Extended Data Figure 5 | SPOP negatively regulates PD-L1 protein stability in a poly-ubiquitination dependent manner. a-c, Immunoblot (IB) analysis of whole cell lysates (WCL) derived from 293T cells transfected with indicated constructs. **d, e,** 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) at indicated time points **(d)**. The PD-L1 protein abundance were quantified by the ImageJ software and plotted **(e)**. **f,** 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. **g,** A schematic illustration of SPOP with MATH and BTB domain to interact with substrate and Cullin 3, respectively. **h,** 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. **i** IB analysis of WCL derived from 293T cells transfected with indicated constructs. **j,** qRT-PCR analysis of relative mRNA levels of PD-L1 from *Spop*$^{+/+}$ and *Spop*$^{-/-}$ MEFs. Data were represented as mean ± s.d, n=5. **k,** IB analysis of WCL derived from PC3 cells infected with indicated lentiviral shRNAs against *SPOP* and selected with puromycin (1 μg/ml) for 72 hours before harvesting. **l-m,** IB analysis of WCL derived from C42 cells with depletion of *SPOP* using sgRNA and treated with cycloheximide (CHX) for indicated time points before harvesting **(l)**. The PD-L1 protein abundance were quantified by the ImageJ software and plotted **(m)**. **n, o,** IB analysis of WCL derived from LNCaP cells stably expressing shAR or shERG as well as shScr as a negative control. **p, q,** IB analysis of WCL derived from DU145 cells stably expressing shTrim24 or shDEK as well as shScr as a negative control. **r-u,** IB analysis of WCL derived from C42 *SPOP WT* and *SPOP*$^{-/-}$ cells that stably expressed shAR, shERG, shTrim24, or shDEK as well as shScr, respectively.