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ARTICLES

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In situ activation of platelets with checkpoint inhibitors for post-surgical cancer immunotherapy

Chao Wang[1,2], Wujin Sun[1,2], Yanqi Ye[1,2], Quanyin Hu[1,2], Hunter N. Bomba[1] and Zhen Gu[1,2,3*]

Introduction

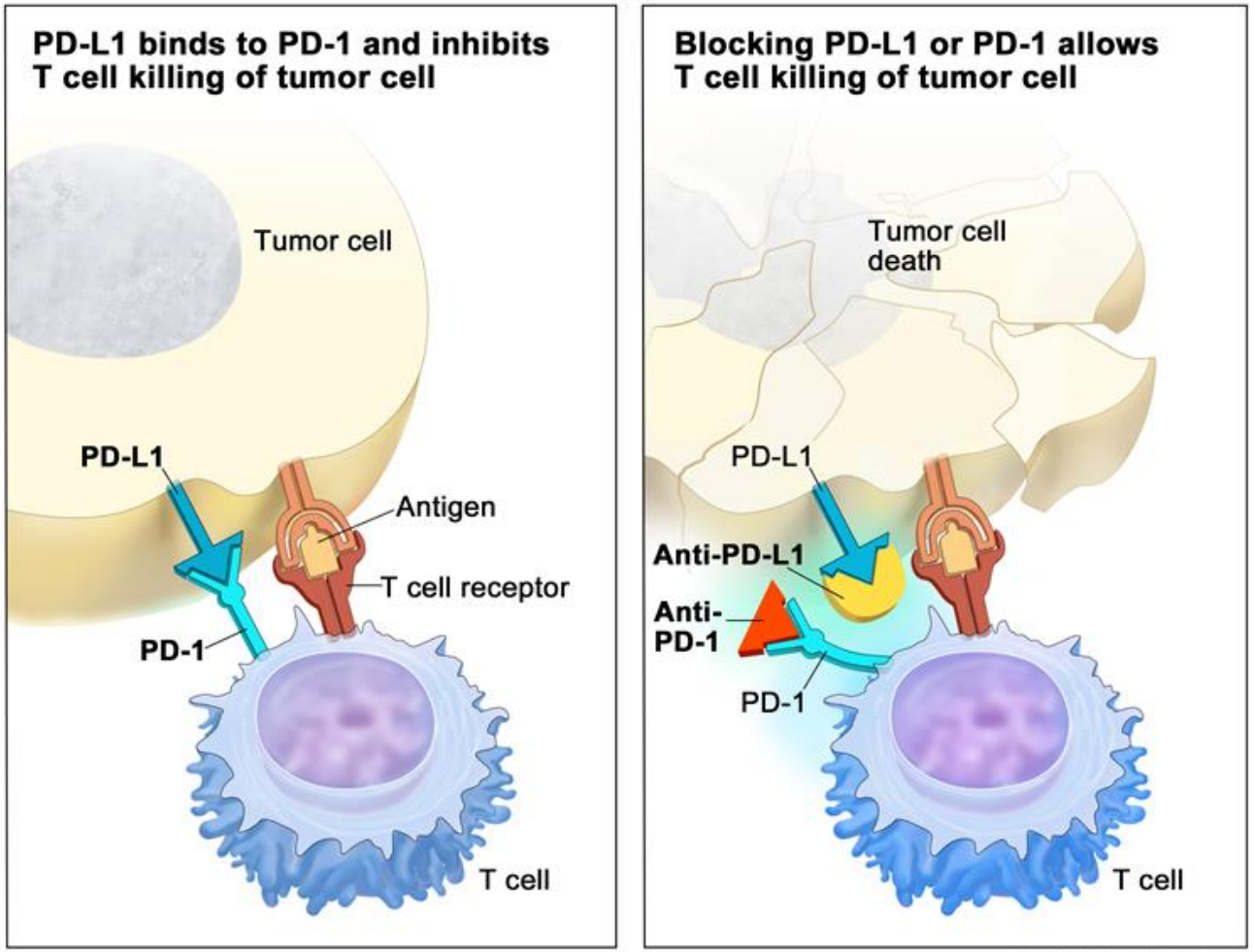
- Surgery – the main treatment option for most solid tumors

- But: residual microtumors & circulating tumor cells (CTCs)

  → and may also induce promotion of cancer metastasis

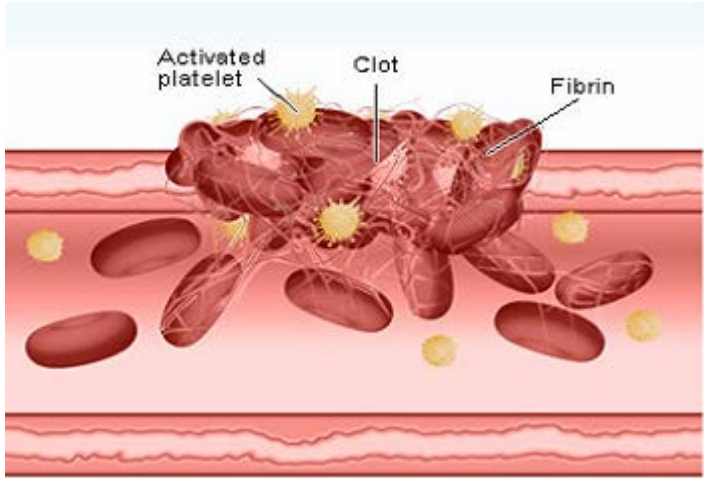
- Immunotherapy may kill residual cancer cells

Immune checkpoint therapy – PD-L1



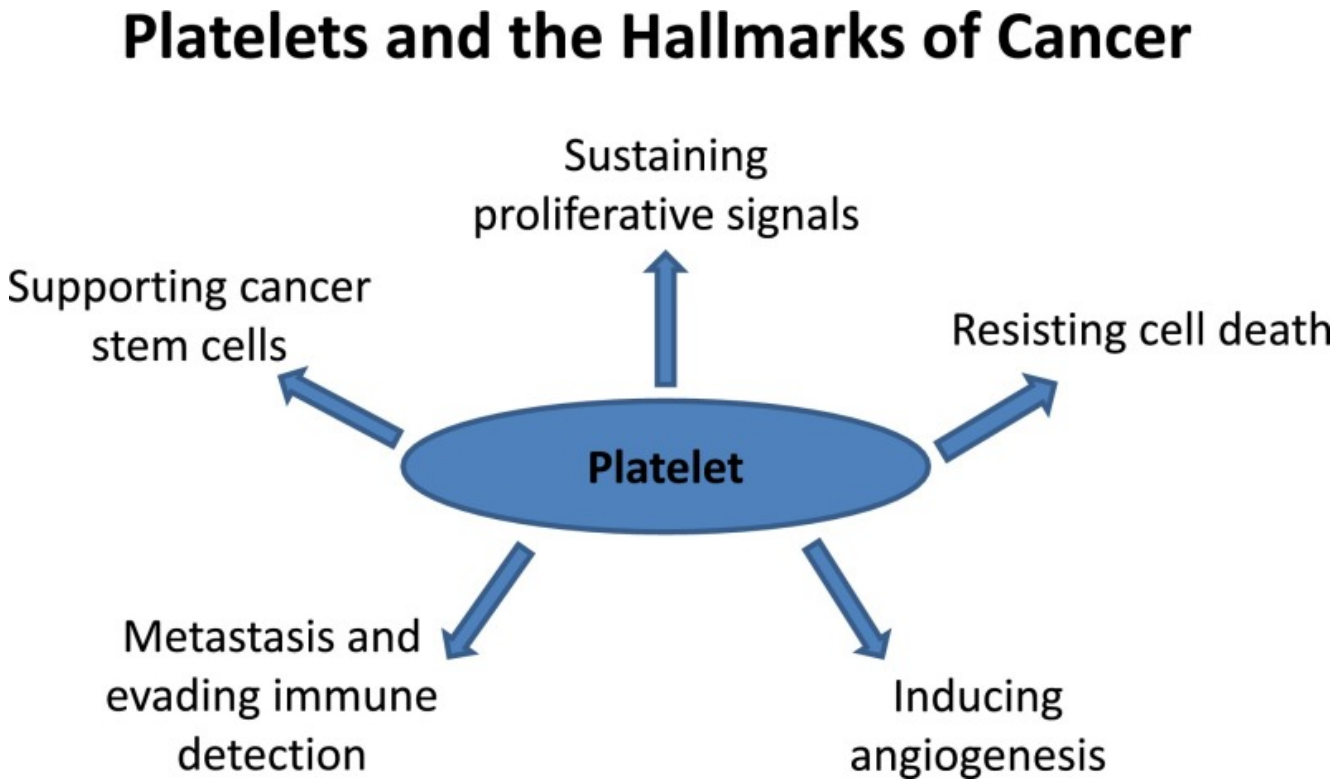
Platelets

- No nucleus
- Derived from megakaryocytes
- Important in hemostasis
- Involved in thrombosis
- Modulated inflammation
- Platelet transfusion in thrombocytopenia



WWW.MED-HEALTH.NET. Last Updated 06 May, 2017.

