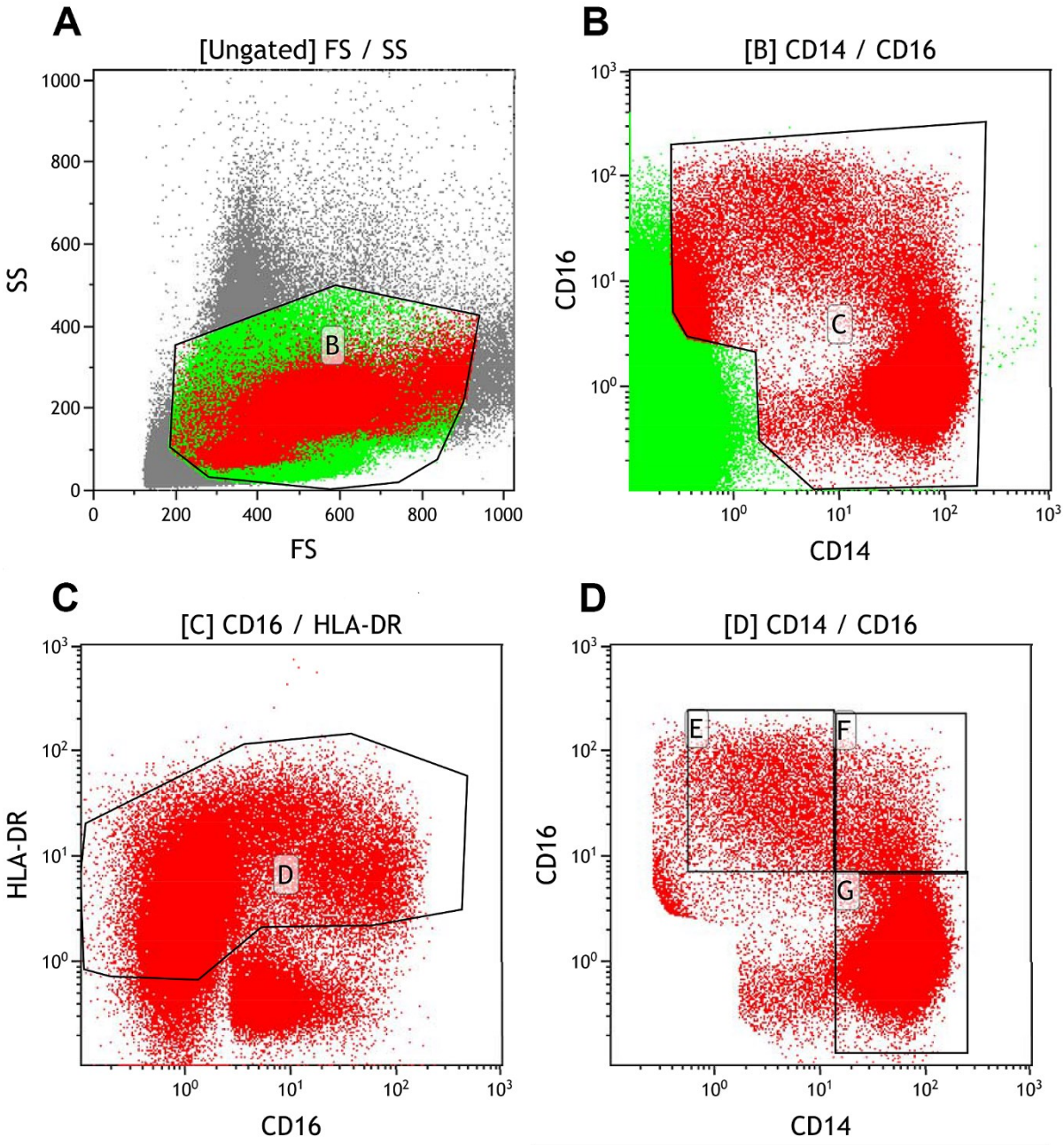
Supplementary Material to Stojkovic et al. "**IL-33 stimulates the release of procoagulant microvesicles from human monocytes and differentially increases tissue factor in human monocyte subsets**" (https://doi.org/10.1160/TH16-10-0784)

Suppl. Methods: *Flow cytometry analysis*

The cultured cells were gently collected using a cell scraper, fixed in 2 ml of cold 1% formaldehyde in PBS, washed twice, and then resuspended in 50 µL of PBS with 2% FBS. The monocytes were then stained using the following fluorochrome labelled antibodies (AB): Fluorescein isothiocyanate (FITC) anti-human CD142 (TF, clone VD8, Sekisui diagnostics, Stamford, CT, USA), Phycoerythrin (PE) anti-human CD14 (clone TK4, Dako, Toronto, ON, Canada), PE-Cyanine 7 (PC7) anti-human CD16 (clone 3G8, Beckman Coulter, Brea, CA, USA), Allophycocyanine (APC) anti-human HLA-DR (clone G46-6, BD Pharmingen, San Diego, CA, USA). Following staining, the cells were washed in PBS and resuspended in FACS buffer and analysed using a FC500 flow cytometer (Beckman Coulter). The human monocyte subsets were characterised according to their surface expression of CD14, CD16 and HLA-DR as classical monocytes (CM, CD14++CD16-), intermediate monocytes (IM, CD14++CD16+) and non-classical monocytes (NCM, CD14+CD16++) using "inclusion gating strategy" as described previously (18, 36). The combination of CD14, CD16 and HLA-DR allows discrimination of monocytes from natural-killer cells and neutrophils. Cells were first visualized on forward vs. side scatter plot and gate was drawn around the monocyte cloud (Supplementary Figure A). Gated cells were then viewed on CD14 vs. CD16 plot and again gate was drawn around monocytes (Supplementary Figure B). Next, remaining cells are viewed on CD16 vs. HLA-DR plot in order to exclude CD14+CD16++ cells which are HLA-DR negative (Supplementary Figure C). Finally, true monocytes are viewed on CD14 vs. CD16 plot, and gates were drawn to divide monocytes into subsets as described above: CM, CD14++CD16-, IM, CD14++CD16+, and NCM, CD14+CD16++ (Supplementary Figure D). Mean fluorescence intensities (MFI) for the treated monocytes were compared to the MFI of untreated cells.

Suppl. Figure 1



Gate		Number	%Total	%Gated	X-AMean
	All	100 319	5,99	100,00	61,04
Non-classical monocytes	E	6 455	0,39	6,43	4,69
Intermediate monocytes	F	4 143	0,25	4,13	46,23
Classical monocytes	G	86 018	5,13	85,74	68,46