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Emerging Roles of DHHC-mediated Protein *S*-palmitoylation in Physiological and Pathophysiological Context

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Abstract: Protein *S*-palmitoylation refers to a post-translational modification (PTM) wherein palmitic acid, a 16-carbon long saturated fatty acid gets covalently attached to Cys sidechain of a protein. It has been known to the literature for almost 50 years and in general, this PTM is believed to facilitate membrane attachments of proteins for the obvious hydrophobicity of the palmitoyl group. But after the discovery of the protein palmitoyl acyltransferases (PATs, also known as DHHC-PATs), a major paradigm shift has been observed in the field of protein *S*-palmitoylation. A family of 23 mammalian DHHC-PATs has been identified and the majority of them are associated with many human diseases spanning from neuropsychiatric diseases to cancers. Novel unique and essential role of DHHC-mediated protein *S*-palmitoylation has been revealed apart from its membrane trafficking role. Biomedical importance of DHHCs has also been reiterated with small molecule inhibitors for DHHCs as well as in DHHC-knockout mice or mouse Xenograft models. In this review, we present recent advances in the field of protein *S*-palmitoylation and the involvement of individual DHHC isoforms in human diseases. In addition, the recent development of the analytical tools to study *S*-palmitoylation and their inhibitors are discussed in detail. We also highlight the issues that need to be addressed in detail to further develop our understanding on protein *S*-palmitoylation and strongly believe that pharmacological modulation of DHHC-mediated protein *S*-palmitoylation has a massive potential to emerge as a novel therapeutic strategy for human diseases. It will not be surprising if reversible protein *S*-palmitoylation prove to be an indispensable PTM that regulates a host of cellular processes, just like protein phosphorylation or ubiquitination.

Keywords: Protein post-translational modification (PTM); Protein *S*-palmitoylation; DHHC-PATs; Palmitic acid; Cancer; Neurodegenerative disorders; Bioorthogonal labeling; Trafficking.

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1. Introduction

Lipids are essential cellular components that structure the cell membranes and provide them the barriers and boundaries needed for the cells to survive and proliferate. In addition to that, lipids can also covalently modify the proteins known as 'protein lipidation' and regulates many aspects of physiology such as protein-protein interactions, protein-membrane interactions, protein stability, membrane trafficking (Chamberlain and Shipston, 2015; Greaves and Chamberlain, 2011; Hancock et al., 1989; Jiang et al., 2018; Linder and Deschenes, 2007; Salaun et al., 2010; Yeste-Velasco et al., 2015). Protein lipidation is a broad term that encompass any post-translational modification (PTM) which results from a covalent attachment of lipid molecules to protein such as *S*-farensylation, N^{ε}-lysine myristoylation, *O*-palmitoleylation, *N*-terminal myristoylation, and *S*-palmitoylation etc. An overview of common protein lipidations are highlighted in Table 1.

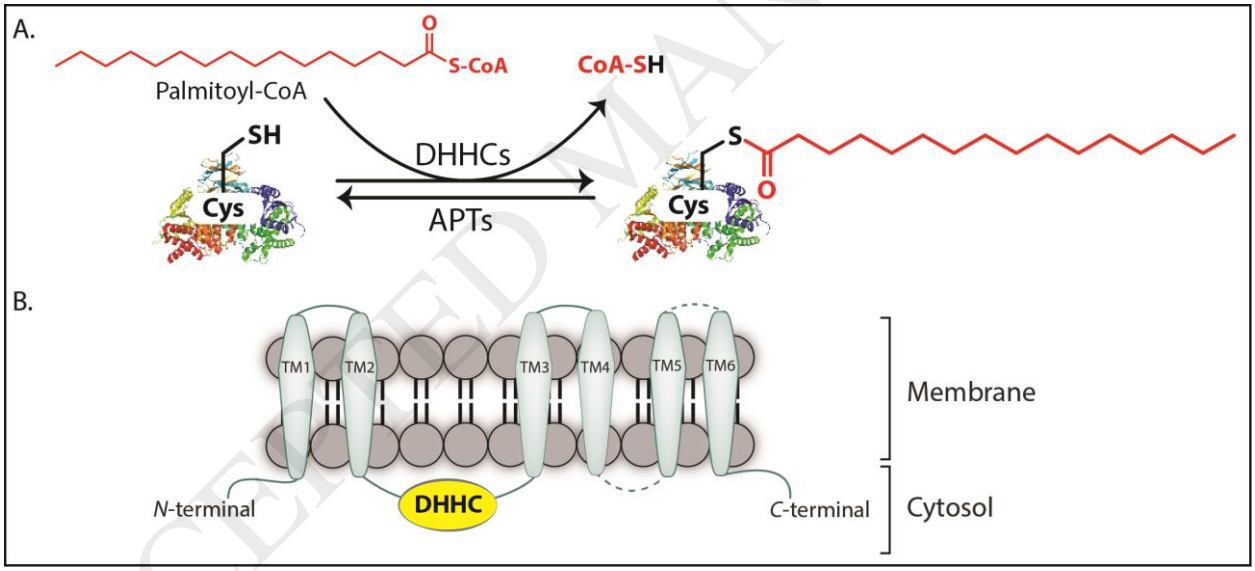


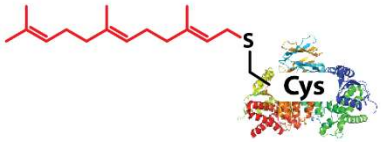
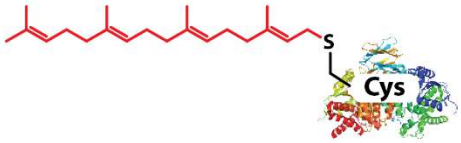
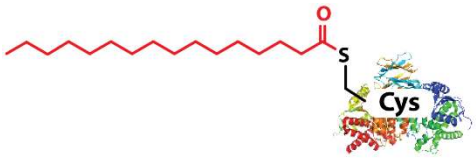
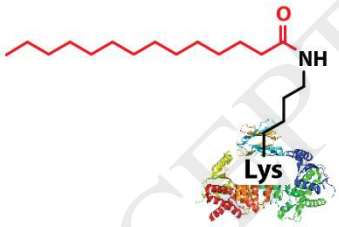
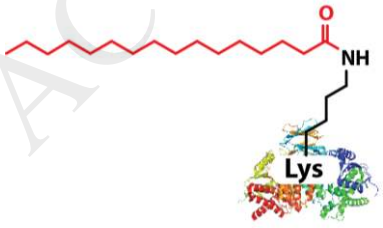
Fig. 1. (A) Protein *S*-palmitoylation occurs on the thiol group of the Cys sidechain and mediated by DHHCs. The palmitoyl group is transferred from palmitoyl-CoA. Acylprotein thioesterases (APTs) can hydrolyze palmitoyl group from the *S*-palmitoylated proteins. (B) A cartoon representation of DHHCs. Humans encode 23 DHHC isoforms and they share the same overall membrane topology, with four to six transmembrane (TM) domains. The *N*- and *C*-terminals are present in the cytosol along with the DHHC catalytic domain.

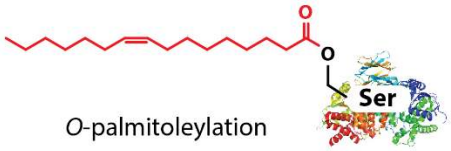
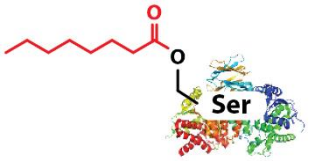
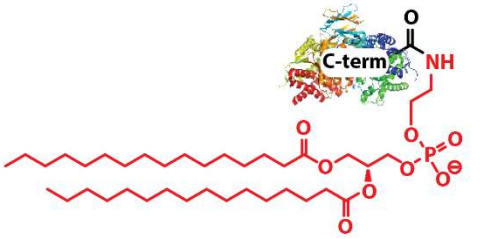
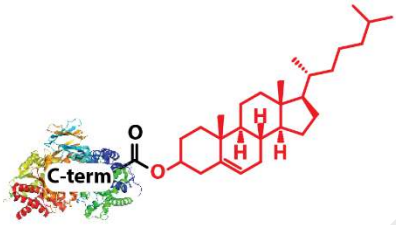
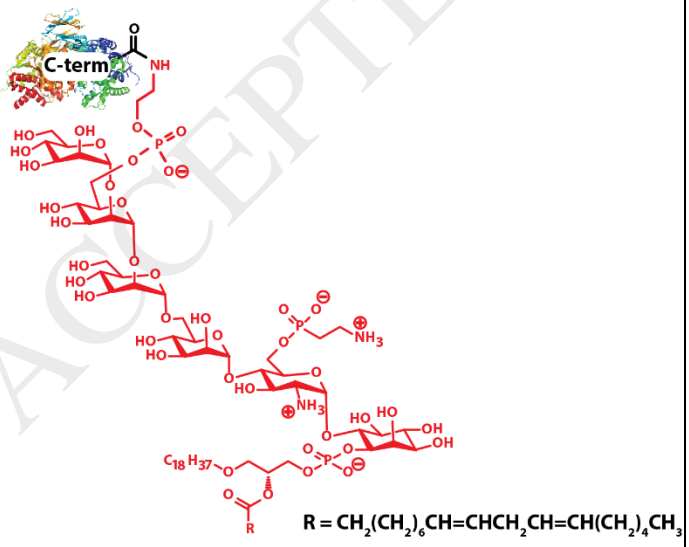
In this review, we will be mainly focusing on the protein *S*-palmitoylation which is unique among various protein lipidations in that it is reversible (Resh, 2006a; Smotrys and Linder, 2004). Protein *S*-palmitoylation is a reversible PTM wherein palmitic acid, a 16-carbon long saturated

fatty acid gets covalently attached onto proteins at cysteine residues *via* a labile thioester bond (Fig. 1A) (Linder and Deschenes, 2007; Resh, 2006b). Although protein *S*-palmitoylation was first discovered back in early 1970s (Braun and Radin, 1969; Schlesinger et al., 1980; Schmidt et al., 1979; Stoffyn and Folch-Pi, 1971), the identification of the enzymes that govern this PTM was only accomplished in 2002 (Lobo et al., 2002; Roth et al., 2002). Protein *S*-palmitoylation is a highly conserved PTM found in all the eukaryotes and plays critical roles in regulating protein stability, interaction with effector proteins, subcellular localization, enzymatic activity, membrane trafficking and many other aspects of the cellular processes (Chamberlain and Shipston, 2015; Greaves and Chamberlain, 2011; Hancock et al., 1989; Linder and Deschenes, 2007; Salaun et al., 2010; Yeste-Velasco et al., 2015). A mounting number of human diseases is associated with a dysregulated protein *S*-palmitoylation starting from neurological disorders to several cancer types (Chavda et al., 2014; Fukata and Fukata, 2010; Greaves and Chamberlain, 2014; Linder and Deschenes, 2007; Yeste-Velasco et al., 2015). *'SwissPalm'* database indicates more than 10% of the entire human proteome is susceptible to protein *S*-palmitoylation (Blanc et al., 2015) and among that over 600 substrates have already been identified and characterized experimentally (Dowal et al., 2011; Kang et al., 2008; Martin et al., 2012).

In the protein lipidation fraternity, prenylation and myristoylation are the other two well-studied PTMs that often precede *S*-palmitoylation at adjacent cysteine residues. The distinct feature that makes *S*-palmitoylation unique is protein prenylation and myristoylation are essentially irreversible, while *S*-palmitoylation is a tightly regulated reversible PTM (Resh, 2006a; Smotrys and Linder, 2004). Protein *S*-palmitoylation is catalyzed by protein palmitoyl acyltransferases (PATs, also known as DHHCs), while acyl protein thioesterases (APTs) take care of the depalmitoylation process (Fig. 1). Despite being such an important PTM, limited substrates of *S*-palmitoylation have been identified and characterized so far and hence, the biomedical importance of *S*-palmitoylation is largely undervalued with respect to their association with human disease and therapeutic potentials. In that context, it is worth mentioning that palmitate group are also found to be attached to proteins by an amide bond via ε-amino group of lysine and can be reversed by selected members of NAD^+-dependent Sirtuins family of enzymes (see Table 1). Physiological importance of protein N^{ε}-palmitoylations have also been demonstrated (Jiang et al., 2013; Jing et al., 2017; Zhang et al., 2017). Nevertheless, protein *S*-palmitoylation has been the far most prevalent palmitoylation occurring in the physiological context.

Table 1: Physiologically relevant protein lipidations and their enzymology

Protein lipidations	Reversibility	Enzymes involved
 *S*-farnesylation	Irreversible	Farnesyltransferase (FTase) (Gomez et al., 1993)
 *S*-geranylgeranylation	Irreversible	Geranylgeranyl transferase I (GGTase I) (Lane and Beese, 2006)
 *S*-palmitoylation	Reversible	DHHC-PATs and APTs *(discussed in the current review)*
 *N*-terminal myristoylation	Irreversible	*N*-myristoyltransferase (NMT) (Wright et al., 2010)
 N^{ε}-myristoylation	Irreversible	Although enzymes that catalyze this PTM are not known yet, several Sirtuins are reported to reverse this PTM (Feldman et al., 2013; Jiang et al., 2013)
 N^{ε}-palmitoylation	Irreversible	Although enzymes that catalyze this PTM are not known yet, several Sirtuins are known to reverse this PTM (Feldman et al., 2013; Jiang et al., 2013)

 *O*-palmitoleylation	Irreversible	Protein-serine *O*-palmitoleoyltransferase (Gao and Hannoush, 2013; Zheng et al., 2016)
 *O*-octanoylation	Irreversible	Ghrelin *O*-acyltransferase (GOAT) (Lemarié et al., 2016)
 *C*-terminal phosphatidylethanolaminylation	Irreversible	Phosphatidylethanolamine *N*-methyltransferase (PEMT) (Xiaonan et al., 2003)
 *C*-terminal cholesterolyation	Irreversible	Carboxyl-terminal of Hedgehog (Hh) proteins during the biosynthesis of Hh (Porter et al., 1996)
 *C*-terminal glycosylphosphatidylinositolyation	Irreversible	Glycosylphosphatidylinositol transamidase (Kinoshita, 2014)

For membrane proteins, the first and foremost role of *S*-palmitoylation is to facilitate the membrane attachment (Salaun et al., 2010). Seminal work from Shahinian et al. demonstrated that single lipidation such as myristoylation or prenylation only provides a transient membrane affinity and a second adjacent lipidation, often *S*-palmitoylation, is essential for a stable membrane-protein interaction (Shahinian and Silvius, 1995). For example, mutation of the palmitoylated Cys in Ras proteins result in weaker membrane attachment while mutation on the farnesylated Cys leads to complete loss of membrane association as farensylation is believed to mediate the second lipidation, *i.e. S*-palmitoylation (Hancock et al., 1989). *S*-palmitoylation has also been shown to protect proteins against premature degradation by blocking ubiquitination (Abrami et al., 2006).

Protein *S*-palmitoylation has been found to be more enigmatic than other lipidations due to the poor sensitivity of existing analytical techniques, lack of a defined motif around the site of modification etc. Nevertheless, recent breakthroughs have identified the family of enzymes that catalyzes the cysteine palmitoylation and that opens a whole new avenue to investigate *S*-palmitoylation. This review is aimed to provide a complete picture on the progress of the protein *S*-palmitoylation field with respect to its functional consequences and implications in human diseases including neurological disorders and cancer. We will start with a brief discussion with the enzymes involved in the process of *S*-palmitoylation and depalmitoylation and then highlight the phenotypes of various PATs and finally talking about the potential druggability of DHHCs by modulating either protein-protein interactions or catalytic activity or both. We will also talk about the budding list of queries that need to be addressed to better understand the significance of DHHCs and protein *S*-palmitoylation in normal physiology as well as pathophysiology.

2. Key players involved in protein *S*-palmitoylation and depalmitoylation process

2.1 Protein palmitoyl acyltransferases or PATs

Protein *S*-palmitoylation is mediated by a family of cysteine rich zinc finger protein acyltransferases that are defined by a conserved Asp-His-His-Cys (DHHC) catalytic domain (Mitchell et al., 2006) (Fig. 1B). The human genome encodes 23 DHHC proteins (Mitchell et al., 2006). Although they are mainly localized to endomembrane compartments such as Golgi, endosomes, endoplasmic reticulum, also found on the plasma membrane (Ohno et al., 2006). The existence of multiple DHHC isoforms strongly advocates differences in substrate specificity and activity (Table 2 and Table 3) which are not fully understood yet. Emerging evidences have shown

that the DHHCs play instrumental role in normal physiology as well as in disease states including schizophrenia, Huntington's disease, and cancer (Greaves and Chamberlain, 2011; Yeste-Velasco et al., 2015). DHHCs exert their catalytic activity via a two-step mechanism. First, they get auto-acylated to form transient acyl-intermediate. Then, the fatty-acyl group is transferred to the DHHC-substrate proteins (Mitchell et al., 2010) (Fig. 2). While almost entire family of the DHHCs are implicated in human diseases, comprehensive substrate identification for each isoform is yet to be uncovered. Although different chemical probes have been reported to detect *S*-palmitoylated proteins (Peng and Hang, 2015; Zheng et al., 2013), much need to be done to get a good grip on the overall understanding of DHHCs and their respective substrates.

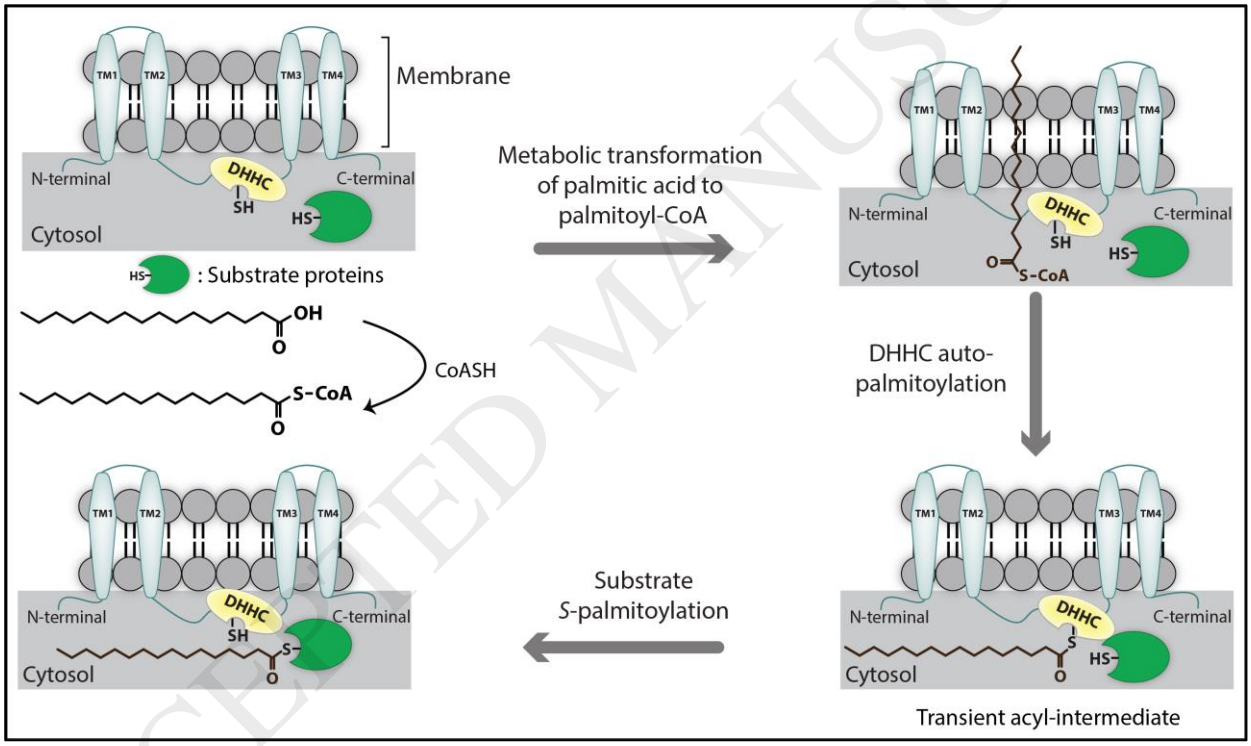


Fig. 2. Mechanism of two-step DHHC-mediated protein *S*-palmitoylation (substrate protein is shown in green).

S-Palmitoylation is a highly conserved PTM found in all eukaryotes and regulated by a DHCC family of enzymes conserved all the way from yeast to humans. While the yeast *Schizosaccharomyces pombe* carries five DHHC isoforms, humans have twenty-three of them designated as DHHC1 to DHHC24 skipping DHHC10. Although the very first *S*-palmitoylated protein was identified as early as in early 1970s (Braun and Radin, 1969; Stoffyn and Folch-Pi, 1971), how this modification is mechanistically installed on the proteins were only revealed in

2002 independently by Deschenes group (Lobo et al., 2002) and Davis group (Roth et al., 2002). The major conclusion from their studies was *S*-palmitoylation is an enzymatic event catalyzed by an evolutionarily conserved family of protein acyltransferases. They found Erf2 and Ark1 as acyltransferase for Ras2 and Yck2 respectively. Strikingly, both Erf2 and Ark1 exhibit homology in a single domain, an Asp-His-His-Cys (DHHC)-cysteine rich domain (CRD) indicating a potential role of DHHC-CRD in the palmitoyl acyltransferase activity (Lobo et al., 2002; Roth et al., 2002). That interesting finding undoubtedly was the key observation which later shaped the whole protein *S*-palmitoylation field.

