Supplementary Material

Image Processing and Analysis

A short description of the image processing and analysis of the raw images are provided here, a more detailed explanation has been published previously by our research group.[1] In short, a database is created to handle the generated images, containing filename, time point, Z-stack and fluorescence channel (this information was obtained from the full filenames provided by the micromanager software used for image acquisition). A difference of Gaussian approach is used for background reduction of the images, this is performed on individual image planes in a three-dimensional (3D) volume (the Z-stack images for each time point are handled as a volume). The images are thresholded to discriminate the platelets from the background, and the threshold is set using a probe to reduce the influence of the operator. Platelets are detected in the volume and their respective *x*, *y* and *z* position is determined. The data from the analysis and in the original database are joined into a data frame from which information and statistics from the experiments can be derived. The script used for image processing and analysis was developed in Python. The above-mentioned steps are built using packages and modules that are readily available for download online and named in the published method. In this article, we have used the positional information about the detected platelets to enable tracking of the platelets throughout the experiment. The platelet positions in 3D for each time frame were used as an input in the Trackpy Python package (v 0.3.2, available online via https://soft-matter.github.io/trackpy/v0.3.2/). The platelets were tracked between consecutive frames provided that the maximum displacement did not exceed 3 µm between two time frames. From this analysis, the tracked platelets were also indexed so it is possible to distinguish the platelet throughout the experiment. With the tracking, it is possible to determine the size of the movement for each tracked platelet along each axis (dvx, dvy, dvz) and the total length (dv). The contractile component was deduced by projecting each platelet movement vector onto a vector towards the thrombus centre of mass. The processing of the data, obtaining mean values, graphs and plots have been performed using several different Python modules, NumPy (for scientific computing), pandas (processing of data frames), matplotlib (2D-plotting) and Seaborn (statistical data visualization).

Supplementary Video S1

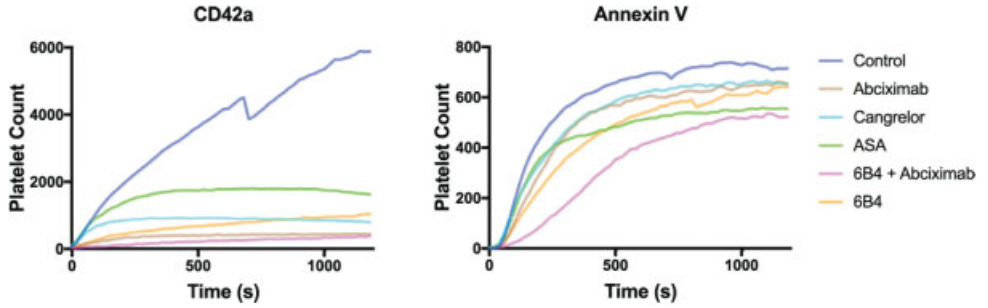
Thrombus formation on collagen at 400 s^{-1}, 10 minutes time-lapse. Experiments were performed in a polydimethylsiloxane (PDMS) flow chamber with 5% CD42a-labelled platelets (red) and annexin V (green). Time-lapse Z-stack images were captured with wide-field fluorescence microscopy. The video shows the first 600 seconds of a 1,200-second experiment and the flow is directed downwards in the video. Online content including video sequences viewable at: www.thieme-connect.com/ejournals/html/doi/10.1055/s-0038-1668151.

