SUPPLEMENTARY DATA

Multimerization of *Drosophila* sperm protein Mst77F causes a unique condensed chromatin structure

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INVENTORY

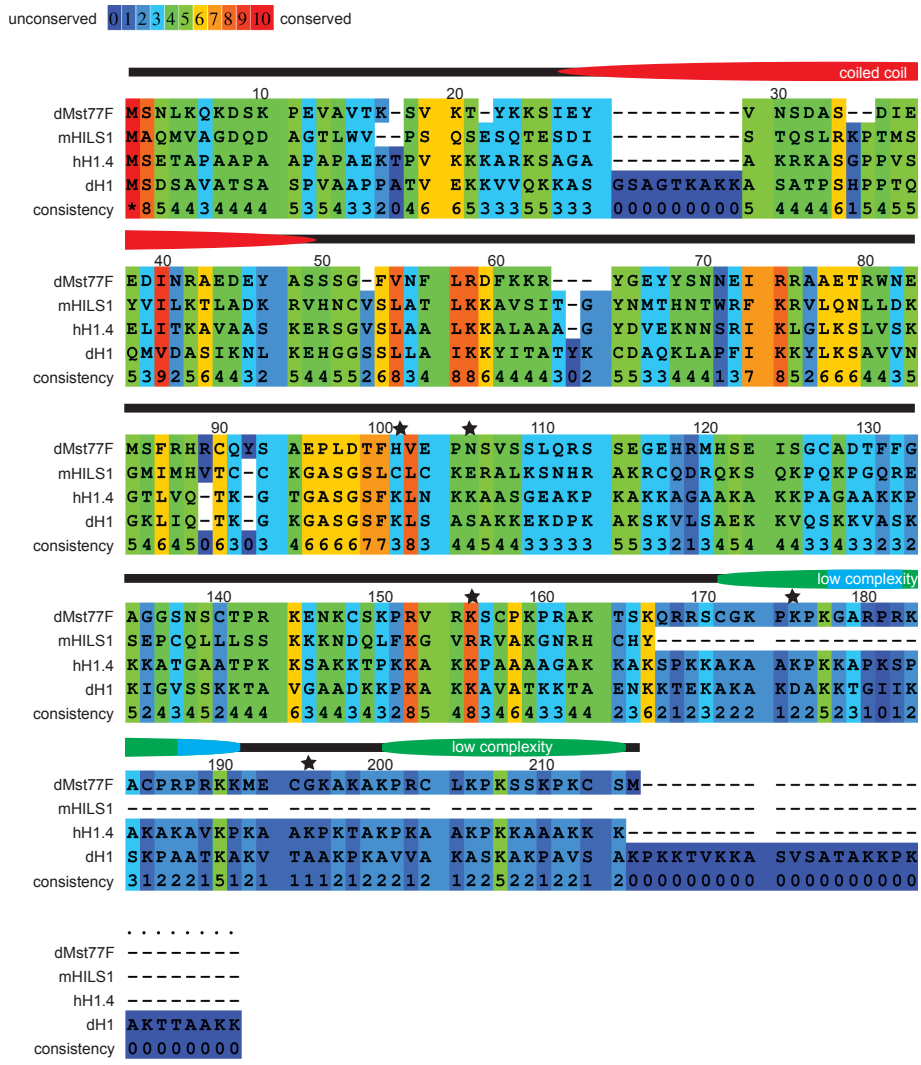
Supplementary Figures:

Supplementary Materials and Methods:

Contains detailed and comprehensive description of all reagents and methods.

