UbiBrowser 2.0: a comprehensive resource for proteome-wide known and predicted ubiquitin ligase/deubiquitinase–substrate interactions in eukaryotic species

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Received September 15, 2021; Revised September 30, 2021; Editorial Decision October 01, 2021; Accepted October 04, 2021

ABSTRACT
As an important post-translational modification, ubiquitination mediates ~80% of protein degradation in eukaryotes. The degree of protein ubiquitination is tightly determined by the delicate balance between specific ubiquitin ligase (E3)-mediated ubiquitination and deubiquitinase-mediated deubiquitination. In 2017, we developed UbiBrowser 1.0, which is an integrated database for predicted human proteome-wide E3–substrate interactions. Here, to meet the urgent requirement of proteome-wide E3/deubiquitinase–substrate interactions (ESIs/DSIs) in multiple organisms, we updated UbiBrowser to version 2.0 (http://ubibrowser.ncpsb.org.cn). Using an improved protocol, we collected 4068/967 known ESIs/DSIs by manual curation, and we predicted about 2.2 million highly confident ESIs/DSIs in 39 organisms, with >210-fold increase in total data volume. In addition, we made several new features in the updated version: (i) it allows exploring proteins’ upstream E3 ligases and deubiquitinases simultaneously; (ii) it has significantly increased species coverage; (iii) it presents a uniform confidence scoring system to rank predicted ESIs/DSIs. To facilitate the usage of UbiBrowser 2.0, we also redesigned the web interface for exploring these known and predicted ESIs/DSIs, and added functions of ‘Browse’, ‘Download’ and ‘Application Programming Interface’. We believe that UbiBrowser 2.0, as a discovery tool, will contribute to the study of protein ubiquitination and the development of drug targets for complex diseases.

INTRODUCTION
Protein ubiquitination is a vital post-translational modification in eukaryotes, which regulates a plethora of intracellular processes, including endogenous protein stability, enzyme activity, DNA damage response, and signal transduction (1). Like most protein post-translational modifications, ubiquitination is a reversible dynamic process. Ubiquitin, a ubiquitously expressed and highly conserved 76-amino acid protein in eukaryotes, can be covalently conjugated to a substrate protein by tight regulation of ubiquitin activating enzyme (E1)-ubiquitin conjugating enzyme (E2)-ubiquitin ligase (E3) cascade and removed from the substrate by deubiquitinase (DUB). The degree of protein ubiquitination is tightly determined by the balance between specific E3-mediated ubiquitination and DUB-mediated deubiquitination (2). Dysregulation of the dynamic ubiquitination process may induce multiple diseases, such as Parkinson’s disease (3) and Alzheimer’s disease (4). Especially, in recent years, E3s/DUBs have been found to be implicated in tumorigenesis at multiple levels (5). Identification of interactions between E3/DUB and substrate has provided novel insights into cancer therapy. For example, the drug of

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†The authors wish it to be known that, in their opinion, the first four authors should be regarded as joint First Authors

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Mitoxantrone (a USP11 inhibitor) has been approved by FDA to treat the hormone-refractory prostate cancer (6).

Several experimental methods have been developed for identifying interactions between E3/DUB and substrates, such as protein microarrays (7), GPS profiling (8), mass spectrometry (9), live phage display library (10) and so on. However, due to the E3/DUB substrates’ low expression levels and their intrinsically weak interactions with enzyme, these methods are often laborious, expensive and low efficient. Therefore, it is an urgent need to identify proteome-wide E3/DUB-substrate interactions by bioinformatics strategy. In 2017, we presented UbiBrowser 1.0 (11), the first bioinformatics database for predicted proteome-wide E3–substrate interactions (ESIs) in human. Since the publication of UbiBrowser, it has received widespread attention (12,13). UbiBrowser has been visited 100 000+ times, making great advancements to the field. Moreover, we have received lots of feedback asking whether we can add the dataset of deubiquitinase–substrate interaction (DSI), and the dataset of ESIs in non-human species. Based on the statistics of 17800 ubiquitination-related papers in PubMed (2019–2021), we found that ~37% of these papers were about DSIs, and ~55% of the ESIs/DSIs were identified in non-human species. Consequently, it is urgent to identify the proteome-wide ESIs/DSIs in multiple species. In fact, many efforts have been devoted to identifying more ESIs/DSIs in multiple organisms. hUbiquitome is the pioneer database for experimentally verified ubiquitination cascades in humans, which contains 344/14 known human ESIs/DSIs (14). Subsequently, Chen et al. manually collected 1806/389 known human ESIs/DSIs from literature (15), and they also predicted 500 high confidence DSIs for 54 human USPs (Ubiquitin-specific-processing proteases) (16). Li et al. developed the database of UbiNet (17), holding 3332 experimentally verified ESIs in 20 eukaryotes. All these efforts present precious E3/DUB-substrate interaction datasets for the field of ubiquitination, however, there is still no database for comprehensively providing proteome-wide known and predicted E3/DUB-substrate interactions in multiple species.