Supplementary Video S2

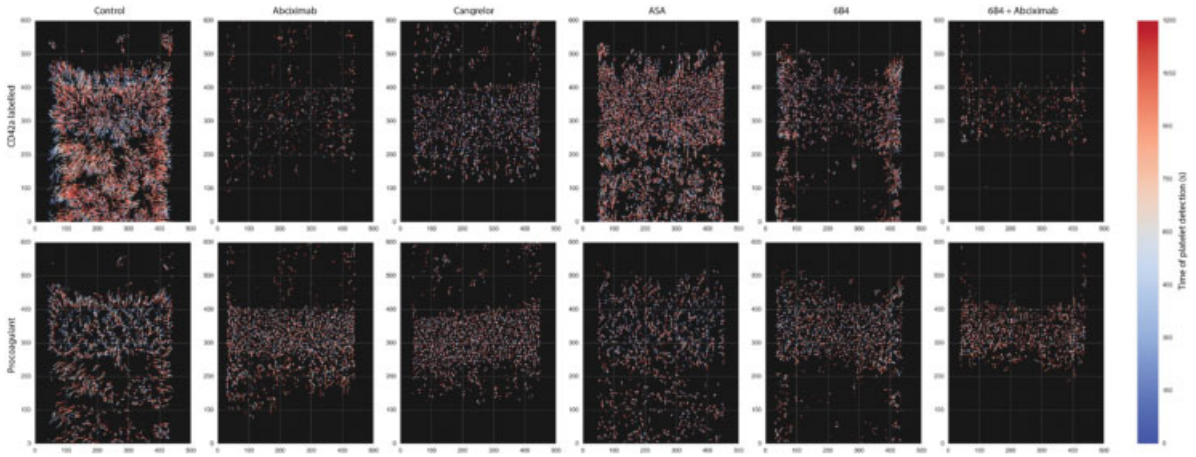
Platelet movement trajectories of CD42a-labelled platelets, obtained from a representative experiment. Thrombus formation was induced by collagen in a polydimethylsiloxane (PDMS) flow chamber at a shear rate of 400 s^{-1} with 5% CD42a-labelled platelets. Time-lapse Z-stack images were captured with wide-field fluorescence microscopy. In order to make individual tracks more visible, tracks shorter than 5 time frames and $>$ 20 µm above the collagen surface were not plotted. Platelet trajectories are given as platelet position in *X*- and *Y*-direction on the representative axis while the timing is represented by the colour of the trajectory. Experiments were carried out for 1,200 seconds where the time scale goes from blue to red. Online content including video sequences viewable at: www.thieme-connect.com/ejournals/html/doi/10.1055/s-0038-1668151.

Reference

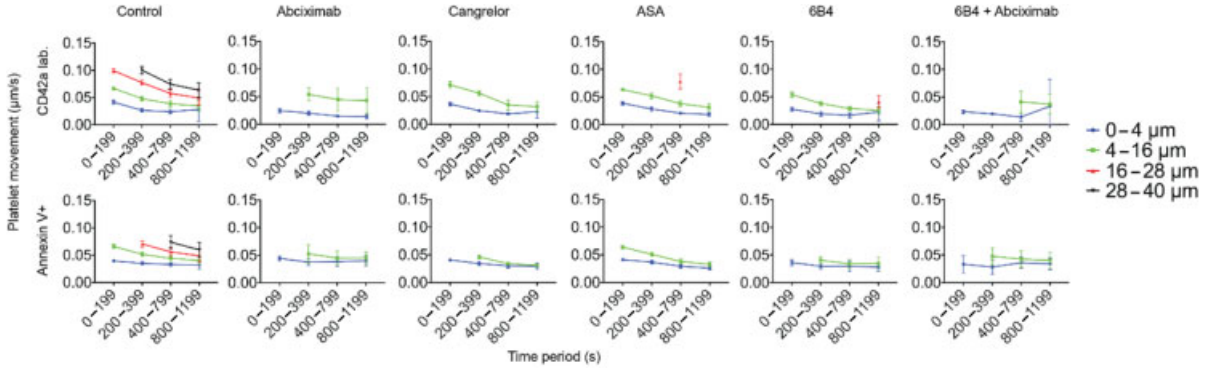
1 Claesson K, Lindahl TL, Faxälv L. Counting the platelets: a robust and sensitive quantification method for thrombus formation. Thromb Haemost 2016;115(06):10–12



