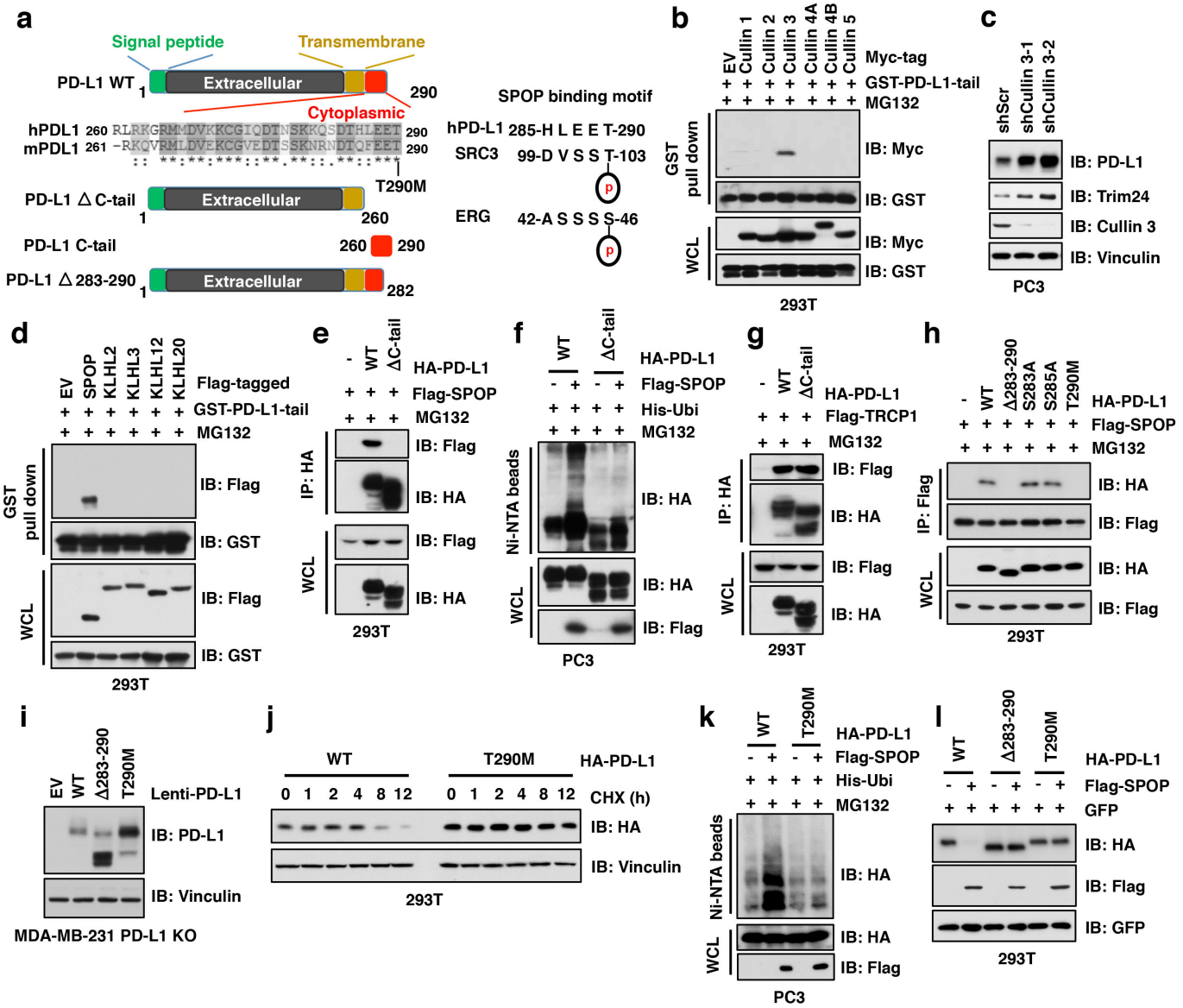

Extended Data Figure 4 | Cullin 3^{SPOP} promotes PD-L1 ubiquitination and subsequent degradation largely through interaction with the cytoplasmic tail of PD-L1. a, A schematic illustration of PD-L1 with N-terminal signal peptide, extracellular domain, trans-membrane domain, cytoplasmic tail and the potential SPOP-binding motif in PD-L1. **b, d,** Immunoblot (IB) analysis of whole cell lysates (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. **c,** IB analysis of WCL derived from PC3 stably expressing shCullin 3. **e, g,** IB analysis of WCL and immunoprecipitation (IP) derived from 293T cells transfected with indicated constructs and treated with MG132 (10 μM) for 12 hours before harvesting. **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 denature buffer. **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 of WCL derived from MDA-MB-231 *PD-L1* KO cells stably expressing PD-L1 WT, delta 283-290, T290M as well as EV as a negative control. **j,** IB analysis of WCL derived from 293T cells transfected with HA-PD-L1 WT and the T290M mutant, which were treated with cycloheximide (CHX) for indicated time points before harvesting. **k,** 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. **l,** IB of WCL derived from 293T cells transfected with indicated constructs.