Materials and Methods:

Preparation of proteins and nucleosomal arrays:

All proteins were expressed and purified via a FLAG epitope tag on PSC as described *(1)*. Proteins were 60-90% homogeneous as determined by Colloidal Blue (Invitrogen) staining (Fig.S1A,C). Active protein concentrations were determined by DNA binding as described in Francis et al. (2001). Preparations of PCC are typically 40-60% active, and PSC preparations 20-30% active. For PSC^{1-572} and PCC assembled with PSC^{1-572}, total protein concentration was used as these preparations have low DNA binding activity (N.J.F., unpublished observation).

Most experiments were carried out on 12-nucleosome array templates, assembled with HeLa histones as described *(2, 3),* but dialyzed into HEN (10mM Hepes, pH 7.9, 0.25mM EDTA, 2.5mM NaCl) or HE. This template (G5E4) consists of two sets of 5 5S nucleosome positioning sequences flanking two unpositioned nucleosomes that encode 5 Gal4 sites and the E4 promoter (described in 4). For experiments with shorter arrays of nucleosomes (Fig.4, Fig.S6), a series of templates consisting of tandem repeats of 5S positioning sequences linked by 25 base pairs were constructed. The centrally located nucleosome was mutated to contain unique PstI and HhaI sites. The short arrays (2N, 3N, 4N, 5N, 6N where N is nucleosomes) were cloned as multimers. Templates were then isolated by restriction enzyme digest followed by Sephacryl-S1000 DNA chromatography. Plasmids and detailed maps are available on request.

Trypsinized histones were prepared essentially as described *(5),* based on the protocol of Ausio et al. *(6)*. Briefly, polynucleosomes were isolated from HeLa nuclear

pellet by limited micrococcal nuclease digestion followed by Sepharose CL-4B (Pharmacia) chromatography. Limited trypsinization (Sigma, cat # T8003) was carried out on concentrated polynucleosome fractions and stopped with a 5-10 fold excess of soybean trypsin inhibitor (Sigma, cat # T9003). Histones were then purified by hydroxyapatite chromatography with the highest NaCl wash at 600mM *(5)* (Fig.S2B). Mock-trypsinized histones were prepared under identical conditions (Fig.S2B). Two preparations of trypsinized histones were prepared and behaved similarly. To analyze histone composition of assembled arrays, ~50µ g array was separated from free histones on a 2ml 10-30% glycerol gradient centrifuged 15 hours at 15,000 RPM in a TL-100 mini ultracentrifuge. 300 µl fractions were collected from the bottom of the gradient, and DNA containing fractions were pooled, TCA precipitated, separated on an 18% SDS-PAGE and stained with Colloidal Blue (Fig. S2B).

Restriction Enzyme Accessibility (REA) and microcentrifuge assays:

REAs were carried out as described *(1)* (Fig.S2D, Fig.S6A). For microcentrifuge assays *(7)*(Fig. S2C), reactions were set up under identical conditions as for EM. Reactions were spun at maximal speed (14,000rpm) in a microcentrifuge at 4 ˚C; pellets and supernatants were digested with proteinase K (as for REAs) and analyzed by Southern blotting with an oligonucleotide probe corresponding to part of the 5S sequence.

Electron microscopy:

Binding reactions were carried out in 15 or 30mM KCl and in the absence of divalent cations unless otherwise indicated. The addition of NP40 to 0.025% was

important to prevent protein aggregation. Nucleosomal arrays were added to reactions to a final concentration of 10-20nM nucleosomes (assuming 12 nucleosomes per array). For analysis of PCC effects on bare DNA, the DNA used to assemble nucleosomal arrays was used at the same template concentration as for arrays. Proteins were centrifuged at full speed for 5 minutes at 4˚C in a microcentrifuge to remove any aggregates, and diluted appropriately into BC300N (20mM Hepes, 0.2mM EDTA, 20% glycerol, 0.05% NP40, 300mM KCl). Binding was carried out for 5-10 minutes at room temperature, typically in 20µl total volume. Glutaraldehyde was added to a final concentration of 0.1% and samples were fixed at least 12 hours on ice before being dialyzed into HEN and mixed with glycerol for shadowing. Concentrations of PCC and PSC indicated refer to active concentrations as determined by fraction active for DNA binding *(1),* except for PSC^{1-572} or PCC made with PSC^{1-572} for which total protein concentrations were used. Most of the EM experiments were carried out at ratios of PCC to nucleosomes expected to produce less than 50% inhibition in solution assays, since higher concentrations tend to induce formation of oligomers that are not retained in the sample processing.

To protect against structural collapse, most fixed samples were dried from glycerol under high vacuum and shadowed with platinum as described *(8, 9)*, although negative staining with uranyl acetate and positive staining with phosphotungstic acid (PTA) were also used (Fig. 2A,B is PTA stained material). Visualization by EM was carried out essentially as described *(10, 11)*. Images were collected digitally with a 2K CCD camera (TVIPS) on a Tecnai 12 TEM at 100kV. Dark field optics were used for all images except Fig.S1B-D.

