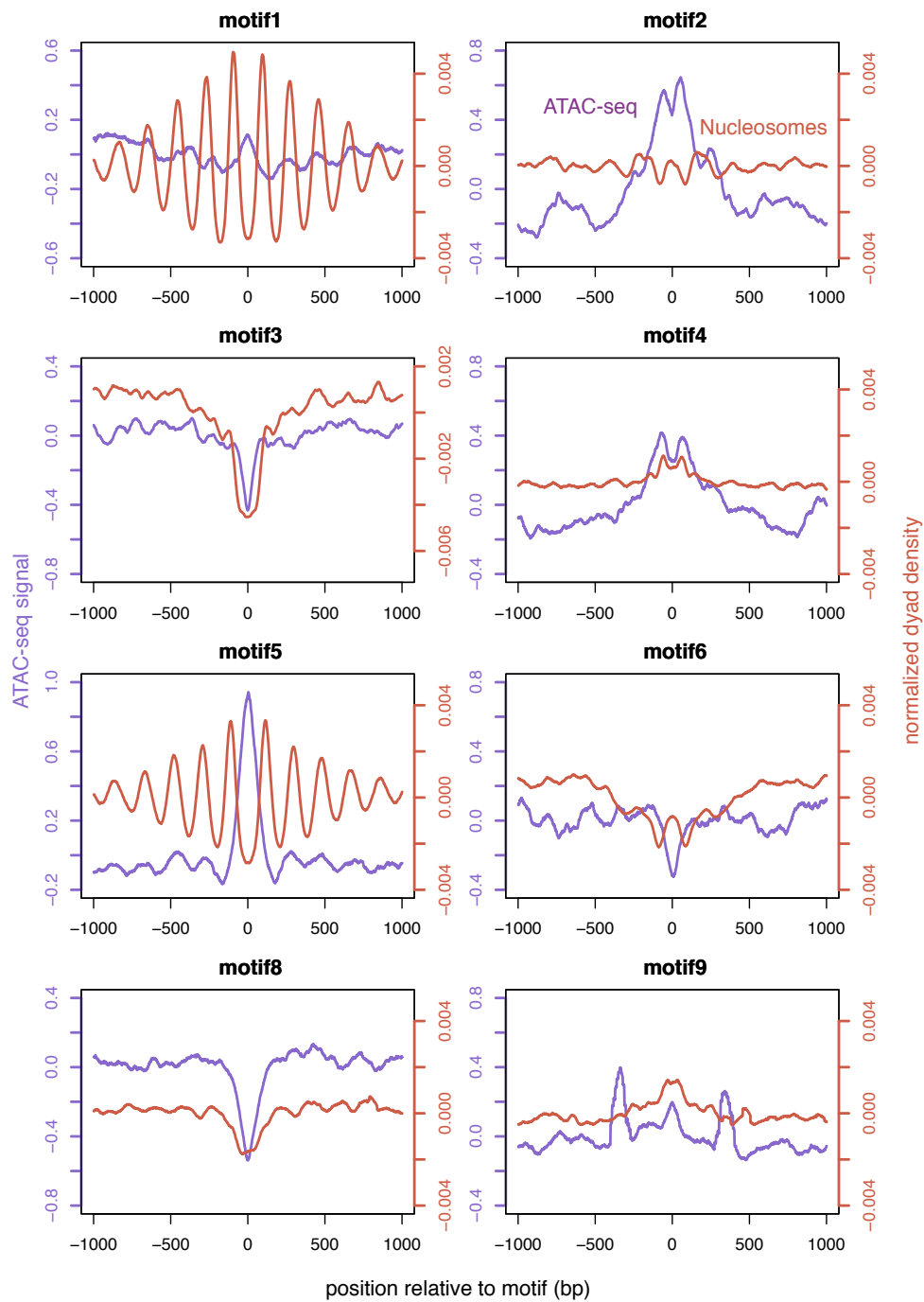