Later in 2004, Luscher and co-workers characterized the first mammalian palmitoyl acyltransferase (Keller et al., 2004). They showed that GODZ (also known as DHHC3), a Golgi-specific protein with the DHHC zinc finger domain can increase *S*-palmitoylation of γ2 subunit of $GABA_A$ receptors upon co-overexpressing the two. Soon after that, Fukata *et. al.* identified 23 DHHC isoforms in human and mouse based on the homology to the DHHC motif found in GODZ (Fukata et al., 2004). All the DHHC family members possess variable extent of palmitoyl acyltransferases activity. They comprise of four to six transmembrane (TM) domains (Fig. 1B). The catalytic DHHC domain is located in the cytosolic part in between the second and third TM. The greatest sequence diversity is observed at the *C*- and *N*-terminal cytoplasmic tails. These variable domains are believed to mediate the protein-protein interaction to bring the substrates closer and thus, facilitate the acyl-transfer process. However, the lack of any crystal structure of DHHC isoforms were the main barrier towards our knowledge on their topology until very recently, Rana et al. solved the crystal structures of human DHHC20, covalent intermediate mimic of DHHC20, and zebrafish DHHC15 (Rana et al., 2018). Although DHHCs were initially assumed to catalyze the addition of palmitoyl group on cysteines, a recent study showed that DHHCs display marked differences in the selection of acyl groups (Greaves et al., 2017). However, palmitate is the major fatty acid incorporated into endogenous proteins in an *S*-acylation process. Among other fatty acids, stearate and oleate are also found in the *S*-acylated proteins but in much lesser extent compared to palmitate (Liang et al., 2001).

As mentioned before, there exists 23 isoforms of DHHC enzyme family in humans and many of them show prominent phenotypes and thus, clearly depict the importance of individual members. A number of human diseases have been correlated with various DHHC isoforms. A

detailed account of each human DHHCs will be discussed in the forthcoming section with respect to their involvement in the physiological and pathophysiological contexts. Being a reversible PTM, protein *S*-palmitoylation can be drugged with small molecules. That can be achieved by modulating either their catalytic activities or by protein-protein interactions or both. But, before one can attempt to exploit the druggability of *S*-palmitoylation to cure diseases, we need to identify and characterize the substrates of DHHC enzymes. Limited number of DHHC-substrates are known so far and that is mainly because of the redundancies among DHHC isoforms, lack of antibodies against *S*-palmitoylation or endogenous DHHCs. Key interacting partners for DHHCs also need to be explored to elucidate their actual cellular functions.

2.2 Acylprotein thioesterases or APTs

The hydrolysis of protein *S*-palmitoylation is also of great interest and is facilitated by a class of acylprotein thioesterases (APTs). The enzymatic palmitoylation and depalmitoylation ensure an efficient dynamic and balanced *S*-palmitoylation cycle. Compared to the large number of palmitoylating enzymes *i.e.* 23 DHHC isoforms, there are mainly three depalmitoylating enzymes namely acyl protein thioesterase 1 (APT1), acyl protein thioesterase 2 (APT2) and acyl protein thioesterase-like 1 (APTL1). These depalmitoylating enzymes are mostly cytoplasmic but can also be translocated onto the membrane upon lipid modifications. In 1993, Camp et al. (Camp and Hofmann, 1993; Muszbek et al., 1999) purified the first depalmitoylase, named as palmitoyl protein thioesterase or PPT1 that can hydrolyze the palmitoyl group from *S*-palmitoylated H-Ras (in native conformation) and α subunits of the heterotrimeric G proteins. But, further studies found that PPT1 is exclusively found in lysosomes and hence, very unlikely to depalmitoylate the cytoplasmic and membrane bound substrates.

Subsequent studies revealed APT1 as the major depalmitoylating enzyme which was originally identified as a lysophospholipase (then known as LYPLA1) but later found to possess a stronger depalmitoylation activity (Duncan and Gilman, 1998) and hence renamed as acyl protein thioesterase 1 or APT1. Apart from its depalmitoylation activity, APT1 can also hydrolyze many other long chain mono-acyl glycerol esters and lysophospholipids but at a much weaker catalytic efficiency. APT1 can get palmitoylated and depalmitoylate itself (Kong et al., 2013). Hence, a regulatory mechanism is in action maintaining the spatial organization and providing access to its substrates. Several physiologically important proteins including G-proteins (Duncan and Gilman,

1998), and H-Ras (Duncan and Gilman, 1998) have been found to be substrates of APT1. Despite the importance of APT1, identification of its substrates has rather been quite slow due to the absence of a well-defined motif around the sites of *S*-palmitoylation.

There exists another depalmitoylase known as APT2, a highly identical analogue of APT1. APT2 is also a potent depalmitoylase (Hedberg et al., 2011) like APT1 and plays critical role in regulating the trafficking of peripheral membrane proteins via *S*-palmitoylation/depalmitoylation mechanism. Growth associated protein-43 (GAP-43) (Kong et al., 2013; Tomatis et al., 2010) has been the very first substrate of APT2, identified during the search for other APTs. Both APT1 and APT2 are ubiquitous across all tissue types (Abrami et al., 2017; Bachovchin et al., 2010). Due to the high similarity (more than 80%) between APT1 and APT2, their substrate specificity is yet to understand clearly and warrant further investigation. Given the high pharmacological value of APTs, researcher have made significant efforts to develop potent inhibitors (Fig. 3) and more importantly, isoform specific inhibitors (Won et al., 2017).

The similar activity of both these enzymes raises a speculation of the need for two isoforms in the cell. Further, these enzymes share several common features such as both the isoforms undergo *S*-palmitoylation at *N*-terminal Cys2 (Kong et al., 2013). But results from experimental data are convincing enough for the presence of a degree of substrate specificity of these enzyme isoforms. The *S*-palmitoylation reversibility of β2-adrenergic receptors is carried out only by APT1 (Adachi et al., 2016) whereas depalmitoylation of DHHC6 is carried out by APT2 (Abrami et al., 2017). Despite of possessing a similar degree of homology between the two isoforms, their accessibility to the substrate pool greatly differs owing to their distinct and unique identity determining regions. The thermodynamic stability observed in presence of APT inhibitors was found to be increased by 4 ˚C in case of both the isoforms (Won et al., 2017). Recent study imparted a substantial knowledge into the crystal structure of APT1 in presence of inhibitor, but the same for APT2 was still lagging until Won et al. solved the structures of both APT1 and APT2 in presence of inhibitors ML348 and ML349 (Fig. 3), respectively (Won et al., 2017). The study highlighted the presence of a β4-β5 sheet and β5-α2 loop. This loop is particularly important as it houses the catalytic triad. Crystal structure comparison of both the isoforms revealed a difference in the chain length of this loop, which may contribute to the substrate specificity by limiting the passage of substrate through the catalytic channel beyond a permissible diameter. This is an important clue for the design of more

efficient, next generation isoform specific inhibitors. A substantial issue associated with the present-day inhibitors is its lack of specificity towards specific isoforms of APTs and hence induces a broad spectrum of inhibition of depalmitoylation. Waldmann and co-workers developed novel small molecule APT1 inhibitors, Palmostatin B and Palmostatin M (Fig. 3), that inhibit Ras depalmitoylation and reverts oncogenic phenotype (Dekker et al., 2010; Hedberg et al., 2011). Although the selectivity of Palmostatin B for APT1 was high compared to the other cellular phospholipases, none of the inhibitors could display any isoform-specificity between APT1 and APT2. Nevertheless, Palmostatin B has gained significant attention as an important anticancer compound for treating melanomagenesis (Chen et al., 2017b).

Recently, Tian et al. identified another depalmitoylase, APTL1 which is a splice variant and homolog of APT1 with around 30% identity (Tian et al., 2012). The large conductance calcium- and voltage-activated potassium (BK) channel has been found to be a substrate of APTL1 along with APT1. The crystal structure of APTL1 reveals a quite similar structure to APT1 with a conserved catalytic site but smaller in size (Bürger et al., 2012). Therefore, the substrate scopes might be a rather limitation for APTL1. Nevertheless, the discovery of APTs opens a whole new avenue to rethink about the substrate specificities or redundancies in APTs.

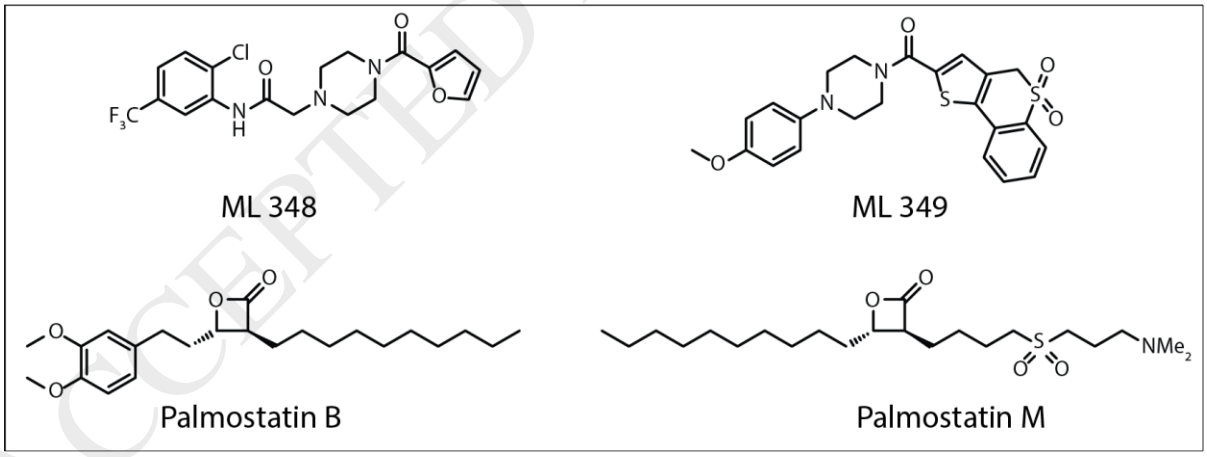


Fig. 3. Chemical structure of reported APT inhibitors.

But the validity of the APTs as a sole depalmitoylase family of thioesterases was in speculation when some striking evidences surfaced regarding the retention of depalmitoylation activities even upon knockdown of APTs. For example, efficient knockdown of APT1/APT2 did not impair the depalmitoylation of R7 regulator of G protein signaling-binding protein (R7BP). But, the treatment

with protein depalmitoylation inhibitors, palmostatin B and hexadecyl fluorophosphonate inhibited R7BP depalmitoylation (Jia et al., 2014). This observation was key in the discovery of α/β hydrolase domain 17 (ABHD17) family of thioesterases as potent depalmitoylating enzymes and Palmostatin B owns the credit owing to its broad spectrum depalmitoylation inhibition activities. Later, ABHD17 was identified as depalmitoylase for N-Ras (Lin and Conibear, 2015) and PSD-95 (Yokoi et al., 2016). ABHD17 hydrolases are found across all the vertebrates and act as functional depalmitoylases.

3. Structural features of DHHCs

A major bottleneck to fully understand the mechanistic aspects of DHHC-mediated *S*-palmitoylation and its implications to the regulation of the function of cellular proteins was the lack of any crystal structure of DHHCs. There were no structural information on the DHHC family of proteins until very recently Rana et al. reported the crystal structure of DHHC20 for the very first time (Rana et al., 2018). The pioneer work sheds light on the mechanism of action for the DHHC family of proteins. According to their proposed structure, the four TM domains of DHHC20 maintains a teppe like organization in the membrane which allows two TM domains to remain closely associated thus creating a certain distance between the other two TM domains. This geometry is important for substrate engagement and catalysis. The structure also proposes that a His^{155} sidechain in the catalytic domain may assist the catalytic Cys for nucleophilic attack. The Asp and first His of DHHC motif may play role in activating the nucleophilic Cys. In the *C*-terminal domain, a conserved TTXE (Thr-Thr-X-Glu) motif makes the intimacy with the DHHC domain and the mutation of TTXE to AAXE (Ala-Ala-X-Glu) results in highly compromised enzymatic activity. As far as the acyl chain length selectivity is concerned, the same has been solved with sufficient clarity. The binding grove of DHHC20 houses a hydrophobic cavity which is constricted by Trp^{158} and Phe^{171}, towards the acyl headgroup. Two other residues, Phe^{174} and Leu^{227} on the membrane, stabilize the acyl chain. Crystal structure of the acyl-intermediate complex formed by DHHC20 and 2-bromopalmitic acid (2-BP) offered invaluable insight on the chain length selectivity of DHHC20. At the tapering end of the acyl binding pocket in the 2-BP-DHHC20 complex, Tyr^{181} was engaged with Ser^{29} by hydrogen bonding interaction eventually closing off the cavity. Thus, the preference of DHHC20 for palmitoyl chain gets switched to

stearoyl-CoA upon mutating Tyr^{181} to Ala (a less bulky amino acid) and altering Ser^{29} to Phe (a bulkier amino acid) shifts DHHC20's preference towards short chain acyl-CoAs.

4. Acyl-chain selectivity of DHHCs

Often *S*-acylation is inappropriately used in the literature as a synonym of *S*-palmitoylation. Protein *S*-acylation is a generic term used to imply DHHC-mediated PTMs on protein Cys sidechains but how and why a given DHHC prefers some acyl chain over another is not completely understood. Acyl groups can vary by their chain length as well as from structural aspects. Many questions abound. Why some DHHCs chose a longer acyl chain? How does the acyl-CoA dynamics control the protein *S*-acylation process? Can we modulate the protein *S*-acylation by simply regulating the diet? Palmitoyl (C16:0) group is just one of the many acyl groups that can be used by DHHCs to modify the proteins at their Cys sidechains. Nevertheless, it is worth mentioning that palmitic acid is present in considerable amount inside the cell and that rightly reflects in the protein *S*-acylation landscape as highlighted by Muszbek et al. identifying more than 70% of the *S*-acylated protein as the *S*-palmitoylated protein in platelet (Muszbek et al., 1999).

In a very diligent study, Muszbek et al. (Muszbek et al., 1999) tried to quantitate the major fatty acids which are covalently bound to proteins Cys residues by thioester linkage in unactivated platelet and they found that from unactivated platelet *S*-acylated proteins 74% of the acyl group were from palmitic acid (C16:0), 22% from stearic acid (C18:0), and oleic acid (C18:1) accounted for only 4%. They also showed that the exogenously added fatty acids can significantly alter protein *S*-acylation dynamics. Other long-chain acyl groups such as palmitoleic acid (C16:1), myristic acid (C14:0), linoleic acid (C18:2), and arachidonic acid (C20:4) have also been found to be incorporated onto proteins via *S*-acylation (Hallak et al., 1994; O'Brien and Zatz, 1984; Veit et al., 1996). While many acyl groups have been explored for protein *S*-acylation, the DHHC isoform(s) that can catalyze the acylation process is/are largely unknown. Different acyl groups on *S*-acylated proteins will have varied membrane affinity and hence, understanding the specificity of DHHC members towards different fatty acids are of immense importance.

In 2012, Jennings et al. for the first time showed that the DHHCs have fatty-acyl-CoA specificity and that governs the dynamics of protein *S*-acylation (Jennings and Linder, 2012). That study was done *in vitro* with purified recombinant DHHC proteins, but a much more desired study would have been to look at the *S*-acylation dynamics inside the cell. A very recent study from

Chamberlain group explored that looking at the differences in the acyl group selectivity of 23 human DHHCs enzymes via an bioorthogonal approach and shed light on its molecular mechanism (Greaves et al., 2017). One of the key facts that came out of their studies is that the fatty acyl-CoA selectivity in DHHC autoacylation step provides a strong correlation to respective substrate *S*-acylation. It was observed that acyl-CoA specificities do not originate from the subcellular localization of DHHC isoforms. For example, DHHC 3, 7 and 23 all are exclusively localized in the Golgi but while DHHC3 and 7 prefer myristic acid (C14:0), and palmitic acid (C16:0) over stearic acid (C18:0), DHHC 23 shows high selectivity towards stearic acid (C18:0). The authors have also found that in some cases a key amino acid in the DHHC protein can be the determinant for the preferred acyl-CoA for example, Ile^{182} in DHHC3 is the critical determinant for limiting the use of C18:0. This fact is further substantiated with the recently published crystal structure of DHHC20 (Rana et al., 2018) as discussed in the earlier section. Rana et al. highlighted the importance of Trp^{158} and Phe^{171} residues in the cavity of DHHC20, serving as a decisive factor for the acyl headgroup of the substrate acyl-CoA. Additionally, they also found that Phe^{174} and Leu^{227} interact with the acyl chain keeping a check on the acyl chain length preference. The selectivity mechanism was further validated by the respective mutants (Rana et al., 2018).

5. Physiological and pathophysiological functions of DHHC isoforms

The mammalian genome encodes 23 DHHC isoforms and their genomic location varies significantly (see Table 1). Almost all the DHHC family members have been correlated with human diseases spanning from neurological disorders to many times of cancer. In this section, we aim to provide a detailed account of the physiological and pathophysiological implications of various DHHC isoforms and their respective substrates (see Table 2 and 3).

5.1 DHHC1

DHHC1 is mainly localized in the Endoplasmic Reticulum (ER) and has high expression in fetal lung, kidney and heart while adult pancreas and lung exhibit lower amount of DHHC1. Though a direct evidence for its physiological function in still unclear, several lines of experimental data had suggested its involvement in many cancer types including prostate cancer (Lin et al., 2013; Sigala et al., 2008). DHHC1 has been predicted to interact with miR-93 which actually suppresses proliferation of human colon cancer stem cells (Yu et al., 2011b) suggesting its potential role in colon cancer as well. Recent studies from Zhou et al. showed that DHHC1 is a

positive regulator of MITA/STING-dependent innate immune responses against DNA viruses (Zhou et al., 2014). DHHC1 has also been reported to be associated with cardiometabolic traits among African population (Ng et al., 2017). Oku and coworkers showed that the *S*-palmitoylation of Neurochondrin (NCDN) is enhanced by DHHC1 and important for the specific endosomal targeting of NCDN (Oku et al., 2013).

5.2 DHHC2

Though ubiquitously expressed, many cancer types including colorectal cancer with liver metastasis, ovarian cancer lymph node metastasis, hepatocellular carcinoma are strongly associated with reduced expression of DHHC2, hence it is often accepted as a potential tumor suppressor (Oyama et al., 2000; Peng et al., 2014; Pils et al., 2005; Planey et al., 2009; Yan et al., 2013). A high level DHHC2 is associated with better survival rates and it further supports its tumor suppressor role (Li et al., 2014). Jiang et al. reported that the lower expression level of DHHC2 is associated with metastasis in case of nasopharyngeal carcinoma (Jiang et al., 2015). Thus, DHHC alone or with other tumorigenic proteins warrant to serve as useful biomarkers for various cancer types. Apart from its role in tumorigenesis, DHHC2 also *S*-palmitoylates tetraspanins CD9 and CD151 and results a physical interaction between them. DHHC2-mediated *S*-palmitoylation of tetraspanins CD9 and CD151 also stabilizes the proteins by protecting them from lysosomal degradation in HEK 293 cells and promotes cell-cell contacts (Sharma et al., 2008). Cytoskeleton associated protein 4 (CKAP) is another well-characterized substrate of DHHC2 and *S*-palmitoylation of CKAP is essential for its proper trafficking to the plasma membrane. DHHC2 mediated *S*-palmitoylation of CKAP also contributes in reversing cancer driven phenotypes.

5.3 DHHC3

DHHC3 is probably one of the well-studied PATs involved in diverse array of cellular physiology. It plays pivotal role in *S*-palmitoylating integrin (ITG) α6 and β4 and this lipidation is important for their proper functioning, expression and stability. Given the well-established role of *S*-palmitoylated ITG α6β4 in cancer cell progression, metastasis and angiogenesis, DHHC3 can be a potential pharmacological target (Sharma et al., 2012). The *S*-palmitoylation is also shown to be essential for the lipid raft localization and signaling activity of ITG β4. Coleman et al. have shown that the *S*-palmitoylation of ITG β4 is significantly inhibited upon curcumin treatment, which may pave a path for targeting DHHCs with phytochemicals as a potential therapeutic (Coleman et al.,

2015). Recently, DHHC3 is reported to mediate *S*-palmitoylation of Endoplasmic Reticulum-Golgi Intermediate Compartment Protein 3 (ERGIC3) which is responsible for breast cancer growth by regulating cellular oxidative stress and senescence (Sharma et al., 2017). They further showed that the DHHC3 knockout in MDA-MB-231 mammary tumor cell xenografts exhibit a decrease in size of primary tumor and metastatic lung colonies.

DHHC3 is also known to *S*-palmitoylate different neuronal proteins including $GABA_A$ receptors and D2 Dopamine Receptor. DHHC3-mediated *S*-palmitoylation of $GABA_A$ receptors represents a novel PTM that is critical for the normal functioning of GABAergic inhibitory synapses (Fang et al., 2006). DHHC3 can also *S*-palmitoylate D2 Dopamine Receptor, a G protein-coupled receptor which gets activated upon this PTM and inhibits adenylate cyclase by coupling to G proteins (Ebersole et al., 2015). Cysteine String Protein (CSP) is a member of the DnaJ/Hsp40 family of chaperones localized on neuronal synaptic vesicles. DHHC3 mediates the *S*-palmitoylation of CSP which further exerts neuroprotective function via stable membrane attachment (Greaves et al., 2008).

