Molecular Cell, Volume *25*

Supplemental Data

Histone H3-K56 Acetylation

Is Catalyzed by Histone

Chaperone-Dependent Complexes

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Supplemental Experimental Procedures:

Nucleosomal arrays - Templates containing complete nucleosomes or only $(H3/H4)_2$ tetramers were generated by salt dialysis as previously described, using chicken histones and a linear DNA fragment containing 11 tandem 5S DNA repeats (Logie and Peterson, 1999). Briefly, 10 μg of DNA templates were mixed with 7.65 μg chicken histone octamers or 6 μg chicken H3/H4 tetramers in 2 M NaCl. Array reconstitution was achieved by stepwise salt dialysis in TE (10 mM Tris pH 8.0, 0.25 mM EDTA) from 2 M NaCl to 2.5 mM NaCl (Logie and Peterson, 1999). The ratio of nucleosomes or H3/H4 tetramers to DNA repeat was sub-stoichiometric (R ~0.8) to ensure negligible free histones in the array reconstitution. Array saturation was monitored by *EcoR*I digestion and analysis on 4% 1x TBE PAGE gels (Logie and Peterson, 1999). Final concentrations of arrays used in acetylation assays were determined by immunoblot comparisons with free H3/H4 tetramers.

Mass spectrometry –

Rtt109/Asf1: 20 pmol of Rtt109, 150 pmol of Asf1, 12.5μM AcCoA, 25μg yeast histone H3/H4 were incubated for 45 min at 30 ºC in 500 μl HAT assay buffer with 10mM NaCl, and proteins were then precipitated with trichloroacetic acid. Liquid Chromatography Mass Spectrometry (LC-MS): Histones H3/H4 were separated by reverse phase HPLC (Discovery Bio wide pore 1.0 mm x 150 mm C18 column, 5 μm, 300 Å, Supelco, USA) at a flow rate of 0.05 ml/min over 60 min using 0.1% TFA in H_2O as mobile phase A and 0.1%TFA in acetonitrile as mobile phase B. The flow from the HPLC was coupled to the

electrospray ionization source of a Micromass LCT time-of-flight mass spectrometer (Waters 2690). Tandem mass spectrometry (LC-MS/MS): Histones H3/H4 were digested with trypsin. The digest was desalted by use of a ziptip, redissolved in water and analyzed by nano-LC tandem mass spectrometry on a Shimadzu capillary LC instrument (Columbia, MD) coupled to a ThermoFinnigan LCQ DECA XP+ ion trap (San Jose, CA). Peptides were separated on a reverse phase C18 nano-column (5 cm, 300 Å, 5 μm, I.D. 75 μm) over a 70 min gradient. The mobile phase A was water with 0.1% formic acid and mobile phase B was acetonitrile with 0.1% formic acid. The data were analyzed by use of an in-house developed database search algorithm, MassMatrix.

Rtt109/Vps75: Enzyme complex (1.15 μM) was incubated with 20 μg (50 μM) of recombinant Xenopus histone H3 with 190 μM acetyl-CoA for 10 minutes in 50 mM Tris, pH 7.5 and 1 mM DTT. Trypsin (1 μg) was then added and reacted for 14 hours at 37 ºC. Samples were then run on Agilent 1100 LC MS and peptides identified in Mascot. The Xenopus histone H3 contains the observed tyrosine at residue 54 rather than the phenylalanine in the yeast H3.

