A Unique Recombinant Fluoroprobe Targeting Activated Platelets Allows *In Vivo* Detection Of Arterial Thrombosis And Pulmonary Embolism Using A Novel Three-Dimensional Fluorescence Emission Computed Tomography (FLECT) Technology

Supplementary Data

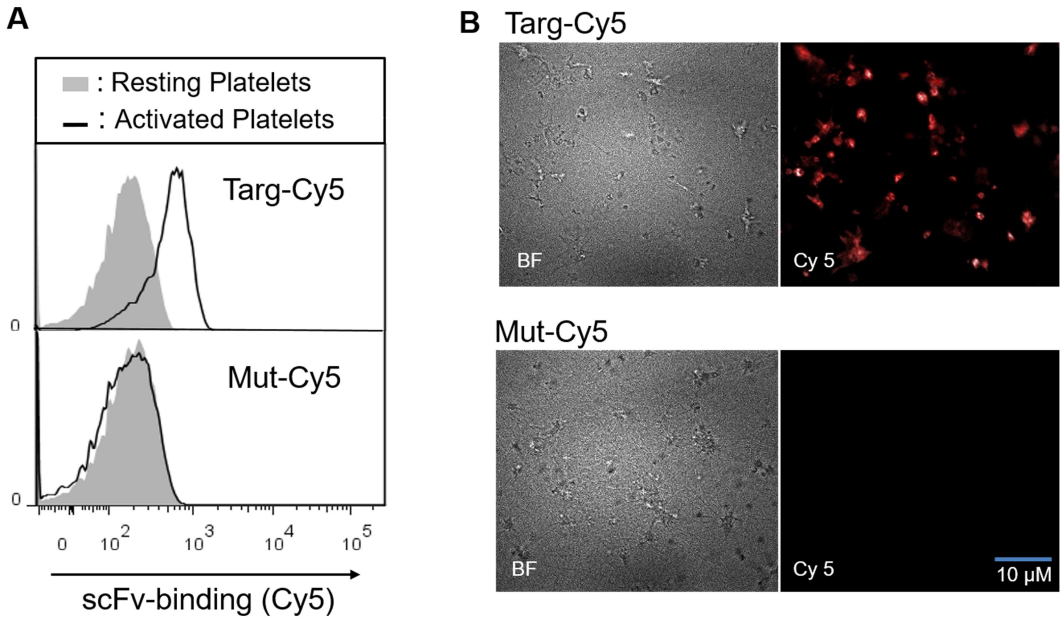

Figure S1. Specific binding of Targ-Cy5 probe to activated platelets shown using flow-cytometry and fluorescence microscopy. Targ-Cy5 and the mutated scFv control (Mut-Cy5) probes were generated based on the same two-step conjugation system used for Targ-Cy7 probe. A) Flow-cytometry was performed on both resting and ADP-activated platelets after the addition of 1 µg/mL of either Targ-Cy5 or Mut-Cy5 fluoroprobe. Following the assay, binding of the fluoroprobe was determined by the levels of detected Cy5 fluorescence. Specific binding of Targ-Cy5 was only observed on activated-platelets and not resting platelets, while Mut-Cy5 control did not bind either. B) Bright-field (BF; left panel) and Cy5-fluorescence (Cy5; right panel) microscopy was performed on ADP-activated platelets in which only Targ-Cy5 probe bound to these cells and not the Mut-Cy5 probe. No binding of both Targ-Cy5 and Mut-Cy5 was noted on resting platelets (data not shown).

