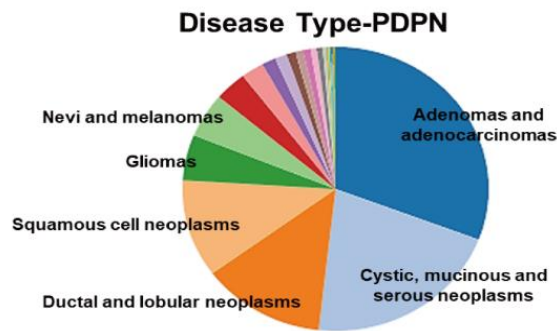
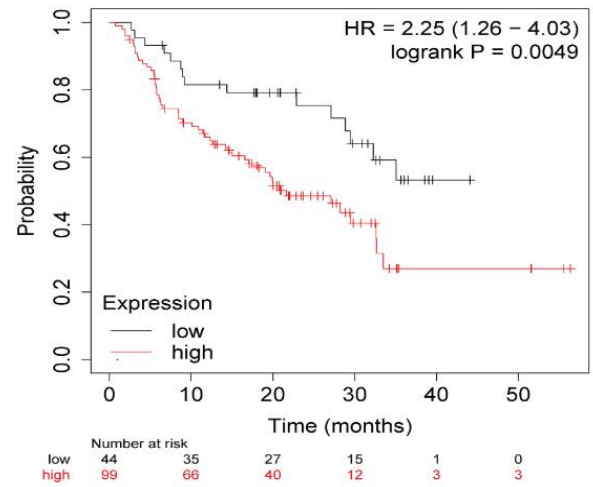


Fig. S1

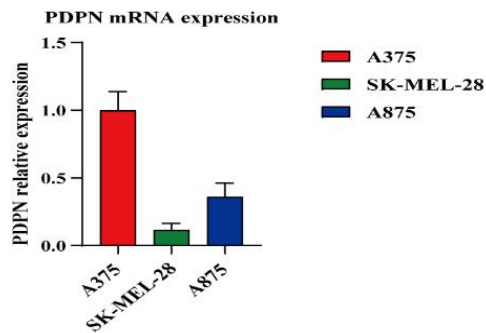
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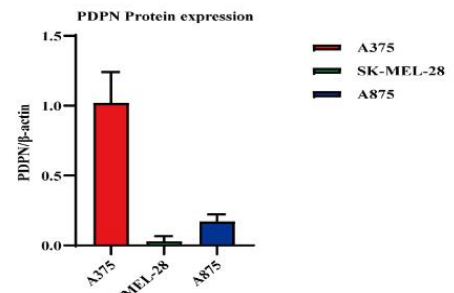
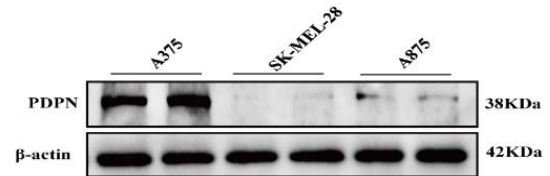
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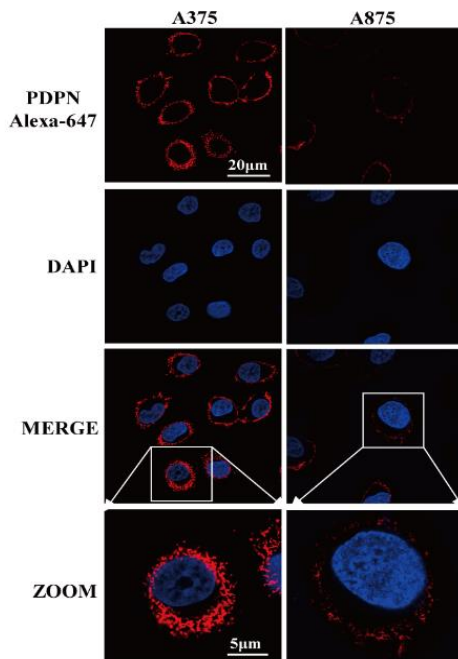
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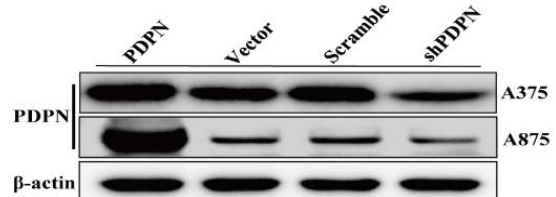
D



