Supplementary Information

Minguez *et al*., Deciphering a global network of functionally associated post-translational modifications

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Supplementary Material

Literature review on co-regulated pairs of PTM types

To assess the current knowledge of PTMs co-regulation we searched co-occurring names of PTM types in Medline abstracts and reviewed them manually. As the information extracted from this procedure served us to evaluate the novelty of the links reported by our own approach, we do not consider trans-regulation between modifications located in different proteins, nor crosstalk of different types of modifications in the same residue. Due to the vast amount of documents retrieved for each PTM (Supplementary Table 6) we cannot ensure we compiled every detail in the relationship of every pair of PTMs although we firmly believe well-stablished events of PTM co-regulation have been addressed. Thus, the following report does not pretend to be a review on the state of art of PTM co-regulation, nor a complete collection of examples where all the possible types of links between the PTMs are commented but just an accurate evaluation of the overlap between current knowledge and our predictions including some overview and highlighting a few significant examples. To the best of our knowledge, pairs of PTMs not reported below did not show any evidence of co-operation in the literature.

Phosphorylation – Ubiquitination. Phosphorylation and poly-ubiquitination are a clear example of positive co-operation. It is reported that they act together in the degradation of short-lived proteins by the ubiquitin proteasome pathway, which is conserved from yeast to mammals. This process is mediated by the enzyme E3 which binds multiple ubiquitin proteins to target for degradation. On top of ubiquitin ligases being phosphorylated themselves, certain types of these enzymes (e.g. SCF E3) require the target protein to be phosphorylated in a particular region, the phosphodegrons, before sent to degradation (Vodermaier, 2004; Hunter, 2007).

Phosphorylation – Acetylation. Phosphorylation and acetylation are both important protein regulators of the transcriptional machinery; they are reported to act as binary switches in histone tails (Fischle *et al*, 2003), i.e. phosphorylation can prevent or promote acetylation and vice versa in H3 (Latham & Dent, 2007).

Other example of direct competition in non-histone proteins is the serine acetylation of MAP2Ks by means of acetyltransferase YopJ encoded in the plasmid of the pathogen *Yersinia pestis (Mukherjee et al, 2006)*. This process inhibits subsequent phosphorylations blocking the nuclear factor κB (NFκB) signaling pathways.

Phosphorylation – Methylation. Besides the close interplay of phosphorylation and methylation in the regulation of histones, where they are extensively reported to work as binary switches (Fischle *et al*, 2003; Latham & Dent, 2007), there is also evidence of crosstalk outside the chromatin regulation, i.e. the methylation of K233 inhibits the phosphorylation of adjacent serines in the Yeast kinetochore protein Dam1 (Zhang *et al*, 2005).

Phosphorylation – Carboxylation. Carboxylation is an important modification of glutamate residues especially in coagulation factors and bone proteins where it is part of the Gamma Carboxyglutamic Acid-rich motif (Gla) and contributes to calcium binding. In Vitamin K-dependent protein MGP serine phosphorylation appears to co-operate with carboxylation to inhibit calcification (Schurgers *et al*, 2007). There is also evidence in trans-regulation in plants crassulacean acid metabolism (Freschi *et al*, 2010).

Phosphorylation – Hydroxylation. Although the DNA binding capacity of the highly hydroxylated hypoxia inducible factor 1 complex (HIF-1) seems to be enhanced by phosphorylation (Richard, 1999), only examples in trans-regulation in different proteins were retrieved on the possible co-operation of phosphorylation and hydroxylation. Thus, cAMP-dependent protein kinase (PKA) seems to control the activity the Phenylalanine hydroxylase PAH (Miranda *et al*, 2004).

Phosphorylation – nitrosylation. As mentioned above, HIF seems to be regulated by both phosphorylation and nitrosylation but no evidences of crosstalk was found.

Phosphorylation – N-linked Glycosylation. Although phosphorylation and N-linked glycosylation have been analyzed together (Hao *et al*, 2011), and they seem to regulate membrane and extracellular proteins, as far as we know no evidence of particular crosstalk has been reported yet. Together with their co-occurrence in proteins, there are few studies reporting trans-regulation in the angiogenesis pathway (Zhang *et al*, 2010) and in nervous system by means of decreasing of phosphorylation of down-stream proteins when altering the N-glycosylation of M(3) muscarinic receptor gene (Romero-Fernandez *et al*, 2011).

Phosphorylation – O-linked Glycosylation. Both phosphorylation and O-linked glycosylation modify serine and threonine amino acids and indeed there is solid evidence for their site competition in the so-called yin-yang sites (Zeidan & Hart, 2010; Butt *et al*, 2011) that act as molecular switches for protein function and localization

even in nuclear proteins. In addition to this, O-linked glycosylation can influence phosphorylation of adjacent sites, e.g. O-GlcNacylation of S149 in p53 reduces phosphorylation of T155 (Forsythe *et al*, 2006).

Phosphorylation – Palmitoylation. Palmitoylation is a reversible protein modification that contributes to the association of proteins to membranes among other functions. A particular subject of study has been the role in proteins involved in synapsis where a few cases of crosstalk with phosphorylation have been reported. For instance, in AMPAR receptors, palmitoylation of the GluR1 C811 residue seems to modulate PKC phosphorylation (Lin *et al*, 2009).

Phosphorylation – Sulfation. Although both modify tyrosines and they are found in the same proteins playing individual regulatory roles (Gulberti *et al*, 2005), to the best of our knowledge, there are no described mechanisms for their possible crosstalk in the literature as some in vitro analysis starts to suggest (Lander *et al*, 2001).

Phosphorylation – SUMOylation. Both PTMs are part of the histone code to regulate chromatin packing and gene transcription. Moreover, they are found to co-regulate the activity of some transcription factors, e.g. MEF2D is phosphorylated in S444 and this is required for subsequent SUMOylation of K439 (Grégoire *et al*, 2006).

Acetylation – Methylation. Acetylation and methylation are lysine modifications, thus they can compete for the same residue with antagonistic effects, e.g. acetylation and methylation of the histone protein H3 (K9) are associated with activation and repression of transcription respectively (Latham & Dent, 2007). Other examples of crosstalk include non-histone proteins and not competing mechanisms, e.g. in p53, methylation of K372 is required for acetylation of other sites (Kurash *et al*, 2008).

Acetylation – Hydroxylation. They are another example of PTM types modifying lysines and they are found to modify the same proteins including the highly regulated HIF. Indeed, acetylation of HIF-1α K532 favors the interaction with pVHL, and thus destabilizes HIF-1α. However, there is not enough evidence for co-regulation with hydroxylation as the acetyltransferase activity is not influenced by hypoxia (Jeong *et al*, 2002).

Acetylation – Nitrosylation. Together with hydroxylation, nitrosylation is the other important regulator of the activity of HIF, although acetylation is also present as mentioned before there is no evidence of a joined mechanism of action between them (Dimova & Kietzmann, 2010). There are however some studies suggesting trans-regulation, e.g. nitrosylation of protein deacetylase HDAC2 makes the enzyme release from chromatin and the histone acetylation is increased (Nott *et al*, 2008).

Acetylation – N-linked/O-linked Glycosylation. Little is known about their possible interplay. In bacteria, the Cj1123c acetyltransferase has also been found to be involved in the N-linked/O-linked glycosylation pathways (Demendi & Creuzenet, 2009). In addition, some trans-regulation of acetylation and O-linked glycosylation being the glycosylation activity regulated by acetylation (Zlocowski *et al*, 2011). However no direct co-regulation has been reported so far.

Acetylation – SUMOylation. Both PTM types modify lysines and they are found to be exclusive modifications in the histone code (Latham & Dent, 2007). In yeast, several Lys in proteins H2B and H4 can be acetylated and SUMOylated, while acetylation is associated to activation, SUMOylation acts as a transcription repressor (Nathan *et al*, 2006). There are also cases of crosstalk outside the histone code, such as the regulation of the transcription factor MEF2A (Shalizi *et al*, 2006).

Acetylation – Ubiquitination. Again a pair of PTM types that modify lysines. Apart from their known co-operation in histones where ubiquitination can promote acetylation (Latham & Dent, 2007), it is in fact one of the few cases where a systematic crosstalk has been studied on a wide scale revealing a high proportion of lysines modified by both PTMs (Danielsen *et al*, 2011).

Methylation – SUMOylation. In spite of the fact that methylation and SUMOylation are PTMs modifying lysine residues and their role in heterochromatin formation, they are only reported to perform trans-regulation of different histones (Uchimura *et al*, 2006).

Methylation – Ubiquitination. Methylation and ubiquitination are a clear example of trans-regulation in histone code by means of the crosstalk of ubiquitinated residues present on the carboxy-terminal tails of histones H2A and H2B and the methylation of histone H3 (Shukla *et al*, 2009). It has also been hypothesize that methylation

through SET7 methyltransferase can lead to proteasome degradation by a co-operation with ubiquitination (Pradhan *et al*, 2009).

Carboxylation – N-linked Glycosylation. N-glycosylation on the Kringle domain interacts with carboxylation of the Gla domain, which increases stability of the prothrombin in the ER (Wu & Suttie, 1999).

Hydroxylation – N-linked Glycosylation. There is some evidence of lack of dependency between these two PTMs; in the protein KCNE1, the mutation of T7 to a non-hydroxylated residue seems to reduce O-linked glycosylations although the protein maintains mono-N-glycosylated residues (Chandrasekhar *et al*, 2011).

Hydroxylation – O-linked Glycosylation. Apart from the example mentioned before, hydroxylation and O-linked glycosylation seem to be clearly co-operating. For instance, the signaling cascade that modulates O_2-signals shows hydroxylation of Skp1 that enhance posterior O-linked glycosylations (Wang *et al*, 2011). In collagen proteins it is well reported the interplay of glycosylated residues close to hydroxylated prolines for their stability and binding activity (Bann & Bächinger, 2000).

Hydroxylation – SUMOylation. Hydroxylation and SUMOylation also regulate the HIF protein although it is reported that they do not cooperate (Cheng *et al*, 2007) probably showing a ubiquitination and SUMOylation crosstalk that could be an alternative mechanism of the action of ubiquitination and hydroxylation that lead to its degradation.

Hydroxylation – Ubiquitination. Both PTMs modify lysine residues and they are found to co-regulate the proteasome degradation mediated by means of E3 ubiquitin ligase VHL complex of HIF-1α (Ke & Costa, 2006).

Nitrosylation – Palmitoylation. There is some evidence that nitrosylation and palmitoylation perform reciprocal processes in protein modifications involved in neurotransmitter signaling (Sen & Snyder, 2010) although no mechanism was proposed.

Nitrosylation – Ubiquitination. The nitrosylation of PTEN phosphatase has been shown to enhance subsequent ubiquitination that has an effect on both the lipid phosphatase activity and the protein stability (Kwak *et al*, 2010).

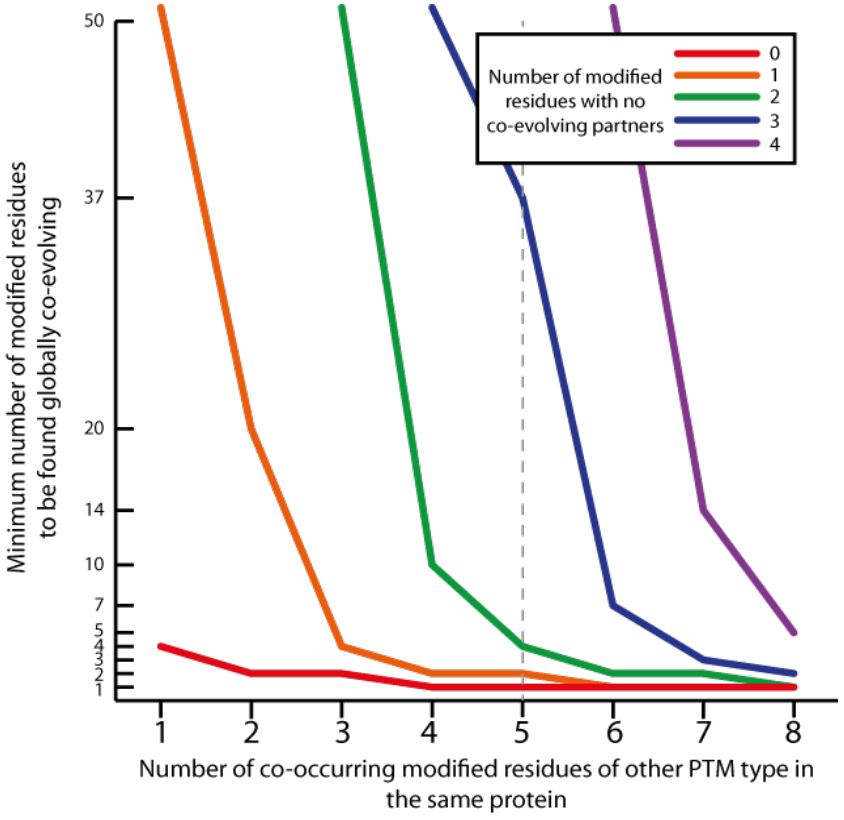
N-linked Glycosylation – O-linked Glycosylation. Little is known about a possible co-operation of both major types of glycosylation. We found only one case where the O-glycosylation of KCNE1 at T7 seems to be required for N-glycosylation at N5 (Chandrasekhar *et al*, 2011).

N-linked Glycosylation – Palmitoylation. In spite of being both membrane associated PTMs, only in Wnt-3a, its glycosylation has been found to show a possible co-regulation as it precedes palmitoylation (Komekado *et al*, 2007).

