

# **Proteome-wide analysis of lysine acetylation suggests its broad regulatory scope in *Saccharomyces cerevisiae***

Peter Henriksen<sup>1,6</sup>, Sebastian A. Wagner<sup>1,6</sup>, Brian T. Weinert<sup>1</sup>, Satyan Sharma<sup>2</sup>, Giedrė Bačinskaja<sup>3</sup>,  
Michael Rehman<sup>4</sup>, André H. Juffer<sup>2</sup>, Tobias C. Walther<sup>4,5</sup>, Michael Lisby<sup>3</sup>, Chunaram Choudhary<sup>1,\*</sup>

<sup>1</sup>Department of Proteomics, The Novo Nordisk Foundation Center for Protein Research, Faculty of Health Sciences, University of Copenhagen, Blegdamsvej 3, DK-2200 Copenhagen, Denmark

<sup>2</sup>Biocenter Oulu and the Department of Biochemistry, PL 3000, University of Oulu, FI-90014, Finland.

<sup>3</sup>Department of Biology, University of Copenhagen, Ole Maaløes Vej 5, Kbh. N., Denmark

<sup>4</sup>Max Planck Institute for Biochemistry, Martinsried, Germany

<sup>5</sup>Yale School of Medicine, Department of Cell Biology, 333 Cedar Street, SHM C425, New Haven , CT 06510, USA

<sup>6</sup>These authors contributed equally to this work

\*To whom correspondence should be addressed: [chuna.choudhary@cpr.ku.dk](mailto:chuna.choudhary@cpr.ku.dk)

## **Supplemental information**

Supplemental Fig. 1-2

Supplemental figure legends

Supplemental Table S1 (provided as Excel workbook)

Supplemental Table S2 (provided as Excel workbook)

Supplemental table legends

## Supplemental figure legends

**Supplemental Fig. S1.** Confirmation of protein acetylation, and schematic of acetylation analysis in *rpd3Δ* cells. (A) Confirmation of yeast protein acetylation. The indicated proteins were immunoprecipitated from yeast strains expressing ORFs of these genes fused to green fluorescent protein (GFP), proteins were resolved on SDS-PAGE and stained with an anti-acetyllysine antibody.

(B) Schematic of SILAC-based acetylation analysis in *rpd3Δ* cells. Wild-type yeast cells were cultured in media supplemented with “light” lysine and *rpd3Δ* strains were cultured in “heavy” lysine containing media. Exponentially growing cells ( $OD_{600} = 0.5$ ) were harvested, mixed, and relative abundances of acetylation sites were quantified using mass spectrometry.

**Supplemental Fig. S2.** Comparative sequence properties of the yeast acetylation sites identified from dataset 1 and 2. (A) The Venn diagram illustrates overlap between the two acetylation datasets acquired in this study. The dataset 1 includes all acetylation sites identified from four experiments shown in Figure 1 (Table S1). The dataset 2 include acetylation sites identified in *rpd3Δ* cells (data shown in Figure 5, Table S2). The dataset 1 and 2 were collected using the two different batches of anti-acetyllysine rabbit polyclonal antibodies (for further details refer experimental procedures section).

(B) Sequence properties of acetylation sites identified in the dataset 1 and 2. The figure shows frequencies of amino acids flanking acetylation sites. Acetylation sites identified in the two dataset showed differences in sequence preferences, likely reflecting sequence preferences of antibodies from the different batches.

### **Supplemental Fig. S3.**

Annotated MS2 spectra of acetylated peptides reported in supplemental Table S1.

### **Supplemental Fig. S4.**

Annotated MS2 spectra of acetylated peptides reported in supplemental Table S2.

### **Supplemental Table legends**

#### **Supplemental Table S1**

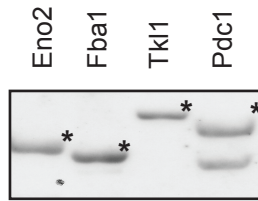
The table lists all acetylation sites identified from four experiments presented in the Fig. 1.

#### **Supplemental Table S2**

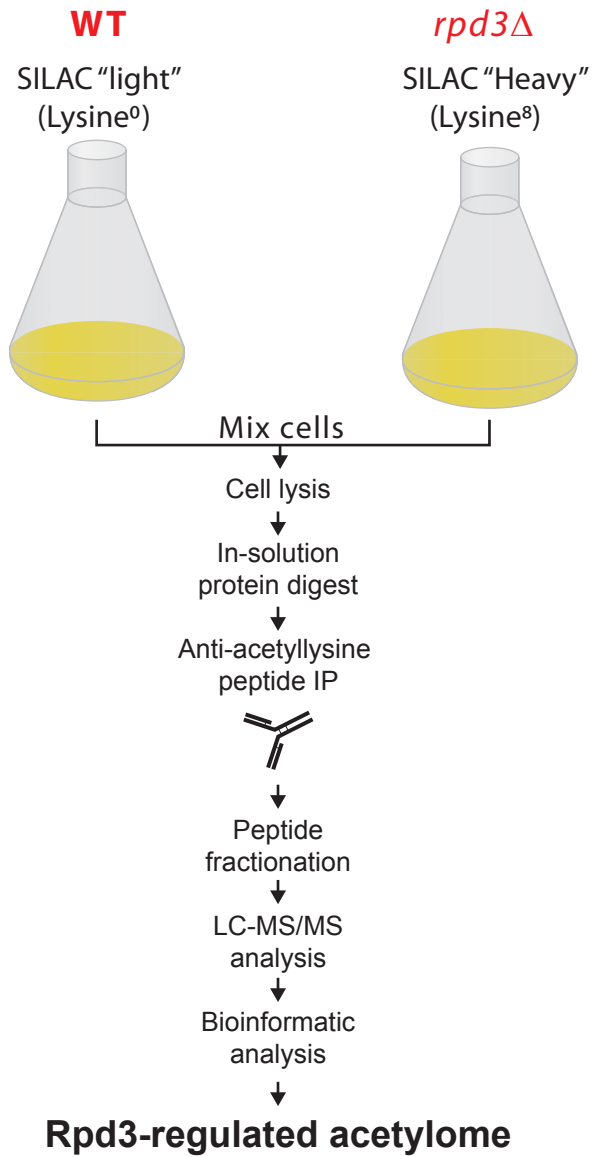
The table lists all acetylation sites quantified in *rpd3Δ* cells. The relevant data are presented in the Fig. 5.

# Supplemental Fig. S1

**A**

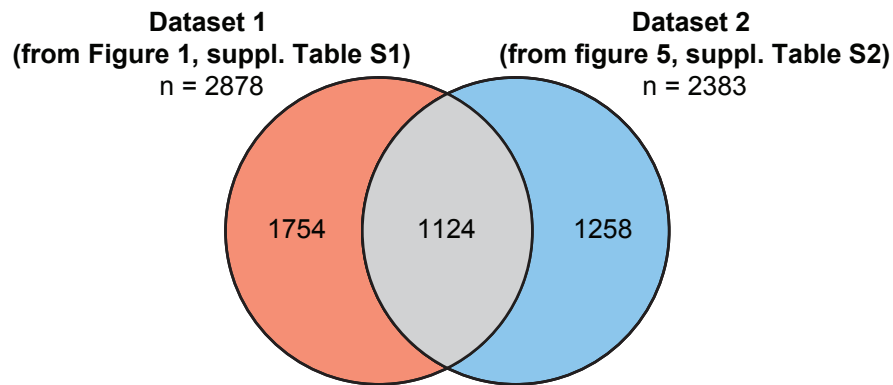


**B**



# Supplemental Fig. S2

A



B

