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## Supplemental information

# Virus-encoded histone doublets are essential and form nucleosome-like structures

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**Table S1.** Primers used in the MV histone knockout generation cloning and analysis. Related to Figure 2.

| Primer ID | Purpose | Sequence |
|---|---|---|
| HB112 | 5' homology arm for recombination 368 | tctcactaagacatatgacagcgtgccttccaaggt |
| HB113 | 5' homology arm for recombination 368 | gcccttgctcaccatgacaaaaccgcggatggga |
| HB114 | 3' homology arm for recombination 368 | cgcgtggtacctctaaaggccgccgagctcatgc |
| HB115 | 3' homology arm for recombination 368 | ccgggtcgactctagagttggatttgaagtttttccagtcg |
| HB120 | 5' homology arm for recombination 369 | tctcactaagacatacgagctgcatcgcaagaa |
| HB121 | 5' homology arm for recombination 369 | gcccttgctcaccatagatttgatggttttagttccctct |
| HB122 | 3' homology arm for recombination 369 | ttcacgcgtggtaccaggaccttggaaggcacgc |
| HB123 | 3' homology arm for recombination 369 | ccgggtcgactctagattagtatccggggctcattcc |
| HB145 | Histone 368 KO genotyping | cgttttcctcagcgaggaggaag |
| HB146 | Histone 368 KO genotyping | ctggtgacggccttggtc |
| HB147 | Histone 369 KO genotyping | atgcatgagctcggcggcc |
| HB148 | Histone 369 KO genotyping | cagaggacgatgcttgttgtctcgtc |
| HB155 | pEF1 (Nourseo) for transcomplementation | taatctagagtcgacccggaac |
| HB156 | pEF1 (Nourseo) for transcomplementation | atgggtggattggtggtgttg |
| HB157 | pEF1-mel368 (Nourseo) for transcomplementation | caccaatccacccatatgtcaaaagcaggcaaaaagg |
| HB158 | pEF1-mel368 (Nourseo) for transcomplementation | gtcgactctagattattatagcggaccatgttccagc |
| HB159 | pEF1-mel369 (Nourseo) for transcomplementation | caccaatccacccatatggcgacacaaaaggaaaccac |
| HB160 | pEF1-mel369 (Nourseo) for transcomplementation | gtcgactctagattattagtatccggggctcattccttg |
| GFP Fo | Histone KO genotyping | aggtgaacttcaagatccgc |
| GFP Rev | Histone KO genotyping | gaacttgtggccgtttacgtc |

**Table S2**. Analysis of AFM data. Samples were imaged by AFM with heights (nm) of nucleosome like particles (NLP) recorded separately from free DNA. Average heights and standard deviations were calculated from the number of incidences (n). In addition to the average height of the NLP, the amount of free DNA (as a percent of particles on the slide) is a good indication of the stability of MV-NLP. When not prepared by GraFix, NLP heights generally correspond with heights of the MV-Tetrasome (p-values from unpaired t-test), whereas GraFix preparation yields heights in agreement with the $eNuc_{147}$ system. Related to Figure 3.

| | **MV-H4-H3-DNA (MV-tetrasome)** | | **$eNuc_{147}$** | | **MV-$NLP_{147}$** | | **MV-$NLP_{207}$** | | **MV-$NLP_{207}$ GraFix** | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Particle Class** | **Free DNA** | **MV-Tetrasome** | **Free DNA** | **eNuc** | **Free DNA** | **MV-NLP** | **Free DNA** | **MV-NLP** | **DNA** | **MV-NLP** |
| **Average Height (nm)** | 0.60 | 1.35 | 0.61 | 2.09 | 0.50 | 1.21 | 0.78 | 1.55 | 0.64 | 2.25 |
| **Heights σ (nm)** | 0.07 | 0.33 | 0.21 | 0.52 | 0.12 | 0.91 | 0.08 | 0.40 | 0.19 | 0.63 |
| **n** | 25 | 26 | 2 | 41 | 29 | 46 | 41 | 37 | 31 | 120 |
| **% Particle.** | 43.9 | 45.6 | 4.3 | 87.2 | 32.6 | 51.7 | 50.6 | 45.7 | 20.0 | 77.4 |
| **p-value (MV-Tet)** | --- | --- | --- | < 0.0001 | --- | 0.4528 | --- | 0.0403 | --- | < 0.0001 |
| **p-value ($eNuc_{147}$)** | --- | < 0.0001 | --- | --- | --- | < 0.0001 | --- | < 0.0001 | --- | 0.1452 |