Data analysis:

For each sample of each experiment, 30-60 images were collected. Subsequently, maximal diameter measurements and number of particles/array were made using Image J software. Once an area with high quality material was identified, the entire area was photographed systematically, and all structures that could be identified as single arrays were used in the analysis.

Scanning Transmission EM:

STEM analysis was carried out at the Brookhaven National Laboratory on the same material used for EM analysis, essentially as described *(12)*. Scattering data were collected at $1nm^2$/pixel and particle masses were measured using TMV as an internal standard *(13)*. For PCC alone, 14% of the particles measured were greater than 500kDa in mass and not included in the analysis (Fig.4C). For 6-nucleosome arrays + PCC, masses above 5MDa (which presumably are oligomers of multiple arrays), were not included (Fig.S6G).

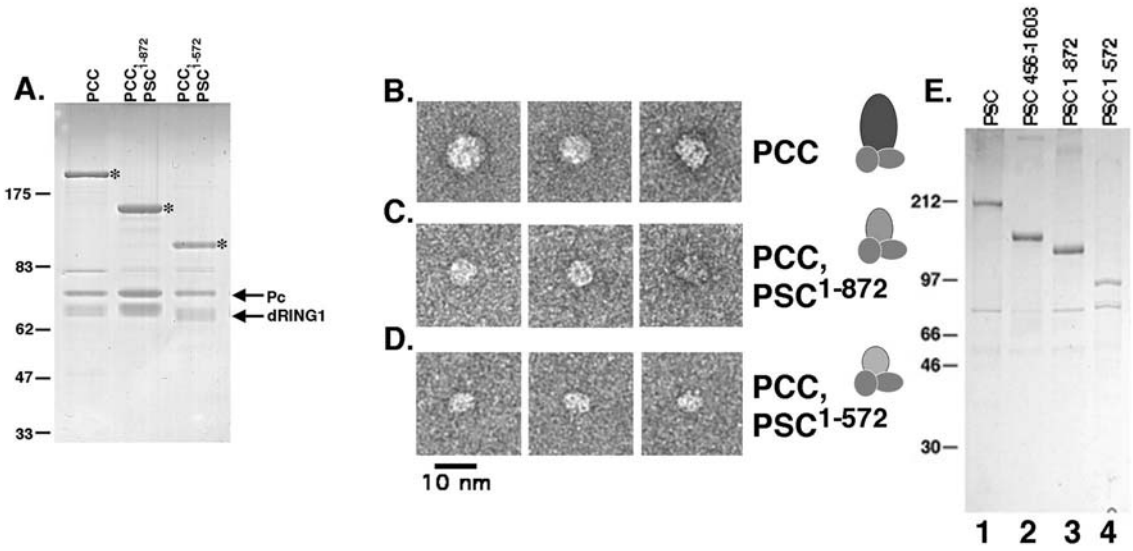
A.
PCC
PCC, PSC1-872
PCC, PSC1-572
175
83
62
47
33
Pc
dRING1
B.
PCC
C.
PCC, PSC1-872
D.
PCC, PSC1-572
10 nm
E.
PSC
PSC 456-1 603
PSC 1-872
PSC 1-572
212
97
66
46
30
1 2 3 4

