Protein S-palmitoylation is the posttranslational attachment of a saturated 16-carbon palmitic acid to a cysteine side chain via a thioester bond. Palmitoylation can affect protein localization, trafficking, stability, and function. The extent and roles of palmitoylation in endothelial cell (EC) biology is not well-understood, partly because of technological limits on palmitoylprotein detection.

Objective: To develop a method using acyl-biotinyl exchange technology coupled with mass spectrometry to globally isolate and identify palmitoylproteins in ECs.

Methods and Results: More than 150 putative palmitoyl proteins were identified in ECs using acyl-biotinyl exchange and mass spectrometry. Among the novel palmitoylproteins identified is superoxide dismutase-1, an extensively studied enzyme that protects all cells from oxidative damage. Mutation of cysteine-6 prevents palmitoylation, leads to reduction in superoxide dismutase-1 activity in vivo and in vitro, and inhibits nuclear localization, thereby supporting a functional role for superoxide dismutase-1 palmitoylation. Moreover, we used acyl-biotinyl exchange to search for substrates of particular protein acyl transferases in ECs. We found that palmitoylation of the cell adhesion protein platelet endothelial cell adhesion molecule-1 is dependent on the protein acyl transferase ZDHHC21. We show that knockdown of ZDHHC21 leads to reduced levels of platelet endothelial cell adhesion molecule-1 at the cell surface.

Conclusions: Our data demonstrate the utility of EC palmitoylproteomics to reveal new insights into the role of this important posttranslational lipid modification in EC biology.

Key Words: endothelium □ palmitoylation □ proteomics

P
rotein S-acylation is the attachment of a lipid to a cysteine residue in a protein via a thioester linkage. Protein S-acylation is often called “palmitoylation” because the lipid is commonly (but not exclusively) the saturated 16-carbon palmitate; thus, “palmitoylation” is used in this report to denote protein S-acylation. Palmitoylated proteins typically are membrane-associated because of other lipid modifications (such as myristoylation or prenylation) or additional membrane-embedded domains. Palmitoylation can affect the trafficking, localization, stability, and function of a protein. Unlike other lipid modifications, palmitoylation is reversible and likely regulated.

Two major developments over the past several years have dramatically changed the study of protein palmitoylation. The first has been the introduction of powerful new methods to detect palmitoylproteins. Classically, palmitoylation was detected primarily by metabolic labeling of proteins with tritiated palmitate followed by fluorography. This approach is time-consuming (requiring weeks to months for film exposures), insensitive, and requires advance knowledge of the palmitoylprotein. More recently, palmitoylproteins have been characterized using acyl-biotinyl exchange (ABE) or metabolic labeling with palmitate analogs amenable to chemical derivatization with reporter groups, typically using copper-catalyzed cycloaddition (“click”) chemistry. ABE involves first blocking free thiols on isolated proteins, followed by cleaving palmitoyl groups with the neutral base hydroxylamine (HA), and the derivatization of the newly formed thiols using reporter groups such as biotin.

The online-only Data Supplement is available with this article at http://circres.ahajournals.org/lookup/suppl/doi:10.1161/CIRCRESAHA.112.269514/-/DC1.

Correspondence to William C. Sessa, Yale University School of Medicine, Vascular Biology and Therapeutics Program, 10 Amistad Street, Rm 437, New Haven, CT 06520. E-mail william.sessa@yale.edu

© 2012 American Heart Association, Inc.
powerful techniques than tritiated palmitate because they are very sensitive and can be performed relatively rapidly. Furthermore, unlike tritiated palmitate, neither approach necessarily requires advanced knowledge of the palmitoylprotein and both can be used to isolate total palmitoylproteins from cells or tissues. These approaches have been applied to the elucidation of all palmitoylproteins from a variety of sources, including yeast, and several mammalian cell types.

The second development has been the discovery of the zinc finger DHHC domain–containing (ZDHHC) family of protein acyl transfersases (PAT). Thus identifying the long-sought enzymatic activity catalyzing protein palmitoylation. There are 23 ZDHHC family members in humans characterized by the presence of a highly conserved tetrapeptide repeat (Asp-His-His-Cys, or DHHC in single-letter code) that lies within a larger conserved cysteine-rich domain. ZDHHC proteins have multiple predicted transmembrane domains and have been found in multiple membrane compartments, including endoplasmic reticulum, Golgi, and plasma membrane. ZDHHC family members are likely responsible for the palmitoylation of most palmitoylproteins, based on experiments in yeast showing that the palmitoylation of nearly all known palmitoylproteins could be attenuated by the deletion of one or more ZDHHC genes. An increasing array of diseases in mice and humans has been associated with mutations in various ZDHHC family members. These data suggest that although there are multiple PAT paralogs, they likely are not functionally redundant and may serve critical independent functions in vivo. Many questions regarding the ZDHHC family of PATs remain unanswered, including the nature of their substrate specificity and regulation.

Protein palmitoylation has not been well-studied in the context of endothelial cells (ECs), the major cell type localized at the interface of blood and tissue representing the largest endocrine organ in the body. Earlier work from our laboratory has shown that endothelial nitric oxide synthase (eNOS), an enzyme that produces the gaseous messenger nitric oxide, is palmitoylated at two cysteines in the amino terminus. Palmitoylation of eNOS is important for trafficking to the plasmalemmal membrane domains, termed caveolae, and for its cellular activity. eNOS can be palmitoylated by several Golgi-localized ZDHHC enzymes, and siRNA-mediated knockdown of one of these (ZDHHC21) leads to mislocalization of eNOS and impaired nitric oxide production.

Because the endothelium is critical for many aspects of vascular health and disease, we used ABE to globally isolate palmitoylproteins from ECs and to begin to search for novel substrates for ZDHHC proteins that regulate EC function. Here, we report the identification of >150 palmitoylprotein candidates, many of which have not been reported previously. In particular, we identify superoxide dismutase-1 (SOD1) as an unexpected palmitoylprotein. Mutation of C6 on SOD1 blocks palmitoylation and diminishes enzymatic activity and nuclear localization, thus supporting an important biological role for SOD1 palmitoylation. In addition, using ABE in combination with siRNA-mediated knockdown of two ZDHHC paralogs enriched in EC, we show that palmitoylation of the adhesion protein platelet endothelial cell adhesion molecule-1 (PECAM1) is dependent on ZDHHC21. Knockdown of ZDHHC21 is shown to affect PECAM1 protein levels. Thus, the application of ABE-based palmitoylproteomics expands the scope of palmitoylation in endothelial cells and suggests that ZDHHC enzymes are likely potential targets for vascular diseases.

Methods

Cell Culture
Detailed Methods are provided in the Online Supplement. EA.hy 926, human umbilical vein endothelial cells, HEK 293T, and COS-7 cells were cultured using standard techniques. HEK 293T cells and COS-7 cells were transfected using either Fugene or Lipofectamine 2000 according to manufacturer’s instructions.

ABE
ABE was performed based on a published protocol with minor modifications. Confluent EA.hy 926 or human umbilical vein endothelial cell (passage ≤5) cells were lysed in 2 mL of lysis buffer (50 mmol/L Tris, 5 mmol/L EDTA, 150 mmol/L NaCl, 1.7% β-octyl glucoside or 1% NP-40, 10% glycerol, 5 mmol/L MgCl₂, 4.5 mg/mL sodium pyrophosphate, 2.1 mg/mL NaF, 0.3 mg/mL AEBSF, 1 mmol/L NaVO₄, 2 mg/mL complete protease inhibitor tablet; Roche; pH 7.4) per C150 plate. Extracted proteins (2–8 mg) were then precipitated using several volumes of ice-cold acetone. After resuspension, free thiols were then blocked by addition of 20 mmol/L methyl methanethiosulfonate (Fluka). After removal of excess methyl methanethiosulfonate via protein precipitation and resuspension, the solution was split in half. Both halves were treated with 1 mmol/L Biotin-HPDP (Thermo Scientific). Simultaneously, one half was treated with HA (NH₂OH; pH 7.4, 1 mol/L final concentration) and the other was treated with Tris buffer pH 7.4 as a control. Biotinylated proteins were then captured on streptavidin-conjugated resin (Sigma Aldrich), washed, and eluted with 1% β-mercaptoethanol.

Subsequently, the proteins were analyzed by Western blotting or by liquid chromatography mass spectrometry (LC-MS)/MS. Raw liquid chromatography mass spectrometry (LC-MS)/MS spectra were assigned to proteins using Mascot software.

Western Blotting
SDS PAGE, electroblotting, and probing with primary and secondary antibodies were performed using standard techniques.

Immunofluorescence of SOD1
COS cells were grown on glass cover slips and transfected with HA-tagged SOD1. SOD1 was detected with anti-HA antibody (Roche 3F10) and goat antirat-488 (Invitrogen), and visualized using a Leica TCS SP5 spectral confocal microscope. Quantification was performed by a blinded independent judge.
Immunopurification of SOD1
SOD1 wild-type (WT) or C6S, labeled with an HA-affinity tag at the carboxyl terminus, was transfected in HEK-293T cells. SOD1 was immunoprecipitated from cell lysates using sepharose resin conjugated with anti-HA antibody (Sigma) overnight, and then eluted with 100 μg/mL anti-HA peptide (Sigma) in wash buffer.