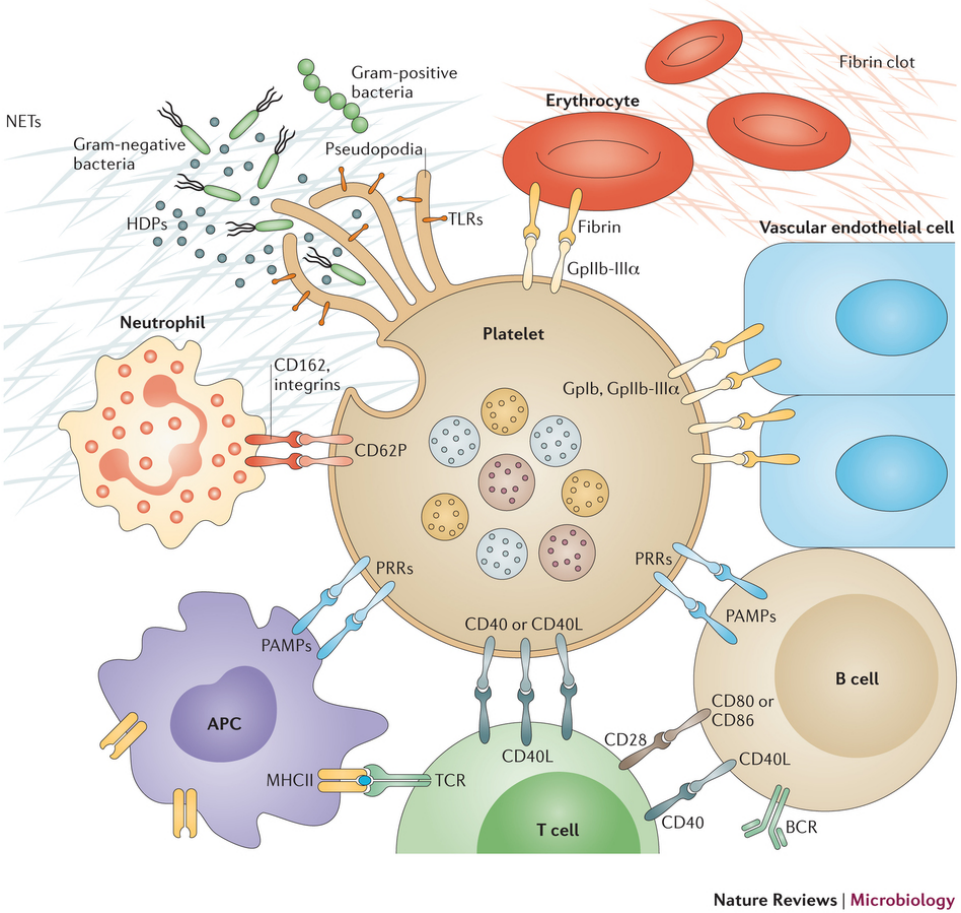
Emerging role of platelets in cancer



Platelets at the interface of thrombosis, inflammation, and cancer
Aime T. Franco, Adam Corken, and Jerry Ware, Blood. 2015 Jul 30; 126(5): 582–588.
Prepublished online 2015 Jun 24. doi: 10.1182/blood-2014-08-531582

Platelets as drug carriers

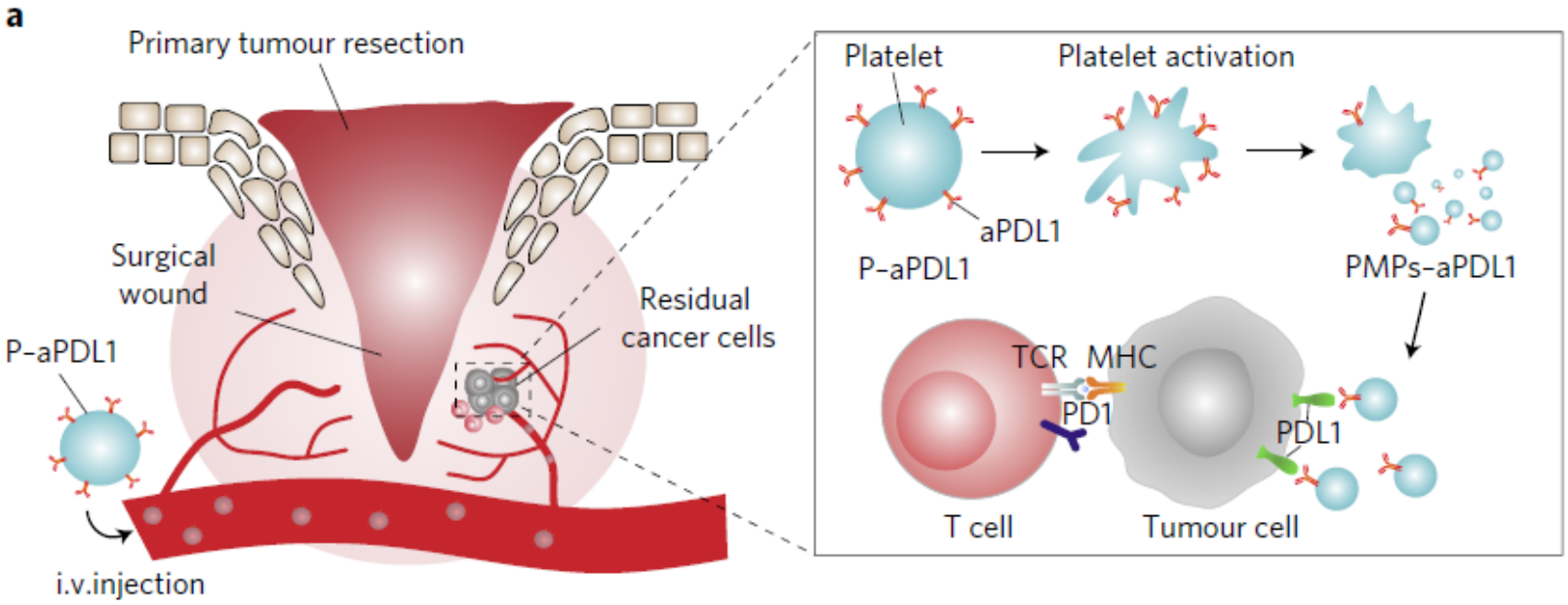
- For increased efficacy
- Longer live span
- Migrate to the surgical wound
- Interact with circulating tumor cells (CTCs)
- Enhances immune response
- Upregulates PD-L1



Platelets: at the nexus of antimicrobial defence
Michael R. Yeaman, Nature Reviews Microbiology 12, 426–437 (2014)
doi:10.1038/nrmicro3269, Published online 16 May 2014

Aim of the study

- Targeting residual as well as circulating tumor cells by binding a PD-L1 antibody to the surface of platelets

