# Supplementary Information

**The PD-L1 cellular nanovesicles carrying rapamycin inhibit alloimmune responses in transplantation**

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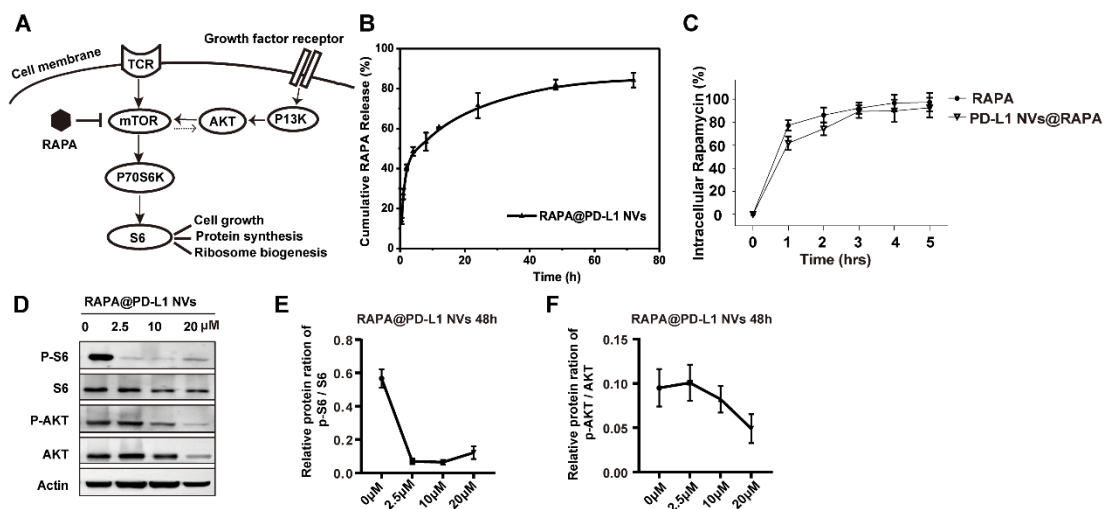
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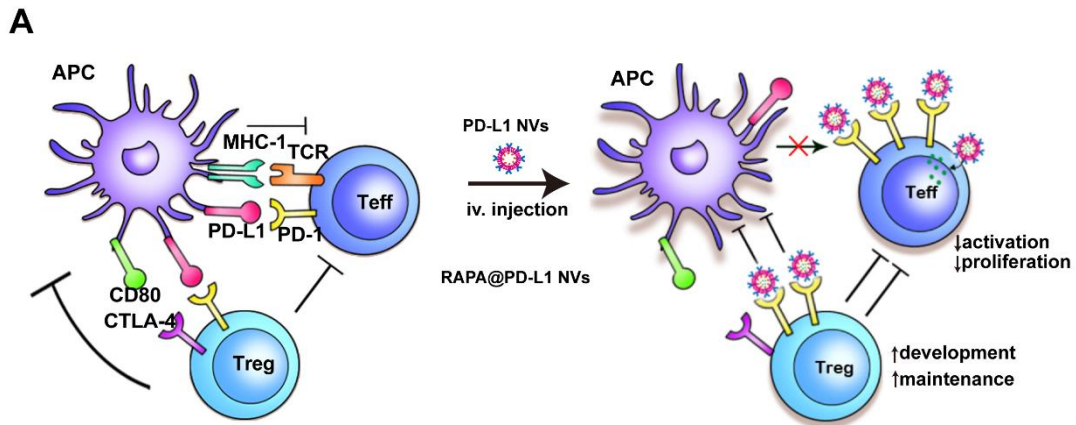
**Figure S1. Schematic diagram of RAPA@PD-L1 NVs.**

(A) Schematic diagram showed the action mechanism of PD-L1 NVs loaded with RAPA (RAPA@PD-L1 NVs).



