Dechassa et al., Supplementary information

Supplementary Information

Supplementary Figure S1: Scm3 mediated Cse4-H4 tetramer formation on DNA is independent of Scm3 DNA binding activity and Scm3 is not stably associated with tetrasome after assembly. A. The N-terminal region of Scm3 is critical for DNA binding activity. Scm3 or $Scm3_{63-189}$ (1-4 µM) were incubated for 30 min at 25°C with 79 bp DNA (0.5 µM) in buffer containing 20 mM Tris-HCl pH 7.5, 300 mM NaCl, 1mM EDTA, 20µg/ml BSA and 4% glycerol. The reactions were analyzed by 5% native PAGE and ethidium bromide staining. Lane 1: DNA only control; lane 2-4: wild type Scm3; lanes 5-7: Scm3 $Scm3_{63-189}$. **B. The DNA binding activity of Scm3 is not important for assembly of tetrasome.** The experiment was conducted as in Figure 2. A preformed complex of Scm3-Cse4ΔN-H4 or Scm3 $Scm3_{63-189}$ -Cse4ΔN-H4, labeled on at H4 either Alexa-488 (green D), or Atto-647N (blue A); or a mixture of the differently labeled complexes (DA) as indicated, was incubated with a five molar excess of 79 bp DNA. Protein-DNA complexes were analyzed by 5% native PAGE followed by fluorescence scanning. Lanes 1: Scm3 $Scm3_{63-189}$ -Cse4ΔN-H4 (D); lanes 2: Scm3 $Scm3_{63-189}$ -Cse4ΔN-H4 (A); lane 3: Scm3 $Scm3_{63-189}$ -Cse4ΔN-H4 (D) and Scm3 $Scm3_{63-189}$ -Cse4ΔN-H4 (A); lane 4: Scm3-Cse4ΔN-H4 (D); lane 5: Scm3-Cse4ΔN-H4 (A); lane 6: Scm3-Cse4ΔN-H4 (D) and Scm3-Cse4ΔN-H4 (A); lane 7: Scm3-Cse4ΔN-H4 (D) and Scm3-Cse4ΔN-H4 (A, Scm3 labeled with Atto-647N); lane 8: Scm3 (Atto-647N labeled). 1 µM protein and 5 µM 79 bp DNA were used in each reaction. Green, blue and red gel scans are for donor, acceptor and FRET, respectively. *- Shows the acceptor label is on Scm3.

Supplementary Figure S2. CENP-A and H4 are compatible to form a heterotypic tetramer. A. Models for a Cse4-H3 four-helical bundle. The coordinates for Cse4 from the *Kluyveromyces lactis* $(Cse4\text{-}H4)_2$ tetramer structure (pdb 2YFW; (1)) were superimposed onto H3 from the yeast nucleosome structure (pdb 1ID3; (2)) using PYMOL. Only regions of H3 and Cse4 that form the four-helix bundle structure are shown. The residues that are critical for four-helical bundle are shown as sticks. **B.** Alignment of the region shown in (A) of H3 and CENP-A from various species highlights conservation of residues that are involved in four-helical bundle formation (H113, D123, L126 and L/I 130; indicated by black dots) among different organisms.

Supplementary Figure S3. In vitro reconstitution of heterotypic tetrasomes and nucleosomes.

