

Meta-analysis fails to show any correlation between protein abundance and ubiquitination changes

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Ubiquitination serves as a key regulatory mechanism in nearly all cellular processes, though it was originally identified as a post-translational modification that targets proteins for degradation. Accordingly, these two molecular events have since been tightly linked, and changes in protein abundance are frequently interpreted as indirect indicators of ubiquitination dynamics. Nevertheless, the relationship between protein abundance and ubiquitination has not been systematically examined across distinct biological systems. Here, we conducted a comprehensive meta-analysis to assess the correlation between protein abundance and ubiquitination levels determined by mass spectrometry in mammals, plants, and yeast. Quantitative proteomics and diGly-ubiquitin peptides data from 19 independent studies encompassing over 50 experimental conditions were analyzed. The findings indicate that alterations in protein abundance cannot reliably be used to infer ubiquitination events – and vice versa – highlighting the need for caution when interpreting proteomic data as a proxy for ubiquitin-mediated regulation.

Cellular protein abundance reflects the dynamic equilibrium among multiple interconnected processes, ranging from gene transcription and mRNA processing to translation, post-translational modification, and protein degradation. Historically, mRNA levels were assumed to be the primary determinant of protein abundance, and transcriptomic data were therefore used to infer protein-level changes. Nevertheless, advances in next-generation sequencing and mass spectrometry have demonstrated that mRNA abundance only partially predicts protein levels [1,2].

Analogously, protein degradation has traditionally been associated with protein ubiquitination. Indeed, ubiquitination was first identified as a signal for proteasomal degradation [3], and the Nobel Prize in

Chemistry in 2004, awarded to Aaron Ciechanover, Avram Herskko, and Irwin Rose, further emphasized the fundamental link between protein ubiquitination and degradation [4].

Ubiquitin is a small, 76-amino acid protein that, through the coordinated action of E1 activating, E2 conjugating, and E3 ligase enzymes, can be covalently attached to target proteins or other ubiquitin molecules already linked to those proteins. Polyubiquitin chains form when the C-terminus of one ubiquitin moiety conjugates to a lysine residue (K6, K11, K27, K29, K33, K48, and K63) or the N-terminal methionine (Met1) on another ubiquitin. Depending on the linkage pattern, ubiquitin chains can adopt homogeneous, heterogeneous, or branched topologies [5]. The

Abbreviations

DDA, data-dependent acquisition; DIA, data-independent acquisition; TMT, tandem mass tag; UPS, ubiquitin proteasome system.

Table 1. Summary of the research papers in which differential proteomics and ubiquitomics studies were carried out, and consequently, were selected for the present study.

Cluster	Author/year	Biological study	Q-MS method	p-values available	Time course	Ref
Plants	Guo <i>et al.</i> (2017)	Ethylene-induced senescence	Label free	✓	–	[20]
	He <i>et al.</i> (2019)	Rice seed germination	Label free	✓	–	[21]
	Liu <i>et al.</i> (2022)	Influence of T ^a on flowering	TMT	✓	–	[22]
	Lu <i>et al.</i> (2019)	Flowering stage 3vs5	Label free	–	–	[23]
Animals	Abreha <i>et al.</i> (2019)	Alzheimer	Label free	✓	–	[24]
	Baher <i>et al.</i> (2019)	MuRF1 overexpression	Label free	✓	–	[25]
	Dybas <i>et al.</i> (2019)	Stimulation of T cells	SuperSILAC	–	–	[26]
Fungi	Iesmantavicius <i>et al.</i> (2014)	Rapamycin treatment	SILAC	–	–	[27]
UPS alterations	Akimov <i>et al.</i> (2018)	Bortezomib and AP15 treatment	Label free	–	–	[28]
	Kim <i>et al.</i> (2011)	Bortezomib treatment	SILAC	–	✓	[29]
Cell lines	Agarwall <i>et al.</i> (2021)	Parkin	SILAC	✓	–	[30]
	Rusilowicz-Jones <i>et al.</i> (2020)	UP30	SILAC	–	–	[31]
	Hansen <i>et al.</i> (2021)	Released from synchronization	DIA	–	✓	[32]
	Li <i>et al.</i> (2018)	Gefitinib resistance	SILAC	–	–	[33]
	Ordureau <i>et al.</i> (2020)	Antimycin/Oligomycin treatment	TMT	✓	✓	[34]
	Rose <i>et al.</i> (2016)	Parkin and variants	TMT	–	–	[35]
	Steger <i>et al.</i> (2021)	FT671 treatment	DIA	–	✓	[36]
	Theurillat <i>et al.</i> (2014)	SPOP E3 ligase	SILAC	–	–	[37]
	Wu <i>et al.</i> (2015)	SAHA treatment	SILAC	–	–	[38]

functional outcome of ubiquitination depends not only on the modified substrate residues but also on the chain architecture [6]. For instance, K63-linked chains participate in DNA repair, endocytosis, and immune signaling [7–9]; while K11-, K27-, and K29-linked chains are involved in cell division, DNA damage, and proteotoxic stress response, respectively [10–12]. Among these, K48-linked chains are the most extensively characterized, serving as the canonical signal for proteasomal degradation [13].

The ubiquitin proteasome system (UPS) is the primary pathway for intracellular protein turnover, responsible for degrading approximately 80–90% of cytosolic proteins in mammalian cells [14]. The UPS primarily targets short-lived regulatory proteins, including cell cycle regulators, transcription factors, and damaged or misfolded proteins. In turn, autophagy degrades long-lived proteins, insoluble aggregates, and organelles [15–18]. Together, the UPS and autophagy work in concert to maintain cellular proteostasis [19].

