Molecular Pharmacology Fast Forward. Published on February 25, 2021 as DOI: 10.1124/molpharm.120.000210
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Title Page

Identification of Celecoxib targeted proteins using label-free thermal proteome profiling on rat hippocampus

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Total number of manuscript pages
28
Total number of figures
5
Total number of tables
0
Total word count of the Abstract
255
Total word count of the Introduction
892
Total word count of the Discussion
1528

Molecular Pharmacology Fast Forward. Published on February 25, 2021 as DOI: 10.1124/molpharm.120.000210
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Running Title Page

Identification of Celecoxib targets by label-free TPP

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Abstract

Celecoxib or Celebrex, an NSAID (non-steroidal anti-inflammatory drug), is one of the most common medicines for treating inflammatory diseases. Recently, it has been shown that celecoxib is associated with implications in complex diseases such as Alzheimer's disease and cancer, as well as with cardiovascular risk assessment and toxicity, suggesting that celecoxib may affect multiple unknown targets. In this project, we detected targets of celecoxib within the nervous system using a label-free TPP (Thermal Proteome Profiling) method. First, proteins of the rat hippocampus were treated with multiple drug concentrations and temperatures. Next, we separated the soluble proteins from the denatured and sedimented total protein load by ultracentrifugation. Subsequently, the soluble proteins were analyzed by nano-liquid chromatography-mass spectrometry to determine the identity of the celecoxib targeted proteins based on structural changes by thermal stability variation of targeted proteins towards higher solubility in the higher temperatures. In the analysis of the soluble protein extract at 67 centigrade, 44 proteins were uniquely detected in drug-treated samples out of all 478 identified proteins at this temperature. Rab4a, one out of these 44 proteins, has previously been reported as one of the celecoxib off-targets in the rat CNS. Furthermore, we provide more molecular details through biomedical enrichment analysis to explore the potential role of all detected proteins in the biological systems. We show that the determined proteins play a role in the signaling pathways related to neurodegenerative disease - and cancer pathways. Finally, we fill out molecular supporting evidence for using celecoxib towards the drug repurposing approach by exploring drug targets.

Keywords: Celecoxib, thermal proteome profiling, rat hippocampus, proteomics, signaling network

Significance Statement

In this study, we determined forty-four off-target proteins of celecoxib, a non-steroidal anti-inflammatory, and one of the most common medicines for treating inflammatory diseases. We showed that these proteins play a role in the signaling pathways related to neurodegenerative disease and cancer pathways. Finally, we provided molecular supporting evidence for using celecoxib towards the drug repurposing approach by exploring drug targets.

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Introduction

Celecoxib is a non-steroidal anti-inflammatory drug (NSAID) with anti-inflammatory, analgesic and antipyretic properties. Celecoxib prevents the synthesis of lipid compounds called prostaglandins, by selectively inhibiting cyclooxygenases-2 (COX-2) (Johnson et al., 2002; Schönthal, 2007). COX has an essential role in the synthesis of prostaglandins (PGs) derived from arachidonic acid (Marnett et al., 1999). There are two isoforms of COX: COX-1, and COX-2. COX-1, as a gastric cytoprotectant, is physiologically constitutive and responsible for renal and platelet homeostasis. COX-2, which is considered to be inductive, is arising only in situations of tissue trauma and infections (Perazella and Tray, 2001; Yaksh et al., 2001). All types of classic NSAIDs can inhibit both COX-1 and COX-2 isoforms with a predominant effect on COX-1 (Smith et al., 2000). Most NSAIDs have broad side effects such as bleeding, ulceration, and perforation on gastrointestinal tract, while celecoxib selectively inhibits COX-2, and does not have side effects on the digestive system (Atukorala and Hunter, 2013; Rahme et al., 2007). Since celecoxib suppresses pain and inflammation, it is one of the most commonly prescribed drugs and accounts for 5-10% of prescriptions per year (Jones, 2001; Onder et al., 2004a; Wongrakpanich et al., 2018). Celecoxib can easily access the central nervous system (CNS), while the mechanism of action (MoA) through its protein targets in CNS has not yet been fully elucidated (Fond et al., 2014).

Determining the affinity of a drug for all its potential targets is the main challenge for understanding the MoA in pharmaceutical sciences. Target-based drug discovery (TDD) starts by identifying molecular targets, which are supposed to have an essential role in the disease of interest (Sams-Dodd, 2005; Schenone et al., 2013; Schmidt, 2010), opposed to phenotypic-based drug discovery (PDD). The mechanism of drug performance, that is essential for designing a drug, is not often considered in PDD investigations (Swinney, 2013). However, also TDD research has its limitations, i.e. proving the presence of a protein target in a particular biological pathway, or its involvement in disease, is a time- and cost-consuming process. Therefore, the development of alternative strategies for target deconvolution is on-demand. Different strategies have been emerged, which are based on changes in target stability upon compound binding (Li et al., 2016; Sjostrom et al., 2015). Some successful options are Stability of Proteins from Rates of OXidation (SPROX) (Strickland et al., 2013; West et al., 2008; West et al., 2010), Drug Affinity Responsive Target Stability (DARTS) (Lomenick et al., 2011), , CEllular Thermal Shift Assay (CETSA) (Molina et al., 2013) and Thermal Proteome Profiling (TPP) (Savitski et al., 2014). In SPROX, proteins are subjected to an increasing concentration of a chemical denaturant (hydrogen peroxide) and then the evaluation of oxidized methionine in unfolded proteins. The folding free-energy (ΔG_f) is then calculated based on the denaturant concentration in the presence and absence of ligands to evaluate protein−ligand affinities. DARTS is based on the modification- or immobilization-free limited proteolysis, following the binding of a ligand to proteins. According to the assumption that protein targets become less susceptible to proteolysis when it is drug-bound, not drug-free, especially the exposed part of the protein, which is

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protected from protease. In CETSA, changes in the thermal stability of proteins are used for studying the ligand-binding process. However, CETSA can only detect a small number of protein changes since it is limited to an antibody readout. However, the CETSA principles can be combined with mass spectrometry-based proteomics to provide an unbiased identification of more comprehensive drug-protein interactions in a single experiment (Aebersold and Mann, 2016; Larance and Lamond, 2015). TPP, a recently suggested method, can be done in high-throughput to identify drug targets (Reckzeh et al., 2019). It can also be applied in living cells in addition to *in vitro* studies without requiring compound labeling. It is an approach combining CETSA and quantitative mass spectrometry, enabling monitoring of changes in protein thermal stability across heat scaling up. Identifying drug targets in TPP is based on changes in the thermal stability of proteins after their binding to the substrates, i.e. drugs (Pace and McGrath, 1980; Vedadi et al., 2006). This stability is mostly related to the protein melting temperature (TM), a temperature in which the process of unfolding will happen (Jarzab et al., 2020).

Thermal stress usually causes some irreversible changes in the structure of a protein leading to unfolding. This process leads to the exposure of the hidden hydrophobic core of a protein, and finally, to its aggregation (Asial et al., 2013; Kurganov et al., 2002). For proteins connected to a ligand (e.g. a drug), more energy is needed for unfolding because the dissociation of a ligand from the protein requires some energy itself (Pace and McGrath, 1980). In other words, binding of a ligand to a protein causes the formation of a complex with increased stability compared to the free protein. Therefore, these proteins are more resistant to the process of unfolding induced by heat, a fact that is the basis of TPP (Becher et al., 2016; Franken et al., 2015; Reinhard et al., 2015; Savitski et al., 2014). TPP can be used to investigate any change in the structure of the protein (Franken et al., 2015). TPP is unique in having the following advantages: While not requiring any labeling, it can be applied to living cells, and it permits an objective search of drug targets (Mateus et al., 2016).

In the present study we have investigated targets of celecoxib, a high prevalence drug, using a label-free TPP method in rat hippocampus. We also provide supporting computational evidence related to biological annotations of the targets to explain the potential repurposing implications of this NSAID (Tanoli et al., 2020; Zagidullin et al., 2019). We further show that several proteins related to cancer and inflammation pathways are the targets of Celecoxib. The results of these experiments are also compared with the available knowledge across all drug-target interaction databases. In addition to reinforcing previous findings, we especially explore more potential off-targets of Celecoxib within the nervous system. Based on these results we suggest a conceivable repurposing strategy of this drug for neuronal inflammation as well as cancer.

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Materials and Methods

Preparation rat brain for protein extraction

Five rats were used as biological replicates, considering not affecting the present study by two crucial variables (i.e., gender and weight). Therefore, five male rats of *Rattus norvegicus* were prepared by the weight of 200+_10 gr. After dissecting the hippocampus under complete anesthesia, tissue was washed two times with cold PBS. Experiments were approved by the local Animal Ethics Committee (National Institute for Medical Research Development Ethics Board, NIMAD, No.964580). Immediately after washing, the hippocampus was homogenized and lysed in RIPA buffer (NaCl, Triton 500, Na deoxycholate, Tris HCl, Protease Inhibitor Cocktail pH~7.4). Then, the homogenates were centrifuged at 20,000 g for 20 min at 4°C in order to separate the protein extracts from precipitates (Pei et al., 2007). Bradford assay was used to measure protein concentrations.

Drug treatment and heating procedure

A solution of celecoxib in dimethyl sulfoxide (DMSO) was added to the protein extracts to have a 0.1% final DMSO concentration. In this study, five concentrations of celecoxib including 20 µM, 10 µM, 5 µM, 1 µM and 0.1µM, were used, based on the pharmaceutical implications as described previously (Dembo et al., 2005; Kang et al., 2009; Paulson et al., 2001; Wang et al., 2017). Two negative controls, i.e., control with DMSO and control with pure DD water were also used. The starting protein amounts in each tube were 1600 µg in total of 400 µl solution. The extracts were incubated for 10 min at 23°C, and then divided into four aliquots of 100 µl.

These 4 aliquots were heated at the following temperatures: 37°C, 47 °C, 57 °C, and 67 °C for 3 min. This was followed by cooling down at room temperature for 3 minutes. Subsequently, the extracts were centrifuged at 60,000 g for 30 min at 4°C and finally, the supernatant which contained soluble targeted proteins was collected and stored at -20°C for further investigations as previously described (Jafari et al., 2014; Savitski et al., 2014).

Sample preparation, proteolytic digestion, and nano LC-ESI-MS/MS

Next, the extracted proteins treated with the highest drug concentration, i.e., 20 µM at the highest temperature, i.e., 67°C was selected for the protein identification step. The highest dosage of Celecoxib and the highest temperature was used to avoid detection of the weak or transient interactions of Celecoxib and the proteins. The same temperature was used to analyze and identify proteins in the control negative samples.

The protein samples were digested in Amicon Ultra-0.5 centrifugal filters using a modified FASP method (Scifo et al., 2015; Wiśniewski et al., 2009). In brief, reduction and alkylation of samples were performed by the addition of tris (2-carboxyethyl) phosphine (TCEP) and iodoacetamide to a final concentration of 2 mM and 50 mM respectively and incubation in the dark for 30 min. The trypsin solution was added in a ratio of 1:50 w/w in 50 mM ammonium bicarbonate and incubate overnight at room temperature. The peptide samples were cleaned using C18-reverse-phase ZipTipTM (Millipore). Dried peptide digest was re-suspended in 1% TFA, and sonicated in a water bath for 1 min before injection. Fractionated protein digests were

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analyzed in nano-LC-Thermo Q Exactive Plus Orbi-Trap MS. Each sample run was followed by two empty runs to wash out any remaining peptides from previous runs. The peptides were separated by Easy-nLC system (Thermo Scientific) equipped with a reverse-phase trapping column Acclaim PepMapTM 100 (C18, 75 µm × 20 mm, 3 µm particles, 100 Å; Thermo Scientific), followed by an analytical Acclaim PepMapTM 100 RSLC reversed-phase column (C18, 75 µm × 250 mm, 2 µm particles, 100 Å; Thermo Scientific). The injected sample analytes were trapped at a flow rate of 2 µl min-1 in 100% of solution A (0.1 % formic acid). After trapping, the peptides were separated with a linear gradient of 120 min comprising 96 min from 3% to 30% of solution B (0.1% formic acid/80% acetonitrile), 7 min from 30% to 40% of solution B, and 4 min from 40% to 95% of solution B.

LCMS data acquisition was done with the mass spectrometer settings as follows: The resolution was set to 140,000 for MS scans, and 17,500 for the MS/MS scans. Full MS was acquired from 350 to 1400 m/z, and the 10 most abundant precursor ions were selected for fragmentation with 30 s dynamic exclusion time. Ions with 2+, 3+, and 4+ charge were selected for MS/MS analysis. Secondary ions were isolated with a window of 1.2 m/z. The MS AGC target was set to 3 x 106 counts, whereas the MS/MS AGC target was set to 1 x 105. Dynamic exclusion was set with a duration of 20 s. The NCE collision energy stepped was set to 28 kJ mol^{-1}.

Proteomic data and bioinformatic analysis

Following LC-MS/MS acquisition, the raw files were qualitatively analyzed by Proteome Discoverer (PD), version 2.4.0.305 (Thermo Scientific, USA). The identification of proteins by PD was performed against the UniProt Rat protein database (release 11-2019 with 8086 entries) using the built-in SEQUEST HT engine. The following parameters were used: 10 ppm and 0.25 Da were tolerance values set for MS and MS/MS, respectively. Trypsin was used as the digesting enzyme, and two missed cleavages were allowed. The carbamidomethylation of cysteines was set as a fixed modification, while the oxidation of methionine and deamidation of asparagine and glutamine were set as variable modifications. The false discovery rate was set to less than 0.01 and a minimum length of six amino acids (one peptide per protein) was required for each peptide hit.

