Supporting Information

Engineering PD-1-Presenting Platelets for Cancer Immunotherapy

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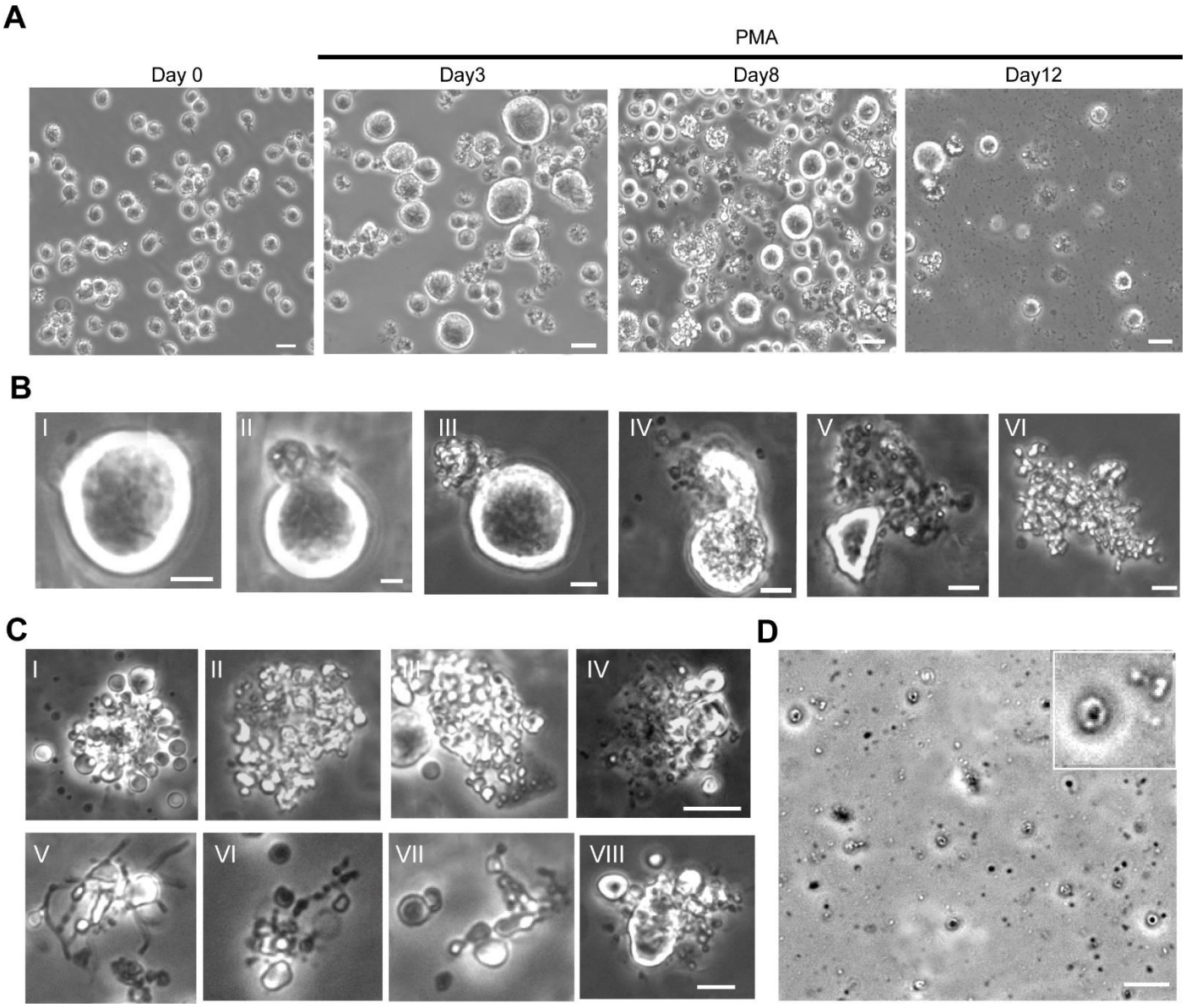

Fig. S1. Process of platelet releasing from L8057 cells. (A) Process of L8057 cell maturation and platelet production by treating the cells with 500 nM PMA (Scale bar: 10 μm). (B) Process of proplatelets extended from L8057 cells after 6 days of stimulation with 500 nM PMA (Scale bar: 10 μm). (C) Morphology of proplatelets released from L8057 cells (Scale bar: 10 μm). (D) Representative image of purified PD-1-expressing platelets (Scale bar: 10 μm).

