

Supplementary Materials and Methods

Reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA of cells was isolated with a TRIzol RNA extraction kit (Invitrogen, Carlsbad, CA), and 3 µg of RNA were subjected to RT-PCR with SuperScript II (Invitrogen). The primers used for PCR for ataxia telangiectasia mutated (ATM) were 5'-GATGATTTTCATGTAGTTTTCAATTC-3' and 5'-CTGGAATAAGTTTACAGGATCTTC-3', and for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were 5'-CCATCACCATCTTCCAGGAG-3' and 5'-CCTGCTTACCACCTTCTTG-3'. The PCR products were separated using 1% agarose gel electrophoresis and visualized with ethidium bromide staining.

Western blot analysis

The protein sample was fractionated using sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred onto a polyvinylidene difluoride membrane (Amersham Bioscience, Piscataway, NJ) using a transfer apparatus according to the manufacturer's protocols (Bio-Rad Laboratories, Hercules, CA). After incubation with 5% nonfat milk in TBST (10 mM Tris, pH 8.0, 150 mM NaCl, and 0.5% Tween 20) for 1 h, the membranes were incubated with anti-Sp1 (1:3000), anti-PP2Ac (1:2000), anti-trimethyl-histone H3 (K27) (1:1000), anti-acetyl-histone H3 (K9/K14) (1:1000; Upstate, Charlottesville, VA), anti-cyclin B1 (1:3000), anti-CDK1 (1:3000), anti-GST (1:1000), anti-myosin IIa (1:500), anti-RNA polymerase II (1:500), anti-Brg-1 (1:500), anti-histone H3 phospho-Ser10 (1:5000), anti-histone H3 (1:1000; Santa Cruz Biotechnology, Santa Cruz, CA), anti-'living colors' GFP (1:2000; Clontech Laboratories, Mountain View, CA), anti- α -tubulin (1:5000 dilution), anti-actin (1:5000; Sigma-Aldrich, St. Louis, MO), or anti-Sp1 phospho-Thr739 antibodies at room temperature for 2 h. The membranes were washed for 5 min three times and incubated with a 1:3000 dilution of horseradish peroxidase-conjugated anti-mouse or anti-rabbit antibodies (Santa Cruz Biotechnology) at room temperature for 1 h. Blots were washed with TBST three times and developed using the enhanced chemiluminescence (ECL) system (Pierce Biotechnology, Rockford, IL) according to the manufacturer's protocols.

Immunoprecipitation

Different truncated GST-Sp1 proteins and cell lysates were prepared using a modified radioimmune precipitation assay (mRIPA) buffer (50 mM Tris, pH 7.5, 150 mM NaCl, 5 mM EDTA, 0.1% Triton-X100, 0.1% Nonidet P-40, and 10 µg/ml each of leupeptin, aprotinin, and 4-(2-aminoethyl) benzenesulfonyl fluoride). The sample was added with anti-Sp1 (1:300 dilution) or anti-CDK1 (1: 200 dilution) antibodies at 4°C for 2 h. Protein-A/G agarose beads (30 µl) were added to the lysate, and the mixture was incubated under shaking at 4°C for 1 h. The beads were collected using centrifugation and washed three times with mRIPA buffer. Proteins binding to the beads were eluted by adding 30 µl of 2x electrophoresis sample buffer.

Protein identification by mass spectrometry analysis

The protein band of interest on the silver staining gel was excised or used. The whole complex of Sp1 was recognized by the specific anti-Sp1 antibodies or IgG as an internal control, ground manually, and transferred into a siliconized 0.5-ml microcentrifuge tube for further identification by mass spectrometry analysis. The in-gel digestion, mass spectrometry analysis, and data base search were performed as described previously (Tyan *et al.*, 2005).

In vitro calf intestinal alkaline phosphatase (CIP) assay

For *in vitro* dephosphorylation of Sp1, the mitotic cell extracts were combined with 5 units of alkaline phosphatase (New England Biolabs) containing 50 mM Tris, pH 7.9, 100 mM NaCl, 10 mM MgCl₂, and 1 mM dithiothreitol in CIP buffer, and the mixture was incubated at 37°C for 30 min.

Immunohistochemistry (IHC), experimental animals and cultured rat primary glial cells

The methods of these experiments were performed as previously described (Chuang *et al.*, 2008).

Apoptosis analysis

Analysis of cell death using alexa 568-labeled annexin V was performed as recommended by the manufacturer (Roche Applied Science, Indianapolis, IN). Cells were seeded onto glass slides and transfected with different expressed plasmids. After 2 days of incubation, the cells were synchronized in mitosis by nocodazole treatment. The cells were then pre-incubated in annexin-V-binding buffer (10 mM

HEPES/NaOH, pH 7.4, 140 mM NaCl, and 2.5 mM CaCl₂). After 5 min, annexin V-alexa 568 and DAPI were added, and cells were incubated for 15 min at room temperature. Finally, cells were fixed with 4% paraformaldehyde and were examined using an Olympus BX51 microscope.

Cell synchronization

HeLa cells were blocked at the G1/S boundary with 2 mM thymidine (Sigma-Aldrich) for 18 h. The cells were then washed three times with PBS and incubated with fresh medium. And 10 h after release, the cells were re-plated onto 6-cm plates with 2 mM thymidine and re-incubated for 16 h. Plates were then washed three times with PBS and fresh medium was added. The time point, corresponding to the G1/S transition, was defined as 0 h. The cells were collected after different time intervals (0, 3, 6, 8, 10, 12 and 14 h). Equal amounts of proteins from these cell extracts were analyzed using immunoblotting. For another kind of cell synchronization, mitotic cells were collected by nocodazole (Sigma-Aldrich) treatment according to a method described previously (Chuang *et al.*, 2008). Cells were then washed three times with PBS and re-plated in fresh medium. The released cells were then collected after different time intervals and then analyzed using immunoprecipitation and immunoblotting.