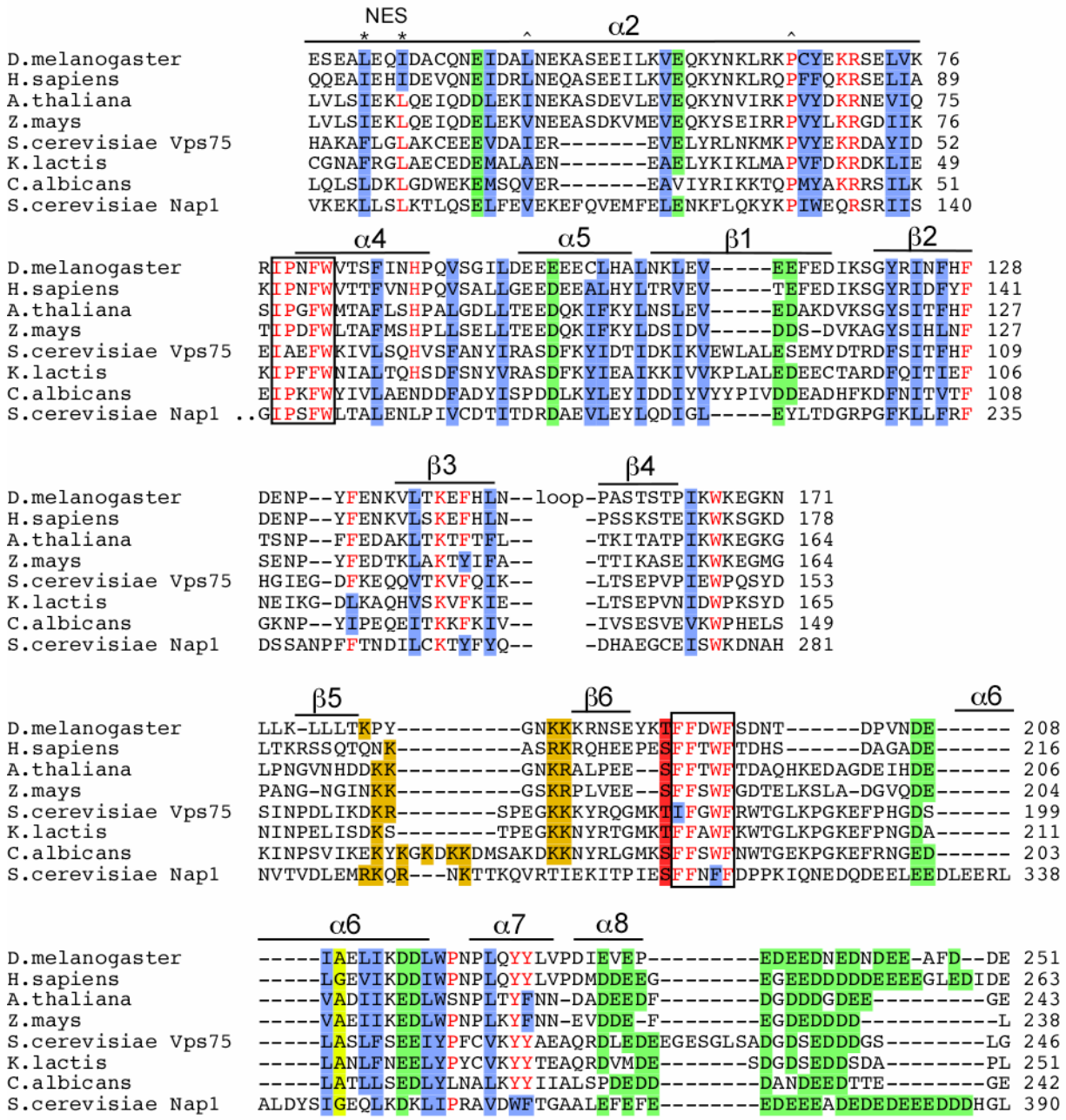

Figure S1. Sequence alignment: Vps75 conservation.
The following protein sequences were aligned using CLUSTAL W (Chenna et al., 2003): *D. melanogaster* NAP/SET protein (NCBI Locus AAA74264); *H. sapiens* NAP/SET (NCBI Locus Q01105); *A. thaliana* NAP/SET (NCBI Locus NP_177596); *Z. mays* NAP/SET (NCBI Locus AAK67145); *S. cerevisiae* Vps75 (NCBI Locus NP_014153); *C. albicans* Vps75 (NCBI Locus XP_712165); *K. lactis* Vps75 (NCBI Locus XP_452333); *S. cerevisiae* Nap1 (NCBI Locus P25293). The secondary structure features listed above the sequences were based on the crystal structure of *S. cerevisiae* Nap1 (Park and Luger, 2006); the Vps75 family appears to lack the α1 and α3 helices. Residues identical in most aligned proteins are typed in red. Conserved hydrophobic/aromatic residues are backshaded in blue, conserved acidic residues are backshaded in green, and small residues in yellow. Conserved basic residues in dark orange are found within the region of *S. cerevisae* Nap1 important for nuclear

localization (Mosammaparast et al., 2005). The two highly conserved, hydrophobic regions of the Nap/Set family important for core packing (Park and Luger, 2006) are boxed. Conserved residues that are essential for nuclear export of yeast Nap1 are marked with an asterisk "*" and the label "NES" (Mosammaparast et al., 2002; Miyaji-Yamaguchi et al., 2003). Conserved residues within the long α2 dimerization helix marked with carets "^" are important for kinks in a long side-by-side helical interaction (Park and Luger, 2006). Not all of the highly acidic C-termini or the non-conserved residues between β3 and β4 are shown.