Following the identification of proteins, for better understanding of the role and importance of proteins, enrichment analysis was used to determine the corresponding biological processes by EnrichR (Chen et al., 2013). Eight different libraries were selected to explore biomedical annotations of drug targets, including gene ontology (GO), molecular function (MF), GO Cellular Component (CC), GO Biological Process (BP), DisGeNet (Piñero et al., 2016), HumanPhen (Köhler et al., 2019), Mouse Genome Informatics (MGI) (Eppig et al., 2017), PheWeb (Taliun et al., 2020) and WikiPathways (Kutmon et al., 2016). We used EnrichR's combined scores and adjusted p-values to sort annotations descendingly. Also, PEIMAN software was used to determine possible enriched post-translational modifications (PTM) in the list of protein targets (Nickchi et al., 2015).

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Statistical analysis

All data were analyzed using R (version 4.0.3) with RStudio (Free Software Foundation Inc., Boston, MA). Unless otherwise noted, data are showed as mean ± s.d. for technical replicates. Statistical significance was calculated by Fisher's exact test and hypergeometric test for enrichment analysis. Multiple testing corrections were done using the Benjamini–Hochberg method (Jafari and Ansari-Pour, 2019). Using the decoy database search feature, the q-value for protein identification was calculated in Proteome Discoverer software. Due to the exploratory nature of this study, we reduced biological variability by pooling the rat samples and focused on the physicochemical effect of celecoxib treatment on extracted proteins from the rat hippocampus. Hence, testing a null hypothesis among rats is irrelevant, and the statistical tests' outcomes are interpretable based on technical replicates in each step of our study.

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Result

The amount of soluble proteins significantly decreased with increasing temperature (Supplementary File 1). The declining pattern was observed for all the five drug concentrations; 20µM, 10 µM, 5 µM, 1 µM, 0.1 µM, and two negative controls, i.e., water and DMSO. Finally, the protein sample treated in 20 µM drug concentration and 67℃ was chosen for further analysis. In fact, proteins start unfolding at high temperature unless the binding energy of any binding partner such as a drug is high enough (Guo et al., 2012; Petsko and Ringe, 2004). We used the highest temperature to avoid detecting the weak and transient interactions among Celecoxib and the proteins. Also, we selected the highest dosage of Celecoxib to detect all potent drug-target interactions.

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A comprehensive comparison of identified proteins in samples treated with celecoxib and two controls is shown in Fig. 2A. These proteins were soluble at 67°C, following the treatment in 20μM celecoxib, water, and DMSO, respectively, and finally detected by nano-LC-Thermo Q Exactive Plus Orbi-Trap MS. Water control treatment contains only protein samples without any other additional substances, and 351 proteins are detected in this subset. Also, 378 proteins were identified in the DMSO treatment (other negative control). Furthermore, 357 proteins were detected in the drug-treated sample, in which 44 proteins were specific to this subset (Supplementary File 2). Fifteen out of all identified proteins are heat shock proteins (HSP), which indicate the intrinsic structural stability of these proteins across the high temperature (Usman et al., 2014). The identified HSPs were shared with other groups, such as Heat shock protein HSP 90-beta and 60 kDa mitochondrial heat shock protein. Thus, we could infer that HSPs are not the particular targets of celecoxib.

We also examined the previously known targets of celecoxib according to five drug-target databases for all species (Fig. 2B) including *Rattus norvegicus* (rat) in particular (Fig. 2C). Then, we compared the TPP-identified proteins with the known targets of this drug in rats. Out of 242 already identified celecoxib targets for 24 species in all five databases, only 21 proteins are found in rat. Figure 2B-C shows the total number of proteins in each set by the horizontal bar plots. The vertical bar plot indicates the number of proteins in each database uniquely and the different set of the intersections, sorted by the frequency of targets. In this analysis, we selected five well-known drug-target databases, i.e., Drug Bank (DB) (Wishart et al., 2018), Super Target (ST) (Hecker et al., 2012), Probes & Drugs portal (PDP) (Skuta et al., 2017), Chembl (Bento et al., 2013), and Drug Target Commons (DTC) (Tang et al., 2018; Tanoli et al., 2018). The DB database shows five targets for celecoxib of which one was related to the rat. The ST database and PDP suggests 41 and 45 proteins as a target of celecoxib of which three and five are expressed in the rat, respectively. Searching in DTC and Chembl databases, introduced 168 and 203 proteins in 24 species as a Celecoxib target and 17, and 16 of them are specified in the rat, respectively. In total, around 70% of the identified targets are related to human proteins and the proportion of rat-specific proteins is much lower, especially if we consider each database independently. It can imply the lack of complete information in rat species databases, avoiding a more comprehensive celecoxib target profile in rats. It should be considered that most of the introduced protein targets are associated with the COX protein family, and are involved in NSAID related pathways, i.e., inflammatory process, which is the explicit indication of this drug.

As shown in Figure 2B, the intersection of all databases contains only two human proteins, i.e., PDPK1, CA2 and one rat protein, Ptgs2, due to the cross-reference of the resources. Chembl and DTC are the most comprehensive drug target bioactivity resources based on manual curation (more than 1.9 million chemicals and 13,000 protein targets); therefore, it was expected that they have the highest number of intersected proteins for Celecoxib. At the same time the other databases used experimental evidence to explore targets of drugs. Only six proteins have been identified as Celecoxib targets using ST, DB, and PDP so far. On the other hand, the main subject of celecoxib studies is to study the effects of this drug on the heart and circulatory

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system; hence researchers focused on exploring new off-targets on related organs and tissues. Although Celecoxib can simply pass through the blood-brain barrier (BBB), its impacts on the brain and CNS have not been well described. Here, we focused on a minute part of CNS, i.e., the hippocampus; hence we did not anticipate to observe a high proportion of intersected protein targets with the other databases. However, we found a Ras-related protein Rab-2A as a shared celecoxib targeted protein between TPP-identified proteins and the PDP database. The high amount of expression of Rab-2A in the whole brain has been previously reported (Palasca et al., 2018), which was helpful for our study (Fig. 2-supplementary File 3). This protein can be a clue to explain the association of Celecoxib with cancer-related pathways since Rab-2A is a cancer driver gene product, and it plays a role in promoting tumorigenesis (Luo et al., 2015).

We also investigated the homology of TPP-identified proteins with reported Celecoxib targets to explore structural similarities (Fig. 2D). The overall similarity of amino acid sequences in both protein groups was represented using a protein homology network. In this graph, the thickness of the edges indicates the amino acid identity percentages. There is a 665 and 3138 pairwise similarity with more than 25% and 10% thresholds. Thus, it can be concluded that several of TPP-identified proteins have a close homology with the previously reported celecoxib targeted proteins.

Furthermore, to characterize the related biological functions of the TPP-identified proteins, we implemented gene enrichment analysis using disease and pathway-related resources available in EnrichR (Fig. 3). The enriched annotations in DisGeNet database include muscular stiffness with the lowest adjusted p-value. Neurodegenerative diseases such as Alzheimer's disease and epilepsy and breast cancer-related annotations are also highly enriched in these proteins. Therefore, it can be a clue for celecoxib to be a potential choice for add-on therapy in these diseases. We also assessed other resources such as MGI, HumanPhen, and PheWeb for exploring enriched phenotypic annotations in the TPP-identified list of 44 proteins. In these databases, terms such as Broad head, increased motor neuron number, Schizophrenia, psychotic disorders, acquired hemolytic anemias, and abnormal thrombopoiesis showed the lowest adjusted p-value. In the perspective of pathway enrichment analysis, mRNA processing, such as cytoplasmic ribosomal proteins and splicing factor Nova regulated synaptic proteins, were also enriched along with cancer-related pathways such as IL-3, PIK3-Akt-mTOR and G protein-mediated signaling pathways which have an importance in cancer, inflammation, and neurodegenerative diseases.

In Figure 4 and Supplementary File 4, the enriched gene ontology annotations i.e., biological processes (BP), cellular components (CC), and molecular functions (MF) were summarized by using semantic similarity. The annotations of BP were divided into six major subsets (Fig. 4-Supplementary File 4A). The SRP−dependent co-translational protein, targeting to membrane processes, contributes to the prominent concept in this analysis. This process is responsible for the targeting of proteins to the cell membrane during translation, and it is dependent on two key components, the signal-recognition particle (SRP) and the SRP receptor. Rab protein signal

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transduction is the second most prevalent annotation in the tree-map of BPs. Rab proteins represent the largest branch of the Ras-like small GTPase superfamily, alternating between GTP- and GDP-bound states and releasing a series of molecular signals within the cell. Nuclear-transcribed mRNA catabolism, nonsense−mediated decay, post-translational protein modification, and neutrophil-mediated immunity are four other groups of annotations in BP similar to the result of pathway enrichment analysis. These terms indicate the long-term effects of Celecoxib by PTM-related mechanisms and G protein-related signaling pathways. At the molecular level, nine groups of MF annotations were illustrated for TPP-identified proteins (Fig. 4-Supplementary File 4B). The activities related to signal transduction in neuronal cells involving transport mechanisms were also highlighted, such as myosin, actin, and cadherin binding, in addition to GDP binding and GTPase activity. The enriched annotations of CC are mainly corresponding to the cytosolic part, which also underscores altering the signaling pathways (Jafari et al., 2013) (Fig. 4-Supplementary File 4C).

The enriched PTMs in TPP-identified proteins were also evaluated by PEIMAN software. It is presumed that soluble proteins at 67C might be enriched in any of PTMs to last longer under temperature changes. We observed fifteen enriched PTMs, which emphasizes the role of PTMs in the thermostability of proteins. For example, Acetylation, prenylation, and phosphorylation are significantly detected in all TPP-identified proteins. Citrullination was the specific PTM for Celecoxib targets which was statistically enriched by adjusted p-value 7.6E-3. All of the enriched PTMs were confirmed by re-searching the proteomic data using these PTM as variable modifications in Proteome Discoverer.

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Discussion

Celecoxib is one of the top-selling NSAID medicines in the world [67]. Also, NSAIDs involve 5%-10% of the remedy of all prescriptions per year (Onder et al., 2004b; Paulson et al., 2001). There are some reports that show the possible indication of celecoxib with the neurodegenerative diseases associated with inflammatory processes (Akiyama et al., 2000; Eikelenboom and Van Gool, 2004; Hirsch et al., 2003; McGeer and McGeer, 2004; Terzi et al., 2018). Though celecoxib can pass through the BBB and access to the CNS, reports about side effects of celecoxib (Goncalves et al., 2010; Nam et al., 2015) are related to cardiovascular diseases rather than the nervous system (Fond et al., 2014). In other words, the major molecular footprints of this medicine on central nervous system (CNS) are not well-described (Fond et al., 2014). Indeed, as we expected, we observed that most of the introduced targets of celecoxib in different databases are not related to CNS. Considering the essential role of celecoxib in the treatment of pain and inflammation, and its influence on the CNS, our study aimed to characterize protein targets of this drug especially in the nervous system.

One of the identified celecoxib-targets is Rab-2A, which is a GTPase required for protein transport from the endoplasmic reticulum to the Golgi complex by regulating COPI-dependent vesicular transport (Gaudet et al., 2011; Haas et al., 2007). This protein was common between TPP-identified targets and the PDP database (Fig. 2C). PDP is a powerful up-to-date web resource that unifies various commercial and public bioactive compound libraries (Skuta et al., 2017). To explore the role of Rab2A in detail, Sugawara *et al.* studied the effect of Rab2A knockdown on glucose-stimulated insulin secretion and the Golgi intermediate compartment in the corresponding cells. They reported that inactivation of Rab2A mitigated glucose-induced ER stress and inhibited apoptosis induced by ER stress through enlarging of the endoplasmic reticulum (ER)-Golgi intermediate compartment (Sugawara et al., 2014). Therefore, it seems that celecoxib is associated with apoptosis by targeting Rab-2A and implicating ER stress. Providing more evidence through testing celecoxib on the same cells, insulinoma cells, to clarify the celecoxib influence on the ER stress is warranted.

Also, TPP-identified proteins were enriched in pathways related to neurodegenerative disease and cancer. Interestingly, the anticancer activity of celecoxib has been reported in various models of animal tumors, and it is proposed that this drug is beneficial for the prevention and treatment of cancer (Dannenberg and Subbaramaiah, 2003; Koehne and Dubois, 2004; Masferrer et al., 2000). The molecular mechanisms of antitumoral effects of celecoxib have become a challenging issue, since some reports showed that the effect of celecoxib on cancer is apart from COX-2 inhibition, meaning that celecoxib has other targets than COX-2 (Grösch et al., 2006; Kashfi and Rigas, 2005; Schönthal, 2007). Several components as intermediate candidates have been proposed for the anticancer effects of celecoxib, the most common of which is the sarco/endoplasmic reticulum Ca2+-ATPase (SERCA) (Johnson et al., 2002; Pyrko et al., 2007; Tanaka et al., 2005). Our CC enrichment analysis also disclosed that the endoplasmic reticulum lumen annotation was statistically enriched in TPP-identified proteins, such that several of the

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proteins involved in the pathways that regulate calcium concentrations, including ERO1A, ARSB, NOL3, STIM1, CALCR, SDF4, and BAX (Figure 4). Interestingly, it has been previously shown that celecoxib increases the intracellular concentration of calcium by inhibiting SERCA (Johnson et al., 2002; Pyrko et al., 2007; Tanaka et al., 2005; Wang et al., 2004) and the long-term leakage of calcium from the endoplasmic reticulum acts as a potent stimulant of ER stress, which finally leads to cell death and exerts its effect on cancer (Kim et al., 2007; Pyrko et al., 2007).