To fill this gap, we updated UbiBrowser to version 2.0, a comprehensive resource for proteome-wide known and predicted ESIs/DSIs in multiple eukaryotic species. Compared with UbiBrowser 1.0, major improvements in UbiBrowser 2.0 include: 4068 known ESIs and 967 known DSIs, proteome-wide predicted DSIs, and significantly increased species coverage (Figure 1). We manually curated known ESIs/DSIs from PubMed abstracts, predicted the proteome-wide human DSIs based on the naïve Bayesian classifier protocol that we built before (11), and inferred proteome-wide ESIs/DSIs in 38 non-human species by homology mapping (Figure 1). To facilitate the usage of the added ESI/DSI datasets, we redesigned the web interface and added functions of ‘Browse’, ‘Download’ and ‘API’.

**DATA STATISTICS**

UbiBrowser 2.0 comprises 4068 known ESIs in 38 species, and 967 known DSIs in eight species (Figure 2A), which is the biggest database of known ESIs/DSIs. The distribution of known E3/DUB-substrate interactions across species is presented in Figure 2B. UbiBrowser 2.0 also predicted high confidence ESIs/DSIs in 39 species (Figure 2C). The number of predicted high confidence ESIs/DSIs significantly increased from 14 419/0 to 1 884 676/303 214, compared with that in UbiBrowser 1.0. As illustrated in Figure 2D, E3s and DUBs, involved in the high confidence predicted ESIs and DSIs, are of high coverage against all genome-encoded E3s and DUBs in most of 39 organisms. The main E3/DUB types (RING, DWD, SINGLE, Other, F-box, SOCS/VHL/BC-box, BTB, HECT, BTB, Other, UBOX, CDC20 for E3; USP, JAMM, OTU, ULP, UCH, MINDY, Josephin for DUB) (18) are covered by UbiBrowser 2.0 predicted DSIs/ESIs in all 39 species (Figure 2E). The predicted E3/DUB substrates of eight well-studied species share the similar functional distribution against the main Gene Ontology (19) categories (metabolic process, response to stimulus, signal transduction, regulation of molecular function, cell cycle process and cell growth) (Figure 2F). The above data statistics suggest that the predicted ESI/DSI datasets of UbiBrowser 2.0 are of no distinct bias.

**EXPANDED DATABASE CONTENT**

**Manual curation for known E3/DUB-substrate interactions**

To collect known ESIs/DSIs, we downloaded all PubMed abstracts from 1982 to 2021, and retained those candidate abstracts containing the following keyword combinations: (‘ubiquitin ligase’ OR ‘ubiquitin ligases’ OR ‘E3 ligase’ OR ‘E3 ligases’) AND (‘deubiquitinase’ OR ‘deubiquitinases’ OR ‘deubiquitinating enzyme’ OR ‘deubiquitinating enzymes’ OR ‘DUB’) AND (‘substrate’ OR ‘substrates’). Next, all candidate abstracts were manually checked to extract known ESIs/DSIs by three experienced researchers independently. Then, these selected candidate ESIs/DSIs and the supporting sentences were manually reviewed by our curation team consisting of three experts. The curation team also integrated known ESIs/DSIs in the published ubiquitination datasets (14–17). Finally, 5035 experimentally validated ESIs/DSIs (4068 ESIs in 38 species and 967 DSIs in 8 species) were deposited in UbiBrowser 2.0 (Supplementary Table S1).

