Supplementary Information

Figure S.1. Schematic representation of production of platelet-derived microparticles (PMP) from activated platelets (AP) in the thrombus milieu; PMPs have active integrin GPIIb-IIIa (CD41a) and P-selectin (CD62P) on their surface which facilitates binding to APs (and leukocytes) via specific interactions with fibrinogen and P-selectin Glycoprotein Ligand-1 (PSGL-1) respectively; PMPs also have pro-coagulant phosphatidylserine rich surface that facilitates complexation and activation of coagulation factors, ultimately augmenting thrombin (Factor IIa) and fibrin generation.

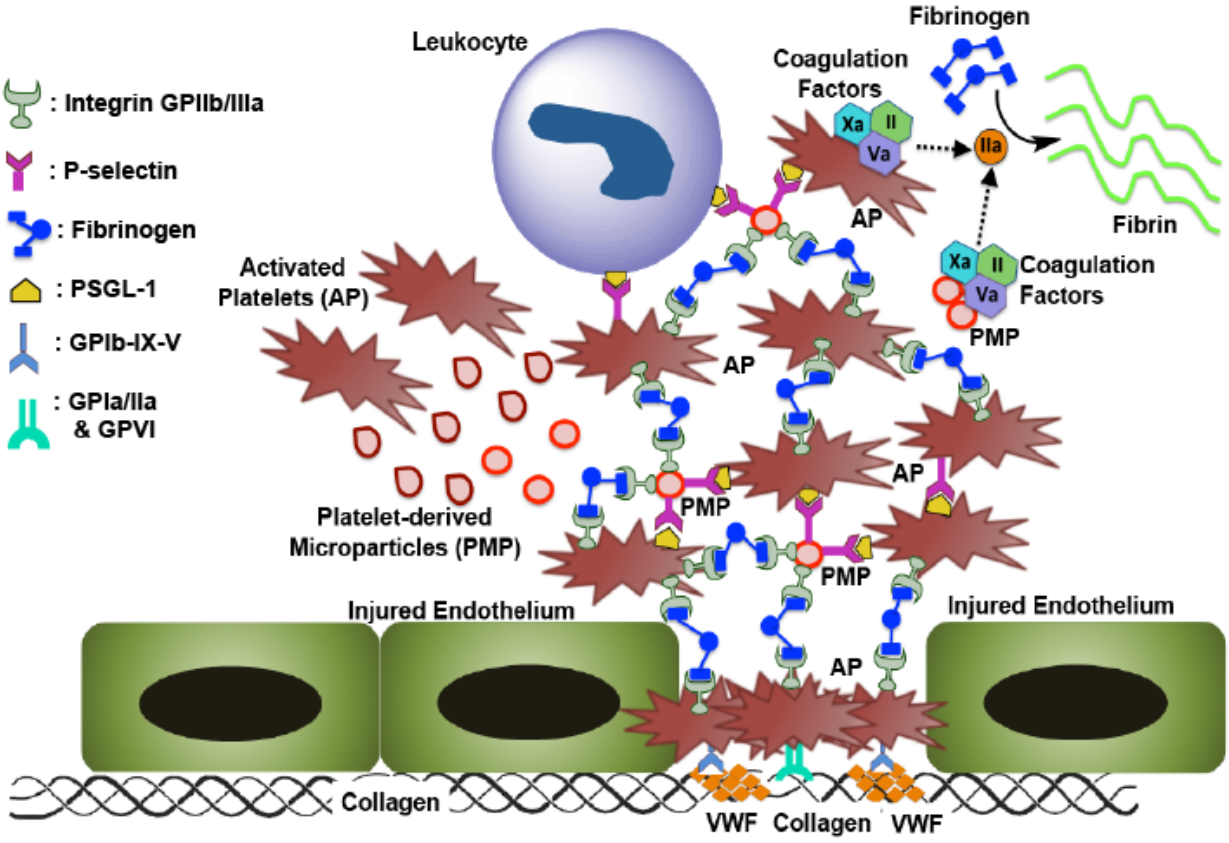


Figure S.2. Representative SEM images of activated platelets adhered on collagen-coated surface upon incubation with 'washed platelet' suspension (left panel) compared to incubation with platelet-rich plasma (PRP). As evident from the images, both incubation approaches result in high density of active platelets on the collagen surface. However the incubation with 'washed platelet' suspension results in adherent platelets adjacent to each other in almost a monolayer with 'spread platelet' pseudopodal morphology clearly showing, while the incubation with PRP results in dense clusters of platelets along with presence of fibrin and plasma artifacts, were the 'spread platelet' pseudopodal morphology are not as clearly visible but the central bodies of platelets are prominently visible. Our in vitro PPFC-based binding studies utilized platelet-rich collagen surfaces where the incubation was done in presence of PRP, because these clots are physiologically/pathologically closer in composition, compared to that from washed platelets.

