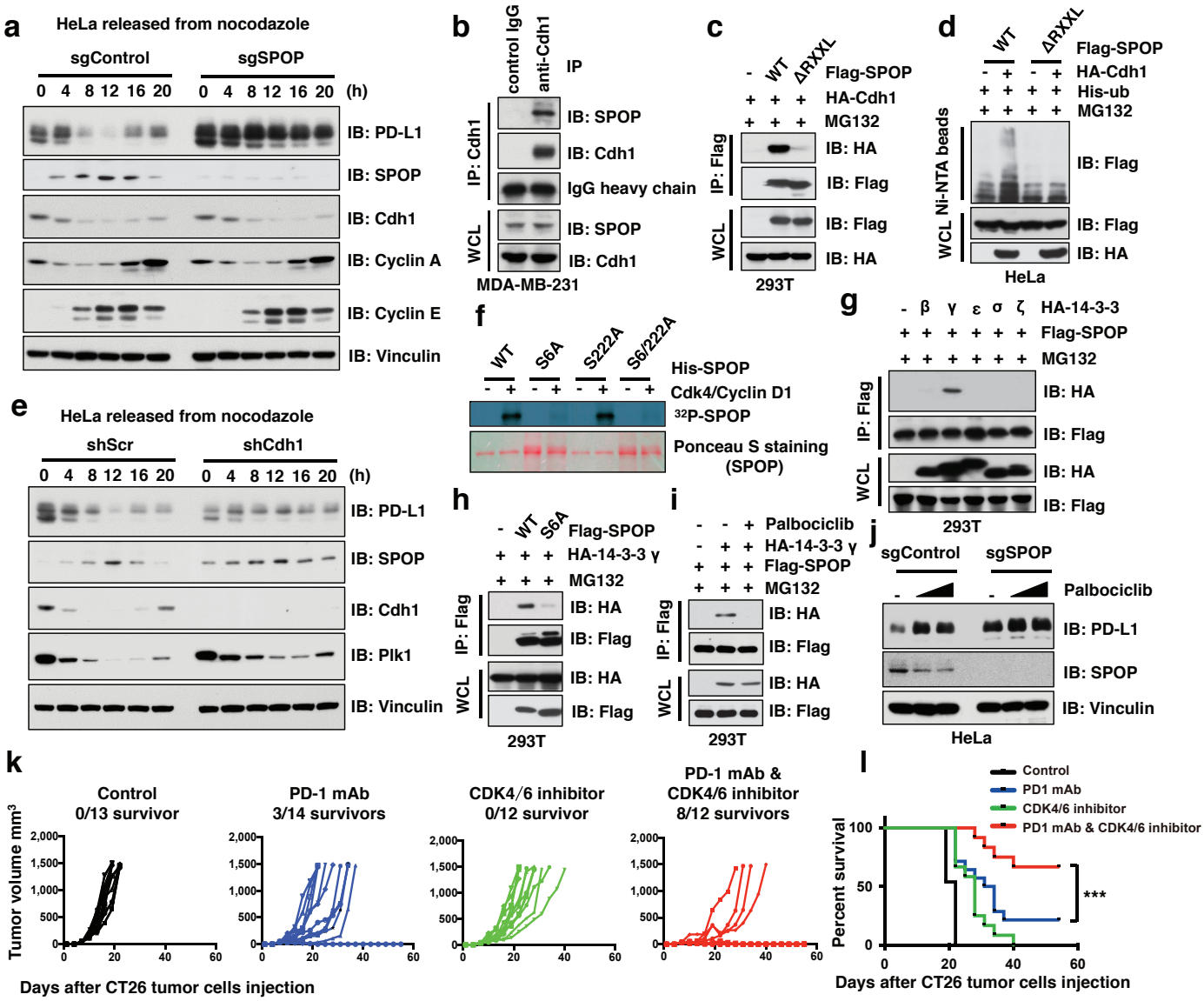

Figure 4 | Cyclin D-CDK4-mediated phosphorylation of SPOP stabilizes SPOP largely through recruiting 14-3-3γ to disrupt its binding with Cdh1. a-e, IB of WCL derived from HeLa cells with/without depletion of *SPOP* (**a**) or *Cdh1* (**e**) synchronized in M phase by nocodazole treatment prior to releasing for the indicated times, IP and WCL derived from MDA-MB-231 (**b**) or 293T (**c**) cells, or Ni-NTA pull-down products derived from HeLa cells transfected with the indicated constructs (**d**). Cells were treated with MG132 (30 μM) for 6 hours in **b**-**d**. **f,** *In vitro* kinase assays showing that cyclin D1/CDK4 phosphorylates recombinant SPOP at Ser6, not Ser222. **g-j,** IB analysis of IP and WCL derived from 293T cells transfected with indicated constructs and treated with MG132 (10 μM) or with/without palbociclib (1 μM) for 12 hours (**g**-**i**), or HeLa cells with/without depletion of *SPOP* treated with palbociclib (0.5, 1 μM) for 48 hours (**j**). **k,** CT26 implanted tumor-bearing mice were enrolled in different treatment groups as indicated. Tumor volumes of mice treated with control antibody (n = 13), anti-PD-1 mAb (n = 14), the CDK4/6 inhibitor, palbociclib (n = 12) or combined therapy (n = 12) were measured every three days and plotted individually. We repeated this experiment twice. **l,** Kaplan-Meier survival curves for each treatment group demonstrate the improved efficacy of combining PD-1 mAb with the CDK4/6 inhibitor, palbociclib. ***$P < 0.001$. (Gehan-Breslow-Wilcoxo test). We repeated this experiment twice.