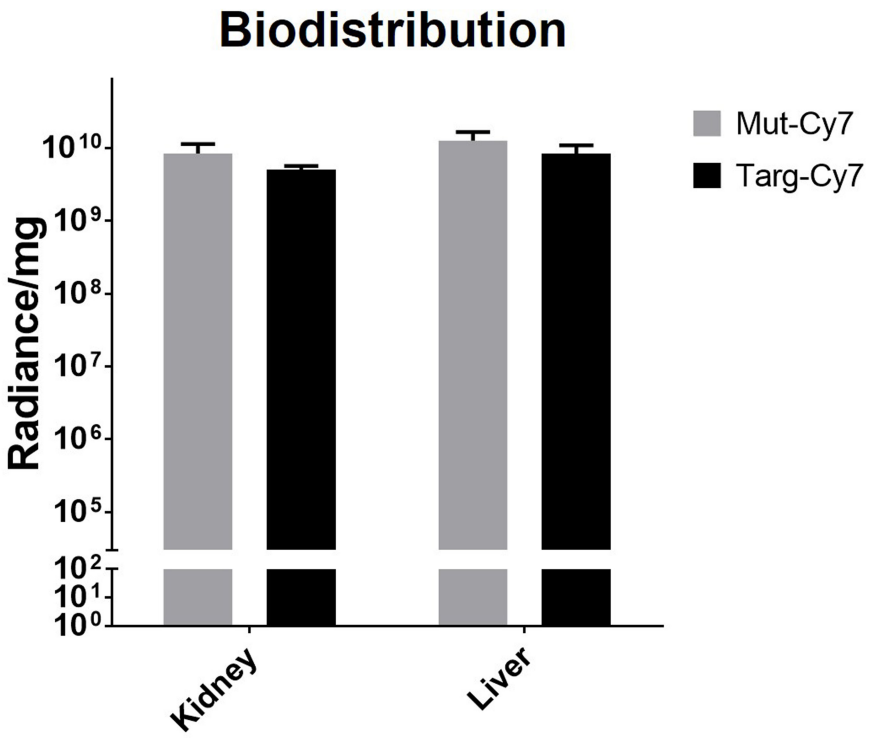

Figure S2. Biodistribution of the NIR fluoroprobe uptake in the kidney and liver of left-carotid injury mice (Figures 3&4) following FLECT scan. IVIS scan was performed on these organs to detect and quantify levels of *ex vivo* Cy7 light radiance. Uptake in each organs was calculated by dividing the detected radiance with the mass of each organ (n=6 for each group). Non-significant differences were noted between Mut-Cy7 and Targ-Cy7 groups in both organs when analyzed using two-way ANOVA analysis followed by Sidak's multiple comparison between these groups where $p = 0.654$ for kidney and $p = 0.5222$ for liver.

