Immunoblotting against c-Myc to detect ubiquitylated proteins in the yeast lysate prior to cobalt-NTA resin purification of ubiquitylated protein (lane 1), the flow-through of the purification (lane 2), and elution from the purification resin (lane 3).
At low pH, a diGly-lysine residue increases peptide solution charge, such that many diGly tryptic peptides are $z=3$. The additional presence of phosphorylation introduces a negative charge, producing a ubiquitylated phosphopeptide with $z=2$. (a) Distribution of peptide solution charge state in each SCX fraction. SCX fractionation effectively separated doubly and triply charged peptides. (b) Ubiquitylated phosphopeptides are enriched in earlier strong-cation exchange fractions, while those peptides that are only ubiquitylated are enriched in later SCX fractions.
For ubiquitylation sites on all proteins or ubiquitylation sites on proteins co-modified with a phosphorylation site, we calculated the ratio of conserved ubiquitylation sites over conserved lysine residues from random sampling of the same number of lysine residues.
(a) For a given protein modified by both phosphorylation and ubiquitylation, the maximum phosphorylation site change correlates with ubiquitylation site abundance increase. (b) The number of phosphorylation sites matching a degron motif does not influence the regulation of ubiquitylation sites.
Supplementary Figure 5. Degradation assays for wild type and phosphorylation site mutants of Swi5 and Gic2 proteins.

Degradation assays for wild type and phosphorylation site mutants of (a-b) Swi5 and (c-d) Gic2 proteins. Cells were treated with galactose to induce protein expression and then incubated with cycloheximide to measure protein degradation. Aliquots where taken at the indicated times and protein expression was monitored by immunoblotting with anti-HA for Swi5 or anti-GST for Gic2 to measure induced protein levels, or anti-α-tubulin for a loading control. Triplicate Western blots for (a) Swi5 T323A or (b) Swi5 WT proteins. (c) Triplicate Western blots for Gic2 WT, S360A, and S360E mutants. (d) Quantitation of the triplicate Gic2 Western blots displayed in panel c.

Degradation assays for wild type and phosphorylation site mutants of (a-b) Swi5 and (c-d) Gic2 proteins.
Supplementary Table 2

The enrichment of PFAM domains in protein isoforms co-modified with ubiquitin and phosphorylation.

<table>
<thead>
<tr>
<th>PFAM Domain</th>
<th>Enrichment Ratio</th>
<th>P-value</th>
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<tr>
<td>AA_permease</td>
<td>4.34</td>
<td>1.90E-07</td>
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<tr>
<td>Cation_ATPase_N</td>
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<td>6.08E-03</td>
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<tr>
<td>Arrestin_N</td>
<td>4.05</td>
<td>6.08E-03</td>
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<tr>
<td>Arrestin_C</td>
<td>3.79</td>
<td>1.06E-03</td>
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<tr>
<td>Pkinase_C</td>
<td>3.61</td>
<td>4.03E-03</td>
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<tr>
<td>ABC_membrane</td>
<td>3.03</td>
<td>4.96E-02</td>
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