Supplemental Materials

Molecular Biology of the Cell

Kumagai and Dunphy

Figure S1. Recombinant protein complexes for add-back experiments. Recombinant versions of full-length human Treslin alone, full-length Treslin in a complex with human MTBP, the Treslin-N fragment in a complex with MTBP, and MTBP alone were purified as described in the Materials and Methods. Preparations were subjected to SDS gel electrophoresis and stained with Coomassie blue.

Figure S2. Properties of the CTM domain. (A) A structural model for the CTM domain from human MTBP (residues 813-904) was generated by the Robetta server (left panel). The right panel depicts the published structure of the C-terminal region from budding yeast Sld7 (residues 178-257)(Itou *et al.*, 2015). (B) Sequence alignment of CTM domain from human MTBP and residues 178-257 from yeast Sld7. Predicted and actual helical regions for MTBP and Sld7, respectively, are indicated. (C) A preparation of GST-CTM was cleaved with AcTEV protease and subjected to gel filtration in a Superdex-200 column. Fractions from the column were subjected to SDS gel electrophoresis and stained with Coomassie blue. Data are representative of two independent experiments.

Figure S3. EMSA experiments. (A) The EMSA data from Figure 4A was quantitated and plotted as indicated. Bars depict mean ± SEM (n=3). (B) The EMSA data from Figure 4D was quantitated and plotted as indicated. Bars depict mean ± SEM (n=3).

Figure S4. Characterization of mutant MTBP protein complexes. Recombinant complexes of the Treslin-N fragment bound to wild-type (WT), ΔC, or 7A versions of MTBP were purified as described in the Materials and Methods. Preparations were subjected to SDS gel electrophoresis and stained with Coomassie blue.

Figure S5. FACS profiles of cells expressing wild-type and mutant MTBP proteins. Different lines of human U2OS Flp-In T-REx cells (control and harboring inducible versions of the indicated forms of MTBP) were incubated with doxycycline and treated with control siRNA (left panels) or MTBP siRNA (right panels) as described in Materials and Methods. Cells were labeled with EdU for 30 min and processed for FACS analysis as described in Materials and Methods. Data are representative of three independent experiments.

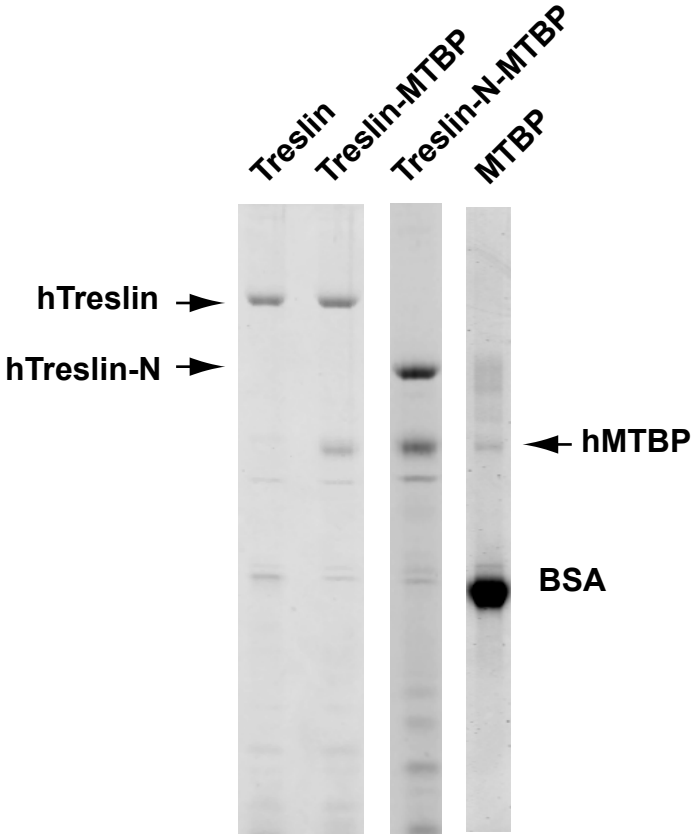
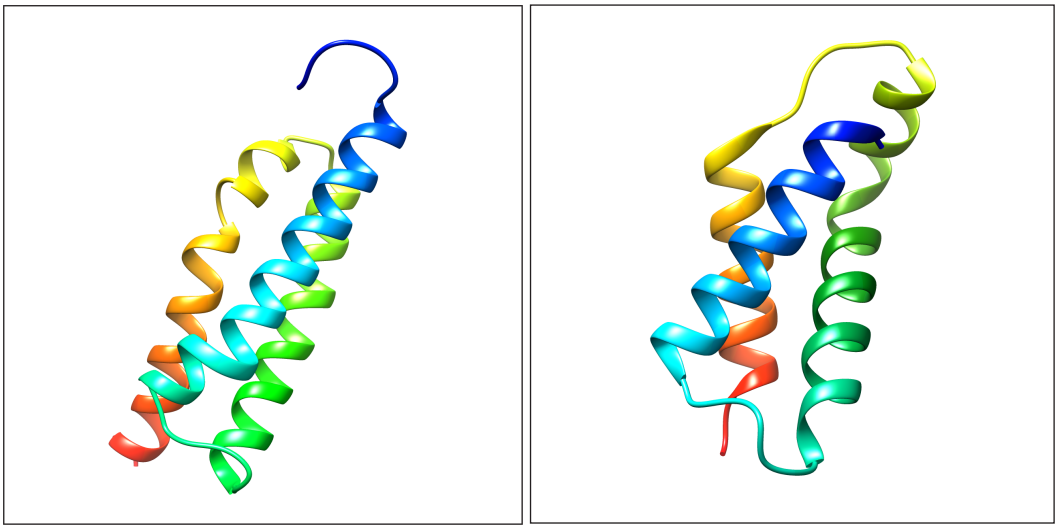