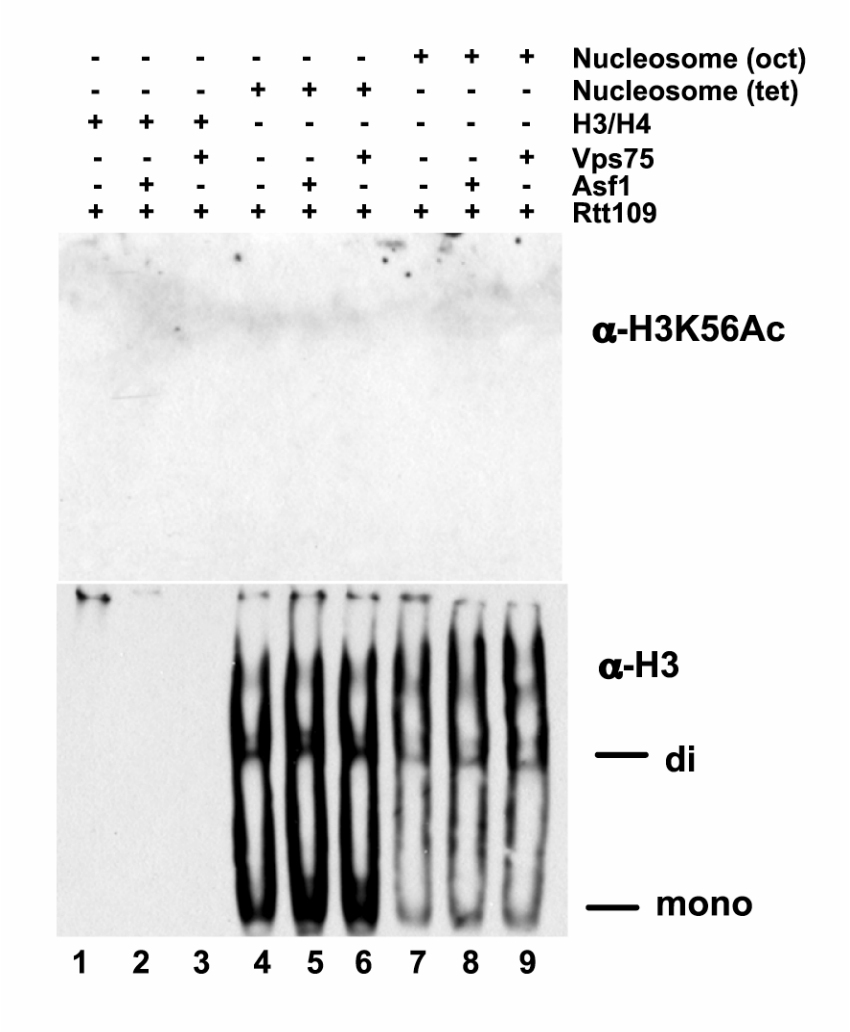

Figure S2. Characterization of histone-DNA complexes. HAT assays were performed with 0.3 pmol of Rtt109 and 3 pmol of Asf1 or Vps75 where indicated. Substrates were 2 pmol of either chicken $(H3/H4)_2$ tetramers in solution (lanes 1-3), tetramers deposited onto arrays of 5S DNA (lanes 4-6), or complete nucleosomes on 5S DNA arrays (lanes 7-9). Products from the same reactions as shown in Figure 3A were partially digested with *Eco*RI, separated on a 4% native PAGE gel and blotted to a membrane prior to immunodetection. The mono- and di-nucleosomes detected with the anti-H3 antibody are indicated (lower panel, lanes 7-9). A ladder of $(H3/H4)_2$ multimers are also observed (lower panel, lanes 4-6). In contrast, these species were not recognized by the anti-H3-K56ac antibody (upper panel).