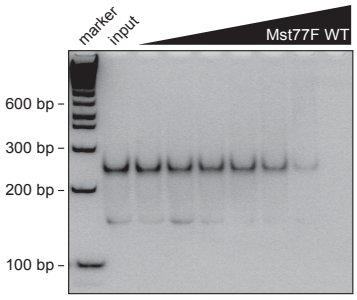
Supplementary References

SUPPLEMENTARY FIGURES



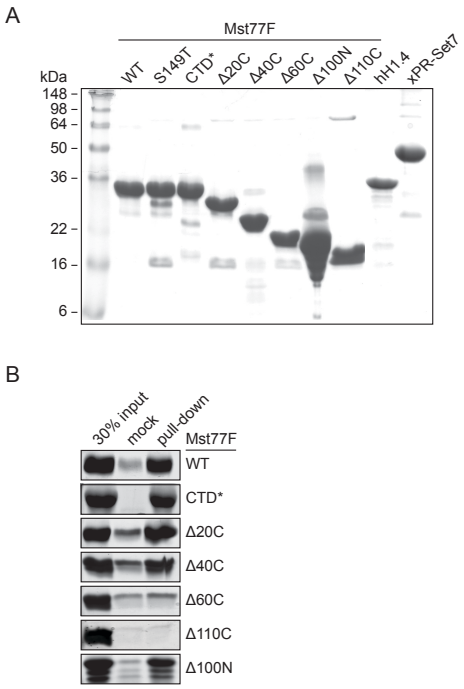
Supplementary Figure S1. Sequence comparison of Mst77F, HILS1 and H1 proteins.

Alignment of Mst77F and histone H1 protein sequences using the PRALINE alignment tool at http://www.ibi.vu.nl/programs/pralinewww/. Predicted domain architecture as shown in Figure 1 is indicated. Numbering is according to dMst77F sequence. Asterisks mark the boundary of the deletion mutants shown in Figure 3.



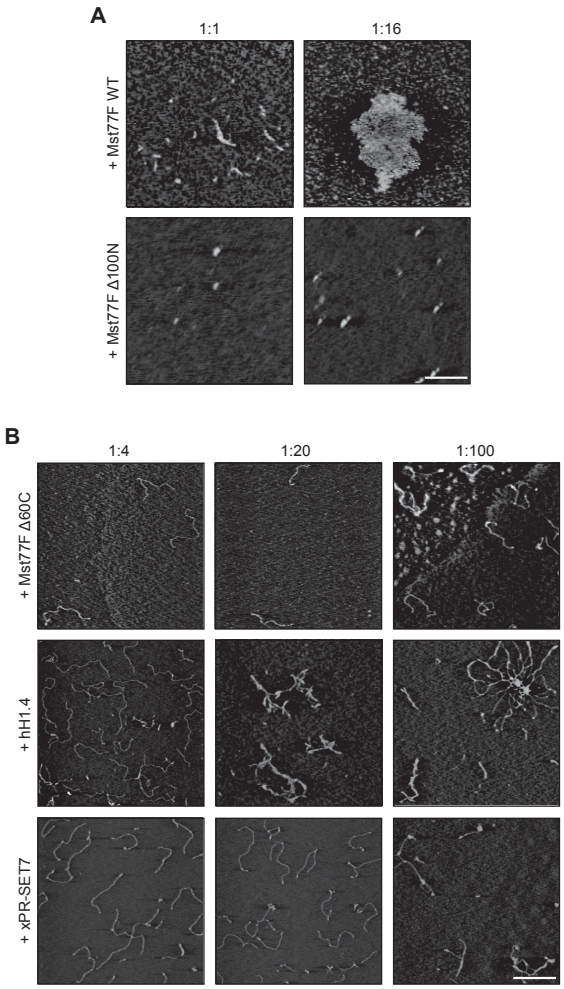
Supplementary Figure S2. MST77F binds nucleosome core particles.

Protein (0.02 μM to 1.28 μM in twofold increments) was incubated with 0.05 μM nucleosome core particles. Samples were separated on a 5% TB-PAGE gel and subsequently stained with EtBr. Input, no protein added.



Supplementary Figure S3. Analysis of DNA-binding of recombinant Mst77F wild-type and mutant proteins.