E



F



G

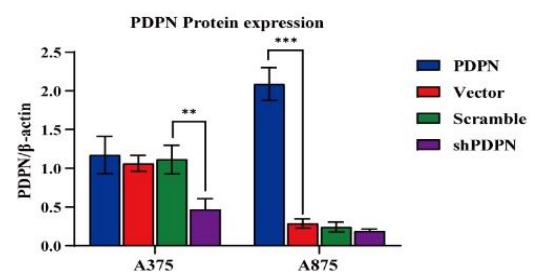


Fig. S2

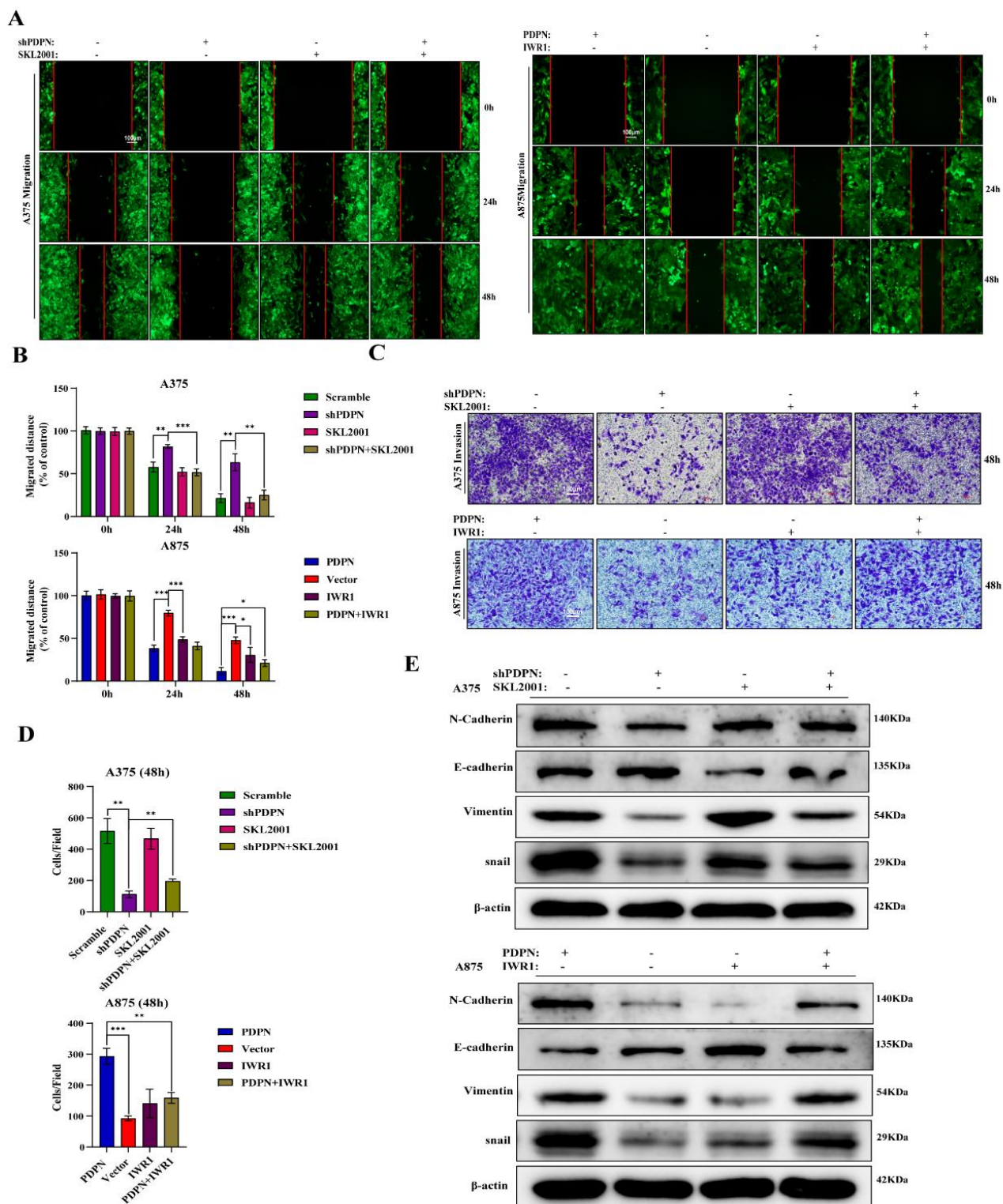


Fig. S3

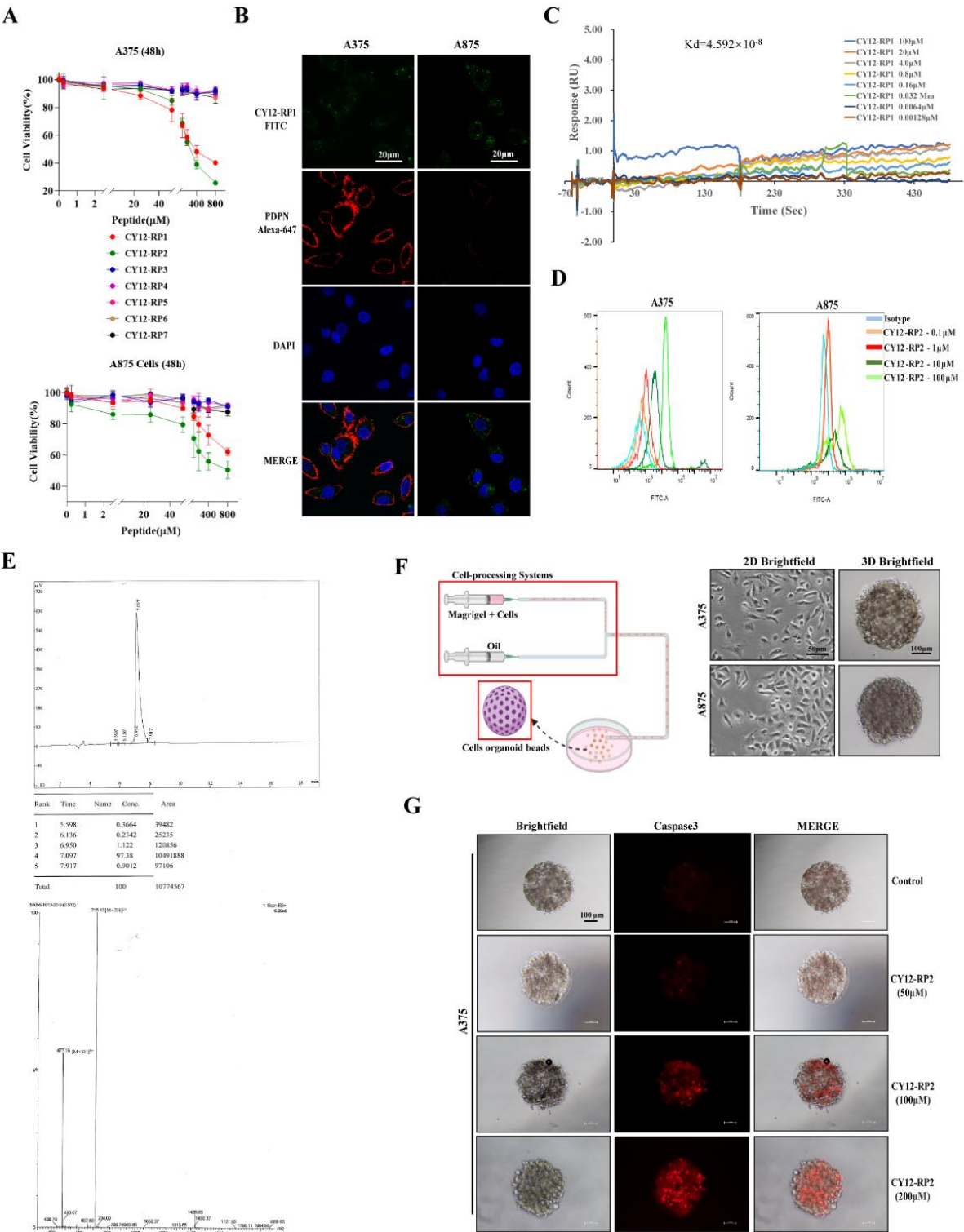
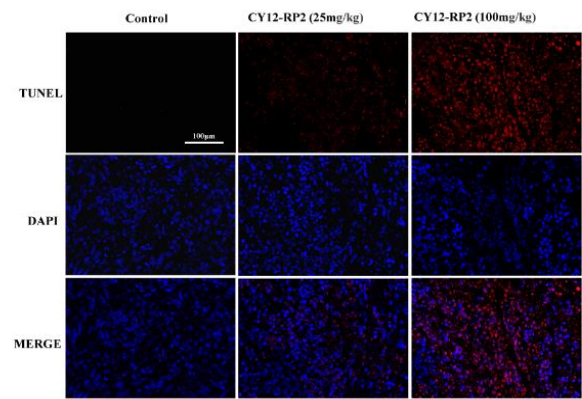
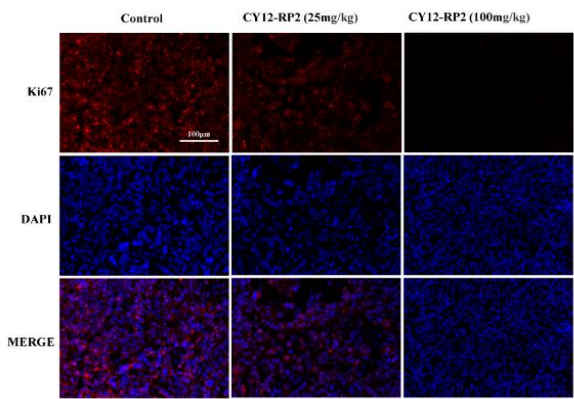


