Splicing factor, proline- and	P23246	SFPQ	76 kDa	6	4	0	0	100%	0

(continued)

Table 1. Continued.

Identified proteins (276)	Accession number	Alternate ID	Molecular weight	Quantitative value (total spectra) PERK-BirA	Exclusive unique peptide count PERK-BirA	Quantitative value (total spectra) Control	Exclusive unique peptide count Control	Protein identification probability PERK-BirA	Protein identification probability Control
glutamine-rich OS = Homo sapiens OX = 9606 GN = SFPQ PE = 1 SV = 2									
Profilin-1 OS = Homo sapiens OX = 9606 GN = PFN1 PE = 1 SV = 2	P07737	PFN1	15 kDa	6	6	0	0	100%	0
Eukaryotic translation initiation factor 4 gamma 2 OS = Homo sapiens OX = 9606 GN = EIF4G2 PE = 1 SV = 1	P78344	EIF4G2	102 kDa	6	6	0	0	100%	0
Coiled-coil domain-containing protein 47 OS = Homo sapiens OX = 9606 GN = CCDC47 PE = 1 SV = 1	Q96A33	CCDC47	56 kDa	6	6	0	0	100%	0
Endoplasmin OS = Homo sapiens OX = 9606 GN = HSP90B1 PE = 1 SV = 1	P14625	HSP90B1	92 kDa	6	6	0	0	100%	0
Eukaryotic translation initiation factor 4B OS = Homo sapiens OX = 9606 GN = EIF4B PE = 1 SV = 2	P23588	EIF4B	69 kDa	6	6	0	0	100%	0
Multifunctional protein ADE2 OS = Homo sapiens OX = 9606 GN = PAICS PE = 1 SV = 3	P22234	PAICS	47 kDa	6	5	0	0	100%	0
Torsin-1A-interacting protein 1 OS = Homo sapiens OX = 9606 GN = TOR1AIP1 PE = 1 SV = 2	Q5JTV8	TOR1AIP1	66 kDa	6	6	0	0	100%	0
Threonylcarbamoyladenosine tRNA methylthiotransferase OS = Homo sapiens OX = 9606 GN = CDKAL1 PE = 1 SV = 1	Q5VV42	CDKAL1	65 kDa	6	5	0	0	100%	0
Receptor of activated protein C kinase 1 OS = Homo sapiens	P63244	RACK1	35 kDa	6	5	0	0	100%	0

(continued)

Table 1. Continued.

Identified proteins (276)	Accession number	Alternate ID	Molecular weight	Quantitative value (total spectra) PERK-BirA	Exclusive unique peptide count PERK-BirA	Quantitative value (total spectra) Control	Exclusive unique peptide count Control	Protein identification probability PERK-BirA	Protein identification probability Control
OX = 9606 GN = RACK1 PE = 1 SV = 3									
Membrane-associated progesterone receptor component 2 OS = Homo sapiens OX = 9606 GN = PGRMC2 PE = 1 SV = 1	O15173	PGRMC2	24 kDa	5	3	0	0	100%	0
Chromodomain-helicase-DNA-binding protein 4 OS = Homo sapiens OX = 9606 GN = CHD4 PE = 1 SV = 2	Q14839	CHD4	218 kDa	5	5	0	0	100%	0
Afadin OS = Homo sapiens OX = 9606 GN = AFDN PE = 1 SV = 3	P55196	AFDN	207 kDa	5	5	0	0	100%	0
Adapter molecule crk OS = Homo sapiens OX = 9606 GN = CRK PE = 1 SV = 2	P46108	CRK	34 kDa	5	5	0	0	100%	0
Nucleophosmin OS = Homo sapiens OX = 9606 GN = NPM1 PE = 1 SV = 2	P06748	NPM1	33 kDa	5	5	0	0	100%	0
Sarcoplasmic/endoplasmic reticulum calcium ATPase 2 OS = Homo sapiens OX = 9606 GN = ATP2A2 PE = 1 SV = 1	P16615	ATP2A2	115 kDa	5	5	0	0	100%	0
Peroxiredoxin-4 OS = Homo sapiens OX = 9606 GN = PRDX4 PE = 1 SV = 1	Q13162	PRDX4	31 kDa	5	2	0	0	100%	0
Eukaryotic translation initiation factor 4 gamma 1 OS = Homo sapiens OX = 9606 GN = EIF4G1 PE = 1 SV = 4	Q04637	EIF4G1	175 kDa	5	4	0	0	100%	0
Cytoskeleton-associated protein 5 OS = Homo sapiens OX = 9606 GN = CKAP5 PE = 1 SV = 3	Q14008	CKAP5	226 kDa	4	4	0	0	100%	0
PEST proteolytic signal-	Q8WW12	PCNP	19 kDa	4	2	0	0	98%	0

(continued)

Table 1. Continued.

Identified proteins (276)	Accession number	Alternate ID	Molecular weight	Quantitative value (total spectra) PERK-BirA	Exclusive unique peptide count PERK-BirA	Quantitative value (total spectra) Control	Exclusive unique peptide count Control	Protein identification probability PERK-BirA	Protein identification probability Control
containing nuclear protein OS = Homo sapiens OX = 9606 GN = PCNP PE = 1 SV = 2									
Desmoplakin OS = Homo sapiens OX = 9606 GN = DSP PE = 1 SV = 3	P15924	DSP	332 kDa	4	4	0	0	100%	0
Ubiquitin-like modifier-activating enzyme 1 OS = Homo sapiens OX = 9606 GN = UBA1 PE = 1 SV = 3	P22314	UBA1	118 kDa	4	4	0	0	100%	0
Fatty aldehyde dehydrogenase OS = Homo sapiens OX = 9606 GN = ALDH3A2 PE = 1 SV = 1	P51648	ALDH3A2	55 kDa	4	4	0	0	100%	0
Creatine kinase B-type OS = Homo sapiens OX = 9606 GN = CKB PE = 1 SV = 1	P12277	CKB	43 kDa	4	4	0	0	100%	0
Syntaxin-5 OS = Homo sapiens OX = 9606 GN = STX5 PE = 1 SV = 2	Q13190	STX5	40 kDa	4	4	0	0	100%	0
Rab-like protein 3 OS = Homo sapiens OX = 9606 GN = RABL3 PE = 1 SV = 1	Q5HYI8	RABL3	26 kDa	4	3	0	0	100%	0
Regulator of chromosome condensation OS = Homo sapiens OX = 9606 GN = RCC1 PE = 1 SV = 1	P18754	RCC1	45 kDa	4	4	0	0	100%	0
Ran GTPase-activating protein 1 OS = Homo sapiens OX = 9606 GN = RANGAP1 PE = 1 SV = 1	P46060	RANGAP1	64 kDa	4	4	0	0	100%	0
Synaptobrevin homolog YKT6 OS = Homo sapiens OX = 9606 GN = YKT6 PE = 1 SV = 1	O15498	YKT6	22 kDa	4	3	0	0	100%	0
Cold shock domain-containing protein E1 OS = Homo sapiens	O75534	CSDE1	89 kDa	4	4	0	0	100%	0

(continued)

Table 1. Continued.

Identified proteins (276)	Accession number	Alternate ID	Molecular weight	Quantitative value (total spectra) PERK-BirA	Exclusive unique peptide count PERK-BirA	Quantitative value (total spectra) Control	Exclusive unique peptide count Control	Protein identification probability PERK-BirA	Protein identification probability Control
OX = 9606 GN = CSDE1 PE = 1 SV = 2									
Vesicle-associated membrane protein-associated protein B/C OS = Homo sapiens OX = 9606 GN = VAPB PE = 1 SV = 3	O95292	VAPB	27 kDa	4	2	0	0	99%	0
Vigilin OS = Homo sapiens OX = 9606 GN = HDLBP PE = 1 SV = 2	Q00341	HDLBP	141 kDa	4	4	0	0	99%	0
Microtubule-associated protein 4 OS = Homo sapiens OX = 9606 GN = MAP4 PE = 1 SV = 3	P27816	MAP4	121 kDa	4	3	0	0	100%	0
TATA-binding protein-associated factor 2N OS = Homo sapiens OX = 9606 GN = TAF15 PE = 1 SV = 1	Q92804	TAF15	62 kDa	3	2	0	0	84%	0
Peptidyl-prolyl cis-trans isomerase A OS = Homo sapiens OX = 9606 GN = PPIA PE = 1 SV = 2	P62937	PPIA	18 kDa	3	3	0	0	100%	0
CAD protein OS = Homo sapiens OX = 9606 GN = CAD PE = 1 SV = 3	P27708	CAD	243 kDa	3	3	0	0	100%	0
Synapse-associated protein 1 OS = Homo sapiens OX = 9606 GN = SYAP1 PE = 1 SV = 1	Q96A49	SYAP1	40 kDa	3	3	0	0	100%	0
Leucine-rich repeat-containing protein 59 OS = Homo sapiens OX = 9606 GN = LRRC59 PE = 1 SV = 1	Q96AG4	LRRC59	35 kDa	3	3	0	0	100%	0
WW domain-containing oxidoreductase OS = Homo sapiens OX = 9606 GN = WWOX PE = 1 SV = 1	Q9NZC7	WWOX	47 kDa	3	3	0	0	99%	0
D-3-phosphoglycerate	O43175	PHGDH	57 kDa	3	3	0	0	100%	0

(continued)

Table 1. Continued.