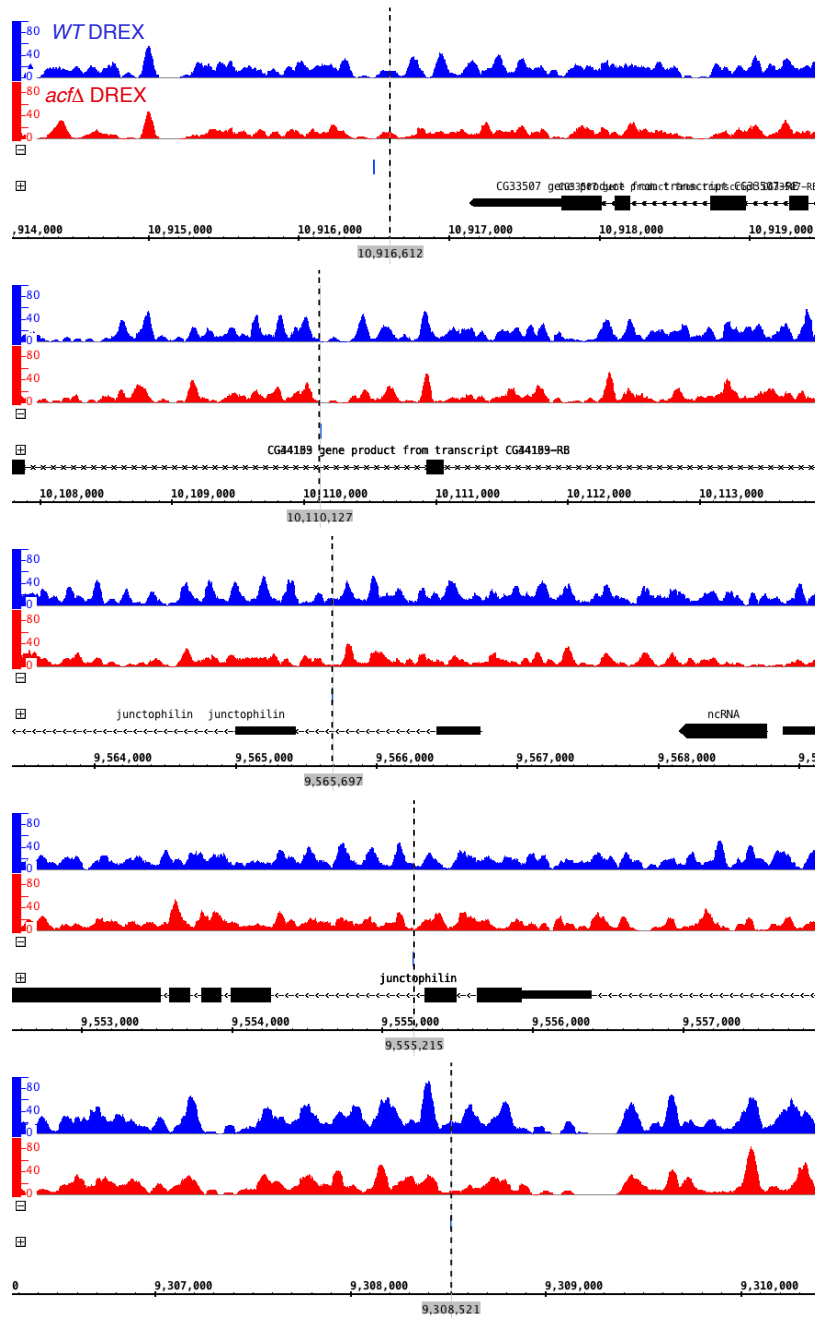


