SUPPLEMENTAL INFORMATION

EXPERIMENTAL PROCEDURES

Protein expression and purification. Nurf55 gene was cloned into pFastBacHT vector (Invitrogen) using standard PCR and restriction cloning techniques. Nurf55 mutants were generated using the Quick Change Site Specific Mutagenesis kit (Stratagene). Recombinant viruses were generated according to the Bac-to-bac manual (Invitrogen). Nurf55 wildtype and mutant proteins, containing an N-terminal TEV-cleavable His_6-tag were expressed in Sf21 insect cells. Cells were harvested 48 hours after infection and lysed in lysis buffer (500 mM NaCl, 150 mM Tris/HCl, pH 7.5, 20% glycerol, 5 mM ß-mercaptoethanol, 4mM $MgCl_2$, DNase I, Complete EDTA-free protease inhibitors (Roche)). Clarified insect cell lysate was incubated with NiNTA sepharose (Qiagen), and the recombinant proteins were recovered by elution with buffer containing 100 mM imidazole, 150 mM NaCl, 50 mM Tris/HCl, pH 7.5, 10% glycerol, 5 mM ß-mercaptoethanol, followed by overnight dialysis and TEV cleavage. Further purification steps comprised ion-exchange chromatography using SP sepharose, where Nurf55 eluted in the flow-through, followed by a MonoQ chromatography (50 mM Tris/HCl, pH 7.5, 5 mM ß-mercaptoethanol , gradient: 50mM – 1M NaCl in 50 column volumes) and size exclusion chromatography using a Superdex 200 column (GE Healthcare) (150 mM NaCl, 10 mM Tris/HCl, pH 7.5, 5 mM ß-mercaptoethanol).

All fragments of the Su(z)12 protein were generated by PCR and cloned into pETM-30 vector using standard restriction cloning techniques. The Su(z)12-$1_{R70A,\ R73A,R75A}$ fragment was generated using Quick Change Site-Specific Mutagenesis kit (Stratagene). Proteins containing an N-terminal TEV-cleavable GST-tag were overexpressed in *E.coli* strain BL21 Star™(DE3) (Invitrogen) co-transformed with pRARE plasmid (Novagen) at 30°C for 5 hours. Cells were lysed in lysis buffer (500mM NaCl, 150mM Tris/HCl, pH 7.5, 20% glycerol, 5mM DTT, 4mM $MgCl_2$, DNaseI, Complete EDTA-free protease inhibitors (Roche)) and clarified bacterial lysate was incubated with glutathione sepharose (GE Healthcare). After washing with lysis buffer the beads were incubated with purified Nurf55 followed by extensive washes with lysis buffer and overnight cleavage with TEV protease. Proteins cleaved from the beads were analysed by Western blot using anti-Nurf55 polyclonal antibodies. Su(z)12 fragments Su(z)12-1, Suz 50-100 and Su(z)12-$1_{R70A,\ R73A,\ R75A}$ for pull-downs and ITC experiments were expressed as described above, bound to glutathione sepharose, washed with wash buffer (150 mM NaCl, 50 mM Tris/HCl, pH 7.5, 10% glycerol, 5 mM ß-mercaptoethanol) and eluted with 15 mM glutathione in the wash buffer, followed by

overnight dialysis. For proteins used for ITC experiments (non-tagged) fusion proteins were mixed with TEV-protease (0.05% w/w) prior to overnight dialysis. Cleaved GST was removed by a second incubation with glutathione beads, and the proteins were further purified using a MonoS column (GE Healthcare) (50 mM Tris/HCl, pH 7.5, 5 mM ß-mercaptoethanol, salt gradient: 50mM – 1M NaCl in 50 column volumes).

