METHODS

Cell culture, transfections and viral infections. HEK293T, HEK293, HeLa, MDA-MB-231, MCF7, Hs578T, WT MEFs, *cyclin D1*$^{-/-}$ MEFs, *cyclin D2*$^{-/-}$ MEFs, *cyclin D3*$^{-/-}$ MEFs, *cyclin D1*$^{-/-}$*D2*$^{-/-}$*D3*$^{-/-}$ MEFs, cyclin *D1*$^{fl/fl}$*D2*$^{-/-}$*D3*$^{fl/fl}$, *Cdk4*$^{+/+}$ and *Cdk4*$^{-/-}$ MEFs, *cyclin A1*$^{+/+}$*A2*$^{+/+}$ and *cyclin A1*$^{-/-}$*A2*$^{-/-}$ MEFs, *cyclin E1*$^{+/+}$*E2*$^{+/+}$ and *cyclin E1*$^{-/-}$*E2*$^{-/-}$, *Spop*$^{+/+}$ and *Spop*$^{-/-}$ MEFs (a kind gift of Dr. Nicholas Mitsiadesa, Baylor College of Medicine, Houston, TX) were cultured in DMEM medium supplemented with 10% FBS (Gibco), 100 units of *penicillin* and 100 μg/ml *streptomycin* (Gibco). HLF, HepG2, Huh1 and Huh7 were cultured in RPMI medium supplemented with 10% FBS. MDA-MB-231 PD-L1 WT and PD-L1 KO cells are kind gift from Dr. Mien-Chie Hung. BT549, T47D, ZR75-1, HCC1954, HCC1937, MDA-MB436, MDA-MB468 and SKBR3 cells were from Dr. Alex Toker laboratory at BIDMC, Harvard Medical School, and cultured in RPMI medium or McCoy's5A (Corning, NY) medium supplemented with 10% FBS. PC3, DU145, 22RV1, LNCaP and C42 were kind gifts from Dr. Pier Paolo Pandolfi group at BIDMC, Harvard Medical School, and cultured in RPMI medium (Corning, NY) with 10% FBS. Mouse tumor derived MC38 cell line was a kind gift from Dr. Arlene Sharpe at Harvard Medical School. Mouse tumor derived 4T1 and B16-F10 cell lines were routinely cultured in Gordon Freeman's laboratory in DMEM medium supplemented with 10% FBS (Gibco), 100 units of *penicillin* and 100 μg/ml *streptomycin* (Gibco). All cell lines were routinely tested to be negative for mycoplasma contamination.

Cells with 80% confluence were transfected using lipofectamine plus reagents in Opti-MEM medium (Invitrogen). 293FT cells were used for packaging of lentiviral and retroviral cDNA expressing viruses, as well as subsequent infection of various cell lines were performed. Briefly, medium with secreted viruses were collected twice at 48 hours and 72 hours after transfection. After filtering through 0.45 μM filters, viruses were used to infect cells in the presence of 4 μg/mL polybrene (Sigma-Aldrich). 48 hours post-infection, cells were split and selected using hygromycin B (200 μg/mL) or puromycin (1 μg/mL) for 3 days. Cells were harvested and lysed in EBC buffer (50 mM Tris pH 7.5, 120 mM NaCl, 0.5% NP40) supplemented with protease inhibitors (Roche) and phosphatase inhibitors (Calbiochem) for immunoblot analysis.

Reagents. Nocodazole (M1404) and Taxol (T7402) were purchased from Sigma. Thymidine (CAS: 50-89-5) and cycloheximide (66-81-9) were purchased from Acros organics. Palbociclib (PD0332991, S1116) and ribociclib (LEE011, S7440) were purchased from Selleckchem. MG132 (BML-PI102-0005) was purchased from Enzo life science. MLN4924 was a kind gift from Dr. William Kaelin (Dana-Farber cancer institute).

