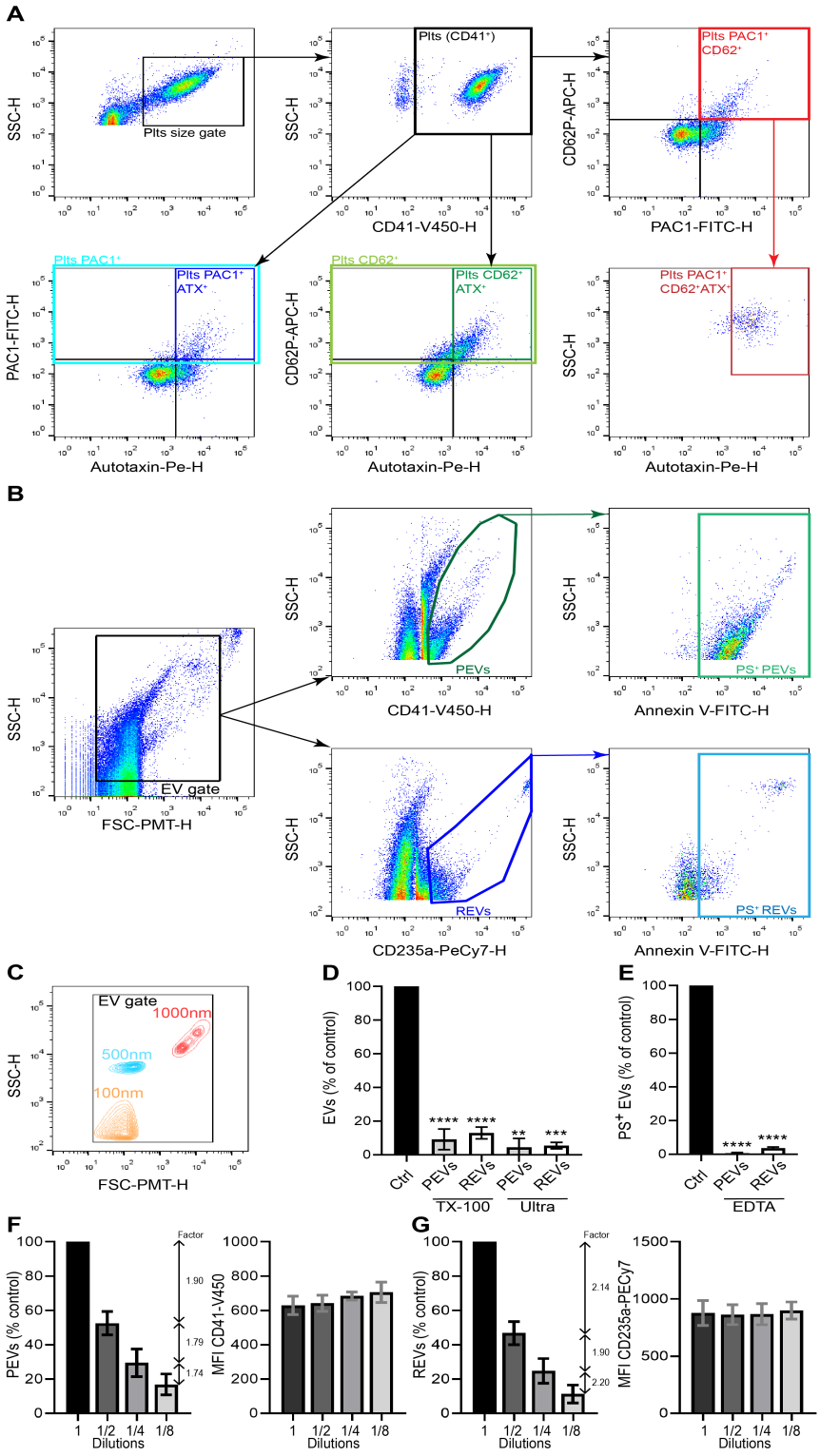
Supplementary figure 1



Supplementary figure 1: Platelet activation and EV detection by high-sensitivity flow cytometry. (A) Plasma samples of SLE patients were assessed by high-sensitivity flow cytometry (BD Canto II Special Order Research Product). Platelet were first gated according to size (SSC) and granularity (FSC), and then for labeling with V450 fluorochrome-conjugated antibodies directed against CD41a (CD41-V450-H), a platelet marker. Platelets where then analyzed for the expression of several activation markers, firstly FITC fluorochrome-conjugated antibodies directed against PAC1 (PAC1-FITC-H) alone or in combination with ATX-PE-H, the APC fluorochrome-conjugated antibodies directed against CD62P (CD62P-FITC-H) alone or in combination with ATX-PE-H, and lastly the combination of PAC1-FITC-H and CD62P-FITC-H. Platelets positive for both PAC1 and CD62P activation markers and labeled with PE fluorochrome-conjugated antibodies directed against ATX (ATX-PE-H) were monitored. (B) PFP samples of SLE patients were assessed by high-sensitivity flow cytometry (BD Canto II Special Order Research Product). Events positive for the EV gate set for relative sizes comprised between 100 nm and 1 000 nm on SSC and FSC-photomultiplier tube (PMT). EVs positive for the expression of CD41-V450-H were considered PEVs and EVs positive for the expression of PECy7 fluorochrome-conjugated antibodies directed against CD235a (CD235a-PECy7-H), a marker of RBC, were considered REVs. Finally, we assessed the fluorescence labelling of PEVs and REVs using FITC-conjugated Annexin V which binds PS (Annexin V-FTIC-H). (C) The EV gate in SSC-H (granularity) and FSC-PMT-H (relative size) were set with polystyrene beads of 100 nm, 500 nm, and 1 000 nm. (D) Specificity of PEV and REV detection was validated by the clearance of PEVs and REVs with 0.05% Triton X-100 (TX-100) treatment (n=6) which destroys EV's lipid bilayer and by 100, 000g ultracentrifugation (ultra) which pellets EVs, (n=3). Data are expressed as mean percentage ± SD relative to an untreated control (Ctrl). Statistical comparisons used the paired t-test. (E) Calcium-free PBS supplemented with 50 μM of EDTA (EDTA) was used for Annexin V labeling of PEVs and REVs to validate the specificity of PS detection at their surface (n=6). Data are expressed as mean percentage ± SD relative to an untreated control (Ctrl). Statistical comparisons used the paired t-test. (F) Two-fold serial dilutions of PEV samples were quantified by high sensitivity flow cytometry using polyester counting beads. PEV concentrations and calculated dilution factors (left panel), and the median (right panel) intensity of fluorescence for each dilution are presented (n=3). Data are expressed as mean ± SD. (G) Two-fold serial dilutions of REV samples were quantified by high sensitivity flow cytometry using polyester counting beads. REV concentrations and calculated dilution factors (left panel), and the median (right panel) intensity of fluorescence for each dilution are presented (n=3). Data are expressed as mean ± SD.