

Supporting Information

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1 M V W L T S I A F I V T L L G A Q Y D I
  ATGGTTGGC TGACGAGTAT CGCGTTTATT GTGACTCTTT TAGGAGCACA ATACGACATA
61 V T A Q A I S V A T S V P S V F S S P S
  GTGACTGCGC AGGCAATFTC AGTGTCAACT TCTGTCCCAT CAGTGTTCAG TAGCCCTAGC
121 L A S G F I G C L T T G I G Q S P D F P
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181 F O E Q O D I D D L A Q V I I S A V T S
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241 N T D T S K S A R A O A L S T A L A S S
  AATACTGACA CCTCAAAGTC AGCGAGAGCA CAAGCCTTGA GCCTGCATT AGCATCTTCC
  L A D I L I S E S S G S S Y O T O T S A
301 TTAGCCGACC TACTGATTTT CGAATCAAGT GSAAGCAGCT ACCAACTCA AATATCTGCC
  L T N I L S D C F V T T T G S N N P A F
361 TCCTAATA TCCTATCCGA TTGTTTTGTC ACAACAAC TGATCAACAA TCCTGCATT
  V S R V O T L I A V L S O S S S N A I S
421 GTATCAAGAG TTCAAACACT TATAGCAATG CTTTCTCAA GCAGCAGTAA TGCAATTTCA
  G A T G G S A F A O S Q A F O O S A S O
481 GCGCAACAG GTGGCTCCGC ATTCCGACAA AGTCAGGCGT TCCAAACAA GGCATCACAA
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541 AGTGTGGCC TAAGTGTTC CCGAGCAGGA TCCACATCCT CTTCCACCAC TACCACCACC
  S C A T S Q A A S O S A S S S V S S A F
601 TCGGAGCAA CAAGTCAAGC AGCAAGCCAG AGTGCAGCA GTTCTTATAG CTCTGCATT
  A Q A A S A L A T S S A T S R A F A S
661 GCACAAGCG CCTCTTCTGC CCTTGCAGC TCCAGTGCTA TCAGCAGAGC CTTCGCTTCT
  V S S A R A S L A V N I G L S A R
721 GTCAGTCCG CCTCTGCGC CTCCAGCCTT GCTTATAACA TAGGCTTATC CGCCGACGG
  S L G I A S D T A L A G A L A Q A V G G
781 TCCCTCGAA TAGCCTCTGA CACAGCCCTC GCTGGTGCCT TAGCTCAAGC TGTGGGTGGA
  V G A G A S A S A Y A N A I A B A A G Q
841 GTAGGCGCG GAGCCTCTGC ATCTGCCTAC GCTAATGCTA TTGCACGTGC CGCTGGACAA
  F L A T Q G V L N A S N A S A L A S S E
901 TTCTTAGCCA CTCAGGTGT TTTGAACGCA GGCAATGCAT CTGCCCTAGC AGGCAGTTC
  A R A L S A S A E S O S E A O S O A Y Q
961 GCCAGAGCCC TCTCGGCTC AGCAGAAATCC CAGTCATTCC CACAGAGTCA AGCCTACCAA
  Q A S
1021 CAGGCATCG
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Fig. S1. N-terminal cDNA sequence of TuSp1. The translated amino acids are indicated above the DNA sequence. The predicted signal peptide is highlighted in bold. NTD (solid line) and RP1 (dashed line) are underlined.

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RP1  SASRAGSTSSSTTTTSGATSQAASQSASSSYSSAFAQAASSALATSSAISRAFASVSSA 60
RP2  SASRAGSTSSSTTTTSGATSQAASQSASSSYSSAFAQAASSLATSSAISRAFASVSSA 60
NTD  QAI SVATSVPSVVFSSPSLASGFLGCLTTGIGQSPDFPFQEQDDLDDLAQVILSAVTSNTD 60
CTD  ---GISVGVPGYLRTPS-----STILAPSNAQII SLGLQTTLAPVLSSSGLSSA 46
      . . . . . : * . . . . . * : . . . . .
      . . . . . : * . . . . . * : . . . . .

