Lu and Pfeffer, http://www.jcb.org/cgi/content/full/jcb.201305158/DC1

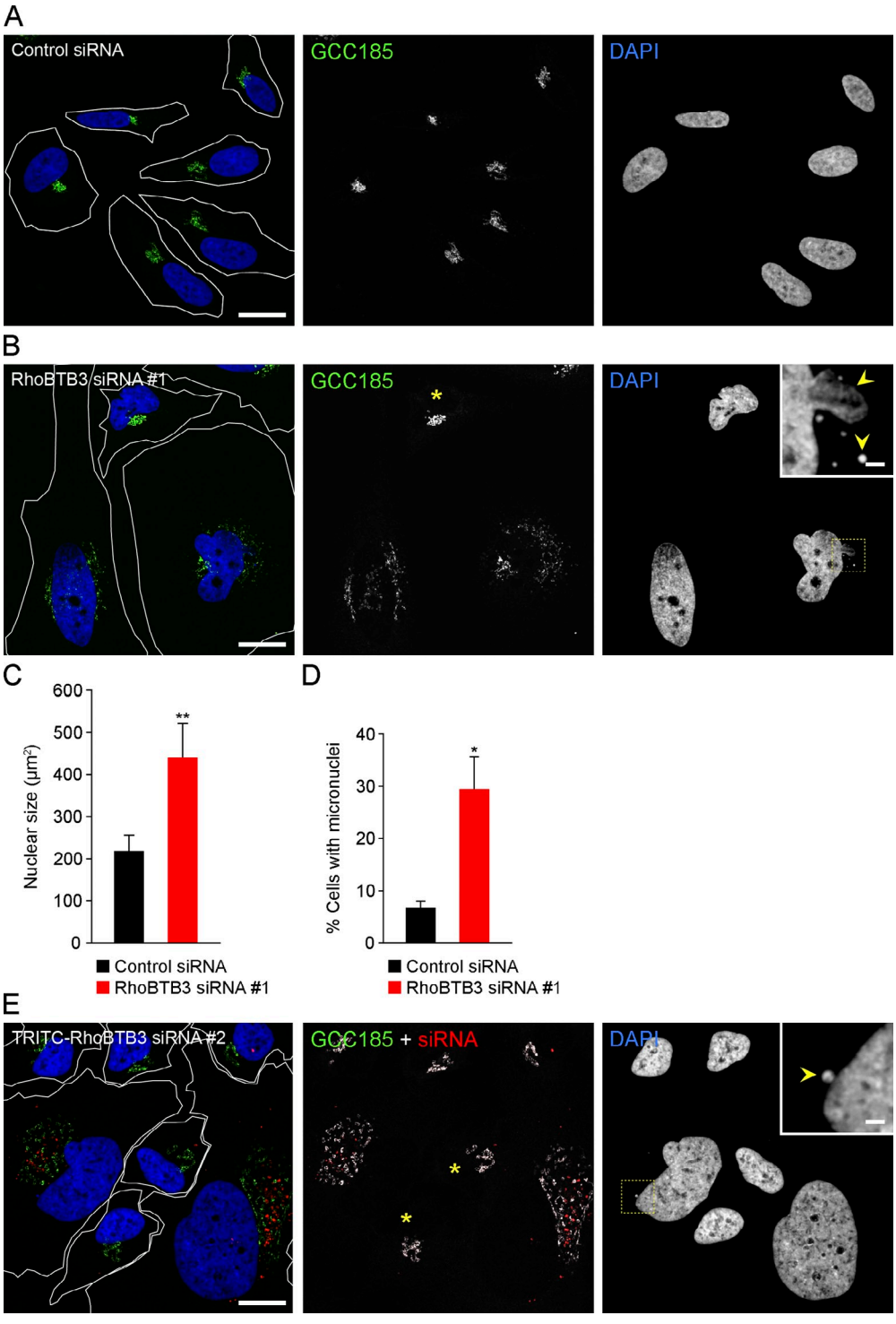


Figure S1. **siRNA depletion of RhoBTB3 increases nuclear size and induces micronuclei formation.** (A–E) RhoBTB3 depletion using two distinct siRNAs increases nuclear size and micronuclei number. siRNA depletion was for 72 h. Asterisks indicate nondepleted HeLa cells displaying a typical Golgi ribbon (detected with anti-GCC185 antibody) and "normal" cell and nuclear size. Arrowheads indicate presence of micronuclei in RhoBTB3-depleted cells. (C and D) Quantification of nuclear size and number of micronuclei; *t* test; *, $P < 0.05$; **, $P < 0.01$; error bars represent SEM of triplicate datasets. Bars: (A, B, and E) 20 µm; (B and E, insets) 2 µm.