Figure S1

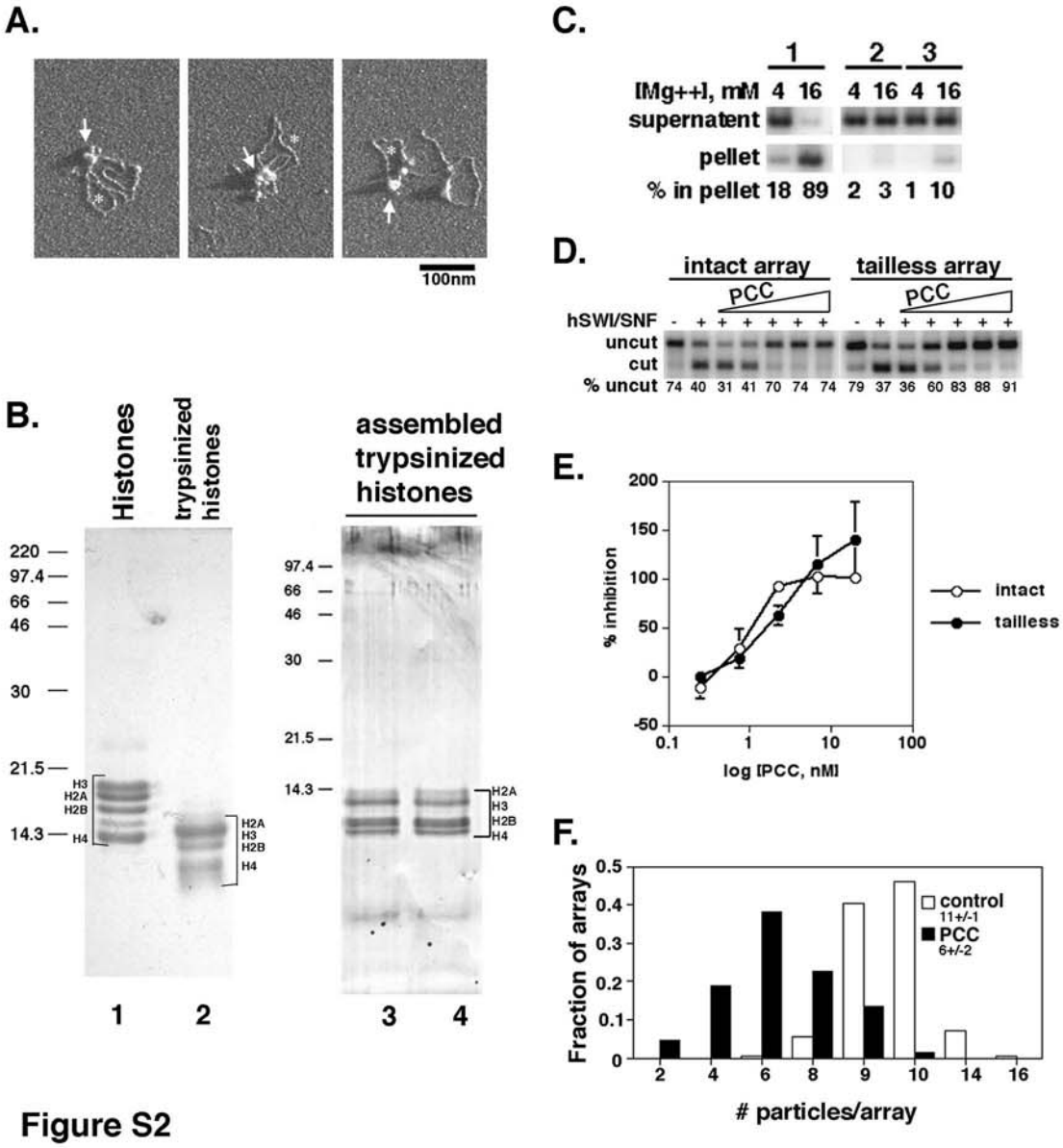
A.
100nm
B.
Histones
trypsinized histones
assembled trypsinized histones
220
97.4
66
46
30
21.5
14.3
H3
H2A
H2B
H4
1
2
3
4
C.
1
2
3
[Mg++], mM 4 16 4 16 4 16
supernatent
pellet
% in pellet 18 89 2 3 1 10
D.
intact array
tailless array
PCC
hSWI/SNF
uncut
cut
% uncut 74 40 31 41 70 74 74 79 37 36 60 83 88 91
E.
% inhibition
log [PCC, nM]
intact
tailless
F.
Fraction of arrays
particles/array
control
11+/-1
PCC
6+/-2