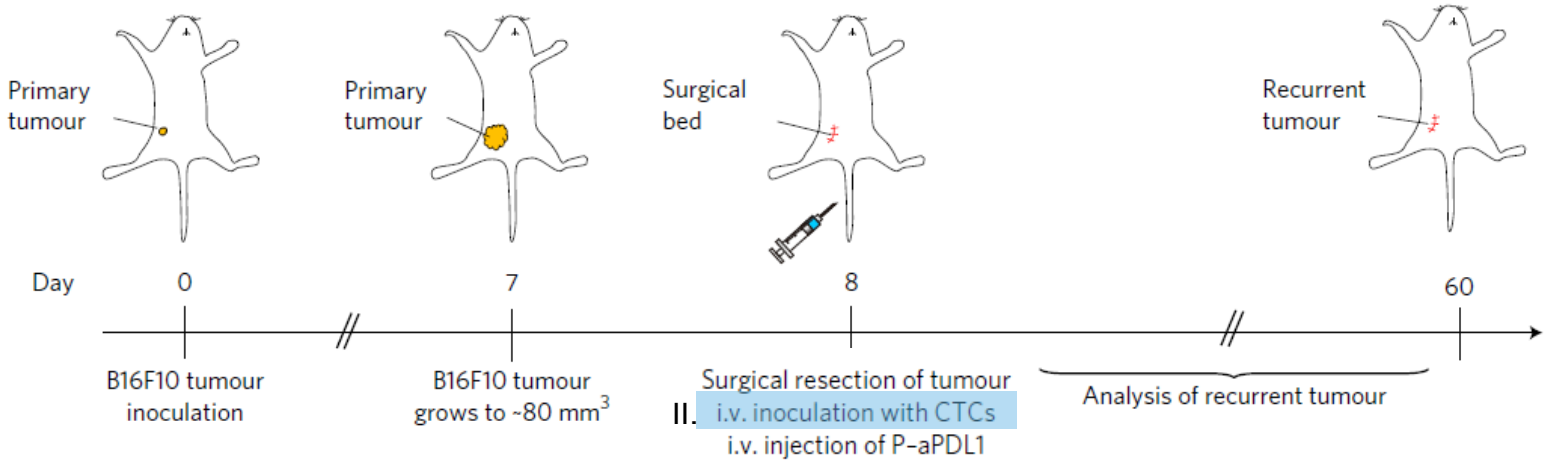


Materials & methods

- Cell lines: mouse melanoma B16F10, mouse mammary carcinoma 4TI (both expressing luciferase and GFP)
- Mice: C57BL/6 and BALB/c (6-10 weeks old)
- Platelet preparation (P-aPDL1): Isolation from whole blood, conjugation of aPDL1 via a maleimide linker, Platelet activation with thrombin
- ELISA for antibody and cytokine detection (aPDL1, IL-1β, TNFα, IL-6, sCD40L)
- TEM
- Fluorescence microscopy and flow cytometry
- *In vivo* bioluminescence and imaging of tumors and antibody
- Tail bleeding assay

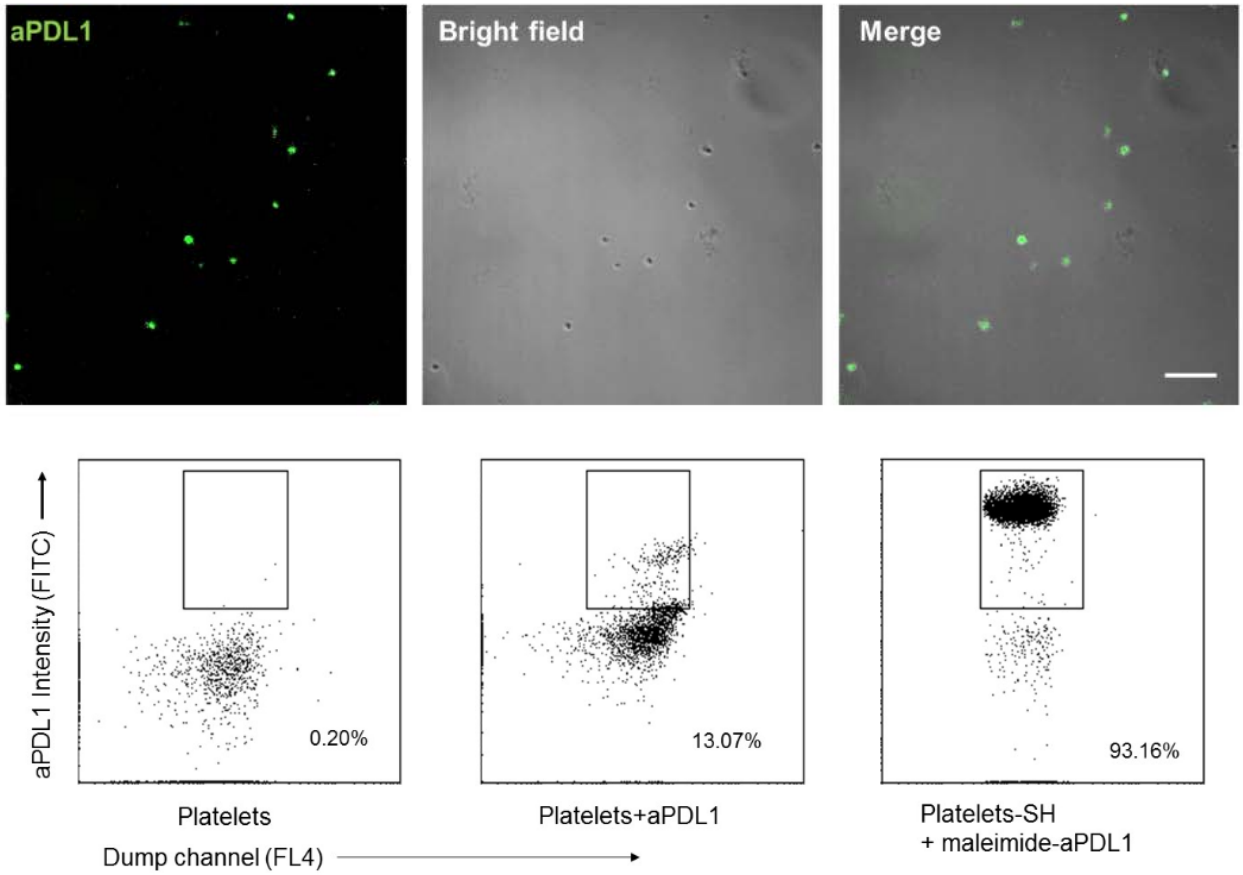
Materials & methods

- *In vivo* experiments

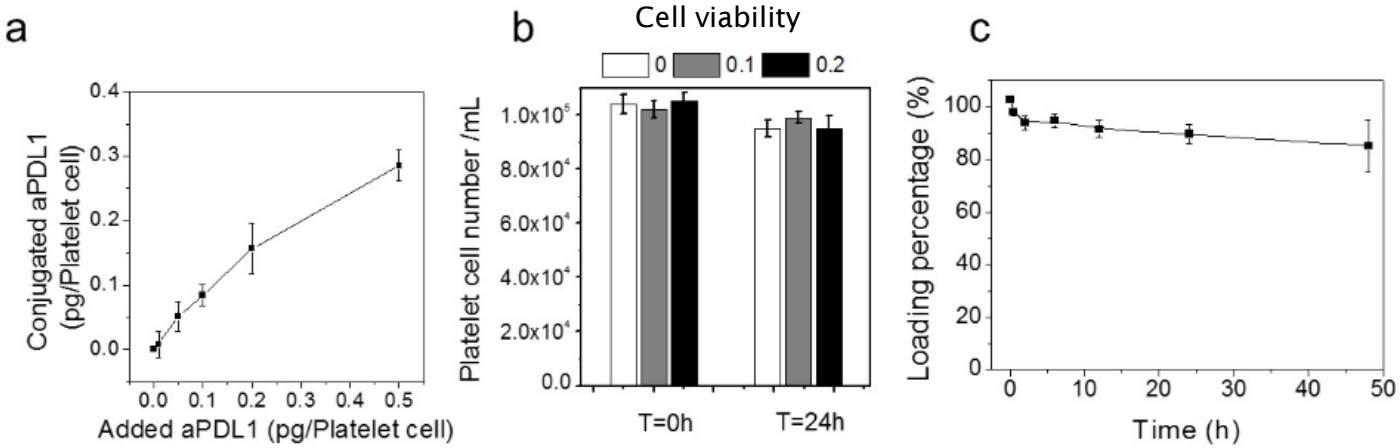


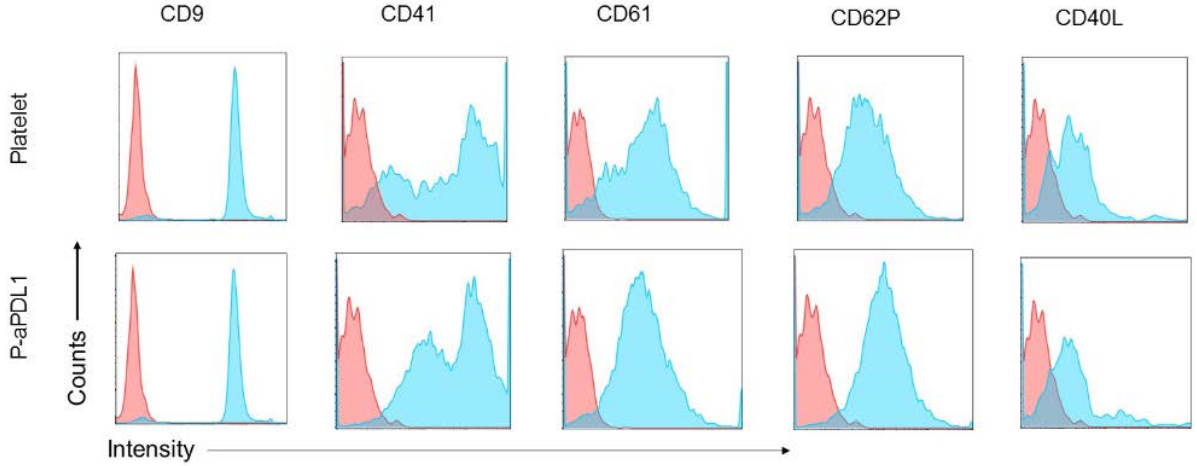
- I. Therapy model of incomplete resection
- II. Therapy model of incomplete resection and metastasis
- III. Therapy model of recurrent triple negative 4T1 tumor