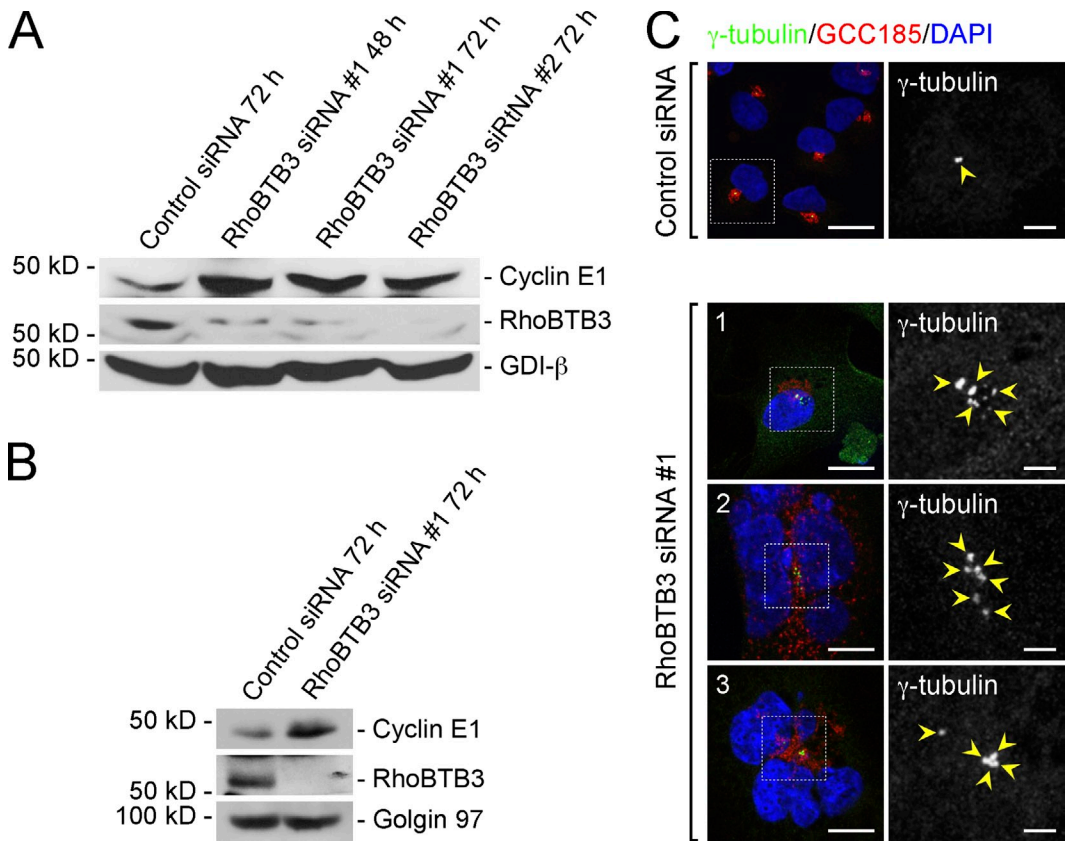


Figure S2. **Increased Cyclin E levels and centrosome overduplication upon RhoBTB3 depletion.** (A) Immunoblot of total HeLa cell lysates shows that Cyclin E1 increases upon depletion of RhoBTB3 with two distinct siRNAs. (B) Immunoblot showing Cyclin E levels in RhoBTB3-depleted U2OS cells. (C) 10% of RhoBTB3-depleted cells (n = 70) show centrosome overduplication (detected with anti–γ-tubulin antibody); three examples are shown. Golgi complexes were identified using anti-GCC185 antibody. Examples 2 and 3 show nuclear atypia and are multinucleated. Bars in merge and inset images are 20 and 5 µm, respectively.