RP1  SAASSLAYNIGLSAARSLGIASDTALAGALAQAVGGVGAGASASAYANAIARAAGQFLAT 120
RP2  SAASSLAYNIGLSAARSLGIASDTALAGALAQAVGGVGAGASASAYANAIARAAGQFLAT 120
NTD  TSKS-----ARAQALSTALASSLADLLI ESSGSSSYQTQISALTNILS---DCFVTT 109
CTD  SASAR-----VSSLAQSLASALSTSRGTLSLSTFNLNLSISSSEIRAS 89
      : : . . . . . : ** * . . . . . * : * : . . . . . : :

RP1  QGVL-NAGNASALAGSFARALSASAESQSFAQSQAYQASA-FQQAAAQSAQ- 171
RP2  QGVL-NAVNASLGSALANALSDSAANS--AVSGNYLGVSNFGRIPVTGGTA 171
NTD  TGSN-NPAFVSRVQTLIAVLSQSSSNAISGATGGSAFAQSQAQQSASQSAGL- 161
CTD  TSLDGTQATVEVLLLEALALLQVINGAQITDVNVSSVPSVNAALVSALVA---- 139
      . . . . . : * . . . . . * : . . . . .

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Fig. S2. Internal aa sequence alignment of TuSp1 domains. The sequence identity among NTD, RP1/RP2, and CTD ranges from 11% to 19%, whereas RP1 and RP2 share 78% sequence identity. The structural regions are underlined and highlighted in bold.

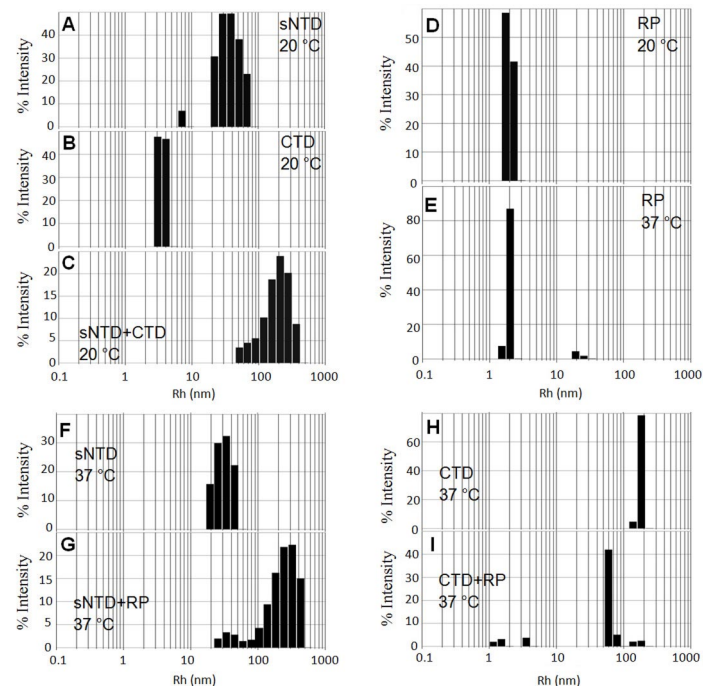


Fig. S3. DLS of TuSp1 domains. (A–C) terminal domains at 20 °C: sNTD (A), CTD (B), sNTD + CTD (C). (D and E) RP domain (RP1/RP2) at 20 °C (D) and at 37 °C (E). RP1 and RP2 gave rise to the same results, and only RP1 is shown here. (F and G) sNTD and RP domain at 37 °C. (F) sNTD. (G) sNTD + RP. (H and I) CTD and RP domain at 37 °C. (H) CTD. (I) CTD + RP. Each protein concentration was adjusted to $\approx 50 \mu\text{M}$ in 10 mM Tris buffer (pH 7.0).

