N-terminal protein acetylation by NatB modulates the levels of Nmnats, the NAD^+ biosynthetic enzymes in *Saccharomyces cerevisiae*

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SUPPORTING INFORMATION

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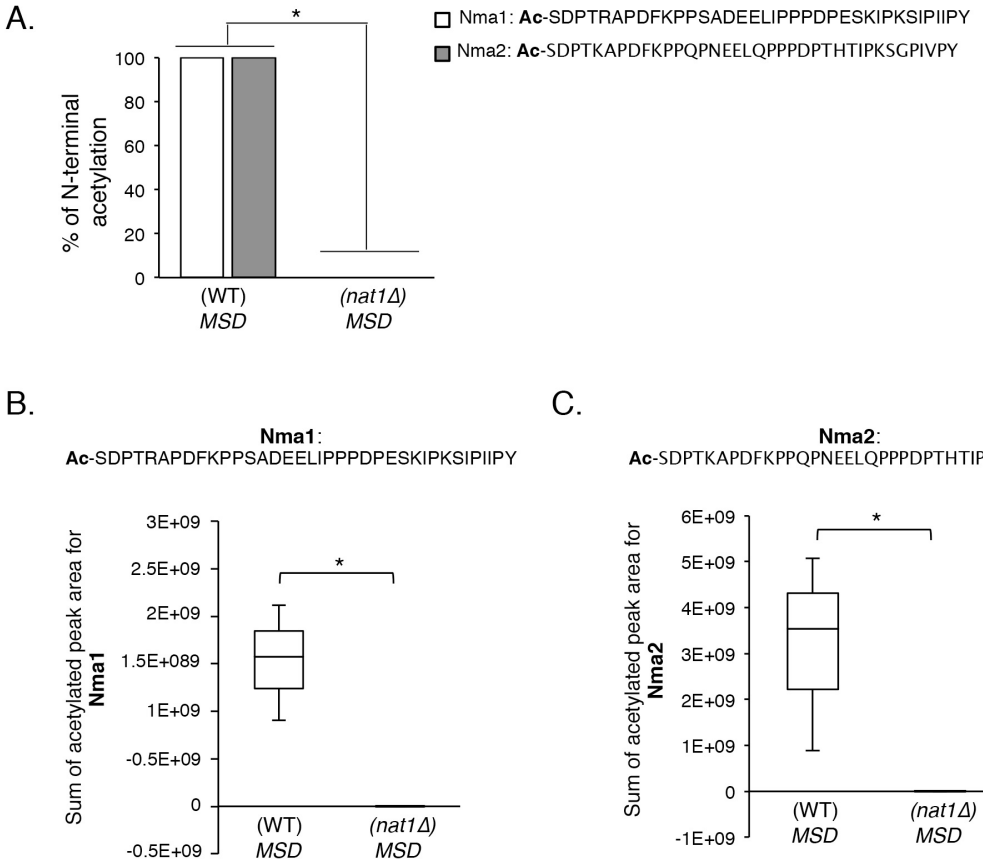
A.
Nma1: **Ac**-SDPTRAPDFKPPSADEELIPPPDPESKIPKSIPIIPY
Nma2: **Ac**-SDPTKAPDFKPPQPNEELQPPPDPTHTIPKSGPIVPY
% of N-terminal acetylation
100
80
60
40
20
0
*
(WT) *MSD*
(nat1Δ) *MSD*
B.
Nma1:
Ac-SDPTRAPDFKPPSADEELIPPPDPESKIPKSIPIIPY
Sum of acetylated peak area for **Nma1**
3E+09
2.5E+09
2E+09
1.5E+089
1E+09
-0.5E+09
0
-0.5E+09
*
(WT) *MSD*
(nat1Δ) *MSD*
C.
Nma2:
Ac-SDPTKAPDFKPPQPNEELQPPPDPTHTIPKSGPIVPY
Sum of acetylated peak area for **Nma2**
6E+09
5E+09
4E+09
3E+09
2E+09
1E+09
0
-1E+09
*
(WT) *MSD*
(nat1Δ) *MSD*

FIGURE *S*1. Mass Spectrometry analysis of Nt-acetylation of Nma1 and Nma2 in the *MSD* (WT) and *MSD* (*nat1*Δ) cells. *A*, among specific Nma1 and Nma2 N-terminal peptides identified from *MSD* (WT) cells, 100% are Nt-acetylated at the first serine residue. The initial first methionine is cleaved, which is expected for all NatA substrates. All identified Nma1 and Nma2 N-terminal peptides from the *nat1*Δ mutant cells are not Nt-acetylated. The percentages of Nt-acetylated Nma1 and Nma2 peptides shown in the graph are calculated using the Scaffold software with the peptide threshold set at 1% (false-discovery rate). A total of three pairs of biological replicates of WT and *nat1*Δ mutant strains are analyzed in three independent experiments. Detailed methods are shown in the *EXPERIMENTAL PROCEDURES* in the main manuscript. E*rror bars* represent data from 3 biological replicates. The *p* values are calculated using Student's *t* test (*, $p<0.05$). *B* and *C*, the sum of Nt-acetylated Nma1 and Nma2 peptides and the distribution of 3 biological replicates analyzed in three independent experiments are shown in box plots. The mean values of the WT samples are significantly higher than that of the *nat1*Δ samples (*, $p<0.05$). The *p* values are calculated by the Student's *t* test.