Supplementary references

Chuang JY, Wang YT, Yeh SH, Liu YW, Chang WC, Hung JJ (2008). Phosphorylation by c-Jun NH2-terminal Kinase 1 Regulates the Stability of Transcription Factor Sp1 during Mitosis. *Mol Biol Cell* **19**: 1139-1151.

Tyan YC, Wu HY, Su WC, Chen PW, Liao PC (2005). Proteomic analysis of human pleural effusion. *Proteomics* **5**: 1062-74.

Supplementary Figure Legends

Figure S1. The localization of Sp1 with cyclin B1 and CDK1 during mitosis. HeLa cells were double-labeled with rabbit anti-Sp1 antibodies and mouse anti-cyclin B1 (A) or mouse anti-CDK1 (B) antibodies, and then stained with secondary antibodies conjugated with Alexa Fluor 488 and Alexa Fluor 568, respectively. DNA was stained with DAPI. Finally, the cells were examined using a confocal laser scanning microscope or immunofluorescence microscope. The corresponding pictures were merged and all pixels which had the same position in both images were considered coincident and are depicted as white spots (co-localization points). The co-localization in both Sp1 and CDK1 was also analyzed and quantified with *softWoRx* software.

Figure S2. Sp1 interacts with CDK1 in mitosis in A549 and CL1-0 lung cancer cell lines. Lung cancer cell lines, A549 (a) and CL1-0 (b), were synchronized in mitosis by nocodazole treatment. Samples were harvested for immunoprecipitation assay with anti-Sp1 antibodies, and then analyzed by immunoblotting using anti-Sp1, anti-CDK1, anti-cyclin B1 and anti-actin antibodies.

Figure S3. Sp1 phosphorylates at Thr739 in mitosis in other cell lines. The other cell lines were synchronized in mitosis with nocodazole treatment. Samples were harvested for immunoblotting using anti-p-Sp1(T739) and anti-actin antibodies.

Figure S4. No interaction of Sp1 with cyclin A and cyclin E in mitosis. Cells synchronized in mitosis by nocodazole treatment were harvested for immunoprecipitation assay using anti-Sp1 antibodies. Samples were analyzed by immunoblotting using anti-Sp1, anti-cyclin E, anti-cyclin A2 and anti-actin antibodies.

Figure S5. The localization of phospho-Sp1 and Sp1 during mitosis. HeLa cells were double-labeled with mouse anti-Sp1 antibodies (green) and rabbit anti-Sp1 phospho-

Thr739 antibodies (red). DNA was stained with DAPI (blue). Finally, the cells were examined using a confocal laser scanning microscope. The images were analyzed with *softWoRx* software.

Figure S6. The localization of phospho-Sp1 with cyclin B1 and CDK1 during mitosis. HeLa cells were double-labeled with rabbit anti-Sp1 phospho-Thr739 antibodies (red) and mouse anti-CDK1 (green) (A) or mouse anti-cyclin B1 (green) (B) antibodies. DNA was stained with DAPI (blue). Finally, the cells were examined using a confocal laser scanning microscope. The images were analyzed with *softWoRx* software.

Figure S7. The effect of Sp1 phosphorylated at Thr278 on its DNA binding activity. Wild type Sp1 and Sp1(T278A) plasmids were transfected into HeLa cells for 24 h, and then cells were synchronized into mitosis by nocodazole. Cell lysates were harvested for performing DAPA assay using Sp1 binding probe from p21 promoter. Samples were analyzed by immunoblotting using anti-Sp1, anti-cyclin B and anti-actin antibodies.

Figure S8. Sp1 phosphorylated at Thr739 reduces its transcriptional activity in other cell lines. Wild type Sp1 and Sp1(T739D) were co-transfected with p21 reporter plasmid into cells for 24 h, and then samples were harvested for determining the luciferase activity.

Figure S9. Sp1-interacting proteins identified in mitosis. Cells were synchronized in mitosis, and cell lysate was harvested for immunoprecipitation assay with anti-Sp1 antibodies and IgG as an internal control. Samples were used for the protein identification by LTQ-ObiTrap MASS. All of the identified proteins shown here have 5 fragments at least to be identified. (A) Sp1-interacting proteins in mitotic stage were analyzed with Ingenuity System. (B) After analysis with ingenuity system, the major group, skeletal and muscular systems are shown.

Figure S10. The localization of Sp1 with cyclin B1 and CDK1 during mitosis. Immunofluorescence was performed with anti-Sp1 (red) antibodies and green fluorescence signal in GFP-myosin expression HeLa cells (A) or with anti-Sp1 (green) antibodies and Alexa Fluor 568-conjugated phalloidin (red) in asynchronous HeLa cells (B). DNA was stained with DAPI (blue).

Figure S11. No dephosphorylation of phospho-Sp1 by PP1 in mitosis. Phospho-Sp1 from mitosis immunoprecipitated by anti-Sp1 antibodies was used for performing the

in vitro dephosphorylation assay of PP1. Samples were then analyzed by immunoblotting using anti-Sp1 and anti-tubulin antibodies.