Another significant DHHC3 substrate is neural cell adhesion molecule, which is a vital component for cell-cell adhesion, neurite outgrowth, synaptic plasticity and in that context, the enzymatic activity of DHHC3 is tightly regulated through tyrosine phosphorylation mechanism mediated by FGF Receptor and Src kinase (Lievens et al., 2016). DHHC3 has also been linked to Retinitis Pigmentosa via *S*-palmitoylation of progressive rod-cone degeneration (PRCD) and without the lipidation, PRCD is mislocalized (Murphy and Kolandaivelu, 2016). DHHC 3, along with DHHC7, has been shown to increase *S*-palmitoylation of phosphatidylinositol 4-kinase IIα (PI4KIIα), responsible for generating a major part of phosphatidylinositol-4-phosphate (PI4P) in the Golgi (Lu et al., 2012). DHHC3 also regulates Golgi integrity via *S*-palmitoylating PI4KIIα and PI4P generation (Lu et al., 2012). The Herpes Simplex Virus 1 (HSV-1) replication in murine embryonic fibroblasts requires a concerted assistance from UL20 with proper targeting and localization. DHHC3 interacts with UL20 in Golgi and helps in its proper trafficking which is critical to carry out the function of UL20 to reduce the infectivity of HSV-1 (Wang et al., 2017a; Wang et al., 2017b). Thus, DHHC3 plays a positive role in abrogating viral replication in host cells.

5.4 DHHC4

DHHC4 is one of the least studied PATs in the DHHC family of enzymes and mainly localized in Endoplasmic Reticulum. With the very few literatures, its physiological role and functions are of debate. Ebersole at al. showed that DHHC4 interacts with and *S*-palmitoylate Dopamine 2 Receptor (D2R) and that S-palmitoylation is important for the stability of D2R (Ebersole et al., 2015). Recently, Hasan et al. reported that DHHC4 is overexpressed in case of experimental autoimmune encephalitis (Hasan et al., 2017). DHHC4 can palmitoylate β-site amyloid precursor protein cleaving enzyme 1 or BACE1 which is a key player that initiates amyloidogenic processing of amyloid precursor protein and involved in Alzheimer disease (Vetrivel et al., 2009). Identification of additional DHHC4-targets will provide us a clearer picture of its role in physiological context.

5.5 DHHC5

DHHC5 is the first PAT identified for a G-protein coupled receptor where the interaction between somatostatin receptor 5 and *N*-terminal region of DHHC5 was studied using the Ras recruitment interaction screening system (Kokkola et al., 2011). Protein *S*-palmitoylation is crucial in maintaining synaptic plasticity which is mainly achieved by dynamic localization of proteins in the synapse. For example, under basal conditions DHHC5 forms a complex with PSD-95 and Fyn kinase at the synaptic membrane. This complex is stabilized via phosphorylation of Tyr533 residue of DHHC5 within the endocytic motif and therefore, inhibiting the DHHC5 endocytosis. Upon neuronal activity, the complex gets dissociated followed by its endocytosis and translocation to dendritic shaft where it interacts and *S*-palmitoylates δ-catenin, a DHHC5 substrate. These series of cellular events highlight an important PTM crosstalk between phosphorylation and *S*-palmitoylation (Brigidi et al., 2015). DHHC5 also *S*-palmitoylates flotillin-2 (Li et al., 2012) which tethers the growth factor receptors involved in signal transduction pathway (Solomon et al., 2002). DHHC5 undergoes clathrin mediated sorting due to the presence of four tyrosine YXXΦ motifs in the *C*-terminal which also helps it to get in intracellular tubular and vesicular structures apart from extensive localization in plasma membrane (Breusegem and Seaman, 2014). Recently Chen et al. reported an interesting evidence that mutant p53 can transcriptionally up-regulate DHHC5 along with nuclear transcription factor NF-Ƴ, which highlights the importance of targeting DHHC5 in abrogating the mutant p53 mediated tumorigenic signaling cascade (Chen et al., 2017c). It is

important to note that DHHC5 is also associated with schizophrenia phenotype along with DHHC18 (Zhao et al., 2018).

5.6 DHHC6

Like most of the other DHHCs, DHHC6 is mainly localized in Endoplasmic Reticulum (ER). DHHC6 *S*-palmitoylates Calnexin, located in ER, that acts as a chaperone is assisting protein folding by interacting with ribosome translocon complex (Lakkaraju et al., 2012). Selenoprotein K (SELK) and DHHC6 both being in the ER form a complex which is critical for stable expression and palmitoylation of inositol 1,4,5-triphosphate receptor (Fredericks et al., 2017; Fredericks et al., 2014). Recently Abrami et al. reported, for the first time, the existence of a palmitoylation cascade where autopalmitoylation of DHHC6 is mediated by upstream DHHC16 (Abrami et al., 2017). Thus, it highlights the importance of the dynamics of the palmitoylated species and their physiological importance and that too governed by the DHHCs itself.

5.7 DHHC7

DHHC7 is known to *S*-palmitoylate many sex steroids receptors such as estrogen, progesterone and androgen receptors for their efficient localization on the plasma membrane (Pedram et al., 2012). DHHC7 helps in sequestering F508 deleted mutant Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) which accounts for more than 90% of clinical Cystic Fibrosis in the Western countries (McClure et al., 2014). The death receptor Fas ligand present on membrane is important for apoptosis induction and is extensively post-translationally modified including *S*-palmitoylation. The stability of Fas is achieved by DHHC-mediated *S*-palmitoylation via protecting it from lysosomal proteolysis (Rossin et al., 2015). DHHC7 is also associated with cancer through scribble (SCRIB) which is a well-established tumor suppressor protein playing an important role in establishing and maintaining epithelial cell polarity. DHHC7 also *S*-palmitoylates SCRIB and the lipidated SCRIB is believed to be the major reason behind its polarity (Chen et al., 2016). Recently Aramsangtienchai et al. reported that the DHHC7 mediated *S*-palmitoylation of Junctional Adhesion Molecule C (JAM-C) is important for its localization to tight junctions (Aramsangtienchai et al., 2017).

5.8 DHHC8

DHHC8 is mainly localized in Golgi but its presence in mitochondria had also been reported (Maynard et al., 2008) and hence, represents as the only DHHC-PAT that may act from mitochondria. The phenotypic role of DHHC8 upon genomic ablation is reported to have implication in Schizophrenia along with Huntington disease. The first evidence of the association of this gene with Schizophrenia was reported by Mukai et al. using a relatively dense genomic mapping of Single Nucleotide Polymorphisms (SNPs) in entire 1.5 Mb locus on chromosome 22q11 that is well known for its association with Schizophrenia (Mukai et al., 2004). But there are several conflicting reports that denies the link of DHHC8 with phenotypes of Schizophrenia in Japanese and European population (Glaser et al., 2005; Otani et al., 2005; Saito et al., 2005).

5.9 DHHC9

DHHC9 forms a complex with GCP16, a Golgi-localized membrane protein which can then *S*-palmitoylate H-Ras and N-Ras. It should be noted here that GCP16 is essential for the fatty acyltransferase activity and stability of DHHC9 (Swarthout et al., 2005). Further to that, Raymond et al. identified several mutations in DHHC9 that alter the expression levels and cellular localizations of H-Ras and N-Ras which are sufficient to cause X linked mental retardation (Raymond et al., 2007). Recently, Mitchell et al. identified two DHHC9 mutants (R148W and P150S) that affect the auto-palmitoylation step of the reaction by lowering the steady state amount of the transient acyl-intermediate of DHHC9 which in turn affects *S*-palmitoylation of target proteins (Mitchell et al., 2014). The mutation-driven dysregulation of DHHC9 PAT activity has implication in intellectual disability, epilepsy, speech and language impairment and neurodevelopment (Baker et al., 2015; Bathelt et al., 2016; Bathelt et al., 2017; Hackmann et al., 2016; Han et al., 2017). The inactivation of DHHC9 is also reported to be involved in mitigating the leukemogenic potential of oncogenic N-Ras (Liu et al., 2016). Interestingly, Meng et al. recently identified Multiple myeloma-associated antigen-1 (MMSA-1), another DHHC9 mutant which is overexpressed in myeloma cell lines as well as primary cells isolated from Multiple Myeloma patients (Meng et al., 2016). This study established MMSA-1 to be a potential independent prognostic marker in Multiple Myeloma patients.

5.10 DHHC11

The precise physiological role of DHHC11 is unclear and the pursuit is still on. Sasaki and coworkers screened the whole genome-wide copy numbers in multiple patients with bladder

cancer and discovered that a gain of 5p15.33 including the genes encoding DHHC11 and Tubulin Polymerization Promoting Protein (TPPP) can serve as a potential biomarker in bladder cancer (Yamamoto et al., 2007). On the contrary, deletion of genomic regions 5p15.33 and 16q12.2 was found to be associated with lower survival rates in case of hepatoblastoma (Wu et al., 2013). In a very recent report, Dzikiewicz-Krawczyk et al. identified a complex involving DHHC11, DHHC11B (a highly homologous variant of DHHC11), MYC, and miR-150 that promotes proliferation of Burkitt Lymphoma cells. DHHC11 and DHHC11B are involved in maintaining the oncogenic MYC-miR-150-MYB network in Burkitt lymphoma (Dzikiewicz-Krawczyk et al., 2017). In brief, the available data strongly suggest a significant role of DHHC11 in cancer, but a concise mode of action is yet to be defined.

5.11 DHHC12

DHHC12 is mainly localized in the Endoplasmic Reticulum and Golgi. The physiological role of DHHC12 is largely unknown. Mizumaru et al. showed that DHHC12 strongly inhibits the metabolism of amyloid precursor protein (APP) and amyloid-beta generation by restricting APP in the Golgi (Mizumaru et al., 2009). But it is not clear whether its protective role is due to APP being a substrate of DHHC12 as APP lacks cytoplasmic cysteine residue. DHHC12 has also been implicated in the progression of Huntington Disease resulting from dysregulated *S*-palmitoylation (Young et al., 2012a).

5.12 DHHC13

Mutation in DHHC13 has extensively been reported to be associated with alopecia, osteoporosis and systemic amyloidosis, increased susceptibility to skin carcinogenesis (Liu et al., 2015; Perez et al., 2015; Saleem et al., 2010). DHHC13 also facilitates bone mass acquisition and regulates the fate specification of ectoderm and mesoderm cell lineages (Chen et al., 2014; Song et al., 2014). It is interesting to note that while Huntingtin protein (HTT) is a substrate of DHHC13, the mutated HTT is not an as good a substrate. This reduced *S*-palmitoylation of the mutant HTT results in accelerated aggregation and increased cellular cytotoxicity (Sanders et al., 2015). Loss of DHHC13 is also shown to increase the chances of lesions and microbial attack due to compromised skin integrity and associated with abnormal liver functions, lipid abnormalities and hypermetabolism (Chen et al., 2017a; Shen et al., 2017). In a separate report, it is also shown that

S-palmitoylation of dynamin-related protein 1 or, Drp1 by DHHC13 helps in brain bioenergetics, anxiety, coordination and motor skills (Napoli et al., 2017).

5.13 DHHC14

DHHC14 is associated with several cancer types with the dual role of both oncogenic and tumor suppressor. DHHC14 is highly expressed or activated in with gastric cancer and acute biphenotypic leukemia (Anami et al., 2010; Oo et al., 2014; Yu et al., 2011a). But there are also reports that supports the importance of this DHHC family member in mitigating cancer such as the expression of DHHC14 is downregulated in case of testicular germ cell tumor and prostate cancer and its expression is also suppressed by miR-574-5p in case of coronary artery disease (Lai et al., 2017; Yeste-Velasco et al., 2014). Overexpression of DHHC14 was also found to reduce the tumor size in a xenograft model (Yeste-Velasco et al., 2014). Hence, DHHC14 can act both as a tumor suppressor and/or oncogenic protein.

5.14 DHHC15

DHHC15 also falls among the list of understudied DHHCs and the clear physiological function is unclear. In a recent study, DHHC15 has been found to be essential for neuronal differentiation in Zebrafish (Wang et al., 2015). Shedding light on the tumorigenic role, Kang et. al. observed an upregulation of DHHC15 in high grade ovarian adenocarcinoma with loss of X chromosome inactivation (Kang et al., 2015). DHHC15 has also been correlated with nonsyndromic X-linked mental retardation (Mansouri et al., 2005).

5.15 DHHC16

DHHC16 is mainly localized in Endoplasmic Reticulum and plays important role in neural stem/progenitor cells proliferation via FGF/ERK modulation (Shi et al., 2016). Protein *S*-palmitoylation is also involved in DNA damage response. Genetic (*via* DHHC16 knockout) or pharmacological inhibition of DHHC16 has been shown to compromise DNA damage-induced activation of ATM, p53 activation, cell cycle arrest in primary mouse embryonic fibroblasts (Cao et al., 2016). In addition, they also showed that DHHC16 physically interacts with c-ABL, a tyrosine kinase involved in DNA damage response (Cao et al., 2016). Overall, a clear connection is made with DHHC16 and DNA damage responses and more work need to be done to better understand the molecular mechanism of DHHC16-mediated *S*-palmitoylation in DNA damage

response. This could also explain why and how some DHHC isoforms exert tumor suppression activities via DNA damage pathway.

5.16 DHHC17

DHHC17 is also known as Huntington Interacting Protein 14 (HIP14). DHHC17 mediates dual function: palmitoyl acyl transferase activity and Mg^{2+} transport (Goytain et al., 2008). Cysteine palmitoylation plays pivotal role in trafficking and transport of Huntingtin protein. As a result, DHHC17 null mice shows phenotype of behavioral, biochemical and neuropathological defects similar to Huntington disease (Singaraja et al., 2011; Yanai et al., 2006). On a separate note, it has been shown that the reduced palmitoylation of GAD65 have an implication in Huntington disease which can be circumvented by over expressing DHHC17 (Rush et al., 2012; Young et al., 2012b). DHHC17 also possesses anti-apoptotic activity which is required for β-cell survival and insulin secretion. It has also been found that DHHC17 is expressed exclusively in insulin positive cells of islet of Langerhans (Berchtold et al., 2011). In another report, Yang et al found that DHHC17 is involved in ischemic stroke by mediating neuronal responses in acute brain injury (Yang and Cynader, 2011). ClipR-59, in a *S*-palmitoylation dependent manner, is known to regulate Akt signaling and Glut4 membrane translocation (Ren et al., 2013). They also identified DHHC17 as the palmitoyltransferase for ClipR-59. DHHC17 is also required in axonal outgrowth (Shi et al., 2015) and DHHC17 deficiency had been found to cause sudden death due to paralysis and synaptic and behavioral deficit (Sanders et al., 2016). From cancer perspective, Ducker et al. initially assigned DHHC17 to be oncogenic and the tumorigenesis is believed to be exerted via Ras palmitoylation as per their reported *in vitro* data (Ducker et al., 2004). But, it is still not clear whether Ras proteins are DHHC17 substrate *in vivo*. The expression level of DHHC17 highly depends upon the type of cancers. In some cases, for example lung, colon, prostate tumors DHHC17 is upregulated while in liver, thyroid gland, bladder tumor DHHC17 is downregulated (Ducker et al., 2006). Hence, DHHC17 can act as both oncogenic and tumor suppressor properties and further studies are needed to dissect its active role in tumorigenesis. Recently, Banerjee et al. reported the crystal structure of the complex between ankyrin repeat domain of human DHHC17 and its substrate, SNAP25B. It revealed the key residues in DHHC17 that are important for its interaction with SNAP25B and the same set of amino acids were found to be crucial for the interaction of DHHC17 with Huntingtin as well (Verardi et al., 2017).

5.17 DHHC18

There is very little data available to conclude a physiological function of DHHC18 but recently Zhao et al. identified DHHC18 as one of the top scored genes associated with Schizophrenia based on their large scale integrative analysis of genome-wide association study and expression quantitative trait loci data with more than 9000 schizophrenia patients (Zhao et al., 2018). Bredt and coworkers showed that when co-transfected with H-Ras, DHHC18 significantly increased the *S*-palmitoylation of H-Ras (Fukata et al., 2004).

5.18 DHHC19

Several small GTPases are known to be *S*-palmitoylated by DHHC family of enzymes. While DHHC19 was unable to alter the *S*-palmitoylation of most of the small GTPases including H-Ras, N-Ras, K-Ras4A, RhoB, Rap2, it significantly increased the *S*-palmitoylation of R-Ras. DHHC19-mediated increased *S*-palmitoylation of R-Ras has been demonstrated to provide enhanced cell viability in NIH3T3 cells that were co-transfected with R-Ras and DHHC19 (Baumgart et al., 2010).

5.19 DHHC20

DHHC20 has mostly been reported as an oncogenic protein as its overexpression was correlated with increased cellular proliferation, and growth in anchorage independent manner (Draper and Smith, 2010). DHHC20 is upregulated in many cancer types including prostate and breast cancers (Draper and Smith, 2010). Runkle et al. have discovered that EGFR is a substrate of DHHC20 and inhibiting the palmitoyltransferase activity of DHHC20 makes the cancer cell survival highly dependent on EGFR signaling. Hence, a combination therapy targeting both DHHC20 and EGFR might be a very effective therapeutic approach to treat cancers that develop resistance towards EGFR inhibitors (Runkle et al., 2016).

5.20 DHHC21

Several Src-family kinases have been identified as DHHHC21 substrates. For example, DHHC21 is known to *S*-palmitoylate Src-family kinase, Fyn which helps in maintaining hair follicle differentiation (Mill et al., 2009) and thus playing a key role in regulating developmental signals in mammalian tissue homeostasis. Src-family kinase Lck, an essential component of the

Fas signaling pathway, is also known to be *S*-palmitoylated by DHHC21 in a very rapid kinetics. This dynamic palmitoylation/depalmitoylation cycle of Lck plays significant role in the activation of the Fas signaling pathway (Akimzhanov and Boehning, 2015). This DHHC21-mediated *S*-palmitoylation of Src-family of kinases highlight a novel PTM-crosstalk between phosphorylation and *S*-palmitoylation. Beard et al. have recently reported a novel physiological role of DHHC21 in mediating endothelial dysfunction. Mice lacking DHHC21 exhibited resistance to injury and improved survival. It has been shown that DHHC21-mediated *S*-palmitoylation of PLCβ1 on Cys17 augments barrier dysfunction and pharmacological inhibition of DHHC21 attenuates the barrier leakage and leukocyte adhesion (Beard et al., 2016). Other important targets of DHHC21 include sex steroids receptors such as progesterone, estrogen and androgen receptors (Pedram et al., 2012), cell adhesion protein platelet endothelial cell adhesion molecule 1 (Marin et al., 2012), and α1D adrenoceptor (Marin et al., 2016). DHHC21 has also been shown to mediate physiological processes required for gut hyperpermeability induced by inflammation signifying the importance of DHHC21 as a novel therapeutic target for treating thermal injury-induced intestinal barrier damage (Haines et al., 2017). Regarding the role of DHHC21 in tumorigenesis, it has been found to be overexpressed in various cancer types including breast cancer, lymphoma and leukemia suggesting DHHC21 as an oncogenic protein (Bergom et al., 2005; Marin et al., 2012; Pedram et al., 2012; Sardjono et al., 2006).

5.21 DHHC22

DHHC22, along with DHHC23, is involved in palmitoylating large conductance calcium-activated potassium (BK) channels facilitating its efficient cell surface expression by regulating the exit of BK channels from trans-Golgi network (Tian et al., 2012). Protein *S*-palmitoylation is also known to assist the normal extracellular secretion process for proteins such as Nephroblastoma overexpressed protein (NOV, also known as CCN3). CCN3 plays an instrumental role in adhesion, migration and differentiation of cells. Recently Kim et al., reported the *S*-palmitoylation of C241 residue by DHHC22 in Thrombospondin type-1 (TSP-1) domain of CCN3 helps in the concerted secretion process of this protein and the inhibition of *S*-palmitoylation process induces intracellular accumulation of CCN3 and neuronal growth inhibition (Kim et al., 2018).

5.22 DHHC23

DHHC23 is one of the understudied PATs with no clear evidence for its actual localization. DHHC23 has been found to be differentially expressed in case of B-precursor acute lymphoblastic leukemia (BP-ALL) with varied level of white blood cells (Edwards et al., 2016). As mentioned before, BK channels is also a DHHC23 substrate along with DHHC22 (Tian et al., 2012).

5.23 DHHC24

The genomic localization for the gene that encodes DHHC24 is on chromosome number 11. DHHC24 is probably the least known and studied PAT with no evidence for its actual localization. The physiological function of DHHC24 is yet to be explored.

6. Analytical methods to identify and characterize protein *S*-palmitoylation

Identification and characterization of protein *S*-palmitoylation has been more enigmatic than any other lipidations. Proteomics of *S*-palmitoylated proteins have always been a challenging task. Despite the lack of suitable antibody against *S*-palmitoylation, scientists have developed several methods to detect palmitoylation which are outlined below.

6.1 Radiolabeled palmitic acid as a probe

The oldest analytical method to study protein *S*-palmitoylation was accomplished by utilizing radio isotope-labeled palmitic acids (Schlesinger et al., 1980; Schmidt and Schlesinger, 1979; Swarthout et al., 2005). Naturally occurring ^{1}H and ^{12}C in palmitic acid were replaced by their radio isotopes ^{3}H and ^{14}C respectively and this does not alter are structural feature of endogenous palmitic acid and, therefore can mimic *S*-palmitoylation quite accurately. In a typical experiment, cells are subjected to the radiolabeled palmitic acid. $^{3}H/^{14}C$-labeled palmitic acids then get metabolically converted to the corresponding acyl-CoA and ultimately incorporated on the substrate proteins as an *S*-palmitoylation modification with the help of DHHCs. Finally, protein *S*-palmitoylation (radiolabeled) can be detected by fluorography (see Fig. 4A). Incorporation of $^{3}H/^{14}C$-labeled palmitic acid into substrate proteins at Cys residues remained a gold standard for defining *S*-palmitoylation for more than two decades. The limitation of this method is technical difficulties associated with handling radioactive samples along with the weak radio signal from ^{3}H and ^{14}C.

