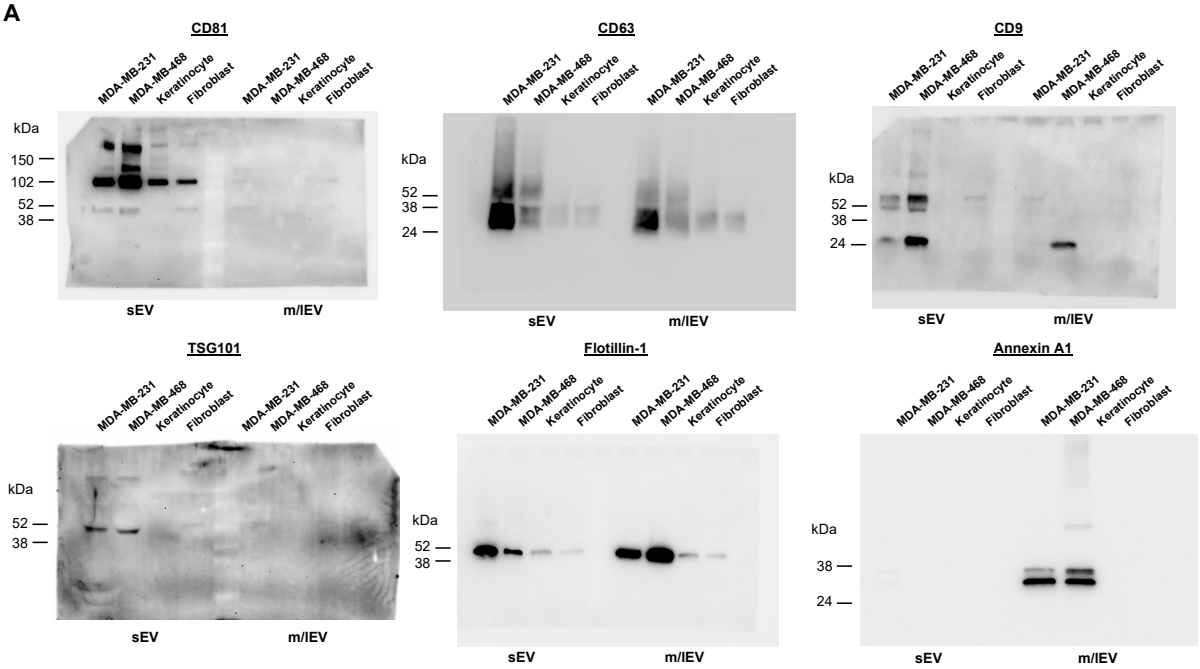
Supplementary Material



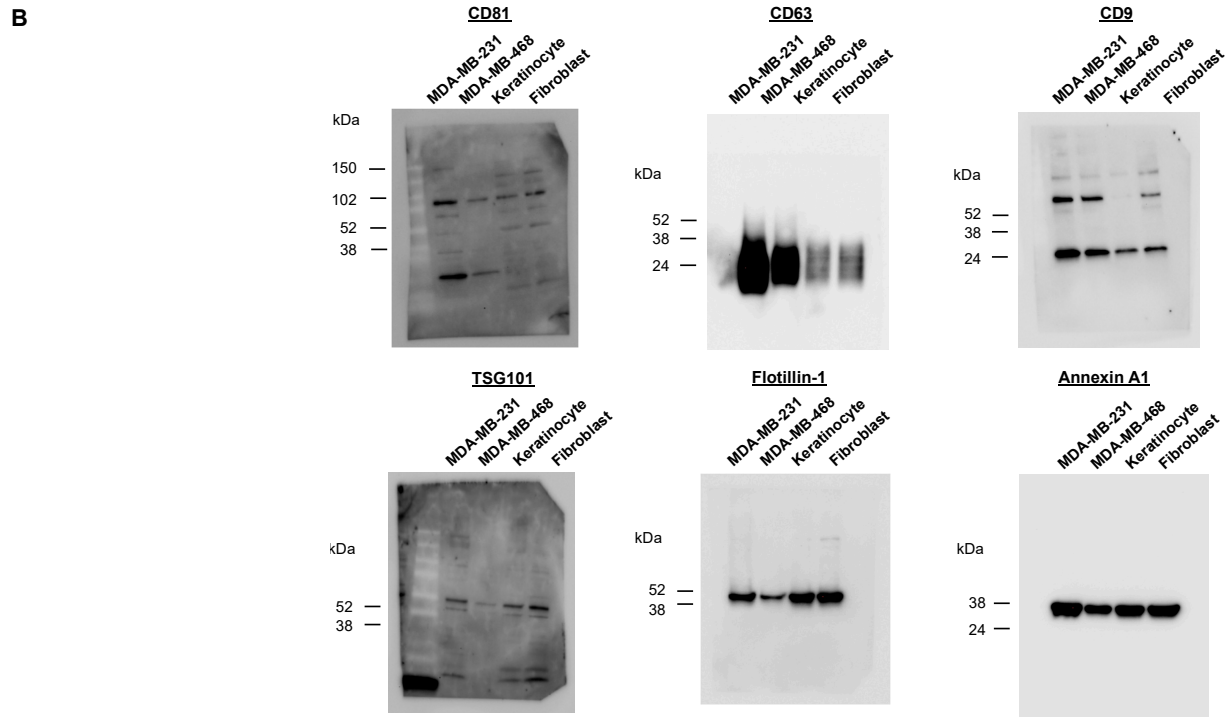


Figure S1. Western blot analysis of EV markers in sEVs and m/lEVs derived from equivalent numbers of parental cells. (A) The expression of EV markers, CD81, CD63, CD9, TSG101, flotillin-1, and annexin A1, in sEV- and m/lEV-enriched fractions. Original images for Figure 1D. (B) EV markers expressed in equal numbers of cancer (MDA-MB-231, MDA-MB-468) and non-cancerous cells (keratinocyte, fibroblast).

