Zou et al., http://www.jcb.org/cgi/content/full/jcb.201206065/DC1

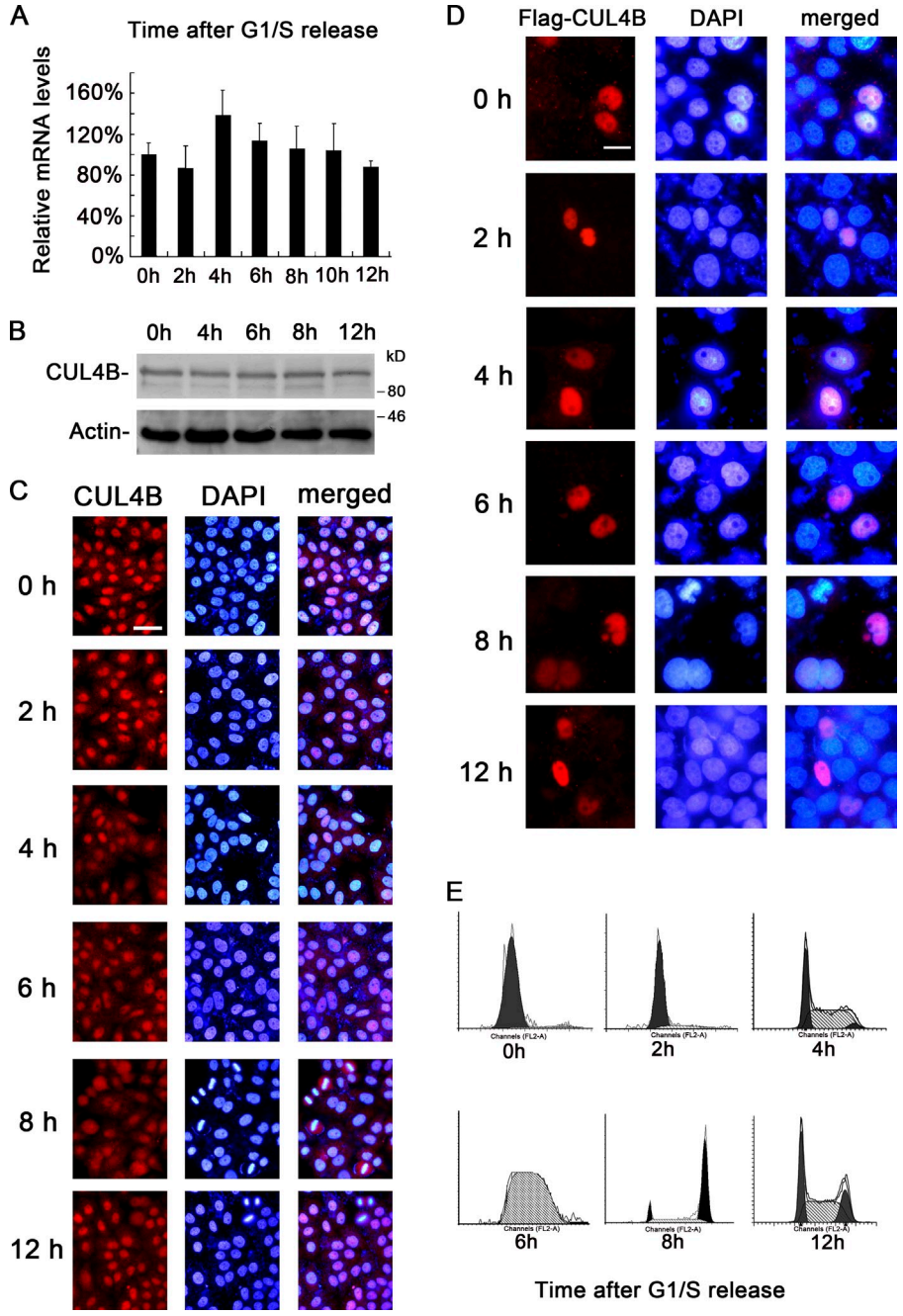

Figure S1. **Expression pattern and subcellular localization of human CUL4B during the cell cycle.** HeLa cells were synchronized with the double-thymidine block and released to progress through the cell cycle. Cells were harvested for isolation of total RNA and protein or immunostained with mouse anti-CUL4B or anti-Flag antibodies at the indicated number of hours after the release. (A) CUL4B mRNA expression was analyzed by real-time PCR. The expression levels were normalized to that of GAPDH in the same sample. The relative gene expression levels were also normalized to calibrator sample (0 h released from G1/S block), the value of which is 100%. The results are expressed as means ± SE of three independent experiments. (B) Cells were synchronized and restimulated to enter the cell cycle as described above. Equal amounts