**Prediction of human proteome-wide E3/Deubiquitinase-substrate interaction dataset**

The ubiquitination status of a protein is determined by a delicate balance between two opposing forces: ubiquitination mediated by E3 ubiquitin ligases and deubiquitination mediated by DUBs (2). In UbiBrowser 1.0, a naïve Bayesian classifier was used to predict the human ubiquitin ligase-substrate interactions (11). UbiBrowser 2.0 inherited the ESI prediction protocol from version 1.0 (11). To verify the reliability, we have implemented the 5-fold cross-validation, independent dataset validation and also the experimental validation in our previous publication of UbiBrowser 1.0 (11). Some of these predicted human ESIs by UbiBrowser 1.0 have been further experimentally validated.
validated by independent studies (12,13). Considering that the identification of DUB-substrate interactions is of equal significance for maintaining ubiquitin homeostasis (2), we took the same protocol as version 1.0 (11) to predict the human DUB-substrate interactions (Supplementary Methods). Four heterogeneous biological evidences were integrated to construct the Bayesian model (11), including Gene Ontology (GO) (19) term pair, domain pair, DUB recognition consensus motif (20) and protein interaction network loop (21) (Figure 3A, Supplementary Figure S1). Both 5-fold cross-validation and independent test were adopted to evaluate the performance of DSI prediction (11). A golden standard positive data set with 495 DSIs by manually curating PubMed before January 2018 was used for five-fold cross-validation, while a data set with 66 DSIs after January 2018 for independent test. We plotted ROC curves (22) in Figure 3B and C by computing the true positive rate and false positive rate against different likelihood ratio (LR) cut-off during cross-validation test and independent test. We found that this Bayesian model has the ROC curve area of 0.85 against the five-fold cross-validation (Figure 3B), and the area of 0.71 against the independent test set (Figure 3C), which suggest that our model (11) is of certain prediction power for new DSIs.

Figure 1. Overview of UbiBrowser 2.0, a comprehensive resource for proteome-wide known and predicted ESIs/DSIs in eukaryotic species. First, known ESIs/DSIs were manually curated from literature. Then, proteome-wide ESIs/DSIs in human were predicted by our published protocol for UbiBrowser 1.0 (11). The ‘interolog’-based approach was employed to transfer ESIs/DSIs from human to 38 non-human species (24). ESI: E3-substrate interaction; DSI: deubiquitinase–substrate interaction; API: application programming interface; ‘interolog’: a conserved interaction between a pair of proteins which are interacting orthologs in another species.

Transferring E3/DUB-substrate interactions from human to 38 non-human species

The ubiquitin signaling system was supposed to be of certain conservation (23). Therefore, we adopted the well-recognized ‘interolog’-based strategy (24) to transfer both the known and predicted E3/DUB-substrate interactions with high confidence from human to 38 non-human species. First, we obtained the ortholog information between human and non-human species (such as Mus musculus, Rattus norvegicus and Drosophila melanogaster) from the InParanoid database (Released version 8.0) (25). Each human protein was mapped to a non-human protein with the highest orthology score. Then, the interactions between human E3s/DUBs and substrates were transferred to orthologous protein pairs in the non-human species (Figure 4A). After the homology mapping, we took the similar protocol as UbiBrowser 1.0 (11) to compute the derived non-human E3/DUB-substrate interactions’ confidence score based on four supporting biological evidences, including domain pair, Gene Ontology term pair, DUB recognition consensus motif and protein interaction network loop (Figure 4A). Based on the protein domain data from Pfam (26) (released version 33.1), the supporting evidence of domain
pairs in human was transferred to 38 non-human species. The \(LRs\) of these domain pairs were recalculated based on the protein domain distribution of target species (11). The \(LR\) calculation of Gene Ontology term pair feature followed the similar protocol as that of domain pair (11). The supporting evidence of human E3/DUB recognition sequence motif of substrates was directly mapped to 38 non-human species after checking whether the motif was matched in the substrate protein sequence of target species (11). As for the supporting evidence of network topology, human protein interaction network (21) was firstly transferred to non-human species by the ‘interolog’-based strategy (24), generating high confidence orthologous protein–protein interaction networks (PPIN) for 38 non-human species, and then for each derived ESI/DSI, the number of involved four-interaction loops in the corresponding PPIN was counted for the \(LR\). Ultimately, \(LRs\) from four biological evidences will be multiplied and transformed into the confidence score (11) for target ESI/DSI (Supplementary Methods, Supplementary Figure S1).

