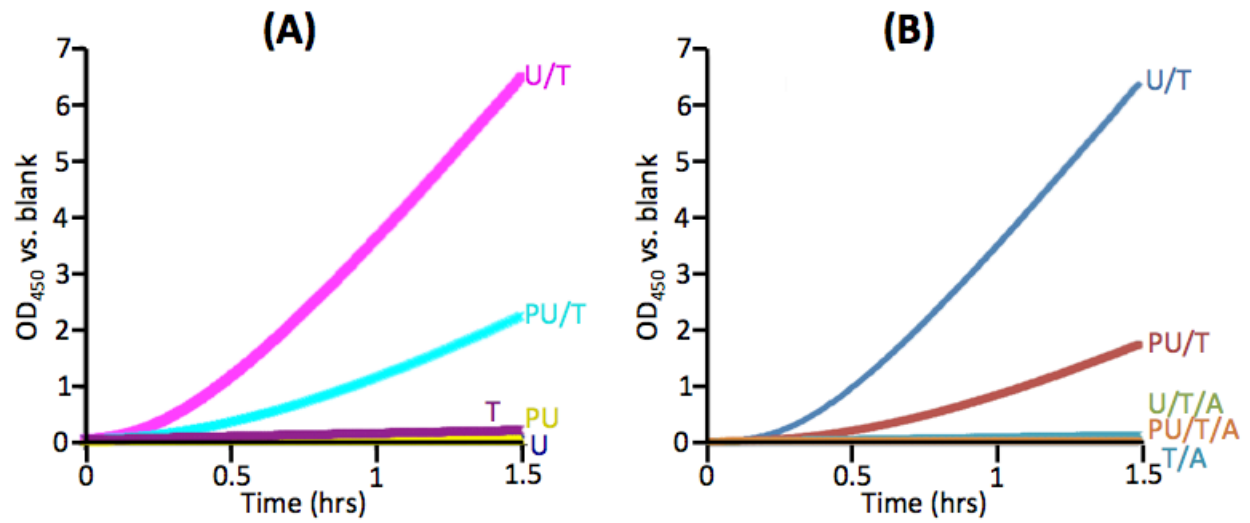


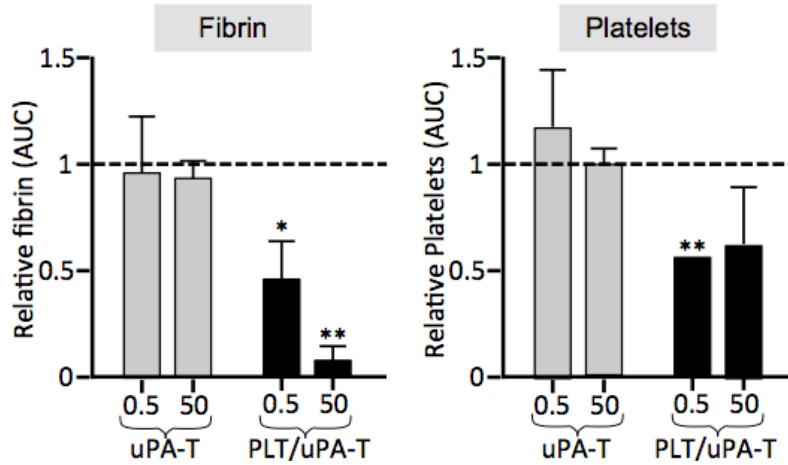
Supplement Figure 1. Characterization of the 312.8 moAb.

(A) A series of moAbs that are anti- α IIb-specific were tested for their ability to bind to platelets. The black line represents the 312.8 moAb used in constructing the PLT/uPA-T. (B) Platelet aggregation studies are shown. 312.8 moAb was added at 2 μ g/ml to platelet-rich plasma prior to the addition of ADP (final concentration, 10 μ M).