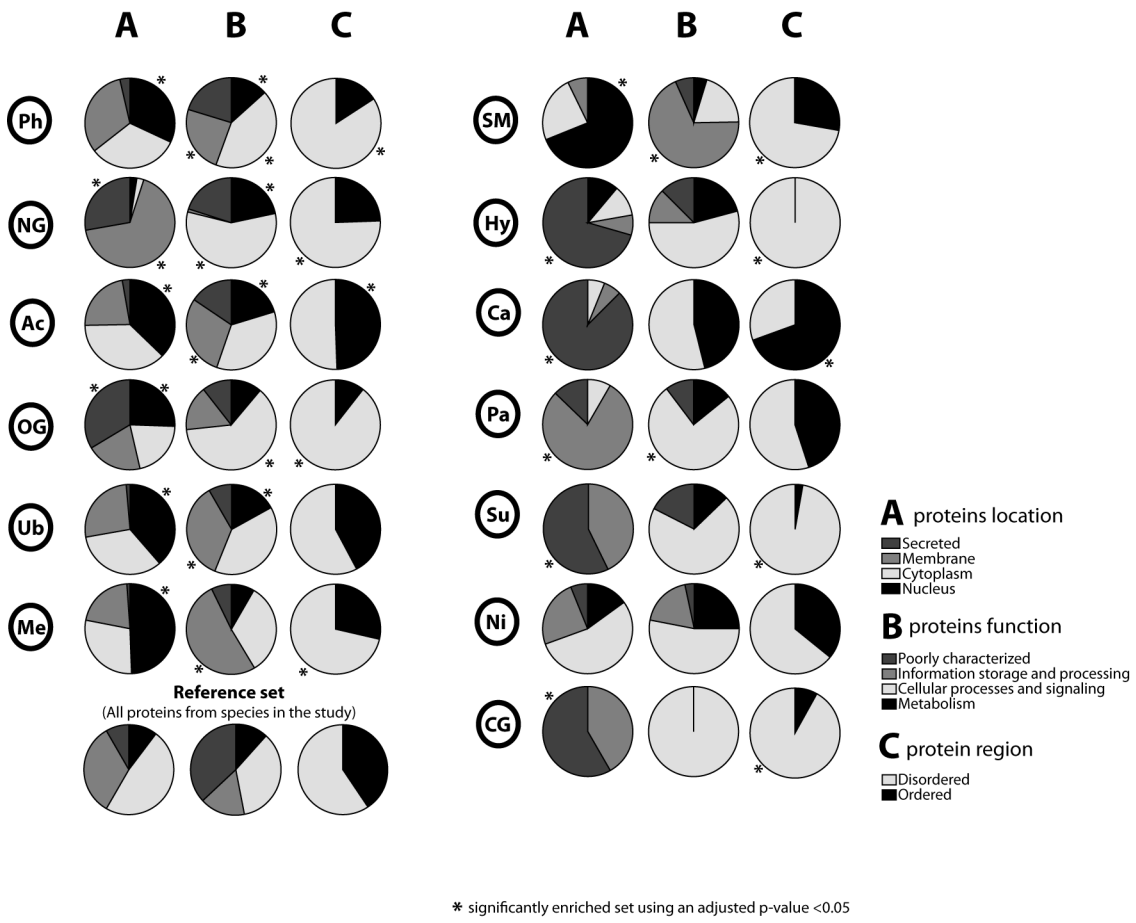
N-linked Glycosylation – Ubiquitination. N-linked glycosylation seems to play a role in the stability of proteins associated to the endoplasmic reticulum opposing ubiquitination as a signal for degradation. An instance of this co-regulation is the misfolded protein ABCG2 which lack glycosylations are then target of ubiquitin ligases that lead to their degradation (Wakabayashi-Nakao *et al*, 2009).

SUMOylation – Ubiquitination. Both PTM types exclusively modify lysine residues and in addition of site competition they can also regulate each other in cis in histones (Latham & Dent, 2007). In non-histone proteins there is also evidence of SUMOylation that leads to proteasomal degradation by means of co-regulation with poly-ubiquitination as it happens in the HIFalpha subunit under hypoxia (Cheng *et al*, 2007).

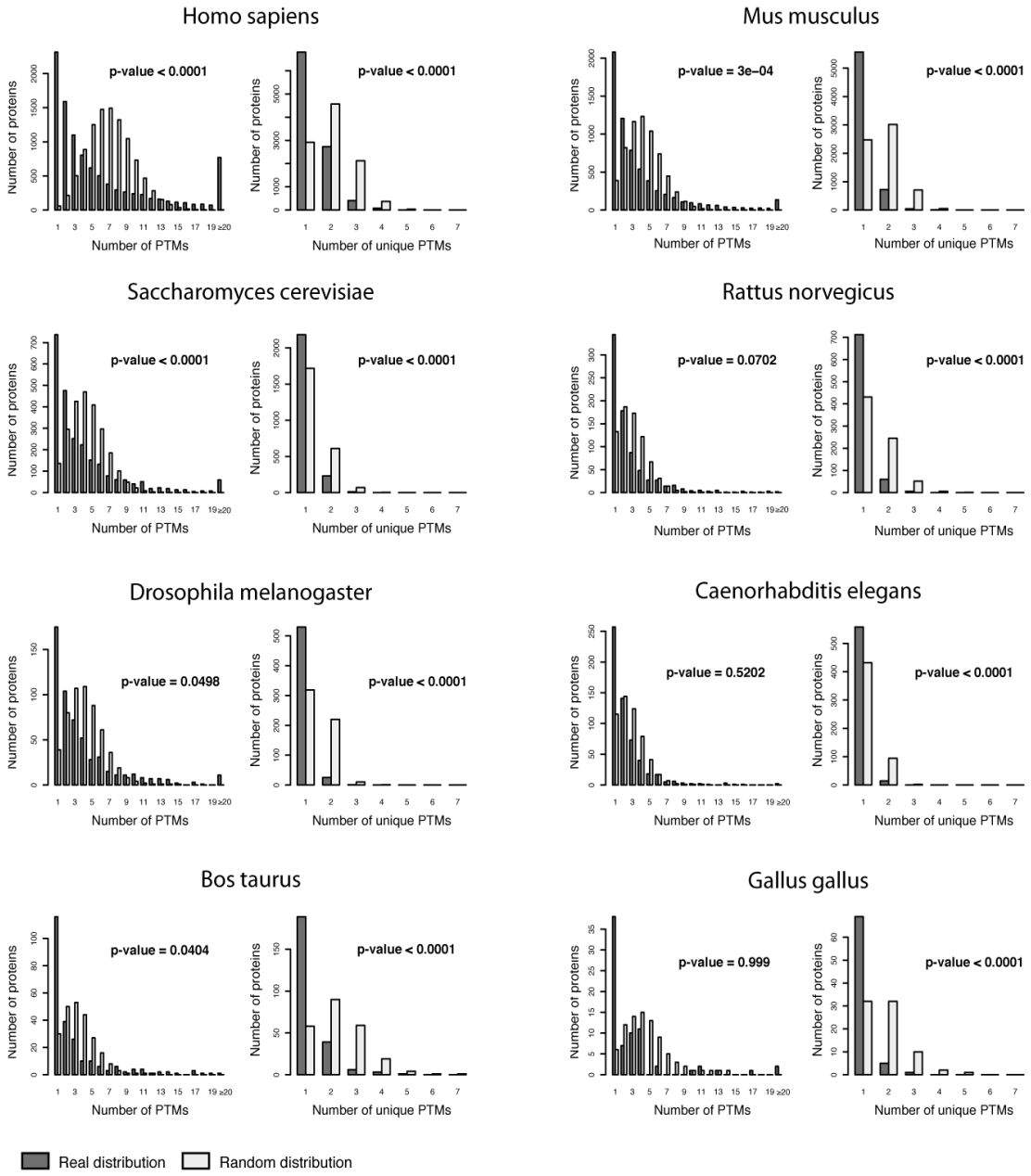
Supplementary Figures



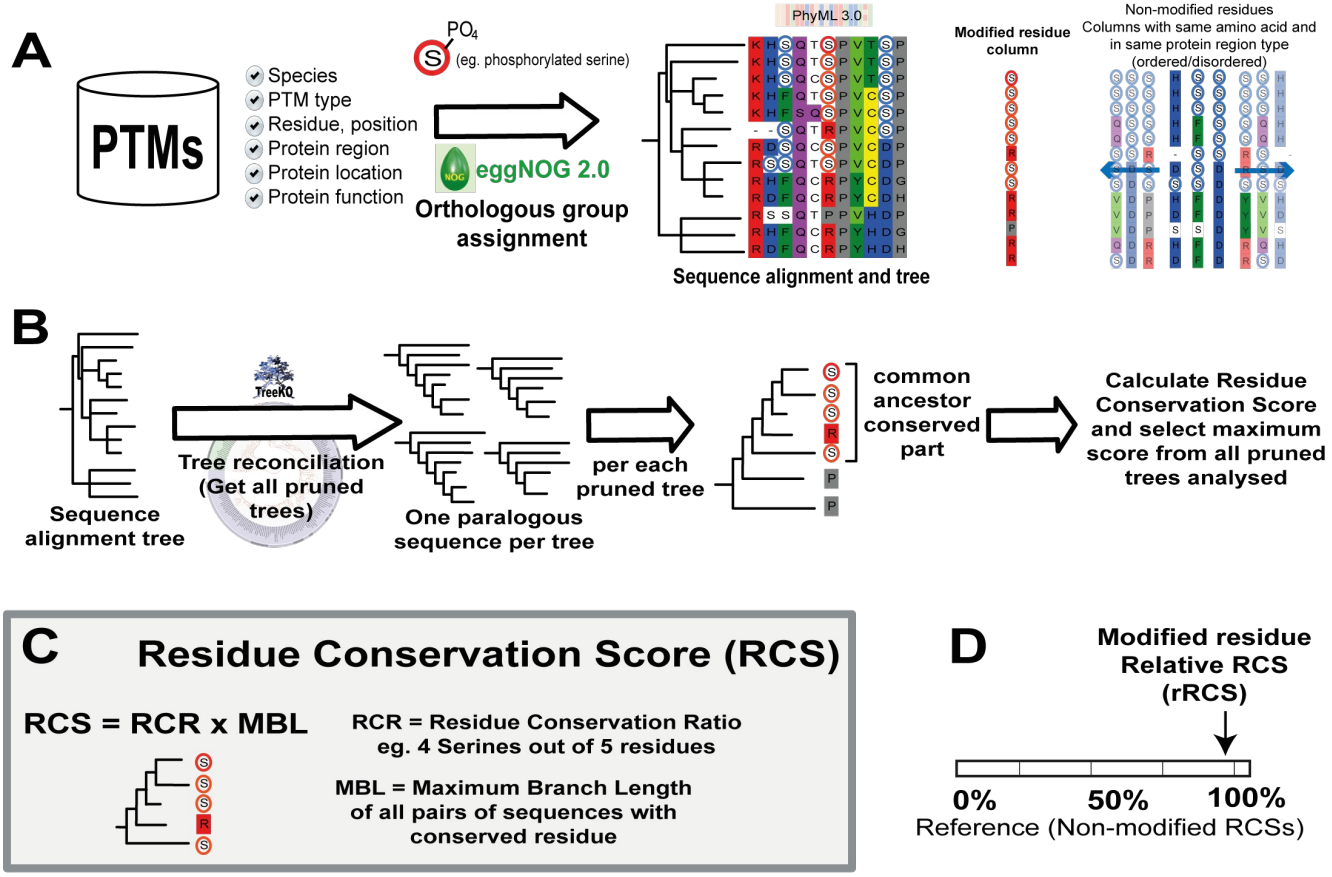
Supplementary Figure 1. Detection of global co-evolution of a PTM type with others depends on its number of sites in the dataset. We performed a simulation to test the capacity of our pipeline to detect global co-evolution between two PTM types. Assuming averaged parameters on the background residues (see Materials and Methods) the limit of the number of residues a PTM type should have to detect statistical significance depends on the number of residues that are co-occurring from another PTM type within the same protein. Thus, even assuming that all the co-occurring residues are found co-evolving, we would need a higher co-occurrence of residues when we have fewer number of residues from one of the PTM types. In addition, a PTM type with more residues in the dataset could have more non co-evolving residues with other residues from other PTM type to be found significantly co-evolving, this also depends on the number of co-occurring residues within one protein. For instance, assuming a co-occurrence with 5 phosphosites (average number for phosphorylated proteins, the by far largest PTM type in terms of residues; grey line) another PTM type would need to have (to be found significantly co-evolving with phosphorylation) in the dataset: only 1 residue if we assume all pairs are found co-evolving (red line), 2 residues if we assume 1 not co-evolving pair per residue (orange line), 4 residues if 2 not co-evolving partners are found (green line) and up to 37 residues if 3 pairs are not found to be not co-evolving per residue (blue line). Therefore, adding more PTM types from our dataset (with less instances than C-linked glycosylation) to the study would not increase the number of globally co-evolving pairs of PTM types reported.



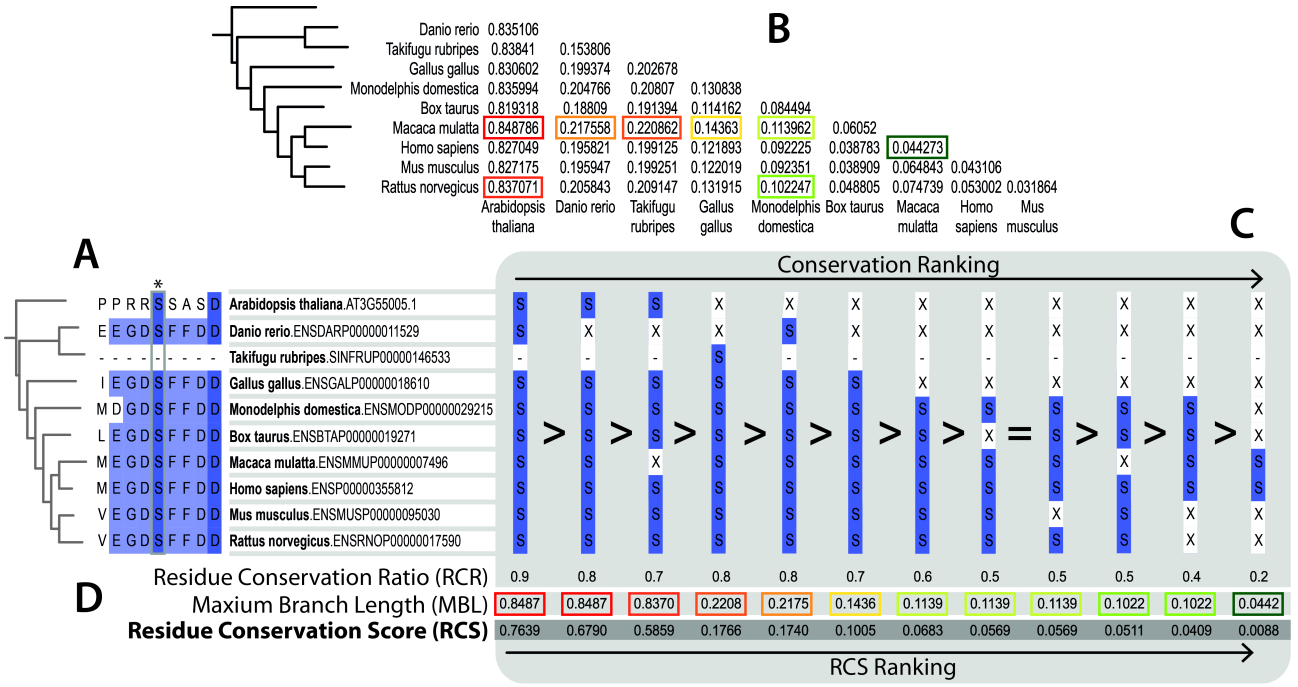
Supplementary Figure 2. Functional enrichment analysis of the proteins having a particular PTM type. The proteins with a particular PTM type show different preferences (measured as enrichment analysis) for certain cell compartments **(A)**, functionalities **(B)** and for regions (ordered and disordered) within the protein **(C)**. All PTM types but nitrosylation were enriched in any of the categories analyzed, significant enrichments (with adjusted p-values <0.05) are highlighted with an asterisk. A differential preference for subcellular location and functionality suggests different roles of the PTM types within the cellular processes in which they participate, thus there are PTM types that preferentially modify nuclear and cytoplasmic proteins (Me, SM, Ub) and others that are focused on secreted and membrane proteins (NG, Ca, Hy, Su, Pa, CG). There are also differences in the protein region type in which the PTMs are found, although most of them are enriched in disordered regions, acetylation and carboxylation seem to be more present in ordered regions.