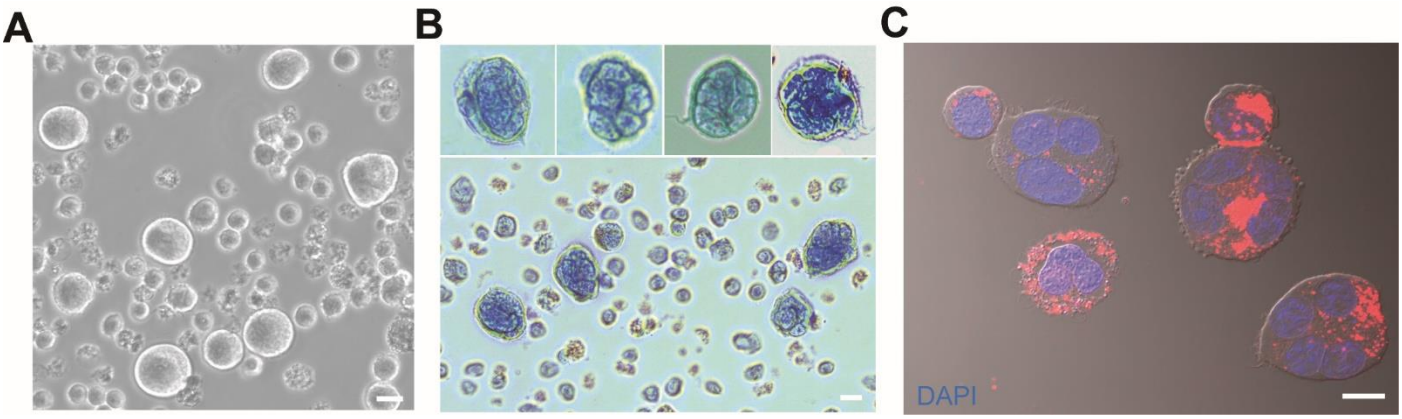

Fig. S2. Mature L8057 cells containing polyploid nuclei. (A) Morphology of mature L8057 cells showing increased cell volume 5 days after treatment with 500 nM PMA. (B) Wright-Giemsa staining indicated the presence of multinucleated MKs 5 days after treatment with 500 nM PMA. (C) Representative confocal image of L8057 cells stained with DAPI to indicate the presence of multiple nuclei. All scale bars: 10 μm.

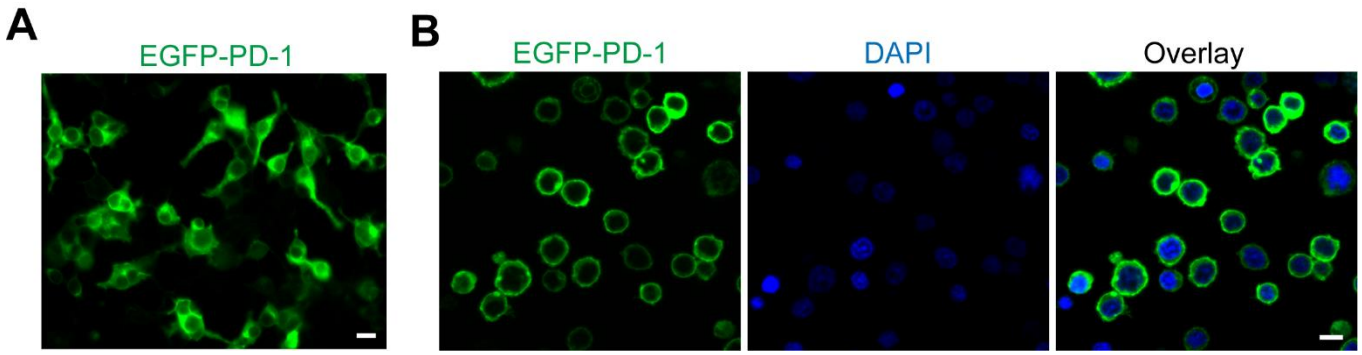

Fig. S3. Establishment of L8057 cell line stably expressing EGFP-PD-1. (A) Confocal image represents HEK293T cells transfected with PD-1 plasmid and packaging plasmids for PD-1 lentivirus packaging. (B) Confocal images represent L8057 cells stably expressing EGFP-PD-1. Scale bars: 10 μm.

