



## RESEARCH ARTICLE

# SwissPalm: Protein Palmitoylation database [version 1; referees: 3 approved]

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## Abstract

Protein S-palmitoylation is a reversible post-translational modification that regulates many key biological processes, although the full extent and functions of protein S-palmitoylation remain largely unexplored. Recent developments of new chemical methods have allowed the establishment of palmitoyl-proteomes of a variety of cell lines and tissues from different species. As the amount of information generated by these high-throughput studies is increasing, the field requires centralization and comparison of this information. Here we present SwissPalm (<http://swisspalm.epfl.ch>), our open, comprehensive, manually curated resource to study protein S-palmitoylation. It currently encompasses more than 5000 S-palmitoylated protein hits from seven species, and contains more than 500 specific sites of S-palmitoylation. SwissPalm also provides curated information and filters that increase the confidence in true positive hits, and integrates predictions of S-palmitoylated cysteine scores, orthologs and isoform multiple alignments. Systems analysis of the palmitoyl-proteome screens indicate that 10% or more of the human proteome is susceptible to S-palmitoylation. Moreover, ontology and pathway analyses of the human palmitoyl-proteome reveal that key biological functions involve this reversible lipid modification. Comparative analysis finally shows a strong crosstalk between S-palmitoylation and other post-translational modifications. Through the compilation of data and continuous updates, SwissPalm will provide a powerful tool to unravel the global importance of protein S-palmitoylation.

## Open Peer Review

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## Introduction

S-palmitoylation is defined as the enzymatic attachment of a 16-carbon-chain palmitic acid to cysteine residues via a thioester bond<sup>1,2</sup>. S-palmitoylation increases the local hydrophobicity of proteins, driving their association with cellular membranes and their segregation to specific membrane domains<sup>3</sup> and also affect protein stability and protein-protein interactions<sup>4-12</sup>. S-palmitoylation appears to occur in all eukaryotes since it was found in yeast, parasites, worm, flies and plants. Importantly, it is the only reversible lipid post-translational modification (PTM) identified to date<sup>13</sup> and thus can dynamically regulate the function of proteins. For example, S-palmitoylation/S-depalmitoylation cycles control the shuttling of the small GTPases H-Ras and N-Ras between the Golgi and the plasma membrane thus regulating cell growth<sup>14,15</sup>. The dynamics of S-palmitoylation are mediated by the opposing activities of two families of enzymes: palmitoyltransferases (PATs), which catalyze the attachment of palmitate to specific cysteine residues while thioesterases detach it<sup>3,16,17</sup> and Acyl Protein Thioesterases (APTS) which remove the acyl chain.

Palmitoylated proteins are involved in key biological processes including ion transport, receptor function, membrane trafficking, signaling, cell growth, development, neuronal plasticity and immune response<sup>5,18-20</sup>. Not surprisingly S-palmitoylation has been linked to a variety of human diseases, including neurological diseases such as Huntington's disease<sup>21</sup>, schizophrenia<sup>22</sup>, Alzheimer's disease<sup>23</sup> and cancer (gastric, bladder, lung, colorectal, carcinoma<sup>24-29</sup>). In addition, pathogens utilize host S-palmitoylation machinery to promote infection<sup>30</sup>.

Despite the growing evidences of the importance of S-palmitoylation and the attractive therapeutic perspectives that it provides, the full extent of the S-palmitoylated proteomes is not well defined and our understanding of the mechanisms that regulates S-palmitoylation and its consequences on cellular and organismal levels function remains incomplete. This is due in part to several factors; technical difficulties in studying lipid modifications, the absence of a consensus sequence for S-palmitoylation identifiable with bioinformatics means and the lack of antibodies that would recognize S-palmitoylated cysteines, in analogy to phosphospecific antibodies. The field has therefore long relied almost exclusively on metabolic labeling with radioactive palmitate, immuno-precipitation of specific proteins, and autoradiography - sometimes requiring months of exposure times.

Novel methods now allow the capture of cellular or tissue palmitoyl proteomes followed by their identification by mass spectrometry. So far two major methods have been used. The first is Acyl Biotin Exchange (ABE), and its derivative Acyl Resin Assisted Capture (Acyl-RAC)<sup>31-33</sup>. It provides a snapshot of the S-palmitoylated proteins in a cell or tissue. It is based on the selective cleavage of thioester bonds, as found between the palmitate moiety and the cysteine residue, using neutral hydroxylamine (HA), and the subsequent capture of the liberated thiol groups with a thiol-specific reagent coupled - directly or indirectly - to beads. The second method is based on the metabolic labeling of S-palmitoylated proteins in live cells with palmitate analogues containing an azide or alkyne group, and thus will reveal the proteins that have undergone S-palmitoylation during the labeling time. After cell lysis, the labeled proteins can be isolated using click chemistry and affinity capture<sup>34</sup>.

So far, 19 palmitoyl-proteomes have been published, identifying thousands of new putative S-palmitoylated proteins<sup>26,31,33-49</sup>, and many more are expected to be reported. Since these palmitoyl-proteomes were obtained from various cell lines and tissues originating from different species, the field is in need of integration of these datasets in order to compare them, identify orthologs, and analyze what conditions lead to changes in S-palmitoylation. Importantly, the above-described capture techniques not only differ in the population of S-palmitoylated proteins that they aim to capture but also in some drawbacks. For example, incomplete alkylation of proteins during ABE/Acyl-RAC isolation will lead to a poor enrichment ratio and thus false negatives, but this step is absent from the *in vivo* labeling followed by click chemistry protocol. Thus comparing studies performed with different techniques increases the confidence that a protein represents a true positive. Moreover, different studies have used different thresholds and criteria to establish their confidence levels. Finally, low-abundance proteins might be missed in studies in cells or tissues, while being enriched in studies on subcellular fractions.

To provide the scientific community with a tool to extract information from the comparison of different palmitoyl-proteomics studies, we have created SwissPalm, which combines results from large-scale palmitoyl-proteomics studies with curation from the literature of small S-palmitoylation studies. SwissPalm is a user-friendly web resource that allows users to search for proteins of interest through all published palmitoyl-proteomes, determine the predicted S-palmitoylation sites, identify orthologues, compare palmitoyl-proteomes and more. Combination of the available data raises the confidence that a protein of interest is indeed palmitoylated, as we have herein validated on the chaperone complex CCT. A palmitoylation database also provides the opportunity to compare with other databases, leading for example to the generalized cross-talk between palmitoylation and ubiquitination.

## Methods

### SwissPalm architecture

The database was designed to offer a general solution for storing knowledge on protein S-palmitoylation obtained from different types of studies: from biochemical studies focusing on a specific protein, to large-scale analyses by mass spectrometry-based proteomics.

**Protein hits and sites.** In SwissPalm, two main objects have been designed to store the information related to S-palmitoylation. First, a *Hit* represents the knowledge that a given protein (or isoform) has been found as S-palmitoylated in a given study. Second, a *Site* is defined by a *Hit* and the position in the related sequence where the S-palmitoylation event was identified. A given *Hit* can have none, one or several associated sites. *Hit* and *Site* records are labeled with a unique identifier SPalmH# and SPalmS#, respectively. The enzyme(s) catalyzing the S-palmitoylation/S-depalmitoylation reactions on a *Hit* or *Site* can also be indicated.

**Protein sequences and annotations.** Protein sequences are the primary and central source of information of the SwissPalm database. The UniProt Knowledge Base (UniProtKB) was chosen because it is a stable and regularly updated resource for protein sequences. One protein entry in UniProtKB can contain one

or several sequences corresponding to alternative products (isoforms) of a single gene. Therefore, in the database, proteins and isoforms represent two distinct reference objects, and the known isoform-specific S-palmitoylation information can be reported to one or the other. The SwissPalm database is built for a set of species for which S-palmitoylation events have been reported for at least one protein. For each of these species, UniProtKB/SwissProt - the manually curated part of UniProtKB - sequences are inserted in the database. UniProtKB/TrEMBL entries are taken into account only if a palmitoyl-proteome hit protein is not found in UniProtKB/SwissProt. Moreover, UniProtKB provides curated functional annotations on proteins, *e.g.* ubiquitination or phosphorylation, which is useful to compare to S-palmitoylation. Information extracted from UniProtKB entries includes subcellular localization and sequence features (topological domains, variants, post-translational modifications, etc.). Post-translational modification information was also retrieved from Phosphosite<sup>50</sup>.

Mappings to other protein databases like RefSeq or genome-specific databases (*e.g.* The Arabidopsis Information Resource (TAIR), Mouse Genome Informatics (MGI), or *Saccharomyces* Genome Database (SGD)) were obtained either through the UniProt mapping API or from the databases themselves and inserted in SwissPalm as protein references. Gene Ontology (GO) terms and annotations were downloaded from the GO website and inserted in our database. A full mapping of UniProtKB entries and GO terms was computed, taking into account the lineage of GO terms and stored in the database. Thus, all UniProtKB entries associated with a given GO term can be found.

### Orthologies and computed data

Orthologs of *Hits* were extracted from OMA (Orthologs Matrix) groups<sup>51</sup> and OrthoDB<sup>52</sup> and included in the database. Orthology relations are indicated in both databases at the level of proteins. For each protein, orthology groups represented in OrthoDB are listed non-redundantly. In OrthoDB, one protein can present several orthologs in the same species but in different orthology groups. To simplify the orthology information from OrthoDB, orthology groups were sorted by their size (number of orthologs in the group) from the smallest to the biggest, and only the first encountered protein for each species was selected. OMA group orthologs were used to complement this resource. These orthologs were used in the application for three purposes: the comparison of palmitoyl-proteomes of different species and the comparison of palmitoyl acyl transferases (PATs) from different organisms, and finally the analysis of conservation of known S-palmitoylation sites across different species.

Due to the increasing number of palmitoyl-proteome studies and of known involved proteins and orthologs, the comparison of palmitoyl-proteomes had to be precomputed to keep the website responding fast. The results of the comparison are stored in a specific table of the database, which is queried to present results in the palmitoyl-proteome comparison tool. For each protein, two types of multiple sequence alignments (MSA) were performed (when relevant) using MAFFT [1]: a MSA of isoform sequences and a MSA of protein orthologs (taking into account only the main isoform if several exist). For each sequence and each cysteine, a prediction of S-palmitoylation sites has been performed using two tools: PalmPred<sup>53</sup> and CSS-Palm 4.0<sup>54</sup>. Results are stored in the database.

### SwissPalm server and administrator interface

SwissPalm is a standard Ruby-on-Rails (RoR) application with a PostgreSQL database backend. The update procedure is fully automated and includes every step of download, parsing and loading in the database of the external and computed data. It is possible to initially update the list of species to load in the system. The application consists of a public website to browse and search the S-palmitoylation data in different contexts and an administration interface to manually add any curated information. The search is powered by a systematic indexation system of all known protein identifiers, all possible key-words from the UniProtKB description of proteins and GO terms. Mass spectrometry data from literature can be loaded through a specific function part of the SwissPalm server used in admin mode. The system uses the database to directly load the lacking sequences and validate the possibility of protein S-palmitoylation. The original file (tab-delimited format) is kept on the server file system, and in the database only internal protein/isoform IDs (corresponding to UniProtKB entries or sequences) are stored. A dedicated interface has been set up to allow the curation of hits and sites extracted from the literature. Several validators help to keep consistency in the database; for example, a systematic check is done at the level of the sequence to verify the presence of a cysteine at the indicated position.

