Supplementary legends

Table S1. Dissociation constants (K_D) of *A. thaliana* NRP1 interaction with either histones or C*c* measured by Surface Plasmon Resonance (SPR). K_D values were calculated from the fits of SPR curves to the binding models described in Figure S6.

Figure S1. Multiple alignment of amino acid sequences of NRP1 (UniProt entry: Q9CA59-1), AtNAP1;1 (UniProt: Q9SZI2-1), AtNAP1;2 (UniProt: Q9ZUP3-1), AtNAP1;3 (UniProt: Q94K07-1) and AtNAP1;4 (UniProt: F4JEI8-1) using Clustal Omega. Sequences are coloured by similarity: fully conserved residue (black), residues with strongly similar properties (light blue) and residues with weakly similar properties (grey).

Figure S2. MALDI-TOF MS analyses of H2B, H3 and H4 histones (*X. laevis*), Cc and NRP1 (*A. thaliana*).

Figure S3. A. MALDI-TOF MS analyses of the H2A, H2B, H3 and H4 histones (*G. gallus*). **B.** *Left*: SDS-PAGE showing the electrophoretic purity of *A. thaliana* Cc and NRP1 (lanes 2-3), *X. laevis* H2B, H3 and H4 histones (lanes 4-6). *Right*: SDS-PAGE of *G. gallus* H2A-H2B and H3-H4 dimers (lanes 2-3). In both gels, lane 1 indicates protein ladder markers.

Figure S4. Far-UV CD spectra of NRP1 (*left*) and X. *laevis* core histones H2B, H3 and H4 (*right*).

Figure S5. ITC measurements of interactions of NRP1 with H2B, H3 and H4 isolated core histones, H2A-H2B, H3-H4 and Cc. Thermograms along with the binding isotherms (*top* and *bottom*, respectively) are shown.

Figure S6. Analysis of Surface Plasmon Resonance curves. SPR response curves – obtained with 0.1 and 1 μ M NRP1 analyte and distinct immobilized ligands – are represented by dots. Red lines correspond to a simultaneous, global fit of the two curves to a one-site binding model. Blue lines represent global fits to a multiple-site binding model. Data is representative of a total of 6 injections.

Figure S7. Multiple alignment of amino acid sequences of core histones H2A, H2B, H3 and H4 from *X. laevis* (UniProt [respectively]: P06897-1, P02281-1, P02302-1, P62799-1) and *A. thaliana* (UniProt [respectively]: Q8GUH3-1, Q1H5F7-1, B9DGR9-1, P59259-1) using Clustal Omega. Sequences coloured by similarity: fully conserved residue (black), residues with strongly similar properties (light blue) and residues with weakly similar properties (grey).

Figure S8. A. Schematic representation of the nucleosome assembly activity of NRP1 by means of plasmid supercoiling assays. **B.** Nucleosome assembly activity by supercoiling assays in presence of isolated NRP1 (lane 3), HeLa core histones (lane 4), Cc (lane 5) or combination of Cc with histones (lane 6) or NRP1 (lane 7). Plasmids previously treated with Topo I. Lane 1 (control) shows supercoiled, untreated DNA plasmid, whereas lane 2 corresponds to DNA plasmid relaxed by Topo I. **C.** MNase assay in presence of isolated HeLa core histones (lane 2),

NRP1 (lane 3), Cc (lane 4) or combination of Cc with histones (lane 5) or NRP1 (lane 6). Plasmid DNA was digested with 30 U/ml MNase. Lane 1 indicates a DNA ladder marker and the size of each band is represented on the left. **D.** 1D ¹H NMR spectra monitoring Met-88 methyl signal of reduced Cc (13 μ M) in absence (—) or presence of 6.5 μ M BSA (—) or 300 μ g calf thymus histones (…).

Figure S9. BiGGER molecular docking of complexes between NRP1 and *A. thaliana* core histones.

500 solutions of docking for histone:NRP1 complexes. Centres of mass for H2A (green spheres), H2B (purple spheres), H3 (red spheres) and H4 (blue spheres) represented. NRP1 ribbon representations (gold) are shown and arrows indicate the two highest-scoring solutions.

Figure S10. Differences in line-width ($\Delta\Delta \nu_{1/2Binding}$) in ¹⁵N dimension of Cc amide NMR signals upon binding to NRP1.

