Supporting Information

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SI Materials and Methods

Protein Production and Purification for NMR. Isotope-labeled full-length cpSRP43 and CD1Ank-BH were overexpressed in BL21(DE3) cells at 37 °C in M9 D_2O media supplemented with $^{15}NH_4SO_4$ and ^{12}C or ^{13}C-glucose. Protein expression was induced at OD_{600} = 0.7 by addition of 1 mM Isopropyl beta-D-1-thiogalactopyranoside (IPTG) for 15 h. Isotope-labeled cpSRP43 and CD1Ank-BH were purified using the same protocol as unlabeled cpSRP43. Proteins were exchanged into NMR buffer (50 mM phosphate, 150 mM NaCl, pH 6.5) using a PD MidiTrap G-25 column (GE Healthcare).

Assignment of cpSRP43 Backbone. Triple labeled (^2H, ^{15}N, ^{13}C) cpSRP43 and CD1Ank-BH (~0.8 mM) were prepared in NMR buffer containing 10% (vol/vol) D_2O. Backbone resonance assignments were made using 3D TROSY-HNCA, TROSY-HN(CO)CA, TROSY-HNCACB, and TROSY-HN(CO)CACB spectra (44, 45). A total of 252 of 323 nonproline backbone residues were assigned using RunAbout in NMRview java. Many of the cross-peaks associated with CD2 are weak, and only a subset (residues 278–287) could be assigned from the triple resonance spectra. Assignments for additional CD2 residues (F267, D273, I275, E277, L288, V289, W291, D293, G294, W299, V300, G302, D308, V309, and K311) were made by transferring published assignments for the isolated CD2 domain (33).

Characterization of the Oligomeric State of cpSRP43. The oligomeric state of cpSRP43 was assessed by two methods. First, cpSRP43 in the presence or absence of equal molar HiLyte-Fluor488–labeled L11 peptide were analyzed by size exclusion chromatography using Superdex 200 column (GE Healthcare). The column was equilibrated in 50 mM KHepes, pH 7.5, with either 200 mM or 50 mM NaCl. The protein elution profile was confirmed by SDS/PAGE, and the molecular mass on the column was further calibrated using the LMW kit (GE Healthcare). Second, velocity sedimentation-type analytical ultracentrifugation was performed using Optima XL-I (Beckman Coulter) with an absorbance optical detection system (280 nm). The sample was spun at 201,600 × *g* at 20 °C with 7 μM of cpSRP43 alone and in complex with equal molar of HiLyte-Fluor488–labeled L11 peptide or cpSRP54M. Buffer viscosity, protein partial specific volumes, and density were calculated using the SEDNTERP (46). The observed sedimentation data were fitted to a single component system by using the SEDFIT software (47), and the sedimentation coefficient distribution was extracted from the fitting.

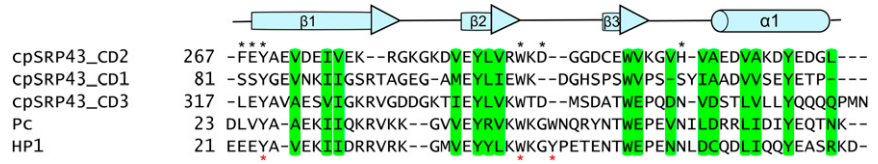

Fig. S1. Sequence alignment of cpSRP43 CD1, CD2, CD3, Polycomb (Pc), and heterochromatin protein 1 (HP1). Green highlights conserved hydrophobic residues in CD2 that were mutated in this study. Red asterisks denote aromatic cage residues in canonical chromodomains. Black asterisks denote aromatic cage residues involved in 54M binding (23). Secondary structure information is labeled above the sequence highlighting β-sheet (β1, β2, β3) and α-helix (α1).