Trinitiated Palmitate Incorporation
HEK 293 cells were transfected with WT eNOS, eNOS C15/C26S, WT SOD1, or SOD1 C6S and metabolically labeled with ^3H palmitate as described. eNOS or SOD1 was immunopurified from lysates of transfected cells, and analyzed by scintillation counting.

Quantitative Reverse-Transcription Polymerase
Chain Reaction
RNA isolation, reverse-transcription, and quantitative polymerase chain reactions were performed as described, except that GAPDH was used for normalization of transcript abundance.

Reactive Oxygen Species Levels and SOD Activity
Reactive oxygen species generation was measured by the oxidation of 2,7-dichlorofluorescein diacetate (Molecular Probes). Transfected cells were incubated with dichlorofluorescein diacetate 5 μmol/L for 30 minutes at 37°C, and subsequently analyzed by flow cytometry.

Activity of purified SOD1 was determined using the SOD assay kit (Dojindo Molecular Technologies) as described by the manufacturer. Purified bovine SOD1 (Sigma) was used to determine a standard curve.

siRNA Knockdown
Knockdown of ZDHHC3 and ZDHHC21 in EA.hy 926 cells was performed as described, except when scaled-up to accommodate larger tissue culture plates. Cells were harvested and analyzed 96 hours after transfection. All-star nonsilencing control siRNA (Qiagen) was used when indicated.

Statistics
The two-tailed Student t test was used to compare treated samples with controls. P<0.05 was considered to be significant.

Results
Proteomic Analysis of Palmitoylated Proteins in ECs
To appreciate the scope and significance of protein palmitoylation in ECs, we globally isolated total palmitoylproteins (in five different experiments) from EA.hy 926 cells (an immortalized human EC line) using ABE2 and subsequently identified the proteins by mass spectrometry (Supplemental Figure I). EA.hy 926 cells are a stable human EC line that retains many of the characteristics of primary ECs, including expression of EC surface proteins such as intercellular adhesion molecule, vascular cell adhesion molecule, PECAM, and factor VIII–related antigen, as well as expression of a novel palmitoylated protein, SOD1. SOD1 has been intensively studied and is an important enzyme that acts on acyl-CoA substrates. SOD1, and (2) the use of ABE to associate activity of individual PAT paralogs with substrate palmitoylproteins.

Identification of SOD1 as a Novel Palmitoylprotein
One protein with strong mass spectrometry evidence supporting its palmitoylation but that has not been described previously is SOD1. SOD1 has been intensively studied and is an important enzyme that protects cells from oxidative damage by converting superoxide anion into hydrogen peroxide. In addition, mutations in SOD1 are associated with the familial variant of amyotrophic lateral sclerosis, also known as Lou Gehrig disease. Examination of the mass spectrometry data revealed that eight different peptides mapping to SOD1 were detected with high Mascot scores (and expectation values <0.05) in the +HA samples, but not in the −HA-treated samples (Figure 1C, D). This was confirmed by Western blotting for SOD1 after ABE (Figure 1A, B). Mutagenesis of C6 to serine (C6S) in SOD1 led to a loss of palmitoylation when expressed in HEK-293T cells (Figure 1E), as determined by ABE and Western blotting. In addition, the C6S mutant SOD1 displayed reduced incorporation of trinitiated palmitate (Figure 1F), demonstrating that C6 is the likely site of palmitoylation in SOD1.

Next, we explored two possible functional consequences of S-acylation of SOD1. Because palmitoylation affects the subcellular localization of many proteins, we examined the all the final proteins to ensure that none appeared to be among the proteins known to be false-positives by ABE, such as ubiquitin conjugating enzymes that use lipochromic cofactors, or enzymes that act on acyl-CoA substrates. Using these criteria, >150 palmitoylated proteins were classified and are shown in Supplemental Table I. Proteins that were identified but are likely to be either contaminants or false-positives are shown in Supplemental Table II. A subset of the identified proteins was confirmed by ABE performed with and without HA, followed by semiquantitative Western blotting (Figure 1A). The recovery of the protein in the absence, but the absence of HA demonstrates that the protein is palmitoylated. As seen in Figure 1A, the known palmitoylated proteins eNOS, PECAM1, calnexin, Y3, R-Ras, total Ras, and caveolin-1 and the novel palmitoylprotein SOD1 are enriched in the presence of HA treatment. Hsp90 and c-Src, which are not palmitoylated, were used as negative controls. Similar, ABE followed by Western blotting confirmed the presence of several palmitoylated proteins, including PECAM1 and SOD1, in primary cultures of human umbilical vein endothelial cells (Figure 1B).

Further analysis of the identified proteins was performed by ascertaining whether the protein was previously identified as palmitoylated by searching published palmitoylproteomes identified via mass spectrometry in other human cell types, by searching for the key word “palmitate” in human protein entries in the Uniprot database (www.uniprot.org), and by using semiautomated batch Pubmed searches (using the Web site pmid.us) for every identified palmitoylprotein in conjunction with the search terms “palmitoylation” or “palmitate” or “palmitoyl transferase.” Of all proteins, nearly one-third (43 total) were not previously reported. We describe two examples of advances that have resulted from applying ABE and mass spectrometry technology to the study of ECs: (1) the identification of novel unanticipated palmitoylproteins, such as SOD1, and (2) the use of ABE to associate activity of individual PAT paralogs with substrate palmitoylproteins.
distribution of WT and C6S SOD1 in transfected cells. SOD1 is mainly soluble and localizes in the cytosol as well as in peroxisomes, mitochondria, and nuclei. Expression of WT SOD1 in COS cells resulted in predominately cytoplasmic and nuclear localization (co-labeled with the nuclear marker DAPI) in approximately 70% of the cells examined, whereas C6S SOD1 was frequently excluded from the nucleus (Figure 2A; quantified in Figure 2B). These results suggest that C6 is involved in the nuclear targeting of SOD1.

We also examined whether palmitoylation affected the enzymatic activity of SOD1. Long-chain acylation previously has been reported to alter activity of certain mitochondrial enzymes. Transfection of WT, but not C6S SOD1, into HEK293T cells reduced the levels of reactive oxygen species by guest on July 9, 2017 http://circres.ahajournals.org/ Downloaded from
as measured by fluorescence-activated cell sorting analysis of cells treated with the reactive oxygen species-sensitive dye dichlorofluorescin diacetate (Molecular Probes; Figure 2C, 2D). Expression levels of WT and C6S were similar, as judged by Western blotting (Figure 2E), suggesting that the activity of the mutant protein is reduced relative to WT. To assess whether this reduction was attributable to changes in the specific activity of SOD1, we purified WT and C6S HA-tagged SOD1 proteins from HEK cells to near-homogeneity using anti-HA resin (Figure 2F) and assayed enzymatic activity directly. C6S SOD1 displayed approximately 30% reduction in specific activity relative to the WT enzyme (Figure 2G). In sum, these data suggest that palmitoylation of SOD1 at C6 is important for the ability of SOD1 to localize to the nucleus and to optimally scavenge superoxide. It is not clear whether these 2 phenomena are linked or if they are unrelated.

Utilization of ABE to Assign ZDHHCs and Substrates in ECs: ZDHHC21 Is Necessary for Optimal Palmitoylation of PECAM1

The discovery of the large family of ZDHHC PATs has raised the issue of which paralogs are responsible for palmitoylating which substrate proteins. Previously, we demonstrated that ZDHHC3 and ZDHHC21 are expressed in ECs, that both can palmitoylate eNOS when co-expressed in HEK cells, and that siRNA knockdown of ZDHHC21, but not ZDHHC3, in ECs impairs eNOS palmitoylation and activity. We sought to discover new substrates for ZDHHC3 and ZDHHC21 by using siRNA to knockdown both of these PATs (Figure 3A). In an initial experiment, ECs were transfected with control siRNA and siRNA targeting both ZDHHC3 and ZDHHC21 and the level of palmitoylation after ABE determined by semiquantitative Western blotting of a group of proteins identified in the EC palmitoylome. As seen in Figure 3B, the knockdown of ZDHHC3 and ZDHHC21 markedly reduced the palmitoylation of PECAM1 (aka CD31) and, to a lesser extent, eNOS, calnexin, and syntaxin-6. The loss of these ZDHHCs did not influence the palmitoylation of MCAM/MUC18, Yes, Gα11, R-Ras, total Ras, or caveolin-1, thus demonstrating the specificity of the effect. PECAM1 is a type I transmembrane protein that is expressed in ECs and several circulating blood cells. In the endothelium, it serves several important roles in cell migration, transendothelial migration of leukocytes, flow sensing, and angiogenesis.
Palmitoylation of PECAM1 on C595 in platelets was recently described, where it was found to be important for localization of PECAM1 to lipid rafts and for mediating inhibition of stimulated apoptosis.