(*A*) The indicated purified, recombinant proteins were run on SDS-PAGE and stained with Coomassie Blue. Running position of molecular weight markers is indicted on the left. (*B*) Biotinylated random 12 bp DNA immobilized on streptavidin-functionalized beads was incubated with the indicated recombinant proteins (pull-down). Recovered material was analyzed by SDS-PAGE stained with Coomassie Blue. Mock, reactions without immobilized DNA.



Supplementary Figure S4. Mst77F aggregates short and long DNA.

The indicated proteins were complexed with 12 bp DNA (A) or 12 x 200 bp x 601 DNA (2434 bp) at different molar excess of the protein over nucleic acid. AFM images of the protein-DNA complexes were recorded in tapping mode and flattened post imaging. Scale bar represents 50 nm.

SUPPLEMENTARY MATERIALS AND METHODS

Bioinformatic analysis

Protein structural domain prediction was carried out using the Simple Modular Architecture Research Tool (SMART) algorithm (http://smart.embl-heidelberg.de/) (1)

Plasmids

Mst77F cDNA was amplified from pUAST-Mst77F-fGFP using a forward primer containing an *Eco*RI site and a reverse primer containing sequences encoding for a SV40 NLS followed by a 3xFLAG-tag sequence as well as a *Xho*I site. The resulting fragments were cloned into the pMT-his/V5 vector. For expression of recombinant proteins, the Mst77F coding sequence was generated by gene synthesis (GeneArt) on the basis of NM_079464. Codons were optimized for bacterial expression. The sequence of the protein with a randomized CTD (CTD*) is: MSNLKQKDSKPEVAVTKSVKTYKKSIEYVNSDASDIEED INRAEDEYASSSGFVNFLRDFKKRYGEYYSNNEIRRAAETRWNEMSFRHRCQYSAEP LDTFHVEPNSVSSLQRSSEGEHRMHSEISGCADTFFGAGGSNSCTPRKENKCSKPRVR KRSMRPGKKQCPARKGPPRTRKCPPPSKEAKKCKAAKCKRCSKSGKSPAKPPLKMC SKRRK. Mst77F WT, deletion mutants and histone hH1.4 were C-terminally fused to a His_6-Tag by PCR and cloned into the *Xba*I/*Bam*HI sites of the pET3a plasmid vector (NEB). Details of cloning and plasmid constructs are available upon request.

Antibodies for western blotting

Anti-FLAG (1:1000, Santa Cruz, Sc 807); anti-Histone H3 (1:5000, Abcam, ab1791); anti-β-Tubulin (1:2000, Sigma T4026); goat-anti-mouse HRP (1:4000, DakoCytomation, PO447); swine-anti-rabbit HRP (DakoCytomation, PO399).

Micrococcus nuclease digestion

Analysis of chromatin after addition of recombinant proteins to purified nuclei was performed as described (2). S2 cells from ten confluent 90 mm dishes were harvested by centrifugation. After briefly washing with PBS, cells were resuspended in 5 mL of hypotonic buffer (10 mM HEPES-KOH pH = 7.9, 1 mM $MgCl_2$, 100 mM KCl, 1 mM DTT and protease inhibitor cocktail (Roche)) and kept on ice for 5 min. Cells were homogenized in a dounce homogenizer (20 strokes with tight pestle) followed by centrifugation at 228 x g for 5 min at 40 °C to pellet the nuclei. The nuclear pellet was resuspended in 3 mL S1 buffer (0.25 M Sucrose, 10 mM $MgCl_2$) and layered over a 3 mL cushion of S3 buffer (0.88 M Sucrose, 0.5 mM $MgCl_2$). After centrifugation at 2800 xg for 10 min, 4°C, the pellet was resuspended in 1 mL of MNase digestion buffer (15 mM Tris-HCl pH = 7.5, 15 mM NaCl, 60 mM KCl, 0.35 M Sucrose, 0.5 mM Spermidine, 0.15 mM Spermine, 0.25 mM PMSF, 0.1% (w/v) β-mercaptoethanol). Approximately 10^7 nuclei were used for each MNase time-point with recombinant proteins added to 10 μM final concentration. 1 mM $CaCl_2$ was added and the suspension was divided into equal aliquots before adding MNase (Takara Biotech) to a final concentration of 2.5 U/mL. Reactions were performed at 20°C and stopped at the indicated times by adding 100 mM EDTA pH = 8.0. DNA was purified by standard phenol chloroform extraction followed by ethanol precipitation.