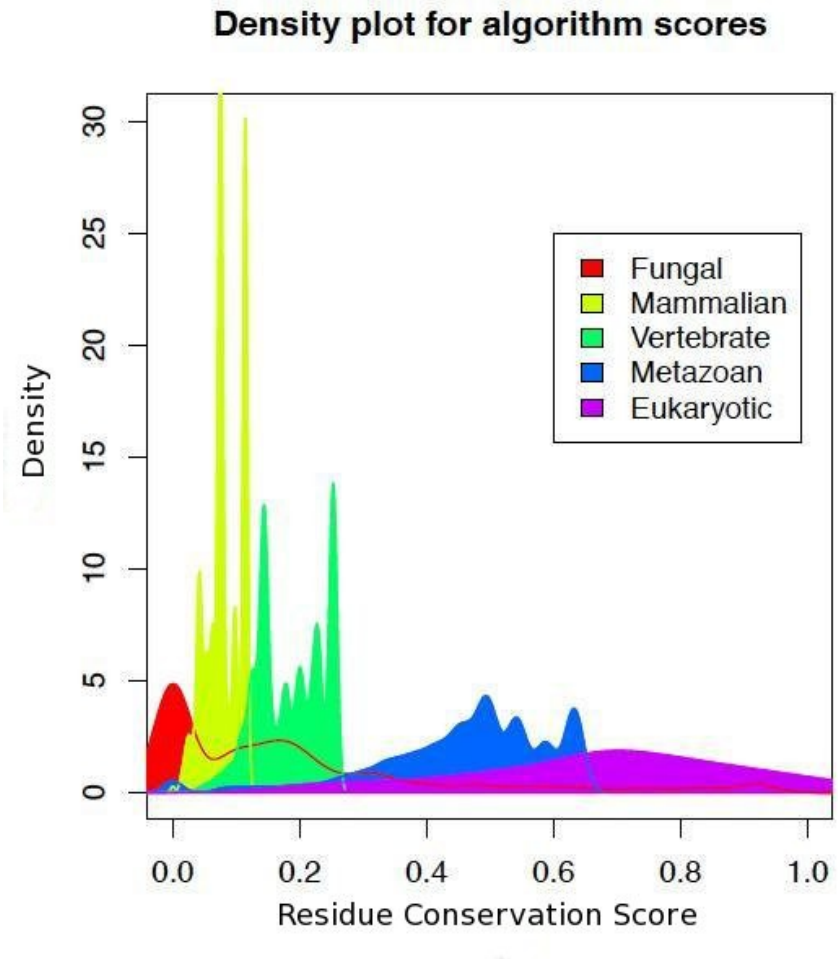
Supplementary Figure 3. Evaluation of the number of PTMs and unique PTM types per protein. We compared the expected distribution of the number of proteins with particular number of PTMs (all PTMs in our dataset randomly distributed over the same number of proteins in our dataset) to the real distribution in our dataset using the non-parametric Kolmogorov-Smirnov test (left graphic of each species). In the species with more experimentally verified PTMs (human, mouse, yeast, drosophila and cow) we see a significant difference between the two distributions. While proteins in general have less PTMs than expected, there are few proteins that have far more PTMs than expected which again suggest the incompleteness of the current knowledge of PTMs as the proteins with more PTMs are surely the more studied ones as shown in Figure 1C. We tested also whether proteins have more unique PTM types than expected (all PTMs in our dataset randomly distributed over the same number of proteins in our dataset). We performed a Fisher exact test to evaluate whether the number of proteins with more than one PTM type in our dataset is more than expected (graphic in the right of every species). Proteins have systematically less PTM types than the random expectation, probably due to either the nature of PTMs that might be co-regulating more among the ones that are of the same type (eg. several phosphorylations are needed to activate a protein) or due to the nature of the experiments as they are obviously focused on a specific type of PTM which again suggests the incompleteness of the dataset.



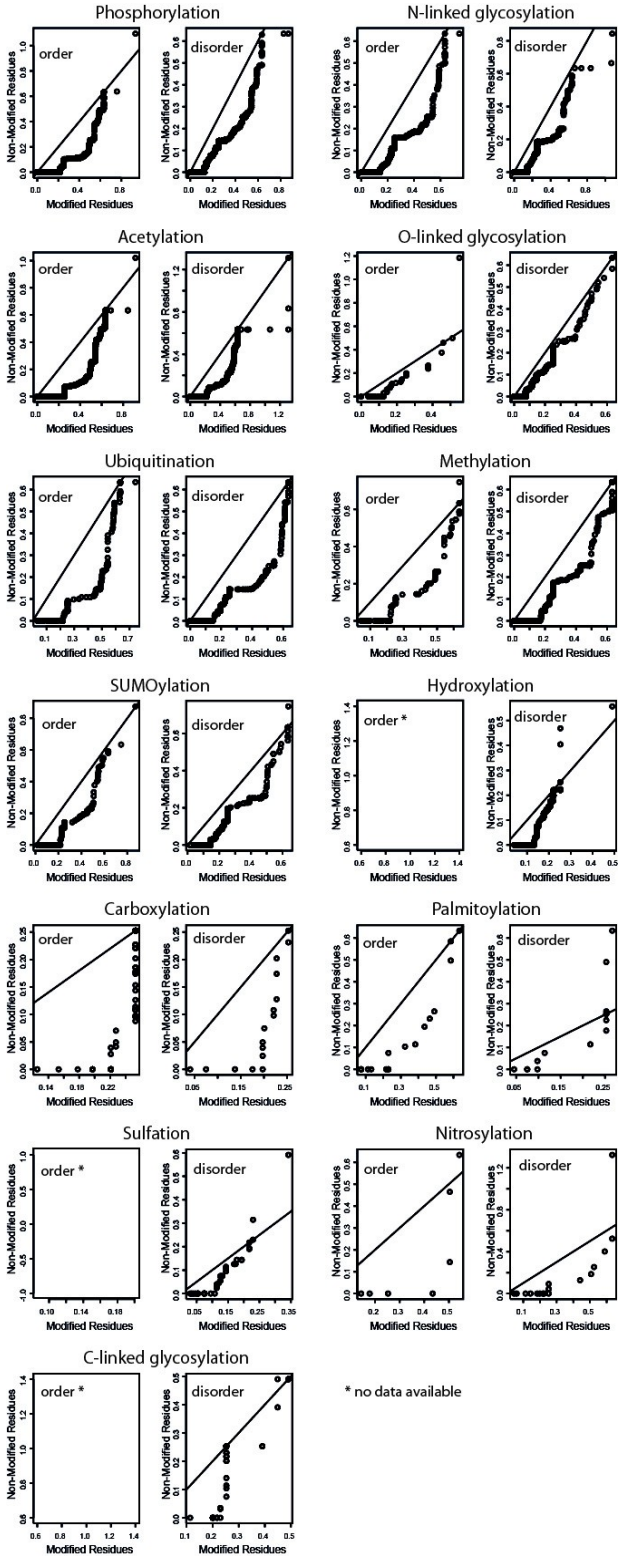
Supplementary Figure 4. Conceptual schema for the calculation of the Residue Conservation Score (RCS). The pipeline starts with the compilation of the available PTMs and their annotation **(A)**, after evaluating the conservation status of every modified residue, we obtain the orthologous group of the protein from which we calculate the multiple sequence alignment (MSA) and its phylogenetic tree. The rest of the analysis is applied to the modified residues and to non-modified residues with the same features that we use as statistical background. From the MSA tree **(B)** we generate a number of pruned trees with no paralogy and branch lengths from the species tree which we use to calculate the two components of the RCS algorithm **(C)**, the RCR that accounts for the conservation ratio of the residue within the subtree rooted in the most recent common ancestor of the species with the residue conserved, and the branch length of most remote pair of species with the residue conserved (MBL). **(D)** Finally the RCS for the modified residues is normalized mapping its value within the percentiles of the distribution of the values from the non-modified residues.



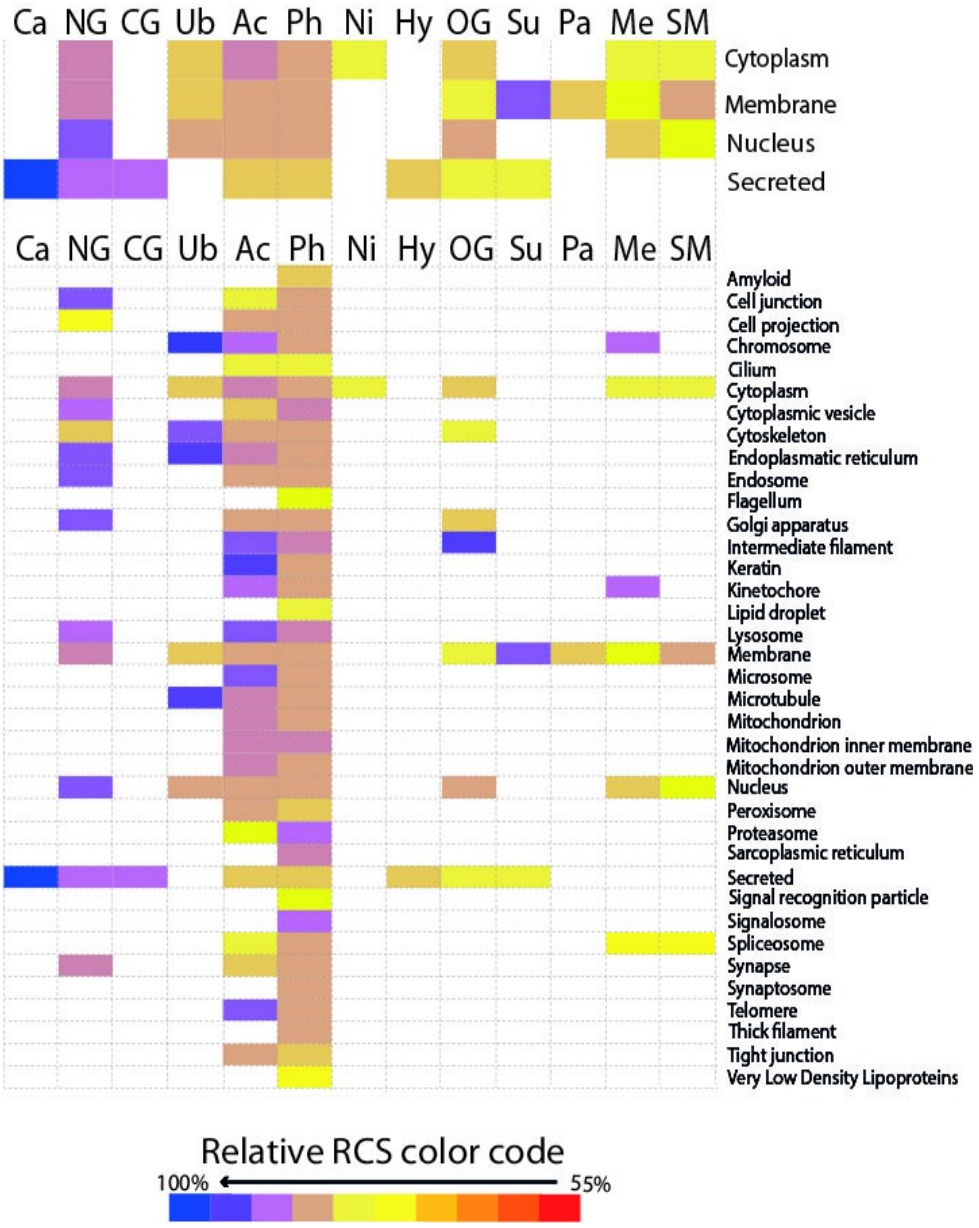
Supplementary Figure 5. Performance of the RCS. The algorithm used to evaluate the conservation of one residue is able to sort different conservation scenarios more accurately than the two elements of the RCS (RCR and MBL) individually. In **(A)** a modified residue marked with an asterisk (*) in the MSA is evaluated according to 12 different situations ranked from more to less conserved. Colors in the matrix of distances **(B)** show the different branch lengths used in the calculation of the RCSs. Neither the RCR, nor the MBL can individually sort the 12 scenarios accurately, in contrast, RCS can do it using information from both **(D).**



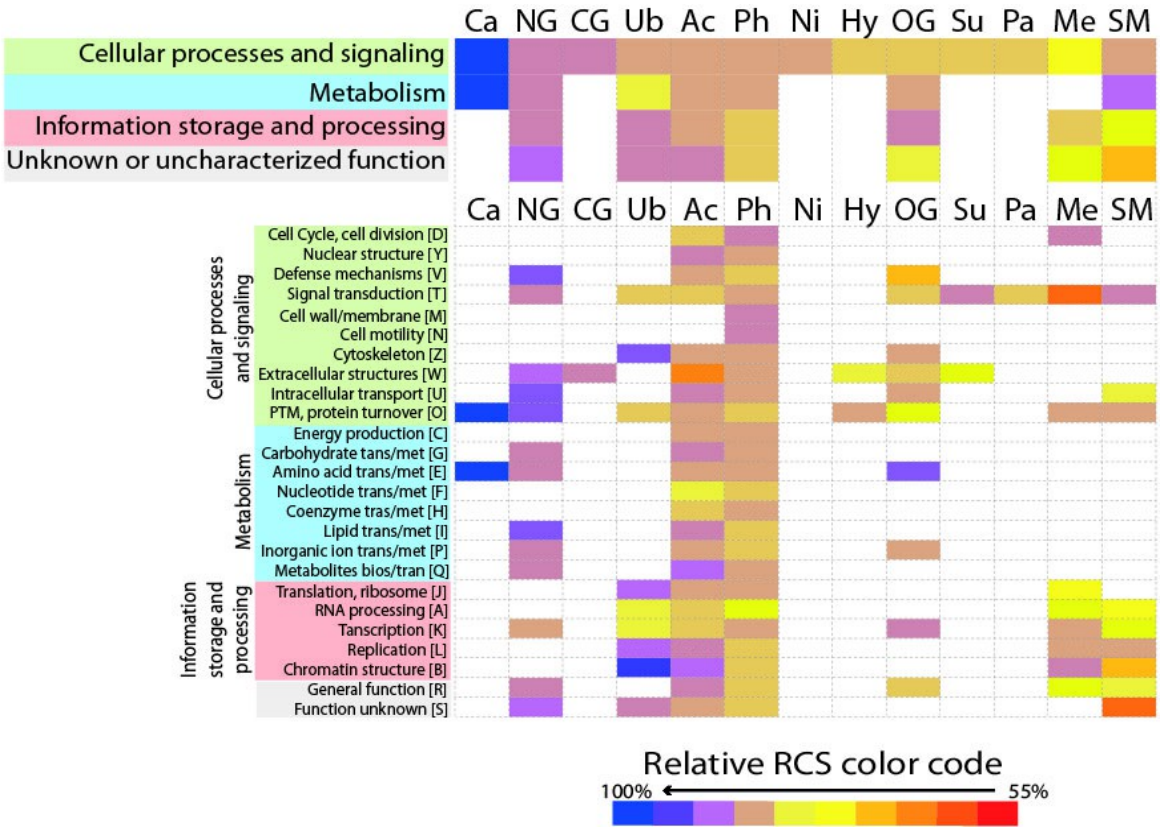
Supplementary Figure 6. Density plot of RCS values for modified residues conserved over different levels in the tree of life. Modified residues get different levels of values for RCS depending on which level in the tree of life they are conserved. We consider in here the oldest taxa category in which the residue is conserved considering conservation in eukaryotes, metazoan, vertebrate, mammalian and fungal. Thus, higher RCS values are given to the residues conserved in eukaryotes, followed to the ones conserved in metazoan but both with a wide variability as they are very general groups. The RCS values for the modifications conserved in vertebrates and mammals are more concentrated. The amino acids conserved in fungal seems to present the lowest values although higher variability than mammals and vertebrates is shown as they are a more fast evolving group.