Fig. S4

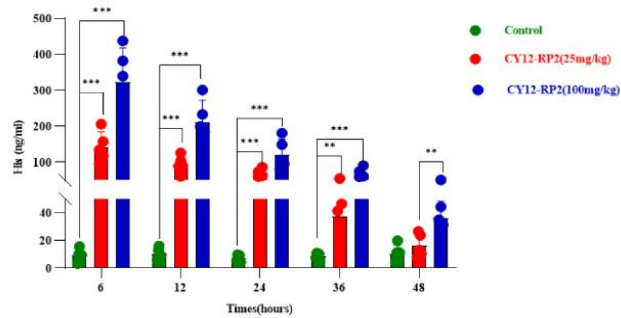
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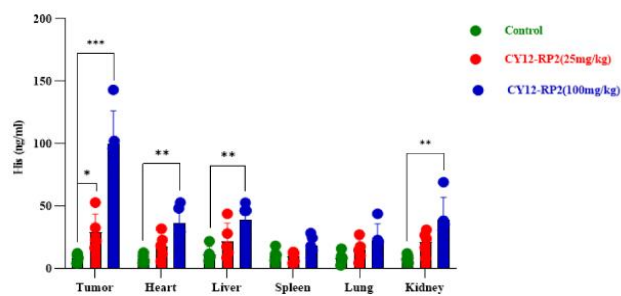
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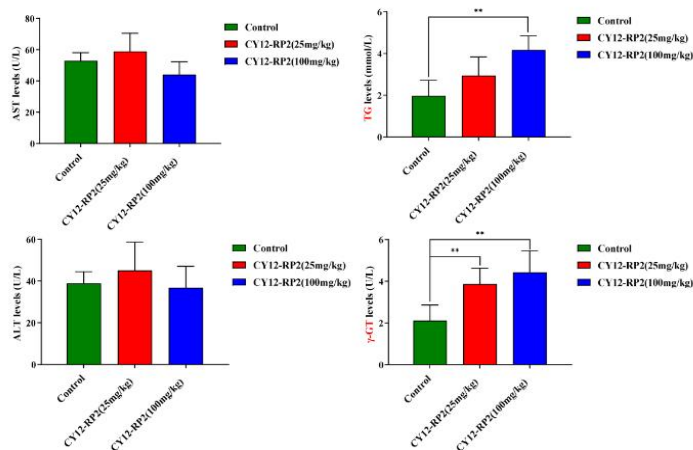
C



