

Supplemental Figure 1: Leukocyte:platelet conjugates persist after purification of peripheral blood mononuclear cells (PBMC): Freshly drawn blood was collected in CPT tubes and spun to separate peripheral blood mononuclear cells (PBMC, Panel A and C). For two-step centrifugation, a portion of the cell suspension was layered on top of discontinuous sucrose gradient (8.125% plus 4.0625%, Figures B and D). PBMC stained with Giemsa show numerous platelets (open arrows), some of which are adherent to leukocytes (solid arrow, Figure A). Sucrose gradient centrifugation removed visible platelets on Giemsa-stained smear (Figure B). PBMC were stained with CD61-FITC and CD45-APC for further characterization by flow cytometry. For each experiment, 50,000 events were acquired on FACSCalibur and analyzed in FlowJo software with no gating. In PBMC separated by CPT tube, the platelet:leukocyte ratio was 12:1 (Figure C), which decreased to 0.45:1 after sucrose gradient separation (Figure D). The proportion of leukocyte/platelet conjugates (quadrant b) was 27% and 21% of the total leukocytes, respectively, with the two methods.