Kinase assay for Rb by immune-purified endogenous CDK4/cyclin D1 from mice tissues. For endogenous kinase assays, the endogenous CDK4 was immunoprecipitated by 6 μg of anti-CDK4 Ab (Santa Cruz, sc-23896 AC) from 2.5 mg of lysates (buffer: 20 mM Tris-HCl pH 8.0, 0.1 M KCl, 5 mM $MgCl_2$, 10% glycerol, 0.1% Tween-20, 0.1% NP40) of livers or brains isolated from C57BL6 mice. The association of cyclin D1 was also confirmed by cyclin D1 antibodies (abcam, ab134175). The immunopurified endogenous CDK4/cyclin D1 was used as kinase, and 0.5 μg of Rb1 C-terminal recombinant protein (SC-4112) was used as the kinase substrate.

***In vivo* ubiquitination assays.** PC3 or HeLa cells with 80% confluence were transfected with His-ubiquitin and the indicated constructs. 36 hours post-transfection, cells were treated with 30 μM MG132 for 6 hours and lysed in buffer A (6 M guanidine-HCl, 0.1 M Na_2HPO_4/NaH_2PO_4, and 10 mM imidazole [pH 8.0]). After sonication, the lysates were incubated with nickel-nitrilotriacetic acid (Ni-NTA) beads (QIAGEN) for 3 hours at room temperature. Subsequently, the His pull-down products were washed twice with buffer A, twice with buffer A/TI (1 volume buffer A and 3 volumes buffer TI), and one time with buffer TI (25 mM Tris-HCl and 20 mM imidazole [pH 6.8]). The pull-down proteins were resolved by 2 × SDS-PAGE for immunoblotting.

Protein half-life assays. Cells were transfected or treated under indicated conditions. For half-life studies, cycloheximide (20 μg/ml, Sigma) was added to the medium. At indicated time points thereafter, cells were harvested and protein abundances were measured by immunoblot analysis.

Cell synchronization and FACS analyses. Cells synchronized with nocodazole arrest and double thymidine treatment as described previously[39]. Cells synchronized with nocodazole or double thymidine-arrest and release were collected at the indicated time points and stained with propidium iodide (Roche) according to the manufacturer's instructions. Cells were fixed by 70% ethanol at -20 °C overnight and washed 3 times using cold PBS. The samples were digested with RNase for 30 minutes at 37 °C and stained with propidium iodide (Roche) according to the manufacturer's instructions. Stained cells were sorted with BD FACSCanto™ II Flow Cytometer. The results were analyzed by ModFit LT 4.1 and FSC express 5 softwares.

BrdU/PI labelling and FACS analyses. Cells were incubated with/without BrdU (75 μM, Sigma) containing medium for 1 hour. Cells were harvested and washed once with cold PBS for centrifuge 5 min at 1200 rpm. Cells were re-suspended in 200 μl cold PBS and added in 5 ml of cold 90% ethanol for fixation overnight. After centrifuge 5 min at 1200 rpm, cells were washed once using 5 ml PBS and added in 0.5 ml 2N HCl-0.5% Triton X-100 for 30 min at room temperature (RT). After adding in 5 ml PBS, samples were centrifuged for 5 min at 1200 rpm and re-suspended in 1 ml Na2B4O7 (pH 8.5). Samples were re-suspended in 200 μl of anti-BrdU diluted (1: 40) in PBS with 0.5% tween 20 and 1% BSA and were incubated 30 min at room temperature. After adding in 5 ml 20 mM Hepes-PBS (pH7.4) with 0.5% tween 20, samples were centrifuged for 5 min at 1200 rpm and were re-suspended in 0.5 ml PBS with propidium iodide (PI, 5 μg/ml, Sigma) and RNAse A (200 μg/ml, Roche). After incubating 30 min at RT, samples were transfered into FACS tube and analyzed by flow cytometry.