Treslin
Treslin-MTBP
Treslin-N-MTBP
MTBP
hTreslin
hTreslin-N
hMTBP
BSA

Figure S1

A



MTBP-CTM Sld7 C-term

B

```
          ----------HHHHHHHHHHHHHHHHHHHHH-------HHHHHHHHHHHHHHHHHHHHH-----------HHHHHHHHHHHHHHHHHHHHHHH---
MTBP-CTM: MHESKTSRQIKESRSQKHTRILKEVVTETLKKHSITETHECFTACSQRLFEISKFYLKDLKT-------SRGLFEEMKKTANNNAVQVIDWVLEKTSKK
                    ::.* * : . *.:::   *: :.*:::   :    :  *: ::*  :*  .       ..  **.:::*.:.  ::::
Sld7-C:   ----------NDKRLQFN-ETLSKLILGGLRLRGISNSITDYQKLYKITFDAAEFTHRDELKRISMGSGEEVSFESLQETVETL-LKLFTKS-------
          -----------HHHHHHHHHHHHHHHHHHH-------HHHHHHHHHHHHHHHHHHHHHHHHHH--------HHHHHHHHHHHHHHHH--
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C

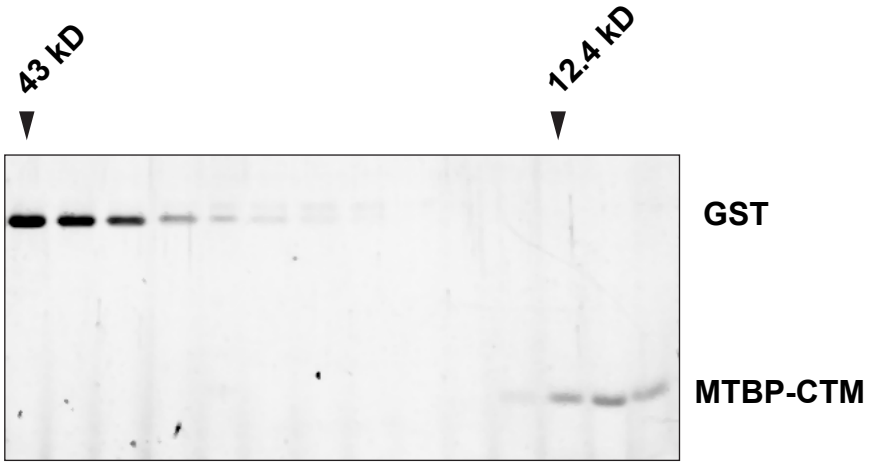
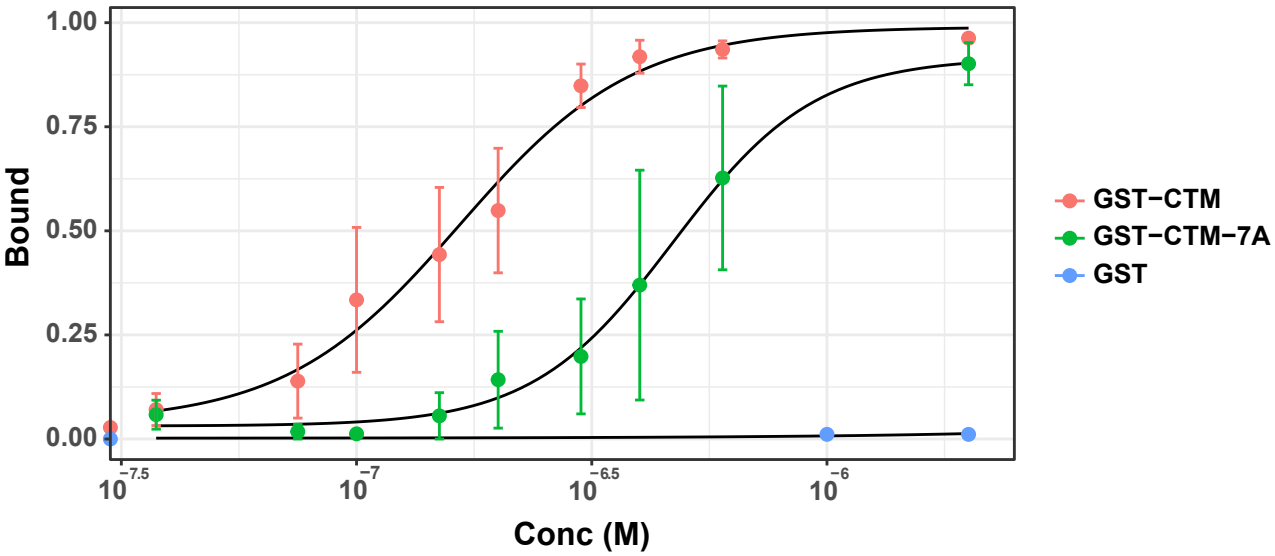


Figure S2

A


1.00
0.75
0.50
0.25
0.00
Bound
10^−7.5
10^−7
10^−6.5
10^−6
Conc (M)
GST−CTM
GST−CTM−7A
GST

B

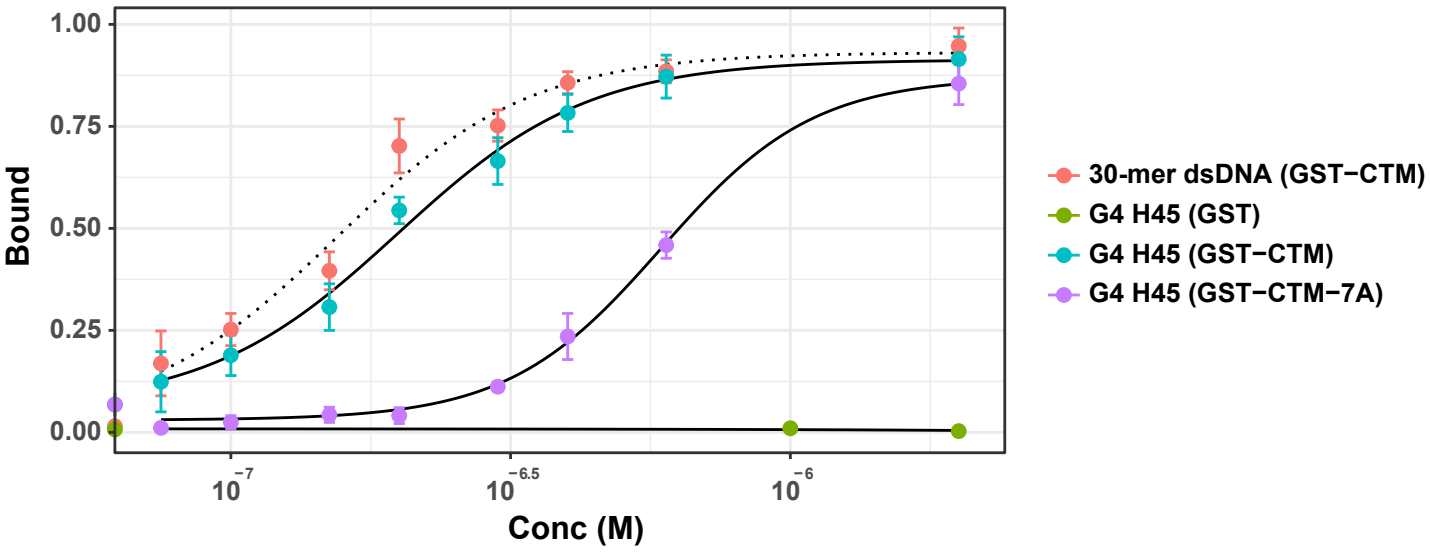

1.00
0.75
0.50
0.25
0.00
Bound
10^−7
10^−6.5
10^−6
Conc (M)
30-mer dsDNA (GST−CTM)
G4 H45 (GST)