Despite extensive research on ubiquitination and protein turnover, a systemic evaluation of the correlation between ubiquitination levels and protein abundance across diverse biological systems has not been performed. Here, we address this gap by performing a meta-analysis of quantitative proteomic and ubiquitomic data from 19 large-scale studies, encompassing over 50 experimental conditions (Table 1). Our analyses reveal a striking lack of correlation between

changes in protein abundance and ubiquitination levels, indicating that alterations in protein levels determined by proteomics cannot be reliably predict alterations in ubiquitination, challenging a long-standing assumption in molecular biology.

Material and methods

Bibliographic search

A comprehensive search of the PubMed® database was conducted in 2023 to identify original research articles that included both quantitative proteomics and ubiquitomics analyses under identical experimental conditions. Only studies providing accessible quantitative data – either deposited in public repositories or presented in supplementary materials – were considered for further analysis. To ensure analytical consistency, papers were included only if they reported complete protein-level and diGly site quantitative data suitable for statistical evaluation (File S1).

Data processing and statistical analysis

Data processing and statistical analysis were performed using either R (version 4.3.0) with *tidyverse* package (version 2.0.0) or using Python (version 3.7.9) with the *scipy* package (version 1.7.3). Quantitative data extracted from supplementary tables of selected studies were consolidated into a unified dataset (File S2) comprising six columns: (i) study identifier and experimental condition, (ii) protein

identifier, (iii) protein-level fold change between conditions, (iv) corresponding *p*-value, (v) fold change of each ubiquitination site (*individual diGly-containing peptide*), and (vi) associated *p*-value. Studies lacking *p*-values when required were excluded. When overall fold change values were not reported, they were computed by averaging replicate intensities. Proteins were retained for analysis only if intensity data were available for more than half of the total biological replicates (rounded up). In cases where a protein was detected in all replicates of one condition but absent in the other, the minimum detected intensity in the condition with missing values was used to calculate the ratio.

From this initial dataset, a secondary table (File S3) was generated by averaging the $\log_2$ ratios of the distinct diGly-modified peptides reported for the same protein within the study (*average diGly-containing peptide ratio*).

Two-sided Pearson correlation coefficients and associated *p*-values were calculated using the *cor.test* function of the R *stats* package with default parameters. Correlations were computed under four different conditions for each study (Table S1): (a) using the averaged ubiquitination values of all identified proteins. (b) Considering only up-ubiquitinated proteins. (c) Considering only down-ubiquitinated proteins. (d) Using all individual (nonaveraged) ubiquitination site values. Unless otherwise specified, threshold values of $\pm \log_2$ (1.5) for fold changes and 0.05 for *p*-values were applied. Hierarchical clustering was performed using group-average method on Euclidean distances implemented in *scipy*. Only proteins displaying fold change values exceeding $\pm \log_2$ (1.5) in the proteome were included in the clustering analysis.

The completed PRISMA checklist is provided as Supporting Information (File S4).

Results

To systematically evaluate whether protein ubiquitination directly influences protein abundance across biological systems, we analyzed 19 independent proteomic datasets that included simultaneous quantitative measurements of both the proteome and ubiquitome under identical experimental conditions. Quantitative data were extracted from each study and subjected to correlation analysis across all proteins for which both protein-level and ubiquitination-level data were available. Specifically, we examined the relationship between changes in protein abundance and corresponding diGly peptide ratios.

We systematically searched for original research articles in which investigators performed both differential proteomics and ubiquitomics analyses under the same experimental conditions. Studies were included only if the complete mass spectrometry (MS) datasets, including quantitative information, were uploaded in public

repositories or provided as supplementary materials. A total of 19 publications met these inclusion criteria.

The selected studies encompassed a wide range of biological systems, and we grouped them according to the organism in which the experiments were performed to account for potential species-specific differences in proteostasis. Accordingly, the 19 studies were classified into four categories: plants (*n* = 4), animals (*n* = 3), yeast (*n* = 1), and human cell lines (*n* = 11). Within the human cell line category, we included an additional subgroup comprising two studies in which the UPS was pharmacologically inhibited, as these interventions could directly influence ubiquitination–protein-level relationships.

In addition to differences in biological systems, the studies also varied in the mass spectrometry (MS)-based approaches employed. Most studies used data-dependent acquisition (DDA), in which the most abundant peptides in each scan are selected for fragmentation, allowing identification and quantification. Two studies – one on circadian biology in HEK293 and U2OS cells [32] and another characterizing USP7 deubiquitinase substrates [36] – used data-independent acquisition (DIA), a method in which all peptides within a defined mass range are fragmented simultaneously, improving reproducibility and coverage.

Regarding labelling strategies, most human cell line [29–31,33,37,38] and yeast [27] studies relied on stable isotope labelling with amino acids in cell culture (SILAC) to quantify changes in both proteome and ubiquitome abundance. Isobaric labelling via tandem mass tag (TMT) was employed in a study investigating PARKIN- and PINK1-dependent mitophagy in HTC116 cells [35], as in another analyzing PARKIN-mediated ubiquitination in induced neurons [34]. Among all cell line studies, only one applied a label-free mass spectrometric approach [28]. Notably, this study did not utilize the conventional anti-diGly antibody, which specifically recognizes the diglycine remnant left on ubiquitinated peptides following trypsin digestion. Instead, the authors developed UbiSite antibody, which targets the C-terminal 13 amino acids of ubiquitin that remain conjugated to peptides after endoproteinase LysC digestion.