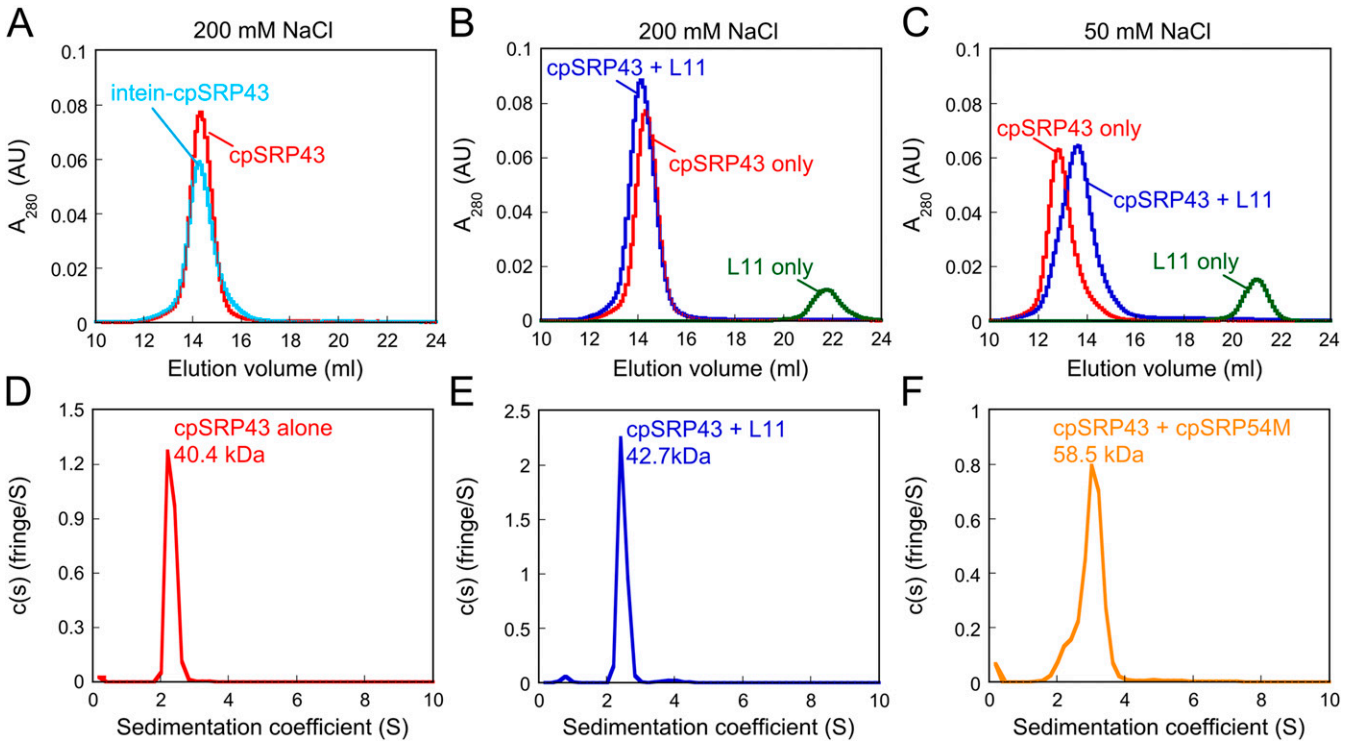

Fig. S2. CpSRP43 is active as a monomer. (*A*) WT (red) and superactive intein-cpSRP43 (cyan) runs as a monomer on Superdex 200 column with buffer containing 200 mM NaCl. (*B*) The complex of WT cpSRP43 and HiLyte-Fluor488–labeled L11 peptide (blue) was eluted as a 1:1 complex. (*C*) At lower ionic strength (50 mM NaCl), cpSRP43 exhibits oligomeric forms (red), but L11 binding shifts cpSRP43 to a lower molecular weight complex (blue). (*D–F*) Sedimentation coefficient distributions calculated from a velocity sedimentation experiment of cpSRP43 alone (*D*), cpSRP43 with HiLyte-Fluor488–labeled L11 peptide (*E*), and cpSRP43 with cpSRP54M (*F*) using buffer containing 200 mM NaCl. The experimental molecular mass is close to the predicted values of cpSRP43 and cpSRP54M (36 and 22 kDa, respectively), suggesting that the active form of cpSRP43 is monomer.