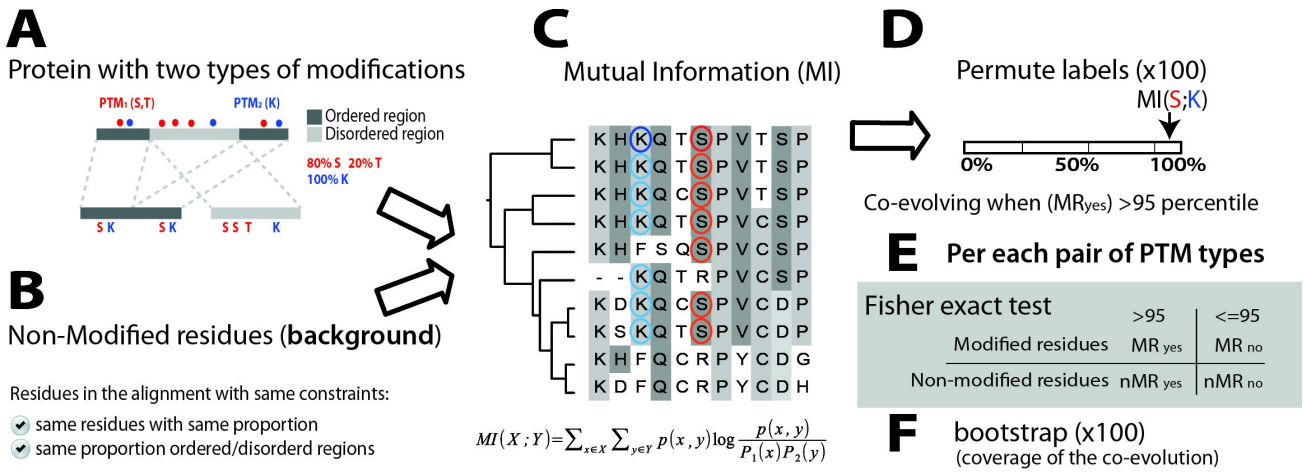
Supplementary Figure 7. Comparison of conservation (RCS values) of modified and non-modified residues over both ordered and disordered regions for each of the PTM types. Quantile-quantile (Q-Q) plots compare graphically two non-parametric distributions, in this case the RCS values for modified and non-modified residues of each of the PTM types under study. Empty plots represent no data for that PTM type in that particular protein region.



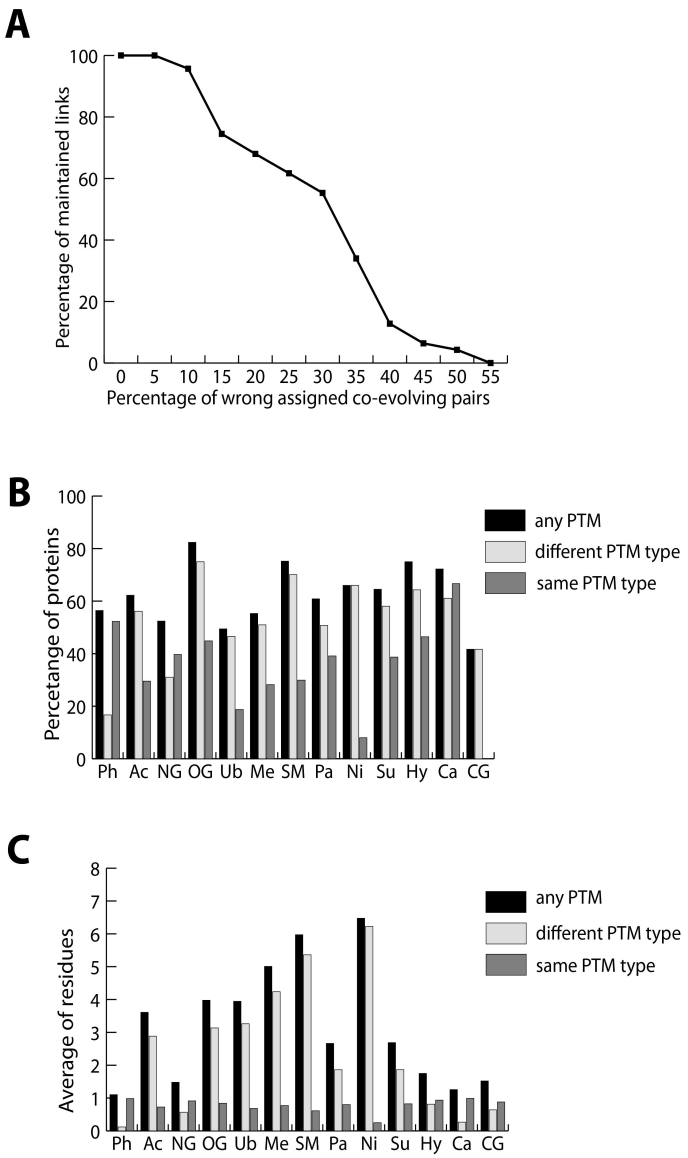
Supplementary Figure 8. Differential conservation of PTM types over different cellular compartments. Relative RCS (rRCS) is shown in color code for each of the sets of human proteins present in particular subcellular locations, we show here the mean of the rRCS for the whole set of modified residues in those proteins. In the upper part we show the four main classes in which more specific locations can be classified. PTM types has not only differences in conservation among themselves but also differential conservation within them according to their subcellular location, for example acetylation (Ac) presents more conservation in cytoplasmic proteins and less in the ones that can be secreted. Thus, highest conservation can be observed in keratin proteins, intermediate filaments and lysosomes while less conserved acetylations take place in cell junction, cilium or synapse.



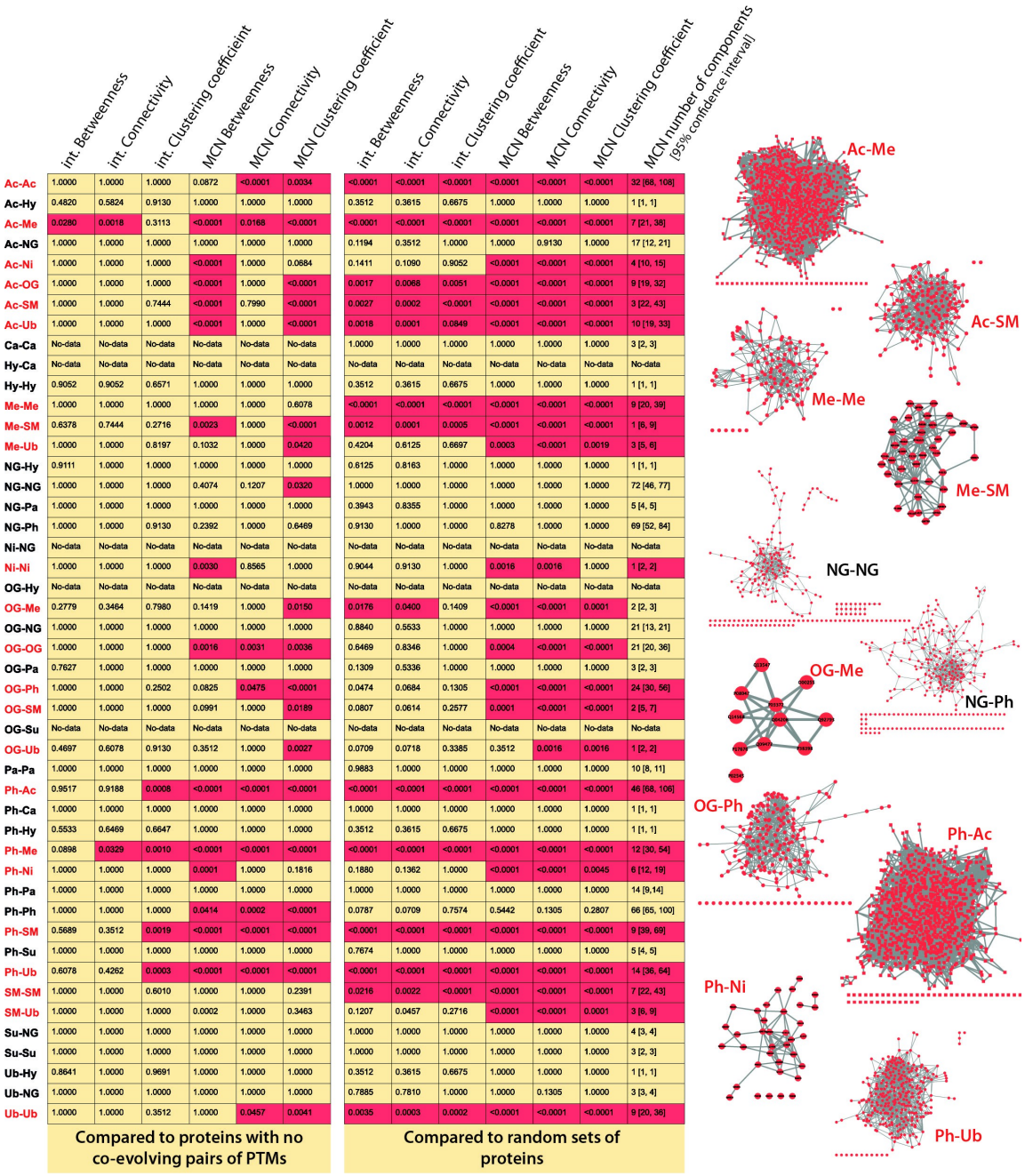
Supplementary Figure 9. Functional specificity in the differential conservation of PTM types. Relative RCS (rRCS) is shown in color code for each of the sets of human proteins present with a particular functionality, we show here the mean of the rRCS for the whole set of modified residues in those proteins. In the upper part we show the four main classes in which more specific functions can be classified. PTM types also present differential conservation according to the protein functionality (as in many cases reflects a distinct subcellular location or vice versa). For example, ubiquitinated residues are more conserved in proteins involved in information storage and processing and less conserved in metabolic proteins, even within those functionalities there are considerable differences as ubiquitinated residues are more conserved in chromatin structure and replication than in translation and RNA processing.



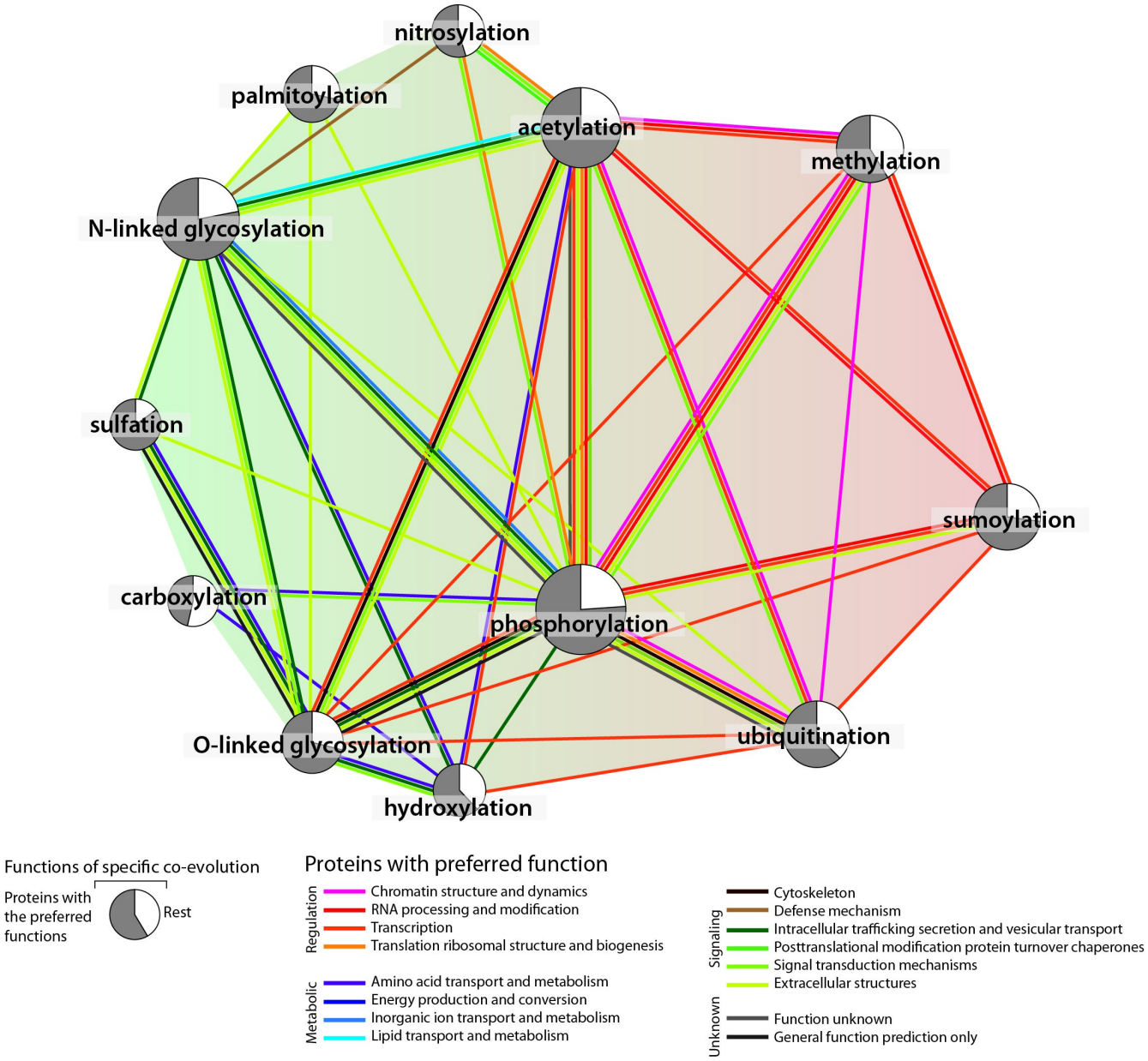
Supplementary Figure 10. Pipeline for the evaluation of co-evolution between two PTM types. For the global evaluation of co-evolution of two PTM types we measure the co-evolution of pairs of modified residues from both types that appear together in a protein **(A)** and compare these values with the co-evolution of non-modified residue under the same conditions **(B)**. For every pair of residues (modified and non-modified pairs), we use mutual information (MI) algorithm, adjusted for differentiate between correlation and anti-correlation, as a measurement of co-evolution of two residue **(C)**, thus, we measure the co-occurrence of both residues in the organisms present in the MSA of the orthologous group where the protein is mapped to applying the MI formula **(B)**. To evaluate the significance of the MI value for each of the pairs of amino acids we generate a reference distribution of values by permuting the organism labels of one of the residues, then the percentile of the MI value over the reference distribution is calculated **(D)**, if the percentile is above 95, the pair of residues is said to co-evolve. We evaluate whether the co-evolution of the modified residues is higher than for the non-modified residues by means of a Fisher test **(E)**. As the pairs of the residues are taken randomly, we measure the global coverage of the co-evolution by repeating the whole analysis 100 times (**F**). Therefore this framework aims to extract global co-evolution status between two types of modifications (step **E**), however the cases where we do not report a general predicted functional link can still show significant co-evolution between two individual residues (step **D**).