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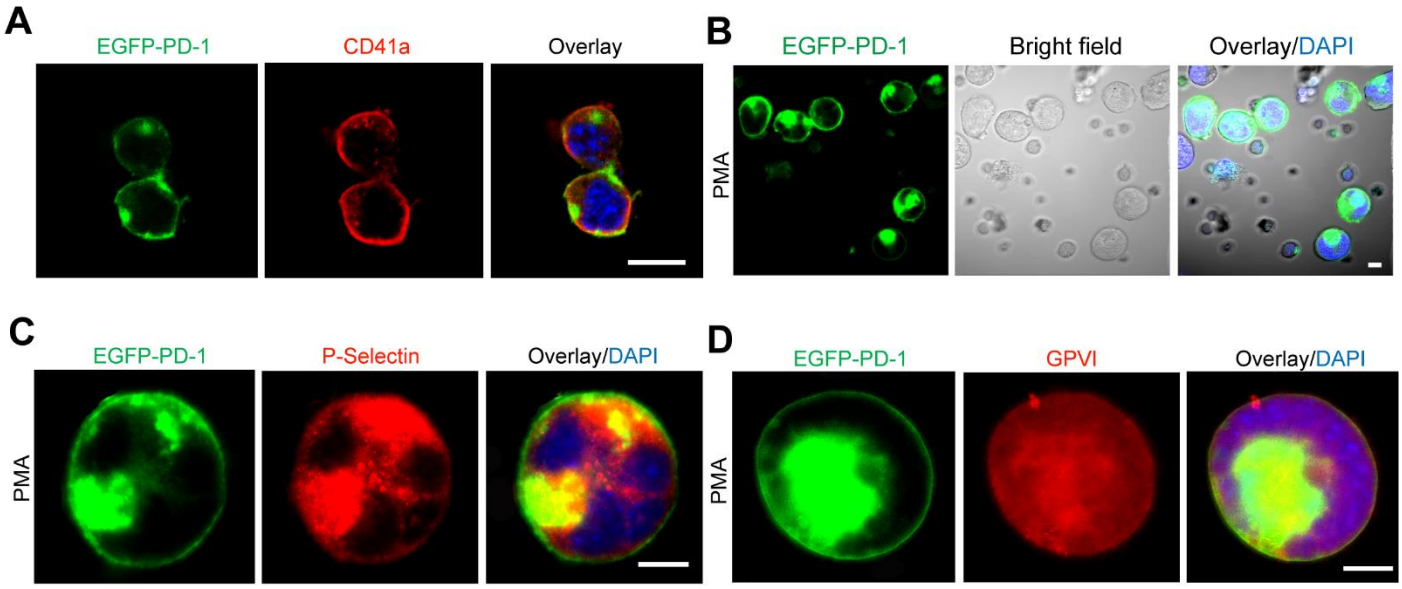

Fig. S4. Maturation and biomarker of PD-1-expressing MKs. (A) Representative confocal images of EGFP-PD-1-expressing L8057 cells stained for the detection of CD41a expression. (B) Confocal image shows mature PD-1-expressing MKs with increased cell volume after treatment with 500 nM PMA for 3 days. (C, D) EGFP-PD-1-expressing L8057 cells stimulated with 500 nM PMA for 3 days, and stained to detect P-Selectin and GPVI expression, respectively. Scale bars: 10 μm.