### Bioinformatics analysis

**GO term, STRING, DAVID and CORUM analysis.** GO terms, coming from RDAVIDWebService<sup>55,56</sup>, with enrichment scores >1.6 and corrected p-values <0.01 (using a Benjamini-Hochberg correction for multiple testing) were selected. In order to cluster GO terms by group of ontology, GO terms with more than ten proteins but fewer than 500 were selected. GO terms sharing the same biological functions enriched in S-palmitoylation Hit proteins were clustered using the R environment and the results were visualized with RCytoscape<sup>57</sup> and Cytoscape version 2.8.3 (Prefused forced direct layout)<sup>58</sup>. The distance between GO terms corresponds to the inverse numbers of proteins common to the two terms. Uniprot IDs corresponding to the human and mouse palmitoyl proteomes were searched against the STRING database version 9.1<sup>59</sup> for protein-protein interactions. Only interactions between the proteins belonging to the palmitoyl proteome dataset were selected using a confidence score  $\geq 0.9$  (high confidence). For CORUM analysis, complexes present in the CORUM database<sup>60</sup> with more than six proteins were selected and classified by the % of S-palmitoylation Hit proteins in these complexes.

**Two sample logo analysis.** Sequence motif analyses were performed with the Two-Sample Logo software<sup>61</sup> using the 535 known sites of S-palmitoylation present in SwissPalm. Sequences collected in this study were compared with a set of 5000 same-length protein sequences containing cysteine residues randomly selected but not annotated or predicted as S-palmitoylated. Two groups of aligned sequences were statistically analyzed by the binomial test ( $p < 0.01$ ) against a binomial distribution.

### Experimental methods

In order to illustrate the potential of the SwissPalm database to increase the confidence level as to whether a given protein is palmitoylated or not, we have validated the palmitoylation of an identified protein complex, CCT. Similarly, the comparison of SwissPalm

with ubiquitination databases suggested that proteins that can be palmitoylated also appear to be ubiquitinated. We therefore investigated this experimentally.

**Cells, Antibodies and Reagents.** HeLa cells (human uterine cervical carcinoma) from ATCC were grown at 37°C in complete modified Eagle's medium (MEM) (Sigma), supplemented with 10% fetal bovine serum (FBS) (Brunschwig), L-glutamine, penicillin and streptomycin. HAP1 knockout cell lines were purchased from Horizon Genomics (Vienna, Austria) and were grown in complete Dulbecco's MEM (DMEM) (Gibco), supplemented with 10% FBS, 2 mM L-glutamine, penicillin and streptomycin. The DHHC2 clone (09818-03) contains a 2bp insertion in exon 1, the DHHC5 clone (30129-12) contains a 10bp insertion in exon 2 and the DHHC6 clone (13474-01) contains a 5bp insertion in exon 2. Human Anti CCT1 (ab109126- Rabbit monoclonal, RRID:AB\_10864216), CCT2 (ab92746- Rabbit monoclonal, RRID:AB\_10565196), CCT3 (ab167559- Mouse polyclonal), CCT4 (ab49151- Rabbit polyclonal, RRID:AB\_2073761), and CCT5 (ab129016- Rabbit monoclonal, RRID:AB\_11154964) were obtained from Abcam. Human anti- $\alpha$  Ubiquitin (P4D1) (mouse monoclonal) was from SantaCruz (sc-8017, RRID:AB\_628423) and anti human  $\alpha$ -actin (Mouse monoclonal) from Millipore (MAB 1501, RRID:AB\_1675188).

**Immunoprecipitation.** HeLa cells were lysed for 30 min at 4°C in IP buffer (0.5% NP-40, 500 mM Tris-HCl pH 7.4, 20 mM EDTA, 10 mM NaF, 2 mM benzamide, and Roche protease inhibitor cocktail) followed by centrifugation for 3 min at 2000 g. The supernatants were pre-cleared with protein G-agarose-conjugated beads (GE Healthcare) and incubated for 16 h at 4°C with antibodies and beads. The beads were washed three times with the immunoprecipitation buffer and resuspended in sample buffer (2 ×) after the final wash. The samples were heated at 95°C for 5 min and migrated on SDS-PAGE. Western blotting was performed using the iBlot (Invitrogen) according to the manufacturer's instructions.

**Acyl-RAC.** Protein S-palmitoylation was assessed by the Acyl-RAC assay as previously described<sup>33</sup>, with some modifications. Briefly, total protein extracts (2 mg/ml) from cultured cells were incubated for 4 h at 42°C in buffer containing 1.25% SDS, 0.75% Triton X-100, Hepes 62.5 mM 1 mM EDTA, 20 mM methyl methanesulfonate (MMS-Sigma), 8M Urea and protease inhibitor cocktail (Roche) at pH 7.4. MMS was removed from the protein extract by chloroform-methanol precipitation followed by five methanol washes. Protein pellets were dried and solubilized in binding buffer (1% SDS, 1 mM EDTA, Hepes 100 mM, Urea 8M and protease inhibitor cocktail at PH 7.4). Supernatants were split into two: one sample was supplemented with 1 M hydroxylamine hydrochloride (pH 7.4) and 16.5 mg thiopropyl beads (Sigma); the other sample contained no hydroxylamine but 1M Tris (pH 7.4) and the same amount of thiopropyl beads. After overnight incubation at RT, beads were washed with washing buffer at least five times, and bound proteins were eluted by incubation of beads with 2 × SDS-PAGE loading buffer at 95°C for 5 min. Finally, samples were submitted to SDS-PAGE and analyzed by immunoblotting.

**Metabolic labeling with <sup>3</sup>H-palmitic acid.** HeLa cells were incubated for 2 h at 37°C in IM (Glasgow minimal essential medium buffered with 10 mM Hepes, pH 7.4) with 200  $\mu$ Ci/ml <sup>3</sup>H-palmitic

acid (9,10-<sup>3</sup>H(N)) (American Radiolabeled Chemicals, Inc.). Cells were washed and the cell lysate was subjected to immunoprecipitation of the protein of interest as described above. After the washes, beads were incubated for 5 min at 95°C in reducing sample buffer (2 ×) prior to SDS-PAGE. After the SDS-PAGE, the gel was incubated with a fixative solution (25% isopropanol, 65% H<sub>2</sub>O, 10% acetic acid), followed by a 30 min incubation with signal enhancer Amplify NAMP100 (Amersham). The dried gels were exposed to a Hyperfilm MP (Amersham). Chemical removal of S-palmitoylation was performed by treating cell extracts for 10 minutes at room temperature with 1 M hydroxylamine hydrochloride (Sigma) pH 7.4.

## Results

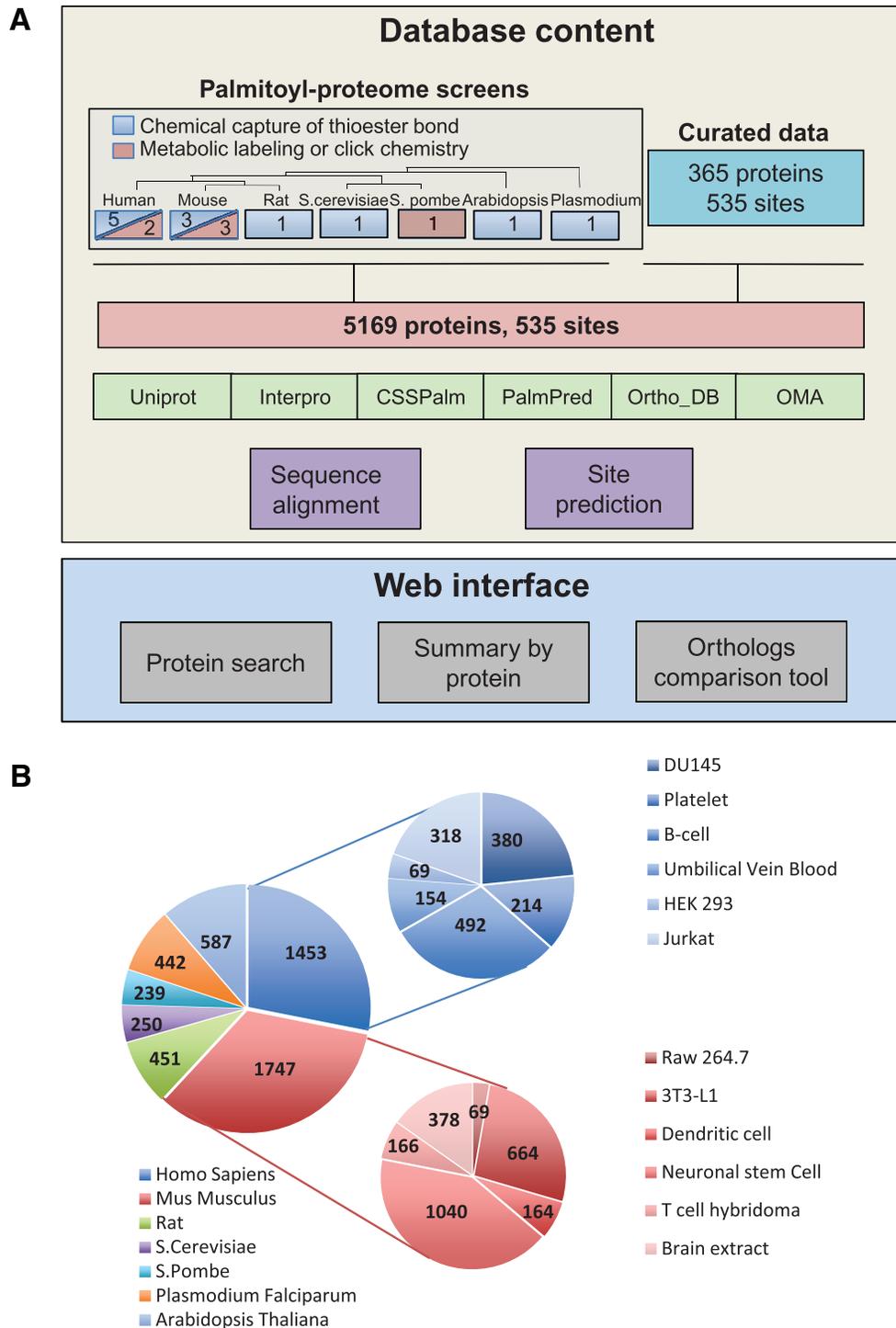
### SwissPalm content

**Curated articles.** By the time of submission, 303 published studies describing 365 S-palmitoylated proteins were incorporated into SwissPalm (<http://swisspalm.epfl.ch/hits>). Strict criteria were applied to consider a protein as S-palmitoylated: proteins must have been demonstrated as S-palmitoylated by two independent methods or by one method and mutagenesis of S-palmitoylation sites. As a result, 535 known S-palmitoylation sites were included in SwissPalm. In addition, palmitoyltransferases and thioesterases demonstrated to be involved in the S-palmitoylation and S-depalmitoylation of those proteins were annotated.