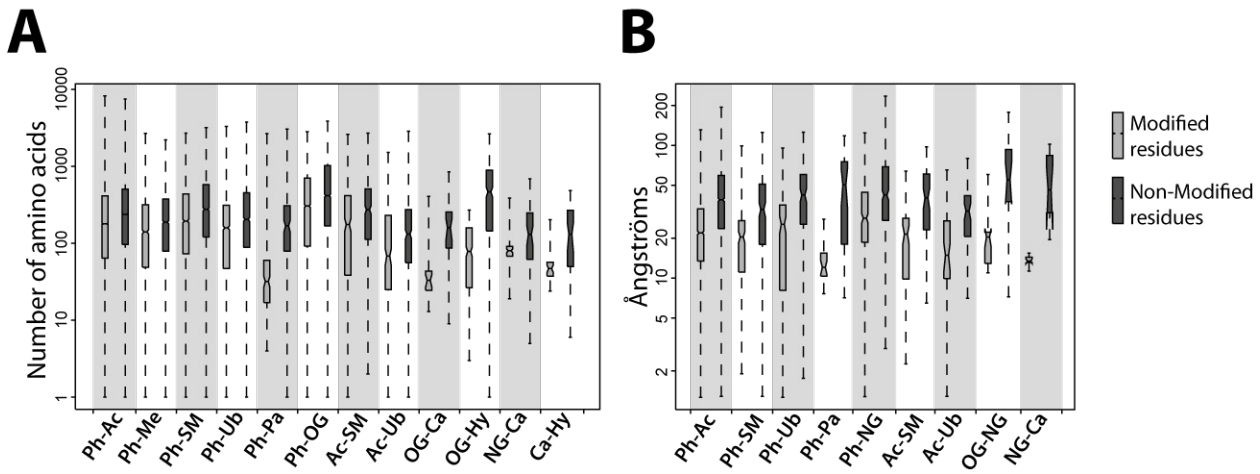
Supplementary Figure 11. Statistics on the global network of co-evolving PTM types. A, We tested the robustness of the edges of the global network of co-evolving PTM types. Thus, for a certain percentage of co-evolving residue hypothetically wrongly assigned (x edge) the y edge represents the percentage of edges that are maintained in the global network, for instance if 10% of the associations are wrongly assigned the global networks would still have 45 of the 47 edges (including self-connections of PTMs) that represent the 96% of the links. Although not shown in the global network of co-evolving PTM types (Figure 3 in the main manuscript), all PTM types (but C-linked glycosylation) presented a significant rate of self-co-evolution, a few statistics including self-co-evolution PTM types are shown in **(B)** and **(C)**. **(B)** Fraction of proteins with a particular PTM type (x edge) that have at least one modified residue co-evolving with another residue of the same kind of modification or with any other type. Phosphorylation, N-linked glycosylation and carboxylation are found to be the most self-co-regulated PTM types as (contrarily to the rest) the fraction of proteins with at least two co-evolving phosphorylations, two N-linked glycosylations or two carboxylations is larger than the fraction of proteins with different modifications co-evolving. **(C)** Average of co-evolving partners for a residue modified with a particular PTM type (x edge) divided into self-PTM type associations (with the same type of modification) and co-evolving with any other type of PTM. For instance, a phosphorylated amino acid co-evolves with 1.1 other modified residues on average, from them only 0.1 is modified by any other PTM type. In opposition to this, nitrosylated residues co-evolve in average with almost 7 other residues, most of them being modified by any other PTM type.



Supplementary Figure 12. Enrichment analysis in protein-protein interactions networks. We first compared de distribution of three topological network parameters (betweenness, connectivity and clustering coefficient) within the human interactome of the set of proteins of each of the pairs with co-evolving PTM types against the distribution of the same parameters of the proteins with the same type of PTMs but not found co-evolving (left columns) and against a set of random proteins (right columns), comparisons marked as “int”. We also calculated the minimal connected network (MCN) of the set of proteins with a particular pair of co-evolving PTM types allowing one external protein to connect any protein in the list, and compared its topological parameters against the distribution of the same parameters of the proteins with the same type of PTMs but not found co-evolving (left columns) and against a set of random proteins (right columns), comparisons marked as “MCN”. All p-values are corrected by FDR and in red we highlight the ones below 0.05. PTM types pairs with higher connectivity also highlighted in red.



Supplementary Figure 13. Preferred functions of co-evolving PTM types. We calculated the preferred general functions of the proteins with each of the co-evolving pairs of PTM types. Pies in the nodes represent the percentage of proteins with that particular function annotated.



Supplementary Figure 14. Comparison of distance between modified co-evolving residues and random sets of non-modified residues. Residues of several co-evolving PTM types are found to be close in sequence **(A)** or structure **(B)** compared to equivalent, but non-modified residues in the same proteins. All PTM types pairs shown in A and B have modified sites significantly closer than control sets. As our reference set is made of random proteins we repeated the comparison 100 times (as bootstrap), we show in here those comparisons that were found significant (adjusted p-value by FDR < 0.05) 100 times. Nuclear and cytosolic PTM type pairs like those in proteasome degradation seem to be frequently interacting physically as they are found in close distance either in sequence, in structure or in both. Also residues with PTM type co-evolution associated to membrane-related or secreted proteins tend to be close in space to perform the interplay, for instance O-linked glycosylation and phosphorylation that it are mainly reported to work as competitive switch for the same S/T amino acid; or carboxylated and hydroxylated residues known to co-regulate in coagulation factors and vitamin k-dependent proteins.

Supplementary Tables

Species (ncbi taxon id)	**Subset** (non-modified residues in the proteins having a particular PTM type)	**Region with higher mean**	**Adjusted p-value**
Mus musculus (10090)	Phosphorylated proteins	Ordered regions	< 0.0001
Mus musculus (10090)	Acetylated proteins	Ordered regions	8.29 e-46
Mus musculus (10090)	Methylated proteins	Ordered regions	6.46 e-10
Mus musculus (10090)	O-linked glycosylated proteins	Ordered regions	1.27 e-09
Mus musculus (10090)	N-linked glycosylated proteins	Disordered regions	1.91 e-32
Mus musculus (10090)	Ubiquitinated proteins	Ordered regions	7.34 e-50
Mus musculus (10090)	SUMOylated proteins	Ordered regions	3.72 e-09
Mus musculus (10090)	Carboxylated proteins	Ordered regions	2.14 e-15
Mus musculus (10090)	All modified proteins	Ordered regions	< 0.0001
Rattus norvegicus (10116)	Phosphorylated proteins	Ordered regions	1.68 e-139
Rattus norvegicus (10116)	Acetylated proteins	Ordered regions	4.75 e-06
Rattus norvegicus (10116)	Methylated proteins	Ordered regions	3.23 e-15
Rattus norvegicus (10116)	O-linked glycosylated proteins	Ordered regions	7.68 e-05
Rattus norvegicus (10116)	N-linked glycosylated proteins	Ordered regions	0.0244
Rattus norvegicus (10116)	Ubiquitinated proteins	Ordered regions	0.0430
Rattus norvegicus (10116)	SUMOylated proteins	Disordered regions	0.1749
Rattus norvegicus (10116)	All modified proteins	Ordered regions	6.07 e-167
Saccharomyces cerevisiae (4932)	Phosphorylated proteins	Ordered regions	3.03 e-210
Saccharomyces cerevisiae (4932)	Acetylated proteins	Ordered regions	7.68 e-05
Saccharomyces cerevisiae (4932)	O-linked glycosylated proteins	Disordered regions	0.0036
Saccharomyces cerevisiae (4932)	N-linked glycosylated proteins	Ordered regions	4.25 e-08
Saccharomyces cerevisiae (4932)	Ubiquitinated proteins	Ordered regions	6.75 e-21
Saccharomyces cerevisiae (4932)	SUMOylated proteins	Ordered regions	8.04 e-11
Saccharomyces cerevisiae (4932)	All modified proteins	Ordered regions	2.48 e-241
Caenorhabditis elegans (6239)	Phosphorylated proteins	Ordered regions	4.65 e-57
Caenorhabditis elegans (6239)	Acetylated proteins	Ordered regions	0.4908
Caenorhabditis elegans (6239)	N-linked glycosylated proteins	Disordered regions	0.1051
Caenorhabditis elegans (6239)	All modified proteins	Ordered regions	4.47 e-53
Drosophila melanogaster (7227)	Phosphorylated proteins	Ordered regions	1.27 e-29
Drosophila melanogaster (7227)	N-linked glycosylated proteins	Disordered regions	1.06 e-08
Drosophila melanogaster (7227)	All modified proteins	Ordered regions	2.89 e-24
Gallus gallus (9031)	Phosphorylated proteins	Ordered regions	1.26 e-25
Gallus gallus (9031)	Acetylated proteins	Disordered regions	0.0717
Gallus gallus (9031)	O-linked glycosylated proteins	Ordered regions	0.4293
Gallus gallus (9031)	N-linked glycosylated proteins	Ordered regions	0.0521

Gallus gallus (9031)	All modified proteins	Ordered regions	1.16 e-17
Homo sapiens (9606)	Phosphorylated proteins	Ordered regions	< 0.0001
Homo sapiens (9606)	Acetylated proteins	Ordered regions	< 0.0001
Homo sapiens (9606)	Methylated proteins	Ordered regions	4.88 e-89
Homo sapiens (9606)	O-linked glycosylated proteins	Ordered regions	8.041 e-13
Homo sapiens (9606)	N-linked glycosylated proteins	Ordered regions	2.55 e-11
Homo sapiens (9606)	Ubiquitinated proteins	Ordered regions	3.87 e-71
Homo sapiens (9606)	SUMOylated proteins	Ordered regions	1.34 e-64
Homo sapiens (9606)	palmitoylation	Disordered regions	2.61 e-05
Homo sapiens (9606)	sulfation	Disordered regions	0.2713
Homo sapiens (9606)	nitrosylation	Ordered regions	0.1882
Homo sapiens (9606)	carboxylated proteins	Ordered regions	2.73 e-26
Homo sapiens (9606)	All modified proteins	Ordered regions	< 0.0001
Bos taurus (9913)	Phosphorylated proteins	Ordered regions	6.37 e-28
Bos taurus (9913)	Acetylated proteins	Ordered regions	0.1321
Bos taurus (9913)	O-linked glycosylated proteins	Ordered regions	0.0967
Bos taurus (9913)	N-linked glycosylated proteins	Ordered regions	0.2684
Bos taurus (9913)	palmitoylation	Disordered regions	0.0310
Bos taurus (9913)	sulfation	Ordered regions	0.6432
Bos taurus (9913)	carboxylated proteins	Ordered regions	7.12 e-29
Bos taurus (9913)	All modified proteins	Ordered regions	7.73 e-77

Supplementary Table 1. Statistical analysis on the conservation of ordered and disordered protein regions. Per each of the species and PTM types included in the analysis (13 PTM types and 8 eukaryotes) we extracted the non-modified residues of the same amino acids that the PTM type can modify, calculate their RCS and performed an statistical analysis to test the differential conservation between ordered and disordered regions. We used the non-parametric Kolmogorov-Smirnov test to asses the statistical significance of the differences of the RCS distributions of each set and adjusted the p-values by False Discovery Rate. The rows in grey show the evaluation of all the modified proteins in the dataset per each species. Although there are a few cases where disordered regions show more conservation that ordered regions in particular sets formed by the proteins modified by a particular PTM type, the evaluation of all proteins together always present ordered regions more conserved than disordered regions.

Species	**PTM type**	**Protein region**	**Type of residues (modified/non-modified) with highest mean**	**Adjusted p-value**
Mus musculus (10090)	Phosphorylation	Ordered regions	Modified residues	< 0.0001
Mus musculus (10090)	Phosphorylation	Disordered regions	Modified residues	< 0.0001
Mus musculus (10090)	Acetylation	Ordered regions	Modified residues	3.56 e-49
Mus musculus (10090)	Acetylation	Disordered regions	Modified residues	4.38 e-73
Mus musculus (10090)	Methylation	Ordered regions	Modified residues	0.0054
Mus musculus (10090)	Methylation	Disordered regions	Modified residues	1.17 e-08