Regarding the studies performed in animals and plants, most relied on label-free quantification to monitor changes in protein and ubiquitination abundance [21,23–25]. A study on the proteome of tree peony (*Paeonia suffruticosa*) cut flowers subjected to different temperatures used TMT labelling to investigate the effect of temperature on senescence and abscission [22]. Similarly, in a study examining the role of ethylene – a plant hormone involved in senescence – on the

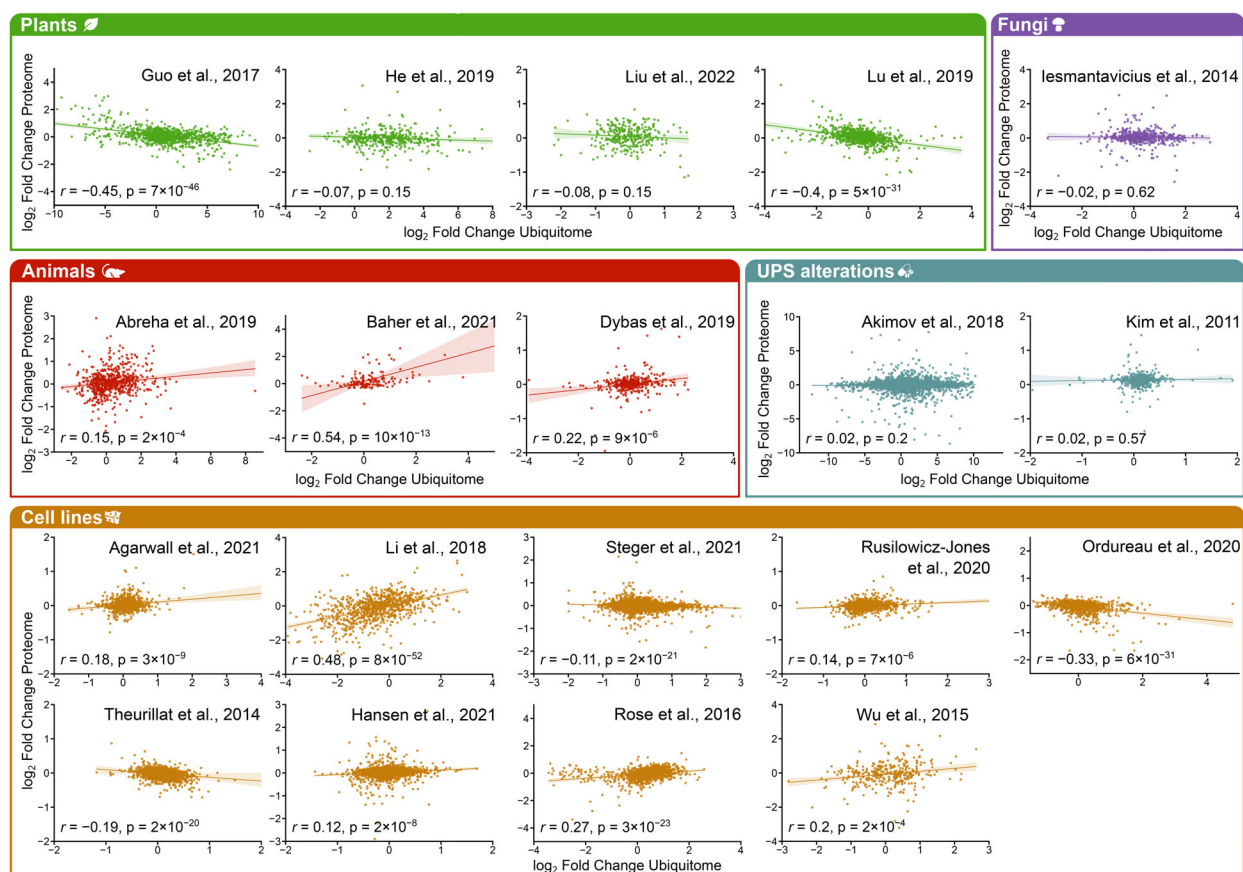


Fig. 1. Proteomics quantified protein abundance changes do not inversely correlate with changes in diGly peptide abundance. Data from 19 independent proteomic experiments on which both protein and ubiquitome abundance levels were analyzed and quantified under two different conditions are shown. Datasets have been grouped in five major clusters: Investigations carried out in plants are colored in green, whereas investigations done in animals, yeast, and cell lines are marked in red, purple, and yellow, respectively. Additionally, two studies performed in cell lines but in which UPS was altered are colored in blue. Each point corresponds to one protein from the dataset, as retrieved from the corresponding papers and data repositories. Ubiquitome data are shown as an average of all diGly peptides quantified for each protein. For each dataset, two-sided Pearson correlation coefficients and associated p -values were calculated. In the case of articles with more than one experimental condition, only the one with the correlation closest to the expected trend is shown.

ubiquitome and proteome of petunia corollas, TMT labelling and label-free quantitative strategy were used, respectively [20]. The superSILAC approach was employed to assess proteome and ubiquitome changes in activated CD4⁺ T cells [26].

Although the selected studies employed distinct MS-based quantitative strategies, all were processed using a unified analytical workflow. When quantitative data of biological replicates were available, proteins were retained only if detected in more than half of the replicates, allowing calculation of an average fold change (File S2). Similar criteria were applied to diGly peptide datasets (File S3). Finally, we computed Pearson correlation coefficients for each of the 57 experimental conditions represented across the 19 studies. For three additional experiments – corresponding to

studies that reported only deregulated proteins – we also calculated Pearson correlation coefficients and p -values (Table S1).

Poor correlation between protein abundance and corresponding diGly-containing peptide ratios

Figure 1 illustrates the correlation between proteome and ubiquitome changes across the 19 research articles analyzed, highlighting the strongest when multiple experimental conditions were tested within a single study. Additional comparisons are provided in Fig. S1.