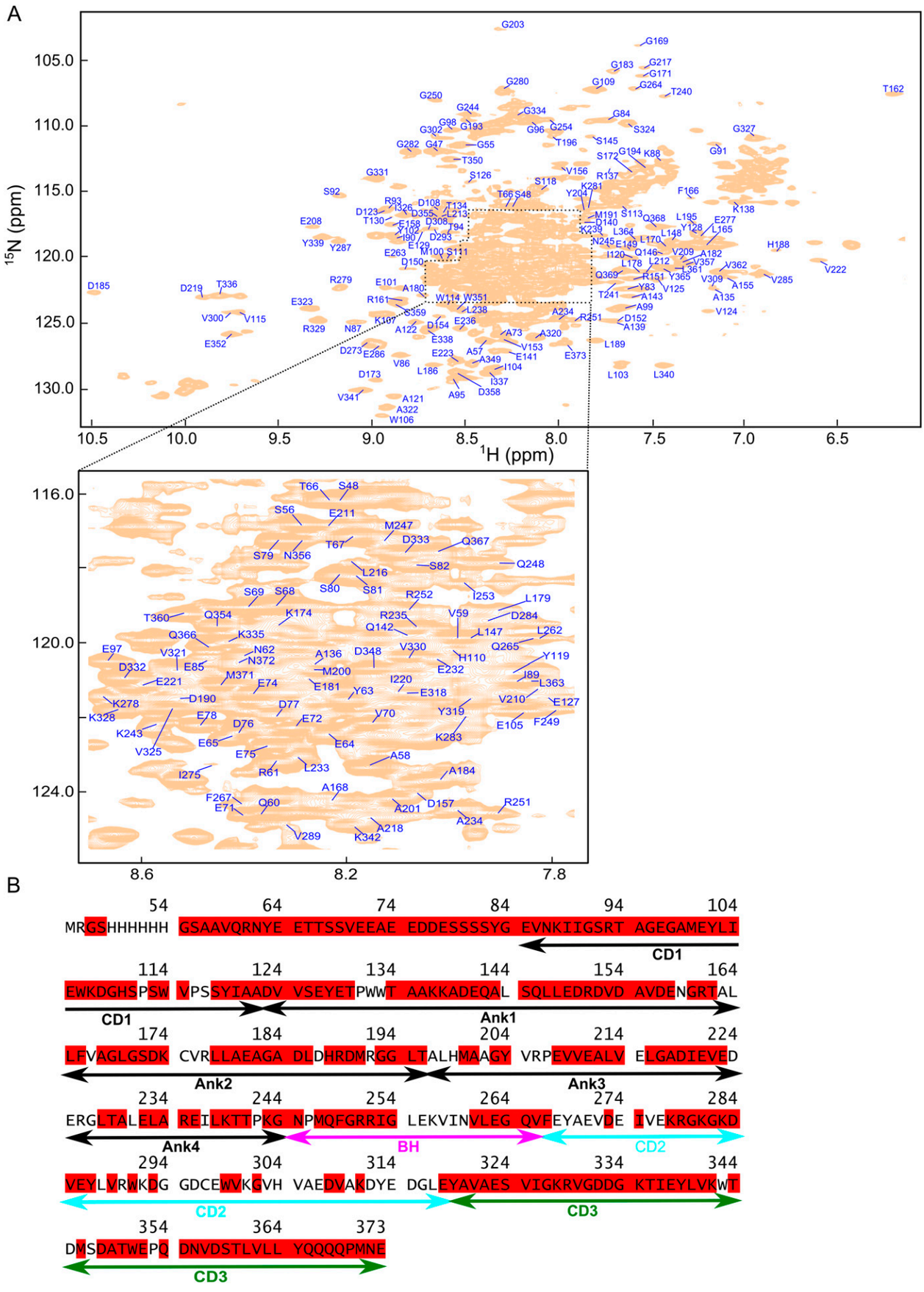

Fig. S3. Assignments of the cpSRP43 spectra. (*A*) 800 MHz ^{1}H-^{15}N TROSY spectrum of full-length ^{15}N, ^{13}C, ^{2}H-labeled cpSRP43 is shown with the assigned residues indicated. The central area of the spectrum is enlarged for clarity. (*B*) Assigned residues are highlighted in red in the cpSRP43 sequence.

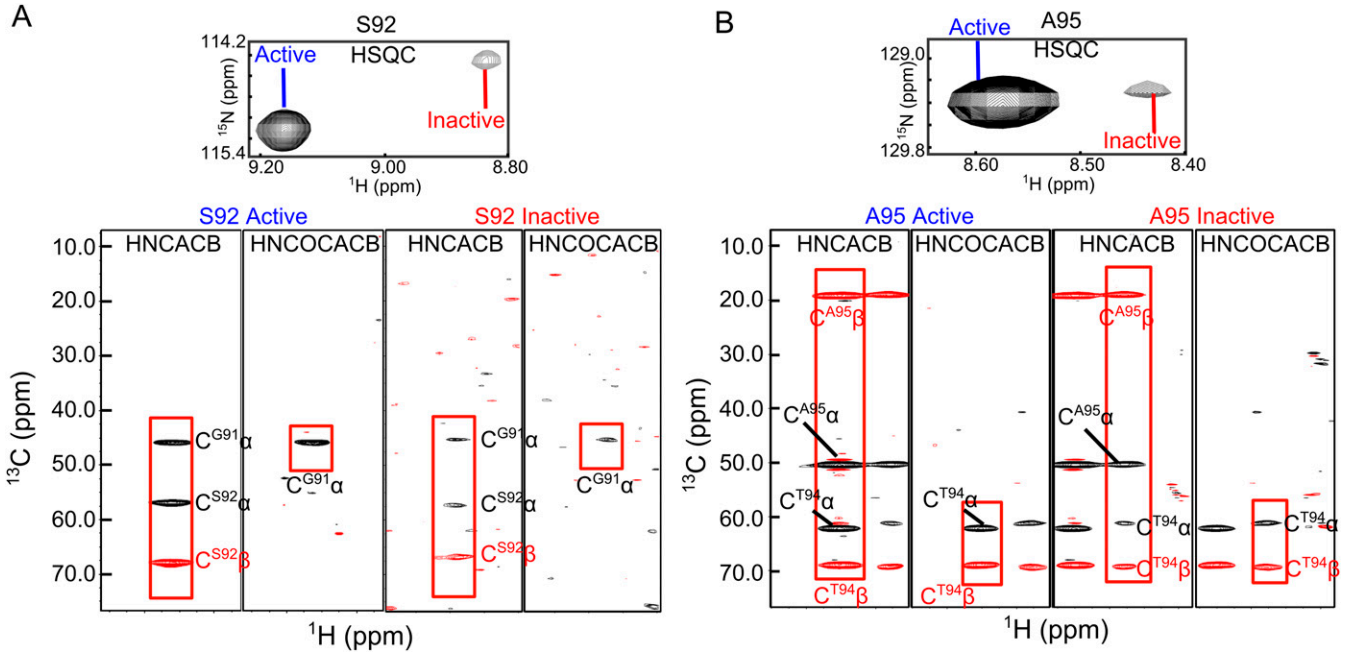

Fig. S4. Pairs of component cross-peaks in CD1 arise from the same amino acids. (*Upper*) Component cross-peaks of residues Ser92 (*A*) and Ala95 (*B*) in the TROSY spectrum of ^{2}H, ^{15}N, ^{13}C-labeled CD1Ank-BH. (*Lower*) Strips from the HNCACB and HN(CO)CACB spectra of ^{15}N, ^{13}C, ^{2}H-labeled CD1Ank-BH for the two component peaks of S92 and A95. For each residue, the two component peaks have connectivities to identical Cα and Cβ chemical shifts for residues i and i-1, indicating that they arise from the same amino acid. The red rectangles highlight the connectivities for the active state (*Left*) and inactive state (*Right*).