Figure S12. No interaction of phospho-Sp1 with p300, Brg1, and RNA pol II, and histone H3 is decreased in acetylation. (A) Interphase and mitotic cell lysates were used for immunoprecipitation assay with anti-Sp1 antibodies in which IgG served as a negative control. The total lysates and products of immunoprecipitation were then analyzed by immunoblotting with anti-Sp1, anti-RNA polymerase II, anti-p300, anti-Brg-1 and anti-cyclin B1 antibodies. (B) Cells were synchronized with nocodazole to accumulate mitotic cells, and samples were harvested for immunoblotting with anti-Sp1, anti-H3, anti-acetyl-histone H3 (K9/K16), anti-tri-methyl-histone H3 (K27), anti-cyclin B1 antibodies. Actin was used as an equal loading control.

Figure S13. *In vitro* kinase assay with CDK1 and JNK1. Truncated GST-Sp1 (618-785 a.a.) protein was synthesized and purified from *E. coli* and then were combined with [γ - 32 P] ATP and activated CDK1/cyclin B1 or activated JNK1 for an *in vitro* kinase assay. Samples were then subjected for SDS-PAGE, gel drying, and X-ray exposure.

Supplementary Table 1. Proteins interact with Sp1 in mitotic stage. Cells were synchronized in mitosis, and cell lysates were harvested for immunoprecipitation assay with anti-Sp1 antibodies. Protein identification was performed by LTQ-ObiTrap (MASS). All of the identified proteins shown here have 5 fragments at least to be identified, and Sp1 determined by immunoblotting with anti-Sp1 antibodies was as an internal control.

Gene name	Protein name
ACTG	Actin, cytoplasmic 2
ACTBL	Beta-actin-like protein 2
ACTC	Actin, alpha cardiac muscle 1
ANXA2	Annexin A2
CALM	Calmodulin
CAPZB	F-actin-capping protein subunit beta
CBP	CREB-binding protein
CC29L	Putative coiled-coil domain-containing protein 29-like
CNTLN	Centlein

CO3A1	Collagen alpha-1(III) chain
CS021	Uncharacterized protein c19 or f21
DESM	Desmin
DREB	Drebrin
FKB15	FK506-binding protein 15
FGOP2	FGFR1 oncogene partner 2
GBP1	Interferon-induced guanylate-binding protein 1
GRP75	Stress-70 protein, mitochondrial
LMO7	LIM domain only protein 7
LUZP1	Leucine zipper protein 1
MAT1	CDK-activating kinase assembly factor MAT1
MRCKB	Serine/threonine-protein kinase MRCK beta
MYH7B	Myosin-7B
MYH9	Myosin-9
MYH10	Myosin-10
MYH11	Myosin-11
MYH14	Myosin-14
MYO1B	Myosin-Ib
MYO1C	Myosin-Ic
MYO1E	Myosin-Ie
MYO6	Myosin-VI
ML12A	Myosin regulatory light chain 12A
MYL9	Myosin regulatory light polypeptide 9
MYL6B	Myosin light chain 6B
MYL3	Myosin light chain 3
NEBU	Nebulin
ODFP2	Outer dense fiber protein 2
PLEC1	Plectin-1
POTEE	POTE ankyrin domain family member E
RSF1	Remodeling and spacing factor 1
SLIM1	SLIT and NTRK-like protein 1
SRRT	Serrate RNA effector molecule homolog
TBA1B	Tubulin alpha-1B chain
TBB3	Tubulin beta-3
TBB5	Tubulin beta-5 chain
TIM	Protein timeless homolog
TITIN	Titin
TNR11	Tumor necrosis factor receptor superfamily member 11A
TPM1	Tropomyosin alpha chain
TPM2	Tropomyosin beta chain
TPM3	Tropomyosin alpha-3 chain
TPM3L	Putative tropomyosin alpha-3 chain
TPM4	Tropomyosin alpha-4 chain
VIME	Vimentin

Note: Search Parameters; Type of search: MS/MS Ion Search; Enzyme: Trypsin; Mass values: Monoisotopic; Protein Mass: Unrestricted; Peptide Mass Tolerance: ± 1 Da; Fragment Mass Tolerance: ± 1 Da; Max Missed Cleavages: 2; Instrument type: ESI-TRAP; Number of queries: 1826