For analysis of recombinant chromatin 2.5 μg 12 x 200 bp x 601 oligonucleosomes were incubated with recombinant proteins (10 μM final concentration) in 500 μL MNase digestion buffer (200 mM Tris-HCl pH = 8.0, 50 mM NaCl, 25 mM $CaCl_2$) for 30 min at 4°C. Alternatively, 5 μg mononucleosomes were incubated (30 min, 4°C) with equal molar amounts of recombinant proteins in 1 mL of MNase buffer (20 mM Tris-HCl pH=7.2, 5 mM $MgCl_2$, 3 mM $CaCl_2$). Digests were performed with 0.2 U/mL MNase at 20°C. 200 μL

aliquots each of the reaction mixture were immediately mixed with 400 µl of binding buffer (taken from the Macherey & Nagel PCR purification kit) to stop the reaction. DNA was purified according to manufacturer's protocol.

Samples from MNase digests of chromatin were analyzed on 1.5% agarose gels in TBE, while samples form digests of mononucleosomes were analyzed on 5% polyacrylamide gels in TBE.

DNA templates

12 bp DNA templates were generated by thermal annealing (10 min at 95°C followed by cooling to RT) of complementary oligonucleotides labeled with fluorescein [Flc] (Sigma). Random: 5'-GTACCACGGTAG[Flc]-3'; poly-AT: 5'-ATATATATATAT[Flc]-3'; poly-GC: 5'-GCGCGCGCGCGC[Flc]-3'. Biotinylated or fluorescinated 234 bp DNA was generated by PCR from a sequence containing the "601" 147 bp nucleosome positioning sequence. 234x601 biotin fwd: 5'-[biotin]GGTTATGTGATGGACCCTATACG-3'; 234x601 fluorescein fwd: 5'-[Flc]GGTTATGTGATGGACCCTATACG-3'; 234x601 rev: 5'-ATGATTACGAATTCGAGCTCGGTAC-3'; 147x601 fwd: 5'-CTGGAGAATCCCGGTGCCGAGGC-3'; 147x601 rev: 5'-ACAGGATGTATATATCTGACACGTGCCTG -3'. The 2434 bp DNA fragment was derived from a pUC 18 plasmid and prepared by restriction digest with *Dde*I, *Hae*II, *Eco*RI, *Bfu*CI and subsequent PEG precipitation (3).

Fluorescence polarization

FP assays were essentially carried out and analyzed as described using FP buffer (10 mM TEA, 150 mM NaCl, pH = 7.4) (4). Titration series of proteins in 10 µL volumes contained 4 nM fluorescein-DNA. Each titration point was read multiple times on a Plate

Chameleon II plate reader (HIDEX Oy). Multiple readings and independent titration series were averaged after data normalization.

Pull-downs

2.5 µg of biotinylated 12 bp random sequence DNA were immobilized on 50 µL of streptavidin coated paramagnetic particles (Promega) O/N at 4°C in binding buffer (10 mM triethanolamine-HCl pH = 7.5, 150 mM NaCl, 0.1 % v/v Nonidet P40, 1 mM DTT). Unbound DNA was removed by three washes with 1 mL binding buffer each. Beads were blocked with 0.5% (w/v) dry non-fat milk (Regilait) dissolved in binding buffer for 1 h at RT. Then, the immobilized 12mer duplex DNA was incubated with 7.5 µg recombinant Mst77F protein for 2 h at RT in blocking buffer. The beads were washed three times with 1 mL washing buffer (10 mM triethanolamine-HCl pH = 7.5, 300 mM NaCl, 0.2 % v/v Nonidet P40, 1 mM DTT) and bound proteins were eluted in SDS-PAGE loading buffer.