G4 H45 (GST−CTM)
G4 H45 (GST−CTM−7A)

Figure S3

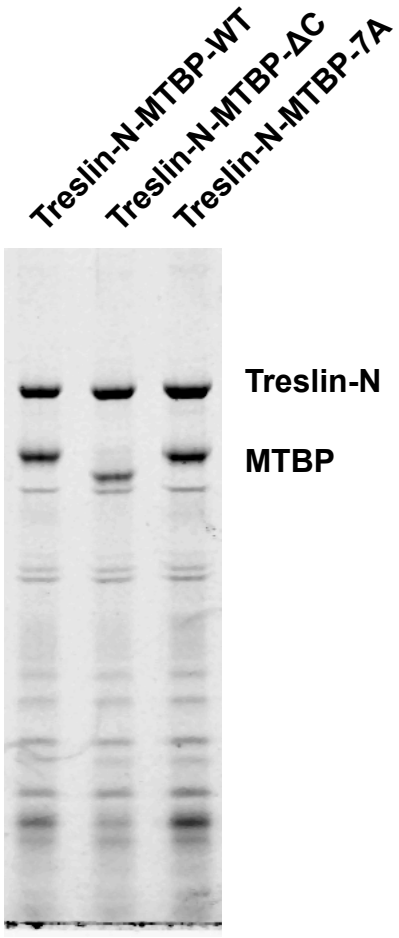

Treslin-N-MTBP-WT
Treslin-N-MTBP-ΔC
Treslin-N-MTBP-7A
Treslin-N
MTBP

Figure S4

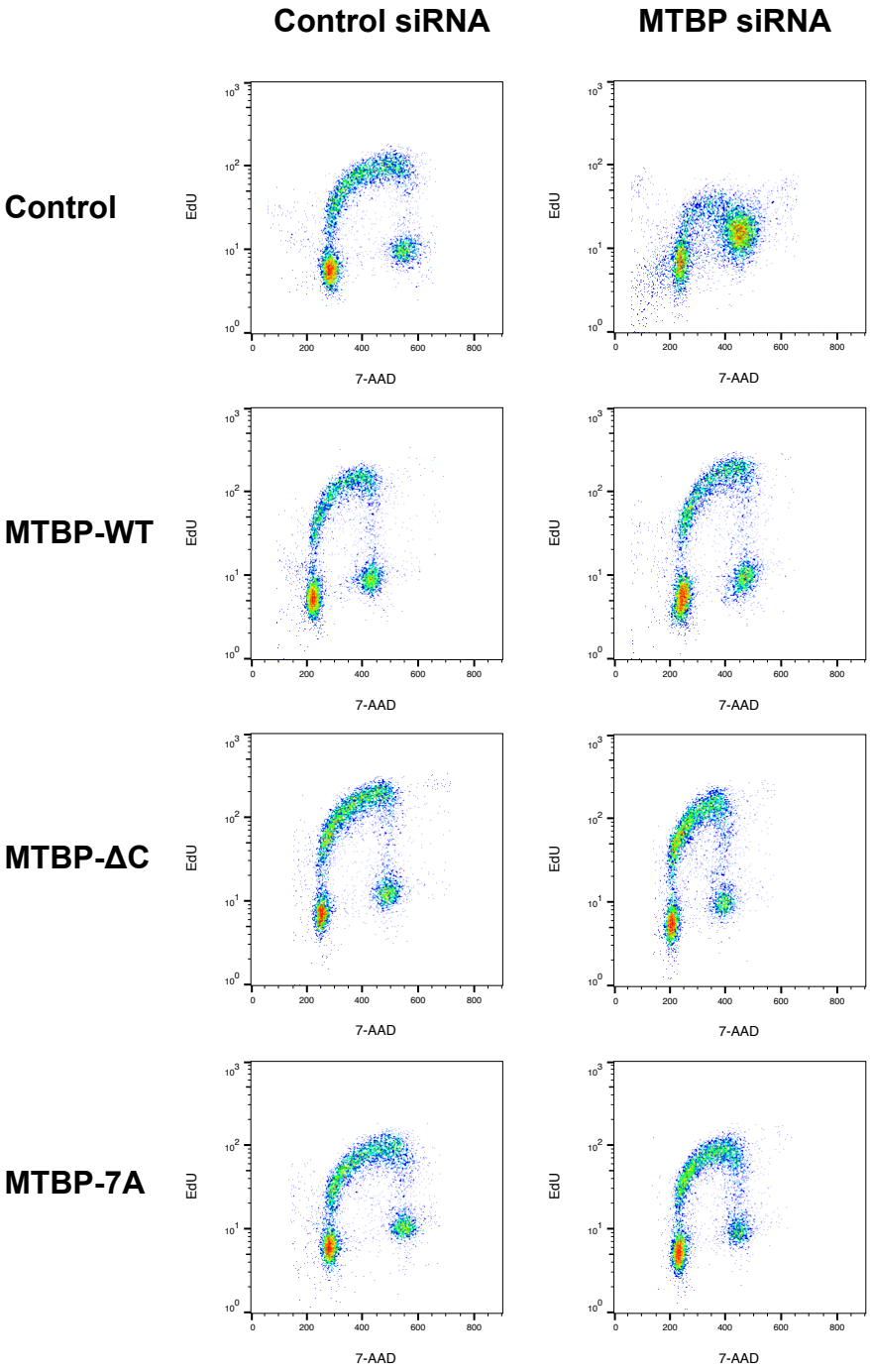

Control siRNA
MTBP siRNA
Control
MTBP-WT
MTBP-ΔC
MTBP-7A
EdU
7-AAD

Figure S5

Table S1. List of Oligonucleotides Used in This Study

siRNA

Control: ON-TARGET plus non-coding siRNA #1 (Dharmacon)
ON-TARGET plus MTBP #1: UCACAUUGUUGGAUGCUAAUU (Dharmacon)
ON-TARGET plus MTBP #2: GAGAGAAACAGUUAGCUAAUU (Dharmacon)

Cloning of *Xenopus* MTBP from *Xenopus* oocyte cDNA library

Forward: TTCTCCGCTTCCCCTGGTGT
Reverse: AGTCCTCTGTGCTTTCTTCAGC

Cloning of MTBP amino acids 436-860 into pH10UE for antibody production

Forward: GGAATTCCATATGAGAGAGCAGATACTAGCC
Reverse: ATAAGAATGCGGCCGCCTCTGTGCTTTCTTCAGC

For Production of siRNA-resistant MTBP

MTBP #1
Forward: GGAAAAGCAACTGGCCAATGTTCAAGTTTTAGCTTTGGAAG
Reverse: GGCCAGTTGCTTTTCCCTCTGTACAATCTGCTCCC

MTBP #2
Forward: TACCCTCCTCGACGCCAAAGAATTGCTGAAGTACTTTACCTC
Reverse: GGCGTCGAGGAGGGTAATTGAGTCCCGAGGACC