D



E



F

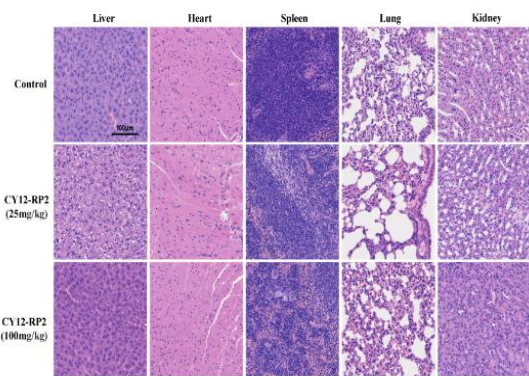


Fig. S5

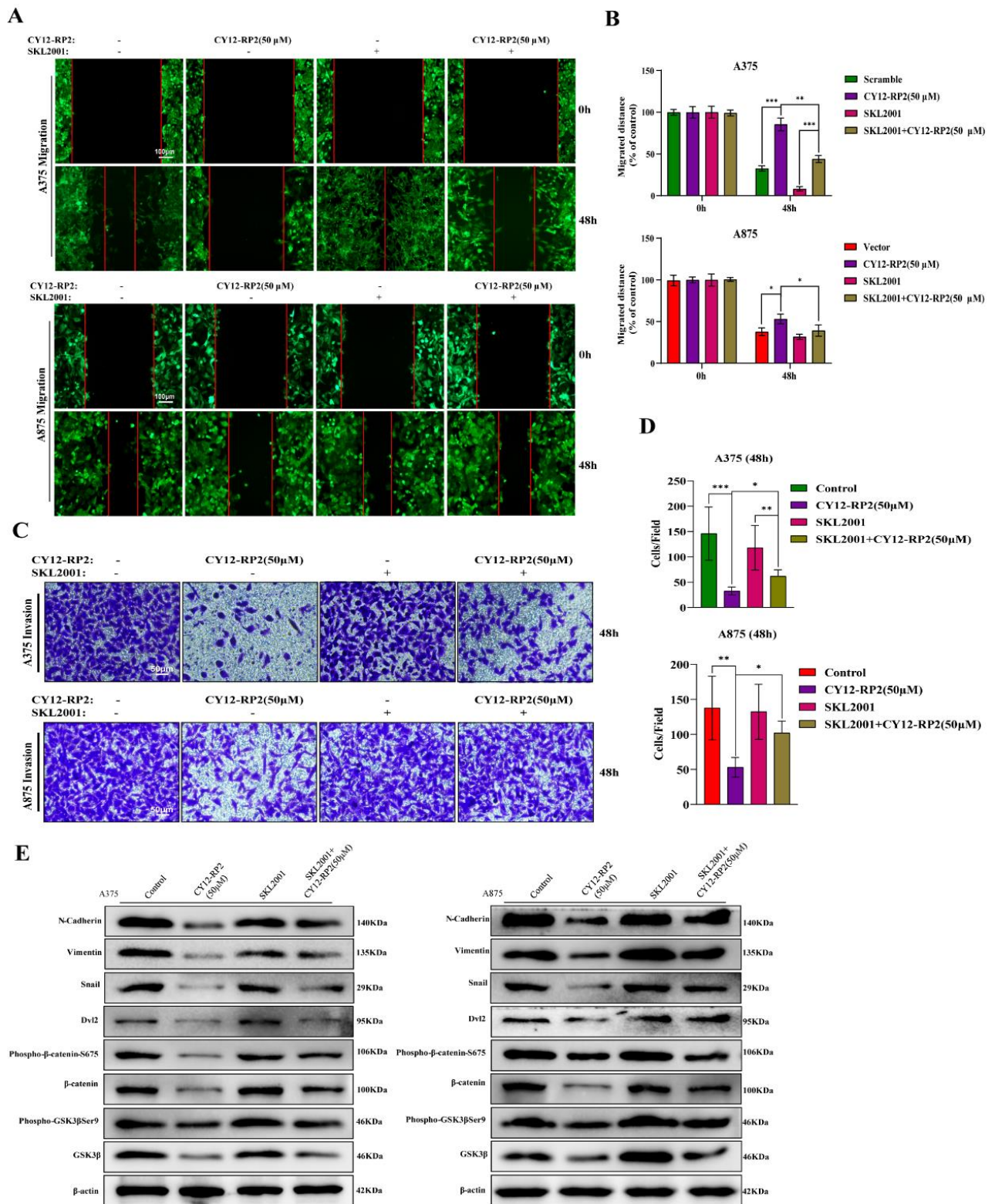
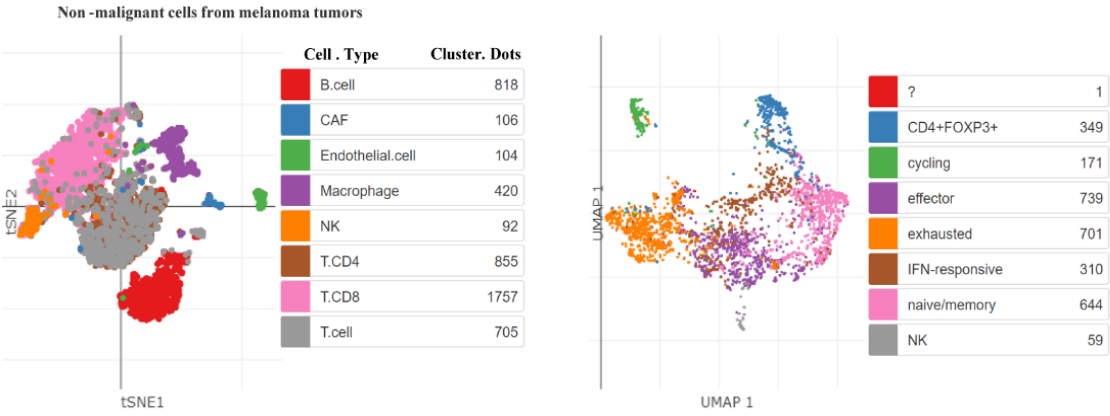
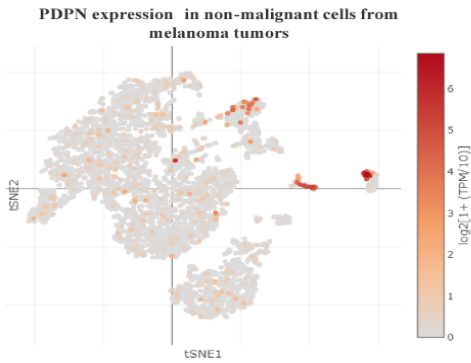


