Chiyoda et al., http://www.jcb.org/cgi/content/full/jcb.201110110/DC1

Table S1 demonstrates mass spectrometric analysis of immunopurified MYPT1 and is provided in an Excel file.

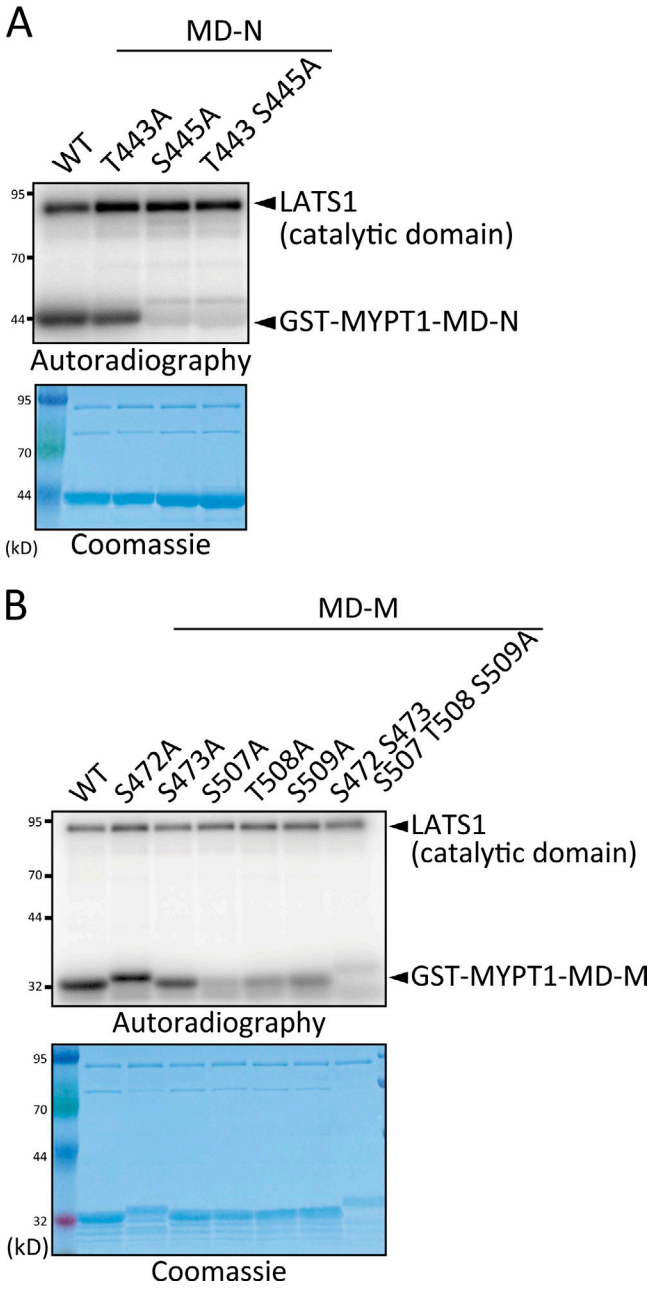

Figure S1. **Mapping of *LATS1* phosphorylation sites within MYPT1.** (A and B) 200 ng active GST-*LATS1* was incubated with the indicated GST-MD-N (A) or GST-MD-M (B) mutants for 30 min. Samples were then subjected to SDS-PAGE followed by autoradiography and Coomassie blue staining. WT, wild type.