Finally, we obtained 1.77/0.28 million high confidence predicted ESIs/DSIs in 38 non-human species based on the above protocol. By manual curation on PubMed, we also obtained 560, 71 and 47 ESIs in \(M\). \(m\)usculus, \(R\). \(n\)orvegicus and \(D\). \(m\)elanogaster, and collected 79 DSIs in \(M\). \(m\)usculus. Interestingly, we found that the number of the overlapping interactions between our predicted ESIs/DSIs and the manual curated ESIs/DSIs is significantly higher than random expectation (Figure 4B), suggesting that our improved identification strategy for ESIs/DSIs in non-human species is of certain reliability.

ENHANCED USER INTERFACE

To facilitate the usage of newly added known and predicted E3/DUB-substrate interaction datasets in multiple
A network view was designed to visualize the retrieved known and predicted ESIs/DSIs, with the central node is the query E3/DUB or substrate, and the surrounding nodes are the retrieved substrates or E3s/DUBs (Figure 5C and D). In the network view, the known and predicted interactions are obviously distinguished: the nodes of the known interactors are painted in red, while the nodes of the predicted interactors in blue. Considering network view for predicted interactions mainly act as discovery tool to find the potential upstream E3s/DUBs for the query protein (12, 13), all known interactors were excluded from prediction results to avoid possible misleading. Clicking the node in the network view will lead to the page of supporting evidence information for the retrieved interactors (Figure 5E and F). To help users focus on certain types of ESIs/DSIs in this network view, two filters were established: one is
for data source allowing users to select known interactions to confirm the existent knowledge (Figure 5C), or to select the predicted interactions for discovery (Figure 5D); the other is for E3/DUB families (18) providing the rapid selection of a more specialized list of E3/DUB according to the user’s needs. If users choose to show only the predicted interactors, UbiBrowser 2.0 provides two visualization modes: confidence mode and evidence mode. In the confidence mode, node size and edge width are positively proportional to the confidence score. In the evidence mode, various coloured edges between the central node and the surrounding nodes represent different types of supporting evidence (Figure 5D). Benefitting from the added ESIs/DSIs in multiple organisms, a function of ‘homology mapping’ was specially added in the network view page to help users investigate the orthologous ESI/DSI network of the query protein in other species (Figure 5C and D).

Detailed information page for the known and predicted ESIs/DSIs

Supporting literature information for the known ESIs/DSIs. All known ESIs/DSIs were manually curated from literature, and the supporting literature information was provided for further study (Figure 5E). A brief introduction about E3/DUB and substrate, original literature PubMed ID and the supporting sentence are shown. The entries of E3/DUB and substrate in the supporting sentences were painted in yellow to provide an intuitive user experience. Furthermore, the user can click the hyperlink of PubMed ID to see the original paper.

Supporting evidences for the predicted ESIs/DSIs. To help users understand the basis for the prediction, we also re-designed the supporting evidence page for each predicted ESI/DSI (Figure 5F). The detailed information for the supporting evidences, including the enrichment domain pair (27), E3/DUB recognizing motif (20), the enriched GO term pair (11) and protein interaction network loop (11), was presented, together with the deriving known ESIs/DSIs with the enriched domain and GO term pair and E3/DUB recognizing motif. For example, the domain pair of ‘SPRY domain-CARD domain’ was predicted to mediate the ESI of ‘TRIM21-MAVS’ based on the real ESI of ‘TRIM25-DDX58’ (28), and this predicted domain pair of ‘SPRY domain-CARD domain’ can be validated by (28). Interestingly, the predicted ‘TRIM21-MAVS’ has been validated by Xue et al., and the SPRY domain of TRIM21 was found to be the key domain for its interaction with MAVS (29). Considering that ESIs/DSIs in the non-human species were derived from human by homology mapping (24), a linkage of ‘Interolog in human’ was particularly added. When querying ESIs/DSIs of the non-human species, users can click on this linkage to explore the ortholog interaction information of these ESIs/DSIs in human.