Fig. S6

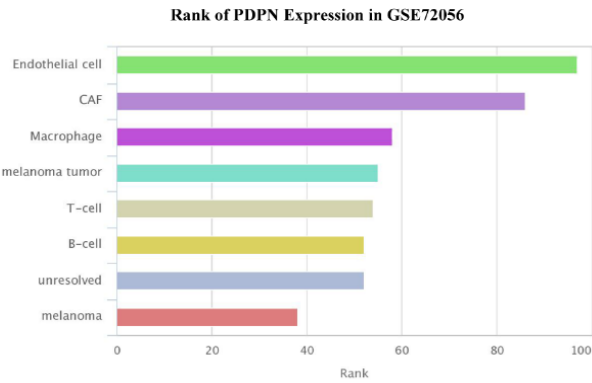
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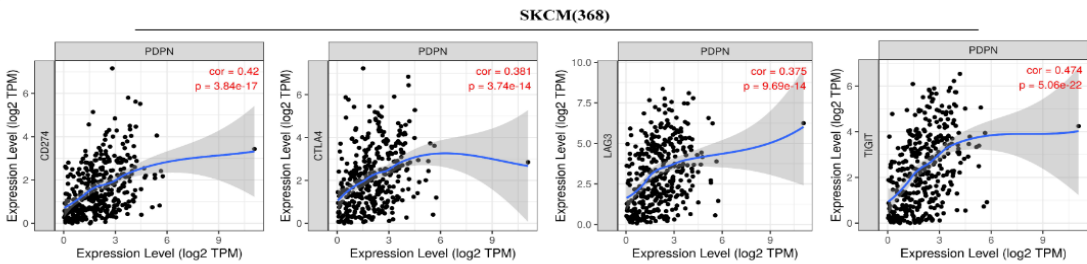
B



