Supporting Information

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SI Materials and Methods

Vector Construction and RNAi. To specifically deplete endogenous p150^{Glued} in HeLa cells, plasmid pBS/U6-p150^{Glued} was constructed. The targeting sequence of human p150^{Glued} (accession no. NM_004082) was GGTGCTGAAGAAATGGTGG, corresponding to the coding region 1303 to 1322 relative to the first nucleotide of the start codon. Plasmid pBS/U6-p150^{Glued}-first half (sense strand) was used as a control vector. The same target sequence was also used to design siRNA against human p150^{Glued}. Plasmid pBS/U6-Plk1 (Polo-like kinase 1) was described previously (1). The following target sequence was used to design siRNA against human Plk1: AAGGGCGGCTTTGCCAAGTGCTT (2).

Cell Culture and Transfection. HeLa and HEK293T cells were cultured in DMEM supplemented with 10% (vol/vol) FBS, 100 units/mL penicillin, and 100 units/mL streptomycin at 37 °C in 8% CO_2. Cells were arrested at mitosis by incubation with 100 ng/mL of nocodazole for 12 h. To synchronize HeLa cells at prophase, cells were treated with 2.5 mM thymidine for 16 h, released for 8 h, and then treated with thymidine a second time for 16 h, followed by release for 11 h. Small interfering RNA was transfected using transmessenger transfection reagent (QIAGEN), whereas plasmid DNA was transfected using lipofectamine 2000 (Invitrogen), as described by the manufacturers. Cells were harvested 36 h after transfection and analyzed by immunoprecipitation (IP) and Western blotting.

Antibodies. The antibodies against Plk1 (sc-17783) and lamin A/C (sc-7292) used in this study were purchased from Santa Cruz Biotechnology. Antibodies against p150^{Glued} (610474) and cyclin B1 (554177) were from BD Transduction Laboratories, and antibodies against actin and α-tubulin were obtained from Sigma. The GFP antibody (A11122) was from Invitrogen. The phospho-specific antibody against Ser179 of p150^{Glued} was generated by Proteintech. Briefly, a peptide containing phospho-S179 (GSASAGELSpSSEPS TPA) was synthesized and used to immunize rabbits. After the antibody was affinity-purified, a series of control experiments was performed to confirm the specificity of the antibody.

Protein Purification, Mutagenesis, and Taxol-Polymerized Microtubule Preparation. Various GST-tagged human p150^{Glued} constructs were subcloned into pGEX-KG, expressed in *Escherichia coli*, and purified. Point mutations were generated using the QuikChange Site-Directed Mutagenesis Kit (Stratagene). Taxol-polymerized microtubules were prepared using the MT-associated protein spin-down assay kit (Cytoskeleton, Inc.).

IP and Immunoblotting. Whole-cell lysates were incubated with different antibodies indicated in each experiment for 1.5 h at 4 °C, followed by 1 h of incubation with protein A/G-Sepharose beads. Immunocomplexes were resolved by SDS/PAGE, and coimmunoprecipitated proteins were detected by Western blotting using antibodies indicated in the specific experiments. Antibodies against Plk1, p150^{Glued}, GFP, actin, cyclin B, and p-H3 were used at 1:1,000 dilution, and antibody against pS179 was used at 1:200.

Immunofluorescence Intensity Analysis. Plk1-depleted HeLa cells or U2OS cells stably expressing GFP-p150^{Glued} were treated with thymidine for 20 h, released for 8 h, and exposed to nocodazole for an additional 3 h. Cells were fixed and processed for immunofluorescence (IF) staining with antibodies against p150^{Glued} or GFP. Multiple same-exposure three-channel digital images were acquired for each set of cultures (FITC for lamin A/C, Texas red for p150^{Glued}, and DAPI to label nuclei). IF intensity analysis was performed as previously described (3). In brief, differences in fluorescence intensity at the NE were measured in two ways. (*i*) Pixel intensities were determined along 4-μm lines that start 3 μm away from the nuclear envelope (NE). The first pixel was set to an intensity of 1, and the remaining pixels were normalized accordingly. The mean intensities were calculated for 20 lines. (*ii*) The mean of p150^{Glued} pixel intensities of 10 to 30 points on the NE was compared with mean pixel intensities of 10 to 30 points in the cytoplasm near the NE. Pixel intensities were obtained using ImageJ software from the National Institutes of Health.

