

Supplementary figure legends

Figure S1. Spindlin1 does not localize to the spindle or centromere in mitotic mouse fibroblast cells. (A) Co-staining for Spindlin1 and Tubulin in prometaphase cells; (B) co-staining for Spindlin1 and Tubulin in anaphase cells; (C) co-staining for Spindlin1 and Tubulin in telophase cells; (D) co-staining for Spindlin1 and Tubulin in interphase cells.

Figure S2. Quality control of the mono-nucleosomes. (A) Mono-nucleosomes on a 1.5% agarose gel; (B) Verification of the MLA reaction products with antibodies specific to H3K4me2 and H3K4me3.

Figure S3. Spindlin1 does not bind to H3K9me3, H3K27me3 and H3K4me0. (A) Pull-down experiments showed no specific interaction between Spindlin1 and mono-nucleosomes containing H3Kc9me3 and H3Kc27me3; (B) ITC experiments showed no interaction between Spindlin1 and H3K4me0 peptide; (C) ITC experiments showed no interaction between Spindlin1 and H3K9me3 or H3K27me3 peptides.

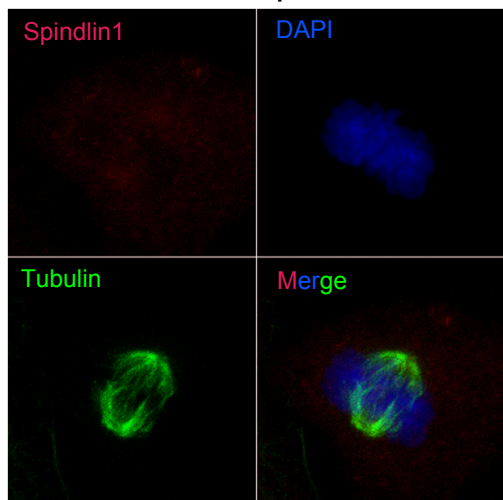
Figure S4. (A) Flag-Spindlin1 Y170A and F141A mutants displayed slightly reduced rDNA occupancy in ChIP experiments; (B) siRNA 3 for Spindlin1 specifically targets Spindlin1, but not Spindlin2A/2B (2A and 2B differ for only two bases in the coding region, which can be amplified using the same primer pair).

Figure S5. Nucleotide sequence alignment for Spindlin family proteins.

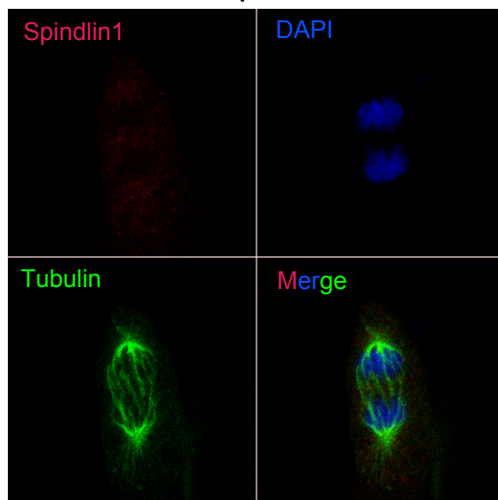
Figure S6. Spindlin1 does not appear to form a stable complex with other proteins in HeLa cells. Silver-stained SDS/PAGE gel of anti-Flag affinity-purified samples from HeLa cells expressing Flag-Spindlin1 and mock HeLa cells. * Co-purified common contaminants were verified using mass spectrometry analysis.