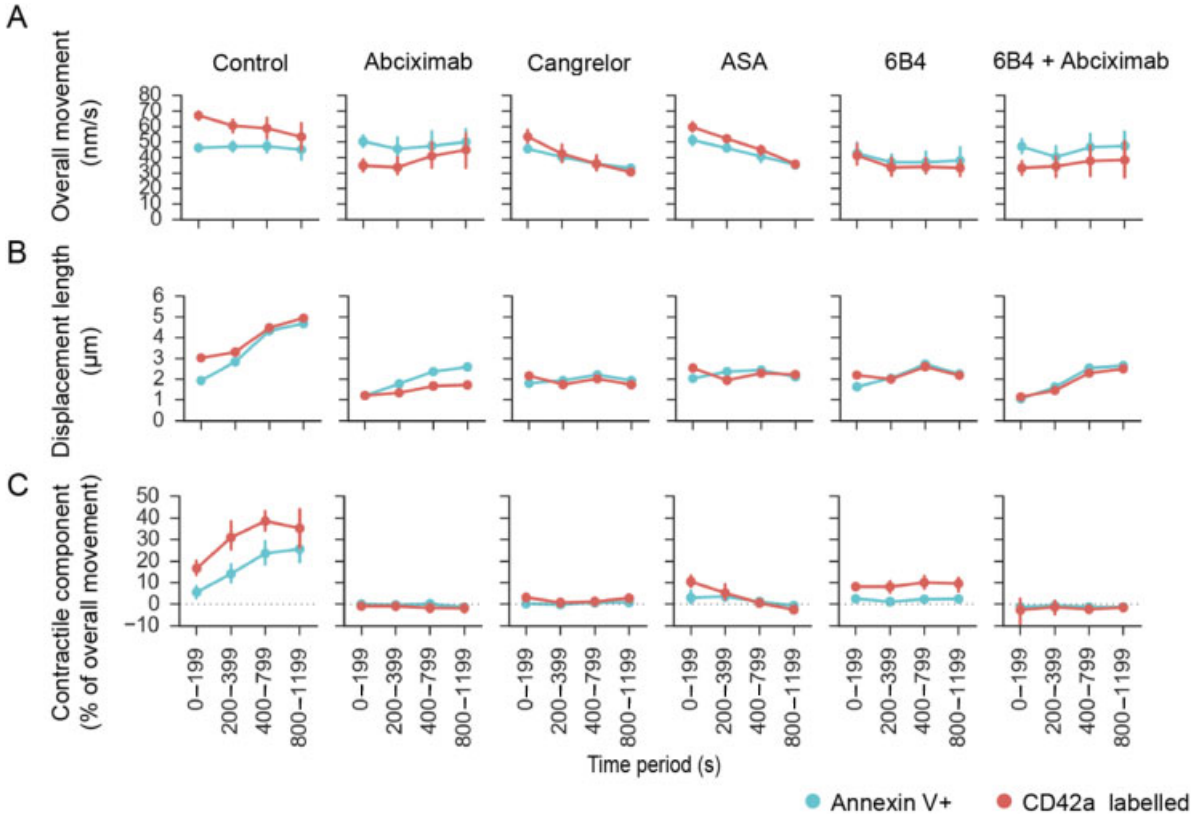
Supplementary Fig. S1 Platelet thrombus formation on collagen. Experiments were performed in a polydimethylsiloxane (PDMS) flow chamber at a shear rate of 400 s^{-1} with 5% CD42a-labelled platelets or platelets labelled with annexin V, w/wo fibrinogen receptor inhibitor abciximab at 10 µg/mL, adenosine diphosphate (ADP)-receptor inhibitor cangrelor at 5 µM, acetylsalicylic acid (ASA) at 25 µM or von Willebrand receptor blocking Ab 6B4 at 8 µg/mL. Time-lapse Z-stack images were captured with wide-field fluorescence microscopy (20× objective, NA 0.8) and analysed with the platelet count method. Mean platelet count plotted over time (n = 6–10).