Figure S2

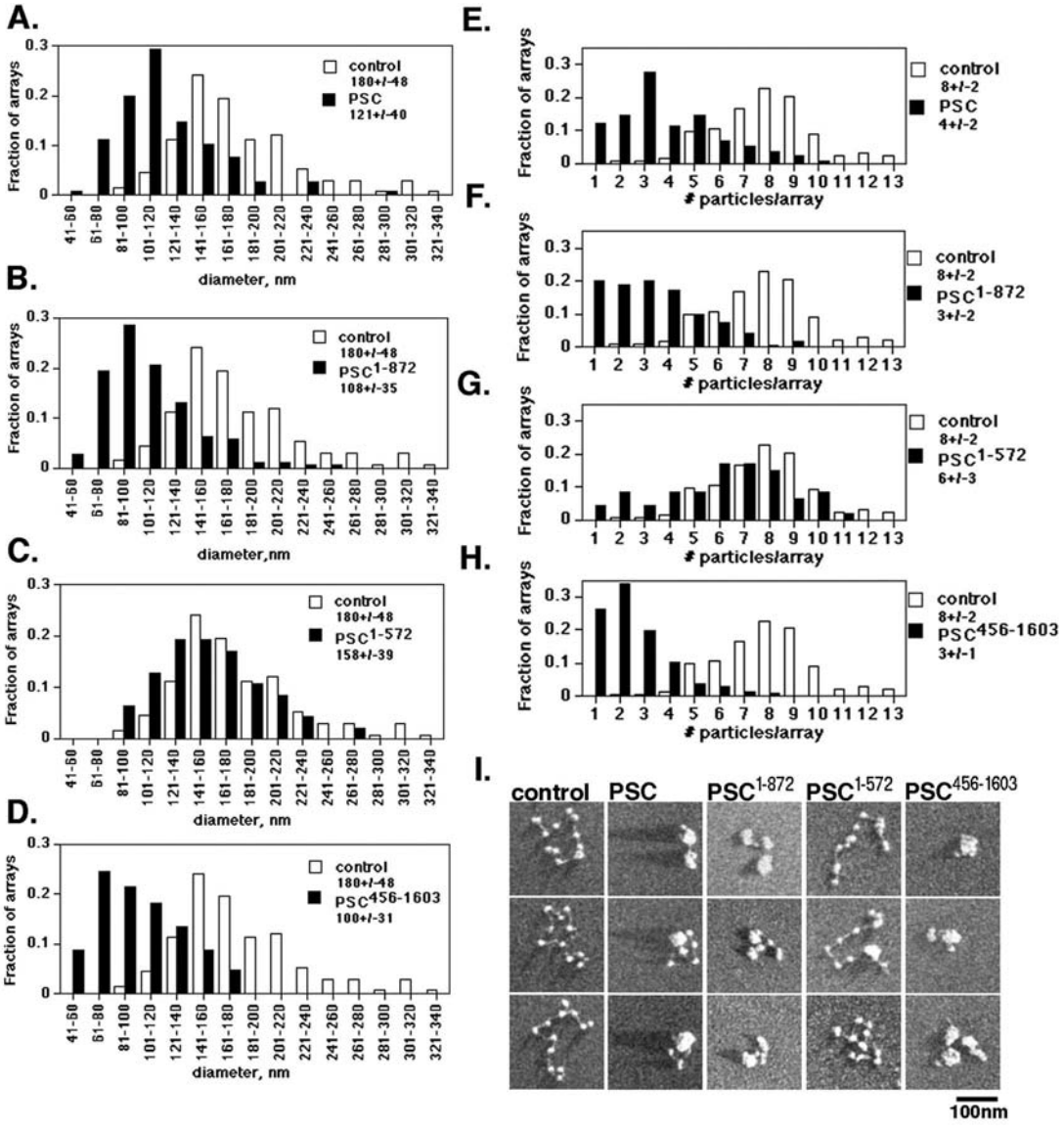
A.
Fraction of arrays
control
180+/-48
PSC
121+/-40
diameter, nm
B.
control
180+/-48
PSC1-872
108+/-35
diameter,nm
C.
control
180+/-48
PSC1-572
158+/-39
diameter, nm
D.
control
180+/-48
PSC456-1603
100+/-31
diameter, nm
41-60
61-80
81-100
101-120
121-140
141-160
161-180
181-200
201-220
221-240
241-260
261-280
281-300
301-320
321-340
E.
control
8+/-2
PSC
4+/-2
F.
control
8+/-2
PSC1-872
3+/-2
G.
control
8+/-2
PSC1-572
6+/-3
H.
control
8+/-2
PSC456-1603
3+/-1
particles/array
I.
control
PSC
PSC1-872
PSC1-572
PSC456-1603
100nm

Figure S3

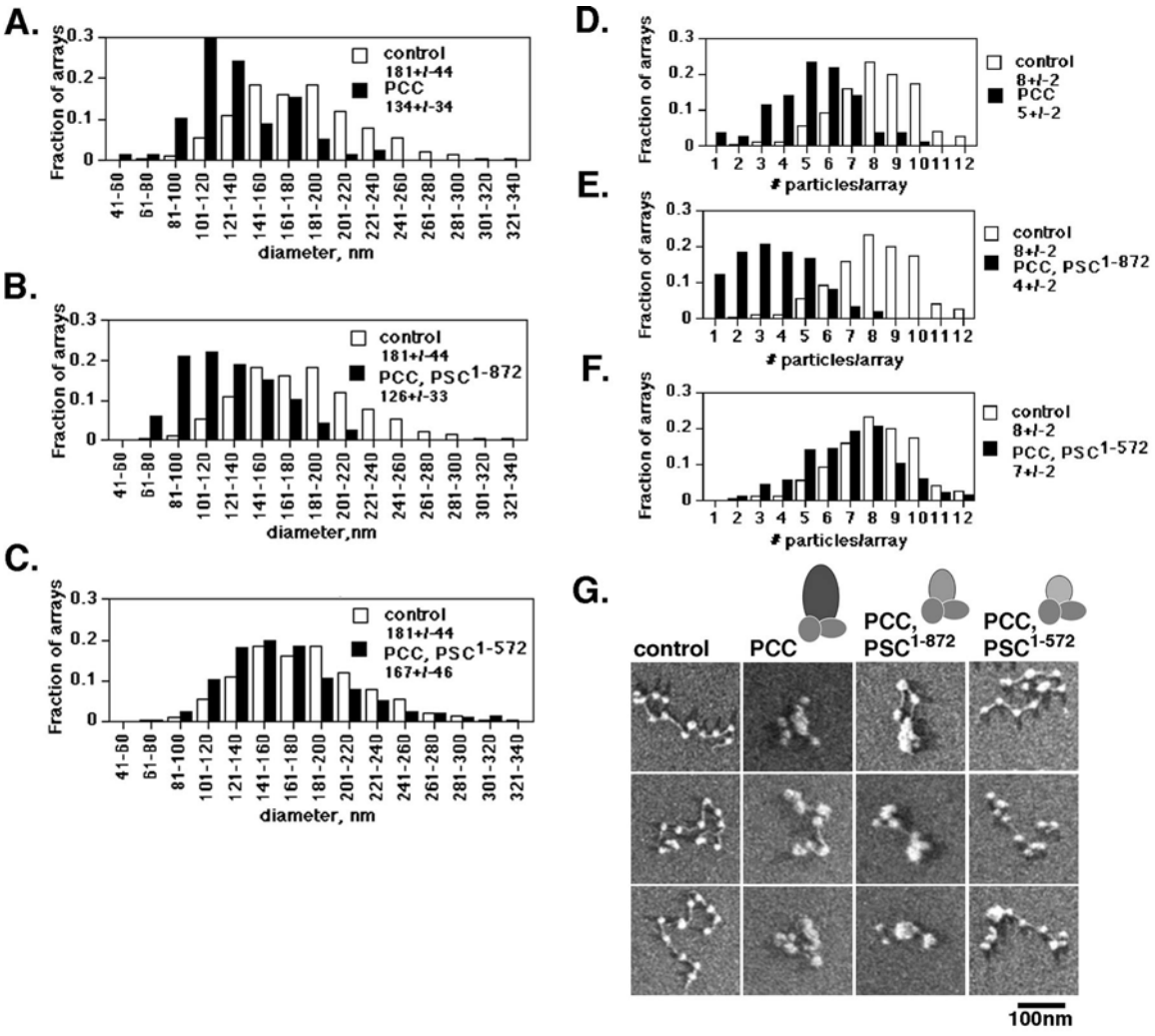
A.
Fraction of arrays
control
181+/-44
PCC
134+/-34
diameter, nm
B.
control
181+/-44
PCC, PSC1-872
126+/-33
diameter,nm
C.
control
181+/-44
PCC, PSC1-572
167+/-46
diameter, nm
D.
control
8+/-2
PCC
5+/-2
particles/array
E.
control
8+/-2
PCC, PSC1-872
4+/-2
particles/array
F.
control
8+/-2
PCC, PSC1-572
7+/-2
particles/array
G.
control
PCC
PCC, PSC1-872
PCC, PSC1-572
100nm

