significant upregulation of PD-L1 in all these cancer models, which was accompanied by a reduction in the number of infiltrating $CD3^+$ TILs (Fig. 2i-k; Extended Data Fig. 3a-c). We also observed that palbociclib treatment significantly elevated PD-L1 protein levels in various organs of normal mice (Extended Data Fig. 3d-h). Collectively, these results demonstrate that cyclin D-CDK4 kinase plays a rate-limiting role in regulating PD-L1 levels *in vivo*.

To understand how cyclin D-CDK4 regulates PD-L1 levels, we first determined that treatment of cells with proteasome inhibitor MG132, or with cullin-based ubiquitin E3 ligase inhibitor, MLN4924[22] elevated PD-L1 protein levels (Fig. 3a). To identify which cullin family E3 ligase(s) regulates PD-L1, we screened the potential interaction of PD-L1 with each cullin family proteins and found that Cullin 3, and to a lesser extent, Cullin 1 interacted with PD-L1 in cells (Fig. 3b, Extended Data Fig. 4a, b). These results indicate that in addition to Cullin 1/β-TRCP[11], Cullin 3-based E3 ligase(s) might play a role in regulating PD-L1 stability. Consistent with this notion, depletion of *Cullin 3* elevated the protein abundance of endogenous PD-L1 (Extended Data Fig. 4c).

Cullin 3-based E3 ubiquitin ligases recognize their downstream substrates through substrate-recruiting adaptor proteins[23]. We found that SPOP, but not other adaptor proteins examined interacted with PD-L1 in cells (Fig. 3c, d). We further determined that deletion of the C-tail, or the last eight amino acids of PD-L1 (283-290), disrupted binding of PD-L1 to SPOP, and rendered PD-L1 resistant to SPOP-meditated poly-ubiquitination (Extended Data Fig. 4d-h), indicating that the 283-290 region of PD-L1 might represent the potential binding motif for SPOP. Importantly, the cancer-derived PD-L1 T290M mutant (cBioPortal) located within the SPOP-binding motif also lost its ability to interact with SPOP and became more stable through decreased SPOP-mediated poly-ubiquitination and degradation (Extended Data Fig. 4i-l). Furthermore, depleting *SPOP* or deleting its substrate-interacting MATH domain elevated and stabilized PD-L1 in cells (Fig. 3e, f; Extended Data Fig. 5a-m). However, depleting known SPOP substrates including *AR*, *ERG*, *Trim24*, or *DEK* in *SPOP-WT* or *SPOP*$^{-/-}$ cells did not lead to obvious changes in PD-L1 levels (Extended Data Fig. 5n-u), arguing against a possibility of secondary effects for the observed elevation of PD-L1 upon *SPOP* depletion.

SPOP mutations occur in 10-15% of human prostate cancers, and are largely clustered within the MATH domain[24,25] (Extended Data Fig. 6a). Notably, these cancer-derived SPOP mutants failed to promote PD-L1 degradation due to their deficiency in binding to PD-L1 and promoting PD-L1 poly-ubiquitination (Fig. 3g-i and Extended Data Fig. 6b, c), which resembles the Elongin C-encoding *TCEB1* hotspot mutants in clear cell renal carcinoma, resulting in deficiencies in the ability of Cullin 2/Elongin B/C/VHL E3 ligase complex to promote HIF1α degradation 26. We also observed that mutations in the PD-L1 C-tail (degron) are mutually exclusive with mutations in the substrate-interacting MATH domain of SPOP (Extended Data Fig. 6d, e).

To further explore the impact of SPOP mutations on tumorigenesis, we generated tumor cell lines expressing SPOP-WT or cancer-derived mutants. We found that cells expressing cancer-derived SPOP mutants displayed elevated levels of endogenous PD-L1 protein, as compared to cells expressing SPOP-WT. (Fig. 3j and Extended Data Fig. 6f-j). Upon inoculation into immunoproficient mice, the growth of implanted tumors expressing cancer-derived SPOP-F102C was faster than tumors expressing SPOP-WT (Fig. 3k and Extended Data Fig. 6k). Tumors expressing cancer-derived SPOP-F102C mutant displayed elevated PD-L1 levels and significantly reduced numbers of $CD3^+$ TIL (Fig. 3l and Extended Data Fig. 6l). Strikingly, the difference in tumor weights between SPOP-WT and SPOP-F102C groups was largely alleviated after treatment with anti-PD-L1 antibody (Extended Data Fig. 6m-p), or when tumor cells were inoculated into T cell-deficient *Tcrα*$^{-/-}$ mice (Extended Data Fig. 6q-s). Hence, enhanced tumorigenic potential of SPOP-mutant cells is largely caused by elevated PD-L1 levels resulting in increased immune evasion.