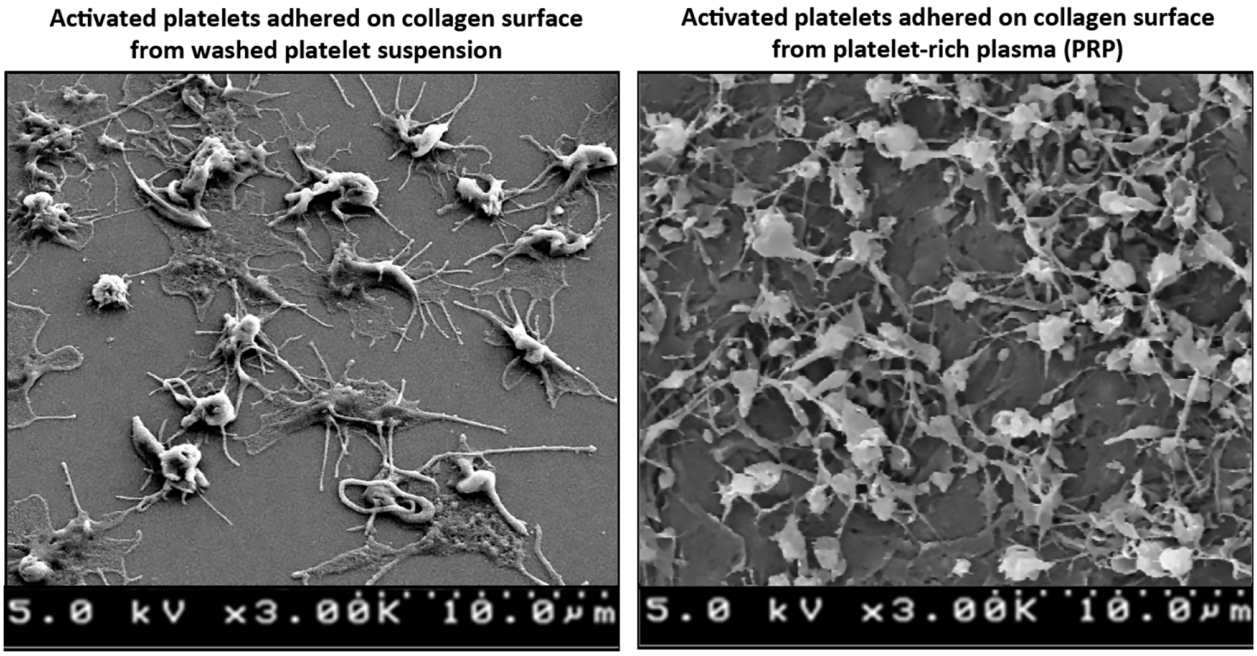
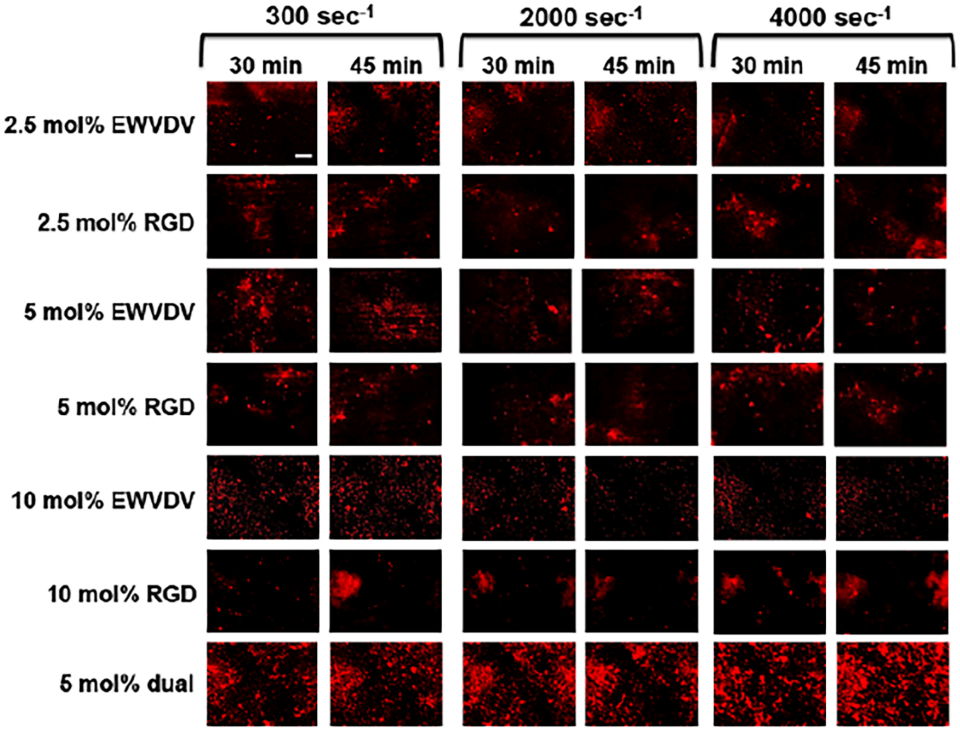


Figure S.3. Representative fluorescence images for Rhodamine-B labeled single peptide modified vesicles (EWVDV-modified or RGD-modified at 2.5 mol%, 5 mol% or 10 mol% peptide-lipid composition with respect to total lipid) compared to dual peptide modified vesicles (combined modification at 50:50 with both EWVDV and RGD peptides at 5 mol% peptide-lipid composition with respect to total lipid), binding to platelet-rich region (simulating platelet-rich thrombi) in parallel plate flow chamber at 300 sec^{-1}, 2000 sec^{-1} and 4000 sec^{-1} shear rate conditions, along with quantitative data of vesicle binding at the 2000 sec^{-1} condition (corresponding data for 300 sec-1 and 4000 sec-1 shear rate conditions shown in main text Figure 3), show that dual modified (heteromultivalent) vesicles significantly enhances binding (30 min time point) and retention (45 min time point) on activated platelet-rich thrombi.



