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Supplementary Information for

SIR proteins create compact heterochromatin fibers

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Supplementary Information Text

SI Materials and Methods

Proteins

FLAG-tagged Sir3 protein and TAP-tagged Sir2/4 complex were individually overexpressed and affinity purified from yeast. Briefly, yeast cultures transformed with plasmids contained tagged proteins under a galactose-inducible promoter were grown to OD 0.6 and induced with 2% galactose for 5 hours. Cultures were pelleted, resuspended in E Buffer (20 mM HEPES pH 7.4, 350 mM NaCl, 10% glycerol, 0.1% Tween 20, and protease inhibitors), and frozen in liquid nitrogen. Pellets were ground using a cold mortar and pestle with frequent additions of liquid nitrogen until approximately 50% of cells appeared lysed under a microscope. Cells were incubated on ice in E buffer for 30 min, then spun at 3,000 rpm for 15 minutes to remove debris. Supernatant was spun down at 40,000 rpm for 1 hour, then the aqueous layer was removed from the lipid layer using a syringe. For Sir3 purification, lysate was incubated with anti-Flag resin from Sigma for three hours at 4°C. Resin was washed in E buffer, then Sir3 was eluted in batch via four 30 minute incubations of resin with E Buffer containing 100 μg/mL 3xFLAG peptide from Sigma. For Sir2/4 purification, lysate was incubated with IgG resin for 2 hours, washed in E buffer, then eluted in batch via the addition of purified TEV protease overnight. Eluted Sir2/4 was then bound in batch to Calmodulin resin for 2 hours in the presence of Ca^{2+}, washed in E buffer, and eluted with EGTA. Concentrations were determined by comparison to known concentrations of BSA electrophoreses on the same Coomassie-stained SDS-PAGE gel. The Sir2/4I1311N plasmid was generated by site-directed mutagenesis, and purified as above. All Sir2/4

was dialyzed into 20 mM sodium phosphate buffer pH 8.0 prior to use in order to maintain moderate concentrations of salt across experiments. Recombinant *Xenopus laevis* histones were expressed in BL21 cells, purified, and assembled into histone octamers according to standard protocols.

DNA

The 601-177-12 nucleosomal array template containing twelve copies of the Widom 601 nucleosome positioning sequence was digested from its plasmid backbone using EcoRV and purified by size-exclusion chromatography. The 601-177-36 fragment was generated by HaeII and XbaI digestion of the 601-177-36 plasmid and purified as above. The 36x601 multinucleosomal array sequence was generated in three assembly steps. First, 12x601 sequences were generated via golden gate assembly[45,46] from individual monomers. Briefly, the 601 nucleosome positioning sequence was first PCR amplified with primers carrying a unique barcode sequence and BsaI cleavage site. Twelve of these 601 PCR amplicons were assembled via golden gate as an array (12x601) into the pFUS-A backbone[47] via BsaI digestion and ligation to generate three different arrays (MA-1, MA-2 & MA-3). Next, two 12x601 sequences were concataned to generate 24x601 sequences as follows: one of the 12x601 plasmids (MA-1) was digested with SpeI and SphI and the backbone was recovered; a second 12x601 plasmid (MA-2) was digested with XbaI and SphI and the released 12x601 array was recovered; then, these two components were ligated. This process was repeated using the MA-3 12x601 array to generate the 36x601 sequence.

Nucleosomal array assembly

Nucleosomal arrays were assembled by combining recombinant histone octamers and 601-177-12 or 601-177-12 DNA template at varying molar ratios of octamer to nucleosome positioning sequence in 2 M NaCl, and step-wise salt dialysis was performed until completion into 20 mM sodium phosphate pH. 8.0 with 0.1 mM EDTA. Array saturation was determined by ScaI digestion followed by analysis via native PAGE and by SV-AUC.

EMSA

300 ng WT or H4-K16Q nucleosomal array was combined with Sir2/4 at a ratio of 1, 2, or 3 molecules per nucleosome to a final concentration of 10 ng/ul array and 5% glycerol. For combined Sir2/4 and Sir3 EMSA, Sir2/4 or Sir2/4I1311N was added at 2 molecules per nucleosome, and Sir3 was titrated at 1, 2, 4, and 6 molecules per nucleosome. Binding reactions were incubated at room temperature for 30 minutes, run on 1% TBE agarose gels, and stained with ethidium bromide.