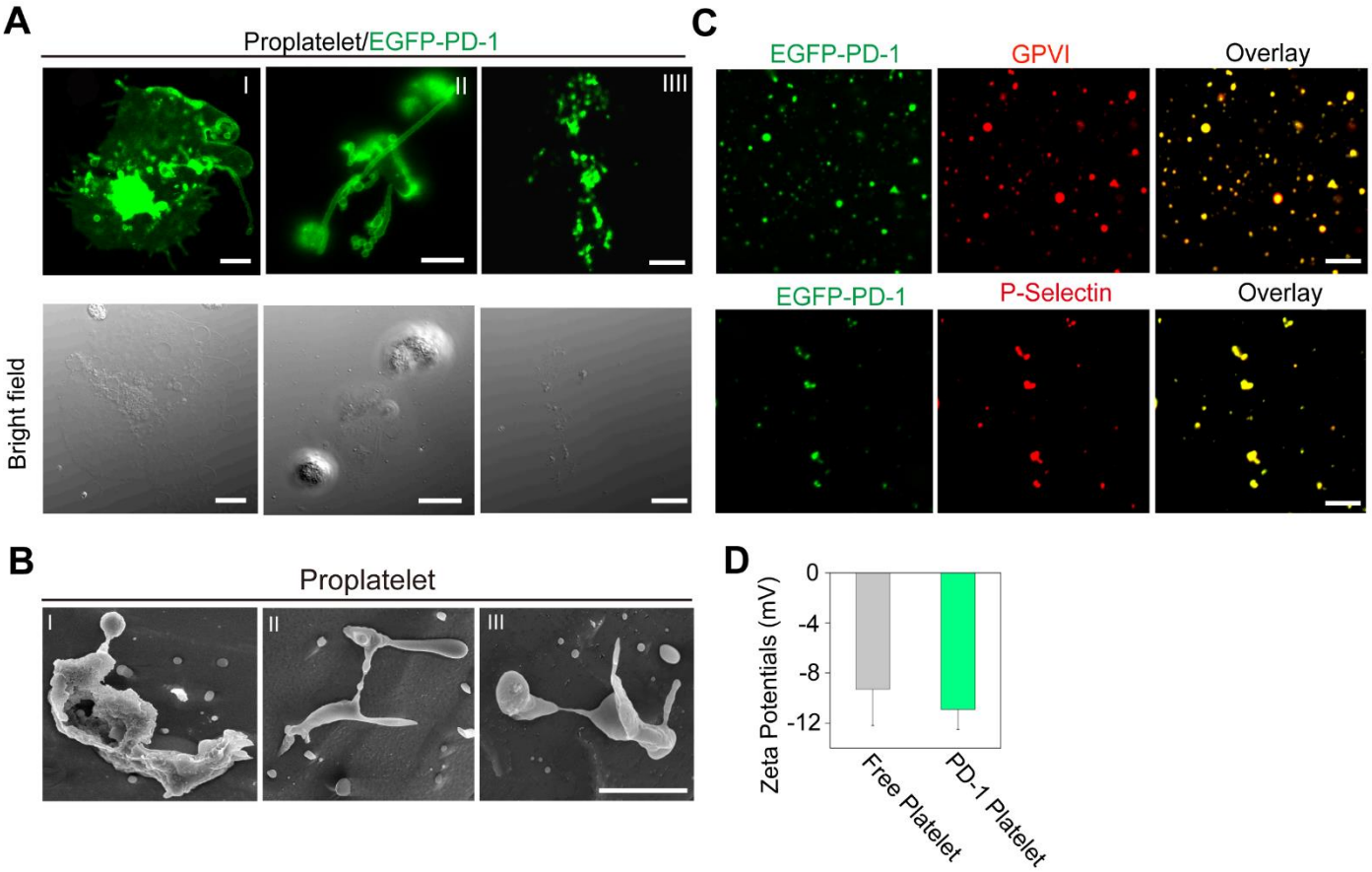

Fig. S5. Production of proplatelets and platelets from PD-1-expressing L8057 cells. (A) Extension and fragmentation of proplatelets. (B) Cryogenic-scanning electron microscopy (CSEM) images showing the morphology of PD-1-expressing proplatelets. (C) Representative confocal images of EGFP-PD-1-expressing platelets stained to detect the expression of GPVI and P-Selectin. (D) Zeta potential of free platelets and PD-1-expressing platelets (*n*=3). Data represent as mean ± s.d. All scale bars: 10 μm.

