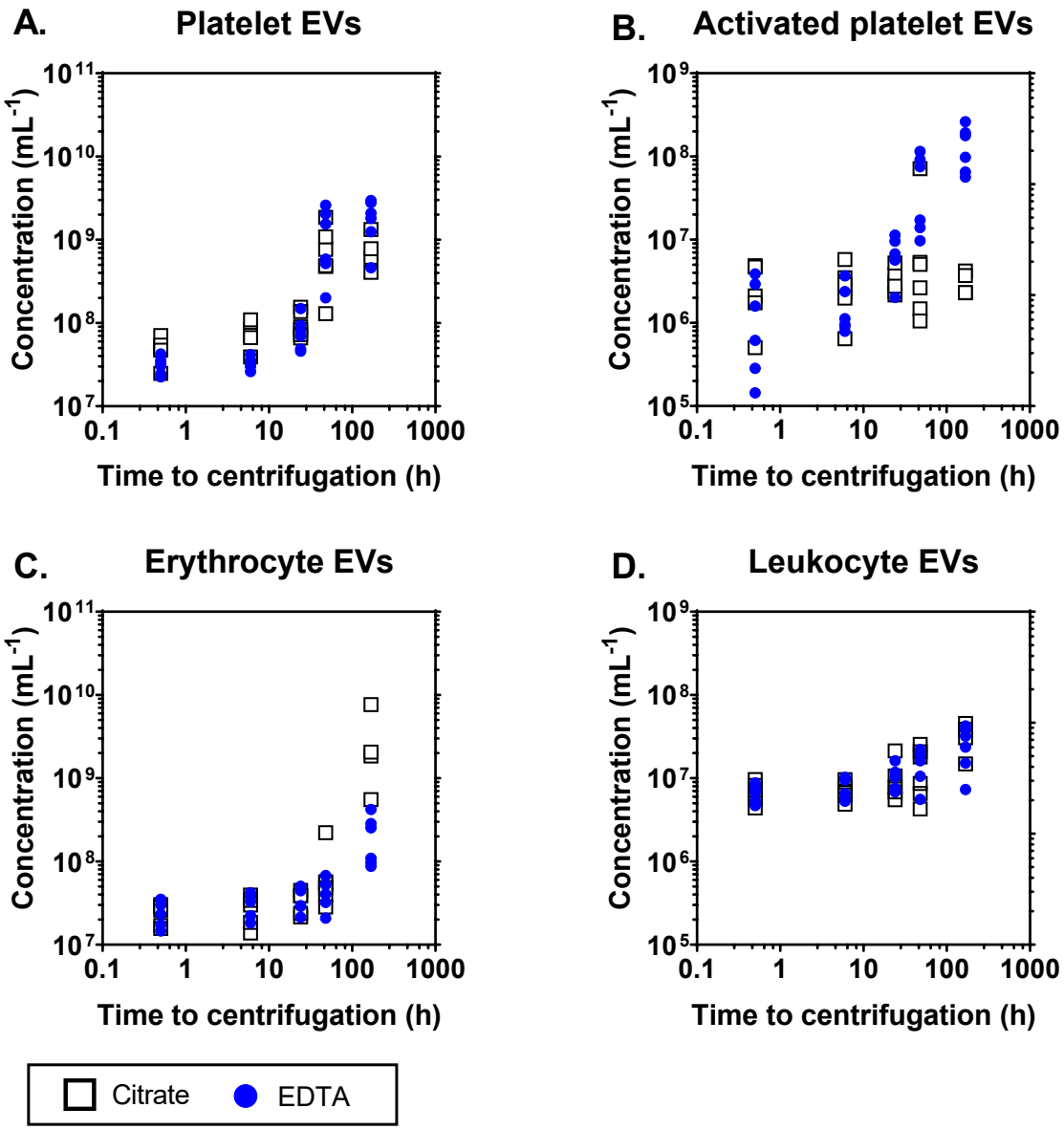
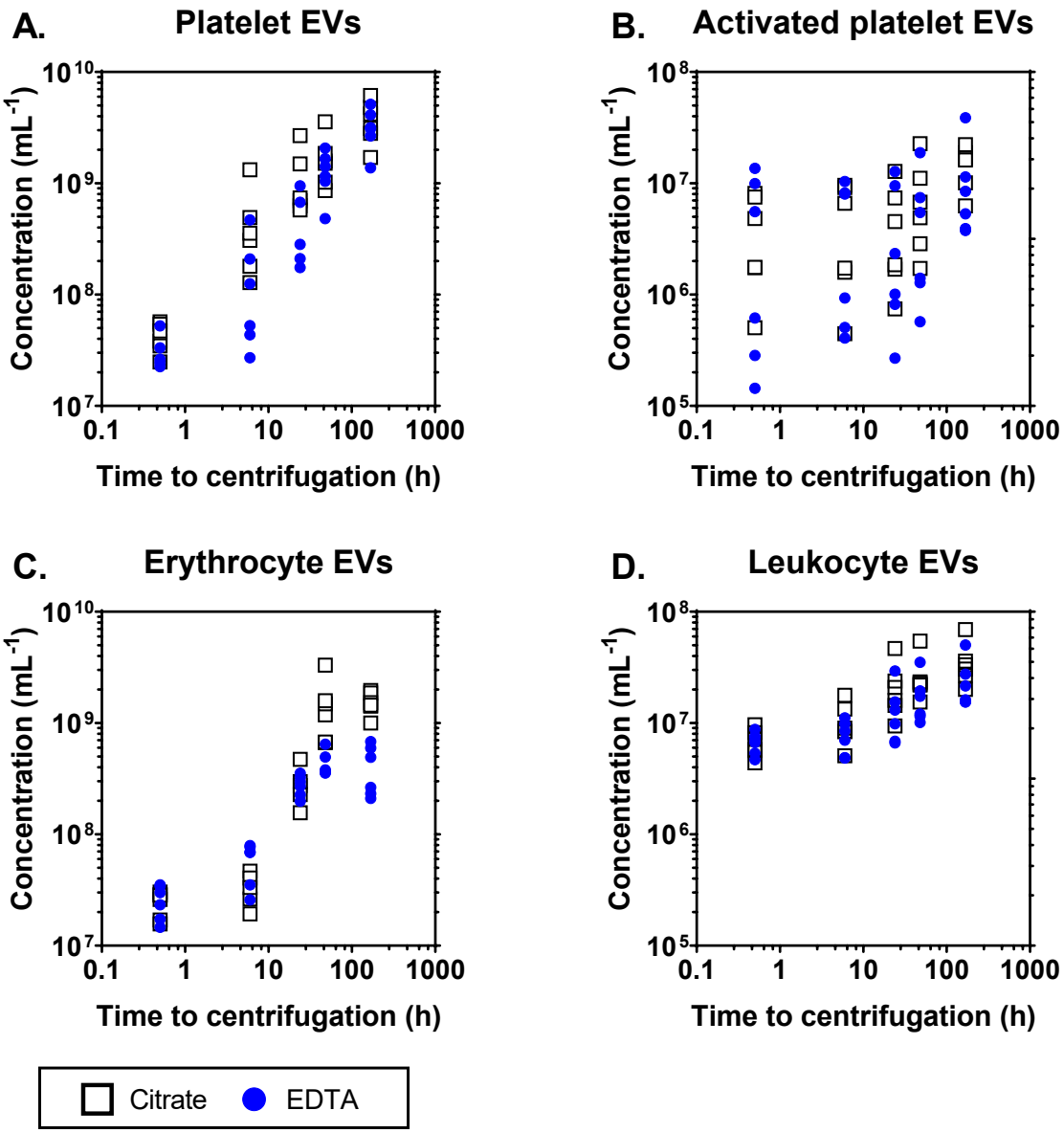
# Supplemental Material of "EDTA stabilizes the concentration of platelet-derived extracellular vesicles during blood collection and handling"