SV-AUC

SV-AUC was carried out using 400 µl sample loaded into two-sector Epon centerpieces in an An60 Ti rotor in a Beckman Optima XL-I analytical ultracentrifuge, and run at 20°C. Measurement was completed in intensity mode. Nucleosomal arrays were run at 10 ng/ul concentrations with the indicated amounts of Sir3 or Sir2/4 at 20,000 RPM, and were measured at 215 nm (for arrays alone) or 260 nm (for samples containing SIR proteins). For experiments containing all three SIR proteins, Sir2/4 was added first,

followed by array, followed by Sir3. For $\bar{\nu}$ determination, three preparations of sample were run as above, with 0, 25, or 50% $H_2{}^{18}O$ (obtained from Cambridge Isotope Laboratories, Andover, MA) added in place of $H_2{}^{16}O$. The obtained S values were then plotted as a function of solvent densities, linear regression was performed, and the $\bar{\nu}$ was calculated by dividing the slope of the resulting line by the y-intercept. Solvent densities and viscosities were obtained from the literature. Linear regression was performed using GraphPad Prism software.

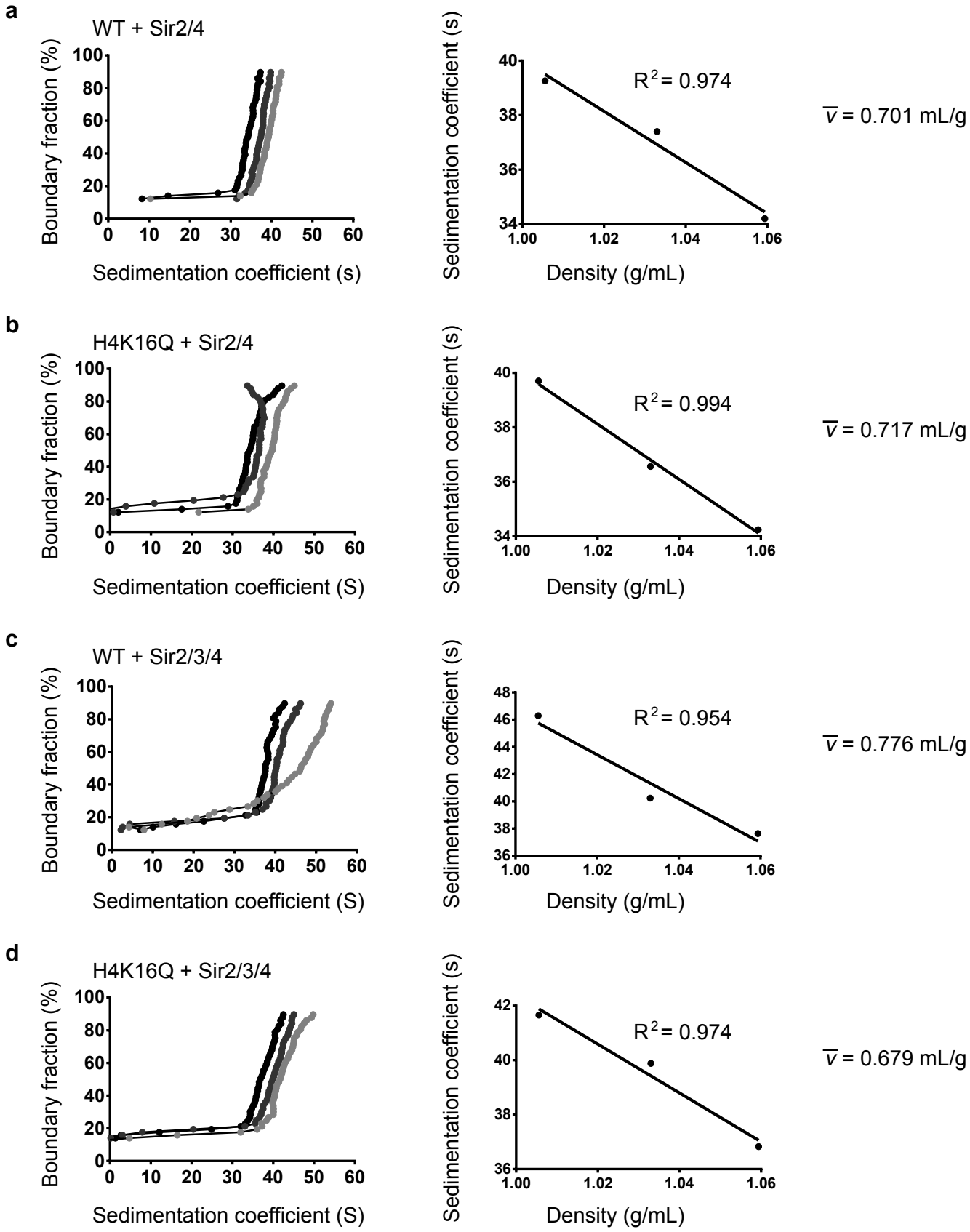
2DSA/GA-MC

All SV-AUC data were analyzed using UltraScan3 software, version 3.3 and release 1977 (http://www.ultrascan3.uthscsa.edu/index.php), and fitting procedures were completed on XSEDE clusters at the Texas Advanced Computing Center (Lonestar, Stampede) and at the San Diego Supercomputing Center (Trestles) through the UltraScan Science Gateway (https://www.xsede.org/web/guest/gateways-listing). Raw intensity data were converted to pseudo-absorbance by using the intensity of the air above the meniscus as a reference and edited. As previously described,[21] partial specific volumes ($\bar{\nu}$) of each of the chromatin fibers were determined experimentally (**Fig. S1**). Next, 2DSA was performed to subtract time-invariant noise and the meniscus was fit using 10 points in a 0.05 cm range. Arrays were fit using an S range of 5-60 S, an f/f_0 range of 1-10 with 100 grid points for each, 10 uniform grid repetitions, 400 simulation points, and meniscus fitting within a 0.6 cm range with 10 points. 2DSA was then repeated at the determined meniscus to fit radially-invariant and time-invariant noise together using 5 iterations. vHW analysis was completed using these noise subtraction profiles to determine S.

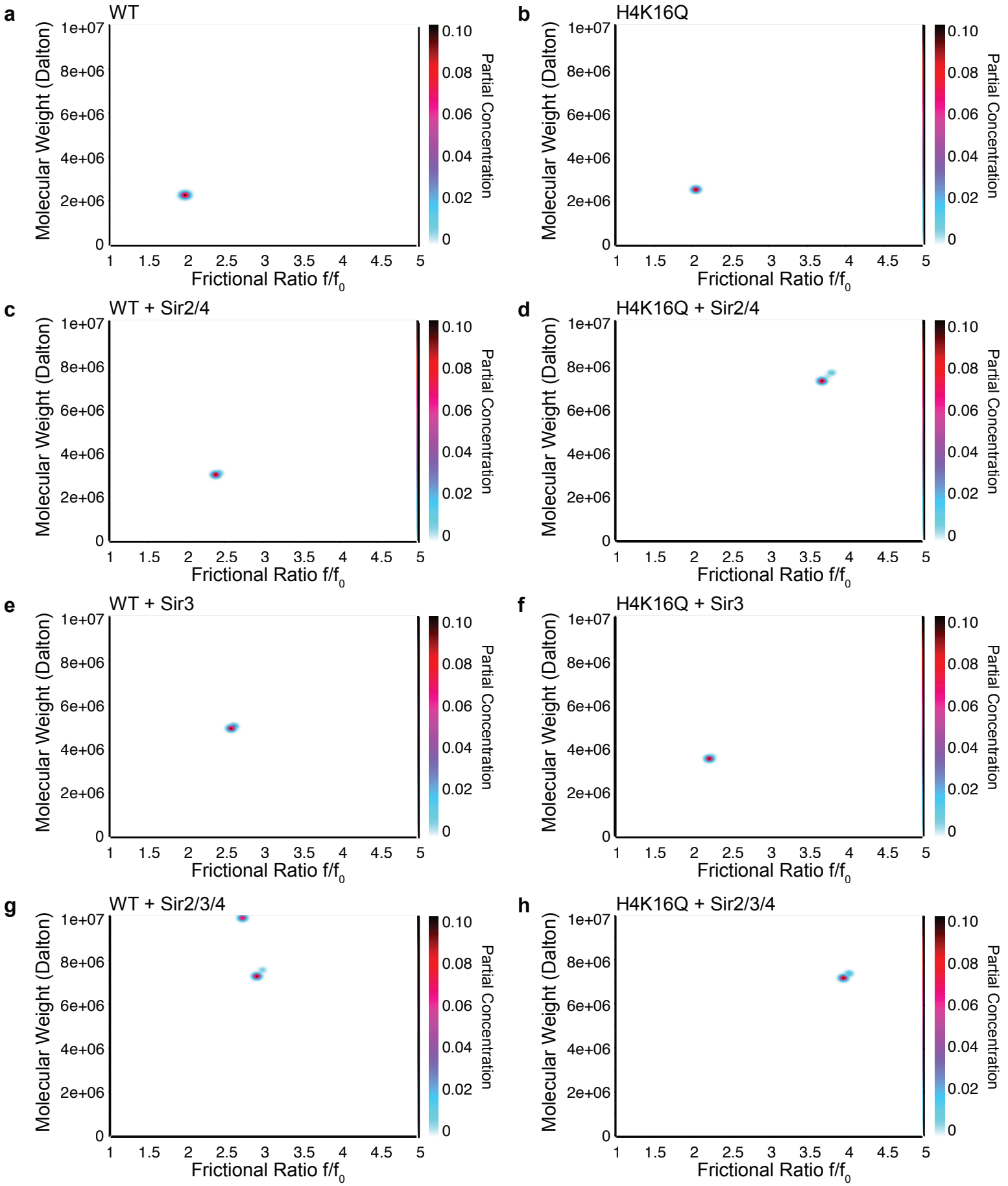
Where indicated, GA was initialized by binning major solutes in the 2DSA dataset, and run via LIMS. Major solutes from GA analysis were then binned and run again using GA with 50 MC iterations.