If protein ubiquitination directly triggered proteasomal degradation, a negative correlation would be expected – that is, reduced protein abundance accompanied by increased ubiquitination. Conversely, under

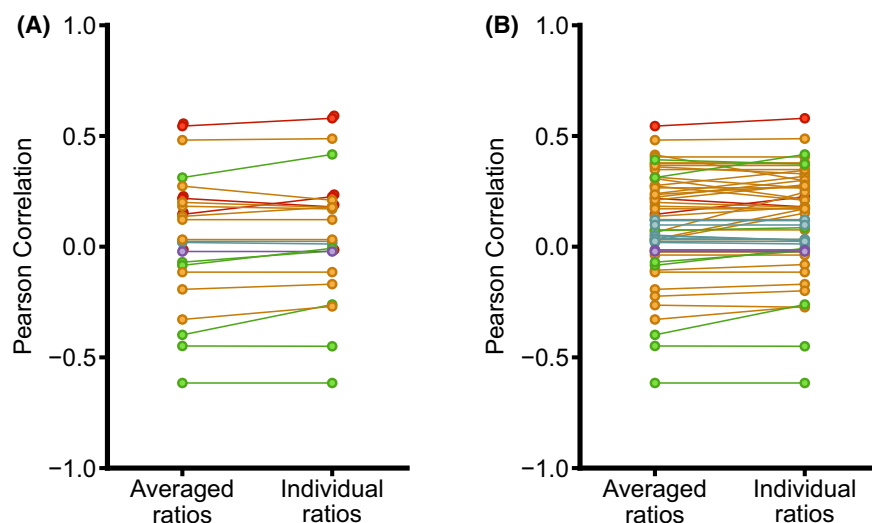


Fig. 2. Changes in protein and ubiquitination abundance do not correlate better considering individual diGly site ratios. Given the possibility that independent diGly peptide behavior would better conform to the predicted correlation with overall protein abundance, data were reanalyzed for all the datasets compiled in this work. Pearson correlation was calculated between changes in protein abundance and each diGly peptide corresponding to the protein for all the experimental conditions analyzed. Changes in Pearson correlation when considering average or individual diGly ratios are shown for (A) the articles and conditions shown in Fig. 1 and (B) the rest of the experimental conditions. No improvement was observed with this further analysis, further confirming that changes in protein abundance cannot be used as a predictor of protein ubiquitination, nor globally, neither for specific ubiquitination sites. *n.s., not statistically significant.

conditions where the UPS is inhibited, a positive correlation might occur due to the simultaneous accumulation of proteins and their ubiquitinated versions. Across all biological systems examined and regardless of the MS-based quantification method used, no consistent correlation was observed between global proteome and ubiquitome changes. Only two plant studies investigating senescence [20,23] exhibited a weak negative correlation. Specifically, ethylene-treated *Petunia hybrid* petals, in which ethylene promotes senescence [20], displayed increased ubiquitination concomitant with reduced protein abundance. Similarly, in *Rosa hybrid*, protein levels were modestly inversely correlated with ubiquitination during late stages of flower opening, coinciding with the onset of senescence [23]. Nonetheless, in both cases, the negative correlations were relatively weak (Pearson $r = -0.45$ and $r = -0.4$, respectively).

Correlation does not improve when considering diGly-containing peptide ratios individually

A single protein can be ubiquitinated at multiple lysine residues, and the modification dynamics of each site may differ substantially [28,34]. Therefore, averaging the ratios of all ubiquitinated peptides corresponding to the same protein could mask the contribution of specific regulatory ubiquitination sites. To explore this

possibility, we compared the fold change ratio of each protein with that of every individual diGly-modified peptide detected for that same protein and recalculated the Pearson correlation coefficient (Table S1).

In a few instances, the correlation became slightly more negative when individual diGly-modified peptide ratios were analyzed (Fig. 2). However, the differences were minor, with Pearson's r values changing by less than 5%. These findings indicate that the overall correlation between global proteome and ubiquitome abundance remains largely unchanged regardless of whether averaged or individual ubiquitinated peptide ratios are used in the calculations. Therefore, in subsequent analyses, average ubiquitinated peptide ratios were used.

Lack of correlation between significantly regulated proteins and corresponding ubiquitination sites

The quality of the quantitative data can influence the observed relationship between protein abundance and corresponding diGly peptide ratios. To minimize noise from statistically insignificant changes, we recalculated Pearson correlation coefficients using only proteins with significant abundance changes ($p < 0.05$).

In the study by Guo *et al.* [20], the previously observed negative correlation (Fig. 1) became slightly

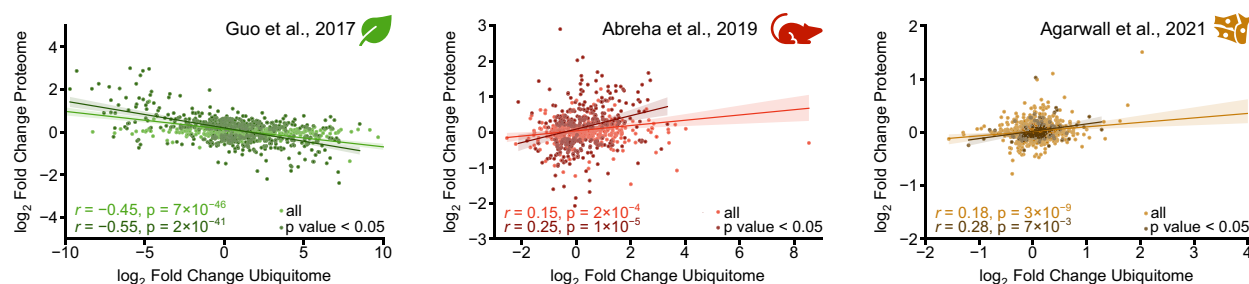


Fig. 3. Statistically significantly regulated proteins neither correlate with changes in ubiquitination. Pearson correlation was calculated between ratios of proteins displaying a p -value < 0.05 and the average ratio of their corresponding diGly sites. The most representative study for each cluster reporting a p -value in the proteomics data is shown. For each comparison, Pearson correlation and p -value are calculated; confidence interval is shown as shaded area.

stronger when only significantly regulated proteins were included (Fig. 3). However, in the remaining investigations, the correlation either remained largely unchanged or shifted in the opposite direction from the expected if ubiquitination were consistently associated with protein degradation (Fig. S2).