Figure S4

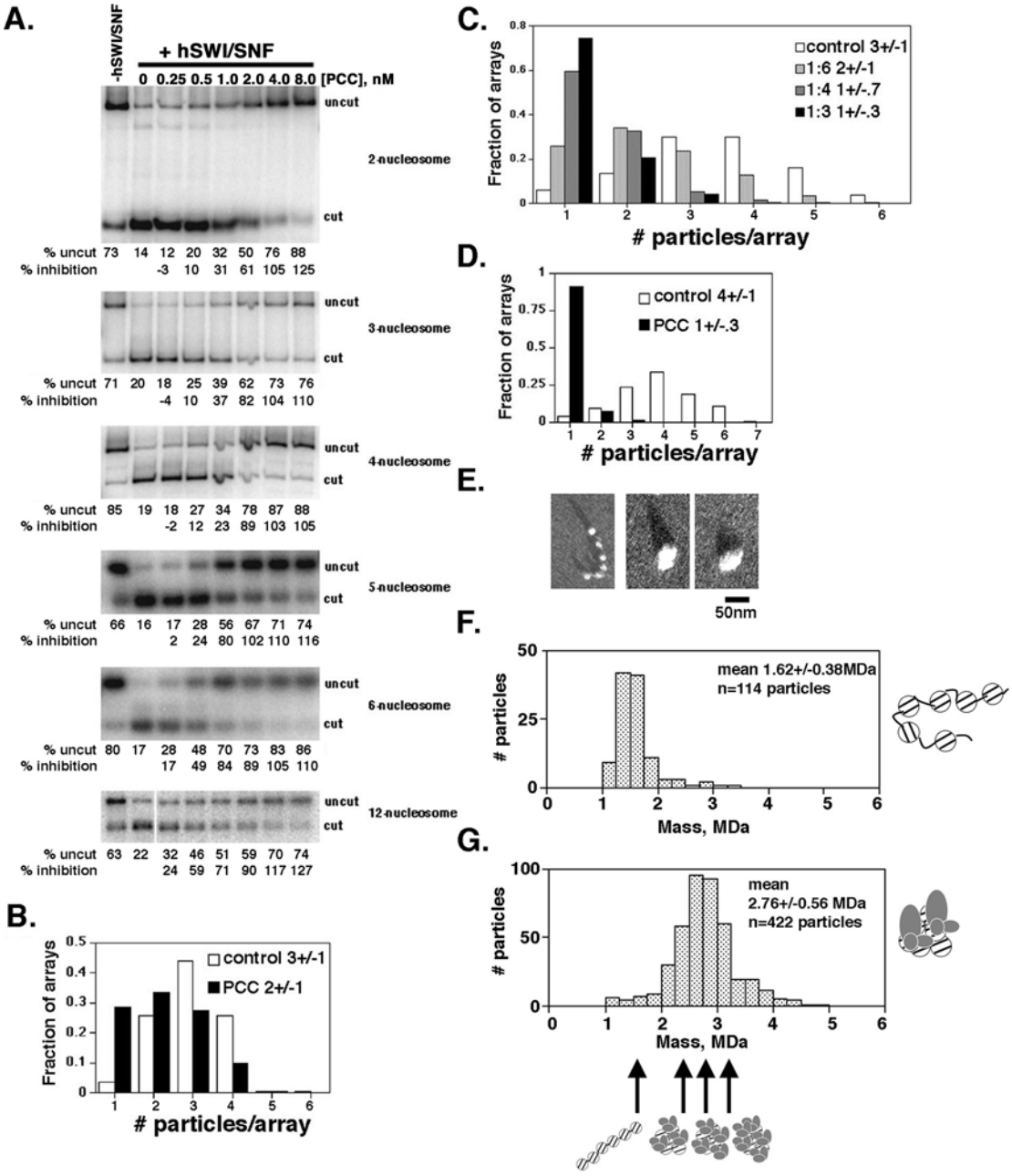
A.
-hSWI/SNF
+ hSWI/SNF
0 0.25 0.5 1.0 2.0 4.0 8.0 [PCC], nM
uncut
cut
2-nucleosome
% uncut 73 14 12 20 32 50 76 88
% inhibition -3 10 31 61 105 125
3-nucleosome
% uncut 71 20 18 25 39 62 73 76
% inhibition -4 10 37 82 104 110
4-nucleosome
% uncut 85 19 18 27 34 78 87 88
% inhibition -2 12 23 89 103 105
5-nucleosome
% uncut 66 16 17 28 56 67 71 74
% inhibition 2 24 80 102 110 116
6-nucleosome
% uncut 80 17 28 48 70 73 83 86
% inhibition 17 49 84 89 105 110
12-nucleosome
% uncut 63 22 32 46 51 59 70 74
% inhibition 24 59 71 90 117 127
B.
Fraction of arrays
control 3+/-1
PCC 2+/-1
particles/array
C.
Fraction of arrays
control 3+/-1
1:6 2+/-1
1:4 1+/-.7
1:3 1+/-.3
particles/array
D.
Fraction of arrays
control 4+/-1
PCC 1+/-.3
particles/array
E.
50nm
F.
particles
mean 1.62+/-0.38MDa
n=114 particles
Mass, MDa
G.
particles
mean
2.76+/-0.56 MDa
n=422 particles
Mass, MDa

