

## Supporting Information

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Platelet Adhesion and Activation in an ECMO Thrombosis-on-a-Chip Model

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# Supporting Information

## **Platelet adhesion and activation in an ECMO thrombosis-on-a-chip model**

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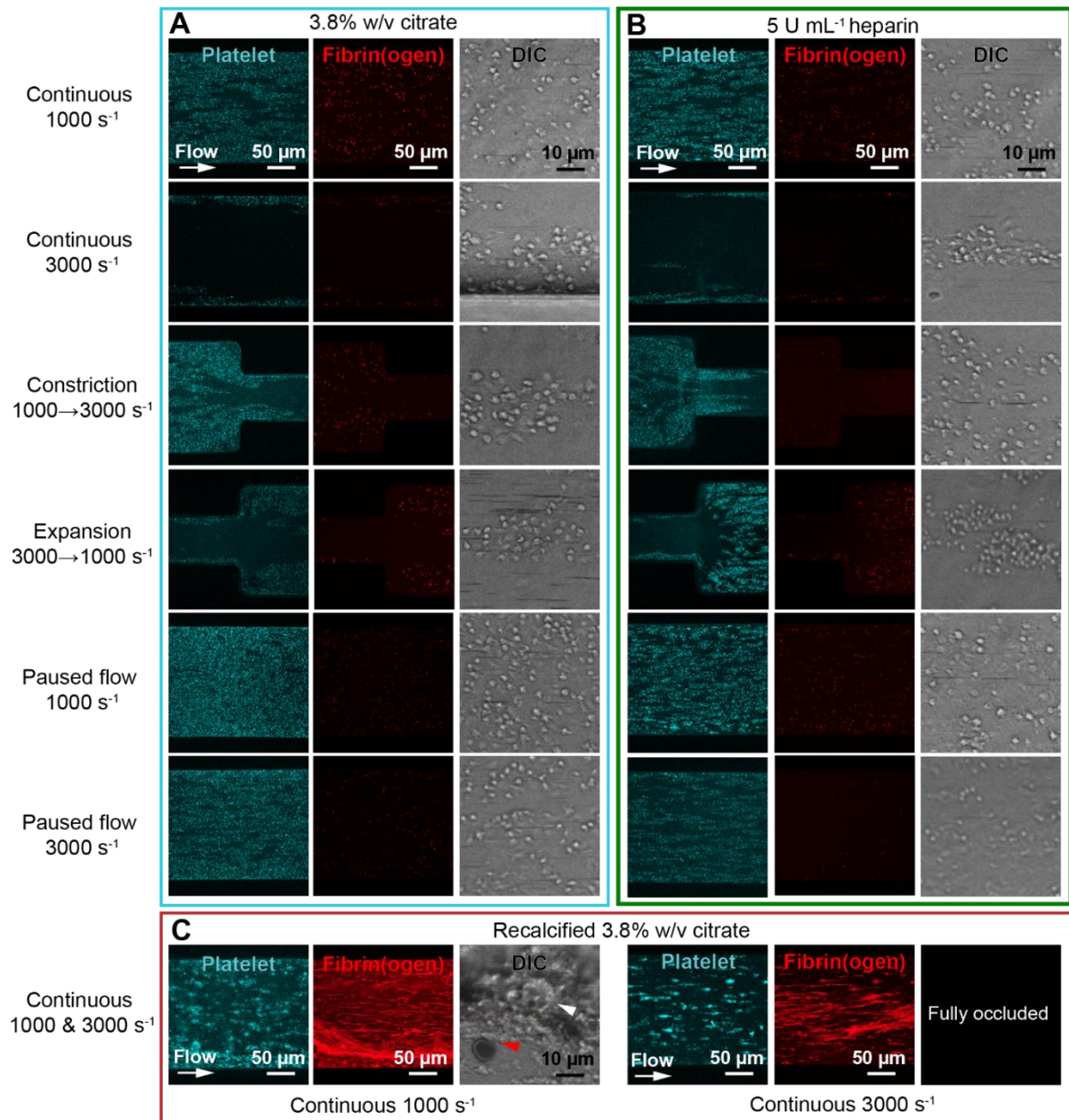