A

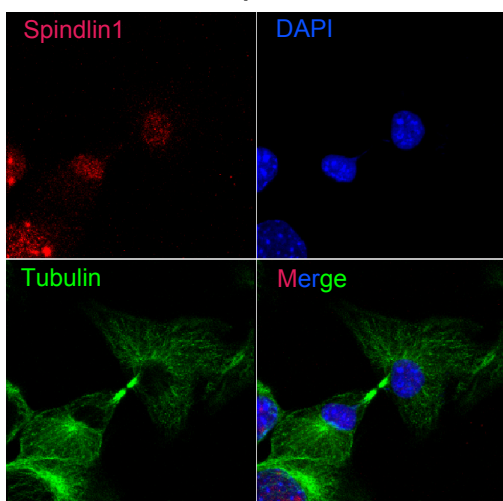
Prometaphase

**B**

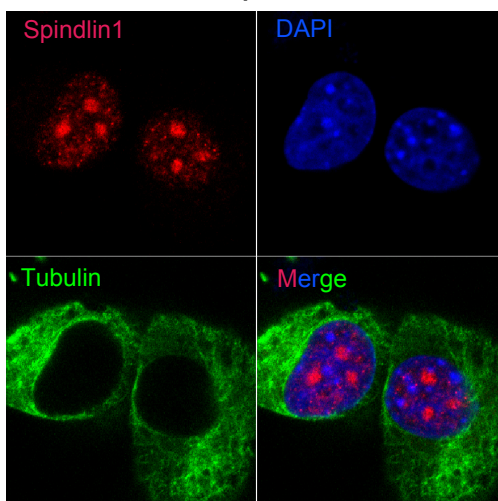
Anaphase

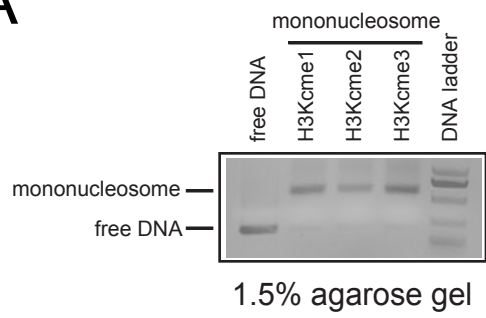
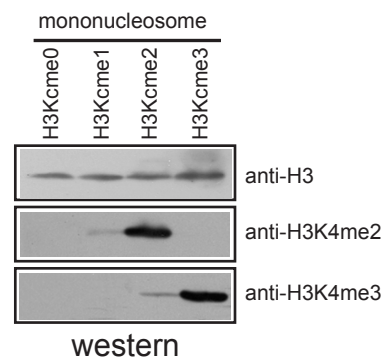
**C**