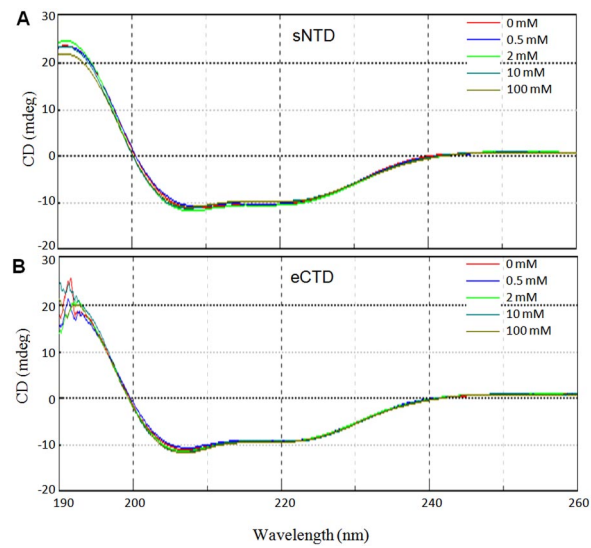


Fig. S4. CD spectra of sNTD (A) and eCTD (B) of TuSp1 in a series of DPC concentrations at 20 °C. The sNTD/eCTD concentration was $\approx 10 \mu\text{M}$, and DPC concentration varied from 0 mM to 100 mM in 10 mM phosphate (pH 6.5).