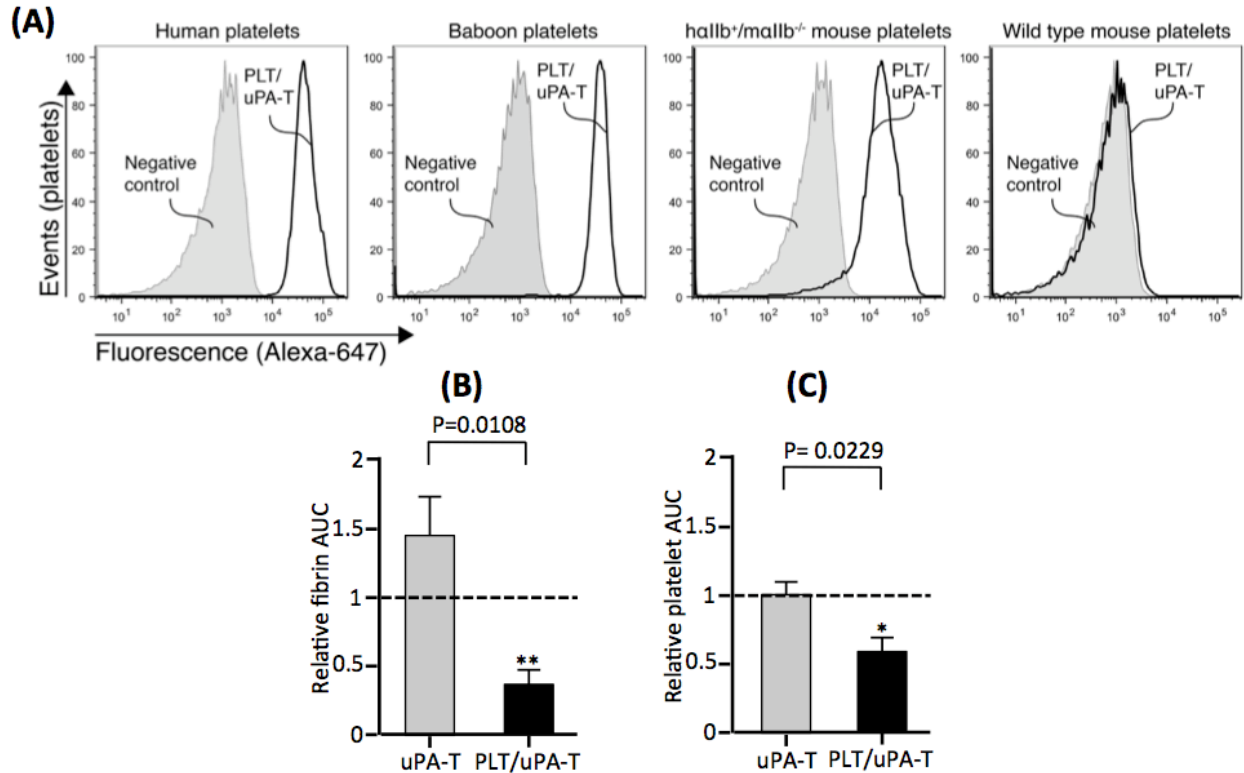
Supplement Figure 2. Thrombin/antithrombin studies of PLT/uPA-T.

(A) Kinetics of 1 nM thrombin (T)-mediated activation of 10 nM uPA-T (U) or PLT/uPA-T (PU) using a chromogenic assay as described in Methods compared to adding thrombin alone. Mean of N = 3 studies is shown. **(B)** Same as A, but with and without added antithrombin (A) at a T:A activity Units ratio of 1:2.



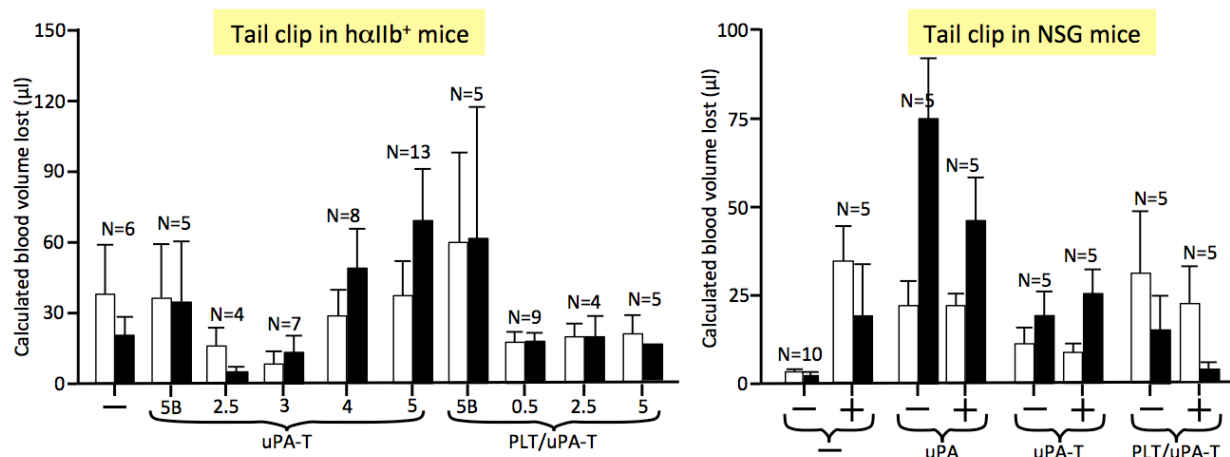
Supplement Figure 3. Microfluidic studies of PLT/uPA-T using HIT activation.