Figure 4. Transferring of ubiquitin ligase/deubiquitinase–substrate interactions from human to non-human species. (A) Homologous mapping and confidence scoring scheme (please refer to text for details). (B) Number of overlapping interactions between the predicted and manually curated interactions in non-human species (observed overlapping links versus random expectation). The p value was obtained by the binomial test (one-sided). ESI: E3–substrate interaction; DSI: deubiquitinase–substrate interaction.
Figure 5. A screenshot of the redesigned UbiBrowser 2.0 interface. (A) Three ‘Search’ pages with the added options for ‘process’ and ‘species’. Users can specify the prediction process as ‘Ubiquitination’ or ‘Deubiquitination’, and designate whether the query protein is ‘Ubiquitin ligase (E3)’, ‘Deubiquitinase (DUB)’ or ‘Substrate’. The current version of UbiBrowser supports queries in 39 organisms. (B) ‘Browse’ page for known (left panel) and predicted (right panel) E3/DUB-substrate interactions across 39 organisms. (C) Network view for the retrieved known and predicted ESIs/DSIs. Red node: known interactor; blue node: predicted interactor. (D) Network view for the retrieved predicted ESIs/DSIs (confidence mode). Node size and edge width are positively proportional to the confidence score. The inset is the evidence mode, where various coloured edges between the central node and the surrounding nodes represent different types of supporting evidence. (E) Supporting literature information page for the known ESIs/DSIs, with the entries of E3/DUB and substrate in abstract texts are highlighted in colour. (F) Supporting evidence page for the predicted ESIs/DSIs. Clicking on each red node for known interactor in the network view (C) will pop up the corresponding supporting literature information (E); meanwhile, the blue nodes for predicted interactors in the network view (C and D) can lead to the supporting evidences page (F). ESI: E3–substrate interaction; DSI: deubiquitinase–substrate interaction.
Download
Some users would like to download the dataset for data mining rather than to query it via the web interface, therefore, we added the download function to improve UbiBrowser 2.0’s data accessibility. All known and predicted E3/DUB-substrate interactions can be downloaded from the ‘Download’ page in bulk, together with their supporting evidences. All files are provided in a tab-delimited text format.

API
To facilitate programmatic access and cross-references, we created an application programming interface (API). The API is called by constructing a URL that contains the selected parameters (including type of the request, the desired output format and the input item), and returns the query result in a computer-readable XML format for program, or a visible hypertext page for external databases cross-references (please refer to the document in our website for the detailed information).

CASE STUDIES
UbiBrowser 1.0 (11) has been used as a discovery tool to study protein homeostasis regulation mechanism (5) from the aspect of E3–substrate interaction. Some of the predicted human ESIs by UbiBrowser 1.0 have been experimentally validated by independent studies. Examples include the discovery of E3 ligase STUB as a novel regulator for ERα protein stability in osteoclasts (12), and the finding of MDM2 as the E3 ligase for ACE2 (the receptor for SARS-CoV-2 to enter host cells) (13). Benefiting from the added ESI/DSI datasets in multiple organisms, users can further explore the delicate protein homeostasis regulation mechanism from two opposing directions of ubiquitination and deubiquitination.

Case study I: exploring the homeostasis regulation mechanism for proteins in two opposing directions
As we know, loss of protein homeostasis can influence disease-related proteins’ abundance (5). The homeostasis regulatory mechanism of proteins in disease signaling cascades (from receptors to signaling proteins and transcriptional factors) can be explored by UbiBrowser 2.0 in two opposing directions (Figure 6).

WWP1-EGFR-STAMBP. Epidermal growth factor receptor (EGFR) controls the growth and survival of epithelial cells. The dysregulation of EGFR degradation was supposed to accelerate tumor initiation and progression, thus exploring the E3/DUB-mediated ubiquitination regulation of EGFR is promising for cancer therapy (30). By searching the potential E3 and DUB for EGFR in UbiBrowser 2.0, we speculated that WWP1 E3 ligase with confidence score of 0.949 might be a negative regulator of EGFR abundance, while the deubiquitinase STAMBP with confidence score of 0.852 might stabilize EGFR (Figure 6A). The recent studies validated these hypotheses: WWP1-induced EGFR ubiquitination was found to be a decisive signal for EGFR recycling (30); meanwhile, the overexpression of STAMBP can promote the stabilization of EGFR, and promote tumor progression (31). Hence, the predicted STAMBP provides novel potential targets for cancer therapy.

