# Supplemental Material

## Mass spectrometry analyses of p300-acetylated c-Myc residues

For mass spectrometry analysis of human c-Myc acetylated by p300 in vitro, recombinant human Myc:Max complex (8 pmol) was acetylated in HAT buffer in the presence of 50 μM acetyl-CoA and 50ng recombinant p300 (full-length) for 30min/ 30 °C. Then, additional p300 and acetyl-CoA (50 ng and 50 μM respectively) were added and the reaction was extended for additional 30 min/ 30 °C. This step was repeated once more and samples were prepared for mass spectrometry as described further below.

For identification of p300-acetylated residues on mouse c-Myc in vivo, HEK293 cells were transfected 3 times (at 4 h intervals) with 3μg pCbS-Flag-Myc and 5μg pCMVβ-p300-CHA. Before lysis, cells were incubated with medium containing HDAC inhibitors (10mM sodium butyrate, 10 mM nicotinamide, 2 μM TSA) for 14 hours. Whole cell extracts were prepared 48 hours after the first transfection by using lysis buffer containing 10 mM nicotinamide and 2μM TSA. Cell extracts equivalent to seven 10-cm plates were adjusted to 500mM NaCl and Flag-Myc was immunoprecipitated by incubation with 35μl Flag M2 resin for 14 hours/4 °C, under constant rotation.

Flag-Myc immunoprecipitated from HEK293 cells and in vitro acetylated Myc proteins were resolved by SDS-PAGE in a 8-16% Tris-Glycine gel (Invitrogen), and stained with Colloidal Blue (Invitrogen). Bands were cut from the gel, in-gel trypsin digested, and analyzed by tandem mass spectrometry (MS/MS) essentially as previously described (Ref. S1).

**Reference S1:**

**Carter C., S. Pan, J. Zouhar, E.L. Avila, T. Girke, and N.V. Raikhel.** 2004. The vegetative vacuole proteome of Arabidopsis thaliana reveals predicted and unexpected proteins. Plant Cell **16:** 3285-3303.

**Figure legends.**

**Fig. S1.** Human c-Myc residue K148 (corresponding to mouse c-Myc K149) is a direct substrate for acetylation by p300 in vitro. MS/MS spectrum of fragmentation ions (y) is shown. The precursor ion and accuracy mass measurement (in ppm) are indicated.

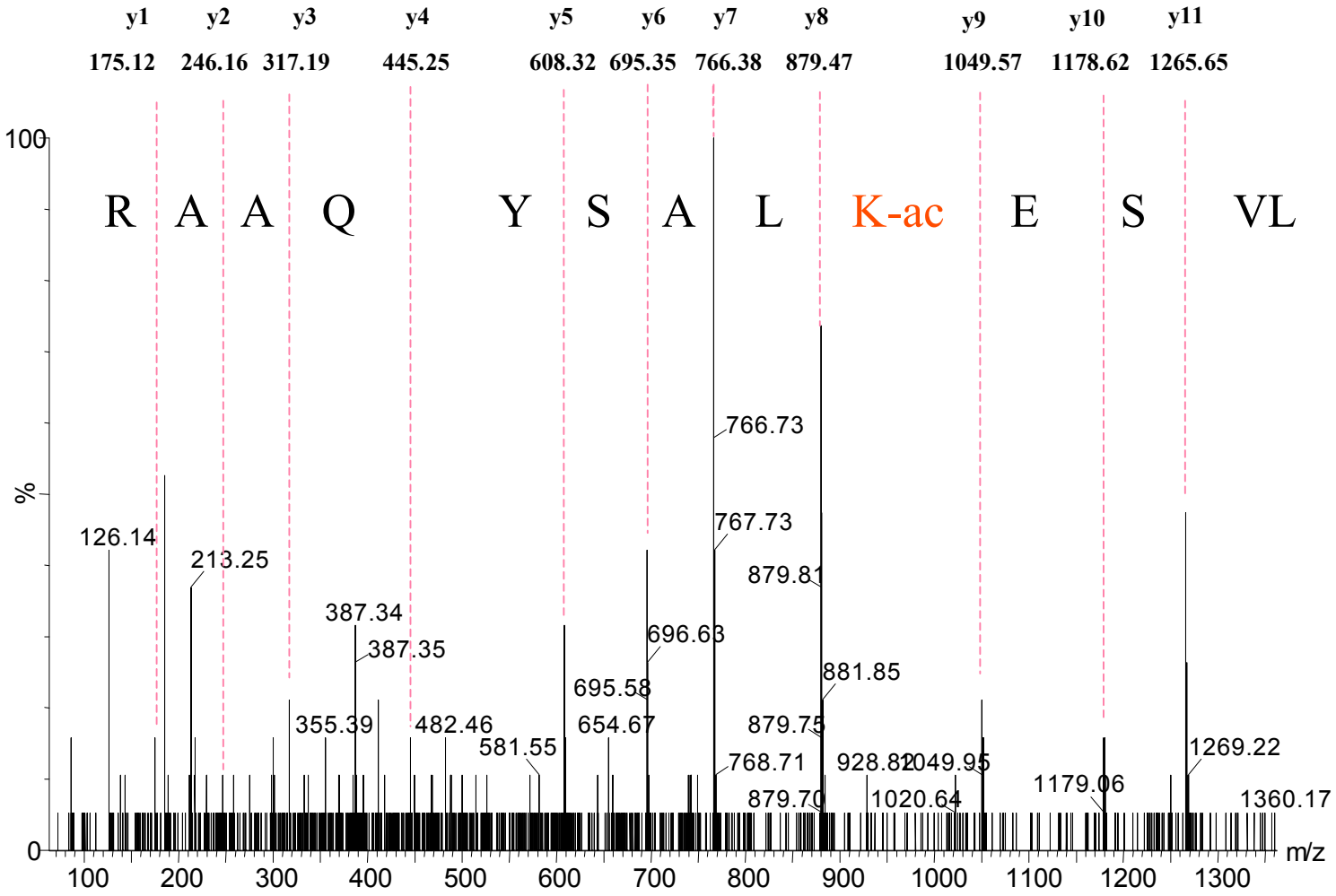
**Fig. S2-S5.** Mouse c-Myc residues K149, K158, K317, and K323 are acetylated in HEK293 cells transfected with a p300 expression vector. The MS/MS spectra of fragmentation ions (y) for the different precursor ions detected are shown. For each precursor ion the sequence and accuracy mass measurement (in ppm) are indicated. Fig. S5 shows a precursor ion in which both K317 and K323 are acetylated.