Supplemental Figures 1 and 2 show the EV concentrations versus sample storage time at room temperature (S1) and at 4°C (S2). For these samples, we determined the dilution factor prior to staining based on the count rate of the sample that was centrifuged within 30 minutes after collection. For some of the samples, this resulted in count rates exceeding 5,000 events/second. Previous experiments have shown that count rates below 10,000 events/second should still result in reliable concentration measurements (data not shown). However, those exceeding 10,000 events/second are most likely affected by swarm. Therefore, measurements with count rates above 10,000 events/second were excluded from analysis. This concerned two citrated anticoagulated plasma samples, centrifuged 168 hours after blood collection.

It can be concluded that EV concentrations substantially increase upon prolonged storage of blood on the lab bench and should therefore be avoided.




**Supplemental Figure 1. Stability of concentrations of extracellular vesicles upon storage of blood at room temperature.** Concentrations of extracellular vesicles (EVs) in plasma prepared from blood stored for different time intervals at room temperature (i.e. before centrifugation to prepare plasma), from A: platelets ($CD61^+$); B: activated platelets ($P\text{-}selectin^+$); C: erythrocytes ($CD235a^+$); D: leukocytes ($CD45^+$). Tubes were standing in a vertical position at room temperature until centrifugation. Concentrations show the number of particles (i) exceeding the side scatter or forward scatter threshold, (ii) having a diameter <1,000 nm, and (iii) exceeding the fluorescent threshold corresponding to the used labels, per mL of plasma (n=6, except citrated samples centrifuged after 168 hours n=4).




**Supplemental Figure 2. Stability of concentrations of extracellular vesicles upon storage of blood at 4 °C.** Concentrations of extracellular vesicles (EVs) in plasma prepared from blood stored for different time intervals at 4 °C (i.e. before centrifugation to prepare plasma), from A: platelets ($CD61^+$); B: activated platelets (P-$selectin^+$); C: erythrocytes ($CD235a^+$); D: leukocytes ($CD45^+$). Tubes were standing in a vertical position at 4°C until centrifugation. Concentrations show the number of particles (i) exceeding the side scatter or forward scatter threshold (ii) having a diameter <1,000 nm, and (iii) exceeding the fluorescent threshold corresponding to the used labels, per mL of plasma (n=3).