of protein lysates were subjected to SDS-PAGE (12%) and then detected using the antibodies against CUL4B and β-actin, respectively. (C) HeLa cells grown on coverslips were immunostained with anti-CU4B antibody and counterstained with DAPI for nuclei. Bar, 50 µm. (D) HeLa cells transiently transfected with Flag-CUL4B construct were immunostained with anti-Flag antibody. Bar, 20 µm. (E) After release from G1/S block, cells were processed at the indicated times for analysis of cell cycle distribution by flow cytometry. The data shown are from a representative of three independent experiments.

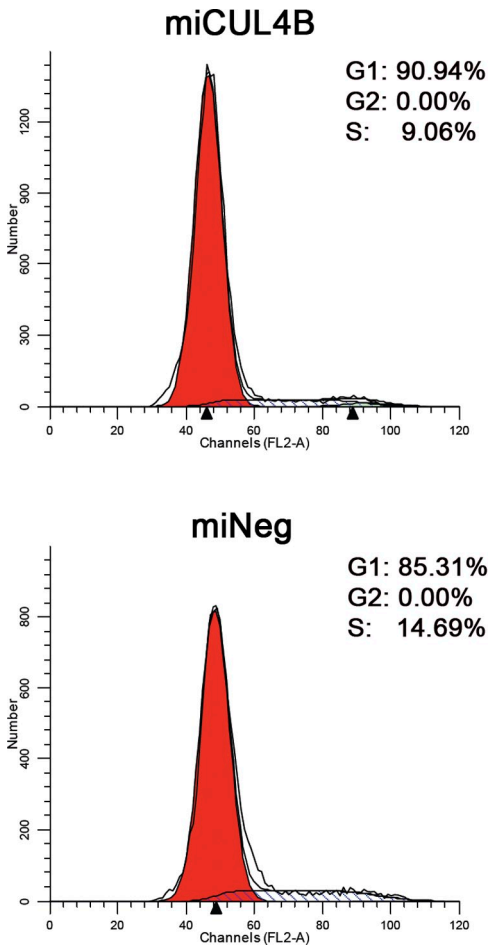

Figure S2. **Cell cycle analysis of synchronized miCUL4B and miNeg cells.** miCUL4B and miNeg cells were synchronized at the G1/S boundary using double-thymidine block and cell cycle distribution was analyzed by flow cytometry. The data shown are from a representative of three independent experiments.

