**Supplemental Methods**

Microscopy: Yeast cells were GFP fixed with 4% paraformaldehyde solution using a protocol from Doug Koshland Lab (UC Berkeley). GFP signal was visualized with the Zeiss AxioImager M1 and captured with Zen Imaging Software.

Co-IP: Cells were grown to mid-log phase in YM-1 + 2% dextrose. Cells were untreated or treated with 0.05% MMS for 2.5 hours. 50 OD600 worth of cells were harvest for each sample, lysed in 600 µL HEPES lysis buffer (25 mM HEPES pH 7.5, 0.2% Triton X100, 250 mM NaCl, 1 mM EDTA, 10% Glycerol, 0.1 mg/mL PMSF, 1 μg/mL leupeptin, 1 μg/mL bestatin, 1 μg/mL pepstatin, 1 μM benzamidine HCl, 5 mM sodium fluoride, 80 mM β-glycerophosphate, and 1 mM sodium orthovanadate) by bead beating in a cold block for nine cycles of 1.5 mins (2 mins on ice in between), and cleared by centrifugation at 4oC. Protein concentrations were quantified using the Bio-Rad Bradford Protein Assay and equal amounts of extract were incubated with 25 µL slurry of anti-Flag M2 Magnetic Beads (Sigma-Aldrich) and rotated at 4oC overnight. Beads were collected on a magnetic rack and washed three times with 500 µL lysis buffer. Proteins were eluted by mild vortexing in high salt lysis buffer (lysis buffer with 0.5 M NaCl) containing 0.5 μg/mL 3XFlag peptide (Sigma-Aldrich) for 30 min at room temperature. Samples were analyzed by Western blotting against FLAG epitope on Xrn1 proteins and GFP epitope on the indicated substrates.

Phosphopeptide ID and SILAC mass spectrometry: Samples were digested by the addition of lys-C and trypsin as previously described (Wohlschlegel 2009). Proteolyzed samples were then fractionated using a Dionex Ultimate 3000 UHPLC coupled to a 75 µM internal diameter fused silica capillary column with an integrated 5 µM electrospray emitter and packed with 25 cm of 1.9 µM C18 reversed phase resin (Dr. Maisch GmbH). MS/MS spectra were collected on a Thermofisher Fusion Lumos mass spectrometer using data-dependent acquisition (Senko *et al.* 2013). For SILAC experiments, data were analyzed using IP2 (Integrated Proteomics Applications). For phosphosite mapping, the raw data was analyzed using MSGF+ and Percolator to generate peptide identifications filtered at a 1% false discovery rate (Käll *et al.* 2007 p.; Kim and Pevzner 2014), phosphoRS to assess phosphosite localization (Breitwieser and Colinge 2013), and Skyline to perform MS1-based label-free quantitation of each peptide across samples (MacLean *et al.* 2010; Schilling *et al.* 2012).

**References**

Breitwieser F. P., and J. Colinge, 2013 Isobar(PTM): a software tool for the quantitative analysis of post-translationally modified proteins. J. Proteomics 90: 77–84. https://doi.org/10.1016/j.jprot.2013.02.022

Käll L., J. D. Canterbury, J. Weston, W. S. Noble, and M. J. MacCoss, 2007 Semi-supervised learning for peptide identification from shotgun proteomics datasets. Nat. Methods 4: 923–925. https://doi.org/10.1038/nmeth1113

Kim S., and P. A. Pevzner, 2014 MS-GF+ makes progress towards a universal database search tool for proteomics. Nat. Commun. 5: 5277. https://doi.org/10.1038/ncomms6277

MacLean B., D. M. Tomazela, N. Shulman, M. Chambers, G. L. Finney, *et al.*, 2010 Skyline: an open source document editor for creating and analyzing targeted proteomics experiments. Bioinforma. Oxf. Engl. 26: 966–968. https://doi.org/10.1093/bioinformatics/btq054

Schilling B., M. J. Rardin, B. X. MacLean, A. M. Zawadzka, B. E. Frewen, *et al.*, 2012 Platform-independent and label-free quantitation of proteomic data using MS1 extracted ion chromatograms in skyline: application to protein acetylation and phosphorylation. Mol. Cell. Proteomics MCP 11: 202–214. https://doi.org/10.1074/mcp.M112.017707

Senko M. W., P. M. Remes, J. D. Canterbury, R. Mathur, Q. Song, *et al.*, 2013 Novel parallelized quadrupole/linear ion trap/Orbitrap tribrid mass spectrometer improving proteome coverage and peptide identification rates. Anal. Chem. 85: 11710–11714. https://doi.org/10.1021/ac403115c

Wohlschlegel J. A., 2009 Identification of SUMO-conjugated proteins and their SUMO attachment sites using proteomic mass spectrometry. Methods Mol. Biol. Clifton NJ 497: 33–49. https://doi.org/10.1007/978-1-59745-566-4\_3