We next explored whether loss-of-function SPOP mutations regulate PD-L1 levels or TILs in primary human prostate cancers. To this end, we identified 15 *SPOP*-mutant and 82 *SPOP*-wild type tumors through large-scale sequencing as described[27,28]. IHC staining results revealed that approximately 80% of *SPOP*-mutant tumors exhibited strong PD-L1 staining, while only approximately 10% of *SPOP*-WT tumors exhibited strong staining for PD-L1 and 70% of *SPOP*-WT cases displayed weak or no PD-L1 staining (Fig. 3m, n; Extended Data Fig. 7a-d). Moreover, the numbers of $CD8^+$ TILs were reduced in samples harboring SPOP mutations, as compared to SPOP-WT tumors (Fig. 3o; Extended Data Fig. 7e-h). These results indicate that SPOP-deficiency correlates with elevated PD-L1 protein abundance and decreased numbers of TILs in primary human prostate cancers.

We further found that SPOP protein abundance fluctuated during the cell cycle and displayed an inverse correlation with PD-L1 protein levels (Fig. 1a and Fig. 4a); depleting *SPOP* resulted in stabilization of PD-L1 across the cell cycle (Fig. 4a and Extended Data Fig. 8a). We noted that the Anaphase-Promoting Complex/Cyclosome (APC/C) E3 ligase adaptor protein Cdh1 displayed an inverse correlation with SPOP protein levels during cell cycle (Figs 1a, 1c and Fig. 4a). Furthermore, depletion of *Cdh1*, but not *Cdc20*, elevated SPOP protein abundance, which was accompanied by a simultaneous reduction in PD-L1 protein levels (Extended Data Fig. 8b, c). Consistent with these results, we detected a physical interaction between the endogenous SPOP and Cdh1 proteins (Fig. 4b and Extended Data Fig. 8d, e), and identified an evolutionarily conserved destruction-box motif (D-box: RxxLxxxxN)[29] in SPOP (Extended Data Fig. 8f). Deleting the D-box motif in SPOP disrupted its binding to Cdh1 and rendered SPOP resistant to Cdh1-mediated poly-ubiquitination and degradation (Fig. 4c, d; Extended Data Fig. 8g-i). Moreover, depletion of *Cdh1* led to SPOP stabilization, which subsequently resulted in a reduction in PD-L1 protein level during cell cycle progression (Fig. 4e). Taken together, these results indicate that Cdh1 is a physiologically important upstream E3 ligase responsible for negatively regulating SPOP protein stability.

To elucidate how the cyclin D-CDK4 kinase affects this mechanism, we established that cyclin D1-CDK4 directly phosphorylates SPOP at Ser6, but not Ser222, the only two conserved serine-proline sites in SPOP (Fig. 4f and Extended Data Fig. 9a-d). Conversely, treatment of cells with CDK4/6 inhibitor, palbociclib, reduced the phosphorylation of SPOP in cells (Extended Data Fig. 9e). We observed that 14-3-3γ protein physically interacted with SPOP in a pSer6-dependent manner and disrupted the interaction of SPOP with Cdh1 in cells (Fig. 4g, h; Extended Data Fig. 9f-h). Inhibition of SPOP-pSer6 decreased the interaction of SPOP with 14-3-3γ and increased its binding to Cdh1, leading to elevated SPOP poly-ubiquitination (Fig. 4i; Extended Data Fig. 9i-p). Consequently, palbociclib treatment decreased SPOP protein abundance and elevated PD-L1 levels in *SPOP*-WT, but not *SPOP*-deficient cells (Fig. 4j). Moreover, depletion of *14-3-3γ* dramatically upregulated PD-L1 levels and stabilized PD-L1 during cell cycle progression (Extended Data Fig. 9q-t).

Recent clinical studies revealed that the success of PD1/PD-L1 blockade correlates with PD-L1 expression levels in tumor cells[6,7]. Given our observation that inhibition of CDK4/6 elevated PD-L1 levels, we hypothesized that inhibitors of CDK4/6 kinase might synergize with anti-PD-1/PD-L1 therapy to elicit an enhanced therapeutic effect. Notably, we observed that treatment of immunoproficient mice bearing CT26 tumors with palbociclib plus anti-PD-1 antibody dramatically retarded tumor progression and resulted in 8 complete responses out of 12 treated mice (Fig. 4k; Extended Data Fig. 10a). Moreover, combining CDK4/6 inhibitor with anti-PD-1 therapy resulted in a significant improvement of overall survival compared to single-agent treated group (Fig. 4l). Similar results were obtained using mice bearing tumors derived from MC38 cells. (Extended Data Fig. 10b, c). As expected from our earlier observations, treatment of tumor-bearing mice with palbociclib decreased the absolute numbers of TILs, including $CD3^+$, $CD4^+$, $CD8^+$, Granzyme B^+ and $IFN\gamma^+$ cells. Importantly, addition of