Identified proteins (276)	Accession number	Alternate ID	Molecular weight	Quantitative value (total spectra) PERK-BirA	Exclusive unique peptide count PERK-BirA	Quantitative value (total spectra) Control	Exclusive unique peptide count Control	Protein identification probability PERK-BirA	Protein identification probability Control
dehydrogenase OS = Homo sapiens OX = 9606 GN = PHGDH PE = 1 SV = 4									
40S ribosomal protein S3 OS = Homo sapiens OX = 9606 GN = RPS3 PE = 1 SV = 2	P23396	RPS3	27 kDa	3	3	0	0	100%	0
Voltage-dependent anion-selective channel protein 2 OS = Homo sapiens OX = 9606 GN = VDAC2 PE = 1 SV = 2	P45880	VDAC2	32 kDa	3	3	0	0	100%	0
Band 4.1-like protein 3 OS = Homo sapiens OX = 9606 GN = EPB41L3 PE = 1 SV = 2	Q9Y2J2	EPB41L3	121 kDa	3	3	0	0	100%	0
Junctophilin-1 OS = Homo sapiens OX = 9606 GN = JPH1 PE = 1 SV = 2	Q9HDC5	JPH1	72 kDa	3	2	0	0	100%	0
Emerin OS = Homo sapiens OX = 9606 GN = EMD PE = 1 SV = 1	P50402	EMD	29 kDa	3	2	0	0	100%	0
Thioredoxin-dependent peroxide reductase, mitochondrial OS = Homo sapiens OX = 9606 GN = PRDX3 PE = 1 SV = 3	P30048	PRDX3	28 kDa	3	3	0	0	100%	0
Eukaryotic translation initiation factor 5B OS = Homo sapiens OX = 9606 GN = EIF5B PE = 1 SV = 4	O60841	EIF5B	139 kDa	3	3	0	0	99%	0
Clathrin heavy chain 1 OS = Homo sapiens OX = 9606 GN = CLTC PE = 1 SV = 5	Q00610	CLTC	192 kDa	3	3	0	0	99%	0
Stromal interaction molecule 1 OS = Homo sapiens OX = 9606 GN = STIM1 PE = 1 SV = 3	Q13586	STIM1	77 kDa	3	2	0	0	98%	0
Splicing factor 3B subunit 3 OS = Homo sapiens OX = 9606 GN = SF3B3 PE = 1 SV = 4	Q15393	SF3B3	136 kDa	3	3	0	0	96%	0

(continued)

Table 1. Continued.

Identified proteins (276)	Accession number	Alternate ID	Molecular weight	Quantitative value (total spectra) PERK-BirA	Exclusive unique peptide count PERK-BirA	Quantitative value (total spectra) Control	Exclusive unique peptide count Control	Protein identification probability PERK-BirA	Protein identification probability Control
Nuclear migration protein nudC OS = Homo sapiens OX = 9606 GN = NUDC PE = 1 SV = 1	Q9Y266	NUDC	38 kDa	3	3	0	0	93%	0
Proliferation marker protein Ki-67 OS = Homo sapiens OX = 9606 GN = MKI67 PE = 1 SV = 2	P46013	MKI67	359 kDa	3	2	0	0	84%	0
Chloride channel CLIC-like protein 1 OS = Homo sapiens OX = 9606 GN = CLCC1 PE = 1 SV = 1	Q96S66	CLCC1	62 kDa	3	3	0	0	99%	0
Far upstream element-binding protein 2 OS = Homo sapiens OX = 9606 GN = KHSRP PE = 1 SV = 4	Q92945	KHSRP	73 kDa	3	3	0	0	96%	0
Clathrin interactor 1 OS = Homo sapiens OX = 9606 GN = CLINT1 PE = 1 SV = 1	Q14677	CLINT1	68 kDa	3	3	0	0	91%	0
Eukaryotic translation initiation factor 2A OS = Homo sapiens OX = 9606 GN = EIF2A PE = 1 SV = 3	Q9BY44	EIF2A	65 kDa	3	3	0	0	61%	0
Junction plakoglobin OS = Homo sapiens OX = 9606 GN = JUP PE = 1 SV = 3	P14923	JUP	82 kDa	3	3	0	0	40%	0
Pre-mRNA-splicing factor ATP-dependent RNA helicase DHX15 OS = Homo sapiens OX = 9606 GN = DHX15 PE = 1 SV = 2	O43143	DHX15	91 kDa	2	2	0	0	100%	0
T-complex protein 1 subunit zeta OS = Homo sapiens OX = 9606 GN = CCT6A PE = 1 SV = 3	P40227	CCT6A	58 kDa	2	2	0	0	100%	0
Cytoplasmic dynein 1 heavy chain 1 OS = Homo sapiens	Q14204	DYNC1H1	532 kDa	2	2	0	0	100%	0

(continued)

Table 1. Continued.

Identified proteins (276)	Accession number	Alternate ID	Molecular weight	Quantitative value (total spectra) PERK-BirA	Exclusive unique peptide count PERK-BirA	Quantitative value (total spectra) Control	Exclusive unique peptide count Control	Protein identification probability PERK-BirA	Protein identification probability Control
OX = 9606 GN = DYNC1H1 PE = 1 SV = 5									
60S ribosomal protein L15 OS = Homo sapiens OX = 9606 GN = RPL15 PE = 1 SV = 2	P61313	RPL15	24 kDa	2	2	0	0	100%	0
Dolichyl-diphosphooligosaccharide–protein glycosyltransferase subunit STT3B OS = Homo sapiens OX = 9606 GN = STT3B PE = 1 SV = 1	Q8TCJ2	STT3B	94 kDa	2	2	0	0	100%	0
Protein SGT1 homolog OS = Homo sapiens OX = 9606 GN = SUGT1 PE = 1 SV = 3	Q9Y2Z0	SUGT1	41 kDa	2	2	0	0	100%	0
Protein 4.1 OS = Homo sapiens OX = 9606 GN = EPB41 PE = 1 SV = 4	P11171	EPB41	97 kDa	2	2	0	0	100%	0
Heterogeneous nuclear ribonucleoprotein D0 OS = Homo sapiens OX = 9606 GN = HNRNPD PE = 1 SV = 1	Q14103	HNRNPD	38 kDa	2	2	0	0	100%	0
Chloride intracellular channel protein 1 OS = Homo sapiens OX = 9606 GN = CLIC1 PE = 1 SV = 4	O00299	CLIC1	27 kDa	2	2	0	0	100%	0
Signal recognition particle subunit SRP68 OS = Homo sapiens OX = 9606 GN = SRP68 PE = 1 SV = 2	Q9UHB9	SRP68	71 kDa	2	2	0	0	100%	0
Signal recognition particle 54 kDa protein OS = Homo sapiens OX = 9606 GN = SRP54 PE = 1 SV = 1	P61011	SRP54	56 kDa	2	2	0	0	100%	0
Polyadenylate-binding protein 1 OS = Homo sapiens OX = 9606 GN = PABPC1 PE = 1 SV = 2	P11940	PABPC1	71 kDa	2	2	0	0	100%	0

(continued)

Table 1. Continued.

Identified proteins (276)	Accession number	Alternate ID	Molecular weight	Quantitative value (total spectra) PERK-BirA	Exclusive unique peptide count PERK-BirA	Quantitative value (total spectra) Control	Exclusive unique peptide count Control	Protein identification probability PERK-BirA	Protein identification probability Control
T-complex protein 1 subunit epsilon OS = Homo sapiens OX = 9606 GN = CCT5 PE = 1 SV = 1	P48643	CCT5	60 kDa	2	2	0	0	100%	0
C-1-tetrahydrofolate synthase, cytoplasmic OS = Homo sapiens OX = 9606 GN = MTHFD1 PE = 1 SV = 3	P11586	MTHFD1	102 kDa	2	2	0	0	100%	0
Voltage-dependent anion-selective channel protein 3 OS = Homo sapiens OX = 9606 GN = VDAC3 PE = 1 SV = 1	Q9Y277	VDAC3	31 kDa	2	2	0	0	100%	0
RNA-binding protein 26 OS = Homo sapiens OX = 9606 GN = RBM26 PE = 1 SV = 3	Q5T8P6	RBM26	114 kDa	2	2	0	0	99%	0
Ran-binding protein 3 OS = Homo sapiens OX = 9606 GN = RANBP3 PE = 1 SV = 1	Q9H6Z4	RANBP3	60 kDa	2	2	0	0	99%	0
60S ribosomal protein L10 OS = Homo sapiens OX = 9606 GN = RPL10 PE = 1 SV = 4	P27635	RPL10	25 kDa	2	2	0	0	99%	0
Protein RCC2 OS = Homo sapiens OX = 9606 GN = RCC2 PE = 1 SV = 2	Q9P258	RCC2	56 kDa	2	2	0	0	98%	0
Extended synaptotagmin-1 OS = Homo sapiens OX = 9606 GN = ESYT1 PE = 1 SV = 1	Q9BSJ8	ESYT1	123 kDa	2	2	0	0	97%	0
PC4 and SFRS1-interacting protein OS = Homo sapiens OX = 9606 GN = PSIP1 PE = 1 SV = 1	O75475	PSIP1	60 kDa	2	2	0	0	94%	0
SAFB-like transcription modulator OS = Homo sapiens OX = 9606 GN = SLTM PE = 1 SV = 2	Q9NWH9	SLTM	117 kDa	2	2	0	0	92%	0
Probable rRNA-processing protein EBP2 OS = Homo	Q99848	EBNA1BP2	35 kDa	2	2	0	0	80%	0

(continued)

Table 1. Continued.