Several members of the Ras-associated binding (Rab) family are obviously expressed in various cancer tissues, and dysregulation of Rab expression could be tumorigenic or tumor-suppressive (Chia and Tang, 2009). The Rab family plays an essential role in multiple aspects of membrane trafficking control. Therefore, vesicle transport regulators play crucial roles in the mediation of cancer cell biology, including uncontrolled cell growth, invasion, and metastasis. The Rabs, like other members of the Ras superfamily, function as molecular switches through changes in its guanine nucleotide-binding status between the active GTP-bound and inactive GDP-bound forms. In its active, GTP-bound form, Rabs could mediate vesicular transport by allowing transport carriers or vesicles to engage specific effectors such as motor proteins and tethering factors, as well as vesicle fusion with the engagement of soluble N-ethylmaleimide sensitive factor (NSF) (Zhao et al., 2007) attachment receptor (SNARE) (Hong, 2005; Ungermann and Langosch, 2005) proteins. Vesicle delivery and dynamics are critical for regulating cell behavior associated with cell migration/invasion and tumorigenesis. Cooperation between Rabs and effectors in mediating vesicle movement pathways has significant influences on tumor progression and malignancy. Therefore, it raises the possibility that targeting a particular trafficking system may provide a new approach to cancer treatment (Tzeng and Wang, 2016). As shown in this study, celecoxib targeted proteins, i.e., RAB2A, RAB10, and RAB11B are notably involved in Rab protein signal transduction. As shown in Figure 4B, TPP-identified proteins are enriched in GDP binding, GTPase activity, and protein phosphatase inhibitor activity that change the GTPases and, as a result, involve in mechanisms associated with cancer. Therefore, it seems that studying the effect of celecoxib on cancer models by TPP provide more supporting evidence.

Neurodegenerative diseases are also assigned to TPP-identified targets of celecoxib as an anti-inflammatory drug. Recent studies demonstrated that neuronal inflammation is a vital trigger of neurological diseases (Terzi et al., 2018), and it exacerbates disorders including Alzheimer 's -, Parkinsons - , Huntingtons diseases, as well as amyotrophic lateral sclerosis and multiple sclerosis (Akiyama et al., 2000; Eikelenboom and Van Gool, 2004; Hirsch et al., 2003; McGeer and McGeer, 2004). In the present study, some of the mentioned neurodegenerative disorders were enriched based on phenotypic-based biological annotations, such as schizophrenia and depression. Twelve of 44 TPP-identified celecoxib targets are involved in Alzheimer's disease metabolism, suggesting a high possibility of celecoxib involvement in the mechanisms of this neurodegenerative disease. Notably, inflammation of the nervous system is observed in these disorders, and it is accompanied by an increase in inflammatory cytokines (Agius, 2012; Morales

Molecular Pharmacology Fast Forward. Published on February 25, 2021 as DOI: 10.1124/molpharm.120.000210
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et al., 2015; Philips and Robberecht, 2011). We also illustrated that celecoxib could be beneficial in treating the diseases mentioned above that are associated with inflammation by affecting the biosynthesis pathway of prostaglandins by the involvement of four identified proteins, i.e., DCTN1, PSIP1, BAX and AMPH.

Finally, we describe the importance of PTMs for the thermal stability of proteins. We show that multiple PTMs are involved in the protein thermostability. For example, acetylation, which significantly affects the life span of intracellular proteins by avoiding intracellular proteases degradation, is enriched in all TPP-identified proteins (Lahusen et al., 2018; Zhou et al., 2016). Citrullination is the specific PTM identified in celecoxib treated sample (See Fig. 5). It is related to the change of arginine to citrulline, which strongly affects the structure and function of proteins in both physiological and pathological processes such as apoptosis, multiple sclerosis, and Alzheimer's disease (Acharya et al., 2012; György et al., 2006; Piran et al., 2020). Interestingly, an important diagnostic tool in the painful inflammatory disease such as Rheumatoid arthritis is to use anti-cyclic citrullinated peptide (anti-CCP) antibodies which detect citrullination levels of the patients and NSAIDs including Celecoxib are usually prescribed for those patients (Gilliam et al., 2013; Kwiatkowska et al., 2017). Our findings highlight the role of citrullinated proteins as a target of Celecoxib.

Although phenotypic-based screens have become increasingly popular in drug discovery, the major challenge of this approach is the mechanistic deconvolution of the putative drug action during screening. The promising TPP approach has been introduced and expanded to tackle such challenges. In the present study, targets of celecoxib within rat hippocampus were characterized using TPP as a high throughput target discovery approach.

We show that celecoxib plays an effector role in several signaling pathways and biological processes, which can be linked to various diseases such as neurodegenerative disorders and cancer. Therefore, in addition to inhibiting COX2, we illustrate that celecoxib might modify also other pathways. Our findings support the pharmaceutical reports related to the repurposing of celecoxib for cancer and neurodegenerative disorders (Assefnia et al., 2014; Chuang et al., 2008; Goldstein et al., 2009). It seems that celecoxib is potentially beneficial for treating cancer by inhibiting SERCA and increasing the intracellular concentration of calcium, which causes ER stress along with cell death. Another proposed mechanism is affecting the trafficking system since transport regulators play essential roles in the mediation of cancer cell biology and especially circulating tumor cells. We found a significant effect of this medicine on proteins involved in the trafficking system of cells.

On the other hand, neuronal inflammation is a major culprit of neurodegenerative diseases, proteins of which were significantly enriched in the present study. Inflammation in CNS starts by stimulation of astrocytes, and it continues with entering environmental immune cells to the brain. This process causes overproduction of cytokines, nitric oxide, active oxygen species, prostaglandins and eventually damage and cause death of neurons (Nam et al., 2015; Philips and Robberecht, 2011; Terzi et al., 2018; Wang et al., 2017). Our findings support the idea of using

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celecoxib for neuronal inflammation due to the explored association of celecoxib targets and the inflammation.

To conclude, we identified several novel Celecoxib protein targets using TPP, which could be of interest in order to modify several pathways in CNS. Our findings provide new molecular evidence for celecoxib to be used as an add-on therapy in neurodegenerative disorders and cancer. However, more preclinical and paraclinical evidence is required to suggest the true drug repurposing potential of celecoxib. In general, the potential drug targets can be corroborated by functional studies. Our findings can be considered in different pathophysiological conditions such as animal models with neurodegenerative disease or cancer with high COX protein family expression and other inflammatory proteins. In the case of providing enough amount of purified protein target, the kinetics and affinities of the drug-target interactions can also be evaluated using various biophysical methods such as isothermal titration calorimetry and surface plasmon resonance. All-told, additional supporting evidence using the possible protein-specific strategies such as antibody-based and activity-based assays can support the rationale of celecoxib repositioning.

Molecular Pharmacology Fast Forward. Published on February 25, 2021 as DOI: 10.1124/molpharm.120.000210
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Acknowledgement

The authors also acknowledge Dr. Rozbeh Jafari and Dr. Farnaz Barneh for helpful comments.

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Contributed new reagents or analytic tools: Khaleghian, Salimi, Gilany, Rezadoost, Tanoli and Baumann.

Performed data analysis: Gholizadeh, Rezadoost, Soliymani and Jafari.

Wrote or contributed to the writing of the manuscript: Gholizadeh, Baumann, Tang, and Jafari.

Molecular Pharmacology Fast Forward. Published on February 25, 2021 as DOI: 10.1124/molpharm.120.000210
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References

.

Acharya NK, Nagele EP, Han M, Coretti NJ, DeMarshall C, Kosciuk MC, Boulos PA and Nagele RG (2012) Neuronal PAD4 expression and protein citrullination: possible role in production of autoantibodies associated with neurodegenerative disease. *Journal of autoimmunity* **38**(4): 369-380.

Aebersold R and Mann M (2016) Mass-spectrometric exploration of proteome structure and function. *Nature* **537**(7620): 347-355.

Agius LM (2012) Neuroinflammation as the proximate cause of signature pathogenic pattern progression in amyotrophic lateral sclerosis, AIDS, and multiple sclerosis. *Pathology research international* **2012**.

Akiyama H, Barger S, Barnum S, Bradt B, Bauer J, Cole GM, Cooper NR, Eikelenboom P, Emmerling M and Fiebich BL (2000) Inflammation and Alzheimer's disease. *Neurobiology of aging* **21**(3): 383-421.

Asial I, Cheng YX, Engman H, Dollhopf M, Wu B, Nordlund P and Cornvik T (2013) Engineering protein thermostability using a generic activity-independent biophysical screen inside the cell. *Nature communications* **4**(1): 1-8.

Assefnia S, Dakshanamurthy S, Guidry Auvil JM, Hampel C, Anastasiadis PZ, Kallakury B, Uren A, Foley DW, Brown ML, Shapiro L, Brenner M, Haigh D and Byers SW (2014) Cadherin-11 in poor prognosis malignancies and rheumatoid arthritis: common target, common therapies. *Oncotarget* **5**(6): 1458-1474.

Atukorala I and Hunter DJ (2013) Valdecoxib : the rise and fall of a COX-2 inhibitor. *Expert Opin Pharmacother* **14**(8): 1077-1086.

Becher I, Werner T, Doce C, Zaal EA, Tögel I, Khan CA, Rueger A, Muelbaier M, Salzer E and Berkers CR (2016) Thermal profiling reveals phenylalanine hydroxylase as an off-target of panobinostat. *Nature chemical biology* **12**(11): 908-910.

Bento AP, Gaulton A, Hersey A, Bellis LJ, Chambers J, Davies M, Krüger FA, Light Y, Mak L, McGlinchey S, Nowotka M, Papadatos G, Santos R and Overington JP (2013) The ChEMBL bioactivity database: an update. *Nucleic Acids Research* **42**(D1): D1083-D1090.

Chen EY, Tan CM, Kou Y, Duan Q, Wang Z, Meirelles GV, Clark NR and Ma'ayan A (2013) Enrichr: interactive and collaborative HTML5 gene list enrichment analysis tool. *BMC bioinformatics* **14**: 128-128.

Chia WJ and Tang BL (2009) Emerging roles for Rab family GTPases in human cancer. *Biochimica et Biophysica Acta (BBA)-Reviews on Cancer* **1795**(2): 110-116.

Chuang HC, Kardosh A, Gaffney KJ, Petasis NA and Schönthal AH (2008) COX-2 inhibition is neither necessary nor sufficient for celecoxib to suppress tumor cell proliferation and focus formation in vitro. *Mol Cancer* **7**: 38.

Dannenberg AJ and Subbaramaiah K (2003) Targeting cyclooxygenase-2 in human neoplasia: rationale and promise. *Cancer cell* **4**(6): 431-436.

Dembo G, Park SB and Kharasch ED (2005) Central nervous system concentrations of cyclooxygenase-2 inhibitors in humans. *Anesthesiology-Hagerstown* **102**(2): 409-415.

Eikelenboom P and Van Gool W (2004) Neuroinflammatory perspectives on the two faces of Alzheimer's disease. *Journal of Neural Transmission* **111**(3): 281-294.

Eppig JT, Smith CL, Blake JA, Ringwald M, Kadin JA, Richardson JE and Bult CJ (2017) Mouse Genome Informatics (MGI): resources for mining mouse genetic, genomic, and biological data in support of primary and translational research, in *Systems Genetics* pp 47-73, Springer.

Fond G, Hamdani N, Kapczinski F, Boukouaci W, Drancourt N, Dargel A, Oliveira J, Le Guen E, Marlinge E and Tamouza R (2014) Effectiveness and tolerance of anti-inflammatory drugs' add-on therapy in major mental disorders: a systematic qualitative review. *Acta Psychiatrica Scandinavica* **129**(3): 163-179.

Franken H, Mathieson T, Childs D, Sweetman GM, Werner T, Tögel I, Doce C, Gade S, Bantscheff M and Drewes G (2015) Thermal proteome profiling for unbiased identification of direct and indirect drug targets using multiplexed quantitative mass spectrometry. *Nature protocols* **10**(10): 1567.

Gaudet P, Livstone MS, Lewis SE and Thomas PD (2011) Phylogenetic-based propagation of functional annotations within the Gene Ontology consortium. *Briefings in bioinformatics* **12**(5): 449-462.

Gilliam BE, Chauhan AK and Moore TL (2013) Evaluation of anti-citrullinated type II collagen and anti-citrullinated vimentin antibodies in patients with juvenile idiopathic arthritis. *Pediatric rheumatology online journal* **11**(1): 31.

Goldstein BI, Kemp DE, Soczynska JK and McIntyre RS (2009) Inflammation and the phenomenology, pathophysiology, comorbidity, and treatment of bipolar disorder: a systematic review of the literature. *The Journal of clinical psychiatry* **70**(8): 1078-1090.

Goncalves MB, Williams EJ, Yip P, Yáñez-Muñoz RJ, Williams G and Doherty P (2010) The COX-2 inhibitors, meloxicam and nimesulide, suppress neurogenesis in the adult mouse brain. *British journal of pharmacology* **159**(5): 1118-1125.

Grösch S, Maier TJ, Schiffmann S and Geisslinger G (2006) Cyclooxygenase-2 (COX-2)–independent anticarcinogenic effects of selective COX-2 inhibitors. *Journal of the National Cancer Institute* **98**(11): 736-747.

Guo M, Xu Y and Gruebele M (2012) Temperature dependence of protein folding kinetics in living cells. *Proceedings of the National Academy of Sciences of the United States of America* **109**(44): 17863-17867.

György B, Tóth E, Tarcsa E, Falus A and Buzás EI (2006) Citrullination: a posttranslational modification in health and disease. *The international journal of biochemistry & cell biology* **38**(10): 1662-1677.

Haas AK, Yoshimura S, Stephens DJ, Preisinger C, Fuchs E and Barr FA (2007) Analysis of GTPase-activating proteins: Rab1 and Rab43 are key Rabs required to maintain a functional Golgi complex in human cells. *J Cell Sci* **120**(Pt 17): 2997-3010.