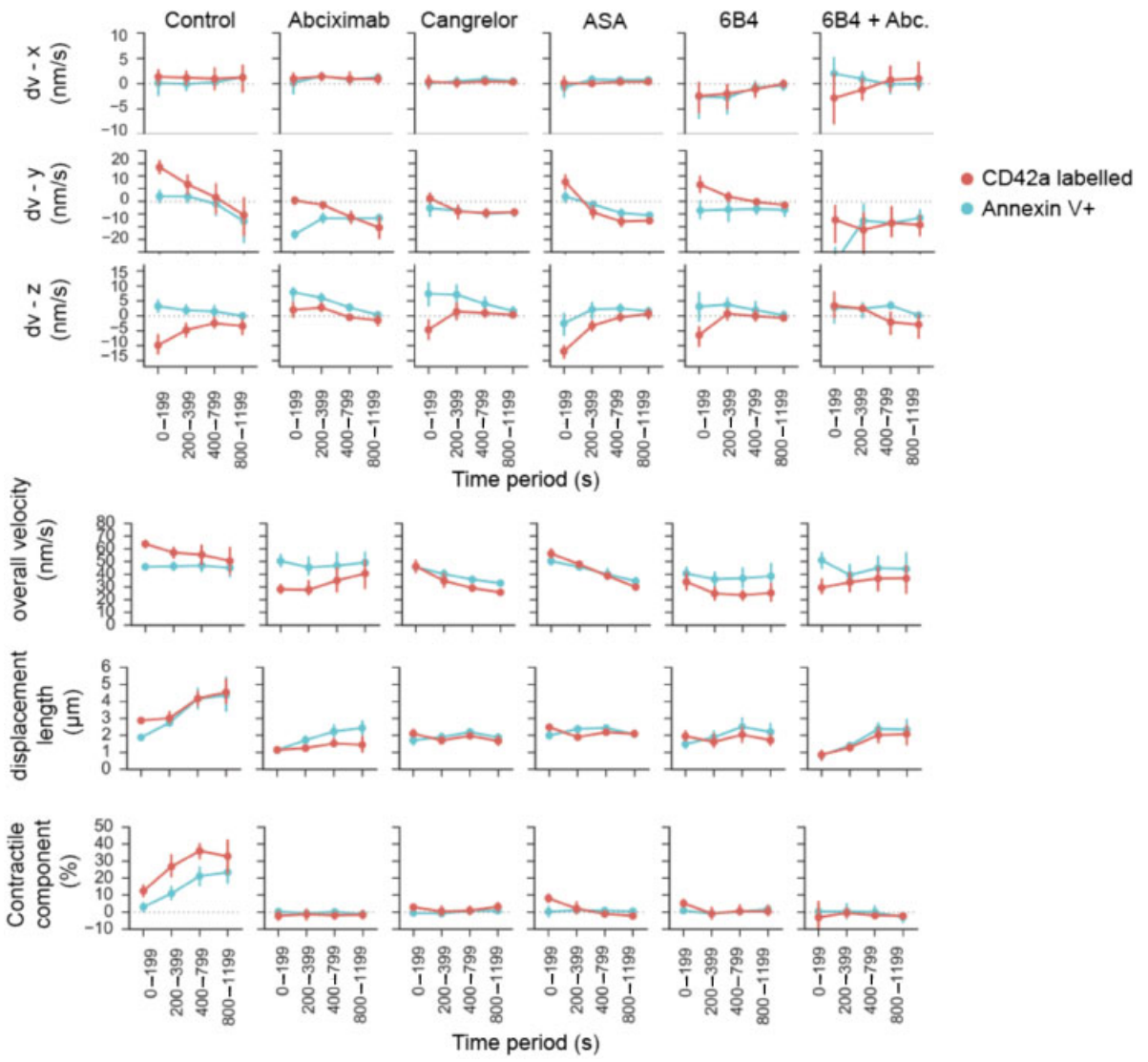
Supplementary Fig. S2 Platelet movement trajectories obtained from a set of time-lapse experiments (without inhibition and with the addition of common inhibitors) performed in a polydimethylsiloxane (PDMS) flow chamber at a shear rate of 400 s^{-1} with 5% CD42a-labelled platelets and annexin V+ platelets. Time-lapse Z-stack images were captured with wide-field fluorescence microscopy (20× objective, NA 0.8). Tracks shorter than 5 time frames and above 20 µm from the collagen surface were not plotted in order to make individual tracks more visible. The colour code represents at which time the platelet was detected at that position during a 1,200-second experiment and these tracks start in blue and are red towards the end of the experiment.