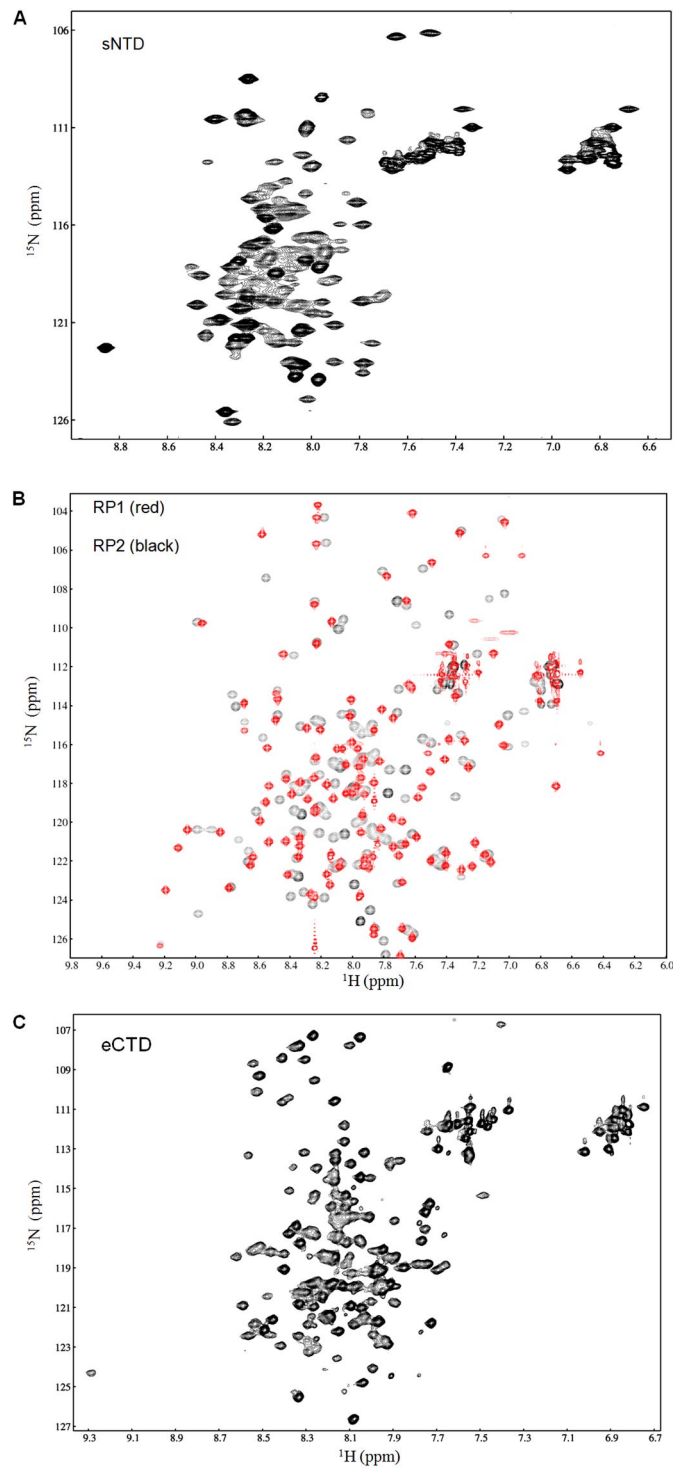


Fig. S5. $[\text{}^1\text{H}, \text{}^{15}\text{N}]$ HSQC spectra of TuSp1 domains. (A) Spectrum of sNTD acquired at 500 MHz, 30 $^\circ\text{C}$, pH 6.0, and 5 mM phosphate, 100 mM DPC. (B) Overlay of RP1 (red) and RP2 (black) spectra recorded at 800 MHz, 17 $^\circ\text{C}$, pH 6.5, 50 mM phosphate, and 100 mM NaCl. (C) Spectrum of eCTD acquired at 800 MHz, 35 $^\circ\text{C}$, pH 6.5, 10 mM phosphate, 100 mM DPC. For all of the samples, the protein concentration was ≈ 1 mM.

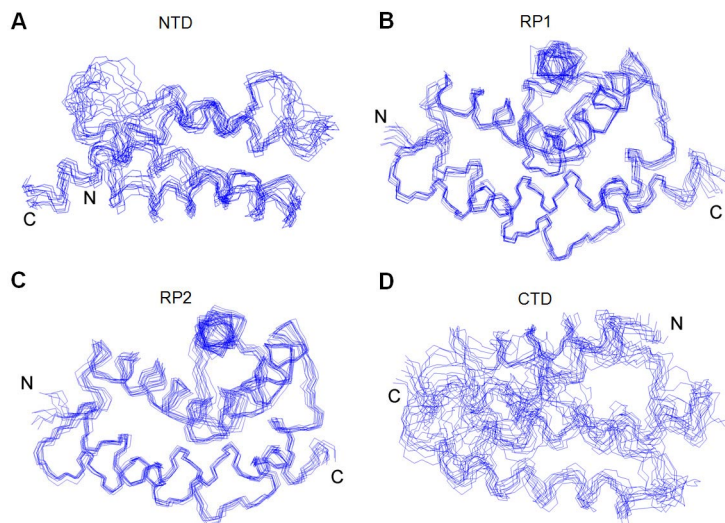


Fig. S6. Overlay of the 10 lowest energy structures of NTD (A), RP1 (B), RP2 (C), and CTD (D). Unstructured N- and C-terminal regions are not shown.

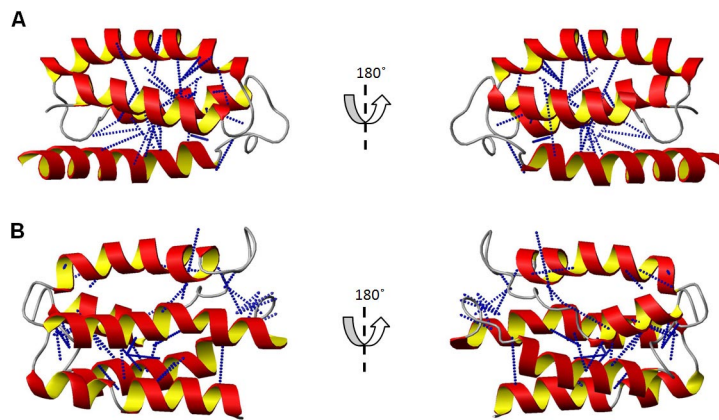


Fig. S7. Detected long-range NOEs (blue dashed lines) in the structural region of NTD (A) and CTD (B).