These results indicate that applying a significance threshold ($p < 0.05$) does not substantially affect the overall relationship between global protein and ubiquitination changes. Nevertheless, we propose that when protein degradation plays a central role in the biological process under investigation – such as in plant senescence – the inclusion of statistical significance as a filtering criteria may provide additional biological insight.

Upregulated and downregulated proteins can be either more or less ubiquitinated

K48-linked polyubiquitinated chains typically serve as a signal for proteasomal degradation [39], and consequently, decreased protein levels are often interpreted as a consequence of ubiquitin-dependent degradation. In this context, all downregulated proteins would be expected to exhibit increased ubiquitination, whereas upregulated proteins should display low levels of ubiquitination. To test this hypothesis, we evaluated the predicted relationship between protein abundance and ubiquitination by hierarchical clustering, color-coding upregulated and downregulated protein fold changes ($FC \geq 1.5$ and $FC \leq 0.67$, respectively) and corresponding average diGly ratios for visual assessment. Consistent with previous observations (Figs 1 and 3), the two studies on plant senescence [20,23] showed a weak trend suggesting that ubiquitination may influence protein abundance to some extent (Fig. 4). However, across the remaining studies with available data, no consistent relationship was observed between

changes in ubiquitination and corresponding changes in protein levels (Fig. 4 and Fig. S3).

We further assessed this hypothesis by focusing specifically on the ubiquitination status of downregulated proteins ($\log_2$ fold change < -0.66). For this analysis, three additional studies were included, as they provided data only for deregulated proteins. Two of these investigations were conducted in plants and examined rose petal abscission [40] and metabolic leaf senescence [41], while the third, performed in human cell lines, analyzed proteins regulated by the deubiquitinase UPS14 [42]. As shown in Fig. 5, with the exception for the two studies on plant senescence [20,23] and one on rice seed germination [21], downregulated proteins were not more ubiquitinated in any of the remaining 19 studies analyzed.

Time delays do not explain the lack of correlation between protein and ubiquitination levels

Having ruled out a direct correlation between protein abundance and ubiquitination levels, we next hypothesized that temporal delays might underlay their apparent lack of association, similar to the well-documented time lag between mRNA and protein expression changes [43]. To evaluate this possibility, we assessed whether nonsynchronous correlations – reflecting potential delays in proteasome-dependent protein degradation – could explain the observed patterns. Specifically, we compared global protein and ubiquitination levels across different time points in studies that included time course experiments.

Among the datasets analyzed, four studies incorporated temporal profiling of both the proteome and ubiquitome. To conceptualize the expected behavior, we first illustrated a theoretical model using heat maps depicting two possible scenarios: (a) a negative

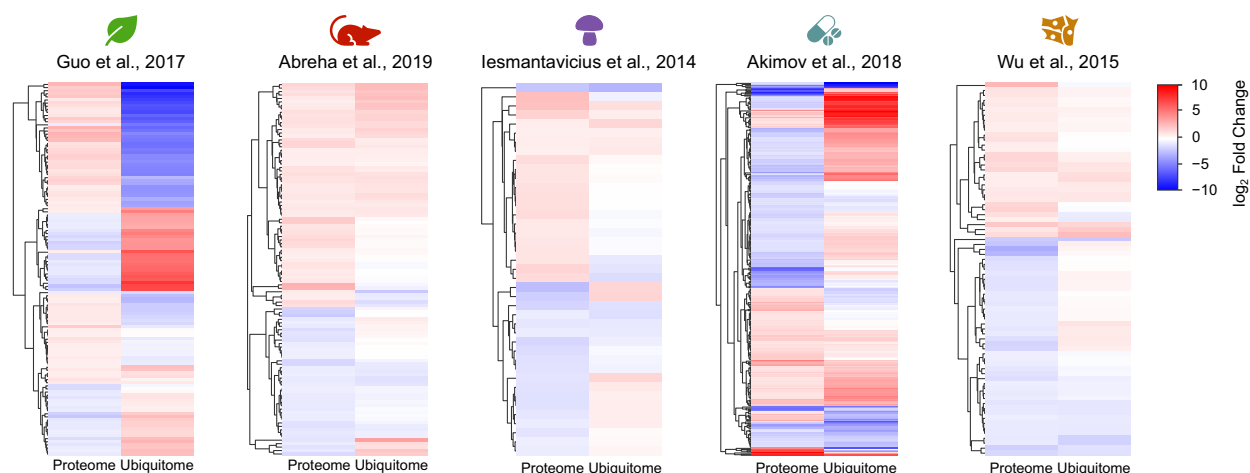


Fig. 4. Downregulated and upregulated proteins are not more and less ubiquitinated, respectively. Hierarchical clustering heatmap of proteome and ubiquitome of proteins displaying a fold change above 1.5 or below 0.67. Each row represents a protein from the study. The color code indicates the protein abundance and ubiquitination levels in $\log_2$ scale. A representative study for each experimental system is shown. Only for the work of Guo and colleagues, distinct groups of downregulated and more ubiquitinated and upregulated and less ubiquitinated can be found.