Identified proteins (276)	Accession number	Alternate ID	Molecular weight	Quantitative value (total spectra) PERK-BirA	Exclusive unique peptide count PERK-BirA	Quantitative value (total spectra) Control	Exclusive unique peptide count Control	Protein identification probability PERK-BirA	Protein identification probability Control
sapiens OX = 9606 GN = EBNA1BP2 PE = 1 SV = 2									
Cysteine and histidine-rich domain-containing protein 1 OS = Homo sapiens OX = 9606 GN = CHORDC1 PE = 1 SV = 2	Q9UHD1	CHORDC1	37 kDa	2	2	0	0	79%	0
ATP-dependent RNA helicase DDX39A OS = Homo sapiens OX = 9606 GN = DDX39A PE = 1 SV = 2	O00148 (+1)	DDX39A	49 kDa	2	2	0	0	100%	0
Heterogeneous nuclear ribonucleoprotein H3 OS = Homo sapiens OX = 9606 GN = HNRNPH3 PE = 1 SV = 2	P31942	HNRNPH3	37 kDa	2	2	0	0	100%	0
Peroxisomal multifunctional enzyme type 2 OS = Homo sapiens OX = 9606 GN = HSD17B4 PE = 1 SV = 3	P51659	HSD17B4	80 kDa	2	2	0	0	98%	0
Eukaryotic translation initiation factor 3 subunit D OS = Homo sapiens OX = 9606 GN = EIF3D PE = 1 SV = 1	O15371	EIF3D	64 kDa	2	2	0	0	96%	0
Tyrosine-protein phosphatase non-receptor type 1 OS = Homo sapiens OX = 9606 GN = PTPN1 PE = 1 SV = 1	P18031	PTPN1	50 kDa	2	2	0	0	92%	0
Jupiter microtubule associated homolog 2 OS = Homo sapiens OX = 9606 GN = JPT2 PE = 1 SV = 1	Q9H910	JPT2	20 kDa	2	2	0	0	90%	0
U5 small nuclear ribonucleoprotein 200 kDa helicase OS = Homo sapiens OX = 9606 GN = SNRNP200 PE = 1 SV = 2	O75643	SNRNP200	245 kDa	2	2	0	0	89%	0
PDZ and LIM domain protein 5	Q96HC4	PDLIM5	64 kDa	2	2	0	0	86%	0

(continued)

Table 1. Continued.

Identified proteins (276)	Accession number	Alternate ID	Molecular weight	Quantitative value (total spectra) PERK-BirA	Exclusive unique peptide count PERK-BirA	Quantitative value (total spectra) Control	Exclusive unique peptide count Control	Protein identification probability PERK-BirA	Protein identification probability Control
OS = Homo sapiens OX = 9606 GN = PDLIM5 PE = 1 SV = 5									
Coatomer subunit alpha OS = Homo sapiens OX = 9606 GN = COPA PE = 1 SV = 2	P53621	COPA	138 kDa	2	2	0	0	86%	0
Activator of 90 kDa heat shock protein ATPase homolog 1 OS = Homo sapiens OX = 9606 GN = AHSA1 PE = 1 SV = 1	O95433	AHSA1	38 kDa	2	2	0	0	76%	0
Asparagine synthetase [glutamine-hydrolyzing] OS = Homo sapiens OX = 9606 GN = ASNS PE = 1 SV = 4	P08243	ASNS	64 kDa	2	2	0	0	69%	0
Transitional endoplasmic reticulum ATPase OS = Homo sapiens OX = 9606 GN = VCP PE = 1 SV = 4	P55072	VCP	89 kDa	2	2	0	0	62%	0
RNA cytidine acetyltransferase OS = Homo sapiens OX = 9606 GN = NAT10 PE = 1 SV = 2	Q9H0A0	NAT10	116 kDa	2	2	0	0	54%	0
Protein arginine N-methyltransferase 5 OS = Homo sapiens OX = 9606 GN = PRMT5 PE = 1 SV = 4	O14744	PRMT5	73 kDa	2	2	0	0	38%	0
60S ribosomal protein L38 OS = Homo sapiens OX = 9606 GN = RPL38 PE = 1 SV = 2	P63173	RPL38	8 kDa	2	2	0	0	37%	0
Angiomotin OS = Homo sapiens OX = 9606 GN = AMOT PE = 1 SV = 1	Q4VCS5	AMOT	118 kDa	2	2	0	0	29%	0
Segment polarity protein dishevelled homolog DVL-2 OS = Homo sapiens OX = 9606 GN = DVL2 PE = 1 SV = 1	O14641	DVL2	79 kDa	2	2	0	0	21%	0

(continued)

Table 1. Continued.

Identified proteins (276)	Accession number	Alternate ID	Molecular weight	Quantitative value (total spectra) PERK-BirA	Exclusive unique peptide count PERK-BirA	Quantitative value (total spectra) Control	Exclusive unique peptide count Control	Protein identification probability PERK-BirA	Protein identification probability Control
DDRGK domain-containing protein 1 OS = Homo sapiens OX = 9606 GN = DDRGK1 PE = 1 SV = 2	Q96HY6	DDRGK1	36 kDa	2	2	0	0	41%	0
Calponin-3 OS = Homo sapiens OX = 9606 GN = CNN3 PE = 1 SV = 1	Q15417	CNN3	36 kDa	2	2	0	0	19%	0
Synaptosomal-associated protein 29 OS = Homo sapiens OX = 9606 GN = SNAP29 PE = 1 SV = 1	O95721	SNAP29	29 kDa	2	2	0	0	16%	0
T-complex protein 1 subunit theta OS = Homo sapiens OX = 9606 GN = CCT8 PE = 1 SV = 4	P50990	CCT8	60 kDa	58	32	1	1	100%	55%
Fatty acid synthase OS = Homo sapiens OX = 9606 GN = FASN PE = 1 SV = 3	P49327	FASN	273 kDa	32	27	1	1	100%	82%
Protein LYRIC OS = Homo sapiens OX = 9606 GN = MTDH PE = 1 SV = 2	Q86UE4	MTDH	64 kDa	24	15	1	1	100%	11%
Plasminogen activator inhibitor 1 RNA-binding protein OS = Homo sapiens OX = 9606 GN = SERBP1 PE = 1 SV = 2	Q8NC51	SERBP1	45 kDa	12	8	1	1	100%	77%
Ubiquitin-40S ribosomal protein S27a OS = Homo sapiens OX = 9606 GN = RPS27A PE = 1 SV = 2	P62979	RPS27A	18 kDa	16	7	1	1	100%	15%
Heterogeneous nuclear ribonucleoprotein L OS = Homo sapiens OX = 9606 GN = HNRNPL PE = 1 SV = 2	P14866	HNRNPL	64 kDa	9	9	1	1	100%	94%
Poly(rC)-binding protein 1 OS = Homo sapiens OX = 9606 GN = PCBP1 PE = 1 SV = 2	Q15365	PCBP1	37 kDa	7	5	1	1	100%	98%
Eukaryotic initiation factor 4A-I	P60842	EIF4A1	46 kDa	6	6	1	1	100%	81%

(continued)

Table 1. Continued.

Identified proteins (276)	Accession number	Alternate ID	Molecular weight	Quantitative value (total spectra) PERK-BirA	Exclusive unique peptide count PERK-BirA	Quantitative value (total spectra) Control	Exclusive unique peptide count Control	Protein identification probability PERK-BirA	Protein identification probability Control
OS = Homo sapiens OX = 9606 GN = EIF4A1 PE = 1 SV = 1									
Heterogeneous nuclear ribonucleoprotein A3 OS = Homo sapiens OX = 9606 GN = HNRNPA3 PE = 1 SV = 2	P51991	HNRNPA3	40 kDa	5	5	1	1	100%	98%
GTP-binding nuclear protein Ran OS = Homo sapiens OX = 9606 GN = RAN PE = 1 SV = 3	P62826	RAN	24 kDa	5	5	1	1	100%	93%
ADP/ATP translocase 2 OS = Homo sapiens OX = 9606 GN = SLC25A5 PE = 1 SV = 7	P05141	SLC25A5	33 kDa	4	4	1	1	100%	92%
Splicing regulatory glutamine/lysine-rich protein 1 OS = Homo sapiens OX = 9606 GN = SREK1 PE = 1 SV = 1	Q8WXA9	SREK1	59 kDa	4	4	1	1	100%	47%
60S ribosomal protein L12 OS = Homo sapiens OX = 9606 GN = RPL12 PE = 1 SV = 1	P30050	RPL12	18 kDa	3	2	1	1	100%	76%
Fructose-bisphosphate aldolase A OS = Homo sapiens OX = 9606 GN = ALDOA PE = 1 SV = 2	P04075	ALDOA	39 kDa	4	4	1	1	100%	20%
60S ribosomal protein L3 OS = Homo sapiens OX = 9606 GN = RPL3 PE = 1 SV = 2	P39023	RPL3	46 kDa	5	4	1	1	100%	8%
Heterogeneous nuclear ribonucleoprotein H OS = Homo sapiens OX = 9606 GN = HNRNPH1 PE = 1 SV = 4	P31943	HNRNPH1	49 kDa	3	3	1	1	100%	98%
Desmoglein-1 OS = Homo sapiens OX = 9606 GN = DSG1 PE = 1 SV = 2	Q02413	DSG1	114 kDa	2	2	1	1	100%	97%
40S ribosomal protein S2 OS =	P15880	RPS2	31 kDa	2	2	1	1	100%	96%

(continued)

Table 1. Continued.