Supp. Fig. 1: Chromatin accessibility at motifs that are associated with non-TSS PNA



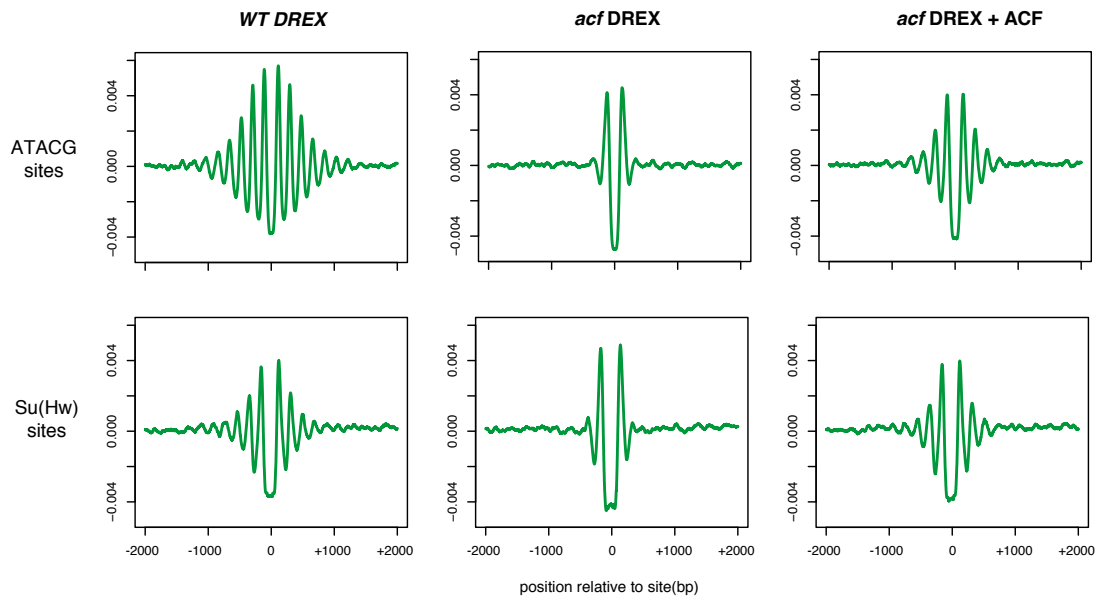
Chromatin accessibility at the DNA motifs identified in Fig. 1A based on ATAC-seq from NC14-18 embryos (Blythe and Wieschaus, 2016) (purple). As a comparison, nucleosome dyad densities from 2-8 h embryos are included (red).

Supp. Fig. 3 Nucleosomes are still present in the vicinity of ATACG sites in chromatin assembled with *Acf* mutant extract



Genome browser screen shots of nucleosome dyad densities around several ATACG sites. Chromatin assembled with *WT* preblastoderm embryo extract in blue, chromatin assembled with *Acf* mutant extract in red. Dashed lines indicate the location of the ATACG motifs.

Supp. Fig. 4 Recombinant ACF rescues loss of phasing in chromatin assembled with *acf* mutant extract



Nucleosome dyad densities aligned at ATACG (top) and su(Hw) sites (bottom) derived from chromatin assembled with *WT* preblastoderm embryo extract, *acf* mutant extract, or *acf* mutant extract supplemented with recombinant ACF.

Supp. Table S1

ID	antigen	source	peaks	overlaps/nonTSS-PNA	overlaps/ctrl	log P-Val	log Q-Val	FE
SRX191909	su(Hw)	S2	5357	861/4867	146/4867	-135,9	-132,5	5,9
SRX101475	su(Hw)	Ovary	4235	814/4867	127/4867	-134,3	-131,1	6,4
SRX191910	su(Hw)	S2	4958	814/4867	129/4867	-133,1	-130,1	6,3
SRX287584	su(Hw)	14-16h embryos	4941	817/4867	155/4867	-119,3	-116,5	5,3
SRX287585	su(Hw)	14-16h embryos	5310	793/4867	157/4867	-112,1	-109,5	5,1
SRX085403	su(Hw)	Kc167	8598	862/4867	196/4867	-110,5	-107,9	4,4
SRX085401	su(Hw)	Kc167	7779	826/4867	181/4867	-108,6	-106,1	4,6
SRX085402	su(Hw)	Kc167	7845	818/4867	185/4867	-104,7	-102,3	4,4
SRX186109	su(Hw)	ML-DmBG3-c2	3109	418/4867	65/4867	-66,4	-64,0	6,4
SRX101478	su(Hw)	Ovary	2367	390/4867	64/4867	-59,8	-57,5	6,1
SRX046654	su(Hw)	S2	13590	834/4867	338/4867	-54,4	-52,2	2,5
SRX046658	su(Hw)	S2	9550	716/4867	280/4867	-48,6	-46,7	2,6
SRX749036	Cp190	Kc167	13340	759/4867	309/4867	-48,6	-46,7	2,5
SRX202127	su(Hw)	S3	2646	325/4867	58/4867	-47,1	-4,5	5,6
SRX749037	Cp190	Kc167	8882	589/4867	204/4867	-47,0	-45,1	2,9
SRX191912	mod(mdg4)	S2	6786	475/4867	144/4867	-4,4	-42,3	3,3
SRX191911	mod(mdg4)	S2	6425	467/4867	147/4867	-41,5	-39,7	3,2
SRX085400	Cp190	Kc167	8661	431/4867	150/4867	-33,6	-32,2	2,9
SRX191907	Cp190	S2	12412	574/4867	254/4867	-31,0	-29,7	2,3
SRX100248	Cp190	Kc167	7918	452/4867	174/4867	-30,5	-29,2	2,6