# Fig. S1: Human c-Myc K148 acetylated in vitro

**Precursor: 739.40, 2+**

**MS/MS mass error: 43 ppm**

**$_{144}$LVSEK-acLASYQAAR$_{156}$**

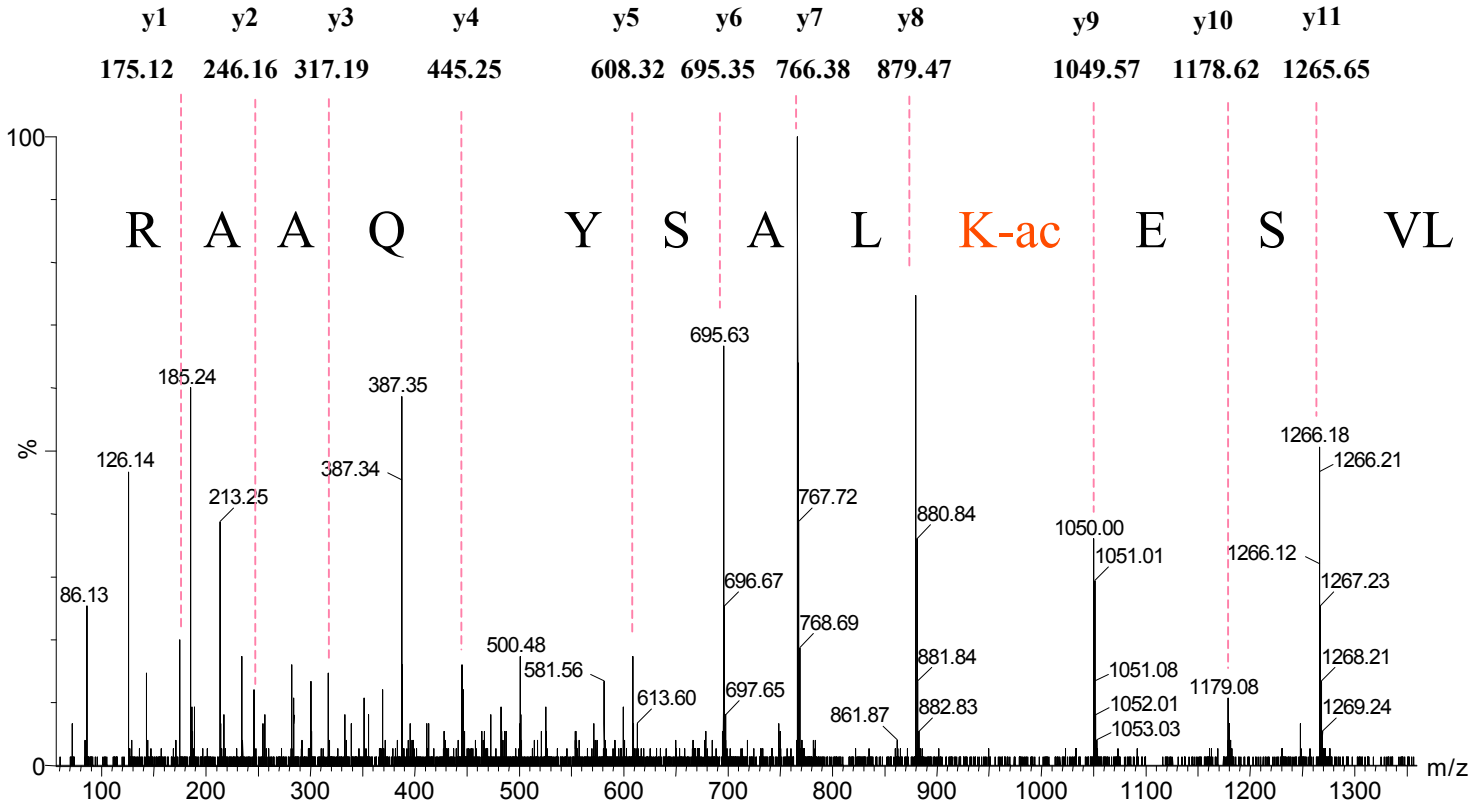


# Fig. S2: Mouse c-Myc K149 acetylated in HEK293 cells

**Precursor: 739.39, 2+**

**MS/MS mass error: 40 