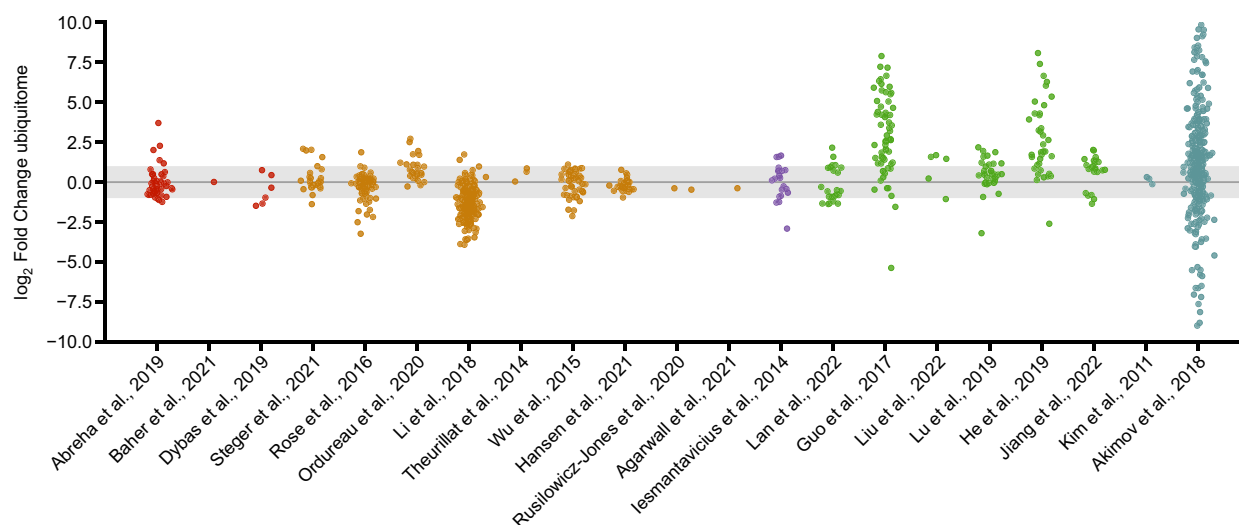


Fig. 5. Downregulated proteins contain either up-, down-, or nonregulated diGly peptides. For each paper analyzed, proteins displaying a fold change below 0.67 were selected and the ratio of each of their corresponding diGly peptide was plotted.

correlation between protein and ubiquitination levels at the same time point (Fig. 6A), and (b) a delayed negative correlation, in which changes in ubiquitination precede changes in protein abundance (Fig. 6B).

Applying this approach to the four available time course datasets revealed only weak evidence of such temporal relationships. A mild pattern consistent with a potential time lag was observed in cells treated with a USP7 deubiquitinase (DUB) inhibitor [36] (Fig. 6C). In contrast, no comparable trends were detected in cells exposed to a USP30 DUB inhibitor [34]

(Fig. 6E), nor in those subjected to proteasome inhibition with bortezomib [29] (Fig. 6F). Likewise, proteome and ubiquitome oscillations measured across the circadian cycle showed no time-dependent association [32] (Fig. 6D).

Discussion

Since the discovery by Chau *et al.* [44] in 1989 that K48-linked polyubiquitin chains target substrates for proteasomal degradation, ubiquitination has been