Results – aPDL1 binding to platelets

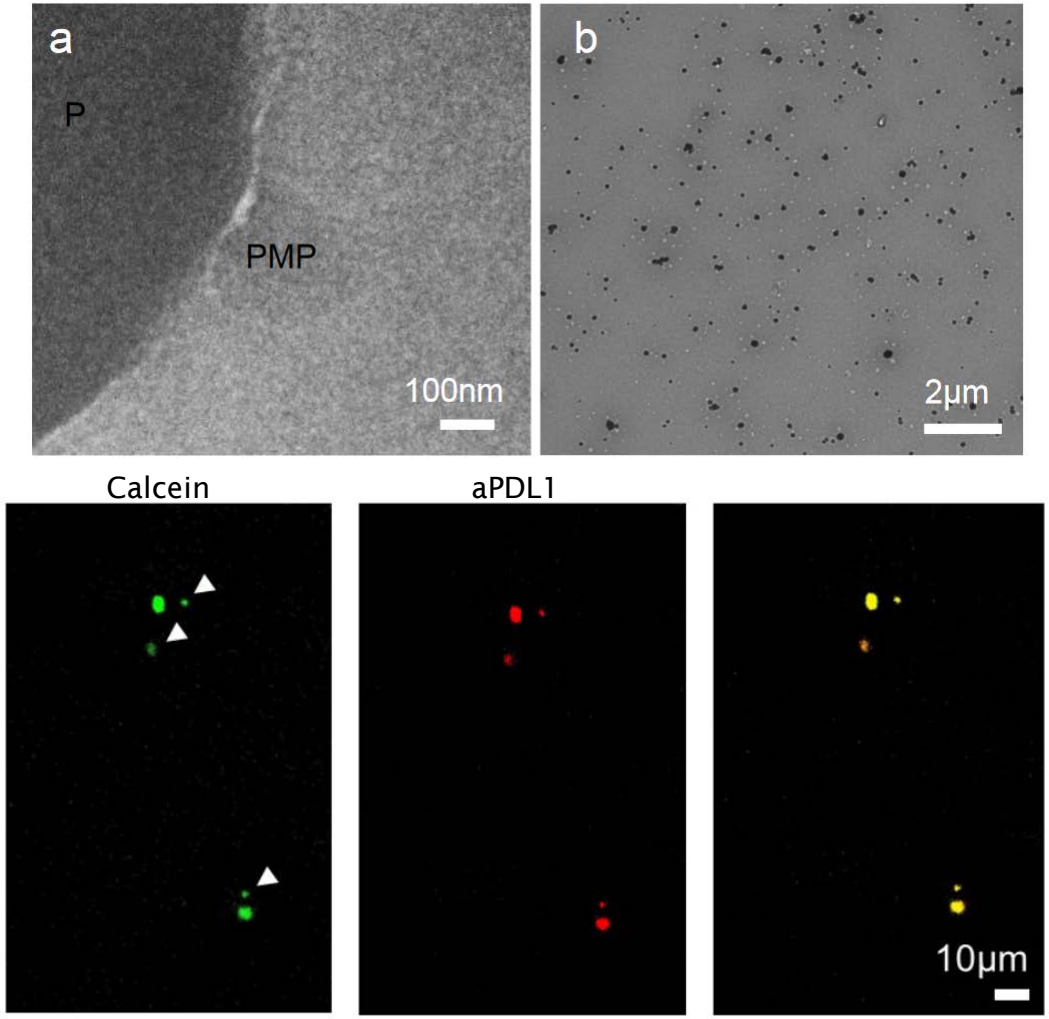


Results – Efficiency, Stability, Surface proteins

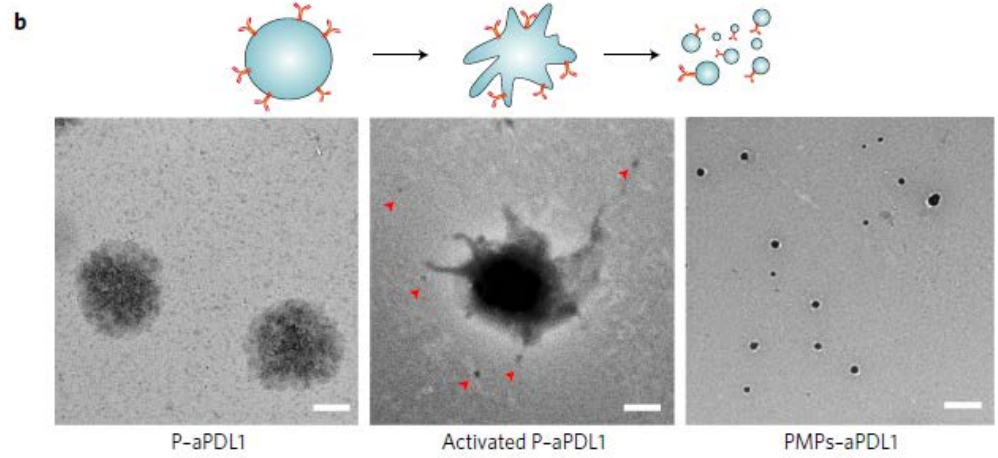


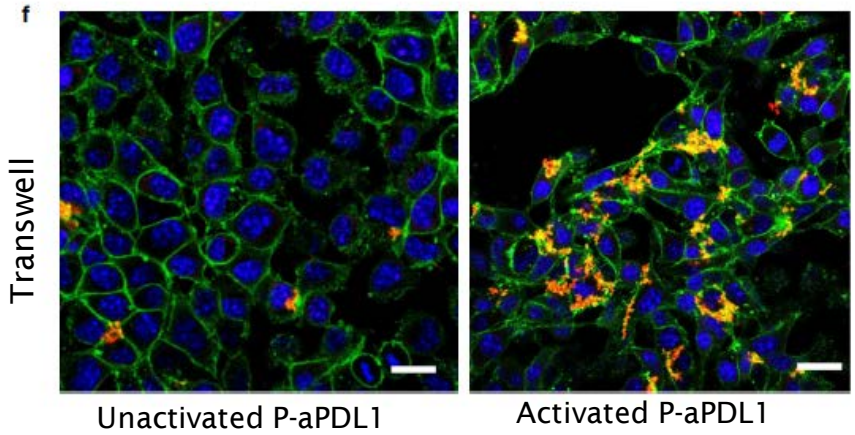


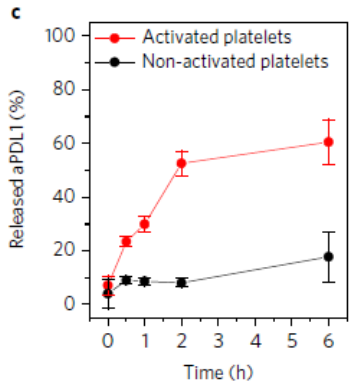
Results – Platelet activation I

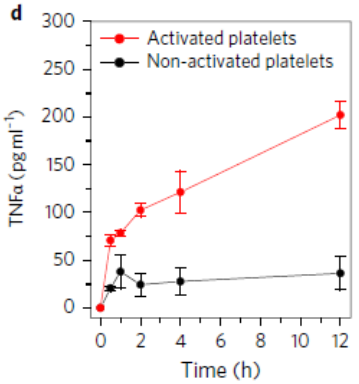


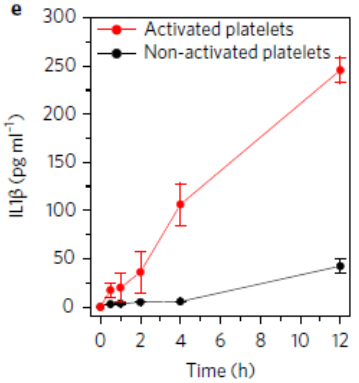
Platelet activation II