AFM

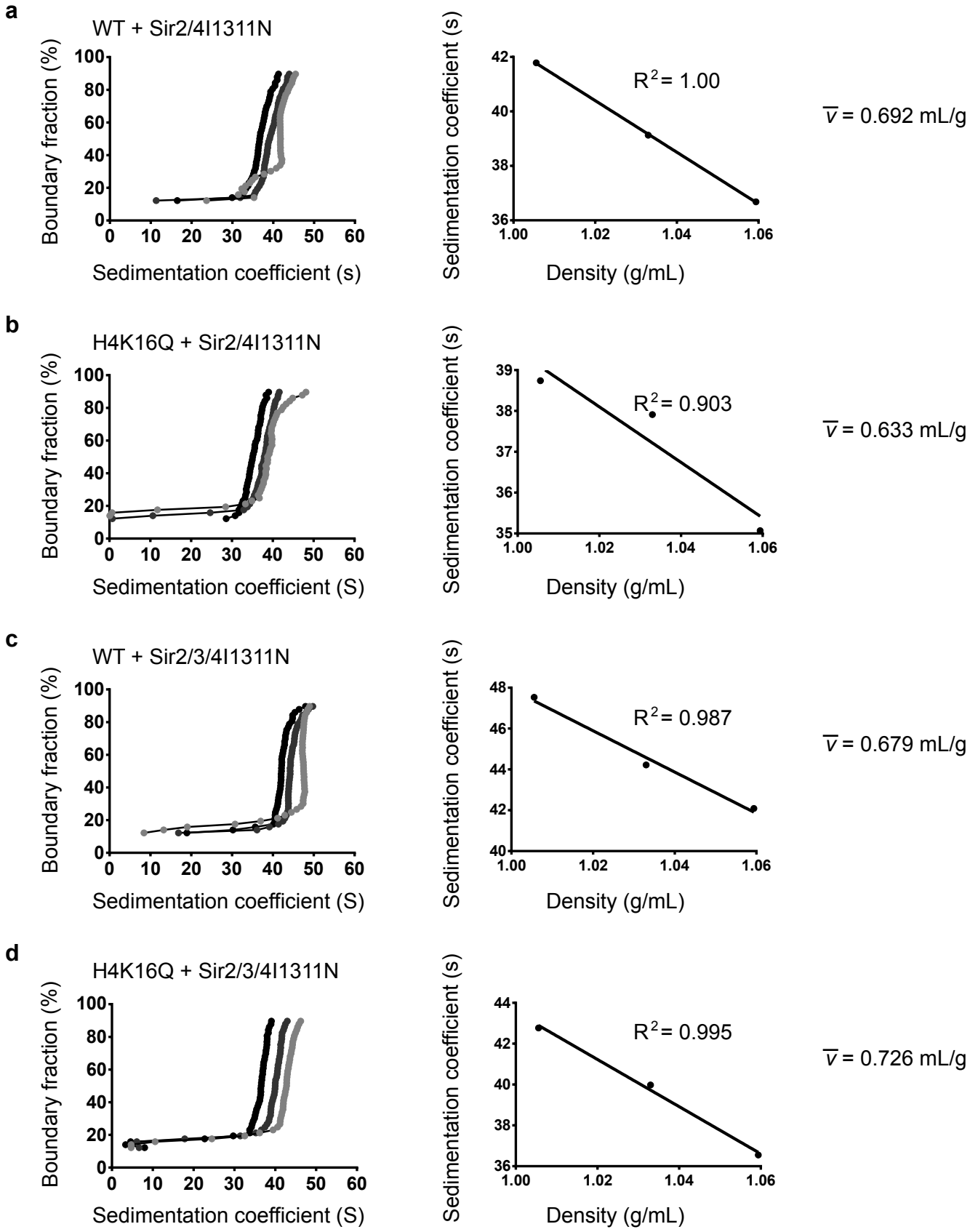
For atomic force microscopic experiments, an Agilent AFM 5500 instrument and silicon nitride cantilevers were used (force constant 25-75 N/m, resonant frequency 332 kHz). Imaging was done in air using the acoustic AC mode with an amplitude of ~10 nm and a set-point reduction of about 10%, scanning at 1 line per second. Immobilization of chromatin arrays on mica surface was done as follows. First, Sir3 or Sir2/4 was added to phosphate buffer followed by addition of 10 ng/ul chromatin array and mixed gently, maintaining a ratio of 4 Sir3 or Sir2/4 molecules/nucleosome. For imaging with both Sir3 and Sir2/4, Sir2/4 was added first, then arrays, followed by Sir3, at a ratio of 2 Sir3's and 2 Sir2/4's per nucleosome. After 30 minutes, 0.5% glutaraldehyde solution (1 μL) was added to this mixture for crosslinking and incubated for 10 minutes. APTES was deposited on freshly cleaved mica substrate using vapor deposition. The crosslinked chromatin solution was diluted to 1 ng/μL and 3 μL was added to this APTES modified mica surface and after 5 minutes the surface was cleaned three times using 400 μL of buffer solution, dried carefully using argon gas and immediately used for imaging. To image only chromatin arrays, the first mixing step with SIR proteins was omitted, and imaging was carried out in the indicated buffer. Nucleosomal heights were measured using Gwyddion software, and then mean heights were computed for each experimental condition.