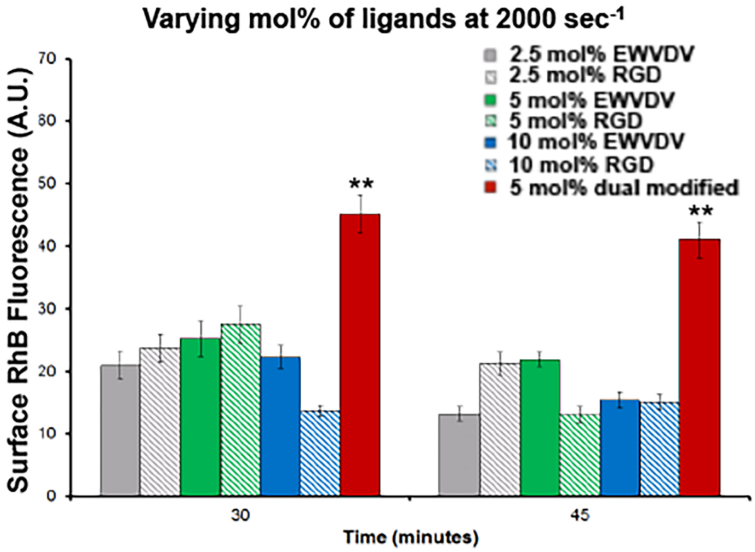


Figure S.4. Surface averaged fluorescence intensity analysis data sets for PPFC-based binding study histograms shown in main manuscript Figure 3C as well as previous Supplementary Figure S.3. The data is shown as Mean ± S.D. for surface fuorescence (Arbitrary Units) analysis for binding studies done at 300 sec-1, 2000 sec-1 and 4000 sec-1 for homomultivalently (2.5 mol%, 5 mol% and 10 mol% of either RGD decoration of EWVDV decoration) and heteromultivalently (total 5 mol% of peptide decoration with 2.5. mol% RGD and 2.5 mol% EWVDV) decorated platelet-targeted nanovesicles.

300 sec^-1	30 min	45 min
2.5% EWVDV	24.33+/-8.13	14.24+/-2.28
2.5% RGD	23.64+/-4.63	16.18+/-3.81
5% EWVDV	20.52+/-3.43	19.95+/-4.77
5% RGD	24.29+/-6.11	17.40+/-4.97
10% EWVDV	34.46+/-6.56	19.37+/-3.90
10% RGD	16.07+/-1.46	21.90+/-2.56
5% dual	53.27+/-13.72	36.69+/-4.30

2000 sec^-1	30 min	45 min
2.5% EWVDV	20.98+/-4.38	13.21+/-2.23
2.5% RGD	23.67+/-4.55	21.21+/-3.47
5% EWVDV	25.17+/-5.67	21.95+/-2.22
5% RGD	27.47+/-5.69	13.06+/-2.63
10% EWVDV	22.29+/-3.63	15.37+/-2.66
10% RGD	13.67+/-1.85	15.03+/-2.50
5% dual	45.18+/-6.10	41.06+/-5.78

4000 sec^-1	30 min	45 min
2.5% EWVDV	19.55+/-6.22	8.83+/-3.95
2.5% RGD	35.79+/-6.07	29.14+/-2.84
5% EWVDV	20.88+/-2.82	22.70+/-3.50
5% RGD	30.80+/-4.31	21.09+/-4.00
10% EWVDV	20.36+/-2.24	20.17+/-4.59
10% RGD	20.92+/-2.45	23.21+/-3.82
5% dual	48.00+/-11.47	38.84+/-7.15

Figure S.5. Representative images of vesicle fluorescence (red from Rhodamine B), brightfield of the surface and platelet fluorescence (green from pre-staining platelets with calcein) of platelet-rich collagen surface in the PPFC experiments exposed to homomultivalently decorated (RGD-only or EWVDV-only) compared to heteromultivalently decorated (both RGD and EWVDV) PMIN vesicles. As seen from brightfield images and green calcein fluorescence, platelets are adhered in clusters on the collagen-coated surface (please refer high resolution SEM in main manuscript Figure 3A and Supplementary Figure S.2. as a further confirmation of such clusters). Yellow arrows show representative one-to-one correspondence of red vesicle fluorescence, brightfield image areas and green platelet fluorescence, confirming that the red vesicle fluorescence is indeed from vesicles specifically bound to clustered platelets and not just non-specific binding to the surface in general. The images further show that homomultivalently decorated (RGD-only or EWVDV-only) vesicles can bind to platelets to some extent (as also shown by our fluorescence intensity histogram data in main manuscript Figure 3C and Supplementary Figure S.4.), but when combined, the resultant heteromultivalently decorated PMINs have a significantly higher extent of vesicles bound to the platelets in cluster (evident from higher intensity of red fluorescence).