Steps in Construction of pcDNA5/TO-MTBP-Twin-Strep and pcDNA5/FRT/TO-MTBP-Twin-Strep

i. Amplification of Human MTBP cDNA

Forward: GTTTAAACTTAAGCTTGGCCACCATGGATCGGTACCTGC
Reverse: CTTTCTTGCTTGTCTTTTCTAATACCC

ii. Amplification of pcDNA5/TO and pcDNA5/FRT/TO

1: CCAAGCTTAAGTTTAAACGC
2: TGAGCAGATATCCAGCACAGTGG

iii. Tagging C-terminal end of MTBP with Twin-Strep

1: Twin-Strep-5
AGACCCACCATGGCTAGCTGGAGCCACCCTCAGTTCGAGAAAGGCGGAGGCTCCGGAGGCG
GATCCGGAGGCAGCGCCTGGAGCCACCCTCAGTTCGAAAAAGGCGCCCTGGAGGTGCTGTT
CCAGGGCCCTGATATC
2: Twin-Strep-3

GATATCAGGGCCCTGGAACAGCACCTCCAGGGCGCCTTTTTCGAACTGAGGGTGGCTCCAGG
CGCTGCCTCCGGATCCGCCTCCGGAGCCTCCGCCTTTCTCGAACTGAGGGTGGCTCCAGCTA
GCCATGGTGGGTCT
3: GGCCCCAAGAAGAAGAGGAAGGTGGAGGACAGCGCTTGGAGCCACCCTCAGTTC
4: CTGGATATCTGCTCATTTTTCGAACTGAGGGTGGC

Mutations of pcDNA5/TO-MTBP-Twin-Strep

MTBP-N (1-532)
1: GGACAAAATTAAAACCTTCGGCGGCGAAAACCTG
2: GAAGGTTTTAATTTTGTCCATCTTTC

MTBP-C (526-904)
1: TTTAAACTTAAGCTTGGCCACCATGGACAAAATTAAAACCTTCAATATATTAAATG
2: CCAAGCTTAAGTTTAAACGC

MTBP-ΔC