(A) Relative fibrin accumulation and (B) platelet deposition in the microfluidic channel of reconstituted “whole” blood activated by inducing a HIT-like state with platelet-bound prodrug is shown compared to pre-incubation with PBS (dotted line). Means \pm 1 SEM are shown. N = 5 independent experiments, each done in duplicate. Data analysis done by Student t-test of drug-treated mice compared to PBS controls. * = $p \leq 0.05$ and ** = $p \leq 0.01$, both compared to PBS.



Supplement Figure 4. Microfluidic studies of PLT/uPA-T using baboon blood.

(A) Flow cytometric studies of PLT/uPA-T binding to human, baboon, $ha11b^+$ and WT mice platelets. The data shown are representative of three studies. (B) and (C) Study as in Figure 3, but with baboon reconstituted blood showing again that platelet-bound PLT/uPA-T can significantly inhibit fibrin deposition and platelet accumulation, respectively, at the site of injury, supporting the potential use of this species for in vivo efficacy studies of PLT/uPA-T. N = 5 independent experiments, each performed in duplicate. Data analysis done by Student t-test of drug-treated mice compared to PBS controls. * = $p \leq 0.05$ compared to PBS. ** = $p \leq 0.01$ compared to PBS.



Supplement Figure 5. Absolute volume of blood loss during tail clip studies.

Absolute data corresponding to the relative blood loss shown in Figure 6B for the hαIIb⁺ mice (left) and in Figure 6D for the NSG mice infused with human platelets (right). Absolute volume of blood lost following tail clipping as described in the paper with the tail held in 2 ml of 37°C water for ten minutes (open bars) after which the prodrug was started and the tail was then placed in a fresh 2 ml bath for 30 minutes (closed bars). The indicated prodrug was infused at the indicated mg/kg dose both as a bolus and as a 30-minute infusion except for the samples labeled “5B” where the animals received a bolus only of 5 mg/kg of PLT/uPA-T with no subsequent 30-min infusion. “-” = no infused human platelets into the mice, while “+” = infused human platelets. Mean ± 1 SEM are shown. Above each pair of bars is the number of mice studied under each condition.

Supplement Videos

In all the videos, cremaster laser arteriolar injuries are shown. Odd-numbered Supplemental videos show calcein-loaded human platelets (green) and murine platelets detected by Alexa Fluor 647–conjugated rat anti-mouse CD41 Fab fragments (red). Even-numbered Supplemental videos show murine platelets labeled by Alexa Fluor 488–conjugated rat anti-mouse CD41 Fab fragments (red). Incorporated fibrin was detected using an Alexa Fluor 647–labeled anti-fibrin Ab (blue) with overlap in magenta. Shown are 3-minute films of the original injuries. Pre-existing clots were revisited 30 minutes after original injury and filmed for an additional 10 seconds for comparative purposes.

SupplVids 1 through 6.

Nascent clot studies. Three minute-videos of laser injuries done following a bolus/infusion of PBS (SupplVids 1 and 2), uPA (5 mg bolus and infusion, SupplVids 3 and 4) and PLT-UPA-T (0.125 mg bolus and infusion, SupplVids 5 and 6).

SupplVids 7 through 18.

Pre-existing clot studies. Videos of laser injuries done prior to a bolus/infusion of PBS (SupplVids 7 and 8) and a shorter second video during the drug infusion phase of the same clot (SupplVids 9 and 10). SupplVids 11–14 are like SupplVids 7–10, respectively, but for uPA studies. Likewise, SupplVids 15–18 are also like SupplVids 7–10, respectively.