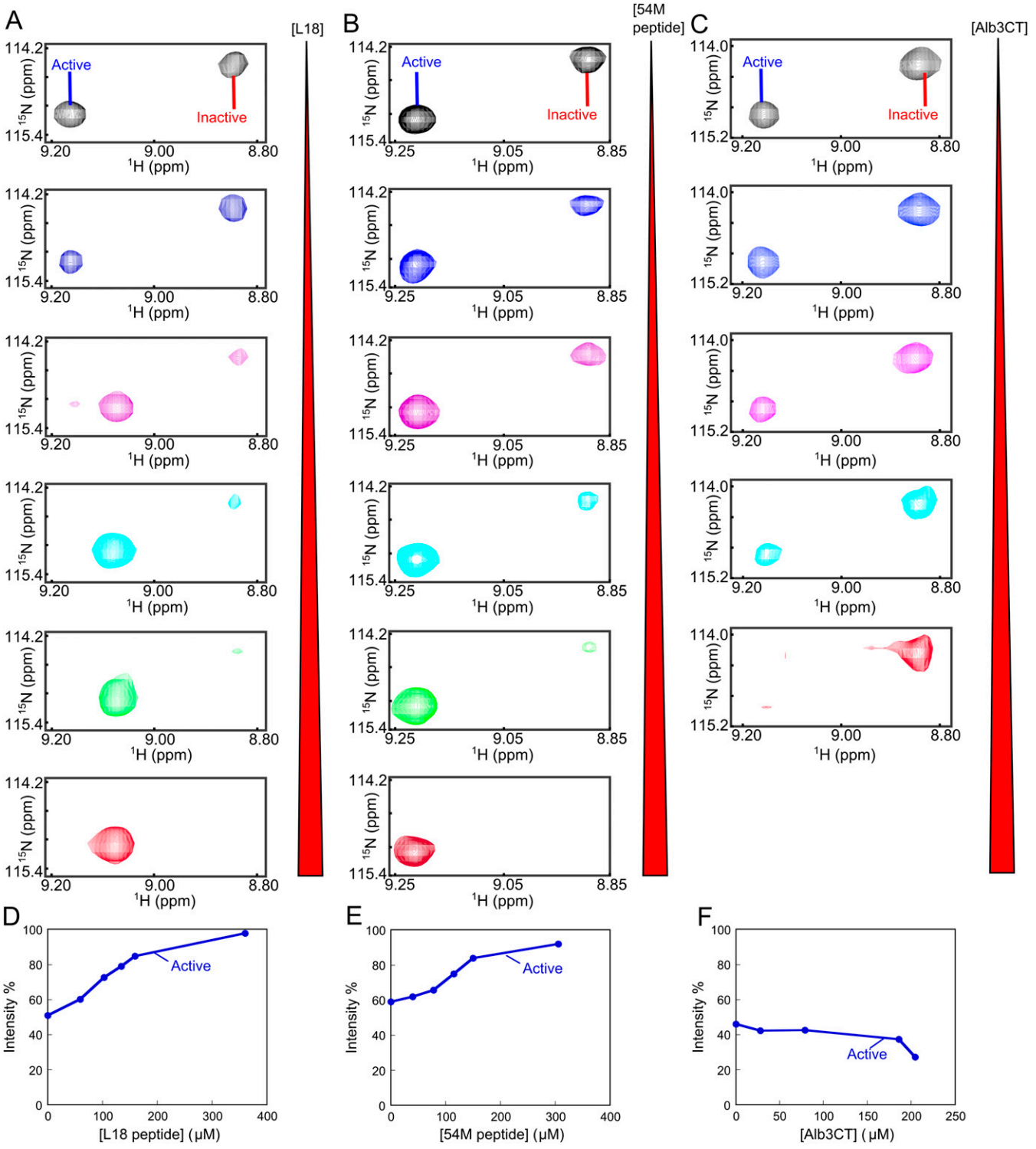

Fig. S5. The conformation of cpSRP43 is regulated by binding partners. (*A*–*C*) Titration of the L18 peptide (*A*), 54M peptide (*B*), and Alb3CT (*C*) into the ^{2}H, ^{15}N-labeled cpSRP43 during the TROSY experiment. Component cross-peaks are shown for Ser92. (*D*–*F*) Quantification of the relative intensity of the component cross-peaks from the data in *A*–*C*.