Top hits of published ChIP profiles resembling the distribution of non-TSS PNAs, sorted by p-value.

Supp. Table S2: Position Probability Matrices for motifs 1-9

#motif1			
0.698925	0.001792	0.299283	0.000000
0.139785	0.851254	0.000000	0.008961
0.032258	0.007168	0.754480	0.206093
0.001792	0.609319	0.000000	0.388889
1.000.000	0.000000	0.000000	0.000000
0.003584	0.001792	0.001792	0.992832
0.948029	0.034050	0.014337	0.003584
0.000000	1.000.000	0.000000	0.000000
0.005376	0.000000	0.994624	0.000000
0.089606	0.856631	0.001792	0.051971
0.385305	0.605735	0.001792	0.007168
0.351254	0.252688	0.362007	0.034050
0.034050	0.553763	0.023297	0.388889
0.071685	0.012545	0.913978	0.001792
0.019713	0.023297	0.043011	0.913978
#motif2			
0.691803	0.075410	0.216393	0.016393
0.101639	0.875410	0.000000	0.022951
0.750820	0.075410	0.088525	0.085246
0.042623	0.727869	0.072131	0.157377
0.862295	0.081967	0.029508	0.026230
0.065574	0.832787	0.059016	0.042623
0.875410	0.032787	0.032787	0.059016
0.160656	0.731148	0.049180	0.059016
0.842623	0.016393	0.098361	0.042623
0.111475	0.770492	0.055738	0.062295
0.819672	0.065574	0.032787	0.081967
0.072131	0.845902	0.022951	0.059016
0.757377	0.072131	0.137705	0.032787
0.124590	0.796721	0.013115	0.065574
0.750820	0.009836	0.127869	0.111475
0.032787	0.780328	0.147541	0.039344
0.842623	0.081967	0.075410	0.000000
0.150820	0.786885	0.013115	0.049180
0.803279	0.009836	0.091803	0.095082
0.075410	0.491803	0.183607	0.249180
0.563934	0.095082	0.186885	0.154098
0.068852	0.645902	0.108197	0.177049
0.583607	0.098361	0.213115	0.104918
0.167213	0.629508	0.114754	0.088525
0.770492	0.104918	0.045902	0.078689
0.255738	0.377049	0.091803	0.275410

0.511475	0.055738	0.236066	0.196721
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#motif3

0.748459	0.106042	0.145499	0.000000
0.921085	0.016030	0.053021	0.009864
0.914920	0.082614	0.000000	0.002466
0.727497	0.091245	0.020962	0.160296
0.508015	0.125771	0.200986	0.165228
0.533909	0.138101	0.196054	0.131936
0.763255	0.155364	0.076449	0.004932
0.951911	0.003699	0.034525	0.009864
0.970407	0.029593	0.000000	0.000000
0.628853	0.128237	0.077682	0.165228
0.406905	0.251541	0.124538	0.217016
0.715166	0.064118	0.220715	0.000000
0.799014	0.093711	0.081381	0.025894
0.881628	0.099877	0.017263	0.001233
0.893958	0.007398	0.000000	0.098644