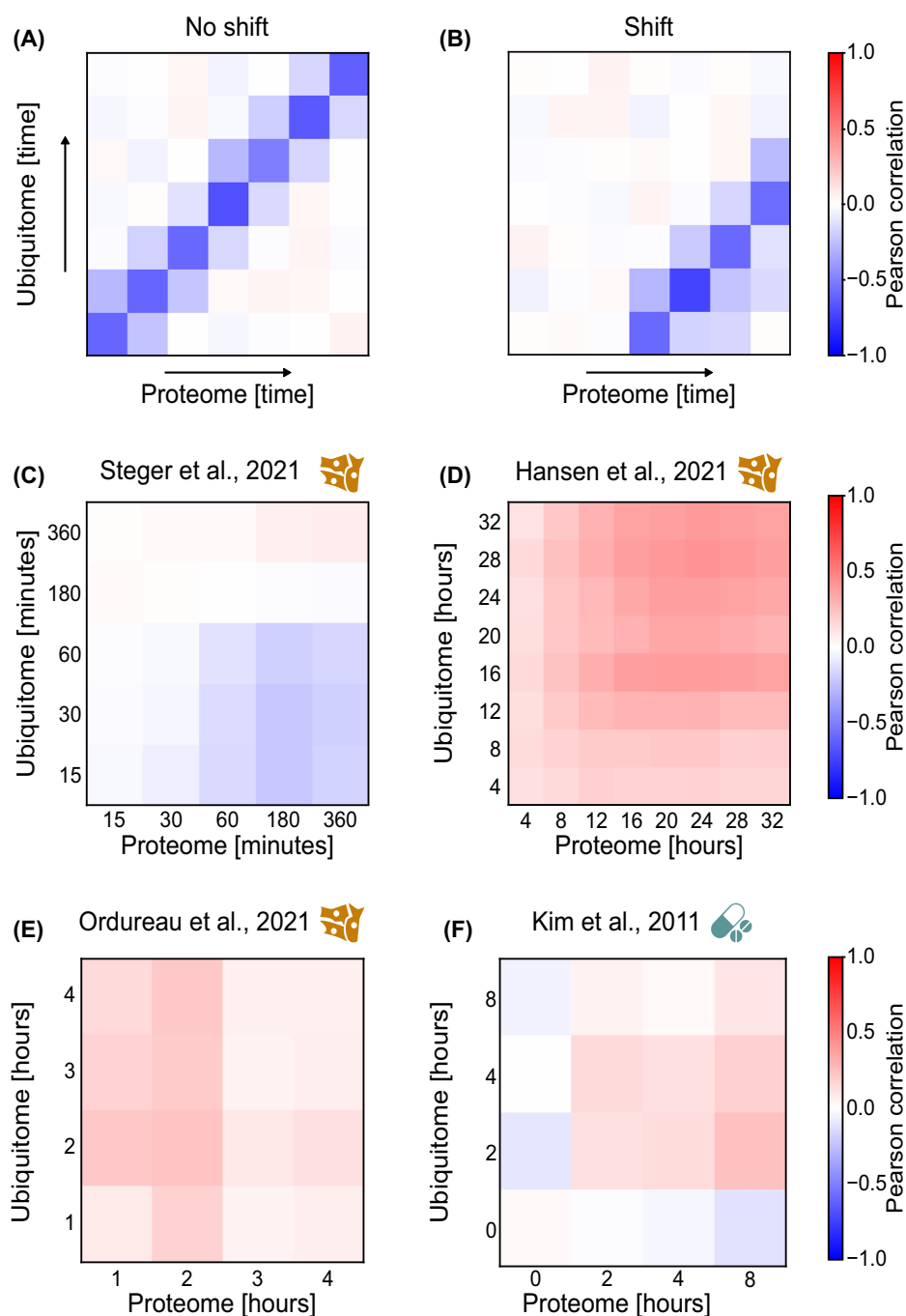


Fig. 6. Heatmap of Pearson correlation coefficient between protein abundance and ubiquitination levels of time course experiments. Theoretical representation of negative correlation between protein and ubiquitin changes with (A) no time shift and (B) with shift. (C–F) Correlation of the time course experiments in which proteomics and ubiquitome data were available.

closely associated with protein turnover. This association has become so entrenched that changes in ubiquitination are often interpreted as indicative of protein degradation, even in the absence of direct experimental evidence. However, accumulating evidence over the

past decades has revealed that ubiquitin serves regulatory roles far beyond proteasomal targeting, including protein–protein interactions, protein localization, and endocytosis [45–47]. In agreement with this expanded view, our meta-analysis demonstrates that, except

under very specific biological conditions, there is no consistent association between changes in proteome and ubiquitome levels.

A key methodological consideration is that most ubiquitome studies included in this meta-analysis is that most of them use the K- ϵ -GG enrichment to capture ubiquitinated peptides. This approach enriches all ubiquitinated peptides regardless of chain type. Consequently, this method does not distinguish between different forms of ubiquitination, such as K48-linked polyubiquitination – which primarily targets proteins for proteasomal degradation – and K63-linked polyubiquitination, which is typically associated with nondegradative processes including signal transduction, DNA repair, and endocytic trafficking [7–9]. Monoubiquitinated peptides are also enriched alongside polyubiquitinated species, preventing assessment of the relative abundance of specific chain linkages. Therefore, while our analysis provides a comprehensive and site-specific overview of global ubiquitination dynamics, results should be interpreted as reflecting general ubiquitination changes rather than specific chain architectures. Future studies using linkage-specific antibodies or targeted MS strategies will be valuable to determine how particular ubiquitin chain architectures contribute to the observed regulatory effects.

Proteins typically contain multiple lysine residues, providing the potential for several ubiquitination sites. However, not all lysines are equally susceptible to modification, as E3 ubiquitin ligases recognize motifs that depend not only on the primary sequence surrounding the target lysine but also on the protein's secondary and tertiary structure. Bioinformatics analyses indicate that ubiquitination sites tend to be surface-exposed and are frequently located within loop regions of the proteins [48]. Despite substantial advances in identifying ubiquitination motifs [49,50], only a few have been well-characterized since the first linear ubiquitination motif was described in 1986 [51,52]. Both large-scale ubiquitome studies [28,34] and single-protein analyses [53] commonly identify multiple ubiquitination sites within a single protein, and the abundance of each site can vary depending on the experimental conditions. For instance, in a previous study, we identified six ubiquitination sites in DDI1, three of which (K77, K133, and K151) were less ubiquitinated in the absence of the E3 ligase UBE3A, whereas the remaining sites were unaffected, indicating site-specific regulation by this enzyme [53].

Importantly, all the studies included in this meta-analysis relied on bottom-up MS, currently the gold standard for large-scale protein analysis. In bottom-up approaches, proteins are digested into peptides prior

to MS analysis [54]. Consequently, protein isoforms cannot be distinguished and the information of multiple modified sites mapping to the same protein does not imply that these modifications co-exist on a single proteoform. Top-down MS, which analyzes intact proteins [55], would be necessary to resolve combinatorial ubiquitination patterns and clarify which proteins are truly targeted for degradation. In the absence of intact protein data, we assessed individual diGly peptide ratios to explore the relationship between protein abundance and site-specific ubiquitination. To ensure our conclusions were not dependent on individual peptide ratios, we also assessed correlations using average diGly peptide ratios. Integrating data from 19 large-scale studies covering over 50 experimental conditions, we consistently observed that changes in the proteome and ubiquitome show little to no significant correlation.

The investigations examined here span highly diverse biological systems – including human cell lines, animal models, yeast, and plants – and address topics such as Alzheimer's disease [24], muscle atrophy [25] cancer [30,37], therapeutic responses [36,38], T-cell receptor stimulation [26], nutrient starvation [27], mitophagy [31,34,35] and proteasome inhibition [28,29]. In several of these, the original authors already reported a lack of correlation between proteome and ubiquitome alterations [26,28,29]. Dybas *et al.* [26], for instance, reported that changes in TCR-induced ubiquitination were largely independent of protein degradation, and pattern similarly observed in *Drosophila* [56]. Our comprehensive re-analysis confirms that this absence of correlation is a widespread phenomenon, independent of the organism or biological process under study, suggesting it represents a general trend within the available data.

Two plant senescence studies represent a notable exception to this trend. Lu *et al.* [23] and Guo *et al.* [20] both report modest but consistent negative correlations between protein and ubiquitination levels ($r = -0.40$ and -0.45). These results imply that ubiquitin-mediated regulation of protein homeostasis may play a more predominant role under specific physiological contexts, such as senescence. In most other conditions, however, ubiquitination serves regulatory or signaling functions independent of protein turnover. Consequently, protein abundance is likely regulated by additional mechanisms, preventing any correlation between ubiquitination and total protein levels.