 $\Delta\Delta v_{1/2Binding}$ of Cc amide groups were calculated from differences between [¹H, ¹⁵N] HSQC spectra of free Cc and Cc in Cc:NRP1 complex at a ratio of 1:0.5. Residues exhibiting significant line broadening beyond threshold marked with asterisk. Threshold (dashed line) corresponds to average plus 2-fold the standard deviations ($\Delta\Delta v_{1/2Binding} \ge \Delta\Delta v_{1/2Binding} + 2S_{n-1}$).

Figure S11. Comparison of complexes formed between NRP1 and Cc or core histones.

A. Ribbon representations for two best models, according to overall score for complex between NRP1 and Cc yielded by NMR-restrained BiGGER molecular docking. NRP1 ribbon representation (gold), Cc (blue) and Cc heme group (red) are shown.

B-E. Ribbon representations for two best models according to overall score for complexes between NRP1 and histones H2A (B), H2B (C) H3 (D) or H4 (E) obtained by BiGGER molecular docking. NRP1 ribbon representation (gold), H2A (green), H2B (purple), H3 (red) and H4 (dark blue) are shown. NRP1 is shown in the same orientation as in A.

Table S1

Protein complex	<i>K</i> ⊳ (μΜ)
H2B:NRP1	18.5
H3:NRP1	57.9
H4:NRP1	28.9
H2A-H2B:NRP1	0.51
H3-H4:NRP1	0.74
Cc:NRP1	12.1

NRP1 AtNAP1;4 AtNAP1;2 AtNAP1;1 AtNAP1;3	KOSFNVSDLTSALKDEDRAGLVNALKNKLQNLAGQHSDVLTS	3 (5 9 5 4 5 4 5 4
NRP1	IEKLQEIQDDLEKINEKASDEVLEVEQKYNVIRKEVYDKRNEVI	74
AtNAP1;4	VLFLKDIQVTHDELEEKFLAEKSALEATYDNLYKELFAKRYEIVNGVVEAE	110
AtNAP1;2	VEFLREIQNQYDEMEAKFFEERAALEAKYQKLYQELYTKRYEIVNGVVEVEGAAEE-VKS	113
AtNAP1;1	VDALRDIQSQHDELEAKFREERAILEAKYQTLYQELYVKRYEIVNGTTEVELAPEDDTKV	114
AtNAP1;3	VEVLREIQGKHDEIETKFREERAALEAKYQKLYQELYNKRYEIVNGATEVEGAPED-AKM	113
NRP1	QSIBGEWMTRFLSHPALGDLLTEEDQKIFKYLNSLEVEDAKDVKSGYSIT	124
AtNAP1;4	AEKEGVENEWLIAMKTNEMLANEITERDEAALKYLKDIRSCRVEDTSRNFKLE	163
AtNAP1;2	EQGEDKSAEEKGVEDEWLIALKNNEITAEEITERDEGALKYLKDIKWSRVEE-PKGFKLE	172
AtNAP1;1	DQGEEKTAEEKGVESEWLTALKNNDVISEEVTERDEGALKYLKDIKWCKIEE-PKGFKLE	173
AtNAP1;3	DQGDEKTAEEKGVESEWLTALKNNDVISEEITERDEGALIYLKDIKWCKIEE-PKGFKLE	172
NRP1	FHFTSNPFFEDAKLTKTFTFLEEGTTKITATPIKWKECKGLPNGVNHDDKKGN	177
AtNAP1;4	FLFDSNLYFKNSVLSKTYHVNDEDGPVLEKVIGTDIEWFPCKCLTHKVVVKKKTKKCPKK	223
AtNAP1;2	FFFDQNPYFKNTVLTKTYHMIDEDEPILEKALGTEIEWYPGKCLTQKILKKK-PKKGS	229
AtNAP1;1	FFFDTNPYFKNTVLTKSYHMIDEDEPLLEKAMGTEIDWYPGKCLTQKILKKK-PKKGS	230
AtNAP1;3	FFFDQNPYFKNTLLTKAYHMIDEDEPLLEKAIGTEIDWYPGKCLTQKILKKK-PKKGA	229
NRP1	KRALPEESFFTWETDAQHKEDAGDEIHDEVADIIKE	213
AtNAP1;4	VNNIPMTKTENCESFFNFEKPPEIPEIDEVDDYDDFDTIMTEELQNLMDQDYDIAVTIRD	283
AtNAP1;2	KNTKPITKTEDCESFFNFESPPQVPDDDEDLDDDMADELQGQMEHDYDIGSTIKE	284
AtNAP1;1	KNTKPITKLEDCESFFNFESPPEVPDEDEDIDEERAEDLQNLMEQDYDIGSTIRE	285
AtNAP1;3	KNAKPITKTEDCESFFNFENPPQVPDDDEDIDEERAEELQNLMEQDYDIGSTIRE	284
NRP1	DLWSNPLTYFNNDADEE-DFDGDDDGDEEGEEDDDDEEEEDGEE	256
AtNAP1;4	KLIPHAVSWFTGEALVDEDDSDDNDDDDN-DEKSDGEEDDDDEEEDDEDDEEEDDE	317
AtNAP1;2	KIISHAVSWFTGEAVEADDLDIEDDDD-EIDEDDDEEDEEDDEDDEEEDDEDDEEEAD	343
AtNAP1;1	KIIPRAVSWFTGEAMEAEDFEIDDEEDDIDEDEDEEDEEDE-EDDDDEDE	335
AtNAP1;3	KIIPHAVSWFTGEAIEGEFEIDNDDEDDIDEDEDEEDEDEDEEE-EDDEDEEE	338
NRP1 AtNAP1;4 AtNAP1;2 AtNAP1;1 a+NAP1:3	QGKKSKKKSSAGHKKAGRSQLA-EGQAGERPPECKQQ 379 EESKTKKKPSIGNKKGGRSQIVGEGKQDERPPECKQQ 372 EVSKTKKKPSVLHKKGGRPOVTD-DOOGERPPECKQQ 374	