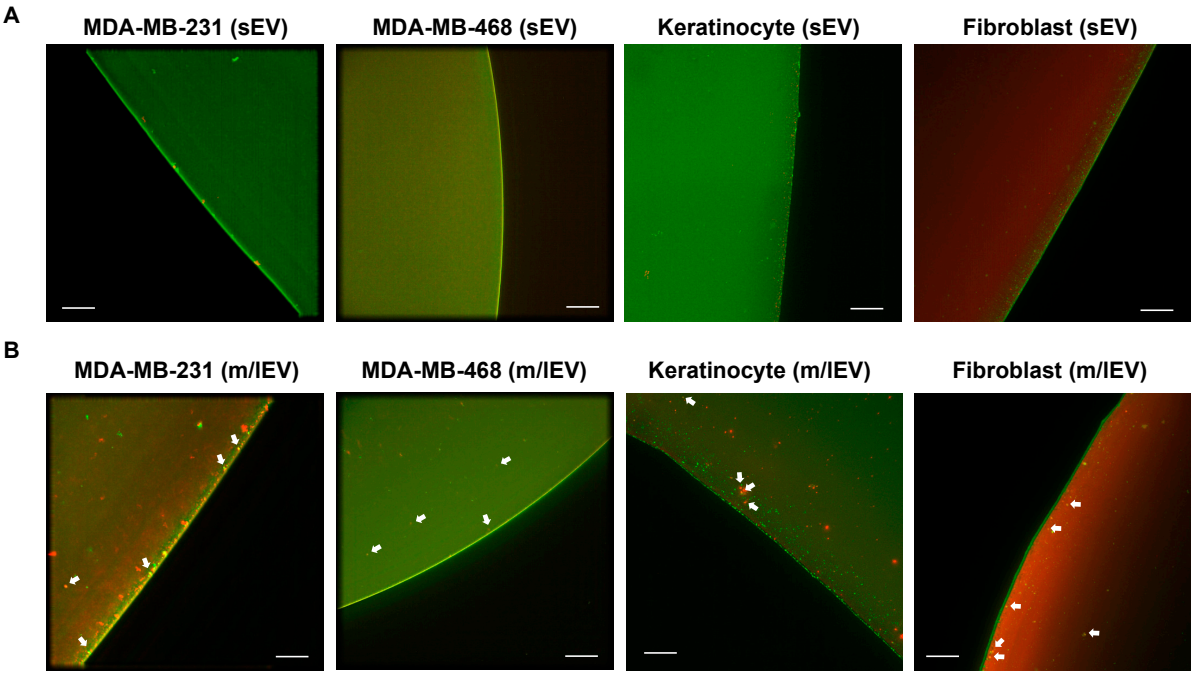


Figure S2. Recognition of EV subpopulations by FITC-GlaS and APC-annexin A5 proteins. (A and B) Dual-staining of sEV- and m/lEV-enriched fractions derived from cancer cells (MDA-MB-231 and MDA-MB-468) and non-cancerous cells (keratinocyte and fibroblast) by FITC-GlaS (green) and APC-annexin A5 (red). Arrows indicate FITC and APC double-positive fluorescent puncta. Scale bar, 10 μm.