**Table S3.** Summary of cryoEM data collection and refinement. Related to Figure 4.

| | MV-NLP$_{147}$ (EMD-24238) (PDB: 7N8N) | MV-NLP$_{207}$ |
|---|---|---|
| **Data collection and processing** | | |
| Magnification | 64,000 | 29,000 |
| Voltage (kV) | 300 | 200 |
| Electron exposure (e–/Å$^2$) | 50 | 80 |
| Defocus range (μm) | 0.8-2.0 | 1.0-2.5 |
| Pixel size (Å) | 1.065 | 1.219 |
| Symmetry imposed | C1 | C1 |
| Initial particle images (no.) | 2,648,493 | 2,131,563 |
| Final particle images (no.) | 34,418 | 377,702 |
| Map resolution (Å) | 3.95 | 6.1 |
| FSC threshold | 0.143 | 0.143 |
| Map resolution range (Å) | 3.9-6.9 | 6.0-10.0 |
| | | |
| **Refinement** | | |
| Initial model used (PDB code) | 1AOI, 3LZ0 | |
| Model resolution (Å) | 4.1 | |
| FSC threshold | 0.143 | |
| Model resolution range (Å) | 4.1-7.3 | |
| Map sharpening *B* factor (Å$^2$) | | |
| Model composition | | |
| Non-hydrogen atoms | 11125 | |
| Protein residues | 789 | |
| Nucleotide | 250 | |
| Ligands | 0 | |
| *B* factors (Å$^2$) | | |
| Protein | n/a | |
| Nucleotide | n/a | |
| Ligand | n/a | |
| R.m.s. deviations | | |
| Bond lengths (Å) | 0.006 | |
| Bond angles (°) | 0.840 | |
| Validation | | |
| MolProbity score | 2.38 | |
| Clashscore | 22.42 | |
| Poor rotamers (%) | 0.00 | |
| Ramachandran plot | | |
| Favored (%) | 90.38 | |
| Allowed (%) | 9.62 | |
| Disallowed (%) | 0.00 | |

**Table S4: Comparison of MV-NLP$_{147}$ with eNuc$_{147}$.** "Global" root-mean-squared deviation (RMSD) values represent changes in positioning throughout the nucleosome-like complex and are calculated for each domain after least-squares fitting the cores of the MV-NLP$_{147}$ and eNuc$_{147}$ complexes, whereas "local" RMSDs are values calculated when least-squares fitting individual domains prior to calculation and thereby represent the degree of internal re-ordering of the domain. Distances are calculated as the separation of geometric centers of backbone positions (C, $C_\alpha$, and N atoms), except for the H2A L1 Loops "closest distance", which defines the smallest distance between heavy atoms in the two moieties. The H2B $\alpha_C$ orientation angle is calculated around the pseudo-dihedral defined in Figure S5A. Related to Figure 5.

| **RMSD Values (Å)** | **Global** | **Local** |
|---|---|---|
| Particle Core | 2.7 | --- |
| DNA (central 120 bp) | 3.1 | 3.1 |
| Octamer Fold | 2.3 | 2.3 |
| H4-H3 Tetramer | 2.1 | 1.2 |
| H3 $\alpha_N$-helix (Chain A) | 2.3 | 0.3 |
| H3 $\alpha_N$-helix (Chain E) | 2.2 | 0.3 |
| H2B-H2A Dimers (both) | 2.5 | 2.1 |
| H2B-H2A Dimer (Chain C,D) | 2.5 | 1.6 |
| H2B-H2A Dimer (Chain G,H) | 2.5 | 1.6 |
| H2B $\alpha_C$-helix (chain D) | 2.6 | 0.5 |
| H2B $\alpha_C$-helix (chain H) | 3.4 | 0.9 |
| H2A Docking Domain (Chain C) | 8.5 | 3.9 |
| H2A Docking Domain (Chain G) | 6.2 | 3.3 |
| | | |
| **Separation Distances (Å)** | **eNuc$_{147}$** | **MV-NLP$_{147}$** |
| H4-H3 Dimers | 32.6 | 33.3 |
| H3-H3' 4HB Interface | 10.1 | 10.4 |
| H2B-H2A Dimers | 36.3 | 38.6 |
| H2B $\alpha_C$-helices | 49.1 | 52.2 |
| H2A L1 Loops (centers) | 7.2 | 9.7 |
| H2A L1 Loops (closest distance) | 2.9 | 5.3 |
| | | |
| **Orientation Angle (°)** | **eNuc$_{147}$** | **MV-NLP$_{147}$** |
| H2B $\alpha_2$-helices | 135.4 | 143.9 |