Identified proteins (276)	Accession number	Alternate ID	Molecular weight	Quantitative value (total spectra) PERK-BirA	Exclusive unique peptide count PERK-BirA	Quantitative value (total spectra) Control	Exclusive unique peptide count Control	Protein identification probability PERK-BirA	Protein identification probability Control
Homo sapiens OX = 9606 GN = RPS2 PE = 1 SV = 2									
Dermcidin OS = Homo sapiens OX = 9606 GN = DCD PE = 1 SV = 2	P81605	DCD	11 kDa	2	2	1	1	100%	94%
40S ribosomal protein S16 OS = Homo sapiens OX = 9606 GN = RPS16 PE = 1 SV = 2	P62249	RPS16	16 kDa	2	2	1	1	100%	92%
Heterogeneous nuclear ribonucleoprotein M OS = Homo sapiens OX = 9606 GN = HNRNPM PE = 1 SV = 3	P52272	HNRNPM	78 kDa	2	2	1	1	100%	92%
Serine/arginine-rich splicing factor 7 OS = Homo sapiens OX = 9606 GN = SRSF7 PE = 1 SV = 1	Q16629	SRSF7	27 kDa	3	3	1	1	99%	77%
40S ribosomal protein S15a OS = Homo sapiens OX = 9606 GN = RPS15A PE = 1 SV = 2	P62244	RPS15A	15 kDa	3	3	1	1	100%	74%
T-complex protein 1 subunit alpha OS = Homo sapiens OX = 9606 GN = TCP1 PE = 1 SV = 1	P17987	TCP1	60 kDa	3	3	1	1	100%	56%
Pre-mRNA-processing factor 40 homolog A OS = Homo sapiens OX = 9606 GN = PRPF40A PE = 1 SV = 2	O75400	PRPF40A	109 kDa	3	3	1	1	99%	50%
Glyceraldehyde-3-phosphate dehydrogenase OS = Homo sapiens OX = 9606 GN = GAPDH PE = 1 SV = 3	P04406	GAPDH	36 kDa	2	2	1	1	100%	31%
RNA-binding protein 39 OS = Homo sapiens OX = 9606 GN = RBM39 PE = 1 SV = 2	Q14498	RBM39	59 kDa	3	3	1	1	99%	28%
Probable ATP-dependent RNA helicase DDX46 OS = Homo	Q7L014	DDX46	117 kDa	3	3	1	1	100%	8%

(continued)

Table 1. Continued.

Identified proteins (276)	Accession number	Alternate ID	Molecular weight	Quantitative value (total spectra) PERK-BirA	Exclusive unique peptide count PERK-BirA	Quantitative value (total spectra) Control	Exclusive unique peptide count Control	Protein identification probability PERK-BirA	Protein identification probability Control
sapiens OX = 9606 GN = DDX46 PE = 1 SV = 2									
Filaggrin-2 OS = Homo sapiens OX = 9606 GN = FLG2 PE = 1 SV = 1	Q5D862	FLG2	248 kDa	3	3	1	1	85%	23%
Cofilin-1 OS = Homo sapiens OX = 9606 GN = CFL1 PE = 1 SV = 3	P23528	CFL1	19 kDa	3	3	1	1	100%	7%
Heterogeneous nuclear ribonucleoprotein A/B OS = Homo sapiens OX = 9606 GN = HNRNPAB PE = 1 SV = 2	Q99729	HNRNPAB	36 kDa	2	2	1	1	98%	98%
Pre-mRNA-processing-splicing factor 8 OS = Homo sapiens OX = 9606 GN = PRPF8 PE = 1 SV = 2	Q6P2Q9	PRPF8	274 kDa	4	4	1	1	85%	31%
Elongation factor Tu, mitochondrial OS = Homo sapiens OX = 9606 GN = TUFM PE = 1 SV = 2	P49411	TUFM	50 kDa	9	8	2	2	100%	95%
RNA-binding protein FUS OS = Homo sapiens OX = 9606 GN = FUS PE = 1 SV = 1	P35637	FUS	53 kDa	11	6	2	2	100%	30%
Peroxiredoxin-2 OS = Homo sapiens OX = 9606 GN = PRDX2 PE = 1 SV = 5	P32119	PRDX2	22 kDa	6	3	2	1	100%	73%
ADP-ribosylation factor-like protein 6-interacting protein 4 OS = Homo sapiens OX = 9606 GN = ARL6IP4 PE = 1 SV= 2	Q66PJ3	ARL6IP4	45 kDa	6	4	2	1	100%	98%
Nucleolar RNA helicase 2 OS = Homo sapiens OX = 9606 GN = DDX21 PE= 1 SV = 5	Q9NR30	DDX21	87 kDa	7	7	2	2	100%	96%
Fibronectin OS = Homo sapiens OX = 9606 GN = FN1 PE = 1 SV = 4	P02751	FN1	263 kDa	4	3	2	2	100%	100%

(continued)

Table 1. Continued.

Identified proteins (276)	Accession number	Alternate ID	Molecular weight	Quantitative value (total spectra) PERK-BirA	Exclusive unique peptide count PERK-BirA	Quantitative value (total spectra) Control	Exclusive unique peptide count Control	Protein identification probability PERK-BirA	Protein identification probability Control
Serine/arginine-rich splicing factor 1 OS = Homo sapiens OX = 9606 GN = SRSF1 PE = 1 SV = 2	Q07955	SRSF1	28 kDa	5	4	2	2	100%	66%
Serine/threonine-protein kinase PRP4 homolog OS = Homo sapiens OX = 9606 GN = PRPF4B PE = 1 SV = 3	Q13523	PRPF4B	117 kDa	4	4	2	2	100%	100%
Nucleolar protein 58 OS = Homo sapiens OX = 9606 GN = NOP58 PE = 1 SV = 1	Q9Y2X3	NOP58	60 kDa	4	4	2	2	100%	100%
Alpha-enolase OS = Homo sapiens OX = 9606 GN = ENO1 PE = 1 SV = 2	P06733	ENO1	47 kDa	4	3	2	2	100%	98%
Histone H2A type 1 OS = Homo sapiens OX = 9606 GN = HIST1H2AG PE = 1 SV = 2	P0C0S8 (+6)	HIST1H2AG	14 kDa	2	2	2	2	100%	100%
Serine/arginine-rich splicing factor 2 OS = Homo sapiens OX = 9606 GN = SRSF2 PE = 1 SV = 4	Q01130	SRSF2	25 kDa	2	2	2	2	100%	100%
60S ribosomal protein L36a OS = Homo sapiens OX = 9606 GN = RPL36A PE = 1 SV = 2	P83881 (+1)	RPL36A	12 kDa	2	2	2	2	99%	93%
Poly(rC)-binding protein 2 OS = Homo sapiens OX = 9606 GN = PCBP2 PE = 1 SV = 1	Q15366	PCBP2	39 kDa	4	1	2	2	40%	41%
Proliferation-associated protein 2G4 OS = Homo sapiens OX = 9606 GN = PA2G4 PE = 1 SV = 3	Q9UQ80	PA2G4	44 kDa	4	4	2	2	100%	71%
40S ribosomal protein S26 OS = Homo sapiens OX = 9606 GN = RPS26 PE = 1 SV = 3	P62854	RPS26	13 kDa	2	2	2	1	100%	67%
Keratinocyte proline-rich protein OS = Homo sapiens OX =	Q5T749	KPRP	64 kDa	2	2	2	2	73%	85%

(continued)

Table 1. Continued.

Identified proteins (276)	Accession number	Alternate ID	Molecular weight	Quantitative value (total spectra) PERK-BirA	Exclusive unique peptide count PERK-BirA	Quantitative value (total spectra) Control	Exclusive unique peptide count Control	Protein identification probability PERK-BirA	Protein identification probability Control
9606 GN = KPRP PE = 1 SV = 1									
ATP-dependent RNA helicase DDX3X OS = Homo sapiens OX = 9606 GN = DDX3X PE = 1 SV = 3	O00571	DDX3X	73 kDa	2	2	2	2	100%	27%
ATP-citrate synthase OS = Homo sapiens OX = 9606 GN = ACLY PE = 1 SV = 3	P53396	ACLY	121 kDa	5	5	2	2	100%	16%
60S ribosomal protein L4 OS = Homo sapiens OX = 9606 GN = RPL4 PE = 1 SV = 5	P36578	RPL4	48 kDa	3	3	2	2	100%	15%
60S ribosomal protein L7a OS = Homo sapiens OX = 9606 GN = RPL7A PE = 1 SV = 2	P62424	RPL7A	30 kDa	2	2	2	1	92%	10%
60S ribosomal protein L8 OS = Homo sapiens OX = 9606 GN = RPL8 PE = 1 SV = 2	P62917	RPL8	28 kDa	1	1	2	2	83%	98%
Uncharacterized protein NKAPD1 OS = Homo sapiens OX = 9606 GN = NKAPD1 PE = 1 SV = 2	Q6ZUT1	NKAPD1	34 kDa	2	2	2	2	60%	66%
Glutathione S-transferase P OS = Homo sapiens OX = 9606 GN = GSTP1 PE = 1 SV = 2	P09211	GSTP1	23 kDa	1	1	2	2	71%	46%
Interleukin enhancer-binding factor 3 OS = Homo sapiens OX = 9606 GN = ILF3 PE = 1 SV = 3	Q12906	ILF3	95 kDa	0	0	2	2	0	100%
Non-histone chromosomal protein HMG-14 OS = Homo sapiens OX = 9606 GN = HMGN1 PE = 1 SV = 3	P05114	HMGN1	11 kDa	1	1	2	2	9%	31%
ATP-dependent RNA helicase A OS = Homo sapiens OX = 9606 GN = DHX9 PE = 1 SV = 4	Q08211	DHX9	141 kDa	20	15	3	2	100%	98%

(continued)

Table 1. Continued.