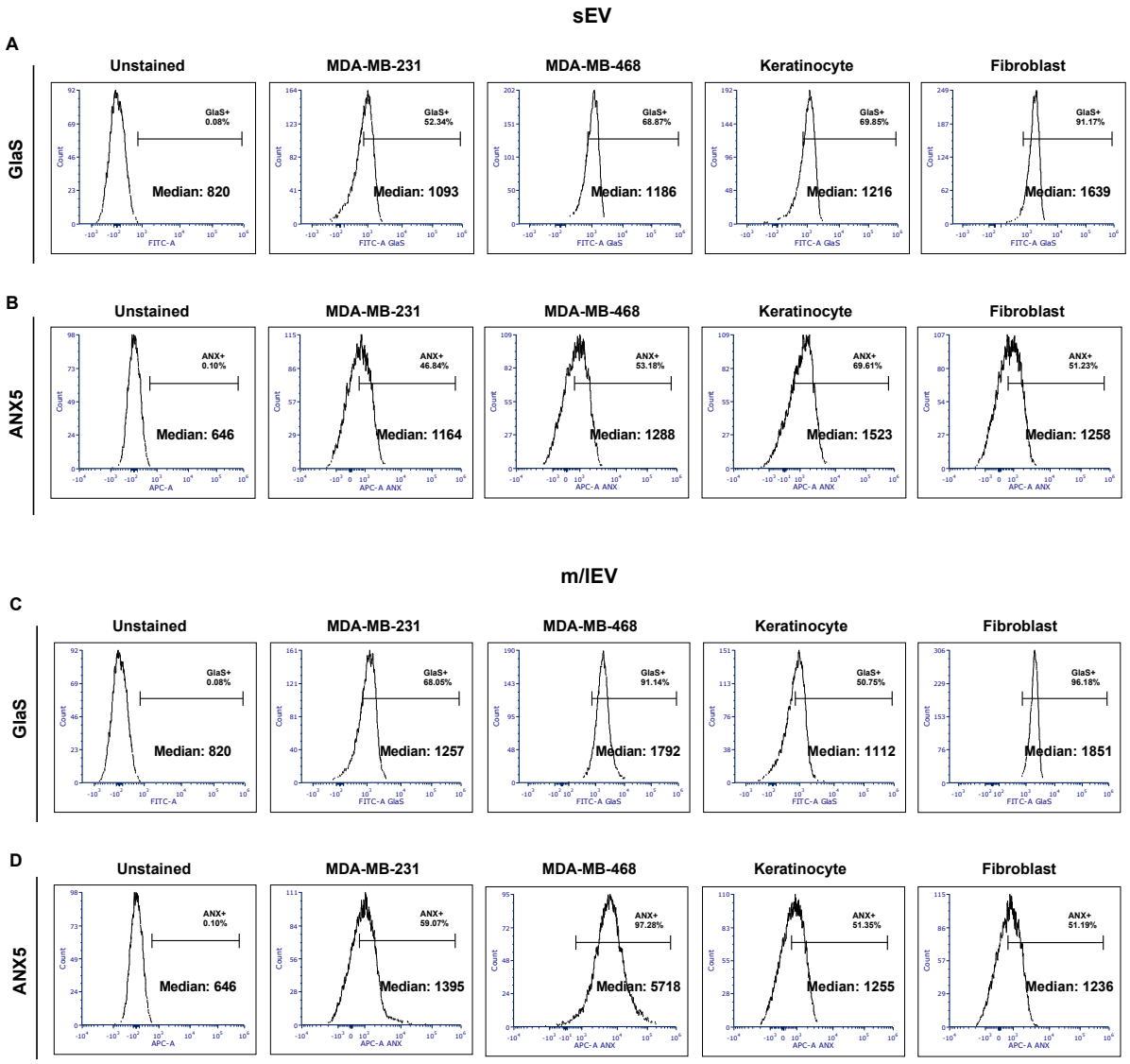
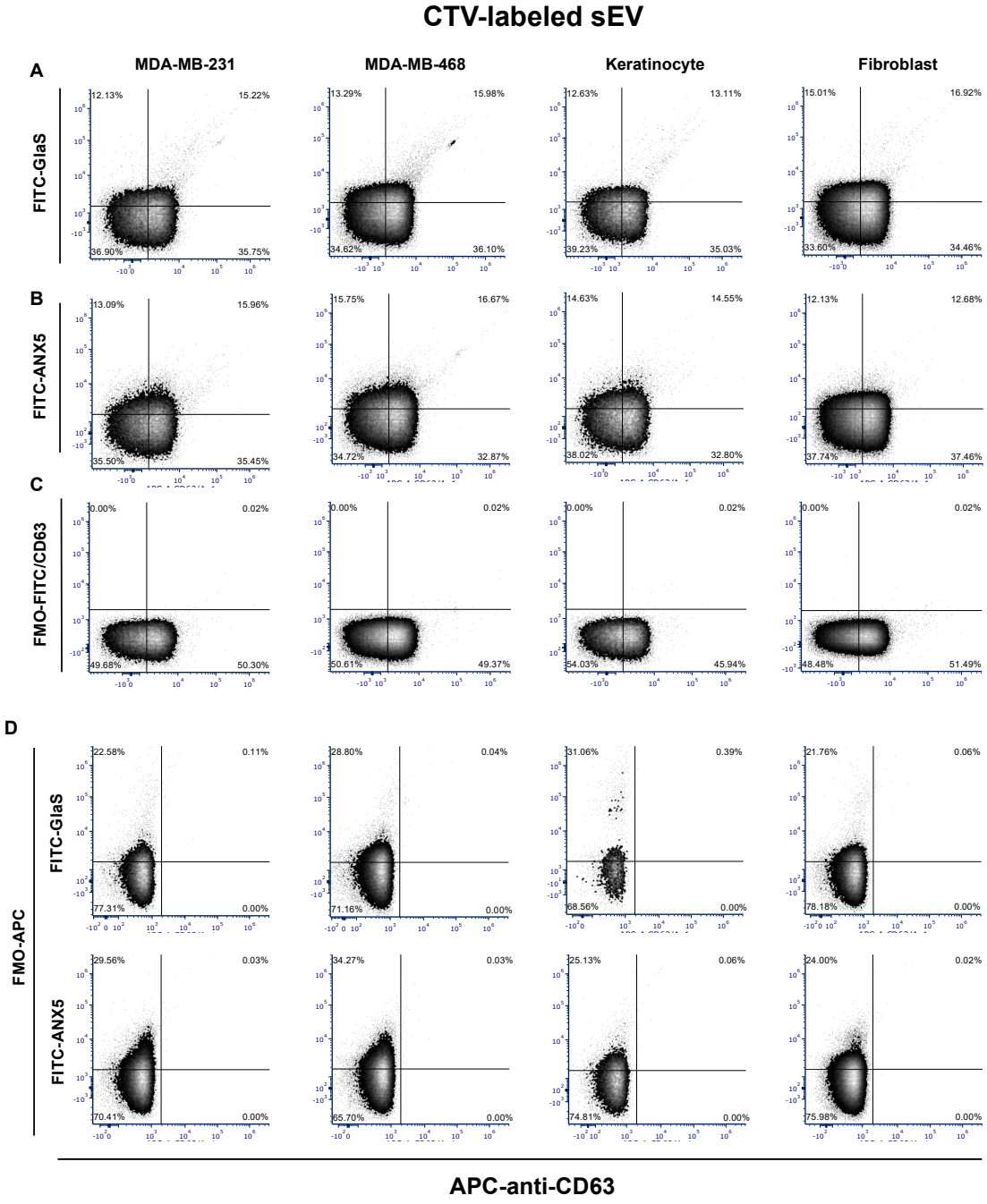


Figure S3. A representative of five analyses of PS exposure on the beads carrying sEVs or m/lEVs derived from cancer and non-cancerous cells. FITC-GlaS or APC-annexin A5 (ANX5) detected PS-exposing sEVs (A and B) or m/lEVs (C and D) on the beads. Marker gates denoting either % annexin A5+ (ANX+) or GlaS+ were set based on FMO control (unstained EV-loaded beads). Each