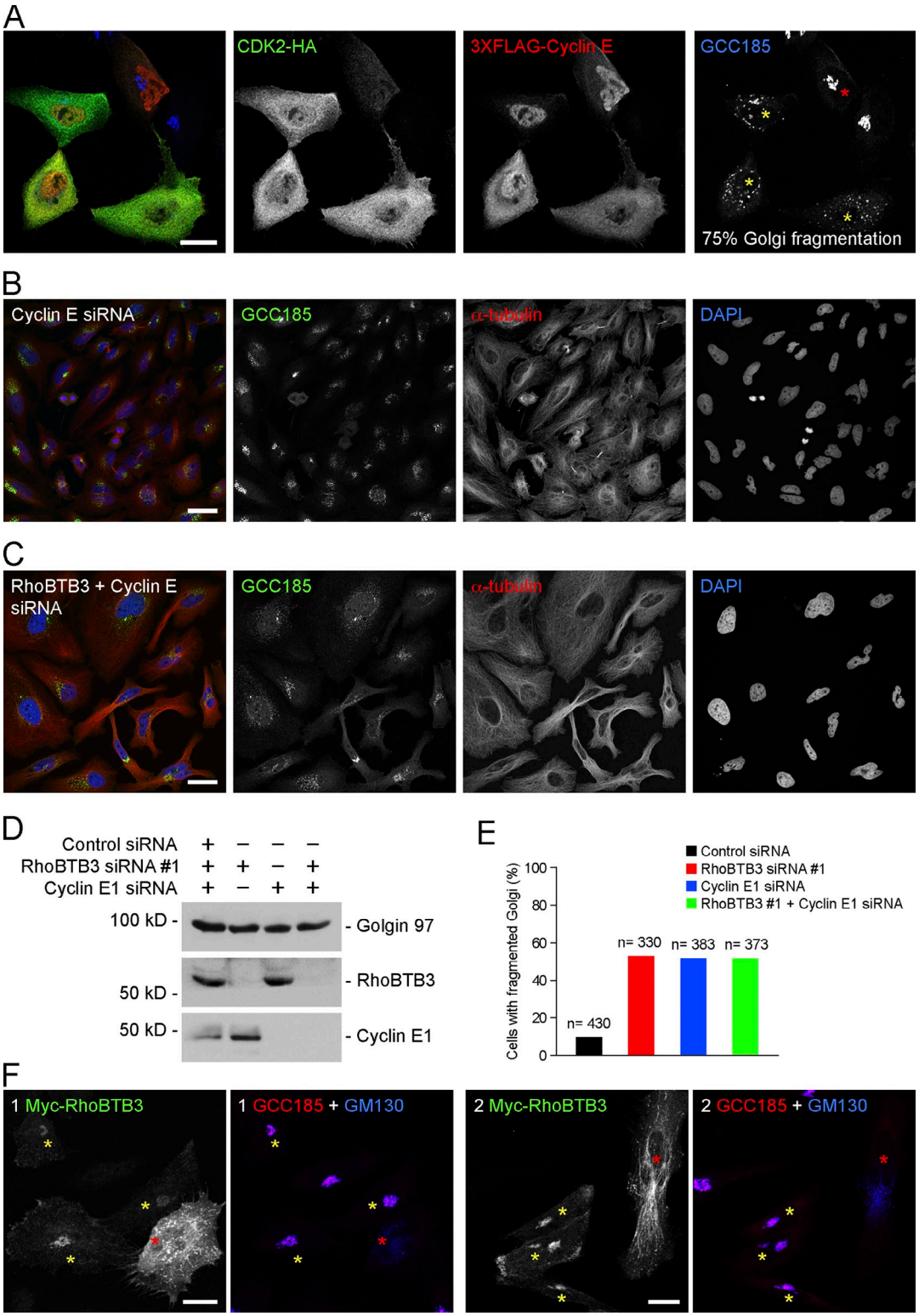


Figure S3. **Involvement of Cyclin E and RhoBTB3 in Golgi structure regulation.** (A) Overexpression of CDK2 with Cyclin E1 causes Golgi fragmentation. Asterisks indicate HeLa cells transfected with both HA-CDK2 and FLAG–Cyclin E1, yielding 75% fragmentation (yellow asterisks); $n = 80$ cells. Golgi complexes of HeLa cells were detected with anti-GCC185. (B–E) Depletion of either Cyclin E1 or RhoBTB3 causes Golgi disruption. HeLa cells were treated with the indicated siRNAs for 72 h and labeled with the indicated antibodies (B and C). (D) Documentation of protein depletion upon siRNA treatment. (E) Quantitation of Golgi fragmentation of Control siRNA (black column), RhoBTB3 siRNA (red column), Cyclin E siRNA (blue column), and RhoBTB3 + Cyclin E siRNA (green column). (F) Myc-RhoBTB3 overexpression disrupts the Golgi. Two fields are shown (1 and 2). Yellow asterisks indicate low expressing, Myc-RhoBTB3-transfected HeLa cells containing "normal" Golgi complexes (detected with anti-GCC185 and anti-GM130 antibodies); red asterisks indicate cells expressing high levels of Myc-RhoBTB3 (see green Myc staining). Bar, 20 μm.