Identified proteins (276)	Accession number	Alternate ID	Molecular weight	Quantitative value (total spectra) PERK-BirA	Exclusive unique peptide count PERK-BirA	Quantitative value (total spectra) Control	Exclusive unique peptide count Control	Protein identification probability PERK-BirA	Protein identification probability Control
Peroxiredoxin-1 OS = Homo sapiens OX = 9606 GN = PRDX1 PE = 1 SV = 1	Q06830	PRDX1	22 kDa	13	11	3	3	100%	100%
Retinitis pigmentosa 9 protein OS = Homo sapiens OX = 9606 GN = RP9 PE = 1 SV = 2	Q8TA86	RP9	26 kDa	11	8	3	3	100%	100%
Non-POU domain-containing octamer-binding protein OS = Homo sapiens OX = 9606 GN = NONO PE = 1 SV = 4	Q15233	NONO	54 kDa	12	9	3	2	100%	100%
L-lactate dehydrogenase B chain OS = Homo sapiens OX = 9606 GN = LDHB PE = 1 SV = 2	P07195	LDHB	37 kDa	7	5	3	2	100%	100%
Vimentin OS = Homo sapiens OX = 9606 GN = VIM PE = 1 SV = 4	P08670	VIM	54 kDa	7	6	3	2	100%	100%
40S ribosomal protein S3a OS = Homo sapiens OX = 9606 GN = RPS3A PE = 1 SV = 2	P61247	RPS3A	30 kDa	3	3	3	3	100%	100%
THO complex subunit 4 OS = Homo sapiens OX = 9606 GN = ALYREF PE = 1 SV = 3	Q86V81	ALYREF	27 kDa	2	2	3	2	100%	100%
Histone H1x OS = Homo sapiens OX = 9606 GN = H1FX PE = 1 SV = 1	Q92522	H1FX	22 kDa	3	2	3	2	100%	100%
40S ribosomal protein S14 OS = Homo sapiens OX = 9606 GN = RPS14 PE = 1 SV = 3	P62263	RPS14	16 kDa	2	2	3	2	100%	97%
60S ribosomal protein L13 OS = Homo sapiens OX = 9606 GN = RPL13 PE = 1 SV = 4	P26373	RPL13	24 kDa	2	2	3	2	100%	100%
Serum albumin OS = Homo sapiens OX = 9606 GN = ALB PE = 1 SV = 2	P02768	ALB	69 kDa	2	1	3	3	96%	100%
Elongation factor 1-gamma OS =	P26641	EEF1G	50 kDa	1	1	3	3	97%	100%

(continued)

Table 1. Continued.

Identified proteins (276)	Accession number	Alternate ID	Molecular weight	Quantitative value (total spectra) PERK-BirA	Exclusive unique peptide count PERK-BirA	Quantitative value (total spectra) Control	Exclusive unique peptide count Control	Protein identification probability PERK-BirA	Protein identification probability Control
Homo sapiens OX = 9606 GN = EEF1G PE = 1 SV = 3									
60S ribosomal protein L10a OS = Homo sapiens OX = 9606 GN = RPL10A PE = 1 SV = 2	P62906	RPL10A	25 kDa	1	1	3	2	41%	98%
ATP synthase subunit alpha, mitochondrial OS = Homo sapiens OX = 9606 GN = ATP5F1A PE = 1 SV = 1	P25705	ATP5F1A	60 kDa	1	1	3	3	8%	100%
Heat shock protein HSP 90-alpha OS = Homo sapiens OX = 9606 GN = HSP90AA1 PE = 1 SV = 5	P07900	HSP90AA1	85 kDa	12	9	4	3	100%	100%
Pyruvate kinase PKM OS = Homo sapiens OX = 9606 GN = PKM PE = 1 SV = 4	P14618	PKM	58 kDa	15	15	4	4	100%	100%
Heat shock protein HSP 90-beta OS = Homo sapiens OX = 9606 GN = HSP90AB1 PE = 1 SV = 4	P08238	HSP90AB1	83 kDa	10	4	4	1	100%	8%
Splicing factor, arginine/serine-rich 19 OS = Homo sapiens OX = 9606 GN = SCAF1 PE = 1 SV = 3	Q9H7N4	SCAF1	139 kDa	8	7	4	3	100%	100%
Heterogeneous nuclear ribonucleoprotein K OS = Homo sapiens OX = 9606 GN = HNRNPK PE = 1 SV = 1	P61978	HNRNPK	51 kDa	5	5	4	4	100%	100%
Trypsin-1 OS = Homo sapiens OX = 9606 GN = PRSS1 PE = 1 SV = 1	P07477	PRSS1	27 kDa	4	2	4	2	100%	100%
40S ribosomal protein S30 OS = Homo sapiens OX = 9606 GN = FAU PE = 1 SV = 1	P62861	FAU	7 kDa	3	1	4	2	97%	100%
DNA topoisomerase 1 OS = Homo sapiens OX = 9606 GN = TOP1 PE = 1 SV = 2	P11387	TOP1	91 kDa	6	5	4	4	100%	97%

(continued)

Table 1. Continued.

Identified proteins (276)	Accession number	Alternate ID	Molecular weight	Quantitative value (total spectra) PERK-BirA	Exclusive unique peptide count PERK-BirA	Quantitative value (total spectra) Control	Exclusive unique peptide count Control	Protein identification probability PERK-BirA	Protein identification probability Control
60S ribosomal protein L7 OS = Homo sapiens OX = 9606 GN = RPL7 PE = 1 SV = 1	P18124	RPL7	29 kDa	4	3	4	3	100%	100%
40S ribosomal protein S8 OS = Homo sapiens OX = 9606 GN = RPS8 PE = 1 SV = 2	P62241	RPS8	24 kDa	3	3	4	2	100%	100%
60S ribosomal protein L5 OS = Homo sapiens OX = 9606 GN = RPL5 PE = 1 SV = 3	P46777	RPL5	34 kDa	1	1	4	2	95%	80%
Ribosomal RNA processing protein 1 homolog B OS = Homo sapiens OX = 9606 GN = RRP1B PE = 1 SV = 3	Q14684	RRP1B	84 kDa	0	0	4	4	0	100%
Elongation factor 2 OS = Homo sapiens OX = 9606 GN = EEF2 PE = 1 SV = 4	P13639	EEF2	95 kDa	31	19	5	5	100%	100%
Tubulin beta chain OS = Homo sapiens OX = 9606 GN = TUBB PE = 1 SV = 2	P07437	TUBB	50 kDa	16	11	5	4	100%	100%
Tubulin alpha-1B chain OS = Homo sapiens OX = 9606 GN = TUBA1B PE = 1 SV = 1	P68363 (+2)	TUBA1B	50 kDa	13	8	5	4	100%	100%
L-lactate dehydrogenase A chain OS = Homo sapiens OX = 9606 GN = LDHA PE = 1 SV = 2	P00338	LDHA	37 kDa	7	6	5	5	100%	100%
E3 ubiquitin-protein ligase RBBP6 OS = Homo sapiens OX = 9606 GN = RBBP6 PE = 1 SV = 1	Q7Z6E9	RBBP6	202 kDa	11	10	5	5	100%	100%
Protein LLP homolog OS = Homo sapiens OX = 9606 GN = LLPH PE = 1 SV = 1	Q9BRT6	LLPH	15 kDa	7	4	5	3	100%	100%
Probable ATP-dependent RNA helicase DDX17 OS = Homo sapiens OX = 9606 GN = DDX17 PE = 1 SV = 2	Q92841	DDX17	80 kDa	4	3	5	4	100%	100%

(continued)

Table 1. Continued.

