

**Supplementary Material:**

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)<sub>2</sub> 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 *EcoRI* 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.

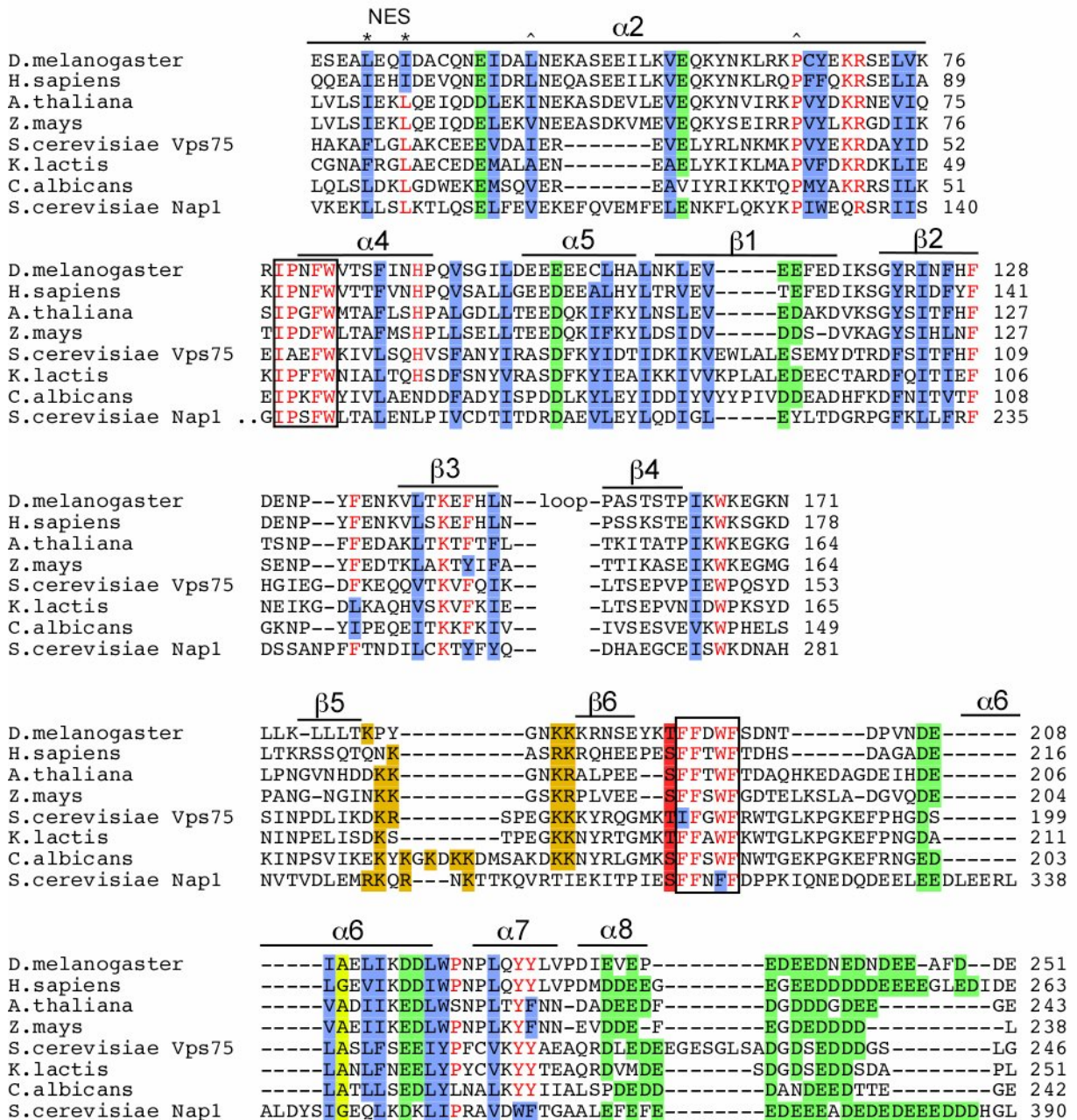
*Mass spectrometry* –

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 °C 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<sub>2</sub>O 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 °C. 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.