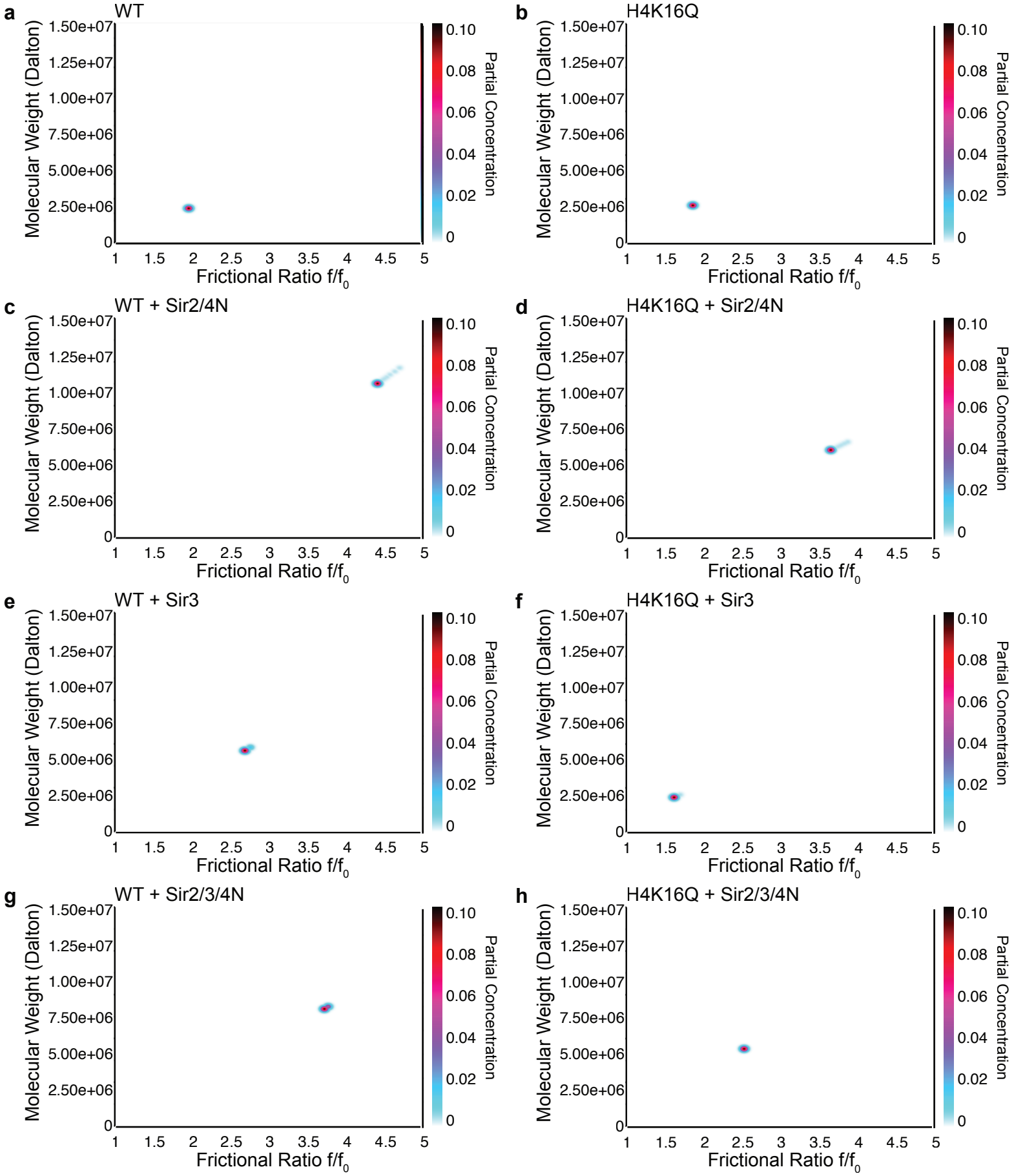
Supplementary Figure 1. The partial specific volume of WT and H4K16Q arrays with Sir2/4. (a-d) vHW plots showing the sedimentation of molecules in 0% (light gray), 25% (dark gray), and 50% H2O18 (black) and plots of sedimentation coefficient vs. density. The $\overline{v}$ is calculated by dividing the slope of the fit line by the y-intercept.



Supplementary Figure 2. Genetic algorithm results for WT and H4K16Q arrays with SIR proteins. (a-h) Pseudo-3D plots showing the f/f_0 ratio and molecular weight modeled by GA-MC analysis for the indicated arrays and SIR proteins.



Supplementary Figure 3. The partial specific volume of WT and H4K16Q arrays with Sir2/4I1311N. (a-d) vHW plots showing the sedimentation of molecules in 0% (light gray), 25% (dark gray), and 50% H2O18 (black) and plots of sedimentation coefficient vs. density. The $\overline{v}$ is calculated by dividing the slope of the fit line by the y-intercept.



Supplementary Figure 4. Genetic algorithm results for WT and H4K16Q arrays with WT and mutant SIR proteins. (a-h) Pseudo-3D plots showing the f/f_0 ratio and molecular weight modeled by GA-MC analysis for the indicated arrays and SIR proteins.

Supplemental Table 1

t-test results comparing the nucleosomal heights measured by AFM under different conditions for WT and H4K16Q arrays

Parameters	P value
WT in Tris vs WT in 1 mM $MgCl_2$	<0.0001
WT vs H4K16Q in phosphate	0.4826
WT+Sir3 vs H4K16Q+Sir3	<0.0001
WT+Sir2/4 vs H4K16Q+Sir2/4	0.5056
WT+Sir2/3/4 vs H4K16Q+Sir2/3/4	<0.0001

* P value <0.05 implies significant statistical differences for the parameters compared