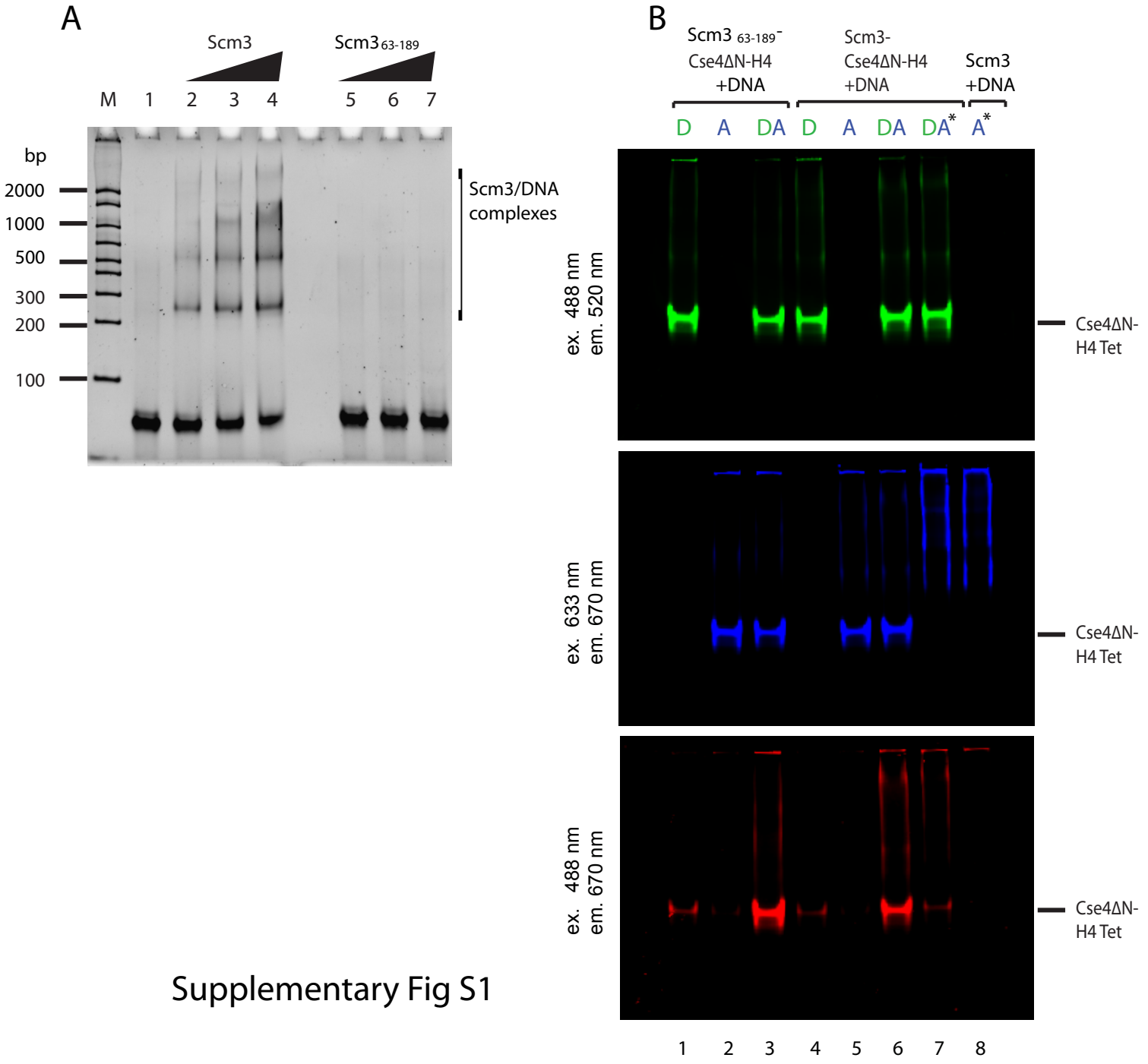
A. Cse4ΔN forms a tetramer with His_6.H3 and two copies of H4. Cse4ΔN, His_6.H3 and H4 were refolded and purified by gel filtration. Fractions 1-5 from the peak were analyzed by SDS-PAGE (inset).

B. Complexes containing Cse4ΔN-His_6.H3-$H4_2$ taken from gel filtration fraction # 3 (A) and control tetramers (His_6.H3-H4)$_2$, (H3-H4)$_2$ and (Cse4ΔN -H4)$_2$ were reconstituted onto 147 bp 601 DNA by salt dilution to form tetrasomes (lanes 2, 5, 8 and 11). Tetrasomes were reconstituted by salt dilution using 1.8 µM 147 bp DNA and 3.0-5.2 µM tetramers (1.7-2.9: 1 tetramer to DNA ratio). Nucleosomes were reconstituted as in tetrasomes assembly except that H2A-H2B dimers (3.7-5.6 µM) were also included in the reactions. Tet- refers to tetrasomes; Nuc- nucleosomes.