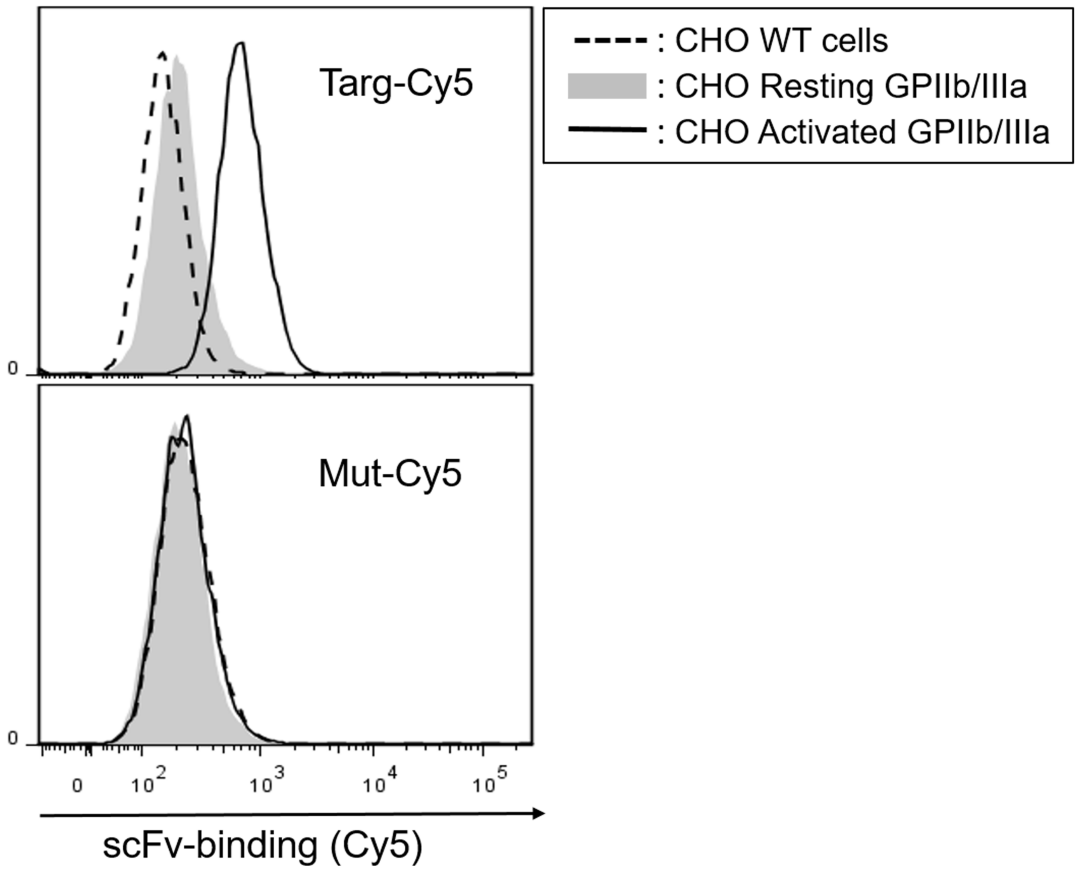

Figure S3: Specific binding of Targ-Cy5 fluoroprobe on transfected CHO cells expressing activated GPIIb/IIIa receptors on their surface as compared to cells expressing the resting receptors and wild-type CHO cells. Flow-cytometry was performed on wild-type CHO and transfected-CHO cells expressing either activated or resting GPIIb/IIIa (see ref 21) on their surface. One million cells were added with 1 µg/mL of either Targ-Cy5 or Mut-Cy5 fluoroprobe for 30 minutes before they were washed 3 times. Flow-cytometry was then performed to examine the levels of Cy5-fluoresence indicating the binding of the fluoroprobe. Binding was only detected on CHO-cells expressing activated GPIIb/IIIa after incubation with Targ-Cy5, while Mut-Cy5 did not bind to any of the cell-types.