#motif4

0.005970	0.110448	0.847761	0.035821
0.000000	0.820896	0.000000	0.179104
0.077612	0.053731	0.098507	0.770149
0.000000	0.101493	0.844776	0.053731
0.000000	0.964179	0.032836	0.002985
0.164179	0.047761	0.080597	0.707463
0.000000	0.020896	0.979104	0.000000
0.000000	0.838806	0.000000	0.161194
0.000000	0.032836	0.032836	0.934328
0.000000	0.128358	0.871642	0.000000
0.000000	0.910448	0.038806	0.050746

#motif5

0.144279	0.144279	0.318408	0.393035
0.059701	0.318408	0.000000	0.621891
0.308458	0.000000	0.651741	0.039801
0.000000	0.124378	0.009950	0.865672
0.378109	0.000000	0.024876	0.597015
0.000000	0.000000	1.000.000	0.000000
0.000000	0.955224	0.009950	0.034826
0.761194	0.223881	0.014925	0.000000
0.000000	0.074627	0.029851	0.895522
0.945274	0.000000	0.054726	0.000000
0.000000	0.975124	0.014925	0.009950
0.134328	0.059701	0.000000	0.805970
0.004975	0.024876	0.004975	0.965174

0.129353	0.154229	0.029851	0.686567
0.114428	0.218905	0.139303	0.527363
0.223881	0.283582	0.328358	0.164179
0.243781	0.059701	0.492537	0.203980
0.004975	0.014925	0.970149	0.009950
0.079602	0.547264	0.348259	0.024876
0.094527	0.039801	0.771144	0.094527
0.124378	0.582090	0.134328	0.159204

#motif6

0.167630	0.028902	0.716763	0.086705
0.164740	0.153179	0.468208	0.213873
0.101156	0.080925	0.497110	0.320809
0.005780	0.083815	0.910405	0.000000
0.176301	0.014451	0.728324	0.080925
0.251445	0.364162	0.210983	0.173410
0.297688	0.052023	0.569364	0.080925
0.115607	0.040462	0.708092	0.135838
0.115607	0.147399	0.297688	0.439306
0.008671	0.060694	0.927746	0.002890
0.037572	0.135838	0.716763	0.109827
0.294798	0.187861	0.323699	0.193642
0.343931	0.052023	0.511561	0.092486
0.271676	0.083815	0.644509	0.000000
0.242775	0.112717	0.283237	0.361272
0.043353	0.008671	0.684971	0.263006
0.040462	0.150289	0.705202	0.104046
0.150289	0.179191	0.453757	0.216763
0.193642	0.040462	0.508671	0.257225
0.202312	0.138728	0.520231	0.138728
0.158960	0.083815	0.471098	0.286127
0.066474	0.130058	0.693642	0.109827
0.066474	0.196532	0.549133	0.187861
0.289017	0.121387	0.335260	0.254335
0.130058	0.002890	0.757225	0.109827
0.026012	0.231214	0.630058	0.112717
0.101156	0.202312	0.306358	0.390173
0.092486	0.112717	0.708092	0.086705
0.187861	0.219653	0.537572	0.054913

#motif7

0.074074	0.022222	0.777778	0.125926
0.000000	0.985185	0.000000	0.014815
0.007407	0.000000	0.992593	0.000000
0.014815	0.014815	0.014815	0.955556
0.977778	0.000000	0.022222	0.000000

0.000000	0.000000	0.000000	1.000.000
0.474074	0.000000	0.525926	0.000000
0.200000	0.711111	0.022222	0.066667
0.022222	0.000000	0.792593	0.185185
0.029630	0.237037	0.000000	0.733333
0.718519	0.044444	0.207407	0.029630
0.977778	0.000000	0.000000	0.022222
0.000000	0.074074	0.000000	0.925926
0.466667	0.022222	0.348148	0.162963
0.051852	0.162963	0.000000	0.785185

#motif8

0.989529	0.000000	0.000000	0.010471
0.668848	0.000000	0.000000	0.331152
0.675393	0.000000	0.000000	0.324607
0.226440	0.003927	0.000000	0.769634
0.456806	0.003927	0.301047	0.238220
0.350785	0.382199	0.000000	0.267016
0.968586	0.000000	0.000000	0.031414
0.437173	0.026178	0.000000	0.536649
0.433246	0.000000	0.061518	0.505236
0.303665	0.069372	0.044503	0.582461
0.655759	0.000000	0.017016	0.327225
0.566754	0.035340	0.000000	0.397906
0.836387	0.000000	0.000000	0.163613
0.430628	0.000000	0.000000	0.569372
0.350785	0.000000	0.036649	0.612565