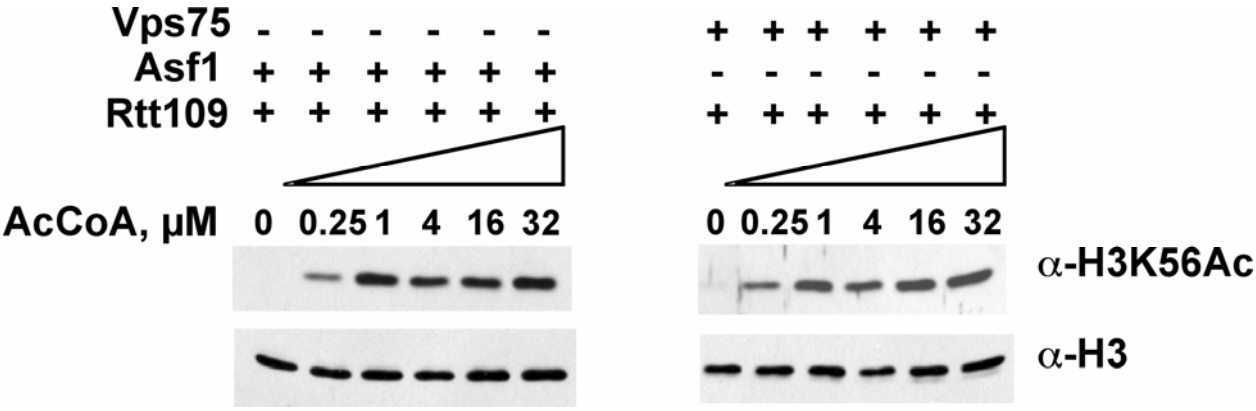

Figure S3. Similar K_m for Ac-CoA for the Rtt109-Asf1 and Rtt109-Vps75 complexes. Ac-CoA was titrated from 0 µM to 32 µM in assays containing 0.4 pmol of Rtt109 and 1.5 pmol of Asf1 or Vps75 as indicated. Products were analyzed by immunoblotting.

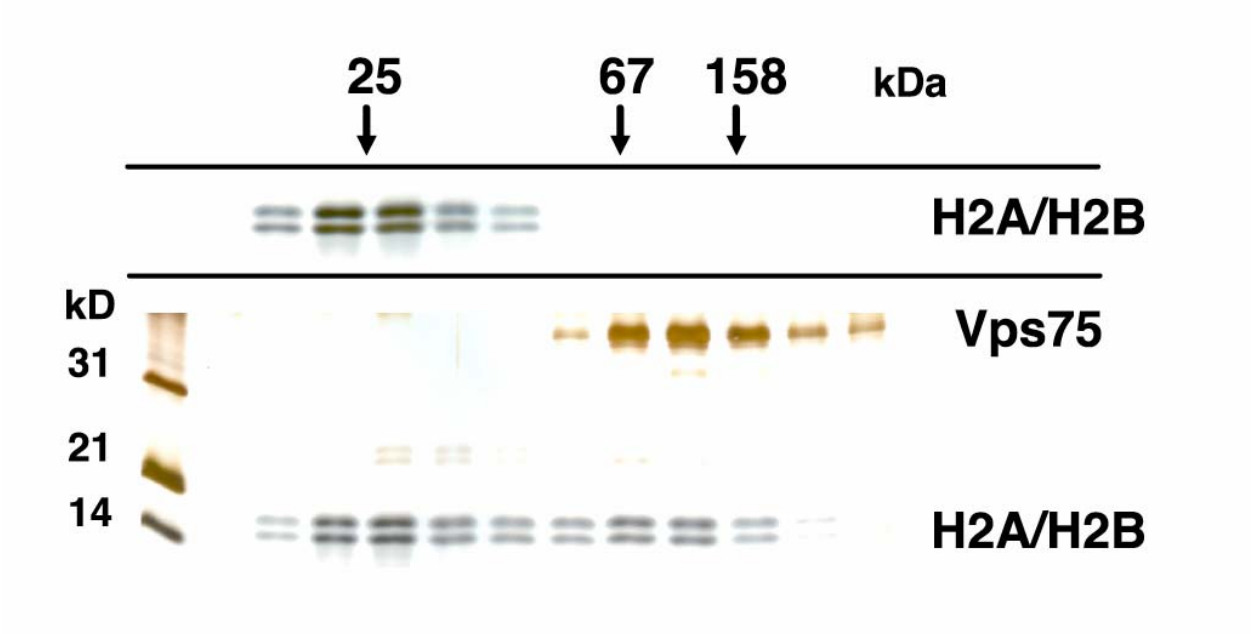

Figure S4. Histone H2A/H2B binding by Vps75. Vps75 and histones H2A and H2B were analyzed by velocity sedimentation on glycerol gradients as described in Figure 2.