Hecker N, Ahmed J, von Eichborn J, Dunkel M, Macha K, Eckert A, Gilson MK, Bourne PE and Preissner R (2012) SuperTarget goes quantitative: update on drug-target interactions. *Nucleic acids research* **40**(Database issue): D1113-D1117.

Hirsch E, Breidert T, Rousselet E, Hunot S, Hartmann A and Michel P (2003) The Role of dial Reaction and Inflammation in Parkinson's Disease. *DOCUMENTATION PAGE* **991**: 214-228.

Hong W (2005) SNAREs and traffic. *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research* **1744**(2): 120-144.

Jafari M and Ansari-Pour N (2019) Why, When and How to Adjust Your P Values? *Cell journal* **20**(4): 604-607.

Jafari M, Mirzaie M, Sadeghi M, Marashi S-A and Rezaei-Tavirani M (2013) Exploring biological processes involved in embryonic stem cell differentiation by analyzing proteomic data. *Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics* **1834**(6): 1063-1069.

Jafari R, Almqvist H, Axelsson H, Ignatushchenko M, Lundbäck T, Nordlund P and Molina DM (2014) The cellular thermal shift assay for evaluating drug target interactions in cells. *Nature protocols* **9**(9): 2100.

Jarzab A, Kurzawa N, Hopf T, Moerch M, Zecha J, Leijten N, Bian Y, Musiol E, Maschberger M, Stoehr G, Becher I, Daly C, Samaras P, Mergner J, Spanier B, Angelov A, Werner T, Bantscheff M, Wilhelm M, Klingenspor M, Lemeer S, Liebl W, Hahne H, Savitski MM and Kuster B (2020) Meltome atlas—thermal proteome stability across the tree of life. *Nature Methods* **17**(5): 495-503.

Johnson AJ, Hsu A-L, Lin H-P, Song X and Chen C-S (2002) The cyclo-oxygenase-2 inhibitor celecoxib perturbs intracellular calcium by inhibiting endoplasmic reticulum Ca2+-ATPases: a plausible link with its anti-tumour effect and cardiovascular risks. *Biochemical Journal* **366**(3): 831-837.

Jones R (2001) Nonsteroidal anti-inflammatory drug prescribing: past, present, and future. *The American Journal of Medicine* **110**(1, Supplement 1): S4-S7.

Kang KB, Zhu C, Yong SK, Gao Q and Wong MC (2009) Enhanced sensitivity of celecoxib in human glioblastoma cells: induction of DNA damage leading to p53-dependent G 1 cell cycle arrest and autophagy. *Molecular cancer* **8**(1): 66.

Kashfi K and Rigas B (2005) Non-COX-2 targets and cancer: expanding the molecular target repertoire of chemoprevention. *Biochemical pharmacology* **70**(7): 969-986.

Kim S-H, Hwang C-I, Juhnn Y-S, Lee J-H, Park W-Y and Song Y-S (2007) GADD153 mediates celecoxib-induced apoptosis in cervical cancer cells. *Carcinogenesis* **28**(1): 223-231.

Koehne C-H and Dubois RN (2004) COX-2 inhibition and colorectal cancer, in *Seminars in oncology* pp 12-21, Elsevier.

Köhler S, Carmody L, Vasilevsky N, Jacobsen JOB, Danis D, Gourdine JP, Gargano M, Harris NL, Matentzoglu N, McMurry JA, Osumi-Sutherland D, Cipriani V, Balhoff JP, Conlin T, Blau H, Baynam G, Palmer R, Gratian D, Dawkins H, Segal M, Jansen AC, Muaz A, Chang WH, Bergerson J, Laulederkind SJF, Yüksel Z, Beltran S, Freeman AF, Sergouniotis PI, Durkin D, Storm AL, Hanauer M, Brudno M, Bello SM, Sincan M, Rageth K, Wheeler MT, Oegema R, Lourghi H, Della Rocca MG, Thompson R, Castellanos F, Priest J, Cunningham-Rundles C, Hegde A, Lovering RC, Hajek C, Olry A, Notarangelo L, Similuk M, Zhang XA, Gómez-Andrés D, Lochmüller H, Dollfus H, Rosenzweig S, Marwaha S, Rath A, Sullivan K, Smith C, Milner JD, Leroux D, Boerkoel CF, Klion A, Carter MC, Groza T, Smedley D, Haendel MA, Mungall C and Robinson PN (2019) Expansion of the Human Phenotype Ontology (HPO) knowledge base and resources. *Nucleic Acids Res* **47**(D1): D1018-d1027.

Kurganov B, Rafikova E and Dobrov E (2002) Kinetics of thermal aggregation of tobacco mosaic virus coat protein. *Biochemistry (Moscow)* **67**(5): 525-533.

Kutmon M, Riutta A, Nunes N, Hanspers K, Willighagen EL, Bohler A, Mélius J, Waagmeester A, Sinha SR and Miller R (2016) WikiPathways: capturing the full diversity of pathway knowledge. *Nucleic acids research* **44**(D1): D488-D494.

Kwiatkowska B, Majdan M, Mastalerz-Migas A, Niewada M, Skrzydło-Radomańska B and Mamcarz A (2017) Status of etoricoxib in the treatment of rheumatic diseases. Expert panel opinion. *Reumatologia* **55**(6): 290-297.

Lahusen TJ, Kim SJ, Miao K, Huang Z, Xu X and Deng CX (2018) BRCA1 function in the intra-S checkpoint is activated by acetylation via a pCAF/SIRT1 axis. *Oncogene* **37**(17): 2343-2350.

Larance M and Lamond AI (2015) Multidimensional proteomics for cell biology. *Nature reviews Molecular cell biology* **16**(5): 269-280.

Li J, Xu H, West GM and Jones LH (2016) Label-free technologies for target identification and validation. *MedChemComm* **7**(5): 769-777.

Lomenick B, Jung G, Wohlschlegel JA and Huang J (2011) Target identification using drug affinity responsive target stability (DARTS). *Current protocols in chemical biology* **3**(4): 163-180.

Luo M-L, Gong C, Chen C-H, Hu H, Huang P, Zheng M, Yao Y, Wei S, Wulf G and Lieberman J (2015) The Rab2A GTPase promotes breast cancer stem cells and tumorigenesis via Erk signaling activation. *Cell reports* **11**(1): 111-124.

Marnett LJ, Rowlinson SW, Goodwin DC, Kalgutkar AS and Lanzo CA (1999) Arachidonic acid oxygenation by COX-1 and COX-2 Mechanisms of catalysis and inhibition. *Journal of Biological Chemistry* **274**(33): 22903-22906.

Masferrer JL, Leahy KM, Koki AT, Zweifel BS, Settle SL, Woerner BM, Edwards DA, Flickinger AG, Moore RJ and Seibert K (2000) Antiangiogenic and antitumor activities of cyclooxygenase-2 inhibitors. *Cancer research* **60**(5): 1306-1311.

Mateus A, Määttä TA and Savitski MM (2016) Thermal proteome profiling: unbiased assessment of protein state through heat-induced stability changes. *Proteome science* **15**(1): 13.

McGeer PL and McGeer EG (2004) Inflammation and neurodegeneration in Parkinson's disease. *Parkinsonism & related disorders* **10**: S3-S7.

Molina DM, Jafari R, Ignatushchenko M, Seki T, Larsson EA, Dan C, Sreekumar L, Cao Y and Nordlund P (2013) Monitoring drug target engagement in cells and tissues using the cellular thermal shift assay. *Science* **341**(6141): 84-87.

Morales I, Guzmán-Martínez L, Cerda-Troncoso C, Farías GA and Maccioni RB (2015) Neuroinflammation in the pathogenesis of Alzheimer's disease. A rational framework for the search of novel therapeutic approaches. *Which New Directions for Alzheimer's Disease*.

Nam SM, Kim JW, Yoo DY, Choi JH, Kim W, Jung HY, Won M-H, Hwang IK, Seong JK and Yoon YS (2015) Comparison of pharmacological and genetic inhibition of cyclooxygenase-2: effects on adult neurogenesis in the hippocampal dentate gyrus. *Journal of veterinary science* **16**(3): 245-251.

Nickchi P, Jafari M and Kalantari S (2015) PEIMAN 1.0: Post-translational modification Enrichment, Integration and Matching ANalysis. *Database (Oxford)* **2015**: bav037-bav037.

Onder G, Pellicciotti F, Gambassi G and Bernabei R (2004a) NSAID-Related Psychiatric Adverse Events. *Drugs* **64**(23): 2619-2627.

Onder G, Pellicciotti F, Gambassi G and Bernabei R (2004b) NSAID-related psychiatric adverse events: who is at risk? *Drugs* **64**(23): 2619-2627.

Pace CN and McGrath T (1980) Substrate stabilization of lysozyme to thermal and guanidine hydrochloride denaturation. *Journal of Biological Chemistry* **255**(9): 3862-3865.

Palasca O, Santos A, Stolte C, Gorodkin J and Jensen LJ (2018) TISSUES 2.0: an integrative web resource on mammalian tissue expression. *Database* **2018**.

Paulson SK, Vaughn MB, Jessen SM, Lawal Y, Gresk CJ, Yan B, Maziasz TJ, Cook CS and Karim A (2001) Pharmacokinetics of celecoxib after oral administration in dogs and humans: effect of food and site of absorption. *J Pharmacol Exp Ther* **297**(2): 638-645.

Pei H, Zhu H, Zeng S, Li Y, Yang H, Shen L, Chen J, Zeng L, Fan J and Li X (2007) Proteome analysis and tissue microarray for profiling protein markers associated with lymph node metastasis in colorectal cancer. *Journal of proteome research* **6**(7): 2495-2501.

Perazella MA and Tray K (2001) Selective cyclooxygenase-2 inhibitors: a pattern of nephrotoxicity similar to traditional nonsteroidal anti-inflammatory drugs. *The American journal of medicine* **111**(1): 64-67.

Petsko GA and Ringe D (2004) *Protein structure and function*. New Science Press.

Philips T and Robberecht W (2011) Neuroinflammation in amyotrophic lateral sclerosis: role of glial activation in motor neuron disease. *The Lancet Neurology* **10**(3): 253-263.

Piñero J, Bravo À, Queralt-Rosinach N, Gutiérrez-Sacristán A, Deu-Pons J, Centeno E, García-García J, Sanz F and Furlong LI (2016) DisGeNET: a comprehensive platform integrating information on human disease-associated genes and variants. *Nucleic acids research*: gkw943.

Piran M, Karbalaei R, Piran M, Aldahdooh J, Mirzaie M, Ansari-Pour N, Tang J and Jafari M (2020) Can We Assume the Gene Expression Profile as a Proxy for Signaling Network Activity? *Biomolecules* **10**(6).

Pyrko P, Kardosh A, Liu Y-T, Soriano N, Xiong W, Chow RH, Uddin J, Petasis NA, Mircheff AK and Farley RA (2007) Calcium-activated endoplasmic reticulum stress as a major component of tumor cell death induced by 2, 5-dimethyl-celecoxib, a non-coxib analogue of celecoxib. *Molecular cancer therapeutics* **6**(4): 1262-1275.

Rahme E, Bardou M, Dasgupta K, Toubouti Y, Ghosn J and Barkun AN (2007) Hospitalization for gastrointestinal bleeding associated with non-steroidal anti-inflammatory drugs among elderly patients using low-dose aspirin: a retrospective cohort study. *Rheumatology (Oxford)* **46**(2): 265-272.

Reckzeh ES, Brockmeyer A, Metz M, Waldmann H and Janning P (2019) Target Engagement of Small Molecules: Thermal Profiling Approaches on Different Levels. *Methods in molecular biology (Clifton, NJ)* **1888**: 73-98.

Reinhard FB, Eberhard D, Werner T, Franken H, Childs D, Doce C, Savitski MF, Huber W, Bantscheff M and Savitski MM (2015) Thermal proteome profiling monitors ligand interactions with cellular membrane proteins. *Nature methods* **12**(12): 1129-1131.

Sams-Dodd F (2005) Target-based drug discovery: is something wrong? *Drug discovery today* **10**(2): 139-147.

Savitski MM, Reinhard FB, Franken H, Werner T, Savitski MF, Eberhard D, Molina DM, Jafari R, Dovega RB and Klaeger S (2014) Tracking cancer drugs in living cells by thermal profiling of the proteome. *Science* **346**(6205): 1255784.

Schenone M, Dančík V, Wagner BK and Clemons PA (2013) Target identification and mechanism of action in chemical biology and drug discovery. *Nature chemical biology* **9**(4): 232.

Schmidt C (2010) GSK/Sirtris compounds dogged by assay artifacts, Nature Publishing Group.

Schönthal A (2007) Direct non-cyclooxygenase-2 targets of celecoxib and their potential relevance for cancer therapy. *British journal of cancer* **97**(11): 1465-1468.

Scifo E, Szwajda A, Soliymani R, Pezzini F, Bianchi M, Dapkunas A, Dębski J, Uusi-Rauva K, Dadlez M and Gingras A-C (2015) Proteomic analysis of the palmitoyl protein thioesterase 1 interactome in SH-SY5Y human neuroblastoma cells. *Journal of proteomics* **123**: 42-53.

Sjostrom M, Ossola R, Breslin T, Rinner O, Malmström L, Schmidt A, Aebersold R, Malmstrom J and Niméus E (2015) A combined shotgun and targeted mass spectrometry strategy for breast cancer biomarker discovery. *Journal of proteome research* **14**(7): 2807-2818.

Skuta C, Popr M, Muller T, Jindrich J, Kahle M, Sedlak D, Svozil D and Bartunek P (2017) Probes & Drugs portal: an interactive, open data resource for chemical biology. *Nature methods* **14**(8): 759-760.

Smith WL, DeWitt DL and Garavito RM (2000) Cyclooxygenases: structural, cellular, and molecular biology. *Annual review of biochemistry* **69**(1): 145-182.