**Figure S2. The action mechanism and uptake of RAPA in cells.**

(A) Mechanisms of RAPA inhibiting AKT/mTOR/p70S6K feedback pathway and impact on T cell proliferation. (B) *In vitro* release of RAPA from the RAPA@PD-L1 NVs. Error bar, mean ± SEM (n=3). (C) Uptake of rapamycin in Jurkat cells. Jurkat cells were incubated with 45 μg /ml of rapamycin at 37 °C during above time. Extracted and purified intracellular rapamycin was detected by HPLC analysis (n=3). (D-F) Representative western blot plots and quantitative analysis of the effect of RAPA at different drug concentrations on the expression of pS6 and pAKT in Jurkat T cells, Error bar, mean ± SEM (n=3).
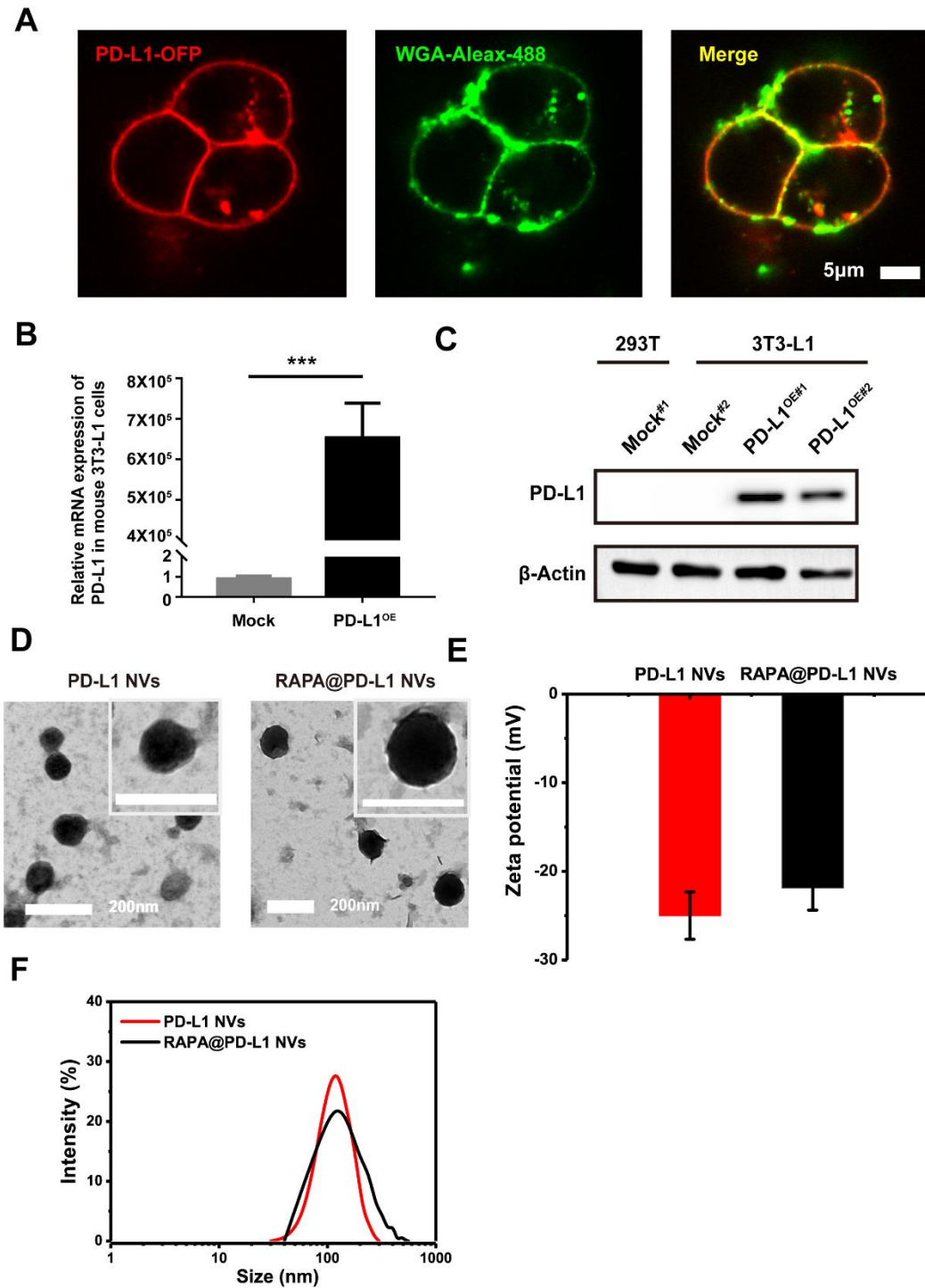
**Figure S3. Construction of mouse 3T3-L1 stable cell line overexpressing PD-L1 proteins on membrane and characterization of PD-L1 NVs.**

(A) Representative confocal images of mouse 3T3-L1 expressing PD-L1-OFP. Scale bar: 5 μm. (B) qPCR analysis of PD-L1 mRNA in 3T3L1-PD-L1-OFP cells. (C) Western blotting of mouse PD-L1-OFP expressed on the membrane of the 3T3-L1 cells. (D) The TEM image of mouse PD-L1 NVs and RAPA@PD-L1 NVs. Scale bar: 200