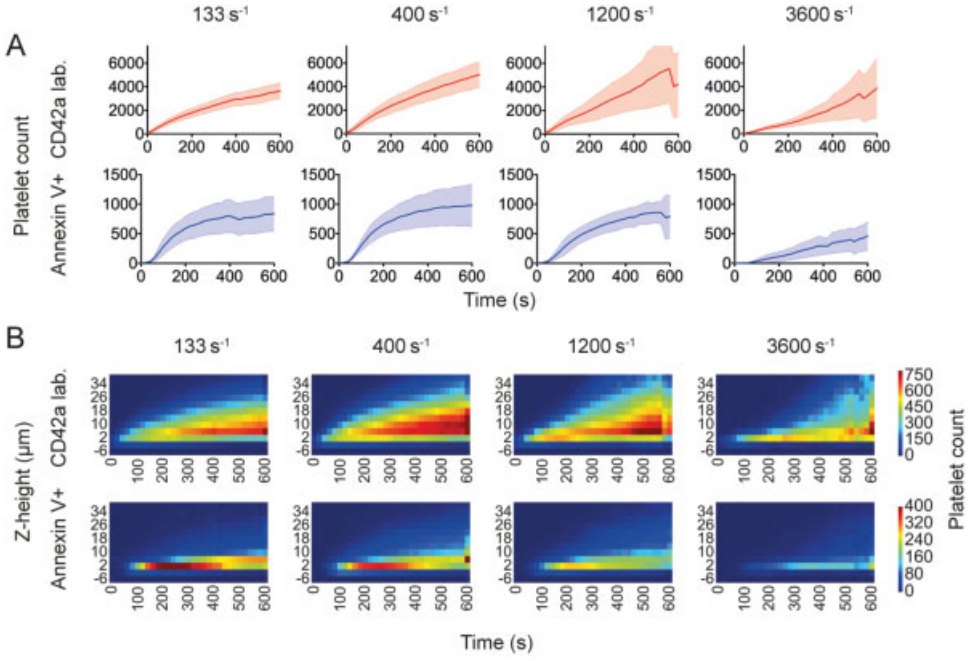
Supplementary Fig. S3 Platelet movement at different height from the surface. The mean platelet movement at different heights throughout the thrombus. Values were calculated from time-lapse data of thrombus formation on collagen at 400 s^{-1} with and without the addition of platelet inhibitors. Performed in a polydimethylsiloxane (PDMS) flow chamber with 5% CD42a-labelled platelets and annexin V+ platelets. Time-lapse Z-stack images were captured with wide-field fluorescence microscopy (20× objective, NA 0.8).



