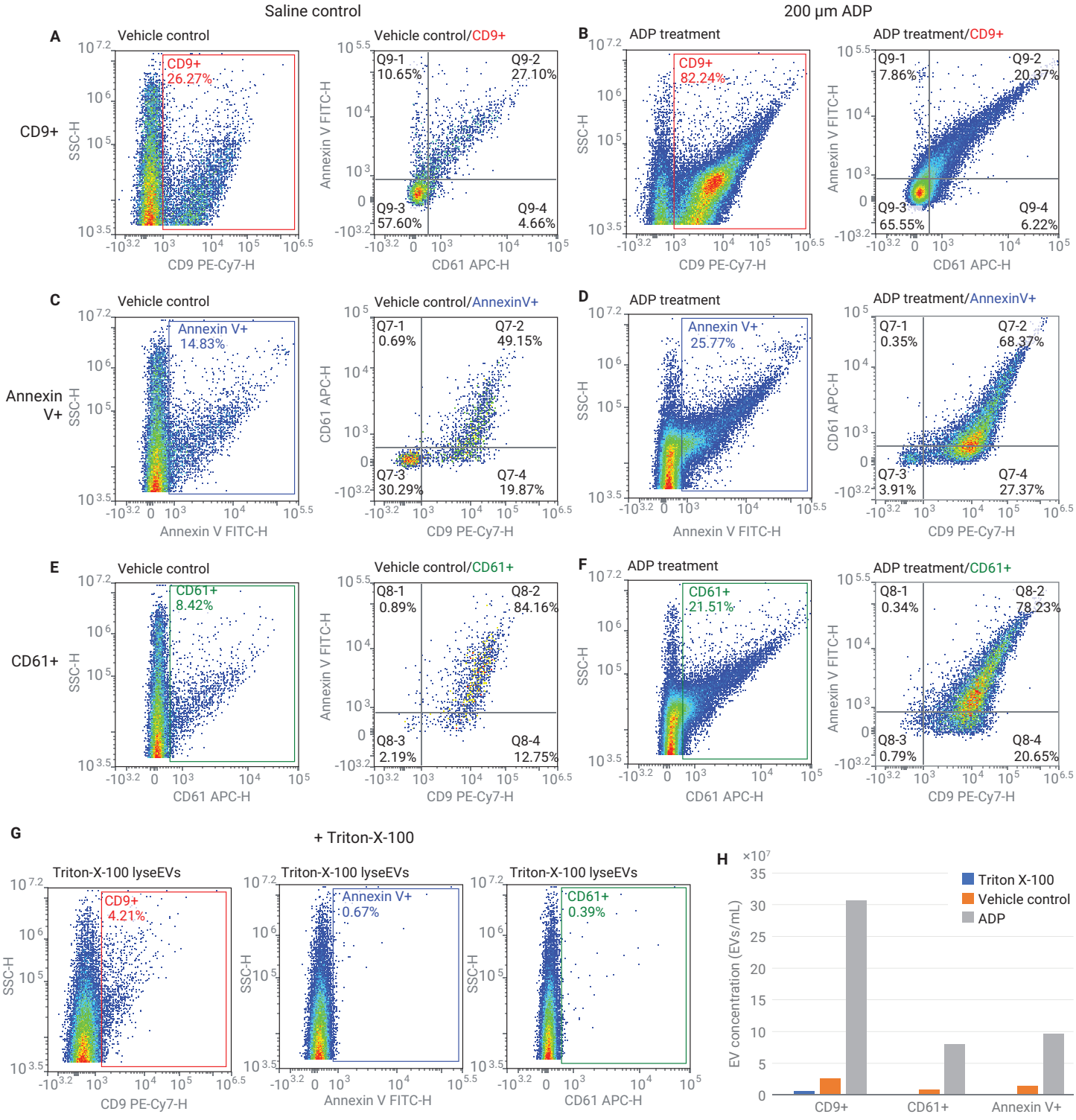

Figure 3. Increased EV release by platelet activation can be resolved by flow cytometry. Donor blood was collected using a sodium citrate blood collection tubes. Whole blood was treated either with 200 µm ADP or saline alone for 30 minutes. EVs were then isolated by centrifugation: 2,500 × g for 15 minutes twice, then the supernatant was centrifuged at 110,000 × g for 90 minutes twice. This was followed by staining with αCD9 PE-Cy7, αCD61 APC antibodies, and Annexin V FITC. EVs were identified by CD9 (A,B) and Annexin V (C,D), and CD61 (E,F) in the saline-treated control (A,C,E) or ADP-treated samples (B, D,F). (G) Identified EVs were analyzed for co-expression of other EV markers (A-F, right plots). A portion of the vesicles was incubated with 1% Triton-X-100 on ice for one hour. (H) Graph with EV concentration under the conditions described in the text. Instrument settings: SSC-H gain 1,000, threshold SSC-H greater than 1,000.