Strickland EC, Geer MA, Tran DT, Adhikari J, West GM, DeArmond PD, Xu Y and Fitzgerald MC (2013) Thermodynamic analysis of protein-ligand binding interactions in complex biological mixtures using the stability of proteins from rates of oxidation. *Nature protocols* **8**(1): 148.

Sugawara T, Kano F and Murata M (2014) Rab2A is a pivotal switch protein that promotes either secretion or ER-associated degradation of (pro) insulin in insulin-secreting cells. *Scientific reports* **4**(1): 1-14.

Swinney D (2013) Phenotypic vs. target-based drug discovery for first-in-class medicines. *Clinical Pharmacology & Therapeutics* **93**(4): 299-301.

Taliun SAG, VandeHaar P, Boughton AP, Welch RP, Taliun D, Schmidt EM, Zhou W, Nielsen JB, Willer CJ and Lee S (2020) Exploring and visualizing large-scale genetic associations by using PheWeb. *Nature Genetics* **52**(6): 550-552.

Tanaka K-i, Tomisato W, Hoshino T, Ishihara T, Namba T, Aburaya M, Katsu T, Suzuki K, Tsutsumi S and Mizushima T (2005) Involvement of intracellular Ca2+ levels in nonsteroidal anti-inflammatory drug-induced apoptosis. *Journal of Biological Chemistry* **280**(35): 31059-31067.

Tang J, Tanoli ZU, Ravikumar B, Alam Z, Rebane A, Vähä-Koskela M, Peddinti G, van Adrichem AJ, Wakkinen J, Jaiswal A, Karjalainen E, Gautam P, He L, Parri E, Khan S, Gupta A, Ali M, Yetukuri L, Gustavsson AL, Seashore-Ludlow B, Hersey A, Leach AR, Overington JP, Repasky G, Wennerberg

Molecular Pharmacology Fast Forward. Published on February 25, 2021 as DOI: 10.1124/molpharm.120.000210
This article has not been copyedited and formatted. The final version may differ from this version.

K and Aittokallio T (2018) Drug Target Commons: A Community Effort to Build a Consensus Knowledge Base for Drug-Target Interactions. *Cell chemical biology* **25**(2): 224-229.e222.

Tanoli Z, Alam Z, Vähä-Koskela M, Ravikumar B, Malyutina A, Jaiswal A, Tang J, Wennerberg K and Aittokallio T (2018) Drug Target Commons 2.0: a community platform for systematic analysis of drug-target interaction profiles. *Database (Oxford)* **2018**: 1-13.

Tanoli Z, Seemab U, Scherer A, Wennerberg K, Tang J and Vähä-Koskela M (2020) Exploration of databases and methods supporting drug repurposing: a comprehensive survey. *Briefings in bioinformatics*.

Terzi M, Altun G, Şen S, Kocaman A, Kaplan AA, Yurt KK and Kaplan S (2018) The use of non-steroidal anti-inflammatory drugs in neurological diseases. *Journal of chemical neuroanatomy* **87**: 12-24.

Tzeng H-T and Wang Y-C (2016) Rab-mediated vesicle trafficking in cancer. *Journal of biomedical science* **23**(1): 70.

Ungermann C and Langosch D (2005) Functions of SNAREs in intracellular membrane fusion and lipid bilayer mixing. *Journal of cell science* **118**(17): 3819-3828.

Usman MG, Rafii M, Ismail M, Malek M, Latif MA and Oladosu Y (2014) Heat shock proteins: functions and response against heat stress in plants. *International Journal Of Scientific and T echnology Research* **3**(11): 204-218.

Vedadi M, Niesen FH, Allali-Hassani A, Fedorov OY, Finerty PJ, Wasney GA, Yeung R, Arrowsmith C, Ball LJ and Berglund H (2006) Chemical screening methods to identify ligands that promote protein stability, protein crystallization, and structure determination. *Proceedings of the National Academy of Sciences* **103**(43): 15835-15840.

Wang J-L, Lin K-L, Chen J-S, Lu Y-C, Jiann B-P, Chang H-T, Hsu S-S, Chen W-C, Huang J-K and Ho C-M (2004) Effect of celecoxib on Ca2+ movement and cell proliferation in human osteoblasts. *Biochemical pharmacology* **67**(6): 1123-1130.

Wang R, Tian S, Yang X, Liu J, Wang Y and Sun K (2017) Celecoxib-induced inhibition of neurogenesis in fetal frontal cortex is attenuated by curcumin via Wnt/β-catenin pathway. *Life sciences* **185**: 95-102.

West GM, Tang L and Fitzgerald MC (2008) Thermodynamic analysis of protein stability and ligand binding using a chemical modification-and mass spectrometry-based strategy. *Analytical chemistry* **80**(11): 4175-4185.

West GM, Tucker CL, Xu T, Park SK, Han X, Yates JR and Fitzgerald MC (2010) Quantitative proteomics approach for identifying protein–drug interactions in complex mixtures using protein stability measurements. *Proceedings of the National Academy of Sciences* **107**(20): 9078-9082.

Wishart DS, Feunang YD, Guo AC, Lo EJ, Marcu A, Grant JR, Sajed T, Johnson D, Li C and Sayeeda Z (2018) DrugBank 5.0: a major update to the DrugBank database for 2018. *Nucleic acids research* **46**(D1): D1074-D1082.

Wiśniewski JR, Zougman A, Nagaraj N and Mann M (2009) Universal sample preparation method for proteome analysis. *Nature methods* **6**(5): 359-362.

Wongrakpanich S, Wongrakpanich A, Melhado K and Rangaswami J (2018) A Comprehensive Review of Non-Steroidal Anti-Inflammatory Drug Use in The Elderly. *Aging Dis* **9**(1): 143-150.

Yaksh TL, Dirig DM, Conway CM, Svensson C, Luo ZD and Isakson PC (2001) The acute antihyperalgesic action of nonsteroidal, anti-inflammatory drugs and release of spinal prostaglandin E2 is mediated by the inhibition of constitutive spinal cyclooxygenase-2 (COX-2) but not COX-1. *Journal of Neuroscience* **21**(16): 5847-5853.

Zagidullin B, Aldahdooh J, Zheng S, Wang W, Wang Y, Saad J, Malyutina A, Jafari M, Tanoli Z, Pessia A and Tang J (2019) DrugComb: an integrative cancer drug combination data portal. *Nucleic Acids Research* **47**(W1): W43-W51.

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Zhao C, Slevin JT and Whiteheart SW (2007) Cellular functions of NSF: not just SNAPs and SNAREs. *FEBS letters* **581**(11): 2140-2149.

Zhou Y, Wu C, Sheng Q, Jiang C, Chen Q, Lv Z, Yao J and Nie Z (2016) Lysine acetylation stabilizes SP2 protein in the silkworm Bombyx mori. *Journal of insect physiology* **91-92**: 56-62.

Molecular Pharmacology Fast Forward. Published on February 25, 2021 as DOI: 10.1124/molpharm.120.000210
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Footnotes

This study was financially supported by the National Institute for Medical Research Development of Iran (NIMAD) as Elite Grants [Grant No.964580], Academy of Finland [Grant No. 317680] and [Grant No. 332454] and European Research Council [Grant No. 716063].

The authors declare that there is no conflict of interest.

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Legends for Figures

Figure legend 1. Schematic representation of TPP-based drug target discovery using samples of rat hippocampus. Then, we extract proteins from the hippocampus following tissue homogenization and cell lysis. Next, the samples were treated with a range of compound concentrations. Each concentration was treated with four serial temperatures, i.e., 37C, 47C, 57C, 67C. Then, soluble proteins were separated and tryptic digested before Mass spectrometry. Protein identification was made using nano LC-ESI-Thermo Q Exactive Plus Orbi-Trap MS followed by Proteome Discoverer software. Finally, data processing and computational analysis were performed to compare with previously identified celecoxib targets in different databases, and to explore the possible enriched biological annotations in the identified protein targets.

Figure legend 2. (A) The Venn diagram of identified proteins of rat hippocampus proteome, recovered by TPP technique in 3 groups: treated with DMSO, H2O, Celecoxib (20μM). (B) Drug-target database comparison based on celecoxib-targeted proteins within diverse species. In this inset plot, intersections between the databases are illustrated. The horizontal bar plot shows the total number of proteins in each database. The vertical bar plot indicates the number of proteins in each database uniquely and the different sets of the intersections. Drug Bank (*DB*), Super Target (*ST*), Drug Central (*DC*), Probes & Drugs portal (PDP), Drug Target Commons (DTC) are represented in this plot. The inset plot displays the characterized species in the mentioned databases. We scaled the word size by their frequency of corresponding protein targets of *celecoxib* in each species independently. (C) Rat-specific drug-target databases comparison based on celecoxib-targeted proteins along with the TPP-identified proteins (D) Homology network of TPP-identified proteins and reported targets of *celecoxib* in drug-target databases. The identified proteins in the *present* study are shown with a red triangle, and the proteins introduced by the databases are displayed with green circles. The thickness of the edges indicates the identity percentage of protein sequences in this study.

Figure legend 3. Enrichment analysis of TPP-identified proteins as the targets of celecoxib in the rat hippocampus. This plot indicates enriched annotations related to disease, phenotypes, and biological pathways of celecoxib targeted proteins. Each panel distinctly represents the annotations of gene-disease associations (DisGeNET), Human Phenotype Ontology (HumanPhen), Mouse Genome Informatics (MGI), UKBiobank PheWeb, and WikiPathway. These annotations are displayed with the negative of logarithmic p-values of Fisher's exact test and combined scores based on EnrichR webtool.

Figure legend 4. REVIGO Gene Ontology representative terms for TPP-identified proteins as the targets of celecoxib in the rat hippocampus. Each lollipop plot contains cluster representative terms of enriched annotations based on semantic similarity analysis in REVIGO. The loosely related annotations are joined together and visualized by the average of uniqueness scores calculated based on semantic similarity of the GO annotations in the biological process, cellular component,

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and molecular function, as more uniqueness, more preferable. The points' size was adjusted to reflect the average adjusted p-values (-log10) of Fisher's exact test in the enrichment analysis by EnrichR webtool.

Figure legend 5. Enriched PTMs in Celecoxib-treated and untreated samples. The PTMs of all identified proteins in our experiment and Celecoxib-targets were shown by two different colors. The size of the points was adjusted to reflect the average of adjusted p-values (-log10) calculated based on hypergeometric test.

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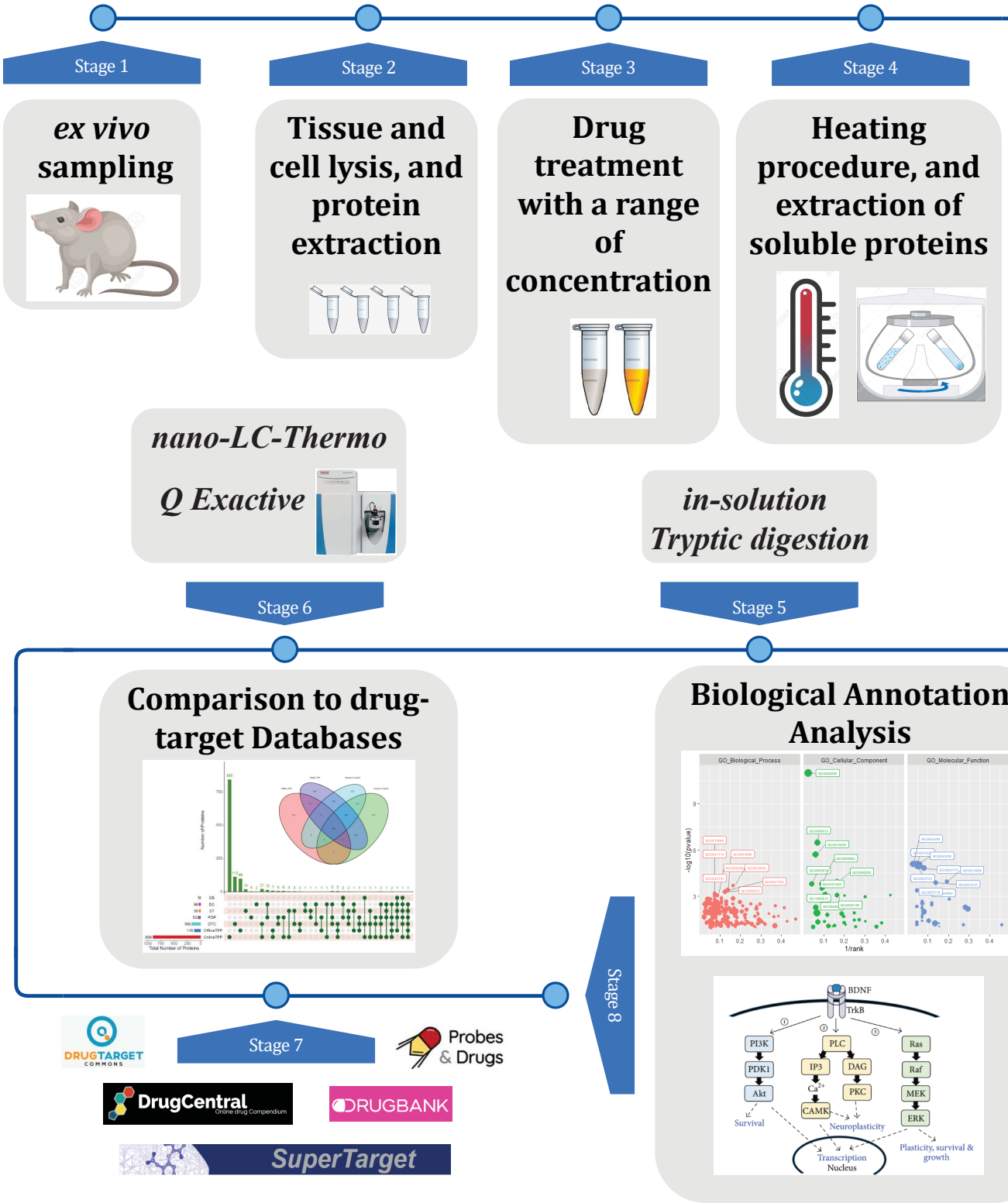