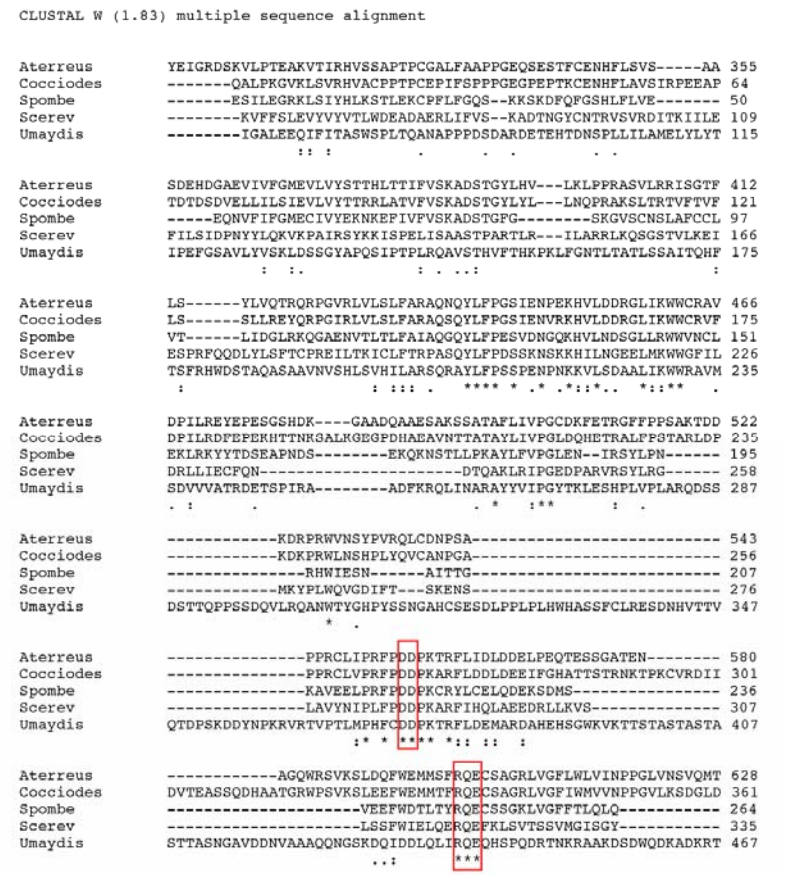

Figure S5. Conservation of Rtt109 among fungal species.
The following protein sequences were aligned using CLUSTAL W (Chenna et al., 2003):
Aterreus = Aspergillus terreus (NCBI Locus XP_001215675); Coccidioides = Coccidioides immitis RS (NCBI Locus EAS31131); Spombe = Schizosaccharomyces pombe SPBC342.06c (NCBI Locus CAB46776); Scerev = S. cerevisiae Rtt109 (ORF YLL002W); Umaydis = Ustilago maydis 521 hypothetical protein UM02284.1 (NCBI Locus XP_758431). The conserved, charged residues that were mutated to alanines are boxed in red (S. cerevisiae Rtt109 amino acids DD287-8, and RQE318-320).

Supplemental References:

Chenna, R., Sugawara, H., Koike, T., Lopez, R., Gibson, T. J., Higgins, D. G., and Thompson, J. D. (2003). Multiple sequence alignment with the Clustal series of programs. Nucleic Acids Res *31*, 3497-3500.

Logie, C., and Peterson, C. L. (1999). Purification and biochemical properties of yeast SWI/SNF complex. Methods Enzymol *304*, 726-741.

Miyaji-Yamaguchi, M., Kato, K., Nakano, R., Akashi, T., Kikuchi, A., and Nagata, K. (2003). Involvement of nucleocytoplasmic shuttling of yeast Nap1 in mitotic progression. Mol Cell Biol *23*, 6672-6684.

Mosammaparast, N., Del Rosario, B. C., and Pemberton, L. F. (2005). Modulation of histone deposition by the karyopherin kap114. Mol Cell Biol *25*, 1764-1778.

Mosammaparast, N., Ewart, C. S., and Pemberton, L. F. (2002). A role for nucleosome assembly protein 1 in the nuclear transport of histones H2A and H2B. Embo J *21*, 6527-6538.

Park, Y. J., and Luger, K. (2006). The structure of nucleosome assembly protein 1. Proc Natl Acad Sci U S A *103*, 1248-1253.