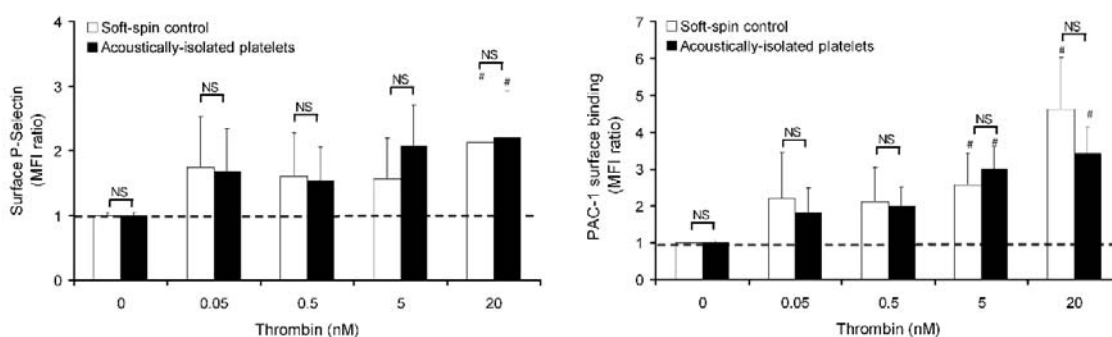


### Supplemental Figure 1. Responsiveness of acoustically-isolated platelets to thrombin.

Platelet reactivity in response to various concentrations of thrombin (0 to 20 nM) was assessed by monitoring the surface expression of the P-selectin (CD62P) (left panel) and PAC1 surface markers (right panel) by flow cytometry. For each donor, the reactivity of platelets was compared after fractionation of diluted whole blood using acoustic (black plots) and soft-spin (white plots) methodology in parallel. The histogram represents the ratio of mean fluorescence intensity (MFI) quantified for each activation marker before and after thrombin stimulation.  $n = 5$  different blood donors. # indicates a significant difference ( $p < 0.05$ ) as compared to non-stimulated control platelets.



**Supplemental movie 1. Acoustophoretic fractionation of blood.** The movie shows the outlet tubing (central and lateral) of the acoustophoretic device during the fractionation process. Without ultrasound, subpopulation concentrations (red blood cells and platelets) are the same in the inlet and in all the outlets. Once the ultrasound is applied, red blood cells are pushed to the midstream while platelets are collected in the lateral outlets. The tubing colour switches from red to a cloudy and yellowish colour, indicating removal of red blood cells.

**Supplemental movie 2. Acoustophoresis-isolated platelets can support transient interactions with the injured vessel wall under flow.** Acoustically-isolated human platelets were labelled with DIOC-6, transfused to immunodeficient (NOD/SCID) mice, and their interactions with FeCl<sub>3</sub>-injured mesenteric vessels were analyzed by intravital microscopy. The movie shows acoustically-isolated human platelets undergoing transient tethering interactions with the FeCl<sub>3</sub>-injured vessel wall. These observations were made within 5 minutes following FeCl<sub>3</sub>-induced injury and are representatives of 5 independent experiments.

**Supplemental movie 3. Acoustophoresis-isolated platelets can incorporate into a growing thrombus.** Accumulation of acoustically-isolated human platelets within a thrombus forming in FeCl<sub>3</sub>-injured mesenteric vessels of immunodeficient (NOD/SCID) mice. These observations were acquired  $\geq 15$  minutes following FeCl<sub>3</sub>-induced injury and are representatives of 5 independent experiments.