Figure S5

Figure S1 Characterization of PCC and PSC used for EM. (A) PCC consisting of PSC, Pc, and dRING1, with full length or truncated PSC; position of PSC is marked with an asterisk. Approximately 1μg of each complex was separated on an 8% SDS-PAGE gel and stained with Colloidal Blue. Negatively stained images of PCC with wild type PSC (B) or truncated PSC (C) PSC $^{1\text{-}872}$, (D) PSC $^{1\text{-}572}$. The complex forms approximately spherical particles slightly larger than 10nm in diameter for wild type and smaller than 10nm for PCC with truncated PSC. (F) Full-length PSC, PSC$^{456\text{-}1603}$, PSC $^{1\text{-}872}$, or PSC $^{1\text{-}572}$ were analyzed by SDS-PAGE and stained with Colloidal Blue.

Figure S2 Effects of PCC on subsaturated and "tailless" nucleosomal arrays. (A) Additional images of effects of PCC on subsaturated arrays (see Fig. 2B). (B) Trypsinized HeLa histones assemble into nucleosomal arrays. 18% SDS-PAGE of HeLa histones (lane 1), trypsinized histones (lane 2) and trypsinized histones assembled into a nucleosomal array and purified over a glycerol gradient (lanes 3, 4 show adjacent fractions). (C) As reported *(14),* control nucleosomal arrays (set 1), but not nucleosomal arrays assembled with two different preparations of trypsinized histones (sets 2,3) are precipitated by Mg++. (D) Restriction enzyme accessibility assay demonstrates that PCC inhibits chromatin remodeling by hSWI/SNF on tailless arrays (right hand panel) as well as on intact ones (left hand panel). Both intact and tailless nucleosomes block access of restriction enzymes (first lane of each set), but DNA becomes accessible in the presence of hSWI/SNF and ATP (second lane of each set), consistent with previous reports*(5, 15)*. (E) Graph demonstrating that PCC inhibits remodeling of arrays assembled with control or trypsinized histones with similar efficiency. Data are from two experiments each carried out with 4 different tailless arrays; error bars (which are too small to be visible in some cases) are standard errors. (F) Distribution of number of particles per array for trypsinized arrays alone or in the presence of PCC demonstrating that PCC compacts trypsinized arrays.

Figure S3 Role of the C-terminal region of PSC in chromatin compaction. Diameter (A-D) or number of particles per array (E-H) for control arrays compared with arrays incubated with full length PSC (A,E), PSC $^{1-872}$ (B,F), PSC $^{1-572}$(C,G), or PSC $^{456-1603}$(D,H). (I) A gallery of EM images demonstrating the effects of PSC and PSC truncations on nucleosomal arrays representative of the data used for quantification.