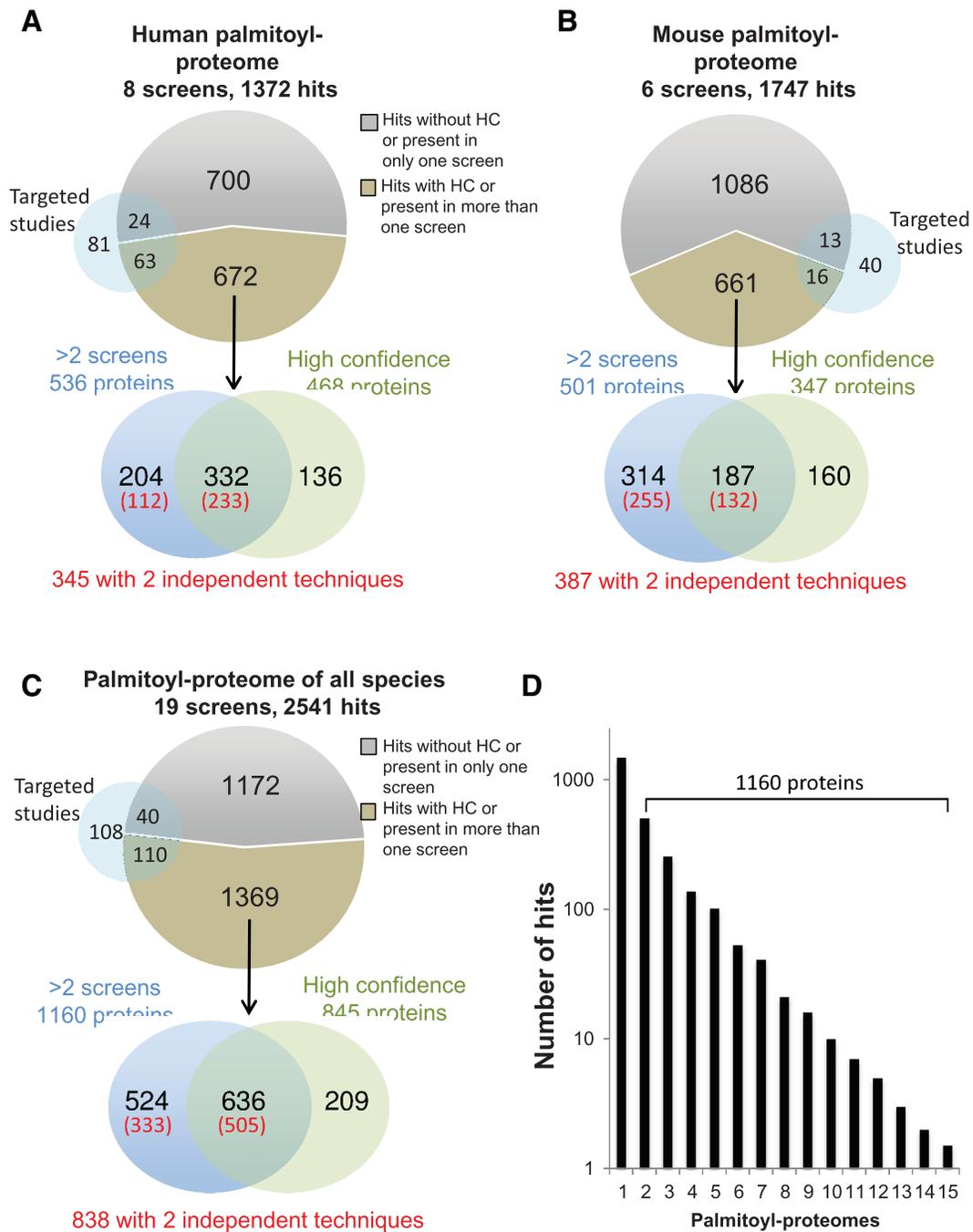
**Palmitoyl-proteomes.** 19 palmitoyl-proteome screens using ABE-, Acyl-RAC- or click chemistry-based studies were selected from published literature (for details and references see: [http://swisspalm.epfl.ch/studies?large\\_scale=1](http://swisspalm.epfl.ch/studies?large_scale=1)). They cover seven species and ten palmitoyl proteomes performed with ABE, 2 with Acyl-RAC and 7 with click chemistry (Figure 1A).

In all cases, the specificity of the capture is controlled by the addition of hydroxylamine (HA). The enrichment ratio (+HA/-HA) for ABE and Acyl-RAC and (-HA/+HA) for click chemistry samples determines the likelihood of proteins to be S-palmitoylated and the cut off in these high-throughput datasets is defined arbitrarily. Accordingly to the vast majority of the palmitoyl-proteomes studies, a ratio (+HA/-HA) greater than two for either ABE, and Acyl-RAC, or a ratio (-HA/+HA) greater than two for click chemistry was selected to add proteins into SwissPalm. In some studies, authors have classified their dataset of S-palmitoylated proteins into high confidence (HC, (+HA/-HA ratio > 20), Medium confidence (MC, 5 < ratio < 20) and low confidence (LC, 2 < ratio < 5) groups. The same classification was kept and annotated in SwissPalm, according to each study. As a result, 5199 unique proteins were incorporated into SwissPalm, all of them showing a ratio +HA/-HA higher than two in each case. The human dataset contains 1453 annotated hits from six different cell types (DU1145, Platelet, B-cell, Umbilical vein blood, HEK 293, Jurkat) while the mouse dataset comprises 1747 proteins from six different cell types or tissues (RAW 264, 3T3-L1, Dendritic cell, Neuronal stem cell, T cell hybridoma, Brain extract) (Figure 1B).

**SwissPalm-mediated improvement of confidence.** The information generated by independent palmitoyl-proteome studies was used to build a filter system that increases the hit confidence. First we annotated the proteins that are defined as high confidence by the authors of the studies (468 for human and 347 for mouse) (Figure 2A and Figure 2B, Supplementary table S1A and Supplementary table S1B).



**Figure 1. Palmitoyl-proteomes integrated in SwissPalm. A:** Database content: Primary data on S-palmitoylation of proteins are extracted from MS large scale experiments on different species. Curated data on S-palmitoylation are obtained from the literature and input together with the MS information in the same data structure. In order to perform complex query related to S-palmitoylation, we have integrated in the database various external sources like orthology databases (Ortho\_DB and OMA), UniProt features and subcellular localization information and Interpro domains. We ran also programs to have additional data, like multiple alignments of orthologous proteins or protein isoforms, and results from existing S-palmitoylation site predictors (CSS-Palm 4.0 and PalmPred). Web interface: The web interface presents the knowledge on S-palmitoylation in protein-centric pages. These pages are accessible through queries on a search engine. Some tools to analyse S-palmitoylation datasets are available online, like the orthologs comparison tool, aiming to perform cross-species S-palmitoylation comparison. **B:** 19 palmitoyl-proteomes from 7 species and various cell types and tissues were selected from published literature and integrated to SwissPalm. In total the dataset includes 5199 proteins.



**Figure 2. Improved confidence in S-palmitoylation protein hits.** **A:** Analysis of the 1372 human hits contained in 8 palmitoyl proteomes: 672 S-palmitoylation hits are present in at least 2 human palmitoyl proteomes or are annotated as “high confident hits” (HC). 204 are only found in at least 2 human palmitoyl proteomes, 136 are only classified as HC and 332 S-palmitoylation hits are both found in more than one palmitoyl-proteome and classified as HC. Out of the 672 hits, 345 are identified with 2 independent techniques. 63 out of the 672 S-palmitoylation hits have been validated in targeted studies, while 24 out of 700 hits only found in 1 human palmitoyl proteome have been validated. **B:** Analysis of the 1747 mouse hits contained in 6 palmitoyl proteomes as described in **A**. **C:** Analysis of the 2541 human orthologous hits contained in 19 palmitoyl proteomes as described in **A**. **D:** Number of the S-palmitoylation hits by the occurrence of palmitoyl-proteomes in which they have been identified.

Second, we assume that the likelihood of a protein to be a true positive increases with its presence in multiple independent palmitoyl-proteomes. It is however important to keep in mind that even if proteins were isolated using the ABE/Acyl-RAC method of labeling followed by click chemistry, all proteins that contain a thioester bond, not related to S-palmitoylation, will be recovered and thus constitute false positives.

536 human proteins and 501 mouse proteins were found in at least two independent palmitoyl-proteomes (Figure 2A and Figure 2B, Supplementary table S1A and Supplementary table S1B). To exploit the information from palmitoyl-proteomes contained in other species, we looked for human orthologs of S-palmitoylated proteins from other species. Out of the orthologs of 2541 human hits in SwissPalm, 1160 were present in at least two independent palmitoyl proteomes (Figure 2C, Supplementary table S2). Well-studied S-palmitoylated proteins such as calnexin (CALX), Thioredoxin-related transmembrane protein 1 (TMX1), Synaptosomal-associated protein 23 (SNAP23), Ras-related protein (RRAS), Phosphatidylinositol 4-kinase II $\alpha$  (P4K2A), transferrin receptor (TFR1), G-proteins (GNAI2, GNAI3), Ras related proteins were identified in multiple palmitoyl-proteome screens (at least in 10 out of 19) in at least four different species (Table 1, Supplementary table S3)<sup>6,50,62-67</sup>.

**Table 1. Top 20 proteins present in the palmitoyl proteome across the seven species.**

UniProt ID	# of palmitoyl-proteome studies/HC hits
CALX_HUMAN	15 / 7
SCAM3_HUMAN	14 / 7
GNAI2_HUMAN	14 / 6
TMX1_HUMAN	13 / 7
SNP23_HUMAN	13 / 6
LYRIC_HUMAN	13 / 6
RAP2B_HUMAN	12 / 5
RAP2C_HUMAN	12 / 6
P4K2A_HUMAN	12 / 4
GNAI3_HUMAN	12 / 5
GNA13_HUMAN	12 / 5
RRAS_HUMAN	11 / 4
TFR1_HUMAN	11 / 3
STX12_HUMAN	11 / 6
GNAQ_HUMAN	11 / 5
VAMP3_HUMAN	11 / 4
ERGI3_HUMAN	11 / 5
CYB5B_HUMAN	11 / 4
RAP2A_HUMAN	10 / 5
RASN_HUMAN	10 / 3

Strikingly,  $\approx 90\%$  (1255 proteins) of the proteins present in more than one screen or annotated as high confidence hits have not yet been validated in targeted studies. In addition, more than 40% (524 proteins) of the proteins present in more than one screen were not annotated as high confidence hits in any of the studies (Figure 2D, Supplementary table S2). Finally, we annotated the proteins that were identified by two distinct independent techniques (metabolic labelling and ABE-based methods), the overlap of false positive proteins generated by these two techniques being very limited. This corresponded to 345 proteins for human and 387 proteins for mouse (Figure 2A and Figure 2B; Supplementary table S1A and Supplementary table S1B).

Altogether, using multiple screens, independent methods and extending the Hits by an ortholog search, we have identified a list of high confidence S-palmitoylated protein hits, which could be useful for further study.

### SwissPalm website

**Search page.** The web-based search tool is accessible from any page of the SwissPalm website: [www.swisspalm.epfl.ch](http://www.swisspalm.epfl.ch) and produces as output a list of proteins with a direct link to detailed information for each protein (Figure 3A). Protein searches are initiated by submitting a protein name, gene name or Uniprot\_ID in the query section and protein IDs that match the string search will be returned in the results sections. Protein searches can be restricted to specific criteria. These include: 'species', 'presence in palmitoyl-proteome screens', 'predicted to be S-palmitoylated' or 'obtained from a third party application/tool' (e.g. from a complex query on the UniProt website). Search can also be restricted to the "reviewed" annotated UniProtKB/SwissProt proteins. Batch searches are possible by submitting a list of identifiers in a tab/csv file (in the first column). An advanced search tool is available to help the user choosing among identifiers recognized by the search engine. Also, it presents controlled vocabularies that can be used to perform complex queries within the database (Motif Search, GO term and Subcellular localization).