NEDD4-TBK1-CYLD. Against viral infection, TANK-binding kinase 1 (TBK1) triggers phosphorylation and translocation of IFN-regulatory factor 3 (IRF3), which then activates transcriptional activation of type I IFNs and initiates antiviral responses (32). In UbiBrowser 2.0, NEDD4 and CYLD were predicted as the high-confidence E3 (score: 0.886) and DUB (score: 0.877) for TBK1 (Figure 6B), respectively. In fact, it has been found that NEDD4 catalyzes ploy-ubiquitination of TBK1 and negatively regulates type I IFN signaling to mediate the homeostasis of innate immunity (32). Meanwhile, CYLD was also observed to remove Lys63-linked polyubiquitin from TBK1, and can negatively regulate innate antiviral response (33).

HECW1-SMAD4-USP4. The transcriptional factor of SMAD4 (SMAD family member 4) can be activated by TGFβ (transforming growth factor-β) and accumulates in the nucleus, which is involved in the tumor invasion and distant metastasis (34). By UbiBrowser 2.0, the E3 ligase HECW1 (score: 0.889) and the deubiquitinase USP4 (score: 0.894) were predicted to be potential regulators for SMAD4 (Figure 6C). Interestingly, these speculations have been confirmed, that HECW1 can promote the proliferation, migration and invasion of cancer cells by inducing the ubiquitination and degradation of SMAD4 (34), while USP4 can mediate the deubiquitination of SMAD4, leading to the expression of apoptotic proteins (35).

Case study II: investigating the delicate regulation of critical proteins in non-human physiological processes
Drosophila melanogaster is a powerful, in vivo system to model Parkinson’s disease (PD) pathobiology for its well-defined nerve system. It was found that ER-mitochondria contacts are altered under PD pathological conditions (36). The mitofusin family proteins were found to mediate mitochondrial fusion, and enrich at contact sites between ER and mitochondria to modulate ER-mitochondria cross-talk in vitro. Interestingly, parkin is predicted to be the highest-ranked E3 of mitofusin in UbiBrowser 2.0 (rank = 1). In fact, researches have shown that parkin can ubiquitinate mitofusin, and promote the formation of ER-mitochondria contacts sites, which was related to the locomotor deficits in PD (37). On the other side, USP8 was predicted to be the deubiquitinase of mitofusin (rank = 12), and the experiment validated that genetic and pharmacological inhibition of USP8 can down-regulate the elevated mitofusin protein levels in parkin deficient fly models (38).

CONCLUSION AND FUTURE
UbiBrowser 2.0 has a great improvement from version 1.0 (11). Extensive manual curation was implemented to extract the known ESIs/DSIs from PubMed abstracts. Two well-recognized algorithms of ‘naive Bayesian classifier’ and ‘interolog mapping’ were integrated to predict proteome-wide ESI/DSI networks (11,24). Using this improved protocol, we identified 5035 known, and more than two million
high confidence predicted ubiquitin ligase/deubiquitinase–substrate interactions in multiple organisms, with >210-fold increase in total data volume. UbiBrowser 2.0 has the following advantages: (i) it comprises the most known ESIs/DSIs compared to the related databases; (ii) it provides proteome-wide E3 ligase/deubiquitinase–substrate predicted interactions in 39 eukaryotes; (iii) it allows studying proteins’ upstream E3 ligases and deubiquitinases simultaneously; (iv) it presents a uniform confidence scoring system to rank the predicted interacting partners of the query protein across multiple organisms; (v) we optimized web interfaces to increase the convenience for users to search, browse and visualize known and predicted ESIs/DSIs. In the future, we will continue to update and maintain our database. E3/DUB-substrate interaction datasets will be expanded to cover all eukaryotic organisms. Cross-references to other public databases will be added to satisfy different requirements. Patient-derived multi-omics datasets will be integrated for the potential biological roles and clinical utility of ubiquitination. We believe that the database of UbiBrowser will be a comprehensive and useful resource for the ubiquitination community.

DATA AVAILABILITY
UbiBrowser 2.0 can be accessed at http://ubibrowser.ncpsb.org.cn.

SUPPLEMENTARY DATA
Supplementary Data are available at NAR Online.

ACKNOWLEDGEMENTS
We gratefully acknowledge UbiNet, hUbiquitome and Di Chen et al. for their generously sharing of the ubiquitination datasets. We also thank the bioinformatics platform at Phoenix Center for the strong and stable IT support.

FUNDING
National Natural Science Foundation of China [32088101, 31871341]; National key Research and Development Program of China [2020YFE002200]; Beijing Talents Foundation [to D.L.]. Funding for open access charge: National Natural Science Foundation of China [32088101].

Conflict of interest statement. None declared.

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