6.2 Bio-orthogonal labeling via Click reaction

Bio-orthogonal labeling is a non-radioactive metabolic labeling strategy and very frequently used analytical tool to identify and characterize protein *S*-palmitoylation. It mainly relies on a highly efficient copper(I)-catalyzed cycloaddition reaction namely 'Click reaction' between a terminal alkyne and azide functional group (Wang et al., 2003). In some way, one can imagine bio-orthogonal labeling as a modification of radiolabeling method as discussed earlier in this section which suffers from low specific activity as well as hazardous chemicals to handle.

In bio-orthogonal labeling method, ω-azido-palmitic acid (Hang et al., 2007) or alkynyl-palmitic acid (Charron et al., 2009) is used as a non-radioactive probe to detect and visualize *S*-palmitoylated proteins in live cells. As alkynyl-palmitic acid (Charron et al., 2009) mimics the actual substrate of *S*-palmitoylation (*i.e.* palmitic acid) more accurately and exhibits less background signal (Speers and Cravatt, 2004) compared to azido-palmitic acid, the former is accepted as a preferred probe for bio-orthogonal labeling. The labeling strategy is outlined in Fig. 4B. In brief, the alkynyl-palmitic acid probe is added to the media and the cells are allowed to uptake it and eventually will get metabolically incorporated. The alkyne tag on the modified proteins can now be covalently attached to Biotin-azide or, BODIPY-azide by Click reaction. Furthermore, Streptavidin beads can be employed to pull-down the *S*-(alkynyl)palmitoylated proteins and analyzed by LC-MS/MS to get a host of information about the substrate proteins including the site of modification. Broncel et al. further modified the azido probe by multi-functionalizing it to carry both biotin group for enrichment and fluorescence tag for in-gel visualization (Broncel et al., 2015).

Gao et al. have reported a major advancement of this bio-orthogonal strategy by developing a method for live-cell imaging for the first time to visualize *S*-palmitoylated proteins (Gao and Hannoush, 2013). The fluorescence imaging method relies on Clickable alkynyl-fatty acids and *in situ* proximity ligation to track the *S*-palmitoylated proteins in live-cell with high spatial resolution.

6.3 Acyl-biotin exchange (ABE)

Acyl-biotin exchange (ABE) is a proteomics tool to identify and characterize protein *S*-palmitoylation via a sequence of chemical labeling strategies (Drisdel and Green, 2004; Wan et al., 2007). The method relies on the following sequences: (a) masking all the free thiols in the Cys sidechain of the proteins with *N*-ethylmaleimide (NEM), (b) cleavage of thioester bond in *S*-palmitoylated Cys residues using hydroxylamine, and (c) capture of newly exposed thiols with

biotinylated probe such as Biotin-*N*-[6-(biotinamido)hexyl]-3'-(2'-pyridyldithio)propionamide or Biotin-HPDP. Subsequent immunoprecipitation with streptavidin conjugated agarose beads allows *S*-palmitoylome characterization via mass-spectrometry as outlined in Fig. 4C. ABE method led to the first global proteomics study on *S*-palmitoylome from yeast cell lysate identifying thirty-fie new S-palmitoylated proteins (Roth et al., 2006) .

6.4 Acyl-PEG exchange (APE) shift assay

Acyl-PEG exchange (APE) shift assay is a modification of the ABE method. After the thioester bond cleavage, the exposed Cys residues are allowed to react with maleimide-functionalized polyethylene glycol reagents (mPEG-Mal) followed by SDS-PAGE or Western blot to detect both unmodified and *S*-palmitoylated proteins (Percher et al., 2016) (Fig. 4D). APE has an added advantage of quantitative measurement of the endogenous levels of *S*-palmitoylation along with the stoichiometry. APE causes a mobility shift on *S*-palmitoylated proteins as a result of incorporating various pre-defined PEG linker of different mass (e.g. 5 or 10 kDa).

6.5 Acyl-resin assisted capture (acyl-RAC)

Acyl- resin assisted capture (RAC) assay is principally also similar to the ABE method. In ARC, the 'biotinylation of originally *S*-palmitoylated Cys' step in ABE is replaced with the direct conjugation of free Cys to thiol-reactive Sepharose resin (Forrester et al., 2011) (Fig. 4E). This method is efficient, rapid and economical with the ability to be applied in complex biological

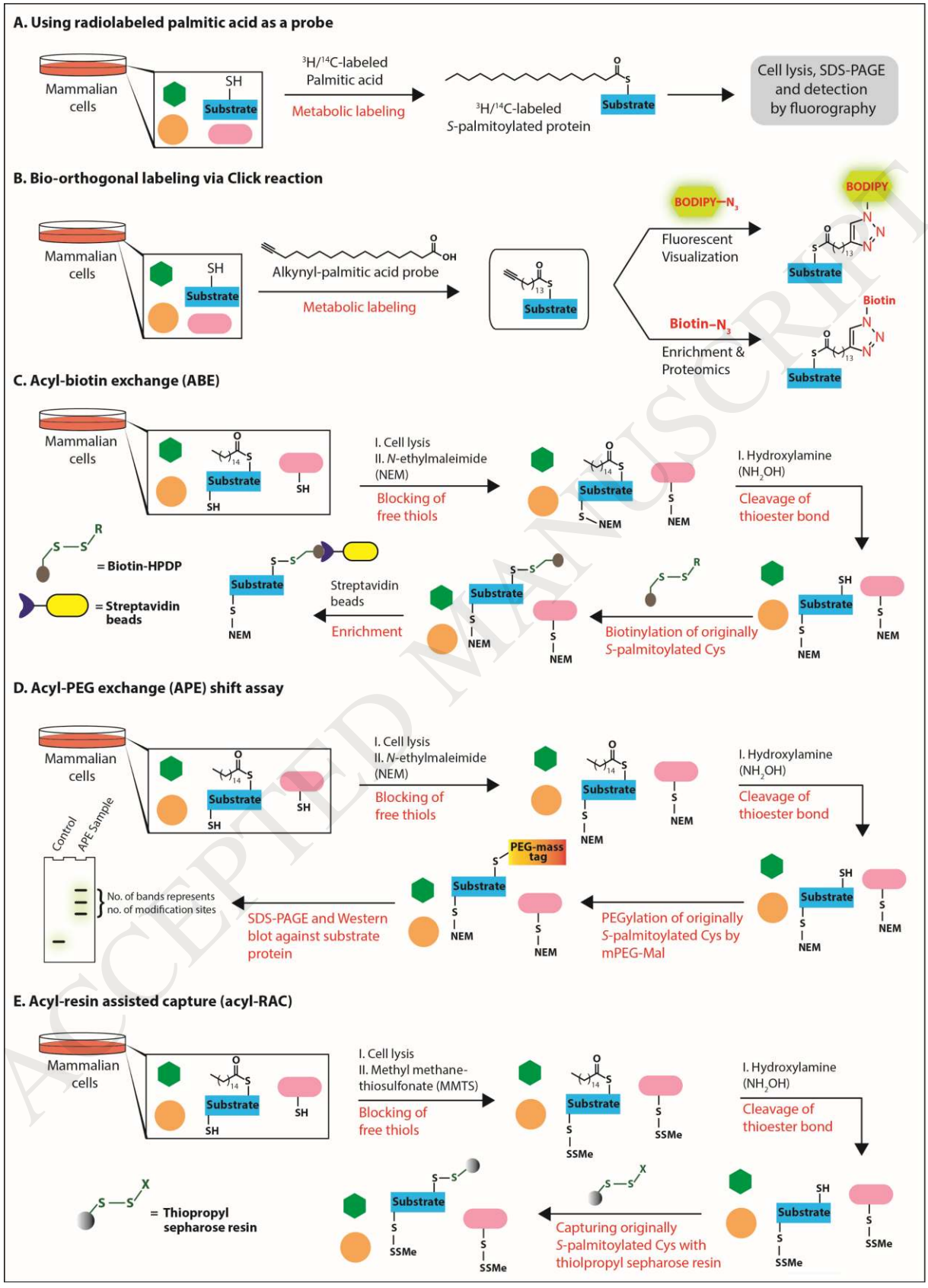


Fig. 4. Different analytical methods to identify and characterize protein *S*-palmitoylation.

samples. Resin-captured proteins can be eluted with reductant such as DTT and analyzed by SDS-PAGE or Western blot. On-bead proteolytic digestion can be further performed to get the proteolytic peptides for mass spectrometry to identify target proteins along with the site of modification.

7. Progress in the development of DHHC inhibitors

The inhibitor development for DHHC family of enzymes, let alone be the isoform specific inhibitors, is probably the least explored area in the field of protein *S*-palmitoylation. This is mainly due to the presence of significant amount of sequence and structural homology among the isoforms. Though it has been proposed that the inhibitor selectivity maybe achieved by exploiting the structural heterogeneity in the *C*- and *N*- terminal domains among the isoforms (Lobo, 2013), it is yet to be achieved experimentally. Very few PAT inhibitors had been reported so far, but they exhibit significant compromise in terms of their potency and specificity. The reported inhibitors can be classified as following:

7.1 Lipid based inhibitors

The well-established, although not specific, lipid based inhibitors include 2-BP, Tunicamycin, and Cerulenin as shown in Fig. 5. 2-BP gets metabolically incorporated inside the cell as 2-BP-CoA and inhibits palmitoyltransferase activity of all the DHHCs tested. The thiol group of Cys in DHHC catalytic domain react with 2-BP irreversibly and inhibits DHHC PAT activity by forming a stable acyl-enzyme intermediate (Jennings et al., 2009). It should be noted that apart from DHHCs, 2-BP also shows potent inhibition to a broad spectrum of metabolic enzymes including fatty acid-CoA ligase, glycerol-3-P-acyltransferase, NADPH cytochrome-c reductase and glucose-6-phosphatase in a concentration dependent manner (Coleman et al., 1992). 2-BP had shown significant inhibition of *S*-palmitoylation in many important proteins such as H-Ras, PSD-95, Rho family proteins, Src family kinases (Chen et al., 2003; Chenette et al., 2005; El-Husseini et al., 2002; Webb et al., 2000).

Other two significant lipid-based DHHC inhibitors are Tunicamycin and Cerulenin. The nucleoside antibiotic Tunicamycin mainly inhibits *N*-linked glycosylation, can also inhibit *S*-palmitoylation. The mode of action for Tunicamycin is still unclear, but it has been suggested that it competes with the palmitoyl-CoA substrate for binding to DHHC-PATs (Patterson and Pate

Skene, 1995). Calcium channels is known to play roles in synaptic transmission and hormone secretion. Tunicamycin had been reported to increase the steady state inactivation of Ca^{2+} channels in chromaffin cells, resulting in dysregulation of its physiological process (Hurley et al., 2000). The natural antibiotic Cerulenin is basically a fatty acid synthase inhibitor (Moche et al., 1999), but has been shown to inhibit *S*-palmitoylation of CD36 (Jochen et al., 1995; Schlesinger and Malfer, 1982). Mechanistically, it is proposed that Cerulenin irreversibly reacts with the catalytic Cys of DHHC or the substrate proteins' Cys residue through its epoxycarboxamide group. Cerulenin caught significant attention among the medicinal chemists as a potential selective DHHC inhibitor as through Structure-Activity Relationship (SAR) studies Lawrence et al. developed an analogue which exhibited selectivity for inhibition of *S*-palmitoylation vs. fatty acid synthase (Lawrence et al., 1999). But it failed at further drug development stage because of its long hydrophobic tail and the presence of highly reactive epoxycarboxamide functionality. The major challenge in developing lipid based inhibitors is the poor solubility of those hydrophobic small molecules. But, recent advances in delivering hydrophobic drugs via liposomal transport could be a potential way out.

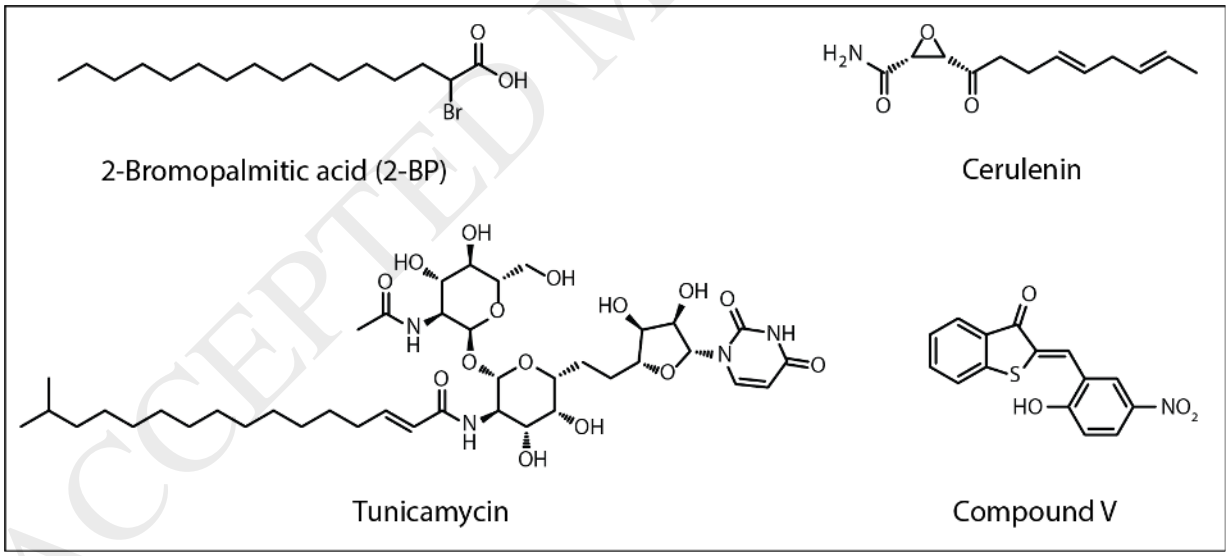


Fig. 5. Chemical structure of reported DHHC inhibitors.

7.2 Non-lipid based inhibitors

The shortcomings with the lipid based non-selective and hydrophobic inhibitors had spurred the exploration for the selective non-lipid based inhibitors. Ducker et al. identified several non-

lipid small molecule DHHC inhibitors from high-throughput screening (Ducker et al., 2006). PAT activities in human cells can be classified by either Class I PATs that palmitoylate farnesyl-dependent palmitoylation motif or Class II PATs that work on myristoyl-dependent palmitoylation motif. Four of the five compounds that they synthesized showed selectivity towards Class I PAT while the fifth compound was specific for class II PAT.(Ducker et al., 2006) However, further studies by Linder group (Jennings et al., 2009) with the abovementioned compounds resulted in some disappointment. Only one compound, Compound V from Ducker et al. (Ducker et al., 2006), was effective but unlike the selectivity reported previously Linder group found compound V to inhibit all the four PATs (Human DHHC2, DHHC9, yeast Pfa3, and yeast Erf2/Erf4) tested. Although Compound V was able to arrest the DHHC autoacylation step but unlike 2-BP, it was reversible and less potent.

Though researchers are still exploring new selective DHHC inhibitors, serendipitously Coleman et al., found curcumin to prevent *S*-palmitoylation of ITGβ4. Curcumin was found to block the autoacylation of DHHC3, which is reported to *S*-palmitoylate ITGβ4 (Coleman et al., 2015). They also showed that the α, β-unsaturated carbonyl group in curcumin is instrumental for its inhibitory activity and that is probably because Cys thiol can react with the α, β-unsaturated carbonyl via a Michael addition and inactivate the enzyme. Nevertheless, this discovery highlights the potential drug-like property of phytochemicals for PAT inhibition.

More potent and selective DHHC inhibitors are needed to further extend the possibility of DHHC druggability to treat human diseases. All the inhibitors reported so far, either suffer from poor solubility or lack of selectivity. We not only need selective DHHC inhibitors but also isoform specific inhibitors. With the recent development of fluorescence-based assay (Hamel et al., 2014), high-throughput screening can be designed to identify new selective/specific DHHC inhibitors. One drawback of that assay is that it targets the autoacylation step and as all the DHHCs show high similarity around the DHHC catalytic domain it could be difficult to get isoform specific inhibitors. To circumvent that issue, allosteric inhibitors might be developed. Another approach could be to develop small molecules that can inhibit the protein-protein interaction between DHHCs and substrate proteins.

8. Perspective

The reversible nature of protein *S*-palmitoylation is probably the most intriguing part of this PTM that makes it unique among all other lipidation. As discussed in this review, almost all the DHHCs, the driver protein *S*-palmitoylation, have been associated with human diseases ranging from neuropsychiatric disorders to cancers. The major advances in the field of *S*-palmitoylation have been achieved after the discovery of DHHC-PATs and the field is still in its infancy with tremendous potential. Technical difficulties are one of the reason for the slow progress of this field. Nevertheless, scientist have developed several analytical tools to identify and characterize protein *S*-palmitoylation. But, all of them suffer from one or more compromizations. For example, radioactive labeling method has a very low signal and needs a longer exposure along with its hazardous nature. On the other hand, bio-orthogonal labeling compromises the structure of palmitic acid, although minimal and acyl-biotin exchange method can only be applied in the cell lysate. Nevertheless, metabolic labeling and acyl-biotin exchange are definitely robust approaches to identify and characterize protein S-palmitoylation but the availability of antibodies against *S*-palmitoylation would greatly complement the experimental findings. The antibodies will possibly simplify the experiments by avoiding multistep labeling and/or enrichment steps in case of acyl-biotin exchange or metabolic labeling.

In last ten years or so, major break throughs have been achieved to understand the importance of DHHC-mediated protein S-palmitoylation in physiological and pathophysiological conditions. There is no doubt that DHHC is a very strong candidate for pharmacological targeting (Planey and Zacharias, 2010). But, for that we need selective and potent DHHC inhibitors. More specifically, we need isoform specific inhibitors. Developing isoform specific DHHC inhibitors so far has been very challenging for the researchers as the catalytic domain of DHHC-PATs are very similar in all the isoforms. Targeting the highly diverse *C*- and *N*-terminals of DHHC could be a potential solution for achieving the isoform specificity. In that context, we can take motivation form protein kinase inhibitor discovery era. Despite the initial difficulties with isoform specific inhibitors, medicinal chemists have come up with highly selective inhibitors for individual kinases and many of them are being used in clinic these days (Fabian et al., 2005; Feng et al., 2008; Karaman et al., 2008). The central questions revolving around the DHHCs, regarding their substrate specificity, acyl chain length selectivity and a sincere endeavor to design DHHC specific inhibitors finally managed to receive an enlightenment with the breakthrough achieved by Banerjee and co-workers recently (Rana et al., 2018). With their novel DHHC structural studies, we will certainly step into

a new era of DHHC with a greater physiological understanding and better set of inhibitors in our tool box that will determine the course of this field

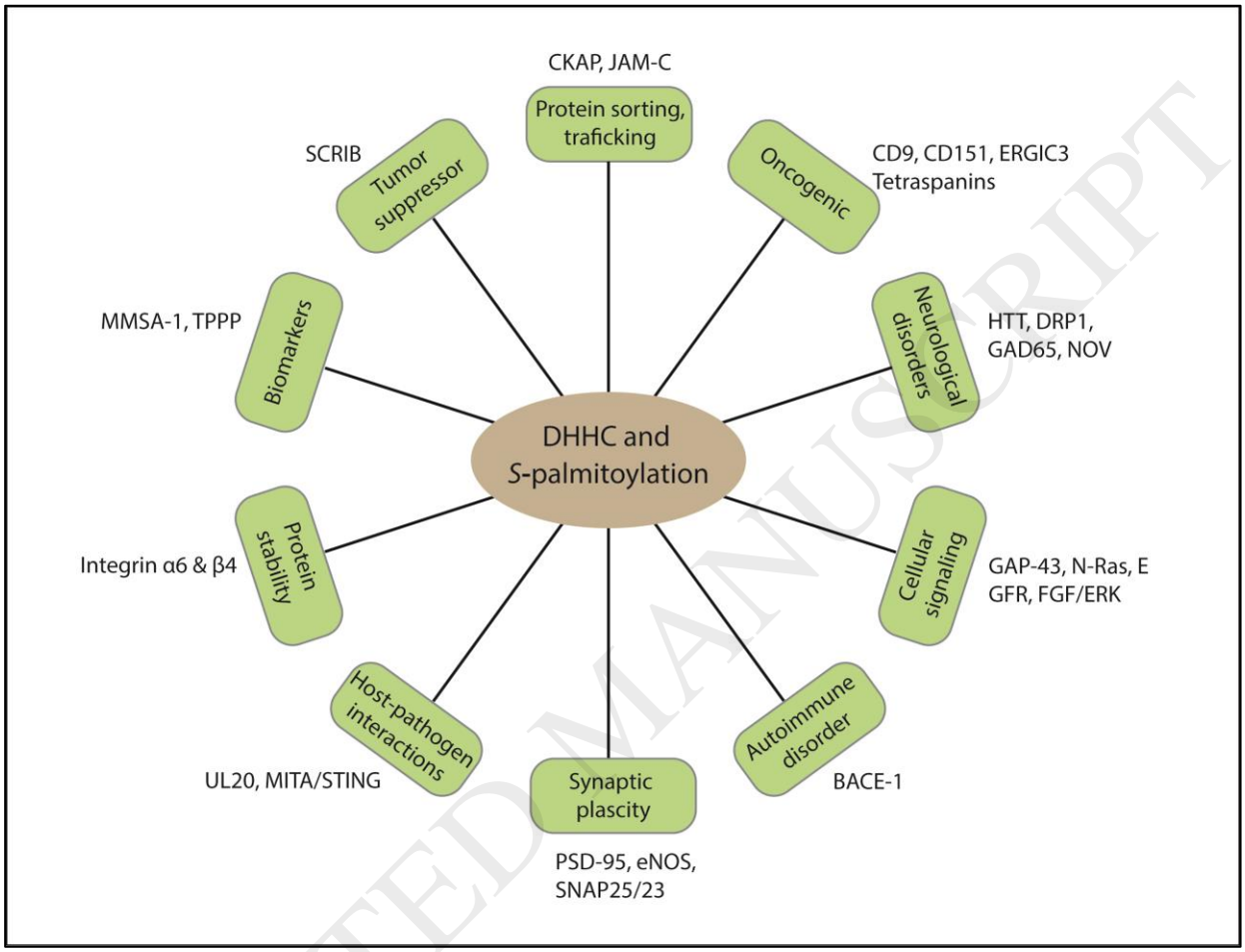


Fig. 6. Diverse physiological and pathophysiological roles of DHHC-mediated protein *S*-palmitoylation.