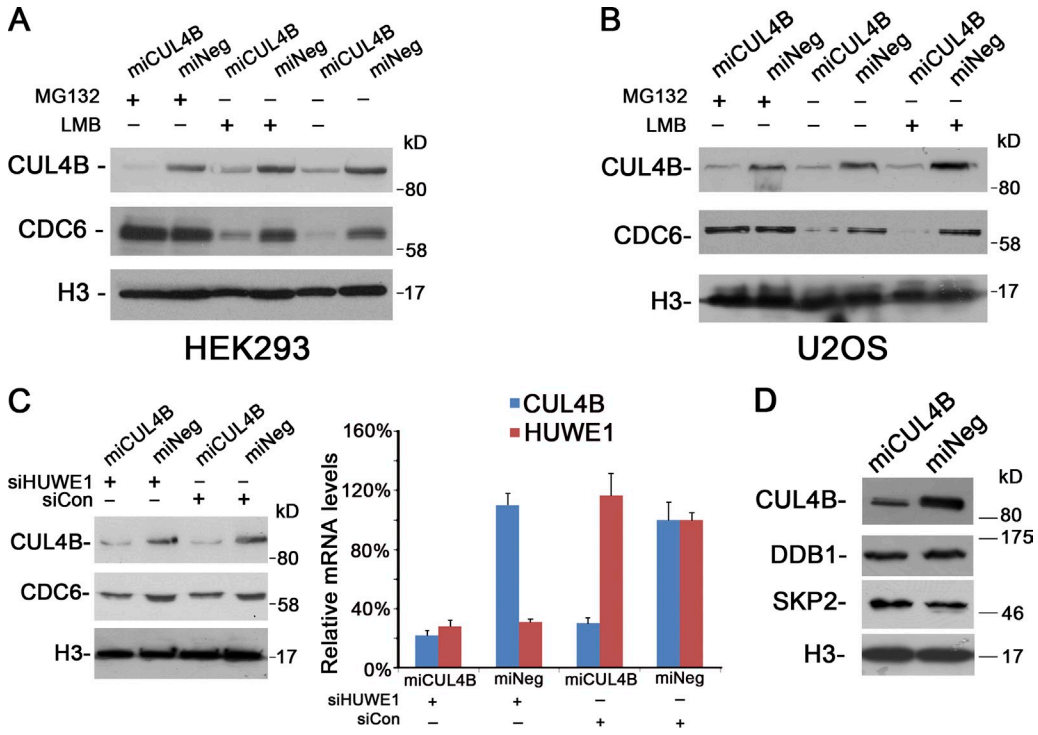

Figure S3. **CUL4B inhibits the proteasomal degradation of CDC6.** (A and B) miCUL4B and miNeg HEK293 (A) and U2OS (B) cells were treated or untreated with 30 μM MG132 for 3 h or 10 μM LMB for 5 h and nuclear proteins were extracted and analyzed by Western blot. (C) miCUL4B and miNeg HeLa cells were transfected with indicated siRNAs. 48 h after transfection, cells were treated with 0.5 mM mimosine for 22 h and nuclear proteins and total RNA was extracted and analyzed by Western blot (left) or real-time PCR (right) . The normalized expression in miNeg cells transfected with siCon was set as 1. The assay was performed in triplicate, and relative means ± SE are shown. (D) Nuclear proteins prepared from miCUL4B and miNeg cells were analyzed by Western blot.

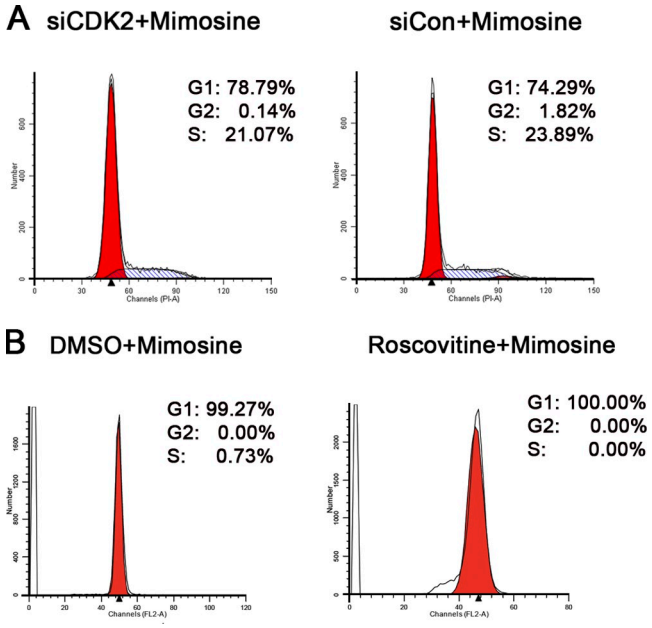

Figure S4. **Flow cytometry analysis of cells treated with mimosine.** (A) HeLa cells were transfected with indicated siRNAs. 48 h after transfection, cells were treated with 0.5 mM mimosine for 22 h and harvested for cell cycle analysis by flow cytometry. (B) HeLa cells arrested in G1 phase by mimosine were incubated further for 2 h in the presence of 0.5 mM mimosine plus 25 μM roscovitine and harvested for cell cycle analysis by flow cytometry. The data shown are from a representative of three independent experiments.