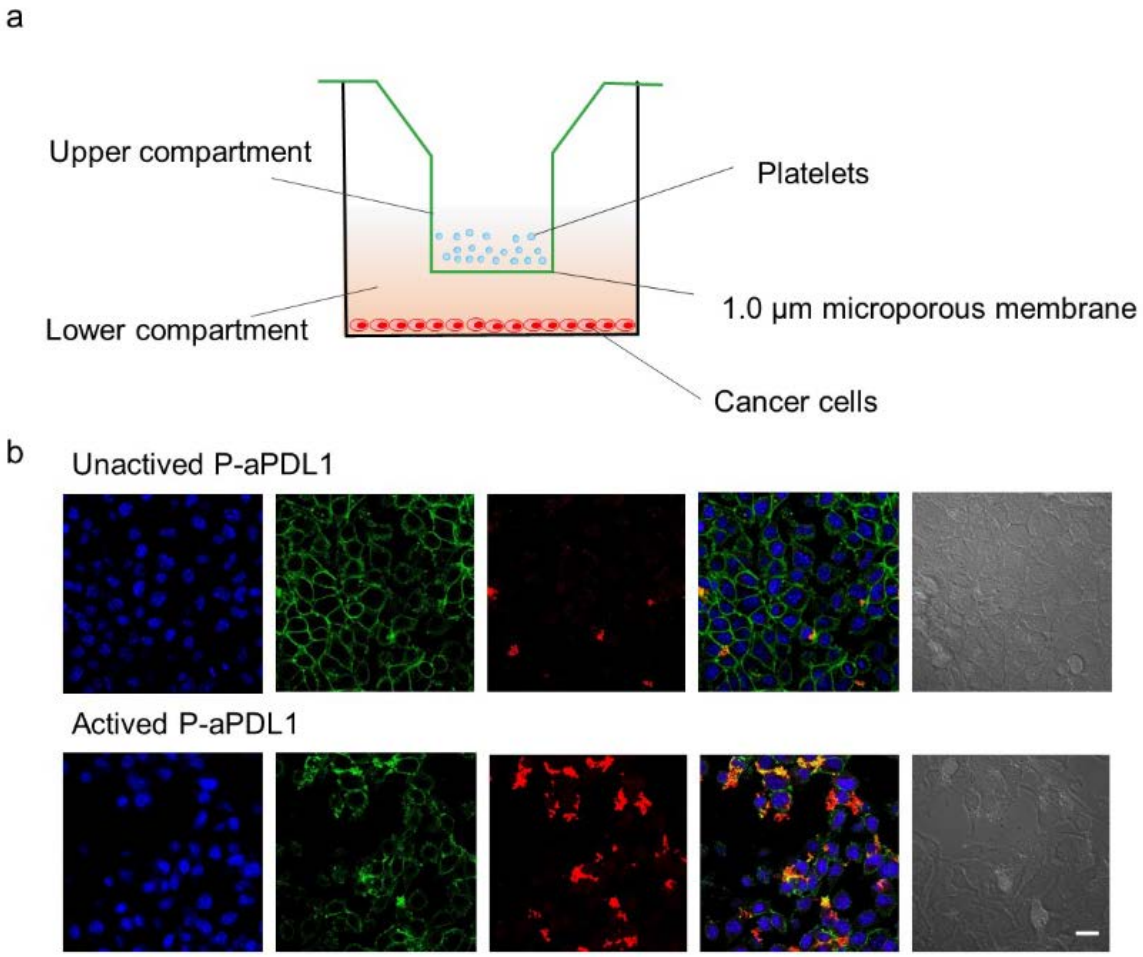




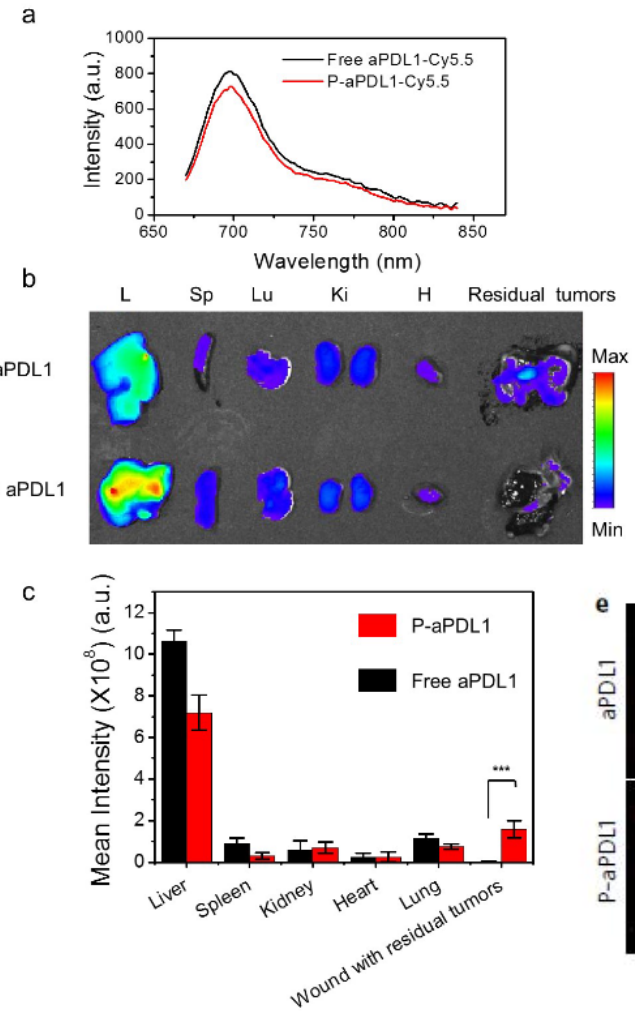


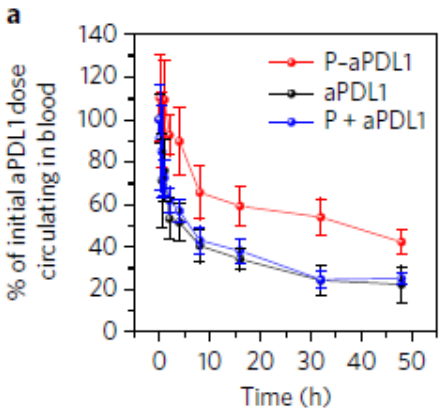


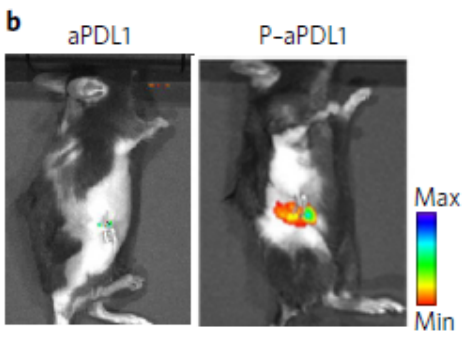
Transwell experiment

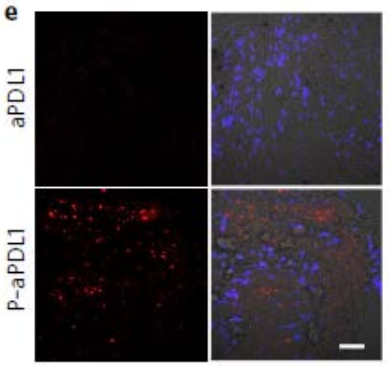


Pharmacokinetics

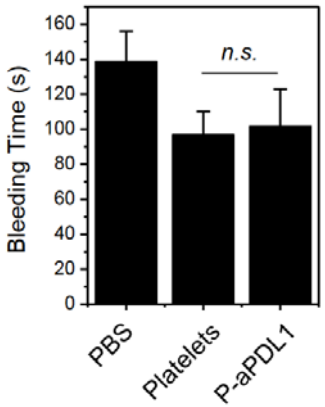




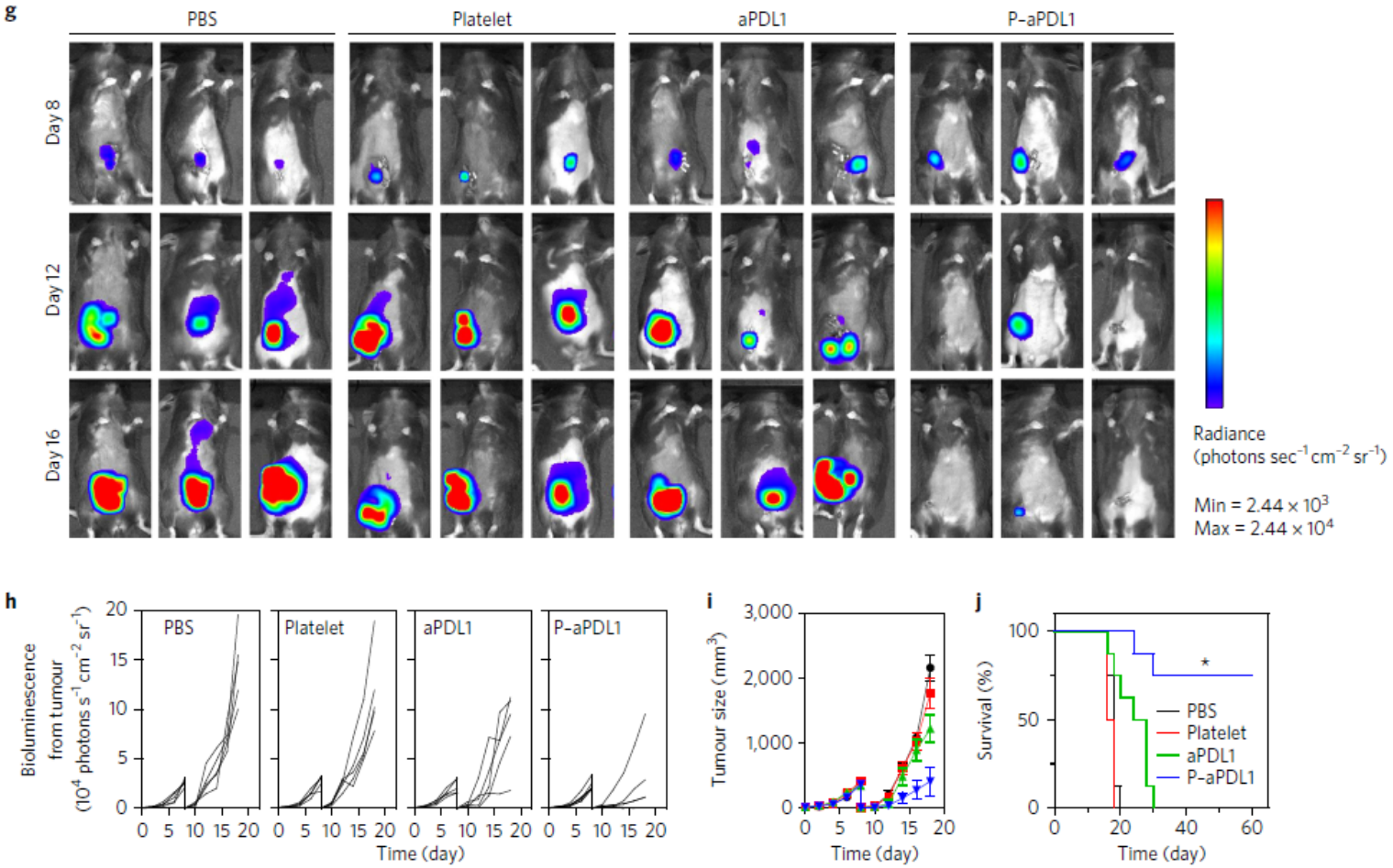




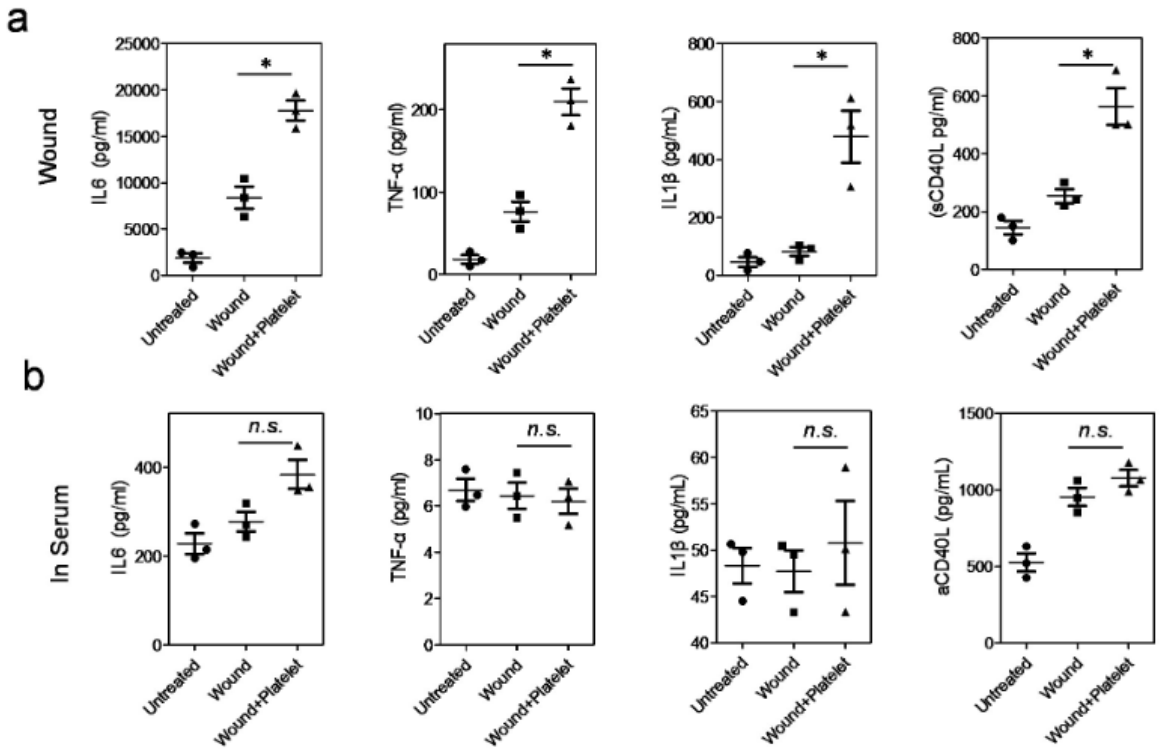