9. Acknowledgment

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10. Conflict of interest

The authors have no competing interests.

Table 2: Characteristics, physiological functions and associated diseases of human DHHCs

DHHC isoforms	No. of amino acids (aa) and molecular weight	No. of transmembrane domain (predicted by Uniprot)	Genomic location (Chromosome number)	Subcellular localizations	Physiological functions/phenotypes	Associated diseases
DHHC1	485 aa; 54,818 Da	4	16	Endoplasmic Reticulum (ER)	Oncogenic (Sigala et al., 2008; Yu et al., 2011b), involved in endosomal targeting (Oku et al., 2013), mediates immune response (Zhou et al., 2014)	Prostate Cancer (Sigala et al., 2008), Colon cancer (Yu et al., 2011b), Cardiometabolic traits (Ng et al., 2017)
DHHC2	367 aa; 42,022 Da	4	8	ER, Golgi	Tumor Suppressor (Li et al., 2014; Peng et al., 2014; Planey et al., 2009), Mediates cell-cell contacts (Sharma et al., 2008)	Ovarian cancer (Pils et al., 2005), Hepatocellular carcinoma (Peng et al., 2014)
DHHC3	299 aa; 34,170 Da	4	3	Golgi	Tumor Suppressor (Choi et al., 2007)	Cancer metastasis (Sharma et al., 2012)
DHHC4	344 aa; 39,787 Da	5	7	ER	*not reported yet*	Autoimmune encephalitis (Hasan et al., 2017)
DHHC5	715 aa; 77,545 Da	4	11	Cytoplasm	Plays role in sorting process mediated by its cytoplasmic tail (Breusegem and Seaman, 2014)	Schizophrenia (Zhao et al., 2018)
DHHC6	413 aa, 47,663 Da	4	10	ER	Stable expression of receptors (Fredericks et al., 2014)	*not reported yet*
DHHC7	308 aa, 35,140 Da	4	16	Golgi	Tumor suppressor (Chen et al., 2016)	Cystic Fibrosis (McClure et al., 2014)
DHHC8	765 aa, 81,443 Da	4	22	Golgi	Important for neuronal growth (Mukai et al., 2015) and cortical volume (Ota et al., 2013)	Schizophrenia (Mukai et al., 2015; Ota et al., 2013; Yoon et al., 2016; Young et al., 2012a)
DHHC9	364 aa, 40,916 Da	4	X	Cytosol, ER, Golgi	Prognostic marker for multiple myeloma (Meng et al., 2016)	X linked intellectual disability (Han et al., 2017), speech and language impairment (Bathelt et al., 2016), myeloma (Meng et al., 2016), Lujan Fryns syndrome (Hackmann et al., 2016), epilepsy (Baker et al., 2015)

DHHC11	412 aa, 45,975 Da	4	5	ER	Potential biomarker for bladder cancer (Yamamoto et al., 2007)	Burkitt Lymphoma (Dzikiewicz-Krawczyk et al., 2017), Hepatoblastoma (Wu et al., 2013)
DHHC12	267 aa, 30,813 Da	4	9	ER, Golgi	Regulates of alpha-secretase activity	Huntington Disease, Alzheimer's Disease, Schizophrenia, mental retardation (Young et al., 2012a)
DHHC13	622 aa, 70,861 Da	6	11	ER, Golgi	Regulator of Bone Morphogenetic Protein (BMP) (Chen et al., 2014)	Involved in anxiety related behaviors (Napoli et al., 2017), amyloidosis, liver anomalies, hypermetabolism (Shen et al., 2017)
DHHC14	488 aa, 53,388 Da	4	6	ER	Tumor suppressor (Yeste-Velasco et al., 2014)	Gastric cancer (Oo et al., 2014), Acute biphenotypic leukemia (Yu et al., 2011a)
DHHC15	337 aa, 39,331 Da	4	X	Golgi	Neuronal differentiation (Wang et al., 2015)	X- chromosome inactivation (XCI) (Kang et al., 2015)
DHHC16	377 aa, 43,633 Da	4	10	ER	Mediates DNA damage response, regulates ATM (Cao et al., 2016)	*not reported yet*
DHHC17	632 aa, 72,640 Da	6	12	Cytosol, Golgi	Anti-apoptotic (Berchtold et al., 2011), requires in axonal growth (Shi et al., 2015)	Huntington disease (Singaraja et al., 2011), Paralysis (Sanders et al., 2016)
DHHC18	388 aa, 42,031 Da	4	1	Golgi	*not reported yet*	Schizophrenia (Zhao et al., 2018)
DHHC19	309 aa, 34,352 Da	4	3	ER	Enhances viability of transfected cells via R-Ras (Baumgart et al., 2010)	*not reported yet*
DHHC20	365 aa, 42,278 Da	4	13	Plasma membrane	Oncogenic, involved in cellular transformation (Draper and Smith, 2010)	Ovarian, Breast and Prostate cancers (Draper and Smith, 2010)
DHHC21	265 aa, 31,385 Da	4	9	Golgi, Plasma membrane	Maintains hair follicle differentiation *via* Fyn (Mill et al., 2009), required for gut hyperpermeability (Haines et al., 2017)	Implication in lung pathology (Beard et al., 2016)
DHHC22	263 aa, 29,100 Da	2*	14	ER, Golgi	Cell surface expression of BK channels (Tian et al., 2012)	*not reported yet*
DHHC23	409 aa, 45,983 Da	6	3	*not reported yet*	*not reported yet*	Leukemia (Edwards et al., 2016)
DHHC24	284 aa, 30,176 Da	5	11	*not reported yet*	*not reported yet*	*not reported yet*

* TMHMM server predicts four transmembrane (TM) domains for DHHC22

Table 3: Experimentally validated DHHC substrates

DHCH isoforms	Substrates
DHHC1	miR-93 (Yu et al., 2011b), Neurochondrin (NCDN) (Oku et al., 2013)
DHHC2	Cytoskeleton-associated protein 4 (CKAP4) /p63 (Zhang et al., 2008), Cytoskeleton-linking membrane protein 63 (CLIMP-63) (Sandoz and van der Goot, 2015), A-kinase anchoring protein (AKAP)79/150 (Woolfrey et al., 2015), Glycoprotein 78 (Gp78) (Fairbank et al., 2012), CD9 and CD151 (Sharma et al., 2008), Leukocyte *C*-terminal Src kinase (Lck) (Zeidman et al., 2011), Postsynaptic density protein 95 (PSD-95) (Fukata et al., 2004), Growth associated protein 43 (GAP-43) (Fukata et al., 2004), Synaptosomal-associated protein 25 (SNAP25)/23 (Greaves et al., 2010), Endothelial nitric oxide synthase (eNOS) (Fernandez-Hernando et al., 2006), RGS7 family-binding protein (R7BP) (Jia et al., 2011)
DHHC3	Neural cell adhesion molecule (NCAM) (Lievens et al., 2016), Dopamine 2 receptor (D2R) (Ebersole et al., 2015), Endothelial nitric oxide synthase (eNOS) (Fernandez-Hernando et al., 2006), Regulator of G-protein signaling 4 (RGS4) (Wang et al., 2010), Palmitoyl-protein thioesterase 1 (PPT1) (Segal-Salto et al., 2016), Integrin β4 and α6 (Sharma et al., 2012), Synaptosomal-associated protein 25 (SNAP25)/23 (Greaves et al., 2010), Neurochondrin (NCDN) (Oku et al., 2013), Protein interacting with C kinase-1 (PICK1) (Thomas et al., 2013), Stress axis-regulated exon (STREX) (Tian et al., 2010), Phosphatidylinositol 4-kinase type IIα (PI4KIIα) (Lu et al., 2012), [Synapse defective-1 (SYDE-1), Transmembrane AMPAR regulatory proteins γ-2 (TARP γ-2), Transmembrane AMPAR regulatory proteins γ-8 (TARP γ-8), Cornichon-2, Calcium/calmodulin-dependent protein kinase type II alpha chain (CaMKIIα), Neurochondrin (NCDN)/Norbin, Zyxin, Transient receptor potential cation channel subfamily M member 8 (TRPM8), Transient receptor potential channel 1 (TRPC1), Orexin 2 receptor] (Oku et al., 2013), GluR1/2 (Huang et al., 2009), Cysteine-string *protein* (CSP) (Greaves et al., 2008), Endoplasmic reticulum-golgi intermediate compartment protein 3 (ERGIC3) (Sharma et al., 2017), UL20 (Wang et al., 2017a; Wang et al., 2017b)
DHHC4	Dopamine 2 receptor (D2R) (Ebersole et al., 2015)
DHHC5	Enhancer of zeste 2 polycomb repressive complex 2 subunit (EZH2) (Chen et al., 2017c), Flotilin2 (Li et al., 2012), Somatostatin receptor 5 (SSTR5) (Kokkola et al., 2011), Stress axis-regulated exon (STREX) (Tian et al., 2010), Phosphoprotein phospholemman (PLM) (Howie et al., 2014), Glutamate receptor interacting protein 1 (GRIP1) (Thomas et al., 2012), δ-Catenin (Brigidi et al., 2014)
DHHC6	Calnexin (Lakkaraju et al., 2012), IP3 receptor (Fredericks et al., 2014), Gp78 (Fairbank et al., 2012)
DHHC7	Junctional adhesion molecule C (JAM C) (Aramsangtienchai et al., 2017), Scribble (SCRIB) (Chen et al., 2016), Fas (Rossin et al., 2015), Cystic fibrosis transmembrane conductance regulator (CFTR) (McClure et al., 2014), Sex steroids receptors (Androgen, Progesterone, Estrogen receptors) (Pedram et al., 2012), Endothelial nitric oxide synthase (eNOS) (Fernandez-Hernando et al., 2006), Palmitoyl-protein thioesterase 1 (PPT1) (Segal-Salto et al., 2016), Synaptosomal-associated protein 25 (SNAP25) (Greaves et al., 2010), Stress axis-regulated exon (STREX) (Tian et al., 2010), Phosphatidylinositol 4-kinase type Iiα (PI4KIIα) (Lu et al., 2012), [SYDE-1, TARP γ-2, TARP γ-8, Cornichon-2, CaMKIIα, NCDN/Norbin, Zyxin, TRPM8, TRPC1, Orexin 2 receptor] (Oku et al., 2013), CSP (Greaves et al., 2008)

DHHC8	Cell division cycle 42 (Cdc42) (Moutin et al., 2017), Dopamine 2 receptor (D2R) (Ebersole et al., 2015), Postsynaptic density protein 95 (PSD-95) (Yoshii and Constantine-Paton, 2014), PDZ domain-containing protein (PICK1) (Thomas et al., 2013), eNOS (Fernandez-Hernando et al., 2006), Glutamate receptor interacting protein 1(GRIP1) (Thomas et al., 2012), ATP binding cassette subfamily A member 1 (ABCA1) (Singaraja et al., 2009), Paralemmin-1 (Huang et al., 2009)
DHHC9	β_2 Adrenergic receptor (β_2AR) (Adachi et al., 2016), N-Ras, H-Ras (Raymond et al., 2007; Swarthout et al., 2005), Stress axis-regulated exon (STREX) (Tian et al., 2010)
DHHC11	Gp78 (Fairbank et al., 2012), Neurochondrin (NCDN) (Oku et al., 2013)
DHHC12	*Substrates not known yet*
DHHC13	Melanocortin-1 receptor (MC1R) (Chen et al., 2017b), Cornifelin (Liu et al., 2015), Drp1(Napoli et al., 2017), Malonyl-CoA-acyl carrier protein transacylase (MCAT), Catenin delta 1 (CTNND1) (Shen et al., 2017), Membrane type1 – Metalloprotease (MT1-MMP) (Song et al., 2014), Gp78 (Fairbank et al., 2012)
DHHC14	β_2 Adrenergic receptor (β_2AR) (Adachi et al., 2016),
DHHC15	Postsynaptic density protein 95 (PSD-95) (Fukata et al., 2004), Growth associated protein 43 (GAP-43) (Fukata et al., 2004), Cysteine string protein (CSP) (Greaves et al., 2008)
DHHC16	c-Abl (Cao et al., 2016), Phospholamban (Zhou et al., 2015), DHHC6 (Abrami et al., 2017), Phospholamban (Zhou et al., 2015)
DHHC17	NAD-synthesizing enzyme nicotinamide mononucleotide adenylyltransferase 2 (NMNAT2) (Milde and Coleman, 2014), Glycoprotein M6A (GPM6A), Sprouty related EVH1 domain containing 1 (SPRED1), SPRED 3 (Butland et al., 2014), ClipR-59 (Ren et al., 2013), Glutamic acid decarboxylase 65 (GAD65) (Rush et al., 2012), Huntingtin (Yanai et al., 2006), SNAP25/23 (Greaves et al., 2010), STREX (Tian et al., 2010), CSP (Greaves et al., 2008), Caspase-6 (Skotte et al., 2017), PSD-95 (Huang et al., 2004), Synaptotagmin I (Huang et al., 2004), Membrane palmitoylated protein 1 (MPP1)/p55 (Lach et al., 2012), c-Jun *N*-terminal kinases 3 (JNK3) (Yang et al., 2013)
DHHC18	β_2 Adrenergic receptor (β_2AR) (Adachi et al., 2016), H-Ras (Fukata et al., 2004)
DHHC19	R-RAS (Baumgart et al., 2010)
DHHC20	*Epidermal growth factor receptor* (EGFR) (Runkle et al., 2016), Calcium homoeostasis modulator 1 (CALMH1) (Taruno et al., 2017)
DHHC21	Fyn (Mill et al., 2009), α1D adrenoceptor (Marin et al., 2016), Estrogen, progesterone and androgen receptors (Pedram et al., 2012), eNOS (Fernandez-Hernando et al., 2006), Phospholipase C beta 1 (PLCβ1) (Beard et al., 2016)
DHHC22	Platelet endothelial cell adhesion molecule (PECAM1) (Marin et al., 2012), Nephroblastoma overexpressed protein (NOV, also known as CCN3) (Kim et al., 2018)
DHHC23	BK channels (Tian et al., 2012)

11. References

Abrami, L., Dallavilla, T., Sandoz, P.A., Demir, M., Kunz, B., Savoglidis, G., Hatzimanikatis, V., Van Der Goot, F.G., 2017. Identification and dynamics of the human ZDHHC16-ZDHHC6 palmitoylation cascade. Elife 6, e27826.

Abrami, L., Leppla, S.H., van der Goot, F.G., 2006. Receptor palmitoylation and ubiquitination regulate anthrax toxin endocytosis. J. Cell. Biol. 172, 309-320.

Adachi, N., Hess, D.T., McLaughlin, P., Stamler, J.S., 2016. S-Palmitoylation of a novel site in the β2-adrenergic receptor associated with a novel intracellular itinerary. J. Biol. Chem. 291, 20232-20246.

Akimzhanov, A.M., Boehning, D., 2015. Rapid and transient palmitoylation of the tyrosine kinase Lck mediates Fas signaling. Proc. Natl. Acad. Sci. USA 112, 11876-11880.

Anami, K., Oue, N., Noguchi, T., Sakamoto, N., Sentani, K., Hayashi, T., Hinoi, T., Okajima, M., Graff, J.M., Yasui, W., 2010. Search for transmembrane protein in gastric cancer by the Escherichia coli ampicillin secretion trap: expression of DSC2 in gastric cancer with intestinal phenotype. J. Pathol. 221, 275-284.

Aramsangtienchai, P., Spiegelman, N.A., Cao, J., Lin, H., 2017. S-Palmitoylation of junctional adhesion molecule c regulates its tight junction localization and cell migration. J. Biol. Chem. 292, 5325-5334.

Bachovchin, D.A., Ji, T., Li, W., Simon, G.M., Blankman, J.L., Adibekian, A., Hoover, H., Niessen, S., Cravatt, B.F., 2010. Superfamily-wide portrait of serine hydrolase inhibition achieved by library-versus-library screening. Proc. Natl. Acad. Sci. USA 107, 20941-20946.

Baker, K., Astle, D.E., Scerif, G., Barnes, J., Smith, J., Moffat, G., Gillard, J., Baldeweg, T., Raymond, F.L., 2015. Epilepsy, cognitive deficits and neuroanatomy in males with ZDHHC9 mutations. Ann. Clin. Transl. Neurol. 2, 559-569.

Bathelt, J., Astle, D., Barnes, J., Raymond, F.L., Baker, K., 2016. Structural brain abnormalities in a single gene disorder associated with epilepsy, language impairment and intellectual disability. Neuroimage. Clin. 12, 655-665.

Bathelt, J., Barnes, J., Raymond, F.L., Baker, K., Astle, D., 2017. Global and local connectivity differences converge with gene expression in a neurodevelopmental disorder of known genetic origin. Cerebral cortex 27, 3806-3817.

Baumgart, F., Corral-Escariz, M., Perez-Gil, J., Rodriguez-Crespo, I., 2010. Palmitoylation of R-Ras by human DHHC19, a palmitoyl transferase with a CaaX box. Biochim. Biophys. Acta. 1798, 592-604.

Beard, R.S., Jr., Yang, X., Meegan, J.E., Overstreet, J.W., Yang, C.G., Elliott, J.A., Reynolds, J.J., Cha, B.J., Pivetti, C.D., Mitchell, D.A., Wu, M.H., Deschenes, R.J., Yuan, S.Y., 2016. Palmitoyl acyltransferase DHHC21 mediates endothelial dysfunction in systemic inflammatory response syndrome. Nat. Commun. 7, 12823.

Berchtold, L.A., Storling, Z.M., Ortis, F., Lage, K., Bang-Berthelsen, C., Bergholdt, R., Hald, J., Brorsson, C.A., Eizirik, D.L., Pociot, F., Brunak, S., Storling, J., 2011. Huntingtin-interacting protein 14 is a type 1 diabetes candidate protein regulating insulin secretion and beta-cell apoptosis. Proc. Natl. Acad. Sci. USA 108, E681-E688.

Bergom, C., Gao, C., Newman, P.J., 2005. Mechanisms of PECAM-1-mediated cytoprotection and implications for cancer cell survival. Leuk. Lymphoma. 46, 1409-1421.

Blanc, M., David, F., Abrami, L., Migliozzi, D., Armand, F., Bürgi, J., van der Goot, F., 2015. SwissPalm: Protein Palmitoylation database.

Braun, P.E., Radin, N.S., 1969. Interactions of lipids with a membrane structural protein from myelin. Biochemistry 8, 4310-4318.

Breusegem, S.Y., Seaman, M.N., 2014. Genome-wide RNAi screen reveals a role for multipass membrane proteins in endosome-to-golgi retrieval. Cell Rep. 9, 1931-1945.

Brigidi, G.S., Santyr, B., Shimell, J., Jovellar, B., Bamji, S.X., 2015. Activity-regulated trafficking of the palmitoyl-acyl transferase DHHC5. Nat. Commun. 6, 8200.

Brigidi, G.S., Sun, Y., Beccano-Kelly, D., Pitman, K., Mobasser, M., Borgland, S.L., Milnerwood, A.J., Bamji, S.X., 2014. Palmitoylation of delta-catenin by DHHC5 mediates activity-induced synapse plasticity. Nat. Neurosci. 17, 522-532.

Broncel, M., Serwa, R.A., Ciepla, P., Krause, E., Dallman, M.J., Magee, A.I., Tate, E.W., 2015. Multifunctional reagents for quantitative proteome-wide analysis of protein modification in human cells and dynamic profiling of protein lipidation during vertebrate development. Angew. Chem. Int. Ed. Engl. 54, 5948-5951.

Bürger, M., Zimmermann, T.J., Kondoh, Y., Stege, P., Watanabe, N., Osada, H., Waldmann, H., Vetter, I.R., 2012. Crystal structure of the predicted phospholipase LYPLAL1 reveals unexpected functional plasticity despite close relationship to acyl protein thioesterases. J. Lipid Res. 53, 43-50.

Butland, S.L., Sanders, S.S., Schmidt, M.E., Riechers, S.P., Lin, D.T., Martin, D.D., Vaid, K., Graham, R.K., Singaraja, R.R., Wanker, E.E., Conibear, E., Hayden, M.R., 2014. The palmitoyl acyltransferase HIP14 shares a high proportion of interactors with huntingtin: implications for a role in the pathogenesis of Huntington's disease. Hum. Mol. Genet. 23, 4142-4160.