Senescence is the final stage of plant organ development, characterized by the systematic breakdown of cellular components to recycle nutrients. Protein

degradation is a central feature of this process, as proteins represent a major reservoir of nitrogen and other essential resources [17,57,58]. The ubiquitin proteasome system (UPS) and autophagy are the primary pathways mediating protein turnover during senescence, selectively removing damaged, misfolded, or regulatory proteins. It is well-documented that the proteasome exhibits high activity in senescing leaves of oilseed rape and wheat [59,60]. In *Arabidopsis*, disruption of proteasome subunits delays senescence, underscoring the essential role of proteasome-dependent degradation [61]. Recent work by Lu *et al.* [62] has revealed that ethylene promotes petal senescence in roses by inducing the E3 ligase RhSAF, which ubiquitinates and degrades the GA receptor RhGID1. Together, these observations, supported by our meta-analysis, reinforce that ubiquitin-dependent protein degradation is a central mechanism driving plant senescence.

Nevertheless, the contribution of ubiquitin-mediated protein degradation varies across species and the specific senescence conditions. For example, in tree peony, heat-induced senescence showed no concordance between proteome and ubiquitome changes [22], and in *Arabidopsis thaliana*, depletion of the senescence-related E3 ligase UPL3 did not result in correlated changes in protein abundance and ubiquitination [41]. Additionally, the moderate correlation observed in the studies by Guo *et al.* and Lu *et al.* suggests that, beyond the UPS, other degradation pathways – most likely autophagy – also play a significant role in protein turnover during petal senescence. Collectively, all these data indicate that protein degradation during plant senescence is orchestrated by multiple, partially independent pathways, with ubiquitin-dependent mechanisms playing a central but context-dependent role alongside other processes such as autophagy.

To further explore the relationship between ubiquitination and protein abundance, our analysis included two studies in which the ubiquitin–proteasome system (UPS) was pharmacologically inhibited [28,29]. In these datasets, cells were treated with proteasome inhibitors (bortezomib and AP15) to block proteasomal degradation. If ubiquitination primarily reflected degradative targeting, one would expect a positive correlation between increased ubiquitination and protein accumulation under these conditions. However, we did not detect any consistent correlation between ubiquitination and protein abundance in either study. Notably, the SILAC-based time course data from Kim *et al.* [29] also showed no correlation even when potential temporal delays were considered. These findings reinforce that global ubiquitination levels are not

necessarily indicative of proteasomal degradation and support the broader view that ubiquitination serves diverse regulatory roles beyond protein turnover.

The temporal relationship between interconnected cellular events represents a critical consideration when interpreting molecular data. Sequential processes, such as gene transcription and protein translation, or protein ubiquitination and ubiquitin-dependent protein degradation, may occur with distinct kinetics. Consequently, correlation between mRNA and protein levels, or between ubiquitination and protein abundance, may only be observable at specific time points. For example, during *Drosophila* embryogenesis, maximal mRNA–protein correlation was detected between mRNA at 12 h and protein levels at 16 h [63]. Consistently, in our analysis of USP7 deubiquitinase inhibition, alterations in ubiquitination at 30 min exhibited a modest inverse correlation with protein-level changes at 3 h, whereas no correlation was observed when both events were compared at the same time point. Notably, in the other three time course experiments analyzed, no temporal effects were detected. Taken together, these findings suggest that the general lack of concordance between changes in protein abundance and corresponding diGyl-modified peptides is not attributable to time lag effects.

In summary, our meta-analysis demonstrates that ubiquitination and protein abundance show little concordance, emphasizing that the presence of ubiquitination does not necessarily imply protein degradation, nor does protein abundance reliably reflect ubiquitination status. Thus, quantification of total protein levels by proteomics or other techniques, such as western blotting, is insufficient on its own to infer or suggest changes in protein ubiquitination status.

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Conflict of interest

The authors declare no conflict of interest.

Peer review

The peer review history for this article is available at <https://www.webofscience.com/api/gateway/wos/peer-review/10.1002/2211-5463.70197>.

Data accessibility

The data that support the findings of this study are available in the supplementary material of this article.

Author contributions

NO, UA, and UM: conceptualization; UA: visualization; GP: data curation and formal analysis; NO: writing-original draft preparation; UM, UA, and GP: writing-review and editing. All authors contributed to the article and approved the submitted version.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

File S1. PRISMA flow diagram.

File S2. Consolidated data obtained from each of the 54 experimental conditions included in the 19 analyzed research papers. Six columns are provided: (i) the name of the paper and the experimental conditions under study, (ii) the protein identifier, (iii) the protein-level fold change between the experimental conditions, (iv) the *p*-value associated with this fold change, (v) the ubiquitination-level fold change between the experimental conditions, (vi) and the *p*-value associated with this fold change.

File S3. Consolidated data after averaging the ubiquitination fold changes in logarithmic scale when different values were provided for the same protein in the same study. Four columns are provided: (i) the name of the paper and the experimental conditions under study, (ii) the protein identifier, (iii) the protein-level fold change between the experimental conditions, and (iv) the ubiquitination-level fold change between the experimental conditions.

File S4. PRISMA 2020 checklist.

Table S1. Two-sided Pearson correlation coefficients and *p*-values.

Fig. S1. Protein – diGly correlation analysis for experimental conditions other than the highest-correlation condition.

Fig. S2. Pearson correlation between proteins and diGly peptides using significantly regulated proteins.

Fig. S3. Relationship between changes in down- and up-regulated proteins and their corresponding diGly ratios.