Kinase Assays. Purified recombinant p150^{Glued} was incubated with purified Plk1 in kinase reaction buffer (50 mM Tris, pH7.5, 10 mM $MgCl_2$, 2 mM EGTA, 0.5 mM Na_3VO_4, 100 mM PNPP, 25 mM DTT, 125 μM ATP) supplemented with 10 μCi of [γ-^{32}P] ATP at 30 °C for 30 min. After the reaction mixtures were resolved by SDS/PAGE, the gels were stained with Coomassie Brilliant Blue, dried, and subjected to autoradiography.

Metabolic Labeling and Phosphopeptide Mapping. HEK293T cells were transfected with various GFP-p150^{Glued} constructs, arrested in mitosis by nocodazole treatment (100 ng/mL) for 12 h, and labeled for 3 h with [^{32}P]orthophosphate (ICN) at 1 mCi/mL in phosphate-free DMEM. After lysates were subjected to anti-GFP IP, phosphoproteins were recovered, digested with trypsin, and resolved by 40% alkaline PAGE, followed by autoradiography.

Generation of U2OS Cells Stably Expressing GFP-p150^{Glued} Constructs. One day after U2OS cells were transfected with RNAi-resistant GFP-p150^{Glued} (WT or S179A) by using MegaTran 1.0, cells were selected with 400 μg/mL of G418 for 6 wk. After at least 10 clones per construct were obtained, Western blotting was performed to isolate the cell lines that express various GFP-p150^{Glued} constructs at protein levels close to those of endogenous p150^{Glued}.

1. Liu X, Erikson RL (2003) Polo-like kinase (Plk)1 depletion induces apoptosis in cancer cells. *Proc Natl Acad Sci USA* 100:5789–5794.
2. Liu X, Erikson RL (2002) Activation of Cdc2/cyclin B and inhibition of centrosome amplification in cells depleted of Plk1 by siRNA. *Proc Natl Acad Sci USA* 99:8672–8676.
3. Hebbar S, et al. (2008) Lis1 and Ndel1 influence the timing of nuclear envelope breakdown in neural stem cells. *J Cell Biol* 182:1063–1071.