Camp, L.A., Hofmann, S.L., 1993. Purification and properties of a palmitoyl-protein thioesterase that cleaves palmitate from H-Ras. J. Biol. Chem. 268, 22566-22574.

Cao, N., Li, J.K., Rao, Y.Q., Liu, H., Wu, J., Li, B., Zhao, P., Zeng, L., Li, J., 2016. A potential role for protein palmitoylation and zDHHC16 in DNA damage response. BMC Mol. Biol. 17, 12.

Chamberlain, L.H., Shipston, M.J., 2015. The physiology of protein S-acylation. Physiol. Rev. 95, 341-376.

Charron, G., Zhang, M.M., Yount, J.S., Wilson, J., Raghavan, A.S., Shamir, E., Hang, H.C., 2009. Robust fluorescent detection of protein fatty-acylation with chemical reporters. J. Am. Chem. Soc. 131, 4967-4975.

Chavda, B., Arnott, J.A., Planey, S.L., 2014. Targeting protein palmitoylation: selective inhibitors and implications in disease. Expert. Opin. Drug Discov. 9, 1005-1019.

Chen, B., Zheng, B., DeRan, M., Jarugumilli, G.K., Fu, J., Brooks, Y.S., Wu, X., 2016. ZDHHC7-mediated S-palmitoylation of Scribble regulates cell polarity. Nat. Chem. Biol. 12, 686-693.

Chen, H.Q., Tannous, M., Veluthakal, R., Amin, R., Kowluru, A., 2003. Novel roles for palmitoylation of Ras in IL-1 beta-induced nitric oxide release and caspase 3 activation in insulin-secreting beta cells. Biochem. Pharmacol. 66, 1681-1694.

Chen, L.Y., Yang-Yen, H.F., Tsai, C.C., Thio, C.L., Chuang, H.L., Yang, L.T., Shen, L.F., Song, I.W., Liu, K.M., Huang, Y.T., Liu, F.T., Chang, Y.J., Chen, Y.T., Yen, J.J.Y., 2017a. Protein palmitoylation by ZDHHC13 protects skin against microbial-driven dermatitis. J. Invest. Dermatol. 137, 894-904.

Chen, S., Zhu, B., Yin, C., Liu, W., Han, C., Chen, B., Liu, T., Li, X., Chen, X., Li, C., Hu, L., Zhou, J., Xu, Z.X., Gao, X., Wu, X., Goding, C.R., Cui, R., 2017b. Palmitoylation-dependent activation of MC1R prevents melanomagenesis. Nature 549, 399-403.

Chen, X., Ma, H., Wang, Z., Zhang, S., Yang, H., Fang, Z., 2017c. EZH2 palmitoylation mediated by ZDHHC5 in p53-mutant glioma drives malignant development and progression. Cancer Res. 77, 4998-5010.

Chen, X., Shi, W., Wang, F., Du, Z., Yang, Y., Gao, M., Yao, Y., He, K., Wang, C., Hao, A., 2014. Zinc finger DHHC-type containing 13 regulates fate specification of ectoderm and mesoderm cell lineages by modulating Smad6 activity. Stem. Cells. Dev. 23, 1899-1909.

Chenette, E.J., Abo, A., Der, C.J., 2005. Critical and distinct roles of amino- and carboxyl-terminal sequences in regulation of the biological activity of the Chp atypical Rho GTPase. J. Biol. Chem. 280, 13784-13792.

Choi, Y.W., Bae, S.M., Kim, Y.W., Lee, H.N., Kim, Y.W., Park, T.C., Ro, D.Y., Shin, J.C., Shin, S.J., Seo, J.S., Ahn, W.S., 2007. Gene expression profiles in squamous cell cervical carcinoma using array-based comparative genomic hybridization analysis. Int. J. Gynecol. Cancer 17, 687-696.

Coleman, D.T., Soung, Y.H., Surh, Y.J., Cardelli, J.A., Chung, J., 2015. Curcumin prevents palmitoylation of integrin beta4 in breast cancer cells. PLoS One 10, e0125399.

Coleman, R.A., Rao, P., Fogelsong, R.J., Bardes, E.S.G., 1992. 2-Bromopalmitoyl-CoA and 2-bromopalmitate: Promiscuous inhibitors of membrane-bound enzymes. Biochim. Biophys. Acta. 1125, 203-209.

Dekker, F.J., Rocks, O., Vartak, N., Menninger, S., Hedberg, C., Balamurugan, R., Wetzel, S., Renner, S., Gerauer, M., Scholermann, B., Rusch, M., Kramer, J.W., Rauh, D., Coates, G.W., Brunsveld, L., Bastiaens, P.I., Waldmann, H., 2010. Small-molecule inhibition of APT1 affects Ras localization and signaling. Nat. Chem. Biol. 6, 449-456.

Dowal, L., Yang, W., Freeman, M.R., Steen, H., Flaumenhaft, R., 2011. Proteomic analysis of palmitoylated platelet proteins. Blood 118, e62-e73.

Draper, J.M., Smith, C.D., 2010. DHHC20: A human palmitoyl acyltransferase that causes cellular transformation. Mol. Membr. Biol. 27, 123-136.

Drisdel, R.C., Green, W.N., 2004. Labeling and quantifying sites of protein palmitoylation. BioTechniques 36, 276-285.

Ducker, C.E., Griffel, L.K., Smith, R.A., Keller, S.N., Zhuang, Y., Xia, Z., Diller, J.D., Smith, C.D., 2006. Discovery and characterization of inhibitors of human palmitoyl acyltransferases. Mol. Cancer. Ther. 5, 1647-1659.

Ducker, C.E., Stettler, E.M., French, K.J., Upson, J.J., Smith, C.D., 2004. Huntingtin interacting protein 14 is an oncogenic human protein: palmitoyl acyltransferase. Oncogene 23, 9230-9237.

Duncan, J.A., Gilman, A.G., 1998. A cytoplasmic acyl-protein thioesterase that removes palmitate from G protein alpha subunits and p21(RAS). J. Biol. Chem. 273, 15830-15837.

Dzikiewicz-Krawczyk, A., Kok, K., Slezak-Prochazka, I., Robertus, J.L., Bruining, J., Tayari, M.M., Rutgers, B., de Jong, D., Koerts, J., Seitz, A., Li, J., Tillema, B., Guikema, J.E., Nolte, I.M., Diepstra, A., Visser, L., Kluiver, J., van den Berg, A., 2017. ZDHHC11 and ZDHHC11B are critical novel components of the oncogenic MYC-miR-150-MYB network in Burkitt lymphoma. Leukemia 31, 1470-1473.

Ebersole, B., Petko, J., Woll, M., Murakami, S., Sokolina, K., Wong, V., Stagljar, I., Luscher, B., Levenson, R., 2015. Effect of C-terminal S-palmitoylation on D2 dopamine receptor trafficking and stability. PLoS One 10, e0140661.

Edwards, H., Rubenstein, M., Dombkowski, A.A., Caldwell, J.T., Chu, R., Xavier, A.C., Thummel, R., Neely, M., Matherly, L.H., Ge, Y., Taub, J.W., 2016. Gene signature of high white blood cell count in b-precursor acute lymphoblastic leukemia. PLoS One 11, e0161539.

El-Husseini, A.E.-D., Schnell, E., Dakoji, S., Sweeney, N., Zhou, Q., Prange, O., Gauthier-Campbell, C., Aguilera-Moreno, A., Nicoll, R.A., Bredt, D.S., 2002. Synaptic strength regulated by palmitate cycling on PSD-95. Cell 108, 849-863.

Fabian, M.A., Biggs Iii, W.H., Treiber, D.K., Atteridge, C.E., Azimioara, M.D., Benedetti, M.G., Carter, T.A., Ciceri, P., Edeen, P.T., Floyd, M., Ford, J.M., Galvin, M., Gerlach, J.L., Grotzfeld, R.M., Herrgard, S., Insko, D.E., Insko, M.A., Lai, A.G., Lélias, J.-M., Mehta, S.A., Milanov, Z.V., Velasco, A.M., Wodicka, L.M., Patel, H.K., Zarrinkar, P.P., Lockhart, D.J., 2005. A small molecule–kinase interaction map for clinical kinase inhibitors. Nat. Biotechnol. 23, 329-336.

Fairbank, M., Huang, K., El-Husseini, A., Nabi, I.R., 2012. RING finger palmitoylation of the endoplasmic reticulum Gp78 E3 ubiquitin ligase. FEBS letters 586, 2488-2493.

Fang, C., Deng, L., Keller, C.A., Fukata, M., Fukata, Y., Chen, G., Lüscher, B., 2006. GODZ-mediated palmitoylation of $GABA_A$ receptors is required for normal assembly and function of GABAergic inhibitory synapses. J. Neurosci. 26, 12758-12768.

Feldman, J.L., Baeza, J., Denu, J.M., 2013. Activation of the protein deacetylase SIRT6 by long-chain fatty acids and widespread deacylation by mammalian sirtuins. J. Biol. Chem. 288, 31350-31356.

Feng, Y., Yin, Y., Weiser, A., Griffin, E., Cameron, M.D., Lin, L., Ruiz, C., Schürer, S.C., Inoue, T., Rao, P.V., Schröter, T., LoGrasso, P., 2008. Discovery of substituted 4-(pyrazol-4-yl)-phenylbenzodioxane-2-carboxamides as potent and highly selective Rho kinase (ROCK-II) inhibitors. J. Med. Chem. 51, 6642-6645.

Fernandez-Hernando, C., Fukata, M., Bernatchez, P.N., Fukata, Y., Lin, M.I., Bredt, D.S., Sessa, W.C., 2006. Identification of Golgi-localized acyl transferases that palmitoylate and regulate endothelial nitric oxide synthase. J. Cell Biol. 174, 369-377.

Forrester, M.T., Hess, D.T., Thompson, J.W., Hultman, R., Moseley, M.A., Stamler, J.S., Casey, P.J., 2011. Site-specific analysis of protein S-acylation by resin-assisted capture. J. Lipid Res. 52, 393-398.

Fredericks, G.J., Hoffmann, F.W., Hondal, R.J., Rozovsky, S., Urschitz, J., Hoffmann, P.R., 2017. Selenoprotein K increases efficiency of DHHC6 catalyzed protein palmitoylation by stabilizing the acyl-DHHC6 intermediate. Antioxidants (Basel, Switzerland) 7, 10.3390/antiox7010004.

Fredericks, G.J., Hoffmann, F.W., Rose, A.H., Osterheld, H.J., Hess, F.M., Mercier, F., Hoffmann, P.R., 2014. Stable expression and function of the inositol 1, 4, 5-triphosphate receptor requires palmitoylation by a DHHC6/selenoprotein K complex. Proc. Natl. Acad. Sci. USA 111, 16478-16483.

Fukata, M., Fukata, Y., Adesnik, H., Nicoll, R.A., Bredt, D.S., 2004. Identification of PSD-95 palmitoylating enzymes. Neuron 44, 987-996.

Fukata, Y., Fukata, M., 2010. Protein palmitoylation in neuronal development and synaptic plasticity. Nat. Rev. Neurosci. 11, 161-175.

Gao, X., Hannoush, R.N., 2013. Single-cell imaging of Wnt palmitoylation by the acyltransferase porcupine. Nat. Chem. Biol. 10, 61-68.

Glaser, B., Schumacher, J., Williams, H.J., Jamra, R.A., Ianakiev, N., Milev, R., Ohlraun, S., Schulze, T.G., Czerski, P.M., Hauser, J., Jönsson, E.G., Sedvall, G.C., Klopp, N., Illig, T., Becker, T., Propping, P., Williams, N.M., Cichon, S., Kirov, G., Rietschel, M., Murphy, K.C., O'Donovan, M.C., Nöthen, M.M., Owen, M.J., 2005. No association between the putative functional ZDHHC8 single nucleotide polymorphism rs175174 and schizophrenia in large european samples. Biol. Psychiatry 58, 78-80.

Gomez, R., Goodman, L.E., Tripathy, S.K., O'Rourke, E., Manne, V., Tamanoi, F., 1993. Purified yeast protein farnesyltransferase is structurally and functionally similar to its mammalian counterpart. Biochem. J. 289, 25-31.

Goytain, A., Hines, R.M., Quamme, G.A., 2008. Huntingtin-interacting proteins, HIP14 and HIP14L, mediate dual functions, palmitoyl acyltransferase and Mg^{2+} transport. J. Biol. Chem. 283, 33365-33374.

Greaves, J., Chamberlain, L.H., 2011. DHHC palmitoyl transferases: substrate interactions and (patho)physiology. Trends Biochem. Sci. 36, 245-253.

Greaves, J., Chamberlain, L.H., 2014. New links between S-acylation and cancer. J. Pathol. 233, 4-6.

Greaves, J., Gorleku, O.A., Salaun, C., Chamberlain, L.H., 2010. Palmitoylation of the SNAP25 protein family: specificity and regulation by DHHC palmitoyl transferases. J. Biol. Chem. 285, 24629-24638.

Greaves, J., Munro, K.R., Davidson, S.C., Riviere, M., Wojno, J., Smith, T.K., Tomkinson, N.C.O., Chamberlain, L.H., 2017. Molecular basis of fatty acid selectivity in the zDHHC family of S-acyltransferases revealed by click chemistry. Proc. Natl. Acad. Sci. USA 114, E1365-E1374.

Greaves, J., Salaun, C., Fukata, Y., Fukata, M., Chamberlain, L.H., 2008. Palmitoylation and membrane interactions of the neuroprotective chaperone cysteine-string protein. J. Biol. Chem. 283, 25014-25026.

Hackmann, K., Rump, A., Haas, S.A., Lemke, J.R., Fryns, J.P., Tzschach, A., Wieczorek, D., Albrecht, B., Kuechler, A., Ripperger, T., Kobelt, A., Oexle, K., Tinschert, S., Schrock, E., Kalscheuer, V.M., Di Donato, N., 2016. Tentative clinical diagnosis of Lujan-Fryns syndrome-A conglomeration of different genetic entities? Am. J. Med. Genet. A 170a, 94-102.

Haines, R.J., Wang, C.Y., Yang, C.G.Y., Eitnier, R.A., Wang, F., Wu, M.H., 2017. Targeting palmitoyl acyltransferase ZDHHC21 improves gut epithelial barrier dysfunction resulting from burn-induced systemic inflammation. Am. J. Physiol. Gastrointest. Liver Physiol. 313, G549-G557.

Hallak, H., Muszbek, L., Laposata, M., Belmonte, E., Brass, L.F., Manning, D.R., 1994. Covalent binding of arachidonate to G protein alpha subunits of human platelets. J. Biol. Chem. 269, 4713-4716.

Hamel, L.D., Deschenes, R.J., Mitchell, D.A., 2014. A fluorescence-based assay to monitor autopalmitoylation of zDHHC proteins applicable to high-throughput screening. Anal. Biochem. 460, 1-8.

Han, J.Y., Lee, I.G., Shin, S., Kim, M., Jang, J.H., Park, J., 2017. The first patient with sporadic X-linked intellectual disability with de novo ZDHHC9 mutation identified by targeted next-generation sequencing. Eur. J. Med. Genet. 60, 499-503.

Hancock, J.F., Magee, A.I., Childs, J.E., Marshall, C.J., 1989. All Ras proteins are polyisoprenylated but only some are palmitoylated. Cell 57, 1167-1177.

Hang, H.C., Geutjes, E.J., Grotenbreg, G., Pollington, A.M., Bijlmakers, M.J., Ploegh, H.L., 2007. Chemical probes for the rapid detection of fatty-acylated proteins in mammalian cells. J. Am. Chem. Soc. 129, 2744-2745.

Hasan, M., Seo, J.E., Rahaman, K.A., Min, H., Kim, K.H., Park, J.H., Sung, C., Son, J., Kang, M.J., Jung, B.H., Park, W.S., Kwon, O.S., 2017. Novel genes in brain tissues of EAE-induced normal and obese mice: Upregulation of metal ion-binding protein genes in obese-EAE mice. Neuroscience 343, 322-336.

Hedberg, C., Dekker, F.J., Rusch, M., Renner, S., Wetzel, S., Vartak, N., Gerding-Reimers, C., Bon, R.S., Bastiaens, P.I.H., Waldmann, H., 2011. Development of highly potent inhibitors of the Ras-targeting human acyl protein thioesterases based on substrate similarity design. Angew. Chem. Int. Ed. Engl. 50, 9832-9837.

Howie, J., Reilly, L., Fraser, N.J., Vlachaki Walker, J.M., Wypijewski, K.J., Ashford, M.L., Calaghan, S.C., McClafferty, H., Tian, L., Shipston, M.J., Boguslavskyi, A., Shattock, M.J., Fuller, W., 2014. Substrate recognition by the cell surface palmitoyl transferase DHHC5. Proc. Natl. Acad. Sci. USA 111, 17534-17539.

Huang, K., Sanders, S., Singaraja, R., Orban, P., Cijsouw, T., Arstikaitis, P., Yanai, A., Hayden, M.R., El-Husseini, A., 2009. Neuronal palmitoyl acyl transferases exhibit distinct substrate specificity. FASEB J. 23, 2605-2615.

Huang, K., Yanai, A., Kang, R., Arstikaitis, P., Singaraja, R.R., Metzler, M., Mullard, A., Haigh, B., Gauthier-Campbell, C., Gutekunst, C.A., Hayden, M.R., El-Husseini, A., 2004. Huntingtin-interacting protein HIP14 is a palmitoyl transferase involved in palmitoylation and trafficking of multiple neuronal proteins. Neuron 44, 977-986.

Hurley, J.H., Cahill, A.L., Currie, K.P.M., Fox, A.P., 2000. The role of dynamic palmitoylation in Ca^{2+} channel inactivation. Proc. Natl. Acad. Sci. USA 97, 9293-9298.

Jennings, B.C., Linder, M.E., 2012. DHHC protein S-acyltransferases use similar ping-pong kinetic mechanisms but display different acyl-CoA specificities. J. Biol. Chem. 287, 7236-7245.

Jennings, B.C., Nadolski, M.J., Ling, Y., Baker, M.B., Harrison, M.L., Deschenes, R.J., Linder, M.E., 2009. 2-Bromopalmitate and 2-(2-hydroxy-5-nitro-benzylidene)-benzo[b]thiophen-3-one inhibit DHHC-mediated palmitoylation in vitro. J. Lipid Res. 50, 233-242.

Jia, L., Chisari, M., Maktabi, M.H., Sobieski, C., Zhou, H., Konopko, A.M., Martin, B.R., Mennerick, S.J., Blumer, K.J., 2014. A mechanism regulating G protein-coupled receptor signaling that requires cycles of protein palmitoylation and depalmitoylation. J. Biol. Chem. 289, 6249-6257.

Jia, L., Linder, M.E., Blumer, K.J., 2011. Gi/o signaling and the palmitoyltransferase DHHC2 regulate palmitate cycling and shuttling of RGS7 family-binding protein. J. Biol. Chem. 286, 13695-13703.

Jiang, H., Khan, S., Wang, Y., Charron, G., He, B., Sebastian, C., Du, J., Kim, R., Ge, E., Mostoslavsky, R., Hang, H.C., Hao, Q., Lin, H., 2013. SIRT6 regulates TNF-αsecretion through hydrolysis of long-chain fatty acyl lysine. Nature 496, 110-113.

Jiang, H., Zhang, X., Chen, X., Aramsangtienchai, P., Tong, Z., Lin, H., 2018. Protein Lipidation: Occurrence, Mechanisms, Biological Functions, and Enabling Technologies. Chem. Rev. 10.1021/acs.chemrev.6b00750.

Jiang, Y.-X., Du, Z.-M., Jiao, L., Shao, Q., Fu, S., Shao, J.-Y., Zhu, X.-F., Ernberg, I., Li, Y.-H., 2015. Inhibition of MiR-155 suppresses cell migration in nasopharyngeal carcinoma through targeting ZDHHC2. Int. J. Clin. Exp. Med. 8, 8472-8484.

Jing, H., Zhang, X., Wisner, S.A., Chen, X., Spiegelman, N.A., Linder, M.E., Lin, H., 2017. SIRT2 and lysine fatty acylation regulate the transforming activity of K-Ras4a. Elife 6, e32436.

Jochen, A.L., Hays, J., Mick, G., 1995. Inhibitory effects of cerulenin on protein palmitoylation and insulin internalization in rat adipocytes. Biochim. Biophys. Acta 1259, 65-72.

Kang, J., Lee, H.J., Kim, J., Lee, J.J., Maeng, L.S., 2015. Dysregulation of X chromosome inactivation in high grade ovarian serous adenocarcinoma. PLoS One 10, e0118927.

Kang, R., Wan, J., Arstikaitis, P., Takahashi, H., Huang, K., Bailey, A.O., Thompson, J.X., Roth, A.F., Drisdel, R.C., Mastro, R., Green, W.N., Yates, J.R., Davis, N.G., El-Husseini, A., 2008. Neural palmitoyl-proteomics reveals dynamic synaptic palmitoylation. Nature 456, 904-909.

Karaman, M.W., Herrgard, S., Treiber, D.K., Gallant, P., Atteridge, C.E., Campbell, B.T., Chan, K.W., Ciceri, P., Davis, M.I., Edeen, P.T., Faraoni, R., Floyd, M., Hunt, J.P., Lockhart, D.J., Milanov, Z.V., Morrison, M.J., Pallares, G., Patel, H.K., Pritchard, S., Wodicka, L.M., Zarrinkar, P.P., 2008. A quantitative analysis of kinase inhibitor selectivity. Nat. Biotechnol. 26, 127-132.