## Supplemental Figures and Legends

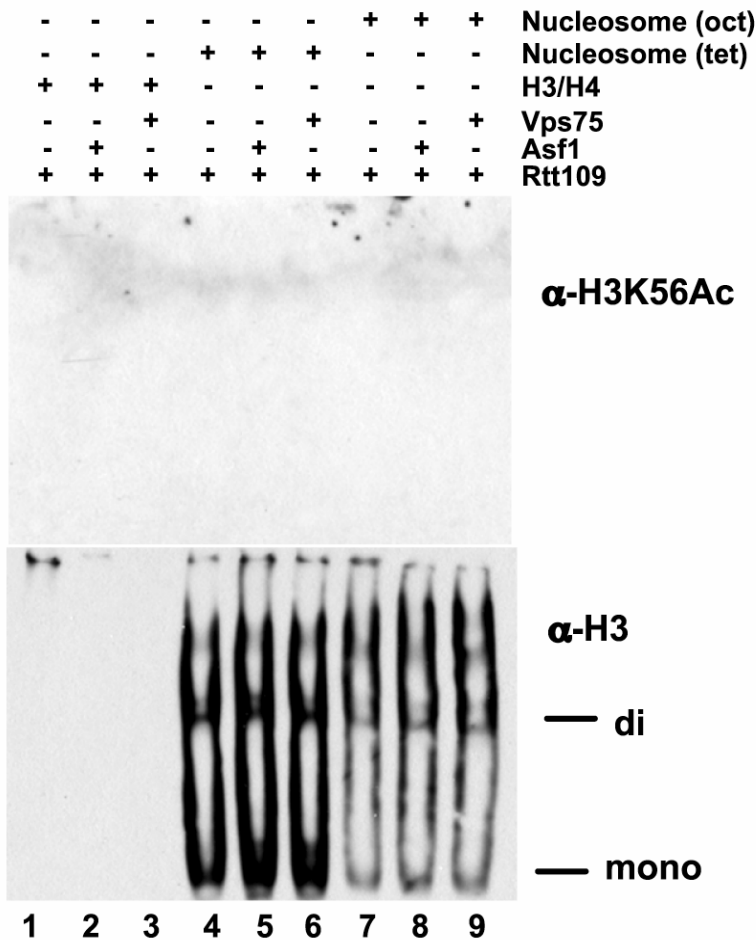


### Supplemental Figure 1. Sequence alignment: Vps75 conservation.

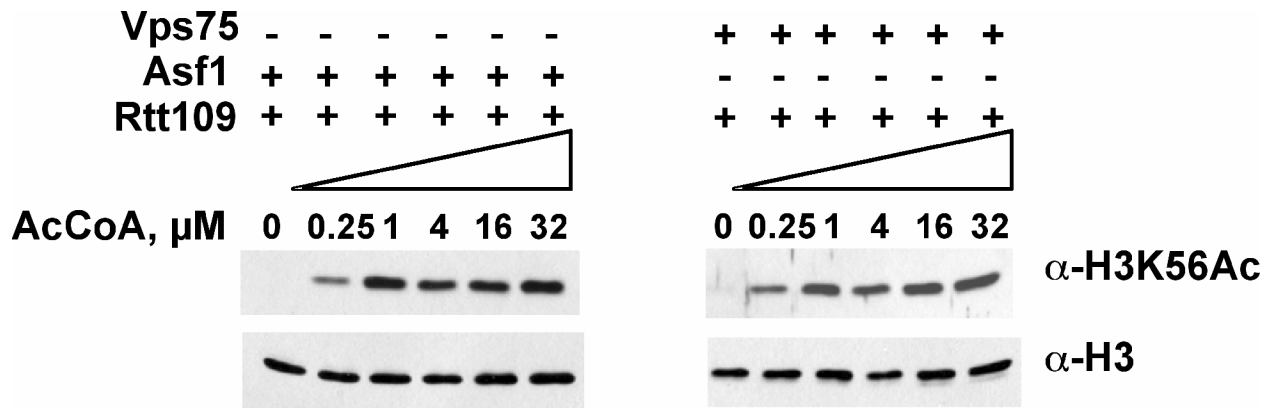
The following protein sequences were aligned using CLUSTAL W (Chenna et al., 2003):