To determine which PAT was necessary for PECAM1 palmitoylation, each gene was knocked-down alone in ECs, followed by ABE and Western blotting for PECAM1. As seen in Figure 3C, palmitoylation of PECAM1 in ECs was primarily dependent on ZDHHC21, not ZDHHC3. To assess the functional consequences of ZDHHC21-dependent PECAM1 palmitoylation in ECs, we examined whether knockdown of ZDHHC21 can decrease total PECAM1 in the lysates, the input of PECAM1 in this experiment was normalized by using increased amounts of total lysate for samples in which ZDHHC21 was knocked-down as shown by blot of Hsp90. Densitometry on Western blots revealed that PECAM1 levels in lysates (normalized to Hsp90) are reduced by approximately 50% after 96 hours of treatment with siRNA targeted to ZDHHC21 but not ZDHHC3. *P<0.05 relative to NS control, n=3 independent experiments. E, Assessment of PECAM1 cell surface expression by fluorescence-activated cell sorting. Treatment with siRNA targeted to ZDHHC21 led to approximately 60% reduction in mean fluorescence intensity as compared with cells treated with NS control siRNA. Results are typical of 3 independent experiments.

One interesting novel palmitoylprotein characterized in the present study is SOD1. SOD1 expressed in ECs is of among other functions. Palmitoylation of PECAM1 on C595 in platelets was recently described, where it was found to be important for localization of PECAM1 to lipid rafts and for mediating inhibition of stimulated apoptosis.

To determine which PAT was necessary for PECAM1 palmitoylation, each gene was knocked-down alone in ECs, followed by ABE and Western blotting for PECAM1. As seen in Figure 3C, palmitoylation of PECAM1 in ECs was primarily dependent on ZDHHC21, not ZDHHC3. To assess the functional consequences of ZDHHC21-dependent PECAM1 palmitoylation in ECs, we examined whether knockdown of ZDHHC21 affected steady-state levels of endogenous PECAM1. The trafficking, breakdown, and cell surface localization of several other integral membrane proteins have been shown to be affected by palmitoylation. Knockdown of ZDHHC21, but not ZDHHC3, reduced levels of PECAM1 in cell lysates by approximately 50% (Figure 3C; quantified in Figure 3D). Similarly, fluorescence-activated cell sorting analysis of cell-surface PECAM1 in nonpermeabilized ECs after knockdown of ZDHHC21 revealed a marked reduction in PECAM1 levels on the cell surface (Figure 3E). Thus, ZDHHC21 regulates the levels of PECAM1 in ECs.

**Discussion**

Palmitoylation has long been recognized as an important posttranslational modification that can affect protein localization, stability, and function. Using ABE followed by an unbiased proteomic approach, we identified >150 candidate palmitoyl proteins in ECs. Confidence in the veracity of the identified candidate palmitoyl proteins is inherent in the fact that more than half of the identified proteins previously have been reported either by direct biochemical analysis or in other global palmitoyl proteomic studies of human cells. In addition, several proteins identified here were confirmed by ABE, followed by Western blotting (Figure 1A, B) and mutagenesis studies (Figure 1D). Thus, our results provide the first global approach in ECs and are an important step forward in the understanding of the role of palmitoylation in this specialized cell type that regulates aspects of cardiovascular disease, cancer, and diabetes.

One interesting novel palmitoyl protein characterized in the present study is SOD1. SOD1 expressed in ECs is of
particular importance in the pathophysiology of variety of diseases associated with elevated oxidative stress such as atherosclerosis, diabetes, and familial variants of amyotrophic lateral sclerosis. SOD1 has been extensively characterized in biochemical and biophysical experiments, including the determination of several crystal structures.44 Thus, our detection of SOD1 as a palmitoylprotein was not anticipated. The failure to detect palmitoylation in previous studies may be attributable to many issues such as the presence of reductant in most purification schemes (ie, the thioester would be reduced) and the usage of inappropriate expression and purification strategies combined with insensitive or unsuitable analytic techniques. Interestingly C6 appears to be buried in the crystal structure of the mature form of SOD1.44 Thus, we suspect that acylation may occur initially in the apoprotein of SOD1, where nuclear magnetic resonance structural data reveal that C6 is solvent-exposed and thus accessible.45 Significant quantities of apoprotein of SOD1 are thought to exist in the cell at the steady state.46 Alternatively, it is conceivable that the acyl group is cleaved during maturation of SOD1.

The cysteines of SOD1 have been extensively studied previously by several laboratories in a number of different contexts. The C6F mutation has been found to be among the many mutations that cause familial variants of amyotrophic lateral sclerosis and destabilize the protein.47 The more conservative C6A mutation was found to slightly enhance rather than inhibit stability.48 Of note, the introduction of the C6S mutation in the context of familial variant of amyotrophic lateral sclerosis-associated mutations inhibits protein aggregation in experimental studies,49 possibly by blocking intermolecular disulfide formation involving C6 (and other cysteines);50–52 thus, palmitoylation of C6 may serve as an endogenous mechanism to mitigate the reactivity of C6, especially in the aggregation-prone apoprotein associated with the causal familial variant of amyotrophic lateral sclerosis mutations.

Confocal microscopy studies of the SOD1 C6S mutant suggest that nuclear localization is impaired (Figure 2A, B). Although nuclear localization of SOD1 has been previously recognized,31 the mechanism by which SOD1 is imported into the nucleus is not known. In general, palmitoylation of nuclear proteins has not been well-described; however, recent data suggest that palmitoylation may be important for the nuclear localization and transcriptional activity of the transcription factor HMGC253 and for the nuclear histone H3.30 The relationship between the decrease in enzyme activity and the alteration of nuclear localization observed in the SOD1 C6S mutant is not clear and is the subject of further exploration. In addition, it is appreciated that SOD1 can exist in the nucleus in many cells, but the functional importance of SOD1 subcellular localization and its role in EC biology are unknown.

Another salient finding in this study is the identification of relationships between individual ZDHHC paralogs and potential substrates in ECs. To date, most success in identifying such relationships has come from studies designed to define the set of ZDHHC paralogs that can augment palmitoylation of a particular substrate protein. These experiments usually involve cotransfection of a substrate protein together with each of the known ZDHHC proteins in HEK 293 cells, followed by determination of the level of palmitoylation using radioactive palmitate.54 However, the inverse experiment, ie, determination of all the substrates of a given ZDHHC paralog, is more relevant to understanding the biological role of ZDHHC enzymes and to dissecting the molecular mechanisms of the growing array of in vivo phenotypes that are caused by defects in particular ZDHHC family members.

To this end, we performed ABE after siRNA-mediated knockdown of ZDHHC21 and ZDHHC3, two paralogs found in ECs that palmitoylate eNOS25 and have identified PECAM1 as a palmitoylprotein sensitive to the loss of ZDHHC21. This knockdown/ABE approach has the advantage of not being subject to artifacts related to overexpression of proteins in a heterologous cell type. Similar approaches have been used in yeast using strains deficient for various PATs,4 using radioactive palmitate.54 However, the inverse experiment, ie, determination of all the substrates of a given ZDHHC paralog, is more relevant to understanding the biological role of ZDHHC enzymes and to dissecting the molecular mechanisms of the growing array of in vivo phenotypes that are caused by defects in particular ZDHHC family members.

In summary, utilizing ABE technology and mass spectrometry in ECs for the first time, we have discovered several new palmitoylproteins and have begun to assign relationships between ZDHHC paralogs and palmitoylproteins. Specifically, we show that SOD1 is palmitoylated, and that palmitoylation may regulate its nuclear localization and activity. In addition, using siRNA knockdown of two ZDHHC enzymes, we have shown that ZDHHC21 is required for the palmitoylation of the junctional adhesion molecule PECAM1 and regulates its residence and/or stability in the plasma mem-
brane. Future studies using ABLE technology will further investigate how growth factors or hemodynamic forces alter the composition of the palmitoylomederinating ZDHHC-substrate pairs as potential targets to regulate EC functions. These experiments will help define the role of S-palmitoylation in EC biology.

Sources of Funding
This work was supported by grants R01 HL64793, R01 HL13171, R01 HL081190, HL096670, and P01 HL70295, and contract N01-HV-28186 (NHLBI-Yale Proteomics Contract) from the National Institutes of Health to W.C. Sessa. It was also supported by a National Kidney Foundation postdoctoral fellowship, the 5T32DK007276-33 Institutional Research Training Grant, and K08 HL103831 Career Development Award to E.P. Marin.

Disclosures
None.

