Supporting Information

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SI Materials and Methods

Preparation of Domain I of Spindlin1 for ITC Measurements. An isolated tudor-like domain I of Spindlin1 (aa 27–120) was expressed in *Escherichia coli* using a pET28a-SUMO vector. The N-terminal His-SUMO tagged fusion protein was first purified with Ni-NTA chelating resins, followed by cleavage of the His-SUMO tag with the SUMO protease during dialysis. The cleaved tag was separated from the domain I protein through Ni-NTA resins, and the domain I protein was further purified using a HiLoad-16/60 Superdex-75 column (GE Healthcare). ITC experiments were carried out following a previously described procedure (1).

Mononuclesome Pull-Down, Analytic Ultracentrifugation, and Size Exclusion Column Chromatography Analyses. Chemical mimetics of histone H3 and H4 methylated at various lysine residues were prepared according to a published protocol (2), and the chemically modified histone H3 or H4 was assembled into mononucleosomes with other core histones and biotin-labeled 601 nucleosome positioning sequence (3). Pull-down experiments with the modified mononucleosomes were carried out following the procedures we previously described (1).

Analytic ultracentrifugation experiments were performed on a Beckman Proteomelab XL-1 machine at 262,000 × *g* (corresponding to 60,000 rpm) for 4 h. Size exclusion chromatography was performed on a Superdex 75 10/300 GL column (GE Healthcare). Both experiments were carried out at 4 °C under the condition of 50 mM Hepes at pH 8.0 and 500 mM NaCl. The concentration of Spindlin1 is at 1 mg/mL, and the H3K4me3 peptide (aa 1–8) was added to 1:1 molar ratio in the protein–peptide complex sample. The protein–peptide complex formation at this salt concentration was assessed by ITC measurements.

1. Wang W, et al. (2011) Nucleolar protein Spindlin1 recognizes H3K4 methylation and stimulates the expression of rRNA genes. *EMBO Rep* 12(11):1160–1166.
2. Simon MD, et al. (2007) The site-specific installation of methyl-lysine analogs into recombinant histones. *Cell* 128(5):1003–1012.
3. Lowary PT, Widom J (1998) New DNA sequence rules for high affinity binding to histone octamer and sequence-directed nucleosome positioning. *J Mol Biol* 276(1): 19–42.