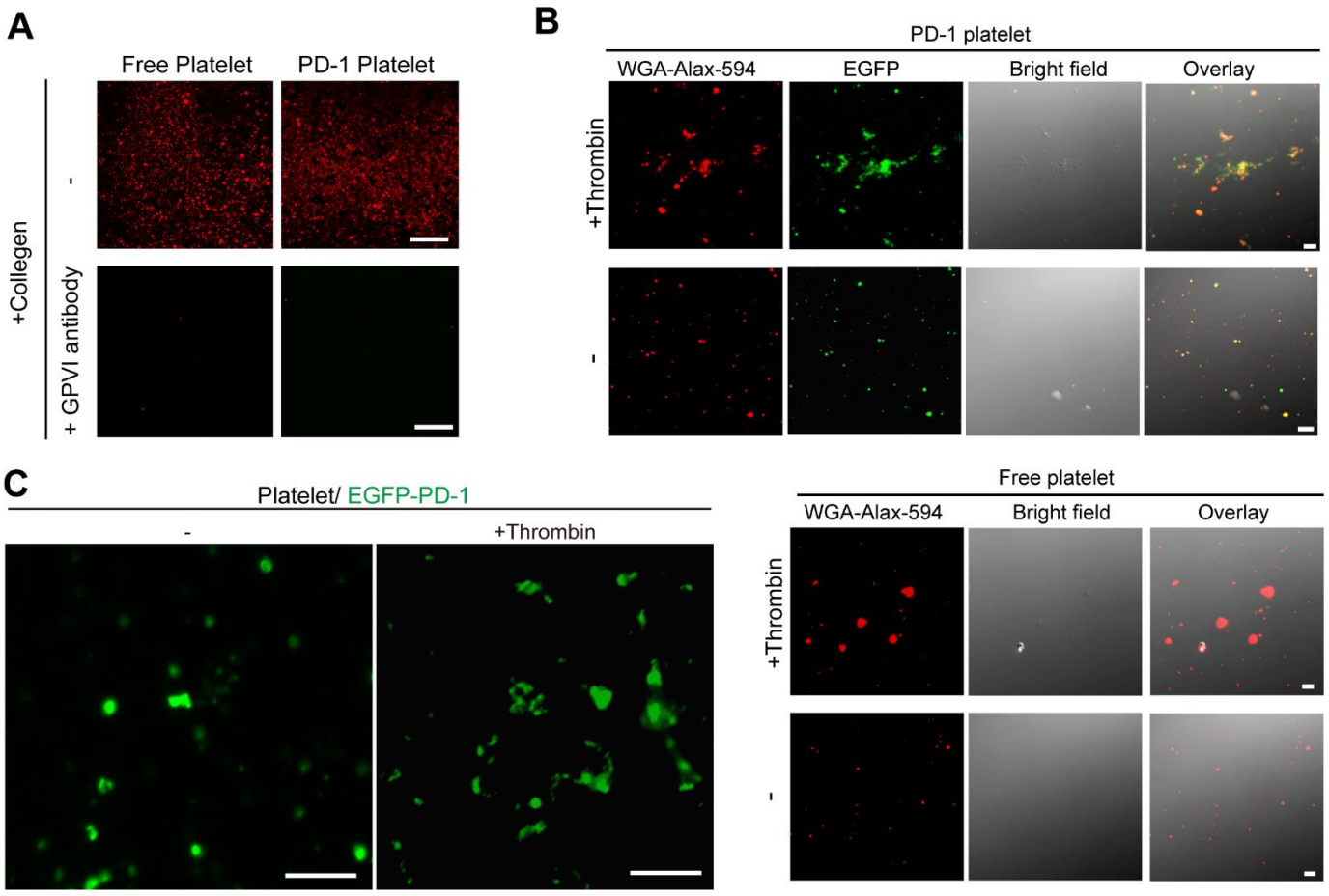

Fig. S6. Activity of platelets released from L8057 cells. (A) Localization of platelets on collagen-coated tissue culture well pretreated or untreated with GPVI antibody for 2 hours. Scale bar, 50 μm. (B) Aggregation of platelets stimulated or non-stimulated with 0.5 U thrombin mL^{-1}. Scale bar: 10μm. (C) Activation of PD-1-expressing platelets after treatment with 0.5 U thrombin mL^{-1}. Scale bar: 10μm.