Plasmids. Myc-tagged Cullin 1, Cullin 2, Cullin 3, Cullin 4A, Cullin 4B, Cullin 5, Flag-tagged SPOP WT, Y87C, F102C, W131G, delta MATH, delta BTB, pLenti-HA-SPOP WT, Y87C, F102C, W131G, pGEX-4T-1-SPOP, Flag-Keap1, Flag-Cop1, shScramble, shCullin 3, shSPOP, and His-ubiquitin constructs were described previously[31]. shAR, shERG, shTrim24, shDEK and sgSPOP constructs were described previously[31,32]. Myc-Cullin 7 construct was kindly offered by Dr. James A. DeCaprio (Dana-Farber Cancer Institute). KLHL2 and KLHL3 constructs were generous gifts from Dr. Shinichi Uchida (Tokyo Medical and Dental University). KLHL12 and KLHL37 constructs were purchased from Addgene. KLHL20 construct was offered by Dr. Ruey-Hwa Chen (Institute of Biological Chemistry, Academia Sinica, Taiwan). The construct of HA-PD-L1 (HA tag in the N-terminus of PD-L1) was kindly provided by Dr. Mien-Chie Hung (The University of Texas MD Anderson Cancer Center). HA-Cdh1, HA-Cdc20, shCdh1, hCdc20 and HA-14-3-3 isoform constructs were described previously[33,34]. pCMV-CDK4 WT, pCMV-CDK4 N158F and shcyclin D3 were described previously[35,36]. pBabe-p16 was a kindly gift from Dr. Charles J. Sherr laboratory. pLKO-shCDK4 (Plasmid #78153 and #78154) and pMLP-shCDK6 (Plasmid #73552 and #73553) were purchased from Addgene. pLKO-sh14-3-3γ (TRCN0000078160, TRCN0000078161, TRCN0000078162), pLKO-shp16 (TRCN0000039748, TRCN0000039751, TRCN0000039782) and pLKO-shCD8a (TRCN0000057583, TRCN0000057587) were purchased from Open Biosystems. pcDNA3-PD-L1, pCMV-GST-PD-L1-tail (cytoplasmic amino acids), HA-PD-L1-ΔC-tail, HA-PD-L1-Δ283-290, HA-PD-L1-S283A, HA-PD-L1-S285A, HA-PD-L1-T290M, pLenti-PD-L1 WT, pLenti-PD-L1-Δ283-290, pLenti-PD-L1 T290M, pET-28a-His-SPOP WT, pET-28a-His-SPOP S6A, pET-28a-His-SPOP S22A, Flag-SPOP with delta D-Box (RxxL), pLenti-HA-c-Myc WT, pLenti-HA-c-Myc T58A/S62A, pLenti-HA-cyclin D1, pLenti-HA-cyclin D2, pLenti-HA-cyclin D3, Flag-SPOP S6A, HA-tagged CDK2, CDK4 and CDK6 were generated in this study.

Antibodies. Anti-PD-L1 (E1L3N) rabbit mAb (13684), anti-pS10-H3 (3377), anti-pS780-Rb (8180), anti-pS807/811-Rb (8516), anti-Rb (9309), anti-cyclin D1 (2978), anti-cyclin D2 (3741), anti-CDK6 (3136), anti-cullin 3 (2759), anti-GST (2625), rabbit polyclonal anti-Myc-Tag antibody (2278) and mouse monoclonal anti-Myc-Tag (2276) antibodies were purchased from Cell Signaling Technology. Mouse PD-L1 antibody (MAB90781-100) was purchased from R&D systems. Anti-mPD-L1 for immunoblotting (clone 298B.8E2), anti-mPD-L1 (clone 298B.3G6) for immunohistochemistry, and anti-human PD-L1 for immunoprecipitation (clone 29E.12B1) were generated in the laboratory of Dr. Gordon J. Freeman. Anti-CDK4 (MS-616-P1) was purchased from Thermo Scientific. Anti-SPOP (16750-1-AP) was purchased from Proteintech. Anti-cyclin A (sc-751), anti-cyclin B (sc-245), anti-cyclin E (SC-247), anti-cyclin D3 (sc-182), anti-Cdh1 (sc56312), anti-Cdc20 (sc-8358), anti-Cdc20 (sc-13162), anti-Plk1 (sc-17783), anti-TRIM24 (TIF1α, SC-271266), anti-HA (sc-805, Y-11), anti-PD-L1 (sc-50298) and anti-GST (sc-459) were obtained from Santa Cruz. Anti-GFP (8371-2) was purchased from Clontech. Anti-Flag (F-2425), anti-Flag (F-3165, clone M2), anti-Vinculin (V9131), anti-Flag agarose beads (A-2220), anti-HA agarose beads (A-2095), peroxidase-conjugated anti-mouse secondary antibody (A-4416) and peroxidase-conjugated anti-rabbit secondary antibody (A-4914) were purchased from Sigma. Anti-HA (MMS-101P) was obtained from BioLegend.