Figure S1

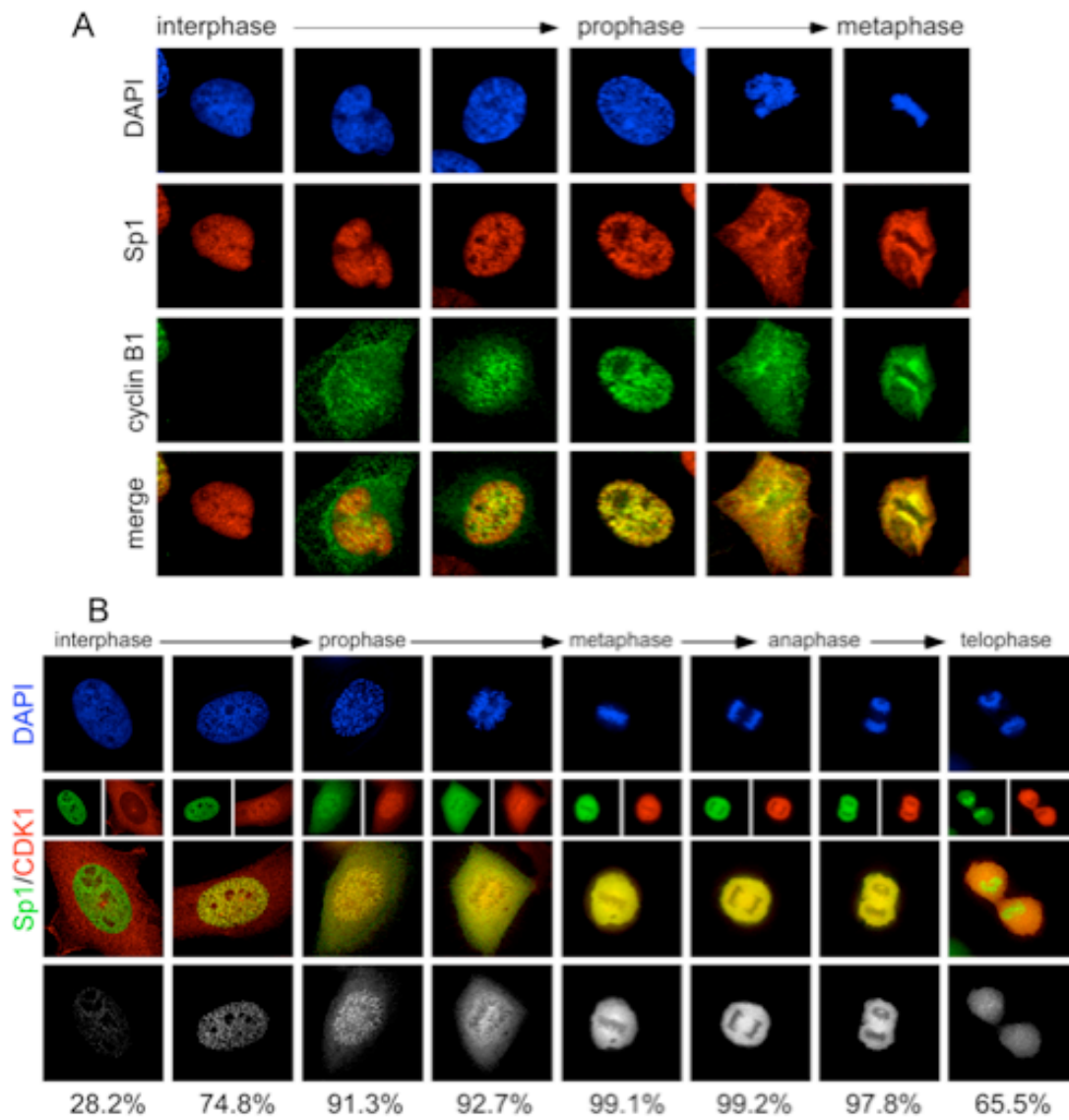
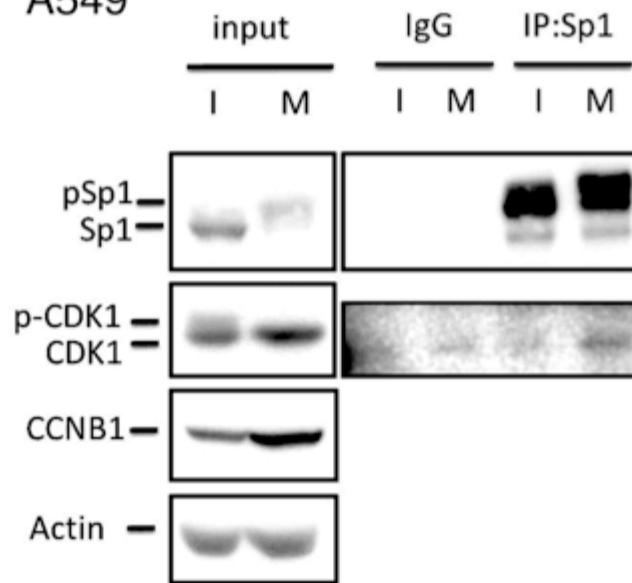