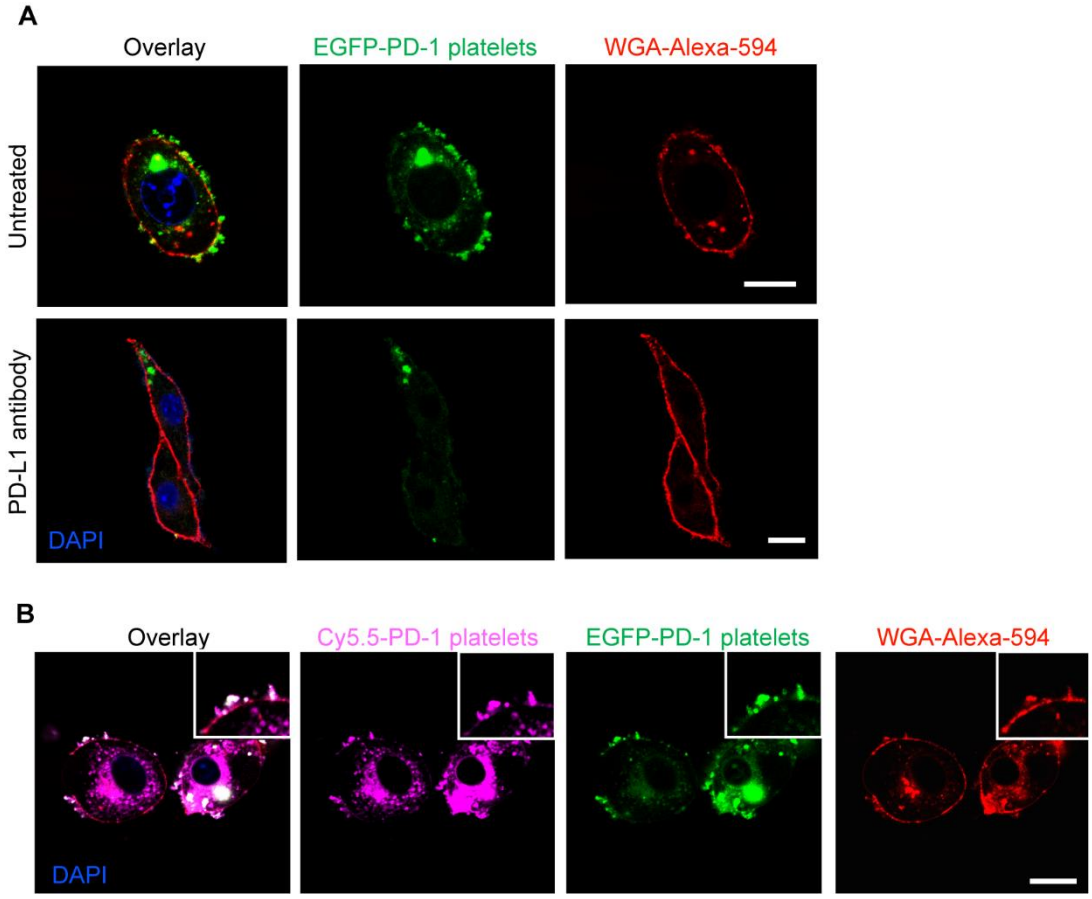

Fig. S7. Platelets binding tumor cells and post-uptake of platelets by tumor cells. (A**)** The confocal images indicate the PD-1 platelets binding with B16F10 cells. PD-1 platelets were incubated with B16F10 cells for 20 h. aPD-L1 antibody (20 μg/mL) were incubated with the cells for 4 h before the PD-1 platelets were added in the culture medium as indicated. Scale bar: 10 μm. **(**B**)** EGFP-PD-1-expressing platelets labeled with Cy5.5 were incubated with B16F10 cells for 20 hours. WGA Alexa-Fluor 594 dye was used to stain the B16F10 cell membrane (Scale bar: 10 μm).

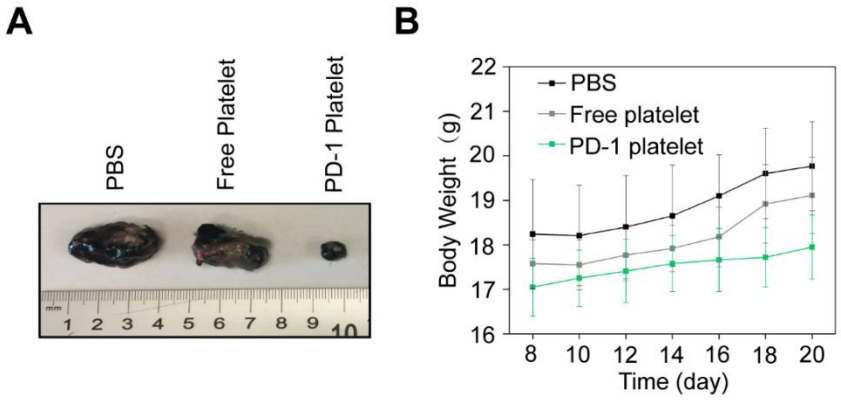

Fig. S8. Tumor size and body weight of mice receiving PD-1-expressing platelet treatment. (A) Tumor collected from euthanized mice. (B) Body weights of treated mice (n=8). Data represent as mean ± s.d.