References
Palmitoylation is known to be important for the function of several EC proteins, but an understanding of the full scope of palmitoylation in endothelial biology has been limited by technical difficulties in identifying which proteins are palmitoylated. Therefore, for the first time using a recently developed unbiased approach, called ABE, we analyzed palmitoylated proteins in ECs. We identified >150 candidate palmitoylated proteins, many of which are novel. We show that SOD1 is palmitoylated and that this modification alters its subcellular localization and its catalytic activity. Additionally, we show that acyl biotinyl exchange can be used to approach an important question regarding identification of specific substrates for individual members of the large family of ZDHHC protein acyl transferases. In particular, we identify a novel relationship between ZDHHC21 and palmitoylation of PECAM1, an important EC adhesion protein. Collectively, these results expand our understanding of the scope of palmitoylation in ECs and identify strategies for further unraveling the role of this modification and the related ZDHHC protein acyl transferase enzymes in ECs.
Endothelial Cell Palmitoylproteomic Identifies Novel Lipid-Modified Targets and Potential Substrates for Protein Acyl Transferases
Ethan P. Marin, Behrad Derakhshan, TuKiet T. Lam, Alberto Davalos and William C. Sessa

Circ Res. 2012;110:1336-1344; originally published online April 10, 2012; doi: 10.1161/CIRCRESAHA.112.269514

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2012 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/110/10/1336

Data Supplement (unedited) at:
http://circres.ahajournals.org/content/suppl/2012/04/10/CIRCRESAHA.112.269514.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/
Online Supplement

Detailed Methods

Cell culture

EA.hy 926, HEK 293T and COS-7 cells were cultured in high glucose DMEM (Invitrogen) supplemented with 10% FBS (Hyclone), penicillin-streptomycin (Invitrogen), glutamine, and HAT (EA.hy926 only), in humidified incubator at 37 °C with 5% CO2. HUVEC were obtained from the Yale University Vascular Biology and Therapeutics Core facility and were cultured in M199 media supplemented with endothelial cell growth supplement, 20% fetal bovine serum, penicillin-streptomycin and glutamine. HEK 293T cells and COS-7 cells were transfected using either Fugene or Lipofectamine 2000 according to manufacturer’s instructions.

Acyl-Biotinyl Exchange

ABE was performed based on a published protocol with minor modifications. Confluent EA.hy 926 cells from 1 or 2 C-150 culture plates were collected and either lysed immediately or stored at -80°C until further processing. Cells were lysed in 2ml of lysis buffer (50mM Tris, 5mM EDTA, 150mM NaCl, 1.7% β octyl glucoside OR 1% NP-40, 10% glycerol, 5mM MgCl2, 4.5 mg/ml sodium pyrophosphate, 2.1 mg/ml NaF, 0.3mg/ml AEBSF, 1 mM NaVO3, 2 mg/ml complete protease inhibitor tablet (Roche), pH 7.4) per C150 plate. Cells were disrupted by dounce homogenization (40 strokes) on ice, and then gently rotated at 4 °C for 45 min. The lysates were clarified by centrifugation at 13,000 rpm for 10min at 4 °C. The supernatants were collected and protein concentrations determined using the DC protein assay kit (Bio-Rad). Proteins (2-8 mg) were then precipitated using several volumes of ice cold acetone for 30 min at -20°C , collected by centrifugation, and then resuspended in warm (37 °C) 4SB buffer (4% SDS/ 5 mM EDTA/ 50 mM Tris/ pH 7.4). The solution was diluted to 1% SDS using lysis buffer. Free thiols were then blocked by addition of methyl methanethiosulfonate (Fluka) to a final concentration of 20 mM followed by incubation at 50°C for 30 min. Proteins were washed by 3 sequential rounds of precipitation in ice cold acetone followed by resuspension in warm 4SB buffer. Following the final resuspension, the buffer was diluted to 1% SDS, then split in half. Both halves were treated with 1 mM Biotin-HPDP (Thermo Scientific) for 60 min at room temperature. Simultaneously, one half was treated with HA (NH2OH), pH 7.4, 1M final concentration, and the other with Tris buffer pH 7.4 as a control. Proteins were recovered and washed again with three sequential rounds of precipitation in acetone and resuspension in 4SB buffer. Finally the solution was diluted with dilution buffer (50 mM Tris, 150 mM NaCl, 5 mM EDTA, 0.2% Triton, pH 7.4) to reach a final SDS concentration of 0.1%. Packed streptavidin-conjugated resin (Sigma Aldrich), prewashed in dilution buffer, was added to each tube, and then rocked for 90 min. The beads were washed 3 times in dilution buffer, and then the captured proteins eluted using dilution buffer with 1% β-mercaptoethanol.

Identification of isolated palmitoyl proteins by MS

Estimated 20 μg of protein pellet was loaded onto a 1D SDS gel (4-12% gradient gel). Proteins on the gel were fixed and silver stained prior to enzymatic processing. Gel lanes were sequentially cut into 20 sections and the silver removed by washing with 5% acetic acid and destaining with 15 mM potassium ferricyanide and 50 mM sodium thiosulfate (prepared fresh). Gel bands were further washed first with 250 μL 50% H2O/50% acetonitrile followed by a 250 μL 50% CH3CN/50 mM NH4HCO3, and a final wash with 250 μL 50% CH3CN/10 mM NH4HCO3 (5 minutes each). Gel pieces were then dried in a Speed Vac and re-hydrated with 124 μL of a 0.007 μg/μL solution of trypsin (Promega Trypsin Gold MS grade) in 10mM NH4HCO3 and incubated at 37 °C for 18 hours. The digested sample is then stored at -20 °C until LC-MS/MS
analysis on a Ultima Q-TOF instrument (experiments #2, 3, 4, and 5) or a LTQ Orbitrap instrument (experiment #1)

LC-MS/MS on the Waters/Micromass Q-Tof Ultima
In gel tryptic digested protein mixtures were analyzed via LC MS/MS on a Waters capLC coupled in-line with a Waters/Micromass Q-TOF Ultima mass spectrometer. The instrument was calibrated and MS/MS conditions optimized with a direct infusion of Glu-fibrinopeptide B (m/z = 785.85 (2+)) at a final concentration of ~ 500 fmol/μl. Following in gel tryptic digestion, 5 μL of the digestion supernatant was injected onto a 100 micron I.D. x 15 cm Atlantis C18 column (Waters) eluted at 500 nl/min. A 95-minute gradient (Initial HPLC conditions were 95% Buffer A and 5% Buffer B with the following linear gradient: 3 min, 5% B; 43 min 37% B; 75 min 75% B; and 85 min 95% B) was used in order to obtain good peptide separation. Buffer A consisted of 98% water, 2% acetonitrile, 0.1% acetic acid, and 0.01% TFA. Buffer B contained 80% acetonitrile, 20% water, 0.09% acetic acid and 0.01% TFA. Data dependent acquisition was enabled so that when the total ion current increased above the 1.5 counts/second threshold, the mass spectrometer switched automatically from MS to MS/MS modes. To ensure optimal fragmentation of selected precursor peaks, a collision energy ramp was set for the different mass sizes and charge states, giving preference to doubly- and triply-charged species for fragmentation.

LC-MS/MS on the LTQ Orbitrap
The LTQ Orbitrap is equipped with a Waters nanoACQUITY™ UPLC system, and uses a Waters Symmetry® C18 180μm x 20mm trap column and a 1.7 μm, 75 μm x 250 mm nanoACQUITY™ UPLC™ column (35ºC) for peptide separation. The flow rate is 300 nl/min with Buffer A: 100% water, 0.1% formic acid and Buffer B: 100% CH₃CN, 0.075% formic acid. A linear gradient (51 minutes) is run with 5% buffer B at initial conditions, 50% B at 50 minutes, and 85% B at 51 minutes. MS (m/z range 400-2000 at average resolving power of 60,000) is acquired in the Orbitrap using a 1 microscan, and in parallel with 6 data dependent MS/MS acquisitions (based on the top 6 most intense MS peaks) in the Linear ion trap (LTQ). Peaks targeted for MS/MS fragmentation by collision induced dissociation (CID) were first isolated with a 2 Da window followed by a normalized collision energy of 35%. Dynamic exclusion was activated where former target ions were excluded for 180 seconds. The total cycle time for both, MS (high resolution, Orbitrap) and 6 MS/MS (low resolution, Linear Ion Trap) acquisitions, was 2.4 seconds.

Database Search
All raw LC-MS/MS spectral data were searched in-house using the MASCOT² algorithm (www.matrixscience.com) with the Mascot Distiller program utilized to generate Mascot compatible files (.mgf). The Mascot Distiller program combines and centroids sequential MS/MS scans from profile data that have the same precursor ion. A charge state of +2 and +3 was preferentially located with a signal to noise ratio of 1.2 or greater and a peak list was generated for database searching. The parameters utilized in the database search were: 1) Searched against the Swiss-Prot database in January, 2011); 2) 2 miscleavages setting for trypsin digestion; 3) Error tolerance were set based on the instrument that was used (i.e. 25 ppm for MS and 0.6 Da for MS/MS on LTQ-Orbitrap); 4) Variable settings of Met Oxidation and propanimide modification (gel); 5) and preferential charge state of +2 and +3 used.

Western Blotting
SDS PAGE, electroblotting, and probing with primary and secondary antibodies was performed using standard techniques. Typically, cells were lysed in 50 mM Tris, 5 mM EDTA, 150 mM NaCl, 1.7% β-octyl glucoside, 10% glycerol, 5 mM MgCl₂, 2 mg/ml protease inhibitors (Roche),
pH 7.4. Lysates were clarified by centrifugation at 13,500rpm for 10min on a table top microcentrifuge. Lysates were boiled with SDS loading buffer for 10min, then loaded (10-50 μg of protein per lane). Proteins were transferred to 0.2 micron nitrocellulose (BioRad) overnight at 30V, 4°C. Primary antibodies used were: PECAM1 mouse monoclonal (Dako); eNOS- mouse monoclonal (BD Pharmingen); SOD1- mouse monoclonal (BD Transduction Laboratories); SOD1—rabbit polyclonal (Stressgen; used only for HUVEC studies); Yes- mouse monoclonal (BD Pharmingen); Calnexin- mouse monoclonal (BD Pharmingen); R-ras (Cell Signaling); total ras- mouse monoclonal (BD Pharmingen); Caveolin1- rabbit polyclonal (Santa Cruz), Hsp90-mouse monoclonal (Santa Cruz), Src, HA- rabbit polyclonal (Santa Cruz), Gαi- rabbit polyclonal (Santa Cruz), MUC18- mouse monoclonal (BD Pharmingen), syntaxin-6- mouse monoclonal (BD Pharmingen). Secondary antibodies were species specific anti-IgG conguated with either AlexaFluor 680 (Invitrogen) or IRDye800 (Rockland); they were used at 1:5000 dilution and incubated for 1h at room temperature. Blots were washed and visualized using a LiCor Odyssey imager.

