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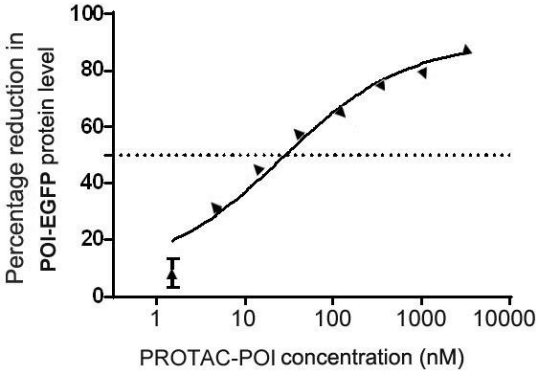
实验报告：PROTAC-POI 体外筛选

以某目标蛋白（protein of interest, POI）为例，评价“内源蛋白-EGFP”293T 稳转株细胞高通量筛选系统对 PROTAC-POI 分子的筛选结果。

首先，利用“POI-EGFP”HEK 293T 细胞稳转株初步检测 PROTAC-POI 对细胞中 POI 蛋白水平的下调情况



	PROTAC-POI
IC50	26 nM

Fig. 1 Percentage reduction in POI-EGFP fusion protein level

HEK 293T cells stably expressing POI-EGFP fusion protein were incubated with PROTAC-POI for 24 h at the concentrations starting from 3333 nM and 1:3 serial dilution. DMSO was used as a solvent control. After lysis of cells, the relative amount of POI-EGFP fusion protein was measured by using Molecular Devices M5 (excitation 488 nm, emission 509 nm). DC50 was determined using the nonlinear regression dose-response equation in GraphPad Prism.

之后，Western Blot 进一步验证 PROTAC-POI 对细胞中 POI 蛋白水平的下调能力

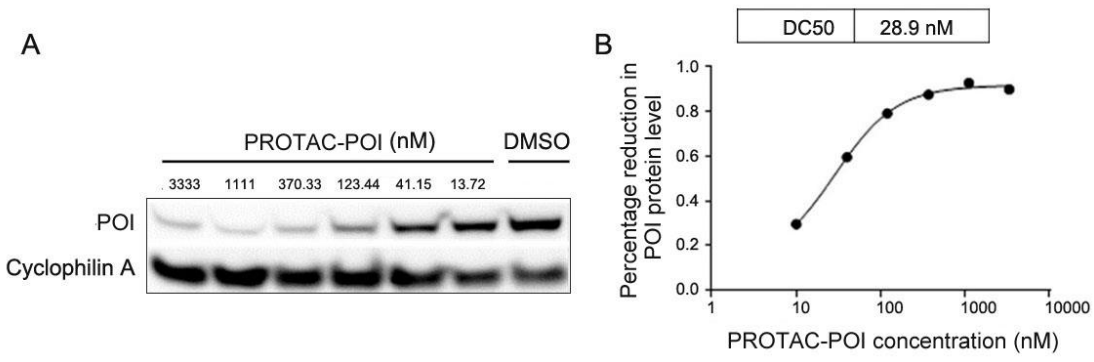


Fig. 2 Western Blot Images showing POI degradation by PROTAC-POI

A. 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.

B. 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.

机制检测 Ⅰ：分析 PROTAC-POI 对 POI 蛋白水平的下调是否由 CRBN E3 Ligase 介导？

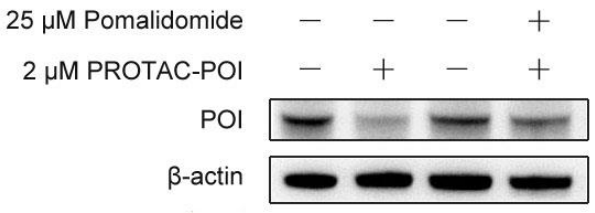


Fig. 3 CUL4-CRBN E3 Ligase mediates PROTAC-POI-induced POI Degradation

HEK293T cells were pretreated with 25 μM of 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.