Figure S2

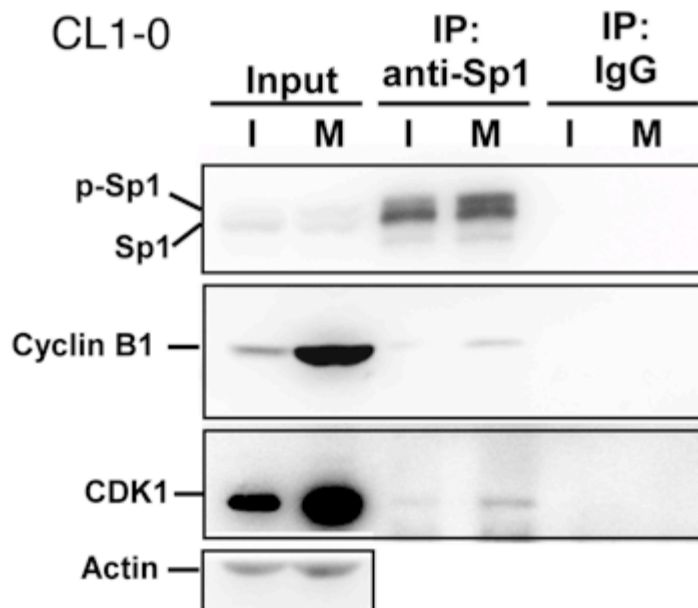
a

A549



b

CL1-0



Supplementary Fig.S3
p-Sp1(T739) in other cell lines

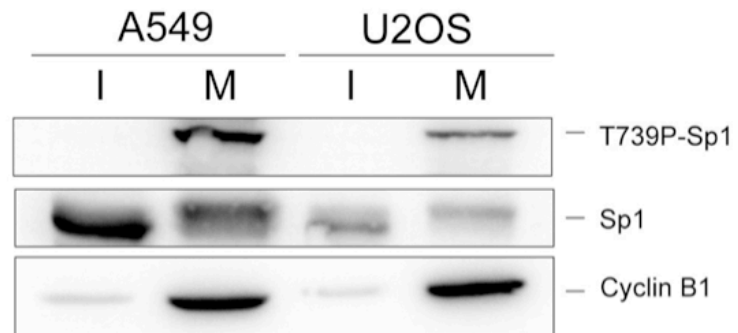


Figure S4

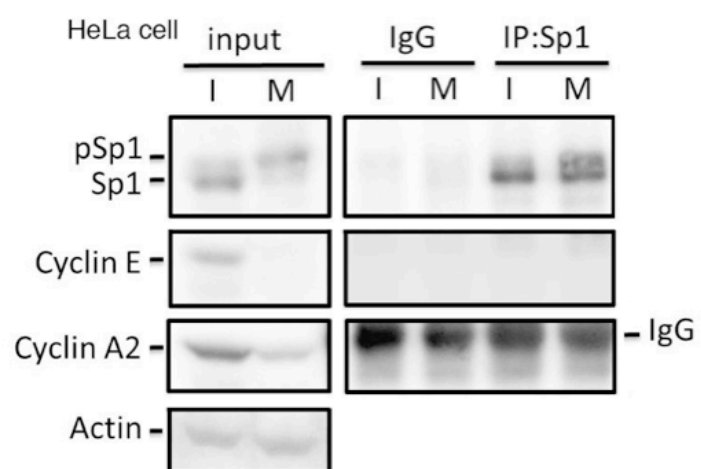


