Supplemental Figure Legends

Figure S1:
Analysis of fibrinolysis involved fibrin network quantification, and RBC movement measurements (JPEG). (A) We created a quantitative mask of the fibrin network at each timepoint using the image processing program Metamorph's Angiogenesis Module employing its Adaptive Background Correction System. We determined the parameters for minimum fiber diameter, maximum fiber diameter, and minimum difference between a fiber and background that generated the most faithful mask of the fibrin network. Then we measured the area covered by this network for each timepoint of the lysis reaction. Examples of both an early (T=7 min) and a late (T=34 min) timepoint are shown. (B) To select RBCs to follow for velocity measurements, we placed a grid over the Z-projection of the RBC channel of the first timepoint of a lysis image. RBCs that contacted the lines of this grid were selected (this helped avoid selection bias). We followed the 3-dimensional coordinates of these RBCs over time. Selected RBCs are shown in the figure with a shaded circle over them. One RBC in the center of the field of view is highlighted with an asterisk. Movement of this RBC from T=5 to T=6 minutes is shown in the overlay to the right. This RBC moved from slice #18 at T=5 to slice #15 at T=6 (3 μm). It also shifted in X-Y space as shown by the arrow. The overlay was created with RBCs from T=5 shown in green and those from T=6 in red.

Figure S2:
Complete movies (AVI) of the spinning disk confocal lysis images shown in Figure 2. Both movies have a 10 second interval between images and play at 4 frames/sec. Images are Z-projections of a 5 μm confocal stack with 0.5 μm slices. Image acquisition for the free PA sample began at 6:10 minutes after clot initiation. That for RBC-PA began at 4:40 minutes.

Figure S3:
Movies (AVI) of laser scanning confocal images shown in Figure 3. Both movies have a 1 minute interval between images and play at 2 frames/sec. Acquisition of both began at 4 minutes. Images are Z-projections of a 20 μm confocal stack with 1 μm slices. We see an increase in the number of RBCs visible at the end of the reaction once the fibrin network has collapsed because all the RBCs trapped in the network above the field of view settle down toward the bottom of the sample.

Figure S4:
Image overlays (JPEG) display how RBC movement differs for clots being lysed by free PA compared to RBC-PA. For each overlay, one timepoint is shown in green and the subsequent point is shown in red. Features that appear yellow are static from one timepoint to the next, whereas those that appear in red and green have moved. Both free PA and RBC-PA overlays are shown at the start of the reaction, at 60% of the time to lysis, and at the point where fibers dissolve. For both, there was almost perfect overlap at the start of the reaction. At the end of the reaction, the RBCs did

not overlap at all. For free PA, there was good overlap midway through the reaction (60% of time to lysis), while for RBC-PA most of the cells had shifted locations.