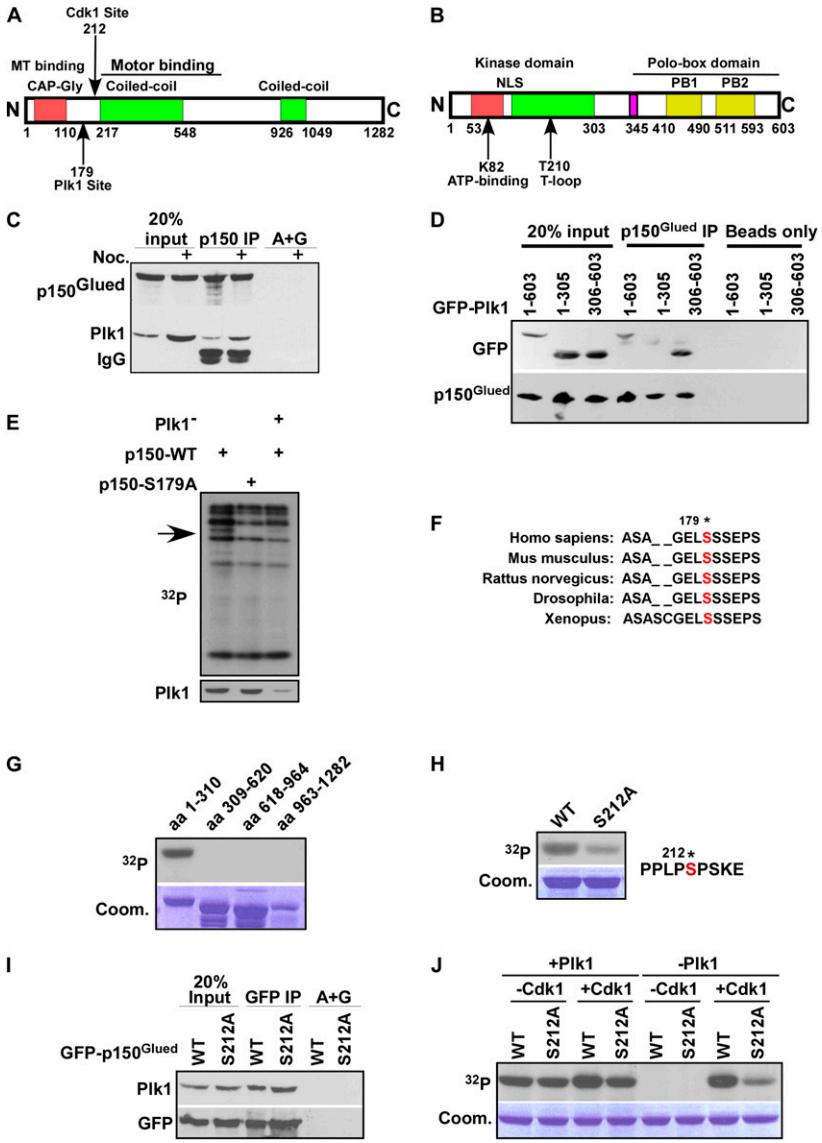

Fig. S1. p150^{Glued} is a Plk1-interacting protein. (*A* and *B*) Schematic representation of p150^{Glued} (*A*) and Plk1 (*B*). (*C*) Coimmunoprecipitation of Plk1 and p150^{Glued}. Lysates from HeLa cells that were treated with or without 100 ng/mL nocodazole (Noc.) for 12 h were immunoprecipitated with p150^{Glued} antibodies, followed by Western blotting with antibodies as indicated on the left. Protein A/G beads-only lanes serve as washing controls. (*D*) HEK293T cells were transfected with GFP-Plk1, GFP-Plk1-aa 1–305, or GFP-Plk1-aa 306–603. After 1 d of transfection, cells were treated with nocodazole for 12 h. Whole extracts were prepared for anti-p150^{Glued} IP, followed by anti-GFP Western blot. (*E*) Metabolic labeling and phosphopeptide mapping of GFP-p150^{Glued}. HEK293T cells were transfected with siRNA to deplete Plk1 (lane 3), incubated overnight, transfected with GFP-p150^{Glued}-WT (lanes 1 and 3) or -S179A (lane 2), treated with nocodazole for 12 h, and metabolically labeled with [^{32}P]orthophosphate. Phosphoproteins were immunoprecipitated with GFP antibodies, resolved by SDS/PAGE. Phosphoproteins were located by autoradiography, excised, digested with trypsin, and resolved on a 40% alkaline PAGE. The arrow indicates the position corresponding to a peptide containing Ser-179. (*F*) Alignment of p150^{Glued} sequences from various species adjacent to the Plk1 phosphorylation site. (*G*) Commercial Cdk1/cyclin B was incubated with four purified GST-p150^{Glued} fragments (amino acids 1–310, 309–620, 618–964, 963–1282) in the presence of [γ-^{32}P]ATP. The reaction mixtures were resolved by SDS/PAGE, stained with Coomassie Brilliant Blue (Coom.), and detected by autoradiography. (*H*) Cdk1/cyclin B was incubated with indicated forms of GST-p150^{Glued}-N (amino acids 1–310) as in *G*. Human amino acid sequence around S212 is shown on the right. (*I*) HEK293T cells were transfected with GFP-p150^{Glued}-WT or S212A. After 1 d of transfection, cells were treated with nocodazole for 12 h. Whole-cell extracts were prepared for anti-GFP IP, followed by anti-Plk1 Western blot. (*J*) Purified GST-p150^{Glued}-WT or S212A proteins were preincubated with or without Cdk1/cyclin B in the presence of unlabeled ATP. All samples were then incubated with or without Plk1 in the presence of [γ-^{32}P]ATP. The reaction mixtures were analyzed as in *H*.

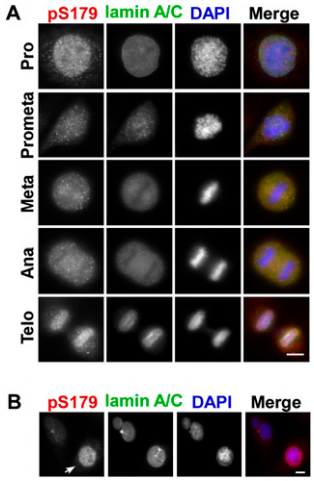

Fig. S2. Spatial regulation of the pS179-p150^{Glued} epitope in HeLa cells. (*A* and *B*) HeLa cells at indicated phases of cell cycle were costained with antibodies against pS179 and lamin A/C. DNA was stained with DAPI. The arrow in *B* indicates a mitotic cell. (Scale bars, 5 μm.)