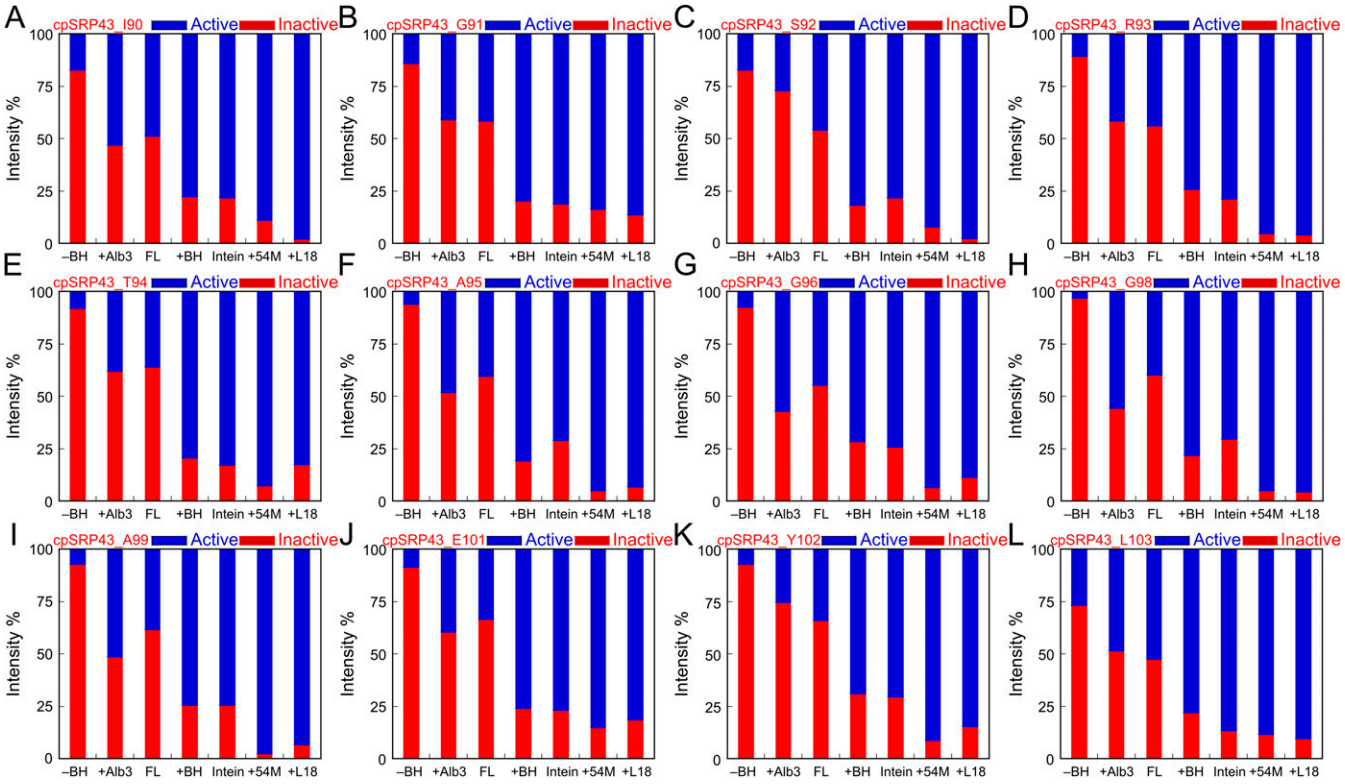

Fig. S6. (*A–L*) Quantification of the relative intensities of component cross peaks for 12 residues in various chaperone constructs and with various ligands bound. –BH, CD1Ank fragment; +Alb3, full-length cpSRP43 + Alb3CT; FL, full-length cpSRP43; +BH, CD1Ank-BH fragment; Intein, Intein-cpSRP43; +54M, full-length cpSRP43 + 54M peptide; +L18, full-length cpSRP43 + L18 peptide.

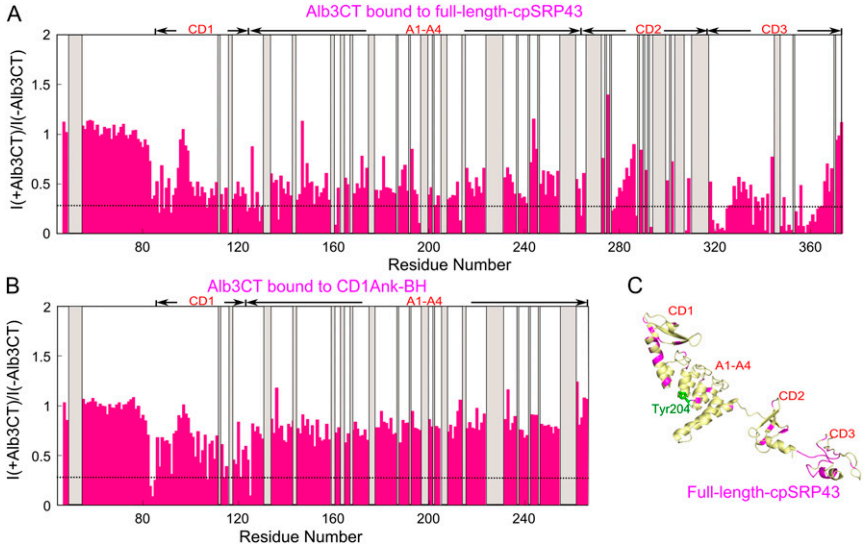

Fig. S7. Summary of the effect of Alb3CT on cross-peak intensities in the ^{1}H-^{15}N TROSY spectra of full-length cpSRP43 (*A*) and the CD1Ank-BH fragment (*B*). The intensities of each cross-peak in the presence and absence of Alb3CT [I(+Alb3CT) and I(–Alb3CT), respectively] were quantified and normalized to those of residues 1–63, a highly unstructured region whose intensities were unaffected by any binding partners, and their ratios were plotted. The gray bars denote unassigned residues (including all of the prolines), and the dashed lines show the cutoff where the peak is broadened ≥70%. (*C*) Structural model for full-length cpSRP43 (19). Dark red highlights residues whose cross-peaks are broadened >70% (from *A*) on binding to Alb3CT. Green highlights Tyr204 that binds L18.