**Protein information page.** A system of summary boxes gives a quick overview on the main information related to S-palmitoylated proteins. This includes the number of times the protein is cited in proteome or targeted studies, information on experimental sites, and high confidence predictions from CSS-Palm 4.0 and PalmPred (Figure 3B). Other information includes a global alignment of isoform sequences highlighting all cysteine residues (Figure 4A), protein topology, disulfide bond positions and prediction scores (Figure 4B), information on orthologous proteins and an alignment of them (Figure 3D), as well as GO terms and references (cell types, techniques, subcellular localization).

### Data set analysis

**Estimation of the human palmitome.** We made use of the information gathered in SwissPalm to obtain a current estimation of the human palmitoyl-proteome. We found using Uniprot annotation that 6.8% of the human (1453 proteins) and 9.5% (1747) of the mouse proteomes may undergo S-palmitoylation (Figure 5A, Supplementary table S3). Since several identified S-palmitoylation sites are conserved across species, we integrated 891 mouse

A

## SwissPalm: protein S-palmitoylation database


[Home](#) [Palmitoyl-proteomes](#) [Curated data](#) [PATs & APTs](#)
[Login](#)
[Search proteins](#) [Palmitoylation literature](#)

Calnexin [Advanced search](#)  Species: All  Dataset: Palmitoylated proteins (palmitoyl-proteome or targeted studies)

or BATCH SEARCH: submit a list of identifiers in a tab/csv file (in the first column) [Browse...](#) No file selected.

6 proteins found (Download complete results).  
\*HC = High Confidence

[Filter all columns](#) (Download):

#?	UniProt AC?	UniProt ID?	UniProt status?	Organism?	Description?	# palmitome studies?	Only in internal studies?	# targeted studies?	# of known sites?	# of distinct techniques?	Predicted cytosolic sites?	Max number of cysteines?	# of isoforms?
1	<a href="#">P29402</a>	CALX1_ARATH	★	Arabidopsis thaliana	Calnexin homolog 1	1/1	No	0	0	1	No	6	1
2	<a href="#">Q38798</a>	CALX2_ARATH	★	Arabidopsis thaliana	Calnexin homolog 2	1/1	No	0	0	1	No	5	1
3	<a href="#">P27824</a>	CALX_HUMAN	★	Homo sapiens	Calnexin (IP90) (Major histocompatibility complex class I antigen-binding protein p88) (p90)	7/8 3 in HC* list	No	1	2	2	Yes	8	1
4	<a href="#">P35564</a>	CALX_MOUSE	★	Mus musculus	Calnexin	6/6 3 in HC* list	No	0	0	2	Yes	7	1
5	<a href="#">P35565</a>	CALX_RAT	★	Rattus norvegicus	Calnexin	1/1 1 in HC* list	No	1	0	1	Yes	7	1
6	<a href="#">Q16094</a>	Q16094_HUMAN	★	Homo sapiens	Calnexin (Fragment)	1/8	No	0	0	1	No	4	1

B

## P27824 - CALX\_HUMAN



## - Palmitoylation summary

Validated by 2 independant techniques or by a targeted study.  
High confidence hit in 3 palmitoyl-proteome studies.



**Figure 3. Search and result page. A:** SwissPalm search page: Example of query for “calnexin” shows that it has been found in palmitoyl-proteomes from several species: human (7 out of 8 screens), mouse (6 out of 6), rat (1 out of 1) and *Arabidopsis thaliana* (1 out of 1). For human, mouse and rat calnexin was classified as a high confident hit and for human and mouse identified by two independent techniques (metabolic labeling and chemical capture). Finally, calnexin S-palmitoylation was also subject to targeted studies and 2 cysteine residues (502 and 503 in human calnexin) were identified. **B:** Results Page from human calnexin display summary boxes containing the main information related to S-palmitoylation: number of occurrences in palmitoyl-proteome screens and targeted studies, sites information, cysteine prediction.

orthologs that were not present in human palmitoyl proteomes. The joint dataset indicates that 11% (2339 proteins) of the human proteome may undergo S-palmitoylation. Extending the analysis to 2649 human orthologs present across all species, 12.47% of the human proteome may be subject to S-palmitoylation (Figure 5A, Supplementary table S3). Moreover, since more than half of the proteins described in published targeted studies were not identified in any of the palmitoyl proteome screens (Figure 5B), the current figure of 9 to 12% is probably an underestimate.

**Protein localization and S-palmitoylation motif.** Using the UniProt annotation on protein localization, 45% of both the human and mouse S-palmitoylation hits (679 and 974 for proteins respectively) were annotated as membrane proteins, 30% as cytosolic (445 and 723 proteins respectively) (Figure 5D). This represents a modest enrichment in membrane proteins, more specifically of Type I membrane proteins, when compared to the total human and mouse proteomes (Figure 5C, Supplementary table S4, Supplementary figure S1). S-palmitoylation hits are found in all major compartments of the

endomembrane system – endoplasmic reticulum, Golgi apparatus, lysosome, endosome – as well as in mitochondria (Figure 5E). The latter finding is intriguing since none of the DHHC palmitoyl transferases have so far been localized to mitochondria<sup>68</sup>.

Additionally, 377/1453 human and 422/1747 mouse proteins identified in palmitoyl proteomes were associated with the nucleus which represents around 25% of the total S-palmitoylated human and mouse proteins (Figure 5D). While these might be cytosolic proteins associated with the outer nuclear membrane, the higher number of nuclear proteins raises the possibility that S-palmitoylation may occur in the nucleus and could be used to reversibly target nuclear proteins to the inner nuclear membrane. Presence of a palmitoyltransferase in the nuclear envelope has been reported for the yeast protein rif1<sup>69</sup>. Palmitoylation of nuclear proteins will clearly require validation.

In order to investigate whether certain amino acids were preferentially enriched around S-palmitoylation sites, we compared the

**A**

- Sequences

```

1      11      21      31      41      51      61      71      81      91
Isoform 1 MEGKWLUML LVLGTAIVEA HDGHDDDDVID IEDDLDDVIE EVEDSKPDTT APPSSPKVTY KAPVPTGEVY FADSFDRGTL SGWLSKAKK DDTDDDEIAKY
101     111     121     131     141     151     161     171     181     191
Isoform 1 DGKWEVEEMK ESKLPGDKGL VLMSRAKHHA ISAKLNKPFLL FDTKPLIVQY EVNFQNGIEEGGAYVKLLSK TPELNLDQFH DKTPTTIMFG PDKEGGEDYKL
201     211     221     231     241     251     261     271     281     291
Isoform 1 HFIFRHKNPKIGIYEEKHAK RPDADLKTYF TDKKTHLYTL ILNPDNSFEI LVDQSVVNSG NLLNDMTPVPVNPSREIEDPE DRKPEDWDER PKI PDPEAVK
301     311     321     331     341     351     361     371     381     391
Isoform 1 PDDWDEDAPAKIPDEEATKP EGWLDDEPEY VPDPAEKPE DWDEDMDGEW EAPCIANPREESAPGEGVWQRPVIDNPNYK GKWKPPMIDN PSYOGIWKPR
401     411     421     431     441     451     461     471     481     491
Isoform 1 KLPNPDFFED LEPFRMTPFSAIGLELWSMT SDIFFDNFIICADRRIVDDW ANDGWGLKKA ADGAAEPGVV GOMIEAAEER PNLWVVYLLT VALPVFLVIL
501     511     521     531     541     551     561     571     581
Isoform 1 FCCGSGKKQTS GMEYKKTDAPQPDVKEEEEE KEEKDKGDE EEEGEEKLEE KOKSDAEEEDG GTVSQEEEDR KPAAEEDELLNRS PRNRKPR RE
    
```

- Cysteines

Pos in alignment	8	160	194	360	366	441	502	503
Topology	U	E	E	E	E	E	T	C
Disulfide		yes	yes	yes	yes			
SS prediction	H	E	C	E	C	E	H	C
SS UniProt	NA	NA	NA	NA	NA	NA	NA	NA
	Cys <sup>8</sup>	Cys <sup>160</sup>	Cys <sup>194</sup>	Cys <sup>360</sup>	Cys <sup>366</sup>	Cys <sup>441</sup>	Cys <sup>502</sup>	Cys <sup>503</sup>
Isoform 1 [Main isoform]	13.084	5.091	1.307	4.216	3.411	1.466	14.644	5.409
	-0.69995953	-0.94820158	-0.73879006	-1.3806969	-0.58638265	-1.293996	-0.15277572	0.42033358

Legend:

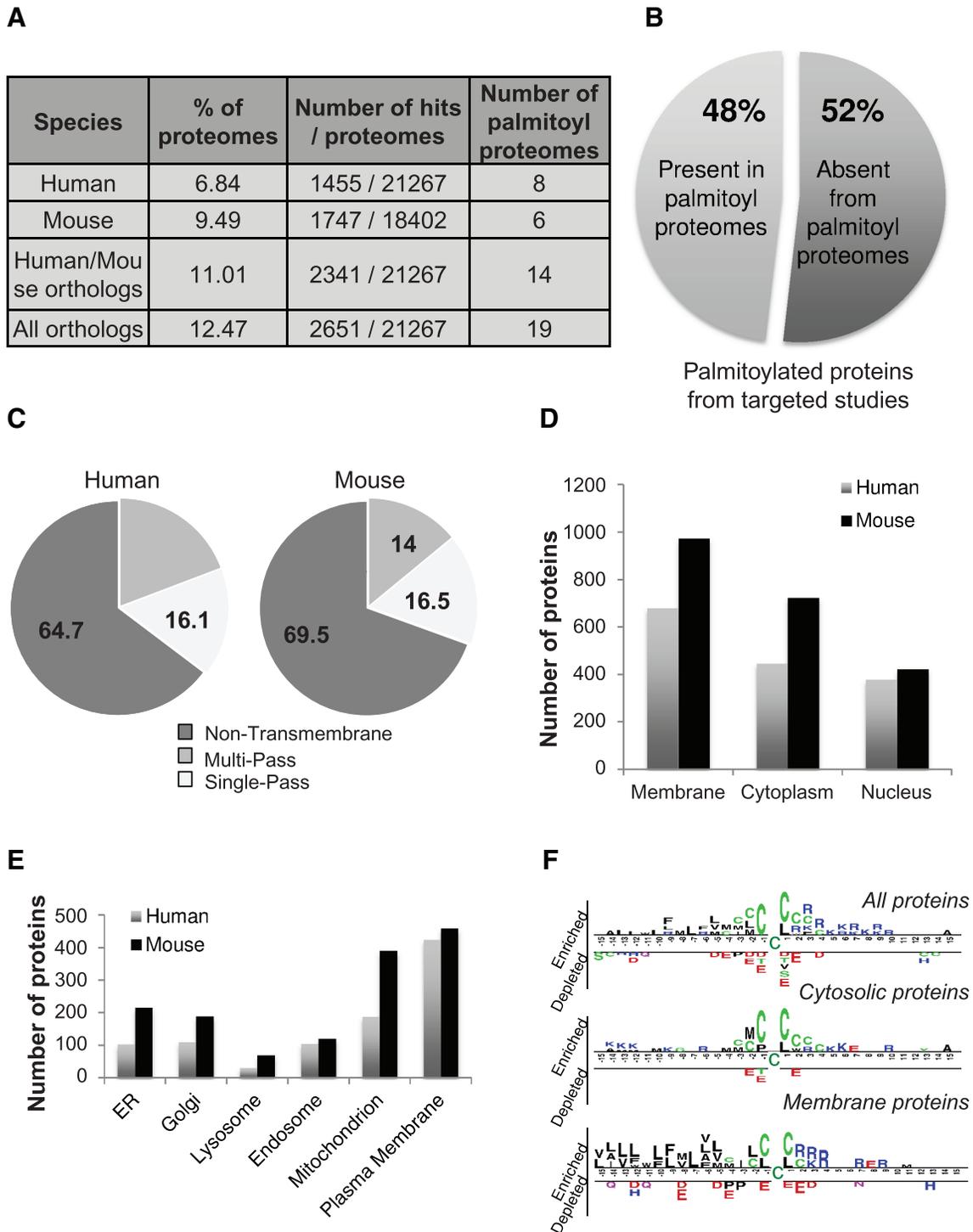
Topology	<ul style="list-style-type: none"> <li>U Undefined</li> <li>E Extracellular/Lumenal</li> <li>T Transmembrane</li> <li>C Cytosolic/Nuclear</li> </ul>
Prediction scores	<p>From top to bottom: Cysteine position, CSS-Palm 4.0 score, PalmPred score</p> <p>Color code for cysteine position: Predicted (high confidence prediction with CSS-Palm or/and PalmPred) / Predicted but not validated (in terms of disulfide bond and cellular location) / Not predicted</p> <p>Color code for CSS-Palm 4.0: High confidence, Medium confidence, Low confidence, Not predicted.</p> <p>Color code for PalmPred: High confidence (score &gt; 0.4), Medium confidence (0.1 &gt; score &gt; 0.4), Low confidence (score &lt; 0.1), Not predicted.</p>