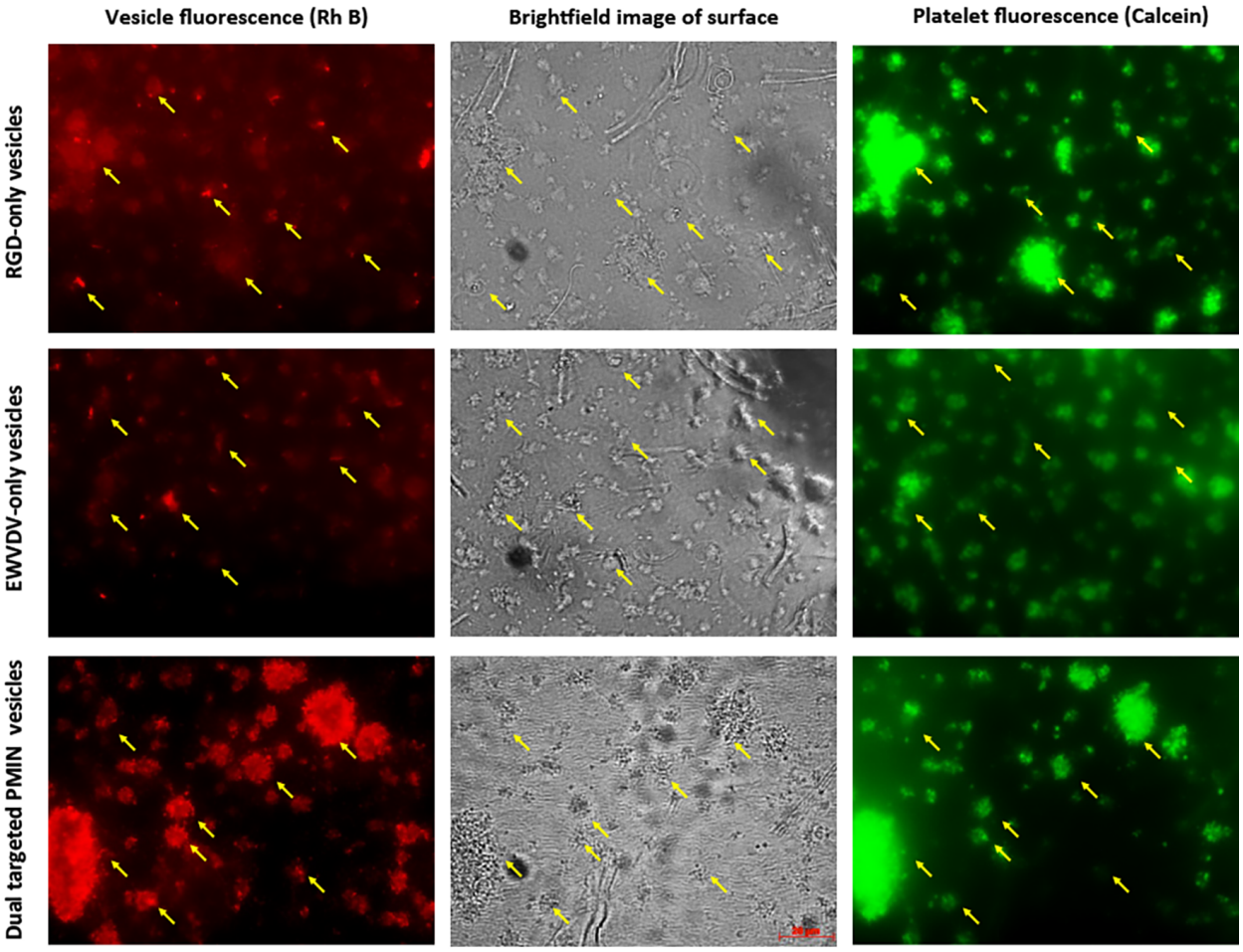


Figure S.6. [A] Representative fluorescence images, and [B]-to-[D] quantitative data for Rhodamine-B labeled dual peptide modified vesicles (combined modification with EWVDV and RGD peptides at 5 mol% peptide-lipid composition with respect to total lipid) bearing varying ratios of the EWVDV:RGD peptide, binding to platelet-rich region (simulating platelet-rich thrombi) in parallel plate flow chamber at 300 sec^{-1}, 2000 sec^{-1} and 4000 sec^{-1} shear rate conditions, indicate that varying the relative ratio of peptides may influence binding at various shear flow conditions but irrespective of shear rate the heteromultivalently decorated systems always have higher binding and retention compared to homomultivalently decorated vesicles.