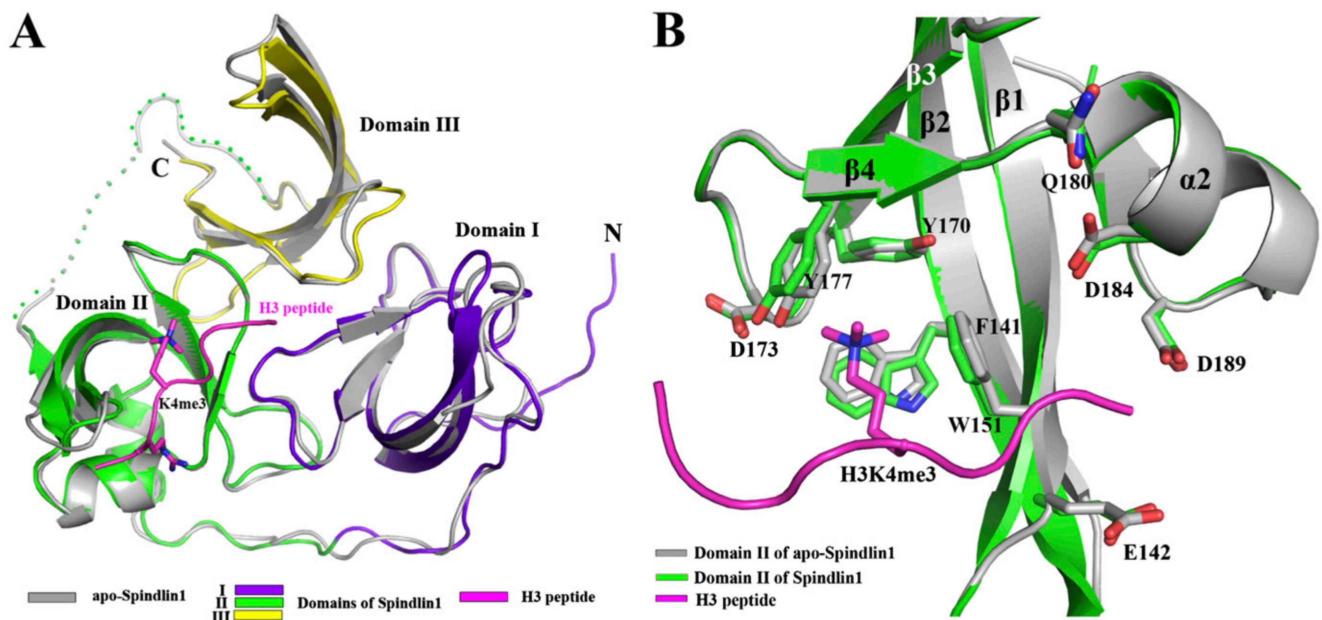

Fig. S1. Comparison of apo and H3K4me3 peptide-bound structures of Spindin1. (*A*) Superposition of the apo and the peptide-bound structures of Spindlin1. The apo structure is colored gray, and the peptide-bound structure is colored the same as in Fig. 1. (*B*) Domain II residues involved in binding the histone H3 peptide, including those forming the methyllysine-binding aromatic cage, show no significant conformational differences between the peptide-bound (green) and the apo (gray) structures.

Fig. S2. Fo-Fc omit map of the H3K4me3 peptide bound to the second tudor domain of Spindlin1. The map is contoured at the 2.5 σ level.

Fig. S3. Analyses of conformational rigidity of Spindlin1 tudor-like domains. (*A*) Analytic ultracentrifugation experiments show that free (red line) and peptide-bound (blue line) Spindlin1 have identical sedimentation profiles. (*B*) Free (dashed line) and peptide-bound (solid line) Spindlin1 elute from the gel-filtration column at the same volume. (*C*) Under the buffer condition used for analytic ultracentrifugation and gel-filtration experiments, the H3K4me3 peptide bound Spindlin1 with a dissociation condition of 1.2 μM, as measured using ITC.

Fig. S4. Domain I of Spindlin1 does not bind methylated histones. (*A*) Mononucleosome pull-down experiments show that, apart from nucleosomes installed with H3Kc4me3 (*Top*, left two lanes), nucleosomes with unmodified or modifications mimicking mono-, di-, or trimethylation on H4K20 (*Top*), H3K79 (*Middle*), and H3K36 (*Bottom*) do not bind Spindlin1. Together with earlier results that Spindlin1 does not bind nucleosomes methylated at H3K9 or H3K27, it appears Spindlin1 is specifically targeted to nucleosomes with H3K4 methylation. (*B*) ITC measurements indicate that domain I of Spindlin1 does not bind H3K4me3, H3K9me3, H3K27me3, H3K36me2, or H4K20me3 peptides.

Table S1. Statistics for crystallographic analysis

Data collection	Spindlin1 + H3K4me3 peptide
Wave length (Å)	0.9789
Space group	$P2_12_12$
Unit cell (Å)	a = 122.43, b = 42.69, c = 50.31
Resolution (Å)	50–2.10 (2.18–2.10)
Rmerge	0.077 (0.387)
I/σI	24.3 (6.6)
Completeness (%)	99.9 (100)
Total/Unique reflections	126368/16119
Refinement Statistics	
R-work/R-free (%)	18.1 (21.8)
Rmsd bonds (Å)	0.007
Rmsd angles (°)	1.064
B factor (overall)	52.9 $Å^2$
Spindlin1 (overall)	52.7 $Å^2$
Main chain	49.9 $Å^2$
Sidechain	55.5 $Å^2$
Peptide (overall)	52.2 $Å^2$
Mainchain	55.6 $Å^2$
Sidechain	49.2 $Å^2$
Water	51.8 $Å^2$
SO_4^{2-}	74.0 $Å^2$
Glycerol	57.1 $Å^2$
CHES	53.7 $Å^2$
Ramachandran plots	
Favored	194 (98.0%)
Allowed	4 (2.0%)
Outlier	0 (0%)

Data collection and refinement numbers in parentheses are that for the highest resolution shell. Rmerge is defined as $\Sigma|I - \langle I\rangle|/\Sigma\langle I\rangle$, where I and <I> are the averaged intensity of multiple measurements of the same reflection. The summation is over all of the observed reflections. R-factors are defined as $\Sigma||F_O| - |F_C||/\Sigma|F_O|$, where F_O denotes the observed structure factor amplitude and F_C denotes the structure factor calculated from the model. R-work was calculated using the diffraction data used throughout the refinement, and R-free was calculated using the 10% of the data set aside during refinement. Rmsd, root-mean-square deviation.