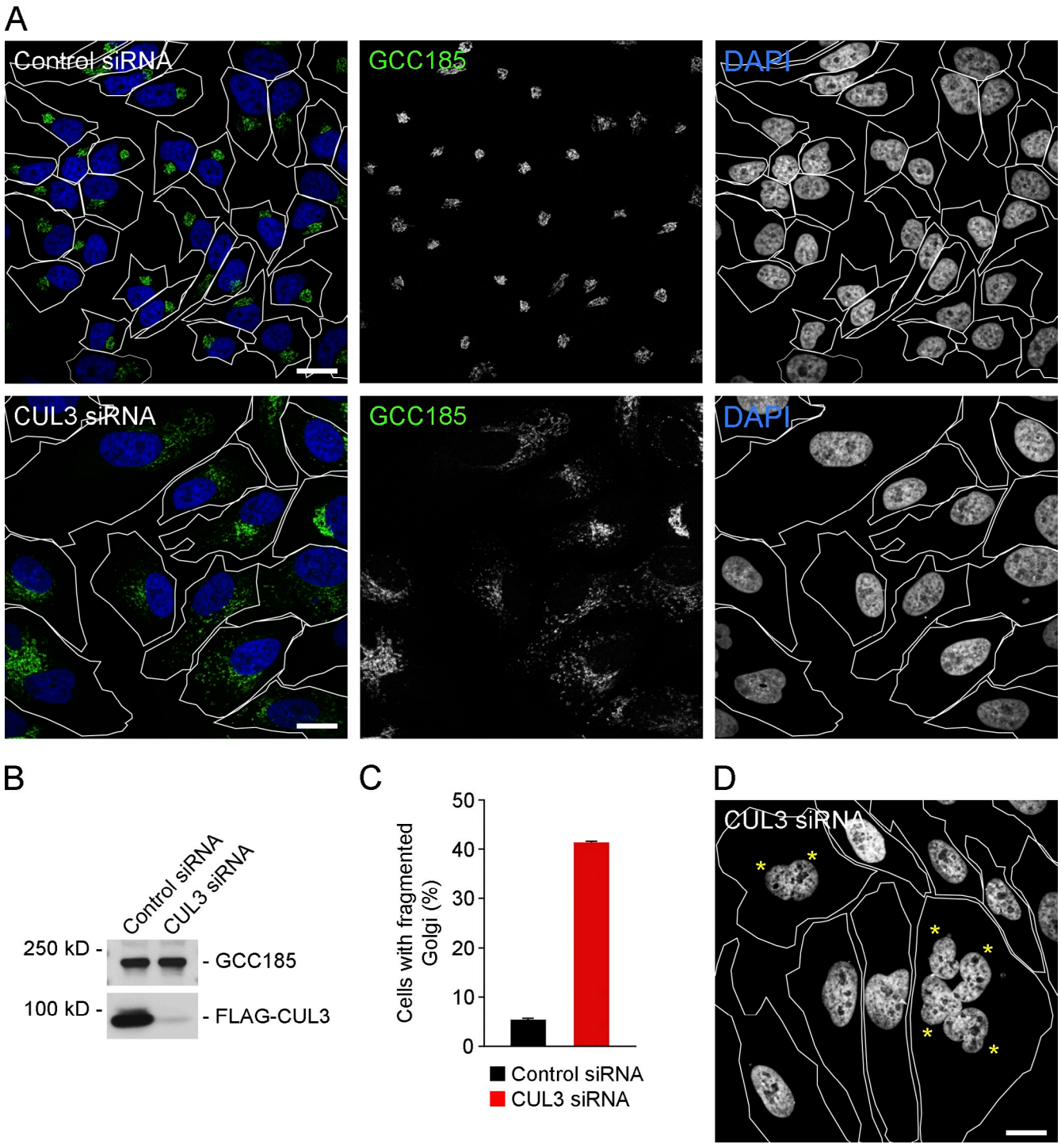


Figure S4. **CUL3 depletion leads to Golgi fragmentation, enlarged cell/nuclear size, and multinuclei formation.** (A) Confocal microscopy of HeLa cells transfected for 72 h with control siRNA or CUL3 siRNAs as indicated and stained for GCC185. DNA was stained with DAPI. (B) Immunoblot detection of CUL3 depletion in HeLa cells. (C) Quantitation of Golgi morphology in control or CUL3-depleted cells. The data represent the mean of two independent data sets in which ≥148 cells were analyzed in both conditions; error bars represent SEM. (D) Presence of multinucleated cells (yellow asterisks indicate nuclei) after 72 h treatment with a CUL3 siRNA. Bar, 20 µm.