Haemostatic effect



I. *In vivo* therapy of incomplete resection

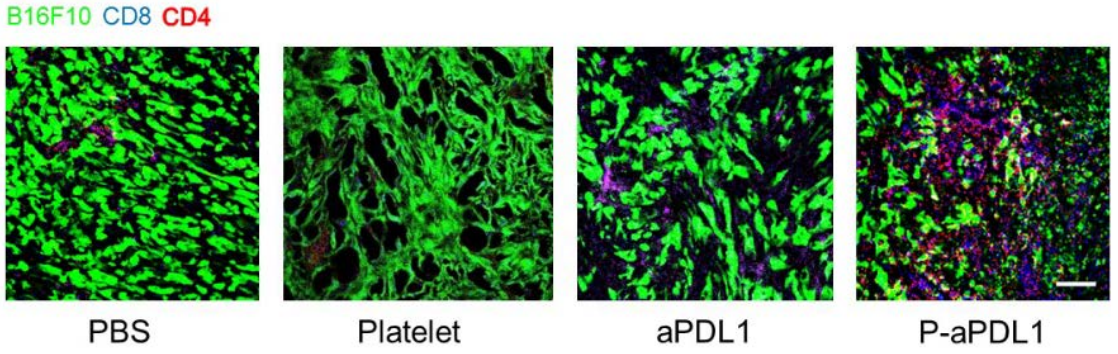


Local and distant inflammation and immune infiltration

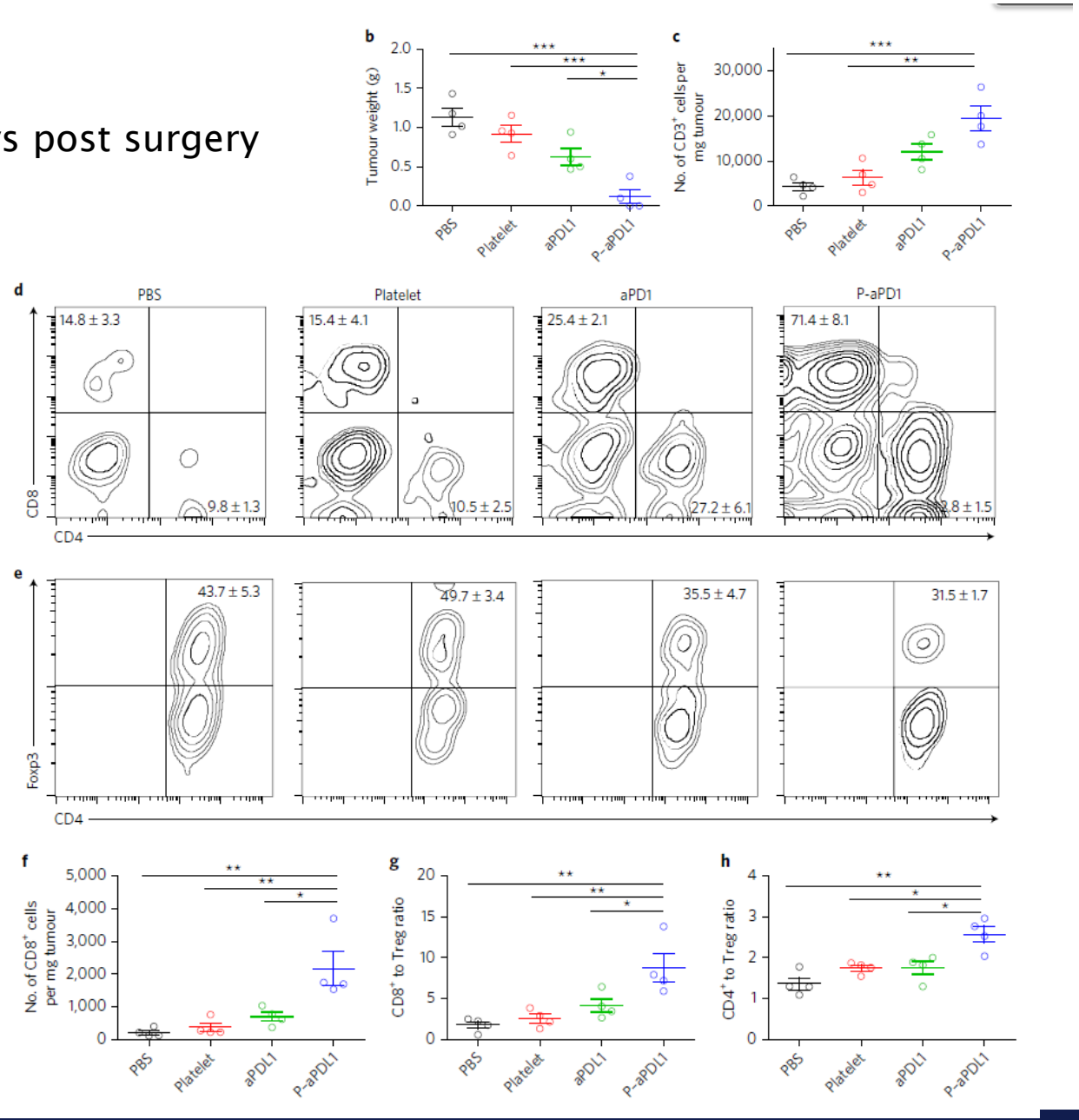


6h post injection

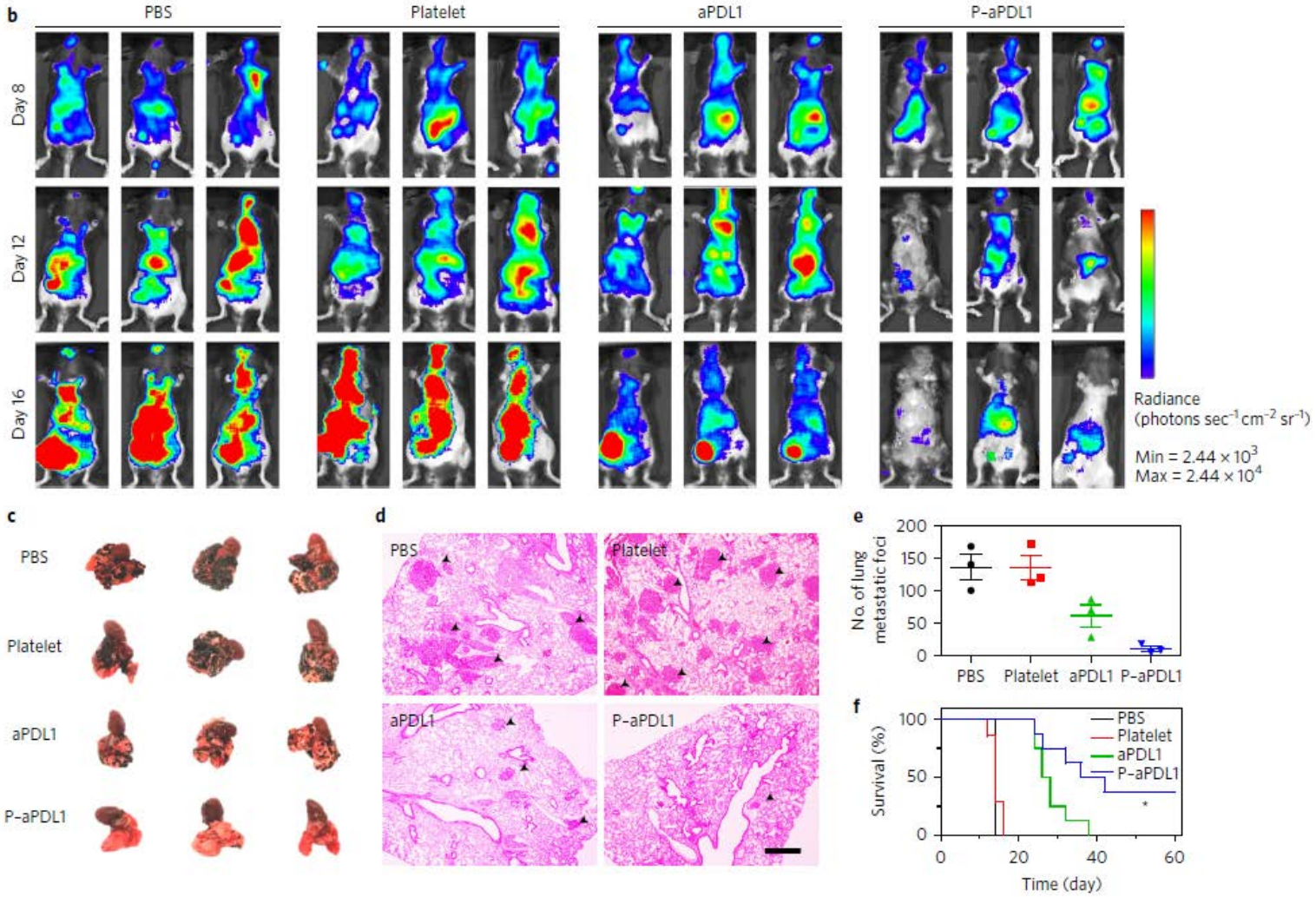
tumor
14 days post surgery

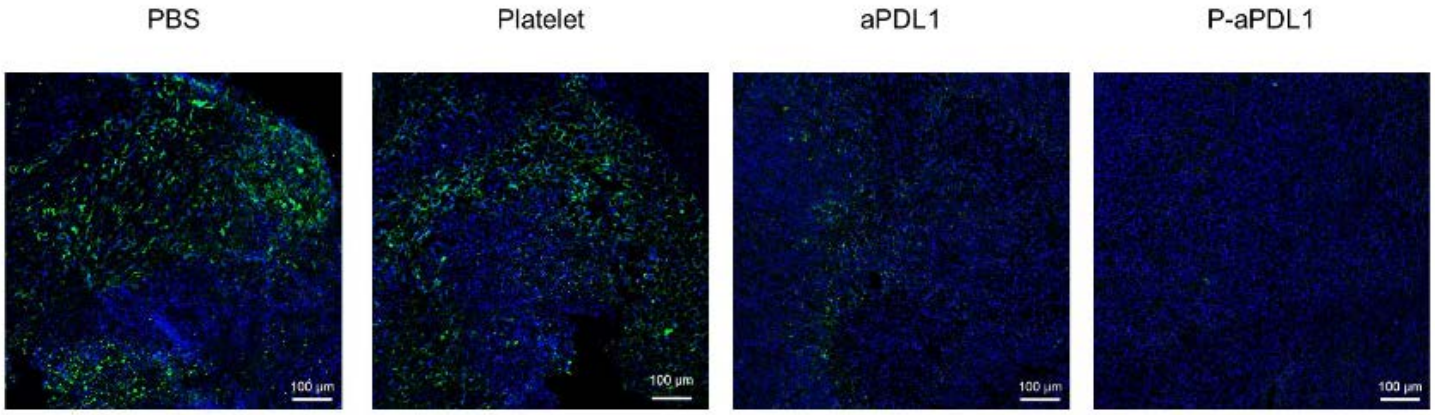


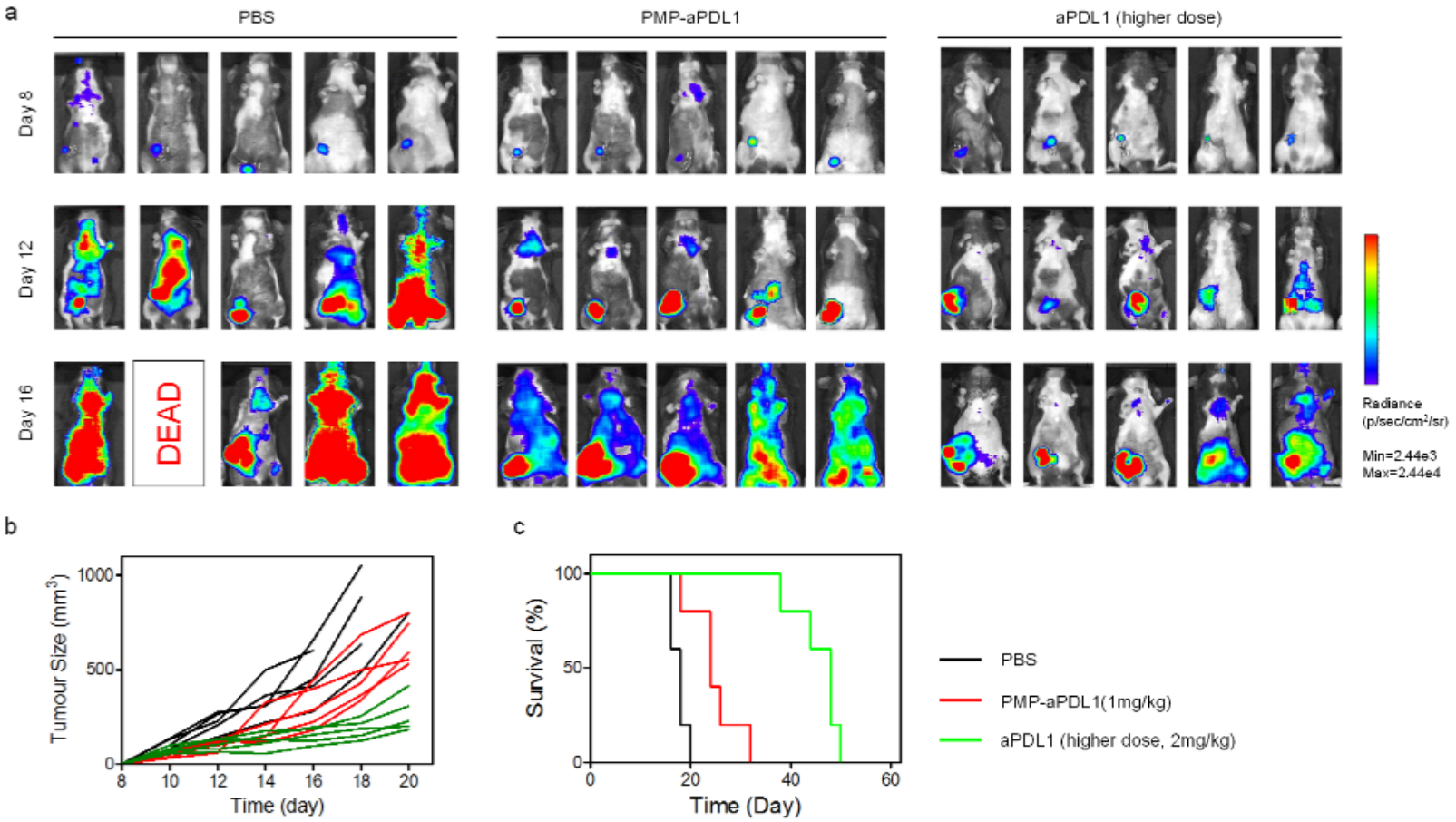
14 days post surgery