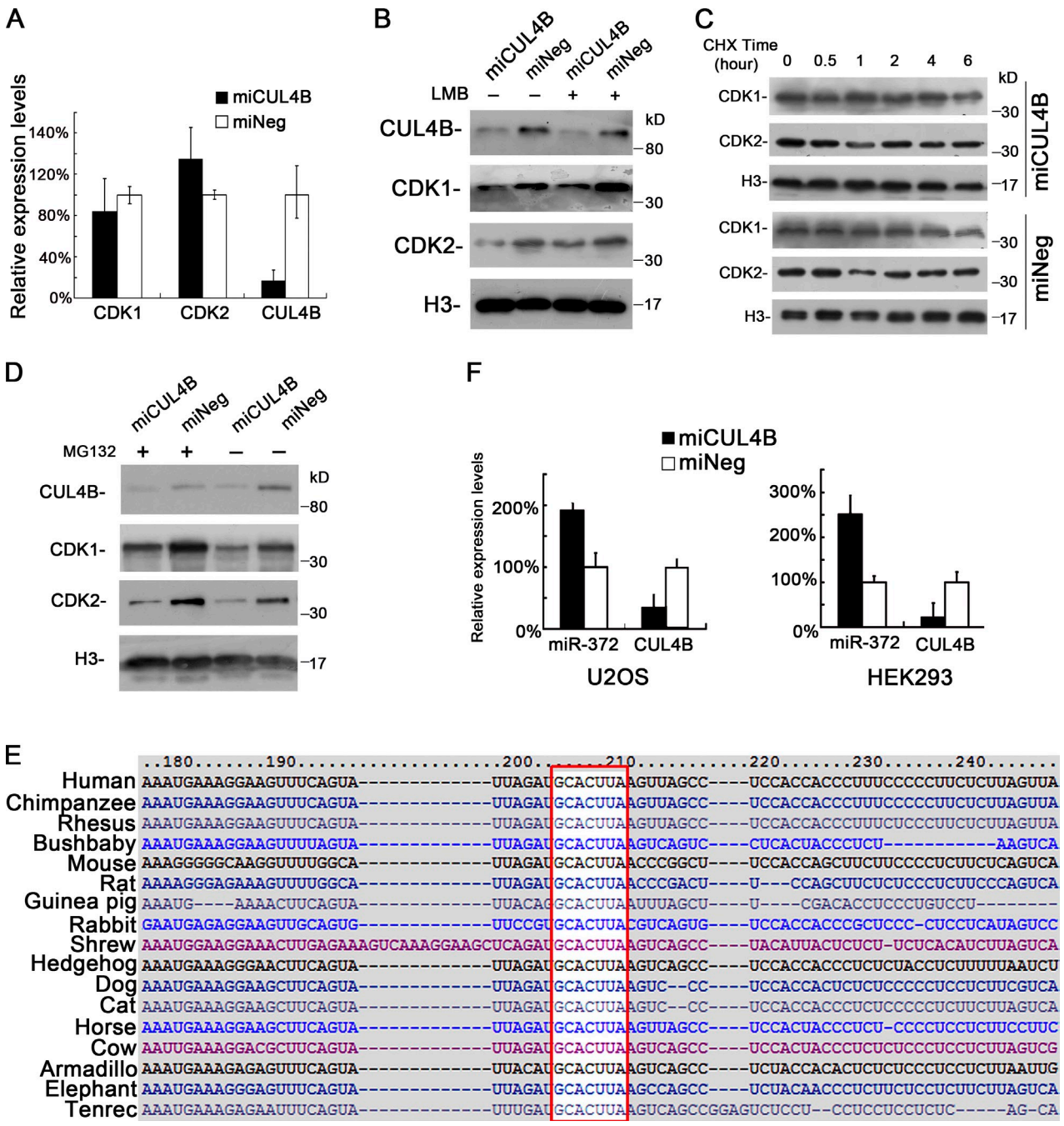

Figure S5. **CUL4B up-regulates CDK2 expression through repression of miR-372/373.** (A) The mRNA levels of CUL4B and CDK1/2 in miCUL4B and miNeg HeLa cells were measured by real-time PCR. The normalized expression in miNeg cells was set as 1. The assay was performed in triplicate, and relative means ± SE are shown. (B) Indicated cells were treated or untreated with 10 µM LMB for 5 h and nuclear proteins were analyzed by Western blot. (C) miCUL4B and miNeg HeLa cells were treated with 50 µg/ml cycloheximide and harvested at the indicated time points. Equal amounts of nuclear proteins of the indicated cells were analyzed by Western blot. (D) miCUL4B and miNeg HeLa cells were treated or untreated with 30 µM MG132 for 3 h and nuclear proteins were analyzed by Western blot. (E) A sequence alignment of the 3′-UTR of CDK2 with orthologues in different species was performed. Missing nucleotides are denoted by hyphens. The miR-372 binding site is boxed, and nucleotide numbers corresponding to the human sequence are also shown. (F) The levels of miR-372 in indicated cells were measured by real-time PCR. The normalized expression in miNeg cells was set as 1. The assay was performed in triplicate, and relative means ± SE are shown.