C



D

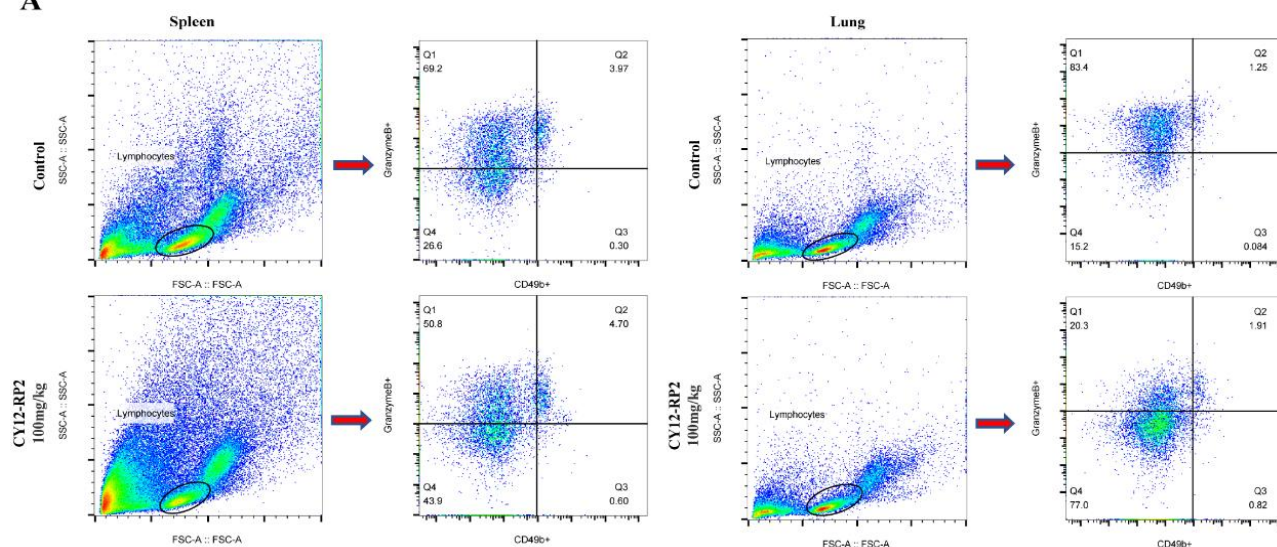


E

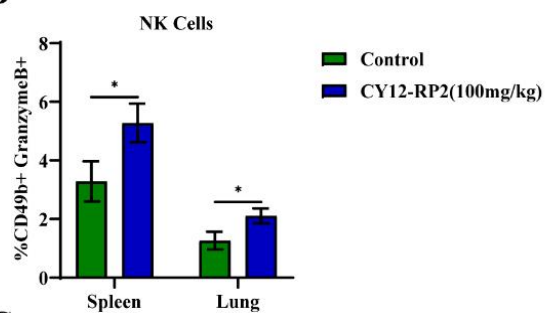
Cancer	CD8+ T cells	CD4+ T cells	M1 Macrophage	M2 Macrophage	Treg cells	NK cells
SKCM (n=471)	-2.988	-0.209	-5.524	6.865	1.457	-2.557
SKCM -Metastasis (n=368)	-2.464	0.03	-5.512	6.32	1.582	-2.161
SKCM -Primary (n=103)	-1.488	-0.05	-0.563	1.691	-0.768	-0.468