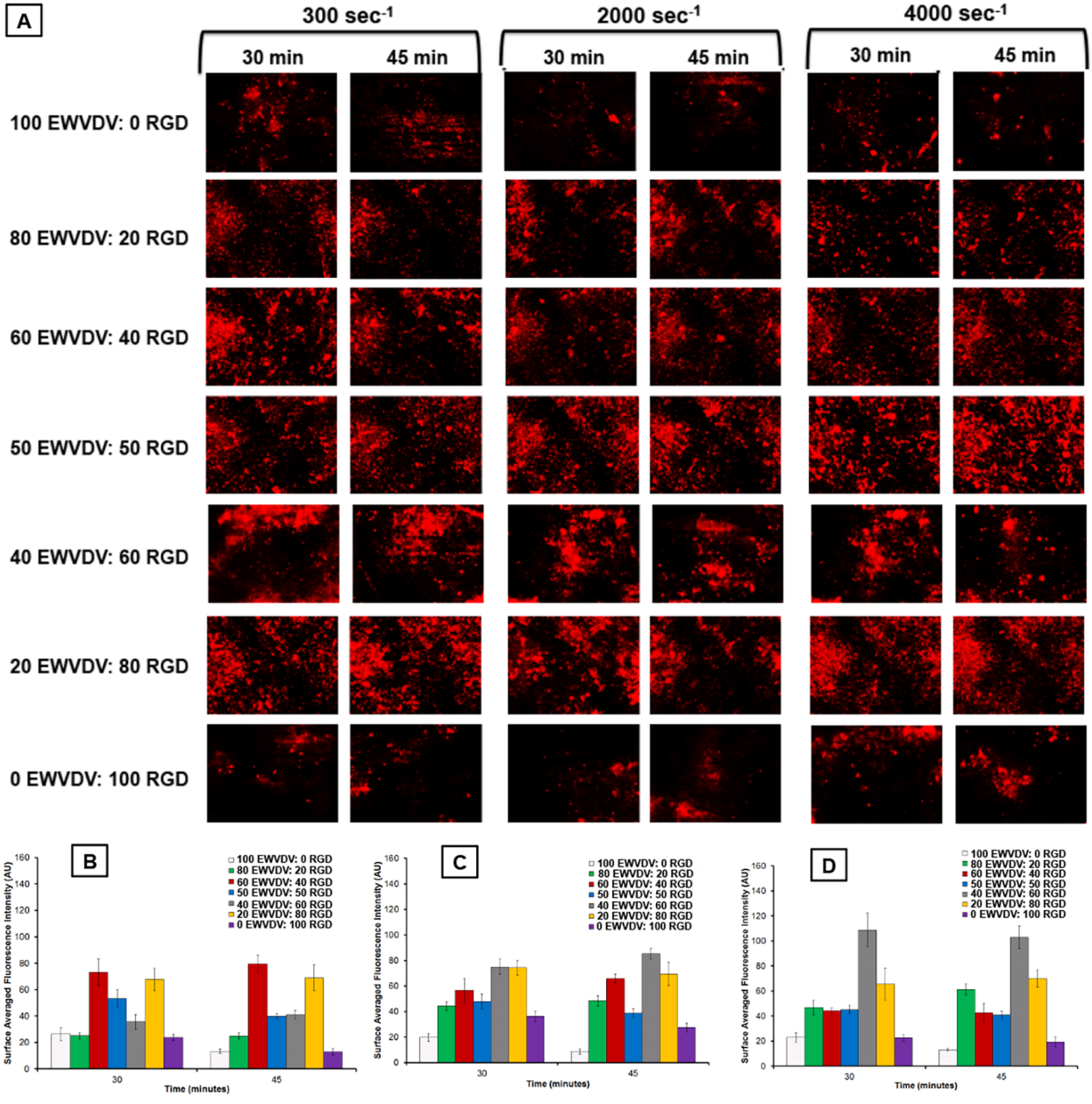
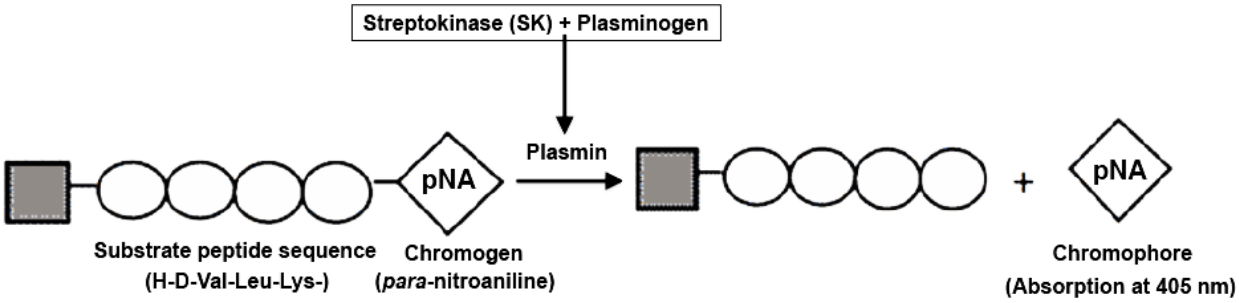
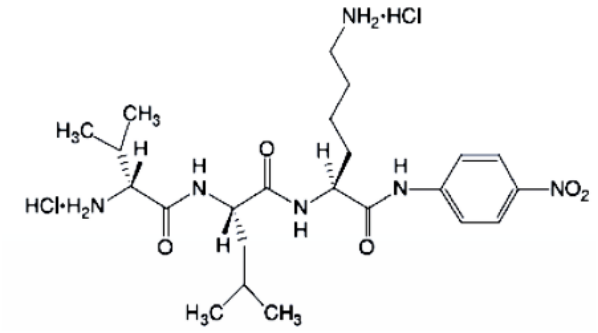


Figure S.7. Reaction schematic of the para-nitroaniline (pNA) based chromogenic assay for analysis of streptokinase (SK) activity and a representative calibration plot developed for this assay based on which the streptokinase release kinetics from the PMINs was determined.





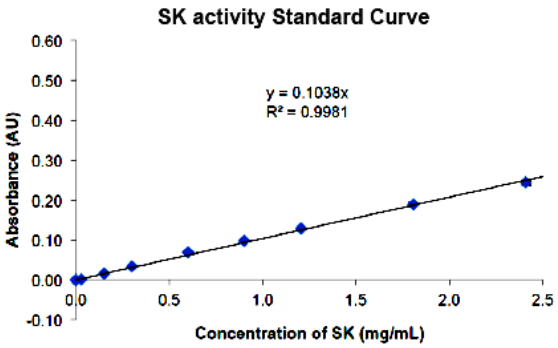


Figure S.8. Representative aggregometry traces for interaction of the peptides (EWVDV peptide or RGD peptide) with mouse platelets show that without agonist (ADP or collagen) addition the peptides themselves show no drastic change in trace compared to saline baseline and the platelet aggregation occurs only after agonist addition, with the aggregation trace for peptide-added samples showing slightly decreased percent (%) aggregation as that for the saline-added sample; this suggests that the peptides themselves do not activate and aggregate resting platelets (hence minimal systemic pro-thrombotic risk) but can interact with receptors of activated platelets to slightly block platelet aggregation possibly due to receptor-inhibiting effect.