Quantitative RT-PCR
Primer sequences used were: GAPDH (accession BC_013310), 5′-CCACCCATGGCCAAATTCCATGGCA-3′ and 5′-TCTAGACGGCAGGTCAGGTCCACC-3′; ZDHHC3 (accession no. NM_016598), 5′-TGTTTGTGTAACGGTGCATTCCG-3′ and 5′-TTGGTCTGGCGTGAAAGG-3′; ZDHHC21 (accession no. NM_178566) 5′-AAGGGTTCCCATCATGCAGC-3′ and 5′-GAACCTGCAGTGTTGCCCTG-3′. The cycling conditions were as follows: 95 deg x 8.5 min, then 40 cycles of 95° x 30 sec, 58° deg x 30 sec, 72° x 60 sec. Melting curve analyses were routinely performed to ensure amplification of a single species.

Cell surface PECAM expression analysis by FACS
EA.hy 926 cells were washed with PBS, collected by trypsinization, and neutralized in 5% BSA. Cells were blocked in 2% BSA for 10 min on ice. PECAM1 on the cell surface was detected by incubating cells with anti-PECAM-PE (BD-Pharmingen) or isotype matched IgG (Beckman Coulter) as a control for 30 min on ice. Following washing in PBS, cells were analyzed on a FACScan cell sorter (Becton Dickinson) and data processed with WinMDI software (http://facs.scripps.edu/software.html).

ROS Levels and SOD activity
ROS generation was measured by the oxidation of 2,7-dichlorofluorescin diacetate (DCF; Molecular Probes). Cells were incubated with DCF 5 μmol/L for 30 min 37°C. Immediately after incubation cells were washed with cold PBS, trypsinized and collected in 0.5 ml of PBS. Cells were transferred to polystyrene tubes with cell-strainer caps (Falcon) and subjected flow cytometry analysis (FACScalibur; Becton Dickinson). Data were analyzed by using CELL QUEST software (version 3.2.1; Becton Dickinson), and the median intensity of fluorescence (MIF) was used to evaluate the fluorescence of each tube.

Immunopurification of SOD1
SOD1 WT or C6S, labeled with an HA-affinity tag at the carboxyl terminus, was transfected in HEK-293T cells. Cells were lysed after 48h in 50 mM Tris, 5 mM EDTA, 150 mM NaCl, 1.7% β-octyl glucoside, 10% glycerol, 5 mM MgCl2, 2 mg/ml protease inhibitors (Roche), pH 7.4. SOD1 was immunoprecipitated using sepharose resin conjugated with anti-HA antibody (Sigma) overnight. The resin was washed three times in wash buffer (50 mM Tris, 150 mM NaCl, 0.2% Triton, 2 mg/ml protease inhibitors, pH 7.4) and then eluted with 100 μg/ml anti-HA peptide (Sigma) in wash buffer. The purity and quantity of the eluted protein was determined by SDS-
PAGE followed by staining with sypro-ruby (Molecular Probes), using purified bovine SOD1 (Sigma) to construct a standard curve. The stained gels were analyzed on a Typhoon fluorescence gel scanner (GE Healthcare Life Sciences). The quantification was confirmed by Western blotting and densitometry.

Immunofluorescence of SOD1
Transfected cells grown on glass coverslips were fixed in 4% PFA, permeabilized with 0.1% Triton for 10 min at RT, then blocked with 5% BSA in PBS at RT for 30 min. Cells were incubated with anti-HA antibody (Roche 3F10) at 1:500 dilution in 5% BSA/PBS overnight at 4°C. Cells were washed 3x in PBS, then incubated with goat anti-rat-488 antibody (Invitrogen), 1:500 dilution in PBS with DAPI for 60 min at RT. Cells were washed 3x with PBS, then set using Vectashield hardset fluorescence mounting media. Cells were visualized using a Leica TCS SP5 Spectral Confocal Microscope. In order to quantify nuclear staining of transfected SOD1 proteins, a blinded independent judge scored cells from randomly collected microscopic fields for the presence of nuclear localization. The nucleus was defined as regions demonstrating positive DAPI staining. Nuclear SOD1 was judged to be present if the intensity of HA staining was comparable to or greater than the intensity in the surrounding cytoplasm.
Supplemental Figure Legends

Supplemental Fig. I: Acyl-Biotinyl Exchange schematic†. Proteins from EA.hy 926 cell lysates were denatured in SDS, and free thiols were blocked with methyl methanethiosulfonate (MMTS) (Step 1). Subsequently, palmitate groups attached via thioesters were cleaved using neutral HA (Step 2); in control reactions, HA was replaced with Tris. Next, newly liberated cysteine sidechains were derivatized with biotin-HPDP (Step 3). Biotinylated proteins were purified on streptavidin resin (Step 4), and eluted with β-mercaptoethanol (Step 5). Subsequently, eluted proteins were analyzed by MS or by Western blotting (Step 6).
Supplemental Figure I

Step 1 - Blocking Free Thiols

Step 2 - Thioester cleavage

Step 3 - Biotinylation of newly formed free thiols

Step 4 - Streptavidin Capture of Biotinylated Proteins

Step 5 - β-ME Elution

Step 6a - Immunoblot analysis

Step 6b - Mass Spectrometry Analysis
Supplemental Table I: Palmitoylated proteins identified in EC by ABE and mass spectrometry.