Fig. S7

A



B



C

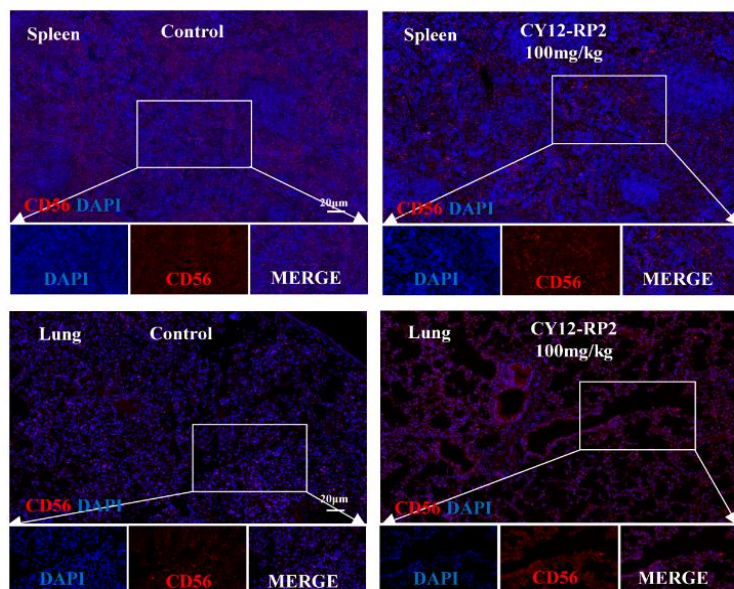


Fig. S8

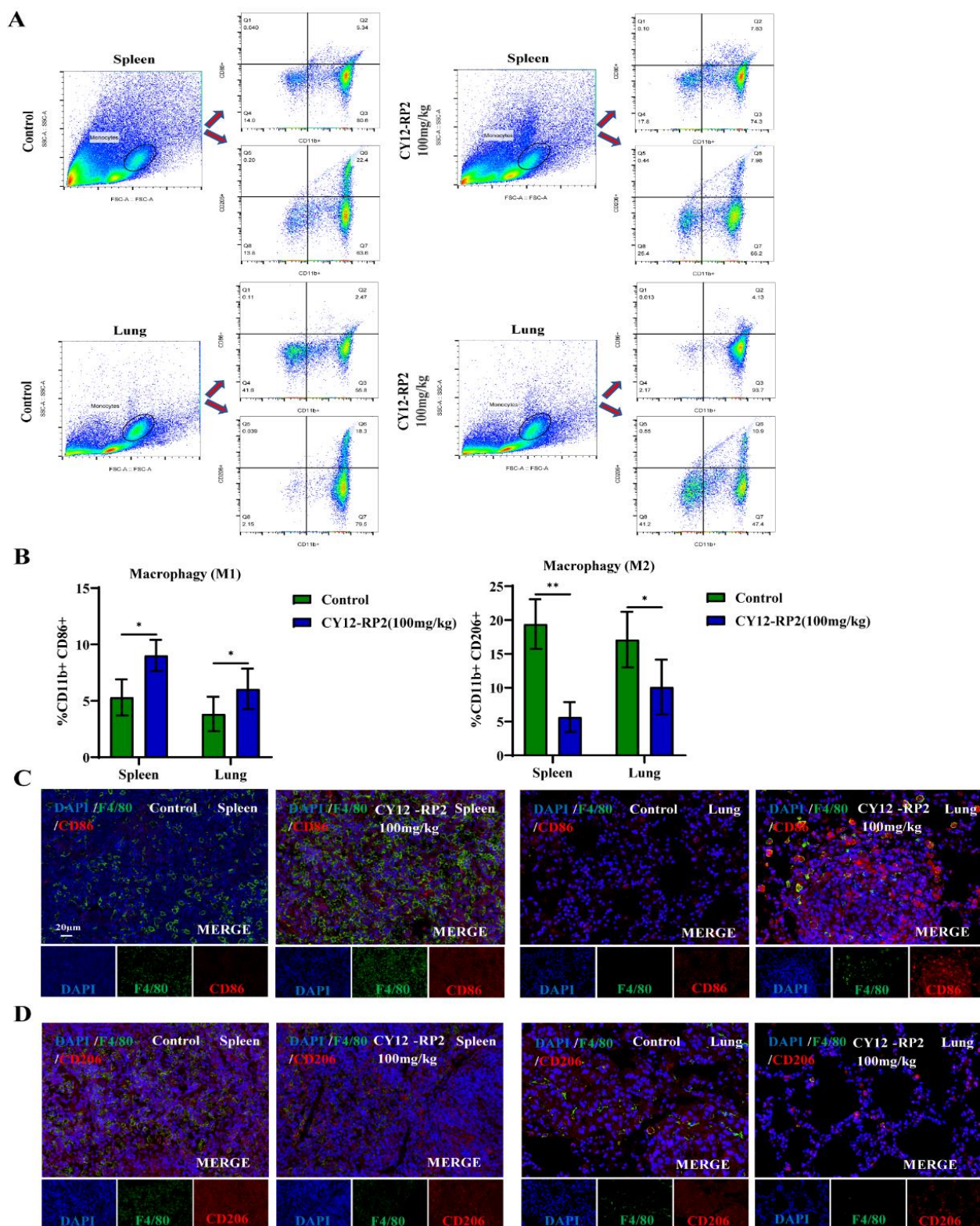


Fig. S1: PDPN was upregulated in melanoma, which was prognostically relevant to melanoma patient survival

(A) PDPN was significantly associated with the development of several types of tumors from the TCGA database. (B) Relationship between PDPN expression and prognosis in melanoma. (C) The mRNA expression of PDPN in melanoma cell lines (A375, SK-MEL-28, A875) were measured by real-time RT-PCR. (D) The protein levels of PDPN in melanoma cell lines (A375, SK-MEL-28, A875) were measured by western blot. (E) Immunofluorescence staining of PDPN in melanoma cell lines (A375, A875). The power field scale bar, 20µm and 5µm. (F, G) The interference and overexpression efficiency of PDPN were determined by western blot (F). The statistical analysis was performed to quantify the relative PDPN levels (G). Data are presented as mean ± SD. *p < 0.05, **p < 0.01, ***p < 0.001.

Fig. S2: The effects of PDPN on migration and invasion could be modulated by SKL2001 and IWR1

(A, B) Effects of PDPN on the migration ability of melanoma cells was detected by wound healing assays using SKL2001 and IWR1 (A). Statistical analysis was performed to determine the migrated distance (B). (C, D) Transwell assays were performed to quantify the modulatory effects of SKL2001 and IWR1 (C). Statistical analysis was performed to evaluate the invasion of cells (D). (E) Western blot assays were also performed on EMT-related proteins to identify the effects of SKL2001 and IWR1. Data are presented as mean ± SD. *p < 0.05, **p < 0.01, ***p < 0.001 versus control.

Fig. S3: The identification and functional analysis of PDPN- binding peptides

(A) Cell viability of melanoma cell lines (A375, A875) treated with 6 peptides except CY12-RP2 at various concentrations for 48h. (B) Colocalization of CY12-RP1 and PDPN protein was performed by immunofluorescence in melanoma cell lines (A375, A875). (C) SPR assay was performed to quantify the binding activity of CY12-RP1 (100 μ M, 20 μ M, 4.0 μ M, 0.8 μ M, 0.16 μ M, 0.032 μ M, 0.0064 μ M, 0.00128 μ M) to PDPN. (D) A representative flow cytometry analysis showed the binding efficiency of CY12-RP2 with PDPN at various concentrations in melanoma cell lines (A375, A875). (E) High-performance liquid chromatography (HPLC) and mass spectrometry (MS) were performed to confirmed the purity of CY12-RP2. (F) Assessing the anti-tumor effects of CY12-RP2 on the viability of melanoma cell spheroids by using cell-processing system, and representative images of 2D cells and 3D cellular sphere were shown. (G) Immunofluorescence staining was performed to measure the caspase3 fluorescence intensity of A375 cell spheroids after treating with CY12-RP2

Fig. S4: Effects and toxicity of CY12-RP2 in vivo

(A) TUNEL analysis of apoptosis was conducted on orthotopic xenografts tumor sections using fluorescence staining. The power field scale bar, 100 μ m. (B) Fluorescence staining of Ki67 was carried out on orthotopic xenograft tumor sections. The power field scale bar, 100 μ m. (C) Serum His-CY12-RP2 levels of the orthotopic xenografts mice treated by various dosage of CY12-RP2 were measured by His-ELISA kit. (D) Tumor, heart, liver, spleen, lung, kidney His-CY12-RP2 levels of the orthotopic

xenografts mice treated by various dosage of CY12-RP2 were measured by His-ELISA kit. **(E)** Serum ALT, AST, TG, and γ -GT levels of the orthotopic xenografts model were measured by ELISA. **(F)** H&E staining of liver, heart, spleen, lung, and kidney treated with CY12-RP2 (25 mg/kg, 100 mg/kg) daily for 15 days. The power field scale bar, 100 μ m. Data are presented as mean $\pm$ SD. * p < 0.05, ** p < 0.01, *** p < 0.001 versus control.

