

Fig. 2 Western Blot Images showing POI degradation by PROTAC-POI

- Images showing POI protein level measured by western blot assay after 24-h treatment with PROTAC-POI at concentrations starting from 3333 nM and 1:3 serial dilution in OCI-LY10 cells.
- Degradation Curve of POI. Relative POI level was quantized using Image J software and normalized to both DMSO and cyclophilin A (a loading control). Then, DC50 was determined using the nonlinear regression dose-response equation in GraphPad Prism.

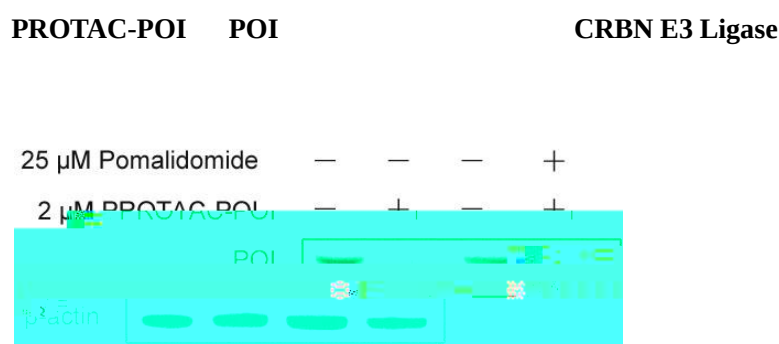


Fig. 3 CUL4-CRBN E3 Ligase mediates PROTAC-POI-induced POI Degradation

CRBN inhibitor pomalidomide for 1 h, then incubated with the combination of PROTAC-POI and pomalidomide for another 24 h. Cells were collected and subjected to western blot assay. β -actin was used as a loading control.

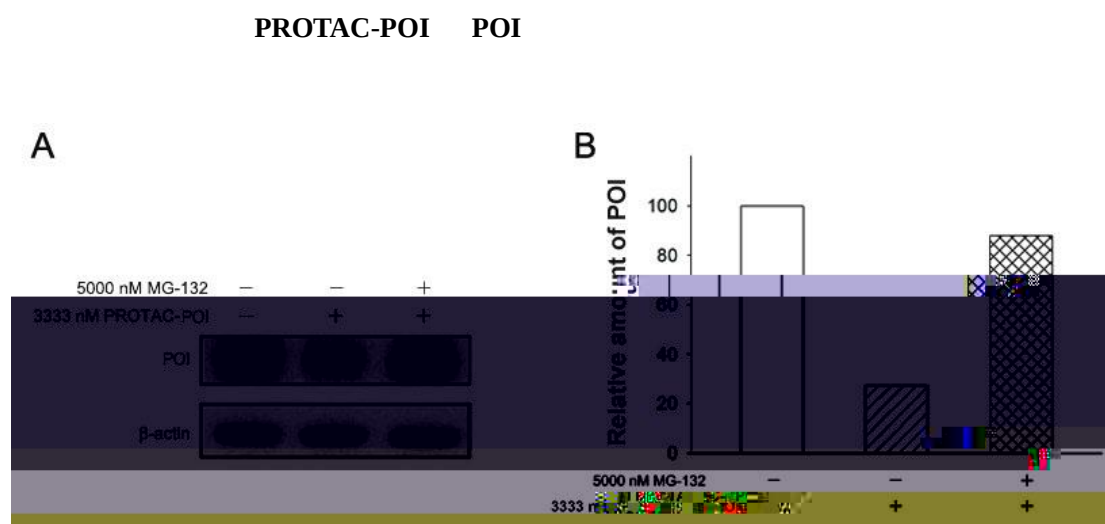


Fig. 4 Proteasome system mediates PROTAC-POI-induced POI Degradation

- Western Blot images showing POI level after 1- and then 24-h treatment with PROTAC-POI in HEK 293T cells. β -actin was used as a loading control.

B. Bar chart shows quantized POI level. Protein level in (A) was quantified by using -actin loading control.

PROTAC-POI

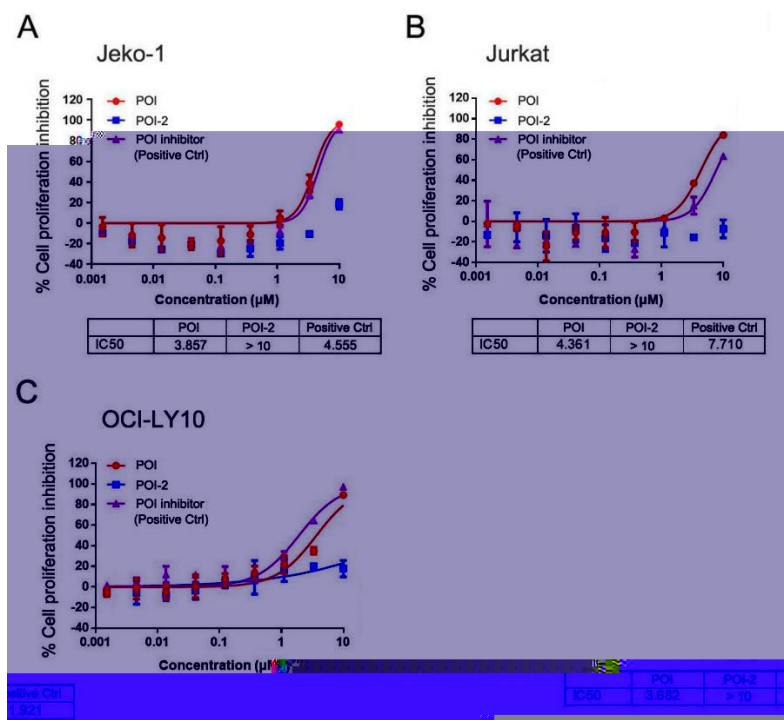


Fig. 5 Proliferation inhibition effects of POI in the indicated cell lines at 120 h after treatment

The cells were treated with the indicated three types of PROTAC molecules for 120 h -fold serial dilution covering 9 different doses. Proliferation inhibition was examined by using the nonlinear regression dose-response equation in GraphPad Prism.

1. A-EGFP Western

Blot POI PROTAC-POI POI
2. POI-EGFP IC50

26 nM; Western Blot PROTAC-POI POI DC50 28.9

nM HEK293T OE POI-EGFP PROTAC-POI POI

HEK293T OE POI-EGFP PROTAC-POI

3.	CRBN	Pomalidomide	PROTAC-POI	POI
	CUL4-DDB1-CRBN E3	PROTAC-POI	POI	
4.		MG-132	PROTAC-POI	POI
	PROTAC-POI		POI	
5.	PROTAC-POI	IC50	POI	
1.	HEK293T POI-EGFP		PROTAC-POI	;
2.	PROTAC-POI	CUL4-DDB1-CRBN E3	-	POI
			POI	