Figure S5

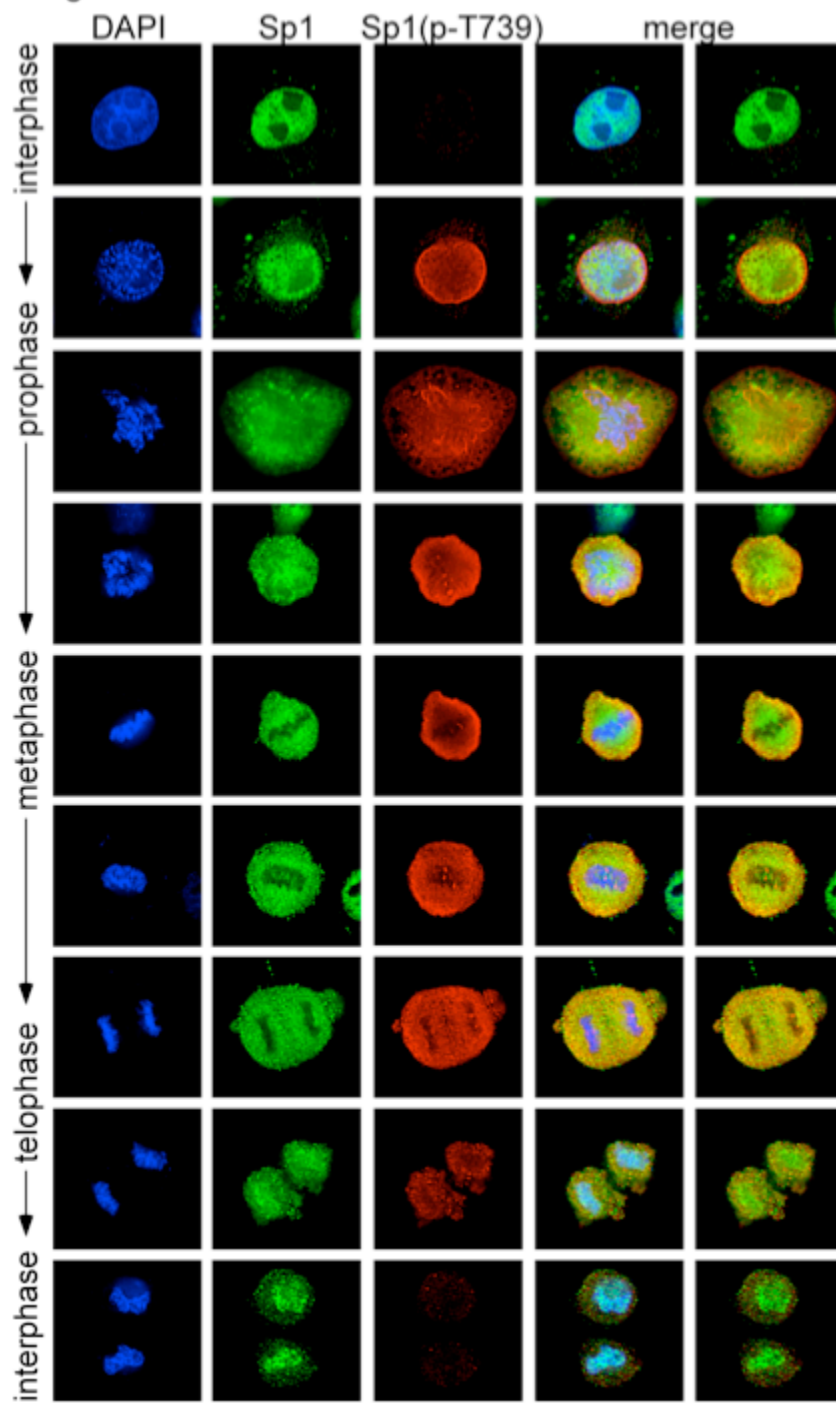


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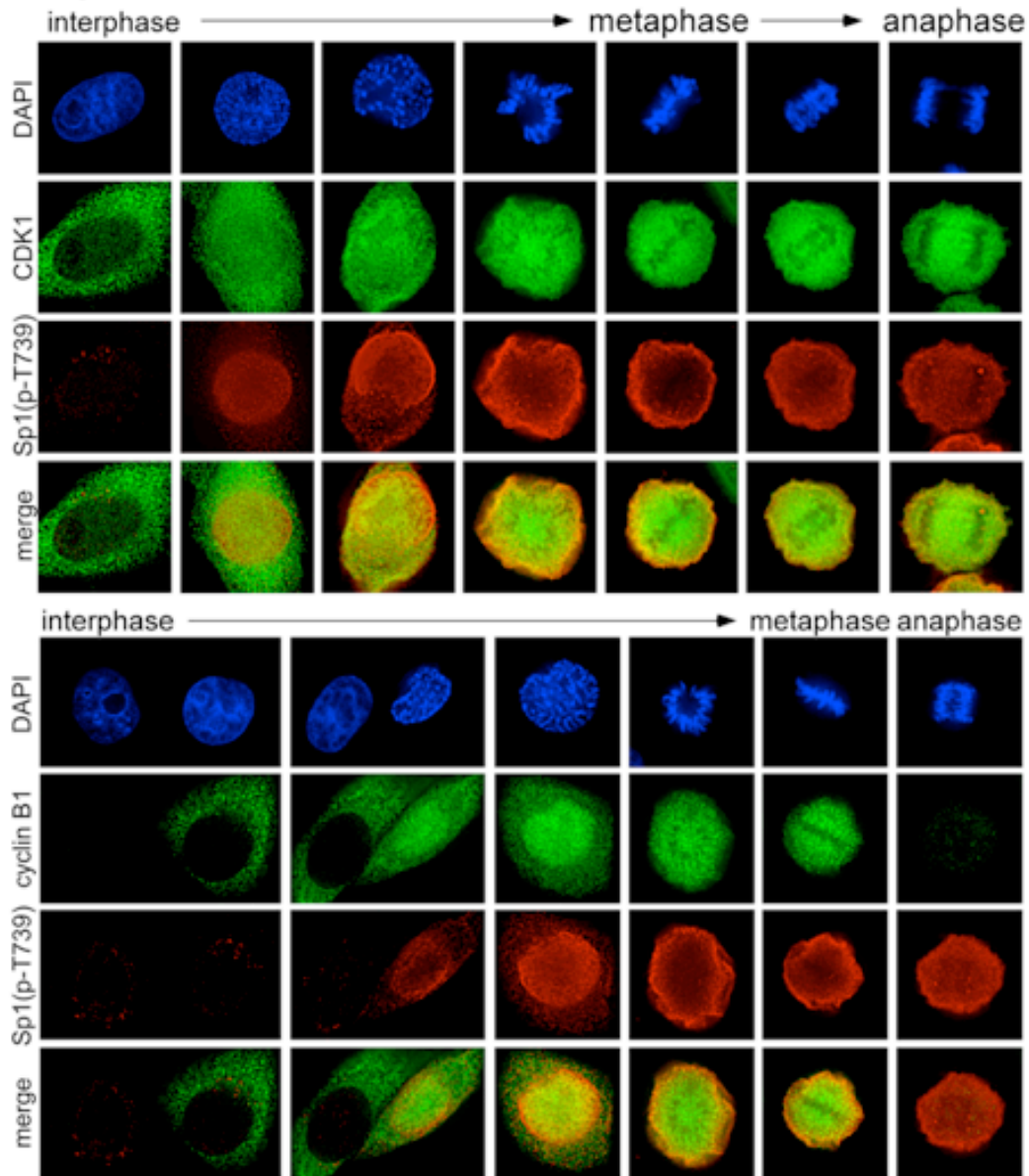
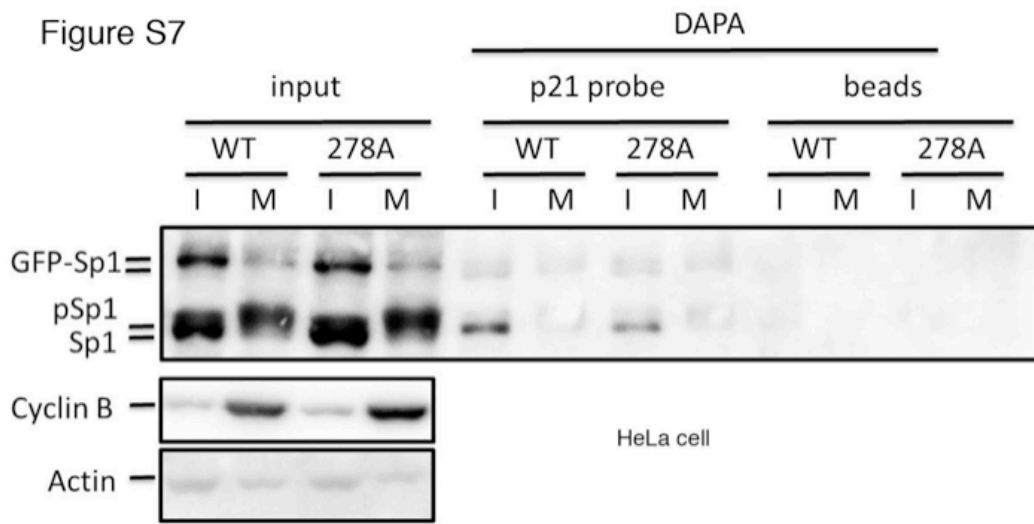