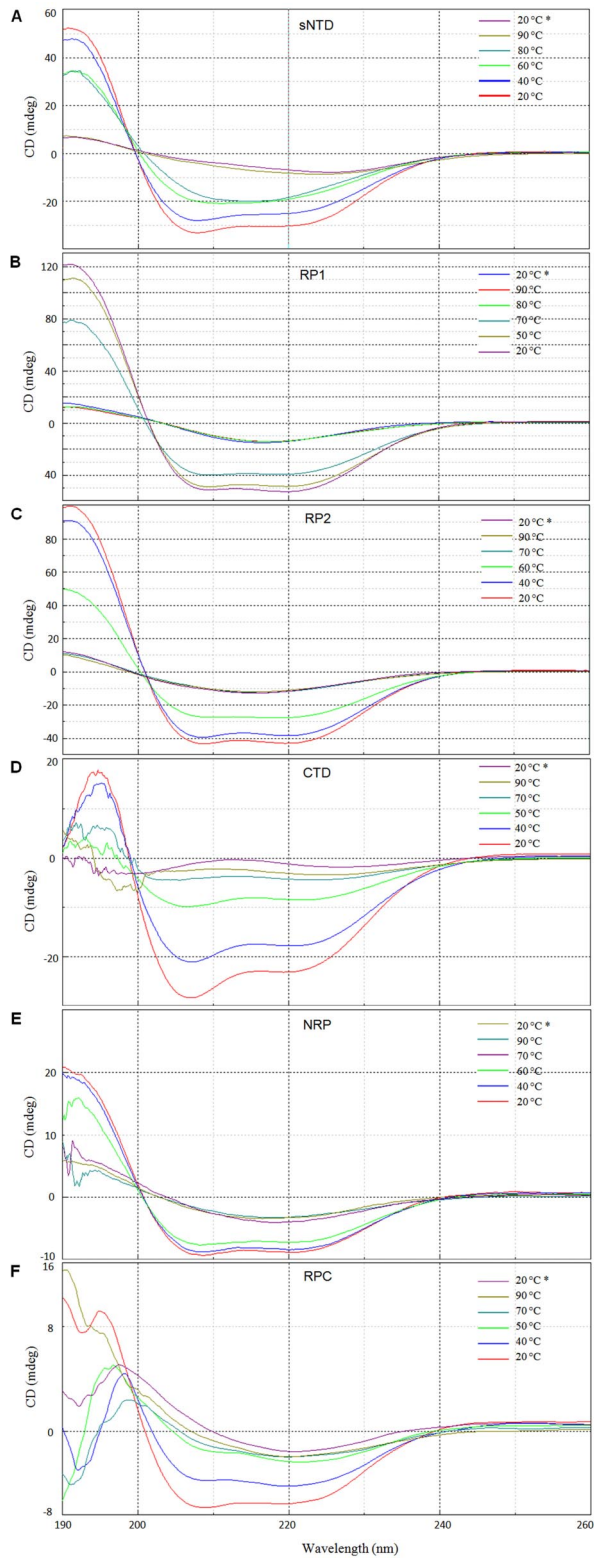


Fig. S8. Temperature-dependent structural transition of TuSp1 domains. (A) sNTD. (B) RP1. (C) RP2. (D) CTD. (E) NRP. (F) RPC. *, CD spectra recorded at 20 °C after cooling down from 90 °C. The protein concentration of each domain was $\approx 40\ \mu\text{M}$ in 10 mM phosphate buffer (pH 6.5). The protein concentration of NRP/RPC was $\approx 10\ \mu\text{M}$ in 5 mM Tris buffer (pH 7.0).

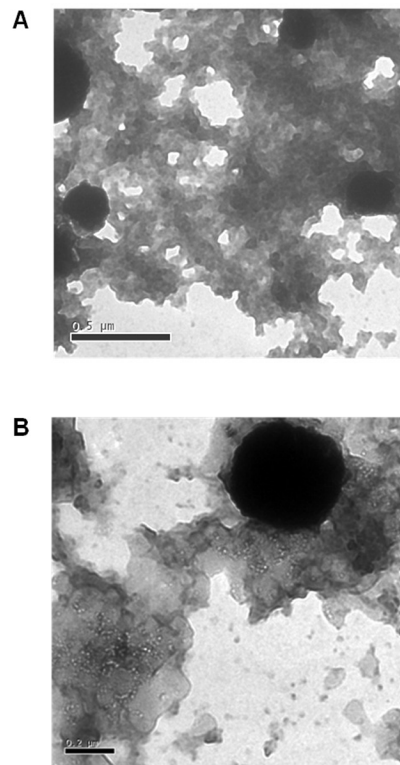


Fig. 59. TEMs of stained sNTD (A) and CTD (B), illustrating irregular morphology. (Scale bars: (A) 500 nm, (B) 200 nm.)

Table S1. Statistics of pairwise structural comparison of TuSp1 structural domains

Parameters	NTD-RP1	NTD-CTD	RP1-RP2	RP2-CTD
Equivalence residues	82	92	115	96
Structural overlay, %	68	79	95	82
SSAP score	70.81	61.65	90	64.90
Sequence identity, %	9	10	90.21	9
Rms deviation	4.38	10.7	1.58	11.67