nm. (E-F) The zeta potentials and size distribution of mouse RAPA@PD-L1 NVs/PD-L1 NVs by dynamic light scattering analysis.
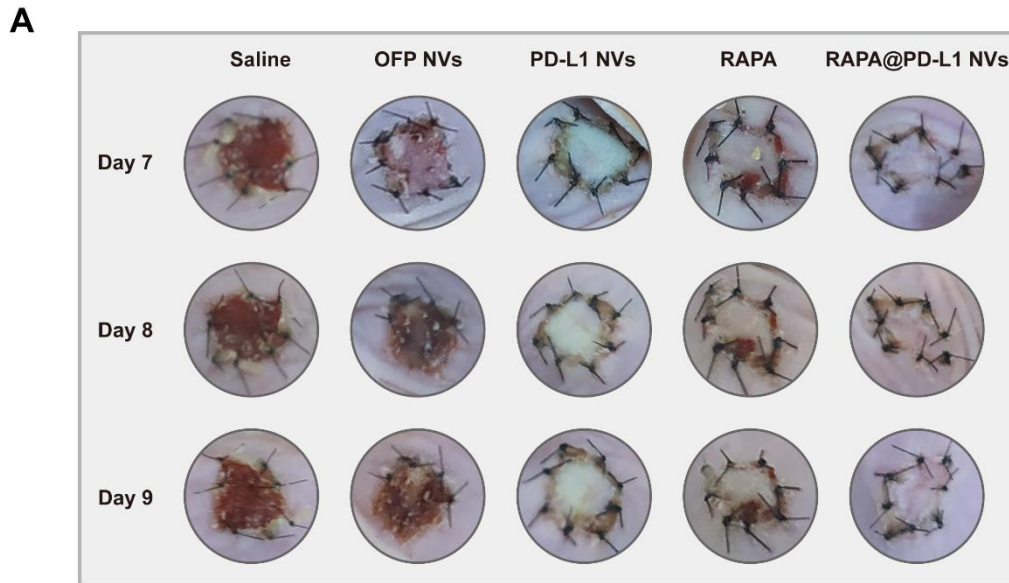


**Figure S4. Time-lapse skin-graft pictures upon treatments.**

(A) The representative skin macro-photographs of mice accepting skin transplantation in Day 7, 8 and 9 upon saline, OFP NVs, PD-L1 NVs, RAPA or RAPA@PD-L1 NVs treatment based on a 5-point skin scoring system. The endpoint of the mouse allograft survival is defined as complete rejection of the allograft (score 5).
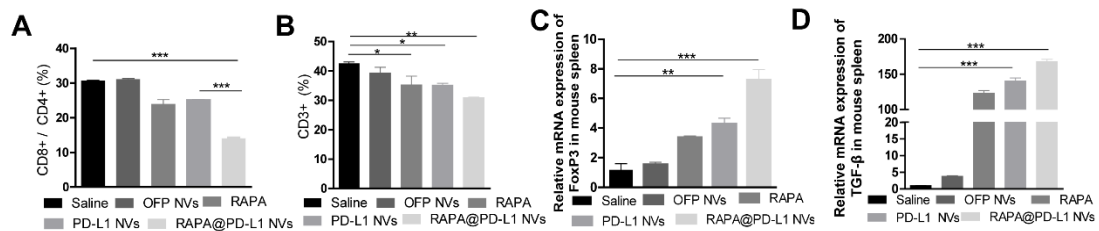


**Figure S5. The quantitative analysis of T cells and related factors levels detected by qpcr.**

(A-B) The quantitative analysis of CD8+/CD4+ T cell ratio and responding T cell ratio from mouse spleen samples of different drug injection groups. (C-D) FoxP3 and TGF-β levels from spleen were measured by qPCR in skin-graft mice. Error bar, mean ±

SEM (n=3). (A-D) One-way ANOVA with Tukey posthoc test analyses were performed. *P < 0.05, **P < 0.01, ***P < 0.001
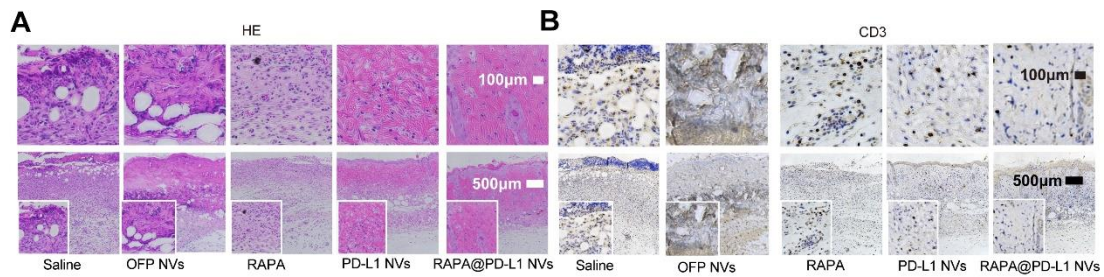


**Figure S6.** *In vivo* **images of graft-skin for HE staining and Immunofluorescence** (A-B) Representative images of HE staining and Immunofluorescence of graft-skin for CD3 antibody. Scale bar: 100 µm.