Identified proteins (276)	Accession number	Alternate ID	Molecular weight	Quantitative value (total spectra) PERK-BirA	Exclusive unique peptide count PERK-BirA	Quantitative value (total spectra) Control	Exclusive unique peptide count Control	Protein identification probability PERK-BirA	Protein identification probability Control
Protein SREK1IP1 OS = Homo sapiens OX = 9606 GN = SREK1IP1 PE = 1 SV = 1	Q8N9Q2	SREK1IP1	18 kDa	4	3	5	3	100%	100%
RNA-binding motif protein, X chromosome OS = Homo sapiens OX = 9606 GN = RBMX PE = 1 SV = 3	P38159	RBMX	42 kDa	8	6	5	4	100%	97%
Putative RNA-binding protein Luc7-like 2 OS = Homo sapiens OX = 9606 GN = LUC7L2 PE = 1 SV = 2	Q9Y383	LUC7L2	47 kDa	2	2	5	4	99%	100%
60S ribosomal protein L37 OS = Homo sapiens OX = 9606 GN = RPL37 PE = 1 SV = 2	P61927	RPL37	11 kDa	0	0	5	2	0	100%
Heat shock 70 kDa protein 1A OS = Homo sapiens OX = 9606 GN = HSPA1A PE = 1 SV = 1	P0DMV8 (+1)	HSPA1A	70 kDa	20	16	6	6	100%	100%
Heat shock cognate 71 kDa protein OS = Homo sapiens OX = 9606 GN = HSPA8 PE = 1 SV = 1	P11142	HSPA8	71 kDa	18	8	6	4	100%	100%
Heterogeneous nuclear ribonucleoproteins A2/B1 OS = Homo sapiens OX = 9606 GN = HNRNPA2B1 PE = 1 SV = 2	P22626	HNRNPA2B1	37 kDa	16	11	6	4	100%	100%
Histone H4 OS = Homo sapiens OX = 9606 GN = HIST1H4A PE = 1 SV = 2	P62805	HIST1H4A	11 kDa	9	7	6	5	100%	100%
AP-3 complex subunit delta-1 OS = Homo sapiens OX = 9606 GN = AP3D1 PE = 1 SV = 1	O14617	AP3D1	130 kDa	9	7	6	4	100%	100%
Nucleolin OS = Homo sapiens OX = 9606 GN = NCL PE = 1 SV = 3	P19338	NCL	77 kDa	8	8	6	6	100%	100%
A-kinase anchor protein 17A OS	Q02040	AKAP17A	81 kDa	11	8	6	6	100%	100%

(continued)

Table 1. Continued.

Identified proteins (276)	Accession number	Alternate ID	Molecular weight	Quantitative value (total spectra) PERK-BirA	Exclusive unique peptide count PERK-BirA	Quantitative value (total spectra) Control	Exclusive unique peptide count Control	Protein identification probability PERK-BirA	Protein identification probability Control
= Homo sapiens OX = 9606 GN = AKAP17A PE = 1 SV = 2									
60S ribosomal protein L6 OS = Homo sapiens OX = 9606 GN = RPL6 PE = 1 SV = 3	Q02878	RPL6	33 kDa	2	2	6	4	95%	100%
Heterogeneous nuclear ribonucleoprotein A1 OS = Homo sapiens OX = 9606 GN = HNRNPA1 PE = 1 SV = 5	P09651	HNRNPA1	39 kDa	16	11	7	4	100%	100%
La-related protein 7 OS = Homo sapiens OX = 9606 GN = LARP7 PE = 1 SV = 1	Q4G0J3	LARP7	67 kDa	6	6	7	6	100%	100%
G patch domain-containing protein 4 OS = Homo sapiens OX = 9606 GN = GPATCH4 PE = 1 SV = 2	Q5T3I0	GPATCH4	50 kDa	5	5	7	6	100%	100%
NF-kappa-B-activating protein OS = Homo sapiens OX = 9606 GN = NKAP PE = 1 SV = 1	Q8N5F7	NKAP	47 kDa	8	6	8	5	100%	100%
60S ribosomal protein L23a OS = Homo sapiens OX = 9606 GN = RPL23A PE = 1 SV = 1	P62750	RPL23A	18 kDa	8	6	8	4	100%	100%
Histone H2B type 1-K OS = Homo sapiens OX = 9606 GN = HIST1H2BK PE = 1 SV = 3	O60814 (+8)	HIST1H2BK	14 kDa	7	5	8	4	100%	100%
Histone H3.1 OS = Homo sapiens OX = 9606 GN = HIST1H3A PE = 1 SV = 2	P68431 (+3)	HIST1H3A	15 kDa	6	4	8	4	100%	100%
Transcription initiation factor TFIID subunit 3 OS = Homo sapiens OX = 9606 GN = TAF3 PE = 1 SV = 1	Q5VWG9	TAF3	104 kDa	6	6	8	7	100%	100%
Keratin, type I cytoskeletal 17 OS = Homo sapiens OX = 9606 GN = KRT17 PE = 1 SV = 2	Q04695	KRT17	48 kDa	12	3	9	2	100%	76%

(continued)

Table 1. Continued.

Identified proteins (276)	Accession number	Alternate ID	Molecular weight	Quantitative value (total spectra) PERK-BirA	Exclusive unique peptide count PERK-BirA	Quantitative value (total spectra) Control	Exclusive unique peptide count Control	Protein identification probability PERK-BirA	Protein identification probability Control
Heterogeneous nuclear ribonucleoproteins C1/C2 OS = Homo sapiens OX = 9606 GN = HNRNPC PE = 1 SV = 4	P07910	HNRNPC	34 kDa	9	6	9	4	100%	100%
Hornerin OS = Homo sapiens OX = 9606 GN = HRNR PE = 1 SV = 2	Q86YZ3	HRNR	282 kDa	12	9	10	7	100%	100%
Heterogeneous nuclear ribonucleoprotein U OS = Homo sapiens OX = 9606 GN = HNRNPU PE = 1 SV = 6	Q00839	HNRNPU	91 kDa	17	12	11	6	100%	100%
Serine/arginine-rich splicing factor 11 OS = Homo sapiens OX = 9606 GN = SRSF11 PE = 1 SV = 1	Q05519	SRSF11	54 kDa	11	8	11	6	100%	100%
Serine/arginine repetitive matrix protein 2 OS = Homo sapiens OX = 9606 GN = SRRM2 PE = 1 SV = 2	Q9UQ35	SRRM2	300 kDa	15	7	11	6	100%	100%
Poly [ADP-ribose] polymerase 1 OS = Homo sapiens OX = 9606 GN = PARP1 PE = 1 SV = 4	P09874	PARP1	113 kDa	29	22	12	12	100%	100%
Elongation factor 1-alpha 1 OS = Homo sapiens OX = 9606 GN = EEF1A1 PE = 1 SV = 1	P68104 (+1)	EEF1A1	50 kDa	21	14	13	9	100%	100%
Nucleolar protein 56 OS = Homo sapiens OX = 9606 GN = NOP56 PE = 1 SV = 4	O00567	NOP56	66 kDa	16	11	13	10	100%	100%
U2 snRNP-associated SURP motif-containing protein OS = Homo sapiens OX = 9606 GN = U2SURP PE = 1 SV = 2	O15042	U2SURP	118 kDa	25	19	14	10	100%	100%
Guanine nucleotide-binding protein-like 3 OS = Homo sapiens OX = 9606 GN = GNL3 PE = 1 SV = 2	Q9BVP2	GNL3	62 kDa	9	7	16	9	100%	100%

(continued)

Table 1. Continued.

Identified proteins (276)	Accession number	Alternate ID	Molecular weight	Quantitative value (total spectra) PERK-BirA	Exclusive unique peptide count PERK-BirA	Quantitative value (total spectra) Control	Exclusive unique peptide count Control	Protein identification probability PERK-BirA	Protein identification probability Control
Actin, cytoplasmic 1 OS = Homo sapiens OX = 9606 GN = ACTB PE = 1 SV = 1	P60709 (+1)	ACTB	42 kDa	22	12	17	9	100%	100%
Multiple myeloma tumor-associated protein 2 OS = Homo sapiens OX = 9606 GN = MMTAG2 PE = 1 SV = 1	Q9BU76	MMTAG2	29 kDa	21	13	17	8	100%	100%
Keratin, type II cytoskeletal 5 OS = Homo sapiens OX = 9606 GN = KRT5 PE = 1 SV = 3	P13647	KRT5	62 kDa	27	15	20	11	100%	100%
Histone H1.0 OS = Homo sapiens OX = 9606 GN = H1F0 PE = 1 SV = 3	P07305	H1F0	21 kDa	16	9	20	9	100%	100%
Nucleolar protein of 40 kDa OS = Homo sapiens OX = 9606 GN = ZCCHC17 PE = 1 SV = 1	Q9NP64	ZCCHC17	28 kDa	19	9	20	10	100%	100%
60S ribosomal protein L29 OS = Homo sapiens OX = 9606 GN = RPL29 PE = 1 SV = 2	P47914	RPL29	18 kDa	10	2	21	5	100%	100%
Keratin, type I cytoskeletal 14 OS = Homo sapiens OX = 9606 GN = KRT14 PE = 1 SV = 4	P02533	KRT14	52 kDa	32	16	22	7	100%	100%
Protein SON OS = Homo sapiens OX = 9606 GN = SON PE = 1 SV = 4	P18583	SON	264 kDa	15	15	23	17	100%	100%
Keratin, type II cytoskeletal 6A OS = Homo sapiens OX = 9606 GN = KRT6A PE = 1 SV = 3	P02538	KRT6A	60 kDa	30	8	25	7	100%	100%
Transcription termination factor 1 OS = Homo sapiens OX = 9606 GN = TTF1 PE = 1 SV = 3	Q15361	TTF1	103 kDa	31	23	25	17	100%	100%
Treacle protein OS = Homo sapiens OX = 9606 GN = TCOF1 PE = 1 SV = 3	Q13428	TCOF1	152 kDa	26	17	27	17	100%	100%
Methylcrotonoyl-CoA	Q96RQ3	MCCC1	80 kDa	50	25	40	19	100%	100%

(continued)

Table 1. Continued.