Immunoblot and immunoprecipitation analyses. Cells were lysed in EBC buffer (50 mM Tris pH 7.5, 120 mM NaCl, 0.5% NP-40) supplemented with protease inhibitors (Complete Mini, Roche) and phosphatase inhibitors (phosphatase inhibitor cocktail set I and II, Calbiochem). Protein concentrations were measured by the Beckman Coulter DU-800 spectrophotometer using the Bio-Rad protein assay reagent. Equal amounts of protein were resolved by SDS-PAGE and immunoblotted with indicated antibodies. For immunoprecipitations analysis, 1000 μg total cell lysates were incubated with the primary antibody-conjugated beads for 4 hours at 4 °C. The recovered immunocomplexes were washed four times with NETN buffer (20 mM Tris, pH 8.0, 100 mM NaCl, 1 mM EDTA and 0.5% NP-40) before being resolved by SDS-PAGE and immunoblotted with indicated antibodies.

Immunohistochemistry (IHC) for cell pellets, xenografted tumors or human prostate tumor specimens. The cultured cells (MDA-MB-231 PD-L1 WT and KO cells; HBP-ALL shScr and shCD8 cells; KE37 shScr and shCD8 cells) were washed and fixed in 4% paraformaldehyde for 20 minutes. Cells pellets or xenografted (MDA-MB-231 PD-L1 WT or KO) tumors were embedded into TFM and frozen. After cryostat sections (10 μM) were placed on Superfrost Plus Stain slides, samples were then permibilized in 0.1% Triton X-100/PBS for 10 minutes. For IHC analysis, we used UltraSensitive^{TM} SP (Mouse) IHC Kit (KIT-9701, Fuzhou Maixin Biotech) following the manufacturer's instructions with minor modification. The sections were incubated with 3% H_2O_2 for 15 min at room temperature to block endogenous peroxidase activity. After incubating in normal goat serum for 1 hour to block non-specific binding of IgG, sections were treated with primary antibody (PD-L1, 298B.3G6, 18 μg/ml; CD8α, sc-53212, clone C8/144B, dilution 1:40) at 4 °C overnight. Sections were then incubated for 30 minutes with biotinylated goat-anti-mouse IgG secondary antibodies (Fuzhou Maixin Biotech), followed by incubation with streptavidin-conjugated HRP (Fuzhou Maixin Biotech). Specific samples were developed with 3'3-diaminobenzidine (DAB-2031, Fuzhou Maixin Biotech). Images were taken using an Olympus microscopic camera and matched software.

The prostate tumor specimens were obtained from Shanghai Changhai Hospital in China. Usage of these specimens was approved by the Institute Review Board of Shanghai Changhai Hospital. For IHC, the paraformaldehyde fixed paraffin embedded prostate tumor samples were deparaffinized in xylene (3 x 10 min), rehydrated through a series of graded alcohols (100%, 95%, 85%, and 75%) to water. Samples were then subjected to heat-mediated antigen retrieval at 95 °C for 20 min. The following IHC steps were the same as described above.

The expression level of PD-L1 in prostate cancer tumor samples was determined according to the intensity of the staining as 0, negative; 1, weak expression; 2, intermediate expression and 3, strong expression. The numbers of intraepithelial CD8$^+$ tumor-infiltrating T lymphocytes (TILs) was counted as described in Hamanishi *et al*[37]. Briefly, three independent areas with the most abundant infiltration were selected under a microscopic field at 200 × magnification (0.0625mm^2). The number of intraepithelial CD8$^+$ TILs was counted manually and calculated as cells per mm^2. The Mann-Whitney test was used to compare the difference in PD-L1 expression between *SPOP* mutated and wide type cases. The Student's t test was used to determine *P* values of the difference in CD8$^+$ TILs between *SPOP* mutated and wide type cases. $P < 0.05$ was considered as significant.

***In vitro* cyclin D/CDK4 kinase assays.** Kinase assays were performed in a final volume of 30 μl of a kinase buffer as described previously[38]: 50 mM HEPES (pH 7.5), 10 mM $MgCl_2$, 1 mM DTT, 1 mM EGTA, 0.1 mM NaF, containing 10 μM ATP and 0.4 mCi [^{32}P]γATP (Perkin Elmer). 0.2 μg of CDK4/cyclin D1 (0142-0143-1, Pro-Qinase), CDK4/cyclin D2 (0142-0375-1, Pro-Qinase), or CDK4/cyclin D3 (0142-0373-1, Pro-Qinase) were used as kinases. 2 μg of His-SPOP, His-SPOP-S6A, His-SPOP-S222A, or His-SPOP-S6A/S222A mutant proteins immobilized on Ni-NTA beads were used as kinase substrates. 0.1 μg of Rb1 C-terminal recombinant protein (Cat. SC-4112, Santa Cruz) was used as a positive control for kinase assays. 2 μg of BSA was used as a negative control. After 60 min incubation at 30 °C, proteins were denatured, resolved on SDS-PAGE, transferred to nitrocellulose membranes and exposed to X-ray films.