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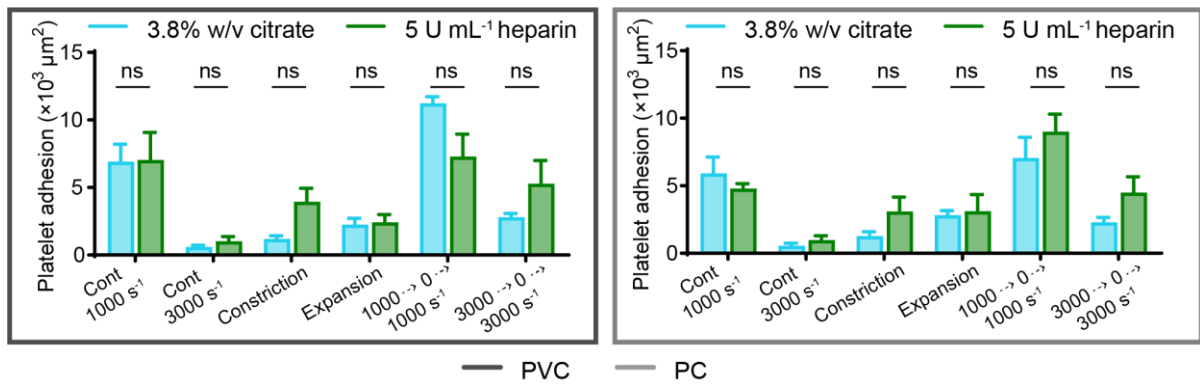
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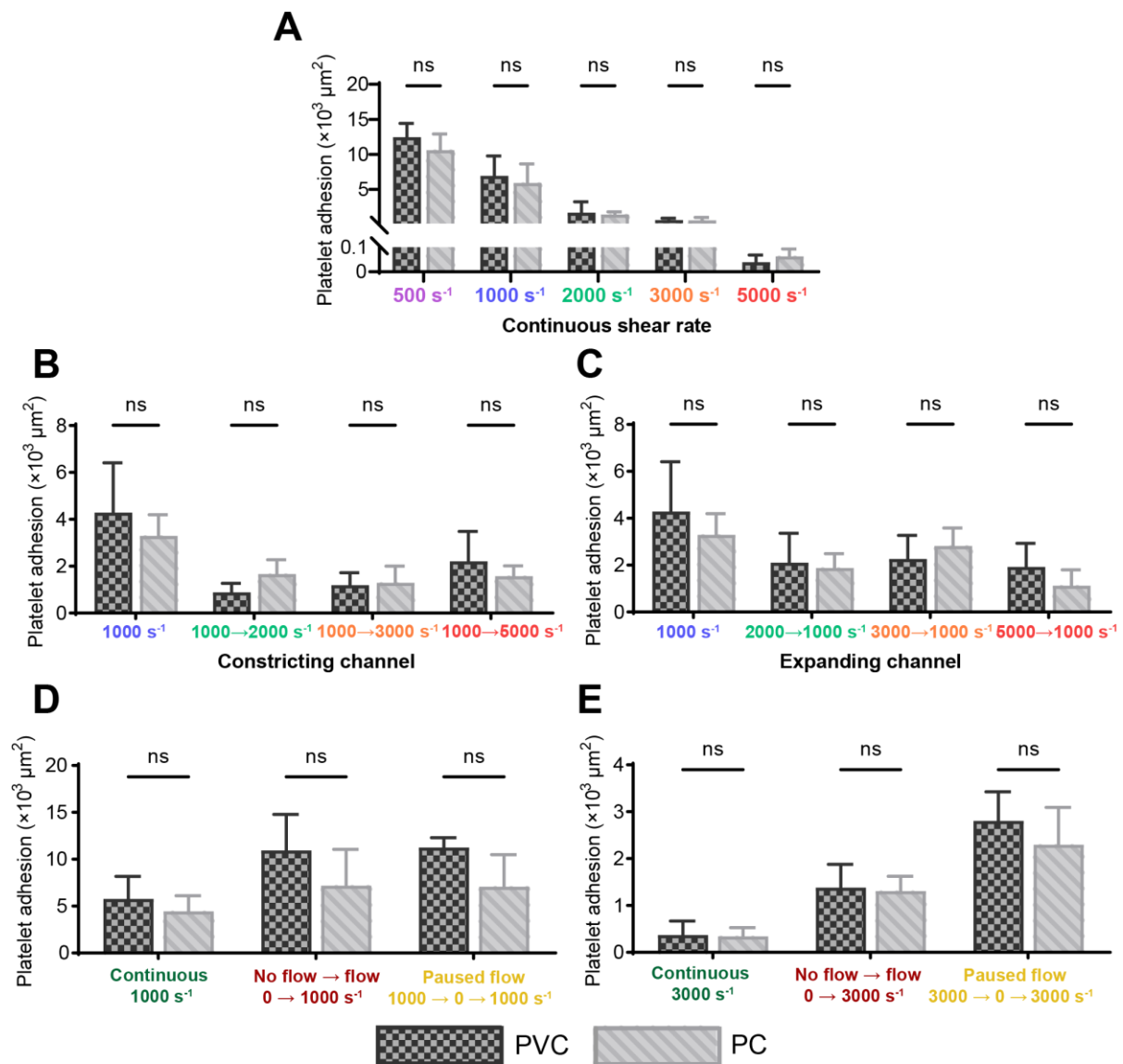
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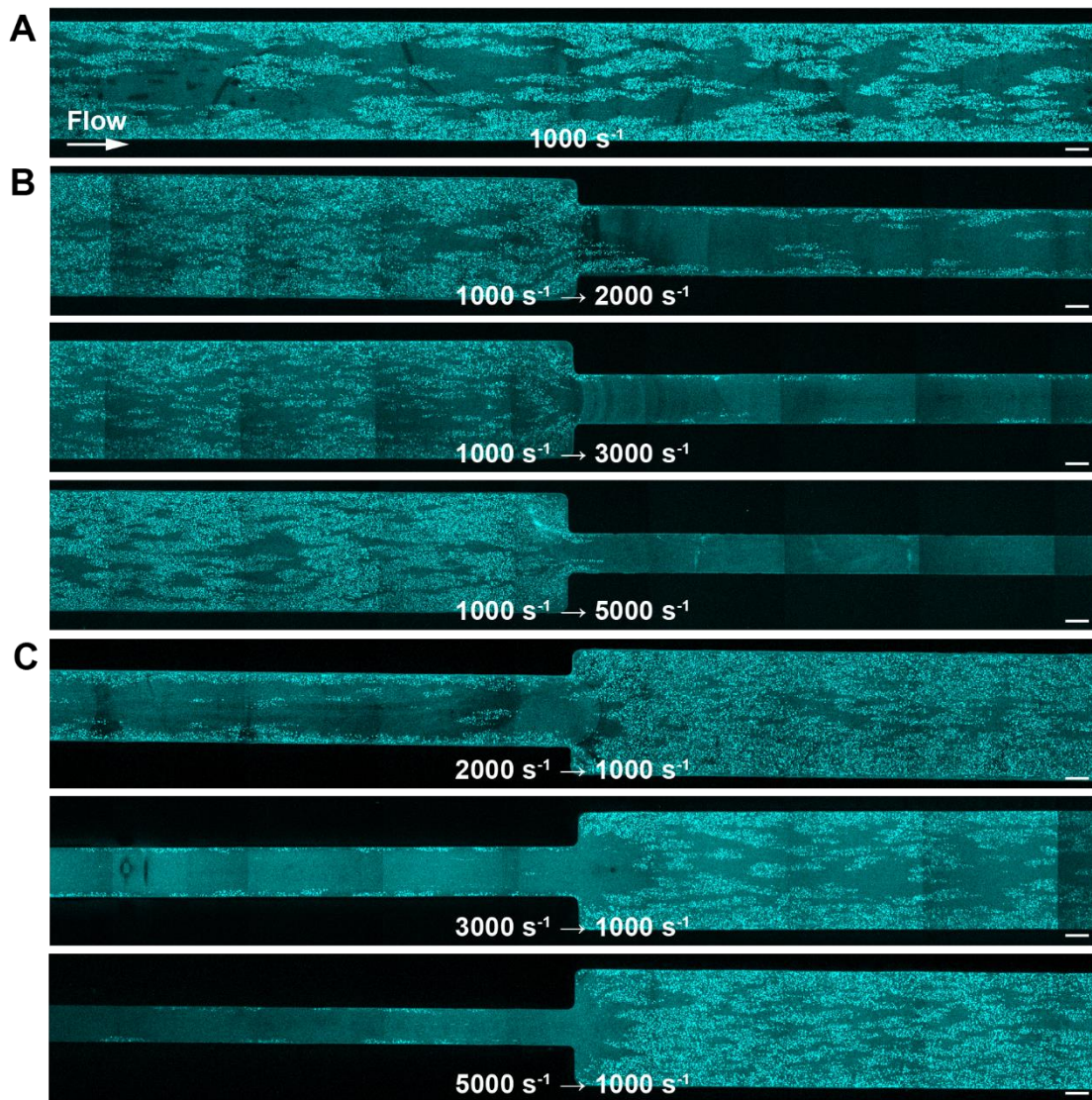
