

Figure S1, Related to Figure 1. Sedimentation Equilibrium of human NatA.

(A) Log plot of sedimentation equilibrium experiments a sedimentation experiment showing theoretical monomer (blue) and theoretical dimer (red) slopes of hNatA ($OD_{A280}=0.8$) at 12,000 rpm (black).

(B) The corresponding residuals for all three speeds associated with A.



Figure S2, Related to Figure 1. $IP_6 F_o - F_c Map$. Human NatA-bound IP_6 density contoured to 3.5 σ (shown in green).



Figure S3, Related to Figure 1. Alignment of Naa15 Homologues. Sequence alignment of Naa15 homologues from *H. sapiens* (Hs); *S. pombe* (Sp); *S. cerevisiae* (Sc); *D. rerio* subunit b (Dr); *G. gallus* (Gg); *S. scrofa* (Ss); *P. troglydtes* Isoform XI (Pt); *M. musculus* (Mm); and *R. norvegicus* (Rn) demonstrating the divergence of the metazoan Naa15 C-termini (highlighted in yellow) from *Sp*Naa15.



Figure S4, Related to Figure 1. Alignment of Naa10 Homologues. Sequence alignment of Naa10 homologues from *H. sapiens* (Hs); *S. cerevisiae* (Sc); *S. pombe* (Sp); *A. sinensis* (As); and *D. rerio* subunit b (Dr). The dashed line indicates the truncation construct used for all experimental studies.



Figure S5, Related to Figure 1. C-terminal Truncation of Naa15 Abrogates NatA Binary Complex Formation. (A) S200 gel filtration chromatogram.

- (B) Corresponding SDS-PAGE gel to the trace of HIS-Naa 15_{FL} /Naa 10_{1-160} complex (red) for A.
- (C) Corresponding SDS-PAGE gel to the trace of HIS-Naa15 $_{\Delta 672-866}$ /Naa10 $_{1-160}$ complex (blue) for A.