1: GAATCAAAAGGCGGCGAAAACCTG
2: GCCGCCTTTTGATTCATGCATACTTTTTTGACCTG

Construction of pGEX4T-CTM

1: GAAGACCCCGAGGCAATGCATGAATCAAAAACATCAAGGC
2: TCAGTCAGTCACGATTCATTTCTTGCTTGTCTTTTCTAATACCCAG

MTBP-824-830 7A
1: CAGCGGCAGCCACACGGATACTGAAAGAAGTA
2: GTGTGGCTGCCGCTGCTGCTGCTGCCTTAATTTGCCTTGATGTTTTTG

MTBP-874-876 3A
1: CTCTAAGTTCTATCTAAAGGATCTTAAAGCTGCAGCGGGTCTATTTGAAGAAATGAAG
2: CTTCATTTCTTCAAATAGACCCGCTGCAGCTTTAAGATCCTTTAGATAGAACTTAGAG

MTBP-881-884 4A
1: CTTCAAGGGGTCTATTTGAAGCAGCGGCGGCAACAGCAAACAACAATGCTGTAC
2: GTACAGCATTGTTGTTTGCTGTTGCCGCCGCTGCTTCAAATAGACCCCTTGAAG

pGEX4T-TEV-CTM
1: GACTGAAAATACAGGTTTTCGGATCCACGCGGAAC
2: AAAACCTGTATTTTCAGTCTATGCATGAATCAAAAACATCAAGG

Oligonucleotides used for EMSA experiments

ds 30-mer
1: ATAGAGTGTTGCCAGTCTCAAGTACCATGC
2: GCATGGTACTTGAGACTGGCAACACTCTAT

AT 30-mer

1: AAAAAATTTAAAAAAAAAAAAAAAAAATT
2: AATTTTTTTTTTTTTTTTTTAAATTTTTT

G4 17-mer (I100-15)
GTGGTGGGTGGGTGGGT

G4 17-mer AS (antisense of G4 17-mer, used for 17-mer dsDNA)
ACCCACCCACCCACCAC

GC 30-mer
1: CCGGGGGGGGGGGGGGGGGGGCCCGGGGGG