Figure S7



Supplementary Fig.S8

MCF-7 cell

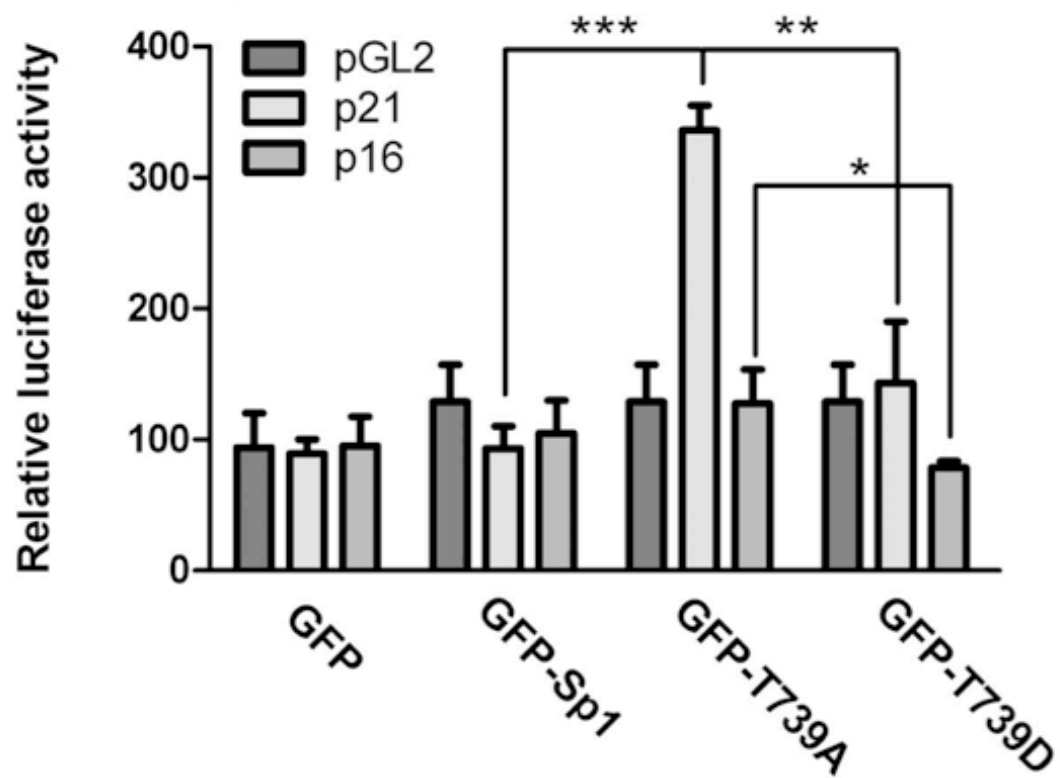
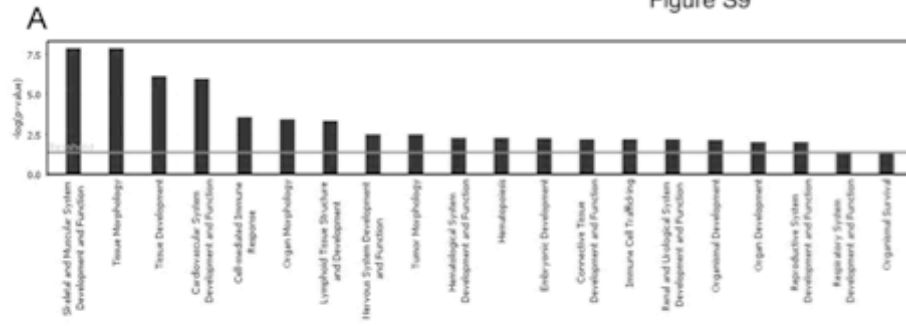


Figure S9



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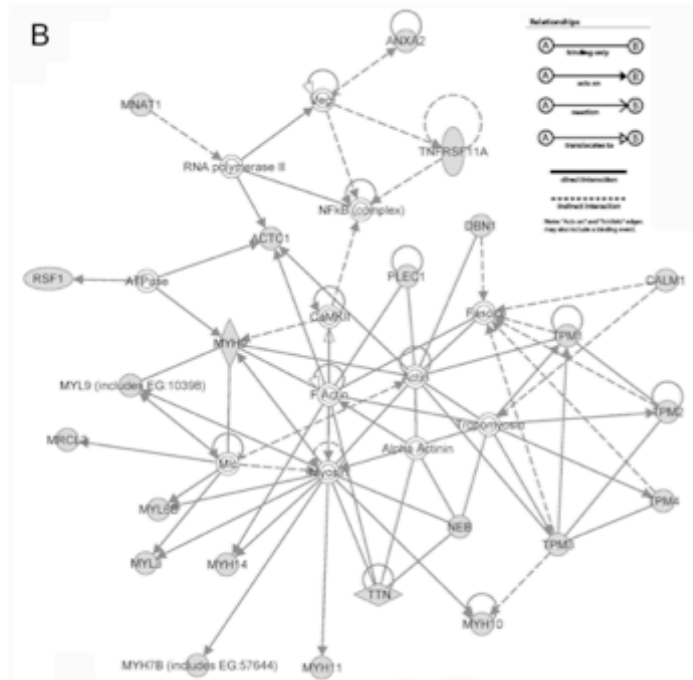