MVADKSKKSKIEEKGEEENLEQIDAELVLSIEKLQEIQDDL EKINEKASDEVLEVEQKYNVIRKPVYDKRNEVIQSIPGFWM TAFLSHPALGDLITEEDQKIFKYLNSLEVEDAKDVKSGYSI TFHFTSNPFFEDAKLTKTFFLEEGTTKITATPIKWKEGKG LPNGVNHDDKKGNKRALPEESFFTWFTDAQHKEDAGDEIHD EVADIIKEDLWSNPLTYFNNDADEEDFDGDDDGDEEGEEDD DDEEEEDGEE

oserved m/z	Peptide
)5.5479	KPVYDKR
73.6633	TFTFLEEGTTK
68.8999	EDAGDEIHDEVADIIK
92.0332	ASDEVLEVEQKYNVIR
37.2988	SGYSITFHFTSNPFFEDAKLTK



MPEPAKSAPAPKKGSKKAVTKTQKKGD **KKRKKSRKESYSIYVYKVLKOVHPDTG** ISSKAMGIMNSFVNDIFERIAGEASRL AHYNKRSTITSREIQTAVRLLLPGELA **KHAVSEGTKAVTKYTSSK**

H2B (G. gallus)

1750

H4 (G. gallus)

1577.8386

1750

Observed m/z	Peptide
816.4587	EIQTAVR
828.4202	HAVSEGTK
901.5073	LAHYNKR
953.5872	LLLPGELAK
1151.5437	ESYSIYVYK
1168.5774	QVHPDTGISSK
1279.6382	KESYSIYVYK
1461.7776	STITSREIQTAVR
1508.8162	VLKQVHPDTGISSF
1775.7592	MGIMNSFVNDIFEF

2000

2000

MSGRGKGGKGLGKGGAKRHRKVLRDNI QGITKPAIRRLARRGGVKRISGLIYEE TRGVLKVFLENVIRDAVTYTEHAKRKT **VTAMDVVYALK**RQGRTLYGFGG

Observed m/z	Peptide
989.5533	VFLENVIR
1134.5123	DAVTYTEHAK
1180.5898	ISGLIYEETR
1325.7170	DNIQGITKPAIR
1336.6814	RISGLIYEETR
1438.7610	KTVTAMDVVYALF
1481.8075	DNIQGITKPAIRF
1577.8386	ISGLIYEETRGVL









Time (s)



Time (s)

Time (s)











H3:NRP1

H4:NRP1