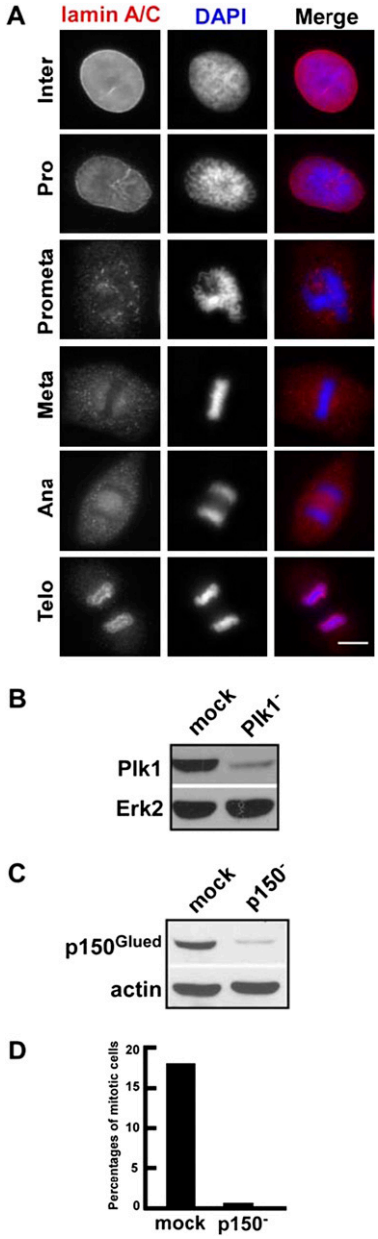

Fig. S3. Plk1 and p150^{Glued} are involved in nuclear envelope breakdown (NEBD) at prophase. (*A*) HeLa cells were synchronized with the double thymadine block (DTB), released for 11 h, and immunostained with antilamin A/C antibodies. DNA was stained with DAPI. (Scale bar, 5 μm.) (*B*) HeLa cells were transfected with siRNA to deplete Plk1. After 24 h of transfection, cells were treated with nocodazole for additional 12 h, and harvested for anti-Plk1 Western blotting. (*C*) HeLa cells were transfected with siRNA to deplete p150^{Glued}. After 2 d of transfection, cells were harvested for anti-p150^{Glued} Western blotting. (*D*) HeLa cells depleted of p150^{Glued} were synchronized with the DTB, released for 11 h, and stained with DAPI. Percentage of mitotic cells was determined based on chromosome morphology.