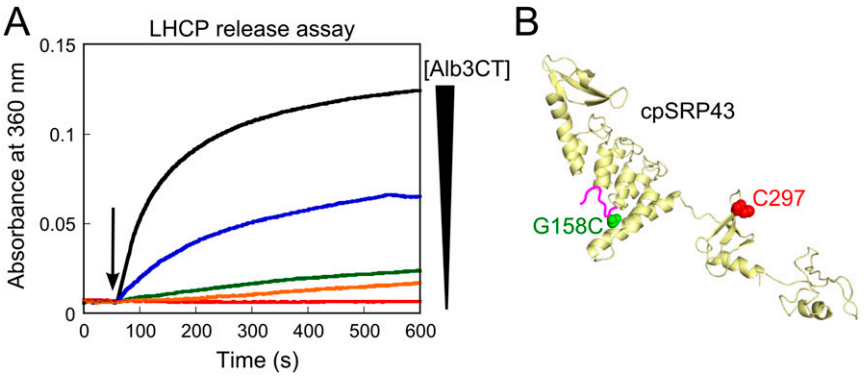

Fig. S8. (*A*) Alb3CT induces dose-dependent release of LHCP TMDs from cpSRP43, performed as outlined in Fig. 5*F* and described in *Materials and Methods*. Red, orange, green, blue, and black denote data obtained with 0, 0.625, 1.25, 2.5, and 5.0 μM Alb3CT, respectively. Errors were estimated to be ±10% (SD) based on three technical replicates. (*B*) Structural model of cpSRP43 highlighting the positions of the FRET probes. Residue G158 in the L18 motif of LHCP (green) was mutated to cysteine and labeled with Atto488 (donor), and the native C297 in cpSRP43 (red) was labeled with TMR.

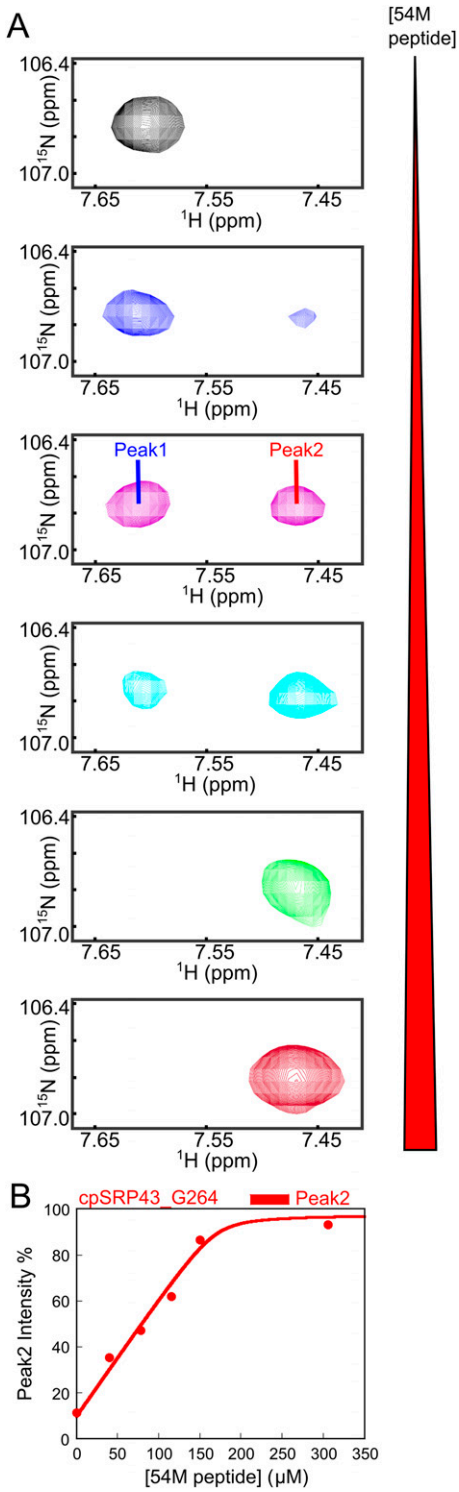

Fig. S9. Effects of 54M binding on the intensity of G264 (in BH). (*A*) Region of the TROSY spectrum of ^{2}H, ^{15}N-labeled cpSRP43 showing the effects of increasing concentrations of the 54M peptide on the G264 cross-peaks corresponding to unbound and 54M-bound cpSRP43 (labeled as peak 1 and peak 2, respectively). (*B*) Quantification of the relative intensities for the 54M-bound peak (peak 2) for G264. The line is a fit of the data to Eq. **1**.