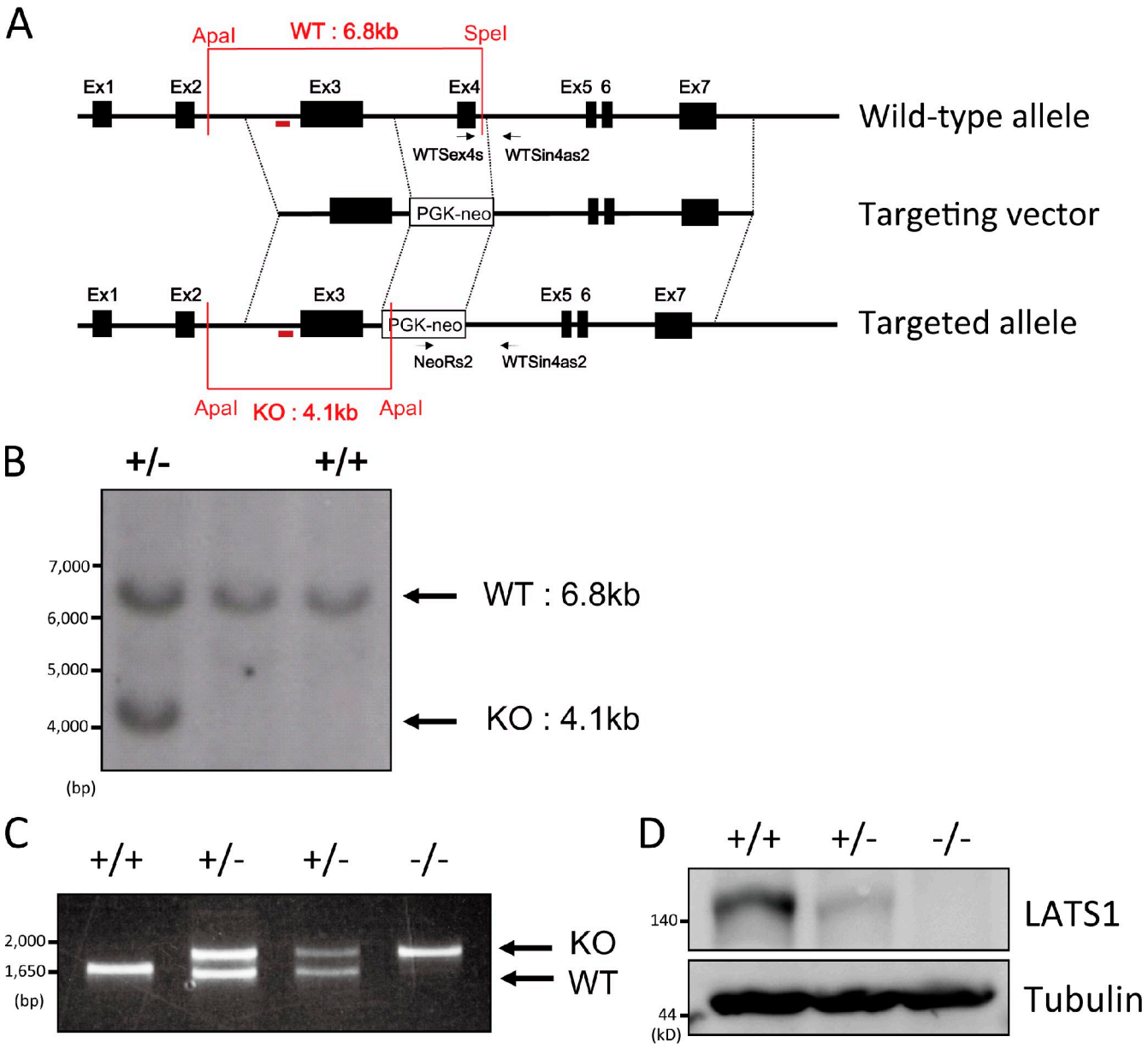

Figure S2. **Characterization of *LATS1* KO mice.** (A) Schematic representation of the wild-type (WT) *LATS1* locus and the targeted locus of C57BL/6J mice. The expected sizes of ApaI–SpeI fragments in Southern blot analysis using the indicated probe (bold red lines) are shown. The arrows indicate the positions of the primers used for genotyping. (B) Southern blot analysis of ApaI–SpeI double-digested DNA from embryos of the indicated genotypes using the probe shown in A. (C) PCR genotyping of embryos obtained from an intercross of *LATS1*$^{+/-}$ mice. (D) Immunoblot analysis of *LATS1* protein in MEFs of the indicated genotypes. The levels of α-tubulin were examined as a protein-loading control.