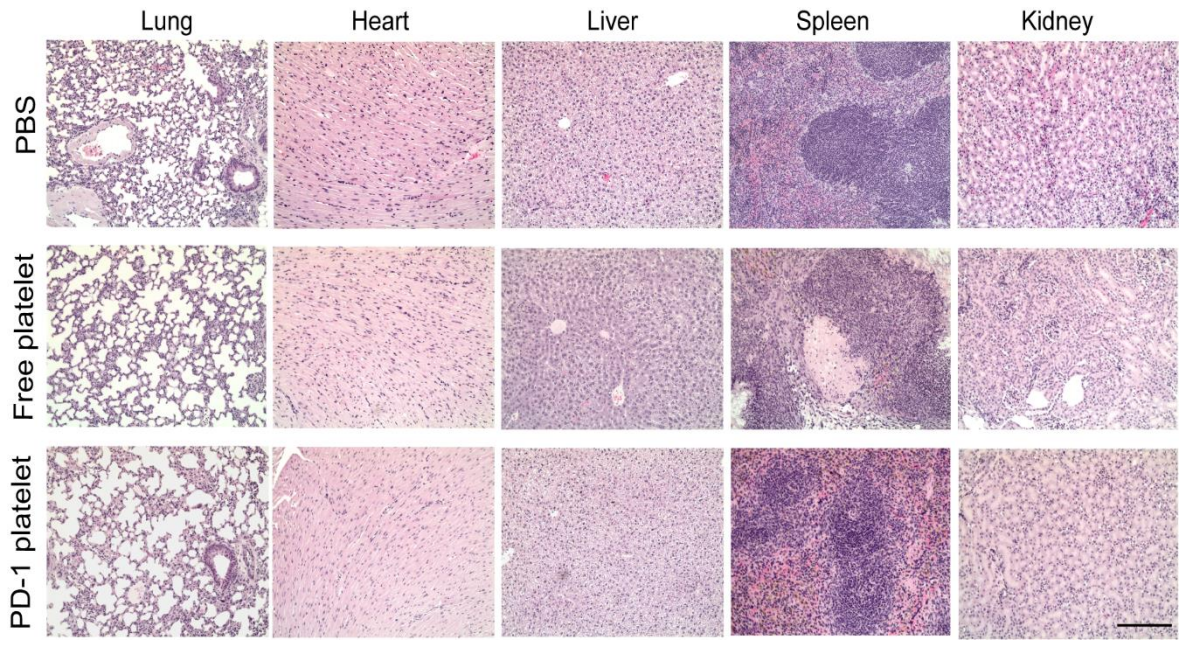

Fig. S9. Histological images for H&E staining of the liver, spleen, kidney, heart and lung of mice with different treatments. Scale bar: 200 μm.

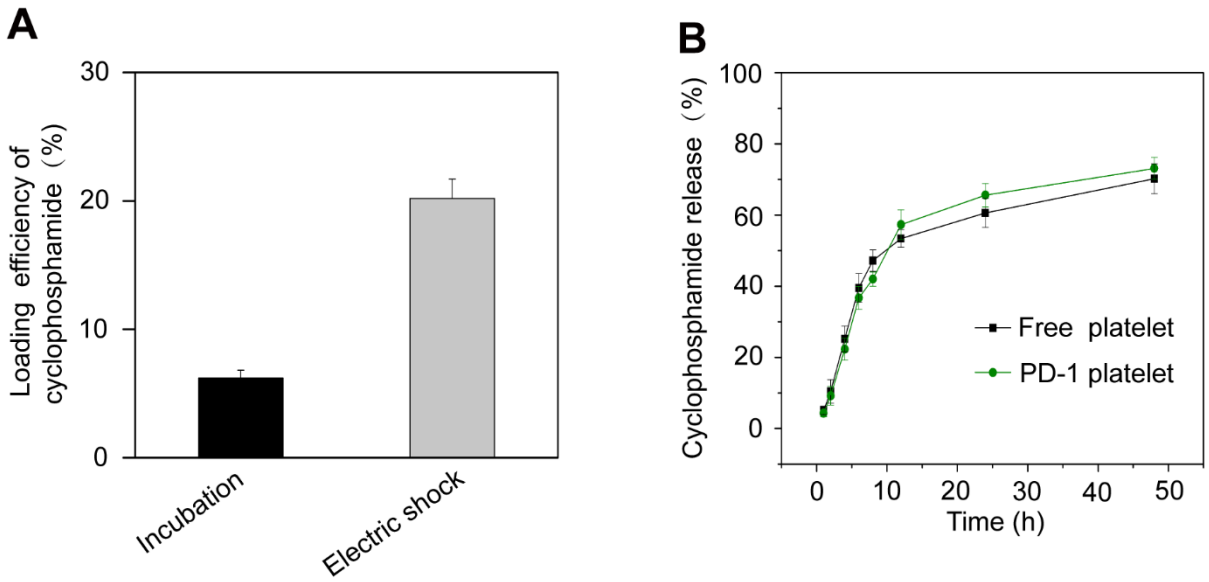

Fig. S10. *In vitro* loading and release of cyclophosphamide by PD-1-expressing platelets. (A) Cyclophosphamide (CP) loading in platelets using different approaches (*n*=3). Error bar, ± s.d. (B) Release profiles of CP from free platelets and PD-1-expressing platelets (*n*=3). Error bar, ± s.d.