Telophase

**D**

Interphase

**Figure S1**

A**B****Figure S2**

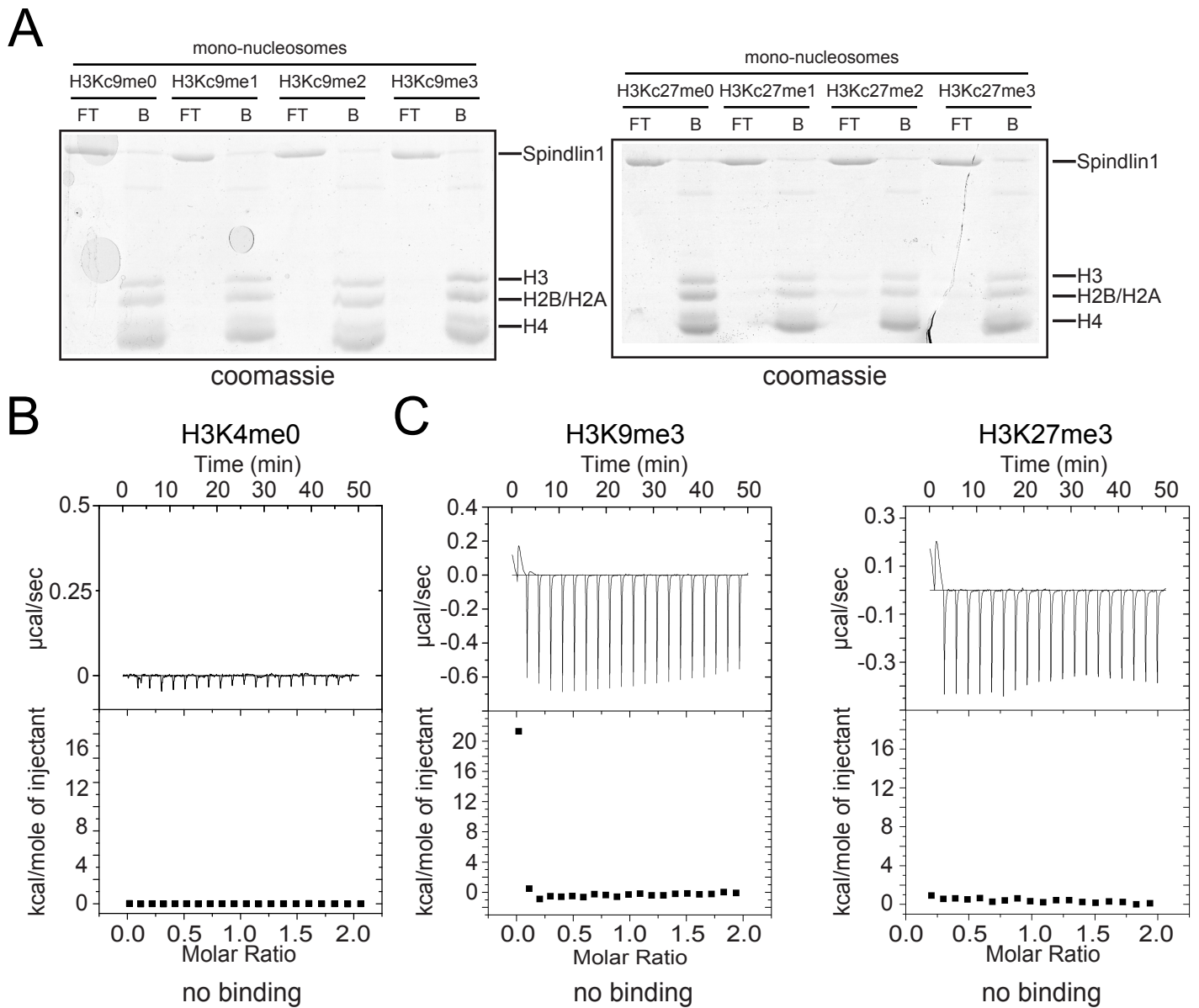


Figure S3

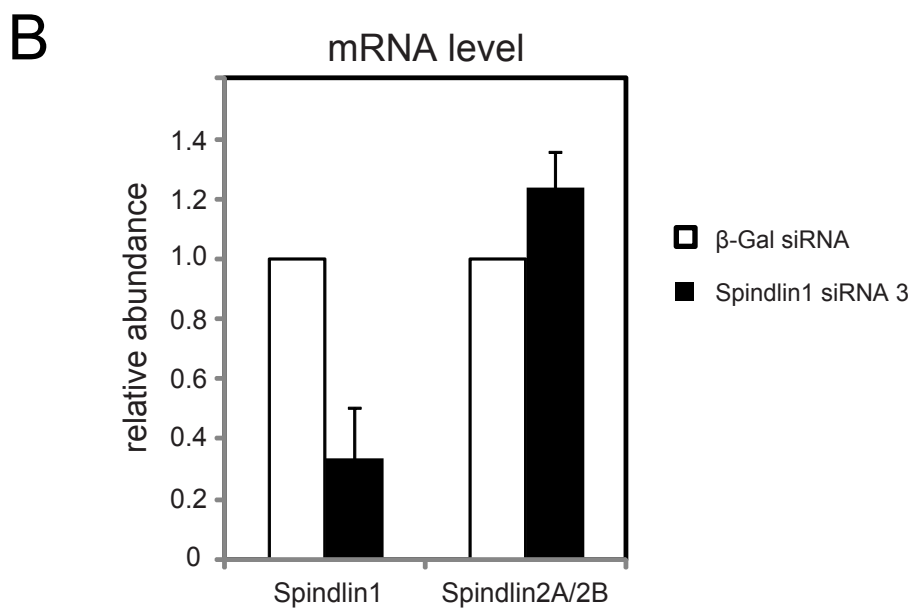
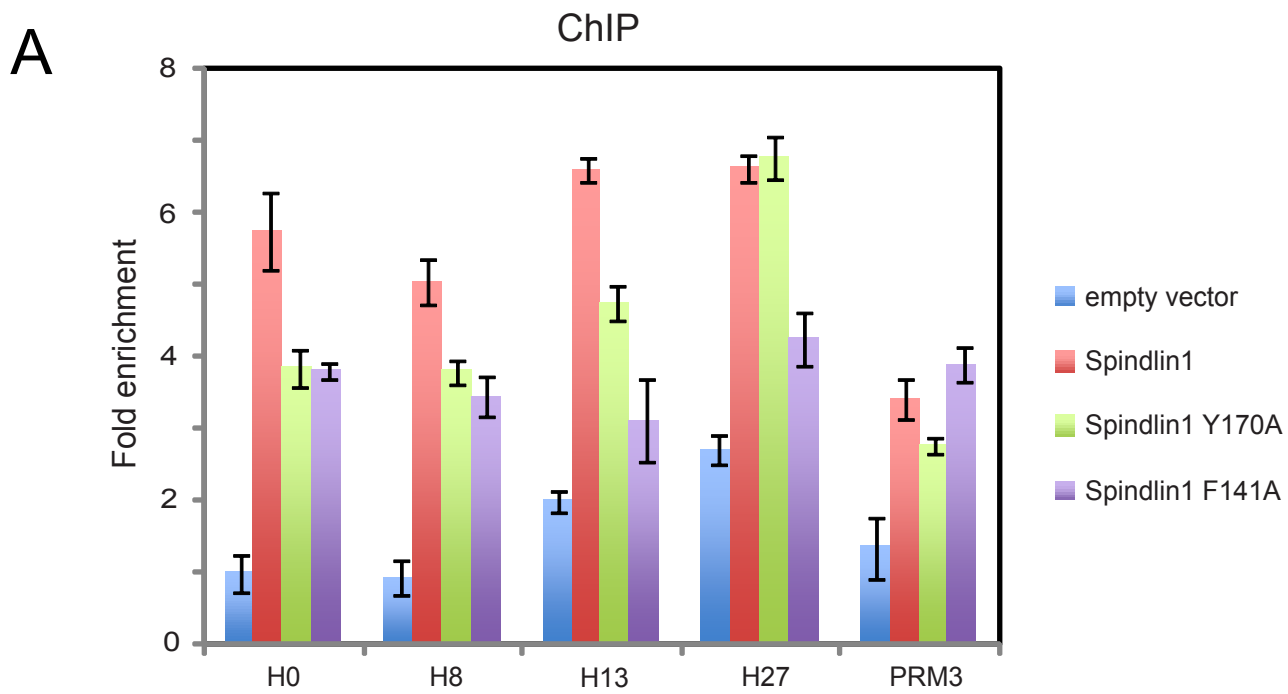