C. Tetrasome samples from (B) were purified by Ni-NTA affinity chromatography and analyzed by SDS-PAGE and Coomassie blue staining. IN- input, UB- Ni-NTA unbound, W3- third wash, W4- fourth/last wash, E- elution. Note that only a relatively weak band of Cse4ΔN is expected (and observed) due to the enrichment of homotypic (His_6.H3-H4)$_2$ tetrasome in addition to heterotypic Cse4ΔN- His_6.H3-$H4_2$ tetrasomes.

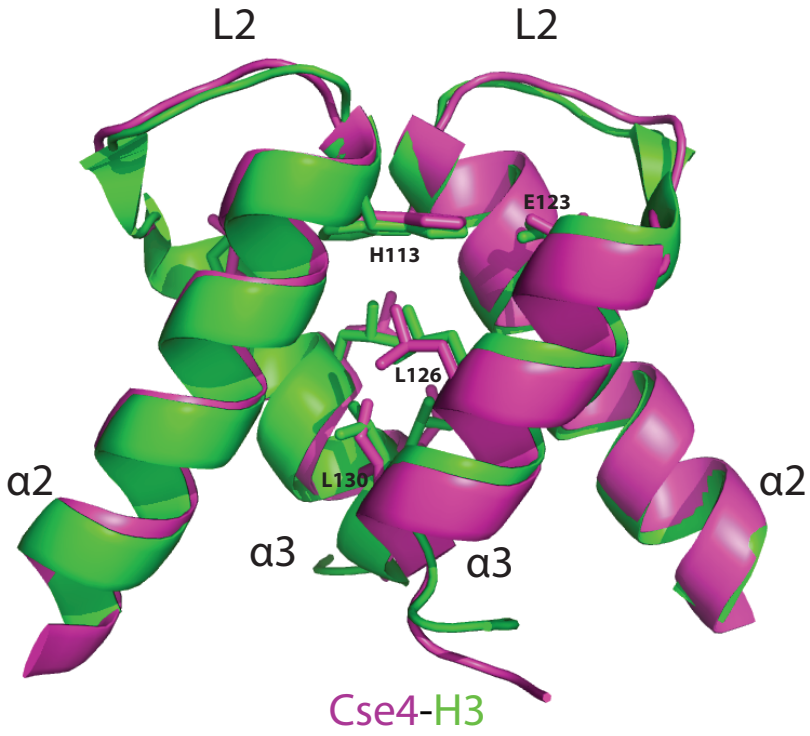
Supplementary References:

1. Cho, U.S. and Harrison, S.C. (2011) Recognition of the centromere-specific histone Cse4 by the chaperone Scm3. *PNAS*, **108**, 9367-9371.
2. White, C.L., Suto, R.K. and Luger, K. (2001) Structure of the yeast nucleosome core particle reveals fundamental changes in internucleosome interactions. *Embo J*, **20**, 5207-5218.



