Supplementary Fig. S1 Overlay of fibrin detected in brightfield and fluorescence microscopic images. Platelet thrombi were generated by perfusion of human blood over a microspot of collagen and 10 pM TF, as described for Fig. 1. Thrombi were superfused with recalci6ed plasma supplemented with AF647 fibrinogen, and brightfield and fluorescence images were recorded from a representative microscopic field every 10 seconds. Shown are brightfield images, subtracted fluorescence images and overlays, recorded at indicated times. Note the full overlap of radially extending fibres detected in the two image sets. Bars = 10 µm.
Supplementary Fig. S2 Localized formation of fibrin fibres on platelet thrombus surface. Fibrin formation (AF647 fibrinogen label) was followed in time during low-shear perfusion of vehicle-treated (A) or TFPI-inhibited plasma (B) over platelet thrombi (DiOC₆ label), as described for ►Fig. 2. Shown are representative confocal images captured after 450 and 650 seconds of plasma flow, as well as line profiles of platelet (green) and fibrin (red) fluorescence intensities. Bars = 25 µm.
Supplementary Fig. S3 Effect of TFPI-α inhibition on kinetics of platelet-dependent fibrin formation in haemophiliac blood. Platelet thrombi were generated by perfusion of blood labeled with DiOC₆ and AF647 fibrinogen from haemophilia A patient A₂ (A, B) and haemophilia B patient B₂ (C, D) over microspots with collagen and indicated amounts of TF, as for Fig. 5. Shown are representative confocal fluorescence images (no TF, time points indicated) after perfusion with vehicle-treated or TFPI-α-inhibited blood. Bars = 25 µm (A, C). In addition, time traces of fibrin formation at platelet thrombi for collagen microspots with 1 and 10 pM TF (n = 3) (B, D).
Supplementary Fig. S4 TF-dependent role of TFPI-α in thrombin generation in controls and haemophilic patients. Platelet-free plasma from male control subjects and patients with haemophilia A (patients A1, A2) or haemophilia B (B1, B2) was used for measurement of calibrated automated thrombin (CAT) generation in a 96-wells plate; triggering was with procoagulant phospholipids and indicated concentrations of TF. (A) Thrombin peak heights, obtained from CAT curves, of antibody treated control plasmas. Means ± SD (n = 3). (B) Thrombin peak heights from CAT curves of control and patient plasma samples (1 or 10 pM TF). Grey bars represent means with confidence intervals for n = 10 control subjects.