Identified proteins (276)	Accession number	Alternate ID	Molecular weight	Quantitative value (total spectra) PERK-BirA	Exclusive unique peptide count PERK-BirA	Quantitative value (total spectra) Control	Exclusive unique peptide count Control	Protein identification probability PERK-BirA	Protein identification probability Control
carboxylase subunit alpha, mitochondrial OS = Homo sapiens OX = 9606 GN = MCCC1 PE = 1 SV = 3									
Lysine-rich nucleolar protein 1 OS = Homo sapiens OX = 9606 GN = KNOP1 PE = 1 SV = 1	Q1ED39	KNOP1	52 kDa	61	33	42	23	100%	100%
Arginine and glutamate-rich protein 1 OS = Homo sapiens OX = 9606 GN = ARGLU1 PE = 1 SV = 1	Q9NWB6	ARGLU1	33 kDa	29	17	46	20	100%	100%
Cell growth-regulating nucleolar protein OS = Homo sapiens OX = 9606 GN = LYAR PE = 1 SV = 2	Q9NX58	LYAR	44 kDa	50	24	50	23	100%	100%
Keratin, type I cytoskeletal 9 OS = Homo sapiens OX = 9606 GN = KRT9 PE = 1 SV = 3	P35527	KRT9	62 kDa	98	34	54	19	100%	100%
Keratin, type I cytoskeletal 10 OS = Homo sapiens OX = 9606 GN = KRT10 PE = 1 SV = 6	P13645	KRT10	59 kDa	60	27	54	24	100%	100%
Keratin, type II cytoskeletal 2 epidermal OS = Homo sapiens OX = 9606 GN = KRT2 PE = 1 SV = 2	P35908	KRT2	65 kDa	64	29	60	24	100%	100%
Histone H1.2 OS = Homo sapiens OX = 9606 GN = HIST1H1C PE = 1 SV = 2	P16403	HIST1H1C	21 kDa	72	2	69	2	100%	100%
Acetyl-CoA carboxylase 1 OS = Homo sapiens OX = 9606 GN = ACACA PE = 1 SV = 2	Q13085	ACACA	266 kDa	41	33	69	45	100%	100%
Histone H1.4 OS = Homo sapiens OX = 9606 GN = HIST1H1E PE = 1 SV = 2	P10412	HIST1H1E	22 kDa	74	13	75	18	100%	100%
Keratin, type II cytoskeletal 1 OS	P04264	KRT1	66 kDa	135	45	94	31	100%	100%

(continued)

Table 1. Continued.

Identified proteins (276)	Accession number	Alternate ID	Molecular weight	Quantitative value (total spectra) PERK-BirA	Exclusive unique peptide count PERK-BirA	Quantitative value (total spectra) Control	Exclusive unique peptide count Control	Protein identification probability PERK-BirA	Protein identification probability Control
= Homo sapiens OX = 9606 GN = KRT1 PE = 1 SV = 6									
Propionyl-CoA carboxylase alpha chain, mitochondrial OS = Homo sapiens OX = 9606 GN = PCCA PE = 1 SV = 4	P05165	PCCA	80 kDa	117	49	114	45	100%	100%
Pyruvate carboxylase, mitochondrial OS = Homo sapiens OX = 9606 GN = PC PE = 1 SV = 2	P11498	PC	130 kDa	122	54	121	49	100%	100%

more studied of the two at MCS, and because both proteins are highly similar, for simplicity, here we focused on VAPB. We tested the possible interaction between VAPB and PERK by co-immunoprecipitation (co-IP) experiments and successfully confirmed that immunoprecipitation of wild type PERK-myc ($PERK^{WT}$) expressed in HEK293-T cells, pulled down VAPB (Figures 2 and S2). In previous studies, we showed that the function of PERK required at the ER-mitochondria and ER-PM contacts was independent of its kinase function (van Vliet et al., 2017; Verfaillie et al., 2012). Here, to investigate whether PERK's kinase activity was dispensable for PERK-VAPB interaction, we performed the IP in cells expressing either a $PERK^{WT}$ or a kinase dead mutant of PERK ($PERK^{KD}$). Interestingly, the expression of the $PERK^{KD}$ mutant yielded no difference in VAPB binding (Figures 2 and S2). Together these observations suggest that PERK interacts with VAPB constitutively, and in the absence of a signal, evoking its UPR activation. It is possible that VAPB could recruit PERK to specific MCS upon certain stresses, or vice versa; further research would be needed to validate this possibility.

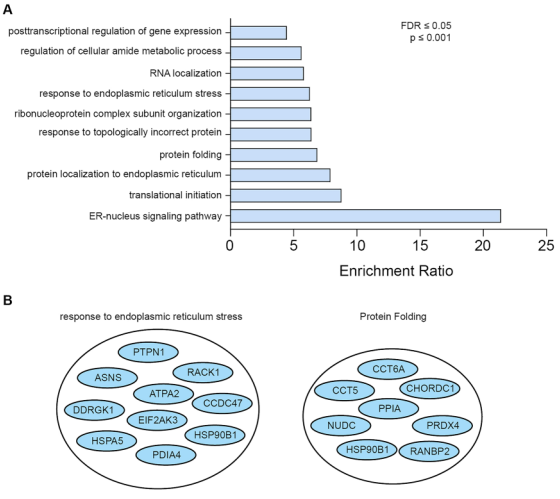

Figure 1. (A) Gene ontology analysis of the protein hits discovered using PERK-BirA* obtained using WEB-based GEne SeT AnaLysis toolkit (http://www.webgestalt.org/). The minimum number of IDs in each category was required to be 5, the maximum was 2000, the false-discovery rate (FDR) correction used was Benjamini and Hochberg (BH). The categories were first ranked based on FDR and after that the topmost significant categories were selected. 10 categories of biological processes were identified, many related to the function of PERK. The enrichment ratio indicates the ratio between the fraction of proteins belonging to each biological process in our dataset over the fraction of these proteins expected If our dataset was completely random. (B) Two specific categories are highlighted, response to endoplasmic reticulum stress, and protein folding, indicating an enrichment in the dataset of proteins and processes linked to PERK's role in the unfolded protein response and ER stress.

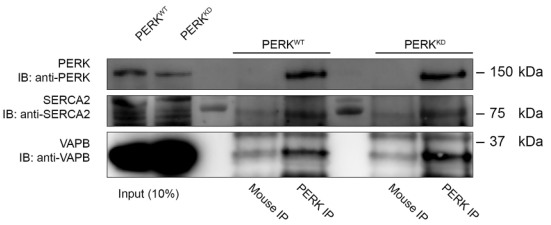

Figure 2. Identification of VAPB and SERCA2 as putative PERK interactors. HEK293-T cells were transfected with myc-tagged expression vectors for PERK WT ($PERK^{WT}$) and PERK Kinase dead ($PERK^{KD}$). After 48 h of transfection, $PERK^{WT}$ and $PERK^{KD}$ were immunoprecipitated (PERK IP) and interactors were detected using antibodies against VAPB and SERCA2 by immunoblot. Input was 10% of the total protein amount used for the IP (50 µg of protein was loaded as an input versus 500 µg of total protein used for the IP). Unspecific anti-mouse antibody was used as a negative control (Mouse IP). Data show are representative of N = 3 (VAPB), N = 2 (SERCA2) biologically independent experiments.

Proteins that interact with VAPs usually do so through a specific domain, termed a FFAT motif (two phenylalanines (FF) in an acidic tract) that binds the major sperm protein domain (MSP) on VAP (Murphy & Levine, 2016). However, using a previously published algorithm (Murphy & Levine, 2016) we did not find any robust FFAT motif in the PERK primary sequence. This suggests that the PERK-VAPB interaction may involve either another FFAT containing protein or may be mediated through another binding *in cis*, as they are both ER membrane proteins. This would leave the MSP of VAPB free to bind FFAT motifs in other proteins. A recent publication has explored the human "VAPome" in a systematic way using BioID and found that PERK was a hit for at least VAPA (Cabukusta et al., 2020). This same study also reports the putative interaction between PERK and MOSDP1 and 3. These proteins are VAP-like but bind to a slight variation of the FFAT motif, the FFNT motif (two phenylalanines (FF) in a neutral tract). Their analysis indicates that PERK contains an FFNT motif at location 283 of the protein, which is located in the lumen of the ER. This study, along with ours, does give support for a functional role between PERK and VAPA/B and other MCS proteins.

PERK Interaction With SERCA2

SERCA1/2 are ATPases located in the ER membrane that pump Ca^{2+} ions from the cytosol into the ER lumen, counteracting the Ca^{2+} leak from various sources (translocon, inositol 1,4,5-trisphosphate receptor) and maintaining a high level of Ca^{2+} in the ER lumen with a steady-state Ca^{2+} concentration of approximately 1 mM (de la Fuente et al., 2013).