Figure 1

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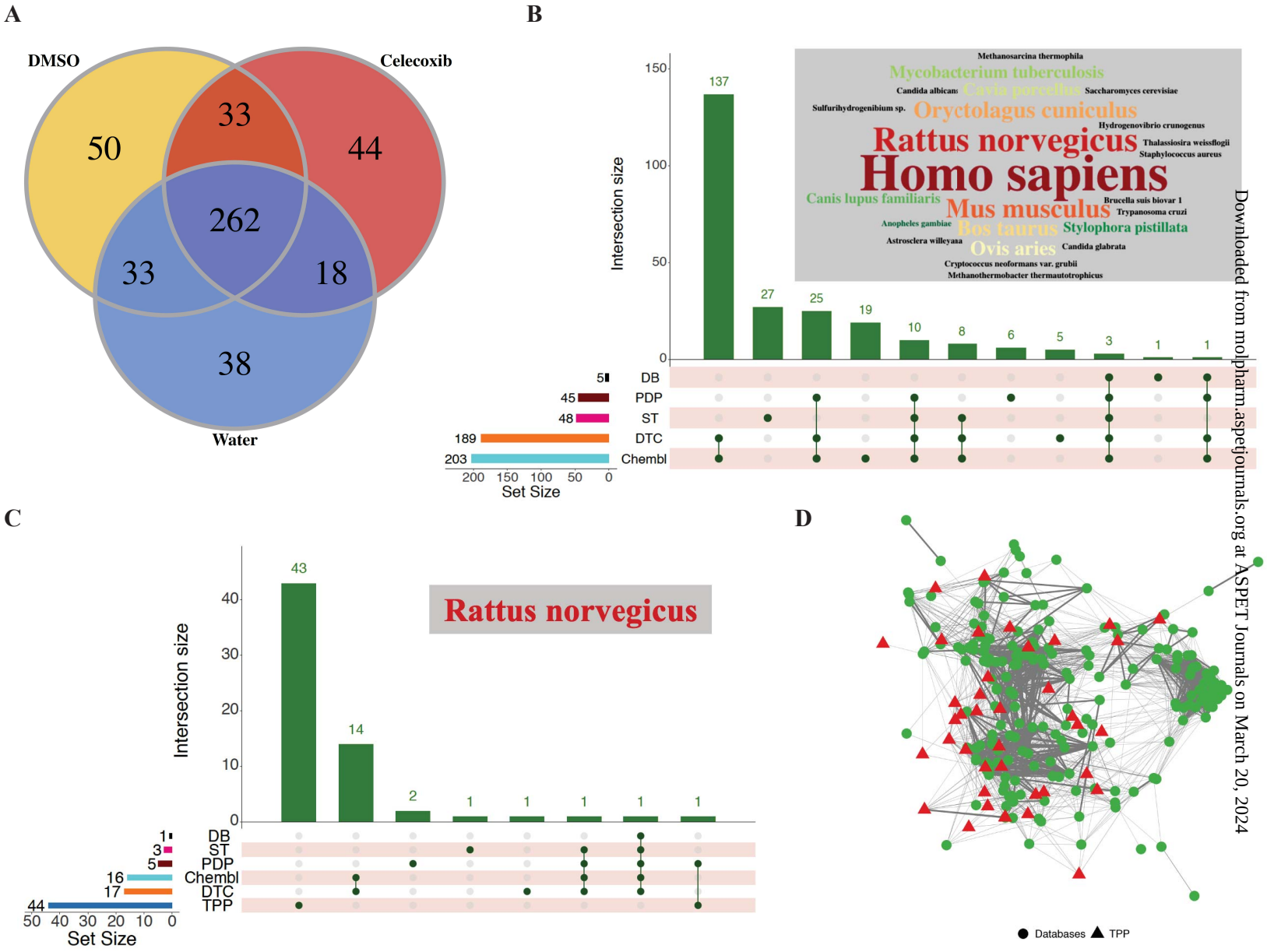

Figure 2

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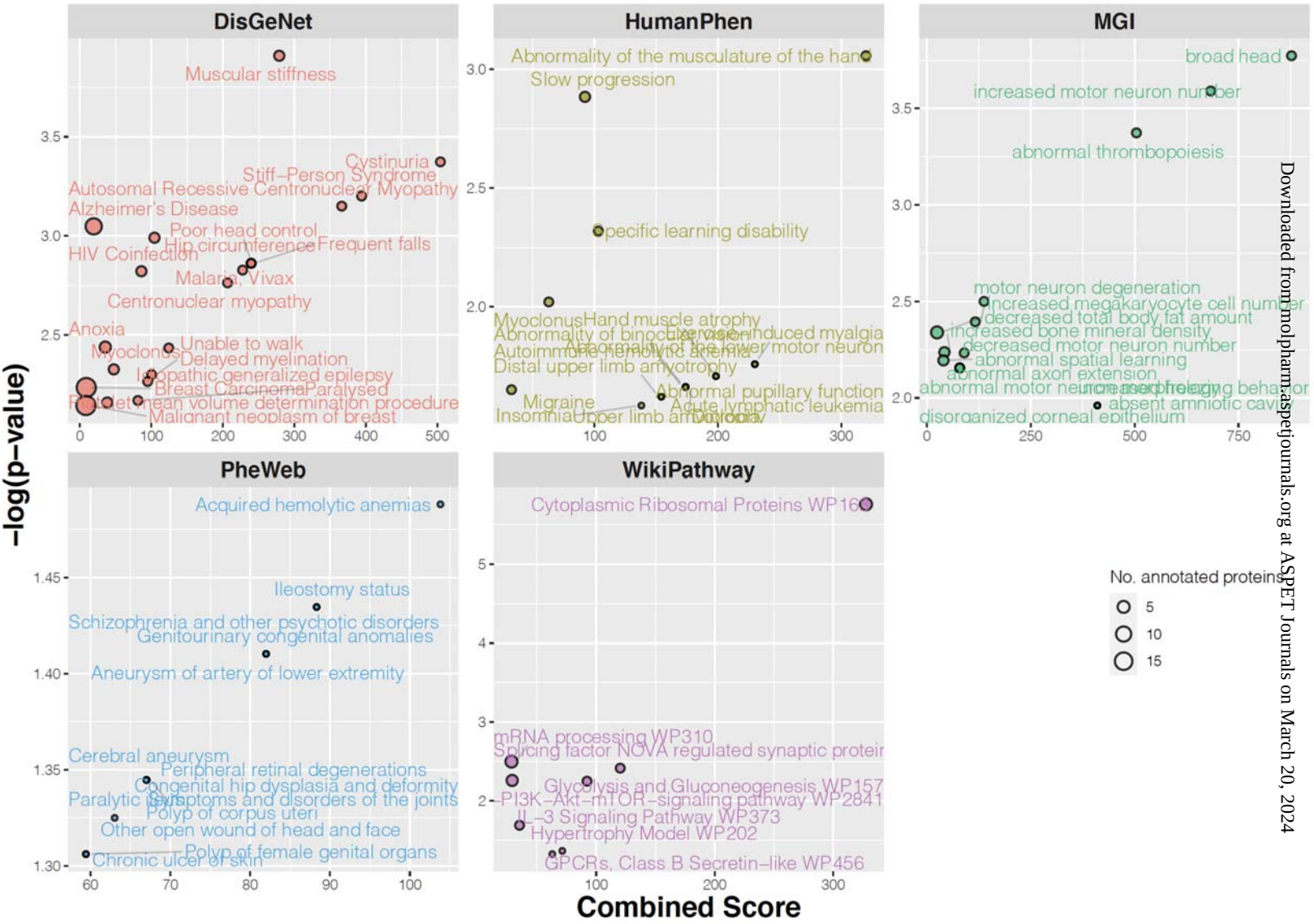

Figure 3

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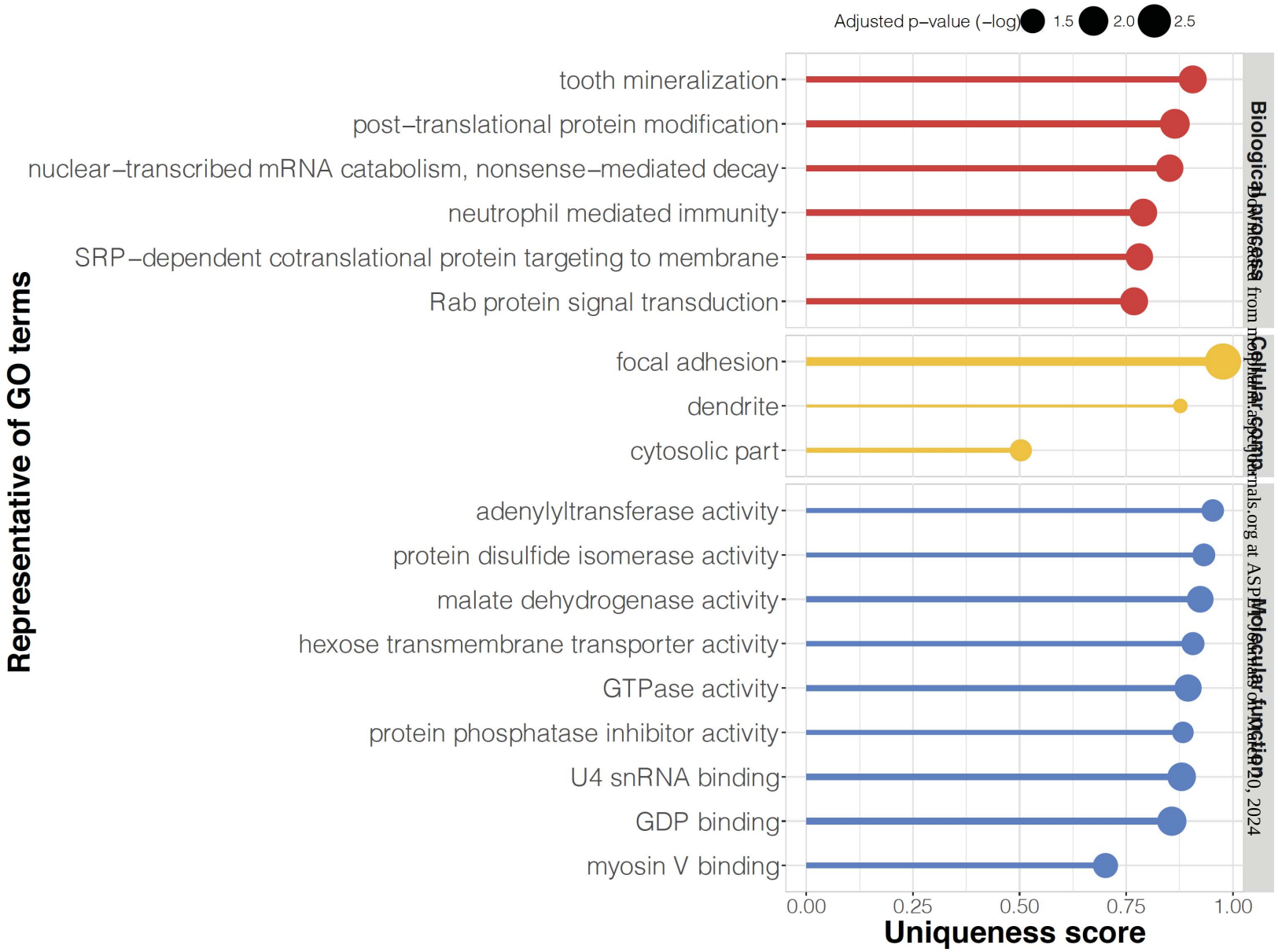