Keller, C.A., Yuan, X., Panzanelli, P., Martin, M.L., Alldred, M., Sassoe-Pognetto, M., Luscher, B., 2004. The gamma2 subunit of GABA(A) receptors is a substrate for palmitoylation by GODZ. J. Neurosci. 24, 5881-5891.

Kim, Y., Yang, H., Min, J.K., Park, Y.J., Jeong, S.H., Jang, S.W., Shim, S., 2018. CCN3 secretion is regulated by palmitoylation via ZDHHC22. Biochem. Biophys. Res. Commun. 495, 2573-2578.

Kinoshita, T., 2014. Enzymatic mechanism of GPI anchor attachment clarified. Cell Cycle 13, 1838-1839.

Kokkola, T., Kruse, C., Roy-Pogodzik, E.M., Pekkinen, J., Bauch, C., Honck, H.H., Hennemann, H., Kreienkamp, H.J., 2011. Somatostatin receptor 5 is palmitoylated by the interacting ZDHHC5 palmitoyltransferase. FEBS Lett. 585, 2665-2670.

Kong, E., Peng, S., Chandra, G., Sarkar, C., Zhang, Z., Bagh, M.B., Mukherjee, A.B., 2013. Dynamic palmitoylation links cytosol-membrane shuttling of acyl-protein thioesterase-1 and acyl-protein thioesterase-2 with that of proto-oncogene H-Ras product and growth-associated protein-43. J. Biol. Chem. 288, 9112-9125.

Lach, A., Grzybek, M., Heger, E., Korycka, J., Wolny, M., Kubiak, J., Kolondra, A., Boguslawska, D.M., Augoff, K., Majkowski, M., Podkalicka, J., Kaczor, J., Stefanko, A., Kuliczkowski, K., Sikorski, A.F., 2012. Palmitoylation of MPP1 (membrane-palmitoylated protein 1)/p55 is crucial for lateral membrane organization in erythroid cells. J. Biol. Chem. 287, 18974-18984.

Lai, Z., Lin, P., Weng, X., Su, J., Chen, Y., He, Y., Wu, G., Wang, J., Yu, Y., Zhang, L., 2017. MicroRNA-574-5p promotes cell growth of vascular smooth muscle cells in the progression of coronary artery disease. Biomed. Pharmacother. 97, 162-167.

Lakkaraju, A.K., Abrami, L., Lemmin, T., Blaskovic, S., Kunz, B., Kihara, A., Dal Peraro, M., van der Goot, F.G., 2012. Palmitoylated calnexin is a key component of the ribosome-translocon complex. EMBO J. 31, 1823-1835.

Lane, K.T., Beese, L.S., 2006. Thematic review series: lipid posttranslational modifications. Structural biology of protein farnesyltransferase and geranylgeranyltransferase type I. J. Lipid Res. 47, 681-699.

Lawrence, D.S., Zilfou, J.T., Smith, C.D., 1999. Structure-activity studies of cerulenin analogues as protein palmitoylation inhibitors. J. Med. Chem. 42, 4932-4941.

Lemarié, F., Beauchamp, E., Legrand, P., Rioux, V., 2016. Revisiting the metabolism and physiological functions of caprylic acid (C8: 0) with special focus on ghrelin octanoylation. Biochimie 120, 40-48.

Li, S.X., Tang, G.S., Zhou, D.X., Pan, Y.F., Tan, Y.X., Zhang, J., Zhang, B., Ding, Z.W., Liu, L.J., Jiang, T.Y., Hu, H.P., Dong, L.W., Wang, H.Y., 2014. Prognostic significance of cytoskeleton-associated membrane protein 4 and its palmitoyl acyltransferase DHHC2 in hepatocellular carcinoma. Cancer 120, 1520-1531.

Li, Y., Martin, B.R., Cravatt, B.F., Hofmann, S.L., 2012. DHHC5 protein palmitoylates flotillin-2 and is rapidly degraded on induction of neuronal differentiation in cultured cells. J. Biol. Chem. 287, 523-530.

Liang, X., Nazarian, A., Erdjument-Bromage, H., Bornmann, W., Tempst, P., Resh, M.D., 2001. Heterogeneous fatty acylation of Src family kinases with polyunsaturated fatty acids regulates raft localization and signal transduction. J. Biol. Chem. 276, 30987-30994.

Lievens, P.M., Kuznetsova, T., Kochlamazashvili, G., Cesca, F., Gorinski, N., Galil, D.A., Cherkas, V., Ronkina, N., Lafera, J., Gaestel, M., Ponimaskin, E., Dityatev, A., 2016. ZDHHC3 tyrosine phosphorylation regulates neural cell adhesion molecule palmitoylation. Mol. Cell. Biol. 36, 2208-2225.

Lin, D.T., Conibear, E., 2015. ABHD17 proteins are novel protein depalmitoylases that regulate N-Ras palmitate turnover and subcellular localization. Elife 4, e11306.

Lin, P.-C., Giannopoulou, E.G., Park, K., Mosquera, J.M., Sboner, A., Tewari, A.K., Garraway, L.A., Beltran, H., Rubin, M.A., Elemento, O., 2013. Epigenomic alterations in localized and advanced prostate cancer. Neoplasia 15, 373-383.

Linder, M.E., Deschenes, R.J., 2007. Palmitoylation: Policing protein stability and traffic. Nat. Rev. Mol. Cell Biol. 8, 74-84.

Liu, K.M., Chen, Y.J., Shen, L.F., Haddad, A.N.S., Song, I.W., Chen, L.Y., Chen, Y.J., Wu, J.Y., Yen, J.J.Y., Chen, Y.T., 2015. Cyclic alopecia and abnormal epidermal cornification in Zdhhc13-deficient mice reveal the importance of palmitoylation in hair and skin differentiation. J. Invest. Dermatol. 135, 2603-2610.

Liu, P., Jiao, B., Zhang, R., Zhao, H., Zhang, C., Wu, M., Li, D., Zhao, X., Qiu, Q., Li, J., Ren, R., 2016. Palmitoylacyltransferase Zdhhc9 inactivation mitigates leukemogenic potential of oncogenic N-Ras. Leukemia 30, 1225-1228.

Lobo, S., 2013. Discovery of selective and potent inhibitors of palmitoylation. InTech.

Lobo, S., Greentree, W.K., Linder, M.E., Deschenes, R.J., 2002. Identification of a Ras palmitoyltransferase in Saccharomyces cerevisiae. J. Biol. Chem. 277, 41268-41273.

Lu, D., Sun, H.Q., Wang, H., Barylko, B., Fukata, Y., Fukata, M., Albanesi, J.P., Yin, H.L., 2012. Phosphatidylinositol 4-kinase IIalpha is palmitoylated by Golgi-localized palmitoyltransferases in cholesterol-dependent manner. J. Biol. Chem. 287, 21856-21865.

Mansouri, M.R., Marklund, L., Gustavsson, P., Davey, E., Carlsson, B., Larsson, C., White, I., Gustavson, K.-H., Dahl, N., 2005. Loss of ZDHHC15 expression in a woman with a balanced translocation t(X;15)(q13.3;cen) and severe mental retardation. Eur. J. Hum. Genet. 13, 970-977.

Marin, E.P., Derakhshan, B., Lam, T.T., Davalos, A., Sessa, W.C., 2012. Endothelial cell palmitoylproteomic identifies novel lipid-modified targets and potential substrates for protein acyl transferases. Circ. Res. 110, 1336-1344.

Marin, E.P., Jozsef, L., Di Lorenzo, A., Held, K.F., Luciano, A.K., Melendez, J., Milstone, L.M., Velazquez, H., Sessa, W.C., 2016. The protein acyl transferase ZDHHC21 modulates alpha1 adrenergic receptor function and regulates hemodynamics. Arterioscler. Thromb. Vasc. Biol. 36, 370-379.

Martin, B.R., Wang, C., Adibekian, A., Tully, S.E., Cravatt, B.F., 2012. Global profiling of dynamic protein palmitoylation. Nat. Methods. 9, 84-89.

Maynard, T.M., Meechan, D.W., Dudevoir, M.L., Gopalakrishna, D., Peters, A.Z., Heindel, C.C., Sugimoto, T.J., Wu, Y., Lieberman, J.A., LaMantia, A.S., 2008. Mitochondrial localization and

function of a subset of 22q11 deletion syndrome candidate genes. Mol. Cell. Neurosci. 39, 439-451.

McClure, M.L., Wen, H., Fortenberry, J., Hong, J.S., Sorscher, E.J., 2014. S-palmitoylation regulates biogenesis of core glycosylated wild-type and F508del CFTR in a post-ER compartment. Biochem. J. 459, 417-425.

Meng, S., Lu, C., Zhang, W., Shen, W., Wei, Y., Su, D., Zhou, F., 2016. MMSA-1 expression pattern in multiple myeloma and its clinical significance. Clin. Exp. Med. 16, 599-609.

Milde, S., Coleman, M.P., 2014. Identification of palmitoyltransferase and thioesterase enzymes that control the subcellular localization of axon survival factor nicotinamide mononucleotide adenylyltransferase 2 (NMNAT2). J. Biol. Chem. 289, 32858-32870.

Mill, P., Lee, A.W., Fukata, Y., Tsutsumi, R., Fukata, M., Keighren, M., Porter, R.M., McKie, L., Smyth, I., Jackson, I.J., 2009. Palmitoylation regulates epidermal homeostasis and hair follicle differentiation. PLoS Genet. 5, e1000748.

Mitchell, D.A., Hamel, L.D., Reddy, K.D., Farh, L., Rettew, L.M., Sanchez, P.R., Deschenes, R.J., 2014. Mutations in the X-linked intellectual disability gene, zDHHC9, alter autopalmitoylation activity by distinct mechanisms. J. Biol. Chem. 289, 18582-18592.

Mitchell, D.A., Mitchell, G., Ling, Y., Budde, C., Deschenes, R.J., 2010. Mutational analysis of saccharomyces cerevisiae Erf2 reveals a two-step reaction mechanism for protein palmitoylation by DHHC enzymes. J. Biol. Chem. 285, 38104-38114.

Mitchell, D.A., Vasudevan, A., Linder, M.E., Deschenes, R.J., 2006. Protein palmitoylation by a family of DHHC protein S-acyltransferases. J. Lipid Res. 47, 1118-1127.

Mizumaru, C., Saito, Y., Ishikawa, T., Yoshida, T., Yamamoto, T., Nakaya, T., Suzuki, T., 2009. Suppression of APP-containing vesicle trafficking and production of beta-amyloid by AID/DHHC-12 protein. J. Neurochem. 111, 1213-1224.

Moche, M., Schneider, G., Edwards, P., Dehesh, K., Lindqvist, Y., 1999. Structure of the complex between the antibiotic cerulenin and its target, β-ketoacyl-acyl carrier protein synthase. J. Biol. Chem. 274, 6031-6034.

Moutin, E., Nikonenko, I., Stefanelli, T., Wirth, A., Ponimaskin, E., De Roo, M., Muller, D., 2017. Palmitoylation of cdc42 promotes spine stabilization and rescues spine density deficit in a mouse model of 22q11.2 deletion syndrome. Cereb. Cortex 27, 3618-3629.

Mukai, J., Liu, H., Burt, R.A., Swor, D.E., Lai, W.S., Karayiorgou, M., Gogos, J.A., 2004. Evidence that the gene encoding ZDHHC8 contributes to the risk of schizophrenia. Nat. Genet. 36, 725-731.

Mukai, J., Tamura, M., Fenelon, K., Rosen, A.M., Spellman, T.J., Kang, R., MacDermott, A.B., Karayiorgou, M., Gordon, J.A., Gogos, J.A., 2015. Molecular substrates of altered axonal growth and brain connectivity in a mouse model of schizophrenia. Neuron 86, 680-695.

Murphy, J., Kolandaivelu, S., 2016. Palmitoylation of progressive rod-cone degeneration (PRCD) regulates protein stability and localization. J. Biol. Chem. 291, 23036-23046.

Muszbek, L., Haramura, G., Cluette-Brown, J.E., Van Cott, E.M., Laposata, M., 1999. The pool of fatty acids covalently bound to platelet proteins by thioester linkages can be altered by exogenously supplied fatty acids. Lipids 34 Suppl, S331-S337.

Napoli, E., Song, G., Liu, S., Espejo, A., Perez, C.J., Benavides, F., Giulivi, C., 2017. Zdhhc13-dependent Drp1 S-palmitoylation impacts brain bioenergetics, anxiety, coordination and motor skills. Sci. Rep. 7, 12796.

Ng, M.C.Y., Graff, M., Lu, Y., Justice, A.E., Mudgal, P., Liu, C.T., Young, K., Yanek, L.R., Feitosa, M.F., Wojczynski, M.K., Rand, K., Brody, J.A., Cade, B.E., Dimitrov, L., Duan, Q.,

Guo, X., Lange, L.A., Nalls, M.A., Okut, H., Tajuddin, S.M., Tayo, B.O., Vedantam, S., Bradfield, J.P., Chen, G., Chen, W.M., Chesi, A., Irvin, M.R., Padhukasahasram, B., Smith, J.A., Zheng, W., Allison, M.A., Ambrosone, C.B., Bandera, E.V., Bartz, T.M., Berndt, S.I., Bernstein, L., Blot, W.J., Bottinger, E.P., Carpten, J., Chanock, S.J., Chen, Y.I., Conti, D.V., Cooper, R.S., Fornage, M., Freedman, B.I., Garcia, M., Goodman, P.J., Hsu, Y.H., Hu, J., Huff, C.D., Ingles, S.A., John, E.M., Kittles, R., Klein, E., Li, J., McKnight, B., Nayak, U., Nemesure, B., Ogunniyi, A., Olshan, A., Press, M.F., Rohde, R., Rybicki, B.A., Salako, B., Sanderson, M., Shao, Y., Siscovick, D.S., Stanford, J.L., Stevens, V.L., Stram, A., Strom, S.S., Vaidya, D., Witte, J.S., Yao, J., Zhu, X., Ziegler, R.G., Zonderman, A.B., Adeyemo, A., Ambs, S., Cushman, M., Faul, J.D., Hakonarson, H., Levin, A.M., Nathanson, K.L., Ware, E.B., Weir, D.R., Zhao, W., Zhi, D., Bone Mineral Density in Childhood Study, G., Arnett, D.K., Grant, S.F.A., Kardia, S.L.R., Oloapde, O.I., Rao, D.C., Rotimi, C.N., Sale, M.M., Williams, L.K., Zemel, B.S., Becker, D.M., Borecki, I.B., Evans, M.K., Harris, T.B., Hirschhorn, J.N., Li, Y., Patel, S.R., Psaty, B.M., Rotter, J.I., Wilson, J.G., Bowden, D.W., Cupples, L.A., Haiman, C.A., Loos, R.J.F., North, K.E., 2017. Discovery and fine-mapping of adiposity loci using high density imputation of genome-wide association studies in individuals of African ancestry: African ancestry anthropometry genetics consortium. PLoS Genet. 13, e1006719.

O'Brien, P.J., Zatz, M., 1984. Acylation of bovine rhodopsin by [^{3}H]palmitic acid. J. Biol. Chem. 259, 5054-5057.

Ohno, Y., Kihara, A., Sano, T., Igarashi, Y., 2006. Intracellular localization and tissue-specific distribution of human and yeast DHHC cysteine-rich domain-containing proteins. Biochim. Biophys. Acta 1761, 474-483.

Oku, S., Takahashi, N., Fukata, Y., Fukata, M., 2013. In silico screening for palmitoyl substrates reveals a role for DHHC1/3/10 (zDHHC1/3/11)-mediated neurochondrin palmitoylation in its targeting to Rab5-positive endosomes. J. Biol. Chem. 288, 19816-19829.

Oo, H.Z., Sentani, K., Sakamoto, N., Anami, K., Naito, Y., Uraoka, N., Oshima, T., Yanagihara, K., Oue, N., Yasui, W., 2014. Overexpression of ZDHHC14 promotes migration and invasion of scirrhous type gastric cancer. Oncol. Rep. 32, 403-410.

Ota, V.K., Gadelha, A., Assunção, I.B., Santoro, M.L., Christofolini, D.M., Bellucco, F.T., Santos-Filho, A.F., Ottoni, G.L., Lara, D.R., Mari, J.J., Melaragno, M.I., Smith, M.A.C., Bressan, R.A., Belangero, S.I., Jackowski, A.P., 2013. ZDHHC8 gene may play a role in cortical volumes of patients with schizophrenia. Schizophr. Res. 145, 33-35.

Otani, K., Ujike, H., Tanaka, Y., Morita, Y., Kishimoto, M., Morio, A., Uchida, N., Nomura, A., Kuroda, S., 2005. The ZDHHC8 gene did not associate with bipolar disorder or schizophrenia. Neurosci. Lett. 390, 166-170.

Oyama, T., Miyoshi, Y., Koyama, K., Nakagawa, H., Yamori, T., Ito, T., Matsuda, H., Arakawa, H., Nakamura, Y., 2000. Isolation of a novel gene on 8p21.3-22 whose expression is reduced significantly in human colorectal cancers with liver metastasis. Genes Chromosom. Cancer 29, 9-15.

Patterson, S.I., Pate Skene, J.H., 1995. Inhibition of dynamic protein palmitoylation in intact cells with tunicamycin, Methods Enzymol. Academic Press, pp. 284-300.

Pedram, A., Razandi, M., Deschenes, R.J., Levin, E.R., 2012. DHHC-7 and -21 are palmitoylacyltransferases for sex steroid receptors. Mol. Biol. Cell 23, 188-199.

Peng, C., Zhang, Z., Wu, J., Lv, Z., Tang, J., Xie, H., Zhou, L., Zheng, S., 2014. A critical role for ZDHHC2 in metastasis and recurrence in human hepatocellular carcinoma. BioMed Res. Int. 2014.

Peng, T., Hang, H.C., 2015. Bifunctional fatty acid chemical reporter for analyzing *S*-palmitoylated membrane protein-protein interactions in mammalian cells. J. Am. Chem. Soc. 137, 556-559.

Percher, A., Ramakrishnan, S., Thinon, E., Yuan, X., Yount, J.S., Hang, H.C., 2016. Mass-tag labeling reveals site-specific and endogenous levels of protein S-fatty acylation. Proc. Natl. Acad. Sci. USA 113, 4302-4307.

Perez, C.J., Mecklenburg, L., Jaubert, J., Martinez-Santamaria, L., Iritani, B.M., Espejo, A., Napoli, E., Song, G., Del Rio, M., DiGiovanni, J., Giulivi, C., Bedford, M.T., Dent, S.Y.R., Wood, R.D., Kusewitt, D.F., Guenet, J.L., Conti, C.J., Benavides, F., 2015. Increased susceptibility to skin carcinogenesis associated with a spontaneous mouse mutation in the palmitoyl transferase Zdhhc13 gene. J. Invest. Dermatol. 135, 3133-3143.

Pils, D., Horak, P., Gleiss, A., Sax, C., Fabjani, G., Moebus, V.J., Zielinski, C., Reinthaller, A., Zeillinger, R., Krainer, M., 2005. Five genes from chromosomal band 8p22 are significantly down-regulated in ovarian carcinoma: N33 and EFA6R have a potential impact on overall survival. Cancer 104, 2417-2429.

Planey, S.L., Keay, S.K., Zhang, C.O., Zacharias, D.A., 2009. Palmitoylation of cytoskeleton associated protein 4 by DHHC2 regulates antiproliferative factor-mediated signaling. Mol. Biol. Cell 20, 1454-1463.

Planey, S.L., Zacharias, D.A., 2010. Identification of targets and inhibitors of protein palmitoylation. Expert Opin. Drug Discov. 5, 155-164.

Porter, J.A., Young, K.E., Beachy, P.A., 1996. Cholesterol modification of Hedgehog signaling proteins in animal development. Science 274, 255-259.

Rana, M.S., Kumar, P., Lee, C.-J., Verardi, R., Rajashankar, K.R., Banerjee, A., 2018. Fatty acyl recognition and transfer by an integral membrane *S*-acyltransferase. Science 359, 10.1126/science.aao6326.

Raymond, F.L., Tarpey, P.S., Edkins, S., Tofts, C., O'Meara, S., Teague, J., Butler, A., Stevens, C., Barthorpe, S., Buck, G., Cole, J., Dicks, E., Gray, K., Halliday, K., Hills, K., Hinton, J., Jones, D., Menzies, A., Perry, J., Raine, K., Shepherd, R., Small, A., Varian, J., Widaa, S., Mallya, U., Moon, J., Luo, Y., Shaw, M., Boyle, J., Kerr, B., Turner, G., Quarrell, O., Cole, T., Easton, D.F., Wooster, R., Bobrow, M., Schwartz, C.E., Gecz, J., Stratton, M.R., Futreal, P.A., 2007. Mutations in ZDHHC9, which encodes a palmitoyltransferase of NRAS and HRAS, cause X-linked mental retardation associated with a Marfanoid habitus. Am. J. Hum. Genet. 80, 982-987.

Ren, W., Sun, Y., Du, K., 2013. DHHC17 palmitoylates ClipR-59 and modulates ClipR-59 association with the plasma membrane. Mol. Cell. Biol. 33, 4255-4265.

Resh, M.D., 2006a. Palmitoylation of ligands, receptors, and intracellular signaling molecules. Science's STKE 2006, re14-re14.

Resh, M.D., 2006b. Trafficking and signaling by fatty-acylated and prenylated proteins. Nat. Chem. Biol. 2, 584-590.

Rossin, A., Durivault, J., Chakhtoura-Feghali, T., Lounnas, N., Gagnoux-Palacios, L., Hueber, A.O., 2015. Fas palmitoylation by the palmitoyl acyltransferase DHHC7 regulates Fas stability. Cell Death Differ. 22, 643-653.