Table S1. **Primers used for real-time PCR**

Name	Primer sequence (5′→3′)
CUL4A forward	CATCAACAAGAGACCCAACAAGC
CUL4A reverse	CCGTGGATAAACCTGAACAGGAT
CUL4B forward	TGGAAGTTCATTTACCACCAGAGATG
CUL4B reverse	TTCTGCTTTTAACACACAGTGTCCTA
GAPDH forward	CCAGGTGGTCTCCTCTGACTT
GAPDH reverse	GTTGCTGTAGCCAAATTCGTTGT
CDC6 forward	ACAAATGTCCAAACCGTAACCT
CDC6 reverse	GGTAAATGGGGAGTGTTGCATA
CDK1 forward	AAACTACAGGTCAAGTGGTAGCC
CDK1 reverse	TCCTGCATAAGCACATCCTGA
CDK2 forward	CCAGGAGTTACTTCTATGCCTGA
CDK2 reverse	AATCCGCTTGTTAGGGTCGTA
HUWE1 forward	AGCTCTTGACTAAGGGGTTATCT
HUWE2 reverse	GCATGAGGTGGTAGGGATACTT
pri-miR-371–373 forward	CCTTCAACAGCTCATCAAGGGCT
pri-miR-371–373 reverse	TACCCGCCCCCTCACCCAATCAA

Table S2. **Primers used for vector construction (restriction sites are underlined and mismatched bases are shown in bold)**

Name	Primer sequence (5′→3′)
pcDNA3.1/Myc-His B-CUL4A forward	CCAAGCTTCCATGGCGGACGAGGCC
pcDNA3.1/Myc-His B-CUL4A reverse	CGGGATCCGGCCACGTAGTGGTACT
pCMV-Tag 2B-CUL4A forward	CGGGATCCATGGCGGACGAGGCCCCGC
pCMV-Tag 2B-CUL4A reverse	CCCAAGCTTGGCCACGTAGTGGTACTG
pcDNA3.1/Myc-His A-CUL4B forward	CGCGGATCCATGTTTCCAACAGGTTT
pcDNA3.1/Myc-His A-CUL4B reverse	CCGCTCGAGTGCAATATAGTTGTACTG
pEGFP-C1-CUL4B 150-895 forward	CGGGGTACCGCTTCGGTGCATCATGCA
pEGFP-C1-CUL4B 150-895 reverse	CAGCCTGGTAGAGTTCTTCTAAAT
pCMV-Tag 2B-CUL4B 150-895 forward	CGCGGATCCGCTTCGGTGCATCATGCA
pCMV-Tag 2B-CUL4B 150-895 reverse	CAGCCTGGTAGAGTTCTTCTAAAT
CUL4B RNAi-resistant plasmid	**Primer sequence (5′→3′)**
Outer primer forward	TATGGCAAGGATGTTTTTGAGGC
Outer primer reverse	CCGCTCGAGTGCAATATAGTTGTACTG
Internal primer forward	TCAGAA**C**GT**A**CC**C**GGAAATATTG
Outer primer reverse	CAATATTTCC**G**GG**T**AC**G**TTCTGA

Table S3. **Primers used for reporter construction (restriction sites are underlined)**

Name	Primer sequence (5′→3′)
Pmir-GLO-CDK1-3′UTR forward	GAGCTCCTTTCTGACAAAAAGTTTC
Pmir-GLO-CDK1-3′UTR reverse	CCGCTCGAGAGTTTAATTCCCAAAG
Pmir-GLO-CDK2-3′UTR forward	GAGCTCCCTTCTTGAAGCCCCCAGC
Pmir-GLO-CDK2-3′UTR reverse	CCGCTCGAGAATGAGCTACAAACTA
Pmir-GLO-CDK2-3′UTR 372-Mut forward	AGTATTAGATATGCAGAAGTTAGCCTC
Pmir-GLO-CDK2-3′UTR 372-Mut reverse	GAGGCTAACTTCTGCATATCTAATACT