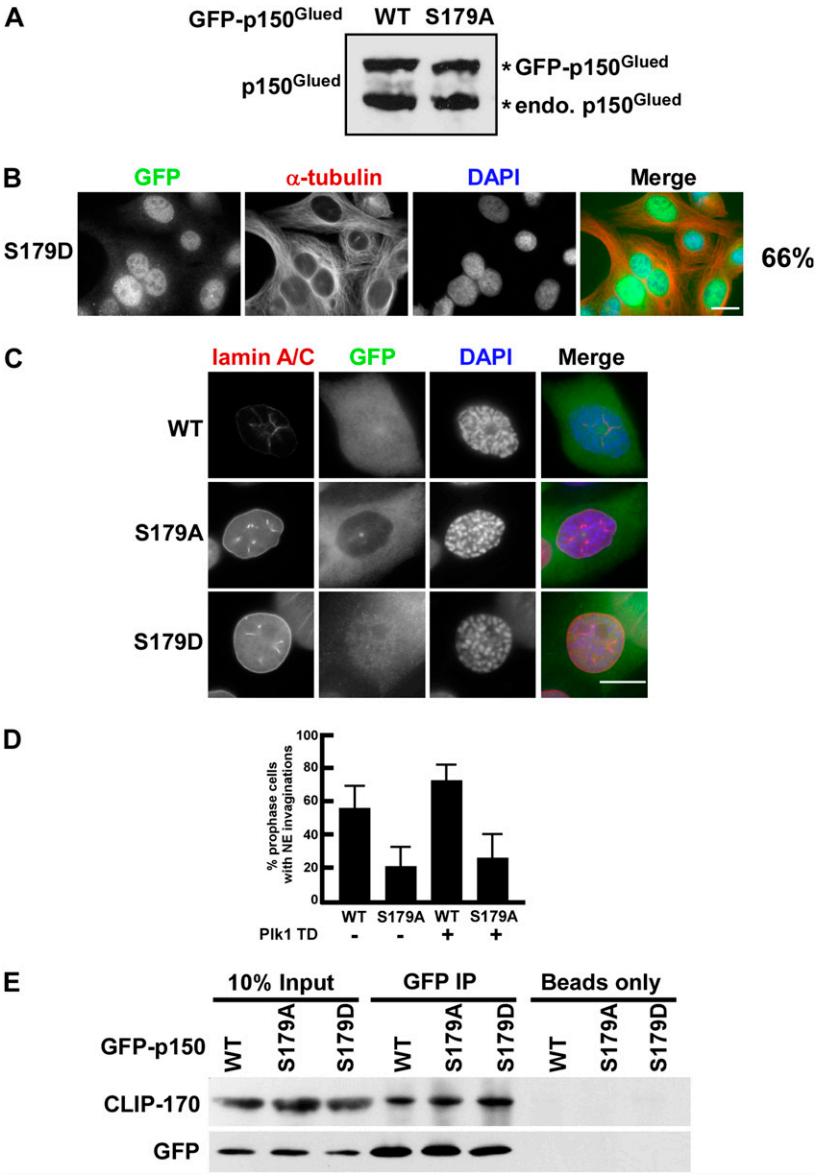

Fig. S4. Phenotypic analysis of overexpression of different forms of p150^{Glued} constructs. (*A*) Expression of various forms of GFP-p150^{Glued} in U2OS cells. Randomly growing U2OS cells stably expressing GFP-p150^{Glued} (WT or S179A) were immunoblotted with antibodies against p150^{Glued}. Both overexpressed and endogenous p150^{Glued} are indicated. (*B*) U2OS cells were transfected with GFP-p150^{Glued}-S179D, stained with α-tubulin antibodies, and analyzed. About 66% of GFP-positive cells showed nuclear envelope localization. (*C*) The S179D mutation of p150^{Glued} did not cause premature NEBD. U2OS cells expressing different forms of GFP-p150^{Glued} constructs (WT, S179A, or S179D) were stained with lamin A/C antibodies. (*D*) Expression of Plk-T210D, a constitutively active construct, promotes NE invagination at prophase. U2OS cells stably expressing GFP-p150^{Glued} constructs (WT or S179A) were transfected with Plk1-T210D and NE invagination was analyzed as in Fig. 3C. (*E*) U2OS cells stably expressing GFP-p150^{Glued} constructs (WT, S179A, or S179D) were subjected to anti-GFP IP, followed by anti-CLIP-170 Western blotting analysis. (Scale bars, 10 μm.)

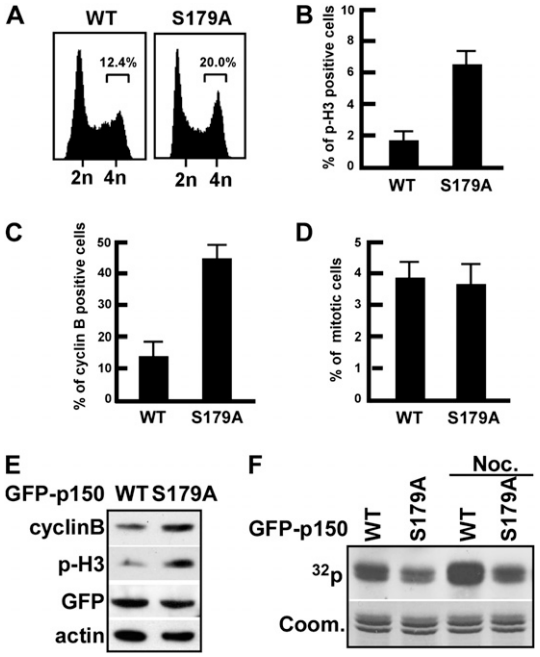

Fig. S5. Expressing of GFP-p150^{Glued}-S179A caused G2/M transition delay. (*A*) Randomly growing U2OS cells stably expressing GFP-p150^{Glued} (WT or S179A) were subjected to FACS analysis. (*B–E*) U2OS cells stably expressing GFP-p150^{Glued} (WT or S179A) were stained with antibodies against phospho-H3 (*B*) or cyclin B (*C*). DNA was stained with DAPI. Percentage of mitotic cells was determined based on chromosome morphology (*D*). (*E*) Randomly growing U2OS cells stably expressing GFP-p150^{Glued} constructs were immunoblotted with antibodies indicated on the left. (*F*) HEK293T cells were transfected with GFP-p150^{Glued} (WT or S179A), treated with or without nocodazole for 12 h, and harvested for anti-Cdc2 IP/kinase assays using histone ^{1}H as a substrate.

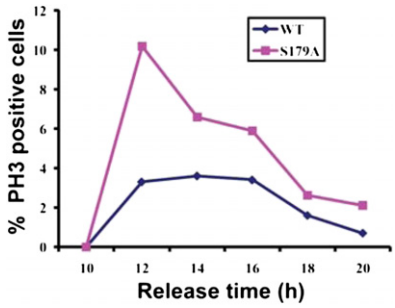

Fig. S6. Cell-cycle analysis of cells expressing p150^{Glued} (WT or S179A). U2OS cells stably expressing GFP-p150^{Glued} constructs (WT or S179A) were synchronized with the DTB protocol, released for different times, and stained with phospho-H3 antibodies.