Figure 4

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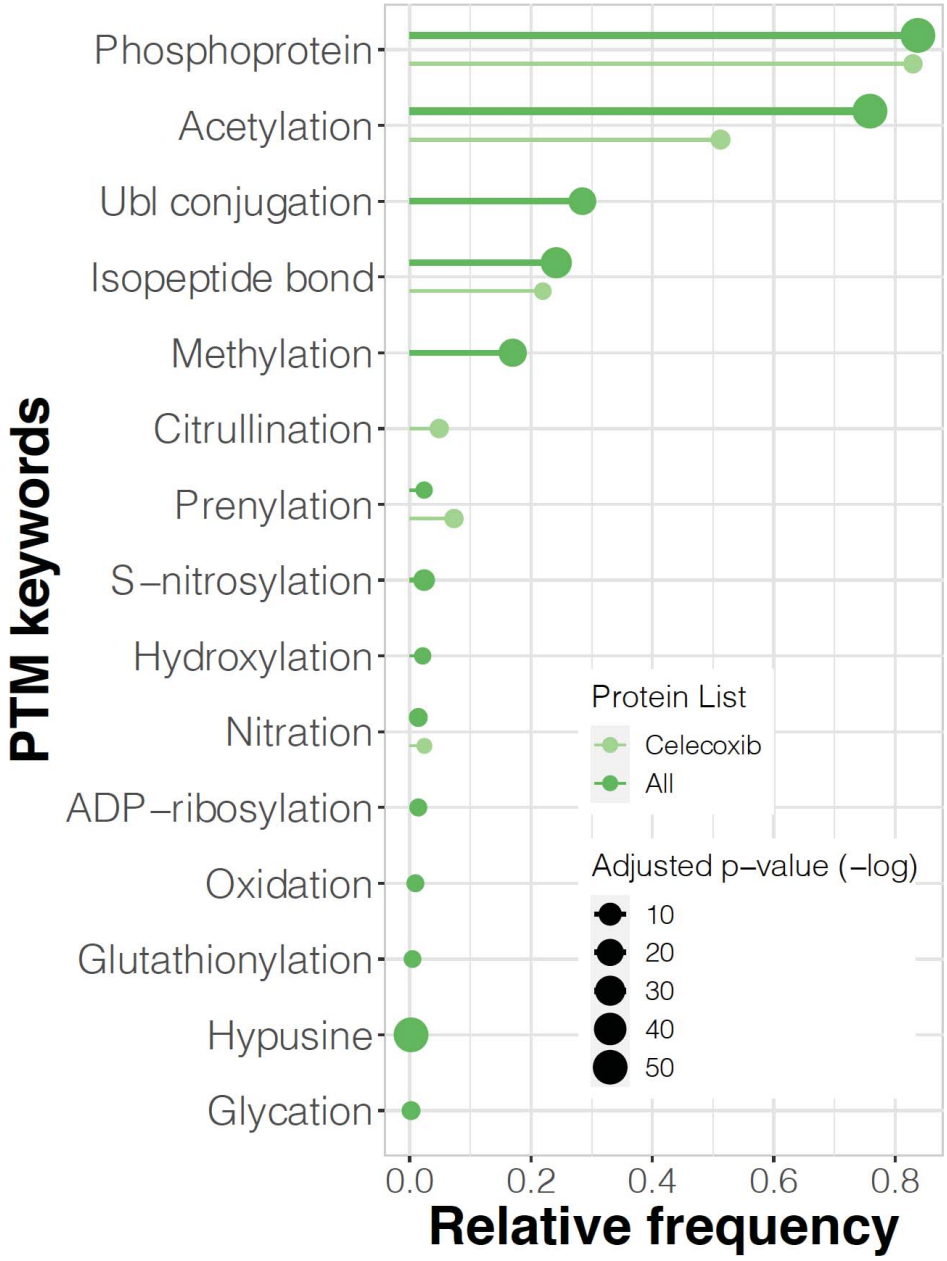
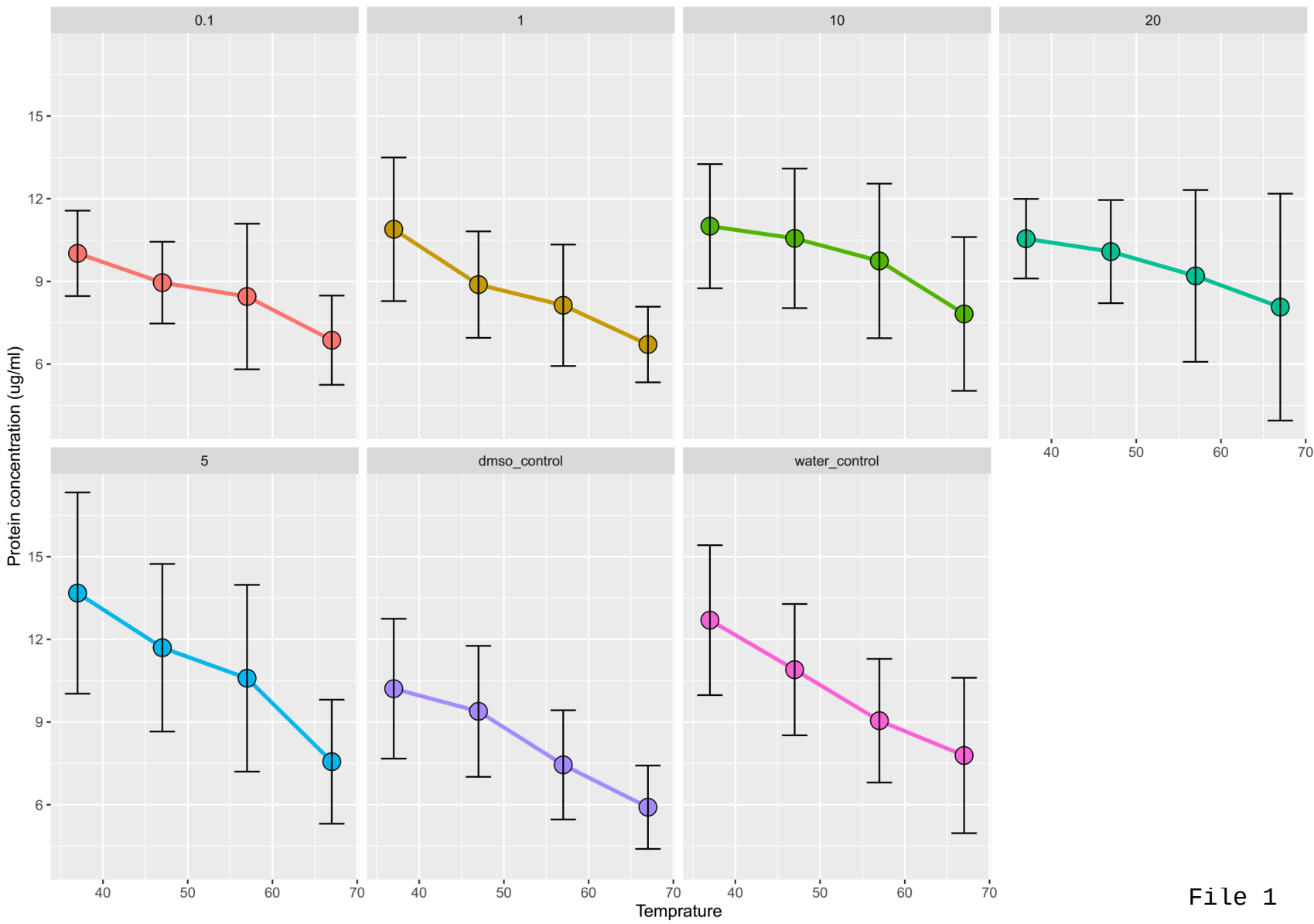


Figure 5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1
10
20
5
dmso_control
water_control
Protein concentration (ug/ml)
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12
9
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40
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60
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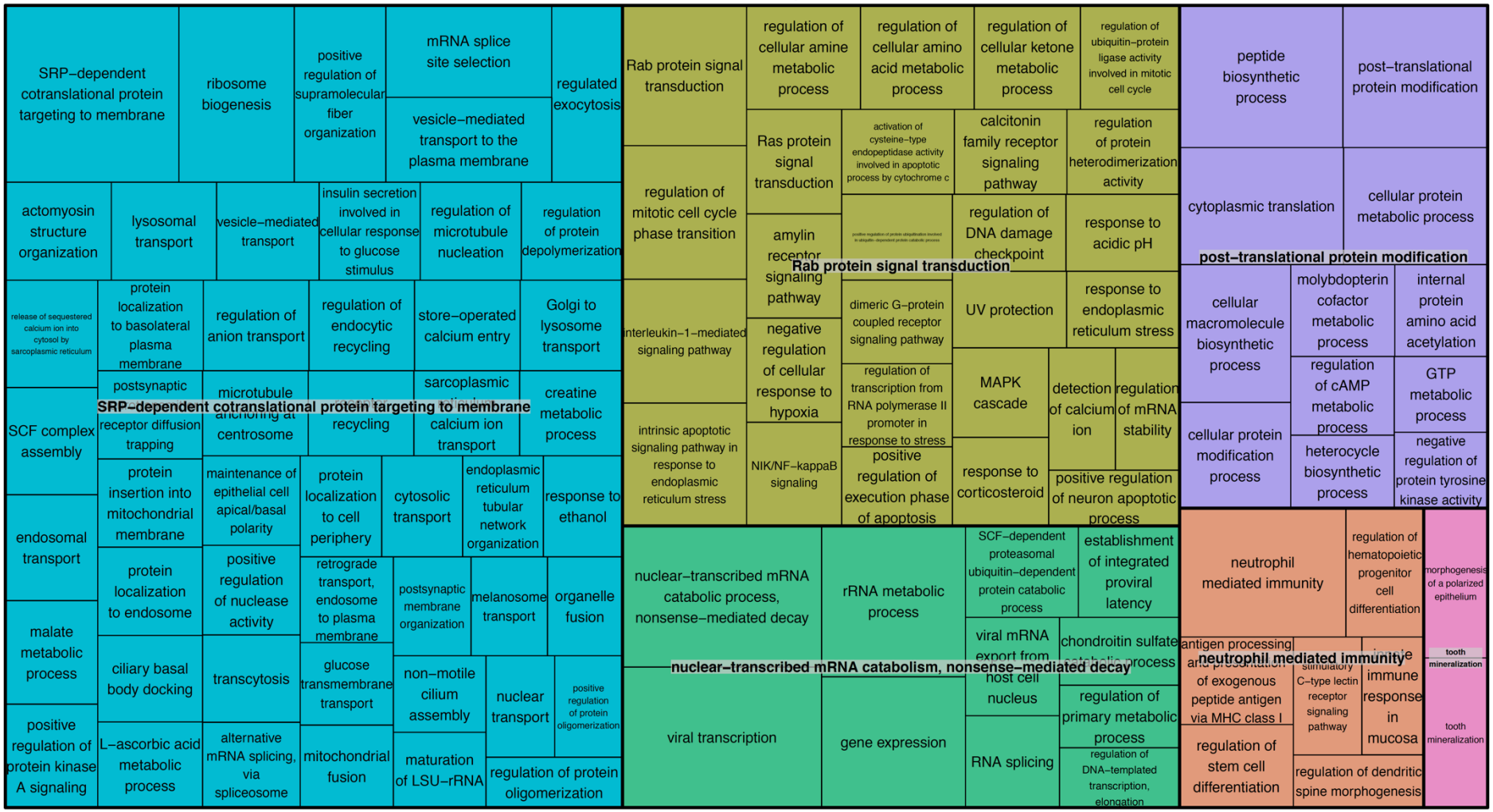
File 1

1

A


SRP−dependent cotranslational protein targeting to membrane
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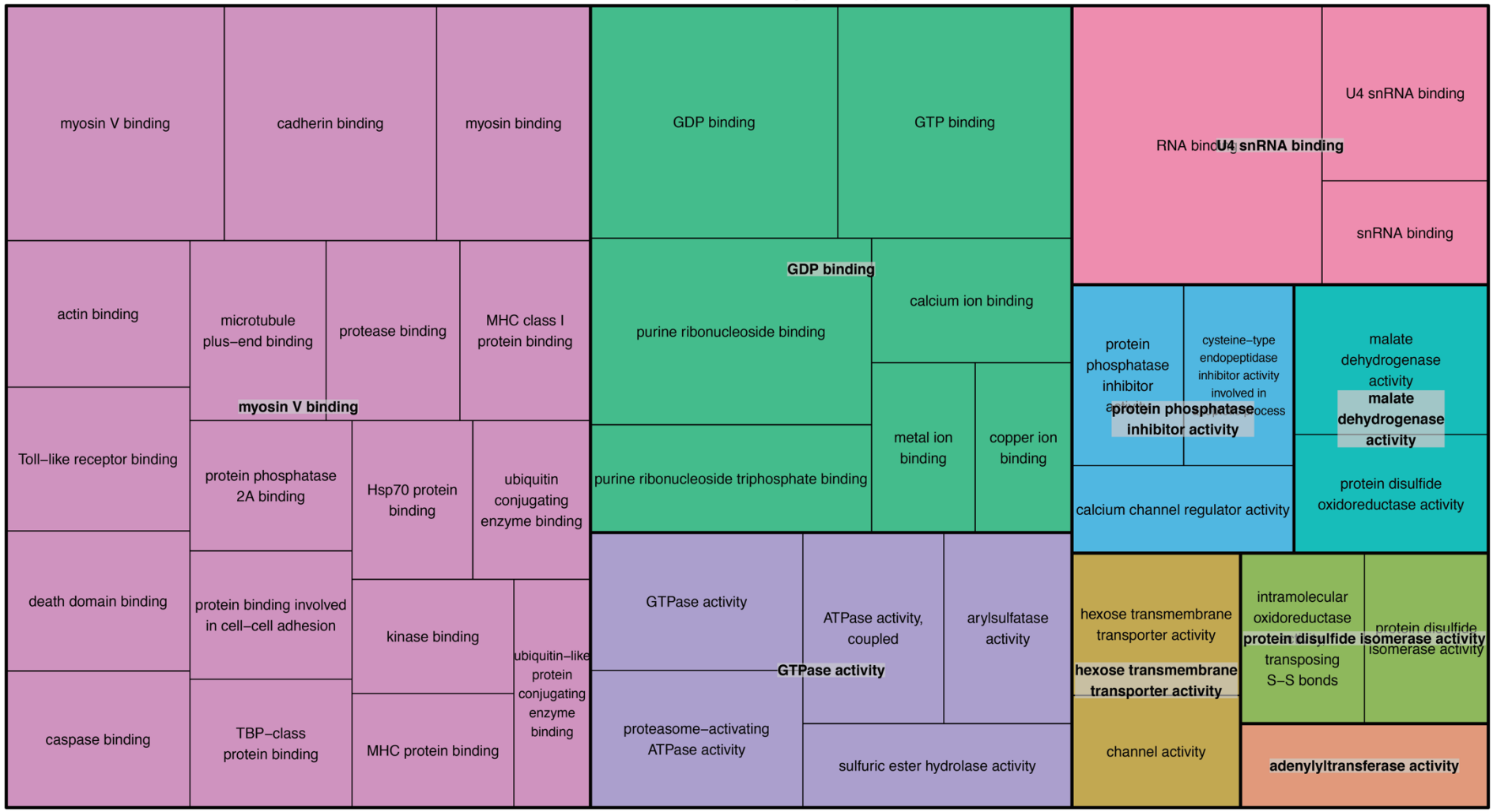
mRNA splice site selection
vesicle−mediated transport to the plasma membrane
regulated exocytosis
actomyosin structure organization
lysosomal transport
vesicle−mediated transport
insulin secretion involved in cellular response to glucose stimulus
regulation of microtubule nucleation
regulation of protein depolymerization
release of sequestered calcium ion into cytosol by sarcoplasmic reticulum
protein localization to basolateral plasma membrane
regulation of anion transport
regulation of endocytic recycling
store−operated calcium entry
Golgi to lysosome transport
SCF complex assembly
postsynaptic receptor diffusion trapping
microtubule anchoring at centrosome
sarcoplasmic calcium ion transport
creatine metabolic process
SRP−dependent cotranslational protein targeting to membrane
endosomal transport
protein insertion into mitochondrial membrane
maintenance of epithelial cell apical/basal polarity
protein localization to cell periphery
cytosolic transport
endoplasmic reticulum tubular network organization
response to ethanol
malate metabolic process
protein localization to endosome
positive regulation of nuclease activity
retrograde transport, endosome to plasma membrane
postsynaptic membrane organization
melanosome transport
organelle fusion
ciliary basal body docking
transcytosis
glucose transmembrane transport
non−motile cilium assembly
nuclear transport
positive regulation of protein oligomerization
positive regulation of protein kinase A signaling
L−ascorbic acid metabolic process
alternative mRNA splicing, via spliceosome
mitochondrial fusion
maturation of LSU−rRNA
regulation of protein oligomerization
Rab protein signal transduction
regulation of cellular amine metabolic process
regulation of cellular amino acid metabolic process
regulation of cellular ketone metabolic process
regulation of ubiquitin−protein ligase activity involved in mitotic cell cycle
Ras protein signal transduction
activation of cysteine−type endopeptidase activity involved in apoptotic process by cytochrome c
calcitonin family receptor signaling pathway
regulation of protein heterodimerization activity
regulation of mitotic cell cycle phase transition
amylin receptor signaling pathway
regulation of DNA damage checkpoint
response to acidic pH
Rab protein signal transduction
dimeric G−protein coupled receptor signaling pathway
UV protection
response to endoplasmic reticulum stress
interleukin−1−mediated signaling pathway
negative regulation of cellular response to hypoxia
regulation of transcription from RNA polymerase II promoter in response to stress
MAPK cascade
detection of calcium ion
regulation of mRNA stability
intrinsic apoptotic signaling pathway in response to endoplasmic reticulum stress
NIK/NF−kappaB signaling
positive regulation of execution phase of apoptosis
response to corticosteroid
positive regulation of neuron apoptotic process
nuclear−transcribed mRNA catabolic process, nonsense−mediated decay
rRNA metabolic process
SCF−dependent proteasomal ubiquitin−dependent protein catabolic process
establishment of integrated proviral latency
viral mRNA export from host cell nucleus
chondroitin sulfate catabolic process
nuclear−transcribed mRNA catabolism, nonsense−mediated decay
viral transcription
gene expression
RNA splicing
regulation of primary metabolic process
regulation of DNA−templated transcription, elongation
peptide biosynthetic process
post−translational protein modification
cytoplasmic translation
cellular protein metabolic process
post−translational protein modification
cellular macromolecule biosynthetic process
molybdopterin cofactor metabolic process
internal protein amino acid acetylation
regulation of cAMP metabolic process
GTP metabolic process
cellular protein modification process
heterocycle biosynthetic process
negative regulation of protein tyrosine kinase activity
neutrophil mediated immunity
regulation of hematopoietic progenitor cell differentiation
morphogenesis of a polarized epithelium
antigen processing and presentation of exogenous peptide antigen via MHC class I
stimulatory C−type lectin receptor signaling pathway
immune response in mucosa
tooth mineralization
neutrophil mediated immunity
regulation of stem cell differentiation
regulation of dendritic spine morphogenesis
tooth mineralization