#motif9

0.000000	0.000000	1.000.000	0.000000
0.000000	0.294118	0.705882	0.000000
0.000000	0.176471	0.000000	0.823529
0.823529	0.176471	0.000000	0.000000
0.000000	1.000.000	0.000000	0.000000
0.000000	0.529412	0.470588	0.000000
0.000000	1.000.000	0.000000	0.000000
0.000000	1.000.000	0.000000	0.000000
0.294118	0.529412	0.000000	0.176471
0.000000	0.000000	1.000.000	0.000000
0.000000	0.823529	0.176471	0.000000
0.294118	0.705882	0.000000	0.000000
0.823529	0.000000	0.000000	0.176471
0.000000	0.176471	0.823529	0.000000
0.823529	0.000000	0.176471	0.000000
1.000.000	0.000000	0.000000	0.000000
0.000000	0.000000	1.000.000	0.000000

0.000000	0.000000	1.000.000	0.000000
0.705882	0.294118	0.000000	0.000000
0.294118	0.705882	0.000000	0.000000
0.000000	0.000000	0.823529	0.176471
0.000000	0.294118	0.705882	0.000000
1.000.000	0.000000	0.000000	0.000000
0.882353	0.000000	0.117647	0.000000
0.000000	0.000000	0.705882	0.294118
0.000000	1.000.000	0.000000	0.000000
0.823529	0.176471	0.000000	0.000000
0.000000	1.000.000	0.000000	0.000000
0.294118	0.705882	0.000000	0.000000
0.176471	0.000000	0.823529	0.000000

#motif10

0.058824	0.241830	0.666667	0.032680
0.032680	0.098039	0.000000	0.869281
0.228758	0.000000	0.771242	0.000000
0.013072	0.117647	0.065359	0.803922
0.098039	0.104575	0.725490	0.071895
0.130719	0.124183	0.013072	0.732026
0.156863	0.039216	0.803922	0.000000
0.052288	0.013072	0.006536	0.928105
0.000000	0.000000	0.993464	0.006536
0.026144	0.026144	0.006536	0.941176
0.143791	0.000000	0.823529	0.032680
0.111111	0.058824	0.000000	0.830065
0.000000	0.000000	0.934641	0.065359
0.091503	0.189542	0.032680	0.686275
0.032680	0.000000	0.830065	0.137255

Supp. Table S3: Primers used

	Forward primer	Reverse primer
CG7372 knockdown, construct 1	TAATACGACTCACTATAGGGTGGAA GAGGCTGAGGTGG	TAATACGACTCACTATAGGGTACCGCCGAT CGTTCTTG TG
CG7372 knockdown, construct 2	TAATACGACTCACTATAGGGGCACA CAGGTGAAAAACC	TAATACGACTCACTATAGGGTGT CATCGAG CTGGACAAAG
su(Hw) knockdown, construct 1	TAATACGACTCACTATAGGGGAGGT TGTACCAGTGGCGTT	TAATACGACTCACTATAGGGCGGCTACCGT AGTCAAGCTC
su(Hw) knockdown, construct 2	TAATACGACTCACTATAGGGAACTG TGGCCACGACCTAAC	TAATACGACTCACTATAGGGTGTGCGGTAA TTGTACGCAT
Cloned ATACG site 1	CATAAATTGCAAGGGAGAACG	TGATGTAGTAATTCACAGACC
Cloned ATACG site 2	AAAATGGAGTGGAACGTTGC	TTGCGGTTGGCTTGCCTTTTTC
Cloned ATACG site 3	TCGATACGATATTCACAAGG	TTTATTCCTCGTCGATTTCC
Dinucleosomal DNA around ATACG site	CCATAAAAATAGACAACAAATTGTT	CAAGTTATAAATTATTTGGCATTTG
Dinucleosomal DNA around su(Hw) site	TAGTTTTCCGACTGTGATTGC	ATAAGGTATTATTATTTATTTTGGTAC