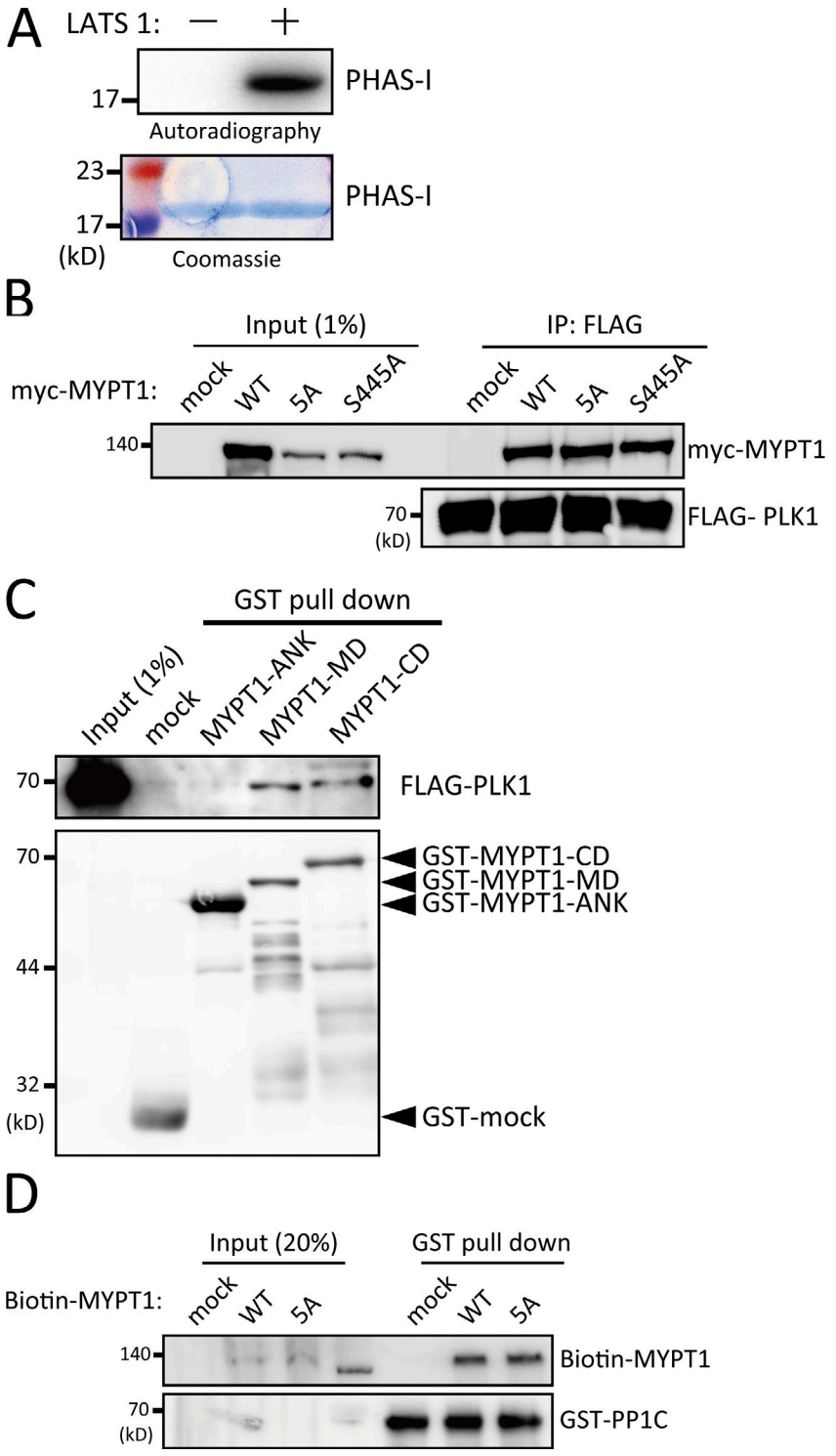

Figure S3. **Interaction between *LATS1* and MYPT1.** (A) In vitro kinase assay. 200 ng recombinant *LATS1* was incubated with 1 µg PHAS-I for 30 min at 30°C, and then, the sample was subjected to SDS-PAGE followed by autoradiography. SDS-PAGE gels were stained with Coomassie blue. (B) *LATS1* interacted with MYPT1 mutants. 293T cells were cotransfected with FLAG-tagged PLK1 expression vector and the indicated Myc-tagged expression vectors or mock vector and treated with Noc for 14 h. The lysates from the resultant cells were immunoprecipitated with the anti-FLAG antibody. The immunoprecipitates were analyzed by immunoblotting with the anti-Myc and anti-FLAG antibodies. (C) MYPT1 could be complexed with PLK1 via its C terminus. FLAG-PLK1 immunoprecipitated from 293T cells treated with Noc (14 h) was incubated with either GST alone, GST-MYPT1-ANK, GST-MYPT1-MD, or GST-MYPT1-CD, each (2 µg of protein) bound to glutathione–agarose beads. The bead-bound proteins (as well as 