histogram shows the median fluorescence intensity of FITC-GlaS or APC-ANX5 for singlet beads.


CTV-labeled sEV
A
MDA-MB-231
MDA-MB-468
Keratinocyte
Fibroblast
FITC-GlaS
12.13%
15.22%
36.90%
35.75%
13.29%
15.98%
34.62%
36.10%
12.63%
13.11%
39.23%
35.03%
15.01%
16.92%
33.60%
34.46%
B
FITC-ANX5
13.09%
15.96%
35.50%
35.45%
15.75%
16.67%
34.72%
32.87%
14.63%
14.55%
38.02%
32.80%
12.13%
12.68%
37.74%
37.46%
C
FMO-FITC/CD63
0.00%
0.02%
49.68%
50.30%
0.00%
0.02%
50.61%
49.37%
0.00%
0.02%
54.03%
45.94%
0.00%
0.02%
48.48%
51.49%
D
FMO-APC
FITC-GlaS
22.58%
0.11%
77.31%
0.00%
28.80%
0.04%
71.16%
0.00%
31.06%
0.39%
68.56%
0.00%
21.76%
0.06%
78.18%
0.00%
FITC-ANX5
29.56%
0.03%
70.41%
0.00%
34.27%
0.03%
65.70%
0.00%
25.13%
0.06%
74.81%
0.00%
24.00%
0.02%
75.98%
0.00%
APC-anti-CD63

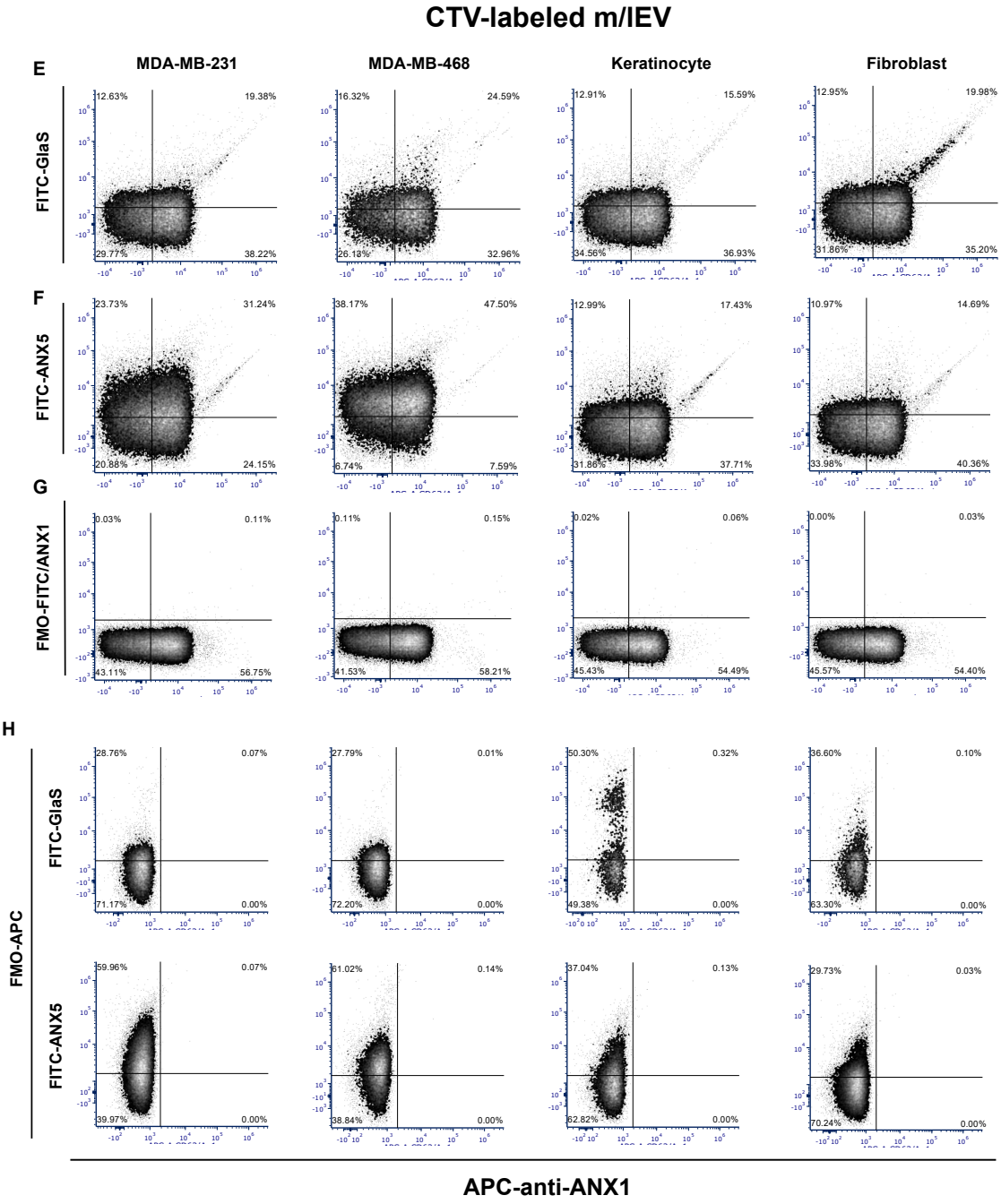


Figure S4. A representative of three independent analyses of PS-exposing sEVs and m/lEVs derived from cancer and non-cancerous cells. EVs were stained with CellTrace Violet (CTV). (A-D) CTV-sEVs were incubated with APC-anti-CD63 antibodies and FITC-GlaS or FITC-annexin A5 (ANX5). (E-H) CTV-m/lEVs were incubated with APC-anti-annexin A1 (ANX1) antibodies and FITC-GlaS or FITC-ANX5. FITC-annexin A5 (ANX5) or FITC-GlaS was evaluated to determine the frequency of individual PS-exposing EVs. Quadrant gates were set based on fluorescence minus one (FMO) controls (C, D, G, and H).