Figure S4

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Spindlin1 : ATGAAGACCCGATTCCGAAAGACACCT---GGCCAGCGGTCCAGAGCTGATGCAGGCCATGTTGGAGTATCTGCCAACATGATGAAGAAAGAGGACATCCACAAAAAACATCCGAGCAGT : 117
Spindlin3 : ATGAAGACCCGTTTGGAAAGCCAGCTGCCAGGCGAGCGGTCCAGGACCGGCGCTGGCCACCGGCAGTGTGTCTGTTACCATGATAAAGAGGAGGGCTGACACAAAGGCATAGGAGCCG- : 119
Spindlin2A : ATGAAGACCCCAACGCACAGGACCGAAAGGGCAACAAAACAGGGCAGCTGCAGGACGGGCCACTGGGTCTGCAAAACATGACAAAGAAAAGAGTCTCCCAAAGAAAGCAGAGAGGCCG- : 119
Spindlin2B : ATGAAGACCCCAACGCACAGGACCGAAAGGGCAACAAAACAGGGCAGCTGCAGGACGGGCCACTGGGTCTGCAAAACATGACAAAGAAAAGTCTCCCAAAGAAAGCAGAGAGGCCG- : 119
      ATGAAGACCC      cG A AgG AgC g aGgCA C      CCAGggC G tGCaGG C      Gcca tG gTCTGc AaCATGA aAAGAA Aa g      tCcCA AAgAaGCA aG      GCcG

      *      140      *      160      *      180      *      200      *      220      *      240
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Spindlin2A : -----ACCTTCAT-----CCCAGCCCGCGGGAACATCGTGGGCTGCAGAAATTCATGGATGGAAAGAAAGGAGATGAGCCCATCACGCAGTGGAAAGGAACCGTTCTGGATCAG : 225
Spindlin2B : -----ACCTTCAT-----CCCAGCCCGCGGGAACATCGTGGGCTGCAGAAATTCATGGATGGAAAGAAAGGAGATGAGCCCATCACGCAGTGGAAAGGAACCGTTCTGGATCAG : 225
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Spindlin2A : GTGCCTATAAATCCCTCTCTTTATCTGGTAAATATGATGGAAATTGACTGTGTCATGGAATTGGAACCTCACAGAGATGAAAGGGTTTTGTCCTTAAATTTCTTTCTGACAGGGTGGCA : 345
Spindlin2B : GTGCCTATAAATCCCTCTCTTTATCTGGTAAATATGATGGAAATTGACTGTGTCATGGAATTGGAACCTCACAGAGATGAAAGGGTTTTGTCCTTAAATTTCTTTCTGACAGGGTGGCA : 345
      GTgCCT TAAATCCcTCTcT TATCT      T AAATAtGATGGA TTGACTGTGT      TATGGAcTgGAACCTcAcAgAGATGAAAG GtTt      tC CTT AA T CTt CTgA AG GT GCA