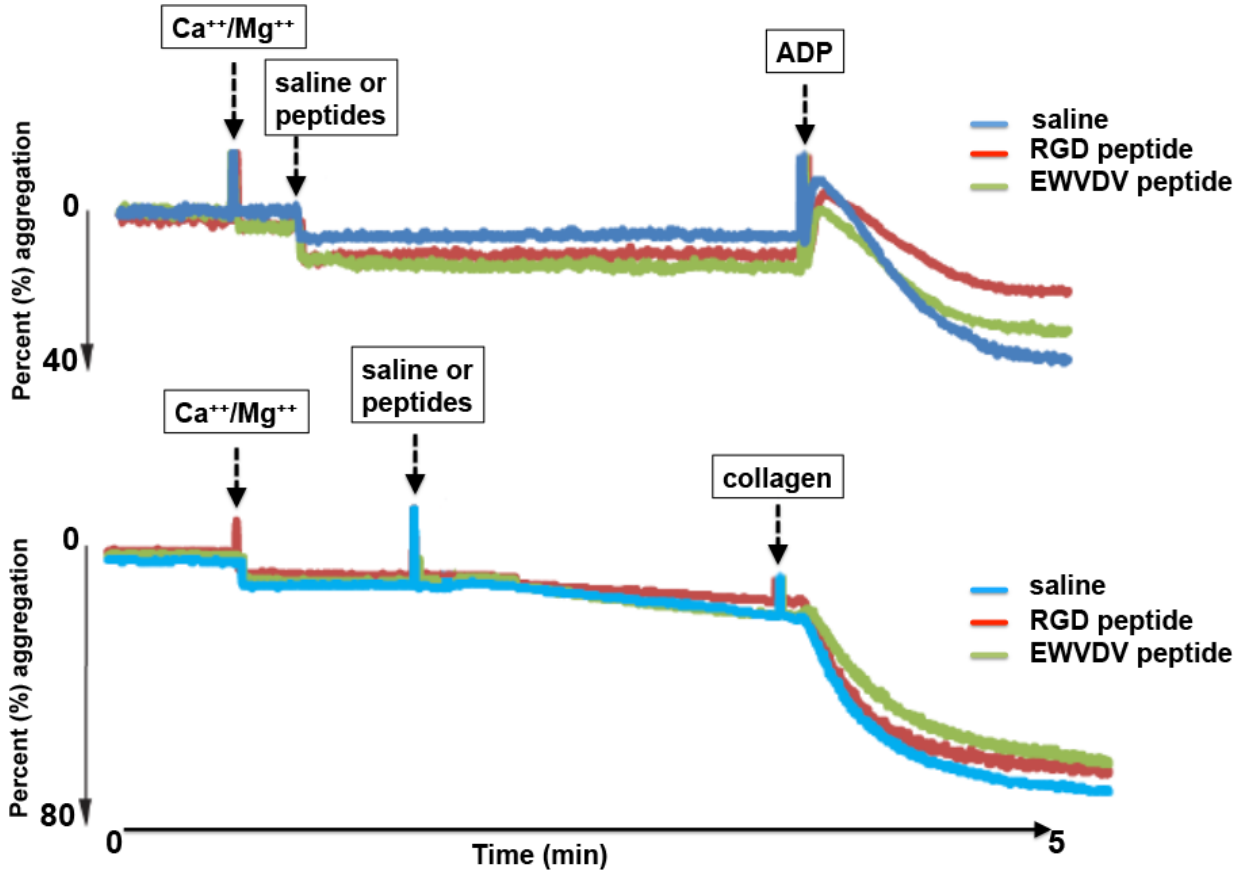


Figure S.9. [A] Representative SEM image of the luminal side of uninjured murine carotid; [B] Representative SEM image of luminal side of murine immediately artery after vascular injury shows dense localization of platelets (appearing as white dots), whose [C] activated states are visible upon magnified view; [D] upon higher magnification, dense fibrin mesh is seen developing on single platelets.