1% of input fraction) were then subjected to immunoblot analysis with anti-FLAG and anti-GST antibodies. (D) In vitro translated full-length MYPT1 (wild type [WT] or 5A) was incubated with GST-PP1C bound to glutathione–Sepharose beads. The bead-bound proteins (as well as 20% of input fraction) were then subjected to immunoblot analysis with anti-GST or the transcend tRNA chemiluminescent nonradioactive detection (Promega). IP, immunoprecipitation.

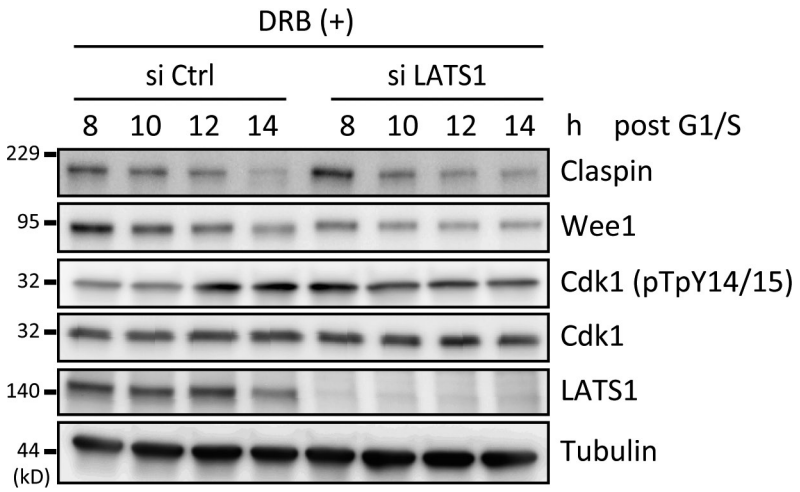

Figure S4. **G2 checkpoint defect in *LATS1*-depleted cells.** HeLa cells were synchronized at the G1/S boundary by a double-thymidine block. siRNA transfection was performed at the time of first release from the thymidine block. The transfected cells were pulsed with solvent or 0.5 μM DRB (1 h) 7 h after release from the G1/S boundary, subsequently cultured in complete medium containing Noc, and collected at the indicated times. The cells were then subjected to immunoblot analysis with the indicated antibodies. Ctrl, control.