      *      380      *      400      *      420      *      440      *      460      *      480
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Spindlin2B : TCATCTCACATTAGTGATGCCAACCTTGCAAATACCATAATTGGCAAAGCAGTGGAAACATATGTTTGAGGACAGAGCATGGTTCTAAGGATGAATGGAGGGGGATGGTCTTAGCTCAAGCA : 465
      tCATCTc AT AGtGATgC AC T GCA A Ac AT aTTGGCAAAGCAGTGGAAACAtATgTTTGAG AGAG AtGGTTcTAA gATGAaTGGAGGGGgATGGTCTTAGCTCa GCA

      *      500      *      520      *      540      *      560      *      580      *      600
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Spindlin3 : CCTGTCATGAACACATGGTTTTACATTACCTATGAGAAAGATCCTGTATTATATATGTACCAGCTCTTAGATGATTATAAAGATGGTGACCTCCGCATCTTCAAGATTCCAATGATTCT : 585
Spindlin2A : CCTATCATGAAGCCTGGTTTTATATTACCTATGAGAAAGATCCTGTCTTGTACATGTACCAGCTCTTAGATGATTATAAAGAAAGGTGACCTCCGCATCATGCCAGAATCCAGTGAAGTCT : 585
Spindlin2B : CCTATCATGAAGCCTGGTTTTATATTACCTATGAGAAAGATCCTGTCTTGTACATGTACCAGCTCTTAGATGATTATAAAGAAAGGTGACCTCCGCATCATGCCAGAATCCAGTGAAGTCT : 585
      CCT TCATGAA C TGGTTTTA ATTACCTATGAGAAAGAtCCTGTcTtGtACATGTACCAGcT TAGATGATTAtAA GAaGgTgACCTcCGCATcaTgCcaGA TCCA TGA TcT

      *      620      *      640      *      660      *      680      *      700      *      720
Spindlin1 : CCTCCAGCAGAAAGGGAAACCAGGAGAGTTCCTGGACAGCCGGTAGGCAACAAAGTGGAAATATGCCAAAGAAAGATGGCTCAGAAAGGACTGGCATGGTCATTTCATCAAGTGAAGCCAAAG : 717
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Spindlin2A : CCTCCAACAGAGAGGGAGCCAGGAGAGTTCATAGATGGCCAAATAGGTAAACCATGTGGAATATACCAAAGAAAGATGGCTCAGAAAGAACTGGCATGGTCATTTCACCAAGTGAAGCCAAA : 705
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      *      740      *      760      *      780      *
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Figure S5

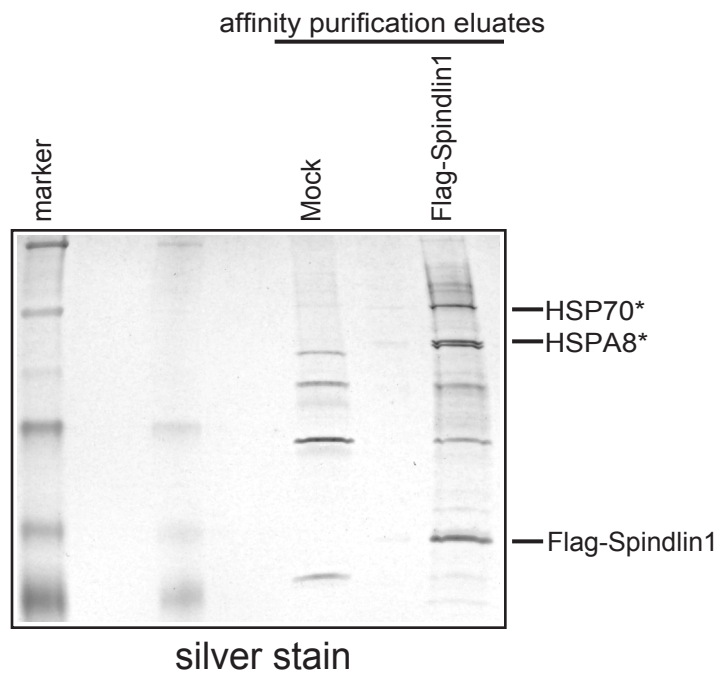


Figure S6