Real-Time RT-PCR analyses. Total RNAs were extracted using the QIAGEN RNeasy mini kit, and reverse transcription reactions were performed using the ABI Taqman Reverse Transcription Reagents (N808-0234). After mixing the generated cDNA templates with primers/probes and ABI Taqman Fast Universal PCR Master Mix (4352042), reactions were performed with the ABI-7500 Fast Real-time PCR system and SYBR green qPCR Mastermix (600828) from Agilent Technologies Stratagene.

Human GAPDH: Forward, 5'-GGAGCGAGATCCCTCCAAAAT-3',
Reverse, 5'-GGCTGTTGTCATACTTCTCATGG-3';
Mouse GAPDH: Forward, 5'-AGGTCGGTGTGAACGGATTTG-3',
Reverse, 5'-GGGGTCGTTGATGGCAACA-3';
Human PD-L1: Forward, 5'-TGGCATTTGCTGAACGCATTT-3',
Reverse, 5'-TGCAGCCAGGTCTAATTGTTTT-3';
Mouse PD-L1: Forward, 5'-GCTCCAAAGGACTTGTACGTG-3',
Reverse, 5'-TGATCTGAAGGGCAGCATTTC-3';

Generation of *cyclin D*-deficient MEFs. *Cyclin* $D1^{-/-}$, $D2^{-/-}$, $D3^{-/-}$ and $D1^{F/F}D2^{-/-}D3^{F/F}$MEFs were derived from E13.5 mouse embryos as described previously[40,41].

Generation of mouse tumors. *Cyclin* $D1^{-/-}$ mice[42] were mated with MMTV-*c-Myc* or MMTV-*Wnt1* mice (from the Jackson Laboratory) yielding *cyclin* $D1^{-/-}$/MMTV-*c-Myc* or *cyclin* $D1^{-/-}$/MMTV-*Wnt1*, as well as control *cyclin* $D1^{+/+}$/MMTV-*c-Myc* or $D1^{+/+}$/MMTV-*Wnt1* mice. Mammary tumors were dissected from multiparous females and snap-frozen.

MMTV-*ErbB2* female mice (from the Jackson Laboratory), bred into a mixed C57BL/6 and 129Sv background, were treated with palbociclib or vehicle only for 6 weeks after detection of palpable tumors. Palbociclib was administered daily by gastric gavage (150 mg/kg of body weight); every two weeks the daily dose was lowered to 100mg/kg for 2–3 days. Control mice were treated with vehicle (10% 0.1N HCl, 10% Cremaphor EL, 20% PEG300, 60% 50 mM citrate buffer pH 4.5) 10 ml/kg by gastric gavage. After 6 weeks, tumors were collected and snap-frozen in OCT.

Treatment of wild-type mice with palbociclib. 6-weeks old C57BL/6 female mice (from the Jackson Laboratory) were treated with palbociclib (150 mg/kg body weight, by gastric gavage) or vehicle only for 7 days. Subsequently, organs were collected and analyzed by immunoblotting.

Mouse tumor implantation. 1 x 10^5 B16-F10 or 2 x 10^5 MC38 cells were injected subcutaneously into 6-weeks old C57BL/6 female mice (from the Jackson Laboratory). Starting one week later, mice were treated daily with palbociclib (150 mg/kg body weight, by gastric gavage) or vehicle only, for 7 days. Subsequently, tumors were collected and analyzed by FACS or immunoblotting.

1 x 10^5 B16-F10 cells stably expressing SPOP WT or F102C mutant were injected subcutaneously into 6-weeks old C57BL/6 female mice (from the Jackson Laboratory). On day 3 after tumor cells were injected, control and PD-L1 mAb treatments were conducted by intra-peritoneal injection (200 μg/mouse in 200 μl HBSS saline buffer) every three days for a total of 3 injections. Subsequently, tumors were collected and analyzed by FACS.

1 x 10^5 B16-F10 cells stably expressing SPOP WT or F102C mutant were injected subcutaneously into 6-weeks old *Tcrα*$^{-/-}$ female mice (from the Jackson Laboratory). After 10 days, tumors were collected and analyzed by FACS.