<table>
<thead>
<tr>
<th>Protein Name</th>
<th>Gene Name</th>
<th>Indisting¹</th>
<th>Novel²</th>
<th>Gene Ontogeny³</th>
</tr>
</thead>
<tbody>
<tr>
<td>14-3-3 protein zeta/delta</td>
<td>YWHAZ</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-oxoacyl-[acyl-carrier-protein] synthase, mitochondrial</td>
<td>OXSM</td>
<td></td>
<td></td>
<td>oxidoreductase activity; methyltransferase activity; acyltransferase activity; hydrolase activity, acting on ester bonds</td>
</tr>
<tr>
<td>40S ribosomal protein S6</td>
<td>RPS6</td>
<td>Y</td>
<td></td>
<td>structural constituent of ribosome; nucleic acid binding</td>
</tr>
<tr>
<td>60 kDa heat shock protein, mitochondrial</td>
<td>HSPD1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60S ribosomal protein L12</td>
<td>RPL12</td>
<td></td>
<td></td>
<td>structural constituent of ribosome; nucleic acid binding</td>
</tr>
<tr>
<td>60S ribosomal protein L7</td>
<td>RPL7</td>
<td></td>
<td></td>
<td>structural constituent of ribosome; nucleic acid binding</td>
</tr>
<tr>
<td>Actin, cytoplasmic 1</td>
<td>ACTB</td>
<td></td>
<td></td>
<td>structural constituent of cytoskeleton</td>
</tr>
<tr>
<td>Actin, cytoplasmic 2</td>
<td>ACTG1</td>
<td></td>
<td></td>
<td>structural constituent of cytoskeleton</td>
</tr>
<tr>
<td>Adenosylhomocysteinase</td>
<td>AHCY</td>
<td></td>
<td></td>
<td>hydrolase activity</td>
</tr>
<tr>
<td>ADP-ribosylation factor 4</td>
<td>ARF4</td>
<td></td>
<td></td>
<td>GTPase activity; protein binding</td>
</tr>
<tr>
<td>Alpha-actinin-1</td>
<td>ACTN1</td>
<td>i4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alpha-actinin-4</td>
<td>ACTN4</td>
<td>i4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alpha-enolase</td>
<td>ENO1</td>
<td></td>
<td></td>
<td>lyase activity</td>
</tr>
<tr>
<td>Anthrax toxin receptor 2</td>
<td>ANTXR2</td>
<td></td>
<td></td>
<td>receptor activity</td>
</tr>
<tr>
<td>ATP synthase subunit alpha, mitochondrial</td>
<td>ATP5A1</td>
<td></td>
<td></td>
<td>hydrolase activity, acting on ester bonds; receptor activity; cation transmembrane transporter activity; hydrogen ion transmembrane transporter activity; anion channel activity; ligand-gated ion channel activity; DNA binding</td>
</tr>
<tr>
<td>ATP-binding cassette sub-family E member 1</td>
<td>ABCE1</td>
<td>Y</td>
<td></td>
<td>ATPase activity, coupled to transmembrane movement of substances; transmembrane transporter activity; protein binding; enzyme inhibitor activity</td>
</tr>
<tr>
<td>Calnexin</td>
<td>CANX</td>
<td></td>
<td></td>
<td>calcium ion binding</td>
</tr>
<tr>
<td>Calpain-5</td>
<td>CAPN5</td>
<td>Y</td>
<td></td>
<td>peptidase activity; calcium ion binding; calmodulin binding; calcium-dependent phospholipid binding</td>
</tr>
<tr>
<td>Caveolin-1</td>
<td>CAV1</td>
<td></td>
<td></td>
<td>structural molecule activity; protein binding; small GTPase regulator activity</td>
</tr>
<tr>
<td>CD44 antigen</td>
<td>CD44</td>
<td></td>
<td></td>
<td>receptor activity</td>
</tr>
<tr>
<td>Protein Name</td>
<td>Gene Name</td>
<td>Indisting$^1$</td>
<td>Novel$^2$</td>
<td>Gene Ontogeny$^3$</td>
</tr>
<tr>
<td>-----------------------------------</td>
<td>-----------</td>
<td>---------------</td>
<td>-----------</td>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>CD63 antigen</td>
<td>CD63</td>
<td></td>
<td></td>
<td>receptor activity; receptor binding</td>
</tr>
<tr>
<td>CD82 antigen</td>
<td>CD82</td>
<td></td>
<td></td>
<td>receptor activity; receptor binding</td>
</tr>
<tr>
<td>CD9 antigen</td>
<td>CD9</td>
<td></td>
<td></td>
<td>receptor activity; receptor binding</td>
</tr>
<tr>
<td>CD99 antigen</td>
<td>CD99</td>
<td></td>
<td></td>
<td>receptor activity; receptor binding</td>
</tr>
<tr>
<td>Cell surface glycoprotein MUC18</td>
<td>MCAM</td>
<td></td>
<td></td>
<td>receptor activity</td>
</tr>
<tr>
<td>Coatomer subunit delta</td>
<td>ARCN1</td>
<td>Y</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cofilin-1</td>
<td>CFL1</td>
<td></td>
<td></td>
<td>structural constituent of cytoskeleton; cytoskeletal protein binding</td>
</tr>
<tr>
<td>Cofilin-2</td>
<td>CFL2</td>
<td>Y</td>
<td></td>
<td>structural constituent of cytoskeleton; cytoskeletal protein binding</td>
</tr>
<tr>
<td>Complement component 1 Q subcomponent-binding protein, mitochondrial</td>
<td>C1QBP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytochrome b5 type B</td>
<td>CYB5B</td>
<td></td>
<td></td>
<td>oxidoreductase activity</td>
</tr>
<tr>
<td>Cytoskeleton-associated protein 4</td>
<td>CKAP4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Desmoglein-1</td>
<td>DSG1</td>
<td>Y</td>
<td></td>
<td>calcium ion binding</td>
</tr>
<tr>
<td>Desmoglein-4</td>
<td>DSG4</td>
<td></td>
<td></td>
<td>calcium ion binding</td>
</tr>
<tr>
<td>Dihydropyrimidinase-related protein 2</td>
<td>DPYSL2</td>
<td>Y</td>
<td></td>
<td>hydrolase activity</td>
</tr>
<tr>
<td>Dystroglycan</td>
<td>DAG1</td>
<td></td>
<td></td>
<td>receptor activity; nucleotidyltransferase activity; GTPase activity; translation factor activity, nucleic acid binding; protein binding; translation initiation factor activity; translation elongation factor activity</td>
</tr>
<tr>
<td>Elongation factor 2</td>
<td>EEF2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endoplasmic reticulum-Golgi intermediate compartment protein 3</td>
<td>ERGIC3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endothelial protein C receptor</td>
<td>PROCR</td>
<td></td>
<td></td>
<td>receptor activity; protein binding</td>
</tr>
<tr>
<td>Endothelin-converting enzyme 1</td>
<td>ECE1</td>
<td></td>
<td></td>
<td>peptidase activity</td>
</tr>
<tr>
<td>Erythrocyte band 7 integral membrane protein</td>
<td>STOM</td>
<td></td>
<td></td>
<td>structural constituent of cytoskeleton</td>
</tr>
<tr>
<td>Protein Name</td>
<td>Gene Name</td>
<td>Indisting</td>
<td>Novel</td>
<td>Gene Ontogeny</td>
</tr>
<tr>
<td>--------------------------------------------------</td>
<td>-----------</td>
<td>-----------</td>
<td>-------</td>
<td>---------------------------------------------------</td>
</tr>
<tr>
<td>Far upstream element-binding protein 2</td>
<td>KHSRP</td>
<td></td>
<td>Y</td>
<td>RNA splicing factor activity, transesterification mechanism; RNA binding</td>
</tr>
<tr>
<td>Fascin</td>
<td>FSCN1</td>
<td></td>
<td>Y</td>
<td>structural constituent of cytoskeleton; cytoskeletal protein binding</td>
</tr>
<tr>
<td>Fatty acid synthase</td>
<td>FASN</td>
<td></td>
<td></td>
<td>oxidoreductase activity; methyltransferase activity; acyltransferase activity; hydrolase activity, acting on ester bonds; ligase activity</td>
</tr>
<tr>
<td>Fatty acid-binding protein, adipocyte</td>
<td>FABP4</td>
<td>i10</td>
<td>Y</td>
<td></td>
</tr>
<tr>
<td>Ferritin light chain</td>
<td>FTL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Filamin-B</td>
<td>FLNB</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flotillin-1</td>
<td>FLOT1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flotillin-2</td>
<td>FLOT2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Galectin-7</td>
<td>LGALS7</td>
<td></td>
<td>Y</td>
<td></td>
</tr>
<tr>
<td>Golgi integral membrane protein 4</td>
<td>GOLIM4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GTPase HRas</td>
<td>HRAS</td>
<td></td>
<td></td>
<td>GTPase activity; protein binding</td>
</tr>
<tr>
<td>GTPase NRas</td>
<td>NRAS</td>
<td></td>
<td></td>
<td>GTPase activity; protein binding</td>
</tr>
<tr>
<td>GTPase HRas</td>
<td>HRAS</td>
<td></td>
<td></td>
<td>GTPase activity; protein binding</td>
</tr>
<tr>
<td>Guanine nucleotide-binding protein G(i) subunit alpha-2</td>
<td>GNAI2</td>
<td></td>
<td></td>
<td>GTPase activity; protein binding</td>
</tr>
<tr>
<td>Guanine nucleotide-binding protein G(k) subunit alpha</td>
<td>GNAI3</td>
<td></td>
<td></td>
<td>GTPase activity; protein binding</td>
</tr>
<tr>
<td>Guanine nucleotide-binding protein G(q) subunit alpha</td>
<td>GNAQ</td>
<td></td>
<td></td>
<td>GTPase activity; protein binding</td>
</tr>
<tr>
<td>Guanine nucleotide-binding protein G(s) subunit alpha isoforms short</td>
<td>GNAS</td>
<td>i8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Guanine nucleotide-binding protein G(s) subunit alpha isoforms Xiias</td>
<td>GNAS</td>
<td>i8</td>
<td></td>
<td>GTPase activity; protein binding</td>
</tr>
<tr>
<td>Guanine nucleotide-binding protein subunit alpha-11</td>
<td>GNA11</td>
<td></td>
<td></td>
<td>GTPase activity; protein binding</td>
</tr>
<tr>
<td>Guanine nucleotide-binding protein subunit alpha-13</td>
<td>GNA13</td>
<td></td>
<td></td>
<td>GTPase activity; protein binding</td>
</tr>
<tr>
<td>Protein Name</td>
<td>Gene Name</td>
<td>Indisting</td>
<td>Novel</td>
<td>Gene Ontogeny</td>
</tr>
<tr>
<td>------------------------------------------------------------------------------</td>
<td>--------------</td>
<td>-----------</td>
<td>-------</td>
<td>------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Guanine nucleotide-binding protein subunit beta-2-like 1</td>
<td>GNB2L1</td>
<td></td>
<td>Y</td>
<td></td>
</tr>
<tr>
<td>Heat shock protein beta-1</td>
<td>HSPB1</td>
<td></td>
<td>Y</td>
<td>structural molecule activity</td>
</tr>
<tr>
<td>Heterogeneous nuclear ribonucleoprotein A1</td>
<td>HNRNPA1</td>
<td>i1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heterogeneous nuclear ribonucleoprotein A1-like 2</td>
<td>HNRNPA1L2</td>
<td>i1</td>
<td></td>
<td>RNA splicing factor activity, transesterification mechanism;DNA binding;RNA binding</td>
</tr>
<tr>
<td>Heterogeneous nuclear ribonucleoprotein K</td>
<td>HNRNPK</td>
<td></td>
<td></td>
<td>RNA splicing factor activity, transesterification mechanism;RNA binding</td>
</tr>
<tr>
<td>Heterogeneous nuclear ribonucleoproteins A2/B1</td>
<td>HNRNPA2B1</td>
<td></td>
<td></td>
<td>RNA splicing factor activity, transesterification mechanism;DNA binding;RNA binding</td>
</tr>
<tr>
<td>Histone H4</td>
<td>HIST1H4A</td>
<td></td>
<td>Y</td>
<td></td>
</tr>
<tr>
<td>HLA class I histocompatibility antigen, Cw-14 alpha chain</td>
<td>HLA-C</td>
<td>i3 i4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ICOS ligand</td>
<td>ICOSLG</td>
<td>i7</td>
<td>Y</td>
<td>ubiquitin-protein ligase activity</td>
</tr>
<tr>
<td>Ig kappa chain C region</td>
<td>IGKC</td>
<td></td>
<td>Y</td>
<td>antigen binding</td>
</tr>
<tr>
<td>Integrin alpha-3</td>
<td>ITGA3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Integrin alpha-6</td>
<td>ITGA6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercellular adhesion molecule 2</td>
<td>ICAM2</td>
<td></td>
<td>Y</td>
<td>receptor binding</td>
</tr>
<tr>
<td>Interferon-induced transmembrane protein 1</td>
<td>IFITM1</td>
<td>i9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interferon-induced transmembrane protein 2</td>
<td>IFITM2</td>
<td>i9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interferon-induced transmembrane protein 3</td>
<td>IFITM3</td>
<td>i9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Junction plakoglobin</td>
<td>JUP</td>
<td></td>
<td></td>
<td>structural constituent of cytoskeleton;receptor binding</td>
</tr>
<tr>