**Figure S1.** Representative confocal and differential interference contrast (DIC) micrographs of platelet adhesion (DiOC6, cyan), fibrin formation (AF647, red), and monitoring for red blood cell, neutrophil or monocyte adhesion (DIC), at 6 screened flow conditions on PVC at  $t = 10$  min, using **(A)** 3.8% w/v citrated blood, **(B)** 5 U mL<sup>-1</sup> heparinised blood and **(C)** recalcified 3.8% w/v citrated blood. Only the recalcified citrated blood condition showed polymerised fibrin formation, red blood cell adhesion (red arrowhead) and neutrophil adhesion (white arrowhead). Scale bar = 50 μm (platelets, fibrin/ogen) or 10 μm (DIC).



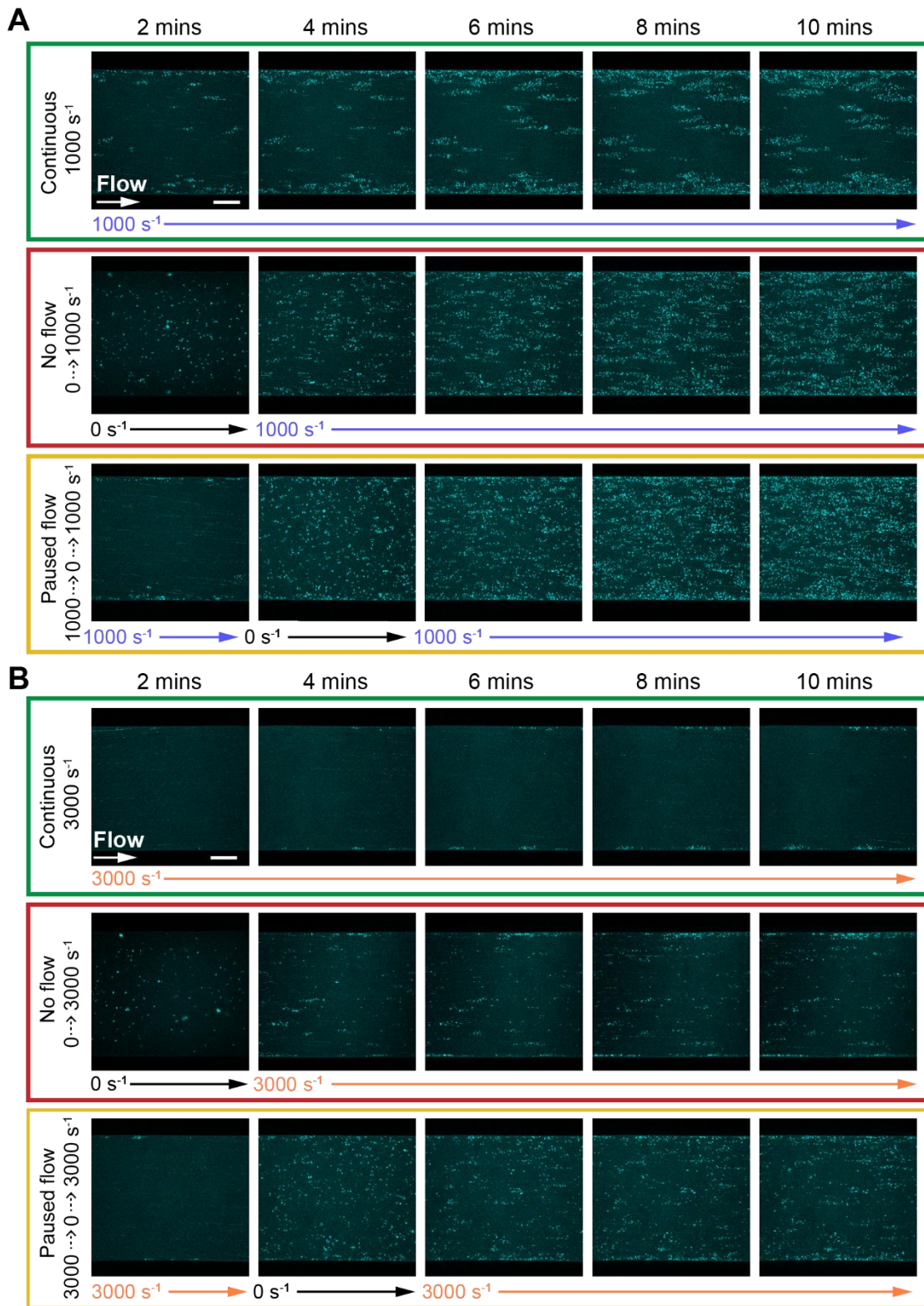
**Figure S2.** Analysis of total platelet adhesion on PVC and PC at  $t = 10$  min, using either 3.8% w/v citrate or 5 U mL<sup>-1</sup> heparin showed no statistical difference at all screened flow conditions. Error bars are mean  $\pm$  SEM,  $n = 5$  donors. Significance comparisons by a two-way ANOVA with Bonferroni's post hoc multiple comparisons test.  $P < 0.05$  was considered statistically significant.