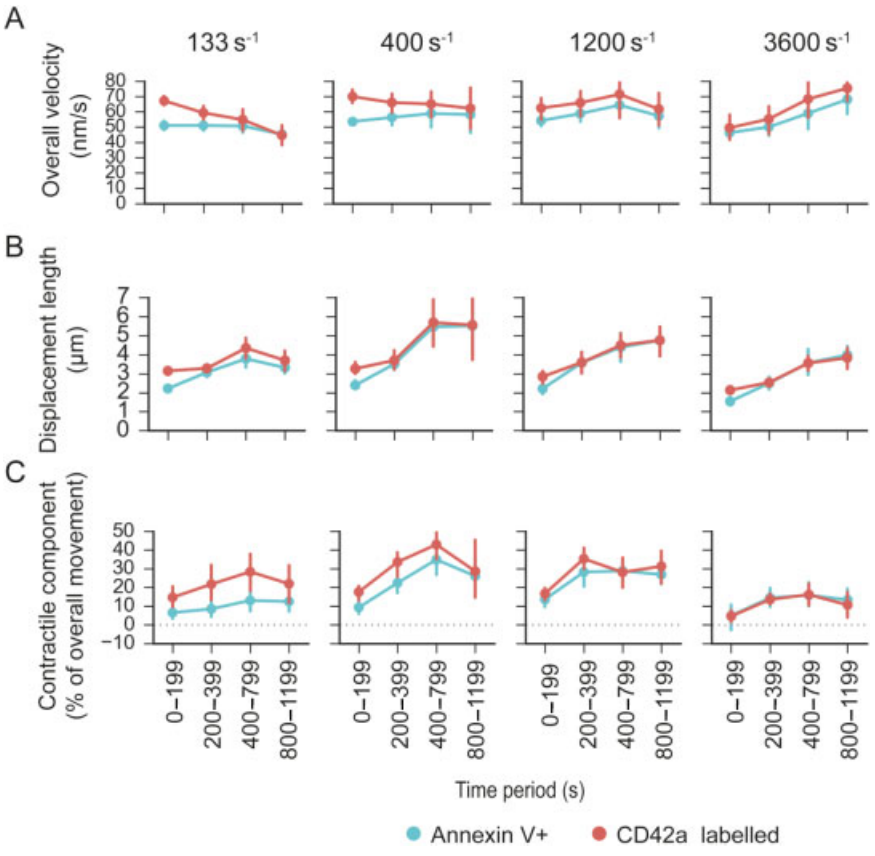
Supplementary Fig. S4 Effects of platelet inhibitors on the mean platelet movement and contractile component. Measured during thrombus formation on collagen at 400 s^{-1}. Performed in a polydimethylsiloxane (PDMS) flow chamber with 5% CD42a-labelled platelets (red) and annexin V+ platelets (blue). Time-lapse Z-stack images were captured with wide-field fluorescence microscopy. (**A**) Mean total movement velocity (µm/s), (**B**) length of displacement and the (**C**) contractile component (%) over four time stages of the movement. Mean ± 95% boot strapped confidence interval (CI) (n = 6–10).



Supplementary Fig. S5 Mean platelet movements and contractile component excluding the 10% closest to each flow chamber wall. Measured during thrombus formation on collagen at 400 s^{-1}. Performed in a polydimethylsiloxane (PDMS) flow chamber with 5% CD42a-labelled platelets (red) and annexin V+ platelets (blue). Time-lapse Z-stack images were captured with wide-field fluorescence microscopy. Mean movement, displacement and contraction over four time stages of the experiment. Mean ± 95% boot strapped confidence interval (CI) (n = 6–10).



Supplementary Fig. S6 Platelet count and thrombus height profile. Platelet thrombus formation on collagen was performed in a polydimethylsiloxane (PDMS) flow chamber at increasing shear rates (133, 400, 1200 and 3600s-1) with 5% CD42a-labelled platelets (red) and annexin V+ labelled platelets (blue). Time-lapse Z-stack images were captured with wide-field fluorescence microscopy and analysed with the platelet count method[18]. (**A**) Platelet count over time, mean ± 95% confidence interval (CI) ($n = 6$) for the CD42a labelled platelets this was 5% of the total volume of platelets. (**B**) Mean thrombus height profile over 600 seconds, the colour map indicates the number of platelets in 4 µm segments.



Supplementary Fig. S7 Effects of different shear rates on the mean platelet movement and contractile component. Measured during thrombus formation on collagen at increasing shear rates. Performed in a polydimethylsiloxane (PDMS) flow chamber with 5% CD42a-labelled platelets (red) and annexin V+ platelets (blue). Time-lapse Z-stack images were captured with wide-field fluorescence microscopy. (**A**) Mean total movement velocity (µm/s), (**B**) length of displacement and the (**C**) contractile component (%) over four time stages of the movement. Mean ± 95% boot strapped confidence interval (CI) (n = 6–10).