Figure S4 The C-terminal region of PSC is important for chromatin compaction by a PcG complex. Diameter (A-C), or number of particles per array (D-F) for control arrays compared with arrays incubated with PCC (A,D), PCC assembled with PSC$^{1-872}$ (B,E) or PCC assembled with PSC$^{1-572}$ (C,F). (G) A gallery of representative EM images demonstrating the effects of PCC and PCC assembled with truncated PSC on nucleosomal arrays.

Figure S5 Effects of PCC on short arrays of nucleosomes. (A) Representative gels of restriction enzyme accessibility assays on templates from 2-12 nucleosomes demonstrating that PCC inhibits chromatin remodeling of longer templates more efficiently. Nucleosomal templates were used at a concentration of ~ 1nM nucleosomes. Differences among templates are most obvious in the range of 0.25-2nM PCC. (B) Number of particles per array for 4-nucleosome arrays alone or in the presence of 1 PCC:5 nucleosomes. (C) Number of particles per array on 6-nucleosomal arrays incubated with increasing ratios of PCC: nucleosomes. Note that at a ratio of 1:4, the majority of arrays are fully compacted (form a single particle). (D) Number of particles per array for 6-nucleosome arrays alone or incubated with 1 PCC:2 nucleosomes. Average number of particles +/- standard deviations are given. (E) EM of 6-nucleosome array alone (left) or in the presence of 1PCC:2 nucleosomes. STEM analysis of 6-nucleosome arrays alone (F), or with an excess of PCC (1PCC:2 nucleosomes) (G).

Table S1 Summary of effects of PCC on nucleosomal arrays. Table shows average maximal diameter, or number of particles per array, +/- standard deviation for control nucleosomal arrays or arrays incubated with PCC. The ratio of active PCC: nucleosomes is 1:8 except for experiments 8, 9, and 11, in which it is 1:4. n is the number of nucleosomal arrays measured in each experiment. Note that in experiments 5-7 nucleosomal arrays were slightly over saturated with nucleosomes, evidenced by the smaller diameters of control arrays. The number of particles per array for controls are underestimated in this case because the higher density of nucleosomes on the arrays results in many particles containing more than one nucleosome. Experiments 1-7 and 13 were carried out in 15mM KCl with no divalent cations; experiments 8, 11, 12 were in 30mM KCl without divalents; experiment 9 was in 1.5mM $Mg(Cl)_2$, 30mM KCl; and experiment 10 was in 2mM $Mg(Cl)_2$, 60mM KCl. In the presence of $Mg(Cl)_2$, control nucleosomal arrays are somewhat compacted (i.e. compare diameters for experiment 8 and 9, which were carried out simultaneously on arrays from the same preparation). *Arrays incubated with PCC had statistically significantly ($P<0.05$, 2-tailed student's t test) reduced diameter and particle number for all experiments except for diameter in experiment 7.

	control diameter, nm	PCC diameter, nm	control particles/array	PCC particles/array	control, n	PCC, n
1	201+/-44	129+/-35	9+/-1	4+/-2	139	95
2	201+/-57	169+/-46	9+/-2	8+/-3	153	60
3	182+/-37	160+/-46	8+/-2	6+/-2	28	97
4	175+/-39	146+/-42	9+/-2	6+/-2	170	273
5	138+/-31	102+/-32	8+/-2	2+/-1	345	177
6	136+/-32	98+/-22	8+/-2	2+/-1	267	124
7	101+/-22	103+/-24	9+/-2	2+/-1	240	153
8	175+/-67	76+/-24	8+/-2	2+/-1	96	83
9	143+/-41	77+/-29	8+/-2	2+/-1	91	73
10	153+/-53	102+/-36	6+/-2	3+/-2	105	64
11	170+/-45	117+/-33	8+/-2	4+/-2	189	118
12	181+/-44	134+/-34	8+/-2	5+/-2	293	78
13	179+/-43	140+/-39	8+/-2	5+/-2	159	157

Table S2 Maximal diameter and number of particles per array for arrays assembled with trypsinized histones. All experiments were carried out at a ratio of 1 PCC:8 nucleosomes.

*Arrays incubated with PCC had statistically significantly ($P \ll 0.001$, 2-tailed student's t test) reduced diameter and particle number for all experiments.

control diameter, nm	20nM PCC diameter, nm	control particles/array	20nM PCC particles/array	control, n	20nM PCC, n
199+/-50	150+/-59	11+/-1	6+/-3	122	99
197+/-51	160+/-44	11+/-1	6+/-2	276	149
221+/-58	197+/-53	9+/-2	7+/-2	226	214
226+/-49	189+/-55	9+/-2	7+/-2	112	100

Table S3 Effect of full length and truncated PSC on nucleosomal array compaction.

PSC or PSC truncations were used at a ratio of total protein to nucleosomes of 1:3 or 1:1.5 (experiment 3). PSC, PSC1$^{1-872}$, and PSC$^{456-1603}$ preparations are 30-50% active, so ratios of active PSC:nucleosomes are ~1:8, 1:4 (experiments 3,7-9) or 1:3 (experiment 10); PSC$^{1-572}$ has low DNA binding activity so that the fraction active could not be determined by this method (see methods). Experiment 6 was carried out in 2mM $Mg(Cl)_2$, 60mM KCl, so that control arrays are partially compacted; experiments 1-5 were carried out in 15mM KCl, and 7-9 in 30mM KCl, all without divalent cations. In cases where multiple entries appear for the same protein in the same experiment, they are independent protein preparations.