For analyzing interaction of Mst77F Δ100N and Mst77F Δ110C beads were loaded with DNA and blocked as described above. 2.5 µg Mst77F Δ100N were immobilized on the immobilized DNA for 30 min at RT. After washing (3x 1 mL washing buffer) 5 µg Mst77F Δ110C were added for 30 min at RT. The beads were washed (3x 1 mL washing buffer) and bound proteins were eluted in SDS-PAGE loading buffer.

Centrifugation fractionation assay

Assays were carried out as described with the following modifications (5). 150 ng of 12 bp DNA was bound to 40 nM to 1.28 µM protein in a total volume of 50 µL (10 mM triethanolamine-HCl pH = 7.4, 150 mM NaCl, 1 mM DTT). Immediatly after addition of the proteins and thorough mixing 20 µL of each sample was removed and saved as ‘input’. Reactions were incubated on ice for 30 min. Then the protein DNA complexes were

recovered by centrifugation for 30 min at 16000 x g, 4°C. Again, 20 µL of the supernatant were removed and saved as 'output'. To correlate the amounts of DNA in the 'input' and 'output' DNA was stained with 0.5 µg/mL ethidium bromide. Fluorescence was measured on a Plate Chameleon II plate reader (HIDEX Oy) equipped with a 360 nm excitation filter and a 612 nm emission filter. For each sample the ratio of 'input' DNA vs. 'output' DNA was determined and plotted vs. the respective protein concentration.

In vitro reconstitution of mono- and oligonucleosomes

Regularly spaced nucleosomes were assembled from recombinant DNA and histone octamers as described (6,7). In brief, the concentration of histone octamer was determined photometrically assuming an OD_{276} = 0.45 corresponds to 1 mg/mL of histone octamer. The reconstituted histone octamers were mixed with the respective DNA in RB high buffer (10 mM Tris-HCl pH = 7.5, 1 mM EDTA, 2 M NaCl, 1 mM DTT) in molar ratios ranging from 0.6 : 1 to 1.3 : 1 in 0.1 increments for mononucleosomal DNA templates and 0.8 : 1 to 1.2 : 1 for 12 x 200 x 601 DNA based oligonucleosomal arrays. The dialysis vessels were placed in 400 mL RB high buffer and the buffer was slowly exchanged against 2 L RB low buffer (RB high but 10 mM NaCl) over a period of 36 hrs using a peristaltic pump. The samples were subsequently dialyzed against TEA20 storage buffer (10 mM triethanolamine-HCl pH = 7.5, 20 mM NaCl, 0.1 mM EDTA), analyzed by native agarose gel electrophoresis and kept at 4°C. The assembly titrations that resulted in saturated but not aggregated material were used for the experiments.

Chromatin co-precipitation

10 ng/µL nucleosomal arrays (OD_{260} = 0.2) were incubated in a titration series with recombinant Mst77F protein (0.08 µM - 1.28 µM in 2 fold increments) for 1 h at 4°C in a

total volume of 100 µL binding buffer (10 mM triethanolamine-HCl pH = 7.4. 150 mM NaCl, 1mM DTT). 5 mM $MgCl_2$ was added and samples were centrifuged for 30 min at 16,000 x *g* and 4°C (5). Pellets were washed once with 1 mL binding buffer containing 5 mM $MgCl_2$ and centrifuged for 15 min at 16,000 x g and 4°C. Recovered material was solubilized in SDS-PAGE loading buffer.

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