In contrast to SERCA2, no unique peptides for SERCA1 could be identified in the BioID data set, so the presence of the latter isoform in the PERK interactome could not be proven. Neither SERCA1 nor SERCA2 have been detected at the ER-mitochondria contact sites, but a truncated version of SERCA1, S1 T (truncated after amino acid 395) has been shown to play a role at ER-mitochondria contact sites (Chami et al., 2008). As with VAPB, we confirmed the interaction of SERCA2 with PERK through co-IP, which was again detected independently of its kinase activity (Figures 2 and S2). Our previous results indicated that PERK is strongly activated by increases in cytosolic Ca^{2+} levels, and we speculated that this is linked to the depletion of ER luminal Ca^{2+} leading to ER stress, as shown also in a recent study (Preissler et al., 2020). Furthermore, we reported that PERK is involved in SOCE, by regulating the ER-PM contacts through its binding to FLNA (van Vliet et al., 2017). SOCE is induced by ER Ca^{2+} depletion, which leads to oligomerization of STIM1 and its translocation to the PM where it interacts with the PM localized ORAI1 protein. The interaction of STIM1 with ORAI1 then allows Ca^{2+} to enter the cytosol from the extracellular medium through the opening of the ORAI1 channel (Zhang et al., 2005). PERK's interaction with SERCA2 is an intriguing link since, although ORAI1 allows Ca^{2+} to enter the cytosol, the refilling of ER-Ca^{2+} store requires the activity of the Ca^{2+} pump SERCA. A previous study has indicated a close relationship between STIM1/ORAI1 driven SOCE and SERCA (Courjaret & Machaca, 2014). We can then speculate that PERK may be located close to the site of STIM1/ORAI1 contact, playing a part in the mechanism that ensures Ca^{2+} entering the cytosol is rapidly internalized into the ER lumen through SERCA2. Further study is needed to investigate a possible role of PERK in regulating SERCA2 activity, and therefore, influencing this mechanism.

Conclusions and Limitations of the Study

In this report, we show a BioID dataset obtained by tagging the promiscuous biotin ligase BirA* to PERK, in order to map its close interactors. We highlight and validate two of these, VAPB and SERCA2, which lead us to further speculate about the role of PERK in ER MCS formation and Ca^{2+} signaling.

Our study was performed when BioID was just emerging as a tool, and at the time of our planning, only the original study had been published (Roux et al., 2012). We, therefore, closely modeled our experimental setup on this study. It is important to note when interpreting this dataset that later studies using BioID (and derivatives of BioID like TurboID, where the BirA* ligase has been mutated to biotinylate at a much higher rate), have implemented more stringent controls to detect false positives (inherent with using BioID as an approach to screen protein-protein interactions). For example, the initial study reporting BioID used only parental cells (mock-transfected) as a control, while some recent studies used BirA* (or TuboID) not tagged to their protein of interest to control for non-specific biotinylation events (Szczesniak et al., 2021; Vermehren-Schmaedick et al., 2021; Zhang et al., 2019). Our reasoning for using only

parental cells as control was that by using free BirA* as a control, there was a risk that this enzyme might biotinylate *bona fide* hits randomly, leading to false negatives. Since the initial study reporting BioID, the original authors have published numerous extensive updates on how to set up a BioID study, incorporating more appropriate or bespoke controls and comparing the different new techniques (May et al., 2020; May & Roux, 2019; Roux et al., 2018; Sears et al., 2019). In summary, we have identified 129 potential interacting partners of PERK, of which we have tested and confirmed two, SERCA2 and VAPB. We hope that this dataset can yield new insights concerning PERK and cellular signaling.

Materials and Methods

Cell Lines and Transfection:

HEK293-T cells have been maintained in Dulbecco's modified Eagle's medium containing 4.5 g/l glucose and 0.11 g/l sodium pyruvate and supplemented with 2 mM glutamine, 100 units/ml penicillin, 100 µg/ml streptomycin, and 10% fetal bovine serum (FBS) (all added, AA medium). Cells were transiently transfected with different PERK constructs encoding $PERK^{K618A}$ (Addgene plasmid 21815) or $PERK^{FL}$ (Addgene plasmid 21814), both myc tagged, using Trans-IT X2 transfection reagent (Mirus Bio LLC, Science Dr.Madison, WI USA).

BioID, Biotinylation Assay, and Mass Spectrometry

BioID was performed as described previously (Roux et al., 2012), with minor modifications. Overexpression of PERK-BioID was achieved by transiently transfecting Hek293-T cells using X-tremegene 9 (Roche, Germany). A 6 µl X-tremegene9 was mixed with a 2 µg PERK-BioID plasmid and dispersed on cultured cells. A 30 h post-transfection medium containing 50 µM biotin was added to the cells for 24 h. After incubation with biotin, cells were lysed in 1 mL lysis buffer (50 mM Tris, pH 7.4, 500 mM NaCl, 0.4% SDS, 5 mM EDTA, 1 mM DTT, and 1x complete protease inhibitor [Roche]) and sonicated. Triton X-100 was added to a 2% final concentration. After further sonication, an equal volume of 4 °C 50 mM Tris (pH 7.4) was added before additional sonication (subsequent steps at 4 °C) and centrifugation at 16,000 g. Supernatants were incubated with 600 µl Dynabeads (50% slurry) (MyOne Streptavidin C1; Invitrogen) overnight. Beads were collected and washed twice for 8 min at 25 °C (all subsequent steps at 25 °C) in 1 mL wash buffer 1 (2% SDS in dH2O). This was repeated once with wash buffer 2 (0.1% deoxycholate, 1% Triton X-100, 500 mM NaCl, 1 mM EDTA, and 50 mM HEPES, pH 7.5), once with wash buffer 3 (250 mM LiCl, 0.5% NP-40, 0.5% deoxycholate, 1 mM EDTA, and 10 mM Tris, pH 8.1), and twice with wash buffer 4 (50 mM Tris, pH 7.4, and 50 mM NaCl). For western blot analysis, bound proteins were removed from the magnetic beads with 50 µl of Laemmli SDS-sample buffer saturated with biotin at 98 °C. For mass spectrometry analysis, beads were washed repeatedly in MQ water containing 50 mM ammonium bicarbonate (AmBic) before being incubated for 30 min at 37 °C in 50 mM AmBic containing 5 mM DTT. Beads were further washed in 50 mM AmBic and incubated with 25 mM iodoacetamide in the dark for 30 min at 37 °C. After reduction/alkylation, beads were washed in 50 mM AmBic and incubated with 1:20 (w/w) modified trypsin (Pierce) ON at 37 °C in 50 mM AmBic containing 5% acetonitrile. After removal of beads by magnetic separation, formic acid was added to the peptide solution (to 2%) before desalting by C18 Micro Spin Columns (Harvard Apparatus). The resulting peptide mixture was analyzed by nano LC-MS on a hybrid quadrupole-orbitrap mass spectrometer (Q Exactive, Thermo Fisher Scientific). Peptides were identified by MASCOT 2.2 (Matrix Science) using the SwissProt database (taxonomy Homo sapiens, 20231 entries) adopting the following MASCOT search parameters: trypsin/P, two missed cleavages allowed, variable modification oxidation (M), fixed modification carbamidomethylation (C). The mascot was searched with a fragment ion mass tolerance of 0,02 Da and a parent ion mass tolerance of 10 ppm.

Scaffold 4 (Proteome Software Inc.) was used to validate MS/MS-based peptide and protein identifications. Peptide and protein identifications were accepted to achieve an FDR less than 10%. Standard protein grouping was adopted. The presence of at least 2 exclusive unique peptides per protein was required.

Immunoprecipitation

After 48 h of transfection with selected plasmids cells, they were collected through scraping and lysed in lysis buffer (1% CHAPS, 100 mM KCl, 150 mM NaCl, 1x protease inhibitor (Pierce Protease Inhibitor Tablets, Thermo Fisher Scientific Inc.)) for 30 min at 4 °C. Cells were centrifuged at 13.000 g for 15 min to remove debris and unbroken cells. From the supernatant, 500 µg of proteins were combined with primary antibodies overnight (ON) at 4 °C, against PERK and using a non-specific Mouse IgG as a control. Protein-antibody complexes were captured by the addition of Protein AG magnetic beads (Pierce) for 1.5 h at room temperature (RT). Protein AG magnetic beads with captured protein-antibody complexes were washed three times with lysis buffer. Proteins were eluted with sample buffer (62.5 µM Tris-HCl, 10% glycerol, 2% SDS, 1x protease inhibitor, 1x phosphatase inhibitor (Pierce phosphatase Inhibitor Tablets, Thermo Fisher Scientific Inc.) in MQ water) and loaded on a gel for western blot analysis.

Western Blotting

Samples were separated by SDS-PAGE on the Criterion system (Bio-Rad Laboratories, Hercules, CA, USA) on a

4%–12% Bis-TRIS gel and electrophoretically transferred to Protran 2 µm-pored nitrocellulose paper (PerkinElmer, Wellesley, MA, USA). The blots were blocked for 1 h at RT in TBS-T buffer (50 mM Tris, pH 7.4, 150 mM NaCl, 0.1% Tween-20) containing 5% nonfat dry milk and then incubated with selected antibody solutions. Samples were processed and enhanced chemiluminescence using Pierce ECL Western Blotting Substrate was used for western blot detection and membranes were scanned using the Bio-Rad Chemidoc Imager (Bio-Rad Laboratories N.V.3, Winninglaan, Temse, Belgium).

Antibodies

Antibodies used were mouse monoclonal anti-c-Myc (Sigma, Cat# M4439), Control normal mouse IgG (sc-2025), anti-ATP2A2/SERCA2 (Cell signaling 388S), anti-VAPB (Invitrogen, PA5-53023), and an HRP-based detection using as a secondary antibody, the Veriblot antibody (#ab131366, Abcam).

Author Contributions

Experiment design, analysis, and manuscript writing: MLS, PA, ARVV. Performing and analyzing western blots: MLS. Mass spectrometry analysis: RD, EW. Project and funding: PA.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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