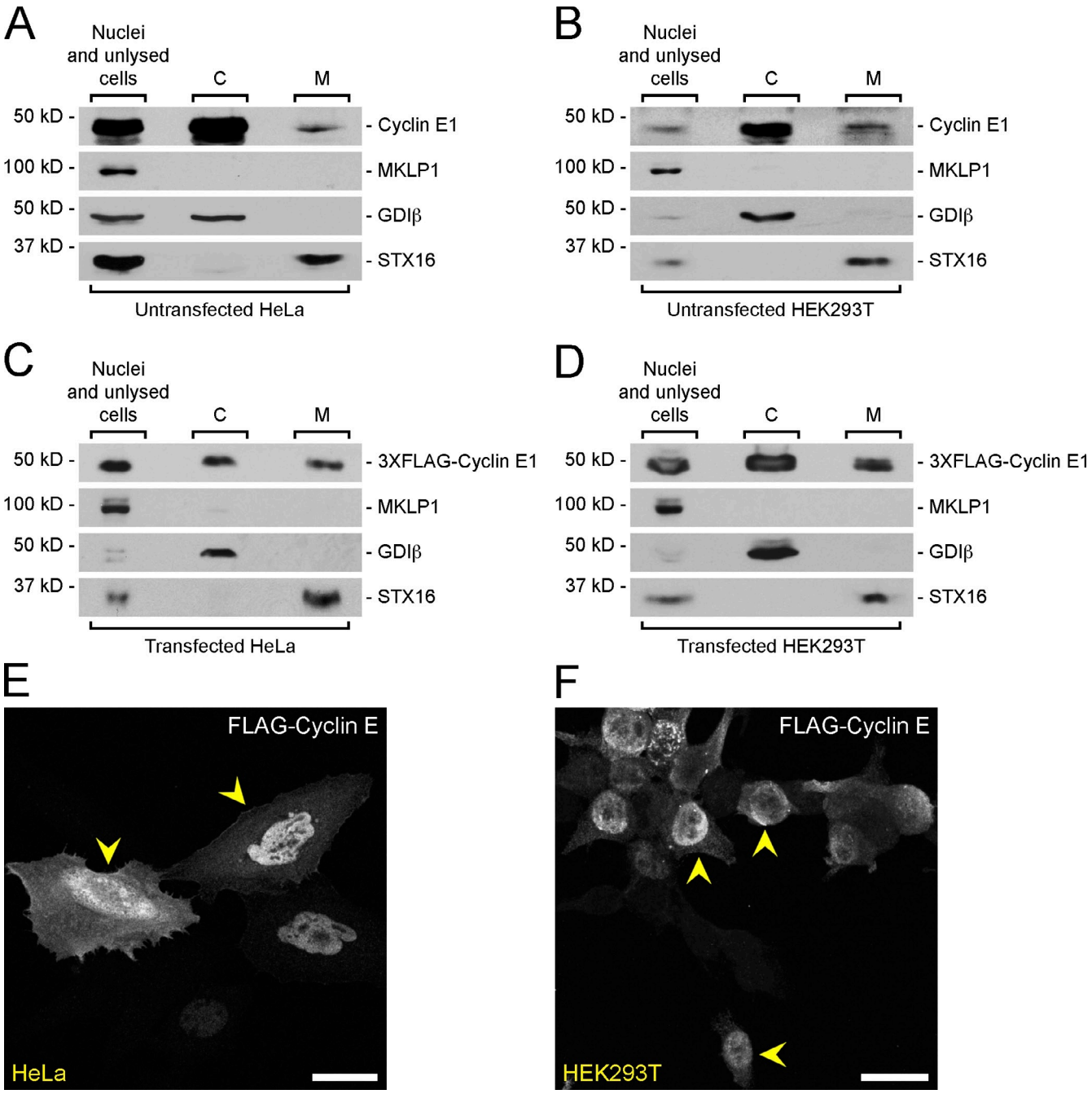
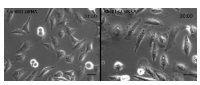


Figure S5. **Subcellular distribution of Cyclin E in human cells.** Subcellular distribution of Cyclin E in HeLa (A and C) or HEK293T cells (B and D), before (A and B) or after (C and D) transfection with Cyclin E1 (A–D). Equal proportions of nuclear, cytosolic (C), or membrane (M) fractions were analyzed. (E and F) Localization of FLAG–Cyclin E in HeLa (E) or HEK293T cells (F) by light microscopy. Arrowheads indicate cells displaying both cytosolic and nuclear pools of protein. Bar, 20 µm.



Video 1. **RhoBTB3-depleted cells do not undergo mitosis in vivo.** Time-lapse phase-contrast video microscopy of HeLa cells after 48 h with control (left frames) or RhoBTB3 siRNA #1 (right frames). Cells were imaged every 3.5 min for 15 h using an inverted microscope (Axio Observer Z1; Carl Zeiss) fitted with an LD Plan-Neofluar 20×/0.4 Korr Ph1 Ph2 objective and a CCD camera (AxioCam MRm; Carl Zeiss) controlled by AxioVision 4.8 software (Carl Zeiss). Black arrows in Control siRNA frames indicate cells undergoing mitosis. White arrows at the beginning and at the end of RhoBTB3 siRNA frames show cells that do not enter mitosis and keep growing in size. Bar, 40 µm.