2: CCCCCCGGGCCCCCCCCCCCCCCCCCCGG

G30
GGGGGGGGGGGGGGGGGGGGGGGGGGGGGG

Pu27
TGGGGAGGGTGGGGAGGGTGGGGAAGG

Pu27 mutant
TGAGGAGAGTGAGGAGAGTGAGGAAGG

Py27
CCTTCCCCACCCTCCCCACCCTCCCCA

Tandem G4 H45
GGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGG

Table S2. List of Antibodies Used in This Study

Antibodies	Company	Catalog Number
Anti-*Xenopus* MTBP	This study	NA
Anti-*Xenopus* Treslin	Kumagai et al. (2010)	NA
Anti-*Xenopus* Orc2	Kumagai et al. (2010)	NA
Anti-*Xenopus* Cdc45	Kumagai et al. (2010)	NA
Anti-*Xenopus* TopBP1	Kumagai et al. (2010)	NA
Anti-Human Treslin	Kumagai et al. (2010)	NA
Anti-Human MTBP (B-5)	Santa Cruz Biotech	sc-137201
Anti- FLAG M2	Sigma-Aldrich	F1804
Anti-Histone H3	Abcam	ab1791
Anti-GAPDH (14C10)	Cell Signaling Technology	#2118
Anti-Human Mcm2 (BM28)	BD Biosciences	610700
Anti-BrdU (MoBU-1)	Thermo Fisher	B35128
Anti-BrdU (B44) (Mouse)	BD Biosciences	347580
Anti-BrdU (Bu1/75 ICR1) (Rat)	Novus	NB500-169
Anti-ssDNA (Rabbit)	IBL	18731
Anti-StrepMAB-Classic	IBA Life Sciences	2-1507-001
Anti-Myc	Abcam	ab9106