Extended Data Figure 9 | Cyclin D/CDK4-mediated phosphorylation of SPOP at the Ser6 residue promotes its binding with 14-3-3γ to reduce its poly-ubiquitination and subsequent degradation by APC/Cdh1. **a,** A sequence comparison of conserved SP sites and putative 14-3-3γ binding motif in SPOP. **b,** Immunoblot (IB) analysis of whole cell lysates (WCL) and immunoprecipitation (IP) derived from 293T cells transfected with indicated constructs and treated with MG132 (10 μM) for 12 hours before harvesting. **c, d,** *In vitro* kinase assays with recombinant Rb and SPOP as substrates and cyclin D1/CDK4, cyclin D2/CDK4 and cyclin D3/CDK4 as kinase complex were performed. BSA was used as a negative control where indicated. **e,** IB analysis of WCL and immunoprecipitation (IP) derived from MDA-MB-231 cells transfected with indicated constructs, which were treated with/without palbociclib (1 μM) for 12 hours. **f,** Streptavidin beads pull-down assay for biotin-labeled SPOP peptide with/without phosphorylation at the Ser6 residue to examine its *in vitro* association with 14-3-3γ. **g,** IB analysis of WCL and GST pull-down precipitates derived from 293T cells transfected with indicated constructs and treated with MG132 (10 μM) for 12 hours before harvesting. **h, i,** IB analysis of WCL and IP derived from 293T cells transfected with indicated constructs and treated with MG132 (10 μM) for 12 hours before harvesting. **j, k,** IB analysis of WCL derived from 293T cells transfected with indicated constructs. 36 h post transfection, cells were treated with 20 μg/ml cycloheximide (CHX) as indicated time points **(j)**. The protein abundance of SPOP-WT and S6A mutant were quantified by the ImageJ software and plotted accordingly **(k)**. **l, p,** IB of WCL and Ni-NTA pull-down products derived from the lysates of PC3 cells transfected with the indicated constructs. Cells were treated with MG132 (30 μM) for 6 hours before harvesting and lysed in the denaturing buffer for following assay. **m-o,** IB analysis of WCL and IP derived from 293T cells transfected with indicated constructs and treated with MG132 (10 μM) and with/without palbociclib (1 μM) for 12 hours before harvesting. **q-s,** IB of WCLs derived from PC3, BT549 and HeLa cells stably expressing *sh14-3-3γ* as well as shScr as a negative control. **t,** IB of WCL derived from HeLa cells stably expressing shScr or sh*14-3-3γ* synchronized in M phase by nocodazole treatment prior to releasing back into the cell cycle for the indicated times.