*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  $\alpha 1$  and  $\alpha 3$  helices. Residues identical in most aligned proteins are typed in red. Conserved hydrophobic/aromatic residues are backshaded in

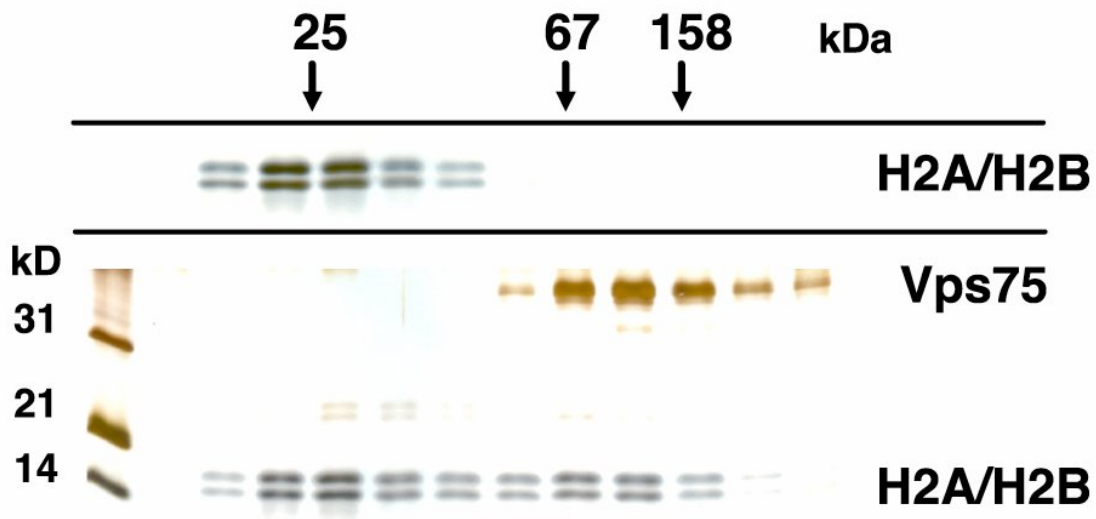
blue, conserved acidic residues are backshaded in green, and small residues in yellow. Conserved basic residues in dark orange are found within the region of *S. cerevisiae* Nap1 important for nuclear localization (Mosammaparast et al., 2005). The two highly conserved, hydrophobic regions of the Nap/Set 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  $\alpha 2$  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  $\beta 3$  and  $\beta 4$  are shown.



**Supplemental Figure 2. 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)<sub>2</sub> 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 *EcoRI*, 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)<sub>2</sub> multimers are also observed (lower panel, lanes 4-6). In contrast, these species were not recognized by the anti-H3-K56ac antibody (upper panel).



**Supplemental Figure 3. Similar  $K_m$  for Ac-CoA for the Rtt109-Asf1 and Rtt109-Vps75 complexes.** Ac-CoA was titrated from 0  $\mu$ M to 32  $\mu$ M in assays containing 0.4 pmol of Rtt109 and 1.5 pmol of Asf1 or Vps75 as indicated. Products were analyzed by immunoblotting.



**Supplemental Figure 4. 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.

CLUSTAL W (1.83) multiple sequence alignment

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Aterreus      YEIGRDSKVLPTAEKVTIRHVSSAPTPCGALFAAPPGEQSESTFCENHFLSVS----AA 355
Coccidiodes  -----QALPKGVKLSVRHVACPTTCEPIFSPFPGEPEPTKCNHFLAVSIRPEEAP 64
Spombe       -----ESILEGRKLSIYHLKSTLEKCPFLFGQS--KSKDFQFGSHLFLVE----- 50
Scerev       -----KVFFSLEVYVYVTLWDEADAERLIFVS--KADTNGYCNTRVSRVDITKILE 109
Umaydis      -----IGALEEQIFI TASWSPLTQANAPPDSDARDETEHTDNSPLLLILAMELYLYT 115
              :: : . . . .