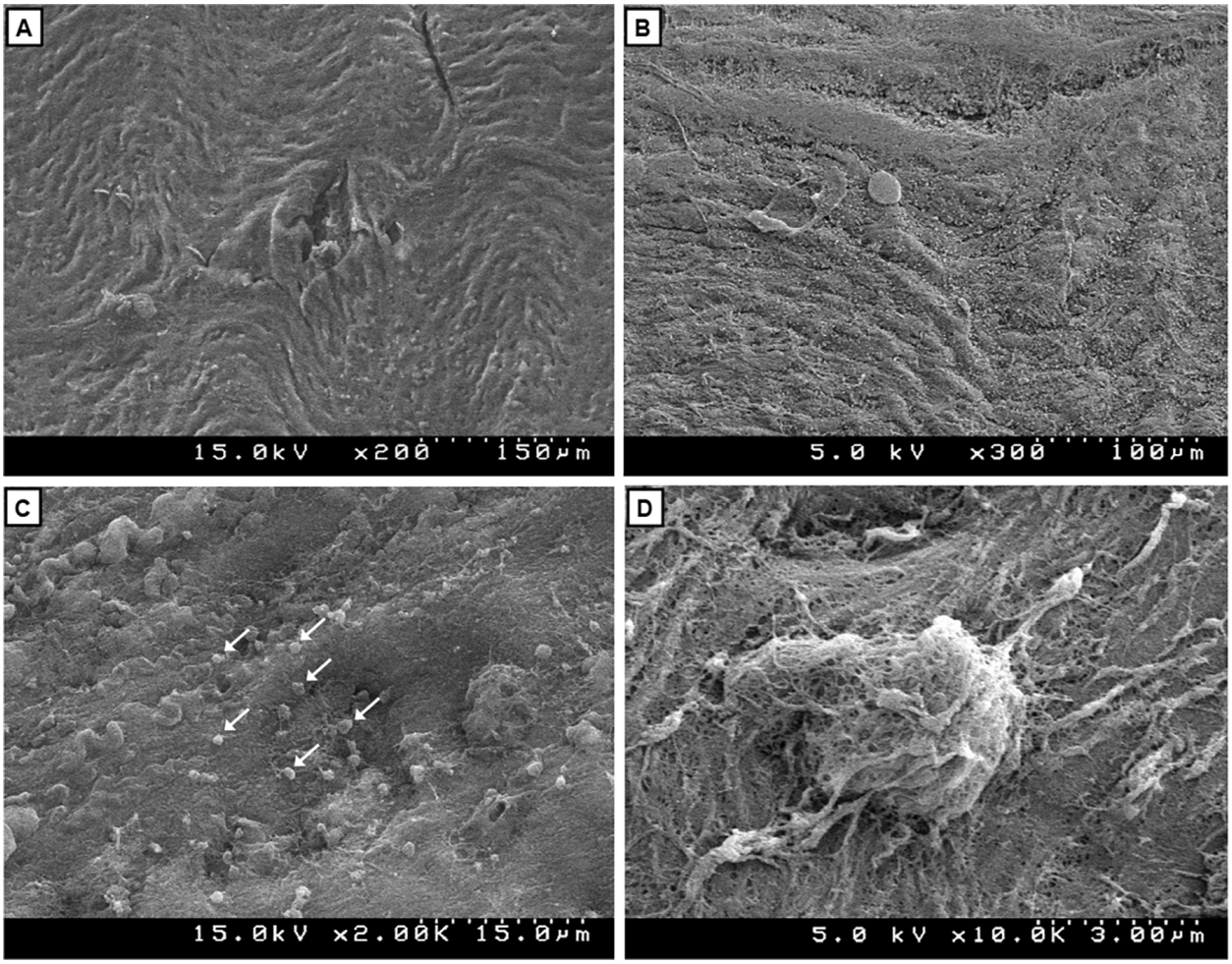


Figure S.10. [A] Tissue homogenate total fluorescence based analysis (Methods described in main manuscript) of localization of targeted PMINs in thrombosed versus non-thrombosed carotid artery in the same mice (n = 3) suggests that the PMINs have a significantly higher level of localization in the thrombosed carotid compared to non-thrombosed, which is also in accordance with our visual observation via intra-vital and ex vivo fluorescence microscopy (main manuscript Figure 5); [B] Organ homogenate based analysis of clearance of PMINs in the same mice, using appropriate RhB fluorescence calibration curve for PMINs, indicate that within the 10-15 min experimental window approximately 20% of total injected dose gets cleared cumulatively in the various organs (liver and spleen being major clearance organs), suggesting that a portion of the injected dose may remain in the vascular compartment (i.e. in circulation), which can be rationalized from the fact that PMINs are built by leveraging a PEG-ylated liposomal platform (e.g. 'Stealth Liposomes'), that are known to have long circulation lifetimes.

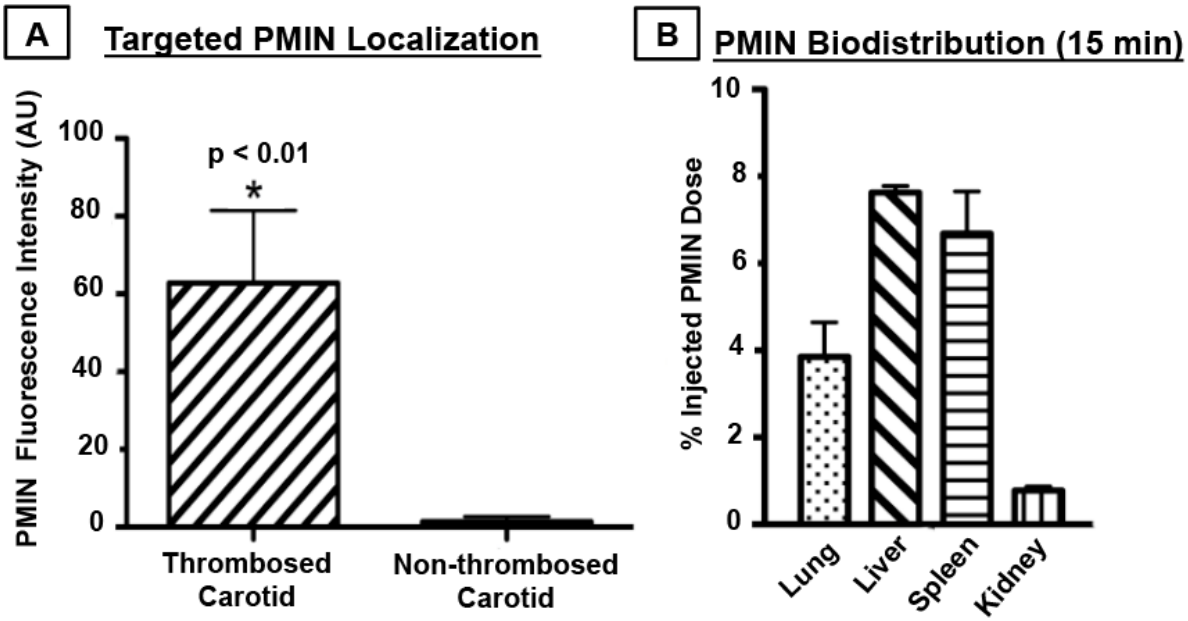
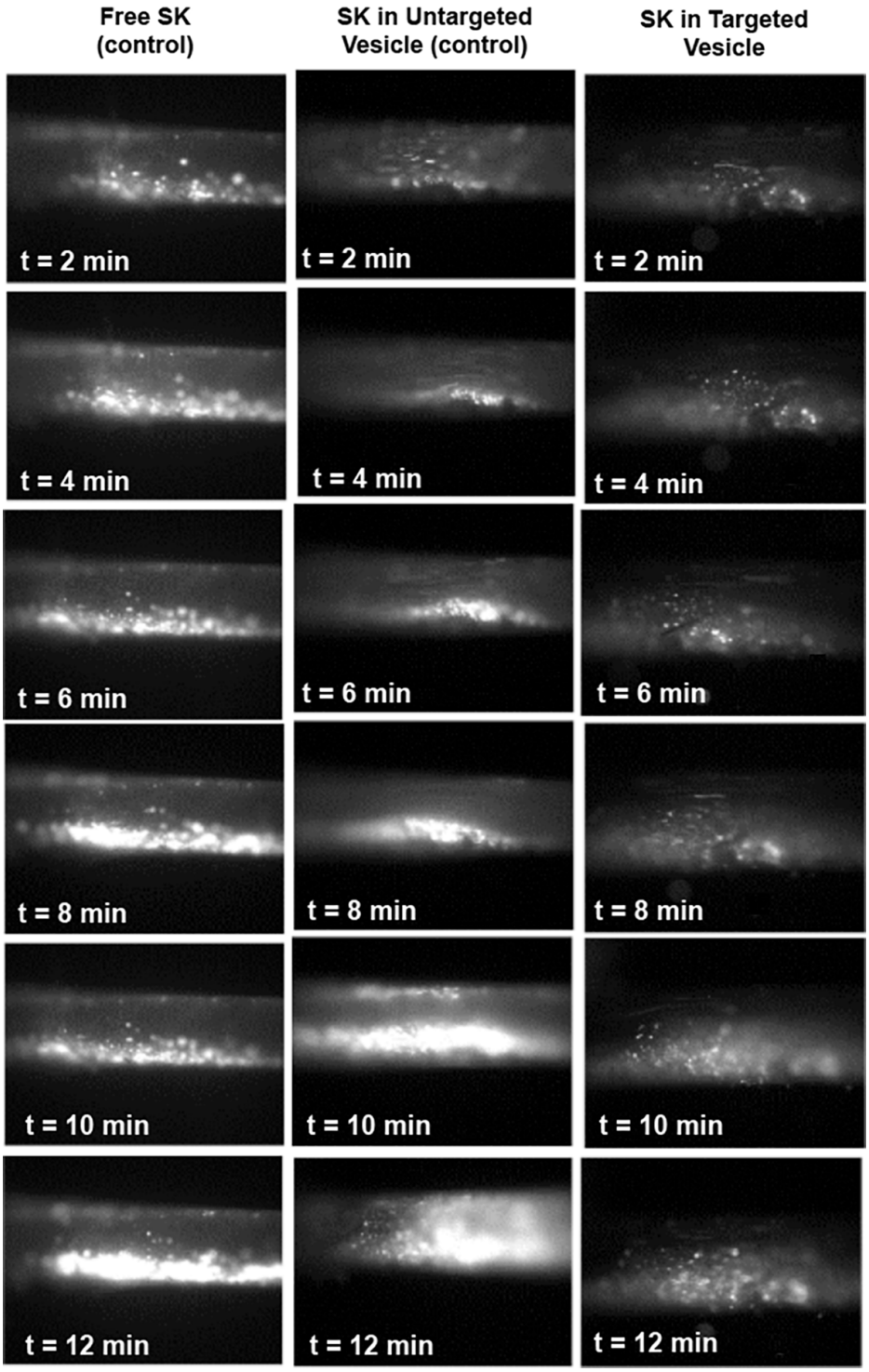