Immunofluorescence staining of cells or tumor tissues. MDA-MB-231 PD-L1 WT and KO cells were seeded in chambers (154534, Thermo Fisher Scientific). Cells were fixed with 4% paraformaldehyde for 20 minutes, followed with 0.1% Triton X-100 in PBS for 10 minutes. Cells were pre-blocked with 2% BSA/PBS for 45 minutes, then incubated with primary antibodies against PD-L1 (PD-L1, 298B.3G6, 1:200), for 2.5 hours at room temperature and followed with secondary anti-mouse antibodies conjugated with Alexa-fluor-568 (Invitrogen, 1:250). Hoechst (life technology, 1:10,000) was used to stain nuclei.

TFM-embedded 10 μM-thick tumor tissue sections were fixed with 2% paraformaldehyde/PBS for 30 min, and permeabilized in 0.1% Triton X-100/PBS for 10 min. Tumor tissue sections were pre-blocked with 2% BSA/PBS for 45 min, then incubated with primary antibodies against PD-L1 (1:200), CD3 (Abcam, 1:250) for 2.5 hours at room temperature and followed with secondary anti-mouse antibodies conjugated with Alexa-fluor-568 (Invitrogen, 1:250) and anti-rabbit antibodies conjugated with Alexa-fluor-488 (Invitrogen, 1:250). Hoechst (life technology, 1:10,000) was used to stain nucleus. Tumor tissues were mounted with fluoromount-G® (SouthernBiotech) at 4 °C overnight. Tissue sections were examined with fluorescent microscope under a 20 × objective lens. $CD3^+$ cell numbers were counted in an area of 5.95×10^5 μm^2.

Single cell generation from tumor tissue and flow cytometry analysis. Tumor tissues were minced and digested with 5 ml of 2 mg/ml collagenase (Sigma) in DMEM for 1 hour at 37 °C. Cells were then collected by centrifuge and filtered through a 70 μm strainer in DMEM. Cell pellets were suspended and lysed in red blood cell lysis buffer for 5 min. The cells were then filtered through a 40 μm strainer in 1 x PBS with 2% BSA. 1 million cells were incubated with antibodies against PD-L1 (564715, BD Biosciences, 1:100) conjugated with APC or antibodies against CD3 (Biolegend, 1:100) conjugated with APC or corresponding isotype IgG1 control at room temperature for 30 min. Cells were washed by 1 x PBS with 2% BSA and analyzed by flow cytometry.

***In vivo* experimental therapy in MC38 and CT26 mice tumor models.** Animal studies were approved by Dana-Farber Cancer Institute Institutional Animal Care and Use Committee (IACUC; protocol number 04-047), and performed in accordance with guidelines established by NIH Guide for the care and use of laboratory animals. MC38 or CT26 tumors were established by subcutaneously injecting 1×10^5 MC38 or CT26 tumor cells in 100 μl HBSS into the right flank of 6-week old C57BL/6 or BALB/c female mice (Jackson Lab, ME). Tumor sizes were measured every three days by caliper after implantation and tumor volume was calculated by length × width^2 × 0.5. On day 7 after tumor cells were injected, animals were pooled and randomly divided into four groups with comparable average tumor size. Moreover, the lab members who measured the mice were blinded to the treatment groups. Mice were grouped into control antibody treatment, PD-1 mAb treatment, CDK4/6 inhibitor treatment, and PD-1 mAb plus CDK4/6 inhibitor treatment. As illustrated in Extended Data Fig. 10a, control and PD-1 mAb treatments were conducted by intraperitoneal injection (200 μg/mouse in 200 μl HBSS saline buffer) every three days for a total of 8 injections. The CDK4/6 inhibitor treatment was given by oral gavage once a day with a dosage of 100 mg/kg for three weeks with a break every week for one day. For survival studies, animals were monitored for tumor volumes every three days for 120 days after initial treatment, until tumor volume exceeded 2000 mm^3, or until tumor had ulcer with diameter reached 1 cm. Statistical analysis was conducted using the GraphPad Prism software (GraphPad Software, Inc., San Diego, CA). Kaplan-Meier