Supplementary Fig S1

A



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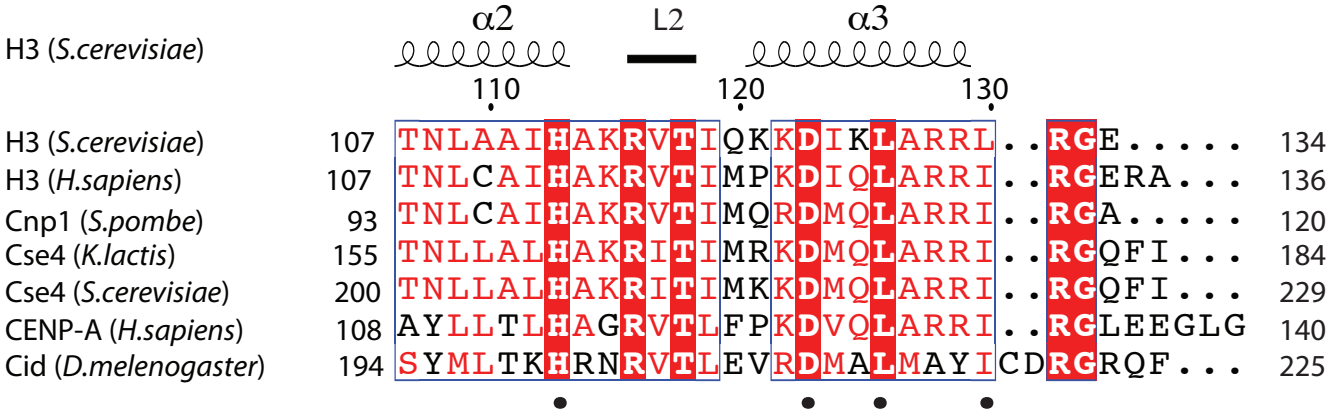
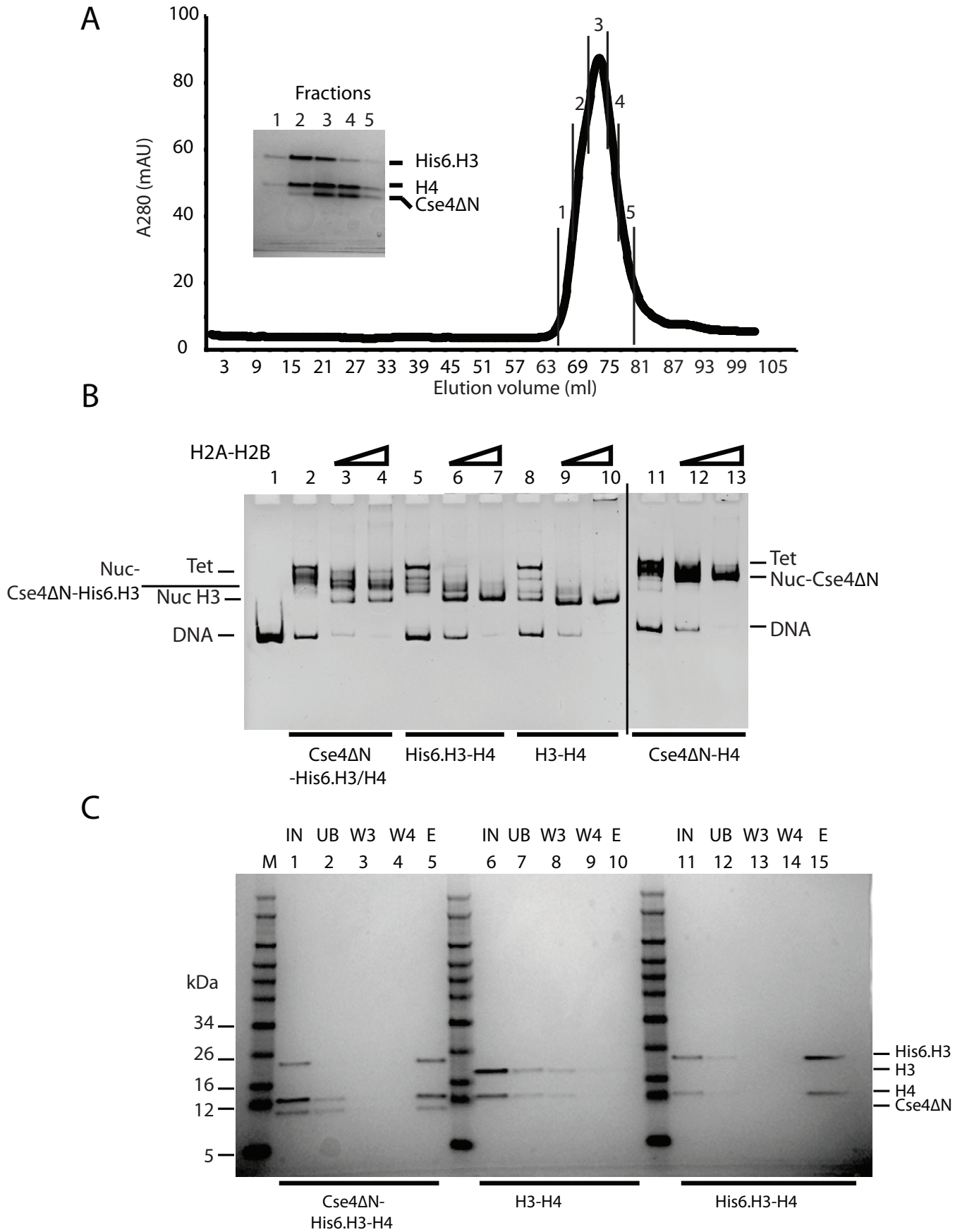


Supplementary Figure ~~2~~



Supplementary Figure 3