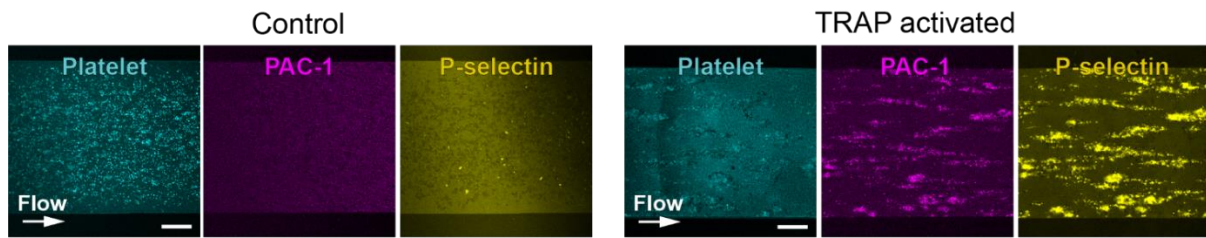
**Figure S3. Comparison of platelet adhesion on PVC and PC showed no significant difference.** Analysis of total fluorescent surface area indicating platelet adhesion at 10 minutes on PVC vs. PC for **(A)** continuous shear at 500, 1000, 2000, 3000 and 5000  $\text{s}^{-1}$ , **(B)** continuous 1000  $\text{s}^{-1}$  versus constricting channels at 1000 to 2000  $\text{s}^{-1}$ , 1000 to 3000  $\text{s}^{-1}$  and 1000 to 5000  $\text{s}^{-1}$  conditions **(C)** continuous 1000  $\text{s}^{-1}$  versus expanding channels at 2000 to 1000  $\text{s}^{-1}$ , 3000 to 1000  $\text{s}^{-1}$  and 5000 to 1000  $\text{s}^{-1}$  conditions **(D)** continuous 1000  $\text{s}^{-1}$ , no flow (0 to 1000  $\text{s}^{-1}$ ) and paused flow (1000 to 0 to 1000  $\text{s}^{-1}$ ), **(E)** continuous 1000  $\text{s}^{-1}$ , no flow (0 to 3000  $\text{s}^{-1}$ ) and paused flow (3000 to 0 to 3000  $\text{s}^{-1}$ ). Error bars are mean  $\pm$  SD,  $n = 5$  donors. Significance comparisons by a two-way ANOVA with Bonferroni's post hoc test.  $P < 0.05$  was considered statistically significant.



**Figure S4. Platelet adhesion at accelerating flow constriction and decelerating flow expansion on PC.** Representative confocal micrographs of platelet adhesion (cyan) at  $t = 10 \text{ min}$ , at (A) continuous (straight) flow, (B) constriction from  $1000 \text{ s}^{-1}$  to  $2000$ ,  $3000$  and  $5000 \text{ s}^{-1}$ , and (C) expansion from  $2000$ ,  $3000$  and  $5000 \text{ s}^{-1}$  to  $1000 \text{ s}^{-1}$ . Scale bar =  $50 \mu\text{m}$ .

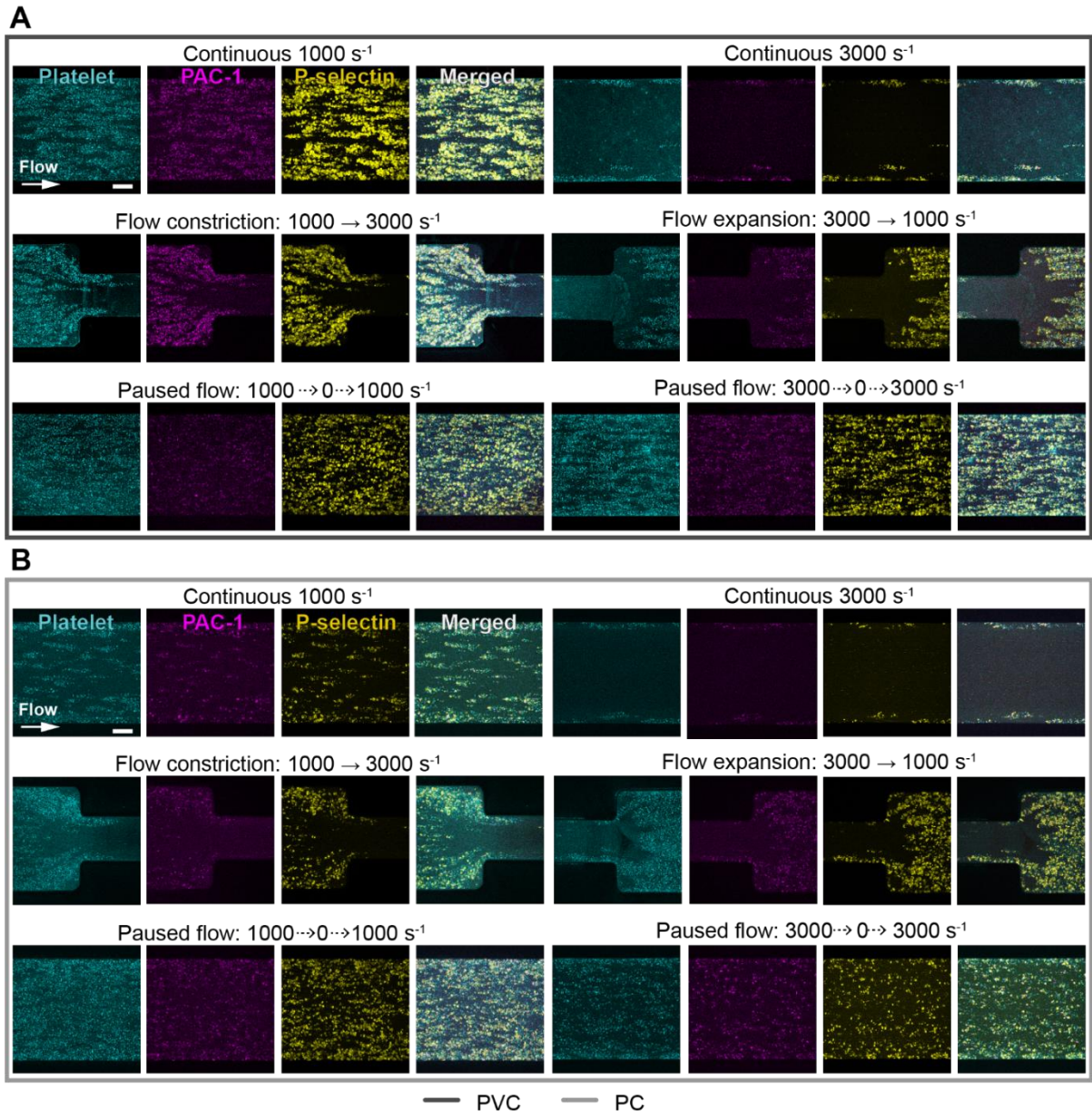


**Figure S5. Platelet adhesion at no flow and paused flow conditions on PC.** Representative confocal micrographs of platelet adhesion (cyan) on PC over 10 minutes of perfusion for **(A)** 1000  $s^{-1}$ , 0 to 1000  $s^{-1}$  and 1000 to 0 to 1000  $s^{-1}$  flow conditions and **(B)** 3000  $s^{-1}$ , 0 to 3000  $s^{-1}$  and 3000 to 0 to 3000  $s^{-1}$  flow conditions. Scale bar = 50  $\mu m$ .

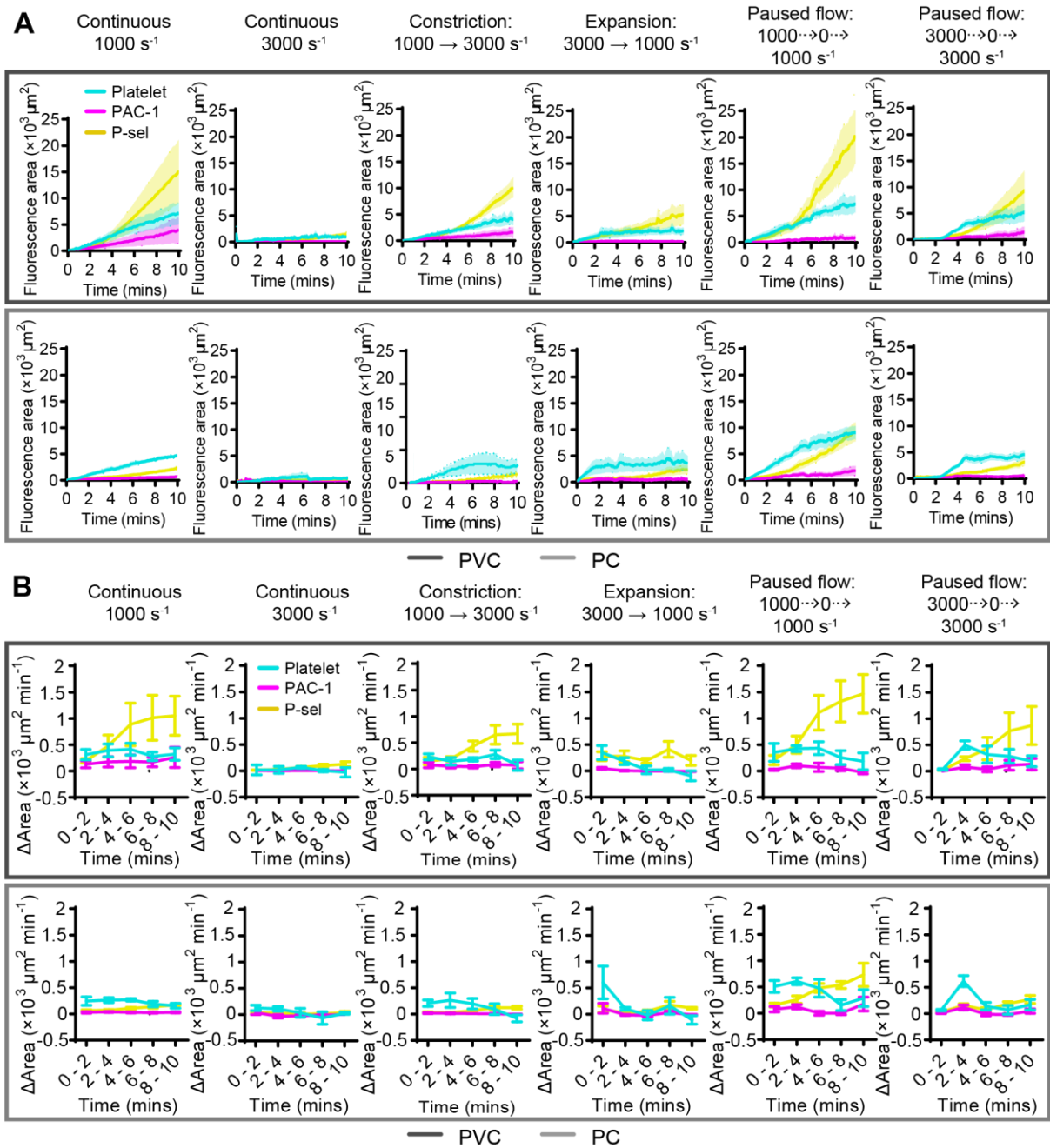


**Figure S6.** Representative confocal micrographs of control (without thrombin receptor activating peptide (TRAP) activation) platelets compared to TRAP (10  $\mu\text{M}$ ) activated platelets, after 2 minutes of flow at  $1000 \text{ s}^{-1}$ . TRAP activated platelets showed platelet aggregation, and high levels of PAC-1 and P-selectin expression. Scale bar = 50  $\mu\text{m}$ .





**Figure S7.** Representative confocal micrographs of platelet adhesion (cyan), PAC-1 (magenta) and P-selectin (yellow) on **(A)** PVC and **(B)** PC after 10 minutes, at various flow conditions. Scale bar = 50  $\mu$ m.



**Figure S8. (A)** Total fluorescent surface area indicating platelet adhesion (cyan), PAC-1 (magenta) and P-selectin (yellow) expression over 10 minutes on PVC (top row) and PC (bottom row) at various flow conditions. **(B)** Platelet adhesion (cyan), PAC-1 (magenta) and P-selectin (yellow) expression rate over 2-minute periods on PVC (top row) and PC (bottom row) at various flow conditions. Error bars are mean  $\pm$  SEM,  $n = 5$  donors.