机制检测 Ⅱ：分析 PROTAC-POI 对 POI 蛋白水平的下调是否通过蛋白酶体系统降解途径？

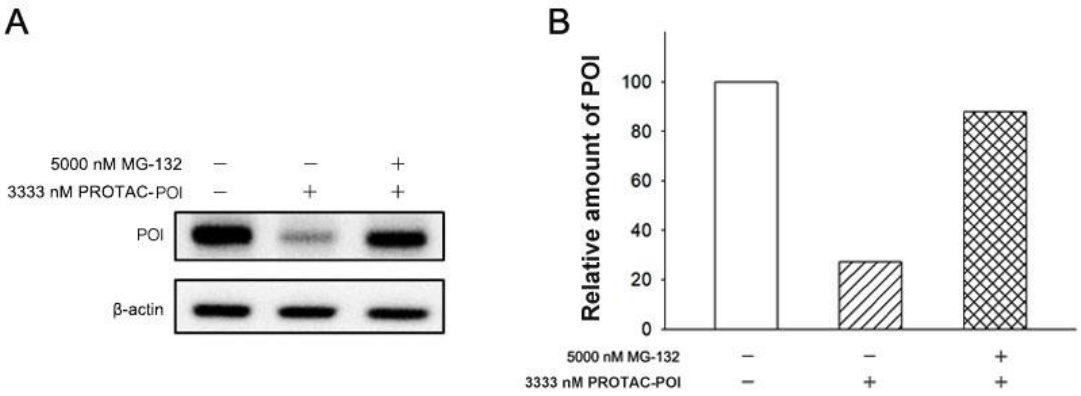
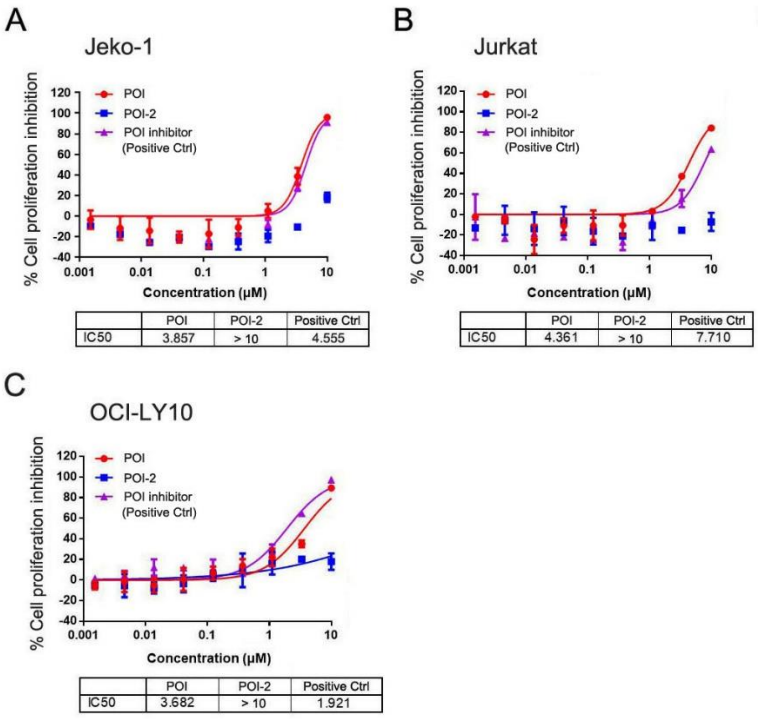


Fig. 4 Proteasome system mediates PROTAC-POI-induced POI Degradation

A. Western Blot images showing POI level after 1-h pretreatment with 5 μM of MG132 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 image J software and normalized by DMSO and β-actin loading control.

最后，检测 PROTAC-POI 对肿瘤细胞的增殖抑制效应



	POI	POI-2	Positive Ctrl
IC50	3.857	> 10	4.555

	POI	POI-2	Positive Ctrl
IC50	4.361	> 10	7.710

	POI	POI-2	Positive Ctrl
IC50	3.682	> 10	1.921

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 respectively, at the concentrations starting with 10 μM and 3-fold serial dilution covering 9 different doses. Proliferation inhibition was examined by using the nonlinear regression dose-response equation in GraphPad Prism.

实验结果及分析

1. 上述实验结果表明，无论通过读荧光值检测 A-EGFP 融合蛋白水平还是通过 Western Blot 法分析 POI 蛋白水平，都检测到 PROTAC-POI 分子对 POI 蛋白的降解。
2. POI-EGFP 融合蛋白荧光值检测，发现 PROTAC-POI 对 POI 蛋白水平抑制的 IC50 值约为 26 nM; Western Blot 法检测到 PROTAC-POI 对 POI 蛋白的 DC50 值约为 28.9 nM。可见，利用 HEK293T OE POI-EGFP 细胞稳转株检测 PROTAC-POI 对 POI 蛋白的降解情况，结果可靠。故，HEK293T OE POI-EGFP 细胞稳转株可用于 PROTAC-POI

的高通量筛选。

3. 利用 CRBN 抑制剂 Pomalidomide，明显抑制了 PROTAC-POI 对 POI 降解。此数据表明 CUL4-DDB1-CRBN E3 介导了 PROTAC-POI 分子对细胞中 POI 蛋白的降解。
4. 利用蛋白酶体抑制剂 MG-132 抑制蛋白酶体功能，则显著抑制了 PROTAC-POI 对 POI 降解。这说明 PROTAC-POI 通过蛋白酶体系统对 POI 进行降解。
5. PROTAC-POI 对肿瘤细胞增殖抑制的 IC50 值，与 POI 抑制剂相当。

实验结论

1. HEK293T POI-EGFP 稳转株细胞可用于高通量筛选 PROTAC-POI 分子;

2. PROTAC-POI 分子通过 CUL4-DDB1-CRBN E3 泛素-蛋白酶体系统靶向降解细胞中 POI 蛋白，由此抑制肿瘤细胞增殖，且其抑制效果可达到 POI 小分子抑制剂水平。