B


myosin V binding
cadherin binding
myosin binding
GDP binding
GTP binding
RNA bind
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U4 snRNA binding
snRNA binding
actin binding
microtubule plus−end binding
protease binding
MHC class I protein binding
myosin V binding
GDP binding
purine ribonucleoside binding
calcium ion binding
protein phosphatase inhibitor
cysteine−type endopeptidase inhibitor activity involved in
malate dehydrogenase activity
protein phosphatase inhibitor activity
malate dehydrogenase activity
Toll−like receptor binding
protein phosphatase 2A binding
Hsp70 protein binding
ubiquitin conjugating enzyme binding
purine ribonucleoside triphosphate binding
metal ion binding
copper ion binding
calcium channel regulator activity
protein disulfide oxidoreductase activity
death domain binding
protein binding involved in cell−cell adhesion
kinase binding
ubiquitin−like protein conjugating enzyme binding
GTPase activity
ATPase activity, coupled
arylsulfatase activity
hexose transmembrane transporter activity
intramolecular oxidoreductase
protein disulfide isomerase activity
transposing S−S bonds
protein disulfide isomerase activity
GTPase activity
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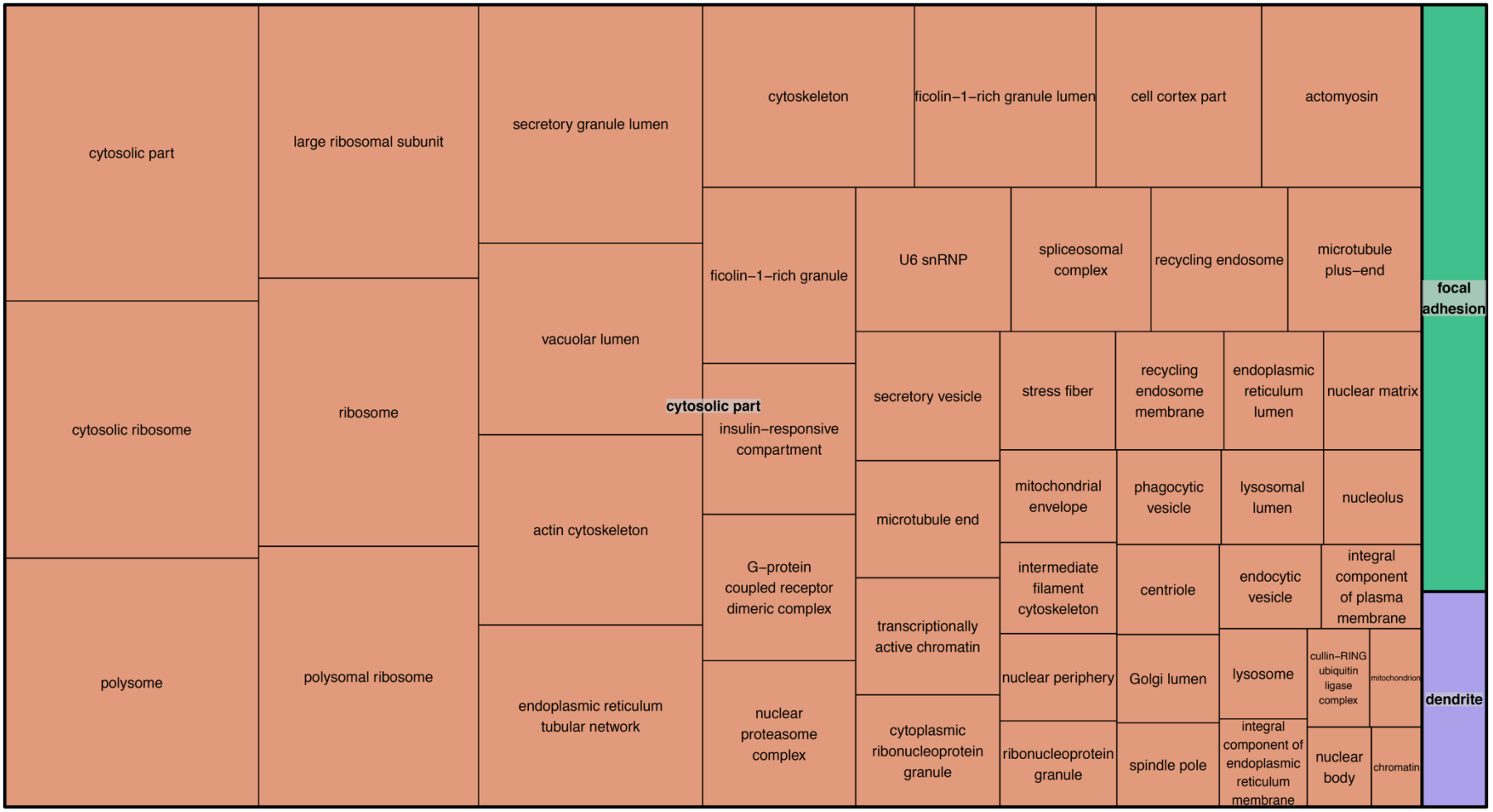
sulfuric ester hydrolase activity
channel activity
adenylyltransferase activity

C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cytoskeleton
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cell cortex part
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spliceosomal complex
recycling endosome
microtubule plus−end
vacuolar lumen
cytosolic part
secretory vesicle
stress fiber
recycling endosome membrane
endoplasmic reticulum lumen
nuclear matrix
cytosolic ribosome
ribosome
insulin−responsive compartment
mitochondrial envelope
phagocytic vesicle
lysosomal lumen
nucleolus
actin cytoskeleton
microtubule end
intermediate filament cytoskeleton
centriole
endocytic vesicle
integral component of plasma membrane
polysome
polysomal ribosome
G−protein coupled receptor dimeric complex
transcriptionally active chromatin
nuclear periphery
Golgi lumen
lysosome
cullin−RING ubiquitin ligase complex
mitochondrion
endoplasmic reticulum tubular network
nuclear proteasome complex
cytoplasmic ribonucleoprotein granule
ribonucleoprotein granule
spindle pole
integral component of endoplasmic reticulum membrane
nuclear body
chromatin
focal adhesion
dendrite

TISSUES

Tissue expression database

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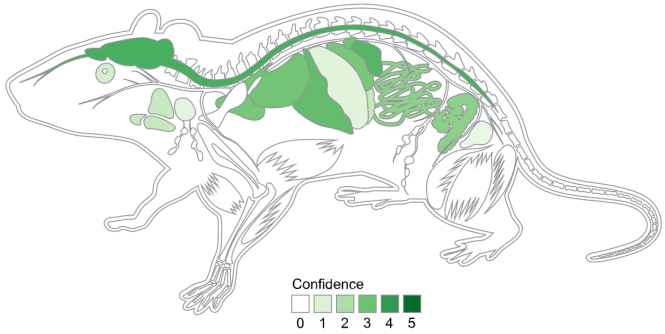
Rab2a tissues

Rab2a [ENSRNOP00000008522]

RAB2A, member RAS oncogene family; Required for protein transport from the endoplasmic reticulum to the Golgi complex.

Synonyms: Rab2a, F1LP82, Rab2ap, F1LP82p, Rab2 ...

Linkouts: STRING



Knowledge

No evidence of this type.

Experiments

Next >

Name	Source	Evidence	Confidence
Brain	BodyMap	123 FPKM	★★★☆☆
Lung	BodyMap	112 FPKM	★★★☆☆
Uterus	BodyMap	104 FPKM	★★★☆☆
Gastrocnemius	BodyMap	86 FPKM	★★★☆☆
Spleen	BodyMap	79 FPKM	★★★☆☆
Thymus	BodyMap	79 FPKM	★★★☆☆
Kidney	BodyMap	75 FPKM	★★★☆☆
Liver	BodyMap	66 FPKM	★★★☆☆
Testis	BodyMap	57 FPKM	★★★☆☆
Heart	BodyMap	56 FPKM	★★★☆☆

Text mining

Next >

Name	Z-score	Confidence
Dorsal nerve cord	4.5	★★★☆☆
Rab9	3.9	★★☆☆☆
HeLa cell	3.8	★★☆☆☆
33B cell	3.6	★★☆☆☆
Ventral nerve cord	3.5	★★☆☆☆
Kidney	3.5	★★☆☆☆
IMCD cell	3.4	★★☆☆☆
Brain	3.4	★★☆☆☆
SW-900 cell	3.2	★★☆☆☆
Kinetoplastid	3.2	★★☆☆☆

Orthologs and paralogs

Next >

Name	Organism	Homology	Correlation
RAB2A	Sus scrofa gene	Ortholog	0.68
RAB2A	Homo sapiens gene	Ortholog	0.65
Rab2a	Mus musculus gene	Ortholog	0.35
Arl16	Rattus norvegicus gene	Paralog	0.94
Trim23	Rattus norvegicus gene	Paralog	0.93
Arl8a	Rattus norvegicus gene	Paralog	0.91
Rhobtb2	Rattus norvegicus gene	Paralog	0.91
Rhot2	Rattus norvegicus gene	Paralog	0.91
Rab2b	Rattus norvegicus gene	Paralog	0.89
Rhot1	Rattus norvegicus gene	Paralog	0.86

The tissue associations are derived from manually curated knowledge in UniProtKB and via automatic text

mining of the biomedical literature, which has not been manually verified. The confidence of each association is signified by stars, where ★★★★★ is the highest confidence and ★☆☆☆☆ is the lowest. Download files from earlier versions are archived on figshare.

Each tissue–gene association is based on a text-mining score, which is proportional to 1) the absolute number of comentionings and 2) the ratio of observed to expected comentionings (i.e. the enrichment). These scores are normalized to z-scores by comparing them to a random background. This is represented by stars, each star corresponding to two standard deviations above the mean of the background distribution.

Developed by Alberto Santos, Oana Palasca, Christian Stolte, Kalliopi Tsafou, Sune Frankild, Janos Binder, Sean O'Donoghue, Jan Gorodkin, and Lars Juhl Jensen from the Novo Nordisk Foundation Center for Protein Research, Center for non-coding RNA in Technology and Health, and the Commonwealth Scientific and Industrial Research Organisation (CSIRO).