**B**

```

501     511     521     531     541     551     561     571     581     591
D3ZFS2  Rat      ERPWLWLMYL VMAGLPIALV TSFCWPRKVK KKYEDIGPKK ADLCQLPSKA ALBQEPEEEKAP-EKPED--LQEEKK---PSETEVVIVEEKEVIGEP--
F1QKS4  Zebrafish ERPWLWIVYV LTVALPLVLI IVFC-----GTGKKSS ASTPAAEYKK TDEPQP-D--VKEEAE---EEEAK---DEEPEAKKSE
J9PAB2  Dog      ERPWLWVVYV LTVALPVFLV ILFC-----CSGKKQS S--FVEYKK TDAPQP-D--VKEEEE---EKEEEKDKG DEEEEGEEEKL
P27824  Human   ERPWLWVVYI LTVALPVFLV ILFC-----CSGKKQTS S--GMEYKK TDAPQP-D--VKEEEE---EKEEEKDKG DEEEEGEEEKL
P28491  Pig      -----KDKQDEEQR-----LKEEEEEKK RKEEEVDKE DEEDKDEDEE
P35564  Mouse   ERPWLWVVYI LTVALPVFLV ILFC-----CSGKKQS N--AMEYKK TDAPQP-D--VK-DEE---GKEEEKNKR DEEEE-EEEKL
P35565  Rat      ERPWLWVVYI LTVALPVFLV ILFC-----CSGKKQS N--AMEYKK TDAPQP-D--VK-DEE---GKEEEKNKG DEEEE-EEEKL
Q3SYT6  Cow     QRPWLWFIYL LTAALPIALI GSPCWPRKVK KKYEDVAFEKLDICKPQTKG ALBQEVKBEKAALEKPVD--LLEEKK---QSDGE--IVEEKEEGEP--
Q9VXF6  Fruit fly ANRAIWGLVI VAIAVPVA-I TIFC-----KFGLGP SRVAAAKKAA A---AAAK TDDPQPDDDL GAEEEEKLEK GKKEEEEKEE EEEEEEEENK
    
```

**Figure 4. Additional information.** **A:** (upper) Global alignment of isoform sequences highlighting all cysteine residues. (lower) When available, information on protein topology, disulfide bond involvement and prediction scores from CSS-Palm 4.0 and PalmPred are provided for each cysteine residue in the different isoform sequences. **B:** Global alignment of orthologs sequences show conserved cysteine residues (502 and 503 in human calnexin) across species.



**Figure 5. Abundance and distribution of S-palmitoylated proteins in the mammalian proteome. A:** Percentage of S-palmitoylation hits combined from 8 human or 6 palmitoyl proteomes in the human and mouse proteomes. The analysis was extended to human orthologs of mouse and all S-palmitoylation hits present in the dataset. **B:** Percentage of targeted studies present in palmitoyl-proteomes. **C:** Topology of human and mouse S-palmitoylation hits. **D** and **E:** Distribution of human and mouse S-palmitoylation hits in cellular compartments. **F:** Enrichment of amino acid nearby validated S-palmitoylated cysteines in all, only cytoplasmic or only membrane proteins.

amino acid environment of the 535 sites present in SwissPalm against a random set of cysteines not predicted as S-palmitoylated (Materials and methods). A two sample logo analysis shows that S-palmitoylated cysteines are preferentially surrounded by other cysteines, have hydrophobic amino acid to the N-terminal side and charged amino acid to C-terminal side of the S-palmitoylated cysteine (Figure 5F). We repeated the analysis, separating soluble from transmembrane proteins. With the exception of neighboring cysteines, no amino acid specificity was found for soluble proteins. For membrane proteins, despite the fact that we did not classify them as type I, II or multispansing, S-palmitoylation sites appear to preferentially locate to the C-termini of transmembrane domains, there appears to be multiple cysteines and these are preferentially followed with charged residues. This residues could contribute to the inside positive rule of membrane proteins and also ensure interaction with the cytosolic domain with negatively charged lipid head groups at the plasma membrane for example, as observed for myristoylated proteins<sup>70</sup>.

This analysis does not exclude the presence of specific motifs that would ensure S-palmitoylation by a given palmitoyltransferase. Such an analysis will require extensive knowledge of the specific target of the DHHC enzymes.

Altogether this analysis indicates that S-palmitoylation is not restricted to a specific compartment and that it is slightly more frequent in membrane proteins.

**Ontology and network analysis.** To search for cellular functions enriched in S-palmitoylated proteins, we performed pathway- and network-based analysis on both human and mouse palmitoyl-proteomes. Gene ontology<sup>56,71</sup> and protein-protein interaction analysis (through the STRING database) were performed on proteins found by two independent techniques or by targeted studies: 470 proteins for human and 443 for mouse (Supplementary figure S2).

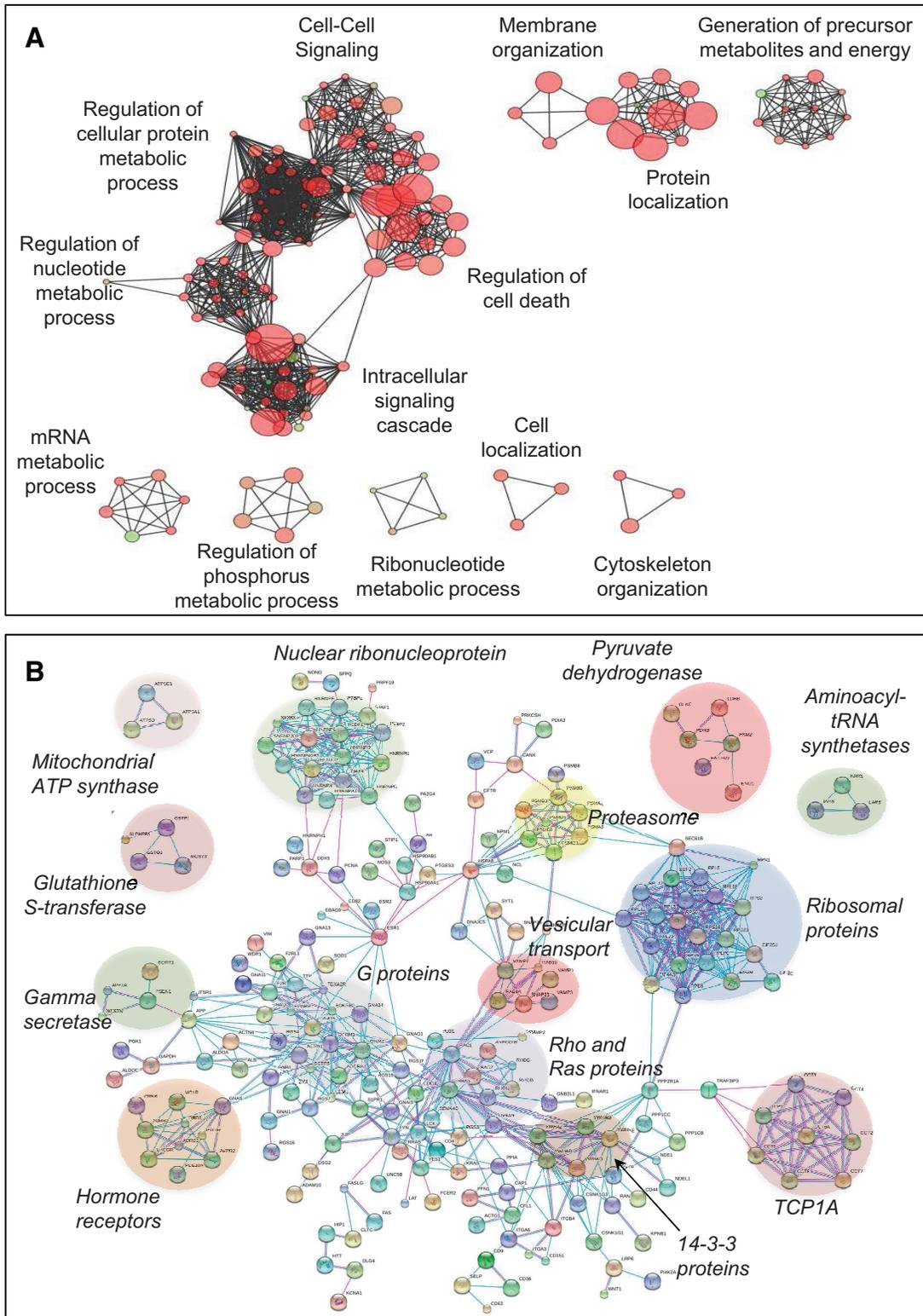
Consistent with the findings reported in some targeted studies, specific biological functions/processes (Figure 6A, Supplementary figure S3 and Supplementary figure S4, Supplementary table S6, methods) are enriched in potentially S-palmitoylated proteins: membrane organisation, cytoskeleton organization, protein localization, cell localization and cell signalling. For example, proteins involved in vesicular trafficking were enriched such as subunits of the coatamer complex, clathrin as well as a significant number of SNARE proteins (Supplementary table S5). We also found 289 hit proteins associated with the GO term Cytoskeleton. These include microfilament- and microtubule-related proteins such as actin subunits, dynein and vimentin but also proteins involved in the formation and regulation of these structures: profilin I, Arp2-Arp3 related proteins. Consistent with a role of S-palmitoylated proteins in cytoskeleton dynamics, tubulin S-palmitoylation was shown to contribute to its association with membrane<sup>72</sup> (Supplementary table S5). More recently, S-palmitoylation of LIM Kinase-I was shown to regulate spine-specific actin polymerization and thereby morphological plasticity<sup>73</sup>. The full view of the role of

S-palmitoylation in cytoskeleton organization will clearly require further studies.