Roth, A.F., Feng, Y., Chen, L., Davis, N.G., 2002. The yeast DHHC cysteine-rich domain protein Akr1p is a palmitoyl transferase. J. Cell Biol. 159, 23-28.

Roth, A.F., Wan, J., Bailey, A.O., Sun, B., Kuchar, J.A., Green, W.N., Phinney, B.S., Yates, J.R., 3rd, Davis, N.G., 2006. Global analysis of protein palmitoylation in yeast. Cell 125, 1003-1013.

Runkle, K.B., Kharbanda, A., Stypulkowski, E., Cao, X.J., Wang, W., Garcia, B.A., Witze, E.S., 2016. Inhibition of DHHC20-mediated EGFR palmitoylation creates a dependence on EGFR signaling. Mol. Cell 62, 385-396.

Rush, D.B., Leon, R.T., McCollum, M.H., Treu, R.W., Wei, J., 2012. Palmitoylation and trafficking of GAD65 are impaired in a cellular model of Huntington's disease. Biochem. J. 442, 39-48.

Saito, S., Ikeda, M., Iwata, N., Suzuki, T., Kitajima, T., Yamanouchi, Y., Kinoshita, Y., Takahashi, N., Inada, T., Ozaki, N., 2005. No association was found between a functional SNP in ZDHHC8 and schizophrenia in a Japanese case-control population. Neurosci. Lett. 374, 21-24.

Salaun, C., Greaves, J., Chamberlain, L.H., 2010. The intracellular dynamic of protein palmitoylation. J. Cell Biol. 191, 1229-1238.

Saleem, A.N., Chen, Y.-H., Baek, H.J., Hsiao, Y.-W., Huang, H.-W., Kao, H.-J., Liu, K.-M., Shen, L.-F., Song, I.w., Tu, C.-P.D., Wu, J.-Y., Kikuchi, T., Justice, M.J., Yen, J.J.Y., Chen, Y.-T., 2010. Mice with alopecia, osteoporosis, and systemic amyloidosis due to mutation in Zdhhc13, a gene coding for palmitoyl acyltransferase. PLoS Genet. 6, e1000985.

Sanders, S.S., Hou, J., Sutton, L.M., Garside, V.C., Mui, K.K.N., Singaraja, R.R., Hayden, M.R., Hoodless, P.A., 2015. Huntingtin interacting proteins 14 and 14-like are required for chorioallantoic fusion during early placental development. Dev. Biol. 397, 257-266.

Sanders, S.S., Parsons, M.P., Mui, K.K., Southwell, A.L., Franciosi, S., Cheung, D., Waltl, S., Raymond, L.A., Hayden, M.R., 2016. Sudden death due to paralysis and synaptic and behavioral deficits when Hip14/Zdhhc17 is deleted in adult mice. BMC Biol. 14, 108.

Sandoz, P.A., van der Goot, F.G., 2015. How many lives does CLIMP-63 have? Biochem. Soc. Trans. 43, 222-228.

Sardjono, C.T., Harbour, S.N., Yip, J.C., Paddock, C., Tridandapani, S., Newman, P.J., Jackson, D.E., 2006. Palmitoylation at Cys595 is essential for PECAM-1 localisation into membrane microdomains and for efficient PECAM-1-mediated cytoprotection. Thromb. Haemost. 96, 756-766.

Schlesinger, M.J., Magee, A.I., Schmidt, M.F., 1980. Fatty acid acylation of proteins in cultured cells. J. Biol. Chem. 255, 10021-10024.

Schlesinger, M.J., Malfer, C., 1982. Cerulenin blocks fatty acid acylation of glycoproteins and inhibits vesicular stomatitis and Sindbis virus particle formation. J. Biol. Chem. 257, 9887-9890.

Schmidt, M.F., Bracha, M., Schlesinger, M.J., 1979. Evidence for covalent attachment of fatty acids to Sindbis virus glycoproteins. Proc. Natl. Acad. Sci. USA 76, 1687-1691.

Schmidt, M.F., Schlesinger, M.J., 1979. Fatty acid binding to vesicular stomatitis virus glycoprotein: a new type of post-translational modification of the viral glycoprotein. Cell 17, 813-819.

Segal-Salto, M., Sapir, T., Reiner, O., 2016. Reversible cysteine acylation regulates the activity of human palmitoyl-protein thioesterase 1 (PPT1). PLoS One 11, e0146466.

Shahinian, S., Silvius, J.R., 1995. Doubly-lipid-modified protein sequence motifs exhibit long-lived anchorage to lipid bilayer membranes. Biochemistry 34, 3813-3822.

Sharma, C., Rabinovitz, I., Hemler, M.E., 2012. Palmitoylation by DHHC3 is critical for the function, expression, and stability of integrin α6β4. Cell. Mol. Life Sci. 69, 2233-2244.

Sharma, C., Wang, H.X., Li, Q., Knoblich, K., Reisenbichler, E.S., Richardson, A.L., Hemler, M.E., 2017. Protein acyltransferase DHHC3 regulates breast tumor growth, oxidative stress, and senescence. Cancer Res. 77, 6880-6890.

Sharma, C., Yang, X.H., Hemler, M.E., 2008. DHHC2 affects palmitoylation, stability, and functions of tetraspanins CD9 and CD151. Mol. Biol. Cell 19, 3415-3425.

Shen, L.F., Chen, Y.J., Liu, K.M., Haddad, A.N.S., Song, I.W., Roan, H.Y., Chen, L.Y., Yen, J.J.Y., Chen, Y.J., Wu, J.Y., Chen, Y.T., 2017. Role of S-Palmitoylation by ZDHHC13 in mitochondrial function and metabolism in liver. Sci. Rep. 7, 2182.

Shi, W., Chen, X., Wang, F., Gao, M., Yang, Y., Du, Z., Wang, C., Yao, Y., He, K., Hao, A., 2016. ZDHHC16 modulates FGF/ERK dependent proliferation of neural stem/progenitor cells in the zebrafish telencephalon. Dev. Neurobiol. 76, 1014-1028.

Shi, W., Wang, F., Gao, M., Yang, Y., Du, Z., Wang, C., Yao, Y., He, K., Chen, X., Hao, A., 2015. ZDHHC17 promotes axon outgrowth by regulating TrkA-tubulin complex formation. Mol. Cell. Neurosci. 68, 194-202.

Sigala, S., Bodei, S., Missale, C., Zani, D., Simeone, C., Cunico, S.C., Spano, P.F., 2008. Gene expression profile of prostate cancer cell lines: effect of nerve growth factor treatment. Mol. Cell. Endocrinol. 284, 11-20.

Singaraja, R.R., Huang, K., Sanders, S.S., Milnerwood, A.J., Hines, R., Lerch, J.P., Franciosi, S., Drisdel, R.C., Vaid, K., Young, F.B., Doty, C., Wan, J., Bissada, N., Henkelman, R.M., Green, W.N., Davis, N.G., Raymond, L.A., Hayden, M.R., 2011. Altered palmitoylation and neuropathological deficits in mice lacking HIP14. Hum. Mol. Genet. 20, 3899-3909.

Singaraja, R.R., Kang, M.H., Vaid, K., Sanders, S.S., Vilas, G.L., Arstikaitis, P., Coutinho, J., Drisdel, R.C., El-Husseini Ael, D., Green, W.N., Berthiaume, L., Hayden, M.R., 2009. Palmitoylation of ATP-binding cassette transporter A1 is essential for its trafficking and function. Circ. Res. 105, 138-147.

Skotte, N.H., Sanders, S.S., Singaraja, R.R., Ehrnhoefer, D.E., 2017. Palmitoylation of caspase-6 by HIP14 regulates its activation. Cell Death Differ. 24, 433-444.

Smotrys, J.E., Linder, M.E., 2004. Palmitoylation of Intracellular Signaling Proteins: Regulation and Function. Annu. Rev. Biochem. 73, 559-587.

Solomon, S., Masilamani, M., Rajendran, L., Bastmeyer, M., Stuermer, C.A., Illges, H., 2002. The lipid raft microdomain-associated protein reggie-1/flotillin-2 is expressed in human B cells and localized at the plasma membrane and centrosome in PBMCs. Immunobiology 205, 108-119.

Song, I.W., Li, W.R., Chen, L.Y., Shen, L.F., Liu, K.M., Yen, J.J., Chen, Y.J., Chen, Y.J., Kraus, V.B., Wu, J.Y., Lee, M.T., Chen, Y.T., 2014. Palmitoyl acyltransferase, Zdhhc13, facilitates bone mass acquisition by regulating postnatal epiphyseal development and endochondral ossification: a mouse model. PLoS One 9, e92194.

Speers, A.E., Cravatt, B.F., 2004. Profiling enzyme activities in vivo using click chemistry methods. Chem. Biol. 11, 535-546.

Stoffyn, P., Folch-Pi, J., 1971. On the type of linkage binding fatty acids present in brain white matter proteolipid apoprotein. Biochem. Biophys. Res. Commun. 44, 157-161.

Swarthout, J.T., Lobo, S., Farh, L., Croke, M.R., Greentree, W.K., Deschenes, R.J., Linder, M.E., 2005. DHHC9 and GCP16 constitute a human protein fatty acyltransferase with specificity for H- and N-Ras. J. Biol. Chem. 280, 31141-31148.

Taruno, A., Sun, H., Nakajo, K., Murakami, T., Ohsaki, Y., Kido, M.A., Ono, F., Marunaka, Y., 2017. Post-translational palmitoylation controls the voltage gating and lipid raft association of CALHM1 channel. J. Physiol. 595, 6121-6145.

Thomas, G.M., Hayashi, T., Chiu, S.L., Chen, C.M., Huganir, R.L., 2012. Palmitoylation by DHHC5/8 targets GRIP1 to dendritic endosomes to regulate AMPA-R trafficking. Neuron 73, 482-496.

Thomas, G.M., Hayashi, T., Huganir, R.L., Linden, D.J., 2013. DHHC8-dependent PICK1 palmitoylation is required for induction of cerebellar long-term synaptic depression. J. Neurosci. 33, 15401-15407.

Tian, L., McClafferty, H., Jeffries, O., Shipston, M.J., 2010. Multiple palmitoyltransferases are required for palmitoylation-dependent regulation of large conductance calcium-and voltage-activated potassium channels. J. Biol. Chem. 285, 23954-23962.

Tian, L., McClafferty, H., Knaus, H.G., Ruth, P., Shipston, M.J., 2012. Distinct acyl protein transferases and thioesterases control surface expression of calcium-activated potassium channels. J. Biol. Chem. 287, 14718-14725.

Tomatis, V.M., Trenchi, A., Gomez, G.A., Daniotti, J.L., 2010. Acyl-protein thioesterase 2 catalizes the deacylation of peripheral membrane-associated GAP-43. PloS one 5, e15045.

Veit, M., Reverey, H., Schmidt, M.F., 1996. Cytoplasmic tail length influences fatty acid selection for acylation of viral glycoproteins. Biochem. J. 318, 163-172.

Verardi, R., Kim, J.S., Ghirlando, R., Banerjee, A., 2017. Structural basis for substrate recognition by the ankyrin repeat domain of human DHHC17 palmitoyltransferase. Structure (London, England : 1993) 25, 1337-1347.

Vetrivel, K.S., Meckler, X., Chen, Y., Nguyen, P.D., Seidah, N.G., Vassar, R., Wong, P.C., Fukata, M., Kounnas, M.Z., Thinakaran, G., 2009. Alzheimer disease aβproduction in the absence of s-palmitoylation-dependent targeting of BACE1 to lipid rafts. J. Biol. Chem. 284, 3793-3803.

Wan, J., Roth, A.F., Bailey, A.O., Davis, N.G., 2007. Palmitoylated proteins: purification and identification. Nat. Protoc. 2, 1573-1584.

Wang, F., Chen, X., Shi, W., Yao, L., Gao, M., Yang, Y., Hao, A., 2015. Zdhhc15b regulates differentiation of diencephalic dopaminergic neurons in zebrafish. J. Cell. Biochem. 116, 2980-2991.

Wang, J., Xie, Y., Wolff, D.W., Abel, P.W., Tu, Y., 2010. DHHC protein-dependent palmitoylation protects regulator of G-protein signaling 4 from proteasome degradation. FEBS Lett. 584, 4570-4574.

Wang, Q., Chan, T.R., Hilgraf, R., Fokin, V.V., Sharpless, K.B., Finn, M.G., 2003. Bioconjugation by Copper(I)-Catalyzed Azide-Alkyne [3 + 2] Cycloaddition. J. Am. Chem. Soc. 125, 3192-3193.

Wang, S., Mott, K.R., Cilluffo, M., Kilpatrick, C.L., Murakami, S., Ljubimov, A.V., Kousoulas, K.G., Awasthi, S., Luscher, B., Ghiasi, H., 2017a. The absence of DHHC3 affects primary and latent HSV-1 infection. J. Virol., 92, e01599.

Wang, S., Mott, K.R., Wawrowsky, K., Kousoulas, K.G., Luscher, B., Ghiasi, H., 2017b. Binding of Herpes simplex virus 1 UL20 to GODZ (DHHC3) affects its palmitoylation and is essential for infectivity and proper targeting and localization of UL20 and Glycoprotein K. J. Virol. 91, e00945.

Webb, Y., Hermida-Matsumoto, L., Resh, M.D., 2000. Inhibition of protein palmitoylation, raft localization, and T cell signaling by 2-bromopalmitate and polyunsaturated fatty acids. J. Biol. Chem. 275, 261-270.

Won, S.J., Cheung See Kit, M., Martin, B.R., 2017. Protein depalmitoylases. Crit. Rev. Biochem. Mol. Biol., 1-16.

Woolfrey, K.M., Sanderson, J.L., Dell'Acqua, M.L., 2015. The palmitoyl acyltransferase DHHC2 regulates recycling endosome exocytosis and synaptic potentiation through palmitoylation of AKAP79/150. J. Neurosci. 35, 442-456.

Wright, M.H., Heal, W.P., Mann, D.J., Tate, E.W., 2010. Protein myristoylation in health and disease. J. Chem. Biol. 3, 19-35.

Wu, J.F., Lee, C.H., Chen, H.L., Ni, Y.H., Hsu, H.Y., Sheu, J.C., Tsuei, D.J., Chang, M.H., 2013. Copy-number variations in hepatoblastoma associate with unique clinical features. Hepatol. Int. 7, 208-214.

Xiaonan, Z., Jiannan, S., Mei-Heng, M., Edwards, L.J., Zeisel, S.H., 2003. Phosphatidylethanolamine N-methyltransferase (PEMT) knockout mice have hepatic steatosis and abnormal hepatic choline metabolite concentrations despite ingesting a recommended dietary intake of choline. Biochem. J. 370, 987-993.

Yamamoto, Y., Chochi, Y., Matsuyama, H., Eguchi, S., Kawauchi, S., Furuya, T., Oga, A., Kang, J.J., Naito, K., Sasaki, K., 2007. Gain of 5p15.33 is associated with progression of bladder cancer. Oncology 72, 132-138.

Yan, S.M., Tang, J.J., Huang, C.Y., Xi, S.Y., Huang, M.Y., Liang, J.Z., Jiang, Y.X., Li, Y.H., Zhou, Z.W., Ernberg, I., Wu, Q.L., Du, Z.M., 2013. Reduced expression of ZDHHC2 is associated with lymph node metastasis and poor prognosis in gastric adenocarcinoma. PLoS One 8, e56366.

Yanai, A., Huang, K., Kang, R., Singaraja, R.R., Arstikaitis, P., Gan, L., Orban, P.C., Mullard, A., Cowan, C.M., Raymond, L.A., Drisdel, R.C., Green, W.N., Ravikumar, B., Rubinsztein, D.C., El-Husseini, A., Hayden, M.R., 2006. Palmitoylation of huntingtin by HIP14 is essential for its trafficking and function. Nat. Neurosci. 9, 824-831.

Yang, G., Cynader, M.S., 2011. Palmitoyl acyltransferase zD17 mediates neuronal responses in acute ischemic brain injury by regulating JNK activation in a signaling module. J. Neurosci. 31, 11980-11991.

Yang, G., Zhou, X., Zhu, J., Liu, R., Zhang, S., Coquinco, A., Chen, Y., Wen, Y., Kojic, L., Jia, W., Cynader, M.S., 2013. JNK3 couples the neuronal stress response to inhibition of secretory trafficking. Sci. Signal. 6, ra57.

Yeste-Velasco, M., Linder, M.E., Lu, Y.J., 2015. Protein S-palmitoylation and cancer. Biochim. Biophys. Acta 1856, 107-120.

Yeste-Velasco, M., Mao, X., Grose, R., Kudahetti, S.C., Lin, D., Marzec, J., Vasiljevic, N., Chaplin, T., Xue, L., Xu, M., Foster, J.M., Karnam, S.S., James, S.Y., Chioni, A.M., Gould, D., Lorincz, A.T., Oliver, R.T., Chelala, C., Thomas, G.M., Shipley, J.M., Mather, S.J., Berney, D.M., Young, B.D., Lu, Y.J., 2014. Identification of ZDHHC14 as a novel human tumour suppressor gene. J. Pathol. 232, 566-577.

Yokoi, N., Fukata, Y., Sekiya, A., Murakami, T., Kobayashi, K., Fukata, M., 2016. Identification of PSD-95 depalmitoylating enzymes. J. Neurosci. 36, 6431-6444.

Yoon, S.C., Jang, Y.L., Kim, J.W., Cho, E.Y., Park, D.Y., Hong, K.S., Lee, Y.S., 2016. Linkage and association analyses of schizophrenia with genetic variations on chromosome 22q11 in koreans. Psychiatry Investig. 13, 630-636.

Yoshii, A., Constantine-Paton, M., 2014. Postsynaptic localization of PSD-95 is regulated by all three pathways downstream of TrkB signaling. Front. Synaptic. Neurosci. 6, 6.

Young, F.B., Butland, S.L., Sanders, S.S., Sutton, L.M., Hayden, M.R., 2012a. Putting proteins in their place: palmitoylation in Huntington disease and other neuropsychiatric diseases. Prog. Neurobiol. 97, 220-238.

Young, F.B., Franciosi, S., Spreeuw, A., Deng, Y., Sanders, S., Tam, N.C., Huang, K., Singaraja, R.R., Zhang, W., Bissada, N., Kay, C., Hayden, M.R., 2012b. Low levels of human HIP14 are

sufficient to rescue neuropathological, behavioural, and enzymatic defects due to loss of murine HIP14 in Hip14$^{-/-}$ mice. PLoS One 7, e36315.

Yu, L., Reader, J.C., Chen, C., Zhao, X.F., Ha, J.S., Lee, C., York, T., Gojo, I., Baer, M.R., Ning, Y., 2011a. Activation of a novel palmitoyltransferase ZDHHC14 in acute biphenotypic leukemia and subsets of acute myeloid leukemia. Leukemia 25, 367-371.

Yu, X.F., Zou, J., Bao, Z.J., Dong, J., 2011b. miR-93 suppresses proliferation and colony formation of human colon cancer stem cells. World J. Gastroenterol. 17, 4711-4717.

Zeidman, R., Buckland, G., Cebecauer, M., Eissmann, P., Davis, D.M., Magee, A.I., 2011. DHHC2 is a protein S-acyltransferase for Lck. Mol. Membr. Biol. 28, 473-486.

Zhang, J., Planey, S.L., Ceballos, C., Stevens, S.M., Keay, S.K., Zacharias, D.A., 2008. Identification of CKAP4/p63 as a major substrate of the palmitoyl acyltransferase DHHC2, a putative tumor suppressor, using a novel proteomics method. Mol. Cell. Proteomics 7, 1378-1388.

Zhang, X., Spiegelman, N.A., Nelson, O.D., Jing, H., Lin, H., 2017. SIRT6 regulates Ras-related protein R-Ras2 by lysine defatty-acylation. Elife 6, e25158.

Zhao, Y., He, A., Zhu, F., Ding, M., Hao, J., Fan, Q., Li, P., Liu, L., Du, Y., Liang, X., Guo, X., Zhang, F., Ma, X., 2018. Integrating genome-wide association study and expression quantitative trait locus study identifies multiple genes and gene sets associated with schizophrenia. Prog. Neuropsychopharmacol. Biol. Psychiatry 81, 50-54.

Zheng, B., DeRan, M., Li, X., Liao, X., Fukata, M., Wu, X., 2013. 2-Bromopalmitate analogues as activity-based probes to explore palmitoyl acyltransferases. J Am. Chem. Soc. 135, 7082-7085.

Zheng, B., Jarugumilli, G.K., Chen, B., Wu, X., 2016. Chemical probes to directly profile palmitoleoylation of proteins. Chembiochem. 17, 2022-2027.

Zhou, Q., Lin, H., Wang, S., Wang, S., Ran, Y., Liu, Y., Ye, W., Xiong, X., Zhong, B., Shu, H.B., Wang, Y.Y., 2014. The ER-associated protein ZDHHC1 is a positive regulator of DNA virus-triggered, MITA/STING-dependent innate immune signaling. Cell Host Microbe. 16, 450-461.

Zhou, T., Li, J., Zhao, P., Liu, H., Jia, D., Jia, H., He, L., Cang, Y., Boast, S., Chen, Y.H., Thibault, H., Scherrer-Crosbie, M., Goff, S.P., Li, B., 2015. Palmitoyl acyltransferase Aph2 in cardiac function and the development of cardiomyopathy. Proc. Natl. Acad. Sci. USA 112, 15666-15671.