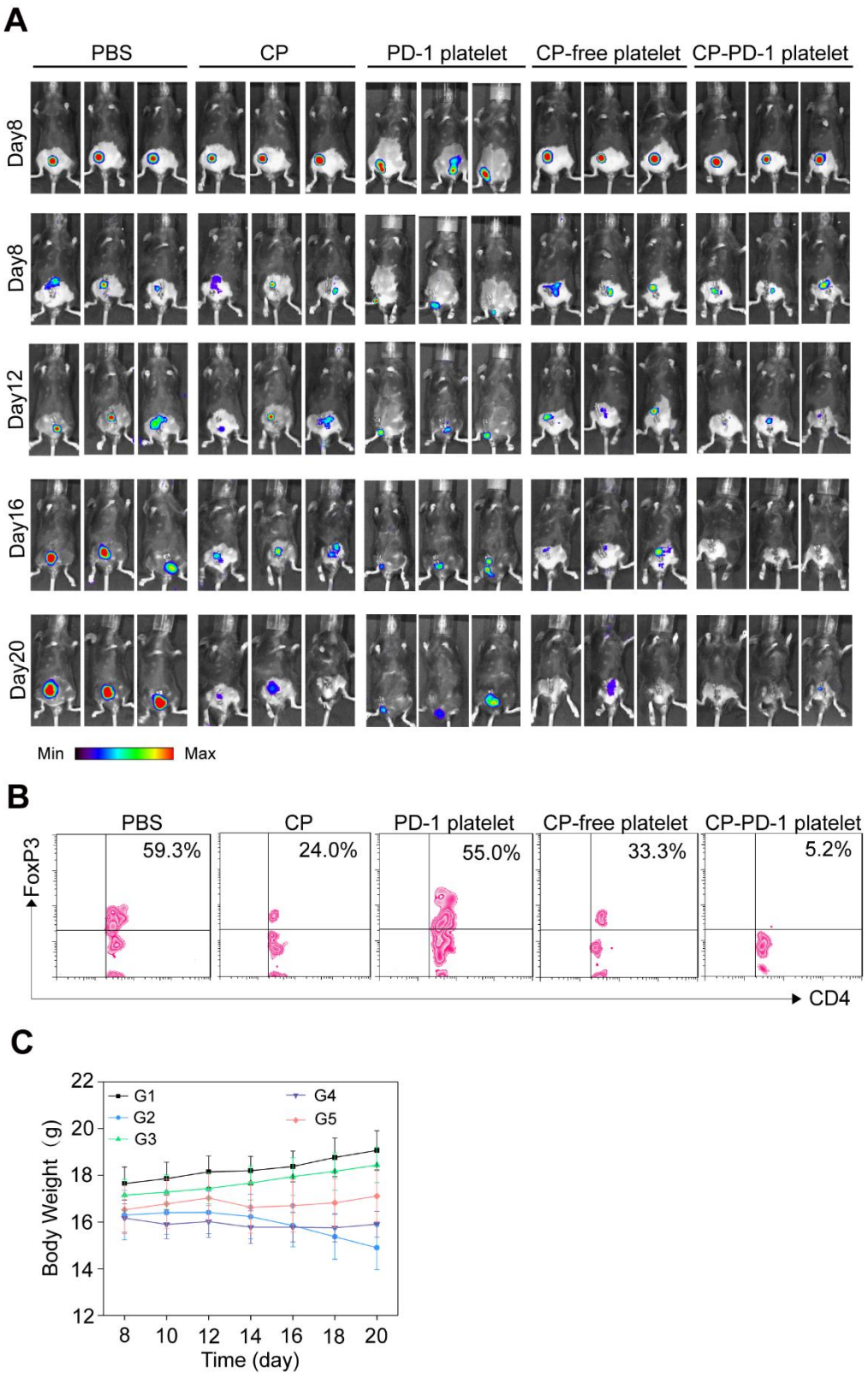

Fig. S11. B16F10 tumor growth in mice treated with PD-1-expressing platelets after partial tumor resection. (A) *In vivo* tumor bioluminescence of B16F10 tumors. (B) Representative plots of FoxP3 expression in $CD4^+$ T cells within tumors analyzed by the flow cytometry (gated on $CD4^+$ T cells) (n=3). (C) Body weights of treated and control mice. Error bar, ± s.d.