More unexpected processes were also found to be enriched: regulation of cell death, metabolism, mRNA metabolic processes, generation of precursor metabolites and energy, ribonucleotides metabolic processes (Figure 6B, Supplementary table S5 and Supplementary table S6). Whether this is due to the abundance of these proteins or a functional requirement for S-palmitoylation remains to be investigated. It is tempting to speculate that S-palmitoylation might regulate the association of large cytosolic protein complexes, such as ribosomes or proteasomes with membranes to ensure an efficient regulation of protein synthesis and degradation (Figure 6B, Supplementary table S5)<sup>74</sup>.

**Enrichment in protein complexes.** Intrigued by the presence of a large number of protein complexes enriched in S-palmitoylated proteins, we analysed the human and mouse palmitoyl proteome dataset using CORUM, a database of manually curated and validated mammalian protein complexes<sup>60</sup>. A clear enrichment of S-palmitoylated proteins in protein complexes was observed: 18% (463/2558) of the human and 23% (217/938) of the mouse S-palmitoylation hits were part of a complex compared to the 6.8% (1455/21267) of human and 9.5% (1747/18402) of S-palmitoylation hits in their corresponding proteomes. 27 human and 20 mouse complexes possess more than 50% of their subunits S-palmitoylated (Figure 7A and Figure 7B). Intriguingly, the majority of the components of the CCT micro complex, also called TCP-1 ring complex (TRiC), appeared to be S-palmitoylated in both human and mouse analyses (Figure 7C). The TRiC complex is a cytosolic molecular chaperone that promotes folding of 10 to 15 percent of cellular proteins<sup>75</sup>. It is composed of two identical stacked rings, each of which contains eight different subunits. We were able to experimentally confirm the S-palmitoylation of CCT components; CCT1 and CCT2 could be labeled with <sup>3</sup>H-palmitate in a hydroxylamine-dependent manner (Figure 7D). These subunits, as well as CCT3, CCT4 and CCT5, also were positive for S-palmitoylation by Acyl-RAC. CCT subunits assemble into the TRiC complex but independent roles as individual subunits proteins have also been proposed<sup>76</sup>. What the functional consequence of this S-palmitoylation is remains to be established.

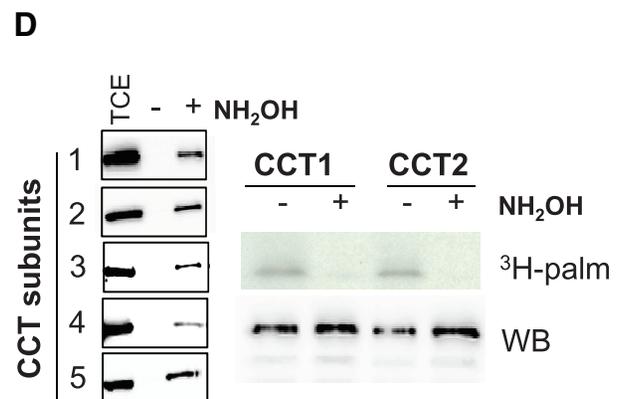
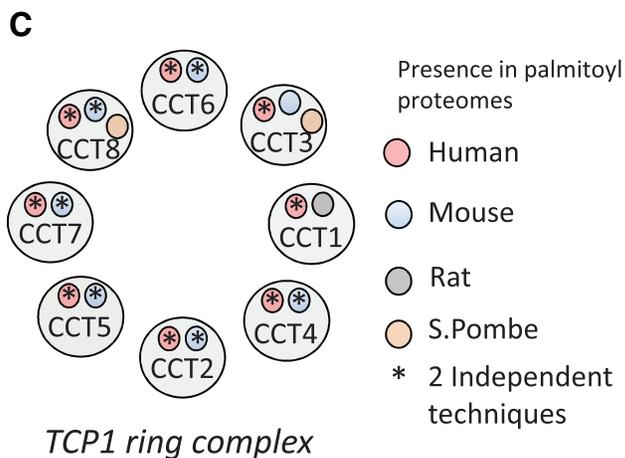
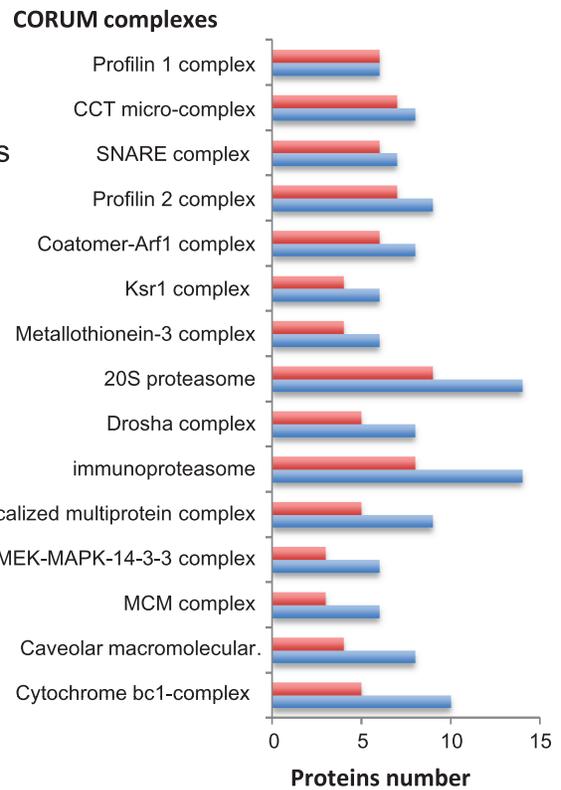
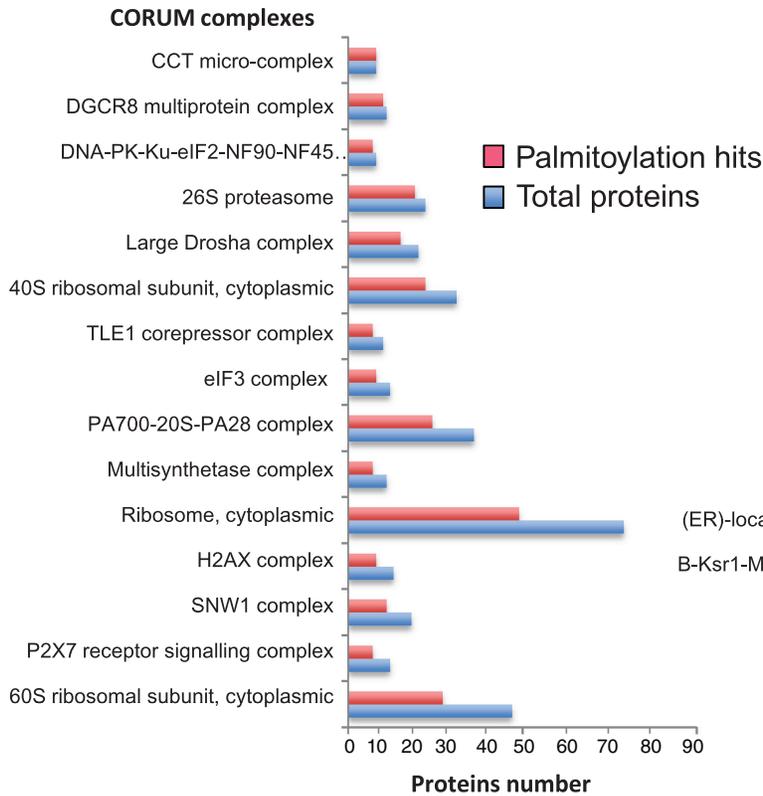
**PTMs and S-palmitoylation.** S-palmitoylation is not the sole PTM that can occur on cysteine residues. They can in particular also undergo other post-translational modifications on cysteine residues such as S-nitrosylation and S-glutathionylation<sup>77,78</sup>. Site competition can thus potentially occur and has been observed<sup>77,78</sup>. To assess the potential amplitude of this competition, we compared the datasets from two available databases, Db\_SNO and Db-GSH, which contain proteomic hits for S-nitrosylation and S-glutathionylation, respectively<sup>79,80</sup>. Comparison of the three datasets shows that more than 40% (1031 out of 2483) of S-palmitoylated protein hits had at least one S-nitrosylated or one S-glutathionylated site and 19% (470 proteins) had both (Figure 8A). This strong overlap suggests that either the isolation methods share the same drawbacks or there is cross-talk between these PTMs.



**Figure 6. Ontology and network analysis of S-palmitoylated proteins. A:** GO term analysis of 470 human S-palmitoylation hits found by 2 independent techniques or by targeted studies. GO terms sharing the same biological functions enriched in S-palmitoylation Hit proteins are clustered. The distance between GO terms corresponds to the inverse numbers of proteins common to the two terms and the size of the circle to the number of proteins associated with the GO term. **B:** Protein-protein interactions networks analysis of 470 human S-palmitoylation hits found by 2 independent techniques or by targeted studies using STRING software. The interactions (high confidence score > 0.9) are shown in evidence view (pink: experimental evidences and blue database evidences).