Aterreus      SDEHDGAEVIVFGMEVLVYSTHLLTTFVSKADSTGYLHV---LKLPPRASVLRRIISGTF 412
Coccidiodes  TDTDSDVELLILSIEVLVYTRRLRATVVFVSKADSTGYLYL---LNQPRAKSLTRTVTFV 121
Spombe       -----EQNVIFVGMCEIVYKNEKFI VVFSKADSTGFG-----SKGVSCNSLAFCC 97
Scerev       FILSIDPNYLLQVKVPAIRSYKKSIPELISAASTPARTLR---ILARLQKSGSTVLKEI 166
Umaydis      IPEFGSAVLYVSKLDDSSGYAPQS IPTPLRQAVSTHVFTHKPKLFGNTLTATLSSAITQH 175
              : . : . . . .

Aterreus      LS-----YLVQTRQRPVGRVRLVLSLFAQAQNYLFPFSGIENPEKHVLLDRGLIKWVCRAV 466
Coccidiodes  LS-----SLLREYQRPGRIRLVLSLFAQAQNYLFPFSGIENVRKHHVLLDRGLIKWVCRAV 175
Spombe       VT-----LIDGLRQGAENVTLTFLFAIAQGYLFPESVDNGQKHVLDNSGLLRWVWVCL 151
Scerev       ESPRFQDQLVLSFTCPRELLTKICLFTRPASQYLFPSKSKNSKHHILNGEELMKWGFIL 226
Umaydis      TSFRHWSDTAQASAAVNVSHLSVHILARSQRAYLFPSSPENPKKVLSDAALIKWVRAVM 235
              : : : : . * * * * . * . * : * . * : * * .

Aterreus      DPILREYEPESGSHDK---GAADQAAESAKSSATAFLVPGCDKFETRGVFFPFAKTDD 522
Coccidiodes  DPILRDFEPEKHTNKGALKGEGPDHAEAVNTTATAYLIVPGLDQHESTRALFFSTARLDF 235
Spombe       EKLRKYITDSEAPNDS-----EKQNSLLEKAYLIVPGLEN--IRSYLPN----- 195
Scerev       DRLLLECFQN-----DTQAKLRIPGEDPARVRSYLRG----- 258
Umaydis      SDVVVATRDETSPIRA-----ADFKRQLINARAYVIVPGYTKLSEHFLVPLARQDSS 287
              . : . . . * * * * : * * .

Aterreus      -----KDRPRVWNSYPVRQLCDNPSA----- 543
Coccidiodes  -----KDKFRWLNSHPLYQVCANPGA----- 256
Spombe       -----RHWIESN-----AITTG----- 207
Scerev       -----MKYPLWQVGDIFT--SKENS----- 276
Umaydis      DSTTQPPSSDQVLRQANWYGHYPYSSNGAHCSSEDLPLPLHWHASSFCLRESDNHVTTV 347
              * .

Aterreus      -----PPRCLIPRFDDPKTRFLIDLDELPEQTESSGATEN----- 580
Coccidiodes  -----PPRCLVPRFDDPKARFLDLDDEEIFGHATTSTRNKTPRCVVDII 301
Spombe       -----KAVEELPRFDDPKCRYLCELQDEKSDMS----- 236
Scerev       -----LAVYNIPLFDDPKARFIHQLAEDRLLKVS----- 307
Umaydis      QTDPKSKDDYNPKRVRTVPTLMPHFCDDEPKTRFLDEMARDADHEHSGWKVKTSTASTASTA 407
              : * * * * * * * * : : :

Aterreus      -----AGQWRSVKSLDQFVEMMSFROECSAGRLVGFELWLVINPPGLVNSVQMT 628
Coccidiodes  DVT EASSQDHAATGRWFSVKSLEEFWEMMTFROECSAGRLVGFIMVNVNPPGLKSDGLD 361
Spombe       -----VEEFWDTLTYROECSGKLVGFETLQ----- 264
Scerev       -----LSSFVIELQEROEKFLSVTSSVMGISGY----- 335
Umaydis      STTASNGAVDDNVAQAQNGSKDQIDDLQLROEQHSPQDRNTKRAAKSDWQDKADKRT 467
              . . : . * * *

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**Supplemental Figure 5. 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:**

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Mosammaparast, N., Del Rosario, B. C., and Pemberton, L. F. (2005). Modulation of histone deposition by the karyopherin kap1 14. *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.