Mus musculus (10090)	O-linked glycosylation	Ordered regions	Modified residues	0.0796
Mus musculus (10090)	O-linked glycosylation	Disordered regions	Modified residues	1.45 e-27
Mus musculus (10090)	N-linked glycosylation	Ordered regions	Modified residues	1.31 e-191
Mus musculus (10090)	N-linked glycosylation	Disordered regions	Modified residues	< 0.0001
Mus musculus (10090)	Ubiquitination	Ordered regions	Modified residues	6.31 e-08
Mus musculus (10090)	Ubiquitination	Disordered regions	Modified residues	1.24 e-09
Mus musculus (10090)	SUMOylation	Disordered regions	Modified residues	2.63 e-08
Mus musculus (10090)	Carboxylation	Ordered regions	Modified residues	3.42 e-06
Mus musculus (10090)	Hydroxylation	Disordered regions	Modified residues	0.0211
Rattus norvegicus (10116)	Phosphorylation	Ordered regions	Modified residues	4.88 e-49
Rattus norvegicus (10116)	Phosphorylation	Disordered regions	Modified residues	3.29 e-259
Rattus norvegicus (10116)	Acetylation	Ordered regions	Modified residues	0.0082
Rattus norvegicus (10116)	Acetylation	Disordered regions	Modified residues	0.0003
Rattus norvegicus (10116)	Methylation	Ordered regions	Modified residues	0.0035
Rattus norvegicus (10116)	Methylation	Disordered regions	Modified residues	2.57 e-05
Rattus norvegicus (10116)	O-linked glycosylation	Disordered regions	Modified residues	1.02 e-08
Rattus norvegicus (10116)	N-linked glycosylation	Ordered regions	Modified residues	4.31 e-06
Rattus norvegicus (10116)	N-linked glycosylation	Disordered regions	Modified residues	1.16 e-13
Rattus norvegicus (10116)	Hydroxylation	Disordered regions	Modified residues	0.0035
Saccharomyces cerevisiae (4932)	Phosphorylation	Ordered regions	Modified residues	1.09 e-103
Saccharomyces cerevisiae (4932)	Phosphorylation	Disordered regions	Modified residues	1.47 e-315
Saccharomyces cerevisiae (4932)	Acetylation	Ordered regions	Modified residues	5.94 e-06
Saccharomyces cerevisiae (4932)	Acetylation	Disordered regions	Modified residues	8.81 e-07
Saccharomyces cerevisiae (4932)	O-linked glycosylation	Disordered regions	Modified residues	0.0001
Saccharomyces cerevisiae (4932)	N-linked glycosylation	Ordered regions	Modified residues	4.67 e-15
Saccharomyces cerevisiae (4932)	N-linked glycosylation	Disordered regions	Modified residues	7.81 e-26
Saccharomyces cerevisiae (4932)	Ubiquitination	Ordered regions	Modified residues	0.0064
Saccharomyces cerevisiae (4932)	Ubiquitination	Disordered regions	Modified residues	4.56 e-06
Caenorhabditis elegans (6239)	Phosphorylation	Ordered regions	Modified residues	2.74 e-05
Caenorhabditis elegans (6239)	Phosphorylation	Disordered regions	Modified residues	1.08 e-26
Caenorhabditis elegans (6239)	N-linked glycosylation	Ordered regions	Modified residues	0.0535
Caenorhabditis elegans (6239)	N-linked glycosylation	Disordered regions	Modified residues	0.0411
Drosophila melanogaster (7227)	Phosphorylation	Ordered regions	Modified residues	8.52 e-09
Drosophila melanogaster (7227)	Phosphorylation	Disordered regions	Modified residues	2.44 e-86
Drosophila melanogaster (7227)	N-linked glycosylation	Ordered regions	Modified residues	5.15 e-05
Drosophila melanogaster (7227)	N-linked glycosylation	Disordered regions	Modified residues	2.75 e-17
Gallus gallus (9031)	Phosphorylation	Ordered regions	Modified residues	6.16 e-05
Gallus gallus (9031)	Phosphorylation	Disordered regions	Modified residues	6.10 e-18
Gallus gallus (9031)	Acetylation	Disordered regions	Modified residues	0.0211

Gallus gallus (9031)	N-linked glycosylation	Disordered regions	Modified residues	0.0255
Homo sapiens (9606)	Phosphorylation	Ordered regions	Modified residues	< 0.0001
Homo sapiens (9606)	Phosphorylation	Disordered regions	Modified residues	0< 0.0001
Homo sapiens (9606)	Acetylation	Ordered regions	Modified residues	< 0.0001
Homo sapiens (9606)	Acetylation	Disordered regions	Modified residues	< 0.0001
Homo sapiens (9606)	Methylation	Ordered regions	Modified residues	1.04 e-10
Homo sapiens (9606)	Methylation	Disordered regions	Modified residues	5.93 e-34
Homo sapiens (9606)	O-linked glycosylation	Ordered regions	Modified residues	2.40 e-09
Homo sapiens (9606)	O-linked glycosylation	Disordered regions	Modified residues	4.48 e-68
Homo sapiens (9606)	N-linked glycosylation	Ordered regions	Modified residues	2.58 e-162
Homo sapiens (9606)	N-linked glycosylation	Disordered regions	Modified residues	< 0.0001
Homo sapiens (9606)	C-linked glycosylation	Disordered regions	Modified residues	1.68 e-12
Homo sapiens (9606)	Ubiquitination	Ordered regions	Modified residues	6.28 e-23
Homo sapiens (9606)	Ubiquitination	Disordered regions	Modified residues	1.86 e-40
Homo sapiens (9606)	SUMOylation	Ordered regions	Modified residues	1.17 e-17
Homo sapiens (9606)	SUMOylation	Disordered regions	Modified residues	3.69 e-53
Homo sapiens (9606)	Palmitoylation	Ordered regions	Modified residues	0.0006
Homo sapiens (9606)	Palmitoylation	Disordered regions	Modified residues	0.0567
Homo sapiens (9606)	Sulfation	Disordered regions	Modified residues	1.20 e-07
Homo sapiens (9606)	Nitrosylation	Disordered regions	Modified residues	3.16 e-05
Homo sapiens (9606)	Carboxylation	Ordered regions	Modified residues	8.76 e-16
Homo sapiens (9606)	Carboxylation	Disordered regions	Modified residues	8.01 e-06
Homo sapiens (9606)	Hydroxylation	Disordered regions	Modified residues	3.85 e-10
Bos taurus (9913)	Phosphorylation	Ordered regions	Modified residues	1.63 e-05
Bos taurus (9913)	Phosphorylation	Disordered regions	Modified residues	1.03 e-34
Bos taurus (9913)	Acetylation	Ordered regions	Modified residues	0.0390
Bos taurus (9913)	Acetylation	Disordered regions	Modified residues	0.0166
Bos taurus (9913)	O-linked glycosylation	Disordered regions	Modified residues	0.0007
Bos taurus (9913)	N-linked glycosylation	Ordered regions	Modified residues	0.0002
Bos taurus (9913)	N-linked glycosylation	Disordered regions	Modified residues	5.41 e-15
Bos taurus (9913)	Carboxylation	Ordered regions	Modified residues	6.23 e-10
Bos taurus (9913)	Carboxylation	Disordered regions	Modified residues	1.42 e-06

Supplementary Table 2. Statistical analysis on the conservation of modified versus non-modified residues. Per each of the species and PTM types included in the analysis (13 PTM types and 8 eukaryotes) we extracted the modified and non-modified residues (the latter from the same amino acids that the modified residues), calculate their RCS and performed an statistical analysis to test the differential conservation between modified and non-modified residues in both ordered and disordered protein regions independently. We used the non-parametric Kolmogorov-Smirnov test to asses the statistical significance of the differences of the RCS distributions of each set and adjusted the p-values by False Discovery Rate. In all the comparisons we got that modified residues are generally more conserved than non-modified residues.

PTM type	Species	Number of residues with rRCS > 95	Number of residues with rRCS < 95	Percentage of residues about 95 rRCS
Acetylation	*Homo sapiens* (9606)	1438	4099	25.97
Acetylation	*Bos taurus* (9913)	7	28	20
Acetylation	*Mus musculus* (10090)	205	658	23.75
Acetylation	*Saccharomyces cerevisiae* (4932)	9	69	11.54
Acetylation	*Rattus norvegicus* (10116)	8	34	19.05
Acetylation	*Gallus gallus* (9031)	7	7	50
Acetylation	*Drosophila melanogaster* (7227)	0	1	0
Acetylation	*Caenorhabditis elegans* (6239)	1	2	33.33
Carboxylation	*Mus musculus* (10090)	10	10	50
Carboxylation	*Homo sapiens* (9606)	43	23	65.15
Carboxylation	*Bos taurus* (9913)	33	13	71.74
C-linked glycosylation	*Homo sapiens* (9606)	16	24	40
C-linked glycosylation	*Mus musculus* (10090)	0	1	0
Hydroxylation	*Homo sapiens* (9606)	36	94	27.69
Hydroxylation	*Mus musculus* (10090)	1	14	6.67
Hydroxylation	*Bos taurus* (9913)	4	4	50
Hydroxylation	*Rattus norvegicus* (10116)	4	13	23.51
Methylation	*Homo sapiens* (9606)	80	268	22.99
Methylation	*Rattus norvegicus* (10116)	11	37	22.92
Methylation	*Mus musculus* (10090)	13	64	16.88
Methylation	*Saccharomyces cerevisiae* (4932)	4	4	50
Methylation	*Gallus gallus* (9031)	1	4	20
Methylation	*Bos taurus* (9913)	0	2	0
Methylation	*Drosophila melanogaster* (7227)	0	1	0
Nitrosylation	*Homo sapiens* (9606)	2	22	8.33
N-linked glycosylation	*Mus musculus* (10090)	753	2956	20.30
N-linked glycosylation	*Homo sapiens* (9606)	633	2397	20.89
N-linked glycosylation	*Saccharomyces cerevisiae* (4932)	46	383	10.72
N-linked glycosylation	*Rattus norvegicus* (10116)	34	114	22.97
N-linked glycosylation	*Bos taurus* (9913)	27	80	25.23
N-linked glycosylation	*Gallus gallus* (9031)	5	12	29.41
N-linked glycosylation	*Caenorhabditis elegans* (6239)	7	63	10
N-linked glycosylation	*Drosophila melanogaster* (7227)	27	195	12.16
O-linked glycosylation	*Homo sapiens* (9606)	80	443	15.3
O-linked glycosylation	*Mus musculus* (10090)	23	179	11.39
O-linked glycosylation	*Rattus norvegicus* (10116)	10	85	10.53

O-linked glycosylation	*Bos taurus* (9913)	9	28	24.32
O-linked glycosylation	*Saccharomyces cerevisiae* (4932)	10	78	11.36
O-linked glycosylation	*Gallus gallus* (9031)	0	5	0
Palmitoylation	*Bos taurus* (9913)	2	4	33.33
Palmitoylation	*Homo sapiens* (9606)	8	24	25
Palmitoylation	*Rattus norvegicus* (10116)	1	2	33.33
Palmitoylation	*Mus musculus* (10090)	0	5	0
Phosphorylation	*Saccharomyces cerevisiae* (4932)	929	8213	10.16
Phosphorylation	*Gallus gallus* (9031)	40	174	18.69
Phosphorylation	*Homo sapiens* (9606)	8467	42989	16.45
Phosphorylation	*Drosophila melanogaste*r (7227)	191	1463	11.55
Phosphorylation	*Mus musculus* (10090)	3074	15574	16.48
Phosphorylation	*Rattus norvegicus* (10116)	399	1163	25.54
Phosphorylation	*Bos taurus* (9913)	72	251	22.29
Phosphorylation	*Caenorhabditis elegans* (6239)	131	772	14.51
Sulfation	*Homo sapiens* (9606)	10	34	22.73
Sulfation	*Mus musculus* (10090)	0	1	0
Sulfation	*Bos taurus* (9913)	0	10	0
SUMOylation	*Homo sapiens* (9606)	66	308	17.65
SUMOylation	*Bos taurus* (9913)	1	0	100
SUMOylation	*Mus musculus* (10090)	11	47	18.97
SUMOylation	*Rattus norvegicus* (10116)	0	3	0
SUMOylation	*Saccharomyces cerevisiae* (4932)	1	13	7.14
Ubiquitination	*Homo sapiens* (9606)	108	295	26.8
Ubiquitination	*Mus musculus* (10090)	48	72	40
Ubiquitination	*Saccharomyces cerevisiae* (4932)	18	71	20.22
Ubiquitination	*Rattus norvegicus* (10116)	3	14	17.65
Ubiquitination	*Gallus gallus* (9031)	1	0	100

Supplementary Table 3. Evaluation of the modified residues overall conservation within the protein. Per every PTM type and species (13 PTM types and 8 eukaryotes) we extracted the number of modified residues that have a relative RCS (rRCS) above 95, i.e. those modified residues that are more conserved than the 95% of the non-modified residues (same amino acids and protein regions, ordered or disordered regions) as a non-parametric approach of measuring the conservation status of the modified residues taking into account the global speed of evolution of the proteins.

	Ph	**SM**	**Me**	**Ub**	**Ac**	**Ni**	**Ca**	**Hy**	**Su**	**NG**	**CG**	**OG**	**Pa**
Ph	189,717												
SM	309	1,418											
Me	1,333	70	55,676										
Ub	1,940	307	245	8,384									

Ac	1,820	138	2,764	348	20,729								
Ni	155	8	4	31	22	1,266							
Ca	65	0	26	3	14	0	2,359						
Hy	183	4	671	64	386	10	45	21,042					
Su	146	2	104	4	126	0	9	123	3,963				
NG	138	0	5	6	3	0	2	1	16	2,768			
CG	1	0	0	0	0	0	0	0	0	1	2		
OG	44	0	1	1	1	1	1	6	5	96	1	469	
Pa	222	9	19	24	22	8	1	3	2	5	0	1	1,445

Supplementary Table 4. Number of Medline abstracts retrieved by a search of co-occurring pairs of terms from a total of 13 types of PTM types. PTM types are abbreviated as Ph (phosphorylation), SM (SUMOylation), Me (methylation), Ub (ubiquitination), Ac (acetylation), Ni (nitrosylation), Ca (carboxylation), Hy (hydroxylation), Su (sulfation), NG (N-linked glycosylation), CG (C-linked glycosylation), OG (O-linked glycosylation and Pa (palmitoylation).

PTM1 - PTM2	PTM type inside the domain	Domain	Subsets (p-values)	Domain function	PTM1-domain regulation	PTM2-domain regulation	PTMs – domain association status
Ph – Ac	Ph	ABC transporter, transmembrane domain (IPR001140)	Whole(0.0088); Membrane(0.0265)	Transmembrane transport. Export or import of a wide variety of substrates ranging from small ions to macromolecules.	ABC transporters are phosphorylated in eukaryotes (Higgins, 1992) regulating its activity (Tsybovsky *et al*, 2011; Alzamora *et al*, 2011).	No evidences found	**Known for PTM1, unknown regulation by PTM2.**
Ph – Ac	Ph	Endonuclease/exonuclease/phosphatase (IPR005135)	Whole(0.0044); Cytoplasm(0.0044)	Domain found in magnesium dependent endonucleases and phosphatases involved in intracellular signalling	Phosphorylation is proved to inhibit endonuclease activity in Xenopus chAPE1 (Borjigin *et al*, 2010).	Acetylation seems also to regulate endonuclease activity as in APE1 (Fantini *et al*, 2010).	**Known for both PTM1 and PTM2 independently.**
Ph – Ac	Ph	High mobility group, HMG1/HMG2 (IPR000910)	Whole(<0.0001); Transcription (<0.0001); Chromosome(0.0429); Nucleus(<0.0001)	Found in a group of non-histone proteins associated to chromatin involve in DNA binding.	Phosphorylation has been described as a source of regulation for several HMG proteins such as HMG1 (Kimura *et al*, 1985) or HMGA2 (Di Agostino *et al*, 2004).	Acetylation is described also as a source for regulation for HMGA1a and HMGA2b proteins (Jiang & Wang, 2006).	Acetylation and phosphorylation of the HMGA1a and HMGA1b proteins are described to regulate interactions with other proteins and with DNA as a mechanism for gene expression regulation (Omary *et al*, 2006). **Known for both PTM1 and PTM2 as a crosstalk**