**A Human**

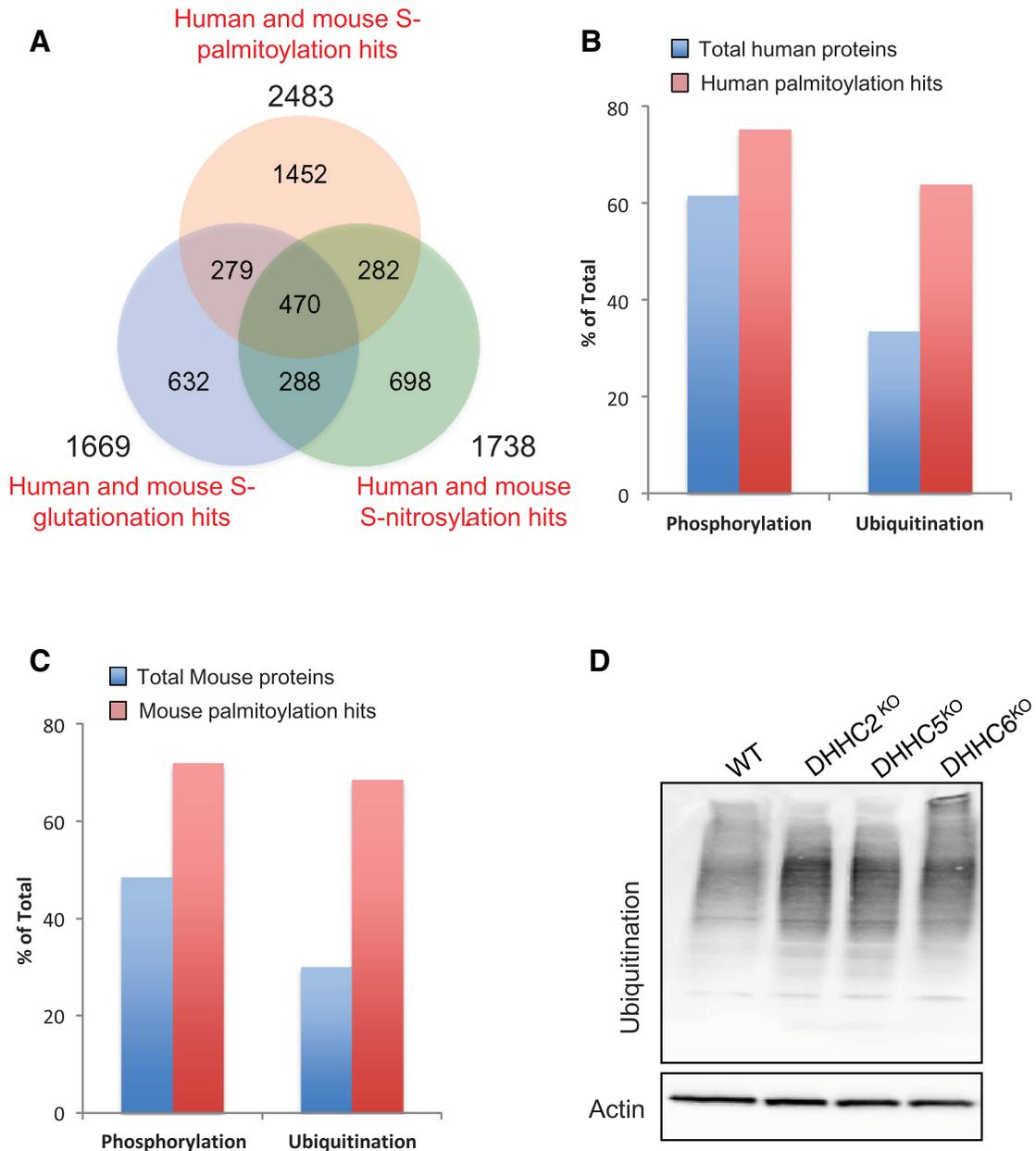
**B Mouse**



**Figure 7. S-palmitoylation of mammalian protein complexes.** **A** and **B**: Human and mouse protein complexes enriched in S-palmitoylation hits using the CORUM database. Proteins complexes containing more than 6 proteins and enriched by at least 50% of S-palmitoylation hits were selected. **C**: Representation of the TRiC complex subunits. Color circles represent the species in which each CCT subunit was identified as S-palmitoylated. The star indicated proteins identified by 2 independent techniques. **D**: Palmitoylation of the subunits was validated by Acyl-RAC on CCT1, CCT2, CCT3, CCT4 and CCT5 subunits and by <sup>3</sup>H-palmitate labelling on CCT1 and CCT2 subunits. (TCE: Total cell extract, NH<sub>2</sub>OH: Hydroxylamine treatment, <sup>3</sup>H-palm: radioactive palmitate signal, WB: Western blot signal).

Several studies, including those from our laboratory, have reported cross-talk between S-palmitoylation and phosphorylation or ubiquitination<sup>7,12,81-88</sup>. To investigate whether this might be a general feature, we used the Phosphosite database to analyse the presence of identified phosphorylation or ubiquitination sites among S-palmitoylation hits<sup>50</sup>. We found using Uniprot annotation that 75% of the human hit proteins contained a phosphorylation site compared to 64% for total proteins. Strikingly 61% of S-palmitoylation hits

contained an ubiquitination site, compare to 33% for the human proteome (Figure 8B). Similar ratios were found for the mouse S-palmitoylated proteins (Figure 8C). The high abundance of ubiquitination sites in S-palmitoylation hits however does not imply that proteins become simultaneously S-palmitoylated and ubiquitinated. On the contrary we have found that S-palmitoylation tends to prevent ubiquitination, protecting membrane proteins for example from lysosomal or endoplasmic-reticulum associated degradation



**Figure 8. S-palmitoylation and other posttranslational modifications.** **A:** Venn diagram displaying the strong co-occurrence of S-palmitoylation, S-nitrosylation and S-glutathionylation modifications within the same proteins. For this analysis human orthologs of mouse proteins were included. **B** and **C:** Percentage of phosphorylation and ubiquitination sites in human and mouse S-palmitoylation hits compared to the total protein population. **C:** Ubiquitination pattern in WT and DHHC2, 5 and 6 HAP1 cells. Total protein extracts from HAP1 cells were subject to Western blot and probe using anti-ubiquitin antibody and actin as loading control.

(ERAD) targeting. Consistent with our observations we found that ubiquitination was markedly increased in HAP1 cell lines knocked-out for the palmitoyltransferases ZDHHC2, ZDHHC5 or ZDHHC6 (using the CRISPR-CAS9 system) when compared to control cells (Figure 8D).

## Conclusion

As the first database dedicated to protein S-palmitoylation, SwissPalm currently assembles 5199 proteins from 19 palmitoyl-proteomes, 7 species and 17 different tissues/cell lines. This database will be updated as new palmitoyl-proteomes appear in the literature and authors are encouraged to directly contact SwissPalm through the website ([webmaster.bbcf@epfl.ch](mailto:webmaster.bbcf@epfl.ch)). By curating information from multiples screens, filters could be established to improve the confidence in positive hits from palmitoyl-proteomics. In addition, SwissPalm provides various tools to ease the study of S-palmitoylation (multiple alignments of orthologous proteins and their isoforms, site prediction score from CSS-Palm and PalmPred). The combined dataset reveals that S-palmitoylation is a widespread post-translational modification that affects key biological processes. SwissPalm, by collecting and sorting information from different sources in a single web page, will accelerate research on S-palmitoylation.

## Software availability

### Software access

The code for the Ruby-on-Rails application for the SwissPalm database can be accessed online at <https://github.com/bbcf/swisspalm>

### Source code as at the time of publication

<https://github.com/F1000Research/swisspalm.git>

### Archived source code as at the time of publication

<http://dx.doi.org/10.5281/zenodo.19106>

## Supplementary materials

### Supplementary table S1

Human palmitoyl-proteome  
[Click here to access the data.](#)

### Supplementary table S2

Mouse palmitoyl-proteome  
[Click here to access the data.](#)

### Supplementary table S3

Orthologs palmitoyl-proteome  
[Click here to access the data.](#)

## Software license

The Ruby-on-Rails application for the SwissPalm database is distributed under the MIT license.

## Author contributions

MB and GVDG conceived the study. MB and LA designed and performed the experiments. FD designed and implements the database. DM curated articles. JB performed the Uniprot topology analysis. FA and MB designed and performed the Gene Ontology analysis. MB, FD and GVDG wrote the manuscript. All authors have seen and agreed to the final content of the manuscript.

## Competing interests

The authors declare no competing interests.

## Grant information

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*I confirm that the funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.*

## Acknowledgments

We thank Alan Bridge, Maria Eugenia Zaballa and Oksana Andrei Sergeeva for helpful advice.

### Supplementary table S4

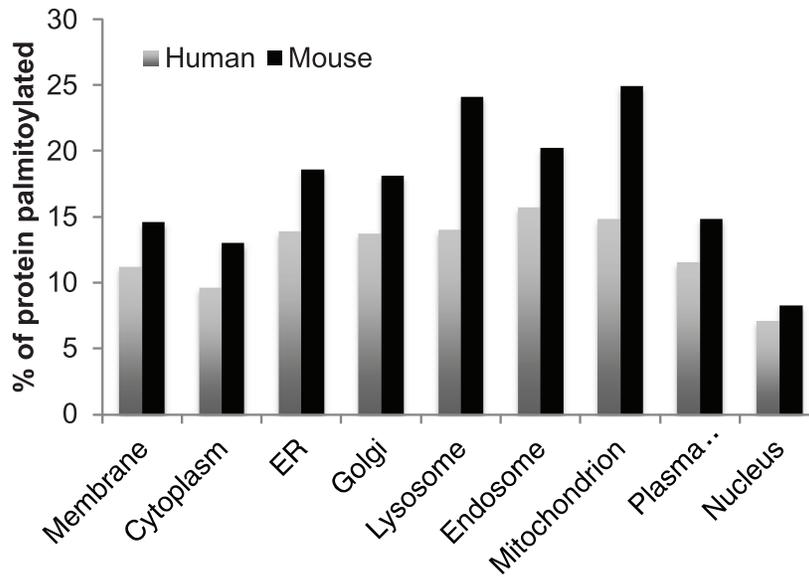
Topology of S-palmitoylated proteins  
[Click here to access the data.](#)

### Supplementary table S5

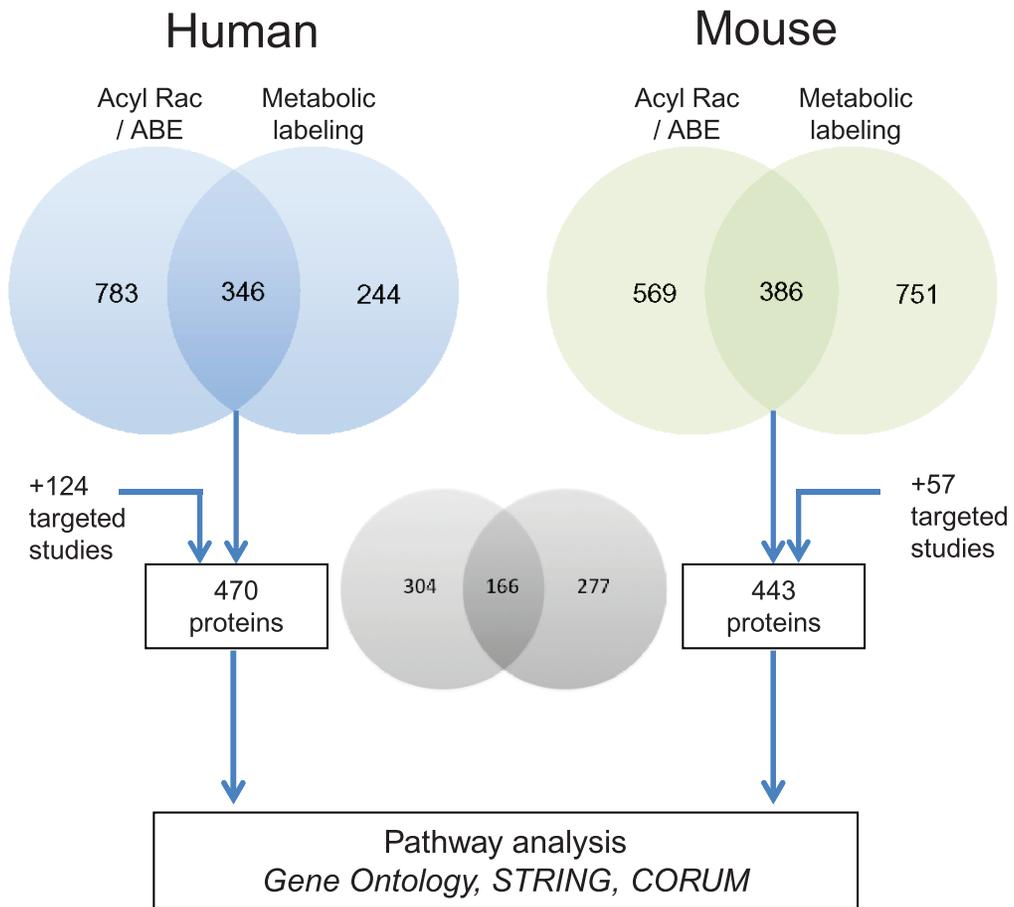
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### Supplementary table S6