* indicates statistically different from control value ($P < 0.05$, students two tailed t-test).

	Protein	diameter, nm	particles/array	n
1	**Control**	138+/-31	8+/-2	345
	PSC	104+/-25*	4+/-2*	245
2	**Control**	136+/-32	8+/-2	265
	PSC	94+/-32*	2+/-1*	87
3	**control**	178+/-45	9+/-2	106
	PSC	89+/-33*	3+/-1*	168
	PSC$^{1-572}$	147+/-44*	6+/-3*	118
	PSC$^{456-1603}$	103+/-32*	3+/-2*	219
4	**control**	163+/-39	8+/-2	311
	PSC	138+/-41*	5+/-3*	369
	PSC$^{1-572}$	162+/-53	7+/-2*	92
	PSC$^{456-1603}$	111+/-41*	3+/-2*	152
5	**control**	103+/-29	8+/-2	109
	PSC	80+/-27*	3+/-2*	253
	PSC$^{1-572}$	121+/-27	4+/-2*	263
	PSC$^{456-1603}$	100+/-26*	3+/-2*	338
6	**control**	153+/-53	6+/-2	105
	PSC	117+/-45*	4+/-2*	57
	PSC$^{1-572}$	140+/-46	5+/-2*	57
7	**control**	141+/-35	6+/-2	154
	PSC	103+/-36*	3+/-2*	156
	PSC$^{1-872}$	123+/-37*	4+/-2*	141
	PSC$^{1-872}$	92+/-30*	2+/-1*	105
	PSC$^{1-572}$	137+/-41	5+/-2*	145
	PSC$^{456-1603}$	125+/-38*	4+/-2*	86
8	**control**	179+/-5	8+/-2	253
	PSC	113+/-41*	3+/-2*	166
	PSC$^{1-872}$	129+/-35*	4+/-2*	274
	PSC$^{1-872}$	145+/-42*	5+/-2*	206
	PSC$^{1-572}$	164+/-50*	6+/-2*	175
	PSC$^{456-1603}$	141+/-35*	5+/-2*	171
9	**control**	180+/-48	8+/-2	133
	PSC	121+/-40*	4+/-2*	115
	PSC$^{1-872}$	113+/-27*	2+/-1*	89
	PSC$^{1-872}$	115+/-31*	3+/-1*	130
	PSC$^{1-872}$	108+/-35*	3+/-2*	175
	PSC$^{1-572}$	158+/-39*	6+/-3*	47
	PSC$^{456-1603}$	100+/-31*	3+/-1*	126

Table S4 The C-terminal region of PSC is important for chromatin compaction by PCC. PCC and PCC with PSC^{1-872} were used at a ratio of 1 active PCC:8 nucleosomes, except for experiment 5 in which the ratio is 1:4. The low DNA binding activity of PCC with PSC^{1-572} precluded active molecule determinations, so PCC with PSC1-572 was used at a similar or higher total protein concentration as PCC. Experiment 3 was carried out in the presence of 2mM $Mg(Cl)_2$, 60mM KCl, so that arrays are partially compacted in the absence of PCC; experiments 4 and 5 were carried out in 30mM KCl, and 1,2,6 in 15mM KCl, all without divalent cations.

* indicates statistically different from control value ($P < 0.05$, students two tailed t-test).

	Protein	diameter, nm	particles/array	N
1	**Control**	201+/-44	9+/-1	139
	PCC	129+/-35*	4+/-2*	95
	PCC, PSC^{1-572}	189+/-48*	8+/-3*	98
2	**Control**	175+/-39	9+/-2	170
	PCC	146+/-42*	6+/-2*	273
	PCC, PSC^{1-572}	175+/-49	8+/-2*	212
3	**Control**	153+/-53	6+/-2	105
	PCC	102+/-36*	3+/-2*	64
	PCC, PSC^{1-572}	137+/-51*	4+/-2*	87
4	**Control**	170+/-45	8+/-2	189
	PCC	117+/-33*	4+/-2*	118
	PCC, PSC^{1-872}	103+/-3-*	2+/-1*	117
	PCC, PSC^{1-572}	132+/-42*	4+/-2*	126
5	**Control**	181+/-44	8+/-2	293
	PCC	134+/-34*	5+/-2*	78
	PCC, PSC^{1-872}	126+/-33*	4+/-2*	205
	PCC, PSC^{1-572}	167+/-46*	7+/-2*	297
6	**Control**	179+/-43	8+/-2	159
	PCC	140+/-39*	5+/-2*	157
	PCC, PSC^{1-872}	135+/-35*	4+/-2*	127
	PCC, PSC^{1-572}	167+/-52	6+/-2*	83

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