Figure 3 | Cullin 3^SPOP is the physiological E3 ubiquitin ligase for PD-L1. a-d, IB analysis of WCL derived from C4-2 cells treated with MG132 (10 μM) or MLN4924 (1 μM) for 12 hours (**a**), immunoprecipitates (IP) and WCL derived from 293T cells transfected with indicated constructs (**b, d**), or anti-PD-L1 IP and WCL derived from PC3 cells (**e**). Cells were treated with MG132 (10 μM) for 12 hours in **b, c. e-g,** IB analysis of WCL derived from *Spop*^+/+ versus *Spop*^−/− MEFs (**e**), C4-2 cells depleted *SPOP* with sgRNAs (**f**), or C4-2 cells stably expressing indicated SPOP WT and mutants (**g**). **h, i,** IB analysis of IP and WCL derived from 293T cells (**h**), or Ni-NTA pull-down products derived from PC3 cells transfected with indicated constructs and treated with 30 μM MG132 for 6 hours.

j-l, FACS analysis for PD-L1 (**j**) or CD3^+ T-cell population (**l**) of the B16-F10 implanted tumors ectopically expressing SPOP-WT or F102C mutant (n = 6 mice each group). Tumor weight were recorded at the time of sacrifice (**k**) (n = 5 mice each group). **m,** Representative images of PD-L1 and CD8 immunohistochemistry (IHC) staining in *SPOP* wild-type or mutant primary human prostate cancer samples. The scale bar: 400 μm or 100 μm. **n, o** Quantification of IHC analysis for PD-L1 (**n**) and CD8^+ T cells (**o**) in *SPOP* wild-type versus mutant human prostate tumor specimens. (n = 15 for *SPOP* mutant, n = 82 for *SPOP* WT). Error bars, ± s.d., two-tailed *t*-test, except (**n**) Mann-Whitney test, *P < 0.05, **P < 0.01, ***P < 0.001.