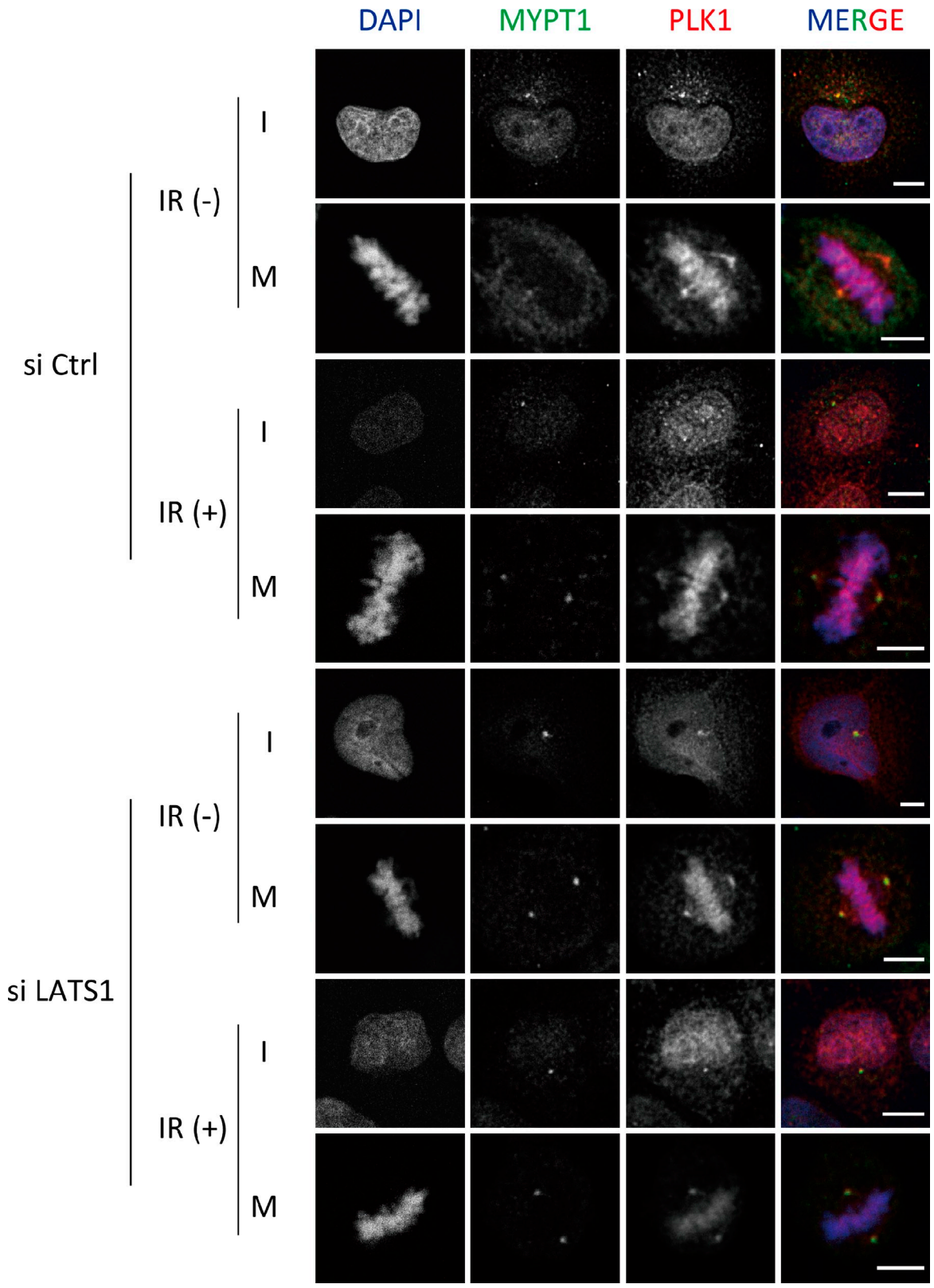

Figure S5. ***LATS1* had no effect on centrosomal localization of PLK1 and MYPT1.** HeLa cells were transfected with control (Ctrl) or *LATS1* siRNAs for 48 h, after which cells were irradiated at 10 Gy. 1 h after IR, cells were preextracted with microtubule-stabilizing buffer and fixed with cold methanol followed by incubation with anti-MYPT1 and anti-PLK1 antibodies. Immunofluorescence staining was performed with Alexa Fluor 448– or Alexa Fluor 555–conjugated secondary antibodies. DNA was stained by DAPI. Bars, 5 µm. I, interphase cells; M, mitotic cells.