Figure S.11. Representative fluorescence intravital microscopy images for various time points (2 min to 12 min) of thrombi forming in mouse carotid artery upon $FeCl_3$ application, showing that SK delivered in targeted vesicles (PMINs) delay occlusive thrombus formation similar to action of free SK, while SK delivered in untargeted vesicles are unable to have such delaying effects, with the artery ~90% occluded by the 12 min time point.



Movie S1. Effect of saline (i.e. no treatment) on platelet-rich fibrin clot; the clot was formed from PRP containing green fluorescent fibrinogen (AlexaFluor-488 Fg), by incubating PRP on collagen-coated surface in presence of thrombin and Ca^{++} inside a parallel-plate microfluidic chamber; flowing saline on the clot did not result in any clot lysis.

Movie S2. Effect of free streptokinase (SK) on platelet-rich fibrin clot; the clot was formed from PRP containing green fluorescent fibrinogen (AlexaFluor 488-Fg), by incubating PRP on collagen-coated surface in presence of thrombin and Ca^{++} inside a parallel-plate microfluidic chamber; flowing SK on the clot resulted in rapid clot lysis.

Movie S3. Effect of SK-loaded unmodified (no peptide decoration) vesicles with $sPLA_2$ presence on platelet-rich fibrin clot; the clot was formed from PRP containing green fluorescent fibrinogen (AlexaFluor 488-Fg), by incubating PRP on collagen-coated surface in presence of thrombin and Ca^{++} inside a parallel-plate microfluidic chamber; unmodified vesicles had red fluorescence (RhB) label on them; particles showed minimal binding to the clot and no discernible clot lysis was found to occur.

Movie S4. Effect of SK-loaded targeted (dual peptide modification) PMINs without $sPLA_2$ presence on platelet-rich fibrin clot; the clot was formed from PRP containing green fluorescent fibrinogen (AlexaFluor 488-Fg), by incubating PRP on collagen-coated surface in presence of thrombin and Ca^{++} inside a parallel-plate microfluidic chamber; targeted vesicles had red fluorescence (RhB) label on them; the vesicles were found to bind the clot effectively but only minimal clot lysis was found to occur, especially at longer time points because the fibrinolytic drug (SK) was not released substantially from the vesicles in absence of $sPLA_2$.

Movie S5. Effect of SK-loaded targeted (dual peptide modification) PMINs with $sPLA_2$ presence on platelet-rich fibrin clot; the clot was formed from PRP containing green fluorescent fibrinogen (AlexaFluor 488-Fg), by incubating PRP on collagen-coated surface in presence of thrombin and Ca^{++} inside a parallel-plate microfluidic chamber; targeted vesicles had red fluorescence (RhB) label on them; the vesicles could bind the clot effectively and rapidly degrade in presence of $sPLA_2$ to release the fibrinolytic drug (SK), resulting in substantial clot lysis, showing a lytic effect comparable to ‘free SK’ treatment (Movie S2).