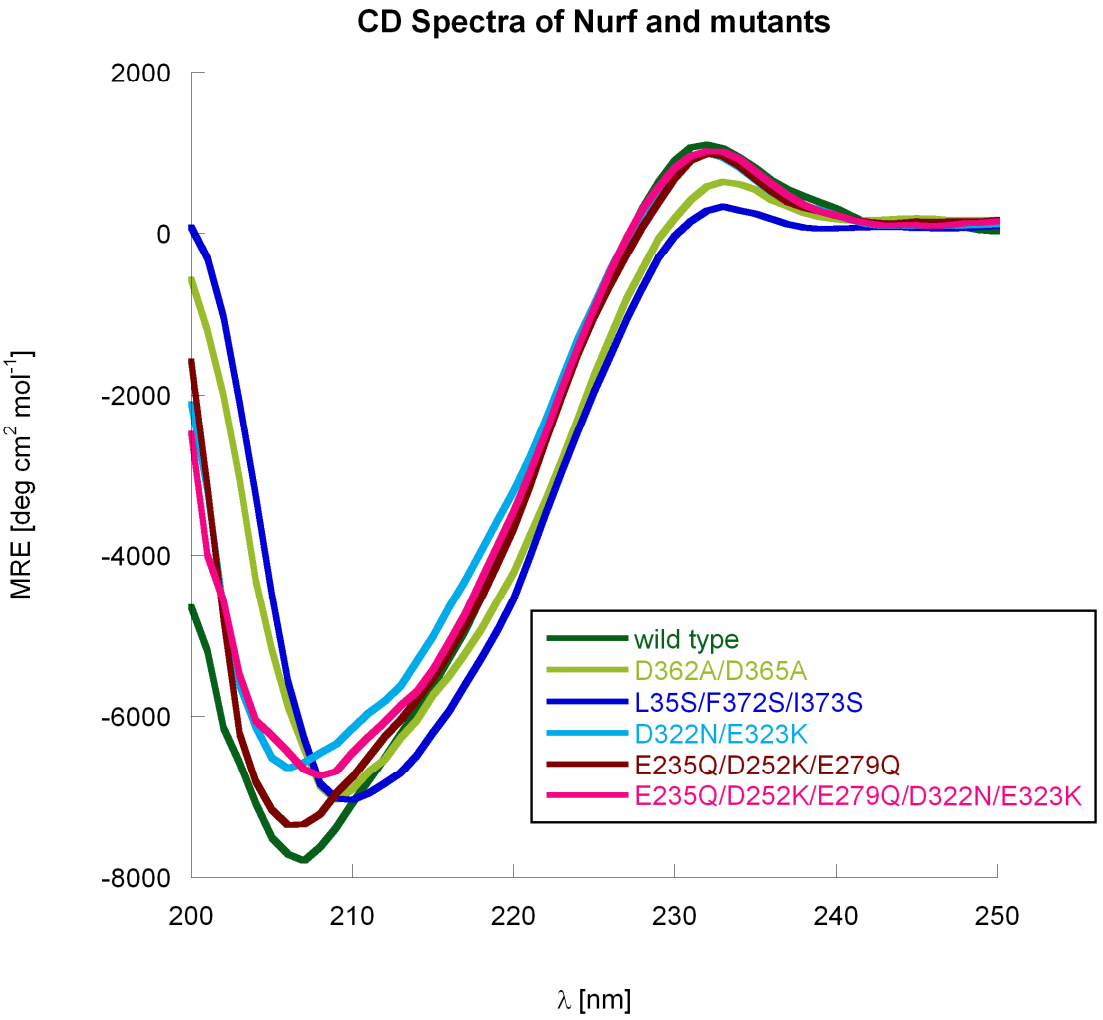
Supplemental Table 1. Crystallographic analysis of the Nurf55-H4 peptide complex

Data collection:	
Resolution range (Å)	25 – 1.75
Radiation source	ESRF Grenoble, beamline ID23-2
Wavelength (Å)	1.0085
Space group	$P12_11$
Unit cell ($Å^3$)	61.51 59.21 65.89
(°)	90.0 99.83 90.0
R_{sym}	4.5 (43.3) [§]
No. of reflections	169411
No. of unique reflections	46472
Redundancy	3.6
Completeness (%)	98.3 (97.3) [§]
Average I/σ	20.1 (3.1) [§]
Wilson B-factor ($Å^2$)	27.2