							regulation.
Ph – Ac	Ph	Intermediate filament head, DNA-binding domain (IPR006821)	Whole(0.0483)	Intermediate filaments are major components of cytoskeleton.	Phosphorylation is essential for the regulation of the head of the intermediate filaments modulating the subcellular localization or the binding activity of the proteins (Omary *et al*, 2006; Takemura *et al*, 2002).	Acetylation of intermediate filaments seems to be associated with their stabilization (Leech *et al*, 2008).	**Known for both PTM1 and PTM2 independently.**
Ph – Ac	Ph	RecF/RecN/SMC (IPR003395)	Whole(0.0163); Nucleus(0.0234); Chromosome(0.0234)	Chromosomal condensation, sister-chromatid cohesion, recombination, DNA repair and silencing of gene expression.	Phosphorylation is found to regulate mobilization of the cohesin complex after DNA damage (Bauerschmidt *et al*, 2011).	Acetylation is found to regulate the replication fork processivity by means of regulating the binding activity of SMC3, a central protein of the cohesin complex (Terret *et al*, 2009).	There are evidences of crosstalk of phosphorylation enhancing acetylation in cohesin proteins (Heidinger-Pauli *et al*, 2009). **Known for both PTM1 and PTM2 as a crosstalk regulation.**
Ph – Ac	Ph	RNA recognition motif domain (IPR000504)	RNA processing and modification (0.0358)	binds single-stranded RNA.	Although proteins with RRM are known to be regulated by means of phosphorylation of the RS domain, no evidences of direct regulation of RRM domain were found (Hagopian *et al*, 2008).	Protein SRSF2 is acetylated in the RNA recognition motif which leads to its degradation (Edmond *et al*, 2011).	In SRSF2 protein acetylation inhibits phosphorylation as a n interplay that regulates protein accumulation (Bignone *et al*, 2007). **Known for both PTM1 and PTM2 as a crosstalk regulation** (Regulation of the RRM domain).
Ph – Ac	Ph	Spectrin repeat (IPR002017)	Whole(0.0159); Cytoplasm(0.0412)	Domain present in cytoskeletal proteins and involve in protein-protein binding regulation.	Phosphorylation is described to regulate several spectrin functions such as neutritogenesis (Bignone *et al*, 2007) or the stability of erythrocytes membrane (Perrotta *et al*, 2001).	The role of acetylation in spectrin proteins is still unknown.	**Known for PTM1, unknown regulation by PTM2.**
Ph – Ac	Ph	Tensin phosphatase, C2 domain (IPR014020)	Whole(0.0044); Signal transduction mechanism(0.009); Cytoplasm(0.0342)	The domain is formed by a lipid phosphatase domain and a Ca2+-dependent membrane-targeting C2-like domain.	Phosphorylation has an important role on PTEN activation and stability (Vazquez *et al*, 2000) and it has been implicated in oncogenesis (Miller *et al*, 2002).	Acetylation of PTEN is found to inhibit its lipid phosphatase activity (Okumura *et al*, 2006; Ikenoue *et al*, 2008).	**Known for both PTM1 and PTM2 independently.**
Ph – Ac	Ph	Treacher Collins syndrome, treacle (IPR003993)	Whole(0.0044); Trancription(0.0109); Nucleus(0.0087)	Treacle (TCOF1) is a protein involved ribosomal DNA gene transcription, mutations in this protein cause the Treacher Collins Syndrome.	These motifs are shared with nucleolar trafficking proteins in other species and are predicted to be highly phosphorylated by casein kinase that have been suggested to be essential for binding nuclear localization as it happen in rat homologous Nopp140 (Wise *et al*, 1997).	There are no evidences of the role of acetylation in TCOF1 in the literature.	**Known for PTM1, unknown regulation by PTM2.**
Ph – Ac	Ph	ATPase,	Whole(<0.00	This domain is	Phosphorylation of the	GCN5-mediated	Acetylation and

		AAA-type, core(IPR003959)	01), Nucelus(<0.001)	present in a wide variety of proteins involved in cell-cycle regulation, protein proteolysis and disaggregation, organelle biogenesis and intracellular transport. The domain is responsible for ATP binding and hydrolysis.	protein CDC6 by Cyclin-dependent kinases regulates its subcellular location (Petersen *et al*, 1999).	acetylation of CDC6 seems to be essential for the relocalization to cytoplasm (Paolinelli *et al*, 2009).	phosphorylation of CDC6 are both required for its cytoplasmic localization during S phase regulating its stability (Petersen *et al*, 1999). **Known for both PTM1 and PTM2 as a crosstalk regulation.**
Ph – Ac	Ph	Calponin homology domain(IPR001715)	Whole(<0.0001), cytoskeleton(<0.0001), Cytoplasm(<0.0001), Membrane(<0.0001), Cell membrane (<0.0001)	Belong to a family of actin-binding domains found in both cytoskeletal proteins and signal transduction proteins.	Phosphorylation of protein Calponin by protein kinase C and Ca2+/calmodulin-dependent kinase II is found to inhibit its binding to actin (Winder *et al*, 1993).	No evidences for the role of acetylation in calponin containing proteins.	**Known for PTM1, unknown regulation by PTM2.**
Ph- Ac	Ph	Cell surface receptor IPT/TIG (IPR002909)	Whole(<0.0001), Transcription (0.0001), Nucleus(0.0001)	Immunoglobulin like domain found in cell surface receptors and transcription factors. It is involved in protein-protein interactions.	Phosphorylation has been proved to play an important role in RelA protein activity, a subunit of the NF-Kappa B complex (Anrather, 1999).	Acetylation of RelA regulates its DNA binding activity (Chen *et al*, 2005).	It is clear that phosphorylation within the Cell surface receptor domain (S276) regulates acetylation activity (Chen *et al*, 2005). **Known for both PTM1 and PTM2 as a crosstalk regulation.**
Ph – Ac	Ph	Conexin, N-terminal (IPR013092)	Whole(0.0004); Signal transduction mechanism(0.0008 Cell membrane(0.0006; Gap junction(0.0006); Membrane(0.00026; Cell junction(0.0006)	N-terminal domain of the conexin a family of proteins. They form channels in cell membrane that play a role in cell-cell contact and molecules transport. The N-terminal is located inside the cytoplasm.	Phosphorylation has been described to play an essential role in conexin proteins (including Cx43) regulating their trafficking, assembly/disassembly and degradation (Lampe & Lau, 2004; Laird, 2005).	Acetylation is involved in altering the gating and permeability properties of connexins (Shearer *et al*, 2008).	**Known for both PTM1 and PTM2 independently.**
Ph – Ac	Ph	Conexin (IPR000500)	Whole(0.0099); Signal transduction mechanism(0.0258); Cell membrane(0.0202); Gap junction(0.0202); Membrane(0.0202); Cell junction(0.0202)	Found in the same family of protein as the Conexin N-terminal domain (IPR013092).	Same as for Conexin, N-terminal domain (IPR013092).	Same as for Conexin, N-terminal domain (IPR013092).	**Known for both PTM1 and PTM2 independently.**
Ph – NG	Ph	Ankyrin repeat (IPR002110)	Whole(0.0063), Signaling transduction	The Ankyrin domain is a very wide	Phosphorylation by CK2 kinase decreases the affinity of Ankyrin for	N-linked glycosylation is involved in the	**Known for both PTM1 and PTM2 independently.**

			mechanism(0.0038), Nucleus(0.0092), Membrane(0.047)	spread domain with a role in the regulation of protein-protein interaction.	spectrin (Lu *et al*, 1985; Ghosh *et al*, 2002).	maturation process of some Ankyrin repeat contining proteins such as Trpv4 and trpv6 (Arniges *et al*, 2006; Hirnet *et al*, 2003) and also to play some role in Notch signaling pathway although O-linked glycosylation has been more studied in this sense (Haines & Irvine, 2003).	
Ph – NG	Ph	ATPase-like, ATP-binding domain (IPR003594)	Whole(<0.0001)	ATP binding domain present in several proteins with a wide range of functionalities.	Phosphorylation of Hsp90beta protein is found to regulate the release of the chaperone from the target protein (Zhao *et al*, 2001).	Although chaperons and N-linked glycosylation play both a role in others proteins folding, little is known about its own regulation by glycosylation apart from that the accumulation of the human alpha-synuclein (α-Syn) protein caused differential phosphorylation and glycosylation of several proteins including Hsp90.	**Known for PTM1, unknown regulation by PTM2.** Preliminary evidences of co-regulation though.
Ph – NG	Ph	Integrin beta subunit, N-terminal (IPR002369)	Whole(<0.0001); Extracellular structures(<0.0001); Membrane(<0.0001)	Integrin domain is involved in cell-cell adhesion as well to extracellular matrix.	Phosphorylation of integrin tails seems to regulate the biding affinity to other proteins and complexes such as talin (Anthis & Campbell, 2011).	Integrins are a major carriers of *N*-glycans, their ability to form functional dimers depends on the N-linked oligosaccharides attached to them affecting receptor-ligand binding and therefore cell migration (Janik *et al*, 2010).	**Known for both PTM1 and PTM2 independently.**
Ph – NG	Ph	Protein-tyrosine phsophatease, receptor/non-receptor type (IPR000242)	Whole(0.0005); Signal transduction mechanism(0.0003)	Tyrosine-specific protein phosphatases remove phosphate groups attached to a tyrosine residues.	Tyrosine phosphatases are found to be activated by phosphorylation (den Hertog *et al*, 1995; *Dadke et al, 2001)*.	Although N-linked glycosylation it is found to be present in phosphatases (Oon *et al*, 1993) little is known about specific regulatory mechanisms.	**Known for PTM1, unknown regulation by PTM2.**
Ph – NG	Ph	DMAP1-binding (IPR010506)	Whole(0.0088)	This domain binds DMAP1, a protein involved in transcription repression and activation.	The protein we have annotated is the yeast YOR093C. No information about the role of phosphorylation in its functionality.	The protein we have annotated is the yeast YOR093C. No information about the role of N-linked glycosylation in its functionality.	**Unknown regulation by both of the PTM types.**

Ph – NG	NG	Immunoglobulin C1-set (IPR003597)	Whole(0.0014)	C1-set domains are found almost exclusively in molecules involved in the immune system.	The protein Sirp-alpha binds SHP-2 in its phosphorylated form through SH2 interactions and acts as its substrate (Kharitonenkov *et al*, 1997).	N-linked glycosylation is found to be important for the binding specificity of Sirp-alpha (van den Nieuwenhof *et al*, 2001).	**Known for both PTM1 and PTM2 independently.**
Ph – NG	NG	Von Willebrand factor, type A (IPR002035)	Whole(0.0368); Membrane(0.0293)	Domain found in glycoproteins expressed in blood plasma and responsible for protein-protein binding interactions.	Phosphorylation activates integrin beta and regulates its binding activity (Anthis *et al*, 2009).	N-linked glycosylation has been found as a major source of regulation of some integrins functionality (Sato *et al*, 2009).	**Known for both PTM1 and PTM2 independently.**
Ph – Ub	Ph	SET domain (IPR001214)	Chromosome(0.0011)	Domain present in a large family of proteins which include histone methyltransferases (SETDB1 in our dataset). The domain is involved in protein-protein interactions.	Nemo-like kinase phosphorylates the histone methyltransferase SETDB1 at the SET domain leading to the formation of a co-repressor complex that inactivates PPAR-gamma function through histone H3-K9 methylation (Takada *et al*, 2007).	Ubiquitination has also been suggested to regulate the activity of methyltransferases and heterochromatin formation (Jia *et al*, 2005).	**Known for both PTM1 and PTM2 independently.**
Ph – Me	Ph	Cell surface receptor IPT/TIG (IPR002909)	Whole(<0.0003), Transcription (0.0019), Cytoplasm(0.0001)	Immunoglobulin like domain found in cell surface receptors and transcription factors. It is involved in protein-protein interactions.	Phosphorylation has been proved to play an important role in RelA protein activity, a subunit of the NF-Kappa B complex (Anrather, 1999).	NF-Kappa B is found to be negatively regulated by means of the methylation of RelA subunit (Yang *et al*, 2009).	Phosphorylation inhibits methylation of RelA and consequently activates NF-Kappa B (Levy *et al*, 2011). **Known for both PTM1 and PTM2 as a crosstalk regulation.**
Ph – Me	Ph	RecF/RecN/SMC (IPR003395)	Whole(<0.0001); Cell cycle control, cell division, chromosome partitioning(<0.0001); Nucleus(<0.0001); Chromosome(<0.0001)	Domain found in SMC proteins (part of cohesin complex) with a chromosomal maintenance role and in RecF and RecN, involved in DNA metabolism and recombination.	Phosphorylation is found to regulate mobilization of the cohesin complex after DNA damage (Bauerschmidt *et al*, 2011).	No details on regulation of cohesin proteins by methylation were found.	**Known for PTM1, unknown regulation by PTM2.**
Ph – Hy	Ph	Proteinase inhibitor I2, Kunitz metazoa (IPR002223)	Whole(0.0004)	Domain involve in serine-type endopeptidase inhibitor activity. We found that this domain might be regulated by phosphorylation and hydroxylation in collagen 6 protein.	No mechanisms of regulation of collagen 6 by means of phosphorylation were found.	Hydroxyproline is a major component of collagen and has been found to be essential for the binding of collagen to platelet glycoprotein VI and to cells by $\alpha_1\beta_1$ (Perret *et al*, 2003).	**Known for PTM2, unknown regulation by PTM1.**
Ph – Ca	Ph	EGF (IPR006209); EGF-like calcium	Whole(<0.0001, <0.0001, <0.0001); Secreted(<0.	Epidermal growth factor (EGF) domain is found in the	Phosphorylation seems to play an important role in the stability of coagulation factor IX as	Carboxylation is performed in the glutamate residues within	**Known for both PTM1 and PTM2 independently.**

		binding (IPR001881) ; EGF-type aspartate/asparagine hydroxylation site (IPR000152) ; Epidermal growth factor-like, type 3 (IPR000742)	0001, <0.0001, <0.0001)	extracellular domain of membrane-bound proteins or in proteins known to be secreted.	non-phosphorylated are not found in plasma (Atoda *et al*, 2006).	the Gla domain of coagulation factors forming gamma-carboxyglutamic. The GLA domain is responsible for the high-affinity binding of calcium ions (Price *et al*, 1987).	
Ac – OG	Ac	Zinc finger, PHD-type (IPR001965)	Whole(0.0048); Nucleus(0.0342)	PHD-type Zinc fingers are found in nuclear proteins, they are thought to be involved in chromatin-mediated transcriptional regulation. This result is found in the Nucleosome-remodeling factor subunit BPTF.	In protein BPTF, the PHD finger binds a methylated site in the histone 3. It has been proved that the acetylation status of the protein and specially of the bromo domain (adjacent to the PHD finger) affect this interaction (Ruthenburg *et al*, 2011).	We did not find proposed regulation of BPTF or Zinc finger domain by means of O-linked glycosylation.	**Known for PTM1, unknown regulation by PTM2.**
NG – Hy	NG	Collagen triple helix repeat (IPR008160)	Whole(0.0176)	Collagen repeats are found in structural proteins involved in formation of connective tissue structure.	N-linked glycosylation is found to be important for the extracellular localization of collagen enhancing collagen binding to the plasma membrane (Franzke *et al*, 2006).	Lysine and proline hydroxylation is essential for the stability of collagen outside the cell (Bella *et al*, 1994). Deficiency in proline hydroxylation may cause scurvy.	Although other types of glycosylation has been shown to participate in the stabilization of collagen in a close interplay with hydroxylation, we did not find any evidence of N-linked glycosylation co-regulating to hydroxylation in the literature (Bann & Bächinger, 2000). **Known for both PTM1 and PTM2 independently.**
NG – Ca	NG	EGF (IPR006209) ; Epiderman growth factor-like, type 3 (IPR000742)	Whole(<0.0001, 0.0014); Posttranslational modification, protein turnover, chaperons(0.025, 0.0004); Secreted(<0.0001)	Epidermal growth factor (EGF) domain is found in the extracellular domain of membrane-bound proteins or in proteins known to be secreted. This result is found in Vitamin K-dependent protein Z.	N-linked glycosylation has been described to play an important role in the enzymatic activity, protein folding and stability of the Vitamin K-dependent proteins (Tie *et al*, 2006). Moreover, N-glycosylation within the EGF domains is found to be essential for epitope accessibility and binding capabilities in CD97 (Wobus *et al*, 2004).	Carboxylation is performed in the glutamate residues within the Gla domain of coagulation factors forming gamma-carboxyglutamic. The GLA domain is responsible for the high-affinity binding of calcium ions (Price *et al*, 1987).	It has been experimentally proven that the degree of initial core glycosylation may affect the efficiency of carboxylation in the Vitamin K-dependent protein HPC (McClure *et al*, 1992). **Known for both PTM1 and PTM2 as a crosstalk regulation.**
NG – Ca	NG	Kringle (IPR000001)	Whole(0.0044)	Kringle domains play a role in the binding	N-linked glycosylation are found within the kringle domain in prothrombin (Pradhan *et*	Carboxylation is performed in the glutamate residues within	**Known for both PTM1 and PTM2 as a crosstalk regulation.**