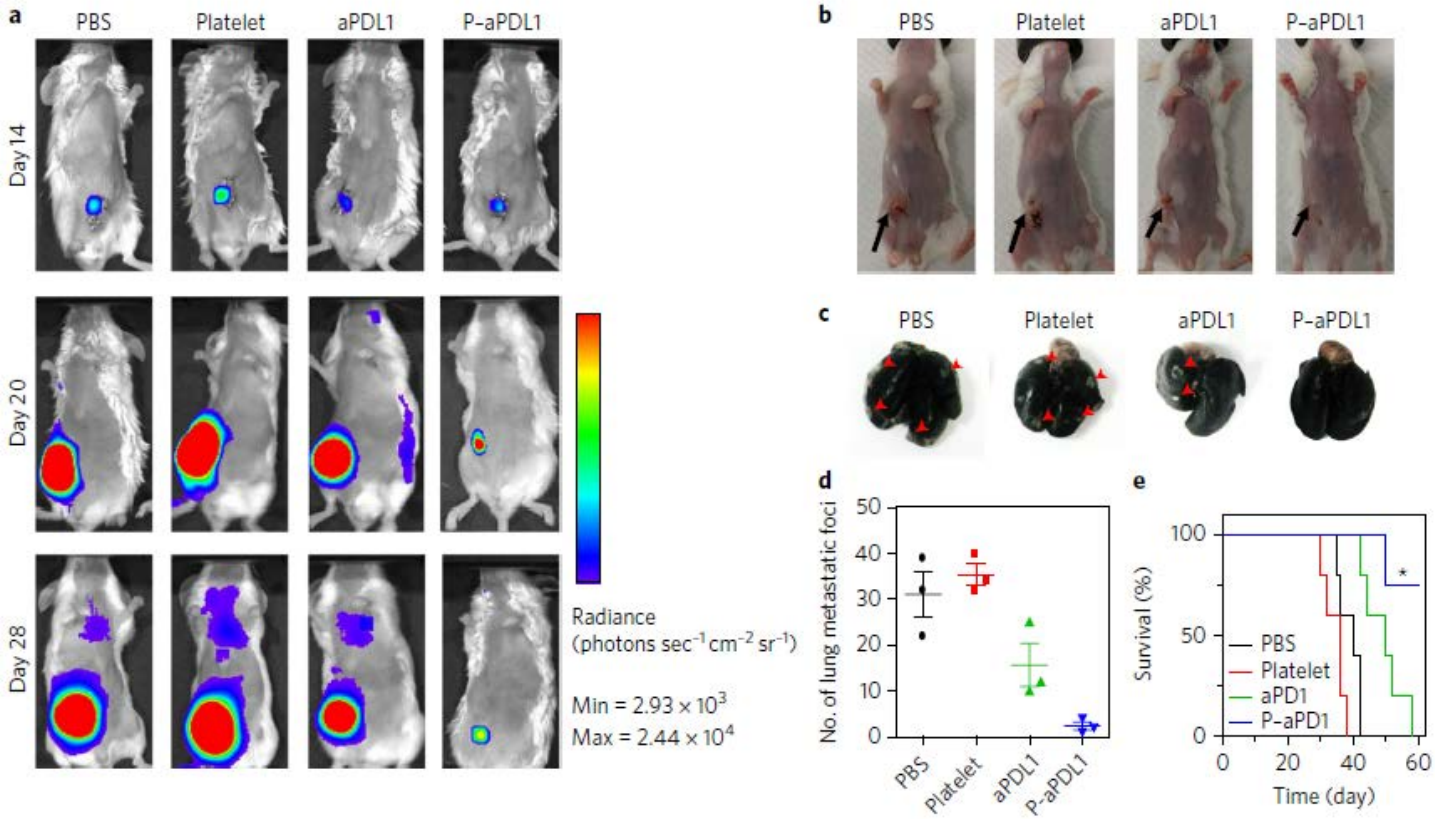
II. Therapy model of incomplete resection and metastasis




PBS
Platelet
aPDL1
P-aPDL1
100 µm
100 µm
100 µm
100 µm
B16F10
Nucleus


a
PBS
PMP-aPDL1
aPDL1 (higher dose)
Day 8
Day 12
Day 16
DEAD
Radiance (p/sec/cm²/sr)
Min=2.44e3
Max=2.44e4
b
Tumour Size (mm³)
1000
500
0
8
10
12
14
16
18
20
Time (day)
c
Survival (%)
100
50
0
0
20
40
60
Time (Day)
PBS
PMP-aPDL1(1mg/kg)
aPDL1 (higher dose, 2mg/kg)

III. Therapy model of a recurrent triple negative 4T1 tumor



Conclusion

- Residual tumor cells after surgery were greatly reduced by platelets conjugated to aPDL1
  - Effective and stable conjugation of platelets to aPDL1
  - Activation of platelets and release of aPDL1 in surgical wound
  - Reduction of residual tumor cells as well as metastasis