Fig. S5: The inhibition of cells metastasis and invasion by CY12-RP2 can be reversed by SKL2001

(A, B) The inhibition of CY12-RP2 on the migration capacity of melanoma cell lines was reversed by SKL2001 (A). Statistical analysis was performed to determine the migrated distance (B). **(C, D)** Effect of CY12-RP2 on the invasive ability of melanoma cell lines were reversed by SKL2001 (C). Statistical analysis was performed to count the invaded cells (D). **(E)** Western blot assay was also performed to detect the proteins related to EMT and Wnt/ β -catenin signaling pathway, including N-cadherin, Vimentin, snail, Dvl2, Phospho- β -catenin-S675, β -catenin, Phospho-GSK3 β -Ser9 and GSK3 β by CY12-RP2 with or without SKL2001 (50 μ M). Data are presented as mean $\pm$ SD. * p < 0.05, ** p < 0.01, *** p < 0.001 versus control.

Fig. S6: The expression of PDPN is associated with immune infiltration of melanoma tumors

(A, B) Single-cell RNA-seq of melanoma immunotherapy outcomes elucidated the role of PDPN related to the proportion of immune cells in the tumor microenvironment.

Immune cell populations from melanoma tumors (A). PDPN expression in immune cells, notably T cells and macrophages (B). (C) Rank of PDPN expression in GSE72056 from TCGA database. (D) The expression correlation between PDPN and immunoinhibitory in SKCM (skin cutaneous melanoma) was investigated using the TISIDB database. (E) The correlation of PDPN expression with immune infiltration level in SKCM was investigated using the TIMER database.

Fig. S7: CY12-RP2 modifies the population of NK cells in the spleen and lung of mice

(A) Flow cytometric analysis of CD49b⁺ and Granzyme B⁺ populations in spleen and lung of BALB/C mice with lung metastases. (B) Statistical analysis was performed to count the percentage of CD49b⁺ and Granzyme B⁺ NK cells in the spleen and lung of BALB/C mice with lung metastases. (C) Fluorescence imaging of NK cells in the spleen and lung of pulmonary metastasis model stained with CD56 antibody.

Fig. S8: The effects of CY12-RP2 on the proportion of macrophages (M1 and M2) in the spleen and lung of mice

(A) Representative flow cytometry images showing the proportion of M1 and M2 macrophages in the spleen and lung of BALB/C mice with lung metastases. (B) Statistical analysis was performed to count the percentage of CD11b⁺ CD86⁺ M1 macrophages and CD11b⁺ CD206⁺ M2 macrophages in the spleen and lung of BALB/C mice with lung metastases. (C, D) Proportion of M1 (F4/80⁺ CD86⁺)

macrophages (C) and M2 (F4/80+ CD206+) macrophages (D) in the spleen and lung of BALB/C mice with lung metastases were detected by representative double immunofluorescence.

Table 1: Antibodies applied for western-blotting, flow cytometry, immunofluorescence and immunohistochemistry analysis

antibodies	sources	catalog No
mouse anti-human pdpn/gp36	BioLegend	127407
Rabbit anti-human pdpn/gp36	ABclonal	A9242
Rabbit anti-human N-Cadherin	ABclonal	A19083
mouse anti-human E-cadherin	ABclonal	A18135
Rabbit anti-human Vimentin	ABclonal	A11952
Rabbit anti-human snail	Abclonal	A5243
Rabbit anti-human Axin1	CST	#2087
Rabbit anti-human Dvl2	CST	#3224
Rabbit anti-human Phospho- β -catenin-S675	ABclonal	AP0795
Rabbit anti-human β -catenin	ABclonal	A0316
Rabbit anti-human Naked1	CST	#2262
Rabbit anti-human Phospho-GSK3 β -Ser9	ABclonal	AP0039
Rabbit anti-human GSK3 β	ABclonal	A6164
Rabbit anti-human LEF1	ABclonal	A4473
Rabbit anti-human TCF1/TCF7	ABclonal	A20835
Rabbit anti-human Caspase3	ABclonal	A2156
Rabbit anti-human Caspase9	ABclonal	A2636
Rabbit anti-human BCL2	ABclonal	A111025
Rabbit anti-human Bax	ABclonal	A0207
mouse anti-human β -actin	ABclonal	AC004
FITC anti-mouse CD3	Biolegend	100203
PE-Cyanine7 Anti-Mouse CD4	Tonbo	60-0042-U025
PerCP-Cyanine5.5 Anti-Mouse CD8a	Tonbo	65-0081-U025
APC anti-mouse CD25	Biolegend	102011
PE Anti-Mouse Foxp3	Tonbo	50-5773-U025
APC/Cyanine7 anti-mouse CD49b	Biolegend	108919
PE anti-human/mouse Granzyme B	Biolegend	372207
APC anti-mouse/human CD11b	Biolegend	101211
FITC anti-mouse CD86	Biolegend	105006
PE anti-mouse CD206	Biolegend	141705