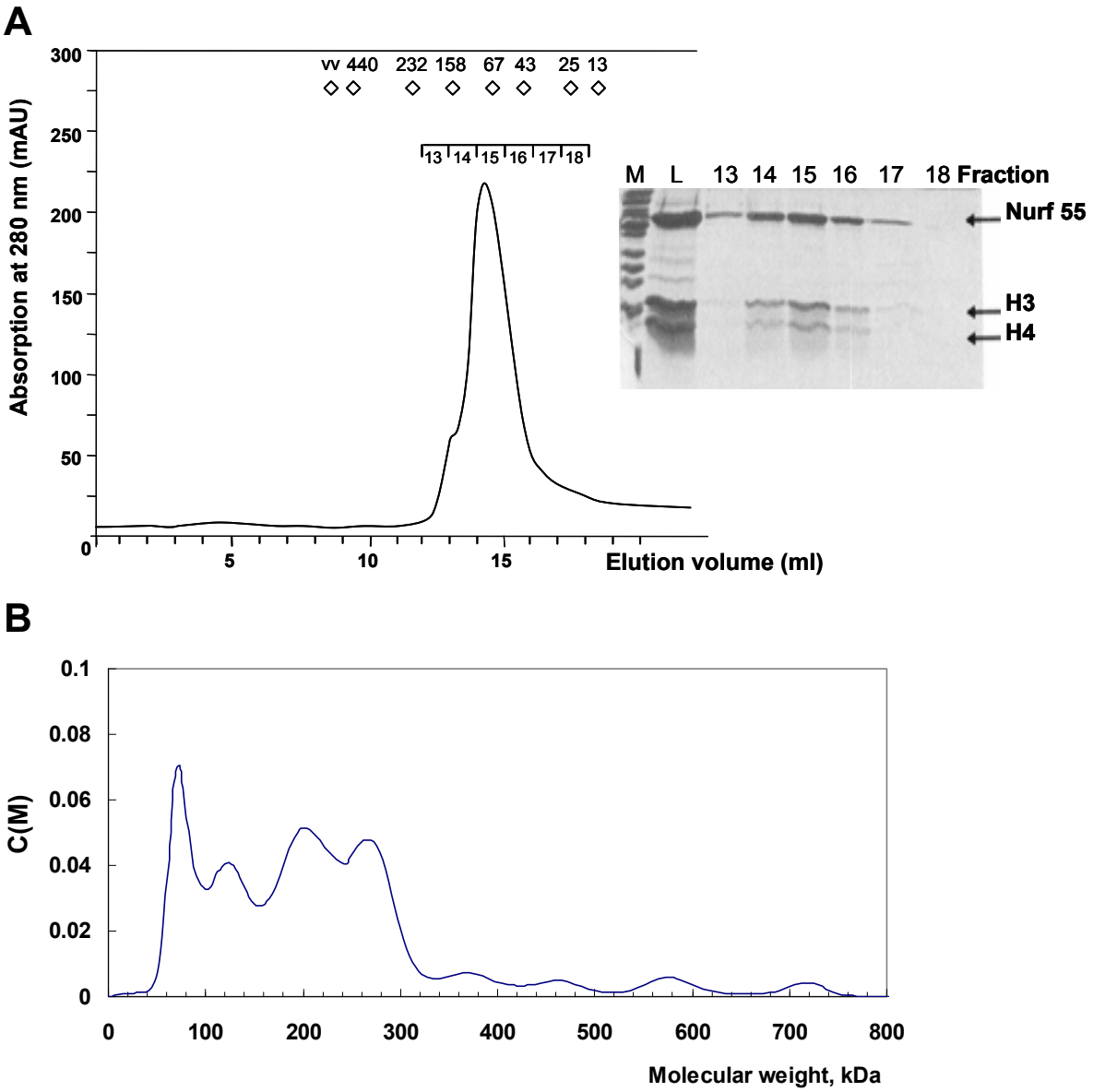
Values in parentheses correspond to the outermost resolution shell : [§] 1.85- 1.75 Å

Refinement statistics:	
Resolution (Å)	25- 1.75
Refinement program	PHENIX v1.3
No. of reflections (No. of test reflections)	46469 (1859)
No. of atoms	3293
No. of water molecules	283
R-factor	0.1735 (0.2303) [Δ]
free R factor	0.2050 (0.2945) [Δ]
R.m.s.d bonds (Å)	0.006
R.m.s.d angles (°)	1.0
Coordinate error (Å)	0.13
Average B factor ($Å^2$)	27.9
Ramachandran Plot regions (%):	
Most favoured	95.7 (355 residues)
Additionally allowed	3.7 (14 residues)
Generously allowed	0.6 (2 residues)