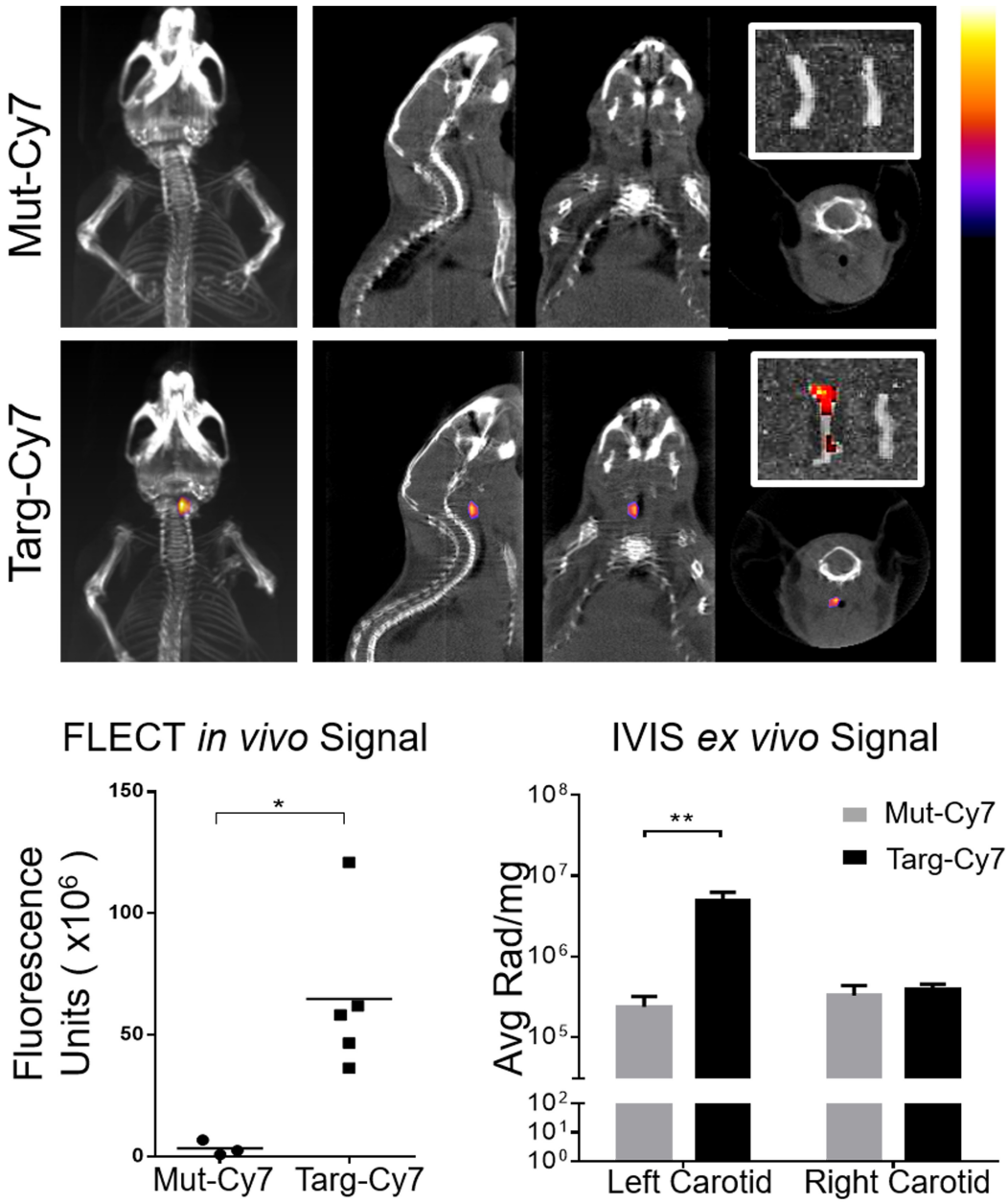

Figure S4: FLECT/CT *in vivo* and IVIS *ex vivo* tissue imaging of mice with fully occluding thrombi in their left carotid artery. Complete thrombotic occlusion was achieved using similar method as the non-occlusive thrombosis model (described in the main report) with the modification of exposing the left carotid artery for 5 minutes to 10% ferric chloride. This method results in the complete occlusion of the injured vessel with a complete flow cessation as determined by a Doppler ultrasound probe

(0.5VB; Transonic, Japan). Following full-occlusion of the left carotid artery, the FLECT-fluroprobe was injected intravenously and allowed to circulate before *in vivo* FLECT/CT scans and *ex vivo* tissue IVIS scans were performed as described for Figures 3 and 4. FLECT/CT scanning detects fluorescence Targ-Cy7 signal on the site of arterial occlusion (n=5) but not in mice injected with Mut-Cy7 (n=3) (top figure). Quantification of the FLECT signals revealed significantly higher levels of fluorescence intensity in mice injected with Targ-Cy7 than Mut-Cy7 (Mann-Whitney nonparametric test, p= 0.0357) (bottom left). After live FLECT/CT scanning, mice were culled and *ex vivo* IVIS imaging of both the completely occluded left carotid and the right healthy carotid arteries were performed. Fluorescence signal was only detected on the left carotid vessel in Targ-Cy7 injected mice (top figure; left boxes for each group of mice). Further quantification of the light radiance showed significantly higher levels detected in the left injured arteries of mice injected with Targ-Cy7 as compared to Mut-Cy7, while the healthy right vessels are similarly low in both groups (two-way ANOVA-Sidak's multiple comparison test between Targ-Cy7 vs Mut-Cy7 where p= 0.0094 and p= 0.9988 for the left and right carotid artery, respectively) (bottom right). For both FLECT *in vivo* and IVIS *ex vivo* signals, there is no significant correlation between the levels of detected Targ-Cy7 in the left artery and the weight of the thrombus (data not shown; Pearson's correlation test was used where r = 0.5921, p = 0.2928 and r = 0.3497, p = 0.564 for FLECT and IVIS signals, respectively.)