<td>KH domain-containing, RNA-binding, signal transduction-associated protein 1</td>
<td>KHDRBS1</td>
<td></td>
<td>Y</td>
<td>RNA splicing factor activity, transesterification mechanism;DNA binding;RNA binding;transcription factor activity;transcription cofactor activity</td>
</tr>
<tr>
<td>Leucine-rich repeat flightless-interacting protein 1</td>
<td>LRRFIP1</td>
<td></td>
<td>Y</td>
<td>DNA binding;RNA binding;transcription factor activity;transcription cofactor activity</td>
</tr>
<tr>
<td>L-lactate dehydrogenase B chain</td>
<td>LDHB</td>
<td></td>
<td>Y</td>
<td>oxidoreductase activity</td>
</tr>
<tr>
<td>Lysosome membrane protein 2</td>
<td>SCARB2</td>
<td></td>
<td></td>
<td>receptor activity</td>
</tr>
<tr>
<td>Protein Name</td>
<td>Gene Name</td>
<td>Indisting&lt;sup&gt;1&lt;/sup&gt;</td>
<td>Novel&lt;sup&gt;2&lt;/sup&gt;</td>
<td>Gene Ontogeny&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td>------------------------------------------------------------------------------</td>
<td>-----------</td>
<td>------------------------</td>
<td>-------------------</td>
<td>------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Malate dehydrogenase, mitochondrial</td>
<td>MDH2</td>
<td></td>
<td></td>
<td>oxidoreductase activity</td>
</tr>
<tr>
<td>Matrix metalloproteinase-14</td>
<td>MMP14</td>
<td></td>
<td></td>
<td>peptidase activity</td>
</tr>
<tr>
<td>Methionine aminopeptidase 2</td>
<td>METAP2</td>
<td>Y</td>
<td></td>
<td>peptidase activity;DNA binding;transcription factor activity</td>
</tr>
<tr>
<td>MHC class II regulatory factor RFX1</td>
<td>RFX1</td>
<td></td>
<td></td>
<td>DNA binding;transcription factor activity</td>
</tr>
<tr>
<td>Myelin P2 protein</td>
<td>PMP2</td>
<td>i10</td>
<td></td>
<td>lipid binding</td>
</tr>
<tr>
<td>Myoferlin</td>
<td>MYOF</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myristoylated alanine-rich C-kinase substrate</td>
<td>MARCKS</td>
<td></td>
<td></td>
<td>structural constituent of cytoskeleton;receptor binding;cytoskeletal protein binding</td>
</tr>
<tr>
<td>NADP-dependent malic enzyme</td>
<td>ME1</td>
<td>Y</td>
<td></td>
<td>oxidoreductase activity;acyltransferase activity;carboxy-lyase activity</td>
</tr>
<tr>
<td>Neuroblast differentiation-associated protein AHNAK</td>
<td>AHNAK</td>
<td>Y</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neutral amino acid transporter B(0)</td>
<td>SLC1A5</td>
<td></td>
<td></td>
<td>cation transmembrane transporter activity</td>
</tr>
<tr>
<td>Nitric oxide synthase, endothelial</td>
<td>NOS3</td>
<td></td>
<td></td>
<td>oxidoreductase activity;calcium ion binding;calmodulin binding</td>
</tr>
<tr>
<td>Nuclear autoantigenic sperm protein</td>
<td>NASP</td>
<td>Y</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peroxiredoxin-6</td>
<td>PRDX6</td>
<td>Y</td>
<td></td>
<td>oxidoreductase activity;peroxidase activity</td>
</tr>
<tr>
<td>Phosphatidylinositol 4-kinase type 2-alpha</td>
<td>PI4K2A</td>
<td></td>
<td></td>
<td>kinase activity</td>
</tr>
<tr>
<td>Phospholipid scramblase 3</td>
<td>PLSCR3</td>
<td></td>
<td></td>
<td>structural constituent of cytoskeleton;cytoskeletal protein binding</td>
</tr>
<tr>
<td>Plakophilin-1</td>
<td>PKP1</td>
<td>Y</td>
<td></td>
<td>structural constituent of cytoskeleton;cytoskeletal protein binding</td>
</tr>
<tr>
<td>Platelet endothelial cell adhesion molecule</td>
<td>PECAM1</td>
<td></td>
<td></td>
<td>receptor activity</td>
</tr>
<tr>
<td>Podocalyxin</td>
<td>PODXL</td>
<td>Y</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poliovirus receptor</td>
<td>PVR</td>
<td></td>
<td></td>
<td>receptor activity</td>
</tr>
<tr>
<td>Poly [ADP-ribose] polymerase 1</td>
<td>PARP1</td>
<td></td>
<td></td>
<td>transferase activity, transferring glycosyl groups</td>
</tr>
<tr>
<td>Profilin-1</td>
<td>PFN1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein LYRIC</td>
<td>MTDH</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein Name</td>
<td>Gene Name</td>
<td>Indisting(^1)</td>
<td>Novel(^2)</td>
<td>Gene Ontogeny(^3)</td>
</tr>
<tr>
<td>--------------------------------------------------</td>
<td>-------------</td>
<td>------------------</td>
<td>-------------</td>
<td>-------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Protein S100-A9</td>
<td>S100A9</td>
<td></td>
<td>Y</td>
<td>calcium ion binding; receptor binding; calmodulin binding</td>
</tr>
<tr>
<td>Proto-oncogene tyrosine-protein kinase Src</td>
<td>SRC</td>
<td>i5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proto-oncogene tyrosine-protein kinase Yes</td>
<td>YES1</td>
<td>i5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Putative heterogeneous nuclear ribonucleoprotein A1-like 3</td>
<td>HNRPA1L3</td>
<td>i1</td>
<td>Y</td>
<td></td>
</tr>
<tr>
<td>Putative Ras-related protein Rab-1C</td>
<td>RAB1C</td>
<td>i2</td>
<td>Y</td>
<td></td>
</tr>
<tr>
<td>Putative tropomyosin alpha-3 chain-like protein</td>
<td>TMP3L</td>
<td></td>
<td></td>
<td>motor activity; structural constituent of cytoskeleton</td>
</tr>
<tr>
<td>Pyruvate kinase isozymes M1/M2</td>
<td>PKM2</td>
<td></td>
<td></td>
<td>kinase activity</td>
</tr>
<tr>
<td>Ragulator complex protein PDRO</td>
<td>PDRO</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ras-related protein Rab-1B</td>
<td>RAB1B</td>
<td>i2</td>
<td></td>
<td>GTPase activity; protein binding</td>
</tr>
<tr>
<td>Ras-related protein Rab-2A</td>
<td>RAB2A</td>
<td></td>
<td>Y</td>
<td>GTPase activity; protein binding</td>
</tr>
<tr>
<td>Ras-related protein Ral-A</td>
<td>RALA</td>
<td></td>
<td></td>
<td>GTPase activity; protein binding</td>
</tr>
<tr>
<td>Ras-related protein Ral-B</td>
<td>RALB</td>
<td></td>
<td></td>
<td>GTPase activity; protein binding</td>
</tr>
<tr>
<td>Ras-related protein Rap-2b</td>
<td>RAP2B</td>
<td></td>
<td></td>
<td>GTPase activity; protein binding</td>
</tr>
<tr>
<td>Ras-related protein Rap-2c</td>
<td>RAP2C</td>
<td></td>
<td></td>
<td>GTPase activity; protein binding</td>
</tr>
<tr>
<td>Ras-related protein R-Ras</td>
<td>RRAS</td>
<td></td>
<td></td>
<td>GTPase activity; protein binding</td>
</tr>
<tr>
<td>Ras-related protein R-Ras2</td>
<td>RRAS2</td>
<td></td>
<td></td>
<td>GTPase activity; protein binding</td>
</tr>
<tr>
<td>Receptor expression-enhancing protein 5</td>
<td>REEP5</td>
<td></td>
<td></td>
<td>receptor activity; transmembrane transporter activity</td>
</tr>
<tr>
<td>Reticulon-4</td>
<td>RTN4</td>
<td></td>
<td>Y</td>
<td></td>
</tr>
<tr>
<td>Retinoic acid-induced protein 3</td>
<td>GPRC5A</td>
<td></td>
<td></td>
<td>G-protein coupled receptor activity</td>
</tr>
<tr>
<td>Protein Name</td>
<td>Gene Name</td>
<td>Indisting</td>
<td>Novel</td>
<td>Gene Ontogeny</td>
</tr>
<tr>
<td>------------------------------------------------</td>
<td>-------------</td>
<td>-----------</td>
<td>-------</td>
<td>------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Rho GDP-dissociation inhibitor 2</td>
<td>ARHGDI B</td>
<td></td>
<td></td>
<td>receptor binding; small GTPase regulator activity</td>
</tr>
<tr>
<td>Ribonuclease inhibitor</td>
<td>RNH1</td>
<td></td>
<td>Y</td>
<td>protein binding; enzyme inhibitor activity</td>
</tr>
<tr>
<td>Ribonucleoside-diphosphosphate reductase large subunit</td>
<td>RRM1</td>
<td></td>
<td>Y</td>
<td>oxidoreductase activity</td>
</tr>
<tr>
<td>Selenium-binding protein 1</td>
<td>SELENBP1</td>
<td></td>
<td>Y</td>
<td></td>
</tr>
<tr>
<td>Serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A alpha isoform</td>
<td>PPP2R1A</td>
<td></td>
<td></td>
<td>hydrolase activity, acting on ester bonds; phosphatase activity</td>
</tr>
<tr>
<td>Serpin B4</td>
<td>SERPINB4</td>
<td></td>
<td>Y</td>
<td>protein binding; peptidase inhibitor activity</td>
</tr>
<tr>
<td>Small VCP/p97-interacting protein</td>
<td>SVIP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stress-induced-phosphoprotein 1</td>
<td>STIP1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Superoxide dismutase [Cu-Zn]</td>
<td>SOD1</td>
<td></td>
<td>Y</td>
<td>oxidoreductase activity, antioxidant activity</td>
</tr>
<tr>
<td>Superoxide dismutase [Mn], mitochondrial</td>
<td>SOD2</td>
<td></td>
<td>Y</td>
<td>oxidoreductase activity</td>
</tr>
<tr>
<td>Synaptosomal-associated protein 23</td>
<td>SNAP23</td>
<td></td>
<td></td>
<td>SNAP receptor activity</td>
</tr>
<tr>
<td>Syntaxin-11</td>
<td>STX11</td>
<td></td>
<td></td>
<td>SNAP receptor activity</td>
</tr>
<tr>
<td>Syntaxin-12</td>
<td>STX12</td>
<td></td>
<td></td>
<td>SNAP receptor activity</td>
</tr>
<tr>
<td>Syntaxin-6</td>
<td>STX6</td>
<td></td>
<td></td>
<td>SNAP receptor activity</td>
</tr>
<tr>
<td>TATA-binding protein-associated factor 2N</td>
<td>TAF15</td>
<td></td>
<td>Y</td>
<td>RNA splicing factor activity, transesterification mechanism; DNA binding; RNA binding; transcription factor activity</td>
</tr>
<tr>
<td>T-complex protein 1 subunit epsilon</td>
<td>CCT5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T-complex protein 1 subunit theta</td>
<td>CCT8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T-complex protein 1 subunit zeta</td>
<td>CCT6A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein Name</td>
<td>Gene Name</td>
<td>Indisting¹</td>
<td>Novel²</td>
<td>Gene Ontogeny³</td>
</tr>
<tr>
<td>---------------</td>
<td>---------------</td>
<td>------------</td>
<td>----------</td>
<td>----------------------</td>
</tr>
<tr>
<td>Thioredoxin-related transmembrane protein 1</td>
<td>TMX1</td>
<td></td>
<td></td>
<td>protein disulfide isomerase activity</td>
</tr>
<tr>
<td>Trafficking protein particle complex subunit 3</td>
<td>TRAPPC3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transferrin receptor protein 1</td>
<td>TFRC</td>
<td></td>
<td></td>
<td>peptidase activity;receptor activity</td>
</tr>
<tr>
<td>Tropomyosin alpha-3 chain</td>
<td>TPM3</td>
<td>Y</td>
<td></td>
<td>motor activity;structural constituent of cytoskeleton</td>
</tr>
<tr>
<td>Tyrosine-protein kinase Fyn</td>
<td>FYN</td>
<td>i5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tyrosine-protein kinase Lck</td>
<td>LCK</td>
<td>i5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uncharacterized protein C6orf125 UPF0160 protein MYG1, mitochondrial</td>
<td>C6orf125</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vesicle-associated membrane protein 1</td>
<td>VAMP1</td>
<td>i11</td>
<td>Y</td>
<td>SNAP receptor activity</td>
</tr>
<tr>
<td>Vesicle-associated membrane protein 2</td>
<td>VAMP2</td>
<td>i11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vesicle-associated membrane protein 3</td>
<td>VAMP3</td>
<td>i11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Voltage-dependent anion-selective channel protein 2</td>
<td>VDAC2</td>
<td></td>
<td></td>
<td>voltage-gated ion channel activity;anion channel activity</td>
</tr>
<tr>
<td>Voltage-dependent anion-selective channel protein 3</td>
<td>VDAC3</td>
<td></td>
<td></td>
<td>voltage-gated ion channel activity;anion channel activity</td>
</tr>
<tr>
<td>V-set and immunoglobulin domain-containing protein 8</td>
<td>VSIG8</td>
<td>Y</td>
<td></td>
<td>receptor activity</td>
</tr>
</tbody>
</table>