Outermost resolution shell: [Δ] 1.80-1.75 Å

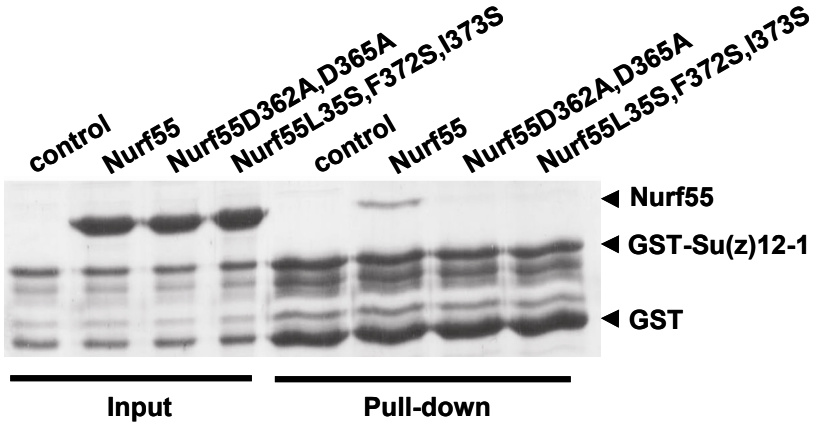


Supplemental Figure S1. Circular dichroism spectra for Nurf55 wildtype and mutant proteins. CD spectra were recorded with a JASCO J810 spectropolarimeter at a protein concentration of ~ 0.5 g/l at 20 °C in 20 mM phosphate/NaOH pH 7.5, 150 mM NaCl, 1mM TCEP using a quartz cuvette with 1mm depth.

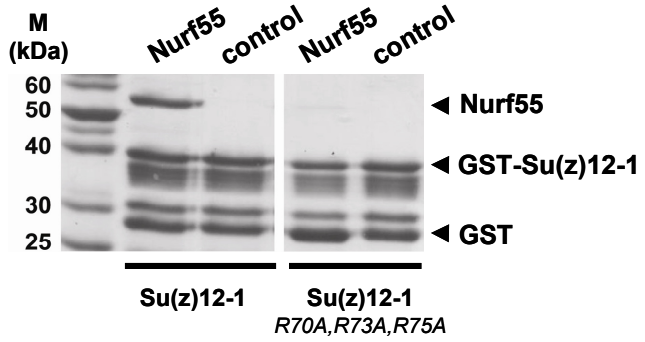


Supplemental Figure S2. Interaction of Nurf55 with histone H3-H4 dimers or tetramers. **(A)** Size exclusion chromatography of the Nurf55/H3-H4 complex with Superdex 200 column (GE Healthcare) in 150 mM NaCl, 25 mM Tris/HCl pH 7.5, 5 mM DTT. The molecular weights of the protein standards used for the calibration of the column are indicated above the elution profile. Amersham Biosciences: ferritin (440 kDa), catalase (232 kDa), aldolase (158 kDa), albumin (67 kDa), ovalbumin (43 kDa), chymotrypsin (25 kDa). Inner panel: Fractions from the size exclusion chromatography (M = Marker, L = Load). **(B)** Analytical ultracentrifugation experiment of the Nurf55-H3-H4 complex. Sedimentation velocity experiments were done at 20^{o}C using 2-channel charcoal centerpieces at 46,000 rpm in a Beckman Optima XL-A centrifuge (Beckman-Coulter) fitted with a four-hole AN-60 Ti rotor. Absorbance of the sample was monitored at 280 nm. Buffer conditions are given above. The plot shows the sedimentation coefficient distribution derived from the sedimentation profile.

A



B



Supplemental Figure S3. Overview of the Nurf55/Su(z)12-1 interaction. (A) Pull-down experiments of wildtype and mutants Nurf55 with GST-Su(z)12-1. N-terminal Su(z)12-1 (like full-length Su(z)12) binds wildtype Nurf55, but not $Nurf55_{D362A,\ D365A}$ and $Nurf55_{L35S,F372S,I373S}$ mutant proteins. No Nurf55 protein was present in the control lane. **B**. Pull-down experiments of Nurf55 protein with wildtype GST-Su(z)12-1 and mutant GST-Su(z)12-$1_{R70A/R73A/R75A}$ proteins. Mutant protein GST-Su(z)12-$1_{R70A/R73A/R75A}$ does no longer interact with Nurf55.