ppm**

**$_{145}$LVSEK-acLASYQAAR$_{157}$**

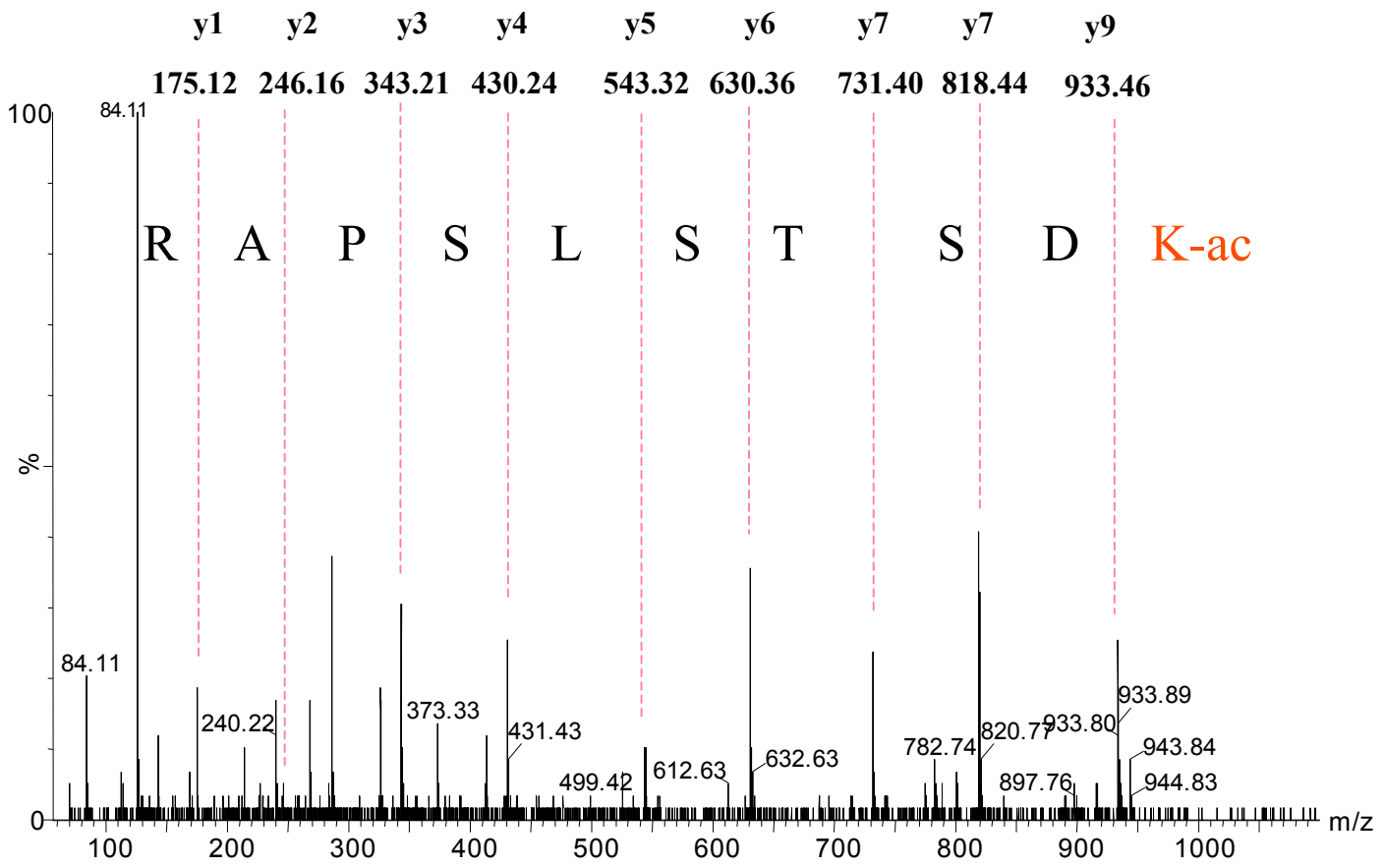


# Fig. S3: Mouse c-Myc K158 acetylated in HEK293 cells

**Precursor: 552.29, 2+**

**$_{158}$K-acDSTSLSPAR$_{167}$**

**MS/MS mass error: 37 ppm**

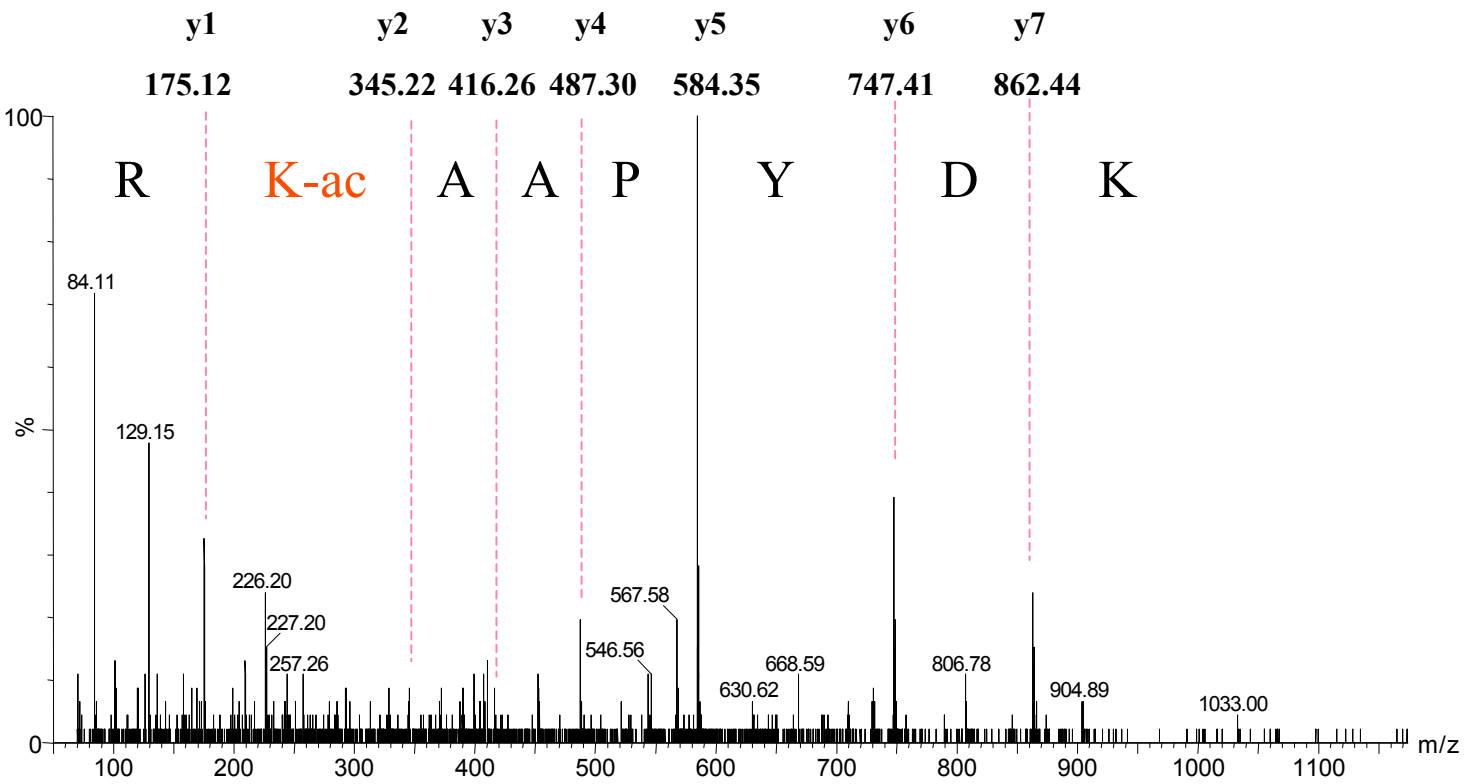


# Fig. S4: Mouse c-Myc K323 acetylated in HEK293 cells

**Precursor: 495.76, 2+**

**$_{317}$KDYPAAK-acR$_{324}$**

**MS/MS mass error: 51 ppm**



# Fig. S5: Mouse c-Myc K317 and K323 acetylated in HEK293 cells

**Precursor: 516.78, 2+**

**$_{317}$K-acDYPAAK-ac$R_{324}$**

**MS/MS mass error: 35 ppm**