Figure S10

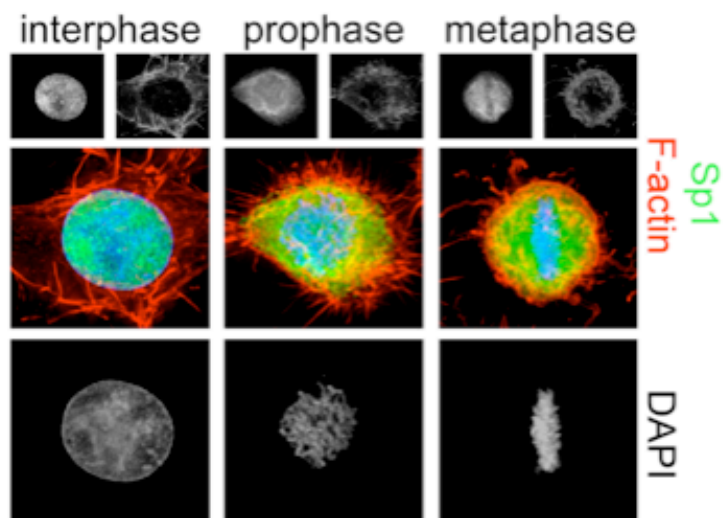
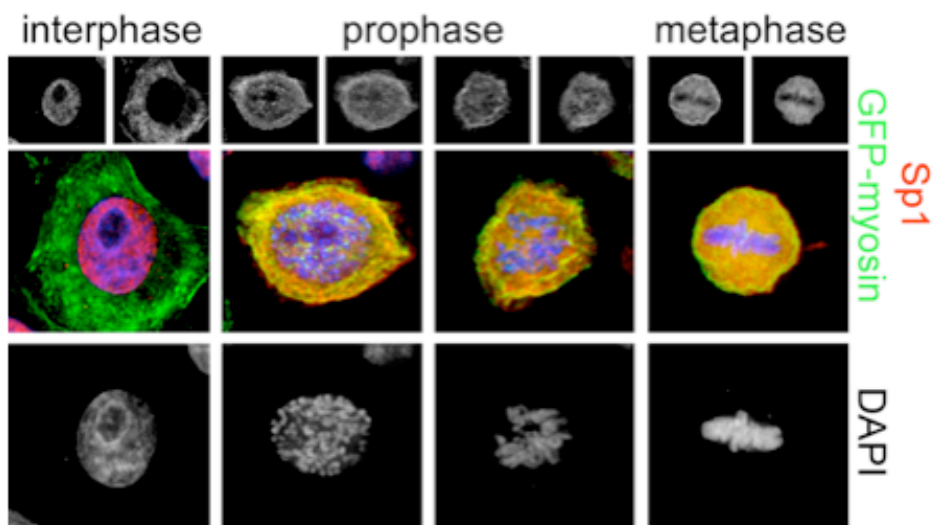


Figure S11

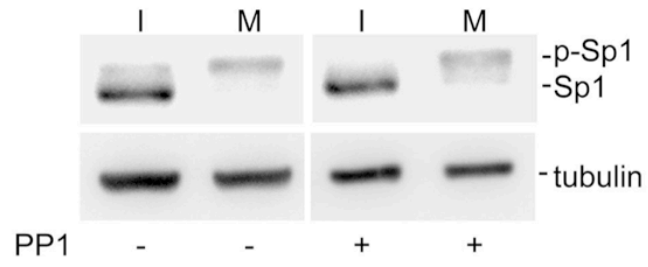
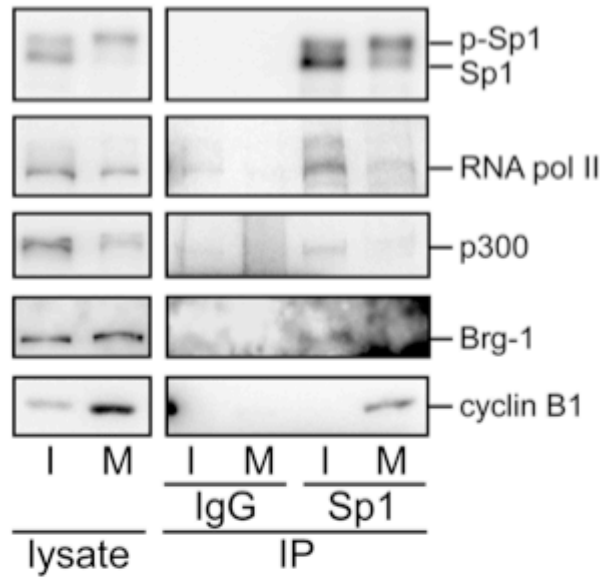


Figure S12

A



B

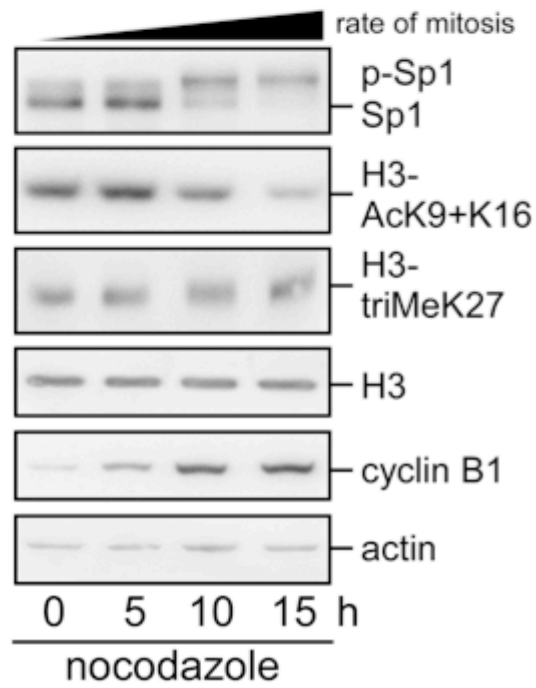


Figure S13