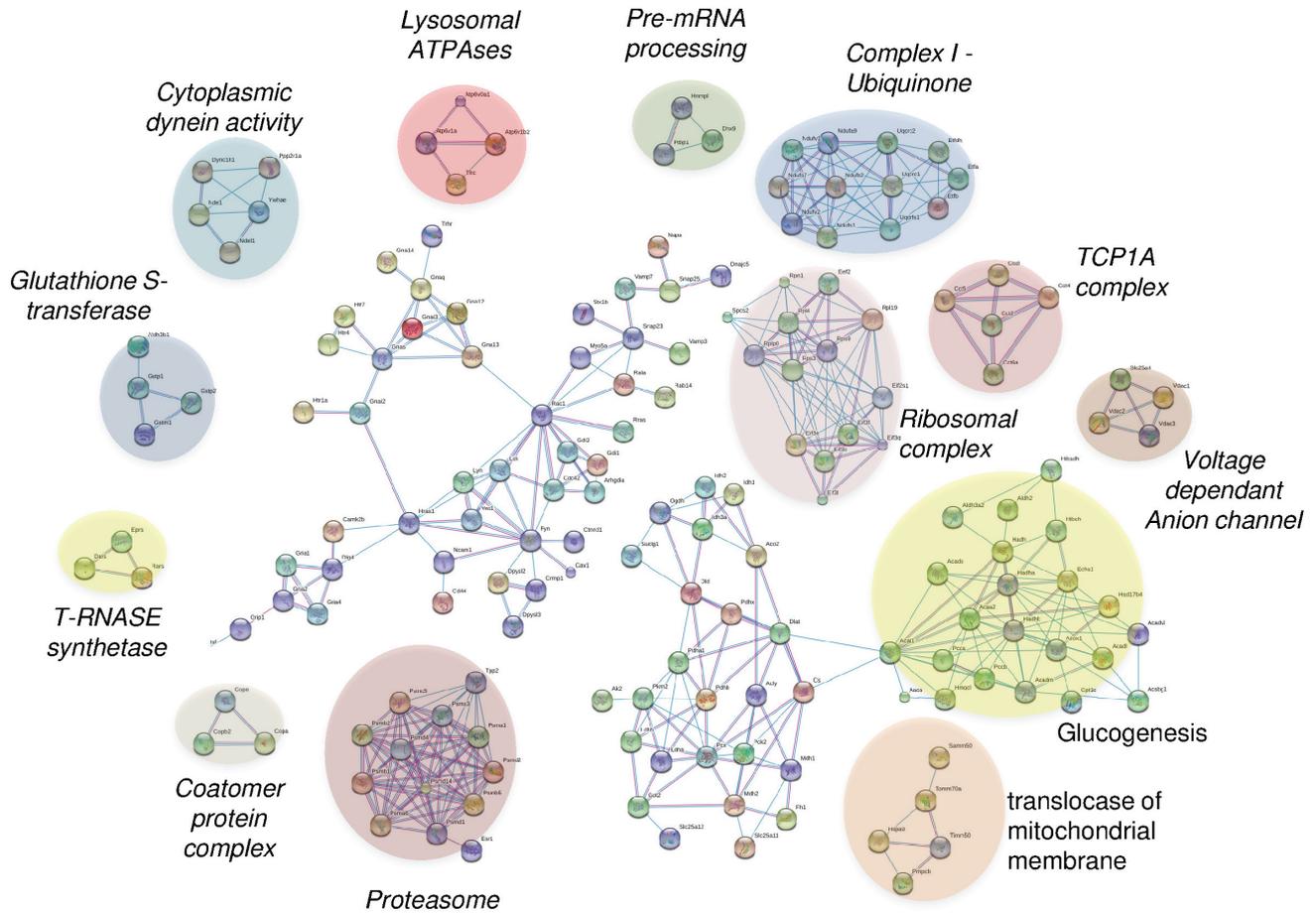
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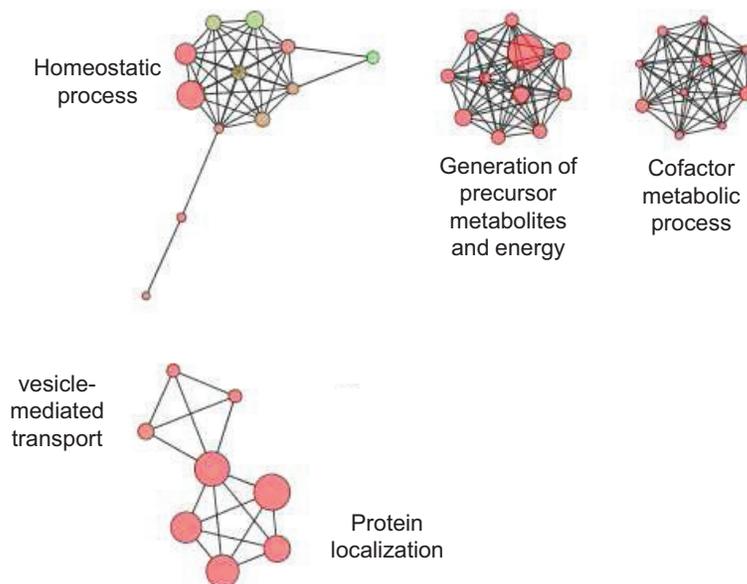
**Supplementary figure S1.** Percentage of human and mouse S-palmitoylation hits in cellular compartments.



**Supplementary figure S2.** Strategy to obtain a high confidence list of 470 human and 443 mouse S-palmitoylation hits to perform ontology and network analysis of S-palmitoylation hits.



**Supplementary figure S3. GO term analysis of mouse palmitoyl-proteome hits.** GO term analysis of 443 mouse S-palmitoylation hits found by 2 independent techniques or by targeted studies. GO terms sharing the same biological functions enriched in S-palmitoylation Hit proteins are clustered. The distance between GO terms corresponds to the inverse numbers of proteins common to the two terms and the size of the circle to the number of proteins associated with the GO term.



**Supplementary figure S4. Protein-protein interaction networks within mouse S-palmitoylated proteins.** Protein-protein interactions networks analysis of 443 human S-palmitoylation hits found by 2 independent techniques or by targeted studies using STRING software. The interactions (high confidence score > 0.9) are shown in evidence view (pink: experimental evidences and blue database evidences).

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# Open Peer Review

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**Anthony Magee**

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This is a very valuable contribution to the field of palmitoylation. It not only provides a wealth of data for researchers but also thoughtful interpretations.

I found the location of cysteine residues in proteins particularly helpful (Fig. 4). Also, the analysis of residues surrounding palmitoylated cysteines is informative, and in essence comes to the same conclusion that I and several others have come to (on a less systematic basis!) that there is no real "consensus" sequence for palmitoylation. This is in contrast to a few papers in the literature which erroneously purport to identify such a consensus.

The enrichment of palmitoylated proteins in complexes is interesting and suggests a possible role of fatty acyl moieties in mediating complex formation. The interplay between palmitoylation, phosphorylation, ubiquitination and other PTMs is also nicely highlighted.

The only major limitation of the paper and the website I have found is the omission of the MBOAT family of protein acyltransferases. Although only one of these is strictly probably a S-palmitoyltransferase (Hedgehog acyltransferase, Hhat), two members of the family (Porcupine and Ghrelin O-acyltransferase, GOAT) are acyltransferases that transfer fatty acyl groups onto proteins. I think it would be helpful if they could be incorporated, and would make the website more comprehensive for investigators interested in protein acylation in its broadest sense. Admittedly, Hhat is thought by some to be a N-palmitoyltransferase, but it is highly likely that it is a S-palmitoyltransferase and that the modified Hedgehog substrates then undergo spontaneous S-to-N acyl transfer resulting in amide-linked palmitate. Porcupine transfers the closely related fatty acyl group palmitoleate to a serine hydroxyl of Wnt proteins and GOAT transfers an octanoyl group to a serine of ghrelin, so these are not "S-palmitoyltransferases" but they share enough in common as protein fatty acyltransferases to warrant inclusion in the database. A minor point, but Hhat has also recently been identified by Konitsiotis et al. to be palmitoylated (Konitsiotis *et al.*, 2015) so should be included in the database at the next revision.

One minor correction I would suggest - the authors generally use the term "palmitoyl proteome" throughout, which was agreed to be the appropriate term at the second dedicated meeting on protein palmitoylation in Edinburgh last year. However, the less suitable term "palmitome" has slipped through in one place, the heading at the bottom right of page 8.

**I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.**

**Competing Interests:** No competing interests were disclosed.

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**Luke Chamberlain**

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SwissPalm represents an extremely valuable resource for the palmitoylation research community, and the authors have undertaken a large volume of work to produce this palmitoylation database. There are also some useful and interesting comparative analyses presented in the paper. As commented by the authors, up to 10% of the proteome might be subject to palmitoylation, emphasising both the importance of this lipid modification and the importance of this new resource. Information has been curated from a growing number of protein-palmitoylome studies and from papers focused on individual palmitoylated proteins, and the database will be continuously updated.

I have only minor comments for improvement:

- Page 3, first paragraph: “while thioesterases detach it and Acyl Protein Thioesterases (APTS) which remove the acyl chain”. There appears to be some repetition here and “while thioesterases detach it” could be removed.
- Figure 1A: text states 19 palmitoyl-proteome screens were selected but first panel of Figure 1A suggests 18? Should there be 6 studies from human using chemical capture? Also text states 5199 proteins were incorporated but figure shows 5169?
- Figure 2A-C: some text is partially obscured.
- Page 8, section on “Protein information page”: the figure numbers given in text of this section do not match with the figures.
- Figure 5A: values of 1455, 2341 and 2651 are given in Figure, whereas corresponding text (pages 8 and 9) states 1453, 2339 and 2649.
- The text corresponding to Figure 7A indicates 27 human and 20 mouse protein complexes - the authors might provide some additional information as to how these values correspond with the bar graph in Figure 7A (which does not show 27/20 complexes).
- The legend of Figure 8 gives “C” rather than “D” for text corresponding to panel D.

**I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.**

**Competing Interests:** No competing interests were disclosed.

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**Howard C. Hang, Emmanuelle Thinon**

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The database developed by Blanc and colleagues is an excellent and valuable resource for researchers working on S-palmitoylation. The database allows to visualize and compare 19 proteomics datasets published in the last few years for protein S-palmitoylation. Proteomics data often generate some false positive hits and SwissPalm-mediated improvement of confidence will help scientists interpret the results of these proteomics studies and could also use as a reference database for future proteomics studies. The other tools added to the database (multiple alignments of orthologous proteins and their isoforms, site prediction score from CSS-Palm and PalmPred) are also valuable. The database interface is well described in the paper. As a result, the database is easy to use and the data easily extracted from the website. The comparative analysis of metabolic labeling and hydroxyl amine-sensitive enrichment methods should encourage researchers to employ both methods for high-quality proteomic analysis of S-fatty-acylated proteins.

**We have read this submission. We believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.**

**Competing Interests:** No competing interests were disclosed.

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