Extended Data Figure 10 | Combination therapy of anti-PD-1 mAb and CDK4/6 inhibitor in MC38 colon cancer mouse model. a, A schematic model that illustrates the treatment plan for mice bearing subcutaneous MC38 tumors. Female C57BL/6 mice were implanted with 0.1×10^6 MC38 cells subcutaneously and treated with four arms: control antibody treatment, anti-PD-1 mAb treatment, CDK4/6 inhibitor treatment, anti-PD-1 mAb plus CDK4/6 inhibitor combination treatment. **b,** MC38 implanted tumor-bearing mice were enrolled in different treatment groups as indicated. Tumor volumes of mice treated with control antibody (n = 15), anti-PD-1 mAb (n = 15), the CDK4/6 inhibitor, palbociclib (n = 14) or combined therapy (n = 12) were measured every three days and plotted individually. We repeated this experiment twice. **c,** Kaplan-Meier survival curves for each treatment group demonstrate the improved efficacy of combining PD-1 mAb with the CDK4/6 inhibitor, palbociclib. $^*P < 0.05$. (Gehan-Breslow-Wilcoxo test). We repeated this experiment twice. **d, e, g, i,** The absolute number of $CD3^+$, $CD4^+$, $CD8^+$, Granzyme B^+, or $IFN\gamma^+$ TILs cells of implanted MC38 tumors treated with indicated agents was analyzed by FACS. Control: n = 8, palbociclib: n = 10, PD-1 Ab: n = 9, Palbociclib & PD-1 Ab: n = 8. **f, h, j,** The percentage of $CD4^+$, $CD8^+$ in $CD3^+$ TILs cells of implanted MC38 tumors treated with indicated agents was analyzed by FACS. Control: n = 8, palbociclib: n = 10, PD-1 Ab: n = 9, Palbociclib & PD-1 Ab: n = 8. **k,** A proposed working model to illustrate how PD-L1 protein stability is regulated by the cyclin D/CDK4-SPOP-Cdh1 signaling pathway. The cyclin D/CDK4 negatively regulates PD-L1 protein stability largely through phosphorylating its upstream physiological E3 ligase SPOP to promote SPOP binding with 14-3-3γ, which subsequently disrupts Cdh1-mediated destruction of SPOP. As such, CDK4/6 inhibitor treatment could unexpectedly elevate PD-L1 protein levels largely through inhibiting cyclin D/CDK4-mediated phosphorylation of SPOP to promote its degradation by APC/C^{Cdh1}. The unexpected rise of PD-L1 could present a severe clinical problem for patients receiving CDK4 inhibitor treatment and could be one of the underlying mechanisms accounting for CDK4 inhibitor resistance via evading immune surveillance checkpoint. Hence, our work provides a novel molecular mechanism as well as the rationale for the combinational treatment of PD-L1 blockage treatment and the CDK4/6 inhibitors as a more efficient anti-cancer clinical option. Error bars, ± s.d., two-tailed *t*-test, $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, NS: no significance.