¹ Proteins designated as “novel” were not reported in previous proteomic analyses of palmitoylproteins in human cells, were not annotated with a post translation modification of “palmitate” in the Swiss Prot database and were not reported as palmitoylated in a semi-automated search of PubMed database (see text).

² When more than one protein matched to the underlying peptides matched by Mascot to the raw MS/MS spectra, the groups of proteins are indicated with an “I” and a number in the “indisting” column.
Gene ontogeny information (retrieved from PantherDB.org) is shown where available.

Peptides mapped HLA class I histocompatibility antigen, Cw-14 alpha chain, were identical to peptides derived from a variety of different HLA I chains; not all are shown in this table.
Supplemental Table II: Likely false positive palmitoylproteins. In addition to likely contaminants (e.g., keratin), other known proteins isolated by ABE such as ubiquitin conjugases were recovered.

<table>
<thead>
<tr>
<th>Protein Name</th>
<th>Gene Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex, mitochondrial</td>
<td>DLAT</td>
</tr>
<tr>
<td>Keratin, type I cuticular Ha1</td>
<td>KRT31</td>
</tr>
<tr>
<td>Keratin, type I cuticular Ha4</td>
<td>KRT34</td>
</tr>
<tr>
<td>Keratin, type I cytoskeletal 16</td>
<td>KRT16</td>
</tr>
<tr>
<td>Keratin, type II cuticular Hb2</td>
<td>KRT82</td>
</tr>
<tr>
<td>Keratin, type II cuticular Hb5</td>
<td>KRT85</td>
</tr>
<tr>
<td>Keratin, type II cytoskeletal 2 oral</td>
<td>KRT76</td>
</tr>
<tr>
<td>Keratin, type II cytoskeletal 5</td>
<td>KRT5</td>
</tr>
<tr>
<td>Keratin, type II cytoskeletal 6B</td>
<td>KRT6B</td>
</tr>
<tr>
<td>Keratin, type II cytoskeletal 79</td>
<td>KRT79</td>
</tr>
<tr>
<td>Prohibitin-2</td>
<td>PHB2</td>
</tr>
<tr>
<td>Ubiquitin/ISG15-conjugating enzyme E2 L6</td>
<td>UBE2L6</td>
</tr>
<tr>
<td>Ubiquitin-40S ribosomal protein S27a</td>
<td>RPS27A</td>
</tr>
<tr>
<td>Ubiquitin-conjugating enzyme E2 D2</td>
<td>UBE2D2</td>
</tr>
<tr>
<td>Ubiquitin-conjugating enzyme E2 D3</td>
<td>UBE2D3</td>
</tr>
<tr>
<td>Ubiquitin-conjugating enzyme E2 K</td>
<td>UBE2K</td>
</tr>
<tr>
<td>Ubiquitin-conjugating enzyme E2 L3</td>
<td>UBE2L3</td>
</tr>
<tr>
<td>Ubiquitin-conjugating enzyme E2 N</td>
<td>UBE2N</td>
</tr>
<tr>
<td>Ubiquitin-conjugating enzyme E2 R2</td>
<td>UBE2R2</td>
</tr>
<tr>
<td>Ubiquitin-conjugating enzyme E2 T</td>
<td>UBE2T</td>
</tr>
<tr>
<td>Ubiquitin-conjugating enzyme E2 variant 1</td>
<td>UBE2V1</td>
</tr>
<tr>
<td>Ubiquitin-like modifier-activating enzyme 1</td>
<td>UBA1</td>
</tr>
<tr>
<td>Ubiquitin-like-conjugating enzyme ATG3</td>
<td>ATG3</td>
</tr>
</tbody>
</table>
Supplemental References