				capabilities of the proteins to membranes, other proteins or phospholipids, and in the regulation of proteolytic activity. This result is found in Prothrombin.	*al*, 2009). he kringle domain and its glycosylation status is critical in determining the metabolic activity of carboxylated prothrombin precursors during processing (Wu *et al*, 1997).	the Gla domain of coagulation factors forming gamma-carboxyglutamic. The GLA domain is responsible for the high-affinity binding of calcium ions (Price *et al*, 1987).	
OG – Ca	OG	EGF (IPR006209) ; Epidermal growth factor-like, type 3 (IPR000742) ; EGF-like calcium binding (IPR001881)	Whole(<0.0001, <0.0001, 0.0038); Secreted(<0.0001, <0.0001, 0.0348)	Epidermal growth factor (EGF) domain is found in the extracellular domain of membrane-bound proteins or in proteins known to be secreted.	O-linked glycosylation has been proved to modify EGF domain in coagulation factors and vitamin K-dependent proteins inhibiting and alters EGF functionality (Shao *et al*, 2002).	Carboxylation is performed in the glutamate residues within the Gla domain of coagulation factors forming gamma-carboxyglutamic. The GLA domain is responsible for the high-affinity binding of calcium ions (Price *et al*, 1987).	**Known for both PTM1 and PTM2 independently.**

Supplementary Table 5. Co-evolving PTM types regulating protein globular domains. We performed and enrichment analysis to extract those Interpro domains that are enriched in a particular pair of co-evolving PTM types when one of the PTM types is inside the domain sequence. As some of the domains were quite similar in functionality (EGF domains) we cluster them as one single group to no over-estimate the findings although they were tested independently. Sets of proteins having a particular pair of co-evolving PTM types were analyzed using standard Fisher exact test and p-value adjustment by FDR. The proteins lists were built for the whole set of proteins with the pair of co-evolving PTM types and for the proteins with the same type of co-evolution and a specific functionality or a particular cellular location. All lists were tested independently for the whole set of Interpro domains, the adjusted p-values are given in brackets. A literature survey was performed to asses the previous knowledge about the regulation of the domain by means of both PTM types within the literature, according to this we classified our predictions as i) *known for both PTM1 and PTM2 as a crosstalk regulation*, ii) *known for both PTM1 and PTM2 independently*, iii) *known for PTM1, unknown regulation by PTM2* or iv) *unknown regulation by both of the PTM types*.

PTM1 – PTM2	**Short linear motif (similar ones are grouped)**	**Proteins (having both PTM types co-evolving an enriched in the motif)**	**Motif in the literature/databases**	**PTM1-motif example of regulation**	**PTM2-motif example of regulation**	***Motif-PTMs knowledge status***
Ph – SM	IK.E [ILV]K.EP IK.EP [IV]K.EP..P IK.EP.D	BACH2_HUMAN CEBPE_HUMAN ELF4_HUMAN GLYC_HUMAN HDAC4_HUMAN HIF1A_HUMAN HSF1_HUMAN IRF2_HUMAN KCNA5_HUMAN KLF3_HUMAN MAML1_HUMAN MBD1_HUMAN MEF2C_HUMAN MITF_MOUSE NFAC2_MOUSE NRIP1_HUMAN OGT1_HUMAN P53_HUMAN PML_HUMAN	Similar to two known motifs: MOD_SUMO: [VILMAFP](K).E LIG_KEPE_1: [VILMFT]K.EP. [DE]	In the protein HSF1 the motif is part of the Phosphorylation-SUMOylation switch motif (Yang & Grégoire, 2006).	Same as the motif.	***Similar to known motif.*** ***Known regulation by both PTM types independently.***

		PPARG_HUMAN PROX1_HUMAN RGPD4_HUMAN RLF_HUMAN RREB1_HUMAN RSBN1_HUMAN SKIL_HUMAN SP100_HUMAN STAT1_HUMAN ZEB1_HUMAN Q6FG41_HUMAN				
Ph – SM	[ST]P..[ST]P	C8AP2_HUMAN CHD8_HUMAN EP300_HUMAN ETV6_HUMAN GATA1_HUMAN HSF1_HUMAN MAFA_HUMAN NCOA2_HUMAN NFAC2_MOUSE NR4A2_HUMAN NRIP1_HUMAN PRGC1_MOUSE RB_HUMAN RGPD4_HUMAN ZN687_HUMAN	Similar to three known motifs: MOD_GSK3_1: ... ([ST])...[ST] LIG_WW_4: ... ([ST])P. (phospho-dependent binding motif) ERK phosphorylation site: [ST]P	No evidence found	In the protein HSF1 the motif is part of the Phosphorylation-SUMOylation switch motif (Yang & Grégoire, 2006).	***Similar to known motif.*** ***Known regulation by one PTM type.***
Ph – SM	KRK KR.R	ARI4A_HUMAN AXIN1_HUMAN BEND3_HUMAN C8AP2_HUMAN CHD8_HUMAN DNMT1_HUMAN DPOD3_HUMAN ELF4_HUMAN ESR1_HUMAN LA_HUMAN MEF2C_HUMAN NCOA2_HUMAN NFAC2_MOUSE P53_HUMAN PROX1_HUMAN PSIP1_HUMAN RREB1_HUMAN RSBN1_HUMAN SAE2_HUMAN SIRT1_HUMAN SP100_HUMAN WDR33_HUMAN ZN462_HUMAN ZN687_HUMAN Q6IBN1_HUMAN	Similar to monopartite NLS: K-K/R-X-K/R	Phosphorylation within an NLS enhances the binding affinity for the isoform importin A5 (Nardozzi *et al*, 2010).	Nuclear localization of the protein Rad52 is pre-requisite for its SUMOylation (Ohuchi *et al*, 2008).	***Similar to known motif.*** ***Known regulation by both PTM types independently.***
NG – OG	[IV].[FH]..E	DAG1_HUMAN ITA2B_HUMAN ITIH4_HUMAN SODE_HUMAN TGBR3_MOUSE THBG_HUMAN	None found	No evidence found	No evidence found	***Predicted new motif.*** ***Not associated to any PTM type.***
NG – OG	FSL	ANT3_HUMAN CSPG5_MOUSE ITA2B_HUMAN TFR1_HUMAN TGBR3_MOUSE TPO_HUMAN	None found	No evidence found	No evidence found	***Predicted new motif.*** ***Not associated to any PTM type.***
Ac – NG	[HK]DE.$ EL$ DEL$	CALR_HUMAN ENPL_HUMAN GLU2B_HUMAN PDIA1_MOUSE RCN1_HUMAN UGGG2_HUMAN	Similar to TRG_ER_KDEL_1: [KRHQSAP][DENQT]EL$	Reversible hyper-acetylation modulates the intracellular and extracellular chaperone function of at shock protein (Hsp90) (Yang *et al*, 2008).	N-linked glycosylation is required for optimal proteolytic activation of membrane-bound transcription factor CREB-H (Chan *et al*, 2010).	***Similar to known motif.*** ***Known regulation by both PTM types independently.***
Ac – Ub	R.R..MF	CTNB1_HUMAN MLL1_HUMAN P53_HUMAN	In the protein p53 the R.R..MF (335-341) is overlapping with the NLS (339-350) motif.	No evidence found	No evidence found	***Predicted new motif.*** ***Not associated to any PTM type.***
Ph – Ni	G.[GS]T	TCP2_HUMAN RL4_HUMAN	Similar to several known motifs that	No evidence found	NMDAR is a calcium channel. It	***Similar to known motif.***

		FAS_HUMAN HIF1A_HUMAN SERA_HUMAN EF1A1_HUMAN HNRL1_HUMAN ANX11_HUMAN	include the phosphodregon motif D(S)G.{2,3} ([ST]), a calcium binding motif (Annexin repeats) and a nucleotide binging motif (overlaps in eEF1A and PGDH3)		is understood that the transient flux of calcium through the NMDAR must be in close proximity to nNOS for efficient Ca2+/CaM binding and NO production (Christopherson *et al*, 1999).	***Known regulation by one PTM.***
Ac – Ni	G.[GS]T	TCP2_HUMAN RL4_HUMAN FAS_HUMAN HIF1A_HUMAN SERA_HUMAN EF1A1_HUMAN HNRL1_HUMAN	The motif is similar to phosphodregon motif D(S)G.{2,3} ([ST]) and a nucleotide binging motif (overlaps in eEF1A and PGDH3)	No evidence found	No evidence found	***Similar to known motif.*** ***Not associated to any of the PTM types.***
Ph – Me	C..M$	LMNA_HUMAN PI2R_HUMAN PP16A_MOUSE PRIC2_HUMAN	Similar to lamin CxxM motif	In the goldfish lamin B3 (GFLB3) post-translational modification of the CaaX box is the most important factor determining nuclear membrane targeting of lamin, after which the phosphorylation status of S28 by p34cdc2 regulates lamin polymerization at the nuclear lamina (Yamaguchi *et al*, 2006).	The CxxM motif is post-translationally processed in the cytoplasm by the attachment of a farnesyl moiety to the thiol group of the cysteine followed by the proteolytic removal of the last three amino acids. Finally the carboxyl group of the cysteine is methylated (Maske *et al*, 2003). These modifications confer a higher hydrophobicity to the lamin C-terminus and are essential for the targeting to the inner nuclear membrane after lamin transport into the nucleus (Hofemeister *et al*, 2000). There is also evidence for its association to methylation (Prüfert *et al*, 2004).	***Similar to known motif.*** ***Known regulation by both PTM types independently.***
Ph – Me	DLF	MRE11_HUMAN CRYAB_HUMAN SART3_HUMAN HNRDL_HUMAN THOC4_HUMAN HS90B_HUMAN K6PF2_YEAST KHDR1_HUMAN STAG1_HUMAN ERCC6_HUMAN PABP1_HUMAN XRCC5_HUMAN TP53B_HUMAN DNJA1_HUMAN GORS2_HUMAN ACINU_HUMAN DAB2_HUMAN A8MVZ6_HUMAN CEBPB_RAT	Involved in the binding of CEBPB and Rb-E2F complex (Dalrymple *et al*, 2001), DNA repair (Darnell *et al*, 2003) and vesicle trafficking (Mills *et al*, 2003).	No evidence found	No evidence found	***Similar to known motif.*** ***Not associated to any of the PTM types.***

Ac – SM	IT..[FY]	DPOD3_HUMAN MBD1_HUMAN TDG_HUMAN ARI1B_HUMAN NUFP2_HUMAN PAPOA_HUMAN	None found	No evidence found	No evidence found	***Predicted new motif.*** ***Not associated to any PTM type.***
Ac – SM	[KR][KR].[KR] KRK [HKR][KR].[KR] [KR][HKR].[KR]	TCRG1_HUMAN ZEB2_HUMAN PSIP1_HUMAN U520_HUMAN LMNA_HUMAN ESR1_HUMAN LA_HUMAN PARP1_HUMAN XRCC5_HUMAN GATA1_HUMAN DDX5_HUMAN PML_HUMAN SOX2_MOUSE NRIP1_HUMAN RBP2_HUMAN PAPOA_HUMAN HNRPM_HUMAN BLM_HUMAN FLI1_HUMAN TOP2B_HUMAN EP300_HUMAN HDAC1_HUMAN TDG_HUMAN WRN_HUMAN DPOD3_HUMAN SAFB1_HUMAN SAFB1_HUMAN NCOA2_HUMAN NUFP2_HUMAN MAML1_HUMAN PP1RA_HUMAN EPAS1_HUMAN SAE2_HUMAN RCOR1_HUMAN ZN148_HUMAN	Similar to monopartite NLS: K-K/R-X-K/R	PCAF and CBP acetylate specific lysine residues within a novel nuclear localization sequence (NLS) of CIITA. Acetylation leads to an increase of CIITA accumulation in the nucleus (Spilianakis *et al*, 2000).	A nuclear localization signal (NLS) suffices to produce a SUMO conjugate in vivo (Seeler & Dejean, 2003), with only a few exceptions (Rodriguez *et al*, 2001).	***Similar to known motif.*** ***Known regulation by both PTM types independently.***

Supplementary Table 6. Co-regulating PTM types associated to short linear motifs. A total of 8 co-evolving pairs of PTM types, i.e. the proteins having those types of co-evolution, were found enriched in one or more short linear motifs. Similar linear motifs were grouped (each column in the table). In here we show the proteins with the co-evolving PTMs, the motif functionality if known and the association of each of the PTM types with the motif. A literature and database (Gould *et al*, 2010; Rajasekaran *et al*, 2009) survey was performed to asses the previous knowledge about the motif and its association with both PTM types within the literature. According to the previous knowledge, the motifs are classified as i) *similar to known motif* or ii) *predicted new motif*. The regulation of the motif by means of the PTM types was also classified based on the literature survey as i) k*nown regulation by both PTM types independently*, ii) *known regulation by one PTM type* or iii) *not associated to any PTM type*.

Supplementary Datasets

Supplementary Dataset 1. Compendium of protein modifications. Attached excel file.

Supplementary Dataset 2. RCS and rRCS scores of all PTMs. Attached excel file.

Supplementary Dataset 3. Co-evolving pairs of PTMs. Attached excel file.

Supplementary Dataset 4. Enriched Gene Ontology terms in each of the co-evolving pairs of PTM types. Attached excel file.

Supplementary Dataset 5. Enriched Gene Ontology terms in each of the co-evolving pairs of PTM types with proteins involved in each of the functions. Attached excel file.

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